


Paramyrothecium eichhorniae sp. nov., Causing Leaf Blight Disease of Water Hyacinth from Thailand

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ABSTRACT

Paramyrothecium eichhorniae sp. nov. was observed and collected from Chiang Mai and Phetchaburi Provinces, Thailand. This new species is introduced based on morphological and molecular evidence. This fungus is characterized by its production of sporodochium conidiomata with a white setose fringe surrounding an olivaceous green to dark green slimy mass of conidia, penicillately branched conidiophores, and aseptate and cylindrical to ellipsoid conidia. Phylogenetic analyses of combined LSU rDNA, ITS rDNA, *tef1*, *rpb2*, *tub2* and *cmdA* sequence data using maximum parsimony, maximum likelihood and Bayesian approaches placed the fungus in a strongly supported clade with other *Paramyrothecium* species in Stachybotryaceae (Hypocreales, Sordariomycetes). The descriptions of the species are accompanied by illustrations of morphological features, and a discussion of the related taxa is presented.

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1. Introduction

Leaf blight disease of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) is distributed in different geographical areas of Thailand. Several fungal species, such as *Alternaria alternata*, *A. geophila*, *A. eichhorniae*, *Ascochyta chartarum*, *Bipolaris zeicola* (syn. *Cochliobolus carbonum*), *Cercospora rodmanii*, *Curvularia lunata*, *Epicoccum nigrum*, *Fusarium chlamydosporum*, *F. equiseti*, *F. pallidoroseum*, *Globisporangium ultimum* (syn. *Pythium ultimum*), *Paramyrothecium roridum* (formerly known as *Myrothecium roridum*) and *Stemphylium vesicarium* have been reported to be pathogens of water hyacinth [1–3]. Leaf blight disease of water hyacinth has been observed in Thailand, and the fungal pathogen causing the disease was identified as *P. roridum* (= *Myrothecium roridum*) using morphological characteristics and ITS rDNA sequence analysis [4–5], as same as the previous report by Okunowo et al. [6] in Nigeria. Moreover, there are many reports that *P. roridum* has the potential to be a mycoherbicide against water hyacinth and other water weeds [2,6,7]. The host range of *P. roridum* strain TBRC 10637 (=KKFC448) was evaluated on

77 plant species (40 families), including water hyacinth. This fungus could not infect 74 economically important plants, while symptoms were observed on water hyacinth plants and severe and slight symptoms were observed on duckweed and water lettuce plants [8].

Lombard et al. [9] revised the genus *Myrothecium* which resulted in the recognition of 13 new genera based on the polyphyletic origin of its species, and more than 15 species have been reported within two renamed genera, *Paramyrothecium* and *Albifimbria*. The genus *Paramyrothecium* was introduced with *P. roridum* (Tode) L. Lombard & Crous as the type species. Species of *Paramyrothecium* are reported as saprobe and weakly pathogenic fungi with a worldwide distribution [9]. *Paramyrothecium* is characterized as follows: sporodochial conidiomata, with or without a white setose fringe surrounding the slimy mass of conidia. Straight to flexuous setae, 1–3(–4)-septate, hyaline conidiophores penicillately branched; conidiogenous cells phialidic or percurrent. Conidia aseptate to 1-septate, cylindrical to ellipsoidal to obovoid, hyaline to pale green, smooth; a sexual

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 Supplemental data for this article can be accessed [here](#).

morph has not been reported. This genus is similar to *Neomyrothecium* except that the pulvinate sporodochia with a white setose fringe [9]. Phylogenetic analysis using the *cmdA*, ITS, *rpb2*, and *tub2* genes showed that members of *Paramyrothecium* formed a highly supported clade distant from the *Myrothecium* s. str. clade [9]. However, Krisai-Greilhuber et al. [10] noted that most of the species of *Paramyrothecium* could not be discriminated morphologically; thus, it was necessary to combine a phylogenetic analysis for accurate taxonomic assignment.

In this study, we introduce a new species in the genus *Paramyrothecium*, which belongs to Stachybotryaceae (Hypocreales, Sordariomycetes), based on morphological and molecular evidence.

2. Materials and methods

2.1. Fungal specimen

Water hyacinth leaves showing blight symptoms were observed and collected from natural water resources in Chiang Mai and Phetchaburi provinces, Thailand.

2.2. Isolation and morphological studies

The fungal pathogen was isolated using the tissue transplanting method on the potato dextrose agar plates (PDA; Difco, Becton, Dickinson and Company, Bangkok, Thailand). The cultures were deposited in the Kasetsart Kamphaengsaen Fungal Collection (KKFC) and Thailand Bioresource Research Center (TBRC), Thailand. The morphological characteristics of the fungi were examined under a light microscope Olympus BX51 (Olympus, Bangkok, Thailand). The sporodochia were collected directly from the substrate using fine forceps or a needle and then placed in a drop of sterilized water on a microscope slide, and a coverslip was added. The specimens were dried by a dehydration machine at 45 °C for 24–36 h and deposited in the BIOTEC Bangkok Herbarium (BBH).

2.3. Pathogenicity test

The healthy water hyacinth plants with 25–50 cm² in size of leaves were prepared for inoculation. The fungal strain TBRC 10637 was subcultured on PDA and incubated at 28 °C. The photoperiods (12 h) were provided by white fluorescent lamps. Inoculation was done by spraying the leaves of water hyacinth plant with 1×10^8 spores per mL; the control treatment was sprayed with 10 mL of sterile distilled water. This experiment was conducted by using a completely randomized design

(CRD), with 10 replications of each treatment. The plants were placed in a growth chamber with 100% relative humidity (RH) for 24 h and then moved to greenhouse conditions. The temperatures in the greenhouse ranged from 26 to 32 °C, with 65–90% RH. The disease symptom was observed at 7 days after inoculation and compared with the leaf blight symptom observed in the nature. Fungal re-isolation was conducted by using the tissue transplanting method. The infected leaves were cut into a 0.5 cm × 0.5 cm size. The samples were surface-disinfected with a 10% sodium hypochlorite solution for 5 min and then washed two times with sterilized distilled water before being plated on the PDA. The cultures were incubated at 28 °C under white fluorescent lamps with a 12 h day per night cycle.

2.4. DNA extraction and PCR amplification

Genomic DNA was extracted from the mycelia on the PDA using a CTAB method [11]. Six nuclear loci, LSU rDNA, ITS rDNA, *tef1*, *rpb2*, *tub2* and *cmdA*, were amplified. The primers used to amplify these regions were LROR/LR5 [12], ITS5/ITS4 [13], EF1-728F/EF2, 5F2/7cR [14], T1/T22 [15] and CAL-228F/CAL2Rd [16–17]. The amplification conditions for the LSU and ITS regions followed the protocol described in Sakayaroj [12], while the amplification conditions for the *tef1*, *rpb2*, *tub2* and *cmdA* genes followed the protocol described in Liang et al. [18]. PCR products were sequenced by Macrogen Inc. (Seoul, South Korea) for Sanger dideoxy sequencing by using the same primers as for amplification.

2.5. Sequence alignment and phylogenetic analyses

Thirty-two sequences (Table 1) were checked for ambiguous bases and assembled using BioEdit v.7.0.5.3 [19]. All the sequences were aligned with MUSCLE [20] and manually edited using BioEdit v.7.0.5.3 [19]. The phylogenetic analyses were performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI).

The maximum parsimony analysis was performed by PAUP v.4.0b10 [21] with 10 replicates of stepwise additions, the heuristic search option, the addition of 1,000 random taxa and the tree bisection reconnection (TBR) branch swapping algorithm. All the characters were given equal weight, and the gaps were treated as missing data. Maxtrees was unlimited, branches of zero length were collapsed, and all the multiple, equally parsimonious trees were saved. The robustness of the

Table 1. Taxa used in the phylogenetic analyses and the new taxa are deposited sequences shown in bold.

Taxa	Strain	GenBank					
		<i>cmdA</i>	ITS	LSU	<i>rpb2</i>	<i>tef1</i>	<i>tub2</i>
<i>Albifimbria verrucaria</i>	CBS 328.52	KU845875	KU845893	KU845912	KU845931	KU845950	KU845969
<i>Albifimbria viridis</i>	CBS 449.71	KU845879	KU845898	KU845917	KU845936	KU845955	KU845974
<i>Albifimbria terrestris</i>	CBS 126186	KU845867	KU845883	KU845902	KU845921	KU845940	KU845959
<i>Alfaria caricicola</i>	CBS 113567	KU845976	KU845983	KU845992	KU846001	KU846008	KU846014
<i>Alfaria putrefolia</i>	CBS 112037	–	KU845985	KU845994	KU846003	–	KU846016
<i>Myrothecium inundatum</i>	CBS 275.48	KU846435	KU846452	KU846474	–	KU846514	KU846533
<i>Myrothecium simplex</i>	CBS 582.93	KU846439	KU846456	KU846478	–	KU846517	KU846537
<i>Myxospora aptrootii</i>	CBS 101263	KU846441	KU846458	KU846480	KU846496	KU846519	KU846539
<i>Myxospora crassiseta</i>	CBS 731.83	KU846442	KU846459	KU846481	KU846497	KU846520	KU846540
<i>Myxospora masonii</i>	CBS 174.73	KU846445	KU846462	KU846484	KU846500	KU846523	KU846543
<i>Paramyrothecium acadense</i>	CBS 123.96	–	KU846288	KU846318	KU846350	KU846379	KU846405
<i>Paramyrothecium breviseta</i>	CBS 544.75	KU846262	KU846289	KU846319	KU846351	KU846380	KU846406
<i>Paramyrothecium cupuliforme</i>	CBS 127789	KU846264	KU846291	KU846321	KU846353	KU846382	KU846408
<i>Paramyrothecium eichhorniae</i>	TBRC 10637	MT975319	MT973996	MT974029	MT977540	MT975321	MT975317
<i>Paramyrothecium eichhorniae</i>	KKFC 474	MT975318	MT973995	MT974028	MT977541	MT975320	MT975316
<i>Paramyrothecium foeniculicola</i>	CBS 331.51	–	KU846292	KU846322	KU846354	KU846383	KU846409
<i>Paramyrothecium foliicola</i>	CBS 113121	KU846266	KU846294	KU846324	–	KU846385	KU846411
<i>Paramyrothecium guiyangense</i>	HGUP 2016-8002	KY196193	KY126418	KY196209	–	–	KY196201
<i>Paramyrothecium humicola</i>	CBS 127295	–	KU846295	KU846325	KU846356	KU846386	KU846412
<i>Paramyrothecium nigrum</i>	CBS 116537	KU846267	KU846296	KU846326	KU846357	KU846387	KU846413
<i>Paramyrothecium pituitipietianum</i>	CBS 146817	MW173100	MW175358	MW175398	–	MW173124	MW173139
<i>Paramyrothecium parvum</i>	CBS 257.35	–	KU846298	KU846328	KU846359	KU846388	KU846415
<i>Paramyrothecium roridum</i>	CBS 357.89	KU846270	KU846300	KU846330	KU846361	KU846390	KU846417
<i>Paramyrothecium salvadorae</i>	CBS 147074	–	MZ064453	MZ064510	MZ078210	MZ078254	MZ078277
<i>Paramyrothecium sinense</i>	CGMCC 3.19212	MH885437	MH793296	–	MH818824	–	MH793313
<i>Paramyrothecium tellicola</i>	CBS 478.91	KU846272	KU846302	KU846332	KU846363	–	KU846419
<i>Paramyrothecium terrestris</i>	CBS 564.86	KU846273	KU846303	KU846333	KU846364	–	KU846420
<i>Paramyrothecium verruridum</i>	HGUP 2016-8006	KY196197	KY126422	KY196213	–	–	KY196205
<i>Paramyrothecium viridiporum</i>	CBS 873.85	KU846278	KU846308	KU846338	KU846369	KU846396	KU846425
<i>Stachybotrys chartarum</i>	CBS 182.80	KU846573	KU846679	KU846792	KU846904	KU847003	KU847115
<i>Stachybotrys chlorohalonata</i>	CBS 109285	KU846623	KU846729	KU846842	KU846954	KU847053	KU847164
<i>Stachybotrys subsylvatica</i>	CBS 126205	KU846634	KU846741	KU846854	KU846964	KU847064	KU847175

CBS: Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; HGUP: Herbarium of the Department of Plant Pathology, Guizhou University, China; KKFC: Kasetsart.Kamphaengsaen Fungal Collection, Thailand; TBRC: Thailand Bioresource Research Center, Thailand.

most parsimonious tree was estimated based on 1,000 bootstrap replications.

The maximum likelihood analysis was performed on the CIPRES supercomputer using the RAXML-HPC2 v.8.2.12 program on XSEDE [22]. One thousand nonparametric bootstrap iterations were run with the GTR model and a discrete gamma distribution.

Bayesian analyses (BA) were conducted in MrBayes v.3.0b4 [23] with a uniform [GTR+I+G] model, Isetnst = 6 rates = invgamma, and prsetstatefreqpr = dirichlet (1,1,1,1). The evolutionary best-fit models of Bayesian analysis (BA) were conducted in MrBayes 3.2.6 [24]. The evolutionary best-fit model was evaluated by means of MrModelTest 2.3 [25] before analysis. Posterior probabilities (PPs) were calculated by the Markov chain Monte Carlo algorithm [26]. Four Markov chains were run for 5,000,000 generations, and trees were sampled every 100 generations. The first 5,000 trees, which represented the burn-in phase of the analysis, were discarded, with 50,000 trees used for calculating the posterior probabilities (BIPP) in the consensus tree.

The matrix and the resulting tree have been deposited at TreeBASE under submission number 29197 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S29197>).

3. Results

3.1. Phylogenetic analyses

The assembled sequences comprised 32 taxa (Table 1). *Alfaria caricicola* (CBS 113567) and *Alfaria putrefolia* (CBS 112037) were used as outgroups. After alignment, the best tree was subjected to maximum parsimony, which combined LSU rDNA, ITS rDNA, *tef1*, *rpb2*, *tub2* and *cmdA*. The dataset consists of 4043 characters, of which 2434 were constant, 366 were variable parsimony-uninformative and 1253 were parsimony informative with a length of 4532 steps (CI = 0.570, RI = 0.687, RC = 0.392 and HI = 0.430). The best scoring RAXML tree had a final optimization likelihood value of -25813.607984 . The bootstrap support values for the maximum parsimony (BSMP, left) and maximum likelihood (BSML, middle) analyses were greater than 50%. The branches with Bayesian posterior probabilities (BPP, right) greater than 0.95 are indicated at the nodes.

The phylogenetic analyses showed that all the collected strains were clustered in the family Stachybotryaceae. The two strains of *P. eichhorniae* sp. nov. (TBRC 10637 and KKFC 474), which were recovered as distinct species, were grouped with *P. foliicola* with bootstrap and posterior probability support (97% BSMP, 99% BSML and 1.00 BPP) in the tree (Figure 1).

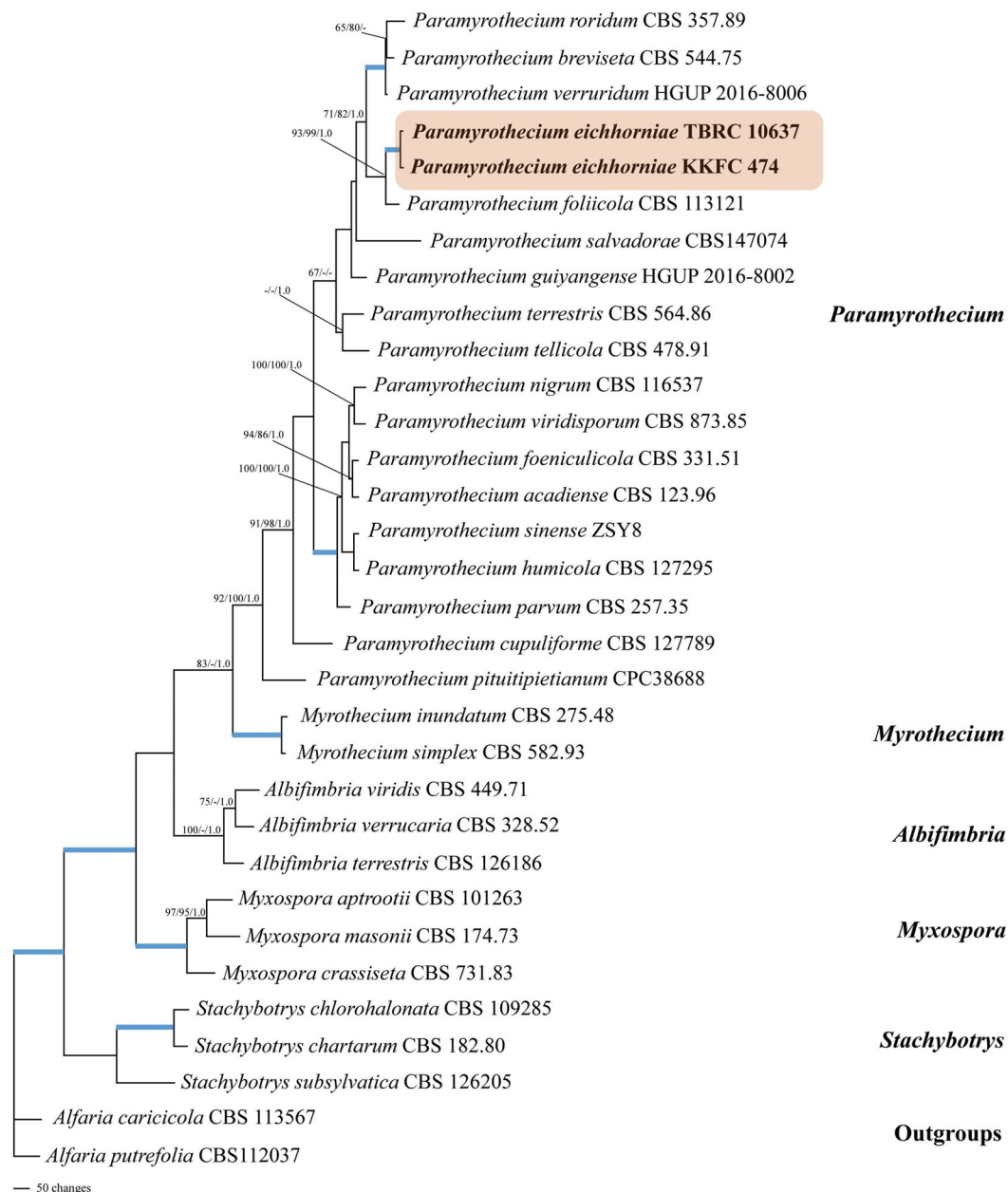


Figure 1. Phylogenetic relationships of *Paramyrothecium* spp. from combined ITS, LSU, *tef1*, *rpb2*, *tub2* and *cmdA* analyses. Bootstrap values (1,000 replicates) over 50% for MP and RAxML and over 0.95 for Bayesian posterior probabilities are added to the left of the nodes (MP/ML/PP), multiplied by 100; the blue lines in the tree represent bootstrap (BSMP and BSML) support of 100% and a posterior probability (BPP) of 1.00.

3.2. Morphological analysis

The genus *Paramyrothecium* was introduced by Lombard et al. [9]. Its original diagnosis was of sporodochial conidiomata, with or without a white setose fringe surrounding the slimy mass of conidia, hyaline conidiophores with penicillately branched, aseptate to 1-septate ellipsoidal to obovoid conidia. It was considered that the species identification using morphology is imprecise because their morphological features cannot clearly differentiate species. We summarized the morphological characters of species of *Paramyrothecium* and provided the details of the host and distribution in Table 2. For the single gene tree of each loci see Supplementary Figures S1–6.

4. Taxonomy

Paramyrothecium eichhorniae J. Unartngam, A. Unartngam & U. Pinruan, *sp. nov.* Figure 2.

Index Fungorum number: IF556554

Etymology: Name refers to *Eichhornia*, the plant genus from which this fungus was collected.

Sexual morph: Unknown.

Holotype: BBH 48295

Asexual morph: *Conidiomata* sporodochial, stromatic, superficial, cupulate, scattered or gregarious; outline oval or irregular in outline, 55 – 500 µm in diam, 60–200 µm deep with a white setose fringe surrounding an olivaceous green to dark green slimy mass of conidia. *Setae* arising

Table 2. Known *Paramyrothecium* species with host, location, and synopsis of morphological characteristics.

Name	Substrate	Country	Conidiophores	Conidia	Setae
<i>Paramyrothecium acadense</i>	leaf of <i>Tussilago farfara</i>	Canada	9–14 × 2–2.5 µm	0–1-septate, 5.5–16.5 × 1.5–2.5 µm	Not observed
<i>Paramyrothecium breviseta</i>	unknown	India	6–9 × 2–4 µm	0-septate, 4–5 × 1–2 µm	Present, 25–40 × 2–3 µm
<i>Paramyrothecium cupuliforme</i>	from soil	Namibia	15–25 × 2–4 µm	0-septate, 6–8 × 1–2 µm	Present, 45–90 × 2–3 µm
<i>Paramyrothecium eichhorniae</i>	leaf of <i>Eichhornia crassipes</i>	Thailand	15–40 × 2–3 µm	0-septate, 5–6.5 × 1.5–2.5 µm	Present, 40–120 × 2–3 µm
<i>Paramyrothecium foeniculicola</i>	leaf sheath of <i>Foeniculum vulgare</i>	The Netherlands	7–17 × 2–3 µm	0-septate, 5–7 × 1–2 µm	Not observed
<i>Paramyrothecium foliicola</i>	rotten leaf of unknown host; from air	Brazil; Cuba	15–25 × 2–3 µm	0-septate, 5–6 × 1–2 µm	Present, 60–100 × 2–3 µm
<i>Paramyrothecium guiyangense</i>	from soil	China	10–60 × 1–3 µm	0-septate, 6.6–9 × 2–3 µm	Present, 60–120 × 1–3 µm
<i>Paramyrothecium humicola</i>	from soil	USA	12–22 × 2–3 µm	0-septate, 6–7 × 1–2 µm	Present, 55–65 × 2–3 µm
<i>Paramyrothecium nigrum</i>	from soil	Spain	25–45 × 2–4 µm	0-septate, 5–6 × 1–2 µm	Present, 60–100 × 2–3 µm
<i>Paramyrothecium parvum</i>	from dune sand; <i>Viola</i> sp.	France; UK	12–26 × 2–4 µm	0-septate, 4–5 × 1–2 µm	Not observed
<i>Paramyrothecium pituitipietianum</i>	on stems of <i>Grikelum humifusum</i>	South Africa	20–35 × 3–4 µm	0-septate, (7–)9–10(–12) × (2–)2.5 µm	Present, 100–300 × 4–5 µm
<i>Paramyrothecium rotidum</i>	<i>Gardenia</i> sp.; twig of <i>Coffea</i> sp.; from water	Italy; Colombia;	15–40 × 2–4 µm	0-septate, (5–)6.5–7.5(–8) × 2 µm	Present, 60–100 × 2–6 µm
		The Netherlands			
<i>Paramyrothecium sinense</i>	from soil	China	20–30 × 2–3 µm	0-septate, 6–7 × 2–3 µm	Present, 45–90 × 1–3 µm
<i>Paramyrothecium salvadorae</i>	on twigs of <i>Salvadora persica</i>	Namibia	20–40 × 3–4 µm	0-septate, (8–)10–12(–13) × 2–2.5 µm	Present, 100–200 × 2.5–3 µm
<i>Paramyrothecium tellicola</i>	from soil	Turkey	15–30 × 2–4 µm	0-septate, (7–)7.5–8.5(–9) × 1–3 µm	Present, 45–80 × 2–3 µm
<i>Paramyrothecium terrestris</i>	from soil	Turkey	15–30 × 2–3 µm	0-septate, (7–)7.5–8.5(–19) × 1–3 µm	Present, 35–70 × 2–3 µm
<i>Paramyrothecium verroridum</i>	from soil	China	20–40 × 1.5–2.5 µm	0-septate, 6.8–7.8 × 2–2.7 µm	Present, 40–120 × 2–3 µm
<i>Paramyrothecium viridisporum</i>	from soil	Turkey; USA	15–35 × 2–3 µm	0-septate, 3–5 × 2 µm	Present, 60–140 × 2–3 µm

from sporodochia, thin-walled, hyaline, 1–3-septate, smooth, unbranched, straight to flexuous, 40–120 µm long, 2–3 µm wide, terminating in an acute rounded apex. *Conidiophores* growing from the basal stroma, consisting of a stipe and a penicillately branched conidiogenous apparatus, stipes unbranched, hyaline, septate, smooth, 15–40 × 2–3 µm, primary branches aseptate, unbranched, smooth, 10–17 × 2–3 µm; secondary branches aseptate, unbranched, smooth, 10–15 × 2–3 µm; terminating in a single whorl of 3–5 conidiogenous cells arising apically. *Conidiogenous cells* phialidic, cylindrical to subcylindrical, hyaline, smooth, straight to slightly curved, (8–)11–17(–20) × 2–3 µm, conspicuous collarettes and periclinal thickenings. Conidia aseptate, hyaline, smooth, cylindrical to ellipsoidal, 5–6.5 × 1.5–2.5 µm ($n=30$, $\bar{x} = 5.6 \times 2.3 \mu\text{m}$), rounded at both ends.

Known distribution: Amphoe Saraphi, Chiang Mai Province, Thailand.

Habit and habitat: on leaf of *Eichhornia crassipes*.

Culture characteristics: Colonies on PDA, Corn meal agar (CMA) and Oat meal agar (OA) approx. 9 cm in diam. after 14 d at 25 °C, circular with entire, white mycelium, hyaline, smooth; reverse on PDA creamy pink, sporulating in culture.

Material examined: THAILAND, Chiang Mai Province, on leaf of *Eichhornia crassipes*, 20 September 2012, O. Piyaboon and J. Unartngam (**holotype** BBH 48295); culture ex-holotype TBRC 10637.

Additional material examined: THAILAND, Phetchaburi Province, on the leaf of *Eichhornia crassipes*, 15 October 2012, O. Piyaboon and J. Unartngam (culture KKFC 474).

Note: Phylogenetically, *P. eichhorniae* is most closely related to *P. foliicola* L. Lombard & Crous (Figure 1). Morphologically, it differs from *P. foliicola* on the longer conidiophore (up to 40 µm long) while in *P. foliicola* it is shorter (up to 25 µm long). The conidia of *P. eichhorniae* (5–6.5 × 1.5–2.5 µm) are slightly larger than those of *P. foliicola* (5–6 × 1–2 µm). The setae of *P. eichhorniae* (40–120 × 2–3 µm) are sometimes slightly longer than those of *P. foliicola* (60–100 × 2–3 µm). Furthermore, *P. foliicola* produces a rosy buff exudate that diffuses into the growth medium, which was not seen on *P. eichhorniae*. However, we found that both species could not be discriminated by morphology, it is greater way that a combined their phylogeny and morphology. Thus, the present strains were identified as the new species *P. eichhorniae*.

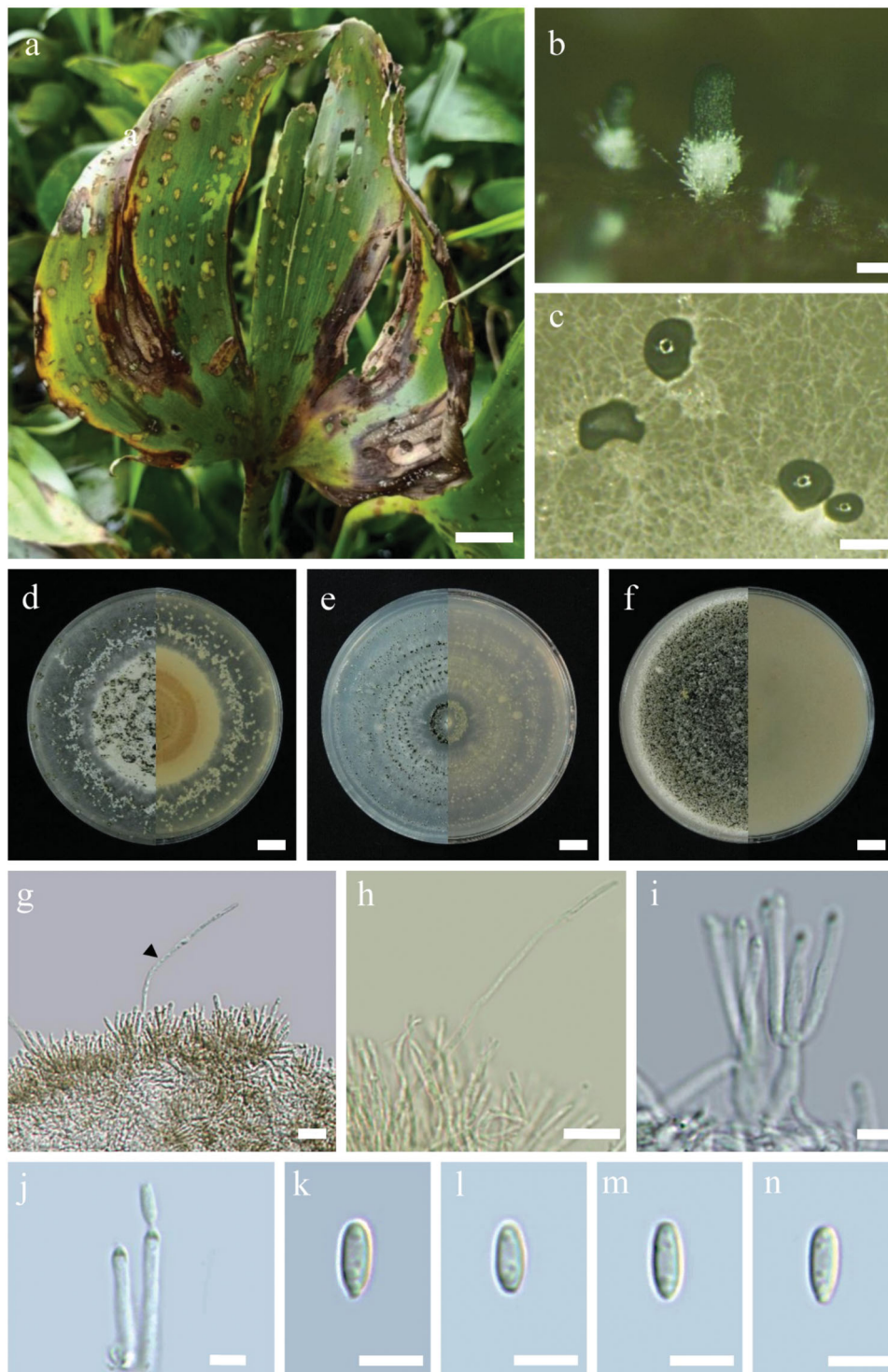


Figure 2. *Paramyrothecium eichhorniae* sp. nov. (BBH 48295, holotype). (a) Leaf blight disease symptom on water hyacinth. (b) Sporodochial conidiomata on substrate. (c) Sporodochial conidiomata on PDA. (d–f) Colonies on PDA, CMA, and OA after 15 days (left, from above; right, from below). (g–h) Setae. (i–j) Conidiogenous cells. (k–n) Conidia. Scale bars: a = 2 cm, b = 100 μm, c = 0.3 mm, d–f = 1 cm, g–h = 10 μm, and i–n = 5 μm.

4.1. Pathogenicity test studies

The characteristics of leaf blight disease of water hyacinth in a natural water source included round-to-teardrop-shaped leaf spots and blights with conidial mass (Figure 3(a)). Pathogenicity test by spraying the spore suspension on water hyacinth leaves showed early leaf blight signs on the water hyacinth leaves and dead tissues appeared. All of the inoculated leaves showed symptoms and the sporodochia

appeared on the leaves after 2 weeks of inoculation similar to the symptoms of leaf blight disease of water hyacinth in nature (Figure 3(b)).

5. Discussion

Taxonomic studies of *Paramyrothecium* have been based on morphological features and molecular analyses. In this study, the fungus causing leaf blight

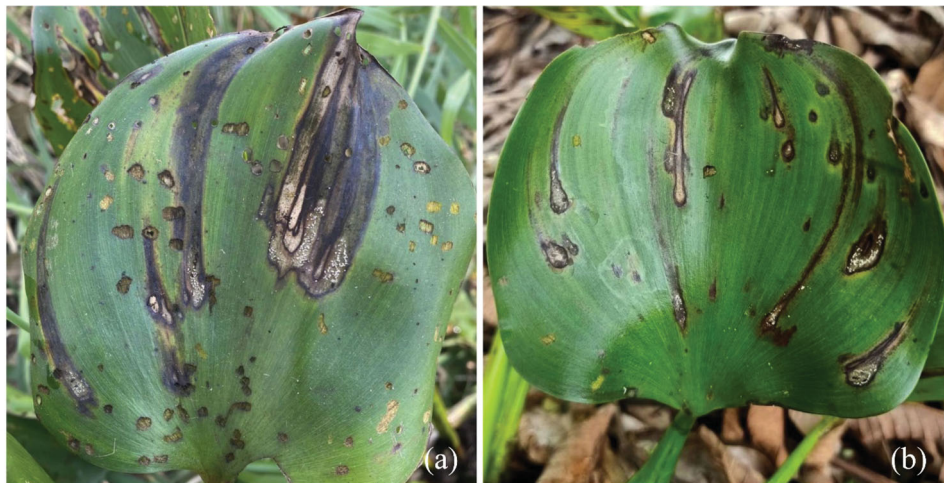


Figure 3. (a) Symptoms of leaf blight disease of water hyacinth in nature. (b) Pathogenicity test by spraying the spore suspension on water hyacinth leaves; all of the inoculated leaves showed symptoms after 2 weeks inoculation.

disease on water hyacinth plants collected in Chiang Mai and Phetchaburi Provinces belongs to the genus *Paramyrothecium*. *P. eichhorniae* is introduced as a new species and is well separated from other species of *Paramyrothecium* in the phylogenetic analyses of combined LSU rDNA, ITS rDNA, *tef1*, *rpb2*, *tub2* and *cmdA* sequence data. This new species group with *P. foliicola*, however, its morphological characters are distinctive, with the conidiophore stipes of *P. foliicola* being shorter than those of *P. eichhorniae*. The conidia of *P. foliicola* are smaller than those of *P. eichhorniae*, and colony on the growth medium produces a rosy buff exudate, which was not seen on the *P. eichhorniae* cultures. Moreover, this is the first report of disease caused by *Paramyrothecium* was on water hyacinth. However, the present isolates on water hyacinth in Chiang Mai had previously been misclassified under *P. roridum* in 2014 using morphological characteristics and ITS rDNA sequence analysis [4–5]. This study supported the comments of Krisai-Greilhuber et al. [10] that the identification of *Paramyrothecium* species using morphology is imprecise because the morphological features cannot clearly differentiate species (Table 2). Combining morphology and analyses of the gene sequence data are needed.

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