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Epitypification of *Broomella vitalbae* and introduction of a novel species of *Hyalotiella*

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Abstract – *Broomella* and *Hyalotiella* are poorly known genera in *Amphisphaeriaceae*. Both genera are known from morphological descriptions, but lack molecular data, thus their generic placement and relationships with other genera are unclear. Three collections of *Amphisphaeriaceae* were made from dead twigs of *Clematis* in Italy, and two from *Spartium* species and were identified as *Broomella* and *Hyalotiella* species. In order to obtain a phylogenetic understanding of *Broomella* and *Hyalotiella* within the family *Amphisphaeriaceae*, we carried out a phylogenetic analysis based on LSU gene data. Results show that these five isolates represent two distinct genera. Based on both morphological and phylogenetic data, the three isolates form *Clematis* are shown to be conspecific with *Broomella vitalbae*. In this paper we designated an epitype with a sexual and asexual morph for *B. vitalbae* to stabilize the understanding of the genus. The two strains from *Spartium* fit within the generic concepts of *Hyalotiella*, but sequence data for *Hyalotiella* species are presently lacking. *Hyalotiella spartii* sp. nov. is introduced based on its host association and morphological characters.

Asexual morphs / *Amphisphaeriaceae* / *Broomella* / *Hyalotiella* / Phylogeny / epitypification

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INTRODUCTION

The family *Amphisphaeriaceae* was introduced by Winter (1887) with *Amphisphaeria* Ces. & De Not. as the generic type (Kang *et al.,* 1999; Maharachchikumbura *et al.,* 2014). *Amphisphaeriaceae* is a large heterogeneous family, with approximately 57 genera (Maharachchikumbura *et al.,* 2015) and apparently comprises several families. Asexual morphs of *Amphisphaeriaceae* are largely coelomycetous with appendaged conidia (Nag Raj, 1993; Jeewon *et al.,* 2002; Liu *et al.,* 2015). Several members of this group are plant pathogens on a broad range of hosts, and responsible for numerous diseases, including leaf-spots, canker lesions, fruit rot and twig dieback (Espinoza *et al.,* 2008, Eken *et al.,* 2009; Maharachchikumbura *et al.,* 2014). They are also endophytes which colonizing plant tissues without causing visible symptoms, or can be saprobic on terrestrial plants (Jeewon *et al.,* 2002; Tanaka *et al.,* 2011; Qadri *et al,*. 2014) and some genera are chemically highly creative (e.g. *Pestalotiopsis,* Xu *et al*., 2014).

The inter- and intrageneric relationships of most amphisphaeriaceous asexual morphs are problematic, due to their simplicity, plasticity and variability of morphological characters (i.e. conidial size, septation, pigmentation, and presence or absence of appendages) (Jeewon *et al.,* 2002). The classification and circumscription of these genera have been controversial in the past decades (Steyaert, 1949; Guba, 1961; Sutton, 1980; Nag Raj, 1993). Steyaert (1949) introduced the genus *Truncatella* with five species to accommodate species having 3-septate conidia, which previously, belonged to *Pestalotia* (Nag Raj, 1993; Lee *et al.,* 2006). Guba (1961) adopted a broader concept by synonymising *Truncatella w*ith *Pestalotia*. Sutton (1980) disagreed with the synonymy and reinstated the genus considering that the species defined by Guba (1961) in *Pestalotia* (sect Quadriloculatae) and *Monochaetia* (sect. Quadriloculatae) should be relocated to *Truncatella* (Sutton, 1980; Lee *et al.,* 2006). Nag Raj (1993) agreed with Sutton (1980) and accepted ten species in *Truncatella*, while another two species, namely *T. pampeana* and *T. suffocata,* still remained in *Pestalotiopsis* (Nag Raj, 1993; Jeewon *et al.,* 2002; Lee *et al.,* 2006). Through a study of LSU and ITS sequence data, and together with existing morphological data, Jeewon *et al.* (2002) showed that species of *Truncatella* appear to be a natural group, and are distinct from other pestalotioid taxa. This was confirmed by Maharachchikumbura *et al.* (2014). A search of Index Fungorum (2015) reveals 21 names in *Truncatella*, although five have been transfered to other genera, many to *Pestalotiopsis*. Maharachchikumbura *et al.* (2014) showed that the broad generic concept of *Pestalotiopsis* of Nag Raj (1993) is problematic and 11 species which have 3-septate conidia should be relocated in *Truncatella*.

The sexual morph of *Truncatella* is associated with *Broomella* (Kang *et al.,* 1999). According to Shoemaker & Müller (1963) and Yuan *et al.* (1992) there are six species of *Broomella* linked to the asexual morph *Truncatella*. The type species *B. vitalbae* (Berk. & Broome) Sacc. and *B. excelsa* Shoemaker & E. Müll. have a conidial morph with single apical and basal appendage, and this does not fit well with the concepts of *Truncatella*. Whether the link between of *Broomella* species and *Truncatella* species is correct requires further assessment using molecular data.

Papendorf (1967) introduced the genus *Hyalotiella* based on *H. transvalensis* Papendorf, which somewhat morphologically resembles *Truncatella*. Both share similar characters in having 4-celled conidia, more than one branched apical appendage, and lack of basal appendages. However,

Hyalotiella is further characterized by vase-shaped pycnidia, with a long neck, and 3-septate, cylindrical conidia, with median cells that are longer than the ends cells. With the exception of the apical cell, the other cells in the conidia are almost colourless to pale brown (Nag Raj, 1993). In addition, species of *Hyalotiella* have conidia bearing 3-4-branched appendages, which arise from the apical cell, and lack a basal appendage (Nag Raj, 1993). According to the Index Fungorum (2015) there are four names in *Hyalotiella*. However, *H. subramanianii* Agnihothr. & Luke was transferred to *Hyalotiopsis* by Nag Raj (1993) based on conidia characters and *H. orientalis* was synonymised under *H. americana* (Speg.) Nag Raj (Nag Raj 1993). Therefore, to date, only two species *H. americana* and *H. transvalensis* are accepted in *Hyalotiella*. However, none of these are presently known from culture or to have DNA sequence data.

The aim of the present paper is to designate an epitype for the type species of *Broomella*, *B. vitalbae,* in order to stabilize the understanding of *Broomella* (*Amphisphaeriaceae*) with morphological and molecular characterization. In addition, a new species of *Hyalotiella* is introduced.

MATERIAL AND METHODS

Collection and examination of specimens

Fresh specimens were collected in Italy from dead stems of *Clematis vitalba* L. and *Spartium junceum* L., dried and sent to Thailand for examination. Type specimen was borrowed from K (M). Samples were examined and pure cultures obtained by single spore isolation, following the method described in Chomnunti *et al.* (2014). The colonies were transferred to 2% potato-dextrose agar (PDA) and incubated at 25°C. The colony characters and growth rates were determined after one to four weeks. The pure cultures from our study are deposited at Mae Fah Luang University Culture Collection (MFLUCC). Duplicate cultures are deposited in International Collection of Microorganisms from Plants, Landcare Research, New Zealand (ICMP). The holotype is deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and the isotype specimens are deposited at the herbarium of Kunming institute of Botany Chinese Academy of Sciences (KUN).

DNA extraction, PCR amplification and sequencing

Isolates were grown on PDA plates in darkness at 25°C until completely covering the agar surface. The mycelium (about 50 mg) was scraped off and collected in a 1.5 ml micro centrifuge tube. Genomic DNA was extracted from fresh mycelium, following the specification of Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). The primer pairs LROR and LR5 as defined by Vilgalys and Hester (1990) were used to amplify a segment of the large subunit rDNA. DNA amplification was performed by polymerase chain reaction (PCR). The sequencing of PCR products were carried on to Beijing Bai Mai Hui Kang Biological Engineering Technology Co. Ltd (Beijing, P. R. China).

DNA sequence data analysis

The newly generated sequences were analyzed with sequences obtained from GenBank (Table 1). Sequences were aligned using Bioedit v. 7.0.9 (Hall, 1999) and Clustal X v. 1.83 (Thompson *et al.*, 1997). The alignments were checked visually and improved manually where necessary. A maximum likelihood (ML) analysis was performed with raxmlGUI version 1.3 (Silvestro & Michalak, 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model. The resulting trees were printed with TreeView v. 1.6.6 (Page, 1996).

RESULTS

Phylogenetic analyses

Partial sequences of the large subunit rRNA (LSU) were used to resolve the generic placement of species of *Amphisphaeriaceae*. The alignment dataset is comprised of 50 taxa, with *Rostrohypoxylon terebratum* (CBS 119137) as the outgroup taxon. The maximum likelihood dataset consists of 795 characters. The best scoring RAxML tree generated from maximum likelihood analysis is shown in Figure 1.

Sequence analysis of LSU data was used to link sexual and asexual morph of *Broomella vitalbae* and to resolve its placement within the family *Amphisphaeriaceae*. The three strains (strain MFLUCC 13-0798 for the sexual morph, strains MFLUCC 14-1000 and MFLUCC 15-0023 for the asexual morph) of *Broomella* formed a well-supported clade (BS=100%). Based on morphology and sequence data, our fresh collection (MFLU 15-0065) from *Clematis vitalba* is epitypified.

Based on morphology and LSU sequence data, a new species *Hyalotiella spartii,* isolated from *Spartium junceum*, is also introduced. ITS (KP757756), SSU (KP757760) and EF1-α (KP757764) sequences data for this taxon have also been deposited in GenBank for the benefit of future study.

TAXONOMY

Broomella vitalbae (Berk. & Broome) Sacc., Syll. fung. (Abellini) 2: 558 (1883) **Fig. 2-4**

≡ *Hypocrea vitalbae* Berk. & Broome, Ann. Mag. nat. Hist., Ser. 33: 363 (1859)

Epitypification identifier: IF551046; *Facesoffungi number*: FoF 00589
Epitype: MFLU 15-0065, designated here.

Table 1. Collection details and GenBank accession number of isolates includes in this study. The newly generated sequences are indicated with an asterisk. T signifies ex-type/ex-epitype isolates

Species	*Culture accession No.*	*Host/Substrate*	*Location*	*GenBank Accession*
				LSU
Adisciso tricellulare	NBRC 32705^{T}	*Rhododendron indicum*	Japan	AB593728
Adisciso yakushimense	MAFF 242774^{T}	*Symplocos prunifolia*	Japan	AB593721
Broomella vitalbae	**MFLUCC 13–0798^{T}**	***Clematis vitalba***	**Italy**	**KP757749***
Broomella vitalbae	**MFLUCC 14–1000**	***Clematis vitalba***	**Italy**	**KP757750***
Broomella vitalbae	**MFLUCC 15–0023**	***Clematis vitalba***	**Italy**	**KP757751***
Amphisphaeria umbrina	HKUCC 994	*Tilia* sp.	Switzerland	AF452029
Bartalinia bischofiae	HKUCC 6534	Unidentified dead leaf	Hong Kong	AF382367
Bartalinia laurina	HKUCC 6537	Unidentified dead leaf	Hong Kong	AF382369
Ciliochorella castaneae	HHUF 28799	–	Japan	AB433277
Discosia artocreas	NBRC 8975	*Poa pratensis*	–	AB593705
Discosia pini	MAFF 410149	*Pinus densiflora*	Japan	AB593708
Discosia sp.	MAFF 238070	*Fallopia japonica*	Japan	AB593720
Discostroma fuscellum	NBRC 32680	*Ribes* sp.	–	AB593739
Discostroma fuscellum	NBRC 32625^{T}	*Rosa canina*	–	AB593726
Discostroma tostum	NBRC 32626	–	–	AB593727
Dyrithiopsis lakefuxianensis	HKUCC 7303	–	–	AF452047
Ellurema indica	ATCC 22062	–	–	MIU43478
Hyalotiella spartii	**MFLUCC 13–0397^{T}**	***Spartium junceum***	**Italy**	**KP757752***
Hyalotiella spartii	**MFLUCC 15–0024**	***Spartium junceum***	**Italy**	**KP757753***
Immersidiscosia eucalypti	MAFF 242781	Decayed leaves	Japan	AB593725
Immersidiscosia eucalypti	NBRC 104195	*Quercus myrsinifolia*	Japan	AB593722
Lepteutypa cupressi	IMI 052255	*Cupressus forbesii*	Kenya	AF382379
Monochaetia kansensis	PSHI2004Endo1031	–	China	DQ534036
Monochaetia kansensis	PSHI2004Endo1030	–	China	DQ534035
Monochaetia kansensis	PSHI2004Endo1032	–	China	DQ534037
Neopestalotiopsis formicarum	CBS 115.83	Plant debris	Cuba	KM116255
Neopestalotiopsis formicarum	CBS 362.72^{T}	Dead Formicidae	Ghana	KM116248

Table 1. Collection details and GenBank accession number of isolates includes in this study. The newly generated sequences are indicated with an asterisk. T signifies ex-type/ex-epitype isolates *(continued)*

Species	*Culture accession No.*	*Host/Substrate*	*Location*	*GenBank Accession*
				LSU
Neopestalotiopsis protearum	CBS 114178^{T}	*Leucospermum cuneiforme*	Zimbabwe	JN712564
Neopestalotiopsis rosae	CBS 101057^{T}	*Rosa* sp.	New Zealand	KM116245
Pestalotiopsis knightiae	CBS 114138	*Knightia* sp.	New Zealand	KM116227
Pestalotiopsis malayana	CBS 102220^{T}	*Macaranga triloba*	Malaysia	KM116238
Pestalotiopsis telopeae	CBS 114137	*Protea neriifolia* × *susannae*	Australia	KM116219
Pseudopestalotiopsis cocos	CBS 272.29^{T}	*Cocos nucifera*	Indonesia	KM116276
Pseudopestalotiopsis theae	MFLUCC 12–0055^{T}	*Camellia sinensis*	Thailand	KM116282
Robillarda sessilis	BCC13393	*Eucalyptus camaldulensis*	Thailand	FJ825378
Rostrohypoxylon terebratum	CBS 119137	*Lithocarpus* sp.	Thailand	DQ840069
Seimatosporium elegans	NBRC 32674	*Melaleuca ericifolia*		AB593733
Seimatosporium eucalypti	CBS 115131	*Eucalyptus smithii*	South Africa	JN871209
Seimatosporium glandigenum	NBRC 32677	*Fagus sylvatica*	–	AB593735
Seimatosporium hypericinum	NBRC 32647	*Hypericum* sp.	–	AB593737
Seiridium cardinale	CBS 172.56	–	–	AF382376
Seiridium cardinale	ICMP 7323	*Cupressocyparis leylandii*	New Zealand	AF382377
Seiridium papillatum	CBS 340.97	–	–	DQ414531
Seiridium phylicae	CPC 19970	*Phylica arborea*	United Kingdom	KC005810
Seiridium phylicae	CPC 19965	*Phylica arborea*	United Kingdom	KC005809
Truncatella angustata	ICMP 7062	*Malus X domestica*	New Zealand	AF382383
Truncatella hartigii	CBS118148	*Restio egregius*	South Africa	DQ278928
Truncatella laurocerasi	ICMP 11214	*Prunus persica*	New Zealand	AF382385
Truncatella restionacearum	CMW 18755^{T}	*Ischyrolepis* cf. *gaudichaudiana*	South Africa	DQ278929
Truncatella sp.	HKUCC 7987	*Leucospermum* sp.	South Africa	AF382382
Zetiasplozna acaciae	CPC 23421	*Acacia melanoxylon*	–	KJ869206

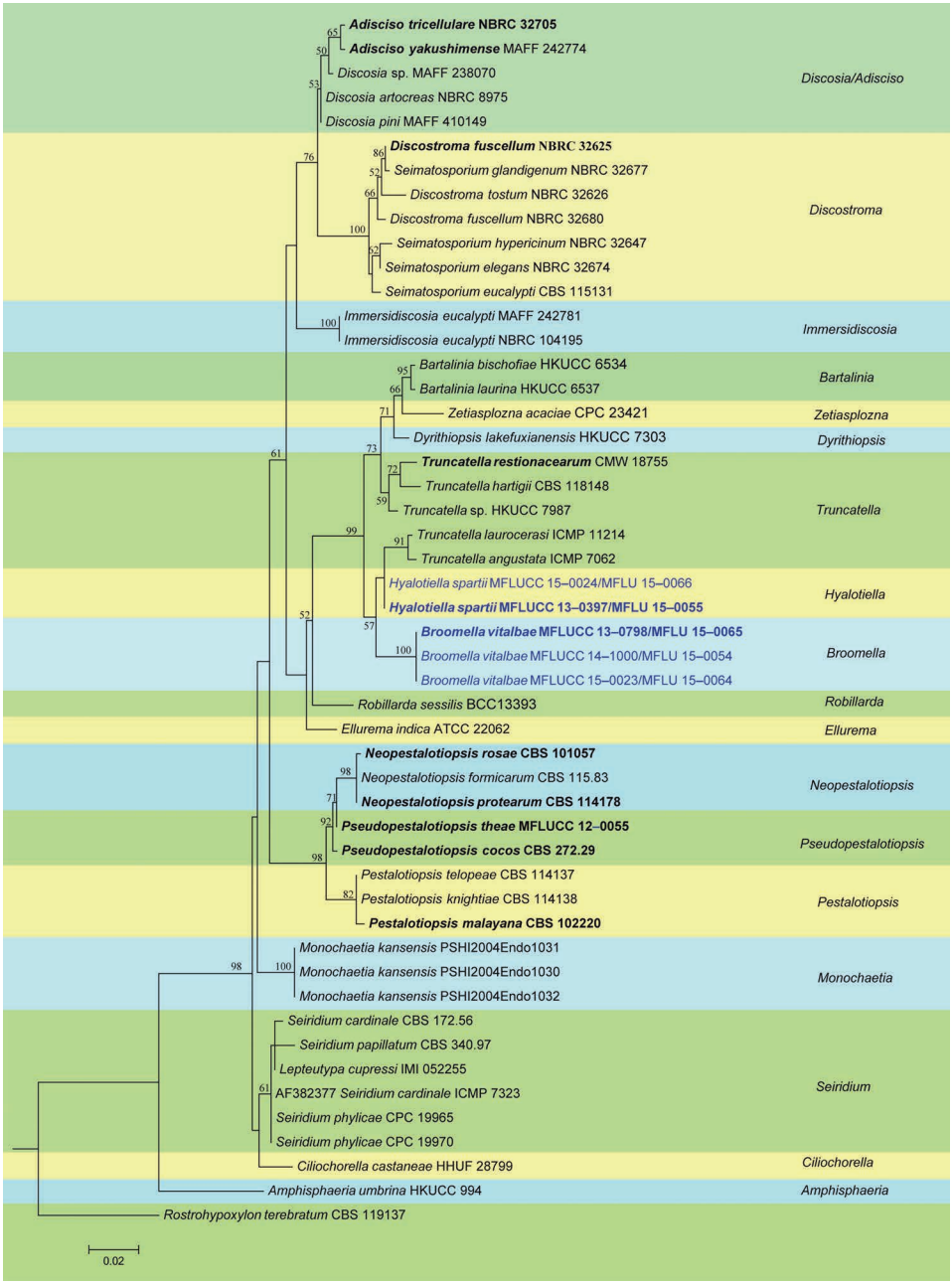

Fig. 1. Best scoring RAxML tree of *Amphisphaeriaceae* strains obtained from dataset of LSU sequence alignment. RAxML bootstrap support values (equal to or greater than 50% based on 1.000 replicates) are shown at the nodes. The ex-types (ex-epitype strain) are in bold; the new isolates are in blue. The tree is rooted to *Rostrohypoxylon terebratum* CBS 119137.

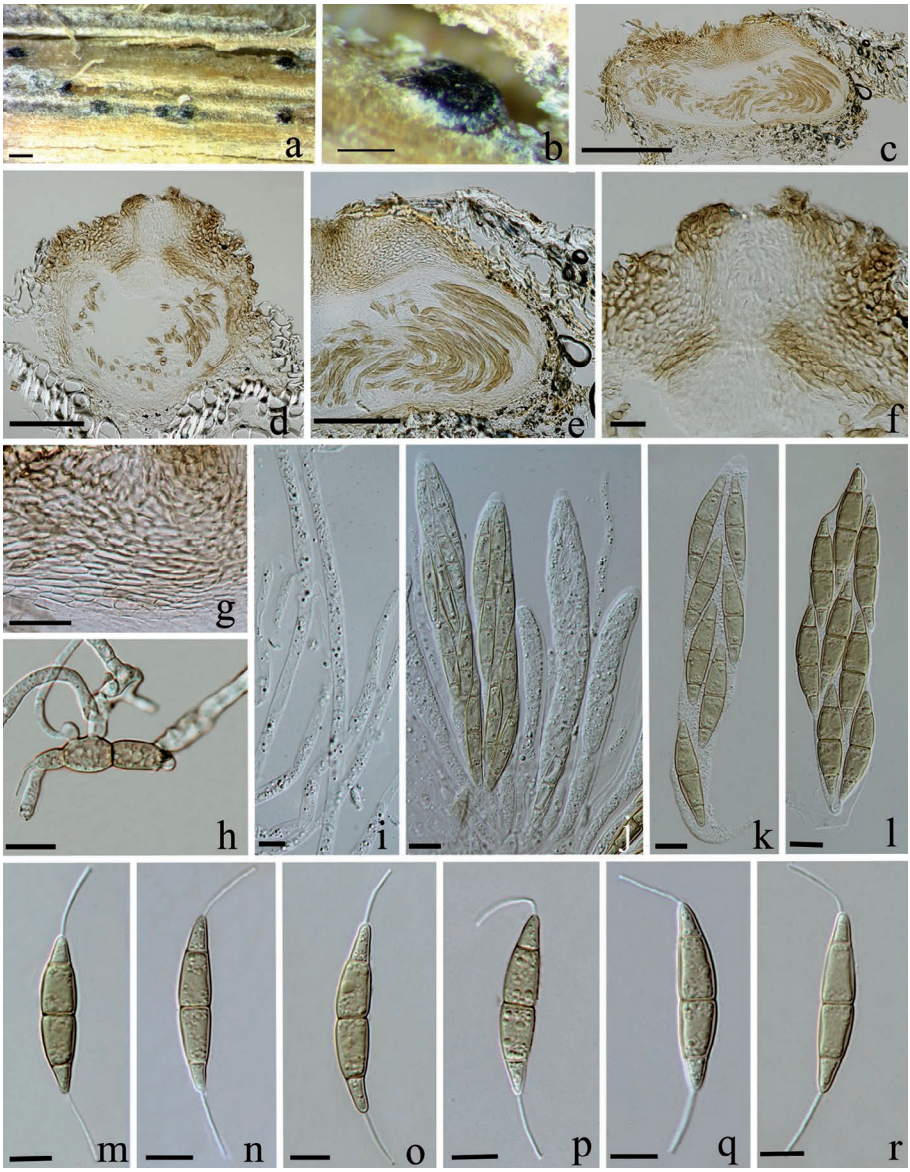

Fig. 2. *Broomella vitalbae* (MFLU 15-0065, epitype) **a-b.** Black ascomata on the host. **c-d.** Vertical section of ascomata. **e, g.** Section of peridium. **f.** Ostiole. **h.** Germinating spore. **i.** Paraphyses. **j-l.** Asci with ascospores. **m-r.** Ascospores. **Scale bars:** a = 500 µm, b-c = 200 cm, d-e = 100 µm, f-g = 20 µm, h-r =10 µm.

Saprobic on the dead stem of *Clematis viltalba* forming conspicuous rounded, black dots, ascomata initially immersed between the vascular stands of the host stem and later becoming partly exposed on twig-surface when the bark shreds out. ***Sexual morph:*** *Ascomata* 300-550 µm high, 250-300 µm diam., solitary to gregarious, uniloculate, glabrous, globose to subglobose, papillate. *Ostiole*

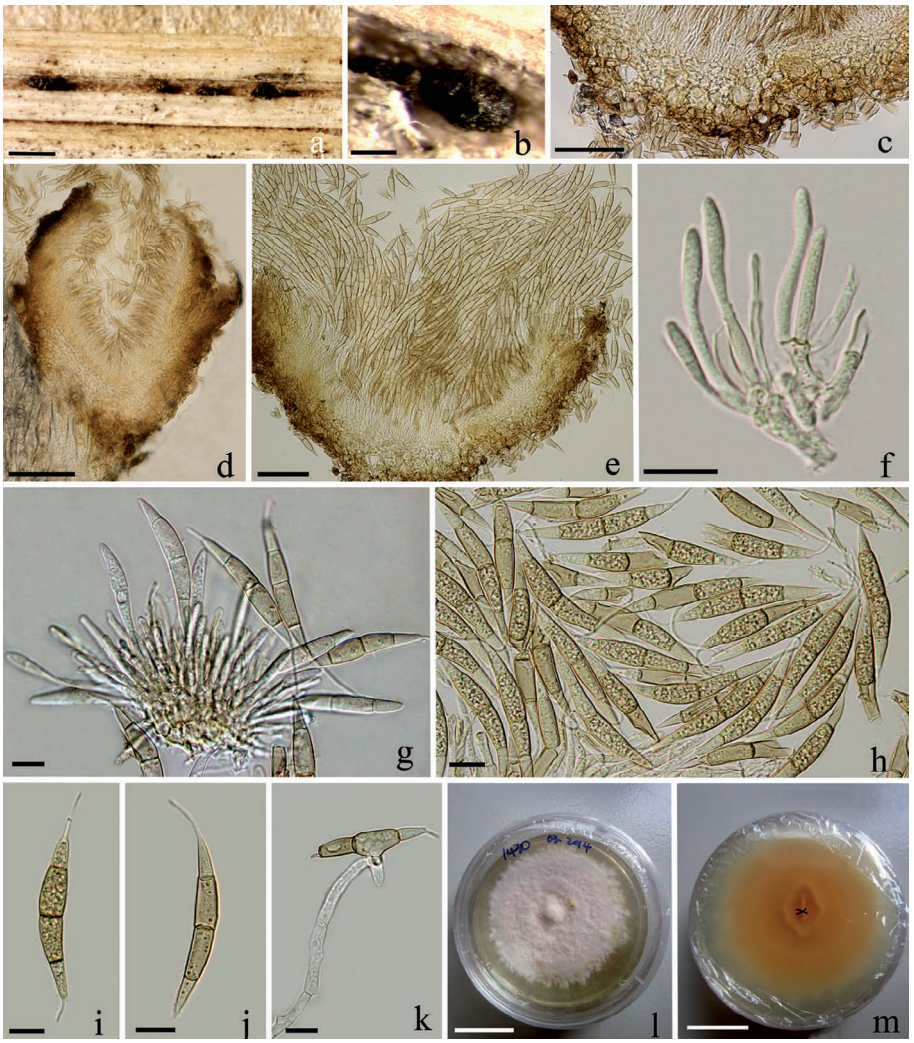

Fig. 3. *Broomella vitalbae* (MFUL 15-0054) **a-b.** Black coniodiomata on the host. **c.** Section of peridium. **d.** Vertical section of conidiomata. **e.** Developing conidia are growing in conidiaomata. **f, g.** Conidiophores, conidiogenous cells and developing conidia. **h-j.** Conidia. **k.** Germinating spore. **l-m.** Culture on PDA. **Scale bars:** a= 200 µm, b = 500 µm, c = 50 µm, d = 100 µm, e = 50 µm, f-k = 10 µm, l-m = 25 mm.

60-90 µm long, 40-80 µm wide, central located, composed of longitudinally aligned cells, and internally lined with hyaline periphyses. *Peridium* 20-80 µm wide, composed of light yellow, thick-walled cells of *textura prismatica* in the upper part and surrounding the ostiole, thin-walled, hyaline to pale brown in the most part. *Hamathecium* comprising numerous, 3-6 µm wide, cylindrical, hypha-like, septate, paraphyses, tapering towards the ends. *Asci* 100-160 × 10-20 µm ($\bar{x}$ = 120 × 15 µm; n = 30), 8-spored, unitunicate, cylindrical to cylindrical-clavate, pedicellate, apically rounded, with a J-, apical area (ring). *Ascospores* 30-40 × 6.5-10 µm

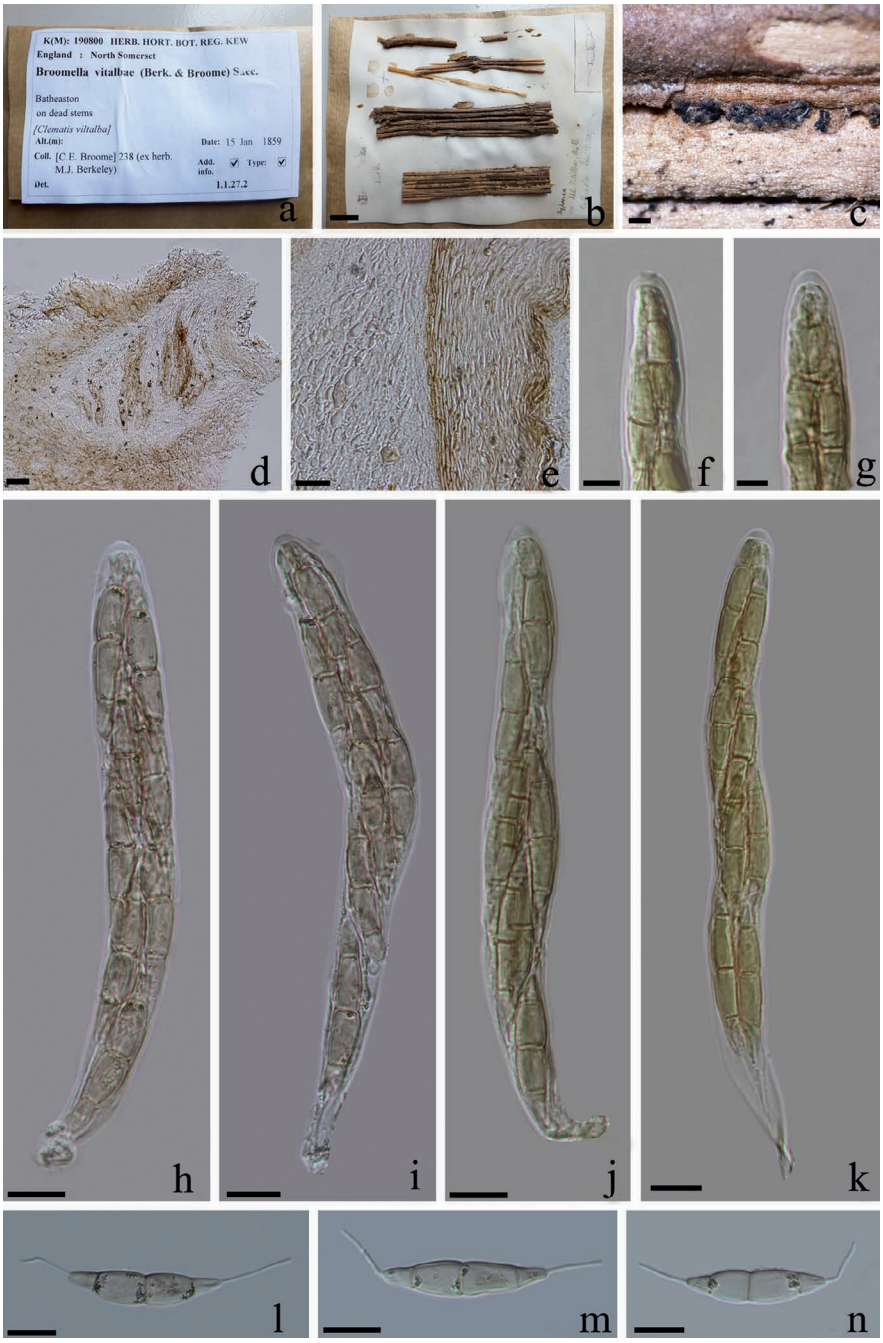

Fig. 4. *Broomella vitalbae* (holotype) **a.** Herbarium package. **b.** Herbarium material. **c.** Ascomata on the surface of host. **d.** Section of ascoma. **e.** Peridium. **f, g.** Ascus apex with a J- subapical ring. **h-k.** Asci with ascospores (j, k stained in Melzer's). **l-n.** Ascospores. **Scale bars:** b = 5 mm, c = 200 µm, d = 20 µm, e = 10 µm, f, g = 5 µm, h-n = 10 µm.

($\bar{x}$ = 35 × 8 µm; n = 50), biseriate or overlapping tri-seriate, fusiform, glabrous, straight or inequilaterally curved, 3-septate, constricted at the septa, thick walled, pale greyish brown, with doliform median cells, with conic, yellowish to pale grayish brown ends cells, each bearing 10-20 µm long, unbranched, terminal appendage.

Asexual morph: Coelomycetous. *Conidiomata* 300-400 µm high, 350-410 µm diam., stromatic, pycnidioid, scattered to gregarious, immersed to semi-immersed, rounded, oval or elongated in outline, black, unilocular, papilate, glabrous. *Peridium* 40-60 µm wide, composed of 8-10-cell layers, with thick-walled cells of *textura globulosa* to *textura angularis*, pale brown to brown in the outer layers, merging with relatively thin-walled and colourless cells in the inner layers. *Conidiophores* 10-15 wide, arising all around the cavity of conidioma, short, branched, septate, hyaline, cylindrical. *Conidiogenous cells* 10-15 µm wide, integrated, cylindrical, long, phialidic, percurrently proliferating 1-2-times, hyaline, smooth. *Conidia* 35-45 × 4-9 µm ($\bar{x}$ = 45 × 6.5 µm; n = 50), fusiform to aciculate, with acute ends, 3-septate, with single apical and basal appendages; conidial cells unequal in size; basal cell obconic, pale brown, with 2 doliform median cells, verruculose, thick walled, brown, constricted at septa, with conic apical cell, thin-walled, pale brown; with an 6-15 µm long tubular, unbranched, filiform, flexuous, hyaline appendage.

Culture characteristics: Colonies fast growing on PDA, reaching 40 mm diam. after one week at 20-25with crenate edge, whitened to pale pink, flattened, felt-like, with filamentous, dense, aerial mycelium on the surface, reverse similar in colour.

Material examined: UK, England, Batheaston, on dead stems of *Clematis vitalba*, 15 January 1859, C.E. Broome & M.J. Berkeley, (K(M) 190800, **holotype**); ITALY. Province of Forlì-Cesena [FC], Modigliana, Montebello, on dead stem of *Clematis vitalba*, 23 February 2013, Erio Camporesi, IT-1079 (MFLU 15-0065, **epitype designated here**); ex-type living culture, MFLUCC 13-0798, ICMP; ITALY. Province of Arezzo [AR], Montemezzano, on dead stem of *Clematis vitalba*, 25 August 2013, Erio Camporesi, IT-1430 (MFLU 15-0054); living culture, MFLUCC 14-1000, ICMP; *ibid.* IT-1430B (MFLU 15-0064); living culture, MFLUCC 15-0023, ICMP.

Notes: *Broomella* was introduced by Saccardo (1883) and typified by *B. vitalbae* (Berk. & Broome) Sacc. It was later placed in *Amphisphaeriaceae* (Kang *et al.,* 1999). The genus is characterized by unitunicate, cylindrical-elongate asci, with a J-, discoid ring at the apex, and ellipsoid-fusiform, straight or inequilaterally curved, 3-septate ascospores with two brown median cells, lighter terminal cells, and single, centric appendages arising from the ends (Shoemaker & Müller, 1963). *Broomella* has been shown to be linked to pestalotiod-like asexual morph (Shoemaker *et al.,* 1989; Yuan *et al.,* 1992; Kang *et al.,* 1999). Shoemaker & Müller (1963) introduced two new species in *Broomella*, namely *B. acuta* Shoemaker & E. Müll. and *B. excelsa* Shoemaker & E. Müll. Subsequently, the genus was expanded to include three more species, viz. *B. montaniensis* (Ellis & Everh.) E. Müll. & S. Ahmad, *B. tianshanica* Z.Q. Yuan & Z.Y. Zhao and *B. verrucosa* Shoemaker *et al.*. Presently, there are 20 species epithets listed under *Broomella* in Index Fungorum (2015), however, none of these are studied using DNA sequence data. The *Broomella* species and their truncatella-like asexual morphs differ in various ways from the type species of *Broomella* and its asexual morph and are probably not congeneric.

Broomella vitalbae was originally collected on a dead stem of *Clematis vitalba* L. in Batheaston (England). We re-examined the type specimen, and

Table 2. Comparison of demension of ascomata, asci and ascospores of type *Broomella vitalbae*.

Location	*Ascomata size (μm)*	*Asci size (μm)*	*Ascoapore size (μm)*	*Reference*
Batheaston	160-210 μm diam., 170-230 μm high	98.5-133.5 μm long × 10-14 μm wide	26-32 μm long × 5-8 μm wide	From this study
Italy	250-300 μm diam., 300-550 μm high	100-160 μm long × 10-20 wide	30-40 μm long × 6.5-10 μm wide	From this study
South France	350-400 μm diam., 200-250 μm high	85-120 μm long × 8-12 μm wide	20-30 μm long × 4-5 μm wide	Shoemaker & Müller 1963

compared it morphologically with our collection (MFLU 15-0065). Our collection (MFLU 15-0065) largely resembles the *B. vitalbae* in form of ascomata, asci and ascospores, and the only distinguishing character is the dimension of the asci and ascospores. It should be noted that this character may not be very reliable in *B. vitalbae*. The three specimens of *B. vitalbae* collected from different areas (i.e. Batheaston, south France and Italy) show different size in asci and ascospores (Table 2). In addition, the asexual morph (i.e. conidiomata structure, the number of apical appendage of conidia) is also used as criteria to distinguish the species in *Broomella* (Shoemaker *et al.,* 1989; Yuan *et al.,* 1992). According to Shoemaker & Müller (1963), the asexual morph of *B. vitalbae* is characterized by fusiform, 3-septate conidia with two doliform, verruculose, thick walled, brown median cells, and hyaline end cells, with one simple appendage arising from the ends, and cylindrical to subcylindrical, annelidic conidiogenous cells (Shoemaker & Müller, 1963). A comparison of morphological characters of the asexual morph of collection (MFLU 15-0054) with previously known species of *Broomella*, shows that the description of collection (MFLU 15-0054) is in accordance with the concept of *B. vitalbae* (Shoemaker & Müller 1963; Yuan *et al.* 1992).

The phylogeny of the *Amphisphaeriaceae* is reconstructed based on sequence data from the LSU gene, showing that strain (MFULCC 13-0798), strain (MFLUCC 14-1000) and strain (MFLUCC 15-0023) form a distinct clade with high support (100%), and is sister to the *H. spartii*, *Truncatella angustata* (Pers.) S. Hughes and *T. laurocerasi* (Westend.) Steyaert (Fig.1). Based on molecular data coupled with morphological information, we confirm that the strain MFULCC 13-0798, MFLUCC 14-1000 and MFLUCC 15-0023 are conspecific with *B. vitalbae,* but they are the asexual morph. Because of the lack of detailed cultures and DNA sequence data from the type specimen, we therefore use our collection to epitypify *Broomella vitalbae*. Furthermore, the sexual and asexual morphs *Broomella vitalbae* are described and illustrated.

Hyalotiella spartii W.J. Li, Camporesi & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF551047 *Facesoffunginumber*: FoF00590, Fig. 5.

Etymology: Named after the host genus, *Spartium,* from which the species was isolated.

Holotype: MFLU 15-0055

Saprobic on dead stems of *Spartium junceum*, forming conspicuous rounded, black conidiomata. *Sexual morph:* Undetermined. *Asexual morph:* coelomycetous. *Conidiomata* 250-300 μm high, 200-250 μm diam., stromatic, pycnidial, vase-shaped, scattered to gregarious, epidermal to subepidermal in origin, globose to subglobose, semi-immersed, unilocular with the locule occasionally convoluted or irregularly divided, glabrous, brown to dark brown,

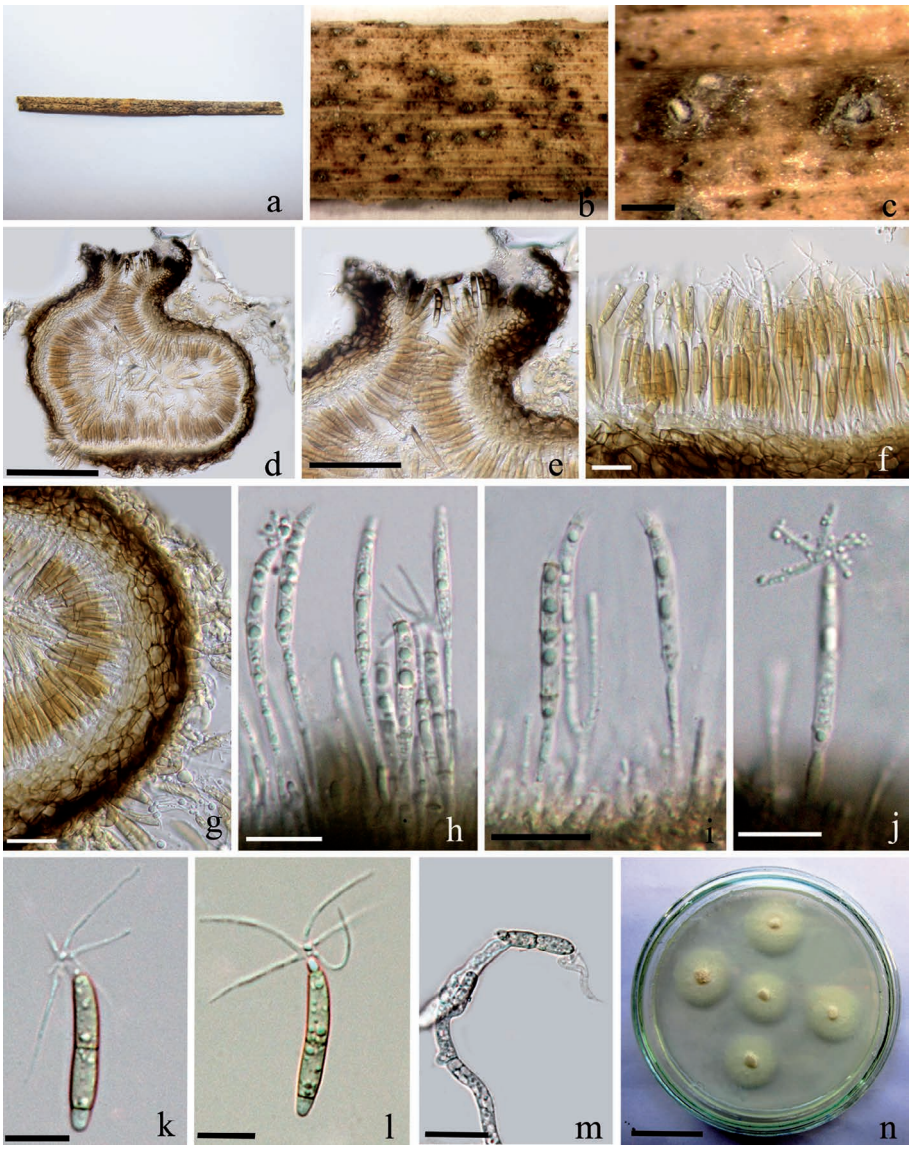

Fig. 5. *Hyalotiella spartii* (MFLU 15-0055, holotype). **a.** Material specimen. **b-c.** Black coniodiomata on the host. **d.** Vertical section of conidioma. **e.** Ostiole. **f, h-j.** Conidiogenous cells and developing conidia. **g.** Section of peridium. **k-l.** Conidia. **m.** Germinating spore. **n.** Culture on PDA. **Scale bars:** c = 200 µm. d = 100 µm, e = 50 µm, f, h-j = 10 µm, g = 10 µm, k-l = 10 µm, n = 25 mm.

ostiolate. *Ostiole* lageniform, single, centrally located, with a well-developed neck, thick-walled. *Peridium* 30-50 µm wide, composed of 7-8-cell layers, with thick-walled cells of *textura angularis*, brown to dark brown. *Conidiophores* arising all around the cavity of the conidioma, short, often reduced to conidiogenous cells. *Conidiogenous* cells 10-15 µm wide, integrated, cylindrical, long, phialidic, hyaline,

smooth. *Conidia* 25-28 × 3-4 µm ($\bar{x}$ = 26 × 3.5 µm; n = 20), fusiform, 3-septate, bearing apical appendages; basal cell obconic with an obtuse base, almost colourless, with 2 cylindrical to subcylindrical, thick-walled, yellowish to brown, median cells constricted at septa, with conic, thin-walled, hyaline apical cell; apical appendages 14-20 µm long, tubular, acellular, often irregularly or dischotomously branched at the base, filiform, flexuous, hyaline.

Culture characteristics: Colonies slow growing on PDA, reaching 15 mm diam. after 2 weeks at 20-25 with circular, whitened to pale yellow, dense, aerial mycelium on the surface, reverse similar in colour.

Material examined: ITALY. Province of Forlì-Cesena [FC], Santa Sofia, Collina di Pondo, on dead stem of *Spartium junceum*, 16 October 2012, Erio Camporesi, IT-812 (MFLU 15-0055 **holotype**); ex-type living culture, MFLUCC 13-0397, ICMP 20788; *ibid.* IT-812B (MFLU 15–0066); living culture, MFLUCC 15-0024, ICMP.

Notes: Hyalotiella is a poorly known genus both in morphology and phylogeny. The genus comprises 2 species, viz. *H. americana* and *H. transvalensis*, and both taxa lack sequence data. In the present phylogenetic study, both strains of *H. spartii* formed a distinct branch (Fig. 1). Comparative morphological study of *H. spartii* with other genera of coelomycetes showed that *H. spartii* fits well within the generic concept of *Hyalotiella* (Nag Raj, 1993). *Hyalotiella spartii* is similar to *H. transvalensis*, type species of the genus, in its conidiogenous cells and conidia, but can be easily distinguished in dimension and shape of conidiomata (Nag Raj 1993). *Hyalotiella spartii* has vase-shaped, ostiolate conidiomata that are smaller than those in *H. transvalensis* which has globose to cupulate, and irregularly-lobed conidiomata. In addition, *H. spartii* is similar to *H. americana* in having vase-shaped conidiomata and ampulliform to cylindrical conidiogenous cells, as well as cylindrical to fusiform conidia. However, recognizable differences between those two species can to be observed in the septation and number of conidial appendages. *Hyalotiella spartii* has 3-septate conidia, bearing 5-6-branched appendages, whereas *H. americana* has 3-septate conidia (occasionally 4-septate) with 2-4, mostly 3, branched appendages. Based on distinct morphology, *H. spartii* is introduced as a new species in *Hyalotiella*.

DISCUSSION

Epitypification is necessary to fix taxonomic problems and to stabilize the understanding of species, genera, families or orders (Hyde *et al.,* 2008; Ariyawansa *et al.,* 2014; Boonmee *et al.,* 2014). Maharachchikumbura *et al.* (2012) epitypified three *Pestalotiopsis* species (*P. adusta* (Ellis & Everh.) Steyaert, *P. clavispora* (G.F. Atk.) Steyaert and *P. foedans* (Sacc. & Ellis) Steyaert), and this helped to resolve the natural classification within the genus. In this study, we use morphology coupled with sequence data from fresh collections, as well as sequence data downloaded from GenBank to place *Broomella* in the family *Amphisphaeriaceae* and link the sexual and asexual morph of *Broomella*.

By designating an epitype with molecular data, we are able to confirm the placement of *Broomella*, so that related species and asexual morphs can be placed in this genus in future studies. Nevertheless, the classification of many genera in *Amphisphaeriaceae* is problematic. For example, our phylogenetic analysis (Fig. 1) suggests that there may be two distinct clades for *Truncatella*. Unfortunately, the ex-type culture of this genus (*T. angustata)* is unavailable, and

therefore it is difficult to recognize the type lineage of *Truncatella*. Thus recollecting material from type localities and isolating the organism into a pure culture are essential in order to provide further taxonomy and phylogeny studies of *Truncatella*.

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