



Sequence data from isolated lichen-associated melanized fungi enhance delimitation of two new lineages within Chaetothyriomycetidae

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Abstract

Lichen thalli provide a long-lived and stable habitat for colonization by a wide range of microorganisms. Increased interest in these lichen-associated microbial communities has revealed an impressive diversity of fungi, including several novel lineages which still await formal taxonomic recognition. Among these, members of the Eurotiomycetes and Dothideomycetes usually occur asymptotically in the lichen thalli, even if they share ancestry with fungi that may be parasitic on their host. Mycelia of the isolates are characterized by melanized cell walls and the fungi display exclusively asexual propagation. Their taxonomic placement requires, therefore, the use of DNA sequence data. Here, we consider recently published sequence data from lichen-associated fungi and characterize and formally describe two new, individually monophyletic lineages at family, genus, and species levels. The Pleostigmataceae fam. nov. and *Melanina* gen. nov. both comprise rock-inhabiting fungi that associate with epilithic, crust-forming lichens in subalpine habitats. The phylogenetic placement and the monophyly of Pleostigmataceae lack statistical support, but the family was resolved as sister to the order Verrucariales. This family comprises the species *Pleostigma alpinum* sp. nov., *P. frigidum* sp. nov., *P. jungermannicola*, and *P. lichenophilum* sp. nov. The placement of the genus *Melanina* is supported as a lineage within the Chaetothyriales. To date, this genus comprises the single species *M. gunde-cimermaniae* sp. nov. and forms a sister group to a large lineage including Herpotrichiellaceae, Chaetothyriaceae, Cyphellophoraceae, and Trichomeriaceae. The new phylogenetic analysis of the subclass Chaetothyriomycetidae provides new insight into genus and family level delimitation and classification of this ecologically diverse group of fungi.

Keywords Black yeast · Fungal lifestyles · *Melanina* · Ribosomal RNA · nucLSU · *Pleostigma*

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Introduction

Lichen symbioses give rise to unique structures. The lichen thallus is that of a shape-determining fungus, the mycobiont, while one or more phototrophic microorganisms, the photobionts (green algae or cyanobacteria), are hosted extracellularly, entangled in a network of fungal hyphae (Hawksworth and Honegger 1994). The lichen thallus is an open, usually long-lived system, which with age provides a habitat for a growing number of additional microorganisms. These comprise other fungi (filamentous and yeast-like), microalgae, and bacteria (Petrini et al. 1990; Hofstetter et al. 2007; Spribille 2018). During the past decade, these complementary microorganisms have become subject of research to understand their role in the lichen symbiosis (e.g., Grube et al. 2009, 2015; Moya et al. 2017; Molins et al. 2018; Muggia and Grube 2018; Spribille 2018).

Lichen-inhabiting fungi have long been recognized in morpho-anatomical analyses (Lawrey and Diederich 2003; Diederich et al. 2018; Muggia and Grube 2018). These lichenicolous fungi cause diagnostic symptoms or formations on their specific lichen hosts, such as recognizable reproductive structures, gall-like hypertrophifications, melanized or discolored patches, or senescence of thallus (Lawrey and Diederich 2003). Interactions may occur with diverse degrees of specificity with the photo- or the mycobiont, or with the intercellular matrix of the host (de los Rios et al., 2002). Recently, some of them were included in molecular phylogenetic studies (e.g., Untereiner et al. 2011; Diederich et al. 2013; Suija et al. 2015; Muggia et al. 2015, 2019; Ertz et al. 2016). Various other types of trophic interactions between these lichen-associated fungi and their hosts are noted; however, the role in the symbiosis of these symptomatic commensals is less easy to understand than that of destructive pathogens. Basidiomycetous yeasts have been suggested to act as ubiquitous third symbionts in the upper cortex of lichen thalli (Spribille et al. 2016), although other authors failed to find them in metagenomic surveys (Lendemmer et al. 2019; Smith et al. 2020). Microscopy, culture-based experiments, and metabarcoding analyses have emphasized that many other fungi use lichens as a habitat in a hidden life cycle (Petrini et al. 1990; Harutyunyan et al. 2008; U'Ren et al. 2012; Muggia et al. 2016; Gueidan et al. 2019). Fungi with these associations have become known as “endolichenic fungi,” as they were isolated from surface-sterilized lichens (Arnold et al. 2009). An unambiguous distinction between endolichenic and lichenicolous lifestyles is difficult since the latter are also known to invade the host thallus. In general, it has become clear that lichens are inhabited by a plethora of other fungi that comprise specific and generalist fractions (Fernandez-Mendoza et al., 2017; Banchi et al. 2018; Muggia and Grube 2018; U'Ren et al. 2019). The lichen mycobiome changes with ecological and growth parameters

of the lichen host, but precise interactions have still to be unraveled.

Fluctuations of temperature and humidity are characteristic for sun-exposed rock surfaces. Despite the extreme conditions, these habitats share species-rich lichen communities. The thalli of the epilithic lichens are commonly colonized by filamentous ascomycetes, which are closely related to rock-inhabiting or pathogenic black yeasts in the classes Dothideomycetes and Eurotiomycetes (Harutyunyan et al. 2008; Muggia et al. 2016, 2017; Muggia and Grube 2018). Typically, these fungi have dark mycelia due to the accumulation of melanin in the cell walls. The pigmentation confers resistance to high radiation and other abiotic stress conditions, such as desiccation and extreme temperatures (Gostinčar et al. 2012, 2018).

In previous studies, Muggia et al. (2016, 2017) provided data on a comprehensive set of strains of culturable fungi isolated from lichens surveyed in an alpine habitat. In addition, an overview was made of their phylogenetic relationships, with special emphasis on members of Dothideomycetes and Eurotiomycetes. Some of the lineages represented earlier described lichenicolous fungi within Chaetothyriomycetidae, specifically in the orders Chaetothyriales and Verrucariales (Muggia et al. 2015, 2019). Others remained unnamed and awaited formal description, anticipating additional knowledge of the extant diversity of these fungi. Relationships within the order Chaetothyriales have been elucidated by Teixeira et al. (2017) and Quan et al. (2020). The enlarged DNA sequence dataset of Chaetothyriomycetidae now available (e.g., Chomnunti et al. 2012; Gueidan et al. 2014; Vasse et al. 2017) facilitates phylogenetic placements of the yet-unnamed lineages of lichen-inhabiting black fungi. This enables fulfillment of a pressing need to establish the nature of yet-unnamed lineages. Here we re-consider these yet-unnamed lineages of lichen-inhabiting black fungi and present a formal description of one new family, one new genus, and four new species. We also reappraise the ancestral lineages within the Chaetothyriales-Verrucariales clade by presenting a phylogenetic analysis for the major orders and families within Chaetothyriomycetidae and discuss on the evolution of their different life styles.

Materials and methods

Origin of samples: sampling and culture isolation

The here analyzed lichen-associated fungi were isolated from lichen thalli collected during a survey done as part of a research project carried out between 2012 and 2015 by LM and MG. The lichens were sampled in an Alpine habitat on siliceous-schist and gneissic boulders and cliffs on the Koralpe mountain range in the south-eastern rim of the

Austrian Alps, between the regions Styria and Carinthia. This mountain region has been characterized for its peculiar flora, as it was not covered by ice during the last ice age (i.e., representing a nunatak; van Husen 1987). Crust-forming lichen thalli were selected (Fig. 1) as reported previously in Fleischhacker et al. (2015) and Muggia et al. (2016, 2017).

The lichen-associated fungi were isolated and cultured following the protocol of Yamamoto et al. (2002) as described in Muggia et al. (2016, 2017). The inocula were placed on six different media in order to promote the growth of different fungi as reported by Muggia et al. (2017). The identity of the isolated fungi was checked by sequencing nuclear and mitochondrial ribosomal RNA gene fragments (Muggia et al. 2016, 2017) and cultures of representative strains were kept alive on agar plates using the same growth medium on which the inoculum was originally isolated successfully. All cultured strains reported in the present study are deposited and are preserved both in a metabolically inactive state and as cryostocks at the University of Graz in the culture collection of the first author (LMCC).

Morphological analyses of cultured strains

Morphological and anatomical characters of the cultured strains included in the phylogenetic inference and representing the two newly recognized lineages (*Melanina* gen. nov. and Pleostigmaceae fam. nov., see below) were analyzed using standard microscopic techniques and documented with digital photographs. Analyses and photographs were performed on

10-month-old to 1-year-old subcultures considering the following characters: form of growth (filamentous vs. yeast-like), form and size of hyphal cells, branching of hyphae, development of conidiogenous cells and formation of conidia, melanization. Small fragments of mycelia were removed, and squashed sections were mounted in water and studied by light microscopy. Images were acquired with a ZeissAxioCam MRc5 digital camera fitted to the microscope. Both images of growth habit and hyphae structure were digitally processed using the CombineZM software (www.hadleyweb.pwp.blueyonder.co.uk). Photos were slightly refined in sharpness and color tone with Adobe Photoshop 7.0, and the figures were prepared with CorelDRAW X4.

Molecular analyses: DNA extraction, amplification, sequencing, and identification

Sequence data of the studied strains were already available in GenBank as they were published previously by Muggia et al. (2016, 2017). Here, we used the data which were generated for the nuLSU fragment, while additional ITS sequences were recently generated and included in the analyses of Quan et al. (2020). The nuLSU fragment was amplified and sequenced in two parts using primers SR6R (<http://www.botany.duke.edu/>) and LR5 (upstream), and primers LR3R and LR7 (Vilgalys and Hester 1990) (downstream), as reported by Muggia et al. (2016, 2017). The preparation of the alignment was done in BioEdit (Hall 1999).

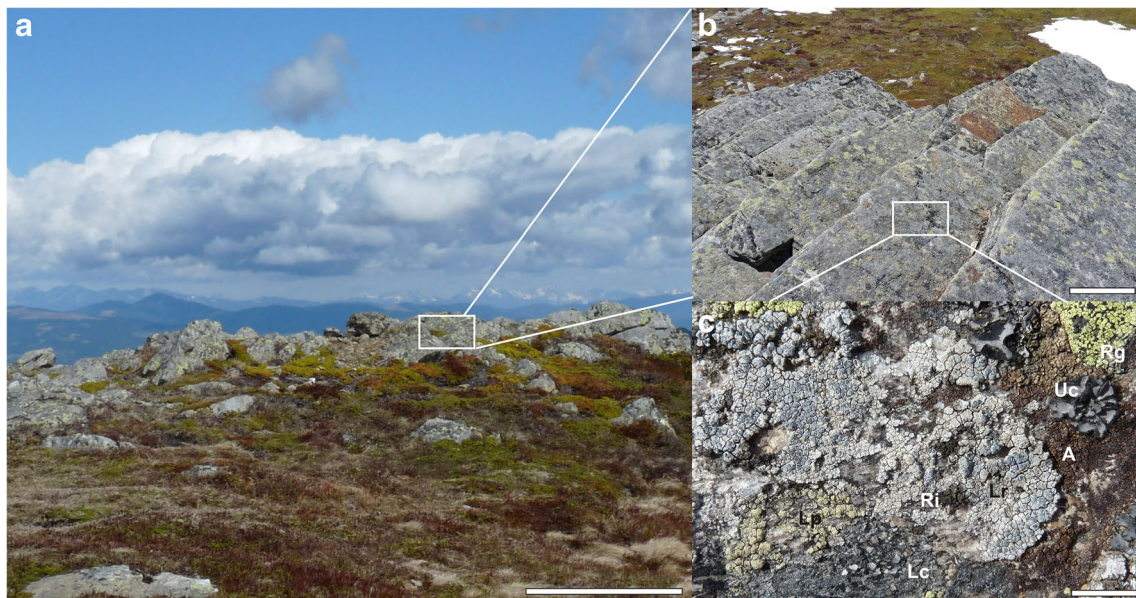


Fig. 1 Ecology of the lichen communities from which the studied chaetothyrialean fungi were isolated. **a** Overview of the alpine environment with siliceous-schist and gneissic rocks scattered in wide areas of pastures or dwarf shrub formations at 2000 m a.s.l. **b** Detailed view of the rock surfaces. **c** Detailed view of the density of the lichen

thalli on rocks; the lichen species are *Acarospora* sp. (A), *Lecidea* sp. (Lc), *Lecanora polytropa* (Lp), *Lecanora rupicola* (Lr), *Rimularia insularis* (Ri), *Rhizocarpon geographicum* (rg), *Umbilicaria cylindrica* (Uc). Scale bars: **a**, 2 m; **b**, 20 cm; **c**, 2 cm

Alignment and phylogenetic analyses

Taxon sampling was that of Muggia et al. (2016, 2017) supplemented with relevant sequences available in GenBank. The selection of taxa also considered phylogenetic analyses of Gueidan et al. (2007, 2008, 2011, 2014), Untereiner et al. (2011), Teixeira et al. (2017), Réblová et al. (2017a,b), and Muggia et al. (2015, 2016, 2017, 2019). The present dataset comprises nucLSU sequences belonging to 207 Eurotiomycetes covering the entire class (Table S1), all taxa being represented by a single nucLSU sequence. Of these, 196 taxa are representatives of the subclass Chaetothyriomycetidae, including its four orders: Chaetothyriales (with families Chaetothyriaceae, Cyphellophoraceae, Epibryaceae, Herpotrichiellaceae, Trichomeriaceae, and the “unnamed lineage 3” sensu Gueidan et al. (2014). Phaeomoniellales, Pyrenulales, and Verrucariales (with its sole family Verrucariaceae) and the closely related “unnamed lineage 2” sensu Gueidan et al. (2014). The order Onygenales (Eurotiomycetidae) is represented by four species, and the Sclerococcales by two species (Sclerococomycetidae; corresponding to the “unnamed lineage 1” sensu Gueidan et al. (2014). The phylogeny was rooted with three taxa belonging to Mycocaliciales (Mycocaliciomycetidae), i.e., *Chaenotheca savonica*, *Sphinctrina turbinata*, and *Stenocybe pullatula*. The selection of the nucLSU locus alone in our phylogenetic analysis is due to the fact that sequence data for the nucSSU and mtSSU loci are missing for the majority of the compared taxa. However, the identity of cultured fungal strains was originally studied also using partial sequences of the nuclear large and nuclear small ribosomal subunits and the mitochondrial small ribosomal subunit (Muggia et al. 2016, 2017).

Introns and ambiguously aligned regions were removed from the alignment. The nucLSU dataset was analyzed with a maximum likelihood (ML) (Mason-Gamer and Kellogg 1996; Reeb et al. 2004) and a Bayesian approach. The final sequence alignment used for the analyses is available as supplementary material. The ML analysis was performed using the program RAxML v8.2 (Stamatakis 2014) applying the GTRGAMMA model and running 1000 bootstrap replicates. The Bayesian analysis was run with the program MrBayes v3.2.5 (Ronquist et al. 2011). Two runs of four simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled every 100th generation. MCMC-heated chain was set with a “temperature” value of 0.15. The distribution of log-likelihood scores was examined using the program Tracer v1.5 (Rambaut and Drummond 2009) to determine that stationary phase for each search was reached and chains had achieved convergence. The first 25% of the sampled topologies were discarded as part of a burn-in procedure, while the remaining trees were used for calculating the posterior probabilities in the majority rule consensus tree. The convergence of the chains was also confirmed by the convergent diagnostic of

the potential scale reduction factor (PSRF), which approached 1 (Ronquist et al. 2011). The phylogenetic trees were visualized in TreeView v1.6.6 (Page 1996).

Results

Phylogeny

ML and Bayesian analyses were found to be topologically concordant with each other (Fig. 2) and with previously and presently published phylogenetic inferences of Eurotiomycetes based on nucLSU and further combined nuclear and mitochondrial loci (Gueidan et al. 2008, 2011, 2014; Teixeira et al. 2017; Muggia et al. 2015, 2016, 2017, 2019; Quan et al. 2020). All major orders in Chaetothyriomycetidae and all families in Chaetothyriales are fully resolved as monophyletic and highly supported (95–100% bootstrap value, PP > 95%). The order Verrucariales is here only represented by the family Verrucariaceae. This family includes lichenized taxa and a small lineage represented by endolichenic fungi, recently reported by Muggia et al. (2019). The latter group likely represents lichenicolous fungal species of the genus *Muellerella*.

The majority of sequences belonging to the endolichenic fungi are resolved into two main lineages, here named Pleostigmaceae fam. nov. and *Melanina* gen. nov., while the five remaining sequences are placed in the *Cladophialophora* clade within Herpotrichiellaceae (A1033, A1044, A1069, A1102 and A1123), one in Epibryaceae (A515), and one in Pyrenulales (A1133). The two new lineages of Pleostigmaceae and *Melanina* correspond to those highlighted in Gueidan et al. (2014) and Réblová et al. (2017a, b) under the names “unnamed lineage 2” and “unnamed lineage 3,” and in Muggia et al. (2016, 2017) under the names “clade IV” and “clade V,” “clade VI,” and “clade VII”, respectively. This correspondence is here evident by the fact that the three sequences of Chaetothyriales TRN242, Chaetothyriales A14, and *Pleostigma jungermannicola* M174—representing the original “unnamed lineage 2” sensu Gueidan et al. (2014)—cluster with the strains recognized as “clade IV” and “clade V” sensu Muggia et al. (2016, 2017), i.e., the new family Pleostigmataceae. Similarly, sequences of the two rock-inhabiting fungi TRN508 and TRN210—originally recognized as “unnamed lineage 3” sensu Gueidan et al. (2014)—cluster with the strains of “clade VI” and “clade VII” sensu Muggia et al. (2016, 2017), i.e., the new genus *Melanina*.

The new family Pleostigmataceae is not statistically supported but is placed sister to the Verrucariales, and both are sister to the Chaetothyriales, although none of these relationships is supported (Fig. 2). Members of the Pleostigmataceae are morphologically highly similar, but sequence diversity allows

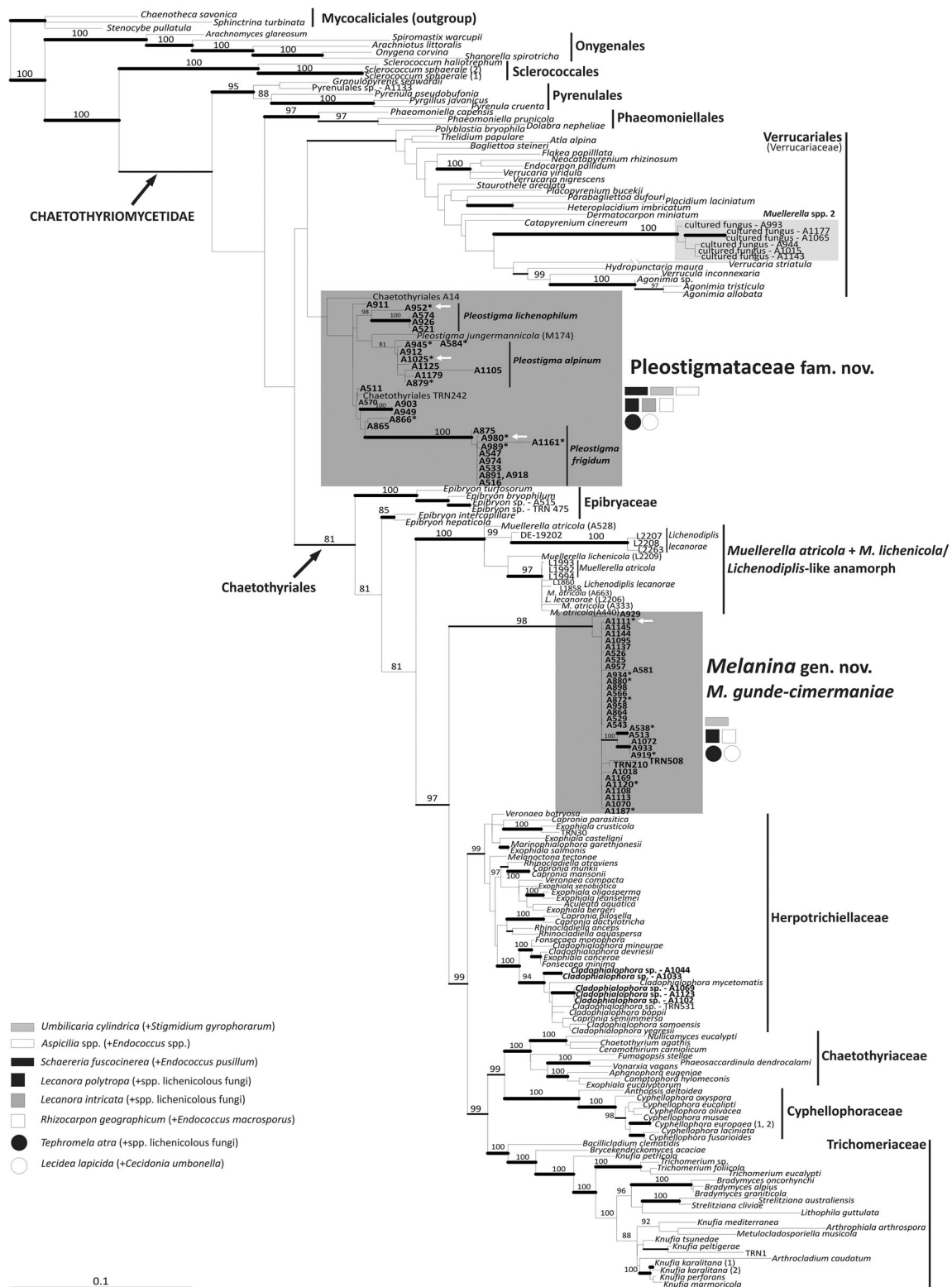


Fig. 2 Maximum likelihood phylogenetic analysis based on the nucLSU sequences of Eurotiomycetes. The ML and Bayesian topology were identical. The newly identified lineages of Pleostigmataceae and *Melanina* are highlighted and strain numbers are reported in bold. The type strains are indicated by a white arrow. Asterisks mark the samples in

the photo tables (Figs. 4, 5, and 6). Posterior probability (PP) values > 80 are reported above branches, while ML bootstrap supports are indicated by the thickness of the branches: the thickest, fully supported 96–100%; middle thick, well-supported 70–95%; no thickness, poorly supported lower than 70%. The scale bar is proportional to the substitution rate

recognizing three highly supported (PP > 98%; bootstrap values > 80) sub-clades, which are here described as new species. The three new species *Pleostigma alpinum*, *P. frigidum*, and *P. lichenophilum* are well-supported either by ML or Bayesian analyses, or both. In addition, six strains of endolichenic fungi and the sample Chaetothyriales TRN242 are basal to *P. frigidum* but do not form a separate monophyletic clade. This placement still prevents either their inclusion in the *P. frigidum* clade, or to recognized them as a separate taxonomic unit. The sequence of *Pleostigma jungermannicola* M174 is basal to *Pleostigma alpinum*, albeit unsupported. The sample Chaetothyriales A14 is an individual branch at the base of the whole Pleostigmataceae lineage. The entire Pleostigmataceae lineage is unsupported, and this might impair its reliable phylogenetic placement inferred here.

The order Chaetothyriales includes (i) the family Epibryaceae, which is paraphyletic and basal to the order, splitting into two highly supported lineages; (ii) the monophyletic clade of the ascus-forming species *Muellerella atricola* + *M. lichenicola* and their asexual form *Lichenodiplis* (as recognized in Muggia et al. 2015, 2019); (iii) the new monophyletic lineage *Melanina* gen. nov. represented by strains which are genetically almost identical and are described as the new species *M. gunde-cimermaniae*; and (iv) the remaining families Chaetothyriaceae, Cyphellophoraceae, Herpotrichiellaceae, and Trichomeriaceae.

Morphology

The new lineages Pleostigmataceae and *Melanina* share similar morphological traits (Figs. 3, 4, 5, and 6), although they are clearly not closely related phylogenetically. Strains of both lineages are characterized by dense, melanized, mainly filamentous mycelia, which grow in a compact form on agar media (Figs. 3a–f; 4 a–o, r–u, z; 5a–k, n–s; and 6a–g, k, l). In some cases, the mycelia grow in a yeast-like form, producing a rather gelatinous mass from which filamentous hyphae extend (Figs. 4n and 6c, g, l). Peripheral hyphae and hyphal tips, being the younger parts of the mycelium, are not homogeneously melanized, sometimes almost hyaline (Figs. 3c, d, f; 4q, w–y; 5l, m; and 6o, q, r) conferring a paler tone to the surface of the mycelium. Intense melanization is invariably observed in the hyphae of the central, older parts of the colonies (e.g., Fig. 5e, g–k, n–s). In Pleostigmataceae, hyphal cells are rather elongated with a rectangular shape, but usually assume a spherical form, in correspondence with the branching (Figs. 4b, f, g, k, m and 5e, g, h, k, n–p, s). These spherical cells either subtend multiple branches or they divide in a meristematic fashion at the site where each cell generates a ramification (Fig. 4b, f, g, k, m). In *Melanina*, hyphae are composed of smaller cells, usually spherical and generated by lateral and apical cell divisions (Fig. 6h, i, m–v) and clumps of conidia have been observed (Fig. 5h, j).

Detailed descriptions of morphological traits of each new lineage are reported below in the “Taxonomy” section.

Taxonomy

Pleostigmataceae Grube, Muggia, de Hoog, **fam. nov.**—Mycobank: MB838771 (Figs. 3, 4, and 5)

Etymology. The family is named after the genus *Pleostigma*, which is here identified to belong to Chaetothyriomycetidae.

Type genus: *Pleostigma* Kirschst.—Mycobank MB4236 (Figs. 3, 4, and 5)

Diagnosis: melanized filamentous fungi, endolichenic and bryophilous, mycelium dark gray to black, hyphae septate, hyaline to heavily melanized, toruloid, yeast-like growth observed in cultured mycelium

Incertae sedis family, phylogenetically close to the orders Verrucariales and Chaetothyriales, Ascomycota, containing the genus *Pleostigma*. Comprising bryosymbiotic, rock-inhabiting and endolichenic melanized fungi; for the latter group, the sexual morph is unknown.

Pleostigma Kirschst.—Annals Mycol. 37(1/2): 91 (1939)—Mycobank: MB4236

Pleostigma alpinum Grube, Muggia, de Hoog, **sp. nov.**—Mycobank: MB838768 (Fig. 3)

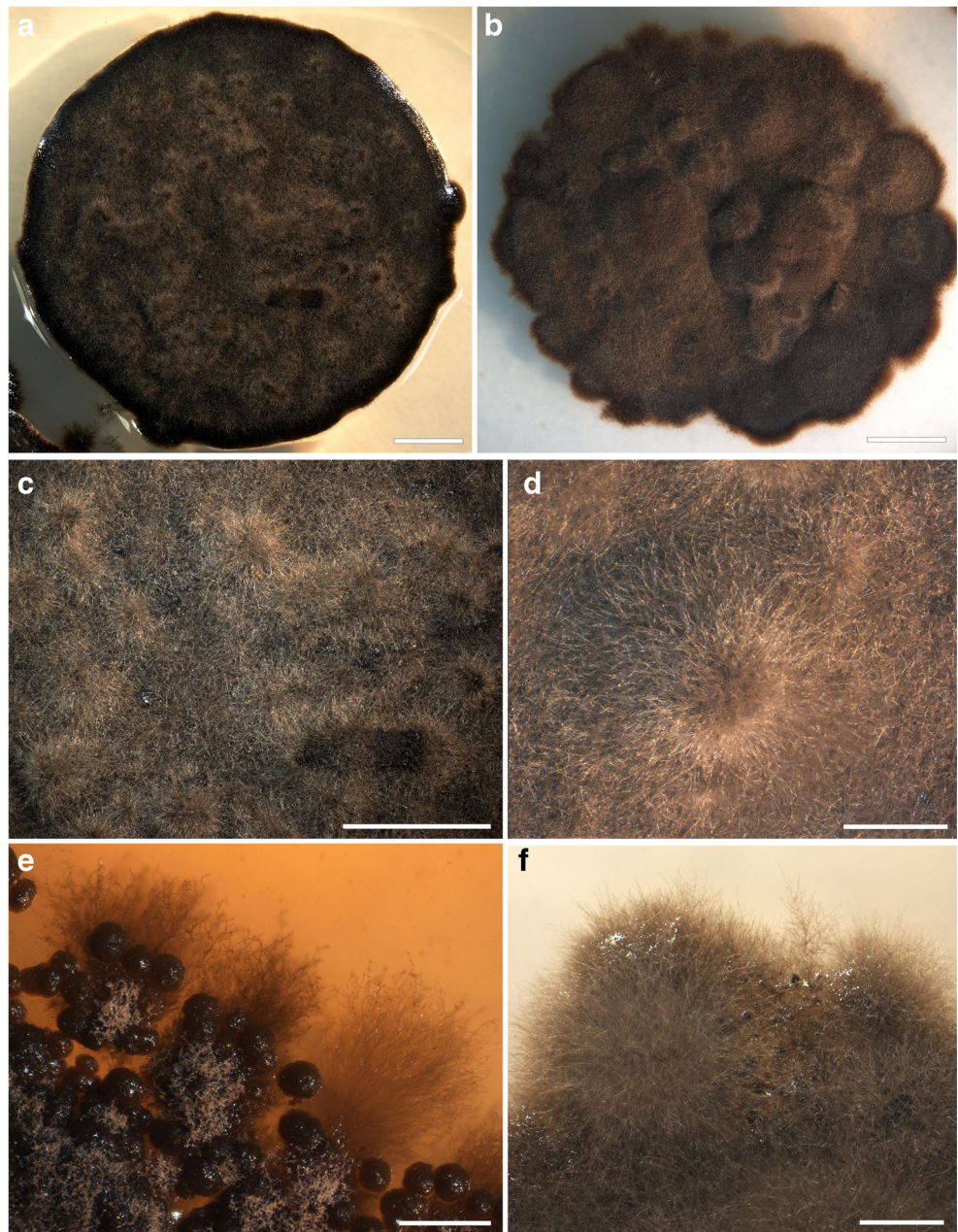
Etymology: The species name refers to the Alpine sampling location.

Holotype: GZU A1025 (LMCC0348), cultured strain, preserved in a metabolically inactive state (20 November 2013, date at which it was first identified from culture isolates, isolated from the thallus of *Rhizocarum geographicum* (GZU A666), Austria, Styria, Central Alps, Steirisches Randgebirge, Koralpe, Ochsenofen, ca. 14 km W above Schwanberg, in the vicinity of the summit, on big boulders NW 46° 46' 29" N/ 15° 00' 52" E, ca. 1760 m, on siliceous rocks, 18.VII.2012, L. Muggia & A. Fleischhacker.

Isotype: GZU A1105.

Diagnosis: Endolichenic (i.e., cryptically present in lichen thalli), asexual fungus, for which the sexual morph is unknown. Axenically cultured colonies, likely derived from asexual spores or hyphal fragments entrapped in the thalline matrix of the lichen hosts, grow in vitro rather slowly; mycelium dark gray to black. Filamentous hyphae at the surface and at the margins of the colony usually hyaline or slightly melanized (Fig. 6c–f); yeast-like growth in the central internal part of the colony; cell walls slightly verrucose. Hyphae septate, hyaline to heavily melanized, toruloid, 3–5 µm thick: hyphal cells rather elongate in a rectangular shape, usually spherical in correspondence of the branching. Spherical cells 7–10 µm wide, at branching either subtending multiple branches or dividing in a meristematic fashion and each cell

Fig. 3 Morphological characters of *Pleostigma alpinum*. **a, c** Strain A1025 (type). **b, d** Strain A879. **e** Strain A584. **f** Strain A945. **a, b** Colony appearance on solid malt yeast medium after one year of growth. **c–f** Less melanized hyphae on the upper/tip hyphae of the mycelium. Scale bars: **a–c** 2 mm; **d, f** 0.5 mm; **e** 0.4 mm



generating a new ramification. *P. alpinum* differs from the type species *P. jungermannicola* by the lack of ascomata and its endolichenic life style. No morphological traits can be compared in cultured strains, as *P. jungermannicola* has never been isolated in culture.

Distribution: Boreal, thus far isolated from alpine lichens, growing on siliceous-schist rocks at about 2000 m a.s.l. and rock from Mediterranean habitats and bryophytes. Isolated from the following lichen species: *Aspicilia caesiocinerea*; *A. simoensis*; *Aspicilia* sp.; *Lecanora intricata*; *L. polytrapa*; *Lecidea lapicida*; *Lecidea* sp.; *Rhizocarpon geographicum*; *Schaereria fuscocinerea*; *Tephromela atra*; and *Umbilicaria cylindrica*.

Material examined: EUROPE, Austria, Koralpe mountain range, on siliceous-schist and gneissic rocks, alt. 1800–2100 m a.s.l., endolichenic fungi isolated from lichen thalli, May–July 2012, L. Muggia and A. Fleischhacker, strain numbers A584, A879, A912, A945, A1105, A1125, and A1179.

Notes: Axenically isolated strains are reported in Table S1 and in Muggia et al. (2016, 2017).

Pleostigma frigidum Grube, Muggia, de Hoog, **sp. nov.**—Mycobank: MB838769 (Figs. 4, 5)

Etymology: The species name refers to the ecology, i.e., cold and Alpine, characteristic of the environment from which the strains were isolated.

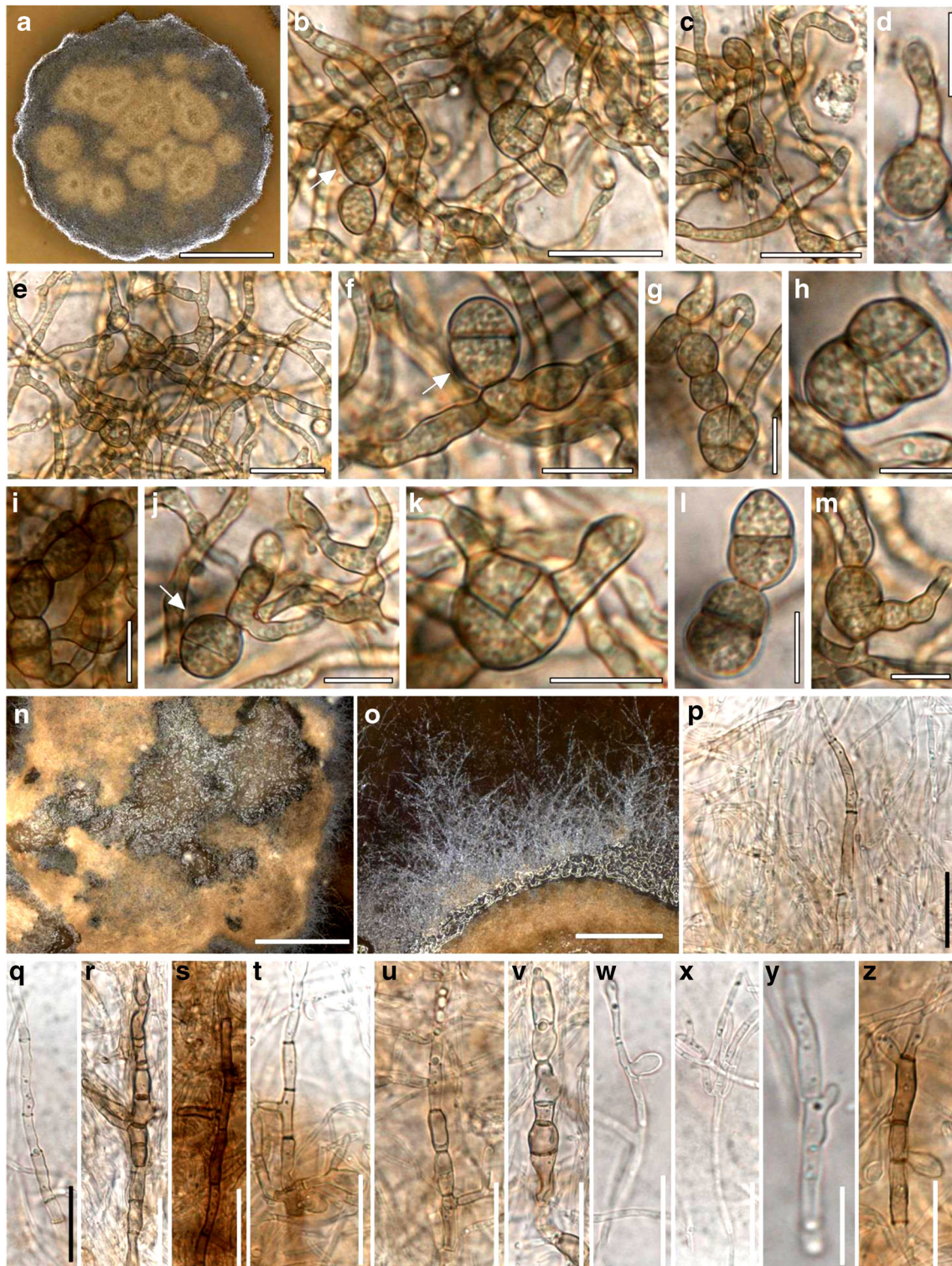


Fig. 4 Morphological characters of *Pleostigma frigidum* and *P. lichenophilum*. **a–m** Strain A952 (type), *Pleostigma lichenophilum*. **n–z** Strain A866, *Pleostigma frigidum*. **a, n, o** Colony appearance on solid malt yeast medium after 1 year of growth: superficial and marginal filamentous hyphae (**a, o**) and yeast-like growth in the central internal part (**n**). **b–m** Melanized, toruloid hyphae with the formation of

septate (**b, f, j, l**) or muriform conidiogenous cells (**g, h, k, m**) often bearing germinating hyphae (**d, g, k, m**). **q–z** Filamentous, septate hyphae with diverse degree of melanization (subhyaline to pale brown) and branching. Scale bars: **a, n** 5 mm; **o** 1 mm; **b, c, e, p–z** 20 μm ; **d, f–m** 10 μm

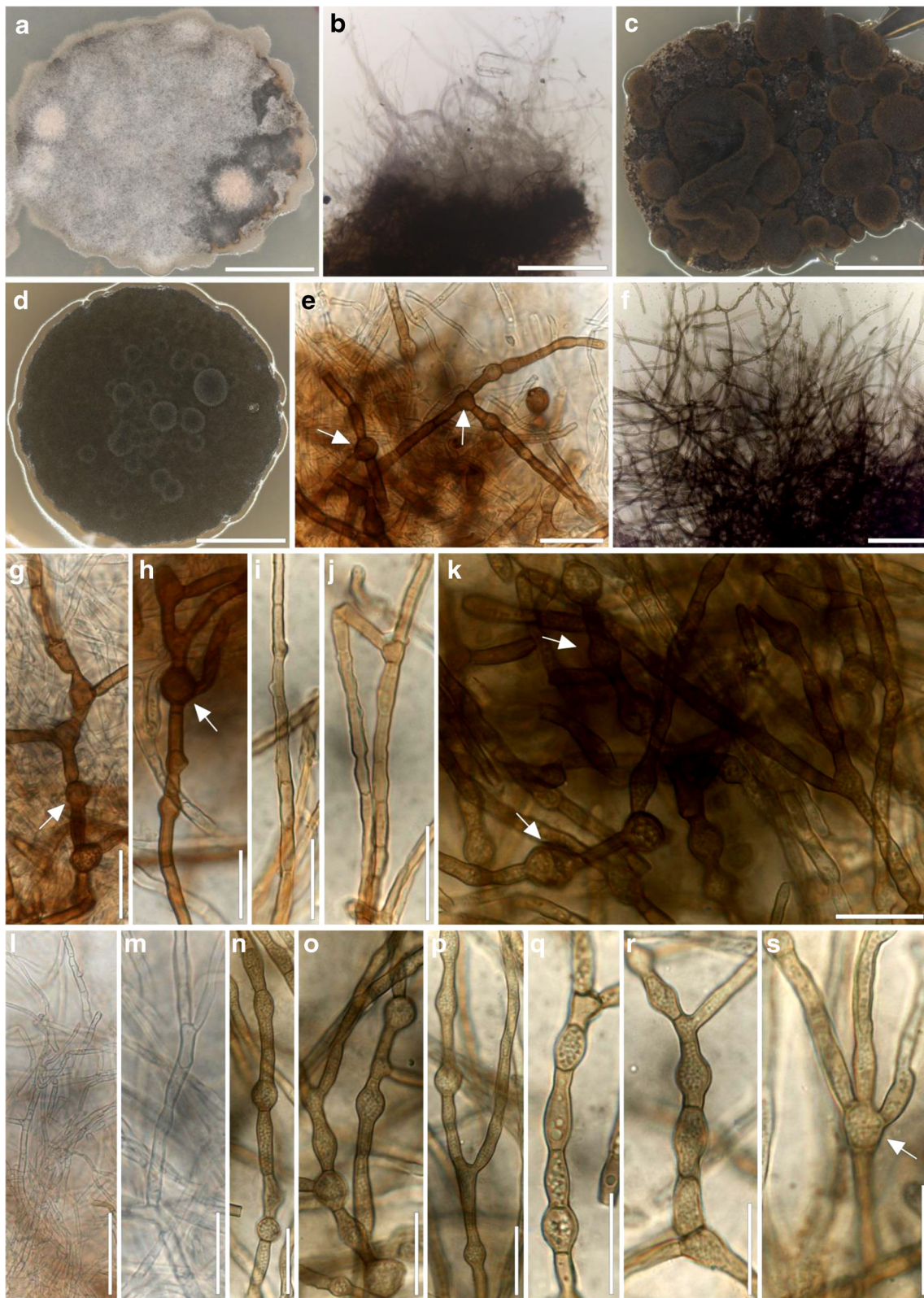


Fig. 5 Morphological characters of *Pleostigma frigidum*. **a, b, e, g, h, l, m** Strain A1161. **c, i, j** Strain A989. **d, f, k, n–s** Strain A980 (type). **a–d** Colony appearance on solid malt yeast medium after one year of growth. **e–s** Hyphae morphology: brown, septate, toruloid hyphae, with

accentuate spherical cell at the branching or interposed along the hyphae length. Scale bars: **a, c, d** 5 mm; **b** 0.2 mm; **f** 100 μm ; **e, g–s** 20 μm

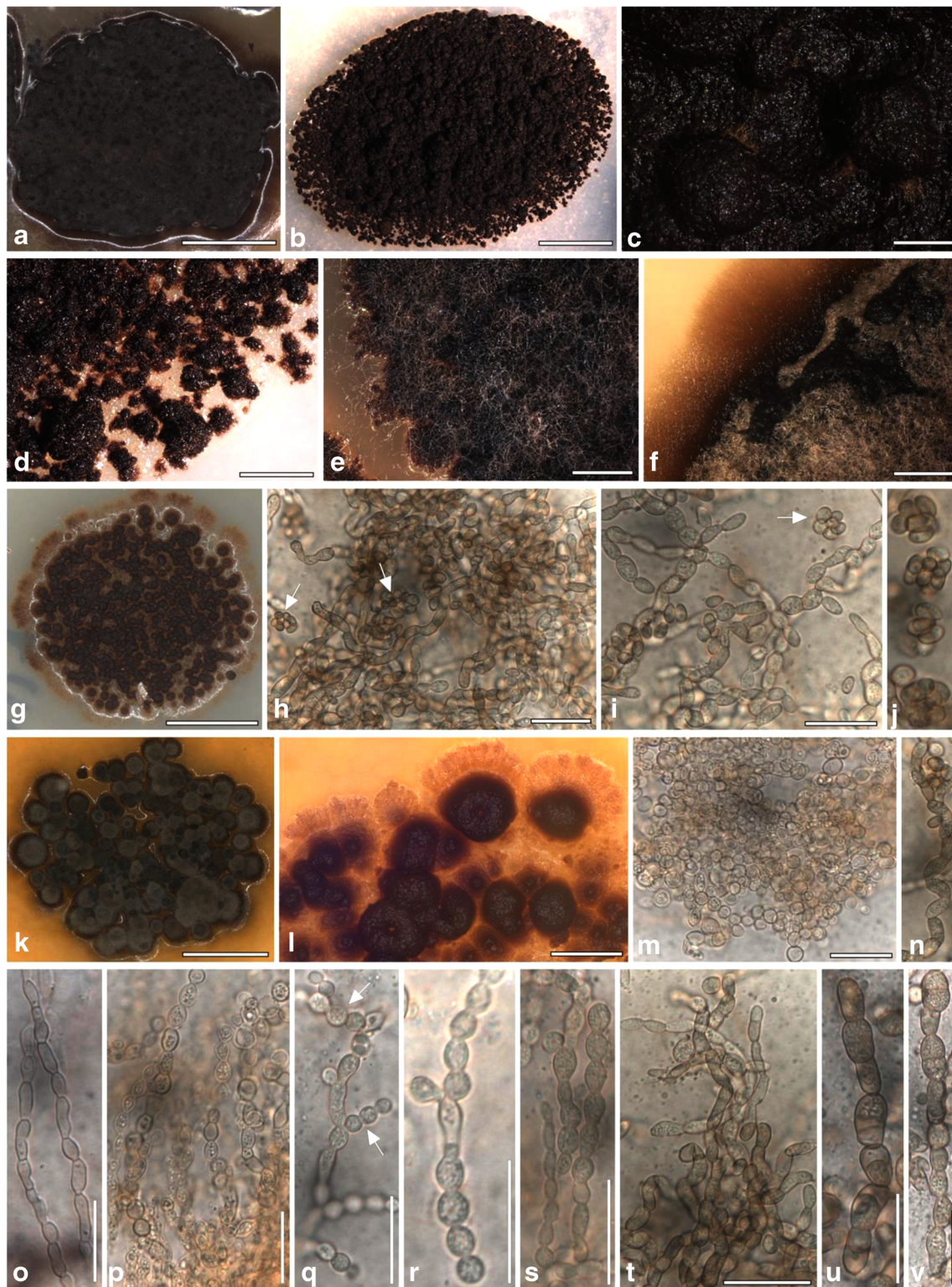


Fig. 6 Morphological characters of *Melanina gunde-cimermaniae*. Strains **a, o** A934; **b, d** A872; **c** A538; **e** A880; **f** A919; **g–j, n, t** A1163; **k, q–s** A1111 (type); **l, m, p** A1187; and **u, v**, A1120. **a–g, l** Colony appearance on solid malt yeast medium after 1 year of growth. **h–j, m–s** Toruloid hyphal organization and filaments of conidia, likely

resulting from a blastic-percurrent ontogeny. **h–j, q** Clumps of conidia (arrows in **H, I, Q**). **t–v** Chain of conidia likely resulting from an arthric ontogeny. Scale bars: **a, k** 5 mm; **b** 4 mm; **c** 0.4 mm; **d, e, l** 1 mm; **f** 0.5 mm; **m–v** 20 μ m

Holotype: GZU-A980 (LMCC0317), cultured strain, preserved in a metabolically inactive state (20 November 2013, date at which they were first identified from culture isolates). Isolated from the thallus of *Tephromela atra* (A675, GZU 000329462, Austria, Styria, Central Alps, Steirisches Randgebirge, Koralpe, Ochsenofen, ca. 14 km W above Schwanberg, in the vicinity of the summit, on big boulders NW 46° 46' 29" N/15° 00' 52" E, ca. 1760 m, on siliceous rocks, 18.VII.2012, L. Muggia & A. Fleischhacker.

Isotype: GZU-A989.

Diagnosis: Endolichenic (i.e., cryptically present in lichen thalli), asexual fungus, for which the sexual morph is unknown. Axenically cultured colonies, likely derived from asexual spores or hyphal fragments entrapped in the thalline matrix of the lichen hosts, grow in vitro rather slowly; mycelium dark gray to black. Filamentous hyphae at the surface and at the margins of the colony usually hyaline or slightly melanized; yeast-like growth in the central internal part of the colony; cell walls slightly verrucose. Hyphae septate, hyaline to heavily melanized, toruloid, 3–5 µm thick (Figs. 4q–z and 5g–j, l–s); hyphal cells rather elongate in a rectangular shape, usually spherical in correspondence of the branching. Spherical cells 7–10 µm wide, at branching either subtending multiple branches or dividing in a muriform fashion and each cell generating a new ramification (Fig. 4m). *P. frigidum* differs from the type species *P. jungermannicola* by the lack of ascomata and its endolichenic life style. No morphological traits can be compared in cultured strains as *P. jungermannicola* has never been isolated in culture.

Distribution: Thus far isolated from lichens and bryophytes. Lichens were growing on siliceous-schist rocks at about 2000 m a.s.l. in the Alpine area and on rock in Mediterranean habitats. Isolated from the following lichen species: *Aspicilia caesiocinerea*; *A. simoensis*; *Aspicilia* sp.; *Lecanora intricata*; *L. polytropa*; *Lecidea lapicida*; *Lecidea* sp.; *Rhizocarpon geographicum*; *Schaereria fuscocinerea*; *Tephromela atra*; and *Umbilicaria cylindrica*.

Material examined: EUROPE, Austria, Koralpe mountain range, on siliceous-schist and gneissic rocks, alt. 1800–2100 m a.s.l., endolichenic fungi isolated from lichen thalli, May–July 2012, L. Muggia and A. Fleischhacker, strain numbers A516, A533, A547, A875, A891, A918, A974, A980, A989, and A1161.

Notes: Axenically isolated strains are reported in Table S1 and in Muggia et al. (2016, 2017).

Pleostigma jungermannicola (C. Massal.) Kirschst. *Annals Mycol.* 37: 91 (1939)—MycoBank: MB268477

Pleostigma lichenophilum Grube, Muggia, de Hoog, **sp. nov.**—MycoBank: MB838770 (Fig. 4)

Etymology: The species name refers to the endolichenic lifestyle of the fungus.

Holotype: GZU-A952 (LMCC0289), cultured strain, preserved in a metabolically inactive state (20 November 2013, date at which they were first identified in culture isolation). Isolated from the thallus of *Tephromela atra* (A675, GZU 000329462, Austria, Styria, Central Alps, Steirisches Randgebirge, Koralpe, Loskogel S der Weinebene, im Gipfelbereich, 46° 48' 23" N/15° 00' 33" E, ca. 1790 m, 15.V.2012, L. Muggia & leg. A. Fleischhacker.

Isotype: GZU-A926 (LMCC0304).

Diagnosis: Endolichenic (i.e., cryptically present in lichen thalli), asexual fungus, for which the sexual morph is unknown. Axenically cultured colonies, likely derived from asexual spores or hyphal fragments entrapped in the thalline matrix of the lichen hosts, grow in vitro rather slowly; mycelium dark gray to black. Filamentous hyphae at the surface and at the margins of the colony usually hyaline or slightly melanized; yeast-like growth in the central internal part of the colony; cell walls slightly verrucose. Hyphae septate, hyaline to heavily melanized, toruloid, 3–5 µm thick; hyphal cells rather elongate in a rectangular shape, usually spherical in correspondence of the branching. Spherical cells 7–10 µm wide, at branching either subtending multiple branches or dividing in a muriform fashion and each cell generating a new ramification (Fig. 5g, k, m). *P. lichenophilum* differs from the type species *P. jungermannicola* for the lack of ascomata and its endolichenic life style. No morphological traits can be compared in cultured strains as *P. jungermannicola* has never been isolated in culture.

Distribution: Boreal, thus far isolated from alpine lichens growing on siliceous-schist rocks at about 2000 m a.s.l. and rock from Mediterranean habitats, and bryophytes. Isolated from the following lichen species: *Aspicilia caesiocinerea*; *A. simoensis*; *Aspicilia* sp.; *Lecanora intricata*; *L. polytropa*; *Lecidea lapicida*; *Lecidea* sp.; *Rhizocarpon geographicum*; *Schaereria fuscocinerea*; *Tephromela atra*; and *Umbilicaria cylindrica*.

Material examined: EUROPE, Austria, Koralpe mountain range, on siliceous-schist and gneissic rocks, alt. 1800–2100 m a.s.l., endolichenic fungi isolated from lichen thalli, May–July 2012, Lucia Muggia and Antonia Fleischhacker, strain numbers A521, A574, A926, and A952.

Notes: Axenically isolated strains as reported in Table S1 and in Muggia et al. (2016, 2017).

Melanina Grube, Muggia, de Hoog, **gen. nov.**—MycoBank: MB838740 (Fig. 6)

Etymology: The genus name refers to the melanized mycelium, and therefore its inclusion in the group of “melanized, black fungi.”

Type species: *Melanina gunde-cimermaniae* Grube, Muggia, de Hoog—MycoBank: MB838767 (Fig. 6)

Monotypic genus in the order Chaetothyriales, Ascomycota. Endolichenic fungi, for which the sexual morph

is unknown. Colonies growing rather slowly in vitro, dark gray to black. Hyphae toruloid, hyaline to heavily melanized. Superficial hyaline hyphae rare; yeast-like growth in the central internal part of the colony, cell walls slightly verrucose. Conidia pale pigmented, organized in chains, likely resulting from a blastic-percurrent or arthric ontogeny.

Melanina gunde-cimermaniae Grube, Muggia, de Hoog, **sp. nov.**—MycoBank: MB 838767 (Fig. 6)

Etymology. The species is named in honor of the Slovenian mycologist and microbiologist Nina Gunde-Cimerman, who has contributed significantly to the knowledge of stress tolerance of black yeasts.

Holotype: GZU-A1111 (LMCC0452), cultured strain, preserved in a metabolically inactive state (20 November 2013, date at which they were first identified from culture isolates). Isolated from the thallus of *Rhizocarpon geographicum* (GZU A697), Austria, Styria, Central Alps, Steirisches Randgebirge, Koralpe, Ochsenofen, ca. 14 km W above Schwanberg, in the vicinity of the summit, on big boulders NW, 46° 46' 30" N/15° 00' 50" E, 18.VII.2012, L. Muggia and A. Fleischhacker.

Isotype: GZU-A1120.

Diagnosis: Endolichenic (cryptically present in lichen thalli), asexual fungi. Colonies growing extremely slowly in vitro (reaching 1.5 cm in a year), dark gray to black. Hyphae toruloid, composed of almost isodiametric cells, 4–5 µm wide, hyaline to heavily melanized. Superficial, hyaline hyphae rare; yeast-like growth present in the central internal part of the colony, with slightly verrucose cell walls. Conidia pale pigmented, 3–4 µm wide organized in chains, likely resulting from a blastic-percurrent or arthric ontogeny.

Distribution: Isolated from alpine lichens growing on siliceous-schist rocks scattered in wide, alpine areas of pastures or dwarf shrub formations, between 1800 m and 2100 m a.s.l., and from calcareous rocks in dry Mediterranean habitat (Spain). Isolated from the following lichen species: *Lecanora polytropa*; *Lecidea lapicida*; *Lecidea* sp.; *Rhizocarpon geographicum*; *Tephromela atra*; and *Umbilicaria cylindrica*.

Material examined EUROPE, Austria, Koralpe mountain range, on siliceous-schist and gneissic rocks, alt. 1800–2100 m a.s.l., endolichenic fungi isolated from lichen thalli, May–July 2012, Lucia Muggia and Antonia Fleischhacker, strain numbers A513, A525, A526, A529, A538, A543, A566, A581, A864, A872, A880, A898, A919, A933, A929, A934, A957, A958, A1018, A1070, A1072, A1095, A1108, A1111, A1113, A1120, A1137, A1144, A1145, A1169, and A1187.

Notes: Axenically isolated strains are reported in Table S1 and in Muggia et al. (2016, 2017).

Discussion

Large sequencing efforts conducted during the past decade have progressively contributed in clarifying and reconsidering evolutionary relationships of fungi in the class Eurotiomycetes. Particularly, study of the diverse evolutionary lineages emerging from less frequently explored ecological niches has led to the discovery of numerous novel taxa and to better understanding of their various lifestyles (Stenroos et al. 2010; Chen et al. 2015; Teixeira et al. 2017). Black fungi that grow asymptotically inside lichen thalli (Harutyunyan et al. 2008; Muggia et al. 2016, 2017; Muggia and Grube 2018), in a similar way as endophytes do (Arnold et al. 2009; U'Ren et al. 2010, 2012, 2019), are also known as endolichenic fungi and form a polyphyletic group in this remarkably ecologically variable class. These fungi have been found to be ancestral to the chaetothyrialean “sooty molds” (colonizers of inert surfaces) and, at larger phylogenetic distance, to opportunists on mammal hosts (Quan et al. 2020).

Because the description of new taxa in this fungal group requires comprehensive sampling, morphological characterization in vitro, and sequencing efforts for inferring their phylogenetic positions, only very few species have been formally described yet. Quan et al. (2020) provided an extensive phylogenetic overview of Chaetothyriales (based on nuLSU and ITS loci), and corroborated the phylogenetic placement of two lineages of black fungi isolated from lichens by Muggia et al. (2016, 2017), and of some bryosymbiotic and rock-inhabiting taxa (Stenroos et al. 2010; Gueidan et al. 2014). Both lineages are now represented by multiple strains isolated from different lichen thalli and are here formally introduced as the new family Pleostigmataceae, close to the order Verrucariales, and the new genus *Melanina* in Chaetothyriales.

Our phylogenetic inference, although based on the single nuLSU locus, is topologically in agreement with previous results based on multiple markers (Gueidan et al. 2014; Muggia et al. 2015, 2016, 2017, 2019; Réblová et al. 2017a,b; Teixeira et al. 2017; Voglmayr et al. 2019; Quan et al. 2020). The use of the single locus nuLSU is due to the fact that sequence data for other loci, such as nucSSU, mtSSU, or RPB1/2, are missing for the majority of the considered taxa. Although our choice to use a single genetic marker departs from the more conventional multilocus approach, in which several markers are combined to find higher support for the final topology, the majority of the taxa included here have been already analyzed in multilocus datasets which showed congruent results.

The new family Pleostigmataceae and basal lineages within Chaetothyriomycetidae

Our phylogenetic inference of the orders in the subclass Chaetothyriomycetidae and the placement of lichen-colonizing black fungi comprised all recognized orders and families (for which sequences of the nucLSU locus are available) and has led to a reappraisal of relationships and altered delimitation of some taxa. Since the pioneering molecular studies of lichenized and non-lichenized rock-inhabiting fungi in Eurotiomycetes by Ruibal et al. (2005, 2008) and Gueidan et al. (2007, 2008, 2011), the class underwent various “adjustments” to reliably place the clades that emerged from sequence data. All these fungi are of great interest because they evolved different lifestyles albeit being closely related and sharing common ancestors (Gueidan et al. 2008, 2011).

Gueidan et al. (2014) recognized four orders in Chaetothyriomycetidae (Celotheliales, Chaetothyriales, Pyrenulales, and Verrucariales) comprising ten families and indicated three unnamed lineages. A more recent outline of Fungi by Wijayawardene et al. (2020) reported the same four orders but 16 families (ten families recognized within Chaetothyriales, four in Verrucariales and one in Phaeomoniellales and Pyrenulales). In the present phylogeny, as well as in previous studies, Pyrenulales and Phaeomoniellales invariably took the most basal positions (Gueidan et al. 2014; Réblová et al. 2017a,b; Muggia et al. 2016, 2017; Voglmayr et al. 2019). While Pyrenulales has been recognized a long time ago, Phaeomoniellales was introduced only recently by Chen et al. (2015) and confirmed by Réblová et al. (2017a,b), to accommodate endophytic and plant-pathogenic fungi of the genera *Celothelium*, *Dolabra*, *Moristoma*, *Phaeomoniella*, and *Xenocylindrosporium*. Phaeomoniellales corresponds to the family/order clade Celotheliaceae/Celotheliales which was proposed ad interim by Gueidan et al. (2014).

Verrucariales, with its single family Verrucariaceae, has been recovered as a fully supported monophyletic group and includes lichenized fungi forming crustose, squamulose, and foliose thalli (Gueidan et al. 2007, 2009; Muggia et al. 2010), associating with multiple lineages of microalgae (Thüs et al. 2011). They colonize various, mainly rocky, habitats ranging from marine and fresh water to arid environments (Gueidan et al. 2007; Muggia et al. 2010). Recently, Muggia et al. (2019) identified a new lineage of endolichenic black fungi within Verrucariaceae, which was provisionally named “*Muellerella* sp. 2,” as the generic type species could not be sequenced so far. This group may represent the lichenicolous genus *Muellerella*.

The “unnamed lineage 1” recognized by Gueidan et al. (2014) included two species of *Sclerococcum* and two of *Dactylospora* and corresponded to “clade C” of Diederich et al. (2013), in which these two genera of lichenicolous fungi

were judged to be closely related. This “unnamed lineage 1” was named “*Dactylospora*-clade” by Chen et al. (2015), while later, Réblová et al. (2017a) introduced the new family Sclerococcaceae for this group, and accommodated it in the new order Sclerococcales and the new subclass Sclerococomycetidae within Eurotiomycetes. Within Sclerococcales, the authors accommodated, together with lichenicolous species of *Sclerococcum* and *Dactylospora*, some marine and lignicolous *Dactylospora* species, the lignicolous genus *Rhopalophora*, and other fungi from the digestive tracts of Neotropical wood-inhabiting beetles, as all these taxa formed a strongly supported, monophyletic clade that is basal to Chaetothyriomycetidae (i.e., Sclerococcales). In a more recent taxonomic revision of lichenicolous fungi by Diederich et al. (2018), the two genus names *Dactylospora* and *Sclerococcum* were recognized to be synonymous and *Sclerococcum* was set as the valid genus name, as it pre-dates *Dactylospora*. Family and order names were maintained as Dactylosporaceae and Sclerococcales, respectively (Diederich et al. 2018). The new subclass Sclerococomycetidae was recognized also by Voglmayr et al. (2019) in their phylogenetic inference supporting the description of the two new classes, Xylobotryomycetes and Candelariomycetes (Ascomycota). The new taxonomy of Sclerococcales seems to have been overlooked by Ekanayaka et al. (2019) who recently introduced the order Dactylosporales and family Dactylosporaceae for a corresponding phylogenetic lineage within Eurotiomycetes. In our phylogenetic reconstruction, we selected samples of the lichenicolous species *Sclerococcum sphaerale* and the marine species *Sclerococcum haliotrephum* to represent the order clade Sclerococcales.

The second “unnamed lineage 2” of Gueidan et al. (2014) consisted of two unidentified rock isolates (A14, TRN242), *Pleostigma jungermannicola* M174 and *Teichospora* sp. M195. The lineage resulted as a sister clade of Verrucariales. The same, well-supported lineage of *Pleostigma jungermannicola* M174 and *Teichospora* sp. M195 was recognized by Stenroos et al. (2010) in their five-gene phylogeny when investigating the multiple origins of symbiosis between ascomycetes and bryophytes. “Unnamed lineage 2” was found to be monophyletic and well-supported by Gueidan et al. (2014), but completely paraphyletic and closely related to Verrucariales by Chen et al. (2015), who maintained the label “unknown lineage.” The sample of *Teichospora* sp. M195 used by Stenroos et al. (2010), Gueidan et al. (2014), and Chen et al. (2015) is likely to represent an unknown taxon or a contamination, because the type species of *Teichospora* and the family Teichosporaceae was revealed to be a member of Pleosporales in the class Dothideomycetes (Jaklitsch et al. 2016). For this reason, we excluded the sequence of *Teichospora* sp. M195 from our analysis. The “unnamed lineage 2,” referred to as

“*Pleostigma*-clade,” was represented by *Pleostigma jungermannicola* only in the study of Ekanayaka et al. (2019). Réblová et al. (2017a) did not include this lineage in their description of the new order Sclerococcales, but they considered it when describing the two new genera *Bradomyces* (Trichomeriaceae) and *Bacillicladium* (undetermined position in Chaetothyriales; Réblová et al. 2017b). Boonmee et al. (2017), when revising genera of ascomycete of *incertae sedis*, reported that “based on sequence data, the genus *Pleostigma* is treated as *incertae sedis* in Chaetothyriales.” In our present analysis, the three taxa representing the “unnamed lineage 2” sensu Gueidan et al. (2014), i.e., Chaetothyriales spp. A14, TRN242 and *Pleostigma jungermannicola* M174, cluster together with strains of lichen-associated black fungi that formed the lineages “clade IV” and “clade V” in Muggia et al. (2016, 2017). This lineage is now formally described as the family Pleostigmaceae with three lichen-associated molecular species, which however lack distinctive morphological traits. The rock-inhabiting fungus Chaetothyriales TRN242 is closely related to six samples of lichen-associated black fungi basal to *P. frigida*. These lichen-associated strains are morphologically identical to *P. frigida*, but due to their significant genetic distance, we refrain to merge them. Chaetothyriales sp. A14 and *Pleostigma jungermannicola* M174 on individual branches represent separate species. The lineage Pleostigmaceae, though individually monophyletic, is not supported in the present analysis and its phylogenetic position might undergo variation if a different taxon sampling is investigated. For this reason, we opt here to define it as a new family with still an uncertain position within Chaetothyriomycetidae. However, we do believe that its establishment as a new family is reasonable and warranted: it now delimits a monophyletic taxonomic entity that has been identified since 2010 and has been confirmed by a substantial number of samples in the present study.

The new genus *Melanina* and basal lineages within Chaetothyriales

The third “unnamed lineage 3” is represented by three strains of rock-inhabiting fungi, TRN210, TRN508, and TRN515 (Gueidan et al. 2014); all isolates originated from natural rocks in the Mediterranean basin (Ruibal et al. 2008). This clade has consistently been recovered within Chaetothyriales (Ruibal et al. 2008; Gueidan et al. 2014; Réblová et al. 2017a; Muggia et al. 2016, 2017), with slight changes in phylogenetic position, probably depending on different taxon samplings. Gueidan et al. (2014) considered “unnamed lineage 3” to be basal to Cyphellophoraceae, Chaetothyriaceae, and Trichomeriaceae, while it was sister to Cyphellophoraceae in Réblová et al. (2017b). This “unnamed lineage 3” corresponds to the lineage “clade VI + VII” of Muggia et al. (2016, 2017),

and is formalized here as the novel genus *Melanina*. The rock-inhabiting fungi (TRN210 and TRN508) of “unnamed lineage 3” cluster with strains isolated from lichens. This suggests that these fungi are widespread in multiple rocky environments, either in association with lichens, or free-living. In our analysis, *Melanina* has a basal position to most Chaetothyriales except for the family Epibryaceae and the lineage composed by *Muellerella atricola*, *M. lichenicola*, and their *Lichenodiplis*-like asexual fungus.

The family Epibryaceae includes the genus *Epibryon*, some species described as *Cladophialophora* (Quan et al. 2020), and some unnamed fungi isolated from rock (TRN475) and from lichens (A515). Depending on taxon sampling, the family Epibryaceae has been recovered as either monophyletic with low support (Gueidan et al. 2014; Muggia et al. 2016, 2017; Réblová et al. 2017b; Teixeira et al. 2017) or paraphyletic (Muggia et al. 2019; Quan et al. 2020). As in this study, Stenroos et al. (2010) and Quan et al. (2020) also found the genus to consist of two separate clades, suggesting that future, in-depth analyses might lead to a reconsideration of the genus in its whole.

The clade containing *Muellerella atricola*, *M. lichenicola*, and a *Lichenodiplis*-like species was recognized by Muggia et al. (2015, 2019) including some perithecia-forming lichenicolous fungi with their asexual, pycnidial state referred to as *Lichenodiplis*. Since fungi ascribed to the genus *Muellerella* were found to be polyphyletic within Chaetothyriomycetidae, while its type species, *M. polyspora* has not been sequenced yet, the name *Muellerella* cannot be applied with certainty to any of these fungi (Muggia et al. 2019).

The remaining four families Chaetothyriaceae, Cyphellophoraceae, Herpotrichiellaceae, and Trichomeriaceae are monophyletic, fully resolved and well-supported, and their species and ecological diversity have been the focus of recent phylogenetic and phylogenomic studies (Teixeira et al. 2017; Gostinčar et al., 2018; Quan et al. 2020). The Trichomeriaceae comprises the “sooty molds” that colonize inert surfaces (Chomnunti et al. 2012). The derived family Herpotrichiellaceae includes black yeasts and relatives, which have very different habitat choices, often characterized by extreme conditions and oligotrophy (de Hoog, 2016). These fungi are unique for their polyextremotolerance and opportunistic pathogenicity in humans (de Hoog et al., 2020). Five strains of lichen-associated black fungi are nested in the *Cladophialophora*-clade in Herpotrichiellaceae.

Lifestyles of lichen-associated fungi and basal lineages within Chaetothyriomycetidae

Trophic interactions between lichen-associated black fungi and their hosts are still poorly understood. This is mainly because microscopic analyses of lichen thalli do not allow to

clearly localize the endolichenic fungi inside the thalli. Furthermore, these fungi neither produce clear infection structures with the host's photobiont, nor they show any mycoparasitic behavior towards the mycobiont. In the cases where black fungal hyphae have been detected in different layers of the lichen thalli, the hyphae were observed to spread randomly without forming any structure inside the lichen (M. Grube, unpubl. results). In most cases, individually melanized hyphae were seen between the hyphae of the upper cortex of epilithic crustose lichens, or growing in between the areoles of the thallus, in which thin fissures are formed due to the discontinuous growth of the thallus. These thin fissures may also act as spaces where airborne spores and fungal fragments remain trapped and might grow and extend inside the lichen thalli. Consequently, we hypothesize that these black fungi are commensals that seem to benefit from compounds exuded into the intercellular matrix of the conglutinate fungal plectenchyma. This does not exclude that the algal partner of the lichen may have some effect on the growth of lichen-associated fungi. Earlier studies demonstrated an affinity of a lichen-inhabiting black fungus to microalgae during axenic co-cultivation with the principal photobiont of the host (Gorbushina et al. 2005; Brunauer et al. 2007). In natural habitats, particularly in calcareous rock environments, black fungi are known to establish communities with green algae and cyanobacteria either along crevices or endolithic in the weathering rinds of the rocks (Gorbushina and Broughton 2009; Gostinčar et al. 2012). The potential for these fungal lineages to associate and grow with microalgae suggests that primitive fungal-algal interactions may be involved (Gostinčar et al., 2012; Ametrano et al. 2017, 2019a).

The two new lineages Pleostigmataceae and *Melanina* have not been tested for their capacity to associate with microalgae. However, their phylogenetic position as inferred in the present paper supports their close affiliation to bryosymbiotic taxa, lichen-forming fungi, and lichen pathogens. Indeed, species of lichen-associated *Pleostigma* are closely related to the bryosymbiotic taxon *Pleostigma jungermannicola*. *Melanina*, on the other hand, is basal to the families Herpotrichiellaceae, Chaetothyriaceae, Cyphellophoraceae, and Trichomeriaceae. Chaetothyriaceae and Trichomeriaceae are surface colonizers of plants and inert substrates, respectively, while the remaining families show a high ecological diversity (Quan et al. 2020). The ancestral clades (i.e., Epibryaceae, *Melanina*, and the *Muellerella atricola* + *M. lichenicola* + *Lichenodiplis*-like asexual state) of these core-families of Chaetothyriales are all associated with rocks, lichens, or bryophytes as a rather strong apomorphic/ancestral character, which is maintained across the evolution of Chaetothyriomycetidae (Gueidan et al. 2008, 2011; Quan et al. 2020).

Interestingly, in both Pleostigmataceae and *Melanina*, a certain heterogeneity of hyphae can be discerned, hyphal cells being either rectangular, elongated, with frequent branching, or subspherical and budding, sometimes forming short chains. Similar morphology is found in other lineages of rock-inhabiting fungi in Dothideomycetes and Chaetothyriales (Egidi et al. 2014; Ametrano et al. 2019b). Poor morphological differentiation is indeed common among these fungi in general, and although these fungi have substantially diversified phylogenetically, they share morphological and functional traits which indicate adaptation to similar types of abiotic stresses.

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Code availability Not applicable.

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Declarations

Conflict of interest The authors declare no competing interests.

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