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Some entomogenous fungi from Wuyishan and Zhangjiajie Nature Reserves 2. Three new species of the genus *Hirsutella**

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Abstract—Three new entomogenous fungi of the genus *Hirsutella*, *H. zhangjiajiensis*, *H. hunanensis* and *H. crinita*, collected from Wuyishan (Wuyi Mountains) and Zhangjiajie Nature Reserves, are described and illustrated. Some problems of the identification of these fungi are briefly discussed.

Key Words—hyphomycete, taxonomy, morphology

Introduction

Hirsutella Pat. is one of the most abundant and important entomogenous fungi. In 1990, we reviewed the development, taxonomic characteristics of the genus, and proposed a key to 62 taxa. A few doubtful species were discussed briefly (Liang 1990a, b). Seifert & Boulay (2004) suggested that *Hirsutella* consists of 65 species. From our study, we refer 90 species around the world to the genus of *Hirsutella*.

The genus *Hirsutella* plays an important role in the natural control of pest insects (Evans 1974, 1982). *H. gigantea* Petch can infect many larvae and pupae of Lepidoptera in the Kuankuoshui Preserve in Guizhou, China (Liang 1991a). Besides this fungus, *H. rhossiliensis* Minter & B. L. Brady, discovered in the early 1980's, has a stronger lethiferous effect on many plant parasitic nematodes, such as *Ditylenchus dipsaci*, *Meloidogyne incognita*, *Aphelenchoides fragariae* and *Criconemella xenoplax* (Jaffee et al. 1982; Cayrol & Frankowski 1986; Cayrol et al. 1986).

It is known that many species in the genus *Hirsutella* are anamorphs of *Cordyceps* Fr. For example, an anamorph of the famous Chinese traditional medicine *C. sinensis* (Berk.) Sacc. is *H. sinensis* (Liu et al., 1989). Some valuable bioactive compounds have been recently discovered from members of *Hirsutella*. A protein toxic to insects, hirsutellin

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A (HtA), has been purified from the fungal mite pathogen, *H. thompsonii* F. E. Fisher (Mazet & Vey, 1995). HtA is considered to be one of many ribonuclease inactivator proteins (RIPs) (Maimala et al. 2002). Vongvanich et al. (2002) found hirsutellide A from a new species of *Hirsutella*, an interesting antimycobacterial cyclohexadepsipeptide. The synthesis of its key precursor has been studied (Xu et al. 2005).

From 1989 to 2001, Chinese mycologists reported seven new species of *Hirsutella* (Table 1). The present paper describes three additional new species of *Hirsutella* and their taxonomic position within the genus.

Table 1. Previously reported species of *Hirsutella* in China

Fungal Name	References
<i>H. leizhouensis</i> H.M. Fang & S.M. Tan	Fang & Tan 1992
<i>H. changbeishanensis</i> Z.Q. Liang	Liang 1991b
<i>H. polycolluta</i> Z.Q. Liang	Liang 1991b
<i>H. yunnanensis</i> Z.Q. Liang & A.Y. Liu	Liu et al., 1993
<i>H. yunnanensis</i> var. <i>tenuisynnemata</i> Z.Q. Liang & A.Y. Liu	Liang et al., 1997
<i>H. sinensis</i> X.J. Liu et al.	Liu et al., 1989
<i>H. longissima</i> C.R. Li et al.	Li et al., 1999
<i>H. huangshanensis</i> C.R. Li et al.	Li et al., 2005

Materials and Methods

All collected specimens were routinely oven-dried at 40°C to prevent growth of contaminant fungi. For examination by light microscopy, slide preparations were made of a snippet of outer layer tissue from the delaminated synnemata by mounting in lactophenol and cotton blue.

Description of new species

***Hirsutella zhangjiajiensis* Z.Q. Liang & A.Y. Liu sp. nov.**

Figs. 1-1, 4

Stromatibus solitariis vel binariis, cylindraceis, simplicibus, 100 × 2 mm. Stipite et capitulo brunneo vel ochraceo. Phialides e ascosporis exorientibus, subulatae graciles, 30-52 × 2-4.5 μm, vel basi inflata ellipsoidea, 4.5 × 3 μm. Conidia lanceolata or leviter curvata, 4.5-7.5 (-10) × 1.5-2.5 μm, mucigeri, limoniformes, 10 × 4 μm. Holotypus: GZDXIFR98-7131.

Stroma single or 2, cylindrical, 100 × 2 mm, not ramified, leathery. Stipe and fertile part brown to snuff-colored. Conidiogenous structure deriving from the microcycle conidiation of ascospores from *Cordyceps zhangjiajiensis*. Phialides slender awl-shaped, 30-52 × 2-4.5 μm or inflated ellipsoidal at basal portion, 4.5 × 3 μm. Conidia lanceolate or the shape of an orange segment, 4.5-7.5(10) × 1.5-2.5 μm, embedded in a mucous sheath, limoniform, 10 × 4 μm.

Specimen studied: GZDXIFR98-7131 was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiyong et al. in VII 1998.

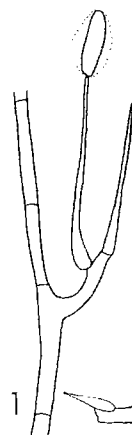


Fig. 1 Synnemata structures and *H. crinita*. 1-3,

Habitat: Pup.
Teleomorph:
Distribution:

Hirsutella

Synnema nigro-brunneum 0 × 1.5-2 μm

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Figs. 1-1, 4

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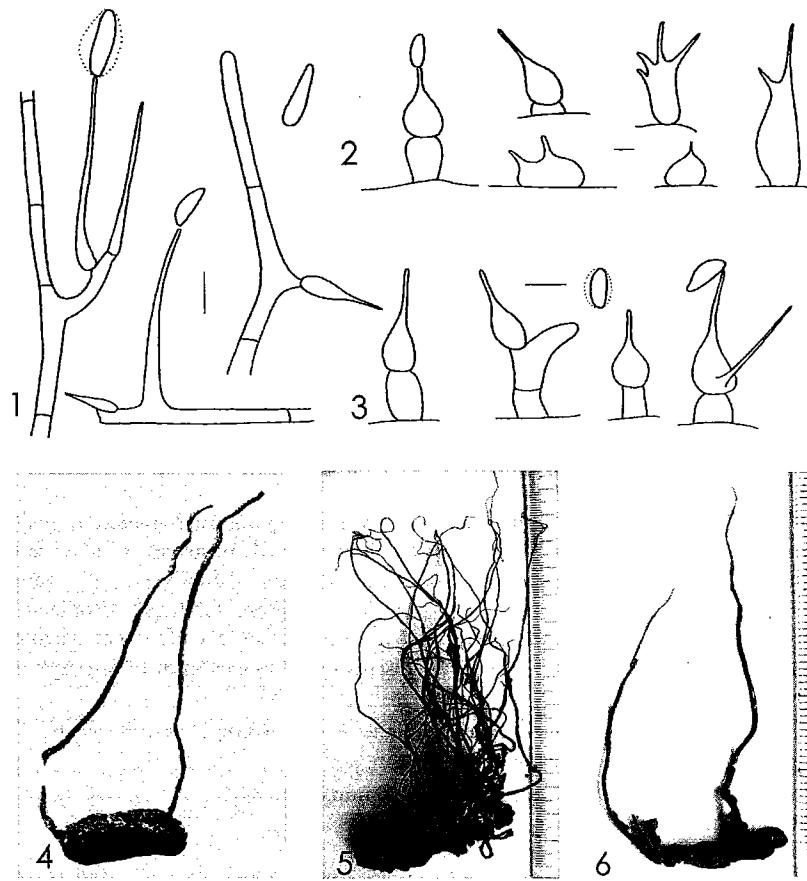


Fig. 1 Synnemata and conidiogenous structures of three species of *Hirsutella*. 1-1, 4: Conidiogenous structures and synnemata of *H. zhangjiajiensis*. 1-2, 5: Conidiogenous structures and synnemata of *H. crinta*. 1-3, 6: Conidiogenous structures and synnemata of *H. hunanensis*

Bars 1, 2 = 10 μm, Bar 3 = 5 μm

Habitat: Pupa of Lepidoptera.

Teleomorph: *Cordyceps zhangjiajiensis* Z.Q. Liang & A.Y. Liu (Liang et al. 2002).

Distribution—Zhangjiajie Nature Reserve: Hunan Province, China.

Hirsutella hunanensis Z.Q. Liang sp. nov.

Figs. 1-3, 6

Synnemata erecta, filiformes, simplices, 5-55 mm longa, 1mm crassa, flexiles vel lignee, nigro-brunneae. *Phialides* 7.5-9 × 4.5-5 μm, e basi inflata cylindrica vel pyriformibus in cillum 0.5-0.8 μm crassum angustatae, prolificis. *Conidia ellipsoidea vel leviter curvata*, 6 × 1.5-2 μm, mucigeri, ellipsoideis, 6 × 4.5 μm. *Holotypus*: GZDXIFR98-7132

Synnemata filiform, erect, unbranched, 5-55 mm long, 1mm wide, flexible to ligneous, dark brown, arising from between head and thorax parts of host insect. *Phialides* solitary

or crowded along synnemata, mostly with cylindrical or pyriform inflated basal portion, $7.5-9 \times 4.5-5 \mu\text{m}$ and with one or 2 slender, thin necks, $7.5-11 \times 0.5-0.8 \mu\text{m}$. Conidia long ellipsoidal to the shape of an orange segment, $6 \times 1.5-2 \mu\text{m}$; embedded in a mucous sheath, ellipsoidal, $6 \times 4.5 \mu\text{m}$. Teleomorph not observed.

Specimen studied: GZDXIFR98-7132. was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiying et al. in VII 1998.

Habitat: A larva of Lepidoptera.

Distribution: Zhangjiajie Nature Reserve—Hunan Province, China.

Hirsutella crinita Z.Q. Liang sp. nov.

Figs. 1-2, 5

Synnemata erecta, filiformes vel cylindrica, simplices, 170 mm longa, 1-1.5 mm crassa, flexiles vel lignae, nigro-brunneae, caespitosa. Phialides $4.5-6(-10) \times 3.5-4.5 \mu\text{m}$, e basi inflata globosa vel cylindrical in collum $0.3-0.5 \mu\text{m}$ crassum angustatae, prolificis. Conidia rara, clavata angusta, $6-9 \times 1-1.5 \mu\text{m}$, exmuca. Holotypus: GZDXIFR98-5231.

Synnemata slender cylindrical or filiform, unbranched, flexible to ligneous, dark brown, caespitose, arising from the head and thorax parts of host insect, very long (up to 170 mm), slightly swollen in the basal region, 1.0-1.5mm wide, in middle part to upside, 0.5-1.0 mm wide. Phialides somewhat scattered, forming a loose hymenium, arising as lateral cells from the outer hyphae of the synnemata, hemispheric to globose or cylindrical, inflated at basal portion, $4.5-6(-10) \times 3.5-4.5 \mu\text{m}$, abruptly narrowing into a short thin neck, $1.5-4 \mu\text{m}$ long and $0.3-0.5 \mu\text{m}$ wide, always proliferating 2-4, sometimes forming inflated, sterile cylindrical hyphae, $15 \times 4.5-5 \mu\text{m}$. Conidia infrequent, narrowly anisomerous obclavate, $6-9 \times 1-1.5 \mu\text{m}$, absence of distinct mucus layer. Teleomorph not observed.

Specimen studied: GZDXIFR98-5231 was collected from Wuyishan, Hujian Province by LIANG Zongqi, LIU Aiying et al. in V 1998.

Habitat: A larva of Lepidoptera. 80-100 \times 50-80 mm.

Distribution: Wuyishan, Fujian Province, China.

Discussion

Evans & Samson (1982a,b, 1984) reported several species with phialides of two types (Table 2): A-phialides, which are lateral and compacted in a layer below the head, and B-phialides, which are terminal, compact, and awl-shaped. The new species, *H. zhangjiajiensis*, forms both A- and B-phialides during the ascospore microcycle conidiation (Fig. 1-1) and produces B-phialides that are solitary and compacted. Species in the genus *Hirsutella* that have awl-shaped phialides more than $40 \mu\text{m}$ long include *H. aphidis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi*, *H. sporodochialis*, *H. darwinii*, *H. guignardii*, *H. sinensis*, and *H. zhangjiajiensis*. Among them, species that have both A- and B-phialides and some phialides greater than $40 \mu\text{m}$ long are *H. sporodochialis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi* and *H. zhangjiajiensis* (Table 2). Possession of rough-walled hyphae and echinate phialides separates the three former species from *H. zhangjiajiensis*.

In the genus *Hirsutella*, some species that have proliferating phialides are *H. besseyi*, *H. guyana*, *H. versicola*, *H. verticillioidea*, and *H. yunnanensis* var. *tenuisynnemata*. All

Table 2. A cor

Species
<i>H. aphidis</i> Petch
<i>H. besseyi</i> F.E. Fisher
* <i>H. crinita</i>
<i>H. darwinii</i> H.C. Evans &
<i>H. guignardii</i> (Samson et al.
<i>H. guyana</i> Minter & B.L.
* <i>H. humanensis</i>
<i>H. minnesotensi</i> S.Y. Chen et al
<i>H. necatrix</i> Minter et al.
<i>H. sinensis</i> Liu et al.,
<i>H. sporodochialis</i> H.C. Evans &
<i>H. stilbelliformis</i> H.C. Evans &
<i>H. stilbelliformis</i> <i>dolichoderi</i> H.C. Evans &
<i>H. versicola</i> Petch
<i>H. verticillioidea</i> Charles
<i>H. yunnanensis</i> <i>tenuisynnemata</i> Z.Q. Liang & * <i>H. zhangjiajiensis</i>

Table 2. A comparison of three new *Hirsutella* with related species

Species	Phialides			Conidia (μm)	Reference
	Shape	Length in μm (max.)	Type		
<i>H. aphidis</i> Petch	Awl	>40	B	Cymbiform 9 \times 1.5-2.5	Petch 1942
<i>H. besseyi</i> F.E. Fisher	Awl or cylindrical	>40	B	Ellipsoid or limoniform 4.1-8.3 \times 2.5-5.8	Minter & Brady 1980
* <i>H. crinita</i>	Base inflated: subglobose	>10	A	Narrowly clavate, 6-9 \times 1-1.5	This work
<i>H. darwinii</i> H.C. Evans & Samson	Awl	>40	B	Fusiform 4.5-11.5 \times 1.5-2	Evans & Samson 1982a
<i>H. guignardii</i> (Maheu) Samson et al.	Awl	>40	B	Ellipsoid or fusiform 7-13 \times 4-6	Samson et al. 1984
<i>H. guyana</i> Minter & B.L. Brady	Base inflated: cylindric	>40	A	Ellipsoid or an orange segment 8-12 \times 3-7	Minter & Brady 1980
* <i>H. humanensis</i>	Base inflated: cylindric to pyriform	>10	A	Ellipsoid or an orange segment 6 \times 1.5-2	This work
<i>H. minnesotensis</i> S.Y. Chen et al.	Base inflated: subglobose	>10	A	Globose 4-6 (in length)	Chen et al. 2000
<i>H. necatrix</i> Minter et al.	Base inflated: subglobose & awl	>5 >10	A & B	Ovoid or ellipsoid 3-4 \times 2.5-3 Globose, -3.5	Minter et al. 1983
<i>H. sinensis</i> Liu et al.,	Awl	>40	B	Reniform or ellipsoid 5.4.-14 \times 3.2-5.4	Liu et al., 1989
<i>H. sporodochialis</i> H.C. Evans & Samson	Base inflated: flask-shaped or awl	>80	A to B	Fusiform 10-27 \times 3.5-4	Evans & Samson 1984
<i>H. stilbelliformis</i> H.C. Evans & Samson	Base inflated: ellipsoid echinate & awl	>10 >100	A & B	Clavate 7-9 \times 1.5-2.5 Ovoid 8-12 \times 4-5	Evans & Samson, 1982b
<i>H. stilbelliformis</i> var. <i>dolichoderi</i> H.C. Evans & Samson	Base inflated: ellipsoid echinate & awl	>40 >100	A & B	Cylindrical to ovoid 6.5-9.5 \times 3.5-4.5	Evans & Samson, 1982b
<i>H. versicolor</i> Petch	Base inflated: ellipsoid	>40	A	Fusiform 4-7 \times 1	Minter & Brady 1980
<i>H. verticillioides</i> Charles	Base inflated: cylindrical	>40	A	Ellipsoid or an orange segment 6-8 \times 3-5	Minter & Brady 1980
<i>H. yunnanensis</i> var. <i>tenuisynnemata</i> Z.Q. Liang & A.Y. Liu	Base inflated: ellipsoid or subglobose	>10	A	Cylindrical or obclavate 4.5-7.2 \times 1.5-1.8	Liang et al., 1997
* <i>H. zhangjiajiensis</i>	Awl & base inflated: ellipsoid	>40	A & B	Lanceolate or an orange segment, 4.5-10 \times 1.5-2.5	This work

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of these species are similar to the new species *H. hunanensis* in fusiform, ellipsoidal and orange conidia. *H. hunanensis* can be distinguished from the above-mentioned species by phialides less than 10µm length and smaller conidia, around 6 × 1.5-2 µm.

Minter et al. (1983) described a new species, *H. necatrix*, that also has phialides with a subglobose or globose swollen base portion. Possession of ovate or ellipsoidal separates this fungus from *H. crinita*, which has narrowly clavate conidia (Table 2). The new species *H. crinita* is also closely related to *H. minnesotensis* and *H. yunnanensis* var. *tenuisynnemata* with similar phialides containing a subglobose basal portion. Globose conidia typically found in *H. minnesotensis* distinguish it from *H. crinita*. *H. yunnanensis* var. *tenuisynnemata* has fusiform or ellipsoidal conidia and can produce yellow caespitose synnemata.

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