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Six new species of *Pythiogeton* in Taiwan, with an account of the molecular phylogeny of this genus

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ABSTRACT

Pythiogeton is a little-studied genus of pythialean Oomycete. The genus is characterized by producing its zoospores outside of the sporangium within an apparently naked protoplasmic mass, which formed from a discharge tube-vesicle complex. A total of nine morphologically distinct *Pythiogeton* species were identified, of which six were new species (*Pythiogeton abundans*, *Pythiogeton microzoosporum*, *Pythiogeton oblongilobum*, *Pythiogeton paucisporum*, *Pythiogeton proliferatum*, and *Pythiogeton puliensis*). A phylogenetic analysis based on internal transcribed spacer sequences revealed that all isolates of *Pythiogeton* formed a highly supported clade, nested within the wider clade of *Pythium* species. Each newly recognized *Pythiogeton* species that was established on the basis of morphological characters was found to occur in a well-supported subgroup within the *Pythiogeton* clade, confirming their assignment to new species. *Pythiogeton* shares a common ancestor with the monophyletic group of *Pythium* species that have predominantly filamentous sporangia rather than with the separate clade of *Pythium* species that have predominantly globose or ovoid sporangia. This study confirms that *Pythium* is an extremely heterogenous and polyphyletic genus containing a number of distinct clades of species, including *Pythiogeton*, which possess morphologically distinguishable characters. A synoptic key to all the described *Pythiogeton* species is provided.

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1. Introduction

The genus *Pythiogeton*, a member of the pythialean line of oomycetes, was first erected by Minden (1916) to describe three species occurring on decaying plant materials in water (*Pythiogeton utriforme* Minden, *Pythiogeton ramosum* Minden, and *Pythiogeton transversum* Minden). An additional six species were subsequently described, namely *Pythiogeton autossytum* Drechsler (Drechsler 1932), *Pythiogeton uniforme* Lund (Lund 1934), *Pythiogeton dichotomum* Tokunaga (Ito and Tokunaga 1935), *Pythiogeton nigrescens* Batko (Batko 1971), *Pythiogeton zeae* Jee et al. (Jee et al. 2000), and *Pythiogeton zizaniae* Ann & Huang (Ann et al. 2006). Of these nine species, only five (*P. autossytum*, Hsieh and Chang 1976; Zebrowska 1976; *P. ramosum*, Watanabe 1974; *P. uniforme*, Cantino 1949; Hsieh and Chang 1976; *P. zeae*, Jee et al. 2000, and *P. zizaniae*, Ann and

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Huang 2006) have been successfully grown in laboratory culture. Descriptions of the other four species were exclusively based only on uncultured material (Minden 1916; Ito and Tokunaga 1935; Batko 1971). Recently, isolates that were presumed new species of *Pythiogeton*, were made from plant roots, ponds, irrigation canals and ditches although they were not formally named (Silva-Rojas et al. 2004; Lodhi et al. 2006).

Pythiogeton is distinguished from other members of the pythialean oomycetes by having asymmetrical sporangia with the long axis of sporangium eccentrically attached to the supporting hyphae (Minden 1916; Fitzpatrick 1930; Sparrow 1960). Protoplasm is discharged through a discharge tube into an elongate transient vesicle, which soon disappears, leaving the apparently naked protoplasm to differentiate into zoospores (Minden 1916; Fitzpatrick 1930; Sparrow 1960), i.e. zoosporogensis occurs extrasporangially in the apparently naked protoplasm. In this respect it seems to differ from other members of oomycetes such as *Pythium*, *Phytophthora* and *Halophytophthora*. In the former zoospores differentiate within a clearly visible extrasporangial vesicle (Webster and Dennis 1967; Van der Plaats-Niterink 1981) whereas in latter two genera fully formed zoospores are discharged from sporangia into transient vesicles which rapidly ruptures allowing the zoospores to swim away (Gisi et al. 1979; Nakagiri et al. 1994; Erwin and Ribeiro 1996). Dick (2001) however considered that even in *Pythiogeton* the discharged protoplasmic mass, which frequently detaches from the discharge tube, remained surrounded by a thin, barely visible vesicle during the period when the zoospores are forming.

The genus *Pythiogeton* has traditionally been placed in the family Pythiaceae (Hawksworth et al. 1995) because of the morphological similarities with the genus *Pythium*. However, a new family Pythiogetonaceae was then introduced to accommodate this genus and a newly described genus *Medusoides* based on subtle morphological and ecological characteristics (Voglmayr et al. 1999; Dick 2001). However, recently Robideau et al. (2011), in a study of DNA barcoding of oomycetes, included a species of *Pythiogeon* (*P. zeae*) in their analysis and confirmed that *Pythiogeton* was located among *Pythium* species in their phylogenetic tree. Other recent phylogenetic analyses have also shown that the genus *Pythium* is not monophyletic and contains several clades each with a different type of sporangium morphology (Levesque and de Cock 2004; Villa et al. 2006; Uzuhasi et al. 2010). Indeed the latter paper introduced a number of new genus names for the different *Pythium* clades. The clade 1 (Uzuhasi et al. 2010), containing mostly species with ovoid sporangia, was attributed to the genus *Ovatisporangium*, whilst the clade 4 (Uzuhasi et al. 2010), containing species with predominantly globose sporangia, was named *Globisporangium*. In this respect the genus *Pythiogeton* is interesting as it produces similar forms of sporangia.

Four species of *Pythiogeton* species (*P. autossytum*, *P. ramosum*, *P. uniforme*, and *P. zizaniae*) have been previously been reported from Taiwan (Watanabe 1974; Hsieh and Chang 1976; Ann et al. 2006). In this current survey conducted from 2004 to 2007 from cultivated paddy field ecosystems a total of nine *Pythiogeton* species were found, six of which appeared to be new species. They were isolated from various crops including water bamboo (*Zizania latifolia*), rice (*Oryza sativa*), water convolvulus (*Ipomoea aquatica*), and water caltrops (*Trapa natans*). Descriptions and illustrations of these species are provided. In an attempt to fully characterize these new species as well helping to resolve the relationship between *Pythiogeton* and *Pythium*, a phylogenetic analysis based on ITS sequences was also carried out on these new Taiwanese isolates. Preferable habitats of *Pythiogeton* are also discussed based on their isolation tendency.

2. Materials and methods

2.1. Isolation and cultivation

Water bamboo, rice, water convolvulus, and water caltrops grown in paddy fields were surveyed. Debris and necrotic tissue of these crops submerged in stagnant water were brought to the laboratory. They were washed in running tap water, rinsed in sterile distilled water, and blotted dry with sterile paper towel. The dried plant tissue was then cut into pieces and placed onto 9 cm Petri dishes (4 pieces per plate) of potato dextrose agar (PDA; Difco, USA) and modified PDA (MPDA). The latter consists of 1% Bacto Agar (WA; Difco, USA), 1% PDA, supplemented with 800 ppm streptomycin (Sigma, USA), 1 ppm benomyl, and 1 ppm prochloraz. The plates were incubated at room temperature (23–30 °C) for 3–5 days. Once hyphae protruding outwards from samples and growing onto agar media, small agar blocks containing hyphal tips were picked up and transferred onto rye-seed medium (RSA) (Ann et al. 2006), with 1 block in each plate. Hyphal tip transfer was sometimes repeated 2–3 times on RSA to ensure the complete removal of contaminants.

2.2. Colonial morphology and mycelial growth

Cultures were grown on RSA at 24–28 °C in darkness for 4 days. To measure the cardinal temperatures and optimal growth rates, agar blocks (8 × 8 × 3 mm) from the mycelial front were removed onto RSA and placed near the edge of plates with 1 block in each plate, with 3 repeats for each species. Plates were incubated at different temperatures (8–40 °C with intervals of 4 °C), and daily growth rates were determined between 24 and 48 h after inoculation.

2.3. Production of sporangia and release of zoospores

Sporulation was induced using a modification of the method described by Chang (1988). Agar blocks (8 × 8 × 3 mm) were cut from the mycelia fronts of 3–7 d old RSA cultures and placed in 10% clarified V-8 juice (one block per dish) and incubated in darkness at 24–26 °C for 48–72 h. Once the mycelium has reached 4–5 cm in diameter, the V-8 medium 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 respectively in SDW or filtered (non-sterile) field water (NSFW). The latter was prepared from water collected from paddy fields growing water bamboo, which was filtered through double-layer filter paper (Whatman No. 1, USA) before use. The plates containing SDW and NDFW were incubated at 24–26 °C for 24–48 h prior

to examining for release of zoospores under the light microscope (Olympus BX-50, Japan). Photographing was conducted with an Olympus PM-30 camera system. Color film of Fujifilm Superia X-tra 400 was used to obtained high resolution.

2.4. Oospore production

Agar blocks (8 × 8 × 3 mm) from mycelial fronts of 3–7 d age cultures on RSA were placed into plates with 10% clarified V-8 juice solution, and then were incubated in darkness at 20–24 °C for 14 d. If oospores could not be obtained by this method, isolates of each species were mutually paired. Two agar blocks from different isolates of the same species were placed in the same plate of 10% V-8 juice agar incubated in darkness at 20–24 °C for 14 d. Plates were examined weekly for the production of oogonia and oospores under light microscope (Olympus BX-50, Japan).

2.5. DNA extraction, PCR amplification, sequencing ITS rDNA and phylogenetic analysis

In total 24 isolates of *Pythiogeton* isolated in this study were sequenced. They underwent DNA extraction, amplification and sequencing, and were deposited in Bioresources Collection and Research Center (BCRC) in Taiwan. Additional 5 *Pythium* isolates and 1 *Phytophthora* isolate obtained in Taiwan were also used, each of which was designated as "Py" or "Ph" followed by a number to represent the isolates of *Pythium* and *Phytophthora* respectively (Table 1). In addition to the above-mentioned isolates, the other sequences applied in the phylogenetic analysis were retrieved from Genbank (Table 1). The only other sequence of *Pythiogeton* incorporated into the phylogenetic analysis is from Genbank.

The total DNA was extracted from mycelia using Plant Genomic DNA Mini Kit (Cat. No.: GP100, Geneaid Co., Taiwan) according to the manufacturer's instructions. The extracted DNA was amplified by PCR reaction with the primer pair ITS5 and ITS4 (White et al. 1990) for the ITS region. PCR reaction mixtures were 25 μl, including 2.5 μL 1×PCR buffer, 0.2 mM dNTP, 0.2 μM forward and reverse primers, 0.5 U Taq DNA polymerase (Geneaid Co., Taiwan), and 0.1 μg template genomic DNA. For initial amplification, PCR reaction mixtures were denatured for 5 min at 96 °C, followed by 35 PCR cycles for 30 s at 95 °C, 30 s at 52 °C, and 90 s at 72 °C. The final extension step was 10 min at 72 °C. The PCR products were purified with the Gel/PCR Fragments Extraction Kit (Cat. No.: DF300, Geneaid Co., Taiwan) following the manufacturer's instructions. Sequencing was conducted by Seening Biotechnology Co. (Taiwan).

In the phylogenetic analysis, ITS sequence (AB217688) of *Saprolegnia parasitica* was selected as an outgroup. Sequences were aligned using Clustal X1.8 software (Thompson et al. 1997) and visual editing the alignments were conducted with Sequence Alignment (Se-Al) BioEditor v.1.0 program (Rambaut 2000). The multiple aligned sequences were analyzed using neighbor-joining (NJ) program under the PAUP 4.0 software (Swofford 2001) with bootstrap analysis of 1000 replicates. The Kimura 2-parameter distance matrix was analyzed by pairwise distances with PAUP 4.0 (Kimura 1980).

3. Taxonomy

Pythiogeton abundans J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 1.

Mycobank no.: MB 563285

Hyphae usque 5 μm latae. Sporangia copiosa, cum formis variis, globosa, 21–33 μm diam., ovoidea, 35–50 × 25–45 μm, vel ellipsoidea, 32–80 × 20–35 μm, raro interne proliferantia. Tubi emittentes 5–75(–107) μm longi et 5 μm lati. Zoosporae incystatae 10–13 μm diam.

Holotype: TAIWAN, Nantou, isolated from stem debris of water bamboo (*Z. latifolia*), Jun. 2006, J.H. Huang, *Pg-144*, BCRC CH30016 (metabolically inactive culture).

Etymology: species epithet in reference to the abundant sporangia.

Colonies on RSA at 28 °C with daily growth rate of 22.3 mm per day, appearing a rosette pattern on RSA and PDA, hyphae up to 5 μm wide, without aerial mycelium. Appressoria club-shaped. Sporangia terminal on supporting hyphae, abundant, various in shape, globose, 21–33 μm in diam., ovoid, 35–50 × 25–45 μm, or ellipsoid, 32–80 × 20–35 μm, occasionally bilobate, rare internally proliferating. Discharge tubes 5–75(–107) μm long and 5 μm wide, protruding from different positions around sporangia. Encysted zoospores 10–13 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 12 °C; optimum, 28 °C; maximum, 36 °C.

Other specimens examined: TAIWAN, Nantou, isolated from debris of water bamboo (*Z. latifolia*). Aug. 2006, J.H. Huang, *Pg-145*, BCRC CH30017 (metabolically inactive culture); Taichung, isolated from leaf debris of rice (*O. sativa*), Aug. 2006, J.H. Huang, *Pg-163*, BCRC CH30026 (metabolically inactive culture).

Note: in *P. abundans* sporangia were produced abundantly both in non-sterile field water and sterile distilled water, and are deciduous 2–3 days after formation. This species is morphologically similar to *P. autossytum*, a species synonymized under *P. ramosum* in the present paper. Both species have globose to ellipsoid sporangia. However *P. autossytum* differs in having larger sporangia (50–150 × 30–54 μm in size), large encysted zoospores (13–17 μm in diam.), and wider hyphae (up to 7 μm in width) (Drechsler 1932; Hsieh and Chang 1976).

Pythiogeton microzoosporum J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 2.

Mycobank no.: MB 563286

Hyphae usque 5 μm latae. Sporangia cum formis variis, globosa, 35–52 μm diam., vel irregulariter lobata, 90–125 × 50–62.5 μm, interdum interne proliferantia. Tubi emittentes 15–55(–350) μm longi et (4–)6–9 μm lati. Zoosporae incystatae 7–10 μm diam.

Holotype: TAIWAN, Nantou, isolated from stem debris of water bamboo (*Z. latifolia*), Jun. 2006, J.H. Huang, *Pg-127*, BCRC CH30013 (metabolically inactive culture).

Etymology: species epithet in reference to the small zoospores.

Colonies on RSA at 32 °C with daily growth rate of 10.0 mm per day, appearing a rosette pattern on RSA, while not appearing special pattern on PDA, hyphae up to 5 μm wide, without aerial mycelium. Sporangia terminal on supporting

Table 1 – List of isolates used in this study.

Species	Isolate number	GenBank accession no.	Reference
Peronosporales			
Hyaloperonospora brassicae	KUS-F22524	EU137726	Hong et al. 2008
Peronospora conglomerate	WU22894	AY919304	Belbahri et al. 2005
Pseudoperonospora cubensis	CDN-278	JF304672	Mitchell et al. 2011
Pythiales			
Phytophthora cactorum	Shakuyaku 1-1	AB217674	Villa et al. 2006
Phytophthora capsici	IFO30696	AB217670	Villa et al. 2006
Phytophthora capsici	Ph-037	JQ610200	The present paper
Phytophthora cinnamomi	IFO33182	AB217675	Villa et al. 2006
Phytophthora citricola	CH98U121C	AB217678	Villa et al. 2006
Phytophthora nicotianae	Phkq 1-1	AB217682	Villa et al. 2006
Phytophthora vignae	IFO30473	AB217687	Villa et al. 2006
Pythiogeton abundance	BCRC-CH30016	JQ610184	The present paper
Pythiogeton abundance	BCRC-CH30026	JQ610189	The present paper
Pythiogeton microzoosporum	BCRC-CH30013	JQ610183	The present paper
Pythiogeton oblongilobum	BCRC-CH30011	JQ610182	The present paper
Pythiogeton oblongilobum	BCRC-CH30022	JQ610187	The present paper
Pythiogeton paucisporum	BCRC-CH30035	JQ610196	The present paper
Pythiogeton paucisporum	BCRC-CH30039	JQ610198	The present paper
Pythiogeton proliferatum	BCRC-CH30037	JQ610197	The present paper
Pythiogeton proliferatum	BCRC-CH30040	JQ610199	The present paper
Pythiogeton puliensis	BCRC-CH30010	JQ610181	The present paper
Pythiogeton puliensis	BCRC-CH30020	JQ610186	The present paper
Pythiogeton puliensis	BCRC-CH30033	JQ610194	The present paper
Pythiogeton ramosum	BCRC-CH30007	JQ610179	The present paper
Pythiogeton ramosum	BCRC-CH30008	JQ610180	The present paper
Pythiogeton ramosum	BCRC-CH30019	JQ610185	The present paper
Pythiogeton ramosum	BCRC-CH30023	JQ610188	The present paper
Pythiogeton ramosum	BCRC-CH30027	JQ610190	The present paper
Pythiogeton ramosum	BCRC-CH30031	JQ610193	The present paper
Pythiogeton ramosum	BCRC-CH30034	JQ610195	The present paper
Pythiogeton uniforme	BCRC-CH30029	JQ610191	The present paper
Pythiogeton uniforme	BCRC-CH30030	JQ610192	The present paper
Pythiogeton zeae	Lev3132	HQ643405	Robideau et al. 2011
Pythiogeton zizaniae	BCRC-CH30001	JQ610176	The present paper
Pythiogeton zizaniae	BCRC-CH30002	JQ610177	The present paper
Pythiogeton zizaniae	BCRC-CH30006	JQ610178	The present paper
Pythium amasculinum	CBS 552.88	AY598671	Levesque and de Cock (2004)
Pythium aphanidermatum	Py-010	JQ610201	The present paper
Pythium catenulatum	CBS 842.68	AY598675	Levesque and de Cock (2004)
Pythium deliense	CBS 314.33	AY598674	Levesque and de Cock (2004)
Pythium diclinum	CBS 664.79	AY598690	Levesque and de Cock (2004)
Pythium grandisporangium	CBS 211.85	AY598716	Levesque and de Cock (2004)
Pythium helicoides	Py-014	JQ610202	The present paper
Pythium hydnosporum	CBS 253.60	AY598672	Levesque and de Cock (2004)
Pythium hypogynum	CBS 234.94	AY598693	Levesque and de Cock (2004)
Pythium irregulare	CBS 250.28	AY598702	Levesque and de Cock (2004)
Pythium mamillatum	CBS 251.28	AY598703	Levesque and de Cock (2004)
Pythium monospermum	CBS 158.73	AY598621	Levesque and de Cock (2004)
Pythium myriotylum	CBS 254.70	AY598678	Levesque and de Cock (2004)
Pythium myriotylum	Py-050	JQ610204	The present paper
Pythium nunn	CBS 808.96	AY598709	Levesque and de Cock (2004)
Pythium ostracodes	CBS 768.73	AY598663	Levesque and de Cock (2004)
Pythium porphyrae	CBS 369.79	AY598673	Levesque and de Cock (2004)
Pythium rostratum	CBS 533.74	AY598696	Levesque and de Cock (2004)
Pythium spinosum	CBS 275.67	AY598701	Levesque and de Cock (2004)
Pythium splendens	Py-017	JQ610203	The present paper
Pythium ultimum	Py-052	JQ610205	The present paper
Pythium uncinulatum	CBS 518.77	AY598712	Levesque and de Cock (2004)
Pythium undulatum	CBS 157.69	AY598708	Levesque and de Cock (2004)
Pythium vexans	CBS 119.80	AY598713	Levesque and de Cock (2004)
Pythium violae	CBS 159.64	AY598706	Levesque and de Cock (2004)
Saprolegniales			
Saprolegnia parasitica	IFO 32780	AB217688	Villa et al. 2006

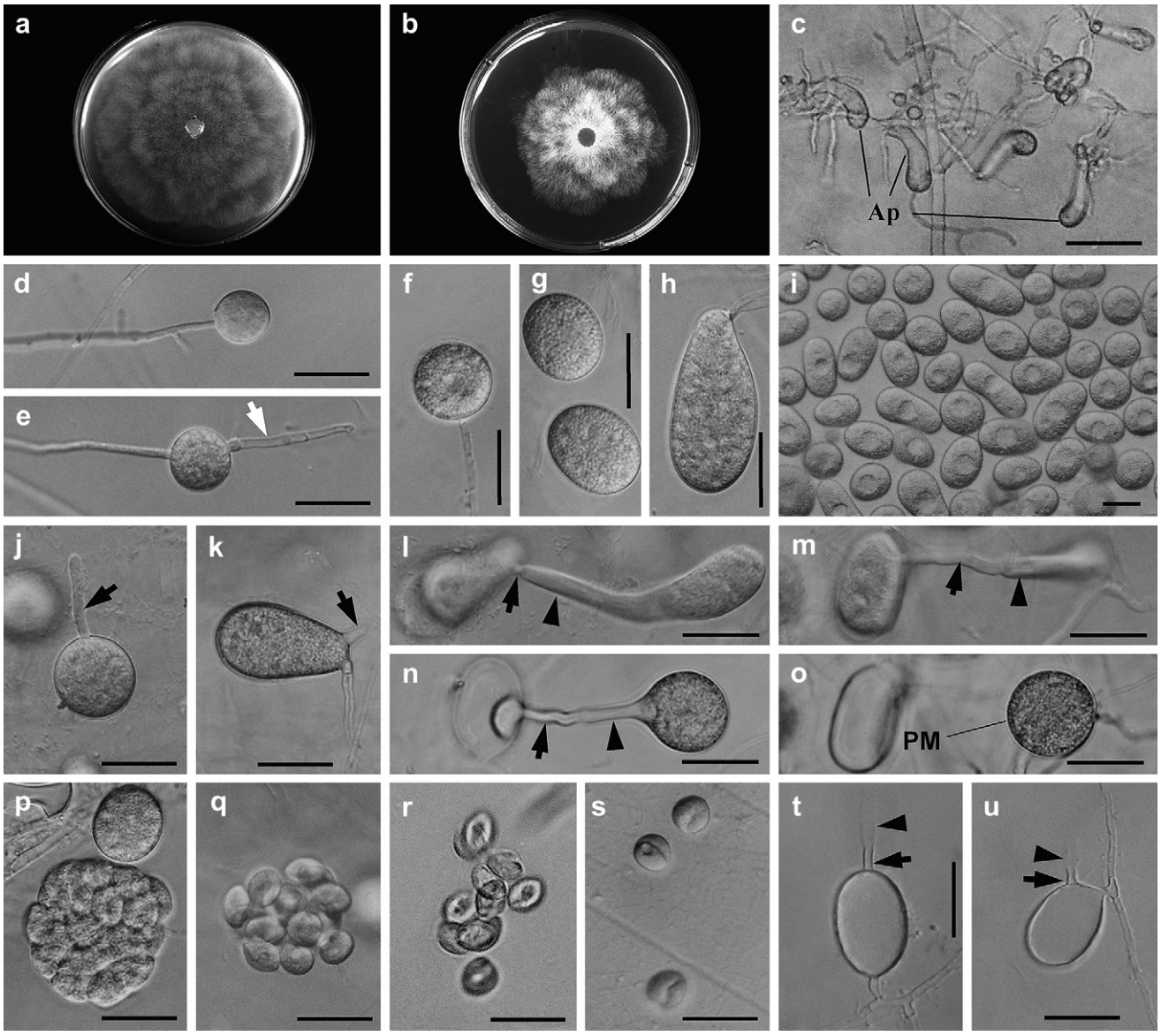

Fig. 1 – *Pythiogeton abundans*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–I, sporangia, showing immature sporangia in d and e, evanescent hyphal residue (arrow) in e, and deciduous sporangia in i; j–o, processes of the discharge of protoplasm from sporangia, showing discharge tubes (arrows) in j and k, discharge tubes (arrows) and vesicles (arrowheads) in l, m, and n, and a protoplasm mass (PM) in o (figures n and o are photographed on the same object with consecutive time); p, q, formation of zoospores from a protoplasm mass; r, s, discharged zoospores; t, u, empty sporangia, showing empty discharge tubes (arrows) and remnant of vesicle residue (arrowheads). *Bars* 25 μm.

hyphae, various in shape, globose, 35–52 μm in diam., or irregularly lobate, 90–125 × 50–62.5 μm, occasionally internally proliferating. Discharge tubes 15–55(–350) μm long and (4–)6–9 μm wide, protruding from different positions around sporangia, becoming narrowed at the opening, 1–2 μm wide. Encysted zoospores 7–10 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 32 °C; maximum, 36 °C.

Note: this species produced many sporangia in non-sterile field water, but few in sterile distilled water. This species is distinct from other *Pythiogeton* species in having small zoospores and broad discharge tubes, which are broader than the hyphae. *P. nigrescens* (Batko 1971), a closely resembling species, also has small zoospores, but differs in that the primary zoospores encyst soon after formation and aggregate in a group.

Pythiogeton oblongilobum J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 3.

Mycobank no.: MB 563287

Hyphae usque 7.5 μm latae. Sporangia cum formis variis, globosa, 20–38 μm diam., ovoidea, 38–45 × 24–40 μm, vel bilobata ad multilobata elongata, 44–90 × 30–40 μm, raro

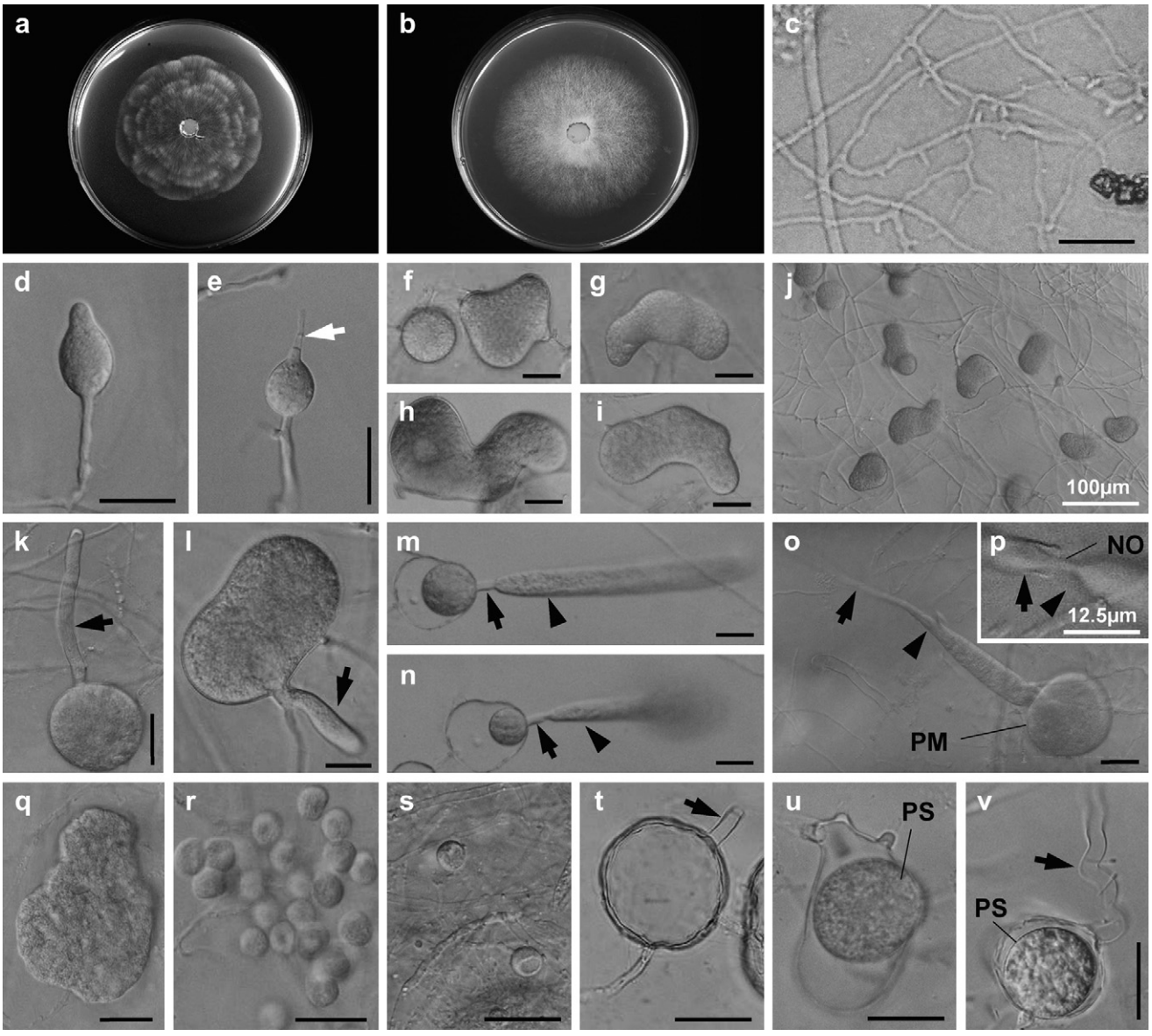

Fig. 2 – *Pythiogeton microzoosporum*. a, Colony on RSA; b, colony on PDA; c, hyphae on RSA; d–j, sporangia, showing immature sporangia in d and e, and evanescent hyphal residue (arrow) in e; k–p, processes of the discharge of protoplasm from sporangium, showing discharge tubes (arrow) in k and l, discharge tubes (arrows) and vesicles (arrowheads) in m–p, an protoplasm mass (PM) in o, and a narrow opening (NO) in p (figures m and n are photographed on the same object with consecutive time); q, r, formation of zoospores from a protoplasm mass; s, the encysted zoospores; t–v, empty sporangia, showing empty discharge tubes (arrows) in t and v, and internal proliferous sporangia (PS) in u and v. *Bars* 25 μm.

interne proliferantia. Tubi emittentes 9–24(–400) longi et 7–8 μm lati, apertura leviter, contracta, 5–6 μm lata. Zoosporae incystatae 12–15 μm diam.

Holotype: TAIWAN, Nantou, isolated from stem debris of water bamboo (*Z. latifolia*), Aug. 2006, J.H. Huang, *Pg-158*, BCRC CH30022 (metabolically inactive culture).

Etymology: species epithet in reference to the oblong shape of sporangia.

Colonies on RSA at 32 °C with daily growth rate of 12.3 mm per day, appearing a coarsely radiate pattern on RSA and PDA, hyphae up to 7.5 μm wide, without aerial mycelium. Sporangia terminal on supporting hyphae, various in shape, globose, 20–38 μm in diam., ovoid, 38–45 × 24–40 μm, or bilobate to elongate multilobate, 44–90 × 30–40 μm, rarely internally proliferating. Discharge tube 9–24(–400) long and 7–8 μm wide, protruding from different positions around sporangia, becoming slightly narrowed at the opening, 5–6 μm wide. Encysted zoospores 12–15 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 32 °C; maximum, 36 °C.

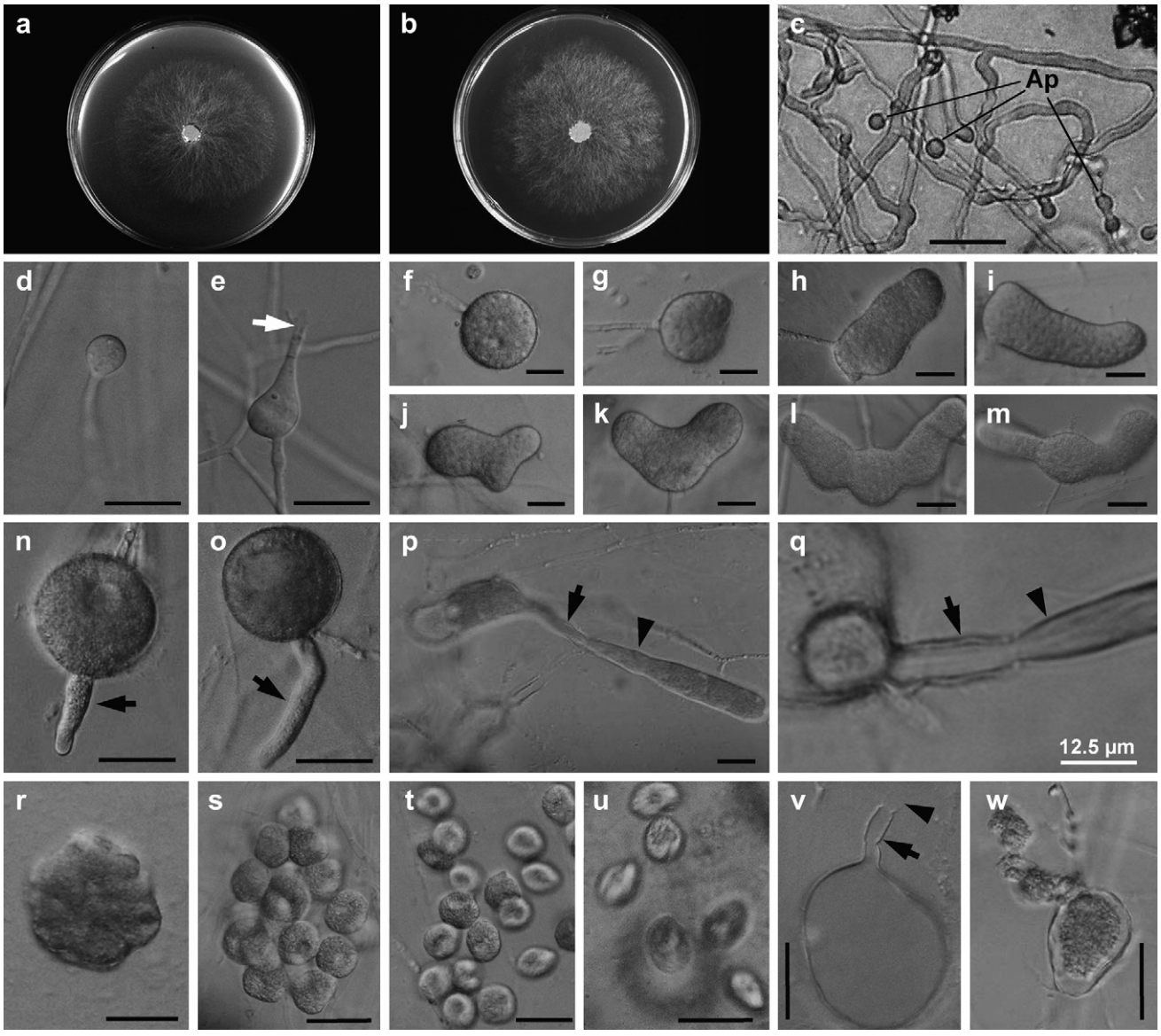

Fig. 3 – *Pythiogeton oblongilobum*. a, colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–m, sporangia, showing immature sporangia in d and e, and evanescent hyphal residue (arrow) in e; n–q, processes of the discharge of protoplasm from sporangia, showing discharge tubes (arrows) in n and o, and discharge tubes (arrows) and vesicles (arrowhead) in p and q; r, s, formation of zoospores from a protoplasm mass; t, u, discharged zoospores; v, empty sporangia, showing an empty discharge tube (arrow) and remnant of vesicle residue (arrowhead); w, immature sporangium. *Bars* 25 μm.

Other specimens examined: Taiwan, Nantou, isolated from stem debris of *Z. latifolia*, Aug. 2006, J.H. Huang, *Pg-121*, BCRC CH30011 (metabolically inactive culture); Nantou, isolated from stem debris of *Z. latifolia*, Aug. 2006, J.H. Huang, *Pg-157*, BCRC CH30021 (metabolically inactive culture).

Note: this species is distinct from other *Pythiogeton* species in having globose and elongate multilobate sporangia. Mycelium is obviously sparse on RSA and PDA. Zoospores in liquid cultures tend to swim toward the bottom, while zoospores of other *Pythiogeton* species tend to swim toward the water surface.

Pythiogeton paucisporum J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 4.

Mycobank no.: MB 563288

Hyphae usque 7.5 μm latae. Sporangia plerumque globosa, 25–32 μm diam, interdum ovoidea, 55–65 × 25–30 μm, interdum interne proliferantia, cum minus quam 10 zoosporis in sporangio singulo. Tubi emittentes 3.8–42.5(–170) μm longi et 5 μm lati. Zoosporae incystatae 17.5–20 μm diam.

Holotype: Taiwan, Tainan, isolated from leaf debris of water caltrops (*T. natans*), Nov. 2007, J.H. Huang, *Pg-192*, BCRC CH30039 (metabolically inactive culture).

Etymology: species epithet in reference to the few zoospores produced in each sporangium.

Colonies on RSA at 32 °C with daily growth rate of 14.3 mm per day, appearing a coarsely radiate pattern on RSA

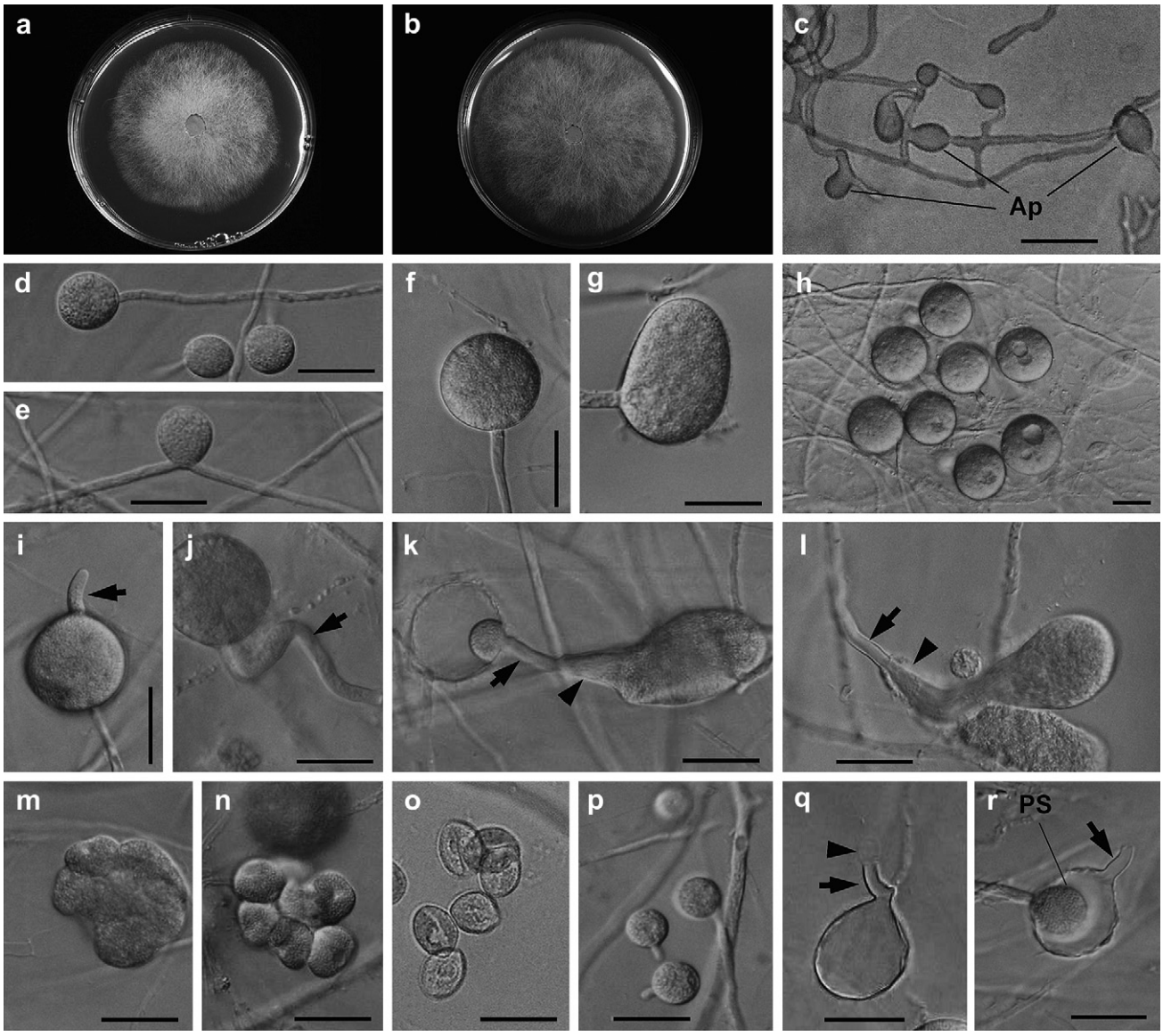

Fig. 4 – *Pythiogeton paucisporum*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–h, sporangia, showing immature sporangia in d and e; i–l, processes of the discharge of protoplasm from sporangia, showing discharge tubes (arrows) in i and j, and discharge tubes (arrows) and vesicles (arrowheads) in k and l; m, n, formation of zoospores from a protoplasm mass; o, p, discharged zoospores, showing the swimming zoospores in o, and encysted and germinating zoospores in p; q, r, empty sporangia, showing an empty discharge tube (arrow) and remnant of vesicle residue (arrowhead) in q, and an internal proliferous sporangium (PS) in the empty sporangium provided with an empty discharge tube (arrow) in r. *Bars* 25 μm.

and PDA, hyphae up to 7.5 μm, without aerial mycelium. Appressoria club-shaped. Sporangia terminal on supporting hyphae, mostly globose, 25–32 μm in diam., occasionally ovoid, 55–65 × 25–30 μm, occasionally internally proliferating, with less than 10 zoospores in each sporangium. Discharge tubes 3.8–42.5(–170) μm long and 5 μm wide, protruding from different positions around sporangia. Encysted zoospores 17.5–20 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 32 °C; maximum, 36 °C.

Other specimens examined: TAIWAN, Tainan, isolated from leaf debris of water caltrops (*T. natans*), Nov. 2007, J.H. Huang, *Pg-185*, BCRC CH30035 (metabolically inactive culture); Tainan, isolated from leaf debris of water caltrops (*T. natans*), Nov. 2007, J.H. Huang, *Pg-186*, BCRC CH30036 (metabolically inactive culture).

Note: this species is characteristic in globose and relatively small sporangia, and relatively large zoospores. Due to the small sporangia, less than 10 zoospores can be accommodated in each sporangium. Other *Pythiogeton* species with globose sporangia contain more than 10 zoospores in each sporangium.

Pythiogeton proliferatum J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 5.

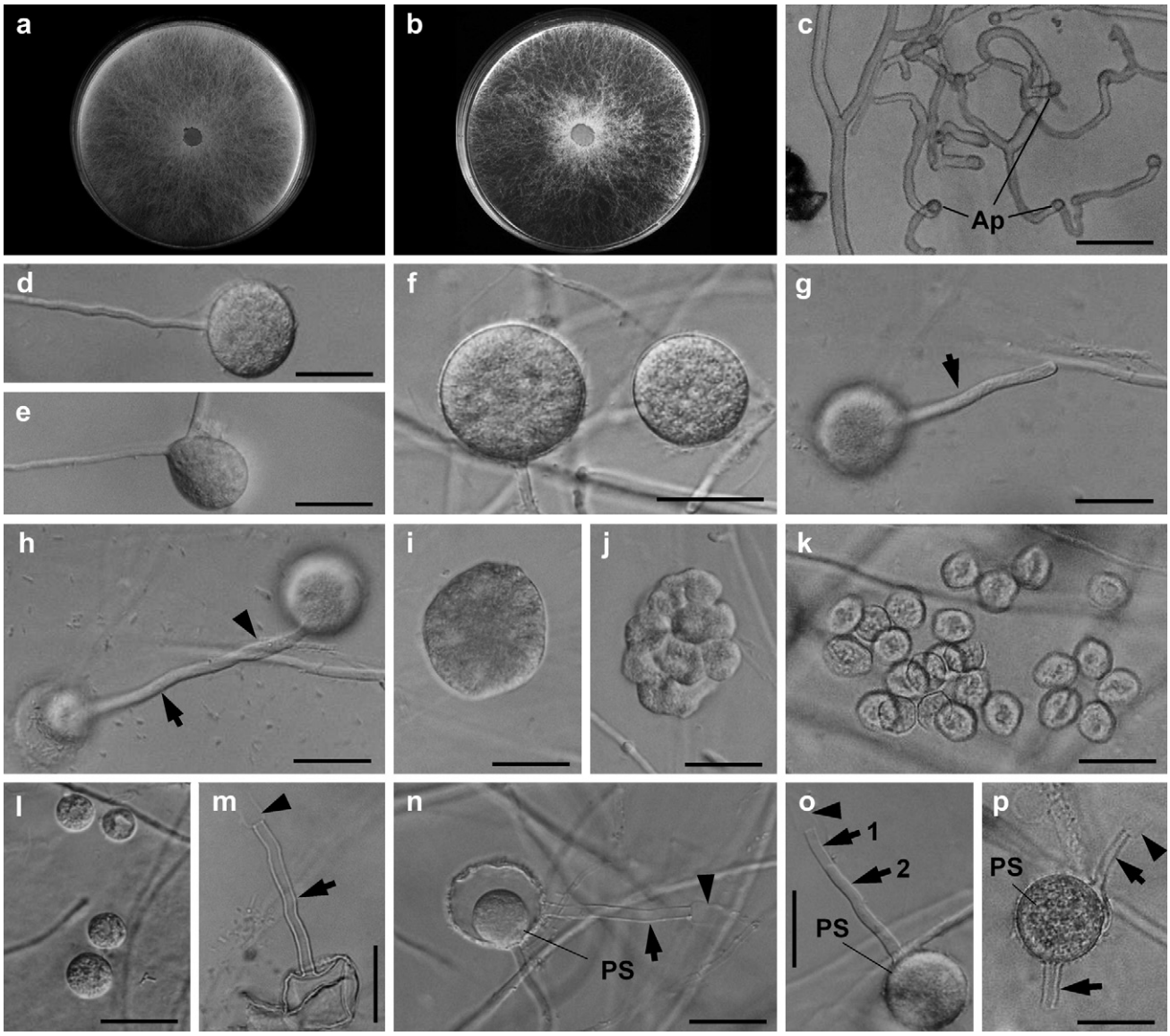

Fig. 5 – *Pythiogeton proliferatum*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–f, sporangia, showing immature sporangia in d and e; g–j, processes of the discharge of protoplasm from sporangia, showing an discharge tube (arrow) in g, and an discharge tube (arrow) and a vesicle (arrowhead) in h; i, j, formation of zoospores from a protoplasm mass; k–l, discharged zoospores, showing the swimming zoospores in k and encysted zoospores in l; m–p, sporangia, showing an empty discharge tube (arrows) and remnant of vesicle residue (arrowheads) in m and n, an internal proliferous sporangium (PS) in n, an internal proliferous sporangium (PS) producing a new discharge tube (arrow 2) through the old one (arrow 1) with a vesicle residue (arrowhead) in o, and a second generation of proliferous sporangium (PS) in an empty sporangium with two old discharge tubes (arrows) and vesicle residue (arrowhead) in p. *Bars* 25 μm.

Mycobank no.: MB 563289

Hyphae usque 7.5 μm latae. Sporangia globosa, 30–42.5 μm diam, interne proliferantia. Tubi emittentes 10–102 μm longi et 4.5 μm lati. Zoosporae incystatae 11.3–12.5 μm diam.

Holotype: TAIWAN, Tainan, isolated from leaf debris of water caltrops (*T. natans*), Nov. 2007, J.H. Huang, *Pg-188*, BCRC CH30037 (metabolically inactive culture).

Etymology: species epithet in reference to the proliferating sporangium.

Colonies on RSA at 32 °C with daily growth rate of 10.3 mm per day, appearing a coarsely radiate pattern on RSA and PDA, hyphae up to 7.5 μm wide, without aerial mycelium. Sporangia terminal on supporting hyphae, globose, 30–42.5 μm in diam., internally proliferating, appearing shrunken after zoospores discharge. Discharge tubes 10–102 μm long and 4.5 μm wide, protruding from different positions around the sporangia. Encysted zoospores 11.3–12.5 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 12 °C; optimum, 32 °C; maximum, 36 °C.

Other specimens examined: TAIWAN, Tainan, isolated from leaf debris of water caltrops (*T. natans*), Nov. 2007, J.H. Huang, *Pg-190*, BCRC CH30038 (metabolically inactive culture); Tainan, isolated from leaf debris of water caltrops (*T. natans*),

Nov. 2007, J.H. Huang, *Pg-195*, BCRC CH30040 (metabolically inactive culture).

Note: this species is characterized by the internally proliferating sporangia. There are usually two to three generations of proliferations.

Pythiogeton puliensis J.H. Huang, C.Y. Chen & Y.S. Lin, sp. nov. Fig. 6.

Mycobank no.: MB 563290

Hyphae usque 7.5 μm latae. Sporangia plerumque globosa, 22–43 μm diam, interdum ovoidea vel obovoidea, 42–50 × 30–40 μm, interdum interne proliferantia. Tubi emittentes 7–43(–250) μm longi et 5–7 μm lati, apertura contracta, 1–2 μm lata. Zoosporae incystatae 11–14 μm diam.

Holotype: TAIWAN, Nantou, Puli town, isolated from stem debris of water bamboo (*Z. latifolia*), Aug. 2006, J.H. Huang, *Pg-118*, BCRC CH30010 (metabolically inactive culture).

Etymology: species epithet in reference to the location of the type species.

Colonies on RSA at 32 °C with daily growth rate of 24.3 mm per day, not appearing special pattern on RSA and PDA, hyphae up to 7.5 μm wide, without aerial mycelium. Sporangia terminal on supporting hyphae, mostly globose, 22–43 μm in diam., occasionally ovoid or obovoid, 42–50 × 30–40 μm, occasionally internally proliferating. Discharge tubes 7–43(–250) long and 5–7 μm wide, protruding

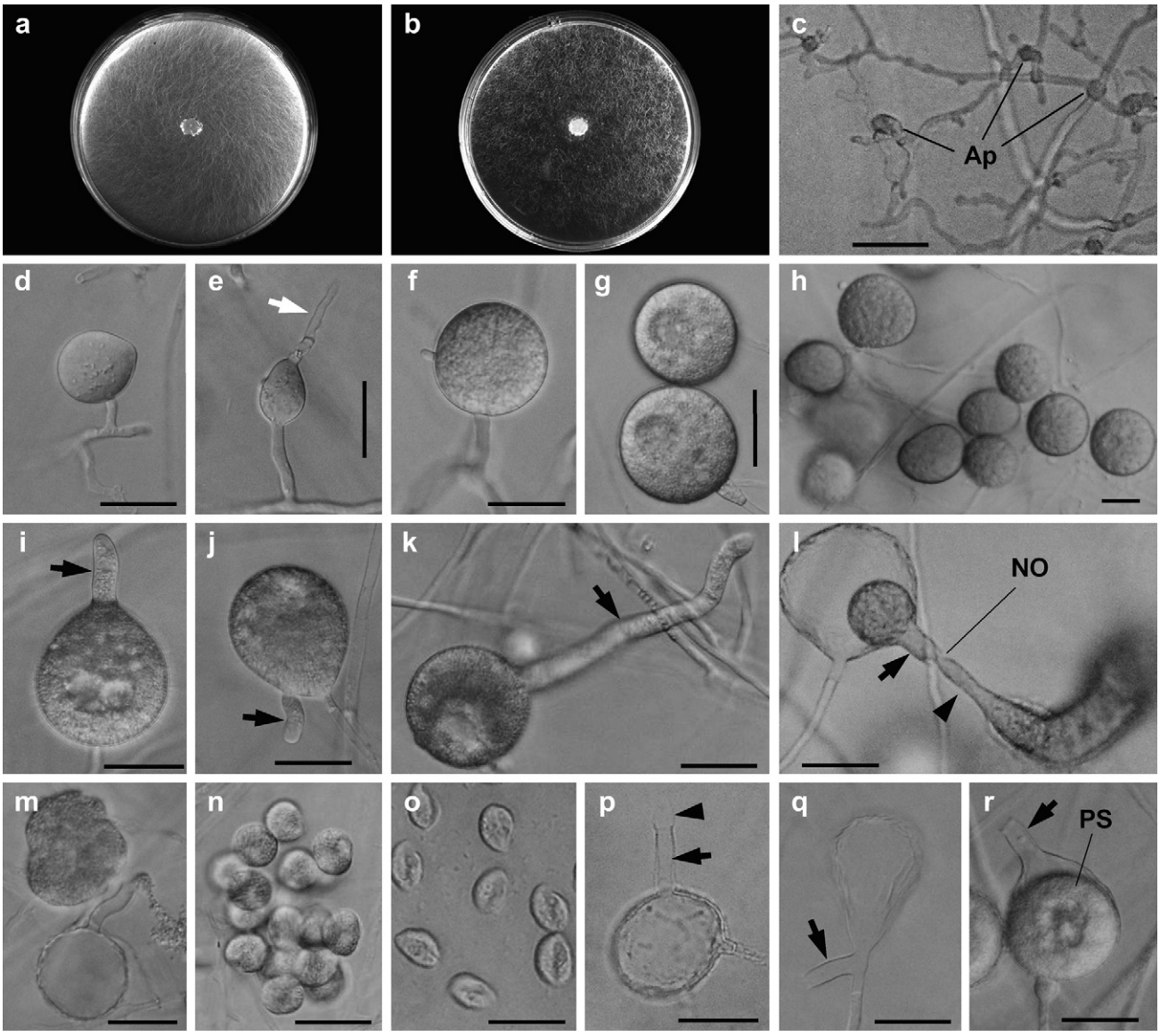

Fig. 6 – *Pythiogeton puliensis*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–h, sporangia, showing immature sporangia in d and e, and evanescent hyphal residue (arrow) in e; i–l, processes of the discharge of protoplasm from sporangium, showing discharge tubes (arrows) in i, j, and k, an discharge tube (arrow), a vesicle (arrowhead), and a narrow opening (NO) in l; m, n, formation of zoospores from a protoplasm mass; o discharged zoospores; p–r, sporangia, showing empty discharge tubes (arrows) and remnant of vesicle residue (arrowhead) in p and q, and an internal proliferous sporangium (PS) in the empty sporangium attached with an empty discharge tube (arrow) in r. *Bars* 25 μm.

from different positions around sporangia, becoming narrowed at the opening, 1–2 μm wide. Encysted zoospores 11–14 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 32 °C; maximum, 36 °C.

Other specimens examined: TAIWAN, Nantou, Puli town, isolated from stem debris of water bamboo (*Z. latifolia*), Aug. 2006, J.H. Huang, *Pg-153*, BCRC CH30020 (metabolically inactive culture); Taoyuan, isolated from leaf debris of water bamboo Nov. 2006, J.H. Huang, *Pg-180*, BCRC CH30033 (metabolically inactive culture).

Note: a narrow opening at the apex of each discharge tube is distinct in this species. Non-sterile field water can significantly enhance the formation of sporangia.

***Pythiogeton ramosum* Minden**, in Falck, Mykol. Untersuch. Ber. **1**: 243 (1916) Fig. 7.

Pythiogeton autossytum Drechsler, J. Wash. Acad. Sci. 22: 447 (1932)

Colonies on RSA at 32 °C with daily growth rate of 31.0 mm per day, not appearing special pattern on RSA and PDA, hyphae up to 7.5 μm wide, aerial mycelia sparse. Sporangia subterminal on supporting hyphae, narrowly bursiform,

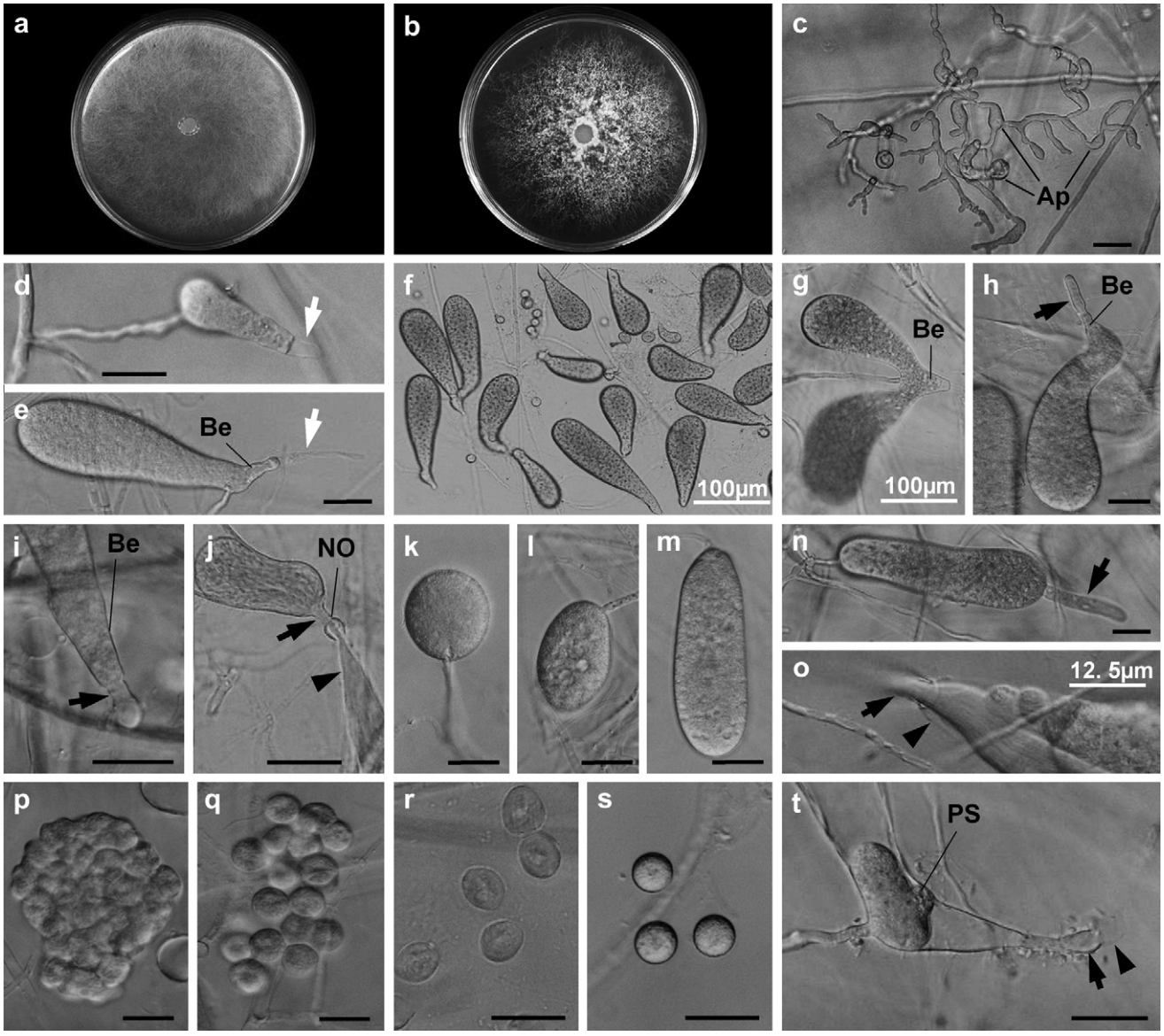

Fig. 7 – *Pythiogeton ramosum*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–g, beaked (Be) sporangia in non-sterile field water, showing evanescent hyphal residue (arrows) on an immature sporangium in d and persistently on an mature sporangium in e; h–j, processes of the discharge of protoplasm from sporangia in non-sterile field water, showing discharge tubes (arrows) in h and i, and an discharge tube (arrow) , a vesicle (arrowhead) and a narrow opening (NO) in j; k–m, non-beaked sporangia in sterile distilled water; n, o, processes of the discharge of protoplasm from sporangia in sterile distilled field water, showing an discharge tube (arrow) in n, and an discharge tube (arrow) and a vesicle (arrowhead) in o; p, q, formation of zoospores from a protoplasm mass; r, s, discharged zoospores, showing the swimming zoospores in r, and encysted zoospores in s; t, an internal proliferous sporangium (PS) producing in an empty sporangia with an empty discharge tube (arrow) and remnant of vesicle residue (arrowhead). *Bars* 25 μm.

65–185(–215) × 25–50 μm, with an apical beak, occasionally bilobate, sometimes internally proliferating, transversely attached to the sporangiophore at the point near the beak, persistent hyphal residue 20–40 μm long (Fig. 7e), extending from the beak. Discharge tubes usually protruding from the apex of beak, 5–22(–74) μm long and 5–6 μm wide, with a flaring opening. Encysted zoospores 14–15 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 32 °C; maximum, 36 °C.

Specimens examined: TAIWAN, Nantou, isolated from debris of water bamboo (*Z. latifolia*). Jun. 2004, J.H. Huang, *Pg-101*, BCRC CH30007 (metabolically inactive culture); Taichung, isolated from leaf debris of rice (*O. sativa*) Aug. 2006, J.H. Huang *Pg-160*, BCRC CH30023 (metabolically inactive culture).

Note: when sporangia of *P. ramosum* were produced in sterile distilled water, their shapes are entirely different from those produced in non-sterile field water. If one observes this species in these two different culture conditions, it is very likely to mistakenly regard the different forms of sporangia as being from different fungi. In non-sterile field water, sporangia are bursiform, and the discharge tube has a flaring opening. Whereas in sterile distilled water, sporangia are globose (34–45 μm) ovoid to ellipsoid (65–200 × 25–60 μm), and the discharge tube has a normal opening. Sporangia in *P. ramosum* were described as being narrowly bursiform according to the observation from raw materials in field water (Minden 1916; Sparrow 1932; Ou 1940) (equivalently non-sterile field water), whereas in *P. autossytum* sporangia were described as being intercalary globose, ovoid to ellipsoid sporangia according to the observation from axenic culture (Drechsler 1932; Hsieh and Chang 1976; Zebrowska 1976) (equivalently sterile distilled water). In this study, bursiform sporangia of this fungus produced in non-sterile field water conform to the description of *P. ramosum*, whereas globose, ovoid to ellipsoid sporangia produced in sterile distilled water conform to the description of *P. autossytum*. It suggests that *P. ramosum* and *P. autossytum* represent the same species, producing different shapes of sporangia in different conditions. Because the name *P. ramosum* predates *P. autossytum*, *P. autossytum* is treated herein as a synonym accordingly.

***P. uniforme* A. Lund**, Mém. Acad. Roy. Sci. Lett. Danemark, Copenhague, Sect. Sci., 9 Série 6: 54 (1934) Fig. 8.

Colonies on RSA at 28 °C with daily growth rate of 22 mm per day, appearing a coarsely radiate pattern on RSA and PDA, hyphae up to 7.5 μm wide, without aerial mycelium. Appressoria club-shaped. Sporangia terminal on supporting hyphae, globose, 37.5–55 μm in diam., occasionally internally proliferating. Discharge tubes 12.5–142.5(–500 μm) long and 6–7 μm wide, protruding from different positions around sporangia, becoming narrowed at the opening, 1–2 μm wide. Encysted zoospores 12.5–16.5 μm in diam. Sexual structures absent. Cardinal temperatures for growth: minimum, 16 °C; optimum, 28 °C; maximum, 36 °C.

Specimens examined: TAIWAN, Taichung, isolated from debris of water convolvulus (*I. aquatica*). Sep. 2007, J.H. Huang, *Pg-176*, BCRC CH30030 (metabolically inactive culture); Taichung, isolated from debris of water convolvulus (*I. aquatica*). Sep. 2007, J.H. Huang, *Pg-175*, BCRC CH30029 (metabolically inactive culture).

Note: six *Pythiogeton* species produce globose sporangia. There are *P. dichotomum*, *P. nigrescens*, *P. paucisporum*, *P. proliferatum*, *P. puliensis*, and *P. uniforme*. Except for *P. uniforme* where the sporangia are uniformly globose, sporangia in other species are "mostly" globose, but intermixed with ovoid sporangia.

P. zizaniae Ann & J.H. Huang, in Ann, Huang, Wang & Ko, Mycologia 98(1): 117 (2006) Fig. 9.

Colonies on RSA at 32 °C with daily growth rate of 13.0 mm per day, not appearing special pattern on RSA and PDA, margin irregular, hyphae up to 11 mm wide, without aerial mycelium. Sporangia terminal on supporting hyphae, bursiform, 75–160 × 46–110 μm, with an apical beak, occasionally ovoid, ellipsoid, or bilolate, sometimes internally proliferating transversely attached to the sporangiophore at the point near the beak. Discharge tubes, 8–15(–110) μm long and 5–8 μm wide, usually protruding from the beak of sporangium, becoming narrowed at the opening, 1–2 μm wide. Encysted zoospores 20–30 μm in diam. Oogonia terminal or rarely intercalary, globose to subglobose, 40–100 × 38–70 μm. Antheridia club-shaped, 8–20 × 10–22 μm, monoclinous, attaching to the base of oogonial stalk. Oospores singly on oogonium, plerotic, globose to subglobose, (36–)45–96 × 34–62 μm, thick-walled, (6–)10–24 μm thick. Cardinal temperatures for growth: minimum, 16 °C, optimum, 32 °C, maximum, 36 °C.

Specimens examined: TAIWAN, Nantou, isolated from basal stalk rot tissue of water bamboo (*Z. latifolia*). Aug. 2005, J.H. Huang *Pg-002*, BCRC CH30001 (metabolically inactive culture); Nantou, isolated from basal stalk rot tissue of water bamboo (*Z. latifolia*) Aug. 2006, J.H. Huang, *Pg-043*, BCRC CH30006 (metabolically inactive culture).

Note: Ann et al. (2006) designed a modified RSA medium (RSA medium amended with sterile extract of water bamboo) to culture this species. It has been proved best suitable for culturing *P. zizaniae* in this study. This species is characterized by bursiform sporangia and large zoospores (cysts larger than 20 μm in diam.). In liquid cultures zoospores tend to swim toward the bottom, a phenomenon also occurring on *P. oblongilobum*. Both *P. zizaniae* and *P. zeae* have been reported to have sexual reproduction, and they are morphologically similar (Jee et al. 2000; Ann et al. 2006). However in *P. zizaniae*, antheridia are usually attached to oogonia at the point adjacent to the oogonial stalk, whereas in *P. zeae*, antheridia are attached to oogonia at the point distant from the oogonial stalk. *P. zizaniae* is the causal pathogen of basal stalk rot of water bamboo (*Z. latifolia*) (Ann et al. 2006), whereas *P. zeae* is the causal pathogen of root and basal stalk rot of corn.

Key to the genus *Pythiogeton* (modified from Batko 1971 and Jee et al. 2000).

1. Sporangia bursiform with beaked appearance, but other variable forms sometimes also present; or multilobate or ellipsoid, but globose, ovoid or bilobate forms also present ---------- 2
1. Sporangia globose, ovoid, or obovoid ---------- 10
2. Sporangia bursiform with beaked appearance, but other variable forms sometimes also present ---------- 3
2. Sporangia multilobate or ellipsoid, but globose, ovoid or bilobate forms also present ---------- 7

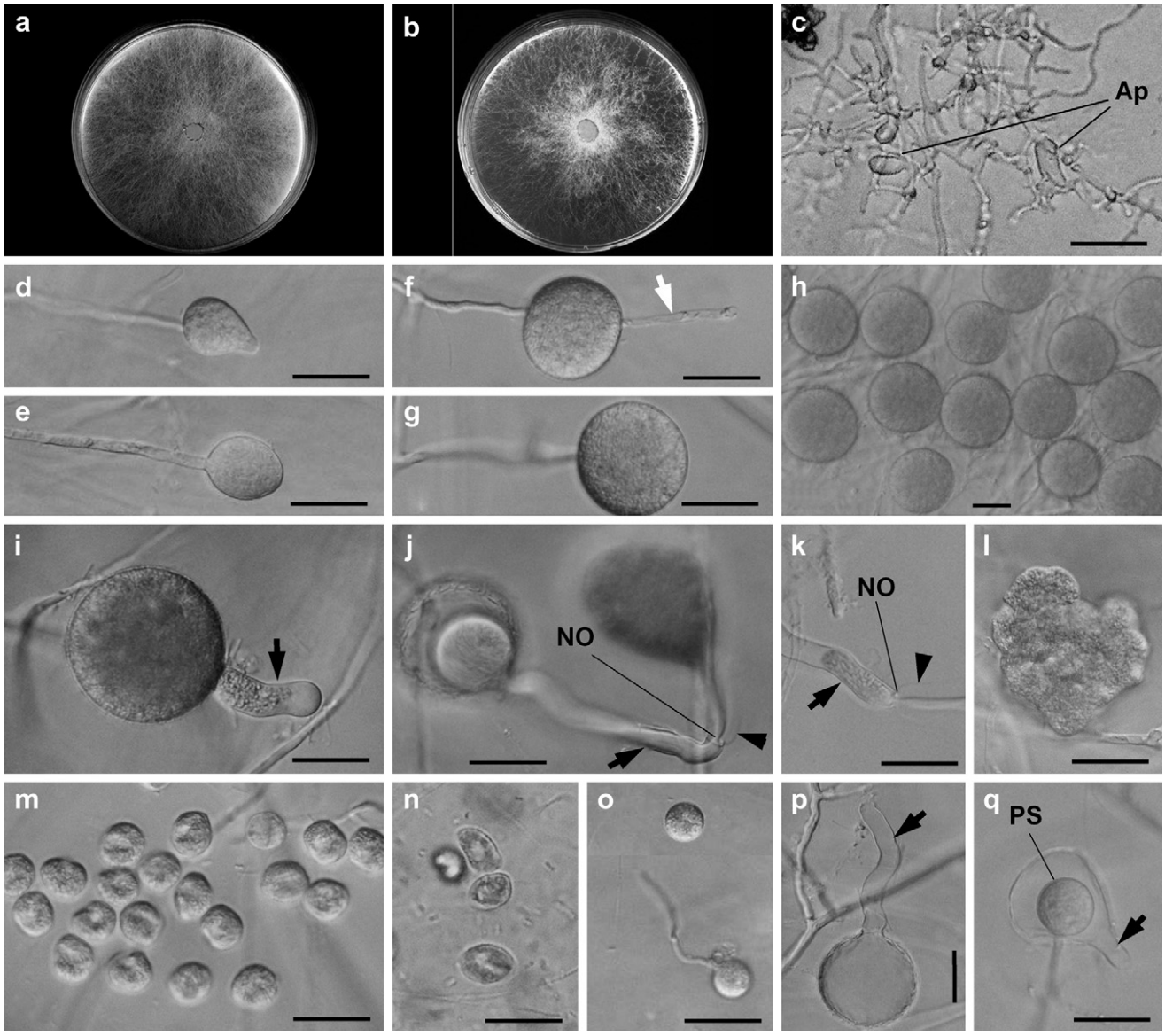

Fig. 8 – *Pythiogeton uniforme*. a, Colony on RSA; b, colony on PDA; c, hyphae and appressoria (Ap) on RSA; d–h, sporangia, showing immature sporangia in d, e, and f, and evanescent hyphal residue (arrow) in f; i –k, processes of the discharge of protoplasm from sporangia, showing an discharge tube (arrow) in i, discharge tubes (arrows), vesicles (arrowheads) and narrow openings (NO) in j and k; l, m, formation of zoospores from a protoplasm mass; n, o, discharge zoospores; showing the swimming zoospores in n, and an encysted zoospore and an germinating cyst in o; p, q, empty sporangia, showing empty discharge tubes (arrows) in p and q, and an internal proliferous sporangium (PS) in q. *Bars* 25 μm.

3. Sporangia mostly intercalary, averaging 154 × 50.5 μm; oogonia polygonal; culture unknown ------*P. transversum* Minden
3. Sporangia mostly terminal ---------- 4
4. Bursiform sporangia narrow, length:width ratio >2.0 ---------- *P. ramosum* Minden
4. Bursiform sporangia broad, length:width ratio <2.0 ---- 5
5. Sporangia uniformly broadly bursiform, 45–97 μm long; oogonia globose, small, 28–57 μm diam., zoospores small, <20 μm ---------- *P. utriforme* Minden
5. Sporangia broadly bursiform, but variable forms also present, up to 160 μm long; oogonia, globose to obovoid, large, up to 72 μm long; zoospores large, >20 μm ---------- 6
6. Sporangia broadly bursiform, but ovoid, or elongate multilobate forms frequently also present; oogonia averaging 52 × 45 μm; antheridia monoclinous or diclinous, attaching distant from the oogonial stalk base; pathogenic to *Zea mays* ---------- *P. zeae* Jee et al.
6. Sporangia broadly bursiform, while ovoid, ellipsoid, or bilolate forms infrequently also present; oogonia averaging 66 × 53 μm; antheridia monoclinous, attaching to the base of oogonial stalk base; pathogenic to *Z. latifolia* ---------- *P. zizaniae* Ann & Huang

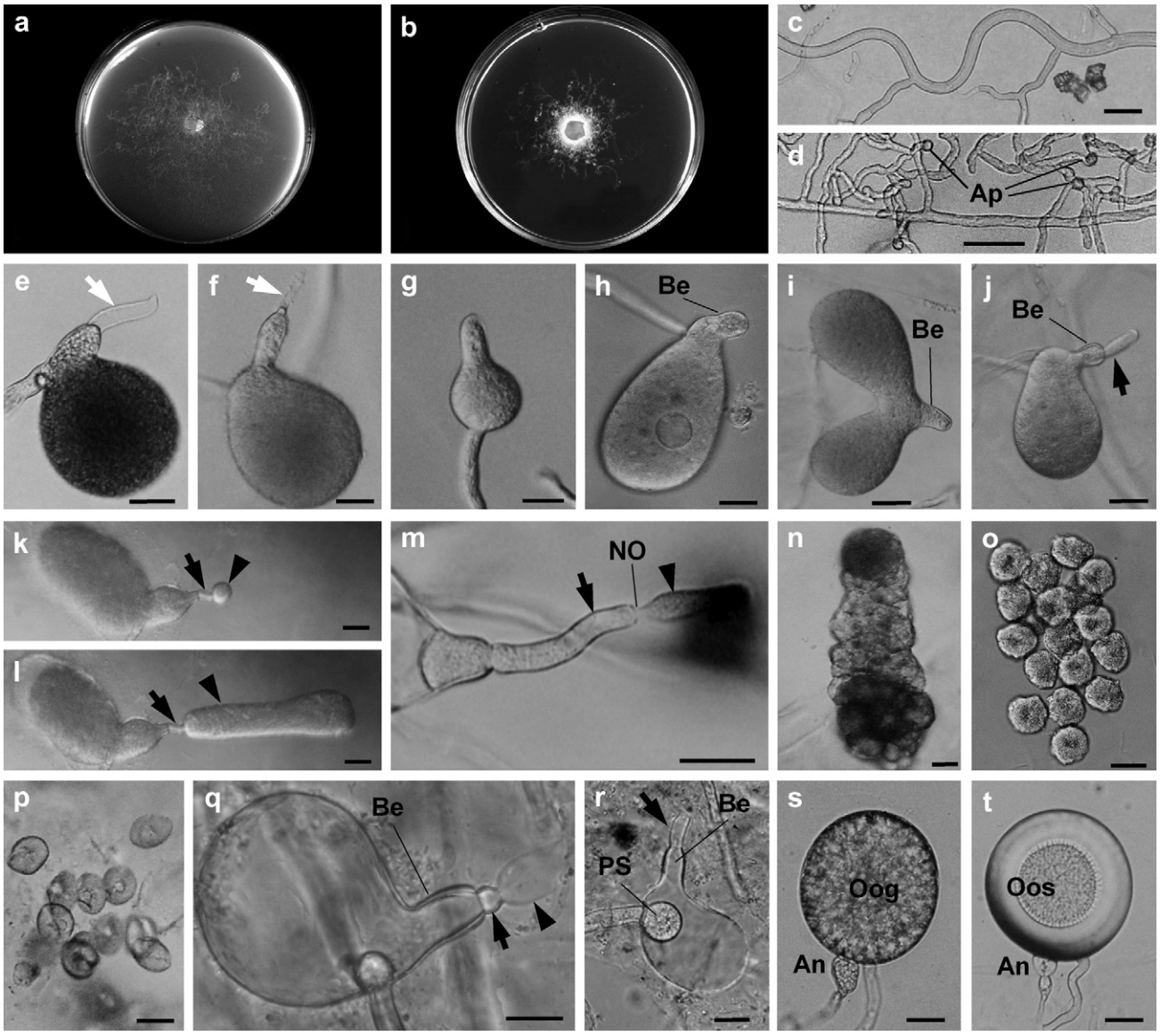

Fig. 9 – *Pythiogeton zizaniae*. a, Colony on RSA; b, colony on PDA; c–d, hyphae and appressoria (Ap) on RSA; e–i, beaked sporangia, showing immature sporangia with a evanescent hyphal residue (arrows) in e and f; j–m, processes of the discharge of protoplasm from sporangia, showing an discharge tube (arrow) in j, discharge tubes (arrows) and vesicles (arrowheads) in k and l, and an discharge tube (arrow), a vesicle (arrowhead) and a narrow opening (NO) in m (figures k and l are photographed on the same object with consecutive time); n ,o, formation of zoospores from a protoplasm mass; p discharged zoospores; q, r, empty sporangia, showing an empty discharge tube (arrow) and remnant of vesicle residue (arrowhead) in q and r, and an internal proliferous sporangium (PS) in r; s–t, sexual structures, showing an oogonium (Oog) and an antheridium (An) in s, and an oospore (Oos) and an antheridium (An) in t. *Bars* 25 μm.

7. Hyphae up to 7.5 μm in width ---------- 8
7. Hyphae up to 5 μm in width ---------- 9
8. Sporangia elongate multilobate, but globose, ovoid, or bilobate forms also present, less than 90 μm in length ----------*P. oblongilobum* Huang et al.
8. Sporangia globose, ovoid, or ellipsoid, averaging 96 × 14 μm in size ---------- *P. autossytum* Drechsler (synonymized under *P. ramosum* Minden)
9. Sporangia irregularly lobate, but globose forms also present; zoospores small, 7–10 μm diam. --------*P. microzoosporum* Huang et al.
9. Sporangia ellipsoid, but globose, ovoid or occasionally bilobate forms also present; zoospores 10–13 μm diam ---------- *P. abundans* Huang et al.
10. Sporangia larger than 40 μm in diam. or in length ---------- *P. uniforme* Lund
10. Sporangia mostly smaller than 40 μm in diam. or in length ---------- 11
11. Vesicle and protoplasmic mass spherical; zoospore becoming inactively swimming then clustering together to form cysts soon after release, actively swimming

zoospores discharged from the cysts; sporangia 25–37 × 25–32 μm; discharge tubes 7.5–20 μm long; antheridia diclinous ---------- *P. nigrescens* Batko

11. Vesicle and protoplasmic mass oblong-ovoid to elongate; zoospores actively swimming after release ---------- 12
12. Hyphae up to 3 μm in width; sporangia 20–43 × 17–28 μm; sporangiophores unbranched or dichotomously branched; discharge tubes 7.5–20 μm long ---------- *P. dichotomum* Tokunaga
12. Hyphae up to 7.5 μm in width ---------- 13
13. Less than 10 zoospores in each sporangium; sporangia 25–32 μm diam.; encysted zoospores 17.5–20 μm diam. ---------- *P. paucisporum* Huang et al.
13. More than 10 zoospores in each sporangium ---------- 14
14. Sporangia globose, 22–43 μm diam., occasionally ovoid or obovoid, 42–50 × 30–40, occasionally internally proliferating; discharge tube with a narrow opening (<2 μm in width) at the apex; encysted zoospores 11–14 μm diam. ---------- *P. puliensis* Huang et al.
14. Sporangia globose, 30–42.5 μm diam.; frequently internal proliferating; discharge tube without a narrow opening; encysted zoospores 11.3–12.5 μm diam. ---------- *P. proliferatum* Huang et al.

3.1. *Molecular phylogenetic analysis*

Based on NJ analysis, the test oomycetes isolates are divided into six clades (clade A, B, C, and D, *Phytophthora* clade and downy mildew clade) (Fig. 10) with high bootstrap value. Clade A corresponds to the genus *Pythiogeton* (100% bootstrap value support). Among clade A, 10 subgroups (A1–A10) can be separated with high bootstrap value. Clade B comprises *Pythium* species that bear largely filamentous sporangia (100% bootstrap value support). "Largely filamentous" refers to the forms of sporangia ranging from inflated filamentous, non-inflated filamentous, to contiguous globose. Clade C represents *Pythium* species that produce globose sporangia (99% bootstrap value support). Clade D represents *Pythium* species bearing papillate ovoid sporangia (100% bootstrap value support). Clade A (*Pythiogeton*) clusters with clade B (*Pythium* with largely filamentous sporangia) with 100% bootstrap value support.

4. Discussion

Species of the genus *Pythiogeton* were little studied in the past. They were generally considered uncommon and difficult to culture (Sparrow 1960; Jee et al. 2000; Ann et al. 2006). Descriptions of some species were exclusively based on observations on the natural substrates (Minden 1916; Ito and Tokunaga 1935; Batko 1971), as pure cultures of them were not obtained. This study reveals that *Pythiogeton* species are actually very common, as long as the right habitats for isolation are realized and the adequate approaches of isolation are applied. WA and PDA media previously used to isolate *Pythiogeton* species (Watanabe 1974; Hsieh and Chang 1976; Jee et al. 2000; Ann et al. 2006) are in fact inappropriate, as they are not suitable for the mycelial growth of *Pythiogeton* or liable to be contaminated (Jee et al. 2000; Ann et al. 2006). The modified PDA (MPDA) was designed in this study. It can efficiently suppress most undesirable microorganisms except *Pythium*, *Pythiogeton* and some saprolegnialean oomycetes. Hyphae of *Pythium* have conspicuous granular particles or large vacuoles, while hyphae of *Pythiogeton* appear glassy and refractive. This significant distinction between *Pythium* and *Pythiogeton* makes the isolation of *Pythiogeton* efficient. During the isolation, *Pythium* species are always the most dominant oomycetes appearing on the medium. Once the characteristic hyphae of *Pythiogeton* can be recognized, they can be picked up and isolated before the hyphae of *Pythium* overgrows and masks the presence of *Pythiogeton*. This characteristic feature in hyphae has been observed in *P. nigrescens* (Batko 1971).

Most *Pythiogeton* species are saprophytic, occurring on plant debris in river, pond, and swamp (Minden 1916; Drechsler 1932; Sparrow 1932, 1933, 1936; Lund 1934; Batko 1971; El-Hissy 1974; Zebrowska 1976; El-Hissy and Khallil 1991; Czeczuga 1994, 1995; Czeczuga et al. 2003, 2005). Only two species, *P. zeae* and *P. zizaniae*, are known to be plant parasitic (Jee et al. 2000; Ann et al. 2006). Emerson and Natvig (1981), and Natvig and Gleason (1983) considered *Pythiogeton* species were facultative anaerobic by their capability of growing in anaerobic conditions. If the anaerobic property of *Pythiogeton* stands, it can be inferred that plant materials submerged in stagnant water during crop cultivation are ideal for the isolation of *Pythiogeton* species. Accordingly, in the survey of *Pythiogeton* species, the samples were mainly collected from paddy fields of water bamboo, rice, water caltrop, and water convolvulus. These kinds of paddy fields are always flooded in the cultivation duration. Present results show that *Pythiogeton* species seem to prefer living in anaerobic condition.

Most *Pythiogeton* species produce hyphae with swellings of various shapes (Figs. 1c, 3c, 4c, 5c, 6c, 7c, 8c, and 9c–d) when grown on solid medium. These swellings firmly attach to the hard bottom surface of Petri dish. An adhering hyphal swelling structure, namely appressorium, has been reported in *Pythium* (Van der Plaats-Niterink 1981), and is generally considered to play a role in infection. Although unclear in the function, the term "appressorium" has been used by Sparrow (1960) to refer to this hyphal swelling structure occurring in *Pythiogeton* due to its adhering property. This term "appressorium" is followed herein.

Sparrow (1960) in defining the genus *Pythiogeton* stated that sporangia may develop in terminal or intercalary positions. Dick (2001) described the sporangia in *Pythiogeton* as being "often unequally dilated from an intercalary subterminal hyphal segment". The observation in this study coincided with that of Sparrow (1960) and Dick (2001). Species obtained in this study, except *P. paucisporum* and *P. proliferatum*, have been observed to have the intercalary development of sporangia. However, the terminal segment of hyphae topping the sporangia will gradually break down during the maturation, leaving an overhanging remnant of hyphae protruding from sporangia at the immature developmental stage of sporangia. This feature is pointed out in Figs. 1e, 2e, 3e, 6e, 7d, 8f, 9e and f, and is designated as evanescent hyphal residue. It should be noted that this feature can only be observed in liquid culture and on immature sporangia. After sporangia have matured, this remnant of hyphae will be evanescent, and then sporangia eventually appear terminal rather than subterminal or intercalary. For this reason in the species

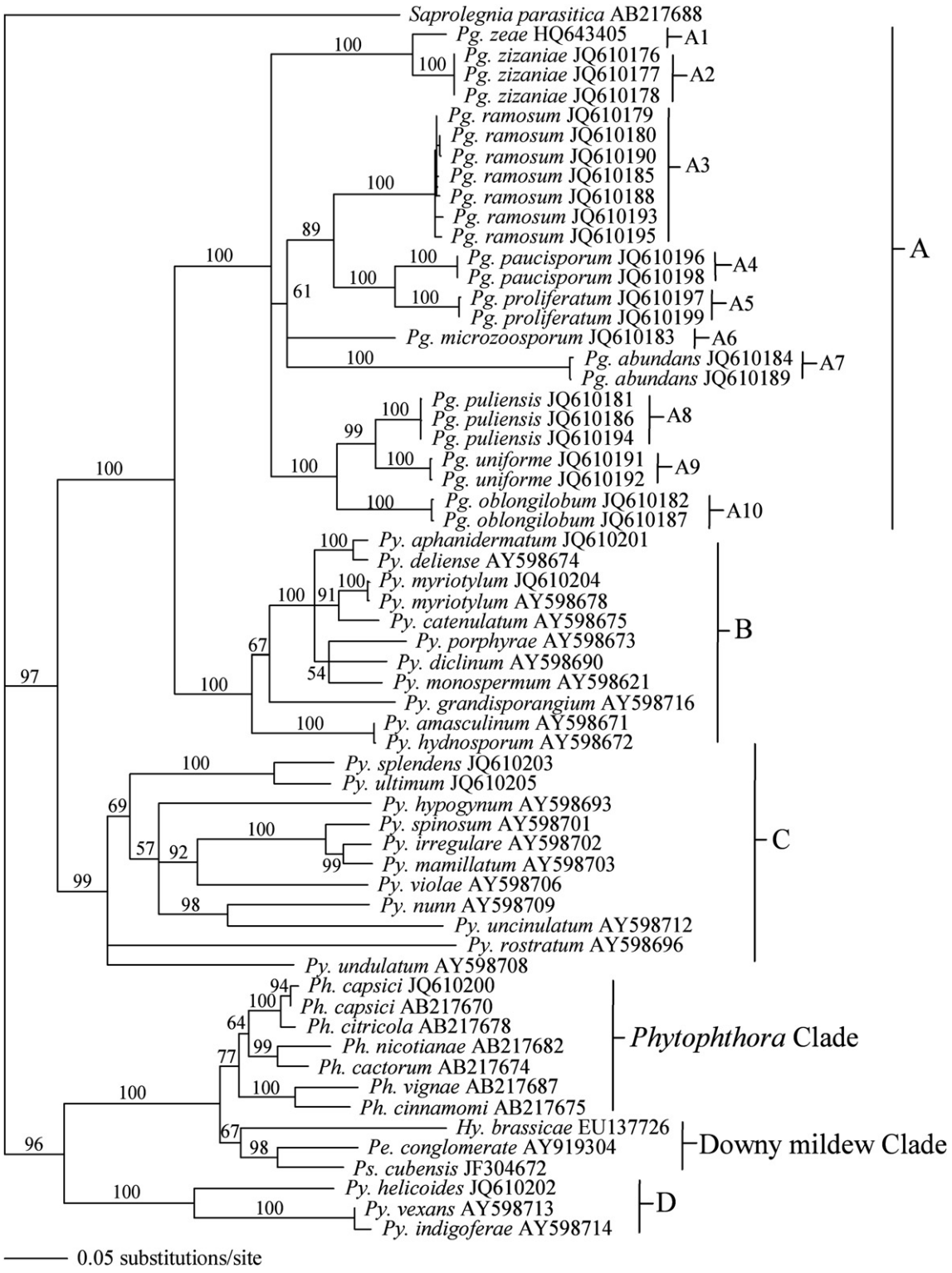

Fig. 10 – Phylogenetic tree of *Pythiogeton* species and related genera based on ITS sequences by using neighbor-joining analysis under PAUP. Numbers on the branches represent bootstrap values obtained from 1000 replications (only values greater than 50% are shown).

diagnosis, sporangia are describe as being terminal on supporting hyphae in terms of mature sporangia. *P. ramosum* is the exception in that a segment of the hyphal residue persists (Fig. 7e) on the top of sporangia until the discharge tube emerges; its sporangia are described as being subterminal. In *P. transversum*, sporangia were described as being intercalary by Minden (1916). This specie has only been reported once, and we do not have this species. However, judging from the

illustration, it is supposed that there is a long hyphal residue persisting on the top of sporangia at maturity, leading to the intercalary appearance of sporangia.

All the *Pythiogeton* species obtained in this study have sporangia releasing their contents as undifferentiating protoplasm through a discharge tube into an elongate transient vesicle. The vesicle, which is continuous with the wall of discharge tube, lasts no more than 30 s, then ruptures to free the apparently naked protoplasm to drift apart. Zoospores are produced from the apparently naked protoplasm. This conforms to the observation of Minden (1916), Fitzpatrick (1930), and Sparrow (1960). After rupture of the vesicle, a sleeve-shaped vesicle residue can be seen situating on the end of discharge tube. This was also observed by Batko (1971) in *P. nigrescens*, who inferred that the vesicle is actually broken instead of evanescent so as to have the residue on the discharge tube. The vesicle residue has never been observed in other oomycetes. This may accounts for the unique composition of vesicle wall in *Pythiogeton*. It requires further TEM sections to resolve vesicle composition. Dick (2001) proposed that the zoosporogenesis of *Pythiogeton* is in a detachable vesicle from a discharge tube, and protoplasm within the vesicle is cleaved into zoospores after the vesicle has detached from the discharge tube. The present study does not support Dick's observation, as a transient vesicle has been observed to be broken with a residue remaining. Unless there is another thin and indiscernible layer of vesicle wall which persists after the outer wall has broken, it is believed that there is unprotected naked protoplasm within which zoospores are produced. We suppose that, before protoplasm is set free from the vesicle, invisible cleavage of zoosporogenesis has begun, which explains why the apparently naked protoplasm can be present without any surrounding membrane or wall. However before carrying out TEM sections on relevant stages, the question remains open.

It was reported that the production of sporangia of *Phytophthora cinnamomi* can be induced by soil extracts (Mehrlich 1935), mineral salts (Chen and Zentmyer 1970; Hwang and Ko 1975) or bacteria and their metabolites (Zentmyer 1965; Ayers and Zentmyer 1971). In *P. microzoosporum*, *P. paucisporum*, *P. proliferatum*, *P. puliensis*, and *P. uniforme*, non-sterile field water can improve the production of large amount of sporangia, whereas only small amount of sporangia is produced in sterile water. Non-sterile field water has been proved useful in promoting the production of sporangia of many oomycetes. It implies that bacterial metabolites or other materials in the field water are probably responsible for the production of sporangia.

In the phylogenetic analysis, all the *Pythiogeton* isolates constitute a single clade A (Fig. 10), within which subgroups A1–A10 can be separated, with each subgroup coinciding with individual *Pythiogeton* species established on morphological characters. It suggests that the genus *Pythiogeton* is well-defined, and the species identification based on morphological characters is accurate. Additionally, it appears that *Pythiogeton* shares a most recent common ancestor with the Clade B *Pythium* species which include most species with filamentous sporangia, and includes the type species *Pythium monospermum* (Fig. 10). *Pythiogeton* and the Clade B *Pythium* species form a sister clade to Clade C *Pythium* species most of which have globose sporangia and which Uzuhasi et al. (2010) have named *Globisporangium*. It is perhaps unexpected and interesting that *Pythiogeton*, with its generally globose to irregular sporangia, should be most closely related to those *Pythium* species with mostly filamentous sporangia. It also confirms that *Pythiogeton* is a member of the Pythiaceae and the family Pythiogetonaceae (Voglmayr et al. 1999; Dick 2001) is not supported by sequence data. Clade D represents *Pythium* species possessing ovoid sporangia with papillate apices which have been renamed both as *Phytopythium* (Bala et al. 2010) and *Ovatisporangium* (Uzuhasi et al. 2010). This clade clusters with and shares a common ancestor with the clades encompassing *Phytophthora*, *Halophytophthora* and the downy mildews (Robideau et al. 2011). The genus *Pythium* has long been considered to be heterogenous. With the advent of *Pythiogeton* species, the proper taxonomic positions of *Pythium*-related species have also been resolved.

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REFERENCES

Ann PJ, Huang JH, Wang IT, Ko WH, 2006. *Pythiogeton zizaniae*, a new species causing basal stalk rot of water bamboo in Taiwan. *Mycologia* 98: 116–120.

Ayers WA, Zentmyer GA, 1971. Effect of soil solution and two soil *Pseudomonads* on sporangium production by *Phytophthora cinnamomi*. *Phytopathology* 61: 1188–1193.

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 & Lévesque, sp. nov. *Persoonia* 24: 136–137.

Batko A, 1971. A new species of *Pythiogeton* showing *Achlya* like diplanetism. *Acta Mycologica* 7: 241–249.

Belbahri L, Calmin G, Pawlowski J, Lefort F, 2005. Phylogenetic analysis and real time PCR detection of a presumably undescribed *Peronospora* species on sweet basil and sage. *Mycological Research* 109: 1276–1287.

Cantino EC, 1949. The growth and nutrition of *Pythiogeton*. *American Journal of Botany* 36: 747–756.

Chang HS, 1988. *Phytophthora* species associated with strawberry fruit rot in Taiwan. *Botanical Bulletin of Academia Sinica (Taipei)* 29: 61–67.

Chen DW, Zentmyer GA, 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycologia* 62: 397–402.

Czeczuga B, 1994. Studies of aquatic fungi 17. Aquatic fungi of Lake Hancza in the Suwalki Scenic Park and of some adjacent lakes (northeastern Poland). *Acta Hydrobiologica* 36: 371–385.

Czeczuga B, 1995. Hydromycoflora of thirty one lakes in Elk Lake District and adjacent waters with reference to the chemistry of the environment. *Acta Mycologica* 30: 49–63.

Czeczuga B, Kiziewicz B, Mazalska B, 2003. Further studies on aquatic fungi in the river Biebrza within Biebrza National Park. *Polish Journal of Environmental Studies* 12: 531–543.
Czeczuga B, Mazalska B, Godlewska A, Muszynska E, 2005. Aquatic fungi growing on dead fragments of submerged plants. *Limnologica* 35: 283–297.
Dick MW, 2001. *Straminipilous Fungi: Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protists, the Plasmodiophorids and Similar Organisms*. Kluwer Academic Publishers, Netherlands.
Drechsler C, 1932. A species of *Pythiogeton* isolated form decaying leaf-sheaths of the common cat-tail. *Journal of the Washington Academy of Sciences* 22: 421–449.
El-Hissy FT, 1974. Fresh water fungi in Egypt. *Egyptian Journal of Botany* 17: 187–189.
El-Hissy FT, Khallil ARM, 1991. Distribution and seasonal occurrence of aquatic Phycomycetes in water and submerged mud in El-Ibrahimia canal (upper Egypt). *Journal of Islamic Academy of Sciences* 4: 311–316.
Emerson R, Natvig DO, 1981. Adaptation of fungi to stagnant waters. In: Wicklow DT, Carroll GC (eds), *The Fungal Community, Its Organization and Role in the Ecosystem*. Marcel Dekker Inc., New York, pp 109–128.
Erwin DC, Ribeiro OK, 1996. Phytophthora *Diseases Worldwide*. The American Phytopathology Society, St. Paul.
Fitzpatrick HM, 1930. *The Lower Fungi*. McGraw-Hill Book Company, Inc., New York.
Gisi U, Hemes DE, Zentmyer GA, 1979. Origin and significance of the discharge vesicle in *Phytophthora*. *Experimental Mycology* 3: 321–339.
Hawksworth DL, Kirk PM, Sutton BC, Pegler DN, 1995. *Ainsworth & Bisby's Dictionary of the Fungi*, 8th edn. CAB International, Wallingford.
Hong SY, Jee HJ, Choi YJ, Shin HD, 2008. First confirmed report of downy mildew caused by *Hyaloperonospora parasitica* on broccoli in Korea. *Plant Pathology* 57: 777.
Hsieh HJ, Chang HS, 1976. Five species of *Pythium*, two species of *Pythiogeton* new for Taiwan and *Pythium afertile*. *Botanical Bulletin of Academia Sinica (Taipei)* 17: 141–150.
Hwang SC, Ko WH, 1975. A simplified method for sporangial production by *Phytophthora cinnamomi*. *Mycologia* 67: 1233–1234.
Ito S, Tokunaga Y, 1935. Notae mycologicae Asiae orientalis I. *Transactions of the Sapporo Natural History Society* 14: 1–33.
Jee HJ, Ho HH, Cho WD, 2000. *Pythiogeton zeae* sp. nov. causing root and basal stalk rot of corn in Korea. *Mycologia* 92: 522–527.
Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.
Levesque CA, de Cock AWAM, 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research* 108: 1363–1383.
Lodhi AM, Shahzad S, Ghaffar A, Levesque CA, 2006. Oomycete species of Pakistan – a morphological and molecular study. *Inoculum* 57 (4): 27.
Lund A, 1934. Studies on Danish freshwater phycomycetes and notes on their occurrence particularly relative to the hydrogen ion concentration of the water. *Memoirs of the Royal Academy of Sciences and Letters of Denmark, Series* 9 (6): 1–97.
Mehrlich FP, 1935. Non-sterile soil leachate stimulating to zoosporangia production by *Phytophthora* sp. *Phytopathology* 25: 432–434.
Minden M von, 1916. Beitrage zur biologie und systematik einheimscher submerser Phycomyceten. *Mykologische Untersuchungen und Berichte von Dr Richard Falck* 1: 146–255.
Mitchell MN, Ocamb CM, Grünwald NJ, Mancino LE, Gent DH, 2011. Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*. *Phytopathology* 101: 805–818.
Nakagiri A, Newell SY, Ito T, 1994. Two new *Halophytophthora* species, *H. tartarea* and *H. masteri*, from intertidal decomposing leaves in saltmarsh and mangrove regions. *Mycoscience* 35: 223–232.
Natvig DO, Gleason FH, 1983. Oxygen uptake by obligately-fermentative aquatic fungi: absence of a cyanide-sensitive component. *Archives of Microbiology* 134: 5–8.
Ou SH, 1940. Phycomycetes of China II. *Sinensia* 11: 427–449.
Rambaut A, 2000. *Se-Al: Sequence Alignment Editor*. Department of Zoology, University of Oxford, Oxford.
Robideau GP, De Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Desaulniers N, Eggertson QA, Gachon CM, Hu CH, Kupper FC, Rintoul TL, Sarhan E, Verstappen EC, Zhang Y, Bonants PJ, Ristaino JB, Levesque AC, 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources* 11: 1002–1011.
Silva-Rojas HV, Creswell TC, Phillips JL, Abad JA, Abad ZG, 2004. Two putative new species of *Pythiogeton* associated with Cypress and English Ivy in North Carolina. *Phytopathology* 94: S95.
Sparrow Jr FK, 1932. Observations of the aquatic fungi of Cold Spring Harbor. *Mycologia* 24: 268–303.
Sparrow Jr FK, 1933. Inoperculate chytridiaceous organisms collected in the vicinity of Ithaca. N. Y., with notes on other aquatic fungi. *Mycologia* 25: 513–535.
Sparrow Jr FK, 1936. A contribution to our knowledge of the aquatic Phycomycetes of Great Britain. *Journal of the Linnean Society of London, Botany* 50: 417–478.
Sparrow Jr FK, 1960. *Aquatic Phycomycetes. Second Revised Edition*. University of Michigan Press, Ann Arbor.
Swofford DL, 2001. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, 4th edn. Sinauer Associates, Sunderland.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882.
Uzuhahsi S, Tojo M, Kakishima M, 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* 51: 337–365.
Van der Plaats-Niterink AJ, 1981. Monograph of Genus *Pythium*. *Studies in Mycology* 21: 1–242.
Villa NO, Kageyama K, Asano T, Suga H, 2006. Phylogenetic relationships of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and beta-tubulin gene sequences. *Mycologia* 98: 410–422.
Voglmayr H, Bonner LJ, Dick MW, 1999. Taxonomy and oogonial ultrastructure of a new aero-aquatic peronosporomycete, *Medusoides* gen. nov. (Pythiogetonaceae fam. nov.). *Mycological Research* 103: 591–606.
Watanabe T, 1974. Fungi isolated from the underground parts of sugar cane in relationship to the poor rationing in Taiwan. (2) *Pythium* and *Pythiogeton*. *Transactions of the Mycological Society of Japan* 15: 343–357.
Webster J, Dennis C, 1967. The mechanism of sporangial discharge in *Pythium middletonii*. *New Phytologist* 66: 307–313.
White TJ, Bruns T, Lee S, Taylo JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., New York, pp 315–322.
Zebrowska E, 1976. Nikoflora kilku zbiornikow wodnych Puszczy Kampinoskiej. *Acta Mycologica* 12: 77–89.
Zentmyer GA, 1965. Bacterial stimulation of sporangium production in *Phytophthora cinnamomi*. *Science* 150: 1178–1179.