Myrothecium-like new species from turfgrasses and associated rhizosphere

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Abstract

Myrothecium sensu lato includes a group of fungal saprophytes and weak pathogens with a worldwide distribution. Myrothecium s.l. includes 18 genera, such as Myrothecium, Septomyrothecium, Myxospora, all currently included in the family Stachybotryaceae. In this study, we identified 84 myrothecium-like strains isolated from turfgrasses and their rhizosphere. Five new species, i.e., Alfaria poae, Alf. humicola, Dimorphiseta acuta, D. obtusa, and Paramyrothecium sinense, are described based on their morphological and phylogenetic distinctions. Phylogenies were inferred based on the analyses of sequences from four DNA loci (ITS, cmdA, rpb2 and tub2). The generic concept of Dimorphiseta is broadened to include a third type of seta, i.e. thin-walled, straight with obtuse apices.

Keywords

Stachybotryaceae, soil fungi, turfgrass disease, multi-locus phylogeny, cup-shaped sporodochia

Introduction

Myrothecium was first introduced by Tode (1790) based on M. inundatum. The typical characters of these fungi are cup-shaped sporodochia covered by a mass of slimy, green to black conidia. The generic concept of Myrothecium has been emended several times

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(Link 1809; von Höhnel 1905; Pidoplichko and Kirilenko 1971). Decock et al. (2008) reported that the genus *Myrothecium* is not monophyletic based on internal transcribed spacer regions and the intervening 5.8S rDNA (ITS). Chen et al. (2015) re-evaluated the phylogeny of *Myrothecium* based on ITS and elongation factor 1-alpha (EF1-α) gene sequences, suggesting the polyphyly of *Myrothecium* within Stachybotryaceae. These studies did not make taxonomic conclusions accordingly. Lombard et al. (2016) constructed a backbone tree of *Myrothecium* s.l. based on a multi-locus phylogeny and resolved *Myrothecium* s.l. to 18 genera including 13 new genera introduced. Under the current concept of *Myrothecium* sensu stricto, only two species were included, *M. inundatum* and *M. simplex* (Lombard et al. 2016).

Most myrothecium-like species are saprobes in soils (Ellis and Ellis 1985). Many species were named referring to their substrates such as *Alfaria terrestris*, *Albifimbria terrestris*, *Simorphiseta terrestris* and *Parvothecium terrestre*. Some species were also reported as weak plant pathogens. For instance, *Paramyrothecium roridum* (syn. *Myrothecium roridum*) can infect coffee plants, causing bark canker (Tullock 1972). *Albifimbria verrucaria* (syn. *Myrothecium verrucaria*) is pathogenic to mulberry causing leaf spot (Murakami et al. 2005). In addition, myrothecium-like species are also well-studied for their natural compounds, which are able to inhibit the activity of liver cancer and tumors (Pope 1944; Okunowo et al. 2010). Some myrothecium-like species can also produce a cocktail of secondary metabolites, which have strong antifungal and antibiotic activity (Kobayashi et al. 2004; Liu et al. 2006; Ruma et al. 2015). Hereto, more than 50 of these bioactive compounds have been reported from *P. roridum* and *Alb. verrucaria* (Wagenaar and Clardy 2001).

In a survey of turfgrass diseases from 2017, a number of myrothecium-like strains were collected from leaves and roots of turfgrasses and their rhizosphere. The aim of this study was to characterize these strains based on morphology and molecular phylogenetic analyses.

**Materials and methods**

**Fungal isolates**

From May 2017 to March 2018, turfgrass diseases were investigated on cold-season species in Beijing and on warm-season species in Hainan Province. A total of 130 samples were collected. Each sample was treated as an underground part of soil sample and a ground part of diseased grasses. Soil samples were isolated following the modified dilution plate method (Zhang et al. 2017). Five grams of each soil sample were suspended in 30 mL sterile water in a 50 mL bioclean centrifuge tube. The suspension was mixed thoroughly using Vortex-Genie 2 (Scientific Industries, New York) with maximum speed and then diluted to a series of concentration, i.e., $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$. The 100 μL suspensions of each concentration were spread on to antibiotic potato dextrose agar (PDA, 4 g potato starch, 5 g dextrose and 15 g agar, 50 mg ampicillin...
and streptomycin sulfate in 1 L sterile water). The first few samples suggested that 10^-2 was the best-diluted concentration for colony pickup. Diseased samples were isolated following a tissue isolation protocol (Chen et al. 2015). All plates were incubated at room temperature (23–25 °C) for 3–4 weeks, and from which all single colonies were picked up and transferred to clean PDA plates. Purified strains were stored at 4 °C for further studies. For phylogenetic analysis, associated sequences of 73 myrothecium-like strains and one outgroup strain were retrieved from GenBank (NCBI, https://www.ncbi.nlm.nih.gov/; Table 1).

Morphology and culture characteristics

Descriptions of macromorphological features are based on 7-d old materials incubated in the dark at room temperature (20–25 °C) and grown on potato dextrose agar (2% w/w; PDA), oatmeal agar (OA), cornmeal agar (CMA) and synthetic low-nutrient agar (SNA; Nirenberg 1981). Color description followed the color guide by Kornerup and Wanscher (1978). Digital images of colonies were made with a Nikon Eclipse 80i light microscope (Tokyo, Japan) with differential interference contrast (DIC) illumination and a LV2000 digital camera (Beijing, China). Slides mounted in clear lactic acid were also prepared to observe conidiogenesis, conidiophores and conidia.

DNA extraction and PCR amplification

Genomic DNA was extracted from 1–2 weeks’ old cultures grown on potato dextrose agar (2% w/w; PDA) incubated at room temperature using a modified Cetyltrimethyl Ammonium Bromide (CTAB) method (Rogers and Bendich 1994). Partial sequences of four genes, ITS, RNA polymerase II second largest subunit (rpb2), β-tubulin (tub2) and calmodulin (cmdA) gene sequences were amplified using the following pairs of primers, ITS1 and ITS4 (White et al. 1990) for ITS, RPB2-5F2 and RPB2-7cR (O’Donnell et al. 2007) for rpb2, Br2a and Br2b (Glass and Donaldson 1995) for tub2 and CAL-228F (Carbone and Kohn 1999) and CAL2Rd (Groenewald et al. 2013) for cmdA. Amplification for each locus followed the PCR protocols as described in Lombard et al. (2016). The PCR was performed in a 25 μL reaction volume including 2.5 μL 10 × PCR Buffer (Dingguo, Beijing, China), 2 mM MgCl₂, 50 μM dNTPs, 0.1 μM of each primer, 0.5 U Taq DNA polymerase and 10 ng genomic DNA. PCR reactions were conducted in ProFlex™ PCR system (Applied Biosystems, California, USA) under the following reaction conditions: predenaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C (for ITS) or 54 °C (for rpb2 and cmdA) or 56 °C (for tub2) for 40 s and elongation at 72 °C for 1 min, a final elongation at 72 °C for 5 min.

The purified PCR products were sequenced in both forward and reverse directions on an ABI-3730 XL DNA Analyzer (Applied Biosystems, California, USA). The se-
**Table 1.** Strains and NCBI GenBank accessions used in the phylogenetic analyses.

| Species                  | Isolate no. * | Host/Substrate | Country         | NCBI accession numbers          |
|--------------------------|---------------|----------------|-----------------|---------------------------------|
| **Mycotoxicium simplex** | CBS 582.93    | Decaying agaric| Japan           | KU846439 NR145079 KU846537 –    |
|                          | CBS 100287    |                | Japan           | KU846440 KU846457 KU846538 –    |
| **M. inundatum**         | CBS 275.48\*  | *Rusula* nigricans| England       | KU846435 KU846452 KU846533 –    |
|                          | CBS 116539    | Agaric         | Canada          | KU846437 KU846454 KU846535 –    |
| **Albosphinria lateralis** | CBS117712    | Unknown        | USA             | KU845865 KU845881 KU845957 KU845919 |
| **Alb. terrestris**      | CBS 1261\*6T  | Soil in mopane woodlands| Namibia    | KU845867 KU845883 KU845959 KU845921 |
|                          | CBS 109378\*  | Dead hardwood  | USA             | KU845866 KU845882 KU845958 KU845920 |
|                          | CBS 127838    | Soil           | Namibia         | KU845868 KU845884 KU845960 KU845922 |
|                          | LC12196       | rhizosphere soils of *Poa* sp. | China  | MK500260 MK478879 MK500277 –    |
| **Alb. verrucaria**      | CBS 328.52\*  | *Solanum* tuberorum| USA         | KU845875 KU845893 KU845969 KU845931 |
|                          | CBS 189.46\*  | *Tubularia* tubulifera| Cyprus     | KU845872 KU845889 KU845965 KU845927 |
|                          | LC12191       | Rhizosphere soils of *Poa* sp. | China  | MK500255 MK478874 MK500272 MK500264 |
|                          | LC12192       | Rhizosphere soils of *Poa* sp. | China  | MK500256 MK478875 MK500273 MK500265 |
|                          | LC12193       | Rhizosphere soils of *Poa* sp. | China  | MK500257 MK478876 MK500274 MK500266 |
|                          | LC12194       | Rhizosphere soils of *Poa* sp. | China  | MK500258 MK478877 MK500276 MK500267 |
|                          | LC12195       | Rhizosphere soils of *Poa* sp. | China  | MK500259 MK478878 MK500275 MK500268 |
| **Alb. viridis**         | CBS 449.71\*  | Unknown        | India           | KU845879 KU845898 KU845974 KU845936 |
|                          | CBS 127346    | Soil           | USA             | KU845880 KU845899 KU845975 KU845957 |
| **Alfaria. uniformis**   | CBS 324.74\* | Prairie soil   | USA             | KU845977 KU845984 KU846015 KU846002 |
| **Alf. humicola sp. nov.** | CGMCC3.19213\* | Rhizosphere soils of *Poa* sp. | Beijing, China | MH885432 MH793291 MH793317 MH818829 |
|                          | LC12144       | Rhizosphere soils of *Poa* sp. | Beijing, China | MH885434 MH793292 MH793318 MH818830 |
| **Alf. poae sp. nov.**   | CGMCC3.19198\* | Leaves of *Poa* sp. | Hainan, China | MH885419 MH793278 MH793314 MH818826 |
|                          | LC12141       | Rhizosphere soils of *Poa* sp. | Hainan, China | MH885420 MH793279 MH793315 MH818828 |
|                          | LC12142       | Rhizosphere soils of *Poa* sp. | Hainan, China | MH885421 MH793280 MH793316 MH818827 |
| **Alf. putrefolia**      | CBS 112037\* | Rotten leaf    | Brazil          | – KU845985 KU846016 KU846003    |
|                          | CBS 112038    | Rotten leaf    | Brazil          | – KU845986 KU846017 KU846004    |
| **Alf. terrestris**      | CBS 477.91\* | Soil           | Turkey          | KU845979 KU845988 KU846019 KU846006 |
| **Alf. thymi**           | CBS 447.83\* | *Thymus* serpyllum| The Netherlands | KU845981 KU845990 KU846021 –     |
| **Capito fimbia compacta** | CBS 111739\* | Decaying leaf  | Brazil          | KU846261 KU846287 KU846404 KU846349 |
|                          | MUCL 50238    | Bark           | Zimbabwe        | – KU878556 KU878559 KU878558    |
| **Dimorphospora terrestris** | CBS 127345\* | Soil collected in tallgrass prairie| USA      | KU846284 KU846314 KU846431 KU846375 |
|                          | CGMCC3.19208\* | Rhizosphere soils of *Poa* pratensis  | Beijing, China | MH885429 MH793288 – MH818815    |
| **D. acuta sp. nov.**    | LC12123       | Leaves of *Digitaria* sanguinalis | Beijing, China | MH885417 MH793276 MH793300 MH818811 |
|                          | LC12124       | Leaves of *Poa* pratensis | Beijing, China | MH885418 MH793277 MH793297 MH818812 |
| Species | Isolate no. | Host/Substrate | Country | NCBI accession numbers |
|---------|-------------|----------------|---------|-----------------------|
| **D. acuta sp. nov.** | | | | cmdA | ITS | tub2 | rpb2 |
| LC12125 | Rhizosphere soils of *Poa pratensis* | Beijing, China | MH885427 | MH793286 | MH793298 | MH818813 |
| LC12126 | Rhizosphere soils of *Poa pratensis* | Beijing, China | MH885428 | MH793287 | MH793299 | MH818814 |
| LC12127 | Rhizosphere soils of *Poa pratensis* | Beijing, China | MH885430 | MH793289 | MH793301 | MH818820 |
| CGMCC3.19206 | *Poa pratensis* | Beijing, China | MH885426 | MH793285 | MH793307 | MH818816 |
| LC12129 | Rhizosphere soils of *Agrostis tenuifolia* | Beijing, China | MH885415 | MH793274 | MH793303 | MH818821 |
| LC12130 | Rhizosphere soils of *Poa pratensis* | Beijing, China | MH885431 | MH793290 | MH793308 | MH818817 |
| LC12131 | Rhizosphere soils of *Poa sp.* | Beijing, China | MH885416 | MH793275 | MH793304 | – |
| LC12132 | Rhizosphere soils of *Festuca arundinacea* | Beijing, China | MH885422 | MH793281 | MH793305 | MH818818 |
| LC12133 | Rhizosphere soils of *Poa pratensis* | Beijing, China | MH885423 | MH793282 | MH793306 | MH818819 |
| LC12134 | Roots of *Poa pratensis* | Beijing, China | MH885424 | MH793283 | MH793307 | – |
| | | | | | | |
| **D. obtusa sp. nov.** | | | | cmdA | ITS | tub2 | rpb2 |
| LC12135 | Roots of *Poa pratensis* | Beijing, China | MH885425 | MH793284 | MH793308 | MH818817 |
| | | | | | | |
| Gregatothecium humicola | CBS 205.96c | Soil | Papua New Guinea | KU846285 | KU846315 | KU846432 | KU846376 |
| Peethambara sundara | CBS 646.77c | Dead twig | India | – | KU846471 | KU846551 | KU846509 |
| Inaequalispora prestonii | CBS 175.73c | Forest soil | Malaysia | KU846286 | KU846316 | KU846433 | KU846377 |
| Myxospora masonii | CBS 148.73c | Leaves of *Glyceria sp.* | England | KU846445 | KU846462 | KU846543 | KU846500 |
| My. graminicola | CBS 116538c | Decaying grass leaf | USA | KU846444 | KU846461 | KU846542 | KU846499 |
| My. aptrootii | CBS 101263c | Leaf litter | China | KU846441 | KU846458 | KU846539 | KU846496 |
| My. mucae | CBS 265.71c | *Musa sp.* | Madagascar | – | KU846473 | KU846544 | KU846501 |
| My. crassieta | CBS 731.73c | Tarsopterid lesion | South Africa | KU846446 | KU846464 | KU846545 | KU846502 |
| Panmyxothecium baniola | CBS 127295c | Soil collected in tallgrass prairie | USA | – | KU846295 | KU846412 | KU846356 |
| P. parvum | CBS 257.35c | *Viola sp.* | United Kingdom | – | KU846298 | KU846415 | KU846359 |
| P. foliicola | CBS 331.51c | *Foeniculum vulgare* leaf sheath | The Netherlands | – | KU846292 | KU846409 | KU846354 |
| P. nigrum | CBS 116537c | Soil | Spain | KU846267 | KU846296 | KU846413 | KU846357 |
| P. terrestris | LC12188 | Rhizosphere soils of *Poa sp.* | China | MK500025 | MK78871 | MK500269 | MK500261 |
| P. papuliferum | CBS 127790T | Surface soil in desert | Namibia | KU846264 | KU846291 | KU846408 | KU846353 |
| P. viridisporum | CBS 873.85c | Soil | Turkey | KU846278 | KU846308 | KU846425 | KU846369 |
| P. foliicola | CBS 113121c | Decaying leaf | Brazil | KU846266 | KU846294 | KU846411 | – |
| | CBS 419.93c | Air | Caba | KU846265 | KU846293 | KU846410 | KU846355 |
| Species               | Isolate no. * | Host/Substrate | Country | NCBI accession numbers |
|----------------------|---------------|----------------|---------|------------------------|
| P. brevistria        | CBS 544.75†   | Unknown        | India   | KU846262               |
|                      | CBS 357.89†   | Gardenia sp.   | Italy   | KU846170               |
|                      | CBS 212.95    | Water          | The Netherlands | KU846260               |
|                      | CBS 372.50 = IMI 140050 | Coffea sp. | Colombia | KU846361               |
| P. guangense         | GUCC 201608501† | Soil         | Guyang, China | KY169193               |
|                      | HGUP 2016-8001 | Soil          | Guyang, China | KY169192               |
| P. verrucatum        | HGUP 2016-8006† | Soil        | Guizhou, China | KY169197               |
| P. sinense sp. nov.  | CGMGCC 3.19212† = LC12136 | Rhizosphere soils of Par in virgin forest | Beijing, China | MH885437               |
|                      | LC12137       | Rhizosphere soils of Par sp. | Beijing, China | MH885436               |
|                      | LC12138       | Rhizosphere soils of Par sp. | Beijing, China | MH885433               |
|                      | LC12139       | Rhizosphere soils of Par sp. | Beijing, China | MH885435               |
| Parroticium terrestris | CBS 198.89†   | Soil in virgin forest | Brazil | KU846449               |
| Neomyrothecium hamicola | CBS 310.96†    | Soil          | Papua New Guinea | KU846448               |
| Gergotosticium hamicola | CBS 205.96†    | Soil          | Papua New Guinea | KU846425               |
| X. jollymannii       | CBS 276.48* = MUCL 11830 | Nicotiana tabacum | Malawi | KU847223               |
|                      | CBS 126168    | Soil          | Namibia | KU847224               |
| X. leucotricha       | CBS 131.64* = IMI 105664 = ATCC 16686 | Soil | India | KU847225               |
|                      | CBS 483.78    | Soil          | Colombia | KU847228               |
| Smagadinieta bitien | CBS 459.82†   | Rotten bark    | India | KU847206               |
| S. brachyporum       | CBS 513.71† = IMI 115293 | Dune sand | Iran | KU847209               |
|                      | CBS 131.71* = IMI 158441 = ATCC 22270 | Soil | Ukraine | KU847207               |
|                      | LC12189       | Rhizosphere soils of Par sp. | Beijing, China | MK500253               |
|                      | LC12190       | Rhizosphere soils of Par sp. | Beijing, China | MK500254               |
| S. cinctum           | CBS 932.69†   | Soil          | The Netherlands | KU847216               |
|                      | CBS 277.48* = IMI 001526 | Soil | New Zealand | KU847213               |
| S. hamicola          | CBS 388.97    | Soil          | Papua New Guinea | KU847217               |
| Tangerinosticium thalictroideae | CBS 317.61* = IMI 034815 | Thalictrum flavum | UK | KU847219               |
|                      | CBS 598.80†   | Halimeda sp.   | Tonga | KU847221               |
| Virgatospora echinofibrosa | MUCL 39092 = ATCC 200437 | Theobroma cacao | Ecuador | KU847220               |
| Fusarium sambucinum   | CBS 146.95    | Solarium taberum | UK | KM231391               |

* ATCC: American Type Culture Collection, Manassas, USA; BCC: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand; CBS: CBS-KNAW Fungal Diversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; GUCC: Guizhou University Culture Collection, Guiyang, China; HGUP: Herbarium of the Department of Plant Pathology, Guizhou University, China; IMI: International Mycological Institute, England, UK; LC: Collection of Lei Cai, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; MUCL: Mycothèque de l'Université Catholique de Louvain, Belgium; NRRL: Northern Regional Research Laboratory, USA.
† Ex-type and ex-epitype cultures.
quences were checked and manually corrected where necessary. A consensus contig was assembled with BioEdit v. 7.0.9 (Hall 1999) and the reference sequences were downloaded from GenBank (Table 1). Sequences were aligned with MAFFT v. 7 (Kazutaka and Standley 2013) and manually trimmed to equal length by cutting the unaligned sequences at both ends.

**Phylogenetic analyses**

Phylogenetic analyses were based on Bayesian inference (BI) and Maximum Likelihood (ML). For BI analysis, the optimal evolutionary model was estimated in MrModeltest v. 2.3 (Nylander 2004) using the Akaike Information Criterion (AIC) for each locus. For the selected substitution models for each locus see Table 2. MrBayes v. 3.2.1 (Ronquist and Huelsenbeck 2003) was used to generate tree topology and a Markov Chain Monte Carlo (MCMC) algorithm of four chains was started with a random seed and a burn in of first 25% trees. The MCMC analysis lasted until the average standard deviation of split frequencies came below 0.01. The ML analysis was performed using RAxML servers (http://phylobench.vital-it.ch/raxml-bb/index.php), with a maximum likelihood bootstrap (LB) of 1,000 replicates, under the GTR-GAMMA model (Stamatakis 2006).

**Results**

In this study, 603 fungal strains were isolated. Based on colony morphologies and preliminary sequence comparison of ITS via BLASTn in GenBank, 84 myrothecium-like strains were selected. Phylogenetic analyses of above 84 strains were performed on single locus and concatenated datasets (ITS, cmdA, tub2 and rpb2), with 70 strains in *Myrothecium* s.l. as reference and *Fusarium sambucinum* (CBS 146.95) as outgroup. After alignment, the concatenated datasets of four loci contained 569 characters (with gaps) for ITS, 318 for tub2, 732 for cmdA and 724 for rpb2. The characters of different alignments and statistics of phylogenetic analyses were shown in Table 2. The four single locus trees of all strains showed essentially similar topology (Supp. materials 1–4), with only minor differences affecting unsupported nodes on the trees. The resulting multi-locus ML tree was presented in Fig. 1 together with BI posterior probability values. Among 84 myrothecium-like strains, 14 strains were identified as four known species, *Albifimbria verrucaria* (10 strains), *Alb. terrestris* (1 strain), *Striaticonidium brachysporum* (2 strains) and *Paramyrothecium nigrum* (1 strain). The rest of them were grouped into five distinct clades with high supported values. Based on the morphological and phylogenetic distinctions, five novel species (i.e. *Alfaria humicola*, *Alf. poae*, *Dimorphiseta acuta*, *D. obtusa* and *Paramyrothecium sinense*) were described in this paper.
Figure 1. The ML consensus tree inferred from a four-locus concatenated alignment (ITS, cmdA, rpb2 and tub2). Bootstrap values (1,000 replicates) over 70% for ML and posterior probability (PP) over 0.95 are added to the left of a node (ML/PP). The type strains are labeled with “T”. Strains obtained from this study are in red. The tree is rooted using *Fusarium sambucinum* (CBS 146.95).
Figure 1. Continued.

Table 2. Characteristics of the different datasets and statistics of phylogenetic analyses used in this study.

| Locus† | Number of sites* | Evolutionary model‡ | Number of tree sampled in B | Maximum-likelihood statistics |
|--------|------------------|---------------------|-----------------------------|-------------------------------|
|        | Total | Conserved Ph | Phylogenetically | B unique | Number of | Best tree optimised | Tree length |
|        |       |         | informative | patterns | tree sampled | likelihood |                        |
| ITS    | 569   | 334    | 193       | 247   | 7501     | -32666.73 | 5.36                    |
| tub2   | 318   | 168    | 140       | 159   | GTR+I+G  |                        |                        |
| cmdA   | 732   | 258    | 381       | 490   | HKY+I+G  |                        |                        |
| rpb2   | 724   | 360    | 367       | 367   | HKY+I+G  |                        |                        |

† ITS, the internal transcribed spacer regions and 5.8S rRNA gene; tub2, β-tubulin; cmdA, calmodulin; rpb2: RNA polymerase II second largest subunit.

* B = Bayesian inference.
‡ G: Gamma distributed rate variation among sites. GTR: Generalised time-reversible. I: Proportion of invariable sites. HKY: Hasegawa-Kishino-Yano.
Taxonomy

*Dimorphiseta* L. Lombard & Crous., Persoonia. 36: 188. 2016. emend. J.M.Liang & L.Cai.

*Dimorphiseta terrestris* L. Lombard & Crous. Persoonia. 36: 188. 2016. (Type species)

**Note.** *Dimorphiseta* was a monotypic genus, introduced based on *D. terrestris*, which showed both type I (thin-walled, flexuous to circinate, narrowing to a sharp apex) and type II (thick-walled, straight to slightly curved, narrowing to a sharp apex) setae. Our study demonstrated that there is a third type of setae (type III: thin-walled, straight, terminating in an obtuse apex) in the genus.

*Dimorphiseta acuta* J.M. Liang, G.S. Li & L. Cai, sp. nov.

MycoBank MB 829693

Fig. 2

**Type.** China, Beijing, isolated from rhizosphere soils of *Poa pratensis*, 26 Aug 2017, J.M. Liang, holotype HMAS 247957, dried culture on PDA, ex-holotype culture CG-MCC3.19208 = LC12122.

**Description.** Colonies on PDA, CMA and OA approx. 7–8 cm diam. after 7 d at room temperature (approx. 25 °C), mycelium white and abundant, with conidiophores forming on the aerial mycelium, carrying slimy olivaceous green to black conidial masses, reverse on PDA buff. *Conidiomata* sporodochial, stromatic, superficial, cupulate to discoid, scattered, rarely gregarious, irregular in outline, 50–300 μm diam., 60–150 μm deep, consisting of bundles of parallel, longitudinal, closely compacted hyphae, terminating in whorls of 3–5 conidiogenous cells, covered by an olivaceous green to black slimy mass of conidia without marginal hyphae. *Stroma* poorly developed, hyaline, of a textura angularis. *Setae* arising from the conidial mass, thick-walled, subhyaline, smooth, 5–15-septate, tapering to sharp apices, 120–370 μm long, 10–13 μm wide at the broadest part, 2–4 μm wide at the apex. *Conidiophores* macronematous, irregularly, unbranched, smooth to lightly verrucose, arising from the basal stroma. *Conidiogenous cells* phialidic, subcylindrical, hyaline, smooth, 10–20 μm long, 2–3 μm wide. *Conidia* aseptate, smooth, hyaline, ellipsoidal, rounded at the base, pointed at the apex with a funnel-shaped appendage, 7–12 × 2–3 μm (av. 10 ± 0.7 × 3 ± 1.3 μm, n = 50).

**Distribution.** China.

**Etymology.** Name refers to the setae with tapered and sharp apices.

**Additional isolates examined.** China, Beijing, from leaves of *Digitaria sanguinalis*, 21 Aug 2017, J.M. Liang, LC12123; China, Beijing, from leaves of *Poa pratensis*, 21 Aug 2017, J.M. Liang, LC12124; China, Beijing, from rhizosphere soils of *P. pratensis*, 21 Aug 2017, J.M. Liang & G.S. Li, LC12125, 21 Jul 2017, J.M. Liang, LC12126, 25 Jul 2017, J.M. Liang, LC12127.
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Figure 2. *Dimorphiseta acuta* (from ex-type strain CGMCC3.19208) a–c colony on PDA, CMA, OA d conidiomata on SNA e conidiophores f conidiogenous cells g setae h–k conidia. Scale bars: 5 μm (e, f, h): 50 μm (g); 2 μm (i, j, k).

Notes. The multi-locus phylogenetic analyses indicated that *D. acuta* formed a sister clade to *D. terrestris*, but differs from the latter in the type and size of setae. *Dimorphiseta terrestris* produces both types of setae, the thin-walled and circinate type (Type I) and the thick-walled sharp-edged type (Type II), whereas *D. acuta* only produces the type I setae. In addition, the setae of *D. acuta* are much longer and wider than that in *D. terrestris* (120–370 μm × 10–13 μm vs. 70–95 × 3–4 μm) (Lombard et al. 2016). Morphologically, *D. acuta* should also be compared with *M. miconiae* and *M. xigazense*, which also produce sharp-edged setae. *Myrothecium miconiae*, however, differs from *D. acuta* in producing 1-septate conidia (Alves et al. 2010), while *M. xigazense* differs in producing conidia that are truncate at both ends (Wu et al. 2014).
**Dimorphiseta obtusa** J.M. Liang, G.S. Li & L. Cai, sp. nov.
MycoBank MB 829694

**Type.** China, Beijing, isolated from rhizosphere soils of *P. pratensis*, 23 Jun 2017, J.M. Liang, holotype HMAS 247954, ex-holotype culture CGMCC3.19206 = LC12128.

**Description.** Colonies on PDA, OA and CMA approx. 5–6 cm diam. after 7 d at room temperature (approx. 25 °C), mycelium white and abundant, with conidiophores forming on the aerial mycelium, carrying slimy olivaceous green to black conidial masses, reverse on PDA pale luteous to buff. Conidiomata sporodochial, stro-

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**Figure 3.** *Dimorphiseta obtusa* (from ex-type strain CGMCC3.19206) **a–c** colony on PDA, CMA, OA **d** conidioma on SNA **e** setae **f** conidiophores **g** conidiogenous cells **h–k** conidia. Scale bars: 50 μm (**e**); 10 μm (**f, g**); 5 μm (**h**); 2 μm (**i, j, k**).
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Matric, superficial, scattered, rarely gregarious, oval to elongate or irregular in outline, 60–280 μm diam., 40–120 μm deep, with a setose fringe surrounding green to black slimy mass of conidia. **Stroma** poorly developed, hyaline, smooth to verrucose, of textura angularis. **Setae** arising from the basal stroma, thin-walled, 3–6-septate, unbranched, hyaline, smooth, 80–250 μm long, 2–4 μm wide at the broadest, terminating in a blunt apex. **Conidiophores** macronematous, irregularly, unbranched, smooth to lightly verrucose, arising from the basal stroma, up to 18 μm long. **Conidiogenous cells** phialidic, hyaline, smooth to verrucose, cylindrical, 7–19 × 2–3 μm, becoming narrowed at the tip with collarette. **Conidia** aseptate, ellipsoidal or cylindrical, hyaline, smooth, rounded both ends, with a funnel-shaped apical appendage, 9–11 × 2–4 μm (av. 10 ± 0.5 × 3 ± 0.3 μm, n = 50).

**Distribution.** China.

**Etymology.** Named refers the setae with obtuse apices.

**Additional isolates examined.** China, Beijing, from rhizosphere soils of *Agrostis stolonifera*, 24 Jul 2017, J.M. Liang, LC12129; China, Beijing, from rhizosphere soils of *P. pratensis*, 25 Aug 2017, J.M. Liang & G.S. Li, LC12130, 19 Jul 2017, J.M. Liang, LC12133; China, Beijing, from rhizosphere soils of *Poa* sp., 19 Jul 2017, J.M. Liang, LC12131; China, Beijing, from rhizosphere soils of *Festuca arundinacea*, 19 Jul 2017, J.M. Liang, LC12132; China, Beijing, from leaves of *P. pratensis*, 23 Jun 2017, J.M. Liang, LC12134, LC12135.

**Notes.** *Dimorphiseta obtusa* formed a highly supported cluster with *D. terrestris* and *D. acuta*, but can be distinguished from the latter two by having setae with erect and obtuse apices. In addition, *D. obtusa* is also morphologically similar to two old un-sequenced *Myrothecium* taxa, i.e. *M. biforme* and *M. dimorphum*, but both of these two taxa have two types of conidia. *Myrothecium biforme* produces short cylindrical and ellipsoidal to navicular conidia (Jiang et al. 2014) and *M. dimorphum* has ovate and ellipsoidal conidia (Watanabe et al. 2003).

**Alfaria humicola** J.M. Liang, G.S. Li & L. Cai, sp. nov.

Mycobank MB 829696

Fig. 4

**Type.** China, Beijing, Olympic Park, from rhizosphere soil of *Poa* sp., 13 Dec 2017, S.Y. Zhou, holotype HMAS 247955, ex-holotype culture CGMCC3.19213 = LC12143.

**Description.** Colonies on PDA, CMA and OA approx. 7–8 cm diam. after 7 d at 25 °C. **Hyphae** hyaline, smooth, branched, 1–2 μm wide. **Conidiomata** sporodochial, stromatic, superficial, cupulate to discoid, scattered to gregarious, oval to elongate or irregular in outline, 50–200 μm diam., 70–150 μm deep, without setose hyphae, covered by a green to black agglutinated slimy mass of conidia. **Stroma** well-developed, hyaline, of textura globulose or textura angularis. **Setae** absent. **Conidiophores** arising from the basal stroma, unbranched or branched, initially hyaline and smooth, becoming pigmented and verrucose with age, 11–25 μm long.
Conidiogenous cells phialidic, cylindrical to allantoid, initially hyaline and smooth becoming pigmented and verrucose with age, 14–33 × 2–3 μm. Conidia aseptate, smooth, hyaline, elongated ellipsoidal to limoniform, straight, 7–9(–10) × 2–3 μm (av. 8 ± 0.6 × 3 ± 0.2 μm, n = 50).

**Distribution.** China.

**Etymology.** Name refers the substrate, soil, from which this fungus was isolated.

**Additional isolate examined.** China, Beijing, Olympic Park, from rhizosphere soil of *Poa* sp., 13 Dec 2017, S.Y. Zhou, LC12144.

**Notes.** *Alfaria humicola* represents another distinct lineage in *Alfaria* (Fig. 1). *Alfaria humicola* lacks setae, distinguishing it from *Alf. caricicola* and *Alf. thymi*. Furthermore, the conidiogenous cells of *Alf. humicola* (14–33 × 2–3 μm) are much longer than that of *Alf. arenosa* (5–10 × 1–2 μm), *Alf. ossiformis* (5–10 × 2–3 μm) and *Alf. terrestris* (5–11 × 1–3 μm). Compared with those old *Myrothecium* taxa lacking sequences, *Alf. humicola* is morphologically similar to *M. atrocarreum* (Berkeley & Broome, 1877), *M. conicum* (Fuckel, 1870), *M. ellipsosporum* (Fuckel, 1866), *M. fragostanum* (Saccardo, 1917), *M. leucomelas* (Höhnel, 1925) and *M. oryza* (Saccardo, 1917), but *Alf. humicola* produces limoniform conidia which makes it distinguishable. In addition, the conidiogenous cells of *Alf. humicola* show conspicuous collarettes which were not described in previous old taxa.
**Alfaria poae** J.M. Liang, G.S. Li & L. Cai, sp. nov.
MycoBank MB 829697

**Fig. 5**

**Type.** China, Hainan Province, Haikou, isolated from leaves of *Imperata cylindrica*, 10 Mar 2018, J.M. Liang and L. Cai, holotype HMAS 247953, ex-holotype culture CGMCC3.19198 = LC12140.

**Description.** Colonies on PDA, CMA and OA with white aerial mycelium, approx. 6–7 cm diam. after 7 d at 25 °C, giving rise to dark green or blank sporodochia scattered or gregarious on the surface, covered by olivaceous green pillars of conidia.
reverse on PDA sienna. *Hyphae* hyaline, smooth, branched, 1–2 μm wide. *Conidiomata* synnematous, solitary, 60–250 μm high, 30–80 μm wide at the base, 60–150 μm at the apex, with setose hyphae surrounding a green agglutinated mass of conidia. *Stroma* well developed, hyaline, of textura angularis. *Setae* absent. *Conidiophores* arising from the basal stroma, branched, initially hyaline and becoming pigmented and verrucose with age covered by an olivaceous green mucoid layer, up to 30 μm long. *Conidiogenous cell* phialidic, clavate to cylindrical, hyaline, smooth, 5–10 × 1–2 μm, becoming pigmented and verrucose with age, with conspicuous collarettes and periclinal thickenings. *Conidia* aseptate, smooth, hyaline, ellipsoidal to fusiform, 6–8 × 2–3 μm (av. 7 ± 0.4× 2 ± 0.2 μm, n = 50).

**Distribution.** China.

**Etymology.** Name refers the host, *Poa* sp., from which this fungus was isolated.

**Additional isolate examined.** China, Hainan, from leaves of *Imperata cylindrica*, 10 Mar 2018, J.M. Liang & Lei Cai, LC12141, LC12142.

**Notes.** *Alfaria poae* formed a well-supported clade in *Alfaria* (Fig. 1). Similar to *Alf. ossiformis* and *Alf. terrestris*, *Alf. poae* does not produce setae surrounding the sporodochia, distinguishing it from *Alf. caricicola* and *Alf. thymi*. *Alfaria poae* produces ellipsoidal to fusiform conidia, which are different from the ossiform conidia produced by *Alf. ossiformis* (Lombard et al. 2016). The conidia of *Alf. terrestris* have basal hilum which was not observed in *Alf. poae*. In addition, *Alf. poae* shares morphological characters with several un-sequenced *Myrothecium* taxa, such as *M. atrocarneum* (Berkeley & Broom, 1877), *M. conicum* (Fuckel, 1870), *M. ellipsosporum* (Fuckel, 1866) and *M. leucomelas* (Höhnel, 1925). Because the descriptions of *M. atrocarneum*, *M. conicum* and *M. ellipsosporum* were not elaborate enough, these old species are not distinct from *Alf. poae* yet. Future comparisons should be made when these old species are epitypified by fresh collections. Although *M. leucomelas* (host: *Sumbaviae rottleroidis*; location: Bulacan, Luzon) had a detailed description, it cannot be epitypified by *Alf. Poae*, because *Alf. poae* was collected from a distinct location and plant host. Taking the above special characters into account, we considered introducing a new species, *Alfaria poae*.

**Paramyrothecium sinense** J.M. Liang, G.S. Li & L. Cai, sp. nov.

*Mycobank MB 829698*

**Type.** China, Beijing, Olympic Park, from rhizosphere soil of *Poa* sp., 13 Dec 2017, S.Y. Zhou, holotype HMAS 247956, ex-holotype culture CGMCC3.19212 = LC12136.

**Description.** Colonies on PDA, CMA and OA approx. 5–6 cm diam. after 7 d at 25 °C. *Hyphae* white, hyaline, smooth, branched, 1–2 μm wide, reverse on PDA pale luteous. *Conidiomata* sporodochial, stromatic, cupulate, superficial, scattered or gregarious, oval or irregular in outline, 80–600 μm diam., 50–150 μm deep, with a white setose fringe surrounding an olivaceous green to black agglutinated slimy mass
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Conidiomata of conidia. Stroma poorly developed, hyaline, of textura angularis. Setae arising from stroma, thin-walled, hyaline, 1–3-septate, straight to flexuous, 45–90 μm long, 1–3 μm wide, tapering to an acutely rounded apex. Conidiophores arising from the basal stroma, consisting of a stipe and a penicillately branched conidiogenous apparatus; stipes unbranched, hyaline, septate, smooth, 20–30 × 2–3 μm; primary branches aseptate, unbranched, smooth, 13–40 × 2–3 μm; secondary branches aseptate, unbranched, smooth, 8–15 × 2–3 μm; terminating in a whorl of 3–6 conidiogenous cells; conidiogenous cell phialidic, cylindrical to subcylindrical, hyaline, smooth, straight to slightly curved, 7–16 × 1–3 μm, with conspicuous collarettes and periclinal thickenings. Conidia aseptate, hyaline, smooth, cylindrical, 6–7 × 2–3 μm (av. 7 ± 0.3 × 2 ± 0.2 μm, n = 40), rounded at both ends.

Distribution. China.

Figure 6. Paramyrothecium sinense (from ex-type CGMCC3.19212) a–c colony on PDA, CMA, OA d conidiomata on SNA e sporodochial conidioma f setae g conidia h conidiogenous cells. Scale bars: 20 μm (e, f); 10 μm (g); 5 μm (h).
**Etymology.** Named after the country of collection, China.

**Additional isolate examined.** China, Beijing, Olympic Park, from rhizosphere soils of *Poa* sp., 13 Dec 2017, S.Y. Zhou, LC12137, LC12138, LC12139.

**Notes.** Lombard et al. (2016) introduced a new genus, *Paramyrothecium*, based on an epitype of *Myrothecium roridum* Tode, 1790. Gams (2016) pointed out that *Myrotheciella catenuligera*, the type species of *Myrotheciella* was listed as a synonym of *P. roridum* by Lombard et al. (2016), thus *Paramyrothecium* is illegitimate and *Myrotheciella* should be the correct name for *Paramyrothecium*. However, the original description of *Myrotheciella catenuligera* suggested that it lacks seta (Spegazzini 1911), thus is clearly different from the morphological circumscription of *P. roridum*. Therefore, we do not agree with the treatment of Lombard et al. (2016) of listing *Myrotheciella catenuligera* as a synonym of *P. roridum*.

*Paramyrothecium sinense* formed a highly supported distinct clade closely related to *P. humicola*. The setae of this species are terminated with obtuse apices, dissimilar to the acute apices in *P. humicola*. In addition, the conidiophore stipes (20–30 μm long) and primary branches (13–40 μm long) of *P. sinense* are much longer than those of *P. humicola* (stipe, 12–22 μm long; primary branches, 7–17 μm long) (Lombard et al. 2016). Among old un-sequenced taxa in *Myrothecium*, only *M. biforme* and *M. dimorphum* show seta with obtuse apices, but both taxa produce two types of conidia (Jiang et al. 2014; Watanabe et al. 2003).

**Discussion**

The ITS has been shown to be insufficient to delineate the myrothecium-like species. With the additions of partial sequences of *rpb2*, *cmdA* and *tub2*, phylogenetic relationships within Stachybotryaceae could be better resolved (Lombard et al. 2016). In this study, we isolated fungi from rhizosphere soils, leaves and roots of several turfgrasses, and our phylogenetic analyses based on concatenated four loci together with the morphological characters supported the recognition of five novel species in Stachybotryaceae.

By comparing the topologies of the four single-locus trees, incomplete lineage sorting was discovered in *Dimorphiseta*. Based on the single-locus trees of ITS and *rpb2*, *D. acuta*, *D. obtusa* and *D. terrestris* grouped together (Supp. materials 1, 4). Whereas in the single-locus phylogenetic analyses based on *tub2* and *cmdA*, *D. obtusa* grouped distantly from *D. acuta* and *D. terrestris*, but close to *Myxospora* and *Albifimbria* species (Supp. materials 2, 3). Three *Dimorphiseta* species are similar in the conidial shape and size (7–19 μm long), which are distinct from the shorter conidia in *Albifimbria* (4–8 μm long) and *Myxospora* (4–6 μm long) species (Tulloch 1972; Lombard et al. 2016). Conidia with a funnel-shaped apical appendage are a distinct feature of three *Dimorphiseta* species, but they are absent in all *Myxospora* species and most *Albifimbria* species (Lombard et al. 2016). Furthermore, the *rpb2* and 28S ribosomal DNA combined dataset, which was suggested to delimit generic boundaries of myrothecium-like
species (Lombard et al. 2016) revealed that the three Dimorphiseta species clustered together (Supp. material 6: Table S1, Supp. material 5).

In the multi-locus sequence analysis of Myrothecium s.l. by Lombard et al. (2016), thirteen new genera were introduced including several monotypic genera, such as Dimorphiseta, Capitofimbria, Gregatothecium and Neomyrothecium. In this study, we reported two new species in Dimorphiseta (D. acuta and D. obtusa). With this addition, the generic concept of Dimorphiseta is slightly expanded for including a third type of setae. Hereto, Dimorphiseta is the genus with the most variable types of setae among Myrothecium s.l., which might be useful in the generic delimitation in Myrothecium s.l. (Lombard et al. 2016).

Lombard et al. (2016) narrowed the concept of Myrothecium s.s. to only include species with sporodochia or mononematous conidiophores producing conidia shorter than 5 μm in green slimy masses without mucoid appendages. Whether or not a conidial size should be defined in the generic concept remained debatable. Because many Myrothecium published recently produced much longer conidia, e.g. M. chiangmaense (4–7 μm) (Dai et al. 2017), M. uttaraditense (10–15 μm) (Dai et al. 2017), M. thailandicum (6.5–10 μm) (Dai et al. 2017), M. septentrionale (8.5–12 μm) (Tibpromma et al. 2017), M. variabile (12.5–16.5 μm) (Wu et al. 2014) and M. xigazense (2.5–15 μm) (Wu et al. 2014). These above species were identified, either based on morphology only or with a single molecular locus (ITS), and should be better confirmed for their generic placement when more data are available. Currently, there are 90 records of Myrothecium in Index Fungorum (Jan 10, 2019), and 25 names have been successively transferred to other genera, i.e., Capitofimbria, Melanconis, Striaticonidium, Xepicula (Lombard et al. 2016), Digitiseta (Gordillo and Decock 2018). Only a limited number of the remaining species in Myrothecium have available molecular data (Dai et al. 2017; Tibpromma et al. 2017), as most of these taxa have no living cultures. We agree with Gams (2016) that these unvisited taxa are still important when the original descriptions are sufficiently clear to recognize a species. They should be epitypified in future studies when fresh collections with living cultures are available, and before that, descriptions of new taxa in this group should be made carefully with the inclusion of these un-sequenced taxa in morphological comparisons.

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**Supplementary material 1**

**Figure S1.** The ML consensus tree inferred based on *ITS* partial sequence with bootstrap values for ML (> 70%) and posterior probability (PP) (PP > 0.95) labeled to the left of a node (ML/PP)
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
Explanation note: The type strains were labeled with “T”. Strains obtained from this study are in red.
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Link: https://doi.org/10.3897/mycokeys.51.31957.suppl1
Supplementary material 2

Figure S2. The ML consensus tree inferred based on *tub2* partial sequence with bootstrap values for ML (> 70%) and posterior probability (PP) (PP > 0.95) labeled to the left of a node (ML/PP)
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
Explanation note: The type strains were labeled with “T”. Strains obtained from this study are in red.
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Link: https://doi.org/10.3897/mycokeys.51.31957.suppl2

Supplementary material 3

Figure S3. The ML consensus tree inferred based on *cmdA* partial sequence with bootstrap values for ML (> 70%) and posterior probability (PP) (PP > 0.95) labeled to the left of a node (ML/PP)
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
Explanation note: The type strains were labeled with “T”. Strains obtained from this study are in red.
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Supplementary material 4

Figure S4. The ML consensus tree inferred based on rpb2 partial sequence with bootstrap values for ML (> 70%) and posterior probability (PP) (PP > 0.95) labeled to the left of a node (ML/PP)
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
Explanation note: The type strains were labeled with “T”. Strains obtained from this study are in red.
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Supplementary material 5

Figure S5. The ML consensus tree inferred based on LSU and rpb2 partial sequences with bootstrap values for ML (> 70%) and posterior probability (PP) (PP > 0.95) labeled to the left of a node (ML/PP)
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
Explanation note: The type strains were labeled with “T”. Strains obtained from this study are in red.
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Link: https://doi.org/10.3897/mycokeys.51.31957.suppl5
Supplementary material 6

Table S1. NCBI GenBank accessions of 28S ribosomal DNA large-subunit sequences (LSU) used in the phylogenetic analyses
Authors: Junmin Liang, Guangshuo Li, Shiyue Zhou, Meiqi Zhao, Lei Cai
Data type: phylogenetic data
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