

Mycosphere 8 (8): 1070–1079 (2017) www.mycosphere.org

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Xylaria spinulosa sp. nov. and X. atrosphaerica from southern China

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Li QR, Liu LL, Zhang X, Shen XC, Kang JC 2017 – *Xylaria spinulosa* sp. nov. and *X. atrosphaerica* from southern China. Mycosphere 8(8), 1070–1079, Doi 10.5943/mycosphere/8/8/8

Abstract

Two species of *Xylaria* collected from southern China are reported. *Xylaria spinulosa* sp. nov. is introduced as a new species based on morphology and sequence data analysis. *Xylaria spinulosa* differs from other species in the genus mainly by its long spines covering the surface of the stroma. *Xylaria atrosphaerica* is a new record for China. Descriptions and illustrations for both species are provided in this paper.

 ${\it Key \ words} - morphology - new \ species - phylogeny - taxonomy - Xylariales$

Introduction

Xylaria belongs in the subclass *Xylariomycetidae* in the order Xylariales, a group which is presently undergoing considerable revision (Daranagama et al. 2015, 2016). It is not presently clear how many families will be accepted in the genus, but *Xylaria* Hill ex Schrank is the largest genus in the family with species having been recorded in most countries worldwide (Læssøe 1987, 1999, Ju & Hsieh 2007, Ju et al. 2009, Liu et al. 2015, Hongsanan et al. 2017). Currently, nearly 300 estimated species are thought to belong in *Xylaria* (Kirk et al. 2008). *Xylaria* differs from other genera in Xylariaceae by producing upright, cylindrical to clavate, multi-peritheciate stromata (Maharachchikumbura et al. 2016). Most *Xylaria* species produce stromata on decayed dicotyledonous wood (Hsieh et al. 2010).

Species of *Xylaria* are difficult to identify and classify especially as stroma of a given species often varies greatly in colour, size and sometimes in general shape (Whalley 1996). Species have mainly been resolved on the basis of morphological characteristics. These characteristics are not suitable for differentiating closely related species of *Xylaria*, due to lack of differentiating characteristics. The genus *Xylaria* has great variation in morphology and may be polyphyletic. Few phylogenetic studies have been conducted to infer the relationships of the taxa of *Xylaria* and there is a need for a comprehensive monograph. ITS sequence data have been used to infer phylogenetic relationships among species of *Xylaria* by Lee et al. (2000). Hsieh et al. (2010) analyzed 114 species of *Xylariaceae*, based on α -actin, β -tubulin and rpb2 sequence data and stated that *Xylaria* is a paraphyletic genus. Hsieh et al. (2010) made an important step to resolve closely related species of *Xylaria*.

Although there has been a high interest in *Xylaria* over the last 30 years (Smith et al. 2003, Tang et al. 2009, Daranagama et al. 2015, 2016, Hashemi et al. 2015, Karun & Sridhar 2015, Srihanant et al. 2015, Kim et al. 2016), these have generally not incorporated molecular data and

have rarely looked at type species. There have been very few reports on the genus from the Chinese mainland (Chou 1935, Tai 1979, Abe & Liu 1995, Ma et al. 2011, Huang et al. 2014a, b). Therefore, the diversity of *Xylaria* in China is still underrepresented and needs further investigation (Ma et al. 2011). In the present paper, we have collected *Xylaria* species from China and introduce *Xylaria spinulosa* sp. nov. and provide a description of *X. atrosphaerica* which is a new record for China.

Materials & Methods

Isolation and morphology

The fungal materials were collected in the forest from Hainan and Guizhou provinces in China. The methodology used for morphological examination of fungi followed that used by Stadler et al. (2004). Materials were mounted in water and Melzer's iodine reagent for examination. Asci and ascospores were examined by light microscopy (BX41, Olympus). Cultures were obtained by placing tissue of freshly collected stromata on Oatmeal agar (Ju et al. 2009). Total DNA extraction and sequencing of ITS rDNA, α -actin gene (act) and RNA polymerase II second largest subunit genes (rpb2) follow Ju & Hsieh (2007). For PCR amplification of β -tubulin gene (tub2), primers T1/T2, Bt2a/Bt2b were used (Ju & Hsieh 2007). The herbarium and living culture are deposited in the Collection of Guizhou University (GZUCC) and Kunming Institute of Botany (KUN), Chinese Academy of Sciences.

Phylogenetic analyses

All sequences used in this paper were gained from recent publications and are listed in Table 1. The alignments are available in TreeBASE (www.treebase.org/treebase-web/home.html) under ID15112 for ITS alignment and ID 20686 for act-rpb2-tub2 alignment. A-actin, β-tubulin and rpb2 sequence data were assembled using the alignment program BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). Alignment was manually adjusted to achieve the maximum alignment and to minimize gaps. Maximum parsimony was applied to the act-tub2-rpb2 dataset. All characters were assessed as independent, unordered, and equally weighted. Gaps were treated as missing characters. *Biscogniauxia arima* was used as the outgroup taxon (Hsieh et al. 2010). Maximum parsimony analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Felsenstein 1985, Swofford 2002).

Results

Using ITS rDNA sequences, our initial BLAST searches suggested that our sequences were most closely related to those of the taxa in *Xylaria polymorpha* complex (Hsieh et al. 2010). Act, Rpb2 and tub2 genes sequences were used to identify those species.

Phylogenetic analyses

A phylogenetic study using three combined loci (Rpb2, act and tub2) was conducted. All characters and gaps have equal weight. A total of 47 taxa were included in the analysis. Total length of the alignment had 1892 characters, in which 144 of those characters were variable, 935 characters were constant, 813 parsimony-informative characters.

The result of the phylogenetic analysis is shown in Fig. 1. The appearance of *Xylaria spinulosa* (100% BS) was reasonably well-supported as sister clade of *X. haemorrhoidalis* Berk. & Broome. The closest relatives to *X. spinulosa*, clustered basal to this subclade, were *X. scruposa* (Fr.: Fr.) Fr., *X. atrosphaerica* (Cooke & Massee) Callan & J. D. Rogers, *X. polymorpha* (Pers.: Fr.) Grev., *X. cf. glebulosa* (Ces.) Y.-M. Ju & J. D. Rogers, *X. ophiopoda* Sacc. *X. schweinitzii* Berk. & M. A. Curtis, *X. globosa* (Spreng. ex Fr.: Fr.) Mont. and the earliest diverging lineage is represented by *Xylaria enterogena* (Mont.) Fr. and *X. telfairii* (Berk.) Fr. supported by the bootstrap analysis (100% BS). *Xylaria atrosphaerica* GZUCC14028 and *X. atrosphaerica* 91111214 HAST clustered at a short branch with high bootstrap support (100%

Taxon	Origin and strains	GenBank accession number			Defeneres
		Rpb2	α-actin	β-tubulin	- References
Amphirosellinia fushanensis	Taiwan, 911111209 HAST	GQ848339	GQ452360	GQ495950	Ju et al. 2004
A. nigrospora	Taiwan, 91092308 HAST	GQ848340	GQ452361	GQ495951	Ju et al. 2004
Astrocystis bambusae	Taiwan, 89021904 HAST	GQ844836	GQ449239	GQ495942	Hsieh et al. 2010
As. mirabilis	Taiwan, 94070803 HAST	GQ844835	GQ449238	GQ495941	Hsieh et al. 2010
As. sublimbata	Taiwan, 89032207 HAST	GQ844834	GQ449236	GQ495940	Hsieh et al. 2010 Ju et al. 1998;
Biscogniauxia arima	Mexico, YMJ 122	GQ304736	AY951784	AY951672	Hsieh et al. 2005
Discoxylaria myrmecophila	Mexico, 169 HAST	GQ844819	GQ438747	GQ487710	Rogers et al. 1995
Stilbohypoxylon elaeicola	Taiwan, 94082615 HAST	GQ844827	GQ438754	GQ495933	Hsieh et al. 2010
S. quisquiliarum	French Guiana, 91111209 HAST	GQ853020	EF025590	EF025605	Rogers & Ju, 1997; Ju et al. 2007
S. quisquiliarum	Taiwan, YMJ 172	GQ853021	EF025591	EF025606	Ju et al. 2007
Xylaria allantoidea	Taiwan, 94042903 HAST	GQ848356	GQ452377	GQ502692	Hsieh et al. 2010
X. apoda	Taiwan, 90080804 HAST	GQ844823	GQ438751	GQ495930	Hsieh et al. 2010
X. atrosphaerica	Taiwan, 91111214 HAST	GQ848342	GQ452363	GQ495953	Hsieh et al. 2010
X. badia	Taiwan, 95070101 HAST	GQ844833	GQ449235	GQ495939	Hsieh et al. 2010
X. berteri	Taiwan, YMJ 90112623	GQ848362	AY951874	AY951763	Hsieh et al. 2005
X. castorea	New Zealand, PDD 47417	GQ853018	GQ455447	GQ502703	Hsieh et al. 2010
X. cf. castorea	Taiwan, 91092303 HAST	GQ853019	GQ455448	GQ502704	Hsieh et al. 2010
X. cf. glebulosa	French West Indies, 431 HAST	GQ848345	GQ452366	GQ495956	Hsieh et al. 2010
X. cf. heliscus	Taiwan, 88113010 HAST	GQ848355	GQ452376	GQ502691	Hsieh et al. 2010
X. crozonensis	France, 398 HAST	GQ848361	GQ455441	GQ502697	Hsieh et al. 2010
X. cubensis	Papua New Guinea, 860 JDR	GQ853017	GQ455446	GQ502702	Gucht, 1995
X. culleniae	Thailand, 189 JDR	GQ844829	GQ438756	GQ495935	Hsieh et al. 2010
X. curta	French West Indies, 494 HAST	GQ844831	GQ449233	GQ495937	Hsieh et al. 2010
X. digitata	Ukraine, 919 HAST	GQ848338	GQ449245	GQ495949	Hsieh et al. 2010
X. enterogena	French Guiana, 785 HAST	GQ848349	GQ452370	GQ502685	Hsieh et al. 2010
X. feejeensis	Taiwan, 92092013 HAST	GQ848336	GQ449243	GQ495947	Hsieh et al. 2010
X. feejeensis	Thailand, 180 JDR	GQ848335	GQ449242	GQ495946	Hsieh et al. 2010
X. frustulosa	Taiwan, 92092010 HAST	GQ844838	GQ449240	GQ495944	Hsieh et al. 2010

 Table 1 Strains used in phylogenetic analyses and their corresponding GenBank accession numbers.

Taxon		GenBank accession number			Defe
	Origin and strains	Rpb2	α-actin	β-tubulin	- References
X. globosa	French West Indies, 775 HAST	GQ848348	GQ452369	GQ502684	Hsieh et al. 2010
X. haemorrhoidalis	Taiwan, 89041207 HAST	GQ848347	GQ452368	GQ502683	Hsieh et al. 2010
X. ianthinovelutina	French West Indies, 553 HAST	GQ844828	GQ438755	GQ495934	Hsieh et al. 2010
X. intracolorata	Taiwan, 90080402 HAST	GQ502690	GQ452375	GQ502690	Hsieh et al. 2010
X. juruensis	Taiwan, 92042501 HAST	GQ844825	GQ438753	GQ495932	Hsieh et al. 2010
X. laevis	French West Indies, 419 HAST	GQ848359	GQ455439	GQ502695	Hsieh et al. 2010
X. luteostromata var. macrospora	French West Indies, 508 HAST	GQ848352	GQ452373	GQ502688	Hsieh et al. 2010
X. ophiopoda	Taiwan, 93082805 HAST	GQ848344	GQ452365	GQ495955	Hsieh et al. 2010
X. oxyacanthae	USA, 93082805 HAST	GQ844820	GQ438748	GQ495927	Hsieh et al. 2010
X. palmicola	New Zealand, 604 PDD	GQ844822	GQ438750	GQ495929	Hsieh et al. 2010
X. phyllocharis	French West Indies, 528 HAST	GQ844832	GQ449234	GQ495938	Hsieh et al. 2010
X. plebeja	Taiwan, 91122401 HAST	GQ848353	GQ452374	GQ502689	Hsieh et al. 2010
X. polymorpha	USA, 1012 JDR	GQ848343	GQ452364	GQ495954	Hsieh et al. 2010
X. regalis	Taiwan, 92072001 HAST	GQ848357	GQ452378	GQ502693	Hsieh et al. 2010
X. schweinitzii	Taiwan, 92092023 HAST	GQ848346	GQ452367	GQ495957	Hsieh et al. 2010
X. scruposa	French West Indies, 497HAST	GQ848341	GQ452362	GQ495952	Hsieh et al. 2010
X. telfairii	French West Indies, 421 HAST	GQ848350	GQ452371	GQ502686	Hsieh et al. 2010
X. spinulosa	Hainan, China, GZUCC13016	KM236098	KM236097	KM236099	This study
X. atrosphaerica	Guizhou, China, GZUCC14028	KY677915	KY677916	KY677917	This study

Table 1 Strains used in phylogenetic analyses and their corresponding GenBan	k accession numbers.
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JDR: Herbarium of Jack D. Rogers; **HAST**: Herbarium, Research Center for Biodiversity, Academia Sinica, Taipei; **YMJ**: Herbarium of Yu-Min Ju; **PDD**: Landcare Research

Taxonomy

Xylaria spinulosa Q.R. Li & J.C. Kang, *sp. nov*.

(Figs 2, 3)

MycoBank – MB513099; Facesoffungi number – FoF 03180 Etymology – in reference to the long thorns covering the stromatal surface. Holotype – GZUH13016

Saprobic on dead tree trunk, forming on the host surface. **Sexual morph:** Stromata upright, usually solitary, unbranched, cylindrical or clavate, fertile at apex, texture soft, internally white, whole stromata up to 1.8–2.5 cm long \times 0.3–0.5 cm broad, 0.5–1 cm long at stipes. Surface of stromata black, covered with long soft hairs. Stalk well defined and easy to distinguish. *Perithecia* 0.3–0.5 mm diam., sphaerical, embedded, closely arranged. Ostioles inconspicuous, not clear. *Asci* 80–214 × 8–12 µm (157 × 10.2 µm, n=20), unitunicate, 8-spored, cylindrical, long-stipitate, the spore-bearing part 60–170 µm long. *Apical ring* 8.5–11.5 µm high × 5–6 µm broad, blue in Melzer's reagent, urn-shaped, 8.5–11.5 µm high × 5–6 µm broad. *Ascospores* 19–23 × 6.5–8.5 µm (21.3 × 7.6 µm, n=30), dark brown to black, unicellular, ellipsoid to inequilateral, with broadly

rounded ends, smooth-walled. Germ slit half spore-length, oblique to sigmoid, lacking sheath and appendage. Asexual morph: Undetermined.

Culture characteristics – Colonies on OA (Fig. 3) cover a 9-cm plate in 3–4 weeks. Mycelia white at first, velvety, soon becoming grey and finally become to black and developing a secondary, aerial mycelium, reverse of cultures a little black.

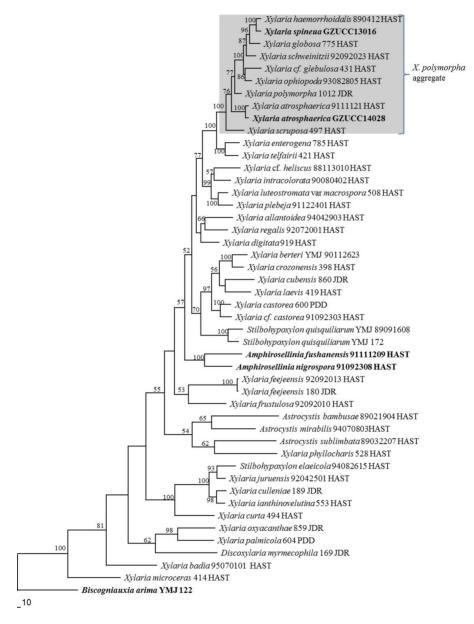


Fig. 1 – MP tree inferred from the analysis of act, rpb2 and tub2 genes sequences. CI=0.319, RI=0.481, RC=0.154, HI=0.681. Bootstrap support values based on 1000 replicates are indicated (above 50%) at the branches at nodes. Sequences labelled in bold were type and our cultures. The tree was rooted to *Biscogniauxia arima* YMJ 122.

No pigments were observed. Stromata arising after 3 weeks of incubation, cylindrical, unbranched, up to 3 cm high and 2 mm diam.

Material examined – CHINA, Hainan Province, Wuzhishan City, Wuzhishan Nature Reserve, on deadwood of unknown plant, March 2013, Q.R. Li, WZS3 (GZUH 13016, holotype; HKAS 99544, isotype); ex-type living culture, GZUCC 13016.

Xylaria atrosphaerica (Cooke & Massee) Callan & J.D. Rogers, Mycotaxon 36 (2): 349 (1990). (Fig. 4) MycoBank MB 127393

Saprobic on dead tree trunk, forming on the host surface. **Sexual morph**: Stromata semiglobular or irregularly globose, pulvinate, 0.5-1.5 mm high $\times 1.5-3$ mm diam., with flattened or slightly convex top, grey dark to black, internally white. Surface cracked into small plates and perithecial mounds inconspicuous.

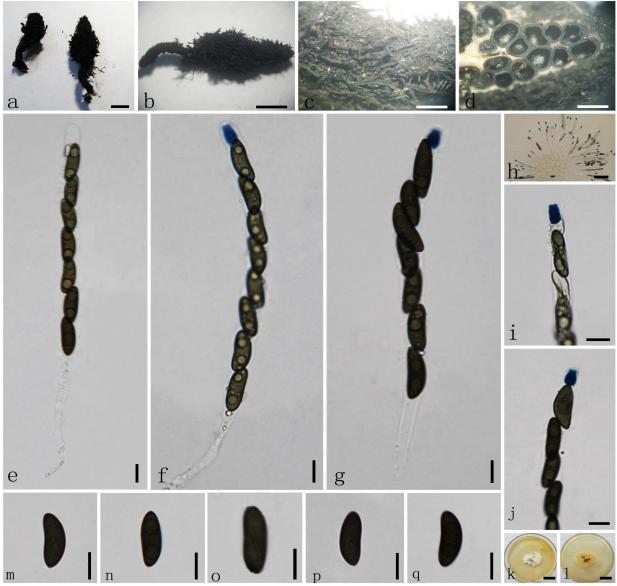


Fig. 2 – *Xylaria spinulosa* (GZUH13016, holotype). **a–c.** Stromata. **d.** Perithecia. **e–h.** Asci. I, J. Ascal apical apparatus (stained in Melzer's reagent). **m–q.** Ascospore. **k**, **l.** Culture on PDA. Scale bars: a,b = 3 mm, c = 1 mm, d = 500 µm, e-g = 10 µm, h = 50 µm, i-q = 10 µm, k, l = 20 mm.

Perithecia 0.2–0.5 mm diam., completely immersed, sphaerical. *Ostioles* inconspicuous. *Asci* 142–215 × 12.5–18.5 μ m (188 × 15.5 μ m, n=30), unitunicate, 8-spored, cylindrical, longstipitate, the spore-bearing part 120–170 μ m long. *Apical ring* bluing in Melzer's iodine reagent, urn-shaped, 7.5–9 μ m high × 5–6.5 μ m broad. *Ascospores* 18.5–24 × 6.5–9.5 μ m (22.5 × 8 μ m, n=30), dark brown to black, unicellular, ellipsoid-inequilateral or crescent to navicular, with ends slightly pinched, smooth, with oblique to spiral germ slit, nearly 1/2 spore-length, lacking sheath and appendage. **Asexual morph**: Undetermined.

Habitat/Distribution – Known from Guiana, Indonesia and China.

Material examined – CHINA. Guizhou Province, Tongren City, Fanjingshan Nature Reserve, on deadwood of unknown plant, July 2014, Q.R. Li, FJS 19 (GZUH14028, HKAS 99545);

living culture, GZUCC14028.

Discussion

Phylogenetic analysis (Fig. 1) showed that *X. spinulosa* clustered with species in the *X. polymorpha* complex (Hsieh et al. 2010).

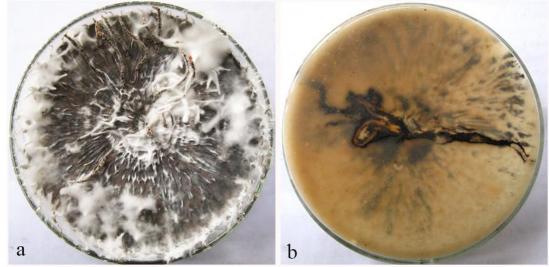


Fig. 3 – Culture of *Xylaria spinulosa* on OA after 4 weeks. a. Culture from above. b. Culture from below.

Taxa within the *X. polymorpha* complex formed a monophyletic group with high bootstrap support. The species were discriminated from the most common known representatives of *Xylaria. Xylaria spinulosa* is most closely related to *X. haemorrhoidalis* Berk. & Broome according to the best-scoring maximum parsimony tree (Fig. 1). Morphologically, *X. haemorrhoidalis* Berk. & Broome has subglobose stromata, and stromatal surface cracked into fine scales without long thorns. *Xylaria spinulosa* has upright, stipitate, woody to leathery stromata. Ascomata of *X. spinulosa* are embedded immediately beneath the surface. Species of *X. polymorpha* complex have a stromatal surface cracked into fine scales and 20–30 µm long ascospores (Ju et al. 2009). *Xylaria spinulosa*, however, differs from other species of the *X. polymorpha* complex in having long thorns on the surface of clavate stromata (Hsieh et al. 2010). Ascospores with half spore-length long germ slit from *X. spinulosa* also separates from other species in the *X. polymorpha* complex which are characterized by bearing a less than spore length germ slit (Rogers and Callan 1986).

The asexual morph of *Penzigia atrosphaerica* collected from Indonesia was described by Rogers et al. (1987). Based on primarily on the nature of cultures, Callan and Rogers (1990) asserted that most species should be accommodated in *Xylaria* and *Hypoxylon* and placed this species in the genus *Xylaria*. GZUCC14028 indicated a rather close relationship to the other sequences included *X. polymorpha* complex. Phylogenetic analysis (Fig. 1) showed GZUCC14028 is much closer to *Xylaria atrosphaerica* 91111214 HAST. Morphologically, our species has same characters with *X. atrosphaerica* and is a new record from China. *Xylaria atrosphaerica* was described from Guiana, Indonesia.

Acknowledgements

The authors would like thank Haixia Ma in Chinese academy of tropical agricultural sciences for her comments. This work was funded by the grants of National Natural Science Foundation of China (Grants Nos. 31460011& 30870009), the agricultural science and technology foundation of Guizhou province, China (Grant No. NY [2013] 3042), the fund of high level innovation talents [No. 2015-4029], the fund of innovation team of Guizhou province [No. 2015-4025], the fund of innovated team of the education department of Guizhou province [No. 2014-31] and the program for new century excellent talents in university [No. NCET-13-0747].

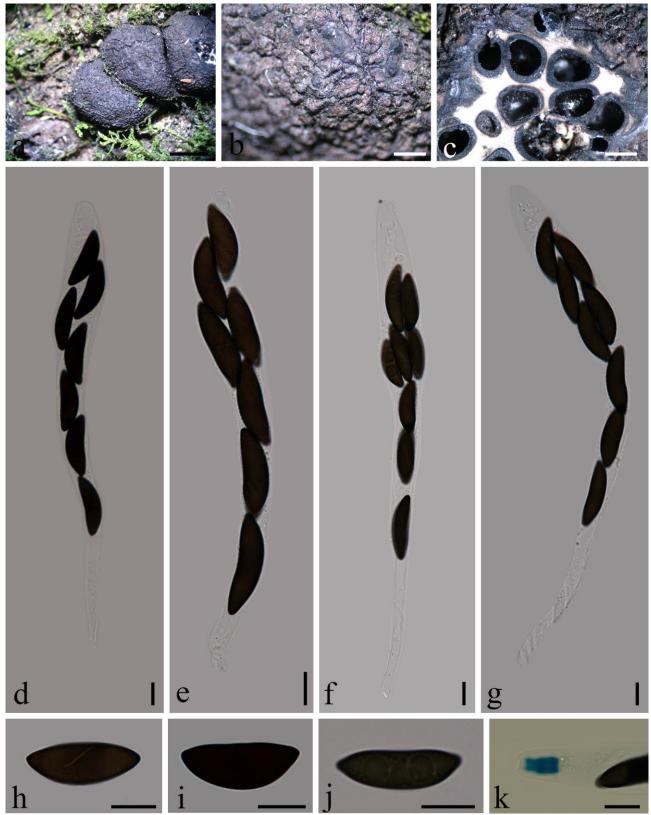


Fig. 4 – *Xylaria atrosphaerica* (GZUH14028). a, b. Stromata on the host. c. Vertical section of stroma. d–g. Asci with ascospores. h–j. Ascospores. k. Ascal apical apparatus and ascospores. – Scale bars: a = 3 mm, b, $c = 200 \mu \text{m}$, d–k = 10 μm .

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