

Keissleriella dactylidis, sp. nov., from *Dactylis glomerata* and its phylogenetic placement

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ABSTRACT: A new species of *Keissleriella* (Lentitheciaeae) is described from a dead stem of *Dactylis glomerata* collected in Italy. The new taxon is supported by morphology and phylogenetic analysis of SSU, LSU, and TEF1α sequence data. The combined analysis of gene data indicate that *Keissleriella dactylidis* sp. nov. forms a distinct clade within the genus *Keissleriella*. The species is characterized by immersed, erumpent, dark grey to black ascomata with papilla containing brown periphyses, and cylindrical ascii with hyaline, ellipsoid, 4–5-septate ascospores with a thin mucilaginous sheath. A synopsis of the characteristics of *Keissleriella dactylidis* and other species is provided and the new species is justified and compared with similar taxa. This is the first report of a *Keissleriella* species from the grass *Dactylis glomerata*.

KEYWORDS: *Dactylidis*, Gramineae, *Keissleriella*, Lentitheciaeae, Poaceae

INTRODUCTION

Dothideomycetes is the largest and most diverse class of Ascomycota comprising an estimated 19 000 species^{1,2} and is characterized by bitunicate, usually fissitunicate ascii^{1,3}. The majority of species in this class are endophytes, epiphytes, or saprobes, with a small number occurring as lichens and hyperparasites^{1,4}. Many of the asexual morphs of dothideomycetous are pathogens of economically important plants and crops^{5,6}.

Pleosporales is the largest order in Dothideomycetes, comprising a quarter of all dothideomycetous species^{7,8} and comprising 41 families¹. This large order comprises two main suborders, namely Pleosporineae and Massarineae. Pleosporineae includes nine families: Cucurbitariaceae, Didymellaceae, Didymosphaeriaceae, Dothidot Thiaceae, Leptosphaeriaceae, Phaeosphaeriaceae, Pleosporaceae⁹, Halojellaceae, and Shiraiaceae^{10,11}, while Massarineae includes six families: Bambusicola-

ceae¹, Montagnulaceae, Lentitheciaeae, Massarinaceae, Morosphaeriaceae, and Trematosphaeriaceae². Ariyawansa et al⁷ have however synonymized Montagnulaceae under Didymosphaeriaceae by giving the priority to the oldest name.

The family Lentitheciaeae was introduced by Zhang et al¹² in the order Pleosporales. Most members in this family are saprobic on stems and twigs of herbaceous and woody plants in terrestrial or aquatic habitats^{12,13}. The family is characterized by the lenticular ascomata, ostioles with or without brown setae, and hyaline, sometimes light yellow and 1-septate to multi-septate ascospores^{1,9}.

Keissleriella was introduced by Höhn¹⁴ and is typified by *Keissleriella aesculi* Sacc. (≡ *Pyrenopeziza aesculi* Höhn.). The genus *Keissleriella* is characterized by ascomata with papilla filled with black setae, and hyaline and 1 to multi-septate ascospores^{15–17}. Previous studies based on morphology and multi-gene analysis have shown that the natural classification of *Keissleriella* is confusing, thus several authors

classified the genus under different families. For example, Munk¹⁸ placed this genus in Lophiostomataceae (including Massarinaceae), Arx and Müller¹⁹ placed it in Pleosporaceae, and Barr¹⁵ referred it to Melanommataceae. Lumbsch and Huhndorf²⁰ classified *Keissleriella* in the family Massarinaceae and Zhang et al¹² placed the genus in Lentitheciaceae.

The aim of this paper is to introduce and describe a new *Keissleriella* species which was discovered as a result of examining saprobic fungi on *Dactylis glomerata* in Italy and discuss its taxonomic placement in Lentitheciaceae based on morphology and phylogeny.

MATERIAL AND METHODS

Sample collection and morphological studies

Dead stems of *Dactylis glomerata* were collected on 5 August 2012 in Marilleva in the Province of Trento, Italy and brought to the laboratory in Ziplock plastic bags where they were examined under a Motic SMZ 168 series dissecting stereo-microscope. Hand sections of ascocarps were mounted in 10% lactoglycerol for microscopic studies and photomicrography. The fungus was examined using a Nikon ECLIPSE 80i compound microscope and photographed by a Canon 450D digital camera fitted to the microscope. Measurements were made by TAROSOFT IMAGE FRAME WORK and images used for figures were made with ADOBE PHOTOSHOP CS3 EXTENDED 10.0 (Adobe Systems Inc., US). Isolation was carried out from single ascospores following the method described by Chomnunti et al²¹ and germinating single spores were transferred directly to malt extract agar (MEA) plates and grown at 15–18 °C. Characters of colonies were observed and measured after 1–2 weeks.

Herbarium specimens were obtained on loan from the Research Institute of Resource Insects (RIRI), Kunming, China.

The type material is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and New Zealand Fungal and Plant Disease Collection (PDD), New Zealand. Cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) and Centraalbureau Voor Schimmelcultures (CBS).

DNA extraction and PCR reaction

Genomic DNA was extracted from mycelium grown on MEA for 1–2 weeks at 15–18 °C using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux) following the manufacturer's protocol. The amplifi-

cation of rDNA regions of the small subunit rDNA (SSU), large subunit (LSU) and translation elongation factor 1-alpha gene (TEF1α) was carried out by using NS1 and NS4²², LROR and LR5²³, and EF1-983F and EF1-2218R²⁴ primer pairs, respectively. The amplification procedure was performed in a 25 µl reaction volume containing 1 µl DNA, 1 µl of primer 1 and primer 2, 12.5 µl of 2× Master Mix and 9.5 µl of ddH₂O. Amplification conditions were setup for initial denaturation of 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 50 s at 55 °C and 1 min at 72 °C, and a final extension period of 10 min at 72 °C. The PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The purification and sequencing of PCR products were sent to Shanghai Majorbio Biopharm Technology Co., Ltd, China.

Sequence alignment and phylogenetic analysis

The newly generated sequences were compared with other sequences in GenBank by nucleotide megablast searches. Multiple sequence alignment was carried out in BIOEDIT²⁵ and CLUSTAL X²⁶. Maximum-parsimony (MP) analysis was processed using PAUP v. 4.0b10²⁷ to obtain the most parsimonious tree. Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with max-trees set to 1000 and other default parameters as implemented in PAUP. The setup detail of the above phylogenetic analysis programs were followed as described by Kishino and Hasegawa²⁸. Maximum trees were viewed in TREEVIEW²⁹.

RESULTS AND DISCUSSION

DNA sequencing—SSU, LSU, and TEF rDNA phylogenies

The combined gene analysis of the SSU, LSU, and TEF dataset comprised 55 taxa including our new stain of *Keissleriella dactylidis* (MFLUCC 13–0751) with *Halojulella avicenniae* as the outgroup taxon (BCC 18422). This analysis comprised 2517 characters, of which 1767 were constant, 542 variable characters were parsimony informative and 208 variable characters are parsimony-uninformative. Six equally parsimonious trees were generated and the first of the most parsimonious tree was selected (Fig. 1). Bootstrap support values of MP (equal to or above 50% based on 1000 replicates) are shown on the upper branches. The Kishino-Hasegawa test shows length = 2161 steps with CI = 0.460, RI = 0.645, RC = 0.296, and HI = 0.540.

Phylogeny

Our strain *Keissleriella dactylidis* (MFLUCC 13-0751) groups with strains of *Keissleriella* in the fam-

ily Lentitheciaceae, but are separated from other species in the genus with relatively high bootstrap support (65%, Fig. 1) and form a robust clade sister to *Keissleriella linearis*.

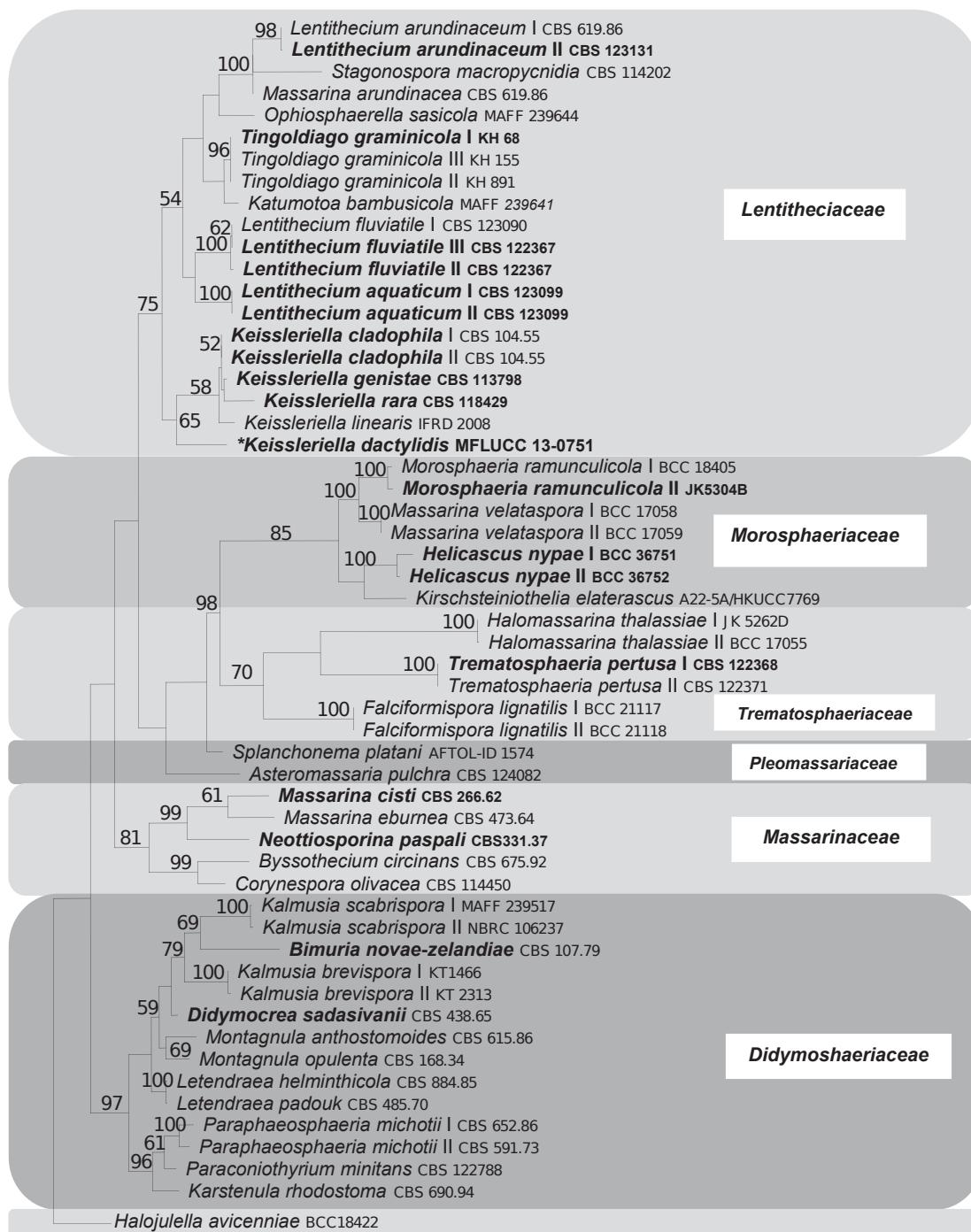


Fig. 1 Gene tree constructed using MP method based on a combined dataset of SSU, LSU, and TEF genes sequence data. *Halojulella avicenniae* is the outgroup taxon. Bootstrap support values > 50% are shown above the branch. All type strains are in bold and the original isolate numbers are noted after the species names.

Table 1 Taxa used in the phylogenetic analysis and their corresponding GenBank Accession numbers.

Species	Culture Accession No.	GenBank Accession No.		
		LSU	SSU	TEF1
<i>Asteromassaria pulchra</i> [†]	CBS 124082	GU301800	GU296137	GU349066
<i>Bimuria novae zelandiae</i>	CBS 107.79	AY016356	AY016338	DQ471087
<i>Byssothecium cincinans</i> [†]	CBS 675.92	AY016357	AY016339	GU349061
<i>Corynespora olivacea</i> [†]	CBS 114450	GU301809	—	GU349014
<i>Didymocrea sadasivani</i>	CBS 438.65	DQ384103	DQ384066	—
<i>Falciformispora lignatilis</i> [†]	BCC 21117	GU371826	GU371834	GU371819
<i>Falciformispora lignatilis</i> [†]	BCC 21118	GU371827	GU371835	GU371820
<i>H. avicenniae</i> [†]	BCC 18422	GU371823	GU371831	GU371816
<i>Halomassarina thalassiae</i> [†]	JK 5262D	GU301816	—	GU349011
<i>Halomassarina thalassiae</i> [†]	BCC 17055	GQ925850	GQ925843	—
<i>Helicascus nypae</i>	BCC 36751	GU479788	GU479754	GU479854
<i>Helicascus nypae</i>	BCC 36752	GU479789	GU479755	GU479855
<i>Kalmusia brevispora</i>	KT1466	AB524600	AB524459	AB539112
<i>Kalmusia brevispora</i> [†]	KT 2313	AB524601	AB524460	AB539113
<i>Kalmusia scabrispora</i> [†]	MAFF 239517	AB524593	AB524452	AB539106
<i>Kalmusia scabrispora</i> [†]	NBRC 106237	AB524594	AB524453	AB539107
<i>Karstenula rhodostoma</i> [†]	CBS 690.94	GU301821	GU296154	GU349067
<i>Katumotoa bambusicola</i> [†]	MAFF 239641	AB524595	AB524454	AB539108
<i>Keissleriella cladophila</i>	CBS 104.55	GU205221	GU205241	—
<i>Keissleriella cladophila</i>	CBS 104.55	GU301822	GU296155	GU349043
<i>Keissleriella dactyliidis</i> [‡]	MFUCC13-0751	KP197668	KP197666	KP197669
<i>Keissleriella genistae</i>	CBS 113798	GU205222	GU205242	—
<i>Keissleriella rara</i>	CBS 118429	GU479791	GU479757	—
<i>Keissleriella linearis</i> [†]	IFRD 2008	FJ795435	—	—
<i>Kirschsteiniothelia elaterascus</i> [†]	A22-5A/HKUCC7769	AY787934	AF053727	—
<i>Lentithecium aquaticum</i>	CBS 123099	FJ795434	FJ795477	—
<i>Lentithecium aquaticum</i>	CBS 123099	GU301823	GU296156	GU349068
<i>Lentithecium arundinaceum</i> [†]	CBS 619.86	GU301824	GU296157	—
<i>Lentithecium arundinaceum</i>	CBS 123131	GU456320	GU456298	GU456281
<i>Lentithecium fluviatile</i> [†]	CBS 123090	FJ795450	FJ795492	—
<i>Lentithecium fluviatile</i> [†]	CBS 122367	FJ795451	FJ795493	—
<i>Lentithecium fluviatile</i> [†]	CBS 122367	GU301825	GU296158	GU349074
<i>Letendraea helminthicola</i> [†]	CBS 884.85	AY016362	AY016345	—
<i>Letendraea padouk</i> [†]	CBS 485.70	AY849951	GU296162	—
<i>Massaria platani</i> [†]	AFTOL-ID 1574	DQ678065	—	—
<i>Massaria arundinacea</i> [†]	CBS 619.86	DQ813509	DQ813513	—
<i>Massaria cisti</i>	CBS 266.62	FJ795447	FJ795490	—
<i>Massaria eburnea</i> [†]	CBS 473.64	GU301840	GU296170	GU349040
<i>Montagnula anthostomoides</i> [†]	CBS 615.86	GU205223	GU205246	—
<i>Montagnula opulenta</i> [†]	CBS 168.34	DQ678086	AF164370	—
<i>Morosphaeria ramunculicola</i> [†]	BCC 18405	GQ925854	GQ925839	—
<i>Morosphaeria ramunculicola</i>	JK5304B	GU479794	GU479760	—
<i>Morosphaeria velataspora</i> [†]	BCC 17058	GQ925851	GQ925840	—
<i>Morosphaeria velataspora</i> [†]	BCC 17059	GQ925852	GQ925841	—
<i>Neottiosporina paspali</i>	CBS 331.37	EU754172	EU754073	GU349079
<i>Ophiosphaerella sasicola</i> [†]	MAFF 239644	AB524599	AB524458	AB539111
<i>Paraconiothyrium minitans</i> [†]	CBS 122788	EU754173	EU754074	GU349083
<i>Paraphaeosphaeria michotii</i> [†]	CBS 652.86	GU456325	GU456304	GU456266
<i>Paraphaeosphaeria michotii</i> [†]	CBS 591.73	GU456326	GU456305	GU456267
<i>Stagonospora macropycnidia</i> [†]	CBS 114202	GU301873	GU296198	GU349026
<i>Tingoldiago graminicola</i>	KH 68	AB521743	AB521726	—
<i>Tingoldiago graminicola</i> [†]	KT 891	AB521744	AB521727	—
<i>Tingoldiago graminicola</i> [†]	KH 155	AB521745	AB521728	—
<i>Trematosphaeria pertusa</i>	CBS 122368	FJ201990	FJ201991	GU456276
<i>Trematosphaeria pertusa</i> [†]	CBS 122371	GU301876	GU348999	GU349085

[†] ex-type sequences.[‡] newly generated sequences.

Taxonomy

Keissleriella dactylidis Singtripop, Camporesi, Hyde, sp. nov.

Index Fungorum number: IF 551020, *Faces of fungi number:* FoF 00567, Fig. 2.

Etymology: The species epithet refers to host genus “*Dactylis*”.

Holotype: MFLU 14–0107.

Saprobic on a dead stem of *Dactylis glomerata*. **Sexual morph:** Ascomata 142–192 µm high × 138–175 µm diam. ($\bar{x} = 161 \times 177$ µm, $n = 5$), immersed in the host tissue, globose to subglobose, dark grey to black, rounded, dimidiate, erumpent through the covering layers of the host tissue, ostiole central. Ostiole 53–74 µm high × 55–79 µm diam. ($\bar{x} = 60 \times 69$ µm, $n = 5$), papillate, dark brown, smooth, containing brown to dark brown setae of periphyses; 47–72 µm long. Peridium 14–48 µm wide, comprising two layers, outer layer heavily pigmented, comprising brown to dark brown cells of *Textura angularis*, inner layer composed of hyaline thin-walled cells of *Textura angularis*. Hamathecium comprising 100–119 µm high, filamentous, occasionally branched, septate, hyaline, pseudoparaphyses. Ascii 58–83 µm × 11–13 µm ($\bar{x} = 68 \times 12$ µm, $n = 10$), 8-spored, bitunicate, fissitunicate, broadly cylindrical, short-pedicellate, apically rounded, smooth-walled, with an ocular chamber. Ascospores 15–20 × 5–6 µm ($\bar{x} = 19 \times 5$ µm, $n = 20$), 2–4-overlapping seriate, broadly ellipsoid to slightly fusiform, somewhat curved, ends usually rounded, hyaline, 4–5-septate, not or only slightly constricted at the septa, surrounding by a thin gelatinous sheath. **Asexual morph:** undetermined.

Colonies in culture: Ascospores germinating on MEA or PDA within 36–48 h. Colonies growing on MEA or PDA, reaching 2.5 cm diam. after 20 days at 18–20 °C, white aerial mycelium on surface and reverse of culture whitish to pale yellow.

Material examined: ITALY, Province of Trento, Marilleva, on a dead stem of *Dactylis glomerata* (Poaceae), 5 August 2012, Erio Camporesi, IT620 (MFLU 14–0107, holotype); *ibid.*, (PDD, isotype); ex-type living culture, MFLUCC 13–0751, CBS.

Keissleriella is characterized by ascomata with papilla filled with black setae, hyaline and 1-septate to multi-septate ascospores^{6,15,16}. Nine species of *Keissleriella* including our new species have been described from the host family Poaceae (Table 2). *Keissleriella dactylidis* differs from other species of *Keissleriella*, based mainly on morphological charac-

ters of ascospores, but is most similar to *K. culmifida* (P. Karst.) S.K. Bose and *K. taminensis* (H. Wegelin) S.K. Bose. Although *K. dactylidis* and *K. taminensis* have hyaline ascospores with 4–5 septa and are surrounded by a mucilaginous sheath³¹, *K. dactylidis* differs from *K. taminensis* in having the ellipsoid to cylindrical ascospores, longer periphyses and smaller ascomata. *Keissleriella culmifida* differs from *K. dactylidis* which has 3-septate, fusiform ascospores and shorter periphyses³¹.

Phylogenetic analysis (Fig. 1) of combined SSU, LSU, and TEF gene data showed that *Keissleriella dactylidis* forms a separate clade within the genus *Keissleriella* sister to *K. linearis* E. Müll. ex Dennis with relatively high bootstrap support (65%). *Keissleriella linearis* differs from *K. dactylidis* in having 1–3-septate ascospores lacking a mucilaginous sheath and larger ascomata³².

Lentithecium lineare (≡ *Keissleriella linearis* E. Müll., Dennis) was introduced by Zhang et al¹² as a new combination in *Lentithecium* as it grouped with *Lentithecium* sp. However, Zhang et al¹² had not included any *Keissleriella* strains in their study. In our study *Lentithecium lineare* groups with *Keissleriella* species (Fig. 1) and is treated as *Keissleriella linearis* (Dennis, 1964) (Fig. 3).

Keissleriella linearis E. Müll. ex Dennis, Kew Bull. 19(1): 120 (1964).

≡ *Lentithecium lineare* (E. Müll. ex Dennis) Hyde et al, Fungal Diversity 38: 236 (2009).

Saprobic on dead stems of *Phragmites australis*. **Sexual morph:** Ascomata 598–752 µm high × 371–491 µm diam. ($\bar{x} = 650 \times 438$ µm, $n = 5$), immersed or erumpent on the host tissue, globose to subglobose, dark grey to black, rounded, dimidiate, erumpent through the covering layers of the host tissue, ostiole centre. Ostiole 91–143 µm high × 73–117 µm diam. ($\bar{x} = 118 \times 98$ µm, $n = 4$), papillate, pale brown to brown, smooth, containing brown to dark brown setae of periphyses; 39–52 µm long. Peridium 35–94 µm wide, comprising brown to dark brown cells of *Textura angularis*. Hamathecium comprising 122–134 µm high, filamentous, occasionally branched, septate, hyaline, pseudoparaphyses. Ascii 117–129 × 16–19 µm ($\bar{x} = 123 \times 17$ µm, $n = 5$), 8-spored, bitunicate, broadly cylindrical, smooth-walled, with an ocular chamber. Ascospores 36–39 × 9–10 µm ($\bar{x} = 37 \times 9$ µm, $n = 10$), 2–4-overlapping seriate, broadly ellipsoid to slightly fusiform, somewhat curved, ends usually rounded, hyaline, 1–3-septate, not or only slightly constricted at the septa, without surrounding by a thin gelatinous sheath. **Asexual morph:** undetermined.

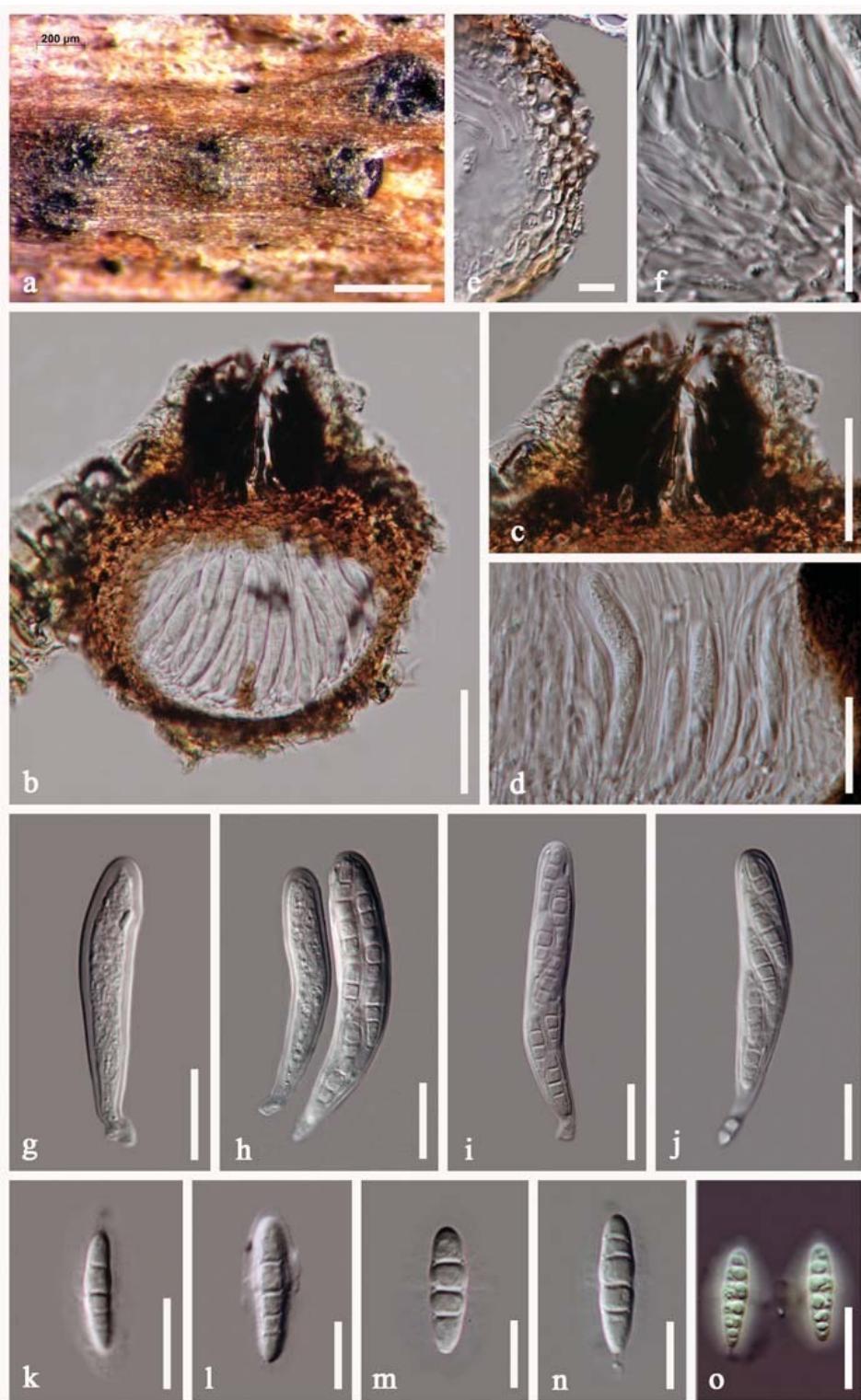


Fig. 2 *Keissleriella dactylidis*: (a) appearance of ascomata on host; (b) vertical section through ascoma; (c) periphyses in ostiole; (d) asci with pseudoparaphyses; (e) section of peridium; (f) pseudoparaphyses; (g–j) immature and mature asci; (k–n) mature ascospores; (o) ascospore in Indian ink. Scale bars: a = 400 µm; b–d, f = 50 µm; e, k–n = 10 µm; g–j, o = 20 µm.



Fig. 2 (Cont.) *Keissleriella dactylidis* (from culture of holotype): (p) germinated ascospore; (q) character of colony on MEA from above; (r) character of colony on PDA medium from below.

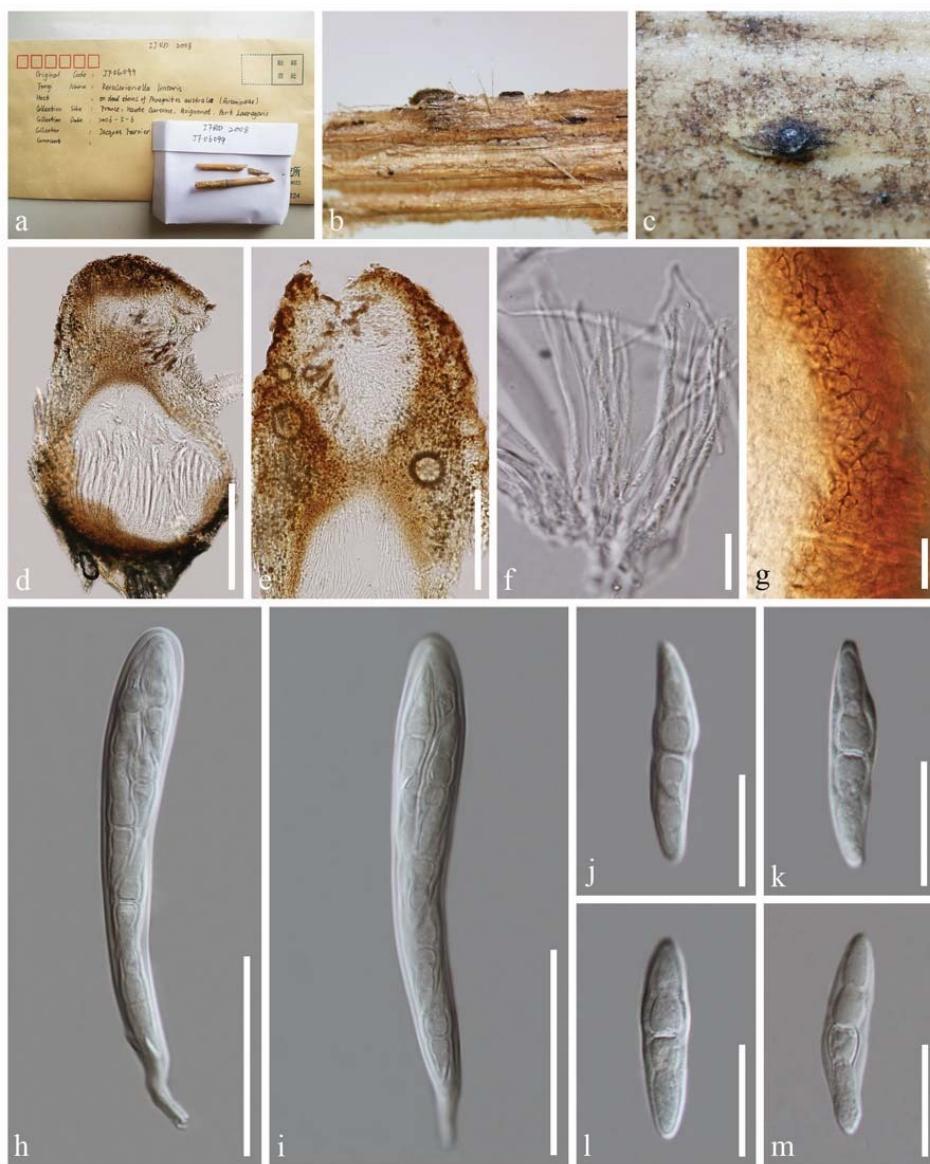


Fig. 3 *Keissleriella linearis*: (a) material label; (b,c) appearance of ascomata on host; (d) vertical section through ascocarp; (e) periphyses in ostiole; (f) pseudoparaphyses; (g) section of peridium; (h,i) mature asci; (j–m) ascospore. Scale bars: d = 200 µm; e = 100 µm; h, i = 50 µm; f, g, j–m = 20 µm.

Material examined: FRANCE, Haute Garonne, Avignonet, Port Laurgaris, on a dead stem of *Phragmites australis* (Gramineae), 6 May 2006, Jacques Fournier, IFRD 2008.

Notes: We examined this type of *Keissleriella linearis* which has characters typical of the genus (Fig. 3). The setae were not illustrated in Zhang et al¹².

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Table 2 Synopsis of the characteristics among *Keissleriella dactylidis* and other species of genus *Keissleriella* reported from Poaceae.

Fungus name	Host	Ascomata	Setae in papilla (periphyses)	Ascospore	Ref.
<i>K. dactylidis</i>	<i>Dactylis glomerata</i>	142–192 × 138–175 µm, globose to subglobose, immersed	47–72 µm long, dark brown	15–20 × 5–6 µm, ellipsoid to cylindrical, hyaline, 4–5-septate, surrounded by a mucilaginous sheath	This study
<i>K. caudata</i> (E. Müll.) Corbaz	<i>Triticum</i> sp.	80–230 µm diameter, flask-shaped, immersed to erumpent	24–40 µm long, light brown	10.5–13 × 4–4.5 µm, fusiform to elliptical, hyaline, 1 septate	30
<i>K. culmifida</i> (P Karst.) S.K. Bose	<i>Agrostis</i> sp., <i>Calamagrostis</i> <i>arundinacea</i> , <i>Leymus arenarius</i> , <i>Nardus stricta</i> , <i>Phleum pratense</i> , <i>Triticum</i> spp., <i>Poa compressa</i> , <i>Poa normalis</i> , <i>Poa pratensis</i>	140–250 × 115–200 µm, globose or pear-shaped, immersed	20–40 × 3–4 µm, dark brown	10–25 × 4.5–6 µm, fusiform, hyaline, 3-septate, surrounded by a mucilaginous sheath	31
<i>K. gloeospora</i> (Berk., Curr.) S.K. Bose	Unidentified grass	200–300 µm diameter, globose or pear-shaped, immersed	60–90 µm long, dark brown	23–33 × 6–8 µm, fusiform to ellipsoid, hyaline, 4–6-septate	31
<i>K. linearis</i> E. Müll. ex Dennis	<i>Phragmites</i> <i>australis</i>	584–762 × 369–495 µm, globose to subglobose, immersed or erumpent	34–48 µm long, brown to dark brown	32–42 × 8–10 µm, ellipsoid to fusiform, hyaline, 1–3-septate	32
<i>K. poagena</i> Crous, Quaedvlieg	<i>Poa</i> sp.	250 µm diameter, erumpent to superficial	60 µm long, dark brown	20–25 × 4–5 µm, fusiform to ellipsoid, hyaline, 3-septate, surrounded by a mucilaginous sheath	33
<i>K. taminensis</i> (H. Wegelin) S.K. Bose	<i>Agropyron repens</i> , <i>Bromus inermis</i> , <i>Festuca</i> spp., <i>Poa nemoralis</i>	180–300 × 150–200 µm, globose or pear-shaped, immersed	20–30 × 3.5–5.5 µm, dark brown	18–23 × 4.5–5 µm, fusiform, hyaline, 4–5-septate, surrounded by a mucilaginous sheath	31

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