



Phylogeny and morphology of *Phaeosphaeriopsis triseptata* sp. nov., and *Phaeosphaeriopsis glaucopunctata*

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Abstract

A collection of the type species of *Phaeosphaeriopsis*, *P. glaucopunctata*, and a new species associated with leaf spots of *Ruscus aculeatus* were collected in Italy. Single ascospore isolates of both species were obtained and formed asexual morphs in culture. Combined ITS, LSU, SSU and RPB2 gene sequence analysis from taxa in Phaeosphaeriaceae showed that *P. glaucopunctata* and the new species cluster in Phaeosphaeriaceae, forming a separate clade from other species in the family. In this study, we designate our new collection of *P. glaucopunctata* as an epitype. The new *Phaeosphaeriopsis* species is morphologically similar to *P. glaucopunctata*, but has 3-septate ascospores. A detailed description and illustration is provided for both species and the new taxon is compared with other *Phaeosphaeriopsis* species. *Phaeosphaeriopsis musae* is excluded from *Phaeosphaeriopsis* based on morphology and multigene phylogenetic analysis and synonymized under *Phaeosphaeria musae*. In present study seven species are accepted in the genus *Phaeosphaeriopsis* and a key to species is provided.

Key words: asexual morph, Phaeosphaeriaceae, *Phaeosphaeriopsis*, phylogeny, taxonomy

Introduction

Phaeosphaeriaceae is one of the largest families in the order Pleosporales, Dothideomycetes, comprising 29 sexual and asexual genera (Hyde *et al.* 2013) and more than 350 species (Kirk *et al.* 2008). Species of Phaeosphaeriaceae are found as pathogens on living plants and as saprobes on decaying organic matter mostly including monocotyledons and especially Poaceae (Hyde *et al.* 2013, Quaedvlieg *et al.* 2013).

Phaeosphaeriopsis was introduced by Câmara *et al.* (2003) based on morphology and 18S rDNA sequence analysis in order to accommodate four species of *Paraphaeosphaeria* (*P. agavensis* (A.W. Ramaley, M.E. Palm & M.E. Barr) M.P.S. Câmara, M.E. Palm & A.W. Ramaley, *P. glaucopunctata* (Grev.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley, *P. nolinae* (A.W. Ramaley) M.P.S. Câmara, M.E. Palm & A.W. Ramaley and *P. obtusispora* (Speg.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley) and one new species (*P. amblyspora* A.W. Ramaley). *Phaeosphaeriopsis* was characterized by immersed, subepidermal, globose to subglobose to pyriform ascomata, cylindrical asci and septate, punctate or verrucose ascospores with *Coniothyrium*-like or *Phaeostagonospora* asexual states. Farr *et al.* (2006) examined *Sphaeria phacidiomorpha* and transferred to *Phaeosphaeriopsis phacidiomorpha*. This species is characterized by bitunicate, obpyriform asci, filiform, branched pseudoparaphyses, four- to five-septate, hyaline ascospores that become distinctly constricted at one of the central septa and yellow at maturity and *Microsphaeropsis*-like asexual morphs of *Phaeosphaeriopsis* (Farr *et al.* 2006). *Phaeosphaeriopsis phacidiomorpha* may not belong in *Phaeosphaeriopsis* because its asci and ascospores differ

from other species of *Phaeosphaeriopsis* and its *Microsphaeropsis*-like asexual morphs. However, recollection, epitypification and molecular data are needed in order to clarify its phylogenetic placement.

Arzanlou & Crous (2006) introduced *Phaeosphaeriopsis musae* associated with leaf spots of *Musa* sp. *Phaeosphaeriopsis musae* is characterized by fusoid-ellipsoidal, 3-septate, brown, verruculose, guttulate, ascospores with obtuse ends, widest in the cell above the primary septum (Arzanlou & Crous 2006). Arzanlou & Crous (2006) accommodated *P. musae* in *Phaeosphaeriopsis* based on its *Phaeoseptoria* asexual state which is similar to those accommodated in *Phaeostagonospora* (asexual morph of *P. nolinae*) and nucleotide sequence data.

In this paper we collected two species of *Phaeosphaeriopsis* from Italy associated with leaf spots of *Ruscus aculeatus*. We examined the holotype (*P. glaucopunctata*) and compared it with our species which proved to be identical and were from the same host. Hence, we designate our specimen as the epitype of *Phaeosphaeriopsis glaucopunctata*. The second species is new to science and introduced as *Phaeosphaeriopsis triseptata* in this paper.

Material and methods

Sample collection, specimen examination and isolation

The specimens were collected from leaves of *Ruscus aculeatus* (Asparagaceae) from Forlì-Cesena and Ravenna provinces, Italy between 8 November 2012 and 2 January 2013. Specimens were observed and examined under a Motic SMZ 168 stereomicroscope. Micromorphological characters of the taxon were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. India ink was added to water mounts to show the presence of gelatinous sheaths of ascospores. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Isolates were derived via single spore isolation following the method of Chomnunti *et al.* (2014). Germinating spores were transferred to potato dextrose agar (PDA) media and incubated at 25°C in the daylight. The cultural characteristics such as mycelium colour, shape, texture and growth rate were determined.

The epitype of *Phaeosphaeriopsis glaucopunctata* and the holotype of *Phaeosphaeriopsis triseptata* are deposited in MFLU and cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in ICMP, New Zealand.

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA at 25°C for 14 days. Genomic DNA from mycelia was extracted as in Udayanga *et al.* (2012). Polymerase chain reaction (PCR) was carried out using four partial gene portions in this study. NS1 and NS4 were used to amplify a region spanning the small subunit rDNA (White *et al.* 1990). LROR and LR5 primer pairs were used to amplify a segment of the large subunit rDNA (Vilgalys & Hester 1990) and internal transcribed spacers was amplified by primer pairs ITS1 and ITS4 (White *et al.* 1990). rRPB2-5f and rRPB2-7cR primer pairs were used to amplify the partial RNA polymerase second largest subunit (RBP2) (Liu *et al.*, 1999). The amplifications were performed in 25 µL of PCR mixtures containing 9.5 µL ddH₂O, 12.5 µL 2×PCR Master Mix (TIANGEN Co., China), 1 µL of DNA template, 1 µL of each primer (10 µM). The amplification conditions for SSU, LSU and ITS consisted of initial denaturation at 94°C for 4 min; followed by 35 cycles of 45 s at 94°C, 45 s at 56°C and 1 min at 72°C, and a final extension period of 10 min at 72°C. The PCR thermal cycle program for the partial RNA polymerase second largest subunit (RBP2) was followed as initially 95°C for 5 mins, followed by 40 cycle of denaturation at 95°C for 45 s, annealing at 55°C for 2 mins, elongation at 72°C for 90 seconds, and final extension at 72°C for 10 mins. The PCR products were observed on 1% agarose electrophoresis gels stained with Ethidium bromide. Purification and sequencing of PCR products were carried at using the above-mentioned PCR primer at Invitrogen Biotechnology Co., China.

Phylogenetic analysis

The generated ITS, LSU, SSU and RBP2 sequences were analyzed using the BLAST search engine of the NCBI in order to obtain a rough identification of the isolates. Sequences were aligned using Bioedit 7.1.3.0 version (Hall 1999) and Clustal X v. 1.83 (Thompson *et al.* 1997) and if necessary, manually improved. The aligned LSU, SSU, ITS and RBP2 datasets were first analysed separately and then the individual datasets were concatenated into

a combined dataset. The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Maximum likelihood analysis was performed by RAxML (Stamatakis 2010) implemented in raxmlGUI 1.3 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTRGAMMAI model of nucleotide substitution (1,000 replicates). The phylogram was visualized in Treeview (Page 1996). The sequences of novel species (*Phaeosphaeriopsis triseptata*) and other sequenced taxa in this study are deposited in GenBank. Novel species and descriptions are deposited in MycoBank.

Results and discussion

Phylogenetic analysis

The combined ITS, LSU, SSU and RBP2 sequence data set comprised 41 strains of 34 taxa in Phaeosphaeriaceae with *Didymella exigua* as the outgroup taxon. All the individual trees were similar in topology and not significantly different with combined tree. Maximum Likelihood analysis used 1000 bootstrap replicates and yielded a tree with the likelihood value of ln: -12330.90314 and the following model parameters: alpha: 0.392200 and invar: 0.645801; $\Pi(A)$: 0.251537, $\Pi(C)$: 0.218113, $\Pi(G)$: 0.270605, and $\Pi(T)$: 0.259745. The best scoring RAxML combined tree is shown in Fig. 1. Maximum Likelihood bootstrap values $\geq 50\%$ are given below or above each node.

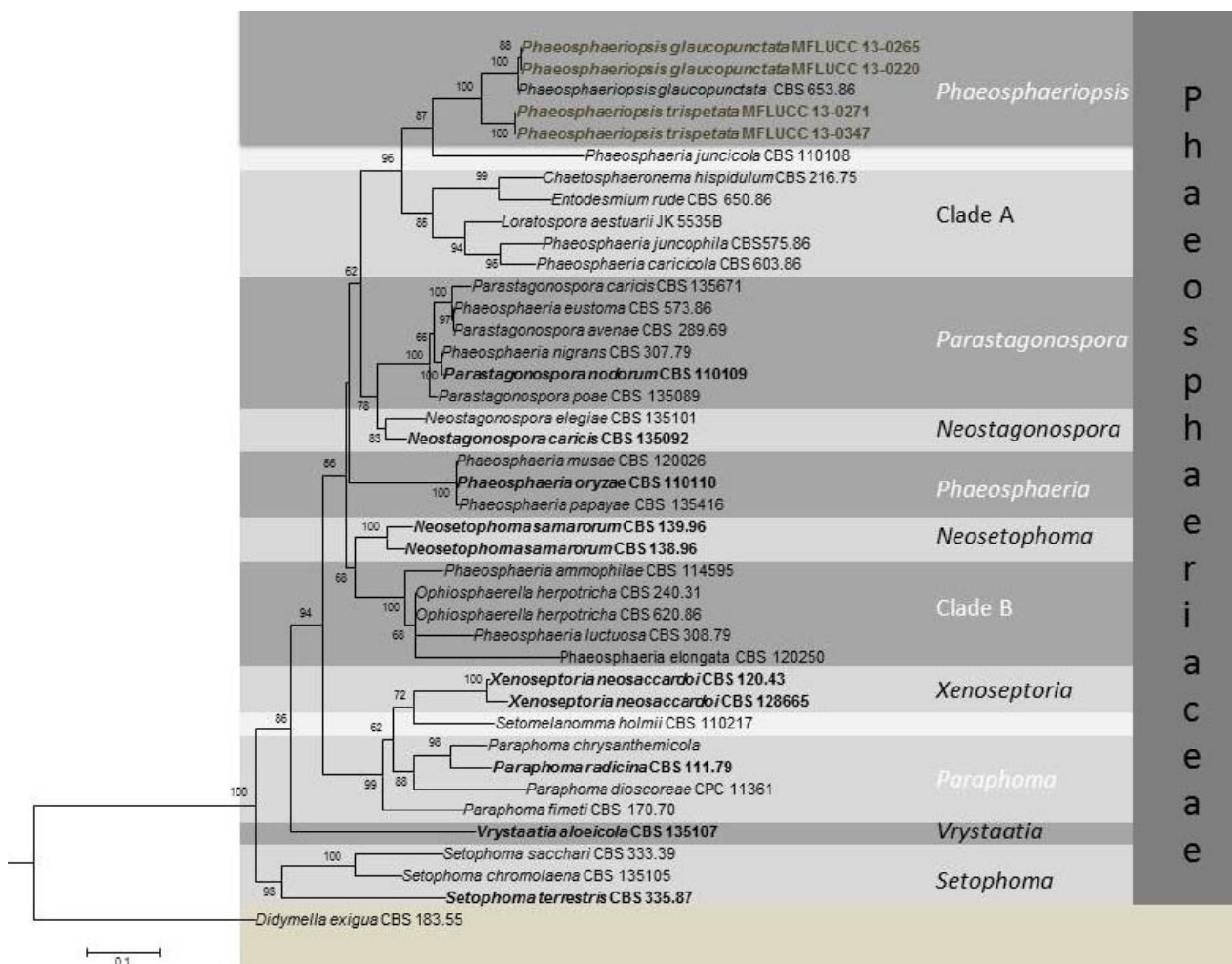


FIGURE 1. RAxML tree based on a combined dataset of LSU, SSU, ITS and RBP2 sequences. Bootstrap support values equal or greater than 50% are given above or below the nodes. The tree was rooted to *Didymella exigua* (CBS 183.55). The original isolate numbers are given after the species names. All sequences from ex-type strains are in bold.

The phylogenetic trees obtained from maximum likelihood gave similar results and are similar to previous studies on Phaeosphaeriaceae (Quaedvlieg *et al.* 2013). The genera *Neosetophoma*, *Neostagonospora*, *Paraphoma*, *Parastagonospora*, *Phaeosphaeria*, *Phaeosphaeriopsis*, *Setophoma*, *Vrystaatia* and *Xenoseptoria* clustered in Phaeosphaeriaceae and formed distinct clades with strong support representing these genera. The novel species clustered in *Phaeosphaeriopsis* clade but separated from *P. glaucopunctata* with 100% bootstrap support. There were also two unnamed clades, A and B. Clade A comprised *Phaeosphaeria caricicola*, *P. juncophila*, *Chaetosphaeronema hispidulum*, *Loratospora aestuarii* and *Entodesmium rude*. This clade was most closely related to the *Phaeosphaeriopsis* clade, but was distinctly separated. *P. juncicola* nested between *Phaeosphaeriopsis* clade and clade A, while *Setomelanomma holmii* clustered between *Xenoseptoria* and *Paraphoma* clades. Clade B comprised *Ophiosphaerella herpotricha*, *Phaeosphaeria ammophilae*, *P. elongata* and *P. luctuosa*. Clades A and B need more strains in order to resolve their phylogenetic placement in Phaeosphaeriaceae and whether they also represent distinct genera.

Phaeosphaeriopsis musae clusters with *Phaeosphaeria oryzae* and *Phaeosphaeria papaya* with 100% bootstrap support (Fig. 1). We consider this clade to be *Phaeosphaeria sensu stricto*. Furthermore, *P. musae* differs morphologically from other species of *Phaeosphaeriopsis* but is more similar to *Phaeosphaeria musae* Sawada. Therefore, we exclude *P. musae* from *Phaeosphaeriopsis* and synonymize *Phaeosphaeriopsis musae* under *Phaeosphaeria musae*.

Taxonomy

Phaeosphaeriopsis M.P.S. Câmara, M.E. Palm & A.W. Ramaley, in Câmara, Ramaley, Castlebury & Palm, Mycol. Res. 107(5): 519 (2003)

Saprobic on dead leaves. Sexual state: *Ascomata* scattered, immersed, subepidermal, usually erumpent at maturity, uniloculate or multiloculate, globose to subglobose to pyriform brown to black, sometime papillate, ostiolate, often surrounded by septate, brown hyphae extending into the host tissues. *Peridium* comprising several layers of brown to dark brown, thick-walled, cells of *textura angularis* or *textura prismatica*. *Hamathecium* comprising cellular, hyaline, filiform, septate, branched or not branched, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, pedicellate or apedicellate and apically rounded, without having a distinct ocular chamber. *Ascospores* uni-triseriate, overlapping, cylindrical to fusiform, 3–5-septate, without constriction or slightly constricted at the basal septum, sometimes apical cells often longer than others, hyaline when immature, becoming yellowish-brown when mature, echinulate, punctate or verruculose, surrounded by a thin mucilaginous sheath. Asexual state: *Conidiomata* pycnidial, pseudoparenchymatous, globose, brown to black, sometimes papillate, ostiolate. *Peridium* comprising several layers of yellowish brown to brown cells of *textura angularis*. *Conidiogenous cells* lining locule, ampulliform, holoblastic, proliferating percurrently resulting in inconspicuous annellations, hyaline, cylindrical to subcylindrical, producing conidium at the tip. *Conidia* cylindrical to oblong to ellipsoid, with rounded ends, 0–3-septate, punctate, smooth or finely verruculose, hyaline to yellow, becoming yellowish-brown at maturity.

Generic type:—*Phaeosphaeriopsis glaucopunctata* (Grev.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley [as 'glauco-punctata'], in Câmara, Ramaley, Castlebury & Palm, Mycol. Res. 107(5): 519 (2003)

Notes:—In the present study we accept seven species in *Phaeosphaeriopsis* including the new species and exclude *P. musae*. A synopsis of the characters of *Phaeosphaeriopsis* are given in Table 1. It seems unlikely that *P. phacidiomorpha* belongs in *Phaeosphaeriopsis* because of its asci and hyaline ascospores *Microsphaeropsis*-like asexual morphs, however we retain it in the genus pending fresh collections.

Key to species of *Phaeosphaeriopsis*

- | | | |
|----|---|----------------------------|
| 1. | Ascospores hyaline, becoming yellow at maturity | <i>P. phacidiomorpha</i> * |
| 1. | Ascospores pigmented, yellowish brown to brown | 2 |
| 2. | Ascospores 3-septate | <i>P. triseptata</i> |
| 2. | Ascospores more than 3-septate | 3 |

| | | |
|----|--|--------------------------|
| 3. | Ascospores mostly 4-septate | 4 |
| 3. | Ascospores 5-septate | 5 |
| 4. | Ascospores cylindrical to clavate 15.8–19 × 3.2–4.6 μm | <i>P. glaucopunctata</i> |
| 4. | Ascospores oblong-cylindric 9–24(–27) × 6.5–9 μm | <i>P. amblyspora</i> |
| 5. | Ascstromata multiloculate | <i>P. agavensis</i> |
| 5. | Ascstromata uniloculate | 6 |
| 6. | Conidia olivaceous brown, 3-septate | <i>P. nolinae</i> |
| 6. | Conidia yellowish brown, nonseptate, rarely 1-septate | <i>P. obtusispora</i> |

* this is unlikely to be a *Phaeosphaeriopsis* species as it has hyaline ascospores which never become brown and different shapes of asci and ascospores.

TABLE 1. Synopsis of *Phaeosphaeriopsis* species.

| Species | Ascomata μm | Asci μm | Ascospores μm | Septa | Host | Source references |
|------------------------------|--|---|--|-----------|--------------------------|---|
| <i>P. agavensis</i> | 270–390 × 325–430(–540) (\bar{X} = 336 × 404) | 80–120 × 9–13 (\bar{X} = 100 × 11) | 15.5–18.5(–20.5) × (2–)4–5.5(–6) (\bar{X} = 18.3 × 4.9) | 5 | <i>Agave harvardiana</i> | Câmara <i>et al.</i> 2001 as <i>Paraphaeosphaeria</i> <i>agavensis</i> |
| <i>P. amblyspora</i> | (150–)200–350 × (170–)250–385 | (116–)120–160 × 12–16 | 19.2–24(–27.2) × 6.4–8.8 | (3–)4(–5) | <i>Yucca baccata</i> | Câmara <i>et al.</i> 2003 |
| <i>P. glaucopunctata</i> | 100–170 × 90–135 (\bar{X} = 131 × 118) | 45–80 × 10–15 (\bar{X} = 59 × 12.2) | 11–19.5 × 2.5–5.5 (\bar{X} = 15.6 × 4.5) | 4 | <i>Ruscus aculeatus</i> | Câmara <i>et al.</i> 2001 as <i>Paraphaeosphaeria</i> <i>glauco-punctata</i> |
| <i>P. glaucopunctata</i> | 110.5–145 × 130–230 | (55–)60–70(–75) × 11–14(–15) (\bar{X} = 68.2 × 13.4) | 18–20(–21.5) × 4–5.5 (\bar{X} = 20 × 4.8) | 4 | <i>Ruscus aculeatus</i> | This study – syntype |
| <i>P. glaucopunctata</i> | 90–165 × 90–180 (\bar{X} = 105 × 120) | 62–85 × 7.5–10 (\bar{X} = 74 × 9) | (–14.5)17–21 × 3.2–4.6 (\bar{X} = 17.5 × 3.8) | 4 | <i>Ruscus aculeatus</i> | This study - epitype |
| <i>P. nolinae</i> | 245–350 × 230–335 (\bar{X} = 297 × 285) | 135–170 × 13.5–17 | 17–25(–34.5) × 6.5–10 (\bar{X} = 24.7 × 8.1) | 5 | <i>Nolina erumpens</i> | Câmara <i>et al.</i> 2001 as <i>Paraphaeosphaeria</i> <i>nolinae</i> |
| <i>P. obtusispora</i> | 250–350 × 200–250 | 90–110 × 11–13 | 17–22 × 5–6 | 5 | Agavaceae sp. | Câmara <i>et al.</i> 2001 as <i>Paraphaeosphaeria</i> <i>obtusispora</i> |
| ? <i>P. phacidiomorpha</i> – | – | 30 | 10 | 4–5 | <i>Phormium tenax</i> | Cesati 1878 as <i>Sphaeria</i> <i>phacidiomorpha</i> / Saccardo 1882 as <i>Didymella</i> <i>phacidiomorpha</i> |
| <i>P. triseptata</i> | 80–110 × 90–130 (\bar{X} = 92 × 110) | 56–70 × 7.5–9 (\bar{X} = 62 × 16) | 14.5–18 × 3–4 (\bar{X} = 16 × 3.7) | 3 | <i>Ruscus aculeatus</i> | This study |

Phaeosphaeriopsis glaucopunctata (Grev.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley [as 'glauco-punctata'], in Câmara *et al.*, Mycol. Res. 107(5): 519 (2003) (Figs 2–4).
Mycobank MB 373148

≡ *Cryptosphaeria glaucopunctata* Grev., Fl. Edin.: 1-478 (1824)

≡ *Leptosphaeria glaucopunctata* (Grev.) Auersw.

≡ *Paraphaeosphaeria glaucopunctata* (Grev.) Shoemaker & C.E. Babc., Can. J. Bot. 63(7): 1286 (1985)

≡ *Sphaeria glaucopunctata* (Grev.) Curr.

Description from syntype

Saprobic on leaves of *Ruscus aculeatus*, visible as orange-brown spots on host surface. Sexual state: *Ascomata* 110.5–145 μm high, 130–230 μm diam, scattered to clustered, immersed, uniloculate, globose to subglobose, brown to dark brown, pseudoparenchymatous, apapillate with central ostiole. *Peridium* 7–20 μm wide, composed of

3–4 layers of pale brown to brown, thin-walled, pseudoparenchymatous cells, arrange in a *textura angularis*. *Hamathecium* comprising 1–3 μm wide, broadly cellular, hyaline, filiform, unbranched, rarely branching, anastomosing, sparse, pseudoparaphyses which are distinctly constricted at the septa, and embedded in a mucilaginous matrix. *Asci* (55–)60–70(–75) \times 11–14(–15) μm (\bar{x} = 68.2 \times 13.4 μm , n = 20), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, sessile to short pedicellate, apically rounded, lacking a distinct ocular chamber, asci arising from the base of the ascoma. *Ascospores* 18–20(–21.5) \times 4–5.5 μm (\bar{x} = 20 \times 4.8 μm , n = 25), uni-seriate at the base to overlapping or bi to tri-seriate at the apex, oblong to cylindrical, narrowing towards the end cells, brown to dark brown, 4-septate, slightly curved and often swollen at the forth cell from the apex, verrucose, echinulate, surrounded by mucilaginous sheath. Asexual state: Not observed.

Material examined:—UK. Scotland: Edinburgh, on dead leaves of *Ruscus aculeatus*, 15 August 1824, *R.K. Greville* (E00074283!, syntype).



FIGURE 2. *Phaeosphaeriopsis glaucopunctata* (E00074283, syntype) a. Herbarium label and specimens of *Phaeosphaeriopsis glaucopunctata*. b. Ascomata on host surface. c. Section through ascoma. d. Section through peridium. e. Squash mount showing asci with pseudoparaphyses. f–j. Asci. k–o. Ascospores. Scale bars: c = 50 μm , d, e, = 20 μm , f–o = 10 μm .



FIGURE 3. *Phaeosphaeriopsis glaucopunctata* (MFLU 14-0029, epitype). a-b. Ascomata on host surface. c-d. Section through ascomata. e. Peridium. f. Pseudoparaphyses. g. Immature ascus. h-j. Mature bitunicate asci. k-m. Ascospores. Scale bars: c-d = 25 µm, e-f = 10 µm, g-j = 25 µm, k-m = 5 µm.

Description from epitype (MFLU 14-0029)

Saprobic on dead leaves of *Ruscus aculeatus*, visible as black colored spots, scattered on the entire leaf surface. Sexual state: *Ascomata* 90–165 μm high, 90–180 μm diam. (\bar{x} = 105 \times 120 μm , n = 10), scattered, immersed, uniloculate, globose to ovoid, dark brown to black, pseudoparenchymatous, apapillate, ostiolate. *Peridium* 12–32 μm (\bar{x} = 19 μm , n = 15), comprising several layers of brown to dark brown, moderately thick-walled, cells of *textura angularis*. *Hamathecium* comprising 1.5–3 μm wide, cellular, hyaline, filiform, septate, branched, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 62–85 \times 7.5–10 μm (\bar{x} = 74 \times 9 μm , n = 20) 8-spored, bitunicate, fissitunicate, cylindrical, very short-pedicellate and apically rounded, lacking a distinct ocular chamber. *Ascospores* (14.5–)17–21 \times 3.2–4.6 μm (\bar{x} = 17.5 \times 3.8 μm , n = 25), uni-biseriate, overlapping, cylindrical to fusiform, 4-septate, without constriction or slightly constricted at the basal septum, sometimes the fourth cell from the apex slightly wider, with basal and apical cells often longer than others, hyaline when immature, becoming yellowish-brown when mature, verruculose, surrounded by a thin mucilaginous sheath. Asexual state: *Conidiomata* 100–500 μm diam, pycnidial, immersed to superficial in agar medium, single, scattered, numerous, globose, brown to black, ostiolate. *Peridium* comprising 2–3 layers of yellowish brown to brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–10 \times 2–3 μm (\bar{x} = 8.5 \times 2.5 μm , n = 30), holoblastic, lining the inner cavity, ampulliform, phialidic, annellidic, with a short collarete and narrow periclinal thickening at the conidiogenous locus, hyaline, subcylindrical, producing conidium at the apex. *Conidia* 4.5–10 \times 2.1–3.5 μm (\bar{x} = 7.5 \times 2.6 μm , n = 50), cylindrical to oblong, with rounded ends, aseptate, smooth to finely verruculose, hyaline to yellow, becoming yellowish-brown at maturity, accumulating in a huge bluish-black slimy mass at the tip of ostiole.

Culture characteristics:—Ascospores germinating on PDA within 18 h and producing germ tubes from one or several septa. Colonies on PDA attaining 56 mm diam. after 14 days at 25–30°C, flat, circular, pinkish white, velvety, with entire to slightly undulate edge. After 14 to 21 days conidiomata formed on PDA.

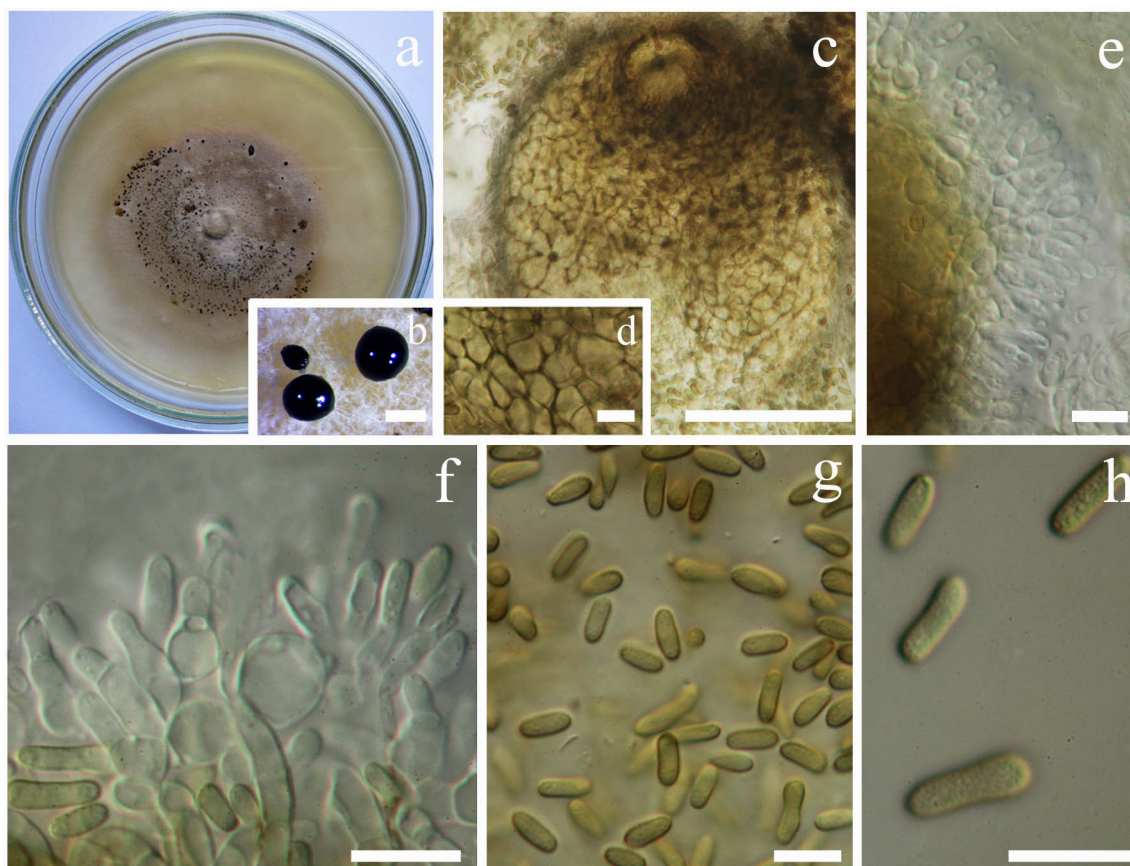


FIGURE 4. *Phaeosphaeriopsis glaucopunctata* (MFLU 14-0029, epitype). a–b. Pycnidia on PDA. c. Squash mount of pycnidia. d. Pycnidial wall. e–f. Conidiogenous cells and developing conidia. g–h. Conidia. Note the verrucose ornamentation in h. Scale bars: b = 500 μm , c = 100 μm , d–h = 10 μm .

Material examined:—ITALY. Forlì-Cesena Province: Farazzano-Forlì, on leaves of *Ruscus aculeatus* (Asparagaceae), 5 November 2012, *Erio Camporesi* (MFLU 14-0029!, **epitype designated here**); extype living culture = MFLUCC 13-0265 = ICMP 20199). ITALY. Forlì-Cesena Province, Bagno di Romagna, on leaves of *Ruscus hypoglossum* (Asparagaceae), 18 November 2012, *Erio Camporesi* (MFLU 14-0030!); living culture = MFLUCC 13-0220 = ICMP 20200).

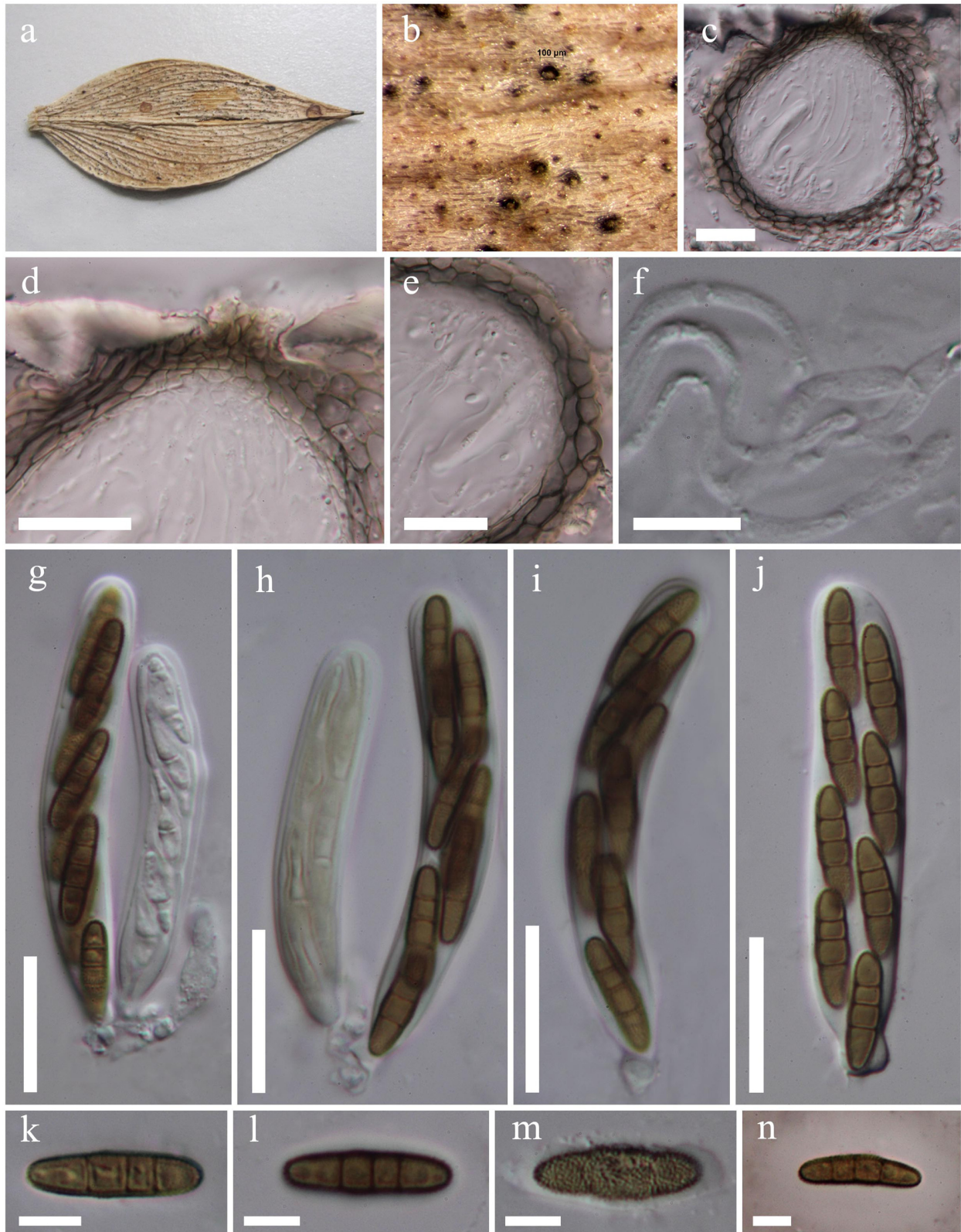


FIGURE 5. *Phaeosphaeriopsis triseptata* (holotype). a–b. Appearance of ascomata on the host surface. c. Section of an ascoma. d. Section of papilla. e. Section of Peridium. f. Pseudoparaphyses. g–j. Bitunicate asci. k–n. Released ascospores. Note the verrucose ornamentation in m. Scale bars: c = 25 µm, d–e = 20 µm, f = 10 µm, g–j = 20 µm, k–n = 5 µm.

Etymology:—The specific epithet *triseptata* is based on the 3-septate ascospores which is characterized only for this species in *Phaeosphaeriopsis*.

Holotype:—MFLUCC 13-0271

Saprobic on dead leaves of *Ruscus aculeatus*, visible as black colored spots, scattered on entire leaf surface, spots not abundant. Sexual state: *Ascomata* 80–110 μm high, 90–130 μm wide (\bar{x} = 92 \times 110 μm , n = 10), scattered, immersed, uniloculate, globose to ovoid, black, pseudoparenchymatous, apapillate, ostiolate. *Peridium* 9–20 μm (\bar{x} = 13 μm , n = 15), comprising several layers dark brown-walled cells of *textura angularis*. *Hamathecium* of 1.5–2.5 μm wide, cellular, hyaline, septate, rarely branching, pseudoparaphyses anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 56–70 \times 7.5–9 μm (\bar{x} = 62 \times 16 μm , n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apically rounded, lacking a distinct ocular chamber. *Ascospores* 14.5–18 \times 3–4 μm (\bar{x} = 16 \times 3.7 μm , n = 25), overlapping 2–3 seriate, cylindrical, 3-septate, without constriction or slightly constricted at the basal septum, hyaline when immature, becoming yellowish brown when mature, verrucose with a larger end cells and surrounded by a thin mucilaginous sheath. Asexual state: *Conidiomata* 600 μm diam, pycnidium, immersed to superficial in agar medium, solitary, scattered, few, brown to black, globose or irregular. *Peridium* comprising 2–3 layers of yellowish brown to brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–5 \times 1.2–1.8 μm (\bar{x} = 4 \times 1.5 μm , n = 30), holoblastic, phialidic, with a short collarete and narrow periclinal thickening at the conidiogenous locus, hyaline, subcylindrical, producing conidium at the apex. *Conidia* 4–6.5 \times 2–2.3 μm (\bar{x} = 5.2 \times 2.1 μm , n = 50), cylindrical to oblong, with rounded ends, aseptate, smooth to finely verrucose, hyaline to yellow becoming yellowish brown at maturity, accumulating in a bluish-black slimy mass at the apex of ostiole.

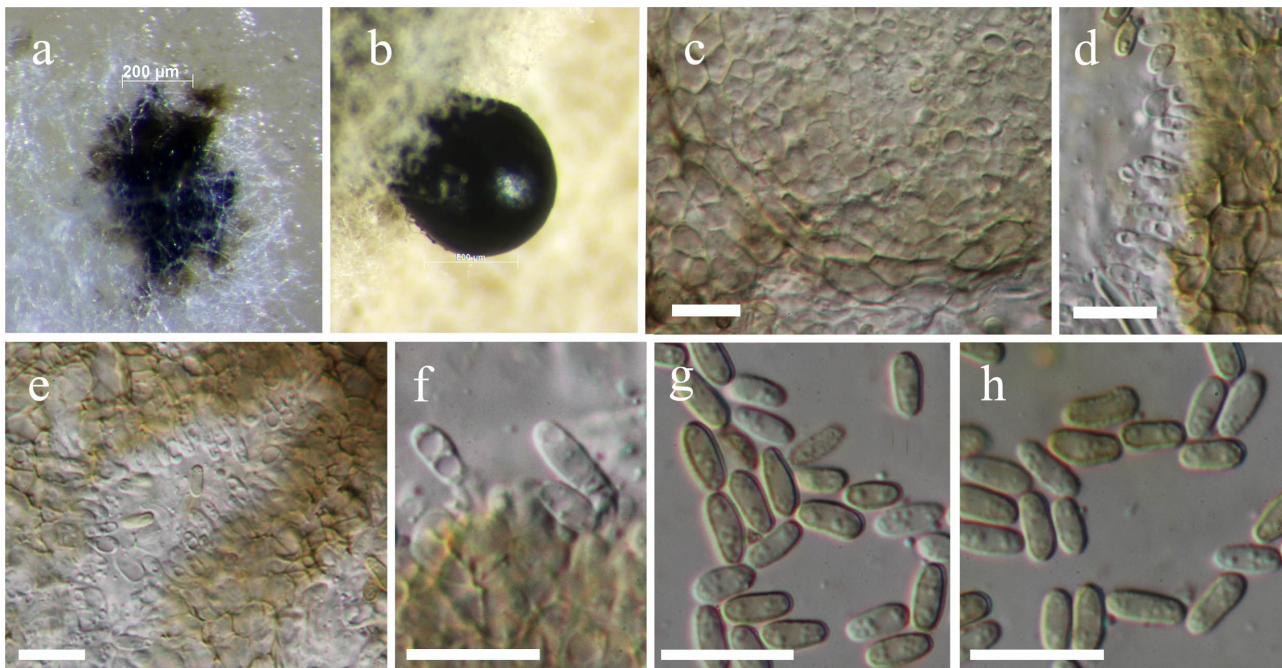


FIGURE 6. *Phaeosphaeriopsis triseptata* (holotype). a–b. Pycnidia on PDA. c. Pycnidial wall d–f= Conidiogenous cells and developing conidia. g–h. Immature and mature conidia. Scale bars: c–h = 10 μm .

Cultural characteristics:—Ascospores germinating on PDA within 18 h and producing germ tubes from one or several septa. Colonies on PDA reaching 52 mm diam after 14 days at 25–30°C, flat, circular, pinkish white, velvety, with entire to slightly undulate edge. After 5–6 weeks conidiomata formed on PDA.

Notes:—*Phaeosphaeriopsis triseptata* is morphologically similar and phylogenetically closely related to the type species of *Phaeosphaeriopsis* (*P. glaucopunctata*). Both species have globose to ovoid ascomata, a peridium composed of several layers dark-brown walled cells of *textura angularis*, cylindrical, very short-pedicellate asci

and yellowish-brown ascospores with larger end cells. *Phaeosphaeriopsis triseptata* differs from *P. glaucopunctata* in processing 3-septate ascospores and in asci and ascospore size.

Material examined:—ITALY. Ravenna Province: Lido di Classe, 24 November 2012, on leaves of *Ruscus aculeatus* (Asparagaceae), *Erio Camporesi* (MFLU 14-0031!, holotype); extype living culture = MFLUCC 13-0271 = ICMP 20201). ITALY. Forli-Cesena Province: Farazzano - Forli, on leaves of *Ruscus aculeatus* (Asparagaceae), 22 December 2012, *Erio Camporesi* (MFLU 14-0032!); living culture = MFLUCC 13-0347 = ICMP 20202).

Phaeosphaeria musae Sawada, Special Publication College of Agriculture, National Taiwan University 8: 66 (1959)

Synonymy: *Phaeosphaeriopsis musae* Arzanlou & Crous, Fungal Planet 9: [1] (2006)

Notes:—We compared *Phaeosphaeriopsis musae* Arzanlou & Crous with the original description of *Phaeosphaeria musae* Sawada. Both species cause leaf spots on *Musa* sp. and share common morphological characters such as 8-spored, cylindrical to fusiform asci and 3-septate, brown, fusiform, ascospores with obtuse ends. We also examined the type specimen of *Phaeosphaeria* (*P. oryzae*). *Phaeosphaeriopsis musae* and *Phaeosphaeria oryzae* share common morphological characters such as a thin peridium comprising 2–3 cells of *textura angularis*, cylindrical, short pedicellate asci and brown, 3-septate, ascospores and a *Phaeoseptoria* asexual state (Arzanlou & Crous 2006, Quaedvlieg *et al.* 2013, Zhang *et al.* 2012). In our phylogenetic tree and also in other phylogenetic studies (Quaedvlieg *et al.* 2013, Zhang *et al.* 2012, Hyde *et al.* 2013) *P. musae* nested with *P. oryzae* in a clade outside of *Phaeosphaeriopsis*. Therefore, we synonymized *Phaeosphaeriopsis musae* under *Phaeosphaeria musae* which was described first (1959) based on morphological and phylogenetic data.

TABLE 2. Isolates used in this study and their GenBank accession numbers. The newly generated sequences are indicated in bold.

| Taxa | Culture Accession No | SSU | LSU | RBP2 | ITS |
|-------------------------------------|----------------------|----------|----------|----------|----------|
| <i>Chaetosphaeronema hispidulum</i> | CBS 216.75 | EU754045 | KF251652 | KF252157 | KF251148 |
| <i>Didymella exigua</i> | CBS 183.55 | EU754056 | EU754155 | EU874850 | GU237794 |
| <i>Entodesmium rude</i> | CBS 650.86 | — | GU301812 | — | KF251278 |
| <i>Loratospora aestuarii</i> | JK 5535B | GU296168 | GU301838 | GU371760 | — |
| <i>Neosetophoma samarorum</i> | CBS 138.96 | GQ387517 | KF251664 | KF252168 | KF251160 |
| <i>Neosetophoma samarorum</i> | CBS 139.96 | GQ387518 | KF251665 | KF252169 | KF251161 |
| <i>Neostagonospora elegiae</i> | CBS 135101 | — | KF251668 | KF252172 | KF251164 |
| <i>Neostagonospora caricis</i> | CBS 135092 | — | KF251667 | KF252171 | KF251163 |
| <i>Ophiosphaerella herpotricha</i> | CBS 620.86 | DQ678010 | DQ678062 | DQ677958 | KF498728 |
| <i>Ophiosphaerella herpotricha</i> | CBS 240.31 | DQ767650 | DQ767656 | — | — |
| <i>Paraphoma dioscoreae</i> | CPC 11361 | — | KF251673 | KF252177 | KF251169 |
| <i>Paraphoma fimeti</i> | CBS 170.70 | — | KF251674 | KF252178 | KF251170 |
| <i>Paraphoma chrysanthemicola</i> | CBS 522.66 | GQ387521 | KF251670 | KF252174 | KF251166 |
| <i>Paraphoma radicina</i> | CBS 111.79 | EU754092 | KF251676 | KF252180 | KF251172 |
| <i>Parastagonospora avenae</i> | CBS 289.69 | — | KF251678 | KF252182 | KF251174 |
| <i>Parastagonospora caricis</i> | CBS 135671 | — | KF251680 | KF252184 | KF251176 |
| <i>Parastagonospora nodorum</i> | CBS 110109 | EU754076 | KF251681 | KF252185 | KF251177 |
| <i>Parastagonospora poae</i> | CBS 135089 | — | KF251682 | KF252186 | KF251178 |
| <i>Phaeosphaeria ammophilae</i> | CBS 114595 | GU296185 | GU301859 | GU371724 | — |
| <i>Phaeosphaeria caricicola</i> | CBS 603.86 | GQ387529 | KF251685 | KF252189 | KF251182 |
| <i>Phaeosphaeria elongata</i> | CBS 120250 | GU456306 | GU456327 | GU456345 | — |

.....continued on the next page

TABLE 2. (Continued)

| Taxa | Culture Accession No | SSU | LSU | RBP2 | ITS |
|---|----------------------|-----------------|-----------------|-----------------|-----------------|
| <i>Phaeosphaeria eustoma</i> | CBS 573.86 | DQ678011 | DQ678063 | DQ677959 | AF439479 |
| <i>Phaeosphaeria juncicola</i> | CBS 110108 | – | KF251686 | KF252190 | KF251183 |
| <i>Phaeosphaeria juncophila</i> | CBS575.86 | GU456307 | GU456328 | – | AF439488 |
| <i>Phaeosphaeria luctuosa</i> | CBS 308.79 | – | GU301861 | – | – |
| <i>Phaeosphaeria nigrans</i> | CBS 307.79 | – | KF251687 | KF252191 | KF251184 |
| <i>Phaeosphaeria oryzae</i> | CBS 110110 | GQ387530 | KF251689 | KF252193 | KF251186 |
| <i>Phaeosphaeria papayae</i> | CBS 135416 | – | KF251690 | KF252194 | KF251187 |
| <i>Phaeosphaeriopsis glaucopunctata</i> | CBS 653.86 | GQ387531 | KF251702 | KF252206 | KF251199 |
| <i>Phaeosphaeriopsis glaucopunctata</i> | MFLUCC 13-0220 | KJ522482 | KJ522478 | – | KJ522474 |
| <i>Phaeosphaeriopsis glaucopunctata</i> | MFLUCC 13-0265 | KJ522481 | KJ522477 | – | KJ522473 |
| <i>Phaeosphaeriopsis triseptata</i> | MFLUCC 13-0271 | KJ522484 | KJ522479 | KJ522485 | KJ522475 |
| <i>Phaeosphaeriopsis triseptata</i> | MFLUCC 13-0347 | KJ522483 | KJ522480 | KJ522486 | KJ522476 |
| <i>Phaeosphaeriopsis musae</i> | CBS 120026 | GU296186 | GU301862 | – | – |
| <i>Setomelanomma holmii</i> | CBS 110217 | GU296196 | GU301871 | GU371800 | – |
| <i>Setophoma chromolaena</i> | CBS 135105 | – | KF251747 | KF252249 | KF251244 |
| <i>Setophoma sacchari</i> | CBS 333.39 | GQ387525 | KF251748 | KF252250 | KF251245 |
| <i>Setophoma terrestris</i> | CBS 335.87 | GQ387528 | KF251750 | KF252252 | KF251247 |
| <i>Vrystaatia aloicicola</i> | CBS 135107 | – | KF251781 | KF252283 | – |
| <i>Xenoseptoria neosaccardoi</i> | CBS 128665 | – | KF251784 | KF252286 | KF251281 |
| <i>Xenoseptoria neosaccardoi</i> | CBS 120.43 | – | KF251783 | KF252285 | KF251280 |

Acknowledgements

We gratefully acknowledge The Mushroom Research Foundation, Chiang Rai, Thailand and Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang City, Guizhou Province, People's Republic of China for providing postgraduate scholarship support to Kasun M. Thambugala. MFLU grant (56101020032) is also thanked for supporting studies on Dothideomycetes. We specially appreciate Professor Jayarama Bhat and Professor Gareth Jones for helpful comments and advice on the manuscript.

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