MycoKeys 41: 1–15 (2018)
doi: 10.3897/mycokeys.41.27536
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
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Beta-tubulin and Actin gene phylogeny supports *Phaeoacremonium ovale* as a new species from freshwater habitats in China

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Academic editor: *Marc Stadler* | Received 15 June 2018 | Accepted 10 September 2018 | Published 11 October 2018

Citation: Huang S-K, Jeewon R, Hyde KD, Bhat DJ, Chomnunti P, Wen T-C (2018) Beta-tubulin and Actin gene phylogeny supports *Phaeoacremonium ovale* as a new species from freshwater habitats in China. MycoKeys 41: 1–15. https://doi.org/10.3897/mycokeys.41.27536

Abstract

A new species of *Phaeoacremonium*, *P. ovale* (Togniniaceae), was isolated during a diversity study of freshwater fungi from Yunnan Province in China. Morphological and cultural studies of the fungus were carried out and its sexual and asexual morphs (holomorph) are introduced herein. This species is characterised by peculiar long-necked, semi-immersed ascomata with oval to ellipsoid ascospores and ellipsoid to ovoid conidia. Phylogenetic analyses of a combined TUB and ACT gene dataset revealed that strains of *P. ovale* constitute a strongly supported independent lineage and are related to *P. griseo-olivaceum* and *P. africanum*. The number of nucleotide differences, across the genes analysed, also supports establishment of *P. ovale* as a new species.

Keywords

1 new species, Togniniales, Sordariomycetes, Morphology, Phylogeny

Introduction

Lignicolous freshwater fungi are important in nutrient recycling (Hyde et al. 2016). A number of taxonomic studies have focused on the diversity of such fungi in the South East Asian region and these investigations have reported a number of novel species (e.g. Jeewon et al. 2003; Cabanela et al. 2007; Zhang et al. 2008; Luo et al. 2018). In this study, we report a new species of *Phaeoacremonium* isolated from decaying wood from a stream in Yunnan Province, China.

Phaeoacremonium (= *Togninia*), introduced by Crous et al. (1996), is typified by *P. parasiticum* and it belongs to Togniniaceae (Gramaje et al. 2015). *Phaeoacremonium* was reported to be the asexual morph of *Togninia* (Mostert et al. 2003, 2006a; Pascoe et al. 2004). Gramaje et al. (2015) proposed *Phaeoacremonium* over *Togninia* as the correct name based on common usage and this has been listed in Réblová et al. (2016) and followed in Wijayawardene et al. (2018). The species are basically characterised by black ascomata with a long neck and clavate to cylindrical asci with oval to ellipsoid, hyaline ascospores and straight or flexuous mononematous conidiophores with oval to reniform phialo-conidia (Marin-Felix et al. 2018; Spies et al. 2018).

Most species of *Phaeoacremonium* are plant or/and human pathogens and some have been recorded on arthropods or in soil (Groenewald et al. 2001; Guarro et al. 2003; Hemashettar et al. 2006; Mostert et al. 2006a; Damm et al. 2008; Gramaje et al. 2015) while others are causal agents of Petri disease and esca of grapevines (Pascoe et al. 2004; Rooney-Latham et al. 2005a; Mostert et al. 2006b). *Phaeoacremonium* species can also infect a wide range of woody hosts, such as cherry, apricot, olive and peach trees (Rumbos 1986; Di Marco et al. 2004; Kubátová et al. 2004). Recent studies have reported the importance of *Phaeoacremonium* species in causing brown wood streaking of *Olea* spp. and *Prunus* spp. (Mostert et al. 2006b; Damm et al. 2008; Gramaje et al. 2012; Nigro et al. 2013; Olmo et al. 2014; Carlucci et al. 2015). Rooney-Latham et al. (2004, 2005a, b) reported that, in the presence of water, spores in some *Phaeoacremonium* species are forcibly discharged from perithecia through the long neck and exit the ostiole to be dispersed by wind, rain or insects in order to colonise other substrates. Recently Hu et al. (2012) introduced a freshwater inhabiting species, *Phaeoacremonium aquaticum* (= *Togninia aquatica*).

Species of Togniniaceae have been reported to colonise substrates in different types of habitats and recent taxonomic studies have revealed additional new species (Gramaje et al. 2015). We have been studying fungi along a north-south gradient in the Asian region (Hyde et al. 2016) and, in this study, we report on two collections of *Phaeoacremonium* from China. The aim here is to characterise these two strains as one novel species based on morphology as well as to investigate their phylogenetic affinities with previously known Togniniaceae species based on partial TUB and ACT genes.

Materials and methods

Sample collection, morphological studies and isolation

Submerged dead wood was collected from Baoshan, Yunnan Province in China in October 2016, brought to the laboratory in zip lock plastic bags and treated in the laboratory following procedures detailed in Luo et al. (2018). Fruiting bodies were found growing on decaying wood in a sterile plastic box after two weeks of incubation and the fungus was subsequently isolated based on the method of Chomnunti et al. (2014). Specimens were examined by a Motic SMZ 168 stereomicroscope. Micromorphological characters were examined using a Nikon ECLIPSE 80i compound microscope and images were captured with a Canon EOS 600D digital camera. Identification of colours was based on Ridgway (1912). The Taro soft Image Framework programme version 0.9.0.7 was used for measurements. Single spores were isolated and grown on water agar (WA) and potato dextrose agar (PDA) media. Ascospores germinated on PDA within 1 week. The colonies were transferred to WA and PDA to promote sporulation (sporulation occurred after 30 days in PDA). The cultures were checked 2 to 3 times per week and all procedures were performed in a sterile environment and at room temperature. The morphological characters of the asexual morph were examined after sporulation. Specimens are deposited in the Kunming Institute of Botany, Academia Sinica (KUN) and duplicated in Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Facesoffungi numbers (FoF) (http://www.facesoffungi.org/) were obtained as stated in Jayasiri et al. (2015) and Index Fungorum numbers (IF) (http://www.indexfungorum.org/names/IndexFungorumRegisterName.asp).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from mycelium using a Trelief Plant Genomic DNA Kit following the instructions of the manufacturer. The genomic DNA was amplified by using polymerase chain reaction (PCR) in a 25 µl reaction mixture. Partial regions of the beta-tubulin (TUB) and Actin (ACT) gene were amplified using the primer pairs T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), ACT-513F and ACT-783R (Carbone and Kohn 1999), respectively. The internal transcribed spacers (ITS) regions of the rDNA (ITS1-5.8S-ITS2) were also amplified using primer pairs ITS5 and ITS4 (White et al. 1990) but no further analyses were done on these due to lack of sequence data. The PCR conditions for these regions were as follows: an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 51 °C (TUB) or 60 °C (ACT) or 55 °C (ITS) for 50 sec and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were then sequenced with the primers mentioned above by a commercial sequencing provider (Tsingke Company, Beijing, P.R. China).

Phylogenetic analysis

The quality of the amplified nucleotide sequences was checked and combined by SeqMan version 7.1.0 (44.1) and Finch TV version 1.4.0 (www.geospiza.com). Sequences used by Marin-Felix et al. (2018), Spies et al. (2018) and the closest matches for our strains were retrieved from the National Center for Biotechnology Information (NCBI) by nucleotide BLAST. Sequences were aligned in MAFFT v. 7.310 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2016) and manually corrected in Bioedit 7.0.9.0 (Hall 1999).

The phylogenetic analyses of combined gene regions (TUB and ACT) were performed using maximum-likelihood (ML) and Bayesian Inference (BI) methods. The best-fit model (GTR+G+I) was obtained using jModelTest 2.1.10 under the Akaike Information Criterion (AIC) calculations (Darriba et al. 2012). The ML analysis was enforced with RAxML-HPC v.8 on XSEDE (Stamatakis 2014; Miller et al. 2015) with 1000 rapid bootstrap replicates. Bayesian inference was implemented by MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003). Four simultaneous Markov chains were run for 5,000,000 generations sampling one tree every 1000th generations and other criteria as outlined by Hongsanan et al. (2017). The temperature value was lowered to 0.15, burn-in was set to 0.25. Gaps were treated as missing data with no differential weighting of transitions against transversions and the partition homogeneity test was performed to assess whether datasets from different genes were congruent. Phylogenetic trees were viewed with FigTree v1.4.0 (http:// tree.bio.ed.ac.uk/software/figtree/) and processed by Adobe Illustrator CS5. Alignment and trees were deposited in TreeBASE (submission ID: 22810). The nucleotide sequence data of the new taxon have been deposited in GenBank (Table 1).

Results

Phylogenetic analyses

The combined TUB and ACT sequence dataset comprised 98 strains of *Phaeoacremonium*. The tree was rooted with *Pleurostoma richardsiae* (CBS 270.33) and *Wuestineaia molokaiensis* (CBS 114877). The alignment comprised 947 total characters including gaps (TUB: 646bp; ACT: 301bp). ML and BI analyses yielded trees which were topologically congruent in terms of species groupings. RAxML analysis yielded a best scoring tree with a final optimisation likelihood value of -15310.399369 (Fig. 1). In the phylogenetic tree, two strains of *Phaeoacremonium ovale* forms a well-supported independent subclade (100%, ML/1.00, PP) and closely related to other *Phaeoacremonium* species in Clade I (83%, ML/0.99, PP).

Table 1. Strains and GenBank accession numbers of the isolates used in this study. Isolates from this study are marked with asterisk (*) and the type strains are in bold.

Species	Voucher/Culture	GenBank accession number	
		TUB	ACT
Phaeoacremonium africanum	**CBS 120863**	**EU128100**	**EU128142**
Phaeoacremonium album	**CBS 142688**	**KY906885**	**KY906884**
Phaeoacremonium alvesii	**CBS 110034**	**AY579301**	**AY579234**
Phaeoacremonium alvesii	CBS 729.97	AY579302	AY579235
Phaeoacremonium amstelodamense	**CBS 110627**	**AY579295**	**AY579228**
Phaeoacremonium amygdalinum	**CBS 128570**	**JN191307**	**JN191303**
Phaeoacremonium amygdalinum	CBS H-20507	JN191305	JN191301
Phaeoacremonium amygdalinum	CBS H-20508	JN191306	JN191302
Phaeoacremonium angustius	**CBS 114992**	**DQ173104**	**DQ173127**
Phaeoacremonium angustius	CBS 114991	DQ173103	DQ173126
Phaeoacremonium argentinense	**CBS 777.83**	**DQ173108**	**DQ173135**
Phaeoacremonium armeniacum	**ICMP 17421**	**EU596526**	**EU595463**
Phaeoacremonium aureum	**CBS 142691**	**KY906657**	**KY906656**
Phaeoacremonium australiense	**CBS 113589**	**AY579296**	**AY579229**
Phaeoacremonium australiense	CBS 113592	AY579297	AY579230
Phaeoacremonium austroafricanum	**CBS 112949**	**DQ173099**	**DQ173122**
Phaeoacremonium austroafricanum	CBS 114994	DQ173102	DQ173125
Phaeoacremonium austroafricanum	CBS 114993	DQ173101	DQ173124
Phaeoacremonium bibendum	**CBS 142694**	**KY906759**	**KY906758**
Phaeoacremonium canadens**e**	**PARC327**	**KF764651**	**KF764499**
Phaeoacremonium cf. mortoniae	**ICMP 18088**	**HM116767**	**HM116773**
Phaeoacremonium cinereum	**CBS 123909**	**FJ517161**	**FJ517153**
Phaeoacremonium cinereum	CBS H-20215	FJ517160	FJ517152
Phaeoacremonium cinereum	CBS H-20213	FJ517158	FJ517150
Phaeoacremonium croatiense	**CBS 123037**	**EU863482**	**EU863514**
Phaeoacremonium fraxinopennsylvanicum	**CBS 101585**	**AF246809**	**DQ173137**
Phaeoacremonium fraxinopennsylvanicum	CBS 110212	DQ173109	DQ173136
Phaeoacremonium fuscum	**CBS 120856**	**EU128098**	**EU128141**
Phaeoacremonium gamsii	**CBS 142712**	**KY906741**	**KY906740**
Phaeoacremonium geminum	**CBS 142713**	**KY906649**	**KY906648**
Phaeoacremonium globosum	**ICMP 16988**	**EU596525**	**EU595466**
Phaeoacremonium globosum	ICMP 17038	EU596521	EU595465
Phaeoacremonium globosum	ICMP 16987	EU596527	EU595459
Phaeoacremonium griseo-olivaceum	**CBS 120857**	**EU128097**	**EU128139**
Phaeoacremonium griseorubrum	**CBS 111657**	**AY579294**	**AY579227**
Phaeoacremonium griseorubrum	CBS 566.97	AF246801	AY579226
Phaeoacremonium hispanicum	**CBS 123910**	**FJ517164**	**FJ517156**
Phaeoacremonium hungaricum	**CBS 123036**	**EU863483**	**EU863515**
Phaeoacremonium inflatipes	**CBS 391.71**	**AF246805**	**AY579259**
Phaeoacremonium inflatipes	CBS 113273	AY579323	AY579260
Phaeoacremonium iranianum	**CBS 101357**	**DQ173097**	**DQ173120**
Phaeoacremonium iranianum	CBS 117114	DQ173098	DQ173121
Phaeoacremonium italicum	**CBS 137763**	**KJ534074**	**KJ534046**
Phaeoacremonium italicum	CBS 137764	KJ534075	KJ534047
Phaeoacremonium italicum	CBS H-21638	KJ534076	KJ534048
Phaeoacremonium junior	**CBS 142697**	**KY906709**	**KY906708**
Phaeoacremonium krajdenii	CBS 110118	AY579324	AY579261
Phaeoacremonium krajdenii	**CBS 109479**	**AY579330**	**AY579267**
Phaeoacremonium longicollarum	**CBS 142699**	**KY906689**	**KY906688**
Phaeoacremonium luteum	**CBS 137497**	**KF823800**	**KF835406**
Phaeoacremonium meliae	**CBS 142710**	**KY906825**	**KY906824**

Species	Voucher/Culture	GenBank accession number	
		TUB	ACT
Phaeoacremonium minimum	**CBS 246.91**	**AF246811**	**AY735497**
Phaeoacremonium minimum	CBS 100397	AF246806	AY735498
Phaeoacremonium mortoniae	CBS 211.97	AF246810	
Phaeoacremonium nordesticola	**CMM4312**	**KY030807**	**KY030803**
Phaeoacremonium novae-zealandiae	**CBS 110156**	**DQ173110**	**DQ173139**
Phaeoacremonium novae-zealandiae	CBS 110157	DQ173111	DQ173140
Phaeoacremonium occidentale	**ICMP 17037**	**EU596524**	**EU595460**
Phaeoacremonium oleae	**CBS 142704**	**KY906937**	**KY906936**
****Phaeoacremonium ovale***	**KUMCC 17-0145**	**MH395327**	**MH395325**
****Phaeoacremonium ovale***	**KUMCC 18-0018**	**MH395328**	**MH395326**
Phaeoacremonium pallidum	**CBS 120862**	**EU128103**	**EU128144**
Phaeoacremonium parasiticum	**CBS 860.73**	**AF246803**	**AY579253**
Phaeoacremonium parasiticum	CBS 113585	AY579307	AY579241
Phaeoacremonium parasiticum	CBS 514.82	AY579306	AY579240
Phaeoacremonium paululum	**CBS 142705**	**KY906881**	**KY906880**
Phaeoacremonium pravum	CBS 142686	KY084246	KY084248
Phaeoacremonium proliferatum	**CBS 142706**	**KY906903**	**KY906902**
Phaeoacremonium prunicola	**CBS 120858**	**EU128095**	**EU128137**
Phaeoacremonium prunicola	CBS 120858	EU128096	EU128138
Phaeoacremonium pseudopanacis	**CPC 28694**	**KY173609**	**KY173569**
Phaeoacremonium roseum	**PARC273**	**KF764658**	**KF764506**
Phaeoacremonium rosicola	**CBS 142708**	**KY906831**	**KY906830**
Phaeoacremonium rubrigenum	**CBS 498.94**	**AF246802**	**AY579238**
Phaeoacremonium rubrigenum	CBS 112046	AY579305	AY579239
Phaeoacremonium santali	**CBS 137498**	**KF823797**	**KF835403**
Phaeoacremonium scolyti	**CBS 113597**	**AF246800**	**AY579224**
Phaeoacremonium scolyti	CBS 113593	AY579293	AY579225
Phaeoacremonium scolyti	CBS 112585	AY579292	AY579223
Phaeoacremonium sicilianum	**CBS 123034**	**EU863488**	**EU863520**
Phaeoacremonium sicilianum	CBS 123035	EU863489	EU863521
Phaeoacremonium sp.	KMU 8592	AB986584	AB986583
Phaeoacremonium spadicum	**CBS 142711**	**KY906839**	**KY906838**
Phaeoacremonium sphinctrophorum	**CBS 337.90**	**DQ173113**	**DQ173142**
Phaeoacremonium sphinctrophorum	CBS 694.88	DQ173114	DQ173143
Phaeoacremonium subulatum	**CBS 113584**	**AY579298**	**AY579231**
Phaeoacremonium subulatum	CBS 113587	AY579299	AY579232
Phaeoacremonium tardicrescens	**CBS 110573**	**AY579300**	**AY579233**
Phaeoacremonium tectonae	**MFLUCC 13-0707**	**KT285563**	**KT285555**
Phaeoacremonium tectonae	MFLUCC 14-1131	KT285570	KT285562
Phaeoacremonium theobromatis	**CBS 111586**	**DQ173106**	**DQ173132**
Phaeoacremonium tuscanicum	**CBS 123033**	**EU863458**	**EU863490**
Phaeoacremonium venezuelense	**CBS 651.85**	**AY579320**	**AY579256**
Phaeoacremonium venezuelense	CBS 110119	AY579318	AY579254
Phaeoacremonium venezuelense	CBS 113595	AY579319	AY579255
Phaeoacremonium vibratile	CBS 117115	DQ649063	DQ649064
Phaeoacremonium viticola	CBS 113065	DQ173105	DQ173128
Phaeoacremonium viticola	CBS 101737	AF246817	DQ173129
Pleurostomophora richardsiae	CBS 270.33	AY579334	AY579271
Wuestneia molokaiensis	CBS 114877	AY579335	AY579272

Abbreviations: **CBS**: CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CMM**: Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes"; **CPC**: Culture collection of Pedro Crous, housed at CBS; **HKUCC**: The University of Hong Kong Culture Collection; **ICMP**: The International Collection of Microorganisms from Plants; **KMU**: Kanazawa Medical University herbarium; **MFLU**: Mae Fah Luang University herbarium, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **PARC**: Pacific Agri-Food Research Centre.

Taxonomy

Phaeoacremonium ovale **S.K. Huang, R. Jeewon & K.D. Hyde, sp. nov.**
Index Fungorum number: IF554786
Facesoffungi number: FoF 04685
Fig. 2

Type. CHINA, Yunnan Province, Baoshan, stream along the roadside; saprobic on dead wood, 21 December 2016; Huang S.K. (KUN HKAS99550, holotype; MFLU MFLU18-1076, isotype); ex-type living culture (KUMCC 17-0145; KUMCC 18-0018). GenBank no. (ITS: MH399732, TUB: MH395327, ACT: MH395325; ITS: MH399733, TUB: MH395328, ACT: MH395326)

Etymology. The name *ovale* refers to the oval shaped ascospores.

Description. Sexual morph: *Ascomata* 225–300 µm (n = 5), on wood, perithecial, solitary, semi-immersed, unilocular, subglobose to globose, black, ostiolate, with ostiolar neck erumpent through bark of host when mature. *Neck* 445–645 × 35–45 µm ($\bar{x}$ = 530 × 40 µm, n = 5), centrally ostiolate, contorted, lined with hyaline periphyses. *Peridium* 17–40 µm diam., membranous, composed of dark brown to hyaline cells of *textura angularis*. *Hamathecium* composed of 2–6 µm wide, hyaline, septate paraphyses, slightly constricted at septa and gradually narrowed towards apex. *Asci* 11–20 × 3–6 µm ($\bar{x}$ = 15.5 × 5 µm, n = 30), 8-spored, unitunicate, clavate, with short pedicel, apically rounded. *Ascospores* 3–5 × 1.5–3 µm ($\bar{x}$ = 3.5 × 2 µm, n = 50), bi-seriate, hyaline, oval to ellipsoid, aseptate, smooth-walled, rounded at the ends. **Asexual morph:** *Mycelium* on culture, partly superficial, composed of septate, branched, hyaline, rarely verrucose, hyphae 1.5–3 µm diam., rarely with adelophialides. *Conidiophores* usually arising from hyaline hyphae, mononematous, unbranched, occasionally constricted at basal septum, hyaline. *Phialides* 8–15 × 2–4 µm ($\bar{x}$ = 9.5 × 3 µm, n = 20), terminal, monophialidic, elongate-ampulliform and attenuated at base. *Conidia* 2.5–6 × 1–2.5 µm ($\bar{x}$ = 4 × 2 µm, n = 30), hyaline, ellipsoid to ovoid, aseptate.

Culture characteristics. Ascospore germinating on PDA within 1 week at 23°C, germ tubes produced from ends. Colonies growing on PDA, reaching 2 cm diam. and sporulating after 30 days. Colonies semi-immersed to superficial, irregular in shape, flat, slightly raised, with undulate edge, slightly rough on surface, cottony to fairly fluffy, colony from above, greyish-brown (5F3–5, Ridgway 1912) at the margin, initially write to cream (5A1–3) in the centre, becoming dark brown (5F7–8) at the margin, orange-white (5B1–3) at the centre; from below, initially, greyish-brown at the margin, white at the centre, becoming dark brown at the margin, orange-white at the centre, producing brown pigmentation in agar.

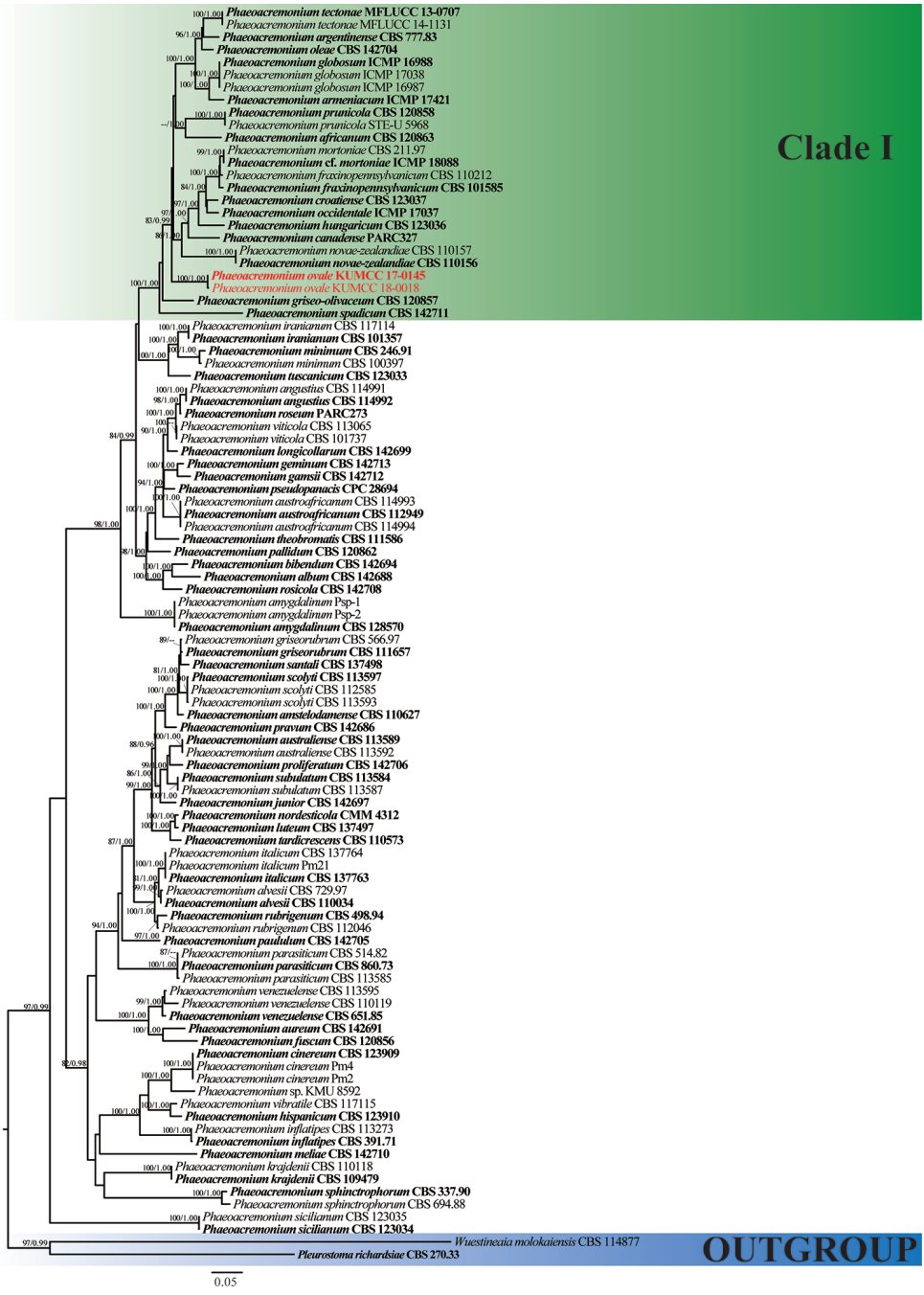

Figure 1. Maximum likelihood phylogenetic tree generated from analysis of a combined TUB and ACT sequences dataset for 98 taxa of Togniniaceae. *Pleurostoma richardsiae* (CBS 270.33) and *Wuestineaia molokaiensis* (CBS 114877) are the outgroup taxa. ML support values greater than 70% (BSML, left) and Bayesian posterior probabilities greater than 0.90 (BYPP, right) are indicated above the nodes. The strain numbers are noted after the species names. Ex-type strains are indicated in **bold**. Isolates from this study are indicated in red.

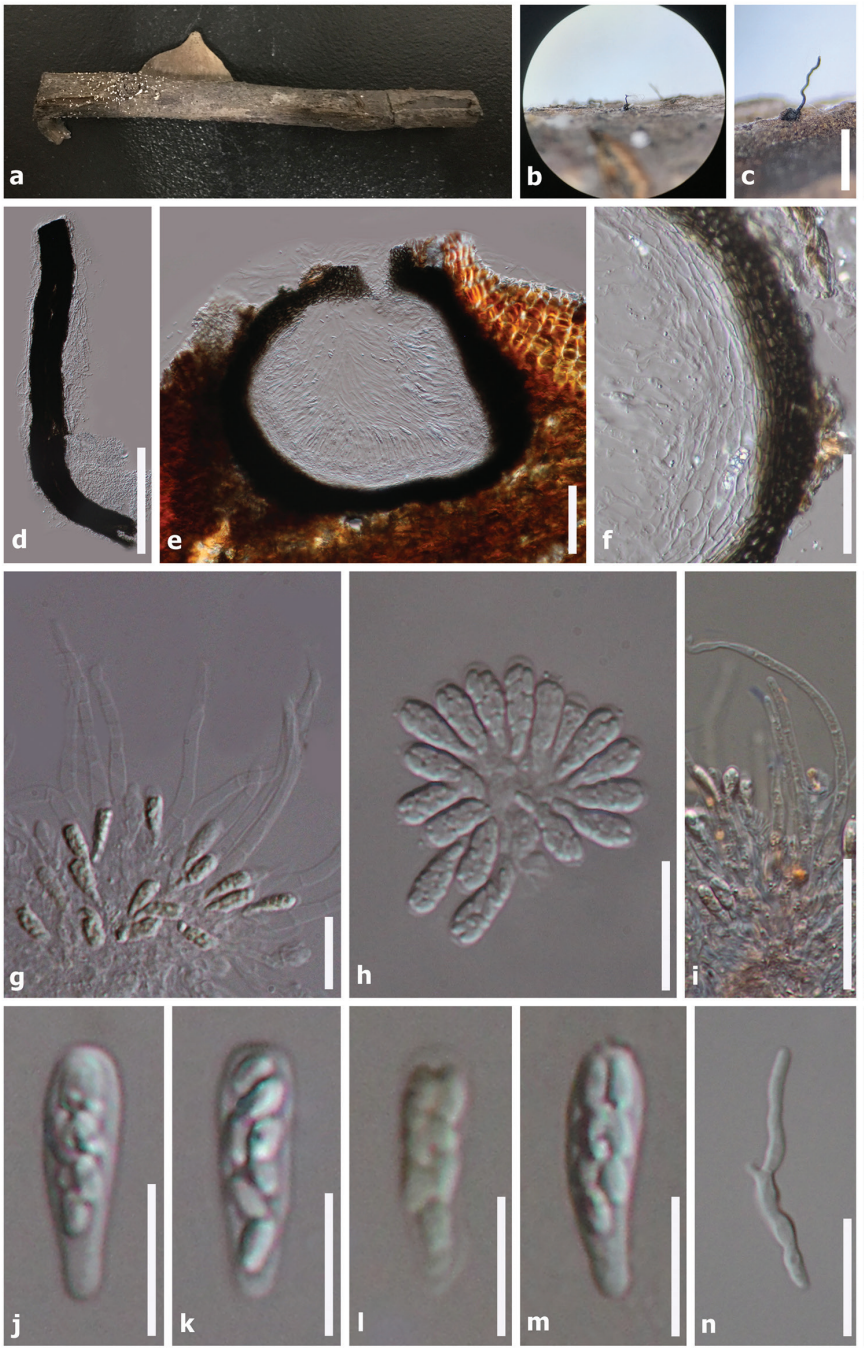

Figure 2. *Phaeoacremonium ovale* (HKAS99550, holotype)**. a** Substrate **b, c** Ascoma on host **d** Squashed neck **e** Ascoma in vertical section **f** Peridium **g** Asci surrounded by paraphyses **h** Asci **i** Septate paraphyses **j–m** Asci with ascospores **n** Germinating ascospores. Note: Fig i stained in Congo red reagent, fig l stained in Melzer's reagent. Scale bars: 500 µm (**c**); 200 µm (**d**); 100 µm (**e**); 50 µm (**f, i**); 30 µm (**n**); 20 µm (**g–h**); 10 µm (**j–m**)

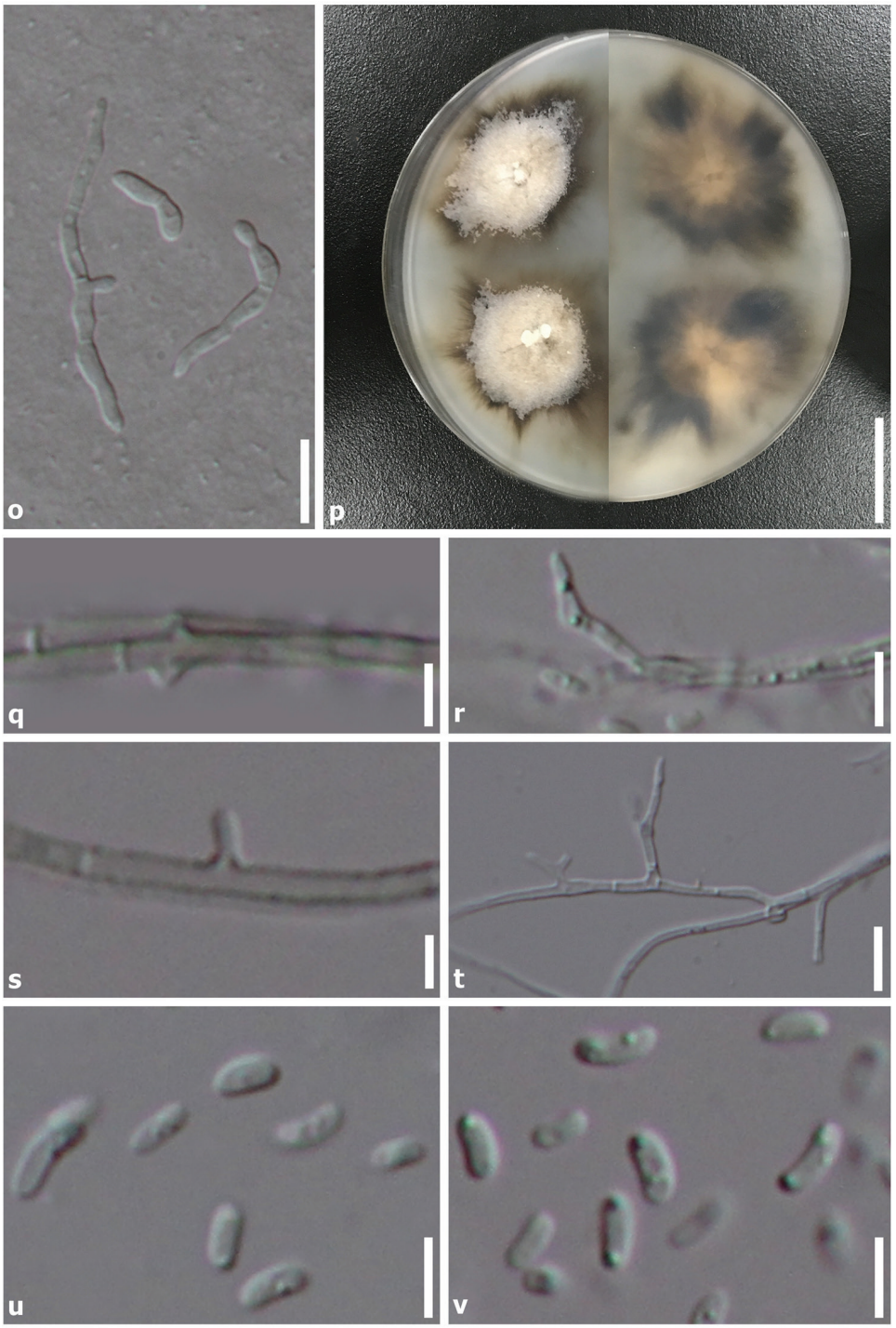

Figure 3. *Phaeoacremonium ovale* (HKAS99550, holotype). **o** Germinating ascospores, **p** 7 weeks of culture plate (above, left/reverse, right), **q** Mycelium with adelophialides **r–t** Branched conidiophores **u–v** Conidia. Scale bars: 20 mm (**p**); 20 μm (**o**); 10 μm (**r, t**); 5 μm (**q, s, u–v**).

Discussion

Phaeoacremonium is currently accommodated in the monogeneric family Togniniaceae (Wijayawardene et al. 2018). To date, 65 species are accepted in this genus (Mostert et al. 2006b; Gramaje et al. 2015; Marin-Felix et al. 2018; Spies et al. 2018). While most of the species are commonly isolated as asexual morphs, some taxa have been recovered in their sexual morph state, viz. *Phaeoacremonium aquaticum* (= *Togninia aquatica*), *P. viticola* (= *T. viticola*), *P. novae-zealandiae* (= *T. novae-zealandiae*) (Hausner et al. 1992; Mostert et al. 2006a; Hu et al. 2012).

In this study, we introduce a novel taxon of *Phaeoacremonium* from dead wood collected in a stream in the Yunnan Province, China and describe its sexual and asexual morph. Examination of morphological characters reveal that our species is sufficiently distinct from extant species to establish it as a new species. Analyses of the combined DNA sequence dataset from partial TUB and ACT genes also support that this taxon is a *Phaeoacremonium* species and phylogenetically distinct from other species (Fig. 1). The two strains of *P. ovale* constitute a strongly supported independent lineage close to other species as depicted in Clade I. Phylogeny also reveals a close relationship to *P. griseo-olivaceum*, but with low support. To further support *P. ovale* as a new species, we compared nucleotide differences with other related species as recommended by Jeewon and Hyde (2016). Comparison of the 533 nucleotides across the TUB region reveals 43 bp (10%) differences, 256 bp of the ACT region reveals 22 bp (8.5%) differences and 517 bp of the ITS region reveals 4 bp (1%) differences compared to *P. griseo-olivaceum* (CBS 120857). Examination of the TUB region reveals 59 bp (11%) difference compared to *P. africanum* (CBS 120863) while the ACT region reveals 19 bp (7%) and ITS region reveals 17 bp (3%) differences, but the latter clusters in a different subclade in our phylogeny and is therefore considered distinct. There are also some morphological similarities between *P. ovale* and *P. africanum* in terms of black ascomata with a long neck, clavate asci and small, oval to ellipsoid ascospores in sexual morph and ellipsoid to ovoid, aseptate conidia in asexual morph (Damm et al. 2008). Despite a morphological resemblance to *P. africanum* and close relationship to *P. griseo-olivaceum*, there are other differences across these species. *Phaeoacremonium ovale* was collected from an aquatic habitat and from dead wood in China whereas the former two species were collected from *Prunus* spp. in South Africa (Damm et al. 2008). In addition, conidial size of *P. africanum* and *P. griseo-olivaceum* are 5–12 × 1.5–2 µm and 5–8 × 1.5–2 µm, whereas conidia of *P. ovale* measure 2.5–6 × 1–2.5 µm (Damm et al. 2008; Fig. 3). No sequence data of the TUB and ACT gene are available for *P. aquaticum* and *P. leptorrhynchum* and therefore we provide ITS sequences of our strains and compare them with those two species. Comparison of ITS regions reveals 61 bp (12%) differences with *P. aquaticum* (IFRDCC 3035) and 11 bp (2%) differences with *P. leptorrhynchum* (UAMH9590). In addition, our new species is also morphologically different from them. *Phaeoacremonium ovale* is morphologically different as ascospores of *P. aquaticum* and *P. leptorrhynchum* are reniform (ascospores of *P. ovale* are oval/ellipsoid) and measure 5–6 × 1–1.5 µm and 7–10 × 1–1.5 µm, respectively. *Phaeoacremonium inconspicuum* as described by Gramaje et al. (2015) also appears morphologically

similar to *P. ovale* in terms of clavate asci and hyaline, aseptate ascospores (Eriksson and Yue 1990), but could not be included in our analyses as DNA sequences are unavailable. However, the ascospore shape and size of *P. inconspicuum* is different (allantoid, measuring 7–10 × 1.5–2 μm) (Eriksson and Yue 1990; Réblová 2011).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31760014), the Science and Technology Foundation of Guizhou Province (No. [2016]2863 & [2017]5788). Dr. Qi Zhao is acknowledged for his support and thanks to Dr. Sajeewa Maharachchikumbura for his valuable help in phylogenetic analyses. Shaun Pennycook is thanked for checking and correcting the Latin name.

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