Phytotaxa 236 (1): 040–052
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.236.1.3

Phylogeny and morphology of *Premilcurensis gen. nov.* (*Pleosporales*) from stems of *Senecio* in Italy

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Abstract

Premilcurensis senecionis gen. et and *sp. nov.* was collected on stems of *Senecio* sp. from Forlì-Cesena Province, Italy and is introduced in *Phaeosphaeriaceae* with the support of a unique combination of morphological characters and combined gene phylogenetic analyses. *Premilcurensis* is distinguished from other genera in *Phaeosphaeriaceae* in having fusiform to cylindrical, hyaline to yellow-green ascospores with 3–5 septa, and mucilaginous, wing-like appendages at the central septum. Phylogenies analyses show *Premilcurensis* as a distinct lineage from other genera in the family *Phaeosphaeriaceae* with high bootstrap support. A comprehensive description and micrographs of *P. senecionis* is provided and the new genus is compared with morphologically similar taxa.

Key words: *Dothideomycetes*, new genus, phylogeny, *Phaeosphaeriaceae*

Introduction

The class *Dothideomycetes* was introduced by Eriksson & Winka (1997) under the subdivision *Pezizomycotina* (Kirk *et al*. 2008), and is the largest class in the Phylum Ascomycota (Kirk *et al*. 2008, Hyde *et al*. 2013). Recent studies on this class have incorporated multigene phylogeny, and the classification of *Dothideomycetes* has been updated accordingly (Schoch *et al*. 2009, Nelsen *et al*. 2011, Zhang *et al*. 2012, Hyde *et al*. 2013). Hyde *et al*. (2013) included 22 orders, 105 families and 249 genera in the class *Dothideomycetes* based on morphology and multigene phylogeny, while Wijayawardene *et al*. (2014) included 23 orders 110 families and 1261 genera.

Pleosporales is the largest order in the class *Dothideomycetes* comprising one fourth of all the described species of *Dothideomycetes* (Kirk *et al*. 2008) and species in this order have various lifestyles, such as epiphytes, endophytes or parasites of living plant leaves or stems (Wang *et al*. 2005, Sánchez Márquez *et al*. 2007, Lawrey *et al*. 2012), hyperparasites on fungi or insects, or are lichenized (Schatz 1984, Barr 1987, Zhang *et al*. 2012), or saprobes of dead plant stems, leaves or bark (Shoemaker 1984, Shoemaker & Babcock 1989, Schoch *et al*. 2006, Zhang *et al*. 2009b, 2012, De Gruyter *et al*. 2010, Hyde *et al*. 2013, Quaedvlieg *et al*. 2013). Twenty families have been accepted in the order *Pleosporales* based on molecular data (Boehm *et al*. 2009a, b, Mugambi & Huhndorf 2009, Schoch *et al*. 2009, Shearer *et al*. 2009, Suetrong *et al*. 2009, Tanaka *et al*. 2009, Zhang *et al*. 2009a, b), but lately based on morphological and molecular analyses Hyde *et al*. (2013) placed 41 families under *Pleosporales*.

 Accepted by Jian-Kui Liu: 17 Oct. 2015; published: 25 Nov. 2015

The family *Phaeosphaeriaceae*, introduced by Barr (1979) with 15 genera, was given a modern morphological and phylogenetic account by Phookamsak *et al*. (2014). Currently, *Phaeosphaeriaceae* includes about 300 species in 35 genera (18 sexual and 17 asexual genera) and it is an important family in the order *Pleosporales* (Kirk *et al*. 2008, Zhang *et al*. 2009b, Hyde *et al*. 2013, Wijayawardene *et al*. 2014). This family is characterized by immersed, erumpent or superficial ascomata with short papillae, bitunicate asci and septate, hyaline, yellowish or brown ascospores (Barr 1987, 1992a, b, Zhang *et al*. 2012; Hyde *et al*. 2013). Many species within *Phaeosphaeriaceae*, especially the asexual taxa, are important plant pathogens that infect major crops (Shoemaker & Babcock 1989, Carson 2005, Stukenbrock *et al*. 2006, Zhang *et al*. 2009b, Quaedvlieg *et al*. 2013, Hyde *et al*. 2014, Phookamsak *et al*. 2014).

In this study we introduce a new monotypic genus in the family *Phaeosphaeriaceae* based on both morphology and phylogeny. It was found as a saprobe on *Senecio* sp. in Italy.

Materials and methods

Sample collection and morphological study

Collections were made in Italy and morphological structures were examined using a Carl Zeiss GmbH (AxioCam ERC 5 S) stereo microscope. Ascomata were rehydrated in water and sectioned by hand. Lactoglycerol was used routinely in preparation of semipermanent slide and 5% KOH (Potassium hydroxide) was used routinely in the rehydration of specimens. Microscopic structures were mounted in water for observation under a Nikon ECLIPSE80i compound microscope. Photographs were made with a Cannon 550D digital camera processed with the microscope. Microscopic structures were measured using Tarosoft® Image Framework program v.0.9.0.7.

Isolation

Single spores were isolated onto potato dextrose agar (PDA) following the method described in Chomnunti *et al*. (2014). Germinated spores were transferred to fresh PDA media and incubated at 16°C. Colonies were observed after 4 weeks. The specimens were deposited in Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand and Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS) China. The living cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC). Duplicated cultures are deposited in Centraalbureau voor Schimmelcultures, Netherlands (CBS). Facesoffungi numbers and Index Fungorum numbers were obtained as detailed in Jayasiri *et al*. (2015) and Index Fungorum (2015).

DNA extraction, PCR amplification and sequencing

Cultures were grown on PDA at 16°C for 2 weeks and the mycelium used for DNA extraction using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer. Phylogenetic analyses were conducted using partial sequences of three genes, the internal transcribed spacers (5.8S, ITS), small subunit rDNA (18S, SSU) and large subunit (28S, LSU). Polymerase chain reaction (PCR) was carried out with the following protocol: the final volume of the PCR reaction was 25 µl and contained 12.5 µl of 2× Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µm dNTP mixture each) (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCL pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, stabilizer and enhancer, 1 µl of each primer, 1 µl of genomic DNA extract (White *et al.* 1990). The amplified PCR fragments were sent to a commercial sequencing provider (Beijing Bai Mai Hui Kang Biological Engineering Technology Co., P.R. China). The nucleotide sequence data are deposited in GenBank.

Molecular phylogenetic analysis

DNA sequence fragments were blasted to investigate the closest taxa in the GenBank database. Newly generated DNA sequences were analyzed together with species in the related families *Coniothyriaceae, Cucurbitariaceae*, *Didymellaceae*, *Dothidotthiaceae, Leptosphaeriaceae*, *Phaeosphaeriaceae*, *Pleosporaceae* and rooted with *Halojulella avicenniae* (*Halojulellaceae*) (Table 1). Each gene sequence dataset was aligned with MAFFT v. 7 (Katoh & Standley 2013) and manually improved with BioEdit v.7.2.5 (Hall 2004). LSU, ITS and SSU sequence dataset were first analyzed separately and then the individual datasets were concatenated into a combined dataset and prepared in BioEdit v.7.2.5 (Hall 2004). Phylogenetic analyses were performed using three methods i.e. Maximum parsimony (MP), Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian posterior probabilities (BYPP).

TABLE 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers (ex-type strains are in bold, the new taxon is indicated with an asterisk).

Taxa	Culture Accessions	GenBank Accessions		
		LSU	ITS	SSU
***Alternaria alternata* (Fr.) Keissl.**	**CBS 916.96**	**-**	**KF465761**	**KC584507**
Amarenomyces ammophilae (Lasch) O.E. Erikss.	CBS 114595	GU301859	KF766146	GU296185
Ampelomyces quisqualis Ces.	CBS 129.79	JX681064	HQ108038	EU754029
Bipolaris maydis (Y. Nisik. & C. Miyake) Shoemaker	CBS 134.39	AY544645	DQ491489	AY544737
***Bipolaris oryzae* Sawada**	**MFLUCC 10-0694**	**JX256381**	**JX256413**	**-**
Coniothyrium carteri (Gruyter & Boerema) Verkley & Gruyter	CBS 105.91	KF251712	KF251209	GQ387533
Coniothyrium carteri	CBS 101633	KF251713	KF251210	GQ387532
Coniothyrium glycines (R.B. Stewart) Verkley & Gruyter	CBS 124141	GQ387598	KF251211	GQ387537
***Cucurbitaria berberidis* Fuckel**	**MFLUCC 11-0384**	**KC506793**	**-**	**KC506797**
Cucurbitaria berberidis	CBS 394.84	JX681088	-	GQ387544
Dematiopleospora mariae* Wanasinghe *et al.	**MFLUCC 13-0612**	**KJ749653**	**-**	**KJ749652**
***Didymella exigua* (Niessl) Sacc.**	**CBS 183.55**	**JX681089**	**GU237794**	**GU296147**
Dothidotthia aspera (Ellis & Everh.) M.E. Barr	CPC 12933	EU673276	-	EU673228
***Dothidotthia symphoricarpi* (Rehm) Höhn.**	**CPC 12929**	**EU673273**	**-**	**EU673224**
***Entodesmium rude* Riess**	**CBS 650.86**	**GU301812**	**-**	**-**
Halojulella avicenniae (Borse) Suetrong, K.D. Hyde & E.B.G. Jones	BCC 20173	GU371822	-	GU371830
***Leptosphaeria doliolum* (Pers.) Ces. & De Not.**	**CBS 505.75**	**GQ387576**	**JF740205**	**GQ387515**
Leptosphaeria errabunda (Desm.) Gruyter *et al*.	CBS 617.75	JF740289	JF740216	-
***Loratospora aestuarii* Kohlm. & Volkm.-Kohlm.**	**JK 5535B**	**GU301838**	**-**	**GU296168**
Muriphaeosphaeria galatellae* Phukhamsakda *et al.	**MFLUCC 14-0614**	**KT438329**	**KT438333**	**KT438331**
***Neostagonospora caricis* Quaedvl *et al*.**	**CBS 135092**	**KF251667**	**KF251163**	**-**
Neostagonospora elegiae Quaedvl *et al.*	CBS 135101	KF251668	KF251164	-
Neosetophoma clematidis N.N. Wijayawardene *et al.*	MFLUCC 14-0746	KP684153	KP744450	KP684154
Neosetophoma italica W.J. Li *et al.*	MFLUCC 13-0388	KP711361	KP711356	KP711366
Nodulosphaeria modesta (Rabenh.) Munk ex L. Holm.	MFLUCC 13-0728	KP744493	-	KP753957
Nodulosphaeria modesta	MFLUCC 11-0461	KM434285	KM434275	KM434294
Ophiobolus cirsii (P. Karst.) Sacc.	MFLUCC 13-0218	KM014662	KM014664	KM014663
***Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White**	**CBS 111.79**	**KF251676**	**KF251172**	**EU754191**
Parastagonospora caricis Quaedvl *et al.*	S615	KF251680	KF251176	-
Parastagonospora nodorum* (Berk.) Quaedvl *et al.	**CBS 110109**	**KF251681**	**KF251177**	**-**
Parastagonospora poae Quaedvl *et al.*	CBS 135089	KF251682	KF251178	-
Phaeosphaeria eustoma (Fuckel) L. Holm	CBS 573.86	DQ678063	-	DQ678011
***Phaeosphaeria oryzae* I. Miyake**	**CBS 110110**	**KF251689**	**KF251186**	**GQ387530**
***Phaeosphaeria papayae* (Speg.) Quaedvl., Verkley & Crous**	**S528**	**KF251690**	**KF251187**	**-**
*Phaeosphaeria nigrans (*Roberge ex Desm.) L. Holm	CBS 307.79	KF251687	KF251184	-
Phaeosphaeria phragmiticola Leuchtm.	CBS 459.84	KF251691	KF251188	-
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	CBS 117487	KF251692	KF251189	-

...Continued on next page

TABLE 1. (Continued)

Taxa	Culture Accessions	GenBank Accessions		
		LSU	ITS	SSU
Phaeosphaeria vagans (Niessl) O.E. Erikss.	CBS 604.86	KF251696	KF251193	-
Phaeosphaeriopsis glaucopunctata* (Grev.) M.P.S. Câmara *et al.	**CBS 653.86**	**KF251702**	**KF251199**	**GQ387531**
Phaeosphaeriopsis glaucopunctata	MFLUCC 13-0265	KJ522477	KJ522473	KJ522481
***Phaeosphaeriopsis triseptata* K.M. Thambugala & K.D. Hyde**	**MFLUCC 13-0271**	**KJ522479**	**KJ522475**	**KJ522484**
Phaeosphaeriopsis triseptata	MFLUCC 13-0347	KJ522480	KJ522476	KJ522483
Phoma herbarum Westend.	CBS 615.75	KF251715	FJ427022	EU754087
***Pleospora herbarum* (Pers.) Rabenh.**	**CBS 191.86**	**GU238160**	**KC584239**	**GU238232**
Premilcurensis senecionis* Tibpromma *et al.*	**MFLUCC 13-0575**	**KT728366**	**KT728365**	-
***Pyrenochaeta nobilis* De Not.**	**CBS 407.76**	**EU754206**	**EU930011**	**EU754107**
Sclerostagonospora phragmiticola* Quaedvl *et al.	**CBS 338.86**	**KF251733**	**KF251230**	-
Scolicosporium minkeviciusii Treigienë	MFLUCC 12-0089	KF366382	KF366383	-
***Setomelanomma holmii* M. Morelet**	**CBS 110217**	**GQ387633**	-	**GQ387572**
Setophoma chromolaena Quaedvl *et al.*	CBS 135105	KF251747	KF251244	-
Setophoma sacchari (Bitanc.) Gruyter *et al.*	MFLUCC 12-0241	KJ476147	KJ476145	KJ476149
Setophoma terrestris (H.N. Hansen) Gruyter *et al.*	CPC 18417	KF251739	KF251236	-
Setophoma terrestris	**CBS 335.87**	**KF251750**	**KF251247**	**GQ387528**
Sulcispora pleurospora* I.C. Senanayake *et al.	**MFLUCC 14-0995**	**KP271444**	**KP271443**	**KP271445**
Vrystaatia aloeicola* Quaedvl *et al.	**CBS 135107**	**KF251781**	**KF251278**	-
Wojnowicia lonicerae N.N. Wijayawardene *et al.*	MFLUCC 13-0737	KP684151	KP744471	KP684152
Wojnowicia dactylidicola N.N. Wijayawardene *et al.*	MFLUCC 13-0738	KP684147	KP744469	KP684148
Xenoseptoria neosaccardoi* Quaedvlieg *et al.	**CBS 128665**	**KF251784**	**KF251281**	-

ABBREVIATIONS: **BCC**: BIOTEC Culture Collection, Bangkok, Thailand; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CPC**: Collection of Pedro Crous housed at CBS, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; Culture and specimen abbreviations; **JK**: J. Kohlmeyer.

Maximum parsimony (MP) analyses were performed in PAUP v. 4.0b10 (Swofford 2003) by using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. The reconstruction of ML analysis was performed using raxmlGUI v.0.9b2 with the model "GTRGAMMA" (Stamatakis 2006; Silvestro & Michalak 2010). A Bayesian analysis was conducted with MrBayes v. 3.1.2 (GTR+I+G model) (Huelsenbeck & Ronqvist 2001) to valuate Posterior probabilities (PP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Phylogenetic trees were sampled every 100th generation in 445,000 generations from the running of six simultaneous Markov chains. The first 890 trees which contained the burn-in phase of the analyses were discarded. The remaining 3,560 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree (Liu *et al*. 2011). The setup details of the phylogenetic analysis follow Liu *et al*. (2012), Phookamsak *et al*. (2014), and Hyde *et al*. (2013) Phylogenetic trees were figured in TreeView (Page 1996).

Results

Phylogenetic analysis

The dataset comprised 59 taxa including the new strain of *Premilcurensis senecionis*, with *Halojulella avicenniae* as

the out group taxon. The combined LSU, ITS and SSU sequence data were analyzed using maximum parsimony (MP) programs with descriptive tree statistics (TL= 2292, CI=0.4250, RI=0.638, RC=0.271 and HI=0.575), the reconstruction of ML analysis with the ML final score is -14608.608832 and Posterior probabilities. The phylogeny showed *Premilcurensis senecionis* grouped with *Ophiobolus cirsii, Chaetosphaeronema hispidulum, Muriphaeosphaeria galatellae, Sulcispora pleospora,* and *Loratospora aestuarii* in clade G (Fig. 1) and *Premilcurensis senecionis* as a distinct lineage with relatively high bootstrap support (76% in ML, 78% in MP and 0.99 in PP). We also compared *P. senecionis* with taxa of clade F and H to confirm its distinctiveness with well supported clade; Clade F comprises species of *Nodulosphaeria* (86% MP/ 81% ML/ 0.99 PP), *Entodesmium* and *Dematiopleospora* (95% MP/ 97% ML/ 1.00 PP). Clade H comprises *Phaeosphaeriopsis* (100% MP/ 100% ML/ 1.00 PP). The other clade in the tree agrees with those in Phookamsak *et al*. (2014) and we do not discuss it in detail.

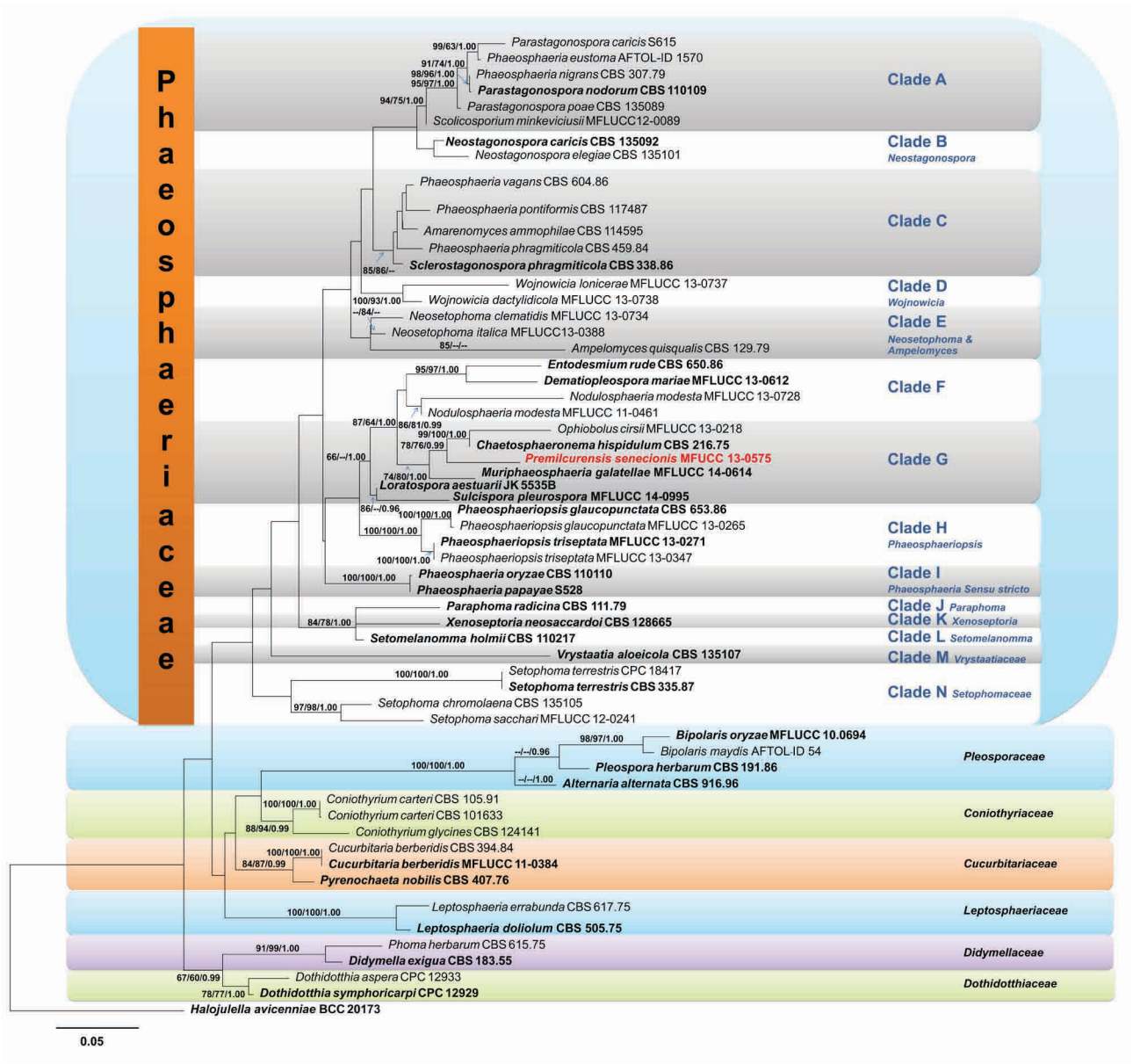

FIGURE 1. Phylogenetic tree obtained from RAxML analysis based on combined LSU, ITS and SSU sequence data. Bootstrap support values for maximum parsimony (MP, left), maximum likelihood (ML, middle) higher than 60% and Bayesian posterior probabilities (BYPP, right) greater than 0.95 are provided at the nodes. The tree is rooted with *Halojulella avicenniae* (BCC 20173). Newly generated sequences and ex-types strains are in bold.

Taxonomy

Premilcurensis Tibpromma, Camporesi & K.D. Hyde, ***gen. nov***.

Index Fungorum number: IF551293, *Facesoffunginumber*: FoF: 00699
Etymology: refers to the name of the municipality (Premilcuore) in Italy where the fungus was collected.

Saprobic on plant stems. Sexual morph: *Ascostromata* superficial, semi-immersed at the base, globose to subglobose, solitary or in small groups, dark brown to black, ostiole central, with minute papilla. *Peridium* thick-walled, multi-layered, surface heavily pigmented, with obviously light brown cells. *Hamathecium* comprising numerous, long cylindrical, cellular, branched, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate to sessile, with a well developed ocular chamber. *Ascospores* overlapping uni- to bi-seriate, initially hyaline, becoming yellow-green to yellow-brown at maturity, cylindrical to elongated fusiform, with rounded ends, usually 3–5-septate, with wing-like appendages. Asexual morph: Undetermined.

Type species: *Premilcurensis senecionis* Tibpromma, Camporesi & K.D. Hyde

Premilcurensis senecionis Tibpromma, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 2

Index Fungorum number: IF551294, *Facesoffungi number*: FoF: 00700
Etymology: refers to the host genus from which the fungus was isolated.
Holotype: MFLU 14-0678

Saprobic on decaying stem of *Senecio* sp. (*Asteraceae*). Sexual morph: *Ascostromata* 160–215 µm high, 230–265 µm diam. ($\overline{x}$ = 195 × 242 µm, n = 5), superficial, semi-immersed at the base, globose to subglobose, solitary or in small groups, glabrous, dark brown to black, scattered on the host surface, base not easily removed from the substrate, ostiole central, papilla minute, thin-walled, shiny, like charcoal. *Peridium* 20–28 µm wide, thick, externally multi-layered, heavily pigmented at the surface, composed of thick-walled light brown cells of *textura angularis*, inwardly cells thin-walled. *Hamathecium* comprising numerous, 1.1–1.5 µm wide, filiform, cellular, branched, hyaline pseudoparaphyses. *Asci* 58–65 × 7–10 µm ($\overline{x}$ = 60 × 10 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, rounded at the apex, with well-developed ocular chamber. *Ascospores* 18–27 × 4–5 µm ($\overline{x}$ = 22 × 4 µm, n = 50), overlapping uni- to bi-seriate, initially hyaline, becoming yellow-green to yellow-brown at maturity, fusiform-cylindrical to elongate fusiform, with rounded ends, usually 3–5-septate, straight to slightly curved, constricted at the central septum, with wing-like appendages, smooth-walled, with small guttules in each cell. Asexual morph: Undetermined.

Culture characteristics: on PDA reaching 2 cm diam. after 2 weeks at 16°C, later with dense mycelium, with circular, rough margin, flattened; upper surface white at first, pale yellow after 4 weeks; reverse cinnamon; hyphae septate, branched, hyaline, thin-walled.

Material examined: ITALY, Province of Forlì-Cesena [FC], Premilcuore, on dead stems of *Senecio* sp., 26 May 2013, Erio Camporesi, IT1310 (MFLU 14-0678, **holotype);** ex-type living culture, MFLUCC 13-0575. (MFLU 15-0186, HKAS 88739, **isotypes**); *Ibid*. (MFLU15-0752bis, MFLU 15-0753tris, MFLU 15-0754tetrakis, MFLU 15-0755pentakis, MFLU 15-0756hexakis, MFLU 15-0757heptakis, MFLU 15-0758enneakis, **paratypes**).

Notes: Barr (1979) introduced the family *Phaeosphaeriaceae* typified by *Phaeosphaeria* with *P. oryzae* (Miyake 1909) as the type species. Fifteen genera were included in this family, although Kirk *et al.* (2008) later accepted 35 genera. Its characteristics are similar to taxa in the family *Leptosphaeriaceae* and the two are often confused (Hyde *et al.* 2013, Phookamsak *et al*. 2014, Ariyawansa *et al*. 2015). The taxa within *Phaeosphaeriaceae* are often associated with monocotyledons, have a peridium of pseudoparenchymatous cells and asexual morph of pycnidial coelomycetes, mostly identified as phoma-like or stagonospora-like (Hyde *et al.* 2013). *Premilcurensis* is similar to some species of *Nodulosphaeria* and *Leptosphaeria* (Table 2), but based on phylogeny *Nodulosphaeria* and *Leptosphaeria* are not congeneric with our taxon (Fig. 1).

TABLE 2. Synopsis of characters of *Premilcurensis senecionis* with similar species in *Phaeosphaeriaceae*.

Name	Ascostromata/ Ascomata	Peridium	Hamathecium	Asci	Ascospores	Material examined	Reference
Premilcurensis senecionis	Superficial, semi–immersed at the base, 230–265 µm wide, 160–215 µm high	*Textura angularis*, thick, multi-layered, thick-walled cells at external layers, towards inner thin-walled cells, heavily pigmented at the surface, light brown cells	1.1–1.5 µm wide, filiform, septa	Cylindrical to cylindric-clavate, 57–65 × 7–9 µm, short stalked	Fusiform to cylindrical, 18–26 × 4–5 µm, usually 3–5-septate, hyaline to yellow-green or yellow-brown, with wing-like appendages	Italy: on dead stems of *Senecio* sp.	This study
Leptosphaeria							
L. ogilviensis (Berk. & Broome) Ces. & De Not.	Immersed, depressed-globose, 250–300 µm wide, 150–200 µm high	*Textura angularis*, 20–25 µm thick, 4 or 5 layers of brown rectangular cells	2–2.5 µm wide, septate, without guttules	Cylindrical, 65–75(–95) × 8–10 µm, short stalked	Cylindrical, 26–35(–38) × 4.5–5.5 µm, 5-septate, yellowish brown, with firm brown septa	Canada: *Solidago* sp.	Shoemaker 1984
L. nanae Shoemaker	Immersed, subepidermal, globose with a flattened base, 300–400 µm wide, 200–250 µm high	*Textura angularis*, 35–40 µm thick, 5 or 6 layers of polygonal brown cells	2–3 µm wide, with thin septa, without guttules	Cylindrical, 100– 120 × 12–15 µm, short stalked	Narrowly fusiform, 36–50 × 6–7 µm, 5(–7)-septate, yellowish brown, with guttules, with globose terminal appendages	Switzerland: Graubünden, Bergün, Palpuognasee.	Shoemaker 1984
L. planiuscula (Riess) Ces. & De Not.	Immersed, subepidermal, globose to laterally compressed with a flattened base, 350–600 µm wide, 300–400 µm high	*Textura angularis*, 40–50 µm thick, 6–8 layers of polygonal brown cells	2–3 µm wide, with thin septa, without guttules	Clavate, 120–135 × 20–24 µm, short stalked	Narrowly fusiform, 45–55(–75) × 6–9 µm, 5-septate, yellowish brown, with guttules, with globose terminal appendages	Canada: *Solidago* sp.	Shoemaker 1984

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TABLE 2. (Continued)

Name	Ascostromata/ Ascomata	Peridium	Hamathecium	Asci	Ascospores	Material examined	Reference
Nodulosphaeria							
N. modesta (Rabenh.) Munk ex L. Holm	Subepidermal becoming exposed, globose with a flattened base, 150–250 µm wide, 130–200 µm high	*Textura angularis*, 7–10 µm thick, 1 or 2 layers of oblong dark brown cells	2–3 µm wide, without guttules, with slime coating	Cylindrical, 60–70 × 13–17 µm, short stalked	Narrowly fusiform, 29–40 × 5–6 µm, 4-septate, yellowish brown, without sheath, with small globose appendages	Canada: *Ranunculus acris*	Shoemaker 1984
N. aquilana (D. Sacc.) L. Holm	Deeply immersed, globose with a flattened base, 275–350 µm wide, 200–250 µm high	*Textura angularis*, 20–25 µm thick, 3–5 layers of oblong brown cells	2–3 µm wide, with thin septa, without guttules, without slime coating	Cylindrical, 70–100 × 13–15 µm, short stalked	Narrowly fusiform, 36–45 × 4.5–6 µm, 5 septate, yellowish brown, with globose appendages	Canada: *Hieracium pratense*	Shoemaker 1984
N. succisae Munk	Subepidermal, ellipsoidal to globose with a flattened base, 250–300 µm wide, 250–300 µm high	*Textura angularis*, 20–25 µm thick, 4 or 5 layers of oblong dark brown cells	2–3 µm wide, with thin septa, without guttules, with slime coating	Cylindrical, 100– 115 × 17–20 µm, short stalked	Narrowly fusiform, 36–46 × 5.5–7.5 µm, (4–)5(–6) septate, yellowish brown, with guttules, without sheath, with small globose caplike appendages	Belgium: *Scabiosa succinea*	Shoemaker 1984

FIGURE 2. ***Premilcurensis senecionis*** **(**MFLU 14-0678, **holotype)**. **a, b, c** Ascostromata on host substrate. **d** Section of ascoma. **e** Section through peridium. **f** Relatively wide cellular pseudoparaphyses. **g–i** Asci. **j** Ocular chamber of ascus. **k–m** Ascospores with wing-like equatorial appendage. Scale bars: a = 200 µm, b–d = 100 µm, e, g, h, i = 20 µm, f = 1 µm, j = 5 µm, k–m = 10 µm.

Discussion

Members of family *Phaeosphaeriaceae* are saprobic, pathogenic or hyperparasitic, typically growing on herbaceous stems or monocotyledonous leaves, culms, or flowers, but also on woody substrates. Ascomata are immersed, erumpent or superficial, globose or conical, short papillate, small to medium-sized, asci are bitunicate, and ascospores are hyaline, yellowish or brown, narrowly or widely obovoid, aseptate or septate (Barr 1987,1992a, b, Zhang *et al*. 2012, Hyde *et al*. 2013, Phookamsak *et al*. 2014).

According to our phylogenetic analysis of LSU, ITS and SSU sequence data (Fig. 1), *Premilcurensis senecionis* groups in a clade with *Chaetosphaeronema*, *Ophiobolus*, *Sulcispora, Loratospora* and *Muriphaeosphaeria* with high bootstrap support, but these related genera have significantly different morphologies as *Premilcurensis senecionis* clearly differs in having ascostromata which are superficial to semi-immersed at the base, an ostiole with a minute papilla, fissitunicate, cylindrical to cylindric-clavate, short pedicellate to sessile asci with a well developed ocular chamber, and fusiform, cylindrical ascospores, which are 3–5-septate, with distinct guttules in each cell and wing-like appendages in the centre. *Premilcurensis senecionis* is compared with morphologically similar species of *Leptosphaeria* and *Nodulosphaeria* (Table 2.) and moreover we compared *Premilcurensis* with clade F*, Entodesmium* and *Dematiopleospora* (95% MP/ 97% ML/ 1.00 PP) which differ morphologically. In *Entodesmium* spores are elliptical to filiform, 4-septate to multi-septate, furnished in some with globose-appendages at each end (Riess, 1854) and *Dematiopleospora* spores are muriform with 5−9-transverse septa and 3−6 vertical septa (Wanasinghe *et al*. 2014). With clade H, *Phaeosphaeriopsis* (100% MP/ 100% ML/ 1.00 PP), which are distinct from *Premilcurensis* with distinctiveness with well supported as the former have immersed, subepidermal ascomata, usually erumpent at maturity through flaps of the epidermis. Their ascospores are cylindrical, brown, 4–5-septate, with the first septum submedian and often constricted (Câmara *et al*. 2001, Thambugala *et al*. 2014). Based on the morphological characteristics and phylogenetic analyses, we introduce *Premilcurensis senecionis* as a new genus and species.

Acknowledgements

The authors extend their sincere appreciations to the Deanship of Scientific Research at King Saud University for funding this Prolific Research group (PRG-1436-09). Kevin D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. Jun-Bo Yang would like to thank Plant Germplasm and Genomics Center in Germplasm Bank of Wild Species, Kunming Institute of Botany for help with molecular work and Mae Fah Luang University for a grants "Taxonomy and Phylogeny of selected families of *Dothideomycetes* (Grant number: 56101020032)" and "Biodiversity, phylogeny and role of fungal endophytes of *Pandanaceae*" (Grant number: 582010200112) for supporting this study. Erio Camporesi thanks Gigi Stagioni for his invaluable field assistance. Saowaluck Tibpromma thanks the Mushroom Research Foundation (MRF), Chiang Rai, Thailand for the support of her study. Rungtiwa Phookamsak thanks the Royal Golden Jubilee Ph. D. Program (PHD/0090/2551) under Thailand Research Fund. Samantha C. Karunarathna, Jian-Kui Liu, Sajeewa Maharachchikumbura, Dhanushka Wanasinghe, Hiran A. Ariyawansa and Jingzu Sun are thanked for their valuable suggestions and help.

REFERENCES

Ariyawansa, H.A., Phukhamsakda, C., Thambugala, K.M., Bulgakov, T.S., Wanasinghe, D.N., Perera, R.H., Mapook, A., Camporesi, E., Kang, J.C., Jones, E.B.G. Bahkali, A.H., Jayasiri, S.C., Hyde, K.D., Liu, Z.Y. & Bhat, D.J. (2015) Revision and phylogeny of Leptosphaeriaceae. *Fungal Diversity*. http://dx.doi.org/10.1007/s13225-015-0349-2

Barr, M.E. (1979) A classification of Loculoascomycetes. *Mycologia* 71: 935–957. http://dx.doi.org/10.2307/3759283

Barr, M.E. (1987) New taxa and combinations in the Loculoascomycetes. *Mycotaxon* 29: 501–505.

Barr, M.E. (1992a) Additions to and notes on the Phaeosphaeriaceae (Pleosporales, Loculoascomycetes). *Mycotaxon* 43: 371–400.

Barr, M.E. (1992b) Notes on the Lophiostomataceae (Pleosporales). *Mycotaxon* 45: 191–221.

Boehm, E., Mugambi, G., Miller, A., Huhndorf, S., Marincowitz, S. & Spatafora, J. (2009a) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15.
http://dx.doi.org/10.3114/sim.2009.64.01

Boehm, E.W.A., Mugambi, G., Miller, A.N., Huhndorf, S., Marincowitz, S., Schoch, C.L. & Spatafora, J.W. (2009b) A molecular phylogenetic reappraisal of the Hysteriaceae, Mytilinidiaceae and Gloniaceae (Pleosporomycetidae, Dothideomycetes) with keys to world species. *Studies in Mycology* 64: 49–83.
http://dx.doi.org/10.3114/sim.2009.64.03

Câmara, M.P.S., Palm, M.E., van Berkum, P. & Stewart, E.L. (2001) Systematics of *Paraphaeosphaeria*, a molecular and morphological approach. *Mycological Research* 105: 41–50.

Carson, M.L. (2005) Yield loss potential of Phaeosphaeria leaf spot of maize caused by *Phaeosphaeria maydis* in the United States. *Plant Disease* 89: 986–988.
http://dx.doi.org/10.1094/PD-89-0986

Chomnunti, P., Hongsanan, S., Hudson, B.A., Tian, Q., Perŝoh, D., Dhami, M.K., Alias, A.S., Xu, J., Liu, X., Stadler, M. & Hyde, K.D. (2014) The sooty moulds. *Fungal Diversity* 66: 1–36.
http://dx.doi.org/10.1007/s13225-014-0278-5

De Gruyter, J., Woudenberg, J.H., Aveskampmm, M.M., Verkley, G.J., Groenewald, J.Z. & Crous, P.W. (2010) Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*. *Mycologia* 102: 1066–1081.
http://dx.doi.org/10.3852/09-240

Eriksson, O.E. & Winka, K. (1997) Supraordinal taxa of Ascomycota. *Myconet* 1 (1): 1–16.

Hall, T. (2004) Bioedit version 6.0.7.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Hyde, K.D., Jones, E.B.G., Liu, J.K, Ariyawansa, H.A., Boehm, E., Boonmee, S., Braun, U., CHomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I.C., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot, A., Bahkali, A., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A, Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yacharoen, S., Yan, J.Y. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.
http://dx.doi.org/10.1007/s13225-013-0263-4

Hyde, K.D., Nilsson, R.H., Alias, S.A., Ariyawansa, H.A., Blair, J.E., Cai, L., De Cock, A., Dissanayake, A.J., Glockling, S.L., Goonasekara, I.D., Gorczak, M., Hahn, M., Jayawardena, R.S., Van, K.J., Laurence, M.H., Lévesque, C.A., Li, X., Liu, J.K., Maharachchikumbura, S.S.N., Manamgoda, D.S., Martin, F.N., McKenzie, E.H.C., Mctaggart, A.R., Mortimer, P.E., Nair, P.V.R., Pawłowska, J., Rintoul, T.L., Shivas, R.G., Spies, C.F.J., Summerell, B.A., Taylor, P.W.J., Terhem, R.B., Udayanga, D., Vaghefi, N., Walther, G., Wilk, M., Wrzosek, M., Xu, J.C., Yan, J.Y. & Zhou, N. (2014) One stop shop: backbones trees for important phytopathogenic genera: I. *Fungal Diversity* 67 (1): 21–125.
http://dx.doi.org/10.1007/s13225-014-0298-1

Index Fungorum (2015) http://www.indexfungorum.org/Names/Names.asp

Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai, Y.C., Abd-Elsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E.B.G., Bahkali, A.H., Karunarathna, S.C., Liu, J.K., Luangsa-ard, J.J., Lumbsch, H.T., Maharachchikumbura, S.S.N., McKenzie, E.H.C., Moncalvo, J.M., Ghobad-Nejhad, M., Nilsson, H., Pang, K., Pereira, O.L., Phillips, A.J.L., Raspé, O., Rollins, A.W., Romero, A.I., Etayo, J., Selçuk, F., Stephenson, S.L., Suetrong, S., Taylor, J.E., Tsui, C.K.M., Vizzini, A., Abdel-Wahab, M.A., Wen, T.C., Boonmee, S., Dai, D.Q., Daranagama, D.A., Dissanayake, A.J., Ekanayaka, A.H., Fryar, S.C., Hongsanan, S., Jayawardena, R.S., Li, W.J., Perera, R.H., Phookamsak, R., De Silva, N.I., Thambugala, K.M., Tian, Q., Wijayawardene, N.N., Zhao, R.L., Zhao, Q., Kang, J.C. & Promputtha, I. (2015) The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity*.
http://dx.doi.org/10.1007/s13225-015-0351-8

Katoh, K. & Standley, K. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution. Available from: http://mbe.oxfordjournals.org/ (accessed 22 June 2013)

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's Dictionary of the Fungi*, 10th edn. CABI, Wallingford, UK.

Lawrey, J.D., Diederich, P., Nelsen, M.P., Freebury, C., Broeck, D.V.D., Sikaroodi, M. & Ertz, D. (2012) Phylogenetic placement of lichenicolous *Phoma* species in the Phaeosphaeriaceae (Pleosporales, Dothideomycetes). *Fungal Diversity* 55: 195–213.
http://dx.doi.org/10.1007/s13225-012-0166-9.

Liu, J.K., Phookamsak, R., Jones, E.B.G., Zhang, Y., Ko-Ko, T.W., Hu, H.L., Boonmee, S., Doilom, M., Chukeatirote, E., Bahkali, A.H., Wang, Y. & Hyde, K.D. (2011) *Astrosphaeriella* is polyphyletic, with species in *Fissuroma gen. nov.*, and *Neoastrosphaeriella gen. nov. Fungal Diversity* 51: 135–154.
http://dx.doi.org/10.1007/s13225-011-0142-9

Liu, J.K., Phookamsak, R., Doilom, M., Wiki, S., Mei, L.Y., Ariyawansa, H.A., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, D.J., Romero, A.I., Xhuang, W.Y., Monkai, J., Jones, E.B.G., Chukeatirote, E., Ko-Ko, T.W., Zhoa, Y.C., Wang, Y. & Hyde, K.D. (2012) Towards a natural classification of Botryosphaeriales. *Fungal Diversity* 57: 149–210.
http://dx.doi.org/10.1007/s13225-012-0207-4

Mugambi, G.K & Huhndorf, S.M. (2009) Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae recircumscribed (Plesporomycetidae, Dothideomycetes, Ascomycota). *Studies in Mycology* 64: 103–121.
http://dx.doi.org/ 10.3114/sim.2009.64.05

Nelsen, M.P., Lücking, R., Mbatchou, J.S., Andrew, C.J., Spielmann, A.A. & Lumbsch, H.T. (2011) New insights into relationships of lichen-forming Dothideomycetes. *Fungal Diversity* 51: 155–162.
http://dx.doi.org/10.1007/s13225-011-0144-7

Page, R.D.M. (1996) Tree View: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357–358.

Phookamsak, R., Liu, J.K., McKenzie, E.H.C., Manamgoda, D.S., Ariyawansa, H.A., Thambugala, K.M., Dai, D.Q., Camporesi, E., Chukeatirote, E., Wijayawardene, N.N., Bahkali, A.H., Mortimer, P.E., Xu, J.C. & Hyde, K.D. (2014) Revision of Phaeosphaeriaceae. *Fungal Diversity* 68: 159–238.
http://dx.doi.org/10.1007/s13225-014-0308-3

Quaedvlieg, W., Verkley, G.J.M., Shin, H.D., Barretto, R.W., Alfenas, A.C., Swart, W.J., Groenewald, J.Z. & Crous, P.W. (2013) Sizing up *Septoria*. *Studies in Mycology* 75: 307–390.
http://dx.doi.org/10.3114/sim0017

Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.

Reiss, M.L.C. (1854) Neue Kernpilze. *Hedwigia* 1 (6): 23–28.

Sánchez Márquez, S., Bills, G.F. & Zabalgogeazcoa, I. (2007) The endophytic mycobiota of the grass *Dactylis glomerata*. *Fungal Diversity* 27: 171–195.

Schatz, S. (1984) The life history, developmental morphology, and taxonomy of *Lautitia danica gen. nov.*, comb. nov. *Canadian Journal of Botany* 62: 28–32.

Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W. & Crous P.W. (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041–1052.
http://dx.doi.org/10.3852/mycologia.98.6.1041

Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W.A., Burgess, T.I., De Gruyter, J., De Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S., Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B.G., Kohlmeyer, J., Kruys, A., Li, Y.M., Lücking, R., Lumbsch, H.T., Marvanová, L., Mbatchou, J.S., Mcvay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L., Nelsen, M.P., Nelson, P., Owensby, C.A., Phillips, A.J.L., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V., Raja, H.A., Rivas Plata, E., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H.C., Yonezawa, H., Zhang, Y. & Spatafora, J.W. (2009) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15.
http://dx.doi.org/10.3114/sim.2009.64.01

Shearer, C.A., Raja, H.A., Miller, A.N., Nelson, P., Tanaka, K., Hirayama, K., Marvanova, L., Hyde, K.D. & Zhang, Y. (2009) The molecular phylogeny of freshwater Dothideomycetes. *Studies in Mycology* 64: 145–153.
http://dx.doi.org/10.3114/sim.2009.64.08

Shoemaker, R.A. (1984) Canadian and some extralimital *Nodulosphaeria* and *Entodesmium* species. *Canadian Journal of Botany* 62: 2730–2753.
http://dx.doi.org/10.1139/b84-367

Shoemaker, R.A. & Babcock, C.E. (1989) *Phaeosphaeria*. *Canadian Journal of Botany* 67: 1500–1599.
http://dx.doi.org/10.1139/b89-199

Silvestro, D. & Michalak, I. (2010) raxmlGUI: a graphical front-end for RAxML Program and documentation. Available from: http://www.sourceforge.com (accessed 22 October 2015)

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Stukenbrock, E.H., Banke, S. & Mcdonald, B.A. (2006) Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*.

Molecular Ecology 15: 2895–2904.
http://dx.doi.org/10.1111/j.1365-294X.2006.02986.x

Suetrong, S., Schoch, C.L., Spatafora, J.W., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sakayaroj, J., Phongpaicht, S., Tanaka, K., Hairayama, K. & Jones, E.B.G. (2009) Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64: 155–173.
http://dx.doi.org/10.3114/sim.2009.64.09

Swofford, D.L. (2003) *PAUP: phylogenetic analysis using parsimony*, version 4.0 b10. Sinauer Associates, Sunderland.

Tanaka, K., Hirayama, K., Yonezawa, H., Hatakeyama, S., Harada, Y., Sano, T., Shirouzu, T. & Hosoya, T. (2009) Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with Tetraploa-like anamorphs. *Studies in Mycology* 64: 175–209.
http://dx.doi.org/10.3114/sim.2009.64.10

Thambugala, K.M., Camporesi, E., Ariyawansa, H.A., Phookamsak, R., Liu, Z.Y. & Hyde, K.D. (2014) Phylogeny and morphology of *Phaeosphaeriopsis triseptata* sp. nov., and *Phaeosphaeriopsis glaucopunctata*. *Phytotaxa* 176 (1): 238–250.
http://dx.doi.org/10.11646/phytotaxa.176.1.23

Wanasinghe, D.N., Jones, E.B.G., Camporesi, E., Boonmee, S., Karunarathna, S.C., Thines, M., Mortimer, P.E., Xu, J. & Hyde, K.D. (2014) *Dematiopleospora mariae* gen. sp. nov., from *Ononis spinosa* in Italy. *Cryptogamie Mycology* 35: 105–117.
http://dx.doi.org/10.7872/crym.v35.iss2.2014.105

Wang, Y., Guo, L. & Hyde, K.D. (2005) Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. *Fungal Diversity* 20: 235–260.
http://dx.doi.org/10.17207/jstc.2005.05.11.235

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, California, USA, pp. 315–322.
http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene, N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., Chomnunti, P., Dai, D.Q., D'Souza, M.J., Diederich, P., Dissanayake, A., Doilom, M., Hongsanan, S., Jones, E.B.G., Groenewald, J.Z., Jayawardena, R., Lawrey, J.D., Liu, J.K., Lücking, R., Madrid, H., Manamgoda, D.S., Muggia, L., Nelsen, M.P., Phookamsak, R., Suetrong, S., Tanaka, K., Thambugala, K.M., Wikee, S., Zhang, Y., Aptroot, A., Ariyawansa, H.A., Bahkali, A.H., Bhat, J.D., Gueidan, C., De- Hoog, G.S., Knudsen, K., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Wanasinghe, D.N., Phillips, A.J.L., Raja, H.A., Slippers, B., Shivas, R.S., Taylor, J.E., Wang, Y., Woudenberg, J.H.C., Pia, T.M., Cai, L., Jaklitsch, W.M. & Hyde, K.D. (2014) Naming and outline of Dothideomycetes. *Fungal Diversity* 69: 1– 55.
http://dx.doi.org/10.1007/s13225-014-0309-2

Zhang, Y., Fournier, J., Crous, P.W., Pointing, S.B. & Hyde, K.D. (2009a) Phylogenetic and morphological assessment of two new species of *Amniculicola* and their allies (Pleosporales). *Persoonia* 23: 48–54.
http://dx.doi.org/10.3767/003158509X472187

Zhang, Y., Schoch, C.L., Fournier, J., Crous, P.W., De Gruyter, J., Woudenbergj, H.C., Hirayama, K., Tanaka, K., Pointing, S.B., Spatafora, J.W. & Hyde, K.D. (2009b) Multi-locus phylogeny of the Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology* 64: 85–102.
http://dx.doi.org/10.3114/sim.2009.64.04

Zhang, Y., Crous, P.W., Schoch, C.L. & Hyde, K.D. (2012) Pleosporales. *Fungal Diversity* 52: 1–225.
http://dx.doi.org/10.1007/s13225-011-0117-x

Zhaxybayeva, O. & Gogarten, J.P. (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3 (1): 4.
http://dx.doi.org/10.1186/1471-2164-3-4