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Pseudodidymosphaeria gen. nov. in Massarinaceae

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Abstract

Didymosphaeria spartii was collected from dead branches of *Spartium junceum* in Italy. Multi-gene phylogenetic analyses of ITS, 18S and 28S nrDNA sequence data were carried out using maximum likelihood and Bayesian analysis. The resulting phylogenetic trees showed this to be a new genus in a well-supported clade in *Massarinaceae*. A new genus *Pseudodidymosphaeria* is therefore introduced to accommodate this species based on molecular phylogeny and morphology. A illustrated account is provided for the new genus with its asexual morph and the new taxon is compared with *Massarina* and *Didymosphaeria*.

Key words: Dothideomycetes, *Spartium*, New genus, Morphology, Phylogeny

Introduction

The genus *Massarina* was introduced by Saccardo (1883), while Clements and Shear (1931) selected *M. eburnea* (Tul. & C. Tul.) Sacc. as the lectotype of this genus. Munk (1956) established *Massarinaceae* in order to accommodate the genera *Keissleriella* Höhn., *Massarina* Sacc., *Metasphaeria* Sacc., *Pseudotrichia* Kirschst. and *Trichometasphaeria* Munk. Von Arx & Müller (1975) synonymized Massarinaceae under Pleosporaceae together with Cucurbitariaceae and Didymosphaeriaceae. Barr (1987) segregated Massarinaceae from Pleosporaceae and synonymized it under Lophiostomataceae based on morphology. Schoch *et al.* (2009) showed Massarinaceae to be a distinct family in Pleosporales based on multigene phylogenetic analysis. Further studies on Pleosporales (Zhang *et al.* 2009, 2012) also recognized Massarinaceae as a distinct lineage based on both morphology and molecular phylogeny. Lumbsch & Huhndorf (2010) included *Byssothecium* Fuckel, *Massarina* and *Saccharicola* D. Hawksw. & O.E. Erikss. in Massarinaceae, while Hyde *et al.* (2013) accepted only *Massarina*. Quaedvlieg *et al.* (2013) epitypified *Stagonospora paludosa* (Sacc. & Speg.) Sacc., the type species of *Stagonospora* (Sacc.) Sacc. and assigned it to Massarinaceae. In addition, several molecular studies have suggested that some species of following genera may belong in Massarinaceae, i.e. *Aquaticheirospora*, *Cheirosporium*, *Corynespora*, *Helminthosporium* and *Neottiosporina* (Kodsueb *et al.* 2007; Suetrong *et al*. 2009; Zhang *et al*. 2012; Hyde *et al.* 2013; Wijayawardene *et al*. 2014). However, further phylogenetic studies on these genera and related species are required in order to clarify their familial placement.

Massarinaceae is characterized by immersed or superficial ascomata with papillate or epapillate ostioles, cellular pseudoparaphyses, bitunicate, fissitunicate, clavate to cylindrical, short pedicellate, asci and ellipsoid to fusoid, hyaline, 1–3-septate ascospores with or without mucilaginous sheaths (Hyde 1995; Zhang *et al*. 2012; Hyde *et al.* 2013). *Stagonospora* (Quaedvlieg *et al.* 2013) and ceratophoma-like (Sivanesan1984) asexual morphs have been reported in Massarinaceae, which are characterized by immersed, globose to pyriform, ostiolate, pycnidial conidiomata, enteroblastic, doliiform, hyaline conidiogenous cells with several percurrent proliferations at the apex and oblong,

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cylindrical or fusoid-ellipsoidal, 1-multiseptate, hyaline, smooth-walled to finely verruculose, guttulate conidia (Hyde *et al.* 2013; Quaedvlieg *et al.* 2013).

Didymosphaeria spartii Fabre was introduced by Fabre (1879) as a new name for *Sphaeria spartii* Castagne since it was a homonym of *Sphaeria spartii* Nees ex Fr. Aptroot (1995a) transferred *Didymosphaeria* (*Sphaeria) spartii* to *Montagnula*, while Chlebicki (2009), accommodated this species in *Didymosphaerella* based on its morphological characters. Aptroot (1995a) listed several synonyms under *M. spartii* based on similar morphological traits. However, the size and shape of ascospores of taxa included under *M. spartii* by Aptroot (1995a) were quite variable. *Montagnula spartii* is abundant on *Spartium junceum* L., while grasses, brooms and *Ephedra* spp. were reported as other hosts (Aptroot, 1995a, b). We collected a species morphologically similar to *Montagnula spartii* on *Spartium junceum* and compared it with the isotype of *Didymosphaeria spartii* (Table 2). The comparison revealed that *D. spartii* and our species are identical. In the multi-gene phylogenetic studies our collection (MFLUCC 13–0273 and MFLUCC 14–1212) and a putatively named strain of *M. spartii* (CBS 183.58) formed a distinct clade in Massarinaceae with high BS/PP support. We therefore introduce a new genus, *Pseudodidymosphaeria* for *Didymosphaeria spartii* in the family Massarinaceae based on its unique morphology and combined ITS, 18S and 28S nrDNA sequence data.

Materials and methods

Sample collection, specimen examination and isolation

The isotype specimens of *Didymosphaeria spartii* Fabre was obtained from G. Fresh material were collected from dead branches of *Spartium junceum* (Fabaceae) from Lago di Corniolo (Province of Forlì-Cesena [FC], Italy. Specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters of the taxon were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Observations and photographs were made from material mounted in water and Indian ink was added to water mounts to show the presence of gelatinous sheaths around the ascospores. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Isolates were derived via single spore isolation following the method of Chomnunti *et al.* (2014). Ascospore germination were examined after 24 h and germinating spores were transferred to potato dextrose agar (PDA) media. The obtained pure culture was incubated at 25°C in the normal light and the cultural characteristics such as mycelium colour, shape, texture and growth rate were determined. The herbarium specimens of the new genus are deposited in the Mae Fah Luang University Herbarium (MFLU) and New Zealand Fungal & Plant Disease Collection (PDD), while cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) and CBS Netherlands.

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelium was grown on PDA at 25°C for 21 days. Extraction of genomic DNA from mycelia was carried out following a modified method of Thambugala *et al.* (2015). Polymerase chain reaction (PCR) was carried out using three partial gene portions in this study. Polymerase chain reaction (PCR) was performed for DNA amplification using the primer combination LROR and LR5 (Vilgalys and Hester, 1990) for the nuclear ribosomal large subunit (LSU); NS1 and NS4 (White *et al.* 1990) for the nuclear ribosomal small subunit (SSU); ITS4 and ITS5 (White *et al.* 1990) for the internal transcribed spacer (ITS). The amplifications were performed in 25 µL of PCR mixtures containing 9.5 µL ddH2O, 12.5 µL 2×PCR Master Mix (TIANGEN Co., China), 1 µL of DNA template, 1 µL of each primer (10 µM). Conditions of amplification for all regions were consisted an initial denaturation step of 5 min at 94 °C and final elongation step of 10 minutes at 72 °C. For the SSU and LSU amplification, the 37 cycles consisted of denaturation at 94°C for 1 minute, annealing at 54°C for 50 seconds and elongation at 72°C for 1 minute; for the ITS amplification the 34 cycles consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 1 minute. The PCR products were observed on 1% agarose electrophoresis gels stained with Ethidium bromide. Purification and sequencing of PCR products were carried at using the abovementioned PCR primer at Invitrogen Biotechnology Co., China.

Phylogenetic analysis

Sequences generated from LSU, SSU and ITS were identified by BLAST analysis in the GenBank database at the National Centre for Biotechnology Information (NCBI) and sequences were analyzed with other sequences

obtained from GenBank (Table 1). Most reliable sequences for taxa in Massarinaceae and representative taxa in Didymosphaeriaceae and Lentitheciaceae were included (Ariyawansa *et al.* 2014a; Hyde *et al.* 2013; Zhang *et al.* 2012). *Pleospora herbarum* (CBS 191.86) was selected as the out group taxon. The sequence data were aligned and combined using Bioedit (Hall 1999) and MEGA 5.0 (Tamura *et al.* 2011) and refined visually. The phylogenetic analysis consisted of two methods: The maximum likelihood analysis was performed at the CIPRES webportal using RAxML v.7.2.8 as part of the "RAxML-HPC2 on TG" tool (Stamatakis *et al.* 2008). The general time reversible model (GTR) using proportion of invariable sites was applied with a discrete gamma distribution and four rate classes. The best scoring tree was selected with a final likelihood value of -8232.350286.

Bayesian analysis was performed using MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003). The nucleotide substitution models were determined with MrModeltest v. 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were defined by Bayesian Markov Chain Monte Carlo (BMCMC) sampling method in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation resulting in 10000 total trees. 8000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree, after discarding the first 2000 trees representing the burn-in phase (20 %) of the analysis. The resulting trees were visualized with TreeView v. 1.6.6 (Page, 1996). The Bayesian posterior probabilities (PP- equal or greater than 0.9) and RAxML bootstrap support values (ML- equal or greater than 50%) are given at the nodes. The sequences generated in this study were deposited in GenBank.

Results and discussion

Phylogenetic analysis

Twenty-eight taxa were included in the combined LSU, SSU and ITS data with *Pleospora herbarum* (CBS 191.86) as the outgroup taxon. Tree topology of the Bayesian analysis (not shown) was almost compatible with the ML tree and the best scoring RAxML tree with a final likelihood value of -8829.280968 is shown in Figure 1.

The taxa belonging to families Didymosphaeriaceae, Massarinaceae and Lentitheciaceae separated into three distinct clades. Taxa in Massarinaceae formed five distinct clades. The clades *Massarina*, *Pseudodidymosphaeria* and *Stagonospora* are well-supported and resolved in the phylogenetic tree (Figure 1). *Corynespora leucadendri* (CBS 135133) clustered in a separate clade, while *Byssothecium circinans* (CBS 675.92) and *Corynespora olivacea* (CBS 114450) formed an indistinct sister clade to the *Massarina* clade. These two clades remain unresolved and need more strains in order to resolve their phylogenetic placement in Massarinaceae. *Neottiosporina paspali* (CBS 331.37) clustered in *Stagonospora* clade and may not belong in *Neottiosporina* but in *Stagonospora*.

Montagnula spartii (CBS 183.58) and our strains (MFLUCC 13–0273 and MFLUCC 14–1212) clustered together and formed a well-supported clade in Massarinaceae (Fig. 1). A new genus *Pseudodidymosphaeria* is therefore introduced to accommodate *D. spartii* (= *Sphaeria spartii*).

Taxonomy

Pseudodidymosphaeria Thambugala & K.D. Hyde, ***gen. nov.***

Etymology: Referring to its similarity with *Didymosphaeria*

Index Fungorum number: IF550959; *Facesoffungi number*: FoF00465

Saprobic on *Spartium junceum* L. and possibly grasses in terrestrial habitats. *Sexual morph*: *Ascomata* scattered, or in small groups, immersed, globose to subglobose, ostiolate. *Peridium* thin-walled, 1-layered, composed of hyaline to brown compressed cells of *textura angularis* and *textura prismatica*, cells towards the inside lighter and somewhat flattened, at the outside, darker, fusing and indistinguishable from the host tissues. *Hamathecium* of dense, long, branched, septate, cellular pseudoparaphyses, anastomosing mostly above the asci, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, rounded at the apex and with an ocular chamber. *Ascospores* uniseriate to obliquely uniseriate, ellipsoid with broadly obtuse ends, brown to reddish brown, 1-septate, constricted at the septum, verrucose, surrounded by mucilaginous sheath. *Asexual morph*: *Conidiomata* solitary or in groups, scattered, globose to subglobose, dark brown to black, semi-immersed on PDA, pulvinate, unilocular.

Conidiomatal wall comprising several cell layers; outer layers composed of brown to lightly pigmented cells of *textura angularis* to *globosa*, becoming thin-walled and hyaline towards the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* formed from the cells lining the inner walls of the conidiomata, phialidic, fusiform to cylindrical, determinate, hyaline. *Conidia* solitary, ovoid, straight, oval to ellipsoidal, producing conidia at their tips, smooth, hyaline, aseptate.

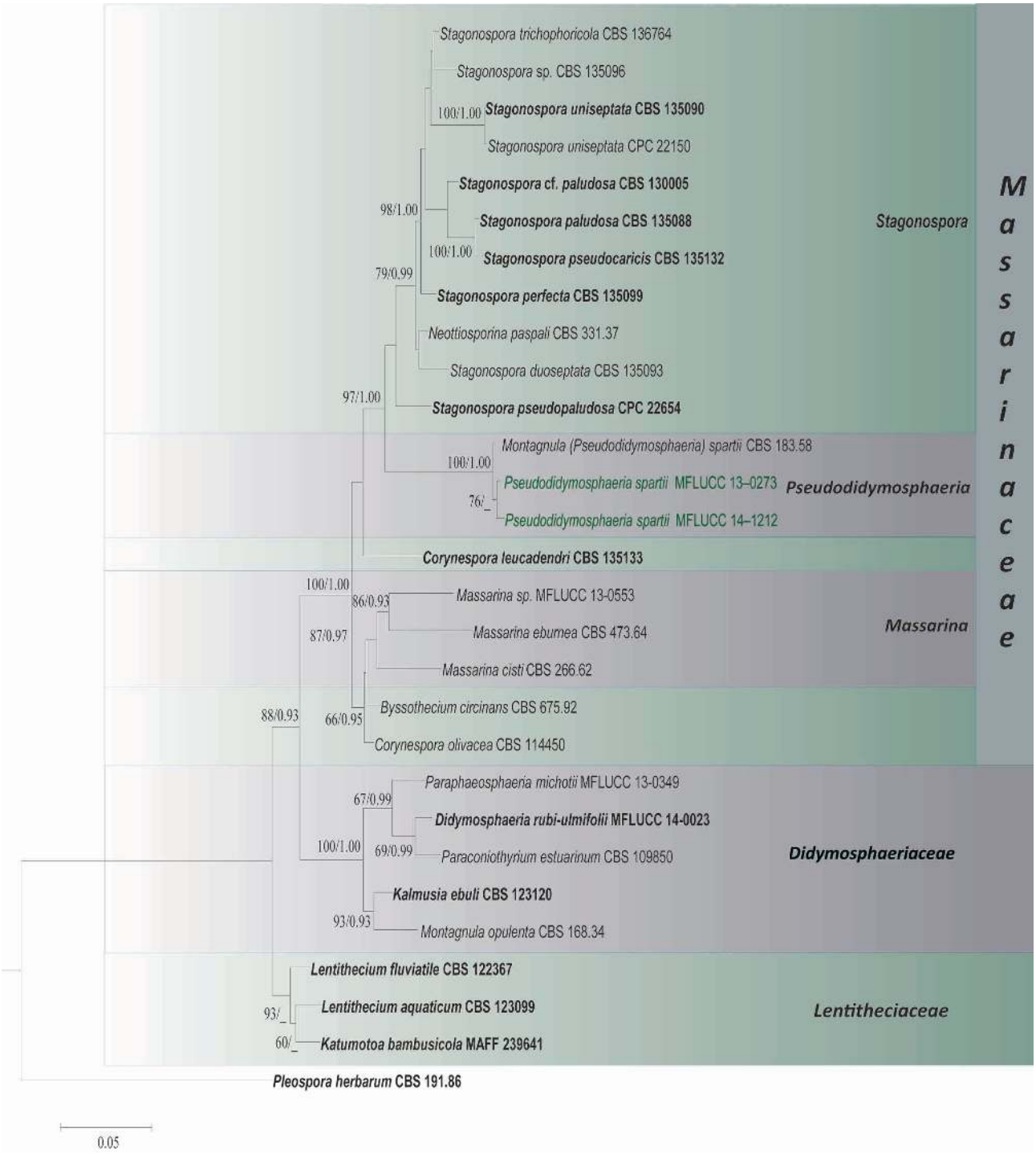

FIGURE 1. Best scoring RAxML tree based on a combined dataset of ITS, SSU and LSU. RAxML bootstrap support values (equal or greater than 50 %) and the Bayesian posterior probabilities (equal or greater than 0.9) and are given at the nodes (ML/PP). The tree was rooted to *Pleospora herbarum* (CBS 191.86). All sequences from ex-type strains are in bold. Newly generated sequences are shown in green.

Type species:—*Pseudodidymosphaeria spartii* (Fabre) Thambugala, E. Camporesi & K.D. Hyde, ***comb. nov***.
Basionym: *Didymosphaeria spartii* Fabre, Annls Sci. Nat., Bot., sér. 6 9: 83 (1879) [1878]

≡ *Didymosphaerella spartii* (Fabre) Chleb., Mycotaxon 110: 444 (2009)
≡ *Microthelia spartii* (Fabre) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 498 (1898)
≡ *Montagnula spartii* (Fabre) Aptroot, Nova Hedwigia 60(3–4): 342 (1995)
≡ *Sphaeria spartii* Castagne, Cat. Pl. Mars.: 169 (1845)
Index Fungorum number: IF550960; *Facesoffungi number*: FoF 00466

Description from isotype

Saprobic on *Spartium junceum* L. *Sexual morph*: *Ascomata* 215–360 × 180–310 µm ($\overline{x}$ = 308 × 262 µm, n = 10), scattered, or in small groups, immersed, globose to subglobose, ostiolate. *Peridium* 10–20 ($\overline{x}$ = 14.1 µm, n = 10) µm wide, 1-layered, composed of hyaline to brown compressed cells of *textura angularis* and *prismatica*, cells towards the inside lighter and somewhat flattened, at the outside, darker, fusing and indistinguishable from the host tissues. *Hamathecium* of dense, 1–2.4 µm wide, long, branched, septate, cellular pseudoparaphyses, anastomosing mostly above the asci, embedded in mucilage. *Asci* 105–150 × 15–20 µm ($\overline{x}$ = 142.8 × 17 µm, n = 15), 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, apex rounded with an ocular chamber. *Ascospores* 18–24 × 9–11 µm ($\overline{x}$ = 22 × 10 µm, n = 25), uniseriate to obliquely uniseriate, slightly overlapping, ellipsoid, with broadly obtuse ends,yellowish when immature, becoming brown to reddish-brown when mature, 1-septate, upper cell slightly broader than the lower one, constricted at the septum, verrucose, surrounded by mucilaginous sheath. *Asexual morph*: Not observed.

Material examined:—FRANCE, Bouches-du-Rhône, Montaud-les-miramas, on *Spartium junceum* L., Castagne, J. L. Martin, G 345707/1, Isotype of *Sphaeria spartii*).

Description from reference specimen (MFLU 14–0578)

Saprobic on *Spartium junceum*. *Sexual morph*: *Ascomata* 200–420 × 195–330 µm ($\overline{x}$ = 300 × 252 µm, n = 10), scattered, or in small groups, immersed, globose to subglobose, ostiolate. *Peridium* 9–20 ($\overline{x}$ = 14.2 µm, n = 10) µm wide, 1-layered, composed of hyaline to brown compressed cells of *textura angularis* and *textura prismatica*, cells towards the inside lighter and somewhat flattened, at the outside, darker, fusing and indistinguishable from the host tissues. *Hamathecium* of dense, 1–2.5 µm wide, long, branched, septate, cellular pseudoparaphyses, anastomosing mostly above the asci, embedded in mucilage. *Asci* 100–175 × 14–20 µm ($\overline{x}$ = 142.8 × 17 µm, n = 15), 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, apex rounded with an ocular chamber. *Ascospores* 18.4–22 × 9–11.5 µm ($\overline{x}$ = 20.4 × 10.2 µm, n = 30), uniseriate to obliquely uniseriate, slightly overlapping, ellipsoid, with broadly obtuse ends, yellowish when immature, becoming brown to reddish-brown when mature, 1-septate, upper cell slightly broader than the lower one, constricted at the septum, verrucose, surrounded by mucilaginous sheath. *Asexual morph*: *Conidiomata* up to 300 µm diam, solitary or in groups, scattered, globose to subglobose, dark brown to black, semi-immersed on PDA, pulvinate, unilocular. *Conidiomatal wall* 45 µm thick, comprising several cell layers; outer layers composed of brown to lightly pigmented cells of *textura angularis* to *globosa*, becoming thin-walled and hyaline towards the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 9.1–18.2 × 1–2.2 µm ($\overline{x}$ = 14.5 × 1.6 µm, n = 25), formed from the cells lining the inner walls of the conidiomata, phialidic, fusiform to cylindrical, determinate, hyaline. *Conidia* 4.5–6.2 × 1.6–2.2 µm ($\overline{x}$ = 5.2 × 1.9 µm, n = 50), solitary, ovoid, straight, oval to ellipsoidal, producing conidia at their tips, smooth-walled, hyaline, aseptate.

Culture characteristics:—Ascospores germinating on PDA within 12–18 h. Germ tubes produced from both ends of the ascospore. Colonies growing on PDA 18 mm diam after 10 days at 16 °C, slow growing; flattened, fairly dense, white, smooth, surface with crenate edge, after a 2–3 weeks conidiomata produced on PDA at 16°C.

Material examined:—ITALY. Province of Forlì-Cesena [FC], Lago di Corniolo, on *Spartium junceum* L. (Fabaceae), 13 October 2012, Erio Camporesi, IT 802 (MFLU 14–0578, PDD 105282, **reference specimen**), living culture, MFLUCC 13–0273, CBS); ITALY. Province of Forlì-Cesena [FC], Cusercoli—Civitella di Romagna, on *Spartium junceum*, 11 September 2014, Erio Camporesi, IT 802–2 (MFLU 14–0928), living culture, MFLUCC 14–1212.

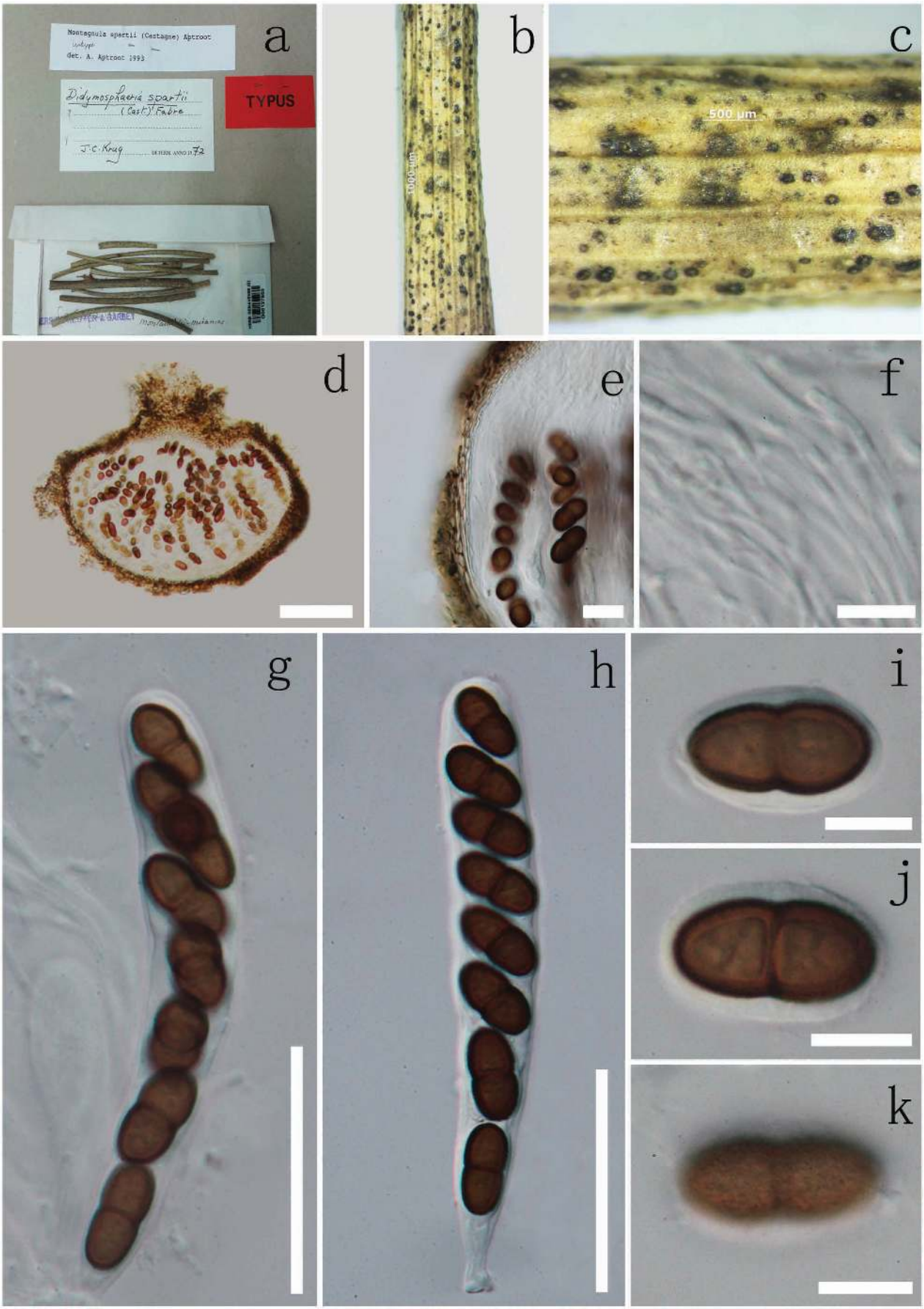

FIGURE 2. *Pseudodidymosphaeria spartii* (**isotype**, G 345707/1). a. Herbarium material b–c. Appearance of ascomata on host surface. d. Section through ascoma. e. Peridium. f. Pseudoparaphyses. g–h. Asci i–k. Ascospores. Scale bars: d = 100 µm, e = 20 µm, g, h = 50 µm, f, i–k = 10 µm.

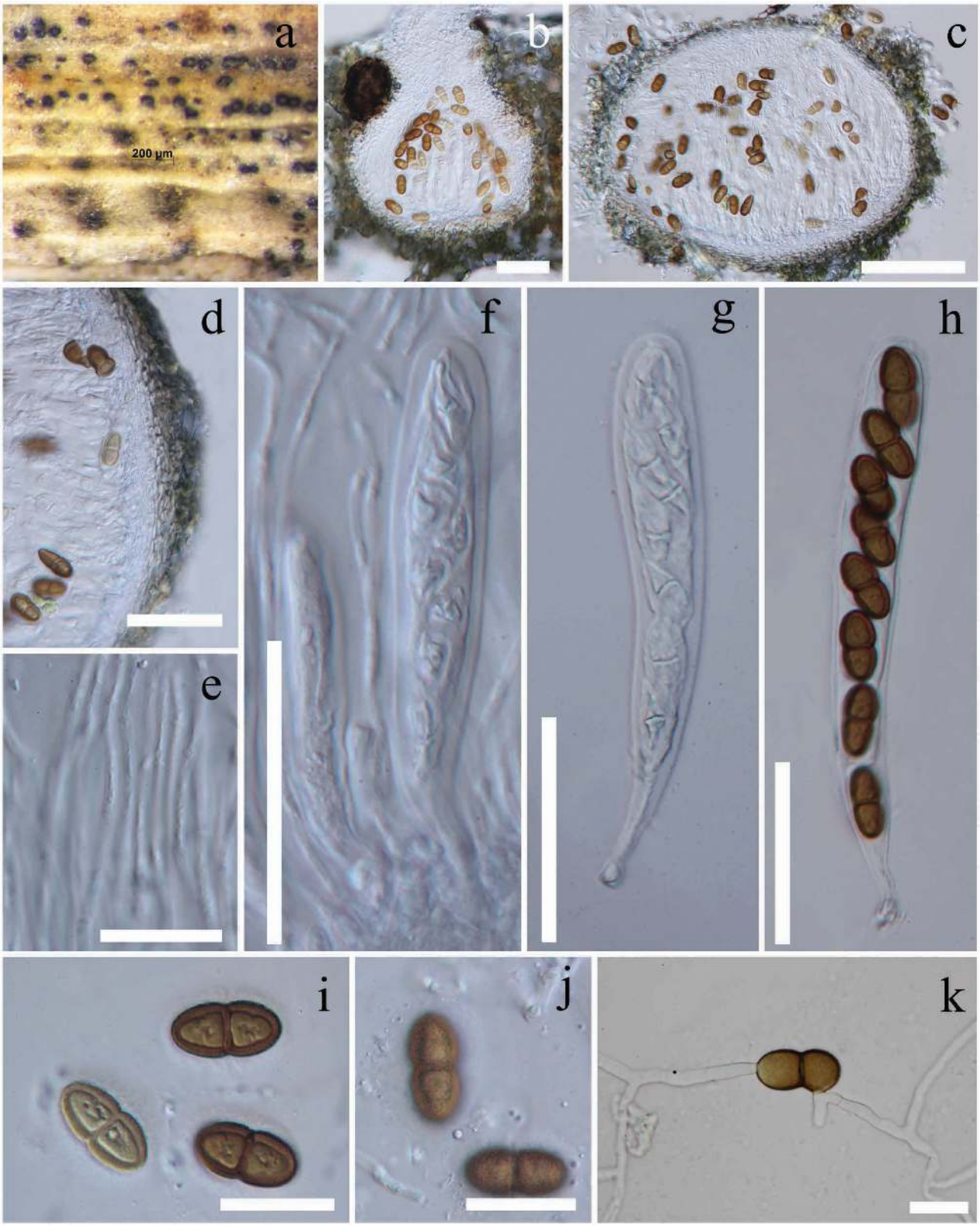

FIGURE 3. *Pseudodidymosphaeria spartii* (MFLU14–0578). a. Ascomata immersed in the host tissue. b–c. Sections through ascomata. d. Section through peridium. e. Pseudoparaphyses. f–g. Immature asci. h. Mature 8-spored, bitunicate ascus. i–j. Ascospores (note the sheaths in i and verrucose ornamentation in j) k. Germinating ascospore. Scale bars: b, d, f–h = 50 µm, c = 100 µm, e, i–k = 10 µm.

Notes:—We re-examined the isotype of *Didymosphaeria spartii* and compared it with other collections by various authors including our specimen (Table 2). There are not significant differences among these specimens. In the phylogenetic analysis two new strains (MFLUCC 13–0273 and MFLUCC 14–1212) and a putatively named strain of *Montagnula spartii* (CBS 183.58) form a well-supported clade in Massarinaceae. We believe that the new specimen is

identical to *Didymosphaeria spartii* and therefore, propose a new genus *Pseudodidymosphaeria* for *Didymosphaeria spartii.* A species of *Dendrophoma* (Scheinpflug, 1958) and a species of *Diplodia* (Moreau 1956) have been reported as the asexual morphs of *M. spartii*, but neither of these reports have yet been verified (Aptroot, 1995a).

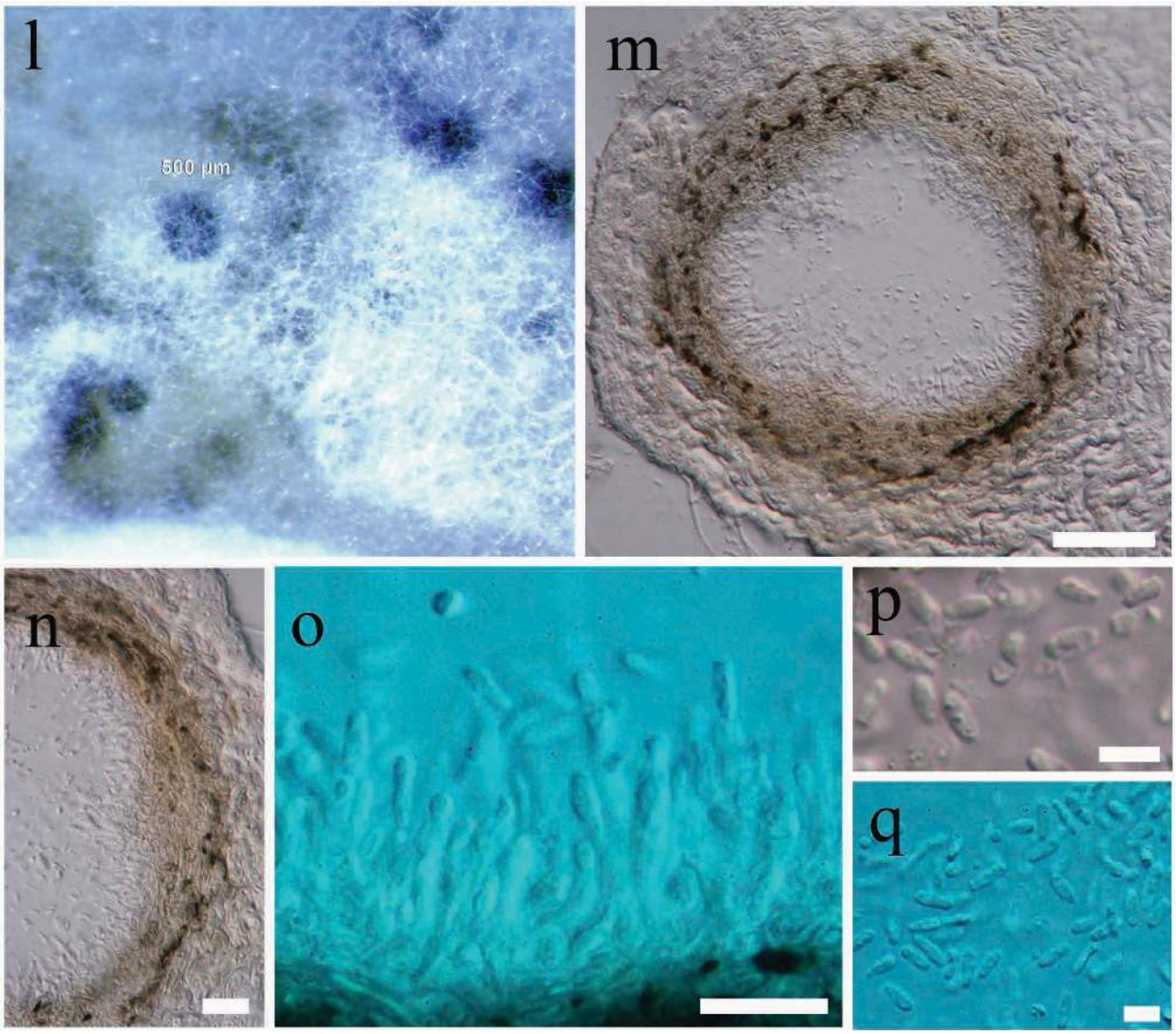

FIGURE 4. *Pseudodidymosphaeria spartii* (asexual morph, from ex-type culture). l. Conidiomata on PDA. m. Section through conidiomata. n. Section through peridium. o. Conidiogenous cells. p. Conidia. Scale Bars: m = 50 µm, n = 20 µm, o = 10 µm, p, q = 5 µm.

Pseudodidymosphaeria shares common morphological characters with *Didymosphaeria* but it is distinct from other *Didymosphaeria* species mainly in having a peridium with brown to hyaline cells of *textura angularis* and *textura prismatica*, cellular pseudoparaphyses and a mucilaginous sheath around the ascospores (Hyde *et al.* 2013; Ariyawansa *et al.* 2014a). *Pseudodidymosphaeria* is distinct from *Massarina*, the generic type of Massarinaceae in having 1-layered peridium composed of hyaline to brown compressed cells of *textura angularis* and *prismatica* and brown, 1-septate, verrucose ascospores (Zhang *et al.* 2012; Hyde *et al.* 2013).

TABLE 1. GenBank and culture collection accession numbers of species treated in the phylogenetic analysis. Bold accession numbers were generated in this study.

species	Culture Accession No.	GenBank accession numbers		
		LSU	ITS	SSU
Byssothecium circinans	CBS 675.92	AY016357	_	GU205235
Corynespora leucadendri	CBS 135133	KF251654	KF251150	_
Corynespora olivacea	CBS 114450	GU301809	_	_
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436587	KJ436588
Kalmusia ebuli	CBS 123120	JN644073	KF796674	JN851818
Katumotoa bambusicola	MAFF 239641	AB524595	_	AB524454
Lentithecium aquaticum	CBS 123099	GU301823	_	GU296156
Lentithecium fluviatile	CBS 122367	GU301825	_	GU296158
Massarina cisti	CBS 266.62	FJ795447	_	FJ795490
Massarina eburnea	CBS 473.64	GU301840	_	GU296170
Massarina sp.	MFLUCC 13–0533	KM875454	_	KM875455
Montagnula opulenta	CBS 168.34	DQ678086	_	AF164370
Montagnula spartii	CBS 183.58	GU205225	_	GU205250
Neottiosporina paspali	CBS 331.37	EU754172	_	EU754073
Paraconiothyrium estuarinum	CBS 109850	JX496129	JX496016	AY642522
Paraphaeosphaeria michotii	MFLUCC 13–0349	KJ939282	KJ939279	KJ939285
Pleospora herbarum	CBS 191.86	DQ247804	NR_111243	DQ247812
Pseudodidymosphaeria spartii	**MFLUCC 13–0273**	**KP325436**	**KP325434**	**KP325438**
	MFLUCC 14–1212	**KP325437**	**KP325435**	**KP325439**
Stagonospora cf. paludosa	CBS 130005	KF251757	KF251254	_
Stagonospora duoseptata	CBS 135093	KF251758	KF251255	_
Stagonospora paludosa	CBS 135088	KF251760	KF251257	_
Stagonospora perfecta	CBS 135099	KF251761	KF251258	_
Stagonospora pseudocaricis	CBS 135132	KF251763	KF251259	_
Stagonospora pseudopaludosa	CPC 22654	KF777239	KF777188	_
Stagonospora sp.	CBS 135096	KF251766	KF251263	_
Stagonospora trichophoricola	CBS 136764	KJ869168	KJ869110	_
Stagonospora uniseptata	CBS 135090	KF251767	KF251264	_
	CPC 22150	KF251769	KF251266	_

CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

TABLE 2. Synopsis of the characteristics of *Pseudodidymosphaeria spartii* and its other collections.

Specimen	Ascomata (μm)	Asci (μm)	Ascospores (μm)	Host	Location	Reference
Didymosphaerella spartii (Fabre) Chleb	240	110–130 × 20–21	(20–)24–27 × 11–12	*Carex griffithii*, *Anthoxanthum alpinum*	Kazakhstan	Chlebicki, 2009

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TABLE 2. (Continued)

Specimen	Ascomata (μm)	Asci (μm)	Ascospores (μm)	Host	Location	Reference
Didymosphaeria spartii Fabre	200–350	110–140 × 14–20	18–24 × 11–13	*Calicothome sp.*, *Spartium junceum*, *Sarothamnus scoparius*	France	Scheinpflug, 1958
	215–360 × 180–310	105–150 × 15–20	18–24 × 9–11	*Spartium junceum*	France	This study/ **isotype**
Montagnula spartii (Fabre) Aptroot	0.4–1.0 mm	_	17–28 × 8–13	*Spartium junceum*, *Genista aspalatoides*, *Calamagrostis epigeios*, *Aeluropus littoralis*, *Festuca sulcata*, *Ephedra ciliata*	Austria, France, Italy, Iran, Russia, Spain, Turkomania	Aptroot, 1995a*
Pseudodidymosphaeria spartii (Fabre) Thambugala, E. Camporesi & K.D. Hyde	200–420 × 195–330	100–175 × 14–20	18.4–22 × 9–11.5	*Spartium junceum*	Italy	This study/ **reference specimen**

***** The description of Aptroot (1995a) was based on several collections that he considered to be synonyms of *Montagnula spartii*. However, comprehensive morphological studies, recollection and molecular sequence data of those collections are essential to ensure correct taxonomy.

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