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RESEARCH ARTICLE

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Pythium subutonaiense, A New Aquatic Oomycete from Southern China Based on Morphological and Molecular Characters

Jia-Jia Chen[a,b] and Xiao-Bo Zheng[a]

[a]Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China; [b]College of Landscape Architecture, Jiangsu Vocational College of Agriculture and Forestry, Zhenjiang, China

ABSTRACT

A new species, *Pythium subutonaiense*, isolated from aquatic environments (lake) in China is being described based on morphological characters and molecular evidence. The isolates grew at temperatures between 5 °C and 38 °C, and the optimum temperature was 30 °C, with a radial growth rate of 17.6 mm at 25 °C per day. It is homothallic and characterized by globose to sub-globose shaped and mostly terminal or sometimes catenulate hyphal swellings, filamentous non-inflated sporangia, and smooth oogonia with hypogynous and monoclinous antheridia that contained one plerotic oospore. In phylogenetic analysis, inferred based on the internal transcribed spacer region of the ribosomal RNA gene and mitochondrial cytochrome c oxidase subunit 1 gene, the new species formed a distinct lineage in *Pythium* clade B. Differences between the new species and phylogenetically related and morphologically similar species are discussed.

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1. Introduction

Pythium [1] (Pythiaceae, Pythiales), typified with *P. monospermum* Pringsh., is characterized by hyaline and coenocytic hyphae without septa, various shaped sporangia, and the development of zoospores in a vesicle which is formed at the tip of a discharge tube derived from a sporangium [2]. *Pythium* spp. are cosmopolitan and represent a range of functional groups, such as saprophytes in natural environments, plant and animal pathogens, and biological control agents protecting against pathogenic fungi [3]. Following recent taxonomic revisions [4,5] and discoveries (e.g., Refs. [6–11]), more than 140 species are currently recorded in the genus *Pythium* [11].

During studies on *Pythium* species diversity in southern China, one new species of *Pythium*, *P. utonaiense* was isolated. Phylogenetic analysis was performed using the internal transcribed spacer (ITS) regions of the ribosomal RNA and mitochondrial cytochrome c oxidase subunit 1 (COI) genes. Combined with the morphological characters, a new species is described in this study. Differences between the new species and phylogenetically related and/or morphologically similar species are also provided.

2. Materials and methods

2.1. Isolation

The samples were recovered from surface water of the lake in Nanjing, Jiangsu Province of China using flowers of *Bougainvillea glabra* Choisy as baits to isolate *Pythium* species [12]. The isolation procedure followed the method described by Benard and Punja [13]. Pieces of tissue 5–10 mm were cut from the baits, washed in tap water and superficially dried on a paper towel, and plated on V8 juice agar (V8A) containing rifampicin (50 mg L^{-1}), phenamacril (5 mg L^{-1}), ampicillin (50 mg L^{-1}), and pentachloronitrobenzene (50 mg L^{-1}) and incubated at 25 °C for 2–3 days. When mycelial growth was observed, purification was carried out twice by transferring a single hyphal tip of colonies onto V8A. Two isolates (Chen 220 and Chen 229) of undescribed *Pythium* species were recovered and deposited in the herbarium of the College of Plant Protection, Nanjing Agricultural University (NJAU).

2.2. Morphology and growth rate

Colony patterns of two *P. subutonaiense* isolates were examined after incubation for 3 days at 25 °C

CONTACT Jia-Jia Chen jiaj.chen19@yahoo.com

in corn meal agar (CMA), potato carrot agar (PCA), and V8A media. Sporulation was induced using a modification of the method described by Chang [14]. Agar blocks (8 mm × 8 mm × 3 mm) were cut from the mycelia fronts of 3-day-old V8A cultures and placed in 10% clarified and sterile V8 juice (one block per dish) and incubated in darkness at 25 °C for 48–72 h. Once the mycelium has reached 4–5 cm in diameter, the V8 juice was pipetted out and the mycelia mats were then rinsed with sterile distilled water (SDW) three times at 20 min intervals. The rinsed mycelia mats were then submerged in SDW. The plates containing SDW were incubated at 25 °C for 24–48 h prior to examining for release of zoospores. Fifty measurements were taken for each morphological feature, such as sporangia, oogonia, and oospores. The cardinal temperatures of growth rates, on PCA media [2], were measured at 24 h of incubation for each isolate at 5–40 °C, with intervals of 5 °C. When no growth was observed, the intervals were reduced from 5 to 2 or 1 °C and the culture was returned to room temperature to check growth revival.

2.3. DNA extraction and sequencing

A cetyl trimethylammonium bromide rapid plant genome extraction kit (Demeter Biotechnologies Co., Ltd., Beijing, China) was used to extract total genomic DNA from purified isolates, and performed the polymerase chain reaction (PCR) according to the study by Chen and Cui [15]. A small piece of dried fungal specimen (about 30 mg) was grounded to powder with liquid nitrogen, transferred to a 1.5-mL centrifuge tube and resuspended in 0.4 mL of lysis buffer (PL), and incubated at 65 °C water bath for 60 min. Then, 0.4 mL phenol–chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer (PQ). The mixture was later transferred to an adsorbing column (AC) for a centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid (IR) was added to the AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer (WB), removing the AC to a clean centrifuge tube, and adding 100 μL elution buffer in the middle of adsorbed film, the genome DNA was eluted in 100 µL elution buffer (EB). The ITS region was amplified with the primers: ITS4 and ITS5 [16]. The COI gene was amplified with the primers: OomCoxI-Levlo (CYTC HGGRTGWCCRAAAAACCAAA) and Oom CoxI-Levup (TCAWCWMGATGGCTTTTTTCAAC) [17]. The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for COI was as follows: initial denaturation at 94 °C for 2–5 min, followed by 35 cycles at 94 °C for 30 s, 52 °C for 30 s and 72 °C for 1–2 min, and a final extension of 72 °C for 5–10 min [18]. The PCR products were purified and sequenced in Genscript Company (Nanjing, China) with the same primers.

2.4. Phylogenetic analysis

Sequences generated in this study were aligned with additional sequences downloaded from GenBank (Table 1) using ClustalX [19] and BioEdit [20].

Table 1. A list of species, cultures, and GenBank accession numbers of sequences used in this study.

			GenBank accession no.	
Species name	Sample no.	Locality	ITS	COI
Pythium adhaerens	CBS 520.74	The Netherlands	HQ643415	HQ708462
P. afertile	Lev 2066	Canada	HQ643416	HQ708463
P. angustatum	CBS 522.74	The Netherlands	HQ643437	HQ708484
P. apleroticum	CBS 772.81	The Netherlands	HQ643444	HQ708491
P. aquatile	CBS 215.80	United Kingdom	HQ643445	HQ708492
P. aristosporum	CBS 263.38	Canada	HQ643447	HQ708494
P. arrhenomanes	CBS 324.62	USA	HQ643452	HQ708499
P. biforme	UZ00796	Japan	KJ995584	KJ995590
P. brachiatum	UZ00736	Japan	KJ995581	KJ995593
P. brachiatum	UZ00746	Japan	KJ995583	KJ995594
P. capillosum	CBS 222.94	France	HQ643483	HQ708529
P. catenulatum	CBS 226.94	France	HQ643490	HQ708536
P. chondricola	CBS 203.85	The Netherlands	HQ643498	HQ708544
P. coloratum	CBS 154.64	Australia	HQ643501	HQ708547
P. conidiophorum	CBS 223.88	United Kingdom	HQ643509	HQ708555
P. contiguanum	CBS 221.94	Algeria	HQ643514	HQ708560
P. diclinum	CBS 526.74	The Netherlands	HQ643523	HQ708569
P. dissimile	CBS 155.64	Australia	HQ643526	HQ708572
P. dissotocum	CBS 166.68	USA	HQ643528	HQ708574
P. flevoense	CBS 234.72	The Netherlands	HQ643538	HQ708582
P. folliculosum	CBS 220.94	Switzerland	HQ643540	HQ708584
P. graminicola	CBS 327.62	Jamaica	HQ643545	HQ708589
P. inflatum	CBS 168.68	USA	HQ643566	HQ708610
P. kashmirense	CBS 122908	India	HQ643671	HQ708715
P. lutarium	CBS 222.88	United Kingdom	HQ643682	HQ708726
P. myriotylum	CBS 254.70	Israel	HQ643701	HQ708745
P. oopapillum	BR 632	–	FJ655174	FJ655178
P. pachycaule	CBS22494	France	HQ643726	HQ708767
P. pectinolyticum	CBS 122643	France	HQ643739	HQ708780
P. periilum	CBS 169.68	USA	HQ643740	HQ708781
P. phragmitis	CBS 117104	Germany	HQ643746	HQ708787
P. plurisporium	CBS 100530	USA	HQ643749	HQ708790
P. pyrilobum	CBS 158.64	Australia	HQ643755	HQ708796
P. rhizo-oryzae	CBS 119169	India	HQ643757	HQ708798
P. salpingophorum	BR 1024	United Kingdom	HQ643770	HQ708811
P. scleroteichum	CBS 294.37	USA	HQ643771	HQ708812
P. subutonaiense	Chen 220[a]	China	MG654703	MG674169
P. subutonaiense	Chen 229[a]	China	MG654704	MG674170
P. sukuiense	CBS 110030	Taiwan	HQ643836	HQ708877
P. sulcatum	CBS 603.73	USA	HQ643837	HQ708878
P. tardicrescens	Lev 1534	USA	HQ643855	HQ708896
P. torulosum	CBS 316.33	The Netherlands	HQ643859	HQ708900
P. tracheiphilum	CBS 323.65	Italy	HQ643862	HQ708903
P. utonaiense	UZ00758	Japan	KJ995586	KJ995588
P. utonaiense	UZ00769	Japan	KJ995587	KJ995589
P. vanterpoolii	CBS 295.37	United Kingdom	HQ643952	HQ708993
P. volutum	CBS 699.83	Japan	HQ643971	HQ709012
P. zingiberis	CBS 216.82	Japan	HQ643973	HQ709014

[a]New sequences determined in the present study.

Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase; submission ID S22483).

Phylogenetic analysis was done as in the study by Chen and Cui [15]. Maximum parsimony (MP) analysis was applied to the combined dataset of ITS and COI sequences. *Pythium adhaerens* Sparrow and *P. chondricola* De Cock were used as outgroups [7]. The tree construction procedure was performed in PAUP* version 4.0b10 [21]. All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap analysis with 1000 replicates [22]. Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree generated.

MrModeltest 2.3 [23] was used to determine the best-fit evolution model for the dataset for Bayesian inference (BI). BI was calculated with MrBayes3.1.2 [24] with a general time reversible (GTR) model of DNA substitution and an invgamma distribution rate variation across sites. Four Markov chains were run for 2 million generations until the split deviation frequency value <0.01, and sampled every 100th generations. The burn-in was set to discard the first 25% of the trees. A majority rule consensus tree of all remaining trees was calculated. Phylogenetic trees were visualized using Treeview [25]. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (MP), and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP) were considered as significantly supported, respectively.

3. Results

3.1. Isolation

Water samples were collected from two lakes, respectively in Zixiahu Park and Soul Valley Temple in China in 2016. The two lakes are located in national park of Dr. Sun Yat-sen's mausoleum of southern China. Two surface water samples were collected at the edge of the lake. Two isolates of the new species, *P. subutonaiense*, were respectively obtained from two water samples and isolated from flowers of *Bougainvillea glabra* as bait on V8A.

3.2. Morphology and growth rate

Both two isolates of *P. subutonaiense* showed similar colony patterns and growth temperature results

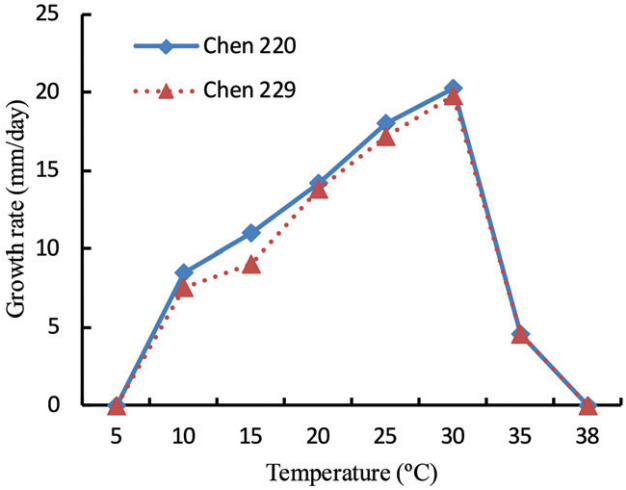

Figure 1. Mecelial growth rate of *P. subutonaiense* isolates Chen 220 and Chen 229 on potato carrot agar at different temperatures.

(Figure 1). They were rosette pattern on PCA, stellate pattern on V8A, and without a special pattern on CMA. The isolates showed maximum growth at 30 °C, and no growth at 5 °C and 38 °C. The growth rate was 17.6 mm per day at 25 °C.

The morphology of asexual and sexual structures was also similar between the two isolates. Hyphal swellings were frequently observed, and zoospores were rarely observed in both isolates. The sexual structures were abundantly produced on V8A. Oogonia were smooth-walled, but sometimes had one outstanding slender projection. Antheridia were hypogynous and monoclinous, produced one or two per oogonium. Oospores were one per oogonium.

P. subutonaiense differs other *Pythium* species from homothallic sexuality, globose to sub-globose shaped, mostly terminal or sometimes catenulate hyphal swellings, filamentous non-inflated sporangia, and smooth oogonia, hypogynous, and monoclinous antheridia with slender antheridial cells and plerotic oospores.

Further differences between the new species and other related ones are listed in Table 2.

3.3. Molecular phylogeny

Two ITS and two COI sequences were newly generated for this study and their accession numbers are available in GenBank (Table 1). BLAST analyses of ITS and COI sequences of the two isolates, described here as *P. subutonaiense*, showed the best phylogenetic matches were with species of *Pythium* clade B.

The combined ITS + COI dataset included sequences from 48 oomycetes representing 45 taxa. The dataset had an aligned length of 1563 characters, of which 1111 characters are constant, 64 are variable and parsimony-uninformative, and 388 are

Table 2. Morphological description of *Pythium subutonaiense* and the most closely related species.

	P. subutonaiense (Chen 218)	*P. brachiatum*	*P. capillosum*	*P. flevoense*	*P. utonaiense*
Width of hyphae (µm)	Up to 5	Up to 5.5	3–5	Up to 6	Up to 5
Hyphal swellings	Present	Absent	Absent	Absent	Absent
Sporangia or inflated structures	Filamentous non-inflated	Filamentous slightly inflated	Filamentous, non-inflated or slightly inflated	Filamentous	Filamentous non-inflated
Oogonia (µm)	17.5–22.5 (av. 20.8), intercalary or rarely terminal	13.3–34.4 (av. 22.7), intercalary or terminal, sometimes in chain	16–35.2 (av. 23.09), mostly intercalary, rarely terminal	17–20 (av. 19), mostly terminal	10–23.3 (av. 17.5), terminal, rarely intercalary
Antheridia	Hypogynous or monoclinous	Monoclinous or diclinous or rarely lacking	Mostly diclinous, rarely monoclinous	Diclinous	Lacking
Oospores	Plerotic	Plerotic, occasionally aplerotic	Plerotic or aplerotic	Aplerotic, occasionally nearly plerotic	Plerotic
Oospore wall thickness (µm)	0.5–2	0.3–3.2	2–3.5	2–3	0.3–3.1
Cardinal temperature	Min 5 °C, optimum 30 °C and max 38 °C	Min 4 °C, optimum 25 °C and max 30 °C	Min 1–2 °C, optimum 25 °C and max 40 °C	Min under 5 °C, optimum 25 °C and max over 35 °C	Min 4 °C, optimum 30 °C and max 40 °C
Daily growth rates on PCA at 25 °C (mm)	17.6/per day	14/per day	7/per day	7–10/per day	23.5/per day
Reference	This study	[7]	[27]	[28]	[7]

parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 1369, CI = 0.455, RI = 0.757, RC = 0.344, HI = 0.545). Best model for the combined ITS + COI sequences dataset estimated and applied in the Bayesian analysis: GTR + I + G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in the same topology with an average standard deviation of split frequencies = 0.006409. The sequences of *Pythium* species in clade B clustered together with strong supports (100% ML, 1 BPP, Figure 2). The phylogeny inferred from the ITS + COI dataset showed that the two newly sequenced isolates formed a lineage within *Pythium* subclade B2 with full statistical supports (100% MP, 1 BPP) and clustered with *P. utonaiense* [7].

3.4. Taxonomy

P. subutonaiense: Jia J. Chen and X.B. Zheng, *sp. nov*. (Figure 3).

MycoBank no.: MB 824731.

Type.—China. Jiangsu Prov., Nanjing, Zixiahu Park, from lake water, November 1, 2016, J.J. Chen, Chen 220 (NJAU, holotype).

Etymology—Subutonaiense (Lat.) referring to the species is somewhat similar to *P. utonaiense* [7].

Cardinal temperatures: minimum 5 °C, optimum 30 °C, maximum 38 °C. Main hyphae hyaline, aseptate, up to 5.0 µm wide. Hyphal swellings globose to sub-globose, mostly terminal or sometimes catenulate, 20–37.5 (mean 26.5) μm in diameter. Sporangia filamentous non-inflated, rarely giving rise to vesicles containing zoospores. Zoospores formed in SDW at room temperature, and encysted zoospores 5–7.5 μm (mean 6.5 μm) in diameter. Homothallic; oogonia globose, smooth or with one outstanding slender projection, intercalary, rarely terminal, 17.5–22.5 µm (mean 20.8 µm) in diameter. Antheridia hypogynous and monoclinous; antheridial stalks unbranched; antheridial cells slender. Oospore one per oogonium, plerotic, globose, 15.5–20.5 μm (mean 18.3 µm) in diameter, wall up to 0.5–2 (mean 1.6) µm thick.

Additional specimens examined.—CHINA. Jiangsu Prov., Nanjing, Soul Valley Temple, from lake water, November 1, 2016, J.J. Chen, Chen 229 (paratype, NJAU).

4. Discussion

P. subutonaiense is characterized by aquatic habit, homothallic sexuality, globose to sub-globose shaped, mostly terminal or sometimes catenulate hyphal swellings, filamentous non-inflated sporangia, and smooth oogonia, hypogynous, and

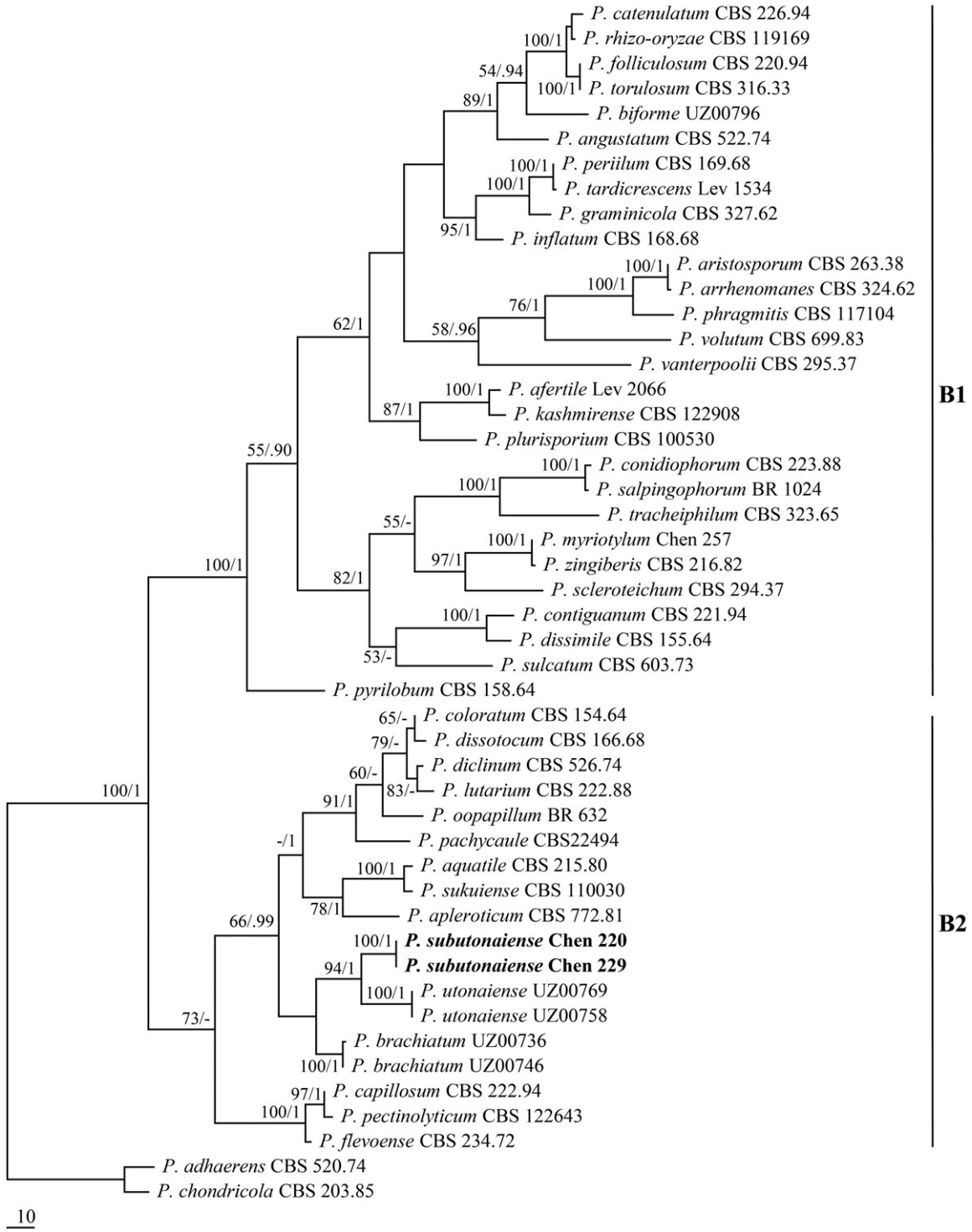

Figure 2. Phylogeny of *Pythium* clade B species inferred from ITS + COI dataset.

monoclinous antheridia with slender antheridial cells, and plerotic oospores.

According to Lévesque and de Cock [26], *Pythium* could be split into 11 clades (A–K), of which the species in subclade B2 are characterized by filamentous non-inflated to slightly inflated sporangia, smooth oogonia mostly smaller than 30 mm in diameter, and a moderate growth rate (mostly 10–20 mm per day). Phylogenetic analysis based on ITS and COI sequences indicated *P. subutonaiense* belongs to *Pythium* subclade B2 with full statistical supports. *P. subutonaiense* shares several morphological characteristics with other *Pythium* subclade B2 species, such as filamentous sporangia, smooth oogonia, and moderate growth rate. However, *P. subutonaiense* can be readily distinguished in having globose to sub-globose shaped and mostly terminal or sometimes catenulate hyphal swellings.

P. subutonaiense has a closer relationship with *P. utonaiense* according to the ITS and COI-based phylogeny (Figure 2). *P. utonaiense* resembles *P. subutonaiense* in having aquatic habit, filamentous

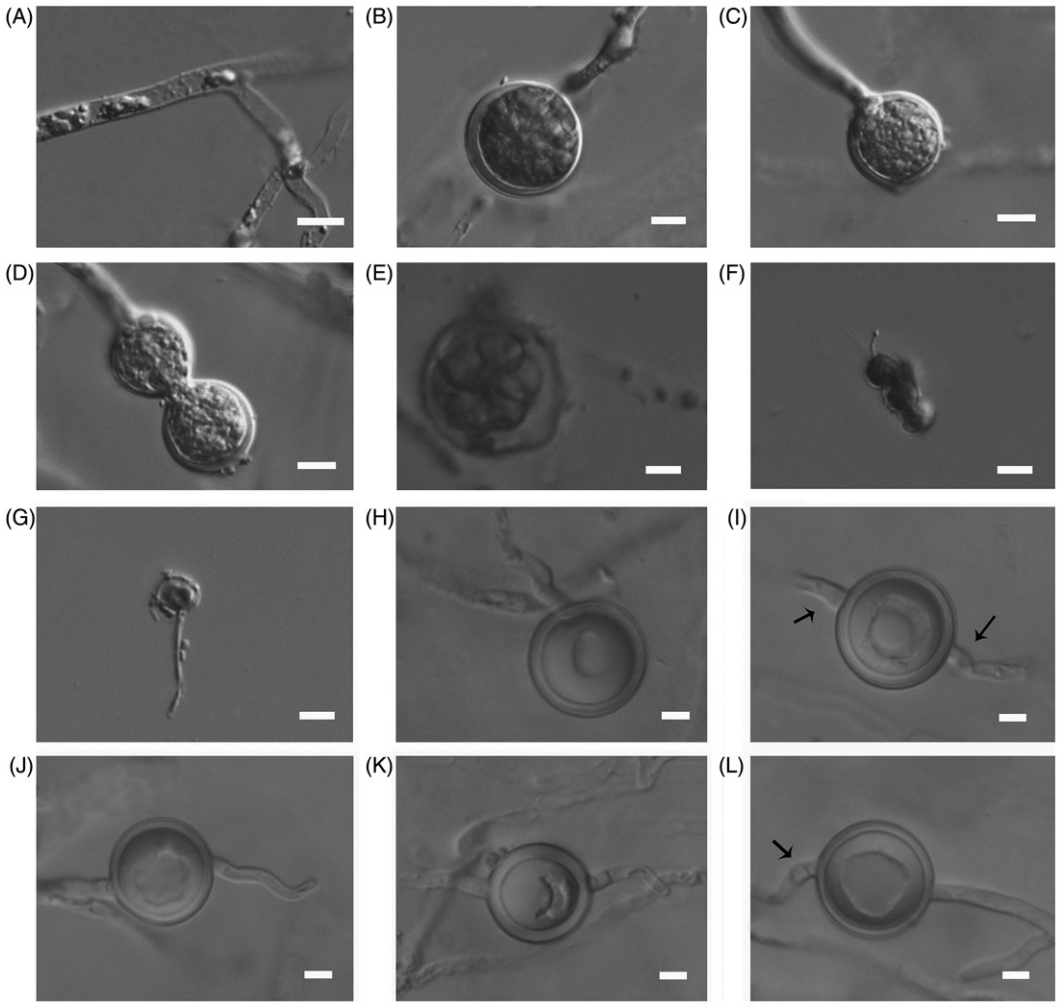

Figure 3. Asexual and sexual reproductive bodies of *P. subutonaiense* (Chen 220). (A) Mycelia; (B,C) Globose, terminal hyphal swellings; (D) Catenulate hyphal swellings; (E) A vesicle with zoospores; (F,G) Germinated encysted zoospore; (H) Terminal oogonium with plerotic oospore; (I) Intercalary oogonium with a plerotic oospore; (J,K) Terminal oogonia with a projection; (L) Plerotic oospore and a hypogynous antheridium. Arrows indicate antheridia are hypogynous. Bars A–F = 10 µm, G–L = 5 µm.

non-inflated sporangia, and plerotic oospores, but it is distinguished in its absence of hyphal swellings, faster growth rate, and the lack of antheridia [7] (Table 2).

P. brachiatum [7] is similar to *P. subutonaiense* by sharing aquatic habit and the formation of projections on oogonia, but the former species has slightly slower growth rate, lower optimum growth temperature, filamentous slightly inflated sporangia, and catenulate oogonia (Table 2). In addition, *P. brachiatum* is distant from *P. subutonaiense* in the ITS + COI sequence-based phylogeny [7] (Figure 2).

Both *P. capillosum* [27] and *P. flevoense* [28] have oogonia with a finger-like projection, and they somehow resemble *P. subutonaiense*; however, except for the lack of hyphal swellings, the two species can be distinguished from *P. subutonaiense* by slower growth rate, and thicker oospore wall [28] (Table 2). Besides, these three species clustered in different lineages in the phylogenetic analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Pringsheim N. Beiträge zur Morphology and Systematik der Algen. 2. Die Saprolegníeen. Jb Wiss Bot. 1858;1:284–306.

[2] van der Plaäts-Niterink AJ. Monograph of the genus *Pythium*. Stud Mycol. 1981;21:1–242.

[3] Ali-Shtayeh MS, Saleh ASF. Isolation of *Pythium acanthicum*, *P. oligandrum*, and *P. periplocum* from soil and evaluation of their mycoparasitic

activity and biocontrol efficacy against selected phytopathogenic *Pythium* species. Mycopathologia. 1999;145:143–153.
[4] Bala K, Robideau GP, Lévesque CA, et al. *Phytopythium sindhum* Lodhi. Shahzad & Levesque, sp. nov. Persoonia. 2010;24:136–137.
[5] Uzuhashi S, Tojo M, Kakishima M. Phylogeny of the genus *Pythium* and description of new genera. Mycoscience. 2010;51:337–365.
[6] Bouket AC, Arzanlou M, Tojo M, et al. *Pythium kandovanense* sp. nov., a fungus-like eukaryotic micro-organism (Stramenopila, Pythiales) isolated from snow-covered ryegrass leaves. Int J Syst Evol Microbiol. 2015;65:2500–2506.
[7] Uzuhashi S, Okada G, Ohkuma M. Four new *Pythium* species from aquatic environments in Japan. Antonie Van Leeuwenhoek. 2015;107:375–391.
[8] Abrinbana M, Badali F, Abdollahzadeh J. Molecular and morphological characterization of three new species of *Pythium* from Iran: *P. ershadii*, *P. pyrioosporum*, and *P. urmianum*. Mycologia. 2016;108:1175–1188.
[9] Ueta S, Tojo M. *Pythium barbulae* sp. nov. isolated from the moss, *Barbula unguiculata*; morphology, molecular phylogeny and pathogenicity. Mycoscience. 2016;57:11–19.
[10] Chen J-J, Lü LI, Ye W-W, et al. *Pythium cedri* sp. nov. (Pythiaceae, Pythiales) from southern China based on morphological and molecular characters. Phytotaxa. 2017;309:135–142.
[11] Veterano ST, Coffua LS, Mena-Ali JI, et al. *Pythium yorkensis* sp. nov., a potential soybean pathogen from southeastern Pennsylvania. Plant Pathol. 2018;67:619–625.
[12] Bush EA, Hong CX, Stromberg EL. Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. Plant Dis. 2003;87:1500–1506.
[13] Benard D, Punja ZK. Role of *Pythium* species in cavity spot development on carrots in British Columbia. Can J Plant Pathol. 1995;17:31–45.
[14] Chang HS. *Phytophthora* species associated with strawberry fruit rot in Taiwan. Bot Bull Acad Sin. 1988;29:61–67.
[15] Chen JJ, Cui BK. Studies on *Wrightoporia* from China 3. *Wrightoporia subavellanea* sp. nov. based on morphological characters and rDNA sequence data. Phytotaxa. 2014;175:225–234.
[16] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, editor. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315–322.
[17] Robideau GP, de Cock AWAM, Coffey MD, et al. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol Ecol Resour. 2011;11:1002–1011.
[18] Blair JE, Coffey MD, Park SY, et al. A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genet Biol. 2008;45:266–277.
[19] Thompson JD, Gibson TJ, Plewniak F, et al. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25:4876–4882.
[20] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–98.
[21] Swofford DL. PAUP*: phylogenetic analysis using parsimony (*and other methods). version 4.0b10. Sunderland: Sinauer Associates; 2002.
[22] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–791.
[23] Nylander JAA. MrModeltest v2. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University; 2004.
[24] Ronquist F, Huelsenbeck JP. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19:1572–1574.
[25] Page RMD. Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 1996;12:357–358.
[26] Lévesque CA, de Cock AWAM. Molecular phylogeny and taxonomy of the genus *Pythium*. Mycol Res. 2004;108:1363–1383.
[27] van der Plaäts-Niterink AJ. The occurrence of *Pythium* in the Netherlands: III. *Pythium flevoense* sp.nov. Acta Bot Neerl. 1972;21:633–639.
[28] Paul B. A new species of *Pythium* with filamentous sporangia from Algeria. Trans Br Mycol Soc. 1987;89:195–198.