

Taxonomy and phylogeny of *Juncaceicola* gen. nov. (*Phaeosphaeriaceae*, *Pleosporinae*, *Pleosporales*)

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Abstract – In order to establish phylogenetic relationships and resolve a natural classification for species of Dothideomycetes, it is necessary to use multi-gene phylogeny as well as morphology. In this paper we introduce a novel genus, *Juncaceicola* collected from Italy, in the family *Phaeosphaeriaceae*, with three new species, *Juncaceicola achilleae* on dead twigs of *Achillea millefolium*, *J. dactylidis* on dead twigs of *Dactylidis* sp. and *J. luzulae* on dead herbaceous stems of *Luzula nivea*. *Juncaceicola* is characterized by globose to subglobose ascomata with a minute papilla, a peridium composed of pseudoparenchymatous cells arranged in a *textura angularis* to *textura prismatica*, cylindrical-clavate asci with a short pedicel and yellowish brown, ellipsoidal to fusiform, 3-4-septate ascospores, with the second cell from the apex enlarged. Descriptions and illustrations for each novel taxon and a key for morphological character differences are provided. Combined analyses of ITS, LSU and SSU sequence data support the validity of the new species with high bootstrap support and the placement of *Juncaceicola* in *Phaeosphaeriaceae*. *Phaeosphaeria alpina*, *P. oreochloae*, *P. padellana* and *P. typharum* are also transferred to the new genus.

Dothideomycetes / ITS / LSU / molecular data / morphology / SSU

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INTRODUCTION

Phaeosphaeriaceae belongs in Pleosporales, which is the largest and most diverse order in class Dothideomycetes (Hyde *et al.*, 2013; Phookamsak *et al.*, 2014; Ariyawansa *et al.*, 2015; Liu *et al.*, 2015). Species in *Phaeosphaeriaceae* mainly occur on monocotyledons (e.g. *Cannaceae*, *Cyperaceae*, *Juncaceae*, *Poaceae*) and some dicotyledons (Shoemaker & Babcock, 1989; Schoch *et al.*, 2006; Zhang *et al.*, 2009, 2012; De Gruyter *et al.*, 2010; Hyde *et al.*, 2013; Wijayawardene *et al.*, 2014; Phookamsak *et al.*, 2014; Jayasiri *et al.*, 2015b). The majority of species in this family are widely distributed on plants as necrotrophic plant pathogens or as saprobes (Shoemaker & Babcock, 1989; Carson *et al.*, 2005; Stukenbrock *et al.*, 2006; Cannon and Kirk, 2007; Jayasiri *et al.*, 2015b). The family *Phaeosphaeriaceae* was introduced by Barr (1979), which is characterized by immersed to superficial, globose to subglobose ascomata, short papilla, bitunicate asci and hyaline, yellowish or brown, fusiform to ellipsoidal, filiform, or muriform, septate ascospores (Barr *et al.*, 1979; Shoemaker *et al.*, 1984; Shoemaker & Babcock, 1989, 1992; Zhang *et al.*, 2012; Hyde *et al.*, 2013; Phookamsak *et al.*, 2014; Phukhamsakda *et al.*, 2015).

More than 30 sexual and asexual genera are presently accepted in *Phaeosphaeriaceae* based on morphology and phylogeny. These include *Allophaeosphaeria*, *Amarenographium*, *Amarenomyces*, *Ampelomyces*, *Bricookea*, *Chaetosphaeronema*, *Dematiopleospora*, *Didymocyrtis*, *Dothideopsella*, *Entodesmium*, *Eudarlucua*, *Gallicola*, *Loratospora*, *Neosetophoma*, *Neostagonospora*, *Nodulosphaeria*, *Ophiobolus*, *Ophiosphaerella*, *Paraphoma*, *Parastagonospora*, *Phaeosphaeria* (= *Phaeoseptoria*), *Phaeosphaeriopsis*, *Phaeostagonospora*, *Populocrescentia*, *Sclerostagonospora*, *Scolecosporella*, *Scolicosporium*, *Septoriella*, *Setomelanomma*, *Setophoma*, *Tiarospora*, *Vagicola*, *Vrystaatia*, *Wojnowicia*, *Wojnowiciella* and *Xenoseptoria* (Phookamsak *et al.*, 2014; Wijayawardene *et al.*, 2014; Ariyawansa *et al.*, 2015).

The aim of this study is to introduce a new genus with three novel species and four novel combinations in *Phaeosphaeriaceae*. Combined gene (ITS, LSU and SSU) analyses using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) clearly showed these species belong in *Phaeosphaeriaceae* with high statistical support (100% ML, 100% MP and 1.00 PP).

MATERIALS AND METHODS

The fresh specimens of dead twigs and herbaceous stems were collected from Italy. Specimens were taken to the laboratory in Zip lock plastic bags and observed under a JNOEC JSZ4 stereomicroscope. Ascomata and ascospores were examined under an OLYMPUS SZ61 compound microscope. Images were taken using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 600D digital camera. Sections were made using a razor blade and kept on a droplet of sterilized water on a glass slide (Ariyawansa *et al.*, 2014). Permanent slides were prepared by mounting fungal material in lactoglycerol and sealed by applying nail-polish around the margins of cover slips. Measurements were obtained using Tarosoft (R) image frame work program. The images needed for photographic plates were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolation was carried out following the method described in Chomnunti *et al.* (2014) and Phookamsak *et al.* (2014, 2015). Ascospore germination was observed after 12 hours and germinated ascospores were transferred aseptically to potato dextrose agar (PDA). Culture characteristics were observed after 3 weeks. Type specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand, and ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi and Index Fungorum numbers are registered as described in Jayasiri *et al.* (2015a) and Index Fungorum (2016).

DNA extraction, Amplification and Sequencing

Genomic DNA was extracted from scraped mycelium using the protocol as described in the manufacturer's instructions of Biospin fungus genomic DNA kit (BioFlux[®], P.R. China). The DNA product was kept at 4°C for the DNA amplification and maintained at -20°C for long term storage. The DNA amplification was by polymerase chain reaction (PCR) using three genes, the large subunit (28S, LSU), internal transcribed spacers (ITS1, 5.8S, ITS2) and small subunit (18S, SSU). The LSU gene was amplified by using the primers LROR and LR5 (Vilgalys & Hester, 1990), nuclear ITS was amplified by using the primers ITS5 and ITS4 (White *et al.*, 1990), the SSU gene was amplified using the primers NS1 and NS4 (White *et al.*, 1990). PCR thermal cycling conditions were carried out following the method described in Wanasinghe *et al.* (2014). PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd after checking the quality on 1% agarose electrophoresis.

Phylogenetic analysis

Phylogenetic analyses were performed on combined ITS, LSU and SSU sequence data. The closest matches of generated DNA sequences were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) by using BLAST searches against the nucleotide database, following recent publications (Phookamsak *et al.*, 2014; Ariyawansa *et al.*, 2015; Li *et al.*, 2015; Liu *et al.*, 2015). The dataset consists of 58 sequences including our newly generated DNA sequences. *Didymella exigua* (CBS 183.55) was selected as the out group taxon. The single gene sequences were initially aligned by MAFFT V.7.036 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley, 2013), and improved manually where necessary using Bioedit v.7.2 (Hall *et al.*, 1999). The multiple alignments were combined using BioEdit v. 7.0.9.0 (Hall *et al.*, 1999).

Maximum likelihood (ML) analysis was performed using CIPRES web portal (Miller *et al.*, 2009) using RAxML v. 8.2.6 – Black Box. Maximum-parsimony (MP) analysis was carried out using PAUP v. 4.0b10 (Swofford, 2002). Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) by Markov Chain Monte Carlo sampling (BMCMC). Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Posada & Crandall, 1998) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and MrModeltest. Phylogenetic reconstructions of combined gene trees were performed using Bayesian inference (BI) and maximum likelihood (ML) and maximum parsimony (MP) criteria.

Phylograms were visualized with FigTree v1.4.0 program (Rambaut, 2012) and reorganized in Microsoft power point (2010) and Adobe Illustrator® CS3 (Version 15.0.0, Adobe®, San Jose, CA).

RESULTS

Phylogenetic analyses

Phylogenetic analyses based on combined ITS, LSU and SSU sequence data were obtained from maximum likelihood (ML), maximum parsimony (MP) and Bayesian analyses (BI). The three analyses were similar in topologies of phylogenetic relationships of genera in *Phaeosphaeriaceae* and did not differ significantly (data not shown). All analyses (ML, MP and BI) gave similar results in the generic placements in agreement with previous studies using multi-gene analyses (Ariyawansa *et al.*, 2015; Li *et al.*, 2015; Liu *et al.*, 2015). *Juncaceicola* species clustered in the family *Phaeosphaeriaceae*, but separate from the other genera of the family with high bootstrap support (100% ML, 100% MP, 1.00 PP, Fig. 1).

The maximum parsimony dataset consists of characters with 1997 characters as constant information, 208 variable characters as parsimony-uninformative, and 331 characters as parsimony-informative characters. The most parsimonious tree showed TL = 2025, CI = 0.417, RI = 0.611, RC = 0.255, HI = 0.583 values. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa, 1989) were performed in order to determine whether trees were significantly different. Three different alignments corresponding to each individual gene and a combined alignment of the three genes were analyzed. A best scoring RAxML tree is shown in Fig. 1, with the value of $-13331.933959(\ln)$.

Taxonomy

The genus *Juncaceicola* typified by *J. luzulae*, is introduced in the family *Phaeosphaeriaceae*. The new taxon is well-differentiated from other genera in the family based on molecular support and morphology. Descriptions and illustrations are provided and each taxon is discussed below.

Juncaceicola Tennakoon, Camporesi, Phookamsak & K.D. Hyde, *gen. nov.*

Facesoffungi number: FoF 021450

Index Fungorum number: IF552126

Etymology: refers to the host family *Juncaceae*, from which the generic type was collected.

Saprobic on dead grasses and woody twigs. **Sexual morph**: *Ascomata* solitary, scattered to clustered, immersed or erumpent through the host surface, visible as raised, black spots, on the host surface, globose to subglobose, uni-loculate, glabrous, with or without papilla. *Peridium* composed of several layers of yellowish brown to dark brown, pseudoparenchymatous cells, arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense, broad, filamentous, distinctly septate, cellular pseudoparaphyses, not constricted at the septa, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 8-spored,

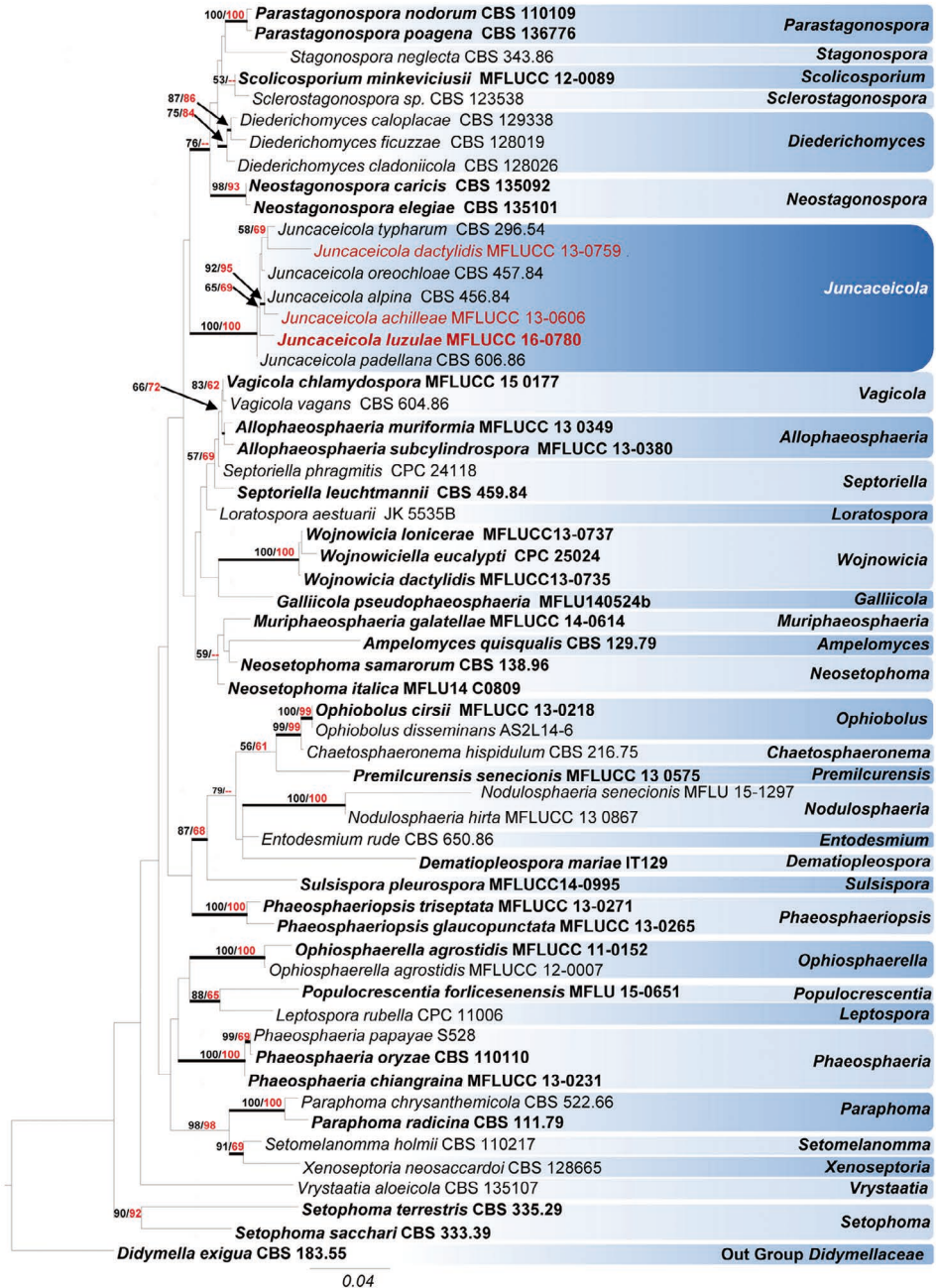


Fig. 1. RAxML tree based on analysis of a combined dataset of ITS, LSU and SSU partial sequences. Bootstrap support values for maximum parsimony (MP, red) and maximum likelihood (ML, black) higher than 50% are defined above the nodes. Bayesian posterior probabilities (BYPP) greater than 0.95 are stated in bold branches. The tree is rooted to *Didymella exigua* (CBS 183.55). All type strains are in bold. New stains are in red.

bitunicate, fissitunicate, broadly cylindrical to cylindrical-clavate, short pedicellate, apically rounded, with a well-developed ocular chamber. *Ascospores* overlapping bi-seriate, ellipsoidal to fusiform with rounded ends, initially hyaline becoming pale yellowish to yellowish brown, 3-4-septate, second cell from apex wider than other cells, slightly constricted at the septum, straight to curved, smooth to rough-walled, guttulate, with or without a mucilaginous sheath. **Asexual morph:** reported as coelomycetous, *stagonospora*-like (from Leuchtmann 1984).

Type: *Juncaceicola luzulae* Tennakoon, Camporesi, Phookamsak & K.D. Hyde

Notes: The genus *Juncaceicola* is introduced to accommodate *Phaeosphaeria sensu lato* species which have been classified in *Phaeosphaeria* subgenus *Ovispora* by Leuchtmann (1984). Species of *Juncaceicola* were mainly collected from *Juncaceae* (based on the type species), and also from *Asteraceae*, *Poaceae* and *Typhaceae*. The genus has unique characters that can distinguish it from other genera of the family *Phaeosphaeriaceae*. This includes broad fusiform to ellipsoidal ascospores, enlarged at the second cell from the apex. Based on morphological characters and phylogenetic analyses, seven species are currently accommodated in this genus.

KEY TO SPECIES OF *JUNCACEICOLA*

1. Ascospores 4-septate.....2
1. Ascospores 3-septate.....3
 2. Ascospores light brown, broadly fusiform, with first septum slightly constricted, not constricted at the other septa *J. luzulae*
 2. Ascospores pale yellowish, ellipsoidal, slightly constricted at the septa
..... *J. dactylidis*
- 3 Ascospores rough-walled, echinulate or punctate.....4
3. Ascospores smooth-walled.....5
 4. Ascospores 21-35 × 8-12 μm, associated with *Typha* spp. and *Spartina* spp. *J. typharum*
 4. Ascospores 41-45 × 15-17 μm, associated with *Trisetum distichophyllum* ...
..... *J. padellana*
5. Ascospores broadly fusiform to clavate, constricted at the first septum, not constricted at the other septa, surrounded by mucilaginous sheath6
5. Ascospores ellipsoidal, slightly constricted at the septum, not surrounded by mucilaginous sheath *J. achilleae*
 6. Ascospores 18-20 × 6-8 μm, golden (in lactic), olive (in water), associated with various hosts *J. alpina*
 6. Ascospores 27-31 × 9-12 μm, yellowish brown, associated with *Sesleria disticha* *J. oreacholae*

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold. The type strains are indicated as superscript T

Species name	Strain	GenBank accession number		
		ITS	LSU	SSU
<i>Allophaeosphaeria muriformia</i>	MFLUCC 13–0349 ^T	KP765680	KP765681	KP765682
<i>Allophaeosphaeria subcylindrospora</i>	MFLUCC 13–0380 ^T	KT314184	KT314183	KT314185
<i>Ampelomyces quisqualis</i>	CBS 129.79 ^T	HQ108038	JX681064	EU754029
<i>Chaetosphaeronema hispidulum</i>	CBS 216.75	KF251148	KF251652	EU754045
<i>Dematiopleospora mariae</i>	MFLUCC 13–0612 ^T	–	KJ749653	KJ749652
<i>Didymella exigua</i>	CBS 183.55 ^T	GU237794	EU754155	EU754056
<i>Diederichomyces caloplacae</i>	CBS-129338	JQ238641	JQ238643	–
<i>Diederichomyces cladoniicola</i>	CBS-128026	JQ238626	JQ268328	–
<i>Diederichomyces ficuzzae</i>	CBS 128019	KP170647	JQ238616	–
<i>Entodesmium rude</i>	CBS 650.86	–	GU301812	–
<i>Galliicola pseudophaeosphaeria</i>	MFLUCC 14–0527 ^T	KT326692	KT326693	–
<i>Juncaceicola achilleae</i>	MFLUCC 13-0606	KX449525	KX449526	–
<i>Juncaceicola alpina</i>	CBS 456.84	KF251181	KF251684	–
<i>Juncaceicola dactylidis</i>	MFLUCC 13-0759	KX449527	KX449528	–
<i>Juncaceicola luzulae</i>	MFLUCC 16-0780	KX449529	KX449530	KX449531
<i>Juncaceicola oreochloae</i>	CBS 457.84	AF 439494	–	–
<i>Juncaceicola padellana</i>	CBS 606.86	AF 439496	–	–
<i>Juncaceicola typharum</i>	CBS 296.54	KF251192	KF251695	–
<i>Leptospora rubella</i>	CPC 11006	DQ195780	DQ195792	DQ195803
<i>Loratospora aestuarii</i>	JK 5535B	–	GU301838	GU296168
<i>Loratospora luzulae</i>	MFLUCC14-0826	KT328497	KT328495	KT328496
<i>Muriphaeosphaeria galatellae</i>	MFLUCC 14-0614 ^T	KT438333	KT438329	KT438331
<i>Neosetophoma italica</i>	MFLUCC14-0826 ^T	KP711356	KP711361	KP711366
<i>Neosetophoma samarorum</i>	CBS 138.96 ^T	FJ427061	KF251664	GQ387517
<i>Neostagonospora caricis</i>	CBS 135092/S616 ^T	KF251163	KF251667	–
<i>Neostagonospora eligiae</i>	CBS 135101 ^T	KF251164	KF251668	–
<i>Nodulosphaeria hirta</i>	MFLUCC 13–0867	–	–	–
<i>Nodulosphaeria senecionis</i>	MFLUCC 15–1297	KT290257	KT290258	KT290259
<i>Ophiobolus cirsi</i>	MFLUCC 13–0218 ^T	KM014664	KM014662	KM014663
<i>Ophiobolus disseminans</i>	AS2L14–6	KP117305	–	–
<i>Ophiosphaerella agrostidis</i>	MFLUCC 11–0152 ^T	KM434271	KM434281	KM434290
<i>Paraphoma chrysanthemicola</i>	CBS 522.66	FJ426985	KF251670	GQ387521
<i>Paraphoma radicina</i>	CBS 111.79 ^T	KF251172	KF251676	EU754092
<i>Parastagonospora nodorum</i>	CBS 110109 ^T	KF251177	KF251681	EU754076
<i>Parastagonospora poagena</i>	CBS 136776 ^T	KJ869116	KJ869174	–
<i>Phaeosphaeria chiangraina</i>	MFLUCC 13–0231 ^T	KM434270	KM434280	KM434289
<i>Phaeosphaeria oryzae</i>	CBS 110110 ^T	KF251186	KF251689	GQ387530
<i>Phaeosphaeria papayae</i>	S528	KF251187	KF251690	–
<i>Phaeosphaeriopsis glaucopunctata</i>	MFLUCC 13-0265 ^T	KJ522473	KJ522477	KJ522481

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold. The type strains are indicated as superscript T (*continued*)

Species name	Strain	GenBank accession number		
		ITS	LSU	SSU
<i>Phaeosphaeriopsis triseptata</i>	MFLUCC 13-0271 ^T	KJ522475	KJ522479	KJ522484
<i>Populocrescentia forlicesesensis</i>	MFLU 15-0651 ^T	KT306948	KT306952	KT306955
<i>Premilcurensis senecionis</i>	MFLUCC 13-0575 ^T	KT728365	KT728366	–
<i>Sclerostagonospora</i> sp.	CBS 123538	FJ372393	FJ372410	–
<i>Scolicosporium minkeviciusii</i>	MFLUCC 12-0089 ^T	–	KF366382	KF366383
<i>Septoriella leuchtmanii</i>	CBS 459.84 ^T	KF251188	KF251691	–
<i>Septoriella phragmitis</i>	CPC 24118 ^T	KR873251	KR873279	–
<i>Setomelanomma holmii</i>	CBS 110217	–	GU301871	GQ387572
<i>Setophoma sacchari</i>	CBS 333.39 ^T	KF251245	KF251748	GQ387525
<i>Setophoma terrestris</i>	CBS 335.29 ^T	KF251246	KF251749	GQ387526
<i>Sulispora pleurospora</i>	MFLUCC14-0995 ^T	KP271443	KP271444	KP271445
<i>Vagicola chlamydospora</i>	MFLUCC 15-0177 ^T	–	KU163654	KU163658
<i>Vagicola vagans</i>	CBS 604.86	KF251193	KF251696	–
<i>Vrystaatia aloecicola</i>	CBS 135107	KF251278	KF251781	–
<i>Wojnowicia dactylidis</i>	MFLUCC 13-0735 ^T	KP744470	KP684149	KP684150
<i>Wojnowicia lonicerae</i>	MFLUCC 13-0737 ^T	KP744471	KP684151	KP684152
<i>Wojnowiciella eucalypti</i>	CPC 25024 ^T	KR476741	KR476774	–
<i>Xenoseptoria neosaccardoii</i>	CBS 128665 ^T	KF251281	KF251784	–
<i>Xenoseptoria neosaccardoii</i>	CBS 120.43	KF251280	KF251783	–

Abbreviations: **CBS**: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; **CPC**: Culture collection of Pedro Crous, housed at CBS; **JK**: J. Kohlmeyer; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **S**: Working collecting of William Quaedvlieg; **ITS**: internal transcribed spacers and intervening 5.8S nrDNA; **LSU**: large subunit (28S) of the nrRNA gene; **SSU**: small subunit (18S) of the nrRNA gene.

Juncaceicola achilleae Wanasinghe, Tennakoon, Camporesi & K.D. Hyde, **sp. nov.**
Fig. 2

Facesoffungi number: FoF 02083

Index Fungorum number: IF552121

Etymology: Name reflects the host *Achillea* sp., from which the holotype was collected.

Holotypus: MFLU 16-0106

Saprobic on dead stems of *Achillea millefolium* L. **Sexual morph**: *Ascomata* 100-150 µm high, 120-150 µm diam., solitary, scattered to clustered, semi-immersed to erumpent, as raised, black spots on the host surface, globose to subglobose, single, glabrous, ostioles central, papillate. *Peridium* of unequal thickness, 10-15 µm wide at the base, slightly thicker at the sides (15-30 µm wide), composed of several layers of brown to dark brown, pseudoparenchymatous cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1.5-3 µm wide, filamentous, cellular pseudoparaphyses, distinctly septate, not constricted at the septa, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 60-80 × 12-18 µm (\bar{x} = 70.1 × 13.9 µm, n = 35), 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, short pedicellate, with obtuse pedicel, apically rounded, with well-developed

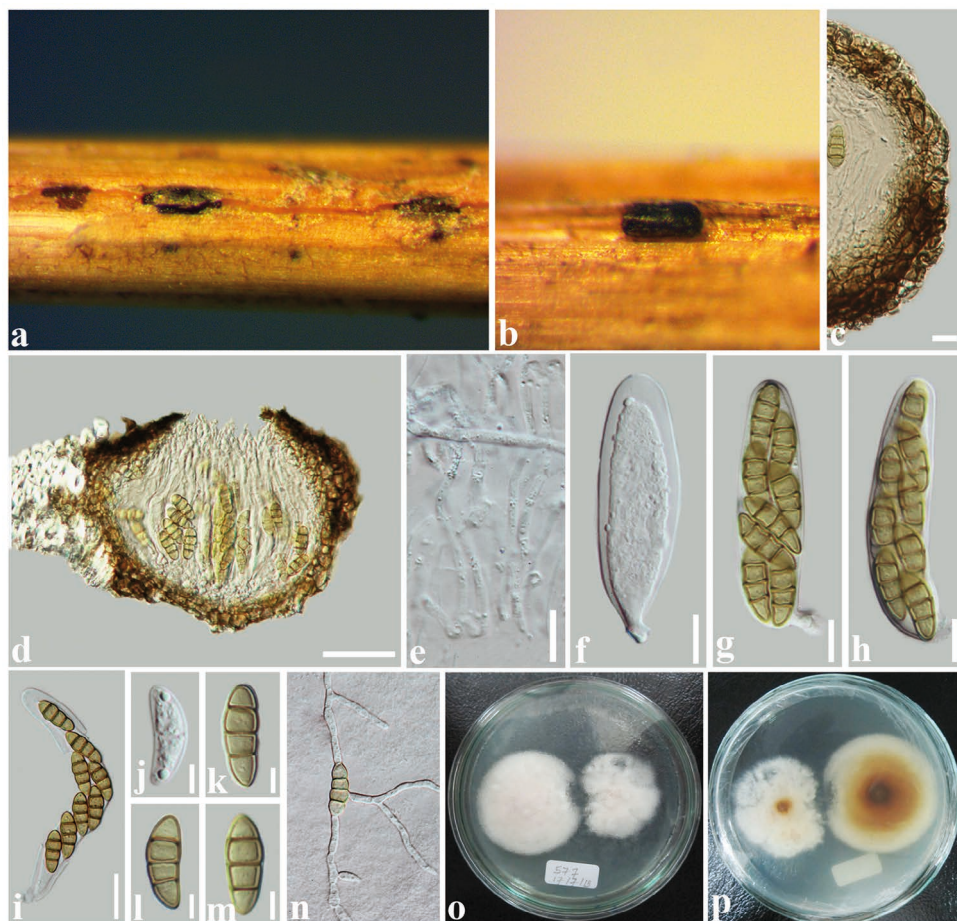


Fig. 2. *Juncaceicola achilleae*. **a**. Appearance of ascomata on host. **b**. Close-up of ascoma. **c**. Section of peridium. **d**. Section of ascoma. **e**. Pseudoparaphyses. **f-i**. Asci. **j-m**. Ascospores. **n**. Germinated ascospore. **o**. Colony from above. **p**. Colony from below. Scale bars: c = 10 μm , d = 50 μm , e-h = 10 μm , i = 20 μm , j-m = 5 μm .

ocular chamber. *Ascospores* 18-22 \times 6-8 μm (\bar{x} = 19.8 \times 6.9 μm , n = 50), overlapping uni- to bi-seriate, brown, ellipsoidal to broadly fusiform with rounded ends, 3-septate, enlarged at the second cell from apex, slightly constricted at the septum, straight to curved, smooth-walled. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA showing moderate growth, reaching 25-30 mm diam. after 2 weeks at 20-25°C, colonies medium dense, circular, flat, with slightly rough surface and entire edge, margin well-defined, cottony to fairly fluffy with sparse aspects, colony from above, white to cream at the margin, white to pinkish at the centre; from below, yellowish brown at the margin, light brown at the centre; mycelium white to cream; not producing pigmentation in PDA media agar.

Recorded host: On dead stem of *Achillea millefolium* L. (Asteraceae), Italy.

Material examined: ITALY, Province of Modena, Sestola, Monte Cervarola, dead and not land stem of *Achillea millefolium* L. (*Asteraceae*), 16 June 2013, E. Camporesi, IT-577 (MFLU 16-0106, **holotype**; HKAS isotype), ex-type living culture, MFLUCC 13-0606, KUMCC.

Notes: *Juncaceicola achilleae* resembles *J. alpina* in sharing the size range of ascospores and asci, but differs in the size of ascomata and host occurrence (see Table 2). *Juncaceicola achilleae* differs from *J. alpina* due to the lack of a mucilaginous sheath surrounding the ascospores. In addition, *J. achilleae* was found on *Achillea* sp., while *J. alpina* was found on *Juncus* sp. (see Table 2). Additionally, we compare our species with *Leptosphaeria* species which were collected from the host *Achillea millefolium*, such as *L. conoidea* (De Not.) Sacc, *L. dolioloides* (Auersw.) P. Karst., *L. doliolum* (Pers.) Ces. & De Not., *L. marginalis* Sacc., *L. millefolii* (Fuckel), *L. multiseptata* (G. winter), *L. ogilviensis* (Berk. & Broome), *L. passerinii* (Sacc.), *L. ptarmicae* P. Karst. and *L. staritzii* Henn. These species differ from *J. achilleae*, in having more than three ascospore septa, except for *L. conoidea* and *L. marginalis*. *Juncaceicola achilleae* can be distinguished from *L. conoidea* and *L. marginalis* in the size of its asci and ascospores (see Table 3). Phylogenetic analyses (Fig. 1) place *Juncaceicola achilleae* in a robust clade closely related to *J. alpina* (92% ML, 95% MP, 1.00 PP).

Juncaceicola alpina (Leuchtm.) Tennakoon, Phookamsak & K.D. Hyde, **comb. nov.**

≡ *Phaeosphaeria alpina* Leuchtm., Sydowia 37: 117 (1984)

Facesoffungi number: FoF 02085

Index Fungorum number: IF552127

Saprobic on various hosts. **Sexual**

morph: *Ascomata* 150-200 µm high, 150-200 µm diam., scattered to clustered, immersed to semi-immersed, subglobose, glabrous. *Ostioles* 10-15 µm diam., 5-10 µm long, 30-40 µm wide, central, with papilla, terete, flush, composed of 4-5 layers of brown, polygonal 4-6 × 4-6 µm cells, without periphyses. *Peridium* 10-12 µm wide, composed of 3-5 layers of polygonal to rectangular, brown,

Table 2. Synopsis of *Juncaceicola* species

Species	Ascomata	Asci	Ascospores	Peridium	Septation	Recorded hosts*
<i>Juncaceicola typharum</i>	130-150 × 90-110	85-110 × 15-20	24-30 × 9-11	10-12	3	<i>Typha angustifolia</i> , <i>Typha latifolia</i>
<i>J. dactylidis</i>	80-110 × 90-110	71-81 × 17.5-18.5	21-23 × 6-7	10-12	4	<i>Dactylidis glomerata</i>
<i>J. oreochloae</i>	125-200 × 125-200	85-110 × 15-20	27-31 × 9-12	15-30	3	<i>Danthonia intermedia</i> , <i>Festuca rubra</i>
<i>J. alpina</i>	150-200 × 150-200	62-75 × 15-27	18-20 × 6-8	10-12	3	<i>Lucula divaricata</i> , <i>Juncus ensifolius</i>
<i>J. achilleae</i>	100-150 × 120-150	60-80 × 12-18	18-22 × 6-8	10-15	3	<i>Achillea millefolium</i>
<i>J. luzulae</i>	90-120 × 140-160	55-65 × 14-15	16-18 × 5-6	20-25	4	<i>Lucula nivea</i>
<i>J. padellana</i>	180-270	120-140 × 35-40	41-45 × 15-17	30-50	3	<i>Trisetum distichophyllum</i>

*Hosts are only listed if proven by molecular data.

Table 3. Synopsis of *Juncaceicola* related species discussed in this study

Species	Ascomata	Size (μm)		Ascospores	Peridium	Septation	Recorded hosts
		Asci	Ascospores				
<i>Leptosphaeria ogilviensis</i>	250-300 × 150-200	65-75 × 8-10	26-35 × 4.5-5.5	20-25	5	<i>Achillea millefolium</i>	
<i>L. conoidea</i>	350-400 × 250-350	90 × 5.5	15-20 × 4	45-55	3	<i>Achillea millefolium</i>	
<i>L. marginalis</i>	–	60 × 10	20 × 4.5	–	3	<i>Achillea millefolium</i>	
<i>L. staritzii</i>	150 × 300-400	70-90 × 12-17	28-35 × 5-6	35-45	5-7	<i>Achillea millefolium</i>	
<i>L. doloboides</i>	240	80-115 × 12-18	35-65 × 4-6	–	7-10	<i>Achillea millefolium</i>	
<i>L. luzulae</i>	160-180	60-70 × 10	19 × 4	20-25	3	<i>Luzula nivea</i>	
<i>Phaeosphaeria caricimella</i>	150-250 × 150-250	85-140 × 20-34	30-41 × 9-12	5-12	3	<i>Luzula</i> sp.	
<i>P. epicalamia</i>	200-280 × 140-240	70-100 × 9-12	22-29 × 5-7	14-17	5	<i>Luzula</i> sp.	
<i>P. herpotrichoides</i>	180-250 × 150-200	75-90 × 12-14	28-33 × 4-5	10-18	8	<i>Luzula</i> sp.	
<i>P. insignis</i>	110-300 × 110-300	120-185 × 25-38	43-60 × 13-15	10-15	5	<i>Luzula</i> sp.	
<i>P. lutea</i>	110-300 × 110-300	60-90 × 60-90	48-60 × 10	6-8	3	<i>Luzula</i> sp.	
<i>P. eustoma</i>	60-200 × 80-200	38-80 × 7-14	17-29 × 4-4.5	6-14	3	<i>Luzula</i> sp.	
<i>P. huronensis</i>	180-220 × 120-140	50-70 × 8-10	16-20 × 4.5-5.5	15-20	5	<i>Dactylidius glomerata</i>	
<i>P. caricis</i>	90-120 × 90-120	65-75 × 10-18	33-40 × 4.5-5.5	5-12	5	<i>Dactylidius glomerata</i>	
<i>P. guttulata</i>	200-240 × 200-240	75-85 × 10-12	26-31 × 3.5-4	–	6-8	<i>Dactylidius glomerata</i>	
<i>P. pontiformis</i>	200-400 × 180-300	85-140 × 9-17	40-50 × 3.5-5.5	20-35	10-13	<i>Dactylidius glomerata</i>	
<i>P. sparsa</i>	120-250 × 150-250	80-110 × 10-14	23-31 × 3.5-5	10-19	7	<i>Dactylidius glomerata</i>	
<i>P. sylvatica</i>	100-220 × 120-300	60-70 × 8-12	18-28 × 4.5-5.5	8-16	5-7	<i>Dactylidius glomerata</i>	

pseudoparenchymatous cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1.5-2 µm wide pseudoparaphyses, with thin septa at 10-15 µm intervals, without guttules, embedded in gelatinous matrix. *Asci* 62-75 × 15-17 µm, 8-spored, bitunicate, not numerous, cylindrical, short pedicellate. *Ascospores* 18-20 × 6-8 µm, overlapping bi-seriate, golden (in lactic), olive (in water), ellipsoidal to clavate, straight or slightly curved, thick-walled, 3-septate, slightly constricted at the first septum, second cell from apex enlarged towards base, smooth-walled, without guttules, surrounded by mucilaginous sheath (2-8 µm wide), laterally bell-shaped at the ends. **Asexual morph:** Coelomycetous, stagonospora-like (description modified from Leuchtmann 1984).

Holotype: SWITZERLAND, Albula, Preda, Kanton Graubünden, Palpuognasee, on *Phleum alpinum* L. (*Poaceae*), 16 June 1982, A. Leuchtmann (ZT-ETH 9229, **type**), ex-type culture, CBS 456.84.

Recorded hosts: (1) *Allium schoenoprasum* L. (*Amaryllidaceae*) (2) *Anthericum* sp. (*Asparagaceae*) (3) *Anthoxanthum odoratum* L. (*Poaceae*) (4) *Carex curvula* ALL. (*Cyperaceae*) (5) *Carex magellanica* (Wahlenb.) Hiitonon (*Cyperaceae*) (6) *Dactylis glomerata* L. (*Poaceae*) (7) *Deschampsia caespitosa* (L.) P. Beauv. (*Poaceae*) (8) *Deschampsia flexuosa* (L.) Trin. (*Poaceae*) (9) *Elyna myosuroides* (Vill.) Fritsch (*Cyperaceae*) (10) *Festuca paniculata* (L.) Schinz & Thell. (*Poaceae*) (11) *Juncus alpinus* Vill. (*Juncaceae*) (12) *Juncus ensifolius* Wikstr. (*Juncaceae*) (13) *Koeleria pyramidata* (Lam.) P. Beauv. (*Poaceae*) (14) *Luzula divaricata* S. Watson (*Juncaceae*) (15) *Nardus stricta* L. (*Poaceae*) (16) *Phleum alpinum* L. (*Poaceae*) (17) *Sesleria caerulea* (L.) Ard. (*Poaceae*) (18) *Stellaria crassipes* L. (*Caryophyllaceae*) (Farr & Rossman, 2016).

Notes: *Juncaceicola alpina* was introduced by Leuchtmann (1984) as *Phaeosphaeria alpina*, which was collected from *Phleum alpinum* L. (*Poaceae*). Leuchtmann (1984) accommodated the species in *Phaeosphaeria* subgenus *Ovispora* due to its 3-septate and ellipsoidal to clavate ascospores and uniform mucilaginous sheath. In the phylogenetic analyses (fig. 1), *Phaeosphaeria alpina* groups in *Phaeosphaeriaceae*, but is unrelated to *Phaeosphaeria sensu stricto* (also shown in Phookamsak *et al.*, 2014, Ariyawansa *et al.*, 2015; Li *et al.*, 2015; Liu *et al.*, 2015). Phookamsak *et al.* (2014) showed *P. alpina* to be a distinct clade in *Phaeosphaeriaceae* as *Phaeosphaeria sensu lato* and this was confirmed in Ariyawansa *et al.* (2015), Liu *et al.* (2015) and Li *et al.* (2015). In this study, *P. alpina* clustered with the new genus *Juncaceicola* with high bootstrap support (92% ML, 95% MP, 1.00 PP, Fig. 1). Therefore, the species is transferred to the genus *Juncaceicola*. The wide range of hosts listed above for *Phaeosphaeria alpina* are based on morphological identifications and this needs confirmation using fresh collections and molecular data.

Juncaceicola dactylidis Wanasinghe, Tennakoon, Camporesi, & K.D. Hyde, **sp. nov.**
Fig. 3

Facesoffungi number: FoF 02084

Index Fungorum number: IF552125

Etymology: Name reflects the host from which the holotype was collected.

Holotypus: MFLU 16-0156

Saprobic on dead stems of *Dactylidis glomerata* L. **Sexual morph:** *Ascomata* 80-110 µm high, 90-110 µm diam., solitary, scattered or sometimes clustered, immersed or erumpent through the host surface, as raised, black spots, globose to subglobose, glabrous, ostioles central, with minute papilla. *Peridium* 10-20 µm wide, thin-walled, of equal thickness, composed of 2-3 layers of dark brown

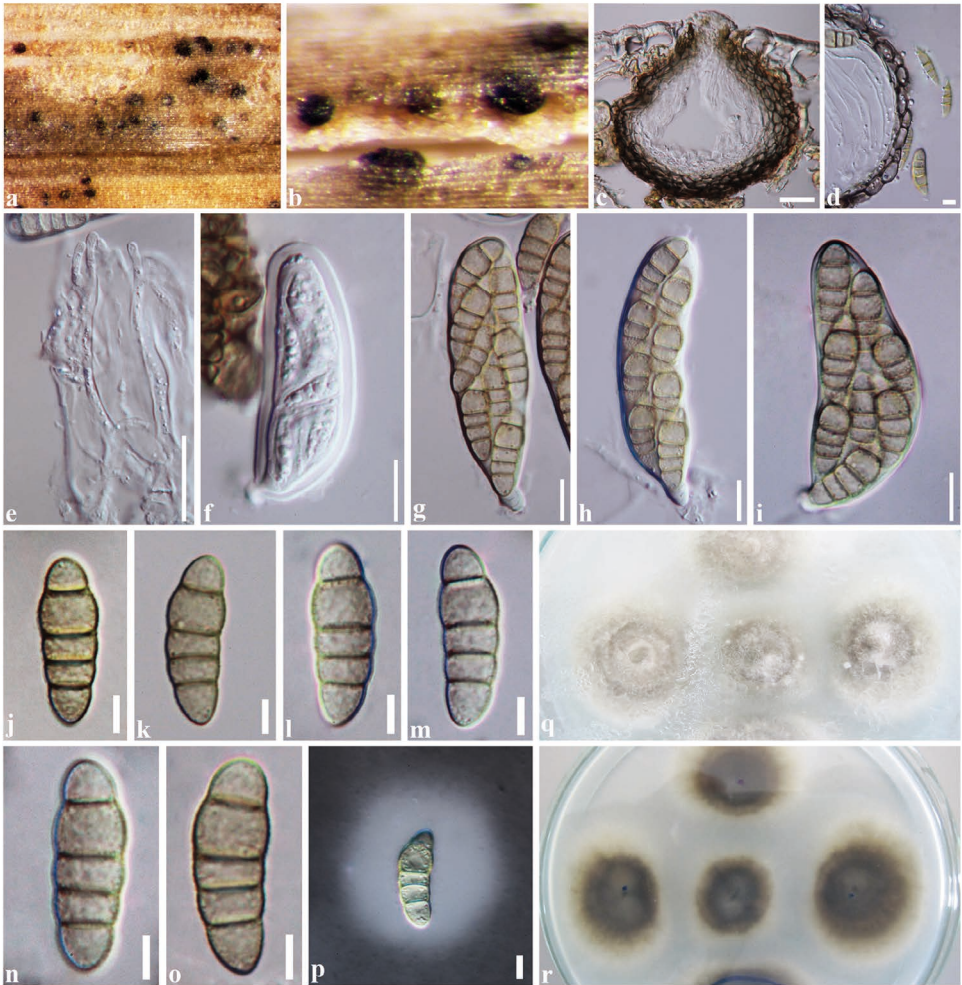


Fig. 3. *Juncaceicola dactylidis*. **a.** Appearance of immersed ascomata on host. **b.** Close-up of ascomata. **c.** Section of ascoma. **d.** Section of peridium. **e.** Pseudoparaphyses. **f.** Young ascus. **g-i.** Asci. **j-o.** Ascospores. **p.** Ascospore stained in Indian ink. **q.** Colony from above. **r.** Colony from below. Scale bars: c = 20 μ m, d = 5 μ m, e = 20 μ m, f-i = 10 μ m, j-o = 5 μ m, p = 5 μ m.

to black pseudoparenchymatous cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1.5-2.5 μ m wide, filamentous, cellular pseudoparaphyses, distinctly septate, not constricted at the septa, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (67-)-71-81(-85) \times (16-)-17.5-18.5(-21) μ m (\bar{x} = 76 \times 18.1 μ m, n = 30), 8-spored, bitunicate, fissitunicate, broadly cylindrical or clavate, short pedicellate, with obtuse pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* (19.5-)-21-23(-24) \times 6-7(-8) μ m (\bar{x} = 22 \times 7 μ m, n = 30), overlapping bi- to tri-seriate, ellipsoidal, yellowish brown, 4-septate, slightly constricted at the septum, enlarged at the second cell from apex, narrow towards the lower end cell, straight to slightly curved, with one side flattened, rough-

walled, echinulate, surrounded by mucilaginous sheath. **Asexual morph:** Undetermined.

Culture characteristics: Colonies on PDA fast growing, reaching 15-20 μm diam. after 2 weeks at 20-25°C, colonies medium sparse, circular, flat, with slightly rough surface with entire edge, margin well-defined, cottony to fairly fluffy with sparse aspects, colony from above, grey to yellowish at the margin, white to grey at the centre; from below, light brown to yellowish at the margin, brown to black at the centre; mycelium white to grey; not producing pigmentation in PDA media.

Recorded host: On dead stem of *Dactylidis glomerata* L. (*Poaceae*), Italy.

Material examined: ITALY, Province of Arezzo, Pratomagno, on dead stems of *Dactylidis glomerata* L. (*Poaceae*), 16 July 2013, E. Camporesi, IT-1381 (MFLU 16-0156, **holotype**; HKAS **isotype**), ex-type living culture, MFLUCC 13-0759, KUMCC.

Notes: *Juncaceicola dactylidis* is most similar to *J. achilleae* in having ellipsoidal ascospores, which are slightly constricted at the septum. However, *J. dactylidis* differs from *J. achilleae* in its echinulate and paler ascospores, while the latter species has smooth-walled ascospores. *Juncaceicola dactylidis* is similar to *J. luzulae* in having 4-septate ascospores, with the last three septa arranged near to the apex, but differs in the size of the asci and ascospores, peridium and pseudoparaphyses (see Table 2). Additionally, we compare our species with other *Phaeosphaeria* species which were collected from *Dactylidis glomerata* (Farr & Rossman, 2016) such as, *Phaeosphaeria alpina* Leuchtm., *P. avenaria* (G.F. Weber) O.E. Erikss., *P. caricis* (J. Schröt.) Leuchtm., *P. culmorum* (Auersw.) Leuchtm., *P. eustoma* (Fuckel) L. Holm, *P. fuckelii* (Niessl) L. Holm, *P. guttulata* Shoemaker & C.E. Babc., *P. huronensis* Shoemaker & Babcock, *P. nigrans* (Roberge ex Desm.) L. Holm, *P. pontiformis* (Fuckel) Leuchtm., *P. rousseiana* (Desm.) L. Holm, *P. sparsa* (Fuckel) Shoemaker & C.E. Babc., *P. silvatica* (Pass.) Hedjar. and *P. vagans* (Niessl) O.E. Erikss. However, *J. dactylidis* differs from these *Phaeosphaeria* species in having ellipsoidal to fusiform, 4-septate ascospores (see Table 3). *Juncaceicola alpina* has also been recorded from *Dactylis glomerata* (Farr & Rossman, 2016), however this identification needs confirmation using molecular data.

Phylogenetic analyses of combined ITS, LSU and SSU sequence data shows that the species clusters with other *Juncaceicola* species with strong support (100% ML, 100% MP, 1.00 PP, Fig. 1) and is most closely related to *J. typharum* with moderate bootstrap support (Fig. 1).

Juncaceicola luzulae Tennakoon, Camporesi, Phookamsak & K.D. Hyde, **sp. nov.**
Fig. 4

Facesoffungi number: FoF 02083

Index Fungorum number: IF552124

Etymology: Name reflects the host genus *Luzula*, from which the holotype was collected.

Holotypus: MFLU 16-1307

Saprobic on dead stem of *Luzula nivea* L. **Sexual morph:** *Ascomata* 90-120 μm high, 140-160 μm diam., solitary, scattered to clustered, immersed or erumpent through the host surface, as raised, black spots, globose to subglobose, glabrous, uni-loculate, ostioles central with minute papilla. *Peridium* 20-25 μm wide, thin to thick-walled, of equal thickness, composed of several layers of flattened, yellowish brown, pseudoparenchymatous cells, arranged in a *textura angularis* to



Fig. 4. *Juncaceicola luzulae* (MFLU 16-1307, holotype). **a.** Appearance of immersed ascomata on host. **b.** Vertical section of ascoma. **c.** Section of peridium. **d.** Pseudoparaphyses. **e-i.** Asci. **j-n.** Ascospores. **o.** Colony (on PDA) from above. **p.** Colony (on PDA) from below. Scale bars: **b** = 50 μ m, **c** = 10 μ m, **d** = 20 μ m, **e-i** = 10 μ m, **j-n** = 5 μ m.

textura prismatica. *Hamathecium* composed of dense, 0.9-1.8 μ m wide, filamentous, cellular pseudoparaphyses, distinctly septate, not constricted at the septa, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (52-)55-65(-68) \times 14-15 μ m (\bar{x} = 59 \times 14.1 μ m, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, with obtuse pedicel, apically rounded, with a well-developed ocular chamber. *Ascospores* 16-18(-19) \times 5-6(-6.5) μ m (\bar{x} = 17.7 \times 5.7 μ m, n = 30), overlapping bi-seriate, ellipsoidal to fusiform with rounded ends, initially hyaline to pale yellowish, becoming yellowish brown at maturity, 4-septate, slightly constricted at the second septa, not constricted at other septa, straight to curved, enlarged at the second cell from apex, rough-walled, echinulate, with guttules. **Asexual morph:** Undetermined.

Culture characteristics: Colonies on PDA showing moderate to slow growth, reaching 75-80 mm diam. after 3 weeks at 20-25°C, colonies medium dense, circular, flat, with slightly rough surface with entire edge, margin well-defined, glabrous to velvety with smooth aspects, colony from above: white to cream, with white tufts; from below, yellowish brown at the margin, light brown at the centre; mycelium white to cream; not producing pigmentation in PDA media.

Recorded host: On dead stem of *Luzula nivea* L. (*Juncaceae*), Italy.

Material examined: ITALY, Province of Forlì – Cesena (FC), Monte Falco, dead stem of *Luzula nivea* L. (*Juncaceae*), 2 June 2015, E. Camporesi, IT 1918 (MFLU 16-1307, **holotype**; HKAS 93700, **isotype**), ex-type living culture, MFLUCC 16-0780, KUMCC 15-0553.

Notes: *Juncaceicola luzulae* resembles to *J. typharum* and *J. oreochloae* in having broadly fusiform, thick-walled, echinulate ascospores which are slightly constricted at the septum. However, *J. luzulae* differs from *J. typharum* and *J. oreochloae* in having 4-septate ascospores, while in *J. typharum* and *J. oreochloae* they are 3-septate (see Table 2). *Juncaceicola luzulae* is similar to *J. dactylidis* in its ascomata, appearing as black spots, immersed or erumpent through host surface and 4-septate ascospores, which are widest at the second cell from the apex. However, *J. luzulae* differs from other *Juncaceicola* species in the size of its asci, and size and shape of its ascospores (see Table 2). Phylogenetic analyses also confirm that they are distinct (65% ML, 69% MP, Fig. 1).

In addition, we compare *Juncaceicola luzulae* with *Leptosphaeria luzulae* and other *Phaeosphaeria* species which were collected from *Luzula*, such as *Phaeosphaeria borealis* Shoemaker & C.E. Babc., *P. caricinella* (P. Karst.) O.E. Erikss., *P. caricis* (J. Schröt.) Leuchtm., *P. culmorum* (Auersw.) Leuchtm., *P. epicalamia* (Riess) L. Holm, *P. eustoma* (Fuckel) L. Holm, *P. fuckelii* (Niessl) L. Holm, *P. herpotrichoides* (De Not.) L. Holm, *P. insignis* (P. Karst.) L. Holm, *P. juncicola* (Rehm ex G. Winter) L. Holm, *P. juncina* (Auersw.) L. Holm and *P. lutea* Leuchtm. (Farr & Rossman, 2016). However, *J. luzulae* differs from *Leptosphaeria luzulae* (see Table 3) and other *Phaeosphaeria* species, in having ellipsoidal to fusiform, 4-septate ascospores. *Juncaceicola alpina* has also been recorded from *Luzula divaricata* (Farr & Rossman, 2016), however this identification needs confirmation using molecular data.

Juncaceicola oreochloae (Leuchtm) Tennakoon, Phookamsak & K.D. Hyde, **comb. nov.**

= *Phaeosphaeria oreochloae* Leuchtm., Sydowia 37: 118,120 (1984)

Facesoffungi number: FoF 02086

Index Fungorum number: IF552128

Saprobic on dead stem of *Danthonia* sp. **Sexual morph:** *Ascomata* 125-200 µm, high 125-200 µm diam., scattered, immersed, subepidermal, globose, glabrous, ostiolate. *Ostioles* 40 µm long, 30-60 µm wide, central, without periphyses. *Peridium* 15-30 µm wide, thin to thick-walled with uniform thickness, composed of 3-5 layers of flattened, brown polygonal (8-15 × 8-10 µm), pseudoparenchymatous cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 2-3 µm wide, cellular pseudoparaphyses, septate, without guttules. *Asci* 85-110 × 15-20, 8-spored, bitunicate, clavate. *Ascospores* 27-31 × 9-12 µm, overlapping tri-seriate, yellowish brown, broadly fusiform, thick-walled, 3-septate, first septum slightly constricted, not constricted at other septa, widest at the second cell from apex, smooth-walled, guttulate, surrounded by mucilaginous sheath. **Asexual morph:** Undetermined (description modified from Leuchtmann 1984)

Holotype: SWITZERLAND, Albula, Preda, Murtel digl, Crap Alv, on *Sesleria disticha* (Wulfen) Pers., 25 August 1980, A. Leuchtmann (ZT-ETH 9231, **type**), ex-type culture, CBS 457.84.

Recorded hosts: (1) *Danthonia intermedia* Vasey (*Poaceae*), (2) *Festuca rubra* L. (*Poaceae*) (3) *Poa epilis* Scribner (*Poaceae*) (Farr & Rossman, 2016).

Notes: *Juncaceicola oreochloae* was described by Leuchtmann (1984) as *Phaeosphaeria oreochloae* and was collected from host family *Poaceae*. Leuchtmann (1984) accommodated the species in the subgenus *Ovispora* due to its 3-septate ascospores surrounded by a uniform mucilaginous sheath and a thick peridium made up of large cells. Phylogenetic analyses place *Phaeosphaeria oreochloae* in *Juncaceicola* with strong bootstrap support (100% ML, 100% MP, 1.00 PP, Fig. 1). Therefore, the species is transferred to *Juncaceicola*. The species is listed from several hosts (Farr & Rossman, 2016), but molecular data is needed to confirm if these were correctly identified.

Juncaceicola padellana (Leuchtm.) Tennakoon, Phookamsak & K.D. Hyde, **comb. nov.**

≡ *Phaeosphaeria padellana* Leuchtm., Mycol. helv. 2(2): 184 (1987)

Facesoffungi number: FoF 02087

Index Fungorum number: IF552129

Saprobic on leaves of *Trisetum distichophyllum* (Vill.) P. Beauv. **Sexual morph:** *Ascomata* 180-270 µm diam., scattered, immersed, without papillate. *Peridium* 30-50 µm wide, thin-walled, composed of several layers of dark brown, small pseudoparenchymatous cells. *Hamathecium* composed of dense, cellular pseudoparaphyses, distinctly septate, embedded in a hyaline gelatinous matrix. *Asci* 120-140 × 35-40 µm, 8-spored, bitunicate, fissitunicate, ellipsoidal, short pedicellate, ends broadly rounded, with well-developed ocular chamber. *Ascospores* 41-45 × 15-17 µm, yellowish brown to brown, 3-septate, slightly constricted at the septum, with large, round inclusions in each segment, second segment slightly swollen, surrounded by a mucilaginous sheath. **Asexual morph:** Undetermined (description modified from Leuchtmann 1987).

Holotype: SWITZERLAND, Kanton Graubünden, Samedan, Piz Padella, on *Trisetum distichophyllum*, 19 July 1985, A. Leuchtmann (ZT-ETH 9655, **holotype**), ex-type living culture, CBS 606.86.

Recorded host: *Trisetum distichophylla* Vill. (*Poaceae*)

Notes: *Juncaceicola padellana* was introduced by Leuchtmann (1987) as *Phaeosphaeria padellana* and was collected from family *Poaceae*. Phylogenetic analyses show that *P. padellana* belongs in *Juncaceicola* with strong bootstrap support (100% ML, 100% MP, 1.00 PP, Fig. 1). Therefore, the species is transferred to *Juncaceicola*.

Juncaceicola typharum (Desm.) Tennakoon, Phookamsak & K.D. Hyde, **comb. nov.**

≡ *Sphaeria scirpicola* var. *typharum* Desm., Pl. cryptog. Fr.: no. 1428 (1849)

≡ *Sphaeria typharum* (Desm.) Rabenh., Klotzschii Herb. Viv. Mycol., Edn 2: no. 731 (1858)

≡ *Sphaerella typharum* (Desm.) Rabenh., Fungi europ. exsicc., Edn nova. Cent. 11(nos 1001-1100): no. 1040 (1866)

≡ *Sphaerella typharum* (Desm.) Rabenh., Fungi europ. exsicc., Edn nova. Cent. 11(nos 1001-1100): no. 1040 (1866) f. *typharum*

≡ *Pleospora typharum* (Desm.) Fuckel, Jb. nassau. Ver. Naturk. 23-24: 137 (1870) [1869-70]

≡ *Leptosphaeria typharum* (Desm.) P. Karst., Bidr. Känn. Finl. Nat. Folk 23: 100 (1873)

≡ *Leptosphaeria typharum* (Desm.) P. Karst., Bidr. Känn. Finl. Nat. Folk 23: 100 (1873) subsp. *typharum*

- ≡ *Leptosphaeria typharum* (Desm.) P. Karst., Bidr. Känn. Finl. Nat. Folk 23: 100 (1873) var. *typharum*
- ≡ *Heptameria typharum* (Desm.) Cooke, Grevillea 18(no. 86): 32 (1889)
- ≡ *Leptosphaeria typharum* f. *scirpi* G. Boyer & Jacz., Annl. Ec. Agric. Montpellier: 29 (1894)
- ≡ *Phaeosphaeria typharum* (Desm.) L. Holm, Symb. bot. ups. 14(no. 3): 126 (1957)

Facesoffungi number: FoF 02088

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Saprobic on *Typha* sp. **Sexual morph:** *Ascomata* 130-150 µm high, 90-110 µm diam., scattered, immersed, subepidermal, as brown spots on the host surface, ovoid, glabrous, uni-loculate, ostioles central with a minute papilla. *Peridium* 10-12 µm wide, composed of 3-4 layers of brown, polygonal, pseudoparenchymatous cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, filamentous, cellular pseudoparaphyses, distinctly septate, without guttules, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 85-110 × 15-20 µm, 8-spored, bitunicate, fissitunicate, cylindrical, short pedicellate. *Ascospores* (21-)24-30(-35) × (8-)9-11(-12) µm, overlapping bi-seriate, reddish brown, broadly fusiform, thick-walled, 3-septate, first septum slightly constricted, not constricted at the remaining septa, second cell from apex enlarged towards base, without guttules, finely echinulate, with a conspicuous sharply delimited sheath (1-1.5 µm wide). **Asexual morph:** reported as *Scolecosporella typhae* (description modified from Holm, 1957).

Holotype: could not be located.

Recorded hosts: (1) *Spartina alterniflora* Loisel. (*Poaceae*) (2) *Spartina patens* (Aiton) Muhl (*Poaceae*) (3) *Typha angustifolia* L. (*Typhaceae*), (4) *Typha australis* L. (*Typhaceae*) (5) *Typha latifolia* L. (*Typhaceae*) (Farr & Rossman, 2016).

Notes: *Juncaceicola typharum* was described by Desmazieres (1849) and Leuchtmann (1984) treated the species in *Phaeosphaeriaceae* as *Phaeosphaeria typharum*. It was collected from *Typhaceae* and *Poaceae*. Leuchtmann (1984) accommodated the species in the subgenus *Ovispora* due to its 3-septate and finely echinulate ascospores and uniform mucilaginous sheath. Our phylogenetic analyses placed *P. typharum* in *Phaeosphaeriaceae*, but unrelated to *Phaeosphaeria sensu stricto* (Phookamsak *et al.*, 2014; Ariyawansa *et al.*, 2015; Li *et al.*, 2015; Liu *et al.*, 2015). Phookamsak *et al.* (2014) mentioned that *P. typharum* formed a distinct clade in *Phaeosphaeriaceae* and treated the species in *Phaeosphaeria sensu lato* and this has been confirmed in subsequent studies (Ariyawansa *et al.*, 2015; Li *et al.*, 2015; Liu *et al.*, 2015). In this study, *Phaeosphaeria typharum* clustered with *Juncaceicola* with strong bootstrap support (100% ML, 100% MP, 1.00 PP, Fig. 1). Thus, we transfer the species to *Juncaceicola*. The species is listed from several hosts (Farr & Rossman, 2016), but molecular data from fresh collections is needed to confirm this.

DISCUSSION

The family *Phaeosphaeriaceae* contains more than 30 genera with various morphological spores, *i.e.* *Amarenographium* (dictyosporous asexual morph), *Muriphaeosphaeria* (dictyosporous sexual morph), *Entodesmium* (scolecosporous), *Nodulosphaeria* (elongated phragmosporous), *Phaeosphaeria* (phragmosporous)

(Zhang *et al.*, 2012; Phookamsak *et al.*, 2014; Ariyawansa *et al.*, 2015; Liu *et al.*, 2015; Phukhamsakda *et al.*, 2015). The 30 genera of *Phaeosphaeriaceae* are mostly monospecific or include 2-3 species. Thus, it is important to reinforce the generic limits, especially in monospecific genera (i.e. *Dematiopleospora*, *Entodesmium*, *Galliicola*, *Leptospora*, *Muriphaeosphaeria*, *Populocrescentia*, *Premilcurensis*, *Setomelanomma*, *Sulsispora*, *Vrystaatia* and *Xenoseptoria*) with additions of species with phylogenetic support.

In this study we have included taxa representing all genera of *Phaeosphaeriaceae* which are available in GenBank (Table 1). The phylogenetic analysis of the combined ITS, LSU and SSU sequence data strongly support that the new genus *Juncaceicola* belongs in *Phaeosphaeriaceae*, and is distinctly separated from other genera with high bootstrap support (100% ML, 100% MP and 1.00 PP, Fig. 1).

Juncaceicola is characterized by globose to subglobose ascomata with a minute papilla, a peridium composed of pseudoparenchymatous cells arranged in a *textura angularis* to *textura prismatica*, short pedicellate asci with a well-developed ocular chamber and 3-4-septate ascospores, whose second segment from the apex is enlarged.

Phookamsak *et al.* (2014) placed *Phaeosphaeria alpina* and *P. typharum* in a distinct clade in *Phaeosphaeriaceae* in their phylogenetic analyses and concluded that these two species belong in *Phaeosphaeria sensu lato*.

In this study we show that *Phaeosphaeria alpina* and *P. typharum* group with our new strains in a sister clade (Fig. 1). Blast searches have shown that *Coniothyrium cereale* (CBS 518.74), *Phaeosphaeria padellana* (CBS 606.86) and *P. oreochloae* (CBS 457.84) are closely related to the new group. Primary analyses however, concluded that *Coniothyrium cereale* cannot be included in *Juncaceicola* (data not shown). Thus, we introduce *Juncaceicola* as a novel genus in *Phaeosphaeriaceae* with three new species (*J. luzulae*, *J. dactylidis* and *J. achilleae*) and four new combinations (*J. alpina*, *J. oreochloae*, *J. padellana* and *J. typharum*). The species may be specific to host species or host genus and this will need confirmation with fresh collections and molecular data.

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