

***Echinospaeria macrospora* sp. nov., teleomorph of *Vermiculariopsiella endophytica* sp. nov.**

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Abstract – *Echinospaeria macrospora* is a novel endophyte isolated from stems of *Centella asiatica* (Apiaceae/Umbelliferae) with its novel, hyphomycetous anamorph, *Vermiculariopsiella endophytica*. The fungus first produced the conidial state, followed by development of its teleomorph after 4 weeks of incubation. *Echinospaeria macrospora* differs from the type of this monotypic genus, *E. canescens*, in having wider asci and larger ascospores. This is the first report of a sexual state amongst the species of *Vermiculariopsiella* and the third asexual stage of *Echinospaeria*.

Anamorph-teleomorph connection / perithecium / Helminthosphaeriaceae / sporodochia / Western Ghats

INTRODUCTION

Fungi are pleomorphic, i.e., they are capable of producing more than one form or type of spore in their life cycle (Sugiyama, 1987; Cai *et al.* 2005; Fernández & Huhndorf, 2004, 2005; Huhndorf & Fernández, 2005). The complete lifecycle of many fungi is poorly understood and therefore anamorph-teleomorph connections as and when established, attain significance. One such anamorphic genus with hitherto unknown teleomorph is *Vermiculariopsiella* Bender (Bender, 1932).

During studies on biodiversity of microfungi of the Western Ghat forests in Goa, India (Pratibha *et al.*, 2005; Shenoy *et al.*, 2005), we isolated a novel species of hyphomycetous, endophytic fungus, *Vermiculariopsiella endophytica*, from living stems of *Centella asiatica*. The fungus in culture produced its sporodochial conidial state in 4 days. After one month of incubation at 23-25 °C, perithecia developed in small groups on tiny, inconspicuous, stromatic base amongst the sporodochia. The ascocarp, asci and ascospores of the teleomorph were similar to *Echinospaeria* A.N. Mill. & Huhndorf (Miller & Huhndorf, 2004). The anamorph and teleomorph distinctly differ from hitherto known species in respective genera and therefore are described as novel taxa, in this paper. This is the first report of sexual state amongst the species of *Vermiculariopsiella* and the third asexual stage of *Echinospaeria*.

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MATERIALS AND METHODS

Isolation of the fungus from host tissue

Fresh stem and leaves of *Centella asiatica* were processed for isolation of endophytic fungi following the procedure described by Petrini & Fisher (1986). The surface sterilized stem and leaf tissues were cut into pieces of 0.5 cm², plated in 2% malt extract agar (MEA) medium and incubated for 7-14 days at 25°C. Fungal mycelium emerging out of cut ends of the tissue was aseptically transferred onto fresh MEA plates. The plates were incubated for over 2 months or until the fungus produced both its anamorphic and teleomorphic forms in the medium.

Confirmation of anamorph-teleomorph connection

The perithecium developed in culture was transferred onto a flame-sterilized slide and carefully dissected in a drop of sterile distilled water to separate individual ascospores. The ascospore suspension when spread on a 2% MEA plate, germinated readily. Germinated ascospores were individually transferred into slants and incubated at 25°C until sporulation effected. The anamorph developed in culture was in conformity with *Vermiculariopsisella endophytica*.

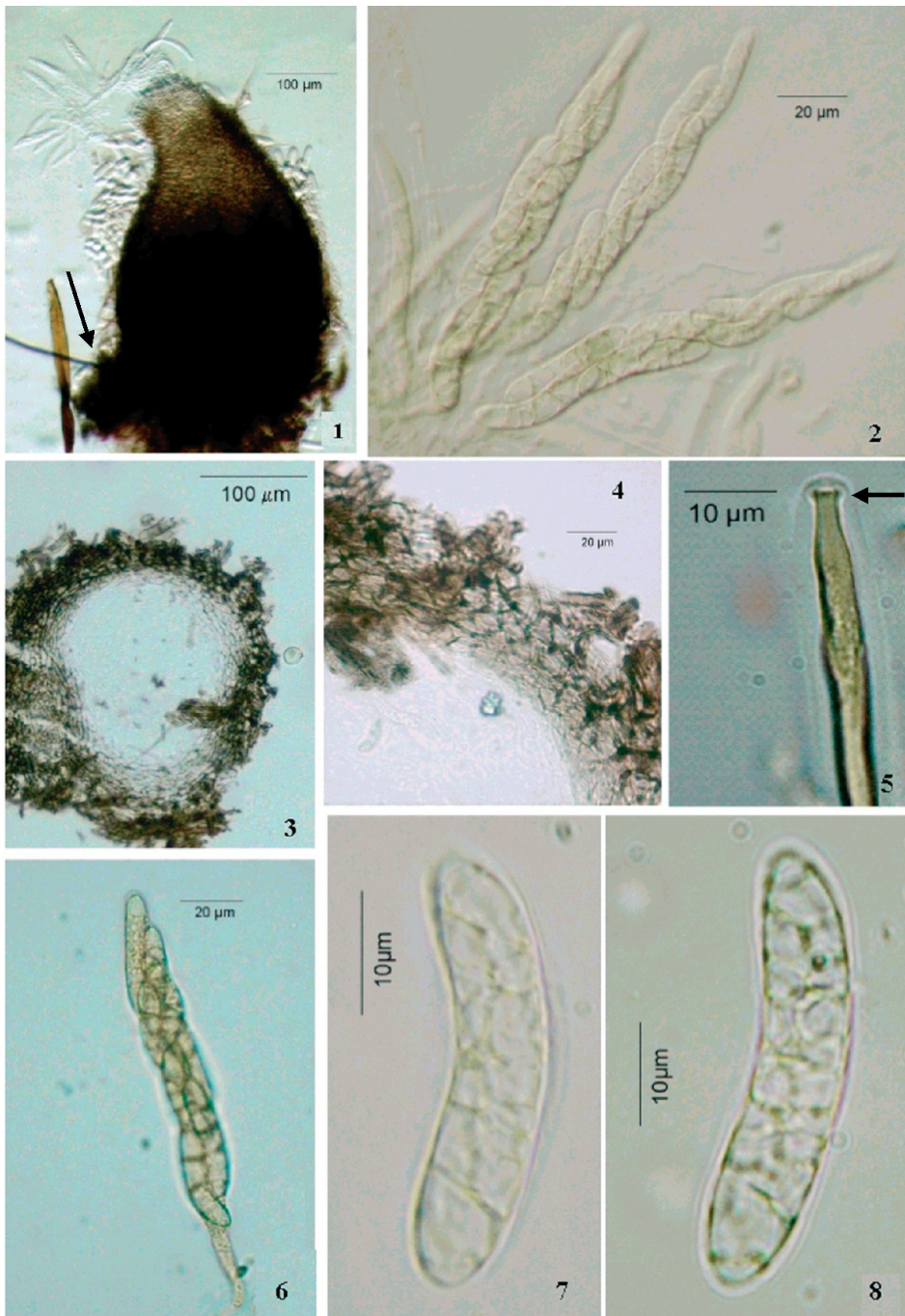
TAXONOMY

Echinospaeria macrospora Puja, Bhat & K.D. Hyde sp. nov. (Figs 1-9)

Ascocarpis peritheciis, pyriformis, gregariis, nigris, velvetis, aggregatis, cupulatis exaresco, 410-490 µm longis, 150-265 µm latit ad medius ora; oriundus brevis stromatic pessum. Ostiolis brevis, conicus, cum centralis apicalis. Peridiumii pseudoparenchymati, duo-layeri, cum angulari, leviter tenuibus cellulae. Extrenus layera atrum brunnea, cum 5-7 rows arto, pariter, profundus, angularis cellulae, 3-7-µm diametro. Penitus layera hyalinis vel subhyalinis, cum 4-6 rows arto, substrictus, parietibus tenuibus cellulae. Paraphyses absens. Asci oriundus penitus peridium pessum cellulae, octospori, clavati, unitunicati, pedicillati, 120-165 × 14-17.5 µm; leviter substricti ad apice, iodo noncoerulescenti provisi, cum emineo apice orbis. Ascosporae 41-45 × 6-11 µm allantoideae vel vermiformae, hyalinae vel subhyalinae, eseptatae, guttulateae, laevia, biseriatae.

Etymology: Larger size of the ascospores as compared to the type.

Ascomata perithecial, pyriform, 410-490 µm high, 150-265 µm wide at the middle broadest region, gregarious, often growing in groups of 2-8 on a small stromatic base, black, velvety, cupulate when dry, with short, conical, centrally located apical ostiole. *Peridium* pseudoparenchymatous, 2-layered, composed of angular, slightly flattened cells. Outer layer dark brown, with 5-7 row of compactly laid, uniformly thickened, angular cells 3-7 µm diam. Inner layer hyaline to subhyaline, with 4-6 rows of closely packed, narrow, thin-walled cells. *Paraphyses* not observed. *Asci* 120-165 × 14-17.5 µm (mean = 150 × 16 µm), arising from the basal cells of inner peridium, 8-spored, clavate, unitunicate, pedicillate, slightly narrower at the tip, nonamyloid, with conspicuous apical ring. *Ascospores* 41-45 × 6-11 µm (mean = 43 × 8 µm) allantoid to vermiform, hyaline to subhyaline, aseptate, guttulate, smooth-walled, biserially arranged in the asci.



Figs 1-8. *Echinosphaeria macrospora* 1. Ascocarp with attached anamorph (arrowed). 2. Asci. 3. Vertical section through ascoma. 4. Peridium. 5. Immature ascus with conspicuous apical ring (arrowed). 6. Ascus with biserially arranged ascospores. 7, 8. Ascospores.

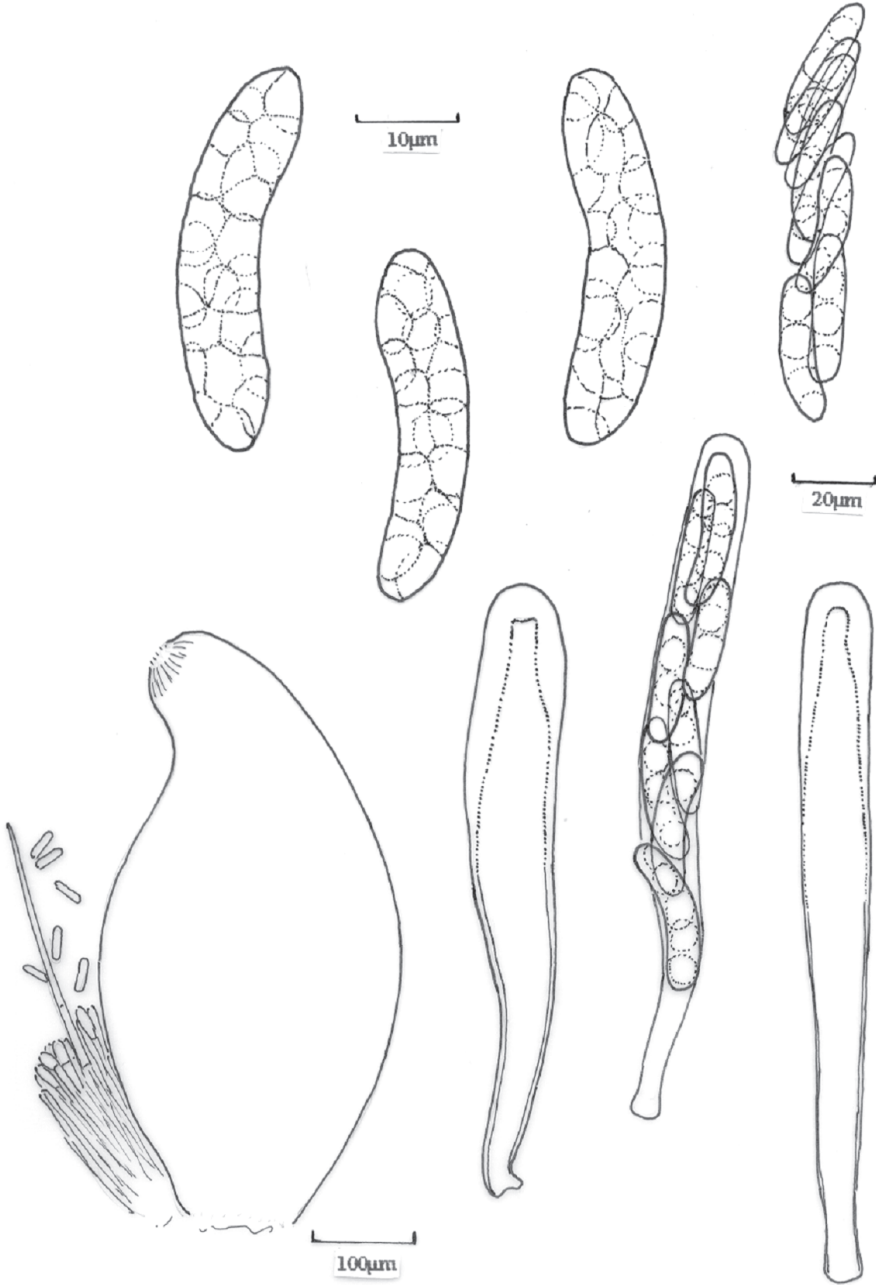


Fig 9. *Echinospaeria macrospora*. Ascocarp, asci and ascospores.

Anamorph – *Vermiculariopsiella endophytica* Puja, Bhat & K.D. Hyde sp. nov.

Habitat – *Centella asiatica*.

Known distribution – India

Holotype: INDIA, Western Ghats, Goa, Colem, endophyte in stems of *Centella asiatica*, 24 January 2005, Puja Gawas, Dried culture mat, GUBH (Goa University Botany Herbarium) No. CaEnC-3.

Vermiculariopsiella endophytica Puja, Bhat, K.D. Hyde sp. nov. (Figs 10-15)

Coloniae in vitro aliquanta vel celer proventa, pervenio 5.5-6 cm diametro in 7 dies, platy, cum irregulari vel rhizoidali labrum, pallens-albo vel pallide brunnea, inverto palide brunnea. Sporodochia oriundus brevis stromatic pessum, sparsa, cream vel peach-coloris, setosae. Conidiophora laevia, septata, parum tremes, hyalinis vel palide-coloris, 75-85 × 6-9 μm. Setae 3-15, laevia, 2-6-septata, haud-tremes, erecta vel leviter curvata ad pessum, atrum brunnea, acuminata ad apicem, 180-318 μm longis, 10-11 μm latit ad pessum, 6-7 μm latit ad medius; oriundus parietibus crassi, brunnea 5-7 μm diametro stromal cellulae. Cellulae conidiogenae monophialideae, integratae vel discretiae, sine emineo collarettae, 22-25 × 10-11 μm. Conidia solitaria, cylindrica, teres ad duo extremitas, laevia, eseptata, hyalina, 32-42 × 10-11 μm, una peach-coloris.

Etymology – Refers to endophytic nature.

Colonies moderate to fast growing in culture, attaining diam of 5.5-6 cm in 7 days, flat, with irregular to rhizoidal margin, off-white to pale brown, reverse pale brown. Sporodochia develop on small stromatic base, scattered, cream to peach-coloured, setose, with smooth, septate, sparsely branched, hyaline to pale-coloured 75-85 × 6-9 μm conidiophores; setae 3-5, smooth, 2-6-septate, unbranched, straight to slightly curved at base, dark brown, pointed at the tip, 180-318 μm long, 10-11 μm wide (mean = 240 × 10 μm) at base, 6-7 μm wide at the center; arising from basal thick-walled, brown 5-7 μm diam stromal cells. Conidiogenous cells monophialidic, integrated to discrete, 22-25 × 10-11 μm, without a conspicuous collarette. Conidia solitary, cylindrical, rounded at both ends, smooth, aseptate, hyaline, 32-42 × 10-11 μm (mean = 36 × 10.5 μm), in mass peach-coloured.

Habitat – *Centella asiatica*.

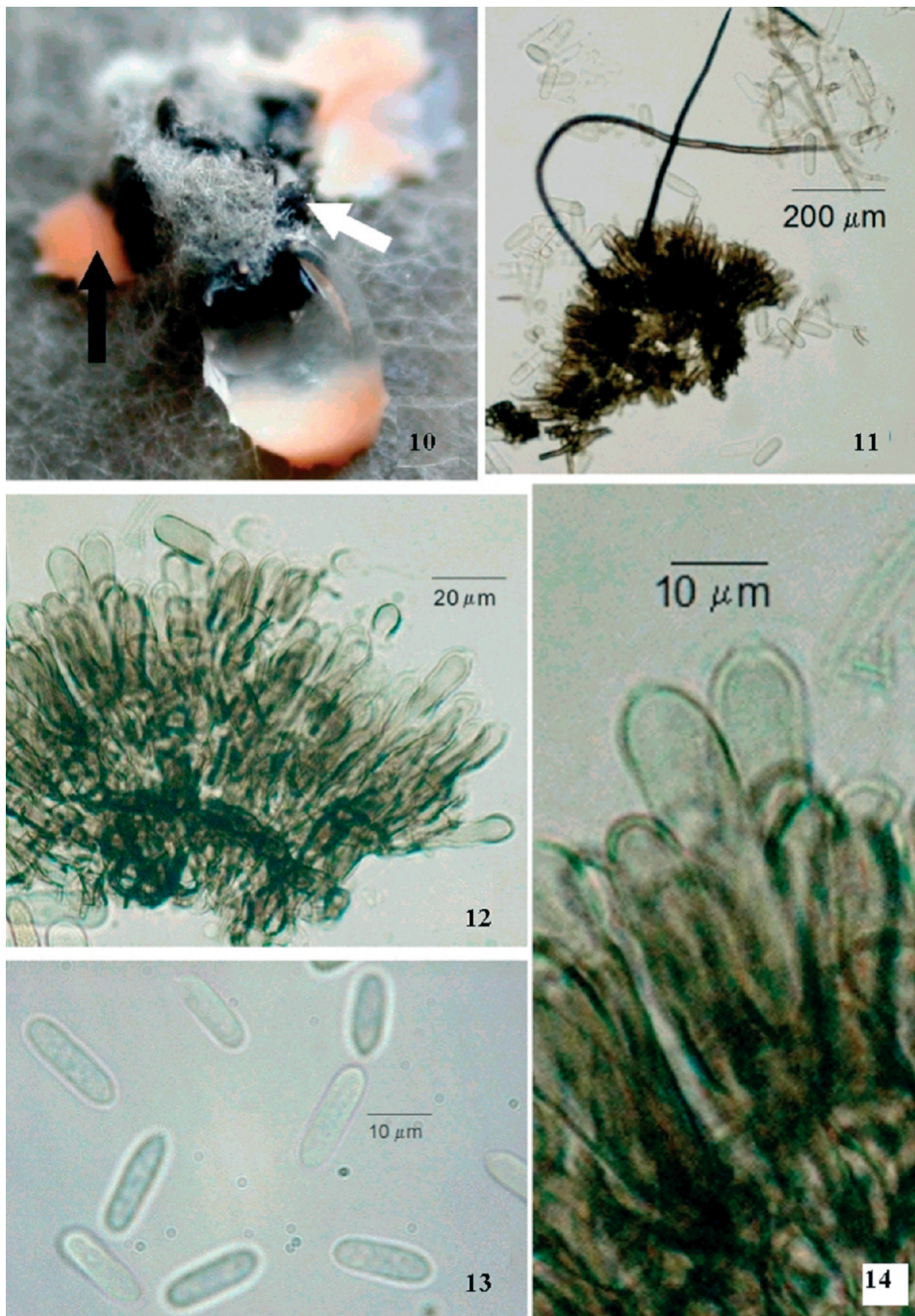
Known distribution – India

Holotype: INDIA, Western Ghats, Goa, Colem, endophyte in stems of *Centella asiatica*, 24 January 2005, Puja Gawas, Dried culture mat, GUBH No. CaEnC-3.

DISCUSSION

The phylogenetic analyses of partial nuclear large subunit (LSU) rDNA sequences have shown the “*Lasiosphaeria*-complex” to be highly polyphyletic in that species segregated into seven monophyletic clades dispersed among several orders (Miller & Huhndorf, 2004). Consequently, the generic circumscription of *Lasiosphaeria* has been narrowed, with an addition of three novel genera, *Echinosphaeria* A.N. Mill. & Huhndorf, *Hiberina* A.N. Mill. & Huhndorf and *Immersiella* (Lasiosphaeriaceae) A.N. Mill. & Huhndorf. *Echinosphaeria* has phylogenetic affinities with the members of family Helminthosphaeriaceae (Miller & Huhndorf, 2004).

The monotypic ascomycetous genus, *Echinosphaeria* is typified by *E. canescens* (Pers: Fr.) A.N. Mill. & Huhndorf. The type species is a basionym of *Lasiosphaeria canescens* (Pers.) Karst. *Mycoth. fenn.* (Helsinki) **2**: 162, 1873



Figs 10-14. *Vermiculariopsiella endophytica*. 10. Stereo-microscopic image with *V. endophytica* (black arrowed) and *E. macrospora* (white arrowed). 11, 12. Sporodochial conidiomata with setae and conidiophores. 13. Conidia 14. Phialidic conidiogenous cells

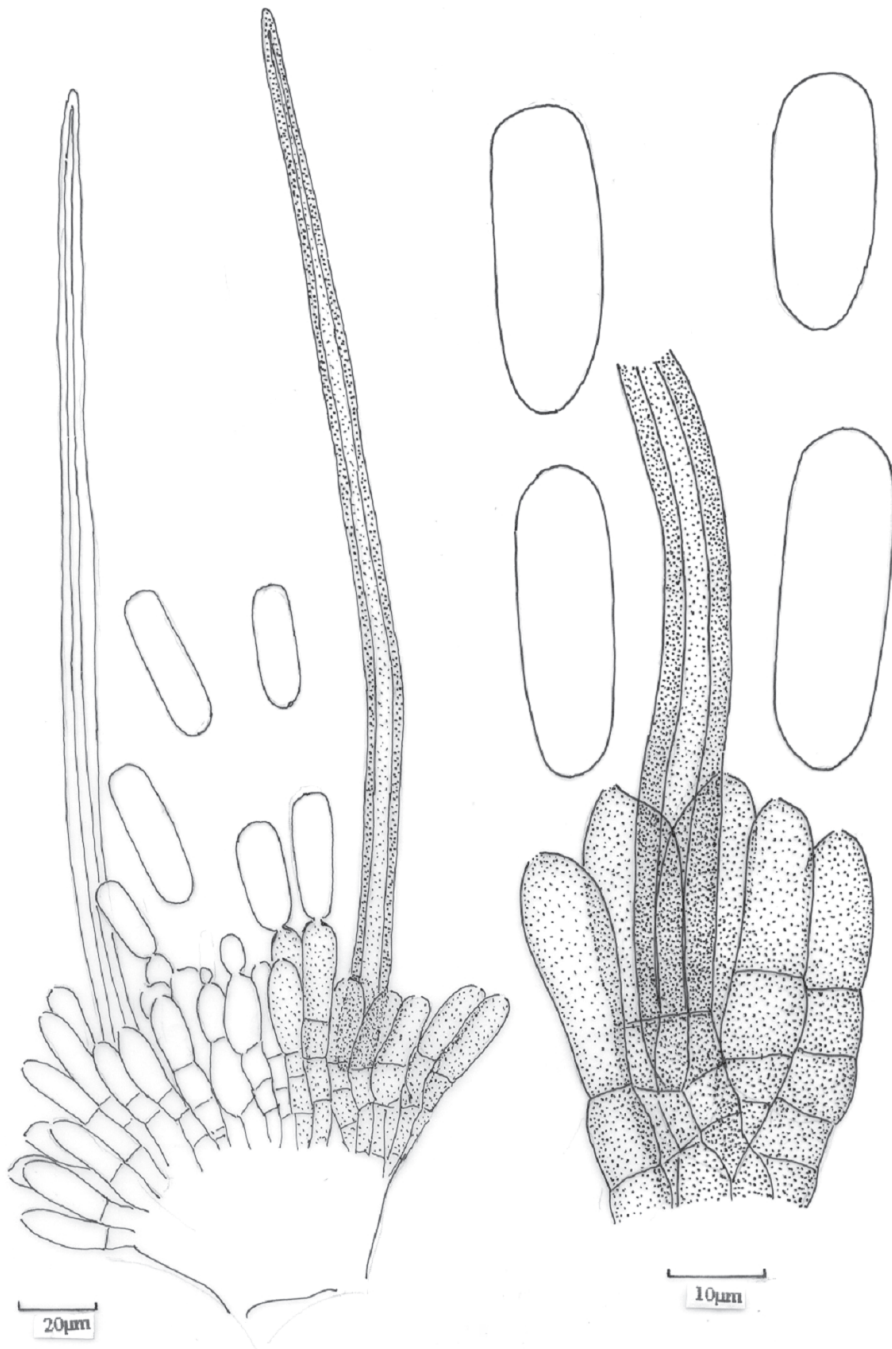


Fig 15. *Vermiculariopsiella endophytica*. Sporodochial conidiomata with setae, conidiophores, conidiogenous cells and conidia.

Table 1. Distinguishing features of *Vermiculariopsisella* species described so far.

Species	Setae	Conidiophores	Conidiogenous cells	Conidia	Ref.
<i>V. arcicula</i> Pasqual. & Zucconi	Unbranched	Branched	Mono- to polyphialidic, lageniform with flared collarette	Aseptate, fusiform, 15-19.5 µm long	5
<i>V. cornuta</i> (Rao & de Hoog) Nawawi, Kuthub. & Sutton	Thrice dichotomously branched	Unbranched	Polypialidic, obclavate to cylindrical	Cylindrical, curved near acuminate apex	4
<i>V. cubensis</i> (Castañeda) Nawawi, Kuthub. & Sutton	Branched with primary and secondary branches	Rarely branched	Monophialidic, subcylindric to lageniform with recurved cylindrical neck recurved with a flared collarette	Data not available	4
<i>V. elegans</i> Keshavaprasad, D'souza & Bhat	Unbranched	Branched	Monophialidic, no conspicuous collarette	Aseptate, 20-27 µm long	3
<i>V. falcata</i> Nawawi, Kuthub. & Sutton	Unbranched	Rarely branched	Monophialidic, cylindrical with distinct collarette	3-septate, guttulate, falcate with pointed and curved apex, truncate to rounded base 36-47 µm long	4
<i>V. immersa</i> (Desm.) Bender.	Unbranched	Rarely branched	Monophialidic, subcylindric to lageniform with recurved cylindrical neck recurved with a flared collarette	Aseptate, guttulate, cylindrical with pointed and curved apex, base obtuse to rounded 13-23 µm long.	1
<i>V. indica</i> Keshavaprasad, D'souza & Bhat	Unbranched	Branched	Monophialidic, no conspicuous collarette	Aseptate, cylindrical 12-15 µm long	3
<i>V. parva</i> Keshavaprasad, D'souza & Bhat	Unbranched	Branched	Monophialidic, no conspicuous collarette	Aseptate, cylindrical 22-30 µm long	3
<i>V. parvula</i> Nawawi, Kuthub. & Sutton	Unbranched	Branched	Monophialidic, subcylindric to lageniform, flared collarette	Aseptate, guttulate, cylindrical with apex slightly curved and pointed, base rounded to obtuse 8-13 µm long	4
<i>V. ramosa</i> (Sutton) Nawawi, Kuthub. & Sutton	Once dichotomously branched	Rarely branched	Monophialidic, subcylindric to lageniform with recurved cylindrical neck recurved with a flared collarette	Data not available	4
<i>V. spiralis</i> Crous, Wingf. & B. Kendr.	Unbranched spirally twisted	Unbranched	Monophialidic, subcylindric to lageniform, with recurved ends, collarette	Aseptate, cylindrical apex curved and pointed, base obtuse rounded 15-19 µm long	2
<i>V. endophytica</i> Puja, Bhat & K.D. Hyde	Unbranched	Rarely branched	Monophialidic, inconspicuous collarette, sub cylindrical	Aseptate, cylindrical, 32-42 µm long and 10-13 µm wide	Present study

1: Bender (1992), 2: Crous *et al.* (1995), 3: Keshavaprasad *et al.* (2003), 4: Nawawi & Kuthubutheen (1990), 5: Pasqualetti & Zucconi (1992).

Table 2. Distinguishing features of known species of *Echinosphaeria*

Species	Ascocarp	Ascus	Ascospore	Refs.
<i>E. canescens</i> (Pers: Fr.) Mill. & Huhndorf	Sub-globose to ovoid	Cylindric-clavate, 10-12 µm wide	Uniseptate, 20-28 × 4-5 µm wide	1, 2
<i>E. macrospora</i> Puja, Bhat & K.D. Hyde	Pyriiform	Clavate, 120-165 µm long	Aseptate, 41-45 µm long and 6-11 µm wide	Present study

1: Saccardo (1883); 2: Miller & Huhndorf (2004)

(= *Sphaeria canescens* Pers., Syn. Meth. Fung.: 72, 1801). The genus is characterised by perithecial ascomata with 8-spored, unitunicate, nonamyloid asci containing allantoid, guttulate, hyaline, smooth-walled, biserially arranged ascospores (Saccardo, 1883; Miller & Huhndorf, 2004). *Echinosphaeria canescens* was previously reported to have *Endophragmiella* anamorph and a *Selenosporella*-like synanamorph (Hughes, 1979; Sivanesan, 1983) and in this study *E. macrospora* was found to have a *Vermiculariopsiella* anamorph.

Echinosphaeria macrospora is typical of the genus in having carbonaceous, shining, soft ascomata, unitunicate, nonamyloid, 8-spored asci and hyaline, allantoid ascospores. *Echinosphaeria macrospora* differs from *E. canescens* in having wider asci (14-17.5 µm vs. 10-12 µm) and greatly larger ascospores (41-45 × 6-11 µm vs. 20-28 × 4-5 µm) (Tab. 2). The length of asci was not indicated in the description of the type species [= *Lasiosphaeria canescens* (Pers.) Karst.] and hence could not be considered for comparison (Saccardo, 1883; Miller & Huhndorf, 2004).

Vermiculariopsiella, typified by *V. immersa* (Desm.) Bender (Bender, 1932) is characterised by setose sporodochia, with hyaline, non-septate conidia produced in slimy mass on compact columns of cylindrical to obclavate phialidic conidiogenous cells. Recently, three new species have been added to the genus from India by Keshavaprasad et al. (2003), who also provided a key to the existing species. The taxa within the genus differ in organization of sporodochia, shape and size of setae, branching of conidiophores and phialides and, shape and size of conidia. An important, notable taxonomic rearrangement associated with *Vermiculariopsiella* is segregation of two species, *V. microsperma* Castañeda & Kendrick and *V. ludoviciana* Castañeda, Cano & Guarro (Pirozynski, 1962; Kirk & Sutton, 1985; Arambarri & Cabello, 1989; Castañeda & Kendrick, 1992; Pasqualetti & Zucconi, 1992; Arambarri et al., 1997; Castañeda et al. 1997; Index Fungorum 2005) from the genus. All recognized species of the genus are listed and compared in Table 1.

Amongst the species described in the genus *Vermiculariopsiella* (Tab. 1), *V. endophytica* is close to *V. falcata* only in conidial dimension. The conidia are 36-47 µm long in *V. falcata* and 31-36 µm long in *V. endophytica*. However, conspicuous phialidic collarettes and 3-septate, falcate conidia of *V. falcata* are not present in *V. endophytica*. Though the shape and architecture of *V. parva*, *V. elegans* and *V. indica* are similar to *V. endophytica*, the conidia in the latter differ markedly in size.

The present study once again exposes the challenges posed by pleomorphism and synanamorphy to systematic mycology (Cannon & Kirk, 2000)

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