NEMATODE DESTROYING FUNGI FROM TENERIFE (CANARY ISLANDS) AND FAYAL, SAO MIGUEL AND PICO (AZORES).

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SUMMARY

8 species of nematode-trapping hyphomycetes and 3 endoparasitic nematode destroying fungi were isolated from soil and plant debris sampled in Tenerife and the islands Fayal, Sao Miguel and Pico of the Azores. The species found underline the mainly cosmopolitan character of most of the species.

RESUMEN

Utilizando el método aprobado de "sprinkled plates" 8 especies de hifomicetos y 3 especies de hongos endoparasíticos, que matan nemátodos han sido aislados de suelos y restos de plantas colectados en Tenerife y las islas de Azores: Fayal, Sao Miguel, y Pico. Las especies halladas subrayan la naturaleza cosmopolita de aquella clase ecológica de hongos microscópicos.

INTRODUCTION

The nematode-destroying fungi are an ecological group consisting of not more tjhan 200 species thought to occur world-wide (LYSEK, 1987), since they have been found in the United States, Europe, South and South-East Russia, India - even in the maritime Antarctic (GRAY and SMITH 1984). Islands have so far not been studied, hence it is not known whether these fungi are capable of reaching these remote locations. This could have been expected because the number of plants introduced as bulbs or living plants would have been effective vectors.

This study provides the first record of the species present on these islands. It may also be regarded as a first step to study the distribution of these fungi in different edaphic and climatic regions - which nowhere are as close together as on islands.

The results given show that there is a considerable number of these species and hence a good basis for further studies on their distribution in the different ecological regions.

MATERIALS AND METHODS

Sampling:

Samples of soil and plant debris were collected in glass vessels (or paper bags) air-dried and brought to the laboratory.

Isolation of nematode-destroying fungi:

The material was sprinkled on to a 2% wateragar (about 1 g material per dish) in Petri dishes with 9 cm diameter. After 4 - 6 days the dishes were checked for fungal growth, presence of nematodes and mites (which were killed individually). If no or only few nematodes were observed, additional eelworms were added as bait from a culture of *Turbatrix aceti L.*. During the following weeks these "sprinkled plates" were examined several times and the fungi, labeled by the killed eelworms, were isolated by transferring conidia, mycelia or the dead nematodes to new media. Endoparasitic species were not isolated but further infections were induced by the eelworms provided.

The isolated fungi were subcultured on water agar to obtain sterile strains. From these permanent cultures on agar slants and soil tubes, permanent microscopic mountos and exsiccates were obtained.

Documentation and identification:

The isolated fungi were identified by using the key of COOKE and GODFREY (1964) and descriptions of the species. Drawings and permanent mounts were made from all the isolated; permanent cultures of the nematode trapping species and herbarium specimens (dried out agar cultures) were kept in the institute; the endoparasitic species were kept as microscopic mounts only.

RESULTS

Table 1 gives the isolated species of predacious fungi, together with their origin. These are the following species:

Arthrobotrys oligospora Fresenius (1852).

This fungus turned out to be the most frequently isolated one, which corresponds to other studies. Most of its isolates resembled the typical form var.*oligospora* (according to van OORSCHOT, 1985), e.g. the isolates from Sao Miguel, Pico and from Tenerife. The latter site, however, also contained forms standing between var.*oligospora* and var. *microspora* or tending more pronounced to this form with smaller conidia. Strain 6-4 from the same origin is - based on its spores - a typical var. *oligospora*. However, it shows heavily branched conidiophores and comparatively large sticky networks (fig. 1).

	Location:					
	La Esperanza Tenerife	Puerto de la Cruz	Volcano Fajal	Pico	Saõ Miguel	Sum
Arthrobotrys oligospora		5		2	5	12
Arthrobotrys superba		2				2
Cephalosporium balanoides			2			2
Harposporium anguillulae			1			1
Monacrosporium cionopagum	3					3
M. cystosporum		1				1
M. gephyropagum	1					1
M. lysipagum			1			1
M. megalosporum		1				1
M. parvicollis	1	1				1
Nematoctonus leiosporus	2		1			3

Table 1: Numbers of isolates of the identified species, together with the location where the samples have been collected.

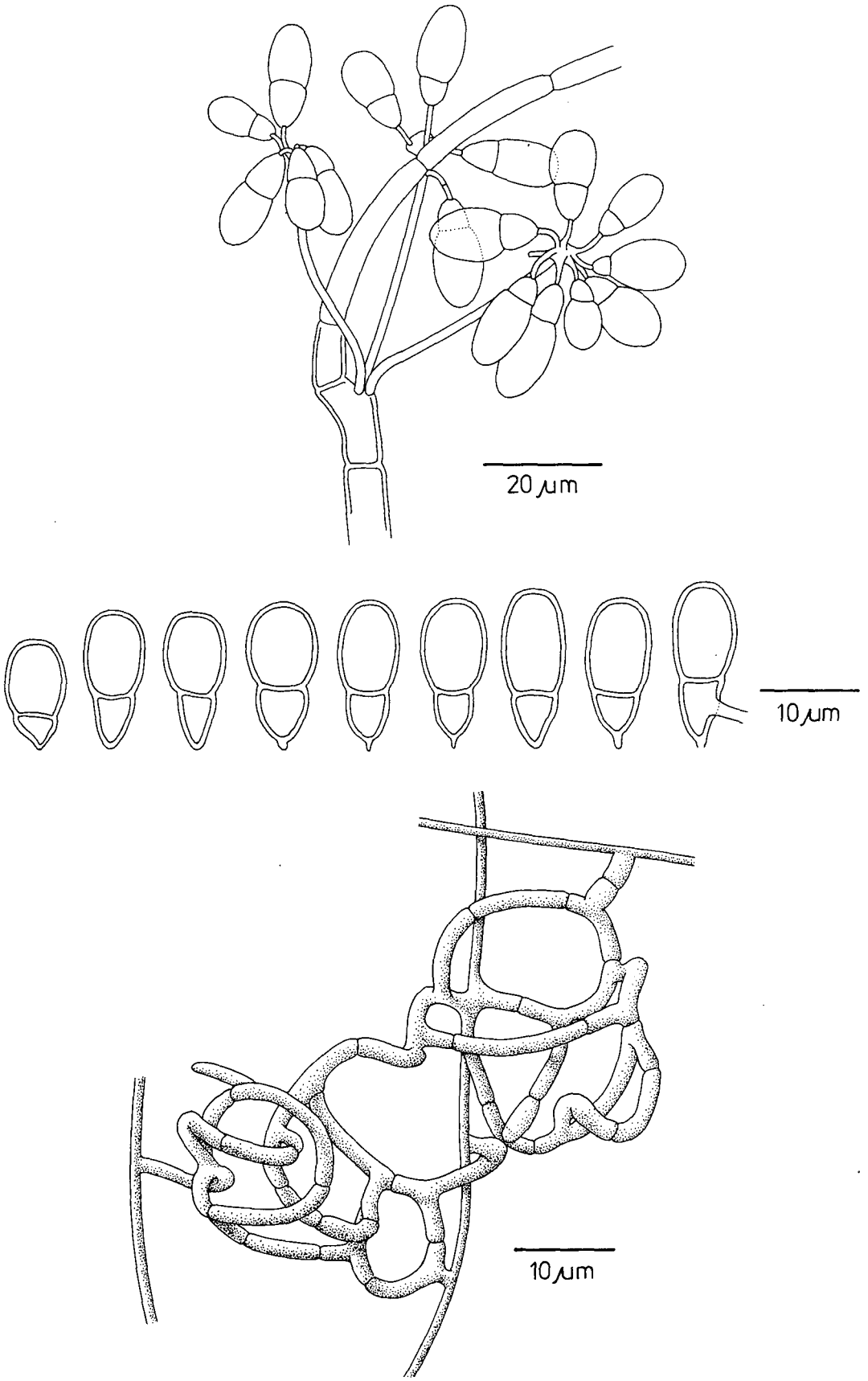

Fig. 1: Conidiophor, conidia and sticky network of *Arthrobotrys oligospora* strain 6-4. The punctuation of the trap may help to show the spatial structure of the network.

In addition the isolates from Sao Miguel and Pico showed variations in spore size according to culture conditions. These were hence not identified to the level of the subspecies.

Arthrobotrys superba Corda (1839)

This species was found in a banana-field near Puerto de la Cruz/Tenerife only. The (two) isolates show the typical form of the species as described by van OORSCHOT (1985): the proximal cell of the conidia is considerably smaller than the distal one. The conidiophores are bent at every whorl and partially branched. The spores measure 20 x 9.7 μm (17.9 - 21.4 x 8.6 - 10.9 μm). The typical conidia and the condiophores which change direction at proliferation are shown in fig. 2.

Cephalosporium balanoides Drechsler (1941).

This endoparasitic nematode destroying fungus was isolated from the *Sphagnum* cushions of the permanently wet and cool regions of Fajal. Two isolates were obtained; it proved, however, impossible to subculture these on additionally added nematodes. Due to resulting lack of material the delimitation against the similar species *Verticillium sphaerosporum and Acrostalagmus obovatus* is not totally certain. The typically long condiophores (up to 500 μm), the small number of 1 to 2 phialides per cell and the acorn-shaped conidia make *Cephalosporium balanoides* most probable.

Harposporium anguillulae (Lohde) Karling (1938)

This endoparasitic species was found in the same *Sphagnum* moss cushions as the former species and *Monacrosporium lysipagum* (see below). The isolated form resembled the descriptions in spore size and formed three conidia per phialide. These remained unliberated - under the conditions of the Petri dish! - until the conidiophore collapsed. The chlamydospores, however, were formed in lower numbers than normally - probably an adaptation to the permanently cool and wet conditions of this site, where no adverse season demanding resting spores occurs.

Monacrosporium cionopagum (Dr.) Subramanian (1963).

The fungus was isolated from plant debris in the canary pine *(Pinus canariensis)* stand of La Esperanza/Tenerife, a substrate which yielded more species than any other site or substrate. The isolate shows the typical broadly spindel shaped conidia which developed singly on the conidiophores (fig.3) After falling to the substrate some of the conidia started to germinate. The trapping organs were typical sticky branches of one to three cells - sometimes two branches formed a small two dimentional loop as shown in fig. 3.

Monacrosporium cystosporum Cooke and Dickinson (1965).

This species was found once in the debris of canary pine needles brought onto the banana-fields near Puerto de la Cruz/Tenerife. This material yielded mostly *Arthrobotrys* of species with few other isolates in between. The isolate had the typical distaly rounded spores (fig.4), which, however, were slightly smaller than described in the literature: 32.6 x 17.2 μm (22.6 - 39.4 x 11.7 - 24.8)

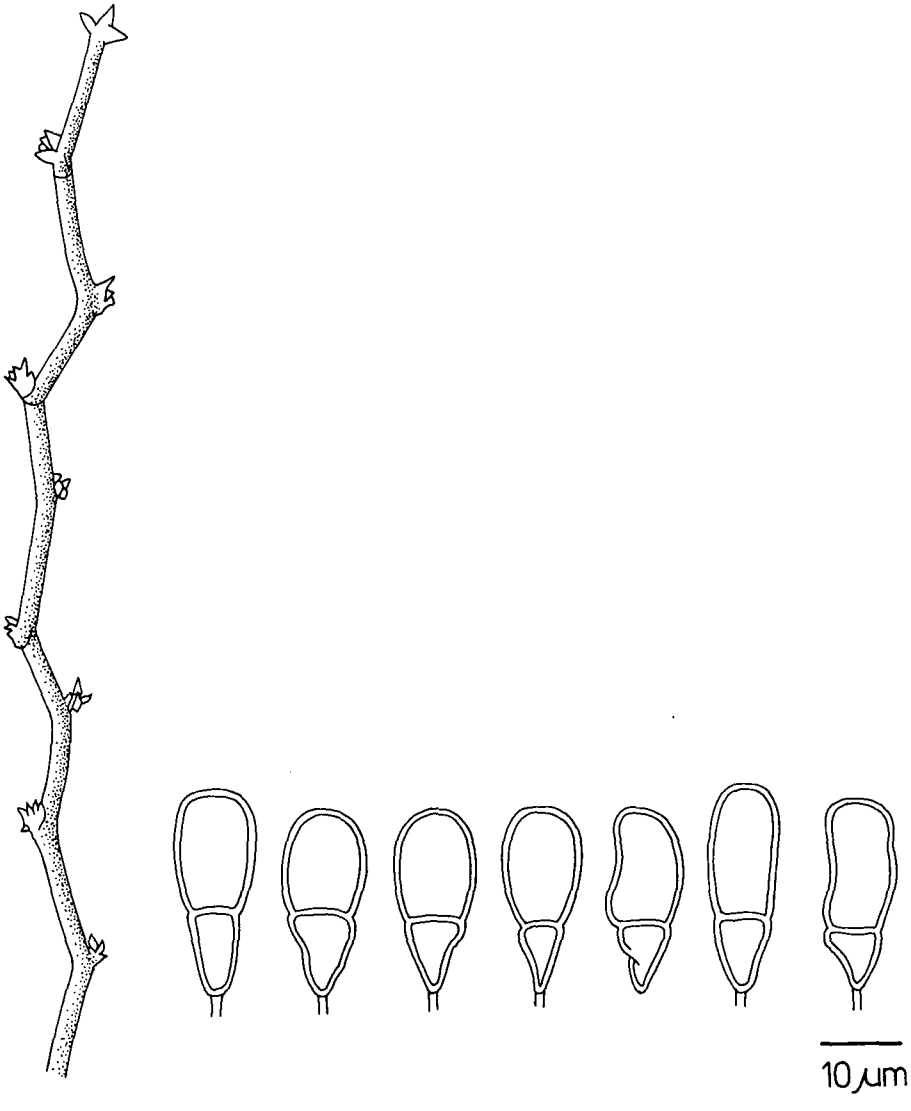

Fig. 2: *Arthrobotrys superba* strain 12-5: Conidia and part of the conidiophor which changes directions at every whorl.

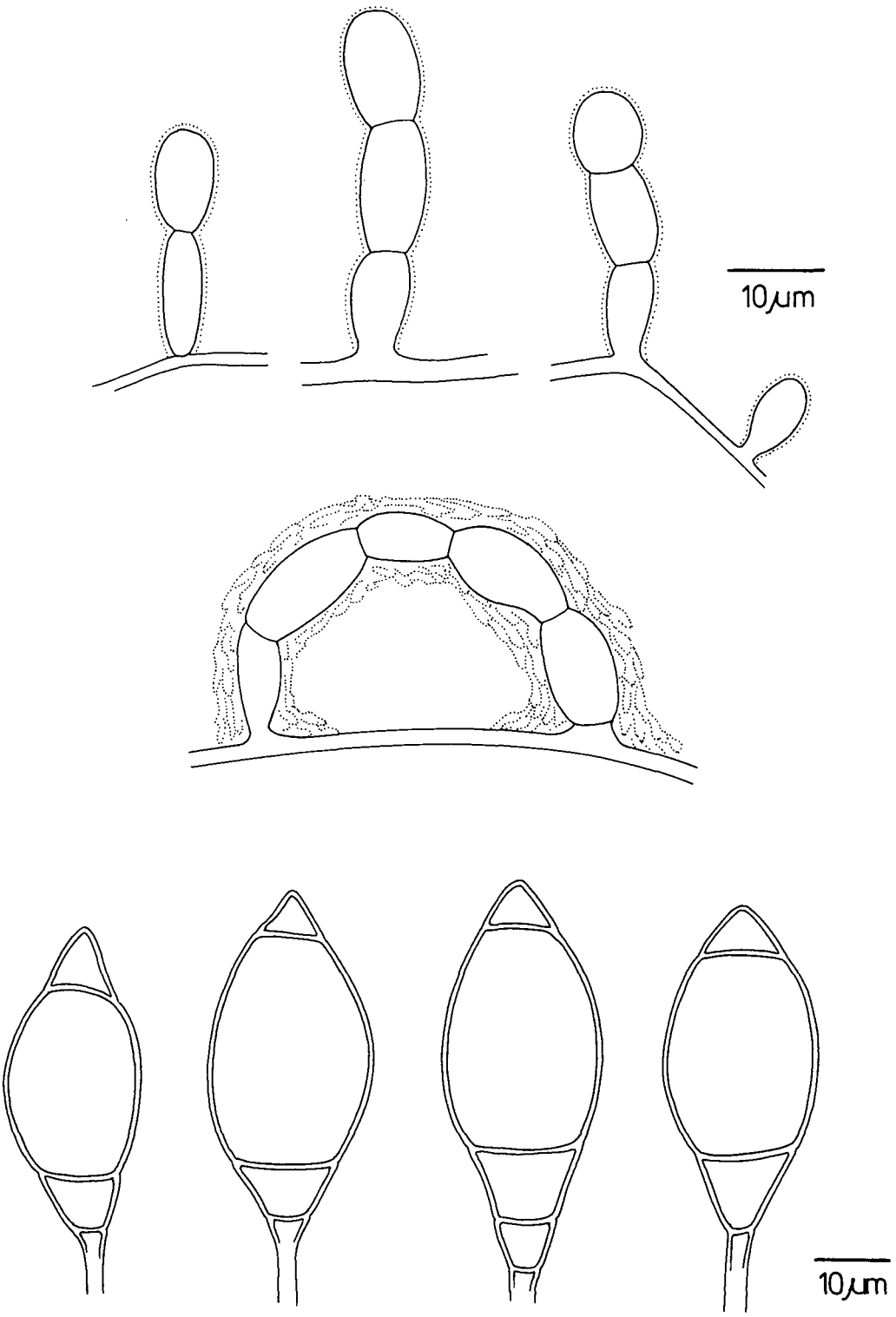

Fig. 3: Monacrosporium cionopagum strain 7-1: Sticky branches (sticky knobs) with one, two or three cells and a sticky loop formed by five cells anastomosing with the original hyphae. The dots mark the sticky layer of the trapping organs.

vs. 32.5 - 50 x 14 - 22.5 μm. The were 1-3 septate. The conidia were borne singly on the conidiophores.

Typical of this species are the very large and complex sticky networks with which it traps nematodes: "saepe in raetia larga auctos" according to COOKE and DICKINSON (1965). Such a network is also given in fig. 4 - unfortunately not in the description by COOKE and DICKINSON (1965).

Monacrosporium gephyropagum (Dr.) Subramanian (1963).

This species - which to some extent is similar to *M. cionopagum.* occurred in a very typical form together with *M.cionopagum* in the debris layer of the canary pine woods of La Esperanza. It differed from the former species by its smaller and obviously narrower conidia - which also started to germinate sometimes on the agar.

Its trapping organs also differed markedly from the former species: The sticky branches were more than three celled and sometimes branched. After fixing and consuming nematodes, the isolate often started again to form more and more complex trapping organs, resulting in multicellular complex structures. Fig. 5 gives the range from simple few-celled sticky branches to complex 3-dimensional structures.

Monacrosporium lysipagum (Dr.) Subramanian (1963).

This predacious fungus was found only in the *Sphagnum* cushions of the permanently wet and totally undisturbed volcanic caldera on Fajal. This species is remarkable as it forms two types of trapping organs: non-constricting stalked rings and sticky knobs (fig.6). The latter, remarkably, were found still active in cultures of one and more years. These old cultures also show typical pearl-like chains of chlamydospores.

The conidiophores are unbranched and form one terminal spindle-shaped conidium of 47 - 58.5 x 8 - 11.3 μm. This length tends to the upper values given by COOKE and GODFREY (1964): 28 - 55 μm, while the width covers the lower part of the range given.

Monacrosporium megalosporum (Dr.) Subramanian (1963).

This isolate is only cf. identified: The conidia are in some cases broader than given by the authors and normally not rounded distally (See fig.7). On the other hand M.*megalosporum* is the species with the widest spores and no other species described has as broad conidia: 1*) The isolate also developed large and complex networks which was not stressed in the descriptions. The question as to whether this isolate resembles a hitherto nor yet found subspecies of *M.megalosporum* cannot be answered here.

1*) 51.8(44.1 - 66.2) x 32.9 (19.7 - 42.8) unobserved vs. 57.5 - 70 x 24 - 35 μm described by COOKE and DICKINSON (1965), while DRECHSLER gives 40 - 75 x 18 - 35 μm. This isolate thus resembled more the description by DRECHSLER (1954) - also in the number of septa per conidium.

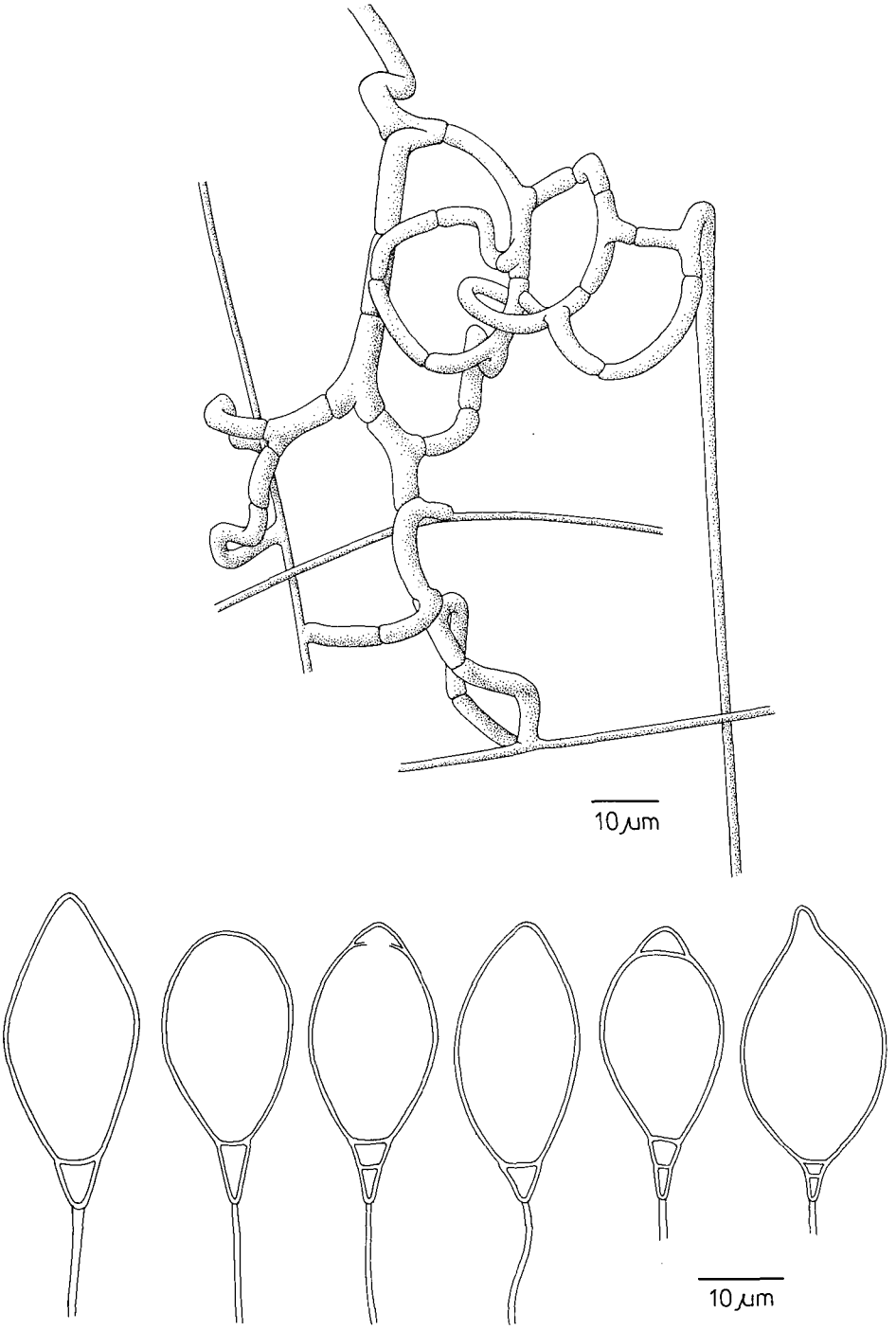

Fig. 4: *Monacrosporium cystosporum* strain 12-3: Given are the complex three dimensional sticky networks extending between different hyphae; and the typical conidia.

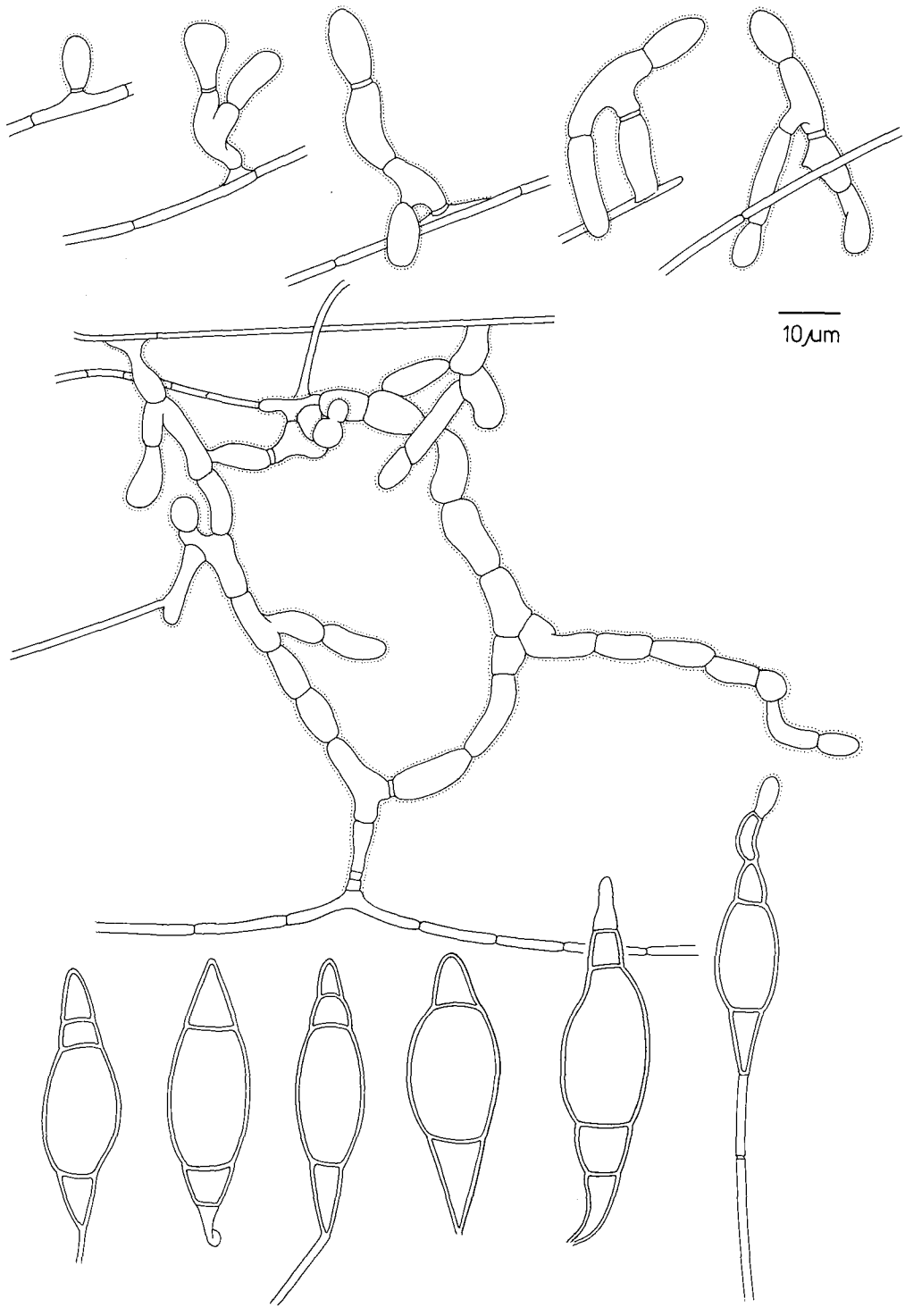

Fig. 5: *Monacrosporium gephyropagum* strain 13-1: Series of sticky branches with one to six cells and a complex network developed after digestion of nematodes (the dots mark the sticky layer) and typical conidia - one germinating with a sticky germ-tube.

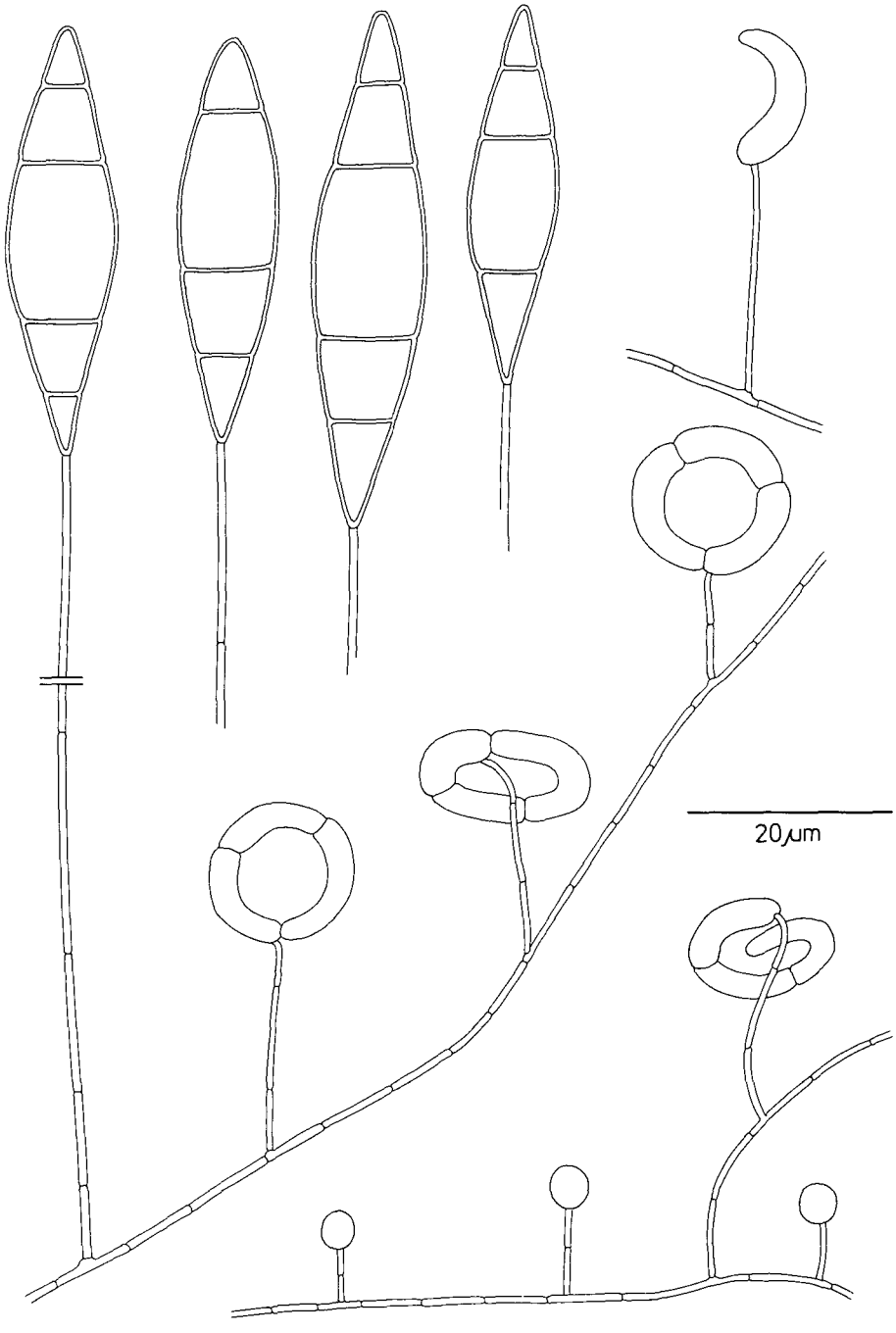

Fig. 6: *Monacrosporium lysipagum*: Conidiophore and the two types of trapping organs: non-constricting rings and sticky knobs.

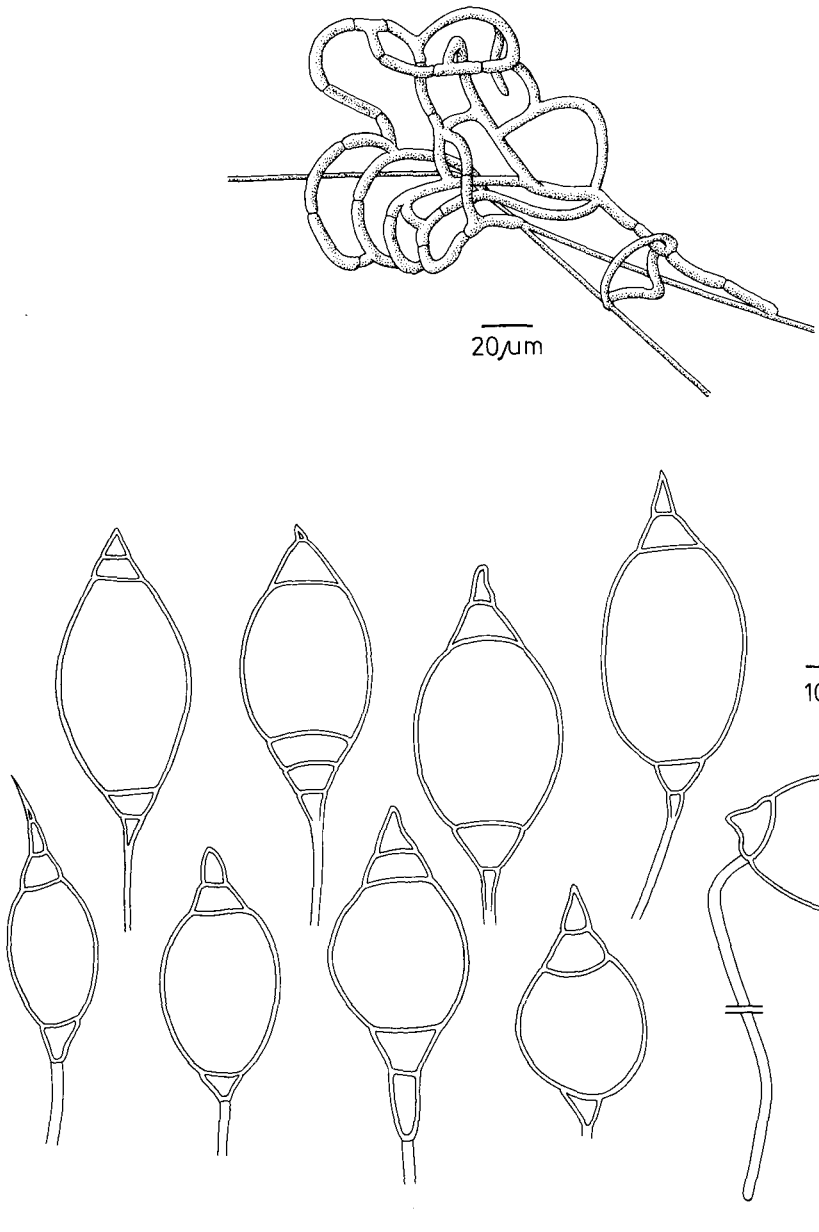

Fig. 7: Three- dimensional sticky network and typical conidia of *Monacrosporium megalosporum* strains 12-1. One conidium shows germination tubes.

Monacrosporium parvicollis (Dr.) Subramanian (1963).

The isolate resembles the form of this species described by DRECHSLER. The narrow four - to six-celled conidia which develop singly on the conidiophore are 59.5 - 70.1 x 11.2 x 14.7 μm large. After being liberated they germinate forming sticky knobs (Fig.8), even when laying on the mycelium of the parent colony This is interpreted as a mode of distribution, since the eelworms to which these sticky germination tubes adhere take them away and hence translocate the fungus. The trapping organs are small, sticky branches of few cells, which sometimes anastomose with their origin hyphae or another one laying nearby. From the microscopic observation it also becomes obvious that often the nematodes are trapped at the front region as shown in fig. 8 and - for *M. cionopagum* - in fig. 3, suggesting an attraction substance.

Nematoctonus leiosporus Drechsler (1941).

This endoparasitic species also occurred several times in the La Esperanza woods, but was also found on Fayal, in the *Sphagnum* cushions near the mouth of the ancient vulcano. Spore form, size and the development of a sticky germ tube after being liberated as well as the clamp connections found in the conidiophores correspond to the descriptions and to the isolates found by other authors (see fig.9).

A second *Nematoctonus* species was also found in La Esperanza. This species, however, demands further studies, as it does not correspond exactly to any species given by THORN and BARRON (l986).

DISCUSSION

The list of species isolated shows a comparatively rich flora of predacious fungi - which doubtless will be enlarged with further studies. Thus these isolates - together with the findings of predacious fungi in the Antarctic by GRAY (1982) show that most of the members of the genera *Arthrobotrys, Monacrosporium,* or *Harposporium* are cosmopolitan - and may occur independently from the climatic or the vegetational character of the studied regions. On the other hand the occurrence of strains/isolates which do not represent the typical form according to the description - e.g. for *Monacorporium megalosporum* - may indicate that slightly different forms may have evolved due to geographic isolation. The species of *Nematoctonus* which could not yet be identified also points into this direction. Further studies hence will pay more attention to this point.

ACKNOWLEDGEMENTS

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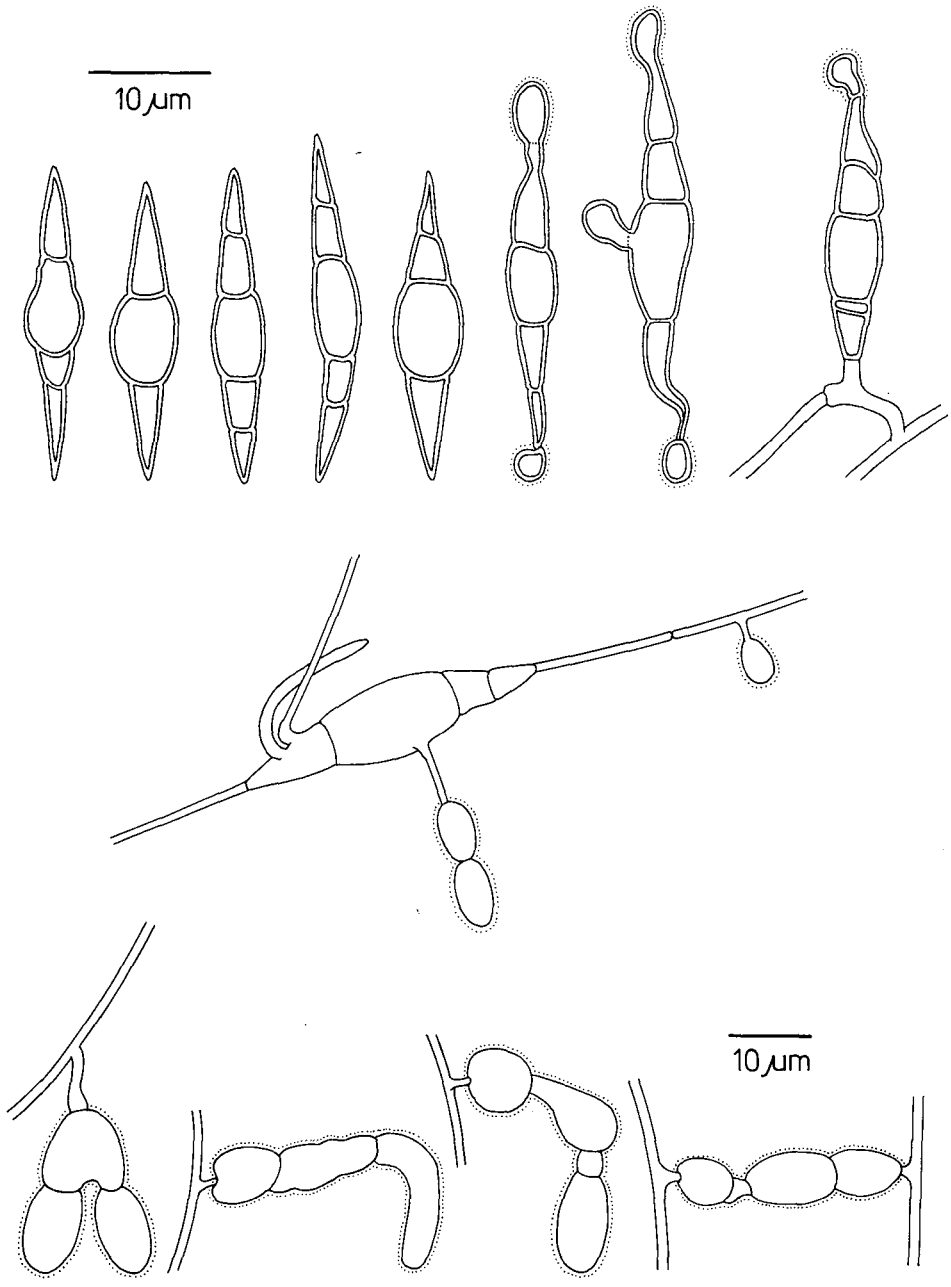

Fig. 8: *Monacrosporium parvicollis* strain 7-5: Range of typical conidia - some with germination tubes forming sticky knobs or branches - and mycelia. In the lower part several sticky knobs ar shown.

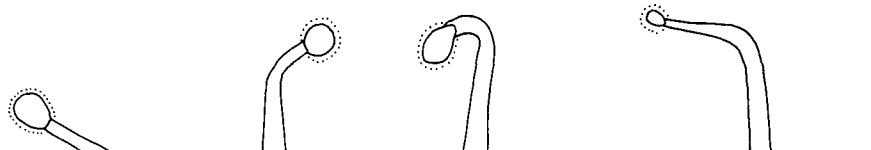

Fig. 9: *Nematoctonus leiosporus* strains 7-4: Typical conidia germinated with a sticky knob - the right one still fixed to the conidiophore, wich also has a clamp connection.

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