MYCOTAXON

Volume 112, pp. 317–323 *April–June 2010*

A new species of *Engleromyces* from China, a second species in the genus

M.A. Whalley[1], A.M.A. Khalil [1], T.-Z. Wei[2], Y.-J. Yao[2*] & A.J.S. Whalley[1*]

[1*] *a.j.whalley@ljmu.ac.uk*
[1]*School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University Byrom Street, Liverpool L3 3AF, UK*

[2*] *yaoyj@im.ac.cn*
[2]*Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology Chinese Academy of Sciences, Beijing 100101, China*

Abstract — *Engleromyces sinensis* is described as new and its distinguishing characteristics are contrasted with those of *E. goetzei* from Africa. Its distribution and bamboo host in China are discussed and its connection to folk medicine noted.

Key words — *Ascomycota*, taxonomy, *Xylariaceae*

Introduction

Engleromyces Henn. was erected for a single species, *E. goetzei*, occurring in East Africa (Hennings 1900). *Engleromyces* has been considered to have affinities to *Sarcoxylon* Cooke and *Thuemenella* Penz. & Sacc. being intermediate between the xylariaceous and hypocreaceous fungi (Saccardo 1902). Von Arx & Müller (1954) placed the genera in synonymy although later they accepted separate status (Müller & Von Arx 1973). Dennis (1961) and Rogers (1981) maintained the separation and agreed that the genus belongs to the *Xylariaceae*. In his review of *Sarcoxylon* and *Entonaema* Möller, Rogers (1981) noted that the key features of *Engleromyces* are

> " ... its polystichous perithecia, whitish flesh, yellowish exterior crust having areas with punctate perithecial ostioles interspersed with sterile areas of tissue. The stroma is apparently rather soft when fresh. Old herbarium material is hard and horny, but becomes soft and somewhat gelatinous when soaked in water."

*Corresponding authors.

Rogers (1981) also stated that he was unable to confirm a massive amyloid apical ring as detailed by Dennis (1961) because of the poor condition of asci in the material he examined, and he suggested that the asci deliquesce at maturity. The ascospores were described as inequilateral and are often crescentric to C-shaped. Furthermore he noted that the ascospores appear to possess a germ pore of variable position and that the presence of truncate apices at one or both ends of the spores is suggestive of cellular appendages that had dehisced.

During a study of the family *Xylariaceae* in the Mycological Herbarium of the Chinese Academy of Sciences, Beijing (HMAS), two collections (five specimens total) from Yunnan Province, China that had been identified as *E. goetzei* were examined. Although providing a clear account of the overall features of this fungus, neither the original description of *E. goetzei* from Africa (Henning 1900) nor subsequent ones by Lloyd (1917), Dennis (1961), and Rogers (1981) provide details on such microscopical characters as the apical apparatus, the asci, and aspects of ascospore morphology. Examination and comparison of the Chinese material with collections from East Africa provided further information on asci and ascospores but also indicated a number of significant differences between the African and Chinese material. We therefore provide additional information on *Engleromyces* from Africa and describe the collections from China as a second species in the genus.

Taxonomy

Engleromyces sinensis M.A. Whalley, A. Khalil, T.Z. Wei, Y.J. Yao & Whalley. **sp. nov.** Figs 1–6.

MycoBank MB 515459.

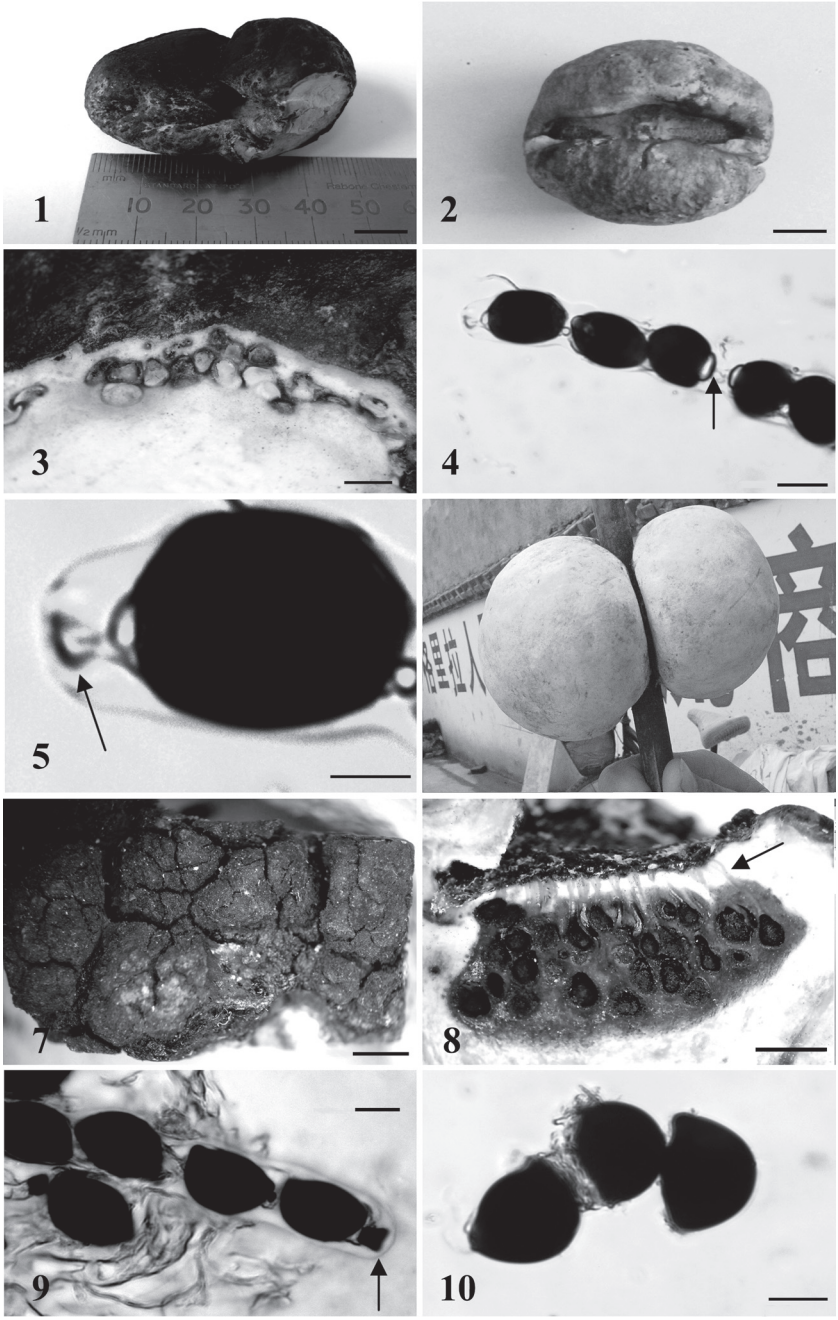
Stromata globosa vel subglobosa, 4.3–4.9 cm crassa × 4–5.5 cm longa et 1.6–4 cm alta, involuta culmi bambusae, pagino bubalina. Ostiola dispersa, plana vel pavum elevate. Annulo apicali in liquore iodata Melzeri cyanescente. Ascosporae atrae, late inequilaterales 15–19 × 11.5–12.5(–14) µm.

Type—Yunnan, China, Yulong County, Yulong mountain, 4 Nov 1958, S.-J. Han & L.-Y. Chen 5058, det. as *Engleromyces goetzei* [as "*goetsii*"] by S.-C. Teng, HMAS 32034 (**Holotype**)

Stromata seated on and partially enveloping bamboo culms forming two lobes, globose to subglobose, 4.3–4.9 × 4–5.5 cm and 1.6–4 cm in height. Surface

Figs 1–6. *Engleromyces sinensis*. Fig. 1. Holotype, partially blackened stroma resulting from fire damage. Fig. 2. Immature specimen showing colour of stroma and position of bamboo culm. Fig. 3. Polystichous perithecial layer. Fig. 4. Ascus, showing ascospores with appendages (arrowed) and apical apparatus. Fig. 5. Funnel-shaped apical apparatus resembling a golf-tee (arrowed). Fig. 6. Fresh specimen in a Yunnan market, China. Figs. 7–10. *Engleromyces goetzei*. Fig. 7. Surface of stroma. Fig. 8. Perithecia in discrete clusters, showing long ostiolar necks (arrowed). Fig. 9. Ascus, showing cuboid apical apparatus (arrowed). Fig. 10. Citriform ascospores.

Bar marker: Figs 1 and 2, 1 cm; Figs 3, 7 and 8, 2 mm; Fig. 4, 9 and 10, 10 µm; Fig. 5, 5 µm.


1
10 20 30 40 50
2
3
4
5
7
8
9
10

buff coloured with a pinkish hue when young, slightly dimpled when young becoming smoother and greyish brown with age. Internal flesh buff coloured, texture firm when fresh becoming woody. Ostioles scattered, slightly papillate becoming punctate with age. Perithecia polystichous, below a crust of ca. 1 mm, unevenly aggregated, spherical to flask shaped, asci 8-spored with apical apparatus blued in Melzer's Reagent, funnel or T-shaped, resembling a golf-tee, c. 4 × 4 µm. Ascospores uniseriate, black, smooth by SEM, broadly inequilateral with one or both ends truncate, with drop-like appendages visible on spores within the ascus, with no germ slit or pore observed, 15–19 × 11.5–12.5 (–14) µm.

Additional collection examined: **CHINA.** YUNNAN: Yulong County, Yulong Mountain. 3000 m. on *Arundinaria*, 4 May 1974, M. Zang 46, det. as *Engleromyces goetzei* [as "*goetzii*"] by M. Zang, HMAS 40511.

Significant characteristic features differentiate the African and Chinese collections of *Engleromyces* and justify their separate taxonomic status. These differences include the overall size of the stromata, ascospore shape and dimensions, and the unique funnel or T-shaped apical apparatus present in the Chinese collections. There are a number of reports on this fungus, as *E. goetzei*, from China that compliment the description and provide additional data on distribution and ecology, e.g. from Muotuo County, Xiang (Tibet) at 2000–3500 m altitude in a coniferous forest with bamboo and also on bamboo culm in Yunnan, Sichuan (Mao et al. 1993, Mao 1998, 2000). Characters cited included stromata 6–10(–20) cm in diameter, spore-containing asci subcylindric and 135–150 × 16–19 µm, ascospores 15–21 × 11–15 µm, and filiform paraphyses. Mao et al. (1993) and Mao (1998, 2000) also reported antibacterial properties and its medicinal use to reduce inflammation. Ying & Zang (1994) cited 120–150 × 14–19 µm asci and 15–21 × 11–15 µm ascospores for collections from Lijiang, Yunnan (HMAS 32034, 40511) and Xizang. Yuan & Sun (1995) provided similar descriptions for collections from Sichuan and Yunnan and pointed out that the fungus contains cytochalasin D, a toxin that inhibits cell division and which can be used to treat skin cancer.

Engleromyces goetzei Henn., Bot. Jahrb. Syst. 28: 327 (1900). Figs 7–10.
≡ *Stromne goetzei* (Henn.) Clem., Gen. fung. (Minneapolis): 44: 173 (1909).

Stromata seated upon and partially enveloping bamboo culms, subglobose up to 30 cm diameter, with an irregularly undulating, roughened surface, dark brown to black, with areas of orange pigmentation especially when young. Flesh solid, white, becoming light brown towards the surface. Perithecia oval to ellipsoid, 0.8–1 mm, compacted at different levels in a layer 3–4 mm deep, with long perithecial necks. Brown punctate ostioles scattered at the surface. Asci 8-spored, 103–121 × 12–15 µm with a large cuboid apical apparatus, c. 4 × 4 µm

blued in Melzer's Reagent. Ascospores uniseriate, black, strongly inequilateral so as to appear citriform, (17.5–) 20–24 × 15–17.5 µm, with no germ slit or pore observed, paraphyses not seen.

Collections examined: AFRICA, KENYA: Kivale, S. Aberdare Mts., 7800 ft., June 1961, I.A.S. Gibson, K(M) 162110. RWANDA (Congo Belge): Kivu, Foret d'Arundinaria alpina, Shamulamda, Massif du Biega, Nov 1951, G. Fontana K(M) 162108. KENYA: Turi, 1958, Baker, K(M) 162109.

Dennis (1961) description of collections of *Engleromyces* from the DR Congo and Rwanda is broadly in line with the one given above, although his ascospore measurements (22–27 × 15–20 µm) are slightly larger than in the material we examined. Rogers (1981), who examined material from Nyassa and Uganda in FH, noted that he was unable to confirm the massive apical ring blued by iodine because of the condition of the asci and indicated that the asci appear to deliquesce at maturity. He did, however, note what appeared to be a germ pore on the ascospores and also referred to the possible presence of cellular appendages. We have examined collections from Kenya and can confirm the presence of a large, cuboid, amyloid apical apparatus 4 × 4 µm, in some cases slightly tapering towards the base. Rogers (1981) also indicated that 'old herbarium material is hard and horny, but becomes soft and somewhat gelatinous when soaked in water'. We, however, did not observe this in *Engleromyces* collections from Kenya; on immersion in water the flesh absorbs water assuming the consistency of a firm bathroom sponge. It was not gelatinous. We consider Kokwaro's (1983) description of the flesh as like a heavy cake resembling the local millet bread 'ugali' as very apt. Thus *Engleromyces* clearly differs from *Entonaema* whose dried stromata readily take up water when submerged and become inflated and gelatinous again.

Discussion

Lloyd (1917) referred to *Engleromyces goetzei* as the largest pyrenomycete. Certainly collections from Africa justify this statement. Kokwaro (1983) stated that 'it is a semi-solid structure which can grow to the size of a football and weigh up to 4 kg. Its Kikuyu name 'Kieha-kia-Murangi' means 'that which sits on bamboo' and it is found only on the upper stems of the mountain bamboo *Arundinaria alpina* K. Schum. It partially envelopes the bamboo stem, often forming two lobes, hence its English name. 'baby's bottom' (Kokwaro, 1983). In a letter to Dr D.A. Reid at Kew on 3 June 1961, Mr I.A.S. Gibson, Forest Pathologist (Kenya), wrote 'I still cannot see where it gets its nutrient from to form such an enormous fruit body. One of the larger ones we have weighed at 4.5 kilos fresh and it was by no means all water!'. The collections from Yunnan that we examined are considerably smaller attaining a size of only 5.5 cm., which is in agreement with Teng (1996) (5–6 cm in the dried state), although Ying &

Zang (1994) recorded up to 20 cm diameter. Two of our authors, YJY and TZW, have seen specimens of *E. sinensis* larger than the holotype commonly for sale on market stalls in Yunnan.

Although possessing many of the features of *E. goetzei, E. sinensis* differs in a number of important characters. The ascospore dimensions are considerably smaller than those of the African collections and do not have the citriform to C-shape of the African material. The apical apparatus is also quite different, being T-shaped or resembling a golf-tee, totally unlike the cuboid apical apparatus found in the African collections. We were unable to observe a germ pore on the ascospores by scanning electron microscopy as spores were shrouded in the remains of the ascus, but we were able to observe appendages on at least one end of the ascospores by light microscopy. However, the ascospores were not in good condition, a problem also encountered by Rogers (1981) and Teng (1996). We found no evidence that the asci of the Kenyan or Chinese material deliquesced at maturity.

The host for both species of *Engleromyces* was recorded as *Arundinaria* in both Africa and China. The African species of bamboo has since been reclassified as *Yushania alpina* (K. Schum.) W.C. Lin and the bamboo from Yulong mountain in Yunnan is now referred to either *Fargesia melanostachys* (Hand.-Mazz.) T.P. Yi or *Fargesia yulongshanensis* T.P. Yi (Professor Nianhe Xia, pers. com.).

Engleromyces goetzei from Kenya has been the subject of chemical analysis and was found to contain a new cytochalasin, engleromycin (Pedersen et al. 1980). Interestingly, *E. goetzei* has been used in traditional African medicine for the treatment of a number of aliments including fever associated with malaria (Kokwaro, 1983). Cytochalasins are produced by many xylariaceous fungi, especially species of *Xylaria* Hill ex Schrank, *Rosellinia* De Not. and *Nemania* Gray (Whalley 1996, Whalley & Edwards 1995). The medicinal uses of *E. sinensis* in China have been recorded mostly under the name *E. goetzei* [as "*goetzii*"]. The fungus has long been used for treating illnesses and has anti-inflammatory and anti-microbial properties (Ying et al. 1987). A study of secondary metabolites from *Engleromyces* from Yunnan revealed a novel compound, neoengleromycin (Liu et al. 2002). These authors also refer to the folk-use of this fungus against infectious diseases and cancer in Tibet, Yunnan, and Sichuan Provinces. The cytochalasins are known to inhibit cell division (Betina 1989) and the link to treatment of cancer is therefore very interesting.

Acknowledgements

Prof. J. Rogers and Dr B.M. Spooner are acknowledged for serving as pre-submission reviewers and for their valuable comments and suggestions. Dr Begoña Aguirre-Hudson at K and Mrs Hong-Mei Lü of HMAS are thanked for assistance in locating the

collections used in this study. Special thanks are due to Prof. Nianhe Xia at the South-China Botanical Gardens, Chinese Academy of Sciences, for providing information on bamboo nomenclature. This work is partly supported by Liverpool John Moores University and the British Mycological Society, and also by the Chinese National Science and Technology Supporting Project (2008BADA1B01) and the Innovation Project of the Chinese Academy of Sciences (KSCX2-YW-G-074-04).

Literature cited

Betina V. 1989. Mycotoxins: chemical, biological and environmental aspects. Elsevier: Amsterdam.

Dennis RWG. 1961. *Xylarioideae* and *Thamnomycetoideae* of Congo. Bulletin Jardin Botanique de l'Etat (Bruxelles) 31: 109–154.

Hennings P. 1900. Fungi Africae orientalis. Engler's botanisheucher fur Systematik, Pflanzengeschichte und Pflanzengeographie 28: 318–529.

Kokwaro JO. 1983. An African knowledge of ethnosystematics and its application to traditional medicine, with particular reference to the medicinal use of the fungus *Engleromyces goetzei*. Bothalia 14: 237–243.

Liu J-K, Tan J-W, Dong Z-J, Ding Z-H, Wang X-H, Liu P-G. 2002. Neoengleromyces, a novel compound from *Engleromyces goetzii*. Helvetica Chimica Acta 85: 1439–1442.

Lloyd CG. 1917. Synopsis of some genera of the large pyrenomycetes. *Camillea. Thamnomyces. Engleromyces.* Cincinnati OH. 16 p.

Mao X-L. 1998. Economic fungi of China. Beijing: Science Press.

Mao X-L. 2000. The macrofungi in China. Henan Science and Technology Press, Zhengzhou, China.

Mao, X-L, Jiang, C-P, Ouzhuciwang. 1993. Economic macrofungi of Tibet. Beijing: Beijing Science and Technology Press.

Müller E, von Arx JA. 1975. *Pyrenomycetes*: *Melioliales*, *Coronophorales*, *Sphaeriales*. In The *Fungi:* an advanced treatise Vol. 4A. (Eds. GC Ainsworth, FK Sparrow, AS Sussman). Pp. 87–132. Academic Press. New York.

Pedersen EJ, Larsen P. Boll PM. 1980. Engleromycin, a new cytochalasin from *Engleromyces goetzii* Hennings. Tetrahedron Letters 21: 5079–5082.

Rogers JD. 1981. *Sarcoxylon* and *Entonaema* (*Xylariaceae*). Mycologia 73: 28–61.

Saccardo PA. 1902. Sylloge Fungorum. Vol. 22 (1). Patavii. 822 p.

Teng S-C. 1996. Fungi of China. Mycotaxon Ltd., Ithaca, New York.

von Arx JA, Müller E. 1954. Die Gattungen der amerosporen Pyrenomyceten. Beitr. Kryptogamenflora Schweiz 11: 1–434.

Whalley AJS. 1996. The xylariaceous way of life. Mycological Research 100: 897–922.

Whalley AJS, Edwards RL. 1995. Secondary metabolites and taxonomic arrangement within the *Xylariaceae*. Canadian Journal of Botany 73(Supplement 1): S802–S810.

Ying J-Z, Mao X-L, Ma Q-M, Zong Y-C, Wen H-A. 1987. Icones of Medicinal Fungi from China. Beijing: Science Press.

Ying J-Z, Zang M. 1994. Economic macrofungi from southwestern China. Beijing: Science Press (in Chinese).

Yuan M-S, Sun P-Q. 1995. Sichuan mushrooms. Chengdu, China: Sichuan Sciences and Technology Press. (in Chinese).