Three new *Xylaria* species (Xylariaceae, Xylariales) on fallen leaves from Hainan Tropical Rainforest National Park

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**Abstract**

Three new species of *Xylaria* on fallen leaves in Hainan Province of China are described and illustrated, based on morphological and molecular evidence. *Xylaria hedyosmicola* is found on fallen leaves of *Hedyosmum orientale* and featured by thread-like stromata with a long sterile filiform apex. Phylogenetically, *X. hedyosmicola* is closely related to an undescribed *Xylaria* sp. from Hawaii Island, USA and morphologically similar to *X. vagans*. *Xylaria lindericola* is found on fallen leaves of *Lindera robusta* and characterised by its subglobose stromata and a long filiform stipe. It is phylogenetically closely related to *X. sicula f. major*. *Xylaria polysporicola* is found on fallen leaves of *Polyspora hainanensis*, it is distinguished by upright or prostrate stromata and ascospores sometimes with a slimy sheath or non-cellular appendages. *Xylaria polysporicola* is phylogenetically closely related to *X. amphithele* and *X. ficicola*. An identification key to the ten species on fallen leaves in China is given.

**Keywords**

Folicolous fungi, Phylogeny, Pyrenomycetes, Taxonomy
Introduction

Species of *Xylaria* Hill ex Schrank are commonly found throughout the temperate, subtropical and tropical regions of the world, associated with wood, fallen fruits or seeds, fallen leaves or petioles and termite nests (Dennis 1956; Rogers 1986; Rogers and Samuels 1986; San Martin and Rogers 1989; Ju and Rogers 1999; Ju and Hsieh 2007; Fournier 2014). Previous studies on *Xylaria* have dealt primarily with species growing on wood and termite nests (Rogers et al. 2005; Ju and Hsieh 2007; Fournier et al. 2020), but the species diversity and distribution of the genus on other substrates, such as fallen fruits or seeds and fallen leaves or petioles, are still poorly studied (Hsieh et al. 2010; Ju et al. 2018). Especially, the study of *Xylaria* species growing on fallen leaves or petioles is far behind those mentioned taxa associated with other substrates and only seven species have been reported on those substrates in China (Dennis 1956; Rogers et al. 1988; Zhu and Guo 2011; Huang et al. 2014, 2015; Ma and Li 2018).

Hainan Province (20°01.04’N, 110°20.95’E) is located in southern China and enjoys a tropical monsoon climate. More than 6036 plant species, 1895 genera and 243 families have been reported in the province (Yang 2015). Different kinds of tropical vegetations (e.g. Moraceae, Euphorbiaceae and Arecaceae) and rainforests are distributed over the vast territory of the province, in which abundant fungi occur (Dai et al. 2009; Dai 2012; Gao and Yang 2016; Cui et al. 2019). Two intensive surveys of xylariaceous fungi were carried out in Hainan province in 2019 and 2020 and about 400 specimens of Xylariaceae were collected. These materials have been carefully studied through both morphological and phylogenetic methods and three new species on fallen leaves were identified. The new taxa are described and illustrated, and an identification key is provided for the 10 known species of *Xylaria* on fallen leaves in China.

Materials and methods

Morphological studies

Voucher specimens are deposited in the Fungarium of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences (FCATAS), Hainan Province, China. Samples for microscopic examination were mounted in distilled water, Melzer’s reagent, India ink or 1% SDS. Microscopic features observation, measurements and photographing were performed by using a Zeiss Axio Imager A2 microscope (Göttingen, Germany) by differential interference contrast microscopy (DIG) and brightfield microscopy (BF). The photographs of stromata, perithecia and ostioles were taken with a VHХ-600E stereomicroscope Keyence Corporation (Osaka Japan). The methods of collecting, preservation and identification of the specimens follow Ma and Li (2018).
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DNA extraction and sequencing

A modified cetyltrimethylammonium bromide (CTAB) extraction kit (Aidlab Bio-technologies, Beijing, China) was employed for total DNA extraction from dried specimens. The ITS region was amplified with the primer pair ITS4 and ITS5 (White et al. 1990) using the following procedure: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 55.8 °C for 45 s and 72 °C for 1 min and a final extension of 72 °C for 10 min. The TUB and RPB2 gene region were amplified with primers T1/T22 (O’Donnell and Cigelnik 1997) and fRPB2-5F/fRPB2-7CR (Liu et al. 1999), respectively, using the following procedure: initial denaturation at 95 °C for 3 min, followed by 35 °C cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1.5 min and a final extension of 72 °C for 10 min (Hsieh et al. 2005). DNA sequencing was performed at BGI tech (Guangzhou, China) and sequences were deposited in GenBank (Table 1).

Table 1. Species, specimens and GenBank accession number of sequences used in this study. New species and sequences are set in bold.

| Taxon                          | Substrate / Origin                 | Specimen No. | GenBank No. | Reference                  |
|-------------------------------|------------------------------------|---------------|-------------|----------------------------|
| *Xylaria acuminatilongissima*  | termite nests / China Taiwan       | HAST 623     | EU178738    | GQ502711                  | GQ853028 | Hsieh et al. (2010) |
| X. adscendens                 | wood / Guadeloupe                  | HAST 570     | GU300101    | GQ487708                  | GQ844817 | Hsieh et al. (2010) |
| X. allantoide                  | trunk / China Taiwan               | HAST 94042903| GU324743    | GQ502692                  | GQ848356 | Hsieh et al. (2010) |
| X. amphithelae                | dead leaves / Guadeloupe           | HAST 529     | GU300083    | GQ478218                  | GQ844796 | Hsieh et al. (2010) |
| X. apoda                      | bark / China Taiwan                | HAST 90080804| GU322437    | GQ495930                  | GQ844823 | Hsieh et al. (2010) |
| X. arbuscula                  | bark / China Taiwan                | HAST 89041211| GU300090    | GQ478226                  | GQ844805 | Hsieh et al. (2010) |
| X. arbuscula var. plenofissura| wood / China Taiwan                | HAST 93082814| GU339495    | GQ478225                  | GQ844804 | Hsieh et al. (2010) |
| X. atrodivaricata             | termite nests / China Taiwan       | HAST 95052001| EU178739    | GQ502713                  | GQ853030 | Hsieh et al. (2010) |
| X. badia                      | bamboo culm / China Taiwan         | HAST 95070101| GU322446    | GQ495939                  | GQ844833 | Hsieh et al. (2010) |
| X. bambusicola                | bamboo culm / Thailand             | JDR 162      | GU300088    | GQ478223                  | GQ844801 | Hsieh et al. (2010) |
| X. berteri                    | bark / USA                         | JDR 256      | GU324750    | GQ502698                  | GQ848363 | Hsieh et al. (2010) |
| X. berteri                    | bark / China Taiwan                | HAST 90112623| GU324749    | AY951763                  | GQ848362 | Hsieh et al. (2010) |
| X. betulicola                 | leaves of *Betula* / China         | FCATAS 750   | MF774330    | –                        | –        | Ma and Li (2018)   |
| X. brunneovinosa              | termite nests / China Taiwan       | HAST 720     | EU179862    | GQ502706                  | GQ853023 | Hsieh et al. (2010) |
| X. castorea                   | wood / New Zealand                 | PDD 600      | GU324751    | GQ502703                  | GQ853018 | Hsieh et al. (2010) |
| X. coccophora                 | termite nests / China Taiwan       | HAST 664     | EU179863    | GQ502707                  | GQ853024 | Hsieh et al. (2010) |
| X. coccophora                 | wood / French                      | HAST 786     | GU300093    | GQ487701                  | GQ844809 | Hsieh et al. (2010) |
| X. crinalis                   | wood / China                       | FCATAS 751   | MF774330    | –                        | –        | Ma and Li (2018)   |
| X. crozomensis                | bark / France                      | HAST 398     | GU324748    | GQ502697                  | GQ848361 | Hsieh et al. (2010) |
| X. cubensis                   | log / Russian Far East             | HAST 477     | –           | GQ502699                  | GQ848364 | Hsieh et al. (2010) |
| X. cullensiae                 | pod / Thailand                     | JDR 189      | GU322442    | GQ495935                  | GQ844829 | Hsieh et al. (2010) |
| X. escharoides                | termite nests / China Taiwan       | HAST 658     | EU179864    | GQ502709                  | GQ853026 | Hsieh et al. (2010) |
| X. fejeensis                  | bark / China Taiwan                | HAST 92092013| GU322454    | GQ495947                  | GQ848336 | Hsieh et al. (2010) |
| X. ficicola                   | fallen leaves and petioles of *Ficus auriculata* / China | HMJAU 22818 | MZ351258    | –                        | –        | This study          |
| X. filiformis                 | herbaceous stem / Iran             | GUM 1052     | KP218907    | –                        | –        | Hashemi et al. (2015) |
| X. fimбриata                  | termite nests / French West Indies | HAST 491     | GU324753    | GQ502705                  | GQ853022 | Hsieh et al. (2010) |
| X. cl. glebulaea              | fruit / French West Indies         | HAST 431     | GU322462    | GQ495956                  | GQ848345 | Hsieh et al. (2010) |
| Taxon | Substrate / Origin | Specimen No. | GenBank No. | Reference |
|-------|-------------------|--------------|-------------|-----------|
| *X. graminea* | wood / China Taiwan | HAST 479 | GU300097 GU487704 QG484813 | Hsieh et al. (2010) |
| *X. griseoepiacea* | termite nests / China Taiwan | HAST 641 | EU179865 QG502714 QG485301 | Hsieh et al. (2010) |
| *X. hedysosmica* | fallen leaves of *Hedyosmum orientale* / China Hainan | FCATAS 856 (HT) | MZ227121 MZ221183 MZ683407 | This study |
| *X. liquidambar* | fruits of *Liquidambar formosana* / China Taiwan | HAST 725 | EU179866 QG502718 QG853035 | Hsieh et al. (2010) |
| *X. longisima* | wood / China | FCATAS 749 | MF743331 – – – | Ma and Li (2018) |
| *X. longisima* | wood / Taiwan | IRAN 16582 F | KP218906 – – – | Hashemi et al. (2015) |
| *X. melacearum* | petioles and infructescence of *Guarea guidonia* / Puerto Rico | JDR 148 | GU300084 GU487821 QG484797 | Hsieh et al. (2010) |
| *X. multiplex* | wood / USA | JDR 259 | GU300099 GU487706 QG484815 | Hsieh et al. (2010) |
| *X. muscida* | dead branch / French West | HAST 520 | GU300087 GU487822 QG484800 | Hsieh et al. (2010) |
| *X. niveipes* | termite nests / China Taiwan | HAST 653 | US247475 QG502710 QG483502 | Hsieh et al. (2010) |
| *X. oxyacanthae* | fallen seeds / USA | JDR 59 | GU322434 QG495927 QG484820 | Hsieh et al. (2010) |
| *X. oxyacanthae* | fruits / Germany | LZ 2010-502 | H4Q14587 – – – | Roensch et al. (2010) |
| *X. palmicola* | fruits / New Zealand | PDD 604 | GU322436 QG495929 QG484822 | Hsieh et al. (2010) |
| *X. phylloecharis* | dead leaves / French West | HAST 528 | US242445 QG495938 QG484832 | Hsieh et al. (2010) |
| *X. plebeja* | trunk / China Taiwan | HAST 91122401 | US242740 QG502689 QG484835 | Hsieh et al. (2010) |
| *X. polymorpha* | wood / USA | JDR 102 | US242246 QG495954 QG484834 | Hsieh et al. (2010) |
| *X. polymorpha* | Stump / Germany | M-M-012599 | FM164944 – – – | Persoh et al. (2009) |
| *X. polysporica* | fallen leaves of *Polyspora bainanensis* / China Hainan | FCATAS 848 (HT) | MZ005592 MZ301976 MZ301980 | This study |
| *X. regalis* | log of Ficus racemosa / India | HAST 920 | US247475 QG502694 QG484835 | Hsieh et al. (2010) |
| *X. schweinitzii* | bark / China Taiwan | HAST 9209203 | US242463 QG495957 QG484836 | Hsieh et al. (2010) |
| *X. sicula f. major* | fallen leaves / China Taiwan | HAST 90071613 | US300081 QG478216 QG447944 | Hsieh et al. (2010) |
| *X. simplicialis sp. 6* | fallen leaves of *Tobuschina semiscenta* / USA | JDR 258 | US300082 QG478217 QG447957 | Hsieh et al. (2010) |
| *X. striata* | branch / China | HAST 304 | GU300089 QG478224 QG484803 | Hsieh et al. (2010) |
| *X. tentaculata* | leaf litter or wood / Korea | KAI1-0530 | KM077162 – – – | Kim et al. (2016) |
| *X. tentaculata* | leaf litter or wood / Korea | KAI1-1324 | KM077163 – – – | Kim et al. (2016) |
| *X. tentaculata* | leaf litter or wood / Korea | KAI1-1325 | KM077164 – – – | Kim et al. (2016) |
| *X. venosula* | twigs / USA | HAST 94080508 | EF026149 EF025617 QG484806 | Hsieh et al. (2010) |
| *X. venosula* | bark / China Taiwan | HAST 88113602 | US300091 QG487699 QG484807 | Hsieh et al. (2010) |
| *X. xylarioides* | wood / Iran | GUM 1151 | KP218909 – – – | Hashemi et al. (2015) |
| *Hypoxylon fragiforme* | bark / France | HAST 383 | JN979420 AY951720 – – | Hsieh et al. (2005) |
| Camillea obularia | – / Puerto Rico | ATCC 28093 | KY610384 KX271243 – – | Wendt et al. (2018) |
Phylogenetic analyses

The molecular phylogeny was inferred from a combined dataset of ITS, TUB and RPB2 sequences. The sequences retrieved from open databases originated from Hsieh et al. (2005), Persoh et al. (2009), Hsieh et al. (2010), Roensch et al. (2010), Hashemi et al. (2015), Kim et al. (2016), Ma and Li (2018) and Wendt et al. (2018) (Table 1). *Hypoxylon fragiforme* (Pers.) J. Kickx f. and *Camillea obularia* (Fr.) Læssøe, J.D. Rogers & Lodge were selected as outgroup taxa. Sequences were aligned using the MAFFT online (http://mafft.cbrc.jp/alignment/server/). Alignments were optimised manually in BioEdit 7.0.5.3 (Hall 1999).

A combined matrix of ITS-RPB2-TUB and ITS-exons of TUB and RPB2 were used to construct phylogenetic analysis by two methods including maximum likelihood (ML) and Bayesian Inference (BI) analysis, respectively. ML tree generation and bootstrap analyses were performed via the programme RAxML7.2.6 (Stamatakis 2006) running 1000 replicates combined with a ML search. Bayesian analysis was performed with MrBayes 3.1 (Huelsenbeck and Ronquist 2005) implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined by MrModeltest 2.3 (Nylander 2004).

Results

Molecular phylogeny

This study used genetic sequences of 57 species, including 69 ITS sequences, 57 TUB sequences and 54 RPB2 sequences. We applied two tree construction methods to improve the reliability of the results.

After the alignment sequence was adjusted using MAFFT, the ITS alignment, shown in BioEdit 7.0.5, consisted of 778 character positions, 2219 in the TUB alignment and 1241 in the RPB2 alignment. After curing, the constructed multigene alignment (MGA) consisted of 3138 characters (523 of which were derived from the ITS alignment, 1550 from TUB alignment, 1065 from RPB2 alignment). Of the MGA, 1354 characters were considered parsimony-informative.

The analysis results show that the phylogenetic tree, generated by ML in RAxML7.2.6, is basically the same as that generated by BI in MrBayes 3.1. Topology of the phylogenetic analyses, based on ITS-RPB2-TUB and ITS-exons of TUB and RPB2, have no significant conflicts. Only the BI tree is shown in Figure 1 with Bayesian posterior probabilities ≥ 0.95 and ML bootstrap values ≥ 50% labelled along the branches. The phylogenetic tree showed that *X. hedyosmica* is clustered with *Xylaria* sp. 6, *X. polysporicola* is clustered with *X. amphithele* F. San Martín & J.D. Rogers and *X. ficicola* Hai X. Ma, Lar.N. Vassiljeva & Yu Li, *X. lindericola* is clustered with *X. sicula* Pass. & Beltr. f. *major* Ciccarone, but were separated from other species, as well as from each other.
Taxonomy

*Xylaria hedyosmicola* Hai X. Ma & X.Y. Pan, sp. nov.
MycoBank No: 839780
GenBank No: MZ227121, MZ221183, MZ683407
Figure 2

**Diagnosis.** Differs from *X. vagans* by its stromata without a black rhizomorphoid mycelium connecting dead leaves, larger ascospores and tubular to slightly urn-shaped apical apparatus. Differs from *X. betulicola* by its smaller stromata and larger ascospores.

**Typification.** **CHINA.** Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of *Hedyosmum orientale* (Chloranthaceae), 31 December 2020, Haixia Ma (holotype, FCATAS 856).

**Etymology.** “*hedyosmicola*” refers to the growth on leaves of *Hedyosmum orientale*.

**Teleomorph.** Stromata upright, solitary to cespitose, thread-like, unbranched or occasionally branched once at top, 2–5.5 cm total length; with a long sterile filiform apex up to 0.5–3 cm long; fertile part 3–17 mm long × 0.5–1 mm diam., usually consisting of closely packed or scattered perithecia; stipe 8–18 mm long × 0.1–0.5 mm diam., glabrous, finely longitudinally striate, the base slightly swollen; surface roughened, with half-exposed to fully exposed perithecium contours and wrinkles. Externally black, interior white. Texture soft. Perithecium subglobose, 200–470 µm diam. Ostioles papillate, 11–22 µm diam. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 105–160 µm total length, the spore-bearing parts 70–100 µm long × 8–12 µm broad, the stipes 25–70 µm long, with apical apparatus bluing in Melzer’s reagent, tubular to slightly urn-shaped, 2.5–4.8 µm high × 2.5–3.5 µm broad. Ascospores brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, smooth, (12–)13–15(–16.7) × (6–) 6.5–7.5 (–8.5) µm (M = 14 × 7 µm, n = 60), straight to slightly sigmoid germ slit spore-length or almost spore-length, with a slimy sheath on ventral side swollen at both ends to form rounded non-cellular appendages visible in Indian ink.

**Additional specimen examined.** **CHINA.** Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of *Hedyosmum orientale*, 31 December 2020, Haixia Ma (FCATAS 857).

**Remarks.** *Xylaria hedyosmicola* closely resembles *X. vagans* Petch by sharing thread-like or long hair-like stromata bearing closely packed or scattered perithecia with a long sterile filiform apex. *Xylaria vagans* was originally described and illustrated by Petch (1915) from Sri Lanka. However, based on comparisons of the descriptions and illustrations, there were some differences between the two species. *Xylaria hedyosmicola* has larger sporiferous part of asci (70–100 µm × 8–12 µm) with tubular to slightly urn-shaped apical apparatus bluing in Melzer’s reagent, brown and larger ascospores with straight (Fig. 2n and p) to slightly sigmoid germ slit (Fig. 2o), with narrowly rounded ends and a slimy sheath on ventral side swollen at both ends to form rounded non-cellular appendages, while *X. vagans* has a black rhizomorphoid mycelium connecting dead leaves, smaller sporiferous part 68–72 µm × 6 µm and black-brown, cymbiform,
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smaller ascospores 9–12 × 5–6 µm, with broadly rounded ends and is without apical apparatus, germ slit and sheath or appendages (Petch 1915). Unfortunately, the molecular sequences of *X. vagans* from Sri Lanka were not available.

*Xylaria betulicola* Hai X. Ma, Lar.N. Vassiljeva & Yu Li is similar to *X. hedyosmica* in stromatal morphology, but differs in having larger stromata 3–7 cm, slightly smaller

**Figure 1.** Phylogenetic tree of *Xylaria* based on multigene alignment of ITS-TUB-RPB2 in the Bayesian analysis. Bayesian posterior probabilities (≥ 0.95, before the slash markers) and RaxML bootstrap values (≥ 50, after the slash markers) are shown. Different clades are indicated as coloured blocks.
Figure 2. Xylaria hedyosmicola (FCATAS 856, holotype) a, b, e stromata on leaves (b, FCATAS 857) c stromatal surface d section through stroma, showing a perithecium f immature asci in water g, h ascals apical ring in Melzer’s reagent i, j ascospores in Melzer’s reagent k ascus in 1% SDS l, m asci and ascals apical ring in Melzer’s reagent n ascospore in Melzer’s reagent showing straight germ slit o ascospore in Melzer’s reagent showing slightly sigmoid germ slit p, q ascospore showing a slimy sheath and non-cellular appendages in India ink. Scale bars: 1 cm (a, b); 0.1 mm (c, d); 0.5 mm (e); 20 µm (f, m); 10 µm (g–l, n–q).
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Ascospores (11.5)12–14(15) × 5–6 µm, without sheath or appendages (Ma and Li 2018). In the phylogenetic tree, *X. hedyosmicola* formed a fully supported clade with *Xylaria* sp. 6 from Hawaiian Islands, USA (Hsieh et al. 2010). Although there are no descriptions on *Xylaria* sp. 6 in the study of Hsieh et al. (2010), we suspected that it is conspecific with *X. hedyosmicola*. The sequences comparison showed that there are 98.7%, 99% and 99.9% maximal percentage identities, respectively in ITS, TUB and RPB2 between *X. hedyosmicola* (FCATAS 856) and *Xylaria* sp. 6 from USA (JDR 258).

*Xylaria lindericola* Hai X. Ma & X.Y. Pan, sp. nov.

MycoBank No: 839554
GenBank No: MZ005635, MZ031978, MZ031982

**Figure 3**

**Diagnosis.** Differs from *X. sicula* f. *major* by its subglobose stromata without a long sterile apex, larger ascospores and host plant. Differs from *X. hypsipoda* by its black stromata, glabrous stipes and smaller apical apparatus.

**Typification.** China. Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of *Lindera robusta* (Lauraceae), 31 December 2020, Haixia Ma (holotype, FCATAS 852).

**Etymology.** “*lindericola*” refers to the growth on leaves of *Lindera robusta*.

**Teleomorph.** Stromata upright or prostrate, solitary to cespitose, unbranched or branched once or more at stipe, 3–26 cm total length; fertile part subglobose on long filiform stipes, 0.1–0.4 cm diam., the stipe 3–25 cm long × 0.1–1 mm diam., glabrous, finely longitudinally striate, the base slightly swollen; surface roughened by wrinkles and barely exposes perithecial contours. External black, interior white. Texture soft. *Perithecia* subglobose, 300–550 µm diam. *Ostioles* black, papillate. *Asci* with eight ascospores in uniseriate manner, cylindrical, 105–165 µm total length, the spore-bearing parts 65–115 µm long × 7.5–10.5 µm broad, the stipes 25–65 µm long, with apical apparatus bluing in Melzer’s reagent, tubular to urn-shaped, 3.9–5.5 µm high × 3–5 µm broad. *Ascospores* brown, unicellular, ellipsoid-inequilateral, with slightly narrowly rounded ends, aberrant ascospores with strongly pinched or beaked ends, smooth, (12.5–)13.5–15.5(--18) × (7–) 7.5–8.5 (--9.5) µm (M = 14.8 × 8 µm, n=60), with straight germ slit spore-length, without sheath or appendages visible in India ink.

**Additional specimen examined.** China. Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of *Lindera robusta*, 31 December 2020, Haixia Ma (FCATAS 853).

**Remarks.** *Xylaria lindericola* is distinguished by its subglobose fertile part of stroma on a long filiform stipe and growing on fallen leaves of *Lindera robusta*. The species is somewhat similar to *X. sicula* f. *major* in morphology of stromatal fertile part. However, *X. sicula* f. *major* has stromata with long sterile apex, slightly smaller ascospores 9–13(--15) × (3–) 4.5–6 (--7) µm and grows on dead *Olea* leaves (Ciccarone 1947;
Figure 3. *Xylaria lindericola* (FCATAS 852, holotype) a, b stromata on leaves c fertile part of stroma d stromatal surface e section through stroma, showing perithecia f ascal apical ring and ascospores with beaked ends in Melzer’s reagent g ascus and ascal apical ring in Melzer’s reagent h ascus in water i, j ascospores in water k, l ascospore in Melzer’s reagent m ascospore in India ink n ascospore in 1% SDS showing germ slit. Scale bars: 1.5 cm (a, b); 0.2 mm (c–e); 10 µm (f–n).
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Graniti 1959; Fournier 2014). In the phylogenetic tree, *X. lindericola* formed a fully supported clade with *X. sicula f. major* (Figure 1).

*Xyliaria hypsipoda* Massee is similar to *X. lindericola* by sharing globose stromata and ascospores dimensions, but differs in having stromata with whitish scales, hairy stipes and urn-shaped, slightly larger apical apparatus 5–8 µm high × 2.9–5 µm broad (Rogers et al. 1987).

*Xyliaria ficicola* resembles *X. lindericola* in stromatal morphology, but differs in having strongly exposed perithecial mounds of stromatal surface, larger ascospores (16–)17.5–21(–22.7) × 6.5–8.5 µm with conspicuous hyaline noncellular appendage and grows on fallen leaves and petioles of *Ficus auriculata* (Ma et al. 2011). *Xyliaria beloidea* Penz. & Sacc. from Indonesia is somewhat similar to *X. lindericola* in stromatal morphology, but the former has obconical, convex stromatal top, larger ascospores (14.5–)15.5–18(–19) × (5–)5.5–6.5(–7) µm (16.7 × 6.1 µm), with a hyaline sheath swelling at both ends to form non-cellular appendages and grows on fallen fruits, twigs, petioles, and leaves of various plants (Ju et al. 2018).

*Xyliaria comosa* (Mont.) Fr. and *X. clusiae* K.F. Rodrigues, J.D. Rogers & Samuels are also somewhat similar to *X. lindericola* in stromatal morphology. However, *X. comosa* has larger ascospores (21)–26–40 × 7–11 µm and larger apical ring 10.5 µm high × 7.5 µm broad (Dennis 1956) and *X. clusiae* has smaller stromata 1–3.5 cm, ascospores broadly ovoida1 to nearly globose (11.6–)12.8–16.7(–18) × 8–15 µm, with colorless appendage at one end (Samuels and Rogerson 1990).

*Xyliaria polysporicola* Hai X. Ma & X.Y. Pan, sp. nov.

MycoBank No: 839552

GenBank No: MZ005592, MZ031976, MZ031980

Figure 4

**Diagnosis.** Differs from *X. phyllocharis* by its half-exposed to fully exposed perithecial contours, the fertile part cylindrical and larger perithecia. Differs from *X. phyllophila* by its smaller ascospores. Differs from *X. amphithelae* by its cylindrical stromata.

**Typification.** CHINA. Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of *Polyspora hainanensis* (Theaceae), 31 December 2020, Haixia Ma (holotype, FCATAS 848).

**Etymology.** “*polysporicola*” refers to the growth on leaves of *Polyspora hainanensis*.

**Teleomorph.** Stromata solitary, upright or prostrate, cylindrical, unbranched or occasionally branched, 1–4 cm total length, with acute sterile apex up to 2 mm long; fertile part 2–15 mm long × 0.5–1.6 mm diam., usually consists of closely packed perithecia and occasionally with scattered perithecia; the stipe 5–30 mm long × 0.3–1 mm diam., glabrous, finely longitudinally striate, the base slightly swollen; surface roughened, with half-exposed to fully exposed perithecial contours and wrinkles. Externally black, interior white. Texture soft. *Perithecia* subglobose, 0.4–0.6 mm diam.
Ostioles papillate. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 115–185 µm total length, the spore-bearing parts 75–100 µm long × 6.5–9 µm broad, the stipes 30–90 µm long, with apical apparatus bluing in Melzer’s reagent, inverted hat-shaped or urn-shaped, 2.5–4.5 µm high × 2–3.2 µm broad. Ascospores brown to

Figure 4. Xylaria polysporicola (FCATAS 848, holotype) a, b stromata on leaves (b, FCATAS 851) c stromatal surface d section through stroma, showing perithecia e, g asci and ascal apical ring in Melzer’s reagent f, i ascal apical ring in Melzer’s reagent h asci in black India ink j ascospore with germ slit in 1% SDS k, l ascospore in water m, n ascospore showing a slimy sheath and non-cellular appendages in India ink (FCATAS 850) o Ascospore in 1% SDS. Scale bars: 1 cm (a, b); 0.2 mm (c, d); 10 µm (e–o).
Three new Xylaria species (Xylariaceae, Xylariales) on fallen leaves

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dark-brown, unicellular, ellipsoidal-inequilateral, with broadly rounded ends, one end slightly pinched sometimes, smooth, (11.5–)12.5–14.5(–15) × 5.5–8 µm (M = 13.2 × 6.4 µm, n=60), with straight germ slit slightly less than spore-length, a slimy sheath or non-cellular appendages visible occasionally in Indian ink.

Additional specimens examined. China. Hainan Province, Lingshui County, Diaoluoshan Natural Reserve, on fallen leaves of Polyspora hainanensis, 31 December 2020, Haixia Ma (FCATAS 849); 5 July 2019, Haixia Ma (FCATAS 850 & 851).

Remarks. Xylaria polysporicola is morphologically similar to X. phyllocharis Mont. However, X. phyllocharis has fully immersed perithecia, the fertile part with peg-like structures and smaller perithecia 0.2–0.3 mm diam (San Martín and Rogers 1989; Fournier et al. 2020). Xylaria polysporicola is similar to Xylaria sp. (80082005) from Taiwan in stromatal morphology, but the latter has slightly smaller stroma (11–14 mm total length × 1 mm diam. vs. 10–40 mm total length × 0.5–1.6 mm diam.), hard texture, slightly larger ascospores 13.5–16.5 × 5–6 µm, with narrowly rounded ends (Ju and Rogers 1999). Xylaria phyllophila Ces. somewhat resembles X. polysporicola in stromatal morphology, but the former has larger ascospores 20 × 10 µm (Cooke 1883).

Xylaria polysporicola is somewhat similar to X. amphithele F. San Martín & J.D. Rogers in shape and size of apical apparatus and ascospores. However, X. amphithele has globose to conical stromata with 3–4 to 20 naked perithecia (San Martín and Rogers 1989). In the phylogenetic tree, X. polysporicola formed a lineage close to X. amphithele and X. ficicola, but is distant from X. phyllocharis.

Discussion

We included ten Xylaria species on fallen leaves in the phylogenetic analyses of the present study. Except for X. phyllocharis, the other nine studied species formed a monophyletic clade with two wood-inhabiting species, X. muscula Lloyd and X. crinalis Hai X. Ma, Lar. N. Vassiljeva & Yu Li, in our phylogenetic tree (Figure 1). In China, only three species have been previously reported with molecular evidence: X. ficicola from tropical Yunnan, X. sicula f. major from tropical Taiwan and X. betulicola from temperate Jilin (Ma and Li 2018). Within the clade, X. meliacearum, associated with petioles and infructescence of Guarea guidonia, formed a separate branch from other Xylaria species on other leaves. In Hsieh et al. (2010), X. phyllocharis grouped with the wood-inhabiting Xylaria species, which did not reveal any contradictions in our tree. Three species, X. polysporicola, X. amphithele and X. ficicola formed a highly supported clade. Morphologically, these species have some similar features, such as ascospores with slimy sheath or non-cellular appendages, inverted hat-shaped or urn-shaped apical apparatus (San Martín and Rogers 1989; Ma et al. 2020). As Xylaria hedyosmicola formed a fully supported clade with Xylaria sp. 6, the two species should be the same, based on the ITS-TUB-RPB2 (Hsieh et al. 2010). Xylaria lindericola, on leaves of Lindera robusta formed a sister lineage to X. sicula f. major on unknown fallen leaves with high bootstrap value 100%. Xylaria muscula, growing on dead branches, formed a weakly supported branch with X. lindericola and X. sicula f. major associated
with fallen leaves in our tree. This may be because our phylogenetic analysis did not include more taxa related to *X. muscula*.

Until now, ten taxa, *X. betulicola*, *X. diminuta* F. San Martín & J.D. Rogers, *X. ficipes* G. Huang & L. Guo, *X. hainanensis* Y.F. Zhu & L. Guo, *X. hedyosmica* G. Huang & L. Guo, *X. jiangsuensis* G. Huang & L. Guo, *X. lindericola*, *X. polysporicola* and *X. sicula* f. *major* have been found on fallen leaves in China (Hsieh et al. 2010; Ma et al. 2011; Zhu and Guo 2011; Huang et al. 2014, 2015; Ma and Li 2018). Amongst these species, *X. diminuta*, originally reported from Mexico, was found in Yunnan province of China in 2013 (Huang et al. 2014). *Xylaria sicula* f. *major* was first described from Sicily in 1878 and then found in Spain, Kenya, Sardinia, and Taiwan province of China (Hsieh et al. 2010; Fournier 2014). Unfortunately, except for the three species in this study, the molecular data of the other *Xylaria* species from China were not available. We anticipate that additional species of *Xylaria* on fallen leaves will be discovered as more studies are conducted.

**Key to species of Xylaria on fallen leaves in China**

1. Stromata with rounded fertile apices ........................................... 2
   - Stromata with acute sterile apices ......................................... 3
2. Stromata associated with leaves and petioles of *Ficus auriculata* (Moraceae), ascospores (16–)17.5–21(–22.7) × 6.5–8.5 µm ......................... *X. ficipes*
   - Stromata associated with leaves of *Lindera robusta* (Lauraceae), ascospores (12.5–)13.5–15.5(–18) × (7–)7.5–8.5(–9.5) µm .......................... *X. lindericola*
3. Stipes tomentose ........................................................................ 6
   - Stipes glabrous ........................................................................ 5
4. Fertile part subglobose ................................................................. *X. sicula* f. *major*
   - Fertile part not subglobose ...................................................... 5
5. Stromata cylindrical ..................................................................... 6
   - Stromata filiform ................................................................... 8
6. Ascospores (5.5–)6–8 × 3–3.5(–4) µm ........................................... *X. diminuta*
   - Ascospores length > 8.5 µm .................................................. 7
7. Stromata with conspicuous perithecial contours, ascospores (11.5–)12.5–14.5(–15) × 5.5–8 µm ....................................................... *X. polysporicola*
   - Stromata with inconspicuous perithecial contours, ascospores (8.5–)9–11 × 4–6 µm ................................................................. *X. foliicola*
8. Ascospores 16.5–20(–21.5) × 4–5(–6) µm ................................... *X. jiangsuensis*
   - Ascospores length < 16.5 µm .................................................. 9
9. Stromata associated with leaves of *Betula* (Betulaceae), ascospores (11.5)12–14(15) × 5–6 µm, with a straight germ slit, without appendages visible in India ink ................................................................. *X. betulicola*
   - Stromata associated with leaves of *Hedyosmum orientale* (Chloranthaceae), ascospores (12–)13–15(–16.7) × (6–)6.5–7.5(–8.5) µm, with straight to slightly sigmoid germ slit, with appendages visible in Indian ink........... *X. hedyosmica*
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