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Two new *Seimatosporium* species from Italy

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Abstract

Two taxa resembling *Seimatosporium* and *Seiridium* were collected from Italy. Mega blast results of ITS and LSU sequence data, showed that new collections are related to *Seimatosporium*. Parsimonious analyses based on LSU and ITS sequence data showed that new taxa reside in *Seimatosporium sensu stricto*. Based on morphological and molecular analyses, the new collections are introduced as new species and compared with taxa with similar morphological characters and host association.

Keywords – Coelomycetes – morphology – multi-gene – phylogeny

Introduction

The genus *Seimatosporium* Corda was introduced by Corda (1833) with *S. rosae* Corda as the type species. The genus is characterised by holoblastic to annellidic conidiogenous cells and cylindrical, fusiform or clavate or obovoid conidia, with brown median cells and (2−)3(−5)-septa (Sutton 1980, Nag Raj 1993, Barber et al. 2011, Tanaka et al. 2011, Senanayake et al. 2015, Norphanphoun et al. 2015, Wijayawardene et al. 2016). Conidia may entirely lack appendages, have only apical or basal appendages or have both apical and basal appendages (Norphanphoun et al. 2015, Wijayawardene et al. 2016). Recent phylogenetic studies showed that *Seimatosporium* resides in *Discosiaceae*, *Amphisphaeriales* (Senanayake et al. 2015, Norphanphoun et al. 2015, Wijayawardene et al. 2016). Tanaka et al. (2011) showed that *Seimatosporium* groups with *Discostroma* as a monotypic clade and this was confirmed by Senanayake et al. (2015), Norphanphoun et al. (2015) and Wijayawardene et al. (2016).

In this paper, we introduce two new species of *Seimatosporium* based on morpho-molecular analyses. Morphological characters and taxonomic keys in Sutton (1980), Nag Raj (1993) and recently published articles (Ariyawansa et al. 2015, Senanayake et al. 2015, Norphanphoun et al. 2015) were used to compare morphological characters. The phylogenetic analyses were carried out based on LSU and ITS sequence data.

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Materials and Methods

Collection, isolation and morphological studies

Decayed plant materials were collected from Italy, and placed in paper bags and/or Zip-lock bags. The samples were observed with a stereo microscope to detect fruit bodies. Sterilized needles were used to pick conidiomata and squash mounts were made to reveal the micro- morphological characters *viz*. conidiophores, conidiogenous cells, conidiogenesis and conidia (Sutton 1980). Vertical sections of conidioma were made using razor blades to examine the shape of conidioma and arrangement of conidiophores and conidiogenous cells. Morphological characters were examined under a compound microscope (Nikon Eclipse E600 DIC microscope and a Nikon DS-U2 camera or a Nikon Eclipse 80i compound microscope fitted with a Canon 450D digital camera).

Isolation was carried out as detailed in Chomnunti et al. (2014) and germinating conidia were transferred aseptically to potato dextrose agar (PDA). Germinating conidia were transferred to PDA plates and incubated at 18 °C for further growth. Colony colour and other characters were assessed after 1 to 2 weeks. The holotype specimens are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. Ex-type cultures are also deposited in Culture Collection at Mae Fah Luang University (MFLUCC) and Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (GUCC). Facesoffungi and Index Fungorum numbers are provided as explained in Jayasiri et al. (2015) and Index Fungorum (2016)

DNA extraction, PCR amplification and sequencing

Colonies generated from germinated single conidia were further grown on PDA for 14 days at 18 °C. Fresh fungal mycelia were scraped from PDA using sterilized scalpels. A BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) was used to extract DNA from the scraped mycelia. The amplification of rDNA regions of the internal transcribed spacers (ITS) and large subunit (LSU) genes was carried out by using primers ITS5 and ITS4 and LROR and LR5 (Vilgalys and Hester 1990, White et al. 1990). Optimum conditions for amplification of ITS and LSU regions are as described in Alves et al. (2004). Amplified PCR fragments were checked on 1% agarose electrophoresis gels stained with ethidium bromide. Purified PCR products (by minicolumns, purification resin and buffer according to the manufacturer's protocols Amersham product code: 27-9602-01) were sent to SinoGenoMax Co., Beijing, China for DNA sequencing. The nucleotide sequence data obtained are submitted to GenBank (Table 1).

Phylogenetic analyses

A megablast search was carried out to confirm the placement of the new strains in *Amphisphaeriales* and therefore phylogenetically related sequences were downloaded from GenBank (Table 1). However, only two strains were successful in single spore isolation, thus, only two strains were used in the phylogenetic analyses. Since both strains show a closer relationship with *Seimatosporium* and *Discostroma*, Ariyawansa et al. (2015), Senanayake et al. (2015) and Norphanphoun et al. (2015) were used to select strains for the phylogenetic analyses. Sequences for each gene region (LSU and ITS) were aligned using MAFFTv6 (Katoh et al. 2002, Katoh and Toh 2008), and online sequence alignment was edited under the default settings (mafft.cbrc.jp/alignment/server/). All absent genes were coded as missing data.

Combined LSU and ITS datasets was performed using maximum parsimony (MP) and Bayesian Posterior Probabilities (BYPP). Maximum-parsimony analyses were performed by PAUP v. 4.0b10 (Swofford 2002) using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. The Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Index (HI) were calculated for each tree generated.

Table 1 Strains used in this study

Taxon	***Culture collection no.***	***GenBank Accession no.***	
		LSU	***ITS***
Adisciso yukushimense	MAFF 242774	AB593721	-
Adisciso tricellulare	NBRC 32705	AB593728	-
Discosia artocreas	NBRC 8975	AB593705	-
Discosia pini	MAFF 410149	AB593708	AB594776
Discosia pseudoartocreas	CPC 21117	KF777214	-
Discosia aff *pleurochaeta*	MAFF 242778	AB593709	AB594777
Discosia aff *pleurochaeta*	MAFF 242779	AB593713	AB594781
Discosia aff. *brasiliensis*	MAFF 237018	AB593719	AB594787
Discostroma botan	HHUF 4642	DQ368629	-
Discostroma fuscellum	MFLUCC 14–0052	KT005514	KT005515
Discostroma fuscellum	NBRC 32680	AB593739	AB594806
Discostroma stoneae	NBRC 32690	AB593729	AB594797
Discostroma tostum	NBRC 32626	AB593727	AB594795
Pseudopestalotiopsis theae	MFLUCC 12−0055	KM116282	JQ683727
Sarcostroma bisetulatum	CBS 122695	-	EU552155
Sarcostroma lomatiae	CBS 118144	DQ278926	DQ278921
Sarcostroma restionis	CBS 118153	DQ278925	DQ278923
Sarcostroma restionis	CBS 118154	DQ278924	DQ278922
Seimatosporium azaleae	MAFF 237478	AB593730	AB594798
Seimatosporium biseptatum	CPC 13584	JN871208	JN871199
Seimatosporium botan	H 4619	AB593731	AB594799
Seimatosporium botan	HMUC 316PD	AB594799	-
Seimatosporium cornii	MFLUCC 14–0467	KR559739	KT162918
Seimatosporium discosioides	H 4621	AB593732	AB594800
Seimatosporium elegans	NBRC 32674	AB593733	AB594801
Seimatosporium eucalypti	CPC 156 / CBS 115131	JN871209	JN871200
Seimatosporium eucalypti	CPC 157 / CBS 110733	JN871210	JN871201
Seimatosporium eucalypti	CPC 158 / CBS 110734	JN871211	-
Seimatosporium eucalypti	CPC 159 / CBS 114876	JN871212	JN871202
Seimatosporium falcatum	CPC 12992	-	JN871203
Seimatosporium falcatum	CPC 13578	JN871213	JN871204
Seimatosporium falcatum	CPC 13580	JN871214	JN871205
Seimatosporium ficeae	MFLUCC 15–0519	KR920686	-
Seimatosporium foliicola	NBRC 32676	AB593734	AB594802
Seimatosporium glandigenum	NBRC 32677	AB593735	AB594803
Seimatosporium grevilleae	ICMP 10981	AF382372	AF405304
Seimatosporium hakeae	NBRC 32678	AB593736	AB594804
Seimatosporium hypericinum	NBRC 32647	AB593737	AB594805
Seimatosporium kriegerianum	NBRC 32679	AB593738	-
Seimatosporium leptospermi	ICMP 11845	AF382373	-
Seimatosporium lichenicola	MFLUCC 14−0623	KT198725	KT198724
Seimatosporium mariae	NBRC 32681	AB593740	AB594807
Seimatosporium obtusum	CPC 12935	JN871215	JN871206
Seimatosporium parasiticum	NBRC 32682	AB593741	AB594808
Seimatosporium physocarpi	MFLUCC 14−0625	KT198723	KT198722
Seimatosporium pistaciae	CBS 138865	KP004491	KP004463
Seimatosporium rhombhisporum	MFLUCC 15–0543	KR092780	KR092792
Seimatosporium rosae	MFLUCC 14−0621	KT198727	KT198726
Seimatosporium vaccinii	ICMP 7003	AF382374	-
Seimatosporium vitis	MFLUCC 14−0051	KR920362	KR920363
Seimatosporium walkeri	CPC 17644	JN871216	JN871207
Seimatosporium cornicola	MFLUCC 14−0448		KU974967
Seimatosporium quercina	MFLUCC 14−1198	KU974964	KU974965

Independent Bayesian phylogenetic analyses were performed in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) using a uniform [GTR+I+G] model. Posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 100th generation (resulting in 10,000 total trees). Phylogenetic trees were visualized with Treeview v. 1.6.6 (Page 1996). Bootstrap values of MP analyses (equal or above 70%) and BYPP with those equal or greater than 0.95 are shown on the upper branches.

Results

Phylogenetic analyses

The combined LSU and ITS data set consists of 67 strains with *Pseudopestalotiopsis theae* (MFLUCC 12−0055) as the outgroup taxon. The data set consists of 1362 characters of which 1103 are constant, 83 are variable parsimony-uninformative characters and 176 are parsimony-informative characters. One of the 16 equally most parsimonious trees is shown in Fig. 1.

Seimatosporium sensu stricto separates from *Discosia sensu stricto* with high bootstrap values and PP values (100% and 1.00). A new strain, MFLUCC 14−0448 grouped with *Seimatosporium pseudocornii* (MFLUCC 13−0529) with high bootstrap values and PP values (86% and 1.00).

Taxonomy

Seimatosporium pseudoglandigenum Wijayaw. & E. Camporesi, ***sp. nov***
Facesoffungi number: FoF 02076
Index Fungorum number: IF552047
Etymology: Named as it morphologically resembles *Seimatosporium glandigenum*
Holotype: MFLU 16−0837

Saprobic or endophytic on leaves of *Quercus cerris* L. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 150−300 μm diam., 100−150 μm high, acervular, unilocular, subglobose to globose, superficial, gregarious, dark brown to black, apapillate ostiolate. *Conidiomata wall* multi-layered, outer wall thick, composed of brown cells of *textura angularis*, inner wall thin, hyaline, composed of hyaline cells of *textura angularis*. *Paraphyses* absent. *Conidiophores* 5−30 × 2−4 μm, long, cylindrical, branched, hyaline, smooth-walled. *Conidiogenous cells* holoblastic, simple, integrated, determinate, hyaline. *Conidia* 15−23 × 5−8 μm ($\bar{x}$ = 19.14 × 6.35 μm, n = 20), obovoid to fusiform, or cymbiform, obtuse apex, straight to slightly curved, with 3 transverse septa, septa dark brown, constricted at the septa or continuous, eguttulate, medium brown to golden brown, with hyaline to sub-hyaline basal and apical cell, smooth-walled.

Material examined − Italy, Forlì-Cesena [FC] Province, near San Paolo in Alpe - Santa Sofia, on decaying leaves of *Quercus cerris* L. (*Fagaceae*), E. Camporesi, 15 December 2013, IT 1577, MFLU 16–0837, **holotype**.

Notes − We made several attempts to isolate this taxon in different media, but were unsuccessful. Hence, we compare our new collection with related species by host association (Sutton 1980, Nag Raj 1993, Farr and Rossman 2016). Taxa reported from *Quercus* species are summarized in Table 2. Moreover, we compared our collection with other *Seimatosporium* species with 3 transverse septa (Sutton 1980, Nag Raj 1993), but our new collection is morphologically distinct.

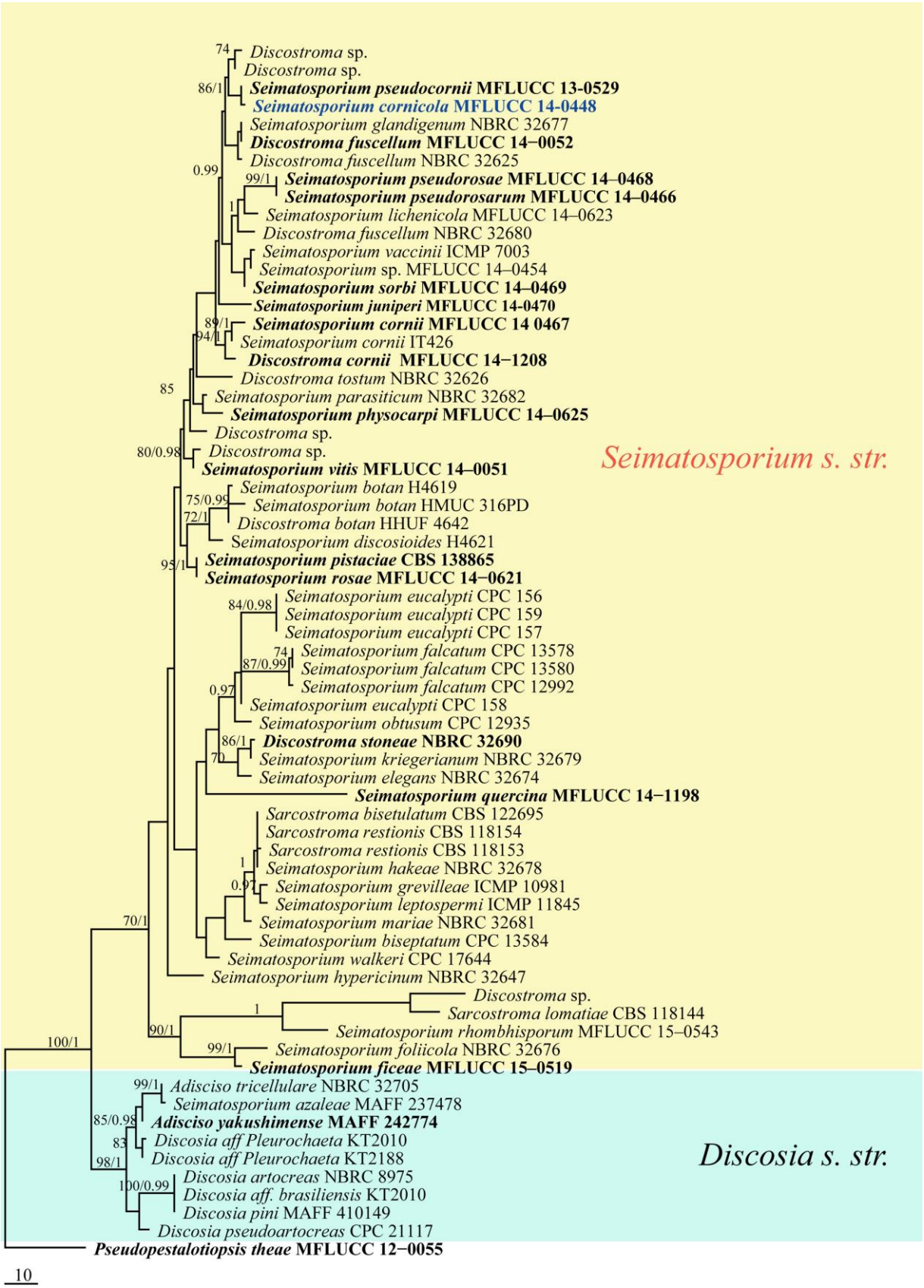

Fig. 1 − One of the 16 equally most parsimonious trees obtained from combined analyses set of ITS and LSU sequence data. (CI=0.557, RI=0.776, RC=0.443, HI=0.443). MP values (>70 %) resulting from 1000 bootstrap replicates and Bayesian posterior probabilities above 0.95 are given at the nodes. The tree is rooted to *Pseudopestalotiopsis theae* (MFLUCC 12−0055). Ex-type strains are in bold and newly introduced species is in blue.

Table 2 *Seimatosporium* species reported from *Quercus* spp.

Seimatosporium spp.	Spore dimensions	Country	Host – *Quercus* spp.	Reference
S. caninum (Brunaud) B. Sutton	9.5−12 μm × 4.5−5.5 μm	India	*Q. incana*	Sutton 1980
S. glandigenum (Bubák & Gonz. Frag.) B. Sutton	15−18 μm × 5−6. 5 μm	Spain	*Q. ballota*	Sutton 1980
S. lichenicola (Corda) Shoemaker & E. Müll.	13−15 μm × 5.5−6.5 μm	Italy	*Q. ilex*	Sutton 1980

Seimatosporium caninum has only 2-septate conidia (Sutton 1980), thus it is distinct from our collection, which has 3-septate conidia. *Seimatosporium glandigenum* (15−18 × 5−6. 5 μm) has a similar conidial morphology with our taxon (15−23 × 5−8 μm). However, our collection has higher variation in both conidial width and length. Sutton (1980) did not mention the slightly curved conidia in *S. glandigenum*, but our new species has slightly curved conidia.

Seimatosporium cornicola Wijayaw. & E. Camporesi, ***sp. nov***
Facesoffungi number: FoF 02077
Index Fungorum number: IF552048
Etymology: Named after the host genus *Cornus*
Holotype: MFLU 16−0701

Saprobic on dead branches of *Cornus sanguinea*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 330−400 μm diam., 220−250 *μm* high, acervular, superficial, solitary to gregarious, black, apapillate ostiolate. *Conidiomata wall* multi-layered, outer wall thick, composed of brown cells of *textura angularis*, inner wall thin, hyaline. *Conidiophores* 25−55 × 2−4 μm, long, cylindrical, branched, hyaline, smooth-walled. *Conidiogenous cells* holoblastic, simple, integrated, determinate, hyaline. *Conidia* 34−51 × 13−18 μm ($\bar{x}$ = 41.86 × 16.1 μm, n = 20), fusiform or obovoid, base truncate, straight, with 3 transverse septa, dark septa brown, constricted at septa, guttulate when immature, medium brown, with hyaline to subhyaline basal cell, smooth-walled, appendage absent.

Culture characteristics − On PDA slow growing, attaining a diam. of 2.5 cm in 7 days at 18 ºC, white to pale brown from above, greyish white from below, with sparse mycelium, flat, margin uneven.

Table 3 *Seimatosporium* spp. reported from *Cornus* spp.

Seimatosporium spp.	Spore dimensions	Country	Reference
S. lichenicola	13–15 × 5.5–6.5 μm *fide* Sutton 1980	Ukraine	Farr and Rossman 2016
S. salicinum (Corda) Nag Raj	11–17 × 4–6 μm *fide* Nag Raj 1993	Ukraine	Farr and Rossman 2016
S. corni Wijayaw. et al.	21–29 × 9–11 μm	Italy	Senanayake et al. 2015
S. pseudocornii Wijayaw. et al.	31−42 × 5−7 μm	Italy	Zhao et al. in prep.
S. cornicola	34−51 × 13−18 μm	Italy	In this study

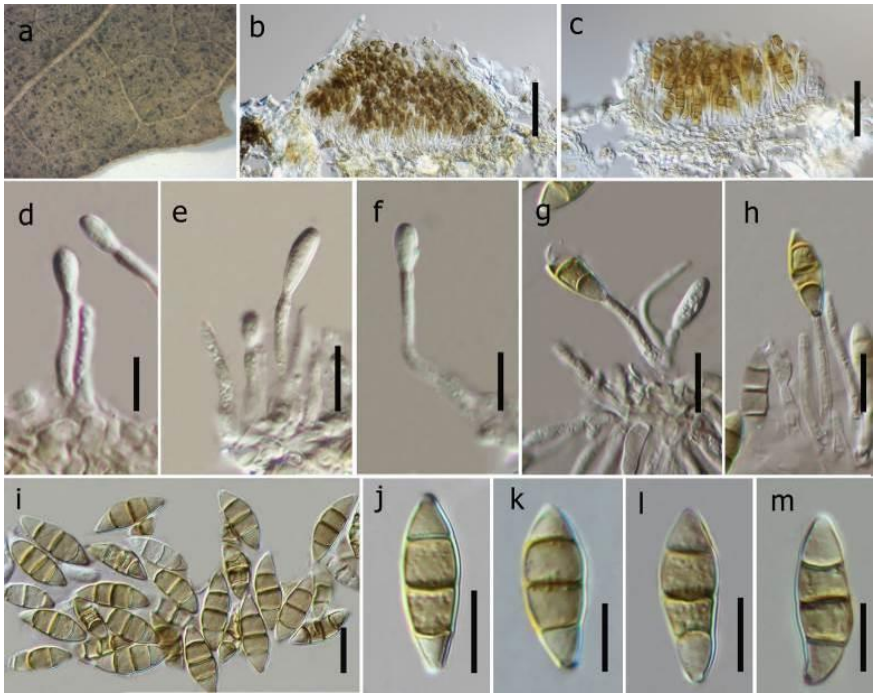

Fig. 2 − ***Seimatosporium pseudoglandigenum*** **(holotype)**. **a** Conidiomata on leaves of *Quercus cerris*. **b, c** Vertical sections of conidiomata. **d-h** Different stages of conidiogenesis. **i-m** Conidia. Scale bars: b, c = 100 μm, d-m = 10 μm.

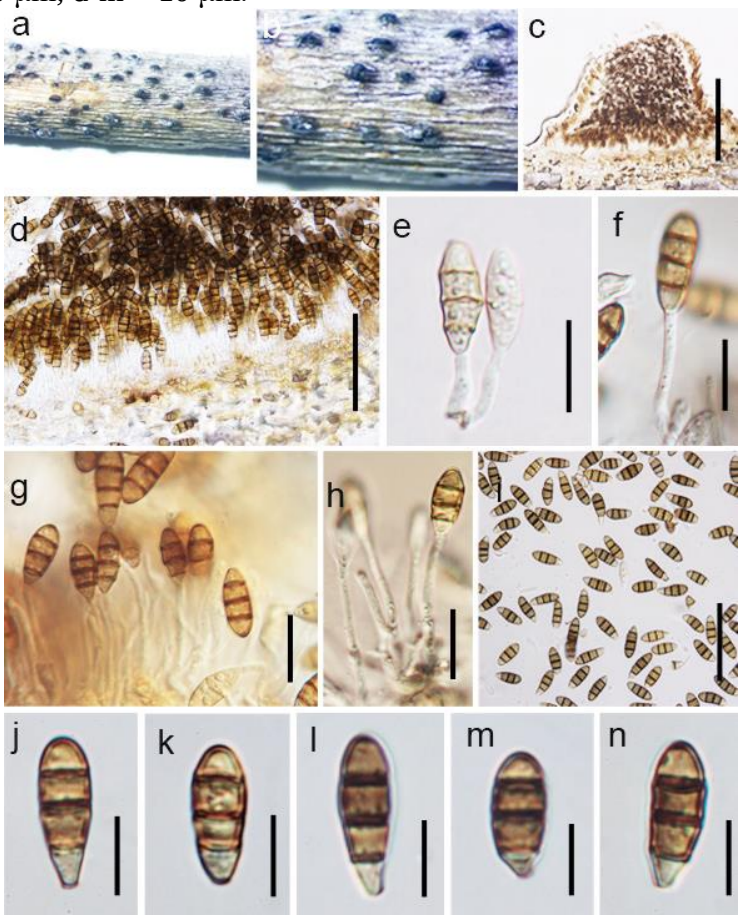

Fig. 3 − ***Seimatosporium cornicola*** **(holotype). a, b** Conidiomata on dead branch of *Cornus sanguinea*. **c, d** Vertical sections of conidiomata. **e-h** Developing conidia attach to conidiogenous. **i-n** Conidia. Scale bars: c = 200 μm, d = 150 μm, e-h = 30 μm, i = 100 μm, j-n = 35 μm.

Material examined − Italy, Forlì-Cesena [FC] Province, Camposonaldo - Santa Sofia, on dead branch of *Cornus sanguinea* L. (*Cornaceae*), E. Camporesi, 17 March 2012, IT 171 (MFLU 16−0701, **holotype**); ex-type living cultures MFLUCC 14−0448, GUCC IT 171.

Notes − Several *Seimatosporium* species have been recorded from *Cornus* spp. (Sutton 1980, Nag Raj 1993, Senanayake et al. 2015, Farr and Rossman 2016) (Table 3).

In phylogenetic analyses, the new collection clusters with *Seimatosporium pseudocornii* (MFLUCC 13−0529) with high bootstrap values and PP values (86% and 1.00 respectively). However, in conidial morphology, both species are distinct (see Table 3). *Seimatosporium pseudocornii* has shorter conidiophores than in *S. cornicola* (5−30 μm vs. 25−55 μm). Hence, we introduce a new scientific name to accommodate our new collection.

Discussion

The genus *Seimatosporium* comprises 86 epithets in Index Fungorum (2016), but only a few species have sequence data. Norphanphoun et al. (2015) designated the epitype (MFLUCC 14−0621) of *S. rosae*, the type species of *Seimatosporium* and phylogenetic analyses confirmed *Seimatosporium* and *Discostroma* are a monophyletic clade. Senanayake et al. (2015) showed that *Seimatosporium sensu stricto* grouped with *Discosia*, thus they introduced *Discosiaceae* to accommodate them. Wijayawardene et al. (2016) also agreed with the findings of Senanayake et al. (2015). Wijayawardene et al. (2016) used a combined LSU, ITS, SSU, β-tubulin and RPB2 data set in their analyses and confirmed the familial arrangements of Senanayake et al. (2015) in *Xylariomycetidae*.

In this study, we introduce two *Seimatosporium* species based on morphology or morpho-phylogenetic analyses. *Seimatosporium pseudoglandigenum* lacks sequence data, thus we have compared it with other taxa in Sutton (1980), Ariyawansa et al. (2015), Senanayake et al. (2015) and Zhao et al. (in prep). *Seimatosporium cornicola* groups with *Seimatosporium pseudocornii* (MFLUCC 13−0529) with high bootstrap and PP support (86% and 1.00 respectively). As they are distinct in morphology and show different branch lengths, we introduce *S. cornicola* as a new species.

Ariyawansa et al. (2015), Norphanphoun et al. (2015) and Goonasekara et al. (2016) used LSU and ITS sequence data in their analyses and are only available for most *Seimatosporium* species in GenBank (Crous et al. 2014, Ariyawansa et al. 2015, Norphanphoun et al. 2015, Goonasekara et al. 2016, Zhao et al. in prep.). However, it is much more reliable to include a protein gene as only LSU and ITS genes do not show high resolution among species. As an example, *Seimatosporium pseudorosarum* Wijayaw. et al. (Ariyawansa et al. 2015) groups with *S. pseudorosae* Wijayaw. et al. (Zhao et al. in prep.). Both species are morphologically distinct, as the former species only has basal appendages, while the latter has both apical and basal appendages. Since most of the species lack protein genes, we recommend relying both on morphology and molecular analyses, prior to introducing new species of *Seimatosporium*.

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