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 mainly on the morphology of their sexual morphs. The genus comprises both lichenicolous 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 PF1022A 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 ascosporo-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. p. 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, *Dematophoraasperata* (Masssee ex Wackel.) Lambert, K. Wittstein & M. Stadler, *Dematophora beccianiana* (Ces.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora boedijnii* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora bothoria* (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 francisci* (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 graniti* (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, *Dematophora hsiiehiae* (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* (Starback) C. Lambert, K. Wittstein & M. Stadler, *Dematophora p. pepo* (Pat.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora pugnax* (Pat.) C. Lambert, K. Wittstein & M. Stadler, *Dematophora pyramidalis* (Lar.N. Vassiljeva) C. Lambert, K. Wittstein & M. Stadler, *Dematophora samueliae* (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 ascosporic 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...
genus Xylaria and other genera of Xylariaceae sensu stricto, in particular Entoleuca, Euepixylon and Nemania. The Hypoxylon fenormaldi complex, which contains some species that were previously included in Rosellinia for their superficially similar stromatal morphology (Kühnert et al. 2015), has been shown to belong to the hypoxoid clade of the Xylariales, which have stromatal pigments and a nodulisporium-like asexual morphs. From polythetic studies employing mult locus molecular phylogenies, this complex now resides in the Hypoxylaceae (Daranagama et al. 2018, Wendl et al. 2018), while Rosellinia is retained in the Xylariales, owing to its phylogenetic affinities to Xylaria. In general, the recent segregation of the stromatic Xylariales proposed in the latter papers demonstrated that ascospor morphology is often incongruent with the lineages defined by mult locus phylogeny than conidiosporic 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 Wendl et al. (2018), Rosellinia buxi and R. necatrix formed a sister clade to a clade containing the type species, R. aquila, R. corticum and Entoleuca mammeta, making Rosellinia sensu Petrini (2013) paraphyletic. This observation motivated 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 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 Agrofood & 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 Wendl 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 Conioalierea, 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 (Wendl 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 (YM) at 23 °C in darkness. Erlenmeyer flasks with 30 mL YM medium were inoculated with four mycelial plugs (0.5 × 0.5 cm²) 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 cm²), 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 strips (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 (Rupic et al. 2018b) [molasses 5 g/L, oatmeal 5 g/L, sucrose 4 g/L, manitol 4 g/L, D-glucose 1.5 g/L, CaCO₃ 1.5 mg/L, edamin (lactalbumin hydrolysate, LP0048 from Oxoid) 0.5 mg/L,
| Species                      | Strain number | Origin      | Status | GenBank accession numbers                        | Reference                      |
|------------------------------|---------------|-------------|--------|-------------------------------------------------|--------------------------------|
| Amphirosellinia fushanensis  | HAST 9111209  | Taiwan      | HT     | GU339496 N/A GQ844339 GQ495950                  | Hsieh et al. (2010)            |
| A. nigrospora                | HAST 91092308 | Taiwan      | HT     | GU322457 N/A GQ848340 GQ495951                  | Hsieh et al. (2010)            |
| Astrocytis mirabilis         | ATCC 66432    | Taiwan      | No sequence data used |                                    | Ju & Rogers (1990)            |
| Coniolearella 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 N/A MN984625 N/A                       | This study                     |
| D. buxi                      | JDR 99        | France      | AY909001 K719204 K624275 K62430                 | Peláez et al. (2006; ITS, LSU), Wendt et al. (2018; RPB2, TUB2) |
| D. necatrix                  | CBS 349.36    | Argentina   | No sequence data used                           | This study                     |
| D. pepo                      | CBS 123592    | Peru        | DF977487 DF977487 DF977459 DF977466            | Shimizu et al. (2018)          |
| Entoleuca mammata            | JDR 100       | France      | GU300072 N/A GQ844782 GQ470230                  | Hsieh et al. (2010)            |
| Euepixylon sphaeriostomum    | JDR 261       | USA         | GU292821 N/A GQ844774 GQ70203                   | Hsieh et al. (2010)            |
| Graphostroma platystomum     | CBS 270.87    | France      | JX658353 DQ836906 K624296                      | Stadler et al. (2014; ITS), Zhang et al. (2006; LSU), Wendt et al. (2018; RPB2, TUB2), TUB2 (Wendt et al. 2018) |
| Hypoxylon fragiforme         | MUCL 51264    | Germany     | ET     | KC477229 KM186295 KM186296 KX271282             | Stadler et al. (2013; ITS), Daranagama et al. (2015; LSU, RPB2), TUB2 (Wendt et al. 2018) |
| Kretzschmaria deusta         | CBS 163.93    | Germany     | KC47723 KY610458 KY624227                       | KX271251                       |
| Nemania abortiva             | BISH 467      | USA         | GU292816 N/A GQ844768 GQ702019                  | Hsieh et al. (2010)            |
| N. beaumontii                | HAST 405      | Martinique  | GU292819 N/A GQ844772 GQ70222                   | Hsieh et al. (2010)            |
| N. beaumontii                | FL 0980       | USA         | 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 GQ70221                   | Hsieh et al. (2010)            |
| N. primolutea                | HAST 91102001 | Taiwan      | EF026121 N/A GQ844767 EF052607                  | Hsieh et al. (2010)            |
| Podosordaria mexicana        | WSP 176       | Mexico      | GU234762 N/A GQ853039 GQ844840                  | Hsieh et al. (2010)            |
| Poronia punctata             | CBS 656.78    | Australia   | KT281904 K624278 K624278                       | 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 KY610641 KY624229 KX271254             | Wendt et al. (2018)            |
| R. corticium                 | STMA 13324    | Germany     | MN984621 MN984627 MN987237 MN987241             | This study                     |
| R. corticium                 | STMA 12170-1520 | 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     | KY204024 KY610495 KY624231 KX271279             | Sir et al. (2016; ITS), Wendt et al. (2018; LSU, RPB2, TUB2) |
| X. polymorpha                | MUCL 49884    | France      | KY610408 KY610646 KY624288 KX271280             | Wendt et al. (2018)            |
| X. bambusicola               | WSP 205       | Taiwan      | EF026123 N/A GQ844802 AY951762                  | Hsieh et al. (2010)            |
(NH₄)₂SO₄ 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 123592 (D. bunodes), CBS 51703 (R. aquila), MUCL 51693 (R. corticium), 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 (200–1000 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), 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, CaCO₃ 3 mg/L, soy peptone (type II, from Marcor) 2 g/L, MgSO₄·7H₂O 2 g/L, NaCl 2 g/L, agar 20 g/L (similar to Seed agar in Harder et al. 2011)], CMD [corn meal

Fig. 1. Inferred phylogenetic tree of selected Xylariaceae, Hypoxylaceae and Graphostromataceae calculated by PhyML with 1000 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.
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 cyclo-depsipeptide PF1022 A, STMA13324 (R. corticium) was cultivated in liquid CS medium [maltose 40 g/L, cottonseed flour 20 g/L, CaCO3 3 mg/L, soy peptone (type II, from Marcor) 2 g/L, MgSO4 • 7H2O 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 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 fatty acids before injection for preparative HPLC.

The following procedures described above the cultivation of D. bundodes (CBS123565) 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 dematophoranes 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), spiranalin A (6, 4.5 mg), libertellenone C (7, 1.4 mg), hymatoxin K (8, 6.5 mg), elaeicolaside C (9, 6.6 mg), 16α-D-glucopyranosyloxyisopimar-7-en-19-oic acid (10, 2.6 mg) and 16b-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):** [α]D260 -13.0 (c 2.1, CH3CN); UV (MeOH) λmax (log ε) 228 nm (3.98), 238 nm (3.98) 334 nm (4.51); 1H NMR (500 MHz, MeOH-d4), 13C NMR (125 MHz, MeOH-d4) (see Table 2); HR-ESI-MS m/z 373.1984 [M+Na]⁺ (calcd for C26H30NaO5, 373.91). (Fig. S3)

**Dematophorane B (2):** [α]D260 +34.1 (c 1.8, CH3CN); UV (MeOH) λmax (log ε) 227 nm (4.19), 268 nm (4.26) 342 nm (4.37); 1H NMR (700 MHz, acetone-d6), 13C NMR (175 MHz, acetone-d6) (see Table 2); HR-ESI-MS: m/z 373.1984 [M+Na]⁺ (calcd for C26H30NaO5, 373.91). (Fig. S3)

### Table 2. 13C and 1H NMR data of dematophoranes A and B (1–2).

| (1) | (2) |
|-----|-----|
| 1   | 2.68, 3.68, (6.6, 7.4) | 3.3, 69.9, 4.26, m |
| 2   | 1.73, 29.7, CH2 2.95, s | 1.70, 29.7, CH2 |
| 3a  | 1.13, 34.9, CH2 (13,0, 3.4) | 1.06, 34.3, CH2 |
| 3b  | 1.61, m | 1.68, m |
| 4   | 1.68, m | 2.06, m |
| 5   | 1.70, m | 1.70, m |
| 6   | 2.66, m | 211.1, C |
| 7   | 76.5, CH, 4.76, m | 4.76, m |
| 8   | 139.6, C | 139.6, C |
| 9   | 75.3, C | 75.3, C |
| 10  | 52.4, C | 52.4, C |
| 11a | 2.34, dt (19.4, 7.5) | 3.13, CH2 |
| 11b | 2.54, ddd (19.2, 7.9, 5.5) | 2.42, td (14.7, 3.4) |
| 12a | 1.41, m | 32.2, CH2 |
| 12b | 1.81, ddd (13.5, 7.9, 6.1) | 1.75, td (14.2, 3.4) |
| 13  | 39.9, C | 39.9, C |
| 14  | 3.99, s | 130.6, CH |
| 15  | 149.4, CH | 149.4, CH |
| 16a | 110.9, CH2 | 110.9, CH2 |
| 16b | 5.00, dd (10.7, 1.4) | 5.00, dd (17.5, 1.4) |
| 17  | 24.1, CH3 | 24.1, CH3 |
| 18  | 1.21, s | 18.8, CH3 |
| 19a | 3.05, m | 70.6, CH3 |
| 19b | 3.46, d (10.8) | 3.54, m |
| 20  | 1.01, s | 13.9, CH3 |
| 21  | 4.35, s | 3.74, l (5.6) |

1 (125 MHz and 500 MHz, MeOH-d4), 2 (175 MHz and 700 MHz, acetone-d6), 3a) (see Table 2); HR-ESI-MS: m/z 373.1984 [M+Na]⁺ (calcd for C26H30NaO5, 373.91). (Fig. S3)

### 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: H2O + 0.1 % formic acid, solvent B: acetonitrile + 0.1 % formic acid, gradient system: 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 (1H 700 MHz, 13C 175 MHz) and a Bruker Avance III 500 (1H 500 MHz, 13C 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 (IC50) 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 Rupic 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 CaCl2 (1 mL), 1M MgSO4 (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 KH2PO4/K2HPO4 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 plasystomum), 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 4176 characters, with 469 and 1273 positions originating from ITS and LSU ribosomal DNA, respectively, and 1101 and 1333 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 Podosoraria mexicana and Poronia punctata (P) are placed as sister clade to other saprotophrotic and pathogenic representatives. Additional clades include Xylaria and Kretzschmaria spp. (X); Xylaria polymorpha clustered within a weakly supported (54 %) Amphirostellina clade (A); and the clade consisting of Nemania spp. (N) forms a well-supported sister clade (94 %) to the Rosellina and Dematophora clades (R, D). Clade N consists of two well supported subclades (100 %) of Euepikylon sphaeroistomum and Nemania beaumontii on the one hand and the type strains of Nemania abortiva and Nemania primolutae 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 Rosellina (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. 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 elaeicoside 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. 2017).
1989, Shimizu et al. 2018), were detected using the described cultivation conditions.

In order to investigate the production of cyclodepsipeptides of the PF1022 family (Scherenkeben 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,$^1$C-HSQC NMR spectra of (1) revealed one olefinic methine proton, one olefinic methylene group with a characteristic carbon at δC 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,$^1$C-HMBC correlations of (1) indicated the presence of a tricyclic diterpene scaffold. Key HMBC correlations include H-7 to Cq-6, Cq-9, H-5 to Cq-6, Cq-9, C-1, C-18, Cq-10, Cq-4, C-3, C-19 and H-14 to Cq-9, Cq-8, C-7, Cq-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 Cq-13, C-17, C-12 and C-14 demonstrated a terminal vinyl group at Cq$_{13}$ 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 B 3.08 (d, 1H), 3.46 (d, 1H), C 70.7 ppm was identified as hydroxylated methylene CH$_2$-19 at δC via $^{1}$H,$^{1}$C Long range correlations of H-19 to C-5, Cq-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, 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]$^+$, calc'd. 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,$^1$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 = 17.0, 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,$^1$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,$^1$C HMBC correlations of this olefinic methine proton H-14 to C-15, Cq-8, C-7, Cq-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 Cq$_{19}$ 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 Muco 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 libterellone 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. marucciana (MUCL 51704 –
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).

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

The cell density was adjusted to 8 × 10⁶ cells/mL, * no complete inhibition; DSMZ: German Collection of Microorganisms and Cell Cultures, Braunschweig.
**Resurrection of Dematophora**

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 of 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.

**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, mamilloata, semiglobose or conical, sessile or broadly stipitate, glabrous, ostiolate, brown, grey, black, uniperticate, sometimes confluent containing a few perithecia, superficial. Perithecia globose, collapsing and usually detached from stromatal wall, rarely remaining attached. Paraphyses filiform, evanescent. Ascii cylindrical, long stipitate, evanescent. Ascus apical plugs cylindrical with bulge at upper rim, rarely without, amyloid, rarely not amyloid. Ascospores unicellular, asymmetrically ellipsoid 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 asc, ascospores or stromata. The species that are retained in the genus Rosellinia comprise the R. aquila, R. mammiformis, R. emergens, R. mammoeidea and R. thelens “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 mammatana and the conifer pathogens of the R. thelens 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-Lectotyp 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 Funqorum 25: 1. 2013.

**Dematophora arcuata** (Petch) C. Lambert, K. Wittstein & M. Stadler, comb. nov. MycoBank MB827529.

**Basionym:** Rosellinia arcuata Petch, Ann. Roy. Bot. Gard. (Peraedieniya) 6 (1): 175. 1916.

**Dematophoraasperata** (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 Funqorum 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. Q. R. canzacotoana (Sir & Hladki 198-LIL). O. R. 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 co-nidiophores (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 (arrow). Q, R. Ascospores showing cellular appendages in 3% KOH (arrow). Scale bars: A = 2 mm; B–F = 1 mm; G, H, L–T = 10 μm; J, I = 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.

Dematophora obregonii (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, comb. nov. MycoBank MB827540.

Rosellinia bunodes (Berk. & Broome) P.M.D. Martin, S. African J. Bot. 42 (1): 72. 1976.

Rosellinia echina Masssee, Bull. Misc. Inform. Kew: 155. 1901.

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 hsiiehiae (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, comb. nov. MycoBank MB827548.

Basionym: Rosellinia hsiiehiae 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 ‘macdonaldii’], Stud. Trent. 7 (1): 66. 1926.

Dematophora obtusioistiolata (L.E. Petrini) C. Lambert, K. Wittstein & M. Stadler, comb. nov. MycoBank MB827542.

Basionym: Rosellinia obtusioistiolata 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. Trimestriell 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, Nizshi Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloasmitsety 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. 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 genus Rosellinia, 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 Conioaliella 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. ascomoida 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. ascomoida (strains CBS 447.89, CBS 448.89 and CBS 450.89), “R. britannica” (current valid name: R. marcucciana, strain CBS 446.89) and R. mammamoraeformis (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 ascospora-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

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REFERENCES

Aldridge DC, Burrows BF, Turner WB (1972). The structures of the fungal metabolites cytochalasin E and F. Journal of the Chemical Society - Chemical Communications 3: 148–149.
Chen YS (1960). Studies on the metabolic products of Rosellinia necatrix Berlese. Bulletin of the Agricultural Chemical Society Japan 24: 372–381.
Chen YS (1964). The metabolic products of Rosellinia necatrix. II. The structure of rosellinic acid. Bulletin of the Agricultural Chemical Japan 28: 431–435.
Conder GA, Johnson SS, Nowakowski DS, et al. (1995). Anthelmintic profile of the cyclodepsipeptide PF1022A in in vitro and in vivo models. Journal of Antimiotics 48: 20–283.
Daranagama DA, Camporesi E, Tian Q, et al. (2015). Anthostomella is polyphyletic comprising several genera in Xylariaceae. Fungal Diversity 73: 203–238.
Daranagama DA, Hyde KD, Sir EB, et al. (2018). Towards a natural classification and backbone tree for Graphostromataceae, Hypoxylaceae, Lopostromataceae and Xylariaceae. Fungal Diversity 88: 1–165.
De Notaris G (1844). Cenni sulla tribù dei Pirenomocicli sferiaci e descrizione di alcuni generi spettanti alla medesima. Giomale Botanico Italiano 1: 322–355.
Guindon S, Dufayard JF, Lefort V, et al. (2010). New algorithms and methods to estimate Maximum-Likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321.
Harder A, Krieger K, Pham TLH, et al. (2012). Novel 24-membered cyclooctadepsipeptides from fungal strains and their use as anthelmintics or endoparasiticides. US Patent Application US 20120302496.
Harder A, Pham TL, Jarling R, et al. (2011). Method for producing optically active, cyclic depsipeptides comprising lactic acid and phenyl lactic and having 24 ring atoms, using fungus strains of Rosellinia type, and further species of Xylariaceae. US Patent Application US 13/133,611.
Hartig R (1983). Rhizomorpha (Dematiophora) necatrix n. sp. Untersuchungen aus dem Volksbotanischen Institut zu München 3: 95–140.
Helay SE, Thongbai B, Stader M (2018). Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascocyste order Xylinales. Natural Products Report 35: 992–1014.
Hsieh HM, Lin CR, Fang MJ, et al. (2010). Phylogenetic status of Xylaria subgenus Pseudoxylaria among taxa of the subfamily Xylanioidae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. Molecular Phylogenetics and Evolution 54: 957–969.
Hsu YM, Nakagawa M, Hirota A, et al. (1988). Structure of myrocin B, a new diterpene antibiotic produced by Myrothecium verrucaria. Agricultural and Biological Chemistry 52: 1305–1307.
Jossang R., Rupcic Z., Pinon J., et al. (1995). Hymatoxins K and L, novel phytotoxins from Hypoxylon mammatum, fungal pathogen of aspens. *Natural Product Letters* **6**: 37–42.

Ju Y.M., Rogers J.D. (1990). Astrocytosis reconsidered. *Mycologia* **82**: 342–349.

Ju Y.M., Rogers J.D. (1996). A revision of the genus Hypoxylon. *Mycologia Memoir* **20**: APS Press, St. Paul, 365 pp.

Kearse M., Moir R., Wilson A., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Kilgaard S., Subko K., Phillips E., et al. (2017). A Dereplication and Bioguided Discovery Approach to Reveal New Compounds from a Marine-Derived Fungus *Stibilia filamenta*. *Marine Drugs* **15**: 253.

Kimura Y., Nakajima H., Hamasaki T. (1986). Structure of rossellichalasin, a new metabolite produced by *Rosellinia necatrix*. *Agricultural and Biological Chemistry* **53**: 1699–1701.

Koukol O., Kelnarová I., Černý K. (2015). Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. *Forest Pathology* **45**: 21–27.

Kuephadungwan H., Welaya SE., Daengrot C., et al. (2017). Akanthopyrones A-D, α-pyrones bearing a 4-O-methyl-β-D-glucopyranosyl moiety from the spider-associated ascomycete *Akanthomyces novoguineensis*. *Molecules* **22**: 1202.

Kuhnt R., Surup F., Sir EB., et al. (2015). Lenornandins A – G, new azaphilones from Hypoxylon lenornandi and Hypoxylon jaklitschi sp. nov., recognised by chemotaxonomic data. *Fungal Diversity* **71**: 165–184.

Lambert C., Wendt L., Hadki AL., et al. (2019). *Hypomontagnella* (Hypoxylaceae): a new genus segregated from Hypoxylon by a polyphasic taxonomic approach. *Mycological Progress* **18**: 187–201.

Lefort V., Longueville JE., Gascuel O. (2017). SMART model selection in *PhyML*, *Molecular Biology and Evolution* **34**: 2422–2424.

Lehr NA., Meffert A., Antelo L., et al. (2006). Antiamoebins, myrocinB and the basis of antifungal activity in the coprophilous fungus *Stibilia erythrophaela* (syn. S. fitremata). *FEMS Microbiology Ecology* **55**: 105–112.

Oh D.C., Jensen PR., Kaufmann CA., et al. (2005). Libellonelones A–D: Induction of cytotoxic diterpenoid biosynthesis by marine microbial competition. *Biorganic & Medicinal Chemistry* **13**: 5267–5273.

Peláez F., González V., Platas G., et al. (2008). Molecular phylogenetic studies within the family Xylariaceae based on ribosomal DNA sequences. *Fungal Diversity* **31**: 111–134.

Petriči LE. (1992). Rosellinia species of the temperate zones. *Sydowiia* **44**: 169–281.

Petriči LE. (2003). Rosellinia and related genera in New Zealand. *New Zealand Journal of Botany* **41**: 71138.

Petriči LE. (2013). Rosellinia - a world monograph. *Bibliotheca Mycologica* **205**: Stuttgart: J. Cramer.

Petriči LE., Petriči O. (2005). Morphological studies in Rosellinia (Xylariaceae): the first step towards a polyphasic taxonomy. *Mycological Research* **109**: 569580.

Rupcic Z., Chepkirui C., Hernández-Restrepo M., et al. (2019a). New nematocidal and antimicrobial secondary metabolites from a new species in the new genus, *Pseudobambusicola thailandica*. *Mycokeys* **33**: 1–23.

Rupcic Z., Rascher M., Kanaki S., et al. (2018b). New cyathane diterpenoids from mycélial cultures of the medicinal mushroom *Hericium erinaceus* and the rare species, *Hericium flagillum*. *International Journal of Molecular Sciences* **19**: 740.

Sasaki T., Takagi M., Yaguchi T., et al. (1992). A new anthelmintic cyclospispeptide, PF1022A. *Journal of Antibiotics* **45**: 692–697 [and Erratum (1996) in *Journal of Antibiotics* 49: C-2].

Senanayake IC., Maharakchikumbura SSN., Hyde KD., et al. (2015). Towards unravelling relationships in *Xylariomycetidae* (Sordariomycetes). *Fungal Diversity* **73**: 73–144.

Sharma N., Kushwaha M., Arora D., et al. (2018). New cytochalasins from *Rosellinia sanctae-cruciana*, an endophytic fungus of Albizia lebbeck. *Journal of Applied Microbiology* **125**: 111–120.

Shinizu T., Kanematsu S., Yaegashi H. (2018). Draft Genome Sequence and Transcriptional Analysis of *Rosellinia necatrix* Infected with a Virulent *Mycovirus*. *Phytopathology* **108**: 1206–1211.

Shiono Y., Matsui N., Imaizumi T., et al. (2013). An unusual spirocyclic iso-pimarane diterpenoid and other isopimarane diterpenoids from fruiting bodies of *Xylaria polymorpha*. *Phytochemistry Letters* **6**: 439–443.

Shiono Y., Motoi S., Koseki T., et al. (2009). Isopimarane diterpene glycosides, apoptosis inducers, obtained from fruiting bodies of the ascomycete *Xylaria polymorpha*. *Phytochemistry* **70**: 935–939.

Sir EB., Lambert C., Wendt L., et al. (2016). A new species of *Daldinia* (Xylariaceae) from the Argentine subtropical montane forest. *Mycosphere* **7**: 596–614.

Scherenbeck J., Jeschke P., Harder A. (2002). PF1022A and related cyclospispeptides—a novel class of anthelmintics. *Current Topics in Medicinal Chemistry* **2**: 759–777.

Stadler M., Kuhnt E., Persich D., et al. (2013). The Xylariaceae as model example for a unified nomenclature following the “One Fungus- One Name” (1F1N) Concept. *Mycology: An International Journal on Fungal Biology* **4**: 5–21.

Stadler M., Lassaa T., Fourrier J., et al. (2014). A polyphasic taxonomy of *Daldinia* (Xylariaceae). *Studies in Mycology* **77**: 1–143.

Surup F., Kuhnt E., Böhm A., et al. (2018). The rickiols, 20-, 22-, and 24-membered macrolides from the ascomycete *Hypoxylon rickii*. *Chemistry - An European Journal* **24**: 2200–2213.

Talavera G., Castresana J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**: 564–577.

U. Ren J., Lutzoni F., Miadlikowska J., et al. (2012). Host and geographic structure of endophytic and endolicthic fungi at a continental scale. *Mycologia* **99**: 898–914.

U. Ren J., Miadlikowska J., Zimmerman NB., et al. (2016). Contributions of North American endophytes to the phylogeny, ecology and taxonomy of *Xylariaeae* (Sordariomycetes, Ascomycota). *Molecular Phylogenetics and Evolution* **90**: 210–232.

Vicente F., Basilo AL., Piatas G., et al. (2009). Distribution of the antifungal agents sorarins across filamentous fungi. *Mycological Research* **113**: 754–770.

Wang GJ., Liang WL., Ju YM., et al. (2012). Inhibitory effects of terpenoids from the fermented broth of the ascomycete *Stilbephytomyces elaeicola* YM173 on nitric oxide production in RAW264.7 macrophages. *Chemistry and Biodiversity* **9**: 131–138.

Wang X., Yu H., Zhang Y., et al. (2018). Bioactive pimarane-type diterpenes from marine Organisms. *Chemistry and Biodiversity* **15**: e1700276.

Wendt L., Sir EB., Kuhnt E., et al. (2018). Resurrection and emendation of the *Hypoxylaceae*, recognised from a multigene phylogeny of the Xylariales. *Mycological Progress* **17**: 115–154.

Yu HB., Wang XL., Zhang YX., et al. (2018). Libellonelones O-S and Eutypellenones A and B, Pimarane diterpene derivatives from the arctic fungus *Eudyptella* sp. D-1. *Journal of Natural Products* **81**: 1553–1560.

Zhang N., Castlebury L., Castlebury M., et al. (2006). An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycolgia* **98**: 1076–1087.