**Echinosopheria macrospora sp. nov., teleomorph of Vermiculariopsisella endophytica sp. nov.**

GAWAS PUJA¹#, B.D. SHENOY², K.D. HYDE² & D.J. BHAT¹*

¹ Department of Botany, Goa University, Goa-403 206, India.
² Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR

**Abstract** – *Echinosopheria macrospora* is a novel endophyte isolated from stems of *Centella asiatica* (Apiaceae/Umbelliferae) with its novel, hyphomycetous anamorph, *Vermiculariopsisella endophytica*. The fungus first produced the conidial state, followed by development of its teleomorph after 4 weeks of incubation. *Echinosopheria 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 *Vermiculariopsisella* and the third asexual stage of *Echinosopheria*.

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 *Vermiculariopsisella* 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, *Vermiculariopsisella 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 *Echinosopheria* 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 *Vermiculariopsisella* and the third asexual stage of *Echinosopheria*.

* Corresponding author: e-mail < bhatdj@rediffmail.com>
# e-mail: < pujabg@yahoo.co.in>
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 Petriani & 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 an amorphic and teleomorphic forms in the medium.

Confirmation of anamorph-teleomorph connection

The perithecia 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 ascospores 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 Vermiculioptiella endophytica.

TAXONOMY

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

Ascocarps peritheciis, pyriformis, gregariis, nigris, velutinis, aggregatis, cupulatis graeco, 410-490 μm longis, 150-265 μm latit ad mediis ora; oriiundus brevis stromatic pessum. Ostiolis brevis, conicis, cum centralis apicalis. Peridiumii pseudoparenchymatis, duo-layeris, cum angularis, leviter tenuibus cellulis. 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 oriiundus 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. Ascosphorae 41-45 × 6-11 μm allantoideae vel vermiformae, hyalinae vel subhyalinae, eseptatae, guttulatae, laevia, biseriatae.

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

Ascomata perithelial, 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 pseudoparenchymatosus, 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. Ascii 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 vermiciform, hyaline to subhyaline, aseptate, guttulate, smooth-walled, biseriately arranged in the asci.
Fig 9. *Echinosphaeria macrospora*. Ascocarp, asci and ascospores.
Anamorph – *Vermiculriopsiella 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.

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

Coloniae in vitro aliquanta vel celer proenta, pervenio 5.5-6 cm diametro in 7 dies, platy, cum irregulari vel rhizoidali labrum, pallens-albo vel pallide brunea, inverte palide brunnea. Sporodochia oriundus brevis stromatic pessum, sparsa, cream vel peach-coloris, setosae. Conidiophora laevia, septata, parum tremes, hali- nis vel palide-coloris, 75-85 × 6-9 µm. Setae 3-15, laevia, 2-6-septata, hauđ-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 parieti- bus crassi, brunnea 5-7 µm diametro stromal cellularae. Cellulae conidiogenae monopodialiae, integrate vel discrete, sine emino collarettae, 22-25 × 10-11 µm. Contidia solitaria, cylindrica, teres ad duo extremitas, laevia, septata, 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 monopodialic, 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


**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, *Echinospaeria* A.N. Mill. & Huhndorf, *Hiberina* A.N. Mill. & Huhndorf and *Immersiella* (Lasiosphaeriaceae) A.N. Mill. & Huhndorf. *Echinospaeria* has phylogenetic affinities with the members of family Helminthosphaeriaceae (Miller & Huhndorf, 2004).

Fig 15. *Vermiculariopsiella endophytica*. Sporodochial conidiomata with setae, conidiophores, conidiogenous cells and conidia.
Table 1. Distinguishing features of *Vermiculariopsiella* species described so far.

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

Echinosopheraia macrospora sp. nov.

Table 2. Distinguishing features of known species of Echinosopheraia

<table>
<thead>
<tr>
<th>Species</th>
<th>Asccarp</th>
<th>Ascus</th>
<th>Ascospore</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. canescens</td>
<td>Sub-globose to</td>
<td>Cylindric-clavate,</td>
<td>Unisepatate, 20-28 × 4-5 µm</td>
<td>1, 2</td>
</tr>
<tr>
<td>(Pers: Fr.) Mill. &amp;</td>
<td>ovoid</td>
<td>10-12 µm wide</td>
<td>wide</td>
<td></td>
</tr>
<tr>
<td>Huhndorf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. macrospora</td>
<td>Pyriform</td>
<td>Clavate,</td>
<td>Aseptate, 41-45 µm long</td>
<td>Present study</td>
</tr>
<tr>
<td>Puja, Bhat &amp;</td>
<td></td>
<td>120-165 µm long</td>
<td>6-11 µm wide</td>
<td></td>
</tr>
<tr>
<td>K.D. Hyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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, uniseriulate, nonamyloid asci containing allantoid, guttulate, hyaline, smooth-walled, biseriately arranged ascospores (Saccardo, 1883; Miller & Huhndorf, 2004). Echinosopheraia 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.

Echinosopheraia macrospora is typical of the genus in having carbonaceous, shining, soft ascomata, uniseriulate, nonamyloid, 8-spored asci and hyaline, allantoid ascospores. Echinosopheraia 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 synanamorph to systematic mycology (Cannon & Kirk, 2000)

Acknowledgements. PG and DJB are indebted to the UGC, CSIR, MOEN, Government of India, for research support grants. BDS thanks The University of Hong Kong for the award of a postgraduate studentship.
REFERENCES


INDEX FUNGORUM, 2005 — www.indexfungorum.org


PRATIBHA S.J., PUJA G., SHENOY B.D., HYDE K.D. & BHAT D.J., 2005 — Chalara indica sp. nov. and Sorocobye indicus sp. nov. from India. Cryptogamie, Mycologie 26: 97-103.


