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Phomatosporales ord. nov. and *Phomatosporaceae* fam. nov., to accommodate *Lanspora, Phomatospora* and *Tenuimurus*, gen. nov.

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Abstract

In an ongoing study on Sordariomycetes from Italy we identified three *Phomatospora*-like species, which we selected for further study. Morphological characterization and phylogenetic analysis, using combined LSU, SSU and ITS sequence data, showed them to be related to other *Phomatospora* species in a distinct clade in Sordariomycetes. The *Phomatospora* species clustered in three clades, including *P. viticola* in *Phomatospora sensu stricto*, *Lanspora coronata*, and the new genus *Tenuimurus*. These new taxa together with *Lanspora coronata* and other *Phomatospora* species form a distinct clade which we introduce as a new family *Phomatosporaceae* and a new order *Phomatosporales*, which is sister to the order *Amplistromatales.* The new genus and species are introduced and compared.

Key words – Diaportheomycetidae – multigene analysis – new taxa – *Phomatospora*-like species

Introduction

We have been carrying out a study of the microfungi in Italy and have described numerous new species of Dothideomycetes (Ariyawansa et al. 2015, Liu et al. 2015) and fewer Sordariomycetes (Daranagama et al. 2015, Li et al. 2015, Senanayake et al. 2015). In the present study we collected three *Phomatospora*-like species and subjected them to morphological and molecular studies.

Phomatospora was introduced based on *Sphaeria phomatospora* Berk. & Broome, and this taxon was renamed as *Phomatospora berkeleyi* (Fournier & Lechat 2010). *Phomatospora* is characterized by immersed ascomata, with a small pseudoparenchymatous-celled peridium, and

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cylindrical, unitunicate asci with a refractive, J-, apical ring. Ascospores are arranged uniseriately and are usually 1-celled, ellipsoidal and hyaline, with longitudinally striate walls, or sometimes with a mucilaginous sheath or variously shaped, bipolar appendages (Barr 1994, Cai et al. 2006, Fournier & Lechat 2010). The asexual morph of this genus reported as *Sporothrix* in culture and Rappaz (1992) proposed *Phomatospora* to be a genus in *Xylariales*, where *Sporothrix* asexual morphs are already known. However phylogenetic studies did not support this and Lumbsch & Huhndorf (2007) placed *Phomatospora* in Sordariomycetes genera *incertae sedis*. Several phylogenetic studies have shown that phylogenetic placement of *Sporothrix* in *Ophiostomataceae*. Currently *Phomatospora* comprises 98 epithets (Index Fungorum, 2016) reported from terrestrial, aquatic and marine habitats (Hyde 1988, 1992, 1993a, Barr 1994, Fallah et al. 1998, Fournier & Lechat 2010).

The aim of the present study is to introduce three *Phomatospora*-like species from Italy. In the combined gene analyses, these isolates cluster with other *Phomatospora* species and *Lanspora coronata* in a distinct lineage. We therefore treat the lineage as *Phomatosporaceae* and *Phomatosporales.* As the taxa in the family cluster in three different clades we also introduce a new genus to accommodate one of the clades, while two are treated as *Phomatospora sensu stricto* and the other as *Lanspora.*

Materials & methods

Specimen collection, morphological examination, photomicrography and single spore isolation

Fresh specimens were collected from Italy during March 2013 to March 2014. Specimens were placed in paper bags and collection details were noted. Specimens were brought to the laboratory and examined under a stereomicroscope to observe the characteristics of ascomata. Macro-morphological characters were photographed with an AxioCam ERc5s digital camera fitted to the ZEISS Discovery V8 stereomicroscope. A few ascomata were transferred to a drop of water mounted on a glass slide using a fine needle and crushed to show internal structures. Cross sections of ascomata were made by razor blade and mounted in a water drop. Morphological characteristics of ascomata, asci, ascospores and other tissues were photographed using a Nikon Eclipse Ni digital camera fitted with the compound microscope. All microphotographs were arranged using Adobe Photoshop CS3 extended (v. 10.0) version and measurements were made with Tarosoft image framework (v. 0.9.0.7). Specimens were preserved and deposited in MFLU herbarium. Facesoffungi and Index Fungorum numbers were registered (Jayasiri et al. 2015, Index Fungorum 2016). Single spore isolates were obtained as detailed in Chomnunti et al. (2014). Colonies were photographed and characters noted. Living cultures are deposited at MFLU culture collection.

DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Fresh fungal mycelia grown on MEA for 4 weeks at 20 °C were scraped from the colony margin and used for genomic DNA extraction using a modified CTAB protocol described by Riethmüller et al. (2002). PCR amplification and sequencing of ITS region using the primer pair ITS4/ITS5, LSU region using primer pair LROR/LR5 and SSU region using primer pair NS1/NS4 was performed (Vilgalys & Hester 1990, White et al. 1990). Each PCR reaction contained 0.3 µl of TaKaRa Ex-Taq DNA polymerase, 12.5 µl of 2 × PCR buffer, 2.5 µl of dNTPs, 1 µl of primer, 1 µl of DNA template and was adjusted with 6.5 µl of double-distilled water to a total volume of 25 mL.

Amplification reactions were performed in a thermal-cycler (BIORAD 1000TM Thermal Cycler, Bio-Rad Laboratories, Hercules, California). The temperature profile for both ITS and LSU was an initial denaturing step for 2 min at 94 °C, followed by 35 amplification cycles of denaturation at 94 °C for 60 s, annealing at 58 °C for 60 s and extension at 72 °C for 90 s and a final extension step of 72 °C for 10 min (Phillips et al. 2008). The temperature profile for the SSU was, initial denaturation at 94 °C for 2 min, followed by 35 amplification cycles of denaturation at

Table 1 Isolates utilized in the phylogenetic tree and their GenBank and culture accession numbers. The newly generated sequences are indicated in bold.

Taxon name	Culture accession number	LSU	SSU	ITS
Amphisphaeria sorbi	MFLUCC 13-0721	KP744475	-	KR092797
Amplistroma caroliniana	CBS 124655	FJ532377	-	-
Amplistroma caroliniana	DOI s n	FJ532376	-	-
Amplistroma erinaceum	AH 43902	KC907374	-	KC907376
Amplistroma guianensis	GJS5740	FJ532380	-	-
Amplistroma hallingii	REH7389	FJ532379	-	-
Amplistroma longicollis	AH37870	HQ901790	-	-
Amplistroma rava	SMH4958	FJ532378	-	-
Annulatascus saprophyticus	MFLUCC 14-0035	KR868947	-	-
Annulusmagnus triseptatus	SMH2359	AY346257	-	-
Aquaticola hongkongensis	HKUCC3703	AF132321	-	AF177156
Ascitendus austriacus	A324-1B	AY590293	-	-
Atractospora reticulata	CBS 138740	KT991661	-	KT991670
Atractospora reticulata	CBS 127884	KT991660	-	KT991669
Barbatosphaeria dryina	CBS 127691	KM492864	KM492852	-
Barrina polyspora	AWR9560A	AY346261	-	-
Brachysporium nigrum	CBS 138741	KT991662	-	KT991673
Bullimyces communis	AF281-3	JF775585	JF758617	-
Catabotrys deciduum	SMH 3436	AY346268	-	-
Cateractispora recepticuli	99709	AF132327	-	AF177153
Ceratocystiopsis minuta	CBS 116963	EU913655	-	EU913696
Ceratostomella cuspidata	ICMP 17629	FJ617558	KT991642	KT991671
Chaetomium globosum	CBS 105.40	KT214597	-	KT214566
Chaetosphaeria innumera	SMH 2748	AY017375	-	AY906956
Clohiesia corticola	HKUCC 3712	AF132329	-	-
Clonostachys buxi	CBS 696.93	KM231721	-	KM231840
Coniochaeta fodinicola	NFR	-	KF857175	JQ904605
Cordana abramovii	PE 0053-24a	KF833358	-	-
Cryptadelphia groenendalensis	SH12	KT991662	KT991643	-
Cumulospora marina	MF46	GU252135	GU252136	-
Cyanoannulus petersenii	R044a	AY316358	-	-
Fragosphaeria purpurea	CBS 133.34	AB278192	AF096176	AB278192
Hydea pygmea	NBRC33069	GU252133	GU252134	-
Lanspora coronata	AFTOL-ID 736	U46889	DQ470996	-
Leotia lubrica	AFTOL-ID 1	AY544644	AY544746	DQ491484
Lindra thalassiae	AFTOL ID 413	DQ470947	DQ470994	DQ491508
Lulworthia fucicola	ATCC 64288	AY878965	AY879007	-
Myrmecridium flexuosum	CBS 398.76	EU041825	-	EU041768
Myrmecridium montsegurinum	JF 13180	KT991664	KT991645	KT991674
Myrmecridium obovoideum	HGUP 0314	KC136139	-	KC136140
Natantiella ligneola	CBS 123470	FJ617556	HQ878598	KT991675
Neopyricularia commelinicola	CBS 128303	KM009151	KM009211	KM009163
Ophiostoma gemellus	CMW23059	DQ821533	-	DQ821562
Papulosa amerospora	AFTOL-ID 748	DQ470950	DQ470998	-
Pesotum australiae	CMW6606	EF408608	-	EF408603
Phomatospora bellaminuta	AFTOL-ID 766	FJ176857	FJ176803	-
Phomatospora biseriata	MFLUCC 14-0832a	KX549448	KX549458	KX549453
Phomatospora biseriata	MFLUCC 14-0832b	KX549449	KX549459	KX549454
Phomatospora striatigera	CBS 133932	KM213618	-	KM213617
Phomatospora viticola	MFLU 16–1973	KX549452	-	KX549457
Pseudoproboscispora caudaesuis	A336-2D	AY094192	-	-
Pyriculariopsis parasitica	HKUCC5562	DQ341514	-	-
Raffaelea lauricola	C2339	KF515710	-	KF515711
Rhamphoria pyriformis	CBS 139033	KT991665	-	KT991677
Rubellisphaeria abscondita	CBS 132078	KT991666	KT991646	KT991678
Sordaria fimicola	HP153	KT323354	-	KT323211
Submersisphaeria aquatica	A354-1C	AY094194	-	-
Tenuimurus clematidis	MFLUCC14-0833a	KX549450	-	KX549455

Tenuimurus clematidis	MFLUCC14-0833b	KX549451	-	KX549456
Thyridium vestitum	AFTOL-ID 172	AY544671	AY544715	-
Trichoderma viride	YNUCC0183	AY291123	-	-
Vertexicola confusa	99709	AF132331	-	AF177151
Vialaea mangiferae	MFLUCC 12-0808	KF724975	-	KF724974
Woswasia atropurpurea	WC	-	-	JX233658
Xylaria hypoxylon	AFTOL ID 51	AY544648	AY544760	DQ491487
Xylochrysis lucida	CBS 135996	KF539911	KF539912	KF747734
Xylomelasma sordida	CBS 131683	KM492871	KM492860	KT991679

94 °C for 30 s, annealing at 55 °C for 30 s and extension at 68 °C for 5 min. (Réblová et al. 2011).All amplified PCR products were determined by electrophoresis at 90 V/cm for 40 min. in 1% agarose gel stained with ethidium bromide (0.5 mg/mL). The gel was visualized under a UV transilluminator to estimate the fragment size. PCR products were purified and sequenced with both primers at the Sunbiotech Company, Beijing, China. Sequences were edited and assembled with DNASTAR.Lasergene (v7.1) and consensus sequences were used. Sequences derived in this study are deposited in GenBank.

The sequences generated in this study were supplemented with additional sequences obtained from GenBank (Table 1) based on blast searches and published literature. Multiple sequence alignments were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html) and the alignment was manually improved with BioEdit v. 7.0.5.2 (Hall 1999).

Maximum likelihood analysis was performed by RAxML 7.4.2 Black Box or RAxMl GUI v.1.3 (Stamatakis et al. 2008, Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis was carried out using the GTRGAMMA model of nucleotide substitution with 1000 replicates. The model of evolution was estimated by using MrModeltest 2.3 (Nylander 2004) and GTR+I+G was selected as the model for Bayesian analyses. Bayesian inference in MrBayes v. 3.2.1 (Ronquist et al. 2012) was performed with default settings, running four chains over 12 million generations and sampling each 100th tree. The first 24000 of the 12000000 saved trees were discarded and the consensus tree was based on the remaining 11976000 trees. Trees were figured in Treeview (Page 1996). The final alignments and the trees obtained were deposited in TreeBASE (https://treebase.org/treebase-web/user/summary.html?id=19567) and are available under study accession no. S19567.

Results

Phylogenetic analysis

The taxa used in the phylogenies were selected from recent publications (Maharachchikumbura et al. 2015, 2016, Réblová et al. 2016). The phylogeny resulting from the analysis of combined LSU, SSU and ITS sequence data of Sordariomycetes is shown in Fig. 1. Overall, the topologies obtained from the different phylogenetic analyses were similar and the best scoring RAxML tree is illustrated (Fig. 1). The separation of *Phomatosporales* from other fungal orders in Sordariomycetes is well-supported (MLB/PP = 94/0.9). This was also supported by single gene phylogenetic trees (results not shown). The new genus *Tenuimurus*, based on *T. clematidis* forms a well-supported clade (MLB/PP = 94/0.9) which is sister to *Phomatospora* and *Lanspora*. *Phomatospora viticola* clusters with *P. striatigera* (CBS133932) with moderate support (MLB/PP = 76/0.9) and *P. biseriata* clusters with *Phomatospora* species with low support values.

Taxonomy

Phomatosporales Senan. Maharachch & K.D. Hyde, **ord. nov**

Index Fungorum number IF552311, Facesoffungi number FoF 02485

Etymology – In reference to the type family *Phomatosporaceae*

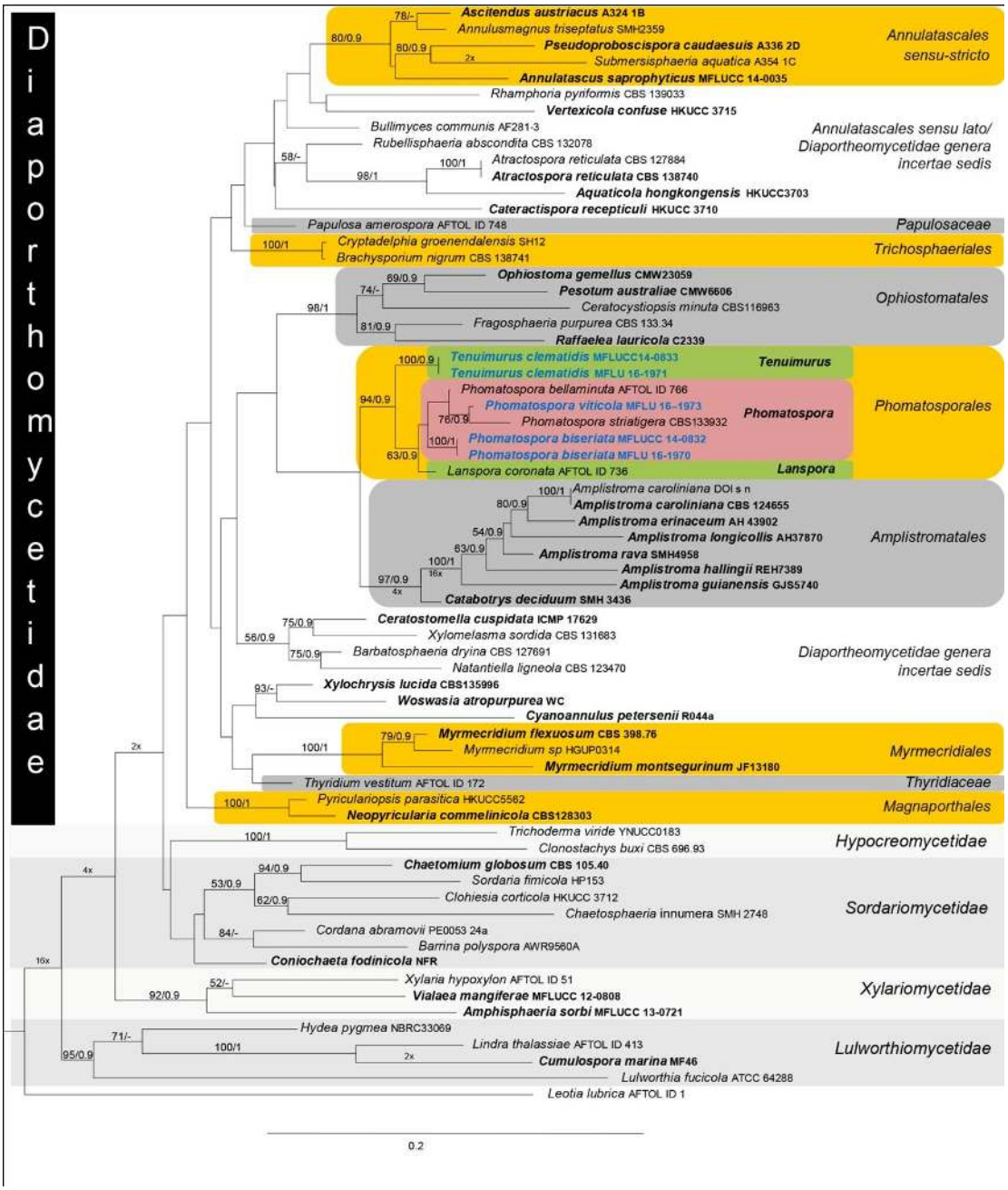

Fig. 1 – Phylogram inferred from analyses of LSU, SSU and ITS sequence data with ML analysis using a GTRGAMMA model of evolution. Maximum likelihood bootstrap support (MLB above 50) and Bayesian posterior probability (PP above 90 %) are indicated at the nodes. Newly introduced strains are in blue bold and type strains are in bold. The tree is rooted to *Leotia lubrica* (AFTOL ID 1).

Saprobic on submerged wood or decaying twigs in terrestrial or aquatic environments. Sexual morph – Ascomata solitary to gregarious, immersed or becoming erumpent with age, globose or subglobose, light brown, dark brown to black, coriaceous, sometimes developing under a small blackened clypeus, ostiolate, papillate. Peridium comprising small, brown pseudoparenchymatous cells. Hamathecium comprising hypha-like, distally tapering, paraphyses. Asci 8-spored, unitunicate, cylindrical, thin-walled, short-pedicellate or sessile, with J- apical ring. Ascospores uniseriate, overlapping uniseriate to biseriate, ellipsoidal to fusiform, septate to aseptate, not constricted at the septum, hyaline, sometimes bi-guttulate, with striations or appendages. Asexual morph–*Sporothrix*-like (Rappaz 1992, Fournier & Lechat 2010).

Type family – ***Phomatosporaceae*** Senan., & K.D. Hyde

Notes – Analyses of combined LSU, SSU and ITS sequence data (Fig. 1) reveals that *Phomatospora, Lanspora,* and *Tenuimurus* group together, forming a distinct clade apart from the known orders in Diaporthomycetidae and this lineage is introduced here as *Phomatosporales* as an order of Diaporthomycetidae. *Amplistromatales* which is the sister clade of *Phomatosporales* was placed in Sordariomycetes order *incertae sedis* by Maharachchikumbura et al. (2016). However this study proved the phylogenetic placement of *Amplistromatales* in Diaporthomycetidae. Members of this clade differ from the sister clades in having thin-walled, long, cylindrical asci with minute apical rings and small, globose, unicellular, hyaline ascospores. Réblová et al. (2016) analyzed combined ITS, LSU, SSU and RPB2 sequence data in Sordariomycetous taxa and also showed *Phomatospora* and *Lanspora* to form a distinct clade with high support (MLB/PP=100/1). However we could not obtain RPB2 sequences from our taxa and LSU, SSU, ITS combined sequences were well separated taxa in the analysis combined (Fig. 1) and single gene analysis.

Phomatosporaceae Senan. & K.D. Hyde, **fam. nov**
Index Fungorum number IF552312
Facesoffungi number FoF 02486

Etymology – In reference to the type genus *Phomatospora*.

Saprobic on submerged wood or decaying twigs in terrestrial or aquatic environments. Sexual morph – Ascomata solitary to gregarious, immersed or becoming erumpent with age, globose or subglobose, light brown, dark brown to black, coriaceous, sometimes developing under a small blackened clypeus, ostiolate, papillate. Peridium comprising small, brown pseudoparenchymatous cells. Hamathecium comprising hypha-like, distally tapering, paraphyses. Asci 8-spored, unitunicate, cylindrical, thin-walled, short-pedicellate or sessile, with J- apical ring. Ascospores uniseriate, overlapping uniseriate to biseriate, ellipsoidal to fusiform, septate to aseptate, not constricted at the septum, hyaline, sometimes bi-guttulate, with striations or appendages. Asexual morph–*Sporothrix*-like (Rappaz 1992, Fournier & Lechat 2010).

Type genus – ***Phomatospora*** Sacc., Nuovo G. bot. ital. 7: 306 (1875)

Notes –The familial name *Phomatosporaceae* was invalidly introduced by von Arx (1951) and *Phomatosporaceae* is formally established here to accommodate *Phomatospora*, *Lanspora* and *Tenuimurus. Phomatospora*, typified by *P. berkeleyi* Sacc., was placed in Sordariomycetes genera *incertae sedis* (Lumbsch & Huhndorf 2007). Réblová et al. (2016) and our molecular analyses (Fig. 1), showed that *Phomatospora* clusters together with *Lanspora.* Hence we establish the new family, *Phomatosporaceae* in *Phomatosporales* (Sordariomycetes) to accommodate these two genera and introduced one additional genus, *Tenuimurus*.

Lanspora K.D. Hyde & E.B.G. Jones, Can. J. Bot. *64(8):* 1581 (1986)

Lanspora was typified by *Lanspora coronata* K.D. Hyde & E.B.G. Jones. This monotypic genus was assigned to *Halosphaeriaceae* (*Microascales*) based on morphology. However, molecular analyses placed *Lanspora* out of *Halosphaeriaceae* (Réblová et al. 2016). Combined gene analyses in this study (Fig. 1) showed that *Lanspora* clustered together with *Phomatospora* species. Morphologically, this genus differs from other genera in *Phomatosporaceae* by subclavate or oblong asci and ascospores with crown-like appendages.

Key to genera of *Phomatosporaceae*

1.....Ascospores ≤10 µm long, globules present at the ends; peridium thin ..... *Tenuimurus*
1.....Ascospores ≥10 µm long, globules present or absent, if present, located at the center; peridium thick..... ...2
2.....Appendages formed by longitudinal fragmentation of the exosporium, crown-like .....*Lanspora*
2.....Appendages do not formed by the exosporium, filamentous or sheet-like..... *Phomatospora*

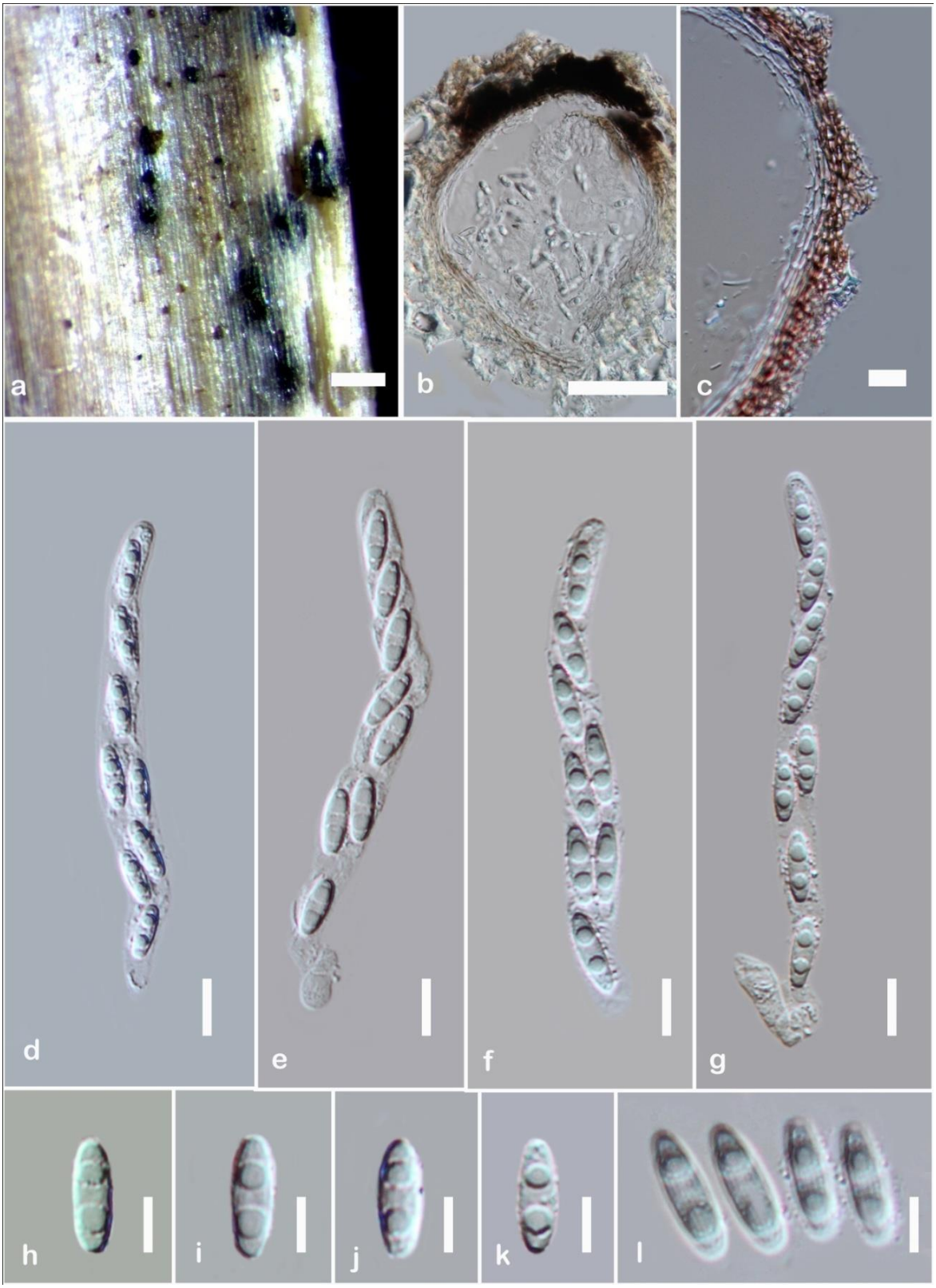

Fig. 2 – *Phomatospora biseriata* (holotype). a. Appearance of ascomata on substrate. b. Cross section of ascoma. c. Peridium. d–g. Asci. h–k. Ascospores. l. Indistinct longitudinal striations. Bars: a = 200 µm, b = 50 µm, c-g = 10 µm, h-l = 5 µm.

Phomatospora Sacc., Nuovo G. bot. ital. 7: 306 (1875)
Index Fungorum number IF4015
Facesoffungi number FoF 02487

Saprobic on submerged wood or decaying twigs. Sexual morph– Ascomata solitary to rarely gregarious, immersed or becoming erumpent with age, globose or subglobose, light brown, dark brown to black, coriaceous, sometimes developing under a small blackened clypeus, ostiolate, papillate. Papilla short or rarely somewhat long, central or eccentric, cylindrical, sometime covered with black, amorphous material around the upper region, periphyses hyaline, short, filiform. Peridium comprising small, brown pseudoparenchymatous cells forming a *textura angularis* to *textura prismatica* or inner, hyaline, thick-walled cells of *textura angularis* and outer, brown, cells of *textura angularis*. Hamathecium comprising hypha-like, filamentous, septate or aseptate, slightly constricted at the septa, distally tapering, hyaline, paraphyses. Asci 8-spored, unitunicate, cylindrical or oblong-fusiform, thin walled, short stalked or sessile, apex oblong with J- apical apparatus. Ascospores uniseriate, rarely biseriate, overlapping uniseriate to biseriate, ellipsoidal to fusiform, 0–3 septa, not constricted at the septum, hyaline, sometimes bi-guttulate, guttules located at the ends of the cell, or longitudinally striate, sometime with filamentous appendages at both ends. Asexual morph – *Sporothrix*-like reported from culture (Rappaz 1992, Fournier & Lechat 2010).

Type species – ***Phomatospora berkeleyi*** Sacc., Nuovo G. bot. ital. 7: 306 (1875)

Notes – *Phomatospora* comprises 116 species epithets (Index Fungorum, 2016) and only 93 species belong to *Phomatospora. Phomatospora bellaminuta* and *P. striatigera* have molecular data in Genbank. *Phomatospora* species are reported from both marine or aquatic and terrestrial habitats. Marine or aquatic *Phomatospora* species shows some morphological adaptation to the habitat such as appendages, or slimy sheaths. These characters help to disperse the ascospores and facilitate subsequent attachment to substrates (Hyde 1993b, Raja & Shearer 2008).

Phomatospora biseriata Senan., Camporesi & K.D. Hyde, **sp. nov.** Fig. 2
Index Fungorum number IF552313
Facesoffungi number FoF 02488

Etymology – Based on the ascospores having longitudinal striations.

Holotype – MFLU 16–1970

Saprobic on *Clematis vitalba* L. Sexual morph – Ascomata 115–170 µm high × 125–200 µm diam., ($\overline{x}$ = 152 × 150 µm, n = 10), solitary to aggregated, immersed and becoming erumpent with age, globose to subglobose, membranous, coriaceous, brown, ostiolate, papillate. Papilla 43–66 µm high, 46–63 µm wide ($\overline{x}$ = 50 × 45 µm, n = 10), short, central or eccentric, broadly conical, periphysate, covered with black. Peridium 5–15 µm wide ($\overline{x}$ = 11 µm, n =10), comprising inner, hyaline, thick-walled elongated cells and outer, brown, thick-walled elongate cells. Hamathecium comprising few, hypha-like, thin-walled, fragile, septate, constricted at septa, hyaline, paraphyses tapering above and shorter than asci. Asci 200–230 × 19–23 µm ($\overline{x}$ = 207 × 22 µm, n =20), 8-spored, unitunicate, cylindrical to fusiform, thin-walled, pedicellate, with a refractive, J-, apical ring. Ascospores 25–29 × 9–11.5 µm ($\overline{x}$ = 27 × 10 µm, n =20), overlapping uniseriate, ellipsoidal, hyaline, unicellular, bi-guttulate, guttules located at the ends of the cell, longitudinally striate. Asexual morph – Undetermined.

Culture characters – Colonies growing on MEA attenuated 1 cm within 14 days incubated at 18°C, slow growing, lacking aerial mycelium, tightly attached to the media, irregular, convex, undulate, cream to olivaceous.

Material examined – ITALY, Province of Forlì-Cesena [FC]), near Premilcuore, dead branch of *Clematis vitalba* L. (*Ranunculaceae*), 1 March 2013, Erio Camporesi, IT 1085, (MFLU 16–1970 holotype), ex-type living cultures, MFLUCC 14–0832.

Notes – *Phomatospora biseriata* clusters in the *Phomatospora* clade as a distinct species. The overlapping uniseriate to biseriate ascospore arrangement is unusual in *Phomatospora*. Hence here we introduce it as a new species. There are no *Phomatospora* species reported from *Clematis* (Index Fungorum 2016, Farr & Rossman 2016).

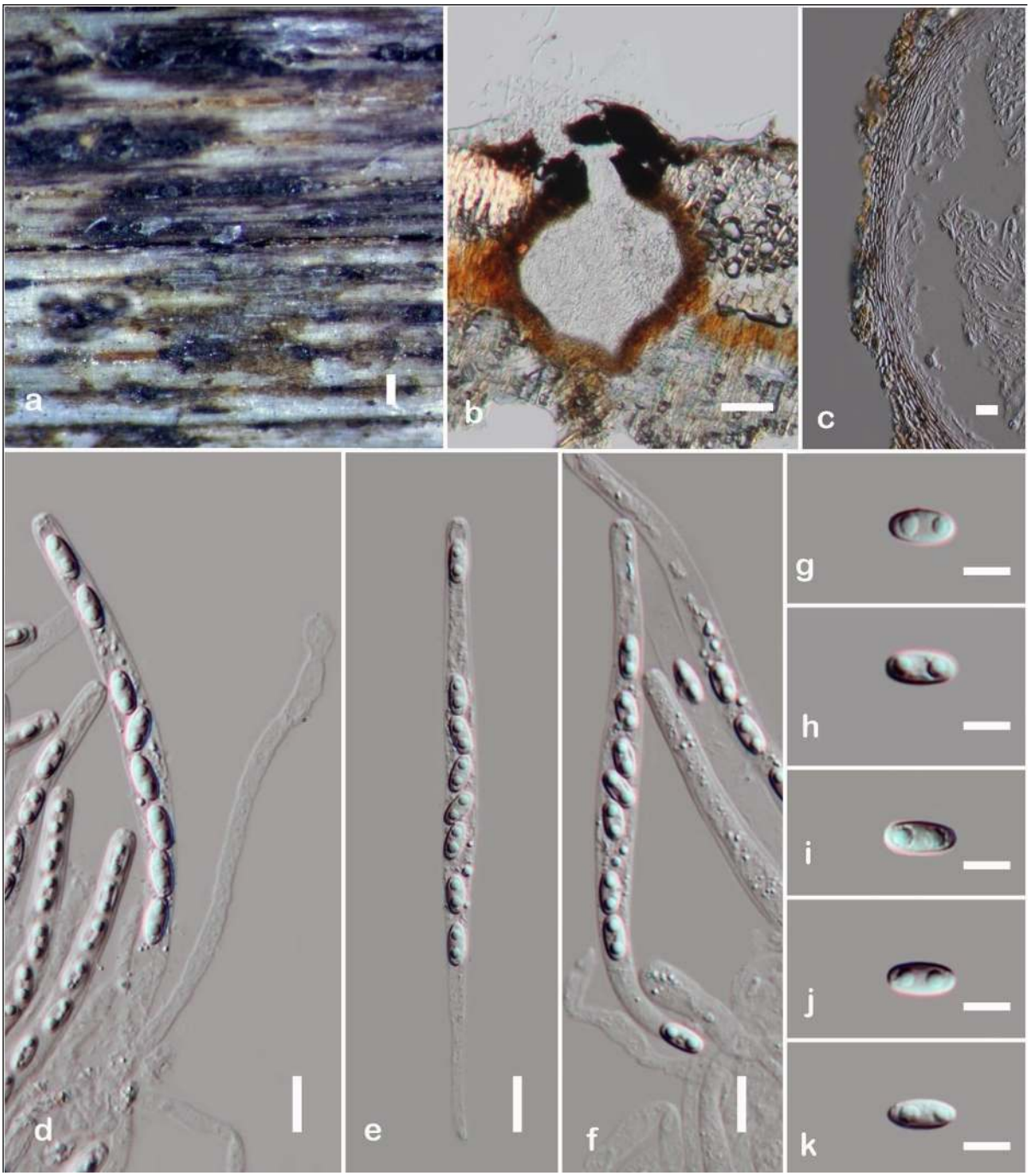

Fig. 3 – *Phomatospora viticola* (holotype). a. Appearance of ascomata on the host. b. Cross section of ascoma. c Peridium. d–f. Asci. g–k Ascospores. Bars: a = 200 µm, b = 50µm, c = 10µm, d-f = 10µm, h-k = 5µm.

Phomatospora viticola Senan., Camporesi & K.D. Hyde, **sp. nov.** Fig. 3

Index Fungorum number IF552314

Facesoffungi number FoF 02489

Etymology – based on two Latin words "*Vitis*" and "cola", meaning "*Vitis* loving".

Holotype – MFLU 16–1973

Saprobic on *Vitis vinifera* L. Sexual morph – Ascomata 420–473 µm high, 320–385 µm diam. ($\overline{x}$ = 450 × 375 µm, n = 10), solitary to aggregated, immersed, globose to subglobose, membranous, coriaceous, brown, ostiolate, papillate. Papilla 140–170 µm high, 165–180 µm wide ($\overline{x}$ = 160 × 175 µm, n = 10), short, central, broadly conical, periphysate, covered with black.

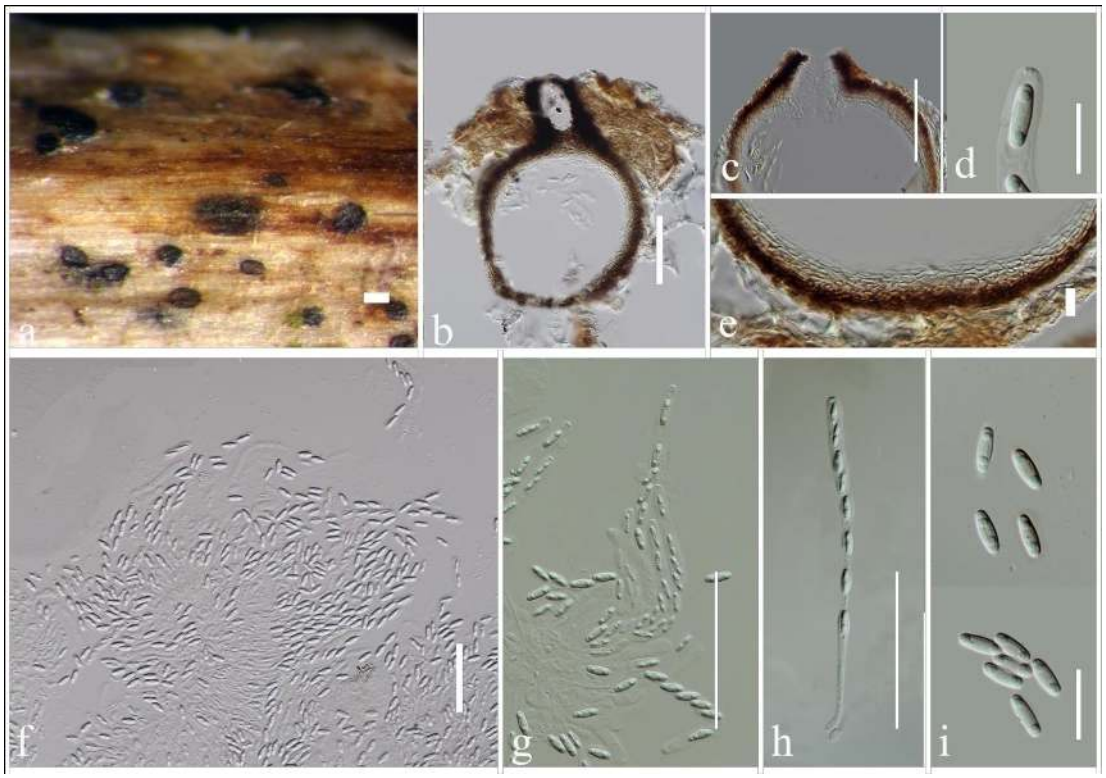

Fig. 4 – *Tenuimurus clematidis* (holotype). a. Appearance of ascomata on substrate. b. Cross section of ascoma. c. Papilla. d. Apical ring. e. Peridium. f–h. Asci. i. Ascospores. Bars: a = 100 µm, b, c, e = 50 µm, f = 20 µm, g, h = 50 µm, i, d = 10 µm.

Peridium 8–14 µm wide ($\bar{x}$ = 10 µm, n =10), comprising inner, hyaline, thick-walled elongated cells and outer, brown, thick-walled elongate cells. Hamathecium comprising hypha-like, aseptate, hyaline, paraphyses. Asci 115–200 × 12–20 µm ($\bar{x}$ = 153 × 16 µm, n =20), 8-spored, unitunicate, cylindrical, thin-walled, pedicellate, with a refractive, J-, apical ring. Ascospores 12–17 × 4.3–5.8 µm ($\bar{x}$ = 13.5 × 5.2 µm, n =20), uniseriate, ellipsoidal, hyaline, unicellular, bi-guttulate, guttules located at each end of the cell. Asexual morph – Undetermined.

Material examined – ITALY, Province of Forlì-Cesena [FC], Predappio, Marsignano, on dead branch of *Vitis vinifera* L. (*Vitaceae*), 7 February 2014, Erio Camporesi, IT 1708 (MFLU 16–1973 holotype).

Notes – There are no *Phomatospora* species reported from *Vitis* prior to this study (Index Fungorum 2016, Farr & Rossman 2016). Hence *Phomatospora viticola* is the first species in this genus reported from *Vitis*. However, we could not obtain a culture from this species and obtained sequence data directly from the ascomata contents. *Phomatospora viticola* differs from other *Phomatospora* species in having ascomata wider than 400 µm, with a black clypeus, long asci with septate, hypha-like paraphyses and globose to ellipsoidal ascospores. Phylogenetically *Phomatospora viticola* has moderate support as being distinct from the other species in the genus that have sequence data.

Tenuimurus Senan., Camporesi & K.D. Hyde,**gen. nov.**
Index Fungorum numberIF552315
Facesoffungi number FoF 02490

Etymology – Based on the Latin words "Tenuis" and "Murus" meaning the delicate, thin peridium.

Saprobic on stems of overwintered plants. Sexual morph –*Ascomata* solitary to aggregated, immersed, globose to subglobose, membranous, coriaceous, brown, developing under a small blackened clypeus, ostiolate, papillate. *Papilla* short, central, periphysate. *Peridium* thin,

comprising very few inner, hyaline, thick-walled elongated cells and outer, brown, thick-walled elongate cells. *Hamathecium* comprising hypha-like, filamentous, septate, distally tapering, hyaline, paraphyses. *Asci* 8-spored, unitunicate, cylindrical, thin-walled, pedicellate, with a refractive, J-, apical ring. *Ascospores* uniseriate, ellipsoidal, hyaline, unicellular, bi-guttulate, guttules located ends of the cell. Asexual morph – Undetermined.

Type species – ***Tenuimurus clematidis*** Senan., Camporesi & K.D. Hyde

Tenuimurus clematidis Senan., Camporesi & K.D. Hyde, **sp. nov.** Fig. 4

Index Fungorum number IF552316

Facesoffungi number FoF 02491

Etymology – In reference to the host genus *Clematis*.

Holotype – MFLU 16–1971

Saprobic on *Clematis vitalba* L. Sexual morph – Ascomata 125–180 µm high × 130–170 µm diam., ($\overline{x}$ = 150 × 156 µm, n = 10), solitary to aggregated, immersed, globose to subglobose, membranous, coriaceous, brown, developing under a small blackened clypeus, ostiolate, papillate. Papilla 58–65 µm high, 35–45 µm wide ($\overline{x}$ = 60 × 40 µm, n = 10), short, central, periphysate. Peridium 10–15 µm wide ($\overline{x}$ = 13 µm, n =10), comprising inner, hyaline, thick-walled elongated cells and outer, brown, thick-walled elongate cells. Hamathecium comprising hypha-like, filamentous, septate, distally tapering, hyaline, paraphyses. Asci 55–80 × 7–7.5 µm ($\overline{x}$ = 68 ×7 µm, n=20), 8-spored, unitunicate, cylindrical, thin-walled, pedicellate, with a refractive, J-, apical ring. Ascospores 9–10 × 3–3.5 µm ($\overline{x}$ = 9.5 ×3.2 µm, n =20), uniseriate, ellipsoidal, hyaline, unicellular, bi-guttulate, guttules located at ends of the cell. Asexual morph – Undetermined.

Culture characteristics – Colonies on MEA, slow growing, reaching 2 cm after 14 days at 18°C, circular, flat, filiform, white, dense colonies, somewhat tightly attached to the media.

Material examined – ITALY, Province of Forlì-Cesena [FC], near Dovadola, on dead branch of *Clematis vitalba* L (*Ranunculaceae*), 19 November 2013, Erio Camporesi, IT 1523 (MFLU 16–1971 holotype), ex-type living culture, MFLUCC 14–0833.

Notes – *Tenuimurus* is a monotypic genus, introduced based on *T. clematidis.* This genus morphologically differs from other genera in *Phomatosporaceae* as *Tenuimurus* has a dark, thin, delicate, peridium with small asci (< 80 µm in high) and smaller ascospores (< 10 µm in length). The phylogenetic analysis in this study (Fig 1) provides high support (MLB/PP=94/0.9) for *Tenuimurus* as a distinct genus. No species of *Phomatospora* are known from *Clematis* (Farr & Rossman 2016).

Discussion

Phomatospora is a widely distributed genus in aquatic, marine and terrestrial habitats. Most species in this genus based on morphological characters. Only two *Phomatospora* species has sequence data previously and here we introduce two new *Phomatospora* species based on morpho-phylogenetic characters. *Phomatospora* and *Lanspora* together with new genus *Tenuimurus* form a distinct clade which is sister to the *Amplistromatales.* Hence we introduce a new order *Phomatosporales* and a new family *Phomatosporaceae* to accommodate these genera.

Our preliminary studies showed that *Paramicrothyrium chinensis* H.X. Wu & K.D. Hyde has 99% similarity to *Phomatospora biseriata.* Wu et al. (2011) introduced *Paramicrothyrium* based on *P. chinensis* using molecular data. However the combined LSU and SSU analysis of that study showed this species morphologically close to *Microthyrium*, but phylogenetically distant from *Microthyrium*. In addition to this, Singtripop et al. (2016) showed that *Paramicrothyrium chinensis* H.X. Wu & K.D. Hyde (IFRDCC 2258) clusters with *Chaetothyrina mangiferae* (*Micropeltidaceae*) with a high support. Hence these sequences might have some errors and we exclude them in our final analysis.

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