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FULL PAPER

Chemotaxonomic and phylogenetic studies of *Thamnomyces* (Xylariaceae)

Marc Stadler · Jacques Fournier · Thomas Læssøe · Andrzej Chlebicki · Christian Lechat · Fabienne Flessa · Gerhard Rambold · Derek Peršoh

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Abstract The tropical genus *Thamnomyces* is characterized by having wiry, black, brittle stromata and early deliquescent asci, lacking an amyloid apical apparatus. *Thamnomyces* is regarded as a member of the Xylariaceae because the morphology of its ascospores and the anamorphic structures are typical for this family. However, its relationship to other xylariaceous genera remained to be clarified. Cultures of three *Thamnomyces* species were obtained and studied for morphological characters, and their secondary metabolite profiles as inferred from high performance liquid chromatography coupled with mass spectrometric and diode array detection (HPLC–MS/DAD) were also compared. Cultures of *Thamnomyces* closely resembled those of the genera *Daldinia* and *Phylacia* and even produced several secondary metabolite families that are known to be chemotaxonomic markers for the aforementioned genera. These findings were corroborated by a comparison of their 5.8S/ITS nrDNA sequences. We conclude that *Thamnomyces*, *Daldinia*, and *Phylacia* are derived from the same evolutionary lineage, despite these genera differing drastically in their stromatal morphology and anatomy. Along with *Entonaema* and *Rhopalostoma*, these fungi comprise an evolutionarily derived lineage of the hypoxyloid Xylariaceae. A new species of *Thamnomyces* is erected, and preliminary descriptions of three further, potentially new taxa are also provided.

Keywords Chemotaxonomy · Fungi · Metabolomics · Phylogeny · Xylariales

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M. Stadler (✉) · F. Flessa · G. Rambold · D. Peršoh
Department of Mycology, University of Bayreuth, Universitätsstraße 30, 95540 Bayreuth, Germany
e-mail: marc.stadler@t-online.de

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

J. Fournier
Las Muros, 09420 Rimont, France

T. Læssøe
Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen Ø, Denmark

A. Chlebicki
Department of Plant Systematics, Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland

C. Lechat
Ascofrance, 64 Route de Chizé, 79360 Villiers-en-Bois, France

D. Peršoh
Systematische Botanik und Mykologie, Ludwig-Maximilians-Universität München, Menzinger Straße 67, 80638 München, Germany

Introduction

Thamnomyces Ehrenb. 1820, based on *T. chamissonis* Ehrenb., is a genus of stromatic pyrenomycetes, known exclusively from the Neotropics and Africa. Their conspicuous brittle stromata are highly melanized, wiry, and unbranched or dichotomously branched, and bear intercalary or terminal stromatized perithecia or clusters of embedded perithecia. Their asci lack an apical discharge apparatus and are early deliquescent; hence, they can rarely be observed even in freshly collected specimens.

Ehrenberg (1820) also described a second species, *T. annulatus* Ehrenb. [=*Xylaria annulata* (Ehrenb.) Sacc.], which appears not to have been studied since. Ehrenberg, furthermore, accepted *T. hippotrichoides* (Sowerby) Ehrenb. [nowadays often accepted as *X. hippotrichoides* (Sowerby) Sacc.] and "*T. capitatus* (Link) Ehrenb." [=? *Sphaeria capitata* Holmsk., *Cordyceps capitata* (Holmsk.) Link] as members of his new genus whereas "*Chaenocarpus setiformis* Rebent." (basionym *Lichen setosus* Roth 1788) was only questionably accepted in *Thamnomyces*. Fries (1830), Montagne (1840), Berkeley (1856), and Cooke and Massee (in Cooke 1888) published further species of *Thamnomyces*. Meanwhile, Saccardo (1882) had proposed to integrate the genus *Thamnomyces* as a section into *Xylaria*, but contemporary mycologists continued to use the name at generic rank.

Thamnomyces species and certain species of *Xylaria*, which were referred to by Lloyd (1917) as "rhizomorphic Xylarias," with filiform, more or less branched stromata, may be easily confused. However, the aforementioned *Xylaria* species have an internally white stroma, are much less brittle, and have persistent asci with typical amyloid apical apparati. In addition, the entire stroma of *Thamnomyces* is externally delimited by a thin crust, composed of colored granules (which are responsible for the chemical reactions observed in KOH and contain the metabolites we detected by HPLC). This situation is much different from *Xylaria* species in which this external crust is composed of pseudoparenchymatous plectenchyma, and appears more or less carbonized, but never bears pigment granules. Such carbonaceous stromata occur in many Xylariaceae and other ascomycetes, and the resulting blackish color is due to the incorporation of large amounts of melanin in the fungal hyphae. The few stromatal secondary metabolites detected from xylarioid Xylariaceae appear to be located on the stromatal surface and are presumably produced by the anamorphs (cf. Stadler et al. 2008a).

The concept of *Thamnomyces* in the current sense (excluding the "rhizomorphic" *Xylaria* spp.) was probably founded by Möller (1901). He reported detailed microscopic characters of *T. chamissonis*, was the first to observe the germ slit of the ascospores, and even included detailed observations on the development of the stromata in the field. Hennings (1897, 1901, 1902, 1904) erected some new taxa in *Thamnomyces*, and defined a section *Scopimyces* Henn., for the unbranched, non-dendroid species. This section was actually referred to as a "subgenus" by Dennis (1957) and Watling (1962). Dennis (1957) provided a concise revision of *Thamnomyces*, based on examinations of type material. His key is still in use today, and no additional taxa have been described since. Dennis (1957, 1961) also dealt with the affinities of *Thamnomyces* to other pyrenomycetes. He eventually introduced a subfamily "Thamnomycetidae" of the Xylariaceae, which was, however, not validly published. Later, Dennis (1970) even transferred *Thamnomyces* into the Diatrypaceae, where he also accepted it as an informal subfamily. One reason for this classification was that Dennis thought that ascospores of *Thamnomyces* were lacking the germ slit that is typically present in Xylariaceae. However, Samuels and Müller (1980) described a typical xylariaceous anamorph referable to *Nodulisporium* Preuss in *T. chordalis* Fr. and confirmed Möller's observations on the presence of an ascospore germ slit.

This observation confirmed Hawksworth's (1977) suspicion on possible affinities of *Rhopalostroma* D. Hawksw. to *Thamnomyces* and *Phylacia* Lév. Hawksworth and Whalley (1985) and Rodrigues and Samuels (1989) later indeed reported *Nodulisporium*-like anamorphs from the aforementioned two genera. Læssøe (1994) listed *Thamnomyces* as a member of the Xylariaceae, and this opinion is now generally accepted. The peculiar stromatal morphology and the reduced ascal morphology of *Thamnomyces* suggest that it is a derived form of this evolutionary lineage, but its phylogenetic affinities remained unclear. No molecular data based on DNA sequences were hitherto recorded. The cultures obtained by Samuels and Müller (1980) did apparently not survive, and no representative of the genus has been cultured and deposited in a public collection.

In their chemotaxonomic study, Stadler et al. (2004a) demonstrated a high similarity of major stromatal secondary metabolites among *Thamnomyces*, *Daldinia* Ces. & De Not., *Phylacia*, and *Rhopalostroma*. However, their data on *Thamnomyces* were mainly based on old herbarium specimens. The results were not conclusive in all cases, but it was still possible to detect certain chemotaxonomic marker compounds in several specimens collected up to 200 years previously by using high performance liquid chromatography (HPLC), coupled with diode array (DAD) and mass spectrometric (MS) detection. The metabolite profiles of several specimens suggested the presence of artifacts in the stromata.

We have studied several freshly collected specimens over the past years and obtained cultures from three *Thamnomyces* species. This study is concerned with their morphology and secondary metabolite profiles in stromata and cultures. Their phylogenetic affinities as inferred from a comparison of 5.8S/internal transcribed spacer (ITS) nrDNA sequence data with those of other representatives of the family are also addressed.

Materials and methods

General methods used for morphological studies, HPLC profiling, polymerase chain reaction (PCR), and generation and comparison of 5.8S/ITS nrDNA sequence data were described by Bitzer et al. (2008) and Stadler et al. (2008a,b). These articles had involved the in-depth characterization of numerous strains and taxa of the Xylariaceae. Their HPLC profiles and DNA sequence data served for comparison with the strains obtained in the present study. For a more detailed account of the procedure used for identification of known metabolites in crude extracts, assisted by a comprehensive HPLC-based spectral library, see Bitzer et al. (2007). Stromatal HPLC profiles of several recently collected mature and immature specimens were compared with type and authentic specimens previously studied by Stadler et al. (2004a) to evaluate possible deviations in secondary metabolite production at different stages of stromatal development and to find correlations between morphological and chemotaxonomic features. Stromatal pigment colors in KOH of *Thamnomyces* were also studied for the first time. Color codes follow Rayner (1970), and data can therefore be compared with those presented in other recent papers on the hypoxyloid Xylariaceae (e.g., Ju et al. 1997; Stadler et al. 2005).

Mycelial cultures of *Thamnomyces* species were obtained from apparently intact perithecia after dissecting them from the stroma using a scalpel, then crushing them on a microscopic slide and diluting the spore masses in 0.9% NaCl. Portions of the suspensions were plated on YMG (yeast–malt–glucose) agar plates. After 2 days, mycelia were transferred to new agar plates. This procedure allowed for isolation of genuine cultures from specimens for which the conventional direct plating of perithecial contents had failed because several other fungi had grown instead of the genuine anamorphs. Interestingly, some of the stromata of *Phylacia poculiformis* (Kunze) Mont. CLL 8105, of which cultures were isolated for comparison from ascomata, were contaminated by a *Xylaria* sp., as had previously been observed by Bitzer et al. (2008) in two other *Phylacia* specimens. This *Xylaria* culture is deposited with CBS and MUCL. All other specimens and cultures used in this study were obtained from or deposited in public herbaria, abbreviated as proposed in the Index herbariorum (http://www.indexherbariorum.org). Details on the addresses of these institutions, including CBS and MUCL, can be obtained from this website.

The cultures were subjected to fermentation in different culture media and compared with numerous other Xylariaceae cultures studied previously by Bitzer et al. (2008). Ethyl acetate extracts were prepared from aliquots of the shake flask fermentations and analyzed by HPLC–UV/Vis and HPLC–MS in a similar manner as in the preceding study (Bitzer et al. 2008). In concordance with this standardized methodology, the cultures were propagated for 7–8 days until the free glucose had been consumed and the secondary metabolites had accumulated.

To obtain stromatal HPLC profiles, perithecia from mature stromata (or stromatal tips of immature stromata, respectively) were extracted with methanol and the extracts studied by HPLC–DAD and HPLC–MS. The resulting spectra and chromatograms were stored in a database. The results were compared to the data previously obtained by Stadler et al. (2004a). Standards and HPLC profiling data, including the retention times, UV–visible spectra as recorded by diode array detection, and mass spectrometry based on electrospray detection were used to identify known metabolites of Xylariaceae. The chemical structures of these compounds, which were available as standards, are depicted in Fig. 1.

We also attempted to identify a characteristic stromatal pigment that was detected in certain *Thamnomyces* spp. and previously in other Xylariaceae. This pigment could not be isolated by HPLC from *Daldinia petriniae* Y.M. Ju, J.D. Rogers & San Martín because of its apparent instability, as previously reported by Stadler et al. (2001). Mass spectrometry revealed this pigment to have a similar molecular mass as the binaphthalene tetrol (**1**). As oxidized binaphthalenes were previously reported from *Daldinia* (Allport and Bu'Lock 1958), a simple oxidation was performed: 1 mg of the pure binaphthalene tetrol (**1**) was added to a solution of 1 M potassium permanganate ($KMnO_4$) in 50% aqueous methanol and incubated overnight. The resulting mixture was analyzed by HPLC. The results are discussed further below.

A selection of nrDNA-ITS sequence data from representative Xylariaceae, the majority of which were also used in the preceding study (Bitzer et al. 2008), have been compiled in a phylogenetic tree with those of the newly obtained data of *Phylacia poculiformis* and *Thamnomyces* species. As representatives of Xylariaceae in the phylogenetic alignment, three strains were newly sequenced and their DNA sequence data deposited in GenBank: accession no. GQ355621; made from *Daldinia gelatinoides* strain MUCL 46173; accession no. FN428829 from *Nemania serpens* strain CBS 533.72, and accession no. FN428832 from *Rosellinia aquila* strain MUCL 51703.

The methodology applied for sequencing was outlined by Triebel et al. (2005). The obtained sequences were added to an existing manually curated alignment (Peršoh et al. 2009). Phylogenetic relationships were reconstructed using RAxML v. 7.0.3 (Stamatakis 2006) based on the reliably alignable positions 26–64, 117–142, 153–165, 172–335, 360–380, 389–439, and 445–461 according to AM993138 (*Xylaria hypoxylon*). Support values resulting

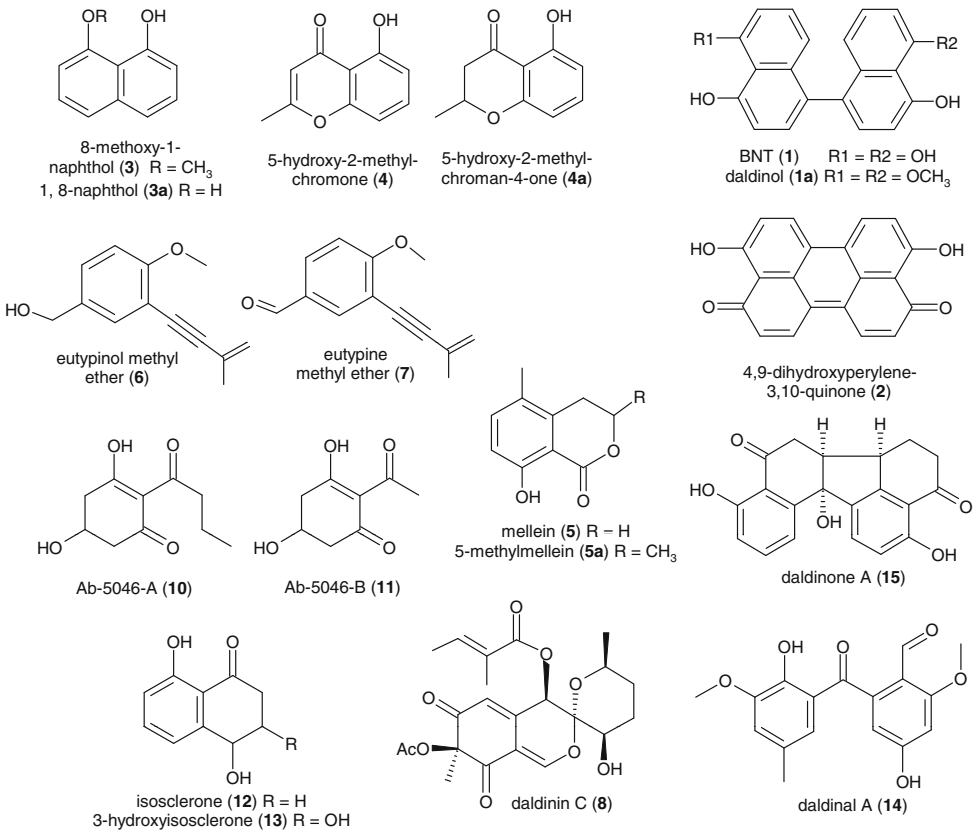

Fig. 1 Chemical structures of characteristic metabolites of Xylariaceae, which are referred to in the text by the *bold* given here. Compounds **5** and **5a** are absent in *Daldinia*, *Phylacia*, and *Thamnomyces*, while compounds **1**, **2**, **8**, and **14**, **15** are frequently encountered in their stromata, and the remainder of the compounds depicted are produced by the cultures of these genera. *BNT*, binaphthalene tetrol

from 500 bootstrap replicates were drawn onto the best-scoring maximum-likelihood (ML) tree found. The GTR-CAT model of substitution was applied for both bootstrap analysis and search for the most likely tree.

Results

Stromatal HPLC profiles of *T. chamissonis* and of fresh material of several other species were recorded for the first time. Moreover, cultures of three *Thamnomyces* species were obtained from perithecial contents of specimens that showed the characteristics of the genus and species as reported by Dennis (1957, 1961). A new taxon of *Thamnomyces* was also found, and a description based on the only known collection is included. This species and three further potentially new *Thamnomyces* spp. are illustrated in supplementary material. A culture of *Phylacia poculiformis*, showing the characteristics of the species as outlined by Medel et al. (2006), was also studied for comparison.

In the following, we first summarize our findings on HPLC profiles (Fig. 2) and the teleomorphic morphology of *Thamnomyces* species, followed by a key. The morphological characteristics of four taxa are also illustrated (Figs. 3, 4, 5, 6). The second part presents the morphological and chemotaxonomic characteristics of the cultures (Figs. 7, 8, 9). HPLC profiling data and ethyl acetate extracts derived from fermentations in yeast–malt–glucose (YMG) and HLX media (Bitzer et al. 2008) are illustrated in Fig. 9, and the results on the individual specimens are summarized in Table 1. Finally, a molecular phylogeny, comparing the 5.8S/ITS nrDNA sequence data of *Thamnomyces* to those of representative species of other Xylariaceae, is presented (Fig. 10).

Morphological descriptions of the teleomorphs and chemotaxonomic characteristics

Thamnomyces chamissonis Ehrenb. 1820

Specimen examined: BRAZIL, Santa Catarina, Blumenau, on wood (ex herb. Möller, label reading "*Thamnomyces chamissoides* Ehrenberg. Material aus dem Schaumuseum") (B700012255).

Notes: One of Möller's specimens of *T. chamissonis* was part of the public exhibition, which was not destroyed by fire during WW II, in contrast to the majority of specimens located in B (cf. Stadler et al. 2008a). Detailed collection data were not given by Möller (1901), who provided an excellent photograph of the stromata but did not cite a particular specimen. Hennings (1902) made an inventory of Möller's Brazilian specimens and cited three collections from Blumenau in Santa Catarina state. Therefore, it is to be assumed that the material still extant was collected there

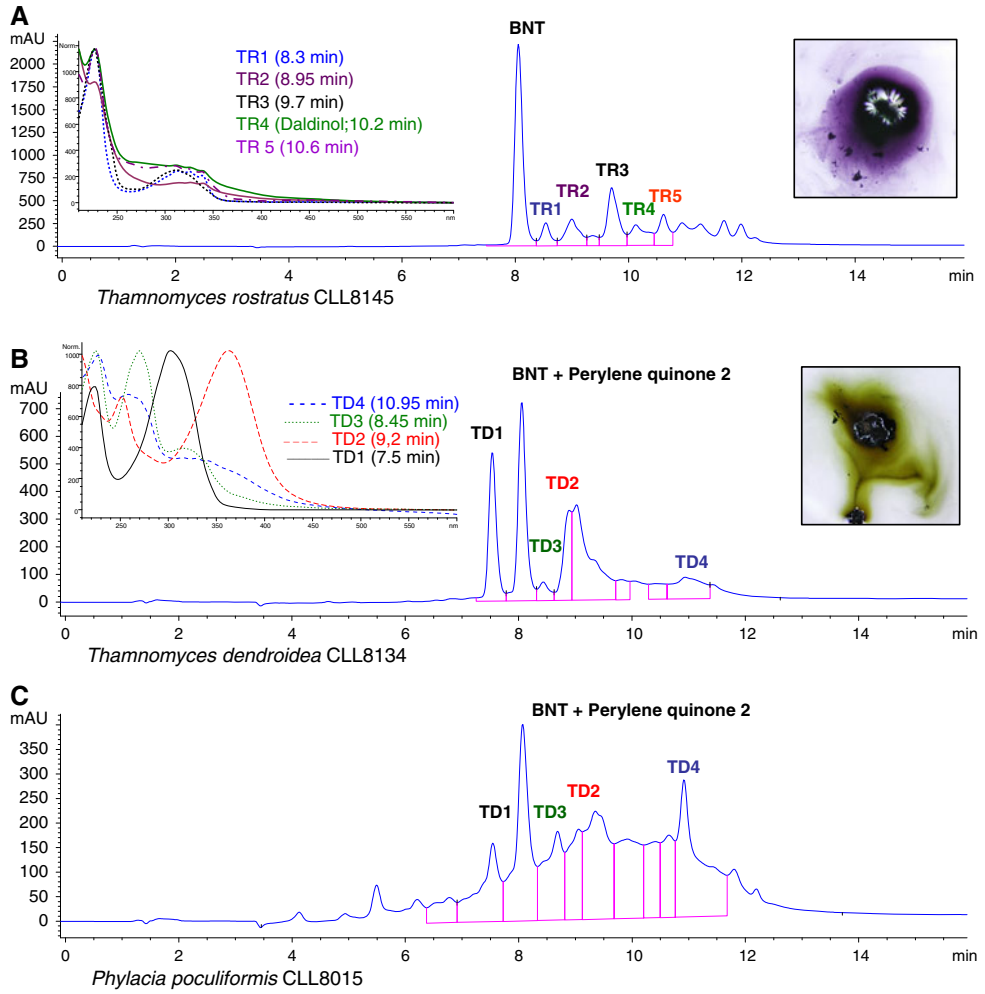

Fig. 2 HPLC-UV chromatograms (210 nm) of stromatal methanol extracts derived from two *Thamnomyces* spp. and *Phylacia poculiformis*, diode array (DAD) spectra of their major detectable components, and color reactions of stromatized perithecia of the respective specimens in KOH. *Thamnomyces chordalis* CLL8145 (**a**) contains the binaphthalene tetrol (**1**) and several derivatives (TR1–TR5) that are probably also naphthalenes as judged from their highly similar DAD spectra. *Thamnomyces dendroidea* CLL8134 (**b**) and *Phylacia poculiformis* CLL8015 (**c**), however, contain compound (**1**) and several other common, yet unidentified metabolites (TD1–TD4), indicating their close chemotaxonomic relationships, which are also reflected by similar stromatal pigment colors in KOH. The stromatal pigments of *P. poculiformis* have a similar color as those of *T. dendroidea* and *T. camerunensis*, and *T. chamissonis* had similar stromatal HPLC profiles as *T. dendroidea* (data not shown)

as well. The specimen we studied was obviously photographed by Lloyd (1917) during a trip to European herbaria. As pointed out by Dennis (1957), Lloyd (1920) later even erected the superfluous taxon "*T. macrospora*" based on the material in B, assuming that the dimensions reported for the spore-bearing parts in the asci of Möller's *T. chamissonis* had actually referred to its ascospore sizes. We did not observe asci in the specimen in B, but the few intact perithecia contained the typical ascospores of *T. chamissonis* as reported by Möller (1901). Unfortunately, fresh material of the type species of *Thamnomyces* has not been encountered for comparison, and the fungus should be searched for in southern Brazil. De Meijer (2006) has, in fact, reported several specimens from the state of Paraná. A specimen reported as *T. chamissonis* from near Iquitos in Peru can probably be referred to *T. dendroidea* (Kobayasi 1982), leaving, at least at present, *T. chamissonis* as an endemic of the "Mata Atlantica" of southeastern Brazil. The HPLC profile of the old Möller specimen was very similar to those of *T. dendroidea* (Fig. 2) and *T. camerunensis.*

Thamnomyces chordalis Fr. 1830 Fig. 3

Syn.: *Thamnomyces rostratus* Mont. 1840

Specimens examined: BRAZIL: Amazonas, Manaus, Jurua-Miry, on dead wood, 1900/1901, coll. E. Ule 2857, *Appendix Mycothecae brasilensis* 29 (M-0057780). ECUADOR: Prov. Pichincha, Los Bancos, Los Bancos—St.

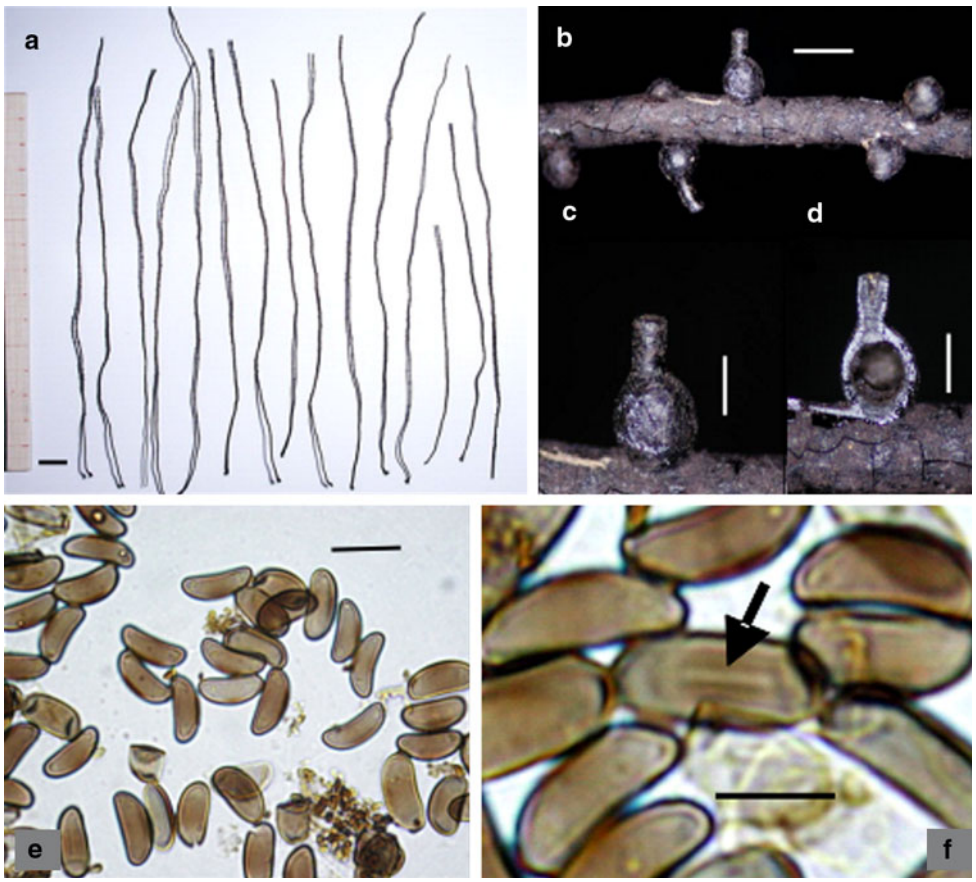

Fig. 3 Morphological characteristics of *Thamnomyces chordalis* specimen SM3 (LIP, French Guiana). **a** Stromatal habit. **b**, **c** Rostrate, stromatized perithecia. **d** Section through stromatized perithecium. **e**, **f** Micrographs. **e** Ascospores. **f** Ascospore showing germ slit, indicated by *arrow*. *Bars* **a** 1 cm; **b** 1 mm; **c**, **d** 200 μm; **e** 10 μm; **f** 5 μm

Domingo Rd, Finca El Encanto, at Río Blanco, 0°0′20″ S, 78°56′30″ W, 770 m, 10 August 2004, coll. T. Læssøe, C. Padilla, T. Sanjuan, M. Villegas & R. Batallas, TL-11801 (QCNE, C; stroma immature). Prov. Tungurahua, Hacienda San Antonio near Baños, on tree trunk, 7 January 1938, coll. H. Sydow, Petrak, Crypt. Exs. 4649 (B700014203, M-005778). FRENCH GUIANA: St. Laurent de Maroni, Balaté, 24 August 1952, coll. R. Heim (PC 0096443). Saul, on trunk, 27 March 2006, coll. J.L. Cheype SM3 (LIP). Sinnamary, Paracou, CIRAD plot, 26 February 2007, coll. C. Lechat CLL 7036 (LIP). Cayenne, Matoury, sentier de La Mirande, on dead wood, 10 May 2008, coll. C. Lechat CLL 8145 (LIP, culture MUCL 51827). GUYANA: ex herb. Cooke [K, designation no. K(M) 110624—holotype]. VENEZUELA: Amazon Territory, Neblina Base Camp on Río Baria (Río Mawarinuma), dead standing tree, 28 January 1985, coll. A. Rossman 2408 (BPI 1100736).

Notes: There is a problem with segregation of *T. chordalis* and *T. rostratus*, which Dennis (1957) could not safely discriminate, based on type and authentic material in public herbaria. He argued that *T. chordalis* was originally described by Fries to have "globose" ascospores (vs. reniform ones in the type material of *T. rostratus*), but still did not key out the two species separately because immature stromata of *T. rostratus* resemble the type of *T. chordalis*. We have therefore decided to follow Samuels and Müller (1980) who referred to a fertile *Thamnomyces* with rostrate perithecia as *T. chordalis*. They synonymized it with *T. rostratus*, even though they clearly showed by scanning electron microscopy (SEM) that the material they studied had reniform ascospores possessing a germ slit. It cannot be excluded that a fungus of this type with globose ascospores as reported by Fries might eventually be found. To finally solve this matter, it could become feasible to destroy one of the intact perithecia mentioned by Dennis (1957), who only superficially studied what was left of the *T. chordalis* type material in UPS. However, as outlined further below, the situation is much more complicated: the *T. chordalis* group apparently represents a species complex, which cannot be resolved in the course of this study. The status of *T. chordalis* could be clarified as additional material of the "aberrant" forms described further below becomes available; this will also afford an on-site study of

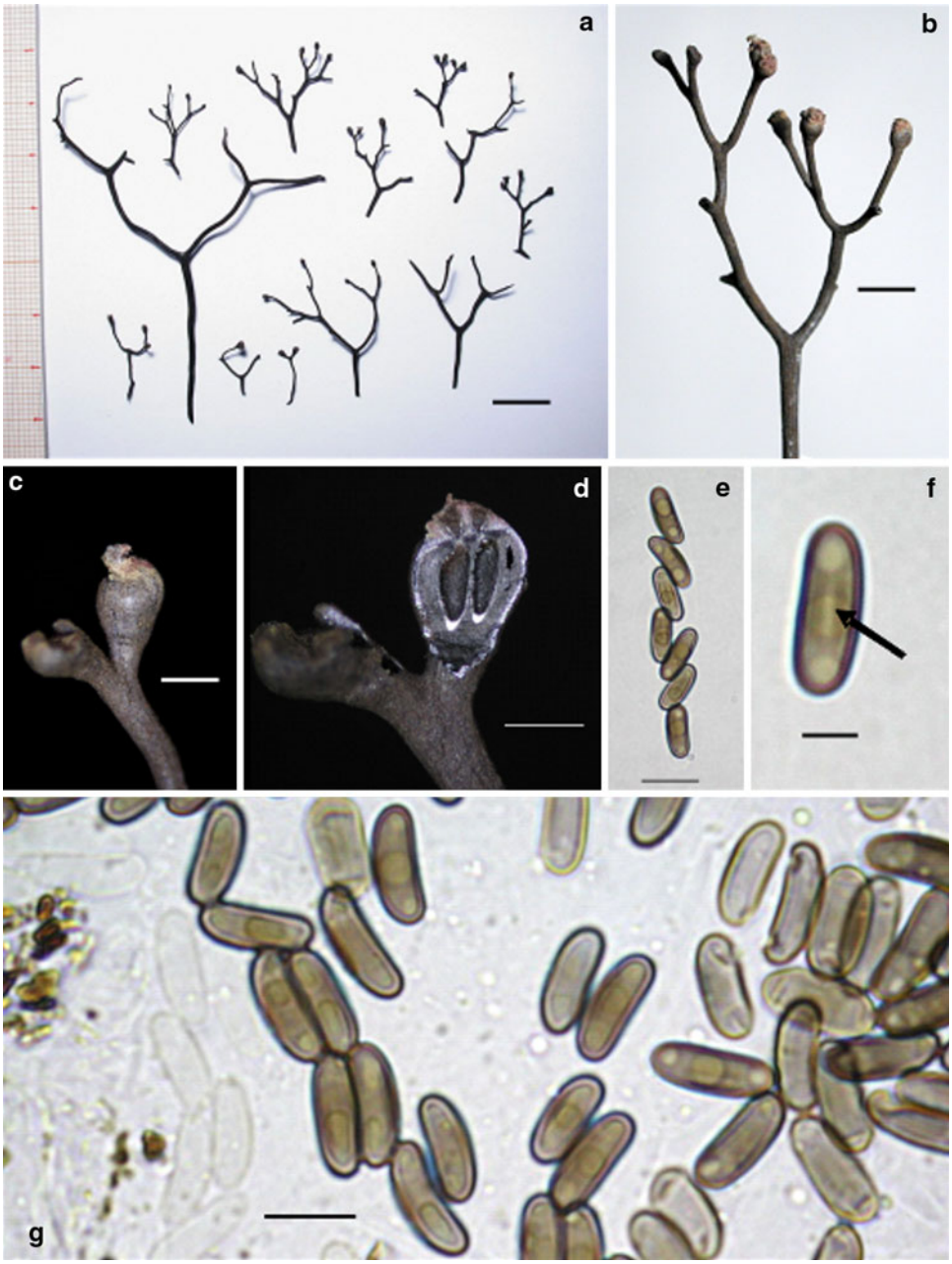

Fig. 4 Morphological characteristics of *Thamnomyces dendroidea* specimen CLL 5134 (LIP). **a**, **b** Stromatal habit. **c** Fertile stromatal tips. **d** Section through multiperitheciate fertile stromatal tip. **e–g** Micrographs. **e** Ascospores in ascus. **f** Ascospore showing a germ slit, indicated by an *arrow*. **g** Ascospores. *Bars* **a** 1 cm; **b** 100 μm; **c**, **d** 100 μm; **e**, **g** 10 μm; **f** 5 μm

the type material, which cannot be received on loan from UPS.

In agreement with a previous study (Stadler et al. 2004a), several additional specimens correspond to the type specimen of *T. rostratus* and to the description by Samuels and Müller (1980) with respect to their morphological characters, as already listed. These specimens contained the binaphthalene tetrol (**1**) and further compounds with similar UV–Vis spectra that are presumably also naphthalene derivatives. Their stromatal pigments in KOH were accordingly purple, or absent in the case of some old specimens. Mature and immature material of this morphotype did not differ much in their HPLC profiles, always revealing binaphthalenes, albeit in fresh specimens the purple stromatal pigments were found more prominent. In old material, such pigments could hardly be observed, but it was still possible to detect the binaphthalenes by HPLC–MS. Cultures, which showed the anamorphic characteristics, including the conidiogenous structures as described by Samuels and Müller (1980), were obtained from all specimens collected in French Guiana (conidia and conidiogenous cells are depicted in Fig. 8). The mycelia lost their viability very soon, unless they were transferred onto new culture medium very frequently, possibly owing

Fig. 5 Morphological characteristics of *Thamnomyces camerunensis* specimen KRAM F 56276. **a** Stromata in natural habitat. **b** Stromatal habit. **c** Fertile stromatal tip. **d** Ascospores (×1000). *Bars* **b** 1 cm; **c** 1 m; **d** 10 μm

to the production of toxic metabolites that they did not tolerate. A culture of specimen CLL 8145 was finally kept stable, which was accomplished by preservation of the young mycelium in 10% glycerol, and deposited with MUCL. San Martín Gonzalez and Rogers (1995) also cultured Mexican material, but no anamorph stages developed, and the culture was apparently not deposited in a public collection as reference.

Some other specimens showing similar stromatal morphology as the above listed material, however, did not have purple stromatal pigments. A closer examination revealed that they also showed certain morphological deviations, as has previously been observed in most cases of divergent chemotaxonomic results in groups of closely related hypoxyloid Xylariaceae (cf. Stadler et al. 2004a,b, 2008b). Our evidence points toward their status as additional, undescribed species. However, the material so far available is scanty, and cultures were not obtained. We refrain from a formal description of these fungi, but give a preliminary report on their morphological characteristics to facilitate collection of further material, allowing for a more precise description. Notably, some of these specimens might also have corresponded to the "aberrant" forms of *Thamnomyces* subgenus *Scopimyces* mentioned by Watling (1962), who gave an excellent overview on the morphological variability of this species complex.

(a) ECUADOR: Orellana, Añangu, Río Napo, tropical rainforest, terra firme, on rotten wood, April–May 1983, coll. T. Læssøe AAU 46385 (C).

Notes: This collection deviates from typical *T. chordalis* by several characters including thinner stromata, 0.6–0.75 mm diameter, smaller perithecial contours that are

Fig. 6 Morphological characteristics of *Thamnomyces chocoënsis* (from holotype). **a** Stromatal habit. **b** Fertile uniperitheciate stromatal tip. **c** Section through apex showing uniperitheciate condition. **d**, **f** Micrographs. **d** Ascospores in ascus (in Melzers reagent, demonstrating lack of amyloid ascal apical apparatus). **e** Ascospores. **f** Ascospores, showing germ slits, indicated by an *arrow* in one of the spores. *Bars* **a** 1 cm; **b**, **c** 200 μm; **d**, **f** 10 μm

less exposed, hazel (88) pigments in 10% KOH, and more slender ascospores, averaging 8.5 × 3 μm, and apparently lacking a germ slit. HPLC profiling revealed a similar profile as in *T. dendroidea* (cf. Fig. 2), which explains the stromatal pigment colors in KOH.

(b) ECUADOR: Orellana, Añangu. south bank of Río Napo, 95 km downstream from Coca, lowland rainforest, 300 m, rotten dicot wood, June–July 1985, coll. T. Læssøe AAU 59782 (C); Los Rios, Río Palenque, 1983?), coll. J. Hedger 810 (C), stroma immature).

Notes: In contrast with the above specimen, these collections deviate from the typical *T. chordalis* in having stouter filiform stromata, 1–1.5 mm diameter, that are at times branched close to the base and bear larger, stromatized perithecial contours 1.2–2.4 mm high × 0.6–0.8 mm diameter; in 10% KOH the stromata yield faintly hazel (88) pigments, and ascospores (8.5–10 × 4.5–5 μm) in the mature material (AAU 59782) are somewhat broader and are short cylindrical versus slightly reniform in *T. chordalis*. The collection J. Hedger 810 likely represents the immature stage of the same taxon. HPLC profiling of both specimens revealed a similar profile as *T. dendroidea* cf. Fig. 2b, with daldinone a (**15** in Fig. 1) being present in the mature AAU 59782 as an additional component. Daldinone A is the typical pigment of *Daldinia placentiformis* (Berk. & M.A. Curtis) Thesis. (Hellwig et al. 2005 as *Hypoxylon placentiforme*), and several species of *Annulohypoxylon* (Quang et al. 2005 as *Hypoxylon* sect. *Annulata*).

(c) ECUADOR: Los Rios, Río Palenque, May 1983, coll. J. Hedger 1059 (C).

Notes: This collection features filiform, unbranched stromata that markedly differ from those of *T. chordalis* in being much shorter (up to 28 mm high), bearing stalked multi-peritheciate heads at tips and uni- to bi-peritheciate heads on the rachis, yielding faint greyish-purple pigments in 10% KOH. Ascospores average 8.5 × 3.5 μm, are short cylindrical with broadly rounded ends, pale to medium brown, smooth, with a fairly conspicuous, straight 3/4 to spore-length germ slit. This combination of characters makes a placement close to any known species of

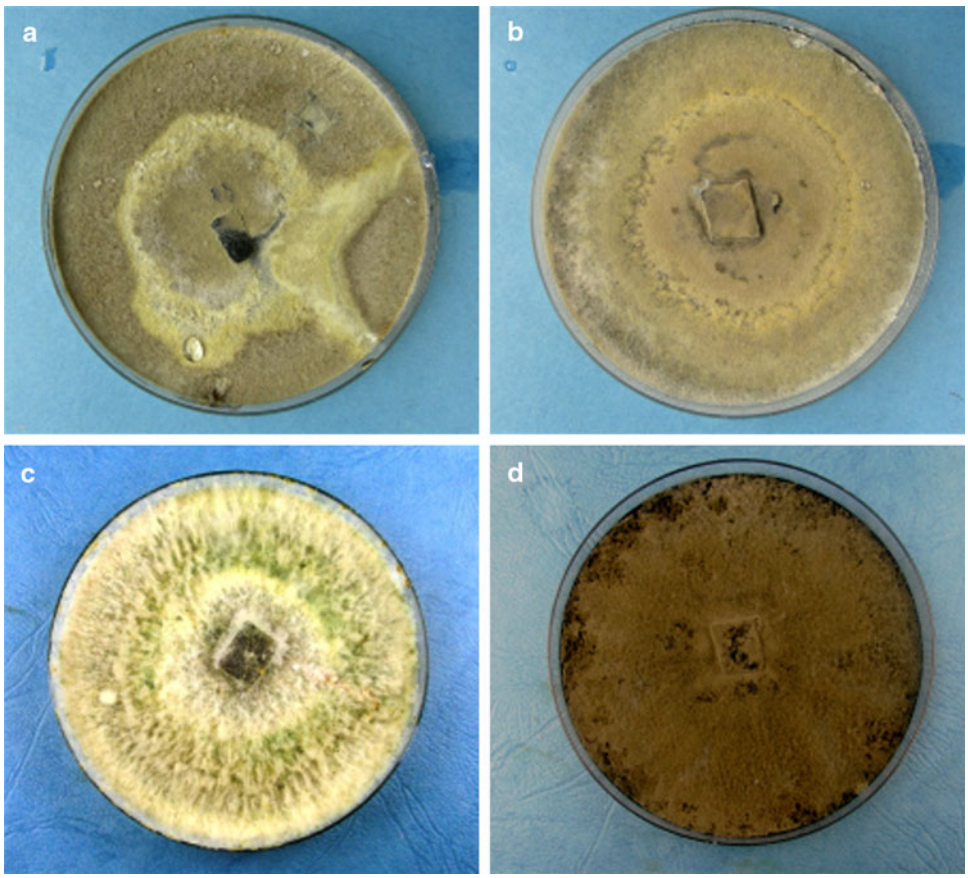

Fig. 7 Morphological characteristics of cultures of *Phylacia* and *Thamnomyces* spp. Difco oatmeal agar (OA) cultures (9-cm plates) **a** *Phylacia poculiformis* strain MUCL 51706 after 2 weeks. **b** *Thamnomyces dendroidea* strain MUCL 51709 after 2 weeks. **c** *Thamnomyces chordalis* strain MUCL 51827 after 10 days. **d** *Thamnomyces camerunensis* strain MUCL 51396 after 3 weeks

Thamnomyces difficult. However, the scantiness of the material does not allow for a formal description.

The morphological characteristics of the specimens described above under (a)–(c) are available as supplementary information.

Thamnomyces dendroidea Cooke & Massee in Cooke 1887 Fig. 4

Specimens examined: Ecuador: Prov. Orellana, Tiputini field station, 0°38′30″ S, 76°90′ W, 190–270 m, on dead wood, 14 July 2004, coll. T. Læssøe, J.H. Petersen, A. Alsgård Jensen, C.A. Padilla & T. Sanjuan, TL-11435 (QCNE, C, stroma immature). FRENCH GUIANA: Roura, Cacao, sentier Molocoï, on dead wood, 8 May 2008, coll. C. Lechat CLL 8134 (LIP, culture CBS 123578, MUCL 51709; GenBank acc. no. FN428831). GUYANA: Upper Demerara River, September 1887, coll. Jenman 4004 ex herb. Cooke [K, designation No. K(M) 110617, holotype].

Notes: This species is peculiar in having many perithecia immersed in the tips of its dendroid stromata; immature material was identified as *T. dendroidea* based on this feature and on a similar HPLC profile to those of the mature specimens. However, it cannot be discriminated by HPLC profiling from other dendroid *Thamnomyces* spp., and has similar olivaceous stromatal pigments in KOH. Microscopically, *T. dendroidea* differs from *T. chamissonis* and *T. camerunensis* in its ascospore size range. Notably, Cooke and Massee (in Cooke 1888) reported subglobose conidia (3–4 µm), which were seen in the recently collected specimens from Ecuador as well.

Thamnomyces fuciformis Berk. 1856

Specimens examined: BRAZIL: Amazonas, Panuré, 1853, Spruce 150, ex herb. Cooke [K, designation no. K(M) 110618, holotype)]. COSTA RICA: San José, Banks of Rio Torres, 4. August 1935, coll. M. Valerio 1988, det. W. W. Diehl (BPI 586718).

Notes: The spores of the Costa Rican material are like those in the holotype: 11–12(–13) × 4–5.5 µm. The HPLC profile of this species resembled those of *T. chordalis* in that the prominent metabolites detected were binaphthalenes. The Costa Rican specimen showed a tinge of purple pigment in KOH, whereas the type specimen yielded none, possibly because of the age of the material. We were unable to find recently collected material of this taxon.

Thamnomyces camerunensis (Henn.) Henn. 1901 Fig. 5

Syn: *T. chamissonis* var. *camerunensis* Henn.

Specimens examined: CAMEROON: Bipindi, on tree trunk, coll. Zenker [comm. P. Hennings, ex herb. Rehm

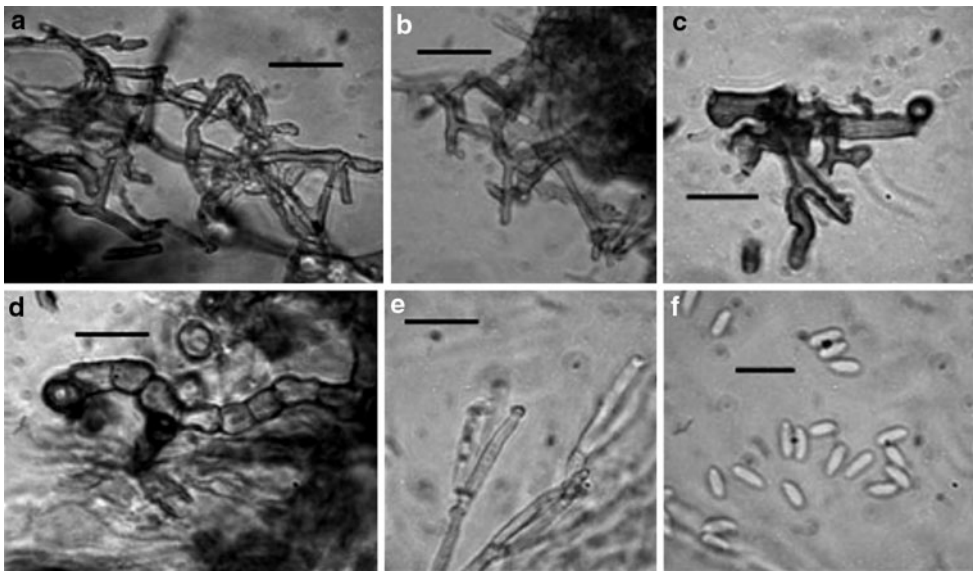

Fig. 8 Microscopic characteristics of cultures of *Phylacia poculiformis* strain MUCL 51706 and *Thamnomyces* spp. **a**–**d** Characteristic inflated hyphae. **a**, **b** *P. poculiformis*. **c**, **d** *Thamnomyces dendroidea*. **e**, **f** Anamorphic structures of *Thamnomyces chordalis*, ex CLL-7036 from Difco OA culture (culture did not survive). **e** Conidiogenous cells of *Nodulisporium*-like conidiophores. **f** Conidia. *Bars* **a**, **b**, **e** 20 μm; **c**, **d**, **f** 10 μm

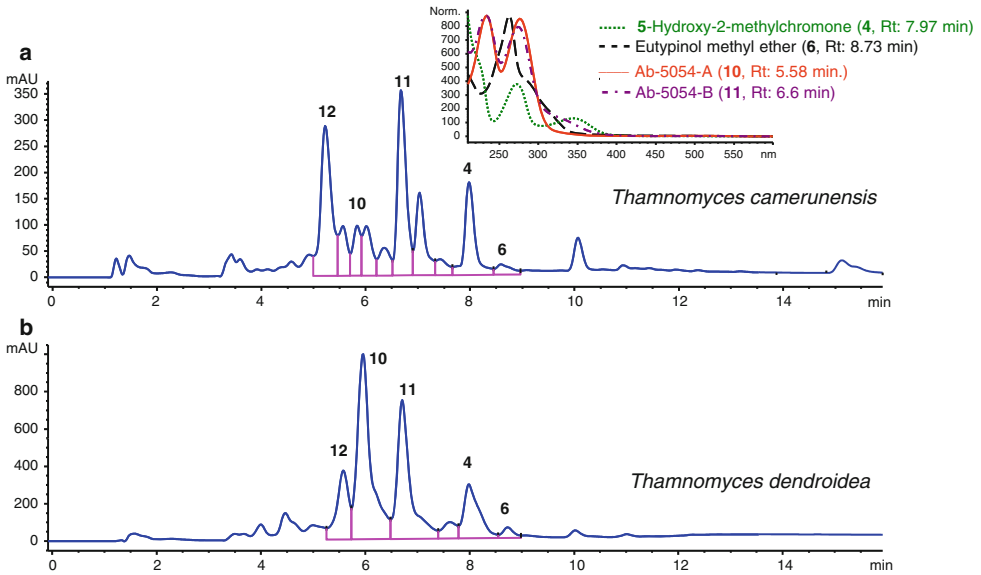

Fig. 9 HPLC chromatograms of ethyl acetate extracts derived from fermentations of *Thamnomyces camerunensis*, strain MUCL 51396 (**a**) (*above*, YMG medium, 144 h) and *Thamnomyces dendroidea*, strain MUCL 51709 (**b**) (*below*, HLX medium, 144 h), and DAD spectra of some metabolites that also occur in cultures of the genera *Daldinia* and *Phylacia*. *Peak numbers* refer to the chemical structures depicted in Fig. 1

1544] (M-0057781; probably type material, see Stadler et al. 2004a); Nikol-Mvon (=Nkol-Melen), forest south of Obala, north of Yaoundé, 4°08′16″ N, 11°31′05″ E, on standing trunk, 3 March 2007, coll. A.L. Njouonkou, J.M. Piatek, and C. & K. Vanky, det. A. Chlebicki (KRAM F 56276, culture MUCL 51396; GenBank acc. no. FN428828). Democratic Republic of the Congo: Bas-Congo district, Kisantu, March 1907, coll. R. Vanderyst (BR, see Dennis 1961 for illustrations of this material).

Notes: This species is apparently restricted in its distribution to the humid climates of tropical Africa. It is the only species of the genus so far not known from the Neotropics. As judged from the collection data of the specimen in M, which basically agrees with the protologue, it is probably a portion of the type specimen. No holotype material of this taxon is extant in B (H. Sipman, personal communication). The HPLC profiles of old and fresh material of *T. camerunensis* were highly similar and also

Table 1 Differences in stromatal morphology and anatomy, and morphology of asci and ascospores that are currently used for definition of generic boundaries in the hypoxyloid Xylariaceae, and characteristic metabolite types produced by representatives of these genera in culture

Genus	Ostioles	Ascal apical ring	Ascospore germ slit	Stromatal anatomy	Metabolites in culture
Phylacia	–	–	–	Essentially homogenous	AB5054 lactones, naphthalenes, chromones and eutypins
Rhopalostroma	+	–	+	Essentially homogenous	AB5054 lactones, naphthalenes, chromones (eutypinol methyl ether only in *D. caldariorum*)
Entonaema	+	+	+	Hollow, liquid filled when fresh	
Daldinia	+	+	+	Internal concentric zones	
Thamnomyces	+	–	+	Wiry, homogeneous	AB5054 lactones, chromones, eutypins
Annulohypoxylon	+	+	+	Essentially homogeneous	Melleins (dihydroisocoumarin derivatives); all other compound classes apparently absent
Hypoxylon	+	+	+		
Pyrenomyxa	–	–	+	Essentially homogeneous	Melleins (dihydroisocoumarin derivatives); all other compound classes apparently absent
Biscogniauxia, *Camillea*	+	+	+	Bipartite	Melleins (dihydroisocoumarin derivatives); all other compound classes apparently absent

+, present; –, absent

agreed with those of *T. dendroidea* (see Fig. 2) and *T. chamissonis*. In all specimens studied, the binaphthalene tetrol (**1**) was present as the major metabolite, overlaid by another unknown, specific pigment (**2**), even in the fresh specimen. Stromatal pigments in KOH were determined to be olivaceous (48) or grey olivaceous (107). As already stated by Stadler et al. (2004a), this HPLC profile is reminiscent of that of certain species in *Daldinia*, such as *D. lloydii* Y.M. Ju, J.D. Rogers & F. San Martín and material referred to as *D. petriniae* by Stadler et al. (2001). A minor metabolite detected only by HPLC–MS in the stromata of all dendroid *Thamnomyces* spp. and in *P. poculiformis* was reminiscent of the azaphilone, daldinin C (**8** in Fig. 1), previously reported by Hashimoto and Asakawa (1998) in a specimen that was later shown to correspond to *D. childiae* J.D. Rogers & Y.M. Ju, by Stadler et al. (2001). Daldinin C is also present as a minor component in stromata of *Hypoxylon rubiginosum* (Pers.) Fr. and represents a major constituent of the stromata of *H. fuscum* (Pers.) Fr. and a range of other *Hypoxylon* spp. (Stadler et al. 2008b).

Thamnomyces chocoënsis Læssøe, sp. nov. Fig. 6

Mycobank: MB 513277

Etymology: For the type locality, the Chocó region of Ecuador and Colombia, which is a well-known center of endemism.

Stromata filiformia, irregulariter ramosa, ad 100 mm longa × 0.4–0.5 mm diametro, apicibus ampullaeformibus (2–2.2 × 0.9–1 mm) uniperitheciatis, cum granulis subsuperficialibus melleis vel isabellinis in KOH dissolutis. Ascosporae unicellulares, brunneae, plusminusqve cylindratae, 8.5–9.5 × 3.8–4.2 µm, rima germinalis recta 1/2–2/3 longitudinis sporae praeditae.

Typus: ECUADOR: Pichincha, Los Bancos, Los Bancos—St. Domingo Rd., Finca El Encanto, at Río Blanco, 0°0′20″ S, 78°56′30″ W, 770 m, 10 August 2004, coll. T. Læssøe, C. Padilla, T. Sanjuan, M. Villegas, & R. Batallas TL-11800 (QCNE, holotype; C, isotype).

Stromata filiform, 0.4–0.5 mm diameter, up to 100 mm high mostly less than 60 mm, more or less contorted, simple to irregularly ramified, with branches very long and with single terminal, swollen, uniperitheciate heads, 2.0–2.2 mm high × 0.9–1.0 mm diameter, more or less flask shaped with a truncate apex, melanized, hard-textured and brittle, with a thin layer just beneath the surface composed of yellow-brown and orange-brown granules yielding dilute hazel (88) to isabelline (65) pigments in KOH. Stromatal surface dull blackish, annellate in places. Stromatized perithecia 0.7–0.8 mm diameter × 0.9–1.0 mm high. Ostioles umbilicate, filled with a cylindrical plug of white tissue. Paraphyses filiform, copious. Asci evanescent, cylindrical to fusiform on a filiform stipe, originating from long ascogenous hyphae, apparently without apical apparatus. Ascospores 8.5–9.5 × 3.8–4.2 µm, pale brown to brown, smooth walled, equilaterally cylindrical with broadly rounded ends to rarely inequilateral with one side slightly convex and the other slightly concave, with a straight to slightly oblique germ slit 1/2–2/3 of spore length, with perispore indehiscent in KOH. No cultures obtained.

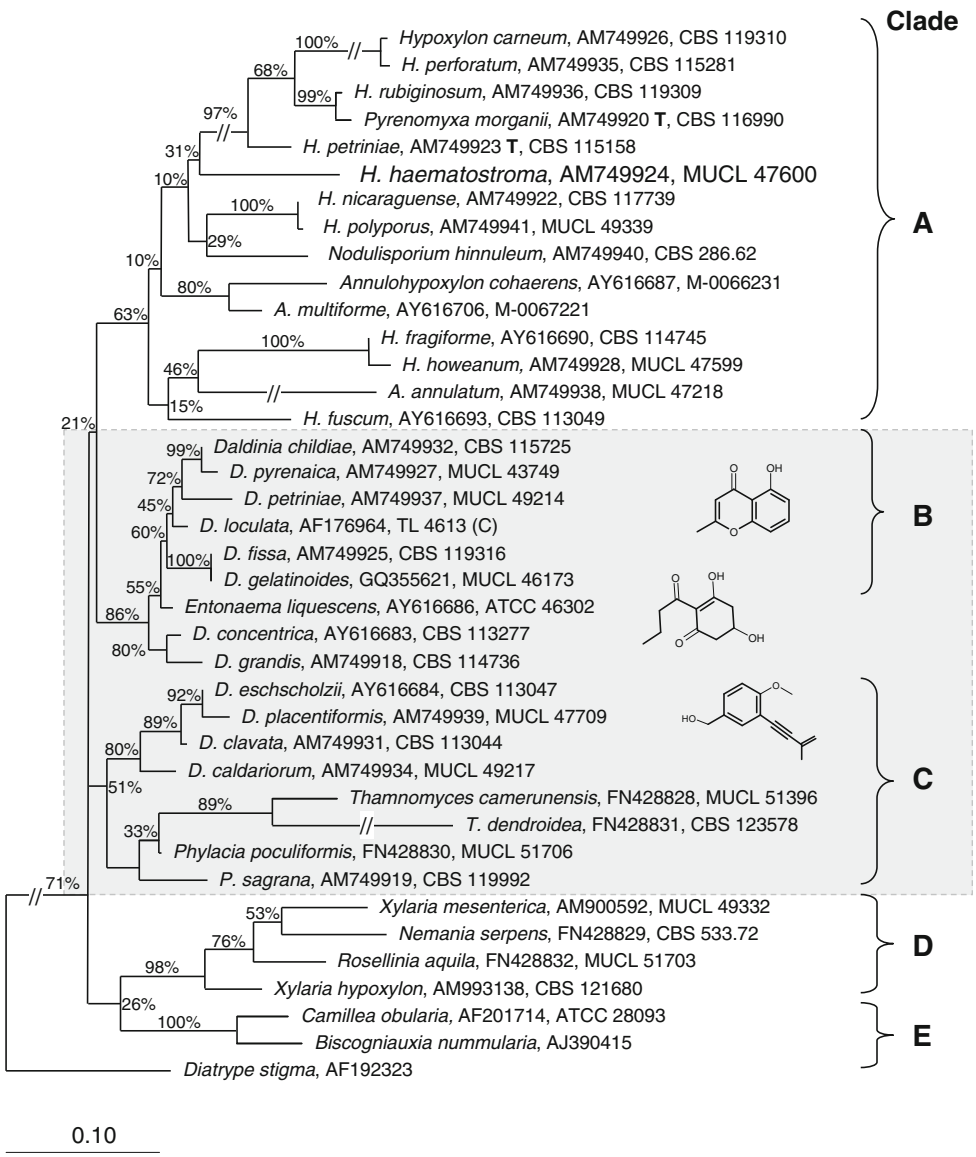

Fig. 10 Phylogenetic relationships among Xylariaceae as inferred from internal transcribed spacer (ITS) nrDNA sequence data. Clades **a**–**e** indicate major groupings, as referred to in the text. Bootstrap support values, from 500 RAxML replicates, are assigned to the tree topology of the most likely tree found by RAxML. Taxon names are followed by the GenBank accession no. of the sequences (**a** "**T**" indicating type strains) and the culture collection and herbarium access numbers (if available). Selected long branches were bisected in length (//). The chemical structures of the characteristic secondary metabolites that occur in clades **b** and **c** (cf. Fig. 1 and Table 1) have been drawn into the figure, and the respective clades are highlighted in *grey background*

Notes: Even though the stromata of *T. chocoënsis* may be occasionally simple as in *T. chordalis*, they markedly differ from this taxon in having fertile heads restricted to the tips of very long, undulating branches, never occurring along the rachis; moreover, the often-branched stromata and chemotaxonomic features (i.e., pigment colors and HPLC profiles) clearly suggest close relationships to the dendroid species. Two species in this group, i.e., *T. dendroidea* and *T. chamissonis*, have ascospores close to the size range recorded on *T. chocoënsis* but both differ in having much higher stromata that are dichotomously and more densely branched. In addition, *T. dendroidea* features multiperitheciate fertile heads, and in *T. chamissonis* the fertile heads are clustered on the densely ramified stromatal tips (Dennis 1957).

Phylacia poculiformis (Kunze) Mont.

Specimen examined: FRENCH GUIANA: Sinnamary, Piste St. Elie, 28 April 2008, on dead wood, coll. C. Lechat CLL 8105 (LIP, culture in CBS 123581, MUCL 51706; GenBank acc. no. FN428830).

Notes: This taxon is included here because its cultures and HPLC profiles were used for comparison with the *Thamnomyces* species. It is the first specimen of this taxon that allowed us to study its stromatal HPLC profiles in the fresh state, as the studies on the old herbarium specimens by Stadler et al. (2004a) had been rather inconclusive. The stromatal HPLC profile revealed a series of unknown compounds, but interestingly, several major metabolites were the same as those detected in dendroid *Thamnomyces* spp. (i.e., the yet unidentified compounds labeled "TD1–TD4" in Fig. 2).

Key to *Thamnomyces* species

1 Stromatal pigments in KOH purple or absent; fertile parts uniperitheciate, scattered along unbranched axis…… **2**

1′ Stromatal pigments in KOH present, other than purple (olivaceous, grey, or brown); fertile parts uni- or multi-peritheciate, at the tips of branched stromata…… **3**

2 Fertile parts short-stalked; ascospores 11–13 × 4.5–5.5 µm…… ***T. fuciformis***

2′ Fertile parts more or less sessile; ascospores (6.6–) 8.5–10.5(–11.4) × 3.8–4.8(–5.4) µm…… ***T. chordalis****

3 Stromata irregularly and sparingly, long-branched; ascospores 8.5–9.5 × 3.8–4.2 µm. Only known from W. Ecuador ……***T. chocoënsis***

3′ Stromata regularly, dichotomously branched in three dimensions…… **4**

4 Tips of the branches multiperitheciate; ascospores 7–10 × 3–4.5 µm…… ***T. dendroidea***

4′ Tips of the branches uniperitheciate, ascospores averaging shorter than 8 µm or longer than 10 µm…… **5**

5 Ascospores 6.5–8 × 3–4 µm. SE Brazil.....…… ***T. chamissonis***

5′ Ascospores 13–26 × 6–9 µm. Tropical Africa…… ***T. camerunensis***

* For specimens with this habit featuring olivaceous stromatal pigments, see Notes on *T. chordalis*.

Cultural characteristics of *Thamnomyces camerunensis*, *T. dendroidea*, and *Phylacia poculiformis* (Figs. 7, 8)

These cultures are described simultaneously as no significant differences were noted among the three species during development of macromorphological characters: colonies on Difco OA at 23°C reaching the edge of 9-cm Petri dish in 7–9 days, at first whitish, felty, azonate, with diffuse margins, becoming smoke gray (105) with olivaceous tone; reverse turning citrine (13). Irregularly branched hyphae as described by Samuels and Müller (1980; Fig. 3e), as well as thickened hyphae from which conidiophores arose in the cultures of *T. chordalis* (cf. fig. 3c in Samuels and Müller 1980) seen in all three species (some examples depicted here in Fig. 8), but no production of conidiophores or stromatal primordia was observed.

Notes: Although the characteristic anamorphic structures reported before from other species of the respective genera were not observed, the macroscopic morphology of cultures in all three species recalls that of the genus *Daldinia*, which was not noted by Samuels and Müller (1980) or Rodrigues and Samuels (1989), who largely restricted their descriptions to anamorphic and other micromorphological characters.

All cultures developed a very characteristic odor, which was also noted before in cultures of *Daldinia* (Petrini and Müller 1986; Van der Gucht 1994). This odor probably does not arise from the nonvolatile metabolites detected in this study, neither of which has a similar odor in pure form, but it is presumably caused by additional, unknown, volatile compounds that were not detected by HPLC. Nevertheless, the metabolites responsible could be chemotaxonomically significant. Gas chromatography rather than HPLC should be conducted to characterize such volatile compounds.

After 4 weeks, the mycelia of all three strains disintegrated to highly melanized hyphal fragments, which could not be revived. Cultures of *Thamnomyces* and *Phylacia* therefore differ from cultures of *Daldinia*, where we have repeatedly been able to reactivate the stromatic structures from dried plates (cf. also results on *D. grandis* Child by Stadler et al. 2004b). The stromata of *Phylacia* and *Thamnomyces* always grow in damp environments, whereas *Daldinia* species may produce their stromata either in humid, shady places or in exposed, sunny, and hence dry areas. Therefore, it is currently not possible to characterize this evolutionary lineage of Xylariaceae as xerophilic, but they may, indeed, have evolved from xerophilic ancestors (Rogers 2000).

At least for *P. poculiformis*, Rodrigues and Samuels (1989) reported similar observations on the lack of conidiogenous structures in culture (they took the description of the anamorph from immature stromata found in the natural environment). Möller (1901) reported the lack of conidiogenous structures also for cultured mycelia of *T. chamissonis.*

The secondary metabolite profiles of these cultures were highly similar to those of *T. chordalis* and even to those of *Daldinia* and *Phylacia* spp. studied previously (Bitzer et al. 2008). As shown in Fig. 9, several different metabolite families that are also characteristic of one or two of the aforementioned genera were also produced by the *Thamnomyces* cultures studied, as well as by the culture of *P. poculiformis*. The major difference between *Thamnomyces* and the other two genera appears to be the apparent lack of 8-methoxy-1-naphthol and its precursor, 1,8-dihydroxynaphthalene (Fig. 1, compounds **3** and **3a**), whereas the compounds **4**, **6–7**, and **10–13** are omnipresent in all three genera studied.

Mellein derivatives (Fig. 1, e.g., **5**, **5a**), which are widely distributed in the genus *Hypoxylon* and other Xylariaceae genera with *Nodulisporium*-like anamorphs, were not encountered in any of the newly obtained cultures. Mellein derivatives were likewise not observed in cultures of *Daldinia* and *Entonaema* (Stadler et al. 2001; Bitzer et al. 2008).

Studies on the identity of a yet unidentified stromatal pigment of *Thamnomyces* and *Daldinia*

It was puzzling to see the resemblances between the stromatal metabolite profiles of the dendroid *Thamnomyces* spp. and certain other Xylariaceae, such as *D. lloydii*, *D. petriniae*, and a collection of *D.* aff. *placentiformis* that keyed out as *H. placentiforme* in Ju and Rogers (1996) but probably represents a different taxon (Bitzer et al. 2008). This apparent chemotaxonomic significance gave impetus to further pursue the problem regarding the identity of this unknown and unstable compound. Allport and Bu'Lock (1958) reported a perylene quinone (see chemical structure **2** in Fig. 1) from the stromatal extract of a fungus they named *D. concentrica* (Bolton) Ces. & De Notaris. Later on, Allport and Bu'Lock (1960) reported (**1**) from stromata of the same fungal material for the first time. HPLC–MS of the respective peak that appeared at slightly higher retention time as (**1**) and revealed a molecular peak at *m/z* 316 (i.e., 2 Da less as compared to **1**), suggesting that it could constitute an oxidation product of the latter compound. When pure binaphthalene tetrol (**1**) was incubated with 1 M $KMnO_4$ in 50% aqueous methanol over night, the compound was, indeed, partly converted into the metabolite that is present in the *Daldinia* and dendroid *Thamnomyces* species with olivaceous pigments. This observation suggests an oxidation of (**1**), similar to that reported by Allport and Bu'Lock (1960), to the corresponding perylene quinone (**2**). The implications of this finding are discussed further below.

Molecular phylogeny based on 5.8S/ITS nrDNA data

In Fig. 10, a selection of DNA sequence data generated from representative Xylariaceae, the majority of which are members of the hypoxyloid Xylariaceae and were also used in the preceding study (Bitzer et al. 2008), have been compiled in a phylogenetic tree with those of the newly obtained ones of *Phylacia poculiformis* and *Thamnomyces* species. In addition, sequence data of *Xylaria hypoxylon* (L.) Grev. (Peršoh et al. 2009), *X. mesenterica* (Möller) M. Stadler, Læssøe & J. Fournier (Stadler et al. 2008a), *Daldinia gelatinoides* Lar.N. Vassiljeva, *Nemania serpens* (Pers.) Gray, and *Rosellinia aquila* (Fr.) Ces. & De Not. have been added.

The phylogenetic analyses revealed two well-supported clades, B and C (86% and 80%, respectively), including members of the genus *Daldinia* and presumably allied Xylariaceae (Fig. 10). One of these clades (clade B) appeared as sister group to a weakly supported (63%) grouping of *Hypoxylon* and *Annulohypoxylon* spp. (clade A), while the other (clade C) appeared as a sister group to a moderately supported (71%) monophylum including species of *Phylacia* and *Thamnomyces*. The latter sister group relationship obtained only very weak bootstrap support (51%), but nevertheless the distance (branch length) between these two clades was clearly shorter than that to any of the other clades. Two further clades clustering apart from these clades included *Xylaria* and other species with *Geniculosporium*-like anamorphs (clade D) and representatives of Xylariaceae with *Nodulisporium*-like anamorphs and bipartite stromata (i.e., *Biscogniauxia* Kuntze and *Camillea* Fr.), which are devoid of stromatal pigments (clade E) and, as do the members of clade A, produce mellein-like metabolites in culture (Bitzer et al. 2008). The representatives of those genera appeared more closely related to one another than to the groups comprising *Daldinia* (clades B and C), *Hypoxylon* (clade A), and their respective allies. These findings are briefly evaluated in the following discussion.

Discussion

As mentioned in the Introduction, the affinities of *Thamnomyces* have so far been rather obscure, and *Xylaria* species had been integrated into the genus from the beginning of its taxonomic history. Dennis (1961) informally proposed a subfamily Thamnomycetoideae for stromatic Xylariaceae with evanescent asci, and Speer (1980) even founded a family, Phylaciaceae, on similar grounds, which was, however, shown to be superfluous (Bitzer et al. 2008; Stadler et al. 2005). In fact, Rogers (1979, 2000) has already hypothesised that gradual reduction of "typical" xylariaceous features (e.g., amyloid asci, ascospore germ slit) have occurred several times during the evolution of the this family. Ju et al. (1997) had already proposed a relationship of *Thamnomyces* (as well as *Rhopalostroma* and *Phylacia*) to *Daldinia*, which was confirmed by Stadler et al. (2004a) based on chemotaxonomic data. The additional results presented here further confirm the hypothesis that all the aforementioned genera are derived from a common lineage, as inferred from a comparison of morphological, chemotaxonomic, and molecular data.

Interestingly, in the current, preliminary molecular phylogeny (Fig. 10), *Daldinia* and allies (clades B and C) did not become nested within the clade A comprising *Annulohypoxylon* and *Hypoxylon*, as in the preceding parsimony analysis (Bitzer et al. 2008), or the phylogenetic study based on α-actin and β-tubulin genes (Hsieh et al. 2005). Other molecular studies that had not included *Phylacia* and *Thamnomyces* (Peláez et al. 2008, Tang et al. 2009) had also suggested paraphylies of *Hypoxylon* with respect to the position of *Daldinia.* As in the present phylogenetic tree, *Annulohypoxylon* and *Hypoxylon* became intermingled in molecular phylogenies based on 5.8S/ITS rDNA data.

Another interesting difference to the aforementioned molecular phylogenies is that the genus *Daldinia* did not appear monophyletic in our preliminary study. Its members rather became split into two different clades, one comprising *Entonaema liquescens* Möller and the second comprising *Phylacia* and *Thamnomyces* species. We think that such results will need to be verified based on a larger number of taxa and therefore refrain from an extensive discussion at this time. In any case, the molecular data strongly supported the hypothesis that *Phylacia* is the closest relative of *Thamnomyces*. The long branch lengths at which *Thamnomyces* species clustered indicate a high variability of 5.8S/ITS nrDNA sequences within the *Phylacia/Thamnomyces* subclade, which may be because sequence data of some of the closest relatives of the species studied have not yet been integrated. It remains to be seen how the phylogenetic tree will be affected by the inclusion of *T. chordalis* and additional species of *Phylacia*, *Daldinia*, and other Xylariaceae.

The metabolites depicted in Fig. 1 are polyketides, derived from the acetate–malonate pathway. From what is known on secondary metabolite biogenesis of fungal polyketides, their carbon skeletons are biosynthesized by specific gene clusters (i.e., polyketide synthases, or PKS), which are composed of modules determining parameters such as chain length, cyclization, and the positions of the oxygen atoms in the rings (cf. Hoffmeister and Keller 2007). Additional genes apparently are involved in the regulation of these processes, as none of the major polyketides of the Xylariaceae cultures can be found in the ascogenous stromata and vice versa (cf. Stadler and Fournier 2006). The fact that the biosynthesis of secondary metabolites has a genetic background needs to be taken into account when comparing phenotype-based data based on the occurrence of these secondary metabolites with molecular phylogenetic data. As summarized in Table 1, cultures of *Daldinia*, *Entonaema*, *Phylacia*, and *Thamnomyces* differ from *Hypoxylon* and *Annulohypoxylon* (i.e., the species in clade A in Fig. 10) by the presence of at least three types of polyketide biosynthetic pathways and apparently lack a fourth one (mellein biosynthesis) that is very characteristic of the hypoxyloid group.

For instance, the naphthols **3** and **3a** are presumably derived from the ubiquitous pathway of melanin biosynthesis, the 1,8-dihydroxynaphthalene or DHN pathway (Bell and Wheeler (1986). Most ascomycetes probably produce polymeric melanin and structurally related molecules in a similar manner (Butler and Day 1998). The dimeric naphthalene (**1**), thought to be biosynthetically derived from condensation of two moieties of compound **3a** (Allport and Bu'Lock 1960), is present in stromata of all species so far examined. Therefore, the *Thamnomyces* cultures might just have lost the ability to accumulate such monomeric naphthols, but definitely the fungi still dispose of the ability to produce naphthalene derivatives. Interestingly, the naphthol (**3**) is a "shunt metabolite" of DHN melanin biosynthesis. Arising from methylation of 1,8-DHN, it accumulates in the cultures as it is not further converted to dimeric naphthalenes and melanin polymers. Modifications such as the introduction of an additional methyl group in DHN (**3a**), leading to naphthol (**3**), could be mediated by an additional methylase gene. According to Turner (1971) and Turner and Aldridge (1983), most of the prevalent secondary metabolite classes in cultures of the hypoxyloid Xylariaceae are probably pentaketides, composed of ten carbon atoms. While *Hypoxylon* and allies developed and retained the dihydroisocoumarin (i.e., mellein) type of polyketide biogenesis, *Daldinia* and allies may either have never attained or eventually abandoned this pathway, but instead attained and/or maintained the naphthol biosynthesis and acquired additional, specific PKS gene clusters. To illustrate this, some prevalent compounds identified from members of the Xylariaceae were redrawn in Fig. 11, according to the classification proposed by Turner (1971). The carbon backbone of the polyketides is drawn beneath the chemical structures of the originally isolated compounds and the carbon–carbon bonds that form the backbone are indicated by a thicker line (in the simplified backbones, all oxygen atoms and all oxygen substituents and cyclizations mediated by esters or lactones have been omitted). The summary implies that up to four different carbon backbones are represented in *Daldinia* and its phylogenetic allies (clades B and C), and at least two different ones in *Hypoxylon* and its closest relatives (clade A), as well as in *Biscogniauxia* and *Camillea* (clade D).

Naphthols and isosclerones could be derived from similar pathways, even though Turner (1971) already demonstrated several possibilities by which the bicyclic naphthalene backbone "B" could theoretically come about by cyclization and de novo formation of a carbon–carbon bond from a monocyclic precursor. The co-occurrence of pairs of chemically similar compounds that differ in one methyl group might be because the respective PKS will accept two different starter units, e.g., malonate and methylmalonate. However, this will eventually need to be confirmed by feeding experiments using labeled precursors. In any case, the hypothetic scheme implies that the mellein derivatives, which are prevalent in *Hypoxylon*, are derived from entirely different carbon skeletons than all of the compounds that are produced by *Daldinia*, *Phylacia*, and *Thamnomyces* instead.

The chromones (**4**, **4a**) and Ab-5046-A (**10**) could arise from the same carbon backbone. However, Ab-5046-B (**11**) is a smaller molecule with backbone **D** and should therefore be synthesized in a different manner. The same is true for the mellein derivatives (**5**, **5a**), which also differ in the

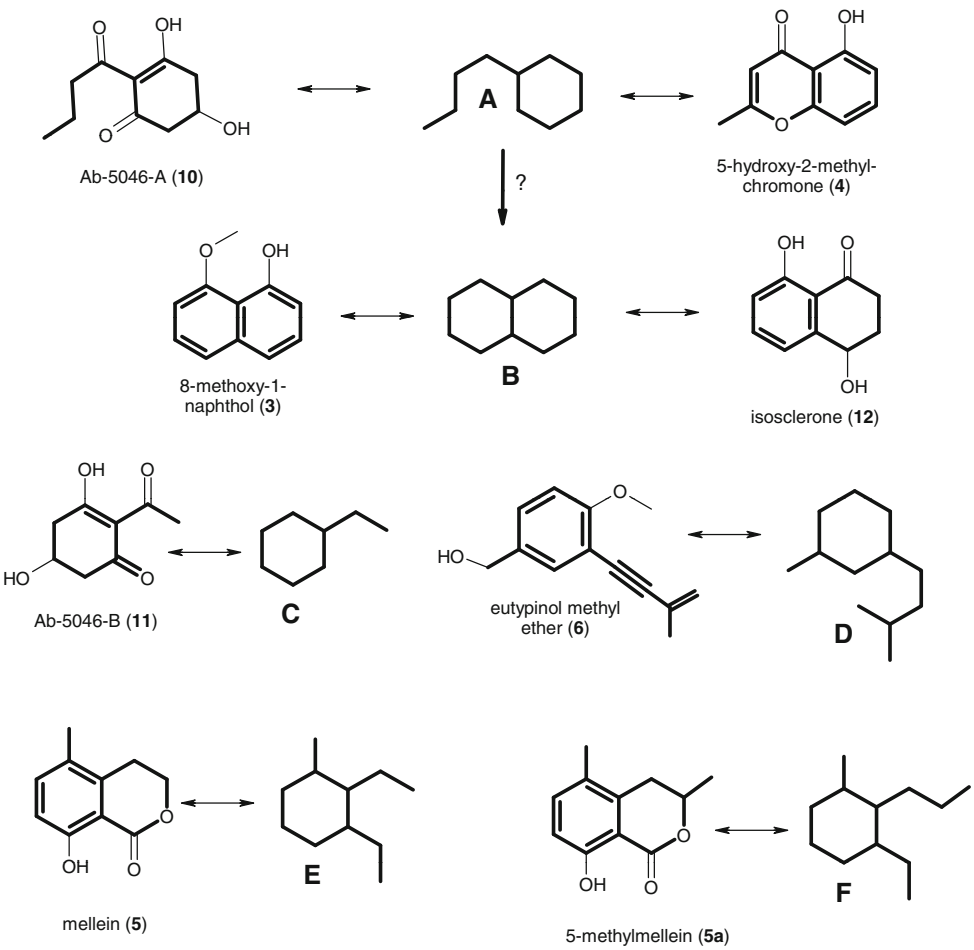

Fig. 11 Differences in the carbon skeletons among the putative polyketides, indicated by *bold letters*, that are prevalent in cultures of the genera *Daldinia*, *Phylacia*, and *Thamnomyces* (**a**–**d**) and *Annulohypoxylon*, *Biscogniauxia*, *Pyrenomyxa*, and *Hypoxylon* (**e**, **f**), respectively. One representative metabolite is depicted and connected by an *arrow* to the respective carbon backbones. For classification of these molecules and theoretical considerations, see Turner (1971)

length of their carbon backbone (**E**, **F**). The eutypinols (e.g., **6**) are larger molecules; their carbon skeleton is composed of 12 carbon atoms, and they are, therefore, possibly hexaketides. It should be interesting to evaluate these differences at the genetic level. Modern genomic methods would facilitate respective studies on the distribution of secondary metabolite genes, as was already proven for other filamentous Ascomycota. However, so far no representative of the Xylariaceae has been subjected to sequencing of its genome, and not a single PKS gene cluster encoding for one of these molecules has been identified and characterised.

The evidence obtained from the alkaline conversion of the binapthalene tetrol (**1**) to the putative perylene quinone (**2**) is still circumstantial because we failed to isolate the reaction product by preparative HPLC and could therefore not confirm its chemical structure by NMR spectroscopy. However, it can be safely assumed that the unknown olivaceous pigments mentioned above most probably correspond to such perylene quinones. Allport and Bu'Lock (1960) had actually treated (**1**) with phenol oxidase from *D. concentrica* and achieved a similar oxidative conversion to compound (**2**), which means that the conversion could also be mediated by the fungus itself. Another possibility would be a slow autoxidation mediated by oxygen from the air, which could explain why the putative perylene quinone (**2**) was detected, even in some old herbarium specimens of Xylariaceae that do not normally contain olivaceous stromatal pigments in the fresh state. In any case, this observation somewhat complicates the chemotaxonomic analyses of Xylariaceae, emphasizing that fresh material should be available for studies.

Daldinia and its closest relatives, as inferred from molecular phylogenetic and chemotaxonomic data, are difficult to circumscribe on the basis of classical morphological concepts. They drastically differ in their stromatal morphology and anatomy as well as in ascal and ascospore morphology, i.e., in the very same criteria used traditionally to discriminate the genera of the lineage. Possibly, this group constitutes an example for adaptive radiation that revealed divergent evolution of stromatal morphology, which may have arisen from ancestral forms that have had either a hypoxyloid habit or even an erect, stipitate stroma such as those currently encountered in *Phylacia*, *Rhopalostoma*, and some *Daldinia* species. *Thamnomyces* could have evolved from common ancestors together with

Phylacia as well, as suggested by the fact that both genera have a predominantly or even exclusively neotropical distribution.

Recent studies on *Pyrenomyxa* Morgan (Stadler et al. 2005) suggested that globose asci with passive dispersal have evolved independently also in the *Hypoxylon rubiginosum* complex. A cladistic approach to a taxonomic revision might call for integration of *Pyrenomyxa* into *Hypoxylon*, as is strictly speaking also the case for *Entonaema* with respect to *Daldinia*. On the other hand, the current generic concepts in *Hypoxylon* and the Xylariaceae in general are certainly not consistent. For instance, several taxa in *Hypoxylon* (e.g., *H. fraxinophilum* Pouzar) are also defined by their reduced ascal apical apparatus, or by their aberrant ascospore morphology (e.g., lack of germ slits, rectangular ascospores in *H. rectangulosporum* Y.M. Ju, J.D. Rogers & Samuels). Molecular data have not yet become available on all these aberrant forms, and it might be wise to deal with this matter in a broader context before premature, drastic taxonomic changes are proposed.

In any case, characters relating to stromatal anatomy are not always reliable for generic segregation, unless corroborated by complementary data. For instance, *Phylacia* and *Thamnomyces* are both well-defined genera that also have numerous morphological characters in common. On the other hand, studies on *Entonaema* (Stadler et al. 2008a) revealed that hollow, liquid-filled stroma arose independently more than once in the tropical Xylariaceae. In this case, results from comparisons of DNA sequences, anamorphic morphology, and HPLC profiles coincided better with one another than morphological traits of the stromata. The ex-type culture of the recently erected genus *Ruwenzoria* J. Fournier et al. (Stadler et al. 2009) also produces similar metabolites in its stromata and cultures as *Daldinia* and appeared most closely related to *Entonaema liquescens* in a molecular phylogeny essentially comprising the same representative Xylariaceae as used in this study. However, its stromata are massive and neither bear concentric zones nor hollow cavities. On the other hand, hollow stromata as in *Entonaema* have also evolved within *Daldinia*, as *D. gelatinoides* is strikingly similar to *D. fissa* Lloyd (Stadler et al. 2004b). A more intensive study on stromatal metabolites of *Daldinia* and allies might also reveal additional chemotaxonomic evidence, because the stromata of *Thamnomyces* and *Phylacia* have several compounds in common, some of which also occur in *Daldinia*. For instance, daldinin C (**8**) was tentatively detected in *T. dendroidea* and *P. poculiformis*, along with some yet unidentified pigments (Fig. 2). Daldinin-type azaphilones are the major yellowish pigments of some *Daldinia* spp. and the *Hypoxylon fuscum* complex (cf. Stadler and Fournier 2006).

In a family of Ascomycota that shows such a high phenotypic plasticity it is certainly not advisable to strictly rely on molecular data, so long as more than 80% of the known Xylariaceae species have not been cultured, sequenced, and their DNA sequence data included in molecular phylogenies. Neither the erection of various new genera for all the aforementioned aberrant forms nor their integration into a mega-genus *Hypoxylon* appears practical at this time, because we still know so little about the biology of these fungi. An integrative, polyphasic approach appears ideal to evaluate an alternative strategy that may eventually solve this problem, and careful morphological studies, as well as extensive fieldwork, will continue to be indispensable.

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