A rare monophialidic fungus, *Taifanglania hechuanensis* gen. & sp. nov., was isolated from soil on the banks of Jialin River, Hechuan, Chongqing City during a survey of soil-borne filamentous fungi from different phytogeographical areas in China. It is described and illustrated in this paper. A further eight monophialidic species of *Paecilomyces* are transferred to the genus. Diagnosis features of the new genus are white, grey, straw yellow or brown to black colonies on Czapek agar. Conidiophores are always absent or simple. Phialides are solitary, consisting of a cylindrical or ellipsoidal swollen basal portion, tapering into a thin neck, directly arising on vegetative hyphae or prophialides, sometimes consisting of a whorl of 2 to 3 phialides on simple conidiophores. Conidia are one-celled, hyaline, smooth-walled, subglobose, ellipsoidal or fusiform, having or no the connective between conidia and being thermotolerant. The new species is characterized by pale yellow to grey-yellow colonies, solitary phialides with an ellipsoidal or fusiform basal portion that arise directly from the vegetative hyphae, big conidia (3.1-)3.9-8.7 × (1.7-)2.1-4.7(-5.1) μm with the connective, and thermotolerant growth. A molecular study based on the nucleotidic sequences of the SSU rDNA and ITS regions support the status of *T. hechuanensis* as a new species and *Taifanglania* as a new genus.

**Key words:** taxonomy, morphology, molecular phylogeny, thermotolerant fungi

**Article Information**
Received January 17, 2008
Accepted April 28, 2008
Published online 15 January 2009

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**Introduction**

The monophialidic species, *Paecilomyces flavescens* A.H.S. Br. & G. Sm. (= *P. inflatus* (Burnside) J.W. Carmich.) was first recorded by Brown and Smith (1957). Later, Onions and Barron (1967) grouped 10 species within the genus *Paecilomyces* as a monophialidic series but did not state whether the main axes of the conidiophore is lacking in these fungi, the sporogenous cell is borne singly either directly on the vegetative hyphae or less frequently in groups of two or three on very short conidiophores. Gams (1971) transferred these monophialidic species with awl-shaped phialides to the genus *Acremonium*, leaving only *P. inflatus* in the genus *Paecilomyces* for the reason that its phialides are flask-shaped with a somewhat inflated basal portion and sometimes clustered in small groups on well-defined, very short conidiophores. Subsequently, Subramanian (1972) placed *P. bacillisporus*, *P. humicola*, *P. roseolus*, *P. striatissporus* and *P. variabilis*, together with *Gliomastix murorum*, in a new genus, *Sagrahamala* Subram.

*Paecilomyces inflatus* can be regarded as an intermediate form between the genera *Paecilomyces* and *Acremonium*, because the phialides are often solitary and orthotrophic; however, the inflated basal portion and distinct neck classify it in *Paecilomyces* (Samson, 1974). Three other monophialidic species, *P. ampullaris* Matsush. (Matushima, 1971), *P. ampulliphorus* Matsush. and *P. riomoteanus* Matsush. (Matushima, 1975) were later reported.

Obornik *et al.* (2001) analyzed sequences of the divergent domain at the 5’ end of the LSU rRNA gene from some entomopathogenic fungi: *Paecilomyces* spp., *Verticillium* spp.,
Beauveria bassiana, Aschersonia spp., and Cordyceps spp. Based on phylogenetic analysis, they proposed polyphyly of the genus Paecilomyces and close relationships among entomopathogenic species of the genera Verticillium, Paecilomyces and Beauveria.

Luangsa-ard et al. (2004, 2005) examined the nuclear-encoded SSR DNA sequence of Paecilomyces strains from Thailand and of some others held in CBS. A phylogenetic analysis based on the 18S nr DNA demonstrated that Paecilomyces is polyphyletic across two subclasses, Sordariomycetidae and Eurotiomycetidae. Paecilomyces variotii and hemophilic relatives belong in the order Eurotiales (Trichocomaceae), while mesophilic species related to Paecilomyces farinosus are in the order Hypocreales (Clavicipitaceae and Hypocreaceae). Subsequently, they used the β-tubulin gene and ITS rDNA and examined the phylogenetic relationships of Paecilomyces sect. Isariodea (Luangsa-ard et al. 2005). Maximum parsimony analysis showed that the section is also polyphyletic within the Hypocreales. However P. inflatus has affinities with the order Sordariales. Its conidio-genous structure is very different from other Paecilomyces.

Fig. 1. The conidiogenous structures of Taifanglania hechuanensis. Bars= 10 μm.
Fungi are important organisms for potential novel compound discovery for medicinal, biofungicide and pesticide use (Huang et al., 2008; Hyde and Soytong, 2008; Rungjindamai et al., 2008). This is particularly true of **Paecilomyces** (Liang et al., 2005, 2007; Song et al., 2007; Wang et al., 2008) and we have been studying this genus in China. During a survey of soil-borne filamentous fungi from 2003-2006 in China, many novel species of the genus **Paecilomyces** were encountered (Table 1). Including the monophialidic and thermotolerant **P. biformis** Z.Q. Liang, H.L. Chu & Y.F. Han, **P. major** (Z.Q. Liang, H.L. Chu & Y.F. Han) Z.Q. Liang, H.L. Chu & Y.F. Han and **P. furcatus** Z.Q. Liang, H.L. Chu & Y.F. Han. Although **P. biformis** and **P. major** were found to produce laccase (Chu et al., 2004; Han et al., 2005a,b,c, 2007; Li et al., 2006; Liang et al., 2006a,b) the relevance of this not clear to determine the taxonomic position of these monophialidic species, we have used both macro- and micro-morphological studies and molecular analyses. Based on these results, we propose that a new genus **Taifanglania** be established, into which we transfer the monophialidic species of **Paecilomyces**.

**Table 1.** Some taxa of **Paecilomyces** newly discovered in China (2003-2006).*

<table>
<thead>
<tr>
<th>Name</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. huaxiensis</em></td>
<td>Han et al., 2005a</td>
</tr>
<tr>
<td><em>P. stipitatus</em></td>
<td>Han et al., 2005c</td>
</tr>
<tr>
<td><em>P. vinaceus</em></td>
<td>Han et al., 2005c</td>
</tr>
<tr>
<td><em>P. parvusporus</em></td>
<td>Han et al., 2005b</td>
</tr>
<tr>
<td><em>P. cylindricosporus</em></td>
<td>Liang et al., 2005</td>
</tr>
<tr>
<td><em>P. biformis</em> [M]</td>
<td>Liang et al., 2007</td>
</tr>
<tr>
<td><em>P. cinereus</em> [M]</td>
<td>Liang et al., 2006b</td>
</tr>
<tr>
<td><em>P. curticatenatus</em> [M]</td>
<td>Han et al., 2007</td>
</tr>
<tr>
<td><em>P. major</em> [M]</td>
<td>Liang et al., 2006a</td>
</tr>
<tr>
<td><em>P. furcatus</em> [M]</td>
<td>Liang et al., 2006b</td>
</tr>
<tr>
<td><em>P. verticillatus</em></td>
<td>Li et al., 2006</td>
</tr>
<tr>
<td><em>T. hechuanensis</em></td>
<td>Han et al., this work</td>
</tr>
</tbody>
</table>

*A species with a bracket containing a letter M is monophialidic.

**Materials and Methods**

**Sample collection and strain isolation**

Strain GZUIFR-H08-1 was isolated from soil samples from Hechuan, Chongqing City. Two grams of soil were added to a flask containing 20 ml sterilized water and some glass beads. Each soil suspension was shaken for about 10 min and then diluted to concentrations of $10^{-1}$-$10^{-2}$. One ml suspension ($10^{-2}$) was mixed with Martin medium in a sterilized Petri dish of 9 cm diam. The cultures were incubated at 40°C for 7 days.

**Strain identification**

The studied strains were transferred to Czapek agar. After incubation at 40 °C for 7 days, the strains were identified based on colony characters and conidiogenous structures according to Brown and Smith (1957) and Samson (1974).

**Reagent and DNA extraction**

Taq enzyme and dNTP was obtained from Shanghai Sangon, An Agarose Gel DNA Purification kit ver 2.0 was from TRKARA Company.

Fresh, sporulating cultures on Czapek agar were used for DNA extraction following Tigano-Milani et al. (1995); the extracted DNA is stored at -20°C.

**PCR amplification and determination of ITS and 18S rDNA sequencing**

To amplify ITS1-5.8S-ITS2 rDNA sequence, the following primers were used: ITS4: 5'-TCCTCCGCTTATTGATATGC-3' and ITS5: 5'-GGAAAGTAAAGTGCTCCTAA CAAGG-3'. Polymerase chain reaction (PCR) amplification was performed as follows: 50 μL reaction system: 10× reaction buffer 5 μL, dNTP 1 μL, primer 1μL each, Pfu buffer 0.5 μl, 2 μL of template DNA and ddH2O 39.5 μL. The amplification program: a first step of 94°C for 5 min, then 35 cycles consisting of 94°C for 40 s, 49°C for 40 s, and 72°C for 1 min, and a final step of 72°C for 10 min. To amplify 18S rDNA sequence, the following primers were used: NS1: 5’-TGAGTCAT ATGCTTTG TCTC-3’ and NS 24: 5’-AAACC TTGTTACGACTTTTA-3’. The amplification program: a first step of 94°C for 4 min, then 35 cycles consisting of 94°C for 40 s, 49°C for 40 s, and 72°C for 1 min, and a final step of 72°C for 10 min. PCR products were purified using Agarose Gel DNA Purification kit ver 2.0 according to instructions (TRKARA Company), 1 μL purification
products were determined in $\rho = 1\%$ Agrose by electrophoresis and sequenced with the above primers by Beijing Sunbiotech Co. Ltd. Sequences of SSU and ITS1-5.8S-ITS2 rDNA regions of this fungus were submitted to GenBank.

**Sequence alignment and phylogenetic analysis**

Table 2 lists the species used in this molecular study from Genbank. The sequence of the new species was aligned with the related species using ClustalX 1.83 computer programme for multiple sequence alignment and manually corrected. The phylogenetic tree was constructed by neighbor-joining method of MEGA version 3.1 (Kumar et al., 2004). Confidence values for individual branches were determined by bootstrap analysis (1000 replications).

**Description of new genus and species**

**Taxonomy**

*Taifanglania* Z.Q. Liang, Y.F. Han, H.L. Chu & R.T.V. Fox, *gen. nov.*

MycoBank No: 512803

Etymology: The genus name *Taifanglania* is dedicated to the late Chinese mycologist Tai, Fanglan.

In agar* Czapekii, coloniae albae, griseae, hubalinae, hinnulae, ochraceae, vel nigricantes, planae. Conidiophora absenita vel simplicia. Phialides singulars, sed interdum in conidiophoris brevibus binate vel ternate aggregatae, e basi inflata ellipsoidea vel cylindrica, in collum distinctum apice inspissato angustatae. Conidia ellipsoidea vel fusiformia, (3.1-) 3.9-8.7 × (1.7-) 2.1-4.7 (-5.1) μm.


Colonies on Czapek agar, attaining a diameter of 56 to 63 mm within 7 days at 40 °C, thermotolerant, flat, loose villiform, pale yellow to grey-yellow, with indistinct zones and sectors. Reverse off-white. Vegetative hyphae hyaline, smooth-walled, short septate, 1.7-3.8 μm wide. Conidiophores simple, inconspicuous and branched (3.1-) 5-8.7 (-11) × 2-4 (-5.8) μm. Phialides solitary, (3.1-) 3.9-19 (-22.1) × (2.2-) 2.5-4.7 (-5.8) μm, consisting of cylindrical or ellipsoidal swollen basal portions, tapering into thin necks, 2.1-12.7 μm long, directly arising on vegetative hyphae or pro-phialides, or consisting of a whorl of 2 to 3 phialides on simple conidiophores. Conidia 1-celled, hyaline, smooth-walled, ellipsoidal or fusiform, connective, (3.1-) 3.9-8.7 × (1.7-) 2.1-4.7 (-5.1) μm, forming divergent long chains.

Material examined: GZUIFR-H08-1 isolated by Y. F. Han & Z. Q. Liang from soil on banks of Jialin River, Hechuan, Chongqing City, China, November 2003.

Teleomorph: Unknown.

Habitat: Soil.

Distribution: Chongqing City, China.

Eight monophialidic species of *Paecilomyces* are transferred to *Taifanglania*:

*Taifanglania hechuanensis* Z.Q. Liang, Y.F. Han, H.L. Chu & R.T.V. Fox, *sp. nov.* (Fig. 1) MycoBank No: 512804

GenBank No: EU053286; DQ185070

In agar* Czapekii, coloniae 56-63 mm diam, 7 diebus ad 40° C, planae humiles. Hyphis septatis, hyalinis, 1.7-3.8 μm crassis. Conidiophora simplicia, (3.1-) 5-8.7 (-11) × 2-4 (-5.8) μm. Phialides singulares, (3.1-) 3.9-19 (-22.1) × (2.2-) 2.5-4.7 (-5.8) μm, e basi inflata ellipsoidea vel cylindrica, in collum distinctum apice inspissato angustatae. Conidia ellipsoidea vel fusiformia, (3.1-) 3.9-8.7 × (1.7-) 2.1-4.7 (-5.1) μm.


Colonies on Czapek agar, attaining a diameter of 56 to 63 mm within 7 days at 40 °C, thermotolerant, flat, loose villiform, pale yellow to grey-yellow, with indistinct zones and sectors. Reverse off-white. Vegetative hyphae hyaline, smooth-walled, short septate, 1.7-3.8 μm wide. Conidiophores simple, inconspicuous and branched (3.1-) 5-8.7 (-11) × 2-4 (-5.8) μm. Phialides solitary, (3.1-) 3.9-19 (-22.1) × (2.2-) 2.5-4.7 (-5.8) μm, consisting of cylindrical or ellipsoidal swollen basal portions, tapering into thin necks, 2.1-12.7 μm long, directly arising on vegetative hyphae or pro-phialides, or consisting of a whorl of 2 to 3 phialides on simple conidiophores. Conidia 1-celled, hyaline, smooth-walled, ellipsoidal or fusiform, connective, (3.1-) 3.9-8.7 × (1.7-) 2.1-4.7 (-5.1) μm, forming divergent long chains.

Material examined: GZUIFR-H08-1 isolated by Y. F. Han & Z. Q. Liang from soil on banks of Jialin River, Hechuan, Chongqing City, China, November 2003.

Teleomorph: Unknown.

Habitat: Soil.

Distribution: Chongqing City, China.

Eight monophialidic species of *Paecilomyces* are transferred to *Taifanglania*:


*Myceliophthora inflata* Burnside, Papers of the Michigan Academy of Science, Arts and Letters 8: 82. 1928.


Table 2. Strains used in the molecular study.

<table>
<thead>
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<th>Species</th>
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<th>ITS GenBank No</th>
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<td>Chaetomium globosum</td>
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<td>AY429054</td>
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<td>Cordyceps takaomontana</td>
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<td>Corynascus verrucosus</td>
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<td>Morchella esculenta</td>
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<tr>
<td>Thielavia terricola var. minor</td>
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</table>

*Paecilomyces cinereus* Z.Q. Liang, H.L. Chu & Y.F. Han, Mycotaxon 97: 16. 2006b


*Taifanglania curticatenata* (Z.Q. Liang & Y.F. Han) Z.Q. Liang, Y.F. Han & H.L. Chu **comb. nov.**, MycoBank No: 512812.

**Fig. 2.** Phylogenetic tree based on nucleotide sequence of the SSU nrDNA sequence by NJ of MEGA3.1 software. Bootstrap values (>50%) from 1000 replicates are included at the internodes. Branch lengths are drawn to scale.

**Taifanglania major** (Z.Q. Liang, H.L. Chu & Y.F. Han) Z.Q. Liang, Y.F. Han & H.L. Chu, **comb. nov.** MycoBank No: 512817.


**Taifanglania ampullaris** (Matsush.) Z.Q. Liang, Y.F. Han & H.L. Chu, **comb. nov.** MycoBank No: 512813.


**Taifanglania ampulliphora** (Matsush.) Z.Q. Liang, Y.F. Han & H.L. Chu, **comb. nov.** MycoBank No: 512814.


**Taifanglania biformis** (Z.Q. Liang, H.L. Chu & Y.F. Han) Z.Q. Liang, Y.F. Han & H.L. Chu, **comb. nov.** MycoBank No: 512815.


**Key to the species of Taifanglania**

1. Sporulating structure of two types, solitary phialide and conidiophore ............................. *T. biformis*
   1. Sporulating structures monophialidic only ........2
   2. Conidia roughened, chains short, consisting of 3–6 conidia ........................................ *T. curticatenata*
   2. Without above characters ........................................3
   3. Phialides with furcate neck ........................ *T. furcata*
   3. Phialides without furcate neck ..........................4
   4. Conidia subglobose or long ellipsoidal ..........5
   4. Conidia fusiform, lemon-shaped or ellipsoidal ....7
   5. Conidia subglobose, 2.2–3.4 × 2.0–2.6 μm ..........
      .................................................................. *T. ampullaris*
   5. Conidia long ellipsoidal ..................................6
   6. Conidia 6–8 × 2.5–3 μm ................................. *T. ampulliphora*
   6. Conidia 3–13 × 1.4–5.4 μm ............................. *T. cinerea*
   7. Conidia fusiform to lemon-shaped, 3.4 × 2.3 μm.  *T. inflata*
   7. Conidia long fusiform .................................8
   8. Conidia fusiform to ellipsoidal, 4.3–7.3 × 2.1–3.5 μm 
      .................................................................. *T. hechuanensis*
   8. Conidia fusiform, larger, 5.2–14.1 × 2.5–5.1 μm .......
      .................................................................. *T. major*

SSU and ITS rDNA sequences of *T. hechuanensis* were aligned with those of related species. The species has close affinities
Fig. 3. Phylogenetic tree based on nucleotide sequence of the ITS-5.8S-ITS2 rDNA region by NJ of MEGA3.1 software. Branch lengths are proportional to distance. Bootstrap replication frequencies (1000 replications) are indicated at the internodes.

to other monophialidic species and the genera *Thielavia* and *Chaetomium, Sordariales* (Figs 2, 3).

**Discussion**

Some species of *Botryotrichum* Sacc. & Marchal and *Humicola* Traaen directly produce phialides and conidial chains on the hyphae as in *Taifanglania.* However, these fungi are characterized by simple, short conidiophores and subglobose or globose brown coloured conidia (Barnett and Hunter, 1998). Another genus with a similar conidiogenous structure is *Monocillium* S.B. Saksena, which is distinguished from *Taifanglania* by the inflated or thickened middle part of the phialide (Arx, 1974; Barnett and Hunter, 1998).

Some species of *Acremonium* Link are similar to the monophialidic species in *Taifanglania.* However, the *Acremonium* species can be distinguished from *Taifanglania* species by their typical awl-shaped phialides (Samson, 1974).

In these monophialidic species, *T. hechuanensis* and *T. major* are two rare species in which conidia are more than 5 μm wide. Moreover, their shapes of phialides, size, surface texture of colonies and reverse color are also basically similar. However, the former species has pale yellow colonies and longer conidia (5.2-14.1 μm) and the later species has off-white colonies, short conidia (3.1-8.7 μm long).

Among the species of *Taifanglania,* the conidia of *T. inflata,* *T. major* and the new species *T. hechuanensis,* are all fusiform. The conidia of *T. inflata* are only 3-4 μm long, distinctly shorter than those of *T. hechuanensis.* The ratio of conidial length: width for *T. major* is greater than for *T. hechuanensis.*

Based on SSU rDNA sequences analyses, the phylogeny tree was split into three mono-
phylectic clades (Fig. 2). Within clade I, are several thermotolerant and thermophilic urotialeous species. These species have more complex conidiogenous structures, which are morphologically very different to those in the monophilic species. T. hechuanensis and several monophilic species including T. inflata were grouped in Clade II together with Chaetomium globosum, Thielavia terricola and Corynascus verrucosus within Chaetomiaceae, Sordariales.

Chaetomium globosum is the most common species of Chaetomium. It can produce several bioactive compounds (Wang et al., 2006, Wijeratne, 2006, Fogle et al., 2007), such as chaetoglobosins A and C. These have been shown to be lethal to various tissue culture cell lines at relatively low levels (Fogle et al., 2007). However the relevance of this is not clear. To date, an anamorph has not been formally identified for C. globosum (Fogle, 2007). Salar and Aneja (2007) also reported that all thermophilic species of Chaetomium did not produce conidia.

Salar and Aneja (2007) reported that the fungus Acremonium albamensis was the imperfect state of Thielavia terricola.

The anamorph of Corynascus thermophilus is Myceliophthora fergusii (Arx, 1974) and some anamorphs of Corynascus species are also placed in Myceliophthora by Salar and Aneja (2007).

Anamorphs of these ascomycetes are members of genera Sepedonium Link, Myceliophthora Costantin and Botryotrichum (Kiffer and Morelet, 1997). They posses awl-shaped phialides, more or less dark-colored hyphae and acrogenous single conidia. The conidiogenous structures of T. hechuanensis, are phialides forming divergent long chains of conidia. No species of Taifanglania are known to produce a teleomorph. Most of the common entomogenous fungi were located in Clade III, which belong in the families Clavicipitaceae and Hypocreaceae, Hypocreales. Their morphological characters are also different from Taifanglania. These entomogenous fungi all produce distinct conidiophores, complex conidiogenous structures and are mesophilic.

Based on ITS sequences analyses (Fig. 3), the phylogeny tree also exhibited as alike phylogenetic tree as SSU. The molecular results based on the nucleotidic sequences of the SSU and ITS rDNA region support the proposal of T. hechuanensis as a new species and Taifanglania as a new genus.

References


