The genus *Xylaria* in the south of China – 7. Two penzigioid *Xylaria* species

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A new xylariaceous fungus, *Xylaria fanjingensis* sp. nov., is described and illustrated from the subtropical forest of Guizhou Province, southern China. The new species is characterized by small, sessile, subglobose stromata attached to the substrate by a narrow connective, the ascal apical ring staining light rust or rust in Melzer's iodine reagent, and the ascospores (37.5)38–44.5(46.5) × 18–20 µm. Another penzigioid *Xylaria* species, *X.* cf. *berteri* is reported in China for the first time. Morphological descriptions and photographs of stromata and microstructures are provided based on the Chinese collections.

Keywords: Ascomycota, xylariaceous fungi, taxonomy.

Xylaria Hill ex Schrank is one of the most complex and difficult genera in the Xylariaceae because of variable stromatal characteristics (Rogers 1985, Van der Gucht 1995). Penzigioid *Xylaria* species have small, sessile to subsessile stromata, attached to the substrate by a narrow connective (Rawla & Narula 1983, Ju *et al.* 2012). These penzigioid fungi have been originally assigned to the genus *Penzigia* Sacc. (Saccardo & Paoletti 1888), which was shown to be synonymous to *Xylaria* (Ju & Rogers 2001, Hsieh *et al.* 2011), and thus they are currently treated as *Xylaria* species (Læssøe 1989, Rogers 1990, Rogers & Ju 2012). This group has been studied by many mycologists, and many new species have been described (Petch 1924; Chardon *et al.* 1940; Martín 1970; Rawla & Narula 1983, 1984; Callan & Rogers 1990; Ju & Rogers 2001; Ju *et al.* 2009, 2012; Rogers & Ju 2012). However the diversity and richness of these fungi are still poorly known and there are still many unidentified specimens of penzigioid fungi from China. During the examination of the specimens from Guizhou province, southern China, an undescribed species was found. In addition, *X.* cf. *berteri* is described for the first time from China based on Chinese collections.

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Materials and methods

Morphological studies

The specimen studied were collected in southern China and are deposited in the Herbarium of the Institute of Mycology, Jilin Agricultural University (HMJAU). Photographs of stromata were taken using a Sony H50x digital camera, and those of stromatal surface were taken with a ZSA30w microscope and S70 Canon camera. Microscopic features and measurements were made from slide preparations mounted in water and Melzer's iodine reagent. The photographs of asci, ascal apical ring, and ascospores were taken by using a VHX-600E microscope of the Keyence Corporation. The methods of collecting, preservation, and identification of the specimens follow Ju & Rogers (1999).

DNA extraction and sequencing

Total DNA from the tissue of stromata was extracted by using a modified cetyltrimethylammonium bromide (CTAB) method. The internal transcribed spacer (ITS) regions were amplified with the primer pair ITS4/ITS5 (White *et al*. 1990). PCR conditions for ITS was an initial denaturation step at 94 °C for 4 min, 30 cycles of 94 °C for 30 s, 56 °C for 30s, 72 °C for 60s and a final extension at 72 °C for 10 min. Reaction components for PCR included approximately 0.1-0.5 ng μL^{-1} of total DNA, 2.5µM of each primer, 200 µM dNTP, 2 mM $MgCl_2$, 0.03 U μL^{-1} of Taq polymerase (Invitrogen, Carlsbad, California), and 1 × standard PCR buffer supplied with the Taq polymerase. DNA sequencing was performed at Beijing Genomics Institute.

Taxonomy

Xylaria fanjingensis H.-X. Ma, Lar. N. Vassiljeva & Yu Li, **sp. nov.** – Fig. 1.
Mycobank no.: MB 804353

Differs from other *Xylaria* species in the ascal apical ring staining light rust or rust in Melzer's iodine reagent and larger ascospores.

Holotypus. – CHINA, Guizhou Prov., Fanjing Mountain, alt. 2000 m, on dead twigs in mixed evergreen and deciduous broad-leaved forest, 21 Aug 2010, *leg*. Haixia Ma, HMJAU 23624.

Stromata gregarious, hemispherical, subglobose to depressed-spherical, attached to substrate with a narrow central connective, 1.5–2.5 mm high × 1.5–3.5 mm in diam., containing 2–3 perithecia; surface plane or with inconspicuous perithecial mounds, blackish, internally white, woody; texture soft. Perithecia embedded in stromata, subspherical, 0.8–1.5 mm in diam.; ostioles papillate. Asci with eight ascospores obliquely arranged in uniseriate occasionally biseriate manner, cylindrical, long-stipitate, (155)240–290(305) × 20–27 µm, the spore-bearing part 190–218 µm long, with an apical ring staining light rust or rust in Melzer's iodine reagent, urn-shaped, 8.5–10.5(11.5)

Fig. 1. *Xylaria fanjingensis,* holotype. **a-c.** Stromata. **d, e.** Vertical section of stroma. **f.** Ascospores and germ slits. **g.** Ascal apical ring. **h.** Asci and ascospores. Scale bars: a, d, e 0.5 mm, b, c 1 mm, f, g 15 µm, h 30 µm.

µm high × (7)8–9.5(10) µm broad. Ascospores dark brown to blackish brown, ellipsoid, almost equilateral, unicellular, with narrowly rounded ends, smooth, (37.5)38–44.5(46.5) × 18–20 µm, with a straight, spore-length germ slit on the less convex side.

Etymology. – Refers to the the mountain name in southern China where the new species was found.

Habitat. – *Xylaria fanjingensis* grows on corticated twigs in mixed evergreen and deciduous broad-leaved forest at the altitude of about 1800 metres in a subtropical monsoon climate.

Distribution. – Known only from the type locality in southern China.

Remarks. – The fungus is easily separated from other *Xylaria* species by its small, subspherical stromata, ascal apical ring staining light rust or rust in Melzer's iodine reagent. Ju *et al.* (2012) described the ascal apical ring of *Xylaria discolor* (Berk. & Broome) Y.-M. Ju, H.-M. Hsieh, J. D. Rogers & Jaklitsch as not staining blue or staining pale blue only at the base in Melzer's iodine reagent, but *X. discolor* can be separated by its smaller stromata 0.5 mm high × 0.5–2 mm in diam., smaller perithecia 0.2–0.3 mm in diam, and smaller ascospores (8.5–)9–12 × 5.5–7(7.5) µm. *Xylaria lechatii* Y.-M. Ju, H.-M. Hsieh, J. D. Rogers & Fournier has a pale blue apical ring staining only at its base in Melzer's iodine reagent; however, it can be recognized by its pulvinate stromata, obovoid and smaller perithecia, 0.4–0.5 mm high × 0.3–0.4 mm in diam., and smaller ascospores 12–15 × 6.5–8 µm (Ju *et al.* 2012).

The new species is similar to *Penzigia indica* Rawla & Narula in perithecial morphology and the same size of perithecia, but the latter has irregularly circular stromata with a short flat or slightly lobed base. In addition, *P. indica* has smaller ascospores 30–37.5 × 12–18 µm. Furthermore, the apical rings in asci of *P. indica* do not stain in Melzer's iodine reagent, whereas those of *X. fanjingensis* stains light rust in Melzer's iodine reagent (Rawla & Narula 1984). Callan & Rogers (1990) described the ascal ring of *P.* cf. *indica* from South America as staining blue in Melzer's iodine reagent and being larger with 12–14 × 6 µm. In addition, the South American collections have slightly smaller ascospores (35)36–40(41) × 12–14(15) µm, with non-cellular appendages on one end. Stromata of the South American material often have remnants of paler, tan to brown, small plates or scales on their surface (Callan & Rogers 1990).

Xylaria fanjingensis somewhat resembles *X. glebulosa* (Ces.) Y.-M. Ju & J. D. Rogers in stromatal morphology, but the latter has smaller perithecia 0.6–0.8 mm in diam. and smaller ascospores 27–31 × 8–10 µm, with oblique germ slits. In addition, the apical ring of asci of *X. glebulosa* stains blue in Melzer's iodine reagent (Ju & Rogers 1999).

Penzigia orientalis Rawla & Narula (Rawla & Narula 1984) from India has similar globose to subglobose stromata, 2 mm high × 3 mm in diam., but its ascospores are small, 21–24 × 7.5–8.5 µm, and the ascal apical ring stains deep blue in Melzer's iodine reagent.

Xylaria cf. ***berteri*** (Mont.) Cooke, Grevillea 11: 126. 1883. – Fig. 2.

Stromata gregarious, peltate to discoid, attached to substrate with a narrow connective, 1.5–2.5 mm high × 0.4–1.5 cm in diam., surface plane or with inconspicuous perithecial mounds, cracked, dark brownish to blackish, external carbonaceous crust hard, interior white, woody. Perithecia embedded

Fig. 2. *Xylaria* cf. *berteri*. **a, b.** Stromata. **c.** Stromatal surface. **d.** Ascospores **e.** Vertical section of stroma. **f.** Asci, apical ring and ascospores. Scale bars: a 1 mm, b 5 mm, c 0.2 mm, d 5 µm, e 0.5 mm, f 10 µm.

in stromata, subspherical, 0.5–0.8 mm. Ostioles slightly papillate. Asci with eight ascospores, obliquely arranged in uniseriate manner, occasionally biseriate, cylindrical, long-stipitate, 130–180 µm total length × 8–9 µm, the spore-bearing part 84–94 µm long, with an apical ring staining blue in Melzer's iodine reagent, discoid-flattened, 1–1.5 µm high × 2–2.5 µm broad.

Ascospores dark brown to blackish brown, ellipsoid, equilateral, unicellular, with narrowly rounded or minutely pinched ends, sometimes with a tiny cellular appendage on one end, smooth, (12.5)13–14(15) × 7–8.5 µm, with a straight germ slit, inconspicuous.

Material examined. – CHINA, Yunnan Province, Hekou County, Dawei Mountain, on the bark of dead wood, 24 Dec 1974, *leg.* Zang M. (HKAS 2585); Hunan Province, Zhangjiajie City, National Forest Park, on the bark of dead wood, 16 Aug 2010, *leg.* He S. H. (HMJAU 23497) .

Habitat. – The fungus was growing on the bark of dead wood in mixed evergreen broad-leaved forests in subtropical monsoon climate.

Remarks. – *Xylaria berteri* is difficult to separate from *Xylaria enteroleuca* (Speg.) P. Martin (Callan & Rogers 1990). *Xylaria enteroleuca* was originally described from Argentina, reported from Brazil, Costa Rica, USA, and is already known from China (Chardon *et al.* 1940, Martín 1970, Callan & Rogers 1990). Martin (1970) reported the species from Sikang, China, but we could not find the collections. Ju & Rogers noted that *X. enteroleuca* is probable a synonym of *X. berteri* (Ju & Rogers 1999, Rogers & Ju 2012). The materials collected in China seem to be typical for *X. enteroleuca* collected in USA (Callan & Rogers 1990). Through a Blast search in the GenBank DNA database, the rDNA-ITS (ITS1-5.8S-ITS2 segment) sequence with 569 bps of *X.* cf. *berteri* (HMJAU 23497) can be comapared with 1029 max scores and 99 % maximal percent identities, 1027 max scores and 99 % maximal percent identities to those of *X. berteri* (JQ936295, GU324749), respectively. So, we designated the Chinese material as *X.* cf. *berteri* following the concept of Rogers & Ju (2012).

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