Cryptogamie, Mycologie, 2008, 29 (4): 333-354

Nematode-Trapping fungi from mangrove habitats

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Abstract – Ten species belonging to, *Arthrobotrys*, *Dactylellina* and *Dreschlerella* were recorded from mangrove habitats in Hong Kong. These nematode-trapping fungi are characterised and illustrated and compared with freshwater and terrestrial stains. Conidial and conidiophore morphology of all the mangrove strains collected in present study fell within the range of their respective species, however, there were also variation in conidiophore and conidial characters.

Habitats / morphology / taxonomy / blast result

INTRODUCTION

Nematode-trapping fungi (NTF) have been relatively well-studied because of their potential in biological control, however most studies have concentrated on nematode-trapping fungi in agriculture, animal husbandry or forestry (Jaffee & Strong, 2005; Waller *et al*., 2006; Su *et al*., 2007) or freshwater environments (Hao *et al*., 2005). Currently, 43 species of predacious hyphomycetes have been recorded from aquatic habitats (Ingold, 1944; Peach, 1950; 1952; Johnson & Autery, 1961; Anastasiou, 1964; Hao *et al.*, 2004, 2005). The first report of marine predaceous fungi was of three zoopagaceous forms discovered in brackish water (Jones, 1958). Although several nematode-trapping fungi have been isolated from freshwater (Hao *et al*., 2004, 2005), *Arthrobotrys dactyloides* appears to be the only species reported from brackish water (Johnson & Autery, 1961).

Original descriptions of NTF species have mostly been based on one isolate. Variation however, has been reported within species, e.g. *A. dactyloides* (Kumar & Singh, 2006), *Monacrosporium drechsleri* (Meyer *et al.*, 2005). Whether this variation represents new forms or species is unclear as variation may also be habitat dependent. Thus there is a need to examine the finer taxonomic resolution of NTF for delimiting species.

In this study, the nematode-trapping fungi on various substrates including decaying wood, leaves and soil from terrestrial, freshwater and mangrove habitats in Hong Kong were surveyed. Given that there is very little information on diversity and morphological studies on nematode-trapping fungi from marine habitats, this paper deals with 1) descriptions of species of mangrove-inhabiting nematode-trapping fungi, 2) morphological comparisons of species with other isolates from terrestrial and freshwater habitats, 3) conidial and other character variation within species from different ecosystems. For each species, the following information is given: habitat, teleomorph, and material examined, and culture code.

MATERIAL AND METHODS

Decaying wood and leaves were collected from various sites in Hong Kong including terrestrial, freshwater and mangroves habitats. Submerged decaying wood and leaves were randomly sampled from freshwater and mangrove habitats, whereas soil was randomly collected from terrestrial habitats. The salinity of the surrounding seawater at the mangrove sites was 10-20‰ (parts per thousand). Samples were placed individually in Zip-lock plastic bags and maintained at 4ºC before examination. The isolation methods used in this study are as described by Liu and Zhang (2003). Fungi were identified to species levels using the keys of Rubner (1996) and Oorschot (1985). Cultures were deposited in the International Fungal Research & Development Centre (IFRD). Observations and photographs were made from material mounted in water. Isolates were cultured in CMA media at 26ºC. After 7 d incubation, morphological characteristics and growth rate were recorded. All microscopic characteristics were measured from 60 individuals and means were calculated. Conidia were measured at their widest point and the range between minimum, mean, and maximum values for microscopic measurements is given. Mean values are in brackets. Statistical analyses were performed in Statistical Programme for Social Sciences (SPSS 13) (SPSS Inc. Chicago, IL).To verify the type of the trapping devices; we used the method as described by Hao *et al*. (2005). The inocula were sub-cultured on CMA plates, and a portion of media (1.5 cm × 1.5 cm) was scraped out to provide a well beside the inoculum. After 7 days of incubation, *Panagrellus redivius* was added to the well and maintained at 26ºC for 2 days and the type of trapping device produced was examined.

DESCRIPTIONS

Arthrobotrys arthrobotryoides (Berl.) Lindau in Rabenhorst, *Rabenh. Krypt.-Fl.* (Leipzig) **1**(8): 371 (1905). (Fig. 1)

Sampling sites: Mangrove; Kei Ling Ha Lo Wai, Sai Kung, 22º 24' 49.32" N 114º 16' 21.47" E. Freshwater; Tai Shing Stream, Tai Po Kung, New Territories 22º 23' 34.01 N 114º 08' 3.32" E.

Culture: IFRD 2015.

Habitat: mangrove, freshwater and terrestrial.

Substrate: unidentified decaying wood.

Teleomorph: unknown.

Colonies white to light yellow from above and below media, relatively fast growing, spreading. Mycelium, hyaline, septate and branched. Conidiophores up to 100-280 µm high, 3-5 µm wide at the base, erect, rarely producing side branches, proliferating up to two times, but sometimes lacking repeated proliferation (Fig. 1C-E), bearing 5-14 conidia, the conidiophores scattered or in tussocks. Conidia broad-ellipsoid, rounded at the ends, with one central septum, 16-(19.6)-22 × 7-(8.2)-9 µm, colourless, sometimes slightly constrict at the septum (Fig. 1A). Forming adhesive network trapping devices (Fig. 1B). Chlamydospores were not observed in the mangrove strain, but present in strain F1.138 from freshwater.

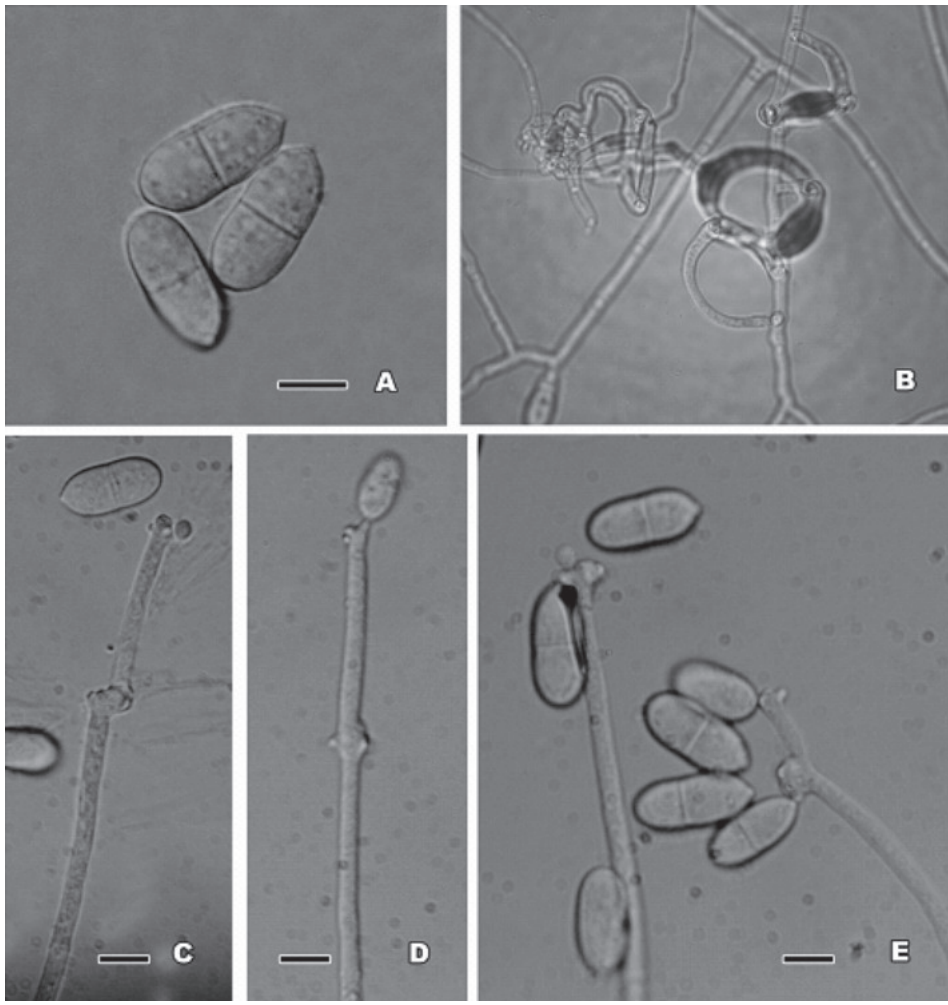

Fig. 1. A-E. *Arthrobotrys arthrobotryoides*. A. Conidia. B. Adhesive network trapping devices. C-E. Different shapes of proliferating swollen conidiophore knots. Bars = 10 µm.

Table 1. Comparison of morphological characteristics of *Arthrobotrys arthrobotryoides* marine strains (M) with other strains from freshwater (F) habitats and terrestrial (T) and description provided by Oorschot (1985).

Strain	*Habitat*	*Conidia (µm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985)	T	20-22 × 9-10	Continuous	1	N/A	AN
M1.137	M	16-(19.6)-22 × 7-(8.2)-9	Lacking or twice	0-1	Absent	AN
F1.138	F	15-(17.2)-21 × 6.5-(8)-9	Lacking or once	1	Present	AN

AN = adhesive net, N/A = not available

Notes: The conidia of the two strains isolated here (Table 1) are relatively smaller when compared to original description of Oorschot (1985). The conidia are produced at the apex of the conidiophores from an irregularly swollen part. The septum is located in the centre of the conidia. The conidia shape and position of septum are slightly different to that in the terrestrial strain *sensu* Oorschot (1985). One-celled conidia were observed in mangrove strain M1.137. This species also has some resemblance to *Arthrobotrys superba*.

Arthrobotrys cladodes* var. *macroides Drechsler, *Mycologia* **36**: 144. (1944). (Fig. 2)

Sampling sites: Mangrove; Kei Ling Ha Lo Wai, Sai Kung, 22º 24' 49.32" N 114º 16' 21.47" E. Freshwater; Lantau North Contry Park, Mui Wo, Lantau, 22º 16' 24.17" N 113º 59' 45.00" E.

Culture: IFRD 2010.
Habitat: mangrove and freshwater.
Substrate: unidentified decaying wood and leaves.
Teleomorph: unknown.

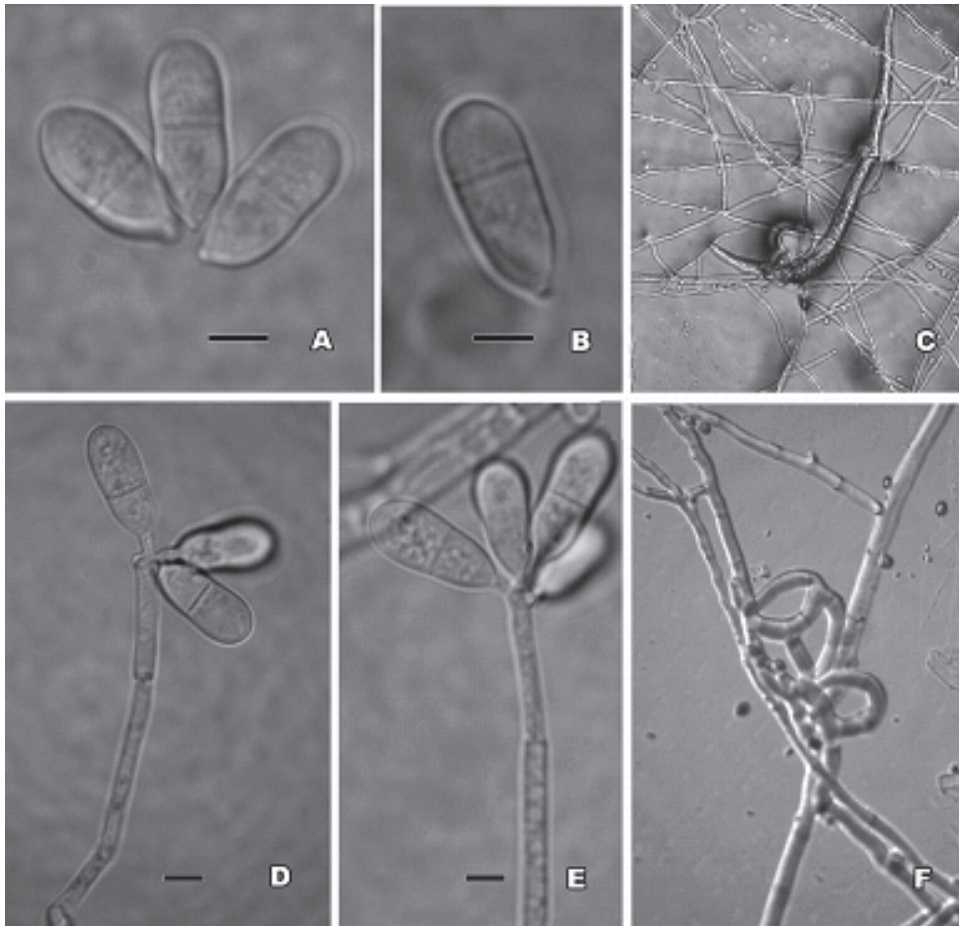

Fig. 2. A-F. *Arthrobotrys cladodes* var. *macroides*. A-B. Differently shaped conidia. C. Nematode trapped by adhesive network trapping device. D-E. Conidiophores, conidia and conidiogenous cells. F. Adhesive network trapping device. Bars = 10 µm.

Table 2. Comparison of morphological characteristics of *Arthrobotrys cladodes* var. *macroides* marine strains (M) with freshwater (F) and terrestrial (T) strains, the latter description provided by Oorschot (1985).

Taxon	*Habitat*	*Conidia (μm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985) CBS 120.54	T	13-26 × 5-8.2	None	1	Present	AN
M1.133	M	13-(18.8)-26 × 7-(8.6)-10	Lacking or once	1	Present	AN
F1.143	F	15-(16.5)-17.5 × 6.3-(7.3)-7.5	None	1	Absent	AN

AN = adhesive net.

Colonies white from both upper and lower surfaces of media. Mycelium spreading, hyaline, septate, branched. Conidiophores up to 850 μm high, 2-5 μm wide at the base, erect, simple, very rarely with side branches, bearing 8-20 conidia. Conidia elongate-obovoidal to ellipsoidal on short, on narrow denticles, straight, 1-septate at or near the middle, 13-(18.8)-26 × 7-(8.6)-10 μm, colourless. Forming adhesive network trapping devices. Chlamydospores present.

Notes: This species was only recorded from mangrove habitats in Hong Kong in this study, whereas, *Arthrobotrys cladodes* has only previously been recorded from terrestrial habitats. Thus this is the first record of the taxon from aquatic habitats. The shape and size of conidia and conidiophores of the three strains isolated in this study (Table 2) are similar to *A. cladodes sensu* Oorschot (1985). The septum however, occurred not only at centre as in the original description, but some were eccentric. Based on conidia size, the mangrove strain M1.133 is identified as variety *macroides* and the freshwater strain F1.136 as variety *cladodes.* Chlamydospores were not observed in freshwater strain (F1.136). Relatively larger conidia were recorded in the mangrove strain (M1.133).

Arthrobotrys musiformis Drechsler, *Mycologia* **29**: 481 (1937). (Fig. 3)

Sampling sites: Mangrove; WWF Mai Po Nature Reserve, Mai Po, New Territories, 22º 29' 11.00" N 114º 02' 24.35" E; Kei Ling Ha Lo Wai, Sai Kung, New Territories, 22º 24' 49.32" N 114º 16' 21.47" E.
Freshwater; Tai Shing Stream, Tai Po Kau, New Territories, 22º 23' 34.01 N 114º 08' 3.32" E. Tai Lam chung Reservoir, New Territories, 22º 23' 51.89" N 114º 02' 48.78".
Terrestrial; Lantau North Contry Park, Mui Wo, Lantau, 22º 16' 39.57" N 113º 59' 45.00" E. Tai Po Kau Natural Reserve, New Territories, 22º 25' 30.72" N 114º 10' 37.46" E.

Culture: IFRD 2005.
Habitat: mangrove, freshwater and terrestrial.
Substrate: unidentified decaying wood, leaves and soil.
Teleomorph: unknown.

Colonies white on both upper and lower sides of the media. Mycelium spreading, hyaline, septate and branched. Growth rates varied depending on strains (10-35 mm d^{-1}) at 25ºC. Conidiophores up to 850 μm high, 2-5 μm wide at the base, erect, rarely producing side branches, proliferating subapically to produce a candelabrum-like branching system, each branch bearing a single, terminal conidium. Conidia elongate-obovoid to ellipsoid, straight or slightly

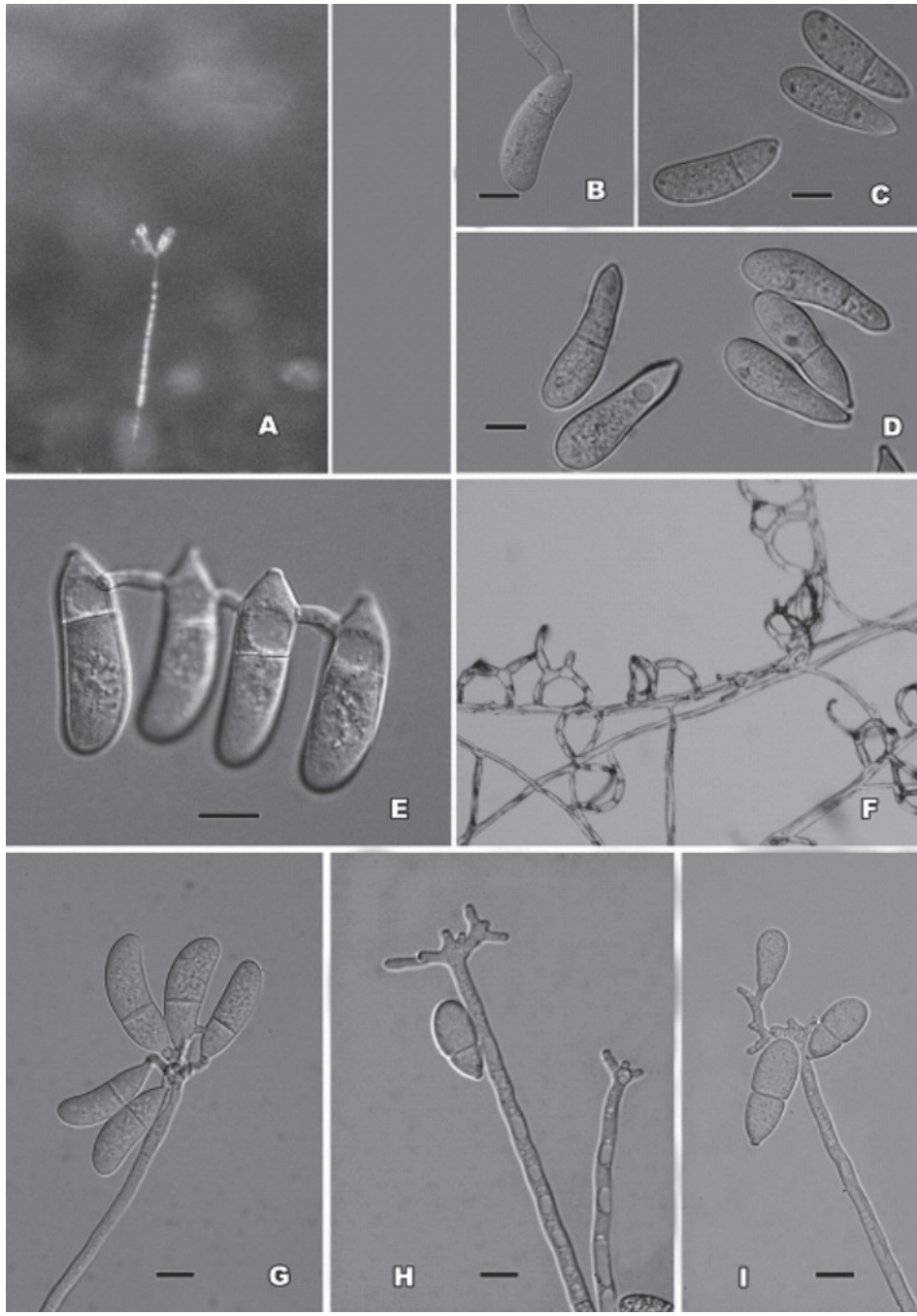

Fig. 3. A-I. *Arthrobotrys musiformis*. A. Conidiophore and conidia under stereomicroscope. B. Germ tube emerging from ellipsoidal conidia. C. Slightly curved conidia as compared with straight conidia. D. Conidia produced at high salinity (20‰). E. Conidia producing germ tubes. F. Adhesive network trapping device. G-I. Different shapes of candelabrum-like conidiophore knots. Bars = 10 μm.

Table 3. Comparison of morphological characteristics of *Arthrobotrys musiformis* marine strain (M) with terrestrial (T), and freshwater (F) strains, the latter from Oorschot (1985).

Taxon	*Habitats*	*Size of conidia (μm)*	*Length: width*	*No. of septa*	*Growth*[a]
Oorschot (1985) CBS 110.37	T	19-42.5 × 7-14	N/A	1	N/A
M1.104	M	25-(30.7)-40 × 8.8-(**10.7**)***-12.5	2.89***	1	46.8
F1.106	F	25-(**32.9**)***-37.5 × 7.5-(**9.7**)***-10	3.4***	1	35.2
T1.132	T	26-30.9-40 × 9-(**11.6**)***-15	2.69***	1	73.6

*** Significant at 99% confidence level. [a]Growth diameter (mm) at 6 days (n = 4) under 0 ppt NaCl.

curved especially in T1.132, 1-septate below the centre mostly at 1/3 from the base, commonly producing up to 9 conidia (mostly 5), 25-30.7-40 × 8.8-10.7-12.5 μm, colourless. Conidial width to ratio of mangrove strain significantly different compared to other strains (Table 3). Forming adhesive network trapping devices. Chlamydospores brown, spherical.

Notes: The shape and size of conidia and conidiophores of all three strains isolated in the present study fell within the ranges given by Drechsler (1937) and Oorschot (1985). However, three populations (terrestrial, freshwater and mangrove) were clearly separated into three groups by average conidia width and length / width ratio, but not by average conidial length (Fig. ••). The results from morphology experiment are shown in Figure 3 and Table 3.

The average length (CL), width (CW) and ratio (length/width) of conidia from different strains were analyzed for morphological variability at different salinities levels. When grown on CMA with increasing levels of NaCl at room temperature (26 ºC) for 7 days, morphological changes occurred; 1) the number of conidia per conidiophore was less (1-3 conidia as compared to 5-9) conidia at lower salinities), 2) conidia were not formed from branching candelabrum-like structures, but from short and narrow denticles, 3) conidia were longer than normal (mean length; 31.5 ± 0.29, n = 180 without NaCl, 34.4 ± 0.35, n = 180 at 25 ‰ NaCl), 4) conidial were thinner at base but the mean was wider than average (mean width; 12.1 ± 0.08, n = 180 without NaCl, 14.3 ± 0.1, n = 180 at 20 ‰ NaCl), 5) conidial shape changed (mean ratio; 2.99 ± 0.04 without NaCl, 3.1 ± 0.03 at 20 ‰ NaCl), and 6) one-celled conidia were observed due to loss of septa (Fig. 3 D).

This study shows that conidial width was most stable characteristic of conidial morphology under salinity stress, because means of conidial width of the three populations did not overlap from one another with increasing salinity.

Arthrobotrys oligospora Fresenius, *Beitr. Mykol.* **1**: 18 (1850). (Fig. 4)

Sampling sites: Mangrove; WWF Mai Po Nature Reserve, Mai Po, New Territories, 22º 29' 11.00" N 114º 02' 24.35" E; Hoi Ha Wan, Sai Kung, New Territories, 22º 28' 10.58" N 114º 20' 07.34" E.

Freshwater; Tai Lam chung Reservoir, New territories, 22º 23' 51.89" N 114º 02' 48.78"; Lantau North Contry Park, Mui Wo, Lantau, 22º 16' 24.17" N 113º 59' 45.00" E.

Terrestrial; Lantau North Contry Park, Mui Wo, Lantau, 22º 16' 39.57" N 113º 59' 45.00" E. Tai Po Kau, New Territories, 22º 24' 42.32" N 114º 07' 00.73" E.

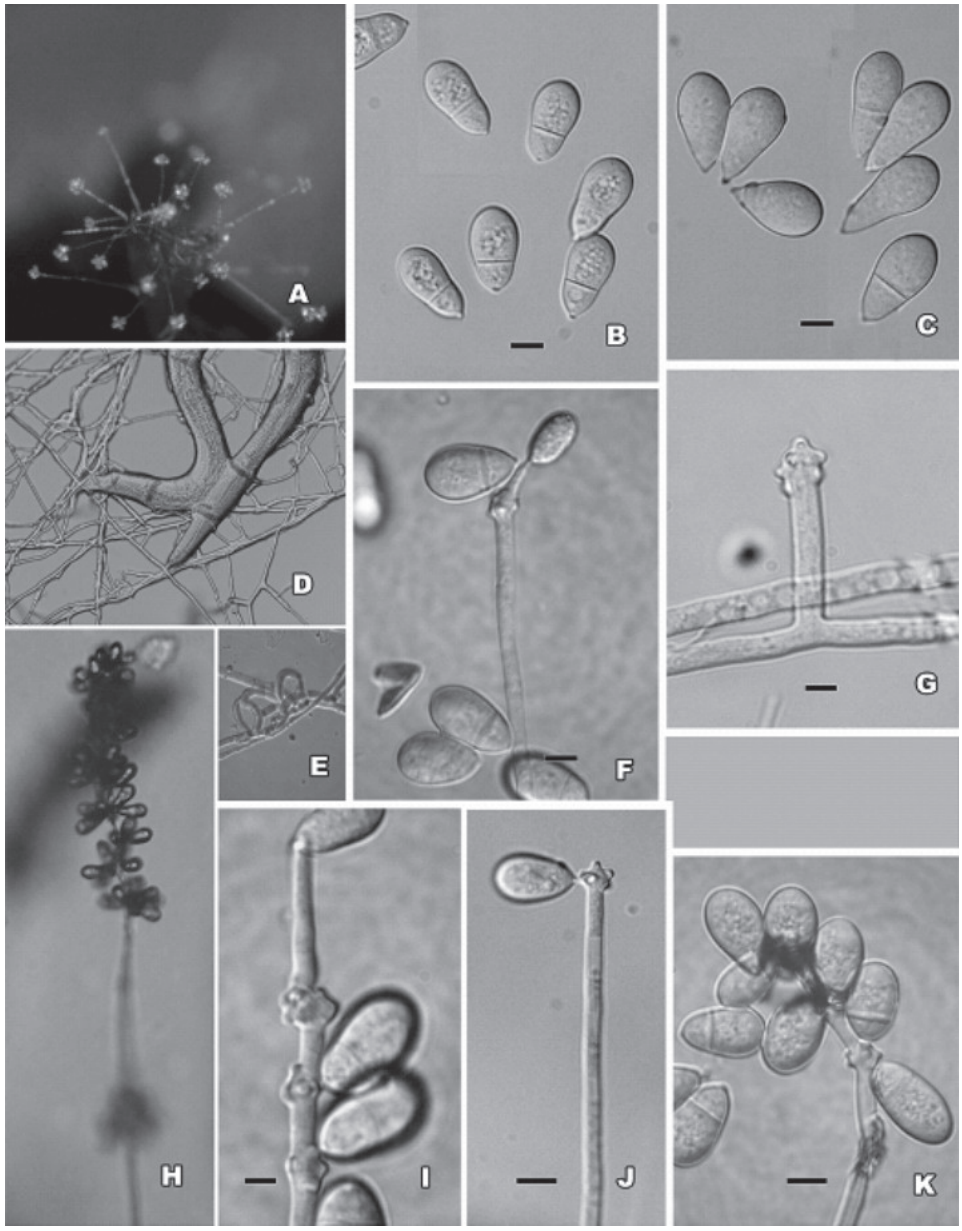

Fig. 4. A-K. *Arthrobotrys oligospora.* A. Conidiophores and conidia under the stereomicroscope. B. Conidia grown in media without NaCl. C. Conidia grown at 20‰ salinity. D-E. Nematode trapped with adhesive network trapping devices. G. Conidiophore produced in media under salinity stress (20‰). F & H-K. Different shapes of proliferating swollen conidiophore knots. F & K. Conidiophore proliferating repeatedly changing direction. H & I. Conidiophore proliferating repeatedly without changing direction. J. Simple conidiophore with no repeated proliferation. Bars = 10 μm.

Culture: IFRD 2001 (M1.101), IFRD2002 (F1.102), IFRD 2003 (M2.109), IFRD 2004 (T1.108)

Habitat: mangrove, freshwater and terrestrial

Substrate: unidentified decaying wood, leaves, dung and soil

Teleomorph: *Orbilia auricolor* (Pfister & Liftik, 1995).

Colonies white both upper and lower sides of the media, farst growing. Mycelium spreading, hyaline, septate and branched. Conidiophores up to 440 µm high, 1-3 µm wide at the base, erect, simple, rarely producing side branches, conidiogenous apices slightly inflated or swollen, bearing 10-15 conidia on narrow, short denticles, repeated proliferations observed in old cultures. Conidia obovoid to pyriform, sometimes slightly constricted at septum, basal scar distinct, one-septate below the middle, 17.5-(24.5)-28.8 × 10-(12.7)-16 µm, colourless. Forming adhesive network trapping devices. Chlamydospores, brownish, spherical to ellipsoid, in chains, smooth.

Notes: The shape and size of conidia and conidiophores of all six strains collected fell within the range of *A. oligospora sensu* Oorschot (1985). However means of conidial length and width of the mangrove strains were significantly different from other strains (Table 4).

Three varieties of *A. oligospora* have been described: *A. oligospora* var. *microspora* (with the range of conidia width of 6-11 µm), *A. oligospora* var. *oligospora* (conidia length up to 25 µm long) and *A. oligospora* var. *sarmatica* (conidial length up to 40 µm long). All strains in this study were identified as *A. oligospora* var. *oligospora.* Interestingly when under salinity stress, conidial length of some strains fell into the range of *A. oligospora* var. *sarmatica*, indicating that delimiting varieties based on conidia size may not be reliable.

Two way ANOVA shows that there is an interaction between habitat and salinity effect on conidial length, width and ratio (CL, $F = 7.093$ $P < 0.001$; CW, $F = 7.989$ $P < 0.001$; L/W Ratio, $F = 3.723$ $P < 0.001$). The dependant variables (conidial length, width and ratio) were significantly different within and between

Table 4. Comparison of morphological characteristics of *A. oligospora,* marine strains (M) with other strains from terrestrial (T), freshwater (F) habitats and description *sensu* Oorschot (1985) without NaCl.

Taxon	*Habitat*	*Size of conidia (µm)*	*Proliferation*	*No. of septa*	*Length: width*	*Growth*[b]
Oorschot (1985)	T	17-25 × 10-24	Repeated	1(2)	N/A	N/A
M1.101	M	17.5-**(24.5)*****-28.8 × 10-**(12.7)***** -13.8	Repeated	1	1.94	74.1
M2.109[a]	M	22-(24.3)-28 × 11-(13.2)-15	Repeated	1	N/A	N/A
M3.128[a]	M	22-(26.2)-30 × 12-(13.6)-16	Repeated	1	N/A	N/A
F1.102	F	17.5-(21.4)-22.5 × 10-(11.8)-12.5	Once	1	1.82***	53.7
T1.108	T	20-(22.2)-26.3 × 10-(11.6)-12.5	Repeated	1	1.90	49.3
T2.127[a]	T	18-(22.7)-28 × 10-(11.3)-13	Twice	1	N/A	N/A

*** Significant at 99% confidence level, [a]isolates were excluded in the statistical analysis, [b] Growth diameter (mm) at 6 days (n = 4) under normal condition, N/A = not available.

strains with response to salinity. In general the number of conidia per conidiophore decreased with increasing salinity concentration and the conidiophores were shorter at higher salinity (20‰) concentrations.

Arthrobotrys pyriformis (Juniper) Schenk, W.B. Kendr. & Pramer, *Can. J. Bot.* **55**: 984 (1977). (Fig. 5)

Sampling sites: Mangrove; Shuen Wang, Sai Kung, New Territories, 22º 27' 49.74" N 114º 12' 33.53 E.

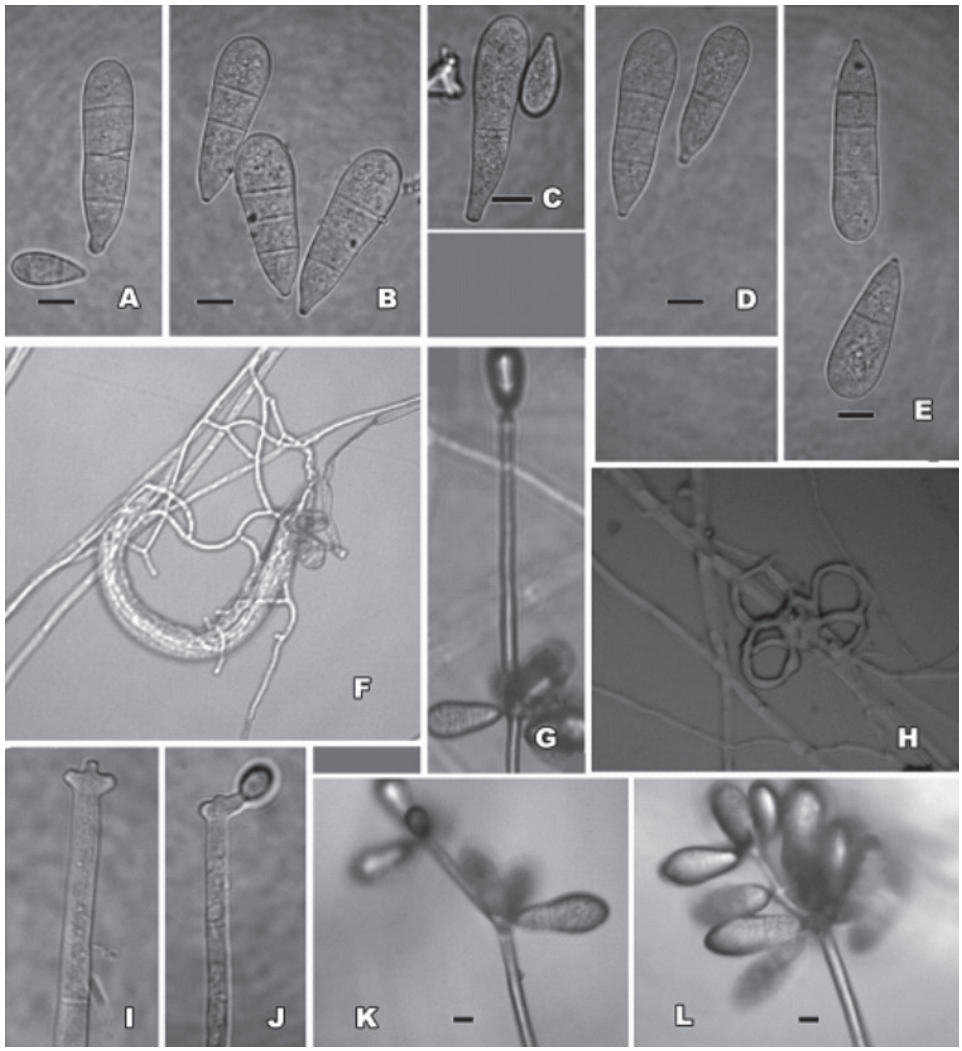

Fig. 5. A-L. *Arthrobotrys pyriformis*. A-E Different shapes of conidia. F. Nematode trapped with adhesive network trapping device. G. Conidiophore proliferation without change of direction. H. Adhesive network trapping device. I-J. Different shapes of proliferating swollen conidiophore apices. K-L. Conidiophore proliferating with changes of direction. Bars = 10 µm.

Table 5. Comparison of morphological characteristics of *Arthrobotrys pyriformis* mangrove strain (M) with terrestrial strain described by Oorschot (1985).

Taxon	*Habitats*	*Size of conidia (μm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985)	T	26-41 × 9-14	Once or twice	2-3	Present	AN
M1.123	M	38-(43)-52 × 10-(13.3)-16	Once or twice	1-3	Absent	AN

AN = adhesive net.

Culture code number: IFRD_M1.123.
Habitat: mangrove.
Substrate: unidentified decaying wood.
Teleomorph: unknown.

Colonies cream colour on upper and lower surfaces of media. Mycelia spreading, hyaline, septate and sticky. Conidiophores erect, simple, 150-500 μm long, no branching, sometimes proliferating one or two times, conidiogenous cell swollen, bearing 3-14 conidia on short, narrow denticles. Conidia broad pyriform or nearly oblong, 1-3-septate, 38-(43)-52 × 10-(13.3)-16 μm, colourless. Forming adhesive network trapping devices. Chlamydospores absent.

Notes: This species occurred only in mangrove habitat of Hong Kong. The conidia are relatively larger than *A. pyriformis sensu* Oorschot (1985) and quite a large number of small one-septate conidia were observed (Fig 5A, C, D and E). The shape and size of conidia however, and their respective conidiophores were almost similar to *A. pyriformis sensu* Oorchot (1985). Oorschot (1985) re-studied the type strain (CBS 204.83) describing "the conidiophores as much branched, showing repeated proliferation, the conidiogenous heads are more extended and less swollen, the conidia remain smaller, 15-25 × 5-8 μm, only 1 septate". In this study, proliferation was observed in old cultures in changing (Fig. 5K and L) and unchanged directions (Fig 5G).

Arthrobotrys superba Corda, *Pracht-Fl. Eur. Schimmelbild.*: **43** (1839). (Fig. 6)

Sampling sites: Mangrove; Shuen Wang, Sai Kung, New Territories, 22º 27' 49.74" N 114º 12' 33.53 E.
Terrestrial; Tai Po Kau Natural Reserve, Tai Po Kung, New Territories, 22º 25' 30.72" N 114º 10' 37.46" E.

Culture number: IFRD 2014.
Habitat: mangrove and terrestrial.
Substrate: unidentified decaying wood and soil.
Teleomorph: unknown

Colonies white from upper and lower side of the media. Mycelium spreading, hyaline, septate and branched. Conidiophores up to 900 μm high, erect, sometimes proliferating one time with or without change of direction (Fig 6F, G), conidiogenous heads swollen, bearing up to 12 conidia on short, narrow denticles. Conidia elongate-obovoid to ellipsoid, one-septate below the middle, 13-(18.6)-23 × 6-(8.5)-10 μm, colorless. Forming adhesive network trapping devices. Chlamydospores brown, globose, smooth, in chains.

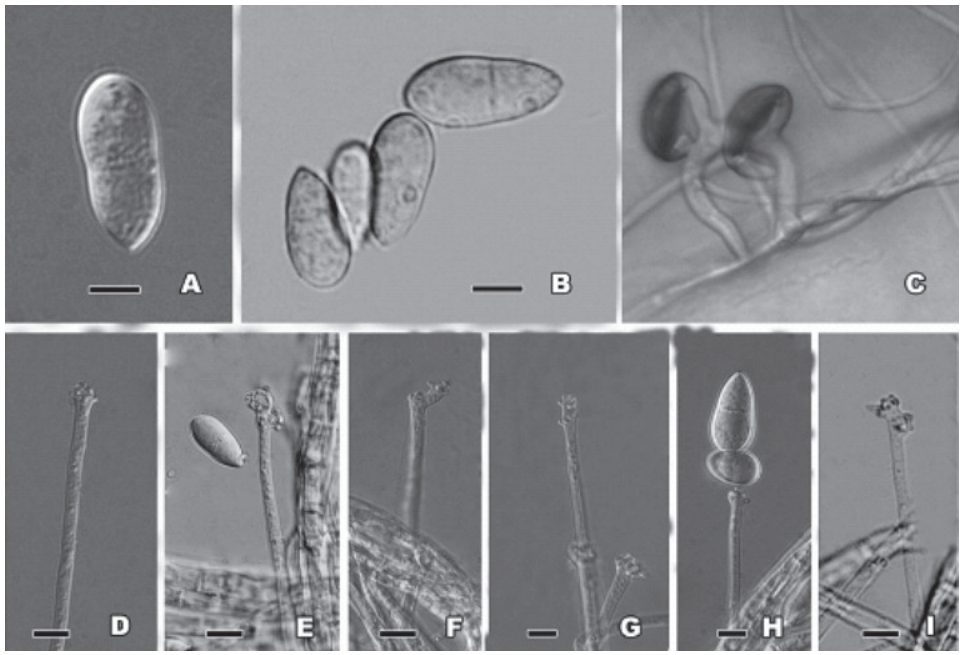

Fig. 6. *Arthrobotrys superba*. A-I. A-B Different shapes of conidia. C. Adhesive network trapping device. D-I. Examples of proliferating swollen conidiophore knots. Bars = 10 µm.

Table 6. Comparison of morphological characteristics of *Arthrobotrys superba* marine strains (M) with other strains from terrestrial (T) habitats, freshwater (F) and terrestrial strain described by Oorschot (1985).

Taxon	*Habitats*	*Size of conidia (µm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985)	T	13-22 × 5-8	Repeated	1-2	Present	AN
M1.135	M	13-(18.6)-23 × 6-(8.5)-10	Lacking or once	1	Present	AN
T1.136	F	12-(17.2)-20 × 5.5-(8)-9	Lacking or once	1	Present	AN

AN = adhesive net.

Notes: The shape and size of conidia and conidiophores of all three strains were similar to *A. superba sensu* Oorschot (1985). Although constricted septa generally distinguish *A. superba*, this was not observed in this study. In fact, Oorschot (1985) pointed out that the conidia as in Oorschot (1985) drawing are constricted at the septum, however this feature is rarely seen in the strains generally accepted as *A. superba* today. Two-septate conidia were also not observed. *Arthrobotrys* was introduced by Oorschot (1985) for *A. superba*, a species isolated from moist soil without testing its ability to trap nematodes. Later, however, it was re-described in detail as a nematode-trapping fungus by Drechsler (1937).

Arthrobotrys thaumasia (Drechsler) S. Schenck, W.B. Kendr. & Pramer *Can. J. Bot.* **55**: 984 (1977). (Fig. 7)

Sampling sites: Mangrove; WWF Mai Po Nature Reserve, New Territories, 22º 29' 11.00" N 114º 02' 24.35" E.
Ting Kok, Sai Kung, New Territories, 22º 28' 08.16" N 114º 13' 01.97" E.
Freshwater; Tai Shing Stream, Tai Po Kau, New Territories, 22º 23' 34.01 N 114º 08' 3.32" E.

Culture: IFRD 2006 (M1.110), IFED 2007 (M1.111), IFEC 2008 (M3.112)
Habitat: mangrove, freshwater and terrestrial
Substrate: unidentified decaying wood, and leaves and soil
Teleomorph: unknown

Colonies dirty white on upper and lower surface of media. Mycelium, hyaline, septate and branched. Conidiophores up to 500 µm high, 2-5 µm wide at the base, erect, producing small perpendicular branches, each branch bearing a single, terminal conidium and up to 4 conidia per conidiophore. Conidia ellipsoid, broadly-obovoid, 1-3-septate, 30-(37.1)-60 × 13-(16.7)-24 µm, colourless. Micro-conidia elongate-obovoid, *ca* 15 × 4.5 µm. Forming adhesive network trapping devices. Chlamydospores present in older cultures, single, more or less globose.

Notes: The delimitation of *A. thaumasia* and *A. eudermata* is not very clear. Drechsler (1950) described conidia of *A. eudermata* as considerably wider than in *A. thaumasia,* and chlamydospores as lacking. However, the shape of conidial terminal end is broader for *A. thaumasia* and sharper for *A. eudermata* and conidial size of *A. thaumasia* are larger than *A. eudermata* (Lui, pers. comm.). Conidial width is the only decisive character which can delimit between these two taxa as the existence of chlamydospores is not a reliable criterion. Conidia sizes of M1.110 were relatively large as compared to other strains. Four-septate conidia were only observed in strain M3.112 whereas two septa were common in all other

Table 7. Comparison of morphological characteristics of *Arthrobotrys thaumasia* marine strains (M) with other strains from freshwater (F) habitat and terrestrial (T) strain described by Rubner (1996).

Taxon	*Habitat*	*Size of conidia (µm)*	*Conidiophores apcies*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Rubner (1996) CBS 176.37	T	36-(41)-45.5 × 15-(18)-19.5	Simple or perpendicular branch	1-3	Present	AN
M1.110	M	32-(37.1)-60 × 13-(16)-24	Simple or perpendicular branch	1-3	Present	AN
M2.111	M	30-(38.7)-51 × 16-(18.3)-22	Simple or perpendicular branch	1-4	Present	AN
M3.112	M	30-(33.6)-50 × 16.3-(17.1)-20	Simple or perpendicular branch	1-3	Present	AN
F1.114	F	37.5-(40.2)-42.5 × 18.8-(20.3)-20	Simple or perpendicular branch	3	Present	AN

AN = adhesive networks.

Fig. 7. A-O. *Arthrobotrys thaumasia*. A-I. Various shapes of conidia; Ellipsoidal, broadly-obovoid. J. Non-septate micro-conidia K, Nematode trapped with adhesive network trapping devices. L. Adhesive networks trapping device. M-O. Different apices of conidiophores. Bars = 10 μm.

strains. Longer conidia were observed in all mangrove strains. One-septate conidia were observed in probable immature conidia. In most cases the middle cell was larger than the other cells. Conidial measurements of marine and freshwater strains are listed in Table 7. Interestingly, only three-septate conidia were observed in freshwater strain.

Arthrobotrys vermicola (R.C. Cooke & Satchuth.) Rifai, *Reinwardtia* **7**: 371 (1968). (Fig. 8)

Sampling sites: Mangrove; WWF Mai Po Nature Reserve, 22º 29' 11.00" N 114º 02' 24.35" E. Pak Sha Wan, Sai Kung, New Territories, 22º 22' 02.91" N 114º 15' 38.33".
Freshwater; Tai Lam chung Reservoir, New Territories, 22º 23' 51.89" N 114º 02' 48.78".

Culture code number: 2011 (M1.125) and IFED 2012 (M2.126)
Habitat: mangrove and freshwater
Substrate: unidentified decaying wood, leaves, and soil
Teleomorph: unknown

Colonies white on upper and lower surfaces of media, first growing. Mycelium, hyaline, septate, branched and sticky. Conidiophores up to 650 µm high, erect, conidiogenous cell sometimes irregularly swollen, proliferating up to two times and changing directions, bearing 3-6 conidia on short denticles. Conidia: ellipsoid to obovoidal, 1-3-septate, 24-(33.3)-40 × 13-(15.7)-20 µm, colourless. Forming adhesive network trapping devices. Chlamydospores absent.

Table 8. Comparison of morphological characteristics of *A. vermicola* marine strains (M) with other strains from terrestrial (T) and freshwater (F) habitats and description by Rubner (1996).

Taxon	*Habitats*	*Size of conidia (µm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985)	T	28.5-48.5 × 17.0-22.5	Repeated	2-3	N/A	AN
M1.125	M	24-(33.3)-40 × 13-(15.7)-20	Lacking or once	1-3	Absent	AN
M2.126	M	24-(32.3)-41 × 13-(15.4)-18	Lacking or once	1-3	Absent	AN
F1.103	F	22-(31.5)-39 × 12-(14.7)-19	Once	2-3	Absent	AN

AN = adhesive networks.

Notes: The shape and size of conidia and conidiophores of all strains were similar to *A. vermicola sensu* Oorschot (1985). One-septate conidia were observed only in the mangrove strains (M1.125 and M2.126). At first a single conidium is formed at the apex (Fig. 8K), later additional conidia from on short denticles below the apex. Proliferation was observed only one time in a young culture, but conidia proliferate progressively along the conidiophores in old cultures. Repeated proliferation however, was rarely observed in the mangrove strains. The adhesive network trapping devices were rarely observed, but nematodes were invaded and were digested well. This species resembles *A. azerbhaidznica* (Mekht.) Oorschot, however, the conidia of the latter are smaller and the conidiogenous head are well differentiated (Oorschot, 1985). *Arthrobotrys vermicola* also resembles *A. megaspora* when this species does not proliferate in culture.

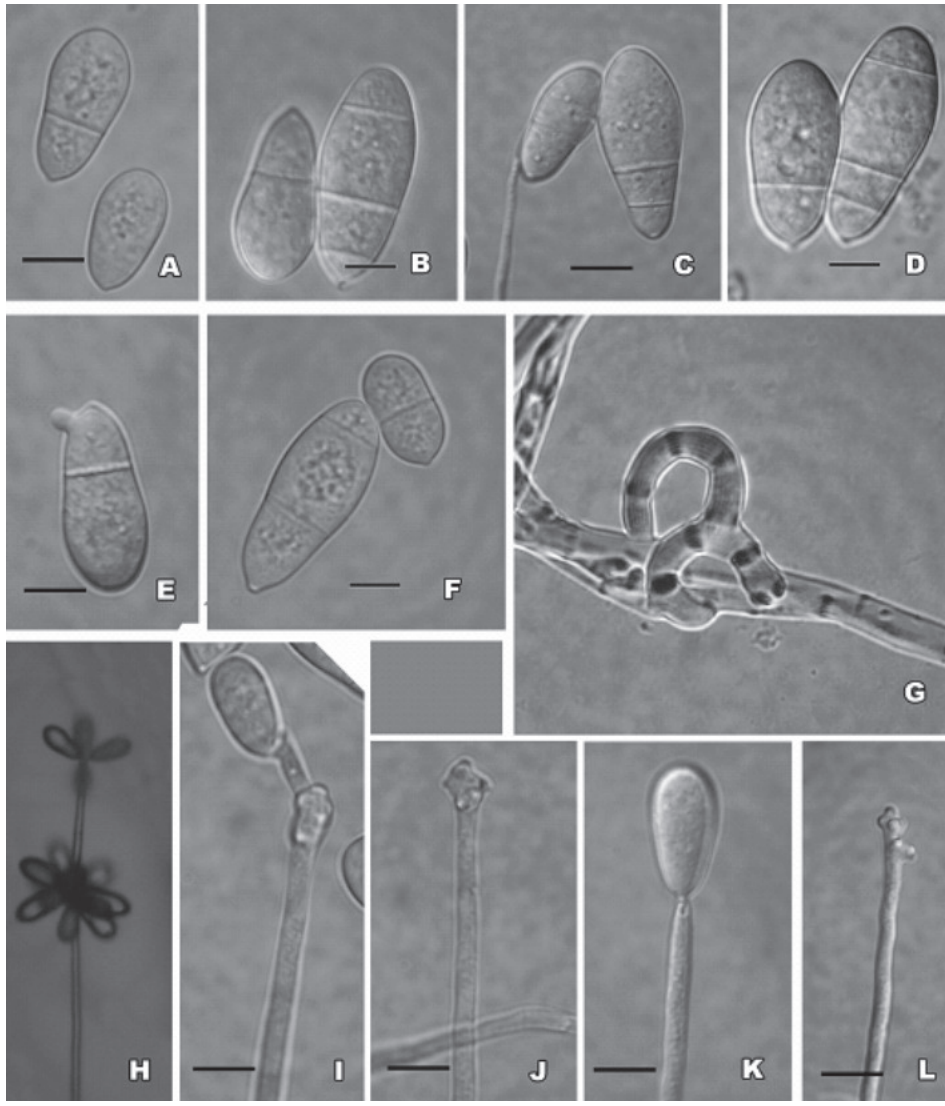

Fig. 8. A-L. *Arthrobotrys vermicola*. A-F. Differently shaped conidia. G. adhesive network trapping device. H-L. Proliferating swollen conidiophore knots. Bars = 10 µm.

Dactylellina lysipaga **(**Drechsler) M. Scholler, Hagedorn & A. Rubner, Sydowia **51**: 111 (1999). (Fig. 9)

Sampling sites: Mangrove; Hoi Ha Wan, Sai Kung, New Territories, 22º 28' 10.58" N 114º 20' 07.34" E.

Terrestrial; Tai Po Kau Natural Reserve, New Territories, 22º 25' 30.72" N 114º 10' 37.46" E.

Culture code number: IFRD 2013.

Habitat: mangrove and terrestrial.

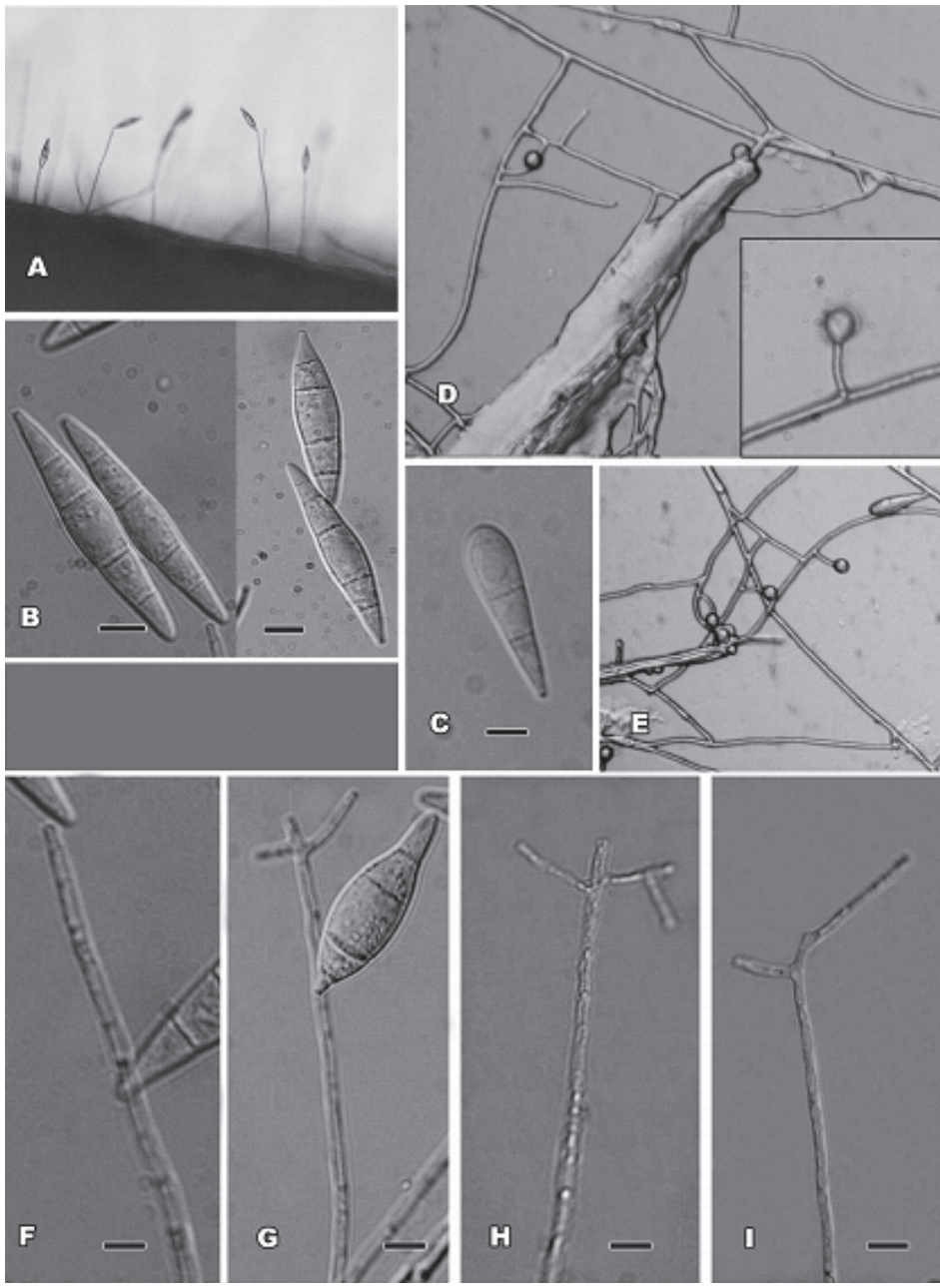

Fig. 9. A-I. *Dactylellina lysipaga*. A. Conidiophores and conidia under stereomicroscope. B-C. Conidia with different shapes. D. Nematode trapped with adhesive knob. E. Adhesive stalked knobs trapping devices. F-I. Different shapes of conidiophore apices. Bars = 10 µm.

Table 9. Comparison of morphological characteristics of *Dactylellina lysipaga* marine strains (M) with other strains from terrestrial (T) habitat and description by Rubner (1996).

Taxon	*Habitats*	*Size of conidia (μm)*	*Proliferation*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Rubner, (1996)	T	29.5-41 × 4.5-7.5	No distinction between the strains	2-4 (3)	Absent	AK
M1.138	M	20-(33.9)-49 × 6-(7.9)-10	No distinction between the strains	3-4	Absent	AK
T1.139	T	25-(38.2)-50 × 6.5-(8)-12	No distinction between the strains	4	Absent	AK

AK = adhesive stalked knobs.

Substrate: unidentified decaying leaves and soil.

Teleomorph: unknown.

Colonies light yellow upper and lower surface of media. Mycelium spreading, hyaline, septate, branched. Conidiophores up to 250 μm high, 2-5 μm wide at the base, erect, bearing first a single conidium at the apex, later additional conidia from on the lateral branches or longer geniculate branches below the apex, commonly producing 1-4 conidia; each long denticle or branch bearing a single conidium. Conidia spindle-shaped, straight, 3 to 4-septate, but commonly 3-septate, 20-(33.9)-49 × 6-(7.9)-10 μm, colourless. Forming adhesive stalked knobs as trapping device. Chlamydospores absent.

Notes: The conidial size of two strains is relatively small as compared to the description of Rubner (1996). Three-septate conidia were observed in the mangrove strain M1.139 and occasionally four septa (Table 9). This species resembles *Dactylellina haptotyla* since both species form the same conidial shape and have long stalked knobs. As *D. haptotyla* forms two types of trapping devices (stalk knobs and non-constricting rings), *D. lysipaga* is easy to differentiate.

Drechslerella dactyloides (Drechsler) M. Scholler, Hagedorn & A. Rubner, *Sydowia* **51**: 99 (1999). (Fig. 10)

Sampling sites: Mangrove; WWF Mai Po Nature Reserve, Mai Po, New Territories, 22º 29' 11.00" N 114º 02' 24.35" E.

Terrestrial; Lantau North Country Park, Mui Wo, Lantau, 22º 16' 39.57" N 113º 59' 45.00" E.

Culture code number: IFRD M1.141

Habitat: Mangrove and terrestrial.

Substrate: Unidentified decaying leave and soil.

Teleomorph: Unknown.

Colonies white both upper and lower view. Mycelium spreading, hyaline, septate, and branched. Conidiophores up to 700 μm high, erect, simple, bearing 1-4 (4 rarely observed) conidia on broad pronounced denticles. Conidia elongate-clavate, mostly straight or sometimes slightly curved, one-septate below the middle, 24-(30.8)-36 × 6-(6.3)-8 μm, colorless. Forming constricting ring as trapping device. Chlamydospores absent.

Notes: Drechsler (1937) described *Arthrobotrys dactyloides* and later it was transferred to *Drechslerella* as it forms constricting rings (Scholler *et al.*, 1999). Morphological variants between five isolates of the species have been

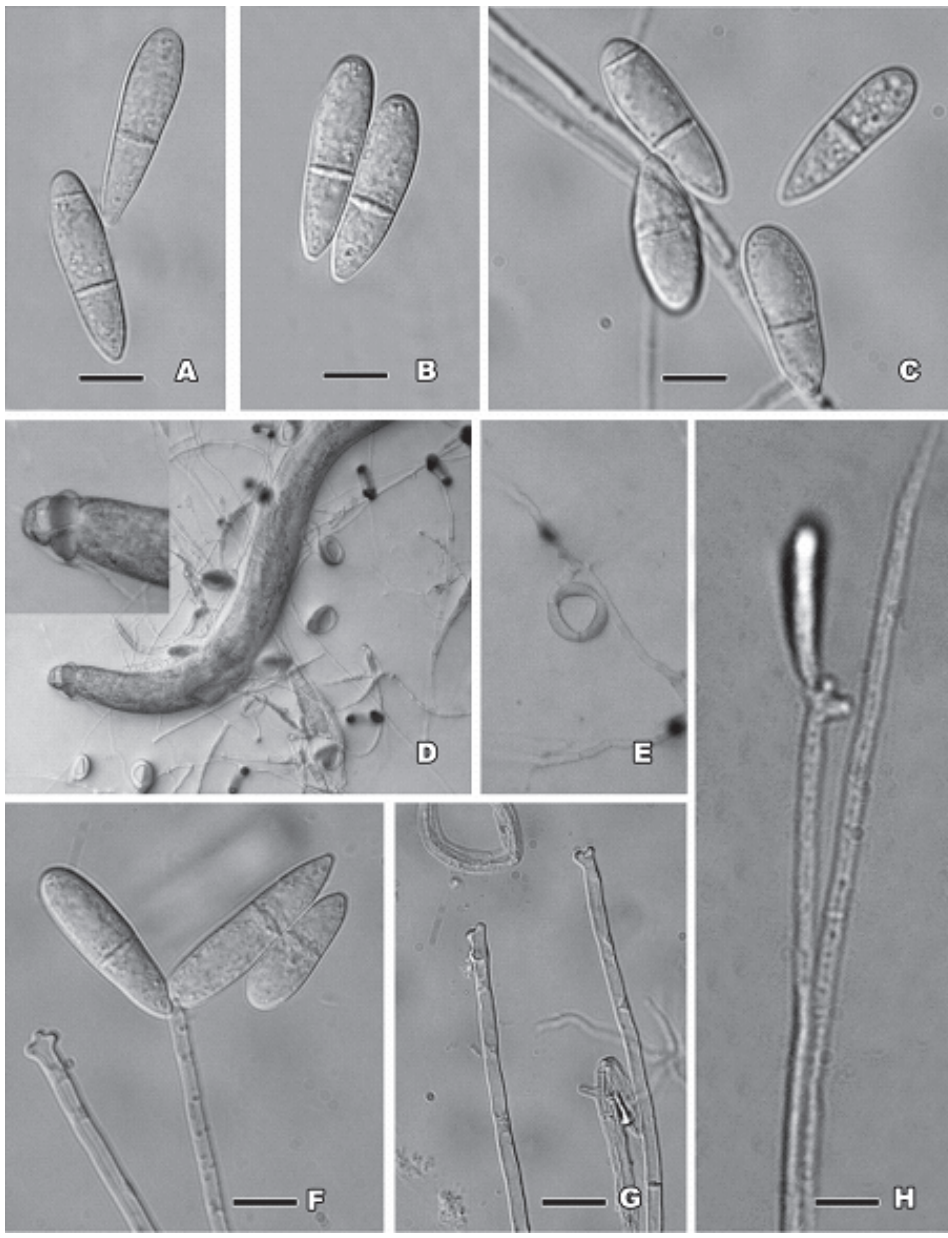

Fig. 10. A-H. *Drechslerella dactyloides*. A-C Different shapes of conidia. D. Nematode trapped by constricting ring. E. Constricting rings. F-H. Different shapes of conidiophore apices. Bars = 10 μm.

Table 10. Comparison of morphological characteristics of *Drechslerella dactyloides* marine strains (M) with other strains from terrestrial (T) habitats and strain described by Rubner (1996).

Taxon	*Habitat*	*Size of conidia (μm)*	*Micro-conidia*	*No. of septa*	*Chlamydo-spores*	*Trapping devices*
Oorschot (1985) CBS 109.37	T	35-60 × 7-9	Present	0-2	N/A	CR
M1.134	M	24-(30.8)-36 × 6-(6.3)-8	Absent	1-2	Absent	CR
T1.135	T	25-(34.2)-40 × 6.5-(7)-9	Absent	1-2	N/A	CR

CR = constricting rings, N/A = not available.

shown (Kumar & Singh, 2006). Macroconidia and chlamydospores were not observed in this study as compared to original description. Conidia of M1.134 were relatively smaller than T1.135 and species described by Oorschot (1985), 35-60 × 7-9 μm. Conidia shorter than 35 μm were observed in both strains (M1.134 and T1.135). Kumar and Singh (2006) also observed morphological variability among 5 isolates of *A. dactyloides* from India. Conidiophores produced up to 4 conidia in this study which is smaller (up to 10) than in the original description (Drechsler, 1937).

DISCUSSION

Conidial morphologies are one of the main characters for delimitating species of nematode-trapping fungi (Rubner, 1996). Details of morphological variation within species have been discussed under each description, and here overviews of morphological variants of nematode-trapping fungi and variations with response to salinity are provided.

Conidial and conidiophore morphology of all the mangrove strains collected in present study fell within the range of their respective species, however, there were also variations. Cai *et al*., (2005) recorded some modified morphological features of specialized adaptations for life in the freshwater environment which included massive ascospores, ascospore appendages and massive ascal apical rings. None of these specialized adaptations characters were observed from the mangrove strains studied here when compared to terrestrial strains. Variations in conidial characters however were observed; for example less number of septa occurred in the mangrove strains.

This study indicated that morphological characters including conidial length, width, length to width ratios, number of septa, number of conidia per conidiophore and shape of conidiophores apex varied with response to salinity. Pugh and Nicot (1964) found that media containing salt increased variation in conidial characters of the marine fungus, *Dendryphiella salina.* The conidia of *Dendryphiella* spp. were shorter when increasing levels of NaCl (Edwards *et al*., 1998). In contrast the mangrove nematode-trapping fungal strains isolated in the present study and grown on CMA with increasing levels of NaCl had longer conidia; e.g. *A. musiformis* (mean length; 31.5 ± 0.29, n = 180 without NaCl, 34.4 ± 0.35, n = 180 at 25 ‰ NaCl), *A. oligospora* (mean length; 22.8 ± 0.18, n = 180

without NaCl, 28.3.4 ± 0.16, n = 180 at 20‰ NaCl). In general, morphological modification were observed under saline stress.

In many cases, less septa were observed in the mangrove strains as compared to freshwater and terrestrial strains of each species. For example one-septate conidia were observed in the mangrove strain of *A. vermicola,* whereas conidia lacked septa in the freshwater and terrestrial strains, and *sensu* Oorschot (1985). Loss of septa was observed at high salinity. The presence of chlamydospores varied depending on stains isolated and thus is not a useful criterion to delimit species of nematode-trapping fungi. Formation of chlamydospores can depend on culture age and condition of nematode-trapping fungi (Scholler *et al.* 1999).

The strains of *A. musiformis* could be separated into three groups by means conidial width and ratio, indicating that these characters may be useful in delimiting strains of *A. musiformis*. Salinity can significantly affect the growth and predacious activity of nematode-trapping fungi. Nematode-trapping fungi are able to produce the same type of trapping device under salinity stress, however some of trapping structures were not well formed at higher salinities.

The nematode-trapping taxa isolated in this study varied morphologically somewhat from their description *sensu* Oorschot (1985) interms of conidial size and shape, ratio, septa, and the production of chlamydospores. The production of chlamydospores, proliferation of conidiophores, existence of microconidia, and position of septa were unstable morphological characters. These morphological characters are not reliable in delimiting species from saline habitats.

The discovery and description of nematode-trapping fungi from different habitats in Hong Kong add to new information concerning their morphological diversity. Moreover, the finer diagnostic characters of species (freshwater, terrestrial and mangrove) and morphological variants within species are described and illustrated, and may aid in future identification purposes.

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