

New species of *Pythium* and *Phytophthora*

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Abstract: In a phylogenetic study of the genera *Pythium* and *Phytophthora* we found isolates with unique ITS sequences, different enough from those of any described species to justify new species status. This paper describes and illustrates the morphology of two new species of *Pythium* and one new species of *Phytophthora*. Their taxonomic position and relationships with other related species in the same genus are presented and discussed.

Taxonomic novelties: *Pythium cystogenes* de Cock & Lévesque sp. nov., *P. rostratifyingens* de Cock & Lévesque sp. nov., *Phytophthora hedraiandra* de Cock & Man in 't Veld sp. nov.

Key words: Oomycetes, *Pythium*, *Phytophthora*, systematics.

INTRODUCTION

Molecular phylogenies have been produced recently for those species from which DNA could be extracted of the genera *Pythium* (Lévesque & de Cock 2004) and *Phytophthora* (Cooke *et al.* 2000, Kroon *et al.* 2004). The ITS sequences have been deposited in GenBank, an internationally available database, and this information can be used to determine the identity or phylogenetic position of unknown *Pythium* and *Phytophthora* isolates. We collected a number of isolates of which the ITS sequence was significantly different from all recognized species. Some of these isolates developed all stages of their life cycle, producing all morphological structures necessary for a species description. In the present paper two new species of *Pythium*, and one species of *Phytophthora* are described.

MATERIALS AND METHODS

Isolates

Table 1 lists accession number, host, geographic origin, and GenBank reference numbers of the isolates used in this study.

Morphology

For morphological studies isolates were cultivated on cornmeal agar (CMA), potato-carrot agar (PCA), potato-dextrose agar (PDA), oatmeal agar (OA) and V8 (for recipes, see Gams *et al.* 1998). Inoculum plugs of 5 × 5 mm were put in the centre of the Petri dish. For studying sporangia and zoospore release, isolates were cultured in water cultures which were prepared as follows: pieces of sterilized grass blades

(*Pythium*) and crushed hemp seeds (*Phytophthora*) were placed on young colonies growing on agar. After 1 d the colonised blades and seeds were placed in sterile soil extract in a Petri dish which was placed on a cool (15–20 °C) bench in the laboratory and exposed to daylight (day length was approx. 16 h). The soil extract was a chemically undefined medium prepared by stirring 100 g of sandy soil in 1 L of demineralized water for 1 min, which was then centrifuged and filtered to remove soil particles and finally autoclaved for 20 min at 121 °C. Observations on sporangia and zoospore discharge were made within 1–2 d in *Pythium* and up to 7 d in *Phytophthora*. Sexual structures were studied between 4–14 d. Fifty sporangia and forty oogonia were measured for each species.

Cardinal temperatures were determined on PDA (*Phytophthora*) or PCA (*Pythium*). Petri dishes were inoculated in the centre and placed at 24 °C. After 24 h two lines were drawn perpendicular to each other on the bottom of the Petri dish which intersected at the place of the inoculum and colony margins were marked on these lines. Dishes were subsequently incubated at temperatures of 3 to 36 °C with intervals of 3 °C. After 24 and 48 h the radial growth was determined in all four directions. If growth stopped before the edge of the Petri dish was reached, the culture was returned to 24 °C to check if growth could resume and if the culture was still viable.

DNA isolation, amplification and sequencing

For DNA studies isolates were cultured in pea broth (de Cock *et al.* 1992). Mycelium from 5–14-d-old cultures was harvested by vacuum filtration and the DNA extracted (Möller *et al.* 1992).

Table 1. Isolates examined in this study. Ex-type strains are printed in bold.

Strains ¹	Species	Host	Locality of origin	GenBank No.*
675.85	<i>Pythium cystogenes</i>	<i>Vicia faba</i>	The Netherlands	AY707985 (ITS)
115459	<i>Pythium rostratifyingens</i>	Leaf litter of <i>Quercus</i> sp.	BC, Canada	–
115464	<i>Pythium rostratifyingens</i>	Soil under apple tree	WA, U.S.A.	AY707986 (ITS)
115465	<i>Pythium rostratifyingens</i>	Soil and roots of <i>Triticum</i> sp.	WA, U.S.A.	–
383.34	<i>Pythium rostratifyingens</i>	Leaf litter	The Netherlands	–
172.68	<i>Pythium rostratifyingens</i>	Roots of <i>Medicago sativa</i>	unknown	–
111725	<i>Phytophthora hedraiaandra</i>	<i>Viburnum</i> sp.	The Netherlands	AY707987 (ITS) AY769115 (<i>Cox1</i>)
444.84	<i>Phytophthora pseudotsugae</i>	<i>Pseudotsuga menziesii</i>	OR, USA	AY707988 (ITS) AY769116 (<i>Cox1</i>)

¹CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. * Sequences deposited in GenBank.

The isolates of *Pythium rostratifyingens* were processed following the protocol described in Mazzola *et al.* (2002). DNA was amplified by PCR in a total volume of 20 µL. For ITS studies primers at 0.08 µM concentration of Un-Up 18S42 (CGTAACAAGGTTT CCGTAGGTGAAC) and Py-Lo 28S22 (GTTTCTTT TCCTCCGCTTATTAATATG) were used with the Taq Titanium (BD Bioscience Clontech, Palo Alto, CA) polymerase (1×) and 0.1 mM of dNTP's. PCR tubes were placed in the Genius thermal cycler (Techne, Princeton, NJ) and processed with the following temperature profile: 1 cycle 3 min at 95 °C; 40 cycles 45 s at 95 °C, 45 s at 68 °C, 1 min 30 s at 72 °C; with a final extension 8 min at 72 °C. *Cox1* sequencing was performed following the protocol of Kroon *et al.* (2004). Visual quantification was made by comparison to a DNA low mass ladder (Invitrogen, Carlsbad, CA) following electrophoresis on a 1 % agarose gel stained with ethidium bromide.

Sequence reactions were made following Big Dye Terminator (BDT) version 2 (Applied Biosystem, Foster City, CA) protocol with four primers to get complete ITS 1 and ITS 2 sequences forward and reverse: Un-Up 18S42, Oom-Up 5.8S55 (TGCGA-TACGTAATG CGAATT) and Py-Lo 28S22, Oom-Lo 5.8S47 (ATTACGTATCGCAGTTCGCAG). The final volume of 20 µL comprised 3 µL of (5×) BDT Seq Buffer, 2 µL of BDT Sequence mix version 2, between 2 and 10 ng of DNA template obtained by PCR and a final concentration of 0.16 µM of the primer. Amplified sequenced products were purified by ethanol 95 % precipitation and resuspended in formamide. *Cox1* sequencing was performed following the protocol of Kroon *et al.* (2004).

Tubes were transferred into an ABI Prism Genetic Analyzer (model 310; Applied Biosystems) for electrophoresis and analysis. Sequences were edited using SeqMan II (version 5.00, DNASTar 2001). The sequence of the putative new *Pythium* species were aligned with all the species within their respective clade (Lévesque & de Cock 2004) whereas the sequence of the new *Phytophthora* species was aligned

to sequences belonging to clade 1a and 1d (Kroon *et al.* 2004). The ITS or *Cox1* sequence alignments done by Megalign (DNASTar) did not need any manual adjustment before the parsimony phylogenetic analysis which was performed with PAUP version 4.062 software (Swofford 2001).

RESULTS AND DISCUSSION

Sequencing

An isolate of *Pythium*, CBS 675.85 related to *P. perplexum* was clearly differentiated from two isolates of this species, one from GenBank and one sequenced by Lévesque & de Cock (2004) (Fig. 1). There was approximately 20 % sequence divergence between the new species and either one of the two *P. perplexum* isolates. It should be pointed out that the GenBank sequence of *P. perplexum* had only the ITS 1 sequence deposited. *Pythium nodosum* and *P. polymastum* (GI 12863073) were the only other incomplete sequences in this data set. The new species is described below as *P. cystogenes*.

For the group of unknown *Pythium* isolates with similar morphology and ITS sequence, GenBank only had partial ITS region sequences of closely related species, therefore, the analysis was done with ITS 1 only. All five isolates were related to *P. rostratum* but formed a unique cluster with a 100 % bootstrap value which also included the GenBank sequence GI 6468683 (Fig. 2). These sequences differed by approximately 30 % from the representative strain of *P. rostratum* that van der Plaats-Niterink (1981) based the description on. There was some intraspecific variation giving bootstrap values above 50 % within the isolates of our putative new species but it is less than 2 % of the entire ITS region. Thus, this species is described below as a new species, *P. rostratifyingens*.

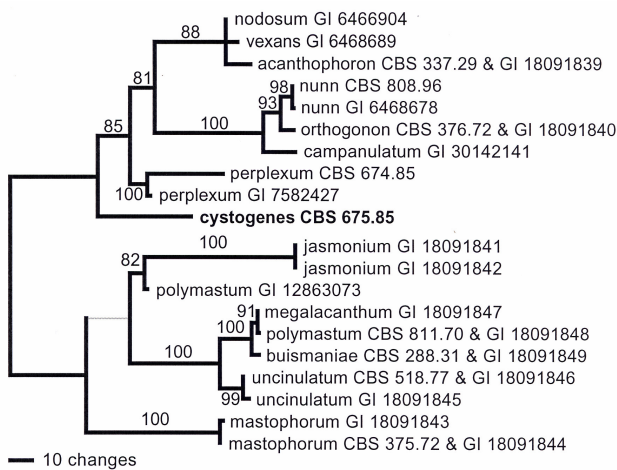


Fig. 1. First of the six equally parsimonious trees resulting from a heuristic search performed on the ITS1, 5.8S and ITS2 rDNA sequences of all *Pythium* species belonging to clade J (see fig. 5J in Lévesque & de Cock 2004). Species from the bottom cluster were used as outgroup. Bootstrap values (1000 reps) are shown at branches when over 50 %, otherwise, branches are represented by thin grey lines if below this threshold. Data deposited in GenBank are represented by GI numbers. Length = 556, CI = 0.84, RI = 0.93.

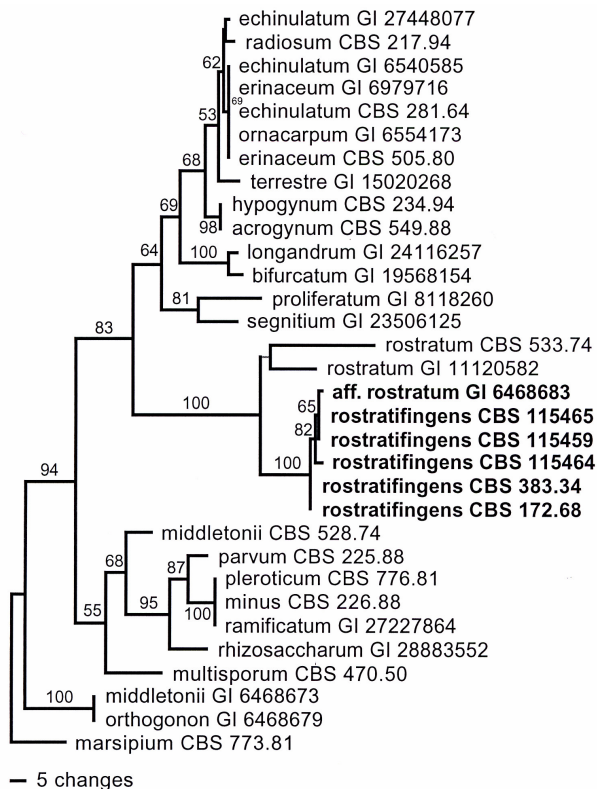


Fig. 2. First of the 143 equally parsimonious trees resulting from a heuristic search performed on the ITS1 rDNA sequences of all *Pythium* species belonging to clade E (see fig. 5E in Lévesque & de Cock 2004). *Pythium marsipium* was used as outgroup. Bootstrap values (1000 reps) are shown at branches when over 50 %, otherwise, branches are represented by thin grey lines if below this threshold. Data already in GenBank are represented by GI numbers. Length = 459, CI = 0.66, RI = 0.86.

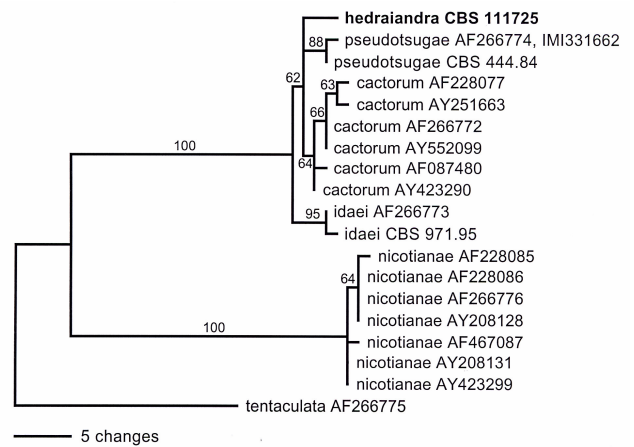


Fig. 3. First of two equally parsimonious trees resulting from a heuristic search performed on the ITS1, 5.8S and ITS2 rDNA sequences of *Phytophthora* species related to *P. hedraiaandra*. *P. tentaculata* was used as outgroup. Bootstrap values (1000 reps) are shown at branches. Data obtained from GenBank are represented by GenBank accession numbers. Length = 90, CI = 0.98, RI = 0.99.

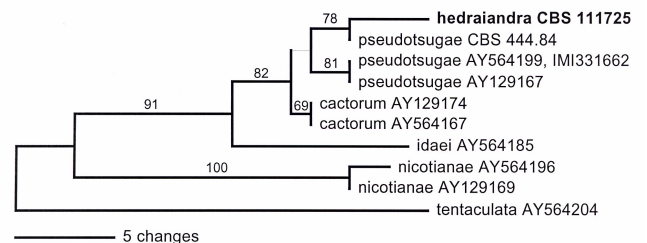


Fig. 4. The only most parsimonious tree resulting from a heuristic search performed on the *Cox1* mitochondrial partial sequences of *Phytophthora* species related to *P. hedraiaandra*. *Phytophthora tentaculata* was used as outgroup. Bootstrap values (1000 reps) are shown at branches when over 50 %, otherwise, branches are represented by thin grey lines if below this threshold. Data obtained from GenBank are represented by GenBank accession numbers. Length = 70, CI = 0.89, RI = 0.85.

GenBank sequences for the ITS region of *Phytophthora* species from clade 1a and 1d (Kroon *et al.* 2004) were complete with an overall length of approximately 800 bp. Two isolates of *P. pseudotsugae* which showed a bootstrap value 88 % differed by 5 or 6 bases from CBS 111725 (Fig. 3). All the isolates of *P. cactorum* showed a bootstrap value of 64 % and differed from CBS 111725 by 4–9 bases. Because of shorter *Cox1* GenBank sequences of *P. pseudotsugae* at the 5' or 3' ends, only 631 bases of alignment were used in the analysis (Fig. 4). CBS 111725 differed from the isolate CBS 444.84 of *P. pseudotsugae* by 5 bases. The putative new species had the longest branch in the *cactorum/pseudotsugae* group in either the ITS or *Cox1* trees. It is described below as a new *Phytophthora* species, *P. hedraiaandra*.

Pythium cystogenes de Cock & Lévesque, **sp. nov.** MycoBank MB500124. Figs 5, 6.

Etymology: ‘*cystogene*’ refers to the direct differentiation of sporangial protoplasma into cysts.

Coloniae in agar CMA submersae, in PCA submersae et structuram radiantem praebentes. Hyphae primariae ad 7 μm latae. Sporangia terminalia, raro intercalaria, interne proliferantia, (sub-)globosa vel ovoidea, saepe papilla plus minusve conspicua praedita, 20–(26.3)–33 μm diam. Zoosporae raro formatae; contentus sporangii per tubum evacuantem liberatus et in cystas divisus. Oogonia terminalia vel lateral, raro intercalaria, (sub-)globosa, levia, hyalina, 28–(30.7)–35 μm diam. Antheridia (1–)2 ad quodque oogonium, diclina, terminalia in stipite saepe ramoso, oogonium circumdantia. Cellula antheridialis cylindrica, 11–18 \times 5–8 μm , secundum oogonium applicata. Oosporae aploeroticae, 21–(23.3)–26 μm diam, paries ad 3 μm crassus. Incrementum diurnum in CMA 25 °C 18 mm, in PCA 17 mm. Temperatura minima crescentiae 3–6 °C, optima 24 °C, maxima 27–30 °C.

Holotypus: CBS H-12855, isolatus e radice *Viciae fabae* in Neerlandia, 1985, in herb. CBS.

Colonies on CMA submerged, on PCA submerged with a slightly radiate pattern. Daily growth at 25 °C on CMA 18 mm, on PCA 17 mm. Minimum temperature for growth 3–6 °C, optimum 24 °C and maximum 27–30 °C (at 30 °C growth stopped on the second day, however, when cultures were placed at 24 °C after 2 d, growth was resumed). Main hyphae up to 7 μm wide. **Sporangia** terminal, occasionally intercalary, internally proliferating; (sub-)globose to ovoid, often provided with a more or less distinct papilla, 20–33 μm diam, av. 26.3 μm . Discharge tubes developed on or near the papilla, mostly short, occasionally, if a sporangium developed inside a grass cell, discharge tubes of > 100 μm might protrude from the grass blade.

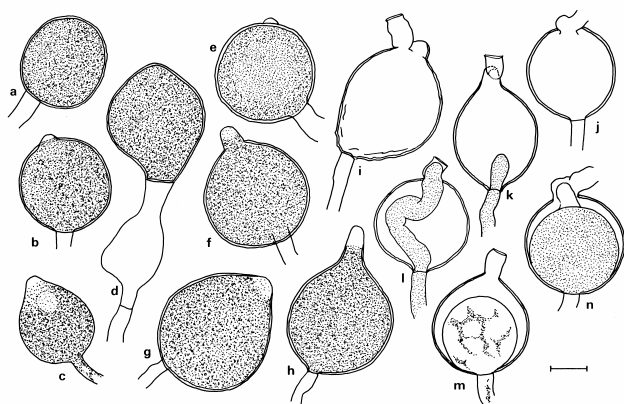


Fig. 5. *Pythium cystogenes* (CBS 675.85). a–h. Young sporangia showing different stages of development of papilla. i–n. Empty sporangia after discharge of zoospores, some showing internal proliferation (k–n). Scale bar = 10 μm .

Zoospores rarely developed: sporangial contents are released through the tip of the discharge tube into a vesicle, where the protoplasm mostly differentiates into cysts directly. After the disappearance of the vesicle wall, the cysts remain at the opening of the discharge tube until they are removed by water currents. **Oogonia** terminal or laterally stalked, occasionally intercalary, (sub-)globose, smooth, colourless, 28–35 μm diam, av. 30.7 μm . **Antheridia** (1–)2 per oogonium, diclinous, terminal on antheridial stalks which are often branched, inflated with constrictions, encircling the oogonium. Antheridial cells cylindrical, 11–18 \times 5–8 μm , applied lengthwise to the oogonium. **Oospores** aploerotic, 21–26 μm diam, av. 23.3 μm , wall up to 3 μm thick.

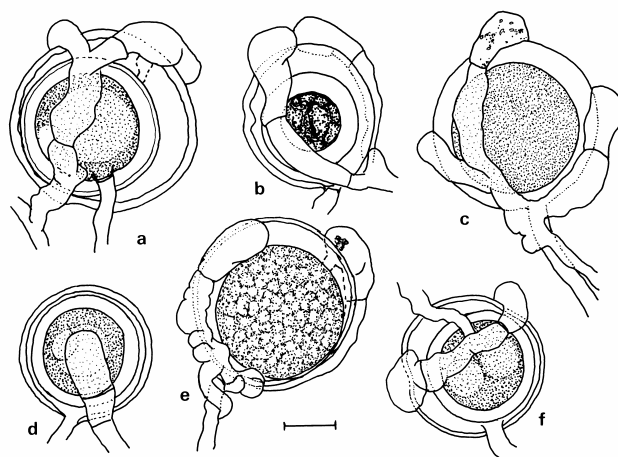


Fig. 6. *Pythium cystogenes* (CBS 675.85). a–f. Oogonia with antheridia. Scale bar = 10 μm .

Specimen examined: **The Netherlands**, on *Vicia faba*, 1985, isolated by Plant Protection Service, Wageningen (PD 85/462-1), **holotype** Herb. CBS H-12855, culture ex-type CBS 675.85.

Pathogenicity: *Pythium cystogenes* was isolated from *Vicia faba*; nothing is known about its pathogenicity.

Notes: Based on ITS sequences, *Pythium cystogenes* is member of clade J (Lévesque & de Cock 2004). Within this clade it belongs to the subclade that contains *P. nodosum*, *P. acanthophoron*, *P. nunn*, *P. orthogonon*, *P. campanulatum*, and *P. perplexum*. This group is morphologically rather heterogeneous and none of the species is similar to *P. cystogenes*; e.g. none of the other members of this group shows internal proliferation of the sporangia. Oogonia and sporangia (if present) are much smaller than those of *P. cystogenes*, and cardinal temperatures are higher in most species in this group.

Morphologically more similar species are *P. pal-lingen*, *P. polytulum* and *P. oedochilum* since they all have proliferating globose sporangia and large oogonia. *Pythium cystogenes* differs from *P. oedochilum* because it produces many globose sporangia, smaller oospores with thinner walls and has much lower

cardinal temperatures. It differs from *P. palingenes* also by the presence of many globose sporangia and by smaller oospores and antheridial stalks which are branched and cover the oogonium, and do not wrap around the oogonial stalk. *Pythium cystogenes* differs from *P. polytulum* by having much smaller antheridia and oospores. Relatedness among *P. cystogenes*, *P. palingenes* and *P. polytulum* based on ITS sequences could not be determined since no cultures of the latter two species are available; *P. oedochilum* is a member of clade K (Lévesque & de Cock 2004), which also includes *P. vexans*. The almost complete absence of zoospores and direct differentiation of sporangial protoplasm into cysts seems to be a unique feature for *P. cystogenes*.

Pythium rostratifengens de Cock & Lévesque, **sp. nov.** MycoBank MB500125. Figs 2, 7, 8.

Etymology: ‘*rostratifengens*’ refers to the similarity with *P. rostratum*.

Coloniae in agar CMA submersae, in PCA structuram angustam *Chrysanthemi* praebentes. Hyphae primariae ad 7 µm latae. Sporangia intercalaria, raro terminalia, globosa, raro ovalia, (11–)16–(21.2)–27 µm diam. Tubus evacuationis at 30 µm longus, 5–10 µm latus. Multa sporangia statim in hyphas germinantia. Oogonia intercalaria, raro terminalia, globosa, levia, hyalina, 11–(17.4)–22 µm diam. Antheridia (1–)2–(4), monoclina, raro diclina, sessilia vel breviter stipitata vel hypogyna. Oosporae pleroticae, paries ad 1.5 µm crassus. Incrementum diurnum in CMA et PCA 25 °C 9 mm. Temperatura minima crescentiae sub 3 °C, optima 27 °C, maxima 33–36 °C.

Holotypus: CBS H-12854, isolatus e terra sub *Malosylvestri*, WA, U.S.A., in herb. CBS.

Colonies on CMA submerged, on PCA with narrow chrysanthemum pattern. Daily growth at 25 °C on CMA and PCA 9 mm. Minimum temperature for growth below 3 °C, optimum 27 °C and maximum 33–36 °C (at 36 °C growth stopped on the second day, however, when cultures were placed at 24 °C after 2 d, growth was resumed). Main hyphae up to 7 µm wide. **Sporangia** intercalary, occasionally terminal, globose, occasionally oval, (11–)16–27 µm diam, av. 21.2 µm. Discharge tubes up to 30 µm long, 5–10 µm wide. Many sporangia do not develop zoospores but may germinate directly by one or more hyphae. **Oogonia** developed on PCA and in water cultures, intercalary, occasionally terminal, globose, smooth, colourless, 11–22 µm diam, av. 17.4 µm. **Antheridia** 1–4, mostly 2 per oogonium, monoclinal, occasionally diclinal, sessile, on a short stalk or hypogynous. **Oospores** plerotic, wall up to 1.5 µm thick.

Specimen examined: U.S.A., WA, in soil under apple tree, 2000, M. Mazzola (CV22), **holotype** Herb. CBS H-12854, culture ex-type CBS 115464 (DAOM 229188).

Additional specimens and cultures examined: **Canada**, British Columbia, leaf litter of *Quercus* sp., 1975, R. Bandoni, CBS 115459 (= DAOM BR197). **U.S.A.**, WA, soil and roots of *Triticum* sp., 2002, T. Paulitz, CBS 115465 (= DAOM 230120). **The Netherlands**, in leaf litter, CBS 383.34. **U.K.**?, roots of *Medicago sativa*, CBS 172.68 (IMI 120413).

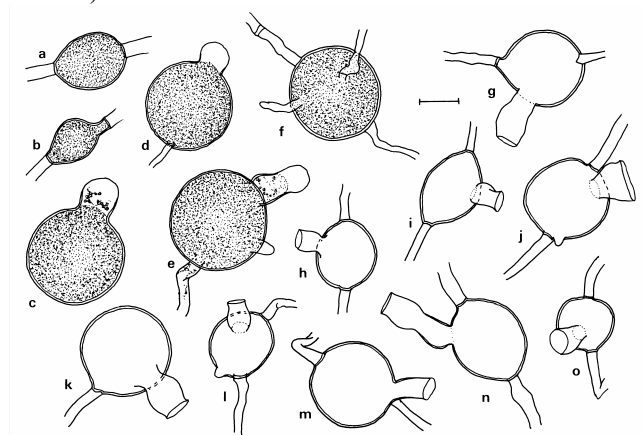


Fig. 7. *Pythium rostratifengens* (CBS 115464). a–e. Young sporangia, some of which with papilla-like protuberance that will develop into a discharge tube. f. Young sporangium germinating by radiating hyphae. g–o. Empty sporangia after discharge of zoospores. Scale bar = 10 µm.

Pathogenicity: *Pythium rostratifengens* was isolated mainly from soil and leaf litter but also from roots of *Triticum* and *Medicago sativa*; nothing is known about its pathogenicity.

Notes: All isolates of *P. rostratifengens* were initially identified as ‘*Pythium rostratum*’ or ‘*P. aff. rostratum*’ (e.g. Mazzola *et al.* 2002). All strains showed a narrow chrysanthemum pattern on PCA, except CBS 172.68 which only showed a vague and coarse chrysanthemum pattern. Growth was slower in CBS 383.34 (6 mm) and somewhat faster in CBS 172.687 (11 mm). Cardinal temperatures were similar, except that 33 °C was lethal for CBS 383.34. Besides the ex-type strain, only CBS 115459 and CBS 115465 produced sexual structures on PCA and/or in grass blade/water cultures and only CBS 172.68 produced zoospores. CBS 383.34 did not produce any propagative structures throughout the time of investigation. Oogonia of CBS 115459 and CBS 115465 were similar to those of the holotype. Zoospore production was more abundant in CBS 172.68 and sporangia were larger (up to 30 µm diam). Those isolates that did not produce zoospores, developed hyphal swellings of a size similar to the size of sporangia in the holotype in water cultures.

Pythium rostratifengens is phylogenetically and morphologically most similar to *P. rostratum*. With the latter species it shares the slow growth and the characteristic intercalary oogonia with monoclinal, sessile or hypogynous antheridia. However, in contrast to *P. rostratum*, the oogonia in *P. rostratifengens* are not catenulate, antheridia are predominantly 2 per oogonium, oogonia and sporangia are smaller and the discharge tubes of the sporangia are wider.

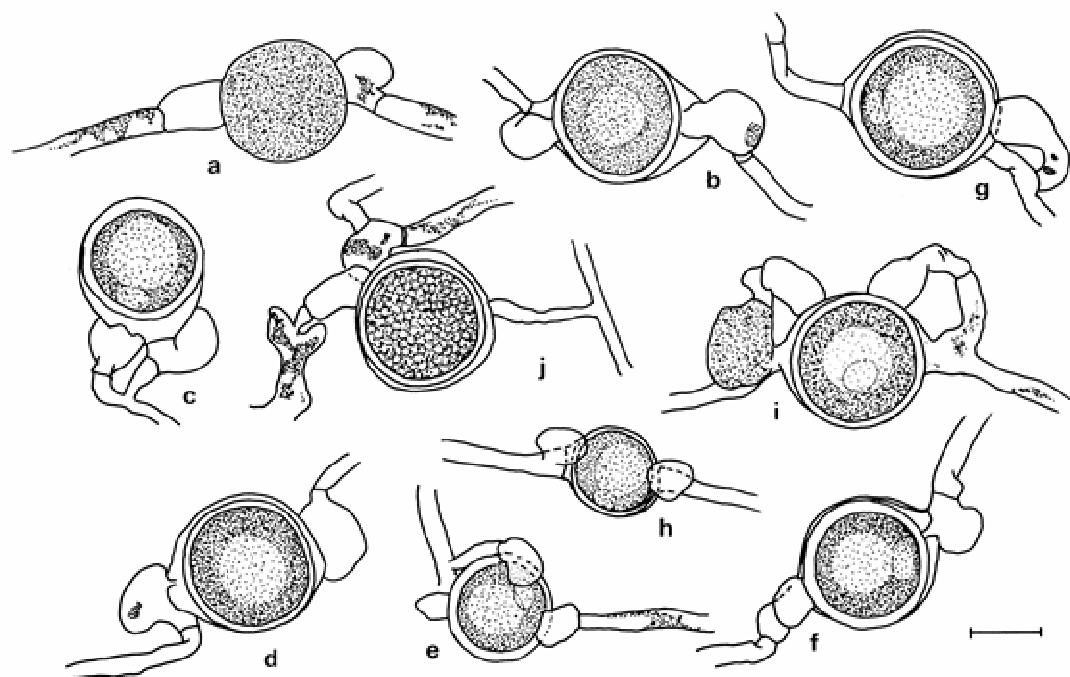


Fig. 8. *Pythium rostratifyingens* (CBS 115464). Oogonia with different types of antheridia: hypogynous (a–f), sessile (g, h), short-stalked (c, e, i), diclinous, intercalary and terminal (j). Scale bar = 10 μ m.

Phytophthora hedraiandra de Cock & Man in 't Veld, **sp. nov.** MycoBank MB500126. Figs 3, 4, 9, 10.

Etymology: ‘*hedraiandra*’ refers to the mainly sessile and short-stalked antheridia.

Coloniae in agar CMA parvos floccos mycelii aerii formantes, in PDA submersae, structuram vagam *Chrysanthemi* similem praebentes, in PCA submersae et concentricae zonatae. Hyphae primariae ad 8 μ m latae. Sporangia in sporangiophoris sympodialiter vel irregulariter elongascentibus formata, raro singula, terminalia vel lateraliter sessilia, globosa vel late ovoidea vel ovalia, longitudo 21–(37)–64, latitudo 16–(28.3)–39 μ m, longitudo : latitudo 1.22–(1.31)–1.41; papilla apicali distincta 5–6 μ m lata et ad 5 μ m crassa praedita; caduca, pedicello brevi. Oogonia terminalia in ramis brevibus vel sessilia, raro terminalia in hypha primaria, globosa, saepe modice applanata, levia, hyalina vel dilute flavida, 26–(30.0)–38 μ m diam in PCA, 33.5 μ m in PDA. Stipes oogonii saepe curvatus vel torquatus. Antheridia plerumque lateraliter sessilia, raro terminalia vel unilateraliter intercalaria, unum ad quodque oogonium, iuxta stipitem oogonii oriunda, plerumque paragyna, raro amphigyna, diclina, raro monoclina, clavata, 9–14–(20) μ m longa. Oosporae apertoticae, 23–(26)–36 μ m diam in PCA, 30.6 μ m in PDA, paries ad 2 μ m crassus. Incrementum diurnum in CMA 25 °C 8.7 mm, in PDA 5.3 mm, in OA 7.6 mm. Temperatura minima crescentiae 3 °C, optima 22 °C, maxima 30 °C.

Holotypus: CBS H-12856, isolatus e folio *Viburni* sp. in *Neerlandia*, in herb. CBS.

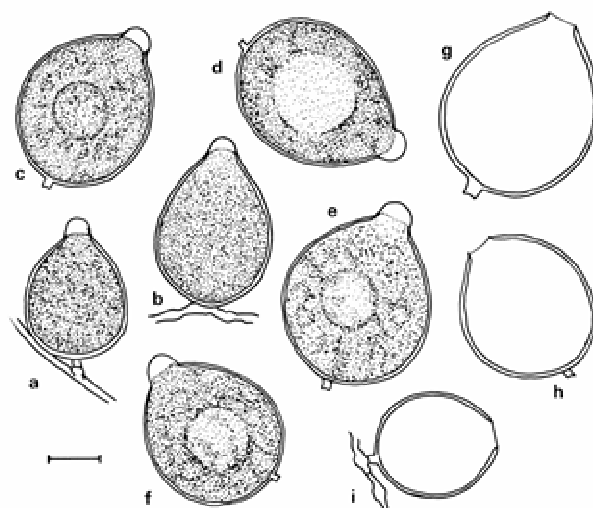


Fig. 9A–I. *Phytophthora hedraiandra* (CBS 111725). a–f. Papillate sporangia. g–i. Empty sporangia after discharge of zoospores. Scale bar = 10 μ m.

Colonies on CMA with small tufts of aerial mycelium, on PDA submerged with a vague chrysanthemum pattern, on OA submerged, on PCA radiate with vague concentric rings. **Daily growth** at 25 °C on CMA 8.7 mm, on PDA 5.3 mm and on OA 7.6 mm. Minimum temperature for growth 3 °, optimum 22 ° and maximum 30 °C. At 30 °C growth rapidly decreased after 3 d. At 33 °C no growth occurred, however, this temperature was not lethal at an incubation of 3 d, in contrast to 36 °C. **Main hyphae** up to 8 μ m wide. **Sporangia** mostly on sympodial or irregularly developing sporangiophores, occasionally single, terminal or laterally sessile; globose to broadly ovoid or oval, with a distinct apical papilla of 5–6 μ m wide and up to 5 μ m thick. Length (21–)30–53–(64) μ m, av. 37 μ m,

width (16–)23–34(–39) μm , av. 28.3 μm , length to width ratio ranging mostly from 1.22–1.41, av. 1.31, caducous with a very short pedicel of up to 2 μm . Oogonia terminal on short side branches or sessile, rarely terminal at the main hyphae, globose, often somewhat flattened, smooth, colourless or slightly yellow, (26–)28–36(–38) μm diam, av. 30 μm on PCA, 33.4 μm on PDA. Oogonial stalk often bent or coiled. Antheridia mostly laterally sessile, occ. terminal or unilaterally intercalary, one per oogonium, attached near the oogonial stalk, mostly paragynous, occasionally amphigynous, diclinous, rarely monoclinal, club shaped, 9–14(–20) μm long. Oospores aplerotic, (23–)24–32(–36) μm , av. 26 μm diam on PCA, 30.6 μm on PDA, wall up to 2 μm thick.

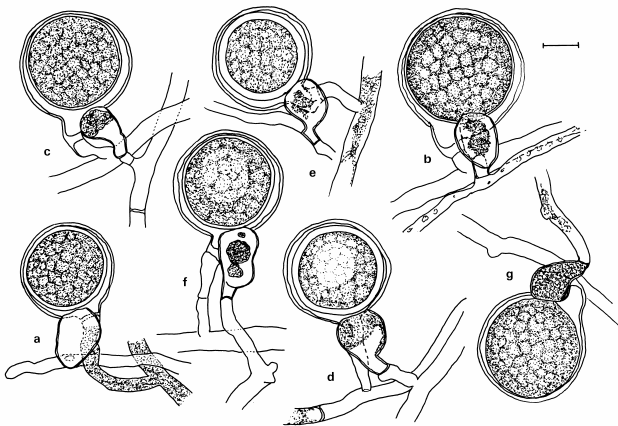


Fig. 10. *Phytophthora hedraiaandra* (CBS 111725). Oogonia with different types of antheridia: sessile (a, b), short-stalked (c, d), unilaterally intercalary (e), terminal (f, g). Scale bar = 10 μm .

Specimen examined: **The Netherlands**, on leaf spots of *Viburnum* sp., 2001, isolated by Plant Protection Service, Wageningen (PD 20017520), **holotype** Herb. CBS H-12856, culture ex-type CBS 111725.

Pathogenicity: *Phytophthora hedraiaandra* was isolated from *Viburnum* sp. in The Netherlands. Nothing is known about its pathogenicity and geographic distribution.

Notes: *Phytophthora hedraiaandra* is a homothallic species that belongs to the group that includes the morphologically similar species *P. cactorum* (Lebert & Cohn) Schröter, *P. pseudotsugae* Hamm & E.M. Hansen, and *P. idaei* D.M. Kenn. It shares the homothallism and the combination of papillate, ovoid sporangia, and mainly paragynous antheridia with the other members of this group. It differs from all these species mainly by the predominantly sessile antheridia. Moreover, it differs from *P. pseudotsugae* and *P. idaei* by its caducous sporangia, from *P. cactorum* by the absence of tangled hyphae below the antheridia and the larger average sizes of oogonia and, particularly, of oospores (Erwin & Ribeiro 1996).

The sequences of *P. hedraiaandra* for the nuclear ribosomal ITS and the mitochondrial *Cox1* gene were

different from the other closely related species (Figs 3, 4). Even though the phylogeny of *P. pseudotsugae* was not congruent between the two analyses, *P. hedraiaandra* showed the longest branch length in the cluster that also included *P. cactorum* and *P. pseudotsugae*.

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