Acta Mycologica

DOI: 10.5586/am.1103

Publication history
Received: 2017-10-17
Accepted: 2018-01-04
Published: 2018-02-12

Handling editor
Maria Rudawska, Institute of Dendrology, Polish Academy of Sciences, Poland

Authors' contributions
RMB and MT conceived the study; RMB and GRD processed all legal documents for collection, conducted field sampling and isolation of strains; RMB and BN conducted DNA extraction, PCR amplification, and sequencing; RMB conducted microscopy and taxonomic analyses; RMB and MT analyzed and interpreted data, and wrote the manuscript with contributions from the other authors

Funding
RMB was funded by the Katholischer Akademischer Ausländer Dienst, Goethe University, and partly by the Studienstiftung Mykologie.

Competing interests
No competing interests have been declared.

Citation
Bennett RM, Nam B, Dedeles GR, Thines M. *Phytopythium leanoi* sp. nov. and *Phytopythium dogmae* sp. nov., *Phytopythium* species associated with mangrove leaf litter from the Philippines. Acta Mycol. 2017;52(2):1103. https://doi.org/10.5586/am.1103

ORIGINAL RESEARCH PAPER

Phytopythium leanoi sp. nov. and *Phytopythium dogmae* sp. nov., *Phytopythium* species associated with mangrove leaf litter from the Philippines

Reuel M. Bennett[1,2], Bora Nam[1,2], Gina R. Dedeles[3], Marco Thines[1,2,4,5]*

[1] Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Georg-Voigt-Str. 14–16, 60325 Frankfurt am Main, Germany
[2] Department of Biosciences, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
[3] UST Collection of Microbial Strains, Research Centre for the Natural and Applied Sciences, College of Science, University of Santo Tomas, Manila, Philippines
[4] Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, 60325 Frankfurt am Main, Germany
[5] Integrative Fungal Research Cluster (IPF), Georg-Voigt-Str. 14–16, 60325 Frankfurt am Main, Germany

* Corresponding author. Email: m.thines@thines-lab.eu

Abstract
The genus *Phytopythium* is a monophyletic taxon of the Peronosporaceae with characteristics intermediate between *Phytophthora* and *Pythium*. In the Philippines, reports of *Phytopythium* are scarce, with the mangrove-swamp-inhabiting species *Phytopythium kandeliae* being the only species recorded to date. It was the aim of the current study to investigate the diversity of *Phytopythium* in mangrove habitats in more detail. Based on culture characteristics, morphology, and molecular phylogenetic position, two new species of *Phytopythium* are described from Philippine mangroves, *P. leanoi* USTCMS 4102 and *P. dogmae* USTCMS 4101. *Phytopythium leanoi* is a species morphologically similar to *P. kandeliae*, but with the ability to develop gametangia in a homothallic fashion. The other new species, *P. dogmae*, is characterized by having a short discharge tube, semipapillate to papillate sporangia and frequently exhibiting a clustering of two sporangia per sporangiogenic hypha. With the addition of the two species described in this study, the genus *Phytopythium* has grown from around 10 to beyond 20 recognized species over the past decade, and it seems likely that several more species of this genus await discovery.

Keywords
mangroves; Oomycetes; Peronosporaceae; *Phytopythium*

Introduction

Oomycetes are fungal-like organisms of the eukaryotic kingdom Straminipila. To date, approximately 2000 species striving in different habitats ranging from the arctic to tropics have been described [1]. The discovery of new lineages that branch outside the major genera of Peronosporales [2–7] has fueled research into the evolution and diversity of cultivable oomycetes of the Peronosporales [8]. Of the recently-described oomycete lineages, the former K-clade *Pythium* species [5,9] have attracted intense research and were segregated taxon from *Pythium* in 2010 [2] as members of the new genus *Phytopythium* on the basis of phylogenetic position and morphology. Uzuhashi et al. [7] as well inferred the polyphyly of *Pythium* based on large ribosomal subunit region (LSU) and *cytochrome oxidase* II (*cox*2) gene sequences and proposed four segregate genera, *Elongisporangium*, *Pilasporangium*, *Globisporangium*, and *Ovatisporangium*.

The genus *Ovatisporangium* was described specifically for the clade K of *Pythium*, however, this genus is a synonym of *Phytopythium*, as the print version of the latter genus description appeared earlier than the former. The characteristic features of *Phytopythium* as described by Bala et al. [2] are: globose to ovoid shaped sporangia that are often papillate, internal proliferation of sporangia, zoospore discharge similar to *Pythium*, smooth and large oogonia, thick-walled oospores and elongate to lobate lateral antheridia. *Phytopythium sindhum* is the type species of this group, isolated from a banana (*Musa paradisiaca* L.) field in Pakistan.

In the Philippines, mangrove oomycetes were reported by Leaño [10], including a single *Phytopythium* species, *P. kandeliae* [1] (basionym *Halophytophthora kandeliae*), which was isolated alongside *Halophytophthora vesicula*, *H. bahamensis*, *H. epistomium*, and *Salispina lobata* (homotypic synonym *H. spinosa* var. *lobata*) [10]. Considering that during the past decade several new species of *Phytopythium* have been discovered and that mangroves have been found to harbor a variety of oomycete species [10–12], it was the aim of this study to evaluate if additional species of the genus *Phytopythium* might be present in Philippine mangroves.

Material and methods

Isolation, growth on solid media, and *Phytopythium* strains

Fallen senescent mangrove leaves from various areas in the Philippines were collected and placed in sealed plastic bags. Leaves were blot-dried, cut into strips of approximately 1–2 mm width, and transferred onto clarified-vegetable juice agar (VJ) [medium No. 15, NBRC (http://www.nite.go.jp/en/nbrc/cultures/media/culture-list-e.html), using V8 Juice (Campbell, USA) or Gemüsesaft (Alnatura, Germany), with or without addition of seawater] with nystatin (500 mg/mL) and rifampicin (30 mg/mL) or streptomycin (0.5 mg/mL). Coenocytic hyphae growing from the edge of the leaf strips were cut and placed on new VJ media with antibiotics until axenic. Cultures were maintained in VJ with or without antibiotics. Incubation was done at room temperature in the dark for 7 days. Additional strains of *Phytopythium kandeliae* NBRC 32620, *Phytopythium* sp. CBS 113.91, *Phytopythium* sp. CBS 111.91, *P. chamaehyphon* CBS 259.30, and *P. helicoides* CBS 286.31 were either purchased from NITE Bioresource Centre (NBRC, Japan) or the Westerdijk Fungal Biodiversity Centre Culture Collection (KNAW, The Netherlands).

The mean radial colony growth of the Philippine strains at 20, 25, 30, and 35°C was assessed on VJ agar [medium No. 15, NBRC; using Gemüsesaft (Alnatura, Germany)] and three media as formulated by CBS [13]: potato carrot agar [PCA; based on Demeter Karotten mit Kartoffeln purée (Alnatura, Germany)], potato dextrose agar (PDA), and peptone yeast-extract glucose agar (PYGA). Colony radial growth was measured for 5 days and values were expressed as mm/day following the method outlined by Solis et al. [14]. For an initial testing at room temperature, isolates were also tested for growth on oatmeal and corn meal agar as formulated by CBS [13].

Induction of sporangia and gametangia

Sporangia and gametangia were induced using 6 mL saline (sea salt) solution at 0, 10, 20, and 30 g/L poured onto 3–7-day-old mycelia in VJ agar plugs in 60-mm Petri dishes. Plates were incubated at room temperature until sporangia and gametangia were formed. Alternatively, sporulation was induced in 90-mm culture plates using 6 mL unsterile soil extract (500 g soil in 500 mL distilled water, settled for 1–2 days and filtered with double-layered cheesecloth) and 6 mL saline solution as above. The set-up was incubated in a climate chamber (CMP 6010; Conviron, Germany) with continuous light and alternating constant temperature at 20°C for 6 h and 23°C for 18 h. Structures were observed and photos were taken using a Canon Digital Camera EOS 500D (Canon, Japan) attached to a Motic AE31 trinocular inverted microscope (Motic, Germany).

Tab. 1 Primer combinations used for PCR amplification.

Loci	Primer	Primer sequence (5′–3′)	Reference
ITS	ITS1-O	CGG AAG GAT CAT TAC CAC	[26]
	LR0	GCT TAA GTT CAG CGG GT	[27]
LSU	LR0R	ACC CGC TGA ACT TAA GC	[27]
	LR6-O	CGC CAG ACG AGC TTA CC	[28]
*cox*1	OomCox1_Levup	GCT TAA GTT CAG CGG GT	[29]
	OomCox1_Levlo	CYT CHG GRT GWC CRA AAA ACC AAA	[29]
*cox*2	cox2-F	GGC AAA TGG GTT TTC AAG ATC C	[30]
	cox2-RC4	TGA TTW AYN CCA CAA ATT TCR CTA CAT TG	[31]

DNA extraction, PCR, and phylogenetics

DNA extraction was performed on a BioSprint 96 Kingfisher flex robot (Thermo Fisher Scientific, USA) using a Qiagen plant tissue DNA extraction kit (Qiagen GmbH, Germany). Primer pairs for PCR amplification of the internal transcribed spacers (ITS), large nuclear ribosomal subunit (nrLSU), *cytochrome oxidase* I (*cox*1), and *cytochrome oxidase* II (*cox*2) are listed in Tab. 1.

The PCR reaction mix contained 1× PCR buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.8 µg BSA, 0.4 µM of each primer, 0.5 U *Taq* polymerase and 10–50 ng DNA. Cycling conditions for ITS were as follows: initial denaturation at 94°C for 4 min, followed by 36 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 20 s, and elongation at 72°C for 60 s. Subsequently, a final elongation at 72°C for 4 min was carried out. For LSU, initial denaturation was at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 53°C for 20 s, and elongation at 72°C for 120 s. Subsequently, a final extension was carried out at 72°C for 7 min. Cycling conditions for the *cox*2 region were: initial denaturation at 94°C for 4 min, followed by 36 cycles of denaturation at 94°C for 40 s, annealing at 51°C for 40 s, and elongation at 72°C for 40 s. Subsequently, a final elongation was carried out at 72°C for 4 min. The *cox*1 locus was amplified with the following cycling conditions: initial denaturation at 95°C for 4 min, followed by 36 cycles of denaturation at 95°C for 40 s, annealing at 51°C for 40 s, and elongation at 72°C for 60 s. Subsequently, a final extension was carried out at 72°C for 5 min. PCR reactions were carried out on an Eppendorf Mastercycler Pro (Eppendorf AG, Germany). PCR products were sequenced at SBiK-F sequencing laboratory with the primers used for PCR. Sequences were analyzed, assembled into contigs, and edited using Geneious version 5.0.4 (Biomatters Ltd., USA). Edited contigs in FASTA format and other sequences obtained from the NCBI webserver (https://www.ncbi.nlm.nih.gov/) (Tab. S1) were uploaded to the TrEase webserver (http://www.thines-lab.senckenberg.de/trease/) for the alignment of sequences and phylogenetic tree construction. The sequence alignment was generated using MAFFT with the G-INS-i algorithm [15]. Phylogenetic trees were constructed for all loci using the following programs: FastTree for minimum evolution (ME) [16] with 1000 bootstrap replicates using the generalized time-reversible (GTR) model; RAxML for maximum likelihood (ML) with 1000 bootstrap replicates using the GTR-GAMMA model [17]; and MrBayes [18] for Bayesian inference and the calculation of Bayesian posterior probabilities (BPP) using the 6 GTR model and four incrementally heated chains run for 1 000 000 generations, sampling every 10 000th generation, with the first 30% of the trees discarded for ensuring sampling from the stationary phase. After confirming that no strongly supported alternate topologies were present, the individual alignments were concatenated into a single FASTA file and the phylogenetic inference was done on the combined dataset as described above. Phylogenetic trees were viewed and taxon labels were edited using MEGA, version 6 or 7 [19].

Results

Phytopythium cultures

The two *Phytopythium* isolates, USTCMS 4102 (Fig. 1) and USTCMS 4101 (Fig. 2), were isolated from mangrove leaf litter in the Philippines. Both isolates were obtained from yellow to brown leaves that were in direct contact with the water surface of the estuarine system.

Mean colony radial growth (Fig. 3) of *Phytopythium* sp. USTCMS 4101 and *Phytopythium* sp. USTCMS 4102 varied in relation to the agar medium used. Specifically, mean colony radial growth on vegetable juice agar was observed to be 11.25 and 6.75 mm/day at 25 and 30°C, respectively, for the former strain, 9.25 and 10.25 mm/day at 25 and 30°C, respectively, for the latter strain. However, USTCMS 4101 was also capable of growing at 35°C with a radial growth similar to the growth observed at 30°C. In contrast, *Phytopythium* sp. USTCMS 4102 showed limited growth on all culture media when incubated at 35°C.

Phytopythium sp. USTCMS 4102 sporulated within 1 day in saline solution at 10–30 g/L when incubated at room temperature (20–25°C) in the dark. *Phytopythium* sp. USTCMS 4101 sporulated in an unsterile soil extract solution with continuous light at 20–23°C.

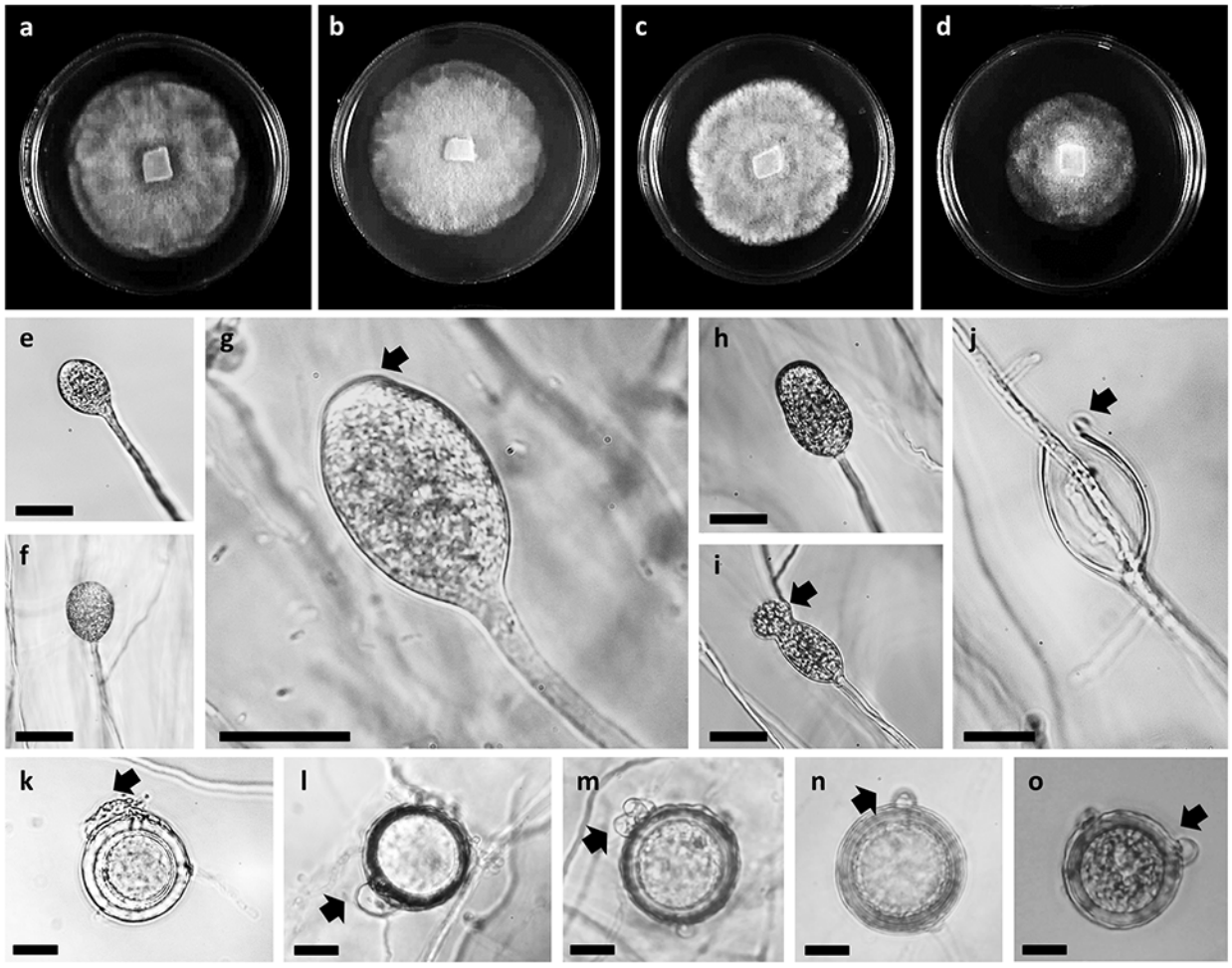

Fig. 1 Morphology of *Phytopythium leanoi* USTCMS 4102. Colony patterns on (**a**) vegetable juice agar, (**b**) potato carrot agar, (**c**) peptone yeast glucose agar, and (**d**) potato dextrose agar. **e,f** Immature sporangia. **g** Semipapillate sporangium, hyaline, and (**h**) a nonpapillate sporangium. **i** Zoospore release, zoospores develop both in the sporangium and vesicle (arrow). **j** Sporangium proliferation, arrow pointing to the operculum, which sometimes curls distally from the sporangium after some time. **k** Elongate, lobate antheridium with constrictions, oospore plerotic. **l** Lobate antheridium. **m** Antheridium with two lobes, oospores thick-walled, plerotic. **n,o** Single-lobed antheridia, oogonium with plerotic oospore. Scale bars: 20 μm.

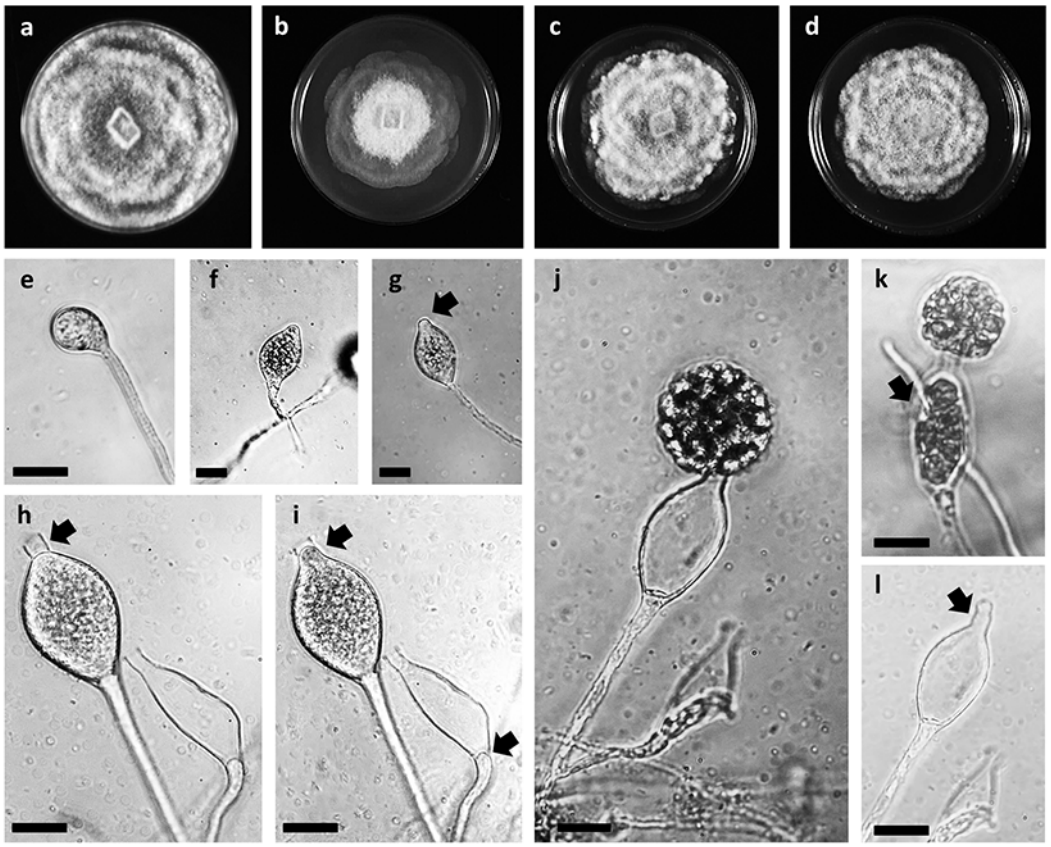

Fig. 2 Morphology of *Phytopythium dogmae* USTCMS 4101. Colony patterns on (**a**) vegetable juice agar, (**b**) potato carrot agar, (**c**) peptone yeast glucose agar, and (**d**) potato dextrose agar. **e** Immature sporangium. **f** Developing sporangium with a developing papilla. **g** Papillate sporangium (arrow). **h,i** Papilla develops into a discharge tube that guides the discharging protoplasma (arrow) forming an external vesicle that will nest at the apex of the sporangia. **i** Convex basal plug (arrow). **j** Development of zoospores in the vesicle. **k** Zoospores can similarly mature and develop inside the sporangia. **l** Empty sporangium; note the presence of the short discharge tube (arrow). Scale bars: 20 µm.

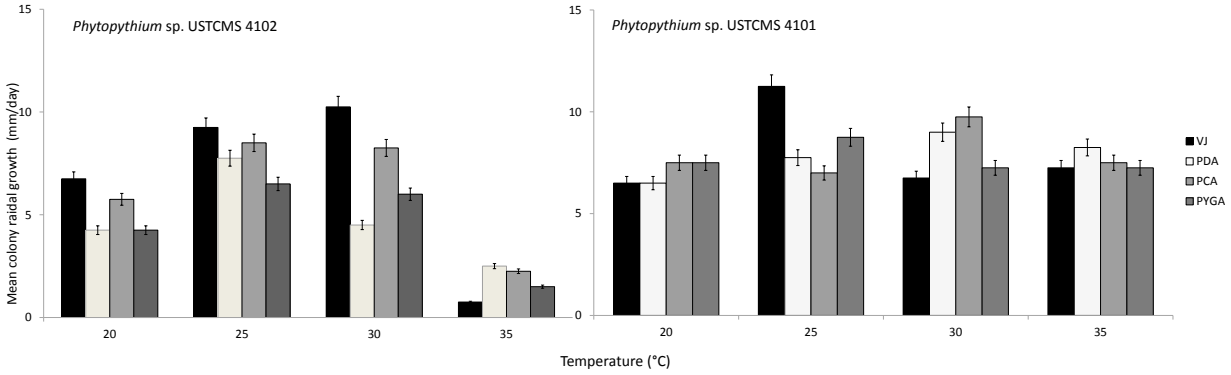

Fig. 3 Mean colony radial growth of *Phytopythium leanoi* USTCMS 4102 and *Phytopythium dogmae* USTCMS 4101.

Morphology

Phytopythium sp. USTCMS 4102 (Fig. 1) was initially identified as *Phytopythium* aff. *kandeliae* based on the mode of zoospore release and shape of sporangia. *Phytopythium* sp. USTCMS 4102 developed non- to semipapillate, obovoid to pyriform sporangia, with a size of 22–34 × 27–43 µm (average 28 × 35 µm; n = 100) within the standard deviation. Proliferation was both internal and external and branching sympodially. Zoospores of this strain developed both within vesicles and sporangia, and, upon

maturity, biflagellate cells moved rapidly inside the vesicle before rupture and release. An operculum was visible once sporangia were empty, which curled distally after some time. Interestingly, USTCMS 4102 is a homothallic species. Oogonia were formed either terminally or intercalary, with a size of 36–46 µm (average 41 µm; n = 100) within the standard deviation; oospores were mostly plerotic with a size of 31–41 µm (average 36 µm; n = 100) within the standard deviation. The antheridia were either lobate with constrictions, elongate, or bilobed and were attached at places all over the oogonial surface.

USTCMS 4102 is distinct from *P. kandeliae* NBRC 32620 (Tab. 2) on the basis of development of intercalary or terminal oogonia with an elongate to cylindrical antheridium and on the process by which sporangia proliferate – internal and external for the former and external for the latter.

Sporangia of USTCMS 4101 were obovoid, ovoid to pyrifom and limoniform, with a size of 25–27 × 29–37 µm (average 26 × 33 µm; n = 100) within the standard deviation. A papilla was formed at maturity, which further developed into a short dehiscence tube (mostly 2–10 µm long). Before zoospore release, the protoplasmic component of the sporangia gradually passed through the apical discharge tube until it came to a rest at

Tab. 2 Morphological differences between *P. kandeliae* and *P. leanoi*.

Characters	*P. leanoi* (USTCMS 4102)	*Phytopythium* sp. (CBS 113.91)	*P. kandeliae* (NBRC 32620 ex-type)	*Phytopythium* sp. (CBS 111.91)
Pattern on VJ plate	Abundant aerial mycelium, forming slightly petaloid colonies on VJ plate	Abundant aerial mycelium, forming slightly petaloid colonies on VJ plate	Abundant aerial mycelium, forming petaloid colonies on VJ plate	Abundant aerial mycelium, forming petaloid colonies on VJ plate
PDA, PYGA, PCA	Petaloid, rosette-like	Petaloid, rosette-like	Petaloid, rosette-like	Petaloid, rosette-like
Mature sporangium shape	Obovoid, pyrifom	Not observed	Turbinate, obovate, pyriform	Turbinate, obovate, pyriform
Papilla/apical projection	Non/semi papillate	Not observed	Non/semipapillate; presence of apical crescent-shaped translucent matrix	Non/semipapillate
Sporangium size	22–34 × 27–43 µm (average 28 × 35 µm)	Not observed	25–49 × 20–36 µm	22–56 × 20–45 µm
Proliferation type	Internal and external proliferation	Not observed	External proliferation	External proliferation
Branching pattern	Sympodial	Not observed	Sympodial	Sympodial
Operculum	Present	Not observed	Present	Present
Basal plug	Present	Not observed	Present	Present
Zoospore release	*Pythium*-like	Not observed	*Pythium*-like	*Pythium*-like
Antheridium	Elongate or cylindrical with constrictions; 1(–2) antheridia per oogonium	Not observed	Not observed	Not observed
Oogonium and oospores	Terminal or intercalary oogonia, 36–46 µm (average 41 µm); oospores plerotic, some are aplerotic; thick-walled, 31–41 µm (average 36 µm)	Not observed	Not observed	Not observed
Chlamydospores	Not observed	Not observed	Not observed	Not observed

its apex. Zoospores developed inside the vesicle and often also inside the sporangium. After some time, zoospores gradually began to move within the vesicle until the rupture of the vesicle and the subsequent release of zoospores took place. No gametangia were observed for USTCMS 4101. In Tab. 3, the morphological differences of species closely related to USTCMS 4101 are summarized.

Tab. 3 Morphology of Clade 2 species of *Phytopythium*.

Characters	*P. dogmae* USTCMS 4101	*P. helicoides* [32]	*P. chamaehyphon* [33,34]	*P. fagopyri* [23]	*P. paligenes* [35]
Pattern on VJ plate	Petaloid, rosette	No specific pattern, dense mycelia	No specific pattern, dense mycelia [33]	*Chrysanthemum*-like	*
Mature sporangium shape	Obovoid, ovoid, pyriform, limoniform	Ovoid to globose	Subspherical	Subglobose, pyriform	Subspherical, ovoid
Papilla/apical projection	Present, papilla develops into discharge tube at maturity (2–10 µm)	Present, papilla could grow approximately 20 µm serving as discharge tubes	Exit tube (dehiscence tube) 5 µm [33]	Present, papilla develops into discharge tube where vesicle exists	Present, develops into an evacuation tube (2–35 µm)
Sporangium size	25–27 × 29–37 µm (average 26 × 33 µm)	30.2–5.5 × 24.5–35.2 µm	15–30 µm [33]; 18–28 µm [34]	27–37 × 17–39 µm	24–42 × 18–36 µm
Proliferation type	External/internal (more frequent) proliferation	Internal proliferation	Internal and internally nested [34]	Internal or internally nested	Internal or internally nested
Operculum	Absent	Absent	*	*	*
Basal plug	Absent	Absent	*	*	*
Zoospore release	Zoospores formed in the vesicle or inside the sporangium (contains ~18–30 zoospores)	*Pythium*-like	*	Formation of vesicles (contained ~20 zoospores)	Zoospores formed in a vesicle, 6–30 zoospores
Antheridium	Not observed	Elongated, smooth, wavy, applied laterally to oogonia; 1–2 antheridia per oogonium	Not observed [33]; monoclinous, diclinous, applied laterally, broadly to the oogonium [34]	1–3 antheridia per oogonium, mainly diclinous	1–4 antheridia, average of 2, per oogonium, diclinous
Oogonium and oospore	Not observed	Terminal, intercalary, or lateral oogonia, 29.2–34.9 µm in diameter; oospores aplerotic, 23.7–32.5 µm in diameter	Not observed in artificial media, only on host [33]; oogonia smooth and globose, 33.5 µm in diameter on average, oospores aplerotic, 30.5 µm in diameter on average [34]	Present, oogonia globose, 28–31 µm in diameter; oospores aplerotic, 23–25 µm in diameter	Terminal, intercalary, or lateral oogonia, 19–41 µm in diameter; oospore 18–37 µm in diameter
Chlamydospores	Not observed	Not observed	Not observed	*	*
Radial growth on potato carrot agar at 25°C (mm/day)	7.75	15 [23]	22 [23]	10	*

* No data available.

Phylogeny

Based on the concatenated sequences of ITS, *cox*1, *cox*2, and LSU, USTCMS 4102 belongs to Clade 1 [20,21] of *Phytopythium* (Fig. 4, Fig. S1–Fig. S4). Interestingly, *P. kandeliae* is split into several distinct lineages. The ex-type specimen *P. kandeliae* NBRC 32620 [22] grouped together with the strain CBS 111.91 in trees based on all loci used, whereas USTCMS 4102 grouped with the strain CBS 113.91. Both CBS strains 111.91 and 113.91 were isolated from Taiwan and are referred to on the KNAW website as an uncharacterized species of *Phytopythium*. The strain *P. kandeliae* AJM 26, which was isolated from Brazil, clustered with the ex-type sequences of *P. kandeliae* in phylogenies based on ITS (Fig. S1), *cox*1 (Fig. S2), and LSU (Fig. S4). *Phytopythium* sp. USTCMS 4101 (Fig. 2) grouped with *P. fagopyri*, *P. palingenes*, *P. chamaehyphon* CBS 259.30, and *P. helicoides* CBS 286.31 (Fig. 4, Fig. S1–Fig. S4), all members of Clade 2 of *Phytopythium* [23,24].

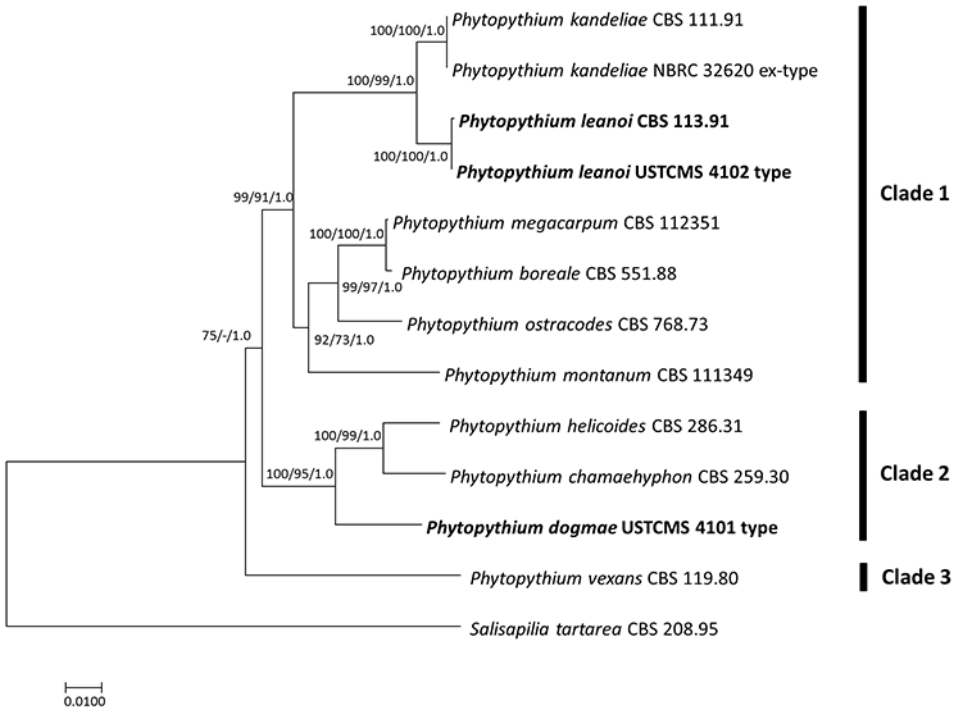

Fig. 4 Minimum evolution phylogenetic reconstruction generated from concatenated sequences of ITS, *cox*1, *cox*2, and LSU. Support values from minimum evolution, and maximum likelihood bootstrapping, as well as Bayesian posterior probabilities are given on the branches in the respective order. "-" denotes support values below 50 or alternate, unsupported topologies. The scale bar indicates the number of nucleotide substitutions per site.

Discussion

According to de Cock et al. [20], key characteristics for *Phytopythium* are the following: predominantly papillate sporangia and internal proliferation in most species as well as often constricted and elongate to cylindrical antheridia. However, there is a large degree of variation in sporangium characteristics. Phylogenetically, the *Calycofera-Phytopythium* lineage [25] is one of the early-diverging branches in the sister clade to *Pythium* that also harbors *Phytophthora* [2]. This is reflected by some morphological features shared with the one or the other of these genera, i.e., a sporangial shape similar to the majority of *Phytophthora* species and a process of zoospore release similar to most *Pythium* species.

The strains USTCMS 4102 and USTCMS 4101 constitute two new records of *Phytopythium* species from the Philippines, adding to *P. kandeliae* [10]. Both strains were isolated from mangrove leaf litter, where they acted as saprotrophs.

The strain USTCMS 4102 was inferred to be a member of the Clade 1 [23,24] group of *Phytopythium* (Fig. 4) and is almost sequence-identical to CBS 113.91 isolated from Taiwan. The ex-type strain of *P. kandeliae* NBRC 32620 is phylogenetically distinct from the aforementioned strains but sequence-identical to CBS 111.91 in all loci included in this study. Sporangia of *P. kandeliae* NBRC 32620 and CBS 111.91, as well as those of USTCMS 4102 and CBS 113.91, are operculate and non- to semipapillate [21] (also, this study). However, sporangia for the former two strains were turbinate, obovate to pyriform, and formed an apical translucent crescent-shaped matrix [22] (also, this study), while the sporangia of the latter two strains were obovoid to pyriform and without an apical crescent-shaped translucent matrix. The proliferation of the sporangia was external for *P. kandeliae* NBRC 32620 and CBS 111.91, while it was both internal and external for USTCMS 4102. With respect to colony morphology, there are also some differences between the groups, as USTCMS 4102 and CBS 113.91 formed rosette-petaloid-like colonies with rounded edges, whereas the colony edges of *P. kandeliae* NBRC 32620 and CBS 111.91 were acute (Tab. 4). Even if these differences might seem minor, they were consistently observed and thus seem stable enough to allow for differentiation.

Tab. 4 Colony patterns of *P. leanoi* and *P. kandeliae*.

Species	PCA	PGYA	VJ	PDA
Phytopythium leanoi USTCMS 4102 ex-type				
Phytopythium leanoi CBS 113.91				
Phytopythium kandeliae NBRC 32620 ex-type				
Phytopythium kandeliae CBS 111.91				

Phylogenetic analyses presented herein are in line with those of Baten et al. [23] and Jesus et al. [24], where *Phytopythium* was divided into three clades based on LSU, ITS, *cox*1, and *cox*2 sequences. Clade 1 includes 15 taxa and these are *P. aichiense*, *P. boreale*, *P. carbonicum*, *P. citrinum*, *P. delawarense*, *P. iriomotense*, *P. litorale*, *P. mercuriale*, *P. megacarpum*, *P. mirpurense*, *P. montanum*, *P. oedochilim*, *P. ostracodes*, *P. sindhum*, and *P. sterilum*. *Phytopythium chamaehyphon*, *P. fragopyri*, *P. palingenes*, and *P. helicoides* were the taxa of the monophyletic Clade 2. *Phytopythium cucurbitacearum* and *P. vexans* are those of Clade 3. Baten et al. [23] construed that the taxa of Clades 1 and 2 have papillate sporangia and proliferate internally (nested and extended) as well as externally; Clade 3 species have nonpapillate sporangia and do not proliferate internally.

USTCMS 4101 is a member of the Clade 2 of *Phytopythium* [23,24], and differs from all other members of the clade by featuring both internal and external proliferation, while other members of this clade are featuring extended or nested-internal proliferation [20]. USTCMS 4101 is similar to *P. chamaehyphon*, *P. fagopyri*, *P. helicoides*, and *P. palingenes*. However, it has a different combination of key morphological characters

(e.g., proliferation of the sporangia and the number of sporangia per sporangiogenic hypha; see also Tab. 3). The sister taxon to USTCMS 4101 is *P. palingenes*, from which it differs in terms of the proliferation of sporangia, the length of discharge tubes (which is longer in *P. palingenes*), and the number of sporangia per sporangiogenic hypha, which are often two in USTCMS 4101 but usually single in *P. palingenes*.

Based on phylogenetic distinctiveness and combination of morphological characters, neither USTCMS 4101 nor USTCMS 4102 can be assigned to any known species of *Phytopythium* and are thus introduced as new species in the "Taxonomy" section of this paper. Considering the recent discovery of some new species of *Phytopythium* from a variety of habitats and that only mangroves were sampled in the current study, it seems likely that additional species of *Phytopythium* await discovery in the Philippines and beyond.

Taxonomy

Phytopythium leanoi R. Bennett et Thines, sp. nov.
Mycobank No.: MB 819795

Type specimen. Isolated from the Philippines, Pagbilao, Quezon, ERDB Pagbilao Mangrove Forest (13.975698° N, 121.725363° E) from mangrove leaf-litter, collected on September 19, 2015, leg. RM Bennett & GR Dedeles, holotype herbarium specimen USTH 013930 (= PQ2YBP293) (University of Santo Tomas Herbarium, Manila, Philippines), ex-type strain USTCMS 4102 (University of Santo Tomas Collection of Microbial Strains).

Etymology. Dedicated to Eduardo Leaño, in recognition of his contributions to the knowledge on oomycetes of the Philippines.

Description. Straminipila, Oomycota, Peronosporales, Peronosporaceae.Hyphae hyaline, aseptate. Sporangiophores undifferentiated, branching sympodially. Sporangia nonpapillate to semipapillate, obovoid to pyriform, noncaducuous, typically 22–34 × 27–43 µm (average 28 × 35 µm), proliferation predominantly external submerged in 10–30 g/L saline solution. Basal plug present, hyaline. Zoospore release *Pythium*-like, operculum present. Homothallic. Oogonia globose, lateral, terminal or intercalary, typically 36–46 µm (average 41 µm) in diameter. Oospores plerotic, sometimes aplerotic, typically 31–41 µm (average 36 µm) in diameter, oospore wall thickness 2–5 µm. Antheridia one(–two) per oogonium, often elongate, cylindrical and constricted, laterally attached to the oogonium, monoclinous or diclinous. Chlamydospores not observed.

Colony characteristics. Grows on corn meal agar (CMA), oatmeal agar (OA), peptone yeast glucose agar (PYGA), potato carrot agar (PCA), and vegetable juice agar (VJ). Growth on VJ with abundant aerial mycelium. Colonies stellate to rosette-like with rounded edges on all culture media used; except OA and CMA, and when old in VJ agar.

Known distribution. Philippines, Taiwan.

Phytopythium dogmae R. Bennett et Thines, sp. nov.
Mycobank No.: MB 819796

Type specimen. Isolated from the Philippines, Padada, Davao del Sur (6.659833° N, 125.380733° E), from decaying mangrove leaf-litter, collected on the September 5, 2015, leg. RM Bennett & GR Dedeles, holotype herbarium specimen USTH 013931 (= P1YBL1144) (University of Santo Tomas Herbarium, Manila, Philippines), ex-type strain USTCMS 4101 (University of Santo Tomas Collection of Microbial Strains, Philippines).

Etymology. Dedicated to Irineo J. Dogma Jr., for his pioneering research on "zoosporic fungi" in the Philippines.

Description. Straminipila, Oomycota, Peronosporales, Peronosporaceae. Hyphae hyaline, aseptate. Sporangiophores undifferentiated, branching sympodially, producing one to two terminal sporangia. Immature sporangia nonpapillate, ovoid to obovoid. Mature sporangia papillate, limoniform, obovoid, obpyriform, pyriform, noncaducuous, typically 25–27 × 29–37 µm (average 26 × 33 µm). Proliferation internal and external. Basal plug convex in empty sporangia. Zoospore release *Pythium*-like, papilla developing into a discharge tube (~2–10 µm); after the dissolution of the tip of the dehiscence tube, the protoplasmic component ejects and nests at the apex of the tube enclosed by a vesicle; zoospores maturing typically inside the vesicle or sometimes inside the sporangium. Operculum absent. Gametangia not observed. Chlamydospores not observed.

Colony characteristics. Petaloid or rosette-like on VJ agar and PCA media with or without marine water. Grows on CMA, OA, PYGA, and PCA. Mycelia on VJ, PDA, and PYGA are typically with abundant aerial mycelium.

Known distribution. Philippines.

Acknowledgments

This research project was supported by the LOEWE excellence program of the government of Hessen, in the framework of the cluster for Integrative Fungal Research (IPF). Collection and transport permits were granted by the Biodiversity and Management Bureau, DENR-NCR Philippines. Support by KAAD and the Studienstiftung Mykologie for RMB is gratefully acknowledged.

Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/am/rt/suppFiles/am.1103/0:

Tab. S1 GenBank accession numbers of various loci used in this study.

Fig. S1 Phylogenetic tree based on minimum evolution using ITS sequence data with support values from ME, ML, and BPP, in the respective order.

Fig. S2 Phylogenetic tree based on minimum evolution using *cox*1 sequence data with support values from ME, ML, and BPP, in the respective order.

Fig. S3 Phylogenetic tree based on minimum evolution using *cox*2 sequence data with support values from ME, ML, and BPP, in the respective order.

Fig. S4 Phylogenetic tree based on minimum evolution using LSU sequence data with support values from ME, ML, and BPP, in the respective order.

References

1. Thines M. Phylogeny and evolution of plant pathogenic oomycetes – a global overview. Eur J Plant Pathol. 2014;138:431–447. https://doi.org/10.1007/s10658-013-0366-5
2. Bala K, Robideau GP, Lévesque CA, de Cock AWAM, Abad ZG, Lodhi AM, et al. *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi and Lévesque, gen. nov. and *Phytopythium sindhum* Lodhi, Shahzad, and Lévesque, sp. nov. Persoonia. 2010;24:136–137. https://doi.org/10.3767/003158510X512748
3. Hulvey J, Telle S, Nigrelli L, Lamour K, Thines M. Salisapiliaceae – a new family of oomycetes from marsh grass litter of southeastern North America. Persoonia. 2010;25:109–116. https://doi.org/10.3767/003158510X551763
4. Lara E, Belbahri L. SSU rRNA reveals major trends in oomycete evolution. Fungal Divers. 2011;49:93–100. https://doi.org/10.1007/s13225-011-0098-9
5. Villa NO, Kageyama K, Asano T, Suga H. Phylogenetic relationship of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and β-tubulin gene sequences. Mycologia. 2006;98:410–422. https://doi.org/10.1080/15572536.2006.11832676
6. Spies CF, Grooters AM, Lévesque CA, Rintoul TL, Redhead SA, Glockling SL, et al. Molecular phylogeny and taxonomy of *Lagenidium*-like oomycetes pathogenic to

mammals. Fungal Biol. 2016;120:931–947. https://doi.org/10.1016/j.funbio.2016.05.005

7. Uzuhashi S, Tojo M, Kakishima M. Phylogeny of the genus *Pythium* and description of new genera. Mycoscience. 2010;51:337–365. https://doi.org/10.1007/s10267-010-0046-7
8. Beakes GW, Honda D, Thines M. Systematics of the Straminipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. In: McLaughlin DJ, Spatafora JW, editors. Systematics and evolution. Berlin: Springer; 2014. p. 39–97. (Mycota; vol 7A). https://doi.org/10.1007/978-3-642-55318-9_3
9. Lévesque CA, de Cock AWAM. Molecular phylogeny and taxonomy of the genus *Pythium*. Mycol Res. 2004;108:1363–1383. https://doi.org/10.1017/S0953756204001431
10. Leaño EM. Straminipilous organisms from fallen mangrove leaves from Panay Island, Philippines. Fungal Divers. 2001;6:75–81.
11. Jesus AL, Marano AV, Jerônimo GH, De Souza JI, Leaño EM, Pires-Zottarelli CLA. The genus *Halophytophthora* (Peronosporales, Straminipila) in Brazil: first descriptions of species. Rev Bras Bot. 2016;39:729–739. https://doi.org/10.1007/s40415-016-0257-z
12. Bennett RM, Thines M. Confirmation that *Phytophthora insolita* (Peronosporaceae) is present as a marine saprotroph on mangrove leaves and first report of the species for the Philippines. Nova Hedwigia. 2017;105:185–196. https://doi.org/10.1127/nova_hedwigia/2017/0404
13. Crous PW, Verkley GJM, Groenewald JZ, Ramson RA. Fungal biodiversity. Utrecht: CBS-KNAW Fungal Biodiversity Centre; 2009. (CBS Laboratory Manual Series).
14. Solis MJL, Draeger S, Dela Cruz TEE. Marine-derived fungi from *Kappaphycus alvarezii* and *K. striatum* as potential causative agents of ice-ice disease in farmed seaweeds. Botanica Marina. 2010;53:587–594. https://doi.org/10.1515/bot.2010.071
15. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30:3059–3066. https://doi.org/10.1093/nar/gkf436
16. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum-evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009;26:1641–1650. https://doi.org/10.1093/molbev/msp077
17. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033
18. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–542. https://doi.org/10.1093/sysbio/sys029
19. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 2013;30:2725–2729. https://doi.org/10.1093/molbev/mst197
20. de Cock AWAM, Lodhi AM, Rintoul TL, Bala K, Robideau GP, Abad ZG, et al. *Phytopythium*: molecular phylogeny and systematics. Persoonia. 2015;34:25–39. https://doi.org/10.3767/003158515X685382
21. Marano AV, Jesus AL, de Souza JI, Leaño EM, James TY, Jerônimo GH, et al. A new combination in *Phytopythium*: *P. kandeliae* (Oomycetes, Straminipila). Mycosphere. 2014;5:510–522. https://doi.org/10.5943/mycosphere/5/4/3
22. Ho HH, Chang HS, Hsieh SY. *Halophytophthora kandeliae*, a new marine fungus from Taiwan. Mycologia. 1991;83:419–424. https://doi.org/10.2307/3760352
23. Baten MA, Asano T, Motohashi K, Ishiguro Y, Rahman MZ, Inaba S, et al. Phylogenetic relationships among *Phytopythium* species, and re-evaluation of *Phytopythium fagopyri* comb. nov., recovered from damped-off buckwheat seedlings in Japan. Mycol Prog. 2014;13:1145–1156. https://doi.org/10.1007/s11557-014-1003-1
24. Jesus AL, Gonçalves DR, Rocha SCO, Marano AV, Jerônimo GH, de Souza JI, et al. Morphological and phylogenetic analyses of three *Phytopythium* species (Peronosporales, Oomycota) from Brazil. Cryptogamie, Mycologie. 2016;37:117–128. https://doi.org/10.7872/crym/v37.iss1.2016.117
25. Bennett RM, de Cock AWAM, Lévesque CA, Thines M. *Calycofera* gen. nov., an estuarine sister taxon to *Phytopythium*, Peronosporaceae. Mycol Prog. 2017;16:947–954. https://doi.org/10.1007/s11557-017-1326-9
26. Bachofer M. Molekularbiologische populationsstudien an *Plasmopara halstedii*, dem falschen mehltau der sonnenblume [PhD thesis]. Stuttgart: University of Hohenheim;

2004.

27. Moncalvo JM, Wang HH, Hseu RS. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia. 1995;87:223–238. https://doi.org/10.2307/3760908
28. Riethmüller A, Voglmayer H, Göcker M, Weiß M, Oberwinkler F. Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large ribosomal DNA sequences. Mycologia. 2001;94:834–849. https://doi.org/10.1080/15572536.2003.11833177
29. Robideau GP, de Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, et al. DNA barcoding of oomycetes with cytochrome *c* oxidase I and internal transcribed spaces. Mol Ecol Resour. 2011;11:1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x
30. Hudspeth DSS, Nadler SA, Hudspeth MES. A *COX2* molecular phylogeny of the *Peronosporomycetes*. Mycologia. 2000;92:674–684. https://doi.org/10.2307/3761425
31. Choi YJ, Beakes G, Glockling S, Kruse J, Nam B, Nigrelli L, et al. Towards a universal barcode of oomycetes – a comparison of the *cox*1 and *cox*2 loci. Mol Ecol Resour. 2015;15:1275–1288. https://doi.org/10.1111/1755-0998.12398
32. Chen XR, Liu BB, Xing YP, Cheng BP, Liu ML, Tong YH, et al. Identification and characterization of *Phytopythium helicoides* causing stem rot of Shatangju mandarin seedlings in China. Eur J Plant Pathol. 2016;146:715–727. https://doi.org/10.1007/s10658-016-0952-4
33. Sideris CP. Taxonomic studies in the family Pythiaceae II. *Pythium*. Mycologia. 1932;24:14–61. https://doi.org/10.2307/3753729
34. van der Plaats-Niterink AJ. Monograph of the genus *Pythium*. Stud Mycol. 1981;21:1–242.
35. Drechsler C. Some new species of *Pythium*. J Wash Acad Sci. 1930;20:399–418.