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Identification of *Rosellinia* species as producers of cyclodepsipeptide PF1022 A and resurrection of the genus *Dematophora* as inferred from polythetic taxonomy

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Abstract: *Rosellinia* (*Xylariaceae*) is a large, cosmopolitan genus comprising over 130 species that have been defined based mainly on the morphology of their sexual morphs. The genus comprises both lignicolous and saprotrophic species that are frequently isolated as endophytes from healthy host plants, and important plant pathogens. In order to evaluate the utility of molecular phylogeny and secondary metabolite profiling to achieve a better basis for their classification, a set of strains was selected for a multi-locus phylogeny inferred from a combination of the sequences of the internal transcribed spacer region (ITS), the large subunit (LSU) of the nuclear rDNA, beta-tubulin (*TUB2*) and the second largest subunit of the RNA polymerase II (*RPB2*). Concurrently, various strains were surveyed for production of secondary metabolites. Metabolite profiling relied on methods with high performance liquid chromatography with diode array and mass spectrometric detection (HPLC-DAD/MS) as well as preparative isolation of the major components after re-fermentation followed by structure elucidation using nuclear magnetic resonance (NMR) spectroscopy and high resolution mass spectrometry (HR-MS). Two new and nine known isopimarane diterpenoids were identified during our mycochemical studies of two selected *Dematophora* strains and the metabolites were tested for biological activity. In addition, the nematicidal cyclodepsipeptide PF1022 A was purified and identified from a culture of *Rosellinia corticium,* which is the first time that this endophyte-derived drug precursor has been identified unambiguously from an ascospore-derived isolate of a *Rosellinia* species. While the results of this first HPLC profiling were largely inconclusive regarding the utility of secondary metabolites as genus-specific chemotaxonomic markers, the phylogeny clearly showed that species featuring a dematophora-like asexual morph were included in a well-defined clade, for which the genus *Dematophora* is resurrected. *Dematophora* now comprises all previously known important plant pathogens in the genus such as *D. arcuata*, *D. bunodes*, *D. necatrix* and *D. pepo*, while *Rosellinia s. str.* comprises those species that are known to have a geniculosporium-like or nodulisporium-like asexual morph, or where the asexual morph remains unknown. The extensive morphological studies of L.E. Petrini served as a basis to transfer several further species from *Rosellinia* to *Dematophora*, based on the morphology of their asexual morphs. However, most species of *Rosellinia* and allies still need to be recollected in fresh state, cultured, and studied for their morphology and their phylogenetic affinities before the infrageneric relationships can be clarified.

Key words: *Xylariaceae*, *Rosellinia*, *Dematophora*, Genus resurrection, Polythetic taxonomy, PF1022A, Isopimarane diterpenoids.

Taxonomic novelties: New combinations: *Dematophora acutispora* (Theiss.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora arcuata* (Petch) C. Lambert, K. Wittstein & M. Stadler, *Dematophora asperata* (Massee ex Wakef.) Lambert, K. Wittstein & M. Stadler, *Dematophora beccariana* (Ces.) C. Lambert, K. Wittstein & M, Stadler, *Dematophora boedijnii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora bothrina* (Berk. & Broome) C. Lambert, K. Wittstein & M. Stadler, *Dematophora bunodes* (Berk. & Broome) C. Lambert, K. Wittstein & M. Stadler, *Dematophora buxi* (Fabre) C. Lambert, K. Wittstein & M. Stadler, *Dematophora compacta* (Takemoto) C. Lambert, K. Wittstein & M. Stadler, *Dematophora francisiae* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora freycinetiae* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora gigantea* (Ellis & Everh.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora grantii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora hsiehiae* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora hughesii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora javaensis* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora macdonaldii* (Bres.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora obregonii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora obtusiostiolata* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora paraguayensis* (Starbäck) C. Lambert, K. Wittstein & M. Stadler, *Dematophora pepo* (Pat.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora puiggarii* (Pat.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora pyramidalis* (Lar.N. Vassiljeva) C. Lambert, K. Wittstein & M. Stadler, *Dematophora samuelsii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora siggersii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler.

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INTRODUCTION

The genus *Rosellinia* was erected by De Notaris (1844) based on *R. aquila* as type species and later synonymised with various other genera of pyrenomycetes, including *Dematophora* (Hartig 1883), which had been typified by *D. necatrix*. The sexual morphs of *Rosellinia* are characterised by uniperithecioid stromata usually growing from a subiculum. The ascospore morphology, as well as the conidiogenous structures and other characteristics of the asexual morphs are rather variable, even though most of the conidiogenous structures of *Rosellinia* spp. known can be assigned to the *Geniculosporium* type. In fact, only some species of the "*R. thelena* group" for which no molecular data are available, have been reported to form nodulisporium-like conidiophores according to the definition of Ju & Rogers (1996). Many species are only known from one or a few collections and have never been cultured or included in molecular phylogenetic analyses. Recent studies of stromatic *Xylariales* by Hsieh *et al.* (2010) and Wendt *et al.* (2018) included some taxa of *Rosellinia* and have clearly showed that the genus has affinities to the

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genus *Xylaria* and other genera of *Xylariaceae sensu stricto*, in particular *Entoleuca*, *Euepixylon* and *Nemania*. The *Hypoxylon lenormandii* complex, which contains some species that were previously included in *Rosellinia* for their superficially similar stromatal morphology (Kuhnert *et al.* 2015), has been shown to belong to the hypoxyloid clade of the *Xylariales*, which have stromatal pigments and a nodulisporium-like asexual morphs. From polythetic studies employing multi locus molecular phylogenies, this complex now resides in the *Hypoxylaceae* (Daranagama *et al.* 2018, Wendt *et al.* 2018), while *Rosellinia* is retained in the *Xylariaceae*, owing to its phylogenetic affinities to *Xylaria*. In general, the recent segregation of the stromatic *Xylariales* proposed in the latter papers demonstrated that ascospore morphology is often incongruent with the lineages defined by multi locus phylogeny than conidiogenous structures or secondary metabolite profiles.

The current study embarks from the comprehensive monograph by Petrini (2013), which compiled all relevant information on the taxonomy of the genus. This work was based on decades of meticulous morphological studies (Petrini 1992, 2003, Petrini & Petrini 2005). The monograph classified the more than 140 currently accepted species into seven informal "Groups" rather than into formal subgeneric taxa. Notably, Petrini (2013) predicted that the genus would need to be further subdivided as data on the phylogeny of its species accumulated. Out of the seven Groups, two, *viz*. the *R. buxi* and the *R. necatrix* groups, are characterised by dematophora-like asexual morphs, which are also often found in nature because of the rather conspicuous synnemata. In the phylogeny of Wendt *et al.* (2018), *Rosellinia buxi* and *R. necatrix* formed a sister clade to a clade containing the type species, *R. aquila*, *R. corticium* and *Entoleuca mammata*, making *Rosellinia sensu* Petrini (2013) paraphyletic. This observation motived a detailed study of the phylogeny and the secondary metabolites of these fungi in an attempt to further unravel their phylogenetic affinities. The results and conclusions are presented in the current paper.

MATERIALS AND METHODS

Fungal material and chemicals

All scientific names of fungi are given without authorities and years of publications, in accordance with MycoBank (*www.mycobank.org*), with the exception of the species discussed in the taxonomic part of this manuscript. Reference cultures are deposited at the Westerdijk Fungal Biodiversity Institute (formerly known as CBS, Utrecht, The Netherlands), BCCM/MUCL Agro-food & Environmental Fungal Collection (Louvain, Belgium) and the STMA culture collection of the HZI (Braunschweig, Germany). Unless indicated otherwise solvents were obtained in analytical grade from J.T. Baker (Deventer, Netherlands) or Merck (Darmstadt, Germany) and media ingredients from Carl Roth (Karlsruhe, Germany) or Sigma Aldrich (St. Louis, US). For glucose monitoring test stripes Medi-Test glucose by Macherey-Nagel (Düren, Germany) were used. In order to study their molecular and chemical diversity 14 strains of the genus *Rosellinia* and closely related species were selected. For molecular phylogenetic analyses additional GenBank sequences were selected. The origin of the fungal strains and their corresponding GenBank accessions are listed in Table 1.

Taxon selection

The general strategy of taxon selection followed the recent study by Wendt *et al.* (2018) and a previous comprehensive study by Hsieh *et al.* (2010), mainly using representatives of the *Xylariaceae s. str.* species of *Rosellinia* and presumably closely related genera like *Coniolariella*, *Entoleuca*, *Euepixylon* and *Nemania* made up the bulk of the taxa. The phylogeny thus covers all relevant taxa that have ever been connected with the genus *Rosellinia* and of which multi locus sequence data are extant in the public domain. In case sequences of the type species or the ex-(epi-)type strains were not available, data derived from vouchers of a related species in the same genus were chosen. As outgroups, members of the *Graphostromataceae* (*Graphostroma platystomum*) and the *Hypoxylaceae* (*Hypoxylon fragiforme*) were chosen (Fig. 1G, H).

Molecular phylogenetic analysis

DNA extraction, primer selection, resulting sequence analysis and processing as well as the alignment calculations were carried out as described previously (Wendt *et al.* 2018, Lambert *et al.* 2019) with Geneious® v. 7.1.9 (*http://www.geneious.com*, Kearse *et al.* 2012). Sequences were aligned via MAFFT v. 7.017 with the G-INS-I algorithm set to default in respect to gap opening and extension penalties. Regions of phylogenetic information were filtered by processing via the Castresana Lab Gblocks Server with stringency set to low (allows smaller final blocks and gap positions within final blocks, Talavera & Castresana 2007). The resulting alignments were concatenated with Geneious and the resulting multigene alignment submitted to the PhyML Online Server (http://www.atgc-montpellier.fr/phyml/) with the PhyML v. 3.0 algorithm for phylogenetic relationship inference and preliminary determination of the best fitting substitution model via smart model selection (SMS, see Guindon *et al.* 2010, Lefort *et al.* 2017). Automatic model selection was carried out by Bayesian Information Criterion (BIC). The tree topology was optimized by subtree pruning and regrafting (SPR). Bootstrap support values (BS) were calculated from 1 000 replicates. The phylogenetic tree was rooted with *Graphostroma platystomum* and *Hypoxylon fragiforme*.

Cultivation procedures

Cultures were grown first on yeast-malt-glucose agar plates (YMG) at 23 °C in darkness. Erlenmeyer flasks with 30 mL YM medium were inoculated with four mycelial plugs (0.5 × 0.5 cm^2) of well-grown plates to obtain seed cultures. For the preparation of submerged cultures flasks with 200 mL medium were inoculated with four to six mycelial plugs (0.5 × 0.5 cm^2), while flasks with 400 mL or 1 000 mL medium were inoculated with well-grown seed cultures (7.5 %). The flasks were kept at 23 °C and 150 rpm in darkness and the consumption of glucose was monitored regularly using glucose test stripes (Macherey-Nagel, Germany). The following liquid media were used: YM [malt extract 10 g/L, yeast extract 4 g/L, D-glucose 4 g/L, pH 6.3], ZM/2 (Rupcic *et al.* 2018b) [molasses 5 g/L, oatmeal 5 g/L, sucrose 4 g/L, mannitol 4 g/L, D-glucose 1.5 g/L, $CaCO_3$ 1.5 mg/L, edamin (lactalbumin hydrolysate, LP0048 from Oxoid) 0.5 mg/L,

Table 1. List of used taxa for chemical analysis and phylogenetic reconstruction. GenBank accession numbers, strain ID of public culture collections or herbaria (if available), origin and reference studies are given. Type specimens are labelled with **HT** (holotype) or **ET** (epitype). Strains included in the chemical study are marked in bold. *This strain was labelled *Rosellinia britannica*, but this is a later erected synonym of *Rosellinia marcucciana* Ces., Atti dell'Accademia di Scienze Fisiche e Matematiche Napoli (1872) **5**:13 *fide* Petrini (2013).

Species	Strain number	Origin	Status	GenBank accession numbers				Reference
				ITS	LSU	RPB2	TUB2	
Amphirosellinia fushanensis	HAST 91111209	Taiwan	**HT**	GU339496	N/A	GQ848339	GQ495950	Hsieh *et al.* (2010)
A. nigrospora	HAST 91092308	Taiwan	**HT**	GU322457	N/A	GQ848340	GQ495951	Hsieh *et al.* (2010)
Astrocystis mirabilis	**ATCC 66432**	**Taiwan**		**No sequence data used**				**Ju & Rogers (1990)**
Coniolarelia limoniispora	MUCL 29409	Japan		MN984615	MN984624	MN987235	MN987240	This study
Dematophora bunodes	CBS 123584	Peru		MN984617	N/A	N/A	MN987243	**This study**
D. bunodes	CBS 123585	Peru		MN984618	N/A	N/A	MN987244	**This study**
D. bunodes	CBS 123597	Peru		MN984619	MN984625	N/A	MN987245	**This study**
D. bunodes	CBS 124028	Peru		**No sequence data used**				**This study**
D. buxi	JDR 99	France		GU300070	N/A	GQ844780	GQ470228	Hsieh *et al.* (2010)
D. necatrix	CBS 349.36	Argentina		AY909001	KF719204	KY624275	KY624310	Peláez *et al.* (2008; ITS, LSU), Wendt *et al.* (2018; *RPB2, TUB2*)
D. necatrix	W 97	Japan		DF977487	DF977487	DF977459	DF977466	Shimizu *et al.* (2018)
D. pepo	CBS 123592	Peru		MN984620	N/A	N/A	MN987246	This study
Entoleuca mammata	JDR 100	France		GU300072	N/A	GQ844782	GQ470230	Hsieh *et al.* (2010)
Euepixylon sphaeriostomum	JDR 261	USA		GU292821	N/A	GQ844774	GQ470224	Hsieh *et al.* (2010)
Graphostroma platystomum	CBS 270.87	France		JX658535	DQ836906	KY624296	HG934108	Stadler *et al.* (2014; ITS), Zhang *et al.* (2006; LSU), Wendt *et al.* (2018; *RPB2*), Koukol *et al.* (2015; *TUB2*)
Hypoxylon fragiforme	MUCL 51264	Germany	**ET**	KC477229	KM186295	KM186296	KX271282	Stadler *et al.* (2013; ITS), Daranagama *et al.* (2015; LSU, *RBP2*), *TUB2* (Wendt *et al.* 2018)
Kretzschmaria deusta	CBS 163.93	Germany		KC477237	KY610458	KY624227	KX271251	Stadler *et al.* (2013; ITS), Wendt *et al* (2018; LSU, *RPB2, TUB2*)
Nemania abortiva	BISH 467	USA	**HT**	GU292816	N/A	GQ844768	GQ470219	Hsieh *et al.* (2010)
N. beaumontii	HAST 405	Martinique		GU292819	N/A	GQ844772	GQ470222	Hsieh *et al.* (2010)
N. beaumontii	FL 0980	USA		N/A	JQ760608	KU684243	KU684161	U'Ren *et al.* (2012; LSU), U'Ren *et al.* (2016; *RPB2, TUB2*)
N. bipapillata	HAST 90080610	Taiwan		GU292818	N/A	GQ844771	GQ470221	Hsieh *et al.* (2010)
N. primolutea	HAST 91102001	Taiwan	**HT**	EF026121	N/A	GQ844767	EF025607	Hsieh *et al.* (2010)
Podosordaria mexicana	WSP 176	Mexico		GU324762	N/A	GQ853039	GQ844840	Hsieh *et al.* (2010)
Poronia punctata	CBS 656.78	Australia		KT281904	KY610496	KY624278	KX271281	Senanayake *et al.* (2015; ITS), Wendt *et al.* (2018; LSU, *RPB2, TUB2*)
Rosellinia aquila	MUCL 51703	France		KY610392	KY610460	KY624285	KX271253	Wendt *et al.* (2018)
R. aquila	**STMA 15208**	**Germany**		**No sequence data used**				**This study**
R. marcucciana*	MUCL 51704	France		MN984616	MN984626	MN987238	MN987238	This study
R. corticium	MUCL 51693	France		KY610393	KY610461	KY624229	KX271254	Wendt *et al.* (2018)
R. corticium	STMA 13324	Germany		MN984621	MN984627	MN987237	MN987241	This study
R. corticium	STMA 12170-15209	Germany		MN984623	MN984629	MN987236	MN987242	This study
R. nectrioides	CBS 449.89	Sweden		MN984622	MN984628	MN987239	N/A	This study
R. quercina	**MUCL52247**	**Germany**		**No sequence data used**				**This study**
Xylaria arbuscula	CBS 126415	Germany		KY610394	KY610463	KY624287	KX271257	Wendt *et al.* (2018)
X. hypoxylon	CBS 122620	Germany	**ET**	KY204024	KY610495	KY624231	KX271279	Sir *et al.* (2016; ITS), Wendt *et al.* (2018; LSU, *RPB2, TUB2*)
X. polymorpha	MUCL 49884	France		KY610408	KY610464	KY624288	KX271280	Wendt *et al.* (2018)
X. bambusicola	WSP 205	Taiwan	**HT**	EF026123	N/A	GQ844802	AY951762	Hsieh *et al.* (2010)

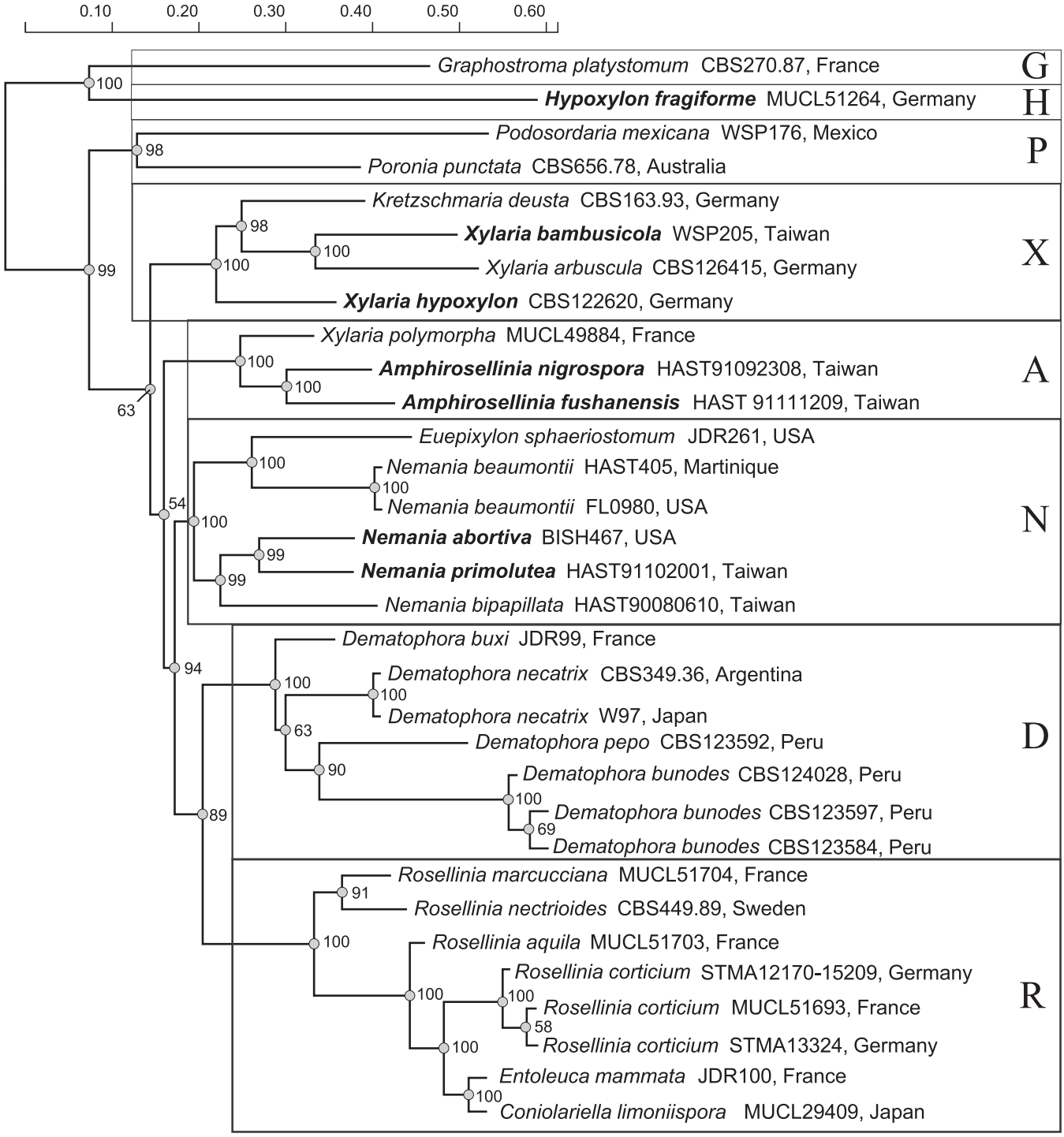

Fig. 1. Inferred phylogenetic tree of selected *Xylariaceae*, *Hypoxylaceae* and *Graphostromataceae* calculated by PhyML with 1 000 bootstrap replicates from a multigene alignment of the ITS-LSU ribosomal DNA region and the TUB2 and RPB2 regions. Bootstrap values above 50% are displayed at their respective branches. Sequence information originating from type strains are highlighted in bold.

$(NH_4)_2SO_4$ 0.5 mg/L, pH 7.2], Q6 [glycerol 10 g/L, cottonseed flour 5 g/L, D-glucose 2.5 g/L, pH 7.2] and CM [corn meal 20 g/L, D-glucose 4 g/L, pH 5.5]. Strains CBS 123585, CBS 123584, CBS 123597, CBS124028 (*D. bunodes*), CBS 123592 (*D. pepo*), STMA 13324, MUCL 51693 (*R. corticium*), MUCL 51703 (*R. aquila*), MUCL 51704 (*R. marcucciana*) and ATCC 66432 (*A. mirabilis*) were selected for investigating their metabolite profiles and cultivated in all four liquid media (200 mL scale, Table S2). For the isolation of compounds the corresponding culture volumes were increased up to 4 L in total (200–1 000 mL per flask). The cultivation was stopped three to four days after total consumption of glucose.

In another attempt, strains MUCL 51703, STMA 15208 (*R. aquila*), MUCL 51704 (*R. marcucciana*), STMA13324, MUCL 51693, STMA 15209/12170 (*R. corticium*), MUCL 52247 (*R. quercina*), CBS449.89 (*R. nectrioides*) and ATCC 66432 (*A. mirabilis*) were cultivated on mPDA, CSA and CMD plates at 23 °C in darkness in order to investigate their production of cyclodepsipeptides (Table S2). mPDA (modified potato dextrose agar) [potato starch 20 g/L, D-glucose 5 g/L, yeast extract 1.5 g/L, agar 20 g/L], CSA (cotton seed agar) [maltose 40 g/L, cottonseed flour 20 g/L, $CaCO_3$ 3 mg/L, soy peptone (type II, from Marcor) 2 g/L, $MgSO_4 \cdot 7H_2O$ 2 g/L, NaCl 2 g/L, agar 20 g/L (similar to Seed agar in Harder *et al.* 2011)], CMD [corn meal

20 g/L, D-glucose 4 g/L, agar 20 g/L, pH 5.5]. Culture plates were extracted when at least two-thirds of the plate surfaces were covered by mycelium (14–21 d). For the isolation of cyclodepsipeptide PF1022 A, STMA13324 (*R. corticium*) was cultivated in liquid CS medium [maltose 40 g/L, cottonseed flour 20 g/L, $CaCO_3$ 3 mg/L, soy peptone (type II, from Marcor) 2 g/L, $MgSO_4 \cdot 7H_2O$ 2 g/L, NaCl 2 g/L] in a total volume of 3 L (200 mL per flask).

Extraction and isolation of secondary metabolites

For the extraction of submerged cultures biomass and culture medium were separated by gauze filtration. The biomass was extracted with acetone in an ultrasonic bath (2 × 30 min) and after removing the organic solvent under reduced pressure at 40 °C the residual aqueous phase was extracted twice with ethyl acetate. The combined ethyl acetate phases were dried over sodium sulphate and the solvent was removed under reduced pressure at 40 °C to yield the crude extract. The culture medium was extracted with ethyl acetate twice and processed as described above. Culture plates were cut into pieces and extracted with 30 mL ethyl acetate twice on a magnetic stirrer for 30 min. After filtration, the organic solvent was removed under reduced pressure at 40 °C to yield the crude extract. The crude extracts were subjected to analytical HPLC-UV/Vis-MS analyses. For the isolation of compounds, the crude extracts were dissolved in 0.5–4 mL methanol and applied to RP solid phase cartridges (Strata-X 33 mm, Polymeric Reversed Phase; Phenomenex, Aschaffenburg, Germany) and eluted with methanol and acetonitrile to remove highly nonpolar contaminants (*e.g.* fatty acids) before injection for preparative HPLC.

Following the procedures described above the cultivation of *D. bunodes* (CBS123585) and *D. pepo* (CBS123592) in CM medium (4 L) provided 1.68 g and 0.27 g of crude extract, respectively. The extracts were subjected to RP-MPLC and/or preparative HPLC (details are given in the Supplementary Information) and finally resulted in the isolation of dematophoranane A (**1**, 21.3 mg), dematophorane B (**2**, 2.1 mg), libertellenone M (**3**, 2.4 mg), γ-lactone of libertellenone M (**4**, 0.3 mg), myrocin B (**5**, 59.6 mg), spiropolin A (**6**, 4.5 mg), libertellenone C (**7**, 1.4 mg), hymatoxin K (**8**, 6.5 mg), elaeicolaside C (**9**, 6.6 mg), 16-α-D-glucopyranosyloxyisopimar-7-en-19-oic acid (**10**, 2.6 mg) and 16-α-D-glucopyranosyloxyisopimar-7-en-19-oic acid (**11**, 15.4 mg). The cultivation of *R. corticium* (STMA13324) in 3 L CS medium and subsequent extraction resulted in 0.48 g of crude extract. After pre-purification via a RP solid phase cartridge (Strata-X 33 mm, Polymeric Reversed Phase; Phenomenex) it was subjected to preparative HPLC to afford PF1022A (**12**, 13.2 mg) (details are given in the Supplementary Information).

Analytical data of the new diterpenoids

*Dematophorane A (**1**)*: $[\alpha]^{25}_D$ -13.0 (*c* 2.1, CH_3CN); UV (MeOH) λ_{max} (log ε) 228 nm (3.98), 238 nm (3.98) 334 nm (4.51); ^{1}H NMR (500 MHz, MeOH-d_4), ^{13}C NMR (125 MHz, MeOH-d_4) (see Table 2); HR-ESI-MS *m/z* 373.1984 $[M+Na]^+$ (calcd for $C_{20}H_{30}NaO_5$, 373.1991). (Fig. S2)

*Dematophorane B (**2**)*: $[\alpha]^{25}_D$ +34.1 (*c* 1.8, CH_3CN); UV (MeOH) λ_{max} (log ε) 227 nm (4.19), 268 nm (4.26) 342 nm (4.37); ^{1}H NMR (700 MHz, acetone-d_6), ^{13}C NMR (175 MHz, acetone-d_6) (see Table 2); HR-ESI-MS: *m/z* 373.1984 $[M+Na]^+$ (calcd for $C_{20}H_{30}NaO_5$, 373.1991).(Fig. S3)

Table 2. ^{13}C and ^{1}H NMR data of dematophoranes A and B (**1–2**).

	(1)		(2)	
1	75.2, CH	3.68, dd (8.6, 7.4)	69.9, CH	4.26, m
2	29.7, CH_2	1.73, m	29.7, CH_2	1.70, m
3a	34.9, CH_2	1.13, dt (13.0, 3.4)	34.3, CH_2	1.06, m
3b		1.61, m		1.68, m
4	38.0, C		37.8, C	
5	55.5, CH	2.95, s	48.9, CH	3.76, s
6	212.4, C	4.66, m	211.1, C	
7	73.8, CH		76.5, CH	4.76, m
8	131.8, C		139.65, C	
9	146.9, C		75.3, C	
10	51.2, C		52.4, C	
11a	24.9, CH_2	2.34, dt (19.4, 7.5)	31.3, CH_2	2.06, m
11b		2.54, ddd (19.2, 7.9, 5.5)		2.42, td (14.7, 3.4)
12a	30.4, CH_2	1.41, m	32.2, CH_2	1.44, dtd (14.2, 4.1,2.8)
12b		1.81, ddd (13.5, 7.9, 6.1)		1.75, td (14.2, 3.4)
13	40.2, C		37.9, C	
14	72.7, CH	3.99, s	130.6, CH	5.84, s
15	145.8, CH	6.10, dd (17.6, 11.0)	149.4, CH	5.86, dd (17.6, 10.6)
16a	113.3, CH_2	5.10, dd (10.2, 1.6)	110.9, CH_2	4.92, dd (10.7, 1.4)
16b		5.10, dd (10.2, 1.6)		5.00, dd (17.5, 1.4)
17	22.8, CH_3	0.95, s	24.1, CH_3	1.01, s
18	18.6, CH_3	1.24, s	18.8, CH_3	1.21, s
19a	70.7, CH_2	3.08, d (10.8)	70.6, CH_2	3.05, m
19b		3.46, d (10.8)		3.54, m
20	18.0, CH_3	1.01, s	13.9, CH_3	0.87, s
OH-1				3.41, d (5.6)
OH-7				3.84, d (4.3)
OH-9				4.35, s
OH-18				3.74, t (5.8)

1 (125 MHz and 500 MHz, MeOH-d_4), **2** (175 MHz and 700 MHz, acetone-d_6),

Analytical HPLC-UV/Vis-MS analyses

All HPLC-MS analyses were performed on Agilent 1260 Infinity or Dionex Ultimate 3000 Systems with diode array detector and C18 Waters Acquity UPLC BEH column (2.1 × 50 mm, 1.7 µm). Solvent A: H_2O + 0.1 % formic acid, solvent B: acetonitrile + 0.1 % formic acid, gradient system: 5 % B for 0.5 min increasing to 100 % B in 19.5 min, maintaining 100 % B for 5 min, flow rate = 0.6 mL/min, detection at 200−600 nm. LC-ESIMS spectra

were recorded on an ion trap MS (amaZon speed, Bruker), and HR-ESIMS spectra on a time-of-flight (TOF) MS (MaXis, Bruker).

Structure elucidation

1D and 2D NMR spectra were recorded on a Bruker Avance III 700 spectrometer with a 5 mm TXI cryoprobe (^{1}H 700 MHz, ^{13}C 175 MHz) and a Bruker Avance III 500 (^{1}H 500 MHz, ^{13}C 125 MHz) spectrometer. UV-Vis spectra were recorded with a UV-2450 Shimadzu UV−Vis spectrophotometer and optical rotations were determined with a PerkinElmer 241 polarimeter.

Bioactivity assays

In vitro cytotoxic effects (IC_{50}) against mouse fibroblast cell line L929 and human carcinoma cell line KB-3-1 of the compounds (1 mg/mL stock solutions in methanol) were determined as described by Surup *et al.* (2018). Minimum inhibitory concentrations (MIC) were obtained in serial dilution assays with pure substances dissolved in methanol and various bacterial and fungal test organisms (Kuephadungphan *et al.* 2017). Nematicidal activity was detected using an assay with *Caenorhabditis elegans* as described by Rupcic *et al.* (2018a). The nematodes were inoculated monoxenically on nematode agar (soy peptone 2 g/L, NaCl 1 g/L, agar 20 g/L, cholesterol (1 mg/mL in EtOH, 0.5 mL), 1M $CaCl_2$ (1 mL), 1M $MgSO_4$ (1 mL) and 40 mM potassium phosphate buffer 12.5 mL; pH 6.8) with living *Escherichia coli* (DSM498) at 21 °C for 4–5 d. For the assay the nematodes were taken up in M9 buffer (0.5 M KH_2PO_4/K_2HPO_4 and 0.1 M NaCl, pH 7.2) and counted using a Malassez counting chamber. A final concentration was adjusted to 500 nematodes per mL and 1 mL of this suspension was added to each well of a 24-well microtiter plate. The pure compounds were dissolved in acetonitrile or ethanol and were tested at different concentrations (100 µg/mL, 50 µg/mL, 20 µg/mL, 10 µg/mL). Ivermectin (in the same concentrations in ethanol) was the positive control. The plate was incubated on a plate shaker for 18 h at 24 °C.

RESULTS

To infer phylogenetic relationships (Fig. 1), 32 different strains were included, *viz*. one representative of the *Graphostromataceae* (**G**, *Graphostroma plastystomum*), one representing the *Hypoxylaceae* (**H**, *Hypoxylon fragiforme*), two representing the coprophilous *Xylariaceae* (**P**) and 28 strains for the remaining *Xylariaceae*, representing nine different genera (**X**, **A**, **N**, **D**, **R**). From these strains, 28 new sequences were generated for their relevant DNA loci, whilst the remaining loci were complemented with 80 sequences from GenBank. The resulting multigene alignment (MGA) consisted of 4 176 characters, with 469 and 1 273 positions originating from ITS and LSU ribosomal DNA, respectively, and 1 101 and 1 333 characters derived from protein coding regions (*RPB2* and *TUB2*). The initial alignments as well as the final inferred phylogenetic tree of the curated and concatenated single-gene alignments are available in the Supplementary Information.

The inferred phylogeny shows six supported (>50 % BS) distinct clades with the Xylariaceae rendered as strongly supported (99 %). The coprophilous *Podosordaria mexicana* and *Poronia punctata* (**P**) are placed as sister clade to other saprotrophic and pathogenic representatives. Additional clades include *Xylaria* and *Kretzschmaria* spp. (**X**); *Xylaria polymorpha* clustered within a weakly supported (54 %) *Amphirosellinia* clade (**A**); and the clade consisting of *Nemania* spp. (**N**) forms a well-supported sister clade (94 %) to the *Rosellinia* and *Dematophora* clades (**R**, **D**). Clade N consists of two well supported subclades (100 %) of *Euepixylon sphaeriostomum* and *Nemania beaumontii* on the one hand and the type strains of *Nemania abortiva* and *Nemania primolutea* as well as a non-type strain of *Nemania bipapillata* on the other hand. The sister clade of clade N consisted of two subclades, the *Dematophora* (**D**) and *Rosellinia* (**R**) clades.

Studies on secondary metabolism of *Rosellinia s. lat*

In parallel to the molecular phylogenetic investigations several strains were cultivated in four different liquid media (see cultivation procedures and Table S2) and their corresponding extracts were analysed by HPLC-UV/Vis-MS. After comparing the secondary metabolite production of the submerged cultures, two *Dematophora* strains, *D. bunodes* CBS 123585 and *D. pepo* CBS 123592, were selected for the cultivation in CM medium in larger scale for the isolation of compounds due to a variety of interesting HPLC-UV/MS profiles in their extracts and in case of CBS 123585 relatively high quantities of substances (Fig. S1). Two new (**1**–**2**) and seven known isopimarane diterpenoids, libertellenone M (**3**) and the γ-lactone of libertellenone M (**4**) (Kildgaard *et al.* 2017), myrocin B (**5**) (Hsu *et al.* 1988), spiropolin

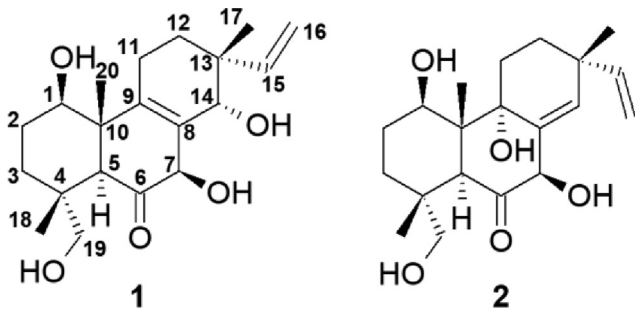

Fig. 2. New isopimarane diterpenoids, dematophoranes A and B (**1**-**2**), isolated from *Dematophora bunodes* (CBS123585).

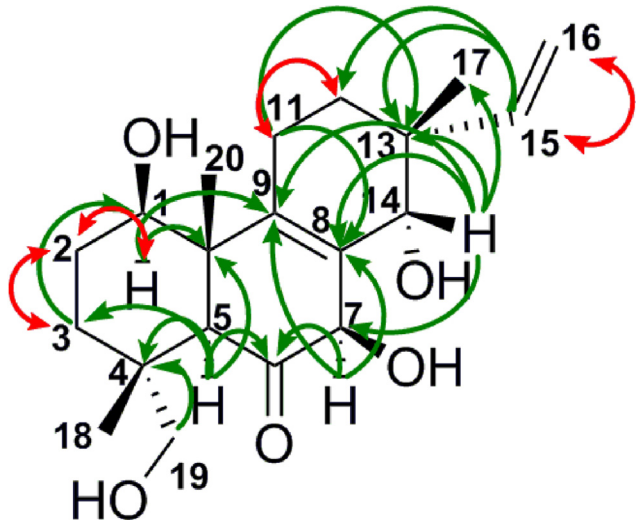

Fig. 3. Relevant ^{1}H,^{1}H-COSY- (red arrows) and ^{1}H-^{13}C-HMBC (green arrows) correlations of dematophorane A (**1**).

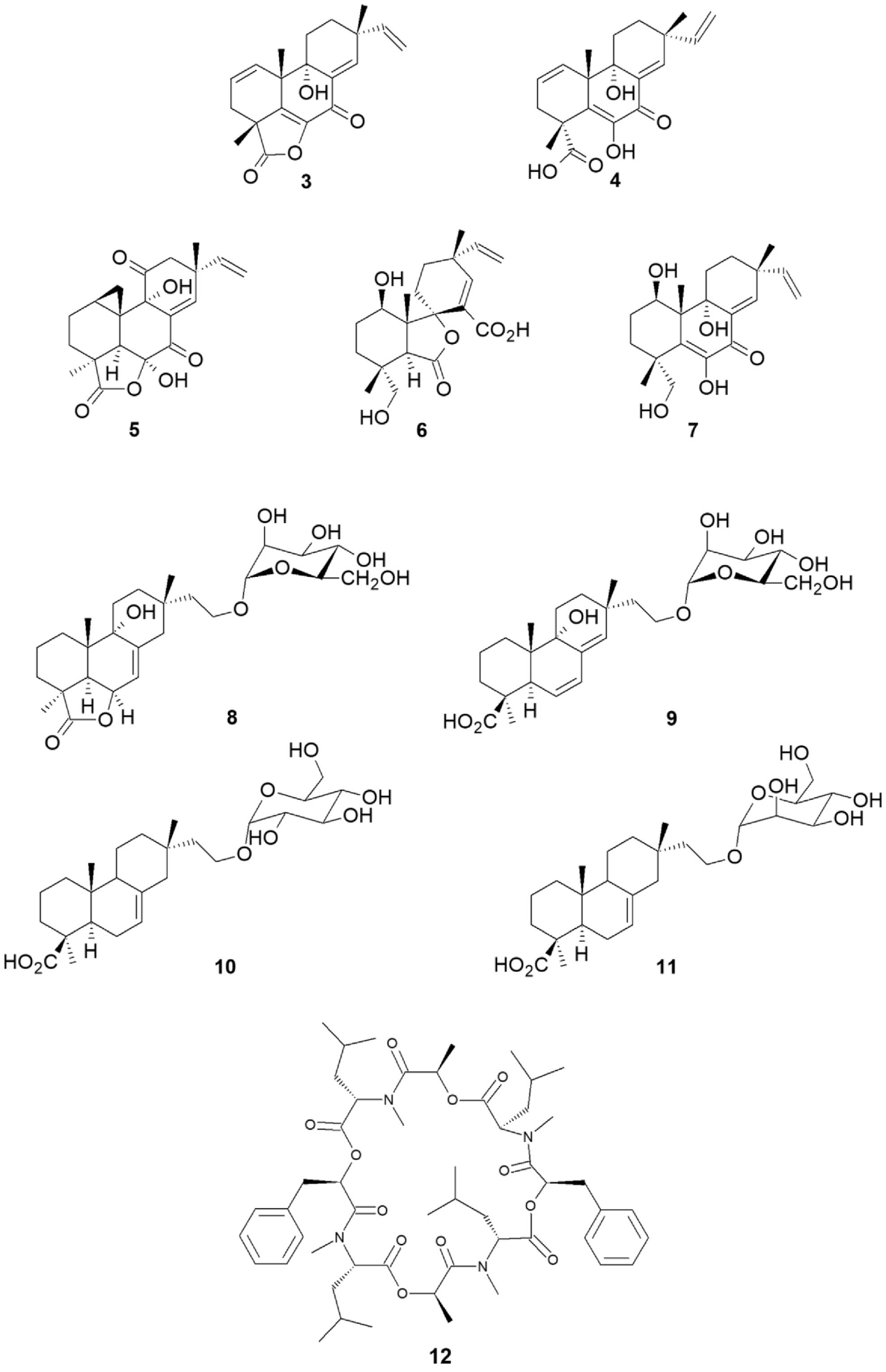

Fig. 4. Isolated secondary metabolites (**3**-**12**), known from different species of the orders *Xylariales* and *Hypocreales*.

A (**6**) (Shiono *et al.* 2013), libertellenone C (**7**) (Oh *et al.* 2005), hymatoxin K (**8**) (Jossang *et al.* 1995) and elaeicolaside C (**9**) (Wang *et al.* 2012), were isolated from a submerged culture (4 L) of *D. bunodes* CBS123585 (Figs 2–4). Further, two known isopimarane diterpene glycosides, 16-α-D-glucopyranosyloxy-isopimar-7-en-19-oic acid (**10**) and 16-α-D-mannopyranosyl-oxyisopimar-7-en-19-oic acid (**11**) (Shiono *et al.* 2009) were obtained from *D. pepo* CBS123592 (4 L) (Fig. 4). All metabolites were characterised by HRMS and NMR. For known compounds, the data were compared with literature values in order to confirm their identity. The isolated isopimarane diterpenoids (**1**–**11**) were the prevailing components within all extracts of the investigated *Dematophora* strains, but some of them could be also detected in extracts of species belonging to the *Rosellinia* clade, like *R. aquila*, *R. corticium* and *R. marcucciana* as well as in extracts of *Astrocystis mirabilis* (Fig. 5). No cytochalasins, as previously reported from *D. necatrix* (Aldridge *et al.* 1972, Kimura *et al.*

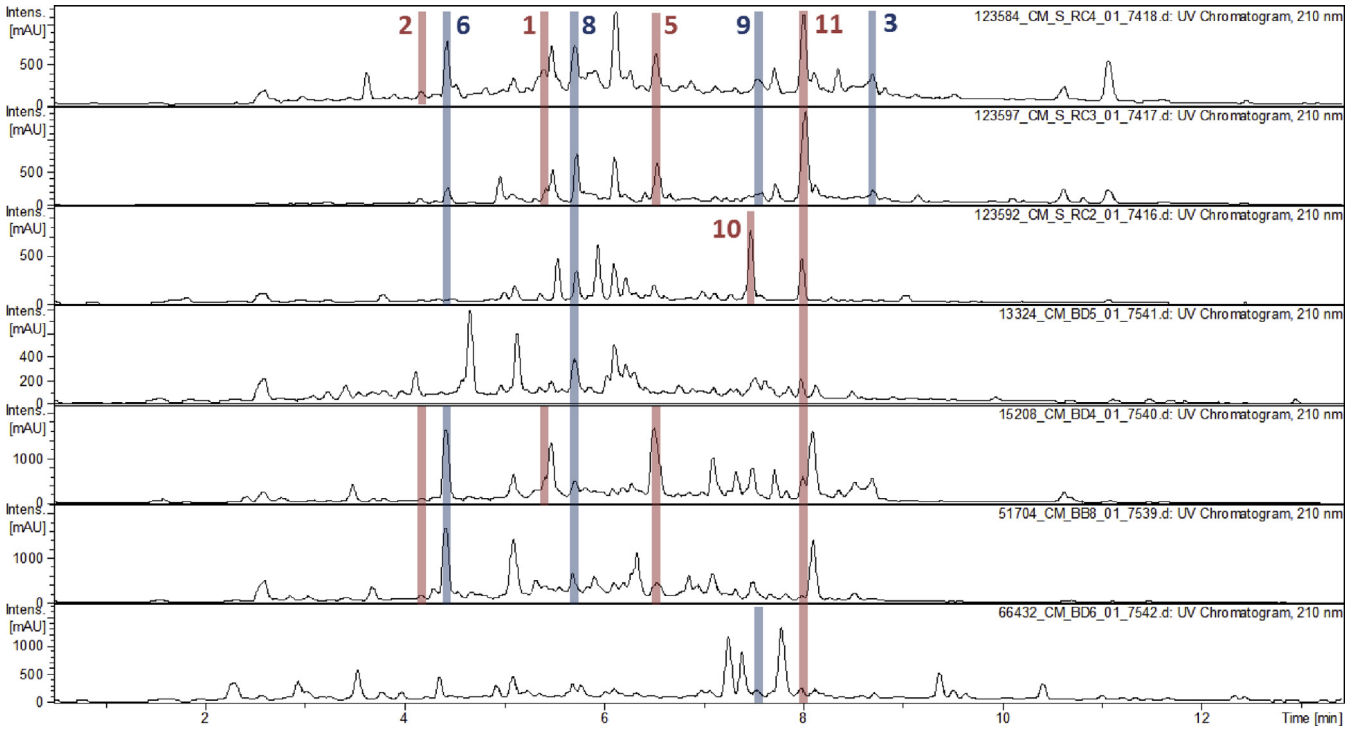

Fig. 5. HPLC-UV chromatograms (210 nm) of crude extracts from *Dematophora bunodes* (CBS123584), *D. bunodes* (CBS123597), *D. pepo* (CBS123592), *Rosellinia corticium* (STMA13324), *R. aquila* (STMA15208), *R. marcucciana* (MUCL51704), and *Astrocystis mirabilis* (ATTC66432) cultivated in liquid CM medium (200 mL). The detected isopimarane diterpenoids (**1**–**3**, **5**–**6**, **8**–**11**) are marked.

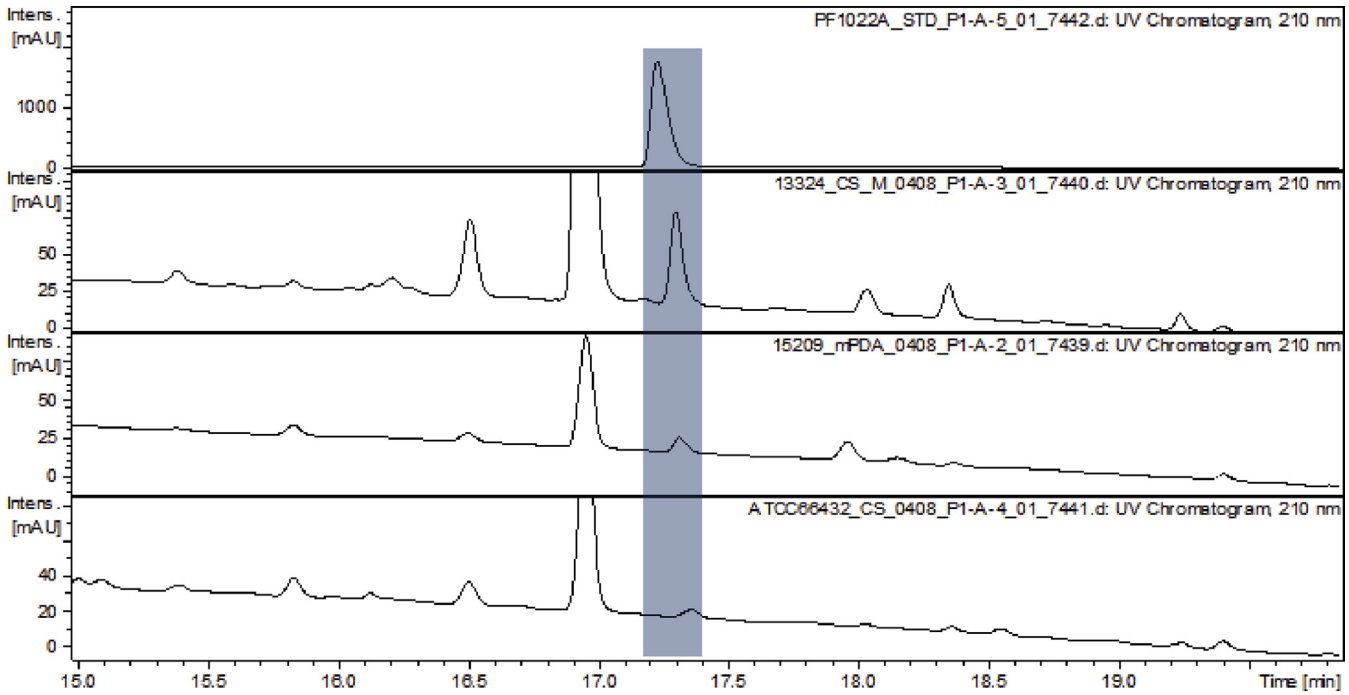

Fig. 6. HPLC-UV chromatograms (210 nm) of crude extracts containing PF1022A (top to bottom: pure PF1022A, *Rosellinia corticium* (STMA13324), *R. corticium* (STMA15209), *Astrocystis mirabilis* (ATTC66432).

1989, Shimizu *et al.* 2018), were detected using the described cultivation conditions.

In order to investigate the production of cyclodepsipeptides of the PF1022 family (Scherkenbeck *et al.* 2002) by species of the genus *Rosellinia*, as indicated in a patent application by Harder *et al.* (2011), several *Rosellinia* strains and *Astrocystis mirabilis* (ATCC 66432) were cultivated on three different solid media (see cultivation procedures and Table S2). PF1022 A (**12**) (Fig. 4) was detected in extracts of *R. corticium* (STMA 15209), *A. mirabilis* (ATCC 66432) and in highest concentration in the extract of *R. corticium* (STMA 13324), (Fig. 6). The compound was isolated from submerged cultures of strain STMA 13324 in CS medium (3 L) and used as standard for the analysis of extracts obtained within this study (details are given in the Supplementary Information). Additionally, compounds with the masses of PF1022 C and PF1022 D with similar retention times to PF1022 A were observed in the CS medium extracts of *R. corticium* (STMA13324). After comparison with a fermentation sample of the original PF1022 producer strain, we concluded that these compounds were most likely cyclodepsipeptides PF1022 C and PF1022 D (Fig. S6).

Structure elucidation

Dematophorane A (**1**) was obtained as a colourless oil (21.3 mg) and its molecular formula was deduced as $C_{20}H_{30}O_5$ by high resolution ESI-MS (Fig. S2), indicating six degrees of unsaturation. The ^{1}H and ^{1}H,^{13}C-HSQC NMR spectra of (**1**) revealed one olefinic methine proton, one olefinic methylene group with a characteristic carbon at δ_C 113.3 ppm, four methines (three of them oxygenated), five methylene groups (one of them oxygenated) and three methyl groups. Additionally, the ^{13}C NMR spectrum exhibited signals of one ketone, two olefinic quaternary carbons and three sp^3 hybridized quaternary carbons. The ^{1}H,^{1}H-COSY correlations between the methylene groups CH_2-11 and CH_2-12 as well as between methine H-1, methylenes CH_2-2 and CH_2-3 and the analysis of the ^{1}H,^{13}C-HMBC correlations of (**1**) indicated the presence of a tricyclic diterpene scaffold. Key HMBC correlations include H-7 to C_q-6, C_q-9, C_q-8, H-5 to C_q-6, C_q-9, C-1, C-18, C_q-10, C_q-4, C-3, C-19 and H-14 to C_q-9, C_q-8, C-7, C_q-13, C-12, C-17 (Fig. 3). Further COSY correlations between olefinic methine H-15 and methylene CH_2-16 and HMBC correlations of H-15 to C_q-13, C-17, C-12 and C-14 demonstrated a terminal vinyl group at C_q-13 between methylene CH_2-12 and oxygenated methine H-14, a structural feature of many pimarane-type diterpenoids (Yu *et al.* 2018). The oxygenated methylene group at δ 3.08 (d, 1H), 3.46 (d, 1H), C 70.7 ppm was identified as hydroxylated methylene CH_2-19 at C_q-4 via ^{1}H,^{13}C long range correlations of H-19 to C-5, C_q-4, C-3 and C-18. The relative stereochemistry of dematophorane A (**1**) was assigned by the analysis of ROESY data, which showed strong correlations between methyl protons H-17, methine H-14 and methylene proton H-11a as well as between methyl protons H-20, H-18 and H-11a, indicating that methyl groups CH_3-17, CH_3-20 and CH_3-18 as well as methine H-14 are positioned at the same face of the molecule. On the opposite side, methine proton H-5 displayed strong correlations to H-7 and H-1 (Figs. S6–11). This stereochemistry is in accordance with the typical structure of an isopimarane-type diterpenoid (Wang *et al.* 2018).

Dematophorane B (**2**) was isolated as colourless film (2.1 mg) and displayed a major peak in the HR-ESIMS spectrum at m/z 373.1984 $[M+Na]^+$, calcd. for $C_{20}H_{30}NaO_5$, 373.1991), consistent with the molecular formula $C_{20}H_{30}O_5$ and indicating six degrees of unsaturation (Fig. S3). The ^{1}H and ^{1}H,^{13}C-HSQC NMR spectra of (**2**) were similar to those of (**1**) and showed signals which were assigned to a terminal vinyl group with CH-15 (δ_H 5.86 (dd, J = 17.6, 10.6), δ_C 149.4) and CH_2-16 (δ_H 4.92 (dd, J = 10.7, 1.4), 5.00 (dd, J = 17.5, 1.4), δ_C 110.9), one olefinic methine, three further methines (two oxygenated), five methylene groups (one of them oxygenated) and three methyl groups. The ^{13}C NMR spectrum exhibited signals of one ketone, one olefinic quaternary carbon, three sp^3 hybridized quaternary carbons and one oxygenated quaternary carbon. Similar to dematophorane A (**1**) a pimarane-type diterpenoid scaffold was assigned due to ^{1}H,^{1}H COSY correlations between CH_2-11 and CH_2-12 as well as between OH-1, H-1, CH_2-2 and CH_2-3 and corresponding ^{1}H,^{13}C HMBC correlations. But in contrast to derivative (**1**) an additional olefinic methine (δ_H 5.84 (s), δ_C 130.6) and an oxygenated quaternary carbon (δ_C 75.3) were detected instead of a second olefinic quaternary carbon. ^{1}H,^{13}C HMBC correlations of this olefinic methine proton H-14 to C-15, C_q-8, C-7, C_q-9 and C-12 indicated a position of the olefin between the terminal vinyl group and hydroxyl group OH-7. HMBC correlations of H-20, H-12, H-11, H-5, H-1 and OH-9 to the quaternary carbon C_q-9 confirmed the assignment of a hydroxyl group at position C-9. The analysis of the NOESY data showed strong correlations between protons H-17, H-11a, H-12a and H-20 as well as between H-20, OH-1 and H-18. Additional NOE correlations between protons H-7, OH-9, H-12b, H-1 and protons H-5, H-7, H-1 demonstrated the same relative stereochemistry as for compound (**1**), (Figs. S12–17). During the purification and analysis processes, the rearrangement of dematophorane B (**2**) in methanol solutions at room temperature was observed. After keeping (**2**) in methanol at 40 °C for 19 d, approximately 70 % of the molecule converted into another major compound, which was identified as libertellenone C (**7**). The slightly basic conditions and/or thermal energy seem to induce a rearrangement of the alpha-hydroxyketone of (**2**) and a final stabilization of the molecule by dehydrogenation.

Biological activity of extracts and pure compounds

Extracts were screened for antimicrobial activity against Gram-positive/Gram-negative bacteria (*Bacillus subtilis*/*Escherichia coli*), a yeast (*Candida albicans*) and the filamentous fungus *Mucor plumbeus* as well as for nematicidal activity against *Caenorhabditis elegans*. The pure compounds were tested against a larger panel of bacteria and fungi (Table 3) and for their cytotoxicity on mouse fibroblast cells L929, cervix carcinoma cells KB-3-1 or primary human umbilical vein endothelial cells HUVEC (Table 3). Due to its instability the γ-lactone of libertellenone M (**4**) was not tested.

Extracts from *D. bunodes* strains (CBS123584 – CM medium, CBS123585 – CM and Q6 medium, CBS 123597 – CM medium, CBS124028 – CM medium) showed antibiotic activity against *B. subtilis* (DSM10). No further antimicrobial activity was detected among the crude extracts. Nematicidal activity was observed for the extracts of *R. corticium* (STMA 15209 – mPDA medium, STMA 13324 – CSA medium, STMA12170 – CSA medium, MUCL 51693 – CMD medium), *R. aquila* (STMA 15208 – CSA and CMD medium), *R. marcucciana* (MUCL 51704 –

Table 3. *In vitro* antibacterial, antifungal and cytotoxic activity of dematophoranes A-B (**1-2**), libertellenone M (**3**), myrocin B (**5**) and libertellenone C (**7**). All compounds were dissolved in methanol (1 mg/ml, test volume: 20 µl). 20 µl of methanol showed no effect on the test organisms. MIC: Minimum inhibitory concentration. Positive controls: [a] Oxytetracyclin hydrochloride, [b] Gentamycin, [c] Nystatin; [d] Epothilon B, n.i.: no inhibition.

Test organisms	MIC [µg/mL]					
	(1)	(2)	(3)	(5)	(7)	Ref [a,b,c]
Gram-positive bacteria						
Bacillus subtilis DSM 10	n.i	67.0	n.i	33.3	n.i.	8.3 [a]
Micrococcus luteus DSM 1790	67.0*	67.0	n.i	67.0	n.i	0.42 - 0.83 [a]
Mycobacterium smegmatis ATCC700084	67.0	67.0	n.i	67.0	n.i.	3.3 [a]
Staphylococcus aureus DSM 346	67.0	67.0	n.i	33.3	67.0*	0.42–0.83 [a]
Gram-negative bacteria						
Chromobacterium violaceum DSM 30191	n.i	n.i	n.i	n.i	n.i	0.83 [a]
Escherichia coli DSM 1116	n.i	n.i	n.i	n.i	n.i	3.3–6.7 [a]
Pseudomonas aeruginosa PA 14	n.i	n.i	n.i	n.i	n.i	4.2 [b]
Yeasts						
Candida albicans DSM 1665	n.i	n.i	n.i	n.i	n.i	8.3 [c]
Wickerhamomyces anomalus DSM 6766	n.i	n.i	n.i	-	n.i	16.6 [c]
Rhodotorula glutinis DSM 10134	n.i.	n.i.	n.i.	n.i	n.i	2.1 [c]
Schizosaccharomyces pombe DSM 70572	n.i	n.i	n.i	n.i	n.i	16.6 [c]
Filamentous fungi						
Mucor hiemalis DSM 2656	n.i.	67.0*	67.0*	n.i	67.0*	8.3 [c]
Cell lines						
Mouse fibroblast cell line L929	28	20	6.3	6.5	9.5	1.1×10^{-3} [d]
Cervix carcinoma cell line KB-3-1	6.5	16	6.1	-	14	0.06×10^{-3} [d]
Primary Human Umbilical Vein Endothelial Cells HUVEC	-	-	-	0.75	-	0.2×10^{-3} [d]

The cell density was adjusted to 8×10^6 cells/mL. * no complete inhibition; DSMZ: German Collection of Microorganisms and Cell Cultures, Braunschweig.

CSA and CMD medium) and *A. mirabilis* (ATCC 66432 – CSA and CMD medium).

The pure compounds dematophorane A (**1**) and B (**2**) showed weak antibiotic activity (MIC: 67 µg/mL) against several Gram-positive test strains (*B. subtilis*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *S. aureus*). Also, myrocin B (**5**) displayed weak to moderate activity (MIC: 67–33 µg/mL) against these Gram-positive strains. Its activity against *B. subtilis* was reported before by Hsu *et al.* 1988 and Lehr *et al.* 2006. For dematophorane B (**2**), libertellenone M (**3**) and libertellenone C (**7**) weak antifungal activity (MIC: 67 µg/mL) against *Mucor hiemalis* was observed. In addition, Libertellenone M (**3**) displayed moderate cytotoxicity on mouse fibroblast cells L929 (6.3 µg/mL) and carcinoma cells KB-3-1 (6.1 µg/mL). Dematophorane A (**1**) also showed cytotoxic effects on carcinoma cell line KB-3-1 (6.5 µg/mL) and myrocin B (**5**) on L929 cells (6.5 µg/mL) as well as on primary HUVEC cells (0.75 µg/mL) (Table 3).

Table 4. Species groups of *Rosellinia sensu* Petrini (2013) and their salient morphological features.

Species group	Conidiophore type	Ratio of ascospore length/width	Ascospore germ slit	Other characteristic features of asci/ascospores
Rosellinia aquila	*Geniculosporum*	<4	straight, spore length	cellular appendages, slimy caps and sheath mostly present
R. mammaeformis	*Geniculosporum*	<4	straight or diagonal, spore length to 2/3 spore length	cellular appendages, slimy caps and sheath mostly present
R. emergens	*Geniculosporum*	≥4	variable	cellular appendages, slimy caps and sheath mostly absent
R. mammoidea	*Geniculosporum*	<4	straight, mostly spore length	cellular appendages, slimy caps and sheath absent; usually smaller than 16 µm
R. necatrix	*Dematophora*	≥4	straight, short	cellular appendages absent, slimy sheath present
R. buxi	*Dematophora*	<4	straight, mostly spore length	cellular appendages absent, slimy caps or sheath present
R. thelena	*Geniculosporum* or *Nodulisporium*	<4	straight, mostly spore length	cellular appendages, slimy caps and sheath present

Taxonomy

The phylogenetic study, in which various species of *Rosellinia* and allies were compared in a multi-locus phylogeny, indicated that the genus should be subdivided. While the majority of species in *Rosellinia sensu* Petrini (2013) remain to be incorporated into a comprehensive molecular phylogeny, we noted a strong correlation between the type of asexual morph and the results of our phylogenetic study.

Table 4 gives a summary on the crucial characteristics of the genus *Rosellinia sensu* Petrini (2013), summarising the most important morphological features of the seven species groups defined in the latter monograph.

All *Rosellinia* spp. that are known to form a dematophora-like asexual morph are included in the "*R. buxi* Group" or the "*R. necatrix* Group", and at the same time, their DNA sequences clustered in clade **D**. On the other hand, sequences of those species studied that have a geniculosporium-like asexual morph, clustered in clade R along with the type species, *R. aquila*. In order to make the systematics of *Rosellinia* more compatible with the evidence obtained from the current molecular study and congruent with the morphological evidence, in particular considering the "One Fungus-One Name" concept (see also Stadler *et al.* 2013 for a respective treatment of the stromatic *Xylariales*), we therefore propose to resurrect the genus *Dematophora* for the two aforementioned "Groups". We propose the following taxonomic rearrangements:

Rosellinia De Not., G. bot. ital. 1(1): 334. 1844. Fig. 7.
Synonyms: *Amphisphaerella* Henn., Hedwigia 41: 18. 1902.
Byssitheca Bonord., Abh. naturforsch. Ges. Halle 8: 82, 156. 1864.
Vrikshopama D. Rao & P. Rag. Rao, Mycopath. Mycol. appl. 23: 289. 1964.

Type species: *Rosellinia aquila* (Fr.) Ces. & De Not. 1844.

Typus: **Sweden**, "Småland", (today Skåne) Lund, E. Fries (UPS, sub *Sphaeria aquila* - **holotype**).

Generic description (modified from Petrini 2013):
Subiculum woolly, wiry, felted, brown, white, yellow, persistent or evanescent. *Stromata* subglobose, mammaeform, semiglobose or conical, sessile or broadly stipitate, glabrous, ostiolate, brown, grey, black, uniperitheciate, sometimes confluent containing a few perithecia, superficial. *Perithecia* globose, collapsing and usually detached from stromatal wall, rarely remaining attached. *Paraphyses* filiform, evanescent. *Asci* cylindrical, long stipitate, evanescent. *Ascus* apical plugs cylindrical with bulge at upper rim, rarely without, amyloid, rarely not amyloid. *Ascospores* unicellular, asymmetrically ellipsoidal to fusoid, light to dark brown, with straight, sigmoid or spiral germ slit extending over the whole spore length or shorter, rarely absent.

Asexual morph: Geniculosporium-like, rarely nodulisporium-like.

Notes: The above generic description is identical to the one by Petrini (2013) except for the definition of asexual morph. The species that remain in *Rosellinia* after segregation of *Dematophora* cannot be segregated by morphological features of the asci, ascospores or stromata. The species that are retained in the genus *Rosellinia* comprise the *R. aquila*, *R. mammaeformis*, *R. emergens*, *R. mammoidea* and *R. thelena* "Groups" (the latter of which has been regarded as subgenus *Corrugata*) as defined by Petrini (2013). A lot of work remains to be done in order to clarify their affinities and the genus may possibly have to be further subdivided once more molecular data and more information about the morphology of the asexual morphs become available (see Figs 7, 8). The segregation of *Dematophora* also has some practical implications, since almost all important pathogens among the rosellinoid *Xylariaceae*, except for *Entoleuca mammata* and the conifer pathogens of the *R. thelena* Group *sensu* Petrini (2013, subgenus *Corrugata*) are included in the genus. We hope that this taxonomic change may facilitate future work on the non-pathogenic species that are retained in *Rosellinia*. Even though the present study does not provide any direct evidence that the "beneficial" compound PF1022 A actually can play a role in the protection of the host plants by the saprotrophic/endophytic producers, there are only few reports on potential, weak pathogenicity of the species that were not transferred to *Dematophora*.

Dematophora R. Hartig, Untersuch. Forstbot. Inst. München 3: 95, 125. 1883. Fig. 7.

Type species: *Dematophora necatrix* R. Hartig, Untersuch. Forstbot. Inst. München 3: 126. 1883.

Typus: **Germany**, Bavaria, Munich, R. Hartig (FOMU-**Lectotype** *fide* Petrini 2013).

Emended generic description: Differs from *Rosellinia* as defined further above in the presence of characteristic synnemata of the *Dematophora* type, while sharing a similar morphology of stromata, asci and ascospores. The asexual morph is geniculosporium-like. Nodulisporium-like conidiogenous structures are not observed.

Notes: The genus comprises the following species (below), all of which were previously retained in *Rosellinia* by Petrini (2013) and are reported to possess a dematophora-like asexual morph.

Dematophora acutispora (Theiss.) C. Lambert, K. Wittstein & M. Stadler, ***comb. et stat. nov.*** MycoBank MB827530.
Basionym: *Rosellinia desmazieri* var. *acutispora* Theiss., Annls mycol. 6(4): 350. 1908.
Synonym: *Rosellinia acutispora* (Theiss.) L.E. Petrini, Index Fungorum 25: 1. 2013.

Dematophora arcuata (Petch) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827529.
Basionym: *Rosellinia arcuata* Petch, Ann. Roy. Bot. Gard. (Peradeniya) 6 (1): 175. 1916.

Dematophora asperata (Massee ex Wakef.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827531.
Basionym: *Rosellinia asperata* Massee ex Wakef., Bull. Misc. Inf., Kew: 209. 1918.

Dematophora beccariana (Ces.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827532.
Basionym: *Rosellinia beccariana* Ces., Accad. Sci. Fis. Napoli 5 (21): 12. 1872.

Dematophora boedijnii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827533.
Basionym: *Rosellinia boedijnii* L.E. Petrini, Index Fungorum 25: 1. 2013.

Dematophora bothrina (Berk. & Broome) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827534.
Basionym: *Sphaeria bothrina* Berk. & Broome, J. Linn. Soc., Bot. 14: 125. 1875.

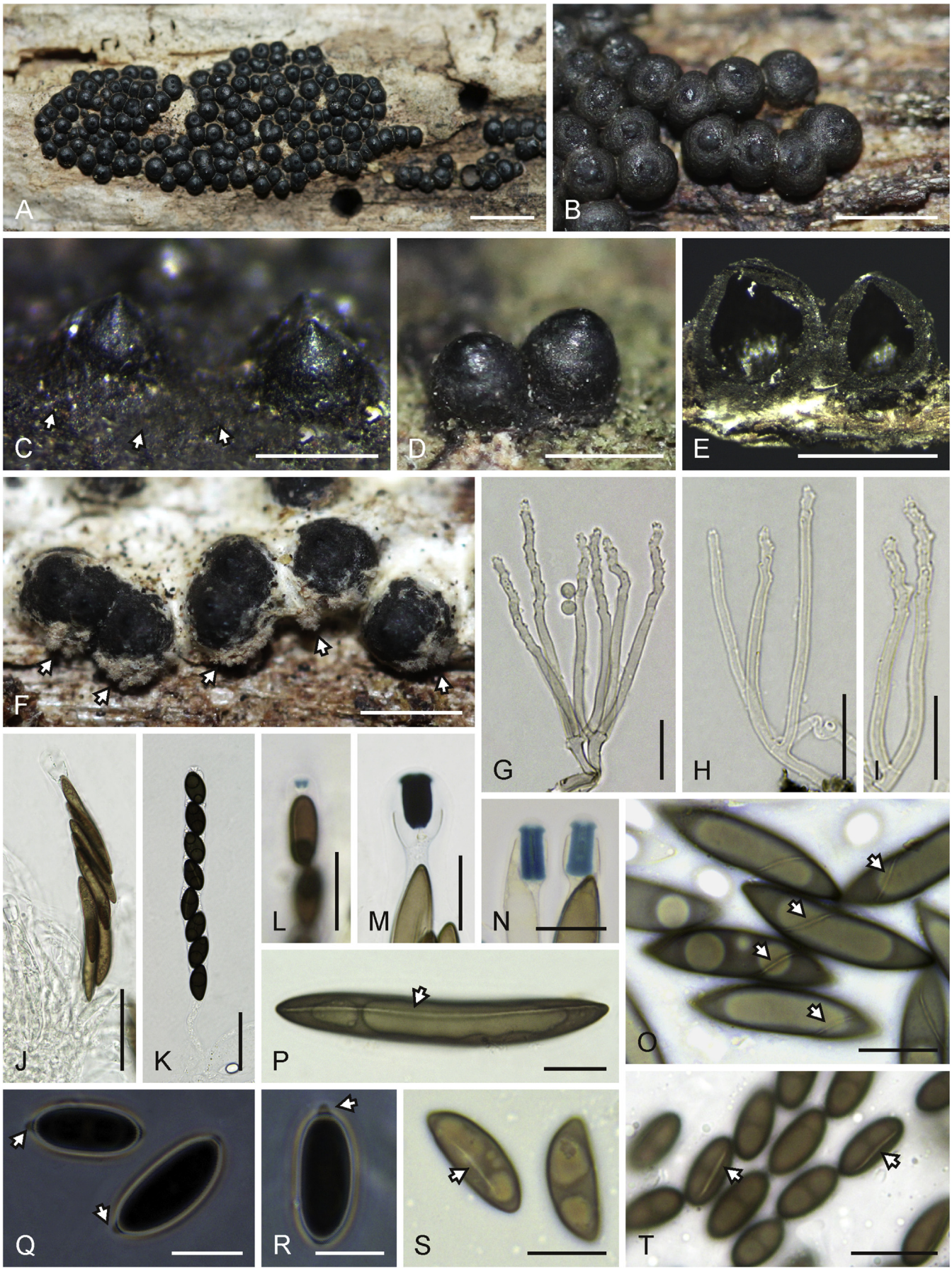

Fig. 7. Sexual and asexual morph structures of different *Rosellinia* species. **A**, **L**, **T**. *R. breensis* (Sir & Hladki 841-LIL). **B**, **S**. *R. hyalospora* (Sir & Hladki 463-LIL). **C**. *R. megalospora* (Sir & Hladki 972-LIL). **D**, **E**, **J**, **M**, **P**. *R. longispora* (Sir & Hladki 939-LIL). **F–I**, **K**. *R. rickii* (Sir & Hladki 062-LIL). **N**, **O**. *R. canzacotoana* (Sir & Hladki 198-LIL). **Q**, **R**. *Rosellinia* sp. (Sir & Hladki 377-LIL). **A, B, D.** Stromata in substrate. **C.** Stromata emerging from the subiculum (arrow). **E.** Cross section of stromata. **F**. Stromata and conidiophores (arrows). **G–I.** Conidiogenous structure in 3% KOH. **J, K.** Ascus in 3% KOH. **L–N.** Ascus apical plugs in Melzer's reagent. **O, P, S, T.** Ascospores showing germ slit in 3% KOH (arrows). **Q, R.** Ascospores showing cellular appendages in 3% KOH (arrows). Scale bars: A = 2 mm; B–F = 1 mm; G, H, L–T = 10 µm; I, J = 50 µm; K = 20 µm.

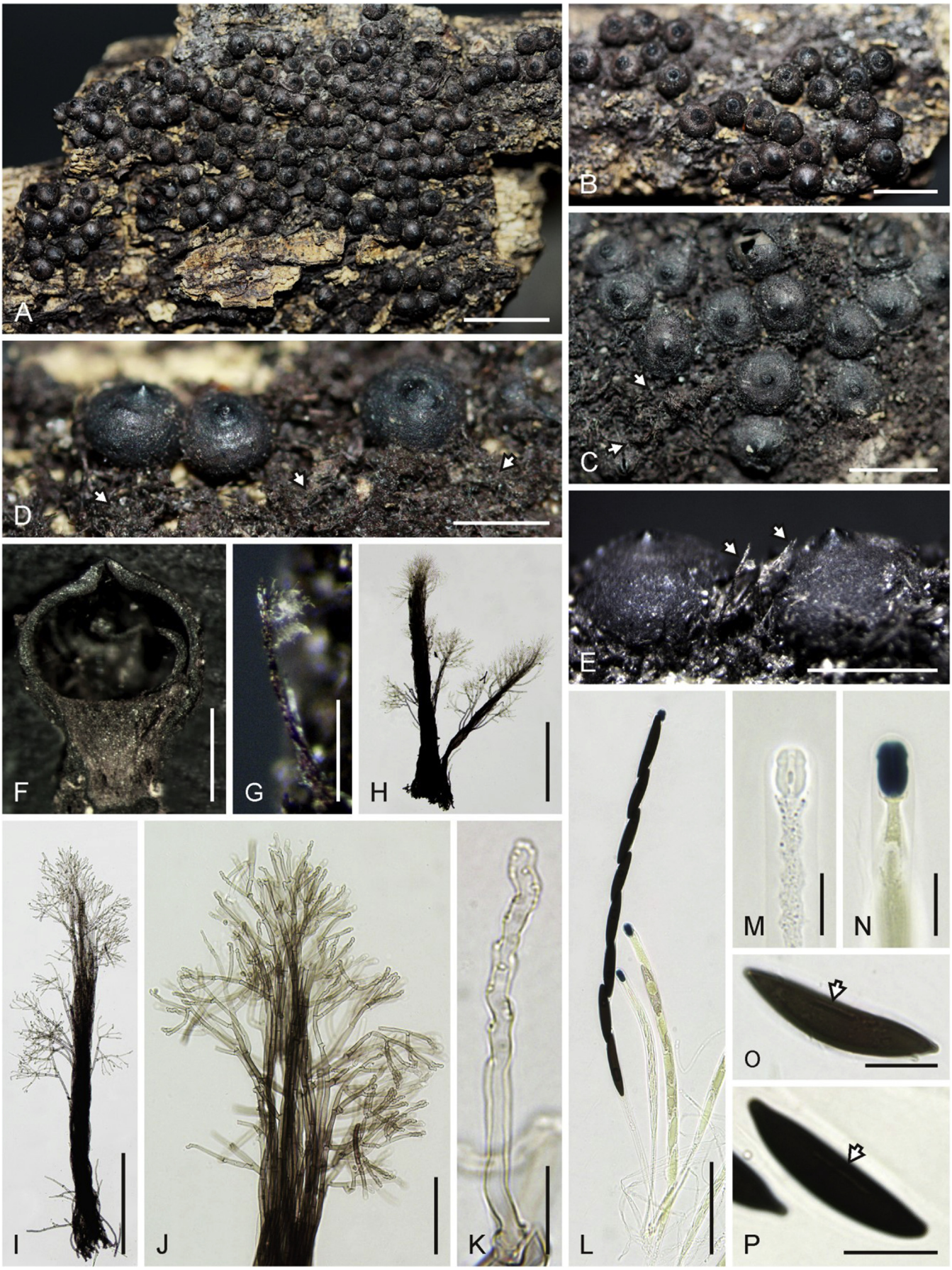

Fig. 8. Asexual and sexual morph structures of different *Dematophora* species. **A**, **B**, **F**, **O**. *D. necatrix* (Hladki 4004-LIL). **C**-**E**, **G**, **H**, **L**–**N**, **P**. *D. paraguayensis* (Sir & Hladki 1098-LIL). **I**–**K**. *D. arcuata* (Sir & Hladki 1098-LIL). **A, B**. Stromata on substrate. **D, C**. Stromata and subiculum (arrows). **E**. Stromata and synnemata (arrows) on substrate. **F**. Short stipitate stroma in cross section. **G**. Synnema on substrate. **H, I**. Synnemata in 3 % KOH solution. **J**. Details of conidiogenous region. **K**. Detail of conidiogenous cell. **L**. Asci in Melzer's reagent. **M**. Ascus apical plugs in 3 % KOH. **N**. Ascus apical plug in Melzer's reagent. **O, P**. Ascospores showing short and central germ slit in 3 % KOH (arrows). Scale bars: A = 5 mm; B, C = 2 mm; D, E = 1 mm; F, G = 500 µm; H, I = 200 µm; J, L = 50 µm; K, M, N, O, P = 10 µm.

Synonym: *Rosellinia bothrina* (Berk. & Broome) Sacc., Syll. Fung. 1: 257. 1882.

Dematophora bunodes (Berk. & Broome) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827535.
Basionym: *Sphaeria bunodes* Berk. & Broome, J. Linn. Soc., Bot. 14 (74): 125. 1873.

Synonyms: *Hypoxylon bunodes* (Berk. & Broome) P.M.D. Martin, S. African J. Bot. 42 (1): 72. 1976.
Rosellinia bunodes (Berk. & Broome) Sacc., Syll. Fung. 1: 254. 1882.

Rosellinia echina Massee, Bull. Misc. Inform. Kew: 155. 1901.
Rosellinia zingiberis F. Stevens & Atienza Philipp. Agric. 20 (3): 174. 1931.

Dematophora buxi (Fabre) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827546.
Basionym: *Rosellinia buxi* Fabre, Ann. Sci. Nat., Bot., sér. 6, 9: 78. 1879.

Dematophora compacta (Takemoto) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827536.
Basionym: *Rosellinia compacta* Takemoto, Mycologia 101 (1): 89. 2009.

Dematophora francisiae (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827547.
Basionym: *Rosellinia francisiae* L.E. Petrini, Index Fungorum 25: 2. 2013.

Dematophora freycinetiae (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler**, *comb. nov.*** MycoBank MB827537.
Basionym: *Rosellinia freycinetiae* L.E. Petrini, New Zealand J. Bot. 41(1): 98. 2003.

Dematophora gigantea (Ellis & Everh.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827538.
Basionym: *Rosellinia gigantea* Ellis & Everh., Bull. Lab. Nat. Hist. Iowa State Univ. 2: 401. 1893.
Synonym: *Hypoxylon giganteum* (Ellis & Everh.) P.M.D. Martin, JI S. African J. Bot. 42(1): 72. 1976.

Dematophora grantii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827539.
Basionym: *Rosellinia grantii* L.E. Petrini, Index Fungorum 25: 2. 2013.

Dematophora hsiehiae (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler**, *comb. nov.*** MycoBank MB827548.
Basionym: *Rosellinia hsiehiae* L.E. Petrini, Index Fungorum 25: 3. 2013.

Dematophora hughesii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827549.
Basionym: *Rosellinia hughesii* L.E. Petrini, New Zealand J. Bot. 41 (1): 102. 2003.

Dematophora javaensis (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827550.
Basionym: *Rosellinia javaensis* L.E. Petrini, Index Fungorum 25: 3. 2013.

Dematophora macdonaldii (Bres.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827551.
Basionym: *Rosellinia macdonaldii* Bres. [as *'macdonaldi'*], Stud. Trent. 7 (1): 66. 1926.

Dematophora obregonii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827540.
Basionym: *Rosellinia obregonii* L.E. Petrini, Index Fungorum 25: 3. 2013.

Dematophora obtusiostiolata (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB82754.
Basionym: *Rosellinia obtusiostiolata* L.E. Petrini, Index Fungorum 25: 4. 2013.

Dematophora paraguayensis (Starbäck) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827542.
Basionym: *Rosellinia paraguayensis* Starbäck, Ark. Bot. 2(5): 15. 1904.

Dematophora pepo (Pat.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827543.
Basionym: *Rosellinia pepo* Pat., Bull. Trimestriel Soc. Mycol. France 24 (1): 9. 1908.

Dematophora puiggarii (Pat.) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827544.
Basionym: *Rosellinia puiggarii* Pat., J. Bot. (Morot) 2: 217. 1888.

Dematophora pyramidalis (Lar.N. Vassiljeva) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827553.
Basionym: *Rosellinia pyramidalis* Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloaskomitsety 202. 1998.

Dematophora samuelsii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov.*** MycoBank MB827552.
Basionym: *Rosellinia samuelsii* L.E. Petrini, New Zealand J. Bot. 41 (1): 124. 2003.

Dematophora* siggersii** (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, ***comb. nov. MycoBank MB827545.
Basionym: *Rosellinia siggersii* L.E. Petrini, Index Fungorum 25: 4. 2013.

DISCUSSION

The secondary metabolite production of the genera *Rosellinia* and *Dematophora* is poorly investigated. So far, there are only a few reports dealing with the metabolites, including cytochalasins from *D. necatrix* (Chen 1960, 1964, Kimura et al. 1989, Shimizu *et al.* 2018), cytochalasins from *R. sanctae-cruciana* (Sharma *et al.* 2018), sordarin and xylarin from various *Rosellinia species* (Vicente *et al.* 2009) and the producer strain of the cyclodepsipeptides "PF1022", which was tentatively assigned to the genus *Rosellinia* (Sasaki *et al.* 1992). During our chemical analyses of the submerged cultures mainly isopimarene diterpenoids were detected and isolated, in particular from *Dematophora* species. But since these compounds are known to be produced by different ascomycetes, especially from *Xylariales* (Helaly *et al.* 2018), and were also detected in extracts of the *Rosellinia* species, they cannot be considered as specific (marker metabolites) for the genus *Dematophora*. All previously reported diterpenoids (**3-11**) were isolated from fungi of the order *Xylariales* or *Hypocreales*, but none of these metabolites have been reported from species of the genus *Rosellinia* (or *Dematophora*) before. Although compounds (**1**–**3**, **5** and **7**) showed weak effects on Gram-positive bacterial strains and *M. hiemalis,* no significant antimicrobial activity could be observed for any of

the isolated isopimarane diterpenoids. Some of them (**1**, **3**, **5**) displayed moderate cytotoxic effects on different cell lines. They remain to be tested for phytotoxic activities in order to evaluate possible natural functions as they were predominant in the extracts of the pathogenic *D. bunodes* and *D. pepo*. However, many compounds detected in the extracts of the submerged cultivations are not yet clearly identified. Further isolation campaigns, along with the modification of culture media, and in particular the inclusion of additional species will shed further light on the true potential of these ascomycetes to produce interesting metabolites, which might be also useful for the taxonomic classification. Cyclodepsipeptide PF1022 A (**12**), which is known for its strong nematicidal activity (Conder *et al.* 1995) was detected in different extracts of *R. corticium* (and *Astrocystis mirabilis*), but in some of them titres were very low (Fig. 6). A patent application by Harder *et al.* (2011) had already indicated that the genera *Rosellinia* (in particular *R. aquila* and *R. corticium*) and *Coniolariella hispanica* were producer organisms of PF1022 A. However, the data were derived from strains that are unavailable in public collections, and the corresponding stromata were likewise not identified by experts or deposited in a public herbarium. Furthermore, the compound was only detected by HPLC-MS. We were unable to find a corresponding publication in a peer-reviewed journal. The patent application mentioned that two strains of *R. abscondita* from the CBS collection were also examined for comparison, but no data were presented suggesting that those strains produced the cyclodepsipeptides. Another patent application by Harder *et al.* (2012) treated the isolation and identification of novel PF1022 derivatives and mentioned that various strains, including *R. abscondita* (strains CBS 447.89, CBS 448.89 and CBS 450.89), "*R. britannica*" (current valid name: *R. marcucciana*, strain CBS 446.89) and *R. mammaeformis* (strain CBS 445.89), *R. nectrioides* (strain CBS 449.89, all the former resulting from the study of Petrini 1992) were studied, as well as *R. millegrana* (strain CBS 111.75), and even strains of *D. necatrix* and *Xylaria hypoxylon*. The patent application does, however, not indicate which, if any, of the organisms studied actually yielded the known and new metabolites. In addition, even though semi-preparative fractionations of the crude extracts were described, the new compounds were only identified tentatively by their HPLC-MS characteristics and not isolated to purity. The authors also reported on "lines" they grew from the CBS cultures, which were able to overproduce the compounds (presumably by some means of classical strain optimisation), but did not state from which of the strains these "lines" were derived. Evidently, the patent application was filed in order to protect the new intellectual property on the newly detected cyclodepsipeptides, rather than to disclose information on their distribution. However confusing these data have been reported, the work gives corroborating evidence that PF1022 derivatives and other cyclodepsipeptides are widespread in *Rosellinia*. In any case, our present study is the first one in which PF1022 A has been isolated to purity from an ascospore-derived isolate that is derived from a taxonomically well-defined specimen and characterised by NMR spectroscopy. Nematicidal activity was observed for extracts of several *Rosellinia* species even though PF1022 A could not be detected in all of them or just in very small amounts, which indicated that PF1022 A is only one active principle in the produced metabolite mixtures. Other components, including derivatives described by Harder *et al.* (2012) may also contribute to the anthelmintic activity of extracts from these species.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.simyco.2020.01.001.

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