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Nematophagous fungi isolated from soil in Oman

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During a survey of the fungus flora of soil and leaf litter in the Sultanate of Oman we isolated six orbiliaceous nematode-trapping fungi, *Arthrobotrys oligospora, A. multiformis, A. oudemansii, A. javanica, Drechslerella brochopaga,* and *Gamsylella gephyropaga.* All isolates are new records for Oman. The isolate of *A. multiformis* is the second record of this species world-wide. The isolates are described in detail. The study indicates that *A. oudemansii, A. multiformis*, and *A. javanica* are morphologically more variable than so far known.

Key word: *Arthrobotrys, Drechslerella, Gamsylella, Dactylella,* mycoflora.

The Sultanate of Oman occupies the southeastern corner of the Arabian Peninsula. Its topography features plains and mountains that are typical of arid and desert habitat. The most important area is the coastal plain where most of the agricultural crops are grown in nutrient-rich irrigated soil. In this and other agricultural areas plant parasitic nematodes cause substantial damage to agricultural farms.

The Middle East, including Oman, is part of the great North African Arabian desert belt that extends from the Atlantic Moroccan coast to the Indian subcontinent. The goal of this study was to find suitable isolates of nematode trapping fungi that could be used for the control of crop nematodes. So far, no orbiliaceous fungi have been reported from Oman, and only four studies from the Middle East, namely from Iraq (Muhsin & Kasim, 1998), Saudi Arabia (Lysek & Kürschner, 1987; Al-Hazmi & Abdul-Razik, 1990), and from Sudan (El Amin, 1980) have described nematode trapping fungi after selective isolation.

The taxonomy of anamorphic nematophagous orbiliaceous fungi and morphologically similar, non-nematophagous species has been studied by e.g. Drechsler (1937), Subramanian (1963), Cooke & Dickson (1965), Rifai & Cooke (1966), Haard (1968), Jarowaja (1970), Schenck & al. (1977), Oorschot (1985), and Rubner (1996). These authors based their genus and species concepts mainly on the mor-

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phology of the conidia and the conidiophores. Recently, Scholler & al. (1999) proposed a new genus concept considering also biochemical, morphological, and molecular features (rDNA sequence data). In this study we follow Scholler & al. (1999) concept by which the type of trapping device is the most important morphological feature for generic delimitation.

Materials and methods

Soil samples were collected in January 2001 from the rhizosphere of plants that were infected by nematodes from Saham, Nizwa, & Sultan Qaboos University, Oman. The sprinkled-plate technique described by Rubner (1996) was used for the isolation of the nematophagous fungi in which 1 g of soil was sprinkled onto Petri dishes containing water agar (WA). Initially, no nematodes were added as bait as most of the soils collected contained nematodes. After five days of growth, 100–200 nematodes (*Panagarellus redivivus* Linne Goodey) were added to the cultures. Trapped nematodes were transferred to half-strength cornmeal agar [CMA: 8.5 g cornmeal agar (Oxoid) and 12.5 g additional agar (Oxoid) and 1 l water; Rubner, 1996]. Using an alcohol-sterilized human hair fixed onto a fine needle, single spores of nematophagous fungi were isolated from the erect conidiophores, cultured on CMA agar plates and challenged with nematodes. For morphological studies, the isolates were also grown on Potato Dextrose Agar (PDA), CMA and WA at 25±2 °C. Measurements (50 conidia and 20 conidiophores) and camera lucida drawings were made from water mounts.

Results

Six species were found during this survey and are described below.

Description of species

Arthrobotrys oudemansii M. Scholler, Hagedorn & A. Rubner, Sydowia 52: 60. 2000. – Fig.1.

For synonyms see Scholler & al. (1999, 2000).

Colonies 4.6 cm diam. after 6 days on half-strength CMA, white to dirty white. – Hyphae hyaline, septate, branched, 2.6–7.8 µm wide. – Conidiophores on WA (70)80–120(140) µm, on CMA (141)200–300 (430) µm long, at base 5.2 µm and at apex 2.8 µm wide, mostly erect with simple apex, usually bearing a single conidium at the tip, forming branches at or near the tip bearing up to 4 conidia

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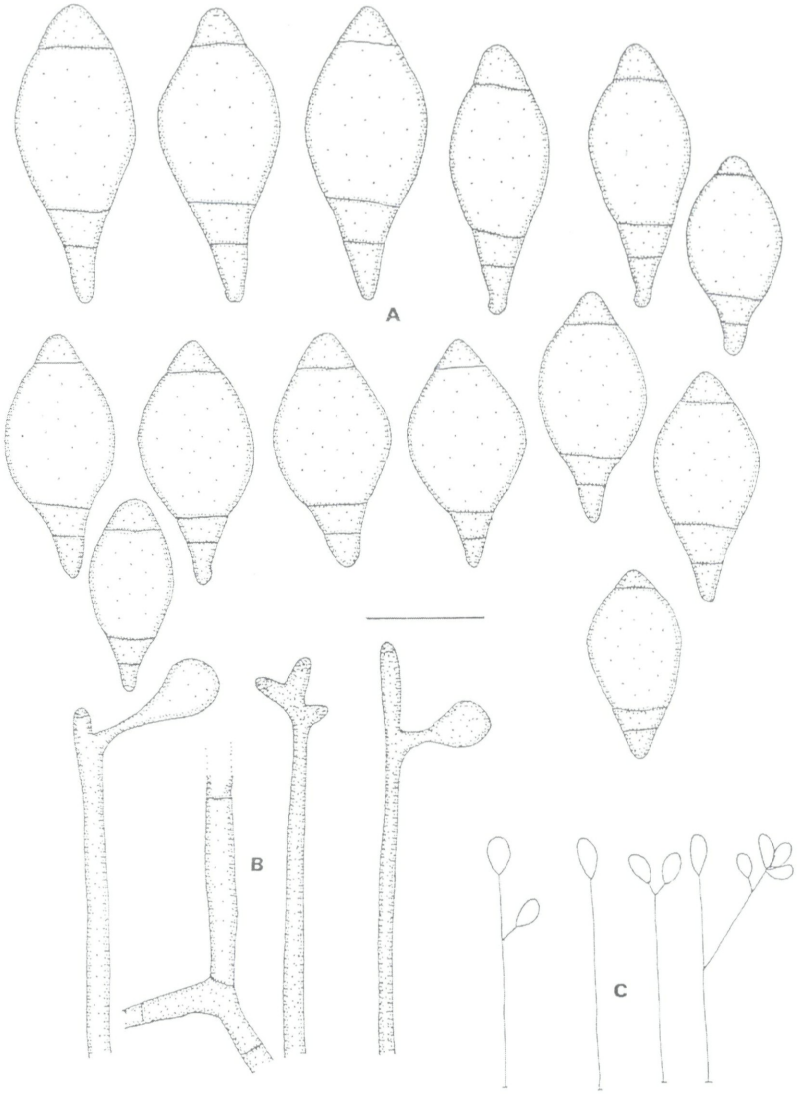

Fig. 1. *Arthrobotrys oudemansii*. – A. Conidia. – B, C. Conidiophores. – Bar: A & B = 25 µm. C: habit sketch of conidiophores.

(Figs. 1B, 1C). – Conidia top-shaped (Fig 1A), 33–57 × 18–26 µm, three-septate, with distinctly inflated middle cell, a parabasal cell and a basal cell that protrudes into a tail-like outgrowth. – Microconidiophores and chlamydospores not produced on WA, CMA or PDA. – Trapping devices three-dimensional networks.

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Material examined. – Saham, North of Oman, isolated from soil of banana plants infected by nematodes Jan. 2001, living culture deposited in CBS, Netherlands (CBS 109510) and in Sultan Qaboos University, Oman (SQU 52).

According to Rubner (1996), this isolate resembles *Monacrosporium thaumasium* (Drechsler) de Hoog & Oorschot [= *A. thaumasia* (Drechsler) S. Schenck, W. B Kendr. & Pramer], *M. psychrophilum* (Drechsler) R. C. Cooke & C. H. Dickinson [= *A. psychrophila* (Drechsler) M. Scholler, Hagedorn & A. Rubner] and *M. elegans* Oudem [= *A. oudemansii* (M. Scholler, Hagedorn & A. Rubner)].

Unlike *A. thaumasia*, this isolate lacks chlamydospores and microconidia and bears a single conidium at the tip of the conidiophore. Additional conidia are formed only on small branches of the conidiophores. *A. psychrophila* differs from this isolate because the median cell of this species is not distinctly inflated and the parabasal and basal cells are gradually tapering toward the truncate basal cell (Rubner, 1996). Furthermore, *A. psychrophila* forms chlamydospores in chains and microconidia in old cultures (Rubner, 1996). Conidial size and number of septa (three to four) seem to be slightly different from those seen in our isolate as well. Our isolate fits best with *A. oudemansii* although its conidia are shorter and wider and it lacks microconidiophores and microconidia. All strains of *A. oudemansii* studied by Rubner (1996), including the type material and the epitype (except CBS 397.93), however, lack microconidia as well, indicating, according to Rubner (1996), that the formation of microconidia is not a constant feature.

Drechslerella brochopaga (Drechsler) M. Scholler, Hagedorn & A. Rubner, Sydowia 51: 99. 1999. – Fig. 2.

Colonies 6.2 cm diam. after two weeks on half-strength CMA, white to pale orange. – Hyphae hyaline, septate, branched, 2.6–3.2 µm wide. – Conidiophores hyaline, septate, erect, (80)120–212(212) µm long, 4.8–6.2 µm wide at the base, tapering gradually upwards to a width of 4.5–5.1 µm near the tip; tip bearing up to 6 conidia (Fig. 2D). Under moist conditions the conidia are attached to each other like closed petals of a flower, and when dry they separate, move apart and open (Fig. 2B). – Conidia hyaline, straight or slightly curved, cylindrical to ellipsoidal, tapering to the base, 24–44 × 5–9 µm (Fig. 2.A). Ninety-six per cent of the conidia are three-septate and only 4% are two-septate. The basal and apical cells are almost equal in size; intermediate cells are smaller than the basal and apical cells and are equal in size. – Trapping devices are stalked, three-celled constricting rings (Fig. 2C).

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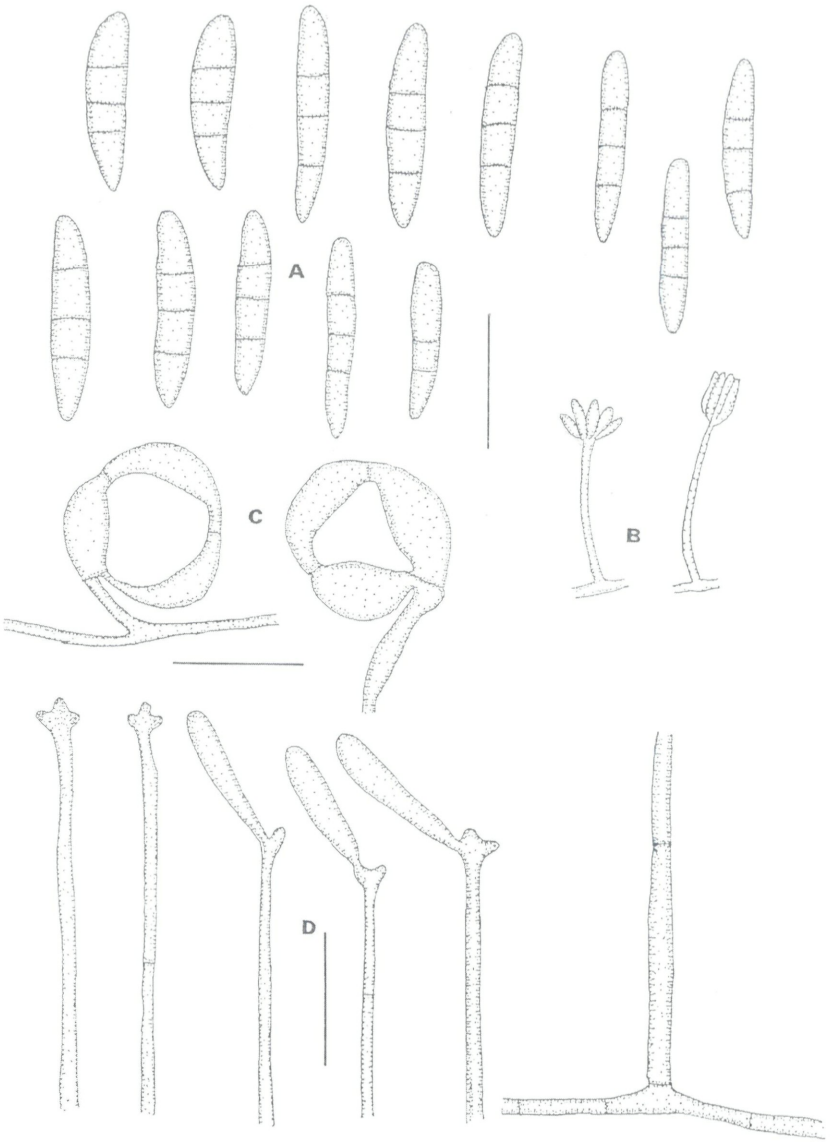

Fig 2. *Drechslerella brochopaga*. – A. Conidia. – B. Conidiophores. – C. Three-celled constricting rings. – D. Conidiophores. – Bar: A, C, D = 25 μm. B: habit sketch of conidiophores.

Material examined. – Nizwa, Oman, isolated from soil of citrus plants infected by nematodes. Very rare, isolated only once from over 80 soil samples surveyed, January-March 2001; living culture deposited in CBS, Netherlands (CBS 109509) and in Sultan Qaboos University, Oman (SQU 50).

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This isolate fits well with *Drechslerella brochopaga*. The fungus has been reported world-wide. It seems also common in the Middle East, e.g. Iraq (Muhsin & Kasim, 1998).

Gamsylella gephyropaga (Drechsler) Subram. M. Scholler, Hagedorn & A. Rubner, Sydowia 5:108. 1999. – Figs. 3–4.

Colonies 5.2 cm diam. after 6 days, after 14 days 6 cm and heavily sporulating on half-strength CMA. – Hyphae hyaline, septate, 4.5–5.4 µm wide. – Conidiophores hyaline, erect, non-branching, septate, (192)242–343(384) µm long, tapering gradually upwards, simple, bearing a single conidium (Fig. 3B, 3C). – Conidia hyaline, top-shaped, rounded at the end and tapering gradually at the truncate base, 39–55 × 18–30 µm, 2–4 septate (84% are 4-septate and very few are 1–2-septate; Fig. 3A). – No microconidia or microconidiophores observed. – Trapping devices one- to three-celled adhesive columns that are slightly constricted at the septa (Fig. 4 D). Later the columns grow out and anastomose with neighboring columns to form a scalariform shape, which becomes more elaborate by growing out and forming three-dimensional structures. Sometimes conidia germinate and form scalariform conidial traps without an intermediate hyphal phase (Fig. 4E).

Material examined. – Nizwa, Oman, isolated from soil of a citrus plant infected by nematodes, not very common, January-March 2001, living culture deposited in the CBS, Netherlands (CBS 109507) and in Sultan Qaboos University, Oman (SQU 46).

Similar observations were made by Persmark & Nordbring-Hertz (1998) on *Arthrobotrys oligospora* and by Rubner (1996) on an isolate of *Monacrosporium gephyropaga* [= *G. gephyropaga* (Drechsler) M. Scholler, Hagedorn & A. Rubner]. *G. gephyropaga* is very variable with respect to the morphology of its adhesive trapping devices. Depending on the strain, it may form traps consisting of simple branches with one to several cells, fused branches forming a two-dimensional net and scalariform shape, and even three-dimensional networks, as in our isolate. Cooke & Cooke (1969) & Rubner (1996) consider *M. gephyropaga* a species complex. Further taxonomic studies are necessary. The record from Sudan [as *Monacrosporium cionopagum* (Drechsler) Subram] reported by El Amin (1980) indicates that this species may be common in arid soils. The isolate reported from Saudi Arabia by Lysek & Kürschner (1987) as *Dactylella gephyropaga*, however, is *Monacrosporium parvicolle* (Drechsler) R. C. Cooke & C. H. Dickinson [= *G. parvicollis* (Drechsler) M. Scholler, Hagedorn & A. Rubner] according to Rubner (1996).

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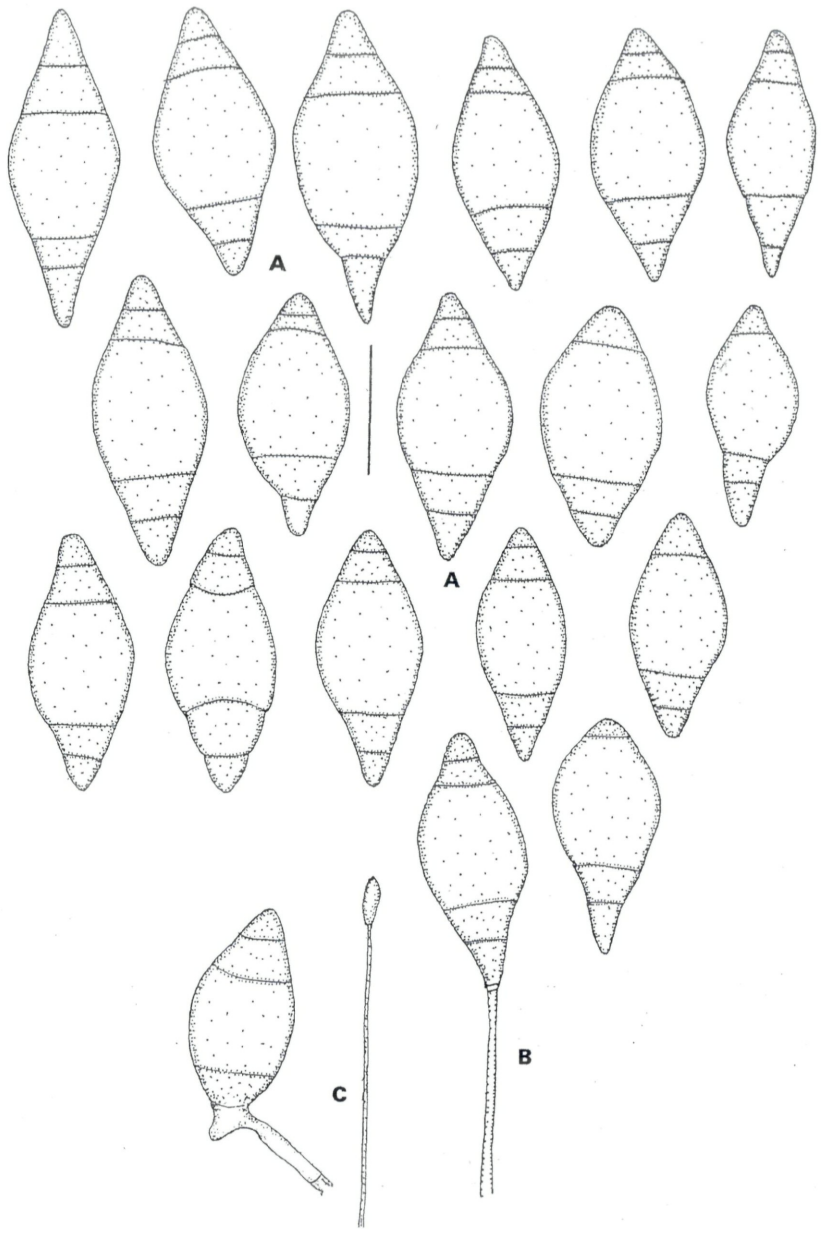

Fig 3. *Gamsylella gephyropaga*. – A. Conidia. – B. conidiophore tip with conidium. – C. Conidiophore with a conidium. – Bar: A, B = 25 µm. C: habit sketch of a conidiophore.

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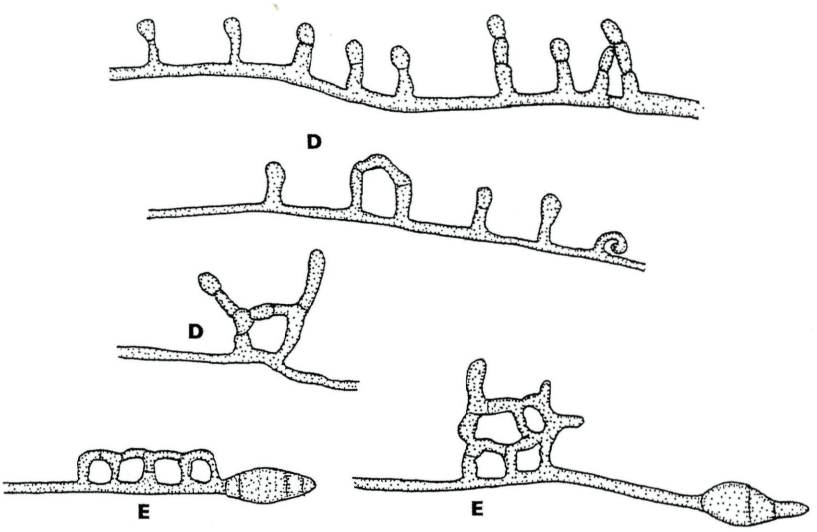

Fig 4. *Gamsylella gephyropaga*: habit sketch of adhesive columns. – D. Adhesive columns, various stages. – E. Adhesive columns formed after conidial germination.

Arthrobotrys oligospora Fres., s. l. Beitr. Mykol. 1–2: 18. 1850. – Fig. 5.

Colonies 9.6 cm diam. after 14 days on half-strength CMA, abundant sporulation, white to pale pink and light yellow to yellow on PDA. – Hyphae hyaline, septate, branched, 2–2.5 µm. – Conidiophores hyaline, erect, not branching, mononematous, straight, up to 600 µm long, 8–9 µm wide at the base and 5.5–6.4 µm wide below the first node (Fig. 5B), proliferating repeatedly, and producing arthobotryoid conidia on denticles on swollen nodular heads carrying 6–12 conidia with 3–7 nodes per conidiophore. – Conidia (Fig. 5A) 15–29 × 10–16 µm, always one-septate, ovoid to pyriform tapering to the base with an apiculate proximal and a swollen distal cell, some constricted at the septa. The septum is below the middle. The proximal and distal cells of the conidia are unequally divided, with the ratio of proximal cell to the distal cell 1:1.2 to 1:2.3 (average ratio of 1:1.7). – Trapping devices are adhesive three-dimensional networks. Conidial traps were not observed.

Material examined. – Nizwa, Oman, isolated from soil of a citrus plant infected by nematodes and from many other soils and compost (common in Oman), January 2001, living culture deposited in Sultan Qaboos University, Oman (SQU 44).

This isolate closely resembles *A. oligospora* Fries. var. *microspora* (Soprunov) van Oorschot and to some extent *A. oligospora* Fries. var. *sarmatica* (Jarowaja) van Oorschot in the morphology of its conidia, conidiophores and the three-dimensional networks. The

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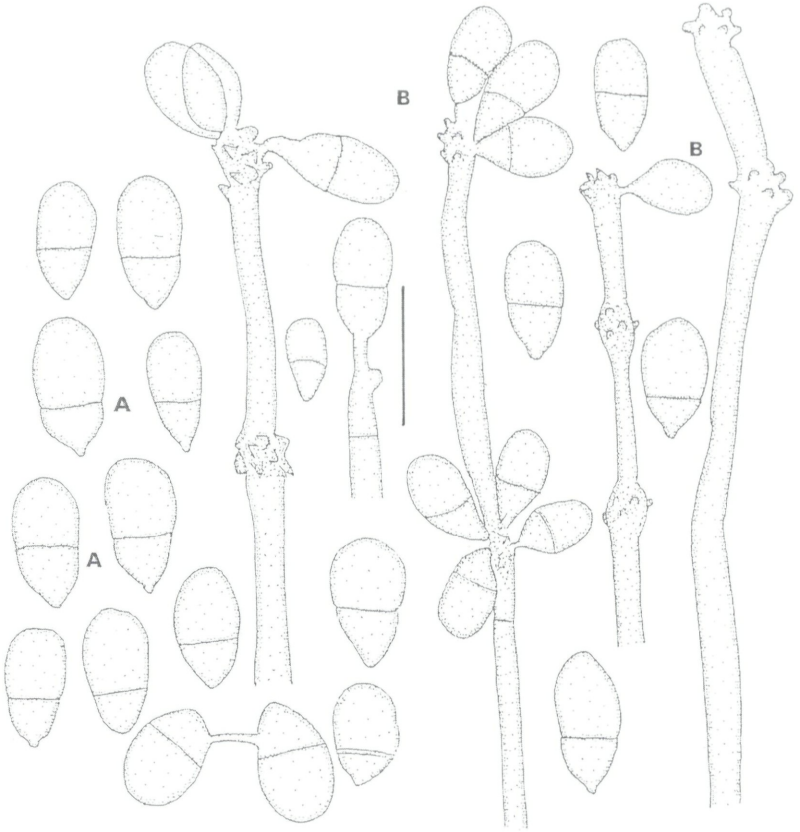

Fig 5. *A. oligospora*. – A. Conidia. – B. Conidiosphores. – Bar: A, B = 25 µm.

conidia of *A. oligospora* var. *microspora*, however, are slightly smaller than in this isolate (15.2–19.6 × 6–11 µm), while those of *A. oligospora* var. *sarmatica* are longer (up to 40 µm) and formed on a non-proliferating conidiophore. Among the three varieties of *A. oligospora* accepted by van Oorschot (1985), this isolate fits best with *A. oligospora* var. *oligospora* as described by Haard (1968) and van Oorschot (1985). The conidiophores, however, are shorter with fewer nodes per conidiophore and fewer conidia per node. Therefore, we do not assign this isolate to any of those varieties.

Arthrobotrys multiformis (Dowsett, J. Reid, & Kalkat) M. Scholler, Hagedorn & A. Rubner. Sydowia 51: 103.1999. – Figs. 6–7.

Colonies 8.5 cm diam. in 10 days on half-strength CMA. Sporulation relatively profuse on WA, conidia germinating on agar

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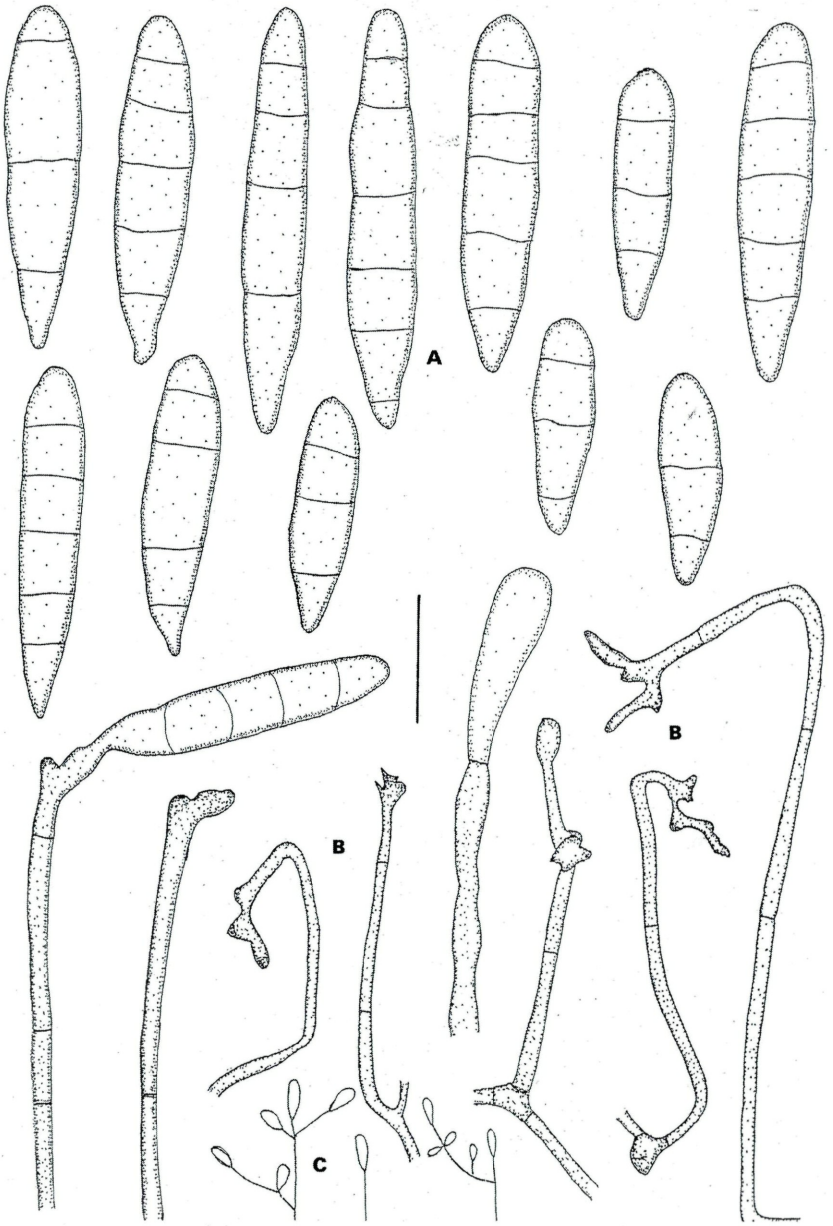

Fig 6. *Arthrobotrys multiformis*. – A. Macroconidia. – B, C. Primary conidiophores. – Bar: A, B = 25 µm. C: habit sketch of conidiophores.

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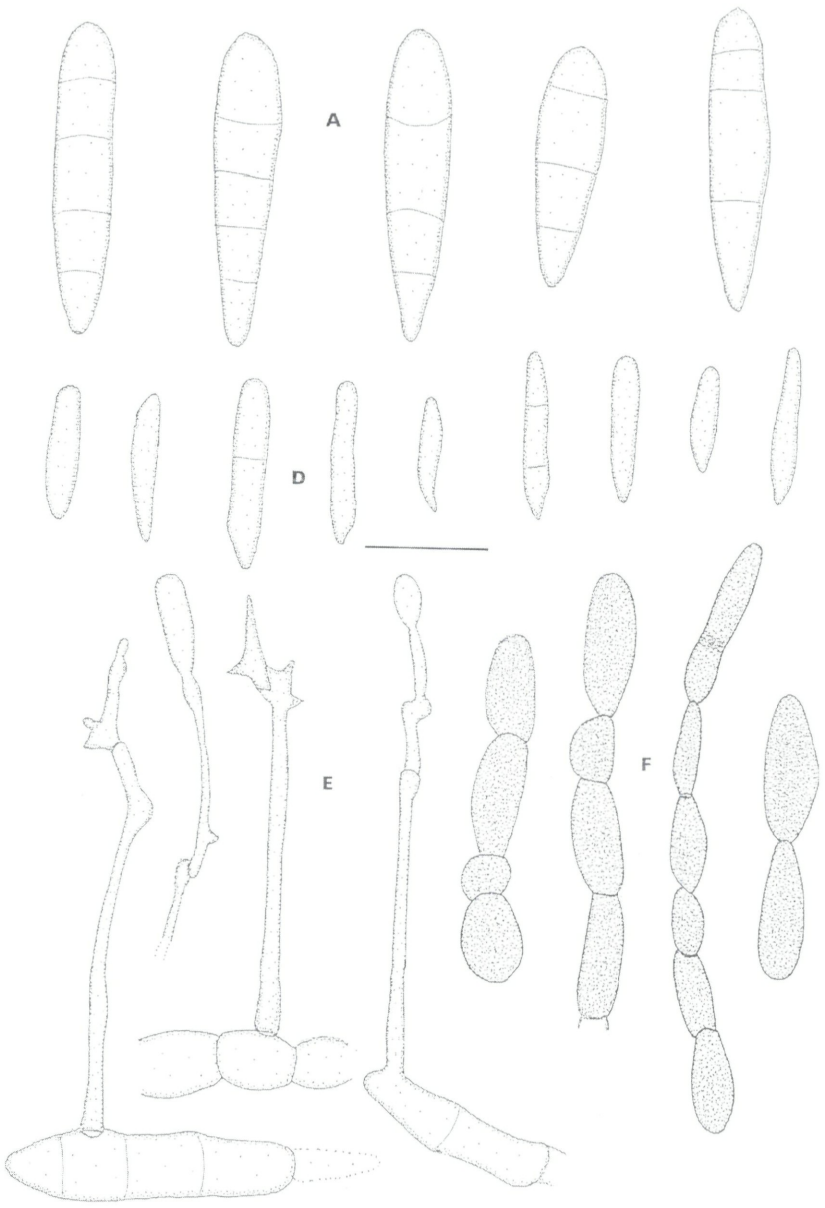

Fig 7. *Arthrobotrys multiformis*. – A. Macroconidia. – D. Microconidia. – E. Secondary conidiophores. – F. Chlamydospores. – Bar: A, D, E, F = 25 µm.

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surface. – Hyphae hyaline, branched, septate, 2.5–4 µm wide. – Primary conidiophores erect, slender, hyaline, arising from hyphae, simple, rarely branched, 65–350 (average 175) µm long, 5–6.5 µm wide at the base, tapering to the apex, 3–4 µm wide, simple with 2–4 septa, geniculate tip and sympodially elongating in one direction carrying up to 3 conidia, sometimes conidiophore tip candelabrelloid (Fig. 6B, 6C). – Secondary conidiophores arising from basal, penultimate or intercalary cells of a macroconidium, 70–150 µm × 3–4.5 µm, septate, with geniculate tip (Fig. 7E). – Macroconidia cylindrical to fusiform (Fig. 6A, 7A), 1–6-septate (mostly 4–5 septa), smooth, hyaline, 41–145 × 8–16 µm. – Microconidia clavate to cylindric-clavate, 0–2-septate (mostly non-septate), smooth, hyaline, 10–39 × 2–8 µm (Fig. 7D). – Chlamydospores (Fig. 7F) in chains of 2–7 formed after 2 months in WA, smooth, thin-walled, 3–8 µm wide. – Trapping devices incomplete adhesive networks, later develop into adhesive two-dimensional and three-dimensional adhesive networks.

Material examined. – Sultan Qaboos University, Oman, from soil and decaying leaves of *Ficus beneghalensis* L. and other plants submerged in an artificial pond, March 2001, living culture is deposited in Sultan Qaboos University (SQU 44).

According to Dowsett & al. (1984), the primary conidiophores of *A. multiformis* produce single conidia on the tip of the conidiophores. The conidiophores of our isolate bear a single conidium but may also have a geniculate tip with up to three conidia (Fig. 6C). This feature was not observed either by Dowsett & al.t (1984) or Rubner (1996). Compared to the Dowsett & al. (1984) strain our isolate has larger conidia (41–145 × 8–16 µm vs. 35–90 × 4–7.5 µm) with fewer septa (1–6 vs. 4–12) and longer secondary conidia (10–39 × 2–8 µm vs. 20–25 × 5 µm). These differences indicate that the species is morphologically more variable than previously known. M. Scholler (Purdue University, USA) checked the isolate and confirmed our identification. This strain is the second record worldwide.

Arthrobotrys javanica (Rifai & R.C. Cooke) Jarowaja, Acta Mycol. 6: 373. 1970. – Fig. 8.

Colonies 8.5 cm diam. after 14 days on half-strength CMA, white to dirty white. – Hyphae hyaline, septate, branched, 3.8–5.2 µm wide. – Conidiophores erect, mostly unbranched, (104)179–236(290) µm long, 2.5–4.4 µm wide. Some conidiophores proliferate at or near the tip producing candelabrum-like branches (Fig. 8E), each branch bearing one to three conidia; conidiophores sometimes

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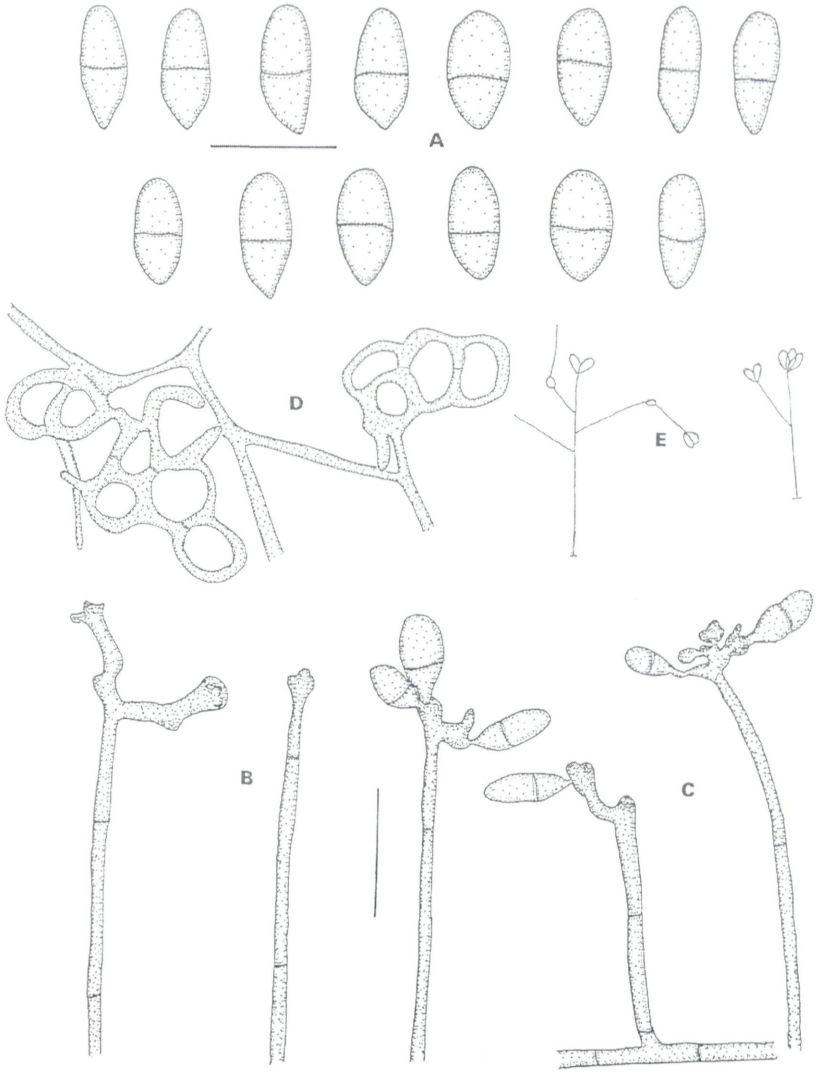

Fig 8. *Arthrobotrys javanica*. – A. Conidia. – B. Conidiophores. – C. Candelabrum-like branching system of conidiophores. – D. Adhesive networks. – E. Conidiophores. – Bar: A, B, C = 25 µm. D, E: habit sketches of adhesive networks & conidiophores.

antler-like (Fig.8B, 8C). – Conidia straight, cylindrical, smooth, obovoid to ellipsoidal, straight, one-septate (rarely non-septate) (Fig. 8A), the majority not constricted at the septum, 17–36 × 7.7–13 µm. Ratio of proximal to longer distal cell 1:1.3. – Trapping devices two- to three-dimensional adhesive networks (Fig. 8D).

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Material examined. – Nizwa, Oman, isolated from citrus plants infected by nematodes (rare, isolated twice from 80 soil samples surveyed), January-March 2001; living culture deposited in CBS, Netherlands (CBS 109508) and in Sultan Qaboos University, Oman (SQU 48).

The species seems to be very variable. Rifai & Cooke (1966) described *A. javanica* (*Candelabrella javanica* Rifai & R. C. Cooke) on sterile rabbit dung agar as having conidia measuring 25–42.5 × 10–15.6 µm, but in pure culture on CMA the conidia were smaller, being only up to 35 × 11.3–13.8 µm. Van Oorschot (1985) measured conidia in the range 22–35 × 7–11 µm. The conidia of this isolate are slightly longer and narrower than reported by Rifai & Cooke (1966) but they are shorter than those of the strain studied by van Oorschot (1985). Rifai & Cooke (1966) and van Oorschot (1985) described the conidia as slightly constricted at the septa but in our isolate the conidia were not constricted.

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