Morphological characteristics and infection processes of nematophagous *Harposporium* with reference to two new species

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Harposporium sinense and *H. multiformis* sp. nov., isolated from soil in Yunnan, China are described and illustrated as endoparasitic parasites of nematodes. The conidia of both species can cause infection of bacterial-feeding nematodes after being ingested. *Harposporium sinense* produces chlamydospores and typical crescent-shaped, *Harposporium*-like conidia with a mucus sheath at the basal end. *Harposporium multiformis* produces four types of spores in pure culture: accessory conidia, arthroconidia, chlamydospores and helicoid infection conidia. The arthroconidia germinate in a peculiar manner to produce *Harposporium* type phialides and helicoid conidia. The infection processes of both species are described and a key to the known species of *Harposporium* is provided.

Key words: accessory conidia, arthroconidia, *Harposporium*, nematode endoparasitic fungi.

Introduction

Nematophagous fungi have been the subject of research over several decades including fundamental studies of their ecology, distribution and systematics, and their potential as biological control agents of nematode pathogens of plants and animals (Li *et al*., 2000; Liu and Zhang, 2003; Dong *et al*., 2004; Mo *et al*., 2005; Zhao *et al*., 2005; Li *et al*., 2005; Xiang *et al*., 2006). Nematophagous fungi comprising a diverse range of species which are able to infect nematodes and can usually be divided into four categories: endoparasitic fungi, nematode-trapping fungi (NTF), fungi which parasitize eggs and sedentary females, and toxin-producing fungi (Barron and Thorn, 1987; Dackman *et al*., 1992).

Harposporium was erected by Lohde (1874) based on the type *Harposporium anguillulae*. The endoparasite produces sickle-shaped conidia

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on slender sterigmata arising from globose conidiogenous cells borne laterally on filamentous hyphae extended from the body of the nematode. Lohde (1874) only provided a very brief species description and a more precise definition for the species was presented by Zopf (1888). So far six pathogens of rotifers and twenty-three endoparasites of nematodes have been reported in *Harposporium*. Among them, only *H. subuliforme* infects nematodes by means of conidia that adhere to the host cuticle (Drechsler, 1950; Glockling, 1998). The rest species parasitize rotifers or nematodes through ingestion of conidia. Conidia are swallowed by hosts during the course of feeding, subsequently germinate and colonize within infected hosts' bodies. So these taxa are capable of infecting bacterial-feeding nematodes (e.g. *Panagrellus redivivus*) rather than the plant-parasitic (stylet-bearing) nematodes.

Only twenty species of *Haprosporium* are reported to have been cultured artificially and can produce different types of conidia on artificial media. Some species in culture also produce accessory conidia, arthroconidia and chlamydospores in addition to curved infection conidia (Barron, 1972; Glockling and Shimazu, 1997; Glockling, 1998). Seven species have been reported to produce chlamydospores within the host bodies. Among them, *H. cycloides* (Drechsler, 1968a; Glockling and Shimazu, 1997) and *H. thaumasium* (Li *et al*., 2005) also produce chlamydospores in pure culture. Nine species have been reported to produce arthroconidia, but only *H. arthrosporum* (Barron, 1979), *H. bysmatosporum* (Drechsler, 1946, 1954) and *H. diceraeum* (Drechsler, 1941; Aoki and Saikawa, 1992) produce arthroconidia from the nematode cadavers. Ten species have also been reported to produce accessory conidia in culture, only *H. microsporum* (Glockling and Dick, 1994) can produce accessory conidia from submerged nematode cadavers. In addition, there are six species which produce a drop of mucoid secretion at the basal end of curved infection conidia (Fig. 1).

During a survey of nematophagous fungi in Yunnan Province, China, two interesting endoparasitic fungi of nematodes were isolated from soil. After morphological comparison with known species, we confirm they are new taxa of the genus *Harposporium*.

Materials and methods

Isolation of the endoparasitic fungi

The nematode endoparasitic fungi were isolated by the technique of centrifugal flotation (Jenkins, 1964). Briefly, about 150-200 g of soil was immersed in water at room temperature for 24 hours and then mixed well in the centrifuge tube and centrifuged for three minutes at 3000 rpm. The supernatant was discarded and the residue was blended with 35% sucrose solution and centrifuged for another three minutes at 3000 rpm. The clear supernatant was passed through a set of sieves composed of 150 µm, 48 µm, and 25 µm. The sieves were rinsed with water and the nematodes on 25 µm sieves were collected in plates. The plates were examined under an inverted microscope. Nematodes with adhering fungal spores or those filled with fungal hyphae were picked out using a hair and placed into a drop of fresh water on a slide. Under an inverted microscope, the nematodes were disinfected for ten seconds in 0.1% sodium hypochlorite solution, rinsed twice in sterile distilled water, and then inoculated on the plates of potato dextrose agar (PDA) amended with 100 µg/l streptomycin sulfate and 50 µg/l benzylpenicillin. The plates were incubated at 26°C for 10-30 days and the pure cultures of fungi were obtained by transferring the mycelia from colonies margin into new PDA plates.

Infection of nematodes

In infection assays, 1 ml (about 500-1000 individuals) of heavy suspension of nematodes (*P. redivivus*) was respectively added to 40-day-old pure culture of *H. sinense* and 15-day-old pure culture of *H. multiformis* and then mixed gently with a glass rod. Several drops of the mixture were placed on a plate of 2% water agar. The nematodes were picked out randomly with a metal filament and placed into a drop of water on a slide at hourly intervals and the infection processes of both species were observed under microscope. Microscopic features were measured using an Olympus BX51 microscope.

Results

Taxonomy

Harposporium sinense C.Y. Wang & K.Q. Zhang, **sp. nov.** (Figs. 2-10)
MycoBank: 510813.

Etymology: The species epithet refers to the country in which the fungus was collected.

Coloniae in PDA ad 0.7 cm diam. post 15 dies 26ºC. *Hyphae* assumentes incoloratae, septatae, mediocriter ramosae, intra vermiculos nematoideos evolutae, plerumque 1.5-3 µm in diametro. *Hyphae* fertiles extra animal moribundum vel emortuum evolutae, erecte vel suberecte, incoloratae, septatae, vulgo simplices, plerumque 84-120 × 1.4-2 µm. *Conidiogenis* globosis, 2-4 µm in diametro, vel ampulliformibus, 9-19 × 1.8-3 µm, ex 1-3 collis brevis praeditis, 1-3 × 0.5-1 µm. *Conidia* hyalina, non-septata, arcuata, 1-2 µm in diametro, 9-29 µm longa, acuta in apice in maturitate, basi rotundata et guttula muci vestitis, 1-5 conidia deinceps in capitulum gerentibus. In cultura, chlamydosporae globosae vel cylindricae, 8-30 µm longe, 4-10 µm late.

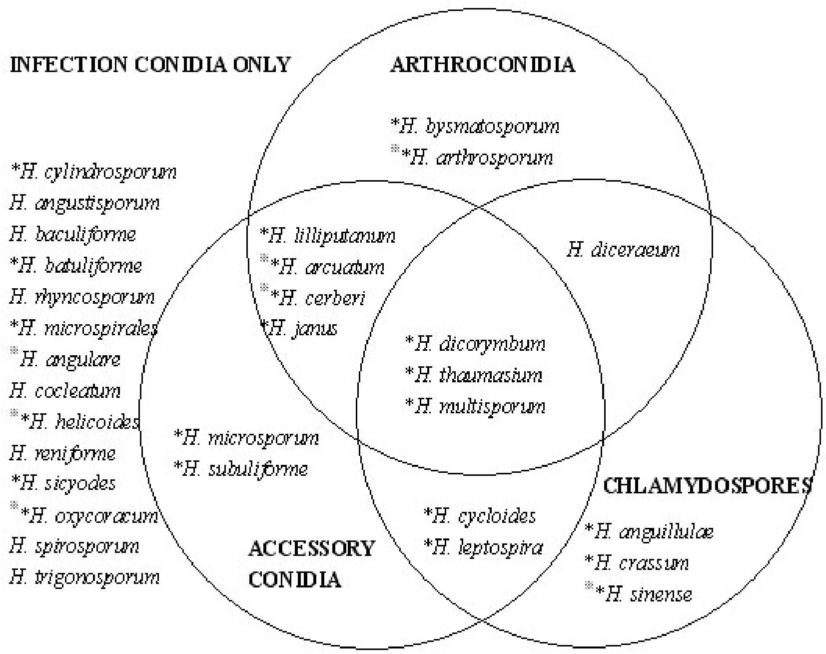
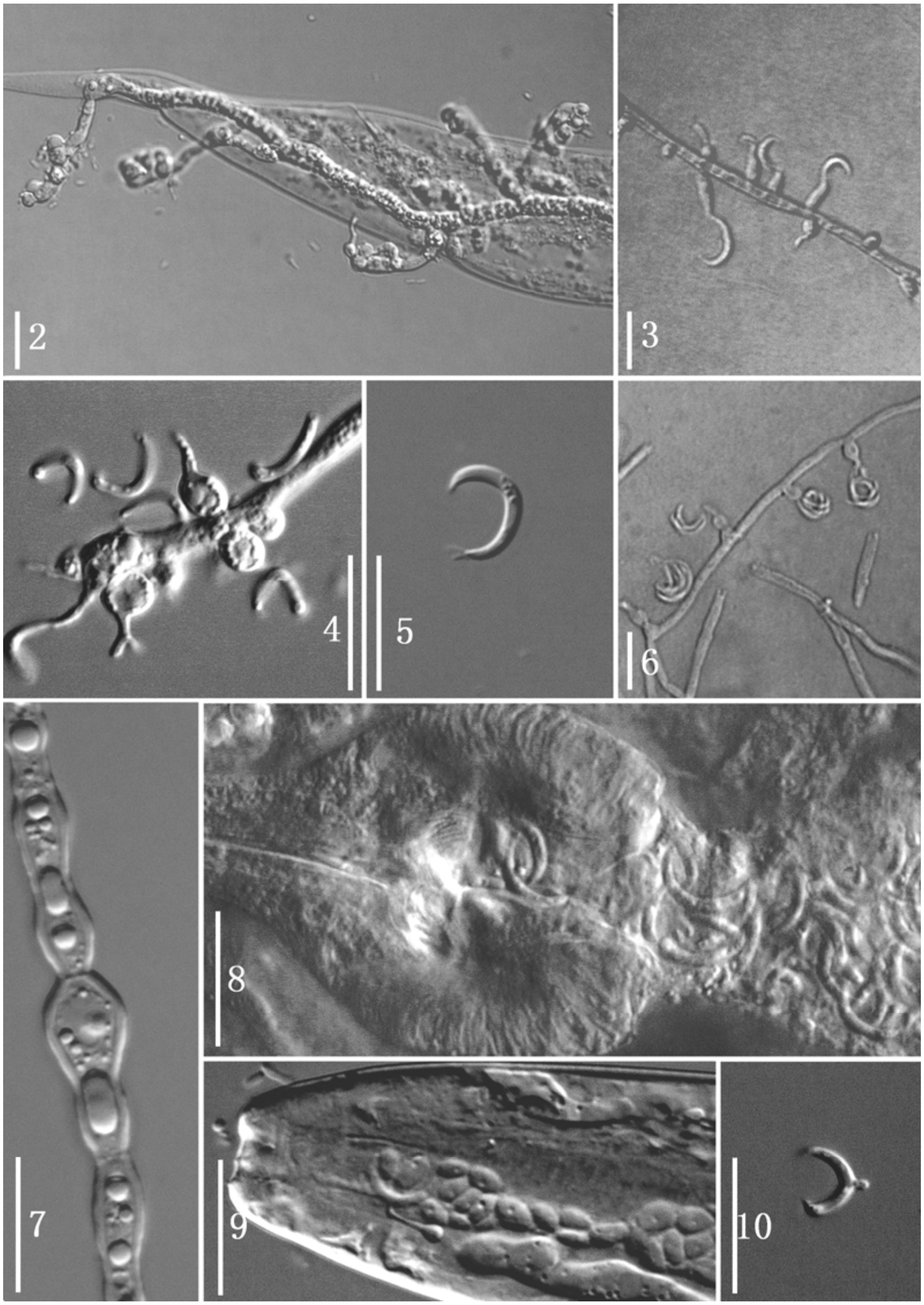


Fig. 1. Diagrammatic summary of relationships between *Harposporium* species (* species cultured; ※ species with mucoid secretion at the basal end of infection conidia; the diagram was established based on Glockling, 1998).

Colonies growing slowly on PDA medium, reaching about 0.7 cm diam. in 15 days at 26ºC, white at first, gradually becoming light yellow, raised at the center, producing red-brown pigments, pale brown in reverse. *Assimilative hyphae* colorless, septate, moderately branched, developing within the nematodes, mostly 1.5-3 µm in diam. *Conidiophores* forming outside of the dead nematode bodies, erect or procumbent, colorless, septate, commonly unbranched, mostly 84-120 × 1.4-2 µm (Fig. 2). In pure culture, *conidiogenous*

Figs. 2-10. *Harposporium sinense* sp. nov. (from holotype: YMF1.01743A). **2.** Conidiophores with phialides and curved conidia developed outside of nematode cadaver. **3.** Two types of conidiogenous cells in pure culture: spherical and flask-shaped phialides. **4.** Spherical phialides and branched sterigmata. **5.** Curved conidia with a sheath of mucus at the basal end. **6.** Curved conidia produced in cluster. **7.** Chlamydospores produced in pure culture. **8.** Curved conidia lodged in the oesophageal bulb and the gut of nematode. **9.** Germinating curved conidia in the buccal cavity of nematode. **10.** A bulb bulge produced by germinating curved conidia from the convex side of the spore. Bars = 10 µm.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cells globose, 2-4 µm ($\bar{x}$ = 3 µm) in diam., or flask-shaped, 9-19 ($\bar{x}$ = 12.5 µm) × 1.8-3 µm ($\bar{x}$ = 2 µm) (Fig. 3), with 1-3 short necks, or a branched neck, 1-3 × 0.5-1 µm (Fig. 4). *Conidia* hyaline, non-septate, arcuate, 1-2 µm ($\bar{x}$ = 1.3 µm) in the greatest width, and 9-29 µm ($\bar{x}$ = 12 µm) in length along the curved axis, sharply pointed at the apex when matured, rounded at the base and surrounded with a droplet of mucus (Fig. 5), 1-5 conidia produced successively and cohering in a head on a short neck (Fig. 6). *Chlamydospores* globose to cylindrical, 8-30 µm long by 4-10 µm broad, seen in pure culture (Fig. 7).

Habitat: Parasitic on free-living nematodes.

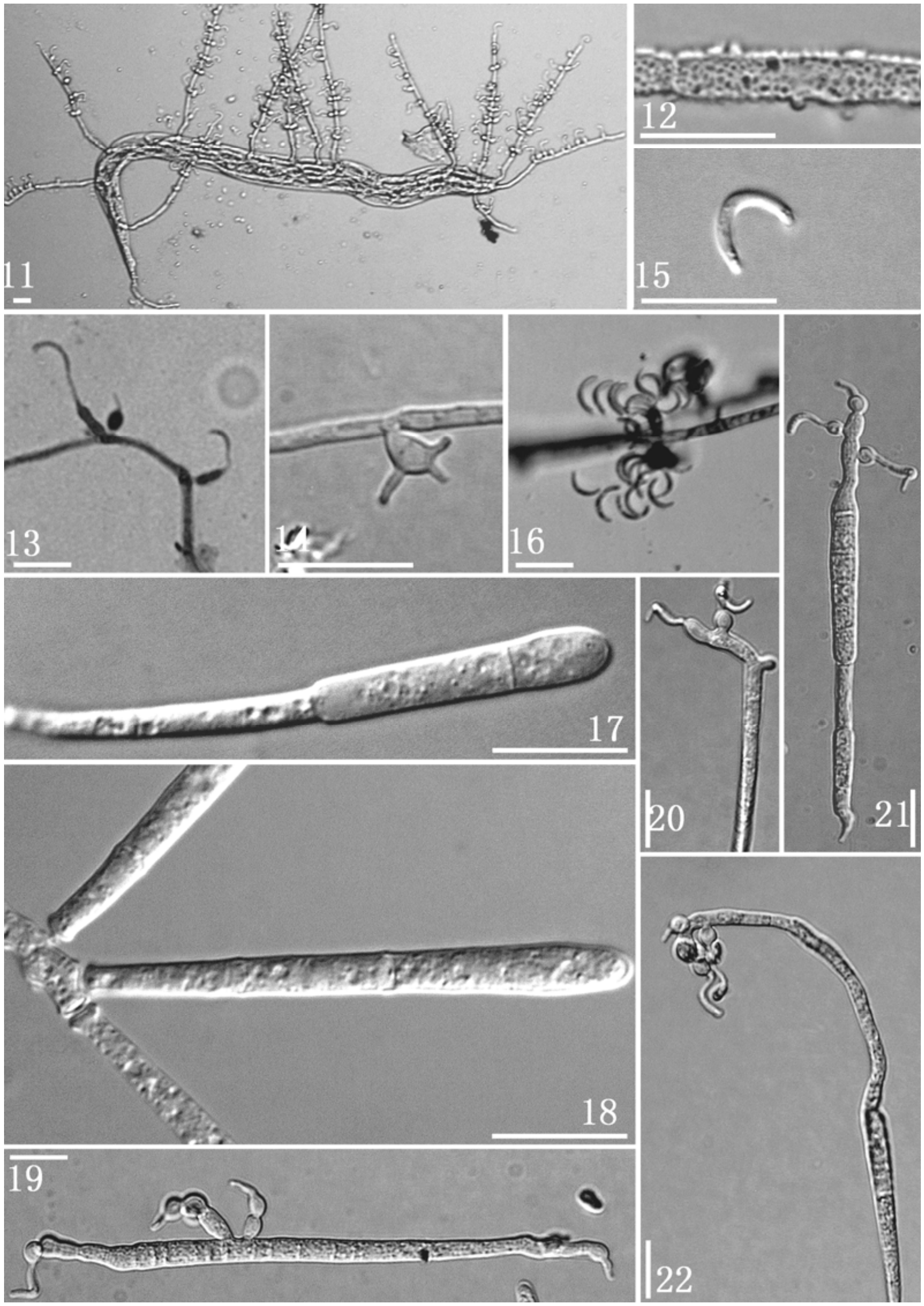
Material examined: CHINA, Yunnan Province, Kunming City, Xiaohe Forest, 10 September 2004, isolated from soil (**holotype**: YMF1.01743A); ex-type culture deposited in the Herbarium of Laboratory for Conservation and Utilization of Bio-resources, Yunnan University (YMF1.01743).

Harposporium sinense is same as the five other species of *Harposporium* which form crescent-shaped conidia, i.e. *H. anguillulae*, *H. crassum* (Shepherd, 1955), *H. janus* (Shimazu and Glockling, 1997), *H. lilliputanum* (Dixon, 1951; Wood, 1973; Glockling and Shimazu, 1997) and *H. thaumasium*. *Harposporium sinense* is characterized by two types of conidiogenous cell and the mucus sheath at the basal end of curved infection conidia. Although the five species share similar shape of conidia to *H. sinense*, they only produce globose conidiogenous cells and their conidia lack mucoid secretions at the base. In addition, *H. anguillulae*, *H. crassum*, and *H. thaumasium* all produce chlamydospores in the nematode cadavers. However, *H. sinense* only produces chlamydospores at a late stage of pure culture and no chlamydospores are observed in the nematode cadavers. *Harposporium janus*, *H. lilliputanum* and *H. thaumasium* produce arthroconidia and accessory conidia in addition to infection conidia and with exception of the latter species, never produce chlamydospores within the nematode or in pure culture.

Harposporium multiformis C.Y. Wang, K.Q. Zhang, **sp. nov.** (Figs. 11-33)
MycoBank: 510814.

Etymology: The species epithet refers to multitype of conidia produced by the fungus.

Figs. 11-22. *Harposporium multiformis* sp. nov. (from holotype: YMF1.01757A). **11.** Conidiophores with phialides and helicoid conidia developed outside of nematode cadaver. **12.** Aged assimilative hyphae produced warty protuberances. **13.** Two types of conidiogenous cells in pure culture: spherical and flask-shaped phialides. **14.** Phialides put forth three sterigmata. **15.** Helicoid conidia. **16.** Helicoid conidia produced in cluster. **17, 18.** Clavate arthroconidia. **19-22.** Germinating arthroconidia. Bars = 10 µm.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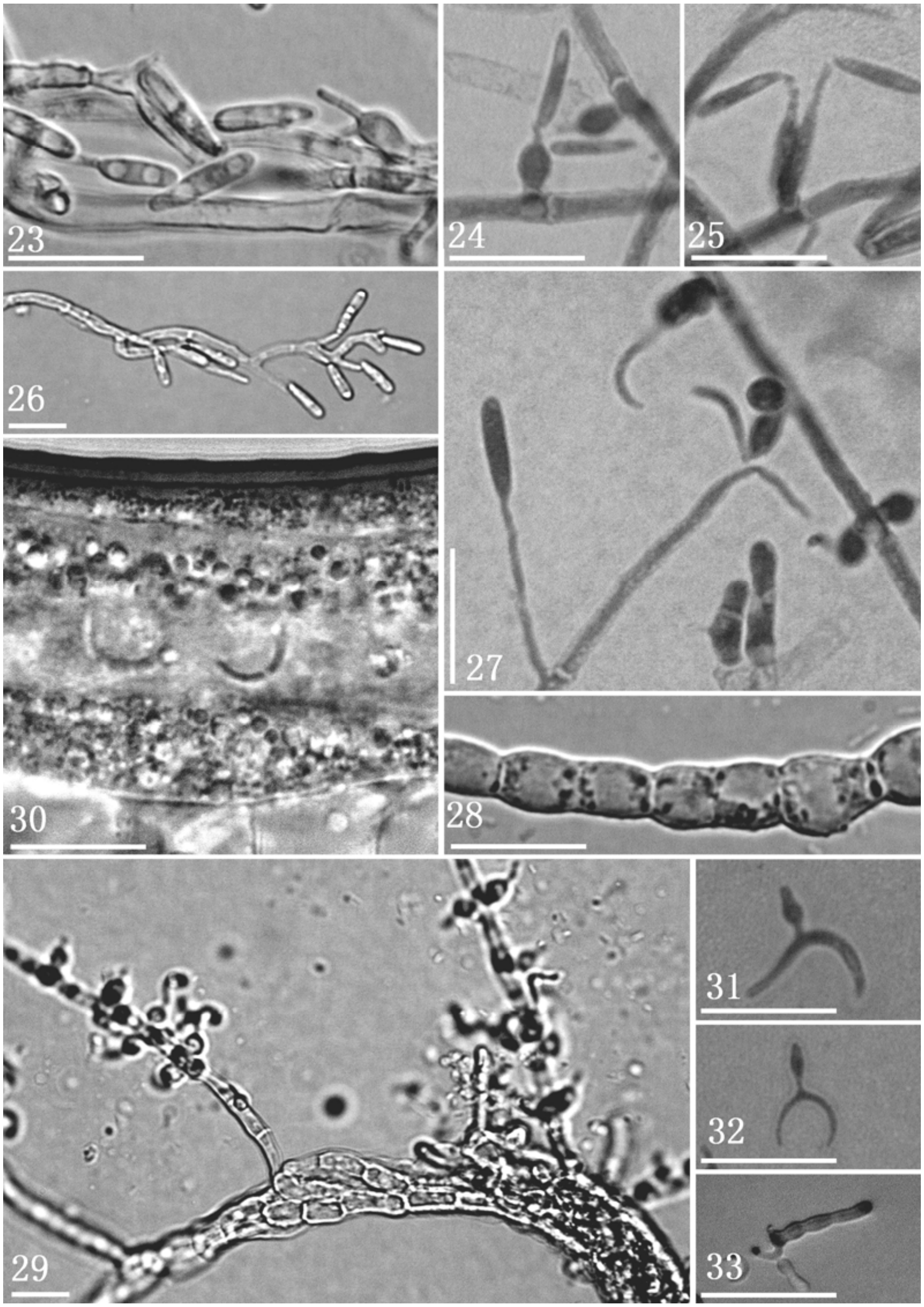
Coloniae in PDA ad 1.8 cm diam. post 15 dies 26ºC. *Hyphae* assumentes incoloratae, intra vermiculos nematoideos viventes evolutae, parce ver mediocriter ramosae, plerumque 3-5.5 µm crassae, primum saepe parce septatae sed deinde vulgo mediocriter septatae. *Conidiophora* incoloratae, suberecta vel recta, extra animal emortuum evolutae, septatae, parce ramosae, plerumque 50-275 µm longae, 1.5-4 µm latae. *Conidiogenis* globosis, 3-4.5 µm in diametro, vel ellipsoideae, 5-13 × 1.8-3.5 µm, ex collo brevi praeditae, 1.8-3.5 × 0.6-1 µm, in singulatim portatae vel in greges irregulars confertae in conidiophora. *Conidia* hyaline, falcate vel helicoidea, non-septata, 8.4-18.6 µm longa × 0.6-1.3 µm lata, plus minusve latitudine. *Constantia* non praecipue aculeate, 1-5 conidia deinceps in capitulum gerentibus. *Arthroconodia* septatae, clavatae, in ramose catenas， ex conidiophorarum apicibus exoriuntur, 25-102 × 2.5-5 µm. In cultura, accessory conidia elongato-ellipsoideae, hyaline, non-septata, 7-12.2 × 1.2-2 µm; conidiogenis globosis, ampulliformibus vel subulatae, 5-36 × 0.9-3 µm. *Chlamydosporae* globosae vel cylindricae, 5-29 × 4-8 µm, intra vermiculos nematoideos evolutae ex in cultura.

Colonies growing more rapidly on PDA medium, reaching about 1.8 cm diam. in 15 days at 26ºC, white at first, gradually becoming yellow, raised at the center, somewhat floccose in the outer regions, producing dark-brown pigments, pale brown in reverse, producing four types of spores. *Assimilative hyphae* colorless, septate, moderately branched, developing within free-living nematodes, often (especially in small host animals) rather sparing branched, moderately septate, mostly 3-5.5 µm ($\bar{x}$ = 4.4 µm) wide (Fig. 11) (in pure culture, aged assimilative hyphae always formed many warty protuberances, Fig. 12). *Conidiophores* colorless, forming outside of the dead nematode body, procumbent or erect, septate, sometimes branched, mostly 50-275 µm ($\bar{x}$ = 122.5 µm) long, 1.5-4 µm ($\bar{x}$ = 2.5 µm) wide (Fig. 11). In pure culture, *conidiogenous cells* globose, 3-4.5 µm ($\bar{x}$ = 3.5 µm) in diam., or long ellipsoid, 5-13 × 1.8-3.5 µm ($\bar{x}$ = 7.4 × 2.5 µm) (Fig. 13), with a short neck (occasionally with three necks) (Fig. 14), 1.8-3.5 × 0.6-1 µm ($\bar{x}$ = 2.24-0.71 µm), borne in single or in irregular cluster on the conidiophores. *Conidia* hyaline, non-septate, crescent-shaped or helicoid, 8.4-18.6 µm ($\bar{x}$ = 12.4 µm) long, 0.6-1.3 µm ($\bar{x}$ = 0.92 µm) broad, more or less uniform in width, not markedly pointed at the ends (Fig. 15), 1-5 conidia produced successively and cohering in a head on a short neck (Fig. 16). *Arthroconidia* septate, clavate, produced in branching chains, produced apically on the conidiophores, 25-102

Figs. 23-30. *Harposporium multiformis* sp. nov. (from holotype: YMF1.01757A). **23.** Accessory conidia. **24.** Accessory conidia produced by spherical phialides. **25.** Accessory conidia produced by flask-shaped phialides. **26, 27.** Accessory conidia produced by subulate phialides. **28.** Chlamydospores produced in pure culture. **29.** Chlamydospores formed within the nematode cadaver. **30.** Helicoid conidia lodged in the lower gut of nematode. **31-33** Germinating helicoid conidia produced 1-2 germ-tubes from the convex side of the spores. Bars = 10 µm.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× 2.5-5 µm ($\bar{x}$ = 52 × 3.6 µm) (Figs. 17-18), germinating to produce typical globose conidiogenous cells and helicoid conidia (Figs. 19-22). *Accessory conidia* elongate-ellipsoid, hyaline, non-septate, 7-12.2 × 1.2-2 µm ($\bar{x}$ = 9 × 1.6 µm) (Fig. 23); phialides globose, flask-shaped or subulate (Figs. 24-27), 5-36 × 0.9-3 µm ($\bar{x}$ = 19.8 × 1.85 µm). *Chlamydospores* globose to cylindrical, 5-29 × 4-8 µm ($\bar{x}$ = 13 × 5.6 µm), produced within nematodes and in pure culture (Figs. 28-29).

Habitat: Parasitic on free-living nematodes.

Material examined: CHINA, Yunnan Province, Wenshan County, Maguan Forest, 5 April 2004, isolated from soil samples (**holotype**: YMF1.01757A) ex-type culture deposited in the Herbarium of Laboratory for Conservation and Utilization of Bio-resources, Yunnan University (YMF1.01757).

Harposporium multiformis can be distinguished from *H. cerberi* (Hodge *et al*., 1997), *H. helicoides* (Drechsler, 1941; Barron, 1970) and *H. leptospira* (Drechsler, 1968b; = *H. drechsleri* Barron, 1972) which produce helicoid conidia. *Harposporium multiformis* produces four types of spores in pure culture and two types within the nematode cadavers. The infection conidia are slender heliciod, blunt at both ends and without mucus secretions at the base, (8.4-18.6 × 0.6-1.3 µm). However, *H. cerberi* and *H. helicoides* produce mucus at the base of infection conidia and never produce chlamydospores within the host cadavers or in pure culture. *Harposporium multiformis* resembles *H. leptospira* (8-12 × 0.5-0.8 µm) greatly in conidial shape and size, but *H. leptospira* does not produce arthroconidia. Moreover, the accessory conidia of *H. leptospira* are two-celled, clavate, 12-15 × 3-4 µm and those of *H. multiformis* are elong-ellipsoid, non-septate, straight or slightly curved, 7-12.2 × 1.2-2 µm. *Harposporium multiformis* also shows difference from *H. leptospira* in the infection process to nematodes. The conidia of *H. leptospira* lodge in the oesophageal lumen of nematodes following ingestion rather than in the lower gut in the same way with *H. multiformis*.

Key to the species of *Harposporium*

1. Parasitic in rotifers 2
1. Parasitic in nematodes.................................... 7

2. Conidia angular or helicoid, sharply pointed at one or both ends 3
2. Conidia cylindrical or triangular with rounded ends.................................... 5

3. Conidia bent at right angles, with a drop of mucus, tapering gradually to an acutely pointed slightly curved apex, 22-28 × 2.2 µm .... ***H. angulare***
3. Conidia without mucus, helicoid 4

4. Conidia filamentous, convolved in a helical turn of right-handed rotation, 25-33 × 1.3-1.7 µm.. ***H. cocleatum***
4. Conidia spiral, with sharply pointed ends, 6-8 × 1.5-2 µm.......................... ***H. spirosporum***

5. Conidia triangular, 6.3-7.2 × 7.2-9 µm ....***H. trigonosporum***
5. Conidia cylindrical, straight or slightly curved...6

6. Conidia narrowly cylindrical, produced in irregular chains, 9-11 × 1.7-2.5 µm.................... ....***H. botuliforme***
6. Conidia long-cylindric, 22-27 × 1.7-2.5 µm .... ***H. cylindrosporum***

7. Infecting nematodes by awl-shaped conidia externally adhering to the host cuticle, 12-26 × 1-1.8 µm; accessory conidia broad cylindrical with rounded ends, 6.5-10 × 2.5-3.5 µm .... ***H. subuliforme***
7. Infecting nematodes by ingested conidia 8

8. Conidia irregular 9
8. Conidia cylindrical (straight or slightly curved), helicoid or arcuate.................................13

9. Only producing infection conidia 10
9. Producing arthroconidia...11

10. Conidia reniform, umbilicate, 3.2-4 µm ....***H. reniforme***
10. Conidia asymmetrically heart-shaped with a spiny outgrowth to one side of their convex surface, 4-5 × 4-5 µm... ***H. rhynchosporum***

11. Conidia like human upper-arm-bone, 4.5-6.5 × 0.8-2.1 µm; arthroconidia cylindrical with rounded ends, 10-20 × 2.3-2.7 µm; not producing chlamydospores........ ***H. bysmatosporum***
11. Producing chlamydospores 12

12. Conidia broadly pod-shaped with minute beaks at both ends, like "tiny high-heeled shoes with upturned pointed toes", 3.4-4 × 1-1.5 µm; arthroconidia cylindrical, 9.8-20.8 × 1.9-3.1 µm...***H. diceraeum***
12. Conidia consisted of a globose proximal part (4-7 µm) and of a columnar outgrowth (3-9 × 2-3 µm) arising from it in an equatorial or somewhat more proximal position; arthroconidia 13-19 × 3 µm; accessory conidia subglobose or obovoid, 3-4 × 2-2.5 µm....... .... ***H. dicorymbum***

13. Conidia cylindrical, straight or slightly curved...14
13. Conidia crescent or helicoids..16

14. Conidiophores very short, club-shaped, non-septate, 4-15 × 0.6-1 µm; conidia cylindrical or tapering slightly toward the apex, with rounded ends, 2.5-5 × 0.7-1.5 µm .... ***H. baculiforme***
14. Conidiophores longer, septate...15

15. Conidiophores developing three short internal basal cells, branched; conidia slender, club-shaped, straight or slightly twisted, 6-9 × 0.3-0.9 µm.............................. ***H. angustisporum***

15. Conidia cylindrical, tapering slightly toward both broadly rounded ends, straight or slightly curved, resembling cucumbers in shape, 3-5 × 0.9-1.2 µm..................... ***H. sicyodes***

16. Conidia helicoid...17
16. Conidia arcuate, crescent or curved 22

17. Conidia with mucus at the base..18
17. Conidia without mucus at the base 19

18. Phialides producing 1-3 sterigmata; conidia filiform, helicoid, 20-45 × 0.7-1.5 µm.............. ....***H. helicoides***
18. Conidia 1-1.5 turns of a left-handed helix, beaked at apices, 12.4-20.5 × 1.2-2 µm; arthroconidia 11.1-23.7 × 3.2-4.7 µm; accessory conidia ellipsoid, 5.6-8.7 × 1.8-3.1 µm .... ***H. cerberi***

19. Conidia spiral, 7-14 × 0.6-0.9 µm; no chlamydospores................................ ***H. microspirale***
19. Producing chlamydospores and accessory conidia 20

20. Conidia helicoid with rounded ends, 8-12 × 0.5-0.8 µm; accessory conidia uniseptate, clavate, 12-15 × 3-4 µm; chlamydospores globose or cylindrical, 4.5-25 × 4-7 µm ....***H. leptospira***
20. Accessory conidia non-septate..21

21. Conidia convolved in a helicoid spiral of approximately one turn, left-handed rotation, 20-30 × 1.3-1.9 µm; accessory conidia clavate, 5-7 × 1.5-2 µm; chlamydospores elongate-ellipsoidal or globose or barrel-shaped, 8-19 × 6-9 µm .... ***H. cycloides***
21. Conidia helicoid with rounded ends, 8.4-18.6 × 0.6-1.3 µm; arthroconidia septate, clavate, 25-102 × 2.5-5 µm; accessory conidia elongate-ellipsoid non-septate, 7-12.2 × 1.2-2 µm; chlamydospores globose to cylindrical, 5-29 × 4-8 µm ....***H. multiformis***

22. Producing accessory conidia..23
22. No accessory conidia produced..27

23. Conidia crescent-shaped with slightly helicoidal twist, 10-16 × 2.5-3 µm; accessory conidia dome-shaped, 1.5-1.8 × 1.5 µm, only produced in non-axenic watery culture; no arthroconidia .... ***H. microsporum***
23. Producing arthroconidia..24

24. Conidia arcuate, very narrow, with sharp point at distal end and mucoid secretion at the base, 18-50 × 0.8-1 µm; arthroconida 8-15 × 2-3 µm; accessory conidia ovoid, 4-5.5 × 0.5-1.2 µm.. ***H. arcuatum***
24. Conidia without mucus at the basal end..25

25. Conidia crescent-shaped, beaked at the apex; truncated at the base, 4-8 × 0.7-1.2 µm; arthroconidia septate, cylindrical or clavate, 52-145 × 4-8.5 µm; accessory conidia globose or ellipsoid, 2-6 × 1-3 µm; chlamydospores elongate-ellipsoidal or globose, 7-16 × 6-10 µm.. ***H. thaumasium***
25. No chlamydospores produced..26

26. Conidia curved to over a semi-circle, 9-16 × 1-2 µm; arthroconidia uniseptate, 14-17 × 4 µm; accessory conidia obovoid, 3-6.5 × 1.5-2 µm..***H. janus***
26. Conidia arcuate, distally pointed, truncated at the base, 4.5-9 × 1-1.5 µm; arthroconidia 10-18×2.5-3 µm; accessory conidia lenticular, 3-6 × 1.2-2 µm ....***H. lilliputanum***

27. Producing chlamydospores 28
27. No chlamydospores produced; conidia with mucus at the base 30

28. Conidia arcuate, sharply pointed at the apex, rounded and with mucoid secretion at the base, 9-29 × 1-2 µm; chlamydospores 8-30 × 4-10 µm ....***H. sinense***
28. Conidia without mucus at the basal end.. 29

29. Conidia hook-shaped, pointed at both ends, 6-18×1-2 µm; chlamydospores 4-6.6×3.5-7 µm..***H. anguillulae***
29. Conidia arcuate, pointed at both ends, 18-22 × 2-3 µm .... ***H. crassum***

30. Conidia crescent-shaped or slightly helicoid, 12.5-35 × 0.8-3 µm; arthroconidia cylindric, 9.5-11.5 × 3-4 µm .... ***H. arthrosporum***
30. Conidia curved distally into hook-shaped, expanding into a knob at the base, a sharp solid cusp at apex, 15-25 × 0.7-1.4 µm..***H. oxycoracum***

Infection tests

Bacterial-feeding nematodes can be infected by the typical *Harposporium*-like conidia of *H. sinense* and *H. multiformis*. After being ingested, the conidia of *H. sinense* lodge and germinate in the buccal cavity, the oesophagus and the gut of nematodes (Figs. 8-9). As to the conidia of *H. multiformis*, they lodge and germinate in the lower gut of nematodes (Fig. 30). The germination of conidia of both species is similar to that of *H. auguillulae* as described by Aschner and Kohn (1958). At first, the conidia forms a bulb-shaped bulge at the convex side about at the central point, then develops a narrow germ tube approximately at right angles from the central point of the spores (Figs. 9-10, 31-33). Sometimes, the conidia of *H. multiformis* produce 2-3 robust germ-tubes from different sites (Fig. 33) as with the conidia of *H. cerberi*. The germ tubes swell gradually to produce infection hyphae and the motility of nematodes decreases rapidly. When the bodies of infected nematodes are filled with a tangled mass of hyphae, some hyphae extend out from nematodes to form conidiophores and produce typical *Harposporium*-like conidiogenous cell and crescent-shaped or helicoid infection conidia (Figs. 2, 29). Chlamydospores do not be produced within the nematode cadavers infected by *H. sinense* (Fig. 2). As far as *H. multiformis* is concerned, chlamydospores are developed within some dead hosts, but arthroconidia and accessory conidia have never be observed on the host cadavers (Figs. 11, 29) and no nematode are attached or infected by accessory conidia.

Arthroconidia and accessory conidia of *H. multiformis* can germinate to produce typical phialides and helicoid infection conidia in the absence of nematodes. Furthermore, the germination manner of arthroconidia is very peculiar. Firstly, they germinate to form short, straight or branched assimilative hyphae at one or both ends, and then produce typical phialides and helicoid infection conidia on the hyphae. Occasionally, phialides and helicoid conidia are produced directly from arthroconidia. It is not found that accessory conidia are produce by germinating arthroconidia (Figs. 19-22).

Discussion

Typical *Harposporium*-like conidia can cause infection of bacterial-feeding nematodes. After being ingested, conidia lodge and germinate in different parts of the digestive tract of nematodes. Some larger conidia, such as *H. helicoides* and *H. oxycoracum* (Drechsler, 1941; Saikawa *et al*., 1983), pass through the buccal cavity, oesophageal bulb and then lodge in the gut of nematodes. On the other hand, some smaller conidia, such as *H. lilliputanum* and *H. sicyodes* (Drechsler, 1959), are blocked in the buccal cavity of nematodes. It may be an adaptation for smaller conidia. It is difficult to anchor themselves inside the gut of nematodes, so that large numbers of them will be drained off along with the egesta of nematodes. In order to increase the probability of infection and complete the parasitic life cycle, they adjust to lodge in the buccal cavity or the oesophagus of the hosts rather than the gut. However, the reason is unclear as to why the conidia of *H. sinense* not only are ingested into the gut of nematodes through the oesophageal bulb but also are blocked in the buccal cavity and the oesophagus.

Generally, accessory conidia produced by *Harposporium* species in pure culture are aseptate, sub-globose, elongate-ovoid, clavate or cylindrical, and their conidiogenous cells are different from those of typical infection conidia and often branched to form several apices. *Harposporium leptospira* is the only species that produces two-celled accessory conidia. As to the role of accessory conidia, Hodge *et al*. (1997) suggested that they are synanamorphs of *Hirsutella* species, which contains many insect pathogens. *Harposporium janus* is the only species which has been reported to infect insect with obovoid accessory conidia. It is isolated from a beetle larva from which *Paraisaria*-like conidiogenous cells are formed on synnematous conidiophores and produce obovoid conidia. This species has two host organisms associated with two different spore types: obovoid accessory conidia infect beetle larvae and only produce this type of conidia on the host cadavers; curved infection conidia parasitize bacterial-feeding nematodes and no obovoid accessory conidia is produced from infected nematodes. Another unusual species, *H. microsporum*, produces large arcuate conidia in pure culture and develops dome-shaped microconidia only in non-axenic water culture or from the submerged infected nematodes. The microconidia can infect nematodes in submerged conditions. This kind of infection mechanism suggests that accessory conidia may be an adaptation to watery environments. In our study, it is not been observed that accessory conidia of *H. multiformis* attach or infect nematodes. So the role of accessory conidia in *Harposporium* is still uncertain.

Arthroconidia often are produced in branching fragile chains on the apices of aerial hyphae or the conidiophores formed from the nematode cadavers. They disarticulate at the septa, so that the cylindrical, non-septate individual segments can be formed with rounded ends. However, the arthroconidia of *H. multiformis* and *H. thaumasium* are clavate, septate and do not disarticulate at the septa to form individual segments. Arthroconidia can germinate and produce typical phialides or cylindrical conidiogenous tubes (which correspond to the upper portion of the typical phialides) and infection conidia. In *H. arthrosporum* and *H. janus*, the germination of arthroconidia needs the stimulation of nematodes. On the other hand, some species, such as *H. cerberi*, *H. lilliputanum*, *H. multiformis* and *H. thaumasium*, their arthroconidia can germinate in the absence of nematodes. Usually, the individual segments of arthroconidia become partitioned by a single septum prior to germination, such as *H. arthrosporum*, *H. cerberi* and *H. diceraeum*. But in *H. multiformis*, arthroconidia germinate firstly to form short assimilative hyphae at one or both ends, and then produce typical phialides and helicoid conidia on them. This kind of germination manner is very peculiar and hitherto no other species has been reported that arthroconidia germinate in this way.

Arthroconidia and chlamydospores are protective modification of hyphae and recognized as an alternative resting spore for fungi to endure through adverse times. Generally, they are not present simultaneously (Zopf, 1888; Barron, 1979). But they are all produced in pure culture of *H. multiformis* and *H. thaumasium*, and within the nematode cadavers infected by *H. diceraeum*. The reason and meaning of the coinstantaneous presence of two types of resting spores are not ascertained. On the other hand, some species, such as *H. helicoides*, *H. oxycoracum* and *H. subuliforme*, never form any types of resting spores from the host cadavers or in pure culture. The way for them to persist through long winter and other unfavorable periods is unclear.

With respect to the function of the mucus at the base of infection conidia, it is yet unknown at present. Barron (1969) conjectured that it can possibly function as an attractant, inducing nematodes to swallow, or it may be related to toxin release after ingestion. Saikawa *et al*. (1983) have ever studied the mucus of *H. oxycoracum* with electron microscope. By using ruthenium red staining en bloc, they observed a network system of fibrils in thin section spreading from a more densely aggregated mass of fibrils apparently originating from the basal part of the conidial cell wall. Without en bloc staining with ruthenium red, the network system of the fibrils was not preserved. Moreover, the fibrous mass began to disappear during the process of conidia traveling to the lower gut through the digestive canal of nematodes after being swallowed. However, they still could not discover the function of the mucus. In *H. sinense,* it seems that the sheath of mucus does not disappear during the process of being ingested. When discharged from the gut of squashed nematodes, the mucus still can be observed under light microscope.

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