Persoonia 32, 2014: 25–51
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

http://dx.doi.org/10.3767/003158514X679191

Novel genera and species of coniothyrium-like fungi in *Montagnulaceae* (*Ascomycota*)

G.J.M. Verkley[1], K. Dukik[1], R. Renfurm[1], M. Göker[2], J.B. Stielow[1]

Key words

γ-actin
β-tubulin
ITS
LSU
Microsphaeropsis
Paraconiothyrium
taxonomy

Abstract Based on analyses of concatenated internal transcribed spacer regions of the nrDNA operon (ITS), large subunit rDNA (LSU), γ-actin and β-tubulin gene sequences the taxonomy of coniothyrium-like fungi belonging in the family *Montagnulaceae*, order *Pleosporales*, was re-assessed. Two new genera are proposed, *Alloconiothyrium*, to accommodate *A. aptrootii* sp. nov., and *Dendrothyrium* for *D. longisporum* sp. nov. and *D. variisporum* sp. nov. One new species is described in *Paraconiothyrium*, viz. *Parac. archidendri* sp. nov., while two species so far classified in *Paraconiothyrium* are transferred to *Paraphaeosphaeria*, viz. *Paraph. minitans* comb. nov. and *Paraph. sporulosa* comb. nov. In *Paraphaeosphaeria* five new species are described based on asexual morphs, viz. *Paraph. arecacearum* sp. nov., *Paraph. neglecta* sp. nov., *Paraph. sardoa* sp. nov., *Paraph. verruculosa* sp. nov., and *Paraph. viridescens* sp. nov. Macro- and micromorphological characteristics are fully described.

Article info Received: 26 November 2012; Accepted: 8 August 2013; Published: 29 January 2014.

INTRODUCTION

Coniothyrium-like fungi are coelomycetous asexual morphs of *Pleosporales* and other *Dothideomycetes* (*Ascomycota*), characterised by pycnidial or stromatic conidiomata producing mostly relatively small, subhyaline to pigmented, 1- or 2-celled conidia. Most species have been classified in the genera *Coniothyrium* or *Microsphaeropsis*. They are often of considerable importance to society, being destructive as plant pathogens or beneficial as effective biological control agents (Carisse et al. 2001, Carisse & Bernier 2002a, b, El-Bassam et al. 2002) or bioremediators (da Silva et al. 2003a, b). They are also being reported from clinical cases with invasive cutaneous infections in immunocompromised or transplant patients (Balajee et al. 2007, Gordon et al. 2012, de Gruyter et al. 2012). The taxonomy of most coniothyrium-like fungi is problematic, due to the simplicity, plasticity and variability of morphological features exhibited by these coelomycetes. Attempts to delimit the genera based on features such as conidiomatal structure, conidiogenesis and conidial morphology have not been successful (Sutton 1980). Species of *Coniothyrium* and *Microsphaeropsis* described from plant material were largely distinguished by host-plant taxonomy (Wollenweber & Hochapfel 1937, Bestagno Biga et al. 1958, Sutton 1974), and for the majority no type or other reference cultures are available to date. Soils are also rich in coniothyrium-like fungi (Domsch et al. 2007), but the small number of species formally described from soil today does not cover the extant diversity, and the variability seen in such isolates hampers reliable identification. DNA sequences are still scarcely available and mostly of doubtful identity (Verkley et al. 2004).

Recent molecular phylogenetic studies focussing on sexual and asexual genera of *Pleosporales* have demonstrated that *Coniothyrium* and *Microsphaeropsis*, and also the ubiquitous and speciose coelomycete genus *Phoma*, are polyphyletic, with species occurring in several clades of the order *Pleosporales*, which are now being used as a firm basis for redefining families (Verkley et al. 2004, 2013, Schoch et al. 2009, Zhang et al. 2009, 2012, Aveskamp et al. 2010, de Gruyter et al. 2010, 2012, Quaedvlieg et al. 2013). The position of the type species of *Microsphaeropsis*, *M. olivacea*, was confirmed within the family *Didymellaceae* and that of *Coniothyrium*, *C. palmarum*, within the *Leptosphaeriaceae*. Several *Coniothyrium* species were grouped in the well-supported clade of *Montagnulaceae*, together with *Paraphaeosphaeria* (including *Paraph. michotii*, the type species of this genus) and the genera *Kalmusia*, *Bimuria*, *Didymocrea*, *Letendraea* and *Montagnula* (Zhang et al. 2009). In early recognition of the genetic distance from *Coniothyrium* s.str., Verkley et al. (2004) introduced the new genus *Paraconiothyrium* for a number of these asexual morphs grouping with *Paraphaeosphaeria*, and described four new *Paraconiothyrium* species, viz. *Parac. estuarinum* (the type species of this genus), *Parac. brasiliense*, *Parac. cyclothyrioides* and *Parac. fungicola*. Based on molecular phylogenetic evidence, the frequently reported soil-borne fungus *Coniothyrium sporulosum* and the important biocontrol agent *C. minitans* were recombined to *Paraconiothyrium*. Damm et al. (2008) described a further two new *Paraconiothyrium* species, *Parac. africanum* and *Parac. variabile*, and also transferred *Microdiplodia hawaiiensis* to *Paraconiothyrium*. Budziszewska et al. (2011) described *Parac. babiogorense*, an endophyte of the clubmoss *Huperzia*. Based on LSU sequence analyses, de Gruyter et al. (2012) transferred the coelomycetes *Phoma falvescens*, *Plenodomus fusco-maculans*, *Asteromella tiliae* and *Phoma lini* to *Paraconiothyrium*, while they also described a new species, *Paraconiothyrium maculicutis*. *Paraconiothyrium* currently holds 15 species, and only one of these, *Parac. fuckelii*, has a known sexual morph (Verkley et al. 2004, Damm et al. 2008, de Gruyter et al. 2012). Other novel genera are sporadically being proposed to accommodate coniothyrium-like fungi in other clades of *Dothideomycetes* as well. For example, the genus *Xenoconiothyrium* Crous & Marinc. was recently erected for coniothyrium-like fungi belonging to *Teratosphaeriaceae* (Crous et al. 2011).

---

[1] CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands; corresponding author e-mail: g.verkleij@cbs.knaw.nl.
[2] DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstrasse 7B, 38124 Braunschweig, Germany.

Table 1 Overview of species and isolates used in this study with their CBS accession numbers, former names and, where applicable, corrected taxon names according to findings in this study.

Species	CBS accession nr.	Former identification	INSDC ITS	INSDC TUB	INSDC LSU	INSDC ACT	Substrate	Host
Alloconiothyrium aptrootii	CBS 980.95^{T}	*Coniothyrium* sp.	JX496121	JX496460	JX496234	JX496347	Soil	~
	CBS 981.95	*Coniothyrium* sp.	JX496122	JX496461	JX496235	JX496348	Soil	~
Ampelomyces quisqualis	CBS 128.79	~	~	~	JX681063	~	Lesion	Cucumber mildew
	CBS 129.79	~	~	~	JX681064	~	Lesion	Cucumber mildew
	CBS 131.31	~	~	~	JX681066	~	Lesion	*Erysiphe cichoracearum* on *Helianthus tuberosus*
	CBS 131.79	~	~	~	JX681065	~	Lesion	Cucumber mildew
	CBS 133.32	~	~	~	JX681067	~	Lesion	*Microsphaera alni* on *Lonicera* sp.
Aplosporella aquifolii	CBS 103.68	~	~	~	JX681068	~	Dead leaf	*Ilex aquifolium*
A. hesperidica	CBS 208.37	~	~	~	JX681069	~	Early stem-end rot	*Citrus sinensis*
A. mali	CBS 519.75	~	~	~	JX681070	~	Fruit	*Malus sylvestris*
A. prunicola	CBS 121167	~	~	~	JX681071	~	Bark	*Prunus persica* var. *nucipersica*
A. ruborum	CBS 117.82	~	~	~	JX681072	~	Dead stem	*Rubus* sp.
A. sterculiae	CBS 342.78	~	~	~	JX681073	~	~	*Sterculia oblonga*
Boeremia exigua var. *exigua*	CBS 431.74	~	~	~	JX681074	~	Tuber with gangrene	*Solanum tuberosum*
Coniothyrina agaves	CBS 470.69	~	~	~	JX681075	~	Spot on dead leaf	*Agave americana*
Coniothyrium cerealis	CBS 157.78	~	~	~	JX681080	~	Stem	*Triticum aestivum*
	CBS 518.74	~	~	~	JX681079	~	~	*Phleum pratense*
Con. juniperi	CBS 610.72	~	~	~	JX681081	~	~	*Juniperus* sp.
Con. nitidae	CBS 111302	~	~	~	JX681082	~	~	*Protea nitida*
	CBS 111321	~	~	~	JX681083	~	~	*Protea nitida*
Con. palmarum	CBS 400.71	~	EU754153	~	JX681084	~	Dead petiole	*Chamaerops humilis*
	CBS 758.73	~	EU040225	~	JX681085 EU754154	~	Leaf spots	*Phoenix dactylifera*
Con. palmicola	CBS 161.37	~	~	~	JX681086	~	Stem	*Pandanus tectoriae*
Coniothyrium sp.	CBS 122.76	~	~	~	JX681077	~	~	*Cocos nucifera*
	CBS 302.72	~	JX496065	JX496404	JX496178	JX496291	Leaf	*Azalea* sp.
	CBS 423.92	~	~	~	JX681078	~	Root	*Hordeum vulgare*
Cucurbidothis pityophila	CBS 149.32	~	~	~	JX681087	~	Root, young tree	*Picea* sp.
Cucurbitaria berberidis	CBS 394.84	~	~	~	JX681088	~	Dead branches	*Berberis julianae*
Dendrothyrium longisporum	CBS 582.83^{T}	*Coniothyrium* sp.	JX496097	JX496436	JX496210	JX496323	~	*Arceuthobium pusillum*
	CBS 824.84	*Coniothyrium cerealis*	JX496115	JX496454	JX496228	JX496341	Leaf spot	*Triticum aestivum*
D. variisporum	CBS 121517^{T}	*Coniothyrium* sp.	JX496030	JX496369	JX496143	JX496256	Declined grape vine	*Vitis vinifera*
	CBS 197.82	*Coniothyrium* sp.	JX496053	JX496392	JX496166	JX496279	~	*Erica carnea*
Didymella exigua	CBS 183.55	~	EU754155.1	~	JX681089	~	~	*Rumex arifolius*
Keissleriella cladophila	CBS 104.55	~	~	~	JX681090	~	~	*Smilax parvifolia*
Leptosphaeria doliolum subsp. *errabunda*	CBS 541.66	~	~	~	JX681093	~	Stem	*Rudbeckia* sp.
Leptosphaeria doliolum var. *doliolum*	CBS 297.51	~	~	~	JX681094	~	~	*Papaver rhoeas*
	CBS 504.75	~	~	~	JX681095	~	Stem	*Urtica dioica*
L. maculans	CBS 260.94	~	~	~	JX681096	~	~	*Brassica oleracea*
L. australis	CBS 100575	~	~	~	JX681099	~	Soil	~
	CBS 939.69	~	~	~	JX681098	~	Soil	~
Massaria platani	CBS 221.37	~	DQ678065	~	JX681100	~	~	*Platanus occidentalis*
‘*Microsphaeropsis arundinis*’	CBS 100243	~	JX496010	JX496349	JX496123	JX496236	Soil	~
M. olivacea	CBS 233.77	~	GU237988	~	JX681103	~	Needle	*Pinus laricio*
	CBS 303.68	~	~	~	JX681101	~	Leaf spots	*Ligustrum vulgare*
	CBS 432.71	~	GU237987	~	JX681102	~	Dead twig and pod	*Sarothamnus* sp.
Neophaeosphaeria filamentosa	CBS 102203	~	~	~	JX681104	~	~	*Yucca rostrata*
Paraconiothyrium africanum	CBS 121166^{T}	~	JX496029	JX496368	JX496142	JX496255	~	*Prunus persica*
Paracon. archidendri	CBS 168.77^{T}	*Coniothyrium* sp.	JX496049	JX496388	JX496162	JX496275	Leaf spot	*Pithecelobium bigeminum*
Paracon. brasiliense	CBS 100299^{T}	~	AY642531	JX496350	JX496124	JX496237	Fruit	*Coffea arabica*
	CBS 115.92	*Coniothyrium* sp.	JX496022	JX496361	JX496135	JX496248	Phyllosphere	*Olea europaea*

	CBS 122319	~	JX496032	JX496371	JX496145	JX496258	~	~
	CBS 122320	~	JX496033	JX496372	JX496146	JX496259	Pruning cut	*Actinidia chinensis* var. Hort16A
	CBS 122321	~	JX496034	JX496373	JX496147	JX496260	Browning wood	*Platanus* × *acerifolia*
	CBS 122851	~	JX496036	JX496375	JX496149	JX496262	~	*Juglans regia*
	CBS 159.60	*Coniothyrium* sp.	JX496044	JX496383	JX496157	JX496270	~	~
	CBS 254.88	*Coniothyrium* sp.	JX496058	JX496397	JX496171	JX496284	~	*Magnolia* sp.
	CBS 395.87	*Coniothyrium* sp.	JX496083	JX496422	JX496196	JX496309	Soil	~
	CBS 587.84	*Coniothyrium* sp.	JX496099	JX496438	JX496212	JX496325	Bark	*Vitis vinifera*
Paracon. cyclothyrioides	CBS 432.75	*Coniothyrium* sp.	JX496088	JX496427	JX496201	JX496314	Soil	*Hevea brasiliensis*
	CBS 972.95^T	~	JX496119	JX496458	JX496232	JX496345	Soil	~
Paracon. estuarinum	CBS 109850^T	~	JX496016	JX496355	JX496129	JX496242	Sediment from estuarine habitat	~
Paracon. fuckelii	CBS 508.94	*Coniothyrium rosarum*	JX496096	JX496435	JX496209	JX496322	Stem cancer	*Rosa* sp.
	CBS 584.69	*Coniothyrium fuckelii*	JX496098	JX496437	JX496211	JX496324	Root of gymnosperm	~
	CBS 653.85	*Coniothyrium* sp.	JX496104	JX496443	JX496217	JX496330	Canker	*Picea abies*
	CBS 764.71B	*Paraconiothyrium minitans*	JX496112	JX496451	JX496225	JX496338	Academic hospital	Human
	CBS 797.95	*Coniothyrium fuckelii*	JX496113	JX496452	JX496226	JX496339	Dead stem	*Rubus* sp.
Paracon. fungicola	CBS 113269^T	~	JX496020	JX496359	JX496133	JX496246	Resupinate polypore fungus	~
Paracon. hawaiiense	CBS 120025^T	~	JX496027	JX496366	JX496140	JX496253	Stem	*Sophora chrysophylla*
Paraconiothyrium sp.	CBS 119485	~	EF055359	~	~	~	Wood	*Actinidia chinensis* var. Hort 16A
	CBS 194.82	*Coniothyrium* sp.	JX496052	JX496391	JX496165	JX496278	~	*Lycopodium annotinum*
Paraconiothyrium sp. 1	CBS 113682	*Microsphaeropsis pseudaspera*	JX496021	JX496360	JX496134	JX496247	Air sample	~
	CBS 251.87	*Coniothyrium* sp.	JX496057	JX496396	JX496170	JX496283	Nail	Human
Paracon. variabile	CBS 112.72	*Coniothyrium* sp.	JX496019	JX496358	JX496132	JX496245	Dead stem ex herbarium specimen	*Dianthus* sp.
	CBS 119486	~	JX496023	JX496362	JX496136	JX496249	Wood	*Actinidia chinensis* var. Hort 16A
	CBS 119633	*Paraconiothyrium* sp.	JX496024	JX496363	JX496137	JX496250	~	*Laurus nobilis*
	CBS 120014	*Paraconiothyrium variabile*	JX496026	JX496365	JX496139	JX496252	Wood	*Actinidia chinensis* var. Hort 16A
	CBS 121163	~	EU295639	~	~	~	~	*Prunus persica*
	CBS 121164	~	JX496028	JX496367	JX496141	JX496254	~	*Prunus persica*
	CBS 121754	~	JX496031	JX496370	JX496144	JX496257	~	*Prunus salicina*
	CBS 122322	*Paraconiothyrium* sp.	JX496035	JX496374	JX496148	JX496261	Pruning cut	*Actinidia chinensis* var Hot16A
	CBS 168.69	*Paraconiothyrium sporulosum*	JX496048	JX496387	JX496161	JX496274	Soil	Acer pseudoplatanus
	CBS 269.74	*Coniothyrium* sp.	JX496060	JX496399	JX496173	JX496286	Desert soil	~
	CBS 413.84	*Coniothyrium* sp.	JX496086	JX496425	JX496199	JX496312	Stem and leaf	*Lepidosperma longitudinale*
	CBS 433.71	*Coniothyrium palmigenum*	JX496089	JX496428	JX496202	JX496315	Dead leaf	*Chamaerops humilis*
	CBS 461.90	*Coniothyrium platani*	JX496093	JX496432	JX496206	JX496319	Pruning wound	*Platanus acerifolia*
	CBS 504.84	*Coniothyrium* sp.	JX496095	JX496434	JX496208	JX496321	Teleutosorus	*Puccinia allii*
	CBS 638.93	*Microsphaeropsis* sp.	JX496102	JX496441	JX496215	JX496328	Disintegrated calcareous sandstone	~
	CBS 680.83	*Coniothyrium* sp.	JX496105	JX496444	JX496218	JX496331	Toe nail	Human
	CBS 882.70	*Coniothyrium* sp.	JX496118	JX496457	JX496231	JX496344	Dead stem	*Spartium junceum*
Paraphaeosphaeria angularis	CBS 167.70^T	*Coniothyrium* sp.	JX496047	JX496386	JX496160	JX496273	Leaf	*Saccharum officinarum*
Paraph. arecacearum	CBS 158.75^T	*Coniothyrium* sp.	JX496043	JX496382	JX496156	JX496269	Soil	*Elaeis guineensis*
	CBS 614.75	*Coniothyrium* sp.	JX496100	JX496439	JX496213	JX496326	Cocos nucifera	*Cocos nucifera*
Paraph. michotii	CBS 340.86	*Coniothyrium* sp.	JX496079	JX496418	JX496192	JX496305	Leaf	*Phragmites australis*
	CBS 652.86	~	JX496103	JX496442	JX496216	JX496329	~	*Typha latifolia*
Paraph. minitans	CBS 111750	*Paraconiothyrium minitans*	JX496017	JX496356	JX496130	JX496243	Sclerotia	*Sclerotinia sclerotorium*, Lucerne
	CBS 111752	*Paraconiothyrium minitans*	JX496018	JX496357	JX496131	JX496244	~	Unknown
	CBS 151.96	*Paraconiothyrium minitans*	JX496042	JX496381	JX496155	JX496268	Sclerotia	*Sclerotinia sclerotiorum*
	CBS 286.81	*Paraconiothyrium minitans*	JX496063	JX496402	JX496176	JX496289	Stem	*Solanum tuberosum*
	CBS 859.71	*Paraconiothyrium minitans*	JX496116	JX496455	JX496229	JX496342	Sclerotium, in soil	*Sclerotinia trifoliorum*
	CBS 860.71	*Paraconiothyrium minitans*	JX496117	JX496456	JX496230	JX496343	Sclerotium, in soil	*Solanum tuberosum*

Table 1 (cont.)

Species	CBS accession nr.	Former identification	INSDC ITS	INSDC TUB	INSDC LSU	INSDC ACT	Substrate	Host
Paraph. neglecta	CBS 119637	*Paraconiothyrium sporulosum*	JX496025	JX496364	JX496138	JX496251	Inner ear	Human
	CBS 124076	*Paraconiothyrium* sp.	JX496037	JX496376	JX496150	JX496263	Wood	*Actinidia chinensis* var. Hort16A
	CBS 124077	*Paraconiothyrium* sp.	JX496038	JX496377	JX496151	JX496264	Wood	*Actinidia chinensis* var. Hort16A
	CBS 124078	*Paraconiothyrium* sp.	JX496039	JX496378	JX496152	JX496265	Wood	*Actinidia chinensis* var. Hort16A
	CBS 180.61	*Coniothyrium fuckelii*	JX496051	JX496390	JX496164	JX496277	Acid mull soil, with very well decomposed leaves	~
	CBS 300.72	*Coniothyrium* sp.	JX496064	JX496403	JX496177	JX496290	Leaf	*Azalea* sp.
	CBS 303.77	*Paraconiothyrium sporulosum*	JX496067	JX496406	JX496180	JX496293	Taxus baccata	*Taxus baccata*
	CBS 305.77	*Paraconiothyrium sporulosum*	JX496070	JX496409	JX496183	JX496296	~	*Taxus baccata*
	CBS 306.77	*Paraconiothyrium sporulosum*	JX496071	JX496410	JX496184	JX496297	~	*Juniperus chinensis*
	CBS 307.77	*Paraconiothyrium sporulosum*	JX496072	JX496411	JX496185	JX496298	~	*Cupressocyparis leylandii*
	CBS 335.78	*Coniothyrium* sp.	JX496076	JX496415	JX496189	JX496302	Decayed wood	~
	CBS 337.78	*Paraconiothyrium sporulosum*	JX496077	JX496416	JX496190	JX496303	Rotten wood	~
	CBS 359.75	*Paraconiothyrium sporulosum*	JX496081	JX496420	JX496194	JX496307	Canker	*Juniperus* sp.
	CBS 431.77	*Paraconiothyrium sporulosum*	JX496087	JX496426	JX496200	JX496313	~	Unknown
	CBS 434.71A	*Paraconiothyrium minitans*	JX496090	JX496429	JX496203	JX496316	~	*Erica carnea*
	CBS 434.71B	*Paraconiothyrium minitans*	JX496091	JX496430	JX496204	JX496317	~	*Pyrola rotundifolia*
	CBS 452.81	*Paraconiothyrium sporulosum*	JX496092	JX496431	JX496205	JX496318	Dead branches	*Pyrus malus*
	CBS 627.94	*Paraconiothyrium sporulosum*	JX496101	JX496440	JX496214	JX496327	Decaying leaf	*Mahonia nervosa*
	CBS 683.83	*Paraconiothyrium sporulosum*	JX496107	JX496446	JX496220	JX496333	Seed	*Quercus robur*
Paraph. pilleata	CBS 102207	~	JX496013	JX496352	JX496126	JX496239	~	*Juncus roemerianus*
Paraph. sardoa	CBS 501.71^{T}	*Coniothyrium* sp.	JX496094	JX496433	JX496207	JX496320	Dead leaf	*Smilax aspera*
Paraphaeosphaeria sp.	CBS 101464	*Microsphaeropsis rugosa*	JX496012	JX496351	JX496125	JX496238	Soil	~
	CBS 978.95	*Microsphaeropsis* sp.	JX496120	JX496459	JX496233	JX496346	Soil	~
Paraph. sporulosa	CBS 105.76	*Paraconiothyrium sporulosum*	JX496014	JX496353	JX496127	JX496240	Root	*Picea abies*
	CBS 109.72	*Coniothyrium* sp.	JX496015	JX496354	JX496128	JX496241	Agricultural soil	~
	CBS 146.69	*Paraconiothyrium sporulosum*	JX496040	JX496379	JX496153	JX496266	Agricultural soil	~
	CBS 150.32	*Coniothyrium rosarum*	JX496041	JX496380	JX496154	JX496267	~	*Rosa canina*
	CBS 162.69	*Coniothyrium* sp.	JX496045	JX496384	JX496158	JX496271	Soil	~
	CBS 163.69	*Coniothyrium* sp.	JX496046	JX496385	JX496159	JX496272	Soil	~
	CBS 177.59	*Paraconiothyrium sporulosum*	JX496050	JX496389	JX496163	JX496276	Artificially inoculated soil	~
	CBS 218.68^{T}	*Paraconiothyrium sporulosum*	JX496054	JX496393	JX496167	JX496280	Wheat-field soil	~
	CBS 221.78	*Coniothyrium* sp.	JX496055	JX496394	JX496168	JX496281	Soil	~
	CBS 245.76	*Coniothyrium* sp.	JX496056	JX496395	JX496169	JX496282	~	~
	CBS 271.78	*Coniothyrium* sp.	JX496061	JX496400	JX496174	JX496287	Rhizosphere of grass	~
	CBS 281.81	*Coniothyrium* sp.	JX496062	JX496401	JX496175	JX496288	~	*Clematis* sp.
	CBS 302.77	*Coniothyrium* sp.	JX496066	JX496405	JX496179	JX496292	~	*Calluna vulgaris*
	CBS 304.80	Coniothyrium sp.	JX496068	JX496407	JX496181	JX496294	Root	*Malus sylvestris*
	CBS 305.68	*Microsphaeropsis olivacea*	JX496069	JX496408	JX496181	JX496295	~	*Opuntia* sp.
	CBS 308.81	*Coniothyrium* sp.	JX496073	JX496412	JX496186	JX496299	Soil, potato field	*Solanum tuberosum*
	CBS 317.81	*Paraconiothyrium sporulosum*	JX496074	JX496413	JX496187	JX496300	River water	~
	CBS 329.76	*Coniothyrium* sp.	JX496075	JX496414	JX496188	JX496301	~	*Picea abies*
	CBS 340.85	*Coniothyrium* sp.	JX496078	JX496417	JX496191	JX496304	Cyst, buried in soil	*Globodera rostochiensis*
	CBS 391.86	*Coniothyrium* sp.	JX496082	JX496421	JX496195	JX496308	~	*Triticum aestivum*
	CBS 401.71	*Coniothyrium* sp.	JX496084	JX496423	JX496197	JX496310	~	*Fragaria vesca*
	CBS 688.70B	*Paraconiothyrium sporulosum*	JX496108	JX496447	JX496221	JX496334	Soil	~
	CBS 688.70C	*Paraconiothyrium sporulosum*	JX496109	JX496448	JX496222	JX496335	Soil	~
	CBS 690.70	*Coniothyrium fuckelii*	JX496110	JX496449	JX496223	JX496336	~	*Secale cereale*
	CBS 764.71A	*Paraconiothyrium minitans*	JX496111	JX496450	JX496224	JX496337	Greenhouse soil	~
	CBS 824.68	*Coniothyrium cydoniae*	JX496114	JX496453	JX496227	JX496340	Leaf spot	*Cydonia oblonga*

Paraph. verruculosa	CBS 263.85	*Coniothyrium* sp.	JX496059	JX496398	JX496172	JX496285	Needle	*Picea abies*
	CBS 354.80	*Coniothyrium* sp.	JX496080	JX496419	JX496193	JX496306	Páramo soil, after burning	~
	CBS 682.84	*Coniothyrium* sp.	JX496106	JX496445	JX496219	JX496332	Wood	*Pinus radiata*
Paraph. viridescens	CBS 854.73ᵀ	*Coniothyrium* sp.	JX496085	JX496424	JX681076	JX496311	Fresh water	~
Parastagonospora nodorum	CBS 272.59	~	~	~	JX681114	~	Grain	*Triticum aestivum*
	CBS 273.59	~	~	~	JX681115	~	Leaf	*Triticum aestivum*
	CBS 287.52	~	~	~	JX681113	~	Decaying straw	*Triticum aestivum*
Peyronellaea glomerata	CBS 528.66	~	~	~	JX681105	~	Wood cutting	*Chrysanthemum*
Phaeocytostroma plurivorum	CBS 113835	~	~	~	JX681106	~	~	*Helianthus annuus*
P. sacchari	CBS 275.34	~	~	~	JX681107	~	~	Unknown
Phaeosphaeria avenaria f.sp. *triticae*	CBS 289.52	~	~	~	JX681108	~	Leaf	*Triticum aestivum*
	CBS 385.86	~	~	~	JX681109	~	~	*Triticum aestivum*
P. brevispora	CBS 120248	~	~	~	JX681110	~	Culms	*Sasa* sp.
P. eustoma	CBS 307.71	~	~	~	JX681111	~	~	*Juncus alpinus*
	CBS 724.92	~	~	~	JX681112	~	~	*Ramalina* sp. (lichen)
P. occulta	CBS 582.86	~	~	~	JX681116	~	~	*Carex hirta*
P. parvula	CBS 260.49	~	~	~	JX681117	~	Dead leaf	*Iris pseudacorus*
	CBS 605.86	~	~	~	JX681118	~	~	*Iris pseudacorus*
Phaeosphaeriopsis obtusispora	CBS 246.64	~	~	~	JX681119	~	Dead leaf	*Aloe arborescens*
Plenodomus lingam (syn. *Leptosphaeria maculans*)	CBS 147.24		~	~	JX681097	~	~	Unknown
Plenodomus biglobosus	CBS 475.81		~	~	JX681091	~	Leaf spots	*Brassica oleracea*
(syn. *Leptosphaeria biglobosa*)	CBS 476.81		~	~	JX681092	~	Black leaf spots	*Brassica oleracea*
Pleospora herbarum var. herbarum	CBS 191.86	~	~	~	JX681120	~	Leaf	*Medicago sativa*
Thyridaria rubronotata	CBS 385.39	~	~	~	JX681121	~	~	*Acer* sp.

* GenBank accession numbers of ITS, TUB, LSU and ACT sequences (starting with ‘JX’ for newly generated sequences in this study), substrate, and host organism. ‘T’ following the CBS accession number indicates ex-type strains.

In the course of many decades strains of coniothyrium-like fungi have been deposited in culture collections world-wide to serve as reference material for important research. These cultures represent a valuable resource of genetic diversity that has thus far been under-investigated. The culture collection of CBS (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands) holds several hundreds of these strains. The main purpose of this study was to assess the genetic diversity of isolates preserved in CBS with special attention to strains grouping in the family of *Montagnulaceae*, and to delimit and formally describe novel species by comparing the obtained molecular phylogenetic and morphological data of cultures and their sporulating structures.

MATERIAL AND METHODS

Culture studies and morphological analyses

Cultures preserved in the CBS-KNAW, Utrecht, The Netherlands were used for the present study. Cultures were activated from lyophilised or cryopreserved material and inoculated on oatmeal (OA) and 3 % malt extract (MEA, Oxoid) agars, prepared according to Crous et al. (2009). For culture studies, 5-d-old cultures were transferred to fresh plates and incubated in the laboratory in diffuse daylight (20 °C), and in an incubator under n-UV light (12 h light, 12 h dark) at 18 °C to promote sporulation. Colony diameter measurements were taken from OA plates placed in the incubator with UV, after 10 d. Colours were described according to Rayner (1970). Sporulating structures obtained from cultures were used for the morphological description. Structures were mounted in water and examined with an Olympus BX 50 microscope mounted with bright field and differential interference contrast (DIC) objectives, and photographed using a mounted Nikon Digital Sight DS-5M camera. Photographs of culture plates were taken after 10 and 14 d on a photo stand with daylight tubes with a Pentax K110 D digital camera. Conidial masses from OA plates were mounted in water and 30 spores measured. Length/width (L/W) ratio was calculated for each spore and average L/W ratio calculated (N = 30). Descriptions and nomenclature of taxonomic novelties were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

DNA isolation, PCR and sequencing

Total genomic DNA was extracted from material preserved in liquid nitrogen or from living cultures, using the Genomed Jetquick general DNA clean-up kit or a high-throughput 96-well plate extraction (Ivanova et al. 2006) following the given protocols. The PCR reactions for amplification of the recently ratified universal fungal barcode ITS1-5.8S-ITS2 of the nuclear ribosomal DNA operon (Schoch et al. 2012), using ITS5/ITS1 and ITS4 were performed under standard or semi-nested conditions (White et al. 1990, Stielow et al. 2010). PCR conditions for amplifying the partial LSU rDNA using the standard primers LR0R and LR5 only differed in their annealing temperature (55 °C instead of 60 °C) and increased cycle extension time (90 s per cycle). Amplification of partial γ-actin (ACT), covering the more variable 5’-end containing two small introns, and partial β-tubulin (TUB), covering the variable 5’-end containing four small introns, followed the protocol of Aveskamp et al. (2009) and Carbone & Kohn (1999) using the primers ACT-512f, ACT783r, TUB4Rd and TUB4Fd, respectively. PCR products were directly purified using FastAP thermosensitive alkaline phosphatase and shrimp alkaline phosphatase (Fermentas, Thermo Scientific). The cycle-sequencing reaction was set up using ABI big dye terminator v. 3.1, using a quarter of the suggested volumes (modified manufacturers’ protocol), followed by bidirectional sequencing with a laboratory capillary electrophoresis system (Life Technologies 3730XL DNA analyser). Sequences were stored, manually corrected for sequencing artefacts and forward and reverse sequences assembled using the Biolomics database (www.bio-aware.com) (Vu et al. 2012). Sequences were deposited at NCBI GenBank under the accession numbers provided in Table 1. Alignments were deposited in TreeBASE.

Sequence alignment and phylogenetic analysis

Sequences were aligned with MAFFT v. 6.850b, using the '–genafpair' option but default settings otherwise (Katoh et al. 2005). All introns and exons were aligned separately. Regions containing many leading or trailing gaps were removed from the ITS and LSU alignments prior to tree building. Phylogenetic analysis under the maximum-likelihood (ML) criterion (Felsenstein 1981) was conducted with RAxML v. 7.2.8, using its novel rapid bootstrap option combined with the autoMRE bootstopping criterion (Pattengale et al. 2009) with subsequent search for the best tree under the GTRMIX approach (Stamatakis et al. 2008). The resulting best-known ML tree was rooted using the midpoint-rooting method (Farris 1972, Hess & de Moraes Russo 2007). Bootstrapping under the maximum-parsimony (MP) criterion (Fitch 1971) was done with PAUP v. 4.0b10 (Swofford 2002), treating gaps as missing data, collapsing branches of zero minimum length, and using, per bootstrap replicate, five rounds of random sequence addition followed by TBR branch swapping, saving only one tree per round. In MP bootstrapping, 1 000 replicates were conducted. Search for the best MP tree(s) was done in the same manner but using 1 000 rounds of random sequence addition, saving no more than ten trees per round, and the strict consensus of tree all most-parsimonious trees determined.

The relative performance of the four loci (ITS, LSU, ACT and TUB) in phylogenetic inference for the group was assessed as follows. ML bootstrap analyses of the four alignments were conducted separately (using the same settings as above), the support values from each gene mapped to the best ML tree from combined analysis using RAxML, and each average bootstrap support determined, both absolute and relative to the number of variable characters per alignment. Under MP, partitioned Bremer support (Baker & DeSalle 1997, Baker et al. 1998) was determined using the 'bremer.tcl' script (Göker et al. 2009b) in

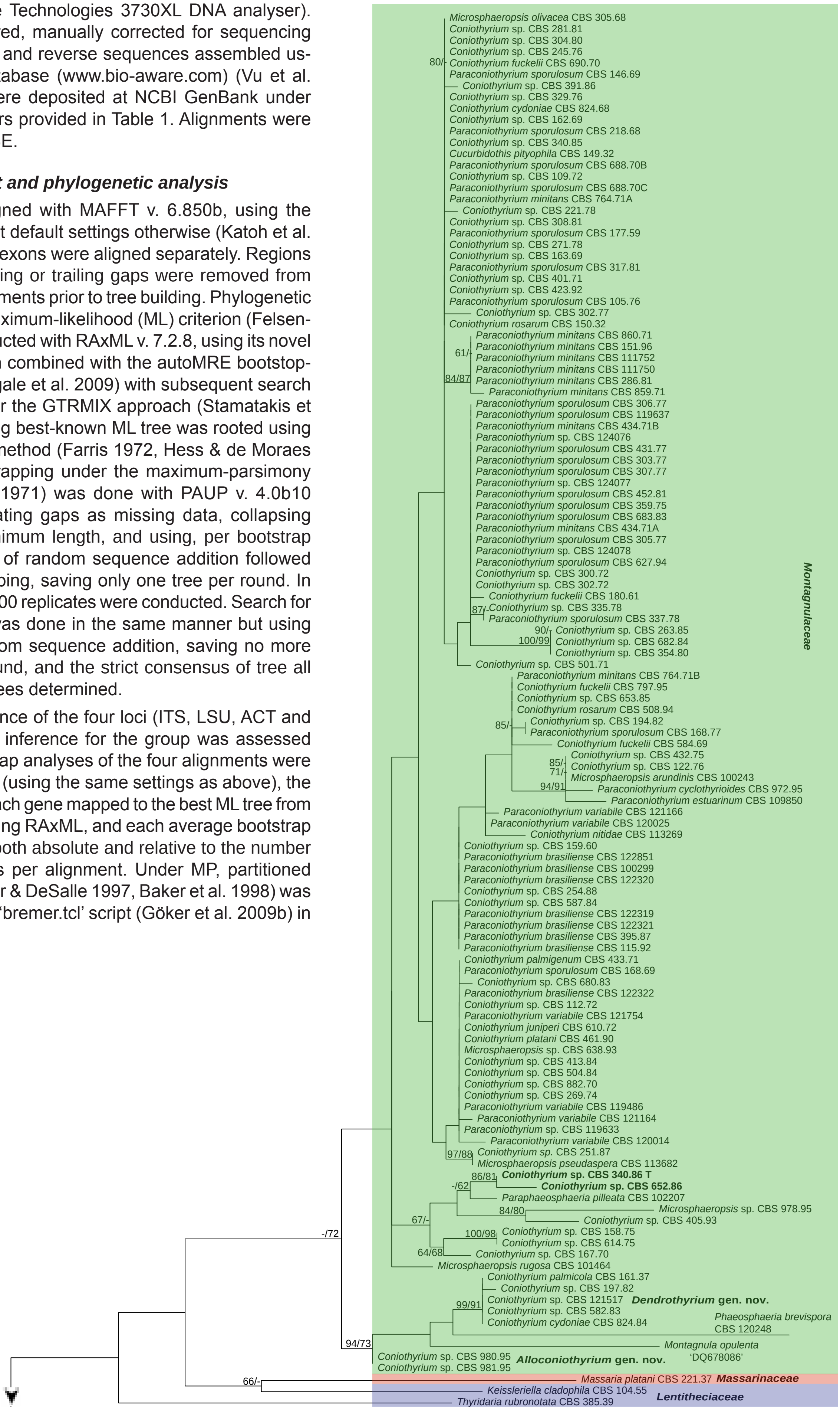
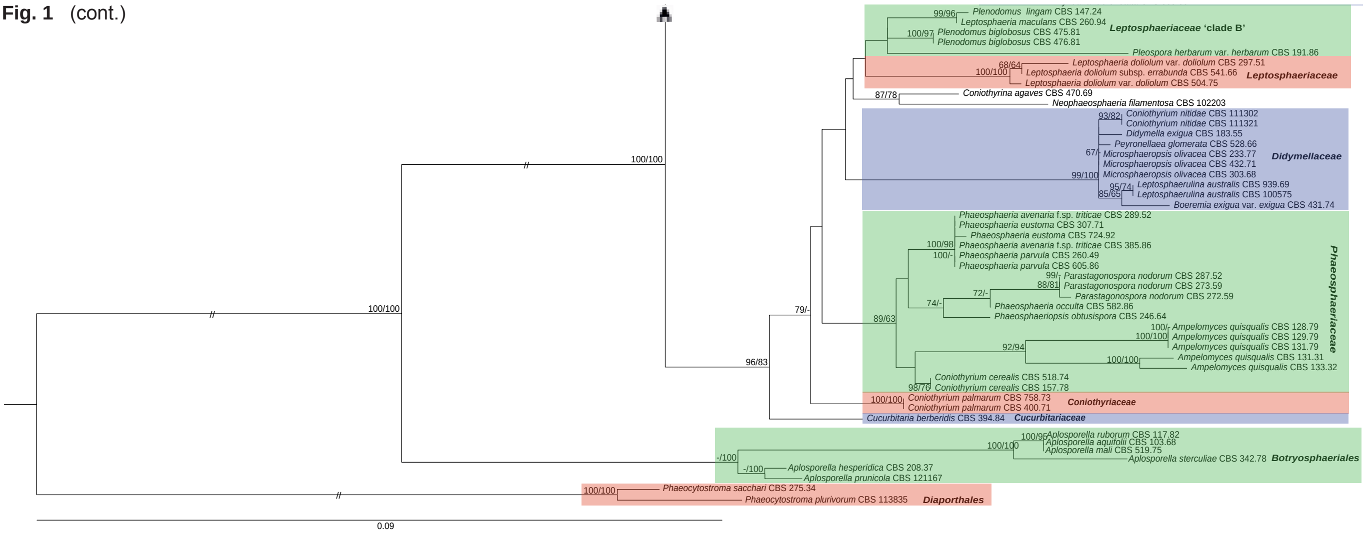


Fig. 1 Midpoint-rooted maximum-likelihood phylogeny of *Coniothyrium*-like *Pleosporales* and their relatives inferred from 890 LSU characters. The numbers abovenext to the branches are ML (left) and MP (right) bootstrap support values. Several large branches (marked by "//") have been scaled to 25 % of original length to better fit the tree on page. Highlighted sections indicate affiliations to families.

Fig. 1 (cont.)



conjunction with PAUP (heuristic-search settings were as above but with 100 rounds for each Bremer search), visualised using a heatmap as implemented in the opm package for R (Vaas et al. 2012) and summed up over all nodes for each gene, both absolute and relative to the number of parsimony-informative characters per alignment (partitioned Bremer support trees are available upon request). The suitability of the four loci for molecular taxonomy of the group was investigated using OPTSIL (Göker et al. 2009a, Stielow et al. 2011) with the revised classification of the group (as detailed below) as reference partition, thus optimizing sequence dissimilarity thresholds for *F* values between 0.0 and 1.0 (with a step width of 0.05), and measuring the resulting best agreement between the clustering and the reference partition. The *F* value determines the shape of the clusters; we here considered the full range between single-linkage (0.0) and complete-linkage (1.0) clustering; see Göker et al. (2009a) for details. The underlying distance matrices were calculated with PAUP, using uncorrected ('p') distances.

RESULTS

Sequence alignment and phylogenetic analyses

The aligned LSU dataset used for determining the relationships between coniothyrium-like members of *Pleosporales* and their relatives comprised 172 organisms and 890 characters, including 290 variable and 248 parsimony-informative characters. The resulting ML tree is presented in Fig. 1 together with ML and MP bootstrap values. Strains representing the dark-spored coelomycete genera *Asplosporella* (*Botryosphaeriales*) and *Phaeocytostroma* (*Diaporthales*) form the outgroup and a small ingroup clade sister to all other ingroup clades, respectively. The pleosporalean taxa that constitute the major part of this tree group in clades that correspond to families that have previously been resolved in other molecular phylogenetic studies of *Pleosporales* (Schoch et al. 2009, Zhang et al. 2009, Aveskamp et al. 2010). One monophyletic group comprising 41 strains representing various families (bootstrap support 96/83 %) includes two strains of *Coniothyrium palmarum*, of the recently reinstated family *Coniothyriaceae* (de Gruyter et al. 2012), and *Cucurbitaria berberidis* (CBS 394.84) of the *Cucurbitariaceae*. Its subclade (89/63 %) representing the family *Phaeosphaeriaceae* comprises four subclades of its own, viz. a clade (98/79 %) of two strains identified as '*Coniothyrium*' *cerealis* (CBS 518.74, 157.78), a second, well-supported (92/94 %) subclade of five strains of *Ampelomyces quisqualis* which reveals at least two distinct genotypes based on LSU. According to de Gruyter et al. (2009) *Ampelomyces* is heterogeneous, with the type species *A. quisqualis* belonging in the *Phaeosphaeriaceae*, and *A. quercinus* in the *Didymellaceae*. Our data indicate that the three strains originating from cucumber mildew in Canada (CBS 128.79, 129.79, 131.79) are specifically distinct from USA strains CBS 131.31 and 133.32, from *Erysiphe cichoracearum* on *Helianthus tuberosus* and *Microsphaera alni* on *Lonicera* sp., respectively. A third, rather weakly supported (74/< 60 %) subclade with *Phaeosphaeriopsis obtusispora* (CBS 246.64), *Phaeosphaeria occulta* (CBS 582.86) and *Parastagonospora nodorum* (CBS 287.52, 272.59, 273.59), and a fourth, strongly supported subclade (100/98 %) with *Phaeosph. avenaria* (CBS 289.52, 385.86), *Phaeosph. parvula* (CBS 260.49, 605.86) and *Phaeosph. eustoma* (CBS 724.92, 307.71). The *Didymellaceae* clade (99/100 %) contains 10 strains, including *Didymella exigua* (CBS 183.51), the type species of the genus *Didymella*, *Microsphaeropsis olivacea* (CBS 233.77, 432.71) and two strains of '*Coniothyrium*' *nitidae* (CBS 111302, 111321). Its unsupported sister group of miscellaneous fungi comprises *Neophaeosphaeria filamentosa* (CBS 102203), *Coniothyrina agaves* (CBS 470.69) (type species of the genus is *C. agavicola*), a well-supported subclade (100/100 %) with *Leptosphaeria doliolum* vars *doliolum* (CBS 297.51, 504.75) and *errabunda* (CBS 541.66), agreeing with *Leptosphaeriaceae* clade B of de Gruyter et al. (2012), and an incompletely resolved clade containing *Plenodomus biglobosus* (syn. *Leptosphaeria biglobosa*) (CBS 475.81, 476.81),

Table 2 Performance of the four loci in separate and combined phylogenetic analyses of *Montagnulaceae* and in molecular taxonomy.

	LSU	ITS	ACT	TUB
# characters	887	615	302	482
# variable	74	194	156	238
# MP-informative	44	159	136	218
SPBrS	62.12	174.90	221.14	266.83
...per character	1.41	1.10	1.63	1.22
ABS, combined	14.34	24.65	31.90	39.80
...per character	0.19	0.13	0.20	0.17
ABS, separate	26.38	34.69	45.12	52.61
...per character	0.36	0.18	0.29	0.22
highest MRI	0.9682	0.9952	0.9795	0.9927
...for *F* value(s)	0.8	0.35–0.5	0.0–1.0	0.0–1.0
...for threshold(s)	0.29 %	1.37 %	3.005–3.64 %	4.585–4.71 %
# clusters	23	25	28	28

Note: SPbrS, sum of partitioned Bremer-support values over all nodes; ABS, average bootstrap support (under ML, either in combined or separate analysis); MRI, modified Rand index (indicating the agreement, at most 1.0, between sequence clustering and proposed classification). Normalization 'per character' was conducted per number of parsimony-informative characters for SPBrS and per number of variable characters for all other measures.

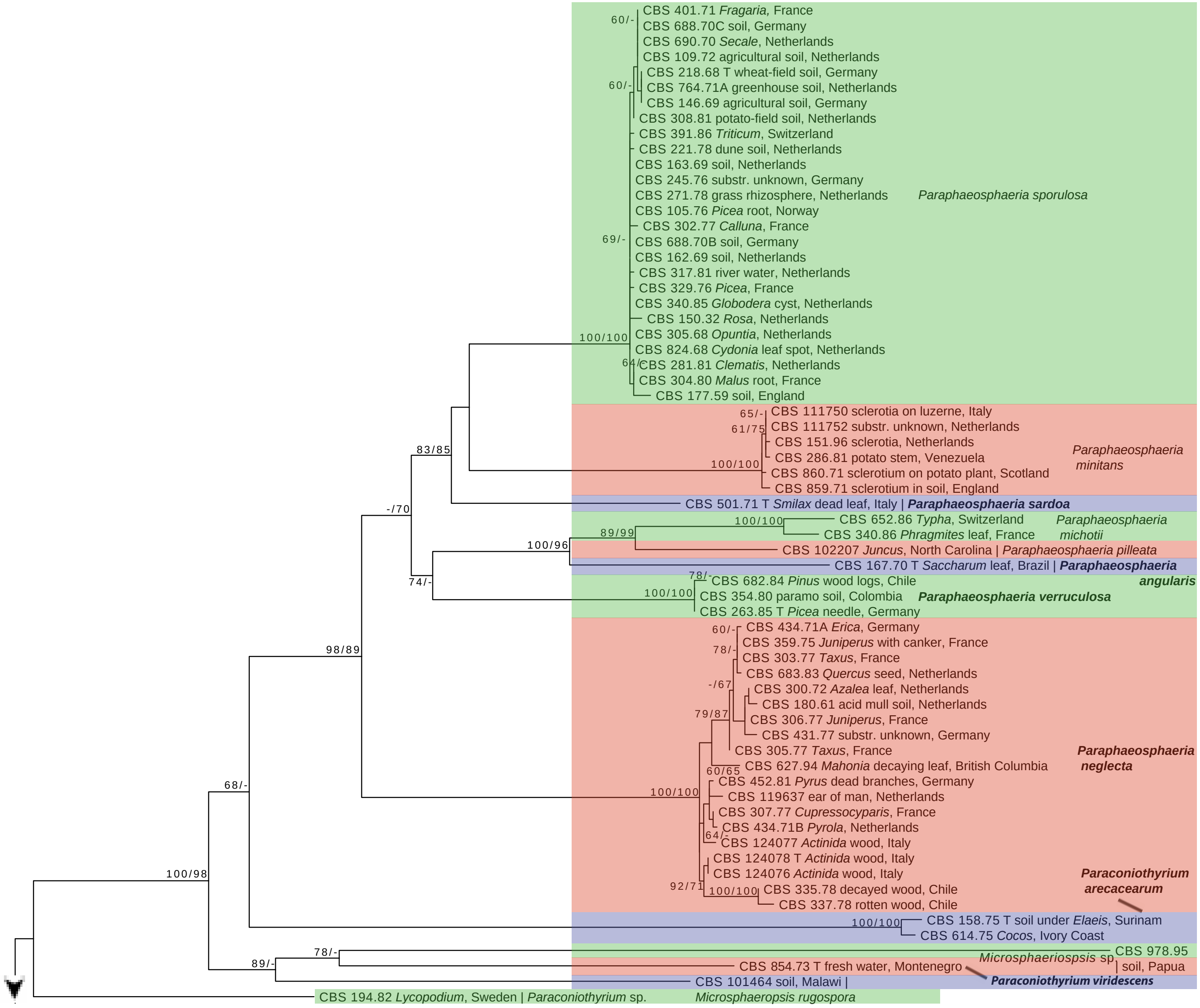


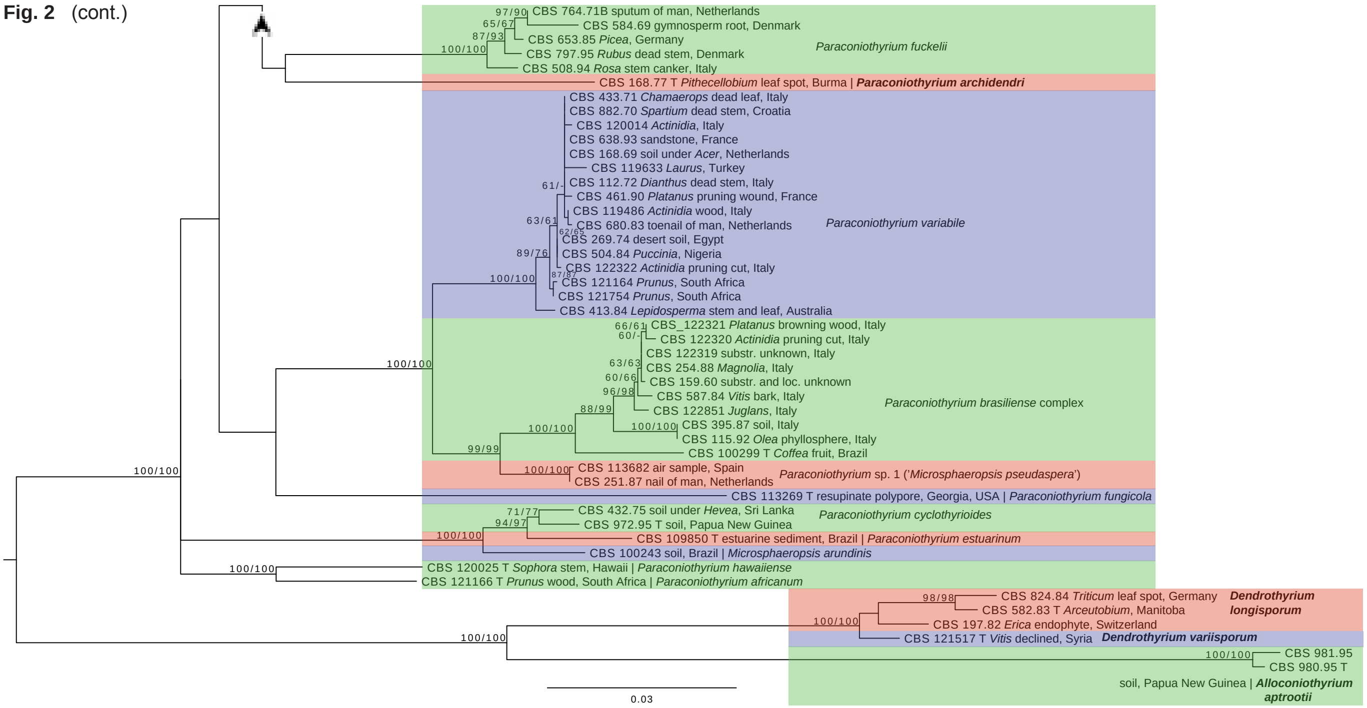
Fig. 2 Midpoint-rooted maximum-likelihood phylogeny of *Montagnulaceae* inferred from four concatenated gene alignments (ITS, LSU, ACT and TUB) yielding a total of 2 286 characters. The numbers next to the branches are ML (left) and MP (right) bootstrap support values. The affiliations to species are highlighted. Species named in **bold** indicate taxa proposed in this study.

Plenodomus lingam (syn. *Leptosphaeria maculans*, *Phoma lingam*) (CBS 147.24, 260.94) and *Pleospora herbarum* (CBS 191.86).

Keisleria cladophila (CBS 104.55) and *Massaria platani* (CBS 221.37) classified in the *Lentitheciaceae*, and *Thyridaria rubronotata* (CBS 385.39) of uncertain familial affinity ('Clade J' in Schoch et al. 2009) form the sister group of the *Montagnulaceae* clade (< 60/72 %), which consist of 120 strains, and the sister group of the latter two clades, respectively. LSU apparently does not provide sufficient variation within *Montagnulaceae*, most inner branches show poor or no support, and thus the genera cannot be sufficiently resolved here. But the following well-supported subclades can be noted: the new monotypic genus *Alloconiothyrium* (CBS 980.95^{T}, 981.95) and the new genus *Dendrothyrium* with *D. longisporum* (CBS 582.83^{T}, 824.84) and *D. variisporum* (CBS 121517^{T}, 197.82). CBS 161.37 preserved as '*Coniothyrium' palmicola* also groups here, and ITS shows 99 % similarity to the type strain of *Dendrothyrium longisporum*. No additional sequences could be obtained for CBS 161.37 and the identity of this strain therefore remains uncertain. CBS 120248 also groups here, confirming the position of *Phaeosphaeria brevispora* in *Montagnulaceae* (Schoch et al. 2009). Furthermore, *Parac. estuarinum* (CBS 109850^{T}), *Parac. cyclothyrioides* (CBS 972.95^{T}), CBS 122.76 and 432.75 '*Coniothyrium* sp.', as well as CBS 100243 '*Microsphaeropsis*' *arundinis* group together (94/91 %).

The performance of the four concatenated gene alignments (ITS, LSU, ACT and TUB) in combined and separate phylogenetic inference is shown in Table 2. The measures agreed that TUB provided overall the most support in combined and separate analysis, followed by ACT, ITS and LSU. Relative to the number of variable and parsimony-informative characters, however, ACT performed best, followed by LSU, TUB and ITS (in this respect, LSU performed even better than ACT when analysed separately). In the multi-locus phylogeny inferred from the combined dataset shown in Fig. 2, several well-supported clades can be identified, which are interpreted as appropriate for the delimitation of genera. The outgroup of the tree is formed by two highly supported clades representing the genera *Alloconiothyrium* (100/100 %) and *Dendrothyrium* (100/100 %). The *Dendrothyrium* clade comprises two species, with two isolates of *D. longisporum* (CBS 824.84, 582.83^{T}) and the type strain of *D. variisporum* (CBS 121517^{T}). A second strain, CBS 197.82, is also assigned to this species based on morphological similarities to the type strain, even though this renders the species paraphyletic in the presently postulated phylogeny, but without

Fig. 2 (cont.)



support. Another well-supported (100/98 %) clade forming the major part of the ingroup of the tree comprises 64 strains assigned to the genus *Paraphaeosphaeria*, with two isolates of *Paraph. michotii*, the type species of the genus, and the highly supported clades of the following species: *Paraph. sporulosa* (26 strains), *Paraph. minitans* (6), the new species *Paraph. sardoa* (1), *Paraph. angularis* (1), which clusters with *Paraph. michotii* and *Paraph. pilleata*, and furthermore *Paraph. verruculosa* (3), *Paraph. neglecta* (19), *Paraph. arecacearum* (2) and *Paraph. viridescens* (1). The intraspecific sequence variability regarding TUB is somewhat higher in *Paraph. neglecta* than in the other species of the genus with multiple strains in the tree, as indicated by partitioned Bremer support values for the interior branches of the *Paraph. neglecta* clade of 1–5 steps for TUB but ≤ 2 for the other genes (data not shown). CBS 101464 from Malawi deposited in CBS as *Microsphaeropsis rugospora* is found within the *Paraphaeosphaeria* clade (close to its base), and is preliminarily re-identified as *Paraphaeosphaeria* sp. The type of *M. rugospora* originated from cultivated soil in southern Japan (Someya et al. 1997).

Paraconiothyrium estuarinum (CBS 109850^{T}), the type species of *Paraconiothyrium*, groups together with *Parac. cyclothyrioides* (CBS 972.95^{T}) and CBS 432.75, regarded conspecific with it, in a well-supported (100/100 %) clade also comprising CBS 100243, identified as *Microsphaeropsis arundinis*. A second *Paraconiothyrium* subclade comprises *Parac. variabile* (16 strains), the *Parac. brasiliense* complex (10), and a group containing CBS 113682 and 251.87, '*Paraconiothyrium* sp. 1'. A third *Paraconiothyrium* subclade comprises CBS 120025 and 121166, the type strains of *Parac. hawaiiense* and *Parac. africanum*, respectively. Two additional clades correspond to *Parac. fuckelii* (5 strains) and the novel species *Parac. archidendri* (CBS 168.77^{T}), respectively.

The results of optimising sequence-clustering parameters for the concatenated alignment and each gene individually with OPTSIL are included in Table 2. Expectedly, LSU performed worst, failing to differentiate between a number of species (see also Fig. 1), but also dividing *Paraconiothyrium fuckelii* and *Paraphaeosphaeria michotii* into two clusters, respectively (details not shown, but compare Fig. 1). ACT and TUB divided *Paraconiothyrium brasiliense* into three or two clusters, respectively; in addition, ACT merged *Parac. cyclothyrioides* and *Parac. estuarinum*. ITS merged these two species and also *Dendrothyrium longisporum* and *D. variisporum*; as ITS divided no species, it thus yielded the highest overall agreement, minimally larger than the one obtained with TUB, as the conflicting species were only represented by few specimens. The best clustering obtained with the entire dataset was identical to the optimal one for ITS. The data also indicate, however, that once the *Parac. brasiliense* complex could convincingly be split into two species, TUB sequence clustering would yield 100 % agreement with the classification for a single choice of sequence dissimilarity threshold applied to all included taxa, independent of the clustering parameter *F* (Table 2). That *F* = 0.0 is included in the optimal values also indicates the presence of a TUB barcoding gap for the species under study.

Taxonomy

Alloconiothyrium Verkley, Göker & Stielow, *gen. nov.* — MycoBank MB800756

Type species. *Alloconiothyrium aptrootii* Verkley, Göker & Stielow.

Etymology. Named after its morphological resemblance to *Coniothyrium* in contrast to the phylogenetic distance between both genera.

Conidiomata pycnidial or eustromatic. *Conidiogenous cells* holoblastic, annellidic. *Conidia* olivaceous-brown and irregular in outline, surface roughened. Sexual morph unknown.

Alloconiothyrium aptrootii Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800757; Fig. 3

Etymology. Named after André Aptroot, who collected the soil sample from which the species was isolated.

Conidiomata pycnidial, 300–450 µm diam and with a single cavity, or eustromatic and consisting of complexes reaching 1 mm diam, with several cavities, the outer surface black, glabrous or covered by grey mycelium. *Conidiomatal wall* composed of an outer layer of brown, thick-walled *textura angularis* and an inner layer of hyaline, thick-walled *textura angularis-globulosa*, the outer surface sometimes covered by a diffuse web of brown hyphae. *Conidiogenous cells* discrete, often positioned on clumps of cells that protrude into the cavity, broadly ampulliform, holoblastic, annellidic, often with an elongated

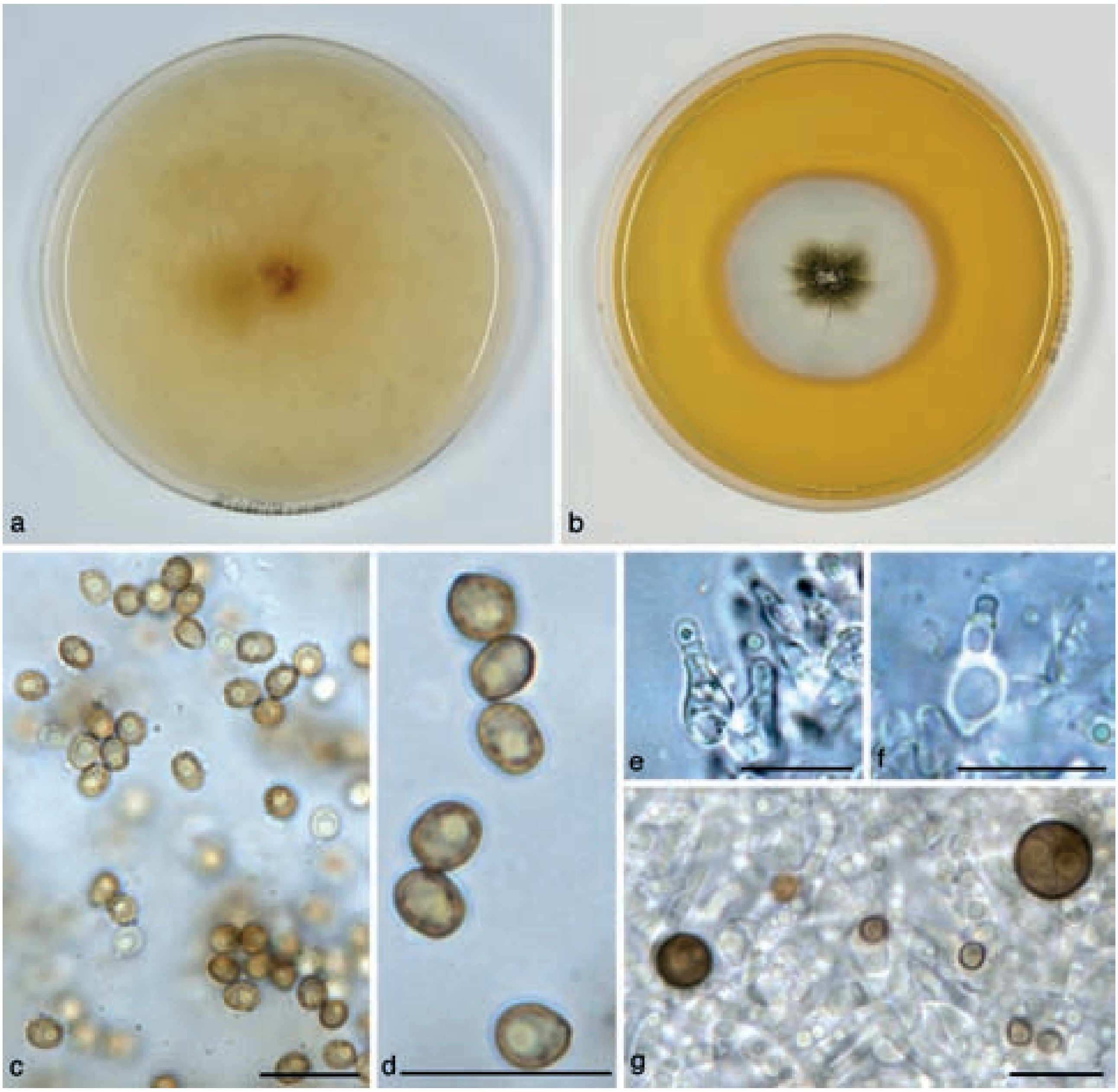


Fig. 3 *Alloconiothyrium aptrootii* (CBS 980.95^T, ex-type culture). a. Colony on OA; b. colony on MEA; c, d. conidia on OA; e, f. conidiogenous cells showing distinct annellations; g. chlamydospores. — Scale bars = 10 µm.

neck showing several distinct percurrent proliferations, 4–9 × 3–4 µm. *Conidia* globose to irregularly ellipsoid, initially hyaline, after secession olivaceous-brown, mature conidial wall orange-brown, the outer surface verruculose giving the conidium an irregular outline, with 1 large oil-droplet 1–1.5 µm diam, 0-septate, 3–4(–5) × 2.5–3(–3.5) µm, average L/W ratio 1.2 ± 0.2. *Chlamydospores* formed in the mycelium, terminal or intercalary, usually solitary, globose, mostly 6–8.5 µm diam, with a smooth brown wall and 1–2 large oil-droplets. Sexual morph unknown.

Colonies on OA reaching 37–40 mm diam in 10 d, with an even, glabrous, colourless margin. Immersed mycelium mostly colourless, but with some faint buff to honey in the centre after 10 d. Aerial mycelium very diffuse, white or absent. Reverse concolourous. Conidiomata developing after 10–15 d. *Colonies* on MEA reaching 36–40 mm diam in 10 d, with an even, buff margin. Immersed mycelium greenish olivaceous to olivaceous, fading to buff at margin, mostly covered by a moderately dense layer of woolly to floccose grey to white aerial mycelium. Reverse in the centre isabelline, fading over cinnamon to buff at the margin.

Specimens examined. Papua New Guinea, Central Province, Varirata Nat. Park near Port Moresby, isolated by A. van Iperen from a soil sample, Oct. 1995, *A. Aptroot*, holotype CBS H-21035, living ex-type culture CBS 980.95; isolated from the same soil sample CBS 981.95.

Notes — The fungus is only known from a soil sample collected in Papua New Guinea, and all other coniothyrium-like fungi studied here are relatively distantly related. The annellidic conidiogenous cells and the verruculose conidia remind of *Coniothyrium palmarum*, the type species of the genus, but that species is characterised by 2-celled conidia and is also genetically distinct, and belongs in the *Leptosphaeriaceae* (de Gruyter et al. 2009).

Dendrothyrium Verkley, Göker & Stielow, *gen. nov.* — MycoBank MB800758

Type species. Dendrothyrium variisporum Verkley, Göker & Stielow.

Etymology. Named after the branched, tree (= dendron)-like conidiophores occurring in the conidiomata of the type species.

Conidiomata pycnidial or eustromatic. *Conidiogenous cells* discrete or integrated in conidiophores that are branched at the base, phialidic, terminal cells of the conidiophore occasionally also percurrently proliferating. *Conidia* 1-celled, olivaceous-brown, thin- and smooth-walled. Sexual morph unknown.

Dendrothyrium longisporum Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800759; Fig. 4

Etymology. Named after the comparatively long conidia of this species.

Conidiomata pycnidial, globose, 140–170 µm diam, with a single, central ostiolum 10–20 µm. *Conidiomatal wall* composed of *textura angularis* with pale yellowish brown cells and darker cells around the ostiolum, sometimes overlaid with a diffuse web of thin-walled brown hyphae. *Conidiogenous cells* discrete or integrated in simple, 1–2-septate, 10–17 µm long conidiophores, phialidic, doliiform to ampulliform, with a distinct periclinal thickening, 3.5–6(–8) × 2–3 µm. *Conidia* consistently cylindrical-ellipsoid, initially hyaline, soon after secession with a olivaceous-brown, thin, smooth wall, with minute granules and no oil-droplets, 0-septate, (3.5–)4–5(–6) × 1.5–2 µm, average L/W ratio 2.8 ± 0.4. Sexual morph unknown.

Colonies on OA reaching 28–32 mm diam in 10 d, with a smooth, glabrous margin. Immersed mycelium colourless, faintly yellowish to ochreous in the centre where scattered pycnidia emerge after 5–7 d. Aerial mycelium only in the centre, fluffy, white. Reverse concolourous. *Colonies* on MEA reaching 23–25 mm diam in 10 d, with an even to slightly undulating buff margin; immersed mycelium buff to ochreous in the centre, where also numerous densely aggregated pycnidia are formed after 5–7 d, colony surface mostly hidden under a mat of pure white, woolly-tufty aerial mycelium. Reverse in the centre chestnut, fading over fulvous to ochreous or buff near the margin.

Specimens examined. Canada, Manitoba, Grand Beach, isolated from *Arceuthobium pusillum*, 25 July 1981, *J. Reid*, holotype CBS H-10965, living ex-type culture CBS 582.83. – Germany, Monheim, from leaf spot in *Triticum aestivum*, June 1984, *M. Hossfeld 111*, living culture CBS 824.84 (preserved as *Coniothyrium cerealis*).

Notes – See following species.

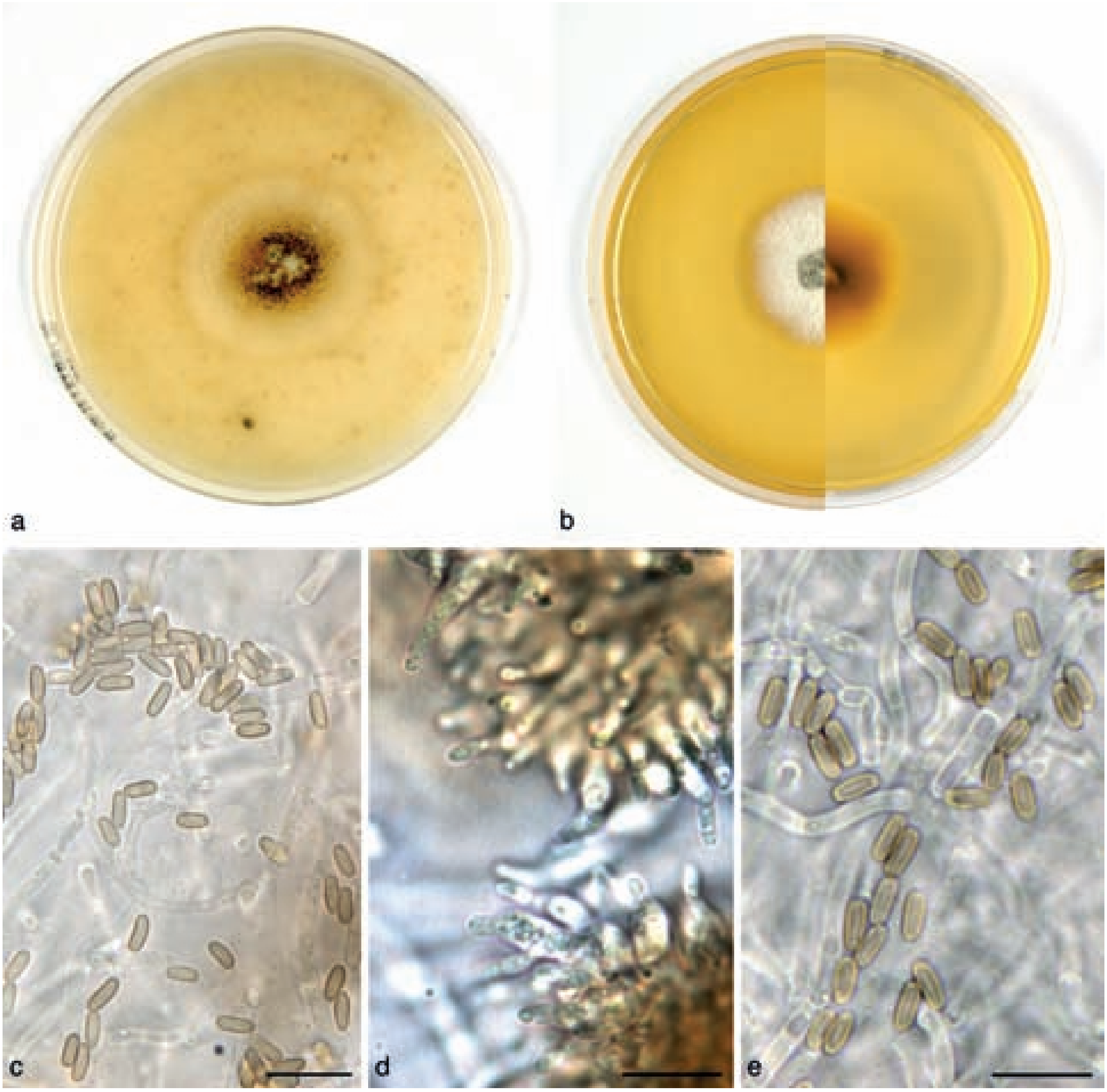


Fig. 4 *Dendrothyrium longisporum* (CBS 582.83^T, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c. conidia on OA; d. conidiogenous cells on OA; e. conidia on OA. — Scale bars = 10 µm.

Dendrothyrium variisporum Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800760; Fig. 5

Etymology. Named after the variation in the shape of the conidia.

Conidiomata eustromatic, often merged to complexes reaching 400–500 µm diam with several discrete or fused cavities, dark brown to black; sporocarps on the agar surface appearing grey due to numerous colourless hyphal outgrowths. *Conidiomatal wall* relatively thick, composed of a single layer of *textura angularis* with hyaline to pale yellow, relatively thick-walled cells 4–7 µm diam. Outer surface sometimes overgrown by a diffuse web of brown, glabrous hyphae oriented parallel to the wall surface. *Conidiogenous cells* integrated in 1–4-septate acropleurogenous conidiophores that are simple or branched at the base, 10–18(–25) × 2.5–4 µm, phialidic, terminal cells cylindrical and slightly attenuating to the apex where sometimes one or more percurrent proliferations can be seen. *Conidia* variable in shape, subglobose, ellipsoid or obovoid, sometimes curved or with a broad, blunt end, initially hyaline, soon after secession with an olivaceous-brown, thin, smooth wall, contents with 1–3 minute oil-droplets, 0-septate, 3–4(–4.5) × 1.5–2.5(–3) µm, average L/W ratio 1.6 ± 0.3. Sexual morph unknown.

Colonies on OA reaching 35–38 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium colourless, aerial mycelium absent. Reverse concolourous. Pycnidia formed after 7–10 d in concentrical zones. *Colonies* on MEA reaching 26–28 mm diam in 10 d, with an even to slightly ruffled, colourless margin. Immersed mycelium buff to ochreous but mostly hidden under a dense mat of woolly-floccose, pure white to buff, later in the centre greyish aerial mycelium. Reverse in the centre umber, fading over sienna to luteous to buff near the margin. Pycnidia formed after 10–14 d.

Specimens examined. Switzerland, Zürich, isolated as endophyte of *Erica carnea*, July 1981, *O. Petrini*, living culture CBS 197.82, CBS H-10964 (dried culture). – Syria, isolate from declined grape vine, *K.A. Halim 35*, holotype CBS H-21036, living ex-type culture CBS 121517.

Notes — The branched, acropleurogenous conidiophores (Fig. 5d) that can be provided with annellidic terminal apertures are the most distinctive feature of this species. *Dendrothyrium longisporum* is a close relative, but morphologically quite distinct from *D. variisporum* by the pycnidial sporocarps with a well-developed ostiolum, more consistently cylindrical-ellipsoid conidia (average L/W ratio 2.8 vs 1.6 in *D. variisporum*) and

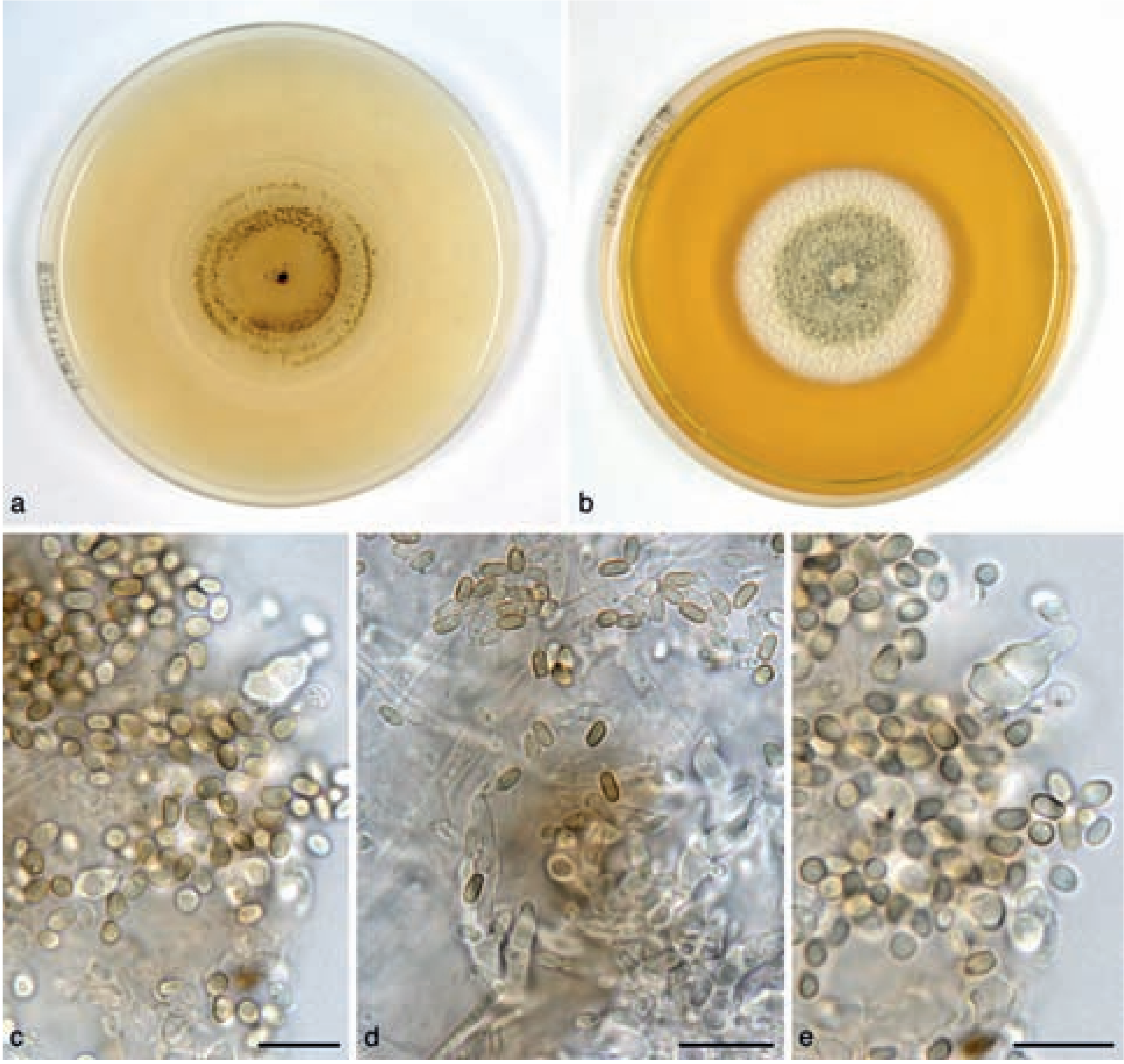


Fig. 5 *Dendrothyrium variisporum* (CBS 121517^T, ex-type culture). a. Colony on OA; b. colony on MEA; c–e. conidia and conidiogenous cells on OA. — Scale bars = 10 µm.

the absence of branched conidiophores or annellidic conidiogenesis. Despite these differences, the multi-locus phylogenetic analysis supports the placement of the two fungi in a single genus. In contrast to *D. longisporum*, the two strains assigned to *D. variisporum* do not group in a monophyletic cluster in the multi-locus phylogeny. CBS 197.82 is nonetheless considered to be conspecific with the ex-type strain, mainly based on good agreement in phenotypic characters and because there is no support for the non-monophyly of *D. variisporum* (Fig. 2). Based on the material available it can be postulated that the genus *Dendrothyrium* is a widely dispersed genus of endophytes and (weak) plant pathogens with a wide host spectrum.

Paraconiothyrium Verkley, Stud. Mycol. 50: 327. 2004

Type species. *Paraconiothyrium estuarinum* Verkley & Manuela Silva, Stud. Mycol. 50: 327. 2004.

A description of the type species was provided by Verkley et al. (2004). Main features of this species are summarised in Table 3.

Conidiomata eustromatic, simple or complex, or pycnidial. *Conidiogenous cells* discrete or integrated, phialidic or holoblastic, annellidic. *Conidia* aseptate, sometimes 1-septate, thin- to relatively thick-walled, smooth-walled or verruculose, hyaline when liberated, later brown.

Paraconiothyrium archidendri Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800761; Fig. 6

Etymology. Named after the host genus, *Archidendron*, from which the species was isolated.

Conidiomata pycnidial, globose, with a single ostiolum 10–30 µm diam, initially glabrous and pale brown, or pilose and appearing grey, later black due to conidia produced inside, 250–350(–400) µm diam, the surface of the wall provided with hyaline to pale brown hyphal outgrowths. *Conidiomatal wall* composed of single layer of relatively thick-walled, pale yellowish *textura angularis* with cells mostly 5–10 µm diam. *Conidiogenous cells* discrete, globose to doliiform, holoblastic, occasionally annellidic with 1–3 percurrent proliferations, 3.5–5(–6.5) × 2.5–4 µm. *Conidia* variable in shape, subglobose or ellipsoid, more rarely obovoid, ends rounded, sometimes one end more or less blunt, initially hyaline, soon after secession olivaceous-brown, contents with several small oil-droplets (< 0.5

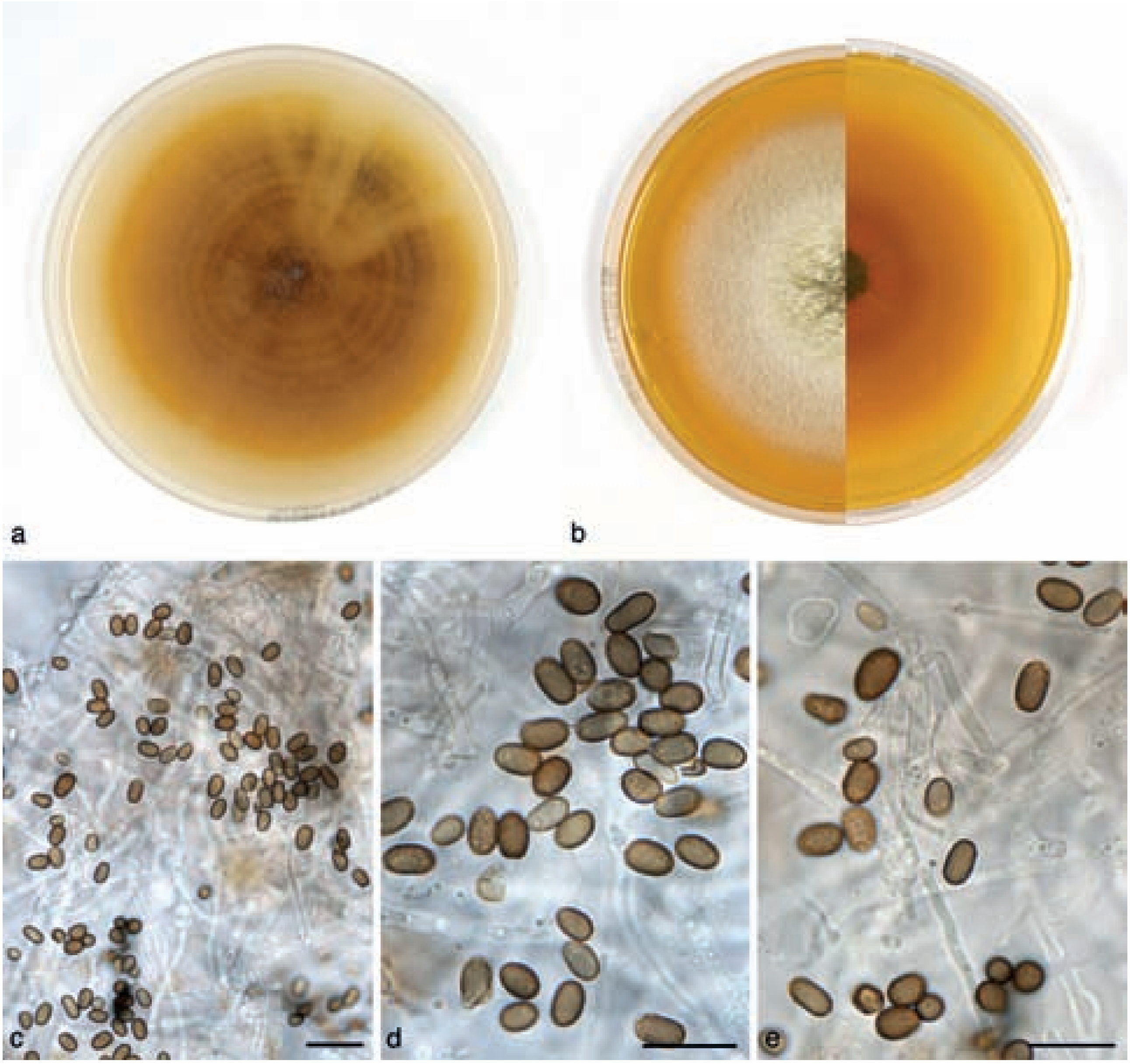


Fig. 6 *Paraconiothyrium archidendri* (CBS 168.77^T, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c–e. conidia on OA. — Scale bars = 10 µm.

Table 3 Overview of morphological characters of investigated asexual species in *Montagnulaceae*.

Species	Conidiogenous cells	Conidial septa and sizes (in µm)	Average L/W ratio conidia	Conidium wall surface	Growth rate on OA (colony diam in mm after 10 d)	Reference
Alloconiothyrium aptrootii	Annellidic, discrete	0-septate, 3–4(–5) × 2.5–3(–3.5) Chlamydospores globose, 6–8.5 diam	1.2 ± 0.2	Verruculose	37–40	This study
Dendrothyrium longisporum	Phialidic, discrete or in simple, 1–2-septate conidiophores	0-septate, (3.5–)4–5(–6) × 1.5–2	2.8 ± 0.4	Smooth	28–32	This study
D. variisporum	Phialidic, integrated in 1–4-septate acropleurogenous conidiophores	0-septate, 3–4(–4.5) × 1.5–2.5(–3)	1.6 ± 0.3	Smooth	35–38	This study
Paraconiothyrium africanum	Phialidic, also proliferating percurrently, discrete	(0–)1(–3)-septate, (4–)6.5–9.5(–12) × (2.5–)3–4(–5)	2.3	Verruculose	44 (7 d)	Damm et al. (2008)
Parac. archidendri	Holoblastic, occasionally annellidic, discrete	0-septate, 3.5–6 × 2.5–3.5(–4)	1.5 ± 0.2	Smooth, or very minutely verruculose	50–55	This study
Parac. babiogorense	Phialidic, discrete	0(–1)-septate, (7–)8–9(–10) × 1–2(–3)	–	Smooth	5 (on PDA after 7 d, darkness, 17 °C)	Budziszewska et al. (2011)
Parac. brasiliense	Phialidic, discrete	0-septate, (3–)3.4–4.6(–5) × (1.8–)2–2.3(–2.5)	1.9 ± 0.2	Smooth	60–68	Verkley et al. (2004)
Parac. cyclothyrioides	Phialidic, occassionally with 1–2 percurrent proliferations, integrated in compact conidiophores, rarely discrete	0-septate, (2.5–)3–4.2(–5) × (1–)1.2–1.5(–1.8)	2.9 ± 0.3	Smooth	60–68	Verkley et al. (2004)
Parac. estuarinum	Phialidic, occassionally with a percurrent proliferation, discrete, sometimes integrated in compact conidiophores	0-septate, (3–)3.2–4(–6) × 1.4–1.7(–2)	2.4 ± 0.4	Smooth	60–68	Verkley et al. (2004)
Parac. flavescens	phialidic, discrete	0-septate, 4–7 × 2–2.5	–	smooth	15 (7 d), 25 (14 d)	Boerema et al. (2004)
Parac. fuckelii (syn. *Coniothyrium fuckelii*)	Annellidic, discrete or integrated in short, simple 1–2-septate conidiophores	0-septate, 3–4 × 2–3(–3.5)	1.4 ± 0.2	Smooth	70–75	This study
Parac. fungicola	Phialidic, occassionally with 1–3 percurrent proliferations, discrete	0–1-septate, (4.2–)4.4–6.2(–7) × (2.7–)3–3.4(–3.6)	1.7 ± 0.2	Smooth	30–35	Verkley et al. (2004)
Parac. hawaiiense	Phialidic, also proliferating percurrently several times near apex, ocassionally polyphialidic, discrete	1(–2)-septate, (10–)12–13 × (4–)5(–5.5)	–	Verruculose	45 (on PDA after 2 wk, 25 °C)	Crous & Groenewald (2006)
Parac. lini	phialidic, discrete	0-septate, 3.5–5.5 × 1.5–2	–	smooth	65 (7 d)	Boerema et al. (2004)
Parac. maculicutis	Phialidic, discrete	0-septate, 1.5–2.5 × 0.5–1.5	1.5–3.2	Smooth	50–52 (7 d)	Gruyter et al. (2012)
Parac. variabile	Phialidic, occassionally with 1–2 percurrent proliferations, integrated in 1–3-celled conidiophores	0-septate, (2.5–)3–4(–5) × 1–2(–2.5)	2.2	Smooth to fine verruculose	43 (7 d)	Damm et al. (2008)
Paraconiothyrium sp.1 (*'Microsphaeropsis pseudaspera'*)	Phialidic, discrete	0-septate, 3–4.5(–5) × 2–3	1.3 ± 0.2	Smooth	41–46	This study

Paraphaeosphaeria angularis	Phialidic, discrete	0-septate, 4.5–7(–8) × 3–4, occasionally 1-septate, 8 × 5	1.9 ± 0.2 (0-septate conidia)	Smooth	53–56	This study
Paraph. arecacearum	Phialidic, discrete	0-septate, (3–)3.5–6(–8.5) × 2–3	2.0 ± 0.4	Smooth	70–75	This study
Paraph. michotii[1]	Phialidic, discrete (?)	0-septate, 4–8 × 2.4–4.4	–	Smooth, with a wrinkled sheath on mature conidia	28 (on CMA after 7 d)	Câmara et al. (2001)
Paraph. minitans (syn. *Paraconiothyrium minitans*)	Phialidic, discrete	0-septate, 4.5–7 × 3.5–4.5(–5)	1.4 ± 0.4	Verrucose	38–45	This study
Paraph. neglecta	Phialidic, discrete	0-septate, (3–)3.5–6(–8.5) × 2–3	1.7 ± 0.4	Smooth to minutely verruculose	45–50	This study
Paraph. pilleata[1]	Phialidic, discrete (?)	0-septate, 3.5–7 × 2–4	–	Smooth	24 (on CMA after 7 d)	Câmara et al. (2001)
Paraph. sardoa	Phialidic, discrete or integrated in short, simple 1–2-septate conidiophores	0-septate, (4.5–)5–6(–7) × (3–)3.5–4.5(–5)	1.4 ± 0.2	Verruculose	40–44	This study
Paraph. sporulosa	Phialidic, occassionally proliferating percurrently, discrete	0-septate, 3.5–5(–6) × 3–4	1.5 ± 0.2	Smooth	42–50	This study
Paraph. verruculosa	Phialidic, discrete	0-septate, (3–)4–5(–6) × (2.5–)3–3.5(–5)	1.3 ± 0.2	Verruculose	50–54	This study
Paraph. viridescens	Phialidic, occassionally proliferating percurrently, discrete	0-septate, (3–)4–4.5(–5) × 1.8–2.2	2.0 ± 0.2	Smooth	52–55	This study

---

[1] For a description of the sexual morph see Câmara et al. (2001).

µm diam) near each end, conidial wall at maturity relatively thick, smooth, sometimes minutely verruculose, 0-septate, 3.5–6 × 2.5–3.5(–4) µm, average L/W ratio 1.5 ± 0.2. Sexual morph unknown.

Colonies on OA reaching 50–55 mm diam in 10 d, with an even, glabrous and colourless margin; immersed mycelium ochreous to cinnamon, aerial mycelium absent. Reverse concolourous. Conidiomata developing after 20–25 d. *Colonies* on MEA reaching 50–53 mm diam in 10 d, with an even, colourless to buff margin; immersed mycelium not visible from above, entirely hidden under a dense moderately high mat of woolly-floccose, white to greyish, in the centre weakly citrine to hazel aerial mycelium; conidiomata not observed. Reverse predominantly ochreous to fulvous, in the centre olivaceous-black with rust patches or circular zones. Conidiomata developing after 20–25 d.

Specimen examined. Burma, E. of Yezin, Kyaukthanbut Village, on leaf spot in *Pithecellobium bigeminum* (= *Archidendron bigeminum*), Oct. 1976, *M.M. Thaung*, isolated by H.A. van der Aa 5654B, holotype CBS H-21037, living ex-type culture CBS 168.77.

Notes — The only strain available of this species sporulated tardily on OA and MEA with small numbers of sporocarps. It was isolated from leaf spots on the leguminose tree *Archidendron bigeminum* in Burma, and more material needs to be collected in order to assess its ecology and geographic distribution. In the multi-locus phylogeny, *Parac. archidendri* clusters with *Parac. fuckelii*, but this grouping is not supported by bootstrapping. The two taxa do share annellidic conidiogenesis (not seen in the other *Paraconiothyrium* species) and a relatively low conidium L/W ratio (1.3–1.5) compared to other members of *Paraconiothyrium* (≥ 1.7).

Paraconiothyrium fuckelii (Fuckel) Verkley & Gruyter, Stud. Mycol. 75: 25. 2012. — Fig. 7

Basionym. *Coniothyrium fuckelii* Sacc., Nuovo Giorn. Bot. Ital. 7: 318. 1875 (asexual morph).

= *Sphaeria coniothyrium* Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 115. 1870 (sexual morph).

≡ *Leptosphaeria coniothyrium* (Fuckel) Sacc., Nuovo Giorn. Bot. Ital. 7: 317. 1875.

More synonyms are provided in Domsch et al. (2007), who described the sexual morph. Below only a description of the asexual morph *in vitro* is given.

Conidiomata pycnidial 300–400 µm diam and with a single cavity, more often eustromatic and consisting of complexes up to 1.2 mm diam, with several cavities, the outer surface black, glabrous, but often covered by a diffuse web of white to greyish hyphae. *Conidiomatal wall* composed of an outer layer of *textura angularis* with somewhat thickened, brown walls, and an inner layer of *textura angularis-globulosa* with somewhat thickened, hyaline walls. *Conidiogenous cells* discrete or integrated in short, simple, 1–2-septate conidiophores, broadly ampulliform to globose, holoblastic, often annellidic with 1 or 2 percurrent proliferations noticeable by the distinct scars on a somewhat elongated neck, hyaline, 4–10(–13) × 3–5 µm. *Conidia* variable in shape, subglobose to ellipsoid or obovoid, rarely more cylindrical, initially hyaline with mostly 1–3(–5) small oil-droplets (< 1 µm diam), soon after secession olivaceous-brown, conidial wall smooth, orange-brown, 0-septate, 3–4 × 2–3(–3.5) µm, average L/W ratio 1.4 ± 0.2.

Colonies on OA reaching 70–75 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium in the centre faintly hazel or ochreous, aerial mycelium absent or diffuse, pure white. Reverse concolourous. *Colonies* on MEA reaching 60–65 mm diam in 10 d, with an even to slightly ruffled colourless margin mostly covered under the aerial mycelium.

Immersed mycelium completely hidden under a dense but not high mat of woolly to woolly-floccose, glaucous grey to pale grey-olivaceous aerial mycelium. Reverse bay, fading over sienna to luteous at the margin.

Specimens examined. Denmark, loc. unknown, isolated from root of gymnosperm, May 1969, *D.S. Malla S 7(45)*, living culture CBS 584.69; Geelskov, on canes of *Rubus* sp., *A.M. Dahl-Jensen*, Dec. 1995, isol. G. Verkley 338, living culture CBS 797.95. – Germany, München, Feldberg, isolated from *Picea abies* with cankers, Oct. 1985, *O. Kandler*, living culture 653.85. – The Netherlands, from sputum of man, Nov. 1971, isolated by M. Luykx, living culture CBS 764.71B.

Notes — Wollenweber & Hochapfel (1937) included the pathogens on *Rosaceae* in their concept of *Coniothyrium fuckelii*, and this is the core of the phylogenetic species here recognised under the name *Paraconiothyrium fuckelii*. In the literature the name for the sexual morph *Leptosphaeria coniothyrium* has mostly been used, but as has been established in previous molecular studies, the species is not congeneric with the type species of *Leptosphaeria*, *L. doliolum*, which resides in the *Leptosphaeriaceae* (Verkley et al. 2004, de Gruyter et al. 2009). According to Domsch et al. (2007) this species has a world-wide distribution.

***Paraconiothyrium* sp. 1** (‘*Microsphaeropsis pseudaspera*’?) — Fig. 8

Conidiomata pycnidial, globose to elliptical in surface view, with one or two ostioli, 8–12 µm diam, dark olivaceous-brown to black, pilose, 200–350(–400) µm diam, the surface provided with brown hyphal outgrowths emerging from a dense web of hyaline to dark brown hyphae growing parallel over the wall surface. *Conidiomatal wall* composed of an outer layer of relatively thin-walled brown *textura angularis* with cells mostly 4–6 µm diam, and an inner layer of similar but smaller, hyaline cells. *Conidiogenous cells* discrete, doliiform to broadly ampulliform, phialidic, with an indistinct periclinal thickening, 4–5(–6) × 3–4 µm. *Conidia* variable in shape, globose to subglobose or ellipsoid, more rarely obovoid, initially hyaline, contents with mostly 1–3(–5) small oil-droplets (< 1 µm diam), soon after secession olivaceous-brown, conidial wall smooth,

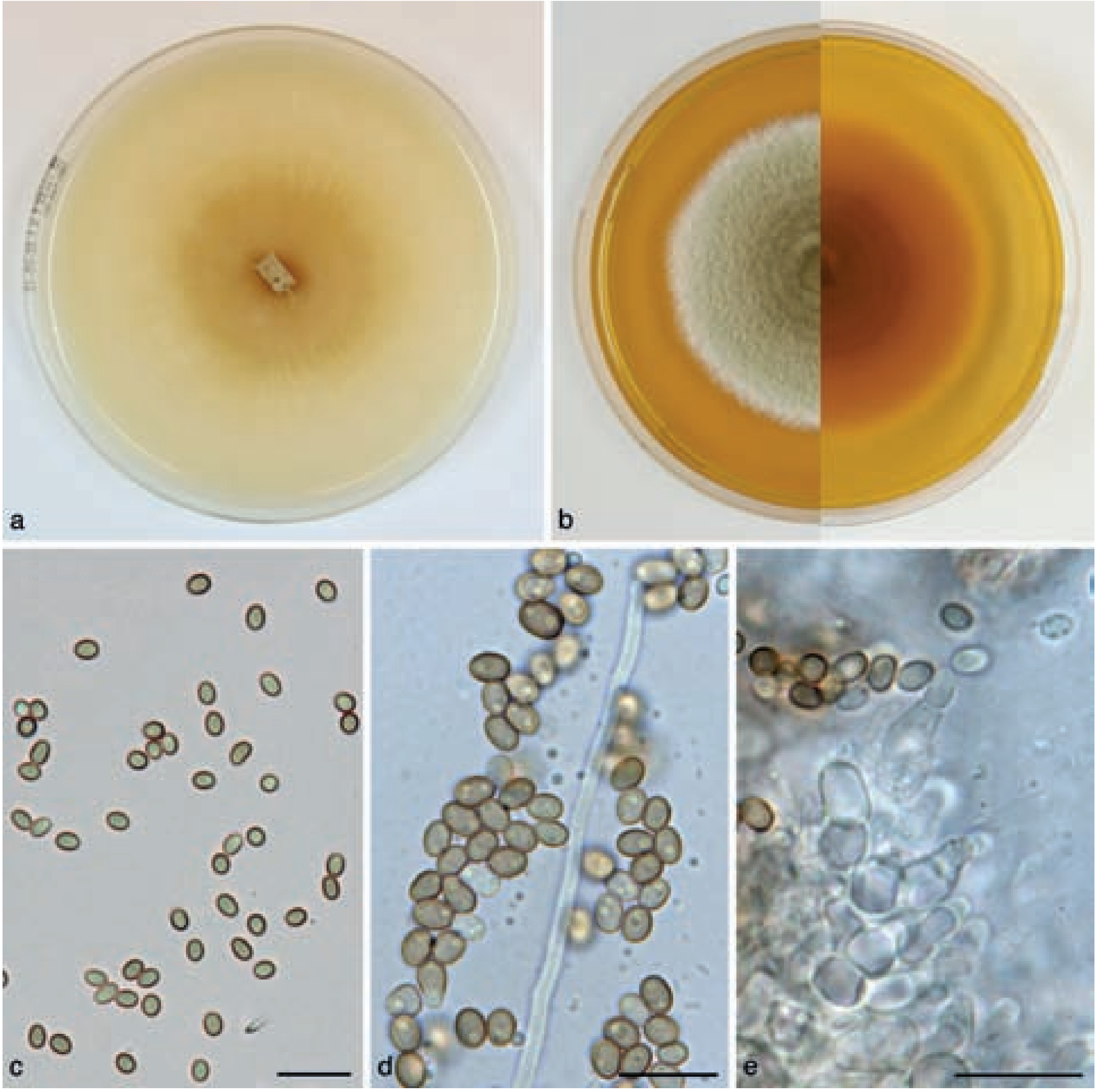


Fig. 7 *Paraconiothyrium fuckelii* (CBS 797.95). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c, d. conidia on OA; e. conidiogenous cells and conidia on OA. — Scale bars = 10 µm.

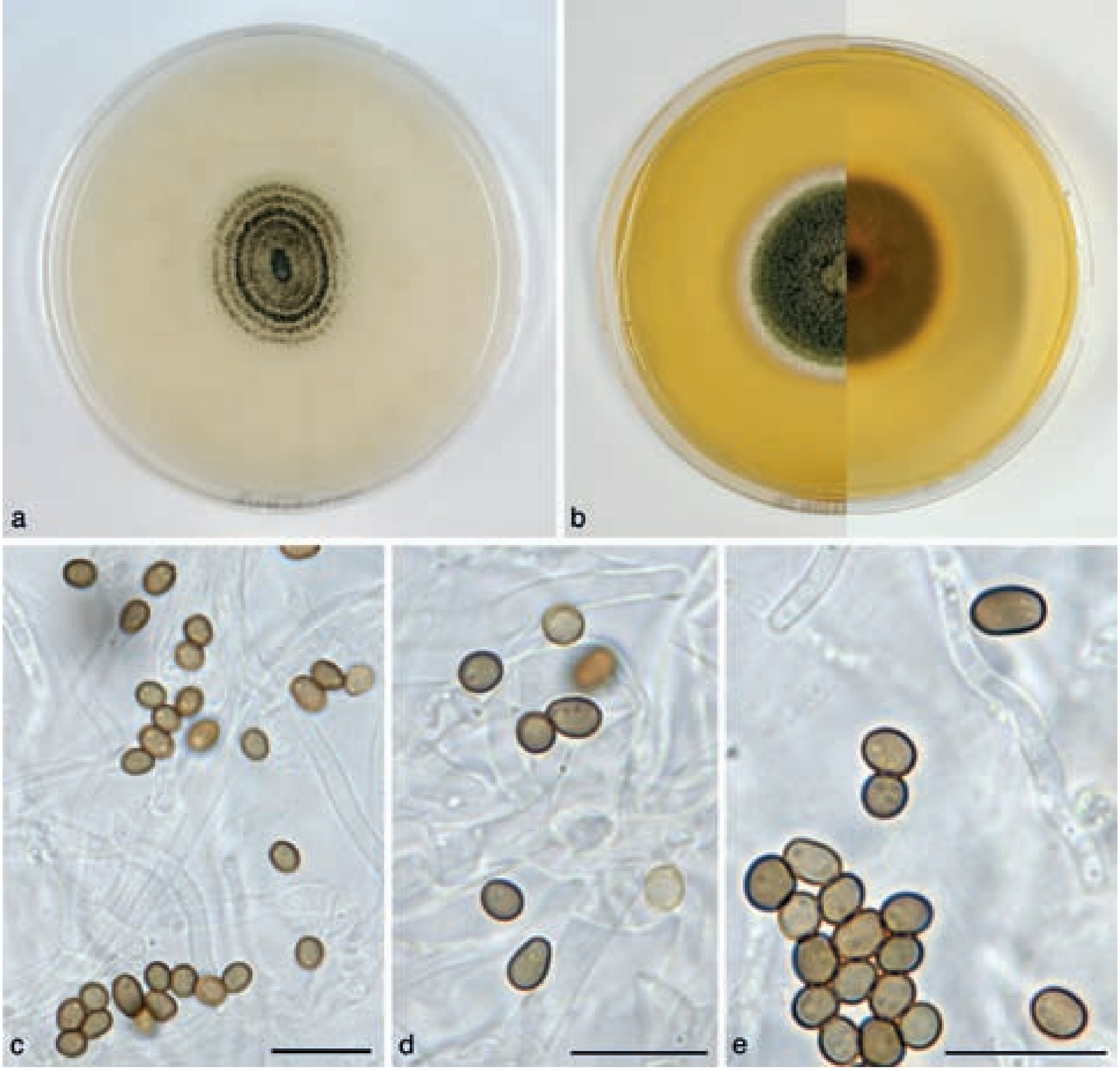


Fig. 8 *Paraconiothyrium* sp. ('*Microsphaeropsis pseudaspera*'?) (CBS 113682). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c–e. conidia on OA. — Scale bars = 10 µm.

orange-brown, 0-septate, 3–4.5(–5) × 2–3 µm, average L/W ratio 1.3 ± 0.2. Sexual morph unknown.

Colonies (CBS 113682) on OA reaching 41–46 mm diam in 10 d, with an even, glabrous colourless margin. Immersed mycelium colourless, with numerous pycnidia formed in distinct concentrical zones after 4–5 d. Reverse concolourous, appearing grey-olivaceous where pycnidia develop. *Colonies* on MEA reaching 32–36 mm diam in 10 d, with an even, glabrous, buff margin. Immersed mycelium in the centre olivaceous to olivaceous-black, buff in a submarginal zone, covered in the centre by olivaceous to olivaceous buff, woolly-floccose aerial mycelium, in a submarginal zone abruptly changing to pure white. Reverse in the centre umber to sienna, with dull luteous areas, fading to pale luteous at the margin.

Specimens examined. Spain, Santiago de Compostela, isolated from air sample, 15 Mar. 2002, *M.J. Aira*, deposited by A.M. Stchigel, living culture CBS 113682 (preserved as *Microsphaeropsis pseudaspera*). – The Netherlands, from nail of human, Apr. 1987, living culture CBS 251.87.

Notes — CBS 113682 was identified as *Microsphaeropsis pseudaspera*, a fungus described by Sutton (1974) from dead branches of *Eucalyptus* in Portugal. The conidiogenous cells and conidia of this isolate agree well with those described for this coelomycete based on material *in planta*. The clinical strain from the Netherlands is very similar in colony characters and other phenotypic traits, as are the sequences of CBS 251.87 generated in this study. Whether the name *M. pseudaspera* definitively applies to this material can only be confirmed by sequencing of the type material or recollecting from *Eucalyptus*.

Paraphaeosphaeria O.E. Erikss.

Type species. *Paraphaeosphaeria michotii* (Westend.) O.E. Erikss., Ark. Bot., ser. 2, 6: 406. 1967.

Câmara et al. (2001) provide descriptions of sexual and asexual morphs of *Paraph. michotii* and *Paraph. pilleata*, while other species treated there under *Paraphaeosphaeria* were transferred later to *Neophaeosphaeria* and *Phaeosphaeriopsis* (Câmara et al. 2003). None of the amerosporic coniothyrium-like fungi associated with these sexual morphs has been assigned a formal name.

Asexual morphs classified in *Paraphaeosphaeria* can be described as follows:

Conidiomata eustromatic or pycnidial. *Conidiogenous cells* discrete or integrated, phialidic, or annellidic with one or two percurrent proliferations. *Conidia* aseptate or 1-septate, smooth to verrucose.

Paraphaeosphaeria angularis Verkley & Aa, *sp. nov.* — MycoBank MB800765; Fig. 9

Etymology. Named after the angular shape of the conidia.

Conidiomata pycnidial, globose, ostiolum absent or with a single undifferentiated ostiolum 15–20 µm diam, pale olivaceous-brown, black due to mature conidia inside, 150–350(–450) µm diam. *Conidiomatal wall* composed of an outer layer of relatively thick-walled, yellowish brown *textura angularis* with cells 5–12 µm diam, and an inner layer of similar structure with hyaline and smaller cells. *Conidiogenous cells* discrete, globose to doliiform, phialidic, with an indistinct periclinal thickening, 4–7.5 × 4–6 µm. *Conidia* ellipsoid or elongated-ellipsoid, with a more or less clear angular outline, initially hyaline with 2–5 small oil-droplets (1–1.5 µm diam), then smoky greyish brown with relative dark, amorphous contents mostly showing no oil-droplets, often a brighter longitudinal band can be seen, conidial wall glabrous and moderately thick, 0-septate, 4.5–7(–8) × 3–4 µm, average L/W ratio 1.9 ± 0.2. Occasionally 2-celled conidia 8 × 5 µm are observed. Sexual morph unknown.

Colonies on OA reaching 53–56 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium colourless, fully covered by a very diffuse mat of pure white finely felted aerial mycelium, and pycnidia developing after 3–5 d in a pattern of radiating and branching rows. Reverse concolourous, greyish where pycnidia are formed. *Colonies* on MEA reaching 43–46 mm diam in 10 d, with an even to slightly undulating, glabrous margin. Immersed mycelium buff, appearing darker and olivaceous or greyish in the centre where pycnidia develop, colony largely covered by a high, tufty to woolly-floccose mat of dirty white, in the centre more greyish, aerial mycelium. Reverse cinnamon to ochreous, darker where pycnidia are formed.

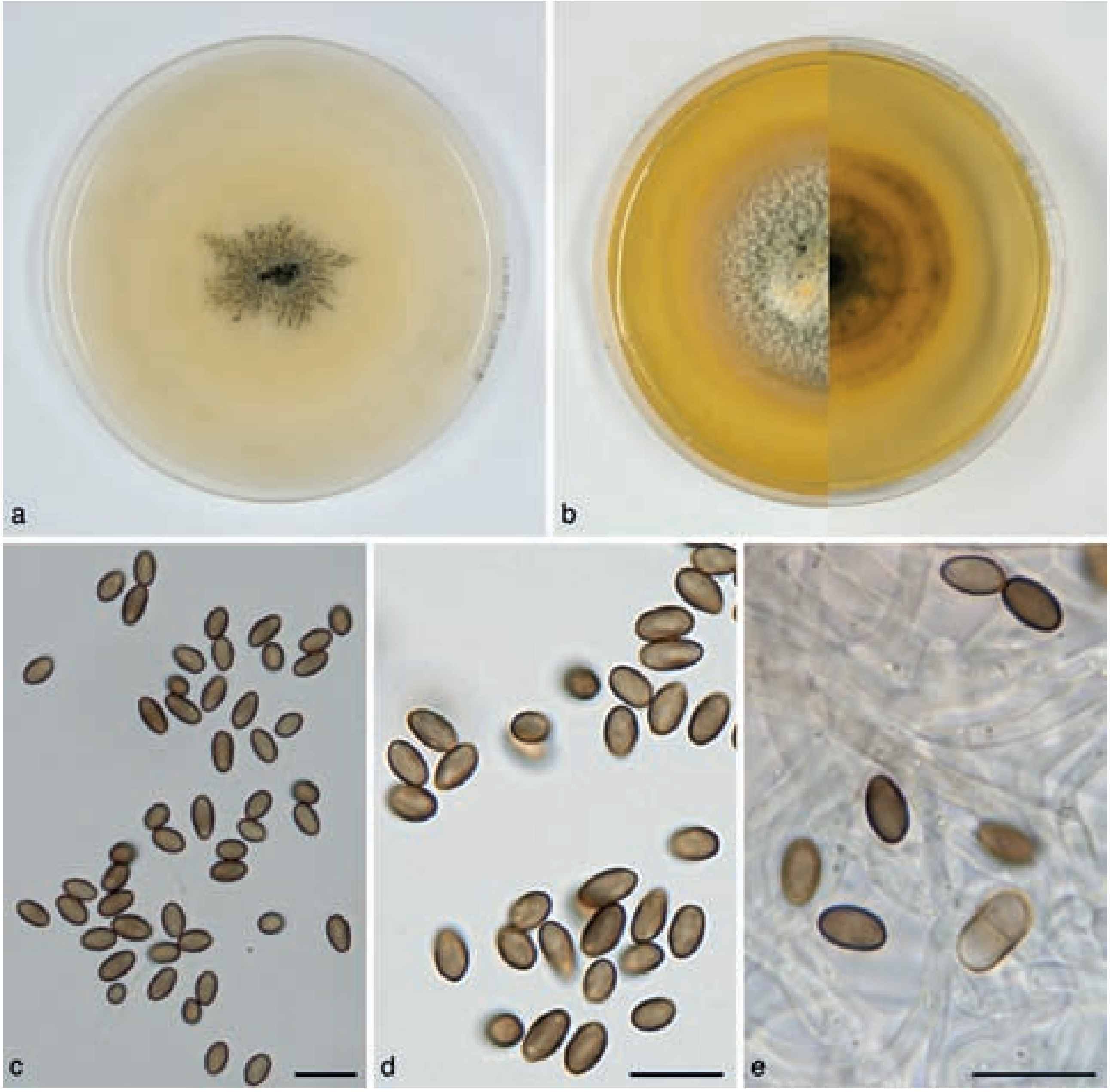


Fig. 9 *Paraphaeosphaeria angularis* (CBS 167.70^{T}, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c–e. conidia on OA. — Scale bars = 10 µm.

Specimen examined. Brazil, Bahia, Salvador, isolated from *Saccharum officinarum*, Oct. 1969, *C. Ram*, isol. H.A. van der Aa no. 1870, holotype CBS H-11085, living ex-type culture CBS 167.70.

Notes — A relatively high average conidial L/W ratio and especially the peculiar bright longitudinal band that can be observed over the mature conidium wall (difficult to record in photomicrographs) characterize this unique species, which is only known from a strain isolated from *Saccharum officinarum* in Brazil. The sexual morph is currently unknown, but since the species groups in a well-supported cluster with the pleomorphic species, *Paraph. michotii* and *Paraph. pilleata*, it would not be unlikely that it exists. The asexual morphs of these two close relatives of *Paraph. angularis* are otherwise highly similar (summarised in Table 3). All three species are associated with monocots.

Paraphaeosphaeria arecacearum Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800762; Fig. 10

Etymology. Named after the occurrence in association with genera of the family *Arecaceae* (= *Palmae*).

Conidiomata pycnidial, globose, glabrous, mostly with a rather undifferentiated single ostiolum, pale olivaceous or greenish, soon black due to mature conidia inside, 130–350 µm diam. *Conidiomatal wall* composed of an outer layer of relatively thick-walled, pale yellow to olivaceous *textura angularis* with cells mostly 4–7.5 µm diam, and an inner layer of hyaline thin-walled *textura angularis-globulosa*. *Conidiogenous cells* discrete, globose, doliiform to broadly ampulliform, phialidic with a distinct periclinal thickening, 4–6.5 × 3–4.5 µm. *Conidia* ellipsoid to obovoid-pyriform, initially hyaline, soon after secession olivaceous-brown, predominantly with two persistent polar oil-droplets (1.5–2 µm diam), conidial wall glabrous, 0-septate, (3–)3.5–6(–8.5) × 2–3 µm, average L/W ratio 2.0 ± 0.4. Sexual morph unknown.

Colonies on OA reaching 70–75 mm diam in 10 d, with an even to slightly ruffled, glabrous and colourless margin. Immersed mycelium colourless, aerial mycelium absent or very scanty, felted, pure white, vegetative hyphae exudating orange-brown material in amorphous masses (up to 25 µm wide) over variable length along the hyphae. Pycnidia developing after 3 d in a pattern of branching and radiating rows (but evenly

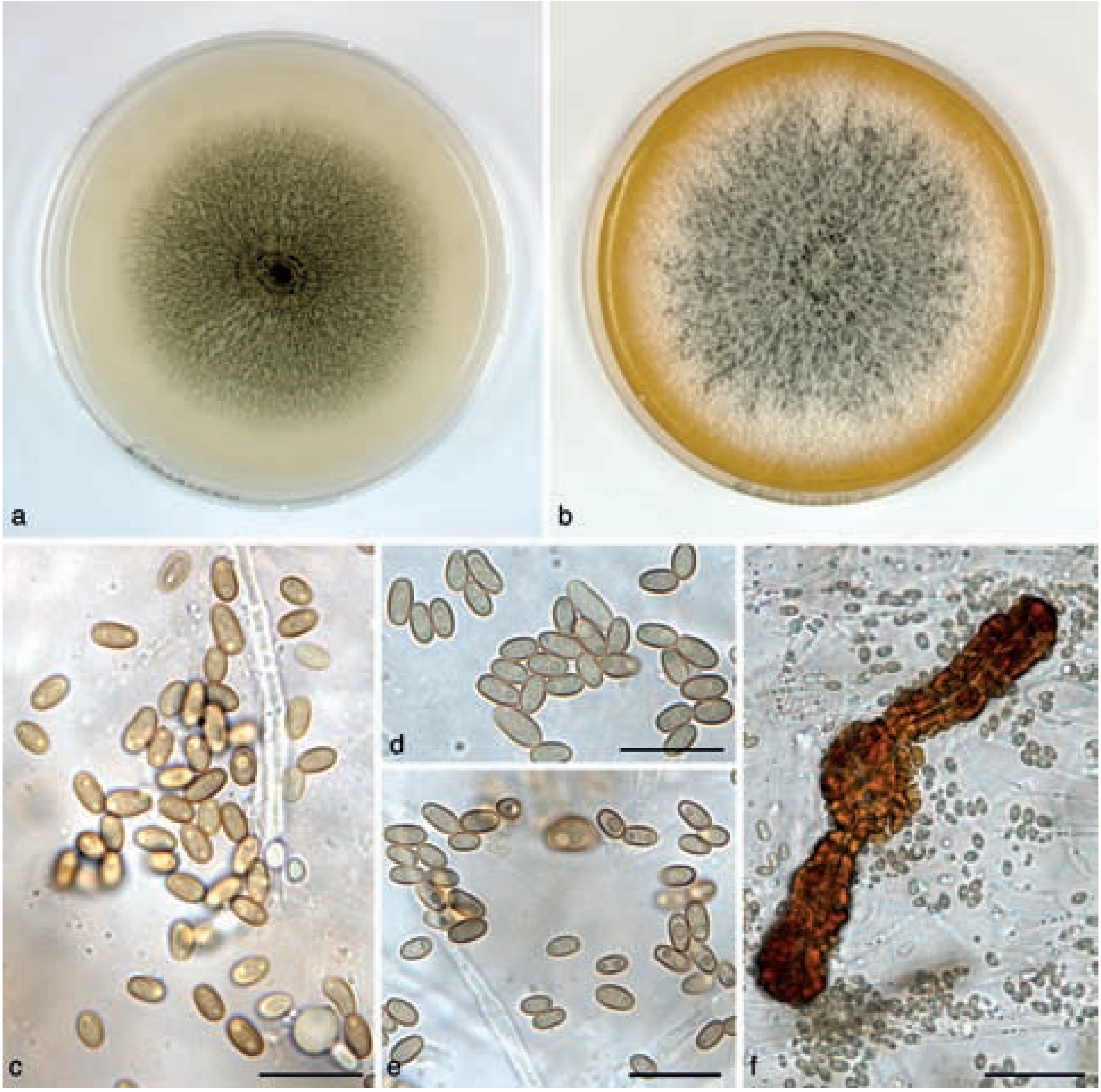


Fig. 10 *Paraconiothyrium arecacearum* (CBS 158.75^T, ex-type culture). a. Colony on OA; b. colony on MEA; c–e. conidia on OA; f. amorphous hyphal exsudate on OA. — Scale bars = 10 µm.

distributed and numerous), in the centre also concentrated in concentrical zones after 10 d. Reverse concolourous, but appearing grey due to pycnidial development. *Colonies* on MEA reaching 65–69 mm diam in 10 d, with a somewhat ruffled, colourless glabrous margin. Immersed mycelium buff, appearing grey due to developing pycnidia that are completely covered by a dense, woolly-floccose to tufty, pure white to faintly greyish or luteous mat of aerial mycelium. Reverse buff to pale luteous, with greyish brown concentrical zones where the pycnidia develop.

Specimens examined. Ivory Coast, isolated from *Cocos nucifera*, Dec. 1975, *J. Mouchacca*, living culture CBS 614.75. – Surinam, isolated from soil under *Elaeis guineensis*, Mar. 1974, *J.H. van Emden*, holotype CBS H-11048, living ex-type culture CBS 158.75.

Notes — This species is notable for its rapid growth rate and sporulation. The two cultures that are known thus far are both associated with tropical palms. *Coniothyrium palmarum*, the type species of *Coniothyrium* (*Leptosphaeriaceae*) is frequently found on palms as well, but that species can easily be distinguished from *Paraph. arecacearum* by the annellidic conidiogenesis and verrucose and 0–1-septate conidia 6–8.5 × 4–5 µm (Sutton 1980).

Paraphaeosphaeria minitans (W.A. Campb.) Verkley, Göker & Stielow, *comb. nov.* — MycoBank MB800766

Basionym. Coniothyrium minitans W.A. Campb., Mycologia 39: 191. 1947.
≡ *Paraconiothyrium minitans* (W.A. Campb.) Verkley, Stud. Mycol. 50: 332. 2004.

A detailed description of the fungus, of which the sexual morph is unknown, is provided by Domsch et al. (2007). In the past a number of CBS strains have been identified as *Coniothyrium minitans* (Table 1, Fig. 1), but the sequence data indicate they belong to a number of different taxa. Most of these strains were obtained from soil samples, and critical characteristics of this species like infective capability of sclerotia of *Sclerotinia*, were not documented.

Paraphaeosphaeria neglecta Verkley, Riccioni & Stielow, *sp. nov.* — MycoBank MB800767; Fig. 11

Etymology. Named for the fact that this fungus was not recognised as distinct within the *Paraphaeosphaeria* (*Paraconiothyrium*) *sporulosum* complex.

Conidiomata pycnidial, globose, glabrous, with a single ostiolum 20–30(–50) µm diam, black due to mature conidia inside, the

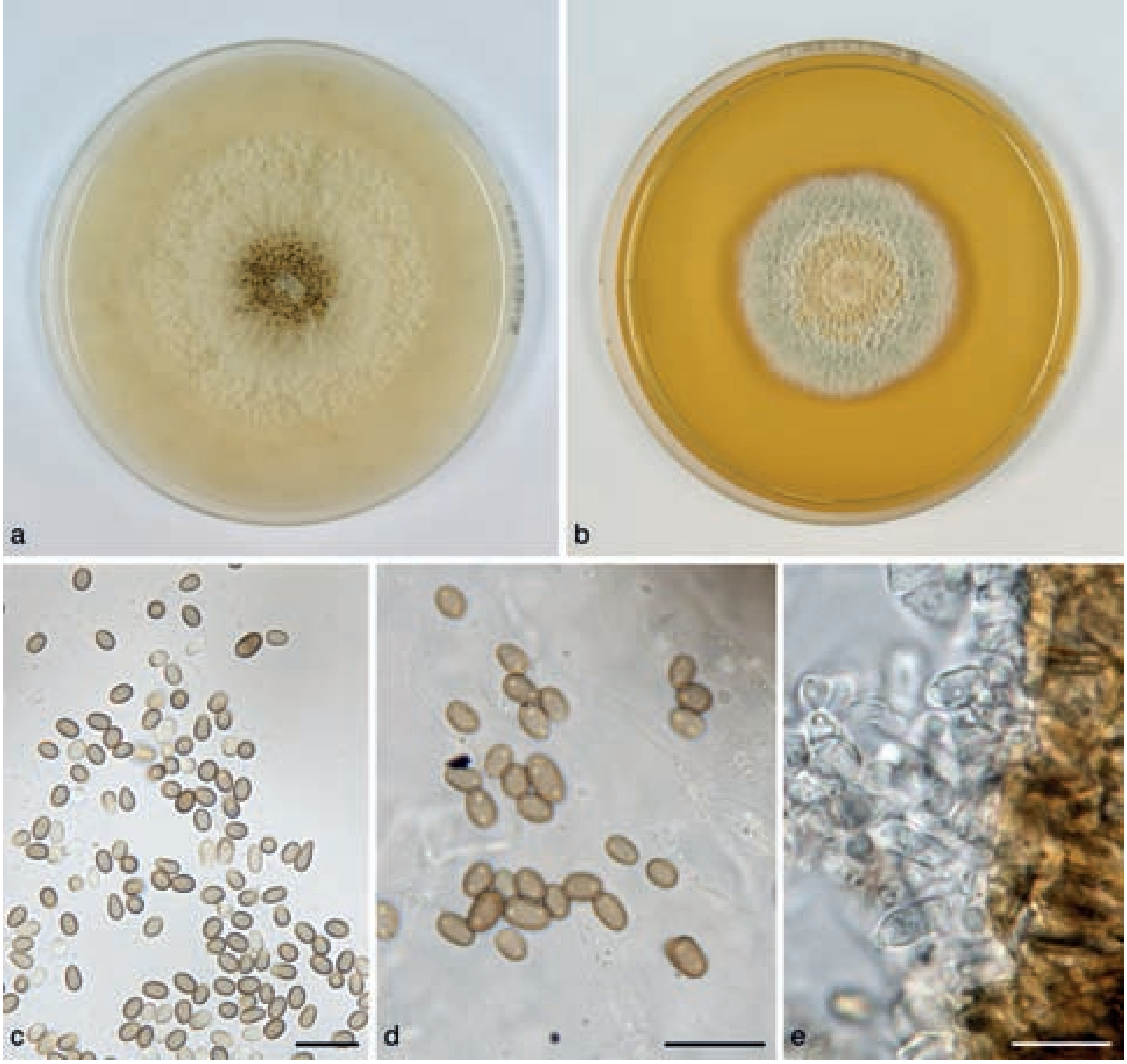


Fig. 11 *Paraphaeosphaeria neglecta* (CBS 124078^T, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse of CBS 124077 on the right; c, d. conidia on OA; e. conidiogenous cells on OA. — Scale bars = 10 µm.

wall yellowish brown but cells surrounding the ostiolum darker, conidiomata 240–350 µm diam. *Conidiomatal wall* composed of an outer layer of yellow-brown, relatively thick-walled *textura angularis*, and an inner layer of similar structure but with hyaline, thin-walled cells. *Conidiogenous cells* discrete or positioned on clumps of cells that protrude into the cavity, globose, doliiform to broadly ampulliform, phialidic with a distinct periclinal thickening, 4–6 × 3–5 µm. *Conidia* highly variable in shape, subglobose, ellipsoid to obovoid-pyriform, or more cylindrical, initially hyaline, soon after secession olivaceous-brown, mostly with two polar oil-droplets (1.5–2 µm diam), and rarely with a few additional smaller ones, conidial wall glabrous or minutely roughened, 0-septate, (3–)3.5–6(–8.5) × 2–3 µm, average L/W ratio 1.7 ± 0.4 (CBS 124078^{T}; 1.5 ± 0.3 for CBS 303.77). Sexual morph unknown.

Colonies on OA reaching 45–50 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium initially colourless, then luteous sometimes with sienna centre, with rather diffuse but high, tufty, pure white aerial mycelium. Pycnidia developing in discontinuous concentrical zones or scattered after 7–10 d. Reverse concolourous. *Colonies* on MEA reaching 34–39 mm diam in 10 d, with an even to undulating, glabrous margin. Immersed mycelium entirely hidden under a dense mat of woolly-floccose, white to pale luteous, sometimes also glaucous to glaucous grey aerial mycelium. Reverse mostly sienna, fading to pale luteous at the margin. Pycnidia absent or developing after 12–15 d.

Specimens examined. CHILE, Valdivia, South Chilean Forest, isolated from rotten wood, June 1978, *A.E. Gonzáles*, living cultures CBS 335.78 and CBS 337.78 (CBS H-10913, H-10923). – FRANCE, Brest, from cankered *Juniperus* sp., July 1975, *M. Morelet*, living culture CBS 359.75; isolated from *Taxus baccata*, 5 Nov. 1975, *I. Vegh*, living culture CBS 303.77; same substrate, 14 May 1976, *I. Vegh 9793*, living culture CBS 305.77; isolated from *Cupressocyparis leylandii*, 1 Apr. 1977, *I. Vegh 10145*, living culture CBS 307.77. – GERMANY, Ülzen, from *Erica carnea*, Aug. 1970, *L. Kiewnick*, living culture CBS 434.71A; Freiburg, *H. Courtois*, living culture 431.77 (CBS H-10916, dried culture); Bavendorf, Ravensburg, on dead branches of *Pyrus malus*, Aug. 1981, *R. Weiler* (CBS H-10915, CBS H-10926), living culture CBS 452.81 isolated by H.A. van der Aa 7867. – ITALY, Latina, from wood of *Actinidia chinensis* var. hort. 16A, *L. Riccioni*, holotype CBS H-21039, living ex-type culture CBS 124078 (ER 1503); isolated from the same material CBS 124077 (ER 1501). – THE NETHERLANDS, Oostvoorne, on *Pyrola rotundifolia*, 3 Apr. 1971, *H.A. van der Aa 2526* (CBS H-10734), living culture CBS 434.71B; Baarn, on leaf of *Azalea* sp., Mar. 1972, *H.A. van der Aa 3012*, living culture CBS 300.72; Wageningen, on *Azalea* sp., Feb. 1972, *H. van Kesteren*, living culture CBS 302.72 isolated by H.A. van der Aa 2998; loc. unknown, *J.C. Went 1021a*, isolated from acid mull soil, with very well decomposed leaves, Feb. 1961, living culture CBS 180.61 (VKM F-2659);

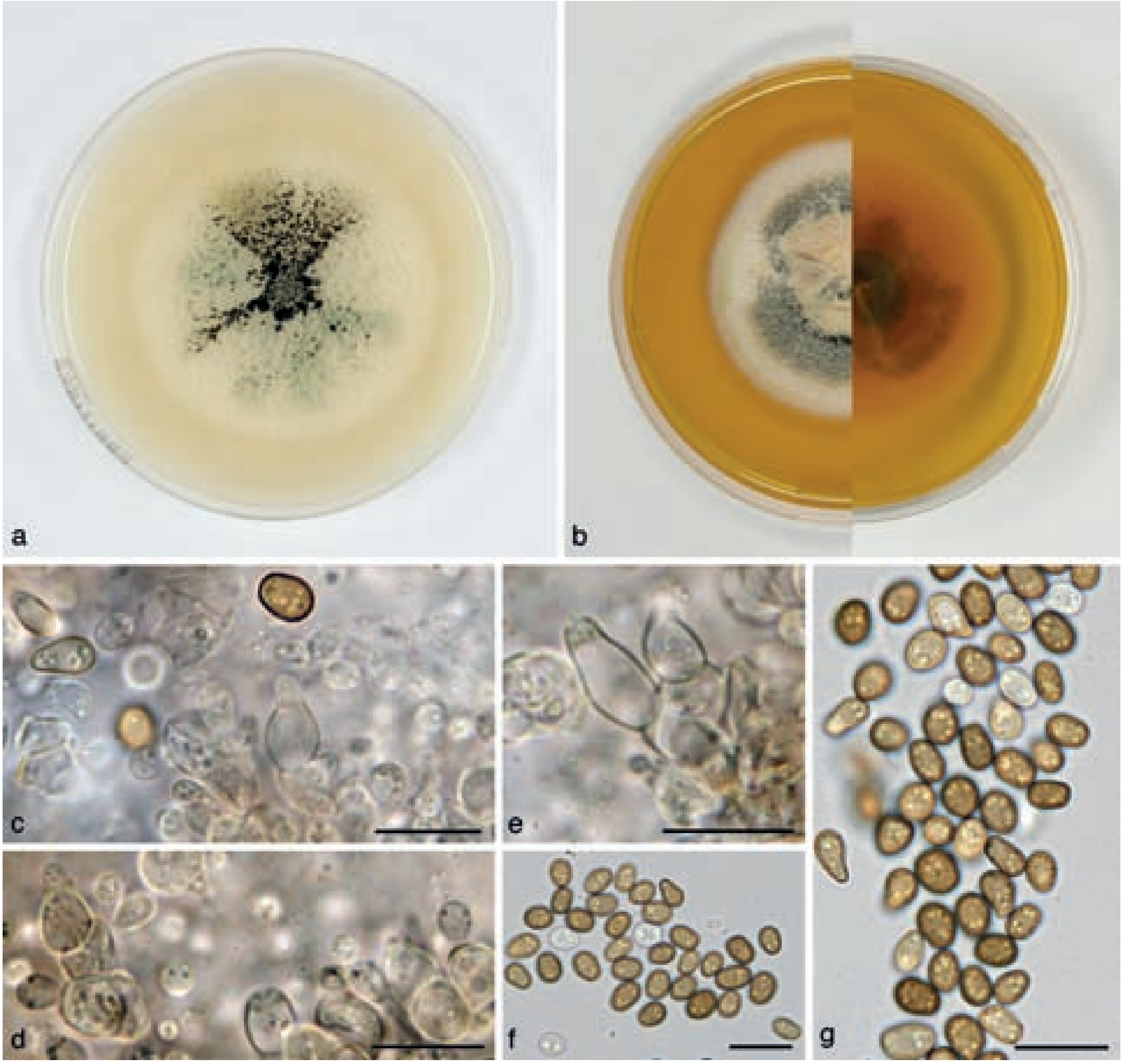


Fig. 12 *Paraphaeosphaeria sardoa* (CBS 501.71^{T}, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c–e. conidiogenous cells on OA; f, g. conidia on OA. — Scale bars = 10 µm.

Eese Estate near Steenwijk, on seed of *Quercus robur*, 13 Oct. 1983, *H.A. van der Aa 8895*, living culture CBS 683.83; Utrecht, ear of human, 15 Dec. 2005, living culture CBS 119637 isolated by J. Vlooswijk.

Notes — Nine of the isolates formerly identified as *Parac. sporulosum* in CBS belong to a new species, for which the name *Paraph. neglecta* is proposed. *Paraphaeosphaeria neglecta* and *Paraph. sporulosa* are not sister taxa and material available in this study suggests that, although both species are ubiquitous fungi occurring in soil and on various plants in different habitats, only *Paraph. neglecta* may be truly cosmopolitan, at least besides Europe also occurring in the Americas. Records of *Paraph. sporulosa* from outside Europe should be confirmed by sequence studies. *Paraphaeosphaeria neglecta* may have a preference for plants, as only one soil isolate belonged to that species. One isolate originated from the human ear. The colonies of *Paraph. sporulosa* and *Paraph. neglecta* look very similar especially on OA, and there is also overlap in conidial sizes. In *Paraph. sporulosa* conidia are 3.5–5(–6) × 3–4 µm, average L/W ratio 1.5 ± 0.2, and in *Paraph. neglecta* (3–)3.5–6(–8.5) × 2–3 µm, average L/W ratio 1.7 ± 0.4. Conidia for some strains of *Paraph. neglecta* show a minutely roughened outer wall surface, a feature not observed in *Paraph. sporulosa*. A further difference pertains to percurrent proliferation in the conidiogenous cells, which was observed in *Paraph. sporulosa* and not in *Paraph. neglecta*, but it should be noted that this character has proven not to be very reliable in coelomycetes.

Paraphaeosphaeria sardoa Verkley, W. Gams & Aa, *sp. nov.* — MycoBank MB800769; Fig. 12

Etymology. Named after Sardinia, where this fungus was collected.

Conidiomata pycnidial, single, or eustromatic and more complex, globose, glabrous, superficial or immersed in the agar, 300–450(–600) µm diam, initially pale but soon appearing dark brown to black due to mature conidia inside. *Conidiomatal wall* composed of hyaline to very pale yellowish *textura angularis* with moderately thickened walls, lined by a layer of globose hyaline thin-walled cells. *Conidiogenous cells* globose to broadly ampulliform, hyaline, discrete, or integrated in short, simple, 1–2-septate conidiophores, but more often positioned on clumps of cells that protrude into the cavity, phialidic, with a distinct periclinal thickening, 5–10 × 4.5–6 µm. *Conidia* variable in shape, subglobose, ellipsoid, obovoid, or pyriform, often irregular in outline, initially hyaline, after secession olivaceous-brown, verruculose, with mostly 6–12 oil-droplets up to 1 µm diam, 0-septate, (4.5–)5–6(–7) × (3–)3.5–4.5(–5) µm, average L/W ratio 1.4 ± 0.2. Sexual morph unknown.

Colonies on OA reaching 40–44 mm diam in 10 d, with an even, glabrous and colourless margin; immersed mycelium colourless, forming a glaucous pigmentation in sectors or irregular patches, or locally faintly olivaceous after 10–14 d; the surface covered by a diffuse layer of woolly to tufty, first pure white, later locally glaucous aerial mycelium. Reverse

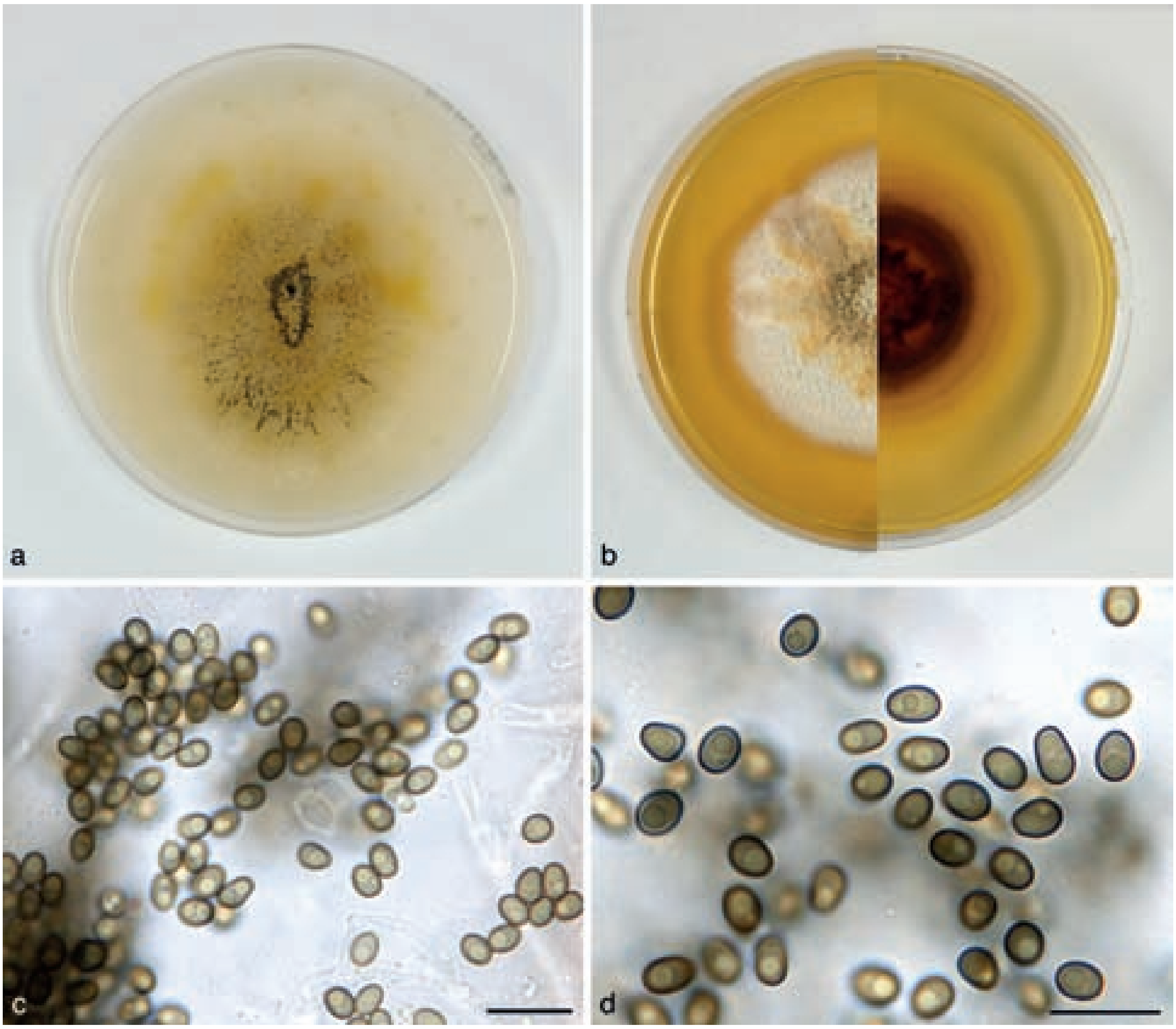


Fig. 13 *Paraphaeosphaeria sporulosa* (CBS 218.68, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c, d. conidia on OA. — Scale bars = 10 µm.

concolourous. Conidiomata developing scattered or in dense clusters mostly colourless sectors after 5–7 d. *Colonies* on MEA reaching 38–40 mm diam in 10 d, with an even, colourless margin; immersed mycelium not visible from above, entirely hidden under a dense but relatively low, woolly-floccose mat of aerial mycelium that shows glaucous and rosy buff to salmon areas; conidiomata developing on the agar surface underneath the aerial mycelium, scattered or in dense clusters. Reverse predominantly ochreous, locally with rust patches, olivaceous black where pycnidia are numerous.

Specimen examined. Italy, Sardinia, on dead leaves of *Smilax aspera*, 18 May 1971, *W. Gams*, isolated by H.A. van der Aa 2568, holotype CBS H-21040, living ex-type strain CBS 501.71.

Notes — Only a single isolate was available of this species. More isolates need to become available in order to assess its ecology and geographic distribution.

Paraphaeosphaeria sporulosa (W. Gams & Domsch) Verkley, Göker & Stielow, *comb. nov.* — MycoBank MB800768; Fig. 13

Basionym. Coniothyrium fuckelii var. *sporulosum* W. Gams & Domsch, Nova Hedwigia 18: 9. 1969.

≡ *Coniothyrium sporulosum* (W. Gams & Domsch) Aa, Verh. Kon. Ned. Akad. Wetensch., tweede sect., 68: 3. 1977.

≡ *Paraconiothyrium sporulosum* (W. Gams & Domsch) Verkley, Stud. Mycol. 50: 332. 2004.

Conidiomata pycnidial, single, or eustromatic and more complex, globose, superficial or immersed in the agar, glabrous, 120–250(–350) μm diam, initially pale but soon appearing dark brown to black due to mature conidia inside. *Conidiomatal wall* composed of hyaline to pale yellowish brown *textura angularis*, lined by a thin layer of globose hyaline cells. *Conidia* released through one, rarely two, well-developed ostioli 15–25 μm diam that are somewhat darker then the surrounding wall and lined with hyaline short clavate periphyses. *Conidiogenous cells* globose to ampulliform, hyaline, discrete or positioned on aggregated clumps of cells that protrude into the cavity, phialidic or with 1–2 percurrent proliferations, mostly 4.5–7 × 3.5–4.5(–5) μm. *Conidia* subglobose, ellipsoid or obovoid-pyriform, initially hyaline, after secession olivaceous-brown, glabrous, with one large (1.5–2 μm diam) and often also 1–2 additional smaller oil-droplets, 0-septate, 3.5–5(–6) × 3–4 μm, average L/W ratio 1.5 ± 0.2. Sexual morph unknown.

Colonies on OA reaching 42–50 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium colourless but in most strains soon showing a pure yellow to pale luteous, more rarely brownish pigmentation, the surface mostly glabrous but in the centre and sometimes also elsewhere with tufts of pure white aerial mycelium. Reverse concolour-

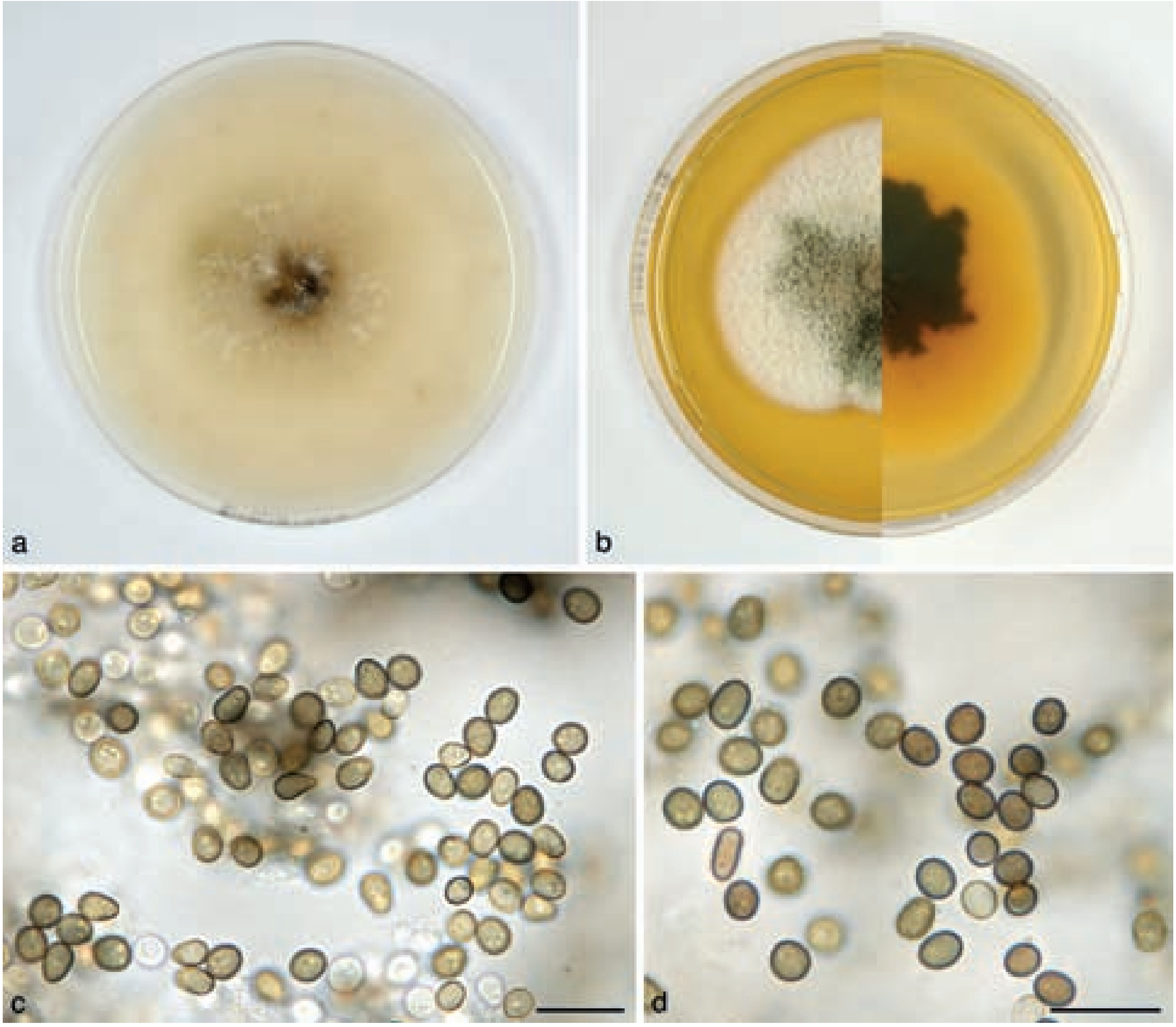


Fig. 14 *Paraphaeosphaeria verruculosa* (CBS 263.85^T, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c, d. conidia on OA. — Scale bars = 10 μm.

ous, appearing olivaceous-brown where numerous pycnidia are formed. Conidiomata developing after 7–10 d. *Colonies* on MEA reaching 30–35 mm diam in 10 d, with an even to slightly ruffled, colourless to buff margin. Immersed mycelium barely visible from above, appearing olivaceous in the centre, covered by a dense mat of woolly-floccose, dirty white to rosy buff or ochreous aerial mycelium. Reverse mostly pale luteous to ochreous, in the centre with darker areas of umber and chestnut.

Specimens examined. GERMANY, Kitzeberg, isolated from wheat field soil, 1963, *W. Gams C353*, living culture ex-isotype CBS 218.68 (CBS H-6956, isotype of *Coniothyrium fuckelii* var. *sporulosum*). Other isolates examined are listed in Table 1.

Notes — In the literature *Paraph. sporulosa* has been reported as a cosmopolitan soil fungus (Domsch et al. 2007), but this study sheds doubt on whether those records were based on correct identifications. Isolates in CBS from outside Europe all proved to belong to *Paraph. neglecta* or to other species outside the *Montagnulaceae*. For morphological differences with the closely related *Paraph. neglecta*, see the note under that species.

Paraphaeosphaeria verruculosa Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800770; Fig. 14

Etymology. Named after the moderately roughened outer wall of the conidia.

Conidiomata pycnidial, globose, ostiolum 10–15 μm diam or absent, black due to mature conidia inside, 140–200 μm diam, predominantly formed in the aerial mycelium (on OA). *Conidiomatal wall* composed of an outer layer of a relatively thick-walled, orange-brown *textura angularis* (cells 4–8 μm diam) and an inner layer of hyaline, thin-walled *textura angularis*. *Conidiogenous cells* discrete, globose to broadly ampulliform, phialidic, 4–6 × 3–4 μm. *Conidia* globose, subglobose to ellipsoid, initially hyaline with mostly 3–7 small oil-droplets (< 1 μm diam), after secession olivaceous-brown and mostly with fewer but larger oil-droplets (1.5–2 μm diam), mature co-

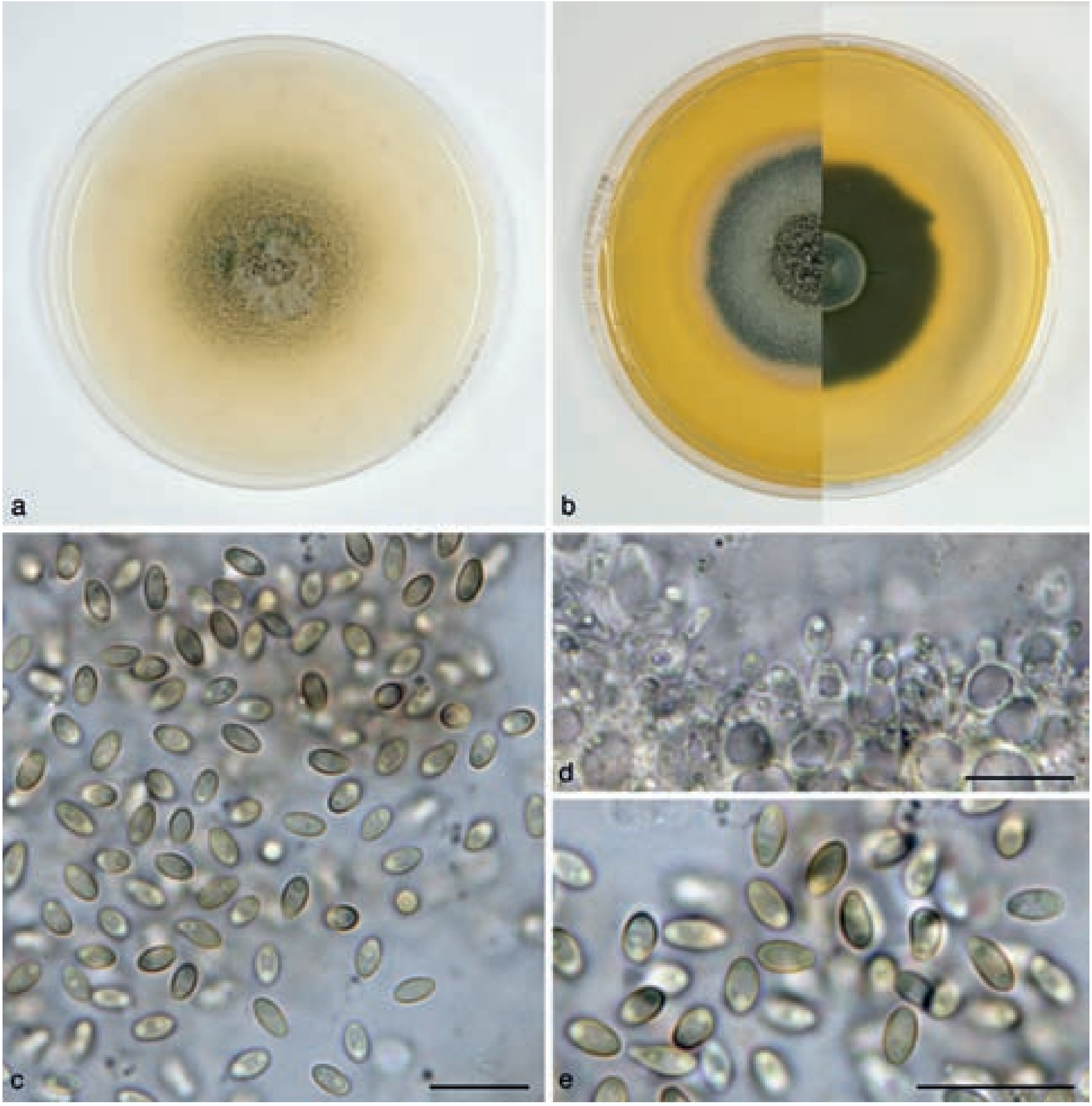


Fig. 15 *Paraconiothyrium viridescens* (CBS 854.73^T, ex-type culture). a. Colony on OA; b. colony on MEA, also showing reverse on the right; c. conidia on OA; d. conidiogenous cells on OA; e. conidia on OA. — Scale bars = 10 μm.

nidial wall orange-brown, verruculose, 0-septate, (3–)4–5(–6) × (2.5–)3–3.5(–5) µm, average L/W ratio 1.3 ± 0.2. Sexual morph unknown.

Colonies on OA reaching 50–54 mm diam in 10 d, with an even, glabrous and colourless margin. Immersed mycelium first colourless, becoming chestnut, often with a greenish haze, in the centre. Aerial mycelium very diffuse, felty, locally also with long, grey tufts. Reverse concolourous. Pycnidia developing after 12–18 d. *Colonies* on MEA reaching 40–44 mm diam in 10 d, with an even, colourless margin. Immersed mycelium entirely hidden under a dense, high mat of floccose aerial mycelium which is for the most pure white, but in an irregularly outlined central area grey to grey-olivaceous. Reverse chestnut in the centre (irregular outline), surrounded by umber fading to ochreous, margin pale luteous.

Specimens examined. CHILE, Valdivia, isolated from wood logs of *Pinus radiata* stored outdoors for 1–8 months, Nov. 1984, *H.L. Peredo*, living culture CBS 682.84. – COLOMBIA, Cundinamarca, Monserrate, isolated from páramo soil, after burning, Mar. 1980, *W. Gams*, living culture CBS 354.80. – GERMANY, Bayerischer Wald, from needle of *Picea abies*, Mar. 1985, *H. Butin*, holotype CBS H-21041, living ex-type culture CBS 263.85.

Notes — This species is characteristic for having globose to subglobose conidia at maturity with an elegant verruculose ornamentation of the outer conidium wall. It may be widely distributed in soils, and shows a preference for conifers. More material needs to become available in order to better understand its ecology.

Paraphaeosphaeria viridescens Verkley, Göker & Stielow, *sp. nov.* — MycoBank MB800764; Fig. 15

Etymology. Named after the green pigment this fungus produces in culture.

Conidiomata pycnidial, with a single cavity but lacking a differentiated ostiolum, black due to mature conidia inside, 250–450 µm diam. *Conidiomatal wall* composed of an outer layer of yellow to very pale orange-brown and thin-walled *textura angularis* with relatively large cells 5–12 µm diam, and an inner layer of hyaline, thin-walled *textura angularis-globosa*. *Conidiogenous cells* discrete, globose, doliiform to broadly ampulliform, phialidic with a distinct periclinal thickening, occasionally percurrently proliferating to form a neck-like protrusion, 5–7.5 × 4–5 µm. *Conidia* consistently ellipsoid, initially hyaline, soon after secession with a greenish yellow, thin, smooth wall, contents with 1–2 oil-droplets (< 1 µm diam) at each end, 0-septate, (3–)4–4.5(–5) × 1.8–2.2 µm, average L/W ratio 2.0 ± 0.2. Sexual morph unknown.

Colonies on OA reaching 52–55 mm diam in 10 d, with an even, glabrous colourless margin. Immersed mycelium colourless to very faintly yellow, later with a green pigment and with numerous pycnidia developing in distinct concentrical zones after 3–5 d. Reverse concolourous. *Colonies* on MEA reaching 40–43 mm diam in 10 d, with an even to slightly ruffled, buff margin. Immersed mycelium in the centre dark herbage green (often with bluish tinges), darkening to dull green, covered by an appressed, diffuse to more dense mat of finely felted to floccose, greyish aerial mycelium. Reverse in the centre greenish olivaceous to olivaceous, quite abruptly changing to buff at the margin.

Specimen examined. MONTENEGRO, Lake of Skadar, isolated from freshwater, Oct. 1973, *M. Muntañola-Cvetkovic*, No. SK 1-32, holotype CBS H-21038, living ex-type culture CBS 854.73.

Notes — The species is noted for producing a green pigment diffusing in the agar and conidia with a consistently ellipsoid shape and relatively high L/W ratio (2.0 ± 0.2), and a relatively faintly green yellowish wall at maturity. CBS 854.73^{T} originates from fresh water and it remains unclear if the fungus also occurs in soils or plants, as do most of its close relatives.

DISCUSSION

By combining multi-locus DNA sequencing with detailed morphological analyses, we were able to delimit and formally propose nine new species and two new genera among the fungi in the *Montagnulaceae* formerly recognisable as coniothyrium-like asexual morphs. The genus *Paraconiothyrium* as accepted here appears paraphyletic, but because the branches that conflict with its monophyly are insufficiently supported, it was decided not to split it up into further genera. We furthermore demonstrated that the diversity of soil-borne fungi in this family is considerable, as 12 out of 24 species studied here are found in soils. Four of these soil-borne fungi are novel species proposed here, viz. *Paraph. arecacearum*, *Paraph. neglecta*, *Paraph. verruculosa* and *Alloconiothyrium aptrootii*, while well-known species such as *Paraph. sporulosa* (*Coniothyrium sporulosum*) and *Paraconiothyrium fuckelii* are now more accurately delimited and described compared to manuals available for identifying soil fungi (Domsch et al. 2007). Many isolates deposited in the CBS collection under these two older names needed to be re-identified (Table 1), including a number of strains proven not to belong in *Montagnulaceae* by LSU and ITS sequencing. These will be treated in forthcoming publications focussing on other families of the *Pleosporales*.

Morphological characters traditionally used to delimit genera in coelomycetes include conidiomatal structure, structure of the conidiophores, conidiogenesis and conidial characters such as pigmentation, septal structure and number, and conidial appendages (Sutton 1980, Nag Raj 1993). Recent molecular studies have shown that these features are not always suitable to delimit genera as natural entities, and they may vary even between sibling species (Crous et al. 2012). Generic boundaries drawn in the present study were based primarily on statistically well-supported branches in the multi-locus phylogeny. Some of the characters mentioned above are thus overlapping between the accepted genera. For example, phialidic and annellidic conidiogenesis occur both in *Paraconiothyrium* and *Paraphaeosphaeria* (Verkley et al. 2004, Damm et al. 2008). Furthermore, in the new genus *Dendrothyrium* the conidiogenous cells are phialidic in both species, but the conidiophores of the type species are acropleurogenous, while those of the other species are acrogenous, a difference that in earlier coelomycete taxonomy would normally not be acceptable in the same genus.

For an accurate species identification of coniothyrium-like fungi, a molecular evaluation has been long overdue, as some species are morphologically very similar and difficult to distinguish based on available literature. Also, with reference to the taxa treated in the present work, it is required to first ascertain the phylogenetic position of the fungus in the *Montagnulaceae*, as similar fungi occur in other *Pleosporales* as well; LSU can be used to determine the order and mostly also the family to which the fungus belongs. ITS alone might suffice for an accurate identification of most species, as it is sufficiently variable among most closely related taxa in *Montagnulaceae*, but it fails to distinguish all species. Better suited for this purpose are, in principle, more variable (Table 2) genes such as ACT and TUB. Whereas ACT did not result that well in *Montagnulaceae* molecular taxonomy, optimised TUB sequence clustering yielded exactly the proposed classification into species with the exception of a single species complex. But when careful morphological analyses are conducted on fresh isolates under standard conditions and media, most species with (almost) identical ITS sequences can also be distinguished by colony features and conidial characters such as ornamentation of the wall, contents and L/W ratio. The main characters are summarised in Table 3.

Apart from occurring in soils, most taxa also colonise tissues of plants that are evolutionary diverse. This study is just one more to show that the host-based taxonomy commonly practised by many (coelomycete) mycologists in the last century was inadequate. Highly host-specific species do exist, but this should be corroborated by infection studies including multiple plant species. It is unlikely that they are more common than generalists.

When Damm et al. (2008) introduced *Parac. variabile*, the species was known from wood necroses and pycnidia on the bark of *Prunus persica* and wood necroses in *P. salicina* in South Africa, leaves of *Laurus nobilis* in Turkey, and wood of decaying trunks and vines of *Actinidia* in Italy (Riccioni et al. 2007). In the present study it was shown that this fungus occurs on more matrices in various habitats, viz. on various distantly related plants in Europe and Africa, including *Chamaerops*, *Spartium*, *Dianthus*, *Platanus* (in pruning wounds) and on sori of *Puccinia* rusts. It also occurs in soils (Egypt, The Netherlands) and sandstone (France). CBS 680.83 was isolated from a toenail of man, but other clinical records are unknown. The closely related *Parac. brasiliense* was introduced by Verkley et al. (2004) for CBS 100299 isolated from coffee fruit in Brazil, but this species has later been reported from various habitats on other continents as well. Most records pertain to woody and herbaceous host plants, especially *Prunus* spp. in South Africa (Damm et al. 2008). Near-identical ITS sequences have been deposited in GenBank for endophytes isolated from trees like *Ginkgo biloba* (DQ094168), *Juniperus virginiana* (Hoffman & Arnold 2008), and *Ulmus davidiana* var. *japonica* (AB665311), and also from the herb *Alliaria petiolata* (EF432267). In the present study, also CBS 395.87 from soil sampled in Italy could be identified as *Parac. brasiliense*. The ACT and TUB sequences of strains available in the present study were more variable than in other related species, suggesting that *Parac. brasiliense* could be a species complex. However, the internal branches of the multi-locus phylogeny were insufficiently supported to split it up.

Acknowledgements We thank the technical staff of the CBS Collections (cultures), Marjan Vermaas (photographic plates) and Hans de Gruyter and Joyce Woudenberg (primers). This work was supported through funding from the European Community's Seventh Framework Programme (FP7, 2007-2013), Research Infrastructures action, under the grant agreement No. FP7-228310 (EMbaRC project).

REFERENCES

Aveskamp MM, Gruyter J de, Woudenberg JHC, Verkley GJM, Crous PW. 2010. Highlights of the Didymellaceae: A polyphasic approach to characterise Phoma and related pleosporalean genera. Studies in Mycology 65: 1–60.

Aveskamp MM, Verkley GJM, Gruyter J de, Murace MA, Perelló A, et al. 2009. DNA phylogeny reveals polyphyly of Phoma section Peyronellea and multiple taxonomic novelties. Mycologia 101: 363–382.

Baker RH, DeSalle R. 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Systematic Biology 46: 654–673.

Baker RH, Yu XB, DeSalle R. 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Molecular Phylogenetics and Evolution 9: 427–436.

Balajee SA, Sigler L, Brandt ME. 2007. DNA and the classical way: Identification of medically important molds in the 21st century. Medical Mycology 45: 475–490.

Bestagno Biga ML, Ciferri R, Bestagno G. 1958. Ordinamento artificiale delle specie del genere Coniothyrium Corda. Sydowia, Annales Mycologici ser. II, 12: 258–320.

Boerema GH, Gruyter J de, Noordeloos ME, Hamers MEC. 2004. Phoma identification manual. Differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, United Kingdom.

Budziszewska J, Szypula W, Wilk M, Wrzosek M. 2011. Paraconiothyrium babiogorense sp. nov., a new endophyte from fir club moss Huperzia selago (L.) Bernh. ex Schrank & Mart. (Huperziaceae). Mycotaxon 115: 457–468.

Câmara MPS, Palm ME, Berkum P van, Stewart EL. 2001. Systematics of Paraphaeosphaeria: a molecular and morphological approach. Mycological Research 105: 41–56.

Câmara MPS, Ramaley AW, Castlebury LA, Palm ME. 2003. Neophaeosphaeria and Phaeosphaeriopsis, segregates of Paraphaeosphaeria. Mycological Research 107: 516–522.

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556.

Carisse O, Bernier J. 2002a. Effect of environmental factors on growth, pycnidial production and spore germination of Microsphaeropsis isolates with biocontrol potential against apple scab. Mycological Research 106: 1455–1462.

Carisse O, Bernier J. 2002b. Microsphaeropsis ochracea sp. nov. associated with dead apple leaves. Mycologia 94: 297–301.

Carisse O, El-Bassam S, Benhamou N. 2001. Effect of Microsphaeropsis sp. strain P130A on germination and production of sclerotia of Rhizoctonia solani and interaction between the antagonist and the pathogen. Phytopathology 91: 782–791.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

Crous PW, Groenewald JZ. 2006. Microdiplodia hawaiiensis. Fungal Planet, no. 7. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, et al. 2011. Fungal pathogens of Proteaceae. Persoonia 27: 20–45.

Crous PW, Verkley GJM, Christensen M, Castañeda-Ruiz RF, Groenewald JZ. 2012. How important are conidial appendages? Persoonia 28: 126–137.

Crous PW, Verkley GJM, Groenewald JZ, Samson RA. 2009. Fungal biodiversity. CBS Laboratory Manuals Series 1. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Damm U, Verkley GJM, Crous PW, Fourie PH, Haegi A, Riccioni L. 2008. Novel Paraconiothyrium species on stone fruit trees and other woody hosts. Persoonia 20: 9–17.

Domsch KH, Gams W, Anderson T-H. 2007. Compendium of soil fungi. Second edition. IHW Verlag, Eching.

El-Bassam S, Benhamou N, Carisse O. 2002. The role of melanin in the antagonistic interaction between the apple scab pathogen Venturia inaequalis and Microsphaeropsis ochracea. Canadian Journal of Microbiology 48: 349–358.

Farris J. 1972. Estimating phylogenetic trees from distance matrices. The American Naturalist 106: 645–667.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17: 368–376.

Fitch WM. 1971. Towards defining the course of evolution: minimal change for a specified tree topology. Systematic Zoology 20: 406–416.

Göker M, García-Blázquez G, Voglmayr H, Tellería MT, Martín MP. 2009a. Molecular taxonomy of phytopathogenic fungi: a case study in Peronospora. PLoS ONE 4: e6319.

Göker M, Voglmayr H, García-Blázquez G, Oberwinkler F. 2009b. Species delimitation in downy mildews: the case of Hyaloperonospora in the light of nuclear ribosomal internal transcribed spacer and large subunit sequences. Mycological Research 113: 308–325.

Gordon RA, Sutton DA, Thompson EH, Shrikanth V, Verkley GJM, et al. 2012. Cutaneous phaeohyphomycosis caused by Paraconiothyrium cyclothyrioides. Journal of Clinical Microbiology 50: 3795–3798.

Gruyter J de, Aveskamp MM, Woudenberg JHC, Verkley GJM, Groenewald JZ, Crous PW. 2009. Molecular phylogeny of Phoma and allied anamorph genera: towards a reclassification of the Phoma complex. Mycological Research 113: 508–519.

Gruyter J de, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW. 2010. Systematic reappraisal of species in Phoma section Paraphoma, Pyrenochaeta and Pleurophoma. Mycologia 102: 1066–1081.

Gruyter J de, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW. 2012, '2013'. Redisposition of Phoma-like anamorphs in Pleosporales. Studies in Mycology 75: 1–36.

Hess PN, Moraes Russo CA de. 2007. An empirical test of the midpoint rooting method. Biological Journal of the Linnean Society 92: 669–674.

Hoffman MT, Arnold AE. 2008. Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycological Research 112: 331–344.

Ivanova NV, deWaard J, Hebert PDN. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6: 998–1002.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.

Nag Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Waterloo, Ontario.

Pattengale ND, Alipour M, Bininda–Emonds ORP, Moret BME, Stamatakis A. 2009. How many bootstrap replicates are necessary? Lecture Notes in Computer Science 5541: 184–200.

Quaedvlieg W, Verkley GJM, Shin H-D, Barreto RW, Alfenas AC, et al. 2013. Sizing up Septoria. Studies in Mycology 75: 307–390.

Rayner RW. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew, UK.

Riccioni L, Manning M, Valvassori M, Haegi A, Casanato S, Spinelli R. 2007. A new disease: leader die-back in Actinidia chinensis Hort16A in Italy. Acta Horticulturae 753: 669–676.

Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, et al. 2009. A class-wide phylogenetic assessment of Dothideomycetes. Studies in Mycology 64: 1–15.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences 109: 6241–6246.

Silva M da, Cerniglia CE, Pothuluri JV, Canhos VP, Esposito E. 2003a. Screening filamentous fungi isolated from estuarine sediments for the ability to oxidize polycyclic aromatic hydrocarbons. World Journal of Microbiology and Biotechnology 19: 399–405.

Silva M da, Umbuzeiro GA, Pfenning LH, Canhos VP, Esposito E. 2003b. Filamentous fungi isolated from estuarine sediments contaminated with industrial discharges. Soil and Sediment Contamination 12: 345–356.

Someya A, Yaguchi T, Udagawa S-I. 1997. Microsphaeropsis rugospora, a new species from Japanese soil. Mycoscience 38: 429–431.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 75: 758–771.

Stielow B, Bratek Z, Orczán KA, Rudnoy S, Hensel G, et al. 2011. Species delimitation in taxonomically difficult fungi: the case of Hymenogaster. PLoS ONE 6: e15614.

Stielow B, Bubner B, Hensel G, Munzenberger B, Hoffmann P, et al. 2010. The neglected hypogeous fungus Hydnotrya bailii Soehner (1959) is a widespread sister taxon of Hydnotrya tulasnei (Berk.) Berk. & Broome (1846). Mycological Progress 9: 195–203.

Sutton BC. 1974. Miscellaneous coelomycetes on Eucalyptus. Nova Hedwigia 25: 161–172.

Sutton BC. 1980. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. CMI, Kew, UK.

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4. Sinauer Associates, Sunderland, Massachusetts.

Vaas LAI, Sikorski J, Michael V, Göker M, Klenk H-P. 2012. Visualization and curve-parameter estimation strategies for efficient exploration of Phenotype Microarray kinetics. PLoS ONE 7: e34846.

Verkley GJM, Quaedvlieg W, Shin HD, Crous PW. 2013. A new approach to species delimitation in Septoria. Studies in Mycology 75: 213–305.

Verkley GJM, Silva M da, Wicklow DT, Crous PW. 2004. Paraconiothyrium, a new genus to accommodate the mycoparasite Coniothyrium minitans, anamorphs of Paraphaeosphaeria, and four new species. Studies in Mycology 50: 323–335.

Vu TD, Eberhardt U, Szöke S, Groenewald M, Robert V. 2012. A laboratory information management system for DNA barcoding workflows. Integrative Biology 4: 744–755.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR Protocols: a guide to methods and applications: 315–322. Academic Press, New York, USA.

Wollenweber HW, Hochapfel H. 1937. Beitrage zur Kenntnis parasitarer und saprophtischer Pilze. IV. Z. Parasitenkunde 9: 600–638.

Zhang Y, Crous PW, Schoch CL, Hyde KD. 2012. Pleosporales. Fungal Diversity 53: 1–221.

Zhang Y, Schoch CL, Fournier J, Crous PW, Gruyter J de, et al. 2009. Multilocus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Studies in Mycology 64: 85–102.