Species Diversity in *Penicillium* and *Acaulium* From Herbivore Dung in China, and Description of *Acaulium stericum* Sp. Nov.

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Abstract

Penicillium and *Acaulium* species are common in the fresh of herbivore dung and can produce abundant secondary metabolism, which play important roles as decomposers of organic materials, food industry, and enzyme factories. Besides, the well-characterized diversity of dung fungi offers accessible systems for dissecting the function of fungi in gut and for exploring potential to produce high cellulases in herbivorous animal. During a survey of intestinal fungi from herbivorous animal in China, more than 400 were isolated, 38 belonging to *Penicillium* and 4 belonging to *Acaulium* were obtained from 12 healthy animals including marmot and chinchilla and selected for detailed study. Putative taxa were characterized by a multi-gene sequencing analysis testing the partial β-tubulin (*TUB*), the internal transcribed spacer rDNA (ITS), calmodulin (*CAM*), and *RPB2*, and a detailed phenotypic study. *Penicillium* strains were identified as six sections, 12 known species. In addition, four *Acaulium* isolates were identified as *Acaulium album* and *Acaulium stericum* sp. nov. based on morphology and phylogeny of multi-gene sequences. This study shows that the species diversity of *Penicillium* on herbivore dung has not been widely studied and that seems to be a good source of offers opportunities for discovery of new cellulases from microbial communities.

Introduction

The gut fungi were recently recognized to possess a wide range of biomass-degrading enzymes that are central to the lignocellulolytic ability of herbivorous animals, while its roles in the herbivore gut microbiome has yet to be investigated (Gruninger et al. 2014; Solomon et al. 2016). *Penicillium* and *Acaulium* species are common in the dung of herbivorous animal (Guevara-Suarez et al. 2020; Su et al. 2019, 2020). *Penicillium* is well known and one of the most common fungi occurring in a diverse range of habitats, which has a worldwide distribution and a large economic impact on human life. *Acaulium* species have been reported from a variety of environments such as skin of horse, decaying meat, and dung in cat and so on.

The genus *Penicillium* was introduced by Link (1809) and described the generic type *P. expansum*. Dierckx (1901) first proposed an infrageneric classification system in *Penicillium*, after that various subgenera, sections, subsections and series were introduced and most of them were based on macro- and microscopic characters, and occasionally supplemented with physiological and/or extrolite data (Biourge 1923; Frisvad and Samson 2004; Pitt 1980; Ramirez 1982; Raper and Thom 1949; Stolk and Samson 1985). Since then, more than 1000 species were introduced in the genus by morphological characters. Herein, many of these names are not recognizable today because descriptions were incomplete by modern criteria, and some names were published invalidly, or are now considered synonyms of other species by the modern taxonomy (Houbraken et al. 2011a, b; Houbraken et al. 2012; Houbraken et al. 2016; Houbraken et al. 2020; Visagie et al. 2014). In recently, Houbraken and Samson (2011) subdivided the genus into two subgenera and 25 sections based on a four-gene phylogeny and accepted 225 species (Pitt et al. 2000). Subsequently, two new sections (Osmophila and Robsamsonia) were introduced based on a combined partial β-tubulin, *CaM* and *RPB2* multigene sequence dataset

(Houbraken et al. 2016). In the most recent list from 2020, totally 483 *Penicillium* species were divided into two subgenera, 32 sections (seven new), 89 series (57 new, six new combinations) were accepted based on combined phylogeny for *TUB*, *CaM* and *RPB2* data, showing the large diversity and high interest in this genera (Guevara-Suarez et al. 2020; Houbraken et al. 2020). These data give a great helpful for researchers to obtain a correct identification using the current taxonomic schemes and also conform that the molecular reassessments for *Penicillium* species into subgenera and sections has been rather stable.

The genus *Acaulium* was established as the sexual morph and the type species is *Acaulium albonigrescens* Sopp (1912), is characterised by annellidic conidiogenesis, guttulate conidia and mycelium forming abundant hyphal fascicles. *Acaulium* has been considered a synonym of *Scopulariopsis* but recently was re-instated as an accepted genus of Microascaceae with three species as *A. acremonium*, *A. albonigrescens* and *A. caviariforme* (Sandoval-Denis et al. 2016). *Acaulium album* is transferred to *Acaulium* and redescribed by Woudenberg et al. (2017) based on morphological, physiological and molecular phylogenetic analyses. In addition, *A. pannemaniae* is introduced in this genus by morphological and phylogenetic analyses of LSU (Crous et al. 2018). Subsequently, two new combinations are introduced, i.e. *Acaulium peruvianum* and *Acaulium retardatum* based on morphological characters and multilocus phylogenetic analysis of the ITS, *LSU*, translation elongation factor 1α (*TEF*) and *TUB* genes by Su et al. 2020. Seven species are currently accepted at present (Crous et al. 2018; Woudenberg et al. 2017; Su et al. 2020).

The species in *Penicillium* play important roles as decomposers of organic materials and with the food industry exploiting some species for the production of speciality cheeses (Giraud et al. 2009) and fermented sausages (Ludemann et al. 2010). Some species are also being screened for the production of novel enzymes (Adsul et al. 2007; Terrasan et al. 2010) or the production of penicillin, which revolutionized medical approaches to treating bacterial diseases (Abraham et al. 1941; Chain et al. 1940; Fleming 1929; Thom 1945). During our investigations of intestinal fungi associated with herbivorous animal in China, 38 *Penicillium* strains were isolated from marmot and chinchilla. Phylogenetic analysis of multi-locus DNA sequences placed the strains in *Penicillium* and belongs to 6 sections, 12 species. In addition, a new species was isolated from fresh dung samples of marmot and belong to *Acaulium* can be morphologically differentiated from related species by absence of sexual morph or presence chlamydospores, and characters of conidia and conidiophores. The multi-locus phylogenetic analysis also showed that these strains represent a distinct species of *Acaulium*. In this paper, we describe and illustrate the new species as *Acaulium stericum* with complete morphological descriptions and phylogenetic data.

Materials And Methods

Strains, media and morphological observation

Fecal samples were collected from six healthy (n = 3 male and n = 3 female) marmot and six healthy (n = 3 male and n = 3 female) chinchilla which had not received oral antibiotic or antifungal drugs for at least

two months (typically > 6 months) in Beijing (E: 116°13’; N: 39°48’; h: 58 m), China, in July, 2020. The samples were transported to laboratory and isolated for intestinal fungi within 24 h (Su et al. 2019). All samples were serially diluted and spread plated in triplicate onto Sabouraud agar (SDA) and Potato dextrose agar (PDA), supplemented with antibiotics and used for isolation with the methods of Huseyin et al. (2017). Agar plates were incubated at 30 ºC, and counted after 48 h incubation and again at 2 weeks to allow for the detection of potentially slow growing species. The pure strains were incubated on different media such as PDA, SDA, Corn meal agar (CMA), and Oatmeal agar (OA) (Becton, Dickinson & Co.) at 25 ºC. Colony morphology and microscopic characteristics were examined, measured and photographed after incubation for 8 days with the methods of Su et al. (2015). All measurements were conducted from 50 individuals in water mounts. The ex-type living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC). The dried culture and microscope slide were deposited in Herbarium Mycologicum, Academia Sinica, Beijing, China (HMAS).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from the fungal mycelia on PDA plates following the protocol described by Guo et al. (2000). Amplification of the partial 28S rDNA (LSU) was performed with LROR/LR5 primers (Vilgalys and Hester 1990)]. The primers ITS1 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) of rDNA; Bt2a and Bt2b (Glass and Donaldson 1995) for the partial β-tubulin gene (*TUB*); CMD5 and CMD6 (Hong et al. 2006) for the partial calmodulin gene (*CaM*); 983F and 2218R (Rehner and Buckley 2005) were used to amplify the elongation factor 1-α gene (TEF); fRPB2-5f and fRPB2-7cR (Liu et al. 1999) for the partial RNA polymerase II largest subunit gene (*RPB*2). Polymerase chain reactions (PCR) were performed in 25 µL reactions containing 1.0 µL DNA template, 1.0 µL of each forward and reverse primers (10 µmol/L), 12.5 µL 2 × MasterMix (Tiangen Biotech Co. Ltd. Beijing, China) and 10.5 µL ddH_2O by using the following parameters: 94°C for 3 min; followed by 35 cycles at 94°C for 40 s, the annealing temperature dependent on the gene amplified (54°C for ITS, 58°C for *TUB*, 55°C for *CaM* and 54°C for *RPB2*) for 60 s and 72°C for 120 s; and a final extension at 72°C for 10 min. PCR products were sequenced by Beijing Sunbiotech Co. Ltd. (Beijing, China) using the same primers. Sequences were compared with accessions in the GenBank database using BLASTn searches to obtain the most likely taxonomic designations.

Phylogenetic analysis

Sequences of the six genes were aligned with Clustal X (Thompson et al. 1997). Reference sequences of related species were retrieved from GenBank. Manual editing of sequences was performed in MEGA6 (Tamura et al. 2013). The concatenated sequences (*RPB2*, ITS, *CaM* and *TUB*) and (ITS, *LSU*, *TUB* and *TEF*) were assembled using SequenceMatrix1.7.8 (Vaidya et al. 2011) and alignments were deposited in TreeBASE (www. treebase.org, submission no.: S28382). The *RPB2* and combined dataset of four loci were analyzed phylogenetically using Bayesian MCMC (Altekar et al. 2004) and Maximum Likelihood (Stamatakis 2014), respectively. For the Bayesian analyses, the models of evolution were estimated by using MrModeltest 2.3 (Nylander 2008). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC), Six

simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation (resulting in 20,000 total trees). The first 4,000 trees represented the burn-in phase of the analyses and were discarded and the remaining 16,000 trees were used for calculating PP in the majority rule consensus tree. For the ML analysis in RAxML (Stamatakis 2014), the GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. Analyses were performed using the CIPRES web portal (Miller et al. 2010). Trees were visualised in TreeView 1.6.6 (Page 1996).

Results

DNA sequence alignment and Phylogenetic analysis

DNA sequences of *Penicillium* and *Acaulium* strains determined in this study is deposited in GenBank and accession numbers are listed in Table 1. *CaM* and *TUB* sequences could not be obtained for all strains. A dataset of partial *RPB2* sequence (*RPB1*: 1–816) for 77 strains, including our isolates and type or reference strains of related species and outgroup, was used for a preliminary phylogenetic analysis. The general time reversible (GTR) model with gamma distributed (+G) and invariant sites (+I) was the most suitable model for ML and BI. *Aspergillus niger* NRRL 326NT was selected as outgroup. The phylogenetic tree was constructed and bootstrap values for likelihood

(ML analysis) (≥ 50%) and branch support values for posterior probability (PP) (≥

0.95) are illustrated. Phylogeny of 38 *Penicillium* strains which isolated from herbivore dung based on *RPB2* sequences revealed that these strains belong to six sections, 12 known species (Fig. 1). Twenty-three *Penicillium* strains isolated from chinchilla and belong to sect. *Fasciculata*, sect. *Citrina* and sect. *Lanata-divaricata*, respectively. Fifteen *Penicillium* strains isolated from marmot and belong to sect. *Chrysogena*, sect. *Citrina*, sect. *Fasciculata*, sect. *Ramigena* and sect. *Roquefortorum*, respectively. Multiple sequence alignment and multi-gene phylogentic analysis were further carried out by testing *TUB*, ITS, *CAM*, *RPB2* and a detailed phenotypic study (Figs. 2, 3). The strains isolated from chinchilla dung could be identified as five species, *P. polonicum*, *P. aurantiogriseum*, *P. steckii*, *P. fructuariae-cellae*, *P. oxalicum*. However, the strains isolated from marmot dung could be identified as eight species, *P. polonicum*, *P. echinulatum*, *P. allii-sativi*, *P. dipodomyis*, *P. paneum*, *P. roqueforti*, *P. steckii*, *P. citrinum*. Besides, *P. polonicum* and *P. steckii* could be isolated from chinchilla and marmot.

In addition, BLAST searches of GenBank using the ITS sequences of four *Acaulium* strains isolated from the healthy marmot revealed that CGMCC 3.20206T (MZ157171), CGMCC 3.20207 (MZ157173) and CGMCC 3.20208 (MZ157172) showed 93% similarity to *Acaulium acremonium* CBS 290.38 (MH855966) (Table 1). The three *Acaulium* strains had 40 variable positions, including 6 transitions, 2 transversions and 32 indels in the ITS regions. The isolate TBS429 showed 99% similarity to *Acaulium album* CBS 539.85 (NR_159559). The dataset of combined 4 loci (ITS: 1–434; *LSU*: 435–1186; *TEF*: 1187–1658; *TUB*: 1659–1866) comprised 1,866 characters. The phylogenetic tree was constructed based on 20 taxa including *Graphium penicillioides* CBS 102632T as outgroup. The terminal branch support value and

additional internal branch support value of the Bayesian posterior probabilities (PP) (≥ 95%) and Bootstrap value (BP) (≥ 50%) are indicated on the nodes. The species belonging to *Acaulium* clustered in a separate clade with a high bootstrap value. *Acaulium stericum* clustered with *Acaulium pannemaniae* CBS 145025T, with Bayesian posterior probability and bootstrap support of 99% and 100% (Fig. 4). Moreover, morphological comparisons showed differences between these species. Therefore we assigned the new species in *Acaulium*. Coupled with analysis of the polymorphisms in the ITS and *TUB* regions and morphological characters, three strains were designated as a new species *A. stericum*.

Taxonomy

Acaulium stericum L. Su, H. Zhu & C. Qin, sp. nov. (Fig. 5).

MycoBank No.: MB 840126

Etymology: The specific epithet 'stericum', indicating the type strain isolated from the dung of animal.

Colonies on PDA reaching 8 mm diameter after 15 days at 25 °C, slow growing, raised centrally, with irregular margin, white. On SDA reaching 10 mm diameter, slow growing, raised centrally, aerial mycelium absent or sparse, white to cream. On CMA reaching 21 mm diameter, moderately growing, planar, white, margin discrete. On OA reaching 30 mm diameter, planar, white, raised centrally. *Hyphae* hyaline to subhyaline, smooth-walled, 2–5 µm ($\bar{x}$ = 3.3 µm) wide. Conidiophores often arising from the substratum or from the aerial mycelium, branched or unbranched, septate, smooth, cylindrical, 3–16 × 2–6 µm ($\bar{x}$ = 8.0 × 4.0 µm). Conidiogenous cells percurrent in conidiophores or produced on hyphae in laterally, flask-shaped, subhyaline and smooth-walled, 6–20 × 2–6 µm ($\bar{x}$ = 11.7 × 3.6 µm). Conidia formed in slimy heads at the apex of the phialides, ellipsoidal to fusiform, with a truncate base and rounded or bluntly pointed apex, smooth, 3–8 × 2–5 µm ($\bar{x}$ = 5.7 × 3.3 µm). Chlamydospores formed short chains by schizolytic secession intercalary or laterally, irregular, thick- walled. Ascomata not observed.

Typification: CHINA, Beijing, Fangshan District, in the North Center for Experimental Animal Resources, Institute of Medical Laboratory of Animal Science, Chinese Academy of Medical Sciences, 116°13¢ E, 39°48¢ N, 58 m above sea level, from fresh fecal samples of healthy *Marmota monax*, 30 July 2020, collected and isolated by Lei Su, HMAS 249146 (holotype), ex-type culture CGMCC 3.20206.

Other cultures examined: CHINA. Beijing: Daxing, E 116°13', N 39°48', 58 m above sea level, isolated as the fresh fecal samples of healthy marmot, 30 July 2020, *Lei Su*. CGMCC 3.20207, CGMCC 3.20208.

Notes: The phylogenetic analysis shows that the ex-type culture of *Acaulium stericum* grouped with statistical support with species of *A. pannemaniae*. However, Compared to *A. pannemaniae*, *A. stericum* produces the short conidiophores. Besides, the dimensions of conidiogenous cells and conidia, and the irregular chlamydospores for two species are apparent different (Fig. 5).

Discussion

Our multi-locus phylogenetic analyses of *Penicillium* species (*RPB*2, ITS, *TUB* and *CaM*) yielded similar trees to those reported earlier (Houbraken et al. 2016; Guevara-Suarez et al. 2020; Houbraken et al. 2020). All *Penicillium* strains isolated from chinchilla and marmot dung in this study were classified in seven sections by *RPB*2 phylogeny. Among these isolates, twelve species were identified based on multi-locus phylogenetic analysis and morphological characters. Multi-locus phylogenetic trees (*RPB*2, ITS, *TUB* and *CaM*) are quite stable in different sections of *Penicillium*, and the combination of multi-locus phylogenetic analysis and morphological characters allows robust delimitation of *Penicillium* species. Besides, four strains isolated from marmot dung could be identified as two species, *Acaulium album* and a new species of *A. stericum* based on multi-locus phylogenetic analysis (ITS, *TUB*, *LSU* and *TEF*) and morphological characters.

This research agree with other studies in which a large set of isolates of penicillium-like fungi could be identified with the *RPB*2, ITS, *TUB* and *CaM* sequence analysis. These barcodes are a good marker, easy to amplify and sequence, and useful for the classification at the sectional and species level, and even for detection of putative undescribed species by multi-gene phylogenetic analyses (Barbosa et al. 2018; Chen et al. 2016; Guevara-Suarez et al. 2016, 2020; Su and Niu 2018; Visagie et al. 2014) Based on this molecular approach, we were able to find a remarkable diversity of *Penicillium* that inhabit on herbivore dung so far. Among the 38 isolates recovered, we identified a total of 12 species, 12 *Penicillium* species belong to seven sections (i.e. sections. *Fasciculata*, *Citrina*, *Lanata-divaricata*, *Chrysogena*, *Citrina*, *Fasciculata*, *Ramigena* and *Roquefortorum*). In addition, of note is that the *Acaulium* and *Kernia* species are frequently isolated. Seven represent stains were selected for detailed study. Phylogenetic trees based on combined sequence datasets of ITS, *TUB*, *LSU* and *TEF* indicated that these strains clustered in two distinct, well supported and two previously unknown subclades within *Acaulium* and *Kernia* clades. Therefore, two new species were described corresponding to those two clades. The species of *Kernia anthracina* was described by Su et al. 2020. The new species of *Acaulium stericum* is described in here and characterized by short conidiophores, the dimensions of conidiogenous cells and conidia, and the irregular chlamydospores and multi-gene phylogeny.

Among *Penicillium* isolates, those of the sections *Fasciculata* and *Lanata-divaricata* were the most frequently isolated, accounting nearly 30 % of the isolates in each section. However, that in *Fasciculata* most isolates identified was of *P. polonicum*, while in *Lanata-divaricata*, we found the most of strains were identified as *P. oxalicum*. These species would seem to be common intestinal fungi in herbivore dung, but they were mainly isolated from chinchilla. The intestinal fungi which isolated from marmot dung mainly belong to *Penicillium* sections, as sect. *Chrysogena*, *Citrina*, *Roquefortorum*. Besides, *Acaulium* and *Kernia* species were also frequently isolated. Section *Fasciculata* was common on stored or manufactured foods as stored cereals, cheese, nuts, and other fat and protein rich substrates. Section *Roquefortorum* was common in symbiotic relationship with lactic acid bacteria and certain acid-tolerant yeasts (Samson et al. 2004). Section *Chrysogena* usually isolated from dry habitats, e.g. desert and Artic or indoor environments (Houbraken et al. 2016). As mentioned before, Guevara-Suarez et al. (2020) proposed the majority of coprophilous *Penicillium* species in sect. *Fasciculata* and sect. *Robsamsonia*. However, our results clearly show that the coprophilous penicillia are distributed across different and

phylogenetically distant sections of the genus (Fig. 1). In sections such as *Fasciculata* and *Lanata-divaricata*, we even found a considerable diversity of *Penicillium* species. In addition, we also identified *P. fructuariae-cellae*, *P. hispanicum*, *P. steckii*, and *P. oxalicum* which belong to sect. *Citrina*, *Lanata-divaricata* and *Ramigena* as first records on dung.

Isolates of section *Citrina* (100 % bs) were identified as *P. citrinum* (n = 2) and *P. steckii* (n = 2). Although we have not found previous records of *P. steckii* from dung, they were very common isolates from marine organisms (Malmstrùm et al. 2000). Nearly 40 species were comprised in this section, but only those identified here were included in the analysis to simplify the four genes phylogenetic tree (Fig. 2). Although section *Lanata-Divaricata* (100% bs) included 14 dung isolates, there were only two species and they were all isolated from chinchilla dung. Besides, these species have not found previous records from dung, but they can produce extracellular enzyme systems with good lignocellulose hydrolysis performance as *P. oxalicum* have been used in commercial production of lignocellulolytic enzymes in China for more than a decade and *P. fructuariae-cellae* (Fig. 6) were isolated from the fruit of *Vitis viniferain* fruit-drying room for grape withering (Lorenzini et al. 2018). Section *Ramigena* was introduced by Thom (1930), which was proposed by Houbraken and Samson (2011) and included five species *Penicillium capsulatum*, *P. cyaneum*, *P. hispanicum*, *P. ornatum*, *P. ramusculum* (Houbraken et al. 2020). The only isolate of section *Ramigena* (100% bs) was identified as *P. hispanicum*, which isolated from marmot dung, a species commonly inhabiting fruit (Ramirez et al. 1978). Other isolates from dung have been records (Guevara-Suarez et al. 2020), but they are being widely used in industry, such as cheese starters *P. roqueforti* (Ropars et al. 2014) and *P. echinulatum* mutants have the potential to produce high cellulases and its enzyme complex with good stability at 50°C (Camassola 2007; Camassola et al. 2010). Therefore, our study highlights that herbivore dung is a good substrate for recovering interesting species of penicillium-like fungi and discovery of new cellulases from microbial communities, although their ecological role on that substrate remains unclear and would merit further investigation.

Declarations

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Author contribution The samples in this study were collected by B. Yang, P. Sun and X. Li. Mophological investigations were observed and illustrated by L. Su. Description of the new and known species were described by L. Su. Molecular data and phylogenetic analyses were performed by L. Su. L. Su wrote the original draft, and review and editing were performed by H. Zhu, H. Gao, Z. Xiang and C. Qin. All authors have read and approved the manuscript.

Data availability

All sequence data generated in this study (see Table 1) are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Alignment files can be accessed via TreeBASE (http://www.treebase.org).

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of interest

The authors declare no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

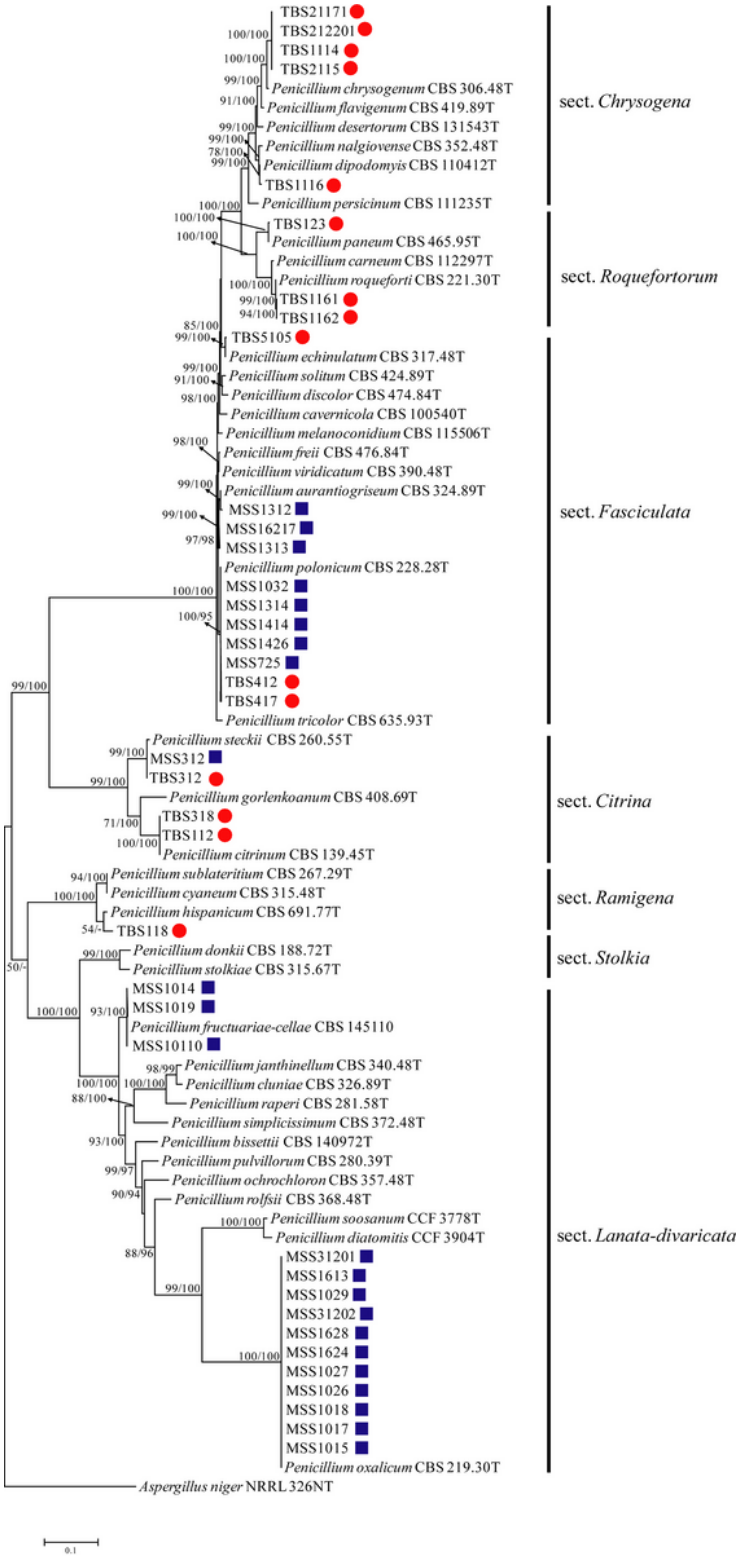

Figure 1

ML tree of Penicillium inferred from RPB2 including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the parallel diagonal lines. Bootstrap support values/Bayesian

posterior probability scores above 50%/95% are indicated on the nodes. Isolates from dung are shown in different shapes (red circles represents as isolates from marmot dung, blue squares represents as isolates from chinchilla dung). The tree is rooted to Aspergillus niger NRRL 326NT. T = ex-type strain.

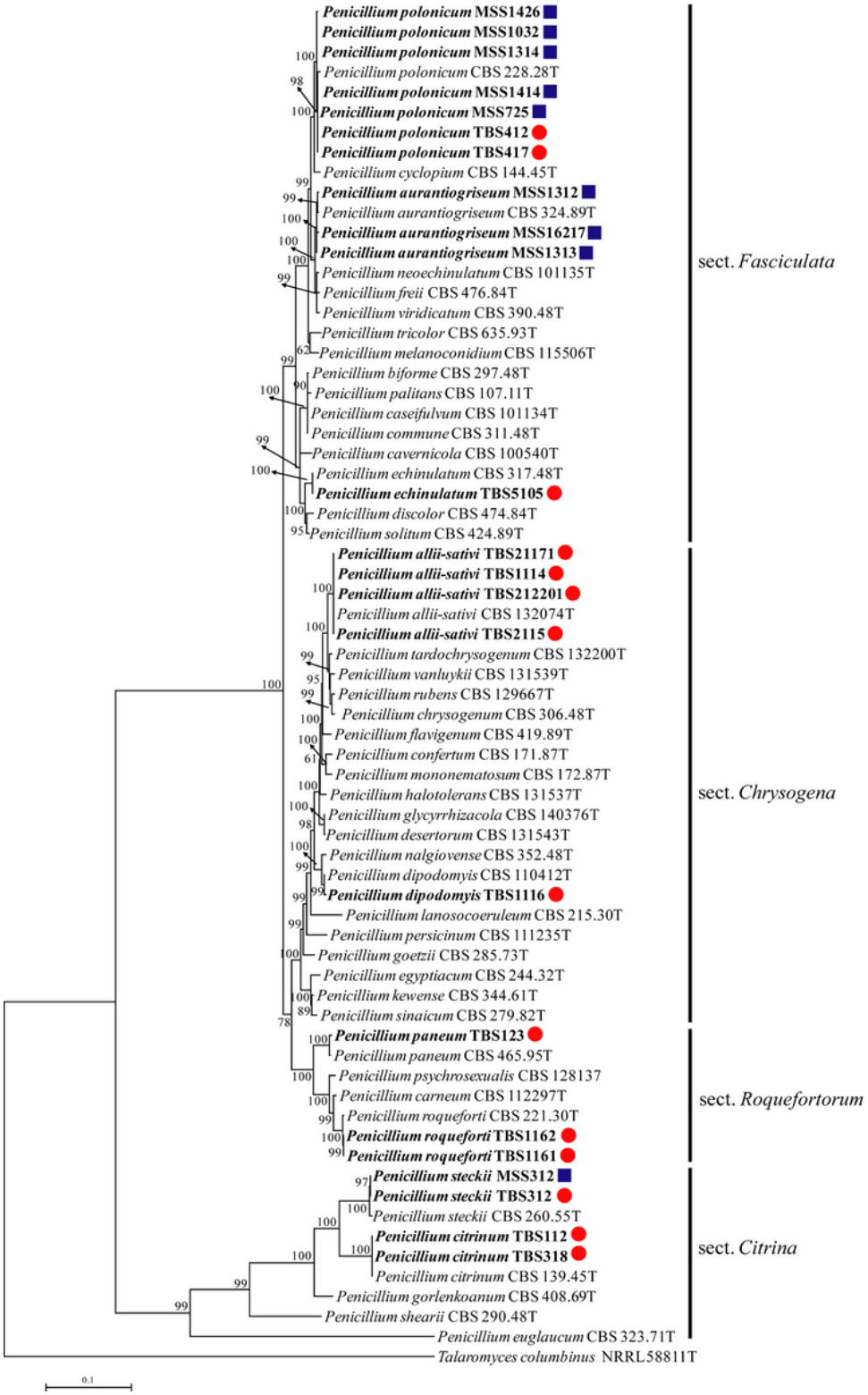

Figure 2

ML tree of Penicillium inferred from ITS, TUB, CAM, and RPB2 loci including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Bootstrap support values

above 50% are indicated on the nodes. Isolates from dung are shown in different shapes (red circles represents as isolates from marmot dung, blue squares represents as isolates from chinchilla dung). The tree is rooted to Talaromyces columbinus NRRL 58811T. T = ex-type strain.

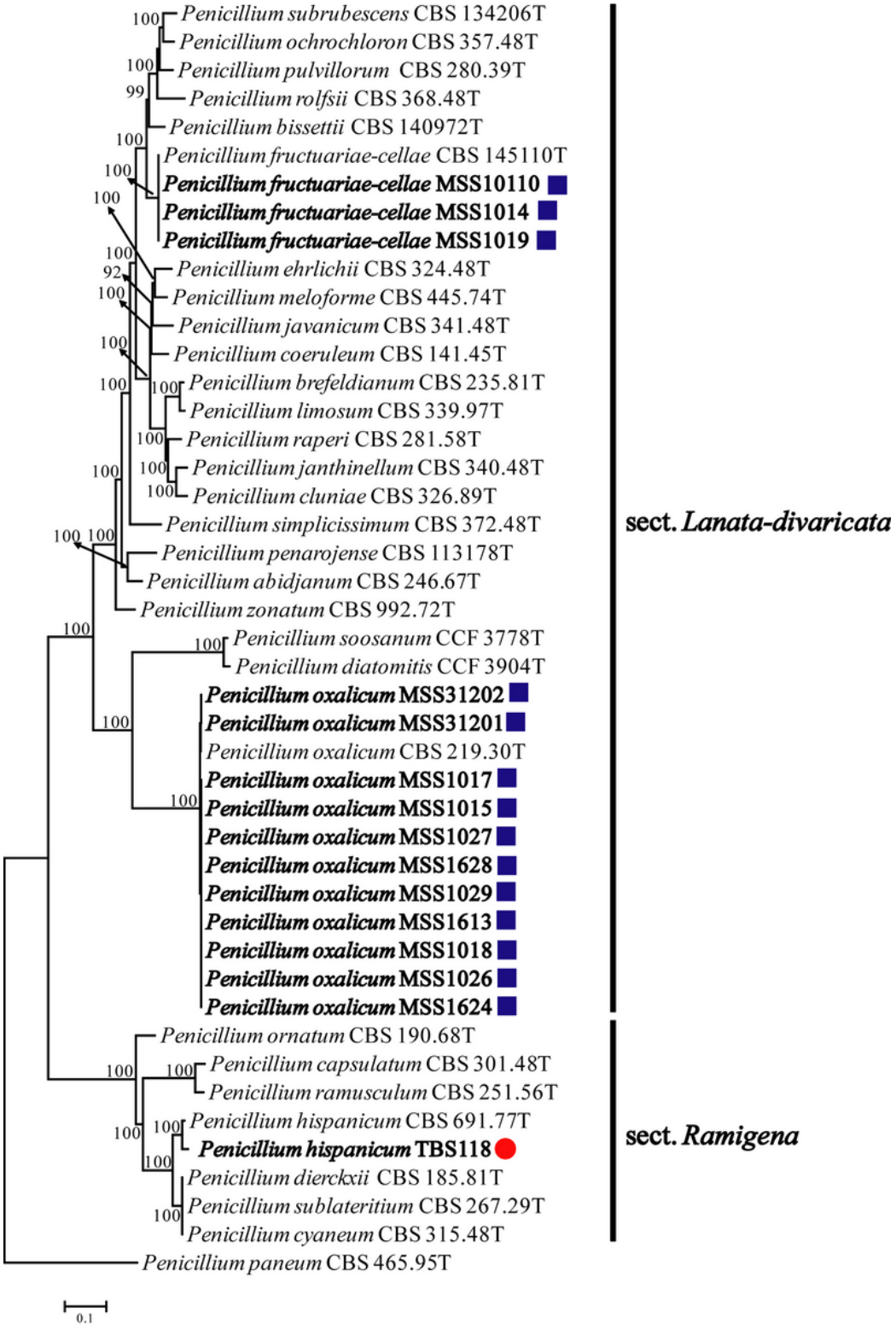

Figure 3

ML tree of Penicillium inferred from ITS, TUB, CAM, and RPB2 loci including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Bootstrap support values

above 50% are indicated on the nodes. Isolates from dung are shown in different shapes (red circles represents as isolates from marmot dung, blue squares represents as isolates from chinchilla dung). The tree is rooted to Penicillium paneum CBS 465.95T. T = ex-type strain.

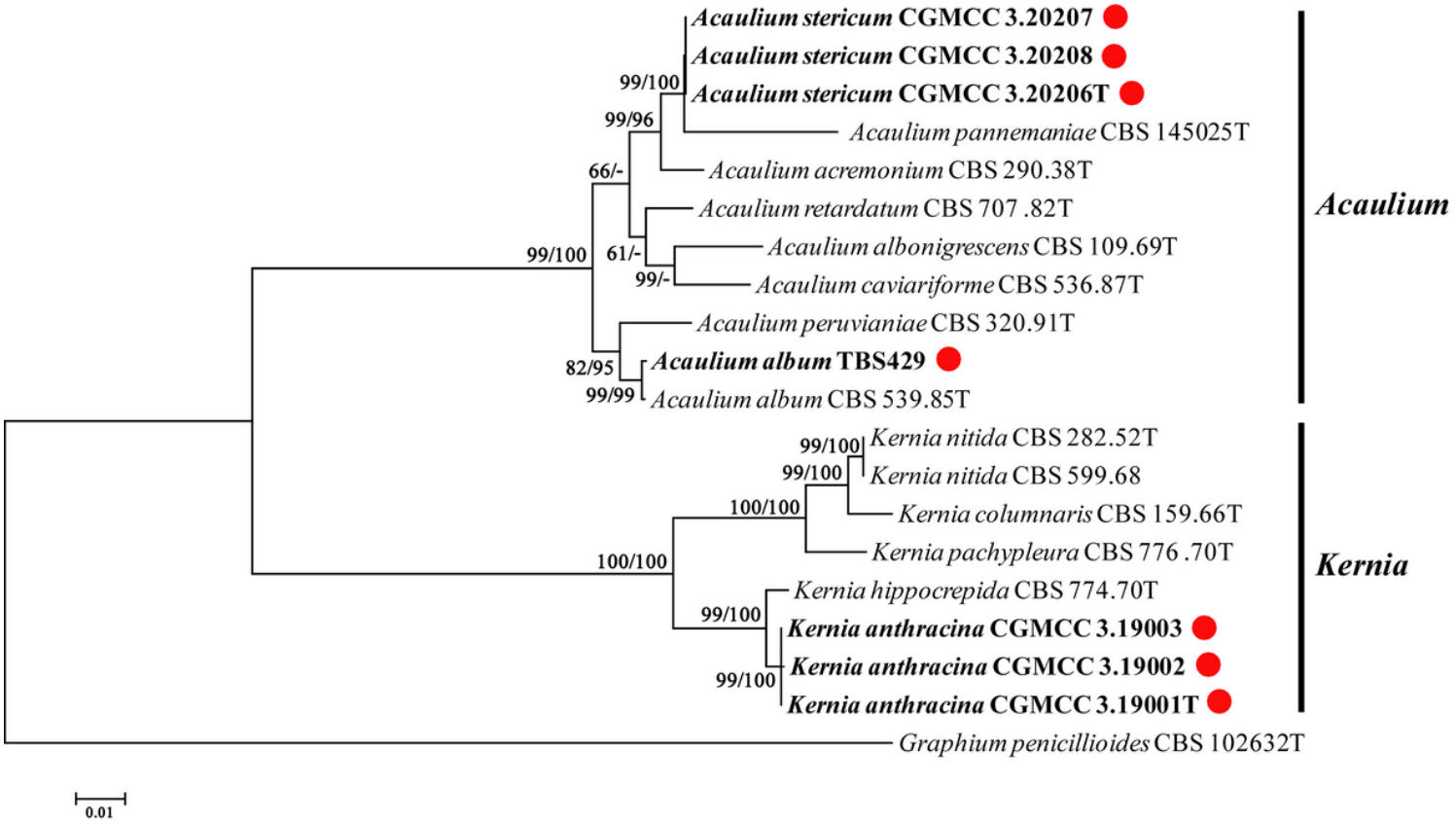

Figure 4

Maximum likelihood (ML) tree obtained from the combined LSU, ITS, TEF and TUB sequences included most of species in the genera of Kernia and Acaulium. Numbers on the branches are ML bootstrap values (bs) above 50% and Bayesian posterior probabilities (pp) above 95%. A dash (–) indicates support value lower than 75% bs or 95% pp. Isolates from marmot dung are shown in red circles. The tree was rooted to Graphium penicillioides CBS 102632T. T = ex-type strain.

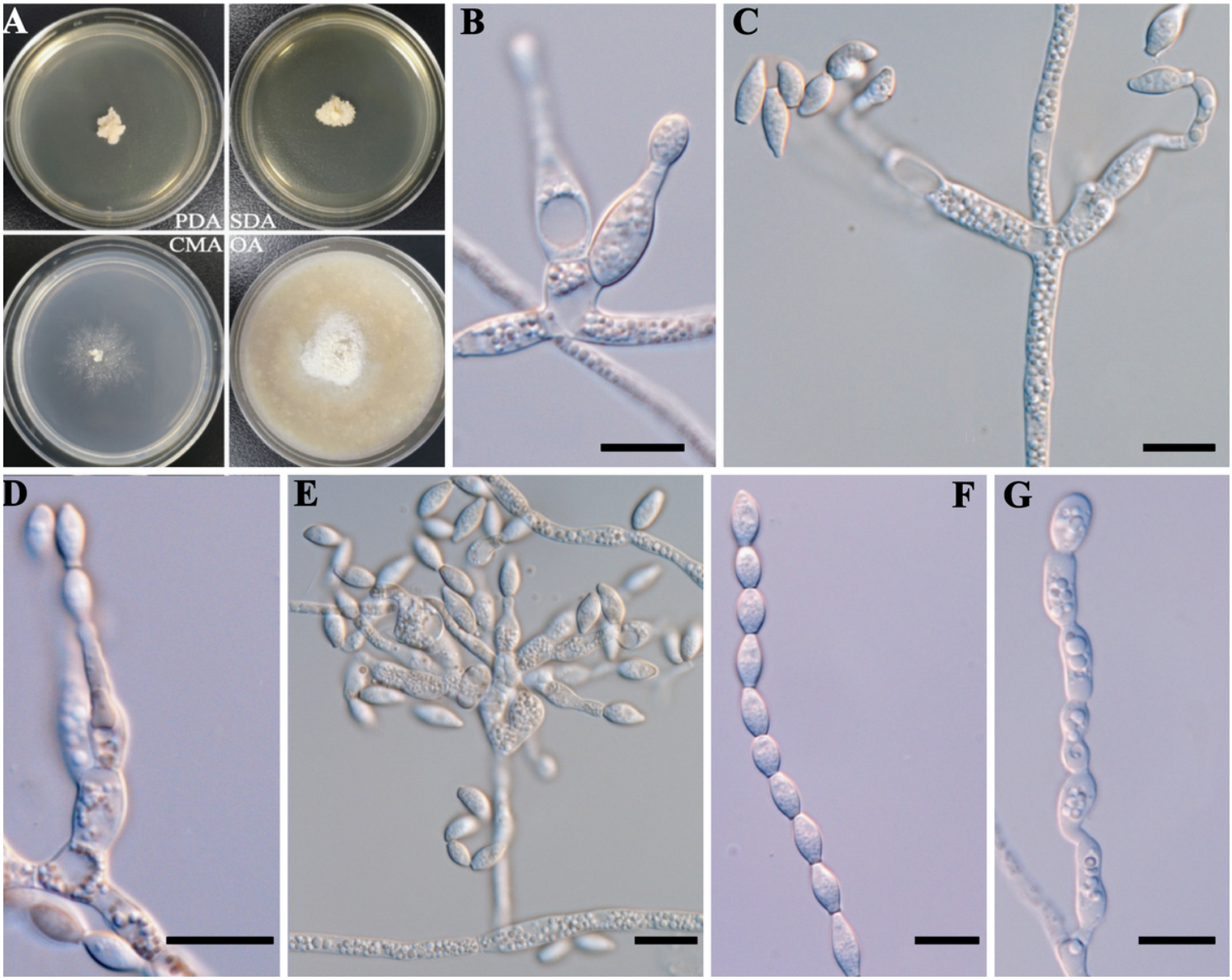

Figure 5

Acaulium stericum (CGMCC 3.20206). A Colonies on different media after 15 days at 25 °C, B-E conidiophores and conidiogenous cells, F conidia, G irregular chlamydospores. Bar = 10 μm.

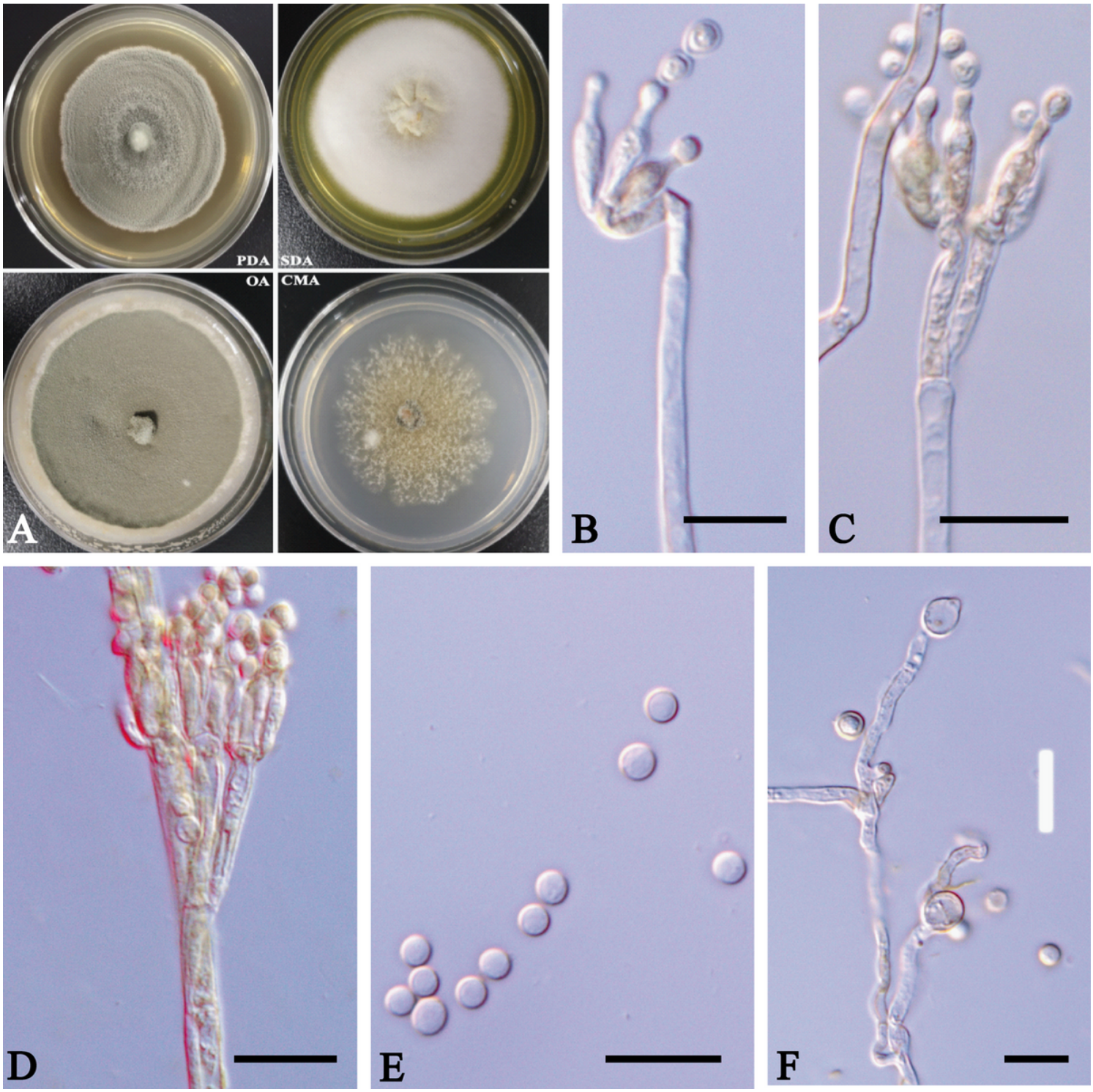

Figure 6

Penicillium fructuariae-cellae (MSS1014). A Colonies on different media after 15 days at 25 °C, B-D conidiophores and conidiogenous cells, E conidia, F chlamydospores. Scale bars: 10 μm (B–F).

Supplementary Files

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- Table1.pdf