



Molecular phylogeny of *Speiropsis pedatospora*

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Abstract

Speiropsis pedatospora, an aero-aquatic fungus, was isolated from submerged plant litter in freshwater streams from India. Based on analysis of combined ITS and LSU sequence data, the species was positioned in the family Weisneriomycetaceae, as a sister group to Tubeufiales in the Dothideomycetes, instead of its current placement in the order Jahnulales. *Speiropsis pedatospora*, is the type species of the genus *Speiropsis*, is morphologically characterised by macronematous, mononematous, erect, branched conidiophores, polyblastic, denticulate, discrete conidiogenous cells and long catenate conidia linearly joined by narrow, small isthmi. All asexual morph members of Weisneriomycetaceae with isthmospores are morphologically similar to the genus *Speiropsis*.

Key words – aquatic fungi – Dothideomycetes – fungal diversity – phylogeny of asexual fungi – Western Ghats

Introduction

A natural classification of asexual morph taxa in families, orders and classes of Ascomycota and Basidiomycota, are presently being undertaken, by analysing molecular sequence data along with morphology, in order to provide a natural classification. Asexual morph are also being linked to their sexual genera by molecular data and major revisions of the ascomycota are taking place (Hyde et al. 2011, Wijayawardene et al. 2014). Our continued work on taxonomy of asexual fungi (Pratibha & Prabhugaonkar 2015a, b, Pratibha et al. 2014a, b) yielded a rare collection of the asexual genus *Speiropsis*, and the phylogenetic study of this taxon is the subject of this paper.

The genus *Speiropsis* Tubaki is typified by *Speiropsis pedatospora* Tubaki (Tubaki 1958). There are eight species listed in the genus (Index fungorum 2016). *Speiropsis* species are characterized by erect, simple, straight, septate, mostly mononematous conidiophores with discrete, denticulate, polyblastic conidiogenous cells and catenate conidia in branched or unbranched chains connected by narrow isthmi (Ellis, 1976). *Speiropsis pedatospora* has often been collected from terrestrial leaf litter and submerged plant litter (Ellis, 1976, Barbosa & Gusmao 2005, Patil et al. 2014, Pratibha et al. 2012). Based on phylogenetic analysis of ITS rDNA of cultures obtained from BIOTEC Culture Collection (BCC, Thailand) and the Centraalbureau voor Schimmelcultures (CBS, Netherlands), Prihatini et al. (2008) have shown that *Speiropsis pedatospora* belonged to order Jahnulales and clustered with *Jahnula* species. However these cultures are no longer available in the catalogues of those culture collections. Based on Prihatini et al. (2008), the genus *Speiropsis* with all its accepted species have been treated in the order Jahnulales (Shearer et al. 2009, Suetrong et al. 2011, Hyde et al. 2013, Wijayawardene et al. 2014).

In the current study, *Speiropsis pedatospora* was isolated from leaf litter collected from a freshwater stream of Western Ghats, Goa, India and subsequently cultured using single spore isolation (Chomnunti et al. 2014). In our phylogenetic study, the taxon was observed to be related to family Weisneriomycetaceae, instead of its present placement in Jahnulales. Weisneriomycetaceae (Suetrong et al. 2014), typified by the genus *Weisneriomyces* Koord. is morphologically similar to the genus *Speiropsis* in having polyblastic conidiogenous cells and catenate conidia in chains connected by narrow isthmi, but differs by sporodochial or synnematous conidiomata and unbranched or rarely branched conidial chains.

Materials & Methods

Collection and culturing

Freshly aquatic litter samples were taken to the laboratory in sealed polythene bags. The sample was observed under stereomicroscope and fungal material picked with a fine-tipped needle and mounted on a slide containing a drop of lactophenol solution. This was examined under a light microscope for morphological details.

The culture of *Speiropsis pedatospora* was obtained by single spore isolation (Chomnunti et al. 2014). Colonies developing from individual conidia were aseptically transferred to new plates (Bhat 2010). DNA isolation and sequencing work was outsourced to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India. Facesoffungi numbers are added (Jayasiri et al. 2015).

DNA isolation and PCR Analysis

Fresh fungal mycelia (20 mg), scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and PCR Analysis was done according to Prabhugaonkar & Bhat (2011). The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS) and 28S nrDNA sequence (LSU) genes were amplified and sequenced using the primer pairs ITS-1F + ITS-4R (White et al. 1990) and LR5 + LROR (Crous et al. 2009) respectively. The sequence quality was checked using Sequence Scanner Software v.1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with Blastn. ITS and LSU data to obtain related taxa. Taxa were also chosen based on recent publications on Tubeufiales (Boonmee et al. 2011, 2014). The sequence data was assembled for each taxon. The combined data matrix was aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and manually adjusted using MEGA 6.06 to allow maximum alignment and maximum sequence similarity. Phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA6.06 (Kumar et al. 2008) with 1,000 bootstrap replicates. The most suitable substitution model Kimura-2-parameter model with Gamma distributed with Invariant sites (G+I) was selected by using MEGA6.06. Gaps were treated as a pair-wise deletion and tree was viewed with MEGA6.06. Newly generated ITS and LSU sequences used are deposited in GenBank.

Results

Phylogenetic analyses

Forty-two taxa are included in the phylogenetic analysis (Table 1, Fig. 1). The result of phylogenetic analysis showed that *Speiropsis pedatospora* has close affinities with Tubeufiales, Dothideomycetes. A dataset of two families of Tubeufiales was assembled. *Pleospora herbarum* from Pleosporaceae was selected as the outgroup taxon. Upon analysis of sequence data, it was observed that *Speiropsis pedatospora* forms a separate basal group to family related to Weisneriomycetaceae and Tubeufiaceae in the Tubeufiales.

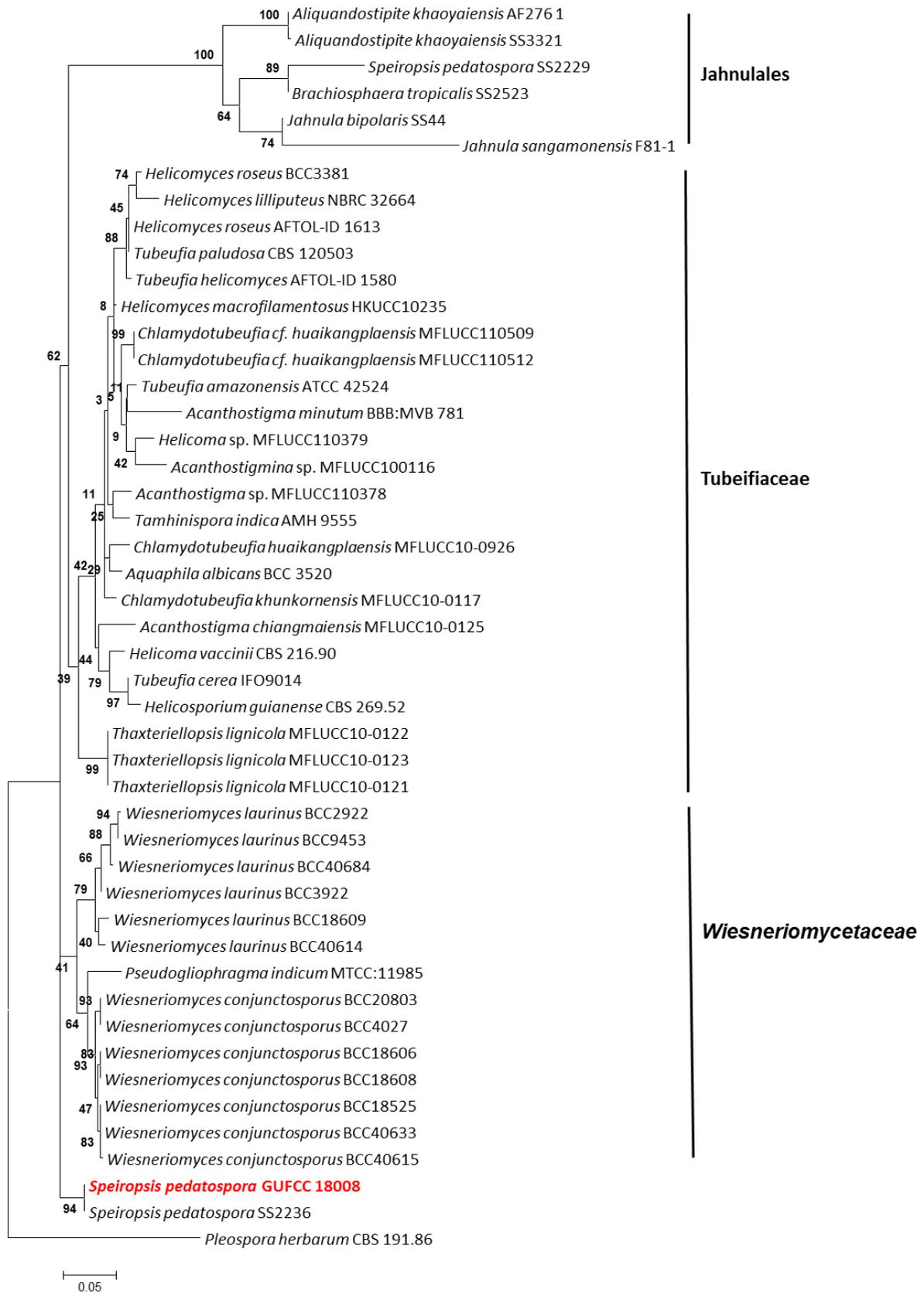


Fig. 1 – Maximum likelihood (ML) tree inferred from, ITS and LSU showing the relationship of *Speiropsis pedatospora* with Wiesneriomycetaceae. The species dealt in the present study is in red.

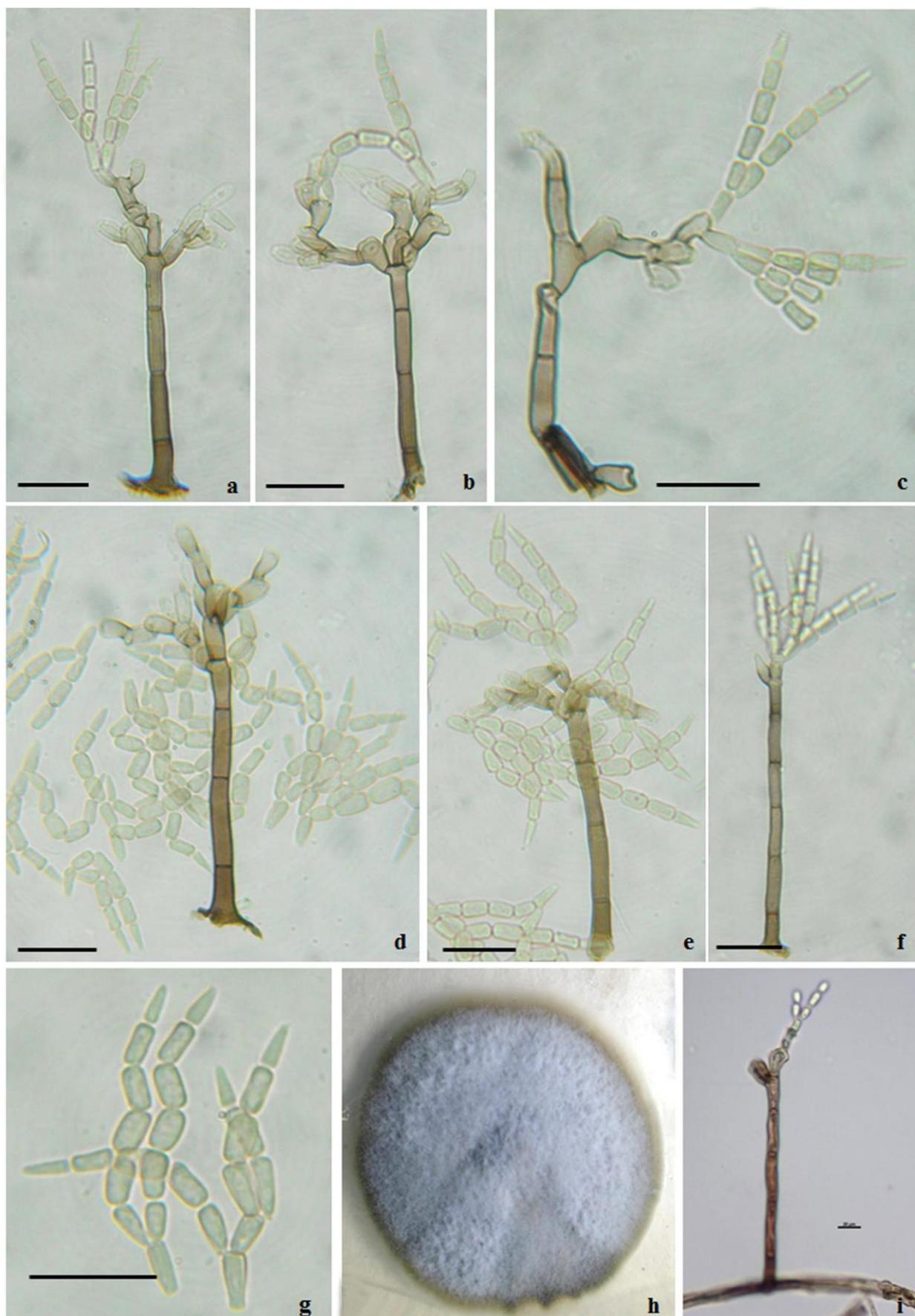


Fig. 2 – *Speiropsis pedatospora* a-f, conidiophore with conidia. g. conidial chains. h. culture. i. conidiogenesis in culture. Scale bars = 10 µm.

Table 1 Sequence data used in combined ITS and LSU analyses. Newly deposited sequences are in bold

Taxon	Accession no.	ITS	LSU
<i>Acanthostigma chiangmaiensis</i>	MFLUCC10-0125	JN865209	JN865197
<i>Acanthostigma minutum</i>	BBB:MVB 781	-	JN127360
<i>Acanthostigma</i> sp.	MFLUCC110378	KF301523	KF301531
<i>Acanthostigmina</i> sp.	MFLUCC100116	KF301526	KF301534
<i>Aliquandostipite khaoyaiensis</i>	AF276_1	JN942357	JN673028
<i>Aliquandostipite khaoyaiensis</i>	SS3321	JN819278	EF175650
<i>Aquaphilaalbicans</i>	BCC 3520	DQ341098	DQ341102
<i>Brachiosphaera tropicalis</i>	SS2523	FJ887923	JN819284
<i>Chlamydotubeufia</i> cf. <i>huaikangplaensis</i>	MFLUCC110509	KF301527	KF301535
<i>Chlamydotubeufia</i> cf. <i>huaikangplaensis</i>	MFLUCC110512	KF301528	KF301536
<i>Chlamydotubeufia huaikangplaensis</i>	MFLUCC10-0926	JN865210	JN865198
<i>Chlamydotubeufia khunkornensis</i>	MFLUCC10-0117	JN865201	JN865189
<i>Helicoma</i> sp.	MFLUCC110379	KF301524	KF301532
<i>Helicoma vaccinii</i>	CBS 216.90	AY916486	AY856879
<i>Helicomyces lilliputeus</i>	NBRC 32664	AY916483	AY856899
<i>Helicomyces macrofilamentosus</i>	HKUCC10235	-	AY849942
<i>Helicomyces roseus</i>	BCC3381	-	AY787932
<i>Helicomyces roseus</i>	AFTOL-ID 1613	-	DQ678083
<i>Helicosporium guianense</i>	CBS 269.52	AY916487	AY856893
<i>Jahnula bipolaris</i>	SS44	JN819281	EF175658
<i>Jahnula sangamonensis</i>	F81-1	JN942351	EF175663
<i>Pleosporaherbarum</i>	CBS 191.86	KC584239	DQ247804
<i>Pseudogliophragma indicum</i>	MTCC:11985	KM052850	KM052851
<i>Speiropsis pedatospora</i>	SS2229	FJ887926	JN819285
<i>Speiropsis pedatospora</i>	GUFCC 18008	KU563769	KU563770
<i>Speiropsis pedatospora</i>	SS2236	FJ887927	-
<i>Tamhinisporaindica</i>	AMH 9555	KC469282	KC469283
<i>Thaxteriellopsis lignicola</i>	MFLUCC10-0122	JN865206	JN865194
<i>Thaxteriellopsis lignicola</i>	MFLUCC10-0123	JN865207	JN865195
<i>Thaxteriellopsis lignicola</i>	MFLUCC10-0121	JN865205	JN865193
<i>Tubeufia amazonensis</i>	ATCC 42524	AY916458	AY787938
<i>Tubeufiacerea</i>	IFO9014	-	AY849964
<i>Tubeufia helicomyces</i>	AFTOL-ID 1580	-	DQ767654
<i>Tubeufia paludosa</i>	CBS 120503	-	GU301877
<i>Wiesneriomyces conjunctosporus</i>	BCC20803	-	KJ425453
<i>Wiesneriomyces conjunctosporus</i>	BCC4027	-	KJ425449
<i>Wiesneriomyces conjunctosporus</i>	BCC18606	-	KJ425451
<i>Wiesneriomyces conjunctosporus</i>	BCC18608	-	KJ425452
<i>Wiesneriomyces conjunctosporus</i>	BCC18525	-	KJ425450
<i>Wiesneriomyces conjunctosporus</i>	BCC40633	-	KJ425455
<i>Wiesneriomyces conjunctosporus</i>	BCC40615	-	KJ425454
<i>Wiesneriomyces laurinus</i>	BCC2922	-	KJ425456
<i>Wiesneriomyces laurinus</i>	BCC9453	-	KJ425458
<i>Wiesneriomyces laurinus</i>	BCC40684	-	KJ425461
<i>Wiesneriomyces laurinus</i>	BCC3922	-	KJ425457
<i>Wiesneriomyces laurinus</i>	BCC18609	-	KJ425459
<i>Wiesneriomyces laurinus</i>	BCC40614	-	KJ425460

Taxonomy

Speiropsis pedatospora Tubaki, J. Hattori Bot. Lab. 20: 171. 1958

Fig. 2

Facesoffungi number: FoF 02652

Colonies on natural substrate effuse, white, flexuous. *Conidiophores* macronematous, mononematous, simple, straight or flexuous, solitary, smooth, brown, branched at the tip, 73–120 × 4–6.5 µm. *Conidiogenous cells* polyblastic, proliferating, sympodial, discrete, smooth, light brown, 8–12.5 × 4–8.5 µm. *Conidia* 6–12.5 × 2.5–5.5 µm, catenate, with 4–8 linearly connected by narrow

isthmi, cylindrical to cuneiform, smooth, pale to mid-brown, often forming branched chains 65–80 µm long.

Colonies on MEA greyish green, planar, irregular in outline, filamentous, with a black reverse, attaining 2.8 cm diameter after 7 days.

Known distribution – widespread in tropical and subtropical regions.

Material examined – INDIA, Goa, Canacona, Cotigao, on an unidentified fallen leaf in a freshwater stream, 30 Oct 2012, Ashish P., (herbarium no. LF-28) – living culture GUFCC 18008; INDIA, Goa, Ponda, Bondla, on an unidentified fallen leaf in freshwater stream, 11 Oct 2015, (collector) Pratibha J.; INDIA, Meghalaya, Jowai, Tyrshi falls, on an unidentified dry leaf in freshwater stream, 13 Jul 2016, (collector) Ashish P. (herbarium no. AVP-102)

Discussion

Suetrong et al. (2014) introduced the family Weisneriomycetaceae as sister to Tubeufiaceae in the Dothideomycetes to accommodate asexual isthmosporous fungus *Wiesneriomyces*. The family Weisneriomycetaceae without any known sexual state is originally characterised by sporodochial setose conidiomata, with setae arising from basal pseudoparenchymatous stalk or mycelium immersed in the substrata. The conidiophores are macronematous, septate and branched. The conidiogenous cells are holoblastic, discrete, determinate, terminate, clavate to cylindrical. The conidia are hyaline, non-septate; the conidium at each end of a chain is tapered, with intermediate ones more or less cylindrical, formed in uniseriate, acropetal chains and connected by narrow isthmi (=phragmoconidia) (Suetrong et al. 2014). Branching of conidial chains was rarely observed. *Pseudogliophragma* Phadke & V.G. Rao, added as second genus in the Weisneriomycetaceae by Pratibha et al 2014, differs from *Wiesneriomyces* by synnematos conidiomata and dry, swollen, much shorter conidia. The genus *Speiropsis* is different from *Pseudogliophragma* and *Wiesneriomyces* in having erect, simple, straight, mostly mononematous conidiophores and catenate conidia in mostly branched chains. Sequence data analysis showed that *Speiropsis pedatospora* forms a separate basal group to family Weisneriomycetaceae and Tubeufiaceae in the Tueufiales. This observation is different from current placement of the genus in the Jahanulales (Prihatini et al. 2008, Shearer et al. 2009, Suetrong et al. 2011, Wijayawardene et al. 2014). It was observed that this classification was adopted based on single-gene phylogenetic study by Prihatini et al. 2008. In our analysis of sequence data of Prihatini et al. 2008, it was observed that one of the sequences, *Speiropsis pedatospora* SS2236, grouped well with our collection placed in Weisneriomycetaceae, whereas the second sequence deposited by authors (*Speiropsis pedatospora* SS2229) clustered in Jahnulales. In this study, we propose correct phylogenetic placement for *Speiropsis* based on study of ITS and LSU regions generated from our collection. This placement is further supported by morphological similarity of genus *Speiropsis* to the family Weisneriomycetaceae.

As discussed by Pratibha et al 2014, in the absence of adequate molecular data on morphologically similar asexual genera such as *Abgliophragma* R.Y. Roy & S. Gujarati, *Gliophragma* Subram. & B.C. Lodha and *Phalangispora* Nawawi & Webster, further collections and morpho-molecular studies are necessary for more stable generic concepts in Weisneriomycetaceae.

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