Mycologia, 101(3), 2009, pp. 363–382. DOI: 10.3852/08-199

DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties

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Abstract: Species of the anamorph genus *Phoma* are commonly isolated from a wide range of ecological niches. They are notoriously difficult to identify due to the paucity of morphological features and the plasticity of these when cultivated on agar media. Species linked to *Phoma* section *Peyronellaea* are typified by the production of dictyochlamydospores and thus have additional characters to use in taxon delineation. However, the taxonomy of this section is still not fully understood. Furthermore the production of such chlamydospores also is known in some other sections of *Phoma*. DNA sequences were generated from three loci, namely ITS, actin, and β-tubulin, to clarify the phylogeny of *Phoma* taxa that produce dictyochlamydospores. Results were unable to support section *Peyronellaea* as a taxonomic entity. Dictyochlamydospore formation appears to be a feature that developed, or was lost, many times during the evolution of *Phoma*. Furthermore, based on the multigene analyses, five new *Phoma* species could be delineated while a further five required taxonomic revision to be consistent with the genetic variation observed.

Key words: actin, β-tubulin, coelomycetes, dictyochlamydospores, ITS, multigene phylogeny, taxonomy

INTRODUCTION

Although the genus *Phoma* Sacc. emend Boerema & Bollen is widely distributed and omnipresent, it is still poorly understood and generally considered to be a taxonomically difficult group of mitosporic ascomycetes. *Phoma* is characterized by the production of single-celled, hyaline conidia in monophialidic, doliiform to flask-shaped conidiogenous cells in thin-walled pycnidia (Boerema and Bollen 1975). The present concept however also includes species that produce thick-walled pycnidia, or form septate conidia in addition to continuous conidia in pure culture (Boerema 1997, Boerema et al 2004). Specimens have been isolated mainly from soil and from a wide range of plant hosts where they reside as primary pathogens, opportunists, saprobes or endophytes (Aveskamp et al 2008). The existing subgeneric classification was defined by Boerema (1997) after a 40 y study of the morphological characters. The genus was divided into nine sections that are based on their morphological appearance: *Phoma*, *Heterospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Sclerophomella*, *Plenodomus*, *Macrospora* and *Pilosa*. Although this subdivision is extremely helpful in identifying strains up to species level, it remains artificial because several taxa exhibit features that are representative of different sections.

One of the most confusing sections in this regard is *Peyronellaea*, even though it has been studied intensively (Boerema 1993, Boerema et al 1965, 1968, 1971, 1973, 1977, Morgan-Jones and Burch 1987, Morgan-Jones and White 1983, White and Morgan-Jones 1983, 1986, 1987). *Peyronellaea* was incorporated into genus *Phoma* in 1990 (van der Aa et al 1990). It accommodates fungi producing pycnidial conidiomata with phialidic conidiogenous cells as well as dictyochlamydospores, having both transverse and longitudinal septa. Three types of dictyochlamydospores are distinguished: (i) alternarioid to irregular botryoid, that is those that resemble the conidia of genus *Alternaria* and that often also develope in chains (Luedemann 1961); (ii) epicoccoid, resembling the

Accepted for publication 20 January 2009.
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conidia of the genus *Epicoccum*: and (iii) pseudosclerotioid, resembling pseudosclerotia, often developing in aggregates of many unicellular chlamydospores (Boerema et al 2004).

The section currently comprises 12 species and five infraspecific taxa (Boerema et al 2004). However, several species accommodated in other sections of *Phoma* are also capable of producing comparable chlamydospores, including *P. gardeniae* (sect. *Paraphoma*), *P. clematidina* and *P. narcissi* (sect. *Heterospora*), *P. multirostrata* and *P. eupyrena* (sect. *Phoma*) and *P. zeae-maydis* and *P. boeremae* (sect. *Macrospora*). Furthermore *P. exigua* had been included erroneously in sect. *Peyronellaea*, but in contrast to the species mentioned above it does not produce multicellular chlamydospores (Boerema et al 1977).

Several taxa that currently are incorporated in *Phoma* sect. *Peyronellaea* have features in common with other sections, or even with other genera. For example two species from North America, *P. americana* and *P. subglomerata*, are characterized by the incidental production of uniseptate conidia, a key character for species placed in *Phoma* sect. *Phyllostictoides* (van der Aa et al 1990). Furthermore one species, *P. epicoccina*, produces thick-walled poroid pycnidia resembling those of *Phoma* sect. *Sclerophomella* (Boerema and de Gruyter 1998). Some strains of the type species of this section, *P. glomerata*, produce conidia that become pigmented after maturation. This feature is uncommon in *Phoma* and actually is regarded as a character of Microsphaeropsis, *Coniothyrium* or *Paraconiothyrium* (Verkley et al 2004, Damm et al 2008).

In the present paper we studied the genetic and morphological diversity among the taxa currently accommodated in *Peyronellaea*. A further aim was to clarify the phylogenetic relation with several chlamydospore-producing species currently accommodated in other sections or that still remain to be described.

MATERIALS AND METHODS

Cultural and morphological studies.—A total of 122 strains (TABLE I) were obtained from the culture collections of CBS (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands), PD (Plant Protection Service, Wageningen, the Netherlands), IMI (International Mycological Institute, Kew, UK) and LEV (Plant Health and Diagnostic Station, Auckland, New Zealand). Freeze-dried strains were revived overnight in 2 mL malt/peptone (50/50%) liquid medium. Cultures were transferred and maintained on oatmeal agar (OA, Gams et al 2007) at 10 C and in complete darkness. Morphological studies of the strains were performed on OA, malt extract agar (MEA) and cherry decoction agar (CHA, Gams et al 2007). Cultures were incubated as described in Boerema et al (2004). Eight days after inoculation colony growth was measured. Colony colors were rated 15 d after incubation with Rayners' color chart (1970). Morphological features were studied after sporulation. Fungal structures were mounted in tap water with a scalpel blade and examined under a Nikon 80i light microscope. Sizes of the various structures were determined by averaging the measurements of 30 examples of each structure, except for conidiogenous cells, of which the size range was estimated based on ca. five structures. Fifth and 95th percentiles were determined for all measurements and are provided in parentheses. The production of metabolite E+ was determined by application of a droplet of 1N NaOH (Dorenbosch 1970, Noordeloos et al 1993). The structure of the pycnidial wall and shape of conidiogenous cells were studied with microtome sections 6 µm thick, prepared with a Leica CM3050 freezing microtome and mounted in lactic acid. Taxonomic novelties and descriptions were deposited in MycoBank (www.mycobank.org, Crous et al 2004).

Molecular studies. DNA extraction, PCR and sequencing.—Actively growing mycelium was scraped from culture plates and transferred to 2 mL collection tubes from the Ultra—Clean™ Microbial DNA Kit (MoBio Laboratories Inc., Carlsbad, California). DNA isolation was carried out according to the manufacturer's recommendations. DNA samples were checked for purity and integrity by gel electrophoresis, after which the samples were diluted 10× and stored at 4 C before further handling. The ITS1-5.8S-ITS2 region (ITS) of the nuclear ribosomal DNA operon was amplified with the V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al 1990) primer pair. The actin gene (ACT) was partly amplified with primer pair ACT-512F and ACT-783R (Carbone and Kohn 1999). Two newly designed primers, TUB2Fd (5′- GTB CAC CTY CAR ACC GGY CAR TG – 3′) and TUB4Rd (5′ - CCR GAY TGR CCR AAR ACR AAG TTG TC – 3′), were used to amplify a part of the β-tubulin (TUB) gene. For the two housekeeping genes ACT and TUB, each PCR reaction had a total volume of 12.5 µL and contained 0.5 µL 10× diluted gDNA, 1× PCR Buffer, 2 mM $MgCl_2$, 100 µM of each of the dNTP, 0.2 µM of each of the primers and 0.5 units *Taq* DNA polymerase (Bioline, Luckenwalde, Germany). The reaction mixture prepared for ITS amplification was similar, except for a double concentration of dNTP. The polymerase chain reactions (PCR) were conducted in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) with an initial denaturation at 95 C for 5 min, followed by 40 cycles of denaturation (95 C for 30 s), annealing (48 to 55 C for 30 s depending on the locus) and extension (72 C for 80 s). The final extension phase was conducted at 72 C for 7 min. Annealing temperatures varied per reaction and were set at 48 C, 52 C, and 55 C for ITS, TUB and ACT respectively.

Both strands of the amplified DNA fragments were sequenced with the same PCR primers and the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations. Sequence products were purified using a 96-well multiscreen HV plate (Millipore, Billerica, Massachusetts) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, the Netherlands). The products were analyzed on an

TABLE I. List of strains used, with their country of origin and the host. Sequences derived from other studies are marked in bold

Species	Collection number[1]	Source	Origin	GenBank accession numbers		
				ACT	ITS	TUB
Phoma americana	CBS 185.85	*Zea mays*	USA	FJ426870	FJ426972	FJ427088
	CBS 256.65	*Phaseolus vulgaris*	Denmark	FJ426871	FJ426973	FJ427089
	CBS 568.97	*Glycine max*	USA	FJ426872	FJ426974	FJ427090
	CBS 112525	*Triticum aestivum*	Argentina	FJ426873	FJ426975	FJ427091
	IMI 361195	—	—	FJ426874	FJ426976	FJ427092
	PD 78/1089	*Zea mays*	South-Africa	FJ426875	FJ426977	FJ427093
	PD 79/58	*Sorghum vulgare*	Nigeria	FJ426876	FJ426978	FJ427094
	PD 80/1143	*Zea mays*	USA	FJ426877	FJ426979	FJ427095
	PD 82/1059	Nematode cyst	—	FJ426878	FJ426980	FJ427096
P. betae	CBS 523.66	*Beta vulgaris*	Netherlands	—	FJ426981	—
	—	*Beta vulgaris* var. *cicla*	Italy	—	**EU003450**	—
P. boeremae	CBS 109942	*Medicago littoralis*	Australia	FJ426879	FJ426982	FJ427097
P. calidophila	CBS 448.83	Desert soil	Egypt	FJ426948	FJ427059	FJ427168
	PD 84/109	*Cucumis sativus*	Europe	FJ426949	FJ427060	FJ427169
P. calorpreferens	CBS 109.92	Food	Netherlands	FJ426880	FJ426983	FJ427098
P. chrysanthemicola	CBS 172.70	*Chrysanthemum morifolium*	Germany	—	FJ426984	—
	CBS 522.66	*Chrysanthemum morifolium*	UK	—	FJ426985	—
	PD 87/153	*Cichorium intybus*	Netherlands	—	FJ426986	—
	PD 92/468	—	—	—	FJ426987	—
P. clematidina	CBS 102.66	*Clematis* sp.	UK	FJ426881	FJ426988	FJ427099
	CBS 108.79	*Clematis* sp.	Netherlands	FJ426882	FJ426989	FJ427100
	CBS 195.64	*Clematis jackmannii*	Netherlands	FJ426883	FJ426990	FJ427101
	CBS 201.49	*Clematis* sp.	Netherlands	FJ426884	FJ426991	FJ427102
	CBS 520.66	*Selaginella* sp.	Netherlands	FJ426885	FJ426992	FJ427103
P. coffeae-arabicae	CBS 123380	*Coffea arabica*	Ethiopia	FJ426886	FJ426993	FJ427104
	CBS 123398	*Coffea arabica*	Ethiopia	FJ426887	FJ426994	FJ427105
P. epicoccina	CBS 125.82	Human	Netherlands	FJ426888	FJ426995	FJ427106
	CBS 173.73	*Dactylis glomerata*	USA	FJ426889	FJ426996	FJ427107
	CBS 505.85	Soil	Germany	FJ426890	FJ426997	FJ427108
	CBS 115825	*Malus* sp.	Netherlands	FJ426891	FJ426998	FJ427109
P. eupyrena	CBS 374.91	*Solanum tuberosum*	Netherlands	FJ426892	FJ426999	FJ427110
	CBS 527.66	Wheat field soil	Germany	FJ426893	FJ427000	FJ427111
P. exigua var. *exigua*	CBS 431.74	*Solanum tuberosum*	Netherlands	**EU880854**	FJ427001	FJ427112
P. exigua var. *exigua*	CBS 118.94	*Phaseolus vulgaris*	Netherlands	—	**EU167567**	—
P. gardeniae	CBS 302.79	Air sample	Netherlands	FJ426894	FJ427002	FJ427113
	CBS 626.68	*Gardenia jasminoides*	India	FJ426895	FJ427003	FJ427114
P. glomerata	CBS 133.72	Church wall-fresco	Romania	FJ426896	FJ427004	FJ427115
	CBS 284.76	*Populus nigra*	Russia	FJ426897	FJ427005	FJ427116
	CBS 287.76	*Rubus idaeus*	Russia	FJ426898	FJ427006	FJ427117
	CBS 288.76	*Populus alba*	Russia	FJ426899	FJ427007	FJ427118
	CBS 289.76	*Allium nutans*	Russia	FJ426900	FJ427008	FJ427119
	CBS 290.76	*Ribes nigrum*	Russia	FJ426901	FJ427009	FJ427120
	CBS 293.36	*Solanum tuberosum*	Germany	FJ426902	FJ427010	FJ427121
	CBS 304.49	*Lycopersicon esculentum*	Netherlands	FJ426903	FJ427011	FJ427122
	CBS 464.97	Indoor (Bathroom)	Netherlands	FJ426904	FJ427012	FJ427123
	CBS 528.66	*Chrysanthemum* sp.	Netherlands	FJ426905	FJ427013	FJ427124
	CBS 834.84	*Hordeum sativum*	Germany	FJ426906	FJ427014	FJ427125
	CBS 120109	*Juniperus* sp.	USA	FJ426907	FJ427015	FJ427126
	CBS 112448	Indoor environment	Germany	FJ426908	FJ427016	FJ427127
	—	*Rosa* sp.	Mexico	—	**AY904060**	—
	PD 73/1415	*Heracleum* sp.	Russia	FJ426909	FJ427017	FJ427128
	PD 74/1023	Air sample	UK	FJ426910	FJ427018	FJ427129
	PD 77/47	*Medicago sativa*	Netherlands	FJ426911	FJ427019	FJ427130

TABLE I. Continued

Species	Collection number[1]	Source	Origin	GenBank accession numbers ACT	ITS	TUB
	PD 81/767	*Cucumis sativus*	—	FJ426912	FJ427020	FJ427131
	PD 83/782	*Capsicum* sp.	—	FJ426913	FJ427021	FJ427132
P. herbarum	CBS 615.75	*Rosa multiflora*	Netherlands	**EU880896**	FJ427022	FJ427133
	—	Rhizosphere of *Picea mariana*	Canada	—	**DQ132841**	—
P. heteromorphospora	CBS 448.68	*Chenopodium album*	Netherlands	—	FJ427023	—
P. infossa	CBS 123394	*Fraxinus pennsylvanica*	Argentina	FJ426914	FJ427024	FJ427134
	CBS 123395	*Fraxinus pennsylvanica*	Argentina	FJ426915	FJ427025	FJ427135
P. jolyana	CBS 463.69	*Mangifera indica*	India	FJ426916	FJ427026	FJ427136
	PD 83/326	*Malus sylvestris*	India	FJ426917	FJ427027	FJ427137
P. lingam	—	*Brassica napus*	Australia	—	**M96384**	—
P. microchlamydospora	CBS 105.95	*Eucalyptus* sp.	UK	FJ426918	FJ427028	FJ427138
	CBS 491.90	Unidentified vegetable crop	UK	FJ426919	FJ427029	FJ427139
P. multirostrata	CBS 110.79	*Cucumis sativus*	Netherlands	FJ426920	FJ427030	FJ427140
	CBS 274.60	Soil from poultry farm	India	FJ426921	FJ427031	FJ427141
	CBS 380.67	*Lilium* sp.	—	FJ426922	FJ427032	FJ427142
P. multirostrata	CBS 368.65	Soil	India	FJ426923	FJ427033	FJ427143
	CBS 120115	Soil	Puerto Rico	FJ426924	FJ427034	FJ427144
	CBS 120116	Soil	Puerto Rico	FJ426925	FJ427035	FJ427145
	PD 77/508	*Philodendron* sp.	Netherlands	FJ426926	FJ427036	FJ427146
	PD 83/48	*Cucumis sativus*	Netherlands	FJ426927	FJ427037	FJ427147
P. narcissi	CBS 251.92	*Nerine* sp.	Netherlands	FJ426928	FJ427038	FJ427148
	PD 71/6	*Ismene* sp.	—	FJ426929	FJ427039	FJ427149
	PD 76/61	*Hippeastrum* sp.	—	FJ426930	FJ427040	FJ427150
	PD 92/1460	*Sprekelia* sp.	Netherlands	FJ426931	FJ427041	FJ427151
P. omnivirens	CBS 341.86	*Phaseolus vulgaris*	Belgium	FJ426932	FJ427042	FJ427152
	CBS 654.77	—	India	FJ426933	FJ427043	FJ427153
	CBS 991.95	Soil	Papua New Guinea	FJ426934	FJ427044	FJ427154
	CBS 992.95	Soil	Papua New Guinea	FJ426935	FJ427045	FJ427155
	CBS 123396	*Chrysanthemum indicum*	Netherlands	FJ426936	FJ427046	FJ427156
	CBS 123397	*Statice* sp.	Tanzania	FJ426937	FJ427047	FJ427157
P. paspali	CBS 560.81	*Paspalum dilatatum*	New Zealand	FJ426938	FJ427048	FJ427158
P. pimprina	CBS 246.60	Soil	India	FJ426939	FJ427049	FJ427159
	PD 77/1028	Soil	India	FJ426940	FJ427050	FJ427160
P. pinodella	CBS 318.90	*Pisum sativum*	Netherlands	FJ426941	FJ427051	FJ427161
	CBS 531.66	*Trifolium pratense*	USA	FJ426942	FJ427052	FJ427162
P. pomorum var. *circinata*	CBS 285.76	*Heracleum dissectum*	Russia	FJ426943	FJ427053	FJ427163
	CBS 286.76	*Allium nutans*	Russia	FJ426944	FJ427054	FJ427164
P. pomorum var. *cyanea*	CBS 388.80	*Triticum* sp.	South Africa	FJ426945	FJ427055	FJ427165
P. pomorum var. *pomorum*	CBS 539.66	*Polygonum tataricum*	Netherlands	FJ426946	FJ427056	FJ427166
	PD 81/592	*Ribes uva-crispa*	Netherlands	FJ426947	FJ427057	FJ427167
P. radicina	CBS 111.79	*Malus sylvestris*	Netherlands	—	FJ427058	—
P. samarorum	CBS 138.96	*Phlox paniculata*	Netherlands	—	FJ427061	—
	CBS 139.96	*Poa* sp.	Netherlands	—	FJ427062	—
P. sancta	CBS 281.83	*Ailanthus altissima*	South Africa	FJ426950	FJ427063	FJ427170
	CBS 644.97	*Opuntia ficus-indica*	Argentina	FJ426951	FJ427064	FJ427171
	LEV 15292	*Gleditsia triacantha*	—	FJ426952	FJ427065	FJ427172
P. schachtii	CBS 502.84	*Heterodera schachtii*	Netherlands	—	FJ427066	—
P. sorghina	CBS 179.80	*Sorghum vulgare*	Puerto Rico	FJ426953	FJ427067	FJ427173
	CBS 180.80	*Zea mays*	South Africa	FJ426954	FJ427068	FJ427174
	CBS 181.80	*Oryza sativa*	Guinea-Bissau	FJ426955	FJ427069	FJ427175

TABLE I. Continued

Species	Collection number[1]	Source	Origin	GenBank accession numbers		
				ACT	ITS	TUB
P. sorghina	CBS 293.72	*Panicum miliare*	India	FJ426956	FJ427070	FJ427176
	CBS 301.89	*Lycopersicon esculentum*	Martinique	FJ426957	FJ427071	FJ427177
	CBS 627.68	*Citrus* sp.	France	FJ426958	FJ427072	FJ427178
	CBS 846.68	*Coffea* sp.	India	FJ426959	FJ427073	FJ427179
	CBS 886.95	*Stellaria* sp.	Papua New Guinea	FJ426960	FJ427074	FJ427180
	CBS 986.95	Soil	Papua New Guinea	FJ426961	FJ427075	FJ427181
	PD 76/1025	*Aspidiotus destructor*	India	FJ426962	FJ427076	FJ427182
	PD 81/721	*Pinus* sp.	USA	FJ426963	FJ427077	FJ427183
	PD 88/549	*Lycopersicon esculentum*	Martinique	FJ426964	FJ427078	FJ427184
	PD 03486771	*Triticum* sp.	Netherlands	FJ426965	FJ427079	FJ427185
P. subglomerata	CBS 110.92	*Triticum* sp.	USA	FJ426966	FJ427080	FJ427186
	PD 78/1090	*Zea mays*	South-Africa	FJ426967	FJ427081	FJ427187
P. violicola	CBS 100272	*Viola tricolor*	New Zealand	—	FJ427082	—
	CBS 306.68	*Viola tricolor*	Netherlands	—	FJ427083	—
P. zantedeschiae	CBS 131.93	*Calla* sp.	Netherlands	FJ426968	FJ427084	FJ427188
	PD 69/140	*Calla* sp.	Netherlands	FJ426969	FJ427085	FJ427189
P. zeae-maydis	CBS 588.69	*Zea mays*	USA	FJ426970	FJ427086	FJ427190
	MA 0027	*Zea mays*	USA	FJ426971	FJ427087	FJ427191
Pyrenochaeta romeroi	IP 571.61	Human mycetoma	Senegal	—	**DQ836802**	—

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; IMI: International Mycological Institute, Kew, UK; IP: Pasteur Institute Collection of Fungi, Pasteur Institute, Paris, France; LEV: Plant Health and Diagnostic Station, Auckland, New Zealand; MA: Culture collection of Maikel Aveskamp, housed at CBS; PD: Plant Protection Service, Wageningen, the Netherlands.

ABI Prism 3700 DNA Sequencer (Applied Biosystems). A consensus sequence was assembled from the forward and reverse sequences with the BioNumerics v4.5 software package (Applied Maths, St-Martens-Latem, Belgium). Sequences were deposited in GenBank (TABLE I).

Phylogenetic analysis.—The consensus sequences were aligned with BioNumerics and adjusted by hand where necessary. The best nucleotide substitution models were determined with MrModeltest v2.2 (Nylander 2004). A Bayesian tree inference (BI) analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). One tree was saved per 100 generations, and the run was automatically ended when the standard deviation of split frequencies was below 0.01. To avoid suboptimal trees being taking into account for the consensus tree, a burn-in of 25% of the saved trees was used. The resulting ''50% majority rule consensus'' trees were printed with TreeView v1.6.6 (Page 1996) and are lodged with TreeBASE (www.treebase.org).

To obtain further evidence for branch supports, a series of neighbor joining (NJ) analyses was conducted in PAUP (phylogenetic analysis using parsimony) v4.0b10 (Swofford 2003) with the uncorrected (''p''), the Kimura-2-parameter and the HKY85 substitution models. Alignment gaps were treated as missing data, and all characters were unordered and of equal weight. Ties were broken randomly.

A third measure of branch support was obtained by conducting a maximum likelihood (ML) analysis using RAxML (randomized axelerated maximum likelihood) software (Stamakis et al 2008) through the CIPRES Website (www.phylo.org). The same three partitions were used as in the BI and NJ tests, but because RAxML implements only the GTR substitution model the symmetrical model for the ITS partition was waived. The robustness of trees in the NJ and ML analyses was evaluated by 1000 bootstrap replications.

To test whether the three different loci could be used in a combined analysis phylogenies were estimated with maximum likelihood analyses for each data partition (ML bootstrap values $> 70\%$) and compared by eye for congruency. Congruence of these trees was further determined with the Shimodaira-Hasegawa test (SH test, Shimodaira and Hasegawa 1999), which is implemented in PAUP. The topology of the concatenated ML tree was compared to the topology of the ML trees obtained for each partition in a one-tailed bootstrap test using 1000 replications with full likelihood maximization to determine whether the trees were significantly different.

The SH test also was used to determine whether the species that currently are linked with *Phoma* section *Peyronellaea* represent a monophyletic group. Therefore a constraint tree in which such a phylogeny was simulated was compared to the consensus tree obtained from the RAxML analysis of the ITS dataset. These trees subsequently were compared as described above.

TABLE II. Log likelihood scores for data partition combinability with the Shimodaira-Hasegawa (SH) test. ($P < 0.05$)

Partition	Constraint	Score (−lnL)	Difference (−lnL)	Probability (*P*)
Actin	ACT	3353.19772	—	—
	Concatenated	323.64131	70.4439	0.104
β-tubulin	TUB	305637488	—	—
	Concatenated	3103.20713	46.83225	0.327
ITS	ITS	1668.99164	—	—
	Concatenated	1690.18063	21.18899	0.073

RESULTS

ITS phylogeny.—Due to alignment difficulties of the housekeeping genes, two alignments of DNA sequences were subjected to phylogenetic analyses. The first alignment consisted of 122 ITS sequences generated in this study and six obtained from GenBank. This ITS alignment consisted of 566 characters including alignment gaps, of which 237 were variable and 329 were constant. A GenBank sequence of *Pyrenochaeta romeroi* (DQ836802) was used as outgroup. The BI analysis was run using the best model and parameters as determined, which were the symmetrical (SYM) substitution model with inverse gamma rates and equal dirichlet base frequencies. The temperature value set at 0.4. The analysis run of the ITS sequence matrix in MrBayes resulted in 11 039 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated. The topology and support values of the BI tree were in congruence with those of the trees obtained by NJ and the optimal tree obtained in the ML analysis. The reconstructed phylogeny with the ITS dataset revealed 16 heterogenous strains to be a paraphyletic basal assemblage to a major clade consisting of 106 strains (FIG. 1). The majority of the taxa belonging to *Phoma* sect. *Peyronellaea* were found in this major clade (support values 1, 100% and 95% for BI posterior probability, NJ and ML bootstrap supports respectively), although several type species of other *Phoma* sections also were accommodated here, such as *P. herbarum* (section *Phoma*), *P. exigua* var. *exigua* (section *Phyllostictoides*) and *P. zeae-maydis* (sect. *Macrospora*). Further the *Peyronellaea* species *P. chrysanthemicola* and *P. violicola* were located amongs the basal lineages, indicating that section *Peyronellaea* does not represent a monophyletic clade. This is supported by the SH test conducted on the ITS dataset, in which the hypothesis that the tree (FIG. 1) is in congruence with monophyly of *Peyronellaea* is rejected ($P < 0.01$).

Concatenated phylogeny.—The second alignment included 104 taxa, including one outgroup taxon (CBS 560.81 *P. paspali*), which was found to be basal to the major clade (FIG. 1). No strongly conflicting nodes were detected in the phylogenies of the separate loci (ML bootstrap values > 70%). Topologies were congruent for each partition, although ITS showed a lower degree of resolution of the terminal taxa. Also the results of the SH tests (TABLE II) suggest that the ITS tree differs most from the concatenated tree, although this is not significant ($P = 0.073$). Based on the similarity in topologies and the nonsignificant SH tests, the partitions used in the second dataset (ITS, ACT, TUB) could be concatenated.

The concatenated alignment had a total length of 1148 characters (ITS: 500, ACT: 300, TUB: 348) including alignment gaps. Of these characters 383 (ITS: 96, ACT: 156, TUB: 131) were variable and 767 (ITS: 406, ACT: 144, TUB: 217) were constant. The SYM+I+G model was found to be optimal for the ITS partition, whereas the best substitution model for the ACT and TUB sequence matrix was determined to be GTR+I+G. The temperature value was set at 0.5 for the BI analysis. The MrBayes run of the second dataset resulted in 3340 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated (FIG. 2). Trees supporting the same clades were obtained irrespective of the analysis method used. Further phylogenetic results are discussed below where applicable.

TAXONOMY

Most *Peyronellaea* taxa and other chlamydospore-forming species studied here appeared to be properly described in the past. However, five novel dictyochlamydospore-forming species of *Phoma* could be identified in the present study. These species are described below. One species, *P. infossa*, was already known to science, but its description is amended as it appeared to produce dictyochlamydospores. Furthermore five new combinations are proposed.

Phoma calidophila Aveskamp, Gruyter & Verkley, nom. nov. pro *Sphaeronema sahariense* Faurel & Schotter. MycoBank MB512566.

≡ *Sphaeronaema sahariense* Faurel & Schotter, Revue

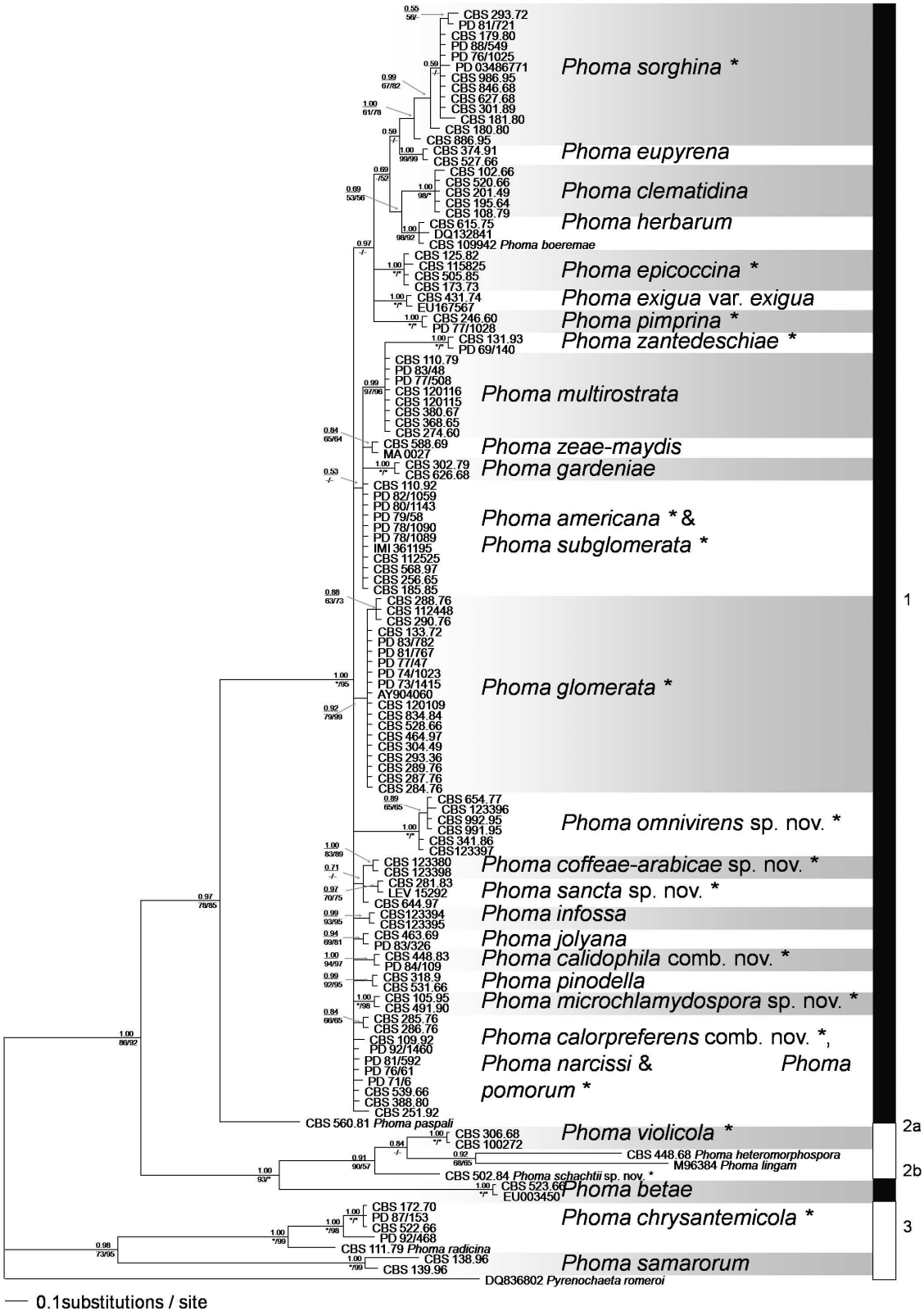

FIG. 1. Fifty percent majority rule consensus tree from a BI analysis of ITS sequences of *Phoma* sect. *Peyronellaea* (n = 122). At the nodes the BI Posterior Probabilities are presented above the branch, and bootstrap percentages of the NJ analysis using the HKY85 substitution model and ML analysis are given below the branch. Branches that were less than 50% supported in the NJ and ML analyses are indicated with a hyphen, whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted with *Pyrenochaeta romeroi*.

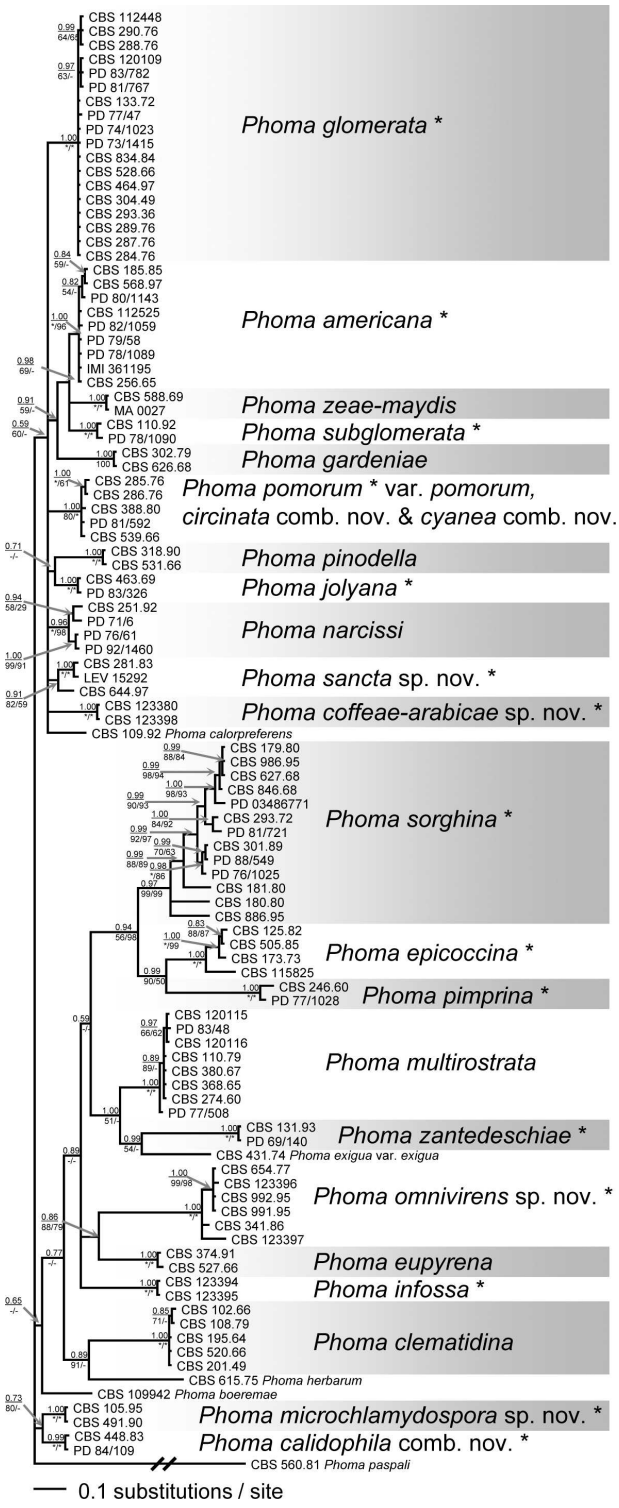

FIG. 2. Reconstructed phylogeny based on a 50% majority rule consensus tree of *Phoma* sect. *Peyronellaea* using a BI analysis of 104 concatenated ITS-ACT-TUB sequences. At the nodes the BI posterior probabilities are presented above the branch and bootstrap percentages of the NJ analysis using the HKY85 substitution model and ML analysis are given below the branch. Branches that were less than 50% supported in the NJ and ML analyses are indicated with a hyphen, whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted to *Phoma paspali* CBS 560.81.

Mycol. 30:156. 1965; not *Phoma sahariensis* Faurel & Schotter, Revue Mycol. 30: 154. 1965.

= *Phoma jolyana* var. *sahariensis* (Faurel & Schotter) Boerema, Dorenb. & Aa apud Boerema, Versl. Med. Pl. ziektenk. Dienst 159:27. 1983 [1982].

For detailed descriptions see Boerema (1983, 1993).

Etymology: Name refers to this species' preference for warmth.

Specimen examined: EGYPT. From desert soil, Feb 1980, *M.I.A. Abdel-Kader* (NEOTYPE designated here, CBS H-20168) (culture CBS 448.83).

Notes. The present species previously was known as a variety of *P. jolyana*. It is elevated to species level here due to the phylogenetic results obtained in the present study. Colony characters are similar to those of *P. jolyana*, although aerial mycelium in OA plates can be yellow-olivaceous, and a yellow discoloration of the agar is present. Pycnidial formation is induced only at temperatures of 28–30 C, indicating the high temperature preference of this species. The pycnidia resemble those of *P. jolyana* in shape and size, but in contrast a pronounced neck may occur in *P. calidophila*. Furthermore the conidia are shorter than those of *P. jolyana*, 4–5.5(–6) × (2–)2.5–3 μm, giving them a somewhat ellipsoidal-obovoid appearance. Boerema (1983, 1993) reports the presence of a halo surrounding these chlamydospores when cultured.

Because a form of the preferred epithet ''*sahariense*'' was already occupied, a new name is proposed here for this species. Type material of *Sphaeronaema sahariense* could not be traced, and therefore neotype material is designated here.

Phoma calorpreferens (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512567.

≡ *Phoma pomorum* var. *calorpreferens* Boerema, Gruyter & Noordel., Persoonia 15:207. 1993.

For a detailed description see Boerema (1993).

Specimen examined: THE NETHERLANDS. From undefined food material, 1973, *G.H. Boerema*, (holotype L990.290 418) (culture CBS 109.92 = CBS 264.74 = PD 73/1405).

Notes. This taxon was considered to be a warmth-preferring variety of *P. pomorum* because it can grow at temperatures above 30 C. The phylogenetic studies however reveal that both taxa are only distantly related. Therefore *P. pomorum* var. *calorpreferens* is elevated to species level here. It shares many characters with *P. pomorum*, but pycnidia are generally smoother

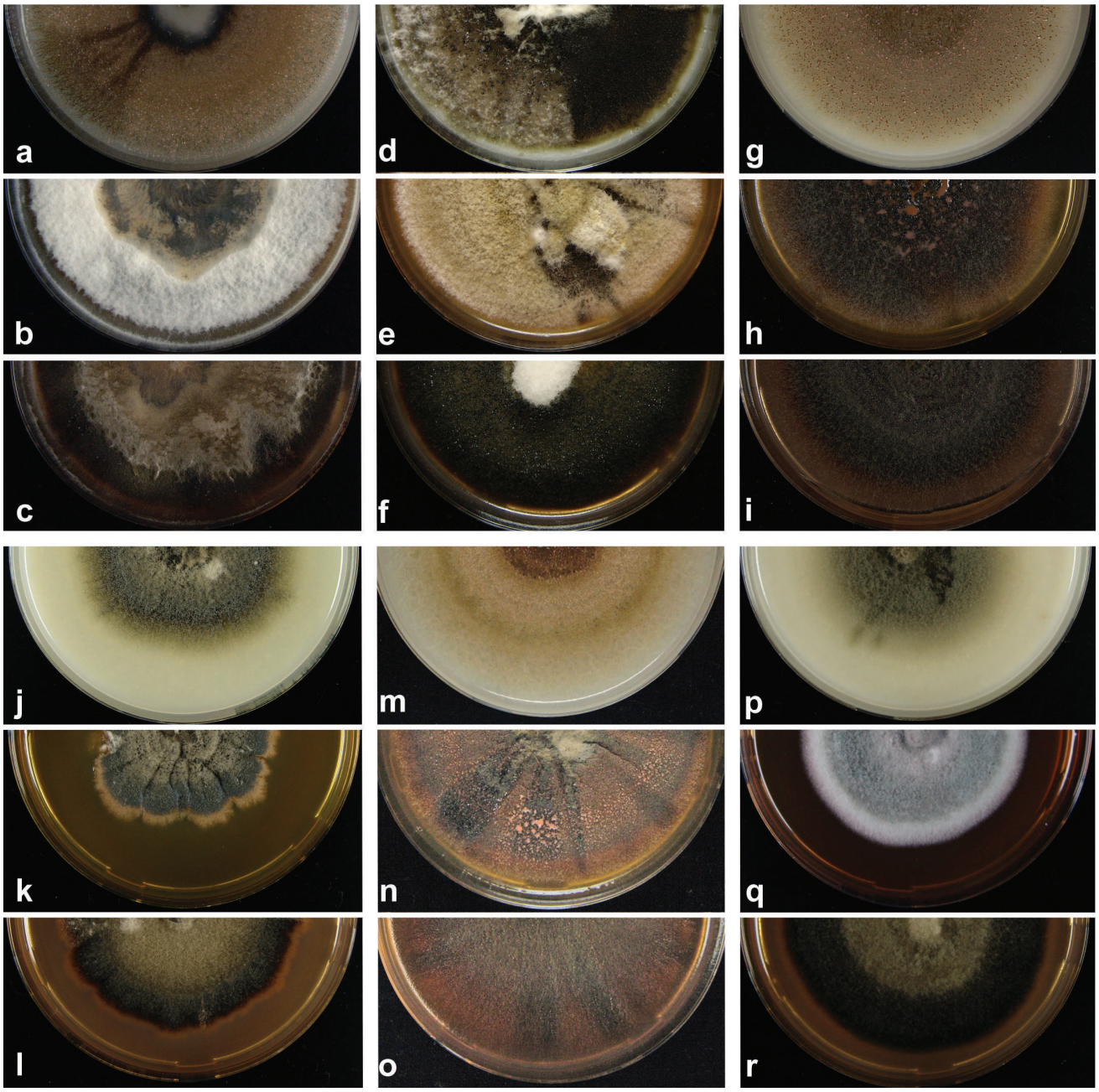

FIG. 3. Two-week old colonies on OA (top), MEA (middle) and CHA (bottom). a–c. *Phoma coffeae-arabicae* CBS 123380. d–f. *P. infossa* CBS 123395. g–i. *P. microchlamydospora* CBS 105.95. j–l. *P. omnivirens* CBS 341.86. m–o. *P. sancta* CBS 281.83. p–q. *P. schachtii* CBS 502.84.

and the conidial matrix is pinkish instead of cream-white (Boerema 1993). Furthermore conidia (4–)5–8.5(–12) × 2–3(–3.5) µm and chlamydopores (< 25 µm) are generally larger than those of *P. pomorum* (Boerema 1993).

Phoma coffeae-arabicae Aveskamp, Verkley & Gruyter, sp. nov. MycoBank MB512568 FIGS. 3a–c, 4

Conidia ellipsoidea usque ovoidea, hyalina, continua, (4–)4.5–6(–7) × (2.5–)3–4(–4.5) µm, eguttulata, vel guttulis polaribus minutis 1–4. Chlamydosporae multicellulares immersae, pseudosclerotioideae, dictyosporae, intercalares, solitariae, (23–)40–100(–190) × (11–)15–30 µm.

Pycnidia mostly solitary or in chains, on the agar surface or submerged, variable in shape and size, mostly ovoid but also (sub)globose or elongated, glabrous, (100–)150–310 × (100–)110–200(–240) µm, papillate or with an elongated neck, mostly uni- or bi-ostiolate. *Ostioles* variable in size, but sometimes

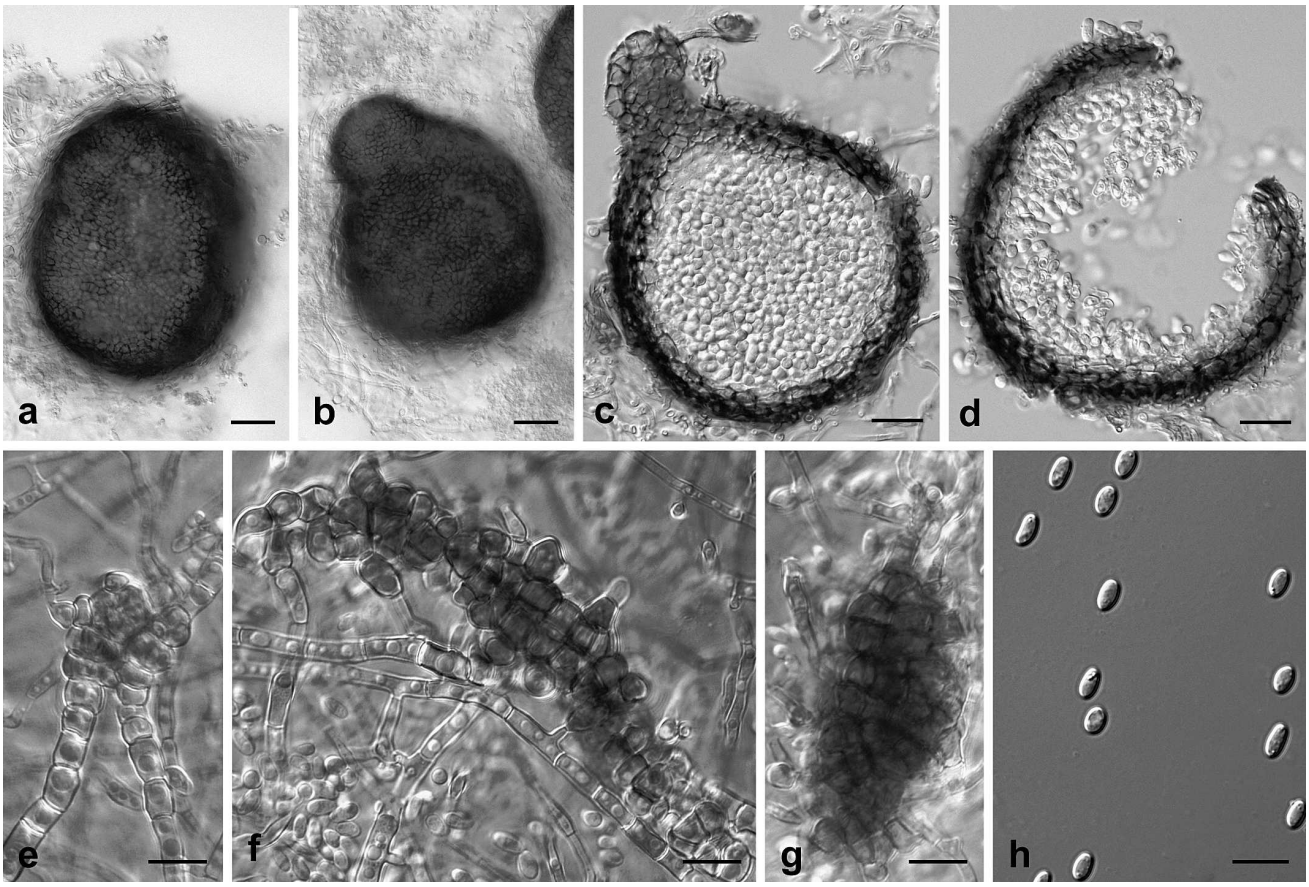

FIG. 4. *Phoma coffeae-arabicae* (ex holotype). a–b. Pycnidia. c–d. Pycnidial section. e–g. Chlamydospores. h. Conidia. Bars: a–b = 50 µm, c–g = 20 µm, h = 10 µm.

relatively wide (< 30 µm diam). *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 10–17 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped to globose, ca. 6–7.5 × 5.5–7 µm. *Conidia* ellipsoidal to ovoid, thin-walled, smooth, hyaline, always aseptate, variable in length, (4–)4.5–6(–7) × (2.5–)3–4(–4.5) µm, eguttulate or with 1–4 minute apolar guttules. *Conidial matrix* salmon to flesh. *Multicellular chlamydospores* immersed, brown, pseudosclerotioid, dictyosporous, intercalary, solitary but often with 2–3 elements on a single hypha, (23–)40–100(–190) × (11–)15–30 µm.

Colonies on OA 61–66 mm diam, with entire, smooth margins. Aerial mycelium sparse or absent, tufted, white. Immersed mycelium hyaline or greenish olivaceous, fuscous-black near center. Reverse concolorous. Colonies on MEA 57–70 mm diam, with entire, smooth, sharp margin. Aerial mycelium condensed, white with rosy-vinaceous tinges. Agar surface iron-gray. Reverse fulvous to amber, but leaden black in zones with abundant pycnidia. Colonies on CHA similar growth rate to MEA. Aerial mycelium compact or tufted, primrose to citrine-green, pale greenish glaucous near center, and leaden-gray near margin. Reverse leaden-black.

Etymology: Named after the host from which it was isolated, *Coffea arabica*.

Specimens examined: ETHIOPIA. From *Coffea arabica*, 1984, *M.M.J. Dorenbosch*, (HOLOTYPE, CBS H-20143) (culture CBS 123380 = PD 84/1013); From *Coffea arabica*, 1984, *M.M.J. Dorenbosch*, (CBS H-20144) (culture CBS 123398 = PD 84/1014).

Notes. Multiple *Phoma* species have been found in association with *Coffea arabica*, such as *P. coffeicola*, *P. coffeiphila*, *P. costarricensis*, *P. excelsa*, *P. pereupyrena* and *P. tarda*. However none of those species produces multicellular chlamydospores, although unicellular, perennial structures have been described in *P. pereupyrena* (de Gruyter et al 1993). Furthermore the conidia of these species are more elongated than those of *P. coffeae-arabicae* (Saccas 1981, Boerema et al 2004).

Although *Phoma coffeae-arabicae* forms pseudosclerotioid chlamydospores, it is phylogenetically related to a group that mainly comprises *Peyronellaea* species forming alternarioid-botryoid chlamydospores (FIG. 2). It is easily recognized by its conspicuously

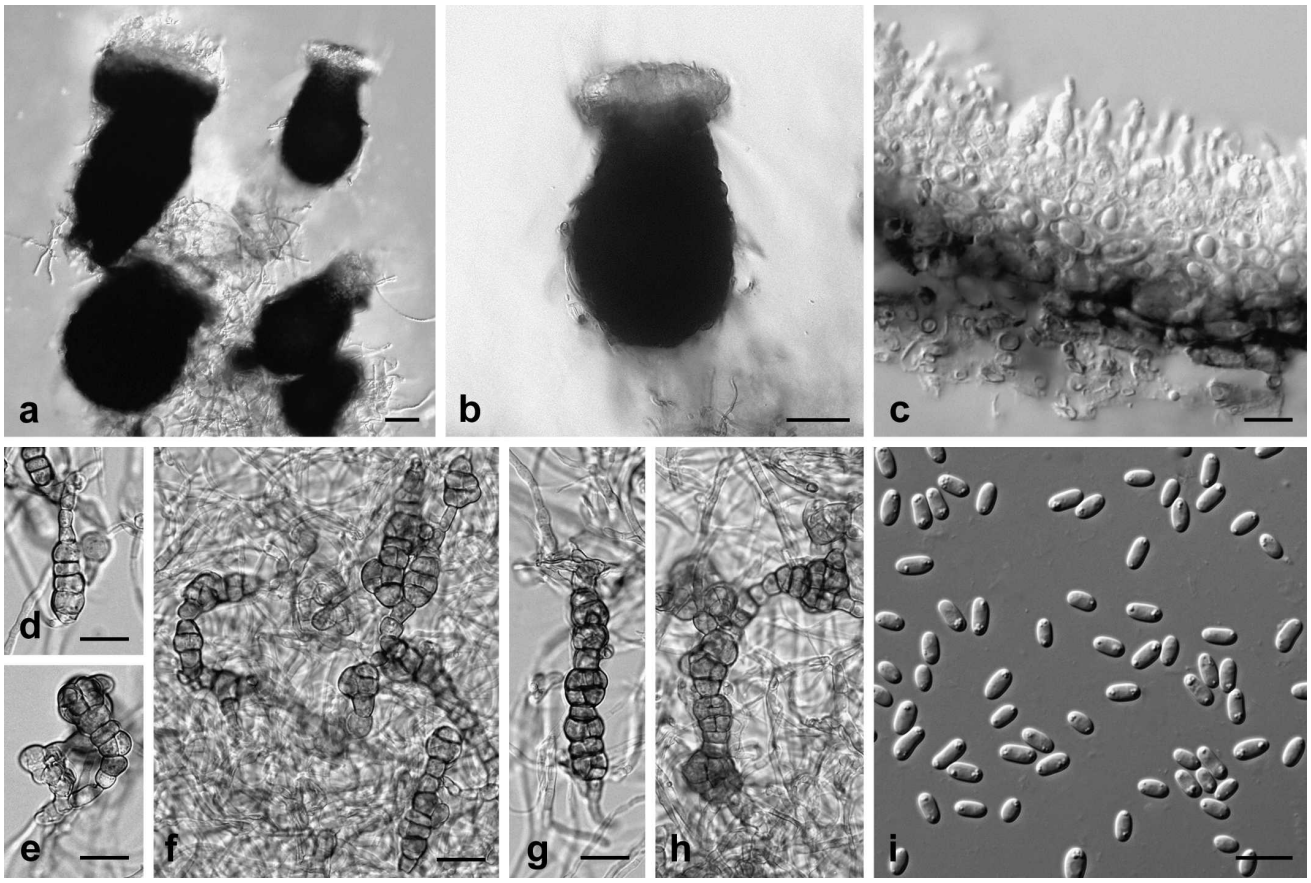

FIG. 5. *Phoma infossa* (ex neotype). a–b. Pycnidia. c. Pycnidial section. d–h. Chlamydospores. i. Conidia. Bars: a–b = 50 µm, c, i = 10 µm, d–h = 20 µm.

wide ostiole, comparable to that of *P. macrostoma* (White and Morgan-Jones 1984).

Phoma infossa Ellis & Everh., J. Myc. 4:102. 1888.

FIGS. 3d–f, 5

Pycnidia mostly solitary on the agar surface, subglobose to elongated, but sometimes somewhat tapering toward the ostiolum, glabrous, (170–)190–250 (–305) × (105–)140–180(–200) µm. *Ostioles* mostly single, (22–)40–75(–105) µm diam, papillate, or with an erumpent and obtusely-conic neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 5–9 layers, 28.5–55 µm thick. *Micropycnidia* sometimes emerge from pycnidia but also solitary, globose to subglobose, 45–80 µm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, ca. 5.5–8(–11) × 5–5.5(–7) µm. *Conidia* from both pycnidial types indistinguishable, ovoid, thin-walled, smooth, hyaline but incidentally brown, aseptate, (4–)4.5–6 × 2.5–3.5 µm, eguttulate, or with (1–)3–6 minute polar guttules. Conidial matrix rosy-buff to salmon. *Multicellular chlamydospores* honey to cinnamon, commonly alternarioid-botryoid, dictyosporous, but sometimes also phragmosporous, solitary or coalescing into long chains of up to five elements, terminal on hyphae, but occasionally intercalary, abundantly in the aerial mycelium, 18–32(–55) × 11.5–17(–22) µm.

Colonies on OA 45–55 mm diam, with entire, smooth margins; aerial mycelium occurring in sections, tufted, floccose, lavender-gray or white, ca. 2–3 mm high; immersed mycelium gray to gray-olivaceous; near colony margin becoming hyaline or citrine, with zones of olivaceous-black mycelium. Reverse slate-blue with dark mouse-gray tinges. Colonies on MEA 42–49 mm diam, with entire, smooth, sharp margins. Aerial mycelium compact, tufted, smoke-gray, but olivaceous or primrose near the center and rosy-vinaceous near the margin; sometimes with zones in which the aerial mycelium is absent and where the surface is covered by abundant black pycnidia. Occasionally sectors occur with more developed white to pale mouse-gray aerial mycelium. Reverse black, but primrose near the center and sienna at the margins. Colonies on CHA similar to MEA, but with moderate aerial mycelium occurring; reverse violaceous-black.

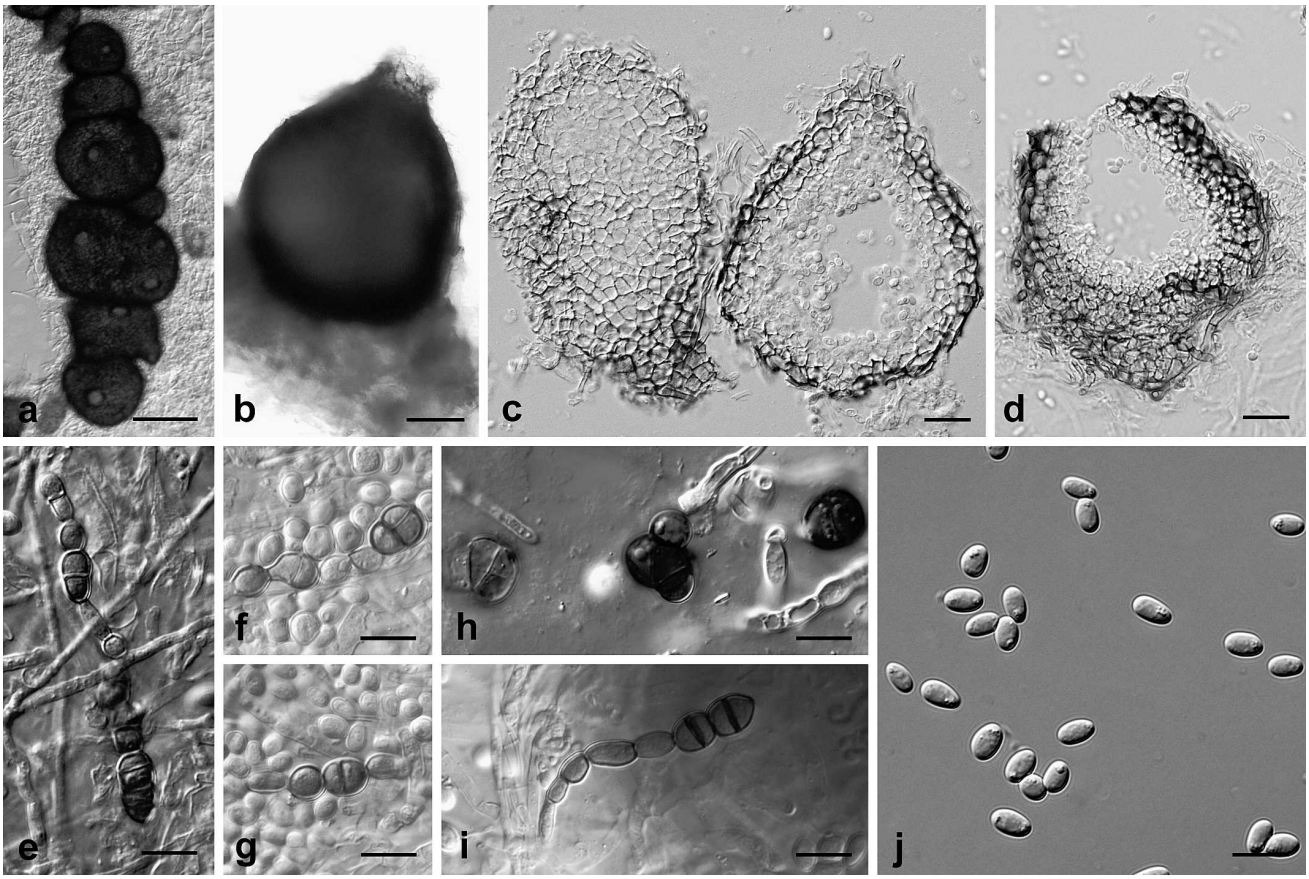

FIG. 6. *Phoma microchlamydospora* (ex holotype). a–b. Pycnidia. c–d. Pycnidial section. e–i. Chlamydospores. k. Conidia. Bars: a = 100 µm, b = 50 µm, c–i = 20 µm, j = 10 µm.

Specimens examined: ARGENTINA, PROVINCIA DE BUENOS AIRES: La Plata. From *Fraxinus pennsylvanica*, 2008, *M.A. Murace*, (NEOTYPE designated here, CBS H-20145) (culture CBS 123395 = CPC 15054); PROVINCIA DE BUENOS AIRES: La Plata, From *Fraxinus pennsylvanica*, 2008, *M.A. Murace*, (CBS H-20146) (culture CBS 123394 = CPC 15052).

Notes. The obtusely-conic, erumpent ostioles that are produced abundantly together with the simple, papillate ones are characteristic for *P. infossa*. This species is only rarely observed and has been found before on dead limbs of *Fraxinus* in New York state (Ellis and Everhart 1888). To our knowledge however this is the first time this species has been cultivated and preserved.

Phoma microchlamydospora Aveskamp & Verkley, sp. nov. MycoBank MB512569 FIGS. 3g–i, 6

Conidia subglobosa usque ellipsoidea, hyalina, continua, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) µm, a *P. pimprina* guttulis majoribus differentia. Chlamydosporae unicellulares (sub)-globosae, 4.5–6.5 µm diam, intercalares, plerumque catenulatae. Chlamydosporae multicellulares sparsae, botryoideae-dictyosporae, e cellulis usque septem compositae, globosae, semper solitariae, 4–13 µm diam.

Pycnidia solitary or confluent, globose, glabrous, dark mouse-gray to black, immersed or superficial on the agar surface, as well as in the aerial mycelium, (110–)150–260(–380) × (110–)150–260(–340) µm. *Ostioles* 1–3(–5), papillate, but often on an elongated neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–5 layers, 10–18 µm thick. *Micropycnidia* abundant, pale brown, solitary, globose to elongated, (27.5–)35.5–71 × (27–)31–62(–70) µm. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped or broadly cymbiform, ca. 11 × 6 µm. *Conidia* from both pycnidial types indistinguishable, subglobose to ellipsoidal, hyaline, smooth, aseptate, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) µm, egutulate or with up to 4(–6) small guttules. Conidial matrix rosy-buff to rosy-vinaceous. *Unicellular chlamydospores* (sub)globose, tan-brown, intercalary, often in chains, relatively small, 4.5–6.5 µm diam, with many small to medium-sized guttules. *Multicellular chlamydospores* sparse, botryoid-dictyosporous, brown, consisting of up to seven cells, globose, intercalary but sometimes laterally branched from hyphal strands, always solitary, 4–13 µm diam, eguttulate or with many medium-sized guttules.

Colonies on OA 36–40 mm diam, with entire, smooth,

sharp margins. Aerial mycelium normally absent, or dark aerial hyphae may appear near center. Immersed mycelium hyaline; reverse olivaceous. Sometimes with a saffron discoloration of the agar due to a diffusible pigment, which persists after application of NaOH. Colonies on MEA 28–34 mm diam, with entire, smooth, sharp margin. Immersed mycelium fuscous-black. Sometimes sectors with white compact aerial mycelium are present; reverse concolorous. Colony on CHA as on MEA, although sometimes a thin, pale olivaceous-gray to iron-gray mycelial mat is covering the surface.

Etymology: Named after its relatively small chlamydospores.

Specimens examined: UNITED KINGDOM. From leaves of *Eucalyptus* sp., 1994, *A.M. Ainsworth* (HOLOTYPE CBS H-20147) (culture CBS 105.95); From an unknown vegetable plant, 1990, *D. Hyall* (CBS H-20148) (culture CBS 491.90).

Notes. The chlamydospores of *Phoma microchlamydospora* are extremely small compared to most other botryoid dictyochlamydospore producing species, which produce on average structures 8–20 µm diam (Boerema et al 2004). Conidia are similar in shape and size to those of *P. pimprina*, but the guttules are larger in the present species. Phylogenetically, this species clusters with *P. calidophila*, although distinctive differences exist in pycnidial and chlamydopore morphology.

Phoma multirostrata (P.N. Mathur, S.K. Menon & Thirum.) Dorenb. & Boerema, Mycopath. Mycol. Appl. 50:255. 1973.

≡ *Sphaeronaema multirostratum* P.N. Mathur, S.K. Menon & Thirum., Sydowia 13:146. 1959 [as *Sphaeronema multirostrata*].

= *Phoma multirostrata* var. *macrospora* Boerema, Versl. Med. Pl. ziektenk. Dienst 164:29. 1986

= *Phoma multirostrata* var. *microspora* (Allesch.) Boerema, Versl. Med. Pl. ziektenk. Dienst 164:30. 1986.

For an extended synonymy see Boerema et al (2004).

Pycnidia solitary or confluent, globose to subglobose or irregular, glabrous, brown to black, superficial or immersed, variable in size, 150–350(–720) µm diam. *Ostioles* multiple, conspicuous (10–25 µm diam), nonpapillate or on elongated necks, up to 260 µm long. *Pycnidial wall* pseudoparenchymatous, composed of oblong to cylindrical or elongated cells, 4–5 layers, ca. 9–14.5 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped. *Conidia* oblong to ellipsoidal, thin-walled, smooth, hyaline, aseptate, highly variable in size, (3.5–)4.5–6.5(–8.5) × (1.5–)2–2.5(–3) µm, with 0–3(–4) polar guttules. Conidial matrix white to buff or rosy-buff. *Chlamydospores* mostly unicellular, 5–15 µm diam, ellipsoidal to oblong to somewhat pyriform, olivaceous or pale brown with greenish guttules, solitary or in chains, intercalary but incidentally also terminal. A bunch of clustered unicellular chlamydospores can be observed regularly, especially in older cultures. These structures are easily mistaken for pseudosclerotioid chlamydospores as in *P. violicola*.

Colonies on OA (60–)65–70(–80) mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse, floccose or tufted, white to gray or absent. Agar surface olivaceous to chestnut with colorless sectors. Reverse concolorous. Colony on MEA 60–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium felty, floccose or wooly, olivaceaous to olivaceous-buff. Agar surface glaucous-gray. Reverse leaden-gray to olivaceous-black. Colony on CHA 65–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium floccose, white to gray, absent near the margin of the colony. Agar surface dark mouse-gray to greenish black. Reverse concolorous.

Specimens examined: INDIA, MAHARASHTRA, Poona, Talegaon. From poultry farm soil, Mar 1959, *M.J. Thirumalachar* (isotype CBS H-7616) (culture CBS 274.60); MAHARASHTRA, Poona, Talegaon. From soil, Mar 1959, *M.J. Thirumalachar* (CBS H-16499) (culture CBS 368.65); THE NETHERLANDS, Hoorn, greenhouse. From the stem of *Cucumis sativus*, Aug 1967, *G.H. Boerema* (CBS H-16502) (culture CBS 110.79).

Notes. The three varieties of *P. multirostrata* recognized by Boerema (1986), var. *multirostrata*, *macrospora* and *microspora* can no longer be retained as separate taxonomic entities. Taxonomic characters distinguish these varieties insufficiently, forcing Boerema et al (2004) already to state that ''intermediate variants commonly occur.'' Furthermore no genetic differences consistent with those distinguishing the varieties were found in the DNA analysis in the present study. Therefore all varieties are synonymized with the original species, *P. multirostrata*.

Phoma omnivirens Aveskamp, Verkley & Gruyter, sp. nov. MycoBank MB512570 FIGS. 3j–l, 7

Conidia subcylindrica usque ellipsoidea, hyalina, continua, (3.5–)4–5.5(–7) × (1.5–)2–2.5(–3) µm, guttulis polaribus 1–2. Chlamydosporae unicellulares oblongae, plerumque in catenas longas positae, 7–14(–20) × (4–)4.5–8.5(–18) µm, pluriguttulatae. Chlamydosporae multicellulares irregulares, dictyosporae, botryoideae, intercalares, in agaro immersae, (12–)15–52.5(–70) µm diam.

Pycnidia solitary or confluent, immersed or on the agar surface, globose to slightly subglobose, with many hyphal outgrowths, dark brown to black, 100–260(–350) × (90–)100–240(–300) µm, uni-ostiolate, nonpapillate, papillate or sometimes with a broad, elongated neck, giving the pycnidium a somewhat

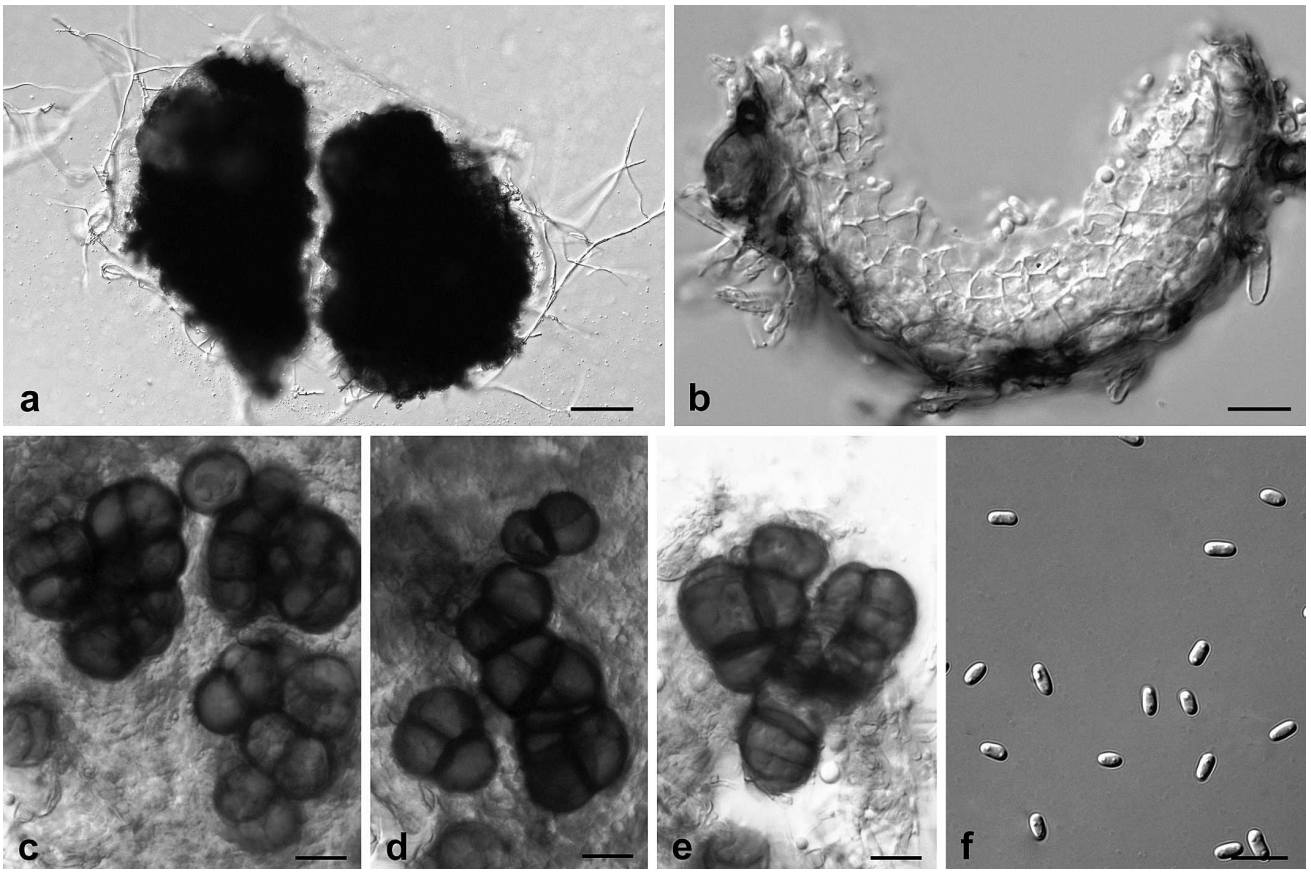

FIG. 7. *Phoma omnivirens* (ex holotype). a. Pycnidia. b. Pycnidial section. c–e. Chlamydospores. f. Conidia. Bars: a = 100 µm, b–f = 10 µm.

ovoid appearance. Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 2–6 layers, 10.5–16.5(–17.5) µm thick. *Micropycnidia* if present, generally darker than the regular pycnidia, solitary or confluent, globose, obpyriform or elongated, (40–)65–120 × (40–)60–100 µm. *Conidiogenous cells* phialidic, hyaline, simple, smooth, globose to flask-shaped, ca. (4.5–)5–6 × 4.5–5.5 µm. *Conidia* from both pycnidial types indistinguishable, subcylindrical to ellipsoidal, thin-walled, smooth, hyaline, aseptate, (3.5–)4–5.5(–7) × (1.5–)2–2.5(–3) µm, with (1–)2 small to medium-sized polar guttules; conidial matrix buff. *Submerged hyphae* smooth, hyaline, thin-walled, but often becoming pigmented and swollen, attaining a width of up to 9.5 µm. *Unicellular chlamydospores* oblong, brownish, often in long chains, 7–14(–20) × (4–)4.5–8.5(–18) µm, with many guttules in each cell. *Multicellular chlamydospores* consisting of agglomerates of unicellular chlamydospores, irregularly shaped, dictyosporous, botryoid, brownish, intercalary, submerged in the agar, (12–)15–52.5(–70) µm diam.

Colony on OA 35–60 mm diam, with entire, smooth, sharp margin. Aerial mycelium tufted, floccose to compact, locally well developed, white to (pale-)olivaceous gray, sometimes greenish olivaceous near margin. Immersed mycelium dark mouse-gray to leaden-black, toward the colony margin the color fades away to dull-green and white. Reverse concolorous or greenish black. Often an amber, primrose or buff diffusible pigment can be observed on the agar. Colony on MEA 28–52 mm diam; margins entire, smooth, sharp, or lobate to crenate. Aerial mycelium, floccose, wooly or compact, white or with various shades of gray (pale mouse-gray, olivaceous-gray, iron-gray). Reverse olivaceous-gray to leaden-black. After application of NaOH the agar color changes to bright green. Colony CHA as on MEA, but aerial mycelium less well developed.

Etymology: Name refers to the omnipresence of this species, which has been isolated from a wide range of hosts and geographical locations.

Specimens examined: BELGIUM, Gembloux. From *Phaseolus vulgaris*, 1968, *L. Obando* (HOLOTYPE CBS H-20151) (culture CBS 341.86); INDIA, Japalbur. From an unknown substrate, 1977, *D.P. Tiwari*, (CBS H-20152) (culture CBS 654.77); PAPUA NEW GUINEA, Varirata National Park. From soil, Aug 1995, *A. Aptroot*, (CBS H-20153) (culture CBS 991.95); Varirata National Park. From

soil, Aug 1995, *A. Aptroot*, (CBS H-20154) (CBS 992.95); THE NETHERLANDS. From *Chrysanthemum indicum*, 1981, *J. de Gruyter* (CBS H-20155) (culture CBS 123396 = PD 81/122); TANZANIA. From *Statice* sp., 1990, *J. de Gruyter* (CBS H-20156) (culture CBS 123397 = PD 90/1555).

Notes. This species has been isolated from a wide variety of substrates and from geographically distinct locations. Isolates have been identified erroneously as *P. sorghina* due to the similarity in shape of the chlamydospores, but this species is distinguishable by the absence of pink or reddish pigments in the colony.

Phoma pomorum var. circinata (Kusnezowa) Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512571.

≡ *Peyronellaea circinata* Kusnezowa, Nov. sist. Niz. Rast. 8:189. 1971.

= *Phoma jolyana* var. *circinata* (Kusnezowa) Boerema, Dorenb. & Kesteren, in Kew Bull. 31:535. 1977 [1976].

= *Peyronellaea nigricans* Kusnezowa, Nov. sist. Niz. Rast. 8:191. 1971.

For detailed descriptions see Boerema et al (1977), Boerema (1993) and Morgan-Jones and Burch (1987).

Specimens examined: RUSSIA, SIBERIA, Novosibirsk, Hortus Botanicus. From *Heracleum dissectum*, 1963, *T.T. Kusnezowa*, isotype CBS H-3747, (culture CBS 285.76 = ATCC 26241 = IMI 176742 = VKM F-1843); SIBERIA, Novosibirsk, Hortus Botanicus. From a leaf of *Allium nutans*, 1963, *T.T. Kusnezowa*, CBS H-16399, (culture CBS 286.76 = ATCC 26242 = IMI 176743 = VKM F-1844).

Notes. This taxon, which was seen as a variety of *P. jolyana*, differs by only one nucleotide in the ITS sequence from *P. pomorum* var. *pomorum* (CBS 539.66), whereas both ACT and TUB sequences do not show any consistent differences. Nevertheless this taxon is distinct morphologically. *Phoma pomorum* var. *circinata* has somewhat larger conidia, (3.5–)5–9 × 2–3.5 µm than the type var., (4–)5–7(–8) × 1.5–2.5(–3) µm (Boerema 1993). Furthermore unicellular chlamydospores are absent in *P. pomorum var. circinata*. Thus far strains have been reported only from Novosibirsk, Russia.

Phoma pomorum var. cyanea (Jooste & Papendorf) Aveskamp, Gruyter & Verkley, comb. nov. MycoBank MB512572.

≡ *Phoma cyanea* Jooste & Papendorf, Mycotaxon 12:444. 1981.

For detailed descriptions see Jooste and Papendorf (1981) and Boerema (1993).

Specimen examined: SOUTH AFRICA, Heilbron. From straw of *Triticum* sp., 1972, *W.J. Jooste*, holotype PREM 45736, (culture CBS 388.80).

Notes. Phoma pomorum var. *cyanea* is a species that thus far has been reported only from South Africa. It is easily distinguishable from *P. pomorum* var. *pomorum* by the production of a bluish pigment in the hyphae, pycnidia and chlamydospores. The remaining morphological characters however fit within the scope of *P. pomorum*. Furthermore the sequence analyses in the present study show a 100% similarity on ITS, ACT and TUB between the two taxa. It is concluded therefore that *P. cyanea* should be reduced to a variety of the older *P. pomorum*, as *P. pomorum* var. *cyanea*.

Phoma sancta Aveskamp, Gruyter & Verkley, sp. nov. MycoBank MB512573 FIGS. 3m–o, 8.

Conidia ovoidea, hyalina, continua, 5–7(–7.5) × 2.5–4 (–4.5) µm, guttulis polaribus 3–9(–12). Chlamydosporae multicellulares alternariodeae, phragmosporae vel dictyosporae, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) µm, solitariae, terminales, in hyphis aereis brevibus formatae.

Pycnidia solitary or confluent, globose, glabrous or covered with short hyphal outgrowth, superficially on the agar and in aerial mycelium, (80–)125–260 µm diam, conspicuously papillate; ostioles 1(–2), 20–40 (–60) µm diam. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–7 layers, relatively thick, 21–43(–51) µm thick. *Micropycnidia* formed in the aerial mycelium, generally paler than regular pycnidia or even hyaline, solitary, (sub)globose, (40–)60–80(–110) µm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, globose to flask-shaped, (5–)6–7 × (5–)5.5–6.5 µm. *Conidia* ovoid, thin-walled, smooth, hyaline, aseptate, 5–7(–7.5) × 2.5–4(–4.5) µm, with 3–9(–12) polar guttules. Conidial matrix salmon. *Chlamydospores* multicellular, alternarioid, phragmosporous or dictyosporous, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) µm, dark brown, terminal on erect aerial hyphae, solitary.

Colonies on OA 45–60 mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse or absent, tufted, gray to white. Immersed mycelium fawn, but fading away to gray-olivaceous, becoming hyaline near margin; reverse concolorous. After application of NaOH the agar near the hyphae becomes inconspicuously reddish brown. Colonies on MEA 52–57 mm diam, with entire, smooth, sharp margins. Aerial mycelium greenish olivaceous to white, floccose and abundant near center, toward the margin less well developed. Immersed mycelium iron-black with or without vinaceous sectors. Reverse concolorous. Colonies on CHA similar to MEA, but aerial mycelium less well developed.

Etymology: Named because of its association with the hosts *Gleditsia triacantha* (Christusdoorn in Dutch, meaning Christ's thorn) and *Ailanthus altissima*, tree of heaven.

Specimen examined: SOUTH AFRICA. From dead

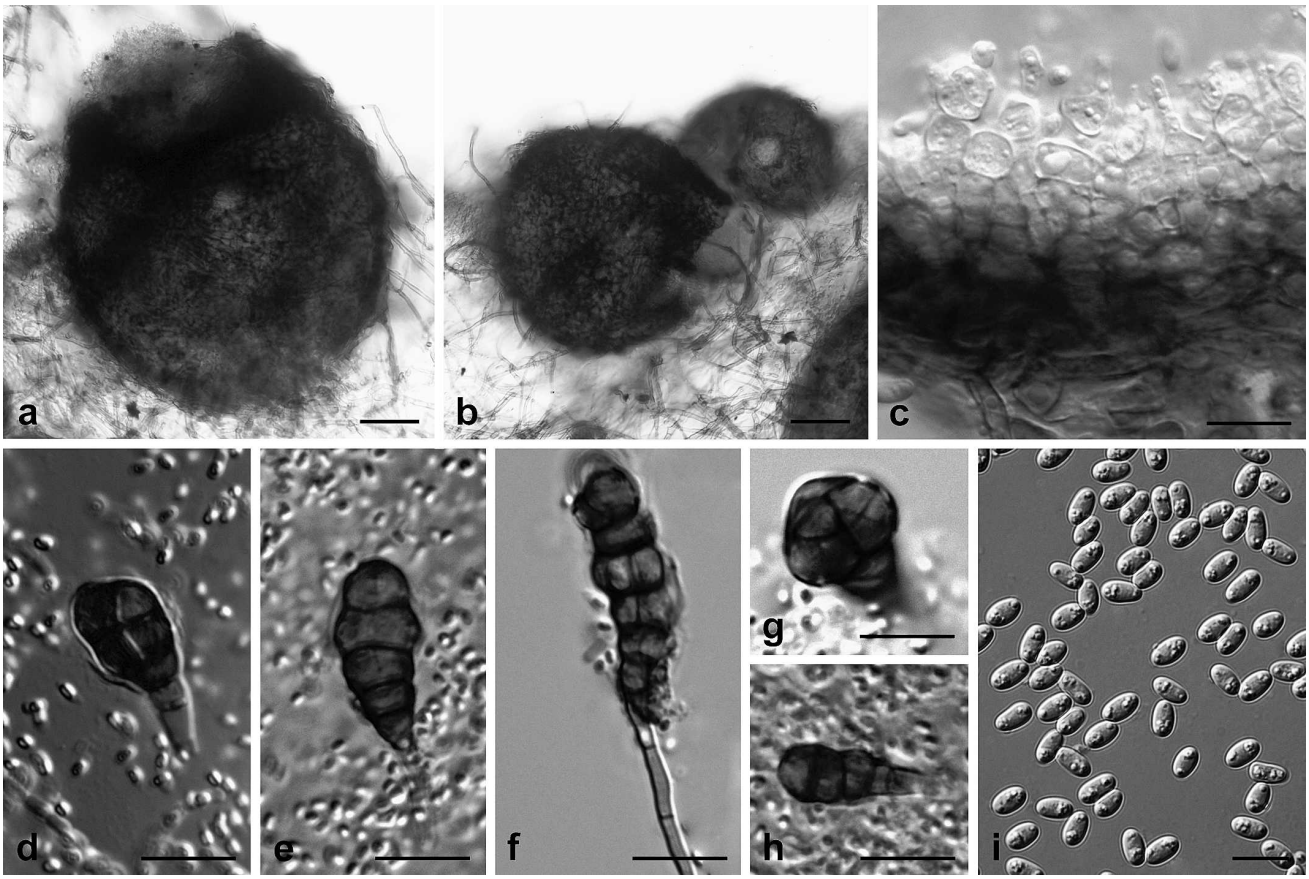

FIG. 8. *Phoma sancta* (ex holotype). a–b. Pycnidia. c. Pycnidial section. d–h. Chlamydospores. i. Conidia. Bars: a = 50 µm, b–i = 10 µm.

branches of *Ailanthus altissima*, Oct 1982, *C. Jansen* (HOLOTYPE, CBS H-16332) (culture CBS 281.83).

Notes. Phoma sancta appears to be widespread, and clusters within a group in which among others *P. glomerata*, *P. pomorum* and *P. jolyana* are accommodated. This species is recognizable by the high percentage of phragmospores that are formed in culture. The latter feature might have been the reason for the previous identification as *P. jolyana*. The latter species produces its chlamydospores mainly in the agar and in the aerial mycelium on a wide range of media, whereas the multicellular chlamydospores of *P. sancta* are formed mainly on OA, and are terminally located on short, erect hyphae emerging from the agar surface.

Phoma schachtii Aveskamp, Gruyter & Verkley, sp. nov. MycoBank MB512574 FIGS. 3p–r, 9

Conidia ellipsoidea, hyalina, continua, (4–)4.5–5.5(–6) × (1.5–)2–2.5 µm, eguttulata, vel guttulis polaribus 2(–3). Chlamydosporae multicellulares dictyosporae, alternariodeae vel botryoideae, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) µm diam, viridulae, terminales, solitariae vel in catenas breves positae, in culturis vestioribus confertim aggregatae et pseudosclerotiodeae.

Pycnidia solitary or confluent, globose, completely covered with hyphal outgrows, submerged in the agar, (180–)220–600(–650) µm diam, papillate, or with an elongated neck, and a single inconspicuous ostiole. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric to oblong cells, 4–9 layers, (22.5–)26.5–37(–41.5) µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, ca. 5–7 × 4–6 µm. *Conidia* ellipsoidal, thin-walled, smooth, hyaline, aseptate, (4–)4.5–5.5(–6) × (1.5–)2–2.5 µm, eguttulate or with 2(–3) polar guttules. Conidial matrix cream white. *Multicellular chlamydospores* developing after several weeks, dictyosporous, alternarioid or botryoid, abundant in the aerial mycelium, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) µm diam, greenish, terminal, single or in short chains with up to three elements, in older cultures aggregating into pseudosclerotioid masses.

Colonies on OA 26–32 mm diam, with entire, smooth, sharp margins; aerial mycelium felted, mostly olivaceous-gray, near center iron-gray and smoke-gray near margins. Reverse olivaceous-gray with some olivaceous zones. Colonies on MEA 20–24 mm diam, with entire, smooth, sharp margins. Aerial mycelium

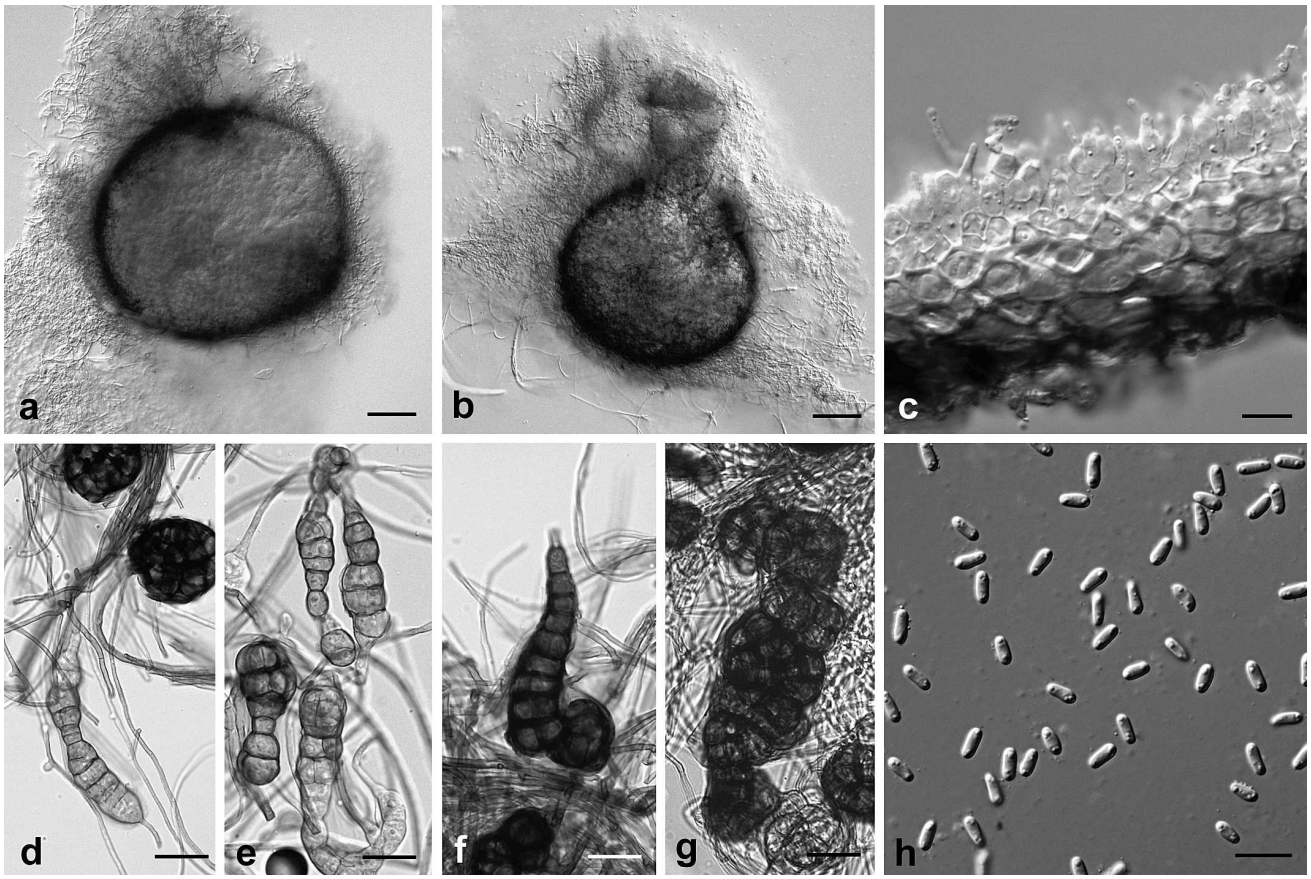

FIG. 9. *Phoma schachtii* (ex holotype). a–b. Pycnidia. c. Pycnidial section. d–g. Chlamydospores. h. Conidia. Bars: a–b = 50 µm, c, h = 10 µm, d–g = 20 µm.

felted or floccose to tufted, greenish gray or pale olivaceous-gray. Reverse olivaceous-black to dark slate-blue, near margin somewhat brown-vinaceous. Colonies on CHA 28–32 mm diam, with entire, smooth, sharp margins, covered by a compact or felted mycelial mat, olivaceous-gray to fuscous, near the center mouse-gray. Reverse concolorous.

Etymology: Named after the host species on which the fungus was found, a cyst of the nematode *Heterodera schachtii*.

Specimen examined: THE NETHERLANDS, Bergen op Zoom. From the cyst of *Heterodera schachtii*, 1984, *W. Heybroek* (HOLOTYPE, CBS H-16188) (culture CBS 502.84).

Notes. At least nine *Phoma* species, of which most are capable of producing chlamydospores, have been isolated from the cysts of *Heterodera* spp. (Chen et al 1996). *Phoma schachtii*, which has been found parasitizing a cyst nematode, has many characters in common with *P. chrysanthemicola*, which explains why it has not been recognized previously as a separate taxon. The two species can be distinguished by the clear alternarioid-botryoid chlamydodospores that are present in fresh cultures. In later stages these will aggregate and form long pseudosclerioid masses. Those masses are generally smoother than in *P. chrysanthemicola*, which has more warty chlamydospore walls.

DISCUSSION

In their final publication after more than 40 y of morphological studies on genus *Phoma*, Boerema and co-workers listed the 223 specific and infraspecific taxa that they recognized (Boerema et al 2004). Since the publication of this identification manual several studies on *Phoma* species have been conducted with DNA sequence phylogenies, revealing *Phoma* to be a more complicated genus than previously considered (Reddy et al 1998; Torres et al 2005a, b). In addition to the unclear generic definition (Aveskamp et al 2008) the current morphology-based subdivision of *Phoma* appears not to be in congruence with its molecular phylogeny.

Two species that are regarded members of section *Peyronellaea* (viz. *P. chrysanthemicola* and *P. violicola*) and the newly described species *P. schachtii* do not group with the majority of the Peyronellaea species in clade 1 but are found among the basal lineages,

together with the type species of *Phoma* sections *Heterospora*, *Paraphoma* and *Plenodomus* (FIG. 1). Characters that are considered to be typical for these sections, namely pluriform conidia, setose pycnidia or scleroplectenchyma respectively, were never observed in *P. chrysanthemicola*, *P. violicola* or *P. schachtii*. These species all are characterized by the formation of chlamydospores in so-called pseudosclerotioid masses.

Most dictyochlamydospore-producing taxa cluster together with *P. herbarum*, the type species of genus *Phoma* and as a consequence also of section *Phoma* in clade 1. Further these taxa cluster with the type species of two other sections, namely *P. exigua var. exigua* (sect. *Phyllostictoides*) and *P. zeae-maydis* (sect. *Macrospora*) (FIG. 2). Chlamydospores produced by the taxa in this cluster represent the botryoid and alternarioid types, except for those of the novel species *P. coffeae-arabicae*, which are pseudosclerotioid. Based on these results the subdivision of genus *Phoma* (Boerema 1997) therefore can be questioned. This observation is in congruence with the study of Torres et al (2005a), who found major inconsistencies between the system of Boerema (1997) and their molecular data and advocated that the current taxonomy of the genus *Phoma* needs to be thoroughly revised.

The *Phoma* anamorph state is found in multiple Pleosporalean teleomorphs, including *Didymella*, *Leptosphaeria* and *Pleospora* (Aveskamp et al 2008). The backbone structure (FIG. 1) can be explained largely by the clustering of *Peyronellaea* species with the different teleomorph groups. Most species studied cluster with *P. zeae-maydis* (teleomorph *D. zeae-maydis*) in clade 1, indicating that *Didymella* would be the most likely teleomorph for those species if a sexual state were encountered. *Phoma violicola* and *P. schachtii* are found in clade 2a, in which *P. lingam* also is accommodated, which has a teleomorph in *Leptosphaeria*. Clade 2b represents the *Pleospora*-associated clade. In clade 3 three species are grouped for which thus far no teleomorph has been recorded. Species in this clade represent sections *Peyronellaea* (*P. chrysanthemicola*), *Heterospora* (*P. samarorum*) and *Paraphoma* (*P. radicina*, type species of its section). Clades found in this study resemble some of the groups found in Schoch et al (2006) in the Pleosporales. The phylogenetic distances between clades 1 and 2 were observed by Reddy et al (1998) and Torres et al (2005b) and forced these authors to advocate the reinstallation of the anamorph genus name *Plenodomus* for the *Leptosphaeria*-associated species. However, such a taxonomic recombination requires further evaluation of all *Phoma* species and associated genera.

Phoma identification is problematic and gives rise to many misidentifications (Bridge et al 2003), but most strains studied here have been classified properly. Strains that could not be identified upon collection due to overlapping species characters now can be delimited and defined with molecular characterization tools. In the present study we recognize five novel dictyochlamydospore-forming species that were preserved in culture collections under incorrect names or as unidentified species. New combinations in a further five taxa were made to ensure consistency with the DNA data obtained in the present study. The species concepts defined in the past appear to be still valid for *P. americana* (Morgan-Jones and White 1983, Boerema 1993), *P. epicoccina* (Boerema 1993, Arenal et al 2000), *P. glomerata*, *P. pomorum* var. *pomorum* (Boerema et al 1965, Boerema 1993), *P. chrysanthemicola*, *P. pimprina*, *P. subglomerata*, *P. violicola* and *P. zantedeschiae* (Boerema 1993). Also *P. sorghina* (Boerema et al 1968, White and Morgan-Jones 1983) appears to be properly described and represents a monophyletic clade, although a high level of infraspecific genetic variation has been observed. Only two strains (CBS 991.95 and CBS 992.95) were morphologically and genetically clearly distinct and are reclassified in the novel species *P. omnivirens* here. The remaining 13 species clustered together in a *P. sorghina* superclade, in which no less than nine different, often well supported subclades are recognized (FIG. 2). The morphological variation was sparse however, and all strains fitted within the scope of the species as described by Boerema et al (1973) and White and Morgan-Jones (1983). Also the host association and the origin are too diverse to provide further information on a possible further classification. The high genetic variety in comparison to *P. glomerata*, for example, might indicate a high recombination rate. Sexual recombination, although a teleomorph has never been observed, might be one of the reasons for this phenomenon.

Much confusion still surrounds the identity of *P. jolyana*. A relatively wide species concept had been applied to this taxon (Boerema et al 1965, Morgan-Jones and Burch 1987), which gave rise to many incorrectly identified isolates. At least two new taxa were encountered among the strains that initially were stored in the CBS and PD culture collections as *P. jolyana* and are renamed *P. coffeae-arabicae* and *P. sancta* in this study. Three varieties previously were recognized within this species, of which the type variety was widespread, whereas var. *circinata* and *sahariensis* had been collected on only a few occasions from isolated places (Boerema et al 2004). In this study both varieties have been recombined: var. *sahariensis* is elevated to species level as *P. calidophila*, whereas var. *circinata* has been recombined to a variety of *P. pomorum*. Given the isolated origins of

these isolates we expect that many more dictyochlamydospore-producing taxa will be encountered once these origins are sampled more.

This study also addresses the problem that single morphological characters cannot always be discriminative between taxa. A good example is the genetic similarity of *P. pomorum* and *P. cyanea*. Although *P. cyanea* was easily distinguishable due to the obvious production of a bluish pigment in its hyphae, pycnidia and chlamydospores, sequence analysis proved it to be highly similar to *P. pomorum*. Because several other morphological characters showed high similarity between the two taxa it was concluded that *P. cyanea* should be reduced to a variety of the older *P. pomorum*.

The taxa that clustered in section *Peyronellaea* resemble a genetically heterogenous group. The ability to produce dictyochlamydospores probably has been lost and gained multiple times in the evolution of the *Pleosporales*. This character is also easily lost in culture, as has been reported in literature (Boerema et al 1965, Dorenbosch 1970). Chlamydospore production in fungi is generally considered to be a survival strategy due to harsh conditions by perennation (Kirk et al 2008). Although the strains used in this study were collected from a wide variety of environments, a relatively high number was retrieved from plant material belonging to the Gramineae. Also many of the chlamydospore-forming species have been found in association with cyst nematodes (Heteroderidae, Chen et al 1996). It is tempting to link the similarity in hosts with the capability to produce chlamydospores. Therefore it very well might be that production of such thick-walled spores serves ecological purposes other than long-term survival. Further research should be conducted on the functioning of these structures.

ACKNOWLEDGMENTS

This research was supported by the Dutch Ministry of Agriculture, Nature and Food Quality through an endowment of the FES program ''Versterking infrastructuur Plantgezondheid''. The authors are extremely grateful for the help of Mrs Karin Rosendahl (PD) and Dr Amy Rossman (Systematic Botany and Mycology Laboratory) who provided strains. We thank Mrs Marjan Vermaas for her help in preparing the photo plates and Dr Cécile Gueidan for her help with the phylogenetic analyses. We are further indebted to Prof Dr Pierre de Wit and Dr Chiel Noordeloos for their critical discussions on the manuscript.

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