Anacacumisporium, a New Genus Based on Morphology and Molecular Analyses from Hainan, China

Author(s): Ying-Rui Ma , Ji-Wen Xia , Jian-Mei Gao , Zhuang Li & Xiu-Guo Zhang

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Anacacumisporium, a new genus based on morphology and molecular analyses from Hainan, China

Ying-Rui MA, Ji-Wen XIA, Jian-Mei GAO, Zhuang LI & Xiu-Guo ZHANG**

Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, China, e-mail: sdau613@163.com

Abstract – The anamorphic taxon *Anacacumisporium appendiculatum* gen. et sp. nov. is described and illustrated from fallen branches of decaying wood in Hainan, China. *Anacacumisporium* is characterized by pigmented, transversely septate, appendaged conidia and conidiophores that are brown, macronematous, mononematous and that bear one (or more) integrated phialide at the tip. Complete sequences of internal transcribed spacer (ITS) and partial sequences of nuc 28S rDNA (28S) genes are provided. The new fungus is compared with morphologically similar genera of hyphomycetes. Phylogenetic analyses revealed its placement in the Chaetosphaeriaceae. A key to the novel genus and morphologically similar genera is given.

Anamorphic fungi / Identification key / ITS / LSU / Phylogeny / Taxonomy

INTRODUCTION

China is considered an important Asian reservoir of biodiversity (Williams *et al*., 2001). During ongoing exploration for saprobic microfungi, many wood-inhabiting species have been described in recent years (Wu & Zhuang, 2005; Zhang *et al*., 2009a, 2010, 2011, 2012a; Ma *et al*., 2011a; 2012a, b; Ren *et al*., 2012; Xia *et al*., 2014). Hainan Island, located in the south of China, harbours an inestimable diversity of fungi (Zhang *et al*., 2009b, c; 2012b; Ma *et al*., 2008, 2011b). The island's humid, subtropical climate, with an average annual temperature of 22 to 27 °C and an average annual precipitation of 1000-2600 mm, favors development of fungi. In our study of the conidial fungi from the Jianfengling National Forest Park, Hainan Province, a dematiaceous species was collected on dead branches of an unidentified broadleaf tree that we were unable to identify to genus or species (Ellis, 1971, 1976; Subramanian, 1971; Matsushima, 1975, 1983, 1985, 1989, 1993, 1995; Carmichael *et al*., 1980; Castañeda, 1986; Castañeda & Kendrick, 1990a, b, 1991; Seifert *et al*., 2011; Zhang, 2013). We propose it here as a new genus and species.

* Corresponding author

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MATERIALS AND METHODS

Sampling and specimen examination. — Samples of decomposed woody debris were placed in separate polyethylene bags and transported to the laboratory. The specimens were incubated in plastic boxes containing damp, sterilized tissue paper at about 25°C for more than 3 weeks and then examined with a dissecting microscope.

Isolations of fungi. — Single-spore cultures of hyphomycetes that could not be identified on natural substrate were isolated from dead branches and incubated on potato-dextrose agar (PDA; 200 g white potatoes boiled and filtered, 30 g dextrose, 15 g agar, 1 L distilled water). The isolates were transferred from PDA to potato-carrot agar (PCA; 200 g white potatoes boiled and filtered, 200 g carrot boiled and filtered, 15 g agar, 1 L distilled water). Isolates were grown at 20-25°C under cool-white fluorescent light 35-40 cm above the culture surface and 8/16 h light/dark cycles for about 2 months. Mycelial plugs cut from leading edges of colonies were stored on PDA slants at 4°C.

Morphological and cultural studies. — Characteristics in culture, including micromorphology, colony characters and growth rate, are taken from the ex-type culture grown on PCA. Characteristics taken from nature are based on our study of the specimen from which the ex-type culture was isolated. Morphological structures were transferred with a needle to a drop of lactophenol on a slide; excess lactophenol was evaporated in a drying oven (55°C). The dried slides were sealed with neutral balsam. All microscopic characteristics were determined on the basis of measurements of 50 mature conidia and 30 conidiophores mounted in lactophenol. Photographs were obtained by use of an Olympus BX53 microscope (Olympus, Japan). The holotype is a dry culture and is deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP). Living cultures were also deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP) and in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences [HMAS; http://hmas.im.ac.cn].

Scanning electron microscopy (SEM). — In order to clearly observe the elongation of conidiophores in our new species *Anacacumisporium appendiculatum*, the samples were taken from PCA after 2 months incubation, glutaraldehyde-fixed samples were post-fixed in 1% aqueous OsO_4, then dehydrated using an ascending series of ethanol (Blanco & Judelson, 2005), and treated in a critical-point dryer (Balzers Union, Liechtenstein; CPD 020) with liquid CO_2. All samples were then mounted on aluminum stubs with two-sided tape, and coated with gold-palladium. Images were obtained with a Phillips XL30 FEG microscope (Phillips; Natick, MA, USA).

DNA extraction, PCR amplification and sequencing. — Total genomic DNA was extracted using the CTAB method (Doyle & Doyle, 1987). Primers ITS4 and ITS5 (White *et al*., 1990) were used to amplify sequences of the internal transcribed spacer region. DNAMAN (version 7.0) software based on sequences of six *Sporidesmium* species: *S. knawiae* (FJ349610), *S. tropicale* (DQ408560), *S. tengii* (DQ408559), *S. parvum* (DQ408558), *S. pachyanthicola* (DQ408557) and *S. obclavatulum* (DQ408556) were used to design primers to amplify 28S rDNA gene: 28S1 (5′-AGTAACGGCGAGTGAAGCG-3′) and 28S3 (5′-ACTCCTTGG TCCGTGTTTCA-3′). Reaction mixtures contained 5 µL of 10× ThermoPol reaction buffer [200 mM Tris-HCl, pH 8.3, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$ and 1% Triton X-100], 20 ng template genomic DNA, 2 pmol of each

primer, 4 μL of 2.5 mM dNTPs, 0.5 U of AmpliTaq polymerase, and total volume was adjusted to 50 μL with deionized water. The PCR thermal cycle for ITS and 28S rDNA gene region amplication were as follows: 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s and elongation at 72°C for 50 s, with a final extension step of 72°C for 10 min. The PCR-amplified DNA fragments were fractionated in 1.0% agarose gels in 0.5×TBE buffer, and UV illumination. The PCR products were purified using a DNA fragment Purification Recovery Kit (BioTeke). Sequencing of both strands of each fragment was performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA, USA). The DNA sequences of ITS and 28S genes generated in this study were submitted to GenBank [www.ncbi.nlm.nih.gov.] Other ITS and 28S sequences used in our analyses were obtained from GenBank (Table I).

Molecular phylogenetic analyses. — A BLAST sequence identity search (Altschul *et al*., 1997) was carried out to compare data from our isolate with those of other fungi deposited in the GenBank database, mostly from studies published by Paulus *et al*., (2004), Jeewon *et al*. (2009), Shenoy *et al*., (2010), Réblová *et al*., (2011), Magyar *et al*., (2011), Crous *et al*., (2012, 2014), Crous *et al*., (2014), Hern.-Restr *et al*. (2014), Hashimoto *et al*., (2014, 2015). Sequences were managed and aligned manually with Sequencher 3.1 (Gene Codes Corp., Ann Arbor, Michigan) and Clustal X 1.81 (Thompson *et al*., 1997). The alignments were checked visually and improved manually where necessary. Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford, 2002). Trees were produced with neighbor joining (NJ) and Maximum parsimony (MP) analyses of combined ITS and 28S sequence datasets. The Kimura two-parameter distance calculation was used in NJ analysis. In the MP analysis trees were inferred with heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions. Maxtrees were 100, branches of zero length were collapsed and all parsimonious trees were saved, 2 parsimonious trees were obtained [tree length (TL) of 1430 steps, consistency index (CI)=0.544056, retention index (RI)=0.748360, rescaled consistency index (RC)=0.407150, homoplasy index (HI)=0.455944]. All characters have equal weight in the analysis, and gaps were treated as missing data. The alignment was deposited in TreeBASE [www.treebase.org, submission number 17725]. Sequences of *Phialophphora hyalina* were used as outgroups.

RESULTS

Phylogenetic analyses. — The size of DNA fragments was of 475-478 bp for ITS, and 498-501 bp for 28S. Alignment of the ITS-28S fragments resulted in a 925-character dataset (including alignment gaps), 36 sequences representing 31 species (including the outgroup sequences) belonging to 4 families were used in the alignment to define the placement of the new species *Anacacumisporium appendiculatum*. Comparisons of bootstrap support for clades inferred in the MP and NJ obtained from the analyses of the combined DNA sequences show no conflicts. Both trees have similar topologies. (Figs 1-2). MP and NJ analyses concur on two major lineages, respectively representing the orders Chaetosphaeriales and Glomerellales. *Anacacumisporium appendiculatum* fell into the Chaetosphaeriales lineage very well. Our results indicate that the Chaetosphaeriaceae is paraphyletic

Table 1. List of taxa used in the molecular analyses. All sequences are from the ITS1-5.8S-ITS2 and 28S gene region

Taxa	*Substrate and Locality*	*Source*[a]	*ITS*[b]	*28S*
Anacacumisporium appendiculatum Y.R. Ma & X.G. Zhang	China (Hainan), decaying broadleaf tree	HMAS 245593	KT001555	KT001553
Anacacumisporium appendiculatum	–	–	KT001556	KT001554
*Brunneodinemasporium brasiliens*e P.W. Crous & R.F. Castañeda	Brazil, decaying leaf	CBS 112007	JQ889272	JQ889288
*Codinaea pin*i P.W. Crous & M.J. Wingf.	Uganda, dead needles of *Pinus patula*	CBS 138866	KP004465	KP004493
*Codinaeopsis gonytrichoi*des (Shearer & Crane) Morgan-Jones	Japan, decaying plant material	CBS 593.93	AF178556	AF178556
Cylindrotrichum hennebertii W. Gams & Hol.-Jech.	Germany, *Symphoricarpos albus*	CBS 570.76	AF178560	AF178560
*Cylindrotrichum gori*i Lunghini	Sweden, dead stem of *Urtica dioica*	CBS 879.85	HM237328	HM237322
Dictyochaeta simplex (S. Hughes & W.B. Kendr.) Hol.-Jech.	Netherlands, acorn of *Quercus* sp.	CBS 966.69	AF178559	AF178559
*Dinemasporium cruciferu*m M.B. Ellis	Japan, dead culms of bamboo	MAFF 244328	AB900896	–
Dinemasporium cruciferum	–	HHUF 30001	–	AB934039
*Dinemasporium ipomoea*e P.W. Crous	Viet Nam (Can Dao Islands), leaves of *Ipomoea pes-caprae*	CBS 138898	KP004446	KP004474
*Dinemasporium strigosu*m (Pers.) Sacc.	USA (Illinois), *Malus*	CPC 18957	JQ889285	AB934040
*Dinemasporium pseudostrigosu*m P.W. Crous	Cuba (Granma), *Stigmaphyllon sagraeanum*	CBS 825.91	JQ889279	JQ889295
*Kylindria peruamazonensi*s Matsush.	Cuba, leaf litter of *Bucida palustris*	CBS 838.91	GU180628	–
Kylindria peruamazonensis.	Cuba, leaf of *Bucida palustris*	CBS 421.95	–	HM237325
*Menispora glauc*a Link ex Pers.	unpublished	FMR 12089	HF678528	HF678538
*Menispora tortuos*a Fresen.	Netherlands, bark of *Acer* sp.	CBS 214.56	AF178558	AF178558
*Monilochaetes dimorphospo*ra M. Réblová & W. Gams	Cuba, decayed wood of a twig	MUCL 40959	HQ609488	HQ609480
*Monilochaetes infuscan*s Halst. ex Harter	South Africa, *Ipomoa batatas*	CBS 869.96	GU180626	–
Monilochaetes infuscans	South Africa, *Ipomoa batatas*	CBS 870.96	–	GU180644
*Monilochaetes guadalcanalensi*s (Matsush.) I.H. Rong & W. Gams	Solomon Islands, leaf of *Musa*	CBS 346.76	GU180625	GU180640
*Neopseudolachnella acutispor*a A. Hashim., Sat. Hatak. & Kaz. Tanaka	Japan (Aomori), dead twigs of *Pleioblastus chino*	MAFF 244358	AB934065	AB934041
*Neopseudolachnella magnispor*a A. Hashim., G. Sato & Kaz. Tanaka	Japan (Aomori), dead twigs of *Sasa kurilensis*	MAFF 244359	AB934066	AB934042
*Neopseudolachnella uniseptat*a A. Hashim., Sat. Hatak. & Kaz. Tanaka	Japan (Aomori), *Sa*sa sp.	MAFF 244360	AB934067	AB934043
Pseudolachnea fraxini P.W. Crous	Sweden, *Fraxinus excelsior*	CBS 113701	JQ889287	–
Pseudolachnea fraxini	Japan (Aomori), dead stems of *Clematis florida*	MAFF 244363	–	AB934045
Pseudolachnea fraxini	Japan (Aomori), dead stems of *Clematis florida*	MAFF 244363	AB934070	–
Pseudolachnea fraxini	Japan (Aomori), overwintered stems of *Artemis*ia sp.	MAFF 244362	–	AB934046
Pseudolachnea hispidula (Schrad.) B. Sutton	Japan (Aomori), dead twigs of *Morus bombycis*	MAFF 244364	AB934071	

Table 1. List of taxa used in the molecular analyses. All sequences are from the ITS1-5.8S-ITS2 and 28S gene region *(continued)*

Taxa	*Substrate and Locality*	*Source*[a]	*ITS*[b]	*28S*
Pseudolachnea hispidula	Japan (Aomori), dead twigs of *Morus bombycis*	MAFF 244365	–	AB934048
Pseudolachnella botulispora A. Hashim., G. Sato & Kaz. Tanaka	Japan (Shizuoka), dead twigs of *Phyllostachys aurea*	MAFF 244367	AB934075	–
Pseudolachnella botulispora	Japan (Iwate), dead twigs of *Phyllostachys pubescens*	MAFF 244368	–	AB934051
Pseudolachnella campylospora A. Hashim., G. Sato & Kaz. Tanaka	Japan (Iroshima), dead twigs of bamboo	MAFF 244370	AB934077	AB934053
Pseudolachnella fusiformis A. Hashim., G. Sato & Kaz. Tanaka	Japan (Nara), dead sheath of bamboo	MAFF 224373	AB934080	AB934056
Pseudolachnella longiciliata (I. Hino & Katum.) Nag Raj	Japan (Hokkaido), dead twigs of *Sasa kurilensis*	MAFF 224374	AB934084	–
Pseudolachnella longiciliata	Japan (Aomori), dead sheath of *Sasa kurilensis*	MAFF 244377	–	AB934060
Phialophora hyaline W. Gams	Germany, soil of wheat field	CBS 177.74	GU727563	GU727563
Phialophora hyaline	Germany, soil of wheat field	CBS 130.74	GU727562	GU727562
Pyrigemmula aurantiaca D. Magyar & R. Shoemaker	Hungary (Noszvaj), cortex of *Vitis vinifera*	CBS 126743	HM241692	HM241692
Pyrigemmula aurantiaca	Hungary (Noszvaj), cortex of *Vitis vinifera*	CBS 126744	HM241693	HM241693
Rattania setulifera Prabhugaonkar & Bhat	India, leaf of *Calamus thwaitesii*	GUFCC15501	GU191794	HM171322
Striatosphaeria codinaeophora Samuels & E. Miill.	Puerto Rico, *Dacryodes excelsa*	CBS 101323	AF178546	AF178546
Striatosphaeria codinaeophora	–	SMH 1524	–	AF466088
Thozetella nivea (Berk.) Kuntze	–	Jeewon et al. 2015	EU825201	EU825200

[a] CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS; GUFCC = Fungus Culture Collection of Goa University, India; HHUF = Herbarium of Hirosaki University, Fungi; HMAS = Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences; MAFF = Ministry of Agriculture, Forestry and Fisheries; MUCL = Mycothèque de Université Catholique de Louvain, (Agro) Industrial Fungi & Yeasts Collection, Louvain-la-Neuve, Belgium; FMR = Facultat de Medicina i Cie`ncies de la Salut, Reus, Spain. Culture and specimen abbreviations: SMH = S.M. Huhndorf.
[b] ITS1-5.8S-ITS2 gene region.

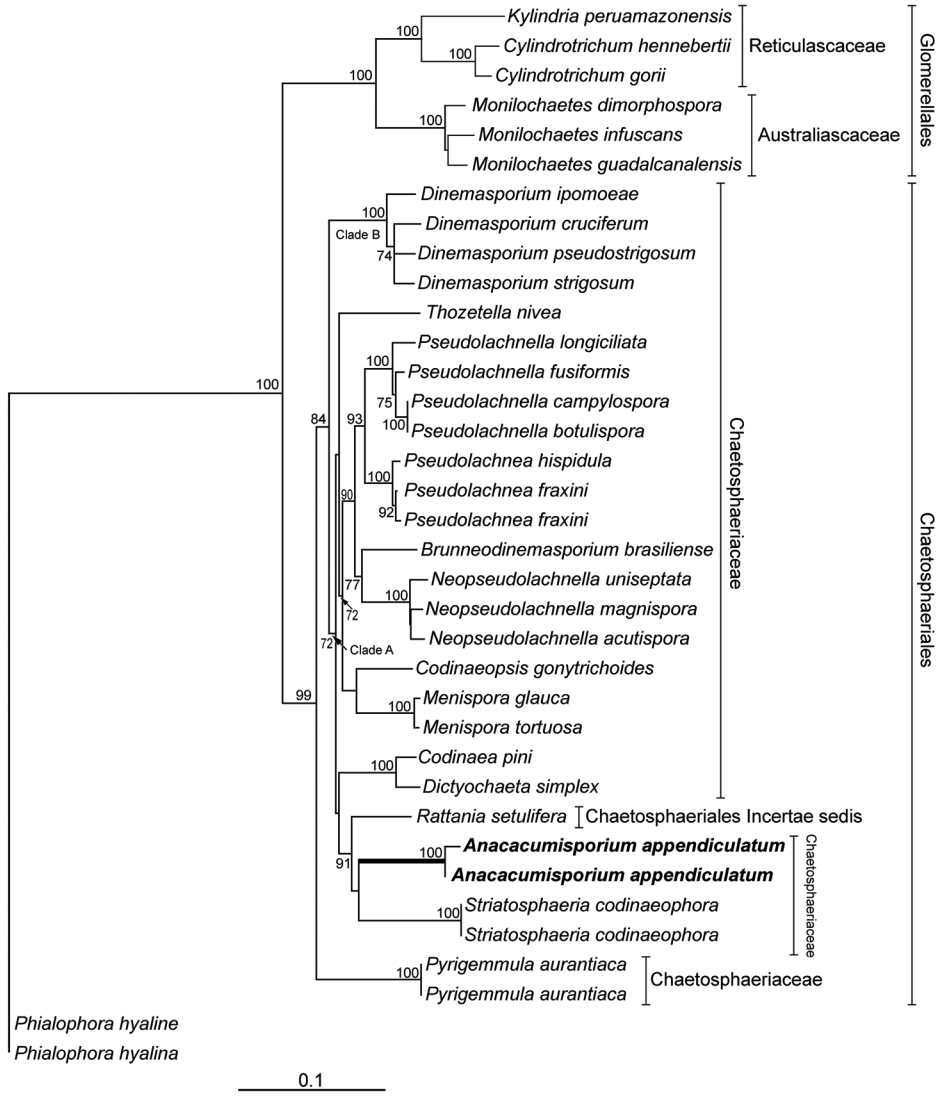

Fig. 1. Neighbor joining tree based on analysis of combined ITS and 28S sequences. Bootstrap support for the nodes is indicated above branches. Bootstrap values < 70% are not shown. The tree is rooted with *Phialophora hyaline*.

with two collections of *Pyrigemmula* forming a sister group to the other genera of the Chaetosphaeriaceae except for *Rattania*. The Chaetosphaeriales lineage contains a well-supported clade of 26 sequences belonging to 13 genera, which is further subdivided into two well supported sister groups: a large clade (A) composed of 12 different genera, and a small clade (B) composed of 1 genera, every genus forms a single lineage with high bootstrap support. BLAST of combined ITS-28S sequences of our unknown fungus did not reveal any close hits although

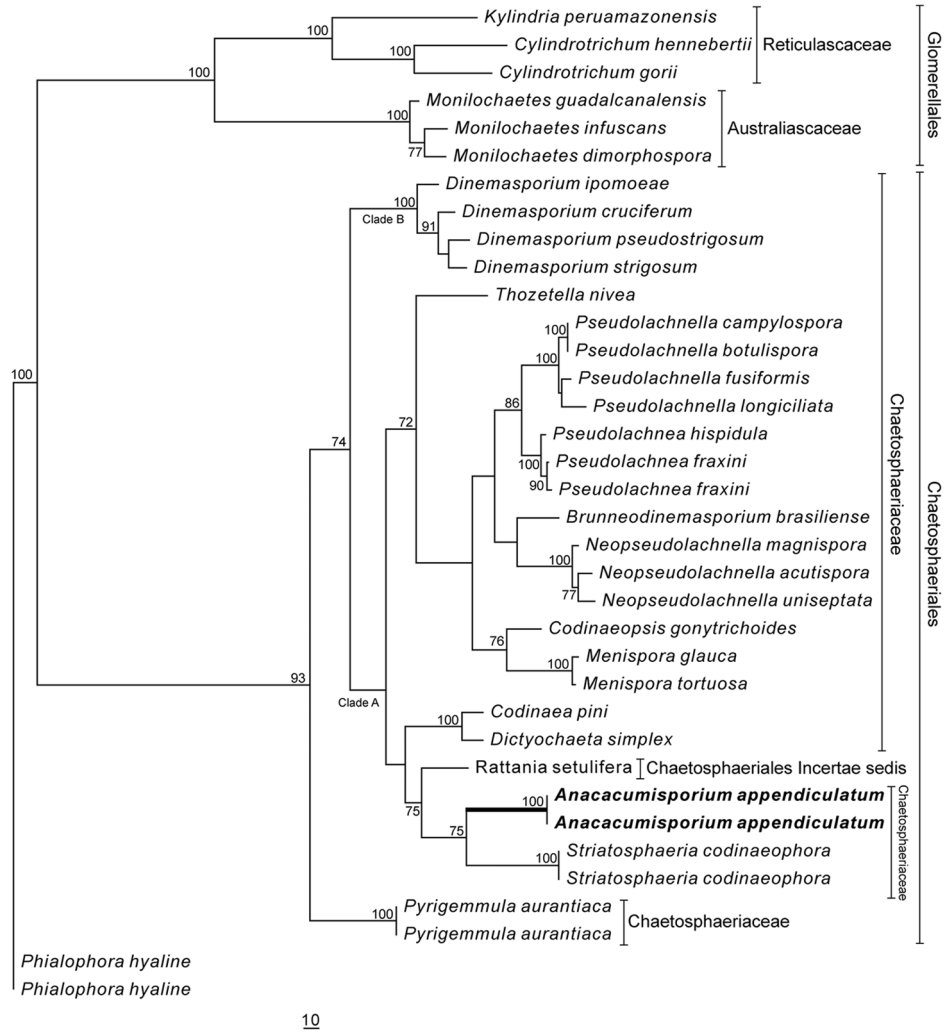

Fig. 2. Maximum parsimony tree derived from analysis of the combined ITS and 28S sequences. Bootstrap support for the nodes is indicated above branches. Bootstrap values < 70% are not shown. The tree is rooted with *Phialophora hyaline*.

Striatosphaeria codinaeaphora was shown to be a close sister lineage. For this reason and because of its distinct morphological characters we propose to describe our unknown isolate as a new species in a new genus: *Anacacumisporium appendiculatum*. Species belonging to the Glomerellales lineage clustered in a well-supported monophyletic sister clade to the Chaetosphaeriales. There they formed into two well-supported branches representing, respectively, the families Australiascaceae and Reticulascaceae.

TAXONOMY

Anacacumisporium Y.R. Ma & X.G. Zhang, gen. nov.

MycoBank MB 811418

Type species: *Anacacumisporium appendiculatum*

Etymology: In reference to its similarity to *Cacumisporium* Preuss.

Diagnosis: *Anacacumisporium* is characterized by mononematous, macronematous conidiophores with inconspicuous phialidic conidiogenous cells and euseptate conidia with an appendage at the tip and base, conidia aggregated in slimy masses at the conidiogenous loci, schizolytic secession.

Colonies effuse, dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum. **Conidiophores** macronematous, mononematous, erect, unbranched, straight or flexuous, cylindrical, thick-walled, smooth, septate, brown at the base, colourless towards the apex, determinate in length. **Conidiogenous cells** integrated, terminal and/or intercalary, cylindrical, brown, pale brown or subhyaline, smooth or verrucose, phialidic, mono-polyblastic; collarette narrow or flaring, not apparently proliferating. **Conidia** solitary, dry, apical and/or lateral, simple, smooth, typically reniform, ellipsoidal to fusiform, but sometimes cylindrical, rarely obclavate, brown to dark brown or bicolorus, with an appendage at each end. Conidial secession schizolytic.

Habitat: Dead branches of unidentified broadleaf tree.

Known distribution: Hainan, China.

Anacacumisporium appendiculatum Y.R. Ma & X.G. Zhang, sp. nov. **Figs 3-5**

MycoBank MB 811419

Typification: CHINA. HAINAN PROVINCE: Ledong, Jianfengling National Forest Park, 18°42'N, 108°52'E, 1412 m elevation, fungus on dead branches of unidentified broadleaf tree, 17 May 2014, Yingrui Ma (HOLOTYPE HSAUP H4589). Ex-type culture HSAUPmyr4589, HMAS 245593 (living cultures).

Etymology: The epithet refers to the conidia ('spora') that have small appendages (appendiculatus).

Known distribution: Hainan, China.

Teleomorph: Unknown.

Characteristics on the natural substrate: Colonies effuse, dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum. Conidiophores macronematous, mononematous, erect, unbranched, straight or flexuous, cylindrical, thick-walled, smooth, 4-9-septate, 115-280 μm long, 5.5-10.5 μm wide at the broadest part, determinate in length, brown at the base, colourless towards the apex, not apparently proliferating. Conidiogenous cells integrated, cylindrical, subhyaline to pale brown, smooth, with one apical and occasionally several lateral phialidic openings distributed over the top region. Conidia solitary, dry, reniform, fusiform or oval, acute at each end, 20-25 μm long, 7-10.5 μm wide in the broadest part, mostly with 3-transversely euseptate, the central cells darker than the terminal cells or uniformly pale brown, smooth, with a single, unicellular appendage at the tip and base, conidia adhering in a slimy mass.

Characteristics in culture: Colonies on PCA 4-6 cm after about 2 months at 20-25°C, floccose, very dark brown to black. Hyphae thick-walled, septate, dark brown, smooth, sometimes verrucose. Conidiophores arising from surface of aerial hyphae, solitary, macronematous, 83-250×3.5-7 μm, determinate in length, dark brown, colourless towards the apex. Conidiogenous cells integrated, terminal and

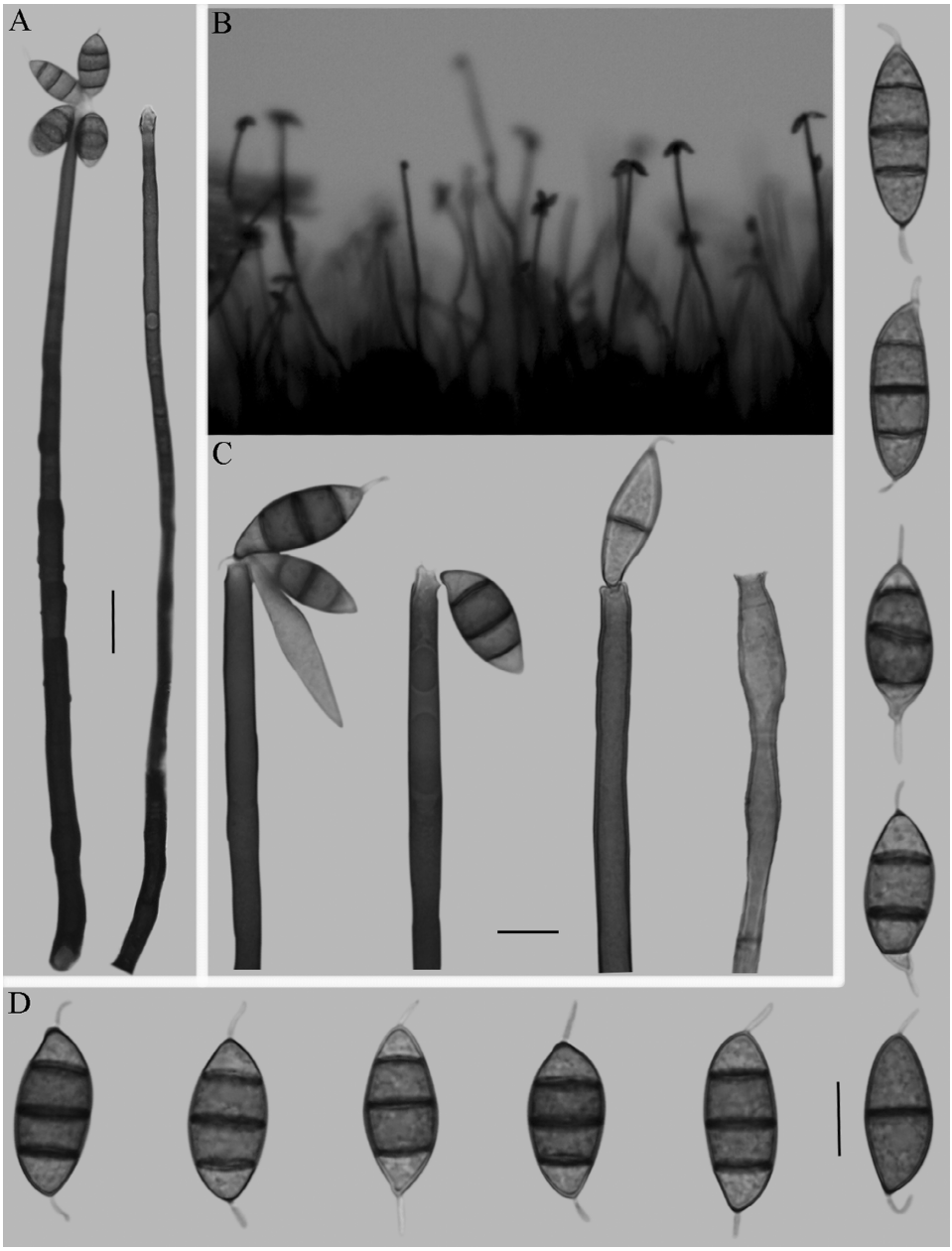

Fig. 3. *Anacacumisporium appendiculatum* from the natural substrate (HSAUP H4589). **A**: Conidiophore with conidia and conidiogenous pores. **B**: Colony on natural substrate. **C**: Conidiogenous cells. **D**: Conidia. Bars A, C 20 µm; D 10 µm.

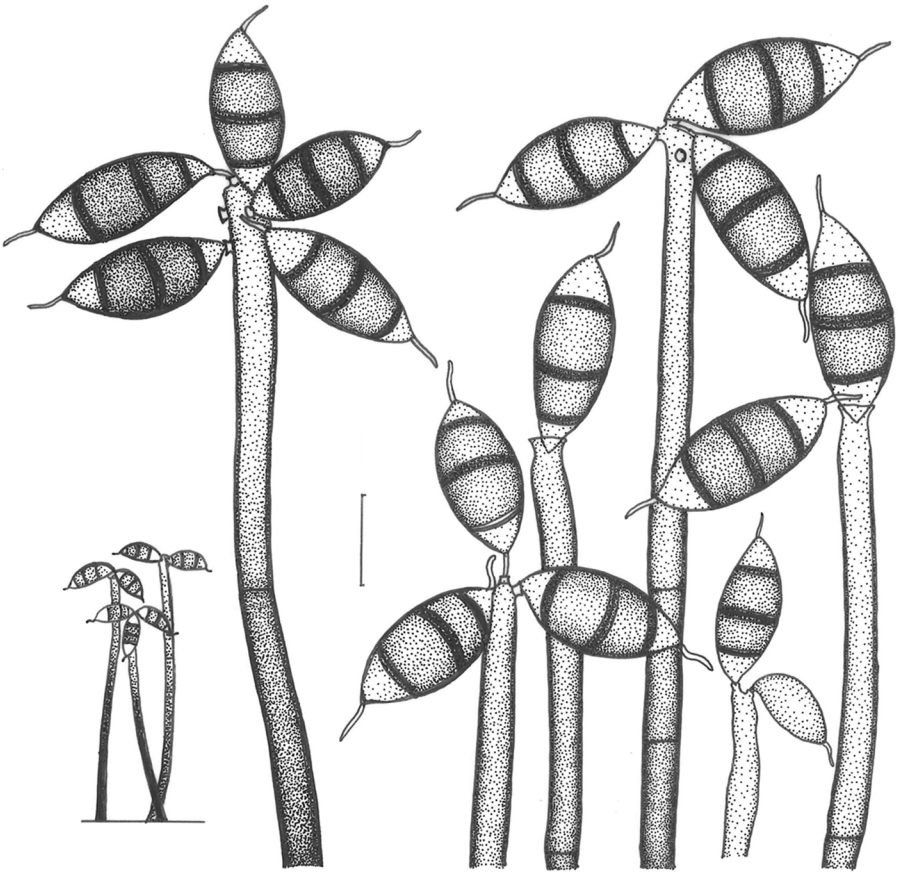

Fig. 4. *Anacacumisporium appendiculatum*. Conidiophores and conidia. Bar 10 μm.

intercalary, cylindrical, pale brown to subhyaline, smooth, swollen at the base and tapering towards the apex, vase-shaped collarette visible. Conidia navicular, oval to fusiform, 1-3-septate, 15-25×7.5-12 μm, brown, terminal cells lighter, smooth, but finely rough when seen with SEM, with a single, unicellular appendage at each end, appendages 2.5-6 μm long.

DISCUSSION

The anamorphs of the Chaetosphaeriaceae (Chaetosphaeriales, Réblová *et al*., 1999, 2011) are macronematous, mononematous conidiophores with the conidiogenous cell terminal, integrated, and phialidic. *Anacacumisporium appendiculatum* fits the general description well. *Anacacumisporum* is distinct in the Chaetosphaeriaceae in having multiseptate, phragmosporous conidia that bear a

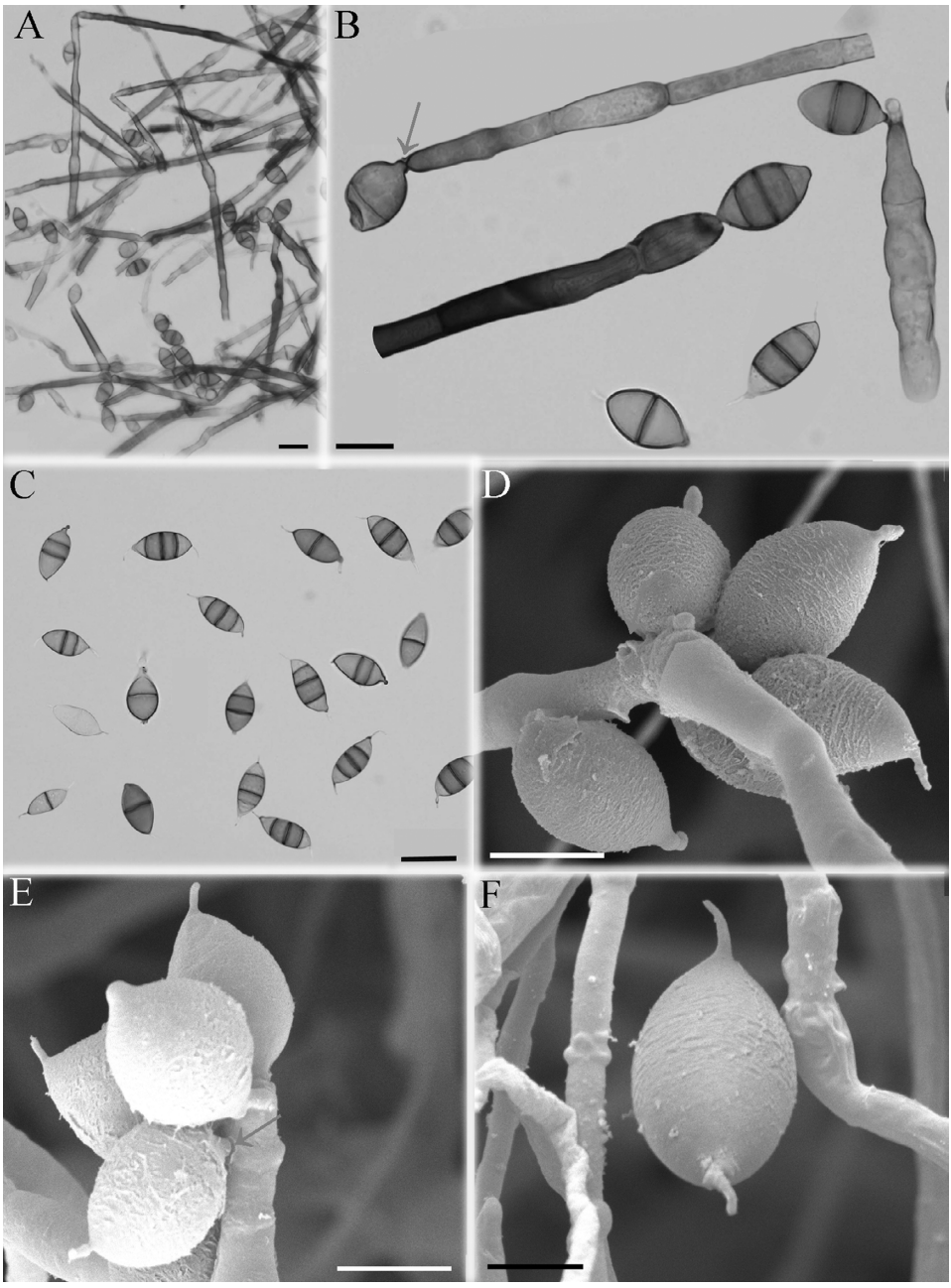

Fig. 5. *Anacacumisporium appendiculatum* from culture on PCA (HSAUPmyr4589). A-C: Photographs from culture. **A**: Conidiophores with conidia. **B**: Conidiogenous cells showing the collarette. **C**: Mature conidia. **D-F**: SEM microphotographs from culture. **D, E**: Conidiogenous cells with conidia; **F**: Conidia. Bars A, C 20 µm; B, D, E 10 µm; F 5 µm.

setula at each end. Conidia of typical members of the family, including *Dictyochaeta* and *Codinaea* (Réblová, 2000, Whitton *et al*., 2000, Cruz *et al*., 2008, Crous *et al*., 2014), are aseptate or 1-septate; they may be setulose or not. Based on our analysis, the closest relative of *A. appendiculatum* is *Striatosphaeria codinaeaphora* Samuels & E. Müll., type of the monotypic genus *Striatosphaeria* Samuels & E. Müll. (Samuels & Müll, 1978). The anamorph of *S. codinaeaphora* was identified as a *Codinaea* species on the basis of its appearance in agar culture; the authors did not find conidiophores on the type or paratype specimens. This anamorph was described as having falcate, bicellular, esetulate, enteroblastic conidia that arise successively from the flared tip of a darkly pigmented, mononematous, macronematous, monoblastic conidiophore. This differs from *A. appendiculatum* in which conidia that form in agar culture and in nature are multiseptate and bear a single seta at each end. In addition, the conidiogenus locus of *A. appendiculatum* proliferates percurrently, whereas the conidiogenous locus of *S. codinaeaphora* does not proliferate. For this reason we consider the two species to represent different genera.

Besides, *Anacacumisporium* is morphologically similar to *Cacumisporium*, *Kylindria*, *Cylindrotrichum* and *Monilochaetes* in that all possess similar conidiophores and their conidia are septate and form enteroblastically (described in Hawksworth *et al*., 1995, Kirk *et al*., 2008). Unlike *A. appendiculatum*, conidia of species in these genera lack setulae (DiCosmo *et al*., 1983, Gams & Holubova, 1976, Castañeda, 2007, Réblová *et al*., 2011, Zhang *et al*., 2010). *Kylindria*, *Cylindrotrichum* and *Monilochaetes* are members of the Glomerellales (Figs 1-2), but no species of *Cacumisporium* has yet been sequenced and its phylogenetic relationships are unknown.

KEY TO *ANACACUMISPORIUM* AND SIMILAR GENERA

1. Conidiophores mononematous or in sporodochia; setae present 2
1. Conidiophores mononematous; setae present or absent 8
2. Conidiophores in sporodochia; conidiogenous cells monoblastic ..... *Rattania*
2. Conidiogenous cells phialidic, mononematous or sporodochial 3
3. Conidiogenous cells arising from stromatic conidiomata 4
3. Conidiophores mononematous, bearing lateral phialides ..... *Dictyochaetopsis*
4. Conidia with one or more appendages at each end 7
4. Conidia bearing a single filiform appendage at each end 5
5. Conidia 0-septate 6
5. Conidia 1-septate ..... *Pseudolachnea*
6. Conidiogenous cells and conidia brown ..... *Brunneodinemasporium*
6. Conidiogenous cells and conidia hyaline to pale brown ..... *Dinemasporium*
7. Conidiomata lacking an excipulum ..... *Neopseudolachnella*
7. Conidiomata with an excipulum ..... *Pseudolachnella*
8. Conidiogenous cells arising directly from hyphae or rarely from delicate conidiophores ..... *Pyrigemmula*
8. Conidiogenous cells arising from well-developed conidiophores 9
9. Conidia with a setula at each end 10
9. Conidia lacking setulae 13
10. Conidiophores determinate in length ..... *Anacacumisporium*
10. Conidiophores indeterminate in length 11

11. Conidiophores branched..... *Menispora*
11. Conidiophores unbranched.....12
12. Conidia curved ..... *Thozetella*
12. Conidia straight ..... *Codinaea*
13. Conidia solitary 14
13. Conidia in basipetal chains or heads ..... *Monilochaetes*
14. Conidia usually with an eccentric protruding basal hilum ..... *Kylindria*
14. Conidia without basal hilum.....15
15. Conidiogenous cells monophialidic ..... *Cylindrotrichum*
15. Conidiogenous cells polyphialidic 16
16. Conidia pigmented ..... *Cacumisporium*
16. Conidia hyaline or pale brown ..... *Dictyochaeta*

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