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Additions to *Sporormiaceae*: Introducing two novel genera, *Sparticola* and *Forliomyces*, from *Spartium*

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Abstract – Members of the family *Sporormiaceae* are mostly saprobic on dung, but sometimes occur on other substrates, including plant debris, soil and wood. They have also been isolated as endophytes. The taxonomy and classification of the family is based on a small number of morphological and ecological characters. Several taxa are easily confused by their shared

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morphological features, and the relationships between genera are inadequately recognized and in need of critical study. In recent treatments, the genera *Chaetopreussia*, *Pleophragmia*, *Preussia*, *Sporormia*, *Sporomiella*, *Sporormin*ula and *Westerdykella* were included in the family. During our survey on various hosts and habitats in Italy we obtained several interesting ascomycetous fungi from *Spartium junceum*. In this paper we introduce *Sparticola* and *Forliomyces gen. nov.* with three new species, namely *Forliomyces uniseptata*, *Sparticola forlicesenae* and *Sparticola junci* in the family *Sporormiaceae* based on multi-locus phylogeny together with morphology. Illustrated accounts are provided for the new taxa, which are compared with morphologically related taxa. Further, we provide an illustrated account of the type of *Massariosphaeria triseptata* and synonymize it under *Sparticola.*

Dothideomycetes / Endophyte / Italy / *Pleosporales* / new genus

INTRODUCTION

Spartium junceum L., commonly known as Spanish broom (Cardoso *et al.*, 2013) or weaver's broom, is native to the Mediterranean, southwest Asia and northwest Africa (Angelini *et al.*, 2000) where it grows in sunny sites, frequently on dry, sandy soils (Gavilán *et al.*, 2015). It is regarded as a noxious invasive weed in places where it has been introduced, such as Australia, California and South Africa (DiTomaso *et al*., 2013). Knowledge on the fungi that occur on this plant in its native range may help guide measures for biological control where it may become a weed species. Currently 56 genera and 72 fungal species have been reported from *Spartium junceum* (USDA, 2016; Index Fungorum, 2016). They are mostly classified under *Dothideomycetes*, *Sordariomycetes*, and *Leotiomycetes*, and some from the division Basidiomycota.

In this paper a collection of isolates from *S. junceum* in Italy were studied in terms of morphology and phylogenetic relationships, based on sequence data from parts of the LSU, SSU, ITS, TEF1-α, and RPB2. This revealed two clades within the *Sporormiaceae* representing two new genera, namely *Sparticola gen. nov.* with two new species and *Forliomyces gen. nov.* with *F. uniseptata*, which are described and illustrated here.

MATERIAL AND METHODS

Sample collection, morphological study and isolation. Fresh materials were collected from dead branches of *Spartium junceum* (Fabaceae) in Italy during 2013-2014. Fresh and herbarium material were examined following the methods outlined by Phukhamsakda *et al.* (2015) and Ariyawansa *et al.* (2015b). Pure cultures were established from single ascospores on 2% potato dextrose agar (PDA; 39 g/L Difco potato dextrose in distilled water) and malt extract agar (MEA; 62 g/L Criterion in distilled water) as described in Chomnunti *et al*. (2014). Cultures were incubated in darkness at 16°C for up to 8 weeks. Type specimens were deposited in Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand. Ex-type living cultures

were deposited at the Mae Fah Luang Culture Collection. Faces of fungi numbers and Index Fungorum numbers are provided (Jayasiri *et al*., 2015; Index Fungorum, 2016).

Herbarium examination. Type specimens were borrowed from ZT. Ascomata were rehydrated in water before examination and sectioning. Hand-cut sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. Samples were examined with a Nikon ECLIPSE 80i compound microscope and photographs recorded with a Canon 600D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS5 Extended version 10.0 (Adobe Systems, United States).

DNA extraction, amplification and sequencing. Isolates were grown on PDA at 16 ± 2°C for 8 weeks. DNA was extracted from mycelium with Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China) following the manufacturer's protocol. Primers LROR and LR5 were used to amplify part of the nuclear ribosomal large subunit 28S rRNA gene (LSU) (Vilgalys & Hester, 1990). The nuclear ribosomal small subunit 18S rRNA gene (SSU) was amplified with primers NS1 and NS4 and the internal transcribed spacer region (ITS) was amplified with primers ITS5 and ITS4 (White *et al.*, 1990). RNA polymerase subunit II (RPB2) was amplified with primers RPB2-5f2 and RPB2-7cr (Sung, 2007). Part of the translation elongation factor 1-alpha (TEF1-α) gene region was amplified with primers EF1-983F and EF1-2218R (Carbone & Kohn, 1999). Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). Amplification reactions for LSU, SSU, and ITS were according to Phukhamsakda *et al.* (2015) and RPB2 follow Liu *et al.* (2014). The PCR thermal cycle program for TEF1-α was an initial denaturation at 95°C for 8 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 58°C for 20 sec and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min (Carbone & Kohn, 1999). The PCR products were checked on 1% Agarose gels stained with ethidium bromide. Purified PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China).

Sequence alignment and phylogenetic analysis. Sequences of closely related strains were retrieved in BLAST searches of GenBank (http://www.ncbi.nlm.nih.gov) together with sequences of representative species used by Kruys & Wedin (2009), Mapperson *et al.* (2014) and Ariyawansa *et al.* (2015b) and these are listed in Table 1. Sequences were aligned with MUSCLE in MEGA 6 (Tamura *et al.*, 2013). The alignments were checked visually and improved manually where necessary. Leading or trailing gaps were removed from the alignments prior to tree building. Phylogenetic analyses were performed with the CIPRES webportal for maximum likelihood (ML) analysis (Miller *et al.*, 2010) and MrBayes v. 3.2.2 for Bayesian analysis (Huelsenbeck & Ronquist, 2001).

A maximum likelihood analysis including 1000 bootstrap replicates was done using RAxML v. 8.2.4 (Stamatakis, 2014), which is part of RAxML-HPC BlackBox tool (Miller *et al.*, 2010). The online tool Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was used to determine the best nucleotide substitution model for the combined gene data. A general time reversible model (GTR) was applied with a discrete gamma distribution (GAMMA) (Stamatakis *et al.*, 2008). The best scoring tree was selected with a final likelihood value of -39419.653716. The resulting replicates were plotted on to the best scoring tree obtained previously. Maximum likelihood bootstrap values (ML) equal to or greater than 50 % are given in black below or above each node (Fig. 1).

Table 1. GenBank and culture collection, and accession numbers used in this study

Species[1]	*Strain number*[2]	*Family*[3]	*GenBank accession numbers*				
			LSU	*SSU*	*ITS*	*RPB2*	*TEF1*
Amorosia littoralis	**NN 6654**	Amo	AM292055	AM292056	AM292047	–	–
Angustimassarina acerina	MFLUCC 14-0505	Amo	KP888637	KP899123	KP899132	–	KR075168
Angustimassarina populi	**MFLUCC 13-0034**	Amo	KP888642	KP899128	KP899137	–	KR075164
Angustimassarina quercicola	MFLUCC 14-0506	Amo	KP888638	KP899124	KP899133	–	KR075169
Arthopyrenia salicis	**CBS 368.94**	Rou	AY538339	–	KF443410	–	KF443404
Asymmetrispora mariae	**CBS 124079**	Flo	JN851819	–	–	–	KR075166
Aurantiascoma minimum	**GKM 169N**	Flo	GU385165	–	–	–	GU327768
Bahusandhika indica	GUFCC 18001	IS	KF460274	–	KF460273	–	–
Berkleasmium crunisia	BCC 17023	IS	DQ280271	–	DQ280265	–	–
Berkleasmium micronesicum	BCC 8141	IS	DQ280272	DQ280268	DQ280262	–	–
Berkleasmium nigroapicale	BCC 8220	IS	DQ280272	DQ280268	DQ280262	–	–
Berkleasmium typhae	BCC 12536	IS	DQ280275	–	DQ280264	–	–
Biatriospora mackinnonii	CBS 674.75	Bia	GQ387613	GQ387552	NR_132037	KF015703	KF407986
Biatriospora marina	**CY 1228**	Bia	GQ925848	GQ925835	–	GU479823	GU479848
Brunneoclavispora bambusae	**MFLUCC 11-0177**	Hal	KT426562		–	–	
Byssosphaeria jamaicana	SMH 1403	Mel	GU385152	–	–	–	GU327746
Corynespora cassiicola	**CBS 100822**	Cor	GU301808	GU296144	–	GU371742	GU349052
Corynespora smithii	CABI 5649b	Cor	GU323201		FJ852597	GU371783	GU349018
Decaisnella formosa	**BCC 25616**	IS	GQ925846	GQ925833	–	GU479825	GU479851
Decaisnella formosa	BCC 25617	IS	GQ925847	GQ925834	–	GU479824	GU479850
Dendryphion europaeum	CPC 22943	Tor	KJ869203	–	KJ869146	–	–
Eremodothis angulata	**CBS 610.74**	Spo	DQ384105	DQ384067	GQ203757	–	GU371821
Exosporium stylobatum	CBS 160.30	Amo	JQ044447	–	JQ044428	–	–
Floricola striata	**JK 5603K**	Flor	GU479785	GU479751	–	–	–
Floricola viticola	MFLUCC 15-0039	Flor	KT305993	KT305995	KT305997	–	–
Forliomyces uniseptata	**MFLUCC 15-0765**	Spo	**KU721762**	**KU721767**	**KU721772**	**KU727897**	–
Fungal endophyte	EL-14	Spo	–	–	JQ316447	–	–
Guttulispora crataegi	**MFLUCC 13-0442**	Lop	KP888639	KP899125	KP899134	–	KR075161

Halotthia posidoniae	**BBH 22481**	Hal	GU479786	GU479752	–	–	–
Herpotrichia juniperi	CBS 200.31	Mel	DQ678080	DQ678029	–	DQ677978	DQ677925
Lignosphaeria fusispora	MFLUCC 11-0377	IS	KP888646	–	KP899140	–	–
Lignosphaeria thailandica	**MFLUCC 11-0376**	IS	KP888645	–	KP899139	–	–
Lophiostoma arundinis	CBS 621.86	Lop	DQ782384	DQ782383	–	DQ782386	DQ782387
Lophiostoma compressum	MFLUCC 13−0343	Lop	KP888643	–	–	–	KR075165
Lophiostoma compressum	**IFRD 2014**	Lop	GU301834	GU296164	–	FJ795457	–
Lophiostoma macrostomum	JCM 13544	Lop	AB619010	AB618691	JN942961	JN993491	–
Massarina corticola	CBS 154.93	Amo	FJ795448	FJ795491	–	FJ795465	–
Mauritiana rhizophorae	**BCC 28866**	Hal	GU371824	GU371832	–	–	GU371817
Melanomma pulvis-pyrius	CBS 124080	Mel	GU456323	GU456302	–	GU456350	GU456265
Misturatosphaeria aurantiinotata	**GKM 1238**	Flo	GU385173	–	–	–	GU327762
Misturatosphaeria radicans	ATCC 42522	Flo	U43479	U43461	–	AY485625	–
Misturatosphaeria radicans	SMH 4330	Flo	GU385167	–	–	–	GU327770
Neocurreya grandicipis	**CBS 114272 = CPC 1852**	Flo	JN712520	–	JN712456	–	–
Neolophiostoma pigmenatum	**MFLUCC 10-0129**	Hal	KT324588	KT324589	–	–	–
Nigrograna mackinnonii	E5202H	Bia	KJ605422	JX264155	JX264157	JX264156	JX264154
Paradictyoarthrinium diffractum	MFLUCC 12-0557	Par	KP744497	KP753960	KP744454	–	–
Paradictyoarthrinium tectonicola	**MFLUCC 13-0465**	Par	KP744500	KP753961	KP744456	–	–
Phaeoseptum aquaticum	**CBS 123113**	IS	JN644072	–	–	–	–
Pleomassaria siparia	**CBS 279.74 T**	Ple	DQ678078	DQ678027	–	DQ677976	DQ677923
Pontoporeia biturbinata	BBH 23338	Hal	GU479796	GU479763	–	GU479837	–
Preussia sp.	ELV3.2	Spo	KF269206	–	JN418773	–	–
Preussia sp.	ELV3.11	Spo	KF269205	–	JN418774	–	–
Preussia flanaganii	CBS 112.73	Spo	AB470528	–	AY943061	–	–
Preussia funiculata	**CBS 659.74**	Spo	GU301864	GU296187	–	GU371799	GU349032
Preussia lignicola	CBS 264.69	Spo	GU301872	GU296197	–	GU371765	GU349027
Preussia lignicola	CBS 363.69	Spo	DQ384098	–	GQ203783	–	–
Prosthemium orientale	JCM 16965	Ple	AB553750	AB553642	AB554081	–	–
Pseudoaurantiacoma kenyense	**GKM 1195**	Flo	GU385194	–	–	–	GU327767
Roussoella nitidula	MFLUCC 11-0182	Rou	KJ474843	–	KJ474835	KJ474859	KJ474852

Table 1. GenBank and culture collection, and accession numbers used in this study *(continued)*

Species[1]	*Strain number*[2]	*Family*[3]	*GenBank accession numbers*				
			LSU	*SSU*	*ITS*	*RPB2*	*TEF1*
Roussoella pustulans	MAFF 239637	Rou	AB524623	AB524482	–	AB539103	AB539116
Roussoellopsis tosaensis	MAFF 239638 = KT 1659	Rou	AB524625	AB524484	–	AB539104	AB539117
Sparticola forlicesenica	**MFLUCC 14-1097**	Spo	**KU721763**	**KU721768**	**KU721773**	–	–
Sparticola forlicesenica	MFLUCC 14-0952	Spo	**KU721764**	**KU721769**	**KU721774**	–	–
Sparticola junci	**MFLUCC 15-0030**	Spo	**KU721765**	**KU721770**	**KU721775**	**KU727900**	**KU727898**
Sparticola junci	MFLUCC 13-0926	Spo	**KU721766**	**KU721771**	**KU721776**	**KU727901**	**KU727899**
Sparticola triseptata	CBS 614.86 = ETH 9494	Spo	EF165031	EF165036		EF165040	–
Splanchnonema pupula	MFU 14-0807 = KT2093	Ple	KP659197	–	KP659196	–	–
Sporormia fimetaria	**UPS:Dissing Gr.81.194**	Spo	GQ203729	–	GQ203769	–	–
Sporormia fimetaria	UPS:Lundqvist 2302-c	Spo	GQ203728	–	GQ203768	–	–
Sporormiella minima	**CBS 524.50**	Spo	DQ468046	–	DQ468026	–	–
Sulcosporium thailandica	**MFLUCC 12-0004**	Hal	KT426563	KT426564	–	–	–
Thyridaria macrostomoides	GKM 1033	IS	GU385190	–	–	–	GU327776
Thyridaria macrostomoides	GKM 1159	IS	GU385185	–	–	–	GU327778
Thyridaria rubronotata	**CBS 385.39**	OG	JX681121	AY642521	–	–	–
Torula herbarum	CBS 220.69	Tor	KF443384	KF443389	NR_132893	KF443393	KF443401
Torula herbarum	**CBS 379.58**	Tor	KF443383	KF443388	–	–	KF443400
Versicolorisporium triseptatum	**JCM 14775 = SH 130**	Bia	NG_042318	AB524501	NR_119392	–	–
Westerdykella dispersa	CBS 297.56	Spo	GQ203753	–	GQ203797	–	–
Westerdykella ornata	CBS 379.55	Spo	GU301880	GU296208	AY943045	GU371803	GU349021

1. Type species from ex-type of each genus are given in bold, new generated in this study in blue.
2. Abbreviations: **ATCC:** American Type Culture Collection, Virginia, USA; **BBH:** BIOTEC Bangkok Herbarium, Thailand; **BCC:** BIOTEC Culture Collection, Bangkok, Thailand; **CABI:** Centre for Agriculture and Biosciences International,Egham, UK; **CBS:** CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; **CPC:** Culture collection of Pedro Crous, housed at CBS; **CY:** City University of Hong Kong Culture Collection, Hong Kong; **E5202H**: Botany, Peabody Museum of Natural History at Yale University, Connecticut, USA; **ETH:** Mycological Plant Collection, ETH Zurich, Switzerland; **GKM:** G.K. Mugambi; **GUFCC:** Goa University Fungus Culture Collection and research Unit, India; **IFRD:** Culture Collection, International Fungal Research and Development Centre, Chinese Academy of Forestry, Kunming, China; **JCM:** The Japan Collection of Microorganisms, Japan; **JK:** J. Kohlmeyer; **KT:** K. Tanaka; **NN:** NovoNordisk culture collection (now Novozymes, Bagsvaerd, Denmark); **MAFF:** Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SMH**: S.M. Huhndorf; **UPS:** The Museum of Evolution Herbarium, SWEDEN.
3. Abbreviation of family names: **Amo**: *Amorosiaceae*; **Bia:** *Biatrosporraceae*; **Cor**: *Corynesporaceae*; **Flo**: *Floricolaceae*; **Hal**: *Halotthiaceae*; **IS:** *Incertae sedis*; **Lop**: *Lophiostomataceae*; **Mel**: *Melanommataceae*; **OG**: Outgroup; **Par**: *Paradictyoarthriniaceae*; **Ple**: *Pleomassariaceae*; **Rou:** *Roussoellaceae*; **Spo**: *Sporormiaceae*; **Tor**: *Torulaceae.*

The model of evolution for the Bayesian inference analysis was determined with MrModeltest 2.3 (Nylander, 2004) and the GTR+I+G nucleotide substitution model was used for each partition. Posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 100th generation and 100,000 trees were obtained. The first 20,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01). Bayesian Posterior Probabilities (PP) equal or greater than 0.90 are given above each node in red (Fig. 1).

Phylogenetic trees and data files were viewed in MEGA v. 5 (Tamura *et al.*, 2011), TreeView v. 1.6.6 (Page 1996) and FigTree v. 1.4 (Rambaut and Drummond 2008). The phylogram with bootstrap values on the branches is presented in Fig. 1 by using graphical options available in Microsoft power point (2013). The sequences generated in this study were submitted to GenBank. The finalized alignment and tree were deposited in TreeBASE, submission ID: 18896 (http://www.treebase.org/).

RESULTS

Phylogenetic analysis

The data for the aligned sequence matrices for the trees obtained in the different analyses are provided below. In the case where alignments of multi-genes were involved, the topologies of the obtained trees for each gene were compared visually to confirm that the overall tree topology of the individual datasets were similar to each other and to that of the tree obtained from the combined alignment. The results of the molecular phylogenetic analyses are given in Fig. 1.

The final *Pleosporales* alignment included 79 strains, representing 12 families and the new genera proposed in the present study. The dataset consisted of 4367 characters (LSU 990 characters, SSU 945 characters, ITS 518 characters, TEF1-α 881 characters, and RPB2 1033 characters). An insertion at positions 417-778 in the SSU region of isolates *Nigrograna mackinnonii* (Borelli) Gruyter *et al.* (E5202H), *Biatriospora mackinnonii* (Borelli) Ahmed *et al.* (CBS 674.75), and *Roussoellopsis tosaensis* Hino & Katumoto (MAFF 239638) was excluded from the analysis prior to tree building. The dataset was rooted with *Thyridaria rubronotata* (Berk & Broome) Sacc. (CBS 385.39).

A best scoring RAxML tree is shown (Fig. 1) with a final ML optimization likelihood value of -39419.653716. Maximum likelihood and Bayesian analyses trees obtained were congruent at the family levels in agreement with previous work based on maximum likelihood analysis and Bayesian analysis (Schoch *et al.*, 2009; Zhang *et al.*, 2012; De Gruyter *et al.*, 2013; Hyde *et al.*, 2013; Wijayawardene *et al.*, 2014; Ariyawansa *et al.*, 2015b; Thambugala *et al.*, 2015) The support values for the different phylogenetic methods varied, with the Bayesian posterior probabilities being higher than the RAxML bootstrap support values.

The two novel genera proposed in this study represented by *Forliomyces* (*F. uniseptata*) and *Sparticola* (*S. junci*) form moderately-supported clades basal to

Table 2. Synopsis of characters of *Sparticola forlicesenae, S. junci*, and *S. triseptata*

Name	*Sparticola forlicesenae*	*Sparticola junci*	*Sparticola triseptata*
Ascomata	Immersed to semi-erumpent, globose to subglobose, 275-325 × 200-250 µm	Immersed, globose to subglobose, 125-200 × 190-230 µm	Immersed, globose, 260-510 × 360-440 µm
Ostioles	80-110 µm × 60-80 µm diam., canal filled with hyaline periphyses	48-76 µm × 87-104 µm diam., canal filled with periphyses	160(-180) µm high, conical, canal filled with periphyses
Peridium	*Textura angularis*, 20-50 µm wide, 7-8 layers	*Textura angularis*, 8-12 µm wide, 2-3 layers	*Textura angularis*, 24-50 µm wide, 7-8 layers
Hamathecium	2-2.5 µm wide, filamentous, branched	1.8-2.6 µm wide, cellular pseudoparaphyses, anamosing,branched, septate	1-2 µm wide, pseudoparaphyses, anastomosing, branched, septate
Asci	Cylindric to clavate, 130-150 × 20-30 µm, short pedicellate, ocular chamber	Clavate to suboblong 91-160 × 14-23 µm, short pedicellate, ocular chamber	Cylindrical, (118-)165-272 × 15-19(-25) µm, short bulbous-pedicel, ocular chamber
Ascospores	Curved-fusoid, 30-40 × 10-15 µm, overlapping 1-2-seriate, 5-6(-9) septate, yellowish brown to brown	Oval to ellipsoid, 19-29 × 7-12 µm, bi-seriate or partially overlapping, 3- septate, yellowish	Ellipsoid, apex and base slightly conical, 20-36 × 8-12 µm, uni-seriate, slightly overlapping, 3- septate, reddish brown to brown
Habitat/ Host	*Spartium junceum* (*Fabaceae*)	*Spartium junceum* (*Fabaceae*)	*Tofieldia calyculata* (*Poaceae*)
References	This study	This study	Leuchtmann 1987

the familial clade of *Sporomiaceae sensu lato*. The genus *Forliomyces* formed a strongly supported clade (100% ML/1.00 PP) sister to the generic clade of *Sparticola* together with three endophytic strains namely, *Preussia* sp. ELV3.11, *Preussia* sp. ELV3.2 and *Preussia* sp. EL-14. *Sparticola junci* formed a strongly supported (93% ML/0.99 PP) clade together with *S. forlicesenae* and *S. triseptata* (100% ML/1.00 PP), sister to the newly proposed genus *Forliomyces*.

TAXONOMY

Forliomyces Phukhamsakda, Camporesi & K.D. Hyde, ***gen. nov***.

Index Fungorum number: IF551778
Facesoffungi number: FoF 01824
Etymology: Named after the place of collection
Saprobic on dead stem of *Spartium junceum* L. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* pycnidial, solitary, unilocular,

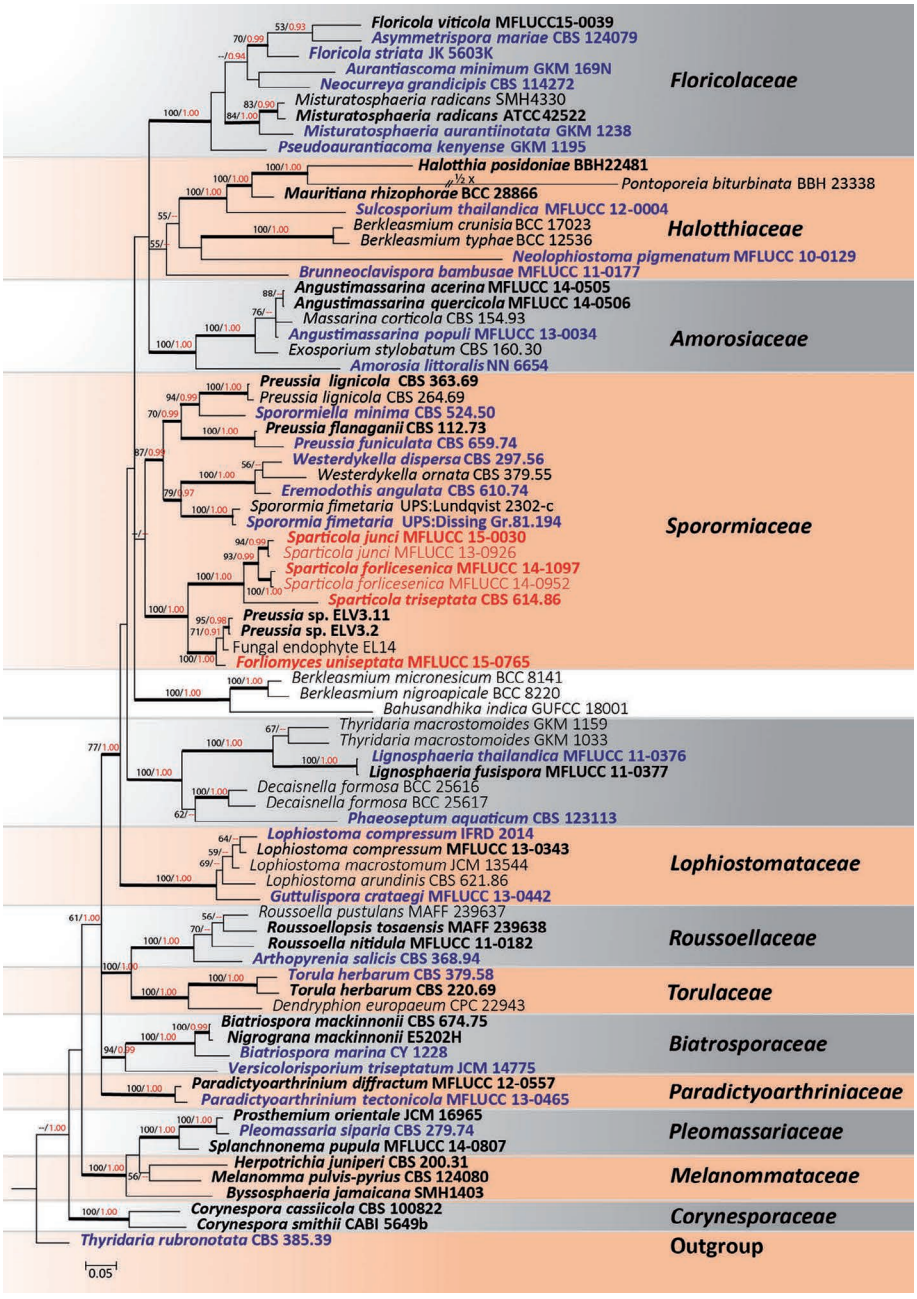

Fig. 1. Bayesian 50% majority rule consensus tree based on combined partial LSU, SSU, ITS, TEF1-α and RPB2 gene datasets. Bootstrap values ≥ 50% from maximum likelihood (ML) are followed by Bayesian posterior probabilities (PP) values ≥ 0.90. The tree is rooted to *Thyridaria rubronotata* (CBS 385.39). Newly generated sequences are indicated in red. The type species of each genus is indicated in blue bold, ex-type strains are in black bold. Hyphen (–) represents support values ≤ 50%/0.90. Bold lines represent significant support values from both analyses (BS ≥ 70%/PP ≥ 0.95).

scattered, immersed, subglobose, dark brown. *Pycnidial wall* composed of cells of *textura angularis*, multi-layered, outer layer composed of irregular, dark brown to light brown cells, inner layer of subhyaline cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, determinate, intergrated, solitary, hyaline, oblong to clavate, and formed from the cells lining inner wall. *Conidia* oblong, subobovoid, slightly curved at the base, with an abscission scar, transversely septate, brown at maturity, granulate, smooth-walled.

Notes: Based on phylogenetic analysis, the new species *Forliomyces uniseptata* forms a clade sister to the generic clade of *Sparticola* (100% ML/1.00 PP) together with three endophytic strains, namely *Preussia* spp. (ELV3.11, ELV3.2, and EL-14). Thus, we tentatively label those *Preussia* spp. with the genus where they cluster in the phylogenetic tree (Fig. 1). Species names are not given because the morphology and identification of these endophytic strains in GenBank cannot be checked, as they are not linked to any herbarium material.

Type species: *Forliomyces uniseptata* Phukhamsakda, Camporesi & K.D. Hyde.

Forliomyces uniseptata Phukhamsakda, Camporesi & K.D. Hyde, ***sp. nov.*** **Fig. 2**

Index Fungorum number: IF551779

Facesoffungi number: FoF 01825

Etymology: Named for the single-septate conidia.

HOLOTYPUS: MFLU 16-0031

Saprobic on dead stem of *Spartium junceum* L. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* pycnidial, 155-190 μm high × 185-320 μm wide, unilocular, solitary, scattered, immersed, subglobose, dark brown. *Pycnidial* wall 18-22 μm wide, composed 5-6 layers, outer layer composed of irregular, dark brown to light brown cells (up to 7 μm diam.) of *textura angularis*, inner layers subhyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2-14 × 1-6 μm ($\bar{x}$ = 7 × 3 μm, n = 50), enteroblastic, phialidic, determinate, discrete, solitary, hyaline, oblong to clavate, and formed from the cells lining the inner wall. *Conidia* 10-15 × 5-8 μm ($\bar{x}$ = 13 × 6 μm, n = 50), oblong, suboboviod, slightly curved at the base, with a faint abscission scar, 1-transverse septum, initially hyaline, brown at maturity, contents granulate, smooth-walled.

Culture characteristics: Spores germinated within 48 hours. Colonies growing on PDA reaching 20 mm diam. after four weeks at 16°C, colonies circular, dense, flat to slightly umbonate, smooth, edge sometimes fimbriate after 8 weeks, with pinkish pigments diffusing into agar. Colonies from above cream at the margin and slightly deep red (11C8) and greyish-green (25E5) in the middle, deep red in center; reverse greyish rose (12B5) at the margin, slightly ruby (12D8) in the center.

Material examined: ITALY, Forlì-Cesena [FC], Collina di Pondo – Santa Sofia, on dead branch of *Spartium junceum* L. (Fabaceae), 27 October 2012, E. Camporesi, IT 859 (MFLU 16-0031, **holotype**), isotype in HKAS91935, ex-type living cultures, MFLUCC 15-0765, KMUCC 15-0549.

Sparticola Phukhamsakda, Ariyawansa, Camporesi, & K.D. Hyde, ***gen. nov.***

Index Fungorum number: IF551921

Facesoffungi number: FoF 01827

Etymology: With reference to the host genus *Spartium.*

Saprobic on dead stem of *Spartium junceum* L. Sexual morph: *Ascomata* immersed in epidermis, solitary, scattered or in small groups, globose to subglobose, smooth, black to dark brown, ostiolate. *Ostioles* centrally located, globose or oblong,

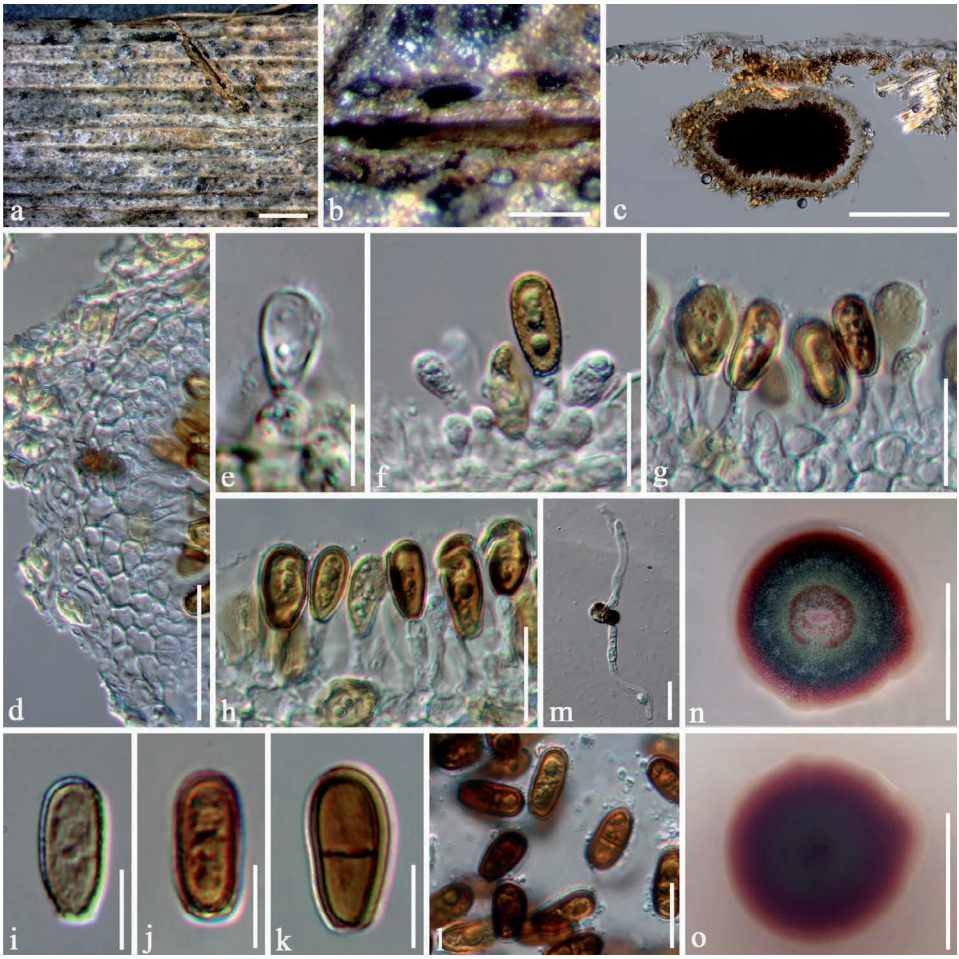

Fig. 2. ***Forliomyces uniseptata*** **(holotype) a.** Habit of *Forliomyces uniseptata* on *Spartium junceum*. **b.** Close up of immersed conidiomata. **c.** Vertical section through a conidioma. **d.** Part of conidioma peridium. **e-h.** Conidiogenous cells and developing conidia. **i-l.** Conidia. **m.** Germinating conidium. **n, o.** Culture characters on PDA media. Scale bars: a = 1 cm, b-c = 200 µm, d = 20 µm, e, i-k = 5 µm, f-h, m, l = 10 µm, n-o = 2 cm.

ostiolar canal filled with periphyses. *Peridium* comprising 2-3 layers of dark brown to light brown cells of *textura angularis*. *Hamathecium* of numerous, dense, long, cellular psedoparaphyses, septate, tapering, branched, anastomosing. *Asci* 8-spored, bitunicate, fisitunicate, short pedicellate, thick-walled, apically rounded, with an ocular chamber. *Ascospores* bi-seriate or partially overlapping, oval to ellipsoidal, slightly conical at the apex, yellowish when mature, 3-transversely septate, rarely 1-2-septate, slightly constricted at the septa, containing granules in the middle cells, smooth walled, surrounded by a wide mucilaginous sheath. Asexual morph: Hyphomycetous. Mycelium smooth, pale brown to brown, septate, branched. *Conidiophores* semi-macronematous to macronematous, erect, septate, branched, slightly constricted at the septa, pale brown to brown. *Conidiogenous cells* annellidic,

holoblastic, doliform, integrated, terminal, indeterminate, smooth to verrucose, pale brown. *Conidia* dictyosporous, coiled, involute, acropleurogenous, brown to dark brown, solitary, with granules.

Notes: *Sparticola* shares similarities with *Sporormiaceae* in having globose, black, coriaceous, ostiolate ascomata and a peridium of thin-walled cells of *textura angularis*. *Sparticola* differs from the other genera of *Sporormiaceae* in having ascospores with transverse septa, lacking a germ slit, being yellowish to light brown, and found on *Spartium junceum* in terrestrial habitats, while in the other genera ascospores are mostly dark brown, multi-septate, often separating in to part spores, with a germ slit, and have a coprophilous habit (Barr, 2000; Kruys *et al.*, 2006; Kruys & Wedin, 2009).

Type species: *Sparticola junci* Phukhamsakda, Ariyawansa, Camporesi & K.D. Hyde

Sparticola forlicesenae Wanasinghe, Phukhamsakda, Camporesi & K.D. Hyde, ***sp. nov.*** **Fig. 3**

Index Fungorum number: IF551922

Facesoffungi number: FoF 01826

Etymology: Named after the province where the species was first found.

HOLOTYPUS: MFLU 16-0033

Saprobic on dead, herbaceous stem of *Spartium junceum* L. **Sexual morph**: *Ascomata* 275-325 μm high × 200-250 μm diam. ($\bar{x}$ = 300 × 215 μm, n = 10), immersed to semi-erumpent, solitary, globose to subglobose, dark brown to black, ostiolate. *Ostioles* 80-110 μm high × 60-80 μm diam. ($\bar{x}$ = 103 × 71 μm, n = 5), with short papilla, black, smooth, ostiolar canal filled with hyaline periphyses. *Peridium* 20-35 μm wide at the base, 30-50 μm wide at the sides, composed of 7-8 layers, brown to dark brown or blackish brown, coriaceous, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* comprising numerous, 2-2.5 μm wide, filamentous, branched, septate pseudoparaphyses. *Asci* 130-150 × 20-30 μm ($\bar{x}$ = 142 × 24 μm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 30-40 × 10-15 μm ($\bar{x}$ = 34 × 11 μm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming yellowish brown to brown at maturity, curved-fusoid, asymmetrical, with one side flattened, 5-6(-9)-transverse septate, slightly constricted at the septa, widest above the central septum, conical and narrowly rounded at the ends, initially guttulate, with rough surface, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinating on MEA within 24 hours. Colonies on MEA reaching 20 mm diam. after 8 weeks at 16°C. Cultures of dense mycelia, embedded in agar. Underneath dark brown, convex with white mycelial papillate surface, with lobate, friable margin, dark brown at margin. Producing dark orange pigment in the media after 4 weeks.

Material examined: ITALY, Forlì-Cesena Province, Modigliana, Montebello, dead and upright stems of *Spartium junceum* L. (Fabaceae), 16 October 2013, E. Camporesi, IT 1480, (MFLU 16-0033, **holotype**), isotype HKAS91934, ex-type living cultures, MFLUCC, 14-1097, KMUCC 15-0548, MFLUCC 14-0952.

Notes: *Sparticola forlicesenae* is introduced based on both morphology and phylogenetical analysis. It formed a clade sister to *S. junci* with high support (93% ML/0.99 PP). The species is typical of the new genus in having immersed, globose to subglobose ascomata, ostioles, yellowish spores and diffusible orange pigment but differs from the generic type of *Sparticola* in having larger ascomata, a wider peridium, and larger 5-6(-9)-septate ascospores.

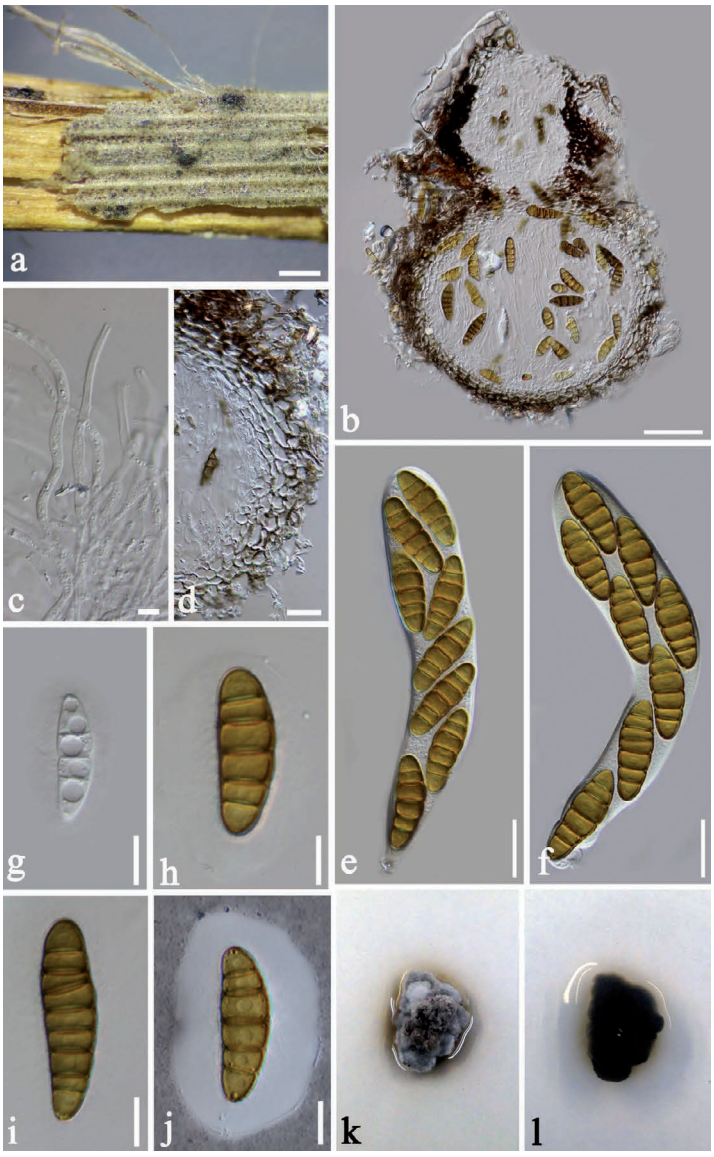

Fig. 3. ***Sparticola forlicesenae*** **(holotype)**. **a.** Appearance of ascomata on host substrate. **b.** Vertical section through ascoma. **c.** Pseudoparaphyses. **d.** Section through peridium. **e, f.** Mature asci and ascospores. **g-i.** Development stages of ascospores. **j.** Ascospore stained in Indian ink to showed thick mucilaginous sheath. **k, l.** Culture character on PDA. Scale bars: a = 500 µm, b = 50 µm, c = 5 µm, e-f = 50 µm, d-e = 20 µm, g-j = 10 µm.

Sparticola junci Phukhamsakda, Camporesi & K.D. Hyde, ***sp. nov.*** **Figs 4, 5**

Index Fungorum number: IF551923
Facesoffungi number: FoF 01828
Etymology: In references to the original host.

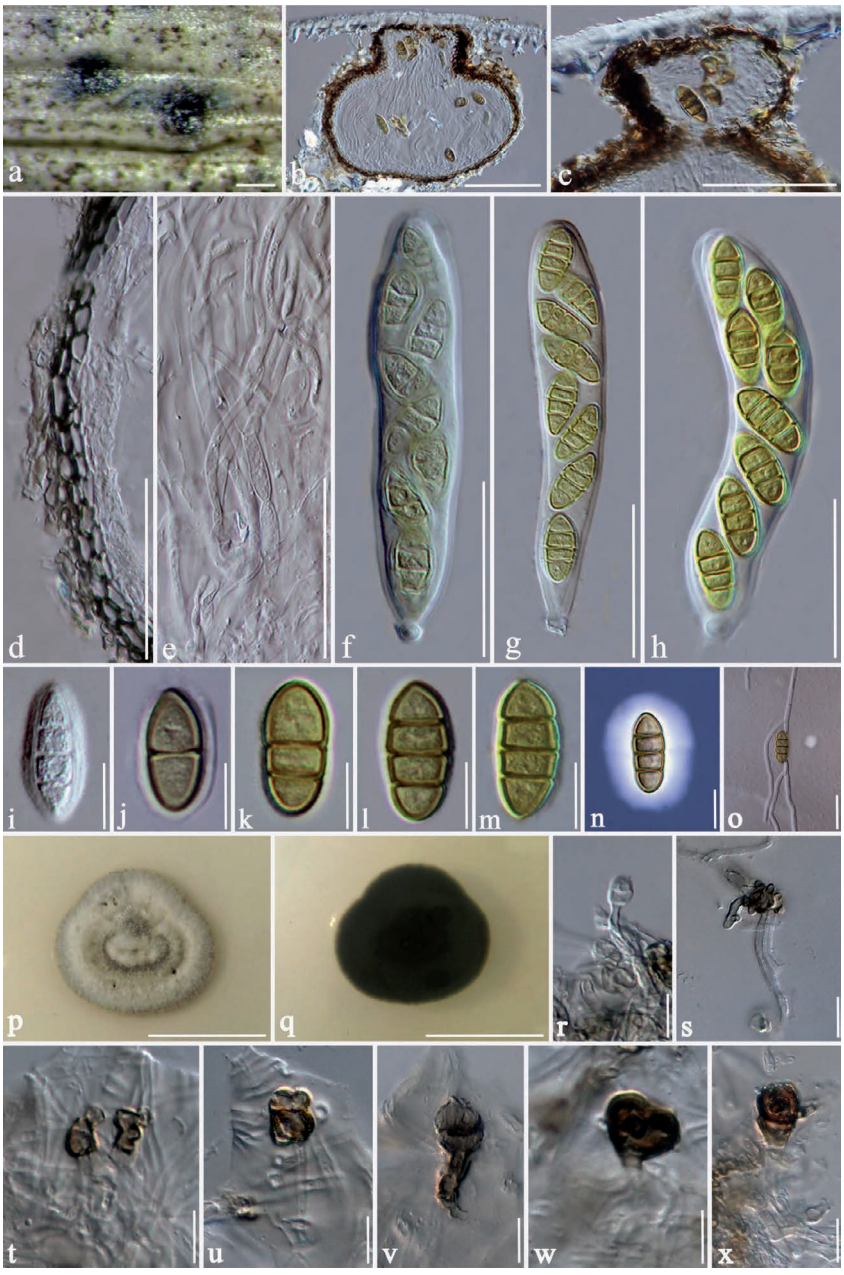

Fig. 4. ***Sparticola junci*** **(holotype) a.** Appearance of ascomata on host surface. **b.** Section of ascoma showing position in substrate. **c.** Globose to subglobose ostiole **d.** Section of peridium comprising cells of *textura angularis*. **e.** Pseudoparaphyses. **f.** Immature asci **g, h.** Mature asci with short pedicels. **i-m.** Development stages of ascospores. **n.** Ascospore negative-stained in Indian ink to show thick mucilaginous sheath **o.** Germinated ascospore **p, q.** Culture characters **r-x.** Appressorium-like structures formed in culture. Scale bars: a = 200 µm, b = 100 µm, c = 50 µm, d-h = 50 µm, p-q = 20 µm, i-o, r-x = 10 µm.

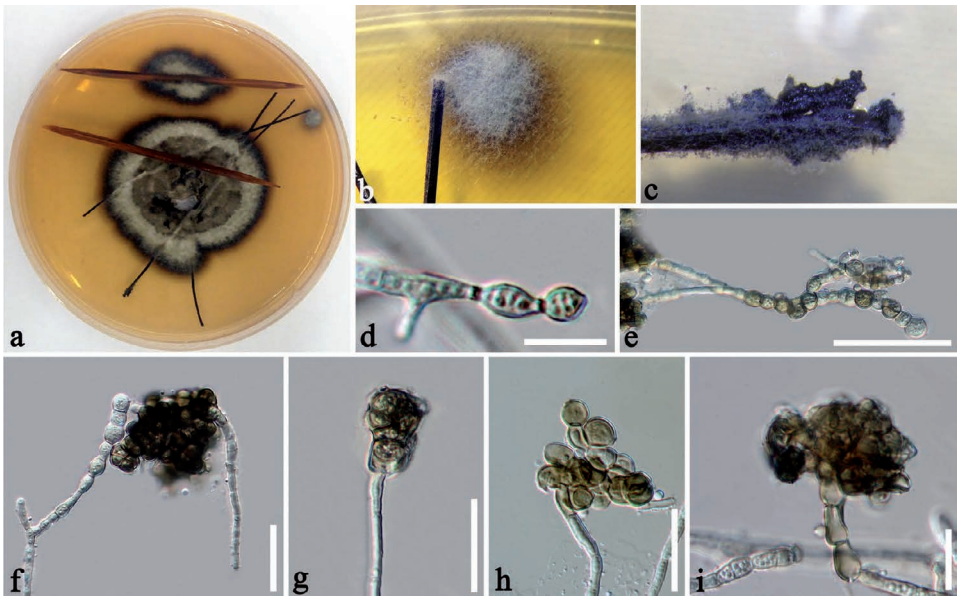

Fig. 5. **Cultures of *Sparticola junci*** (ex-type culture) **a.** Colony on MEA after 4 months with sterile bamboo pieces and sterile pine needles. **b, c**. Mycelium formed on end of pine needle. **d.** Development of conidiophores. **e.** Immature conidial chains. **f-i.** Mature conidiogenous cells with attached conidia. Scale bars: d = 10 µm, e = 50 µm, f-i = 20 µm.

HOLOTYPE: MFLU 15-1405

Saprobic on dead branch of *Spartium junceum* L. **Sexual morph**: *Ascomata* 125-200 µm high × 190-230 µm diam. ($\bar{x}$ = 165 × 192 µm, n = 10), immersed in epidermal layer, with only ostioles visible, coriaceous, solitary, scattered or in small groups, globose to subglobose, smooth walled, black to dark brown, ostiolate. *Ostioles* 48-76 µm high × 87-104 µm diam. ($\bar{x}$ = 59 × 90 µm, n = 10), centrally located, globose or oblong, ostiolar canel filled with periphyses. *Peridium* 8-12 µm wide, composed of 2-3 layers of dark brown to light brown, cells of *textura angularis*, inner layer composed of subhyaline gelatinous cells, thin, easy to break. *Hamathecium* of numerous, dense, long, 1.8-2.6 µm wide ($\bar{x}$ = 2 µm, n = 20), broad, transversely septate, branched, anastomosing, cellular pseudoparaphyses, surrouding asci and along the inner layer of peridium. *Asci* 91-160 × 14-23 µm ($\bar{x}$ = 117 × 20 µm, n = 20), 8-spored, bitunicate, fisitunicate, clavate to suboblong, short-pedicellate, thick-walled, apically rounded with ocular chamber up to 1-2 µm wide × 1-2 µm high. *Ascospores* 19-29 × 7-12 µm ($\bar{x}$ = 23 × 10 µm, n = 50), bi-seriate or partially overlapping, oval to ellipsoid, slightly conical at apex, hyaline when immature, yellowish when mature, 3-transversely septate, rarely 1-2-transversely septate, slightly constricted at the septa, guttulate in the middle, rough-walled, surrounded by a wide mucilaginous sheath. **Asexual morph**: Produced on pine needles placed on MEA agar after 4 months. Mycelium smooth, pale brown to brown, septate, branched 2-4 µm diam. (up to 7 µm in enlarged cells). *Conidiophores* up to 35 µm long, 4-7 µm wide, semi-macronematous to macronematous, erect, septate, branched, slightly constricted at the septa, pale brown to brown. *Conidiogenous cells* holoblastic, doliform-like, integrated, terminal, sometimes intercalary, indeterminate, smooth to

verrucose, pale brown. *Conidia* (15-)17-46 × (19-)22-40(-46) µm diam. ($\bar{x}$ = 28 × 31 µm, n = 30), mono- to dictyosporous, besipetal, initially enlarged cell globose, with 1-2 cells, becoming oval to irregular, with several cells, coiled, involuted, tacropleurogenous, brown to dark brown, solitary, with granules. *Appressoria* 9-21 × 5-17 µm ($\bar{x}$ = 12 × 10 µm, n = 10) solitary, short chains or rarely long chain, reddish to brown, lobate, bullet or irregular in shape, thick-walled.

Culture characteristics: Ascospores germinating on PDA within 24 hours, with germ tubes developing from apical and basal cells of ascospores. Colonies on PDA reaching 40 mm diam. after 8 weeks at 16°C. Colonies of white, dense mycelia, white at first becoming pale orange, after two weeks, dark brown below mycelium embedded in agar, convex, with some papillate surface, with circular, friable margin, dark brown at margin, turning to brown after six weeks. Brown appressorium-like structures forming in culture, producing orange pigment in the media after four weeks.

Material examined: ITALY, Forlì-Cesena Province, Monte Mirabello – Predappio, on dead branch of *Spartium junceum* (Fabaceae), 15 September 2014, E. Camporesi, IT 1454 (MFLU 15-1405, **holotype**), isotype in HKAS91933, ex-type living cultures, MFLUCC 15-0030, KMUCC 15-0547 ; *ibid*., 10 October 2013 (MFLU 14-0167), ex-paratype living culture, MFLUCC 13-0926.

Sparticola triseptata (Leuchtm.) Phukhamsakda, & K.D. Hyde, ***comb. nov.*** **Fig. 6**

Index Fungorum number: IF551924

Facesoffungi number: FoF 01829

Basionym: *Massariosphaeria triseptata* Leuchtm., Mycol. Helv. 2(2): 187 (1987)

Saprobic on leaves, sheath and dry branches of *Tofieldia calyculata* (L.) Wahlenb. **Sexual morph**: *Ascomata* 260-510 µm high × 360-440 µm diam., scattered or sometimes gregarious, immersed, globose, with attached hyphae radiating through substrate, coriaceous, black and shiny, wall dark brown to black, sub-carbonaceous, roughened, ostiolate. *Ostiole* apex well developed, up to 160(-180) µm long, dark brown to black, conical, sometimes globose with central pore, ostiolar canal filled with periphyses. *Peridium* 24-50 µm diam. at the sides, composed of 7-8 layers, outer layer composed of irregular, dark brown to brownish gray (6F8) cells, inner layer composed of light brown to subhyaline cells of *textura angularis*, cells wide up to 12 µm, merging with pseudoparaphyses. *Hamathecium* of numerous, dense, long, 1-2 µm wide ($\bar{x}$ = 1.6 µm, n = 20), transversely septate, branched, filamentous pseudoparaphyses, anastomosing between and above asci, embedded in mucilage. *Asci* (118-)165-275 × 15-20(-25) µm ($\bar{x}$ = 212 × 18 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, with short bulbous pedicel, apically rounded, with an obvious ocular chamber. *Ascospores* 20-36 × 8-12 µm ($\bar{x}$ = 28 × 10 µm, n = 50), uni-seriate, slightly overlapping, ellipsoid, apex and base slightly conical, 3-septate, rarely 2- or 4-septate, slightly constricted at the septa, hyaline when immature, reddish brown to brown when mature, smooth or rough walled, with thick mucilaginous sheath. **Asexual morph**: Undetermined.

Material examined: SWITZERLAND, Graubünden, Albula, Murteldigl Crap Alv, on *Tofieldia calyculata* (Tofieldiaceae), 25 August 1980**,** P. Crivelli (ZT, Myc 55271, **holotype**); ex-type living culture, CBS 614.86, ETH 9494.

Notes: *Massariosphaeria* was reported to be polyphyletic in the *Pleosporales* (Wang *et al.*, 2007; Mugambi & Huhndorf, 2009; Ariyawansa *et al.*, 2015a). *Massariosphaeria triseptata* was introduced by Leuchtmann (1987) and placed in the family *Lophiostomataceae*. Based on LSU, SSU, ITS and RPB2 data, Wang

Fig. 6. ***Sparticola triseptata*** (*Massariosphaeria triseptata*, **holotype**). **a.** Specimen and herbarium label of *Massariosphaeria triseptata*. **b.** Appearance of ascomata on the host surface. **c.** Vertical section through ascoma. **d.** Ostiole part showing periphyses. **e.** Section through peridium. **f.** Pseudoparaphyses. **g.** Cellular pseudoparaphyses branching and anastomosing between and above asci. **h, i.** Mature asci with ocular chamber and short pedicel. **j-n.** Development of ascospores. **o.** Ascospore with visible mucilaginous sheath. Scale bars: b = 500 µm, c = 200 µm, d, g-i = 100 µm, e-f = 50 µm, o = 20 µm, j-n = 5 µm.

et al. (2007) suggested that *M. triseptata* has a close relationship with *Sporormiaceae* and *Melanomma radicans* (ATCC 42522). However, Thambugala *et al.* (2015) placed *Melanomma radicans* in *Floricolaceae*. Ariyawansa *et al.* (2015a) transferred the generic type, *Massariosphaeria phaeospora*, to *Thyridariaceae* based on both

morphology and phylogeny. Literature reviews coupled with our molecular data have shown that *Massariosphaeria triseptata* resides within the family *Sporormiaceae*. In the present study, *Massariosphaeria triseptata* forms a well-supported clade within the generic clade of *Sparticola* in the family *Sporomiaceae*. Therefore we synonymise *M. triseptata* under the genus *Sparticola* based on morphology and phylogenetic analyses.

DISCUSSION

In this paper we introduce two new genera namely *Sparticola* and *Forliomyces* in the family *Sporormiaceae*. Within the genera we include three new species, *Forliomyces uniseptata*, *Sparticola forlicesenae*, and *Sparticola junci*, all of which were isolated from *Spartium junceum* collected in Italy. We further transfer *Massariosphaeria triseptata* to *Sparticola*, as *S. triseptata.* The genera are introduced based on multi-locus phylogeny coupled with morphology. All phylogenetic analyses support *Sparticola* and *Forliomyces* as a separate lineage within the order *Pleosporales*, but the closest relatives of the family could not be revealed due to lack of backbone support. Therefore, for the present *Sparticola* and *Forliomyces* are treated as separate genera in family *Sporormiaceae*.

The family *Sporormiaceae* was introduced by Munk (1957) and typified by *Sporormia*, with the type species *S. fimetaria* (Rabenh.) De Not. Members of this family occur worldwide and occur as saprobes on various substrates including herbivore dung, plant debris, soil and wood (Cain, 1961; Dissing, 1992; Kruys *et al.*, 2006; Sue *et al.*, 2014). Most of the taxa are coprophilous and their ascospores are valuable tools in paleoecological studies when reconstructing animal abundances in the past (Burney *et al.*, 2003; van Geel *et al.*, 2003, 2006). Others have been reported as saprobes on submerged wood, endophytes, and human angioinvasive fungal infections (Asgari & Zare, 2010; Arenal *et al.*, 2007; Mapperson *et al.*, 2014; Sue *et al.*, 2014).

Morphologically *Sporormiaceae* is well circumscribed. Ascospores are mostly thick-walled and dark brown, strongly constricted at septa and often fragment into part-spores at maturity (Barr, 2000). Germ slits in the spore walls are also common. The spores develop in fissitunicate asci within pseudothecioid ascomata (Kruys *et al.*, 2006). Recent studies had included *Chaetopreussia, Pleophragmia, Preussia, Sporormia, Sporormiella, Sporormin*ula and *Westerdykella* in *Sporormiaceae* (Kruys & Wedin, 2009; Hyde *et al.*, 2013; Ebead *et al.*, 2012). *Chaetopreussia* and *Pleophragmia* were included in *Sporormiaceae* based on morphology, however, molecular data are needed to confirm their natural classification (Zhang *et al.*, 2012).

The asexual morphs of the *Sporormiaceae* can be coelomycetous or hyphomycetous. In *Preussia polymorpha* it is chrysosporium-like (Asgari & Zare, 2010), while *Westerdykella dispersa* has a phoma-like asexual morph (Sue *et al.*, 2014). In the present study *Forliomyces* also produced a coelomycetous asexual morph in agreement with the previous studies of the asexual morphs of *Sporormiaceae* (Zhang *et al.*, 2012; Ebead *et al.*, 2012; Sue *et al.*, 2014). However, *Forliomyces* differences from other phoma-like asexual morphs in the family in having brown, pyriform conidia and a single transverse septum.

Our new genus *Sparticola* shares similarities with *Sporormiaceae* in having coriaceous, ostiolate ascomata, a peridium with thick-walled cells of *textura*

angularis, clavate or cylindrical asci, multi-celled ascospores, with a wide gelatinous sheath and cultures producing diffusible pigments (Barr, 2000; Kruys *et al.*, 2006). *Sparticola* differs from the other genera of the family based on the terrestrial habitat, while other genera are regularly found on dung.

In our phylogenetic tree the generic type of *Forliomyces*, *F. uniseptata* clusters with three strains of fungal endophytes, named *Preussia* spp.. *Preussia* sp. ELV3.11 and *Preussia* sp. ELV3.2 which were reported by Mapperson *et al*. (2014) and *Preussia* sp. EL-14 reported in Zaferanloo *et al*. (2012). These endophytic strains were sterile in culture, thus no morphological data could be obtained for species level identification. Literature reviews coupled with molecular data have shown that *Preussia* sp. are paraphyletic in *Sporormiaceae* (Kruys & Wedin 2009; Mapperson *et al.* 2014) and we observed similar results in our phylogenetic analysis with *Preussia* spp. forming a clade sister with the generic type of *Forliomyces* (Fig. 1).

Santos *et al*. (2015) reported an endophyte from *Indigofera suffruticosa* Miller (Fabaceae), which is in the same family as *Spartium junceum*. Remarkably our new species *Sparticola junci* produced appressoria in culture, which are similar to those found in other genera of endophytic pathogens such as *Colletotrichum*, *Pyricularia*, *and Pestalotiopsis* (Emmett & Parbery, 1975; Hyde *et al.*, 2009; Maharachchikumbura *et al.*, 2012; Woloshuk *et al.*, 1983). Molecular evidence also indicates that *Forliomyces* has a close relationship with endophytic isolates. Promputtha *et al.* (2007, 2010) state in their study that endophytic fungal species can be found as saprobes during early stages of leaf decomposition. It may be that *Sparticola* species are endophytes that become saprobes when the host dies. The production of appressoria may facilitate endophytic infection.

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