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ORIGINAL ARTICLE

Paraphoma species associated with Convolvulaceae

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Abstract
Substantial difficulties in the morphological identification of phoma-like fungi, including *Paraphoma* spp., have resulted in poor understanding of the generic and species boundaries in this group of organisms. This study was devoted to the reidentification and taxonomic revision of phoma-like isolates derived from Convolvulaceae leaves collected from different geographical locations in Russia and territories of neighboring countries. The study was based primarily on sequencing phylogenetically informative loci (ITS, LSU, *TUB*, and *RPB2*) and on traditional morphological approaches. The resulting phylogenetic tree revealed three well-supported monophyletic clades, corresponding to three *Paraphoma* species. The new species *Paraphoma melnikiae* Gomzhina M. M. & Gasich E. L. was described, and a new taxonomic combination, *Paraphoma convolvuli* (Dearn. & House) Gomzhina M. M. & Gasich E. L., was established for *Stagonospora convolvuli*. Several isolates were preliminarily identified as *Paraphoma* cf. *convolvuli* and are likely new species of the genus *Paraphoma*, but this requires further verification.

Keywords Phaeosphaeriaceae · Phoma-like fungi · Taxonomy · Multilocus phylogeny · New species · New combination

Introduction

The genus *Paraphoma* Morgan-Jones & J. F. White (Phaeosphaeriaceae) was established in 1983 with *Paraphoma radicina* (McAlpine) Morgan-Jones & J. F. White (≡ *Pyrenochaeta radicina* McAlpine) as the type species (Morgan-Jones and White 1983). Initially, it was suggested that the most informative taxonomic feature for members of this genus was setose pycnidia. However, the presence of such pycnidia in the fungal life cycle is specific for both *Paraphoma* and *Pyrenochaeta* De Not. species. Thus, it has been proposed to use ultrastructural features of conidiogenesis to distinguish the species of these two genera. According to those data, *Paraphoma* species have been placed in the genus *Phoma* Sacc. as a part of the appropriate section *Paraphoma* (Boerema et al. 2004).

Substantial difficulties in morphological identification have resulted in poor understanding of the generic and species boundaries generally in coelomycetes and particularly in the genus *Phoma* and its sections (sensu Boerema et al. 2004). Additionally, classification systems based on morphological features are highly artificial and do not represent evolutionary relationships. Molecular phylogenetic studies based on DNA sequencing have shown that *Paraphoma* is not a sister clade to phoma-like fungi transferred to the family Didymellaceae, but it is closely related to other genera affiliated with the families Phaeosphaeriaceae (de Gruyter et al. 2010), Cucurbitariaceae, and Coniothyriaceae (Chen et al. 2015). Currently, MycoBank categorizes eight species in the genus *Paraphoma*, and this group of organisms is actively being investigated. In the last 6 years, at least four new *Paraphoma* species have been described (Quaedvlieg et al. 2013; Crous et al. 2017; Moslemi et al. 2017).

Convolvulus arvensis and *Calystegia sepium* are perennial, soboliferous plants and two of the most harmful weeds. Controlling these weeds requires intense tillage and the use of a considerable amount of herbicides (Stetsov and Sadovnikova 2012; Nadtochiy 2008). Consequently, the potential application of biological weed control alternatives, particularly phytopathogenic fungi, has been studied more intensively in recent years. Several members of Phaeosphaeriaceae, including *Paraphoma* species, can be applied as living

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mycoherbicides and produce bioactive compounds with herbicide characteristics (Guntli et al. 1998; Poluektova et al. 2018).

The taxonomical diversity of phytopathogenic fungi, which can infect plants of the family Convolvulaceae, was examined in Russia and other countries. Several phoma-like species were revealed on these plants and are listed in Table 1. Three of those species produce several compounds with mycoherbicide activity against *C. arvensis*: *Phoma proboscis* (Heiny and Templeton 1991, 1995), *Phomopsis convolvuli* (Ormeno-Nuñez et al. 1988a, b; Watson et al. 1993; Vogelsang et al. 1998), and *Stagonospora convolvuli* (Pfirter and Defago 1998; Pfirter et al. 1999; Defago et al. 2001).

Due to extensive analysis of fungal biodiversity on weeds in Russia and neighboring countries in 1990–2010, pure cultures of fungi isolated from Convolvulaceae were collected. This collection is stored in the laboratory of Mycology and Phytopathology of the All-Russian Institute of Plant Protection. It includes 70 isolates of phoma-like fungi obtained from *C. arvensis* and *C. sepium*. All isolates in this collection were identified based on morphological features. A considerable number of isolates in this collection were not identified at the species level and have unclear definitions, such as *Ascochyta* sp., *Mycosphaerella* sp., and *Phoma* sp. According to preliminary molecular phylogenetic data (Gomzhina et al., unpublished), the collection consists of at least ten genera of phoma-like fungi. Among them, eleven isolates were identified as species of the genus *Paraphoma*.

The aim of this study was to correctly reidentify Russian *Paraphoma* isolates collected from Convolvulaceae and to taxonomically revise the fungal isolates, based primarily on a molecular phylogenetic approach and traditional morphological analysis.

Materials and methods

Isolates

As a result of the extensive studies of fungal biodiversity on Convolvulaceae weeds carried out in 1990–2010 in different geographical locations in Russia and territories of neighboring countries, eleven *Paraphoma* isolates (Table 2) were collected by authors from the leaves of *C. arvensis* and *C. sepium* that exhibited typical leaf spot symptoms. To isolate a pure culture of fungus from the leaves, fragments of infected material were surface sterilized with 20 ml of 5% sodium hypochlorite (NaClO) solution. Firstly washed for 2 min with 0.1% sodium dodecyl sulfate (SDS), washed with 5% sodium hypochlorite, and then washed three times with 20 ml of sterile water. After surface sterilization, the samples were placed on potato-sucrose agar (PSA) (Samson et al. 2000) containing antibiotics (100 μg/ml ampicillin, streptomycin, penicillin, HyClone, GE Healthcare Life Science, Austria) and 0.4 μl/l Triton X-100 (PanReac, Spain) to restrict the growth of fungi. The Petri dishes were incubated at 24 °C in the dark and were analyzed on days 7–10 of cultivation. Samples of infected leaves were deposited in the Mycological Herbarium (LEP) of All-Russian Institute of Plant Protection (VIZR). All *Paraphoma* isolates were stored in plastic microtubes on PSA at + 4 °C in the VIZR pure culture collection.

DNA isolation, PCR, and sequencing

Mycelium was scraped from 20-day-old cultures on oatmeal agar (OA, Boerema et al. 2004) and macerated with 0.3-mm glass sand on a Retsch MM400 mixer mill (Retsch, Germany). Genomic DNA was then extracted according to the standard CTAB/chloroform method (Doyle and Doyle 1990).

Table 1 Phytopathogenic phoma-like fungi that can infect plants of the family Convolvulaceae

Pathogenic fungus	Host	References
Phoma sepium Brunaud	*Calystegia sepium* (L.) R. Br.	Saccardo 1895
P. minuta Wehm.	*C. sepium*	Alcalde 1952
P. macrocollum Alcalde	*C. sepium*	Alcalde 1952
P. convolvuli Wehm.	*Convolvulus glomeratus* Choisy	Wehmeyer 1946
P. capsularum Cooke & Harkn.	*Ipomoea purpurea* (L.) Roth.	Saccardo 1895
P. proboscis Heiny	*Convolvulus arvensis* L.	Heiny 1990
Phomopsis calystegiae (Cooke) Petr. & Syd.	*C. sepium*	Alcalde 1952
Diaporthe convolvuli (Ormeno-Nuñez, Reeleder & A.K. Watson) R.R. Gomes, C. Glienke & Crous	*Convolvulus arvensis*	Ormeno-Nuñez et al. 1988a, b
Phyllosticta batatas (Thüm.) Cooke	*Ipomoea batatas* (L.) Lam.	Punithalingam 1982

Table 2 Information of the geographical location and dates of sample collections, from which the investigated isolates were obtained

Isolate/culture collection no.	Location	Substrate	Date of collection
MF-9.88. Ex-type	Russia, Saint Petersburg	*Convolvulus arvensis*	17 September 2002
MF-9.95. Ex-type	Russia, Saint Petersburg	*C. arvensis*	17 September 2002
MF-9.182.1	Ukraine, Tchernigovskaya oblast	*C. arvensis*	01 August 2004
MF-9.222	Kazakhstan, Almatinskaya oblast	*C. arvensis*	16 June 2006
MF-9.240	Russia, Vladivostok	*C. arvensis*	07 September 2006
MF-9.294	Russia, Saint Petersburg	*C. arvensis*	02 October 2009
MF-9.296.1	Russia, Saint Petersburg	*C. arvensis*	18 August 2009
MF-9.265	Russia, Saint Petersburg	*Calystegia sepium*	14 September 2007
MF-9.298.1	Russia, Saint Petersburg	*C. sepium*	07 September 2009
MF-9.300.1	Russia, Saint Petersburg	*C. sepium*	05 August 2009
MF-9.301.1	Russia, Saint Petersburg	*C. sepium*	Summer 2009

The ITS and LSU regions of rDNA were amplified and sequenced for all 11 isolates. The RNA polymerase II (*RPB2*) and β-tubulin (*TUB*) genes were sequenced for 10 and 9 isolates, respectively. All obtained sequences were deposited into GenBank, and accession numbers are listed in Table 3.

The primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and the primers LR0R (Rehner and Samuels 1994) and LR5 (White et al. 1990) were used to amplify the ITS and LSU regions of the ribosome genes, respectively. The primer T1/T2 (O'Donnell and Cigelnik 1997; Saleh and Leslie 2004) was used to amplify part of the *TUB* gene, and fRPB2-7cR/fRPB2-5f2 (Liu et al. 1999) was used to amplify part of the *RPB2* gene.

The amplification reactions had a total reaction volume of 25 μl, which was composed of dNTPs (200 μM), each of the forward and reverse primers (ITS1F/ITS4, LR0R/LR5, T1/T2, fRPB2-7cR/fRPB2-5f2) (0.5 μM), Taq DNA-polymerase (5 U/μl), 10× PCR buffer with Mg^{2+} and NH_4^+ ions, and 1–10 ng of total genomic DNA.

Table 3 Collection details and GenBank accession numbers of isolates

Isolate	Isolate/culture collection no.	Location	Substrate	GenBank accession number			
				ITS	LSU	TUB	RPB2
Paraphoma melnikiae	MF-9.88. Ex-type	Russia, Saint Petersburg	*Convolvulus arvensis*	MG764063	MG764065	MG779456	MG779466
P. melnikiae	MF-9.95. Ex-type	Russia, Saint Petersburg	*C. arvensis*	MG764054	MG764067	–	MG779462
P. melnikiae	MF-9.182.1	Ukraine, Tchernigovskaya oblast	*C. arvensis*	MG764058	MG764068	MG779454	MG779463
P. convolvuli	MF-9.222	Kazakhstan, Almatinskaya oblast	*C. arvensis*	MG764055	MG764069	–	–
P. melnikiae	MF-9.240	Russia, Vladivostok	*C. arvensis*	MG764061	MG764070	MG779453	MG779464
P. melnikiae	MF-9.294	Russia, Saint Petersburg	*C. arvensis*	MG764059	MG764072	MG779455	MG779471
P. melnikiae	MF-9.296.1	Russia, Saint Petersburg	*C. arvensis*	MG764056	MG764073	MG779458	MG779465
Paraphoma cf. *convolvuli*	MF-9.265	Russia, Saint Petersburg	*Calystegia sepium*	MG764062	MG764071	MG779457	MG779467
Paraphoma cf. *convolvuli*	MF-9.298.1	Russia, Saint Petersburg	*C. sepium*	MG764057	MG764074	MG779459	MG779468
Paraphoma cf. *convolvuli*	MF-9.300.1	Russia, Saint Petersburg	*C. sepium*	MG764064	MG764066	MG779460	MG779469
Paraphoma cf. *convolvuli*	MF-9.301.1	Russia, Saint Petersburg	*C. sepium*	MG764060	MG764075	MG779461	MG779470

Table 4 GenBank accession numbers of reference isolates

Isolate	Strain/culture collection no.	GenBank accession number				References
		ITS	LSU	TUB	RPB2	
Neosetophoma samarorum	CBS 138.96	KF251160.1	KF251664	KF252655.1	KF252168.1	Quaedvlieg et al. 2013
Neostagonospora caricis	CBS 135092	KF251163.1	KF251667	KF252658.1	KF252171.1	Quaedvlieg et al. 2013
Paraphoma chlamydocopiosa	UMPc01; BRIP 65168	KU999072	–	KU999084	–	Moslemi et al. 2017
P. chrysanthemicola	CBS 172.70	KF251165.1	KF251669	KF252660.1	KF252173.1	Quaedvlieg et al. 2013
P. dioscoreae	CBS 135100	KF251167.1	KF251671	KF252662.1	KF252175.1	Quaedvlieg et al. 2013
P. fimeti	CBS 170.70	KF251170.1	KF251674	KF252665.1	KF252178.1	Quaedvlieg et al. 2013
P. pye	UMPp04; BRIP 65171	KU999075	–	KU999087	–	Moslemi et al. 2017
P. radicina	CBS 111.79	KF251172.1	KF251676	KF252667.1	KF252180.1	Quaedvlieg et al. 2013
P. rhaphiolepidis	CBS 142524	KY979758.1	KY979813.1	KY979924.1	KY979851.1	Crous et al.
P. vinacea	UMPV004	KU176887.1	KU176891.1	KU176895.1	–	Moslemi et al. 2016, Moslemi et al. 2017
Parastagonospora nodorum	CBS 110109	KF251177.1	KF251681	KF252672.1	KF252185.1	Quaedvlieg et al. 2013
Phaeosphaeriopsis glaucopunctata	CBS 653.86	KF251199.1	KF251702	KF252693.1	KF252206.1	Quaedvlieg et al. 2013
Phaeosphaeria oryzae	CBS 110110	KF251186.1	KF251689	KF252680.1	KF252193.1	Quaedvlieg et al. 2013
Setophoma terrestris	CBS 335.29	KF251246.1	KF251749	KF252729.1	KF252251.1	Quaedvlieg et al. 2013
Stagonospora convolvuli	12–039	KC634206.1	–	–	–	Not published
S. convolvuli	01–634	HQ677906	–	–	–	Not published
Vrystaatia aloeicola	CBS 135107	KF251278.1	KF251781	KF252759.1	KF252283.1	Quaedvlieg et al. 2013
Xenoseptoria neosaccardoi	CBS 120.43	KF251280.1	KF251783	KF252761.1	KF252285.1	Quaedvlieg et al. 2013

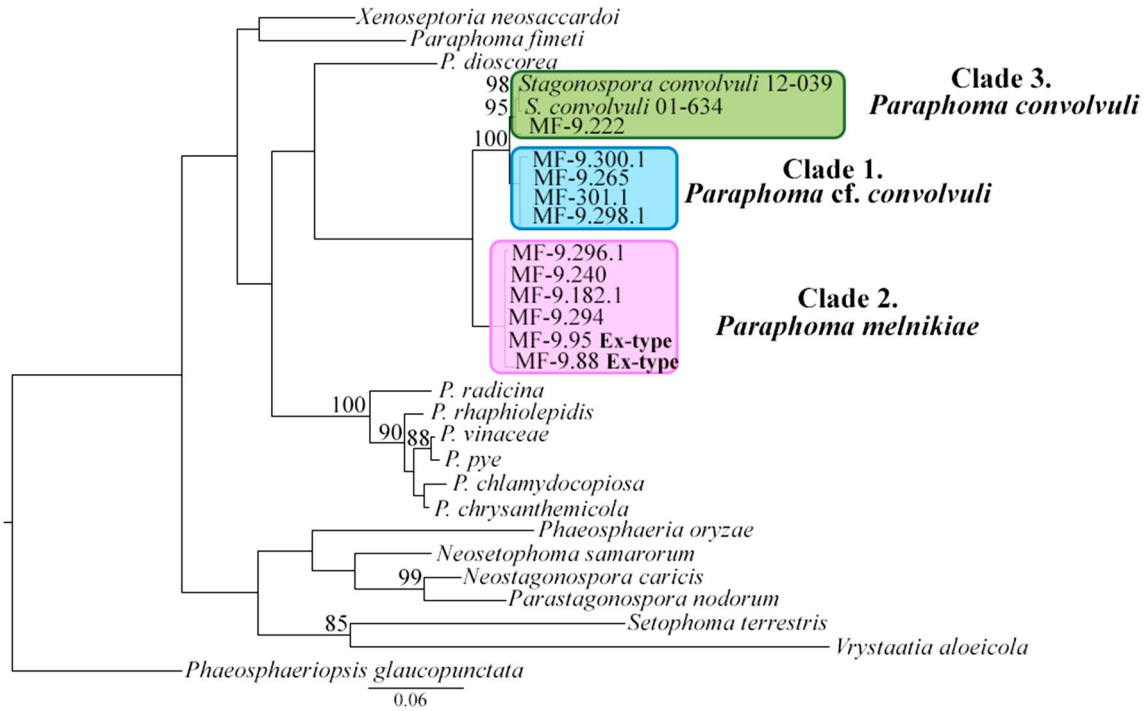

Fig. 1 Maximum-likelihood phylogenetic tree inferred from ITS, representing all *Paraphoma* species and reference of *Stagonospora convolvuli*

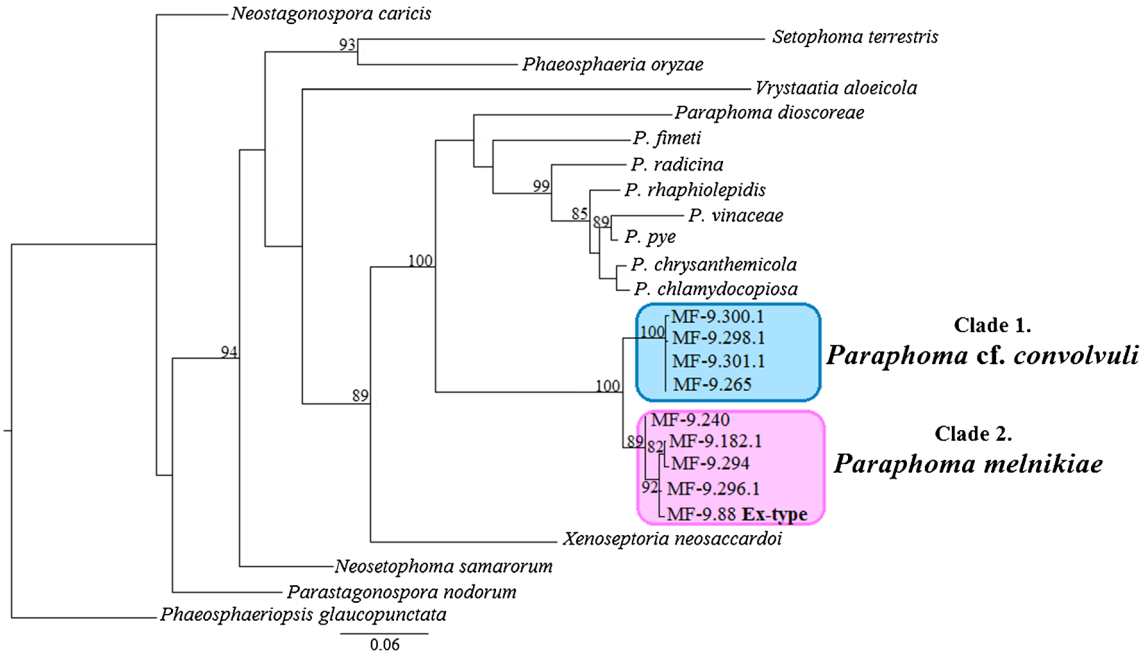

Fig. 2 Maximum-likelihood phylogenetic tree inferred from ITS and *TUB*

The PCR conditions were as follows: predenaturation of DNA at 95 °C for 5 min; 35 cycles of denaturation at 92 °C for 50 s, annealing at 55 °C for 40 s (ITS1F/ITS4 and T1/T2) or at 55 °C for 50 s (LR0R/LR5), and elongation at 72 °C for 75 s, followed by a final elongation step for 5 min at 72 °C. The *RPB2* gene was amplified according to the touchdown program. All steps were the same as described below, but the annealing temperature consequently declined from 5 cycles of 60 °C for 40 s and 5 cycles of 58 °C for 40 s to 30 cycles of 54 °C for 40 s.

After PCR, amplicons were purified according to a standard method with a DNA-binding silica matrix (Boyle and Lew 1995). Visualization and concentration measurements of the purified PCR products were implemented by electrophoresis in 1% agarose gel stained with ethidium bromide and MassRuler 100 bp as a marker of concentration.

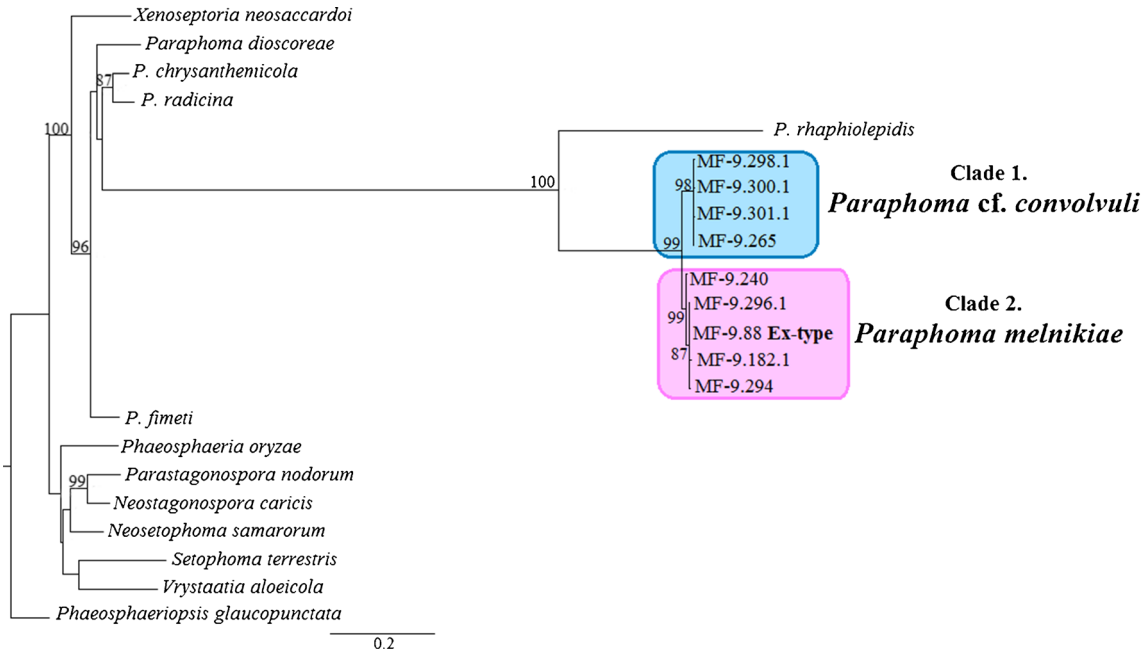

Fig. 3 Maximum-likelihood phylogenetic tree inferred from ITS, *TUB*, and *RPB2*

Amplicons were sequenced by Sanger's method (1977) on ABI Prism 3500 (Applied Biosystems, Hitachi, Japan), with the Big Dye Terminator v3.1 Cycle Sequencing Kit (ABI, Foster City, USA), according to the manufacturer's instructions.

Phylogenetic analysis

Sequences were assembled using Vector NTI advance v. 11.0 (Invitrogen, Thermo Fischer Scientific, Waltham, USA) and aligned with ClustalX 1.8 (Thompson et al. 1997). All known representative *Paraphoma* strains and type species of all genera in the family Phaeosphaeriaceae, with *Phaeosphaeriopsis glaucopunctata* as an outgroup, were obtained from GenBank (Table 4) and included in the analysis. The phylogenetic trees were inferred with RAxML (randomized accelerated maximum likelihood) software (v. 7.2.8, Stamatakis 2006) by the maximum-likelihood (ML) method. Bootstrap support values with 1000 replications were calculated for tree branches.

Morphological analysis

Pure cultures were incubated on PSA and OA amended with 200 mg/ml streptomycin sulfate for up to 2 weeks under standard conditions (Boerema et al. 2004). Petri dishes were placed for 1 week in darkness and then for a week under 12-h near-ultraviolet light/12-h dark to stimulate sporulation. Colony diameter was measured after 7 days, and colony morphology was examined after 14 days of incubation. Colony colors on the surface and underside of the inoculated Petri dishes were assessed according to the color charts of Bondartsev (1953). Isolates that did not produce pycnidia on agar medium were cultivated on autoclaved grain (millet, oat, and pearl barley) under near-ultraviolet conditions for 14 days. Observations and measurements of 50 replicates of conidia and conidiomata were conducted with a stereomicroscope Olympus SZX16 (Olympus, Japan) and microscope Olympus BX53.

Results

Phylogeny

The adjusted and aligned sequences in the phylogenetic analysis had the following lengths: ITS region, 483 bp; LSU, 845 bp; *TUB*, 490 bp; and *RPB2*, 736 bp; the number of polymorphic sites per genome locus was 44 (9.1%), 2 (0.2%), 28 (5.7%), and 32 (4.3%), respectively.

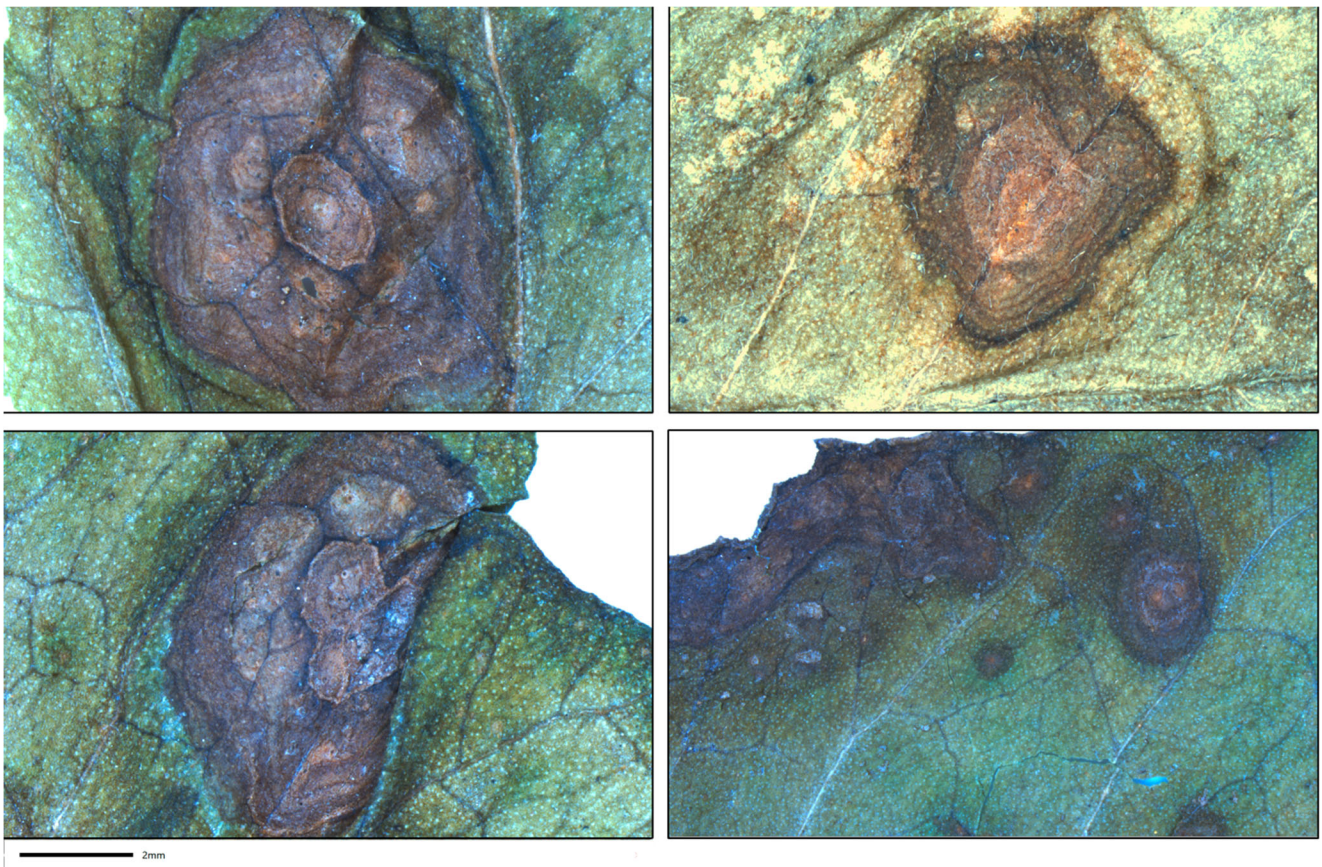

Fig. 4 Leaf spots on *Convolvulus arvensis* caused by *Paraphoma melnikiae* sp. nov. from the type material

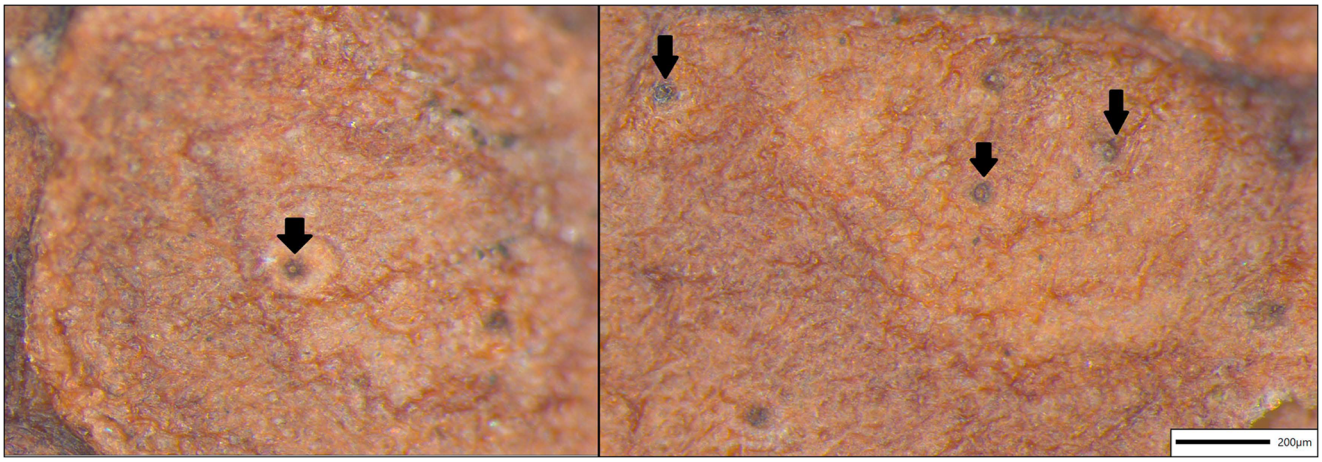

Fig. 5 Immersed pycnidia of *Paraphoma melnikiae* sp. nov. on leaves of *Convolvulus arvensis* from the type material

Nucleotide sequences of 11 isolates were used for 2-gene phylogenetic analysis (sequences of ITS and LSU regions). Sequences of 10 isolates in the 3-gene phylogenetic analysis (ITS, LSU, and *RPB2*) and sequences of only nine isolates in the 4-gene phylogenetic analysis (ITS, LSU, *TUB*, and *RPB2*) were suitable for analysis. Three phylogenetic trees were developed: an individual tree of the ITS region, a combined tree for the ITS and *TUB* genes, and a combined phylogram for all studied loci.

All currently known species of *Paraphoma* and all our isolates formed a common monophyletic group. Within this group, all our isolates clustered as three distinct clades in all phylogenetic trees with a maximum value of bootstrap support (100%). The composition of these clades was identical in all trees, and these clades did not cluster with any *Paraphoma* species (Figs. 1, 2, 3). The first clade consisted of four isolates (MF-9.301, MF-9.298.1, MF-9.265, MF-9.300.1). The second clade included six isolates (MF-9.296.1, MF-9.88, MF-9.294.1, MF-9.240, MF-9.95, MF-9.182.1) (Figs. 2, 3). The third clade included only the isolate MF-9.222 (Fig. 1).

In the tree of the ITS region (Fig. 1), the isolate MF-9.222 clustered within the same clade with the two reference strains of *S. convolvuli* (12–039; 01–634), which are represented in GenBank only by these two sequences. Thus, according to the obtained molecular phylogenetic data, isolate MF-9.222 was identified as *S. convolvuli*. However, due to its position in the *Paraphoma* cluster, the new taxonomical combination *Paraphoma convolvuli* has been proposed for *S. convolvuli*.

The other isolates were divided into two subclades in all phylogenetic trees. The topologies of the combined phylogenetic trees were more detailed, including the

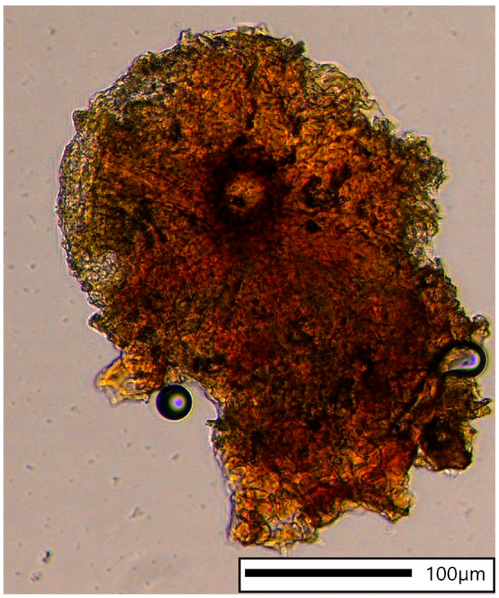

Fig. 6 Pycnidia of *Paraphoma melnikiae* sp. nov. on leaves of *Convolvulus arvensis* from the type material

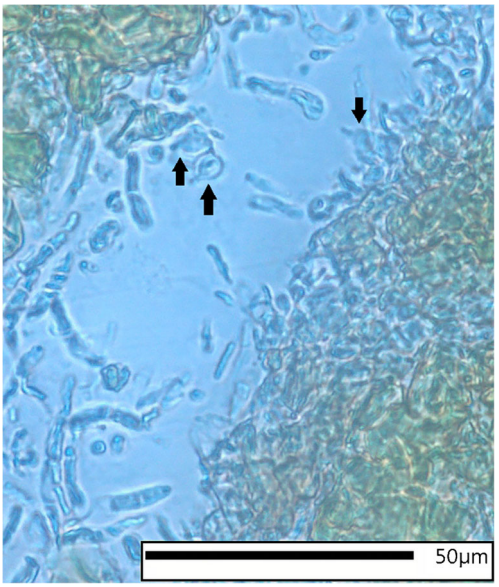

Fig. 7 Conidiogenous cells of *Paraphoma melnikiae* sp. nov. from the type material

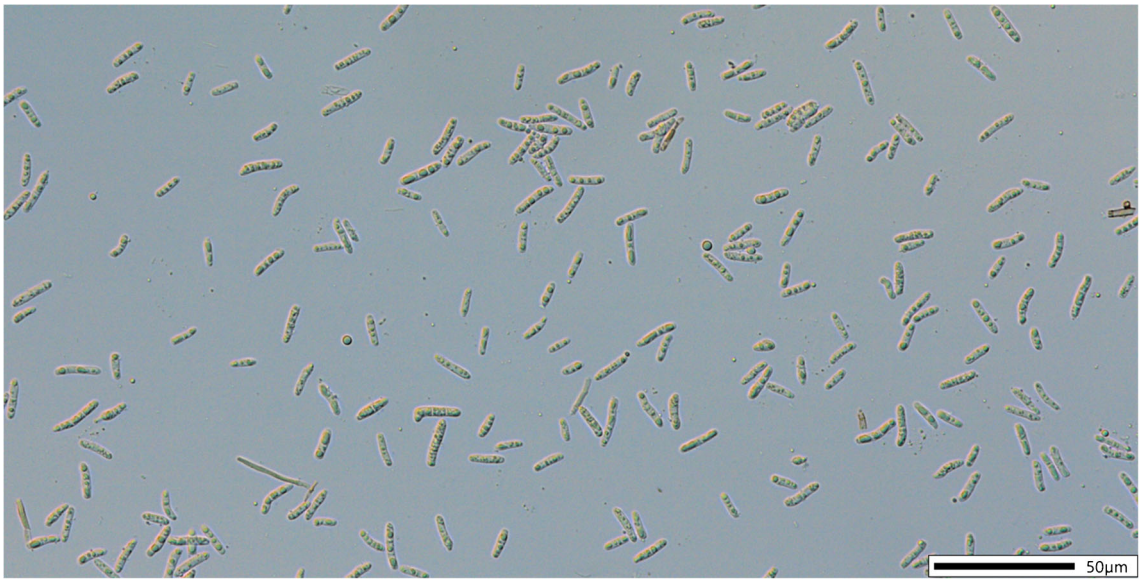

Fig. 8 Conidia of *Paraphoma melnikiae* sp. nov. from the type material

topology of clade 2, which was more explicit and allowed the division of isolates MF-9.182, MF-9.299, and MF-9.240 into two subclades (Figs. 2, 3). This clade did not include any type or representative isolate. It is monophyletic and, in all trees, has a maximum value of bootstrap support. Therefore, the isolates of this clade are considered a new species, *Paraphoma melnikiae.*

Morphology and Taxonomy

Paraphoma convolvuli (Dearn. & House) Gomzhina M. M. & Gasich E. L. comb. nov.

MycoBank MB823867

Basionym *Stagonospora convolvuli* Dearn. & House

Paraphoma melnikiae Gomzhina M. M. & Gasich E. L., sp. nov.

MycoBank MB823800

Type specimen LEP 131845. The type specimen represents dried leaves of *C. arvensis* with leaf spots collected in Saint Petersburg on February 17, 2002.

Etymology: Named after Dr. V. A. Melnik (1937–2017), an outstanding Russian mycologist and taxonomist who dedicated his work to different fungi, including phoma-like species.

This fungus causes leaf spots on *C. arvensis*. Spots are incorrectly rounded with concentric zones and some pycnidia (Fig. 4). Pycnidia are diffuse, semisubmerged, rounded, dark brown, and 100–250 µm (Figs. 5, 6). Conidiophores are reduced to phialidic conidiogenous cells formed from the inner cells of the pycnidial wall, hyaline, discrete, flask-shaped, and 7.29–10.25 × 3.6–4.24 µm (Fig. 7). Conidia are cylindrical with rounded tips, straight or slightly curved, with 0–2 transverse septa, 9–22.5 × 1.5–3.8 µm (Fig. 8). On OA (Fig. 9), the colony diameter is 25–34 mm after 7 days and 42–54 mm after 14 days. Felty-velvet or flocculose felty-velvet aerial mycelia are not abundant, pale-olivaceous. The colors of the colonies on the upper and lower parts are from pale to dark brown, sometimes with dark brown, reddish, and fallow sectors. The color of the colonies could also be from pale to dark vinaceous shades, sometimes with pale-olivaceous sectors. Margins are regular and slightly curved. Pycnidia are sparse, scattered, immersed and

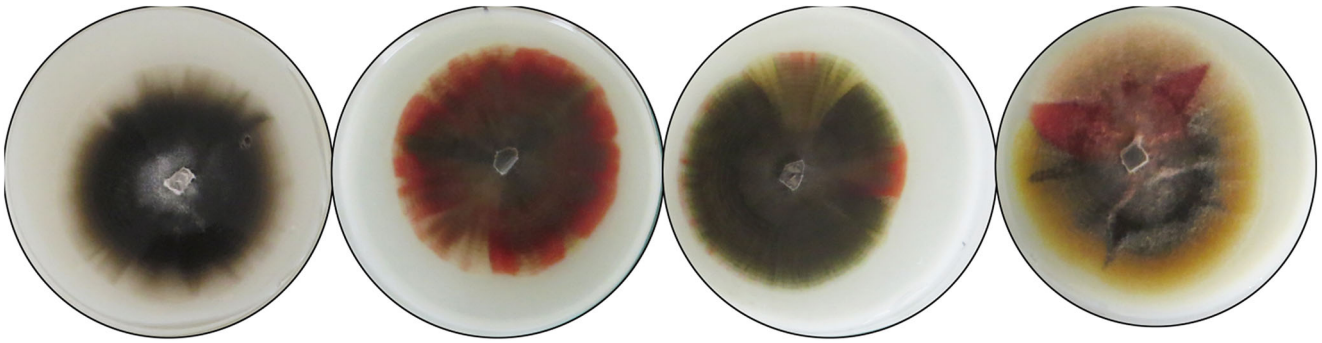

Fig. 9 Morphology of *Paraphoma melnikiae* sp. nov. colonies on OA (ex-type isolate MF–9.88)

semi-immersed, rare in aerial mycelium, dark brown, rounded, hairy, 40–420 μm, with 1–3 ostioles. Conidia are hyaline, multiguttulate, cylindrical with rounded tips, straight or slightly curved, with 0–2 transverse septa 7–16 (10.4 ± 0.5) × 1.5–2.5 (2.0 ± 0.1) μm.

Chlamydospores were absent. Perithecia were not observed.

Note: Morphologically, the conidia of *P. melnikiae* differ from conidia of the closely related species *P. convolvuli* in shape and size. The conidia of *P. convolvuli* are longer (15–18 μm) and have more transverse septa (2–3) (Saccardo 1931).

Discussion

Based on the morphological characteristics, all isolates were primarily identified as *S. convolvuli* by the authors. However, the conidia of the studied isolates were broader and less elongated than the conidia of *Stagonospora* species. Our isolates did not possess typical morphological features of pycnidia and conidia to identify them as members of the genus *Paraphoma*. The pycnidia of the studied isolates were not setose, and the conidia were longer than typical *Paraphoma* conidia. It is known that such morphological characteristics are highly variable and do not represent phylogenetic relationships among fungi in this group.

Unlike the traditional morphological approach, molecular-phylogenetic methods allow the identification of all isolates as members of the genus *Paraphoma*. Based on molecular data, a new combination, *P. convolvuli*, was proposed for *S. convolvuli*. Isolate MF-9.222 should be identified as *P. convolvuli*, whereas isolates from clade 1 should be identified as *Paraphoma* cf. *convolvuli*. All *Paraphoma* isolates from clade 1 shared similar morphological features with *P. convolvuli* isolate MF-9.222 but differed from it by a single deletion in the ITS sequence and one insertion in the LSU sequence. Clade 1 was monophyletic and well supported; all isolates were obtained from *C. sepium*, not from *C. arvensis*, as was isolate MF-9.222 and the reference *P. convolvuli*. Apparently, these isolates are new species of the genus *Paraphoma*, but this requires subsequent validation.

Isolates of the second phylogenetic clade were treated as a new species of *Paraphoma*, *P. melnikiae.* This new taxon was proposed according to the polyphasic approach to species recognition (Consolidated Species Concepts) and based on phylogenetic, morphological, and biological characteristics.

To construct phylogenetic hypotheses for closely related phoma-like species, the most informative loci are ITS, *TUB*, and *RPB2*. The sequencing of these loci was implemented in this study and resulted in robust, well-supported phylogenetic clades in the phylograms. Thus, to resolve phylogenetic relationships among *Paraphoma* species, this set of loci is also taxonomically informative. Despite this being used in non-taxonomic studies, the molecular identification of phoma-like fungi is often based only on sequences of the ITS region. Thus, data on sequences of phylogenetic informative loci of particular phoma-like species in GenBank are often presented one-sidedly and scantly. The implementation of phylogenetic studies in such cases becomes difficult. Although it was previously suggested not to identify phoma-like fungi only by morphological features and to take into account molecular traits, now it is not recommended to identify these fungi only by sequencing the ITS loci, as the most popular region for phylogenetic studies, but to include sequences of other informative regions of DNA in the phylogenetic analysis.

A majority of *Paraphoma* species are widely distributed, occurring as soil-borne fungi causing diseases of aboveground parts of plants. Analysis of the pure culture collection of phoma-like fungi derived from Convolvulaceae showed that species of the genus *Paraphoma* were detected in Russia, Kazakhstan, and Ukraine. *P. convolvuli* was found on *C. arvensis* only in Kazakhstan, whereas closely related isolates of *Paraphoma* cf. *convolvuli* (probably a new species) were detected only in one location in Russia, in Saint Petersburg, on *C. sepium*. The new species *P. melnikiae* was found on *C. arvensis* in two locations in Russia (Saint Petersburg and Vladivostok) and in Ukraine.

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