Multigene phylogeny and taxonomic revision of Atheliales s.l.: Reinstatement of three families and one new family, Lobuliciaceae fam. nov.

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1. Introduction

Atheliales Jülich (1982) is a fungal order in the subclass Agaricomycetidae, class Agaricomycetes, phylum Basidiomycota (Hibbett et al., 2007; Wijayawardene et al., 2020). Unlike most other orders within the extremely diverse Agaricomycetes, members of Atheliales are generally inconspicuous with relatively simple gross morphology, possessing few diagnostic features. Members of the order are generally corticioid and athelioid, producing effused, crust-like fruiting bodies that are loosely attached to the substrate and with non-differentiated margins (Eriksson et al., 1978, 1981, 1984). Atheliales species prefer moist habitats on the forest floor, and they are mostly documented in temperate regions (Dai, 2011; Ezhou et al., 2017; Ginn, 1998; Gorjón and Bernicchia, 2013; Gorjón and Hallenberg, 2013; Ordynets et al., 2017; Rosenthal et al., 2017). The order encompasses approximately 100 species in 20 genera (He et al., 2019; Index Fungorum, 2020), mostly composed of described...
species from Europe (Duhem, 2013; Kotiranta et al., 2011), but also from Argentina (Gorjon et al., 2012), Chile (Gorjón and Hallenberg, 2013), Bhutan (Dhingra and Singh, 2018), and India (Singh et al., 2010; Prasher, 2015).

Despite their simple gross morphology, members of _Atheliales_ exhibit a remarkable diversity of ecological strategies, which include: ectomycorrhizal (Amphinema, Byssocorticium, Piloderma and Tylospora; Tedersoo et al., 2010), white rot saprotrophic (_Athelia, Athelopsis, Fibulomyces, Leptosporomyces_; Tedersoo et al., 2014), lichenicolous (_Athelia arachnoides_; Yurchenko and Olubkov, 2003), and putatively parasitic on termites involving mimicry of termite eggs (_Athelia termitiphila_; Maekawa et al., 2020; Matsuura et al., 2000; Yashiro et al., 2011). Sequences obtained from the roots of achlorophyllous orchids Lecanorchis spp. and Erythrorchis altissima also suggest that members of _Atheliales_ are associated with mycoheterotrophy (Ogura-Tsujita et al., 2018; Okayama et al., 2017), and are potentially diverse in the tropics (Tedersoo and Varga, 2015). Recent additions of new taxa, such as _Dhingra et al._ (2019), _Lichinae_

Over the years, a number of genera have been described and added to _Atheliales_, based on morphological characters alone (Hjortstam and Ryvarden, 2004, 2010) or combined with molecular phylogenetic evidence (Kotiranta et al., 2011). Sequence-based studies have found some of these genera to be polyphyletic, sometimes with members clustering within other orders (Binder et al., 2010; Erz et al., 2008; Hibbett et al., 2007). Genera of _Atheliales sensu lato_ are summarized in Table 1, as well as significant sources indicating their presumed affinities. Well-annotated molecular data in public databases are scarce for _Atheliales_, and a phylogeny of the order is lacking.

In this study, we present the first comprehensive phylogenetic treatment of the order _Atheliales_ with two specific aims. First, we aimed to delimit _Atheliales_ by sampling the type species of the genera listed in _Atheliales sensu lato_ (Table 1) as well as representatives of various orders within _Agaricomycetes_. Due to the taxonomic breadth of this analysis, we used molecular data from 5.8S and LSU of the nuclear ribosomal DNA as well as the protein coding regions of _rpb2_ and _tef1_ excluding the third codon position to reconstruct the phylogeny of _Agaricomycetes_. Second, we aimed to delineate phylogenetic lineages within the order. For this aim, we assembled a dataset composed of taxa belonging to _Atheliales sensu stricto_. This dataset was based on the nuclear ribosomal ITS1, 5.8S, ITS2, and LSU, as well as _rpb2_ and _tef1_ including the third codon position.

## 2. Materials and methods

### 2.1. Taxon sampling, fungal isolates, and DNA extraction

We targeted taxa in _Atheliales sensu lato_ as summarized in Table 1, with emphasis on the type species of each genus. Specimens were retrieved from the herbarium of Uppsala University Museum of Evolution (UPS), the herbarium of University of Gothenburg (GB), and the Farlow Herbarium at Harvard University (FH), as well as the private collections of B.P. Sulistylo (BPS) and M. Ryberg (MR) (Table 2). Several specimens from GB (Table 2: E. Bendiksen 645 07, E. Bendiksen 523 07, E. Bendiksen 580 07, KHL 13899, E. Bendiksen 573 07, KHL 13496b, V. Spini 8810a) were extracted using the DNeasy Plant Mini Kit (Qiagen, Stanford, CA), whereas specimens from FH were extracted using the Extract-N-Amp Plant PCR Kit (Sigma–Aldrich, St. Louis, MO) according to Haelewaters et al. (2018), or the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Alpharetta, GA) according to Aldrich, St. Louis, MO) according to Haelewaters et al. (2018), or the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Alpharetta, GA).

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**Fig. 1.** Comparison of relationships among orders within _Agaricomycetidae_ according to: A) Nagy et al. (2015), B) Chen et al. (2019), Liu et al. (2018), and Zhao et al. (2017), C) Varga et al. (2019).
Norcross, GA) following the manufacturer’s instructions. Specimens from UPS, BPS, and MR, as well as the rest of the specimens from GB were extracted using a modified CTAB/chloroform-isooamyl alcohol DNA extraction (Cubero et al., 1999). Approximately 5 × 5 mm hymenium was picked from the substrate and grinded using a micropestle in 500 μl of 2% CTAB extraction buffer (100 mL Tris, 20 mM Na₂EDTA, 1.4 M NaCl, pH 8.0) with 1% β-mercaptoethanol. The resulting mixture was then incubated at 65 °C for up to 2 h. Subsequently, 500 μl of chloroform: isooamyl alcohol (24:1) was added and the mixture was shaken horizontally at low speed for 1 h before centrifugation at 12,000 rpm for 14 min. Following this, 360 μl of the upper phase was transferred into a new tube and 240 μl of cold isopropanol was added. After the sample precipitated overnight at cold temperature, it was centrifuged and the resulting DNA pellet was washed using wash buffer (76% EtOH, 10 mM Tris, 1 mM EDTA, pH 8.0), and its concentration and purity were determined by means of Qubit Fluorometric Quantitation (Invitrogen, Carlsbad, CA) and gel electrophoresis.

### 2.2. PCR amplification, sequencing, and sequence analyses

Six molecular markers were used in this study: nuclear ribosomal regions of ITS1, 5.8S, ITS2, and LSU, as well as the protein coding regions of rpb2 and tef1 (Binder et al., 2010; Matheny et al., 2007; Miettinen et al., 2012; Zhao et al., 2017). Amplification of rpb2 targeted the region between conserved domains 5 and 7 (Liu et al., 1999), whereas for tef1 the target region was between exons 4 and 8 (Woodland and Kothe, 1997). Primers used for PCR and sequencing of these target regions are listed in Table 3. Modifications to the primers used in previous studies were also done to facilitate amplification and sequencing of Atheliales taxa. LB-W-R is a reverse-complement of LB-W (Tedersoo et al., 2008), used to bridge the gap in the sequencing of LSU PCR products. Additionally, EF1-1577Fa was based on EF1-1577F (Rehner and Buckley, 2005) with one nucleotide difference for better priming in Atheliales, and this primer was designed using the dataset of Binder et al. (2010).

Cycling conditions for the amplification of ITS began with initial denaturation at 95 °C for 3 min, followed by 35–40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, concluded by final extension at 72 °C for 10 min. LSU amplification used similar cycling conditions as ITS, but with annealing temperature of 48 °C. Cycling conditions for the amplification of rpb2 and tef1 were based on the methods of Rehner and Buckley (2005) and Matheny et al. (2007). The program started with denaturation at 94 °C for 2 min, 8 cycles of denaturation at 94 °C for 40 s, annealing at 60 °C for 40 s with 1 °C decrease/cycle, and extension at 72 °C for 1–2 min, followed by 36 cycles of denaturation at 94 °C for 45 s, annealing at 53 °C for 90 s, and extension at 72 °C for 1–2 min, concluded by final extension at 72 °C for 10 min. All PCR products were cleaned by means of ExoSAP-IT (Applied Biosystems, Foster City, CA) and sent for sequencing to Macrogen Europe (Amsterdam, the Netherlands). Purification and sequencing of PCR products for samples obtained from FH were outsourced to Geneuwiz (South Plainfield, NJ). Sequencing reads were assembled, assessed, and edited using CodonCode Aligner (CodonCode Corporation, Centerville, MA) or Sequencher 4.10.1 (Gene Codes Corporation, Ann Arbor, MI). To confirm their identities and filter out contaminations, ITS sequences of all samples were blasted against the UNITE database (Kölgj et al., 2013; https://unite.ut.ee/).

### 2.3. Dataset assembly and multiple sequence alignment

We constructed two datasets: an Agaricomycetes-wide dataset and an Atheliales sensu stricto dataset. The Agaricomycetes dataset
| Order/Species                        | ID                  | Country of origin (ISO code) | GenBank accession number   | Source                                      |
|-------------------------------------|---------------------|------------------------------|----------------------------|---------------------------------------------|
|                                     |                     |                              | ITS           | LSU          | rpb2         | tef1         |
| Agaricales                          |                     |                              |                |              |              |              |
| Anthracophyllium archeri           | AF81073             |                              | DQ404387      | AY745079    | DQ385877     | DQ028586     |
| Aphanopsidium pseudotusque         | HHP-822             | US                           | GU187509      | GU187567    | GU187781     | GU187965     |
| Chondrostereum purpureum           | AF81044             | US                           | DQ000929      | AF518607    | AY218477.2   | DQ457632     |
| Digitatispora marina *             | 3027C               | NO                           | KM272371      | KM272362    |              |              |
| Gymnopus piceus                    | ZR2015011           | CN                           | LT716066      | KY418882    | KY419027     | KY419077     |
| Henningsomyces candidis            | AF81046             | US                           | AY571043      | AF267864    | AY218513.2   | AY883424     |
| Lepiota crisata                    | ZR20151133          | CN                           | LT716026      | KY418841    | KY418992     | KY419048     |
| Lepista irina                      | AF81081             | US                           | DQ221109      | DQ234538    | DQ385885.2   | DQ028591     |
| Mycophyllum corneipes              | AF81097             |                             | DQ404393      | AY745707    | DQ408110     | DQ029197     |
| Amylocorticium molle               | KHL 13500           | SE                           | GU187667      |              |              |              |
| Amylocorticium subilleaquatum      | KHL 8493            |                             | AY463411      | AY586679    |              |              |
| Amylocorticium cebennense          | HHP-2808            | US                           | GU187505      | GU187561    | GU187770     | GU187675     |
| Amylocorticium subsulphureum       | HHP-13817           | SE                           | GU187506      | GU187562    | GU187773     | GU187680     |
| Leptosporomyces septentrionalis + 1| G8-0090937          | SE                           | LR694203      | LR694181    | LR689276     | LR694219     |
| Leptosporomyces septentrionalis + 2| JS 16122            | NO                           | GU187497      | GU187664    |              |              |
| Plicaturopsis crispa               | MR000462            |                             | LR694209      | LR694187    | LR689281     | LR694225     |
| Atheliales                         |                     |                              |                |              |              |              |
| Amphinema byssoides 1             | EL 11/98            | EE                           | AY463375      | AY586266    |              |              |
| Amphinema byssoides 2              | M. Ryberg           | SE                           | GQ162810      | GQ162810    |              |              |
| Amphinema byssoides 3              | MR00333             |                             | LR694190      | LR694167    |              |              |
| Amphinema diadema                  | JS 25999            | NO                           | GQ162811      | GQ162811    |              |              |
| Athelia arcahiaris                  | B1710028            | SE                           | LR694191      | LR694168    | LR694266     | LR694212     |
| Athelia arcahiaris 2                | GB0087426           | SE                           | LR694192      | LR694169    | LR694267     | LR694213     |
| Athelia bombacina 1                 | E. Bendiksen        | 645/07                       | MT305993      | MT305993    | LR794094     | LR794095     |
| Athelia bombacina 2                 | E. Bendiksen        | 523/07                       | MT305995      | MT305995    | LR794099     | LR797834     |
| Athelia decipiens 1                 | GB0090493           | SE                           | LR694193      | LR694170    | LR694268     | LR694215     |
| Athelia decipiens 2                 | JS 4930             | NO                           | AY463381      | AY586632    |              |              |
| Athelia decipiens 3                 | L-10567             | US                           | GU187537      | GU187592    | GU187802     | LR797877     |
| Athelia epiphylla 1                 | GB0090655           | SE                           | LR694194      | LR694171    |              |              |
| Athelia epiphylla 2                 | KHL 13889           |                              | MT305996      | MT305996    | LR794100     | LR794100     |
| Athelia fibulata                    | E. Bendiksen        | 573 07                       | MT305997      | MT305997    | LR794101     | LR794102     |
| Athelia neuohaoffii                 | GB0087199           | SE                           | LR694195      | LR694172    | LR694269     | LR694214     |
| Athelia spinulsa                    | JS 25630            | NO                           | GQ162813      | GQ162813    |              |              |
| Athelia sp. 1                       | BHI-F636            | US                           | MK958813      | MK958817    | MK983172     | MK983174     |
| Athelia sp. 2                       | BHI-F645            | US                           | MK958814      | MK958818    | MK983173     | MK983175     |
| Athelia sp. 3                       | BHI-H1595           | US                           | GU187502      | GU187565    | GU187766     | GU187678     |
| Athelopsis glauca 1                 | GB0058723           | SE                           | LR694196      | LR689417    |              |              |
| Athelopsis glauca 2                 | KHL 13901           | SE                           | LR694197      | LR694174    | LR694270     | LR738852     |
| Athelopsis subincopisica            | BS1710033           | SE                           | LR694198      | LR694175    | LR694271     | LR694214     |
| Byssocorticium atrovirens 1         | GB0078129           | SE                           | LR694199      | LR694176    | LR694272     | LR694215     |
| Byssocorticium atrovirens 2         | RS 09400            | FI                           | GQ162814      | GQ162814    |              |              |
| Byssocorticium caeruleum            | GB0078135           | SE                           | LR694200      | LR694177    | LR694273     | LR694216     |
| Byssocorticium pulchrum 1           | KHL 11710           | FI                           | AY463388      | AY586639    |              |              |
| Byssocorticium pulchrum 2           | GB0107231           | 242                          |                |              |              |              |
| Fibulomyces mutabilis               | HG-C 5753           | DE                           | GQ162817      | GQ162817    |              |              |
| Leptosporomyces galzinii            | GB0107231           | SE                           | LR694202      | LR894180    | LR694275     | LR694218     |

Table 2: Species names, voucher information, GenBank accession numbers and references of taxa included in this study. Taxon followed by an asterisk (*) indicates that it was placed in Atheliales sensu lato but placed elsewhere according to this study, double asterisks (**) indicates the opposite.
| Order/Species | ID | Country of origin (ISO code) | GenBank accession number | Source |
|---------------|----|-----------------------------|-------------------------|--------|
| **Leptosporomyces sp.** | GB0087510 | SE | LR694204 LR694182 LR694277 LR694220 | This study |
| **Lobulicicum octactum** | KHL 13496b | | MT340827 MT340827 | This study |
| **Piloderma bicalcaratum** | BS1710030 | NO | LR694205 LR694183 LR694278 LR694221 | This study |
| **Piloderma byssinum 1** | GB0121002 | SE | LR694206 LR694184 LR694279 | This study |
| **Piloderma byssinum 2** | JS 20399 | NO | DQ469281 DQ469282 | Larsson, unpublished |
| **Piloderma croceum** | KHL 8456 | | AY463454 AY38669 | Larsson et al. (2004) |
| **Piloderma falax** | MR00338 | NO | LR694207 LR694185 LR738853 LR694223 | This study |
| **Piloderma lanatum 1** | JS 22149 | NO | DQ469288 DQ469288 | Larsson, unpublished |
| **Piloderma lanatum 2** | JS 24861 | NO | AY463454 AY386700 | Larsson et al. (2004) |
| **Piloderma olivaceum** | BS1710031 | SE | LR694208 LR694186 LR694280 LR694224 | This study |
| **Stereopsis vitellina** | F03241 | SE | LR694211 LR694189 LR694283 | This study |
| **Stereopsis vitellina** | Gilsenius | | JN649374 JN649374 | Spokivst et al. (2012) |
| **Stereopsis vitellina** | | NO | GQ162680 GQ162680 | Kotiranta et al. (2011) |
| **Stereopsis microspora** | GB0090789 | FI | GQ162681 | Kotiranta et al. (2011) |
| **Tylospora asterophora** | KHL 8566 | SE | AY463480 | Larsson et al. (2004) |

**Auriculariales**

| Boletales | AFTOL-676 | US | DQ200918 AY634277 QD366278 DQ408143 | Matheny et al. (2007) |

| Coniophora arida | FP-104367 | US | GU187510 GU187573 GU187775 GU187684 | Binder et al. (2010) |
| Coniophora marmorata | MUCL 31667 | BE | GU187515 GU187571 GU187780 GU187688 | Binder et al. (2010) |
| Comphalidus roseus | AFTOL-1780 | DE | DQ534570 DQ346669 GU187818 GU187702 | Binder et al. (2010) |
| Hygrodor-dus saracini | MUCL 40589 | GF | GU187822 GU187579 GU187764 GU187703 | Binder et al. (2010); Binder and Hibbett, 2006 |
| Hydnomera lurida | MD312 | US | GU187523 GU187580 GU187787 GU187708 | Binder et al. (2010) |
| Leucogyrophana licheniola | DAOM 04172 | CA | GU187531 GU187583 GU187789 GU187715 | Binder et al. (2010) |
| Leucogyrophana olivaceus | HHB-11134 | US | GU187532 GU187587 GU187790 GU187717 | Binder et al. (2010) |
| Pseudomerulius aureus | FP-103859 | US | GU187534 GU187590 GU187799 GU187731 | Binder et al. (2010) |
| Pseudomerulius cartiisi | REH912 | AU | GU187533 GU187589 GU187796 GU187725 | Binder et al. (2010) |

**Cantharellales**

| Clavulinopsis | AFTOL-667 | US | DQ202266 AY745694 DQ366286 DQ408143 | Matheny et al. (2007) |
| Hydnellum albonema | AFTOL-471 | US | DQ218305 AY700199 DQ234553 DQ234568 | Matheny et al. (2007) |

**Corticiellales**

| Punctularia | AFTOL-1248 | US | DQ398958 AY518642 DQ381843 DQ408147 | Matheny et al., unpublished; (Hibbett and Binder, 2002) |
| Vaiulmina comedens | AFTOL-1247 | US | DQ398959 AY518666 DQ381844 | Matheny et al., unpublished; (Hibbett and Binder, 2002) |

**Dacrymycetes**

| Calocera conria | AFTOL-438 | US | AY799083 AY701526 AY356286 AY881019 | Matheny et al., unpublished |
| Gloeophyllales | ARIZ | | HM536092 HM536063 HM640259 HM536111 | Garcia-Sandoval et al. (2011) |
| Gloeophyllum striatum | AN027866 | | HM536094 HM536067 HM536112 HM536113 | Garcia-Sandoval et al. (2011) |
| Gloeophyllum trabecum | IBU 9930 | MX | HM536095 HM536069 HM536114 HM536115 | Garcia-Sandoval et al. (2011) |
| Helvocybe sakata | DAOM 214911 | | HM536096 HM536071 HM536116 HM536117 | Garcia-Sandoval et al. (2011) |

**Gomphales**

| Gasteria cerithi | AFTOL-466 | US | AF377072 AF930358 AY218486 AY883434 | (Bidartondo and Bruns, 2002; Wang et al., 2004) |
| Hymenochaetales | AFTOL-447 | US | DQ234559 AY827854 AY218526 AY885147 | Matheny and Hibbett, unpublished; (Hibbett et al., 2000; Wang et al., 2004) |
| Cotylidia sp. | AFTOL-700 | US | AY854079 AY629317 AY383422 AY885148 | Wang et al., unpublished; Matheny and Hibbett, unpublished |
| Fomitiporia gabonensis | MUCL 47576 | GA | GU461971 GU461990 JQ087972 GU461923 | (Anafi et al., 2010, 2012) |
| Fomitiporia mediterranea | AFTOL-488 | US | AY854080 AY614517 AY803748 AY885149 | Matheny, unpublished |
| Fomitoporia sonae | MUCL 47689 | US | JQ087893 JQ087920 JQ088006 JQ087947 | Anafi et al. (2012) |
| Lycoperdon lactaz * | MUCL 47689 | US | MT305998 MT305998 | This study |
| Rescinicum bicolor | AFTOL-810 | US | | (Binder and Hibbett, 2002; Matheny et al., 2007) |
| Rickenella fubula | AFTOL-486 | | | Matheny et al., unpublished; |
| Jaapia argillacea | CBS 252.74 | NL | GU187524 GU187581 GU187788 GU187711 | Binder et al. (2010) |
| Lepidodendromatiales | RV-MX16 | MX | JN699807 JN699808 | Hodkinson et al. (2012) |
| Lepidodendromatiales | Sulphurachromyces caotingae | BR | KC170320 KC170318 | Sulzbacher et al. (2012) |

| Phallales | AFTOL-683 | US | DQ403885 AY885165 DQ408114 DQ435792 | Matheny et al., unpublished |
| Phallus hadrian | AFTOL-700 | US | DQ403885 AY885165 DQ408114 | Matheny et al., unpublished |
| Polyporales | AFTOL-770 | US | DQ403885 AY885165 DQ408114 | Matheny et al., unpublished; (Matheny et al., 2007) |
| Phallus hirsutus | AFTOL-771 | US | DQ403885 AY885165 DQ408114 | Matheny et al., unpublished; (Matheny et al., 2007) |

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comprised representatives of each order in the class (except Hysterangiales and Geastrales in the Phallomycetidae), with Dacrymycetes as outgroup. This Agaricomycetes dataset was used to identify the members of Atheliales sensu stricto and to ascertain the phylogenetic position of several taxa that had not yet been considered in a phylogenetic context. Based on the result of this dataset's
phylogenetic analysis, we then constructed the *Atheliales sensu stricto* dataset, with *Atheliales* as ingroup, and Lepidostromatales and Boletales as outgroups.

In total, 108 sequences were newly generated during this study: 31 ITS, 32 LSU, 26 *rpb2*, and 19 *tef1* sequences. These were supplemented with 310 sequences downloaded from NCBI GenBank for phylogenetic analyses. The complete list of taxa and GenBank accession numbers can be found in Table 2. For both datasets, taxa without LSU were excluded from subsequent analyses to avoid indistinguishable branches in the tree (Sanderson et al., 2010). The molecular markers for the *Agaricomycetes* dataset were LSU, 5.8S, *rpb2*, and *tef1*. The ITS regions (ITS1 and ITS2) were excluded from the *Agaricomycetes* dataset to avoid erroneous alignment due to the large numbers of indels (Tedorsoo et al., 2018). Furthermore, we also excluded the third codon positions of both *rpb2* and *tef1* from the *Agaricomycetes* dataset since this position is prone to saturation under broad taxonomic range (Binder et al., 2010; Matheny et al., 2007). This finding was supported by preliminary analyses, which showed that the exclusion of third codon positions increased overall support values. However, the opposite was true for the *Atheliales* dataset due to its narrower taxonomic breadth; as a result, third codon positions were included in this dataset. All three ITS regions (ITS1, 5.8S, and ITS2) were also included in the *Atheliales* dataset since direct comparison suggested that they contained significant phylogenetic signals as marked by the change in overall support values.

Alignments and overall data management were done in Geneious v10.2 (Biomatters, Auckland, New Zealand). Sequences were grouped according to their respective region and aligned separately. Full-length ITS1, 5.8S, and ITS2 regions were identified and separated using ITSx (Bengtsson-Palme et al., 2013). Multiple sequence alignments were carried out using MAFFT v7.388 (Katoh et al., 2002; Katoh and Standley, 2013). LSU, *rpb2*, and *tef1* were aligned using the MAFFT E-INS-i algorithm, whereas G-INS-i was used for aligning full-length 5.8S, ITS1, and ITS2 sequences. Afterwards, alignments were improved by realigning several challenging regions, manual adjustments, including trimming the ends of sequences and the removal of introns from *rpb2* and *tef1* alignments.

### 2.4. Phylogenetic analyses

Prior to phylogenetic analyses, the topological congruence among different regions was evaluated using RAxML v8.2.12 (Stamatakis, 2014), with the GTRGAMMA model and 500 rapid bootstrap replicates. Since no well-supported conflict (BS ≥ 75) was found among the topologies of each region, the sequences were concatenated for further analyses. For each dataset, partitioning schemes and substitution models were determined using PartitionFinder2 (PF2) (Kainer and Lanfear, 2015). Phylogenetic trees were estimated based on maximum likelihood (ML) using RAxML v8.2.12 (Stamatakis, 2014) and Bayesian inference (BI) with MrBayes v3.2.7a (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). All PF2, ML, and BI analyses were done on the CIPRES Science Gateway webserver (Miller et al., 2010).

Input for PF2 was the concatenated alignment with user-defined data blocks for each region as well as each codon position for *rpb2* and *tef1* (only the 1st and 2nd for the *Agaricomycetes* dataset and all three for the *Atheliales* dataset), analyzed using the greedy algorithm (Lanfear et al., 2012) and based on Bayesian information criterion (BIC), with options for models according to those that are available in either RAxML or MrBayes. Consequently, phylogenetic analyses were done following the partitioning scheme and substitution model recommended by PF2 (Table 4).

For RAxML analyses, a phylogenetic tree was inferred through 1000 rapid bootstrap replicates. Since the specification of different models among partitions is not possible with RAxML, GTRGAMMA + I was selected as it fitted most of the partitions. As for MrBayes analyses, the MrBayes block appended to the PF2 output was directly used to define the partitions as well as their respective substitution models, with independent estimation of substitution rate matrix, gamma shape parameter, transition/transversion rate ratio, proportion of invariable sites, and character state frequencies for each partition. Each MrBayes analysis was performed with two separate runs and four chains for each run, for 100,000,000 generations with a stop rule based on max standard deviation of split frequencies below 0.01 and sampling of trees every 1000 generations. Tracer v1.7 (Rambaut et al., 2018) was used to assess the convergence of parameter values for each run. FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/), TreeGraph2 (Stöver and Müller, 2010), and Inkscape (https://inkscape.org/en/) were used to visualize trees and edit the final figures.

### 3. Results

#### 3.1. Phylogenetic analyses of the *Agaricomycetes* dataset

Alignment statistics for the *Agaricomycetes* dataset are summarized in Table 5. The final concatenated alignment for the *Agaricomycetes* dataset contained 3254 total characters, 2841 of which were variable (87.30%), with mean coverage of 80.22%. Furthermore, based on subsequent RAxML searches the corresponding alignment contained 1997 distinct alignment patterns and a proportion of gaps and completely undetermined characters of 36.30%. Partitioned RAxML analyses resulted in the best scoring tree with a likelihood value of −42267.72. In addition to this, MrBayes analyses, which consisted of two runs, converged into a stable distribution with mean likelihood value of −42251.59 and −42253.66, respectively. There was no significant conflict between the RAxML and MrBayes analyses. The topology shown in Fig. 3 is based on the ML tree, with bootstrap (BS) support and posterior probability (PP) values from the BI consensus tree. To facilitate discussion, support values are mentioned in the text as (BS/PP).

The reconstructed phylogeny of *Agaricomycetes* (Fig. 3) recovered species that had been classified in *Atheliales* s.l. in the following major groups with strong support (BS ≥ 75/PP ≥ 0.95): *Byssoriasis terrestris* in *Russulales* (96/1.00), *Digitatispora marina* in *Agaricales* (98/1.00), *Lyoathelia laxa* in *Hymenochaetales* (87/1.00), and *Pteridomyces galzinii* in *Trechisporales* (100/1.00). Two additional species were retrieved in other orders, receiving weak to moderate BS support but strong PP values: *Hypochnellia violacea* in *Polyporales* (60/0.99), and *Leptosporomyces septentrionales* in *Amylocorticales* (55/1.00). The order *Lepidostromatales* was inferred as sister group to *Atheliales*, but without strong support in the ML analysis (49/0.96), and *Boletales* as sister to the *Atheliales*-*Lepidostromatales* clade (34/0.96). *Atheliales* was strongly supported (89/0.99) and contained *Stereopsis vitellina* in *Amylocorticales*, in congruence with previous results by Šjökvist et al. (2012).

#### 3.2. Phylogenetic analyses of the *Atheliales sensu stricto* dataset

The *Atheliales* dataset consisted of 59 ingroup taxa and 11 outgroup taxa (2 Lepidostromatales and 9 Boletales taxa). Various alignment statistics for the *Atheliales* dataset are summarized in Table 5. The final concatenated alignment added up to 3713 total characters, 3130 of which were variable (84.30%), with mean coverage level of 71.85%. Based on successive RAxML searches, the alignment consisted of 2036 distinct alignment patterns with the
Partitioned RAxML analyses of the dataset yielded a proportion of gaps and completely undetermined characters of 40.53%. Partitioned RAxML analyses of the dataset yielded a final optimized likelihood value of $-33904.79$, whereas the two MrBayes runs each converged into a stable distribution with mean likelihood of $-33449.37$ and $-33451.04$, respectively. Similar to the Agaricomycetes dataset, the best tree from RAxML and the consensus tree from MrBayes analyses showed congruent topology without any strongly supported conflict. The reconstructed ML phylogeny of *Atheliales* is shown in Fig. 4 with ML BS and Bayesian PP values.

In the resulting *Atheliales* phylogeny (Fig. 4), *Lobulicium occultum* was placed as sister to the rest of *Atheliales* taxa, which formed a clade with relatively strong support (72/1.00). Because of its unique and isolated phylogenetic position in combination with a distinct spore morphology and ecology, we propose to place *Lobulicium* in a new family, *Lobuliciaceae*. *Atheliales* was further divided into four major clades with moderate to strong support,

### Table 4
Partition scheme and substitution models of *Agaricomycetes* dataset and *Atheliales sensu stricto* dataset for RAxML and MrBayes analyses based on PF2.

#### Agaricomycetes dataset

| ML Subset | Regions | no. of sites | Best Model  |
|-----------|---------|--------------|-------------|
| 1         | 5.8S, LSU | 1164         | GTR + I + G |
| 2         | rpb2 1st codon | 726          | GTR + I + G |
| 3         | rpb2 2nd codon, tef1 2nd codon | 1045         | GTR + I + G |
| 4         | tef1 1st codon | 319          | GTR + I + G |

| MB Subset | Regions | no. of sites | Best Model  |
|-----------|---------|--------------|-------------|
| 1         | LSU     | 997          | GTR + I + G |
| 2         | 5.8S    | 167          | K80 + G     |
| 3         | rpb2 1st codon | 726          | GTR + I + G |
| 4         | rpb2 2nd codon, tef1 2nd codon | 1045         | GTR + I + G |
| 5         | tef1 1st codon | 319          | GTR + I + G |

#### Atheliales sensu stricto dataset

| ML Subset | Regions | no. of sites | Best Model  |
|-----------|---------|--------------|-------------|
| 1         | rpb2 1st codon, LSU | 1271        | GTR + I + G |
| 2         | 5.8S, rpb2 2nd codon, tef1 2nd codon | 818         | GTR + I + G |
| 3         | ITS1, ITS2 | 660          | GTR + I + G |
| 4         | rpb2 3rd codon | 348          | GTR + I + G |
| 5         | tef1 1st codon | 308          | GTR + I + G |
| 6         | tef1 3rd codon | 308          | GTR + I + G |

| MB Subset | Regions | no. of sites | Best Model  |
|-----------|---------|--------------|-------------|
| 1         | rpb2 1st codon, LSU | 1271        | GTR + I + G |
| 2         | 5.8S    | 162          | K80 + G     |
| 3         | ITS1, ITS2 | 660          | GTR + I + G |
| 4         | tef1 2nd codon, rpb2 2nd codon | 656        | GTR + I + G |
| 5         | rpb2 3rd codon | 348          | GTR + I + G |
| 6         | tef1 1st codon | 308          | GTR + I + G |
| 7         | tef1 3rd codon | 308          | GTR + I + G |

### Table 5
Summary of various statistics for the alignment of LSU, 5.8S, rpb2, tef1, ITS1, ITS2, as well as the final concatenated alignment.

|                  | LSU | 5.8S | rpb2 | tef1 | ITS1 | ITS2 | concatenated |
|------------------|-----|------|------|------|------|------|--------------|
| **Agaricomycetes dataset** |
| sequence lengths before excluding characters | --  | --  | 387–2231 | 465–1126 | --  | --  | --  |
| sequence lengths in final alignment | 526–916 | 84–162 | 224–1420 | 266–638 | --  | --  | 685–3046 |
| final alignment length | 997 | 167 | 1452 | 638 | --  | --  | 3254 |
| number of sequences | 123 | 116 | 93 | 84 | --  | --  | 123 |
| mean coverage in final alignment | 97.97% | 97.83% | 51.06% | 61.85% | --  | --  | 80.22% |
| pairwise identity in final alignment | 87.30% | 95.00% | 87.10% | 63.50% | --  | --  | 74.10% |
| variable sites in final alignment | 65.60% | 56.90% | 59.40% | 36.50% | --  | --  | 87.30% |

| **Atheliales sensu stricto dataset** |
| sequence lengths before excluding characters | --  | --  | 395–1118 | 467–1093 | --  | --  | --  |
| sequence lengths in final alignment | 530–899 | 84–160 | 346–1044 | 396–924 | 168–267 | 180–285 | 689–3481 |
| final alignment length | 923 | 162 | 1044 | 924 | 311 | 349 | 3713 |
| number of sequences | 59 | 56 | 34 | 31 | 44 | 46 | 59 |
| mean coverage in final alignment | 96.72% | 91.81% | 46.66% | 47.35% | 74.37% | 77.53% | 71.85% |
| pairwise identity in final alignment | 91.10% | 97.20% | 78.10% | 86.40% | 54.00% | 56.60% | 74.50% |
| variable sites in final alignment | 44.30% | 32.70% | 52.60% | 37.70% | 91.60% | 94.80% | 84.30% |
proposed to correspond to families sensu Jülich (1982): Atheliaceae (92/1.00) with Athelia and Fibulomyces; Byssocorticaceae (55/1.00) with Athelopsis, Byssocorticium, and Leptosporomyces; Pilodermataceae (83/1.00) with Piloderma, Tretomyces, and Stereopsis vitellina; and Tylosporaceae (89/1.00) with Amphinema and Tylospora. A number of genera were found to be non-monophyletic, including Amphinema, Athelia, Athelopsis, and Leptosporomyces. In addition to this, Athelopsis subinconspicua and Leptosporomyces raunkiaeri could not be placed with confidence in any of the proposed families. These two taxa formed a maximum supported clade (100/1.00), which was placed sister to Pilodermataceae with only high PP support (45/0.99).

4. Taxonomy

4.1. Atheliales Jülich (1982) emend. Sulisty, K.H. Larss., Haelew., & M. Ryberg

Bibliotheca Mycologica 85: 343 (1982).

Type family: Atheliaceae Jülich.

Description: Basidiomata annual, resupinate or spathulate, soft to tough, byssoid, pellicular or membraneous; hymenium smooth; hyphal system monomitic, septa with or without clamps, subicular hyphae sometimes with encrustation, hyphal strands present or absent; cystidia present or absent; basidia clavate to pedunculate,
Fig. 3. Phylogenetic relationships of Agaricomycetes based on LSU, 5.8S rpb2, and tef1 excluding the third codon position. Topology and branch lengths originated from RAxML analyses. Color shading indicates different orders. Thickened branches are strongly supported with BS ≥ 75 and PP ≥ 0.95. Branches supported by only either BS or PP have their
with 2 or 4 sterigmata; basidiospores smooth or verrucose, globose, elliptic, cylindrical, or lobed, thin-to slightly thick-walled, never amyloid or dextrinoid, sometimes cyanophilous. Saprotrophic, ectomycorrhizal, or parasitic on plants and lichens.

Confirmed genera: *Amphinema*, *Athelia*, *Athelopsis*, *Byssocorticium*, *Fibulomyces*, *Leptosporomyces*, *Lobulicium*, *Piloderma*, *Tretozymes*, *Tylospora*.

**Remarks**: Jülich (1982) included four families in the order: *Atheliaceae*, *Byssocorticaceae*, *Pilodermataceae*, and *Tylosporaceae*. Here we confirm that all four families should be accepted and that a fifth family, *Lobulicium* fam. nov., should be added. *Athelopsis subinconsipicua* and *Leptosporomyces raunkaierii* are currently left as *Atheliales incertae sedis*.

Our results showed that *Pteridomyces* (type: *P. galzinii* (Bres.) Jülich) should be placed in *Trechisporales* and *Lyoathelia* (type: *L. laxa* (Burt) Hjortstam) in *Hymenochaetales* (Fig. 3). *Pteridomyces galzinii* differs from *Atheliales* through the presence of sterile hyphal pegs and adnate, slightly ceraceous basidiomata (Gorjon et al., 2012; Jülich, 1979; Nakasone, 2017). *Lyoathelia laxa* has pellicular-membranous basidiomata like most species in *Atheliales* but differs by having doliform-pedunculate basidium and well-differentiated, capitate cystidia (Hayashi, 1974; Hjortstam and Ryvarden, 2004; Jülich, 1972).

4.2. *Atheliaceae* Jülich (1982) emend. Sulisty, K.H. Larss., Haelew., & M. Ryberg

**Type genus**: *Athelia* Pers., Traité champ. Comest. (Paris): 57 (1818) – Fig. 2A.

**Description**: Basidiomata annual, resupinate, effused, loosely adnate, pellicular, undifferentiated margin; hymenium smooth, sometimes slightly plicate when fresh, light colored; hyphal system adnate, pellicular, undifferentiated margin; hymenium smooth, continuous, white, blue or greenish blue; hyphal system monomitic, septa with or without clamps, hyphae thin-to slightly thick-walled, in subiculum loosely arranged, hyphal strands present in some species; cystidia absent; basidium clavate, rarely pedunculate, arranged in clusters, with 2–8 sterigmata; basidiospores thin-walled, hyaline, smooth, subglobose to elliptic or fusoid, neither amyloid, dextrinoid nor cyanophilous. Saprotrophic on various substrates, rarely parasitic on plants or lichens.

**Genera**: *Athelia*, *Fibulomyces* Jülich.

**Remarks**: Jülich (1982) included also the genera *Athelopsis*, *Caerulicum*, *Confortobasidium*, *Leptosporomyces*, and *Luellia* in *Atheliaceae*. Here we show that *Athelopsis* and *Leptosporomyces* belong in *Byssocorticaceae*. *Caerulicum* seems to be closely related to *Byssocorticium* but this can only be confirmed after sequencing of the type species, *C. neomexicanum*. *Confortobasidium* (type: *C. olivaceoalbum*) has its place in *Russulales* (Larsson and Larsson, 2003), and *Luellia* seems to belong in *Trechisporales* (Larsson, 2007).

*Athelia* and *Fibulomyces* share a pellicular basidioma and basidia arranged in clusters (Eriksson and Ryvarden, 1973, 1975). The decision to keep the two genera separate has been questioned (Eriksson and Ryvarden, 1973). Our analyses show that if the current concept of *Athelia* is to be maintained, then *Fibulomyces* must be reduced to synonymy. If, on the other hand, we want to keep *Fibulomyces* separate, then *Athelia bombacina* and *Athelia singularis* must be transferred to a new genus. Morphologically, *A. bombacina*, *A. singularis*, and *Fibulomyces mutabilis* are united by their consistently clamped hyphae (Eriksson et al., 1978, 1984; Kunttu et al., 2016). Eriksson and Ryvarden (1973) stated that *A. bombacina* resembles *F. mutabilis* morphologically and even ecologically.

4.3. *Byssocorticaceae* Jülich (1982) emend. Sulisty, K.H. Larss., Haelew., & M. Ryberg

**Type genus**: Byssocorticium Bondartsev & Singer, Mycologia 36: 69 (1944) – Fig. 2B.

**Description**: Basidiomata annual, resupinate, effused, soft, hyssoid to membranous, easily detached, margin undifferentiated; hymenium smooth, continuous, white, blue or greenish blue; hyphal system monomitic, septa with or without clamps, hyphae thin-walled or slightly thick-walled, hyphal strands present or absent; cystidia absent; basidia clavate or pedunculate, with four sterigmata; basidiospores smooth, globose, elliptic or cylindrical, thin- or slightly thick-walled, neither amyloid, dextrinoid nor cyanophilous. Saprotrophic or ectomycorrhizal.

**Genera**: *Athelopsis* Oberw. ex Parmasto, *Byssocorticium* Jülich.

**Remarks**: Jülich (1982) included three genera in this family, beside the type genus also *Byssosporia* and *Hypochnopsis*. However, *Byssosporia* has its place near *Albatrellus* in *Russulales* (Bruins et al., 1998; Larsson, 2007; Smith et al., 2013), and *Hypochnopsis* is a synonym of *Amaurodon* and belongs to *Thelephorales* (Köjalg, 1996). In addition to the type genus, *Byssocorticaceae* now includes *Athelopsis* and *Leptosporomyces*, both of which are in need of revision as they are non-monophyletic. This has been shown in the present study as well as in previous work (Hodkinson et al., 2014; Larsson, 2007).

*Athelopsis* was introduced to accommodate four species with an *Athelia*-like basidioma but having pedunculate instead of clavate basidia (Parmasto, 1968). Of these four species, only the type, *Athelopsis glaucina*, remains. Several other species have subsequently been added to the genus but a great majority of them are probably placed elsewhere. In our *Atheliales* analysis, *A. glaucina* was placed as sister to the rest of *Byssocorticaceae* with moderate ML support (50 < BS < 75) but strong PP support (Fig. 4). However, its placement seems to be unstable and affected by dataset composition, as it clustered with *Athelia* in the *Agaricomycetes* dataset with strong support (Fig. 3). More data is needed to ascertain its position within the *Byssocorticaceae*.

Jülich (1982) introduced *Leptosporomyces* to accommodate species with *Athelia*-like basidioma but with short-cylindrical instead of clavate basidia. The genus was introduced with five species although subsequent additions have raised the number of species to 15 (He et al., 2019; Index Fungorum, 2020). For most of these, DNA sequences are currently not available. In our analyses, only the type species was retrieved in *Byssocorticaceae*, whereas *Leptosporomyces raunkaierii* was placed in a separate clade with *Athelopsis subinconsipicua*, both currently ranked as *Atheliales incertae sedis*.

Support values noted above the branches as (BS/PP). Thickened species name denotes the type species of genera within *Atheliales sensu lato*, an asterisk (*) indicates that the corresponding taxon used to belong to *Atheliales sensu lato*, double asterisk (**) indicates that the taxon used to be placed outside of *Atheliales*. Arrow points to the clade corresponding to subclass *Agaricomycetes*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
4.4. Lobuliciaceae Sulistyo, K.H. Larss., & M. Ryberg, fam. nov

**Mycobank no.** MB 835270.

**Type and single genus:** Lobulicum K.H. Larss. & Hjortstam, Mycotaxon 14: 69 (1982).

**Description:** Basidiomata annual, resupinate, thin and soft, easily detached, margin very finely fibrillose; hymenium smooth, porulose, white; hyphal system monomitic, septa with clamps, hyphae thin-walled; cystidia absent; basidia small, clavate, with four sterigmata, basally clamped; basidiospores strongly lobed, neither amyloid, dextrinoid nor cyanophilous. Presumably saprotrophic on coniferous trees.

**Remarks:** Lobulicum is a monotypic genus and contains only *L. occultum*, a saprotrophic species that produces small pellicular fruiting bodies with a soft and loose hymenial construction, typical of most *Atheliales* members. However, it is notable for its peculiar basidiospores (Fig. 2C), which are strongly lobed but still bisymmetric. *Lobulicum occultum* also has a specialized habitat, growing in the cracks formed when trunks of *Abies* or *Picea* are subjected to brown cubic-rot decay by *Fomitopsis pinicola* (Hjortstam and Larsson, 1982; Nordén et al., 1999). This habitat preference is somewhat similar to that of *A. subinconspicua* (Kotiranta and Saarenkoska, 2005). Despite always being associated with brown-rot, *Lobulicum* is very likely not performing brown-rot but rather feeds from organic molecules left by other organisms. This species could be an interesting candidate for genome sequencing, in relation to its nutritional mode.

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**Fig. 4.** Phylogenetic relationships of *Atheliales* sensu stricto based on LSU, 5.8S, ITS1, ITS2, rpb2, and tef1 including the third codon position, with *Lepidostromatales* and *Boletales* as outgroups. Topology and branch lengths originated from RaxML analyses. Within *Atheliales*, colored shading indicates different families, while non-shaded groups are incertae sedis. Thickened branches are strongly supported with BS ≥ 75 and PP ≥ 0.95. Branches well-supported by only either BS or PP have their support values noted above the branches as (BS/PP). Thickened species name denotes the type species of genera within *Atheliales*, while an asterisk (*) indicates the type genus of the family. Symbols before the names indicate the nutritional mode: empty circle (○) for saprotrophic, filled circle (●) for ectomycorrhizal, and diamond (◊) for lichenicolous.
4.5. Pilodermaporaceae Jülich (1982) emend. Sulistyö, K.H. Larss., Haelew., & M. Ryberg

Type genus: Piloderma Jülich, Ber. dt. bot. Ges. 81: 415 (1969) — Fig. 2E.

Description: Basidiomata annual, resupinate and effused or spathulate, soft, byssoid to membranous, margin undifferentiated; hymenium smooth, continuous or porulose, white, yellowish or olivaceous brown; hyphal system monomitic, septa with or without clamps, hyphal strands present or absent, subicular hyphae often with crystals; cystidia absent; basidiate clavate, with 2–4 sterigmata; basidiospores smooth, subglobose to elliptic, slightly thick-walled, neither amyloid nor dextrinoid, slightly cyanophilous. Saprotrophic or ectomycorrhizal.

Genera: Piloderma, Tretomyces, Stereopsis vitellina.

Remarks: Only one genus, Piloderma, was mentioned in the original description of this family (Jülich, 1982). Tretomyces lutescens, the type species of Tretomyces, was described by Byssocor- cium lutescens in Eriksson and Ryvarden (1973), but shares micromorphological characters with Piloderma (Kotiranta et al., 2011). The major difference in relation to the original circumscription is the inclusion of Stereopsis vitellina, which produces stipitate sterigmata in the hyloma and thus deviates from all other species in Atheliales. The type species of Stereopsis (Stereopsis radicans) belongs to the order Stereopsidales, and is characterized by a spored basidium and spores that become slightly angular upon drying (Sjökvist et al., 2012, 2014). These features are lacking in S. vitellina. In addition, S. vitellina becomes brittle upon drying (Eriksson et al., 1984) similar to most other Atheliales members, whereas S. radicans becomes tough (Reid, 1965).

Piloderma species are completely devoid of clamps whereas Tretomyces species have clamps at all septa in hymenium and subhymenium and variable on subicular hypha. Ecologically, both Piloderma and Tretomyces are ectomycorrhizal (Aucina et al., 2019; Erland and Söderström, 1990; Kyaschenko et al., 2017; Pedersoo et al., 2010). The nutritional mode of Stereopsis vitellina on the other hand, has been reported to be saprotrophic (Maaroufi et al., 2019), preferring very old pine-dominated forests (Kunttu et al., 2018). Piloderma was found to be monophyletic in our analyses, although this monophyly lacked strong support in both ML and BI analyses, whereas the Tretomyces clade was strongly supported. The interrelationships among the three lineages are still unresolved.

4.6. Tylosporaceae Jülich (1982) emend. Sulistyö, K.H. Larss., Haelew., & M. Ryberg

Type genus: Tylospora Donk, Taxon 9: 220 (1960) — Fig. 2D.

Description: Basidiomata annual, resupinate, effused, soft to tough, byssoid to hypnophloid, margin undifferentiated; hymenium smooth, porulose to continuous, white to yellowish or brownish; hyphal system monomitic, septa with clamps, hyphae thinned-out to firm-walled, hyaline to yellowish, subicular hyphae often with encrustation; cystidia present or absent; basidiate clavate, with 2–4 sterigmata, always with a basal clamp; basidiospores elliptic, cylindrical, trilobate, thin-walled to slightly thick-walled, neither amyloid nor dextrinoid, sometimes cyanophilous. Ectomycorrhizal.

Genera: Amphinema, Tylospora.

Remarks: The family was introduced with only one genus (Jülich, 1982). Whereas our concept of the family corresponds to the amphinema-tylospora lineage of Tedersoo et al. (2010), the close relationship of Amphinema and Tylospora is surprising when considering the differences in morphology. Stalpers (1993) suggested that Tylospora, with its lobed and ornamented basidiospores, had affinities to Telephorales. Amphinema has smooth basidiospores but stands out by having cystidia, contrary to all other Atheliales. Eriksson (1958) suggested a relationship with Hyphodontia, an idea that prevailed until DNA data became available (Eriksson and Ryvarden, 1973; Jülich, 1982; Parmasto, 1968). Both genera form ectomycorrhizas with Picea (Danielson and Pruden, 1989; Eberhardt et al., 1999; Taylor and Alexander, 1991).

In our phylogenetic analyses, Amphinema was not recovered as monophyletic (Fig. 4). Tylospora asterophora was placed between Amphinema byssoids (type) and Amphinema diadema, indicating that the latter species should be moved out of Amphinema. However, this arrangement was not strongly supported by either BS or PP, and thus more data are needed before any taxonomic changes are made. Additionally, Tylospora was also found to be non-monophyletic in Tedersoo and Smith (2013).

4.7. Atheliales incertae sedis

Athelopsis subinconspicua (Litsch.) Jülich.

Leptosporomyces raunkiaeri (M.P. Christ.) Jülich.

Two species included in our analyses did not cluster with any of the recognized families. Athelopsis subinconspicua and Leptosporomyces raunkiaeri formed a strongly supported clade, which was also found in previous studies (Binder et al., 2010; Hodkinson et al., 2014; Liu et al., 2018). This clade seems to be closely related to the Pilodermaporaceae clade but this relationship was only supported by PP (Fig. 4). These two species are reported to be saprotrophic (Ambrosio et al., 2014; Kubartova et al., 2012) but are morphologically rather different, which is obvious from their generic placement. It is doubtful that they should be united in the same genus. Leptosporomyces raunkiaeri is rather similar to the type of Leptosporomyces. It differs primarily by somewhat larger basidiospores and by growing on dead angiosperm leaves whereas Leptosporomyces galzinii is above all found on decaying conifer wood. Athelopsis subinconspicua has typical pedunculate basidia but is in other respects not so similar to A. glaucina, the type species of Athelopsis. We believe the classification on genus level should first be disentangled before a clade name on family level is introduced.

5. Discussion

The class-wide phylogeny (Fig. 3) of this study made it possible to circumscribe Atheliales sensu stricto. Each order included in this dataset received either strong support from both ML BS (>75) and Bayesian PP (>0.95), or moderate support from ML BS (50–74) and strong support by PP. The resulting topology of our four-locus Agaricomycetes dataset (Fig. 3) lacked support on several deep nodes, although it is largely congruent with previous studies (Binder et al., 2010; Chén et al., 2019; Hodkinson et al., 2014; Liu et al., 2018; Nagy et al., 2015; Sjökvist et al., 2014; Zhao et al., 2017). Atheliales was placed within Agaricomycetidae (96/100), as sister to Lepidostromatales, although only with strong support from PP. The Atheliales–Lepidostromatales clade was placed as sister to Boletales with weak ML BS support but strong PP support. Close relationships among Atheliales, Boletales, and Lepidostromatales mirrors results of previous studies using similar sets of genes (Chén et al., 2019; Liu et al., 2018; Zhao et al., 2017). Unlike these studies, however, a sister relationship between Amylocorticulae and Agaricaleae was not recovered in our analyses (Fig. 3), further highlighting the uncertainty of the placement of this order.

Only two members of Atheliales (Fibulorhizoctonia sp. — anamorph of Athelia sp., and Piloderma olivaceum) and five members of Amylocorticulae (Amylocorticum subincarnatum, Anomoloma alboluteolens, Anomoporia bombycina, A. myceliosa, and Plicaturopsis
crispa) have their genomes sequenced, while no genomes are available for any representative of Lepidostromatales according to JGI’s MycoCosm (https://mycocosm.jgi.doe.gov/mycocosm/home, accessed 1 October 2020; Grigoriev et al., 2012). Compared to the number of genomes of Agaricales and Boletales (147 and 59, respectively), this number is very low. Future phylogenomic studies on the relationships among orders within Agaricomycetidae should focus on sampling more taxa from Amylocorticatales, Atheliales, and Lepidostromatales and carefully sort out the signal for different placements of Amylocorticatales.

Out of 23 described genera of Atheliales sensu lato (Table 1), 15 were included in our analyses, of which five genera fell outside of Atheliales and are phylogenetically placed in other orders (Fig. 3, taxa in bold with an asterisk): Byssospora (type: B. terrestris), Digitatispora (type: D. marina), Hypochnella (type: H. violacea), Lyoathelia (type: L. laxa), and Pteridomyces (type: P. galzinii). Byssospora terrestris is placed within Russulales, specifically in Albatrellaceae according to previous studies (Chen et al., 2016; Chen and Cui, 2014; Larsson, 2007; Smith et al., 2013; Zhou and Dai, 2013), making it the only corticoid taxon in this family. Digitatispora marina, a marine species, is placed in the Agaricales. Previous studies placed it within Naeaceae, closely related with other marine genera such as Calatharthus, Haplophanea, and Nia (Abdel-Wahab et al., 2019; Larsson et al., 2018). In our results (Fig. 3), Leptosporomycetes septentrionalis clustered with Amylocorticatales. This was also shown in previous studies (Binder et al., 2010; Song et al., 2016; Zhou et al., 2016), where it is found to be closely related to other corticoid taxa with smooth hymenium: Amyloxyenasma allantosporum and Serpulomyces borealis. Hypochnella violacea, Lyoathelia laxa, and Pteridomyces galzinii clustered with Polyporales, Hymenoaetiales, and Trechisporales, respectively. Blasting the sequences against UNITE’s database indicated that H. violacea closely resembles Australothyphnum dregeanum (Phanerochaetaceae), another corticoid taxon with purple fruiting body, whereas L. laxa is closely related to Poriodontia subvinosa (Schizoporaceae). For the aforementioned taxa of Atheliales sensu lato, their prior morphological association within Atheliales sensu lato largely stemmed from soft and pellicular fruiting bodies with microscopic characteristics that resemble core Atheliales taxa such as Athelia, Byssocorticium, and Piloderma (Erikssson and Ryvarden, 1973, 1975; Hjortstam, 1991; Jülich, 1972; Larsen and Zak, 1976). However, several authors have previously also expressed doubts regarding their association with Atheliales sensu lato (Erikssson et al., 1984; Gorjon et al., 2012; Hayashi, 1974; Larsson, 2007; Nakasone, 2013). On the other hand, the stipitate-sclerotoid species S. vitellina clustered within the Atheliales clade, as was also shown by Sjokvist et al. (2012). The phylogenetic placement of S. vitellina within Pilodermaetaceae is strongly supported, but not its relationship with other lineages within the family. This makes it difficult to infer the evolution of fruiting body type and nutritional modes within Pilodermaetaceae.

Athelopsis and Leptosporomycetes were found to be non-monophyletic, and the monophyly of Amphihema, Athelia, and Piloderma was unsupported. This can probably be attributed to incomplete taxon sampling in combination with low molecular data coverage. Although previous studies have found members of Athelia, Athelopsis, and Leptosporomycetes to be phylogenetically affiliated with other orders (Binder et al., 2010; Ertz et al., 2008; Larsson, 2007; Miettinen and Larsson, 2011), this study confirmed that their respective type species belong to Atheliales. Notwithstanding, revisions of these genera is necessary especially for Athelia, the type genus of the family. Corticioid fungi are particularly prone to misidentification (Binder et al., 2005). To minimize this problem, we used specimens that were identified by known experts of corticioid fungi. In addition, we utilized multiple collections for each species whenever possible. Future studies should build on this work by including type specimens.

Atheliales is a suitable group to study the evolutionary patterns of different nutritional modes because of the remarkable diversity observed within the group (Adams and Kropp, 1996; Matsuura et al., 2000; Stokland and Larsson, 2011; Tedersoo et al., 2010; Wenneker et al., 2017; Yurchenko and Olubkov, 2003). Atheliales is dominated by saprotrophic taxa, with one lichenicolous species (Athelia arachnoidea), while Byssocorticaceae, Pilodermaetaceae, and Tylosporaceae are dominated by ectomycorrhizal taxa. Within Byssocorticaceae, the earlier branching taxa (Athelopsis glaucina, L. galzinii, and Leptosporomycetes sp.) are saprotrophic, which seemed to be the plesiomorphic state of the family. Although the overall relationships among taxa within Pilodermaetaceae are still lacking in support, this seems to also be the case within the family, as the earliest branching taxon likely is Stereopsis vitellina, a non-corticioid and reportedly saprotrophic species (Maaroufi et al., 2019). Tylosporaceae, on the other hand, seems to consist of strictly ectomycorrhizal species. However, our sampling only represents a fraction of the true Atheliales diversity, thus it is possible that Tylosporaceae also contains saprotrophic members. Based on our analyses, the earliest branching taxon in Atheliales is Lobulicium ochromum, a saprotrophic species (Fig. 4). It is likely that the plesiomorphic state for nutrition in Atheliales is saprotrophic, and that ectomycorrhizal evolved multiple times in different groups. Ectomycorrhizal symbiosis arose several times from saprotrophic ancestors within fungi (Kohler et al., 2015; Tedersoo and Smith, 2013), as well as within smaller groups (Sánchez-Garcia and Matheny, 2017; Sato and Toju, 2019; Veldre et al., 2013). To make conclusive statements on the evolution nutritional modes, more data, better phylogenetic resolution on key nodes, and more comprehensive analyses of ancestral states are needed.

Compared with most other orders within Agaricomycetidae except Amylocorticatales, Atheliales is relatively understudied and undersampled (Rosenthal et al., 2017). It is possible that Atheliales contains undiscovered lineages. This gives hope to add taxa in the future that can break up long branches and pinpoint evolutionary relationships that are currently unresolved (e.g., the placement of A. subinconspicua and L. raunkiaeri, and the relationships among lineages). Additionally, the different placement of A. glaucina depending on the composition of the dataset and its relatively long branch, as well as the weakly-supported placement of the A. subinconspicua—L. raunkiaeri clade suggest that these lineages might be composed of many more taxa.

The classification proposed here is only a first step in improving the taxonomy of Atheliales, and further refinement will be needed as more taxa will continue to be included in phylogenetic analyses. Future systematic studies of Atheliales should include genera of Atheliales s.l. that were not included in our study (Table 1): Athelicium, Athelocystis, Buttelliera, Elaphosepha, Hypochniciellum, Melzericum, and Mycostigma.

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