

Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*

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INTRODUCTION

Asexual ascomycetes producing ostiolate pycnidia with hyaline conidia have been classified in a number of coelomycetous genera. These genera are separated mainly on a combination of conidiomatal anatomy, conidiogenesis and conidial morphology. The genus *Phoma* takes a prominent position among these genera because it incorporates many important plant pathogenic species. The nearly 220 *Phoma* species that are recognized currently (Boerema et al. 2004) were classified in nine sections according to pycnidial, conidial and cultural characters. Some of these sections are highly artificial from an evolutionary perspective, and overlapping characters among sections as well as with other genera of coelomycetes do occur (Boerema et al. 2004, Aveskamp et al. 2008).

The genus *Phoma* is characterized by hyaline, unicellular conidia that may become septate due to secondary septation, phialidic, ampulliform to doliform conidiogenous cells and (sub)globose, glabrous to pilose or setose, pseudoparenchymatous or scleroplectenchymatous pycnidia. Six *Phoma* sections include species with a known teleomorph in the genera *Didymella*, *Leptosphaeria* or *Pleospora*, indicating the genetic diversity in genus *Phoma* (van der Aa et al. 1990, Boerema 1997). A recent molecular study demonstrated that the type species of all *Phoma* sections grouped in the Pleosporales. The representatives of five sections clustered in the new family Didymellaceae, including *Phoma herbarum*, the type species of genus *Phoma* (de Gruyter et al. 2009).

Phoma radicina, the type species of *Phoma* sect. *Paraphoma*, clustered with species classified in the Phaeosphaeriaceae (de Gruyter et al. 2009). *Phoma* sect. *Paraphoma* is delimited in the genus *Phoma* by its setose, pseudoparenchymatous pycnidia. The characteristics of the setae (viz. stiff or more hyphal-like) and their length and position on the pycnidial surface are important for further species delimitation (de Gruyter and Boerema 2002). Species with setose pycnidia producing hyaline conidia also were classified in other genera, particularly *Pyrenochaeta*, for which the branched, filiform, septate, acropleurogenous conidiophores were considered the most important delimitating character (Schneider 1979). However *Pyrenochaeta* species have been described with setose pycnidia but with a *Phoma*-like conidiogenesis such as the *Pyrenochaeta romeroi*, which in rare cases causes human infections (Borelli 1959). Setose

Abstract: Sequence data from the 18S nrDNA (SSU) and 28S nrDNA (LSU) regions of isolates of *Phoma* section *Paraphoma* were compared with those of representative isolates of the morphologically similar anamorph genera *Pleurophoma* and *Pyrenochaeta* and of the type species of *Phoma* sections *Phoma*, *Pilosa* and *Plenodomus*. *Phoma* section *Paraphoma* was found to be highly polyphyletic within the Pleosporales and only distantly related to *Phoma* section *Phoma*. The genus *Paraphoma*, which is based on *Paraphoma radicina*, is reintroduced in the Phaeosphaeriaceae with two additional taxa. The new genera *Setophoma* and *Neosetophoma*, type species *Setophoma terrestris* comb. nov. and *Neosetophoma samarorum* comb. nov., are introduced and represent species that are closely related to *Paraphoma* but differ based on morphological characters and molecular phylogeny. *Phoma coonsii* is transferred to genus *Chaetosphaeronema* that also belongs to the Phaeosphaeriaceae. *Pyrenochaetopsis* gen. nov. is introduced to accommodate the type species *Pyrenochaetopsis leptospora* comb. nov., as well as several other species formerly accommodated in *Phoma* and *Pyrenochaeta*. *Pyrenochaetopsis* is closely related to *Pyrenochaeta* and classified in the Cucurbitariaceae. *Pleurophoma cava* is transferred to genus *Pyrenochaeta*. The new genera elucidate the confusing taxonomy of species in genera *Phoma*, *Pyrenochaeta* and *Pleurophoma* and recognize monophyletic genera with distinct teleomorph affinities.

Key words: coelomycetes, 18S nrDNA, molecular phylogeny, Pleosporales, *Pyrenochaetopsis* gen. nov., *Setophoma* gen. nov., 28S nrDNA, *Neosetophoma* gen. nov.

Submitted 29 Sep 2009; accepted for publication 10 Dec 2009.

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pycnidia also have been reported rarely in other *Phoma* sections, such as in the generic type species *Phoma herbarum* and in *Phoma glomerata* (Boerema et al. 2004). Furthermore *Phoma* species such as *Phoma clematidina*, producing pycnidia with hyphal outgrowths resembling setose pycnidia, have been described and classified in the Didymellaceae (Woudenberg et al. 2009). In the related genus *Pleurophoma* both types of conidiogenesis may be present but species attributed to this genus produce glabrous pycnidia.

The classification of several species in the genera *Pyrenochaeta*, *Pleurophoma* and *Phoma* sect. *Paraphoma* has been disputed because of the overlapping characters of conidiogenesis and setose pycnidia (Schneider 1979, Grondona et al. 1997). The teleomorphs of *Phoma* section *Paraphoma* and *Pleurophoma* are still unknown, whereas in the genus *Pyrenochaeta* two species have been associated with a teleomorph, *Py. parasitica* (teleom. *Herpotrichia parasitica*, von Freyer and van der Aa 1975) and *Py. berberidis* (teleom. *Cucurbitaria berberidis* [Pers.] S.F. Gray, Sivanesan 1984). *Pyrenochaeta berberidis* was excluded from the genus by Schneider (1979) because the pycnidial characteristics were different and setose pycnidia proved not to be a stable character. The multiple teleomorphs and the overlapping morphological characters suggest that these anamorph genera are polyphyletic.

To clarify the systematics of these groups, DNA sequence data of the SSU and LSU regions of 61 isolates representing the species currently classified in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma* were compared with those of 19 isolates representing related genera. The taxonomy of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma* is redefined, a classification at family level indicated and related teleomorph genera identified.

MATERIALS AND METHODS

Isolate selection, cultural studies and DNA extraction.—Generic abbreviations in this study include *Paraphoma* (*Pa*), *Phoma* (*Ph*), *Pleurophoma* (*Pl*), *Pyrenochaeta* (*Py*), *Pyrenochaetopsis* (*Pyr*) and *Setophoma* (*S*). Freeze-dried isolates were obtained from the culture collections of Centraalbureau voor Schimmelcultures (CBS) and the Dutch National Reference Laboratory of the Plant Protection Service (PD) (TABLE I). Isolates were revived overnight in 2 mL malt/peptone (50/50%) liquid medium. Isolates were transferred and maintained on oatmeal agar (OA) (Crous et al. 2009). Cultures growing on OA, malt extract agar (MEA) and cherry-decoction agar (CHA) were studied morphologically as described in Boerema et al. (2004). DNA extraction was conducted with the Ultraclean Microbial DNA Isolation

Kit (Mo Bio Laboratories, Carlsbad, California) according to manufacturer instructions. All DNA extracts were diluted 10 times in milliQ water and stored at 4 °C before use.

PCR and sequencing.—The SSU region was amplified with primers NS1 and NS4 (White et al. 1990), and the LSU region was amplified with primers LR0R (Rehner and Samuels 1994) and LR7 (Vilgalys and Hester 1990). PCR was performed and analyzed as described by de Gruyter et al. (2009). Sequencing of the PCR amplicons was conducted with the same primer combinations, although LR5 was used as an additional sequencing primer for LSU (Vilgalys and Hester 1990). Sequence products were purified with Sephadex G-50 Superfine (Amersham Biosciences, Rosendaal, the Netherlands) and analyzed with an ABI Prism 3730 × L Sequencer (Applied Biosystems) according to manufacturer instructions. Consensus sequences were computed from both forward and reverse sequences with the Bionumerics 4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium) and were lodged with GenBank.

Phylogenetic analyses.—Sequence data of 61 cultures generated in this study were aligned with sequences of 19 cultures obtained from GenBank (TABLE I). The alignment was calculated automatically by the BioNumerics software package, but manual adjustments for improvement were made by eye where necessary. The phylogeny was rooted to *Sporormiella minima* (CBS 524.50). The phylogenetic analyses were done for each dataset and based on similar tree topologies obtained, as well as with a combined alignment consisting of both SSU and LSU regions. A neighbor joining (NJ) distance analysis was conducted with PAUP* 4b10 (Swofford 2003) with the uncorrected “p”, Jukes-Cantor and Kimura 2-parameter substitution models. The robustness of the trees was evaluated by 1000 bootstrap replications. A Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) in two parallel runs, using the default settings but with these adjustments: GTR model with gamma-distributed rate was selected for both partitions with Findmodel freeware (<http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html>), and a MCMC heated chain was set with a temperature value of 0.05. The number of generations, sample frequencies and burn-in ratio were set respectively at 5 000 000, 10 and 0.1; the run was stopped automatically as soon as the average standard deviation of split frequencies fell below 0.01. Trees were printed with TreeView 1.6.6 (Page 1996) and alignments and trees are lodged with TreeBase (www.treebase.org), including those trees that were obtained for the single SSU and LSU datasets.

RESULTS

The aligned sequence matrix obtained for the SSU and LSU regions had a total length of 2633 nucleotide characters, 1332 (positions 1–1332 in the TreeBase alignment) and 1301 (positions 1333–2633 in the TreeBase alignment) respectively. In the alignment an insertion in the SSU at positions 440–796 was observed for two cultures of *Phialophora*

TABLE I. Fungal isolates included for SSU and LSU analysis

Species name, final identification	Formerly identified	Strain number	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Chaetodiplotidia</i> sp.		CBS 568.88	EU754043	EU754142	Rock	Israel
<i>Chaetopyrena penicillata</i>		CBS 498.72	GQ387512	GQ387573	<i>Medicago sativa</i> (<i>Leguminosae-Papilionoideae</i>)	South Africa
<i>Chaetosphaeronema coonsii</i> comb. nov.	<i>Phoma coonsii</i>	CBS 199.89 CBS 141.84 (T), CECT 20047, PD 78/241, ATCC 56513 CBS 216.75	GQ387513 GQ387514	GQ387574 GQ387575	soil <i>Malus sylvestris</i> (<i>Rosaceae</i>)	Turkey Japan, Mazoika
<i>Chaetosphaeronema hispidulum</i> (TS)		CBS 216.75	EU754045	EU754144	<i>Anthyllis vulneraria</i> (<i>Leguminosae-Papilionoideae</i>)	Germany
<i>Leptosphaeria dohioi</i> subsp. <i>dohioi</i> (teleom.)		CBS 505.75	GQ387515	GQ387576	<i>Urtica dioica</i> (<i>Urticaceae</i>)	Netherlands
<i>Neophaeosphaeria filamentosa</i> (teleom.)		CBS 102202	GQ387516	GQ387577	<i>Yucca rostrata</i> (<i>Agavaceae</i>)	Mexico
<i>Neosetophoma samarorum</i> comb. nov.	<i>Phoma samarorum</i> <i>Phoma samarorum</i> <i>Phoma samarorum</i>	CBS 568.94 CBS 138.96, PD 82/653 CBS 139.96, PD 82/905 CBS 240.31, ATCC 12279	GQ387519 GQ387517 GQ387518	GQ387580 GQ387578 GQ387579	<i>Urtica dioica</i> (<i>Urticaceae</i>) <i>Phlox paniculata</i> (<i>Polemoniaceae</i>) (<i>Graminae</i>)	Netherlands Netherlands Netherlands
<i>Ophiosphaerella herpotricha</i> (teleom.)		CBS 122788, PD 07/03486739	DQ767650	DQ767656	unknown	France
<i>Paraconiothyrium minitans</i>		CBS 101461	EU754074	EU754173	unknown	United Kingdom
<i>Paraconiothyrium</i> sp.		CBS 652.86	EU754101	EU754200	man, cutaneous lesions	USA, Texas
<i>Paraphaeosphaeria michoti</i> (teleom.)	<i>Pleurophoma pleurospora</i>	CBS 522.66 (NT)	GQ387520	GQ387581	<i>Typha latifolia</i> (<i>Typhaceae</i>)	Switzerland
<i>Paraphoma chrysanthemicola</i> nov.	<i>Phoma chrysanthemicola</i> <i>Phoma chrysanthemicola</i>	CBS 172.70	GQ387521	GQ387582	<i>Chrysanthemum morifolium</i> (<i>Asteraceae</i>)	United Kingdom
<i>Paraphoma fimeti</i> comb. nov.	<i>Phoma fimeti</i> <i>Phoma fimeti</i>	CBS 170.70 (NT), IMI 163514, ATCC 22707 CBS 368.91, PD 78/1096	GQ387522	GQ387583	<i>Chrysanthemum morifolium</i> (<i>Asteraceae</i>)	Germany
<i>Paraphoma radicina</i> (TS)	<i>Phoma radicina</i> <i>Phoma radicina</i>	CBS 111.79, IMI 386094, PD 76/437 CBS 102875, PD 78/ 1097	GQ387523	GQ387584	<i>Apium graveolens</i> (<i>Apiaceae</i>)	Netherlands
		CBS 377.52, ATCC 11321 CBS 335.87	GQ387524	GQ387585	<i>Juniperus communis</i> (<i>Cupressaceae</i>)	Switzerland
			EU754092	EU754191	<i>Malus sylvestris</i> (<i>Rosaceae</i>)	Netherlands
			EU754091	EU754190	<i>Lycopersicon esculentum</i> (<i>Solanaceae</i>)	Germany
			GQ387527	GQ387588	<i>Allium cepa</i> (<i>Alliaceae</i>)	Unknown
			GQ387528	GQ387589	<i>Allium cepa</i> (<i>Alliaceae</i>)	Senegal

TABLE I. Continued

Species name, final identification	Formerly identified	Strain number	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Phaeosphaeria caricicola</i> (teleom.)		CBS 603.86	GQ387529	GQ387590	<i>Carex pendula</i> (Cyperaceae)	Switzerland
<i>Phaeosphaeria nodorum</i> (teleom.)		CBS 110109	EU754076	EU754175	<i>Lolium perenne</i> (Gramineae)	Denmark
<i>Phaeosphaeria oryzae</i> (teleom.)		CBS 110110	GQ387530	GQ387591	<i>Oryza sativa</i> (Oryzaceae)	South Korea
<i>Phaeosphaeriopsis glaucopunctata</i> (teleom.)		CBS 653.86	GQ387531	GQ387592	<i>Ruscus aculeatus</i> (Ruscaceae)	Switzerland
<i>Phoma betae</i> , teleom. <i>Pleospora betae</i>		CBS 109410, PD 77/113	EU754079	EU754178	<i>Beta vulgaris</i> (Chenopodiaceae)	Netherlands
<i>Phoma carteri</i>		CBS 101633, PD 84/74	GQ387532	GQ387593	<i>Quercus</i> sp. (Fagaceae)	Netherlands
<i>Phoma gardeniae</i>		CBS 105.91	GQ387533	GQ387594	<i>Quercus robur</i> (Fagaceae)	Germany
		CBS 626.68, IMI 108771	GQ387534	GQ387595	<i>Gardenia jasminoides</i> (Rubiaceae)	India
<i>Phoma glycinicola</i>		CBS 302.79, PD 79/1156	GQ387535	GQ387596	airborne	Netherlands
		CBS 124455, IMI 294986	GQ387536	GQ387597	<i>Glycine max</i> (Leguminosae- <i>Papilionoideae</i>)	Antilles Zambia
<i>Phoma herbarum</i> (TS)		CBS 124141, PG-1	GQ387537	GQ387598	<i>Glycine max</i> (Leguminosae- <i>Papilionoideae</i>)	Zimbabwe
		CBS 615.75, IMI 199779	EU754087	EU754186	<i>Rosa multiflora</i> (Rosaceae), <i>Brassica</i> sp. (Brassicaceae)	Netherlands unknown
<i>Phoma lingam</i> , teleom. <i>Leptosphaeria maculans</i>		DAOM 229267	DQ470993	DQ470946		
<i>Phoma septicidialis</i>		CBS 188.71	GQ387538	GQ387599	air	Finland
		CBS 856.97	GQ387539	GQ387600	mineral wool	Finland
<i>Pyrenochaeta acicola</i>		CBS 101636, PD 86/1186	GQ387540	GQ387601	<i>Glycine max</i> (Leguminosae- <i>Papilionoideae</i>)	Zimbabwe
		CBS 812.95	GQ387541	GQ387602	waterpipe	Netherlands
<i>Pyrenochaeta berberidis</i> , teleom. <i>Cucurbitaria</i> <i>berberidis</i>		CBS 101634, PD 76/416	GQ387542	GQ387603	<i>Fragaria</i> (x) <i>ananassa</i> (Rosaceae)	Netherlands
		CBS 124142, PD 70/407	GQ387543	GQ387604	<i>Fragaria</i> (x) <i>ananassa</i> (Rosaceae)	Netherlands
<i>Pyrenochaeta cava</i> comb. nov.		CBS 394.84	GQ387544	GQ387605	<i>Berberis julianae</i> (Berberidaceae)	Netherlands
		CBS 363.93	GQ387545	GQ387606	<i>Berberis vulgaris</i> (Berberidaceae)	Netherlands
<i>Pyrenochaeta cava</i> comb. nov.		CBS 257.68, IMI 331911	EU754100	EU754199	wheat field soil	Germany
		CBS 115953	GQ387546	GQ387607	<i>Quercus cerris</i>	Italy

TABLE I. Continued

Species name, final identification	Formerly identified	Strain number	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Pyrenochaeta corni</i>	<i>Phialophorophoma litoralis</i> (TS)	CBS 248.79	GQ387547	GQ387608	<i>Fraxinus excelsior</i> (Oleaceae)	Netherlands
		CBS 234.92	EU754077	EU754176	<i>Olea europaea</i> (Oleaceae)	Italy
<i>Pyrenochaeta dolichi</i>	<i>Phialophorophoma litoralis</i>	CBS 102828	GQ387548	GQ387609	<i>Olea europaea</i> (Oleaceae)	Italy
		CBS 124143, IMI 217261	GQ387549	GQ387610	<i>Dolichos biforus</i> (Leguminosae-Papilionoideae)	India
<i>Pyrenochaeta lycopersici</i>		CBS 124140, IMI 217262	GQ387550	GQ387611	<i>Dolichos biforus</i> (Leguminosae-Papilionoideae)	India
		CBS 267.59	GQ387551	GQ387612	<i>Lycopersicon esculentum</i> (Solanaceae)	Netherlands
<i>Pyrenochaeta mackinnonii</i>		CBS 306.65	EU754106/ DQ898289	EU754205	<i>Lycopersicon esculentum</i> (Solanaceae)	Germany
		CBS 674.75 (T)	Q387552	GQ387613	black grain mycetoma, man	Venezuela
<i>Pyrenochaeta nobilis</i> var. <i>ilicis</i>		CBS 110022	GQ387553	GQ387614	mycetoma of patient	Mexico
		CBS 292.74	GQ387554	GQ387615	<i>Ilex aquifolium</i> (Aquifoliaceae),	Netherlands
<i>Pyrenochaeta nobilis</i>		CBS 566.75	GQ387555	GQ387616	<i>Buxus sempervirens</i> (Buxaceae)	Netherlands
		CBS 407.76 (NT)	EU754107/ DQ898287	EU754206	<i>Laurus nobilis</i> (Lauraceae)	Italy
<i>Pyrenochaeta parasitica</i> , teleom <i>Herpotrichia parasitica</i>		CBS 451.73 (T)	GQ387556	GQ387617	<i>Abies alba</i> (Pinaceae)	Germany
		CBS 218.77	GQ387557	GQ387618	<i>Abies alba</i> (Pinaceae)	Germany
<i>Pyrenochaeta quercina</i>	<i>Phialophorophoma litoralis</i>	CBS 115095	GQ387558	GQ387619	<i>Quercus robur</i> (Fagaceae)	Italy
		CBS 297.74	GQ387559	GQ387620	Sea water	Montenegro
<i>Pyrenochaeta romeroi</i>		CBS 252.60 (T), ATCC 13735	EU754108	EU754207	maduromycosis in man	Venezuela
		CBS 122784, PD 84/1022	EU754109	EU754208	<i>Hordeum vulgare</i> (Gramineae)	unknown
<i>Pyrenochaeta unguis-hominis</i>	<i>Phoma septicidialis</i>	CBS 378.92, IMI 227230	GQ387560	GQ387621	man, fingernail	United Kingdom
		CBS 112.79, IMI 386095, PD 74/1018	GQ387561	GQ387622	airborn	United Kingdom
<i>Pyrenochaetopsis decipiens</i> comb. nov.	<i>Phoma leveillei</i> var. <i>leveillei</i>	CBS 111112	GQ387562	GQ387623	<i>Agapornis</i> sp. (Aves), lung	Netherlands
		CBS 343.85, IMI 386097	GQ387563	GQ387624	<i>Globodera pallida</i> , cyst	Netherlands
<i>Pyrenochaetopsis indica</i> comb. nov.	<i>Phoma terricola</i>	CBS 165.89	GQ387564	GQ387625	<i>Hordeum vulgare</i> (Gramineae)	Netherlands
		CBS 124454, IMI 062569(b) (T)	GQ387565	GQ387626	<i>Saccharum officinalium</i> (Poaceae)	India

TABLE I. Continued

Species name, final identification	Formerly identified	Strain number	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Pyrenochaetopsis leptospora</i> comb. nov.	<i>Phoma briardii</i>	CBS 101635 (T), PD 71/1027	GQ387566	GQ387627	<i>Secale cereale</i> (<i>Gramineae</i>)	Europe
	<i>Pyrenochaeta aciicola</i>	CBS 122789, PD 07/ 03486800	EU754105	EU754204	<i>Hordeum vulgare</i> (<i>Gramineae</i>)	unknown
	<i>Phoma leveillei</i> var. <i>leveillei</i>	CBS 536.66	GQ387567	GQ387628	wheat field soil	Germany
	<i>Phoma leveillei</i> var. <i>leveillei</i>	CBS 131.69	GQ387568	GQ387629	dung of sheep	Netherlands
<i>Pyrenochaetopsis microspora</i> comb. nov.	<i>Phoma leveillei</i> var. <i>microspora</i>	CBS 101333	GQ387569	GQ387630	<i>Buellia</i> (lichen, ascomata)	China
	<i>Phoma leveillei</i> var. <i>microspora</i>	CBS 102876 (T), PD 75/911	GQ387570	GQ387631	water	Montenegro
	<i>Phoma leveillei</i> var. <i>microspora</i>	CBS 119739	GQ387571	GQ387632	<i>Coffea arabica</i> (<i>Rubiaceae</i>)	Brazil
<i>Setamelanomma holmii</i> (<i>teleom.</i>) nov.	<i>Phoma setariae</i>	CBS 110217	GQ387572	GQ387633	<i>Picea pungens</i> (<i>Pinaceae</i>)	USA, Wisconsin
<i>Setophoma sacchari</i> comb. nov.	<i>Phoma setariae</i>	CBS 333.39	GQ387525	GQ387586	<i>Saccharum officinarum</i> (<i>Poaceae</i>)	Brazil
<i>Setophoma terrestris</i> comb. nov.	<i>Phoma terrestris</i>	CBS 335.29	GQ387526	GQ387587	<i>Allium sativum</i> (<i>Alliaceae</i>)	USA
<i>Sporormiella minima</i>		CBS 524.50	DQ678003	DQ678056	dung of goat	Panama

T, Ex-type strain.

TS, Type species of the genus.

NT, Ex-neotype strain.

litoralis, *Phoma samarorum* and *Pyrenochaeta mackinnonii*, and a culture of *Ophiosphaerella herpotricha* (= *Ophiobolus herpotrichus*), *Phoma coonsii* and *Chaetosphaeronema hispidulum*. This insertion was excluded from further phylogenetic analyses. The combined dataset in the analyses included 80 taxa and contained 2276 characters with 76 and 193 unique site patterns for SSU and LSU respectively.

The PAUP* NJ analyses with the three substitution models showed similar tree topologies.

The analysis run in MrBayes resulted in 408 202 trees after 2 041 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 188 452 trees. For individual SSU and LSU alignments trees were compared by eye and the tree topology of the individual datasets was similar to each other and to the tree obtained from the combined alignment. The results of the PAUP* NJ analyses were congruent with those obtained in the Bayesian analysis.

The phylogenetic tree based on the combined LSU and SSU sequence data calculated with MrBayes (FIG. 1) demonstrated that *Phoma* sect. *Paraphoma* was highly polyphyletic in that the taxa involved segregated into four distinct clades in the Pleosporales. The type species *Ph. radicina* grouped with *Ph. chrysanthemicola* and *Ph. fimeti* (support > 70%) in a main clade B that represents the Phaeosphaeriaceae. In the same clade *Phoma terrestris* and *Ph. setariae*, two other species currently ascribed to *Phoma* sect. *Paraphoma*, clustered with *Phoma samarorum*, currently classified in *Phoma* section *Heterospora*. However the support (52%) of this subclade in the Phaeosphaeriaceae is low. *Phoma coonsii*, although being classified in *Phoma* section *Plenodomus*, also fits in the Phaeosphaeriaceae and is most related to *Chaetosphaeronema hispidulum* and the teleomorph *Phaeosphaeria caricicola*. The *Phoma* species that group in these different clades in the Phaeosphaeriaceae are related only distantly to *Phoma* species recently classified in the Didymellaceae (de Gruyter et al. 2009) and therefore *Phoma* sect. *Paraphoma* is elevated here to generic level by reinstatement of genus *Paraphoma* previously described by Morgan-Jones and White (1983). The only member of *Phoma* sect. *Paraphoma* clustering with *Phoma herbarum* in the Didymellaceae was *Phoma gardeniae*, which produces pycnidia with short setae. Also *Chaetopyrena penicillata*, characterized by setose pycnidia and *Phoma*-like conidiogenous cells, grouped with *Ph. herbarum* in the Didymellaceae.

The other seven species currently classified in *Phoma* sect. *Paraphoma* clustered in clades that are not related to the Phaeosphaeriaceae or to the Didymellaceae. *Phoma carteri* clustered in clade C

with an isolate identified as *Chaetodiplodia* sp. being the most related species. *Pyrenochaeta glycinicola* and *Py. dolichii*, both pathogens on Leguminosae and of tropical origin, and *Ph. septicidalis* also grouped in this clade.

Phoma leveillei var. *leveillei* has been recorded as a cosmopolitan soil fungus, a heterogeneous species with much variability in morphological and physiological characters (Boerema et al. 2004). Our molecular data revealed that *Ph. leveillei* var. *leveillei* is indeed a species complex and moreover included several isolates preserved under an incorrect name. Two isolates obtained respectively from wheat field soil and sheep dung (CBS 536.66, 131.69) were phylogenetically identical to *Phoma briardii* (clade A), a morphologically similar species distinguished only by a minor difference in conidial length (de Gruyter and Boerema 2002). An isolate preserved as *Pyrenochaeta acicola* (CBS 122789), a synonym of *Ph. leveillei* var. *leveillei*, was similar to *Ph. briardii*. *Phoma leveillei* var. *microspora*, *Ph. terricola* and *Ph. indica* also could be attributed to this group but both are slightly different morphologically and genetically. Most species in this group are soilborne organisms and frequently found in association with members of the Gramineae. This group formed a distinct subclade in clade A, which mainly includes *Pyrenochaeta* species, among those the type species of the genus, *Py. nobilis*. Three other *Ph. leveillei* isolates (CBS 812.95, 101634, 124142), one isolated from a waterpipe and two from *Fragaria* (×) *ananassa* respectively, also could be recognized in clade A, which further include the *Pyrenochaeta* species *Py. lycopersici*, *Py. parasitica* and *Py. berberidis*.

Isolate CBS 111112, preserved as *Phoma leveillei* var. *leveillei* and obtained from a lung of a lovebird (*Agapornis* sp.), was genetically similar to the human pathogen *Pyrenochaeta unguis-hominis*. Both species share in vitro characters, but their conidiogenesis was considered to be significantly different and therefore the species were classified respectively in *Phoma* (de Gruyter and Boerema 2002) and *Pyrenochaeta* (Punithalingam and English 1975). However re-examination of this *Ph. leveillei* var. *leveillei* isolate revealed that in addition to the *Phoma*-like conidiogenous cells filiform, septate, acropleurogenous conidiophores are produced in pycnidia that are covered by relatively short setae. Similar setose pycnidia with both types of conidiogenesis also were observed in the *Pyrenochaeta unguis-hominis* isolate IMI 227230 = CBS 378.92 (FIGS. 2–4). Branched, septate conidiophores also have been described as “sometimes present” in *Phoma septicidalis* (Kinsey 2002). Isolate IMI 386095 = CBS 112.79, obtained from an air sample and originally identified as *Phoma septicidalis* (de Gruyter

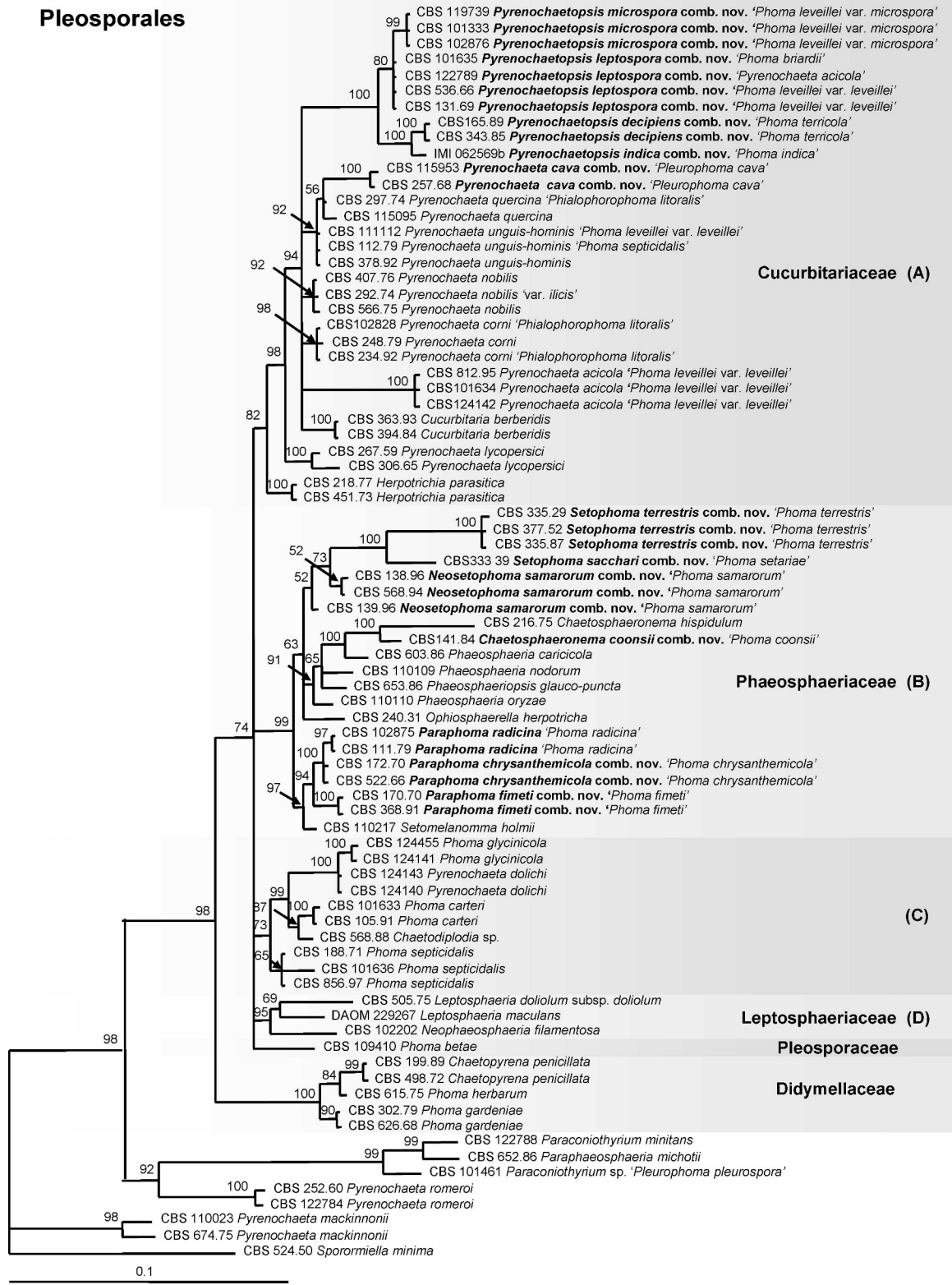
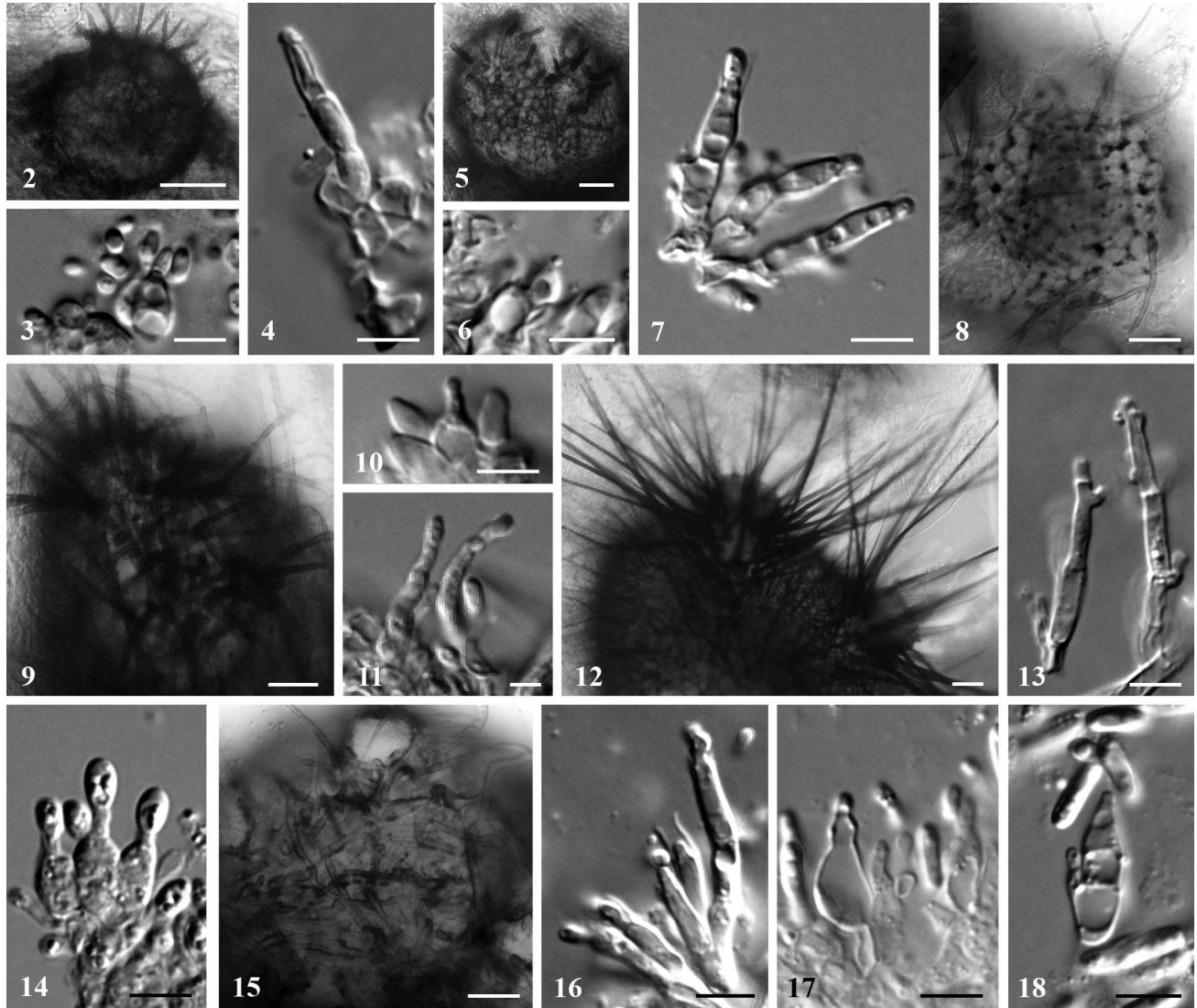


FIG. 1. The phylogenetic relationships of species in genus *Paraphoma*, *Pyrenochaeta*, *Pyrenochaetopsis* gen. nov. and related genera based on the strict consensus tree from a Bayesian analysis of 80 LSU/SSU sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted with *Sporormiella minima* (CBS 524.50).



FIGS. 2–4. *Pyrenochaeta unguis-hominis* IMI 227230 = CBS 378.92. 2. Setose pycnidium. 3. *Phoma*-like conidiogenous cells. 4. Elongate, septate conidiophore.

FIGS. 5–7. *Pyrenochaeta unguis-hominis* IMI 386095 = CBS 112.79, originally identified as *Phoma septicialis*. 5. Setose pycnidium. 6. *Phoma*-like conidiogenous cells. 7. Elongate, septate conidiophores with acropleurogenous conidiogenesis and a discrete collarette.

FIG. 8. *Pyrenochaeta cava* comb. nov., CBS 115953. Pycnidium with setae-like mycelial hairs.

FIGS. 9–11. *Pyrenochaeta corni*. CBS 248.79. 9. Setose pycnidium. 10. *Phoma*-like conidiogenous cells. 11. Elongate conidiogenous cells.

FIGS. 12–13. *Pyrenochaeta nobilis* CBS 292.74. 12. Setose pycnidium. 13. Elongate, septate conidiophores with acropleurogenous conidiogenesis.

FIG. 14. *Pyrenochaeta acicola*, CBS 101634. *Phoma*-like conidiogenous cells.

FIGS. 15–16. *Pyrenochaeta corni*, CBS 234.92, originally identified as *Phialophorophoma litoralis*. 15. Setose pycnidium. 16. Elongate, septate conidiophores, with acropleurogenous conidiogenesis, and discrete collarette.

FIGS. 17–18. *Pyrenochaetopsis leptospora* comb. nov. CBS 101635. 17. *Phoma*-like conidiogenous cells. 18. Elongate conidiogenous cells.

Bars: 2, 12 = 50 μ m; 3–4, 6–7, 10–11, 13–14, 16–18 = 5 μ m; 5, 8–9, 15 = 20 μ m.

and Boerema 2002), was congruent with *Py. unguis-hominis* in the present study and indeed produced branched, septate, acropleurogenous conidiophores (FIGS. 5–7). *Pyrenochaeta unguis-hominis* has been

reported from human nails thus far, but these findings suggest that *Py. unguis-hominis* is a more common species that might be dispersed through air. *Pyrenochaeta quercina* and an isolate that originally

was identified as *Phialophorophoma litoralis* (CBS 297.74) grouped in the same subclade, whereas both isolates of *Pleurophoma cava* clustered in a closely related subclade. *Pleurophoma cava* has been described as producing glabrous pycnidia with two types of conidiogenesis similar to *Pyrenochaeta* or more *Phoma*-like (Boerema et al. 1996). However in a recently obtained isolate, CBS 115953, the development of pycnidia with setae-like mycelial hairs has been observed in vitro (FIG. 8). The combined characters of two types of conidiogenesis produced in setose pycnidia also have been observed further in *Pyrenochaeta corni* (FIGS. 9–11). Based on these findings *Pl. cava* is transferred to *Pyrenochaeta* in TAXONOMY. Two isolates identified as *Phialophorophoma litoralis* (CBS 234.92, 102828) clustered with the reference isolate of *Pyrenochaeta corni* (CBS 248.79) in clade A. The human pathogens *Pyrenochaeta romeroi* and *Py. mackinnonii* clustered in separate clades only distantly related to the type species, *Py. nobilis*, and therefore have to be excluded from the genus *Pyrenochaeta*, as was suggested based on in vitro characters by Schneider (1979). Isolate CBS 101461, identified as *Pleurophoma pleurospora*, grouped in the *Paraconiothyrium/Paraphaerosphaeria* cluster as described by de Gruyter et al. (2009) and therefore is treated here as a species of *Paraconiothyrium*.

TAXONOMY

A modified classification of *Phoma* species currently included in *Phoma* sect. *Paraphoma* is provided below. The genus *Paraphoma* is reintroduced, and two new related genera, *Setophoma* and *Neosetophoma*, are described. *Pyrenochaetopsis* gen. nov. is introduced here to accommodate the species closely related to *Pyrenochaeta* in the Cucurbitariaceae.

Chaetosphaeronema coonsii (Boerema & Loer.)

Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514647

Basionym: *Phoma coonsii* Boerema & Loer., Trans. Br. mycol. Soc. 84:289. 1985.

Specimens examined: JAPAN, Mazioka. On bark of *Malus pumila* (Starking[®] Delicious), 1978, *H. Koganezana* (HOLOTYPE CBS-H 141.84, culture ex-holotype CBS 141.84 = PD 78/241 = ATCC 56513 = CECT 20047).

Notes: The ex-type culture was obtained from *Malus pumilae* (Starking[®] Delicious), an American apple tree variety, growing in Japan. The fungus was considered to be *Plenodomus fusco-maculans* (Sacc.) Coons (= *Phoma fusco-maculans* Sacc. = *Aposphaeria fuscomaculans* (Sacc.) Sacc. However the type material of *A. fuscomaculans*, preserved in Saccardo's herbarium (PAD), is a species of *Aposphaeria* (Boerema and

Loerakker l.c.), and therefore it was concluded that Coons' *Plenodomus fusco-maculans* was a misidentification. The name *Phoma coonsii* was introduced for "*Plenodomus fusco-maculans*" sensu Coons, J. Agric. Res. 5:714. (1916), Rep. Mich. Acad. Sci. 17:122 (1916) (Boerema and Loerakker, l.c.). LSU and SSU sequences of *Phoma coonsii* CBS 141.84 (= CBS 559.78, deposited as *Phoma fusco-maculans*) obtained in this study were different from those of isolate CBS 116.16 ex *Malus* sp. USA, deposited by Coons (de Gruyter et al. 2009), so the Japanese isolate is described as *Chaetosphaeronema coonsii*, a distinct species.

Neosetophoma Gruyter, Aveskamp & Verkley, gen. nov. Mycobank MB514648

Pycnidia solitaria vel confluentia, superficialia in agar, globosa vel irregularia, eminentiis hypharum vestita, ostioliis papillatis, interdum collis longis, mellea vel olivacea/olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discretatae. Conidia pallide lutea, continua vel septata, ellipsoidea vel cylindrica, plerumque una extremitate attenuata, saepe guttulata.

Pycnidia solitary to confluent, on upper surface of agar, globose to irregular, with mycelial outgrowths, with papillate ostioles, sometimes developing long necks, honey to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete. Conidia slightly yellowish, aseptate, or septate, ellipsoidal to cylindrical, usually attenuate at one end, often guttulate.

Etymology: *neo* Lat. = new. Refers to the similarity to *Setophoma*.

Type species: *Neosetophoma samarorum* (Desm.) Gruyter, Aveskamp & Verkley.

Neosetophoma samarorum (Desm.) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514649

Basionym: *Phoma samarorum* Desm., Pl. cryptog. N. France [ed. 1] Fasc. 7, No. 349. 1828.

For a full synonymy see Boerema et al. (2004).

Note: A pycnidial dimorph with large stagonosporoid conidia that are 1–3-septate, up to 25 µm long and 3.5 µm wide, has been described as *Stagonosporopsis fraxini* (Allesch.) Died.

Specimens examined: FRANCE, *Fraxinus excelsior*, 1828, *Desmazières*, Pl. cryptog. N. France [ed. 1] Fasc. 7, No. 349 P (HOLOTYPE); THE NETHERLANDS, Bladel. On *Phlox paniculata*, 1982, *G.H. Boerema* (EPITYPE designated here CBS H-20319, culture ex-epitype CBS 138.96).

Paraphoma Morgan-Jones & J.F. White, Mycotaxon 18:58. 1983.

= *Phoma* sect. *Paraphoma* (Morgan-Jones & J.F. White) Boerema, van der Aa et al., Stud. Mycol. 32:7. 1990.

Type species: *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White.

Paraphoma radicina (McAlpine) Morgan-Jones & J.F. White, Mycotaxon 18:60. 1983.

Basionym: *Pyrenochaeta radicina* McAlpine, Fung. Dis. Stone-fruit-trees Melb. 127. 1902.

= *Phoma radicina* (McAlpine) Boerema, Boerema & Dorenbosch, Versl. Meded. plziektenk. Dienst Wageningen 153 (Jaarb. 1978):20. 1979.

Specimens examined: AUSTRALIA, Shepparton, Victoria. On roots of *Prunus cerasus*, 21 Oct 1901, *Piscott*, 2064.3 VPRI (HOLOTYPE); THE NETHERLANDS, Wanssum. On *Malus sylvestris*, grafting base "M 106", Jun 1976, *G.H. Boerema* (EPITYPE designated here CBS H-16560, culture ex-epitype CBS 111.79 = IMI 386094 = PD 76/437); GERMANY. On *Lycopersicon esculentum*, 1976, *G.H. Boerema* (culture CBS 102875 = PD 76/1097).

Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley, comb. nov.

MycoBank MB514650

Basionym: *Phoma chrysanthemicola* Hollós, Anns hist.-nat. Mus. natn hung. 5:456. 1907.

= *Phoma radialis-oxycocci* Ternetz, Jb. wiss. Bot. 44:365. 1907.

Specimens examined: UNITED KINGDOM, Kent. On stem of *Chrysanthemum morifolium*, 1963, *H.J. Wilcox* (NEOTYPE CBS H-5161, culture ex-neotype CBS 522.66); GERMANY, Berlin. On root of *Chrysanthemum morifolium*, Oct 1967, *R. Schneider* (culture CBS 172.70).

Note: Dorenbosch (1970) selected a neotype because the original herbarium material had been lost.

Paraphoma fimeti (Brunaud) Gruyter, Aveskamp & Verkley, comb. nov.

MycoBank MB514651

Basionym: *Phoma fimeti* Brunaud, Bull. Soc. bot. Fr. 36 [= Vol II, ser. 11]: 338. 1889.

Specimens examined: THE NETHERLANDS, Zwijndrecht, from soil, Dec 1966, *M.A. de Waard* (NEOTYPE CBS H-2011, culture ex-neotype CBS 170.70); SWITZERLAND, on *Juniperus communis*, Jun 1991, *G.H. Boerema* (culture CBS 368.91 = PD 78/1096).

Note: The original herbarium material was lost so a neotype was designated (Dorenbosch 1970).

Pyrenochaeta acicola (Moug. & Lév.) Sacc., Sylloge Fung. 3:220. 1884 [as '(Lév.) Sacc.'].]

Basionym: *Vermicularia acicola* Moug. & Lév. apud Léveillé, Anns Sci. nat. (Bot.) III, 9:259. 1848 (as 'Moug. Lév. '; not *Phoma acicola* sensu Saccardo), Sylloge Fung. 3:100. 1884 [as '(Lév.) Sacc. '; = *Sclerophoma pythiophila* (Corda) Höhn.].

= *Phoma leveillei* Boerema & G.J. Bollen, Persoonia 8:115. 1975, var. *leveillei*.

For a full synonymy see Boerema et al. (2004).

Specimens examined: THE NETHERLANDS, Someren. On waterpipe, Dec 1995, *Y. Driessen* (NEOTYPE designated here CBS H-20314, culture ex-epitype CBS 812.95); THE NETHERLANDS, Arnhem. On *Fragaria* (×) *ananassa*, 1976, *M.M.J. Dorenbosch* (culture CBS 101634 = PD 76/416); THE NETHERLANDS. On *Fragaria* (×) *ananassa*, 1970, *M.M.J. Dorenbosch* (culture CBS 124142 = PD 70/407).

Note: The original herbarium material of *Vermicularia acicola* Moug. & Lév. could not be located and is presumed missing so a neotype was designated herein.

Pyrenochaeta cava (Schulzer) Gruyter, Aveskamp & Verkley, comb. nov.

MycoBankMB514652

Basionym: *Phoma cava* Schulzer, Verh. Zool.-bot. Ges. Wien 21:1248. 1871.

= *Pleurophoma cava* (Schulzer) Boerema, Loerakker & Hamers, Persoonia 16:172. 1996.

For full synonymy see Boerema and Dorenbosch (1973) and van der Aa and Vanev (2002).

Specimens examined: CROATIA, Vinkovci, from *Cydonia vulgaris*, 1871, *Schulzer von Muggenburg*, illustrated plate 13, fig 28 (HOLOTYPE); GERMANY, Kiel-Kitzeberg, from wheatfield soil (EPITYPE designated here CBS H-20320, culture ex-epitype CBS 257.68 = IMI 331911); ITALY, on branch of *Quercus cerris* (culture CBS 115953).

Pyrenochaetopsis Gruyter, Aveskamp & Verkley, gen. nov.

MycoBank MB514653

Pycnidia solitaria vel confluentia, superficialia vel in agaro immersa, globosa vel subglobosa, setosa, ostiolis papillatis vel non-papillatis, mellea/citrina vel olivacea/olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discretiae, vel integratae in conidiophoris acropleurogenis septatis. Conidia continua, cylindrica/ellipsoidea vel allantoidea, guttulate.

Pycnidia solitary to confluent, on upper surface or submerged in agar, globose to subglobose, setose, with non-papillate or papillate ostiole, honey/citrine to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete, or integrated in septate, acropleurogenous conidiophores. Conidia aseptate, cylindrical/ellipsoidal to allantoid, guttulate.

Etymology: *-opsis* Greek = resembling, refers to the resemblance to *Pyrenochaeta*.

Type species: *Pyrenochaetopsis leptospora* (Sacc. & Briard) Gruyter, Aveskamp & Verkley.

Pyrenochaetopsis leptospora (Sacc. & Briard) Gruyter, Aveskamp & Verkley, comb. nov.

MycoBankMB514654

Basionym: *Pyrenochaeta leptospora* Sacc. & Briard, Revue Mycol. 11:16. 1889; not *Pyrenochaeta leptospora* Speg., Ann. Mus. Buenos Aires 20:354. 1910; not *Phoma leptospora* Speg., Fungi Chilens. 145. 1910, nor *Phoma leptospora* Sacc., Annls Mycol. 11:553. 1913.

≡ *Pyrenochaeta spagazziniana* Trotter, Sylloge Fungi. 25: 190. 1931 (illegitimate: superfluous name, Boerema et al. 2004).

≡ *Phoma briardii* Gruyter & Boerema, Persoonia 17: 555. 2002 ['2001'].

Specimens examined: FRANCE, near Troyes. On stem pieces of *Milium effusum*, May 1888, HOLOTYPE coll. P.A. Briard 'no. 6' PAD; THE NETHERLANDS, on *Secale cereale* (EPITYPE designated here CBS H-20313, culture ex-epitype CBS 101635 = PD 71/1027); GERMANY, Kiel, Kitzberg. On wheatfield soil (CBS 536.66); THE NETHERLANDS. On sheep dung, 27 Oct 1968, van der Aa H.A. (culture CBS 131.69); THE NETHERLANDS. On *Hordeum vulgare*, J.W. Veenbaas (culture CBS 122789 = PD 07/03486800).

Pyrenochaetopsis decipiens (Marchal) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514655

Basionym: *Pyrenochaeta decipiens* Marchal, Bull. Soc. Roy. Bot. Belg. 30:139. 1891, not *Phoma decipiens* Mont., Fl. chil. cell. 7:488. 1852.

= *Phoma terricola* Boerema, Versl. Meded. plziektenk. Dienst Wageningen 163 (Jaarb. 1984):38. 1985. Not *Phoma terricola* 'Agnihotrudu', Soil Sci. 91: 135. 1961 [nomen nudum erroneously adopted in Mathur, Coelom. India:185. 1979].

Specimens examined: THE NETHERLANDS, Hoofdorp. On cyst of *Globodera pallida*, May 1985, D. Hugo, No. 727 (NEOTYPE CBS H-20315, culture ex-neotype CBS 343.85 = IMI 386097); THE NETHERLANDS, Marknesse. On roots of *Hordeum vulgare*, M. Barth, nr. 830, 1988 (culture CBS 165.89).

Note: The original herbarium material is lost, so a neotype was designated by Schneider (1979).

Pyrenochaetopsis indica (T.S. Viswan.) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514656

Basionym: *Pyrenochaeta indica* T.S. Viswan., Curr. Sci. 26(4):118. 1957.

≡ *Phoma indica* (T.S. Viswan.) Gruyter & Boerema, Persoonia 17:556. 2002.

Specimen examined: INDIA, Poona. On leaf spot of *Saccharum officinarum* (HOLOTYPE AMH-11, culture ex-holotype IMI 062569 = CBS 124454).

Pyrenochaetopsis microspora (Gruyter & Boerema) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514657

Basionym: *Phoma leveillei* var. *microspora* Gruyter & Boerema, Persoonia 17:553. 2002 ['2001'].

Specimens examined: MONTENEGRO. Lake of Skadar, water, 1975 (HOLOTYPE HLB 999-242399, culture ex-type CBS 102876 = PD 75/911); CHINA, Hong Kong. Lichen *Buellia*, Jun 1998, A. Aptroot (culture CBS 101333); BRAZIL, Minas Geras, Lavras. On leaf of *Coffea arabica*, L.H. Pfenning (culture CBS 119739).

Setophoma Gruyter, Aveskamp & Verkley, gen. nov. Mycobank MB514658

Pycnidia solitaria vel confluentia, superficialia vel in agar immersa, globosa vel subglobosa, setosa, ostioli papillatis, mellea vel olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discrete. Conidia continua, ellipsoidea vel subcylindrica/subfusioidea, guttulate.

Pycnidia solitary to confluent, on upper surface or submerged in agar, globose to subglobose, setose, with papillate ostioles, honey to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete. Conidia aseptate, ellipsoidal to subcylindrical/subfusoid, guttulate.

Etmology: *seto* Lat. = bristle. Refers to the setose pycnidia.

Type species: *Setophoma terrestris* (H.N. Hansen) Gruyter, Aveskamp & Verkley.

Setophoma terrestris (H.N. Hansen) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514659

Basionym: *Phoma terrestris* H.N. Hansen, Phytopathology 19:699. 1929.

≡ *Pyrenochaeta terrestris* (H.N. Hansen) Gorenz, J.C. Walker & Larson, Phytopathology 38:838. 1948; not *Phoma terrestris* R.K. Saxena, Nand & A.K. Sarbhoy, Mycopath. Mycol. Appl. 29:86. 1966 (= *Phoma multirostrata* Boerema).

Specimens examined: NORTH AMERICA, on root of *Allium sativum*, Dec 1929, H.N. Hansen (LECTOTYPE designed here CBS H-20311, culture ex-lectotype CBS 335.29); NORTH AMERICA, on root of *Allium cepa*, Aug 1952, R.H. Larson (culture CBS 377.52); SENEGAL, Dakar, on *Allium cepa*, May 1987 (culture CBS 335.87).

Note: Type material was not indicated in the original description (Hansen 1929) and therefore a dried specimen of isolate CBS 335.29, deposited by the author, is designated here as lectotype.

Setophoma sacchari (Bitancourt) Gruyter, Aveskamp & Verkley, comb. nov.

Mycobank MB514660

Basionym: *Pyrenochaeta sacchari* Bitancourt, Arquivos Inst. Biol., Sao Paulo 9:301. 1938.

= *Pyrenochaeta setariae* H.C. Greene, Trans. Wis. Acad. Sci. Arts Lett. 53:211. 1964.

= *Pyrenochaeta penniseti* J. Kranz, Sydowia 22:360. 1968.

= *Phoma setariae* (H.C. Greene) Gruyter & Boerema, *Persoonia* 17: 559. 2002 ['2001'].

Specimens examined: BRAZIL, Cantareira, Sao Paulo. On leaves of *Saccharum officinarum*, 11–13 Oct 1937, nr. 2769, IBI, coll. A.A. Bitancourt (HOLOTYPE). On leaves of *Saccharum officinarum* var. EK28, Jul 1939, nr 3064, A.A. Bitancourt (EPITYPE designed here, CBS H-20312, culture ex-epitype CBS 333.39).

DISCUSSION

Phoma section *Paraphoma* was shown to be polyphyletic in the Pleosporales based on the SSU and LSU nrDNA phylogeny obtained in this study. *Phoma radicina*, the type species of section *Paraphoma* (Morgan-Jones and White 1983), grouped with two related *Phoma* species in a subclade being assigned to the Phaeosphaeriaceae. The anamorph genus *Paraphoma* is re-introduced here and comprises *Pa. radicina*, *Pa. chrysanthemicola* and *Pa. fimeti*. The conidiogenous cells are typical for *Phoma*: phialidic, ampulliform to doliiform. *Paraphoma radicina* and *Pa. chrysanthemicola* produce pycnidia with long setae, also interpreted as mycelial hairs, exceeding 200 µm. However this character is variable in the genus *Paraphoma* because *Pa. fimeti* produces glabrous pycnidia. The phylogenetic relationship of *Pa. radicina* and *Pa. chrysanthemicola* is congruent with a similar ecological niche as soilborne organisms that often are associated with root surfaces in temperate zones in Australia, Eurasia and North America. *Paraphoma fimeti* is also a soilborne fungus often isolated from dead tissue of herbaceous and woody plants. *Paraphoma chrysanthemicola* originally was described in *Phoma* section *Peyronellaea* based on its formation of aggregates chlamydo-spores and pseudo-scleroid masses (Boerema 1993). However the type species of this section, *Ph. glomerata*, belongs in the Didymellaceae (de Gruyter et al. 2009), as do the majority of the other species classified in *Phoma* sect. *Peyronellaea* (Aveskamp et al. 2009). The closest teleomorph we could find for these three species was the monotypic genus *Setomelanomma* (Rossman et al. 2002). *Setomelanomma holmii* recently was re-described and placed in the Phaeosphaeriaceae, supported by SSU rDNA phylogeny (Rossman et al. 2002). This species with unknown anamorph is associated with needle chlorosis and needle drop of *Picea pungens* and *P. glauca* in Europe and North America.

The genera *Setophoma* and *Neosetophoma* are introduced here, although the subclade that comprises both genera is weakly supported (52%). The species in both genera are related only distantly to the well supported *Paraphoma* clade. A relation with other coelomycetes or with a teleomorph could not

be found, and the species certainly could not be maintained in the genus *Phoma*, classified in the Didymellaceae. Therefore we introduced both genera to accommodate these species in the Phaeosphaeriaceae, although more phylogenetic relatives need to be found for a better understanding of this clade. *Setophoma terrestris* and *S. sacchari* have been recorded associated with monocotyledonous plants. *Neosetophoma samarorum*, type species of the genus, is closely related genetically to these species but morphologically distinct based on its typically yellowish conidia that are attenuate at one end and the stagonosporoid synanamorph, *Stagonosporopsis fraxini*. The phylogeny of the *Neosetophoma samarorum* isolates suggest a species complex to be studied in more detail. The genera *Paraphoma*, *Setophoma* and *Neosetophoma* grouped in sister clades closely related to the teleomorph genera *Ophiosphaerella*, *Phaeosphaeria* and *Phaeosphaeriopsis*, mainly recorded on Poaceae. The *Phaeosphaeria* species included in this study represent “clade B” in an 18S rDNA sequence analysis by Câmara et al. (2002). The associated anamorphs in this clade are variable and described as “*Stagonospora*-like”, “*Coniothyrium*-like”, or as *Microsphaeropsis* sp. (Câmara et al. 2002, 2003). In some species an *Aposphaeria*-like or *Phoma*-like synanamorph has been observed in vitro (Leuchtmann 1984).

Although *Chaetosphaeronema hispidulum* and *C. coonsii* proved to be closely related to teleomorphs *Phaeosphaeria* and *Phaeosphaeriopsis*, both species are recorded on dicotyledons. The genus *Chaetosphaeronema* is characterized by setose, dark brown pycnidia with thick-walled outer cell layers, producing hyaline, septate conidia (Sutton 1980). In addition the thick-walled cells of the pycnidia of *Chaetosphaeronema coonsii* may become scleroplectenchymatous and the conidia produced are always aseptate (Boerema et al. 2004). Re-examination of the ex-type culture of *C. coonsii* revealed that the pycnidia are covered by brown mycelial hairs, resembling setae.

Pyrenochaeta species with distinct elongated, septate, acropoleurogenous conidiophores produced in pycnidia usually covered by long setae, 200 µm or more, grouped in several related subclades in clade A, such as *Py. nobilis*, the type species of the genus (FIGS. 12, 13), *Py. lycopersici* and *Py. parasitica* (teleom. *Herpotrichia parasitica*). Furthermore *Py. berberidis* (teleom. *Cucurbitaria berberidis*) also grouped among these species. The teleomorph genera *Cucurbitaria* and *Herpotrichia* have been classified respectively in the Cucurbitariaceae and Lophiostomataceae (Barr 1987). In molecular studies it was demonstrated that *C. berberidis*, being the type species of *Cucurbitaria*, was only distantly related to

Herpotrichia juniperi and *Herpotrichia diffusa*, both species with an unknown anamorph (Berbee 1996, Silva-Hanlin and Hanlin 1999). In a subsequent molecular study *H. juniperi* and *H. diffusa* were classified in the Melanommataceae (Câmara et al. 2003). The basal clustering of *H. parasitica* with *Cucurbitaria berberidis* in the Cucurbitariaceae demonstrate that both species with a *Pyrenochaeta* anamorph are related and that *H. parasitica* does not fit in genus *Herpotrichia*. *Phoma leveillei* var. *leveillei* proved to be a species complex, and three isolates, all producing pycnidia covered by long setae, group in *Pyrenochaeta*; the correct name for these isolates is *Pyrenochaeta acicola*. The conidiogenesis is *Phoma*-like (FIG. 14), and it is likely that the further development into branched, filiform, septate conidiophores is a lost character in *Py. acicola*. Therefore this species morphologically has more affinity with the species described here in the genus *Paraphoma* because the latter produce only *Phoma*-like conidiogenous cells. In clade A two subclades comprise *Pyrenochaeta* species with pycnidia covered by relatively short setae, up to 100 µm, producing branched, filiform, septate, acropleurogenous conidiophores as well as *Phoma*-like conidiogenous cells. Species included are *Pyrenochaeta unguis-hominis*, *Py. quercina* and *Py. corni*.

Two cultures preserved as *Phialophorophoma litoralis* (CBS 234.92, 102828) were similar to *Py. corni* according to the SSU/LSU sequences and morphological characters. This finding was confirmed by sequence analyses of the Actin, β-tubulin genes and ITS 1 and 2 regions (data not shown). The characters observed in both isolates, including the formation of setose pycnidia and conidiogenesis (FIGS. 15–16), confirmed that these isolates belong to *Py. corni*. A third *Phialophorophoma litoralis* culture (CBS 297.74) clustered with *Py. quercina*. It is likely that *Phialophorophoma litoralis*, producing glabrous pycnidia (Linder 1944, Sutton 1980), refers to a different species.

The presence of two types of conidiogenesis is also a key character for genus *Pleurophoma* (Boerema et al. 2004). Therefore it was suggested that *Py. corni* could be considered a “setose” species of *Pleurophoma*, having more affinity with *Phoma* than with true species of *Pyrenochaeta* (de Gruyter and Boerema 2002). However the present study demonstrated that both *Py. corni* and *Pleurophoma cava* can be attributed to genus *Pyrenochaeta*.

The novel genus *Pyrenochaetopsis* is characterized by setose pycnidia similar to those of the genus *Pyrenochaeta*, but the conidiogenesis is usually *Phoma*-like because only incidentally elongated, septate conidiophores may be observed (FIGS. 17, 18). The genus *Pyrenochaetopsis* morphologically resembles the genus

Paraphoma based on *Phoma*-like conidiogenesis. However both genera are only distantly related based on the molecular phylogeny and grouped in two distinct families (i.e. the Cucurbitariaceae and Phaeosphaeriaceae respectively). Morphological criteria for delimitation of both genera are limited, and the genus *Pyrenochaetopsis* so far includes only species that were formerly described in *Phoma* section *Paraphoma*, such as *Phoma leveillei* var. *microspora*, *Ph. briardii*, *Ph. terricola*, *Ph. indica* and *Ph. leveillei* var. *leveillei*. In general the conidia of species in the genus *Pyrenochaetopsis* are relatively narrow, usually not exceeding 2 µm, similar to those of the *Pyrenochaeta* species included in this study but narrower than those observed in *Paraphoma*. These species are reclassified here in the new genus *Pyrenochaetopsis* as *Pyr. microspora*, *Pyr. leptospora*, *Pyr. decipiens* and *Pyr. indica*. These species are all soilborne and mainly associated with gramineous plants. The species accommodated in the subclades in clade A share ecological and morphological characters. The *Pyrenochaeta* species *Py. corni*, *Py. quercina* and *Py. cava* are found mostly as endophytes or saprobes on dicotyledons, trees and shrubs in particular. *Pyrenochaeta nobilis*, *Py. acicola*, *Py. berberis*, *Py. lycopersici* and *Py. parasitica* are recognized as plant pathogens on dicotyledons. These data demonstrate a parallel evolution on gramineous plants of the *Pyrenochaetopsis* species in clade A and *Setophoma* spp. in clade B.

The phylogeny generated here demonstrates that the presence and appearance of some morphological characteristics, such as setose pycnidia and conidiogenous cells can be variable in *Pyrenochaeta* and *Pyrenochaetopsis*, probably due to degeneration or even loss of characters. The combination of elongated conidiogenous cells and setose pycnidia is not found in all species involved. Two other *Pyrenochaeta* species in this study, the human pathogens *P. romeroi* and *P. mackinnonii*, were found in distinct clades in the Pleosporales, unrelated to the type species of *Pyrenochaeta*. Both species can be differentiated from genus *Pyrenochaeta* by non-septate phialidic conidiogenous cells and conidia that are catenulate or in chains, small, not exceeding 3 × 2 µm (Borelli 1959, 1976). A GenBank BLAST analysis of LSU and ITS sequences (data not shown) of *Py. romeroi* and *Py. mackinnonii* revealed only a relatively high similarity for the LSU sequence with *Versicolorisporium triseptatum* (AB330081), a morphologically different coelomycete recently described from bamboo in Japan (Hatakeyama et al. 2008). New genera to accommodate both species have to be introduced, but their phylogenetic relatives first need to be clarified.

Phoma gardeniae, producing pycnidia with short setae, clustered with *Phoma herbarum* in the Didymellaceae. In *Phoma herbarum* occasionally setae-like

outgrowths from outer wall cells have been recorded on older pycnidia in vitro, such as in the case of the Didymellaceae-associated species *P. glomerata* and *P. sorghina* (Boerema et al. 2004). Also *Chaetopyrena penicillata*, producing setose pycnidia and *Phoma*-like conidiogenous cells grouped with *Phoma herbarum* in the Didymellaceae. *Phoma carteri*, *Phoma septacidalis*, *Phoma glycinicola* and *Pyrenochaeta dolichii* grouped in a distinct clade (Clade C), but their taxonomic position remains to be elucidated. Due to their close association with the Leptosphaeriaceae, a subsequent study dealing with *Phoma* species currently classified in *Phoma* section *Plenodomus* is required before the taxonomy of these four setose species can be redefined properly.

ACKNOWLEDGMENTS

The Dutch Ministry of Agriculture, Nature and Food Quality has supported this research through an endowment of the FES program "Versterking infrastructuur Plantgezondheid". We thank Karin Rosendahl for technical assistance, Marjan Vermaas for helping with the photoplate and Machiel E. Noordeloos for critical reading of the manuscript. Paul Tooley kindly supplied two isolates of *Phoma glycinicola*.

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