Eight new *Arthrinium* species from China

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Abstract
The genus *Arthrinium* includes important plant pathogens, endophytes and saprobes with a wide host range and geographic distribution. In this paper, 74 *Arthrinium* strains isolated from various substrates such as bamboo leaves, tea plants, soil and air from karst caves in China were examined using a multi-locus phylogeny based on a combined dataset of ITS rDNA, TEF1 and TUB2, in conjunction with morphological characters, host association and ecological distribution. Eight new species were described based on their distinct phylogenetic relationships and morphological characters. Our results indicated a high species diversity of *Arthrinium* with wide host ranges, amongst which, Poaceae and Cyperaceae were the major host plant families of *Arthrinium* species.

Keywords
Ascomycota, Morphology, Phylogeny, Systematics, Taxonomy

Introduction

*Arthrinium* Kunze is an anamorph-typified genus, which has been traditionally linked to the teleomorph-typified genus *Apiospora* Sacc. (Ellis 1971, Seifert et al. 2011). It is strikingly different from other anamorphic genera for the presence of basauxic conidiophores (Hughes 1953, Minter 1985). The traditional generic circumscription of *Arthrinium* was primarily based on morphological characters (e.g. conidial shape, conidiophores, sterile cells and the presence of setae) but has been regarded as too narrow (Ellis 1971, 2011).
Minter 1985, Crous et al. 2013). It is now recognised that, at the generic level, conidial shape and the presence of setae are not reliable characters to infer phylogenetic relationships (Crous et al. 2013). For example, *Arthrinium* was regarded as being different from *Cordella* Speg. (1886) by the absence of setae amongst the clusters of specialised hyphae and different from *Pteroconium* Sacc. (1892) by the absence of sporodochia and pseudoparenchyma (Minter 1985). However, both genera have been reduced to the generic synonyms of *Arthrinium*, based on molecular phylogenetic data (Crous et al. 2013).

*Arthrinium* species are geographically widely distributed in various hosts. Many species of *Arthrinium* are associated with plants as endophytes or saprobes, as well as plant pathogens on some important ornamentals, e.g. *A. phaeospermum* causing culm rot on *Phyllostachys viridis* (Li et al. 2016); *A. arundinis* causing brown culm streak of *Phyllostachys praecox* (Chen et al. 2014). Moreover, *A. phaeospermum* has been reported for causing cutaneous infections of humans (Rai 1989, Zhao et al. 1990, de Hoog et al. 2000, Crous et al. 2013). Many *Arthrinium* species are also known to produce bioactive compounds with pharmacological and medicinal applications, such as *A. arundinis* and *A. saccharicola* isolated from a brown alga *Sargassum* sp., with good antifungal activities against some plant pathogenic fungi (Hong et al. 2015). *Arthrinium saccharicola*, *A. sacchari* and *A. phaeospermum* isolated from *Miscanthus* sp. are known to produce industrially important enzymes (Shrestha et al. 2015).

In this paper, eight new *Arthrinium* species are described and characterised based on morphological characters and phylogeny inferred from the combined ITS rDNA, TEF1 and TUB2 sequences dataset. Comparisons were made with morphologically similar and phylogenetically related species. Fungus-host distribution of *Arthrinium* species are summarised based on data from literature and this study.

**Materials and method**

**Isolates**

Diseased and healthy tissues of bamboo leaves and other plant hosts were collected from six provinces or municipalities in China (Chongqing, Guangxi, Guangdong, Guizhou, Jiangxi, Hunan). Tissue pieces (5 mm × 5 mm) were taken from the margin of leaf lesions and the surface sterilised with 75% ethanol for 1 min, 5% NaClO for 30 s, followed by rinsing in sterile distilled water for 1 min. The pieces were dried with sterilised paper towels and then placed on 1/4 PDA (potato dextrose agar) (Cai et al. 2009).

All cultures were preserved in the LC culture collection (personal culture collection of Lei Cai housed in the Institute of Microbiology, Chinese Academy of Sciences). Type specimens were deposited in Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), with ex-type living cultures deposited in China General Microbiological Culture Collection Center (CG-MCC). Taxonomic information of the new taxa was deposited in MycoBank (www. MycoBank.org; Crous et al. 2004).
Morphology

Cultures were incubated on PDA for 7 d at 25 °C to measure the growth rates and on 2% malt agar with bamboo leaves to enhance sporulation. Morphological descriptions were based on cultures sporulating on MEA (malt extract agar) medium at room temperature (ca. 25 °C). Shape and size of microscopic structures were observed using a light microscope and colonies were assessed according to the colour charts of Rayner (1970). At least 50 conidiogenous cells and conidia were measured to calculate the mean size.

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia were taken from 7-d-old cultures growing on PDA and ground with the organisation disruptor FastPrep-48. Genomic DNA was extracted following the modified CTAB protocol as described in Guo et al. (2000).

Phylogenetic analyses were conducted using partial sequences of three loci, 5.8S nuclear ribosomal gene with the two flanking transcribed spacers (ITS), part of the translation elongation factor 1-alpha (TEF1) and beta-tubulin (TUB2). The ITS locus was amplified using the primer pair ITS1/ITS4 (Vilgalys and Hester 1990, White et al. 1990); TEF1 using EF1-728F/EF-2 (O’Donnell et al. 1998, Carbone and Kohn 1999); and TUB2 using T1 (O’Donnell and Cigelnik 1997) and Bt-2b (Glass and Donaldson 1995).

PCR was performed in a 25 ml reaction containing 18.95 µl double distilled water, 2.5 µl 10 × PCR buffer, 0.3 µl dNTP mix (2.5 mM), 1 µl of each primer (10 mM), 1 µl DNA template and 0.25 µl Taq DNA polymerase (Genstar). The annealing temperatures were adjusted to 52 °C for ITS and TUB2, and 56 °C for TEF1. Purification and sequencing of the PCR amplicons were done by SinoGenoMax, Beijing.

Phylogenetic analysis

Sequences generated from the forward and reverse primers were used to obtain consensus sequences using MEGA v. 6.0 (Tamura et al. 2013). The concatenated tree was inferred based ITS, TUB2 and TEF1 sequences (Figure 1) using Bayesian and Maximum-likelihood analyses. Sequences were aligned using an online version of MAFFT v. 7 (available at http://mafft.cbrc.jp/alignment/server/). Ambiguous regions were excluded from the analyses and gaps were treated as missing data. Maximum-likelihood (ML) analysis was performed in RAxML v. 7.2.6 (Stamatakis and Alachiotis 2010), employing GTR models of evolution settings of the programme and bootstrap support obtained by running 1000 pseudo replicates. Maximum Likelihood bootstrap values (ML) equal to or greater than 70% are given above each node.

Bayesian analysis was conducted using MrBayes v. 3.2.1 (Ronquist et al. 2012) and the best nucleotide substitution model for each locus was calculated with jModelTest v. 2.1.4 (Posada 2008). Posterior probabilities (PP) (Zhaxybayeva and Gogarten 2002) were de-
Figure 1. Phylogenetic tree based on the combined ITS, TEF1 and TUB2 sequences alignment generated from a Maximum likelihood phylogenetic analysis. Bootstrap support values (>70%) and posterior probabilities (>0.9) are given at the nodes (ML/PP). The tree is rooted with *Nigrospora gorlenkoana* CBS 480.73. The novel species were highlighted (* indicates the ex-type cultures).
average standard deviation of split frequencies fell below 0.01. The first 25% trees, which represented the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating PP in the majority rule consensus tree. Sequences generated in this study were deposited in GenBank (Table 1) and the final matrices used for the phylogenetic analyses in TreeBASE (www.treebase.org; accession number: 21341).

Fungus-host distribution of *Arthrinium* species

To determine the distribution of *Arthrinium* species on host/substrate, the number of species occurred on each host (based on family level) was counted based on data from this study, relevant literature and the USDA fungal database (https://nt.ars-grin.gov/fungaldatabases/). The proportion account for the known 66 species in *Arthrinium* (Index Fungorum) was illustrated in a histogram. Four species with an unknown host range were not included in this analysis.

Results

Phylogeny

The combined ITS, TUB2 and TEF1 dataset contained 75 strains, with *Nigrospora gorlenkoana* CBS 480.73 as the out group. For the Bayesian analyses, the best-fit models TrN+I+G, GTR+I+G, HKY+I+G were selected for ITS, TUB2 and TEF1 loci, respectively. The ML analysis showed the same tree topology as that obtained in the Bayesian analysis. All the *Arthrinium* strains in this study separated into 13 clades, representing five known (*A. arundinis*, *A. hydei*, *A. rasikravindrii*, *A. thailandicum*, *A. xenocordella*) and eight new species (Figure 1). The eight new species clustered in distinct clades with high bootstrap supports (Figure 1). Phylogenetic analyses based on an individual locus were also conducted (not shown) and the generated trees are similar to the one generated from the combined multi-locus dataset (Figure 1).

Host associated with *Arthrinium* species

The histogram in Figure 2 shows that *Arthrinium* species were widely distributed amongst 17 plant families, including Brassicaceae, Bromeliaceae, Cornaceae, Cyperaceae, Euphorbiaceae, Fagaceae, Juncaceae, Lauraceae, Myrsinaceae, Oleaceae, Pinaceae, Poaceae, Restionaceae, Rosaceae, Tiliaceae, Urticaceae and Vitaceae. *Arthrinium* species were also isolated from air, dust, soil and sand. The proportion of species occurring on each host family was assessed (Figure 2). Poaceae and Cyperaceae were the major host families for *Arthrinium*, which accounted for 42.42% and 24.24% of species in *Arthrinium* respectively.
Table 1. Strains included in the phylogenetic analyses.

| Species               | Strain numbers | Hosts                  | Countries | GenBank accessions |
|-----------------------|----------------|------------------------|-----------|-------------------|
|                        |                |                        |           | ITS               |
| Arthrinium arundinis   | CBS 114316     | Leaf of *Hordeum vulgare* | Iran      | KF144884          |
|                        | CBS 124788     | Living leaves of *Fagus sylvatica* | Switzerland | KF144885          |
|                        | LC4477         | Unknown host           | China     | KY494688          |
|                        | LC4493         | *Phyllostachys* sp.    | China     | KY494689          |
|                        | LC4650         | *Osmanthus* sp.        | China     | KY494695          |
|                        | LC4951         | *Dichotomanthus tristianiae*carpa | China     | KY494698          |
|                        | LC4959         | *Bothrocaryum controversum* | China     | KY494699          |
|                        | LC5311         | Air in karst cave      | China     | KY494706          |
|                        | LC5312         | Air in karst cave      | China     | KY494707          |
|                        | LC5332         | Air in karst cave      | China     | KY494710          |
|                        | LC5394         | Soil in karst cave     | China     | KY494711          |
|                        | LC5416         | Water in karst cave    | China     | KY494712          |
|                        | LC7118         | Leaf of bamboo         | China     | KY494723          |
|                        | LC7122         | Leaf of bamboo         | China     | KY494726          |
|                        | LC7160         | Leaf of bamboo         | China     | KY494738          |
|                        | LC7211         | Leaf of bamboo         | China     | KY494739          |
|                        | LC7216         | Leaf of bamboo         | China     | KY494741          |
|                        | LC7218         | Leaf of bamboo         | China     | KY494742          |
|                        | LC7243         | Leaf of bamboo         | China     | KY494744          |
|                        | LC7252         | Leaf of bamboo         | China     | KY494747          |
|                        | LC7277         | Leaf of bamboo         | China     | KY494750          |

Arthrinium

A Histogram to show fungus-host distribution of *Arthrinium* species.

Figure 2.
| Species                  | Strain numbers1 | Hosts                  | Countries | GenBank accessions |
|--------------------------|-----------------|------------------------|-----------|-------------------|
| **A. aureum**            | CBS 244.83*     | Air                    | Spain     | AB220251 KF144981 KF145023 |
|                          | LC7106* = CGMCC 3.18335 | Leaf of bamboo        | China     | KY494718 KY705186 KY806204 |
|                          | LC7107          | Leaf of bamboo         | China     | KY494719 KY705187 KY705117 |
|                          | LC7113          | Leaf of bamboo         | China     | KY494720 KY705188 KY806205 |
|                          | LC7124          | Leaf of bamboo         | China     | KY494727 KY705195 KY806206 |
|                          | LC7125          | Leaf of bamboo         | China     | KY494728 KY705196 KY705124 |
|                          | LC7128          | Leaf of bamboo         | China     | KY494730 KY705198 KY705126 |
|                          | LC7246          | Leaf of bamboo         | China     | KY494745 KY705213 KY705141 |
| **A. bambusae**          | LC5007* = CGMCC 3.18333 | Camellia sinensis    | China     | KY494704 KY705173 KY705103 |
|                          | LC8181          | Brassica capes              | China     | KY494761 KY705229 KY705157 |
| **A. camelliae-sinensis**| LC4950* = CGMCC 3.18332 | Dichotomanthus tristaniacarpae | China   | KY494697 KY705167 KY705096 |
|                          | LC8175          | Dichotomanthus tristaniacarpae | China   | KY494755 KY705223 KY705151 |
|                          | LC8176          | Dichotomanthus tristaniacarpae | China   | KY494756 KY705224 KY705152 |
| **A. euphorbiae**        | IMI 285638b     | Bambusa sp.              | Bangladesh | AB220241 AB220288 – |
| **A. guizhouense**       | LC5318          | Air in karst cave        | China     | KY494708 KY705177 KY705107 |
|                          | LC5322* = CGMCC 3.18334 | Air in karst cave    | China     | KY494709 KY705178 KY705108 |
| **A. gutiae**            | CBS 135835      | Gut of a grasshopper    | India     | KR011352 KR011350 KR011351 |
| **A. hispanicum**        | IMI 326877*     | Maritime sand           | Spain     | AB220242 AB220289 – |
| **A. hydei**             | CBS 114990*     | Culms of Bambusa tuldoides | Hong Kong | KF144890 KF144982 KF145024 |
|                          | LC7103          | Leaf of bamboo          | China     | KY494715 KY705183 KY705114 |
|                          | LC7105          | Leaf of bamboo          | China     | KY494717 KY705185 KY705116 |
| **A. hyphopodii**        | MFLUCC 15-0003* | Culms of Bambusa tuldoides | Thailand   | KR069110 – – |
| **A. japonicum**         | IFO 30500       | Carex despalata (dead leaf) | Japan     | AB220262 AB220309 – |
|                          | IFO 31098       | Carex despalata (leaf)  | Japan     | AB220264 AB220311 – |
| **A. garethjonesii**     | KUMCC 16-0202   | Dead culms of bamboo    | China     | KY356086 – – |
| **A. jatrophae**         | MM1 00052* = MCC 1014 | Healthy petiole of Jatropha podagrica | India | JQ246355 – – |
|                          | LC2831          | Leaf of bamboo          | China     | KY494686 KY806201 KY705085 |
|                          | LC4494          | Phyllostachys sp.       | China     | KY494690 KY705160 KY705089 |
|                          | LC4541          | Maesa sp.               | China     | KY494691 KY705161 KY705090 |
|                          | LC4547          | Machilus sp.            | China     | KY494692 KY705162 KY705091 |
|                          | LC4577* = CGMCC 3.18381 | Maesa sp.            | China     | KY494693 KY705163 KY705092 |
| **A. jiangxiense**       | LC4578          | Camellia sinensis       | China     | KY494694 KY705164 KY705093 |
|                          | LC4993          | Phyllostachys sp.       | China     | KY494700 KY806203 KY705099 |
|                          | LC4997          | Phyllostachys sp.       | China     | KY494701 KY705170 KY705100 |
|                          | LC5001          | Phyllostachys sp.       | China     | KY494702 KY705171 KY705101 |
|                          | LC5004          | Phyllostachys sp.       | China     | KY494703 KY705172 KY705102 |
|                          | LC5015          | Imperata cylindrica     | China     | KY494705 KY705174 KY705104 |
| Species          | Strain numbers | Hosts                     | Countries     | GenBank accessions |
|------------------|----------------|---------------------------|---------------|--------------------|
|                  |                |                           |               | ITS                |
|                  |                |                           |               | TUB                |
|                  |                |                           |               | TEF                |
| A. jiangxiense   | LC7104         | Leaf of bamboo            | China         | KY494716          |
|                  | LC7154         | Leaf of bamboo            | China         | KY494736          |
|                  | LC7156         | Leaf of bamboo            | China         | KY494737          |
|                  | LC7275         | Leaf of bamboo            | China         | KY494749          |
| A. kogelbergense | CBS 113333*    | Dead culms of Restionaceae | South Africa  | KF144892          |
| A. longistromum  | MFLUCC 11-0481*| Decaying bamboo culms     | Thailand      | KU940141          |
|                  | MFLUCC 11-0479 | Decaying bamboo culms     | Thailand      | KU940142          |
| A. malaysianum   | CBS 102053*    | Macaranga hullettii stem colonised by ants | Malaysia      | KF144896          |
| A. marii         | CBS 497.90*    | Air                        | Spain         | AB220252          |
| A. mediterranei  | IMI 326875*    | Air                        | Spain         | AB220243          |
| A. mytilomorphpum| DAOM 214595*   | Dead blades of Andropogon sp. | India         | KY494685          |
| A. neougblobosa  | JHB006         | Dead culms of bamboo       | China         | KY356089          |
|                  | KUMCC 16-0203  | Dead culms of bamboo       | China         | KY356090          |
| A. obovatum      | LC4940*        | Lithocarpus sp.            | China         | KY494696          |
|                  | LC8177         | Lithocarpus sp.            | China         | KY494757          |
|                  | LC8178         | Lithocarpus sp.            | China         | KY494758          |
| A. ovatum        | CBS 115042*    | Arundinaria hindsii       | Hong Kong     | KF144903          |
| A. paraphaeospermum | MFLU 16-1974  | Dead clumps of Bambusa sp. | Thailand      | KX822128          |
|                  | CBS 114314     | Leaf of Hordeum vulgare    | Iran          | KF144904          |
| A. phaeospermum  | CBS 114315     | Leaf of Hordeum vulgare    | Iran          | KF144905          |
|                  | CBS 114317     | Leaf of Hordeum vulgare    | Iran          | KF144906          |
|                  | CBS 114318     | Leaf of Hordeum vulgare    | Iran          | KF144907          |
| A. phragmites    | CPC18900*      | Culms of Phragmites australis | Italy        | KF144909          |
| A. pseudoparenchymaticum | LC7234* | Leaf of bamboo | China | KY494743 |
|                  | LC8173         | Leaf of bamboo             | China         | KY494753          |
|                  | LC8174         | Leaf of bamboo             | China         | KY494754          |
| A. pseudosinense | CPC 21546*     | Leaf of bamboo             | The Netherlands | KF144910         |
| A. pseudospegazzinii | CBS 102052* | Macaranga hullettii stem colonised by ants | Malaysia | KF144911 |
| A. pterospermum  | CPC 20193*     | Leaf lesion of Machaerina sinclatrii | Australia    | KF144913          |
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| Species          | Strain numbers¹ | Hosts                  | Countries          | GenBank accessions |
|------------------|-----------------|------------------------|--------------------|--------------------|
|                  |                 |                        |                    | ITS    | TUB   | TEF   |
| *A. puccinioides*| CBS 549.86      | *Leaf of Lepidosperma gladiatum* | Germany            | AB220253 | AB220300 | –     |
|                  | CBS 337.61      | *Cissus sp.*            | The Netherlands     | KF144914 | –      | –     |
|                  | CPC 21602       | Rice                    | Thailand            | KF144915 | –      | –     |
|                  | MFLUCC 15-0203  | Dead bamboo culms       | Thailand            | KU940143 | –      | –     |
|                  | MFLUCC 11-0616  | Dead bamboo culms       | Thailand            | KU940144 | –      | –     |
|                  | NFCCI 2144*     | Soil                    | Svalbard            | JF326454 | –      | –     |
|                  | LC5449          | *Soil in karst cave*    | China               | KY494713 | KY705182 | KY705112 |
|                  | LC7115          | *Leaf of Cissus sp.*    | China               | KY494721 | KY705189 | KY705118 |
|                  | LC7117          | *Leaf of bamboo*        | China               | KY494722 | KY705190 | KY705119 |
|                  | LC7119          | *Leaf of bamboo*        | China               | KY494724 | KY705192 | KY705121 |
|                  | LC7120          | *Leaf of bamboo*        | China               | KY494725 | KY705193 | KY705122 |
|                  | LC7126          | *Leaf of bamboo*        | China               | KY494729 | KY705197 | KY705125 |
|                  | LC7129          | *Leaf of bamboo*        | China               | KY494731 | KY705199 | KY705127 |
|                  | LC7135          | *Leaf of bamboo*        | China               | KY494732 | KY705200 | KY705128 |
|                  | LC7139          | *Leaf of bamboo*        | China               | KY494733 | KY705201 | KY705129 |
|                  | LC7141          | *Leaf of bamboo*        | China               | KY494734 | KY705202 | KY705130 |
|                  | LC7142          | *Leaf of bamboo*        | China               | KY494735 | KY705203 | KY705131 |
|                  | LC7251          | *Leaf of bamboo*        | China               | KY494746 | KY705214 | KY705142 |
|                  | LC7254          | *Leaf of bamboo*        | China               | KY494748 | KY705216 | KY705144 |
|                  | LC8179          | *Brassica capestris*    | China               | KY494759 | KY705227 | KY705155 |
|                  | LC8180          | *Brassica capestris*    | China               | KY494760 | KY705228 | KY705156 |
| *A. raskravindrii*|                 |                        |                    |        |        |       |
|                  | CBS 212.30      | *Phragmites australis*  | United Kingdom      | KF144916 | KF145005 | KF145047 |
|                  | CBS 301.49      | Bamboo                 | Indonesia           | KF144917 | KF145006 | KF145048 |
| *A. sacchari*    | CBS 191.73      | Air                    | The Netherlands     | KF144920 | KF145009 | KF145051 |
|                  | CBS 334.86      | Dead culms of *Phragmites australis* | France | AB220257 | KF145010 | KF145052 |
|                  | CBS 463.83      | Dead culms of *Phragmites australis* | The Netherlands | KF144921 | KF145011 | KF145053 |
| *A. serenense*   | IMI 326869*     | Food, pharmaceutical excipients, atmosphere and home dust | Spain | AB220250 | AB220297 | – |
| *A. subglobosum* | MFLUCC 11-0397* | Dead bamboo culms       | Thailand            | KR069112 | –      | –     |
| *A. subroseum*   | LC7215          | *Leaf of bamboo*        | China               | KY494740 | KY705208 | KY705136 |
|                  | LC7291          | *Leaf of bamboo*        | China               | KY494751 | KY705219 | KY705147 |
|                  | LC7292*         | *Leaf of bamboo*        | China               | KY494752 | KY705220 | KY705148 |
|                  | MFLUCC 15-0202* | Dead bamboo culms       | Thailand            | KU940145 | –      | –     |
| *A. thailandicum*| LC5630          | *Rotten wood*           | China               | KY494714 | KY806200 | KY705113 |

¹ Strain numbers and GenBank accessions are provided for each species. The table includes the species name, strain numbers, host, country, and GenBank accessions for ITS, TUB, and TEF genes.
**Species** | **Strain numbers** | **Hosts** | **Countries** | **GenBank accessions** |
--- | --- | --- | --- | --- |
A. xenocordella | CBS 478.86* | Soil from roadway | Zimbabwe | KF144925 KF145013 KF145055 |
 | LC3486 | Camellia sinensis | China | KY494687 KY705158 KY705086 |
A. yunnanum | MFLUCC 15-0002* | Decaying bamboo culms | China | KU940147 – – |
N. gorlenkoana | CBS 480.73 | Vitis vinifera | Kazakhstan | KX986048 KY019456 KY019420 |

* = type strains, strains and sequences generated in this study are shown in **bold**.

1 CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection; CPC: Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; IFO: Institute for Fermentation, Osaka; LC: Working collection of Lei Cai, housed at CAS, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MCC: Microbial Culture Collection of India; NFCCI: National Fungal Culture Collection of India.

## Taxonomy

**Arthrinium bambusae** M. Wang & L. Cai, sp. nov.

MycoBank: MB824906

Figure 3

**Type.** CHINA, Guangdong Province, on bamboo leaves, 10 Jul. 2016, D.W. Xiao, (holotype: HMAS 247187; culture ex-type: CGMCC 3.18335 = LC7106).

**Etymology.** Named after the host of the holotype.

**Description.** Hyphae hyaline, branched, septate, 1.5–5.0 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliiform to ampulliform, or lageniform, 4.0–12.0 × 3.0–7.0 µm (x̄ = 6.6 ± 1.8 × 4.8 ± 0.9, n = 30). Conidia olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 µm (x̄ = 13.2 ± 0.8 × 11.4 ± 1.2, n = 50).

**Culture characteristics.** On PDA, colonies flat, spreading, margin circular, with abundant aerial mycelia, surface and reverse white to grey. On MEA, colonies flat, spreading, surface and reverse brown to black.

**Additional specimens examined.** CHINA, Jiangxi Province, on bamboo leaves, 10 Jul. 2016, Q. Xiong, living culture LC7246; Guangdong Province, on bamboo leaves, 10 Jul. 2016, D.W. Xiao, living culture LC7107; ibid. living culture LC7113; ibid. living culture LC7124; ibid. living culture LC7125; ibid. living culture LC7128.

**Notes.** Seven strains representing *A. bambusae* clustered in a well-supported clade closely related to *A. subroseum* (98% sequence similarity in ITS; 92% in TUB2; 96% in TEF1). *Arthrinium bambusae* differs from *A. subroseum* in the morphology of conidiophore (reduced to conidiogenous cells in *A. bambusae* vs. erect or ascending, clustered in groups in *A. subroseum*). Moreover, *A. bambusae* does not produce pigment on the PDA.
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Figure 3. *Arthrinium bambusae* (from ex-holotype strain CGMCC 3.18335) A–B 7 d old cultures on PDA C Colony on MEA producing conidia masses D–F Conidiogenous cells giving rise to conidia G Conidia. Scale bars = 10 µm.

*Arthrinium camelliae-sinensis* M. Wang, F. Liu & L. Cai, sp. nov.
MycoBank: MB824907
Figure 4

**Type.** CHINA, Jiangxi Province, on *Camellia sinensis*, 22 Apr. 2013, Q. Chen, (holotype: HMAS 247186; culture ex-type: CGMCC 3.18333 = LC5007).

**Etymology.** Named with the host plant of the type.

**Description.** Hyphae hyaline, branched, septate, 2.0–4.5 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters, hyaline to pale brown, smooth, doliiform to ampulliform, 4.0–9.5 × 3.0–6.0 µm (\( \bar{x} = 6.1 \pm 1.4 \times 4.4 \pm 0.9, n = 30 \)). Conidia brown to dark brown, smooth, globose to subglobose, 9.0–13.5 × 7.0–12.0 µm (\( \bar{x} = 11.1 \pm 0.9 \times 10.1 \pm 1.0, n = 50 \)).

**Culture characteristics.** On PDA, colonies flat, margin circular, initially white, becoming greyish on surface, reaching 9 cm in 7 days at 25 °C. On MEA, with sparse aerial mycelia, surface dirty white, reverse pale luteous.

**Other specimens.** CHINA, Hubei Province, on *Brassica campestris*, 31 Mar. 2016, Y.Z. Zhao, living culture LC8181 = LF1498.

**Notes.** Two strains representing *A. camelliae-sinensis* clustered in a well-supported clade and appeared closely related to *A. jiangxiense* (97% sequence similarity in ITS; 94% in TUB2; 94% in TEF1) and *A. obovatum* (98% sequence similarity in ITS; 95% in TUB2; 93% in TEF1). While *A. camelliae-sinensis* is distinct from *A. jiangxiense* in its larger conidia (globose or subglobose, 9.0–13.5 × 7.0–12.0 µm in *A. camelliae-sin-
Figure 4. *Arthrinium camelliae-sinensis* (from ex-holotype strain CGMCC 3.18333) A–B 7 d old cultures on PDA C Colony on MEA with bamboo leaves producing conidia masses D–F Conidiogenous cells giving rise to conidia G Conidia. Scale bars = 10 µm.

*ensis* vs. surface view 7.5–10.0 µm diam, side view 4.5–7.0 µm diam in *A. jiangxiense*) and conidiogenous cell arrangement (aggregated irregularly on hyphae vs. scattered on hyphae in *A. jiangxiense*) and distinct from *A. obovatum* in the lack of obovoid conidia (see the note under *A. obovatum*).

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*Arthrinium dichotomanthi* M. Wang & L. Cai, sp. nov.
MycoBank: MB824908
Figure 5

**Type.** CHINA, Chongqing, on *Dichotomanthus tristaniae-carpa*, 20 Dec. 2012, L. Cai, (holotype: HMAS 247185; culture ex-type: CGMCC 3.18332 = LC4950).

**Etymology.** Named after the host from which it was isolated.

**Description.** Hyphae hyaline, branched, septate, 1.5–5.0 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliiform to clavate or lageniform, 5.5–11.0 × 3.0–5.0 µm (x̄ = 7.9 ± 1.4 × 4.0 ± 0.5, n = 30). Conidia brown to dark brown, smooth to finely roughened, globose, subglobose to lenticular, with a longitudinal germ slit, 9.0–15.0 × 6.0–12.0 µm (x̄ = 12.0 ± 1.4 × 8.5 ± 1.1, n = 50).

**Culture characteristics.** On PDA, colonies umbonate, margin irregular, with sparse aerial mycelia. Colonies creamy-white to greyish without patches reverse, reaching 9 cm in 7 days at 25 °C. On MEA, colonies flat, spreading, surface and reverse pale luteous.
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**Notes.** Three strains representing *A. dichotomanthi* formed a distinct clade closely related to *A. phaeospermum* (Corda) M.B. Ellis (99% sequence similarity in ITS; 96% in TUB2; 96% in TEF1), *A. serenense* Larrondo & Calvo (99% sequence similarity in ITS; 95% in TUB2) and *A. saccharicola* F. Stevens (99% sequence similarity in ITS; 95% in TUB2; 97% in TEF1). *Arthrinium dichotomanthi* differs from *A. phaeospermum* and *A. saccharicola* in its larger conidia (globose or subglobose, 9.0–15.0 × 6.0–12.0 µm in *A. dichotomanthi* vs. surface view (9–)10(–12) µm diam, side view 6–7 µm diam in *A. phaeospermum*, surface view (7–)8–9(–10) µm diam, side view (4–)5(–6) µm diam in *A. saccharicola*) and from *A. serenense* by the absence of odour on the MEA colony (Larrondo 1990).

*Arthrinium guizhouense* M. Wang & L. Cai, sp. nov.
MycoBank: MB824909

**Type.** CHINA, Guizhou Province, from the air in karst cave, 23 Jul. 2014, Z.F. Zhang, (holotype: HMAS 247188; culture ex-type: CGMCC 3.18334 = LC5322).

**Etymology.** Named after the province where type was collected, Guizhou province.

**Description.** Hyphae hyaline, branched, septate, 1.5–5.0 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters on hy-
phae, pale brown, smooth, subglobose, ampulliform or doliiform, 3.5–8.0 × 3.0 – 4.5 µm ($\bar{x} = 5.1 \pm 1.08 \times 3.7 \pm 0.49, n = 30$). Conidia dark brown to black, smooth to finely roughened, globose or subglobose, occasionally elongated to ellipsoidal, with a longitudinal, hyaline, thin, germ slit, 5.0–7.5 × 4.0–7.0 µm ($\bar{x} = 6.1 \pm 0.5 \times 5.5 \pm 0.6, n = 50$).

**Culture characteristics.** On PDA, colonies flat, woolly, margin circular, with moderate aerial mycelia, surface initially white, becoming greyish and reverse with black patches, reaching 9 cm in 9 days at 25 °C. On MEA, surface dirty white with patches of olivaceous-grey and reverse greyish.

**Other specimens examined.** CHINA, Guizhou Province, from the air in karst cave, 23 Jul. 2014, Z.F. Zhang, living culture LC5318.

**Notes.** *Arthrinium guizhouense* is closely related to *A. sacchari* (Speg.) M.B. Ellis (99% sequence similarity in ITS; 99% in TUB2; 94% in TEF1). Morphologically, *A. guizhouense* and *A. sacchari* are very similar in conidial size, but *A. guizhouense* produces relatively shorter conidiogenous cells (3.5–8.0 µm in *A. guizhouense* vs. 5–12 µm in *A. sacchari*).

*Arthrinium jiangxiense* M. Wang & L. Cai, sp. nov.
MycoBank: MB824910
Figure 7

**Type.** CHINA, Jiangxi Province, on *Maesa* sp., 05 Sept. 2013, Y.H. Gao, (holotype: HMAS 247183; culture ex-type: CGMCC3.18381 = LC4577).
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**Figure 7.** *Arthrinium jiangxiense* (from ex-holotype strain CGMCC 3.18381)  
(A–B) 5 d old cultures on PDA  
(C) Colony on MEA producing conidia masses  
(D–F) Conidiogenous cells giving rise to conidia  
(G) Elongated conidia  
(H) Conidia. Scale bars = 10 µm.

**Etymology.** Named after the province where the most strains of this species were collected, Jiangxi.

**Description.** Hyphae hyaline, branched, septate, 1.5–5.0 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, scattered or aggregated in clusters on hyphae, hyaline to pale brown, smooth, ampulliform, 6.0–15.0 × 2.5–5.0 µm (x = 9.7 ± 2.6 × 3.7 ± 0.6, n = 30), apical neck 2.5–6.0 µm long, basal part 3.0–9.0 µm long. Conidia brown, smooth to finely roughened, granular, globose to ellipsoid in surface view, 7.5–10.0 µm diam (x = 8.7 ± 0.6, n = 50), lenticular in side view, with longitudinal, pale germ slit, 4.5–7.0 µm diam (x = 5.8 ± 0.6, n = 50). Sterile cells forming on solitary loci on hyphae, brown, finely roughened, subcylindrical to clavate.

**Culture characteristics.** On PDA, colonies flat, woolly, margin circular, with sparse aerial mycelia, initially white, becoming greyish due to sporulation, reaching 9 cm in 10 days at 25 °C, on MEA, sienna with patches of luteous, reverse luteous to sienna.

**Other specimens examined.** CHINA, Hunan Province, on bamboo, 22 Sept. 2010, L. Cai, living culture LC2831; Jiangxi Province, on *Phyllostachys* sp., 05 Sept. 2013, Y.H. Gao, living culture LC4494; on *Phyllostachys* sp., 22 Apr. 2013, Q. Chen, living culture LC4993; ibid. living culture LC4997; ibid. living culture LC5001; ibid. living culture LC5004; on *Imperata cylindrical*, 22 Apr. 2013, Q. Chen, living culture LC5015; on *Maesa* sp., 05 Sept. 2013, Y.H. Gao, living culture LC4541; on *Machilus* sp., 05 Sept. 2013, Y.H. Gao, living culture LC4547; on *Camellia sinensis*, 05 Sept. 2013, Y.H. Gao, living culture LC4578; on bamboo, 01 Jul. 2016, J.E. Huang, living
culture LC7104; ibid. living culture LC7154; ibid. living culture LC7156; ibid. living culture LC7275.

**Notes.** Two strains representing *Arthrinium jiangxiense* clustered in a well-supported clade and appeared closely related to *A. camelliae-sinensis* (97% sequence similarity in ITS; 94% in TUB2; 94% in TEF1). While *A. jiangxiensis* is distinct from *A. camelliae-sinensis* in its smaller conidia (surface view 7.5–10.0 µm diam, side view 4.5–7.0 µm diam in *A. jiangxiensis* vs. globose or subglobose, 9.0–13.5 × 7.0–12.0 µm in *A. camelliae-sinensis*) and conidiogenous cell arrangements (conidiogenous cells scattered on hyphae vs. aggregated irregularly on hyphae in *A. jiangxiense*).

**Arthrinium obovatum** M. Wang & L. Cai, sp. nov.
MycoBank: MB824911

*Figure 8*

**Type.** CHINA, Chongqing, on *Lithocarpus* sp., 20 Dec. 2012, L. Cai, (holotype: HMAS 247184; culture ex-type: CGMCC 3.18331 = LC4940).

**Etymology.** Referring to the production of the large obovoid conidia.

**Description.** Hyphae hyaline to pale brown, branched, septate, 1.5–5.0 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters on hyphae, pale brown, smooth, subcylindrical or clavate, 5.5–13.5 × 2.5–5.0 µm (x̄ = 8.7 ± 2.4 × 3.6 ± 0.6, n = 30). Conidia dark brown,
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roughened, globose to subglobose, 11.0–16.5 µm ($\bar{x} = 13.8 \pm 1.5$, n = 50) in diam.; obovoid, 16.0–31.0 × 9.0–16.0 µm ($\bar{x} = 23.0 \pm 2.7 \times 12.7 \pm 1.4$, n = 50), occasionally elongated to ellipsoidal.

**Culture characteristics.** On PDA, colonies flat, spreading, margin circular, initially white, becoming olivaceous-grey on surface, reverse smoke-grey with patches of olivaceous grey, reaching 9 cm in 7 days at 25 °C. On MEA, surface olivaceous grey in the central and luteous around, reverse with patches of olivaceous grey.

**Other specimens examined.** CHINA, Chongqing, on *Lithocarpus* sp., 20 Dec. 2012, L. Cai, living culture LC8177; ibid. living culture LC8178.

**Notes.** *Arthrinium obovatum* is the only species that produces obovoid conidia (Figure. 8F) in this genus, a character distinctly different from other species (Ellis 1965, 1976, Gjaerum 1967, Pollack and Benjamin 1969, Hudson et al. 1976, Calvo and Guarro 1980, Khan and Sullia 1980, Samuels et al. 1981, von Arx 1981, Koskela 1983, Kirk 1986, Larrondo and Calvo 1990, 1992, Müller 1992, Bhat and Kendrick 1993, Hyde et al. 1998, Jones et al. 2009, Singh et al. 2012, Crous et al. 2013, 2015, Sharma et al. 2014, Senanayake et al. 2015, Senanayake et al. 2015, Hyde et al. 2016, Dai et al. 2016a, b).

*Arthrinium pseudoparenchymaticum* M. Wang & L. Cai, sp. nov.

MycoBank: MB824912

Figure 9

**Type.** CHINA, Guangdong Province, on bamboo, Jul. 2016, D.W. Xiao, (holotype: HMAS 247189; culture ex-type: CGMCC 3.18336 = LC7234).

**Etymology.** Referring to the pseudoparenchymatous hyphae.

**Description.** Hyphae hyaline to pale brown, branched, septate, 1.5–5.0 µm diam., pseudoparenchymatous. Conidiophores aggregated in hyaline to light brown sporodochia, smooth, usually unbranched, up to 40 µm long, 3–6 µm width. Conidiogenous cells hyaline to pale yellow, smooth to finely roughened, subcylindrical to doliiform, 8.0–18.5 × 3.0–8.5µm ($\bar{x} = 13.7 \pm 3.2 \times 5.4 \pm 1.2$, n = 30). Conidia pale to dark brown, smooth, finely guttulate, globose to subglobose, 13.5–27.0 × 12.0–23.5 µm ($\bar{x} = 20.2 \pm 2.5 \times 17.1 \pm 2.4$, n = 50). Sometimes lobed or dentate, polygonal or irregular in surface view.

**Culture characteristics.** On PDA, colonies flat, spreading, margin circular, with moderate aerial mycelia, initially white, becoming grey on surface, reverse smoke-grey without patches, reaching 9 cm in 8 days at 25 °C. On MEA, surface pale luteous to grey with abundant mycelia, reverse greyish without patches.

**Other specimens examined.** CHINA, Guangdong Province, on bamboo, Jul. 2016, D.W. Xiao, living culture LC8173; ibid. living culture LC8174.

**Notes.** *Arthrinium pseudoparenchymaticum* is closely related to *A. hyphopodii* (94% sequence similarity in ITS), but differs in its much larger conidia (13.5–27.0 × 12.0–23.5 µm vs. 5–10 × 4–8 µm), the absence of hyphopodia and the presence of dentate conidia.
Figure 9. *Arthrinium pseudoparenchymaticum* (from ex-holotype strain CGMCC 3.18336) A–B 8 d old cultures on PDA C Colony on MEA producing conidia masses D–E Conidiogenous cells giving rise to conidia F–G Dentate conidia H Globose conidia. Scale bars = 10 μm.

*Arthrinium subroseum* M. Wang & L. Cai, sp. nov.  
MycoBank: MB824913  
Figure 10

**Type.** CHINA, Jiangxi Province, on bamboo, 1 Jul. 2016, J.E. Huang, (holotype: HMAS 247190; culture ex-type: CGMCC3.18337 = LC7292).

**Etymology.** Named after the colour of colony on PDA, pinkish.

**Description.** Hyphae hyaline to pale brown, branched, septate, 1.5–6.0 μm diam. Conidiophores hyaline to pale brown, smooth, erect or ascending, simple, flexuous, subcylindrical, clustered in groups. Conidiophores aggregated in brown sporodochia, smooth, hyaline to brown, up to 20 μm long, 2–4.5 μm width. Conidiogenous cells pale brown, smooth, doliiform to subcylindrical, 3.0–6.5 × 2.0–5.0 μm (μ = 4.7 ± 1.2 × 3.7 ± 0.9, n = 30). Conidia pale brown to dark brown, smooth, globose to subglobose or ellipsoidal, 12.0–17.5 × 9.0–16.0 μm (μ = 14.9 ± 1.4 × 11.8 ± 1.8, n = 50).

**Culture characteristics.** On PDA, colonies flat, spreading, margin circular, with moderate aerial mycelia, initially white, becoming light pink on surface, reverse peach-puff without patches, reaching 10 cm in 8 days at 25 °C. On MEA, surface blackish-green with abundant mycelia, reverse with patches of greyish.

**Other specimens.** CHINA, Jiangxi Province, on bamboo, 1 Jul. 2016, J.E. Huang, living culture LC7215; ibid. living culture LC7291.

**Notes.** Three strains representing *A. subroseum* clustered in a well-supported clade, closely related to *A. garethjonesii* (94% sequence similarity in ITS) and *A. bambusae* (98% sequence similarity in ITS; 92% in TUB2; 96% in TEF1). However, *A. subro-
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*Arthrinium subroseum* differs from *A. bambusae* in the morphology of conidiophores (erect or ascending, clustered in groups in *A. subroseum* vs. reduced to conidiogenous cells in *A. bambusae*). *Arthrinium subroseum* is not morphologically comparable to *A. garethjonesii*, whose asexual morph is undetermined (Dai et al. 2016b).

**Discussion**

*Arthrinium*, *Cordella* and *Pteroconium* share similar morphological characters, e.g. basauxic conidiophores with terminal and intercalary polyblastic conidiogenous cells and brown, unicellular conidia with a pallid germ slit (Ellis 1971, Hyde et al. 1998). Crous et al. (2013) reduced both *Cordella* and *Pteroconium* as generic synonyms of *Arthrinium* based on molecular phylogenetic data and regarded traditionally applied morphological characters in distinguishing these genera as phylogenetically insignificant. This study added eight novel species and our data are in good accordance with that of Crous et al. (2013). For example, *A. pseudoparenchymaticum* is sporodochial and pseudoparenchymatous, which would be classified as *Pteroconium* in the traditional taxonomy. However, the multi-locus (ITS, TEF1 & TUB2) tree (Figure. 1) shows that *A. pseudoparenchymaticum* is phylogenetically distant from *A. pterospermum* (syn. *P. pterospermum*, the type of “*Pteroconium*”).

Currently there are 70 recognised species in *Arthrinium* (Index Fungorum), occurring on a wide variety of both living and decaying plant materials. It is noteworthy that *Arthrinium* species showed distinct preference for growing on two graminaceous families, Poaceae

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**Figure 10.** *Arthrinium subroseum* (from ex-holotype strain CGMCC3.18337) A–B 10 d old cultures on PDA C Colony on MEA producing conidia masses D–E Conidiogenous cells giving rise to conidia F–G Conidia. Scale bars = 10 µm.
and Cyperaceae, amongst which, *Bambusa* (Poaceae) and *Carex* (Cyperaceae) are two of the most common host genera for *Arthrinium* species. For example, seven species have been recorded from *Carex* spp., i.e. *A. austriacum* Petr. (1959), *A. cariciola* Kunze (1817), *A. globosum* Koskela (1983), *A. kamtschaticum* Tranzschel & Woron (1914), *A. morthieri* Fückel (1870), *A. muelleri* Ellis (1976) and *A. naviculare* Rostr. (1886). Bamboo has been widely known as a favourable host for *Arthrinium*, e.g. *A. hyphopodii*, *A. longistromum*, *A. subglobosum*, *A. thailandicum* and *A. yunnanum* (Senanayake et al. 2015, Dai et al. 2016). In this study, three new species (*A. bambusae*, *A. subroseum* and *A. pseudoparenchymaticum*) were also isolated from bamboo. In addition, three species (*A. arundinis*, *A. guizhouense*, and *A. rasikravindrii*) were isolated from air and soil from karst caves, where have been shown to encompass a high fungal diversity (Jiang et al. 2017, Zhang et al. 2017).

In addition to the *Arthrinium* species from China, we also tried to resolve the phylogenetic status of *Arthrinium mytilomorphum* Bhat & W.B. Kendr. (Bhat and Kendrick 1993) in the current study. DNA extraction from the type specimen of *A. mytilomorphum* (DAOM 214595) was prohibited but DAOM provided a DNA sample. Unfortunately, we only managed to obtain an ITS sequence from this DNA sample, while the amplifications of all other protein coding genes were unsuccessful. The ITS phylogenetic tree (not shown here) shows that *A. mytilomorphum* is closely related to *A. subroseum* (99 % sequence similarity in ITS), while the morphology of these two species are very different from each other. Conidia of *A. mytilomorphum* are dark brown, fusiform or navicular, measuring 20–30 × 6–8.5 µm, slightly bowed down and asymmetric (Figure 11), while those of *A. subroseum* are pale brown to dark brown, globose or subglobose, measuring 12–17.5 × 9–16 µm.

![Figure 11](image_url)

**Figure 11.** *Arthrinium mytilomorphum* (from holotype DAOM 214595) **A–B** Overview of the type specimen **C–F** Conidiogenous cells giving rise to conidia **G** Conidia. Scale bars = 10 µm.
Teleomorph-typified genus *Apiospora* was treated as a synonym of anamorph-typified genus *Arthrinium* on the basis that *Arthrinium* is older and more commonly used in literature (Crous et al. 2013). However, only three of the 58 recorded *Apiospora* species have been properly linked to their known *Arthrinium* counterparts, i.e. *Arthrinium hysterinum* (syn. *Ap. bambusae*) (Sivanesan 1983, Kirk 1986); *Arthrinium arundinis* (syn. *Ap. montagnei*) (Hyde 1998); *Arthrinium sinense* (syn. *Ap. sinensis*) (Réblová et al. 2016). In addition, molecular data of only four *Apiospora* species (*Ap. bambusae*, *Ap. montagnei*, *Ap. setosa* and *Ap. sinensis*) are available, in which only *A. bambusae* and *A. sinensis* have type-derived sequences. A comprehensive taxonomic revision of this taxonomic group awaits fresh collection and epitypification of many *Apiospora* species and, based on which, phylogenetic links with *Arthrinium* species could be established.

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**References**

von Arx JA (1981) The genera of fungi sporulating in pure culture (3rd edn). Cramer Vaduz, 424 pp.

Bhat DJ, Kendrick WB (1993) Twenty-five new conidial fungi from the Western Ghats and the Andaman Islands (India). Mycotaxon 49: 19–90.

Cai L, Hyde KD, Taylor PW, Weir B, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 204.

Calvo A, Guarro J (1980) *Arthrinium aureum* sp. nov. from Spain. Transactions of the British Mycological Society 75: 156–157. https://doi.org/10.1016/S0007-1536(80)80208-7

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556. https://doi.org/10.2307/3761358

Chen K, Wu XQ, Huang MX, Han YY (2014) First report of brown culm streak of *Phyllostachys praecox* caused by *Arthrinium arundinis* in Nanjing, China. Plant Disease 98: 1274. https://doi.org/10.1094/PDIS-02-14-0165-PDN

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.
Crous PW, Groenewald JZ. (2013) A phylogenetic re-evaluation of *Arthrinium*. IMA fungus 4: 133–154. https://doi.org/10.5598/imafungus.2013.04.01.13

Crous PW, Wingfield MJ, Le Roux JJ, et al. (2015) Fungal planet description sheets: 371–399. Persoonia 35: 264–327. https://doi.org/10.3767/003158515X690269

Dai DQ, Jiang HB, Tang LZ, Bhat DJ. (2016b) Two new species of *Arthrinium* (*Apiosporaceae, Xylariales*) associated with bamboo from Yunnan, China. Mycosphere 7: 1332–1345. https://doi.org/10.5943/mycosphere/7/9/7

Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote E. (2017a) Bambusicolous fungi. Fungal Diversity 82: 1–105. https://doi.org/10.1007/s13225-016-0367-8

Ellis MB. (1965) Dematiaceous Hyphomycetes. VI. Mycological Papers 103: 1–46.

Ellis MB. (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, 608 pp.

Ellis MB. (1976) More Dematiaceous Hyphomycetes. CAB International Mycological Institute, Kew, 507 pp.

Farr DF, Rossman AY. (2017) Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/

Gjaerum HB. (1967) *Arthrinium morthieri*, *A. fuckelii* n. sp., and *A. ushuvaiense*. Nytt magasin for Botanikk 14: 1–6.

Glass NL, Donaldson GC. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Guo LD, Hyde KD, Liew ECY. (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytologist 147: 617–630. https://doi.org/10.1046/j.1469-8137.2000.00716.x

de Hoog GS, Guarro J, Gene J, et al. (2000) Atlas of Clinical Fungi (2nd edn). Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, 1–1160.

Hong JH, Jang S, Heo YM, Min M, Lee H, Lee YM, Lee H, Kim JJ. (2015) Investigation of marine-derived fungal diversity and their exploitable biological activities. Marine Drugs 13: 4137–4155. https://doi.org/10.3390/md13074137

Hudson HJ, McKenzie EHC, Tommerup IC. (1976) Conidial states of *Apiospora* Sacc. Transactions of the British Mycological Society 66: 359–362. https://doi.org/10.1016/S0007-1536(76)80075-7

Hughes SJ. (1953) Conidiophores, conidia, and classification. Canadian Journal of Botany 31: 577–659. https://doi.org/10.1139/b53-046

Hyde KD, Fröhlich J, Taylor JE. (1998) Fungi from palms. XXXVI. Reflections on unitunicate ascomycetes with apiospores. Sydowia 50: 21–80.

Hyde KD, Hongsanan S, Jeewon R, et al. (2016) Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270. https://doi.org/10.1007/s13225-016-0373-x

Jiang JR, Cai L, Liu F. (2017) Oligotrophic fungi from a carbonate cave, with three new species of *Cephalotrichum*. Mycology 8(3): 164–177. https://doi.org/10.1080/21501203.2017.1366370
Eight new *Arthrinium* species from China

Jones EBG, Sakayaroj J, Suetrong S, et al. (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Diversity 35: 1–187.

Khan KR, Sullia SB (1980) *Arthrinium phaeospermum* var. *indicum* var. nov., a new market pathogen of cowpea, garden pea and french bean. Acta Botanica Indica 8: 103–104.

Kirk PM (1986) New or interesting microfungi. XV. Miscellaneous hyphomycetes from the British Isles. Transactions of the British Mycological Society 86: 409–428. https://doi.org/10.1016/S0007-1536(86)80185-1

Koskela P (1983) *Arthrinium glahosum*, a new hyphomycetous species. Karstenia 23: 13–14. https://doi.org/10.29203/ka.1983.218

Larrondo JV, Calvo MaA (1990) Two new species of *Arthrinium* from Spain. Mycologia 82: 396–398. https://doi.org/10.2307/3759915

Larrondo JV, Calvo MaA (1992) New contributions to the study of the genus *Arthrinium*. Mycologia 84: 475–478. https://doi.org/10.2307/3760203

Li BJ, Liu PQ, Jiang Y, Weng QY, Chen QH (2016) First report of culm rot caused by *Arthrinium phaeospermum* on *Phyllostachys viridis* in China. Plant Disease 100: 1013. https://doi.org/10.1094/PDIS-08-15-0901-PDN

Minter DW (1985) A re-appraisal of the relationships between *Arthrinium* and other hyphomycetes. Plant Sciences 94: 281–308.

Müller E (1992) A new parasitic species of *Apiospora*. Boletin de la Sociedad Argentina de Botanica, La Plata 28: 201–203.

O’Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116. https://doi.org/10.1006/mpev.1996.0376

O’Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95: 2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Pollack FG, Benjamin CR (1969) *Arthrinium japonicum* and notes on *Arthrinium kamtschaticum*. Mycologia 61: 187–190. https://doi.org/10.2307/3757360

Posada D (2008) jModelTest: phylogenetic model averaging. Molecular biology and evolution 25: 1253–1256. https://doi.org/10.1093/molbev/msn083

Rai MK (1989) Mycosis in man due to *Arthrinium phaeospermum* var. *indicum*. First case report: mykose durch *Arthrinium phaeospermum* var. *indicum* beim Menschen. Erstbericht. Mycoses 32: 472–475. https://doi.org/10.1111/j.1439-0507.1989.tb02285.x

Rayner RW (1970) A Mycological Colour Chart. Commonwealth Mycological Institute, Kew, 34 pp.

Réblová M, Miller AN, et al. (2016) Recommendations for competing sexual-asexually typified generic names in Sordariomycetes (except Diaporthales, Hypocreales, and Magnaporthales). IMA Fungus 7: 131–153. https://doi.org/10.5598/imafungus.2016.07.01.08

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029
Samuels GJ, McKenzie EHC, Buchanan DE (1981) Ascomycetes of New Zealand 3. Two new species of *Apiospora* and their *Arthrinium anamorphs* on bamboo. New Zealand Journal of Botany 19: 137–149. https://doi.org/10.1080/0028825X.1981.10425113

Seifert K, Morgan-Jones G, Gams W, Kendrick B (2011) The genera of hyphomycetes. CBS Biodiversity Series 9, Utrecht, the Netherlands, 1–997.

Senanayake IC, Maharachchikumbura SS, Hyde KD, Bhat JD, Jones EG, McKenzie EH, Dai DQ, Daranagama DA, Dayarathne MC, Goonasekara ID, Konta S (2015) Towards unraveling relationships in *Xylariomycetidae* (Sordariomycetes). Fungal Diversity 73: 73–144. https://doi.org/10.1007/s13225-015-0340-y

Sharma R, Kulkarni G, Sonawane MS, Shouche YS (2014) A new endophytic species of *Arthrinium* (Apiosporaceae) from *Jatropha podagrica*. Mycoscience 55: 118–123. https://doi.org/10.1016/j.myc.2013.06.004

Shrestha P, Ibáñez AB, Bauer S, Glassman SL, Szaro TM, Bruns TD, Taylor JW (2015) Fungi isolated from *Miscanthus* and sugarcane: biomass conversion, fungal enzymes, and hydrolysis of plant cell wall polymers. Biotechnology for Biofuels 8: 1. https://doi.org/10.1186/s13068-015-0221-3

Singh SM, Yadav LS, Singh PN, Hepat R, Sharma R, Singh SK (2012) *Arthrinium rasikravindrii* sp. nov. from Svalbard, Norway. Mycotaxon 122: 449–460. https://doi.org/10.5248/122.449

Sivanesan A (1983) Studies on ascomycetes. Transactions of the British Mycological Society 81: 313–332. https://doi.org/10.1016/S0007-1536(83)80084-9

Stamatakis A, Alachiotis N (2010) Time and memory efficient likelihood-based tree searches on phylogenomic alignments with missing data. Bioinformatics 26: 132–139. https://doi.org/10.1093/bioinformatics/btq205

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee SJ, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1): 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang ZF, Liu F, Zhou X, Liu XZ, Liu SJ, Cai L (2017) Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. Persoonia 39(1): 1–31. https://doi.org/10.3767/persoonia.2017.39.01

Zhao YM, Deng CR, Chen X (1990) *Arthrinium phaeospermum* causing dermatomycosis, a new record of China. Acta Mycologica Sinica 9: 232–235.

Zhaxybayeva O, Gogarten JP (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3: 4. https://doi.org/10.1186/1471-2164-3-4
Architrypezethelium murisporum (Ascomycota, Trypetheliaceae), a remarkable new lichen species from Thailand challenging ascospore septation as an indicator of phylogenetic relationships

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Abstract
Architrypezethelium murisporum Luangsuphabool, Lumbsch & Sangvichien is described for a crustose lichen occurring in dry evergreen forest in Thailand. It is characterised by a green to yellow-green corticated thallus, perithecia fused in black pseudostromata with white rim surrounding the ostiole and small, hyaline and muriform ascospores. Currently, all species in the genus Architrypezethelium have transversely septate ascospores, hence the discovery of this new species indicates that ascospore septation is variable within the genus, similar to numerous other groups of lichen-forming ascomycetes. Phylogenetic analyses of two loci (mtSSU and nuLSU) supported the position of the new species within Architrypezethelium. This is the first report of the genus in Southeast Asia.

Keywords
Lichens, taxonomy, phylogeny, tropical diversity, Southeast Asia, Trypetheliaceae

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Introduction

The genus *Architrypethelium* Aptroot (Ascomycota, Dothideomycetes, Trypetheli-ales) includes crustose lichens with perithecioid ascomata growing on tree bark in the tropics (Aptroot 1991, Aptroot et al. 2008, Aptroot and Lücking 2016). The genus accommodates species with a corticate thallus, solitary or aggregate ascomata with apical or eccentric ostioles, a clear or inspersed hymenium and hyaline or brown, 3–5 septate, transversely septate ascospores (Aptroot et al. 2008, Aptroot and Lücking 2016). Although, *Architrypethelium* is morphologically similar to *Astrothelium* species, the two genera have been shown to be distantly related. The latter genus fell into two clades (Lücking et al. 2016) with one being a sister group to *Architrypethelium*. Phenotypically *Architrypethelium* differs from *Astrothelium* in having predominantly large ascospore without diamond-shaped lumina when mature (Aptroot 1991, Aptroot et al. 2008, Nelsen et al. 2014, Aptroot and Lücking 2016, Lücking et al. 2016b). Another genus with muriform ascospores is *Aptrootia*, which also shares an astrothelioid stage in the young ascospores (Lücking et al. 2016) and the genus formed a sister-group to a clade including *Architrypethelium* and *Astrothelium* p.p.t. further calling the generic delimitation in the family in question. Morphologically, *Aptrootia* differs from *Astrothelium* in having dark brown ascospores with a hard outer shell (Lücking et al. 2016). While most genera in Trypetheliaceae, such as *Astrothelium* s.str., *Bathelium*, *Polymeridium* and *Viridothelium* include species with various ascospore types (Hyde et al. 2013, Nelsen et al. 2014, Aptroot and Lücking 2016, Lücking et al. 2016b), the species of *Architrypethelium* shared a similar ascospore morphology (Nelsen et al. 2014, Lücking et al. 2016b).

Previously, three species were accepted in *Architrypethelium* (Aptroot 1991, Aptroot et al. 2008). Recently, the numbers of species increased with the description of two new species and two combinations into the genus (Aptroot and Lücking 2016, Flakus et al. 2016, Lücking et al. 2016a). Currently, seven species are accepted in *Architrypethelium*, viz. *Architrypethelium columbianum* (Nyl.) Aptroot & Lücking, *Architrypethelium grande* (Kremp.) Aptroot & Lücking, *Architrypethelium hyalinum* Aptroot, *Architrypethelium lauropaluanum* Lücking, Nelsen & Marcelli, *Architrypethelium nitens* (Fée) Aptroot, *Architrypethelium penuriixanthum* Flakus & Aptroot, and *Architrypethe- lium uberinum* (Fée) Aptroot (Aptroot 1991, Aptroot et al. 2008, Aptroot and Lücking 2016, Flakus et al. 2016, Lücking et al. 2016a). All species are known from the Neotropics, except *A. uberinum*, which is also known from Oceania (Aptroot and Lücking 2016, Flakus et al. 2016, Lücking et al. 2016a), suggesting a pantropical distribution (Aptroot and Lücking 2016). Until now, the genus *Architrypethelium* has not been known from southeast Asia. Here we describe a new species from Thailand, which has a rich pyrenocarpous lichen flora (Buaruang et al. 2017), with muriform ascospores, confirming its presence in southeast Asia. Further, we provide phylogenetic evidence to support its placement in the genus *Architrypethelium* and hence demonstrating that the ascospore septation is also variable in this genus.
Material and methods

Specimen collection and phenotypical studies

The material of the new species was found in a dry evergreen forest of the north-eastern region in Thailand. Morphology was studied using an Olympus SZ11 dissecting microscope and free hand sections were mounted in distilled water and studied using an Olympus BX53 compound microscope with differential interference contrast (DIC) (Olympus U-DICT), connected to a Canon EOS650 digital camera. Secondary metabolites were studied using thin-layer chromatography (TLC) with standard solvent A (Orange et al. 2001, Lumbsch 2002).

Molecular data

Genomic DNA of the holotype was extracted from the dried lichen thallus using the CTAB method with chloroform precipitation (Cubero and Crespo 2002). DNA amplification was performed for mitochondrial small subunit ribosomal DNA (mtSSU) and nuclear large subunit ribosomal DNA (nuLSU) using primer pairs mrSSU1 (Zoller et al. 1999) with MSU7 (Zhou and Stanosz 2001) and LR0R with LR3 (Vilgalys and Hester 1990), respectively. PCR reaction mixture was prepared in a total volume of 50 µl, consisting of 5 µl of 10× Pfu Buffer with MgSO₄, 2 mM of dNTP mix, 20 µM of each primer, 1·25 U of Pfu DNA Polymerase (Thermo Fisher Scientific Inc.) and 5 µl of 1/10 dilution of DNA solution. PCR was performed using a thermal cycler Life ECO (Hangzhou Bioer Technology Co., China) as follows: initial denaturation for 1 min at 94 °C and 38 cycles of 94 °C for 1 min, 52 °C for 45 s (LR0R/LR3) and 53 °C for 45 s (mrSSU1/MSU7), followed by an extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. DNA purification and sequencing methods followed Luangsaphabool et al. (2016).

Phylogenetic analysis

The new sequences were aligned with other species of *Architrypethelium* and other Trypetheliaceae from GenBank (Table 1). *Aptrootia* and *Astrothelium* s. lat. have been shown to be the sister groups to *Architrypethelium* (Lücking et al. 2016b) and two taxa of *Bathe lia madreporiforme* were used as the outgroup. The DNA datasets (mtSSU and nuLSU) were aligned separately using MUSCLE (Edgar 2004) and improved manually using MEGA v.7 (Kumar et al. 2016). The nucleotide substitution model for maximum likelihood (ML) and Bayesian inference (BI) analyses was chosen using jModelTest v.2.1.4 (Darriba et al. 2012) with the Akaike Information Criterion (AIC). The ML tree was performed on the CIPRES supercomputer using the programme RAxML-HPC2 v.8.2.10 on XSEDE (Miller et al. 2010) and bootstrap values were estimated with 1000 pseudo-
Table 1. Species, location, voucher information and GenBank accession numbers for samples used in this study. Newly obtained sequences in bold and missing data are indicated by [–].

| Species              | Isolate      | Country     | Voucher information | GenBank accession No. |
|----------------------|--------------|-------------|---------------------|-----------------------|
|                       |              |             |                     | mtSSU                 | nuLSU                 |
| Aptrootia elatior     | MPN560B      | New Zealand | Knight O61815 (OTA)  | KM455821              | KM455754              |
| A. robusta            | MPN235B      | Australia   | Lumbsch 20012 (F)   | KM455822              | KM455755              |
| A. terricola          | DNA1501      | Costa Rica  | Lücking 17211 (F)   | DQ328995              | KM4553756             |
| Architrypethelium     |              |             |                     |                       |                       |
| latropaluanum         | MPN48        | Peru        | Nelsen Cit1P (F)    | XX215566              | XX215605              |
| A. nitens             | MPN257       | Panama      | Lücking 27038 (F)   | KM455823              | KM455757              |
| A. uberinum           | MPN489       | Brazil      | Nelsen s. n. (F)    | [–]                   | KM4553758             |
| A. muresporum         | UBN215       | Thailand    | Luangsuphabool 031332 (RAMK) | [–] | [–] |
| Astrothelium endochryseum | MPN436     | Brazil      | Lücking 31088 (F)   | KM455837              | KM455772              |
| A. subendochryseum    |              | El Salvador | Lücking 28121 (F)   | [–]                   | XX215659              |
| A. scorizum           | MPN336       | Brazil      | Lücking 29814 (F)   | KM455872              | KM455808              |
| A. obtectum           | MPN422       | Brazil      | Lücking 31242 (F)   | KM455832              | KM455767              |
| A. laevithallinum     | MPN442       | Brazil      | Lücking 31061 (F)   | KM455836              | KM455771              |
| A. subinterjectum     | MPN157       | Brazil      | Nelsen B15 (F)      | XX215583              | XX215660              |
| Bathelium madreporiforme | NAN95        | Thailand    | Luangsuphabool 027903 (RAMK) | LC128029               | LC127414               |
| B. madreporiforme     | UBN147       | Thailand    | Luangsuphabool 027904 (RAMK) | LC128028               | LC127413               |

replicates. Bayesian inference analysis and posterior probabilities were calculated using MrBayes v.3.2.1 (Ronquist and Huelsenbeck 2003) with the Markov chain Monte Carlo (MCMC) algorithm. Four chains and two independent runs were carried out with 10 million generations. Every 100th tree was saved into a file and aborting the analysis was set at the mean standard deviation < 0.01. Tree topology of both ML and BI analyses was illustrated using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

Two new DNA sequences of mtSSU and nuLSU were generated for this study (Table 1). The alignment matrix contained 609 unambiguously aligned nucleotide position characters, including 200 mtSSU and 409 nuLSU positions. The GTR+I+G model was chosen as the best-fit model for phylogenetic analyses. The topology of single locus analyses did not show any conflicts and hence the combined data set was used for the analysis. The posterior probabilities of the BI analysis together with the ML bootstrap values are both shown in the ML tree (Fig. 1).

The tree topology supported the fact that the new species is part of the genus Architrypethelium with strong support values (Fig. 1). Although the morphological characters of the new species would place it in the genus Astrothelium (Fig. 2), the shape of ascospore lumina is somewhat different from Astrothelium in having rounded-shaped...
**Figure 1.** Phylogenetic relationships of *Architrypethelium* and sister genera based on a combined data set of two DNA loci (mtSSU and nuLSU rDNA). Bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 are shown at above and below branches.

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Lumina (Fig. 2C) (Aptroot and Lücking 2016). This new species seems to be closer related to species with hyaline ascospore (*Architrypethelium lauropaluanum*) than brown ascospores (*A. nitens* and *A. uberinum*) (Aptroot 1991, Aptroot et al. 2008, Lücking et al. 2016a). So far, all species in *Architrypethelium* had large, transversely septate ascospores (Aptroot and Lücking 2016). However, our new species has small, muriform ascospores (Fig. 2B–C). The ascospore ontogeny in the new species resembles that of *Architrypethelium* spp. (Sweetwood et al. 2012), but continues septation to form muriiform spores and the endospore is reduced when mature.

The variation of ascospore size and septation in *Architrypethelium* is not surprising given the variation of ascospores in other genera of Trypetheliaceae. This phenomenon is also commonly found in many genera in families of non-lichenised ascomycetes, viz. Lophiostomataceae and Melanommataceae (Mugambi and Huhndorf 2009) and lichenised families, such as Graphidaceae and Pyrenulaceae (Lücking 2009, Aptroot 2012, Weerakoon et al. 2012, Aptroot and Lücking 2016, Gueidan et al. 2016), which supports the fact that ascospore characters are often poor predictors of phylogenetic relationships (Nelsen et al. 2014, Lücking et al. 2016b).
Taxonomic treatment

*Architrypethelium murisporum* Luangsuphabool, Lumbsch & Sangvichien, sp. nov.
MycoBank: MB823970

Figure 2

**Type.** THAILAND. Ubon Ratchathani Province: Na Pho Klang, Khong Chiam District, 15°31’N, 105°35’E, ca. 130 m alt., dry evergreen forest, on tree bark, 27 November 2012, *T. Luangsuphabool* RAMK 031332 (holotype: RAMK).

**Diagnosis.** Characterised within the genus by having small, hyaline and muriform ascospores.

**Etymology.** The specific epithet refers to the muriform ascospore character of the new species.

**Description.** Thallus crustose, corticate, thick, green to yellow-green, smooth to uneven, with cortex 40–125 µm thick, medulla 20–75 µm thick, prothallus black. Algae Trentepohlioid, cells 18–65 µm wide. Ascomata perithecia, pyriform, black, 0.45–0.60 mm diam., erumpent to prominent, fused into a pseudostroma, not covered by thallus. Ascoma wall carbonised, up to ca. 145 µm thick. Ostiole apical, black, not shared, with a white annulus surrounding the ostiolar region. Pseudostroma forming raised black lines, irregular in shape or forming a partial network on the thallus. Hamathecium hyaline, not inspersed with droplets or granules, consisting of branched and anastomosing paraphyses, 1.5–2.5 µm thick. Asci clavate to cylindrical, 150–200 × 32–50 µm. Ascospores 8 per ascus, hyaline, muriform with 6–9 transverse and 1–2 longitudinal septa per tier near centre of spore in optical section, narrowly ellipsoid, 35–50 × 13–15.5 µm. Pycnidia not observed.

**Secondary chemistry.** Thallus UV–, K–, C–, KC–, PD–; pseudostroma UV–, K–, C–, KC–, PD–. TLC: no substances detected.

**Distribution and ecology.** The new species was found in north-eastern Thailand, growing in a dry evergreen forest on tree bark. It is only known from the type locality.

**Notes.** *Architrypethelium murisporum* is morphologically similar to *Astrothelium keralense* (Upreti & Ajay Singh) Aptroot & Lücking and *A. variatum* (Nyl.) Aptroot & Lücking in having hyaline, small and muriform ascospores, but differs in having ascomata fused into a pseudostroma and not covered by the thallus (ascomata solitary, covered by the thallus in *A. keralense* and ascomata covered by thallus except ostiole regions in *A. variatum*), narrowly ellipsoid ascospores ( fusiform in both *Astrothelium* spp.). Also the ascospore size (35–50 × 13–15.5 µm) differs from *A. keralense* (50–60 × 15–20 µm) and *A. variatum* (24–35 × 11–13 µm). The placement of the new species in *Architrypethelium* is supported by molecular evidence (Fig 1), but it is unlikely to be confused with any of the currently accepted species in that genus due to the differences in ascospore size and septation (Aptroot et al. 2008, Aptroot and Lücking 2016, Flakus et al. 2016, Lücking et al. 2016a). The new taxon has muriform and relatively small ascospores (ca. 50 µm, long) (Fig 2), whereas other *Architrypethelium* species have transversely septate ascospores (3–5 septate), that are longer than 90 µm (Aptroot 1991, Aptroot et al. 2008, Aptroot and Lücking 2016, Flakus et al. 2016, Lücking et al. 2016a).
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References

Aptroot A (1991) A monograph of the Pyrenulaceae (excluding Anthracothecium and Pyrenula) and the Requienellaceae, with notes on the Pleomassariaceae, the Trypetheliaceae, and
Mycomicrothelia (lichenized and non-lichenized ascomycetes). Bibliotheca Lichenologica 44: 1–178.

Aptroot A (2012) A world key to the species of Anthracothecium and Pyrenula. Lichenologist 44: 5–53. https://doi.org/10.1017/S0024282911000624

Aptroot A, Lücking R (2016) A revisionary synopsis of the Trypetheliaceae (Ascomycota: Trypetheliales). Lichenologist 48: 763–982. https://doi.org/10.1017/S0024282916000487

Aptroot A, Lücking R, Sipman HJM, Umaña L, Chaves JL (2008) Pyrenocarpous lichens with bitunicate asci. A first assessment of the lichen biodiversity inventory in Costa Rica. Bibliotheca Lichenologica 97: 1–162.

Buaruang K, Boonpragob K, Mongkolsuk P, Sangvichien E, Vongshewarat K, Polyiam W, Rangsiruji A, Saipunkaew W, Naksuwanukul K, Kalb K, Parnmen S, Kraichak E, Phruechamnong P, Meesim P, Luangsuphabool T, Nirongbut P, Poengsungnoen V, Duangphui N, Sodamuk M, Phokaeo S, Molsil M, Aptroot A, Kalb K, Lücking R, Lumbsch HT (2017) A new checklist of lichenized fungi occurring in Thailand. Mycokeys 23: 1–91. https://doi.org/10.3897/mycokeys.23.12666

Cubero OF, Crespo A (2002) Isolation of nucleic acids from lichens. In: Kranner I, Beckett R, Varma A (Eds) Protocols in Lichenology. Springer-Verlag Berlin Heidelberg, 381–391. https://doi.org/10.1007/978-3-642-56359-1_23

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Flakus A, Kukwa M, Aptroot A (2016) Trypetheliaceae of Bolivia: an updated checklist with descriptions of twenty-four new species. Lichenologist 48: 661–692. https://doi.org/10.1017/S0024282915000559

Gueidan C, Aptroot A, Cáceres MEEdS, Binh NQ (2016) Molecular phylogeny of the tropical lichen family Pyrenulaceae: contribution from dried herbarium specimens and FTA card samples. Mycological Progress 15: 7. https://doi.org/10.1007/s11557-015-1154-8

Hyde KD, Jones EBG, Liu J-K, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai D-Q, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li Y-M, Liu Y-X, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang K-L, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambubala KM, Wijayarawatene NN, Wikee S, Wu H-X, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bakhali AH, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog SD, Kang J-C, Knudsen K, Li W-J, Li X-H, Liu Z-Y, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu J-C, Yacharoen S, Yan J-Y, Zhang M (2013) Families of Dothideomycetes. Fungal Diversity 63: 1–313. https://doi.org/10.1007/s13225-013-0263-4

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054
Architrypethelium murisporum (Ascomycota, Tryptetheliaceae)...

Luangsuphabool T, Lumbsch HT, Aptroot A, Piaipukiew J, Sangvichien E (2016) Five new species and one new record of Astrothelium (Tryptetheliaceae, Ascomycota) from Thailand. Lichenologist 48: 727–737. https://doi.org/10.1017/S0024282916000499

Lücking R (2009) The taxonomy of the genus Graphis sensu Staiger (Ascomycota: Ostropales: Graphidaceae). Lichenologist 41: 319–362. https://doi.org/10.1017/S0024282909008524

Lücking R, Nelsen MP, Aptroot A, Benatti MN, Binh NQ, Gueidan C, Gutiérrez MC, Jungbluth P, Lumbsch HT, Marcelli MP, Moncada B, Naksuwankul K, Orozco T, Salazar-Allen N, Upreti DK (2016a) A pot-pourri of new species of Tryptetheliaceae resulting from molecular phylogenetic studies. Lichenologist 48: 639–660. https://doi.org/10.1017/S0024282916000475

Lücking R, Nelsen MP, Aptroot A, Klee RB, Bawingan PA, Benatti MN, Binh NQ, Bungartz F, Cáceres MES, Canèz LS, Chaves JL, Ertz D, Esquivel RE, Ferraro LI, Grijalva A, Gueidan C, Hernández JE, Knight A, Lumbsch HT, Marcelli MP, Mercado-Díaz JA, Moncada B, Morales EA, Naksuwankul K, Orozco T, Parnmen S, Rivas Plata E, Salazar-Allen N, Spielmann AA, Ventura N (2016b) A phylogenetic framework for reassessing generic concepts and species delimitation in the lichenized family Tryptetheliaceae (Ascomycota: Dothideomycetes). Lichenologist 48: 739–762. https://doi.org/10.1017/S0024282916000505

Lumbsch HT (2002) Analysis of phenolic products in lichens for identification and taxonomy. In: Kranner I, Beckett R, Varma A (Eds) Protocols in Lichenology. Springer-Verlag Berlin Heidelberg, 281–295. https://doi.org/10.1007/978-3-642-56359-1_17

Miller M, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 1–8. https://doi.org/10.1109/GCE.2010.5676129

Mugambi GK, Huhndorf SM (2009) Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae re-circumscribed (Pleosporomycetidae, Dothideomycetes, Ascomycota). Studies in Mycology 64: 103–121. https://doi:10.3114/sim.2009.64.05

Nelsen MP, Lücking R, Aptroot A, Andrew CJ, Cáceres M, Plata ER, Gueidan C, Canèz LdS, Knight A, Ludwig LR, Mercado-Díaz JA, Parnmen S, Lumbsch HT (2014) Elucidating phylogenetic relationships and genus-level classification within the fungal family Tryptetheliaceae (Ascomycota: Dothideomycetes). Taxon 63: 974–992. https://doi.org/10.12705/635.9

Orange A, James PW, White FJ (2001) Microchemical methods for the identification of lichens. British Lichen Society, London, 101 pp.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sweetwood G, Lücking R, Nelsen MP, Aptroot A (2012) Ascospore ontogeny and discharge in megalosporous Tryptetheliaceae and Graphidaceae (Ascomycota: Dothideomycetes and Leccanoromycetes) suggest phylogenetic relationships and ecological constraints. Lichenologist 44: 277–296. https://doi:10.1017/S0024282911000740

Vigalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
Weerakoon G, Aptroot A, Lumbsch HT, Wolseley PA, Wijeyaratne SC, Gueidan C (2012)
New molecular data on Pyrenulaceae from Sri Lanka reveal two well-supported groups
within this family. Lichenologist 44: 639–647. https://doi:10.1017/S0024282912000333
Zhou S, Stanosz GR (2001) Primers for amplification of mtSSU rDNA, and a phylogenetic
study of Botryosphaeria and associated nanomorphic fungi. Mycological Research 105:
1033–1044. https://doi.org/10.1017/S0953756201004592
Zoller S, Scheidegger C, Sperisen C (1999) PCR primers for the amplification of mitochondrial
small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist 31: 511–516.
https://doi.org/10.1006/lich.1999.0220
Diversity of polypores in the Dominican Republic: *Pseudowrightoporia dominicana* sp. nov. (Hericiaceae, Russulales)

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Abstract

The new species *Pseudowrightoporia dominicana* is described from the Dominican Republic based on morphological and molecular data (nrITS and nrLSU sequence analyses). It is mainly characterised by pileate basidiomata with a bright pinkish context and a di-trimitic hyphal system. Phylogenetically, it is sister to the African species *P. gillesii* and to the Asiatic *P. japonica*.

Keywords

Basidiomycota, Agaricomycetes, Caribbean Islands, Polypores, Phylogeny, Taxonomy

Introduction

The genus *Wrightoporia* Pouzar, typified with *W. lenta* (Overh. & J. Lowe) Pouzar (Pouzar 1966), is traditionally characterised by resupinate to pileate basidiomata, annual to perennial habit, small to medium pores and cottony to hard texture. Hyphal system monomitic to di-trimitic, generative hyphae clamped or rarely with simple septa, skeletal hyphae dextrinoid, partially dextrinoid (only in the tubes) or not dextrinoid. Basidiospores small, cylindrical to globose, smooth to finely asperulate, amyloid
(Ryvarden 1982, 2016; David and Raichenberg 1987, Stalpers 1996, Núñez and Ryvarden 2001, Hattori 2008). To date, there are 52 species transferred to or described in the genus (Index Fungorum 2018). This genus belongs to the Hericiaceae, in the Russulales (Larsson and Larsson 2003, Chen et al. 2016).

Chen et al. (2016), on the basis of combined nrITS/nrLSU phylogenetic analyses and morphological data, indicated that the genus \textit{Wrightoporia}, as currently circumscribed, is strongly polyphyletic and recognised six clades in \textit{Wrightoporia} s.l. Consequently, species previously treated in \textit{Wrightoporia} were transferred to \textit{Amylonotus} Ryvarden, \textit{Amylosporus} Ryvarden and to the three new genera \textit{Larssoniporia} Y.C. Dai, Jia J. Chen & B.K. Cui, \textit{Pseudowrightoporia} Y.C. Dai, Jia J. Chen & B.K. Cui and \textit{Wrightoporiopsis} Y.C. Dai, Jia J. Chen & B.K. Cui. In particular, the genus \textit{Pseudowrightoporia} was established by Chen et al. (2016) to accommodate \textit{Wrightoporia clyndrospora} Ryvarden (the generic type), \textit{W. japonica} Núñez & Ryvarden, \textit{Pseudowrightopora crassihypha} Y.C. Dai, Jia J. Chen & B.K. Cui, \textit{P. hamata} Y.C. Dai, Jia J. Chen & B.K. Cui and \textit{P. oblongispora} Y.C. Dai, Jia J. Chen & B.K. Cui, species causing white rot and mostly characterised by soft corky to corky basidiomes, shining pores, dimitic hyphal structure with clamped generative hyphae and skeletal hyphae, ellipsoid, finely asperulate and amyloid basidiospores and a subtropical to tropical distribution. Based only on these morphological characteristics, the following species were transferred to \textit{Pseudowrightoporia}: \textit{Wrightoporia africana} Johans. & Ryvarden, \textit{W. aurantipora} T. Hatt., \textit{W. gillesii} A. David & Rajchenb., \textit{W. solomonensis} (Corner) T. Hatt. and \textit{W. straminea} T. Hatt.

During the species diversity study of wood-inhabiting macromycetes in the Dominican Republic, a pileate \textit{Pseudowrightoporia} was discovered. The aim of this investigation was to identify and to analyse the \textit{Pseudowrightoporia} specimens using both morphological and molecular techniques.

**Materials and methods**

**Morphology**

Photographs of fresh basidiomata were taken \textit{in situ} by a Nikon Coolpix 8400 digital camera and then dried, while the photos of the microscopical structures were obtained through a Olympus BH-2 light microscope and a Nikon D7100 digital camera. For microscopical analysis, tiny fragments from dried material were mounted in Melzer’s anionic reagent for testing amyloid and dextrinoid reactions of spores and other microscopical elements. All microscopic measurements were carried out with a ×1000 oil immersion objective. Basidiospores were measured from hymenophores of mature basidiomes, dimensions are given as: (minimum–) average minus standard deviation – average – average plus standard deviation (–maximum) of length × (minimum–) average minus standard deviation – average – average plus standard deviation (–maximum) of width; Q = (minimum–) average minus standard deviation – average – average plus standard deviation.
standard deviation (–maximum) of the length/width ratio. Spore statistics were produced using R version 3.4.4 (R Core Team 2018). Herbarium acronyms follow Thiers (2018, continuously updated) with the exception of ANGE that refers to the personal herbarium of C. Angelini.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 10 mg of a dried voucher specimen (JBSD 127410), using the DNeasy Plant Mini Kit (Qiagen, Milan) according to the manufacturer’s instructions. Primers LR0R/LR6 (Vilgalys and Hester 1990, Vilgalys lab. http://www.botany.duke.edu/fungi/mycolab) were used for the nrLSU (28S) DNA amplification and universal primers ITS1F/ITS4 for the ITS region amplification (White et al. 1990, Gardes and Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems, Norwalk) in 25 ml reaction mixtures using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 mM of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega, Madison). The PCR programme was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C for 35 cycles, 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1% agarose gel and visualised by staining with ethidium bromide. The PCR products were purified with the AMPure XP kit (Beckman Coulter, Pasadena) and sequenced by MACROGEN (Seoul). The sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and their accession numbers are reported in Figs 1–2.

Sequence alignment, dataset assembly and phylogenetic analysis

Sequences were checked and assembled with Geneious 5.3 (Drummond et al. 2010) and compared to those available in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/) using the BLASTN algorithm (Altschul et al. 1990). Based on BLASTN results, sequences were selected according to the recent monographic work on *Wrightoporia* s.l. by Chen et al. (2016).

Two phylogenetic analyses were performed: the first, based on a combined nrITS and nrLSU sequences dataset, to focus on the phylogenetic position of the new species in the Russulales (Russuloid clade); the second, based only on a nrITS dataset was restricted to the taxa closely related to *P. dominicana* according with the previous combined data analysis. Alignments were generated for each nrITS and nrLSU dataset using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The two alignments were imported into MEGA 6 (Tamura et al. 2013) for manual adjustment. The best-fit substitution model for each single alignment was estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 2 (Darriba et al. 2012). The GTR + G...
Figure 1. Bayesian phylogram obtained from the combined nrITS-nrLSU sequence alignment of Russulales taxa selected according to Chen et al. (2016). Sistotrema brinkmannii, S. coronilla, S. muscicola and S. sernanderi were used as outgroup taxa. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP) and Maximum likelihood (ML bootstrap percentage, MLB) analyses are indicated. BPP values (in bold) above 0.70 and MLB values above 50% are given above/below branches. The newly sequenced collection is in bold.

model was chosen for both the nrITS and nrLSU alignments. The sequences of Sistotrema brinkmannii, S. coronilla, S. muscicola and S. sernanderi were used as outgroup taxa (Larsson and Larsson 2003, Chen et al. 2016) in the combined analysis; Dentipelo-
Figure 2. Bayesian phylogram obtained from the nrITS sequence alignment of Pseudowrightoporia and Wrightoporiopsis species. *Dentipellis coniferarum*, *D. fragilis* and *Hericium alpestre* were used as outgroup taxa. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP) and maximum likelihood (ML bootstrap percentage, MLB) analyses are indicated. BPP values (in bold) above 0.70 and MLB values above 50% are given above/below branches. The newly sequenced collection is in bold.

*lis coniferarum*, *D. fragilis* and *Hericium alpestre* were selected as outgroup taxa in the nrITS analysis. The ITS dataset was not partitioned into ITS1, 5.8S and ITS2 subsets. Phylogenetic hypotheses were constructed under Bayesian inference (BI) and Maximum likelihood (ML) criteria. The BI was performed with MrBayes 3.2.6 (Ronquist et al. 2012) with one cold and three incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 million generations, under the selected evolutionary model. Two simultaneous runs were performed independently. Trees were sampled every 1,000 generations, resulting in overall sampling of 10,001 trees per single run; the first 2,500 trees (25%) were discarded as burn-in. For the remaining trees of the two independent runs, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). ML estimation was performed through RAxML 7.3.2 (Stamatakis 2006) with 1,000 bootstrap replicates using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP and MLB values over 0.70 and 50%, respectively, are reported in the resulting trees (Figs 1–2). Branch lengths were estimated as mean values over the sampled trees.
Results

The combined nrITS and nrLSU data matrix comprised 118 sequences (including 117 from GenBank) and includes 2132 positions. The nrITS data matrix comprises a total of 25 sequences (including 24 from GenBank) and includes 687 positions. As both Bayesian and Maximum likelihood analyses produced comparable topologies, only the Bayesian trees with both BPP and MLB values are shown (Figs 1–2). In the combined two-gene phylogeny of Russulales taxa (Fig. 1), the new species falls, as an independent phylogenetic branch, in the Hericiaceae within the Pseudowrightoporia cluster. *Pseudowrightoporia dominicana* is sister (BPP = 1.00, MLB = 95) to *P. japonica*. *Pseudowrightoporia* is shown to be sister (BPP = 1.00, MLB = 100) to a well-supported clade (BPP = 1.00, MLB = 80) consisting of *Wrightoporiopsis* and *Dentipellicula*, as previously highlighted by Chen et al. (2016). The small ITS analysis restricted to species of *Pseudowrightoporia* and *Wrightoporiopsis* (Fig. 2) supports *P. dominicana* as a new species and indicates *P. gillesii* and *P. japonica* as its phylogenetically closest species.

Taxonomy

*Pseudowrightoporia dominicana* Angelini, Losi & Vizzini, sp. nov.
MycoBank MB824844

Fig. 3

**Holotype.** Dominican Republic. La Vega (Province), Jarabacoa (Municipality), Montaña (Locality), 19°06’39”N, 70°37’57”W, on an unidentified live trunk of a deciduous tree, in a mixed mountain forest with several broadleaved species and pines (*Pinus occidentalis*), 17 December 2016, Claudio Angelini, (JBSD 127410, isotype ANGE 789).

**Etymology.** The epithet refers to the country, The Dominican Republic, where this species was found.

Basidiomata annual, pileate, sessile, single or in small clusters, fibrous-tough (Fig. 3a and b). Pileus broadly attached to dimidiate, up to 25 mm wide and 15 mm deep, 5–10 mm thick; upper surface white to cream with pinkish tint, velutinate to glabrous, azonate, smooth; margin rounded, even or slightly lobed; pore surface concolorous with the pileus surface, pores round to angular, at first cupulate, 6–8 per mm, dissepiments thick and entire; tube layer 2–4 mm thick, whitish to cream; context pinkish (Fig. 3c), homogenous, tough-fibrous, up to 6 mm thick. Hyphal system di-trimitic; generative hyphae clamped, hyaline, thin-walled, 2.2–4.8 µm wide; skeletal hyphae thick-walled, rarely branched, 2.4–5.6 µm wide, dextrinoid especially in the trama (Fig. 3d); contextual binding hyphae thick-walled, short-branched, 1.6–2.4 µm wide, weakly dextrinoid (Fig. 3e). Cystidia none. Basidia densely united, clavate, 4-sterigmate, 8–12 × 4–5 µm. Basidiospores (2.6–)2.98–3.2–3.43(–3.6) ×
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**Figure 3.** *Pseudowrightoporia dominicana* (JBSD 127410) **a, b** fresh basidiomes in situ **c** cut side of the basidiome **d** dextrinoid skeletal hyphae **e** binding hypha **f** amyloid spores. Microscopical elements observed in Melzer’s anionic reagent. Scale bars: 10 mm (**a–c**); 10 µm (**d–f**).

(1.8–)1.96–2.2–2.44 (–2.8 ) µm (n = 40), Q = (1.14–)1.28–1.44–1.6 (–1.89), broadly ellipsoid to ellipsoid, finely asperulate, thin- to slightly thick-walled, distinctly amyloid (Fig. 3f).

**Habit, habitat and distribution.** Pileate, gregarious on a live trunk of deciduous tree, so far known only from the type locality.
Discussion

All the phylogenetic analyses show *P. dominicana* to be a distinct lineage in the genus *Pseudowrightoporia* (Figs 1–2). The new species displays a unique combination of outstanding characters such as pileate basidiomes, pink context, very small spores and di-trimitic hyphal system (Fig. 3). In particular, the presence of binding hyphae (only in the context) is quite unusual in *Pseudowrightoporia* as well as in the other genera of *Wrightoporia* s.l. (Ryvarden 1982, 1987, 2000, 2016; David and Rajchenberg 1987, Dai 1995, Núñez and Ryvarden 2001, Dai and Cui 2006, Hattori 2008, Chen and Cui 2012, 2014; Chen and Yu 2012, Jang et al. 2013, Westphalen et al. 2014, Chen et al. 2016, Drechsler-Santos et al. 2016, Campi et al. 2017); binding hyphae have so far been reported only in *P. aurantipora* (Hattori 2008), *W. brunneo-ochracea* A. David & Rajchenb. (David and Rajchenberg 1985), *W. trimitica* (Corner) Stalpers (Corner 1989, Stalpers 1996) and *Larssoniporia tropicalis* (Cooke) Y.C. Dai, Jia J. Chen & B.K. Cui, (Núñez and Ryvarden 2001).

*Pseudowrightoporia gillesii* and *P. japonica* are the species phylogenetically most closely related to *P. dominicana* (Figs 1–2). *Pseudowrightoporia gillesii*, originally described from Africa (Gabon), is characterised by an effused-reflexed basidiome, chestnut ochraceous context, dimitic context, skeletal hyphae dextrinoid only in the pore mouths and presence of lageniform to mucronate cystidiola (David and Rajchenberg 1987). *Pseudowrightoporia japonica* (= *Wrightoporia luteola* B.K. Cui & Y.C. Dai according with Jang et al. 2013 and Chen et al. 2016) shows a basidiome shape ranging from pileate (and then with a zoned pileus) to resupinate, a pore surface cream to wood-coloured, a dimitic hyphal system and more elongated spores, up to 4 × 2.6 µm (Núñez and Ryvarden 1999, 2001; Jang et al. 2013).

Amongst the morphologically most similar species to *P. dominicana*, *Wrightoporia dimidiata* A. David & Rajchenb. from Asia (Singapore) is distinguished by a hymenophore with 3–4 pores per mm, dimitic hyphal system, spores measuring 3.5–4 × 3 µm and presence of cystidiola, gloecystidia and gloeopleurous hyphae (David and Rajchenberg 1987). From above, the new species may resemble the pileate basidiomes of *Wrightoporia cremea* Ryvarden from Brazil, but the latter has larger pores (3–4 per mm) and spores (subglobose, 3–4 µm in diam.), dimitic hyphal system, in addition to a cream to pale ochre context (Ryvarden 1987, 2017 and pers. comm.). Finally, *P. aurantipora* from Japan, *W. brunneo-ochracea* from Guadeloupe, *W. trimitica* from Malay and the pantropical *W. tropicalis* share with *P. dominicana* the presence of binding hyphae, but *P. aurantipora* differs in having resupinate basidiomes with light orange to brown orange 4–6/mm pores, context orange without pinkish hues, tramal skeletal hyphae strongly covered with granules near the tip and longer spores, 3–4.2 × 2–3 µm (Hattori 2008); *W. brunneo-ochracea* differs in having effused-reflexed basidiomes with ochraceous, irregular to angular pores, 3–4 per mm, a thin ochraceous context, non-dextrinoid skeletal hyphae and narrower spores, 3–3.5 × 2 µm (David and Rajchenberg 1985, Ryvarden 2016); *W. trimitica* has dimidiate basidiomes, with a short resupinate foot, ochraceous to wood-coloured pores and up to 4 µm long spores (Corner 1989, Stalpers 1996); *Larssoniporia
Diversity of polypores in the Dominican Republic: *Pseudowrightoporia dominicana*... tropicalis has resupinate, applanate to pulvinate, widely effused, grey to black perennial and very woody basidiomes, grey to brown pore surface, thick-walled and heavily ensutted cystidia, blunt at the apex, presence of gloeocystidia and subglobose spores 3–4 × 2–3 µm (Ryvarden and Johansen 1980, Núñez and Ryvarden 2001, Ryvarden 2016).

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**References**

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. http://dx.doi.org/10.1016/S0022-2836(05)80360-2

Campi M, Maubet Y, Grassi E, Robledo GL (2017) *Amylosporus guaraniticus* sp. nov. (Wrightoporaceae, Russulales) a new neotropical species from Paraguay. Mycosphere 8(6): 1060–1070. https://doi.org/10.5943/mycosphere/8/6/6

Chen JJ, Cui BK (2012) Studies on *Wrightoporia* from China 2. A new species and three new records from South China. Mycotaxon 21: 333–343. https://doi.org/10.5248/121.333

Chen JJ, Cui BK (2014) Studies on *Wrightoporia* from China 3. *Wrightoporia subavellanea* sp. nov. based on morphological characters and rDNA sequence data. Phytotaxa 175: 225–234. http://dx.doi.org/10.11646/phytotaxa.175.4.4

Chen JJ, Yu HY (2012) Studies on the genus of *Wrightoporia* from China 1. A new species described from Hunan Province, South China. Mycotaxon 120: 295–300. http://dx.doi.org/10.5248/120.295

Chen JJ, Cui BK, Dai YC (2016) Global diversity and molecular systematics of *Wrightoporia* s.l. (Russulales, Basidiomycota). Persoonia 37: 21–36. https://doi.org/10.3767/003158516X689666

Corner EJH (1989) Ad Polyporaceas V. Beihefte zur Nova Hedwigia 96: 1–218.

Cui BK, Dai YC (2006) *Wrightoporia* (Basidiomycota, Aphyllophorales) in China. Nova Hedwigia 83: 159–166. https://doi.org/10.1127/0029-5035/2006/0083-0159

Dai YC (1995) A new species of *Wrightoporia* (Basidiomycetes) from China. Karstenia 35: 85–89. https://doi.org/10.29203/ka.1995.312

Dai YC, Cui BK (2006) Two new species of *Wrightoporia* (Basidiomycota, Aphyllophorales) from southern China. Mycotaxon 96: 199–206.
Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109
David A, Rajchenberg M (1985) Pore fungi from French Antilles and Guiana. Mycotaxon. 22(2): 285–325.
David A, Rajchenberg M (1987) A revaluation of *Wrightoporia* and *Amylonotus* (Aphyllophorales, Polyporaceae). Canadian Journal of Botany 65: 202–209. https://doi.org/10.1139/b87-027
Drechsler-Santos ER, Salvador-Montoya CA, Ryvarden L (2016) Studies in neotropical polypores 41. A new species of *Amylosporus* from Caatinga dry woodlands, Brazil. Synopsis Fungorum 35: 4–8.
Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v5.3. Available from http://www.geneious.com/
Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x
Index Fungorum (2018) http://www.indexfungorum.org [Accessed 25 March 2018]
Hattori T (2008) *Wrightoporia* (Basidiomycota, Hericaceales) species and their allies collected in Japan. Mycoscience 49: 56–65. https://doi.org/10.1007/s10267-007-0389-x
Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. https://doi.org/10.1093/nar/gkf436
Jang Y, Lee SW, Lim YW, Lee JS, Hattori T, Kim J-J (2013) The genus *Wrightoporia* in Korea. Mycotaxon 123: 335–341. https://doi.org/10.5248/123.335
Larsson E, Larsson KH (2003) Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. Mycologia 95: 1037–1065. https://doi.org/10.2307/3761912
Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, 1–8. https://doi.org/10.1109/gce.2010.5676129
Núñez M, Ryvarden L (1999) New and interesting polypores from Japan. Fungal Diversity 3: 107–121.
Núñez M, Ryvarden L (2001) East Asian Polypores 2. Synopsis Fungorum 14: 170–522.
Pouzar Z (1966) Studies in the taxonomy of the polypores I. Česká Mykologie 20: 171–177. https://doi.org/10.1007/BF02854587
R Core Team (2018) R: a language and environment for statistical computing, version 3.4.4. http://www.R-project.org
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029
Ryvarden L (1982) Synopsis of the genus *Wrightoporia*. Nordic Journal of Botany 2: 145–149. https://doi.org/10.1111/j.1756-1051.1982.tb01174.x
Diversity of polypores in the Dominican Republic: *Pseudowrightoporia dominicana*...

Ryvarden L (1987) New and noteworthy polypores from tropical America. Mycotaxon 28(2): 525–541.

Ryvarden L (2000) Studies in neotropical polypores 7. *Wrightoporia* (Hericaceae, Basidiomycetes) in tropical America. Karstenia 40: 153–158. https://doi.org/10.29203/ka.2000.366

Ryvarden L (2016) Neotropical polypores Part 3. Polyporaceae, *Obba-Wrightoporia*. Synopsis Fungorum 46: 445–613.

Ryvarden L, Johansen I (1980) A preliminary polypore flora of East Africa. Oslo, Fungiflora.

Stalpers JA (1996) The aphyllophoraceous fungi II. Keys to the species of the Hericiiales. Studies in Mycology 40: 1–185.

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Thiers B (2018, continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [Accessed 25 March 2018]

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Westphalen MC, Reck MA, da Silveira RMB (2014) Studies on *Wrightoporia* (Basidiomycota) from southern Brazil. Phytotaxa 166(1): 94–100. https://doi.org/10.11646/phytotaxa.166.1.7

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press Inc., New York, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1
Two novel species of *Neoaquastroma* (Parabambusicolaceae, Pleosporales) with their phoma-like asexual morphs

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Abstract

The monotypic genus *Neoaquastroma* (Parabambusicolaceae, Pleosporales) was introduced for a microfungus isolated from a collection of dried stems of a dicotyledonous plant in Thailand. In this paper, we introduce two novel species, *N. bauhiniae* and *N. krabiense*, in this genus. Their asexual morphs comprise conidiomata with asperate and hyaline conidia. *Neoaquastroma bauhiniae* has ascomata, asci and ascospores that are smaller than those of *N. krabiense*. Descriptions and illustrations of *N. bauhiniae* and *N. krabiense* are provided and the two species compared with the type species of the genus, *N. guttulatum*. Evidence for the introduction of the new taxa is also provided from phylogenetic analysis of a combined dataset of partial LSU, SSU, ITS and *tef1* sequence data. The phylogenetic analysis revealed a distinct lineage for *N. bauhiniae* and *N. krabiense* within the family Parabambusicolaceae.

Keywords

Dothideomycetes, holomorph, Massarineae, saprotrophs, Southeast Asia
Introduction

Thailand is a highly biodiverse country in the tropics with hot and humid climate (MacKinnon et al. 1986, Marod and Kutintara 2012). Although the fungal diversity in Thailand has been relatively well-studied (Rostrup 1902, Schumacher 1982, Hyde 1989, Jones 2000, Jones et al. 2006, Suetrong et al. 2009), the number of species being discovered is steadily growing due to increasing activities in studying microfungi in a large variety of terrestrial and aquatic ecosystems (Mapook et al. 2016, Phukhamsakda et al. 2016, Dai et al. 2017, Doilom et al. 2017, Phukhamsakda et al. 2017).

The family Parabambusicolaceae was introduced for a distinct phylogenetic lineage in the suborder Massarineae (Pleosporales) (Tanaka et al. 2015). Species of Parabambusicolaceae are characterised by pseudothecioid ascomata with or without stromatic tissues, papillate to apapillate ostioles, clavate to fusiform asci and hyaline or brown phragmospores (Liu et al. 2015, Tanaka et al. 2015, Li et al. 2016, Wanasinghe et al. 2017). The asexual morphs are sporodochial or Monodictys-like (Tanaka et al. 2015, Ariyawansa et al. 2015). Currently, there are seven known genera in this family; Aquastroma, Monodictys-like spp., Multilocularia, Multiseptospora, Neoaquastroma, Parabambusicola (with P. bambusina as generic type) and Pseudomonodictys (Tanaka and Harada 2003, Wijayawardene et al. 2017, 2018). The genus Neoaquastroma Wanas., E.B.G. Jones & K.D. Hyde, has been introduced from a dead twig of a herbaceous plant collected in Northern Thailand and been typifed with N. guttulatum Wanas., E.B.G. Jones & K.D. Hyde (Wanasinghe et al. 2017). The genus is characterised by immersed, glabrous pseudothecia, short, papillate, fissitunicate, clavate asci and ellipsoidal to sub-fusiform, multi-septate hyaline phragmospores, surrounded by a mucilaginous sheath (Wanasinghe et al. 2017). Molecular phylogenetic analysis using ribosomal DNA (LSU, SSU and ITS) and translation elongation factor 1-alpha (tef1) sequence data support it as a distinct genus in Parabambusicolaceae.

The purpose of this study is to describe two new species of Neoaquastroma from collections of dicotyledonous plants in Thailand. Phylogenetic analysis of combined of LSU, SSU, ITS and tef1 sequence data are provided.

Materials and methods

Sample collection, morphological study and isolation

Fresh specimens were collected from northern and southern part of Thailand during 2015–2017. The specimens were packed into brown paper bags for transport to the laboratory. Pure cultures were obtained from single ascospores on malt extract agar (MEA; 62 g/l) in distilled water following the method of Chomnunti et al. (2014). Cultures were incubated at 25 °C for up to 8 weeks. Induction of asexual reproduction has been adapted from Tanaka and Harada (2003) by placing agar squares with mycelia on water agar placed with sterile rice straw pieces. The plates were incubated at room tempera-
Two novel species of *Neoaquastroma* (Parabambusicolaceae, Pleosporales)...

ture (25 °C) with the standard light cycles, 12 hrs in the light followed by 12 hrs in the dark for about eight weeks until the fructifications were produced. Type specimens are deposited in Mae Fah Luang University (MFLU) herbarium and isotypes are deposited at the Kunming Institute of Botany, Academia Sinica Herbarium (HKAS), China. Ex-type living cultures are deposited at the Mae Fah Luang Culture Collection (MFLUCC) and duplicates at the International Collection of Microorganisms and Plants (ICMP), New Zealand. Faces of fungi numbers (Jayasiri et al. 2015) and MycoBank number (http://www.MycoBank.org) are provided. Samples were examined under a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made using Tarosoft (R) Image Frame Work programme and photo-plates were made by using AdobePhotoshop CS6 Extended version 10.0 software (Adobe Systems, United States).

**DNA extraction, amplification and sequencing**

DNA was extracted from mycelium by using Biospin Fungus Genomic DNA Extraction Kit (BioFlux) (Hangzhou, P. R. China) and gene extraction kit (Bio Basic Inc., Canada). PCR amplification was carried out using primers LROR/LR5 for the nuclear ribosomal large subunit 28S rDNA gene (LSU), NS1/NS4 for the nuclear ribosomal small subunit 18S rDNA gene (SSU) and ITS5/ITS4 for internal transcribed spacer rDNA region (ITS1, 5.8S rDNA and ITS2); partial fragments of the translation elongation factor 1-alpha (*tef1*) gene region was amplified using primers EF1-983F and EF1-2218R (Vilgalys and Hester 1990, White et al. 1990, Carbone and Kohn 1999). Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). Amplification reactions for LSU, SSU and ITS followed Phukhamsakda et al. (2016). The PCR thermal cycle programme for EF1-983F and EF1-2218R (Carbone and Kohn 1999) for translation elongation factor 1-alpha (*tef1*) was set for denaturation at 96 °C for 2 min, followed by 40 cycles of denaturation at 96 °C for 45 sec, annealing at 54 °C for 30 sec and extension at 72 °C for 1.30 min, with a final extension step at 72 °C for 5 min. Genomic DNA and PCR amplification products were checked on 1% agarose gel. PCR products were purified as described in Wendt et al. (2017), sequences were generated by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China) and sequencing services at Helmholtz Centre For Infection Research (HZI, Braunschweig, Germany).

**Sequence alignment and phylogenetic analysis**

SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used to assemble consensus sequences. Sequences of closely related strains were retrieved using BLAST searches against GenBank (http://www.ncbi.nlm.nih.gov). We also included the strains from Wanasin-ghe et al. (2017) and these are listed in Table 1. Sequences were aligned with MAFFT
Table 1. Taxa used in the phylogenetic analysis and their corresponding culture collections, and accession numbers used in this study.

| Taxon                          | Culture accession number(s) | GenBank accession numbers | References               |
|--------------------------------|----------------------------|---------------------------|--------------------------|
| *Aquastroma magistiostellata* | CBS 139680<sup>T</sup> = MAFF 243824 | AB807510 AB797722 LC014540 AB808486 | Tanaka et al. 2015       |
| *Aquilomyces patris*          | CBS 135661<sup>T</sup>     | KP184041 KP184077 KP184002 – | Knapp et al. 2015        |
| *Aquilomyces rebunensis*      | CBS 139684<sup>T</sup>     | AB807542 AB797252 AB809630 AB808518 | Tanaka et al. 2015       |
| *Bambusicola masarinia*       | MFLUCC 11-0389<sup>T</sup> | JX442037 JX442041 NR_121548 – | Dai et al. 2012          |
| *Clupeoloculus akitaensis*    | CBS 139681<sup>T</sup>     | AB807543 AB797253 AB809631 AB808519 | Tanaka et al. 2015       |
| *Corynespora cassicola*       | CBS 100822<sup>T</sup>     | GU301808 GU296144 – GU349052 | Schoch et al. 2009       |
| *Corynespora smithii*         | CABI 5649b                 | GU323201 – FJ852597 GU349018 | Schoch et al. 2009       |
| *Falciformispora lignitilis*  | BCC 21117                  | GU371826 GU371834 KF432942 GU371819 | Schoch et al. 2009       |
| *Falciformispora senegalensis*| CBS 196.79<sup>T</sup>     | KF015631 KF015636 KF015673 KF015687 | Ahmed et al. 2014        |
| *Falciformispora tompkinsii*  | CBS 200.79<sup>T</sup>     | KF015625 KF015639 NR_120401 KF015685 | Ahmed et al. 2014        |
| *Helicascus elaterascus*      | A22-5A = HKUCC 7769        | AY787934 AF053727 – – | Tanaka et al. 2015       |
| *Massarina eburnea*           | CBS 473.64                 | GU301840 GU296170 – GU349040 | Zhang et al. 2009        |
| *Monodictys*                  | KH 331 = MAFF 243826      | AB807553 AB797263 – AB808529 | Tanaka et al. 2015       |
| *Monodictys*                  | JO 10 = MAFF 243825       | AB807552 AB797262 – AB808528 | Tanaka et al. 2015       |
| *Morosphaeria ramunculicola*  | BCC 18404                  | GQ925853 GQ925838 – – | Suetrong et al. 2009     |
| *Morosphaeria velatisspora*   | BCC 17059<sup>T</sup>     | GQ925852 GQ925841 – – | Suetrong et al. 2010     |
| *Multilocularia bambusae*     | MFLUCC 11-0180<sup>T</sup> | KU693438 KU693442 KU693446 – | Li et al. 2016           |
| *Multiseptospora thaiandica*  | MFLUCC 11-0183<sup>T</sup> | KP744490 KP753955 KP744447 – | Liu et al. 2015           |
| *Multiseptospora thailandica* | MFLUCC 11-0204             | KU693440 KU693444 KU693447 KU705659 | Liu et al. 2015           |
| *Multiseptospora thaiandica*  | MFLUCC 12-0006             | KU693441 KU693445 KU693448 KU705660 | Liu et al. 2015           |
| *Multiseptospora thyanolaeae* | MFLUCC 11-0238<sup>T</sup> | KU693439 KU693443 – KU705658 | Li et al. 2016           |
| *Neoaquastroma baubinia*      | MFLUCC 16-0398<sup>T</sup> | MH023319 MH023315 MH025952 MH028247 | This study               |
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| Taxon                        | Culture accession number(s) | GenBank accession numbers | References              |
|------------------------------|-----------------------------|---------------------------|-------------------------|
| *Neoaquastroma bauhiniae*    | MFLUCC 17-2205              | MH023320 MH023316 MH025953 MH028248 | This study              |
| *Neoaquastroma krabiense*    | MFLUCC 16-0419†             | MH023321 MH023317 MH025954 MH028249 | This study              |
| *Neoaquastroma guttulatum*   | MFLUCC 14-0917†             | XX949740 XX949741 XX949739 XX949742 | Wanasinghe et al. 2017  |
| *Palmiascoma gregariascomum*  | MFLUCC 11-0175†             | KP744495 KP753958 KP744452 – | Liu et al. 2015        |
| *Parabambusicola bambusina*  | KH 139 = MAFF 243823        | AB807537 AB797247 LC014579 AB808512 | Tanaka et al. 2015     |
| *Parabambusicola bambusina*  | H 4321 = MAFF 239462        | AB807536 AB797246 LC014578 AB808511 | Tanaka et al. 2015     |
| *Parabambusicola bambusina*  | KT 2637 = MAFF 243822       | AB807538 AB797248 LC014580 AB808513 | Tanaka et al. 2015     |
| *Pseudomonodictys tectonae*  | MFLUCC 12-0552              | KT285573 KT285574 – – KT285571 | Ariyawansa et al. 2015 |
| *Stagonospora pseudocaricis* | CBS 135132                  | KF251762 – KF251259 – – | Quaedvlieg et al. 2013 |
| *Trematosphaeria pertusa*    | CBS 122368†                 | FJ201990 FJ201991 NR_132040 KF015701 | Zhang et al. 2008      |

1 Abbreviations: **BCC**: BIOTEC Culture Collection, Bangkok, Thailand; **CABI**: Centre for Agriculture and Biosciences International, Egham, UK; **CBS**: CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CPC**: Culture collection of Pedro Crous, housed at CBS; **HKUCC**: The University of Hong Kong Culture Collection; **HHUF**: Herbarium of Hirosaki University, Fungi; **JCM**: The Japan Collection of Microorganisms, Japan; **JK**: J. Kohlmeyer; **JO**: J. Onodera; **KH**: K. Hirayama; **KT**: K. Tanaka; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLU**: Mae Fah Luang University herbarium, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

2 Status of the strains: (T) ex-type, (ET) ex-epitype. The strains generated in this study are given in bold.

version 7.220 (Katoh et al. 2013) online sequence alignment tools (mafft.cbrc.jp/alignment/server), with minimal adjustment of the ambiguous nucleotides by visual examination and manually corrected in AliView programme (Larsson 2014). Leading or trailing gaps exceeding from primer binding site were trimmed from the alignments prior to tree building and alignment gaps were treated as missing data. The concatenation of the multigene alignment was created in MEGA 6 (Tamura et al. 2013).

Maximum likelihood analyses (ML), including 1,000 bootstrap replicates, was performed using RAxML (Stamatakis 2014) as implemented in raxmlGUI version v.1.3.1 (Silvestro and Michalak 2011). The search strategy was set to rapid bootstrapping. The analysis was carried out with the general time reversible (GTR) model for nucleotide substitution and a discrete gamma-distributed with four rate categories (O’Meara et al. 2006, Stamatakis et al. 2008). The bootstrap replicates were summarised on to the best scoring tree.

The best fitting substitution model for each single gene partition and the concatenated data set was determined in MrModeltest 2.3 (Nylander 2004) for Bayesian
inference posterior probabilities (PP). In our analysis, GTR+I+G model was used for each partition. The Bayesian inference posterior probabilities (PP) distribution (Zhaxybayeva and Gogarten 2002) was estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation, thus 10,000 trees were obtained. The suitable burn-in phases were determined by traces inspected in Tracer version 1.6 (Rambaut et al. 2014). Based on the tracer analysis, the first 1,000 trees representing 10% of burn-in phase of the analyses were discarded. While the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01).

Phylogenetic trees and data files were visualised in FigTree v. 1.4 (Rambaut and Drummond 2008). The phylogram with bootstrap values and/or posterior probabilities on the branches are presented in Fig. 1 by using graphical options available in Adobe Illustrator CS v. 6. All sequences generated in this study were submitted to GenBank. The finalised alignment and tree were deposited in TreeBASE, submission ID: 22419 (http://www.treebase.org/). Maximum likelihood bootstrap values equal to or greater than 70% with Bayesian Posterior Probabilities (PP) equal or greater than 0.90 are presented below or above each node (Fig. 1).

Results
Phylogenetic analyses

The phylogenetic tree included 32 taxa representing six families from the suborder Massarineae. The phylogenetic trees from each individual data sets were initially generated, these were not significantly different (data not shown) and therefore combined data sets were performed. The combined dataset consisting 3,554 nucleotide characters, of which 1,001 characters corresponded to LSU, 1,038 characters to SSU, 508 characters to ITS and 929 characters to \textit{tef1}. \textit{Corynespora smithii} (CABI 5649b) and \textit{C. cassiicola} (CBS 100822) are used as outgroup taxa. An insertion in the SSU rDNA region of isolates \textit{Aquilomyces rebunensis} Tanaka & K. Hiray. (CBS 139684), \textit{Clypeoloculus aki-taensis} Tanaka & K. Hiray. (CBS 139681) and \textit{Trematosphaeria pertusa} Fuckel (CBS 122368) were excluded from the analysis prior to tree building. The best scoring tree from maximum likelihood analysis was selected with a final likelihood value of – In: 23014.934293 and the result is presented in Fig. 1. Phylogenetic trees obtained from maximum likelihood and Bayesian analyses yielded trees with similar overall topology as that of previous work (Tanaka et al. 2015, Liu et al. 2015, Wanasinghe et al. 2017).

In this study, the family Parabambusicolaceae received high support in the phylogenetic analysis. While within the family, the taxa are separated into three subclades (Fig. 1). \textit{Parabambusicola bambusina}, the generic type, clustered with \textit{Multiseptospora} with high support. However, \textit{M. thysanolaenae} (MFLUCC 11-0202) formed a sister
Figure 1. The best scoring RAxML tree based on a combined partial LSU, SSU, ITS and tef1 gene datasets. Bootstrap values (BS) from maximum likelihood (ML, left) of more than 70% BS and Bayesian posterior probabilities (PP, right) greater than 0.90 are given above or below the nodes. The tree is rooted with Corynespora smithii (CABI 5649b) and C. cassiicola (CBS 100822) in Corynesporaceae. The species, determined in this study, are indicated in blue. The ex-type and references strains are indicated in bold. Hyphens (-) represent support values less than 70% BS/0.90 PP. Thick branches represent significant support values from all analyses (BS ≥ 70%/PP ≥ 0.95).

taxon with Parabambusicola bambusina (Clade A). Aquastroma magniostiolata (CBS 139680) and Multilocularia bambusae (MFLUCC 11-0180) formed a clade with the hyphomycetes strains of Monodictys spp. and Pseudomonodictys tectonae (MFLUCC 12-0552) with high support in all computational methods (Clade B). Neoaquastroma formed a basal clade (Clade C), with N. bauhiniae (MFLUCC 16-0398, 17-2205) and N. krabiense (MFLUCC 16-0423) clustered with the type species N. guttulatum, with...
strong support (100% ML /1.00 PP). We describe the new taxa based on agreement in support for all computational methods (Jeewon and Hyde 2016). The new sequence data is deposited in GenBank (Table 1).

Taxonomy

*Neoaquastroma baubiniae* C. Phukhams. & K.D. Hyde, sp. nov.

MycoBank: MB824673

Facesoffungi number: FoF04371

Figure 2

**Etymology.** Name refers to the host from which this fungus was isolated.

**Type material.** THAILAND. Phrae Province: Song District, on dead twigs of *Bauhinia variegata* L. (Fabaceae), 25 July 2015, C. Phukhamsakda, S1-11, MFLU 17-0002 (holotype), MFLUCC 16-0398 = ICMP 21572 (ex-type living culture).

**Description.** Saprobic on dead twigs of *Bauhinia variegata* L. Sexual morph. Ascomata 113–190 µm high × 170–307 µm diam. (⁎ = 160 × 260 µm, n = 10), semi-immersed to immersed, solitary, scattered, subglobose to compressed, coriaceous, brown to dark brown, rough-walled, with short hyphae projecting from peridium, ostiolate. Ostiole 33 × 85 µm diam., centrally located, papillate, periphysoid. Peridium 8–25 µm wide (⁎ = 17, n = 30), with cells 3–8 µm wide, composed of 3 layers of reddish-brown to dark brown, cells of *textura angularis*, inner layer composed of hyaline gelatinous cells. Hamathecium composed of numerous, dense, long, 1–2.4 µm (⁎ = 1.7 µm, n = 50), narrow, filiform, transversely septate, branched, anastomosing, cellular pseudoparaphyses. Asci 53–116 × 26–43 µm (⁎ = 98 × 37 µm, n = 30), 8-spored, bitunicate, fissitunicate, obvoid to oblong, with furcate pedicel, with ocular chamber visible when immature. Ascospores 37–46 × 9–16 µm (⁎ = 43 × 13 µm, n = 50), bi-seriate or overlapping, broad fusiform, narrow towards the apex, initially hyaline, becoming brown to dark brown at maturity, 4–7-transversely euseptate, constricted at the septa, with cell above central septum wider, rough-walled, indentations present, surrounded by 7–12 µm wide, mucilaginous sheath. Asexual morph coelomycetous. Pycnidia produced on mycelium in water agar. Conidiomata 33–49 µm high × 92–108 µm wide diam., pycnidial, dark brown to black, covered by dense vegetative hyphae, globose, in agar immersed to superficial, uniloculate, solitary to scattered, ostiolate. Conidiomatal wall thin, brown to black-walled with cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 3–4 × 2–3.5 µm, enteroblastic, phialidic, integrated, oblong, hyaline, formed from the inner layer of pycnidium wall. Conidia 2–4 × 1.5–2 µm (⁎ = 3 × 1.7 µm, n = 100), broad-oblong to oval, hyaline, aseptate, smooth-walled.

**Culture characteristics.** Colonies on MEA, reaching 50 mm diam. after 4 weeks at 25 °C. Culture dark olive-green with black centre, with dense mycelia, circular, flat, umbonate, rough surface, dull, fimbriate, radially furrowed, covered with white aerial
Two novel species of *Neoaquastroma* (Parabambusicolaceae, Pleosporales).

Figure 2. *Neoaquastroma bauhiniae* (MFLU 17-0002, holotype) a Appearance of ascomata on host surface b Close up of ascoma c Section of ascoma d Ostiolar canal e Section of partial peridium layer f Pseudoparaphyses g–j Development state of asci j Asci produced in culture k–p Development state of ascospores; n, o Senescent spores m, p ascospores in 5% of KOH reagent; q Ascospores stained with India ink, sheath surrounding the entire ascospore r Germinated ascospore s, t Culture character on MEA u Conidiomata forming on agar on rice straw media after 8 weeks v Immature conidiomata w Conidiomatal wall x, y Conidiogenous cells and developing conidia z Conidia j, m Asci and ascospore in culture (on rice straw). Scale bars: 500 µm (b); 100 µm (c, v); 50 µm (d–j); 20 µm (k–r, w); 5 µm (x–z).
mycelium; mycelium strongly radiating into agar, yellow pigment diffusing in the agar; reverse black with radiating brown mycelium. Sexual and asexual morphs formed in culture. Morphology of sexual phase similar to those on substrate.

**Additional material examined.** THAILAND, Phrae Province, Song District, on dead twigs of Bauhinia variegata L. (Fabaceae), 25 July 2015, C Phukhamsakda, S1-11 (isotype in HKAS, under the code of HKAS 99513); ibid., on dead twigs of Bauhinia purpurea L. (Fabaceae), 5 May 2016, C Phukhamsakda, S1_03_16, ex-paratype living culture, MFLUCC 17-2205.

**Distribution.** Phrae Province, Thailand.

**Notes.** Neoaquastroma bauhiniae is similar to *N. krabiense*, but the ascomata, asci and ascospores are smaller and the species also has a thinner peridium with 4–7 septate hyaline ascospores. Thus, *Neoaquastroma bauhiniae* is introduced as a second species in *Neoaquastroma* based on its unique morphology coupled with high support values from the phylogenetic analysis (100% ML/1.00 PP, Fig. 1). Tanaka et al. (2015) only described the asexual morph in *Parabambusicola* to produce spermatia. We now obtained a single spore isolate which produces both sexual and asexual morphs in culture. The asexual morph of *Neoaquastroma bauhiniae* produced pycnidial conidiomata with hyaline conidia (Fig. 2, u–z).

**Neoaquastroma krabiense** C. Phukhams. & K.D. Hyde, sp. nov.
MycoBank: MB824674
Facesoffungi number: FoF04372
Figure 3

**Etymology.** Name refers the location where this fungus was collected.

**Type material.** THAILAND, Krabi Province: Meuang district, on dead twigs of Barringtonia acutangula (L.) Gaertn. 16 December 2015, C. Phukhamsakda, Kr015, MFLU 17-0003 (holotype), MFLUCC 16-0419 = ICMP 21572 (ex-type living culture).

**Description.** Saprobic on dead twigs of Barringtonia acutangula (L.) Gaertn. Sexual morph. Ascomata 404–498 μm high × 290–319 μm diam. (x̄ = 426 × 300 μm, n = 10), immersed in bark, solitary, scattered or sometimes gregarious, compressed globose, with a flattened base, coriaceous, black to dark brown, smooth, papillate, ostiolate. Ostiole 137–146 μm high × 117–154 μm diam. (x̄ = 143 × 137 μm, n = 10), centrally located, oblong, filled with hyaline periphysoid. Peridium 45–73 μm wide (x̄ = 56, n = 30), cell width 3–12 (x̄ = 8 μm, n = 40) composed of 6–10(–13 at base) layers of blackish-brown to dark brown, with cells of textura angularis, outer layer heavily pigmented, inner layer composed of hyaline gelatinous cells. Hamathecium composed of numerous, dense, long, 1.6–2.4 μm (x̄ = 2 μm, n = 50), broad, filiform, transversely septate, branched, anastomosing, cellular pseudoparaphyses. Asci 95–169 × 29–45 μm (x̄ = 135 × 35 μm, n = 25), 8-spored, bitunicate, fisitunicate, oboviod to clavate, with furcate pedicel, ocular chamber clearly visible when immature. Ascospores 50–64 × 9–18 μm (x̄...
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**Figure 3.** *Neoaquastroma krabiense* (MFLU 17-0003, holotype) **a** Barringtonia acutangula (L.) Gaertn specimens **b** Appearance of ascomata on host surface **c** Close up of ascomata **d** Ascomata forming on rice straw on WA after 8 weeks **e**, **f** Section of ascoma **g** Ostiolar canal **h** Section of partial peridium layer **i** Hyaline pseudoparaphyses **j**–**m** Asci **n**–**s** Hyaline ascospores with visible mucilaginous sheath **q** Ascospores stained in Indian ink to show sheath **u** Germinated ascospore **v**, **w** Culture characteristics on MEA **x**, **y** Conidiomata forming in culture after 8 weeks **z** Conidiomatal wall **aa**–**ad** Conidiogenous cells and developing conidia **ae** Conidia **n**–**p** Ascospores in 5% of KOH reagent **m**, **r** Asci and ascospore in culture (on rice straw). Scale bars: 500 µm (**c**–**e**); 200 µm (**f**, **x**); 50 µm (**g**–**m**, **y**), 20 µm (**n**–**u**, **z**); 5 µm (**aa**–**af**); 20 mm (**v**–**w**).
= 57 × 13 µm, n = 50), bi-seriate or overlapping, fusiform, narrow towards the apex, hyaline, 5–8-transversely septate, constricted at the septa, cell above central septum slightly wider, rough-walled, indentations present when mature, granulate when stained with India ink, surrounded by 3–9 µm wide, mucilaginous sheath. Asexual morph coelomycetous, formed on rice straw agar. Conidiomata 84–90 µm high × 73–89 µm wide, pycnidial, uniloculate, confluent or scattered, superficial, covered with dense vegetative hyphae, globose, dark brown to black. Conidiomatal wall thin, brown to black-walled cells of textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 3–5 × 1.5–4 µm, enteroblastic, phialidic, integrated, broad-cylindrical to oblong, hyaline, formed from the inner layer of pycnidium wall. Conidia 2–4 × 1.5–2.5 µm (x = 3 × 2 µm, n = 60), ellipsoidal to oblong, hyaline, aseptate, smooth-walled.

Culture characteristics. Colonies on MEA, reaching 50 mm diam. after four weeks at 25 °C. Culture grey, becoming dark-olive brown after four weeks, of dense mycelia, colonies circular, flat, umbonate, raised from the agar in the centre, surface rough, dull, covered with aerial mycelium, white mycelium radiating into the agar, pale orange pigment diffusing in the agar; reverse black, dense, circular, with irregular, fimbriate margin. Sexual and asexual morphs formed in culture. Morphology of sexual phase similar to those on the substrates.

Additional material examined. THAILAND, Krabi Province, Meuang district, on dead twigs of Barringtonia acutangula (Lecythidaceae), 16 December 2015, C. Phukhamsakda, Kr015, (isotype in HKAS, under the code of HKAS 99512).

Distribution. Krabi Province, Thailand

Notes. Neoaquastroma krabiense was collected in the southern part of Thailand on dead twigs of Barringtonia acutangula. It is placed in Neoaquastroma based on its morphology of both sexual and asexual morph and close phylogenetic affinity to other species of Neoaquastroma. Neoaquastroma krabiense is distinct in that it has a flattened ascomata base and larger and more slender asci and ascospores than N. guttulatum and N. baubinae. The species formed an asexual morph in culture (Fig. 3, m) as pycnidial conidiomata with hyaline conidia (Fig. 3, x-ae).

Discussion

In the present study, we introduce two new species of Neoaquastroma, as N. baubinae and N. krabiense. The descriptions were made from fungi isolated from dicotyledonous plants in Thailand. The new species are introduced based on multi-locus phylogeny coupled with morphology that support their placement within Parabambusicolaceae. Parabambusicolaceae is typified with Parabambusicola Tanaka & K. Hiray. The type of the genus was described originally as Massarina bambusina Teng (Teng, 1936) from bamboo. The family is characterised by ascomata surrounded by stromatic tissues and multiseptate, clavate to fusiform and hyaline ascospores (Tanaka and Harada 2003, Tanaka et al. 2015). The asexual morph in Parabambusicolaceae can be coelomycetous or hyphomycetous. Sporodochia or pycnidia with hyaline conidia are formed in
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Parabambusicola and Neoaquastroma (Tanaka et al. 2015, this study), while hyphomycetous structures are known from Pseudomonodictys and Monodictys spp. (Ariyawansa et al. 2015, Tanaka et al. 2015).

Neoaquastroma was introduced as a distinct genus in Parabambusicolaceae, with N. guttulatum as the type species (Wanasinghe et al. 2017). The genus resembles Parabambusicola and Multiseptospora, but form distinct lineages in phylogenetic studies (Liu et al. 2015, Tanaka et al. 2015, Wanasinghe et al. 2017). Parabambusicola and Neoaquastroma are similar in their morphology. The differentiation between Multiseptospora, Neoaquastroma and Parabambusicola is predominantly based on the morphology of ascospores, particularly with the size and number of septa.

In the phylogenetic analyses of Wanasinghe et al. (2017), Parabambusicolaceae clustered into three clades, where Neoaquastroma guttulatum (MFLUCC 14-0917) clustered with Aquastroma magnioistiolata (KT 2485), Multilocularia bambusae (MFLUCC 11-0180), Monodictys sp. (JO 10, KH 331) and Pseudomonodictys tectonae (MFLUCC 12-0552) with high statistical support. In this study, Neoaquastroma forms a separate clade, sister to Multiseptospora and Parabambusicola. This is probably due to limited taxon sampling.

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References

Ahmed SA, Van De Sande WW, Stevens DA, Fahal A, Van Diepeningen AD, Menken SB, de Hoog GS (2014) Revision of agents of black-grain eumycetoma in the order Pleosporales. Persoonia 33: 141–154. http://doi.org/10.3767/003158514X684744
Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Chethana T, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Luecking R, Ghobad-Nejhad M (2015) Fungal diversity notes 111–252 – taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75(1): 27–274. http://dx.doi.org/10.1007/s13225-015-0346-5
Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. http://doi.org/10.2307/3761358
Dai DQ, Bhat DJ, Liu JK, Chukatirote E, Zhao RL, Hyde KD (2012) Bambusicola, a new genus from bamboo with asexual and sexual morphs. Cryptogamie Mycologie 33(3): 363–379. https://doi.org/10.7872/crym.v33.iss3.2012.363
Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote E (2017) Bambusicolous fungi. Fungal Diversity 82(1): 1–105. https://doi.org/10.1007/s13225-016-0367-8

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hyde KD (1989) Ecology of tropical marine fungi. Hydrobiologia 178: 199–208. https://doi.org/10.1007/BF00006027

Jaktlitsch WM, Olariaga I, Voglmayr H (2016) Teichospora and the Teichosporaceae. Mycological Progress 15(31): 1–20. https://doi.org/10.1007/s11557-016-1171-2

Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J, Buyck B, Cai L, Dai YC, Abd-Elsalam KA, Ertz D, Hidayat I, Jeewon R (2015) The Faces of fungi database: fungal names linked with morphology, molecular and human attributes. Fungal Diversity 74(1): 3–18. https://doi.org/10.1007/s13225-015-0351-8

Jeewon R, Hyde KD (2016) Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7(11): 1669–1677. https://doi.org/10.5943/mycosphere/7/11/4

Jones EBG (2000) Marine fungi: some factors influencing biodiversity. Fungal Diversity 4: 53–73.

Jones EBG, Pilantanaapak A, Chatmala I, Sakayaraj J, Phongpaichit S, Choeyklin R (2006) Thai marine fungal diversity. Songklanakarin Journal of Science and Technology 28: 687–708.

Katoh K, Standley K (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. http://dx.doi.org/10.1093/molbev/mst010

Knapp DG, Kovács GM, Zajta E, Groenewald JZ, Crous PW (2015) Dark septate endophytic pleosporalean genera from semiarid areas. Persoonia 35: 87–100. https://doi.org/10.3767/003158515X687669

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30(22): 3276–3278. http://doi.org/10.1093/bioinformatics/btu531

Li GJ, Hyde KD, Zhao RL, Hongsanan S, Abdel-Aziz FA, Abdel-Wahab MA, Alvarado P, Alves-Silva G, Ammirati JF, Ariyawansa HA, Baghela A (2016) Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 78(1): 1–237. http://doi.org/10.1007/s13225-016-0366-9

Liu JK, Hyde KD, Jones EG, Ariyawansa HA, Bhat DJ, Boonmee S, Maharachchikumbura SS, McKenzie EH, Phookamsak R, Phukhamsakda C, Shenoy BD (2015) Fungal Diversity Notes 1–110: Taxonomic and phylogenetic contributions to fungal species. Fungal Diversity 72(1): 1–197. http://doi.org/10.1007/s13225-015-0324-y

MacKinnon J, MacKinnon K, Child G, Thorsell J (1986) Managing Protected Areas in the Tropics IUCN, Gland, Switzerland and Cambridge, UK, 295.

Mapook A, Hyde KD, Dai DQ, Li J, JONES EG, Bahkali AH, Boonmee S (2016) Muyocopronales, ord nov, (Dothideomycetes, Ascomycota) and a reappraisal of Muyocopron species from northern Thailand. Phytotaxa 265: 225–237. http://doi.org/10.11646/phytotaxa.265.3.3

Marod D, Kutintara U (2012) Biodiversity observation and monitoring in Thailand. The Biodiversity Observation Network in the Asia-Pacific Region. Springer, Japan, 53–63. https://doi.org/10.1007/978-4-431-54032-8_5
Two novel species of *Neoaquastroma* (Parabambusicolaceae, Pleosporales)...
Suetrong S, Schoch CL, Spatafora JW, Kohlmeyer J, Volkman-Kohlmeyer B, Sakayaroj J, Phongpaichit S, Tanaka K, Hirayama K, Jones EB (2009) Molecular systematics of the marine Dothideomycetes. Studies in Mycology 64: 155–173. https://doi.org/10.3114/sim.2009.64.09

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (* and other methods) Sunderland, MA. https://doi.org/10.1002/0471650129.dob0522

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 60. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/msr176

Tanaka K, Harada Y (2003) Pleosporales in Japan (1): the genus Lophiostoma. Mycoscience 44(2): 85–96. https://doi.org/10.1007/s10267-002-0085-9

Tanaka K, Harada Y (2003) Pleosporales in Japan (3) The genus Massarina. Mycoscience 44(3): 173–185. https://doi.org/10.1007/s10267-003-0102-7

Tanaka K, Hirayama K, Yonezawa H, Sato G, Toriyabe A, Kudo H, Hashimoto A, Matsumura M, Harada Y, Kurihara Y, Shirouzu T (2015) Revision of the Massarineae (Pleosporales, Dothideomycetes). Studies in Mycology 82: 75–136. https://doi.org/10.1016/j.simyco.2015.10.002

Teng SC (1936) Additional fungi from China II. Sinensia 7: 490–527.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wanasinghe DN, Hyde KD, Konta S, To-Anun C, Jones EG (2017) Saprobic Dothideomycetes in Thailand: Neoaquastroma gen. nov. (Parabambusicolaceae) introduced based on morphological and molecular data. Phytotaxa 302: 133–144. http://doi.org/10.11646/phytotaxa.302.2.3

Wendt L, Sir EB, Kuhnert E, Heitkämper S, Lambert C, Hladki AI, Romero AI, Luangsa-ard JJ, Srikitikulchai P, Peršoh D, Stadler M (2018) Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. Mycological Progress 17(1–2): 115–154. http://doi.org/10.1007/s11557-017-1311-3

White TJ, Bruns T, Lee SJ, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1): 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK, Maharachchikumbura SS, Ekanayaka AH, Tian Q, Phookamsak R (2018) Outline of Ascomycota: 2017. Fungal Diversity 88(1): 167–263. http://doi.org/10.1007/s13225-018-0394-8

Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H, Kirk PM, Braun U, Singh RV, Crous PW, Kukwa M, Lücking R (2017) Notes for genera-Ascomycota. Fungal Diversity 86(1): 1–594. http://doi.org/10.1007/s13225-017-0386-0

Zhang Y, Fournier J, Pointing SB, Hyde KD (2008) Are Melanomma pulvis-pyrius and Trematosphaeria pertusa congeneric?. Fungal Diversity 33: 47–60. http://doi.org/10.13140/2.1.3875.1364

Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R, Pointing SB, Hyde KD (2009) Towards a phylogenetic clarification of Lophiostoma/Massarina and morphologically similar genera in the Pleosporales. Fungal Diversity 38: 225–251.

Zhaxybayeva O, Gogarten JP (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC genomics 3(1): 1–4. https://doi.org/10.1186/1471-2164-3-4
New *Fusarium* species from the Kruger National Park, South Africa

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Abstract

Three new *Fusarium* species, *F. convolutans*, *F. fredkrugeri*, and *F. transvaalense* (Ascomycota, Hypocreales, Nectriaceae) are described from soils collected in a catena landscape on a research supersite in the Kruger National Park, South Africa. The new taxa, isolated from the rhizosphere of three African herbaceous plants, *Kyphocarpa angustifolia*, *Melhania acuminata*, and *Sida cordifolia*, are described and illustrated by means of morphological and multilocus molecular analyses based on sequences from five DNA loci (CAL, EF-1\(\alpha\), RPB1, RPB2 and TUB). According to phylogenetic inference based on Maximum-likelihood and Bayesian approaches, the newly discovered species are distributed in the *Fusarium buharicum*, *F. fujikuroi*, and *F. sambucinum* species complexes.

Keywords

Natural parks, phylogeny, fungi, multigene, morphology, diversity

Introduction

Fungi are common colonisers of the plant rhizobiome and endosphere, where they play a key role in modulating the interactions between plant roots and soil (Zachow et al. 2009; Visioli et al. 2014). The direct and indirect interaction between fungal growth in the rhizosphere and its effect on plant growth and health is well docu-
mented (Havlicek and Mitchell 2014; Hargreaves et al. 2015; Lareen et al. 2016). Such effects include either a positive feedback by producing plant growth promoting factors, solubilising and stimulating nutrient uptake by plant roots or by inhibiting the growth of concomitant pathogenic organisms (Schippers et al. 1987; Mommer et al. 2016). Conversely, deleterious effects have also been observed, either related to the presence of pathogenic fungal species or caused by fungal-induced modifications of plant root functions, impeding root growth or negatively altering nutrient availability (Schippers et al. 1987; Mommer et al. 2016). Likewise, plants can select and harbour a particular fungal community on its roots via root exudates (Lareen et al. 2016; Sasse et al. 2018), while abiotic influences including water availability, climate and season, soil type, grazers and other animals, orchestrate the development of a unique fungal diversity (Philippot et al. 2013; Havlicek and Mitchell 2014; Hargreaves et al. 2015; Lareen et al. 2016).

The genus *Fusarium* Link (Hypocreales, Nectriaceae) includes a vast number of species, commonly recovered from a variety of substrates including soil, air, water and decaying plant materials; being also able to colonise living tissues of plants and animals, including humans; acting as endophytes, secondary invaders or becoming devastating plant pathogens (Nelson et al. 1994). In addition to their ability to colonise a multiplicity of habitats, *Fusarium* is a cosmopolitan genus, present in almost any ecosystem in the world, including human-made settings such as air and dust in the indoor environment or even in hospitals (Perlroth et al. 2007; Aydogdu and Asan 2008; Pinheiro et al. 2011).

Being common inhabitants of plant root ecosystems, fusaria and, particularly *Fusarium graminearum* Schwabe, *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. verticillioides* (Sacc.) Nirenberg (Syn. *F. moniliforme* J. Sheld.), *F. oxysporum* Schltdl., as well as species recently segregated from *Fusarium*, including *Neocosmospora phaseoli* (Burkh.) L. Lombard & Crous (Syn. *Fusarium phaseoli* Burkh.) and *N. virguliforme* (O’Donnell & T. Aoki) L. Lombard & Crous (Syn. *F. virguliforme* O’Donnell & T. Aoki), have been regularly studied for their interactions with the rhizobiome, motivated mainly by the importance of these organisms as soil-borne plant pathogens and the need to develop effective control mechanisms (Larkin et al. 1993; Hassan Dar et al. 1997; Pal et al. 2001; Fravel et al. 2003; Idris et al. 2006; Díaz Arias et al. 2013). Similarly, abundant data is available regarding the ecology and distribution of plant-associated fusaria, particularly related to pathogenic species or commonly isolated endophytes (Leslie and Summerell 2006). Little attention has however been given to the occurrence of non-pathogenic fungal species, including *Fusarium* spp. in root microbial communities (Zakaria and Ning 2013; Jumpponen et al. 2017; LeBlanc et al. 2017), while comprehensive DNA sequence-based surveys have been directed mostly to the study of highly relevant and abundant rhizosphere fungal genera such as *Trichoderma* Pers., *Verticillium* Nees or mycorrhizal fungi (Zachow et al. 2009; Bent et al. 2011; Ruano-Rosa et al. 2016; Saravanakumar et al. 2016).
The Kruger National Park (KNP) in South Africa is one of the largest natural reserves in Africa, encompassing a number of non-manipulated landscapes, with almost no human alteration (Carruthers 2017). Recently, four research “supersites” have been identified and established in KNP, each of these supersites representing unique geological, ecological and climatic features of the park (Smit et al. 2013). A multidisciplinary study was conducted in KNP aimed to determine functioning and interaction between abiotic and biotic components, as well as soil properties, hydrology and other processes that determine the structure, biodiversity and heterogeneity of a catena or hill slope ecosystem on one of these “supersites”, located deep inside the KNP (data not published). In order to assess the microbial soil population and community dynamics, mainly focused on bacteria, several rhizosphere samples were obtained from diverse African plants on one of these exceptional protected savannah landscapes. From these collections, interesting fusaria were isolated from the root ecosystem of three native African herbaceous plants i.e. *Kyphocarpa angustifolia* (Moq.) Lopr. (Amaranthaceae), *Melhania acuminata* Mast. (Malvaceae) and *Sida cordifolia* Linn. (Malvaceae). According to their unique morphological traits and clear phylogenetic delimitations, these isolates are described here as three new *Fusarium* species.

**Methods**

**Study site and sampling**

During March 2015, rhizosphere soil from three herbaceous plants was collected in the Southern Granites “supersite” catena (Stevenson-Hamilton supersite) in the KNP, between 25°06’28.6S, 31°34’41.9E and 25°06’25.7S, 31°34’33.7E (Fig. 1). A catena consists of different soil types observed from a crest to a valley bottom with a wetland or drainage exhibiting different water retention capabilities due to the slope or aspect (topography) and the depth of underlying geological rocks (Brown et al. 2004, Van Zijl and Le Roux 2014). The main characteristics of the Stevenson-Hamilton supersite are described in detail by Smit et al. (2013). Briefly, in this site, a single catena landscape covers approximately 1 km from top to bottom and consists of a hill slope, a sodic site (or grazing lawn), a riparian and floodplain area and a dry drainage line. Three species of plants were selected for sampling occurring at the two extremes of the catena. Two of these species (*Kyphocarpa angustifolia* and *Sida cordifolia*) occurred at both top and bottom sites while *Melhania acuminata* only occurred at the top site. The soil (100 mm depth) at the top of the slope is Clovelly with a high percentage of sand (90%) and a low cation exchange capacity (CEC) (mean sodium concentration of 1062 mg/kg) and pH (mean 5.85). The soil at the bottom of the slope is of the Sterkspruit type, with higher clay content thus higher CEC (mean sodium concentration of 3802 mg/kg) and higher pH (mean
6.4). Rhizosphere soil of 10 plants of the same species occurring at each top or bottom site was sampled using a core soil sampler. A total of 50 samples consisting of ca. 200 g of soil from the roots of each plant were taken, deposited in zip-lock plastic bags and kept on ice in a cool bag at approximately 5 °C until analysed in the laboratory.

**Isolation of *Fusarium* strains**

Soil samples were mixed thoroughly and sieved to remove large elements. Fine soil particles were uniformly spread and distributed over the surface of pentachloronitrobenzene agar (PCNB; also known as the Nash-Snyder medium, recipe in Leslie and Summerell 2006) supplemented with streptomycin (0.3 g/l) and neomycin sulphate (0.12 g/l) and malt-extract agar (MEA; recipes on Crous et al. 2009) on 9 mm Petri dishes and incubated at 24 °C for 10 d under a natural day/night photoperiod. Each soil sample was processed in duplicate. Fungal growth was evaluated daily and growing colonies were transferred to fresh Potato Dextrose Agar (PDA;
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Colony growth rates and production of diffusible pigments were evaluated on PDA, colony features were also recorded on corn-meal agar (CMA; recipe in Crous et al. 2009) and OA. Colour notations followed those of Rayner (1970). For the study of micro-morphological features, cultures were grown for 7–10 d at 24 °C, using a 12 h light/dark cycle with near UV and white fluorescent light. Aerial and sporodochial conidiophores and conidia and formation of chlamydospores were evaluated on Synthetic Nutrient-poor Agar (SNA; Nirenberg 1976) and on Carnation Leaf Agar (CLA; Fisher et al. 1982). Measurements and photomicrographs were recorded from a minimum of 30 elements for each structure, using sterile water as mounting medium and a Nikon Eclipse 80i microscope with Differential Interference Contrast (DIC) optics and a Nikon AZ100 dissecting microscope, both equipped with a Nikon DS-Ri2 high definition colour digital camera and the Nikon software NIS-elements D software v. 4.30.

**Morphological characterisation**

*Fusarium* isolates were characterised morphologically according to procedures described elsewhere (Aoki et al. 2013; Leslie and Summerell 2006, Sandoval-Denis et al. 2018). Colonial growth rates and production of diffusible pigments were evaluated on PDA, colony features were also recorded on corn-meal agar (CMA; recipe in Crous et al. 2009) and OA. Colour notations followed those of Rayner (1970). For the study of micro-morphological features, cultures were grown for 7–10 d at 24 °C, using a 12 h light/dark cycle with near UV and white fluorescent light. Aerial and sporodochial conidiophores and conidia and formation of chlamydospores were evaluated on Synthetic Nutrient-poor Agar (SNA; Nirenberg 1976) and on Carnation Leaf Agar (CLA; Fisher et al. 1982). Measurements and photomicrographs were recorded from a minimum of 30 elements for each structure, using sterile water as mounting medium and a Nikon Eclipse 80i microscope with Differential Interference Contrast (DIC) optics and a Nikon AZ100 dissecting microscope, both equipped with a Nikon DS-Ri2 high definition colour digital camera and the Nikon software NIS-elements D software v. 4.30.

**DNA isolation, amplification and sequencing**

Isolates were grown for 7 d on MEA at 24 °C using the photoperiod described above. Fresh mycelium was scraped from the colony surface and subjected to total DNA extraction using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer’s instructions. Fragments of five DNA loci were amplified using primers and PCR conditions described by O’Donnell et al. (2009) for calmodulin (*CAL*), O’Donnell et al. (2010) for the RNA polymerase largest subunit (*RPB1*) and second largest subunit (*RPB2*), O’Donnell et al. (1998) for the translation elongation factor 1-alpha (*EF-1α*) and Woudenberg et al. (2009) for
Table 1. Origin, strain and GenBank/ENA accession number of strains and DNA sequences included in this study.

| Species name | Strain\textsuperscript{T} | Country | Host | Sequence accession number\textsuperscript{b} |
|--------------|-----------------|---------|------|-----------------------------------|
| *Fusarium agapanthi* | NRRL 54463\textsuperscript{T} | Australia | Agapanthus sp. | KU900611 KU900630 KU900620 KU900625 KU900635 |
| *Fusarium ananatum* | CBS 118516\textsuperscript{T} | South Africa | Ananas comosus fruit | LT996175 LT996091 LT996188 LT996137 LT996112 |
| *Fusarium andiyazi* | CBS 119857\textsuperscript{T} = NRRL 31727 | South Africa | Sorghum bicolor soil debris | LT996176 LT996092 LT996189 LT996138 LT996113 |
| *Fusarium anthophilum* | CBS 737.97 = NRRL 13602 | Germany | Hippeastrum sp. | LT996177 LT996093 LT996190 LT996139 LT996114 |
| *Fusarium armeniacum* | NRRL 6227 | USA | Fescue hay | JX171446 JX171560 |
| *Fusarium asiaticum* | CBS 110257 = NRRL 13818 | Japan | Barley | JX171459 JX171573 |
| *Fusarium bactridioides* | NRRL 20476 | USA | Cronartium conigenum | AF158343 AF160290 Not public Not public U34434 |
| *Fusarium begoniae* | CBS 403.97\textsuperscript{T} = NRRL 25300 | Germany | Begonia elatior hybrid | AF158346 AF160293 LT996191 LT996140 U61543 |
| *Fusarium buharicum* | CBS 796.70 = NRRL 13371 | USSR | Gossypium rotting stem base | KX302912 KX302920 KX302928 |
| *Fusarium bulbicola* | CBS 220.76\textsuperscript{T} = NRRL 13618 | Germany | Nerine bowdennii | KF466327 KF466415 KF466394 KF466404 KF466437 |
| *Fusarium brachygibbosum* | NRRL 13829 | Japan | River sediments | JX171460 JX171574 |
| *Fusarium circinatum* | CBS 405.97\textsuperscript{T} = NRRL 25331 | USA | Pinus radiata | KM231393 KM231943 JX171510 HM068354 KM232080 |
| *Fusarium coicis* | NRRL 66233\textsuperscript{T} | Australia | Coix gasteenii | LT996178 KP083251 KP083269 KP083274 LT996115 |
| *Fusarium concentricum* | CBS 450.97\textsuperscript{T} = NRRL 25181 | Costa Rica | Musa sapientum fruit | AF158335 AF160282 LT996192 JF741086 U61548 |
| *Fusarium continua* | F201128 | China | Zanthoxylum bungeanum stem | KM236720 KM520389 KM236780 |
| *Fusarium convolutans* | CBS 144207\textsuperscript{T} = CPC 33733 | South Africa | Kyphocarpa angustifolia thizophere | LT996094 LT996193 LT996141 |
| *Fusarium culmorum* | CBS 144208 = CPC 33732 | South Africa | Kyphocarpa angustifolia thizophere | LT996095 LT996194 LT996142 |
| *Fusarium denticulatum* | CBS 417.86 = NRRL 25475 | Denmark | Moldy barley kernel | JX171515 JX171628 |
| *Fusarium denticulatum* | CBS 735.97 = NRRL 25302 | USA | Ipomoea batatas | AF158322 AF160269 LT996195 LT996143 U61550 |
| *Fusarium doliicinii* | CBS 119860\textsuperscript{T} = NRRL 13164 | South Africa | Soil debris in cornfield | AF158330 AF160277 KU171681 KU171701 U34430 |
| *Fusarium falciforme* | CBS 137234\textsuperscript{T} | Colombia | Pinus maximonii stem | LT996179 KJ541059 LT996196 LT996144 KJ541051 |
| *Fusarium fraxifolium* | NRRL 28852\textsuperscript{T} | Japan | Cymbidium sp. | AF158341 AF160288 Not public LT570564 AF160315 |
| *Fusarium fraxinellum* | NRRL 26152 | Niger | Unknown | AF160306 AF160321 |
| *Fusarium fraxifolium* | CBS 144209\textsuperscript{T} = CPC 33747 | South Africa | Melhania acuminata thizophere | LT996180 LT996097 LT996199 LT996147 LT996117 |
| *Fusarium fraxifolium* | CBS 144210 = NRRL 26061 | Madagascar | Striga hermonthica | AF158356 AF160303 LT996197 LT996145 AF160319 |
| *Fusarium fraxifolium* | CBS 144495 = CPC 33746 | South Africa | Melhania acuminata thizophere | LT996180 LT996096 LT996198 LT996146 LT996116 |
| Species name                  | Strain / Conf. / Country / Host                     | Sequence accession number | EF-Ts | RRPI | TUB  |
|------------------------------|----------------------------------------------------|---------------------------|-------|------|------|
| *Fusarium fujikuroi*         | NRRL 13566, China                                  | AF160279, KF466447        |       |      |      |
| *Fusarium globosum*          | CBS 6250 = NRRL 51084                              | KF466439, KF466466        |       |      |      |
| *Fusarium konzom*            | NRRL 13822, USA                                   | KF466417, KF466370        |       |      |      |
| *Fusarium longipes*          | NRRL 13368, Australia                              | LT996183, LT996204        |       |      |      |
| *Fusarium maccaronei*        | CBS 11949 / NRRL 13694                             | AF160272, JX171457        |       |      |      |
| *Fusarium oxysporum*         | CBS 749.97 / NRRL 34064, Germany                  | AF160281, AF160273,       |       |      |      |
| *Fusarium pseudograminearum* | CBS 109956 / NRRL 13592, Nigeria                  | AF160263, AF160263,       |       |      |      |
| *Fusarium pseudonygamai*     | CBS 41797 / NRRL 22946, Ghana                      | AF160271, AF160271,       |       |      |      |
| *Fusarium pseudophylophilum* | CBS 21776 / NRRL 22944, Belgium                   | LT996204, LT996204        |       |      |      |
| *Fusarium pseudosolani*      | CBS 227346 / NRRL 13617, Australia                | LT996183, LT996204        |       |      |      |
| *Fusarium pseudocircinatum*  | CBS 749.97 / NRRL 34064, Germany                  | AF160281, AF160273,       |       |      |      |
| *Fusarium pseudocoloniale*   | CBS 13726, Italy                                   | AF160263, AF160263,       |       |      |      |
| Species name | Strain$^{1,2}$ | Country | Host | Sequence accession number$^5$ |
|--------------|----------------|---------|------|-----------------------------|
| **Fusarium ramigenum** | CBS 418.98$^T$ = NRRL 25208 | USA | *Ficus carica* | CAL: KF466335, EF-1α: KF466423, RPB1: KF466401, RPB2: KF466412, TUB: KF466445 |
| **Fusarium sacchari** | CBS 223.76 = NRRL 13999 | India | Saccharum officinarum | CAL: AF158331, EF-1α: AF160278, RPB1: JX171466, RPB2: JX171580, TUB: U34414 |
| **Fusarium sambucinum** | NRRL 22187 = NRRL 20727 | England | Solanum sp. | CAL: AF158331, EF-1α: AF160278, RPB1: JX171466, RPB2: JX171580, TUB: U34414 |
| **Fusarium sarcochroum** | CBS 745.79 = NRRL 20472 | Switzerland | *Viscum album* | CAL: JX171472, EF-1α: JX171472, RPB1: JX171472, RPB2: JX171586, TUB: HL154472 |
| **Fusarium sibiricum** | NRRL 53430$^T$ | Russia | *Avena sativa* | CAL: LT996184, EF-1α: KJ541067, RPB1: LT996206, RPB2: LT996153, TUB: KJ541057 |
| **Fusarium sororuli** | CBS 137242$^T$ | Colombia | *Pinus patula stems* | CAL: LT996184, EF-1α: KJ541067, RPB1: LT996206, RPB2: LT996153, TUB: KJ541057 |
| *Fusarium* sp. | NRRL 66179 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | NRRL 66180 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | NRRL 66181 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | NRRL 66182 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | NRRL 66183 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | NRRL 66184 | USA | *Hibiscus moscheutos* | CAL: KX302913, EF-1α: KX302921, RPB1: KX302929, RPB2: KX302930, TUB: KX302934 |
| *Fusarium* sp. | CBS 201.63 = NRRL 36351 | Portugal | *Anachis hypogaesta stored nut* | CAL: GQ915484, EF-1α: HQ154454, RPB1: HQ154454, RPB2: HQ154454, TUB: HQ154454 |
| *Fusarium spororrichoiodes* | NRRL 3299 | USA | Corn | CAL: JX171444, EF-1α: HQ154454, RPB1: HQ154454, RPB2: HQ154454, TUB: HQ154454 |
| *Fusarium sterilbysomum* | NRRL 25623 | South Africa | Mango | CAL: AF158353, EF-1α: AF160300, RPB1: Not public, RPB2: Not public, TUB: AF160316 |
| *Fusarium silboides* | NRRL 20429 | Nyasaland | Coffee bark | CAL: JX171468, EF-1α: JX171582, RPB1: JX171582, RPB2: JX171582, TUB: JX171582 |
| *Fusarium subglutinans* | CBS 747.97 = NRRL 22016 | USA | Corn | AF158342, AF160289, JX171468, JX171599, U34417 |
| *Fusarium sublunatum* | CBS 190.34 = NRRL 20897 | Unknown | Unknown | CAL: KX302919, EF-1α: KX302927, RPB1: KX302935, RPB2: KX302935, TUB: KX302935 |
| *Fusarium succisae* | CBS 189.34$^T$ = NRRL 13384 | Costa Rica | Soil of banana plantation | CAL: JX171451, EF-1α: JX171565, RPB1: JX171565, RPB2: JX171565, TUB: JX171565 |
| *Fusarium sudanense* | CBS 219.76 = NRRL 13613 | Germany | *Succisa pratensis flower* | AF158344, AF160291, LT996207, LT996154, U34419 |
| *Fusarium temperatum* | CBS 454.97$^T$ = NRRL 25451 | Sudan | *Striga hermonthica* | LT996185, KU711697, LT996208, LT996155, KU603909 |
| *Fusarium terricola* | NRRL 25622 = NRRL 26616 | South Africa | *Zea mays* | AF158354, AF160301, Not public, Not public, AF160317 |
| *Fusarium thapsinum* | CBS 483.94$^T$ | Australia | Soil | KU603951, KU711698, LT996209, LT996156, KU603908 |
| *Fusarium thapsinum* | CBS 733.97 = NRRL 22045 | South Africa | *Sorghum bicolor* | LT996186, AF160270, JX171487, JX171600, U34418 |
| *Fusarium tjaetaba* | NRRL 66243$^T$ | Australia | *Sorghum interjectum* | LT996187, KP083263, KP083267, KP083275, LT996119 |
| *Fusarium torreyae* | NRRL 54149 | USA | *Torrey sp.* | HM068337, JX171548, HM068359 |
### New Fusarium species from the Kruger National Park, South Africa

| Species name | Strain | †‡ | Country | Host | Sequence accession number § |
|--------------|--------|----|---------|------|-----------------------------|
| Fusarium transvaalense | CBS 144211 = CPC 30932 | † | South Africa | Sida cordifolia rhizosphere | LT996099 LT996100 LT996101 LT996102 |
| Fusarium transvaalense | CBS 144212 = CPC 33751 | † | South Africa | Melhania acuminata rhizosphere | LT996123 LT996124 LT996125 LT996126 |
| Fusarium transvaalense | CBS 144213 = CPC 30919 | † | South Africa | Sida cordifolia rhizosphere | LT996103 LT996104 LT996105 LT996106 |
| Fusarium transvaalense | CBS 144220 = CPC 30918 | † | South Africa | Sida cordifolia rhizosphere | LT996167 LT996168 LT996169 LT996170 |
| Fusarium transvaalense | CBS 144221 = CPC 33723 | † | South Africa | Melhania acuminata rhizosphere | LT996131 LT996132 LT996133 LT996134 |
| Fusarium transvaalense | CBS 144222 = CPC 30932 | † | South Africa | Melhania acuminata rhizosphere | LT996135 LT996136 LT996137 LT996138 |
| Fusarium transvaalense | CBS 144223 = CPC 30933 | † | South Africa | Melhania acuminata rhizosphere | LT996139 LT996140 LT996141 LT996142 |
| Fusarium transvaalense | CBS 144224 = CPC 30934 | † | South Africa | Melhania acuminata rhizosphere | LT996143 LT996144 LT996145 LT996146 |
| Fusarium transvaalense | CBS 144225 = CPC 33750 | † | South Africa | Melhania acuminata rhizosphere | LT996147 LT996148 LT996149 LT996150 |

† CBS: Westerdijk Fungal Biodiversity Institute. CPC: Collection of Pedro W. Crous, held at CBS. F: College of Forestry, Northwest A&F University, Taicheng Road, Yangling, Shaanxi China. NRRL: Agricultural Research Service, Peoria, IL, USA.

‡ IT: ex-isotype culture. PT: ex-paratype culture. T: ex-type culture. NT: ex-neotypic culture.

§ † CAL: Calmodulin. EF-1α: Translation elongation factor 1-alpha. RPB1: RNA polymerase largest subunit. RPB2: RNA polymerase second largest subunit. 71β: Tubulin. New sequences are shown in bold. Sequences marked as “Not public” were obtained from Kerry O’Donnell’s alignment datasets.

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144211 = CPC 30932
- **Country:** South Africa
- **Host:** Sida cordifolia rhizosphere
- **Sequence accession number:** LT996099, LT996100, LT996101, LT996102

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144212 = CPC 33751
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996123, LT996124, LT996125, LT996126

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144213 = CPC 30919
- **Country:** South Africa
- **Host:** Sida cordifolia rhizosphere
- **Sequence accession number:** LT996103, LT996104, LT996105, LT996106

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144220 = CPC 30918
- **Country:** South Africa
- **Host:** Sida cordifolia rhizosphere
- **Sequence accession number:** LT996167, LT996168, LT996169, LT996170

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144221 = CPC 33723
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996131, LT996132, LT996133, LT996134

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144222 = CPC 30932
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996135, LT996136, LT996137, LT996138

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144223 = CPC 30933
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996139, LT996140, LT996141, LT996142

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144224 = CPC 30934
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996143, LT996144, LT996145, LT996146

### Fusarium transvaalense
- **Species name:** Fusarium transvaalense
- **Strain:** CBS 144225 = CPC 33750
- **Country:** South Africa
- **Host:** Melhania acuminata rhizosphere
- **Sequence accession number:** LT996147, LT996148, LT996149, LT996150
beta-tubulin (TUB). Sequencing was made in both strand directions using the same primer pairs as for PCR amplification on an Applied Biosystems, Hitachi 3730xl DNA analyser (Applied Biosystems Inc., Foster City, California, USA). Consensus sequences were assembled using Seqman Pro v. 10.0.1 (DNASTAR, Madison, WI, USA). All DNA sequences generated in this study were lodged in GenBank and the European Nucleotide Archive (ENA) (Table 1).

**Molecular identification and phylogenetic analyses**

A first analysis was based on pairwise alignments and blastn searches on the *Fusarium* MLST (http://www.westerdijkinstitute.nl/fusarium/) and NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) databases, respectively, using EF-1α and RPB2 sequences in order to resolve the position of the KNP isolates amongst the different species complexes recognised in *Fusarium* (O’Donnell et al. 2013). Sequences from individual loci were aligned using MAFFT (Katoh and Standley 2013), on the web server of the European Bioinformatics Institute (EMBL–EBI; http://www.ebi.ac.uk/Tools/msa/mafft/) (Li et al. 2015).

Phylogenetic analyses were based on Maximum-likelihood (ML) and Bayesian (B) analyses, both algorithms run on the CIPRES Science Gateway portal (Miller et al. 2012). Evolutionary models were calculated using MrModelTest v. 2.3 using the Akaike information criterion (Nylander 2004; Posada and Crandall 1998). For ML, RAxML-HPC2 v. 8.2.10 on XSEDE was used (Stamatakis 2014), clade stability was tested with a bootstrap analysis (BS) using the rapid bootstrapping algorithm with default parameters. The B analyses were run using MrBayes v. 3.2.6 on XSEDE (Ronquist and Huelsenbeck 2003) using four incrementally heated MCMC chains for 5M generations, with the stop-rule option on and sampling every 1000 trees. After convergence of the runs (average standard deviation of split frequencies below 0.01) the first 25% of samples were discarded as the burn-in fraction and 50% consensus trees and posterior probabilities (PP) were calculated from the remaining trees.

Phylogenies were first made individually for each locus dataset and visually compared for topological incongruence amongst statistically supported nodes (ML-BS ≥ 70% and B-PP ≥ 0.95) (Mason-Gamer and Kellogg 1996, Wiens 1998), before being concatenated for multi-locus analyses using different locus combinations according to strains and DNA sequences currently available in public databases, in addition to previously published phylogenies (O’Donnell et al. 2000, 2013; Herron et al. 2015; Lupien et al. 2017; Moussa et al. 2017, Sandoval-Denis et al. 2018). A further 232 sequences representing 72 taxa were retrieved from GenBank and included in the phylogenetic analyses, while an additional 58 DNA sequences were obtained from 24 fungal strains requested from the CBS and NRRL (Agricultural Research Service, Peoria, IL, USA) culture collections (Table 1). All alignments and trees generated in this study were uploaded to TreeBASE (https://treebase.org).
Results

Phylogenetic analyses

Pairwise DNA alignments and BLAST searches using EF-1α and RPB2 sequences showed that the 19 isolates from KNP belonged to three different species complexes of the genus Fusarium i.e. the F. buharicum Jacz. ex Babajan & T eterevn.-Babajan species complex (FBSC; two isolates), the F. fujikuroi Nirenberg species complex (FFSC; two isolates) and the F. sambucinum Fuckel species complex (FSAMSC; 15 isolates). According to these results, sequences of related taxa and lineages were retrieved from GenBank and incorporated into individual phylogenetic analyses for each species complex.

Multi-locus analyses were carried out in order to further delimit the KNP Fusarium isolates amongst the known diversity in their respective species complexes. With the exception of the FFSC, the topologies observed from ML and B analyses of single and multi-locus datasets were highly congruent, with only minor differences affecting unsupported nodes on the trees (all trees available in TreeBASE). The characteristics of the different alignments and tree statistics for all the species complexes are shown in Table 2.

The analysis of the FBSC included sequences of EF-1α, RPB1 and RPB2 loci from 18 isolates representing 10 taxa, including members of the Fusarium torreyae T. Aoki, J.A. Sm., L.L. Mount, Geiser & O’Donnell species complex (FTYSC) and Fusarium lateritium Nees species complex (FLSC) as outgroup (Fig. 2). The four ingroup taxa resolved with high statistical support. Two KNP isolates from K. angustifolia obtained from the bottom site of the catena (CBS 144207 and 144208) clustered in a sister relationship with the clade representing Fusarium sublunatum Reinking, but were genetically clearly delimited.

The phylogeny of the FFSC included sequences of CAL, EF-1α, RPB1, RPB2 and TUB loci from 48 strains and 44 taxa, including two outgroups (F. oxysporum CBS 716.74 and 744.97) (Fig. 3). The phylogeny showed a clear delimitation between the biogeographic clades recognised in this species complex (African, American and Asian clades sensu O’Donnell et al. 1998). Both American and Asian clades were shown as monophyletic with high ML-BS and B-PP support; in contrast, the African clade was resolved as polyphyletic, comprising two distinct and highly supported lineages. A terminal, speciose clade (African A) encompassing 17 taxa and a basal clade (African B), close to the American clade which included the ex-type of Fusarium dlaminii Marasas, P.E. Nelson & Toussoun (CBS 119860) and a sister terminal clade (ML-BS=100, B-PP=1) comprising two KNP isolates from M. acuminata (CBS 144209 and 144495) and two unidentified African Fusarium isolates (CBS 144210 and NRRL 26152). From the loci used here, only TUB resolved both African clades as sister groups; however, its monophyly was not supported by clade stability measurements (data not shown). Conversely, individual CAL, EF-1α and RPB2 phylog-
Table 2. Characteristics of the different datasets and statistics of phylogenetic analyses used in this study.

| Analysis† | Locus‡ | Number of Sites§ | Evolutionary model| Number of trees sampled in B | Maximum-likelihood statistics | Tree length |
|-----------|--------|------------------|-------------------|----------------------------|--------------------------------|-------------|
|           | Total  | Conserved | Phylogenetically informative | B unique patterns |                                 |             |

|       |       |       |                   |                       |                                |             |
| Fusarium buharicum SC | EF-1α | 495  | 300  | 119  | 198 | GTR+G | 414 | -11313.23702 | 0.598675 |
|       | RPB1  | 930  | 682  | 203  | 211 | SYM+G |       |                |          |
|       | RPB2  | 1663 | 1251 | 330  | 310 | GTR+I+G |      |                |          |
| Fusarium fujikuroi SC | CAL   | 545  | 423  | 67   | 167 | SYM+G | 282 | -20603.30043 | 0.567054 |
|       | EF-1α | 677  | 428  | 127  | 295 | GTR+I+G |       |                |          |
|       | RPB1  | 1534 | 1219 | 185  | 137 | SYM+I+G |      |                |          |
|       | RPB2  | 1551 | 1211 | 227  | 315 | GTR+I+G |      |                |          |
|       | TUB   | 488  | 351  | 66   | 336 | SYM+G |      |                |          |
| Fusarium sambucinum SC | RPB1  | 854  | 594  | 201  | 213 | SYM+I+G | 241 | -9871.793718 | 0.740271 |
|       | RPB2  | 1580 | 1128 | 346  | 396 | GTR+G |      |                |          |

† SC: Species complex.
‡ CAL: Calmodulin. EF-1α: Translation elongation factor 1-alpha. RPB1: RNA polymerase largest subunit. RPB2: RNA polymerase second largest subunit. TUB: Tubulin.
§ B: Bayesian inference.
|| G: Gamma distributed rate variation among sites. GTR: Generalised time-reversible. I: Proportion of invariable sites. SYM: Symmetrical model.

Figure 2. Maximum-likelihood (ML) phylogram obtained from combined EF-1α, RPB1 and RPB2 sequences of 18 strains belonging to the Fusarium buharicum (FBSC), Fusarium tricinctum (FTSC) and Fusarium lateritium (FLSC) species complexes. Numbers on the nodes are ML bootstrap values above 70% and Bayesian posterior probability values above 0.95. Branch lengths are proportional to distance. Ex-type strains are indicated with T. Strains corresponding to new species described here are shown in bold.
New *Fusarium* species from the Kruger National Park, South Africa

Figure 3. Maximum-likelihood (ML) phylogram obtained from combined *CAL, EF-1α, RPB1, RPB2* and *TUB* sequences of 48 strains belonging to the *Fusarium fujikuroi* (FFSC) and *Fusarium oxysporum* (FOSC) species complexes. Numbers on the nodes are ML bootstrap values above 70% and Bayesian posterior probability values above 0.95. Branch lengths are proportional to distance. Ex-type, ex-neotype and ex-paratype strains are indicated with *T*, *NT* and *PT*, respectively. Strains corresponding to new species described here are shown in **bold**.

...enies resolved African B as basal to the ingroup, while *RPB1* allocated this clade as basal to the American clade. Nonetheless, all the individual phylogenies, in addition to the combined dataset, clearly demonstrated genealogical uniqueness of the terminal clade encompassing KNP isolates.
The FSAMSC was studied using combined RPB1 and RPB2 sequences. The phylogeny included 35 isolates from 20 taxa, including the two outgroups *Fusarium circinatum* Nirenberg & O’Donnell (CBS 405.97) and *Fusarium fujikuroi* Nirenberg (NRRL 13566) (Fig. 4). Fifteen KPN *Fusarium* isolates from the three sampled plant species (three isolates from *K. angustifolia*, four isolates from *M. acuminata* and eight isolates from *S. cordifolia*), all obtained from the top site of the catena, clustered with an unidentified *Fusarium* isolate (NRRL 31008) in a distinct clade (ML-BS=100, B-PP=1), close to *Fusarium brachygibbosum* Padwick (strain NRRL 13829).
The clades including KNP isolates and corresponding to previously undisclosed lineages of *Fusarium* are described in the taxonomy section as the three novel species, *F. convolutans*, *F. fredkrugeri* and *F. transvaalense*.

**Taxonomy**

*Fusarium convolutans* Sandoval-Denis, Crous & W.J. Swart, sp. nov.
MycoBank: MB825102
Fig. 5

**Diagnosis.** Different from *F. circinatum*, *F. pseudocircinatum* O’Donnell & Nirenberg and *F. sterilhyphosum* Britz, Marasas & M.J. Wingf. by the absence of aerial conidia (microconidia) and the presence of chlamydospores. Different from *F. buharicum* Jacz. ex Babajan & Teteren.-Babajan and *F. sublunatum* by its shorter, less septate and less curved conidia and by the presence of sterile hyphal coils.

**Type.** South Africa, Kruger National Park, Skukuza, Granite Supersite, 25°06’33.9"S, 31°34’40.9E, from rhizosphere soil of *Kyphocarpa angustifolia*, 23 Mar 2015, W.J. Swart, holotype CBS H-23495, dried culture on OA, ex-holotype strain CBS 144207 = CPC 33733.

**Description.** Colonies on PDA growing in the dark with an average radial growth rate of 2.1–4.8 mm/d, 4.4–5.8 mm/d and 4.6–6.3 mm/d at 24, 27 and 30 °C, respectively; reaching 11–28 mm diam. in 7 d at 24 °C and a maximum of 23–37 mm diam. in 7 d at 30 °C. Minimum temperature for growth 12 °C, maximum 36 °C, optimal 27–33 °C. Colony surface white to cream coloured, flat and highly irregular in shape, velvety to felty, with scant and short aerial mycelium; colony margins highly irregular to rhizoid, with abundant white to grey submerged mycelium. Reverse white, straw to yellow diffusible pigment produced between 21–33 °C, scarcely produced and turning luteous to orange at 36 °C. Colonies on CMA and OA incubated in the dark reaching 40–48 mm diam. in 7 d at 24 °C. Colony surface white to cream coloured, flat or slightly elevated at the centre, velvety to dusty; aerial mycelium abundant, short and dense, concentrated on the colony centre; margins membranous and regular, buff to honey coloured, without aerial mycelium. Reverse ochreous without diffusible pigments. Sporulation scant from conidiophores formed on the aerial mycelium, sporodochia not formed. Conidiophores on the aerial mycelium straight or flexuous, smooth- and thin-walled, simple, mostly reduced to conidigenous cells borne laterally on hyphae or up to 50 µm tall, bearing terminal single or paired monophialides; phialides subulate to subcylindrical, smooth- and thin-walled, 15.5–22 µm long, (3.5–)4–5 µm at the widest point, with inconspicuous periclinal thickening and a short-flared collarette; conidia clustering in discrete false heads at the tip of monophialides, lunate to falcate, curved or somewhat straight, tapering gently toward the basal part, robust; apical cell often equal in length or slightly shorter than the adjacent cell, blunt
Figure 5. *Fusarium convolutans* sp. nov. A–D Colonies on PDA, SNA, OA and CMA, respectively, after 7 d at 24 °C in the dark E–I Conidiophores, phialides and conidia J–M Chlamydospores N–P Sterile hyphal projections Q Conidia. Scale bars: 20 µm (E, F); 5 µm (G–I); 10 µm (J–Q).
New Fusarium species from the Kruger National Park, South Africa

Fusarium convolutans Sandoval-Denis, Crous & W.J. Swart, sp. nov.
MycoBank: MB825103
Fig. 6

Diagnosis. Differs from Fusarium dlaminii Marasas, P.E. Nelson & Toussoun by producing only one type of aerial conidia, shorter sporodochial conidia and the absence of chlamydospores.

Type. South Africa, Kruger National Park, Skukuza, Granite Supersite, 25°06’48.6”S, 31°34’36.5”E, from rhizosphere soil of Melhania acuminata, 23 Mar 2015, W.J. Swart, holotype CBS H-23496, dried culture on OA, culture ex-holotype CBS 144209 = CPC 33747.

Description. Colonies on PDA growing in the dark with an average radial growth rate of 4.7–5.8 mm/d and reaching 22–35 mm diam. in 7 d at 24 °C, filling an entire 9 cm Petri dish in 7 d at 27 and 30 °C. Minimum temperature for growth 12 °C, maxi-
Figure 6. *Fusarium fredkrugeri* sp. nov. A–D Colonies on PDA, SNA, OA and CMA, respectively, after 7 d at 24 °C in the dark E–G Sporodochia formed on the surface of carnation leaves H–N Aerial conidiophores, phialides and conidia O, P Aerial conidia Q Sporodochial conidiophores and phialides R Sporodochial conidia. Scale bars: 100 µm (E–G); 10 µm (H–R).
New Fusarium species from the Kruger National Park, South Africa

Maximum 36 °C, optimal 27–30 °C. Colony surface at first white to cream coloured, later turning bay to chestnut with pale luteous to luteous periphery; flat, felty to cottony with abundant erect- aerial mycelium forming white patches; colony margins regular and filiform with abundant submerged mycelium. Reverse pale luteous, a blood sepa to chestnut coloured diffusible pigment is scarcely produced at 24 °C, pigment production is markedly enhanced at 27–30 °C, becoming greyish-sepia at 33 °C. Colonies on CMA and OA incubated at 24 °C in the dark reaching 65–67 mm diam. or occupying an entire 9 cm Petri dish in 7 d, respectively. Colony surface pale bay coloured, flat, felty to velvety, aerial mycelium scant, forming white to cream patches; margins regular. Reverse pale bay to pale vinaceous. Sporulation abundant from conidiophores formed on the substrate and aerial mycelium and from sporodochia. Conidiophores on the aerial mycelium straight or flexuous, erect or prostrate, septate, smooth- and thin-walled, often appearing rough by accumulation of extracellular material, commonly simple or reduced to conidiogenous cells borne laterally on hyphae or up to 200 µm tall and irregularly branched at various levels, branches bearing lateral and terminal monophialides borne mostly single or in pairs; phialides subulate, ampulliform, lageniform to subcylindrical, smooth- and thin-walled, (8.5–)9.5–17.5(–24.5) µm long, 2–3(–3.5) µm at the widest point, without periclinal thickening, collarets inconspicuous; conidia formed on aerial conidiophores, hyaline, obovoid, ellipsoidal to slightly reniform or allantoid, smooth- and thin-walled, 0-septate, (4.5–)5–8.5(–12.5) × (1.5–)2–3.5(–6) µm, clustering in discrete false heads at the tip of monophialides. Sporodochia pale orange to pink coloured, often somewhat translucent, formed abundantly on the surface of carnation leaves and on the agar surface. Conidiophores in sporodochia 26–46 µm tall, densely aggregated, irregularly and verticillately branched up to three times, with terminal branches bearing 2–3 monophialides; sporochial phialides doliiform to subcylindrical, (9–)11.5–15.5(–18.5) × (2.5–)3–4(–4.5) µm, smooth- and thin-walled, with periclinal thickening and an inconspicuous apical collarette. Sporochial conidia falcate, tapering toward the basal part, robust, moderately curved and slender; apical cell more or less equally sized than the adjacent cell, blunt to slightly papillate; basal cell papillate to distinctly notched, (1–)3–4-septate, hyaline, thin- and smooth-walled. One-septate conidia: 13–17(–18) × (2.5–)3–4 µm; two-septate conidia: 15 × 4.5 µm; three-septate conidia: (16–)28.5–39(–45) × (3–)4–5(–5.5) µm; four-septate conidia: 39.5–40(–41) × 4.5–5 µm; overall (13–)27.5–39.5(–45) × (3–)3.5–5.5 µm. Chlamydospores absent.

Distribution. Madagascar, Niger and South Africa.

Etymology. In honour and memory of Dr. Frederick J. Kruger, pioneer of forest hydrology, fynbos ecology and invasive species and fundamental for the collections included in this study.

Additional isolates examined. Madagascar, from Striga hermonthica, unknown date, A.A. Abbasher, CBS 144210 = NRRL 26061 = BBA 70127. South Africa, Kruger National Park, Skukuza, Granite Supersite, 25°06’48.6”S, 31°34’36.5”E, from rhizosphere soil of Melhania acuminata, 23 Mar 2015, W.J. Swart, CBS 144495 = CPC 33746.

Notes. This species is genetically closely related to F. dlaminii, both species having similar colonial morphology, optimal growth conditions and biogeography. Moreo-
ver, both species exhibit relatively short aerial phialides producing conidia in heads, somewhat resembling those produced by *F. oxysporum* rather than most members of the FFSC (Leslie and Summerell 2006; Marasas et al. 1985). However, besides exhibiting much faster growth rates, *F. fredkrugeri* presents clearly distinctive morphological features such as the production of only one type of aerial conidia (vs. two types in *F. dlaminii*: allantoid to fusiform and 0-septate; and napiform 0–1-septate); orange to pink sporodochia, produced on carnation leaves but also abundantly on the agar surface (vs. orange sporodochia, produced only on the surface of carnation leaves in *F. dlaminii*) (Leslie and Summerell 2006). Additionally, *F. fredkrugeri* produces shorter and less septate sporodochial conidia ((1–)3–4-septate and up to 45 µm long in the latter species vs. mostly 5-septate and up to 54 µm long in *F. dlaminii*) while chlamydo- spores are not produced. The latter feature, coupled with the somewhat more complex conidiophores also clearly differentiates *F. fredkrugeri* from *F. oxysporum*.

*Fusarium transvaalense* Sandoval-Denis, Crous & W.J. Swart, sp. nov.
MycoBank: MB825104
Fig. 7

**Diagnosis.** Different from most species in FSAMSC by its slender sporodochial conidia with tapered and somewhat rounded apex; its smooth- to tuberculate, often pigmented chlamydospores and the formation of large mycelial tufts on OA.

**Type.** South Africa, Kruger National Park, Skukuza, Granite Supersite, 25°06’45.5”S, 31°34’35.0”E, from rhizosphere soil of *Sida cordifolia*, 23 Mar 2015, W.J. Swart, holotype CBS H-23497, dried culture on SNA, culture ex-holotype CBS 144211 = CPC 30923.

**Description.** Colonies on PDA growing in the dark with an average radial growth rate of 8.5–9.3 mm/d, reaching 34–37 mm diam. in 7 d at 24 °C, filling an entire 9 cm Petri dish in 7 d at 27–33 °C. Minimum temperature for growth 12 °C, maximum 36 °C, optimal 27–30 °C. Colony surface at first white, turning coral to dark vinaceous with white periphery and abundant yellow hyphae at the centre; flat, velvety to woolly, with abundant aerial mycelium and erect hyphal strings reaching several mm tall; colony margins regular and filiform. Reverse with yellow, coral or dark vinaceous patches, coral diffusible pigments strongly produced between 15–30 °C, turning scarlet to orange at 33–36 °C. Colonies on CMA and OA incubated at 24 °C in the dark occupying an entire 9 cm Petri dish in 7 d. Colony surface coral, rust to chestnut coloured in irregular patches, flat, felty to woolly, aerial mycelium scarce on CMA, mostly as radially dispersed white patches, on OA aerial mycelium abundant, especially on the periphery of the colony, forming dense, pustule-like, white mycelial tufts, formed by abundant intermingled hyphae and chlamydospores, 1–1.5 cm tall, with flesh to coral coloured stipes; margins on CMA and OA regular. Reverse pale luteous with red to coral periphery. Sporulation abundant from conidiophores formed on the aerial mycelium, at the agar level and from sporodochia. **Conidiophores** on the aerial mycelium straight or flexuous, septate, smooth- and thin-walled, up to 150 µm tall, sometimes
New Fusarium species from the Kruger National Park, South Africa

Figure 7. Fusarium transvaalense sp. nov. A–D Colonies on PDA, SNA, OA and CMA, respectively, after 7 d at 24 °C in the dark E Pustule-like growth on OA F, G Sporodochia formed on the surface of carnation leaves H–L Aerial conidiophores phialides and conidia M Aerial conidia N, O Chlamydospores P Sporodochial conidiophores and phialides Q Sporodochial conidia. Scale bars: 2 mm (E); 20 µm (F–J); 5 µm (K); 10 µm (L–Q).
emerging from irregular, swollen, pigmented and rough-walled cells on the hyphae; simple or sparingly and irregularly branched, branches bearing terminal, rarely lateral monophialides or reduced to conidiogenous cells borne laterally on hyphae; phialides on the aerial conidiophores short ampulliform, subulate to subcylinndrical, smooth- and thin-walled, (7–)9–14(–15) µm long, (3–)4–5 µm at the widest point, without periclinal thickening and with a minute, inconspicuous collarette; conidia formed on aerial conidiophores of two types: a) hyaline, obovoid, ellipsoidal to clavate, smooth- and thin-walled, 0–1-septate, 2–14 × 2–4 µm; b) lunate to short falcate with a pointed apex and a somewhat flattened base, smooth- and thin-walled, 3–5-septate. Three-septate conidia: (16–)18–27(–29) × 5–6 µm; four-septate conidia: 21–24(–25) × 5–6 µm; five-septate conidia: (25–)27–33 × 5–6 µm. Sporodochia cream to orange coloured, formed abundantly on the surface of carnation leaves and rarely on the agar surface, at first very small and sparse later becoming aggregated. Conidiophores in sporodochia 22–31 µm tall, irregularly branched, bearing clusters of 3–6 monophialides; sporodochial phialides doliiform to ampulliform, (5–)9–14(–18) × (3–)4–5 µm, smooth- and thin-walled, with periclinal thickening and a short apical collarette. Sporodochial conidia falcate, wedge-shaped, tapering towards both ends, markedly curved and robust; apical cell longer than the adjacent cell, pointed; basal cell distinctly notched, sometimes somewhat extended (1–)3–5(–6)-septate, hyaline, smooth- and thick-walled. One-septate conidia: 19 × 4 µm; three-septate conidia: 20–27(–28) × 5–7 µm; four-septate conidia: (29–)30–32 × 5–7 µm; five-septate conidia: (26–)29–41(–53) × 4–5(–6) µm; six-septate conidia: 36 × 7 µm; overall (19–)25.9–40(–53) × (3.5–)4–6(–7) µm. Chlamydospores abundant, hyaline or pigmented, smooth- to rough-walled or tuberculate, 7–8 µm diam., terminal or intercalary, solitary, in chains or in clusters.

**Distribution.** Australia and South Africa

**Etymology.** After Transvaal, the name of a former colony and Republic located between the Limpopo and Vaal rivers, currently a province of South Africa and where this species was found. From Latin *trans* meaning “on the other side of” and Vaal a South African river.

**Additional isolates examined.** South Africa, Kruger National Park, Skukuza, Granite Supersite, 25°06′48.6″S, 31°34′36.5″E, from rhizosphere soil of *Melhania acuminata*, 23 Mar 2015, W.J. Swart, CBS 144224 = CPC 30928, CBS 144212 = CPC 30929); 25°06′45.6″S, 31°34′37.7″E, CBS 144496 = CPC 33750, CBS 144213 = CPC 33751; 25°06′48.8″S, 031°34′36.6″E, from rhizosphere soil of *Sida cordifolia*, 23 Mar 2015, W.J. Swart, CBS 144214 = CPC 30946; 25°06′45.7″S, 31°34′35.1″E, CBS 144215 = CPC 33723; 25°06′45.5″S, 31°34′35.0″E, CBS 144216 = CPC 30918, CBS 144217 = CPC 30919, CBS 144218 = CPC 30922, CBS 144219 = CPC 30926, CBS 144220 = CPC 30927); 25°06′51.4″S, 31°34′37.5″E, from rhizosphere soil of *Kyphocarpa angustifolia*, 23 Mar 2015, W.J. Swart, CBS 144221 = CPC 33740; 25°06′51.8″S, 31°34′38.1″E, CBS 144222 = CPC 30939, CBS 144223 = CPC 30941.

**Notes.** *Fusarium transvaalense* exhibits a sporodochial conidial morphology typical of members of FSAMSC with marked dorsiventral curvature and tapered ends. Several species in FSAMSC form comparable conidia in culture i.e. *F. crookwellense*
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L.W. Burgess, P.E. Nelson & Toussoun, *F. sambucinum*, *F. sporotrichioides* Sherb., *F. venenatum* Nirenberg and *F. culmorum* (Wm.G. Sm.) Sacc. However, with the exception of *F. sporotrichioides*, the conidia of most species above-mentioned, differ by being more robust and often more pointed apically. *Fusarium transvaalense* differs from *F. sporotrichioides* by the absence of pyriform aerial conidia.

Two strains NRRL 13829 and NRRL 31008, previously identified as *F. brachygibbosum* Padwick showed different degrees of genetic similitude with the new species. While NRRL 31008 clustered within *F. transvaalense*, NRRL 13829 formed a clearly delimited sister lineage. Morphologically, *F. transvaalense* exhibits significant differences allowing its separation from *F. brachygibbosum*. Both species produce sporodochial conidia with similar septation and sizes; however, *F. brachygibbosum* commonly exhibits a bulge in the middle portion of the conidia (Padwick 1945), a feature not present in *F. transvaalense*. In addition, the latter species produces comparatively larger sporodochial conidia, when elements with the same degree of septation are compared; its chlamydospores are smaller, smooth-walled to markedly tuberculate and pigmented (7–8 µm vs. 10.7–15.3 µm, smooth-walled and hyaline in *F. brachygibbosum*) and has a distinctive colonial growth on OA, forming large, pustule-like hyphal tufts, a feature not reported for *F. brachygibbosum* (Padwick 1945).

Discussion

In this study, three new *Fusarium* spp. were introduced, isolated from rhizosphere soils of three native African shrubs in a protected savannah ecosystem deep inside the Kruger National Park, South Africa.

Some remarkable differences were noted regarding the distribution of the novel fungal species and their respective hosts on this particular site. For instance, *F. transvaalense*, which exhibited the greatest relative abundance, was found in high quantities from the rhizospheres of the three hosts sampled, showing a considerable genetic diversity. Interestingly, this species was only on the top of the catena, even when two of its hosts, *K. angustifolia* and *S. cordifolia*, were found and sampled either at the top and bottom sites. Similarly, *F. fredkrugeri* was recovered only from soils under *M. acuminata*, a host species which occurred only at the top location. In contrast, *F. convolutans* was found in the rhizosphere of *K. angustifolia*, occurring only at the bottom of the catena, while none of the three fungal species was found associated with *S. cordifolia* at the bottom of the site. Nevertheless, not being an objective of this work, it was not possible to categorically assign these new species to specific hosts or locations. Likely, these fungi could be in low abundance and thus not detectable using the current methods. However, plant species composition varies considerably through a catena ecosystem, in relation to the different soil characteristics, pH gradient and water availability, which also greatly influence microbial and animal biodiversity (Lareen et al. 2016; Mohammadi et al. 2017). However, the full patterns of variation between locations on this particular catena still need to be systematically assessed and compared. As evidenced
here, certain differences do exist between the soils at the upper and bottom locations of the Stevenson-Hamilton supersite, which might explain the fungal diversity variation observed here. The cation exchange capacity (CEC; capacity of a soil to hold exchangeable cations) varies considerably between sampling sites, basically depending on the proportion of sand versus clay content of each soil type (Ketterings et al. 2007; Van Zijl and Le Roux 2014). It is known that CEC greatly impacts the soil's ability to retain essential nutrients and prevents soil acidification (Ketterings et al. 2007). Nutrient content also increased from the top to the bottom of the slope which is consistent with the increase in CEC. Nutrient poor soils are also a driver of biological diversity and most likely influenced fungal diversity in these particular locations (Havlicek and Mitchell 2014, Mapelli et al. 2017).

The three *Fusarium* species, described here, were not associated with any visible symptomatology on their hosts. However, they cannot be ruled out as pathogens since they were not assessed for pathogenicity against the sampled plants nor any other putative host species at the same locations. Likewise, it is unknown if these fungi exert any beneficial or deleterious effect on their ecosystems. These are important unsolved questions that need further evaluation. However, as shown by phylogenetic analyses, each of the three new species was in close genetic proximity with well-known plant pathogenic *Fusarium* spp. on their respective species complexes, which could suggest a potential pathogenic role. *Fusarium convolutans* clustered within the FBSC, together with three known plant pathogenic *Fusarium* spp. i.e. *F. buharicum*, a pathogen of *Hibiscus cannabinus* L. and *Gossypium* L.; *F. sublunatum*, known to affect banana and *Theobroma cacao* L. in Central America (Gerlach and Nirenberg 1982, Leslie and Summerell 2006) and a newly discovered although unnamed phylogenetic species causing wilt, crown and root rot of *Hibiscus moscheutos* L. (Lupien et al. 2017). *Fusarium transvaalense* belonged to the FSAMSC, a genetically diverse group common in temperate and subtropical zones (Leslie and Summerell 2006). *Fusarium sambucinum*, the conserved type species of the genus (Gams et al. 1997) being an aggressive plant pathogen and one of the most important agents of potato dry rot (Peters et al. 2008); while the latter species and several others in the complex have been reported causing disease on diverse crops, including many cereals and fruits (Leslie and Summerell 2006).

*Fusarium fredkrugeri* is here recognised and formally proposed as a new species. Although the clade representing this taxon had already been identified as a distinct unnamed phylogenetic species by O’Donnell et al. (2000), it had not been given a formal description pending the collection of additional isolates. Two other African isolates previously determined to belong to this clade i.e. CBS 144210 from *Striga hermonthica* (Del.) Benth. in Madagascar and NRRL 26152 from an unknown substrate in Niger, were incorporated into the analyses, although the latter strain is not viable anymore (NRRL, pers. comm.), thus not available for morphological assessment. Strain CBS 144210, however, is known as a pathogen of the ‘purple witchweed’, a parasite plant common to sub-Saharan Africa and known to devastate *Sorghum bicolor* (L.) Moench and *Oryza sativa* L. plantations (O’Donnell et al. 2000; Yoshida et al. 2010). As previously demonstrated by O’Donnell et al. (2000), our phylogenetic results showed that
the clade comprising *F. fredkrugeri* and its sister species *F. dlaminii* does not cluster within the main African core of species in the FFSC. Thus, despite the African origin of our isolates, the predicted biogeographic patterns did not match the observed phylogeny. It has been hypothesised that this should not be the result of genetic markers tracing different phylogenies, but the consequence of losing the phylogenetic signal due to saturated sites and introns (O’Donnell et al. 2000). However, the inclusion in our analysis of additional, highly informative and slowly evolving loci such as *RPB1* and *RPB2* yielded similar results, which points out the need to re-evaluate the phylogeographic arrangement of this important species complex including the vast new data generated during the last 20 years that challenges the established assumptions (Kvas et al. 2009; Walsh et al. 2010; O’Donnell et al. 2013; Laurence et al. 2015). Nevertheless, although rather unlikely, alternative factors such as anthropogenic dispersion of *F. fredkrugeri*, its host or additional invasive alternative hosts, cannot be rejected as an explanation for the discordance between biogeography and phylogenetic results. However, these scenarios are difficult to imagine given the characteristics of the sampled site, not being an agroecosystem but a protected, isolated zone, with minimal human intervention (Smit et al. 2013).

This study is a new example of how easily new *Fusarium* spp. can be found when mycological studies are directed to neglected natural ecosystems of minimal anthropogenic disturbance (Phan et al. 2004; Leslie and Summerell 2011; Summerell et al. 2011; Burgess 2014, Laurence et al. 2015). Although irrelevant for some researchers, finding and properly describing new species, regardless of whether they have little or no pathogenic or mycotoxigenic potential, is of utmost importance to improve our understanding on the diversity, biogeographic and phylogeographic patterns of such a complex and heterogeneous genus as *Fusarium*. In addition, this study remarks on the significance and need to further stimulate the exploration of conserved, non-manipulated natural environments (supersites) and their potential impact on biodiversity research on the fungal kingdom.

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References

Aoki T, Smith JA, Mount LL, Geiser DM, O’Donnell K (2013) *Fusarium torreyae* sp. nov., a pathogen causing canker disease of Florida torreya (*Torreya taxifolia*), a critically endangered conifer restricted to northern Florida and southwestern Georgia. Mycologia 105: 312–319. https://doi.org/10.3852/12-262

Aydogdu H, Asan A (2008) Airborne fungi in child day care centers in Edirne City, Turkey. Environmental Monitoring and Assessment 147: 423–444. https://doi.org/10.1007/s10661-007-0130-4

Bent E, Kiekel P, Brenton R, Taylor DL (2011) Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in interior Alaska and correlation of different fungi with host growth responses. Applied and Environmental Microbiology 77: 3351–3359. https://doi.org/10.1128/AEM.02575-10

Brown DJ, Clayton MK, McSweeney K (2004) Potential terrain controls on soil color, texture contrast and grain-size deposition for the original catena landscape in Uganda. Geoderma 122: 51–72. http://doi.org/10.1016/j.geoderma.2003.12.004

Burgess LW (2014) 2011 McAlpine Memorial Lecture – A love affair with *Fusarium*. Australian Plant Pathology 43: 359–368. https://doi.org/10.1007/s13313-013-0261-8

Carruthers J (2017) National Park Science: A Century of Research in South Africa (Ecology, Biodiversity and Conservation). Cambridge University Press, 554 pp. https://doi.org/10.1017/9781108123471

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (2009) Fungal Biodiversity. CBS Laboratory Manual Series (CBS-KNAW Fungal Biodiversity Centre, Utrecht) 1: 1–270.

Díaz Arias MM, Leandro LF, Munkvold GP (2013) Aggressiveness of *Fusarium* species and impact of root infection on growth and yield of soybeans. Phytopathology 103: 822–832. https://doi.org/10.1094/PHYTO-08-12-0207-R

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72: 151–153. https://doi.org/10.1094/Phyto-72-151

Fravel D, Olivain C, Alabouvette C (2003) *Fusarium oxysporum* and its biocontrol. New Phytopathologist 157: 493–502. https://doi.org/10.1046/j.1469-8137.2003.00700.x

Gams W, Nirenberg HI, Seifert KA, Brayford D, Thrane U (1997) (1275) Proposal to conserve the name *Fusarium sambucinum* (Hyphomycetes). Taxon 46: 111–113. https://doi.org/10.2307/1224298

Gerlach W, Nirenberg HI (1982) The genus *Fusarium* – a pictorial atlas. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 209: 1–406.

Hargreaves SK, Williams RJ, Hofmockel KS (2015) Environmental filtering of microbial communities in agricultural soil shifts with crop growth. PLoS One 30: e0134345. https://doi.org/10.1371/journal.pone.0134345

Hassan Dar GH, Zargar MY, Beigh GM (1997) Biocontrol of Fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microbial Ecology 34: 74–80. https://doi.org/10.1007/s002489900036
Havlicek E, Mitchell EAD (2014) Soils supporting biodiversity. In: Dighton J, Krumins JA (Eds) Interactions in Soil: Promoting Plant Growth, Biodiversity, Community and Ecosystems. Springer, Dordrecht, 27–28. https://doi.org/10.1007/978-94-017-8890-8_2
Herron DA, Wingfield MJ, Wingfield BD, Rodas CA, Marincowitz S, Steenkamp ET (2015) Novel taxa in the Fusarium fujikuroi species complex from Pinus spp. Studies in Mycology 80: 131–150. https://doi.org/10.1016/j.simyco.2014.12.001
Idris HA, Labuschagne N, Korsten L (2007) Screening rhizobacteria for biological control of Fusarium root and crown rot of sorghum in Ethiopia. Biological Control 40: 97–106. https://doi.org/10.1016/j.biocontrol.2006.07.017
Jumpponen A, Herrera J, Porras-Alfaro A, Rudgers J (2017) Biogeography of root-associated fungal endophytes. In: Tedersoo L (Ed.) Biogeography of Mycorrhizal Symbiosis. Ecological Studies 230 (Springer), 195–222. https://doi.org/10.1007/978-3-319-56363-3
Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010
Ketterings Q, Reid S, Rao R (2007) Cation Exchange Capacity (CEC), Agronomy Fact Sheet Series (22). Cornell University Cooperative Extension.
Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp ET (2009) Diversity and evolution of Fusarium species in the Gibberella fujikuroi complex. Fungal Diversity 34: 1–21.
Lareen A, Burton F, Schäfer P (2016) Plant root-microbe communication in shaping root microbiomes. Plant Molecular Biology 90: 575–587. https://doi.org/10.1007/s11103-015-0417-8
Larkin RP, Hopkins DL, Martin FN (1993) Effect of successive watermelon plantings on Fusarium oxysporum and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83: 1097–1105. https://doi.org/10.1094/Phyto-83-1097.
Laurence MH, Walsh JL, Shuttleworth LA, Robinson DM, Johansen RM, Petrovic T, Vu TTH, Burgess LW, Summerell BA, Liew ECY (2015) Six novel species of Fusarium from natural ecosystems in Australia. Fungal Diversity 77: 349–366. https://doi.org/10.1007/s13225-015-0337-6
LeBlanc N, Essarioui A, Kinkel L, Kistler HC (2017) Phylogeny, plant species, and plant diversity influence carbon use phenotypes among Fusarium populations in the rhizosphere microbiome. Phytobiomes 1: 150–157. https://doi.org/10.1009/PBIOMES-06-17-0028-R
Leslie JF, Summerell BA (2006) The Fusarium laboratory manual. Blackwell Publishing, Ames. 1–388. https://doi.org/10.1002/9780470278376
Leslie JF, Summerell BA (2011) In search of new Fusarium species. Plant Breeding and Seed Science 63: 94–101. https://doi.org/10.2478/v10129-011-0020-3
Li W, Cowley A, Uludag M, Gur T, McWilliam H, Squizzato S, Park YM, Buso N, Lopez R (2015) The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Research 43: W580–584. https://doi.org/10.1093/nar/gkv279
Lupien SL, Dugan FM, Ward KM, O’Donnell K (2017) Wilt, crown, and root rot of common rose mallow (Hibiscus moscheutos) caused by a novel Fusarium sp. Plant Disease 101: 354–358. https://doi.org/10.1094/PDIS-05-16-0717-RE
Mapelli F, Marasco R, Fusi M, Scaglia B, Tsiamis G, Rolli E, Fodelianakis S, Bourtzis K, Ventura S, Tambone F, Adani F, Borin S, Daffonchio D (2017) The stage of soil development modulates rhizosphere effect along a High Arctic desert chronosequence. The ISME Journal: 1–11. https://doi.org/10.1038/s41396-017-0026-4

Marasas WFO, Nelson PE, Toussoun TA (1985) Fusarium dlamini, a new species from Southern Africa. Mycologia 77: 971–975. https://doi.org/10.2307/3793311

Mason-Gamer R, Kellogg E (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Systematic Biology 45: 524–545. https://doi.org/10.1093/sysbio/45.4.524

Miller MA, Pfeiffer W, Schwartz T (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond, Association for Computing Machinery, Chicago, USA, 1–8. https://doi.org/10.1145/2335755.2335836

Mohammadi MF, Jalali SW, Kooch Y, Theodore TA (2017) Tree species composition, biodiversity and regeneration in response to catena shape and position in a mountain forest. Scandinavian Journal of Forest Research 32: 80–90. https://doi.org/10.1080/02827581.2016.1193624

Mommer L, Kirkegaard J, van Ruijven J (2016) Root-root interactions: towards a rhizosphere framework. Trends in Plant Science 21: 209–217. https://doi.org/10.1016/j.tplants.2016.01.009

Moussa TAA, Al-Zahrani HS, Kadasa NMS, Ahmed SA, de Hoog GS, Al-Hatmi AMS (2017) Two new species of the Fusarium fujikuroi species complex isolated from the natural environment. Antonie Van Leeuwenhoek 110: 819–832. https://doi.org/10.1007/s10482-017-0855-1

Nelson PE, Dignani MC, Anaissie EJ (1994) Taxonomy, biology, and clinical aspects of Fusarium species. Clinical Microbiology Reviews 7: 479–504. https://doi.org/10.1128/CMR.7.4.479

Nirenberg HI (1976) Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium-Sektion Liseola. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 169: 1–117. https://doi.org/10.1002/jpln.19771400220

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

O’Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America 95: 2044–2049. https://doi.org/10.1073/pnas.95.5.2044

O’Donnell K, Nirenberg HI, Aoki T, Cigelnik E (2000) A multigene phylogeny of the Gibberella fujikuroi species complex: detection of additional phylogenetically distinct species. Mycoscience 41: 61–78. https://doi.org/10.1007/BF02464387

O’Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, Frandsen RJ, Lysoe E, Rehner SA, Aoki T, Robert VA, Crous PW, Groenewald JZ, Kang S, Geiser DM
New *Fusarium* species from the Kruger National Park, South Africa

(2013) Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genetics and Biology 52: 20–31. https://doi.org/10.1016/j.fgb.2012.12.004

O’Donnell K, Sutton DA, Rinaldi MG, Gueidan C, Crous PW, Geiser DM (2009) Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum* – *F. equiseti* and *F. chlamydosporum* species complexes within the United States. Journal of Clinical Microbiology 47: 3851–3861. https://doi.org/10.1128/JCM.01616-09

O’Donnell K, Sutton DA, Rinaldi MG, Sarver BA, Balajee SA, Schroers HJ, Summerbell RC, Robert VA, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM (2010) Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. Journal of Clinical Microbiology 48: 3708–3718. https://doi.org/10.1128/JCM.00989-10

Padwick GW (1945) Notes on Indian fungi III. Mycological Papers 12: 1–15.

Pal KK, Tilak KVBR, Saxcna AK, Dey R, Singh CS (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. Microbiological Research 156: 209–223. https://doi.org/10.1078/0944-5013-00103

Perlroth J, Choi B, Spellberg B (2007) Nosocomial fungal infections: epidemiology, diagnosis, and treatment. Medical Mycology 4: 321–346. https://doi.org/10.1080/13693780701218689

Peters JC, Lees AK, Cullen DW, Sullivan L, Strouda GP, Cunnington AC (2008) Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathology 57: 262–271. https://doi.org/10.1111/j.1365-3059.2007.01777.x

Phan HT, Burgess LW, Summerell BA, Bullock S, Liew ECY, Smith-White JL, Clarkson JR (2004) *Gibberella gaditjirri* (*Fusarium gaditjirri*) sp. nov., a new species from tropical grasses in Australia. Studies in Mycology 50: 261–272.

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 11: 789–799. https://doi.org/10.1038/nrmicro3109

Pinheiro AC, Macedob MF, Jurado V, Saiz-Jimenez C, Viegas C, Brandão J, Rosado L (2011) Mould and yeast identification in archival settings: preliminary results on the use of traditional methods and molecular biology options in Portuguese archives. International Biodeterioration & Biodegradation 65: 619–627. https://doi.org/10.1016/j.ibiod.2011.02.008

Posada D, Crandalld KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rayner RW (1970) A Mycological Colour Chart. CMI and British Mycological Society, Kew, Surrey, 34 pp.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ruano-Rosa D, Prieto P, Rincón AM, Gómez-Rodríguez MV, Valderrama R, Barroso JB, Mercado-Blanco J (2016) Fate of *Trichoderma harzianum* in the olive rhizosphere: time course of the root colonization process and interaction with the fungal pathogen *Verticillium dahliae*. BioControl 61: 269–282. https://doi.org/10.1007/s10526-015-9706-z
Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW (2018) Symptomatic Citrus trees reveal a new pathogenic lineage in Fusarium and two new Neocosmospora species. Persoonia 40: 1–25. https://doi.org/10.3767/persoonia.2018.40.01

Saravanakumar K, Fan L, Fu K, Yu C, Wang M, Xia H, Sun J, Li Y, Chen J (2016) Cellulase from Trichoderma harzianum interacts with roots and triggers induced systemic resistance to foliar disease in maize. Scientific Reports 6: 35543. https://doi.org/10.1038/srep35543

Sasse J, Martinoia E, Northen T (2018) Feed your friends: do plant exudates shape the root microbiome? Trends in Plant Science. 23: 25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Schippers B, Bakker AW, Bakker PAHM (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annual Review of Phytopathology 25: 339–358. https://doi.org/10.1146/annurev.py.25.090187.002011

Smit IPJ, Riddell ES, Cullum C, Petersen R (2013) Kruger National Park research supersites: establishing long-term research sites for cross-disciplinary, multiscaled learning. Koedoe – African Protected Area Conservation and Science 55: Art. 1107 https://doi.org/10.4102/koedoe.v55i1.1107

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Summerell B, Leslie J, Liew E, Laurence M, Bullock S, Petrovic T, Bentley AR, Howard CG, Peterson SA, Walsh JL, Burgess LW (2011) Fusarium species associated with plants in Australia. Fungal Diversity 46: 1–27. https://doi.org/10.1007/s13225-010-0075-8

Van Zijl G, Le Roux P (2014) Creating a conceptual hydrological soil response map for the Stevenson Hamilton Research Supersite, Kruger National Park, South Africa. Water SA 40: 331–336. http://doi.org/10.4314/wsa.v40i2.15

Visioli G, D’Egidio S, Sanangelantoni AM (2014) The bacterial rhizobiome of hyperaccumulators: future perspectives based on omics analysis and advanced microscopy. Frontiers in Plant Science 5: 752. https://doi.org/10.3389/fpls.2014.00752

Walsh J, Laurence M, Liew E, Sangalang A, Burgess L, Summerell B, Petrovic T (2010) Fusarium: two endophytic novel species from tropical grasses of northern Australia. Fungal Diversity 44: 149–159. https://doi.org/10.1007/s13225-010-0035-3

Wiens JJ (1998) Testing phylogenetic methods with tree congruence: phylogenetic analysis of polymorphic morphological characters in phrynosomatid lizards. Systematic Biology 47: 427–444. https://doi.org/10.1080/106351598260806

Woudenberg JHC, Aveskamp MM, De Gruyter J, Spiers AG, Crous PW (2009) Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 22: 56–62. https://doi.org/10.3767/003158509X427808

Yoshida S, Maruyama S, Nozaki H, Shirasu K (2010) Horizontal gene transfer by the parasitic plant Striga hermonthica. Science 328: 1128. https://doi.org/10.1126/science.1187145

Zachow C, Berg C, Müller H, Meinecke R, Komon-Zelazowska M, Druzhinina IZ, Kubicek CP, Berg G (2009) Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. The ISME Journal 3: 79–92. https://doi.org/10.1038/ismej.2008.87

Zakaria L, Ning CH (2013) Endophytic Fusarium spp. from roots of lawn grass (Axonopus compressus). Tropical Life Sciences Research 24: 85–90.