Looking for the undiscovered asexual taxa: case studies from lesser studied life modes and habitats

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Abstract

Fungi are vital functional members of the biosphere, playing a crucial role in sustaining ecosystems by maintaining the nutrient balance. Many studies have verified the abundance of fungi across all-natural ecosystems and habitats, such as in forests, fresh-water (including both lentic or lentic or...
lotic), marine environments and deserts. With the focus previously on temperate regions and to a lesser extent biodiversity hotspots, the fungi in other areas remain overlooked. Therefore, it is imperative for mycologists to focus on taxa from these less-studied habitats, those dwelling on a vast number of hosts, and fungi that co-exist with other life forms. Molecular tools have been vital for species identification, in phylogeny, and linking sexual and asexual morphs. Identification of taxa based on the phylogenetic species concept, which relies on multiple loci and concordance of more than one gene genealogy, reduces subjectivity when determining the limits of a phylogenetic species. Large numbers of fungi inhabit biodiversity hotspots; however, they are underexplored owing to the vast diversity present and lack of studies. As examples of illustrating the undiscovered asexual fungi, this paper reports one new genus (Uniiappendiculata Tibpromma), six new species (Caprettia lichexanthotricha Apto & M.F. Souza, Hermatomyces maharashtraense Rajeshkumar et al., Lichenconium hawksworthii Flakus et al., Phaeobotryon spiraeeae L.X. Zhang & X.L. Fan, Rachicladosporium aridum L. Selbmann & C. Coleine and Uniappendiculata kunmingensis Tibpromma) and one new host and country record (Apiculosepora spartii Wijayaw. et al.). The paper discusses the biodiversity rich areas of South-Western China, South America and India, less-studied habitats (rock inhabiting fungi, lichens with conidiomata and lichenicolous fungi), and geographically widespread, but lesser studied hosts to show substantial studies are needed to reveal the extent of fungal diversity. The impact of discovering cryptic species on cataloguing fungal species numbers is also discussed. Each section exemplifies the status of the current research in that genus and future work that is needed.

**Keywords** – 7 new taxa – Ascomycota – DNA sequences – fungal diversity – habitat – life modes – phylogeny – taxonomy

**Introduction**

Predicting the number of species in the Kingdom *Fungi* is one of the many challenges of mycologists. Different studies have addressed this subject using different techniques (Hyde et al. 2018). Several studies have questioned “where are the missing fungi”, looking at the trends that are being followed in order to discover novel taxa (Hyde 2001, Jeewon & Hyde 2007, Hawksworth & Lücking 2017, Hyde et al. 2020). An estimated 20% of taxa reproduce asexually, and are reported as asexually typified or as pleomorphic species (Wijayawardene et al. 2021c). Thus, based on the predictions of Hawksworth & Lücking (2017) only 4% to 6.8% of estimated asexually reproducing species are presently known (Fig. 1). However, currently ca. 3653 genera (ca. 30,000 morphological species) are known from asexual reproduction (1388 coelomycetes and 2265 hyphomycetes) in their life cycle, while 687 genera are pleomorphic (305 coelomycetous; 378 hyphomycetous and four genera show both coelomycetous and hyphomycetous morphs) (Wijayawardene et al. 2021b).

Hawksworth (1991) forecasted that missing taxa could be discovered from tropical regions and poorly studied habitats, aquatic and lichenicolous fungi being provided as examples. This prediction was supported in recent studies with the discovery of hundreds of new taxa (e.g. aquatic fungi *fide* Luo et al. 2019, Dong et al. 2020, lichenicolous taxa *fide* Diederich et al. 2018, Baldrian et al. 2021). Hyde et al. (2018) demonstrated that up to 96% of fungi newly isolated in northern Thailand might be new to science, supporting the predictions of Hawksworth (1991) that many of the undiscovered taxa occur in the tropics. Hawksworth & Lücking (2017) and Hyde et al. (2020) suggested that cryptic species and reference collections could harbour even more undescribed species. Undescribed species within already extant, but cryptic species have been reported in several publications (e.g. species complexes in Collettotrichum, Phyllosticta and Trichoderma *fide* Damm et al. 2009, Jayawardena et al. 2016, Norphanphou et al. 2020, Bhunjun et al. 2021, Cai & Druzhinina 2021). We have observed that many new taxa have been introduced from fungi-rich host genera/ families (such as Arecaceae, Clematis, Eucalyptus, Rosa, Salix, Tilia) during the last decade (e.g. Wanasinghe et al. 2018a for fungi on Rosaceae, Tibpromma et al. 2018a for fungi on
*Pandanaceae*; Crous et al. 2019 for fungi on *Eucalyptus*, Mapook et al. 2020 fungi on Siam weed; Phukhamsakda et al. 2020 for fungi on *Clematis*).

This study introduces a new genus and six new species from: 1. Biodiversity rich, understudied regions (i.e. from South Western China, South America, and India); 2. Lesser studied habitats – e.g. lichens with conidiomata, lichenicolous taxa and rock inhabiting taxa, and 3. Fungi-rich, geographically widespread hosts (and their families). Furthermore, cryptic species of *Diaporthe* Nitschke are discussed since they could comprise numerous undescribed new species.

<p>| Estimated species as in Hawksworth &amp; Lücking (2017) |</p>
<table>
<thead>
<tr>
<th>Lower estimation</th>
<th>Upper estimation</th>
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<tr>
<td>2.2 M</td>
<td>3.8 M</td>
</tr>
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</table>

20% produce asexually as in Wijayawardene et al. 2021c

<table>
<thead>
<tr>
<th>Number of species to be discovered (currently only ca. 30,000 are known as in Wijayawardene et al. 2021c)</th>
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<td>410,000 (93.2%)</td>
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**Figure 1** – Estimated species number and numbers of species to be discovered.

**Materials & Methods**

**Sample collecting and incubating**

Living plant material with disease-like symptoms (for collecting pathogens) and dead plant material were randomly collected from different countries or regions (i.e. Antarctica, Bolivia, Brazil, China [Beijing and Yunnan Province], and India). Date, time, elevation and humidity were recorded. The samples were kept in Ziploc plastic bags and transported to the laboratory. Samples were sealed and incubated at room temperature in moist chambers using sterile distilled water.

**Isolation, examination and maintenance of specimens and cultures**

Single spore isolation method was used to isolate the fungus (Chomnunti et al. 2011). Conidiomata were sectioned with a razor blade, and the centrum tissue containing conidia was removed with a sterile needle and placed in sterile water. For hyphomycetous taxa, conidiophores were picked off and placed in sterilised water. A drop of conidial suspension was placed on water agar (WA) 1.5% and incubated overnight at room temperature. The germinated spores were transferred to potato dextrose agar (PDA). The dry specimens were deposited at well-known fungaria of the respective countries, and the cultures were deposited at the culture collections in the respective countries.

Morphological characteristics were captured with a digital camera (Nikon ECLIPSE 80i) mounted on a Nikon ECLIPSE Ni compound microscope equipped with DIC optics. A digital
camera (HDMI 200C) on an Olympus SZH10 stereomicroscope was used to capture images of macro-morphological characters.

Squash mount preparations were used to determine micromorphology (conidiophores, conidiogenous cells, conidia) (Sutton 1980). Free-hand sections were made to observe the shape of conidiomata and cell arrangement of the conidiomata wall. Conidiophores attached with conidiogenous cells and conidia were placed in water drop to determine the morphological characters of hyphomycetous taxa. Observed characteristics were presented as photo plates that were edited and combined in Adobe Photoshop version CS5 (Adobe Systems Inc., United States). Morphological characteristics were measured using Tarosoft (R) Image Frame Work version 0.9.7.

**DNA extraction, PCR and sequencing**

Genomic DNA was extracted from fresh mycelia grown on PDA at 25–27°C using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, and P.R. China) according to the manufacturer’s instructions. LSU, SSU, ITS, tefI-α and rpb2 genes were amplified by polymerase chain reaction (PCR) using LR0R/LR5, NS1/NS4 and ITS5/ITS4 primers, respectively (Table 1). PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). Newly generated sequences were deposited in GenBank.

**Table 1 Loci, PCR primers, references and protocols used in this study.**

<table>
<thead>
<tr>
<th>Gene region</th>
<th>Primers</th>
<th>Thermal cycles</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>ITS</td>
<td>ITS5/ITS4</td>
<td>(95°C: 30 s, 55°C: 50 s, 72°C: 90 s) × 35 cycles</td>
<td>White et al. (1990)</td>
</tr>
<tr>
<td>LSU</td>
<td>LR0R/LR5</td>
<td>(95°C: 30 s, 55°C: 50 s, 72°C: 90 s) × 35 cycles</td>
<td>Vilgalys et al. (1990), Rehner &amp; Samuels (1994)</td>
</tr>
<tr>
<td>SSU</td>
<td>NS1/NS4</td>
<td>(95°C: 30 s, 55°C: 50 s, 72°C: 90 s) × 35 cycles</td>
<td>White et al. (1990)</td>
</tr>
<tr>
<td>tefI-α</td>
<td>EF1-983F/EF1-2218R</td>
<td>(94°C: 45 s, 56°C: 50 s, 72°C: 60 s) × 40</td>
<td>Rehner (2001)</td>
</tr>
<tr>
<td>rpb2</td>
<td>RPB2-5F/RPB2-7cR</td>
<td>(Touch up PCR; 50°C: 30 s, 72°C: 90 s; 30 cycles with 95°C: 1 min, 52°C: 30 s, 72°C: 90 s; nine cycles of 95°C: 1 min, 55°C: 30 s, 72°C: 90 s)</td>
<td>Liu et al. (1999)</td>
</tr>
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**Phylogenetic analyses**

Phylogenetic analyses were based on the combined multiple loci following the methods reported in the literature. Single locus alignments were carried out to compare tree topologies. The combined locus sequence matrices comprised newly generated sequences and related sequences obtained from GenBank. Sequences were combined and aligned in Mega 6.0.5 (Tamura et al. 2013) and MAFFT multiple sequence alignment software version 7.215 (Katoh et al. 2019), and were manually improved when necessary. Phylogenetic analyses were made using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI).

ML was performed in RaxmlGUI v.1.3 (Silvestro & Michalak 2012) with 1000 thorough bootstrap replicates. A generalized time-reversible (GTR) for nucleotides was applied with a discrete gamma distribution (Silvestro & Michalak 2012). Rapid bootstrap analysis (Stamatakis 2014) and a search for a best-scoring ML tree were applied (Silvestro & Michalak 2012).

BI was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) and the best-fit model of sequences evolution was estimated with MrModeltest 2.2 (Nylander et al. 2004). Markov chain Monte Carlo sampling (MCMC) was used to determine the posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva et al. 2006). Six simultaneous Markov chains were run for 1000000 to 5000000 generations and halted automatically when the standard deviation of split frequencies was less than 0.01. Trees were sampled every 1000th generation. The first 20% of trees
were discarded as the burn-in phase. The remaining trees were used to calculate the posterior probability (PP).

Results & discussion

In this section, we introduce one new genus and nine new species from lesser-studied habitats, biodiversity rich tropical regions and under-explored regions, lesser studied habitats and from a fungi-rich host family (e.g. Rosaceae). Each section provides the status of the current research, and predictions for future species numbers.

Biodiversity-rich, tropical and under explored regions

Biodiversity comprises three main components, ecosystem diversity, species diversity and genetic diversity (DeLong 1996). Smith et al. (2003) noted that the discovery of novel taxa is hampered by the fact that much of the world’s biodiversity resides in developing countries that lack proper funding for taxonomic studies. However, the development of fungal diversity research in biodiversity-rich, Asian countries (such as China, India, Japan, Malaysia and Thailand) is remarkable in the last two decades (Hyde et al. 2020). Long term, organised studies in some regions in tropical countries, confirm higher fungal diversity than was expected (e.g. Northern Thailand fide Hyde et al. 2018). Nevertheless, in some biodiversity-rich countries such as Sri Lanka (Myers et al. 2000), the number of taxonomic studies are few, and thus, knowledge is poor. As an example, Wijayawardene et al. (2021d) suggested Sri Lanka may harbour more than 33,000 species (per new host: fungi ratio; 1:9.8 fide Hawksworth & Lücking 2017) but currently only ca. 3000 are known (Adikaram & Yakandawala 2020). Moreover, fungi associated with insects in Sri Lanka has never been documented, thus, a higher number of species is expected (Wijayawardene et al. 2021c). Hyde et al. (2020) emphasized the importance of experts for carrying out taxonomic studies and surveys. Some of these biodiversity-rich, developing countries lack taxonomists or working groups like other developed countries or even similar to India, Thailand and China (Hyde et al. 2020). This was accepted by Wijayawardene et al. (2021d), who showed that out of 1716 species originally described in Sri Lanka, 1511 were described by British mycologists prior to 1958. Studies which are supported by DNA sequence analyses, are also lacking in most of these tropical, developing countries thus, cryptic diversity is overlooked.

Biodiversity rich areas have been recognized as important locations to discover fungi (Hawksworth 1991, Hawksworth & Lücking 2017, Hyde et al. 2020). The number of plants inhabiting fungi (i.e. host-fungi ratio) in tropical biodiversity rich areas may be higher than those currently known in other regions (Hawksworth & Lücking 2017). Several studies from biodiversity rich areas (South-Western China, India, Brazil, Sri Lanka) have revealed hundreds of novel species in the last decade (Luo et al. 2019, Rajeshkumar & Singh 2012, Rajeshkumar et al. 2019, 2021, Fernandez et al. 2021). These examples indicate that many new taxa will be discovered in such regions. However, it is essential to rely on DNA sequence-based species identification since some taxa could potentially represent other morphs of extant taxa (see Wijayawardene et al. 2021c).

In this section, three new taxa are introduced from biodiversity rich areas and underexplored regions, namely one new genus, Uniappendicipulata Tibpromma (typified by U. kunmingensis Tibpromma), and one new host and country record, Apiculospora spartii from South-Western China (Yunnan Province); one new species, Hermatomyces maharashtraense Rajeshkumar et al. from India.

Index Fungorum number: IF551761; Facesoffungi number: FoF 01425

Notes – Wijayawardene et al. (2016) introduced this genus with A. spartii Wijayaw. et al. from dead branches of Spartium junceum. Apiculospora was included in Leotiomycetes, generas incertae sedis by Ekanayaka et al. (2019), although Hyde et al. (2020) transferred Apiculospora to Rhytismatales genera incertae sedis based on phylogenetics. Karunarathna et al. (2021) introduced A. penniseti from dead leaves of Pennisetum purpureum. Apiculospora currently includes two
species (Index Fungorum 2021). Our new strain from Yunnan Province is morphologically and phylogenetically related to *Apiculospora spartii* (Figs 6, 7). *Apiculospora* species are currently reported from three hosts, *Spartium junceum* (Fabaceae; Italy), *Pennisetum purpureum* (Poaceae; Taiwan, China) and *Yucca gigantea* (Asparagaceae; mainland China). All these host genera have been reported with a large number of fungi (*Spartium* with 172 records; *Pennisetum* with 1891 records; *Yucca* with 665 records) (Farr & Rossman 2021). *Pennisetum* contains 83 species and *Yucca* 49 species (worldflora.org.) and show cosmopolitan distribution. *Spartium* comprises only one species but it is reported worldwide (including Europe, Mediterranean, the middle east, tropical and temperate Asia, Africa, Australasia, USA, Central America and South America [https://worldflora.org.; https://www.cabi.org]). We assume that many *Apiculospora* species are yet to be discovered since its members are not restricted to one host genus or family and are reported from temperate (Italy) and subtropical to tropical regions (Taiwan and mainland China).

**Figure 2** – The best scoring RAxML tree with a final likelihood value of -11985.843083 for combined dataset of LSU and ITS sequence data. The tree is rooted with *Dactylaria dimorphospora* (CBS 256.70) and *Calloria urticae* (MFLU 18-0697). The matrix had 627 distinct alignment patterns with 18.04% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.238013, C = 0.236238, G = 0.285800, T = 0.239949; substitution rates: AC = 1.711489, AG = 2.120742, AT = 1.705688, CG = 0.816113, CT = 6.984196, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.558240$. The newly generated sequence is in blue. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.90 are given above and below the nodes, respectively.


Index Fungorum number: IF551762; Facesoffungi number: FoF 01426
Saprobic on dead leaves of *Yucca gigantea*. Sexual morph: Undetermined. Asexual morph: Conidiomata 125–155 × 145–175 μm, (x̅ = 137 × 161 μm, n = 5), immersed, solitary, scattered, unilocular, subglobose, black dots, masses of spores all over the leaf surfaces of the host. Conidiomata wall 15–25 μm, composed of thick-walled, orange-brown to brown cells of *textura angularis*; inner cell layers thin-walled, almost reduced to a conidiogenesis region. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 3–10 × 1.5–3 μm, subcylinndrical to ovoid, enteroblastic, with percurrent proliferation, hyaline, smooth-walled. Conidia 15–25 × 5–10 μm (x̅ = 22.2 × 8.9 μm, n = 40), subcylinndrical to ellipsoid, slightly curved, conical at apex, aseptate when immature and later become 1-septate, sometimes with a dark band at septum, sometimes constricted at septum, hyaline to pale brown when immature and later dark brown, guttulate, thick-walled non-mucilaginous.

Culture characteristics – culture on PDA, colonies slow growing, circular, spreading, flattened, flossy, smooth with entire edge, brown; reverse brown.

Material examined – Mainland, China, Yunnan Province, Kunming Institute of Botany garden (*Camellia* garden), dead leaves of *Yucca gigantea* Lem. (*Asparagaceae*), 7 May 2020, S. Tibpromma, ST11 (HKAS 115529); living cultures KUMCC 21-0090.

GenBank accession numbers – LSU: MZ822226; ITS: MZ822228; SSU: MZ822225.

Notes – Our collection fits with the characteristics of *Apiculospora* in having subcylinndrical to ellipsoid conidia with a dark band at septum (Wijayawardene et al. 2016, Ekanayaka et al. 2019, Karunaratnha et al. 2021). In the phylogenetic tree, our collection clusters together with *A. spartii* (MFLU 15-3556, MFLU 18-1812, MFLU 18-1813) with high statistical support (Fig. 2). Our collection is similar to *A. spartii* but only conidia size is different (Wijayawardene et al. 2016). ITS base pair differences shown only 1 bp, which suggests that our collection is identical to *A. spartii*. *Apiculospora spartii* was isolated from dead branch of *Spartium junceum* from Italy (Wijayawardene et al. 2016, Ekanayaka et al. 2019). We introduce our new isolate, *A. spartii* as a new host record from *Yucca gigantea* and geographical record from China.

**Hermatomyces** Speg., Anal. Mus. nac. B. Aires, Ser. 3 13: 445 (1910) [1911]

Hermatomyces is typified by *H. tucumanensis* Speg, found on fallen rotten branches of *Smilax campestris* and *Celtis* sp. in Tucumán, Argentina. It was introduced by Spegazzini (1911) with key distinguishing features of sporodochial conidiomata and muriform, lenticular, hyaline or dematiaceous, monomorphic or dimorphic conidia. Currently, the genus is only known from asexual morph characters (Spegazzini 1911, Chang 1995, Leão-Ferreira et al. 2013, Koukol et al. 2018, Tibpromma et al. 2016, 2018b, Hyde et al. 2019, Phukhamsakda et al. 2020). *Hermatomyctaceae* was introduced by Locquin (1984) and formalized by Hashimoto et al. (2017) based on distinctive characteristics, such as sporodochial conidiomata and two conidium types. *Hermatomyctaceae* was recently validated with robust phylogenetic data by Doilom et al. (2017) and Hashimoto et al. (2017) for a distinct clade of *Hermatomyces* species in the order Pleosporales, with *Hermatomyces* Speg. as the generic type. A collection of *Hermatomyces* from India is introduced as *Hermatomyces maharashtraense* sp. nov. based on morpho-molecular analyses.

Currently, 25 species (including the new species in this study) are recognized in *Hermatomyces* (Koukol & Delgado 2019, Hyde et al. 2019, Nuankaew et al. 2019, Delgado et al. 2020, Phukhamsakda et al. 2020, Ren et al. 2021). We expect more species from other understudied regions and even in the regions where the species are reported.

**Hermatomyces maharashtraense** Rajeshkumar, Wijayaw., N. Ashtekar, S. Lad & G. Anand, sp. nov.

Index Fungorum number: IF558559; Facesoffungi number: FoF 10419

Etymology – named after Maharashtra State, where this fungus is native.

Holotype – AMH 10303, on unidentified decaying wood.
Figure 3 – *Apiculospora spartii* (HKAS 115529, new host and geographical record).
a Conidiomata on dead leaves of *Yucca gigantea*. b Longitudinal section of conidioma.
c, d Different stages of developing conidia attach to conidiogenous cells. e–h Conidia. i, j Colony characteristics on PDA medium (14 days old culture). Scale bars: b, e, f = 20 μm, c, g, h = 5 μm, d = 10 μm.

Mycelium mostly superficial or immersed, composed of a loose or compact network of repent, branched, septate, rough and thick-walled, pale to dark brown hyphae. Sexual morph: Undetermined. Asexual morph: Colonies on natural substrate forming sporodochial conidiomata,
subiculate, superficial, scattered, circular or oval, blackish brown, velvety. *Conidiophores* macronematous, mononematous, simple, straight or flexuous, septate, smooth, hyaline to pale brown, often corresponding to conidiogenous cells, 33–53 μm long, 5–10.5 μm wide. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, cylindrical, smooth, hyaline. *Conidia* morphogenic, lenticular, thick-walled, globose or subglobose in front view, broadly ellipsoidal in lateral view, smooth, solitary, dry, muriform, divided longitudinally into two halves, constricted at both ends, occasionally slightly constricted at some septa, often carrying remnant of conidiogenous cell at base, 49–60 μm long, 35–55.5 μm wide. *Conidia* are bunched in a layer of hyaline globose or bubble-like basal cells of sporodochia, single or in chain, 20–27.5 μm diam. Culture characteristics – Colonies on MEA at 25±2 °C after 7 days, grey to grey-brown, 15–25 mm diam., reverse dark brown to black.

Material examined – India, Maharashtra, Mulshi, on dead wood, 23 July 2018, Rajeshkumar & Sneha Lad, holotype AMH 10303; ex-type living culture NFCCI 4879; *ibid.*, NFCCI 4880. GenBank accession numbers – NFCCI 4879; LSU: MZ099917 ITS: MZ147016 *tef1*-α: MZ130659 *rpb2*: MZ130660. NFCCI 4880; LSU: MZ147042 ITS: MZ147019 *tef1*-α: MZ130661 *rpb2*: MZ130662. Notes – *Hermatomyces maharashtraense* is similar to *H. trangensis* in having a single type of lenticular conidia, globose or subglobose in front view and broadly ellipsoidal or oblong in lateral view, smooth-walled and sporulating profusely in culture. The conidial dimensions of *H. maharashtraense* are however, significantly larger than *H. trangensis* (49–60 × 35–55.5 μm vs. 27.5–35 × 25–32.5 μm). *Hermatomyces clematidis* is dimorphic and differs from *H. maharashtraense* in its smaller, 30–45 × 24–31 μm conidia. Phylogeny of *H. maharashtraense* is based on ITS, LSU (not shown), and secondary barcode genes *tef1*-α and *rpb2*. Even though the ITS region poorly resolves *Hermatomyces* species, LSU and combined *tef1*-α and *rpb2* analyses established an independent lineage of the new species closely allied to *H. clematidis* and *H. trangensis* (Fig. 5).

**Uniappendiculata** Tibpromma, gen. nov.

Index Fungorum Number: IF558615; Facesoffungi number: FoF 10421

Etymology – Referring to the single appendage conidia.

Type species – *Uniappendiculata kunmingensis*


Notes – *Sulcatisporaceae* was introduced by Tanaka et al. (2015) to accommodate *Magnicamarosporium*, *Neobambusicola* and *Sulcatispora* in *Pleosporales*. Sexual morphs of *Sulcatisporaceae* are characterized by immersed to erumpent, subglobose to hemisphaerical ascocata, short ostiolar necks, trabeculate pseudoparaphyses, clavate, broadly fusiform ascospores with hyaline, septate and mucilaginous appendages (Liew et al. 2000, Tanaka et al. 2015). The asexual morph has pycnidial conidiomata with various conidial characteristics (Tanaka et al. 2015, Phukhamsakda et al. 2017, 2020, Rupcic et al. 2018). Phylogenetic analysis of ITS, *tef1*-α and LSU sequence data indicates that one new collection from Yunnan, China, is a distinct genus in *Sulcatisporaceae*, which forms a clade sister to *Pseudobambusicola* (Fig. 6). However, *Pseudobambusicola* forms micro and macroconidia but our new genus forms only macroconidia. Based on morphology show macroconidia of *Pseudobambusicola* in having fusoid-ellipsoid, prominently guttulate, hyaline, smooth, 0–3-septate which differ from our new genus (Rupcic et al. 2018). A comparison of ITS, LSU and *tef1*-α gene regions indicate 50 bp (ITS), 12 bp (LSU), 52 bp (*tef1*-α) differences between the type species of *Pseudobambusicola* (BCC 79462) with our new
genus. Hence, the new genus, *Uniappendiculata* is herein introduced. *Uniappendiculata* can be distinguished from other members in *Sulcatisporaceae* by subcylindrical, multi-septate, hyaline conidia with one appendage at each end.

*Acer* species are widely grown as ornamental plants and reported with numerous fungi (Sutton 1980, Nag Raj 1993, Farr & Rossman 2021). Currently, 367 records of microfungi inhabiting *Acer* have been reported from China (Farr & Rossman 2021). However, we predict that a large number of novel taxa are yet to be discovered from *Acer* species in South-Western China.

Figure 4 – *Hermatomyces maharashtraense* (AMH 10303, holotype). a–c Sporodochial conidiomata (c inset conidia front view). d Conidia with hyaline globose basal cells. e–i Mature conidia front view with basal attachment. Scale bars = 10 μm.
Figure 5 – The phylogram was generated from RAxML analysis based on a combined *tef1*-α and *rpb2* sequence data for the genus *Hermatomyces* (*Hermatomycetaceae*) with a final likelihood value of -4508.434349. The matrix had 285 distinct alignment patterns with 1.50% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.265347, C = 0.277374, G = 0.244731, T = 0.212548; substitution rates: AC = 0.758148, AG = 5.092386, AT = 0.713346, CG = 0.713346, CT = 11.538352, GT = 1.000000; gamma distribution shape parameter \( \alpha = 2.403317 \). Bootstrap support values for ML greater than or equal to 56% are given above the nodes. The tree is rooted to *Aquasubmersa japonica* (KT 2862, KT 2863). The new taxon is shown in bold and blue.
**Figure 6** – The best scoring RAxML tree with a final likelihood value of -9124.516643 for combined dataset of LSU, tef1-α and ITS sequence data. The tree is rooted with *Montagnula aloes* (CBS 132531) and *Didymosphaeria rubi-ulumfolii* (MFLUCC 14-0024). The matrix had 547 distinct alignment patterns with 24.64% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.228527, C = 0.263570, G = 0.278846, T = 0.229057; substitution rates: AC = 1.094587, AG = 1.973297, AT = 1.123227, CG = 0.720850, CT = 5.866173, GT = 1.000000; gamma distribution shape parameter α = 0.165842. The newly generated sequence is in blue. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.90 are given above and below the nodes, respectively.

*Uniappendiculata kunmingensis* Tibpromma, sp. nov.  

Index Fungorum number: IF558616; Facesoffungi number: FoF 10422  

Etymology – Named after Kunming, from where the species was first collected.  

Holotype – HKAS 115530  

*Saprobic* on dead stem of *Acer palmatum*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* acervuli, superficial, solitary, scattered, setae formed on cushions of brown to black, masses of spores all over the dead stem surfaces of the host. *Conidiophores* 5–20 × 1–3 μm (\(\bar{x}\) = 16.35 × 2.14 μm, n = 40), hyaline, simple or septate, branched, smooth-walled. *Conidiogenous cells* 3–8 × 1–3 μm (\(\bar{x}\) = 5.35 × 2.12 μm, n = 40), enteroblastic, phialidic, produce only one spore each conidiogenous cells, hyaline, smooth-walled, cylindrical, ellipsoidal, sometimes extending to form new conidiogenous loci (percurrent). *Conidia* 20–35 × 2.5–5 μm (\(\bar{x}\) = 13.94 × 3.85 μm, n = 40), subcylindrical, slightly curved, obtuse at apex, 3–7-septate, sometimes constricted at septum, hyaline, guttulate, thick-walled, with one appendage at each end, 4–10 μm long and without mucilaginous.  

Material examined – China, Yunnan Province, Kunming Institute of Botany garden, dead stem of *Acer palmatum* Thunb. (Sapindaceae), 25 May 2020, S. Tibpromma, ST57 (HKAS 115530,

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*Didymosphaeria rubi-ulmifolii* MFLUCC 14-0024

*Uniappendiculata kunmingensis* HKAS 115530

*Magnicamarosporium diospyricola* MFLUCC 16-0419

*Magnicamarosporium iromotense* KT 2822

*Parasulcatispora clematidis* MFLUCC 17–2082

*Anthosulcatispora brunnia* MFLUCC 18–1393

*Anthosulcatispora subglobosum* MFLUCC 17–2065

*Neobambusicola streltziae* CBS 138869

*Sucatispora acoirina* KT 2982

*Sucatispora berchelae* KT 1607

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*Montagnula aloes* CBS 132531

Outgroup

Notes – Maximum likelihood analysis using the alignment of LSU, tefl-α and ITS rDNA sequences shows the new isolate as a well-separated lineage from other genera in Sulcatisporaceae with 97% in ML, 1.00 PP support (Fig. 6). The results from blast searches of the LSU regions of our new isolate matched 98.3% with Pseudobambusicola thailandica (MG926560), the ITS regions matched 89.71% with Pseudobambusicola thailandica (MG92655) and the tefl-α regions matched 93.55% with Sulcatispora acerina (LC014615). We consider our new isolate as a new species based on such significant differences in morphology and molecular data.

Figure 7 – Uniappendiculata kunmingensis (HKAS 115530, holotype). a Conidiomata on substrate. b Conidiomata with setae. c, d Conidiophore and conidiogenous cell. e–g Developing conidia attach to conidiogenous cells. h–k Conidia (j, k conidia under a SEM). Scale bars: a = 1 mm, b, d = 40 μm, c = 20 μm, e–i = 10 μm, j, k = 2 μm.

Lesser studied habitats
Hawksworth (2001) stated that some fungal groups had been extensively studied as they affect humans either directly (human pathogens) (e.g. Zalar et al. 2011, Chaturvedi & de Hoog 2020) or indirectly (plant pathogens, post-harvest colonisers) (e.g. Jayawardena et al. 2019). Moreover, some taxa have been studied as they are easily accessible, especially saprobic taxa in
temperate regions (e.g. Phukhamsakda et al. 2020, Pem et al. 2021). Hyde et al. (2020) emphasized that some habitats and life modes are overlooked even in extensively studied ecosystems. We agree with this opinion; for example, marine ecosystems have been studied over five decades but mainly focus on a few habitats such as fungi inhabiting mangroves, salt marshes, driftwoods, seaweeds. Jones et al. (2019) stated the importance of focusing on other less studied habitats and life modes such as in deep seawater and hydrothermal vents. Hyde et al. (2020) further list ‘Karst fungi, caves, forests (especially pristine rainforests), extreme environments, volcanoes, mountains, deserts, freshwater aquatic systems, lakes, grasslands, indoor environment’ as other important but less studied habitats to discover novel taxa. Many asexual genera are still monotypic and regarded as orphaned; we assume this is due to ignoring their original habitats and life modes.

In the last decade, frequent studies of saprobic, pathogenic and endophytic microfungi have revealed numerous new taxa (e.g. Thambugala et al. 2017, Jayawardena et al. 2018). Fungi from the soil, lichenicolous taxa and ectomycorrhizal taxa have also been introduced as novel taxa (Etayo et al. 2020, Guivin et al. 2020, Suija et al. 2020), but in lower numbers. Hence, extensive collections of less studied life modes and habitats may reveal a higher number of unknown species.

Here three habitats are used as examples viz. a. Lichens that produce conidiomata; b. Rock inhabiting taxa; c. Lichenicolous taxa that can be studied to reveal undescribed conidial taxa.

A. Lichens reported with conidiomata

Lichen-forming fungi (lichenized fungi or lichens) were always the exception to what is now a rule for all fungi, since dual nomenclature was not allowed for sexual and asexual states (Turland et al. 2018). Pycnidia with conidia are common in lichens and known from over half of the described species. The pycnidia are often partly carbonized and the conidia simple, small, acrogenic and hyaline (Smith et al. 2009). Pycnidia and conidia are often uniform within a lichen genus, insomuch that the details are hardly described, however there are exceptions and conidia can be used to separate species within such genera as Bacidina Vězda (Czarnota & Guzow-Krzemińska 2012) or Strigula Fr. (Aptroot et al. 2008, 2014). Pycnidia typically originate in a rather early developmental state, on the same superficial thallus on which later ascomata are formed. For instance, all species of Astrothelium Eschw., a large genus of tropical lichens, go through a stage with pycnidia. Nonetheless, pycnidia are not reported for most species, because at the time a thallus has pycnidia, it cannot be identified yet to species level (Aptroot & Lücking 2016).

In the fruticose genus Cladonia P. Browne, pycnidia are almost always present, and apothecia, when present, develop later and often without producing ascospores. Conidia are also not frequently seen, but the diagnostic character within the genus is the colour of the conidial slime, which can be hyaline or reddish, and can vary even between similar species (Ahti 2000). Propagation in this genus is mostly by fragmentation or asexual vegetative diaspores (usually soredia) (Ahti 2000). In the foliose genus Xanthoparmelia (Vain.) Hale (the most speciose lichen genus), pycnidia are formed on the marginal lobes, but only in species that do not form soredia or isidia. Thus, the presence of pycnidia on a small thallus of members of this genus helps in identifying the otherwise sterile specimen, as it can be concluded that it will never form vegetative lichenized diaspores (mostly isidia or soredia), but only apothecia. Given the development of the pycnidia before the apothecia, they have generally been interpreted as spermatia, and probably do not serve for direct propagation. The pycnidial states are not separately named, as this would be illegitimate under the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018), but sometimes the pycnidia were thought to be parasitic fungi.

Some lichens have pycnidia with different conidia. Simple, septate and/or curved conidia occur in, e.g. Bacidina, Opegrapha Ach. and Micarea Fr. (e.g. Coppins 1983, Smith et al. 2009, Czarnota & Guzow-Krzemińska 2012). Brown conidia, so common in free-living asexual taxa, are in lichens only known in Eopyrenula R.C. Harris (Harris 1973, Smith et al. 2009) and Savoronala Ertz et al. (Ertz et al. 2013). Larger conidia are usually called macroconidia, to differentiate from the simple microconidia. They usually occur in addition to microconidia, and in genera such as Anisomeridium (Müll. Arg.) M. Choisy and the afore-mentioned genera, two or sometimes even
three different conidia can occur in the same species or even on one thallus (Coppins 1983, Smith et al. 2009, Czarnota & Guzow-Krzemińska 2012, Harris 1995). In lichens, conidia with gelatinous appendages are only known in Strigulaceae. Previously, such thalli were treated in separate fungal nonlichenized genera, Discosia Syd. & P. Syd. and Shanoria Anahous. Those names have been coined as thalli with such pycnidia by mistake as the algae were missed (Lücking 2008). Some Strigula species have very characteristic conidia and are known only in conidial states, viz. *S. lauriformis* Aptroot & Lücking (Aptroot et al. 2008), *S. muriconidiata* Aptroot, L.I. Ferraro & M. Cáceres (Aptroot et al. 2014) and *S. pyrenuloides* Aptroot (Aptroot et al. 2020). Palmate conidia are known only in Cheiromycina B. Sutton (Printzen 2007, Muggia et al. 2017) and Psammina Sacc. & M. Rousseau (Earland-Bennett & Hawksworth 2005, Cáceres & Aptroot 2016). The most unusual conidia are known in the monotypic genus *Savoronala* (Ertz et al. 2013). They are produced in sporodochia and their cells are wrapped around a single green algal cell, thus resembling soredia (Ertz et al. 2013).

There are dozens of lichen species that usually, or only occur in a state with pycnidia. The most useful and most used key to those species is presented by Smith et al. (2009). The key treats species belonging to more than 25 unrelated genera occurring in the British Isles but can also be used in other regions with a similar climate.

Sterile lichens with only pycnidia are often left unidentified or even undescribed in case they seem unrecognizable, because it is difficult to assess which genus they might belong to without molecular data. Recently, however, several characteristic species have been recognized and have been assigned to extant or new genera based on sequencing and comparisons with related taxa. Particularly, these are species in the genus Neosergipea M. Cáceres, Ertz & Aptroot (Aptroot & Cáceres 2017). Molecular data allowed some conidial species to also be placed within extant genera, that otherwise would have remained undescribed, e.g., *Inoderma sorediatum* Ertz et al. (Ertz et al. 2018). It is predicted here that more morphologically characteristic sterile lichens with pycnidia will be described as new to science in the forthcoming years, especially from the tropics, with using molecular tools.

Because lichens with conidia do not need to have separate names, only a few generic names have been introduced for conidial lichens. Some of those were described by mistake by authors missing the algae in the thalli, or the absence of ascomatal stages. An example is *Sarcinulella* Sutton & Alcorn introduced for Anisomeridium species in which the simple conidia agglutinate into packets. This asexual genus was described without recognizing that it was a lichen and it is now regarded as synonymous with Anisomeridium (Harris 1995). Other examples are the above-mentioned Discosia and sterile specimens of *Bacidina* species, which have been described as Lichingoldia D. Hawksw. & Poelt and Woessia D. Hawksw. & Poelt (Hawksworth & Poelt 1986, Ekman 1996).

Conidia on lichens can be formed in organs other than pycnidia, sometimes even in structures only known from lichens. These structures can be categorized as sporodochia, hyphophores and campyliidia.

Lichen thalli with only sporodochia are rare. They are most commonly found in the genus *Tylophoron* Nyl. ex Stizenb. (Ertz et al. 2011). The genus Sporodochiolichen Aptroot & Sipman (Aptroot & Sipman 2011) has been described to accommodate only sporodochial lichens without other known affinities. The type species has been shown to be a *Tylophoron* (Ertz et al. 2013), however the remaining three species are still orphaned and waiting to be transferred to other genera. Additionally, *Blarneya* D. Hawksw., Coppins & P. James described originally as a sporodochial species is subsumed under *Tylophoron* (Ertz et al. 2011). Other sporodochial lichens are found in the small genus *Cheiromycina* (Aptroot & Schiefelbein 2003, Printzen 2007, Muggia et al. 2017), two lichenized species in the typically lichenicolous-saprotrophic genus *Psammina* (Earland-Bennett & Hawksworth 2005, Cáceres & Aptroot 2016), one species of *Micarea* (Coppins 1983), *Savoronala* (Ertz et al. 2013), a species of *Eremothecella* Syd. (Cáceres et al. 2014), part of *Sporodophoron* Frisch, Y. Ohmura, Ertz & G. Thor and *Glomerulophoron* Frisch, Ertz & G. Thor (Frisch et al. 2015), *Sprucidea penicillata* (Aptroot, M. Cáceres, Lücking & Sparrius) M. Cáceres,
Aptroot & Lücking (Cáceres et al. 2017) and a single species within the typically non-lichenized *Sclerococcum* Fr. (Smith et al. 2009, Diederich et al. 2018).

Hyphophores are most common in foliicolous lichens and display a huge variation of shapes, from threads with hanging conidia to umbrella- or shortly stalked shell-shaped structures with conidia forming underneath. The families *Asterothyriaceae* and *Gomphillaceae* are particularly rich in variously shaped hyphophores. Species without known ascomata have not been described, or at least none have been accepted to date. Batista and co-workers described several species in the last century, but this was usually based on incomplete information, as algae were usually not observed. Lücking et al. (1998) presented an evaluation of these taxa. Even though the genera described by Batista & Maria (1965) are not currently accepted, they should be kept in mind when genera are being delineated again, as is commonly being done now. Keys and descriptions to lichens with hyphophores can be found in e.g. Ferraro (2004), Kalb & Vězda (1988), Lücking (2008), and Lücking et al. (2006).

Campylidia are three-dimensional structures with conidia, occurring in *Calopadia* Vězda and related genera. They are triangular to ear-shaped, sometimes with fringes. Their function is to facilitate the dispersal of conidia, as it catches rain droplets and the conidia disperse with the water. Malme (1935) was the first to understand the function. Later, many authors, notably Santesson (1952), got it wrong and interpreted the conidia as parasitic fungi, accepting the genus name *Pyrenotrichum* Mont. for them. Sérusiaux (1986) showed the nature of the campylidia again, and Aptroot & Sipman (1993) found these structures in a totally unrelated group of lichens, viz. *Musaespora* Aptroot & Sipman.

Apparently, many asexually reproducing species can be revealed in lichens. It is necessary to explore this in geographic areas that have not been studied systematically (e.g. biodiversity rich, tropical regions) as previously discussed.


The genus *Caprettia* was introduced by Batista & Silva-Maia (1965) with *C. amazonensis* Bat. & H. Maia as the type species. Subsequent studies by Sérusiaux & Lücking (2003), Lücking (2008) and Yeshitela et al. (2009) introduced six taxa from Costa Rica, Ethiopia, and Papua New Guinea. Currently, all the species lack DNA sequences, thus identification is based on only morphology. Herein, we introduce a new lichen species, *Caprettia lichexanthotricha*, from Brazil.

We assume that the species of *Caprettia* are mainly restricted to Central and South America and in some countries of Africa. Our new collection indicates hidden diversity in Brazil, which has enormous fungal diversity. However, tropical countries or regions which have not been thoroughly studied (e.g. Western Ghats in India, coastal regions of Madagascar) could also harbour more novel taxa.

**Caprettia lichexanthotricha** Aptroot & M.F. Souza sp. nov.

- Index Fungorum number: IF 551453; Facesoffungi number: FoF 10423
- Etymology – From lichexanthone and tricha (hair).
- Holotype – CGMS 82022
- *Thallus* pale greenish grey, dull, almost dusty, thin, covering an area of up to 10 cm diam., without prothallus. *Algae* trentepohlioid, green, c. 10–17 × 7–12 µm, partly escaping from the thallus and becoming filamentous. Sexual morph: Undetermined. Asexual morph: *Conidiomata* pycnidial, densely covering the thallus, superficial, tubular, not tapering, with a dull black, partly (especially often at the tips) white pruinose, often branched, hair-like beak of c. 0.3–0.5 × 0.1 mm. *Conidiophores* hyaline, c. 1 µm wide. *Conidia* hyaline, bacillar, c. 4–4.5 × 1.5 µm, mostly agglutinated together in gelatinous masses with c. 100 conidia.
- Chemistry – Thallus patchily UV+ yellow, with lichexanthone.
- Material examined – Brazil, Paraná, Guaraqueçaba, Tagaçaba, 10 m alt., on tree bark, October 2020, A. Aptroot & M.F. Souza (82022, CGMS, holotype).
Notes – *Caprettia* is a small genus in the *Monoblastiaceae* Walt. Watson (Hongsanan et al. 2020). So far eight species are known, all exclusively from South America. Most of the species are foliicolous and have a very inconspicuous thallus. The new species is the first with lichexanthone and the first reported with secondary metabolites.

**Figure 8** – *Caprettia lichexanthotricha* (CGMS 82022, holotype). A Thallus under 365nm UV light. B Thallus under normal light.

**B. Lichenicolous taxa**

Lichenicolous taxa (lichenicolous fungi or lichenicolous lichens) are exclusively parasitic on lichens and over 2,350 species have been described (Diederich et al. 2018). Hawksworth (1991) predicted that the diversity of lichenicolous taxa has been overlooked, and this prediction was confirmed in Hawksworth & Lücking (2017) who provided statistics for newly described species from the mid-1970s to the present. However, most of the known lichenicolous taxa have been reported from temperate regions, thus the species diversity of these fungi in tropical regions is unclear. Although, during the last decade, a large number of lichen species have been described across tropical and sub-tropical regions, the surveys on lichenicolous fungi have been carried out with less intensity, and only in selected countries, among others, in Bolivia, Colombia, Costa Rica, Ecuador, Kenya, New Guinea or USA (Florida) (e.g. Matzer 1996, Diederich 1997, Etayo 2002, 2017, Etayo et al. 2015, Suija et al. 2018, Diederich et al. 2019, Flakus et al. 2019 a, b). Boonmee et al. (2017) revised old genera which were reported as lichenicolous (e.g. *Myxophora amerospora* Döbbeler & Poelt and *Opegrapha reactiva* (Alstrup & D. Hawksw.) Etayo & Diederich) and further discussed about the necessity of future studies to reveal the hidden taxa. Thus, a higher number of undiscovered, lichenicolous species could be reported in the future in tropical countries that have high lichen diversity (Diederich 1997, Flakus et al. 2016).


The genus *Lichenoconium* was introduced by Petrak & Sydow (1927) with *L. lichenicola* (P. Karst.) Petr. & Syd. as the type species. Twenty-seven epithets are listed in Index Fungorum (2021) but only 20 species are accepted in Species Fungorum (2021). Nevertheless, Diederich et al. (2018) accepted only 15 species. Since Petrak & Sydow (1927), several studies revisited the genus which indicated the members of *Lichenoconium* show widespread distribution (e.g. Alstrup & Cole 1998, Cole & Hawksworth 2004, Lawrey et al. 2011). During the present study, a new *Lichenoconium* taxon was isolated growing on the thallus of *Heterodermia comosa* from Bolivia, which is herein presented as the new lichenicolous species *L. hawksworthii*. As far as we know *L. cargillianum* (Linds.) D. Hawksw. (from Bolivia, Chile, Ecuador *fide* Etayo & Sancho 2008, Flakus & Kukwa 2012, Etayo 2017), *L. echinosporum* D. Hawksw. (from Ecuador *fide* Etayo 2017), *L. erodens* M.S.
Lichenoconium hawksworthii Flakus, Etayo, Kukwa & Rodr. Flakus, sp. nov.

MycoBank number: MB838684; Facesoffungi number: FoF 10424

Holotype – KRAM-L 72092

Etymology – The new species is named in honour of Professor David L. Hawksworth, eminent British mycologist and lichenologist, for his great contribution to our knowledge of lichenicolous asexual taxa and to the Lichenoconium genus.

Mycelium indistinct, completely immersed in the host, composed of hyaline to pale brown hyphae, septate, uneven in thickness, about 2–3 μm thick. Sexual morph: Undetermined. Asexual morph: Conidiomata pycnidial, arising singly or in groups, at maturity the uppermost part evidently exposed, convex, black and matt, subglobose to obpyriform, (150–) 200–250 μm high, (70–)150–200(–230) μm wide, ostiole conspicuous, apical, covered by accumulating, dark-brown conidial mass. Conidiomatal wall pseudoparenchymatous composed of 3–6 layers of cells, 8–25 μm thick, with cells irregularly polyhedral to almost subglobose in shape, lumen 3–10 × 2–4 μm. Wall thickened in the upper part, pale to dark brown below and dark purple in the upper and lateral part, apical part usually incrusted by hyaline crystals, the purple pigment located in the exciple becoming strongly aeruginose in KOH. Conidiogenous cells forming a single layer lining the pycnidial cavity, subcylindric, phialidic, with visibly narrower necks, hyaline, (6–)9–12 × (2–)2.5–3.5 μm. Conidia arising from the apices of the conidiogenous cells, abundant, accumulating in a dry mass in the conidiomatal cavity, globose to subglobose or rarely slightly obovoid, not attenuated, apically rounded, basally sometimes narrower, the base usually truncated, yellow-brown to dark-brown, almost black in mass, delicately warted, aseptate, (3–)3.5–4(–4.5) × (2.5)3–3.5(–4) μm.

Material examined – Bolivia, Dept. Tarija, Prov. Burnet O’Connor, close to Entre Ríos, new road between Tarija and Entre Ríos, 21°30’47”S, 64°11’49”W, 1338 m, disturbed Tucumano-Boliviano forest with shrubs and Tillandsia, on thallus of Heterodermia comosa, 28 July 2015, A. Flakus 27364 (KRAM-L 72092, holotype, LPB – isotype). GenBank No.: MW315198

Additional specimens examined – Lichenocinum cf. cargillanum: Bolivia, Dept. Tarija, 22°02’38”S, 64°35’47”W, on apothecial disc of Punctelia sp., 2015, A. Flakus 27023 (KRAM, LPB); GenBank No.: MW315196; Lichenoconium erodens: Bolivia, Dept. Chuquisaca, 18°45’53”S, 64°49’57”W, on thallus of Parmotrema reticulatum, 2015, A. Flakus 26452 (KRAM, LPB); GenBank No.: MW315197.

Ecology and distribution – Lichenocinum hawksworthii is known from the type locality in Tucumano-Boliviano forests in Bolivian Andes and grows on epiphytic Heterodermia comosa.

Notes – The phylogenetic placement of Lichenocinum in Dothideomycetes was shown by Lawrey et al. (2011). Ertz & Diederich (2015) recently found that Lichenocinum is closely related to Abrothallus, however, to fully understand the phylogenetic relationship between those two genera additional analyses with larger datasets are necessary.

The new species occurs on discolored or brownish infected parts of the host thalli, and is characterized by only partly immersed, large conidiomata (150–250 × 70–230 μm), large conidiogenous cells, small conidia (3–4.5 × 2.5–4 μm) and the presence of purple pigment in apical and lateral part of exciple becoming strongly aeruginose in KOH. The two most morphologically similar species are Lichenocinum aeruginosum and L. pyxidatae growing mainly on Cladonia. Lichenocinum aeruginosum differs in smaller conidiomata, 80–100(–140) μm diam., with bluish pigment (turning aeruginose in KOH), and larger conidia, (3.4–)3.8–4.6(–5.4) × (3.0–)3.4–3.8–
4.3) μm. *Lichenconium pyxidatae* also differs in smaller conidiomata, (60–120(–150) μm diam., with brown pigment in exciple (becoming darker and olivaceous in KOH), and slightly smaller conidia, (2–)2.5–3.5(–4) × 2–3 μm (Hawksworth 1977, Cole & Hawksworth 2004, Lawrey et al. 2011).

Our phylogenetic analyses based on nuLSU sequences (Fig. 10) show that *L. hawksworthii* is placed in a highly supported clade that includes six species of *Lichenconium*. Although we included all *Lichenconium* sequences available in GenBank in our analyses, the relationship of our new species to other species within the genus remains uncertain due to the lack of statistical support. However, it seems to place together with the Bolivian sample of *L. cf. cargillanum* (growing on apothecial discs of *Punctelia*). The significant phylogenetic distance between *L. hawksworthii* and *L. aeruginosum* together with morphological discrepancies and different host preferences seems to justify the description of the new species.

![Figure 9](image)

**Figure 9** – *Lichenconium hawksworthii* (KRAM-L 72092, holotype). A–C Habit of conidiomata growing in thallus of *Heterodermia comosa*. D–E Transversal section of conidiomata showing

**Figure 10** – Phylogenetic reconstruction of the systematic position of *Lichenoconium hawksworthii* within *Lichenoconium* inferred from ML analyses of nuLSU rDNA dataset. The outgroup is represented by *Botryosphaeria dothidea*, *Phoma herbarum* and *Stemphylium vesicarium*. Bold branches represent either bootstrap values ≥ 70 and/or Bayesian posterior probabilities ≥ 0.95.

**C. Rock inhabiting fungi (RIF)**

Fungi found on rocks can be separated into two ecologically and taxonomically different groups: (i) hyphomycetes of soil and epiphytic origin (Sterflinger & Prillinger 2001) and (ii) black (highly melanized) fungi that are slow growing, typically with meristematic development, (i.e. Ascomycota, mainly within orders Capnodiales and Dothideales in Dothideomycetes and Chaetothyriales in Eurotiomycetes) that form peculiar and compact microcolonies (Wollenzien et al. 1997, Quan et al. 2020) (Fig. 11).

Rock-inhabiting hyphomycetes, proliferating during milder and humid seasons, such as *Aureobasidium pullulans* or hormonema-like fungi reported on marble artworks in Sicily, have been described as coming from surrounding vegetation (leaves, barks or soils) (De Leo et al. 1996). *Phoma* species have frequently been isolated from rock surfaces in humid as well as in semi-arid areas (Sterflinger & Prillinger 2001), and also reported together with *Epicoccum* species from surfaces of monuments in Vienna and from the historical quarry in a rural area near there (Sterflinger et al. 1999, Sterflinger & Prillinger 2001). The presence of these hyphomycetes on monument surfaces has a considerable impact on monument alteration, causing evident surface discoloration (Diakumaku et al. 1995).

Black meristematic fungi prevail under harsh and hostile conditions where they are not outcompeted by fast growing fungi (Selbmann et al. 2005). They are variously named as black yeasts, meristematic, microcolonial or rock-inhabiting fungi (RIF), to evoke the idea of organisms perfectly adapted to life on rocks and are among the most competent colonizers of this substratum, providing exceptional occasions for species diversification. In fact, it is now clear that rocks worldwide serve as enduring reservoirs of new RIF species, which are consistently encountered as soon as a new stone surface is sampled and studied (Ruibal et al. 2005, 2009, Selbmann et al. 2005, 2008) (Fig. 12).

Figure 12 – Examples of natural rocks where black meristematic fungi are found: A Battleship Promontory, McMurdo Dry Valleys, Southern Victoria Land, Antarctica (Photo credit: Italian National Program for Antarctic Researches). B Atacama Desert, Chile. C The Karst landform area in Guizhou province (Sun et al. 2020). D, E Temple of Hephaestus in Athens, Greece. F Monument in Beijing, China (Sun et al. 2020). G Black biofilms on the horizontal parts of the Khitrovo tombstone, 18th century necropolis, the Alexander Nevsky Abbey, Saint Petersburg, Russia.

RIF are invariably asexual morphs. They were overlooked for a long time in routine studies due to their very slow growth rate and poor competitive abilities. Often, they are even confused with ash, soot or dust on building and monument surfaces. With improvements in isolation procedures, it has become clear that they are much more common and widespread than previously believed. Yet, their sparse morphology, morphological plasticity, and scarce metabolic
competences has hampered identification, and it is impossible to use only morphology to support species identification and description. Indeed, the high degree of rock-inhabiting species biodiversity only became apparent when molecular techniques became routine in fungal systematics.

Taxonomically, RIF belong to lineages of Dothideomycetes, Eurotiomycetes (specifically in the order Chaetothyriales) (Ruibal et al. 2009, Egidi et al. 2014, Su et al. 2015), and Arthoniomycetes (Muggia et al. 2021). In Dothideomycetes, rock-inhabiting species belong mainly to the order Capnodiales, but a few lineages are also found in Pleosporales, Dothideales and Myriangiales. However, some clusters are still unclassified at the order level.

Notably, some rock-inhabiting species show a worldwide distribution, while others seem to occur in specific restricted areas. For instance, the black fungal species Elasticomyces elasticus and Recurvomyces mirabilis have been reported from Antarctica, the Andes and the Himalaya, and from Antarctica and the Alps, respectively. On the other hand, most species show a more restricted distribution and occur in specific areas; for instance, Bradymyces alpinus has been recorded only from Alpine rocks at high altitude and B. yunannensis only from rocks in China. Lithothypha guttulata occurs in the Mediterranean area and L. catenulata from Tibet. The species Knufia petricola, K. marmoricola, K. karalitana, K. vaticanii, K. perforans occur in the Mediterranean area, while K. separata and K. calcareola are from limestone in Beijing and from sandstone in Yunnan province (China), respectively. Cryomyces antarcticus and C. minteri were found exclusively in the Antarctic desert, while C. funicularus and C. montanus are only retrieved on rocks above 3,000 meters in the Alps. In some cases, this limited distribution encompasses the whole genus, as for Friedmanniomycyes (Antarctica), Monticola (Alps), Perusta (Spain), Saxomyces (Alps), and Spissiomyces, Rupestrionymyes and Anthragina (China).

Studies on Antarctic ice-free areas revealed a new RIF species in the genus Rachicladosporium based on morpho-molecular analyses (Figs 13, 14). Thus, it is herein introduced as Rachicladosporium aridum sp. nov.


The genus *Rachicladosporium* was introduced by Crous et al. (2007) with *R. luciliae* as the type species. Most of the species have been reported from both rocks or plant materials. Members of *Rachicladosporium* have been reported as species associated with leaf spots (e.g. *R. luciliae fide* Crous et al. 2007), as saprobes (e.g. *R. americanum* Cheew. & Crous *fide* Cheewangkoon et al. 2009, *R. iridis* (Auersw.) Crous *fide* Crous et al. 2020), and as RIF species (e.g. *R. antarcticum* Egidi & Onofri *fide* Egidi et al. 2014).

It is plausible that there could be a large number of species to be discovered from different localities as different life modes.

**Rachicladosporium aridum** Selbmann & Coleine, sp. nov.

MycoBank number: MB 839200; Facesoffungi number: FoF 10416

Holotype – MUT6494

Etymology – named after the extremely dry conditions of the natural environment of the fungus (McMurdo Dry Valleys).

Description based on 12-week-old cultures grown on malt extract agar (MEA) at 15°C. Slow growing colonies attaining up to 15 mm in diam. in 12 weeks, black in surface and reverse, compact, cerebriform, lobed with irregular margin crusty and hard, brittle in texture. Hyphae dark brown, septate, thick-walled, with apical or lateral germination producing elongated, cylindrical, regular hyphae 2.5-3 μm wide; torulose hyphae often present, composed of swollen cells, 4.4–5.5 μm wide, with or without transverse septa, brown, thick-walled, smooth, easily evolving in meristematic growth. Torulose hyphae showing polar growth by enteroblastic proliferation and branching by laterally enteroblastic elongation in acropetal chains, liberating by arthric secession, 3.5–4.5 μm long scared ramosioidia with truncated ends. Sexual morph: Undetermined. Asexual morph: Conidiophores micronematous 5.5–7.5 μm wide. Conidiogenous cells (hyphae) holothallic,
integrated or discrete, determinate. Conidial secession schizolytic. *Conidia* globose, doliiform, brown to dark brown, 0–3-septate, very thick walled, coated with fragmented incrustations, 5.5–7.5 μm wide, forming by thallic-arthric disarticulation of the apical and lateral branches of the conidiogenous hyphae. Terminal or intercalary chlamydospore-like cells sometimes present.

Material examined – Antarctica, Mt. Elektra, McMurdo Dry Valleys, collected by Laura Selbmann, MUT6494, holotype = MNA-CCFEE 6514, Culture preserved at -150°C and in dried condition.

GenBank accession numbers – *Rachicladosporium aridum* MNA-CCFEE 6514T (ITS, MW834577) and *Rachicladosporium aridum* MNA-CCFEE 6480 (ITS, MW834584; rpb2, 2446081).

![Image of fungal structures](image)


**Fungi-rich, geographically widespread hosts (and their families) and less studied hosts (e.g. endemic plants)**

Collecting fungi based on the host is one of the popular methods in mycology, thus most of the studies provide host-fungi indices in their publications (e.g. Ellis 1971, Sutton 1980, Nag Raj 1993). Farr & Rossman (2021) is continuously updating, host-fungi index, which is one of the important resources in current mycology. In traditional, morphological based mycology, the identification and naming of fungi was based on the host (Jayawardena et al. 2019). Hence, giving two names for one taxon is common, but DNA sequence-based studies suggested to regard these morphologically-defined species as synonyms of other species (Phillips et al. 2013, Groenewald et
al. 2013). Nevertheless, DNA sequence-based phylogenetic studies reveal cryptic species that show overlapping morphological characters but inhabit the same host or different hosts (Bensch et al. 2015). Besides, studies that used polyphasic approaches (Maharachchikumbura et al. 2021) reveal hundreds of novel taxa even from extensively studied localities and hosts in the last decade (e.g. Wijayawardene et al. 2016, Wanasinghe et al. 2018a, Hyde et al. 2019, Li et al. 2020).

**Figure 14** – ITS-LSU-rpb2 (ITS: 1–530; LSU: 531–1417; rpb2: 1418–1686) multi-locus tree constructed using the maximum-likelihood (ML) criterion. The best scoring RAxML tree with a final likelihood value of -19053.72 (2015 sites). The tree was rooted with *Pseudocercospora eucalypti* (CBS 110777). Estimated base frequencies were as follows: A = 0.2458414, C = 0.240345, G = 0.2749433, T = 0.2388703; substitution rates: AC = 0.1429208, AG = 0.1356144, AT = 0.1416212, CG = 0.09844051, CT = 0.3933481, GT = 0.08805502; gamma distribution shape parameter \( \alpha = 0.03569929 \). The bootstrap values have been calculated on 1000 pseudo-replicated and values above 80% are shown. New species is shown in bold red.

The extensive collection from fungi-rich host plants (e.g. *Eucalyptus*, *Rosa*, *Clematis*, palms and bamboo species) would help to reveal new taxa and old taxa that need to be epitypified. Here, we suggest making new collections from the host families for which the fungi-rich hosts are included. In the case of *Eucalyptus* hosts, collections can be extended to family *Myrtaceae* instead of focusing only on *Eucalyptus*, as well as to other families proven to be fungi-rich hosts, such as *Areceae* (Hyde et al. 1998, Fröhlich & Hyde 1999, Taylor et al. 1999, 2000, Konta et al. 2016, 2020, Thambubogala et al. 2017, Wanasinghe et al. 2018b). Moreover, the fungi-rich hosts that have worldwide distribution could also be prioritized for seeking new taxa. For example, in Sri Lanka, a large area of reforested land is occupied by *Eucalyptus* species (mainly *E. camaldulensis*, *E. grandis*, *E. robusta* fide Bandaratillake 1993), however, no research has been carried out to
investigate the fungi inhabiting them, including pathogens. Some host species that are geographically restricted (i.e. endemic) could be recognized as a valuable source of new species.

Wanasinghe et al. (2018a) studied the fungi on genus *Rosa* (*Rosaceae*) and recognized it as a fungi-rich host genus. Farr & Rossman (2021) listed 767 and 581 records of coelomycetous and hyphomycetous taxa from *Rosa*, respectively. Hence, we assume that there could be a large number of undiscovered species from *Rosa* and its family, *Rosaceae*. Besides, members of *Rosaceae* are important in agriculture and in ornamental plant industry thus, cultivated as economic crops. Most edible species are originally temperate crops (e.g. strawberry, apple) but introduced and cultivated in some tropical countries (e.g. strawberry cultivations in Sri Lanka). Even though these species are important as economic crops, only a few studies have been carried out to study their associated fungi, except pathogens (e.g. Cloete et al. 2011). Thus, we conclude that *Rosaceae* is an important host family to reveal more taxa. Moreover, we predict that there would be interesting relationships between hosts (as an exotic species) with native fungal species in tropical regions.

We have selected *Spiraea salicifolia*, a member of *Rosaceae*, from which to collect conidial taxa during our survey. A new taxon, which morphologically resembles asexual taxa in *Botryosphaeriaceae*, was collected. Morpho-molecular analyses (Figs 18, 19) confirmed that the new collection belongs in *Phaeobotryon* and formed a distinct clade, and thus it is introduced as a new species, *Phaeobotryon spiraeae*. Many *Rosaceae* plants are cultivated as economic crops (e.g. strawberry, apple, pear, loquats) in China, and occur in natural vegetation. Hence, many species may yet to be discovered from China, which could be important as opportunistic pathogens in the future.

### Phaeobotryon Theiss. & Syd., Anns mycol. 13(5/6): 664 (1915)

Theissen & Sydow (1915) introduced this genus with *P. cercidis* (Cooke) Theiss. & Syd. as the type species. The genus was re-visited based on both morphology and phylogeny, which established clear generic boundaries with the members of *Botryosphaeriales* (Phillips et al. 2013). Species boundaries are also well established and several novel species have been recently introduced (Daranagama et al. 2016, Chen et al. 2019, Pan et al. 2019). The genus is holomorphic and recently introduced species from China are represented by asexual morphs. We assume that novel taxa of *Phaeobotryon* can be expected from unexplored regions in China and other South East Asia countries which have higher fungal diversity (e.g. Thailand).

### Phaeobotryon spiraeae L.X. Zhang & X.L. Fan sp. nov.

Index Fungorum number: IF558666; Facesoffungi number: FoF 10495

**Holotype** – CF 20186828

**Etymology** – Named after the host genus on which it occurs, *Spiraea*.

**Sexual morph**: Undetermined. Asexual morph: *Conidiomata* pycnidial, stromatic, scattered to gregarious, multiloculate, immersed to erumpent from bark surface. *Locules* multiple, irregular arrangement with common walls, (430–)530–695–(780) μm (av. = 580 μm, n = 30) in diam., ecastrophomycetous disc and ostiole inconspicuous. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, thin-walled, cylindrical to doliform, holoblastic, phialidic, formed from the cells lining the inner walls. *Conidia* initially hyaline, becoming dark brown, aseptate, smooth with granular contents, guttulate, thick-walled, oblong to cylindrical, straight, both ends broadly rounded, (21.0–)23.5–28.5 × 8.5–13.5 μm (av. = 26.5 × 10.5 μm, n = 50).

Material examined – China, Beijing City, Huairou District, Labagoumen Primeval Forest, from *Spiraea salicifolia*, June 2018, X.L. Fan, CF 20186828 holotype, ex-type CFCC 53925.

Other material examined – China, Beijing City, Huairou District, Labagoumen Primeval Forest, from *Spiraea salicifolia*, June 2018, X.L. Fan, CF 20186829 paratype, ex-paratype CFCC 53926; *ibid*. CF 20186830, living culture CFCC 53927.

GenBank accession numbers: ITS: OM049420-OM049422; LSU: OM049432-OM049434

**Notes** – The new collections from *Spiraea salicifolia* is morphologically and phylogenetically distinct from extant species, thus we introduce it as a new species (Figs 15, 16).
New taxa from genera that are reported with cryptic species

The term “cryptic species” is frequently mentioned in systematic works in mycology, but the term has not always been applied correctly, and other times the term has not been mentioned at all, even when the study clearly involves cryptic species. For these reasons, it is important to define the term before discussing how it can impact the estimates of extant species of fungi.

It is well known that cryptic species exist in all groups of organisms. While cryptic species have been variously defined, and often synonymised with sibling species, a good discussion of the concept has been provided by Bickford et al. (2007). Although Bickford et al. (2007) discussed this term in relation to animal species their definition applies equally to all organisms. In that respect they considered that the term “cryptic species” describes distinct species that are erroneously classified (and hidden) under one species name (Bickford et al. 2007). Thus, the term is often applied when species cannot be distinguished reliably by morphology even if they are known to be distinct. Although this is a relatively simple definition, applying the concept in practice can be fraught with difficulties.

For many years, the morphological species concept was the most commonly applied concept for fungi. In some genera, species were also defined according to the host with which they were associated. However, both approaches are now known to lead to misinterpretations of the number of species. Host-association is a misleading character to define a species and application of this concept has led to a gross over-estimation of species. Thus, this practice has long been considered as unsuitable for species definition. On the other hand, the morphological species concept is considered to underestimate species numbers because morphological characters can be very plastic (phenotypic plasticity), and they frequently overlap between species. In this way, the

**Figure 15** – *Phaeobotryon spiraeae* (CF 20186828, holotype) from *Spiraea salicifolia*. A, B Pycnidia on a twig of *Spiraea salicifolia*. C, D Transverse section of conidiomata. E Longitudinal section through conidioma. F, G Conidia attached to conidiogenous cells. H Conidia. I. Cultures on PDA, surface and reverse views. Scale bars: A = 2 mm, B = 500 μm, C = 100 μm, D–E = 150 μm, F–H = 10 μm.
morphological species concept, as well as the use of host-association as a character to define species, often define groups of cryptic species (Burnett 2003).

Figure 16 – The best scoring RAxML tree with a final likelihood value of -22601.460384 for combined dataset of ITS, LSU, tef1-α sequence data. The topology and clade stability of the combined gene analyses were compared to the single gene analyses. Numbers above the branches indicate ML bootstraps (left, ML BS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.90). The tree is rooted with *Fusicladium effusum* (STE-U 4525) and *Fusicladium oleagineum* (CBS 113427). The matrix had 929 distinct alignment patterns with 27.43% undetermined
characters and gaps. Estimated base frequencies were as follows: 

\[
\begin{align*}
\text{A} & = 0.228527, \\
\text{C} & = 0.263570, \\
\text{G} & = 0.278846, \\
\text{T} & = 0.229057; \\
\text{AC} & = 1.250159, \\
\text{AG} & = 2.344965, \\
\text{AT} & = 1.178390, \\
\text{CG} & = 1.445313, \\
\text{CT} & = 4.419963, \\
\text{GT} & = 1.000000;
\end{align*}
\]

gamma distribution shape parameter \( \alpha = 0.259595 \). The newly generated sequence is in blue. Ex-type strains are in bold. “.” indicates ML BS < 50% or BI PP < 0.90.

The genus *Diaporthe* is a notable example where these species complexes occur frequently due to the definition of species based on host-association. While some *Diaporthe* species are relatively host-specific (generally pathogenic for their respective hosts and less variable at infraspecific level), most of them are species with a broad host range (generally opportunistic pathogens or secondary invaders) (Udayanga et al. 2014a, b). These species often have a high degree of genetic diversity, insomuch as they are frequently regarded as species complexes. Critical analysis of these complexes, well supported with suitable DNA sequence data, often unveils hidden cryptic species (Gomes et al. 2013, Udayanga et al. 2012, 2014a, b). Udayanga et al. (2014b) recognised nine distinct phylogenetic species in the *Diaporthe eres* species complex (*D. alleghaniensis*, *D. alnea*, *D. bicincta*, *D. celastrina*, *D. eres*, *D. helicis*, *D. neilliae*, *D. pulla* and *D. vaccinii*) occurring on an extensive range of hosts. Therefore, several isolates of *Diaporthe* that were formerly identified based on their host were shown to represent different taxa. Several other well-known and important species of phytopathogenic fungi represent complexes of cryptic species, to be found in genera such as *Calonectria*, *Dothiorella*, *Fusarium* and *Phyllosticta* (Cai et al. 2011, Shivas & Cai 2011). Thus, their accurate identification is of particular importance to national biosecurity agencies that promote plant diseases control and prevent the introduction of exotic phytopathogens.

In many genera of microfungi, the number of morphological characters that can be used to distinguish a species is small and the ranges of differences within each character are also small. Not only that but the phenotypic plasticity of the characters confounds the value of their use. Thus, only a few species can be distinguished on morphology and for this reason taxonomists rely increasingly on a phylogenetic concept for species definitions. In that respect it is clear that mycologists have been applying the concept of cryptic species even if the concept is not actually stated. Returning to the previous *Diaporthe* example, many *Diaporthe* species that are morphologically similar have proven to be genetically distinct, which shows how cryptic species can be hidden under the same morphological species. For example, *D. rudis* and *D. australafricana* are two closely related species associated with grapevines that, although morphologically similar, occur on different continents, which probably allowed them to accumulate genetic differences due to their geographical isolation (occupation of non-overlapping areas separated by geographical barriers) (van Niekerk et al. 2005, Gomes et al. 2013). In fact, *Diaporthe* represents a highly complex genus comprising numerous cryptic species, which resulted from either host-association identification or morphology-based identification. However, both of these approaches do not reflect the natural evolutionary history due to the simple and plastic morphological characters. While DNA-based phylogenetic studies on fungi have been applied since the 1980s, the concept of using a DNA-barcoding system was first introduced by Hebert et al. (2003). The original idea was that identifications based on morphology rely on specialist knowledge accumulated over many years of study by individual taxonomists. When those taxonomists retire, their knowledge would be lost. However, an identification system based on a DNA barcode can be applied by anyone with a basic training in working with DNA and who has access to a sequencer. The concept was soon adopted by fungal taxonomists who ultimately chose the ITS cluster as the barcoding locus, with the proviso that other loci would be necessary to resolve cryptic species complexes and these extra loci would not necessarily be the same for all groups of fungi.

Taylor et al. (2000) summarized the challenges associated with the phylogenetic species concept (PSC) and its operational version, the phylogenetic species recognition (PSR), namely the fact that “individuals are grouped very well, but the decision about where to place the limit of the species is subjective”. Through the analysis of variable nucleic acid characters, PSC and PSR are
the concepts that closer recognize species consistent with the Evolutionary Species Concept (ESC), since changes in gene sequences occur and can be noticed before any changes in mating behaviour or morphology. Nevertheless, using DNA barcodes to diagnose species can be challenging for polymorphic genes within certain species since one would not know if the gene is polymorphic within a species, or fixed for alternate alleles in two species. For this reason, mycologists have increasingly relied on phylogenetic species concepts based on multiple loci, since by relying on the concordance of more than one gene genealogy, PSC and PSR can prevent the subjectivity of determining the limits of a phylogenetic species. This concept, which was proposed by Avise & Ball (1990) and named by Mayden (1997), is known in its operational version as the Genealogical Concordance Phylogenetic Species Recognition (GCPSR). GCPSR relies on essentially three aspects: 1) comparison of more than one gene genealogy; 2) parts of certain genes can be used to construct genealogies since recombination does not occur within the gene; 3) the concordances on the topology of different gene trees result from the fixation of formerly polymorphic loci. Thus, concordant branches on the genealogy of different genes define the limits of a phylogenetic species (Taylor et al. 2000). Several examples of cryptic species in different genera, besides the Diaporthe examples referred to above, were described based on GCPSR. The application of this concept has great implications for accurate species recognition and resolution of species complexes, namely in common plant pathogenic genera, such as Calonectria (Lombard et al. 2010), Cercospora (Groenewald et al. 2005, Crous et al. 2006), Fusarium (Aoki et al. 2005, Summerell et al. 2010, 2011, Summerell & Leslie 2011), besides other genera where phylogenetic cryptic species are consistent with allopatric speciation as discussed in Diaporthe. Such genera include Cladosporium (Bensch et al. 2012), Colletotrichum (Crouch et al. 2009, Phoulivong et al. 2010, Damm et al. 2012a, b), Harknessia (Crous et al. 2012), Ilyonectria (Cabral et al. 2012a, b) and Phyllosticta (Glienke et al. 2011, Wikee et al. 2011, Wang et al. 2012) amongst others.

Although several studies have shown that multigene analyses are important for aiding in species recognition, in so much that they often identify cryptic species. However, interpretation of the outcomes of such studies can be challenging, especially if species variation is simply due to natural polymorphism within gene sequences. As stated many years ago by Darwin (1859), variation within a species is essential and is one of the key factors at the base of his theory of evolution. Thus, individuals in a population must vary significantly from one another and much of this variation must be heritable so that individuals less suited to the environment are less likely to survive and less likely to reproduce; individuals more suited to the environment are more likely to survive and more likely to reproduce and leave their heritable traits to future generations. This is the basis of natural selection, which in turn is the basis of speciation events. Unfortunately, in fungi, this aspect of variation within a population has often been ignored by fungal taxonomists and minor variations have been interpreted as indicating species.

Conclusion

The question, ‘what is the number of species in Kingdom Fungi?’ is always followed by the question ‘where and how do we find the missing species? Hawksworth (1991) predicted that molecular tools, e.g. DNA sequence data, are vital in species identification, in phylogeny and in linking sexual and asexual morphs. As the usage of DNA sequences in species identification has become more popular in the last decade, a large number of cryptic species have been introduced. Moreover, many novel taxa have been introduced from tropical and subtropical regions such as Asia (Hyde et al. 2020) (Fig. 17). These records indicate that extensive studies in tropical and subtropical regions are essential to discover missing taxa, and it is important to epitypify the already-described taxa.

Different life modes from different habitats are also essential to study using extensive collections. We have introduced one new genus and nine new species from different life modes that are regarded as overlooked or less studied, viz. lichens with pycnidia, rock-inhabiting fungi and lichenicolous taxa. In addition, fungicolous fungi, indoor fungi, coprophilous fungi and fungi associated with lower plants (e.g. Bryophytes) are also important to study thoroughly. It is
necessary to expand the research on well-studied life modes and habitats. For example, Sri Lanka is an island with rich mangrove diversity, but the knowledge of fungal taxa inhabiting those mangroves is lacking. However, in other regions, fungi associated with mangroves are well studied.

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**Figure 17** – Number of novel taxa described from tropical countries

**Lesser studied habitats**

Fungi play significant roles in different ecosystems. Many studies have focused on natural ecosystems or habitats such as natural forests, fresh-water ecosystems (including both lentic or lotic) and marine environments (e.g. estuaries). In addition to the fungi in natural ecosystems, considerable research has focused on agricultural lands and forest plantations. Hence, a large number of species have been reported from these ecosystems or habitats. Extensively studied ecosystems still contain a large number of undiscovered species. For example, Luo et al. (2019) introduced three new genera and 47 new species of *Sordariomycetes* from freshwater bodies in China. Rashmi et al. (2019) mentioned that foliar endophytic species are the broadly studied group among other endophytic fungi. Asian countries (such as China, India), European countries (such as Germany, Spain and the UK), Brazil and the USA regions have been thoroughly studied (Rashmi et al. 2019). However, the fungi colonize in other parts of the plants, unculturable taxa and slow growing taxa need to be studied based on proper methods, such as culture-independent methods, such as high-throughput sequencing (Wijayawardene et al. 2021a). Sun et al. (2019) comprehensively reviewed the current status of fungicolous taxa and listed 1552 species. Moreover, Sun et al. (2019) regarded that it is essential to carry out further studies to ‘understand their biology, ecological aspects, origin and divergence, host-specificity and application in biocontrol’.

Large ecosystems may also harbour different microhabitats that are distinct from their surroundings. These habitats might be important in revealing undescribed species. For example, swamps or seasonal ponds in evergreen rain forests provide distinct environmental conditions, and so they may be home for different species that are distinct from those inhabiting the neighbouring environments. Moreover, the concept of endemism (or endemic fungal species) has largely been ignored by mycologists, and so species inhabiting particular microhabitats would be an interesting topic for future studies.

**Environmental sequencing and its importance in new species detection**

Characterization of microbial diversity is a key issue, since the composition of natural microbial communities is known to be ecologically relevant. In the past, investigations of the
diversity and functioning of microbial communities have been based on isolation and cultivation of microbes and their characterization through direct observation of distinct morphologies (e.g. Guba 1961). These studies have allowed for a preliminary taxonomical classification. However, by showing that the number of cells observed microscopically far outweighed the number of colonies growing on a petri plate (Aman 1911), it was confirmed that this type of analysis cannot be considered an accurate measure of microbial diversity. This fact clearly hinders our ability to characterize them taxonomically, and thus our knowledge of the microbial world.

Environmental genomics or metagenomics is the term applied to the study of complex fungal communities sampled directly from the environment. There is no need to perform previous culturing or isolation of inhabiting taxa thus directly used to screen the taxa. Several metabarcoding techniques have been developed since the introduction of high-throughput sequencing (HTS) techniques (Margulies et al. 2005, Shendure et al. 2005), which offer high-speed and low-cost massive sequencing services. Numerous pipelines such as PIPITS (Gweon et al. 2015), CloVR-ITS (White et al. 2013), PipeCraft (Anslan et al. 2017), and FindFungi (Donovan et al. 2018) have been developed to generate sequence data linked to the environmental samples. Baldrian et al. (2021) discussed the future possibilities of using HTS in fungal diversity studies and emphasized the magnitude of undiscovered, voucher less taxa which are important to reveal missing species.

The possibility of obtaining DNA sequence information from environmental samples has allowed for the identification of uncultured microbes, regardless of their viability (Aman et al. 1995, Hongsan 2017). Such analyses have revealed an unexpected hidden diversity that had never been seen before through traditional cultivation methods. Metabarcoding studies of soil (Tedersee et al. 2014, 2017, 2021, Bahram et al. 2018), deep sea water and sediments (Singh et al. 2010, Luo et al. 2020) and plant litter (Balint et al. 2014, Duarte et al. 2020) revealed ‘dark taxa’ which have not been discovered before. Tennakoon et al. (2021) strongly suggested that future studies to discover plant litter fungi must depend on high-throughput sequencing.

Nevertheless, naming the sequences or operational taxonomic units (OTUs) generated from environmental studies (and voucher less species) have been a controversial topic. Hibbett et al. (2011), Lücking & Hawksworth (2018) and Lücking et al. (2020) encouraged to proceed with sequence-based nomenclature since it would be the best solution to address the sequences generated from environmental sequencing. However, Hongsan et al. (2018) and Thines et al. (2018) discussed its disadvantages such as the short-length sequences and acceptable value for similarity threshold. However, Tedersso et al. (2020) broadly discussed about the perspectives of long read sequences in future ecological studies and mentioned that techniques such as PacBio and nanopore sequencing are still relatively costly, require large amounts of high-quality starting material, and commonly need specific solutions in various analysis steps. Despite these challenges, long-read sequencing technologies offer high-quality, cutting-edge alternatives for testing hypotheses about microbiome structure and functioning as well as assembly of eukaryote genomes from complex environmental DNA samples. A provisional name system similar to bacterial nomenclature would be the most suitable system for future studies (Hibbett et al. 2011, Lücking et al. 2021).

Murray & Stackebrandt (1995) suggested a provisional category for classifying uncultured taxa, which is called as Candidatus. Nevertheless, this rank has not been widely accepted by the scientific community, claiming the technical limitations and lack of priority for Candidatus names in the official nomenclature (Konstantinidis et al. 2017). Currently, cutting-edge sequencing techniques and computational methods for genome assembly make it feasible for high-quality taxonomic descriptions of uncultivated microbes, as well as for more realistic microbial diversity analyses.

Consequently, a new genome-based taxonomy is emerging based on these “uncultured genome sequences”, although there is a clear need for standards that establish widely adopted procedures and standards. Publication of explicit and well-documented guidelines will facilitate the convergence of traditional and genome-based taxonomies to develop a unique, comprehensive
taxonomic classification system that will include uncultivated microbes with validly published names. However, even if some characters can be inferred from genome sequences, it should be noted that the lack of living cultures limits the information traditionally required for full taxonomic descriptions (e.g., morphologically, physiologically, biochemically). Moreover, the International Code of Nomenclature for Algae, Fungi and Plant (ICNaFp) demands the preservation of type material (Turland et al. 2018).

Additionally, considering that all organisms are “culturable” in their natural environment, genomes mined from environmental samples could assist to improve cultivating efforts through the coded information found on these sequences. For instance, specific nutrient requirements or other growing condition determinants could be extracted from genomic data. In conclusion, this genome-guided approach has the potential to enhance current isolation and cultivation strategies for taxa classified as unculturable, enriching our repertoire of microbiological techniques, and providing access to previously hidden metabolic diversity.

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