



APPENDIX

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
SP1	GROUP	2.954	8	0.369	100.428	0.000
	SEASON	0.191	5	3.821E-02	10.391	0.000
	SUBSTRATE	.277	3	9.248E-02	25.151	0.000
	2-Way Interactions (Combined)	2.585	79	3.273E-02	8.900	0.000
	GROUP *					
	SEASON	.369	40	9.213E-03	2.506	0.000
	GROUP *					
	SUBSTRATE	2.066	24	8.610E-02	23.416	0.000
	SEASON *					
	SUBSTRATE	.150	15	1.002E-02	2.726	0.000
	3-Way Interactions					
	GROUP *					
	SEASON *	1.048	120	8.731E-03	2.374	0.000
SUBSTRATE						
Residual		1.986	540	3.677E-03		
Total		9.041	755	1.198E-02		

SP1 by GROUP, SEASON, SUBSTRATE

C.congesta

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR1	PART	3.641E-02	2	01.821E-02	3.891	0.030
	SEASON	1.489E-02	2	7.444E-03	1.591	0.218
2-Way Interactions	PART *					
	SEASON	5.6953E-02	4	1.424E-02	3.042	0.029
Residual		0.168	36	4.679E-03		
Total		0.277	44	6.289E-03		

GR1 by PART, SEASON

C.congesta

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR2	PART	2.2986	2	1.493	86.017	0.000
	SEASON	2.262	2	1.131	65.162	0.000
2-Way Interactions	PART *					
	SEASON	1.945	4	0.486	28.008	0.000
Residual		0.625	36	1.736E-02		
Total		7.818	44	0.178		

GR2 by PART, SEASON

C.congesta

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR4	PART	0.372	2	0.186	60.350	0.000
	SEASON	0.160	2	8.023E-02	26.040	0.000
2-Way Interactions	PART *					
	SEASON	4.013E-02	4	1.003E-02	3.257	0.022
Residual		0.111	36	3.081E-03		
Total		0.683	44	1.553E-02		

GR4 by PART, SEASON

C.congesta

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR8	PART	5.093E-02	2	2.546E-02	13.491	0.000
	SEASON	4.519E-03	2	2.260E-03	1.197	0.314
2-Way Interactions	PART *					
	SEASON	5.968E-03	4	1.492E-03	0.791	0.539
Residual		6.794E-02	36	1.887E-03		
Total		0.129	44	2.940E-03		

GR8 by PART, SEASON

C.congesta

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR10	PART	2.822	2	1.411	227.508	0.000
	SEASON	3.341E-02	2	1.670E-02	2.693	0.081
2-Way Interactions	PART *					
	SEASON	0.105	4	2.613E-02	4.212	0.007
Residual		0.223	36	6.202E-03		
Total		3.183	44	7.235E-02		

GR10 by PART, SEASON

F.benghalensis

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR2	PART	0.314	2	0.157	16.904	0.000
	SEASON	0.255	2	0.128	13.720	0.000
2-Way Interactions	PART *					
	SEASON	9.513E-02	4	2.378E-02	2.557	0.055
Residual		0.335	36	9.302E-03		
Total		1.000	44	2.272E-02		

GR2 by PART, SEASON

F.benghalensis

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR7	PART	7.464E-02	2	3.732E-02	25.609	0.000
	SEASON	6.790E-03	2	3.395E-03	2.330	0.112
2-Way Interactions	PART *					
	SEASON	1.258E-02	4	3.146E-03	2.159	0.093
Residual		5.246E-02	36	1.457E-03		
Total		0.146	44	3.329E-03		

GR7 by PART, SEASON

F.benghalensis

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR9	PART	0.805	2	0.402	118.180	0.000
	SEASON	2.562E-02	2	1.281E-02	3.761	0.033
2-Way Interactions	PART *					
	SEASON	2.519E-02	4	6.298E-03	1.849	0.141
Residual		0.123	36	3.406E-03		
Total		0.978	44	2.224E-02		

GR9 by PART, SEASON

F.benghalensis

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR4	PART	0.348	2	0.174	31.075	0.000
	SEASON	0.240	2	0.120	21.477	0.000
2-Way Interactions	PART *					
	SEASON	8.552E-02	4	2.138E-02	3.819	0.011
Residual		0.202	36	5.599E-03		
Total		0.876	44	1.990E-02		

GR4 by PART, SEASON

F.benghalensis

ANOVA^a

		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
GR6	PART	0.106	2	5.308E-02	3.710	0.034
	SEASON	4.858E-02	2	2.429E-02	1.698	0.197
2-Way Interactions	PART *					
	SEASON	0.107	4	2.683E-02	1.875	0.136
Residual		0.515	36	1.431E-02		
Total		0.777	44	1.766E-02		

GR6 by PART, SEASON

Two new endophytic conidial fungi from India

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(Received, accepted)

Abstract — Two new taxa of endophytic conidial fungi isolated from fresh young leaves of *Carissa carandas* L. are described and illustrated. These include a new anamorph-genus *Kumbhamaya* and a new species of *Gonatobotryum*. © 2000 Adac / Éditions scientifiques et médicales Elsevier SAS

Anamorphs / new conidial fungi / hyphomycetes / taxonomy / endophytes / biodiversity / India

INTRODUCTION

The discovery of endophytic fungi has attained significance in recent years because they sometimes elaborate secondary metabolites useful in biocontrol, or in industry or as pharmaceuticals (Bills, 1995). During our investigation of the taxonomy and biology of endophytic fungi occurring in leaves of *Carissa carandas* L. (*Apocynaceae*), an evergreen shrub growing on lateritic soils on the west coast of India, several fungi were isolated in culture from fresh leaves gathered from Taleigao and Verna Plateau in Goa, among which the following two taxa of conidial fungi are believed to be new to science. They are described and illustrated below.

MATERIALS AND METHODS

Methods discussed by Petrini (1986) for isolation of endophytic fungi were followed in this work. Five randomly chosen disease-free mature and young leaves of *C. carandas*, freshly gathered and thoroughly washed in tap water, were surface sterilized, first in 70 % ethanol for 30 s, then in 4 % Sodium hypochlorite for 1 min. and finally in 70 % ethanol for 30 s. The surface sterilized leaves were cut into 1 mm² size pieces using a sterile razor blade. About 12 pieces were aseptically placed equidistant in rows in a 10 cm diam. Petri plate containing antibiotic incorporated malt extract agar medium (composition in 1 L : 5 g malt extract, 20 g agar, 1000 ml distilled water, 0.02 g each of bacitracin, neomycin, penicillin, polymixin, streptomycin and tetracycline). The process was repeated for other leaves. The plates were incubated at 23 °C. As the fungal colonies appeared from the edges of the leaf bits and extended into agar medium, generally after 5 – 7 days, these were aseptically transferred to agar slants.

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RESULTS AND DISCUSSION

Several interesting and rare fungi were recovered from leaves and the following two conidial fungi are described as new taxa.

Kumbhamaya* M.Jacob et D.J. Bhat *anam. gen. nov.

(Etym. Sanskrit: *Kumbha* = kettle or pitcher-shaped [= pyriform] phialide; *maya* = numerous)

Ad fungos conidiales, hyphomycetes pertinens. Coloniae effusae, brunneae ad atro-brunneae. Mycelium densum, ramosum, septatum, ex hyphis brunneis ad atro-brunneis constans. Conidiophora mononematosa, indistincta, septata. Cellulae conidiogae monophialidicae, in conidiophoris non-ramosis terminales incorporatae, lageniformes, vel laterales, pyriformes, ad basim elongatae, erectae ad curvatae, crassitunicatae, laeves, brunneae vel atrobunneae, collari prominente et expanso, ad basim constrictae. Conidia solitaria, fusiformia, curvata vel sigmoidea, utrinque acutata, sursum rostrata, saepe ad basim recurvata, hyalina, crassitunicata, laevia, 1–3-septata, in massis mucosis aggregata.

Typus *Kumbhamaya indica* M.Jacob & D.J. Bhat.

Conidial fungi, hyphomycetes. Colonies effuse, medium to dark brown. Mycelium dense, with branched, thickly septate, medium to dark brown hyphae. Conidiophores mononematous, indistinct, septate. Conidiogenous cells monophialidic, integrated, unbranched, terminal or intercalary, kettle- to pitcher-like (= pyriform), often with an elongated base, straight to curved, thick-walled, smooth, medium to dark brown, with a distinct and flared collarete, constricted and narrow at the base. Conidia solitary, fusiform, curved to sigmoid, pointed at both ends, sharply beaked at the tip, often slightly recurved at the base, hyaline, thick-walled, smooth, colourless, 1–3-septate, aggregating in a slimy mass.

Several genera of mononematous hyphomycetes with conspicuous phialides bearing flared collarettes and producing elongate and nonsetulate conidia in slimy heads are known (Bhat and Kendrick, 1993; Carmichael *et al.* 1980; Ellis, 1971, 1976; Matsushima, 1971, 1975). These include *Craspedodidymum* Holubova-Jechova (1972), *Dischloridium* Sutton, *Fusarium* Link, *Fusariella* Sacc., *Phialophora* Medlar and *Phialomyces* Misra & Talbot (Carmichael *et al.*, 1980). In *Craspedodidymum*, the apically inflated phialides with large funnel-shaped collarete, produce brown, non-septate conidia. In *Dischloridium*, the phialides lack a conspicuous collarete and the conidia are non-septate. In the moniliaceous genus *Fusarium*, the branched conidiophores terminating in apical or lateral phialides produce globose to obclavate, nonseptate, hyaline microconidia and fusiform, curved, septate, hyaline, apically and basally pointed macroconidia which superficially resemble those of *Kumbhamaya*. The hyaline phialides in *Fusarium* however are inconspicuous. In *Fusariella*, the phialides lack a conspicuous collarete and the conidia are fusiform, septate, curved, medium brown and produced in linearly arranged false chains. In *Phialophora*, the phialides are small and the conidia are nonseptate, ellipsoidal and inconspicuous. In *Phialomyces*, although the phialides typically have a flared collarete, the conidia are not only globose but also in chains. *Kumbhamaya* differs from all these genera in that the kettle or pitcher-shaped (= pyriform) phialides bearing conspicuous and flared collarete produce fusiform, curved septate, hyaline conidia which are pointed at both ends and they are never in chains.

***Kumbhamaya indica* M.Jacob et D.J. Bhat *anam. sp. nov.* (Figs 1, 2)**

Coloniae in agarco maltoso effusae, reverso atrobunneo. Mycelium aeri densum, hyphae ramosae, crassiseptatae, medio-brunneae vel atrobunneae, 2.2–7.5 latae. Conidiophora mononematosa, indistincta, septata, 2.2–2.5 µm diam. Celli

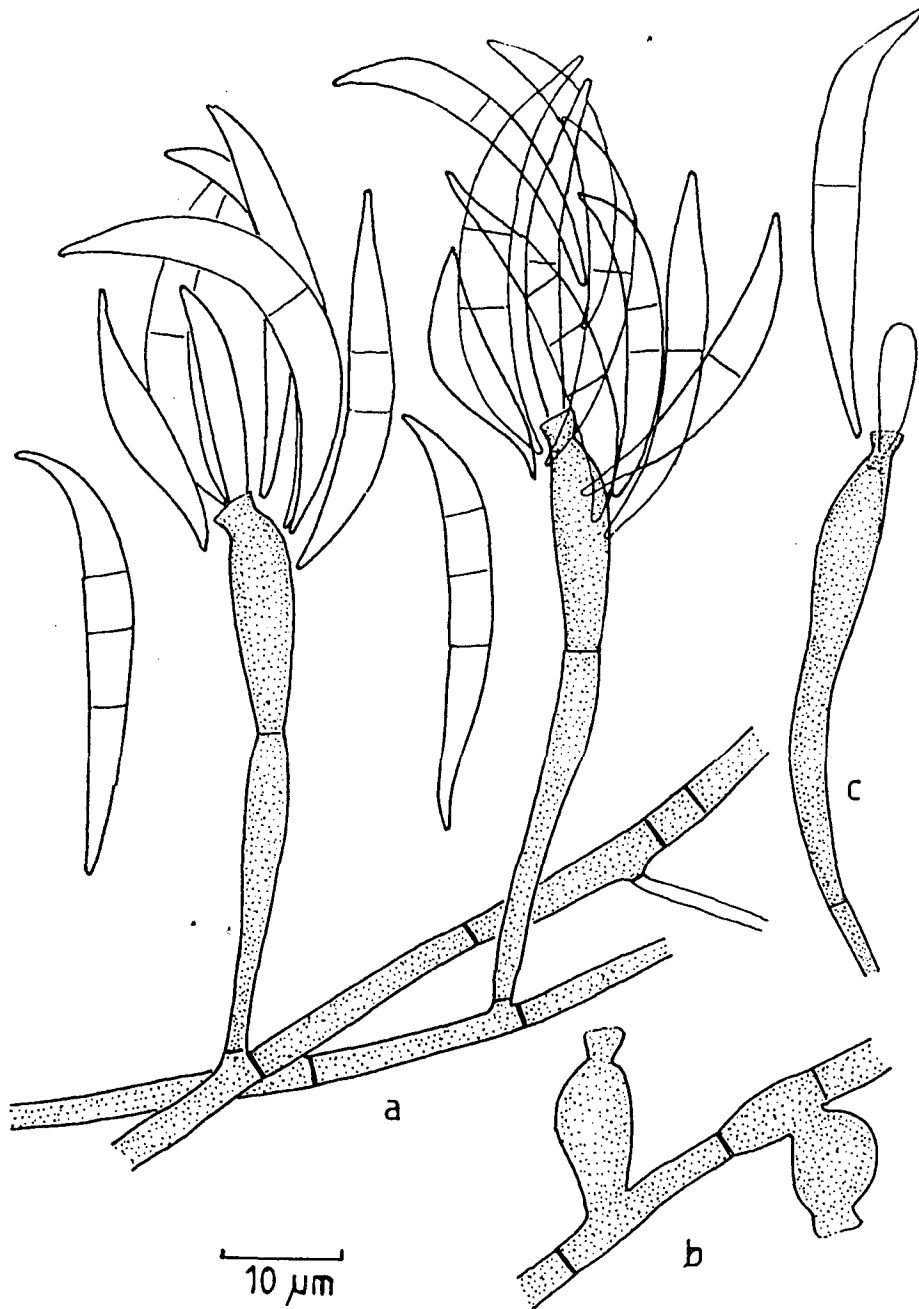


Fig. 1.a – c : *Kumbhamaya indica*. a : Note the slimy conidial mass at the tip of the phialides. b : Conidiophores. c : Conidial development.

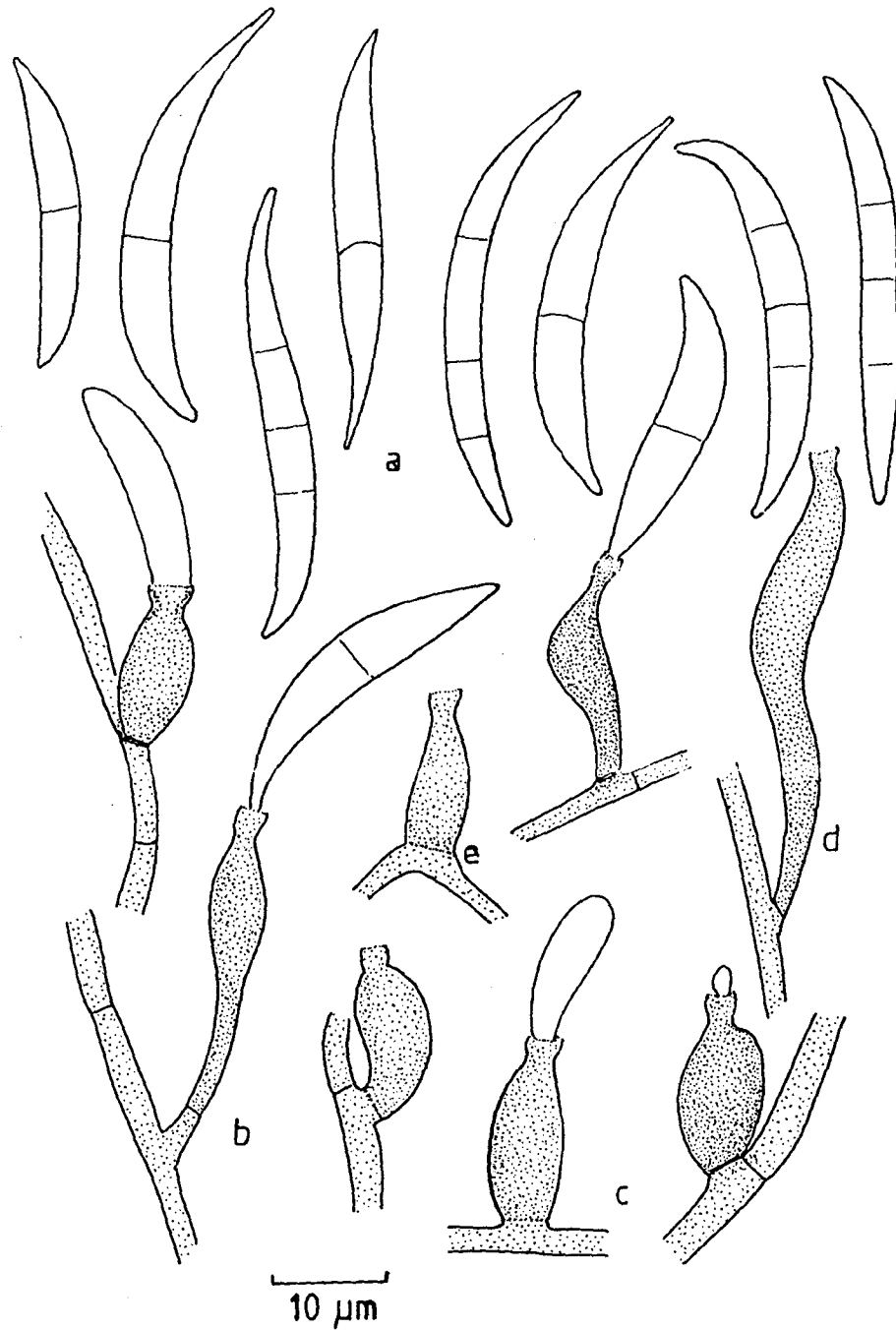


Fig. 2.a - e: *Kumbhamaya indica*. a: Conidia. b: Conidiophore with mature conidia. c - d: Conidiophores showing variations in size. e: Phialide showing wide collarette.

conidiogenae monophialidicae, in conidiophoris incorporatae, terminales vel laterales, pyriformes ad vermiformes, ad basim elongatae, rectae vel curvatae, crassitunicatae, laeves, brunneae vel atrobrunneae, 12 – 50 µm longae et ad basim 2.5 – 4.5 µm latae, in medio 5.5 – 8.5 µm latae, collari prominente et expanso 2 – 3.5 × 1.5 – 2.5 µm. Conidia solitaria, fusiformia, curvata vel sigmoidea, utrinque acuta, falciformia, sursum rostrata, ad basim saepe recurvata, hyalina, crassitunicata, laevia, 1 – 3-septata, 25 – 40 × 3.5 – 5.5 µm, in massis mucosis aggregata.

Colonies on malt extract agar effuse, with moderately dense aerial mycelium, irregular at margin, medium brown at the centre, pale brown towards the periphery, 2 – 3.5 cm diam. in 7 days; reverse of the colony dark brown. **Mycelium** densely branched, with thick septa, medium to moderately dark brown hyphae 2.2 – 7.5 µm wide. **Conidiophores** mononematous, indistinct, septate, 2.2 – 2.5 µm diam. **Conidiogenous cells** monophialidic, integrated, terminal or lateral, vase-like (= pyriform) to vermiform, often with an elongate base, straight to curved, thick-walled, smooth, medium to dark brown, 12 – 50 µm long and 2.5 – 4.5 µm wide at the base, 5.5 – 8.5 µm wide in the middle, with a distinct and flared collarete 2 – 3.5 × 1.5 – 2.5 µm, constricted and narrow at the neck region. **Conidia** solitary, fusiform, curved to sigmoid, pointed at both ends, sharply beaked at the tip, occasionally recurved at the base, hyaline, thick-walled, smooth, mostly 3-septate, rarely 1-septate, 25 – 40 × 3.5 – 5.5 µm, aggregating in a slimy mass at the apex of the phialide.

Holotypus: Slide prepared from a dried culture grown on MEA medium and isolated from tip of young leaf of *Carissa carandas*, 14.11.1997, Verna Plateau, Goa, M. Jacob Herb. No. GUFCC-0233, PC; **Paratype**: Dried culture of the fungus grown on MEA medium and isolated from mature leaf of *Bambusa sp.*, 24.2.1999, Mollem, Goa, Maria D'Souza, Herb. No. GUFCC-0238. Living cultures of same are also maintained at Goa University Culture Collection.

Gonatobotryum himorphosporum M. Jacob et D.J. Bhat sp. nov. (Fig. 3)

Coloniae in agar maltoso effusae, atrobrunneae, floccosae. **Mycelium** partim superficiale, partim immersum, hyphis marginem versus modo rhizoideorum compactis, septatis, ramosis, laevibus, 4.5 – 10 µm latis. **Conidiophora** distincta, mononematosa, recta vel flexuosa, brunnea, septata, non-ramosa, laevia, 175 – 550 µm longa, 6.2 – 10 µm lata, percurrenter elongascentes, in nodis terminalibus vel intercalariibus echinulata, ampullae conidiogenae 13.5 – 45 × 15 – 22 µm. **Cellulae conidiogenae** polyblasticae, integratae, terminales vel intercalares, sphaericae vel subsphaericae, loci conidiogeni prominentes truncati. **Conidia** catenata, 0-septata, laevia, subhyalina vel brunnea, ad basim cicatricem ferentia, duarum formarum: conidia primaria e cellulis conidiogenis oriunda, elongato-ellipsoidea vel elongato-obclavata, 1 – 3 locos conidiogenos ferentia, 7.5 – 11.5 × 3 – 4.5 µm; conidia secundaria ex conidiis primariis oriunda, ellipsoidea, basipetalia, 3 – 6.2 × 2.5 – 3.5 µm.

Colonies in malt extract agar effuse, moderately fast growing, dark brown, with irregular margin, with rough surface, with faint concentric zones, attaining 4 – 4.5 cm diam. after 7 days. **Mycelium** partly immersed, partly superficial, often compacted into root-like aggregates towards the periphery of the colony, with septate, repeatedly branched, smooth-walled, pale brown, hyphae 4.5 – 10 µm wide. **Conidiophores** distinct, mononematous, flexuous to erect, brown, septate, unbranched, smooth, 175 – 550 µm long, 6.2 – 10 µm wide, percurrently regenerating, nodose and distinctly echinulate in the terminal and intercalary conidiogenous ampullae 13.5 – 45 × 15 – 22 µm. **Conidiogenous cells** polyblastic, integrated, terminal or intercalary, spherical to subspherical, ampullae with slightly raised and truncate conidiogenous loci. **Conidia** catenate, 0-septate, smooth, pale to moderately brown, cicatrized at the base, of two types. First-formed conidia

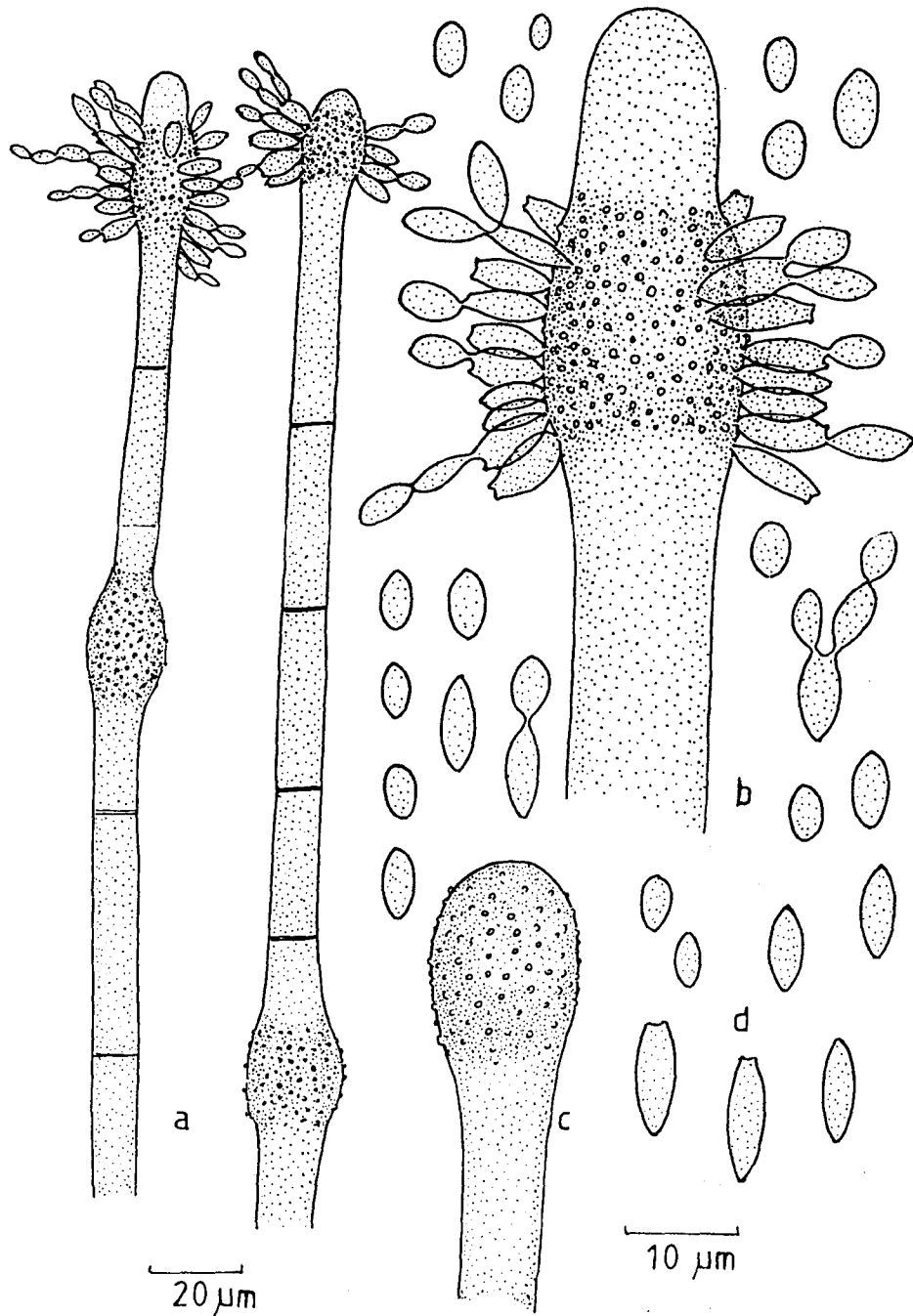


Fig. 3.a - d: *Gonatobotryum bimorphosporum*. a: Conidiophore with conidia. b: Polyblastic conidiogenous cells with 2 distinct type of conidia. c: Conidiogenous ampullae showing cicatrized scars. d: First formed and later formed conidia

arising synchronously and directly from conidiogenous cells, elongate-ellipsoidal to elongate-obclavate, with 1–3 apical conidiogenous loci, $7.5 - 11.5 \times 3 - 4.5 \mu\text{m}$; later formed conidia arise from first-formed conidia, ellipsoidal, in branched basipetal chains, $3 - 6.2 \times 2.5 - 3.5 \mu\text{m}$.

Holotypus : Slide prepared from dried agar culture mat of the fungus isolated from fresh leaves of *Carissa carandas*, 22.11. 1998, Taleigao Plateau, M. Jacob Herb. No. GUFCC 0398, PC. Living culture of same maintained at Goa University Culture Collection.

The genus *Gonatobotryum* Saccardo, with *G. fuscum* (Sacc.) Sacc. as type species, is characterized by mononematous conidiophores producing integrated, terminal or intercalary, percurrent and polyblastic conidiogenous cells and catenate conidia (Carmichael *et al.*, 1980; Ellis, 1971). The species so far described in the genus, viz. *G. fuscum* (Ellis, 1971), *G. apiculatum* (Peck) Hughes (Ellis, 1971) and *G. indicum* Manjal and Gill (Manjal and Gill, 1963) are compared with *G. bimorphosporum* (Tab. 1). *G. apiculatum*, *G. fuscum* and *G. indicum* are saprophytic, parasitic or hyperparasitic and produce only one type of conidia whereas *G. bimorphosporum* is endophytic and produces two morphologically distinct types of conidia.

Tab. 1. Comparison of morphology and habitat of the species of *Gonatobotryum*

Species	Conidiophore	Ampullae	Conidia	Habitat Substrate/Host Distribution
<i>G. fuscum</i> (Sacc.) Sacc.	up to 700 μm long and 12–15 μm wide; Percurrently proliferating	up to 28 μm diam.	One type ; 10–25 \times 6–13 μm , Catenate, ellipsoidal to oblong, rounded at both ends.	Saprophytic on rotten wood of <i>Quercus</i> and <i>Fagus</i> ; Hyperparasitic on <i>Ceratocystis</i> and other fungi ; Europe and America.
<i>G. apiculatum</i> (Peck) Hughes	200–300 \times 6–9 μm	9–15 μm diam.	One type ; 5–12 \times 2.5–6 μm In branched chains, limoniform.	Parasitic on leaves of <i>Hamamelis</i> ; also recorded on <i>Rhus</i> and soil ; N. America.
<i>G. indicum</i> Manjal & Gill	250–420 \times 7–9 μm	11–17 μm diam.	One type ; 5.5–9 \times 3.5–5 μm Ovate to oblong, One end broadly obtuse, the other tapering.	Saprophytic on dead twigs ; India.
<i>G. bimorphosporum</i> <i>sp. nov</i>	175–560 \times 6–10 μm	15–22 μm diam.	Two types : First formed conidia elongate–ellipsoidal to obclavate ; 7.5–11.2 \times 2.9–3.7 μm . Later-formed conidia ellipsoidal ; 3.2–6.2 \times 2.5–3.7 μm	Endophyte in leaves of <i>Carissa carandas</i> <i>L.</i> ; India.

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