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

KASUN M. THAMBUGALA[1,2,3], ERIO CAMPORESI[4], HIRAN A. ARIYAWANSA[2,3], RUNGTIWA PHOOKAMSAK[2,3], ZUO-YI LIU[1*] & KEVIN D. HYDE[2,3]

[1] *Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Xiaohe District, Guiyang City, Guizhou Province 550006, People's Republic of China*
[2] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3] *School of Science, Mae Fah Luang University, Chiang Rai. 57100, Thailand*
[4] *A.M.B. Gruppo Micologico Forlivese "Antonio Cicognani", Via Roma 18, Forlì, Italy; A.M.B. Circolo Micologico "Giovanni Carini", C.P. 314, Brescia, Italy; Società per gli Studi Naturalistici della Romagna, C.P. 144, Bagnacavallo (RA), Italy*
* *Corresponding author: gzliuzuoyi@163.com*

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. glauco-punctata* (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, cylindric 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

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from other species of *Phaeosphaeriopsis* and its *Microsphaeropsis*-like asexual morphs. However, recollection, epitypifycation 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). fRPB2-5f and fRPB2-7cR primer pairs were used to amplify the partial RNA polymerase second largest subunit (RPB2) (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 (RPB2) 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: -12330.90314and the following model parameters: alpha: 0.392200 and invar: 0.645801; Π(A): 0.251537, Π(C): 0.218113, Π(G): 0.270605, and Π(T): 0.259745.The best scoring RAxML combined tree is shown in Fig. 1. Maximum Likelihood bootstrap values ≥ 50% are given below or above each node.

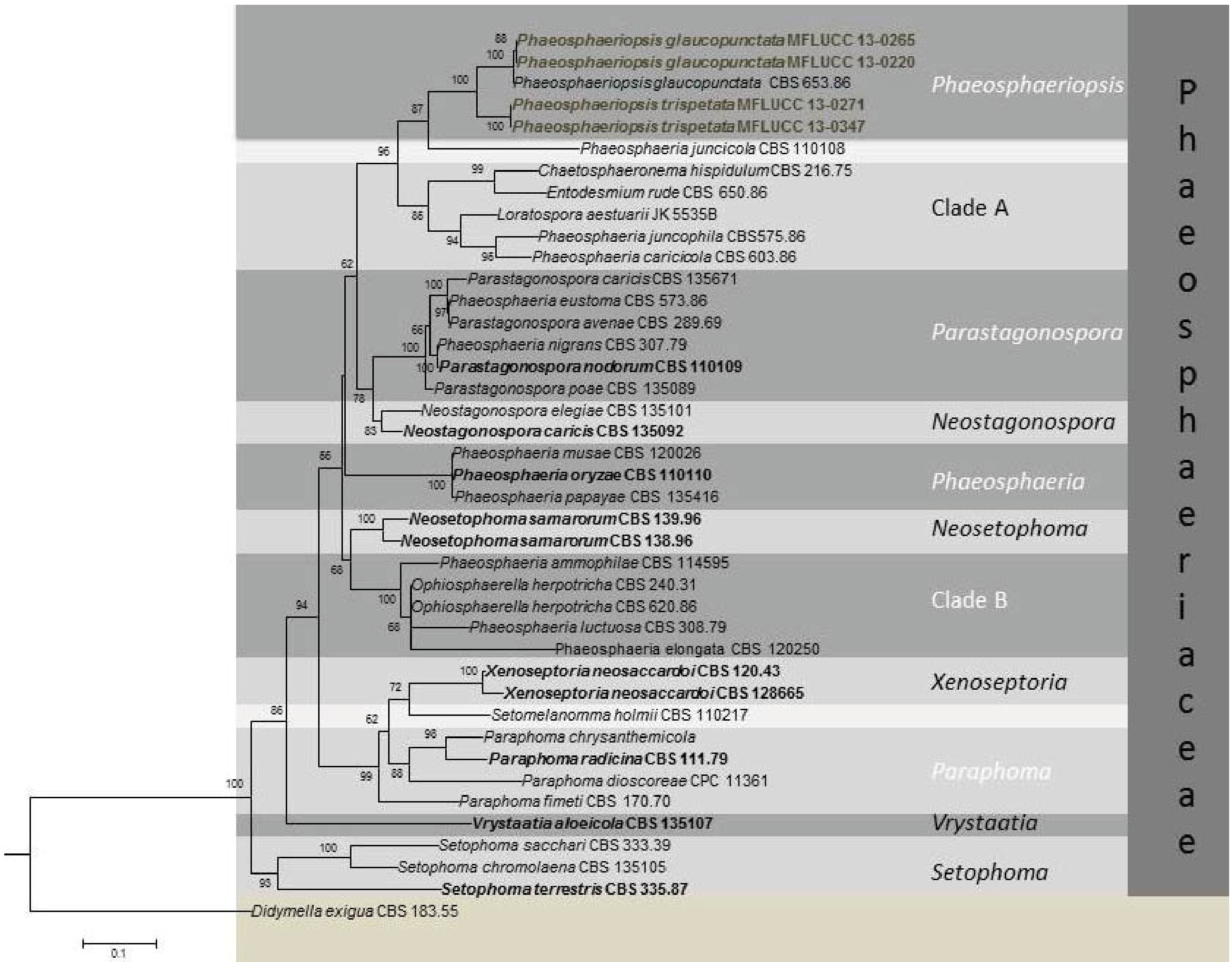

FIGURE. **1**. RAxML tree based on a combined dataset of LSU, SSU, ITS and RBP2 sequences. Bootstrap support values equal or greater than 50% are given above or below the nodes. The tree was rooted to *Didymella exigua* (CBS 183.55). The original isolate numbers are given after the species names. All 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 sevaral 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 .... *P. phacidiomorpha**
1. Ascospores pigmented, yellowish brown to brown 2

2. Ascospores 3-septate .... *P. triseptata*
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 .... *P. glaucopunctata*
4. Ascospores oblong-cylindric 9–24(–27) × 6.5–9 µm .... *P. amblyspora*

5. Ascostromata multiloculate .... *P. agavensis*
5. Ascostromata uniloculate 6

6. Conidia olivaceous brown, 3-septate .... *P. nolinae*
6. Conidia yellowish brown, nonseptate, rarely 1-septate .... *P. obtusispora*

* 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
P. agavensis	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	*Agave harvardiana*	Câmara *et al.* 2001 as *Paraphaeosphaeria agavensis*
P. amblyspora	(150–)200–350 × (170–)250–385	(116–)120–160 × 12–16	19.2–24(–27.2) × 6.4–8.8	(3–)4(–5)	*Yucca baccata*	Câmara *et al.* 2003
P. glaucopunctata	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	*Ruscus aculeatus*	Câmara *et al.* 2001 as *Paraphaeosphaeria glauco-punctata*
P. glaucopunctata	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	*Ruscus aculeatus*	This study – syntype
P. glaucopunctata	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	*Ruscus aculeatus*	This study - epitype
P. nolinae	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	*Nolina erumpens*	Câmara *et al.* 2001 as *Paraphaeosphaeria nolinae*
P. obtusispora	250–350 × 200–250	90−110 × 11−13	17−22 × 5−6	5	Agavaceae sp.	Câmara *et al.* 2001 as *Paraphaeosphaeria obtusispora*
?*P. phacidiomorpha*	−	30	10	4−5	*Phormium tenax*	Cesati 1878 as *Sphaeria phacidiomorpha*/ Saccardo 1882 as *Didymella phacidiomorpha*
P. triseptata	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	*Ruscus aculeatus*	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, psedoparenchymatous, 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) × 11–14(–15) µm ($\overline{x}$ = 68.2 × 13.4 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate, subsessile to short pedicellate, apically rounded, lacking a distinct ocular chamber, asci arising from the base of the ascoma. *Ascospores* 18–20(–21.5) × 4–5.5 µm ($\overline{x}$ = 20 × 4.8 µm, n = 25), uniseriate 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.

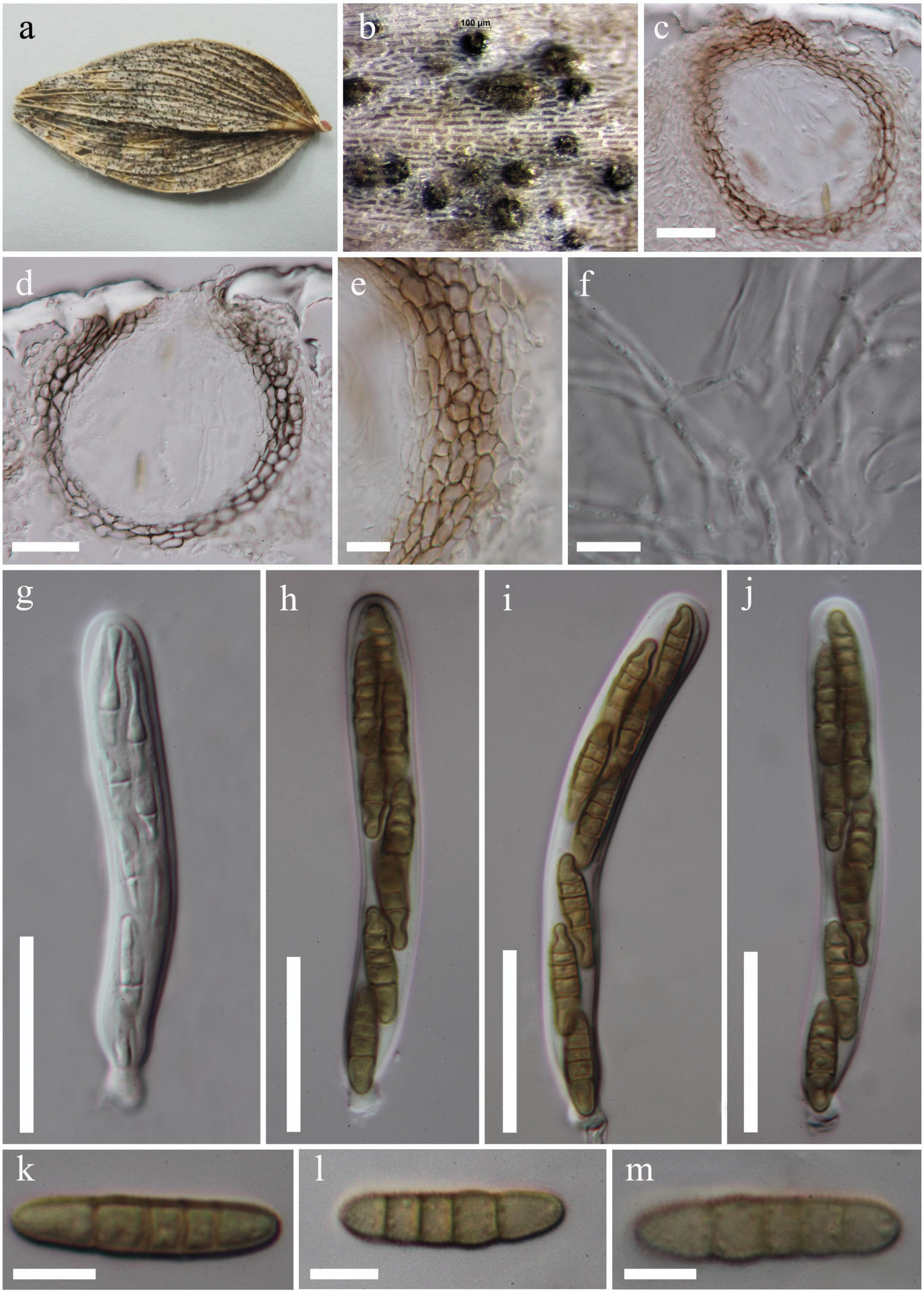

FIGURE 3. *Phaeosphaeriopsis glaucopunctata* (MFLU 14-0029, epitype). a-b. Ascomata on host surface. c–d. Section through ascomata. e. Peridium. f. Pseudoparaphyses g. Immature ascus. h–j. Mature bitunicate asci. k–m. Ascospores. Scale bars: c–d = 25 µm, e–f = 10 µm, g–j = 25 µm, k–m = 5 µm.

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. ($\overline{x}$ = 105 × 120 µm, n = 10), scattered, immersed, uniloculate, globose to ovoid, dark brown to black, psedoparenchymatous, apapillate, ostiolate. *Peridium* 12−32 µm ($\overline{x}$ = 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 × 7.5–10 µm ($\overline{x}$ = 74 × 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 × 3.2−4.6 µm ($\overline{x}$ = 17.5 × 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 × 2−3 µm ($\overline{x}$ = 8.5 × 2.5 µm, n = 30), holoblastic, lining the inner cavity, ampulliform, phialidic, annellidic, with a short collarette and narrow periclinal thickening at the conidiogenous locus, hyaline, subcylindrical, producing conidium at the apex. *Conidia* 4.5−10 × 2.1−3.5 µm ($\overline{x}$ = 7.5 × 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.

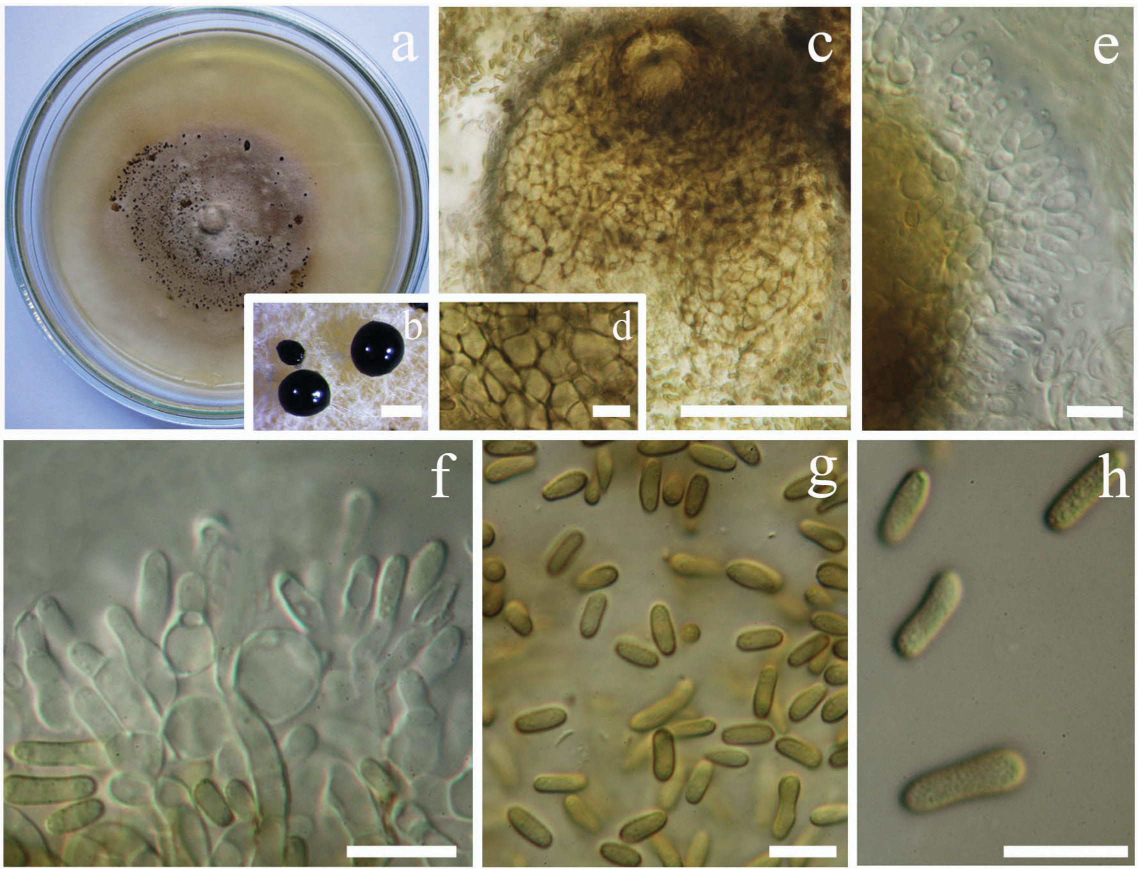

FIGURE 4. *Phaeosphaeriopsis glaucopunctata* (MFLU 14-0029, epitype). a–b. Pycnidia on PDA. c. Squash mount of pycnidia. d. Pycnidial wall. e–f. Conidiogenous cells and developing conidia. g–h. Conidia. Note the verrucose ornamentation in h. Scale bars: b = 500 µm, c = 100 µm, d–h = 10 µm.

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).

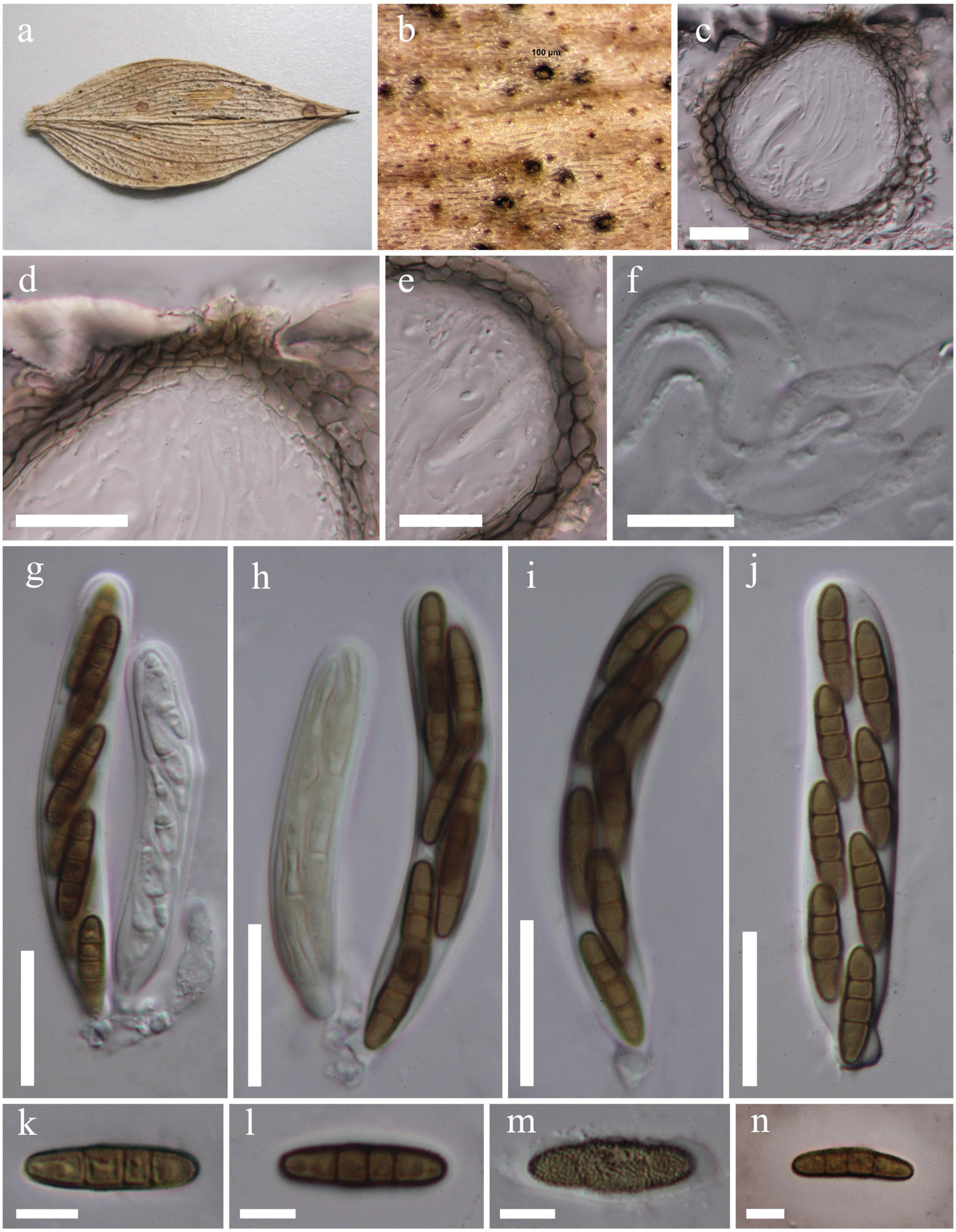

FIGURE 5. *Phaeosphaeriopsis triseptata* (holotype). a–b. Appearance of ascomata on the host surface. c. Section of an ascoma. d. Section of papilla. e. Section of Peridium. f. Pseudoparaphyses. g–j. Bitunicate asci. k–n. Released ascospores. Note the verrucose ornamentation in m. Scale bars: c = 25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

Phaeosphaeriopsis triseptata K.M. Thambugala & K.D. Hyde, *sp. nov.* (Figs 5, 6)
MycoBank MB 808205

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 × 110 µm, n = 10), scattered, immersed, uniloculate, globose to ovoid, black, psedoparenchymatous, 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 × 7.5−9 µm ($\bar{x}$ = 62 × 16 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apically rounded, lacking a distinct ocular chamber. *Ascospores* 14.5−18× 3−4 µm ($\bar{x}$ = 16 × 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 × 1.2−1.8 µm ($\bar{x}$ = 4 × 1.5 µm, n = 30), holoblastic, phialidic, with a short collarette and narrow periclinal thickening at the conidiogenous locus, hyaline, subcylindrical, producing conidium at the apex. *Conidia* 4−6.5 × 2−2.3 µm ($\bar{x}$ = 5.2 × 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.

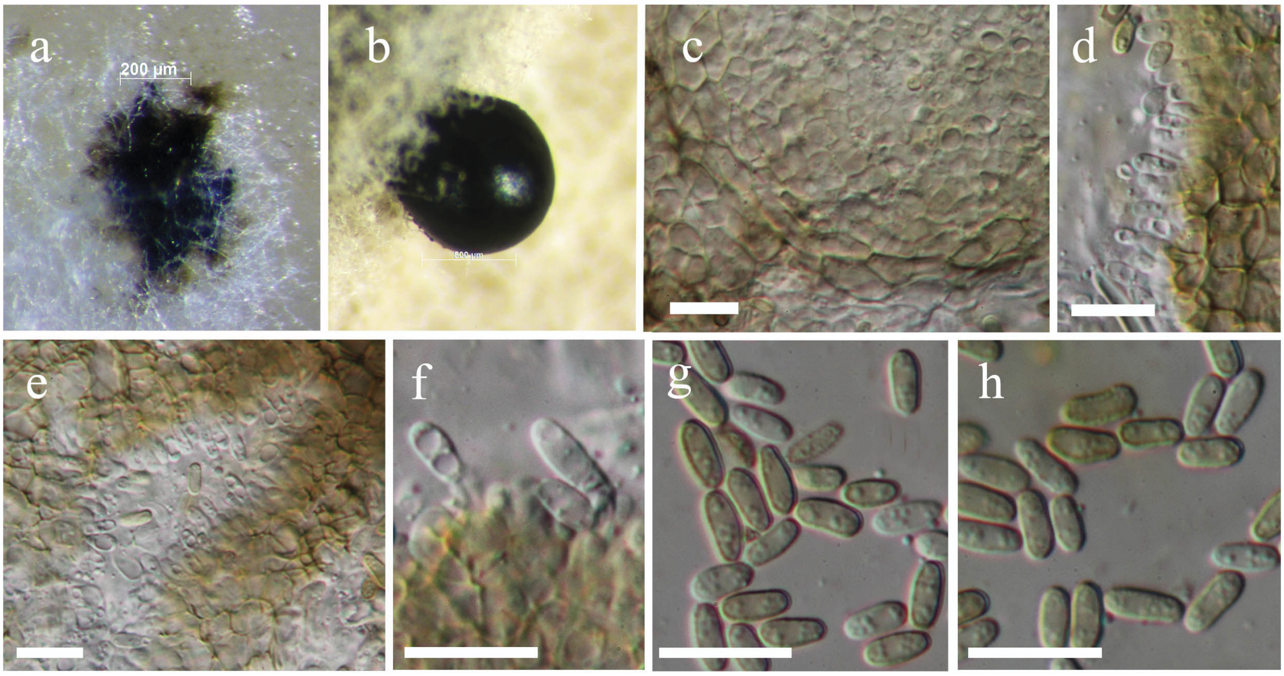

FIGURE 6. *Phaeosphaeriopsis triseptata* (holotype). a–b. Pycnidia on PDA. c. Pycnidial wall d–f= Conidiogenous cells and developing conidia. g–h. Immature and mature conidia. Scale bars: c–h = 10 µm.

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. Forlì-Cesena Province: Farazzano - Forlì, 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
Chaetosphaeronema hispidulum	CBS 216.75	EU754045	KF251652	KF252157	KF251148
Didymella exigua	CBS 183.55	EU754056	EU754155	EU874850	GU237794
Entodesmium rude	CBS 650.86	_	GU301812	_	KF251278
Loratospora aestuarii	JK 5535B	GU296168	GU301838	GU371760	_
Neosetophoma samarorum	CBS 138.96	GQ387517	KF251664	KF252168	KF251160
Neosetophoma samarorum	CBS 139.96	GQ387518	KF251665	KF252169	KF251161
Neostagonospora elegiae	CBS 135101	_	KF251668	KF252172	KF251164
Neostagonospora caricis	CBS 135092	_	KF251667	KF252171	KF251163
Ophiosphaerella herpotricha	CBS 620.86	DQ678010	DQ678062	DQ677958	KF498728
Ophiosphaerella herpotricha	CBS 240.31	DQ767650	DQ767656	_	_
Paraphoma dioscoreae	CPC 11361	_	KF251673	KF252177	KF251169
Paraphoma fimeti	CBS 170.70	_	KF251674	KF252178	KF251170
Paraphoma chrysanthemicola	CBS 522.66	GQ387521	KF251670	KF252174	KF251166
Paraphoma radicina	CBS 111.79	EU754092	KF251676	KF252180	KF251172
Parastagonospora avenae	CBS 289.69	_	KF251678	KF252182	KF251174
Parastagonospora caricis	CBS 135671	_	KF251680	KF252184	KF251176
Parastagonospora nodorum	CBS 110109	EU754076	KF251681	KF252185	KF251177
Parastagonospora poae	CBS 135089	_	KF251682	KF252186	KF251178
Phaeosphaeria ammophilae	CBS 114595	GU296185	GU301859	GU371724	_
Phaeosphaeria caricicola	CBS 603.86	GQ387529	KF251685	KF252189	KF251182
Phaeosphaeria elongata	CBS 120250	GU456306	GU456327	GU456345	_

......*continued on the next page*

TABLE 2. (Continued)

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

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