Mycosphere 5 (4): 510-522 (2014)
www.mycosphere.org
Copyright © 2014

Article

ISSN 2077 7019
Mycosphere
Online Edition

Doi 10.5943/mycosphere/5/4/3

A new combination in *Phytopythium: P. kandeliae* (Oomycetes, Straminipila)

Marano AV[1], Jesus AL[1], de Souza JI[1], Leaño EM[2], James TY[3], Jerônimo GH[1], de Cock AWAM[4] and Pires-Zottarelli CLA[1]

[1]*Instituto de Botânica, Núcleo de Pesquisa em Micologia, Av. Miguel Stéfano 3687, CEP 04301-012, São Paulo, SP, Brazil. *agosvm@hotmail.com*
[2]*Network of Aquaculture Centres in Asia-Pacific, Suraswadi Building, Kasetsart University Campus, Jatujak, Ladyao, Bangkok 10900, Thailand*
[3]*Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA*
[4]*CBS-KNAW, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands*

Marano AV, Jesus AL, de Souza JI, Leaño EM, James TY, Jerônimo GH, de Cock AWAM, Pires-Zottarelli CLA 2014 – A new combination in *Phytopythium*: *P. kandeliae* (Oomycetes, Straminipila). Mycosphere 5(4), 510-522, Doi 10.5943/mycosphere/5/4/3

Abstract

Phytopythium is a new segregate genus of the diverse and polyphyletic oomycete genus *Pythium*. We analysed the morphology and phylogeny (partial large and small subunits and the complete internal transcribed regions of the ribosomal DNA and the cytochrome *c* oxidase subunit I region of the mitochondrial DNA) of an authentic strain and three recent isolates of *Halophytophthora kandeliae* collected from a mangrove area in Brazil. Maximum likelihood trees showed that all isolates clustered within the clade provisionally named *Phytopythium*, which includes 13 species of *Pythium* from clade K, and the type species of the genus *Phytopythium*, *P. sindhum*. Based on the results presented in this paper and previous studies, we consider that *H. kandeliae* should be transferred to the genus *Phytopythium* and therefore we proposed a new combination, *P. kandeliae*.

Key words – *Halophytophthora kandeliae*, morphology, phylogeny, *Pythium* clade K, taxonomy

Introduction

Traditionally, genera and species in Oomycetes have been distinguished and defined based on morphological characteristics. The genus *Pythium* is characterized by its well developed mycelial thallus and the discharge of an undifferentiated mass of protoplasma from the sporangia through a tube into an evanescent vesicle. Subsequently, this protoplasm differentiates into zoospores inside the vesicle. Although this way of zoospore discharge is shared by all *Pythium* species, *Pythiogeton* and *Lagenidium* display the same way of discharge and are considered different genera based on other characteristics. On the other hand, *Pythium* is heterogeneous with regard to other morphological characters like, e.g., the sporangium shape, which appears to be evolutionary significant and ranges from filamentous, filamentous-inflated, contiguous, globose to globose internally proliferating (van der Plaats-Niterink 1981).

Submitted 8 June 2014, Accepted 18 June 2014, Published online 6 August 2014
Corresponding Author: Agostina Marano – e-mail – agosvm@hotmail.com

Since its original description (Pringsheim 1858), new classifications of *Pythium* have been proposed based on morphological characteristics (e.g. Fischer 1892, Schröter 1897). Particularly in the last few years, *Pythium* has been subjected to several phylogenetic analyses and many new species have been described (e.g., Lévesque & de Cock 2004, Broders et al. 2009, Karaca et al. 2009, Senda et al. 2009, Bala et al. 2010, Uzuhashi et al. 2010). Many studies have shown that this genus is not monophyletic and that species are grouped together according to sporangial morphology. Results from Lévesque & de Cock (2004) first showed two major clades corresponding to *Pythium* species with filamentous sporangia (inflated or non inflated) or globose sporangia and a small clade between these two, represented by the species with contiguous sporangia. Using sporangial morphology and phylogeny of the regions LSU rDNA and COII mDNA, Uzuhashi et al. (2010) showed that *Pythium* is polyphyletic and composed of five monophyletic clades, each characterized by a particular type of sporangium. Consequently, the genus was emended and four new genera were split off: *Ovatisporangium, Globisporangium, Elongisporangium,* and *Pilasporangium*. Analysis of rDNA regions and mDNA (COI) showed that a clade within *Pythium* (=*Pythium* clade K from Lévesque & de Cock 2004) that was already included in the genus *Ovatisporangium* (clade 1 from Uzuhashi et al. 2010), is actually more closely related to *Phytophthora* than to *Pythium* (Bala et al. 2010, Uzuhashi et al. 2010, Robideau et al. 2011, Marano et al. in press). Members of this clade, which was provisionally named *Phytopythium* (Bala et al. 2010), appear to be morphologically and phylogenetically between *Pythium* and *Phytophthora*. Their diagnostic characteristics are the presence of globose to ovoid sporangia, which often have a papilla and proliferate internally like occur in the genus *Phytophthora,* and have zoospore discharge as in *Pythium*. Most species have large, smooth oogonia with thick-walled oospores, and 1–2 elongate or lobate and laterally applied antheridia (Bala et al. 2010). Currently, this genus contains only one species formally described, the type species of the genus, *P. sindhum*, which was isolated from the rhizosphere of *Musa paradisiaca* L. in Pakistan (Bala et al. 2010).

Main morphological characters have also shown to be not homogeneous and well- defined for all members of other genera that are closely related to *Pythium* and *Phytophthora*, such as *Halophytophthora*. For example, the type species *H. vesicula*, was first described in *Phytophthora*, since both genera share the same type of zoospore discharge, with full differentiation of zoospores within the sporangium (Anastasiou & Churchland 1969, Fell & Master 1975, Pegg & Alcorn 1982, Gerrettson-Cornell & Simpson 1984). Subsequently, based mainly on their ecological preference (estuarine and brackish habitats, mostly saprophytic) and morphological characteristics (apical structure of the sporangia, mode of zoospore emission), this and another eight species of *Phytophthora* were transferred to *Halophytophthora* (Ho & Jong 1990). As a consequence, this genus appears to be highly polyphyletic (Marano et al. in press). More recently, one species of *Halophytophthora*, *H. tartarea,* was transferred to the newly described genus *Salisapilia* on the basis of oospore formation (Hulvey et al. 2010). This latter genus differs from *Halophytophthora* in a few morphological characteristics (absence of a vesicle during zoospore discharge, the presence of a plug of material at the apex of the discharge tube and oospore formation) and was mainly supported by molecular evidence (Hulvey et al. 2010, Nigrelli & Thines 2013). Although *Salisapilia* appears as a well-supported monophyletic clade in ITS and LSU phylogenies (Hulvey et al. 2010, Nigrelli & Thines 2013), morphological characters that circumscribe this genus are in need of revision. For example, oospore formation has been documented in other species of *Halophytophthora* when first described, such as in *H. epistomia* (Ho et al. 1990) and *H. exoprolifera* (Ho et al. 1992). In addition, in *Salisapilia nakagirii* (ex-type CBS 127947), no zoosporangia were observed by Hulvey et al. (2010) and therefore, the absence of vesicle in this species could not be confirmed.

Considering this and previous molecular results (Hulvey et al. 2010, Nigrelli & Thines 2013), *Halophytophthora* does not appear to be a natural grouping. Moreover, recent results have shown that one species, *Halophytophthora kandeliae,* clustered within the *Phytopythium* clade

(Hulvey et al. 2010, Lara & Belbahri 2011, Nigrelli & Thines 2013, Marano et al. in press) and shares some morphological characteristics that are common to members of this clade.

The aim of this study is therefore to clarify the generic placement of the sequences deposited in GenBank as *Halophytophthora kandeliae* Ho, Chang & Hsieh, (including the sequence of the ex-type strain). These sequences were placed within the *Phytopythium* clade in previous studies (Bala et al. 2010, Robideau et al. 2011, Nigrelli & Thines 2013, Marano et al. in press), and exhibited high levels of molecular similarity with two voucher specimens deposited at the CBS-KNAW Fungal Biodiversity Centre as *H. kandeliae* and with three isolates tentatively assigned to *H. kandeliae* recovered from mangrove swamps in Brazil, based on morphological and phylogenetic analyses based of the partial LSU and SSU regions and the complete ITS region of the rDNA and the COI region of the mDNA.

Materials & Methods

Study area

The "Parque Estadual da Ilha do Cardoso" (PEIC), 25°03'05'-25°18'18''S; 47°53'48''-48°05'42''W, is an island located in the Atlantic Forest Domain (Ab'Saber 1977), which belongs to the estuarine complex called "Iguape-Cananéia-Paranaguá". The weather at the island is mega-thermal and super humid, with no defined dry season or excess of rainfall in summer (Funari et al. 1987). These conditions and the complex geographical configuration of the island lead to the establishment of a well-developed Atlantic Rain Forest and other vegetation types such as "restinga" (mainly composed by grasses, lichens, bromeliads and shrubs) and mangroves.

Sampling

Sampling was carried out in August and November 2012 at the Perequê river. Five sampling points (S0-S4) with different salinities were chosen along the river from typically freshwater to seawater (Table 1). Salinity (%) was measured with a Horiba® U-10 and U-51.

Table 1 Salinities (%) measured at each sampling point (S0-S4) of the Perequê river, "Parque Estadual da Ilha do Cardoso" (PEIC), Cananéia, SP. (*) indicates the salinities under which *Phytopythium kandeliae* was recovered.

	August 2012					**November 2012**				
Sampling points	S0	S1	S2	S3	S4	S0	S1	S2	S3	S4
Salinity	0.05*****	0.72	1.62	2.91	3.00	0.10*	0.79*****	1.67	2.17	2.72

At each sampling point, we collected samples consisting of standardized amounts of water (approximately 500 ml), and floating mangrove fallen leaves, especially of *Rhizophora mangle* L. and *Laguncularia racemosa* L. Gaertn (approximately 400 g). Samples of fallen leaves were collected with a pool leaf rake and placed in plastic bags while water samples were collected in sterile plastic containers. Both types of samples were transported to the laboratory in a cooler (4 °C) to prevent desiccation and excessive temperature.

Laboratory analysis

Leaves were separated according to the species (*R. mangle* / *L. racemosa*) and cut with a cork borer into discs of 1 cm diam. Leaf discs of each species were processed as follows: *(i)* placed into petri dishes with 30 ml of diluted seawater (prepared according to the salinity registered in the field) and baited with five *Sorghum* spp. seeds (Marano et al. 2008, Nascimento et al. 2011); *(ii)* placed onto petri dishes with PYGs solid culture medium (Meat peptone: 1.25 gl^{-1}, Yeast Extract: 1.25 gl^{-1} , Glucose: 3 gl^{-1}, Agar: 6 gl^{-1}) prepared with 50% sterile seawater with 0.5 gl^{-1} of each penicillin G and streptomycin sulphate (Newell & Fell 1994). In addition, 30 ml of sampled water were placed into petri dishes and baited with five *Sorghum* spp. seeds.

Dishes with culture medium were incubated in alternating periods of 12 h of light and 12 h of dark to stimulate the production of zoosporangia (Kohlmeyer et al. 2004). After 4-7 days of incubation, petri dishes were examined under the microscope to check for the presence of mycelia tentatively corresponding to Oomycetes. Afterwards, fragments of culture media containing mycelia from these dishes were placed in new petri dishes with 50% sterile seawater to stimulate sporulation. On the other hand, baited dishes were incubated at room temperature (± 20 °C) and observed at 4, 7, 10, 14, 21 and 30 days. The following culture media supplemented with antibiotics were used for isolation and purification of species: PYG (peptone-glucose agar), V8 (V8 juice agar), CMA (corn-meal agar) prepared with sterile deionized water (Fuller & Jaworski 1987) and PYGs, V8s and CMAs prepared with 50% filtered seawater.

Mycelium production, DNA extraction, amplification and sequencing

Mycelium for DNA extraction was obtained by cultivating each isolate in three 2 ml microfuge tubes containing 1 ml of PYGs liquid medium with 0.5 gl^{-1} of each streptomycin sulphate and penicillin G. After incubation for 5-10 days at 25°C, the mycelium was harvested by centrifugation at 13000 rpm for 15 min in order to obtain mycelial pellets. The mycelial pellets of the three replicates were aseptically combined in order to obtain enough biomass for DNA extraction. The supernatants were discarded and 1 ml of sterile deionized water was added to the tubes followed by vortexing at 2500 rpm. Tubes were again centrifuged and the supernatant discarded. Pellets were treated according to the protocol described in the PureLink Genomic DNA kit (Invitrogen®). Electrophoresis was performed using 1% (p/v) agarose gel applying 3-5 µl of the sample + 2 µl of Gel Loading Buffer Type I (Sigma®) and a standard of 2 µl of 123 bp DNA Ladder (Invitrogen®) + 2 µl of Gel Loading Buffer. Electrophoresis conditions were 100 mV for 40 min. The partial rDNA of the LSU and SSU regions and the complete ITS region were amplified by PCR with the forward/reverse primers LR0R and LR6-O, SR1R, NS4 and SR6.1, and ITS6 and ITS4, respectively (http://www.phytophthoradb.org, White et al. 1990) and the COI mDNA region with primers OomCoxI-Levup and OomCoxI-Levlo (Robideau et al. 2011). Alternatively, because the ITS region in the voucher specimen CBS 113.91 could not be amplified with primers ITS6 and ITS4, the primers UN-up18S42 and UN-lo28S22 were used (Robideau et al. 2011). DNA was amplified with the PCR SuperMix kit (Invitrogen®) for a final volume of 25 µl in a C1000 Touch™ Thermal Cycler Bio-Rad. PCR amplification of the LSU, SSU and ITS regions was as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 54 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min; while the conditions for amplification of the COI region were: initial denaturation at 95 °C for 2 min followed by 35 cycles of 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72° C for 1 min and a final extension at 72 °C for 10 min (Robideau et al. 2011). PCR products were analysed by electrophoresis on a 1% agarose gel using 1-2 µl Low DNA Mass Ladder (Invitrogen®) + 2-4 µl of Gel Loading Buffer as standard and 2-3 µl of DNA. Amplicons were purified with AxyPrep PCR Clean-up kit (Axygen®). Sequencing was performed using the primers LR0R, LR6-O and LSURint for the LSU region (http://www.phytophthoradb.org), SR1R, NS4 and SR6.1 for the SSU region, ITS6 and ITS4 for the ITS region (White et al. 1990) or alternatively UN-up18S42 and UN-lo28S22 (Robideau et al. 2011), and OomCoxI-Levup and OomCoxI-Levlo for the COI region (Robideau et al. 2011), in a ABI 3730 DNA Analyser (Life Technologies™) at the "Centro de Estudos do Genoma Humano", Instituto de Biociências, USP, São Paulo, Brazil (http://genoma.ib.usp.br).

Phylogenetic analysis

Editing and contig assembly of the DNA sequences were performed using Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA**)**. For phylogenetic reconstruction, the LSU, SSU and ITS rDNA and COI mDNA sequences of the isolates (Table 2) were compared with published sequences of species of closely related genera: *Halophytophthora, Pythium* (clades B, C, D, E, F, H and K) and *Phytophthora* (clades 1, 4, 6, 8 and 9) and, whenever available, each genus type

species deposited in GenBank. Sequences were aligned using MAFFT, and the ambiguously aligned characters removed using Gblocks 0.91b (Castresana 2000) with default parameters except gaps allowed in half of the sequences. This led to a remaining 1289 bp, 1672 bp, 420 bp and 737 bp for analysis for the LSU, SSU, ITS and COI regions, respectively. The best fitting model of evolution was then selected using the Akaike Information Criterion in jModeltest 2.1.4 (Posada 2008). The Maximum Likelihood (ML) phylogenies for each region were then created using PhyML 3.1 (Guindon & Gascuel 2003) using the best model for nucleotide substitution, branch swapping by best of NNI and SPR, and support for nodes obtained using 1,000 bootstrap pseudo-replicates.

Table 2 Origin, CCIBt culture collection number (CCIBt: culture collection of the "Instituto de Botânica", São Paulo State, Brazil) and GenBank accession number of the isolates of *Phytopythium kandeliae* sequenced in this study. NA: not available.

Isolate No		**Isolate Origin**		**GenBank Accession Number**			
	CCIBt culture collection	Substrate	Place	LSU	SSU	ITS	COI
AJM 26	4023	leaves of *Laguncularia racemosa*	Perequê river (S0), Brazil	KJ399965	KJ399967	KJ399962	NA
AJM 85	4024	leaves of *Laguncularia racemosa*	Perequê river (S0), Brazil	KJ399964	KJ406205	NA	KJ690247
AJM 95	4025	leaves of *Laguncularia racemosa*	Perequê river (S1), Brazil	KJ399966	KJ399968	NA	KJ690248
CBS 113.91*	4004	leaves of *Kandelia candel*	Taiwan	KJ399963	NA	KJ399961	KJ690245

*voucher specimen

Results

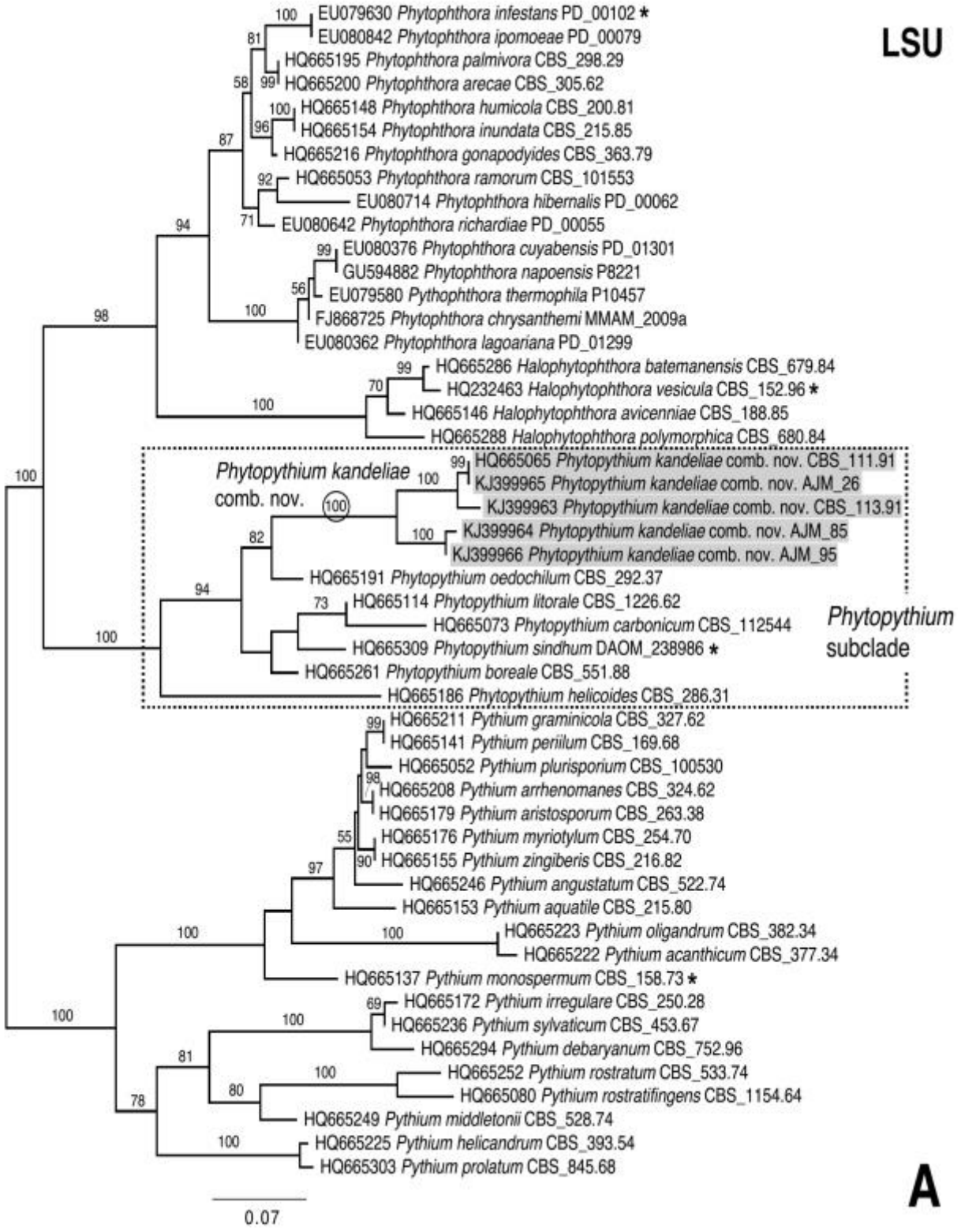
Phylogenetic placement

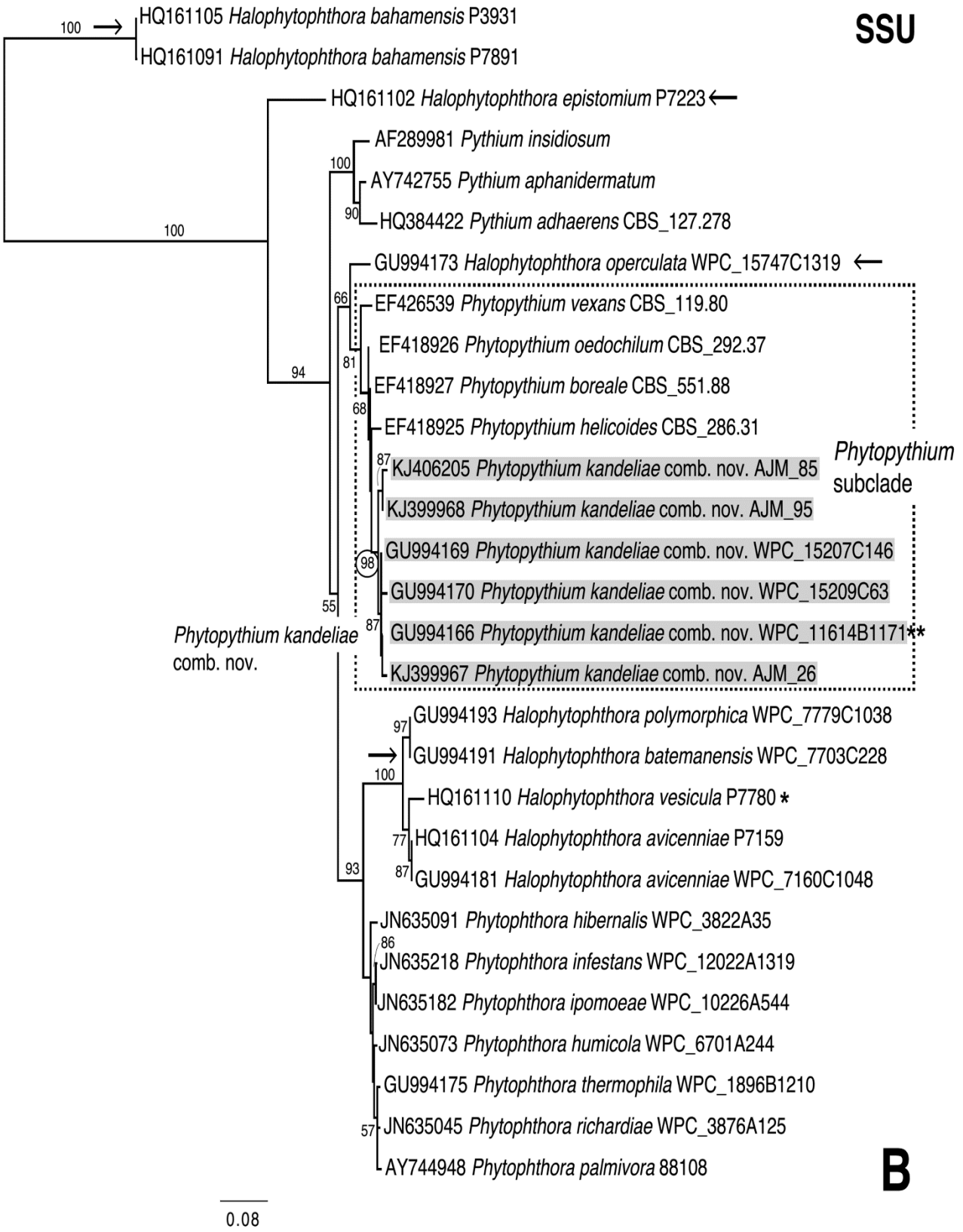
LSU, SSU and ITS maximum likelihood trees (Fig. 1A-C) showed two major clades: the Peronosporales (100%, 55% and 100% branch support, respectively), and the Pythiales (100% in each). The Peronosporales were composed by two major subclades: *(i)* the *Phytopythium* subclade (ex *Pythium* clade K), containing the genus type species *P. sindhum* (100%, 81% and 93%, respectively), and *(ii)* the *Halophytophthora sensu stricto*, containing the genus type species *H. vesicula* + *Phytophthora*, containing the genus type species *P. infestans* (98%, 93% and 91%, respectively). The Pythiales were composed only by *Pythium*, containing the genus type species *P. monospermum.*

Some *Halophytophthora* isolates fall into a different clade than the core clade of *Halophytophthora* and therefore, this genus appears to be polyphyletic and composed of at least four lineages (see arrows in Fig. 1B). Our isolates (AJM 26, AJM 85 and AJM 95) together with the ex-type strain (ATCC 11614; Ho et al. 1991), two voucher specimens from the CBS culture collection (CBS 111.91 and CBS 113.91) and other isolates deposited in GenBank as *Halophytophthora kandeliae* (GU994169, GU994170, GU258976 and HQ171176) form a well-supported subclade (100%, 98% and 99% branch support, Fig. 1A-C) that was placed within the *Phytopythium* clade ex *Pythium* clade K, as previously reported (Bala et al. 2010, Nigrelli & Thines 2013, Marano et al. in press). The SSU tree (Fig. 1B), also shows that another species currently in *Halophytophthora*, *H. operculata*, might belong to *Phytopythium*, but its morphology should be carefully examined before taking a decision and therefore, we did not yet consider it as part of the *Phytopythium* subclade.

The COI phylogeny of the *Phytopythium* clade (Fig. 2) also showed that the clade composed by our sequences, the two voucher specimens from CBS and two sequences from GenBank deposited as *H. kandeliae* is well-defined and supported (90%).

The voucher specimens have been deposited at the CBS culture collection as *H. kandeliae* and referred as belonging to *Phytopythium* by Bala et al. (2010), Robideau et al. (2011) and Marano et al. (in press). Finally, after a revision made in November 2013 they were recognized as belonging to *Phytopythium* (http://www.cbs.knaw.nl/Collections/Biolomics.aspx?Table=CBS+strain+database). Therefore, based on its multigene phylogenetic position and morphological characters, we decided to transfer *H. kandeliae* to the genus *Phytopythium*.




SSU
HQ161105 Halophytophthora bahamensis P3931
HQ161091 Halophytophthora bahamensis P7891
HQ161102 Halophytophthora epistomium P7223
AF289981 Pythium insidiosum
AY742755 Pythium aphanidermatum
HQ384422 Pythium adhaerens CBS_127.278
GU994173 Halophytophthora operculata WPC_15747C1319
EF426539 Phytopythium vexans CBS_119.80
EF418926 Phytopythium oedochilum CBS_292.37
EF418927 Phytopythium boreale CBS_551.88
EF418925 Phytopythium helicoides CBS_286.31
KJ406205 Phytopythium kandeliae comb. nov. AJM_85
KJ399968 Phytopythium kandeliae comb. nov. AJM_95
GU994169 Phytopythium kandeliae comb. nov. WPC_15207C146
GU994170 Phytopythium kandeliae comb. nov. WPC_15209C63
GU994166 Phytopythium kandeliae comb. nov. WPC_11614B1171**
KJ399967 Phytopythium kandeliae comb. nov. AJM_26
Phytopythium subclade
Phytopythium kandeliae comb. nov.
GU994193 Halophytophthora polymorphica WPC_7779C1038
GU994191 Halophytophthora batemanensis WPC_7703C228
HQ161110 Halophytophthora vesicula P7780 *
HQ161104 Halophytophthora avicenniae P7159
GU994181 Halophytophthora avicenniae WPC_7160C1048
JN635091 Phytophthora hibernalis WPC_3822A35
JN635218 Phytophthora infestans WPC_12022A1319
JN635182 Phytophthora ipomoeae WPC_10226A544
JN635073 Phytophthora humicola WPC_6701A244
GU994175 Phytophthora thermophila WPC_1896B1210
JN635045 Phytophthora richardiae WPC_3876A125
AY744948 Phytophthora palmivora 88108
B
0.08

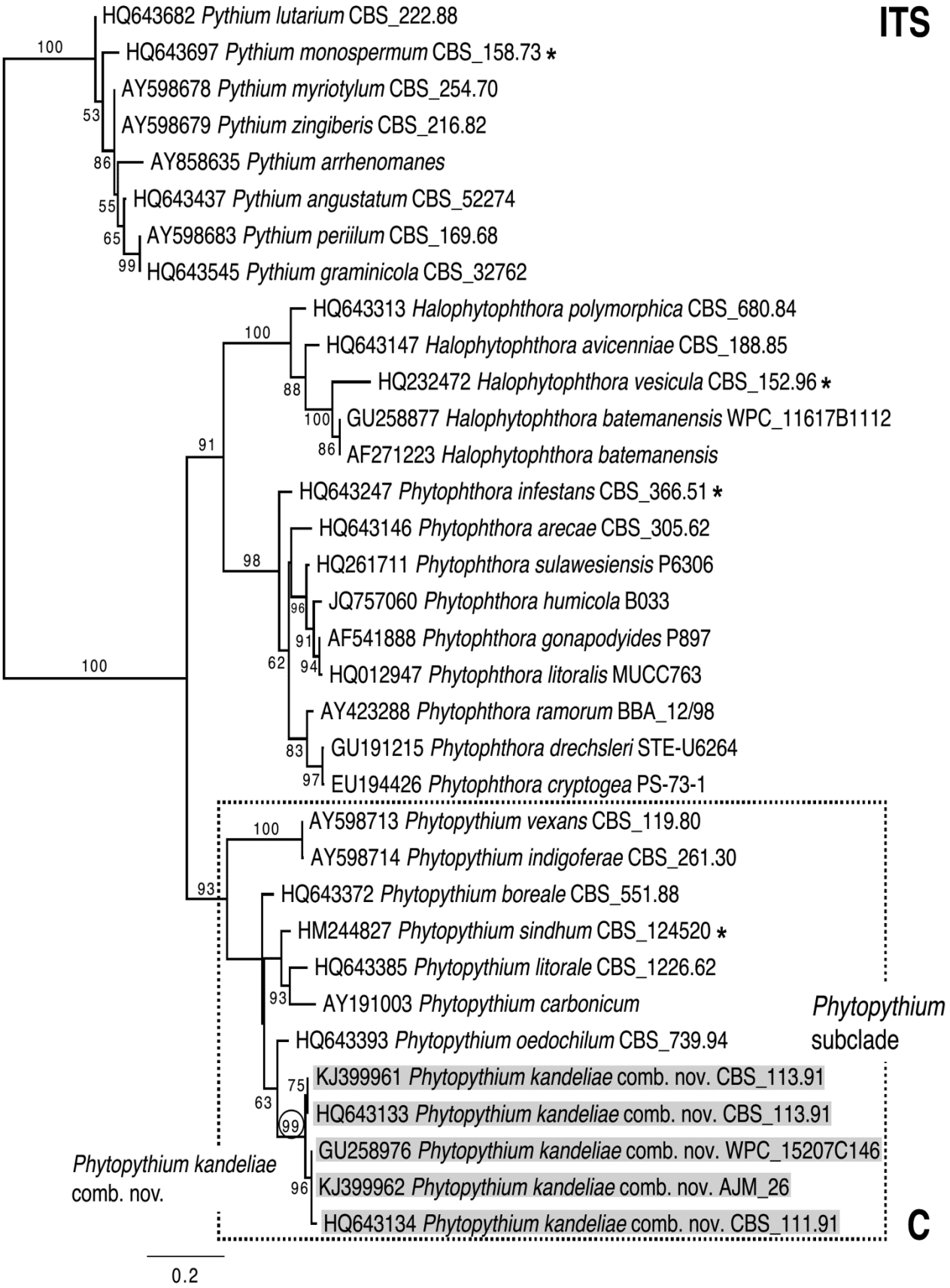


Fig. 1 Maximum likelihood tree inferred from LSU (**A**), SSU (**B**) and ITS (**C**) rDNA sequences of isolates of *Phytopythium kandeliae* and related genera. References: numbers next to branches indicate bootstrap support (%) and the bar shows the number of substitutions per site. Only branches with > 50% of bootstrap support are shown. (*) indicates genus type species; (**) indicates ex-type strain; (←) indicates polyphyly in *Halophytophthora*

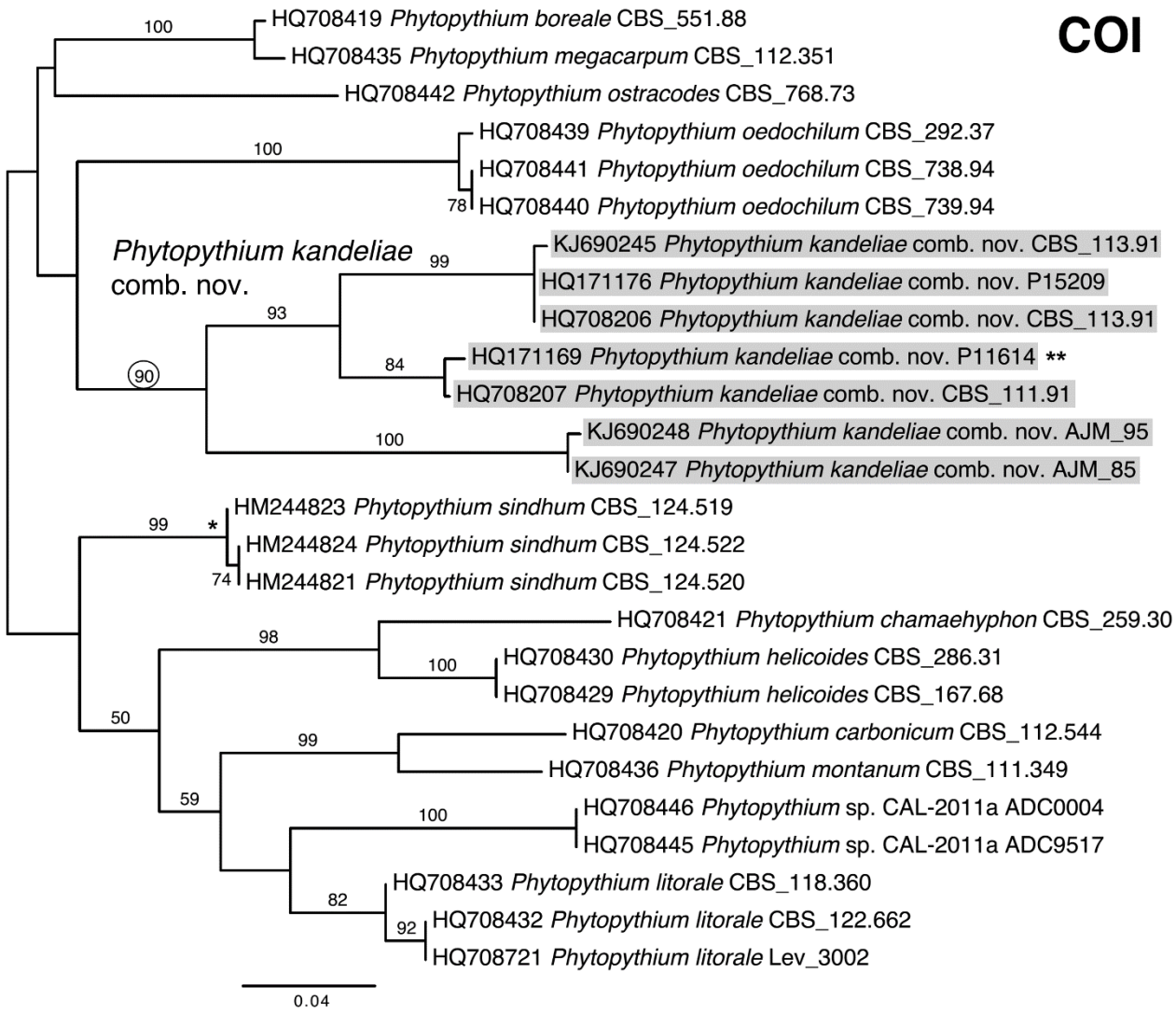


Fig. 2 Maximum likelihood tree inferred from COI mDNA sequences of isolates of *Phytopythium kandeliae* and other species provisionally assigned to *Phytopythium* (*Pythium* clade K). References: numbers next to branches indicate bootstrap support (%) and the bar shows the number of substitutions per site. Only branches with > 50% of bootstrap support are shown. (*) indicates genus type species, (**) indicates ex-type strain.

Species description

Phytopythium kandeliae (H.H. Ho, H.S. Chang & S.Y. Hsieh) A.V. Marano, A.L. Jesus & C.L.A. Pires-Zottarelli, **comb. nov.** Fig. 1

Mycobank MB807745

Basionym – *Halophytophthora kandeliae* H.H. Ho, H.S. Chang & S.Y. Hsieh, Mycologia 83: 419 (1991)

Mycelium abundant, hyaline, branched or unbranched, non septate or septate in old cultures; aerial mycelium scanty. Hyphae thin, 3.75-5 µm. Hyphal swellings absent. Sporangiophore non differentiated, simple, with one terminal zoosporangium, or branched sympodially. Zoosporangia globose to (ob) ovate, semipapillate, non-deciduous, (22-) 27-55 (-56) × (17-) 20-42 (-45) µm. Internal proliferation of zoosporangia occasionally observed. Zoosporangial basal plug present. Discharge typically "*Pythium*-like" (zoospore differentiation inside a vesicle, outside the

sporangium) or "mixed *Pythium*- and *Phytophthora*-like" (part of the plasma is moving out in a vesicle through the exit pore and zoospore development takes place inside the sporangium and in the extruded vesicle), (15-) 17-32 (-35) × 17-38 (-45) µm. Rest of the vesicle remaining attached to the zoosporangium after zoospore discharge, like a "collar" or "operculum-like" . Discharge pore wide, 10-20 (-25) µm. Secondary zoosporangial basal plug frequently formed after zoospore discharge. Encysted zoospores, 6-9 (-10) µm. Zoospore germination through a single germination tube. Sexual reproduction not observed.

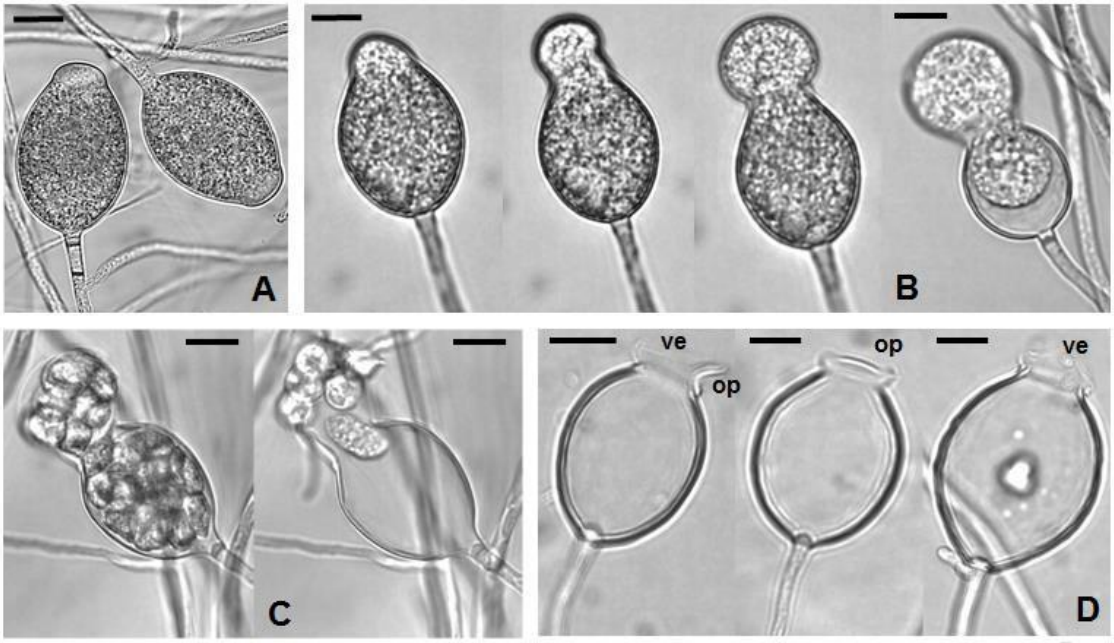


Fig. 3 *Phytopythium kandeliae*. **A.** General aspect of the semipapillate zoosporangia. **B-D:** Different stages during zoospore development and discharge**. B**. Part of the protoplasma is moving out in an external vesicle**. C.** Zoospores are developed inside the sporangium and the external vesicle and released by rupture of the vesicle. **D.** Empty zoosporangia showing rest of the vesicle (**ve**) as a collar or looking like an operculum (**op**). – Bars = 10 µm

Known distribution – Brazil (material examined), Japan (Nakagiri 2000), Taiwan (Ho et al. 1991).

Material examined – TAIWAN, *Kandelia candel, H. kandeliae* CBS 113.91 (CCIBt 4004), and CBS 111.91; BRAZIL, São Paulo State, Cananéia: Ilha do Cardoso, Perequê river. Isolate *H. kandeliae* AJM 26 (CCIBt 4023), 30-VIII-2012, S0 (salinity 0.05%), isolate *H. kandeliae* AJM 85 (CCIBt 4024), 07-XI-2012, S0 (salinity 0.10%), from leaves of *Laguncularia racemosa*, on *Sorghum* spp. seeds; isolate *H. kandeliae* AJM 95 (CCIBt 4025), 07-XI-2012, S1 (salinity 0.79%) from *Laguncularia racemosa*, onto PYGs culture medium; *leg & det.* A.L. Jesus & A.V. Marano

Culture growth – Colony petalloid ("chrysanthemum like"), with scant aerial mycelium. Growth of the isolates after 96 hrs at 21 °C: *(i)* AJM 26: on PYG: 1.3 cm, on PYGs: 2.4 cm, on V8s: 2.7 cm, on CMA: 2.8 cm; *(ii)* AJM 85, on PYG: 1.1 cm, on PYGs: 1.8 cm, on V8s: 2 cm, on CMA: 2.7 cm; *(iii)* AJM 95, on PYG: 1.2 cm, on PYGs: 2.6 cm, on V8s: 3 cm, on CMA: 2.9 cm.

Ecology – This species was first isolated from submerged leaves of *Kandelia candel* (L.) Druce from a mangrove swamp (Ho et al. 1991) and subsequently from submerged leaves of *Rhizophora stylosa* Griff. from a river near its connection with the sea (Nakagiri 2000). In agreement with Nakagiri (2000), who found *H. kandeliae* always upstream in almost freshwater sites, we observed that this species prefer lower salinity levels since our isolates were also always collected upstream, under salinities that ranged from 0.05-0.10%. Nevertheless, it grew well on

half strength seawater medium (salinity 1.25-1.50%), which is in agreement with the salinity optimum for this species (1-2%) documented by Nakagiri (2000).

Notes – Zoosporangia were produced abundantly 24 hrs after being transferred to petri dishes with 50% sterile seawater, at room temperature (24-25 °C). The complete differentiation of zoospores inside the vesicle might take more than 3 hrs. Oogonia and antheridia were not produced in culture media (PYG, PYGs, V8, CMA, CMAs, CMA with sitosterol) or on *Sorghum* spp. seeds.

Discussion

Most of the characteristics observed in the three isolates agreed with the original description of *H. kandeliae* (Ho et al. 1991). The mixed *Pythium-/Phytophthora*-like discharge was not originally described for *Phytopythium*, therefore, we consider that the diagnostic morphological characters for *Phytopythium* are in need of further revision.

Acknowledgements

We are indebted to "Instituto Florestal" for the permission given to collect samples at "Ilha do Cardoso", Cananéia, São Paulo, SP and to Manoel Osorio Neves Junior for his valuable help during sampling. We also wish to thank CAPES ("Coordenação de Aperfeiçoamento de Pessoal de Nível Superior") for the fellowship and support given to A.V. Marano ("Ciência Sem Fronteiras" Program, "Atração de Jovens Talentos" DRI- CAPES Process No. 006/2012) and FAPESP ("Fundação de Amparo à Pesquisa do Estado de São Paulo") for the fellowship given to A.L. Jesus (Process No. 2013/01409-0), and for the financial support given to C.L.A. Pires-Zotarelli (Process No. 12/50222-7). CNPq ("Conselho Nacional de Desenvolvimento Científico e Tecnológico") is also acknowledged for the grant given to C.L.A. Pires-Zottarelli (Process No. 304411/2012-4)

References

Ab'Saber NA. 1977 – Os domínios morfoclimáticos na América do Sul: primeira aproximação. Geomorfologia 52, 1–21.

Anastasiou CJ, Churchland LM. 1969 – Fungi on decaying leaves in marine habitats. Canadian Journal of Botany 47, 251–257.

Bala K, Robideau GP, Lévesque CA, de Cock AWAM, Abad ZG, Lodhi AM, Shahzad S, Ghaffar A, Coffey MD. 2010 – *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi & Lévesque, gen. nov. and *Phytopythium sindhum* Lodhi, Shahzad & Levésque, sp. nov. Persoonia 24, 136–137.

Broders KD, Lipps PE, Ellis M, Dorrance AE. 2009 – *Pythium delawarii* – a new species isolated from soybean in Ohio. Mycologia 101, 232–238.

Castresana J. 2000 – Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular and Biological Evolution 17, 540–552.

CBS-Knaw Fungal Biodiversity Centre – 2013. http://www.cbs.knaw.nl.

Centro de Pesquisa sobre o Genoma Humano e Células-Tronco – 2013. http://genoma.ib.usp.br.

Fell JW, Master IM. 1975 – Phycomycetes (*Phytophthora* spp. nov. and *Pythium* sp. nov.) associated with degrading mangrove (*Rhizophora mangle*) leaves. Canadian Journal of Botany 53, 2908–2922.

Fischer A. 1892 – Phycomycetes. Rabenhorst, Kryptogamenflora 1: 505.

Fuller MS, Jaworski A. 1987 – Zoosporic fungi in teaching and research, Southeastern Publishing Corp: USA.

Funari FL, Struffaldi-De-Vuono Y, Salum ST. 1987 – Balanço hídrico de duas áreas de Mata Atlântica: Reserva Biológica de Paranapiacaba e Parque Estadual da Ilha do Cardoso (Estado de São Paulo). In: Pereira MFA, Massei MAS (eds.). Anais do 6° Congresso da Sociedade de Botânica de São Paulo. Sociedade Botânica de São Paulo: Campinas, pp. 95-101.

Gerrettson-Cornell L, Simpson J. 1984 – Three new marine *Phytophthora* species from New South Wales. Mycotaxon 19, 453–470.
Guindon S, Gascuel O. 2003 – A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. Systematic Biology 52, 696–704.
Ho HH, Jong SC. 1990 – *Halophytophthora*, gen. nov., a new member of the family Pythiaceae. Mycotaxon 36, 377–382.
Ho HH, Chang HS, Hsieh SY. 1991 – *Halophytophthora kandeliae*: a new marine fungus from Taiwan. Mycologia 83, 419–424.
Ho HH, Nakagiri A, Newell SY. 1992 – A new species of *Halophytophthora* from Atlantic and Pacific subtropical islands. Mycologia 84, 548–554.
Hulvey J, Telle S, Nigrelli L, Lamour K, Thines M. 2010 – Salisapiliaceae – A new family of oomycetes from marsh grass litter of southeastern North America. Persoonia 25, 109–116.
Karaca G, Jonathan R, Paul B. 2009 – *Pythium stipitatum* sp. nov. isolated from soil and plant debris taken in France, Tunisia, Turkey, and India. FEMS Microbiology Letters 295, 164–169.
Kohlmeyer J, Volkmann-Kohlmeyer B, Newell SY. 2004 – Marine and estuarine mycelia Eumycota and Oomycota. In: Müeller GM, Bills GF, Foster MS (eds). Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier Academic Press: San Diego, pp. 533-545.
Lara E, Belbahri L. 2011 – SSU rRNA reveals major trends in oomycete evolution. Fungal Diversity 49, 93–100.
Lévesque CA, de Cock AWAM. 2004 – Molecular phylogeny and taxonomy of the genus *Pythium*. Mycological Research 108, 1363–1383.
Marano AV, Barrera MD, Steciow MM, Donadelli JL, Saparrat CMN. 2008 – Frequency, abundance and distribution of zoosporic organisms from Las Cañas stream (Buenos Aires, Argentina). Mycologia 100, 691–700.
Marano AV, Jesus AL, Pires-Zottarelli CLA, James TY, Gleason FH, de Souza JI. In press. – Chapter 8: Phylogenetic relationships of Pythiales and Peronosporales (Oomycetes, Straminipila) within the "peronosporalean galaxy". In: Gareth Jones EB, Hyde KD, Pang K-L (eds.). Freshwater fungi. de Gruyter: Germany.
Nakagiri A. 2000 – Ecology and diversity of *Halophytophthora* species. Fungal Diversity 5, 153–164.
Nascimento CL, Gomes EPC, Pires-Zottarelli CLA. 2011 – Occurrence and distribution of zoosporic organisms in water bodies from Brazilian Cerrado areas. Mycologia 103, 261–272.
Newell SY, Fell JW. 1994 – Parallel testing of media for measuring frequencies of occurrence for *Halophytophthora* spp. (Oomycota) from decomposing mangrove leaves. Canadian Journal of Microbiology 40, 250–256.
Nigrelli L, Thines M. 2013 – Tropical oomycetes in the German bight: climate warming or overlooked diversity? Fungal Ecology 6, 152–160.
Pegg KG, Alcorn JL. 1982 – *Phytophthora operculata* sp. nov., a new marine fungus. Mycotaxon 16: 99-102.
Phytophthora database – 2013. http://www.phytophthoradb.org.
Plaats-Niterink AJ van der. 1981 – Monograph of the genus *Pythium*. Studies in Mycology 21. Centraalbureau Voor Schimmelcultures: Baarn.
Posada D. 2008 – jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25, 1253.
Pringsheim N. 1858 – Beitraege zur morphologie und systematic algae. 1. Die Saprolegnieen. Jb Wiss Bot 1, 284–306.
Robideau GP, de Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, Eggertson QA, Gachon CMM, Hu CH, Küpper FC, Rintoul TL, Sarhan E, Verstappen ECP, Zhang Y, Bonants PJM, Ristaino JB, Lévesque AC. 2011 – DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular and Ecological Resources 11, 1002–1011.
Schröter J. 1897 – Pythiaceae. Engler & Prantl, nat PflFam. 1, 104–105.

Senda M, Suga H, Levésque, GA. 2009 – Two new species of *Pythium*, *P. senticosum* and *P. takayamanum*, isolated from cool-temperate forest soil in Japan. Mycologia 101, 439–448.
Uzuhashi S, Tojo M, Kakishima M. 2010 – Phylogeny of the genus *Pythium* and description of new genera. Mycoscience 51, 337–365.
White TJ, Bruns T, Lee S, Taylor SW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M A, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: a guide to methods and applications. Academic Press: New York, pp. 315–322.