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RESEARCH ARTICLE

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Phytopythium: molecular phylogeny and systematics

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Key words

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Abstract The genus *Phytopythium* (*Peronosporales*) has been described, but a complete circumscription has not yet been presented. In the present paper we provide molecular-based evidence that members of *Pythium* clade K as described by Lévesque & de Cock (2004) belong to *Phytopythium*. Maximum likelihood and Bayesian phylogenetic analysis of the nuclear ribosomal DNA (LSU and SSU) and mitochondrial DNA cytochrome oxidase subunit 1 (COI) as well as statistical analyses of pairwise distances strongly support the status of *Phytopythium* as a separate phylogenetic entity. *Phytopythium* is morphologically intermediate between the genera *Phytophthora* and *Pythium*. It is unique in having papillate, internally proliferating sporangia and cylindrical or lobate antheridia. The formal transfer of clade K species to *Phytopythium* and a comparison with morphologically similar species of the genera *Pythium* and *Phytophthora* is presented. A new species is described, *Phytopythium mirpurense*.

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INTRODUCTION

The genus *Pythium* as defined by Pringsheim in 1858 was divided by Lévesque & de Cock (2004) into 11 clades based on molecular systematic analyses. These clades are generally well supported by morphological features. In particular, *Pythium* species belonging to clade K were observed to be phylogenetically distinct from the rest of the *Pythium* spp. and showed combined features of both *Pythium* and *Phytophthora*. The unique phylogenetic placement of species belonging to clade K has been recognised since the beginning of sequence-based phylogenetics. Briard et al. (1995) and Cooke et al. (2000) showed that *Pythium vexans* was clearly different from other *Pythium* spp. and *Phytophthora* using the ribosomal large subunit (LSU) and internal transcribed spacer (ITS), respectively. In a multigene study, Villa et al. (2006) showed that *Pythium* species belonging to clade K were closely related to *Phytophthora*. The uniqueness of this clade was also supported by Bedard et al. (2006) by analysis of the organisation of the 5S gene family. In species in clade K, the 5S rRNA genes were predominantly linked to the rDNA repeat mostly in tandem arrays in the same orientation as the rRNA genes.

Phytopythium is a new genus in the family *Pythiaceae*, order *Peronosporales* that was described with *Phytopythium sindhum* as the type species by Bala et al. (2010b). They showed that *Phytopythium sindhum* is a member of clade K. Uzuhashi et al. (2010) divided *Pythium* into five new genera and assigned the name *Ovatisporangium* to the members of clade K, this name, however, is a later synonym of *Phytopythium*. *Phytopythium* therefore has priority. The objective of the present study is to establish which species belong to clade K and to make new taxonomic combinations for these species. To achieve this goal, phylogenies based on nuclear LSU rRNA (28S), SSU rRNA (18S) and mitochondrial DNA cytochrome oxidase1 (COI) as well as statistical analyses of the pairwise distances from these datasets were prepared with an extensive coverage of the oomycetes containing almost all *Pythium* and *Phytophthora* species available in culture. The ITS gene region was also used to ascertain the position of all possible species in clade K but not for phylogeny since it is too variable to align sequences between *Pythium* and *Phytophthora*. Diagnostic morphological features of the group are also presented and discussed.

MATERIALS AND METHODS

Morphological studies

The strains used for the phylogenetic study were morphologically examined to verify their identity and to find the characteristic features of the group. The methods used for cultivation of the strains for study of morphology and zoospore development are the same as described by de Cock & Lévesque (2004).

DNA extraction, amplification and sequencing

Almost 300 strains of *Pythium*, *Phytopythium*, *Phytophthora*, *Halophytophthora* and *Albugo* were used in this study (Table 1). DNA was extracted using the protocols as described in Bala et al. (2010a). PCR amplifications for the rDNA LSU and ITS1-5.8S-ITS2 regions and mitochondrial DNA COI were done using the protocols and primer sequences as provided in Robideau et al. (2011). The SSU region was amplified using forward primer NS1 (5’-TAGTCATATGCTTGTCTC-3’) (White et al. 1990) and reverse primer OomLo5.8S47B (3’-CGCATTACG-TATCGCAGTTCGCAG-5’) (Mazzola et al. 2002), with an initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 45 s, elongation at 72 °C for 2 min and final elongation at 72 °C for 8 min. Sequencing primers used for the SSU region were NS1, NS2 (5’-GGCT-GCTGGCACCAGACTTGC3’), NS3 (5’-GCAAGTCTGGTGC-CAGCAGCC), NS4 (5’-CTTCCGTCAATTCCTTTAAG3’), NS5

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Table 1 Species and isolates included in the study, showing GenBank accession numbers for each gene.

Species	Strain Number	Clade	GenBank Accessions					
			SSU_ITS_28S	SSU_ITS	SSU	COI	LSU	ITS
Albugo candida	AC2V		–	–	–	HQ708184	HQ665049	–
	AC7A		–	–	HQ643110	HQ708183	HQ665050	–
	ACCS		–	–	KF853245	–	–	–
Halophytophthora avicenniae	CBS188.85	Halophytophthora	–	–	–	HQ708219	HQ665146	–
Halophytophthora operculata	CBS241.83	Phytopythium	–	–	GU994173	KF853238	KJ128038	KJ128038
Halophytophthora polymorphica	CBS680.84	Halophytophthora	–	–	–	–	HQ665288	–
Phytophthora alni	P10564	Clade 7	–	–	JN635200	–	–	–
Phytophthora alticola	P16053	Clade 4	–	–	JN635264	–	–	–
Phytophthora andina	P13660	Clade 1	–	–	JN635253	–	–	–
Phytophthora arecae	CBS305.62	Clade 4	–	–	–	HQ708218	HQ665200	–
Phytophthora austrocedrae	P16040	Clade 8	–	–	JN635271	–	–	–
Phytophthora batemanensis	CBS679.84	Halophytophthora	–	–	–	HQ708220	HQ665286	–
Phytophthora bisheria	P10117	Clade 2	–	–	–	–	EU080746	–
	P11311	Clade 2	–	–	JN635246	HQ261249	–	–
Phytophthora boehmeriae	CBS291.29	Clade 10	–	–	–	HQ708221	HQ665190	–
	P1257	Clade 10	–	–	JN635228	–	–	–
	P6950	Clade 10	–	–	–	–	EU080166	–
Phytophthora botryosa	P1044	Clade 2	–	–	JN635168	–	–	–
Phytophthora brassicae	CBS178.87	Clade 8	–	–	–	HQ708225	HQ665144	–
	P10155	Clade 8	–	–	JN635172	–	–	–
	P3273	Clade 8	–	–	JN635066	–	–	–
Phytophthora cactorum	CBS108.09	Clade 1	–	–	–	KJ128035	KJ128036	–
	P0714	Clade 1	–	–	JN635210	–	–	–
	P10365	Clade 1	–	–	JN635194	–	–	–
Phytophthora capsici	CBS554.88	Clade 2	–	–	–	HQ708250	HQ665266	–
	P6522	Clade 2	–	–	JN635061	–	–	–
Phytophthora captiosa	P10719	Clade 9	–	–	JN635227	–	–	–
	P10720	Clade 9	–	–	JN635229	–	–	–
Phytophthora cinnamomi	CBS144.22	Clade 7	–	–	–	HQ708257	HQ665126	–
Phytophthora cinnamomi var. *parvispora*	CBS411.96	Clade 7	–	–	–	HQ708268	HQ665231	–
Phytophthora cinnamomi var. *robiniae*	P16351	Clade 7	–	–	JN635269	–	–	–
Phytophthora citricola	CBS221.88	Clade 2	–	–	–	HQ708269	HQ665161	–
Phytophthora citrophthora	CBS950.87	Clade 2	–	–	–	HQ708272	HQ665305	–
	P1212	Clade 2	–	–	JN635223	–	–	–
Phytophthora clandestina	P3942	Clade 1	–	–	JN635111	–	–	–
Phytophthora colocasiae	P6102	Clade 2	–	–	JN635058	–	–	–
Phytophthora cryptogea	P16165	Clade 8	–	–	JN635259	–	–	–
	CBS468.81	Clade 8	–	–	–	HQ708276	HQ665238	–
Phytophthora drechsleri	P10331	Clade 8	–	–	–	–	EU079511	–
	P1087	Clade 8	–	–	–	HQ261299	–	–
	P1087	Clade 8	–	–	JN635260	–	–	–
Phytophthora erythroseptica	CBS129.23	Clade 8	–	–	–	HQ708286	HQ665121	–
	P1693	Clade 8	–	–	JN635249	–	–	–
Phytophthora europaea	P10324	Clade 7	–	–	JN635189	–	–	–
Phytophthora fallax	P10722	Clade 9	–	–	JN635219	–	–	–
Phytophthora foliorum	P10969	Clade 8	–	–	–	HQ261307	EU079704	–
Phytophthora fragariae	CBS209.46	Clade 7	–	–	–	HQ708294	HQ665150	–
	P1435	Clade 7	–	–	JN635233	–	–	–
Phytophthora frigida	P16051	Clade 2	–	–	JN635162	–	–	–
Phytophthora gonapodyides	CBS363.79	Clade 6	–	–	–	–	HQ665216	–
	CBS554.67	Clade 6	–	–	–	HQ708297	HQ665265	–
	P10337	Clade 6	–	–	JN635201	–	–	–
	P3700	Clade 6	–	–	JN635141	–	–	–
Phytophthora hedraiandra	CBS118732	Clade 1	–	–	–	HQ708300	–	–
	PDA331	Clade 1	–	–	–	–	EU080880	–
Phytophthora heveae	CBS296.29	Clade 5	–	–	–	HQ708301	HQ665194	–
	P10167	Clade 5	–	–	JN635090	–	–	–
Phytophthora hibernalis	P3822	Clade 8	–	–	JN635091	–	–	–
Phytophthora himalayensis	CBS357.59	Clade 8	–	–	–	–	HQ665215	–
Phytophthora humicola	CBS200.81	Clade 6	–	–	–	–	HQ665148	–
	P3826	Clade 6	–	–	JN635108	–	–	–
Phytophthora idaei	P6767	Clade 1	–	–	JN635116	–	–	–
Phytophthora ilicis	P3939	Clade 3	–	–	JN635092	–	–	–
Phytophthora infestans	CBS366.51	Clade 1	–	–	–	HQ708309	HQ665217	HQ643247
Phytophthora insolita	P6703	Clade 9	–	–	JN635140	–	–	–
Phytophthora inundata	CBS215.85	Clade 6	–	–	–	HQ708311	HQ665154	–
	P8478	Clade 6	–	–	JN635083	–	EU079946	–
Phytophthora ipomoeae	P10225	Clade 1	–	–	JN635181	–	–	–
Phytophthora iranica	CBS374.72	Clade 1	–	–	–	HQ708314	HQ665219	–
Phytophthora katsurae	CBS587.85	Clade 5	–	–	–	HQ708315	HQ665278	–
	P10187	Clade 5	–	–	JN635173	–	–	–
Phytophthora kelmania	P10613	Clade 8	–	–	JN635103	–	–	–
Phytophthora kernoviae	P10958	Clade 10	–	–	–	HQ261349	EU080057	–
	P10958	Clade 10	–	–	JN635237	–	–	–
Phytophthora lateralis	CBS168.42	Clade 8	–	–	–	–	KJ128037	–
	Lev1213	Clade 8	–	–	–	HQ708320	–	–
Phytophthora macrochlamydospora	P1026	Clade 9	–	–	JN635190	–	–	–
Phytophthora meadii	CBS219.88	Clade 2	–	–	–	HQ708324	HQ665159	–
Phytophthora medicaginis	P7029	Clade 8	–	–	JN635096	–	–	–
Phytophthora megakarya	P1672	Clade 4	–	–	–	HQ261357	–	–
	P1672	Clade 4	–	–	JN635250	–	–	–
	P8516	Clade 4	–	–	–	–	EU079974	–
Phytophthora megasperma	CBS402.72	Clade 6	–	–	–	HQ708329	HQ665228	–

Table 1 (cont.)

Species	Strain Number	Clade	GenBank Accessions					
			SSU_ITS_28S	SSU_ITS	SSU	COI	LSU	ITS
Phytophthora megasperma	P10340	Clade 6	–	–	JN635176	–	–	–
Phytophthora melonis	CBS582.69	Clade 7	–	–	–	HQ708336	HQ665274	–
	P3609	Clade 7	–	–	JN635049	–	–	–
Phytophthora mengei	P10139	Clade 2	–	–	JN635038	–	–	–
Phytophthora mirabilis	CBS678.85	Clade 1	–	–	–	HQ708339	HQ665285	–
	P10231	Clade 1	–	–	JN635179	–	–	–
Phytophthora multivesiculata	CBS545.96	Clade 2	–	–	–	HQ708340	HQ665257	–
Phytophthora multivora	P1233	Clade 2	–	–	JN635155	–	–	–
Phytophthora nemorosa	P10288	Clade 3	–	–	JN635183	–	–	–
Phytophthora nicotianae	CBS303.29	Clade 1	–	–	–	HQ708352	–	–
	P10297	Clade 1	–	–	JN635184	–	–	–
	P7146	Clade 1	–	–	–	–	EU079560	–
Phytophthora palmivora	CBS298.29	Clade 4	–	–	–	HQ708357	HQ665195	–
	P0113	Clade 4	–	–	JN635188	–	–	–
	P0255	Clade 4	–	–	JN635186	HQ261382	EU080343	–
Phytophthora parsiana	P21281	Clade 9	–	–	JN635161	–	–	–
	P21282	Clade 9	–	–	JN635160	HQ261384	–	–
Phytophthora phaseoli	CBS556.88	Clade 1	–	–	–	HQ708359	HQ665267	–
	P10145	Clade 1	–	–	JN635167	–	–	–
Phytophthora pinifolia	P16100	Clade 6	–	–	–	HQ261390	–	–
	P16100	Clade 6	–	–	JN635272	–	–	–
Phytophthora polonica	P15004	Clade 9	–	–	–	HQ261394	EU080268	–
	P15005	Clade 9	–	–	JN635240	–	–	–
Phytophthora porri	CBS567.86	Clade 8	–	–	–	HQ708368	HQ665271	–
	P10728	Clade 8	–	–	JN635236	–	–	–
Phytophthora primulae	P10220	Clade 8	–	–	JN635180	–	–	–
	P10333	Clade 8	–	–	JN635187	HQ261397	EU080403	–
Phytophthora pseudosyringae	P10443	Clade 3	–	–	–	–	EU080026	–
	P16355	Clade 3	–	–	JN635257	HQ261399	–	–
Phytophthora pseudotsugae	CBS444.84	Clade 1	–	–	–	HQ708381	HQ665234	–
	P10218	Clade 1	–	–	JN635207	–	–	–
Phytophthora quercetorum	P15555	Clade 4	–	–	–	HQ261404	–	–
	PD01105	Clade 4	–	–	–	–	EU080905	–
Phytophthora quercina	P10334	Clade 4	–	–	JN635198	–	–	–
Phytophthora quininea	CBS407.48	Clade 9	–	–	–	HQ708386	HQ665230	–
	P3247	Clade 9	–	–	JN635110	–	–	–
Phytophthora ramorum	CBS101553	Clade 8	–	–	–	HQ708387	HQ665053	–
	P10301	Clade 8	–	–	JN635185	–	–	–
Phytophthora richardiae	P3876	Clade 8	–	–	JN635045	–	–	–
Phytophthora rosacearum	P8048	Clade 6	–	–	JN635062	–	–	–
	P8049	Clade 6	–	–	JN635057	–	–	–
Phytophthora rubi	CBS967.95	Clade 7	–	–	–	–	HQ665306	–
Phytophthora sansomea	P3163	Clade 8	–	–	JN635047	–	–	–
Phytophthora sinensis	CBS557.88	Clade 7	–	–	–	–	HQ665269	–
Phytophthora siskiyouensis	P15122	Clade 2	–	–	–	HQ261421	HQ665311	–
	P15123	Clade 2	–	–	–	–	HQ665312	–
Phytophthora sojae	CBS382.61	Clade 7	–	–	–	–	HQ665224	–
Phytophthora sp aacrimae	P15880	Clade 6	–	–	JN635255	–	–	–
Phytophthora sp asparagi	P10707	Clade 6	–	–	JN635226	–	–	–
Phytophthora sp canalensis	P10456	Clade 6	–	–	JN635174	–	–	–
Phytophthora sp cuyabensis	P8213	Clade 9	–	–	JN635084	–	–	–
Phytophthora sp lagoriana	P8220	Clade 9	–	–	JN635085	–	–	–
Phytophthora sp napoensis	P8225	Clade 9	–	–	JN635082	–	–	–
Phytophthora sp niederhauserii	P10617	Clade 7	–	–	JN635212	–	EU080247	–
Phytophthora sp novaeguinee	P3389	Clade 5	–	–	JN635067	–	–	–
Phytophthora sp ohioensis	P16050	Clade 4	–	–	JN635265	–	–	–
Phytophthora sp personii	P11555	Clade 6	–	–	JN635134	–	–	–
Phytophthora sp sulawesiensis	P6306	Clade 6	–	–	JN635095	–	–	–
Phytophthora syringae	CBS132.23	Clade 8	–	–	–	HQ708404	HQ665123	–
	P10330	Clade 8	–	–	JN635193	–	–	–
Phytophthora tabaci	CBS305.29	Clade 1	–	–	–	HQ708411	HQ665198	–
Phytophthora tentaculata	CBS552.96	Clade 1	–	–	–	HQ708413	HQ665264	–
	P10363	Clade 1	–	–	JN635192	–	–	–
Phytophthora thermophilum	P1896	Clade 9	–	–	JN635117	–	–	–
Phytophthora trifolii	P1462	Clade 8	–	–	JN635065	–	–	–
Phytophthora tropicalis	CBS434.91	Clade 2	–	–	–	HQ708417	HQ665233	–
Phytophthora tropicalistype	P10329	Clade 2	–	–	JN635099	–	–	–
Phytophthora uliginosa	P10328	Clade 7	–	–	JN635175	–	–	–
	P10413	Clade 7	–	–	JN635202	–	–	–
Phytopythium boreale	CBS551.88	Phytopythium	AY598662	–	–	HQ708419	–	–
Phytopythium carbonicum	CBS112544	Phytopythium	HQ643373	–	–	HQ708420	–	–
Phytopythium chamaehyphon	CBS259.30	Phytopythium	AY598666	–	–	HQ708421	–	–
Phytopythium citrinum	CBS119171	Phytopythium	HQ643375	–	–	HQ708422	–	–
Phytopythium delawarense	OH382/ CBS123040	Phytopythium	KF853241	–	–	KF853240	–	EU339312
Phytopythium helicoides	CBS286.31	Phytopythium	AY598665	–	–	HQ708430	–	–
Phytopythium kandeliae	CBS113.91	Phytopythium	–	–	–	HQ708206	HQ665079	HQ643133
	ATCC66501/P11614	Phytopythium	–	–	GU994166	–	–	–
Phytopythium litorale	CBS118360	Phytopythium	HQ643386	–	–	HQ708433	–	–
	CBS122662	Phytopythium	–	–	–	–	HQ665114	HQ643385
Phytopythium mercuriale	A89 (GENBANK)	Phytopythium	–	–	–	–	–	JN630486
	CBS122443	Phytopythium	KF853243	–	–	KF853239	KF853236	–
Phytopythium mirpurense	CBS124523	Phytopythium	KJ831613	–	–	KJ831612	–	–
	CBS124524	Phytopythium	–	–	–	–	KJ831614	KJ831614

Table 1 (cont.)

Species	Strain Number	Clade	GenBank Accessions					
			SSU_ITS_28S	SSU_ITS	SSU	COI	LSU	ITS
Phytopythium montanum	CBS111349	Phytopythium	HQ643389	–	–	HQ708436	–	–
Phytopythium oedochilum	CBS292.37	Phytopythium	AY598664	–	–	HQ708439	–	–
Phytopythium ostracodes	CBS768.73	Phytopythium	AY598663	–	–	HQ708442	–	–
Phytopythium sindhum	CBS124518	Phytopythium	HQ643396	–	–	HQ708443	–	–
Phytopythium vexans	CBS119.80	Phytopythium	HQ643400	–	–	HQ708447	–	–
Pythium abappressorium	CBS110198	Clade F	HQ643408	–	–	HQ708455	–	–
Pythium acanthicum	CBS377.34	Clade D	AY598617	–	–	HQ708456	–	–
Pythium acanthophoron	CBS337.29	Clade J	AY598711	–	–	HQ708460	–	–
Pythium acrogynum	CBS549.88	Clade E	–	–	–	–	HQ665258	–
Pythium adhaerens	CBS520.74	Clade B	AY598619	–	–	HQ708462	–	–
Pythium amasculinum	CBS552.88	Clade D	AY598671	–	–	HQ708481	–	–
Pythium anandrum	CBS285.31	Clade H	AY598650	–	–	HQ708482	–	–
Pythium angustatum	CBS522.74	Clade B	AY598623	–	–	HQ708484	–	–
Pythium aphanidermatum	CBS118.80	Clade A	AY598622	–	–	HQ708485	–	–
Pythium apiculatum	CBS120945	Clade E	HQ643443	–	–	HQ708490	–	–
Pythium apleroticum	CBS772.81	Clade B	AY598631	–	–	HQ708491	–	–
Pythium aquatile	CBS215.80	Clade B	AY598632	–	–	–	HQ665153	–
Pythium aristosporum	CBS263.38	Clade B	AY598627	–	–	HQ708494	HQ665179	–
Pythium arrhenomanes	CBS324.62	Clade B	–	–	–	HQ708499	HQ665208	–
Pythium attrantheridium	DAOM230383	Clade F	–	–	–	HQ708524	HQ665308	–
	DAOM230386	Clade F	HQ643476	–	–	–	–	–
Pythium buismaniae	CBS288.31	Clade J	AY598659	–	–	–	HQ665188	–
Pythium camurandrum	CBS124096	Clade E	–	–	–	HQ708527	–	–
Pythium canariense	CBS112353	Clade G	–	–	–	HQ708528	HQ665069	–
Pythium capillosum	CBS222.94	Clade B	AY598635	–	–	HQ708529	HQ665164	–
Pythium carolinianum	CBS122659	Clade E	–	–	–	HQ708530	HQ665111	–
Pythium catenulatum	CBS842.68	Clade B	AY598675	–	–	HQ708540	HQ665302	–
Pythium chondricola	CBS203.85	Clade B	–	–	–	HQ708544	HQ665149	–
Pythium coloratum	CBS154.64	Clade B	AY598633	–	–	HQ708547	HQ665128	–
Pythium conidiophorum	CBS223.88	Clade B	AY598629	–	–	HQ708555	HQ665166	–
Pythium contiguanum	CBS221.94	Clade B	–	–	–	HQ708560	HQ665162	–
Pythium cryptoirregulare	CBS118731	Clade F	HQ643515	–	–	HQ708561	HQ665083	–
Pythium cylindrosporum	CBS218.94	Clade F	AY598643	–	–	HQ708562	HQ665157	–
Pythium cystogenes	CBS675.85	Clade J	HQ643518	–	–	HQ708564	HQ665284	–
Pythium debaryanum	CBS752.96	Clade F	AY598704	–	–	HQ708565	HQ665294	–
Pythium deliense	CBS314.33	Clade A	AY598674	–	–	HQ708568	HQ665204	–
Pythium diclinum	CBS664.79	Clade B	–	–	–	HQ708570	HQ665282	–
Pythium dimorphum	CBS406.72	Clade H	AY598651	–	–	HQ708571	HQ665229	–
Pythium dissimile	CBS155.64	Clade B	AY598681	–	–	HQ708572	HQ665130	–
Pythium dissotocum	CBS166.68	Clade B	AY598634	–	–	HQ708574	HQ665139	–
Pythium echinulatum	CBS281.64	Clade E	AY598639	–	–	HQ708577	HQ665183	–
Pythium emineosum	BR479	Clade F	–	–	–	GQ244423	–	–
Pythium erinaceus	CBS505.80	Clade E	–	–	–	HQ708578	HQ665243	–
Pythium flevoense	CBS234.72	Clade B	AY598691	–	–	HQ708580	HQ665170	–
	CBS278.81	Clade B	–	–	–	–	HQ665182	–
Pythium folliculosum	CBS220.94	Clade B	–	–	–	HQ708584	HQ665160	–
Pythium glomeratum	CBS120914	Clade I	HQ643543	–	–	–	HQ665091	–
Pythium graminicola	CBS327.62	Clade B	AY598625	–	–	HQ708589	HQ665211	–
Pythium grandisporangium	CBS286.79	Clade C	AY598692	–	–	HQ708590	HQ665187	–
Pythium helicandrum	CBS393.54	Clade H	AY598653	–	–	HQ708592	HQ665225	–
Pythium heterothallicum	CBS450.67	Clade I	AY598654	–	–	HQ708597	HQ665235	–
Pythium hydnosporum	CBS253.60	Clade D	AY598672	–	–	HQ708608	HQ665175	–
Pythium hypogynum	CBS234.94	Clade E	AY598693	–	–	HQ708609	HQ665171	–
Pythium inflatum	CBS168.68	Clade B	AY598626	–	–	HQ708610	HQ665140	–
Pythium insidiosum	ATCC 58643	Clade C	AF289981	–	–	–	–	–
	CBS574.85	Clade C	–	–	–	HQ708614	HQ665273	–
Pythium intermedium	CBS266.38	Clade F	AY598647	–	–	HQ708616	HQ665180	–
Pythium irregulare	CBS250.28	Clade F	AY598702	–	–	HQ708640	HQ665172	–
Pythium iwayamai	CBS156.64	Clade G	AY598648	–	–	HQ708713	HQ665131	–
Pythium kashmirense	ADC0819	Clade B	–	HQ643671	–	–	–	–
	CBS122908	Clade B	–	–	–	HQ708715	HQ665118	–
Pythium kunmingense	CBS550.88	Clade F	AY598647	–	–	–	HQ665259	–
Pythium longisporangium	CBS122646	Clade E	–	–	–	HQ708724	HQ665099	–
Pythium lucens	CBS113342	Clade F	HQ643681	–	–	HQ708725	HQ665077	–
Pythium lutarium	CBS222.88	Clade B	–	–	–	HQ643682	HQ665163	–
Pythium lycopersici	CBS122909	Clade D	–	–	–	HQ708727	HQ665119	–
Pythium macrosporum	CBS574.80	Clade F	AY598646	–	–	HQ708728	HQ665272	–
Pythium marsipium	CBS773.81	Clade E	–	–	–	HQ708734	HQ665297	–
Pythium mastophorum	CBS375.72	Clade J	AY598661	–	–	HQ708735	HQ665220	–
Pythium megacarpum	CBS112351	Phytopythium	–	–	–	–	–	HQ643388
Pythium middletonii	CBS528.74	Clade E	–	–	–	HQ708738	HQ665249	–
Pythium minus	CBS122657	Clade E	–	–	–	HQ708739	HQ665109	–
	CBS226.88	Clade E	AY598698	–	–	HQ643696	–	–
Pythium monospermum	CBS158.73	Clade A	HQ643697	–	–	HQ708741	HQ665137	–
Pythium multisporum	CBS470.50	Clade E	AY598641	–	–	HQ708744	HQ665239	–
Pythium myriotylum	CBS254.70	Clade B	AY598678	–	–	HQ708745	HQ665176	–
Pythium nagaii	CBS779.96	Clade G	AY598705	–	–	HQ708749	HQ665299	–
Pythium nodosum	CBS102274	Clade J	–	–	–	HQ708753	HQ665055	–
Pythium nunn	CBS808.96	Clade J	AY598709	–	–	HQ708755	HQ665300	–
Pythium okanoganense	CBS315.81	Clade G	AY598649	–	–	–	HQ665205	–
Pythium oligandrum	CBS382.34	Clade D	AY598618	–	–	HQ708759	HQ665223	–
Pythium oopapillum	BR632	Clade B	–	–	–	FJ655178	–	–
Pythium ornacarpum	CBS112350	Clade E	HQ643721	–	–	HQ708762	HQ665066	–

Table 1 (cont.)

			GenBank Accessions					
Species	Strain Number	Clade	SSU_ITS_28S	SSU_ITS	SSU	COI	LSU	ITS
Pythium ornamentatum	CBS122665	Clade D	–	–	–	HQ708763	HQ665117	–
Pythium orthogonon	CBS376.72	Clade J	–	–	–	HQ708764	HQ665221	–
Pythium pachycaule	CBS227.88	Clade B	–	–	–	HQ708765	HQ665169	–
Pythium paddicum	CBS698.83	Clade G	AY598707	–	–	HQ708769	HQ665290	–
Pythium paroecandrum	CBS157.64	Clade F	AY598644	–	–	–	HQ665133	–
Pythium parvum	CBS225.88	Clade E	AY598697	–	–	HQ708779	HQ665167	–
Pythium pectinolyticum	CBS122643	Clade B	HQ643739	–	–	HQ708780	HQ665096	–
Pythium periilum	CBS169.68	Clade B	–	–	–	HQ708781	HQ665141	–
Pythium periplocum	CBS289.31	Clade D	AY598670	–	–	HQ708784	HQ665189	–
Pythium perplexum	CBS674.85	Clade J	AY598658	–	–	HQ708785	HQ665283	–
Pythium pleroticum	CBS776.81	Clade E	AY598642	–	–	HQ708789	HQ665298	–
Pythium plurisporium	CBS100530	Clade B	AY598684	–	–	HQ708790	HQ665052	–
Pythium polymastum	CBS811.70	Clade J	AY598660	–	–	HQ708793	HQ665301	–
Pythium porphyrae	CBS369.79	Clade A	AY598673	–	–	HQ708794	HQ665218	–
Pythium prolatum	CBS845.68	Clade H	AY598652	–	–	HQ708795	HQ665303	–
Pythium pyrilobum	CBS158.64	Clade B	AY598636	–	–	HQ708796	HQ665136	–
Pythium radiosum	CBS217.94	Clade E	–	–	–	–	HQ665156	–
Pythium rhizooryzae	CBS119169	Clade B	HQ643757	–	–	HQ708798	HQ665087	–
Pythium rhizosaccharum	CBS112356	Clade E	–	–	–	HQ708801	HQ665072	–
Pythium rostratifingens	CBS115464	Clade E	HQ643761	–	–	HQ708802	HQ665080	–
Pythium rostratum	CBS533.74	Clade E	AY598696	–	–	HQ708808	HQ665252	–
Pythium salpingophorum	CBS471.50	Clade B	AY598630	–	–	HQ708809	HQ665240	–
Pythium scleroteichum	CBS294.37	Clade B	AY598680	–	–	HQ708812	HQ665192	–
Pythium segnitium	CBS112354	Clade E	HQ643772	–	–	HQ708813	HQ665070	–
Pythium senticosum	CBS122490	Clade H	HQ643773	–	–	HQ708814	HQ665093	–
Pythium sp balticum	CBS122649	Clade F	–	–	–	HQ708525	–	–
Pythium sp	CBS113341	Clade F	KF853244	–	–	–	–	–
Pythium sp CAL-2011a	CBS122647	Clade D	–	–	–	HQ708815	–	–
Pythium sp CAL-2011e	CBS122648	Clade E	–	–	–	HQ708770	HQ665101	–
Pythium sp CAL-2011f	CBS101876	Clade J	HQ643778	–	–	HQ708819	–	–
Pythium spiculum	CBS122645	Clade F	KF853242	–	–	–	HQ665098	–
Pythium spinosum	CBS275.67	Clade F	AY598701	–	–	HQ708834	HQ665181	–
Pythium splendens	CBS462.48	Clade I	AY598655	–	–	HQ708836	HQ665237	–
Pythium sterilum	B09	Phytopythium	–	–	–	–	–	EU240096
Pythium sukuiense	CBS110030	Clade B	–	–	–	HQ708877	HQ665059	–
Pythium sylvaticum	CBS453.67	Clade F	AY598645	–	–	HQ708886	HQ665236	–
Pythium takayamanum	CBS122491	Clade E	HQ643854	–	–	HQ708895	HQ665094	–
Pythium terrestris	CBS112352	Clade F	–	–	–	HQ708898	HQ665068	–
Pythium torulosum	CBS316.33	Clade B	AY598624	–	–	HQ708900	HQ665206	–
Pythium tracheiphilum	CBS323.65	Clade B	–	–	–	HQ708903	HQ665207	–
Pythium ultimum var. *sporangiiferum*	CBS219.65	Clade I	AKYB02045405	–	–	HQ708920	HQ665158	–
Pythium ultimum var. *ultimum*	CBS398.51	Clade I	AY598657	–	–	HQ708906	HQ665227	–
Pythium uncinulatum	CBS518.77	Clade J	AY598712	–	–	HQ708985	HQ665244	–
Pythium undulatum	CBS157.69	Clade H	AY598708	–	–	HQ708987	HQ665134	–
Pythium vanterpoolii	CBS295.37	Clade B	AY598685	–	–	HQ708993	HQ665193	–
Pythium viniferum	CBS119168	Clade F	HQ643956	–	–	HQ708997	HQ665086	–
Pythium violae	CBS132.37	Clade G	AY598717	–	–	–	–	–
	CBS159.64	Clade G	AY598706	–	–	HQ708999	HQ665138	–
Pythium volutum	CBS699.83	Clade B	AY598686	–	–	HQ709012	HQ665291	–
Pythium zingiberis	CBS216.82	Clade B	–		–	HQ709014	HQ665155	–

(5'-AACTTAAAGGAATTGACGGAAG3') and NS8 (5'-TCCGCA-GGTTCACCTACGGA3') (White et al. 1990) as well as Oom_Lo-5.8S47 (5'-ATTACGTATCGCAGTTCGCAG3') (Man in 't Veld et al. 2002) for full bidirectional coverage. Sequencing reactions were prepared using the Big Dye Terminator (BDT) v. 2 protocols (Applied Biosystems, Foster City, CA). Sequencing of the PCR product was performed in an Applied Biosystems Prism Genetic Analyzer model 3130XL.

Phylogenetic analyses

Sequences were edited manually using the DNAStar Lasergene 9 Suite (Bioinformatics Pioneer DNAStar, Inc., WI) or Geneious v. 6.1.6 (Biomatters http://www.geneious.com/). Multiple alignments of each gene region were generated using MAFFT (Katoh et al. 2005). The genera included in the phylogenetic analyses were *Albugo*, *Halophytophthora*, *Phytophthora*, *Phytopythium* and *Pythium*. Isolates of *Albugo candida* from the order *Albuginales* were included as an outgroup.

In order to include the maximum molecular data for clade K *Pythium* the invalid species *Pythium sterile* and *Pythium megacarpum* as well as two strains of the novel species *Phytopythium mirpurense* are considered in a cladogram generated based on ITS sequence data. *Pythium ultimum* from clade I and *Pythium dimorphum* from clade H are outgroups in these analyses and representatives of *Phytophthora*, *P. infestans*, *P. ramorum* and *P. sojae* are included. The aligned data matrix from 23 strains contained 1 096 characters from the ITS1, ITS2 and the 5.8S gene.

The aligned data matrices were assessed to find the best-fit model of nucleotide substitution using jMODELTEST (Posada 2008). In each case this was identified as General Time Reversible (GTR+I+G). Redundant sequences were identified and those with 100 % identity to other included taxa were removed from the analyses. These duplicates are catalogued in Table 2. The aligned data matrices contained 1 374 bp of D1–D3 regions of LSU with 176 strains, 1 724 bp of SSU rRNA with 159 strains and 680 bp of COI with 174 strains. The sequence alignments were subjected to maximum likelihood analysis using the GTR+I+G substitution model and the Best option for tree topology search with PhyML v. 3.0 (Guindon & Gascuel 2003) to obtain ML trees which were rooted to *Albugo* (LSU, COI and SSU) or *Pythium* (ITS). Nonparametric ML bootstraps were calculated with 1 000 bootstrap replicates. Bayesian inferences (BI) were generated using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) with Markov Chain Monte Carlo (MCMC) methodology to calculate posterior probabilities of the phylo-

Table 2 Species and isolates not included in the study for strains that were 100 % identical for certain genes and therefore not included in the phylogenetic analyses.

Sequence included in phylogeny				Identical sequences not included in phylogenies			
Species	Strain	Clade	GenBank	Species	Strain	Clade	GenBank
SSU							
Phytophthora alticola	P16053	Clade 4	JN635264	*Phytophthora frigida*	P16051	Clade 2	JN635162
Phytophthora asparagi	P10707	Clade 6	JN635226	*Phytophthora rosacearum*	P8048	Clade 6	JN635062
Phytophthora cactorum	P0714	Clade 1	JN635210	*Phytophthora cactorum*	P10365	Clade 1	JN635194
Phytophthora captiosa	P10719	Clade 9	JN635227	*Phytophthora captiosa*	P10720	Clade 9	JN635229
Phytophthora cryptogea	P16165	Clade 8	JN635259	*Phytophthora pseudosyringae*	P16355	Clade 3	JN635257
Phytophthora erythroseptica	P1693	Clade 8	JN635249	*Phytophthora gonapodyides*	P3700	Clade 6	JN635141
				Phytophthora richardiae	P3876	Clade 8	JN635045
				Phytophthora sansomea	P3163	Clade 8	JN635047
				Phytophthora trifolii	P1462	Clade 8	JN635065
Phytophthora europaea	P10324	Clade 7	JN635189	*Phytophthora uliginosa*	P10328	Clade 7	JN635175
				Phytophthora uliginosa	P10413	Clade 7	JN635202
Phytophthora lagoriana	P8220	Clade 9	JN635085	*Phytophthora lagoriana*	P8223	Clade 9	JN635086
				Phytophthora parsiana	P21282	Clade 9	JN635160
Phytophthora palmivora	P0113	Clade 4	JN635188	*Phytophthora palmivora*	P0255	Clade 4	JN635186
Phytophthora primulae	P10220	Clade 8	JN635180	*Phytophthora primulae*	P10333	Clade 8	JN635187
Pythium flevoense	CBS23472	Clade B	AY598691	*Pythium pectinolyticum*	CBS122643	Clade B	HQ643739
Pythium minus	CBS22688	Clade E	AY598698	*Pythium pleroticum*	CBS776.81	Clade E	AY598642
				Pythium parvum	CBS225.88	Clade E	AY598697
Pythium porphyrae	CBS36979	Clade A	AY598673	*Pythium adhaerens*	CBS520.74	Clade B	AY598619
Pythium salinum	CBS113341	Clade F	KF853244	*Pythium attrantheridium*	DAOM230386	Clade F	HQ643476
Pythium spinosum	CBS27567	Clade F	AY598701	*Pythium violae*	CBS132.37	Clade G	AY598717
				Pythium lucens	CBS113342	Clade F	HQ643681
				Pythium kunmingense	CBS55088	Clade F	AY598647
Pythium uncinulatum	CBS51877	Clade J	AY598712	*Pythium buismaniae*	CBS288.31	Clade J	AY598659
LSU							
Phytophthora arecae	CBS30562	Clade 4	HQ665200	*Phytophthora palmivora*	CBS29829	Clade 4	HQ665195
Phytophthora boehmeriae	CBS29129	Clade 10	HQ665190	*Phytophthora boehmeriae*	P6950	Clade 10	EU080166
Phytophthora brassicae	CBS17887	Clade 8	HQ665144	*Phytophthora brassicae*	CBS178.87	Clade 8	HQ665144
Phytophthora erythroseptica	CBS12923	Clade 8	HQ665121	*Phytophthora himalayensis*	CBS35759	Clade 8	HQ665215
Phytophthora fragariae	CBS20946	Clade 7	HQ665150	*Phytophthora rubi*	CBS96795	Clade 7	HQ665306
Phytophthora gonapodyides	CBS55467	Clade 6	HQ665265	*Phytophthora gonapodyides*	CBS36379	Clade 6	HQ665216
Phytophthora inundata	P8478	Clade 6	EU079946	*Phytophthora humicola*	CBS20081	Clade 6	HQ665148
				Phytophthora inundata	CBS21585	Clade 6	HQ665154
Phytophthora melonis	CBS58269	Clade 7	HQ665274	*Phytophthora sinensis*	CBS55788	Clade 7	HQ665269
Phytophthora sp "*niederhauserii*"	P10617	Clade 7	EU080247	*Phytophthora sojae*	CBS38261	Clade 7	HQ665224
Phytophthora siskiyouensis	P15123	Clade 2	HQ665312	*Phytophthora siskiyouensis*	P15122	Clade 2	HQ665311
Pythium amasculinum	CBS55288	Clade D	HQ665263	*Pythium lycopersicum*	CBS122909	Clade D	HQ665119
				Pythium oligandrum	CBS38234	Clade D	HQ665223
Pythium apleroticum	CBS77281	Clade B	HQ665296	*Pythium aquatile*	CBS21580	Clade B	HQ665153
Pythium buismaniae	CBS28831	Clade J	HQ665188	*Pythium polymastum*	CBS81170	Clade J	HQ665301
Pythium capillosum	CBS22294	Clade B	HQ665164	*Pythium flevoense*	CBS27881	Clade B	HQ665182
				Pythium flevoense	CBS23472	Clade B	HQ665170
Pythium catenulatum	CBS84268	Clade B	HQ665302	*Pythium rhizo-oryzae*	CBS119169	Clade B	HQ665087
Pythium viniferum	CBS119168	Clade F	HQ665086	*Pythium debaryanum*	CBS75296	Clade F	HQ665294
COI							
Phytophthora arecae	CBS30562	Clade 4	HQ708218	*Phytophthora palmivora*	CBS29829	Clade 4	HQ643307
Pythium amasculinum	CBS55288	Clade D	HQ708481	*Pythium lycopersicum*	CBS122909	Clade D	HQ643683
				Pythium ornamentatum	CBS122665	Clade D	HQ708763
Pythium conidiophorum	CBS22388	Clade B	HQ708555	*Pythium salpingophorum*	CBS47150	Clade B	HQ643768
Pythium debaryanum	CBS75296	Clade F	HQ708565	*Pythium viniferum*	CBS119168	Clade F	HQ643956
Pythium diclinum	CBS66479	Clade B	HQ708570	*Pythium lutarium*	CBS22288	Clade B	HQ643682
Pythium erinaceus	CBS50580	Clade E	HQ708578	*Pythium ornacarpum*	CBS112350	Clade E	HQ643721
Pythium folliculosum	CBS22094	Clade B	HQ708584	*Pythium torulosum*	CBS31633	Clade B	HQ643859
Pythium minus	CBS122657	Clade E	HQ708739	*Pythium pleroticum*	CBS77681	Clade E	HQ643748
Pythium myriotylum	CBS25470	Clade B	HQ708745	*Pythium zingiberis*	CBS21682	Clade B	HQ643973

genetic trees. The program was run for 20 M generations for the LSU, 40 M generations for the COI, 50 M generations for the SSU and 10 M for the ITS datasets with the GTR+I+G model of evolution for each gene. The first 25 % of the iterations were discarded as burn-in and every 1 000th iteration was sampled from the remainder. The trees were considered to be fully converged when the average standard deviation of split frequencies reached a level less than 0.01. FigTree v. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) was used to view and edit ML and Bayesian phylogenetic trees. Consensus trees were generated using the 50 % majority rule tree criteria and rooted to *Albugo* (LSU, COI and SSU) or *Pythium* (ITS).

Statistical analyses of pairwise distances

The alignments of COI, LSU and SSU used for phylogeny were also used to generate pairwise distance as was done for DNA barcode analyses (Robideau et al. 2011, Schoch et al. 2012). Statistical analyses and plots were performed with R (R Development Core Team, 2011). All pairwise distances involving a *Phytopythium* species against *Pythium* or *Phytophthora* were extracted, i.e. all pairwise distances involving any two *Phytopythium* species were excluded. An arcsine transformation of the distances was done to improve the variance homogeneity. ANOVA using 'lm' was done with markers (COI/LSU/SSU), genera (*Phytophthora*/*Pythium*) or clades (clade 1–10 and A–J) as variables. Plots were generated with ‘ggplot’ for R. The 0.05 confidence interval for 60 multiple comparisons was adjusted using the Bonferoni method. The average pairwise distance by marker was normalised to remove the bias from the difference in number of species between *Pythium* and *Phytophthora*.

Isolation and identification of Phytopythium mirpurense

Stagnant water was collected and immediately brought to the laboratory for the isolation of oomycetous fungi by the baiting technique of Harvey (1925). Grass blades, dicot leaves, hemp seeds, sesame seeds, lemon leaf and young cucumber stems were used as baits. Plates were incubated at room temperature, between 22–25 °C. Hyphae were observed on the baits after 5–8 days of incubation. The baits were rinsed in sterilised water to remove excess contaminants and transferred to fresh plates half-filled with sterile water. New fresh baits were then added and monitored daily for colonisation by oomycetes. After 2 d of incubation, the baits colonised by oomycetous fungi were transferred onto corn-meal agar (CMA) medium for purification by hyphal tip transfer. To obtain a pure culture a small disc of the CMA culture was placed into the centre of water agar plates. After 15–24 h growing apical hyphae were cut with the aid of a microscope in the laminar flow hood and transferred onto the surface of a fresh plate containing culture media.

For the assessment of cardinal temperatures, the isolates from this study were sub-cultured in two replicates on CMA in 90 mm Petri plates, and incubated at 10, 15, 20, 25, 30, 35 and 40 °C for 5 d. Radial growth was measured daily along

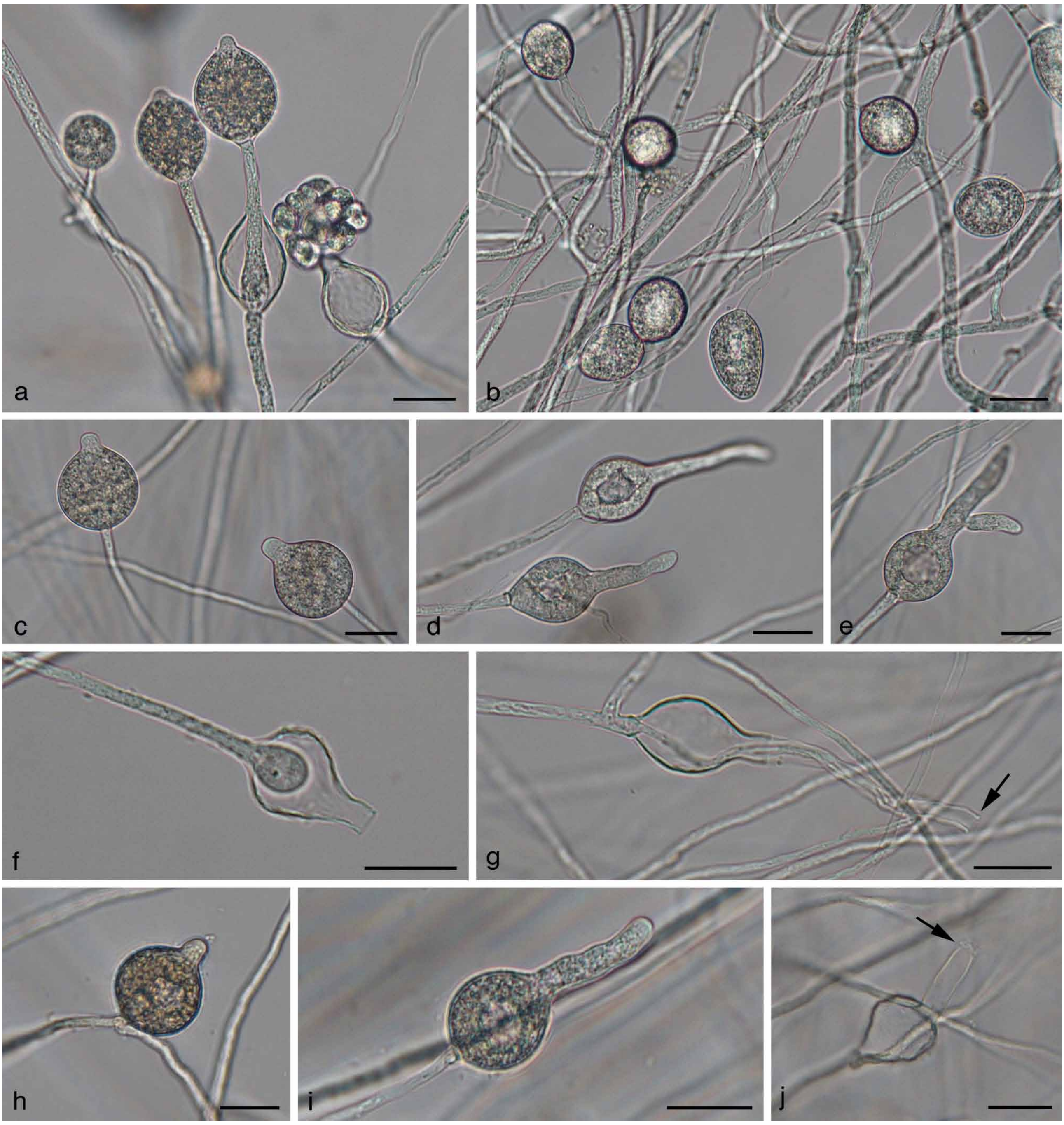

Fig. 1 Sporangia of *Phytopythium* species. a. *P. sindhum*, four stages of sporangium development showing a young, globose sporangium, a mature, papillate sporangium, internal proliferation and pythium-like zoospore development; b. *P. vexans*, subglobose, non-papillate sporangia; c–g. *P. citrinum*: c. normal sporangia; d. outgrowing papillae; e. outgrowing and branching papilla; f. empty sporangium with internal proliferation and short discharge tube; g. empty sporangium with internal proliferation and long discharge tube (arrow indicating tip); h–j. *P. helicandrum*: h. sessile, globose, papillate sporangium; i. outgrowing papilla; j. empty sporangium with intermediate sized discharge tube (arrow indicating tip). — Scale bars = 20 µm.

two lines intersecting the centre of the inoculum. Isolates were also grown on potato dextrose agar (PDA), potato carrot agar (PCA), CMA and corn meal dextrose agar (CMDA) in 90 mm Petri plates (recipes according to Crous et al. 2009), and colony characteristics were assessed after incubation for 5 d at 25 °C.

Water cultures for zoospore and sporangial production were prepared by adding an inoculum disc and a grass blade to sterile water in a Petri plate and incubating at 25 °C. Biometric values i.e aplerotic index, ooplast index and wall index were determined for 20 oogonia with the method described by Shahzad et al. (1992).

RESULTS AND DISCUSSION

Morphological comparison of Phytopythium with Phytophthora and Pythium

Most species in the genus *Phytopythium* produce papillate, internally proliferating sporangia (Fig. 1). The shape of the sporangia is more or less similar to the shape of papillate *Phytophthora* sporangia: (sub-)globose to ovoid and papillate (Fig. 1). However, in *Phytophthora* the papillate sporangium type never shows internal proliferation. The combination of internal proliferation and papillation (Fig. 1) is unique to sporangia of *Phytopythium* and some *Pythium* species (see below). Also, the papillae in *Phytopythium* are different from the papillae in *Phytophthora* sporangia. In *Phytopythium* the sporangia are initially non-papillate, and the papillae develop at maturity and do not consist of a hyaline 'apical thickening' as in *Phytophthora* (Blackwell 1949). They may grow out to form a shorter or larger discharge tube (Fig. 1d, f, g, i, j), which does not occur in *Phytophthora*. In some species the papilla is not the place where the plasma flows out, rather one or more discharge tubes are formed more basally of the sporangium. In some species the papilla grows out and develops branches (Fig. 1e). Another difference with *Phytophthora* is the zoospore discharge which is pythium-like in *Phytopythium*: the plasma flows out of the sporangium through a discharge tube to form a plasma-filled vesicle at the tip. Zoospores are developed outside the sporangium, within the vesicle membrane and are released after rupture of the membrane (Fig. 1a). According to Marano et al. (2014), *Phytopythium kandeliae* has zoospore release mostly like *Pythium* and occasionally in between *Pythium* and *Phytophthora*: zoospores developed (partly) inside a sporangium and partly in a vesicle.

Another unique characteristic of *Phytopythium* is the shape of the antheridium (Fig. 2). In most species the antheridia are elongate, cylindrical, often with constrictions. The fertilisation tube is mostly not apical but in 'navel position' (Fig. 2a–d, arrows). Occasionally club-shaped antheridia with apical attachment occur. In *P. vexans*, the antheridia are often very broadly attached to the oogonium and lobed (Fig. 2e, f).

Papillate sporangia with internal proliferation also occur in a small number of *Pythium* species: three members of clade E (*P. marsipium*, *P. middletonii*, *P. multisporum*), one member of clade G (*P. nagaii*) and clade C (*P. grandisporangium*) and all members of clade H (*P. anandrum*, *P. dimorphum*, *P. helicandrum*, *P. prolatum*, *P. undulatum*). However, none of these species except three has elongate, cylindrical or lobate antheridia. Only *P. helicandrum* has elongate antheridia, however, this species has ornamented oogonia and much bigger sporangia than any of the species in *Phytopythium*. *Pythium marsipium* has bell-shaped antheridia as they occur in *Phytopythium vexans*, however, its sporangia are utriform instead of ovoid. *Pythium grandisporangium* has lobate antheridia but this is a marine species with extremely large sporangia with a tapering neck rather than a distinct papilla.

Phylogenetic position of Phytopythium

Maximum likelihood analyses of nuclear (LSU and SSU) and mitochondrial DNA (COI) with Bayesian probability values mapped onto the trees are shown (Fig. 3A–C). These cladograms place all the strains belonging to the genus *Phytopythium* as a monophyletic group with bootstrap support (85–100 %) and high probabilities (0.99–1.00). Phylogenetic trees of the LSU

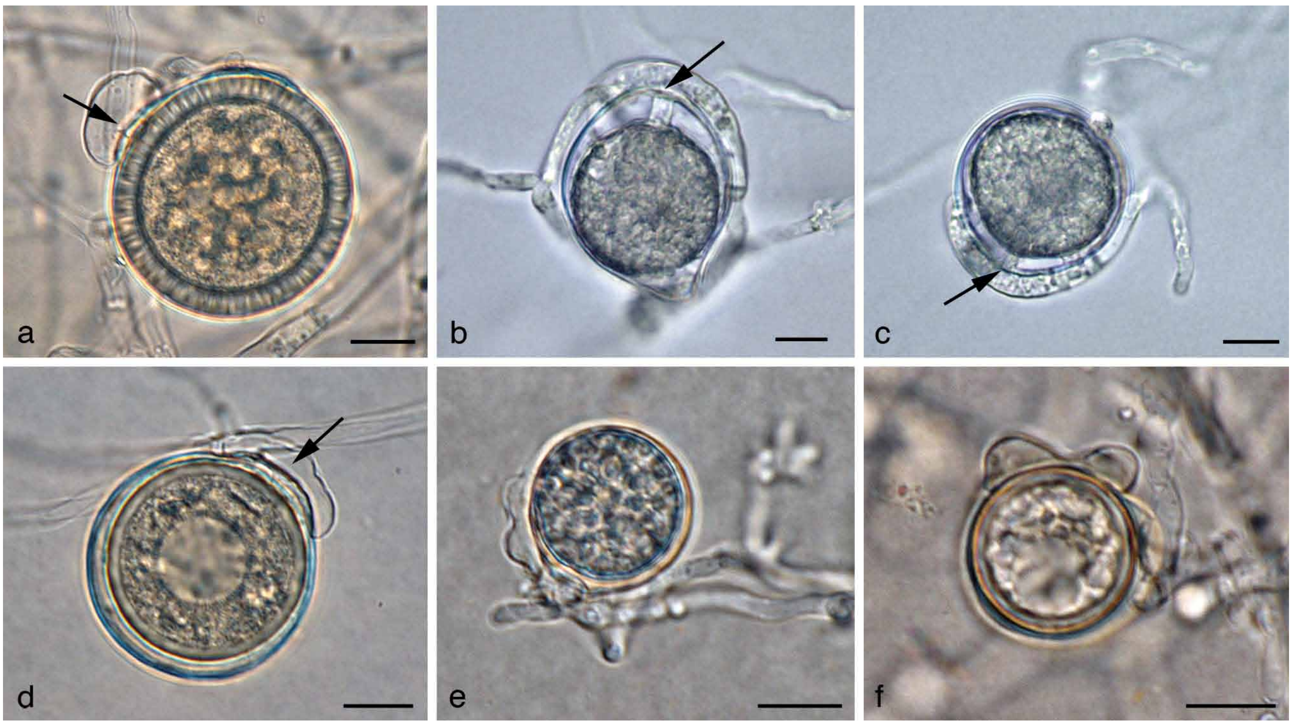

Fig. 2 Oogonia and antheridia of *Phytopythium* species. a. *P. sindhum*, slightly elongated antheridium; b–c. *P. oedochilum*, long cylindrical antheridia; d. *P. mirpurense*, elongate antheridium with slight constriction; e–f. *P. vexans*: e. elongate antheridium with distinct constrictions; f. antheridium with two lobes. Arrows indicate the fertilisation tube in navel position (a–d). — Scale bars = 10 μm.

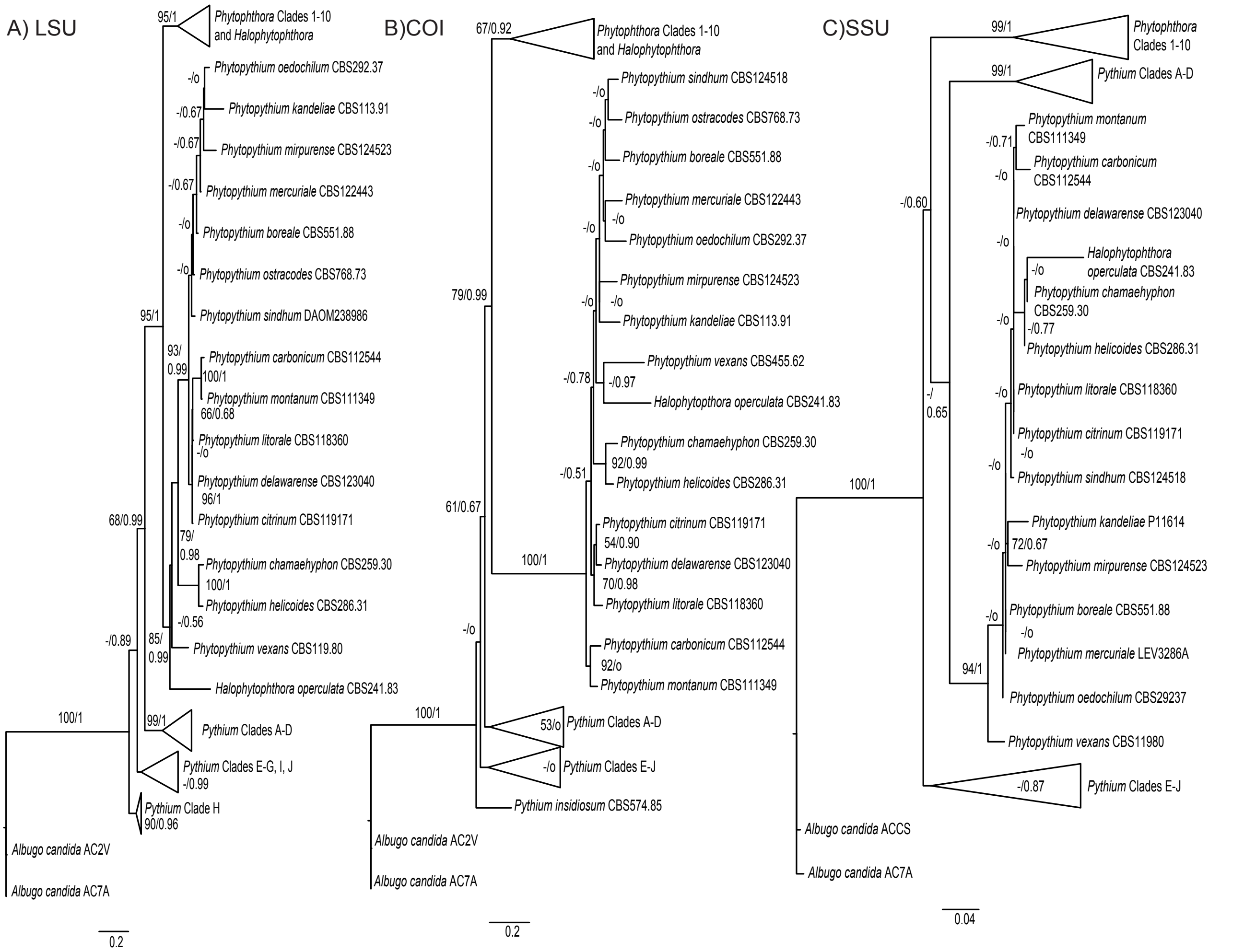

Fig. 3 Maximum likelihood phylogenetic trees of: a. LSU ribosomal RNA region; b. mitochondrial COI; c. SSU ribosomal RNA region. Maximum likelihood bootstrap support values larger than 50 % are indicated numerically, those under 50 % are marked with (–). Posterior probability values larger than 0.50 are labelled numerically, those under 0.50 are marked (–) on each branch, those clades which were not present in the Bayesian trees are marked as (o), the scale bars represent the average number of substitutions per site.

and COI regions support this group as intermediary between *Phytophthora* and *Pythium*. There is phylogenetic support with two of the genes to group *Phytopythium* with *Phytophthora* (95 % / 1.00 for LSU and 79 % / 0.99 for COI). The SSU tree has *Pythium* clades A–D as grouping closer to *Phytophthora* and *Halophytophthora*, with very low bootstrap support and probabilities (< 50 % / 0.65). This suggests that given the SSU dataset, the major clades are unresolved in relation to the outgroup.

Our results from phylogenetic analysis of nuclear (LSU and SSU) and mitochondrial (COI) genes with all available species of *Pythium* and *Phytophthora* support that *Phytopythium* is a distinct genus. Its placement as intermediate between *Pythium* and *Phytophthora* is supported by two of these datasets. In the three gene trees, this new genus clade was strongly supported by both ML bootstrap replicates and Bayesian probability values, which unambiguously confirmed the status of *Phytopythium* as a novel monophyletic genus. The maximum likelihood and Bayesian analyses did not clearly delineate the relationships between the different groups in the part of the oomycete evolutionary tree we focused on. Inclusion of some of the more basal groups such as the *Salisapiliaceae* (Hulvey et al. 2010) and additional markers in future analyses would likely lead to greater resolution of these relationships.

The ITS tree (Fig. 4) shows that the two strains of species *P. mirpurense* are both well embedded within *Phytopythium* with strong support (91 % / 0.96) and demonstrated the close relationships between *P. litorale* and *Pythium sterile* (100 / 1) as well as *Phytopythium boreale* and *Pythium megacarpum* (99 / 1).

Statistical analyses of pairwise distances

Markers, genera and clades as well as interactions between them all had a significant effect on pairwise distances of *Phytopythium* against *Pythium* and *Phytophthora* species ($p < 10^{-15}$). The average pairwise distance of all *Phytophthora* species against all *Phytopythium* species using COI was 13.7 % whereas it was 14.5 % for all *Pythium* species against all *Phytopythium*, showing that *Phytopythium* is significantly closer to *Phytophthora* than *Pythium* ($p < 10^{-16}$). For LSU, these differences were 10.4 % and 10.9 %, respectively, and were also significant ($p < 10^{-16}$). For SSU, the trend was reversed, still significant, with the average pairwise distance between *Pythium* and *Phytopythium* being 2.5 % whereas the average between *Phytophthora* and *Phytopythium* was 2.7 %. The clade effect was significant, including a significant interaction with markers; therefore, the results are presented by clades and markers in Fig. 5. Each clade is compared against *Phytopythium* to show clades that have a significant difference from the average pairwise distance. The significant trend of *Phytopythium* being closer to *Phytophthora* clades than *Pythium* clades can be seen with COI and LSU whereas it is more difficult to visualise the reverse trend in SSU. With all markers, *Pythium* clades H and I were significantly closer to *Phytopythium* than were the other *Pythium* clades but for SSU there were three additional clades (B, F and G) that were significantly closer to *Phytopythium* than were the other clades.

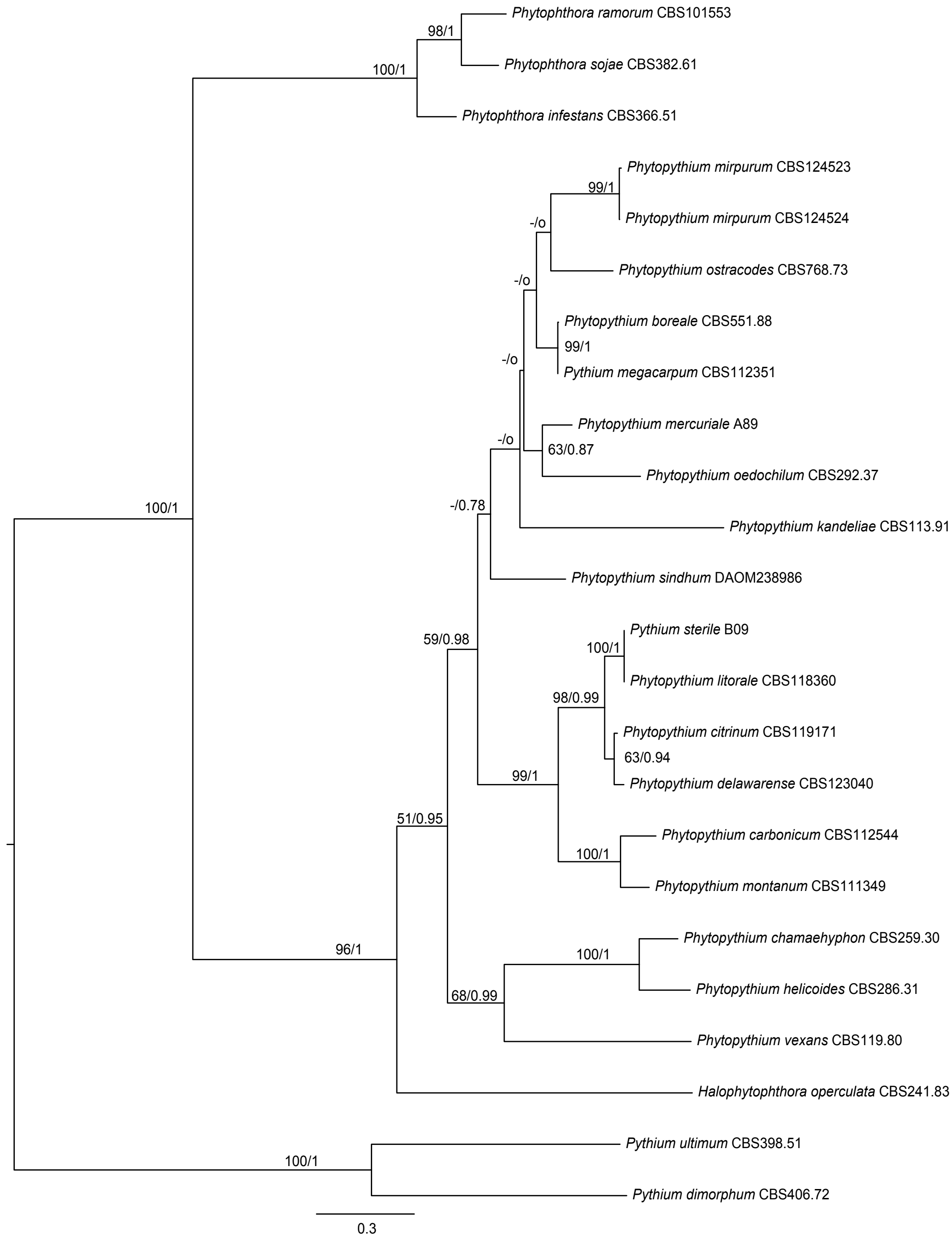

Fig. 4 Maximum likelihood tree of internal transcribed spacer (ITS) region of *Phytopythium* spp., *Pythium* spp. and *Phytophthora* spp. Maximum likelihood bootstrap support values larger than 50 % are indicated numerically, those under 50 % are marked with (–). Posterior probability values larger than 0.50 are labelled numerically, on each branch those clades which were not present in the Bayesian trees are marked as (o), the scale bars represent the average number of substitutions per site.

Strains used in circumscription of the genus

There are two invalid species that were investigated for the sake of examining the complete range of *Pythium* species from clade K, namely *Pythium megacarpum* and *P. sterile. Pythium megacarpum* is an invalid species because no type was indicated at the time of publication. Lévesque & de Cock (2004) placed it as potentially synonymous with *Phytopythium boreale* and in the barcode analyses of Robideau et al. (2011) these two species were only distinguishable through COI sequence data analysis, not by ITS. *Pythium sterile* is an invalid taxon based on the nomination of two herbarium specimens as the type of this species; this contravenes Art. 40.3 of the Melbourne convention (McNeill et al. 2012). *Pythium sterile* possesses identical ITS sequences to *Phytopythium litorale*. Other sequences from this organism could not be compared since no strain of *Pythium sterile* is available. Both species do not produce sexual stages. A more extensive study of these pairs of species, namely, *Phytopythium boreale / Pythium megacarpum* and *Phytopythium litorale / Pythium sterile* including more isolates and more DNA regions should reveal whether *P. sterile* and *P. megacarpum* should be validated as legitimate species.

There were some clade K species which were not included in the phylogenetic analyses presented here. In the studies by Lévesque & de Cock (2004) and Robideau et al. (2011) the species *Pythium indigoferae* appeared in clade K, which is now the genus *Phytopythium*. In stark contrast to the other species in clade K, *Pythium indigoferae* produces filamentous sporangia according to its original description (Butler 1907). The strain of *Pythium indigoferae* in the study of Lévesque & de Cock (2004) was the strain CBS 261.30 which was used by

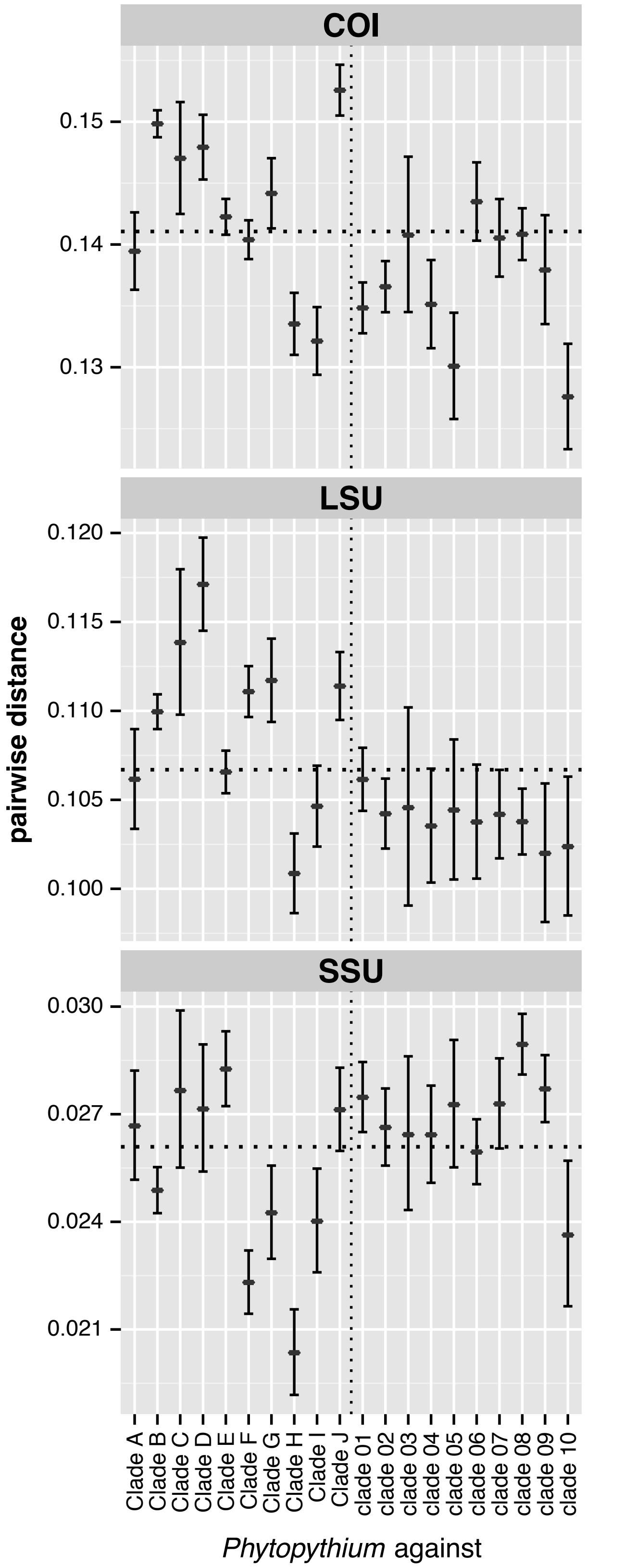

Fig. 5 Analysis of all pairwise distances containing only one representative of each *Phytopythium* sp. The dotted lines represent the average of all these pairwise distances for each marker, adjusted to remove the bias for the difference in species number between *Pythium* and *Phytophthora*. The bars represent 95 % confidence intervals corrected by the Bonferoni method for 60 comparisons. The analysis was done with arcsine transformation, therefore, the averages as well as the upper and lower boundaries of the intervals were transformed back to actual pairwise distances for the plot. Intervals that are not touching the average dotted line are significantly below or above the average, i.e. closer to or more distant to *Phytopythium*, respectively.

van der Plaats-Niterink (1981) in her publication 'Monograph of the genus *Pythium'*, as the ex-type strain was no longer available. However, CBS 261.30 is also no longer viable. Under observation by van der Plaats-Niterink and more recently while it was still culturable, this strain did not sporulate. The identity of this strain can therefore not be confirmed. Other strains with DNA sequences very close to CBS 261.30 have been identified (unpubl. data) which produced, however, subglobose, proliferating, papillate sporangia. These findings agree with Spies et al. (2011) who suggested that this strain be re-identified as *Pythium vexans*. CBS 261.30 and related strains are clearly part of a *Phytopythium vexans* complex that needs to be resolved through further phylogenetic study. This *P. vexans* complex also contains the invalid taxon *Pythium cucurbitacearum*, which was not included in our analyses. This taxon is invalid as it is missing a Latin diagnosis and based on Art. 36 of the Melbourne convention (McNeill et al. 2012). The representative strain of *P. cucurbitacearum* CBS 748.96 is no longer viable. The ITS sequence of this strain was reported by Spies et al. (2011), to be related yet distinct from a novel strain isolated from *Acacia* which was very different among the isolates in the monophyletic *Phytopythium vexans* complex studied. Most likely strain CBS 748.96 represents a distinct species from the *P. vexans* complex, which as of yet is not validly described. Once this complex is resolved it is likely that it will represent a number of new species for the genus *Phytopythium*.

Two other *Pythium* species not included in the phylogenetic analyses are *P. palingenes* and *P. polytylum*. Because no living strains of these species are available, they could not be included in the DNA studies. Morphological data for *P. palingenes* and *P. polytylum* show the typical characters of *Phytopythium*: ovoid, papillate, internally proliferating sporangia and cylindrical antheridia. Therefore we consider *P. palingenes* and *P. polytylum* as members of *Phytopythium*.

A new species of *Phytopythium* was isolated from water samples collected in District MirpurKhas of Sindh province, Pakistan. It is described and illustrated here as *P. mirpurense* (see section New Species). Genetically, *Phytopythium mirpurense* is shown to nestle within the genus *Phytopythium*, in all of the phylogenetic trees presented. The most obvious morphological characters of this new species are the proliferating, subglobose sporangia, terminal and intercalary oogonia, antheridia with lengthwise application to oogonia over their entire length, aplerotic to nearly plerotic oospores, and high optimum temperature for growth. These characters are shared with many other members of *Phytopythium*. The main differentiation of this species is shown through the molecular analyses of DNA sequences and the phylogenetic trees (Fig. 3, 4).

Halophytophthora s.l. is a heterogenous, polyphyletic genus (Hulvey et al. 2010) with species of marine origin. Two species of this genus clustered within the clade of *Phytopythium*: *H. operculata* (originally described as *Phytophthora operculata*) and *H. kandeliae*. Further, only species of *Halophytophthora* s.str. (Hulvey et al. 2010) show some morphological similarity to *Phytopythium*. However, their sporangia are in average two or more times the size of sporangia in the *Phytopythium* species (length av. 64–117 µm, resp. 20–40 µm). They develop zoospores inside the sporangium and not in a vesicle like *Pythium*, though the formation of a vesicle may be part of the release process. Moreover, no internal proliferation was observed in these species. *Halophytophthora kandeliae* was previously transferred to *Phytopythium* (Marano et al. 2014, Thines 2014). The strains of *Halophytophthora kandeliae* used in barcode analyses of ITS and COI regions were CBS 111.91 and CBS 113.91 and they were both found to be associated with the *Phytopythium* clade (Robideau et al. 2011). However, neither of these strains is the type strain of this species. Marano et al. (2014) have published the ITS sequence of the type strain of *H. kandeliae* from ATCC and this sequence was identical to that of CBS 111.91 and 113.91. We have then included data from strain CBS 113.91 in our analyses here and are certain that it well represents the systematic placement of *Phytopythium kandeliae.* There are some difficulties with *Halophytophthora operculata*'s lack of fit in this clade by morphological measures and we have decided not to rename it at this time. This marine species has zoospore development fully within the sporangium; no vesicle occurs. Zoospore discharge is unique, via an operculum at the apex of the sporangium and no internal proliferation was observed. The size of the sporangia is significantly much larger than those of the *Phytopythium* species (up to 175 um). The strain CBS 241.83, which is the ex-type strain of *H. operculata*, did not sporulate during our investigations, so the identity of the strain could not be confirmed. However the current molecular data available about this strain, the sequence data presented here and the organisation of the 5S gene family as reported by Bedard et al. (2006), does indicate that it belongs in a monophyletic circumscription of *Phytopythium*. More investigation of this species is clearly required in order to confirm its identity.

New combinations were deposited in MycoBank (see below in section Taxonomic and Nomenclatural Changes; Crous et al. 2004).

CONCLUSIONS

The genus *Phytopythium* was first proposed to the community in 2008 (see www.phytophthoradb.org/pdf/O8LevesquePM.pdf) and it was formally published in June 2010 (Bala et al. 2010b), with *Phytopythium sindhum* as the type species. In 2010, Uzuhashi et al. (2010) proposed another name *Ovatisporangium* for clade K using a partial sampling of *Pythium* and *Phytophthora* species and published their findings in September of 2010. Comparison of their circumscription of the genus *Ovatisporangium* to our molecular analyses clearly shows that the type of *Phytopythium*, *P. sindhum* is a member of the group described as *Ovatisporangium* (Fig. 1, 2). *Ovatisporangium* is thus recognised as a synonym of *Phytopythium.*

We demonstrated with three different phylogenetic markers that all species belonging to *Pythium* clade K represent a monophyletic genus that includes the type species of the previously described genus *Phytopythium*. The taxonomic circumscription of other *Pythium* clades remains unresolved. The species with filamentous and globose sporangia are well separated as reported before in many studies, however, both LSU and COI suggest that clades A–J could be divided into subgroups but provide no support for any particular arrangement. The inclusion of species from other genera closely related to *Pythium* such as *Pythiogeton*, *Lagenidium* or *Myzocytiopsis* can change these conclusions but clade support remains very low (Schroeder et al. 2013, Hyde et al. 2014). Therefore, we recommend avoiding any further changes in the generic status of *Pythium* Pringsheim species belonging to clade A–J until better phylogenetic markers are found and multigene phylogenies are available with the closely related genera.

TAXONOMIC AND NOMENCLATURAL CHANGES

Phytopythium Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, Persoonia 24: 137. 2010

Type species. *Phytopythium sindhum*, Lodhi, Shahzad & Lévesque, Persoonia 24: 137. 2010.

Etymology. Named after combined features of the genera *Phytophthora* and *Pythium*.

Common morphological characteristics of the species of *Phytopythium* are globose to ovoid shape of sporangia, often with a more or less distinct papilla or non-papillate and often proliferating internally like those in *Phytophthora* with non-papillate sporangia. Zoospore discharge is like *Pythium*. Most species have large, smooth oogonia, thick-walled oospores, and 1–2 elongate or lobate antheridia, laterally applied to the oogonium. Cultures are mostly homothallic, occasionally sterile.

Notes — *Phytopythium* (Bala et al. 2010b) is emended to include species of *Pythium* in clade K from Lévesque & de Cock (2004) and described after that. It is morphologically and phylogenetically between *Pythium* and *Phytophthora*.

NEW COMBINATIONS

Phytopythium boreale (R.L. Duan) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563326

Basionym. *Pythium boreale* R.L. Duan, Acta Mycol. Sin. 4: 1. 1985 (as '*borealis*') (MB105742).

≡ *Ovatisporangium boreale* (R.L. Duan) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517560).

Representative strain — China, soil under *Brassica caulorapa*, CBS 551.88 (ex-type strain not available).

Phytopythium carbonicum (B. Paul) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563328

Basionym. *Pythium carbonicum* B. Paul, FEMS Microbiol. Lett. 219: 270. 2003 (MB489329).

≡ *Ovatisporangium carbonicum* (B. Paul) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517561).

Representative strain — France, soil on top of spoil heap, CBS 112544 (ex-type strain).

Phytopythium chamaehyphon (Sideris) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563329

Basionym. *Pythium chamaehyphon* Sideris, C.P, Mycologia 24: 33. 1932 (as '*chamaihyphon*') (MB260414).

≡ *Ovatisporangium chamaehyphon* (Sideris) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517562).

Representative strain — USA, Hawaii, *Carica papaya*, CBS 259.30 (ex-type strain).

Phytopythium citrinum (B. Paul) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563330

Basionym. *Pythium citrinum* B. Paul, FEMS Microbiol. Lett. 234: 273. 2004 (MB368597).

≡ *Ovatisporangium citrinum* (B. Paul) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517563).

Representative strain — France, Marsaunay la cote, vineyard soil, CBS 119171 (ex-type strain).

Phytopythium delawarense (Broders, P.E. Lipps, M.L. Ellis & Dorrance) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB807542

Basionym. *Pythium delawarense* Broders, P.E. Lipps, M.L. Ellis & Dorrance, Mycologia 104: 789. 2012 (MB563353).

Representative strain — USA, Ohio, Delaware county, *Glycine max*, CBS 123040 (ex-type strain).

Phytopythium helicoides (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563332

Basionym. *Pythium helicoides* Drechsler, J. Wash. Acad. Sci. 20: 413. 1930 (MB266912).

≡ *Ovatisporangium helicoides* (Drechsler) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517559).

= *Phytophthora fagopyri* S. Takim. ex S. Ito & Tokun., Trans. Sapporo Nat. Hist. Soc. 14: 15. 1935 (MB472184).

Representative strain — USA, *Phaseolus vulgaris*, CBS 286.31 (authentic strain).

Phytopythium litorale (Nechw.) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563335

Basionym. *Pythium litorale* Nechw., FEMS Microbiol. Lett. 255: 99. 2006 (MB521454).

≡ *Ovatisporangium litorale* (Nechw.) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517566).

Representative strain — Germany, Lake Konstanz, rhizosphere soil (*Phragmites australis*), CBS 118360 (ex-type strain).

Phytopythium mercuriale (Belbahri, B. Paul & Lefort) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563337

Basionym. *Pythium mercuriale* Belbahri, B. Paul & Lefort, FEMS Microbiol. Lett. 284: 20. 2008 (MB511433).

≡ *Ovatisporangium mercuriale* (Belbahri, B. Paul & Lefort) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517568).

Representative strain — South Africa, Limpopo Province, ex rhizosphere *Macadamiae integrifoliae*, CBS 122443 (ex-type strain).

Phytopythium montanum (Nechw.) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563338

Basionym. *Pythium montanum* Nechw., Mycol. Progr. 2: 79. 2003 (MB373239).

≡ *Ovatisporangium montanum* (Nechw.) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517569).

Representative strain — Germany, Bavarian Alps, wet soil under *Picea abies*, CBS 111349 (ex-type strain).

Phytopythium oedochilum (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563339

Basionym. *Pythium oedochilum* Drechsler, J. Wash. Acad. Sci. 20: 414. 1931 (MB272763).

≡ *Ovatisporangium oedochilum* (Drechsler) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (as '*oedichilum*') (MB517570).

Representative strain — USA, CBS 292.37 (authentic strain).

Phytopythium ostracodes (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB563340

Basionym. *Pythium ostracodes* Drechsler, Phytopathology 33: 286. 1943 (MB290364).

≡ *Ovatisporangium ostracodes* (Drechsler) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517571).

Representative strain — Spain, clay soil, CBS 768.73 (strain used by van der Plaats-Niterink (1981), ex-type strain not available).

Phytopythium palingenes (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov*. — MycoBank MB807543

Basionym. *Pythium palingenes* Drechsler, J. Wash. Acad. Sci. 20: 416. 1930 (MB273284).

Representative strain — None available.

Phytopythium polytylum (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov.* — MycoBank MB807544

Basionym. *Pythium polytylum* Drechsler, J. Wash. Acad. Sci. 20: 415. 1930 (MB275012).

Representative strain — None available.

Phytopythium vexans (de Bary) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *comb. nov.* — MycoBank MB563322

Basionym. *Pythium vexans* de Bary, J. R. Agric. Soc. 12 (Ser. 2,1): 255. 1876 (MB174427).

≡ *Ovatisporangium vexans* (de Bary) Uzuhashi, Tojo & Kakish., Mycoscience 51: 360. 2010 (MB517573).

= *Pythium complectens* M. Braun, J. Agric. Res. 29: 415. 1924 (MB261556).

= *Pythium allantocladon* Sideris, Mycologia 24: 27. 1932 (MB256394).

= *Pythium ascophallon* Sideris, Mycologia 24: 29. 1932 (MB257476).

= *Pythium polycladon* Sideris, Mycologia 24: 32. 1932 (MB274913).

= *Pythium euthyhyphon* Sideris, Mycologia 24: 34. 1932 (MB536649).

= *Pythium piperinum* Dastur, Proc. Indian Acad. Sci., B 1, 11: 803. 1935 (MB274563).

Representative strain — Iran, soil, CBS 119.80 (strain used by van der Plaats-Niterink (1981) ex-type strain not available).

NEW SPECIES

Phytopythium mirpurense Lodhi, De Cock, Lévesque & Shahzad, *sp. nov*. — MycoBank 809691; Fig. 6

Etymology. Name refers to the District MirpurKhas of Sindh province, Pakistan from where this species was frequently isolated.

Main *hyphae* up to 6 µm wide. *Sporangia* papillate, proliferating, subglobose, limoniform, obovoid or ovoid 20–25 µm diam. Discharge tube short 5–8 × 5–6 µm diam. *Oogonia* large smooth globose, terminal, intercalary, occasionally unilaterally intercalary, (27–)34–37(–40) (av. 34) µm diam. *Antheridia* 1–3 per oogonium, mostly monoclinous or distantly monoclinous, occasionally diclinous. Oogonia and antheridial stalk originate from same hyphae. Antheridia apply lengthwise to the oogonium producing lateral or occasionally apical fertilisation tubes. *Oospores* aplerotic or nearly plerotic (22–)29–32(–34) (av. 29.45) µm diam. Oospore wall thickness is 2.5–3 (av. 2.8) µm. *Ooplast* 13–16 µm diam (Fig. 2, 3). Aplerotic index 66.7 %, ooplast index 23 % and wall index 47 %.

Colony characteristics — *Phytopythium mirpurense* produces profuse white cottony growth on PDA and CMDA, on PCA submerged without any patterns, and on CMA with a rosette pattern. The optimum growth occurred at 30 °C. Daily growth at 25 °C on PDA 19 mm, PCA 20 mm, CMA 23.5 mm and CMAD 26 mm. The maximum growth temperature was 35 °C.

Material examined. Pakistan, Sindh, District MirpurKhas, MirWah, N25°23' E69°02', stagnant water, 12 Jan. 2006, *A.M. Lodhi* (holotype CBS 124523, maintained in inactive state. Culture ex-type also deposited as DAOM 238991 in CCFC).

Additional material examined. Pakistan, Sindh, from water pond at Sindhri, District MirpurKhas (DAOM 238992, CBS124524) (N25°37' E69°12').

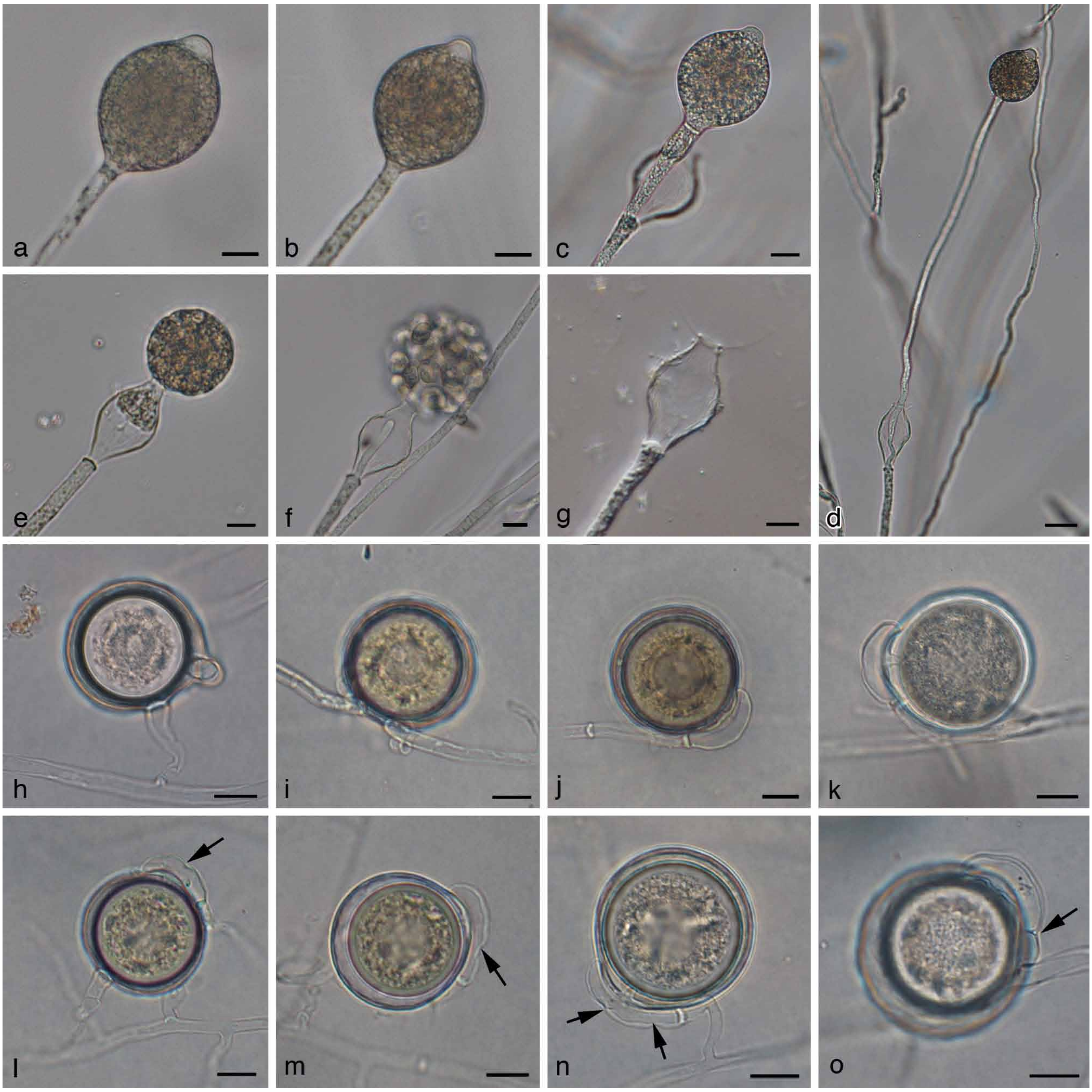

Fig. 6 *Phytopythium mirpurense* sp. nov. sporangia (a–g) and gametangia (h–o). a–b. Papillate sporangia; c. sporangium proliferation outside of empty sporangium; d. internally proliferating sporangium; e. early stage of vesicle formation; f. vesicle with zoospore development inside; g. empty sporangium with remnants of vesicle membrane still attached; h. oogonium on short lateral stalk; i. unilaterally intercalary oogonium; j–o. oogonia with aplerotic oospores and long, cylindrical antheria. Arrows indicate constrictions in antheridia. — Scale bar in all figures 10 µm, except panel d (20 µm).

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