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Further investigations on the occurrence and distribution of endophytic fungi in tropical plants

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Abstract

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Endophytic fungi were isolated from 36 tropical plants belonging to the Pteridophyta, Araceae, Bromeliaceae, Orchidaceae, Piperaceae and Crassulaceae. A list of the fungus taxa isolated is presented and some noteworthy isolates are briefly described.

Introduction

Endophytic fungi have hitherto been isolated from a number of evergreen and annual phanerogams (Petrini *et al.* 1979). The presence of endophytes in tropical climbing or epiphytic plants, e.g. representatives of the families Araceae, Bromeliaceae and Orchidaceae, was demonstrated by Petrini & Dreyfuss (1981). As a continuation of the latter study, we extended our investigations to other tropical members of the same plant families; two representatives of the genus *Peperomia* Ruiz et Pav., one *Kalanchoe*-species (Crassulaceae) as well as some fern species have also been included in the present investigation. The aim was to broaden our knowledge on the occurrence, ecology, and taxonomy of endophytic fungi.

Material and methods

All plants considered in this study were collected either in the highlands of Columbia or from the Amazon Basin near Leticia (Columbia) (Tab. 1).

The samples were collected in polyethylene bags and immediately mailed to the laboratory, where they were tagged and photographed for later identification. The isolation of endophytes was carried out five to eight days after collection. Healthy leaves, stems, and in some instances roots, were removed and processed according to the methods described by Petrini & Dreyfuss (1981). The incubation of the surface-sterilized plant fragments on 2% malt extract agar [MA, supplemented with 50 mg/l Terramycin (Pfizer)] was carried out at 21 °C and outgrowing fungal colonies were transferred onto MA-slants and incubated at 21 °C. From several hundred isolates, a relatively small number was selected arbitrarily for further investigations. The selected

Tab. 1. Tentative taxa and collecting sites of the plants studied.

Sample, nr.	Tentative taxon, family	Growing habitat	Collecting site
	Pteridophyta		
1	*Asplenium serratum* L.	epiphytic	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
2	*Elaphoglossum* sp. J. Sm.	ground	Columbia, near Bogota, moist Paramo vegetation
3	*Polypodium astrolepis* Liebm.	epiphytic	Columbia, Cundinamarca, La Union
4	*P. ciliatum* Willd.	epiphytic	Leticia, Columbia
5	*P. lanceolatum* L.	epiphytic	Columbia, Cundinamarca, La Union
	Araceae		
6	*Anthurium* sp. Schott	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
7	*Anthurium* sp. Schott	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
8	*Philodendron* sp. Schott	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
9	*Philodendron* sp. Schott	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
10	*Philodendron* sp. Schott	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
11	undet.	tree-climbing	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
12	undet.	tree-climbing	Columbia, Restrepo Valley, Departemento de Valle
	Bromeliaceae		
13	*Guzmania* sp. Ruiz et Pav.	epiphytic	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
14	*Tillandsia* sp. L.	epiphytic	Columbia, Cundinamarca, La Union
15	*Vriesea* sp. Lindl.	epiphytic	Columbia, Cundinamarca, La Union
16	*Tillandsia complanata* Benth.	epiphytic	Columbia, La Cumbre, Departemento de Valle
17	*Tillandsia complanata* Benth.	epiphytic	Columbia, Restrepo Valley, Departemento de Valle
	Crassulaceae		
18	*Kalanchoe* sp. Adans.	ground	Columbia, Cundinamarca, La Union
	Orchidaceae		
19	*Epidendron porpax* Reichb.	epiphytic	Columbia, Restrepo Valley, Departemento de Valle
20	*Epidendrum* sp. L.	epiphytic	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
21	*Epidendrum* sp. L.	epiphytic	Leticia, Columbia
22	*Maxillaria* sp. Ruiz et Pav.	ground	Columbia, near Bogota, moist Paramo vegetation
23	*Maxillaria* sp. Ruiz et Pav.	epiphytic	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
24	*Maxillaria* sp. Ruiz et Pav.	epiphytic	Brazil, Quebrada Urumatum, Tabatinga (near Leticia)
25	*Maxillaria* sp. Ruiz et Pav.	epiphytic	Leticia, Columbia
26	*Pleurothallis* sp. R. Br.	epiphytic	Columbia, Restrepo Valley, Departemento de Valle

Continued Tab. 1

Sample, nr.	Tentative taxon, family	Growing habitat	Collecting site
27	undet.	epiphytic	Columbia, Cundinamarca, La Union
28	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
29	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
30	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
31	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
32	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
33	undet.	epiphytic	Columbia, La Cumbre, Departemento de Valle
34	undet.	epiphytic	Columbia, Restrepo Valley, Departemento de Valle
	Piperaceae		
35	*Peperomia* cf. *reflexa* A. Dietr.	epiphytic	Columbia, Cundinamarca, La Union
36	*Peperomia* sp. Ruiz et Pav.	epiphytic	Columbia, Restrepo Valley, Departemento de Valle

strains were simultaneously propagated on fresh MA-slants, 90 mm Petri dishes with 20 ml MA and potato-dextrose-agar-slants (PDA, Difco) and incubated at 15 °C and 24 °C in the dark or under near UV-light. Microscopic examination of the fungal strains was carried out after two to six weeks of incubation.

Results and discussion

A synopsis of the fungal species isolated, from their various hosts, is presented in tab. 2. As we considered only an arbitrary selection of isolates, and only those sporulating under a limited number of culture conditions, we will not attempt to evaluate our findings statistically and we will only point out the most evident distribution patterns.

The presence of endophytic fungi in ferns is noteworthy, but this finding is not unexpected. Boullard (1957, 1979) discussed the endophytism within ferns, and Peterson et al. (1981) recently studied the possible significance of a fungal endophyte in *Psilotum*. The occurrence of endophytes is, thus, not confined to the phanerogams: they seem to be quite common also in pterydophytes, and Schuster (1966) already discussed their presence in some liverworts.

Some fungi (e.g. *Acremonium* spp., *Fusarium* spp.) are confined almost exclusively to the roots, while others (e.g. *Pestalotia* spp., *Colletotrichum* spp.) can be isolated only from aerial plant organs: this supports the assumption that the endophytic fungi are specialized to some extent for different parts of the infected plant (Oberholzer 1982).

Colletotrichum gloeosporioides, Colletotrichum spp., *Fusarium solani,* other *Fusarium* spp., and *Lasiodiplodia theobromae* are known pathogens of tropical plants, and strains belonging to these species can cause severe damage and losses in forests and plantations (Gray 1981). Their occurrence in symptomless plants may be an important but overlooked aspect of plant pathology and epidemiology (Petrini & Dreyfuss 1981).

Tab. 2. A synopsis of the fungal species with regard to their host origin. Isolates from the roots are marked by an asterisk. Numbers refer to the host plants cited in tab. 1: nr. 1–5, Pterydophyta; nr. 6–12, Araceae; nr. 13–17, Bromeliaceae; nr. 18, Kalanchoe sp. (Crassulaceae); nr. 19–34, Orchidaceae; nr. 35, 36, Piperaceae.

Fungus taxa	Recorded host(s)
Ascomycetes	
Anthostomella aracearum Petrini & Dreyfuss	3, 18, 36
Guignardia sp.	3, 19*
Phaeosphaeria eustoma (Fuck.) Holm	3
Phomatospora berkeleyi Sacc.	2, 3, 25
Deuteromycetes	
a) Hyphomycetes	
Acremonium furcatum (F. & R. Moreau) Gams	5
Acremonium pteridii Gams & Frankland	8, 12*
Acremonium strictum Gams	6, 9, 11, 23, 29*, 34*
Acremonium Anam. *Wallrothiella subiculosa* v. Höhn.	33*
Acremonium sp.	6, 29
Chrysosporium sp.	2
Cladosporium tenuissimum Cooke	5
Cylindrocarpon didymum (Hartig) Wollenw.	18
Cylindrocarpon lucidum Booth	18
Daldinia sp. (Anamorph)	28
Dicyma cf. olivacea (Emoto & Tubaki) v. Arx	36
Fusarium oxysporum Schl.	19*, 28*, 29*, 32*, 35
Fusarium sambucinum Fuck.	22*, 31*
Fusarium solani (Mart.) Sacc.	9
Fusarium trichothecioides Wollenw.	28, 28*
Gliocladium roseum Bain.	29*
Hadronema sp.	32*
Hypoxylon mummularium Bull. ex Fr. (Anamorph)	8, 25, 32
Nigrospora sphaerica (Sacc.) Mason	36
Nodulisporium spp.	1, 4, 9, 25
Phialophora lignicola (Nannf. ap. Melin & Nannf.) Goidanich	4
Phialophora spp.	9, 35
Rhizoctonia spp.	24
Scopulariopsis candida (Guéguén) Vuill.	2
Tubercularia sp.	23*
Verticillium cf. psalliotae Treschow	2
Xylaria spp. (Anamorph)	1, 3, 4, 5, 6, 13, 17, 18, 20, 23, 25, 26, 27, 29, 31, 33, 35, 36
Zygosporium cf. echinosporum Bunt. & Mason	18
b) Coelomycetes	
Ascochyta sp.	18, 21
Botryodiplodia piperina Syd.	35
Clypeopycnis sp.	3
Colletotrichum gloeosporioides (Penz.) Sacc.	7, 10, 11, 20
Colletotrichum spp.	4, 8, 13, 14, 15, 20, 33*
Coniothyrium sp.	4, 29
Corniculariella cf. harpographoidea (Dearn.) DiCosmo	15
Cryptocline spp.	3, 7, 10, 15, 16, 20, 26, 31, 32
Cryptosporiopsis sp.	18, 36
Diplodia sp.	14

Continued Tab. 2

Fungus taxa	Recorded host(s)
Epicoccum purpurascens Ehrenb. ex Schlecht	18
Harknessia sp. 36	36
Lasiodiplodia theobromae (Pat.) Griff. et Maub.	4, 20, 25, 30*
Leptodothiorella sp.	10
Libertella sp.	3
Pestalotia calabae West.	18, 35, 36
Pestalotia cf. heterocornis Guba	6, 8, 9, 10, 13, 20, 23, 29, 31, 33
Phaeoseptoria cf. vermiformis Pun. et Wall.	21
Phoma sp.	2, 3, 18, 21
Phomopsis filicina H.C. Greene	3
Phomopsis sp.	3, 6, 8, 13, 23, 36
Pleurophomopsis sp.	35
Sporonema sp.	14

Considerations on some noteworthy or taxonomically interesting isolates

1. *Botryodiplodia piperina* Syd. = *Macrophoma piperina* Syd. (Fig. 1c)

Colony fast growing, attaining 80 mm diameter in 14 d at 15 °C on MA; aerial mycelium abundant, olive-black; colony reverse black. Conidiomata are formed within three weeks and are spherical, brown, pycnidial or stromatic, thin-walled, with or without opening, solitary or gregarious, variable in size. Conidiophores cylindrical, hyaline, unbranched, and aseptate, up to 15 × 5 µm. Conidiogenous cells holoblastic. Conidia large, hyaline, very thick-walled, 27–37 × 13–16 µm. On *Peperomia* cf. *reflexa* A. Dietr.

This fungus fits quite well the description given by Petrak & Sydow (1927) for *Botryodiplodia piperina* Syd., although the conidia of our isolate are larger. Our fungus clearly belongs to *Botryodiplodia* sensu Sydow; however, it is questionable, whether or not *B. piperina* Syd. and our isolate can be assigned to the form-genus *Botryodiplodia* sensu Sutton (1980). *Cytosphaera* Died. could be an alternative, but a careful study of the types is required before any decision can be made.

2. *Dicyma* cf. *olivacea* (Emoto & Tubaki) von Arx (Fig. 1b)

Colony very fast growing, up to 80 mm in 14 d at 15 °C on MA, readily sporulating, aerial mycelium grey to green with white patches. Colony reverse pale yellow to grey. Conidiophores mononematous, long, brown, regularly branched, with a long sterile hyphal elongation terminating as a clavate, sterile process.

Conidiogenous cells on branches, polyblastic, integrated, terminal, or discrete, sympodial, cylindrical to clavate, denticulate. Conidia solitary, hyaline to subhyaline, slightly rough, dry, simple, ellipsoidal, 2–5 × 2–3 µm. On *Peperomia* sp.

A characterization of the genus *Dicyma* Boul., as well as some taxonomic considerations on related genera can be found in von Arx (1982).

3. *Hadronema* sp. (Fig. 1a)

Colony fast growing, attaining 80 mm diameter in 14 d at 15 °C on MA, readily sporulating, aerial mycelium grey, abundant; conidiomata acervular, small, discoidal, dark brown, up to 0.5 mm across. Conidiogenous cells holoblastic, integrated, terminal. Conidia solitary, simple, coarsely verrucose, dark brown, spherical to ellipsoidal, dry, 13–16 × 11–16 µm. On roots of undetermined Orchidaceae. This isolate could well

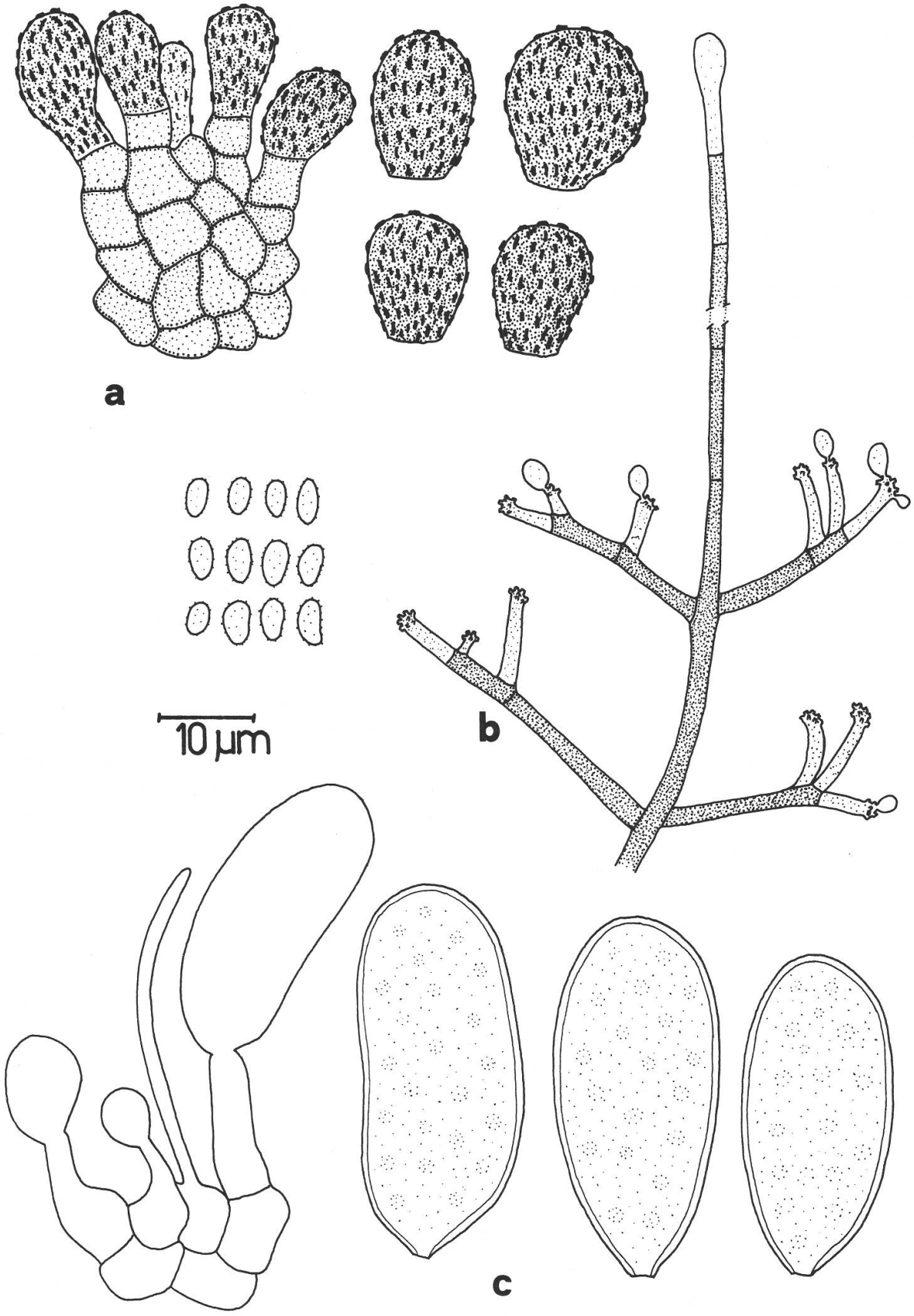

Fig. 1. Conidiophores and conidia of
a) *Hadronema* sp., b) *Dicyma* cf. *olivacea* and c) *Botryodiplodia piperina*.

be a new species; its rare occurrence (it was isolated only once) as well as the absence of conidiomata on the host are the reasons why we refrain from describing a new species.

4. Zygosporium cf. *echinosporum* Bunting et Mason (Fig. 2)

Colony moderately fast growing, attaining 50 mm diameter in 14 d at 15 °C on MA, at 24 °C 80 mm in 14 d. Conidiophores scattered, unbranched, brown, smooth, with one or two vesicles borne on long stalks, with a sterile apical region terminating in a swollen, hyaline bulb 3–5 µm diam. Vesicles 9–13 µm long, 5.5–8 µm thick in their broadest part. Conidiogenous cells holoblastic, polyblastic, ampulliform, often slightly curved, sympodial, hyaline, with distinct scars on a short rachis. Conidia hyaline, ellipsoidal to guttulate, with a truncate base, 4–6 × 2.5–4 µm. On *Kalanchoe* sp.

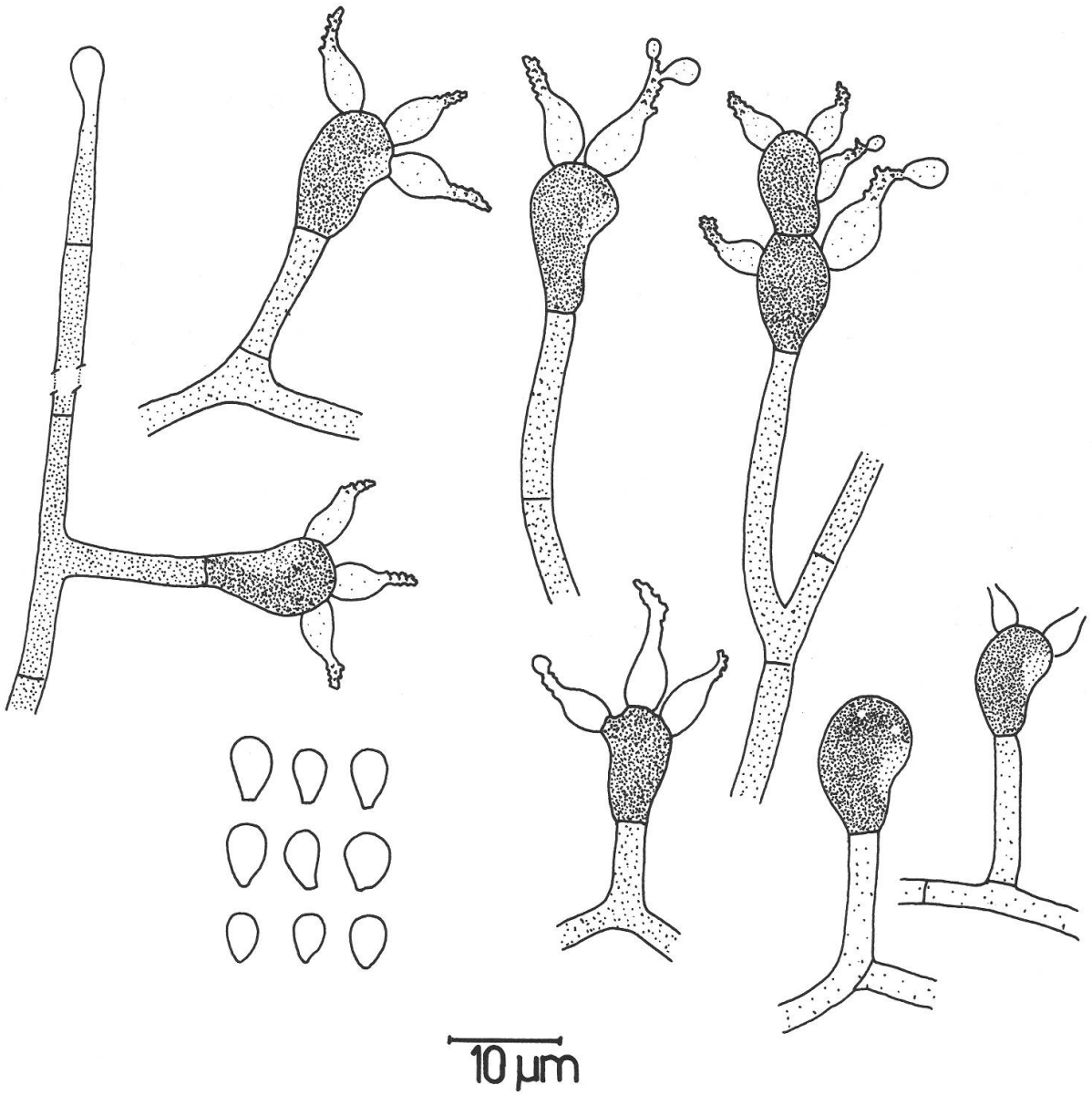

Fig. 2. Conidiophores and conidia of *Zygosporium* cf. *echinosporum*.

This fungus was constantly associated with *Anthostomella aracearum* Petrini et Dreyfuss. Any attempt to produce cultures from single spores of *A. aracearum* or *Zygosporium* failed. Petrini & Dreyfuss (1981) described *Acremoniula*-like chlamydospores in cultures of *A. aracearum* which are strongly reminiscent of the vesicles of *Zygosporium* cf. *echinosporum* Bunting & Mason. An anamorph-teleomorph connection between *Zygosporium* cf. *echinosporum* and *Anthostomella aracearum* is thus very likely, although not directly proven.

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