Muellerella, a lichenicolous fungal genus recovered as polyphylectic within Chaetothyriomycetidae (Eurotiomycetes, Ascomycota)

Lucia Muggia¹*, Sergio Pérez-Ortega² & Damien Ertz³,⁴

Abstract. Molecular data and culture-dependent methods have helped to uncover the phylogenetic relationships of numerous species of lichenicolous fungi, a specialized group of taxa that inhabit lichens and have developed diverse degrees of specificity and parasitic behaviors. The majority of lichenicolous fungal taxa are known in either their anamorphic or teleomorphic states, although their anamorph-teleomorph relationships have been resolved in only a few cases. The pycnidium-forming Lichenodiplis lecanorae and the perithecioid taxa Muellerella atricola and M. lichenicola were recently recovered as monophyletic in Chaetothyriales (Eurotiomycetes). Both genera are lichenicolous on multiple lichen hosts, upon which they show a subtle morphological diversity reflected in the description of 14 species in Muellerella (of which 12 are lichenicolous) and 12 in Lichenodiplis. Here we focus on the teleomorphic genus Muellerella and investigate its monophyly by expanding the taxon sampling to other species occurring on diverse lichen hosts. We generated molecular data for two nuclear and one mitochondrial loci (28S, 18S and 16S) from environmental samples. The present multilocus phylogeny confirms the monophyletic lineage of the teleomorphic M. atricola and M. lichenicola with their L. lecanorae-like anamorphs, but places the rest of the Muellerella species studied in two different monophyletic lineages with strong support. The first, Muellerella spp. 1, is nested within some new lineages of black fungi isolated from different epilithic lichen thalli, while the second, Muellerella spp. 2, is closely related to the Verrucariales. Based on these results, we reappraise the phylogenetic placement of Muellerella and suggest its polyphyly within Chaetothyriomycetidae.

Key words: diversity, multilocus analysis, parasitic, phylogeny, Verrucariales.

Introduction

In the past decade, molecular data have increasingly helped to resolve the phylogenetic position of many fungal taxa, filling numerous gaps in our current knowledge of the fungal tree of life. Many genera have been tested for their monophyly, either confirming it (e.g., see review by Tedersoo et al. 2018) or not (e.g., Aveskamp et al. 2009; Rai et al. 2014; Ertz et al. 2015a, b). Additionally, comparisons of anamorphic and teleomorphic states, sometimes complemented by axenic cultures, have allowed researchers to establish the connections between sexual and asexual states in numerous fungi (e.g., Pérez-Ortega et al. 2011; Ertz et al. 2014; Muggia et al. 2015). Together, these findings have led to important taxonomic revisions, including the introduction and invalidation of several species names (Hawksworth 2011). However, fungal taxa characterized by inconspicuous mycelia or specialized ecological niches have often been neglected due to difficulties encountered in obtaining molecular data from their thalli.

Among these poorly investigated fungal groups are the lichenicolous fungi, the majority of which are Ascomycota. They are known to inhabit lichen thalli or the apothecia of the mycobiont, upon which they are detectable by their symptomatic infections and their sexual or asexual spore-producing structures (Lawrey & Diederich 2003; Diederich et al. 2018). Lichenologists distinguish these fungi from those that inhabit the lichen thalli asymptotically, that is, the ‘endolichenic fungi’ (Arnold et al. 2009)
that are detectable only by molecular analyses or culture isolation. The lichenicolous lifestyle has multiple origins in the fungal kingdom, from both lichenized and non-lichenized ancestors (Arnold et al. 2009; Pino-Bodas et al. 2017). Lichenicolous fungi have been reported in seven classes of Ascomycota, but the majority of taxa have been placed in the three big classes Dothideomycetes, Eurotiomycetes and Lecanoromycetes (Pino-Bodas et al. 2017; Diederich et al. 2018; Muggia & Grube 2018). Though 2000 species of lichenicolous fungi are known worldwide (Diederich et al. 2018), only a few taxa have been the focus of molecular analyses, while the majority of the described species are still classified according to morphological or anatomical characters. Lichenicolous fungi have evolved diverse degrees of specificity towards their hosts, ranging from parasites to commensals (Lawrey & Diederich 2003). Many species seem to have a very narrow host range and to be highly dependent on their lichen hosts, which makes it particularly difficult to isolate and grow them in axenic culture (Crittenden et al. 1995) or to retrieve a reasonable number of environmental samples for molecular investigation.

Recently, Muggia et al. (2015) clarified the phylogenetic relationship between two lichenicolous fungi that frequently co-occurred on thalli of the host lichen Tephromela atra: the pycnidium-forming Lichenodiplis lecanorae and the perithecioid Muellerella atricola. Using molecular data obtained from environmental samples and culture isolates, the authors revealed the anamorph-teleomorph relationship of the two species. An in-depth screening of herbarium collections confirmed the co-occurrence of Lichenodiplis and Muellerella species on other lichen hosts. In particular, the phylogenetic analysis of Muggia et al. (2015) indicates that M. lichenicola also has L. lecanorae as anamorphic state. These first results of Muggia et al. (2015) hint that Lichenodiplis lecanorae represents several cryptic taxa that are the asexual state of at least two Muellerella species (viz. M. atricola and M. lichenicola). Because of this, we use the phrase ‘L. lecanorae-like anamorphic state’ to refer to the anamorphic state of Muellerella species included in the present study.

The genus Muellerella in particular is one of the most widespread and frequently collected lichenicolous fungi. At present, 12 accepted lichenicolicous species have been described from a wide range of lichen hosts growing mainly on calcareous and siliceous rocks and on trees (von Brackel 2014; Diederich et al. 2018). Muellerella species are easily recognizable due to the conspicuous black, sometimes slightly shiny perithecia that are immersed or sessile on the thallus and/or on the apothecia of the host lichens, polyspored asci usually containing 0–1-septate, ellipsoid, brown ascospores (Fig. 1, 2). Triebel (1989) and Triebel & Kainz (2004) classified the genus in the family Verrucariaceae, while the phylogenetic inference of Muggia et al. (2015) suggested that the genus forms a new monophyletic lineage sister to Epiphyaceae within Chaetothyriales. Muellerella species can indeed be bryophilous, lichenicolous or saprophytic (Döbler & Triebel 1985; Triebel 1989; Triebel & Kainz 2004). When occurring on lichens, species of Muellerella present a continuum of morphological variation and subtle character diversity (e.g., variation in ascospore size and their number per ascus), which has been correlated with its host specificity. Because of this, some species have been described according to their occurrence on only certain lichen host species or genera (e.g., M. antarctica from Hypogymnia antarctica, M. atricola from Tephromela atra, M. lecanactidis from Sigridia californica, M. stictinae from species of the genus Sticta, M. vesicularea from species of the genus Toninia). Their genetic diversity has never been assessed, however.

In this study we extend the original taxon sampling of Muggia et al. (2015) by including Muellerella species from different host lichens, and we consider the previous dataset (Muggia et al. 2015) as a framework for testing the monophyly of this genus.

Materials and methods

Sampling

Fresh samples and herbarium vouchers (from BR, TSB and MA-Lichen) of Muellerella erratica, M. ventosicola, and three specimens not fitting the currently accepted Muellerella species were used for molecular and morphological analyses (Table 1, Table S1). The specimens were identified following Triebel (1989) and Hafellner (2007), and are named according to the current nomenclature presented by Diederich et al. (2018).

The final molecular dataset (Table 2) includes (i) the newly sequenced specimens of Muellerella erratica, M. ventosicola, and three specimens not fitting the current Muellerella species concepts, infecting a total of six different lichen host species (Table 1); (ii) sequences of Muellerella atricola, M. lichenicola and their Lichenodiplis lecanorae-like anamorphic state published by Muggia et al. (2015); (iii) representatives of orders of Chaetothyriomycetidae, viz. Chaetothyriales, Phaeomoniellales, Pyrenulales and Verrucariales (Verrucariaceae), and within Chaetothyriales the families Chaetothyriaceae, Cyphellophoraceae, Epiphyaceae, Herpotrichiellaceae and Trichomeriaceae, selected from the recent phylogenetic studies of Gueidan et al. (2014) and Teixeira et al. (2017); and (iv) selected isolates of cultured endolithic fungi obtained from different epilithic lichen thalli and representing new lineages (clade I, clade II, clade IV, clade V, clade VI+VII) in Chaetothyriomycetidae, as published by Muggia et al. (2016, 2017). Some of the latter fungal strains were isolated from lichen thalli infected by Muellerella species (Muggia et al. 2016, 2017; Table 2). The sequences of these cultured endolithic fungi were selected to test whether our newly generated sequences correspond to any of these lineages, and thereby to evaluate whether they ought to be included in Muellerella.

DNA extraction, amplification and sequencing

Perithecia of Muellerella were carefully dissected under a stereomicroscope and prepared for DNA extraction, taking care to remove the lichen thallus and perithelial wall.
A single perithecium was taken per sample and transferred to a 1.5 ml tube. The material was first frozen and then pulverized with metal beads using a TissueLyserII (Retsch) or with an iron pestel. The DNA was extracted using a ZR Fungal/Bacterial DNA MicroPrep™ Kit (Zymo Research) or an EZNA Forensic DNA kit (Omega Bio-Tek), following the manufacturers’ instructions (standard protocol). We also used hand-made sections of the perithecia for direct PCR as in Ertz et al. (2015a) at the Meise Botanic Garden. Fragments of the hymenium, rarely also with tiny fragments of the perithecial wall, were placed directly in microtubes with 20 µl H2O. Amplification reactions were prepared for a 50 µl final volume containing 5 µl 10× DreamTaq Buffer (Thermo Fisher Scientific, Waltham, MA), 1.25 µl of each of the 20 µM primers, 5 µl of 2.5 mg ml⁻¹ bovine serum albumin (Thermo Fisher Scientific, Waltham, MA), 4 µl of 2.5 mM each dNTPs (Thermo Fisher Scientific, Waltham, MA), 1.25 U DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA), and the tiny fragments of the lichenicolous fungus.

The phylogenetic placement of Muellerella was studied by sequencing the same loci as in Muggia et al. (2015, 2016, 2017) in order to allow comparison of the results and verification of coherency in the extended analysis. We amplified the partial nuclear large (28S) and small (18S) subunits ribosomal DNA and the mitochondrial...
were sequenced by Macrogen sequences.

Alignment and phylogenetic analyses

A BLAST search in GenBank was performed for a preliminary taxonomic assignment of each sequence, confirming their matches with taxa of *Chaetothyriomycetidae* (see Results below). First phylogenetic inferences (not shown), based on each individual locus, were performed with a sequence dataset that included members of the class *Eurotiomycetes* representing the orders *Chaetothyriales*, *Corynemeliales*, *Onygenales*, *Pyrenulales* and *Verrucariales*; three species of *Myccocaliciales* (*Chaeontheca savonica*, *Sphinctrina turbinata* and *Stenocybe pullatula*) were chosen as outgroups to allow direct comparison with the previous results of Muggia et al. (2015). This first dataset was reduced to the final dataset (Table 2), as all newly obtained sequences were consistently placed within or basal to *Chaetothyriales* or *Verrucariales*. The final dataset therefore included a selection of representatives of *Chaetothyriomycetidae* only, viz. *Pyrenulales* (selected as outgroup), *Phaeomoniellales*, *Ferrariales*, and within *Chaetothyriales* the families *Chaetothyriaceae*, *Cyphellophoraceae*, *Epipharyngaceae*, *Herpotrichiellaceae* and *Trichomeriaceae* selected from the phylogenetic studies of Gueidan et al. (2014), Muggia et al. (2015, 2016, 2017), Teixeira et al. (2017), Vasse et al. (2017) and from a preliminary dataset of *Eurotiomycetes* in preparation by Muggia et al. (unpublished). The single-locus sequence alignments were prepared manually in BioEdit 7.0 (Hall 1999). Introns and ambiguous aligned regions were removed manually from the alignments.

Combined data of different loci, whether fully or partially congruent, have been commonly considered by inferring organismal phylogeny (Dettman et al. 2003). As in previous studies (Midiłkowska et al. 2006; Muggia et al. 2014, 2016; Pino-Bodas et al. 2017), we also considered both single-locus and combined datasets. Both the

Table 1. Newly sequenced specimens of *Muellerella* spp. from different lichen hosts, and NCBI accession numbers for the corresponding new sequences.

| DNA extr. N. | Specimen type – voucher no. | Origin of environmental samples | Loci sequenced |
|--------------|-----------------------------|---------------------------------|----------------|
| DP946        | *Muellerella erratica* – specimen Ertz 20485 | Austria, Carinthia, Glockner-Gruppe, above Hochtor Pass, on *Xanthoria elegans*, 2670 m a.s.l., 12.VII.2015. | MN241079 MN241075 MN241086 |
| DP855        | *Muellerella ventosicola* – specimen Reider 150307 | Norway, Sor-Trondelag, Oppdal, Grombakken S of Kongsvid fjellstue, on *Ophioparma ventosa*, 960 m a.s.l., 08.V.2015 | MN241080 MN241076 MN241087 |
| DP956        | *Muellerella erratica* – specimen Ertz 20470 | Austria, Carinthia, Glockner-Gruppe, above Hochtor Pass, on *Lecanora polytricha*, 2620 m a.s.l., 12.VII.2015. | MN241081 – MN241088 |
| DP951        | *Muellerella sp.* – specimen Ertz 20419 | Austria, Styria, Hochschub-Gruppe, NW of Tragöß-Oberort, N of Hochturm Mt., on *Protoblastenia rupestris*, ~1050 m a.s.l., 06.VII.2015. | MN241082 MN241077 MN241089 |
| DP953        | *Muellerella ventosicola* – specimen Ertz 20489 | Austria, Carinthia, Glockner-Gruppe, above Hochtor Pass, on *Rhizocarpon geographicum*, 2670 m a.s.l., 12.VII.2015. | MN241083 MN241078 MN241090 |
| DP806        | *Muellerella sp.* – specimen Ertz 17847 | Reunion Island, Saint-Denis, sentier de la Roche Erite, Plaine des Chicots, on cf. *Trapelus*, 1935 m a.s.l., 06.XII.2012. | – – MN241091 |
| S6004        | *Muellerella sp.* – specimen SPO-8778 | Spain, Madrid, Miraflores, Puerto de la Morcuera, on *Lecanora polytricha*, 2001 m a.s.l., 17.II.2019. | MN241084 – MN241092 |
| S6005        | *Muellerella ventosicola* – specimen SPO-8775 | Spain, Madrid, Miraflores, Puerto de la Morcuera, on *Rhizocarpon geographicum*, 2001 m a.s.l., 17.II.2019. | MN241085 – MN241093 |
| A405         | *Muellerella ventosicola* – specimen Muggia-A405 | Austria, Steiermark, Koralpe massif, Krakaberg, S of summit, on *Rhizocarpon geographicum* 2040 m a.s.l, 17.VII.2012. | – – MN241094 |
| Taxon                     | Sample ID          | 28S    | 18S    | 16S    |
|--------------------------|--------------------|--------|--------|--------|
| *Agonimia allobata*      | L467               | FJ455771 | –      | GU121589 |
| *Agonimia tristicula*    | L469 (Hafellner 66664) | FJ455772 | –      | GU12159 |
| *Agonimia sp.*           | –                  | –      | –      | –      |
| *Aphanophora eugeniae*   | CBS 124.105        | FJ396527 | –      | –      |
| *Capronia munkii*        | AFTOL 656          | EF413604 | EF413603 | JF225723 |
| *Capronia parasitica*    | CBS 123.88         | FJ358225 | FJ358293 | JF225724 |
| *Capronia peltigerae*    | –                  | HQ613813 | HQ613815 | HQ613814 |
| *Capronia pilosella*     | AFTOL 657          | DQ823099 | DQ823106 | JF225725 |
| *Capronia semimemoria*   | AFTOL 658          | FJ358226 | FJ358294 | JF225726 |
| *Ceramothryium carniciicum* | AFTOL 1063    | EF413628 | EF413627 | –      |
| *Cladophialophora arxii* | IFM 52022 / CBS 306.94 | –      | –      | –      |
| *Cladophialophora devriesii* | CBS 147.84  | AFTOL 668 | DQ823097 | JF225674 |
| *Cladophialophora minourae* | CBS 556.83    | FJ358235 | FJ358303 | JF225734 |
| *Cladophialophora parmeliae* | –                  | Ertz 16591 | –      | JX081675 |
| *Cyphellophora fusarioides* | AFTOL 1063   | –      | –      | –      |
| *Cyphellophora olivacea* | –                  | MUC 44033 | KC455252 | KC455298 | – |
| *Cyphellophora oxyspora* | –                  | CBS 698.73 | KC455262 | KC455305 | – |
| *Dolabra nepheliae*      | –                  | CBS 122.120 | GU332517 | –      | GU332519 |
| *Endocarpon pallidum*    | AFTOL 661          | DQ823100 | JQ823104 | JF225739 |
| *Epybryon bryophilum*    | M2                 | –      | –      | –      |
| *Epybryon hepaticola*    | M10                | –      | –      | –      |
| *Epybryon intercapillare*| M125               | –      | –      | –      |
| *Epybryon turfosorum*    | M292               | –      | –      | –      |
| *Fonsecaea brasiliensis* | CBS 119.710       | –      | –      | –      |
| *Fonsecaea monophora*    | CBS 102.243        | –      | –      | –      |
| *Granulopyrenis seawardii* | –                  | –      | –      | –      |
| *Heteroplectidium imbricatum* | AFTOL 2281 | –      | –      | –      |
| *Hydropunctaria maura*   | AFTOL 2263         | –      | –      | –      |
| *Knufia karalitana* (1)  | CC005 5465         | –      | –      | –      |
| *Knufia karalitana* (2)  | CC005 5466         | –      | –      | –      |
| *Knufia marmorica*       | CC005 5467         | –      | –      | –      |
| *Knufia mediterranea*    | CC005 5467         | –      | –      | –      |
| *Knufia petrilica*       | CBS 101157         | FJ358249 | FJ358313 | JF225743 |
| *Neocatapyrenium rhizosum* | AFTOL 2282 | –      | –      | –      |
| *Parabaglione dufourii*  | AFTOL 2254         | –      | –      | –      |
| *Phaeomoniella capsensis* | CBS 123.535   | –      | –      | –      |
| *Phaeomoniella prunicola* | STEU.6119   | –      | –      | –      |
| *Phialophora europaea*   | CBS 129.96         | FJ358248 | FJ358317 | JF225750 |
| *Placopyrenium buceki*   | AFTOL 2238         | –      | –      | –      |
| *Pylenula aspistea*      | AFTOL 2281         | –      | –      | –      |
| *Pyrenula cruenta*       | AFTOL 2254         | –      | –      | –      |
| *Pyrenula macropora*     | CG1520a            | –      | –      | –      |
| *Pyrenula pseudobufonia* | –                  | –      | –      | –      |
| *Pyrgillus javanicus*    | AFTOL 342          | –      | –      | –      |
| *Staurothele areolata*   | AFTOL 2291         | –      | –      | –      |
| *Thelidium papulare*     | AFTOL 2249         | –      | –      | –      |
| *Trichomerium foliicola* | MFLUCC10-0054     | –      | –      | –      |
| *Trichomerium sp.*       | –                  | –      | –      | –      |
| *Verrucaria viridula*    | AFTOL 2299         | –      | –      | –      |
| *Verrucula inconnexaria* | AFTOL 307          | –      | –      | –      |
| *Vonarxia vagans*        | CBS 123533         | –      | –      | –      |
| rock isolate TRN1        | –                  | FJ358250 | FJ358319 | JF225754 |
| rock isolate TRN14       | –                  | –      | –      | –      |
| rock isolate TRN30       | –                  | –      | –      | –      |
| rock isolate TRN107      | –                  | –      | –      | –      |
| rock isolate TRN15       | –                  | –      | –      | –      |
| rock isolate TRN210      | –                  | –      | –      | –      |
| rock isolate TRN214      | –                  | –      | –      | –      |
Table 2. Continued.

| Taxon                                              | Sample ID          | 28S        | 18S        | 16S        |
|----------------------------------------------------|--------------------|------------|------------|------------|
| rock isolate TRN475                                | –                  | FJ358260   | FJ358329   | FJ225764   |
| rock isolate TRN488                                | –                  | FJ358262   | –          | FJ225766   |
| rock isolate TRN493                                | –                  | FJ358263   | FJ358331   | FJ225767   |
| rock isolate TRN497                                | –                  | –          | FJ358332   | FJ225768   |
| rock isolate TRN508                                | –                  | FJ358265   | FJ358333   | FJ225770   |
| rock isolate TRN531                                | –                  | FJ358267   | FJ358335   | FJ225772   |
| Cultured fungus from Tephromela atra infected by Taeniiolella atricerebrina | A573               | KT263034   | KT263047   | KT263060   |
| Cultured fungus from Lecanora polytropa infected by Lichenococcus lecanorae | A859               | KT263036   | KT263049   | KT263062   |
| Cultured fungus from Lecidea sp. infected by Muellerella erratica | A526               | KT263136   | KT263180   | KT263224   |
| Cultured fungus from Lecanora polytropa infected by Lichenococcus lecanorae | A529               | KT263138   | KT263182   | KT263226   |
| Cultured fungus from Lecidea lapicida infected by Cecidonia umbonella | A872               | KT270601   | KT270689   | KT270771   |
| Cultured fungus from Lecidea sp. infected by Muellerella erratica | A875               | KT270604   | KT270692   | KT270774   |
| Cultured fungus from Aspicilia sp. infected by Endococcus verrucosus | A926               | KT270637   | KT270726   | KT270806   |
| Cultured fungus from Lecanora polytropa infected by Cercedospora epipolytropa | A945               | KT270649   | KT270735   | KT270818   |
| Cultured fungus from Aspicilia sp. infected by Endococcus verrucosus | A952               | KT270655   | –          | KT270824   |
| Cultured fungus from Aspicilia sp. infected by Endococcus verrucosus | A949               | KT270653   | KT270723   | KT270822   |
| Cultured fungus from Lecanora polytropa infected by Muellerella erratica | A974               | KT270668   | KT270751   | KT270837   |
| Cultured fungus from Tephromela atra infected by Taeniiolella atricerebrina | A980               | KT270672   | KT270754   | KT270841   |
| Cultured fungus from Lecanora intricata infected by Muellerella erratica | A989               | KT270678   | KT270760   | KT270847   |
| Cultured fungus from Lecanora polytropa infected by Lichenococcus lecanorae | A1161              | MF071427   | –          | MF085488   |
| Cultured fungus from Aspicilia sp. infected by Endococcus verrucosus | A1125              | MF071409   | MF071350   | MF085468   |
| Cultured fungus from Rhizocarpon geographicum infected by Muellerella ventosicola | A1113              | MF071402   | MF071345   | MF085462   |
| Cultured fungus from Lecanora polytropa infected by Cercedospora epipolytropa | A1120              | MF071405   | MF071347   | MF085464   |
| Cultured fungus from Rhizocarpon geographicum (A97) infected by Muellerella ventosicola | A944               | KT263072   | KT263094   | KT263110   |
| Cultured fungus from Rhizocarpon geographicum (A263) infected by Muellerella ventosicola | A993               | KT263073   | KT263095   | KT263111   |
| Cultured fungus from Lichenodiplis lecanorae infected by Muellerella ventosicola | A1015              | KT263076   | KT263096   | KT263114   |
| Lichenodiplis lecanorae                            | L1858              | KT263086   | KT263100   | KT263118   |
| Lichenodiplis lecanorae                            | L1860              | KT263087   | KT263101   | KT263119   |
| Muellerella atricola                               | L1992              | KT263083   | –          | KT263120   |
| Muellerella atricola                               | L1993              | KT263084   | KT263102   | KT263121   |
| Muellerella atricola                               | L1994              | KT263085   | KT263103   | KT263122   |
| Lichenodiplis lecanorae                            | L2206              | KT285901   | KT285921   | KT285910   |
| Lichenodiplis lecanorae                            | L2207              | KT285902   | KT285922   | KT285911   |
| Lichenodiplis lecanorae                            | L2208              | KT285903   | KT285923   | KT285912   |
| Lichenodiplis lecanorae                            | L2263              | KT285905   | KT285928   | KT285916   |
| Muellerella atricola                               | A333               | KT285906   | KT285929   | KT285917   |
| Muellerella atricola                               | A440               | KT285907   | KT285930   | KT285918   |
| Muellerella atricola                               | A528               | KT263088   | KT263104   | KT263123   |
| Muellerella atricola                               | A663               | KT285908   | KT285931   | KT285919   |
| Muellerella licheniola                             | L2209              | KT285904   | KT285924   | KT285913   |
| Lichenodiplis lecanorae                            | DE19202            | KT285909   | KT285932   | KT285920   |
| Lichenodiplis lecanorae                            | L2254              | –          | KT285925   | KT285914   |
| Lichenodiplis lecanorae                            | L2256              | –          | KT285926   | –          |
| Lichenodiplis lecanorae                            | L2257              | –          | KT285927   | KT285915   |
single-locus and the combined dataset were analysed with a Maximum Likelihood (ML) approach using RAxML v. 8.2 (Stamatakis 2014) with the user interface. The GTR-GAMMA model was used for both the single-locus and the combined datasets (treating the combined dataset into partition by gene). Node support was assessed by running 1000 bootstrap replicates. We analysed the three single-locus datasets for their topological incongruence by assuming a conflict significant when two different relationships (one monophyletic and the being non-monophyletic) for the same set of taxa were both supported with bootstrap values ≥70% (Mason-Gamer & Kellogg 1996; Reeb et al. 2004). Based on this criterion we detected partial conflict among the three loci (Table S2), so here we show the single-locus and the combined phylogenetic inferences.

Results

Phylogenetic analysis

We obtained 22 new sequences (seven for nuclear 28S, four for nuclear 18S and nine for mitochondrial 16S loci; Table 1). Among the newly sequenced Muellerella specimens, four are represented by all three loci and three by two loci, while two specimens are represented by the single mitochondrial 16S sequences (Table 1). We performed DNA extraction and amplification for another 15 Muellerella samples also, but due to unsuccessful PCR amplification and/or failure in the sequencing process, we did not obtain molecular data to include here. Also, for the newly sequenced Muellerella specimens we included only data from their thalli (environmental samples), as culture isolates prepared for three Muellerella samples (SPO-4576, SPO-4598, SPO-4599) turned out to represent the lichen host Lecidea spp.

The new sequences showed their closest matches with representatives of the order Chaetothyriales and with the three cultured endolichenic fungal strains representing clade II (A944, A993 and A1015), which were isolated from thalli of Rhizocarpon geographicum infected by M. ventosicola s.lat. (as reported in Muggia et al. 2016, 2017). None of the new sequences matched the previously published sequences of Muellerella atricola, Muellerella lichenicola and their Lichenodiplis lecanorae-like anamorphic state.

Due to the missing data in the taxon samplings of the single-locus alignments, some topological differences have been recovered among the inferred single-locus phylogenies (Fig. 3A–C). The major incongruences are given by (i) the paraphyly of Herpotrichiellaceae in the phylogeny based on nuclear 28S (Fig. 3A), (ii) the position of Cyphellophoraceae nested in Herpotrichiellaceae in the phylogeny based on nuclear 18S (Fig. 3B), and (iii) the position of Verrucariales within Chaetothyriales and the splitting of Chaetothyriales into three paraphyletic lineages in the phylogeny based on mitochondrial 16S (Fig. 3C). Phaeomoniellaceae is always monophyletic; it includes...
Figure 3. Single-locus (A–C) and multilocus (D) phylogenetic inferences of Muellerella taxa. The ML phylogenetic hypotheses were inferred from the individual datasets of the nuclear 28S (A), nuclear 18S (B) and mitochondrial 16S (C) loci and the combined dataset of these three loci (D). Branches supported by ML bootstrap support values >98% and 98% < 70% are bolded with two different thicknesses, respectively. The newly sequenced samples are bolded and are reported with the Muellerella species names and the lichen hosts. Culture isolates derived from lichen thalli infected by Muellerella spp. (Muggia et al. 2016, 2017) are asterisked (*); see Table 2 for further details on these specimens and Table S2 for detailed description of topological congruence/incongruence of phylogenetic inferences A–C.
Figure 3. Continued.
Figure 3. Continued.
Figure 3. Continued.
Clade I of endolichenic fungi and is recovered as basal in whole Chaetothyriomycetidae. Epiphyaceae is always paraphyletic, forming two well-supported lineages [here labeled Epiphyaceae (1) and (2)] always basal to Chaetothyriales. Trichomeriaceae is always monophyletic and within Chaetothyriales, representing Chaetothyriales (2) in the phylogeny based on the mitochondrial 16S locus.

The newly generated Muellerella sequences belong to two lineages that are labeled Muellerella spp. 1 and Muellerella spp. 2. The lineage Muellerella spp. 1 groups samples of M. erratica, M. ventosicola and unidentified Muellerella species, and is always recovered either as sister lineage of clades IV and V of cultured endolichenic fungi, or nested within them, but these phylogenetic relationships are only partly supported. Lineage Muellerella spp. 2, alternatively, groups three specimens of M. ventosicola from both R. geographicum and Ophioparma ventosa, and three cultured strains of clade II of Muggia et al. (2016, 2017; i.e. strains A944, A993, A1015) isolated from thalli of Rhizocarpon geographicum infected by M. ventosicola s.l.t. The sample Muellerella sp. DE17847, obtained from a thallus of Trapelia sp., is represented only by the 16S sequence and is recovered as basal in Muellerella spp. 2. This Muellerella spp. 2 lineage is nested within Verrucariales in the 28S phylogeny (Fig. 3A), is nested in Chaetothyriales in the 18S phylogeny (Fig. 3B), and is closely related to Phaeomoniellales in a supported sister relationship in the 16S phylogeny (Fig. 3C). The previously recognized lineage of M. atricola+M. lichenicola and their L. lecanorae-like anamorph is recovered as monophyletic, and is fully supported within Chaetothyriales in all three single-locus analyses.

Clades IV, V and VI+VII represent black melanized fungi isolated from diverse lichen species; originally these three lineages were recovered inside Chaetothyriales by Muggia et al. (2016, 2017). In the present analyses, instead, only clade VI+VII is confirmed to be placed within Chaetothyriales, whereas clades IV and V are placed outside Chaetothyriales (see above), being closely related to the clades of Muellerella spp. 1 and Verrucariales (Fig. 3A–D).

The multilocus phylogenetic hypothesis (Fig. 3D) recovered relationships among the families and the orders of Chaetothyriomycetidae that were congruent with previous studies (e.g., Diederich et al. 2013; Gueidan et al. 2008, 2014; Muggia et al. 2015, 2016, 2017; Teixeira et al. 2017; Vasse et al. 2017). The backbone phylogeny and the individual families and order lineages received full support. The fully supported monophyly of the clade M. atricola+M. lichenicola and their L. lecanorae-like anamorph within Chaetothyriales, as recognized by Muggia et al. (2015), is again confirmed; however, its sister relationships with Epiphyaceae – as suggested by Muggia et al. (2015) – is not recovered. The new lineages Muellerella spp. 1 and Muellerella spp. 2 are also recovered with the same groupings of samples identified in the single-locus phylogenies. Here, Muellerella spp. 1 is supported as sister lineage of the endolichenic fungal clade IV, and both are sister to four samples forming clade V. Muellerella spp. 2 is, instead, the fully supported sister lineage of Verrucariales, and the sample Muellerella sp. DE17847 is again basal within it.

Discussion

In this study we expanded the taxon sampling of Muellerella species to investigate the monophyly of the genus, as speculated in a previous study by Muggia et al. (2015). Muellerella samples were selected from a number of localities from Europe and Reunion Island as well as from six different lichen hosts, which are among the most common species to be parasitized by this lichenicolous fungal genus. Further, we could consider in this study Muellerella species that are commonly found on lichens: Muellerella erratica is indeed one of the best-known lichenicolous fungi reported from more than a hundred host species (Triebel 1989).

The present results suggest that the genus Muellerella is not monophyletic, as our sequences belong to three major lineages within Chaetothyriomycetidae. The first lineage is represented by the monophyletic Muellerella atricola+M. lichenicola complex (including the asexual Lichenodiplosis-like states), corroborating previous results by Muggia et al. (2015). The second and the third clades are the newly recovered lineages Muellerella spp. 1 and Muellerella spp. 2, each of them monophyletic and fully supported. Muellerella spp. 1 is related to two lineages of melanized fungi isolated from lichen thalli, viz. clades IV and V (Muggia et al. 2016, 2017). The phylogenetic placement of these two melanized fungal lineages is discordant from that originally inferred (Muggia et al. 2016). Indeed, they were originally recovered within Chaetothyriales, closely related to clade VI+VII (which is here still recovered within Chaetothyriales), but in the present analyses they form together with Muellerella spp. 1 a fully supported lineage (Fig. 3D) at the base of Chaetothyriales and Verrucariales. Although Muellerella spp. 1 and clades IV and V are closely related, and clade IV (but also clade VI+VII) contains isolates of endolichenic fungi obtained from lichen thalli infected by Muellerella spp., it is unlikely that any of these strains correspond to Muellerella. The isolates recovered in clades IV and VI+VII are melanized fungi morphologically very similar to each other (Muggia et al. 2016, 2017) and highly similar to the melanized rock-inhabiting fungi (RIF) isolated from rocks (Ruibal et al. 2009) and lichen thalli from arid Mediterranean habitats (Harutyunyan et al. 2008, Selbmann et al. 2013).

The position of the third clade Muellerella spp. 2, nested within Verrucariales in the 28S-based phylogeny and sister of this order in the combined analysis, was statistically supported. This placement agrees with the systematic position of Muellerella hypothesized by Triebel (1989). The main morphological characters that could support a relationship with the Verrucariales are the interascal filaments disappearing in an early stage of development but with persisting periphyssoids. However, these characters are also shared by Muellerella spp. 1 and M. atricola+M. lichenicola, rendering morphological synapomorphies for these lineages difficult to infer with
the few data currently at hand. The representatives of Muellerella spp. 2 are Muellerella species amplified directly from their hymenium and three fungal strains isolated from different thalli of R. geographicum infected by M. ventosicola s.lat. These cultured fungi formed clade II in Muggia et al. (2015, 2017), which was already recovered as sister to Verrucariales. The present results suggest that these three strains (A944, A993, A1015) likely represent a species of Muellerella. However, as we recovered one sample of M. ventosicola s.lat. also in clade Muellerella spp. 1, we cannot be certain that these strains belong to M. ventosicola. To confirm this hypothesis, a careful study of the species M. ventosicola, including sequences of its holotype, if possible, will be necessary. These cultured isolates A944, A993 and A1015 are paler than those of M. atricola, M. lichenicola and their L. lecanorae-like anamorph, and so far we have not observed the formation of pycnidia and conidiospores in them, as we did for the cultured M. atricola, M. lichenicola and their L. lecanorae-like anamorph (Muggia et al. 2015). Obtaining further new culture isolates of these new Muellerella lineages would be needed to test whether these other Muellerella taxa also share an asexual state. An asexual state was not observed in the sequenced specimens of M. erratica and M. ventosicola, suggesting that it is absent or very rare in this group. Interestingly, Muellerella atricola and M. lichenicola are characterized by ~100-spored asci, in contrast to M. erratica and M. ventosicola which have ~64-spored asci (Triebel 1989, Hafellner 2007). The degree of polyspor and the presence of a Lichenodiplis asexual state appear to be correlated with our phylogenetic results, supporting the M. atricola+M. lichenicola group as a lineage distantly related to the Muellerella spp. 1 and spp. 2 clades.

The polyphyly of the genus Muellerella leaves open the question of its family placement. This placement will be determined by the phylogenetic position of the generic type, M. polyspora, a species recorded mainly from the corticolous lichen Arthonia radiata. Unfortunately, this species is very rare, and fresh material was not available for sequencing, hampering progress in the taxonomy of the group. Muellerella polyspora has simple ascospores, unlike most species of Muellerella that have 1-septate ascospores (e.g., Hawksworth 1979; Ihlen & Wedin 2008) as well as all specimens of Muellerella that have been sequenced so far.

Interestingly, the close relationship of non-lichenized fungal lineages (clades IV, V and VI+VII of endolichec fungi) and lichenicolous fungi (Muellerella spp. 1 and spp. 2 clades) with a lineage of lichenized fungi (Verrucariales), recovered in the phylogenies based on the 28S and the combined datasets, recalls a pattern already observed in other fungal groups. This is observed also for lichenicolous fungi placed in Polycoccaceae and recovered as sister to the lichenized family Trypetheliaceae (Ertz et al. 2015a) within Dothideomycetes, and for the order Lichenostigmatales sister group of the lichenized lineage Arthoniales/Arthoniomycetes (Ertz et al. 2014).

It is now amply acknowledged that lichens with and without obvious symptoms of fungal infections harbor numerous fungal species in their microbiomes (U’Ren et al. 2010, 2012, 2014; Fleischhacker et al. 2015; Muggia et al. 2016, 2017; Fernández-Mendoza et al. 2017; Banchi et al. 2018) and that their identification is complemented by study of their corresponding axenic isolates. Comparing DNA sequences from the original lichen host sample and from the culture isolates helps determine the identity of these fungi, as found by Muggia et al. (2015) in studies of M. atricola and L. lecanorae. Unfortunately, we were not able to retrieve culture isolates of the Muellerella species for which we obtained sequences from the lichen thallii. The lack of corresponding culture isolates complicates an assessment of the identity of the Muellerella fungi amplified from the lichen thalli. However, the cultures we obtained from three of those 15 specimens chosen for molecular analyses that failed (see above) were not affected by fungal contamination, and only the host mycobiont (Lecidea sp.) grew axenically after a year and a half. It is likely that the mycobiont grew out from a tiny thallus fragment that remained attached to the perithelial hyphae.

The reduced number of molecular data and the multiple attempts that are usually needed to obtain reliable sequences to be included in phylogenetic analyses represent problems that still have to be overcome in future studies of lichenicolous fungi. The environmental material is usually difficult to find, and morphology-based species identification is usually required before performing molecular analyses. Although perithecia of Muellerella are usually abundant in infected lichen thalli, they are nonetheless very tiny structures and the only ones from which DNA extraction and culture isolation can be reliably performed. In general, lichenicolous fungi build inconspicuous reproductive structures (e.g., perithecia, apothecia, pycnidia) on the host thalli, and their removal typically consumes the material while often yielding insufficient DNA for successful amplification. Though PCR biases are well documented and often depend on the level of primer matching in different taxa (Green et al. 2015), to our knowledge there is no report with respect to lichenicolous fungi about bias introduced by direct PCR instead of traditional DNA extraction and amplification. Previously, Muggia et al. (2015) gained their data from environmental samples by performing traditional DNA extraction followed by PCR amplification. In the present study, the new sequences were generated mainly by direct PCR of perithelial material. The single exception is the sample Lichenodiplis lecanorae DE19202, of which sequences were obtained by direct PCR and are included in the monophyletic clade M. atricola+M. lichenicola/ Lichenodiplis-like anamorph. We may therefore exclude any amplification bias generated by direct PCR that could have led to the amplification of a species not belonging to Muellerella corresponding to the newly recovered Muellerella spp. 1 and spp. 2 lineages. In light of these considerations, amplifying Muellerella atricola by direct PCR would likely rule out whether amplification biases might be an issue in detecting certain lichenicolous taxa in lichens. For this reason we also used the Muellerella-specific primers designed by Muggia et al. (2015) to minimize potential amplification biases. However, when
using these primers for the 28S and 18S regions we did not recover any band, or else the sequencing was unsuccessful. This seems to confirm that the primers are indeed very specific to *M. atricola* + *M. lichenicola* and their *L. lecanorae*-like anamorph lineage and do not work for the other lineages of *Muellerella* recovered here. The new sequences representing the new clades *Muellerella* spp. 1 and spp. 2 will now be used to design additional species-specific primers to target *Muellerella* taxa on their lichen hosts with greater precision.

In the present context, the amount of molecular data is still too small to be correlated with the morphological variation within *Muellerella* species, although the degree of polypory and the absence/presence of a *Lichenodiplis*-like anamorphic state appear to be congruent with our phylogenetic results. Indeed, the *Lichenodiplis* asexual state may be confined to the *M. atricola* species, with the exception of the morphologically similar *M. lichenicola* clade. In addition to the species forming asci with ~100 spores. The clades *Muellerella* spp. 1 and spp. 2 likely represent distinct genera characterized by *Muellerella* species with fewer ascospores per ascus (up to ~64 spores) and by the absence of a *Lichenodiplis* anamorphic state. The results also hint at genetic diversity potentially shaped by host specificity. *Muellerella atricola* and *M. lichenicola*, with their *L. lecanorae*-like anamorphic states, both form fully supported clades. *M. ventosicola* s.lat. appears paraphyletic, with the specimen *M. ventosicola* SPO-8775 nested in the *Muellerella* spp. 1 clade, while all other specimens identified as *M. ventosicola* are part of the *Muellerella* spp. 2 clade. Whether the genetic diversity of *Muellerella* spp. could also depend on geographical differentiation still needs to be tested.

To confirm these hypotheses and to obtain a comprehensive understanding of *Muellerella* species diversity, much wider taxon sampling is required, including multiple samples representing the same *Muellerella*-lichen host combination from both the same and different geographic origins.

### Acknowledgements

Elisa Banchi (Trieste) and Mónica García-Gallo (Madrid) are thanked for help in the lab work. The Spanish Ministry of Science, Innovation and Universities supported SPO through a ‘Ramón y Cajal’ contract (RYC-2014-16784).

### Supplementary electronic material

**Table S1.** Characters analysed in the *Muellerella* spp. specimens included in the molecular analysis of this study. [Download file](#)

**Table S2.** Description of the topological congruence/incongruence of the phylogenetic inferences shown in main Fig. 3A–D. [Download file](#)

### References

Arnold, A. E., Miadlikowska, J., Higgins, K. L., Sarvate, S. D., Gugger, P., Way, A., Holstetter, V., Kauff, F. & Lutzoni, F. 2009. A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Systematic Biology* 58: 283–297.

Aveskamp, M. M., Murace, M. A., Wounderberg, J. H. C., Groenwald, J. Z. & Crous P. W. 2009. DNA phylogeny reveals polyphyly of *Phoma* section *Pyronellae* and multiple taxonomic novelties. *Mycologia* 101: 363–382.

Banchi, E., Stankovic, D., Fernandez-Mendoza, F., Gionechetti, F., Palavicini, A. & Muggia L. 2018. ITS2 metabarcoding analysis complements lichen mycobiome diversity data. *Mycological Progress* 17: 1049–1066.

von Brackel, W. 2014. Kommentierter Katalog der flechtenbewohnenden Pilze Bayerns. *Bibliotheca Lichenologica* 109: 1–476.

Crittenden, P. D., David, J. C., Hawksworth, D. L. & Campbell, F. S. 1995. Attempted isolation and success in the culturing of a broad spectrum of lichen-forming and lichenicolous fungi. *New Phytologist* 130: 267–297.

Dettman, J. R., Jacobs, D. J. & Taylor, J. W. 2003. A multigene phylogenetic approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* 57: 2703–2720.

Diederich, P., Ertz, D., Lawrey, J. D., Sikaroodi, M. & Unterreiner, W. A. 2013. Molecular data place the hyphomycetous lichenicolous genus *Sclerococcum* close to *Dactylospora* (Eurotiomycetes) and *S. parmeliae* in *Cladosiphialophora* (Chaetothyriales). *Fungal Diversity* 58: 61–72.

Diederich, P., Lawrey, J. D. & Ertz, D. 2018. The 2018 classification and checklist of lichenicolous fungi, with 2000 nonlichenized, obligately lichenicolous taxa. *The Bryologist* 121: 340–425.

Döbbler, P. & Triebel, D. 1985. Hepaticole Vertreter der Gattung *Muellerella* und *Dactylospora* (Ascomycetes). *Botanisches Jahrbuch der Systematischer 107: 503–519.

Ertz, D., Diederich, P., Lawrey, J. D. & Berger, F. 2015a. Phylogenetic insights resolve *Dacampiaceae* (Pleosporales) as polyphyletic: *Daldymycris* (Pleosporales, *Pleosphaeraceae*) with *Phoma*-like anamorphs resurrected and segregated from *Polycoccum* (*Trypetheliae*, *Polycoccaceae* fam. nov.). *Fungal Diversity* 74: 53–89.

Ertz, D., Lawrey, J. D., Common, R. S. & Diederich, P. 2014. Molecular data resolve a new order of *Arthoniomycetes* sister to the primarily lichenized *Arthoniales* and composed of black yeasts, lichenicolous and rock-inhabiting species. *Fungal Diversity* 66: 113–137.

Ertz, D., Teehler, A., Irestedt, M., Frisch, A., Thor, G. & van den Boom, P. 2015b. A large-scale phylogenetic revision of *Roccellales* (*Arthoniales*) reveals eight new genera. *Fungal Diversity* 70: 31–53.

Fernández-Mendoza, F., Kopun, T., Fleischhacker, A., Grube, M. & Muggia, L. 2017. ITS1 metabarcoding highlights low specificity of lichen mycobionts at local scale. *Molecular Ecology* 26: 4811–4830.

Fleischhacker, A., Grube, M., Kopun, T., Hafellner, J. & Muggia, L. 2015. Community analyses uncover high diversity of lichenicolous fungi in alpine habitats. *Microbial Ecology* 70: 348–360.

Green, S. J., Venkataramanan, R. & Naqib, A. 2015. Deconstructing the Polymerase Chain Reaction: understanding and correcting bias associated with primer degeneracies and primer-template mismatches. *PLoS ONE* 10: e0128122.

Gueidan, C., Villaseñor, R., de Hoog, G. S., Gorbuschina, A. A., Unterreiner, W. A. & Lutzoni, F. 2008. A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology* 61: 111–119.

Gueidan, C., Apteoot, A., Da Silva Cáceres, M. E., Badali, H. & Steenroos, S. 2014. A reappraisal of orders and families within the subclass *Chaetothyriomycetidae* (Eurotiomycetes, *Ascomycota*). *Mycological Progress* 13: 1027–1039.

Hafellner, J. 2007. The lichenicolous fungi inhabiting *Tephromela* species. *Bibliotheca Lichenologica* 96: 103–128.

Hall, T. A. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposia Series* 41: 95–98.

Harutyunyan, S., Muggia, L. & Grube, M. 2008. Black fungi in lichens from seasonally arid habitats. *Study in Mycology* 61: 83–90.

Hawksworth, D. L. 1979. Studies in the genus *Endococcus* (*Ascomycotina*, *Dothideales*). *Botaniska Notiser* 132: 283–290.
Hawksworth, D. L. 2011. A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. *IMA Fungus* 2: 155–162.

Ihlen, P. G. & Wedin, M. 2008. An annotated key to the lichenicolous Ascomycota (including mitosporic morphs) of Sweden. *Nova Hedwigia* 86: 275–365.

Kauff, F. & Lutzoni, F. 2002. Phylogeny of the Gyalectales and Ostragales (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetic and Evolution* 25: 138–156.

Lawrey, J. D. & Diederich, P. 2003. Lichenicolous fungi: Interactions, evolution, and biodiversity. *The Bryologist* 106: 80–120.

Mason-Gamer, R. J. & Kellogg, E. A. 1996. Testing for phylogenetic relationships. *American Journal of Botany* 83: 915–935.

Muggia, L., Kopun, T. & Grube, M. 2017. Effects of growth media on the lichenicolous, anamorphic genus *Vouauxiomyces* (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* 98: 1088–1103.

Muggia, L., Kopun, T. & Ertz, D. 2015. Phylogenetic placement of the lichenicolous, anamorphic genus *Lichenodiplis* and its connection to *Muellerella*-like teleomorphs. *Fungal Diversity* 64: 233–251.

Pérez-Ortega, S., Suija, A. & de los Ríos, A. 2011. The connection of enzymatically amplified ribosomal DNA from several *Dothideomycetes* (Pezizomycotina, Ascomycota) and evolution of polysporous fungi. *Molecular Phylogenetic and Evolution* 32: 1036–1060.

Rai, M. K., Vaibhav, V., Tiwari, V. V., Irinyi, L. & Kővics, G. J. 2014. Advances in taxonomy of genus *Phoma*: polyphyletic nature and role of phenotypic traits and molecular systematics. *Indian Journal of Microbiology* 54: 123–128.

Reeb, V., Lutzoni, F. & Roux, C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polysporous. *Molecular Phylogenetic and Evolution* 32: 1036–1060.

Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A. A., Crous, P. W., Groenewald, J. Z., Muggia, L., Grube, M., Isola, D., Schoch, C. L., Staley, J. T., Lutzoni, F. & de Hoog, G. S. 2009. Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*. *Study in Mycology* 64: 123–133.

Selbmann, L., Grube, M., Onofri, S., Isola, D. & Zaczon, L. 2013. Antarctic epilithic lichens as niches for black meristematic fungi. *Biology* 2: 784–797.

Stamatakis, A. 2014. RAxML Version 8.2: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, open access link: http://bioinformatics.oxfordjournals.org/content/early/2014/01/21/bioinformatics.btu033.abstract?keytype=ref&jkey=VTegUUYCD6f0K

Tedesco, L., Sanchez-Ramirez, S., Koljalg, V., Bahram, V., Döring, M., Schigel, D., May, T., Ryberg, M. & Abarenkov, K. 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity* 90: 135–159.

Teixeira, M. M., Moreno, L. F., Stiełow, B. J., Muszewska, A., Hainaut, M., Gonzaga, L. & Abouelleil A., et al., 2017. Exploring the genomic diversity of black yeasts and relatives (*Chaetothyriales*, Ascomycota). *Studies in Mycology* 86: 1–28.

Triebel, D. 1989. Lecideicole Ascomyceten . Eine Revision der obligat lichenicolen Ascomyceten auf lecideoiden Flechten. *Bibliotheca Lichenologica* 35: 1–278.

Triebel, D. & Kainz, C. 2004. *Muellerella*. In: Nash, T. H., Ryan, B. D., Diederich, P., Gries, C. & Burgantz, F. (eds), *Lichen Flora of the Greater Sonoran Desert Region*, Vol. 2., pp. 673–675. Lichens Unlimited, Arizona State University, Tempe, Arizona.

U’Ren, J. M., Lutzoni, F. M., Miadlikowska, J. & Arnold, A. E. 2010. Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *Microbial Ecology* 60: 340–53.

U’Ren, J. M., Lutzoni, F., Miadlikowska, J., Laetsch, A. D. & Arnold, A. E. 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany* 99: 899–914.

U’Ren, J. M., Riddle, J. M. & Monacell, J. T. et al. 2014. Tissue storage and primer selection influence pyrosequencing-based inferences of diversity and community composition of endolichenic fungi. *Molecular Ecology Resources* 14: 1032–1048.

Vasse, M., Voglmayr, H., Mayer, V., Gueidan, C., Nepel, M., Moreno, L., de Hoog, S. G., Selosse, M. A., McKey, D. & Blatrix, R. 2017. A phylogenetic perspective on the association between ants (*Hymenoptera: Formicidae*) and black yeasts (*Ascomycota: Chaetothyriales*). *Proceedings of the Royal Society B* 284: 20162519.

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptothecium* species. *Journal of Bacteriology* 172: 4238–4246.

Zhou, S. & Stanosz, G. R. 2001. Primers for amplification of mtSSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* 105: 1033–1044.

Zoller, S., Scheidegger, C. & Sperisen, C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* 31: 511–516.