Hirsutella vermicola sp. nov., a new species parasitizing bacteria-feeding nematodes

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The phylogeny of nematode-endoparasitic Hirsutella species was studied based on morphology and DNA sequence analysis. Hirsutella vermicola is proposed as a new species that preys on bacteria-feeding nematodes and nonpathogenic or weakly pathogenic to plant-parasitic nematodes. It is distinguished from H. rhossiliensis by having shorter conidiogenous cells with a swollen base, helical necks and slightly wider conidia. Phylogenetic trees based on sequences of the ITS region, MAPK gene fragment, and the combined data revealed two clusters corresponding to these two species and thus supported the establishment of this new species.

Key words: biological control, Hirsutella rhossiliensis, Hirsutella vermicola, nematophagous, taxonomy

Introduction

There has been a surge in interest in nematode trapping fungi in recent years because their potential use in biological control. This has resulted in descriptions of several new species (Liu et al., 2005; Mo et al., 2005) and studies on nematicidal effects and virulence factors (Dong et al., 2004; Zhao et al., 2005). Hirsutella rhossiliensis Minter & Brady (=Hirsutella heteroderae Sturhan & Schneider) is endoparasite on vermiciform nematodes (Sturhan and Schneider, 1980; Jaffee and Zehr, 1982). It parasitizes 80% of Mesocriconema xenoplax in California peach orchard soils (Jaffee et al., 1988) and 90% of Heterodera schachtii second-stage juveniles (J2s) in oil-radish fields in Germany (Müller, 1982). The fungus is widely distributed and has been isolated from Heterodera humuli (Sturhan and Schneider, 1980), H. schachtii (Müller, 1984), H. avenae (Stirling and Kerry, 1983), H. glycines (Chen, 1982).

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1997), *Meloidogyne javanica* (Cayrol et al., 1986), *M. xenoplax* (Jaffee and Zehr, 1982), *Rotylenchus robustus* (Jaffee et al., 1991), *Xiphinema diversicacaudatum* (Ciancio et al., 1986), *Hoplolaimus galeatus*, bacterial-feeding nematodes, soil mites and soil in different areas of the world (Tedford et al., 1994; Ma et al., 2005). *Hirsutella rhossiliensis* has been extensively studied and has shown potential as a biological control agent (Jaffee and Zehr, 1982).

Although the fungus is generally isolated from only one species of nematodes in the field, at a time, at any site (Sturhan and Schneider, 1980; Jaffee and Zehr, 1985; Jaffee et al., 1991; Timper and Brodie, 1993; Velvis and Kamp, 1995; Liu and Chen, 2000), Tedford et al. (1994) noted that variability among 25 isolates of the species occurred in morphology and their pathogenicity against *Heterodera schachtii*, *Meloidogyne javanica* and *Steinernema glaseri* on agar and in soil. Isolates from bacteria-feeding nematodes collected from soybean fields in Minnesota, USA, did not infect soybean-cyst nematode J2s within three days on agar plates (Liu and Chen, 2001). A recent study also showed that isolates from bacteria-feeding nematodes grew slower than those from plant-parasitic nematodes and were not or weakly parasitic to plant-parasitic nematodes (Xiang et al., unpublished). Preliminary observations indicated that the morphology of isolates from bacteria-feeding nematodes was different from plant-parasitic nematodes. A detailed study based on morphology and molecular data revealed that the isolates from bacteria-feeding nematodes represent a new species, described herein.

**Materials and methods**

**Morphological features**

Seven isolates formerly identified as *Hirsutella rhossiliensis*, including three from bacteria-feeding nematodes and four from different plant-parasitic nematodes, and one isolate of *H. minnesotensis* Chen, Liu & Chen (Chen et al., 2000) from *Heterodera glycines* were used in this study (Table 1). All fungi were maintained on potato dextrose agar (PDA; Oxoid Ltd., Basingstoke, Hampshire, England) slants at 4°C and cultured on PDA plates at room temperature. For morphological measurements of conidia and phialides, a PDA agar block (ca. 4 × 4 mm² and 4 mm thick) inoculated with fungus was placed on a sterile slide with a coverslip (Coetzee and Eicker, 1990). The slide culture was placed into a 90 mm Petri dish. After 7-10 days of incubation at room temperature, all of microscopic characteristics were measured from 30
Table 1. Details of of *Hirsutella* isolates used in this study.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Taxon</th>
<th>Hosts</th>
<th>Isolated from</th>
<th>GeneBank No. (ITS/MAPK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS 3.7878</td>
<td><em>H. vermicola</em></td>
<td>Bacteria-feeding nematode</td>
<td>Watonwan, MN, USA</td>
<td>DQ345592 DQ452362</td>
</tr>
<tr>
<td>AS 3.7877</td>
<td><em>H. vermicola</em></td>
<td>Bacteria-feeding nematode</td>
<td>Jackson, MN, USA</td>
<td>DQ345589 DQ452358</td>
</tr>
<tr>
<td>AS 3.7879</td>
<td><em>H. vermicola</em></td>
<td>Bacteria-feeding nematode</td>
<td>Faribault, MN, USA</td>
<td>DQ345581 DQ452349</td>
</tr>
<tr>
<td>ARSEF 2006</td>
<td><em>H. rhossiliensis</em></td>
<td><em>Criconemella xenoplax</em></td>
<td>Erie, PA, USA</td>
<td>DQ345566 DQ452333</td>
</tr>
<tr>
<td>CBS 113535</td>
<td><em>H. rhossiliensis</em></td>
<td><em>Heterodera glycines</em></td>
<td>Beian, Heilongjiang, China</td>
<td>DQ345584 DQ452352</td>
</tr>
<tr>
<td>ARSEF 3755</td>
<td><em>H. rhossiliensis</em></td>
<td><em>Rotylenchus robusta</em></td>
<td>San Mateo, CA, USA</td>
<td>DQ345575 DQ452342</td>
</tr>
<tr>
<td>ARSEF 2894</td>
<td><em>H. rhossiliensis</em></td>
<td><em>Heterodera humuli</em></td>
<td>Hallertau, Germany</td>
<td>DQ345573 DQ452340</td>
</tr>
<tr>
<td>AS 3.7880</td>
<td><em>H. minnesotensis</em></td>
<td><em>Heterodera glycines</em></td>
<td>Redwood, MN, USA</td>
<td>DQ345591 DQ452361</td>
</tr>
</tbody>
</table>

individuals in water mounts at 1000× magnification. Observations, measurements, and photographs were taken with Nikon 80i microscope with differential interference contrast (DIC).

**DNA extraction, amplification, and sequencing**

Axenic mycelia (0.05-0.1 g) of all tested fungi were harvested from PDA plates and transferred into 1.5 ml Eppendorf tubes for genomic DNA extraction (Wu *et al*., 2001). The ribosomal RNA gene ITS region was amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*., 1990), and mitogen-activated protein kinase (MAPK) gene fragment amplified using the primers P1835 (5′-GAGGAGAATGCGCCGTTACATGAC-3′) and P2370 (5′-CTCGTCAGATGCATCGTGCCA-3′). P1835 and P2370 were designed according to the highly conserved amino acid sequences at C-terminal of MAPK gene. PCR amplification was conducted as follows: 3 min at 95°C and 35 cycles of 95°C for 40 s, 53°C for 40 s (56°C for MAPK gene), 72°C for 60 s, followed by elongation at 72°C for 10 min. PCR products were purified using 3S PCR Products Purification Kit (Shenergy Biocolor BioScience & Technology Company, Shanghai, China) and sequenced.


**Phylogenetic analysis**

Nucleotide sequences were aligned using Clustal X 1.81 (Thompson et al., 1997) and were manually realigned using BioEdit version 5.0.6 (Tom Hall, Department of Microbiology, North Carolina State University, Raleigh, NC 27695). Phylogenetic analysis was performed using PAUP* 4.0 beta 10 (Swofford, 2001) with gaps treated as missing data and all characters equally weighted. The robustness of branches was assessed by bootstrap analysis with 1000 replicates. *Hirsutella minnesotensis*, another nematode-endoparasitic, was selected as outgroup.

**Results**

**Taxonomy**

The isolates from bacteria-feeding nematodes were formerly identified as *Hirsutella rhossiliensis* based on colony and conidial morphologies. A detail examination however, revealed that the morphological and molecular characteristics of those isolates were different from those of isolates infecting plant-parasitic nematodes. Strains AS3.7877 and AS3.7878 of bacteria-feeding nematodes were selected for morphological study and compared with *H. rhossiliensis* isolates ARSEF2006 from *M. xenoplax* in California and CBS113353 from *Heterodera glycines* in Beian County, Heilongjiang, China. The features of AS3.7877 and AS3.7878 are not identical to any known species of *Hirsutella* and related genera. For reasons elaborated below, a new species of *Hirsutella* is proposed.

**Hirsutella vermicola** M.C. Xiang & X.Z. Liu, *sp. nov.*  (Fig. 1)

*MycoBank number:* MB500924.

*Etymology:* Species epithet in reference to the eelworm host.

*Coloniae* in PDA agaro lente crescentes, albae, 12-17 mm diam post 21 dies. *Mycelium* superficiale, hyalinum, septatum, laeve. *Conidiophoris* ad cellulas conidiogenas sessilibus, reductis, singulatim producentis ex hyphis vegetatavis. *Cellulis conidiigenis* monophilalidicis, rarius polyphilalidicis, 14-20-26 μm longis, basi parte inflatis, 7-10.5-15 × 3-4.5-5 μm, apice 1-2 μm latis, attenuatis. *Conidiis* aseptatis, levibus, singulatim vel 2-3 aggregate ad colli apicem facientibus, plus minusve ellipsoideis, 6-6.5-8 μm longis, 3-4-5 μm latis, in muco involutis.

*Holytypus:* HMAS B4501 (cultura exsiccata) isolatus ex nematodo bacteriivoro, X.Z. Liu, in Herbario Mycologico Academiae Sinicae (HMAS) in Beijing conservatus.

*Colonies* on PDA growing much slowly, attaining a diam. of 12-17 mm within 3 weeks. *Mycelium* moderate, superficial, hyaline, septate, smooth. *Conidiogenous cells* arising singly, or occasional in pairs oppositely, more or
Fig. 1. *Hirsutella vermicola*. **a.** Colony on PDA for 3 wks. **b-e.** Conidia and one or two in a mucous sheath. **f-j.** Monophialides of conidiogenous cells with or without conidia. **k-l.** Twisted neck of conidiogenous cell apex. Bars are the same in b-e, f-j, k and l respectively and represent 10 µm.
**Fig. 2. Hirsutella rhossiliensis.**  
**a.** Colony on PDA for 3 wks.  
**b-d.** Conidia with 0-1 septum, one or two in a mucous sheath.  
**e-f.** Germinating conidia.  
**g-i.** Monophialides or polyphialides of conidiogenous cells with conidia.  
**j-m.** Magnification of conidiogenous cells. Bars are the same in c-d, b and e-f, g-i, and j-m respectively and represent 10 µm.
Fungal Diversity

less right angles from vegetative hyphae, monophialidic or polyphialidic, hyaline, smooth, 14-20 (average)-26 µm long, significantly swollen to 3-4.5-5 µm wide towards the base, tapering to 1-2 µm wide and 5-9.5-15 µm long neck that twists in a helix towards the apex. Conidia hyaline, aseptate, smooth, more or less ellipsoid (often the shape of an orange segment), arising from the apex of the neck singly or in a group of 2-3, 6-6.5-8 µm long, 3-4-5 µm wide, enveloped in a hyaline mucous sheath.

Habitat: Parasitizing bacteria-feeding nematodes.

Known distribution: Minnesota, United States

Material examined: Strains AS3.7877 and AS3.7878 isolated from bacteria-feeding nematodes by X.Z. Liu in USA, Minnesota, Jackson and Watonwan Counties in 1998. The living cultures were deposited in China General Microbiological Culture Collection Center.

Notes: Due to the similarity between H. vermicola and H. rhossiliensis in morphology, a comparative description of H. rhossiliensis is provided as follows.

Hirsutella rhossiliensis Minter & Brady

Colonies on PDA growing slowly, attaining a diam. of 16-24 mm within 3 weeks. Mycelium abundant, superficial, hyaline, septate, smooth. Conidiogenous cells arising singly, more or less right angles from vegetative hyphae, monophialidic, occasional polyphialidic, hyaline, smooth, 22-32.5-42 µm long, 2-4-5 µm wide at the base, tapering to a straight neck, 1 µm wide at the apex. Conidia hyaline, aseptate or occasionally with 1 septum, smooth, more or less ellipsoid (often the shape of an orange segment), arising from the apex of the neck singly or 2 in a group, 6-7-10 µm long, 3-4.5-6 µm wide, enveloped in a hyaline or pigmented mucous sheath.


DNA sequence analysis

A neighbor-joining tree was constructed based on sequences from the ITS region, MAPK gene fragment and the combined data of seven isolates of H. rhossiliensis and H. vermicola with H. minnesotensis as the outgroup. The resultant phylogenetic tree was mainly divided by two clades with 100% bootstrap values, one of which encompassed the isolates from plant-parasitic nematodes and the other included isolates from bacteria-feeding nematodes (Fig. 3). The molecular data were consistent with the morphological characteristics and supported the establishment a new species.
Fig. 3. Dendrogram constructed from neighbor-joining analyses based on sequences of rDNA ITS region and MAPK gene fragment and their combination of Hirsutella rhossiliensis, H. vermcola and H. minnesotensis. Bootstrap values are placed on the tree nodes.

Discussion

The new species is distinguished from Hirsutella rhossiliensis by the strongly swollen base and a conspicuous helical twist at the apex of conidiogenous cell. Hirsutella nodulosa and H. brownorum are the only species of Hirsutella with conidiogenous cells with a twisted neck (Minter and Brady, 1980). The new species resembles these two species in the helical neck and size of conidiogenous cell, but its smooth conidiogenous cells differ from H. nodulosa (with tiny warts) and the polyphialidic conidiogenous cell from H. brownorum (versus only monophialidic in H. vermcola) (Minter and Brady, 1980). Both H. nodulosa and H. brownorum are parasites of insects, while the new species is a parasite of bacteria-feeding nematodes.

Hirsutella rhossiliensis was described by Minter and Brady (1980) based on the fungus isolated from soil in Glamorgan, Wales in 1953. In the same year, H. heteroderae was described by Sturhan and Schneider (1980) based on a fungus isolated from H. humuli juveniles in Hallertau, Germany in 1976. Due to the morphological similarity between those two species, H. heteroderae was considered to be a synonym of H. rhossiliensis (Jaffee and Zehr, 1985). Hirsutella rhossiliensis has been isolated from various nematodes, soil mites, springtails and soils from a wide geographic range (Tedford et al., 1994; Liu and Chen, 2000). Although Tedford et al. (1994) found that the isolates from Hoplolaimus galeatus and Rotylenchus robustus grew slower in culture, produced larger conidia and were weakly parasitic to Heterodera schachtii, Meloidogyne javanica and Steinernema glaseri on agar than the other nematodes, there were no significant divergence of sequences of ITS and MAPK gene among isolates from various hosts except bacteria-feeding.
nematodes (Xiang et al., unpublished). The isolates of the new species did not
or only weakly infected these six nematodes, e.g. Heterodera glycines, H.
avenae, Meloidogyne hapla, Bursaphelenchus xylophilus (Steiner & Buhrer)
Nickle, Heterorhabditis bacteriophora Poinar and Steinernema sp.), which
further supports the establishment of the new species.

Hirsutella rhossiliensis has a broad host range and wide distribution
(Sturhan and Schneider, 1980; Jaffee and Zehr, 1985; Timper and Brodie,
1993; Velvis and Kamp, 1995; Liu and Chen, 2000), probably is an obligate
parasite in nature (Jaffee et al., 1991), can parasitize a high percentage of
nematode population and may be responsible for nematode natural suppression
in certain locations (Müller, 1982; Jaffee and Zehr, 1985; Jaffee et al., 1991;
Chen, 1997; Ma et al., 2005). The isolate OWVT-1 was highly effective in
suppressing soybean cyst nematode population density in the soil under
greenhouse conditions, and reduced the nematode egg density by 95% and J2
population density by 98% when compared with the control (Liu and Chen,
2001). These characteristics make the fungus an attractive candidate in
nematode biological control. The investigation on the differentiation of the
fungus may result in discovery of effective biological control agents against
plant-parasitic nematodes.

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