

Novel insights into the phylogenetic and metabolic diversity of preussia-like fungi

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Abstract: The genus *Preussia* (*Sporormiaceae*, *Pleosporales*) represents one of the most diverse and metabolically active groups of ascomycetes. The genus is characterized by a complex taxonomic history involving the genera *Sporormiella* and *Spororminula*, which some authors consider synonyms of *Preussia*. However, the circumscription of these genera remains controversial, and the status of numerous species is unresolved. The present study aims to characterize strains of *Preussia* s.l. recovered during diversity surveys on marine and xerophytic substrates, explore their secondary metabolite profiles, and improve our understanding of the diversity within this group. The obtained strains, as well as strains from the CBS culture collection and exsiccata from different fungaria, were characterized morphologically, and molecular phylogenetic analyses were conducted on strains available in culture. Metabolite profiles were developed for representative strains using mass spectrometry data (MS/MS). Molecular alignments comprised the nuclear ribosomal subunits, consisting of partial sequences for the 18S small subunit (SSU) nrDNA, the internal transcribed spacer regions and intervening 5.8S nrDNA (ITS), as well as partial sequences for the 28S large subunit (LSU) nrDNA. Additional regions within protein-encoding genes were used, including the DNA-directed RNA polymerase II second-largest subunit (*rpb2*) and translation elongation factor 1- α (*tef1- α*) regions. These data were used to reconstruct a robust phylogenetic framework for *Preussia* s.l. and to delineate lineage structure and generic boundaries. Ten well-supported lineages were resolved and integrated with metabolite profiles to inform taxonomic decisions. Our results support the recognition of *Preussia* and *Sporormiella* as distinct genera, the re-establishment of *Spororminula*, and the introduction of four new genera, 11 new species, 18 new combinations, and one new name. Moreover, epi- and lectotypes have been designated to stabilize the taxonomy of some recognized species. This study provides a comprehensive framework for future taxonomic, phylogenetic, and chemical investigations within *Sporormiaceae*.

Key words: chemotaxonomy, integrative taxonomy, mass spectrometry, morphology, multi-locus phylogeny, new taxa, *Preussia*, secondary metabolites, *Sporormiella*, typification.

Taxonomic novelties: **New genera:** *Chaetosporormiella* Guerra-Mateo, Gené & Cano, *Pleosporormiella* Guerra-Mateo, Gené & Cano, *Sporormyces* Guerra-Mateo, Gené & Cano, *Spororvela* Guerra-Mateo, Cano & Gené. **New species:** *Chaetosporormiella monodictyoides* Guerra-Mateo, Gené & Cano, *Pleosporormiella aculeata* Guerra-Mateo, Gené & Cano, *Pleosporormiella compacta* Guerra-Mateo, González-Menéndez, Gené & Cano, *Pleosporormiella polyspora* Guerra-Mateo, González-Menéndez, Gené & Cano, *Pleosporormiella suttonii* Guerra-Mateo, Gené & Cano, *Preussia gypsicola* Guerra-Mateo, González-Menéndez, Cano & Gené, *Sporormiella jejuensis* H. Noh, H.U. Cho & S.H. Kim ex Guerra-Mateo, Gené & Cano, *Sporormiella koreensis* H. Noh, H.U. Cho & S.H. Kim ex Guerra-Mateo, Gené & Cano, *Sporormiella solicola* Guerra-Mateo, Cano & Gené, *Spororminula irregularis* Guerra-Mateo, Cano & Gené, *Sporormyces anthraquinoniformans* Guerra-Mateo, González-Menéndez & Cano. **New combinations:** *Chaetosporormiella pilosella* (Cain) Guerra-Mateo, Gené & Cano, *Pleosporormiella citrullina* (R.M.F. Silva *et al.*) Guerra-Mateo, Gené & Cano, *Pleosporormiella polymorpha* (Asgari & Zare) Guerra-Mateo, Gené & Cano, *Pleosporormiella terricola* (Cain) Guerra-Mateo, Gené & Cano, *Sporormiella aegilopsis* (M. Mehrabi *et al.*) Guerra-Mateo, Gené & Cano, *Sporormiella africana* (Arenal *et al.*) Guerra-Mateo, Gené & Cano, *Sporormiella bezerrensensis* (Ferreira-Sá *et al.*) Guerra-Mateo, Gené & Cano, *Sporormiella elegans* (D.C. Sandberg & A.E. Arnold) Guerra-Mateo, Gené & Cano, *Sporormiella isabellae* (Arenal *et al.*) Guerra-Mateo, Gené & Cano, *Sporormiella japonica* (Udagawa, K. Tominaga & Hamaoka) Guerra-Mateo, Gené & Cano, *Sporormiella lignicola* (W. Phillips & Plowr.) Guerra-Mateo, Gené & Cano, *Sporormiella mediterranea* (Arenal *et al.*) Guerra-Mateo, Gené & Cano, *Sporormiella persica* (Asgari & Zare) Guerra-Mateo, Gené & Cano, *Sporormiella procaviae* (Crous) Guerra-Mateo, Gené & Cano, *Sporormiella procaviicola* (Crous) Guerra-Mateo, Gené & Cano, *Spororminula subcinerensis* (Mouton) Guerra-Mateo, Cano & Gené, *Sporormyces septenarius* (S.I. Ahmed & Cain) Guerra-Mateo, Gené & Cano, *Spororvela tela* (S. Jakob & M.J. Richardson) Guerra-Mateo, Cano & Gené. **New names:** *Sporormiella isopartis* Guerra-Mateo, Gené & Cano. **New synonyms:** *Preussia apgariae* Y.P. Tan *et al.*, *Preussia arizonica* D.C. Sandberg & A.E. Arnold, *Preussia crataegi* Eisvand & M. Mehrabi, *Preussia cylindricalis* F. Liu *et al.*, *Preussia flanaganii* Boylan, *Preussia isomera* Cain, *Preussia mariae* D.C. Sandberg & A.E. Arnold, *Preussia mayoae* Y.P. Tan *et al.*, *Preussia pianica* Eisvand & M. Mehrabi, *Preussia pistaciae* Eisvand & M. Mehrabi, *Preussia tenerifae* (Arx & Aa) Krays. **Typification (basionyms):** *Epitypification:* *Perisporium funiculatum* Preuss; **Lectotypification:** *Sporormia intermedia* Auersw.

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INTRODUCTION

The family *Sporormiaceae* (*Pleosporales*) represents a diverse group of saprobic ascomycetes that primarily inhabit dung, but also colonize soil, plant debris, wood, and even behave as endophytes

(Zhang *et al.* 2012b, Phukhamsakda *et al.* 2022). Morphologically, the family is characterized by sexual morphs with bitunicate, fissitunicate asci, and thick-walled, predominantly multi-septate, dark brown ascospores, as well as pycnidial, chrysosporium- or monodictys-like asexual morphs (González-Menéndez



et al. 2017, Wanasinghe et al. 2018, Crous et al. 2020a, b). Currently, *Sporormiaceae* comprises the genera *Chaetopreussia*, *Forliomyces*, *Pleophragmia*, *Preussia*, *Sparticola*, *Sporormia*, *Sporormiella*, *Sporormispora*, *Trichophoma*, *Westerdykella*, and *Xenomonodictys* (Hyde et al. 2024). However, the taxonomic circumscription of *Preussia* and *Sporormiella* remains controversial, as phylogenetic analyses have shown that species from both genera are intermingled (Phukhamsakda et al. 2016, González-Menéndez et al. 2017).

The genus *Preussia* (Pr.) was established in 1866 to accommodate the basionym *Perisporium funiculatum*, currently *Pr. funiculata*, based on its saprobic behaviour and the production of ascomata with clavate asci, long stipes, and 4-celled ascospores (Fuckel 1867). The examination of additional *Perisporium* collections led to novel *Preussia* combinations, confining *Perisporium* to plant parasites producing pycnidial asexual morphs (Cain 1961). Before the establishment of *Preussia*, the genus *Sporormia* (Sp.) was described in 1849 based on *Sp. fimetaria*, a coprophilous fungus displaying ≥ 16 -celled ascospores (De Notaris 1849). Later, the genus *Sporormiella* (Sm.) was described based on *Sm. nigropurpurea*, another coprophilous fungus, but displaying ascomata with short stipitate asci and 4-celled ascospores (Ellis & Everhart 1892). Over the years, fungi displaying 4–13-celled ascospores were additionally included in *Sporormia*. However, lignicolous species with 4-celled ascospores were later transferred to *Preussia*, while coprophilous species displaying between 4–13-celled ascospores were transferred to *Sporormiella*, restricting *Sporormia* to ≥ 16 -celled ascospores (Cain 1961, Ahmed & Cain 1972). Using this approach, the number of *Preussia* and *Sporormiella* species increased considerably, including the description of novel species (Khan & Cain 1979). Other genera like *Ohleriella* and *Sporormiopsis* were eventually considered synonyms of *Sporormiella* (Ahmed & Cain 1972), and the genus *Spororminula* (Sn.) was ultimately considered a synonym of *Preussia* (Kruys & Wedin 2009).

The circumscription of *Preussia* and *Sporormiella* has been a long-standing topic of debate (Mungai et al. 2012). *Preussia* was reserved for saprobic fungi growing on plant debris, wood, and soil, producing cleistothecial ascomata with clavate asci and 4-celled ascospores, while *Sporormiella* comprised coprophilous fungi displaying perithecial ascomata with cylindrical-clavate asci and 4–13-celled ascospores (Guarro et al. 1997b, Kruys & Wedin 2009). These features lacked consistency across species, with *Pr. funiculata* frequently occurring on dung, several species producing both cleistothecia and perithecia depending on growth conditions in culture, and the absence of phylogenetic monophyly (Guarro et al. 1997b, Kruys & Wedin 2009). Although public databases such as Index Fungorum and MycoBank currently treat these genera as different entities, many authors have considered *Sporormiella* a synonym of *Preussia* (Kruys & Wedin 2009, Guarro et al. 2012, Zhang et al. 2012b, Kruys 2015). In fact, a large number of *Sporormiella* species have already been transferred to *Preussia* (Valldoserra & Guarro 1990, Guarro et al. 1997a, Abdullah et al. 1999, Arenal et al. 2004, Kruys & Wedin 2009).

The genus *Preussia* s.l. represents a heterogeneous group of fungi. This variability has also been acknowledged through secondary metabolites. These molecules represent low-molecular-weight compounds that are not essential for growth, but indicate the fungus's ability to communicate, compete, and cooperate with other microbes in the environment (Macheleidt et al. 2016). More than 50 different metabolites have been recovered from *Preussia* s.l. species (González-Menéndez et al. 2017). Although some of these compounds were also reported in different microbes, up

to ten secondary metabolites have been exclusively recovered from *Preussia* s.l., including australifungin, sporminarin A and B, and zaragozic acid B (González-Menéndez et al. 2017). These natural compounds predominantly display antifungal activity, with australifungin used against fungal causal agents of infection in humans (Mandala & Harris 2000), sporminarin A applied in the control of phytopathogens (Mudur et al. 2006, Rodríguez Sabina et al. 2025), and zaragozic acid B as a cholesterol-lowering medication inhibiting the squalene synthase (Bergstrom et al. 1993, Hornby et al. 2003). In addition, secondary compounds have been used to resolve species boundaries, an approach known as chemotaxonomy that has been successfully used in other fungal groups like *Cortinarius*, *Lepiota*, and *Penicillium* (Huymann et al. 2024, Overy et al. 2025, Sarawi et al. 2025).

The chemotaxonomic study of *Preussia* s.l. revealed 16 chemotypes characterizing different species; i.e., the exclusive production of microsphaerone B by strains of *Pr. lignicola* or sporminarins by different strains of *Pr. africana* (González-Menéndez et al. 2017). The combination of secondary metabolites with phylogenetic analyses enabled the characterization of lineages producing similar compounds and the precise identification of species, highlighting the production of unique molecules. For example, australifungin was obtained from a strain originally identified as “*Sporormiella australis*” (Mandala et al. 1995). However, phylogenetic analyses determined this strain as *Pr. intermedia* and, in addition, the metabolic profile revealed that strains of *Pr. intermedia* and *Pr. australis* produce different sets of metabolites (González-Menéndez et al. 2017). Therefore, the study of metabolites represents a powerful tool to explore the diversity of *Preussia* s.l. in depth, enhancing polyphasic taxonomic decisions.

Despite *Preussia* s.l. chemotypes, the relationships among major lineages remain unresolved with current ITS-LSU phylogenies (Kruys & Wedin 2009, González-Menéndez et al. 2017, Jakob et al. 2024). Protein-coding regions like *tub2* and *tef1-α* have been used to discern phylogenetically close species (Sandberg et al. 2022, Eisvand & Mehrabi-Koushki 2025). However, these analyses were limited in scope due to the small number of sequences available for these regions and a focus on assessing the *Pr. lignicola* and *Pr. minima* lineages, which have been considered species complexes (González-Menéndez et al. 2017, Sandberg et al. 2022). Moreover, a large number of *Preussia* s.l. species lack molecular data, and the identification of many sequenced taxa relies exclusively on morphology, with type specimens not specified or only available as fungarium material. Aside from this, there has been a recent increase in the description of *Preussia* s.l. novel species overlooking these taxonomic limitations (Eisvand & Mehrabi-Koushki 2025, Noh et al. 2025, Phurbu et al. 2025). Thus, the taxonomy of this genus requires the re-evaluation of available strains in culture, including fungarium material when possible.

We recovered several *Preussia* s.l. strains as part of diversity surveys on marine and xerophytic substrates. These underexplored substrates represent sources of rare taxa that produce specialized metabolites (Imhoff 2016, Sayed et al. 2020). In this work, we aimed to revise the taxonomy and metabolic profile of these *Preussia* s.l. strains. To achieve this, we examined type and reference specimens and strains of the genus and allied fungi preserved at the Westerdijk Fungal Biodiversity Institute, covering the widest range possible of species with molecular data. Herein, we provide a concatenated phylogeny with protein-coding regions assessing the diversity of *Preussia* s.l., expand the metabolite profile of this group focused on underexplored lineages, and revise strain identification under a polyphasic approach combining phylogeny, morphology, and

metabolites. In addition, we clarify the relationship between *Preussia* s.str. and *Sporormiella*, re-erect *Spororminula*, and propose four novel genera to stabilize the taxonomy of *Sporormiaceae*. Among these genera, we also propose 11 novel species, 18 novel combinations, and one new name.

MATERIALS AND METHODS

Strains and material examined

The strains included in this work comprised type and reference *Preussia* s.l. strains preserved in the CBS culture collection of the Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands), strains recovered from gypsum soil, xerophytic plants and lichens available in the CF collection of the MEDINA Foundation (Granada, Spain), and strains recovered from marine sediments available in the FMR culture collection of the Universitat Rovira i Virgili (Faculty of Medicine and Health Sciences, Reus, Spain). The CF and FMR strains were initially identified as members of *Preussia* s.l. based on morphological features. However, additional strains displaying humicola-like, monodictys-like, or pycnidia asexual morphs were also included based on preliminary DNA sequence data (Supplementary material Table S1).

In addition, we examined type and reference fungarium specimens to assess strain identification when type material lacked molecular data or was not properly designated. Identification was confirmed when cultural features matched those displayed in the species protologue. The specimens examined are specified in the taxonomy section, and digital images of exsiccatae are available as Supplementary material Figs S1–S6.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from 7-d-old fungal colonies growing on malt extract agar (MEA) at room temperature. Genomic DNA was extracted using a chloroform/isopropanol protocol (Tripathy & Maharana 2017) and quantified with a Nanodrop 2000 (Thermo Scientific, Madrid, Spain). Five loci were amplified following previous works on *Pleosporales*: SSU, ITS, LSU, a fragment of the translation elongation factor 1- α (*tef1-a*) gene, and a fragment of the second-largest subunit of the RNA polymerase 2 (*rpb2*) gene (Thambugala *et al.* 2015, Hongsanan *et al.* 2021). The ITS and LSU regions were amplified using the primer pair ITS5/LR5 (Vilgalys & Hester 1990, Schoch *et al.* 2012); the SSU region with NS1/NS4 (Schoch *et al.* 2012); *tef1-a* with EF-983F/EF-2218R (Rehner & Buckley 2005), *rpb2* with RPB2-5F2/RPB2-7CR (Liu *et al.* 1999). The PCR amplifications were performed in a total volume of 25 μ L. The amplification conditions were set as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 53 °C for 1 min in ITS, LSU, and *rpb2*; and 57 °C for 90 s in *tef1-a*; followed by an extension at 72 °C for 2 min, and a final extension step at 72 °C for 7 min. In the case of SSU, the annealing step was structured in a decreasing gradient of temperature, with two sets of 5 cycles at 60 °C and 58 °C for 45 s, and 30 cycles at 54 °C for 45 s. The PCR products were subsequently purified and sequenced using either an Applied Biosystems, Hitachi 3730xl DNA analyser (Applied Biosystems Inc., Foster City, California, USA) or at MacroGen Corp. Europe (Madrid, Spain). The consensus sequences were assembled from forward and reverse sequences using Geneious Prime v. 2022.0.2 (<http://www.geneious.com>, Kearse *et al.* 2012). Novel sequences

generated in this study were deposited in GenBank (<http://www.ncbi.nlm.nih.gov>, Table S1).

Phylogenetic analyses

Sequence alignments were initially performed using the ClustalW algorithm in MEGA v. 6.0 (Thompson *et al.* 1994, Tamura *et al.* 2013); then, refined using MUSCLE or manually adjusted as needed (Edgar 2004). Each genomic DNA region was aligned separately before being concatenated into a combined dataset. Phylogenetic concordance among individual gene trees was visually assessed to identify any topological conflicts within strongly supported clades (Supplementary material Figs S7–S10). Upon confirmation of overall concordance, the alignments were merged into a single data matrix using MEGA.

Maximum likelihood (ML) analyses were conducted with IQ-TREE v. 2.3.6, employing ultrafast bootstrap approximation to estimate branch support (Minh *et al.* 2013, 2020, Nguyen *et al.* 2015, Hoang *et al.* 2018). The optimal evolutionary model for each data partition was selected using ModelFinder based on the Bayesian information criterion (Chernomor *et al.* 2016, Kalyaanamoorthy *et al.* 2017). Bootstrap support values $\geq 95\%$ were regarded as statistically significant (Minh *et al.* 2013, Hoang *et al.* 2018). Bayesian inference (BI) analyses were performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012). The best-fit substitution model for each locus was determined using jModelTest v. 2.1.3 under the Akaike information criterion (Guindon & Gascuel 2003, Darriba *et al.* 2012). Markov chain Monte Carlo (MCMC) simulations were run for 10 million generations with four chains (one cold and three heated), starting from a random tree topology. Trees were sampled every 1000 generations, and the run was automatically terminated once the average standard deviation of split frequencies dropped below 0.01. The first 25 % of sampled trees were discarded as burn-in, and the remaining trees were used to construct a majority-rule consensus tree. Posterior probabilities (pp) ≥ 0.95 were considered statistically significant (Hespanhol *et al.* 2019). Resulting phylogenetic trees were visualized using FigTree v. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>, accessed during November 2025). The alignments generated in this study were submitted to Zenodo (<https://doi.org/10.5281/zenodo.19625618>).

Species delimitation analyses

Species delimitation analyses were used to resolve the taxonomy of phylogenetically close species represented by several strains. We performed a coalescent-based method, Bayesian Poisson Tree Processes, bPTP (Zhang *et al.* 2013), and a distance-based method, Assemble Species by Automatic Partitioning, ASAP (Puillandre *et al.* 2021). These analyses were performed exclusively with the ITS and LSU regions to include as many strains as possible. The input trees for the PTP analyses were inferred in IQ-TREE, as explained above. The analyses were conducted on the web server for bPTP (<https://species.h-its.org/ptp/>) with 500000 MCMC generations, thinning set to 100, burn-in of 10 % and seed value of 123456. Convergence of the MCMC iterations was assessed by visualising the log-likelihood trace plot, where the chain's predominant exploration of high-likelihood locations was used as indicative of convergence. The ASAP analyses were also performed for the concatenated ITS–LSU alignment using the matrix of pairwise nucleotide distances. This matrix was computed in MEGA v. 6.0 using the K2+G nucleotide substitution model. These analyses were conducted locally (<https://github.com/iTaxoTools/ASAPy>, accessed during January 2026).



The resulting partition was determined by the lowest asap-score. Hypothetical species delimitation was established by evaluating the phylogenetic analyses and the delimitation obtained through PTP and the ASAP partition.

Morphological characterization

Macroscopic characteristics of fungal colonies were recorded after cultivation on oatmeal agar (OA), potato dextrose agar (PDA), and cornmeal agar (CMA). Colony diameters and morphological features were documented after 14 d of incubation in darkness at 25 °C. Colony colours in descriptions were based on Kornerup & Wanscher (1978). The microscopic analysis of the specimens was conducted from colonies grown on OA under the above-mentioned conditions, unless stated otherwise in species descriptions. Microscopic preparations of the reproductive structures were mounted in Shear's solution (300 mL 2 % KCH_3CO_2 in water, 120 mL of glycerine, and 180 mL of 95 % ethanol; Chupp 1940) for long-term storage, or in water when structural features required hydration (i.e., spore release, gelatinous sheath, and germ slit). Microscopic traits were examined using an Olympus BH-2 bright-field microscope (Olympus Corporation, Tokyo, Japan), and sizes in descriptions were based on a minimum of 30 measurements per structure to establish size ranges. Photomicrographs were captured with a Zeiss Axio Imager M1 light microscope (Carl Zeiss, Oberkochen, Germany) using Nomarski differential interference contrast and phase contrast, coupled with a DeltaPix Infinity digital camera. Photoplates were compiled using Adobe Photoshop CS6 v. 13.0.

The holotypes (dried colonies) and ex-type cultures were deposited in the Westerdijk Fungal Biodiversity Institute, and nomenclatural novelties were registered in MycoBank (<https://www.mycobank.org/>; accessed on 7th November 2025).

Fermentation, preparation of extracts and metabolomic analyses

A subset of 28 preussia-like fungal strains was selected based on phylogenetic relationships for metabolomic characterization. Strains were grown in duplicate using EPA vials filled with 10 mL of culture media. We used five media formulations, including different carbon and nitrogen sources, and combined solid- and liquid-state fermentation conditions to promote the production of secondary metabolite diversity. For solid-state fermentations, we used rice-based medium (BRFT) and corn-based medium (F1); while for liquid-state fermentations, we used Potato Dextrose Broth (PDB), Mannitol Murashige and Skoog salts medium (MMK2), and Yeast extract Sucrose medium (YES) (González-Menéndez *et al.* 2014). Strains were incubated at 22 °C for 14 d in liquid-state fermentations, and 21 d in solid-state fermentations. Fermentations were harvested and extracted with acetone to generate crude extracts at 2× whole broth equivalent (WBE) final concentration following previous described protocol (González-Menéndez *et al.* 2014).

Fungal extracts (2 µL) were analysed by Liquid Chromatography coupled with Ultraviolet and High-Resolution Mass Spectrometry (LC-UV-HRMS). Liquid chromatography (LC) was performed on an Agilent 1200 (Santa Clara, CA), using a Zorbax SB-C8 column (2.1 × 30 mm) and 3.5 µm particle size, maintained at 40 °C with a flow rate of 300 µL/min and 210 nm UV detection. Solvent A consisted of 10 % acetonitrile and 90 % water with 0.01 % trifluoroacetic acid and 1.3 mM ammonium formate, while solvent B was 90 % acetonitrile and 10 % water with 0.01 % trifluoroacetic acid and 1.3 mM ammonium formate. Elution consisted of a gradient that shifted from 10 % solvent B to 100 % in six minutes, remained at 100 % solvent B for two minutes and returned to 10 % solvent B for two additional minutes to initialize the system. The resulting compounds were

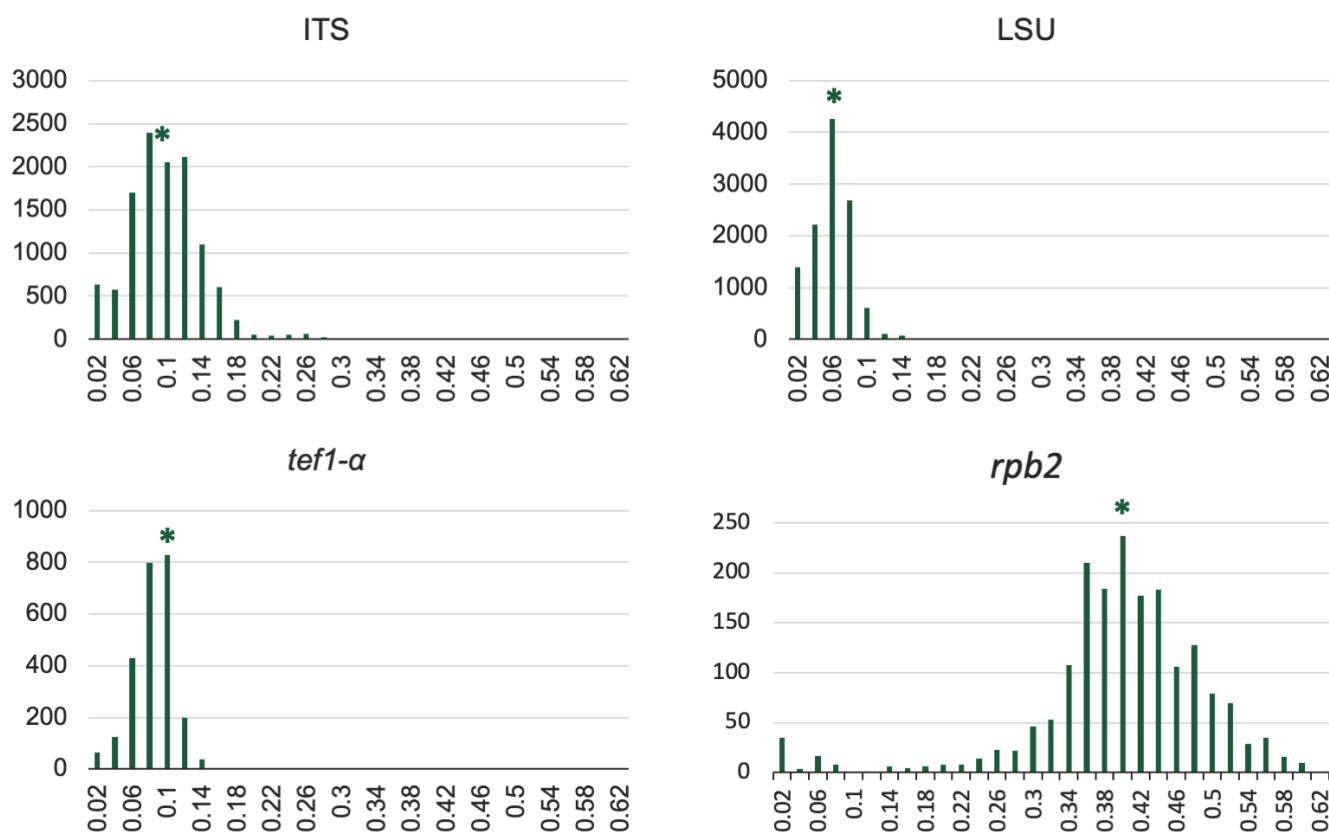


Fig. 1. *Sporormiaceae* inter-specific distances for the loci studied. Mean inter-specific distance is represented with *.



characterized through mass spectrometry on a Bruker maXis High-Resolution Quadrupole Time-of-Flight (HR-QTOF) spectrometer (Bruker Daltonics GmbH, Bremen, Germany). Ionization of the eluting solvent was obtained using the standard electrospray ionization (ESI) sources adjusted to a drying gas flow of 11 L/min at 200 °C and a nebulizer pressure of 40 psig. The capillary voltage was set to 4000 V, and the mass spectrometer was operated in data dependent positive ion mode, automatically switching between full scan MS and MS/MS acquisitions. Fragmentation spectra (*m/z* 50–2000) were collected in the TOF selecting the six most intensive ions above 1000 counts for fragmentation using collision-induced dissociation energy.

Chemical profiles were carried out by comparing the data generated with the Bruker maXis; i.e., retention time, UV spectrum, and exact mass of extracted components. These data were compared to the metabolite data stored in the MEDINA Foundation internal database, which were acquired under the same exact LC-HRMS conditions explained above (Pérez-Victoria *et al.* 2016). The compounds that could not be identified in the database of standards were isolated by semi-preparative high-performance liquid chromatography (HPLC), following the same methodology for characterization through HR-QTOF and ESI. For these compounds, the predicted molecular formulas were compared to the Chapman & Hall Dictionary of Natural Products (v. 25.1) to identify compounds already described in the literature (González-Menéndez *et al.* 2017).

The metabolites detected were clustered through molecular networks based on structural similarity. The MS/MS spectra were converted to mzXML file format through Bruker Data Analysis software. The molecular network was created using the online workflow on the GNPS website (<http://gnps.ucsd.edu>). The dataset was filtered to exclude MS/MS noise fragment ions within +/- 17 Da of the precursor *m/z*, retaining the top 6 fragment ions in the +/- 50 Da window, and using a precursor ion mass tolerance of 2.0 Da, and a MS/MS fragment ion tolerance of 0.5 Da. The resulting network was refined to contain reliable spectral relationships between compounds. Edges were refined to a cosine score above 0.7 and more than 6 matched peaks. Edges connecting nodes were retained only if both appeared in each other ten most similar matches. Molecular family size was set to 100, removing low-score edges based on this threshold. This way, nodes represented compounds and edges represented similarity between fragmentation patterns, suggesting structural relatedness. Spectra included in the network were searched against GNPS libraries, with matches requiring a score above 0.7 and at least 6 matched peaks. The mzXML files

were processed with MZmine v. 2.53 to develop a feature-based molecular network, including mass detection, chromatogram building, chromatogram deconvolution, isotopic peaks grouping, feature alignment, gap filling, filtering, and direct submission/export for GNPS. The resulting networks were analysed online on the GNPS platform and exported to Cytoscape v. 3.6.1 for graphical representation. GNPS data are accessible through the following links: Molecular Network (<https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp>) and Feature-based Molecular Networking (FBMN; <https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=daff1c06c13e4dbda064d25a2c39478f>).

RESULTS

Barcode distances in *Sporormiaceae*

Individual ITS, LSU, *tef1-α*, and *rpb2* alignments were used to determine intra- and inter-specific distances for each locus (Fig. 1). For all loci, the mean inter-specific distance was bigger than the intra-specific distance, without overlapping. Therefore, all loci were suitable barcodes for identification at different taxonomic levels. Among them, *tef1-α*, ITS, and *rpb2* represented appropriate barcodes for species identification with 8 %, 9 %, and 40 % inter-specific distances, respectively. The LSU region was the most conserved region with a 6 % inter-specific distance, which was indicative of different major lineages within *Sporormiaceae*.

Sporormiaceae phylogeny

An initial ITS-LSU phylogenetic analysis was developed to infer the relationships among members of *Sporormiaceae* with available molecular data; i.e., *Forliomyces*, *Preussia* s.l., *Sparticola*, *Sporormia*, *Sporormurispora*, *Trichophoma*, *Westerdykella*, and *Xenomonodictys* (Fig. 2). The alignment comprised 1050 characters (583 bp for ITS, 467 bp for LSU). Among these, 606 character-sites were conserved (ITS: 275, LSU: 331), 418 were variable (ITS: 285, LSU: 133), and 301 were parsimony informative characters (ITS: 207, LSU: 94). The remaining positions included ambiguous or gap-only sites that were excluded from these categories. The models selected for the ML analysis were TIM2e+I+G4 for ITS and TNe+R3 for LSU, while for the BI analysis, the best models were GTR+I+G for ITS and TrNef+I+G for LSU. The outgroups selected were *Lophiostoma rosae-ecae* MFLUCC 17-0807 and

Table 1. Percentage of similarity between *Preussia* s.l. lineages for the LSU region.

Lineages	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7	Clade 8	Clade 9
Clade 1 <i>Sporormiella</i>									
Clade 2 <i>Sporormyces</i>	95–97								
Clade 3 <i>Pleosporormiella</i>	93–96	95–97							
Clade 4 <i>Preussia</i> s.str.	93–95	93–95	92–96						
Clade 5	95–97	94–95	95–97	92–94					
Clade 6 <i>Spororvela</i>	93–96	92–94	93–95	91–93	95–96				
Clade 7	95–97	94–95	92–95	92–94	94–95	92–94			
Clade 8 <i>Chaetosporormiella</i>	94–97	95–96	95–97	92–95	95–97	93–96	93–95		
Clade 9 <i>Spororminula</i>	92–95	92–94	93–95	91–93	94–95	93–94	92–95	95	
Clade 10	90–93	91–92	90–93	91–92	93–95	93–95	92–94	94–95	93–95

Teichospora thailandica MFLUCC 13-0284. We represented the maximum-likelihood tree with the bootstrap support values of the ML analysis and the BI posterior probabilities at the nodes. The species of *Preussia* s.l. were labelled based on the most recent name combinations.

The strains examined in this work clustered within *Preussia* s.l., revealing ten independent clades with unresolved relationships (Fig. 2). These clades were defined based on the phylogenetic distances revealed by the LSU region (Fig. 1, Table 1). A threshold of 97 % LSU similarity was used to define clades, with around 92–96 % similarity between them. The relationships among clades were further explored through a concatenated analysis including protein-coding regions.

Only ITS and LSU sequences were available for many type and reference strains (Supplementary material Table S1). Based on ITS inter-specific mean distance (Fig. 1), we used the analysis of these two gene markers to confirm strain identification at the species level (Fig. 2). The strains examined in this work represented 19 known species and nine independent lineages that could not be assigned to any known species with molecular data available. Relationships at the species level were explored in detail in the following sections.

Concatenated *Preussia* s.l. phylogeny

The concatenated alignment comprising SSU, ITS, LSU, *tef1-α*, and *rpb2* sequences was constructed to provide a more robust evaluation of the phylogenetic relationships among *Preussia* s.l. clades. Since *Pr. pulchella* E: 216605 and *Pr. dakotensis* UPS: 2570-g (clade 10 in Fig. 2; Table 1) showed a great phylogenetic distance from other lineages in the previous ITS and LSU analysis, they were excluded from subsequent phylogenetic analyses.

Individual alignments were concatenated after confirming the congruence between the five regions. The final alignment contained a total of 3451 characters (868 bp for SSU, 567 bp for ITS, 467 bp for LSU, 837 bp for *tef1-α*, and 712 bp for *rpb2*). Among these, 2426 character-sites were conserved (SSU: 822, ITS: 308, LSU: 361, *tef1-α*: 610, *rpb2*: 326), 1004 were variable (SSU: 45, ITS: 243, LSU: 103, *tef1-α*: 226, *rpb2*: 386), and 851 were parsimony informative characters (SSU: 28, ITS: 187, LSU: 85, *tef1-α*: 185, *rpb2*: 366). The remaining positions included ambiguous or gap-only sites that were excluded from these categories. The models selected for the ML analysis were K2P+I for SSU, TIM2e+I+G4 for ITS, K2P+I+G4 for LSU, TIM+F+I+G4 for *tef1-α*, and TN+F+I+G4 for *rpb2*, while for the BI analysis, the best models were K80+I for SSU, SYM+I+G for ITS, TrNef+I+G for LSU, TIM1+I+G for *tef1-α*, and TrN+I+G for *rpb2*. The outgroups selected were *Forliomyces uniseptatus* MFLUCC 15-0765 and *Sparticola junci* MFLUCC 15-0030. We represented the maximum-likelihood tree with the bootstrap support values of the ML analysis and the BI posterior probabilities at the nodes (Fig. 3). Clades and species names were updated through a polyphasic taxonomic analysis, combining this phylogeny with the morphological traits and metabolite profiles explained in the following sections. In addition, chemotypes and the most representative metabolites were also printed in the tree as explained in the section concerning metabolites.

The resulting phylogenetic tree resolved the relationships among *Preussia* s.l. clades, defining nine monophyletic lineages (Fig. 3). Clades 3–9 revealed similar relationships to the ITS-LSU tree; however, clades 1 and 2 were displayed as sister lineages in the concatenated analysis (Figs 2, 3). The great phylogenetic distance between clades suggested independent evolutionary histories.

Thus, each clade could be representing a different genus. This hypothesis was further tested through metabolite and morphology analyses in the following sections. In addition, we followed a similar procedure to resolve the taxonomy of the nine independent lineages. It is noteworthy that seven of the independent lineages were represented by strains of marine and xerophytic origin. In fact, additional marine strains represented the species *Sm. irregularis*, *Sm. koreensis*, *Sm. persica*, and *Sn. subticinensis*.

Dereplication of known compounds and identification of chemotypes

The study of metabolite production was primarily focused on clades 2–4, 8, and 9 (Figs 2, 3). Clade 1 was the main object of study in a previous work (González-Menéndez *et al.* 2017), while clades 5–7 remained to be assessed due to limited access to specimens. The dereplication of the fungal extracts obtained through liquid- and solid-state fermentations revealed 33 compounds. These compounds were classified into networks representing groups of similar molecular structure, with molecules produced across *Preussia* s.l. and molecules exclusively produced by certain clades. The complete list of metabolites identified in this work is available in Table 2, where molecules like $C_{19}H_{28}O_5$, $C_{32}H_{42}N_4O_{10}$, and $C_{11}H_{10}ClO_5$ could not be assigned to any known compound and may represent novel metabolites. Putative hits are included in Supplementary material Table S2.

Among the networks representing structural groups, the flavipucine network revealed up to nine structural variants, with some of them detected across all media tested (Fig. 4). Flavipucines were only detected in members of clades 3 and 8. It is noteworthy that dihydroisoflavipucine was recovered from strains displaying humicola-like (CBS 217.38, FMR 20838; clade 3), monodictys-like (CBS 100.07, CBS 101.60; clade 8) or pycnidia asexual morphs (FMR 20458; clade 3). In a similar fashion, the monocerin group comprised three variants, one of them recovered in all culture media (Fig. 5A). This group of molecules were produced by members of clade 1 (CBS 468.71), clade 3 (FMR 19842, FMR 20458 and FMR 20838), and clade 9 (FMR 19850), representing unique features for each clade (Fig. 3). The compound 7-chloro-6-methoxymellein (Fig. 5B) was detected across clade 2 (CF-277801), clade 4 (CBS 318.81) and clade 9 (CBS 125.66, CBS 354.86, FMR 19850), while $C_{11}H_{10}Cl_2O_4$ was exclusively detected in strains from clade 9 (Fig. 5B). Other molecules were exclusively recovered from single clades. A group of unknown molecules with eight structural variants, including $C_{19}H_{28}O_4$, was exclusively recovered from clade 3 (Fig. 6A). The molecule 4-methoxy-6-pentyl-2H-pyran-2-one was only produced by members of clade 2 (Fig. 6B), while the compound 4-hydroxy-5-methylmellein was only detected in members of clade 9 across all five media (Fig. 7A). Two leptosin analogues ($C_{30}H_{28}N_6O_7S_4$ and $C_{32}H_{34}N_6O_7S_4$) were exclusively detected in the strains CBS 100.07 and CBS 101.60, in clade 8, across all five media tested, cyperin was only recovered from the strains CF-277822 and CBS 318.81 in clade 4, and preussochromone F from FMR 19091 in clade 1 (Fig. 7B–D).

In addition, we used these metabolites to define chemotypes based on the production/absence patterns displayed by the strains examined. We defined 19 chemotypes (A–S) that represented different species (Table 3). These structural network patterns and chemotypes were compared with the concatenated phylogenetic tree to support a polyphasic taxonomic decisions on *Preussia* s.l. clades, as detailed in the following section (Fig. 3).

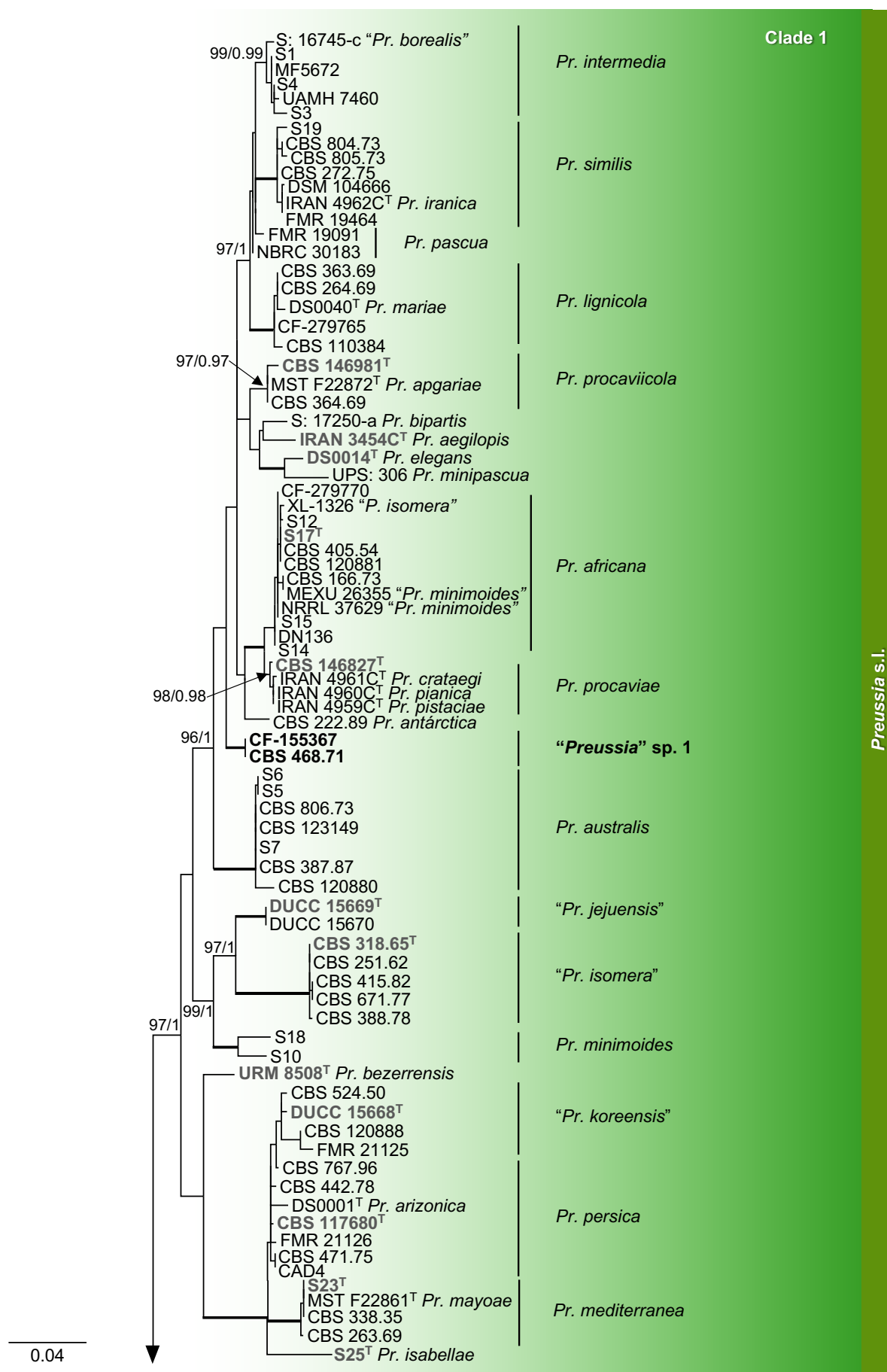


Fig. 2. Phylogenetic tree inferred from a concatenated ITS–LSU alignment of 165 strains representing the family *Sporormiaceae* in *Pleosporales*. The tree is rooted to *Lophiostoma rosae-ecae* MFLUCC 17-0807 and *Teichospora thailandica* MFLUCC 13-0284. Numbers at the branches indicate support values (IQ-TREE-UFBS/BI-PP) above 90 % / 0.95. Fully supported branches (100/1) are highlighted in bold. Quotation marks indicate strains with unresolved taxonomy. Ex-type strains are indicated with ^T, ex-isotypes ^{IT}, reference strain ^R, and those corresponding to the accepted species are highlighted in grey. The scale bar represents the expected number of changes per site.

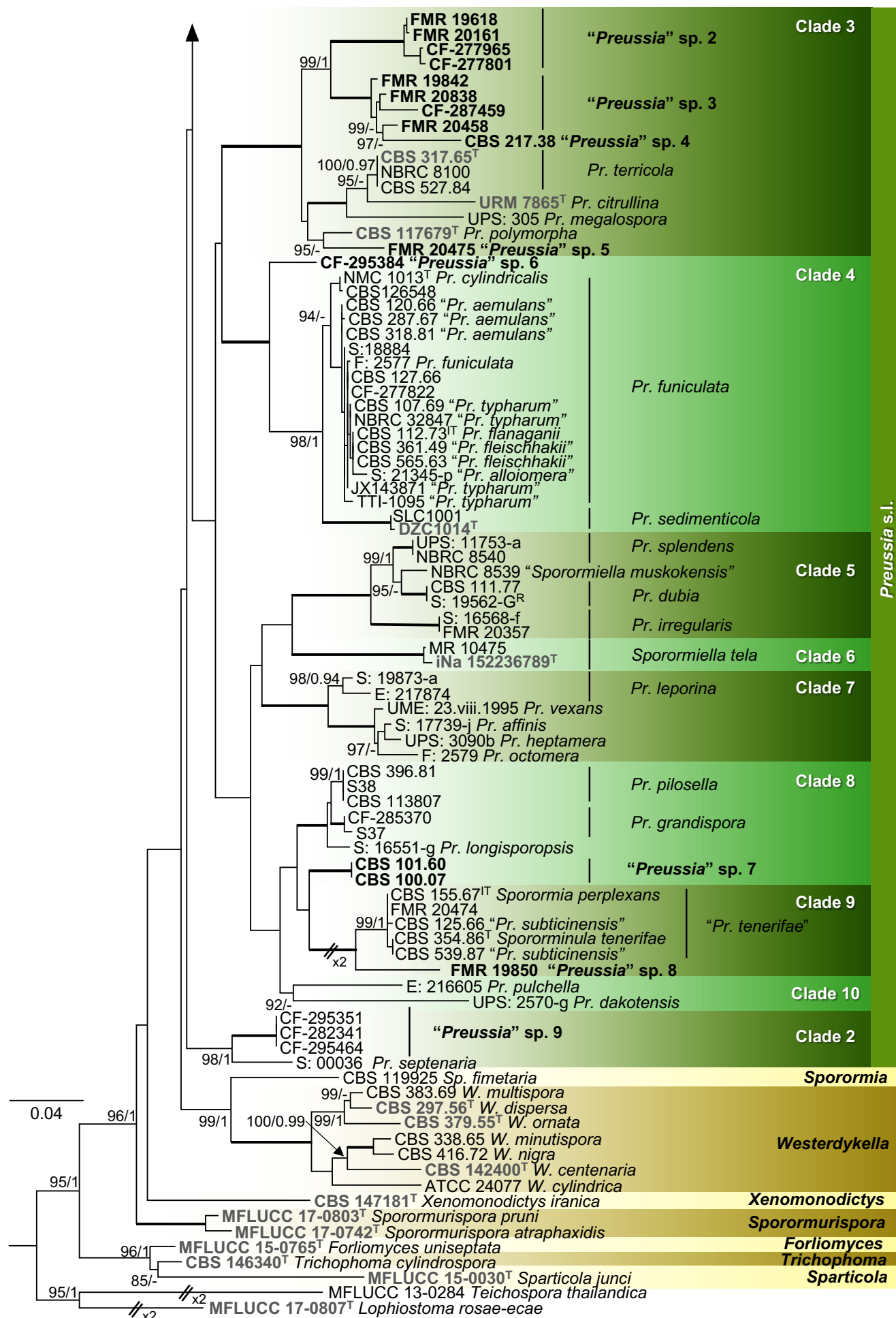


Fig. 2. (Continued).



Table 2. Chemical characterization of the metabolites recovered.

No.	RT ¹ (min)	[M + H] ⁺	Proposed ion	Main secondary ions	Media	Proposed formula	Proposed compound ²
1	2.42	3.070.813	C ₁₅ H ₁₅ O ⁺ ₇	209.0443; 289.0706; 325.0918	PDB	C ₁₅ H ₁₄ O ₇	Preusochromone F
2	3.91	3.010.702	C ₁₆ H ₁₃ O ⁺ ₆	283.0596; 284.06; 302.0735	YES, BRFT, F1	C ₁₆ H ₁₂ O ₆	2,8-Dihydroxy-3-methylxanthone-1-carboxylic acid
3	4.40	1.971.179	C ₁₁ H ₁₇ O ⁺ ₃	1.981.205	PDB, MMK2, YES, BRFT, F1	C ₁₁ H ₁₆ O ₃	4-Methoxy-6-pentyl-2H-pyran-2-one
4	4.27	3.212.065	C ₁₉ H ₂₉ O ⁺ ₃	303.1954; 316.1924; 322.2092	YES, MMK2, BRFT, F1	C ₁₉ H ₂₈ O ₄	Nineteen possible hits in DBs
5	7.77	7.545.823	C ₄₃ H ₈₀ NO ⁺ ₉	391.2842; 755.5958; 776.5647	YES, MMK2, BRFT, F1	C ₄₃ H ₇₉ NO ₉	Glucopyranoside
6	2.30	337.201	C ₁₉ H ₂₉ O ⁺ ₅	319.1903; 338.2042; 354.2273	YES, BRFT, MMK2, F1	C ₁₉ H ₂₈ O ₅	Seventeen possible hits in DBs
7	3.73	2.090.811	C ₁₁ H ₁₃ O ⁺ ₄	191.0701; 210.0839	YES, PDB, MMK2, BRFT, F1	C ₁₁ H ₁₂ O ₄	4-Hydroxy-5-methylmellein
8	4.29	3.091.353	C ₁₆ H ₂₁ O ⁺ ₆	223.06; 291.1226; 310.1365	PDB, BRFT, MMK2, F1	C ₁₆ H ₂₀ O ₆	Monocerin
9	3.19	2.811.746	C ₁₆ H ₂₅ O ⁺ ₄	245.1534; 263.1639; 561.3421	YES, PDB, BRFT, F1	C ₁₆ H ₂₄ O ₄	Brefeldin A
10	2.62	3.271.435	C ₁₆ H ₂₃ O ⁺ ₇	291.1225; 309.1332;	BRFT, F1	C ₁₆ H ₂₂ O ₇	Fifteen possible hits in DBs
11	3.79	3.490.916	C ₁₇ H ₁₇ O ⁺ ₈	287.0914; 299.055; 331.0818	BRFT, MMK2, F1	C ₁₇ H ₁₆ O ₈	Asterric acid
12	3.44	2.090.452	C ₁₇ H ₁₇ O ⁺ ₇	231.1376; 333.0966	BRFT, MMK2, F1	C ₁₇ H ₁₆ O ₇	Bisdechlorodihydrogeodin
13	2.22	2.401.246	C ₁₂ H ₁₈ NO ⁺ ₄	241.1262; 479.2391	YES, BRFT, MMK2, F1	C ₁₂ H ₁₇ NO ₄	Dihydroisoflavipucine
14	4.91	318.282	C ₂₁ H ₃₆ NO ⁺ ₃	300.2682; 319.2821	YES, PDB, BRFT, MMK2, F1	C ₂₁ H ₃₅ NO	Preussin
15	4.95	2.850.755	C ₁₆ H ₁₃ O ⁺ ₅	253.0493; 286.0786	BRFT, F1	C ₁₆ H ₁₂ O ₅	Monodictyquinone
16	4.22	2.902.476	C ₁₉ H ₃₂ NO ⁺ ₃	291.2507; 272.2347	YES, BRFT, MMK2, F1	C ₁₉ H ₃₁ NO	Preussin B
17	0.98	2.781.478	C ₁₆ H ₂₄ NO ⁺ ₃	260.164; 279.1772	PDB, MMK2	C ₁₆ H ₂₃ NO ₃	Six possible hits in DBs
18	4.16	2.761.956	C ₁₇ H ₂₆ NO ⁺ ₂	277.1986; 551.3836	YES, PDB, BRFT, MMK2	C ₁₇ H ₂₅ NO ₂	No match in DBs
19	1.80	3.091.331	C ₁₆ H ₂₁ O ⁺ ₆	291.1223; 310.1362; 331.1148	BRFT	C ₁₆ H ₂₀ O ₆	11-hydroxycurvularin
20	3.23	2.911.242	C ₁₆ H ₁₉ O ⁺ ₅	273.112; 292.1259; 581.2379	BRFT, F1	C ₁₆ H ₁₈ O ₅	Resorcyliide
21	2.35	4.131.265	C ₁₉ H ₂₅ O ⁺ ₈	245.084; 430.153	BRFT, F1	C ₁₉ H ₂₄ O ₈	Sumalarin C
22	3.88	2.611.127	C ₁₅ H ₁₇ O ⁺ ₄	2.621.156	YES, BRFT, F1	C ₁₅ H ₁₆ O ₄	Cyperin
23	4.70	4.131.595	C ₂₃ H ₂₅ O ⁺ ₇	275.0913; 414.163; 842.3377	BRFT, F1	C ₂₃ H ₂₄ O ₇	Six possible hits in DBs
24	4.08	2.430.416	C ₁₁ H ₁₂ ClO ⁺ ₄	245.0389; 244.0447	YES, PDB, BRFT, MMK2, F1	C ₁₁ H ₁₁ ClO ₄	7-Chloro-6methoxymellein
25	1.07	280.118	C ₁₄ H ₁₈ NO ⁺ ₅	262.1073; 281.121	BRFT, MMK2, F1	C ₁₄ H ₁₇ NO ₅	Spirostaphylotrichin A
26	4.26	7.130.967	C ₃₀ H ₂₉ N ₆ O ₇ S ⁺ ₄	277.2159; 714.0993	YES, BRFT, MMK2, F1	C ₃₀ H ₂₈ N ₆ O ₇ S ₄	Leptosin analogue
27	4.95	6.432.973	C ₃₂ H ₄₃ N ₄ O ⁺ ₁₀	429.202; 644.3001; 874.4223	BRFT, F1	C ₃₂ H ₄₂ N ₄ O ₁₀	No match in DBs



Table 2. (Continued).

No.	RT ¹ (min)	[M + H] ⁺	Proposed ion	Main secondary ions	Media	Proposed formula	Proposed compound ²
28	4.21	2.421.172	C ₁₅ H ₁₆ NO ₂ ⁺	240.1016; 275.0911	YES, MMK2	C ₁₅ H ₁₆ NO ₂	TMC120A
29	4.80	3.711.851	C ₂₂ H ₂₇ O ₅ ⁺	297.2417; 372.1885	BRFT, F1	C ₂₂ H ₂₆ O ₅	Four possible hits in DBs
30	4.34	7.431.435	C ₃₂ H ₃₅ N ₆ O ₇ S ₄ ⁺	7.441.461	YES, PDB, BRFT, F1	C ₃₂ H ₃₄ N ₆ O ₇ S ₄	Leptosin analogue
31	3.12	2.770.028	C ₁₁ H ₁₁ ClO ₂ ⁺	258.9922; 279.001	YES, PDB, MMK2, BRFT, F1	C ₁₁ H ₁₀ ClO ₂	Antibiotic LL-N 313ε
32	2.61	3.690.972	C ₂₀ H ₁₇ O ⁺	351.0865; 370.1004	BRFT, F1	C ₂₀ H ₁₆ O ₇	Palmarumycin
33	4.64	3.031.011	C ₂₀ H ₁₅ O ⁺	285.0904; 304.1044	YES, BRFT, F1	C ₂₀ H ₁₄ O ₃	No match in DBs

¹Retention time.

²DBs: databases.

Diversity of *Preussia* s.l. using a polyphasic approach

Based on the relationships revealed by the multi-locus phylogenetic analyses, morphological features, metabolite production, and substrate preference, we divided *Preussia* s.l. into nine genera, including *Preussia* s.str., *Sporormiella*, and re-erecting *Spororminula* (Fig. 3). Table 4 summarizes the major phenotypic differences among them.

Clade 1 comprised members of *Sporormiella*, displaying short-stipitate asci with 4–13-celled ascospores, and primarily pycnidial asexual morphs. This lineage comprised 22 known species, including 11 proposed new combinations into this genus, as well as one new name and one novel species, *Sm. solicola*. It is noteworthy that this clade included the species complexes of “*Preussia lignicola*” and “*Preussia minima*”. The first complex was determined as a single taxon, while the latter represented a set of five species (Figs 2, 3), including the recent addition to the genus *Preussia*, *Pr. japonica* (Li et al. 2025; Supplementary material Fig. S11).

Clade 2 represented an independent lineage that we proposed as *Sporormyces* (Sc.), typified by the novel combination *Sc. septenarius*. This genus was characterized by short-stipitate asci with 7-celled ascospores, and producing 4-methoxy-6-pentyl-2H-pyran-2-one among other compounds. In addition, we proposed the novel species, *Sc. anthraquinoniformans*, represented by strains of xerophytic origin.

We proposed clade 3 as the novel genus *Pleosporormiella* (Ps.), characterized by short-stipitate asci with 4-celled ascospores, pycnidial and humicola-like asexual morphs, and producing preussins and C₁₉H₂₈O₅. Three new combinations were proposed for this genus, along with four novel species, *Ps. aculeata*, *Ps. compacta*, *Ps. polyspora*, and *Ps. suttonii*, the last three species described from strains of marine and xerophytic substrates.

Clade 4 included the type species of the genus *Preussia*, *Pr. funiculata*, another known species, and a novel species of xerophytic origin, *Pr. gypsicola*. We defined this lineage as *Preussia* s.str., which is characterized by displaying predominantly long-stipitate asci with 4-celled ascospores, pycnidial and chrysosporium-like asexual morphs. In addition, *Pr. funiculata* was characterized by producing cyperin.

Clade 6 represented an independent lineage that we proposed as *Spororvela* (Sv.), characterized by long-stipitate asci with 7–12-celled (primarily 8-celled) ascospores. Only one species is known, the novel combination *Sv. tela*.

We also proposed clade 8 as the novel genus *Chaetosporormiella* (Cp.), characterized by hairy ascomata, short-stipitate asci with 4-celled ascospores, pycnidial and monodictys-like asexual morphs, and producing two leptosin analogues. A known species was recombined into this genus, and we proposed an additional novel species, *Cp. monodictyoides*.

Clade 9 accommodated the type species of the genus *Spororminula*, *Sn. tenerifae* (proposed here as *Sn. subticiensis*), and one novel species of marine origin, *Sn. irregularis*. This lineage is characterized by short-stipitate asci with 8-celled ascospores, pycnidial asexual morph, and producing 7-chloro-6-methoxymellein.

The complete morphological descriptions and discussions on synonyms and novel species are provided in the taxonomy section. Unfortunately, we could not examine the strains included in clades 5, 7, and 10, so they remained as unresolved lineages in *Sporormiaceae*.

Taxonomy

We resolved the classification of *Preussia* s.l. using an integrative taxonomic approach that combined phylogenetic, morphological, ecological, and secondary metabolite data. In this section, we provide the information necessary to stabilize the nomenclature of the recognized taxa, including comments on the unresolved clades. Our taxonomic decisions are further supported by the examination of type-specimen exsiccatae. When the original authors did not clearly designate type specimens, or when these were lost or poorly preserved, we addressed the issue by examining original collections and designating lectotypes and/or epitypes when needed. In cases where typification was not possible (i.e., when available specimens differed in substrate and/or geographic origin), and in accordance with best-practice recommendations, we designated a reference strain whose characters matched the protologue of the species undergoing nomenclature stabilization (Lendemer 2020). In addition, two non-sporulating species were proposed based only on DNA sequence data, following the procedures established by previous authors (Gomes *et al.* 2013, Lombard *et al.* 2016, Hou *et al.* 2023). Genera are presented here according to their position in the phylogenetic tree (Fig. 3), and species are arranged alphabetically.

Clade 1. *Sporormiella* Ellis & Everh., *N. Amer. Pyrenomyc.*: 136. 1892.

Mycelium immersed and superficial, composed of hyaline, septate, branched, smooth-walled, sometimes anastomosed hyphae. *Sexual morph*: *Ascomata* perithecial, sometimes cleistothecial, immersed to semi-immersed, solitary or aggregated in small groups, globose to subglobose or pyriform, glabrous, carbonaceous-membranaceous to coriaceous; peridium smooth, dark brown to black, *textura angularis*; neck short, papilliform to rostrate, long, cylindrical, sometimes 2–3 necks. *Paraphyses* filiform, septate, intermixed with asci and longer. *Asci* 8-spored, bitunicate, cylindrical-clavate, sometimes subglobose, tapering into a short stipe. *Ascospores* predominantly biseriate, subcylindrical, 4–13-celled, septa transverse to oblique, deeply constricted, frequently cells easily separable at maturity, and variable in size and shape, thick-walled, hyaline when young, dark brown at maturity; germinal slit elongated extending the full length of each cell, parallel to diagonal, or transverse to the longitudinal axis of the ascospore; gelatinous sheath hyaline, subglobose, surrounding each ascospore. *Coelomycetous asexual morph*: *Conidiomata* pycnidial, solitary or aggregated, immersed to erumpent, globose to subglobose or irregularly shaped, *textura angularis*, brown, ostiolate, with up to 4 ostioles. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, hyaline, smooth. *Conidia* aseptate, solitary, ellipsoid with obtuse ends, hyaline, smooth, guttulate, aggregated in mucous masses. *Chlamydospores* aseptate, solitary or sometimes in short chains, intercalary or terminal, globose to ellipsoidal or variable in shape, grey to brown, smooth-walled.

Type species: *Sporormiella nigropurpurea* Ellis & Everh., *N. Amer. Pyrenomyc.*: 136. 1892.

Habitat: Predominantly on dung, but also on soil, wood, and as endophytes in leaves.

Notes: The genus *Sporormiella* was described based on a coprophilous fungus growing on cow dung in Newfield (New Jersey, USA), *Sm. nigropurpurea* (Ellis & Everhart 1892). The

type specimen is available in the New York Botanical Garden (NY 01047198) without available living cultures or DNA sequences. Despite this, the fungus remains recognizable and displays the main morphological features of the genus; i.e., short-stipitate asci with 4-celled ascospores covered by a gelatinous sheath. In fact, an additional specimen of this species was recently identified based on morphology (Wergen 2025), but cultures or DNA sequence data were not generated.

In our phylogenetic analyses (Fig. 3), clade 1 formed a well-supported monophyletic lineage comprising predominantly coprophilous species, including species with 4-celled (e.g., *Sm. minimoides* and *Sm. similis*), 8-celled (e.g., *Sm. bipartis* and *Sm. minipascua*), and 13-celled ascospores (i.e., *Sm. antarctica*). This circumscription matched the definition of the genus in the sense of Ahmed & Cain (1972). Although we could not include *Sm. nigropurpurea* in the analyses, we recognize this clade as the genus *Sporormiella* based on morphology. The species *Sm. lignicola*, formerly in *Preussia*, also clustered here as anticipated by Kruys & Wedin (2009). This way, *Sporormiella* is defined as a different lineage from *Preussia* s.str. and *Sporormia*, comprising 22 phylogenetic species. Among them, three species are only known for their pycnidial asexual morph.

It is worth mentioning that the genus *Ohleriella* was considered a synonym of *Sporormiella* based on the morphological resemblance between its type species, *Ohleriella neomexicana*, and *Sm. octomera* (Ahmed & Cain 1972). Our phylogenetic analyses resolved the specimen of *Sm. octomera* with available molecular data (strain F: 2579) as a member of clade 7 (Fig. 3). Although we could not examine this specimen, our results suggest that *Ohleriella* should not be considered a synonym of *Sporormiella*. In addition, *Ohleriella* is characterized by producing long trabeculate pseudoparaphyses (Earle 1902). Therefore, we follow previous works that considered this genus as a member of *Delitschiaceae* (Zhang *et al.* 2012b).

Sporormiella aegilopsis (M. Mehrabi *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862060.

Basionym: *Preussia aegilopsis* M. Mehrabi *et al.*, *Nova Hedwigia* **114**: 152. 2022.

Typus: Iran, Kermanshah province, Sonqor, 34°50'20.65" N, 47°09'52.08" E, seed endophyte of *Aegilops umbellulata*, Aug. 2018, M. Mehrabi (**holotype** IRAN 17713F, ex-type strain IRAN 3454C).

Description and illustration: Mehrabi *et al.* (2022).

Notes: Our phylogenetic analyses revealed the basionym *Pr. aegilopsis* as a member of *Sporormiella*, sister to *Sm. australis* and *Sm. bipartis* (Figs 2, 3). *Sporormiella aegilopsis* is only known by its coelomycetous asexual morph, while *Sm. australis* and *Sm. bipartis* display sexual morphs with ascospores that differ in being 4-celled and 8-celled, respectively (Ahmed & Cain 1972, Mehrabi *et al.* 2022).

Sporormiella africana (Arenal *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862061.

Basionym: *Preussia africana* Arenal *et al.*, *Fungal Diversity* **20**: 6. 2005.

Typus: Spain, Canary Islands, Tenerife, Llano de los Viejos, on leaves of *Viburnum tinus*, 2 Apr. 1995, A. Santos (**holotype** AH32767, ex-type strain S17).

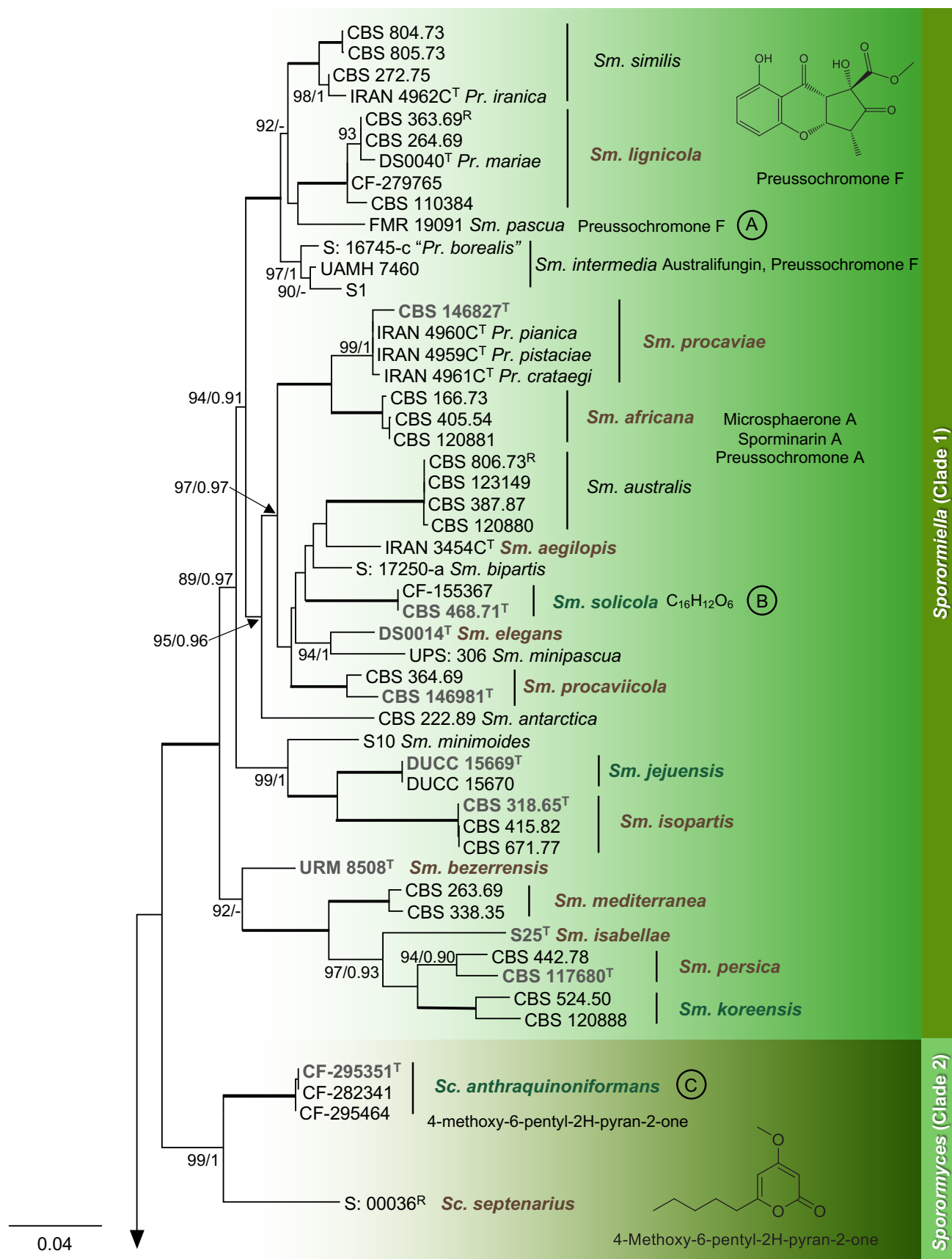


Fig. 3. Phylogenetic tree inferred from a concatenated SSU-ITS-LSU-*tef1-rpb2* alignment of 111 strains representing *Preussia* s.l. and associated genera. The tree is rooted to *Forliomyces uniseptatus* MFLUCC 15-0765 and *Sparticola junci* MFLUCC 15-0030. Numbers at the branches indicate support values (IQ-TREE-UFBS/BI-PP) above 90 % / 0.95. Fully supported branches (100/1) are highlighted in bold. Novel combinations are highlighted in brown, and novel species in green. Quotation marks indicate strains with unresolved taxonomy. Ex-type strains are indicated with ^T, ex-epitypes with ^{ET}, ex-isotypes ^{IT}, reference strains selected in the present study ^R, and those corresponding to the accepted species are highlighted in grey. The scale bar represents the expected number of changes per site. Most representative metabolites per clades are also included in the tree, and chemotypes are indicated within circles (See Table 3).

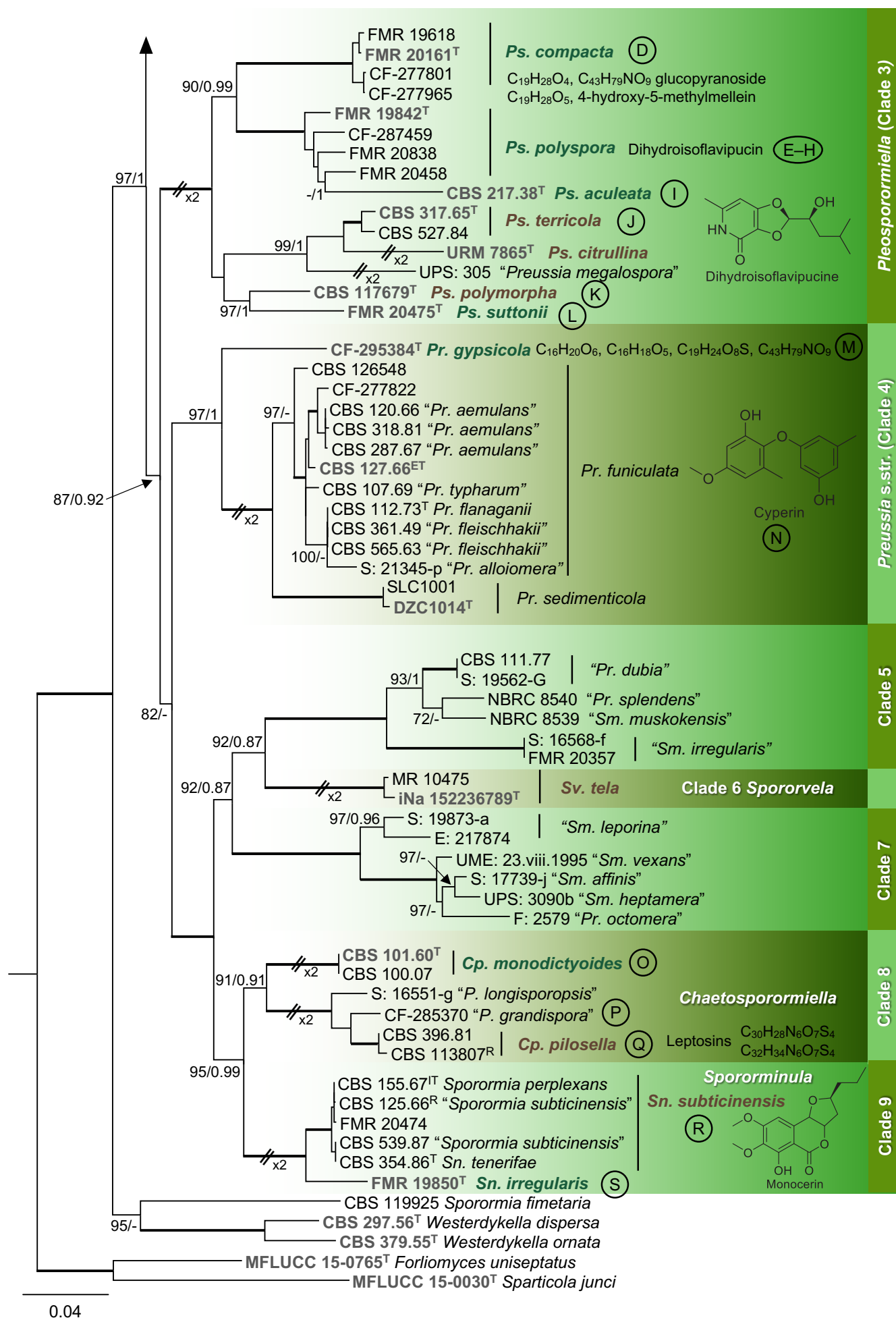


Fig. 3. (Continued).



Description and illustration: Arenal et al. (2005).

Additional materials examined: **Kenya**, on elephant dung, Jan. 1973, R.F. Cain (CBS 166.73). **Mozambique**, Inhaca Island, from mangrove mud, Nov. 1953, H.J. Swart (CBS 405.54). **South Africa**, Limpopo province, Mookgophong (Naboomspruit), from *Prunus salicina*, Aug. 2008, U. Damm (CBS 120881).

Notes: The ex-type strain of the basionym *Pr. africana* clustered as a member of *Sporormiella*, forming a lineage with *Sm. procaviae* (Figs 2, 3). Only the sexual morph is known for *Sm. africana*, while *Sm. procaviae* only produces a pycnidial asexual morph (Crous et al. 2020a).

Sporormiella antarctica (Speg.) S.I. Ahmed & Cain, *Canad. J. Bot.* **50**(3): 434. 1972.

Basionym: *Sporormia antarctica* Speg., *Bol. Acad. Nac. Ci.* **11**(2): 224. 1888.

Synonym: *Preussia antarctica* (Speg.) J.H. Chang & Y.Z. Wang, *Nova Hedwigia* **88**: 252. 2009.

Typus: **Argentina**, Tierra del Fuego, Ushuaia, on dung of *Bernicia antarctica*, Jun. 1882, C. Spegazzini (**holotype** LPS 3500).

Description and illustration: Ahmed & Cain (1972).

Additional material examined: **Norway**, Spitsbergen, from soil, May 1989, F. Seigle-Murandi (CBS 222.89).

Notes: *Sporormiella antarctica* was transferred to *Preussia* based on morphology, with both genera considered as synonyms (Chang & Wang 2009). Our phylogenetic analyses support this species as a member of *Sporormiella*, characterized by producing 13-celled ascospores (Fig. 3, Supplementary material Fig. S1).

Sporormiella australis (Speg.) S.I. Ahmed & Cain, *Canad. J. Bot.* **50**: 434. 1972.

Basionym: *Sporormia australis* Speg., *Bol. Acad. Nac. Ci.* **11**: 50. 1887.

Synonym: *Preussia australis* (Speg.) Arx, *Proc. Kon. Ned. Akad. Wetensch.*, C **76**: 294. 1973.

Typus: **Argentina**, Santa Cruz, Misioneros area, on dung of *Cavia australis*, Aug. 1882, C. Spegazzini (**holotype** LPS 3521).

Description and illustration: Ahmed & Cain (1972).

Additional materials examined: **Kuwait**, from soil, unknown collection date and collector (CBS 123149); *ibid.*, from saline desert soil, Sep. 1973, A.F. Moustafa (CBS 806.73; proposed here as reference strain). **Morocco**, from soil, Jul. 1987, F. Seigle-Murandi (CBS 387.87). **South Africa**, Western Cape Province, Bonnievale, from wood symptoms of *Prunus armeniaca*, Aug. 2005, U. Damm (CBS 120880).

Notes: Our phylogenetic analyses clustered the strains examined in this work with strains previously used to characterize *Sm. australis* (Fig. 2; Arenal et al. 2005, Kruys & Wedin 2009, González-Menéndez et al. 2017). We compared size ranges with the protologue and confirmed the identification (Supplementary material Fig. S2). Since DNA data of the type specimen is not available and metadata of the examined specimens do not match with that of the protologue, we propose CBS 806.73 as reference strain for the species in phylogenetic analyses.

Sporormiella bezerrensis (Ferreira-Sá et al.) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862062.

Basionym: *Preussia bezerrensis* Ferreira-Sá et al., *Persoonia* **49**: 315. 2022.

Typus: **Brazil**, São Domingos, Goiás, Lapa do Bezerra cave, from air, Dec. 2020, A.S. Ferreira-Sá, et al. (**holotype** HUEG 15380/SXS 693, ex-type strain URM 8508).

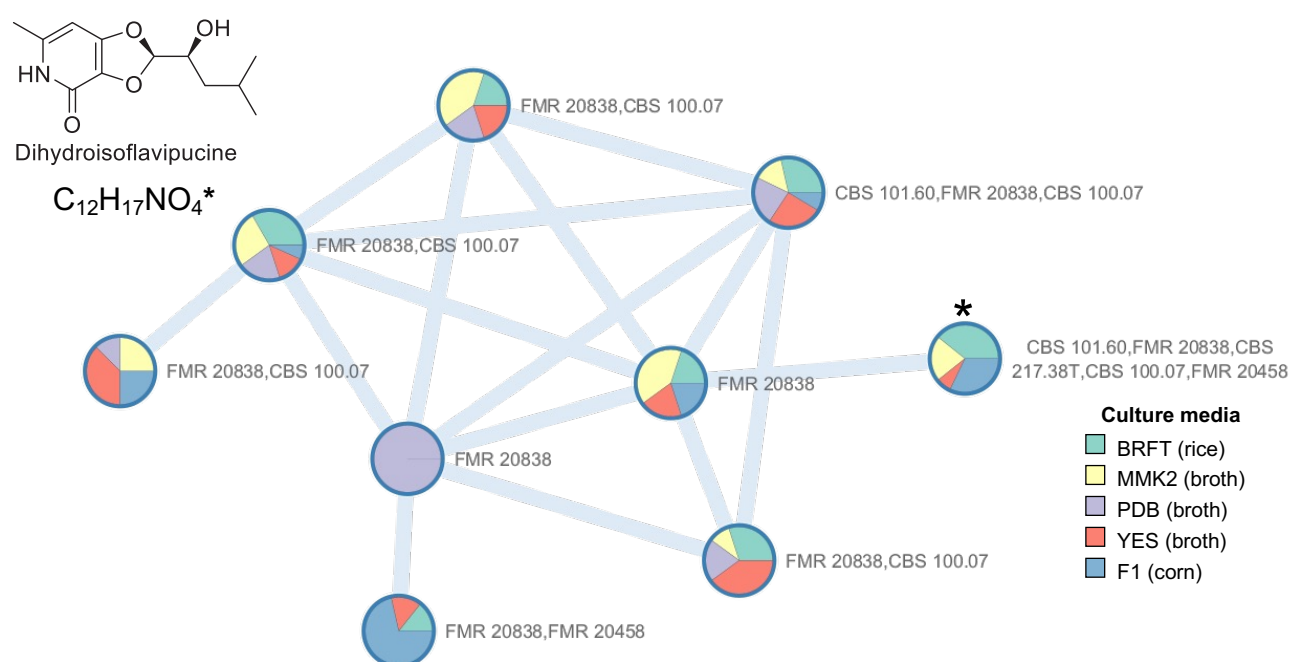


Fig. 4. GNPS network representing flavipucine compounds together with the strains that produced them. Each node includes the fermenting media where the compound was detected. The most abundant compound is highlighted with *.



Description and illustration: Tan et al. (2022).

Notes: *Sporormiella bezerrensis*, formerly known as *Pr. bezerrensis*, formed an independent lineage sister to the *Sm. minima* species complex (Figs 2, 3). For a detailed morphological comparison with related species, see Tan et al. (2022).

Sporormiella bipartis (Cain) S.I. Ahmed & Cain, *Canad. J. Bot.* **50**: 435. 1972.

Basionym: *Sporormia bipartis* Cain, *Stud. Sphaer. Ontario*: 106. 1934.

Synonym: *Preussia bipartis* (Cain) Kruys, *Syst. Biodiv.* **7** (4): 476. 2009.

Typus: **Canada**, Ontario, Nipissing District, Lake Timagami, on rabbit dung (**holotype** TRTC 5320).

Description and illustration: Ahmed & Cain (1972).

Notes: Based on the phylogeny of the strain S: 17250-a, which had been morphologically identified as *Sm. bipartis*, this species was transferred to *Preussia* following the synonymization of the two genera (Kruys & Wedin 2009). Our concatenated phylogenetic analysis confirmed this strain as a member of *Sporormiella* (Fig. 3). This species is characterized by producing 8-celled ascospores (Ahmed & Cain 1972).

Sporormiella elegans (D.C. Sandberg & A.E. Arnold) Guerra-Mateo, Gené & Cano **comb. nov.** MB 862063.

Basionym: *Preussia elegans* D.C. Sandberg & A.E. Arnold, *Pl. Fungal Syst.* **67**: 69. 2022.

Typus: **USA**, Arizona, Pima County, Tucson Mountains, as endophyte in healthy photosynthetic stems of *Ephedra trifurca*, 2009, D.C. Sandberg & A.E. Arnold (**holotype** ARIZ-AEA-DS0014, ex-type strain DS0014).

Description and illustration: Sandberg et al. (2022).

Notes: Our phylogenetic analyses determined the basionym *Pr. elegans* as a member of *Sporormiella* (Figs 2, 3), forming a well-supported clade with *Sm. minipascua* (UPS: 306). These species differ in ascospore cell number, with *Sm. elegans* producing 4-celled ascospores, while *Sm. minipascua* displays 8-celled ascospores (Kruys & Wedin 2009, Sandberg et al. 2022).

Sporormiella intermedia (Auersw.) S.I. Ahmed & Cain ex Kobayasi, *Bull. Natl. Sci. Mus., Tokyo n.s.* **12**: 339. 1969.

Basionym: *Sporormia intermedia* Auersw., *Hedwigia* **7**: 67. 1868.

Synonym: *Preussia intermedia* (Auersw.) S. Ahmad, *Monogr. Biol. Soc. Pakistan* **8**: 49. 1979.

Typus: **Germany**, isolated from cow, deer, hare, and rabbit dung (**holotype** was not clearly indicated); **lectotype** designated here MBT 10031132: **UK**, near Batheaston, on rabbit dung, Oct. 1863, C.E. Broome, No. 644 (under *Sphaeria stercoris*), Rabenhorst, *Fungi Eur. Exs.*, examined by Auerswald, included in the protologue of *Sp. intermedia*, and preserved at the Martin-Luther-Universität Halle-Wittenberg (HAL), Germany.

Description and illustration: Ahmed & Cain (1972).

Additional materials examined: **Greece**, Cephalonia Island, on goat dung, unknown collection date, F. Arenal, et al. (S4; proposed here as reference strain); *ibid.*, (S3). **USA**, Minnesota, Cook County, on moose dung, unknown collection date and collector (MF5672).

Notes: The taxonomic history of the basionym *Sm. intermedia* is based on non-type specimens recovered worldwide (Ahmed & Cain 1972, Arenal et al. 2005). In addition, it is important to highlight that the holotype specimen was not properly indicated in the protologue of the species. Auerswald (1868) described *Sp. intermedia* based on several collections that were distributed in exsiccatal series and

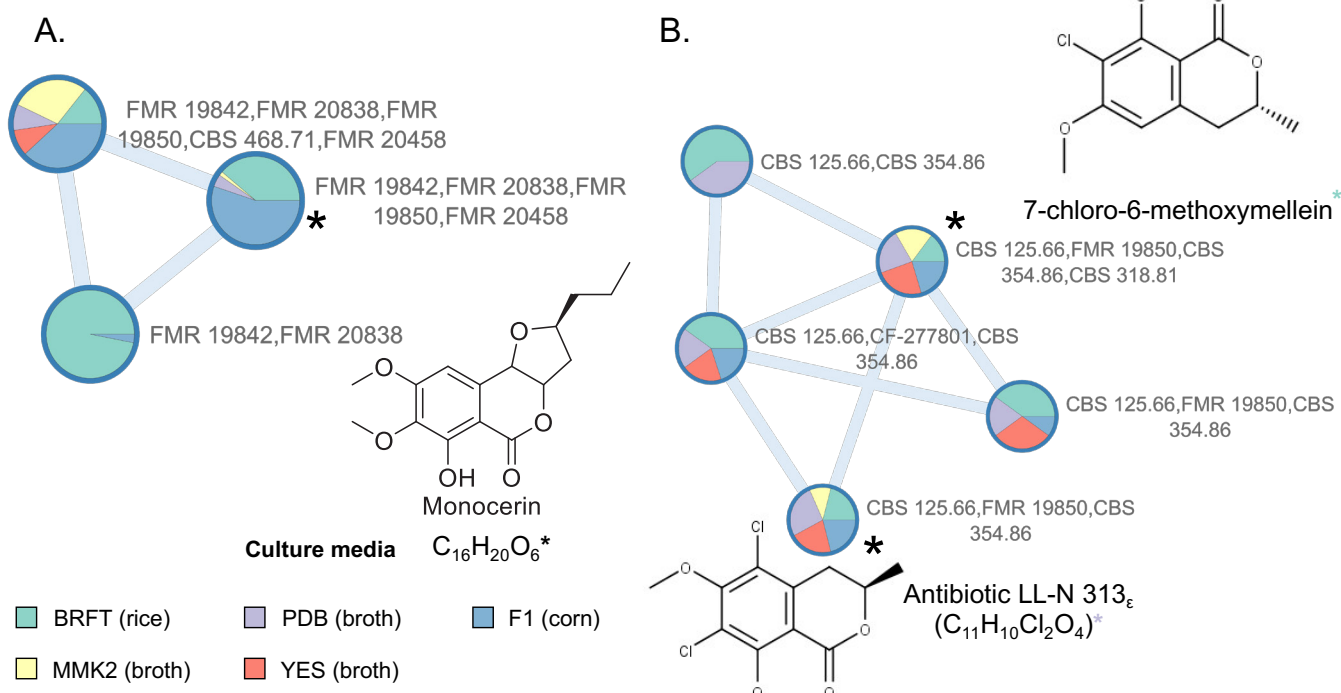


Fig. 5. GNPS networks representing different mellein clusters together with the strains that produced them. The most abundant compounds are highlighted with *. **A.** Monocerin. **B.** 7-chloro-6-methoxy-mellein and Antibiotic LL-N 313_e.

deposited in different herbaria under other names. Consequently, these materials can be regarded as original specimens suitable for lectotypification. At the Martin-Luther-Universität Halle-Wittenberg (HAL, Germany), we located one of Auerswald's specimens (No. 644), preserved in good condition, but incorrectly labelled as *Sp. stercoris*, which is here designated as the lectotype of the species (Supplementary material Fig. S3). Although the cultures examined in this study are morphologically consistent with *Sm. intermedia*, none of them is suitable for designation as an epitype. Therefore, we propose strain S4 as a reference strain to stabilize the phylogenetic framework of this species. We selected this strain based on its morphological resemblance to the protologue and its long history of use as a representative strain of *Sm. intermedia* in phylogenetic analyses (Arenal *et al.* 2005, González-Menéndez *et al.* 2017).

The species has been transferred to both *Sporormiella* and *Preussia* (Kobayasi *et al.* 1969, Ahmad 1979). However, morphologically it resembles *Sporormiella*, and our phylogenetic analyses confirmed *Sm. intermedia* as a monophyletic species (Figs 2, 3). It is noteworthy that the original producer of australifungin and australifunginol, the strain MF5672, was formerly identified as *Sm. australis*, but phylogenetic analyses using both strains (MF5672 and S4) determined the former strain as *Sm. intermedia* (Fig. 2; González-Menéndez *et al.* 2017).

Furthermore, our phylogenetic analyses revealed the strain S: 16745-c, labelled as *Pr. borealis* (Kruys & Wedin 2009) as conspecific with *Sm. intermedia* (Figs 2, 3). These species differ in ascospore length, 48–59 µm in *Sm. intermedia* vs 92–114 µm in *Pr. borealis* (Ahmed & Cain 1972).

Sporormiella isabellae (Arenal *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862064.

Basionym: *Preussia isabellae* Arenal *et al.*, *Fungal Diversity* **20**: 7. 2005.

Typus: Puerto Rico, from woody plant debris, Jan. 1996, J. Guarro (**holotype** AH32771, ex-type strain S25).

Description and illustration: Arenal *et al.* (2005).

Notes: The ex-type strain of *Pr. isabellae* was determined as a member of *Sporormiella*, forming an independent lineage within the *Sm. minima* species complex (Figs 2, 3). Morphological differences among members of this group are subtle and *Sm. isabellae* is primarily distinguished through phylogenetic analyses (Fig. 3).

Sporormiella isopartis Guerra-Mateo, Gené & Cano, **nom. nov.** MB 862065.

Replaced synonym: *Preussia isomera* Cain, *Canad. J. Bot.* **39**: 1643. 1961.

Etymology: Greek “iso-“, equal, and latin “part”, portion, referring to the ascospores' septation in equal portions.

Typus: USA, Florida, near Tallahassee, on unidentified dung, Mar. 1952, S.L. Meyer (**holotype** TRTC 32089, ex-type strain CBS 318.65).

Description and illustration: Cain (1961).

Additional materials examined: Italy, from *Avena sativa* grain, Jul. 1962, G. Vegni (CBS 251.62). Japan, Miyazaki Prefecture, Toimisak, on horse dung, Jul. 1974, K. Furuya (CBS 671.77). Venezuela, Edo

Monagas, 5 km North of El Zamuro, along route 10, on cow dung, Jul. 1972, J.C. Krug (CBS 415.82); *ibid.*, Jusepín, on cow dung, Jun. 1978, J.C. Krug (CBS 388.78).

Notes: The name *Sm. isopartis* is a replacement name for *Pr. isomera* (Cain 1961), as the epithet “isomera” is unavailable in *Sporormiella* due to prior valid publication of *Sm. isomera* (Ahmed & Cain 1972). Therefore, a new name is proposed for *Pr. isomera*.

Although *Sm. isomera* and *Sm. isopartis* differ in ascospore size, 32–38(–40) × 5.5–7(–8) µm vs 30–33 × 4–4.5 µm, respectively, both fungi were described from dung collected in North America (Cain 1961, Ahmed & Cain 1972). Given that ascospore size may vary within a species, they may represent a single taxon. However, no molecular data are available for *Sm. isomera* for comparison. Therefore, we regard this species as taxonomically uncertain (Supplementary material Fig. S2) until further studies are conducted.

Sporormiella isopartis formed an independent lineage sister to *Sm. jejuensis* and *Sm. minimoides* (Figs 2, 3), differing from *Sm. minimoides* by producing smaller asci, 43–55 µm vs 90–110 µm in length, respectively (Cain 1961, Ahmed & Cain 1972). For *Sm. jejuensis* only a coelomycetous asexual morph is known (Noh *et al.* 2025).

Sporormiella japonica (Udagawa *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862375.

Basionym: *Scytalidium japonicum* Udagawa *et al.*, *Mycotaxon* **25**(1): 281. 1986.

Synonym: *Preussia japonica* (Udagawa *et al.*) D.W. Li & N.P. Schult., *Fungal Biol.* **130** (1, no. 101691): 15. 2025.

Typus: Japan, Yamaguchi, Kagawa-ninowari, Yamaguchi-ken Chubu livestock Hygiene service Center, isolated from bronchiole lesions on domestic cow (*Bos taurus* var. *domesticus*), Jun. 1984, K. Tominaga (**holotype** NHL 2954, ex-type strain CBS 494.88).

Description and illustration: Udagawa *et al.* (1986).

Additional descriptions and illustrations: De Hoog *et al.* (2020).

Notes: *Sporormiella japonica* is an opportunistic pathogen originally described as *Scytalidium japonicum* from bronchiole lesions on a domestic cow (Udagawa *et al.* 1986, De Hoog *et al.* 2020). The basionym was recently transferred to *Preussia* based on phylogenetic analyses; however, the authors acknowledged the complex taxonomy of *Preussia* s.l. and the need for further studies (Li *et al.* 2025). Although this species is not represented in Figs 2 and 3, an additional LSU-ITS analysis (Supplementary material Fig. S11) allowed us to resolve the taxonomic position of the type strain (CBS 494.88) as a member of *Sporormiella*, forming an independent lineage within the *Sm. minima* species complex. *Sporormiella japonica* is characterized by producing holothallic, cylindrical, 0–1-septate, hyaline arthroconidia with truncated ends, which may sometimes be clavate to pyriform, as well as dark brown chlamydospores arranged in chains (Udagawa *et al.* 1986). This species represents the first report of holoarthric conidiogenesis in *Sporormiella*.

Sporormiella jejuensis H. Noh, H.U. Cho & S.H. Kim ex Guerra-Mateo, Gené & Cano, **sp. nov.** MB 862066.

[originally described as *Preussia jejuensis* H. Noh *et al.*, *Mycobiology* **53**(2): 209. 2025. **nom. inval.**, Art. F.5.2 (Madrid)].



Typus: Republic of Korea, Jeju Island, Seopjikoji (33°25'48.5"N 126°56'03.5"E), on horse dung, Oct. 2023, unknown collector (**holotype** NIBRFGC000512615, ex-type strain DUC 15669).

Description and illustration: Noh et al. (2025).

Notes: The species *Pr. jejuensis* was invalidly published without citing an identifier for the new name (Noh et al. 2025). Herein, we validated this lineage as a new species of *Sporormiella*, sister to *Sm. isopartis* and *Sm. minimoides* (Figs 2, 3). *Sporormiella jejuensis* is characterized by the production of pycnidia, while the asexual morph remains unknown in the other two species (Cain 1961, Kruys & Wedin 2009).

Sporormiella koreensis H. Noh, H.U. Cho & S.H. Kim ex Guerra-Mateo, Gené & Cano, **sp. nov.** MB 862067.

[originally described as: *Preussia koreensis* H. Noh et al., *Mycobiology* 53(2): 210. 2025. *nom. inval.*, Art. F.5.2 (Madrid)].

Typus: Republic of Korea, Jeju Island, Seopjikoji (33°25'48.5"N, 126°56'03.5"E), on horse dung, Oct. 2023, unknown collector (**holotype** NIBRFGC000512614, ex-type strain DUC 15668).

Description and illustration: Noh et al. (2025).

Additional materials examined: Panama, Santa Rosa, on goat dung, Apr. 1948, S.L. Mayer (CBS 524.50). South Africa, on *Podocarpus falcatus* fruit, unknown collection date, U. Damm (CBS 120888). Spain, Catalonia, Mediterranean coast, Estarlit, discharging area of the Ter River, from sediments at 27 m depth, Oct. 2023, P. Rojas & D. Guerra-Mateo (FMR 21125).

Notes: The species *Pr. koreensis* was invalidly published without citing an identifier for the new name (Noh et al. 2025). Our phylogenetic analyses confirmed this taxon as a member of *Sporormiella*, forming an independent lineage within the *Sm. minima* species complex and we thus validate the name here (Figs. 2, 3). Morphological differences among members of this group are subtle and *Sm. koreensis* is primarily distinguished through phylogenetic analyses.

Sporormiella lignicola (W. Phillips & Plowr.) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862068.

Basionym: *Sporormia lignicola* W. Phillips & Plowr., *Grevillea* 6 (37): 29. 1877.

Synonyms: *Preussia lignicola* (W. Phillips & Plowr.) Kruys, *Syst. Biodiv.* 7 (4): 476. 2009.

Preussia mariae D.C. Sandberg & A.E. Arnold, *Pl. Fungal Syst.* 67 (2): 70. 2022, *syn. nov.*

Typus: England, Norfolk, King's Lynn, Tilney St Lawrence, from wood of *Fraxinus excelsior*, Mar. 1877, unknown collector (**holotype** K(M) 131910).

Description and illustration: Phillips & Plowright (1872).

Additional materials examined: Ethiopia, from seeds of wheat, unknown collection date and collector (CBS 110384). Netherlands, Texel, De Muy area, on rabbit dung, May 1969, unknown collection date, G.S. de Hoog (CBS 363.69; proposed here as reference strain); *ibid.*, Texel, De Geul area, unknown collection date and collector (CBS 264.69). Spain, Granada, from leaves of *Retama sphaerocarpa*, Mar. 2012, G.F. Bills (CF-279765).

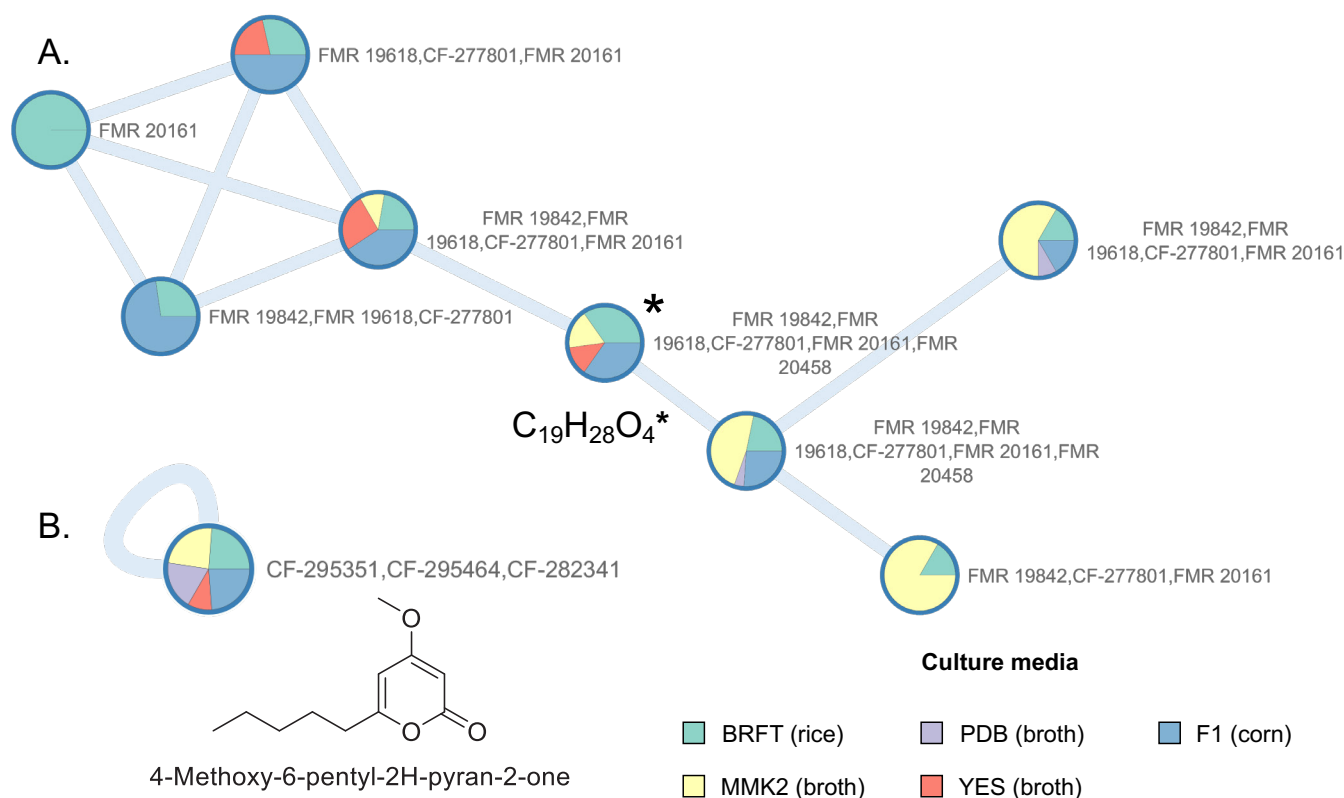


Fig. 6. GNPS networks representing different clusters together with the strains that produced them. The most abundant compounds are highlighted with *. **A.** C₁₉H₂₈O₄. **B.** 4-methoxy-6-pentyl-2H-pyran-2-one.

Notes: *Sporormia lignicola* was transferred to *Preussia* based on phylogenetic analyses using the strain CBS 363.69 (Kruys & Wedin 2009). Although those authors considered the genera *Preussia* and *Sporormiella* as synonyms, they stated that this species displayed the morphological characters of *Sporormiella*. Our phylogenetic analyses confirmed this strain as a member of the latter genus (Figs 2, 3).

We examined the holotype of *Sp. lignicola* and could see that the morphological features of the species match those of CBS 363.69, confirming the identity of the strain (Supplementary material Fig. S4). We tried to obtain DNA from the herborized material but, unfortunately, poor quality hampered further analyses. Therefore, we propose CBS 363.69 as a reference strain to stabilize nomenclature until suitable material is found for epityfication.

In previous works, the *Sm. lignicola* clade was referred to as a species complex (González-Menéndez *et al.* 2017, Sandberg *et al.* 2022). However, our phylogenetic analyses displayed a single taxon with minor intraspecific variability (Fig. 3). Moreover, the ex-type strain of the recently described species *Pr. mariae* (Sandberg *et al.* 2022) clustered in this lineage with a 99 % ITS identity with CBS 363.69. Therefore, we consider this species a synonym of *Sm. lignicola*.

Sporormiella mediterranea (Arenal *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862069.

Basionym: *Preussia mediterranea* Arenal *et al.*, *Fungal Diversity* **25**: 10. 2007.

Synonym: *Preussia mayoae* Y.P. Tan *et al.*, *Index Austral. Fungi* **42**: 14. 2024, *syn. nov.*

Typus: **Spain**, Cáceres, Monfragüe National Park, from leaves of *Cistus albidus*, Nov. 1996, F. Arenal (**holotype** AH 32774, ex-type strain S23).

Description and illustration: Arenal *et al.* (2007).

Additional materials examined: **Netherlands**, Texel, on sheep dung, Oct. 1969, unknown collector (CBS 263.69). **UK**, no additional data available (CBS 338.35).

Notes: The ex-type strain of *Pr. mediterranea* clustered in the *Sporormiella* clade, forming an independent lineage within the *Sm. minima* species complex (Figs 2, 3). Although morphological features are conserved among members of this group, *Sm. mediterranea* differs by producing the largest ascospores in the group, 32–50 × 6–10 µm (Arenal *et al.* 2007). In addition, our phylogenetic analysis resolved *Pr. mayoae* as conspecific with *Sm. mediterranea* (Fig. 2). Of note is that Tan *et al.* (2024) avoided the morphological characterization of this species and reported a 98 % ITS similarity to other members of the *Sm. minima* complex.

Sporormiella minimoides S.I. Ahmed & Cain, *Canad. J. Bot.* **50** (3): 450. 1972.

Synonym: *Preussia minimoides* (S.I. Ahmed & Cain) Valldos. & Guarro, *Bol. Soc. Micol. Madrid* **14**: 88. 1990.

Typus: **Canada**, Ontario, Algoma District, near Aubinadong River, on wolf dung, Aug. 1960, R.F. Cain (**holotype** TRTC 36242).

Description and illustration: Ahmed & Cain (1972).

Notes: *Sporormiella minimoides* was considered a member of *Preussia*, accepting both genera as synonyms (Valldosera & Guarro 1990). This species is currently represented in phylogenetic analyses through two strains, S10 and S18 (Arenal *et al.* 2005). Here, we determined these strains as members of *Sporormiella* as originally stated in the protologue (Figs 2, 3).

Sporormiella minipascua S.I. Ahmed & Cain, *Canad. J. Bot.* **50** (3): 451. 1972.

Synonym: *Preussia minipascua* (S.I. Ahmed & Cain) J.H. Chang & Y.Z. Wang, *Nova Hedwigia* **88** (1–2): 250. 2009.

Typus: **Canada**, Ontario, Nipissing District, Pickerel Lake Portage from Lake Timagami, on deer dung, Aug. 1933, R.F. Cain (**holotype** TRTC 5390).

Description and illustration: Ahmed & Cain (1972).

Notes: *Sporormiella minipascua* was transferred to *Preussia* based on the phylogenetic analysis of the strain UPS: 306, accepting both genera as synonyms (Kruys & Wedin 2009). However, our analyses confirmed this strain as a *Sporormiella* species (Figs 2, 3).

Sporormiella pascua (Niessl) S.I. Ahmed & Cain, *Canad. J. Bot.* **50**(3): 454. 1972.

Basionym: *Sporormia pascua* Niessl, *Oesterr. bot. Z.* **28**: 45. 1878.

Synonym: *Preussia pascua* (Niessl) Valldos. & Guarro, *Bol. Soc. Micol. Madrid* **14**: 88. 1990.

Typus: **Czech Republic**, Brno, Lellekowitz, on sheep dung, Jun. 1874, Niessl (**holotype** M-0378428).

Description and illustration: Ahmed & Cain (1972).

Additional material examined: **Spain**, Balears Islands, Palma de Mallorca, Secar de la Real, on horse dung, Mar. 2021, J.F. Cano-Lira (FMR 19091).

Notes: *Sporormiella pascua* is a poorly represented species in living cultures. The best representative is the strain NBRC 30183, which was identified based on morphological features (Ahmed & Cain 1972; Udagawa & Furuya 1976). Unfortunately, only the LSU sequence is available for phylogenetic analyses (Supplementary material Table S1). Here, we also included the strain FMR 19091, which remained sterile in all culture media. Although the type specimen of this species is well preserved in the Bavarian State Collection for Botany in Munich (M-0378428), no living culture is available for sequencing. We chose to retain this lineage as *Sm. pascua* based on the phylogenetic placement of NBRC 30183 within *Sporormiella* (Fig. 2), which is consistent with the morphological features of the holotype (Supplementary material Fig. S5). Future studies should aim to stabilize the taxonomy of this species by incorporating additional metabolically active strains and barcode sequences.

Sporormiella persica (Asgari & Zare) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862070.

Basionym: *Preussia persica* Asgari & Zare, *Nova Hedwigia* **90** (3–4): 541. 2010.

Synonym: *Preussia arizonica* D.C. Sandberg & A.E. Arnold, *Pl. Fungal Syst.* **67** (2): 66. 2022, *syn. nov.*



Typus: Iran, East Azerbaijan, Sarab, from dead barley leaves, Jan. 2003, *B. Asgari* (**holotype** IRAN 12397 F, ex-type strain CBS 117680).

Description and illustration: Asgari & Zare (2010).

Additional materials examined: **Germany**, Giessen, from colon content of freshly shot deer, Jun. 1975, *R. Hadlok* (CBS 471.75). **Papua New Guinea**, Northern Prov., Owen Stanley Range, Myola, near guesthouse along Iora creek, on sheep dung, Oct. 1995, *A. Aptroot* (CBS 767.96). **Spain**, Catalonia, Mediterranean coast, Estarlit, discharging area of the Ter River, from sediments at 27 m depth, Oct. 2023, *P. Rojas & D. Guerra-Mateo* (FMR 21126). **Venezuela**, Monagas state, Caripe, on donkey dung, Jul. 1978, *J.C. Krug* (CBS 442.78).

Notes: Our phylogenetic analyses revealed *Pr. persica* as a member of *Sporormiella*, forming an independent lineage within the *Sm. minima* species complex (Figs 2, 3). Although morphological features are conserved among members of this group, *Sm. persica* differs by producing ascomata with up to three necks (Asgari & Zare 2010). Moreover, the ex-type strain of the recently described species *Pr. arizonica* (Sandberg *et al.* 2022) clustered in this lineage with a 99 % ITS identity with CBS 117680. Therefore, we consider this species a synonym of *Sm. persica*.

Sporormiella procaviae (Crous) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862071.

Basionym: *Preussia procaviae* Crous, *Persoonia* **45**: 321. 2020.

Synonyms: *Preussia crataegi* Eivsand & M. Mehrabi, *Antonie van Leeuwenhoek* **118** (52): 6. 2025, *syn. nov.*

Preussia pianica Eivsand & M. Mehrabi, *Antonie van Leeuwenhoek* **118** (52): 10. 2025, *syn. nov.*

Preussia pistaciae Eivsand & M. Mehrabi, *Antonie van Leeuwenhoek* **118** (52): 12. 2025. *svn. nov.*

Typus: Namibia, Gobabeb-Namib Research Institute, Mirabib Rock, on dung of *Procavia capensis*, Nov. 2019, *P.W. Crous* (**holotype** CBS H-24446, ex-type strain CBS 146827).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–2 µm wide hyphae. **Sexual morph** not observed. **Coelomycetous asexual morph:** *Conidiomata* pycnidial, solitary or aggregated in clusters of 2- to several pycnidia, immersed to erumpent, rarely superficial or on aerial mycelium, pale brown to brown, globose, sub-globose or ellipsoidal, sometimes irregular in shape, glabrous or with some hyphal outgrowths, 22–200 µm diam., papillate or non-papillate, ostiolate, with 1–4 ostioles, 10 µm diam.; conidiomatal wall of 3–6 layers, formed by brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 4–5 × 2.5–3 µm, hyaline, smooth-walled. *Conidia* aseptate, solitary, ellipsoid with obtuse ends, 3–4 × 2 µm, hyaline, smooth, with one to two guttules. *Chlamydospores* unicellular, solitary or sometimes in short chains, intercalary or terminal, globose to ellipsoidal or variable in shape, 4–14 × 4–11 µm, grey to brown, thick- and smooth-walled. (Adapted from Crous *et al.* 2020, Eivsand & Mehrabi-Koushki 2025).

Culture characteristics (after 14 d at 25 °C). Colonies on PDA reaching 50 mm diam., flat, surface folded, with sparse aerial mycelium or floccose in the centre, olivaceous grey to cream towards the margin, margin lobate, smooth to filiform; reverse iron-grey to cream. On OA, 50 mm diam., flat, with sparse aerial mycelium or floccose to cottony, pale brown with cream margin, often yellow-pigmented, with paler concentric zones of sporulation; reverse brown to cream.

Notes: The ex-type strain of *Pr. procaviae* clustered with the recently described species *Pr. crataegi*, *Pr. pianica*, and *Pr. pistaciae* (Eivsand & Mehrabi-Koushki 2025), forming a well-supported clade

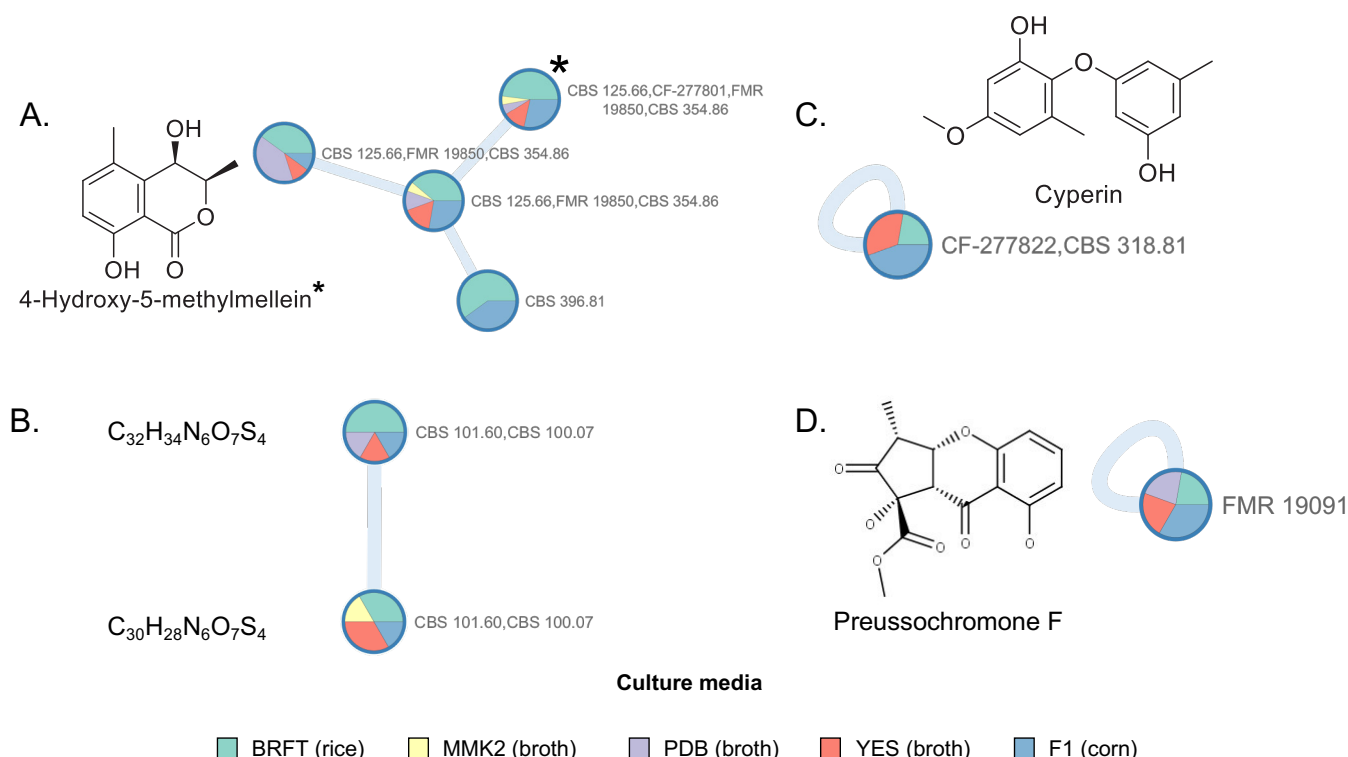


Fig. 7. GNPS networks representing different clusters together with the strains that produced them. The most abundant compounds are highlighted with *. **A.** 4-hydroxy-5-methylmellein. **B.** Leptosin analogues **C.** Cyperin. **D.** Preussochromone F.

in *Sporormiella* (Figs 2, 3). The ITS barcode sequences of the four species showed a 100 % of identity. In addition, the original authors of those species also provided partial sequences of the *tef1-α* region, revealing a 99 % of identity among them; therefore, they are considered conspecific. Since *Pr. procaviae* is the earliest described species, it is proposed as a new combination into *Sporormiella*. Here, we provide an updated description of *Sm. procaviae*, incorporating subtle morphological variations reported in the protologues of the synonymised species (Eisvand & Mehrabi-Koushki 2025).

Sporormiella procaviicola (Crous) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862072.

Basionym: *Preussia procaviicola* Crous, *Persoonia* **46**: 387. 2021.

Synonym: *Preussia apgariae* Y.P. Tan *et al.* [as ‘*apgarae*’], *Index Austral. Fungi* **42**: 13. 2024, *syn. nov.*

Typus: **Namibia**, Gobabeb Namib Research Institute, Mirabib, on dung of *Procavia capensis*, Nov. 2019, P.W. Crous (**holotype** CBS H-24493, ex-type strain CBS 146981).

Description and illustration: Crous *et al.* (2021).

Additional material examined: **Netherlands**, Texel, De Muy area, from rabbit dung, Oct. 1968, G.S. de Hoog (CBS 364.69).

Notes: *Preussia procaviicola* and *Pr. apgariae* clustered together forming a well-supported clade in *Sporormiella* (Fig 2). We consider this clade to represent a single species that is proposed as *Sm. procaviicola*. It is of note that the authors of *Pr. apgariae* did not provide a morphological characterization and reported a 99 % ITS identity with *Pr. procaviicola* (Tan *et al.* 2024).

Sporormiella similis R.S. Khan & Cain, *Canad. J. Bot.* **57**(10): 1178. 1979.

Synonym: *Preussia similis* (R.S. Khan & Cain) Arenal, *Mycotaxon* **89**(1): 140. 2004.

Typus: **Kenya**, Lake Amboseli, 2°33'S, 37°10'E, on rabbit dung at 1200 m above sea level, Aug. 1966, R.F. Cain, *et al.* (**holotype** TRTC 66.1777a).

Description and illustration: Khan & Cain (1979).

Additional materials examined: **Kuwait**, from saline desert soil, Sep. 1973, A.F. Moustafa (CBS 804.73); *ibid.*, (CBS 805.73). **Spain**, Catalonia, Mediterranean coast, Tarragona, Miracle Beach, from sediments at 27 m depth, Jun. 2021, G. Quiroga-Jofre & D. Guerra-Mateo (FMR 19464). **USA**, Wyoming, Albany Co., Bamforth Lake, from saline soil, May 1975, D.E. Davidson (CBS 272.75).

Notes: *Sporormiella similis* was transferred to *Preussia* based on the morphological features of the strain S19, which was later used for phylogenetic analyses (Arenal *et al.* 2004, Kruijs & Wedin 2009). However, our phylogenetic analyses confirmed this strain as a member of *Sporormiella* (Fig 2). In addition, the ex-type strain of the recently described species *Pr. iranica* (IRAN 4962C; Eisvand & Mehrabi-Koushki 2025) clustered into this lineage with a 98.5 % identity to S19. These two species seem to be conspecific, but due to the lack of DNA sequence data for the type of *Sm. similis*, further studies are necessary to confirm this synonymy. Therefore, *Pr. iranica* is included in this work among the list of *Sporormiella* species with uncertain status (Supplementary material Table S3).

Sporormiella solicola Guerra-Mateo, Cano & Gené, **sp. nov.** MB 862040.

Etymology: Referring to the substrate where the species has been reported, soil.

Typus: **USA**, Alaska, from soil, Jun. 1971, P.W. Flanagan (**holotype** CBS H-18421, ex-type strain CBS 468.71 = FMR 21346).

Culture sterile. *Sporormiella solicola* differs from *Sm. bipartis*, represented by the culture: S: 17250-a, by unique fixed alleles in two of the loci studied. ITS, 16 positions: 94 (gap), 95 (gap), 98 (A), 103 (A), 122 (A), 130 (A), 134 (A), 139 (gap), 345 (C), 349 (T), 357 (T), 390 (A), 429 (A), 430 (C), 435 (C), 437 (T). LSU, six positions: 99 (T), 411 (T), 431 (C), 432 (C), 449 (T), 451 (T). It differs from *Sm. australis* (culture: CBS 806.73) by unique fixed alleles in four of the loci studied. ITS, 33 positions: 58 (gap), 97 (A), 108 (C), 120 (T), 121 (A), 133 (A), 141 (gap), 226 (A), 328 (T), 344 (C), 350–353 (gap), 358–363 (gap), 366 (T), 381 (A), 399 (A), 426 (gap), 435 (C), 436 (C), 438 (G), 439 (A), 443 (A), 444 (T), 447 (T), 454 (G). LSU, 5 positions: 432 (C), 440 (T), 450 (T), 455 (T), 456 (A). *tef1-α*, 32 positions: 22 (C), 43 (T), 46 (C), 121 (C), 124 (C), 128 (C), 130 (G), 154 (C), 220 (C), 235 (C), 238 (C), 243 (G), 265 (C), 284 (T), 298 (C), 304 (C), 346 (C), 382 (G), 418 (A), 430 (T), 487 (A), 499 (C), 521 (G), 522 (T), 664 (T), 697 (C), 715 (C), 730 (G), 760 (T), 763 (C), 775 (T), 811 (T). *rpb2*, 132 positions: 9 (A), 12 (G), 42 (A), 48 (C), 57 (C), 66 (G), 69 (G), 72 (T), 78 (A), 84 (C), 90 (T), 99 (T), 102 (A), 129 (T), 141 (C), 144 (G), 153 (C), 156 (T), 177 (C), 183 (T), 184 (A), 186 (C), 195 (T), 201 (C), 207 (C), 222 (T), 231 (T), 235 (C), 237 (T), 246 (C), 249 (C), 252 (G), 255 (A), 258 (G), 264 (T), 279 (C), 282 (C), 288 (C), 294 (G), 303 (T), 315 (T), 318 (C), 319 (T), 320 (C), 321 (A), 331 (T), 333 (T), 336 (G), 339 (G), 348 (C), 351 (G), 354 (G), 363 (G), 375 (G), 384 (T), 385 (A), 387 (C), 390 (C), 405 (T), 423 (C), 426 (T), 429 (C), 432 (T), 444 (C), 448 (T), 450 (G), 453 (T), 456 (G), 459 (G), 462 (A), 464 (C), 465 (G), 467 (A), 471 (A), 474 (T), 475 (A), 477 (A), 479 (C), 481 (gap), 483 (A), 484 (C), 490 (A), 491 (G), 493 (T), 498 (T), 499 (A), 508 (G), 511 (A), 517 (T), 518 (G), 519 (G), 521 (T), 522 (A), 523 (T), 529 (C), 531 (C), 532 (T), 535 (C), 537 (A), 541 (C), 543 (T), 544 (T), 550 (C), 558 (A), 562 (T), 565 (C), 574 (G), 579 (T), 580 (C), 581 (A), 583 (G), 592 (A), 601 (A), 604 (C), 611 (A), 612 (G), 613 (T), 622 (T), 625 (G), 637 (C), 649 (G), 661 (T), 670 (T), 673 (G), 674 (G), 684 (C), 685 (G), 688 (A), 693 (A), 697 (A), 700 (C), 706 (A).

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 28–38 mm diam., slightly elevated, crateriform, glabrous, radially sulcate, yellowish white (2A2), margin slightly lobated and entire; reverse concolourous. On OA, 35–37 mm diam., flat, glabrous, colourless, pale red (11A3) to colourless in CF-155367, margin diffusing in medium; reverse colourless. On CMA, 45 mm diam., flat, glabrous, colourless, margin diffusing in medium; reverse colourless.

Additional material examined: **Spain**, from soil, 2002, unknown collector (CF-155367 = FMR 21494).

Cardinal temperatures for growth: Minimum 5 °C (10 mm), optimum 25 °C (36–38 mm), maximum 30 °C (1 mm).

Secondary metabolite: C₁₆H₁₂O₆, (possible 2,8-dihydroxy-3-methylxanthone-1-carboxylic acid).

Notes: We crossed the strains CBS 468.71 and CF-155367 in culture, but unfortunately, no sporulation was obtained in any of the media tested. Strain CBS 468.71 was in the past morphologically identified as *Sm. australis*. This suggests that at that time the culture produced 4-celled ascospores resembling *Sm. australis*. However, our phylogenetic analyses revealed these strains as different taxa (Figs 2, 3). In addition, CBS 468.71 and CF-155367 produced $C_{16}H_{12}O_6$, a possible 2,8-dihydroxy-3-methylxanthone-1-carboxylic acid that has not been detected in other *Sporormiella* species. Based on this data, we propose both strains as representatives of a novel *Sporormiella* species.

Clade 2. *Sporormyces* Guerra-Mateo, Gené & Cano, **gen. nov.** MB 862043.

Etymology: Referring to the morphological similarity to *Sporormiella* and Greek *múkēs* (μύκης), fungus.

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled hyphae. **Sexual morph** perithecial, immersed to semi-immersed, solitary or aggregated in small groups, subglobose to nearly pyriform, glabrous; peridium thin, membranaceous, dark brown to nearly black, with cells of *textura angularis*; neck small, papilliform. **Paraphyses** filiform, hyaline, longer than asci. **Asci** 8-spored, bitunicate, clavate, tapering into a short stipe. **Ascospores** bi- or tri-seriate above, uni- or bi-seriate below, subcylindrical, 7-celled, thick-walled, septa transverse, constrictions at septa moderate, segments separable, rounded at the ends, hyaline at first, ranging through yellowish brown to dark brown when mature; third cell from the upper end larger than the remaining cells; lower end cells longer than the other cells; germ slit oblique; gelatinous sheath hyaline, narrow. **Asexual morph** not observed.

Type species: *Sporormyces septenarius* (S.I. Ahmed & Cain) Guerra-Mateo, Gené & Cano

Habitat: Dung, soil, and plant endophytes.

Notes: *Sporormyces* represents a lineage sister to *Sporormiella* (Fig. 3). The morphological features of *Sporormyces* overlap with those displayed by different species of *Sporormiella*. Despite this, these genera can be distinguished through phylogeny (Figs 2, 3) and metabolite production, with *Sporormyces* producing anthraquinones and 4-methoxy-6-pentyl-2H-pyran-2-one, compounds that have not been detected across species of *Sporormiella* (González-Menéndez *et al.* 2017). Unfortunately, the second species included in *Sporormyces* remained sterile.

Sporormyces anthraquinoniformans Guerra-Mateo, González-Menéndez & Cano, **sp. nov.** MB 862045.

Etymology: Referring to the production of 1,7-dihydroxy-3-(hydroxymethyl) anthraquinone.

Typus: **Spain**, Andalusia, Granada, Escúzar quarry, from gypsum soil associated with *Squamarina lentigera*, Nov. 2016, V. González-Menéndez (holotype FMR H-20558, ex-type (CF-295351 = FMR 20558).

Culture sterile. *Sporormyces anthraquinoniformans* differs from its closest phylogenetic species *Sporormyces septenarius* (specimen:

S: 00036) by unique fixed alleles in two of the loci studied. ITS, 31 positions: 45 (gap), 54 (G), 56 (C), 83 (A), 89 (C), 90 (A), 95 (A), 100–103 (gap), 105 (T), 106 (T), 108 (T), 112 (C), 116 (gap), 131 (A), 139 (C), 141 (A), 306 (T), 335 (C), 345 (T), 351 (C), 352 (G), 356 (G), 358 (T), 426 (G), 428 (G), 433 (T), 434 (T), 439 (T). LSU, 12 positions: 96 (C), 101 (G), 166 (C), 171 (G), 382 (A), 387 (T), 390 (C), 394 (A), 401 (T), 430 (C), 431 (C), 433 (A).

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 42–60 mm diam., crateriform, radially sulcate, velvety, white (1A1), margin fimbriate, slightly lobated; reverse concolourous. On OA, 50–65 mm diam., flat, velvety, white (1A1), margin submerged, entire, slightly lobated; reverse colourless. On CMA, 38–50 mm diam., flat, velvety, white (1A1), margin submerged, entire, slightly lobated; reverse colourless.

Additional materials examined: **Spain**, Andalusia, Granada, Alhendin business park, from *Ditrichia viscosa*, Jul. 2012, V. González-Menéndez (CF-282341); *ibid.*, Escúzar quarry, from gypsum soil associated with *Squamarina lentigera*, Nov. 2016, V. González-Menéndez (CF-295464 = FMR 20557).

Cardinal temperatures for growth: Minimum 5 °C (6–8 mm), optimum 25 °C (42–60 mm), maximum 35 °C (1 mm).

Secondary metabolites: 4-Methoxy-6-pentyl-2H-pyran-2-one, 1,7-dihydroxy-3-(hydroxymethyl) anthraquinone, SPF 32629B.

Notes: The three strains representing *Sc. anthraquinoniformans* were crossed *in vitro* on different media, but cultures remained sterile. This species can be distinguished through phylogenetic analyses and is characterized by the production of 4-methoxy-6-pentyl-2H-pyran-2-one (Figs. 2, 3). The ex-type strain of the species, CF-282341, was also reported as a producer of SPF 32629B and 7-dihydroxy-3-(hydroxymethyl) anthraquinone (González-Menéndez *et al.* 2017). Future studies should address the morphological description of the new fungus.

Sporormyces septenarius (S.I. Ahmed & Cain) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862073. Fig. 8.

Basionym: *Sporormiella septenaria* S.I. Ahmed & Cain, *Canad. J. Bot.* **50**: 458. 1972.

Typus: **Mexico**, Villa Hidalgo, on dung of *Capra hircus*, Aug. 1960, R.F. Cain (holotype TRTC 36550).

Description adapted from Ahmed & Cain (1972) and the exsiccatal specimen of S: 00036. **Mycelium** immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–2 µm wide hyphae. **Sexual morph:** **Ascomata** perithecial, immersed to semi-immersed, solitary or aggregated in small groups, subglobose to nearly pyriform, glabrous, 270–310 × 225–250 µm; peridium thin, membranaceous, dark brown to nearly black, with cells of *textura angularis*; neck small, papilliform. **Paraphyses** filiform, hyaline, longer than asci. **Asci** 8-spored, bitunicate, clavate, tapering into a short stipe, 130–160 × 18–20 µm. **Ascospores** bi- or tri-seriate above, uni- or bi-seriate below, subcylindrical, 43–54 × 8–10 µm, 7-celled, thick-walled, septa transverse, constrictions at septa moderate, segments separable, rounded at the ends, hyaline at first, ranging through yellowish brown to dark brown when mature; third cell from the upper end larger than the remaining cells, 7–8 × 8.5–10 µm; lower end cell longer than the other cells, 10–11.5



× 6.7–7 µm; germ slit oblique; gelatinous sheath hyaline. Asexual morph not observed.

Additional material examined: **Argentina**, Río Negro, Pilcomayo, on sheep dung, Feb. 1987, L. Espigores (S: 00036; proposed here as reference material).

Notes: Sequences of the Argentinian specimen, S: 00036, have been used to represent *Sm. septenaria* in previous phylogenetic analyses (Kruys & Wedin 2009, González-Menéndez et al. 2017). Notably, no living cultures of this species are available in any public collections. In our phylogeny, this specimen was resolved as phylogenetically distant from the genus *Sporormiella* (Fig. 3); in



Fig. 8. *Sporormyces septenarius* (S: 00036). **A.** Fungarium label and collection data. **B.** Preserved specimen. **C.** Perithecia. **D.** Peridium. **E.** Asci and paraphyses. **F.** Ascus. **G, H.** Ascospores at the ascus bottom. **I.** Released ascospore. Scale bars: D, G–I = 10 µm; E, F = 20 µm.

fact, it represents a novel genus as proposed here. We examined the exsiccate deposited in the Swedish Museum of Natural History, and confirmed that its morphology exactly matches the protologue, including shape and arrangement of the ascospore cells (Fig. 4). Although no culture exists for the specimen, DNA sequence data are available in GenBank (Supplementary material table S1). Therefore, sequence data together with the fungarium material of S: 00036 is designated as reference material for the species, and we introduce the new combination *Sc. septenarius*. Further collections from the type locality are needed for nomenclatural stabilization of the species.

Clade 3. *Pleospormiella* Guerra-Mateo, Gené & Cano, **gen. nov.** MB 862049.

Etymology: Referring to the morphological resemblance of the sexual morph to the genus *Sporormiella* and the production of different asexual morphs.

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled hyphae. **Sexual morph:** *Ascomata* perithecial or cleistothecial, solitary or aggregated, immersed to semi-immersed, globose to subglobose; peridium with polyhedral to isodiametric cells (*textura angularis* and *globosa*) and slightly coriaceous; neck short, papilliform, to rostrate. *Paraphyses* filiform, sometimes branched, hyaline, and longer than asci. *Asci* 8-spored, bitunicate, subcylindrical to clavate, tapering into a short stipe. *Ascospores* bi- to tetra-seriate, subcylindrical, 4-celled, thick-walled, transversely septate, deeply constricted, cells subcylindrical to subglobose, at first hyaline, dark brown in maturity; germ slit parallel to slightly diagonal; gelatinous sheath hyaline, subglobose, surrounding each ascospore. **Asexual morph:** pycnidial, humicola-like or chrysosporium-like. *Pycnidia* solitary, immersed to erumpent, brown, globose, with a central ostiole; wall brown, with cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, hyaline, smooth. *Conidia* aseptate, ellipsoid with obtuse ends, hyaline, smooth, guttulate, aggregated in mucous masses. *Humicola*-like *conidiophores* usually reduced to conidiogenous cells that laterally extend to form a single conidium, sessile or arising from a short lateral pedicel on the hyphae. *Conidiogenous cells* intercalary, lateral or terminal, integrated, monoblastic, cylindrical or slightly swollen as doliiform, hyaline. *Conidia* solitary, sometimes forming clusters, terminal or lateral, 1–2-celled, or multi-celled, olivaceous brown with hyaline to sub-hyaline basal cell, sometimes upper cell tightly coiled in three dimensions resembling dictyospores; conidial secession rhexolytic. *Chrysosporium*-like *conidiophores* reduced to conidiogenous cells arising laterally or on branched supporting cells. *Conidiogenous cells* holoblastic. *Conidia* solitary, clavate to obovate, smooth, hyaline, with a truncated base.

Type species: *Pleospormiella compacta* Guerra-Mateo, González-Menéndez, Gené & Cano

Habitat: Primarily on soil and marine sediments, but also on dung, on dead leaves, or as endophytes.

Notes: *Pleospormiella* represents a well-supported, independent lineage in *Sporormiaceae* (Fig. 3). This genus is characterized by short-stipitate asci with 4-celled ascospores, and the production of three different asexual morphs, pycnidia, humicola-like and chrysosporium-like. Pycnidia resemble those produced by other

Preussia s.l. lineages, but differ on longer conidiogenous cells (6–10 µm vs 4–5 µm in *Sporormiella*). The chrysosporium-like morph has only been observed in *Preussia* s.str. (See below), displaying similar subglobose to obovoid conidia (Asgari & Zare 2010, Phurba *et al.* 2025). The humicola-like morph is reported here for the first time in association with the family *Sporormiaceae*. Our phylogeny reveals that *Pleospormiella* comprises seven species.

The strain UPS: 305 “*Preussia megalospora*” formed an additional independent lineage that probably represents another species of *Pleospormiella*. However, we could not examine this strain.

Pleospormiella aculeata Guerra-Mateo, Gené & Cano, **sp. nov.** MB 862051. Fig. 9.

Etymology: Latin “*aculeus*”, spine, referring to the spiny gelatinous sheath covering the conidia.

Typus: Netherlands, Rotterdam, from soil, Jan. 1938, N.V. Unilever (**holotype** CBS H-25952, ex-type strain CBS 217.38 = FMR 20561).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 2–4.5 µm wide hyphae. **Sexual morph** not observed. **Asexual morph** humicola-like. *Conidiophores* often reduced to conidiogenous cells that form lateral sessile conidia or arising from a short lateral pedicel on the hyphae, hyaline; sometimes slightly differentiated conidiophores present as branched supporting conidial cells. *Conidiogenous cells* intercalary, lateral or terminal, integrated, monoblastic, cylindrical or slightly swollen as doliiform, hyaline, smooth. *Conidia* solitary, 1–2-celled, 12–18 × 6–10 µm, hyaline at first, then upper cell dark brown and basal cell hyaline to subhyaline; upper cell, globose to subglobose, 8–11 × 6–10 µm, thick-walled, smooth, with gelatinous sheath forming mucilaginous spikes in mature conidia, hyaline; basal cell cylindrical or slightly swollen as doliiform, 4.5–7 × 3–6.5 µm, often remaining attached to the supporting cells.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 80–85 mm diam., slightly raised, velvety, medium grey (1E1) with violet brown (10E8) patches to white (1A1) at periphery, margin diffusing in the medium; reverse dark grey (1F1) to white (1A1). On OA, 80–85 mm diam., flat, velvety to glabrous, colourless, margin diffusing in medium; reverse colourless. On CMA, 85 mm diam., flat, velvety, greyish white (1B1) to colourless, margin diffusing in medium; reverse colourless.

Cardinal temperatures for growth: Minimum 5 °C (2 mm), optimum 30 °C (78 mm), maximum 37 °C (12–14 mm).

Secondary metabolite: Dihydroisoflavipucidin.

Notes: The strain representing *Ps. aculeata* was previously labelled as *Humicola brunnea* var. *brunnea* based on the morphology of the conidia. Previous phylogenetic analyses determined that an additional variety of this species, *Humicola brunnea* var. *africana*, was related to *Lophiotremataceae*; while the genus *Humicola* s.str. represented a member of *Chaetomiaceae* (Wang *et al.* 2019). *Pleospormiella aculeata* represents another case of misidentification based on the conserved and cryptic morphological features displayed by these fungi. Our phylogenetic analyses determined *Ps. aculeata* as a novel species in *Pleospormiella* (Figs 2, 3). This genus is characterized by the production of pycnidia, chrysosporium-like and humicola-



like asexual morphs. Another species in the genus with a similar humicola-like morph is *Ps. polyspora*. However, it produces tightly coiled conidia without sheath (Fig. 11), while *Ps. aculeata* exhibits subglobose conidia covered by a smooth or spiny gelatinous sheath (Fig. 9).

Pleosporormiella citrullina (R.M.F. Silva *et al.*) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862074.

Basionym: *Preussia citrullina* R.M.F. Silva *et al.*, *Persoonia* **40**: 313. 2018.

Typus: **Brazil**, Petrolândia municipality, Pernambuco state, as endophyte from leaves of *Citrullus lanatus* (*Cucurbitaceae*), Jul.

2016, R.M.F. Silva (**holotype** URM 91190, ex-type strain URM 7865).

Description and illustration: Crous *et al.* (2018).

Notes: *Pleosporormiella citrullina*, formerly known as *Pr. citrullina*, is only known to produce a pycnidial asexual morph (Crous *et al.* 2018). Although morphological features among pycnidia are conserved, *Ps. citrullina* can be distinguished through phylogenetic analyses, forming an independent and distant lineage in the genus (Fig. 3).

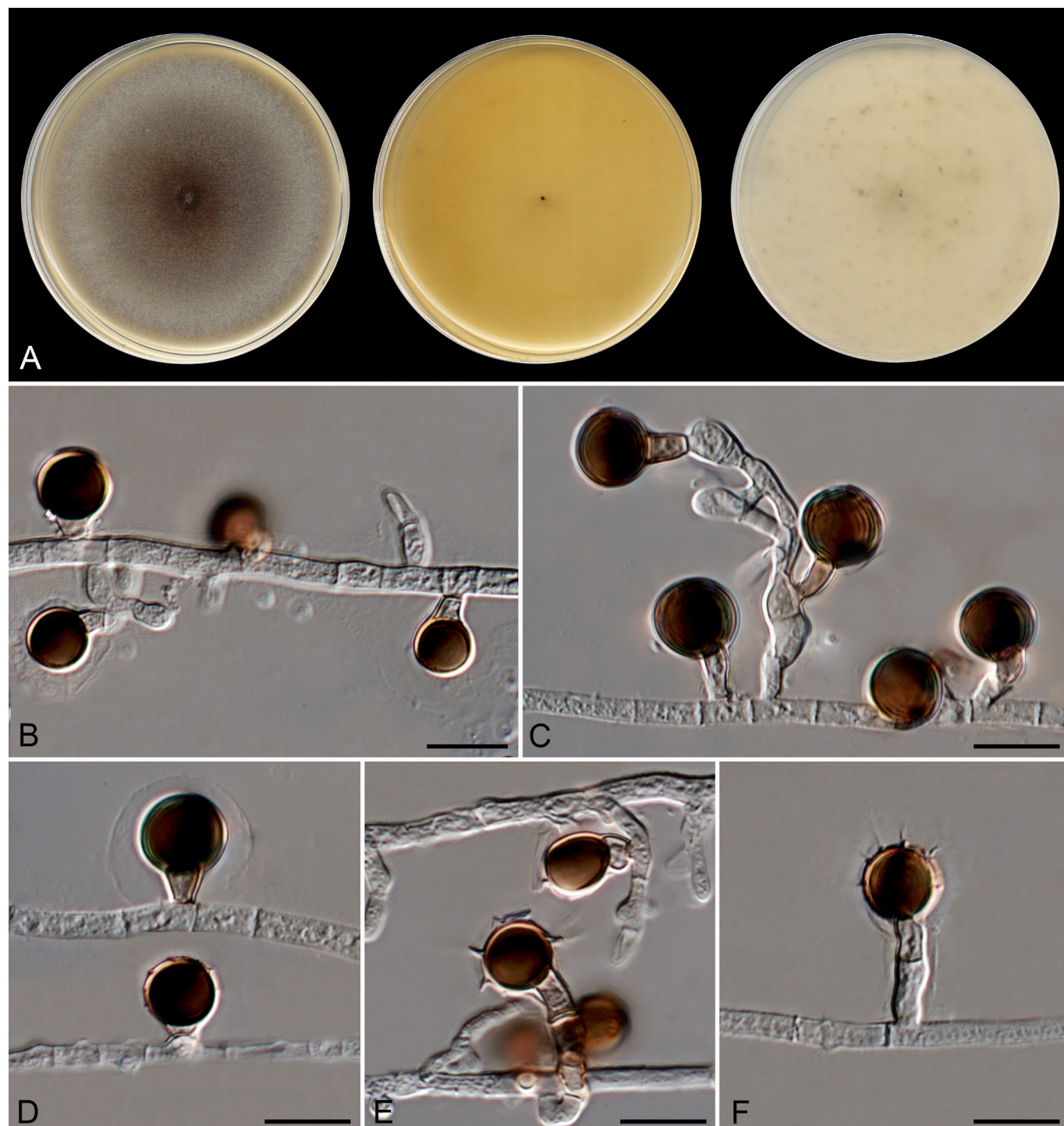


Fig. 9. *Pleosporormiella aculeata* (ex-type CBS 217.38). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B, C.** Conidiophore disposition. **D.** Sessile conidia. **E, F.** Conidia with spiky mucoid sheath. Scale bars = 10 µm.

Pleosporormiella compacta Guerra-Mateo, González-Menéndez, Gené & Cano, **sp. nov.** MB 862052. Fig. 10.

Etymology: Referring to the compacted disposition of the ascospores inside the asci.

Typus: **Spain**, Catalonia, Mediterranean coast, Tarragona, Platja de l'Arrabassada, from sediments at 20 m depth, Jun. 2022, G. Quiroga-Jofre & D. Guerra-Mateo (**holotype** CBS H-25953, ex-type strains FMR 20161 = CF-308079).

Mycelium immersed and superficial, composed of hyaline, septate, branched, smooth-walled, 1.5–2 µm wide hyphae. **Sexual morph:** **Ascomata** perithecial, solitary or aggregated, immersed to semi-immersed, subglobose, 270–500 × 230–360 µm, black, ostiole 55–80 µm diam.; peridium 5–8-layered, brown, with cells of *textura angularis*, 5–6 × 4–5 µm. **Paraphyses** filiform, sometimes branched, longer than asci, 1–1.5 µm wide, hyaline, smooth. **Asci** 8-spored, bitunicate, subcylindrical, often curved, 80–110 × 7–9 µm, with short stipe, 10 µm in length. **Ascospores** biseriate and compacted within asci, cylindrical, straight or slightly curved, 4-celled, transversely septate, septa constricted, broad and shallow, 36–42(–45) × 3.5–5 µm, hyaline at first then pale green, dark brown in maturity, thick- and smooth-walled; middle cells barrel-shaped with obtuse angles; terminal cells tapering apically in obtuse angles, generally longer than middle cells (10–13.5 vs 7–10 µm in length); germ slit parallel to slightly diagonal; gelatinous sheath hyaline. **Coelomycetous asexual morph:** **Conidiomata** pycnidial, solitary, immersed to erumpent, brown, globose, 90–150 µm diam., ostiolate, central ostiole 20–28 µm diam.; wall of 3–6 layers, brown, with cells of *textura angularis*. **Conidiophores** reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 6–8 × 2.5–3.5 µm, hyaline, smooth. **Conidia** aseptate, ellipsoid with obtuse ends, 2.5–4.5 × 2 µm, hyaline, smooth, 1-guttulate, aggregated in pink mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 16–28 mm diam., umbonate, velvety, pastel red (9A4) to yellowish white (4A2) and white (1A1) at periphery, margin entire, slightly lobated; reverse pinkish white (9A2) to white (1A1); in CF-277965 and CF-277801 radially sulcate, funiculate at centre to glabrous towards periphery, greyish orange (5B4) to brownish grey (5C2), white (1A1) at periphery and reverse orange grey (5B2). On OA, 23–30 mm diam., flat, aerial mycelium scarce, arranged in white threads of long aerial mycelium, colourless to pinkish white (9A2) over time, margin submerged, entire and slightly lobated; reverse concolourous. On CMA, 18–27 mm diam., flat, aerial mycelium scarce, arranged in long white hyphae, pinkish white (13A1) or yellowish grey (4B2), margin submerged, entire; reverse concolourous.

Additional materials examined: **Spain**, Catalonia, Mediterranean coast, Tarragona, Platja del Miracle, from sediments at 27 m depth, Jun. 2021, G. Quiroga-Jofre & D. Guerra-Mateo (FMR 19618); *ibid.*, Andalusia, Granada, Fuente Vaqueros, agricultural soil, Oct. 2010, J. Siles (CF-277801, CF-277965).

Cardinal temperatures for growth: Minimum 5 °C (4 mm), optimum 25 °C (16–28 mm), maximum 30 °C (4–10 mm).

Secondary metabolites: 4-Hydroxy-5-methylmellein, C₁₉H₂₈O₄, C₄₃H₇₉NO₉, C₁₉H₂₈O₅.

Notes: *Pleosporormiella compacta* represents a phylogenetic independent lineage that produces both a perithecial sexual morph and a pycnidial asexual morph. The sexual morph resembles those of other species with 4-celled, cylindrical ascospores distributed among *Preussia* and *Sporormiella*, like *Pr. constricta*, *Sm. capybarae*, *Sm. cylindrospora*, *Sm. ovina* and *Sm. teretispora*. Although the taxonomic position of these species remains obscure due to the lack of genetic barcodes for phylogenetic comparison, they differ in ascospore cell morphology. The above-mentioned species display ascospores with round-edge cells, while *Ps. compacta* is characterised by ascospores with barrel-shaped middle cells and terminal cells tapering apically in obtuse angles (Ahmed & Cain 1972, Guarro *et al.* 1997a). The morphology of the ascospore cells also distinguishes *Ps. compacta* from other species of *Pleosporormiella* that produce ascospores with round-edge cells, *Ps. polymorpha* and *Ps. terricola* (Cain 1961, Asgari & Zare 2010). In addition, these species differ in ascospore size, with *Ps. polymorpha* displaying bigger ascospores [45–50(–58) × 10.5–12.5 µm], followed by *Ps. compacta* [36–42(–45) × 3.5–5 µm], and *Ps. terricola* with the smallest ascospores (26–32 × 5–5.5 µm). For the remaining species of *Pleosporormiella* only the asexual morph is known; i.e., *Ps. citrullina*, *Ps. compacta*, and *Ps. suttonii* produce pycnidial conidiomata with subtle morphological differences, while *Ps. aculeata* and *Ps. polyspora* display humicola-like morphs, and *Ps. polymorpha* was described as chrysosporium-like (Asgari & Zare 2010, Crous *et al.* 2018). In addition, *Ps. compacta* differs metabolically from other species of the genus by the production of C₁₉H₂₈O₄, C₄₃H₇₉NO₉, and C₁₉H₂₈O₅ (Table 3).

It is possible that *Ps. compacta* has also been recovered from dung under the provisional name “*Sporormiella catenatispora*” (Wergen 2025). This identification is based on the characteristic compacted disposition of the ascospores inside asci.

Pleosporormiella polymorpha (Asgari & Zare) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862075.

Basionym: *Preussia polymorpha* Asgari & Zare, *Nova Hedwigia* 90(3–4): 539. 2010.

Typus: **Iran**, East Azerbaijan Province, Osku, from dead barley leaves, Mar. 2003, B. Asgari (**holotype** IRAN 12398 F, ex-type strains CBS 117679 = IRAN 845 C).

Description and illustration: Asgari & Zare (2010).

Secondary metabolite: Brefeldin.

Notes: We introduce *Ps. polymorpha*, formerly known as *Pr. polymorpha*, based on phylogenetic analyses (Figs 2, 3). This species is characterized by asci variable in shape and producing a chrysosporium-like morph (Asgari & Zare 2010). For a detailed comparison with other *Pleosporormiella* species, see notes section under *Ps. compacta*.

Pleosporormiella polyspora Guerra-Mateo, González-Menéndez, Gené & Cano, **sp. nov.** MB 862053. Fig. 11.

Etymology: Referring to the production of different asexual morphs.

Typus: **Spain**, Catalonia, Mediterranean coast, Tarragona, Platja del Miracle, from sediments at 20 m depth, Jun. 2021, G. Quiroga-Jofre & D. Guerra-Mateo (**holotype** CBS H-25954, ex-type strains FMR 19842 = CF-308080).

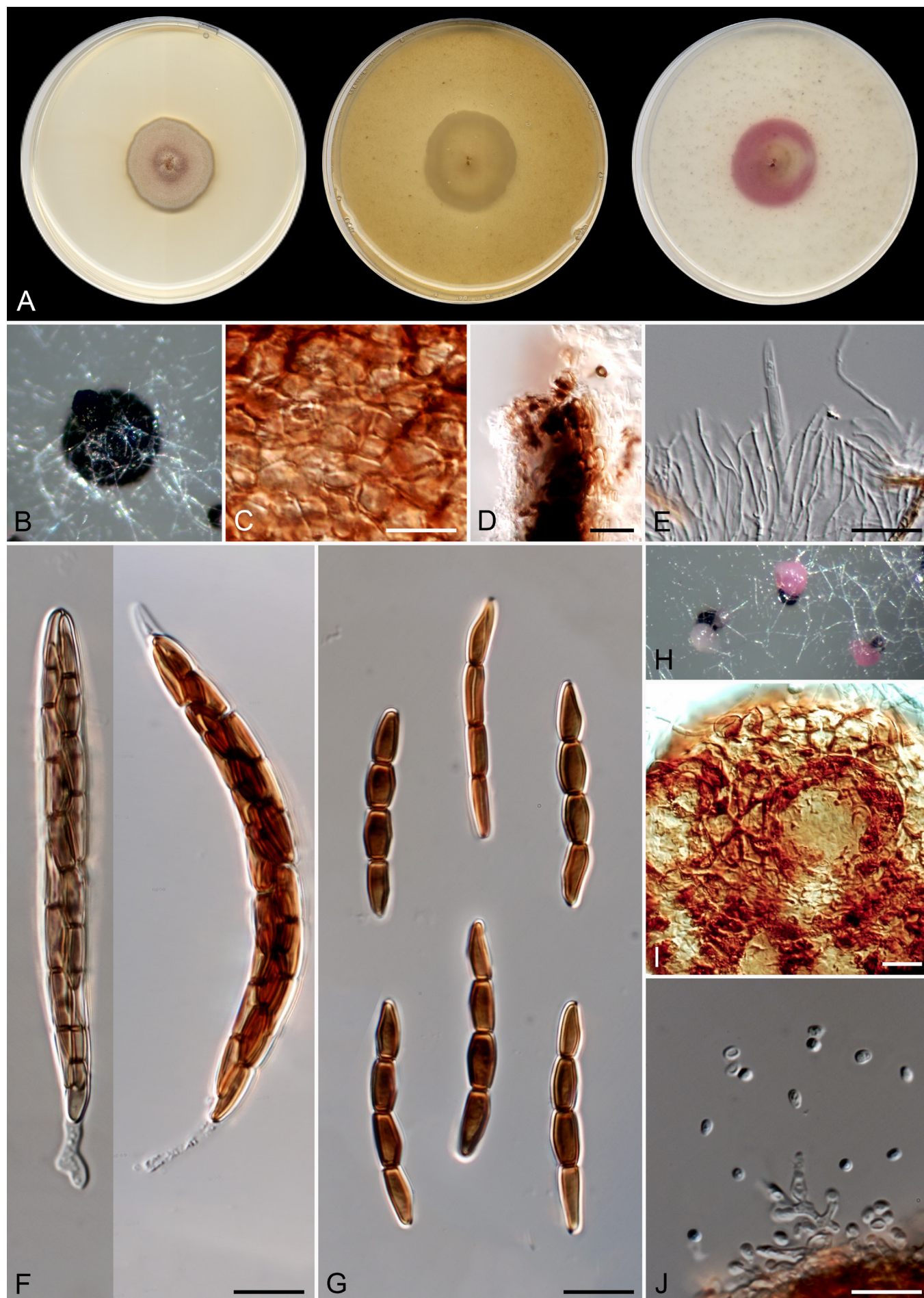


Fig. 10. *Pleosporormiella compacta* (ex-type FMR 20161). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B.** Perithecium. **C.** Peridium with *textura angularis*. **D.** Peridial wall layers. **E.** Paraphyses. **F.** Straight and curved asci. **G.** Ascospores. **H.** Pycnidia. **I.** Pycnidial peridium with *textura angularis*. **J.** Conidiogenous cells and conidia. Scale bars = 10 µm.



Table 3. Secondary metabolites detected for each strain. Chemotypes were defined based on patterns of metabolite production. Asterisks assigned to chemotypes denote minor variations in metabolite production relative to the metabolite profiles of closely related strains.

Strain	Compounds in Table 2																																Chemotype		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		33	
FMR 19091	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	
CF-155367	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	
CBS 468.71	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	
CF-295464	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	
CF-295351	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	
CF-282341	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	
FMR 19618	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
FMR 20161	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
CF-277801	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D*	
FMR 19842	-	-	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	
CF-287459	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F	
FMR 20838	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	
FMR 20458	-	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	
CBS 217.38	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	
CBS 317.65	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	J	
CBS 527.84	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	J	
CBS 117679	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	
FMR 20475	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	L
CF-295384	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	M	
CF-277822	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	N	
CBS 318.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	N*	
CBS 101.60	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	O	
CBS 100.07	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	O	
CF-285370	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	P	
CBS 396.81	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	Q	
CBS 354.86	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	R	
CBS 125.66	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	R	
FMR 19850	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	S	



Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 2–3 µm wide hyphae. *Sexual morph* not observed. *Asexual morph* pycnidial or humicola-like. *Pycnidia* solitary or aggregated, immersed to erumpent, globose, 120–200 µm diam., ostiolate, central ostiole, 9–18 µm diam; wall of 3–6 layers, brown, with cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 8 × 3.5 µm, hyaline, smooth. *Conidia* aseptate, ellipsoid with obtuse ends, 2.5–4.5 × 1.5–2 µm, hyaline, smooth, 2-guttulate, aggregated in white mucous masses. *Humicola-like conidiophores* reduced to conidiogenous cells forming lateral sessile conidia or arising as short lateral pedicels on hyphae, hyaline, smooth. *Conidiogenous cells* intercalary, lateral or terminal, monoblastic, cylindrical or slightly swollen as doliiform, 5–8 × 2.5–3 µm, hyaline. *Conidia* solitary, sometimes two in chain or several in cluster, usually 1–2-celled, sometimes multi-celled, 10–13.5(–15.5) × 9–11 µm, hyaline at first, then olivaceous brown with hyaline or subhyaline basal cell, smooth; upper cell globose to subglobose, very tightly coiled in three dimensions resembling dictyosporous, 9–11.5 × 9–11 µm, thick- and smooth-walled; basal cell when present cylindrical or slightly swollen as doliiform, 3.5–7.5 × 3.5–6 µm, thin-walled; conidial secession rhexolytic.

Culture characteristics (after 14 d at 25 °C): On PDA, reaching 22–44 mm diam., crateriform, radially sulcate, velvety, pale violet (15A3) to yellowish white (4A2), margin entire, slightly lobated; reverse olive brown (4B8) to yellowish grey (4B2); white (1A1) in FMR 19842, CF-287459, and greyish yellow (4B3) in FMR 20838. On OA, reaching 40–60 mm diam., flat, velvety, pink (12A4) to colourless towards periphery, margin submerged, entire and lobated; reverse concolourous. On CMA 34–50 mm diam., flat, velvety in patches, pink (12A4) to colourless towards periphery, margin submerged, entire and lobated; reverse concolourous. FMR 20838 with abundant pale grey (1B1) aerial sporulation in the two latter culture media.

Additional materials examined: **Spain**, Andalusia, Almería, Tabernas, associated with *Launaea arborescens*, May 2014, V. González-Menéndez (CF-287459); *ibid.*, Catalonia, Mediterranean coast, discharging area of the Ebro River, from sediments at 13 m of depth, Oct. 2022, Q. Roca & D. Guerra-Mateo (FMR 20458); *ibid.*, from sediments at 27 m depth, May 2023, Q. Roca & D. Guerra-Mateo (FMR 20838).

Cardinal temperatures for growth: Minimum 15 °C (2 mm), optimum 30 °C (35–38 mm), maximum 18–20 °C (40 mm).

Secondary metabolites: Asteric acid, bisdechlorodihydrogeodin (C₁₇H₁₆O₇), brefeldin, dihydroisoflavipucin, monocerin, C₁₉H₂₈O₄, C₄₃H₇₉NO₉, C₁₉H₂₈O₅, C₁₆H₂₂O₇, C₂₁H₃₅NO, C₁₆H₁₂O₅.

Notes: Our phylogenetic analyses resolved FMR 19842, FMR 20458, FMR 20838, and CF-287459 as a single species (Fig. 2). Nevertheless, these strains exhibited distinct asexual morphs; *i.e.*, pycnidia in FMR 20458 and 19842, humicola-like in FMR 20838 and CF-287459 remained sterile. Their phylogenetic divergence ranged from 7–9 % for *rpb2*, and less than 2–3 % for ITS, LSU, and *tef1-α*. Moreover, the speciation analysis based on the ITS-LSU regions determined these strains as a single evolutionary lineage (Supplementary material Fig. S12). The metabolite profile revealed this group is characterized by the production of brefeldin; additionally, FMR 20838 and FMR 20458 produced dihydroisoflavipucin, a

molecule predominantly detected in strains with humicola- and monodictys-like strains (Fig. 3, Table 3). Altogether, the available phylogenetic evidence supports their recognition as a single species exhibiting intraspecific phenotypic variability.

Pycnidia have been observed in other species of *Pleosporormiella*, *Ps. citrullina*, *Ps. compacta*, and *Ps. suttonii*, but the morphological differences among their conidiomata are subtle. Humicola-like asexual morph has also been observed in *Ps. aculeata*, but *Ps. polyspora* produces tightly coiled conidia without a gelatinous sheath, while the conidia in *Ps. aculeata* are covered with a spiky gelatinous sheath (Fig. 9E, F).

We attempted to induce the sexual morph through *in vitro* crossings among the aforementioned strains, but no ascomata were observed.

Pleosporormiella suttonii* Guerra-Mateo, Gené & Cano, *sp. nov.
MB 862054. Fig. 12.

Etymology: Named in honour of Brian Charles Sutton for his enormous contributions to our knowledge of coelomycetous fungal diversity.

Typus: **Spain**, Catalonia, Mediterranean coast, discharging area of the Ebro River, 40°43'26" N, 0°54'35" E, from sediments at 6 m depth, Oct. 2022, Q. Roca & D. Guerra-Mateo (**holotype** CBS H-25955, ex-type strains FMR 20475 = CF-309230).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–2 µm wide hyphae. *Sexual morph* not observed. *Coelomycetous asexual morph:* *Conidiomata* pycnidial, solitary, immersed to erumpent, globose, 100–120 µm diam., ostiolate, with central ostiole 10–20(–30) µm diam.; wall of 3–6 layers, with brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 8–10(–11) × 2.5–3.5 µm, hyaline, smooth-walled. *Conidia* aseptate, ellipsoid with obtuse ends, 3.5–4.5 × 2 µm, hyaline, smooth, 2-guttulate, aggregated in white mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 12–15 mm diam., flat, cottony, white (1A1) to pale yellow (4A3), margin submerged irregular; reverse greyish yellow (4B5) to white (1A1). On OA, reaching 10–18 mm diam., flat, glabrous and colourless with velvety, dark grey (1F1) patches, margin submerged and entire; reverse dark grey (1F1) and colourless. On CMA, 20 mm diam., flat, velvety, greyish white (1B1), margin submerged, entire and lobated; reverse colourless.

Cardinal temperatures for growth: Minimum 15 °C (10 mm), optimum 25 °C (12–15 mm), maximum 30 °C (8 mm).

Secondary metabolites: C₂₁H₃₅NO, C₁₆H₂₃NO₃, C₁₇H₂₅NO₂.

Notes: *Pleosporormiella suttonii* represents a novel species that, in our phylogeny, formed an independent lineage sister to *Ps. polymorpha* (Fig. 3). These species differ in asexual morph morphology, with *Ps. suttonii* producing pycnidial conidiomata, while *Ps. polymorpha* displays a chrysosporium-like morph (Asgari & Zare 2010). Other species in the genus that display pycnidia are *Ps. citrullina*, *Ps. compacta* and *Ps. polyspora*. *Pleosporormiella suttonii* can be identified through phylogenetic analyses and by the production of C₁₇H₂₅NO₂.

Table 4. Major morphological differences between genera in *Sporormiaceae*.

Genera	Ascomata		Asci		Ascospores			Asexual morph			Main habitat(s)
	Shape	Hairs	Shape	Stipe	Cells	Sheath	Pycnidia	C-like ¹	H-like ²	M-like ³	
<i>Sporormiella</i> (Clade 1)	Perithecia or Cleistothecia	Absent	Cylindrical-clavate (subglobose)	Short	4–13-celled	Subglobose	Present	NA	NA	NA	Dung, soil, and as endophytes
<i>Sporormyces</i> (Clade 2)	Perithecia	Absent	Cylindrical-clavate	Short	7-celled	Subglobose	NA	NA	NA	NA	Dung, soil, and as endophytes
<i>Pleosporormiella</i> (Clade 3)	Perithecia or Cleistothecia	Absent	Subcylindrical	Short	4-celled	Subglobose	Present	Present	Present	NA	Soil and sediments
<i>Preussia</i> s.str. (Clade 4)	Cleistothecia or Perithecia	Absent	Subglobose or broadly clavate	Long (short)	4-celled	Absent or subglobose	Present	Present	NA	NA	Soil and sediments
Clade 5	Perithecia	Absent	Cylindrical-clavate	Short	4–8-celled	Subglobose	Present	NA	NA	NA	Dung, soil, and sediments
<i>Spororvela</i> (Clade 6)	Perithecia	Absent	Cylindrical-clavate	Long	8-celled	Subglobose	NA	NA	NA	NA	Dung
Clade 7	Perithecia	Absent	Cylindrical-clavate	Long (short)	4–8-celled	Subglobose	NA	NA	NA	NA	Dung
<i>Chaetosporormiella</i> (Clade 8)	Perithecia	Present or absent	Subcylindrical	Short	4-celled	Subglobose	Present	NA	NA	Present	As endophyte, dung, and soil
<i>Spororminula</i> (Clade 9)	Perithecia	Absent	Subcylindrical	Short	8-celled	Absent	Present	NA	NA	NA	Dung, soil and sediments
<i>Sporormia</i>	Perithecia	Absent	Subcylindrical	Short	16-or more celled	Lobated	NA	NA	NA	NA	Dung
<i>Westerdykella</i>	Perithecia or cleistothecia	Absent	Globose to subcylindrical	Short	1–4-celled	Absent	Present	NA	NA	NA	Soil, mud, and plant material
<i>Sporormurispora</i>	Cleistothecia	Absent	Subcylindrical	Short	1-celled	Absent	NA	NA	NA	NA	Plant material
<i>Sparticola</i>	Perithecia	Absent	Cylindrical-clavate	short	4–8-celled	Subglobose	NA	NA	NA	Present	Plant material
<i>Forliomyces</i>	NA	NA	NA	NA	NA	NA	Present	NA	NA	NA	Plant material
<i>Trichophoma</i>	NA	NA	NA	NA	NA	NA	Setose	NA	NA	NA	Plant material
<i>Xenomonodictys</i>	NA	NA	NA	NA	NA	NA	NA	NA	Present	NA	Wood

¹Chrysosporium-like asexual morph.²Humicola-like asexual morph.³Monodictys-like asexual morph.⁴Holoblastic conidiogenous cells.⁵Holothallic conidiogenesis.

NA: not available.



Pleosporormiella terricola (Cain) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862076.

Basionym: *Preussia terricola* Cain, *Canad. J. Bot.* **39**: 1641. 1961.

Typus: Honduras, Olnollita farm, from banana rhizosphere soil, Jul. 1958, R.D. Goos (**holotype** TRTC 36955, ex-type strain CBS 317.65).

Description and illustration: Cain (1961).

Additional material examined: Tanzania, Mount Kilimanjaro, North of Lyamungu at 2000 m altitude, on elephant dung, Aug. 1966, R.F. Cain, et al. (CBS 527.84).

Secondary metabolite: C₂₁H₃₅NO.

Notes: The ex-type strain of *Pr. terricola* from Honduras and the isolate from Tanzania formed an independent lineage in *Pleosporormiella*, representative of a single species (Figs 2, 3). This species is included in a well-supported clade together with *Ps. citrullina*, *Ps. polymorpha* and *Ps. suttonii*. *Pleosporormiella terricola* is only known through its sexual morph, which differs from that of *Ps.*

polymorpha in ascospore size (26–32 × 5–5.5 µm vs 45–50 × 13–15 µm, respectively). On the contrary, *Ps. citrullina* and *Ps. suttonii* are only known by their pycnidial asexual morphs (Cain 1961, Asgari & Zare 2010, Crous et al. 2018).

Clade 4. *Preussia* Fuckel, *Fungi Rhen. Exs., Suppl. Fasc. 3*: no. 1750. 1866.

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled hyphae. **Sexual morph:** *Ascomata* cleistothecial, rarely perithecial, immersed to semi-immersed, solitary or aggregated in groups, globose to subglobose, glabrous, carbonaceous-membranaceous to coriaceous; peridium dark brown to black, *textura angularis*. *Paraphyses* filiform, septate, usually present in early stages of development but disappearing at maturity. *Asci* 8-spored, bitunicate, subglobose or broadly clavate with long, rarely short stipe. *Ascospores* arranged in a parallel fascicle or irregularly disposed in a subglobose mass, subcylindrical, 4-celled, septa transverse, deeply constricted, segments easily separable at maturity, at first hyaline, dark brown in maturity; germinal slit elongated, extending the full length of each cell; gelatinous sheath rarely present, hyaline, surrounding each ascospore. **Asexual morph**

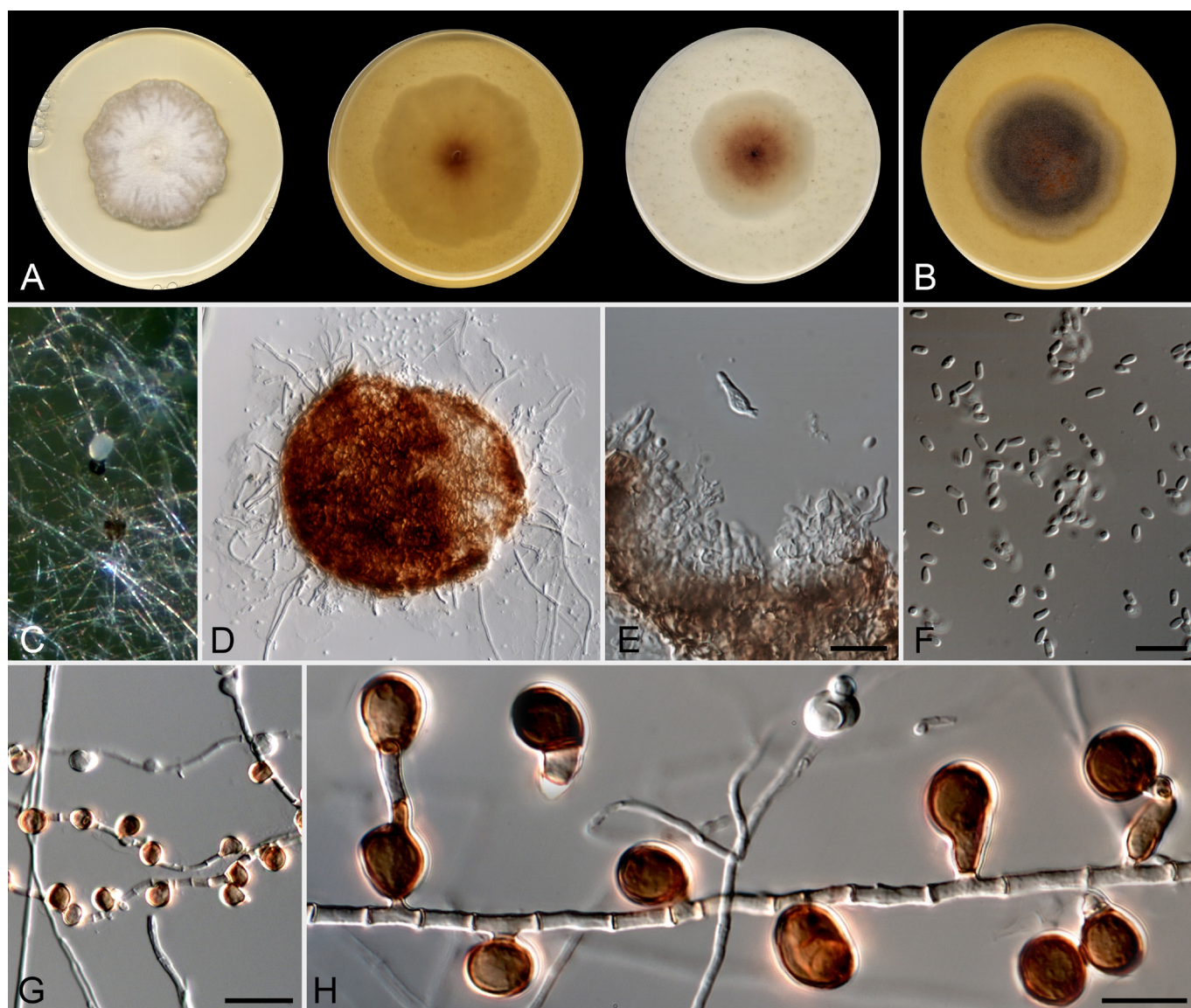


Fig. 11. *Pleosporormiella polyspora* (A, C–F. Ex-type FMR 19842. G, H. FMR 20838). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B.** Colony on OA after 14 d at 25 °C. **C, D.** Pycnidia. **E.** Pycnidial conidiogenous cells. **F.** Pycnidial conidia. **G, H.** *Humicola*-like conidia disposition. Scale bars: E, F, H = 10 µm; G = 20 µm.



pycnidial or chrysosporium-like. *Pycnidia* solitary or aggregated, immersed to erumpent, globose to subglobose; wall brown, with cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, hyaline, smooth. *Conidia* aseptate, ellipsoid with obtuse ends, hyaline, smooth, guttulate, aggregated in mucous masses. *Chrysosporium-like conidiophores* reduced to conidiogenous cells arising laterally or on branched supporting cells. *Conidiogenous cells* holoblastic. *Conidia* solitary, clavate to obovate, smooth, hyaline, with a truncated base.

Type species: Preussia funiculata (Preuss) Fuckel, *Fungi Rhen. Exs., Suppl. Fasc. 3*: no. 1750. 1866.

Habitat: Predominantly on soil and marine sediments, but also on dung.

Notes: We defined clade 4 as *Preussia* s.str. based on morphology and phylogeny. Our phylogenetic analyses determined this clade as a distant, independent, and well-supported lineage displaying long-stipitate asci, rarely short-stipitate, that produce 4-celled ascospores. These features match the original and later morphological circumscription of *Preussia* before considering *Sporormiella* a synonym (Fuckel 1867, Cain 1961). In addition, we emended the generic description to include pycnidia and chrysosporium-like asexual morphs in the genus (Figs 2, 3; von Arx & Storm 1967, Phurbu et al. 2025).

Although *Preussia* s.str. comprises reference and ex-type strains of several species, we recognise only three species (Figs

2, 3). See *Pr. funiculata* notes section for a detailed discussion on strain identification.

Preussia funiculata (Preuss) Fuckel, *Fungi Rhen. Exs., Suppl. Fasc. 3*: no. 1750. 1866. Fig. 13.

Basionym: *Perisporium funiculatum* Preuss, *Linnaea* **24**: 143. 1851.

Synonyms: *Sporormia aemulans* var. *ostiolata* Arx, *Persoonia* **4**(4): 411. 1967, syn. nov.

Preussia cylindricalis F. Liu, S. Song & L. Cai, *Mycology* **16**(2): 676. 2024 [2025], syn. nov.

Preussia flanaganii Boylan, *Canad. J. Bot.* **48**(1): 163. 1970, syn. nov.

Typus: **Germany**, Hoyerswerda, on rotten branches of angiosperms, 1851, *Preuss* (**holotype** B 700132488); **epitype** designated here MBT 10031131: **Germany**, Kiel-Kitzeberg, from wheat field soil, Jan. 1966, *W. Gams* (**epitype** CBS H-25950, ex-epitype strain CBS 127.66).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–2 µm wide hyphae. *Sexual morph*: *Ascomata* cleistothecial or perithecial, solitary or aggregated, immersed to semi-immersed, subglobose, slightly depressed from above, black, glabrous, 120–700 µm diam.; peridium carbonaceous-membranaceous to slightly coriaceous when young and fresh, becoming very fragile and brittle when dry, 5–8-layered, black, with cells of *textura angularis*, 3–10 µm diam. *Paraphyses* septate, filiform, 1–1.5 µm wide, hyaline, abundant in young ascomata and disappearing at maturity, with cylindrical or swollen cells, 3–8 µm

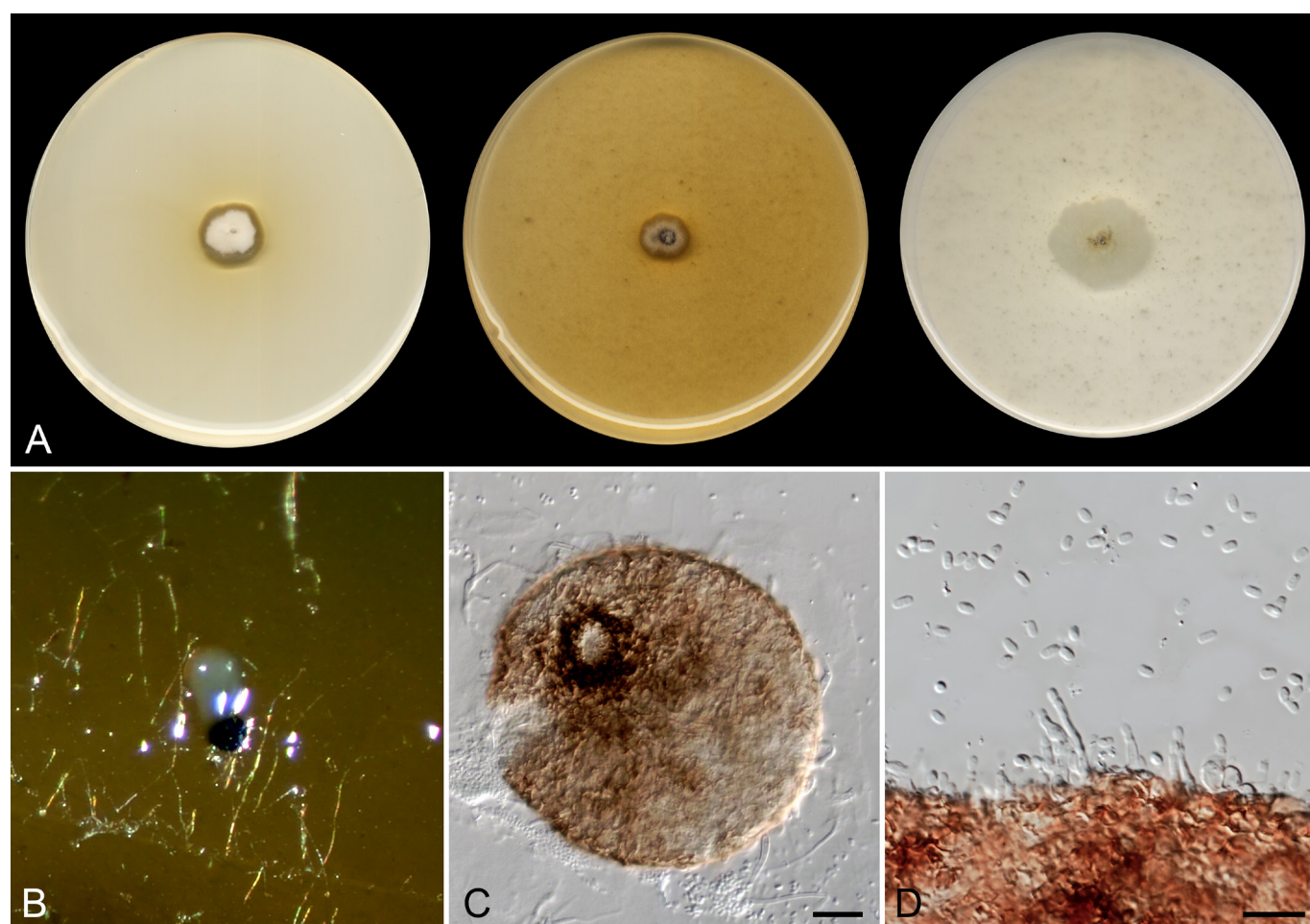


Fig. 12. *Pleosporormiella suttonii* (ex-type FMR 20475). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B, C.** Pycnidia. **D.** Conidiogenous cells and conidia. Scale bars: C = 20 µm; D = 10 µm.

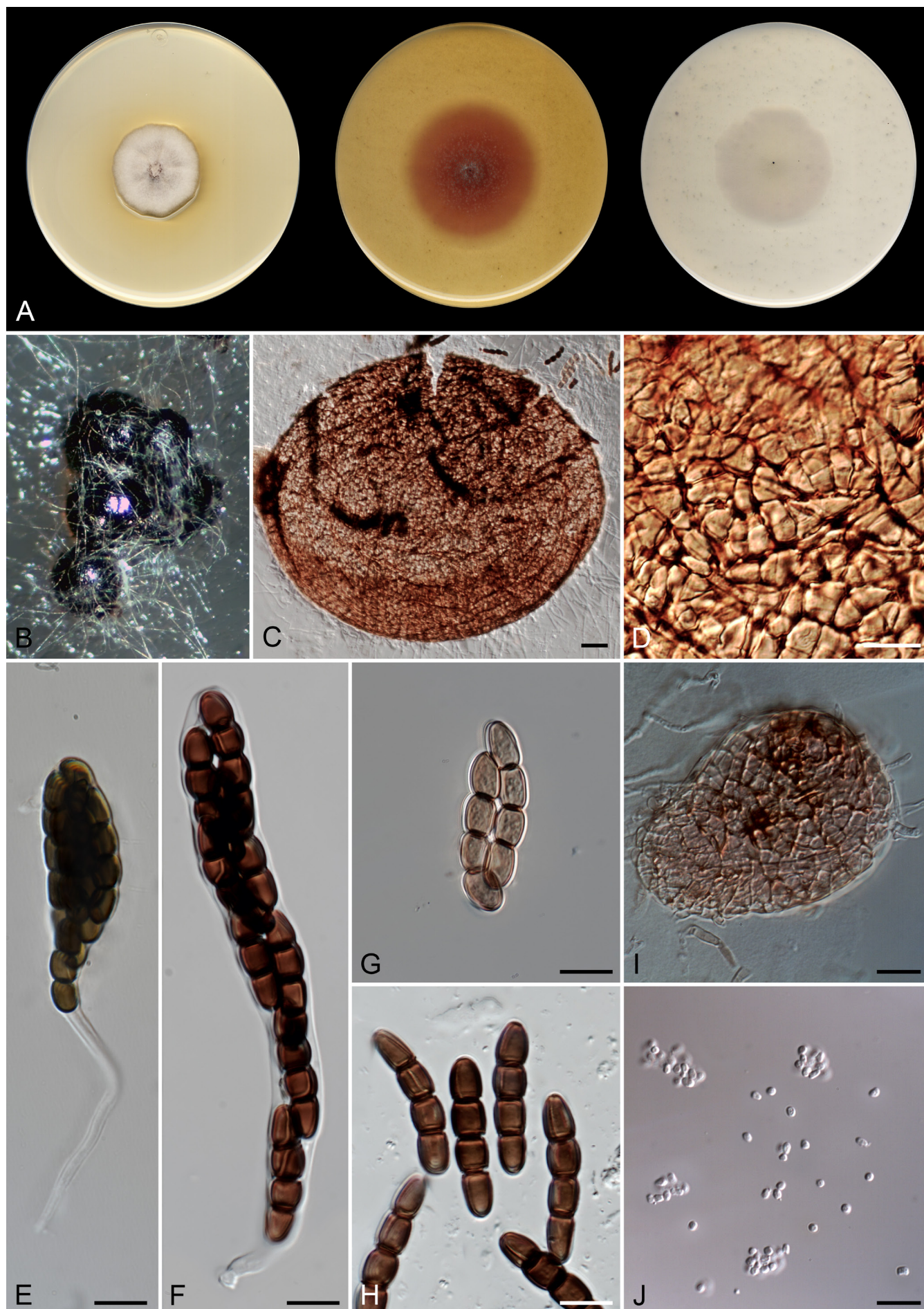


Fig. 13. *Preussia funiculata* (ex-epitype CBS 127.66). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B.** Cleistothecia. **C.** Cleistothecium under light microscopy. **D.** Peridium with *textura angularis*. **E.** Subglobose long-stipitate ascus. **F.** Sub-cylindrical short-stipitate ascus. **G.** Ascospores with oblique septa. **H.** Ascospores with straight septa. **I.** Pycnidia with peridium of *textura angularis*. **J.** Conidia. Scale bars: C = 25 µm; D–J = 10 µm.

diam. Asci 8-spored, subglobose or broadly clavate, 75–200 × 20–28 µm, with long stipe, 15–60(–130) µm in length. Ascospores overlapping, lying parallel with ascus, nearly cylindrical, straight or slightly curved, 4-celled, 22–38 × 5–7 µm, thick-walled, transverse to obliquely septate, deeply constricted, segments easily separable at maturity, hyaline at first, then yellow and finally very dark brown and opaque at maturity; middle cells subglobose, 6–8 × 6.5–7 µm, equal in size and shape, only slightly broader and slightly shorter than end-cells; terminal cells conical, 7–11 × 6 µm, narrowed toward the end and broadly rounded; germ slit parallel to slightly oblique; gelatinous sheath absent; ascospores rarely with fused cells. *Coelomycetous asexual morph*: pycnidial, solitary or aggregated, globose, 50–170 µm diam.; wall 3–6-layered, with cells of brown *textura angularis*. Conidiophores reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 2.5–4 × 1.5–3.5 µm, hyaline, smooth. Conidia aseptate, ellipsoid with obtuse ends, 2–3 × 1.5 µm, hyaline, smooth, 1-guttulate, aggregated in pink mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 20–26 mm diam., slightly umbonate, radially sulcate, velvety, white (1A1), margin entire and slightly lobate; reverse orange white (5A2); diffusible pigment orange white (5A2). On OA, attaining 45–50 mm diam., flat, with sparse, white aerial mycelium, and abundant dark grey sporulation (1F1), pink (12A4) to hyaline at margin, margin diffusing in medium; reverse concolourous. On CMA, reaching 35–50 mm diam., flat, glabrous, subtly pinkish white (12A2) to hyaline at margin, margin slightly lobate; reverse concolourous.

Cardinal temperatures for growth: Minimum 5 °C (2–4 mm), optimum 25 °C (20–26 mm), maximum 30 °C (2 mm).

Secondary metabolites: Cyperin, 7-chloro-6-methoxymellein, C₂₃H₂₄O₇, C₁₄H₁₇NO₅.

Additional materials examined: **Germany**, Kiel-Kitzeberg, from wheat field soil, unknown collection date, K.H. Domsch (CBS 565.63 = CBS 126.66, received as *Preussia fleischhakei*); *ibid.*, W. Gams (CBS 120.66, type of *Sporormia aemulans* var. *ostiolata*). **Japan**, Hokkaido, Otaru-shi, from deer dung, Aug. 1967, S. Udagawa (CBS 107.69, received as *Preussia typharum*). **Mexico**, southwest of Pueblo, from sandy soil in a dry irrigation ditch, Apr. 1967, unknown collector (CBS 112.73, isotype of *Preussia flanaganii*). **Netherlands**, Wageningen, from greenhouse soil, unknown collection date and collector (CBS 287.67, received as *Preussia aemulans*); *ibid.*, from steamed soil from potato field, Oct. 1980, A. Cholí (CBS 318.81, received as *Preussia aemulans*); *ibid.*, Groningen, from human nail, unknown collection date, A.E. Beute (CBS 361.49, received as *Preussia fleischhakei*). **Spain**, Granada, Fuente Vaqueros, from agricultural soil, Oct. 2010, J. Siles (CF-277822, received as *Preussia funiculata*). **USA**, Wyoming, 11 km west of Rock Springs, A1 horizon soil, sagebrush, shrubland, 1978, unknown collector (CBS 126548, received as *Preussia funiculata*).

Notes: *Perisporium funiculatum* was described from rotten branches of angiosperms collected in Hoyerswerda, Germany (Preuss 1851). Although Preuss did not indicate a type specimen, his fungarium was rediscovered in the Berlin herbarium (Jülich 1974). A single collection is preserved in this collection under the name *Perisporium funiculatum* (<https://herbarium.bgbm.org/object/B700132488>), which represents original material and can be interpreted as the

holotype (Supplementary material Fig. S6). This species was later erected as the type of the genus *Preussia*, and *Pr. funiculata* has been represented in phylogenetic analyses with several strains (Figs 2, 3; Kruys & Wedin 2009, González-Menéndez *et al.* 2017, Sandberg *et al.* 2022, Noh *et al.* 2025). Among these, CBS 127.66 was recovered from angiosperm field soil in Kiel-Kitzeberg, Germany, and displayed morphological features typical of *Pr. funiculata*, such as subglobose, long-stipitate asci with 4-celled ascospores with oblique septa (Fig. 13). Therefore, to stabilize the nomenclature of the species and, ultimately, the genus *Preussia*, this strain is selected here as epitype based on its morphological resemblance to the protologue.

In accordance with previous works, our phylogenetic analyses clustered CBS 127.66 with representatives of *Pr. aemulans*, *Pr. alloiomera*, *Pr. cylindricalis*, *Pr. flanaganii*, and *Pr. typharum* (Figs 2, 3). The phylogenetic distance within this clade was around 3–5 % in *rpb2*, 1–3 % in *tef1-α*, and smaller than 1 % for ITS, LSU, and SSU. The speciation analysis of the ITS-LSU regions determined the clade as a single species (Supplementary material Fig. S13). This analysis included the ex-type strains of *Sp. aemulans* var. *ostiolata*, *Pr. cylindricalis*, and *Pr. flanaganii*; therefore, they are considered conspecific with *Pr. funiculata*. Although *Pr. aemulans* s.str., *Pr. alloiomera*, *Pr. fleischhakei* and *Pr. typharum* likely also represent *Pr. funiculata*, the lack of sequence data from their type specimens prevents the resolution of their taxonomy. Consequently, these taxa are regarded as species of uncertain status in the genus until further studies are conducted.

In this context, *Pr. funiculata* encompasses strains that are phylogenetically closely related, but exhibit morphological variability. The strain CBS 107.69, received as *Pr. typharum*, showed asci with long stipes and ascospores bearing transverse septa. CBS 361.49 was identified as *Pr. fleischhakei*, a species often confused with *Pr. funiculata*, and characterized by short-stipitate asci and ascospores with transverse septa (Cain 1961). Similarly, *Pr. flanaganii* was described as a species resembling *Pr. fleischhakei* in the mentioned characters (Boylan 1970). In addition, the ex-type strain of *Pr. flanaganii* (CBS 112.73) clustered with strains displaying *Pr. funiculata* morphology (Figs 2, 3). The strain S: 21345-p was identified as *Pr. alloiomera* by producing perithecia, short-stipitate asci and ascospores that exhibit oblique septa (Ahmed & Cain 1972, Kruys & Wedin 2009). The type of *Sp. aemulans* var. *ostiolata* (CBS 120.66) was characterized by forming perithecia with subcylindrical long-stipitate asci, ascospores exhibiting transverse septa, and pycnidial conidiomata as asexual morph (von Arx & Storm 1967). Moreover, the strain NMC 1013, which exclusively produces pycnidia, was recently described as *Pr. cylindricalis* (Phurba *et al.* 2025). Therefore, based on our phylogenetic analyses, we have updated the morphological description of *Pr. funiculata*.

Finally, the metabolite profile on strains CF-277822 and CBS 318.81 revealed the production of cyperin, a molecule exclusively detected in this lineage (Table 3). It is noteworthy that the strain TTI-1095, previously identified as “*Pr. typharum*”, clustered within *Pr. funiculata* (Fig. 2). This strain was characterized by the production of leptosin C (Perlatti *et al.* 2021), a molecule that in the present work has not been detected.

Preussia gypsicola Guerra-Mateo, González-Menéndez, Cano & Gené, *sp. nov.* MB 862055. Fig. 14.

Etymology: Referring to the substrate where the species has been reported, gypsum soil.



Typus: Spain, Andalusia, Granada, Escúzar quarry, from gypsum soil associated with *Acarospora reagens*, Nov. 2016, V. González-Menéndez (**holotype** CF-H-295384, ex-type strains CF-295384 = FMR 20555).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–3 µm wide hyphae. *Sexual morph* not observed. *Coelomycetous asexual morph*: *Conidiomata* pycnidial, solitary, immersed to erumpent, globose to subglobose, 50–100 × 50–100 µm, ostiolate, with central ostiole, 10–35 µm diam.; wall of 2–3 layers, with cells of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 4–5 × 2.5–3 µm, hyaline, smooth. *Conidia* aseptate, ellipsoid with obtuse ends, 3 × 1.5–2 µm, hyaline, smooth, 1-guttulate, aggregated in pale yellow mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 23–26 mm diam., crateriform, radially sulcate, velvety, white (1A1), margin entire and lobated; reverse greyish red (7B3) to yellowish white (3A2). On OA, 30 mm diam., flat, velvety, pink (12A4) to colourless, margin submerged, entire and slightly lobated; reverse concolourous. On CMA, 26–28 mm diam., flat, velvety, pink (12A4) to colourless, margin submerged, entire and slightly lobated; reverse concolourous.

Cardinal temperatures for growth: Minimum 15 °C (9 mm), optimum 25 °C (23–26 mm), maximum 30 °C (18 mm).

Secondary metabolites: C₄₃H₇₉NO₉, C₁₆H₂₀O₆, C₁₆H₁₈O₅, C₁₉H₂₄O₈S.

Notes: *Preussia gypsicola* represents an independent lineage in the *Preussia* s.str. clade (Figs 2, 3). This species produced pycnidial conidiomata that are difficult to discern from those of *Pr. funiculata*, but differs from *Pr. sedimenticola*, which produces a chrysosporium-like asexual morph (Phurbu *et al.* 2025). These species can be distinguished through phylogenetic analyses, with *Pr. gypsicola* showing a 96–97 % ITS identity to both *Pr. funiculata* and *Pr. sedimenticola*.

Preussia sedimenticola F. Liu, S. Song & L. Cai, *Mycology* **16** (2): 678. 2025.

Typus: China, Xizang, Dagze Co lake (31°49'25.32" N, 87°28'16.68" E), from lake sediment, Aug. 2021, J.E. Huang (**holotype** HMAS 352881, ex-type strain CGMCC 3.27276).

Description and illustration: Phurbu *et al.* (2025).

Notes: This species is characterized by the production of a chrysosporium-like morph, and represents the first report of this asexual morphology in *Preussia* s.str. A similar asexual morph has been observed in *Ps. polymorpha*; however, *Pr. sedimenticola* differs in producing smaller conidia (2.5–4.5 µm vs 3–7.5 µm in length, respectively) (Asgari & Zare 2010, Phurbu *et al.* 2025).

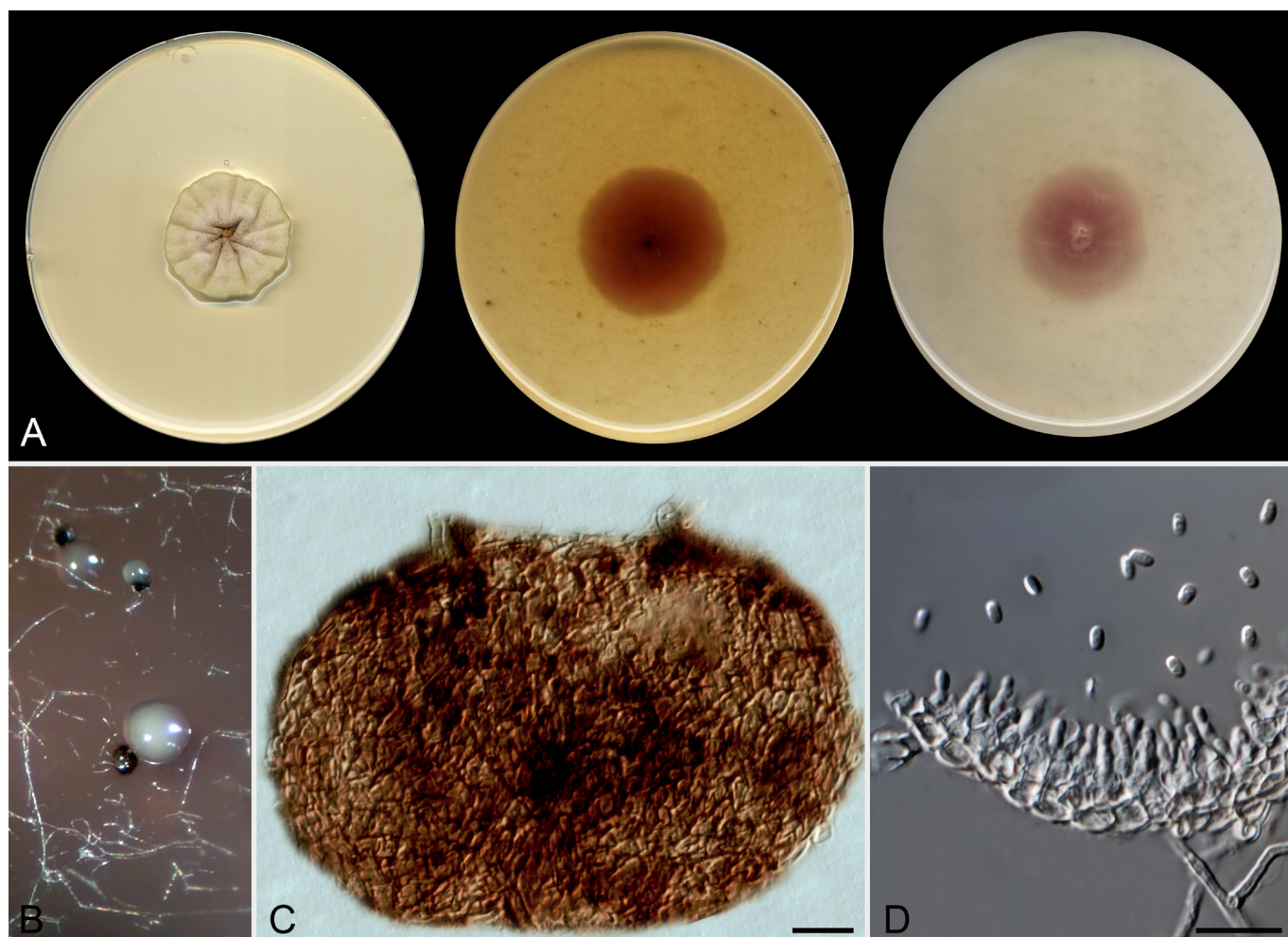


Fig. 14. *Preussia gypsicola* (ex-type CF-295384). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B, C.** Pycnidia. **D.** Conidiogenous cells and conidia. Scale bars: C = 20 µm; D = 10 µm.

Clade 5.

Notes: Clade 5 comprised strains identified as “*Pr. dubia*”, “*Pr. irregularis*”, “*Pr. splendens*”, and “*Sm. muskokensis*”, but lacked ex-type strains for these species (Fig. 3). Based on these identifications, the clade would be characterized by short, sometimes long-stipitate asci producing 4–8-celled ascospores (Ahmed & Cain 1972). The combination of these morphological features with the great phylogenetic distance to other *Preussia* s.l. lineages, suggest that clade 5 represents another novel genus. However, we could only examine two of their strains, CBS 111.77 and FMR 20357, and preferred to maintain this lineage without a formal name until additional strains can be examined.

According to our phylogeny (Figs 2, 3), these two strains are placed in different lineages. Strain FMR 20357, recovered from marine sediments of the Mediterranean Sea, remained sterile in culture but clustered with the strain S: 16568-f identified as “*Sm. irregularis*”. Interestingly, CBS 111.77 was received as “*Sm. australis*”, but clustered with S: 19562-G, a reference strain representative of “*Pr. dubia*” (Kruys & Wedin 2009). Both strains were recovered from horse dung in the Netherlands and Iceland, respectively. *Preussia dubia* was characterized by producing short-stipitate asci with 4-celled ascospores (Ahmed & Cain 1972). Strain CBS 111.77 produced a similar sexual morph in culture, but also a coelomycetous asexual morph characterized by subglobose pycnidial conidiomata and phialidic conidiogenous cells that produce conidia aggregated in white mucous masses.

Clade 6. *Spororvela* Guerra-Mateo, Cano & Gené, **gen. nov.** MB 862056.

Etymology: The name refers to the morphological resemblance to the genus *Sporormiella* and Latin *velum* (plural *vela*), veil, referring to the production of ascomata under a mycelial veil or weft.

Sexual morph: Ascomata perithecial, semi-immersed, covered by a mycelial weft, globose to subglobose, glabrous, coriaceous; peridium smooth, brown-black, thick-walled, with cells of *textura angularis*, sometimes with spikes of hyphae and occasional setae at the base appearing anchor hairs; neck long. *Pseudoparaphyses* filiform to moniliform, septate, hyaline, longer than the asci and mixed with them. Asci 8-spored, bitunicate, cylindrical-clavate, tapering into a long stipe. Ascospores fusiform, bi- to triseriate, fusiform, straight or slightly curved, primarily 8-celled, sometimes 7–12-celled, septa transverse, deeply constricted, segments variable in size and shape, end cells conical and rounded at the tip; at first hyaline, dark brown in maturity, thick-walled; germinal slit diagonal, extending the full length of each cell; gelatinous sheath hyaline, subglobose, surrounding each ascospore. **Asexual morph** not observed.

Type species: *Spororvela tela* (S. Jakob & M.J. Richardson) Guerra-Mateo, Cano & Gené

Habitat: Only known from dung.

Notes: We propose the novel genus *Spororvela* to accommodate *Sm. tela*, which represents a monotypic, independent lineage in *Sporormiaceae* (Fig. 3). *Spororvela* is characterized by ascomata covered by a mycelial weft and long-stipitate asci that produce up to 12-celled ascospores (Jakob & Richardson 2024).

Spororvela tela (S. Jakob & M.J. Richardson) Guerra-Mateo, Cano & Gené, **comb. nov.** MB 862077.

Basionym: *Sporormiella tela* S. Jakob & M.J. Richardson, *N. E. Naturalist* **31** (3, C1): 1. 2024.

Typus: **USA**, Van Cortlandt Park, New York, 40°53'24" N, 73°53'24" W, on dung of *Branta canadensis* (Canada Goose) in grassy field, Feb. 2023, S. Jakob (**holotype** NY 04449436, ex-type strain iNa152236789).

Description and illustration: Jakob et al. (2024).

Notes: *Spororvela* represents a monotypic genus phylogenetically related to clades 5 and 7 in *Preussia* s.l. (Figs 2, 3). The species *Sv. tela* is characterized by long-stipitate asci producing predominantly 8-celled, but also 7–13-celled ascospores, while the species included in clades 5 and 7 display short-, sometimes long-stipitate asci producing 4–7(–8)-celled ascospores (Ahmed & Cain 1972, Jakob & Richardson 2024).

Clade 7.

Notes: Clade 7 comprised strains identified as “*Sm. affinis*”, “*Sm. heptamera*”, “*Sm. leporina*”, “*Sm. octomera*”, and “*Sm. vexans*” (Figs 2, 3). Based on these identifications, the clade would be characterized by long-, sometimes short-stipitate asci producing primarily 7–8-celled ascospores (Ahmed & Cain 1972). Our phylogeny, together with these morphological features, suggests clade 7 as a novel genus in *Sporormiaceae*. However, since we could not examine any specimens, this clade remains unresolved pending further studies.

Additional species without molecular data, and displaying short- to long-stipitate asci producing 8-celled ascospores like *Pr. alpina*, *Pr. octocylindrospora*, *Pr. octonalis*, and *Sm. corynespora* could also represent members of this group (Ahmed & Cain 1972, Kruys 2015). However, this hypothesis needs to be tested through molecular analyses.

Clade 8. *Chaetosporormiella* Guerra-Mateo, Gené & Cano, **gen. nov.** MB 862057.

Etymology: Greek *chaítē* (χαίτη), long hair, referring to the production of hairy ascomata and the morphological resemblance to *Sporormiella* in microscopic features.

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled hyphae. **Sexual morph:** Ascomata perithecial, solitary or aggregated, immersed to semi-immersed, subglobose to pyriform, bare or hairy, dark brown to black; neck short or long, papilliform, conical or cylindrical, stout, bare or hairy, black; peridium membranaceous, covered by dark brown hairs. *Paraphyses* septate, filiform, occasionally branched, longer than the asci and mixed with them, hyaline. Asci 8-spored, cylindrical-clavate, tapering into a short stipe. Ascospores bi- or tri-seriate, 4-celled, fusiform-cylindrical, curved, septa transverse, constrictions at septa shallow to deep, dark brown, smooth-walled; cells almost equal in size, or terminal cells longer and narrower than the middle cells; germ slit parallel to diagonal; gelatinous sheath hyaline. **Asexual morph:** pycnidial or monodictys-like. *Pycnidia* solitary, immersed to erumpent, brown, globose, with a central ostiole; wall brown,



with cells of *textura angularis* and hairs. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, hyaline, smooth. *Conidia* aseptate, ellipsoid with obtuse ends, hyaline, smooth, guttulate, aggregated in mucous masses. *Monodictys-like conidiophores* reduced to conidiogenous cells that laterally extend to form sessile or pedicellate conidia on hyphae. *Conidiogenous cells* lateral or terminal, integrated, cylindrical or slightly swollen as doliiform, monoblastic, hyaline. *Conidia* solitary, bi-cellular to muriform, black, globose to subglobose, rarely subcylindrical or pyriform, dark brown, thick-walled, conspicuously verrucose, basal cell paler and verrucose: secession rhexolytic.

Type species: Chaetosporormiella pilosella (Cain) Guerra-Mateo, Gené & Cano

Habitat: Predominantly as plant endophyte, but also on dung and soil.

Notes: *Chaetosporormiella* represents an independent lineage in *Sporormiaceae* characterized by hairy or bare ascomata, short-stipitate asci with 4-celled ascospores, and monodictys-like asexual morph. Our phylogeny determined four lineages within the genus representing different species (Figs 2, 3). However, the singleton lineage with the strain S: 16551-g identified as “*Pr. longisporopsis*”, and that with the strains S37 and CF-285370 identified as “*Pr. grandispora*”, remain unresolved because we were not able to examine these fungi in the present study.

Chaetosporormiella monodictyoides Guerra-Mateo, Gené & Cano, *sp. nov.* MB 862058. Fig. 15.

Etymology: Referring to conidial morphology, which resembles the genus *Monodictys*.

Typus: **Netherlands**, from a pillow, Jun. 1960, unknown collector (**holotype** CBS H-25951, ex-type strain CBS 101.60 = FMR 20560).

Mycelium immersed and superficial, composed of hyaline, septate, branched, anastomosed, smooth-walled, 1.5–3 µm wide hyphae. *Sexual morph* not observed. *Asexual morph* monodictys-like. *Conidiophores* reduced to a hyphal cell that laterally extends to form a sessile conidium or arising as short lateral pedicels on the hyphae, sometimes branched, 10–50 × 1.5–2 µm, hyaline, finely warted. *Conidiogenous cells* lateral or terminal, integrated, monoblastic, cylindrical or slightly swollen as doliiform, 2–6 × 1.5–2 µm, hyaline, smooth-walled. *Conidia* solitary, bi-cellular to muriform, globose to subglobose, pyriform, rarely subcylindrical, 12–20(–50) × 12–16 µm, hyaline at first, then dark brown to black in mass, thick-walled, conspicuously verrucose, basal cell pale, thin-walled, smooth to verrucose; secession rhexolytic. *Chlamydospores* in chains, composed of aseptate to muriform cells, brown, thick-walled, and verrucose.



Fig. 15. *Chaetosporormiella monodictyoides* (ex-type CBS 101.60). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B.** Conidiophore disposition. **C.** Conidia in chains. **D, E.** Single-celled conidia. Scale bars: B, C = 10 µm; D, E = 5 µm.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 22–30 mm diam., slightly elevated, woolly, growth in rings, orange white (5A2) with light grey (1C1) areas due to sporulation, margin entire; reverse dark orange (5A8) to light yellow (4A4); diffusible pigment yellowish white (4A2). On OA, 45–55 mm diam., flat, woolly, dark grey (1F1) to white (1A1), margin entire; reverse concolourous; diffusible pigment pale red. On CMA, 28 mm diam., flat, slightly floccose with rings of growth, dark grey (1F1) to white (1A1), margin entire; reverse colourless.

Additional material examined: Unknown origin, 1907, C.A.J.A. Oudemans (CBS 100.07).

Cardinal temperatures for growth: Minimum 5 °C (1 mm), optimum 20–25 °C (22–38 mm), maximum 30 °C (6 mm).

Secondary metabolites: Dihydroisoflavipucic, leptosins ($C_{30}H_{28}N_6O_7S_4$ and $C_{32}H_{34}N_6O_7S_4$), $C_{32}H_{42}N_4O_{10}$.

Notes: *Chaetosporormiella monodictyoides* was originally identified as *Monodictys castaneae* based on the morphological similarity to the protologue of this species (Wallroth 1833). However, several phylogenetic analyses revealed *Monodictys* as a polyphyletic genus, and many species have been transferred to different orders in *Dothideomycetes*, *Sordariomycetes*, and *Leothiomycetes* (Han et al. 2014, Tanaka et al. 2015, Réblová et al. 2020). *Monodictys*-like asexual morphs have been reported throughout the order *Pleosporales*, comprising genera in different families such as *Alpestrisphaeria* in *Lophiostomataceae*, *Pleomonodictys* in *Pleomonodictyaceae*, or *Xenomonodictys* in *Sporormiaceae* (Zhang et al. 2012b, Hernández-Restrepo et al. 2017, Yeh & Kirschner 2019). *Chaetosporormiella monodictyoides* resembles the conidia displayed by the species *Ohleria brasiliensis* (*Ohleriaceae*), *Antealophiotrema brunneosporum* (*incertae sedis*), and the genus *Paradictyoarthrinium* (*Paradictyoarthrinaceae*). These fungi display globose to pyriform dictyoconidia that differ in size and ornamentation. *Antealophiotrema brunneosporum* produces smooth and large conidia of up to 85 µm diam. (Hashimoto et al. 2017). Species of *Paradictyoarthrinium* and *Cp. monodictyoides* produce smooth to verrucose, medium sized conidia, around 20 µm in length (Htet et al. 2023), while *O. brasiliensis* is characterized by verruculose and small conidia of 15 µm diam. (Samuels 1980). In *Sporormiaceae*, the only species displaying monodictys-like conidia is *Xenomonodictys iranica*. *Chaetosporormiella monodictyoides* differs in conidial cell organization, showing three rows of cells, while *X. iranica* only produces two rows of cells (Crous et al. 2020a). In addition, these two species can be distinguished through phylogenetic analyses (Figs 2, 3).

Chaetosporormiella pilosella (Cain) Guerra-Mateo, Gené & Cano, **comb. nov.** MB 862078. Fig. 16.

Basionym: *Sporormia pilosella* Cain, *Stud. Sphaer. Ontario*: 93. 1934.

Synonyms: *Sporormiella pilosella* (Cain) S.I. Ahmed & Cain, *Canad. J. Bot.* **50**(3): 455. 1972.

Preussia pilosella (Cain) Guarro & Gené, *Mycol. Res.* **101**(3): 308. 1997.

Typus: **Canada**, Ontario, Muskoka District, Stoneleigh, from porcupine dung (**holotype** TRTC 5316).

Description adapted from Ahmed & Cain (1972, under *Sporormiella pilosella*). **Mycelium** immersed and superficial, composed of septate, branched, anastomosed, hyaline, smooth-walled, 2–3.5 µm wide hyphae. **Sexual morph:** *Ascomata* perithecial, solitary or aggregated, immersed to semi-immersed, subglobose to pyriform, 450–550 × 300–450 µm, black; ostiolar beak short, papilliform or conical; peridium 5–8-layered, dark brown, covered with septate, thick-walled, flexuous, dark brown hairs. **Paraphyses** septate, filiform, occasionally branched, 2–3 µm wide, hyaline. **Asci** 8-spored, subcylindrical-clavate, (160–)190–230 × 17–20.5 µm, with short stipe, 20–30 µm in length. **Ascospores** 4-celled, fusiform-cylindrical, curved, (29–)32–37 × 8–9 µm, septa transverse, constrictions at septa moderately shallow, hyaline at first, dark brown and thick-walled in maturity; terminal cells ovoid-conical, comparatively longer and narrower than the middle cells; germ slit diagonal; gelatinous sheath hyaline. **Coelomycetous asexual morph:** *Conidiomata* pycnidial, solitary, immersed to erumpent, brown, globose, 170–200 µm diam., ostiolate, central ostiole 23–25 µm diam.; wall of 3–6 layers, brown, with cells of *textura angularis*, covered by septate, erect, thick-walled setae, 150–180 × 3–4 µm, brown, smooth. **Conidiophores** reduced to conidiogenous cells lining the inner cavity, phialidic, ampulliform, 7.5–12 × 2.5–3 µm, hyaline, smooth. **Conidia** aseptate, ellipsoid with obtuse ends, 2.5–3 × 1.5–2.5 µm, hyaline, smooth, 1-guttulate, aggregated in white mucous masses.

Additional descriptions and illustrations: Ahmed & Cain (1972).

Additional materials examined: **Spain**, Murcia, Sierra Espuña, from soil, Sep. 2002, D. Garcia (CBS 113807; proposed here as reference strain). **Switzerland**, Montorge, from needles of *Juniperus communis*, 1978, O. Petrini (CBS 396.81, received as *Sporormiella cymatomera*).

Secondary metabolite: $C_{22}H_{26}O_5$.

Notes: In our phylogeny, *Cp. pilosella* is represented by two strains, CBS 113807 and CBS 396.81 (Fig. 3). Strain CBS 113807, identified as *Pr. pilosella*, exhibits the typical features described in the protologue of the species, namely hairy ascomata with short-stipitate asci, producing 4-celled ascospores (Ahmed & Cain 1972). Given the absence of ex-type cultures and the good preservation of the holotype in the Royal Ontario Museum (TRTC 5316), we select this strain (CBS 113807) as the reference strain for the species and typified *Chaetosporormiella* with *Cp. pilosella*. Nevertheless, cultures of the other strain, CBS 396.81, produced bare ascomata and hairy pycnidia, suggesting that *Cp. pilosella* can actually produce both hairy and bare sexual morphs, together with a hairy coelomycetous asexual morph. These features have been incorporated into the concept of the new genus.

Chaetosporormiella pilosella differs from other species with hairy ascomata by showing longer asci (190–230 × 17–20.5 µm vs 130–174 × 16–19 µm in *Pr. aquilirostrata*), and smaller ascospores (32–37 × 8–9 µm vs 62–76 × 10–12 µm in *Pr. lasiocarpa*) (Lorenzo 1994, Guarro et al. 1997a). Moreover, hairy pycnidia in *Sporormiaceae* have only been observed in *Trichophoma cylindrospora*. However, the conidia of this latter species are cylindrical and considerably larger (18–20 × 2–3 µm; Crous et al. 2020b) than those produced by *Cp. pilosella*.

Clade 9. *Spororminula* Arx & Aa, *Trans. Brit. Mycol. Soc.* **89**(6): 117. 1987.



Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled hyphae. *Sexual morph*: *Ascomata* perithecial, immersed to semi-immersed, solitary or aggregated in small groups, globose to subglobose, glabrous, carbonaceous-membranaceous to coriaceous; peridium smooth, dark brown to black, with cells of *textura angularis*; neck papilliform. *Paraphyses* filiform, septate, hyaline, longer than the asci and mixed with them. *Asci* 8-spored, bitunicate, subcylindrical, tapering into a short stipe. *Ascospores* biseriate, 8-celled, subcylindrical, curved, septa transverse, deeply constricted, cells easily separable at maturity, at first hyaline, brown in maturity, thick- and smooth-walled; germinal slit diagonal; gelatinous sheath absent. *Coelomycetous asexual morph*: *Conidiomata* pycnidial, solitary or aggregated, globose to subglobose or irregularly shaped; peridium dark brown, with cells of *textura angularis*. *Conidiogenous cells* lining the inner cavity, phialidic, hyaline, thin- and smooth-walled. *Conidia* aseptate, ellipsoid or cylindrical, with obtuse ends, hyaline, smooth, guttulate, aggregated in mucous masses.

Type species: *Spororminula subticinensis* (Mouton) Guerra-Mateo, Cano & Gené

Habitat: On dung, soil, and marine sediments.

Notes: The genus *Spororminula* was established to accommodate the species *Sn. tenerifae* based on morphological features (von Arx & van der Aa 1987). Although this species was later transferred to *Preussia* (Kruys & Wedin 2009), our phylogenetic analyses resolved this lineage as an independent clade (Figs 2, 3). Therefore, we consider *Spororminula* as a valid genus and accept two species, *Sn. irregularis* and *Sn. subticinensis*. The latter species comprises *Sn. tenerifae* as a synonym; for a detailed discussion, see the species notes section.

Additional *Preussia* s.l. species exhibiting short-stipitate asci with 8-celled ascospores could be representatives of this genus; i.e., *Pr. octocylindrospora*, *Sm. insignis* and *Sm. platymera* (Kruys 2015). However, these species lack molecular data, and this hypothesis needs to be evaluated in future works.

Spororminula irregularis Guerra-Mateo, Cano & Gené, *sp. nov.* MB 862059. Fig. 17.

Etymology: Referring to the irregular shape of the pycnidial conidiomata.

Typus: **Spain**, Catalonia, Mediterranean coast, Tarragona, Platja del Miracle, 41°6'19" N, 1°15'37" E, from sediments at 20 m depth, Jun. 2021, G. Quiroga-Jofre & D. Guerra-Mateo (**holotype** CBS H-25956, ex-type strains FMR 19850 = CF-308081).

Mycelium immersed and superficial, composed of septate, branched, hyaline, smooth-walled, 1.5–2.5 µm wide hyphae. *Sexual morph* not observed. *Coelomycetous asexual morph*: *Conidiomata* pycnidial, solitary or aggregated, immersed to erumpent, dark brown, globose to subglobose or irregular in shape, 45–150 × 45–100 µm, ostiolate, ostiole 18–23 µm diam.; wall of 2–3 layers, with brown cells of *textura angularis*. *Conidiogenous cells* phialidic, cylindrical, 13 × 3.5 µm, hyaline, thin- and smooth-walled. *Conidia* aseptate, cylindrical with obtuse ends, 3.5–4.5 × 2 µm, hyaline, smooth, 2-guttulate, aggregated in white mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 26–30 mm diam., slightly crateriform, radially sulcate, velvety, white (12A1) with pinkish white (9A2) areas, margin entire; reverse reddish grey (9B3) to yellowish white (4A2). On OA, 38–49 mm diam., flat, filamentous, white (1B1), with abundant pycnidia, margin submerged, entire; reverse pale grey (1B1) to colourless; diffusible pigment pale red in old cultures. On CMA, 42–45 mm diam., flat, filamentous, pale grey (1B1) to white (1A1), margin submerged, entire; reverse colourless.

Cardinal temperatures for growth: Minimum 5 °C (4 mm), optimum 25 °C (26–30 mm), maximum 40 °C (4 mm).

Secondary metabolites: Antibiotic LL-N 313ε, monocerin, palmarumycins, 4-hydroxy-5-methylmellein, 7-chloro-6-methoxymellein, C₁₁H₁₀ClO₅, C₂₀H₁₄O₃.

Notes: *Spororminula irregularis* represents the second species described in the genus (Fig. 3), but unlike *Sn. subticinensis*, it only produces a pycnidial asexual morph in culture. This differs from the pycnidia produced by *Sn. subticinensis* in showing slightly longer conidiogenous cells (13 µm vs 8.5 µm in length) and conidia (3.5–4.5 µm vs 2.5–4 µm in length). In addition, *Sn. irregularis* is particularly characterized by the production of monocerin (Table 3).

Spororminula subticinensis (Mouton) Guerra-Mateo, Cano & Gené, *comb. nov.* MB 862079. Fig. 18.

Basionym: *Sporormia subticinensis* Mouton, *Bull. Soc. Roy. Bot.*

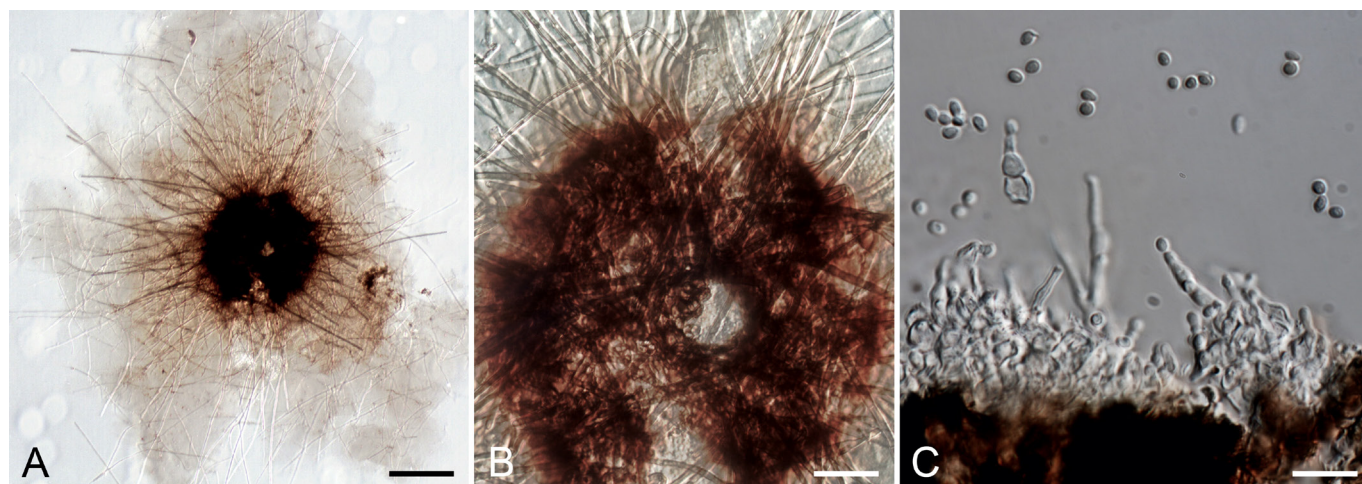


Fig. 16. *Chaetosporormiella pilosella* (CBS 396.81). **A.** Pycnidium. **B.** Pycnidial wall and hairs. **C.** Conidiogenous cells and conidia. Scale bars: A = 100 µm; B = 50 µm; C = 10 µm.



Belgique 36: 14. 1897.

Synonyms: *Preussia subticinensis* (Mouton) Arx, *Proc. Kon. Ned. Akad. Wetensch.*, C 76(3): 294. 1973.

Sporormiella subticinensis (Mouton) Dugan & R.G. Roberts, *Mycologia* 87(5): 717. 1995.

Sporormia perplexans Nicot & Rouch, *Compt. Rend. Hebd. Séances Acad. Sci., Sér. D* 262: 1694. 1966.

Spororminula tenerifae Arx & Aa, *Trans. Brit. Mycol. Soc.* 89(6): 117. 1987.

Preussia tenerifae (Arx & Aa) Kruys, *Syst. Biodiv.* 7(4): 476. 2009, *syn. nov.*

Typus: **Belgium**, Beaufays (Vierzet), near Liège, from wood, 1897, V. Mouton 427 (**holotype** BR 5020105315705).

Description adapted from Mouton (1897, under *Sporormia subticinensis*). *Mycelium* immersed and superficial, composed of septate, branched, anastomosed, hyaline, smooth-walled, 2–3.5 μm wide hyphae. *Sexual morph:* *Ascomata* perithecial, solitary or aggregated, immersed to semi-immersed, pyriform, 260–400 μm , black; ostiolar beak 120–160 $\times$ 200–250 μm ; peridium 5–8-layered, smooth, dark brown, with cells of *textura angularis*, 2.5–5 μm , sometimes covered with some pigmented hyphae. *Paraphyses* septate, filiform, 2 μm wide, hyaline. *Asci* 8-spored, subcylindrical-clavate, 130–160 $\times$ 20–30 μm (150–220 $\times$ 30–40 μm in CBS 354.86), with short stipe, 12–19 μm in length. *Ascospores* 8-celled, subcylindrical, curved, 60–70 $\times$ 10 μm (72–108 $\times$ 14–19 μm in CBS 354.86), septa transverse, constrictions at septa deep, easily

separating into single cells, hyaline at first, brown and thick-walled in maturity; central cells rectangular in vertical section, triangular in transverse section, 10 $\times$ 8 μm ; apical cells longer than broad, 12 $\times$ 9 μm (15–18 $\times$ 12–25 μm in CBS 354.86); germ slit diagonal, faint, slightly sinuous; gelatinous sheath absent; sometimes cells remain aseptate, reaching up to 20 μm in length. *Coelomycetous asexual morph:* *Conidiomata* pycnidial, solitary, immersed to erumpent, brown, subglobose, 150 μm diam., with central ostiole, 10–15 μm diam.; wall of 3–6 layers, with cells of brown *textura angularis*. *Conidiogenous cells* phialidic, cylindrical, 8.5 $\times$ 3 μm , hyaline, smooth. *Conidia* aseptate, subglobose, 2.5–4 $\times$ 2 μm , hyaline, smooth, guttulate, aggregated in white mucous masses.

Culture characteristics (after 14 d at 25 °C): Colonies on PDA reaching 28 mm diam., crateriform, velvety, light grey (1D1) to white (1A1) towards periphery, margin entire; reverse concolourous. On OA, attaining 33–35 mm diam., flat, floccose, dark grey (1F1) to greyish green (1D3) and colourless towards periphery, margin submerged and entire; reverse concolourous. On CMA, reaching 34–38 mm diam., flat, velvety, dark grey (1F1) to greyish green (1D3) and colourless towards periphery, margin submerged and entire; reverse concolourous.

Additional descriptions and illustration: Von Arx & van der Aa (1987).

Additional materials examined: **Bolivia**, from soil, Oct. 1987, J.B. Speakman (CBS 539.87, received as *Sn. tenerifae*). **France**, Toulouse region, from vineyard soil with copper accumulation,

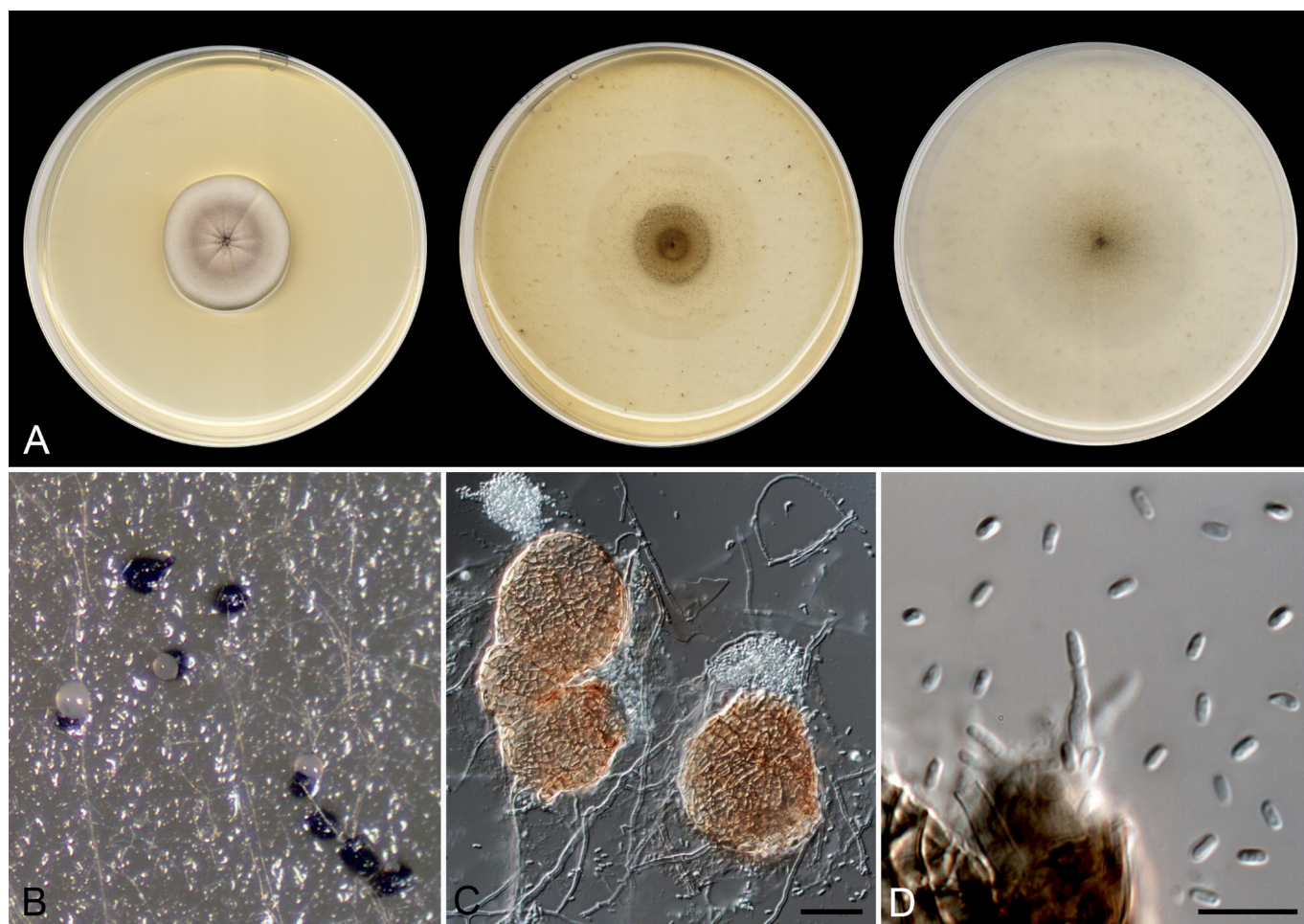


Fig. 17. *Spororminula irregularis* (ex-type CF-295384). **A.** Colonies on PDA, OA, and CMA after 14 d at 25 °C. **B, C.** Pycnidia. **D.** Conidiogenous cells and conidia. Scale bars: C = 25 μm ; D = 10 μm .

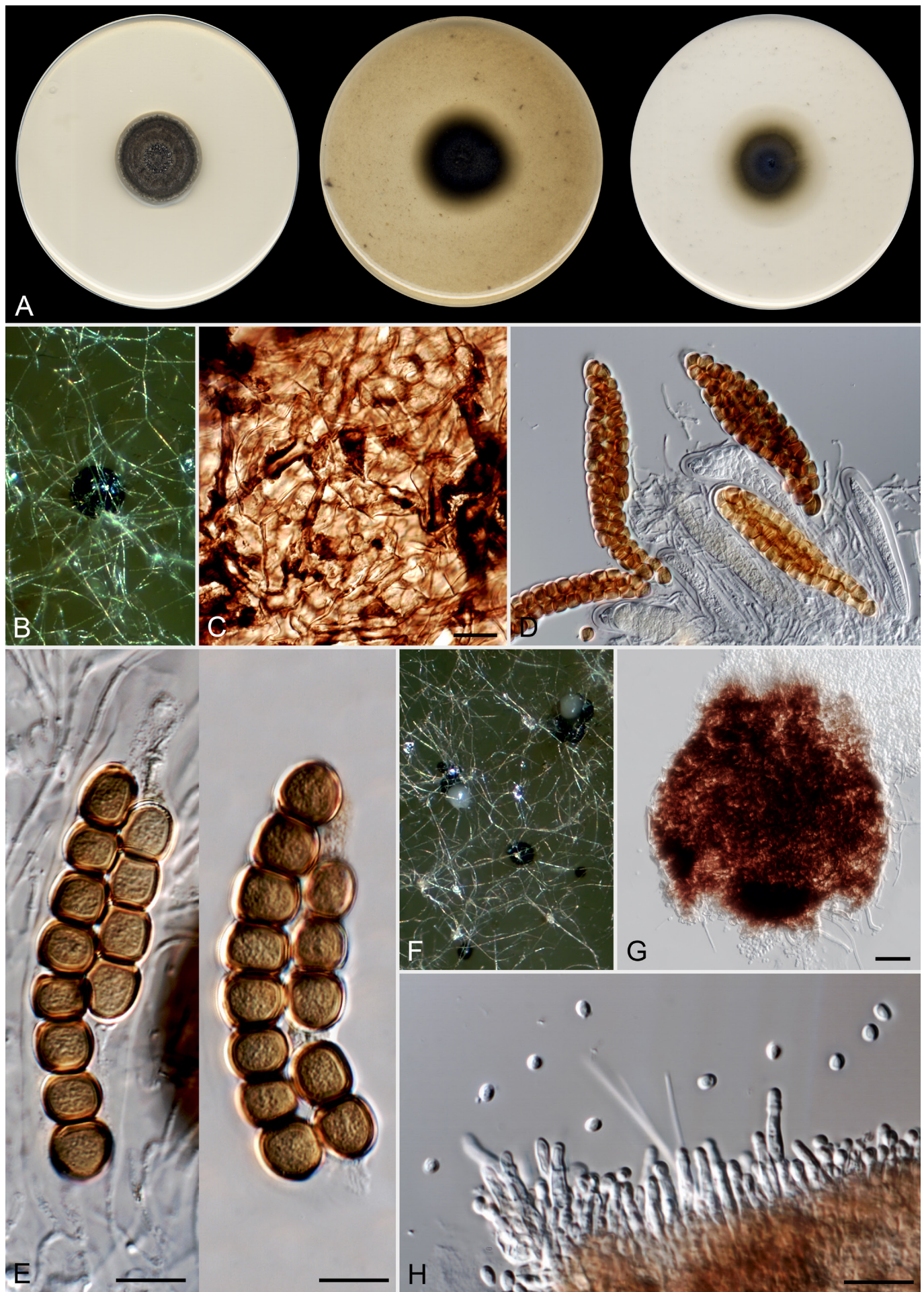


Fig. 18. *Spororminula subticinensis* (FMR 20474). A. Colonies on PDA, OA, and CMA after 14 d at 25 °C. B. Perithecium. C. Peridium with *textura angularis*. D. Asci and paraphyses. E. Ascospores. F, G. Pycnidia. H. Conidiogenous cells and conidia. Scale bars = 10 µm.



Table 5. Bioactive secondary metabolites detected in *Preussia* s.l. with updated species identification.

Metabolite	Current ID	Original ID	Major nutrient source	Fermentation state	Biological activity	Strain ¹	Barcode available	Reference
Asterric acid	<i>Sporormiella persica</i>	<i>Preussia</i> sp.	Rice-based	Liquid	Endothelin binding inhibitor	CAD4	Yes	Talontsi et al. (2014)
Preussiaturan A, B	<i>Sporormiella persica</i>	<i>Preussia</i> sp.	Rice-based	Liquid	Cytotoxic	CAD4	Yes	Talontsi et al. (2014)
Cisetin	<i>Sporormiella persica</i>	<i>Preussia</i> sp.	Rice-based	Liquid	Antibacterial	CAD4	Yes	Talontsi et al. (2014)
Auranticins A, B	<i>Sporormiella intermedia</i>	<i>Preussia aurantiaca</i>	Potato Dextrose Broth	Liquid	Antifungal and antibacterial	ATCC 14745	No	Poch & Gloer (1991)
7-Chloro-6-methoxymellein		<i>Preussia affinis</i>	Corn starch and molasses	Liquid	Antifungal	Lederle culture N313	No	McGahren & Mitscher (1968)
Antifungal FR 173945		<i>Sporormiella minima</i>	Starch flour	Liquid	Antifungal	15604	No	Hatori et al. (2004)
Antifungal FR 171456		<i>Sporormiella minima</i>	Starch flour	Liquid	Antifungal	15604	No	Hatori et al. (2004)
Australifungin	<i>Sporormiella intermedia</i>	<i>Sporormiella australis</i>	Corn-based	Solid	Antifungal	MF5672	Yes	Hensens et al. (1995)
C ₂₈ H ₄₂ N ₄ O ₈		<i>Sporormiella minimoides</i>	Corn-based	Liquid	Antifungal	MF5867	No	Clapp-Shapiro et al. (1998)
Cryptosporopsin		<i>Sporormia affinis</i>	Corn starch and molasses	Liquid	Antifungal	N313	No	McGahren et al. (1969)
Cyperin		<i>Preussia fleischhakkii</i>	Potato Dextrose Broth	Liquid	Antifungal	NRRL 6460	No	Weber & Gloer (1988)
Hyalopyrone	<i>Sporormiella intermedia</i>	<i>Sporormiella teretispora</i>	Potato Dextrose Broth	Liquid	Antifungal	JS 148	No	Wang et al. (1995)
Terezines A, B, C, D		<i>Sporormiella teretispora</i>	Soybean flour	Liquid	Antifungal and antibacterial	JS 148	No	Wang et al. (1995)
Leptosin A, C	<i>Preussia funiculata</i>	<i>Preussia typharum</i>	MMK2	Liquid	Cytotoxic and antifungal	TTI-1095	Yes	Perlati et al. (2021)
Preussiadin A, B	<i>Preussia funiculata</i>	<i>Preussia typharum</i>	Cheerios breakfast cereal	Solid	Cytotoxic	JX143871	Yes	Du et al. (2014)
Microspphaeropsone A	<i>Sporormiella intermedia</i>	<i>Sporormiella minima</i>	Rice-based	Solid	Antifungal and antibacterial	66342	No	Xiong et al. (2014)
Preussin		<i>Preussia</i> sp.	Soluble starch and glucose	Liquid	Antifungal	NA	NA	Johnson et al. (1989)
Preusochromone A, B, C, D		<i>Preussia africana</i>	Rice-based	Solid	Cytotoxic	DN136	Yes	Zhang et al. (2012)
Preussomerin A, D, E, F		<i>Preussia isomera</i>	Potato Dextrose Broth	Liquid	Antifungal and antibacterial	CBS 415.82	Yes	Weber & Gloer (1991)
Similin A, B	<i>Sporormiella africana</i>	<i>Preussia similis</i>	Soybean flour	Liquid	Antifungal	DM 87	No	Weber et al. (1992)
Spiropreussione		<i>Preussia</i> sp.	Wheat bran and glucose	Liquid	Cytotoxic	CGMCC 2022	No	Chen et al. (2009)
Sporminarin A, B		<i>Sporormiella minimoides</i>	Rice-based	Solid	Antifungal	NRRL 37629	Yes	Mudur et al. (2006)
Sporostatin		<i>Sporormiella</i> sp.	Glucose and peptone broth	Liquid	Cytotoxic	M5032	No	Kinoshita et al. (1997)
Sporovexins A, B, C	<i>Sporormiella intermedia</i>	<i>Sporormiella vexans</i>	Potato Dextrose Broth	Liquid	Antifungal and antibacterial	JS 306	No	Soman et al. (1999)
Zaragozic acid B		<i>Sporormiella intermedia</i>	Cerelose and cotton seed	Liquid	Antifungal	Several strains	No	Bills et al. (1994)



Table 5. (Continued).

Metabolite	Current ID	Original ID	Major nutrient source	Fermentation state	Biological activity	Strain ¹	Barcode available	Reference
5-hydroxy-2,7-dimethoxy-8-methylnaphthoquinone	<i>Sporormiella africana</i>	<i>Sporormiella minimoides</i>	Rice-based	Solid	hCaM inhibition	MEXU 26355	Yes	Leyte-Lugo et al. (2013)
C ₂₆ H ₂₂ O ₈		<i>Preussia</i> sp.	Potato Dextrose Broth	Liquid	Antioxidant and antibacterial	NA	NA	Paudel et al. (2018)
Preusisolactone A	<i>Sporormiella africana</i>	<i>Preussia isomera</i>	Malt extract broth	Liquid	Antifungal and antibacterial	XL-1326	Yes	Xu et al. (2019)
Verbalide A–F	<i>Sporormiella africana</i>	<i>Preussia africana</i>	Potato Dextrose Broth	Liquid	Antiviral	CPC 400972	No	Zhang et al. (2023)
Preussilides A, C	<i>Sporormiella similis</i>	<i>Preussia similis</i>	Oatmeal and molasses	Liquid	Cytotoxic and antifungal	DSM 104666	Yes	Noumeur et al. (2017)

¹ NA: not available.

unknown collection date, *J. Nicot* (CBS 155.67, ex-isotype strains of *Sp. perplexans*). **Germany**, Kiel-Kitzeberg, from wheat field soil, Jan. 1966, *W. Gams* (CBS 125.66, received as *Sp. subticinensis*, proposed here as reference strain). **Spain**, Canary Islands, Tenerife, Las Cañadas, from dung of unknown origin, Mar. 1986, *J.A. von Arx* (CBS 354.86, ex-type strain of *Spororminula tenerifae*); *ibid.*, Catalonia, Mediterranean coast, discharging area of the Ebro River, from marine sediments at 27 m of depth, Oct. 2022, *J. Gené & D. Guerra-Mateo* (FMR 20474).

Cardinal temperatures for growth: Minimum 5 °C (4 mm), optimum 25 °C (28–30 mm), maximum 40 °C (4 mm).

Secondary metabolites: Antibiotic LL-N 313ε, palmarumycins, 4-hydroxy-5-methylmellein, 7-chloro-6-methoxymellein, C₁₁H₁₀ClO₅, C₂₀H₁₄O₃.

Notes: *Sporormia subticinensis* was erected to represent a fungus characterized by subcylindrical, curved, 8-celled ascospores (Mouton 1897). This species was later transferred to *Preussia* based on morphology (von Arx 1973). Although molecular data from the ex-type strain of this fungus are not available, early phylogenetic analyses of *Preussia* s.l. using representative strains of *Pr. subticinensis* suggested a close relationship with the ex-type strain of *Sn. tenerifae* (Kruys & Wedin 2009). Despite morphological differences between strains of *Pr. subticinensis* examined in this study and the ex-type strain of *Sn. tenerifae* (von Arx & van der Aa 1987), our concatenated phylogenetic analysis both clustered species as a single conspecific taxon (Fig. 3). In addition, the isotype of *Sp. perplexans* (CBS 155.67) also clustered within this lineage, confirming they are the same species (von Arx & Storm 1967, Ahmed & Cain 1972). Since *Sp. subticinensis* represents the oldest name for this taxon, we propose the novel combination *Sn. subticinensis* in the benefit of nomenclatural stability and the preservation of extant names. In addition, we propose the strain CBS 125.66 as the reference strain for the species in phylogenetic analyses.

Here, we updated the morphological description of *Sn. subticinensis* with FMR 20474, which resembled the protologue in asci and ascospores shape and size ranges and, in addition, produced a pycnidial conidiomata in culture. See *Sn. irregularis* notes section for a detailed morphological comparison of asexual morphs. The ex-type strain of *Sn. tenerifae* (CBS 354.86) exhibited larger asci and ascospores and, according to von Arx & van der Aa (1987), they did not show a germinal slit. However, the ITS comparison of this strain with other strains in this lineage revealed a 100 % identity. Therefore, we interpret these morphological differences as intraspecific variability.

It is worth mentioning that *Sn. tenerifae* was transferred to the genus *Preussia* as *Pr. tenerifae* (Kruys & Wedin 2009). In Index Fungorum, the same name appears as *Pr. tenerifae* (von Arx & Guarro) Kruys, representing an update on the species *Asordaria tenerifae*. However, this synonym is incorrect as *Asordaria tenerifae* is a sordariomycetous species typically characterized by dark brown, aseptate ascospores (von Arx et al. 1987). Therefore, this should be regarded as a mistake resulting from the identical epithet and similar authorship of the two species.

Species of uncertain status

We have compiled a brief morphological description of the *Preussia* s.l. species with uncertain taxonomic status due to lack of molecular

data or additional representative strains (Supplementary material Table S3). In particular, we have referred to the *Sporormiella minima* species complex throughout the manuscript. Our phylogenetic analyses confirmed the species *Sm. isabellae*, *Sm. koreensis*, *Sm. mediterranea*, and *Sm. persica*; however, we could not define *Sm. minima* in the phylogeny. Accordingly, the taxonomic status of this species remains unresolved until further strains are examined.

DISCUSSION

This work aimed to characterize *Preussia* s.l. strains of marine and xerophytic origin and assess the diversity within the genus through a polyphasic approach, combining morphology, phylogeny, and metabolite profiles. The five-region concatenated phylogeny confirmed the genera *Preussia*, *Sporormiella*, and *Spororminula* as different lineages. In addition, we described four novel genera and 11 novel species in *Sporormiaceae*. The marine and xerophytic strains primarily represented novel species, provided additional asexual morphs, and expanded metabolite profiles. These results highlight that the diversity associated with unusual substrates can help resolve complex taxonomic groups.

Unravelling the diversity within *Preussia* s.l.

The confusing circumscription of *Preussia* and *Sporormiella* led to databases organizing species under both genera regardless of synonyms. In consequence, novel species were recently described in either genus indistinctively; i.e., *Pr. jejuensis*, *Pr. koreensis*, and *Sm. tela* (Jakob *et al.* 2024, Noh *et al.* 2025). Our results agree with recent phylogenies addressing *Sporormiaceae*, where *Preussia* s.l. was displayed as a polyphyletic genus (Thambugala *et al.* 2015, Crous *et al.* 2020a, Hongsanan *et al.* 2021). However, the limited representation of type specimens in the phylogeny hampered taxonomic decisions. We addressed this common situation in fungal taxonomy, aiming for the most natural classification possible. For this reason, we defined the genus *Sporormiella* based on the consistency of morphological features in clade 1, and delimited clade 4 as *Preussia* s.str. based on CBS 127.66, which we designated as the epitype culture of *Pr. funiculata* based on morphology and geographical origin (Cain 1961, Ahmed & Cain 1972). Among the remaining clades of *Preussia* s.l., clade 9 was determined as *Spororminula* based on its type species *Sn. subticinensis*, and we managed to describe four additional genera; i.e., *Chaetosporormiella*, *Pleospormiella*, *Sporormyces*, and *Spororvela*. In particular, the genera *Pleospormiella* and *Sporormyces* were described based on the strains recovered from marine and xerophytic substrates, which filled phylogenetic gaps in *Preussia* s.l. (Fig. 3). This way, our phylogenetic tree updated *Preussia* s.l. diversity and enabled the recognition of traditional and novel genera.

The taxonomic rank of genus indicates definable units with biological meaning that result from evolutionary processes (Crous *et al.* 2021). In this sense, genera can be recognized through morphological, metabolic, and ecological patterns. In Table 4, we provide a polyphasic characterization of the genera proposed in this work. This classification provides a robust backbone to understand *Preussia* s.l. diversity and stabilizes nomenclature. It is worth noting that these taxonomic decisions resulted in 18 novel combinations of species and one new name. Although best practices indicate that taxonomic decisions should lead to as few name changes as possible, we think that the complex taxonomic history of this group and its limited circumscription in previous phylogenetic analyses

support these nomenclatural changes (Kruys & Wedin 2009, González-Menéndez *et al.* 2017, Aime *et al.* 2021).

Up to 13 novel species of *Preussia* s.l. have been proposed in the last couple of years (Sandberg *et al.* 2022, Jakob *et al.* 2024, Eisvand & Mehrabi-Koushki 2025, Li *et al.* 2025, Noh *et al.* 2025, Phurbu *et al.* 2025). We proposed one of them as the novel genus *Spororvela*, five represented novel *Sporormiella* and *Preussia* s.str. species, while the rest clustered with representative strains of known species: i.e., *Pr. funiculata*, *Sm. lignicola*, *Sm. procaviae* and *Sm. similis* (Fig. 3). Most of these species lack molecular data derived from type specimens, which promoted the description of superfluous novel taxa taking advantage of intraspecific variability. Our phylogenetic analyses revealed the ITS region as a suitable barcode for species identification in *Sporormiaceae* (Figs 1, 2). Interestingly, the authors of some of the recent novel species overlooked the ITS region and relied on *tef1-α* despite more than 98 % similarity between strains (Sandberg *et al.* 2022, Eisvand & Mehrabi-Koushki 2025). Based on these minor phylogenetic distances accounting for intraspecific variability (Figs 2, 3), we defined reference strains to stabilize the taxonomy of older species and prevent the description of further superfluous taxa. Following best practices and previous works on different fungal groups, we refrained from designating unnecessary epitypes (Ariyawansa *et al.* 2014, Lendemer 2020, Bradshaw *et al.* 2024). Reference strains were selected based on morphological similarity to the protologue, availability of the culture, and historical use in phylogenies. We selected a lectotype or designated an epitype culture only when the original material was not indicated in the protologue, or when only herbarium material was available from the ex-type strain (See *Sm. intermedia* and *Pr. funiculata* taxonomy section).

This conservative approach, favouring nomenclature stability, also helped understand morphological diversity and ecological roles across lineages. Aside from sexual morphological differences, each genus produced different sets of asexual structures, with coelomycetous conidiomata as the most common across lineages (Table 4). It is noteworthy that *Pleospormiella* comprised species with chrysosporium- and humicola-like asexual morphs, and *Sporormiella* included *Sm. japonica*, a holothallic species that, to the extent of our knowledge, represents the first report of an opportunistic animal pathogen in *Preussia* s.l. (Li *et al.* 2025). Animal pathogens are rare in *Sporormiaceae*, with only the species *Westerdykella reniformis* reported as a causal agent of skin lesions in dogs that can disseminate to the kidney and vertebral disks (Hurst 2016).

Despite our current effort to disentangle the diversity within *Preussia* s.l., more than 70 known species remain to be assessed with molecular data. For now, we treat these species as taxa of uncertain classification (Supplementary material Table S3). Hopefully, projects targeting the sequencing of fungarium material will provide novel insights in the near future.

Chemotaxonomy: secondary metabolites across genera and species

Secondary metabolite profiles represent a powerful tool to aid in disentangling complex taxonomic groups, supplementing morphological and phylogenetic evidence. Metabolites have been successfully applied to confirm genus circumscriptions, like the genus *Pochonia* (*Clavicipitaceae*) by the production of pochonins, *Conoideocrella* (*Clavicipitaceae*) by zeorins, and *Pseudorhizophila* (*Naviculisporaceae*) by zopfinol (Stadler *et al.* 2003, Isaka *et al.* 2011, Harms *et al.* 2021). This approach has also been useful

to resolve species boundaries in cryptic groups like *Amesia*, *Ascochyta*, *Aspergillus*, *Cladobotryum*, *Cortinarius*, *Penicillium*, and *Talaromyces* (Frisvad 2015, Kim *et al.* 2016, Milic *et al.* 2022, Charria-Girón *et al.* 2023, Huymann *et al.* 2024). In this work, we combined metabolite profiles with morphology and phylogeny to understand the diversity within *Preussia* s.l. Since this group has been extensively assessed in search for novel bioactive molecules, we compiled these compounds in Table 5, where we updated species identification when possible. Overall, more than 30 compounds have been detected across *Preussia* s.l. lineages, many of them biologically active, while others still await a characterization (Table 5). In fact, here we recovered up to 15 molecules that should be studied in depth in future works.

The metabolite profiles developed in this work relied on the GNPS platform and MS/MS data, which enabled the creation of networks representing metabolite production across strains. This way, metabolites could be traced across the phylogeny to uncover patterns of production. Melleins represented the most common group of polyketide metabolites detected across the lineages studied (Table 3). We detected monocerin, 4-hydroxy-5-methylmellein, and 7-chloro-6-methoxymellein across *Chaetosporormiella* (clade 8), *Pleosporormiella* (clade 3), *Preussia* (clade 4), and *Spororminula* (clade 9). In particular, 7-chloro-6-methoxymellein was the only compound already associated with *Preussia* s.l., recovered from a strain identified as “*Sporormiella affinis*” (McGahren & Mitscher 1968, Reveglia *et al.* 2020). Although we detected this molecule in the aforementioned genera, our phylogeny located *Sm. affinis* in clade 7, suggesting a transversal production across lineages 3–9. It is also noteworthy that monocerin was primarily obtained from strains of marine origin in *Pleosporormiella* and *Spororminula* (Fig. 5A). These compounds are typically associated with common plant pathogens displaying phytotoxic activity, but also antifungal and antibacterial properties (Reveglia *et al.* 2020, Gomes *et al.* 2023). However, the production of this compound *in vivo* should be tested before drawing ecological conclusions.

Dihydroisoflavipucine represented another compound detected across lineages (Fig. 4). This molecule was described from a phoma-like fungus (Loesgen *et al.* 2011). Interestingly, we exclusively recovered it from strains of *Chaetosporormiella* and *Pleosporormiella* displaying phoma-like, humicola-like, and monodictys-like asexual morphs (Table 3). Sporulation processes affect the production of metabolites, and the asexual stage of these strains may have triggered the production of this molecule across lineages (Calvo *et al.* 2002).

Networks also enabled the identification of metabolites exclusively produced by certain genera that could potentially represent chemotaxonomic markers. Regarding the genus *Sporormiella*, we included three strains in the metabolite profile, and one of them produced preussochromone F (Fig. 7D). The previous chemotaxonomic study of *Preussia* s.l. detected preussochromones as common compounds across *Sm. africana*, *Sm. isopartis*, and *Sm. koreensis* using the MMK2 medium (González-Menéndez *et al.* 2017). Other compounds with similar patterns were microsphaerones, detected in *Sm. africana* and *Sm. lignicola* with the same medium. We think these compounds could be used to characterize members of *Sporormiella* (Fig. 3). In fact, we also used MMK2 in the profiling of sister genera, but none produced these molecules (Table 3). In a similar manner, we only detected the production of the antibiotic LL-N 313ε, and the compounds C₁₁H₁₀ClO₅ and C₂₀H₁₄O₃ in *Spororminula* species. Moreover, *Sporormyces* could be characterized by the

production of 4-methoxy-6-pentyl-2H-pyran-2-one (Fig. 6B), but also 1,7-dihydroxy-3-(hydroxymethyl)-anthraquinone, which have been exclusively obtained in this lineage (González-Menéndez *et al.* 2017). The remaining genera produced different sets of secondary compounds, without a clear characteristic metabolite at the genus level. However, *Pleosporormiella* outstands as a metabolically active group with 16 different metabolites (Table 3).

We defined chemotypes to analyse the sets of metabolites produced by different strains (Table 3). In some cases, these chemotypes could be related to phylogenetic species to support species boundaries; i.e., *Pr. gypsicola* produced C₁₆H₂₀O₆, C₁₆H₁₈O₅, C₁₉H₂₄O₈S and C₄₃H₇₉NO₉, molecules that were not detected in *Pr. funiculata*, *Ps. suttonii* produced C₁₆H₂₃NO₃ and C₁₇H₂₅NO₂, while these molecules were absent in *Ps. polymorpha* fermentations, and *Sn. irregularis* differed from *Sn. tenerifae* by the production of monocerin (Table 3). We stated these metabolite relationships of production or absence of production based on the methodology followed here, which ensured that all strains fermented under the same conditions, ensuring the possibility of comparison (Frisvad 2012). However, some species were particularly prolific in metabolite production, like *Ps. polyspora*, which produced up to 12 different metabolites by different strains with only three of them produced simultaneously across strains. A similar pattern was observed in other species like *Pr. funiculata* and *Sm. africana*, with up to 17 secondary metabolites obtained from different strains of *Sm. africana* (Mudur *et al.* 2006, Zhang *et al.* 2012a, 2023, Leyte-Lugo *et al.* 2013, González-Menéndez *et al.* 2017).

In the case of *Pr. funiculata*, ten different metabolites have been detected, including leptosin C (Table 3, Du *et al.* 2014, González-Menéndez *et al.* 2017, Perlatti *et al.* 2021). In this work, we expected to obtain this compound across representatives of *Pr. funiculata* based on the strain TTI-1095 (Fig. 2), which was used to define the gene cluster of the compound (Perlatti *et al.* 2021). In fact, leptosin C had also been recovered from an additional strain, JX143871 (Du *et al.* 2014). Surprisingly, we detected cyperin and 7-chloro-6-methoxymellein, among two other undetermined molecules, from the strains of *Pr. funiculata* included in the metabolite profile, but only recovered leptosin analogues from strains of *Cp. monodictyoides* (Table 3). Leptosin C was detected in strains of *Pr. funiculata* using MMK2 and Cheerios breakfast cereal (Du *et al.* 2014, Perlatti *et al.* 2021). Although we also used MMK2 under similar incubation conditions, the substrate of isolation and intraspecific variability may have played a key role. The strains producing leptosin C were recovered from dung, while the strains examined in this work were obtained from soil samples. In addition, the clade of *Pr. funiculata* is characterized by morphological variability in asci and ascospore septa (See taxonomy section). We do not consider these morphological differences as indicative of a species complex, but they evidence intraspecific diversity, which may also be associated with metabolite production.

Finally, the strains of xerophytic and marine habitats examined here confirmed previous works promoting the exploration of these unusual substrates (Imhoff 2016, Sayed *et al.* 2020). These strains expanded the phylogenetic diversity associated with *Preussia* s.l., but also yielded a wide array of secondary metabolites. Assessing the biological activity of these compounds was beyond the scope of this work. However, we think that these novel insights into the diversity of *Preussia* s.l. represent the first step towards a more natural classification that ultimately will enable metabolite prediction and ease the search for novel bioactive compounds.

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DECLARATION ON CONFLICT OF INTEREST

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- Fig. S1.** *Sporormiella antarctica* (holotype LPS 3500). **A.** Fungarium label and collection data. **B.** Original drawings. **C.** Dung of *Bernicla antarctica* colonized by *Sm. antarctica*. **D.** Perithecia. **E.** Asci with mature ascospores.
- Fig. S2.** *Sporormiella australis* (holotype LPS 3521). **A.** Fungarium label and collection data. **B.** Dung of *Cavia australis* colonized by *Sm. australis*. **C.** Original drawings.
- Fig. S3.** *Sporormiella intermedia* (lectotype Rabenhorst Fungi Europaei No. 644). **A.** Fungarium label and collection data. **B.** Rabbit dung.
- Fig. S4.** *Sporormiella lignicola* (holotype K(M)131910). **A.** Fungarium label and collection data and wood of *Fraxinus excelsior* colonized by *Sm. lignicola*. **B.** Ascus. **C.** Ascospores. Scale bars: B = 25 µm; C = 10 µm.
- Fig. S5.** *Sporormiella pascua* (holotype M-0378428). **A.** Fungarium label, collection data, and dung colonized by *Sm. pascua*. **B.** Perithecia. **C.** Ascospores on dung. **D–E.** Ascospores. Scale bars: D = 25 µm; E = 10 µm.
- Fig. S6.** *Perisporium funiculatum* (holotype B700132488). **A.** Fungarium label. **B.** Ascomata on angiosperm pieces. **C–D.** Detail of the ascomata. Scale bars = 2 µm.
- Fig. S7.** Phylogenetic tree inferred from an ITS alignment of 165 strains representing the family *Sporormiaceae* in *Pleosporales*. The tree is rooted to *Lophiostoma rosae-ecae* MFLUCC 17-0807 and *Teichospora thailandica* MFLUCC 13-0284. Numbers at the branches indicate support values (IQ-TREE-UFBS) above 90 %. The scale bar represents the expected number of changes per site.
- Fig. S8.** Phylogenetic tree inferred from the LSU alignment of 165 strains representing the family *Sporormiaceae* in *Pleosporales*. The tree is rooted to *Lophiostoma rosae-ecae* MFLUCC 17-0807 and *Teichospora thailandica* MFLUCC 13-0284. Numbers at the branches indicate support values (IQ-TREE-UFBS) above 90 %. The scale bar represents the expected number of changes per site.
- Fig. S9.** Phylogenetic tree inferred from a *tef1-α* alignment of 111 strains representing *Preussia* s.l. and associated genera. The tree is rooted to *Sparticola junci* MFLUCC 15-0030. Numbers at the branches indicate support values (IQ-TREE-UFBS) above 90 %. The scale bar represents the expected number of changes per site.
- Fig. S10.** Phylogenetic tree inferred from a *rpb2* alignment of 111 strains representing *Preussia* s.l. and associated genera. The tree is rooted to *Sparticola junci* MFLUCC 15-0030. Numbers at the branches indicate support values (IQ-TREE-UFBS) above 90 %. The scale bar represents the expected number of changes per site.

Fig. S11. Phylogenetic tree inferred from a concatenated ITS–LSU alignment of 28 strains, representing the *Sporormiella minima* species complex and related taxa. The tree is rooted to representative strains of the genus *Westerdykella*. Numbers at the branches indicate support values (IQ-TREE-UFBS) above 90 %. Ex-type strains are indicated with ^T, and those corresponding to the accepted species are highlighted in grey. Novel combinations are highlighted in brown. The scale bar represents the expected number of changes per site.

Fig. S12. Speciation analysis of *Pleosporormiella*. The tree represents the bPTP analyses of the concatenated ITS–LSU alignment. Blue lines indicate speciation events and red lines comprise species. The topology obtained through the ASAP analysis is represented on the side. Incongruent topologies are highlighted in red.

Fig. S13. Speciation analysis of *Preussia* s.str. The tree represents the bPTP analyses of the concatenated ITS–LSU alignment. Blue lines indicate speciation events and red lines comprise species. The topology obtained through the ASAP analysis is represented on the side. Incongruent topologies are highlighted in red.

Table S1. Collection details from the strains studied in the present work and GenBank accession numbers of the barcodes used for identification.

Table S2. Potential metabolite hits obtained in the Chapman & Hall Dictionary of Natural Products (v. 25.1) for those compounds unassigned in Table 2.

Table S3. Species of *Preussia* s.l. with uncertain taxonomic status.