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Phylogenetic Trees of Aecial-Stage Rust Fungus, *Puccinia paederiae* (Dietel) Gorlenko Causing Gall on *Paederia linearis* Hook f.

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Abstract

A rust fungus, *Puccinia paederiae* (Dietel) Gorlenko causing galls on the stem of the skunk vine (*Paederia linearis* Hook. f. var. *linealis* and *P. linealis* var. *palida* (Craib) Puff) was collected for phylogenetic study as no molecular data was exclusively available for this fungus. Three regions of ribosomal DNA sequences, small subunit (SSU), large subunit (LSU) and internal transcribed spacer region 1 (ITS1) were employed. The results of maximum parsimony and Bayesian methods suggested that among the trees with these sequences, this fungus was nested in Pucciniaceae clades and Puccinia species with supportive statistical values. This is the first report on the phylogenetic analysis using multiple genes of the rust, *P. paederiae*.

Keywords: *Aecium*, basidiomycetes, galling rust, pucciniomycetes, skunk vine

Introduction

Skunk vine (*Paederia spp*.) is a climbing tree generally found in Thailand. It is categorized in Rubiaceae with the 3 most common species, *Paederia foetida* L., *Paederia linearis* Hook. f. and *Paederia pilifera* Hook. f. This fast-growing plant is also used as an ornamental plant, vegetable and traditional herbal medicine for different treatments such as colic, cramps, flatulence, dysentery, rheumatism and gout [1]. The root of skunk vine, *P. linearis*, is additionally an important ingredient of a local rice cracker or Khao-pong in the north eastern region of Thailand and it was found to have an antioxidative activity and acetylcholinesterase-inhibitory activity [2-5]. Because of the usefulness, it can be said that this plant is one of the versatile and essential plants in terms of its potential and is related to local wisdom. Despite the rapid growth, which likely occurred without any problems associated with pathogens, there were galling structures on the plant stems with yellow powder covering the surface of the galls. They were then collected for investigation and it was found that the galls were caused by a rust fungus, *Puccinia paederiae* (Dietel) Gorlenko. However, according to the database, no DNA sequences for this fungus are available.

Because of host specificity, the rust, *P. paederiae* is a basidiomycete which infects different *Paederia* species such as *P. pilifera* and *P. scandens* [6,7]. During the aecial phase, it exhibits a unique structure called aecium containing necklace-like aeciospores. The collected galls on the stem of skunk vine were fully covered by aecia with aeciospores. Taxonomically, *P. paederiae* was described and reported as *Aecidium paederiae* Dietel and *Endophyllum paederiae* (Dietel) F. Stevens & Mendiola [8,9]. However, the molecular data on its taxonomy has not been documented. Hence, this research aimed to identify and study the phylogeny of the fungus using different ribosomal DNA sequences which were small subunit (SSU), large subunit (LSU) and internal transcribed spacer region (ITS) to confirm that it was one of *Puccinia* species.

Materials and methods

Sample collection and aeciospore isolation

The galled stems were collected from 2 different types of skunk vine at Non-Muang village, Sila sub-district, Muang district, Khon Kaen province from April to May 2015. The skunk vine plants were finally identified as *P. linearis* Hook. f. var. *linearis* and *P. linearis* var. *pilosa* (Craib) Puff. The gall samples, aecia and aeciospores were measured and kept at −20 °C at the Mycology Laboratory, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University with category numbers, KKUPUC01, KKUPUC02, KKUPUC03,…., KKUPUC0X. The aeciospores were directly isolated from the sample using a sterile needle and micro spatula under stereomicroscope for DNA extraction.

DNA extraction

The extraction method followed that of White *et al*. [10]. The collected aeciospores, 0.2 g were suspended in 95 % ethanol for 1 min in a 1.5 mL tube and briefly centrifuged to collect the spore mass. Then, it was re-suspended and rinsed with sterile distilled water 3 times. Then the spores were ground in liquid nitrogen by using a sterile mortar and pestle. Lysis buffer (200 mmol/L Tris-HCl, pH 8.0; 250 mmol/L NaCl, 25 mmol/L EDTA, pH 8,0; 1 % sodium dodecyl sulfate), 700 µL was added with 3 µL of ß-mercaptoethanol before being incubated at 60 °C for 1 h. After that, the sample was added to chloroform: isoamyl alcohol (24:1; 700 µL) and then centrifuged at 12,000 rpm for 5 min. Only the supernatant was transferred to the new tubes. Then, cold isopropanol, 0.7× the collected supernatant volume was added, and the solution placed at −20 °C for 30 min. The tubes were spun at 12,000 rpm for 5 min to get DNA pellets then they were washed twice with 70 % ethanol, 500 µL, and air-dried. TE buffer, 50 µL (10 mmol/L Tris-HCl, 1 mmol/L EDTA) was added to dissolve the pellet then RNase A, 1 µL (10 ng/µL) and Proteinase K, 1 µL (10 ng/µL) were respectively added and incubated for 20 min. After that, to purify the DNA, 100 µL of chloroform: isoamyl alcohol (24:1) was added before centrifuging at 12,000 rpm for 4 min. The supernatant was collected and transferred to a new tube before adding 3 µL of 3 mol/L sodium acetate and 150 µL of absolute ethanol. The tubes were kept at −20 °C for 20 min and centrifuged at 12,000 rpm for 10 min to derive the cleaned DNA pellets. Finally, the tubes were washed with 70 % ethanol, air-dried and re-suspended in TE buffer. The genomic DNA in TE buffer was stored at −20 °C.

Polymerase chain reaction and sequencing

The gDNA were 10-fold diluted for the polymerase chain reaction (PCR) using these following primer pairs, SSU region using primers NS1-GTAGTCATATGCTTGTCTC and NS4-CTTCCGTCAATTCCTTTAAG [10], LSU sequence amplified with primers NL1-GCATATCAATAAGCGGAGGAAAAG and NL4-GGTCCGTGTTTCAAGACGG [11] and ITS1 region via rust specific primers, ITS1rustF10d-TGAACCTGCAGAAGGATCATTA and ITS1rustR3c-TGAGAGCCTAGAGATCCATTGTTA [12]. The final PCR reaction volume was 50 µL containing 1-µL of diluted gDNA, 2.5 mmol/L $MgSO_4$, 0.6 mmol/L dNTPs, 1× PCR buffer (Thermo Scientific), 1 µL of each 20 pmol primer, and 1.5 U of Taq polymerase (Thermo Scientific). The thermo cycles for PCR were as follows. For NS1/NS4 primers, predenaturation was at 95 °C for 5 min and then 30 cycles of 95 °C for 1 min followed by an annealing process at 54 °C for 1 min then extension at 72 °C for 1 min and final extension at 72 °C for 7 min [10]. For the primer pair NL1/NL4, the initial denaturation was at 94 °C for 5 min then followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s then 72 °C for 1 min and final extension at 72 °C for 7 min [11]. To amplify the ITS region, the first denaturation temperature was 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s [12]. Then, successful PCR products were checked in 1 % agarose gel through the electrophoresis in the TBE buffer (1 mol/L Tris, 0.9 mol/L boric acid, and 0.01 mol/L EDTA, pH 8.3) then stained with ethidium bromide solution and visualized in gel documentation. The in-gel purification and sequencing process of PCR products were achieved by First BASE Laboratories, Seri Kembangan, Selangor, Malaysia.

Phylogenetic analysis

Before the conduction of the phylogenetic analysis, Sequence Scanner Software v2.0 was used to visualize unclear chromatogram signals of the sequences before aligning. The DNA sequences of the fungus were deposited in the database and their accession numbers are shown in **Table 1**. The SSU, LSU and ITS DNA sequences of other fungi were obtained from GenBank (www.ncbi.nlm.nih.gov) (**Table 1**). The selected sequences were aligned using ClustalW and manually edited using MEGA 6.06 [13]. Three datasets e.g. dataset 1, 2 and 3, respectively for SSU, LSU and ITS alignments were constructed. In each dataset, parameters of the analysis were applied as follows. For the maximum parsimony method, Tree-Bisection-Reconnection (TBR) was set to a maximum parsimony search. The number of initial trees was 10 with random addition. Complete deletion was used for gap/missing data treatment. One thousand replicates of bootstrap were applied to all analyzed datasets [14]. The software to perform the phylogenetic trees and to view the obtained trees was MEGA 6.06 [13]. For Bayesian inference, SSU dataset (3,000,000 generations), LSU dataset (2,000,000 generations) and ITS dataset (700,000 generations) were performed with 1,000 generations to sample trees. The general time-reversible model with invariant sites and gamma distribution and the 25 % burn-in were applied to estimate the statistical vales, and posterior probabilities [15]. The derived trees were viewed using FigTree v1.4.2 [16]. The tree files were then converted into Newick format to view them in MEGA 6.06 [13].

Table 1 Accession numbers of SSU, ITS and LSU sequences in dataset 1, 2 and 3 respectively of selected fungi available in GenBank.

Dataset 1 (SSU)		Dataset 2 (LSU)		Dataset 3 (ITS)	
Taxa	**Accession no.**	**Taxa**	**Accession no.**	**Taxa**	**Accession no.**
This study		**This study**		**This study**	
Puccinia paederiae	KU532274	*Puccinia paederiae*	KU532270	*Puccinia paederiae*	KU532266
Puccinia paederiae	KU532275	*Puccinia paederiae*	KU532271	*Puccinia paederiae*	KU532267
Puccinia paederiae	KU532276	*Puccinia paederiae*	KU532272	*Puccinia paederiae*	KU532268
Puccinia paederiae	KU532277	*Puccinia paederiae*	KU532273	*Puccinia paederiae*	KU532269
Pucciniales		**Pucciniales**		***Puccinia* species**	
Family Incertae sedis		**Family Incertae sedis**		*Puccinia boroniae*	AY348710
Aecidium guatteriae	KM217376	*Aecidium kalanchoe*	AY463163	*Puccinia boroniae*	AY348712
Aecidium guatteriae	KM217377	*Aecidium klufaistianum*	HQ699078	*Puccinia boroniae*	AY348715
Aecidium guatteriae	KM217380	*Aecidium sp.*	DQ917721	*Puccinia boroniae*	AY348716
Aecidium kalanchoes	DQ354524	*Aecidium sp.*	FJ669219	*Puccinia chrysanthemi*	EU014034
Aecidium sp.	KM217381	*Aecidium sp.*	KF528007	*Puccinia chrysanthemi*	EU014035
Family Phakopsoraceae		**Family Phakopsoraceae**		*Puccinia chrysanthemi*	EU014037
Batistopsora crucis-filii	KF528024	*Batistopsora crucis-filii*	KF528017	*Puccinia chrysanthemi*	EU014038
Batistopsora pistila	KF528029	*Batistopsora pistila*	KF528028	*Puccinia komarovii*	KC430812
Batistopsora pistila	KF528043	**Family Coleosporiaceae**		*Puccinia komarovii*	KC430851
Phakopsora argentinensis	KF528039	*Chrysomyxa empetri*	DQ917750	*Puccinia komarovii*	KC430852
Phakopsora cherimoliae	KF528040	*Chrysomyxa ledi*	AF426246	*Puccinia komarovii*	KC430854
Phakopsora phyllanthi	KF528025	*Coleosporium euodiae*	KP017567	*Puccinia melanocephala*	KP744147
Family Cronartiaceae		*Coleosporium phlomidis*	KP017563	*Puccinia melanocephala*	KP744148
Cronartium ribicola	M94338	*Coleosporium senecionis*	AY512840	*Puccinia melanocephala*	KP744149
Family Pucciniaceae		*Coleosporium tussilaginis*	AF426242	*Puccinia psidii*	KM282159
Gymnoconia nitens	U41565	**Family Cronartiaceae**		*Puccinia psidii*	KM282160
Gymnoconia peckiana	DQ521422	*Endocronartium harknessii*	AY700193	*Puccinia psidii*	KM282161
Gymnosporangium asiaticum	KJ720161	*Endoraecium tierneyi*	KJ862335	*Puccinia psidii*	KM282162
Gymnosporangium asiaticum	KP308394	*Endoraecium violae-faustiae*	KJ862342	*Puccinia psidii*	KP863477
Gymnosporangium clavipes	AY12330	**Family Pucciniastraceae**		*Puccinia psidii*	KP863478
Gymnosporangium confusum	KJ720166	*Hyalopsora polypodii*	AY512852	*Puccinia psidii*	KT590039
Gymnosporangium ellisii	KJ720156	*Melampsoridium alni*	KF031534	*Puccinia sp.*	EU014042
Peridermium harknessii	M94339	*Melampsoridium betulinum*	DQ35456	*Puccinia sp.*	EU014060

Dataset 1 (SSU)		Dataset 2 (LSU)		Dataset 3 (ITS)	
Taxa	**Accession no.**	**Taxa**	**Accession no.**	**Taxa**	**Accession no.**
Puccinia cardui-pycnocephali	AY125410	*Melampsoridium betulinum*	KF031549	*Puccinia sp.*	EU014062
Puccinia convolvuli	DQ354511	*Melampsoridium hiratsukanum*	KF031546	*Puccinia sp.*	EU014065
Puccinia coronata	DQ354525	*Naohidemyces vaccinii*	AF426238	*Puccinia sp.*	EU014066
Puccinia graminis	AY125409	*Pucciniastrum agrimoniae*	AF426234	*Puccinia tanaceti*	EU014058
Puccinia hemerocallidis	DQ354518	*Thekopsora guttata*	AF426231	*Puccinia tanaceti*	EU400584
Puccinia hordei	DQ415278	*Uredinopsis filicina*	AF426237	*Puccinia tanaceti*	HQ201323
Puccinia menthae	AY123315	**Family Raveneliaceae**		*Puccinia tanaceti*	HQ201324
Puccinia pelargonii-zonalis	AY123316	*Kernkampella breyniae*	KJ862346	*Puccinia wolgensis*	AY956566
Puccinia physalidis	DQ354523	*Ravenelia neocaledoniensis*	KJ862347	**Outgroup**	
Puccinia popowiae	JF263511	*Ravenelia sp.*	KJ862349	*Gymnosporangium ellisii*	KJ720156
Puccinia violae	DQ354508	*Sphaerophragmium sp.*	KJ862350		
Uromyces appendiculatus	DQ354510	**Family Phragmidiaceae**			
Uromyces appendiculatus	AY123307	*Kuehneola uredinis*	AF426218		
Uromyces ari-triphylli	DQ354528	*Kuehneola uredinis*	AY745696		
Family Uropyxidaceae		*Phragmidium fragariae*	AF426217		
Prospodium lippiae	DQ831024	*Trachyspora intrusa*	AF426220		
Order Uredinales		**Family Pucciniaceae**			
Uredo rolliniae	KF528033	*Puccinia allii*	AF511076		
Uredo rolliniae	KF528034	*Puccinia argentata*	KC433400		
Uredo sp.	KF528008	*Puccinia canaliculata*	HQ412647		
Outgroup		*Puccinia carthami*	AY787782		
Ustilago maydis	KJ081758	*Puccinia coronata*	EU851141		
		Puccinia dioicae	GU058019		
		Puccinia emaculata	EU915294		
		Puccinia helianthi	KF214725		
		Puccinia hordei	DQ354527		
		Puccinia magnusiana	GU058000		
		Puccinia malvacearum	EF561641		
		Puccinia physalidis	DQ354522		
		Puccinia poarum	DQ831028		
		Puccinia silvatica	AY222048		
		Puccinia sparganioidis	GU327649		
		Puccinia sporoboli	GU058003		
		Puccinia striiformis	GU058005		
		Puccinia triticina	GU058007		
		Uromyces acuminatus	GU058004		
		Uromyces plumbarius	KP313731		
		Uromyces scillarum	AY302495		
		Family Uropyxidaceae			
		Tranzschelia fusca	AF426225		
		Tranzschelia pruni-spinosae	DQ363329		
		Tranzschelia pruni-spinosae	AF426224		
		Family Sphaerophragmiaceae			
		Triphragmium ulmariae	AF426219		
		Outgroup			
		Ustilago maydis	FJ644528		

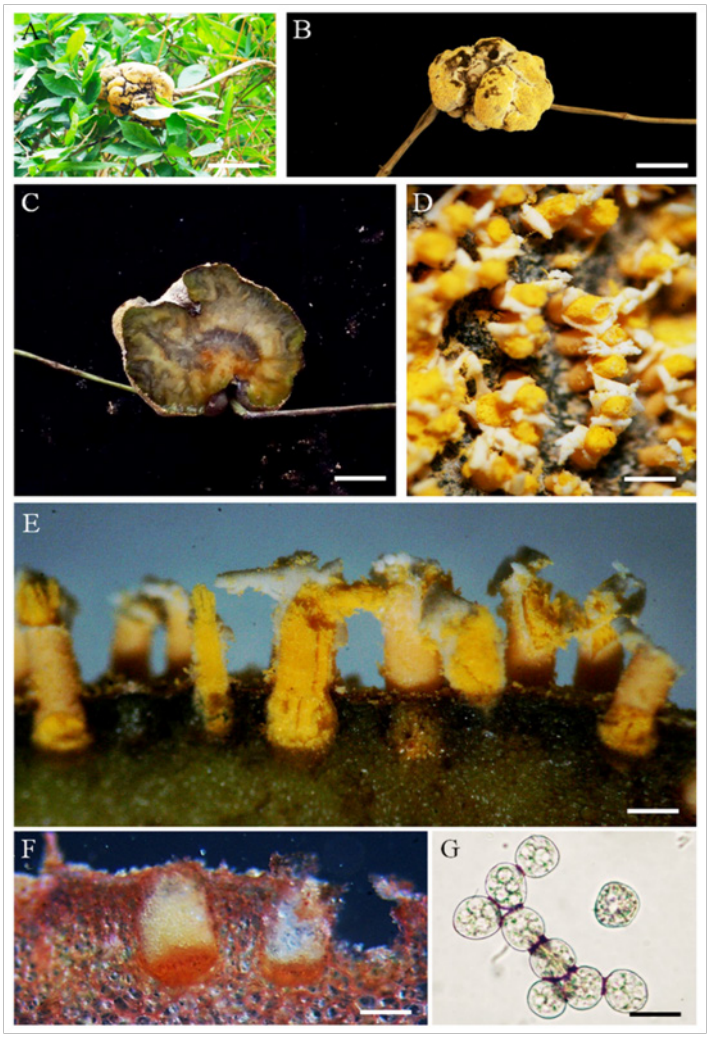

Figure 1 Stem galls of *Paederia linealis* (A and B), dissected gall showing succulent tissues of the plant (C), aecia (D-F), aeciospores (G). Scale bars: A and B = 5 cm, C = 2 cm, D = 500 µm, E and F = 200 µm, G = 10 µm.

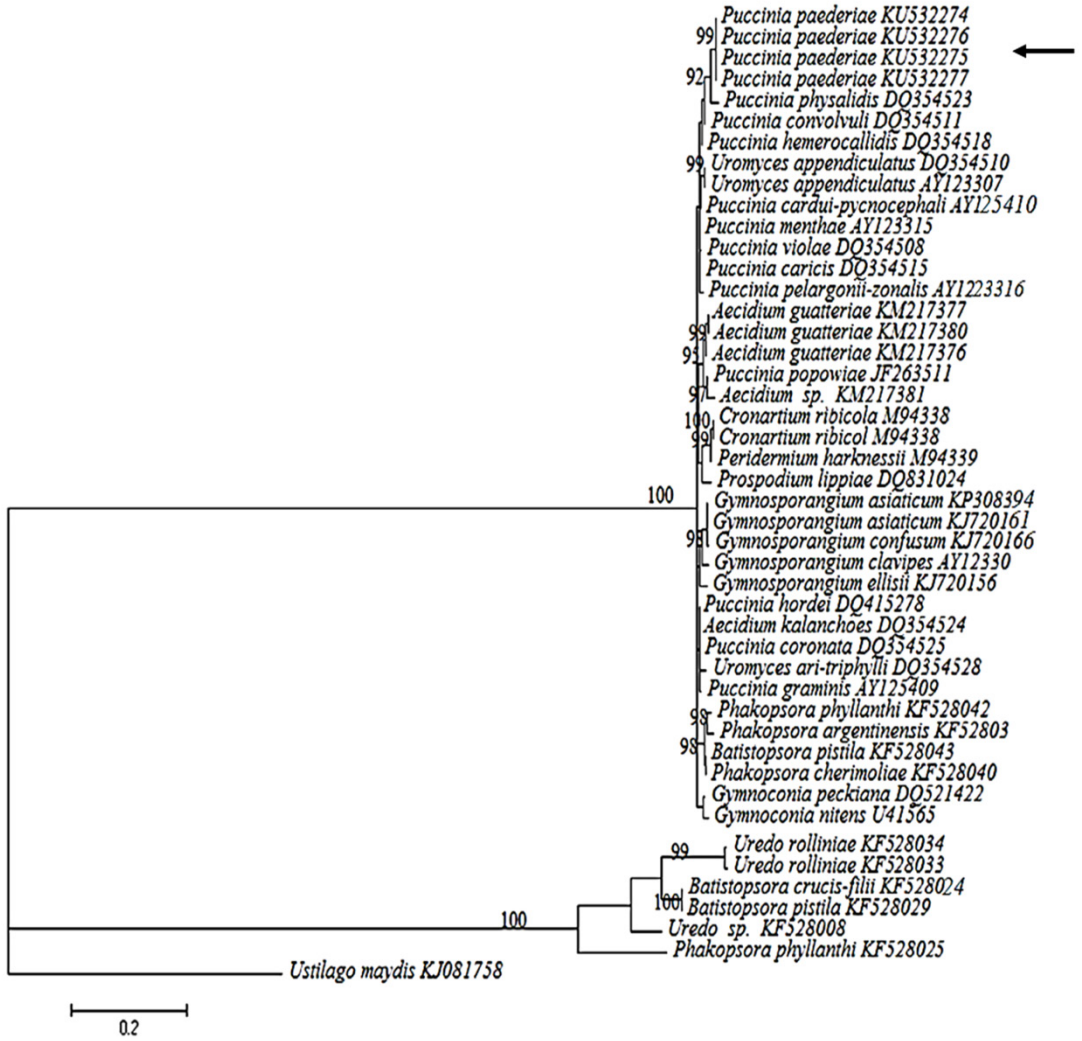

Figure 2 Phylogenetic tree obtained from Bayesian inference using SSU regions of species in Pucciniales indicates *Puccinia paederiae* is clustered in the same clade with *Puccinia physalidis* with posterior probability score at 92 (arrow head). Posterior probability values greater than or equal to 90 are shown at nodes.

Results and discussion

Morphology

This fungus caused succulent galls on the stem of the plant. The morphological features of the fungus in the aecial stage were described and it had been named as *A. paederiae* which was found on the leaves of *P. thorelii* Pitard. and *E. paederiae* described by Stevens and Mendiola [8]. Then, they turned out to be similar to *P. paederiae* which has currently been used [17]. The galls were various in size and succulent with a unique smell of the plant. Additionally, the sizes of aecia, (221-225.5) - (441-445) × (363-370.6) - (485.4-494) µm, L/W=1.24 and aeciospores, (7-7.6) - (13.8-15) × (10-10) - (15.4-16) µm, L/W = 1.25 were measured. The surface of the galls was covered by aecia containing aeciospores exhibiting yellow powder (**Figure 1**).

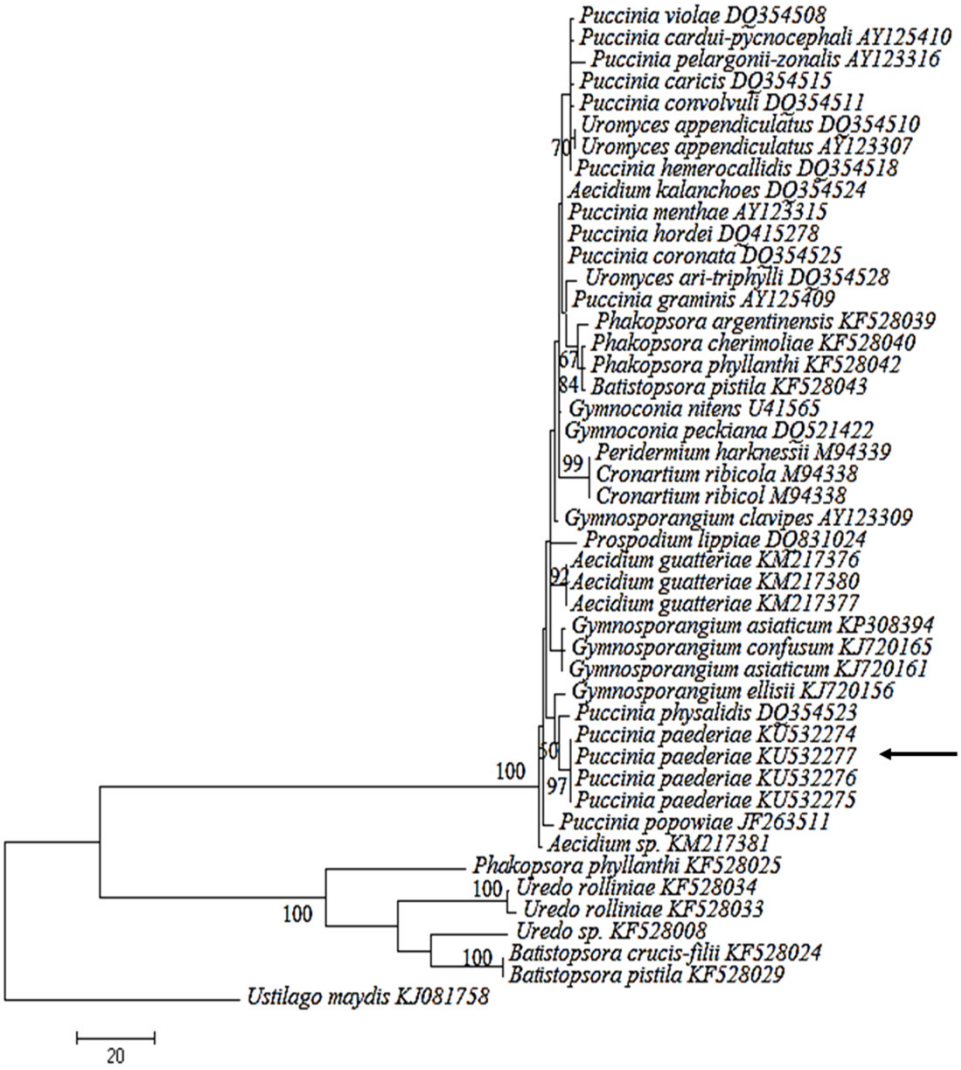

Figure 3 Phylogenetic tree obtained from maximum parsimony using SSU regions of species in Pucciniales indicates *Puccinia paederiae* is clustered in the same clade with *Puccinia physalidis* with bootstrap score at 50 (arrow head). Bootstrap values greater than or equal to 50 are shown at nodes.

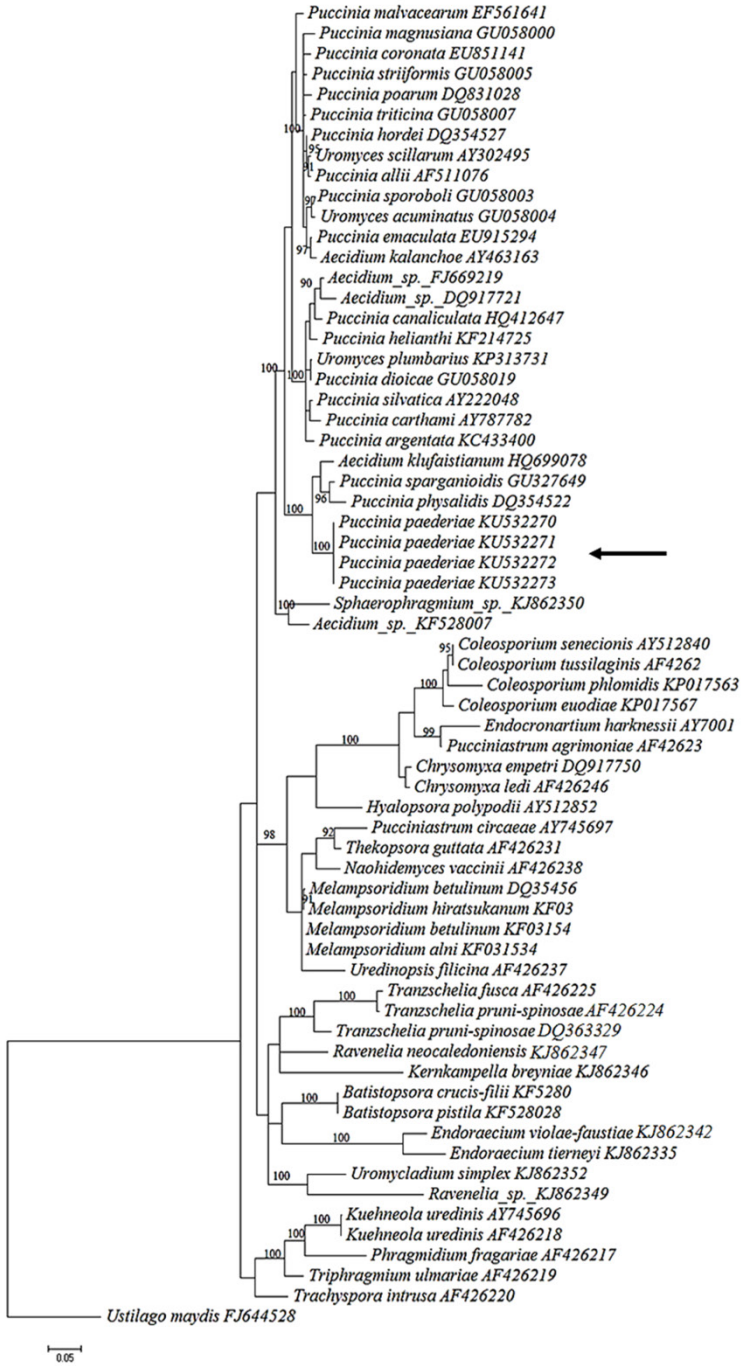

Figure 4 Phylogenetic tree obtained from Bayesian inference using LSU regions of species in Pucciniales indicates *Puccinia paederiae* is clustered in the same clade with Pucciniaceae and *Puccinia physalidis* and *Puccinia sparganioidis* with posterior probability score at 100 (arrow head). Posterior probability values greater than or equal to 90 are shown at nodes.

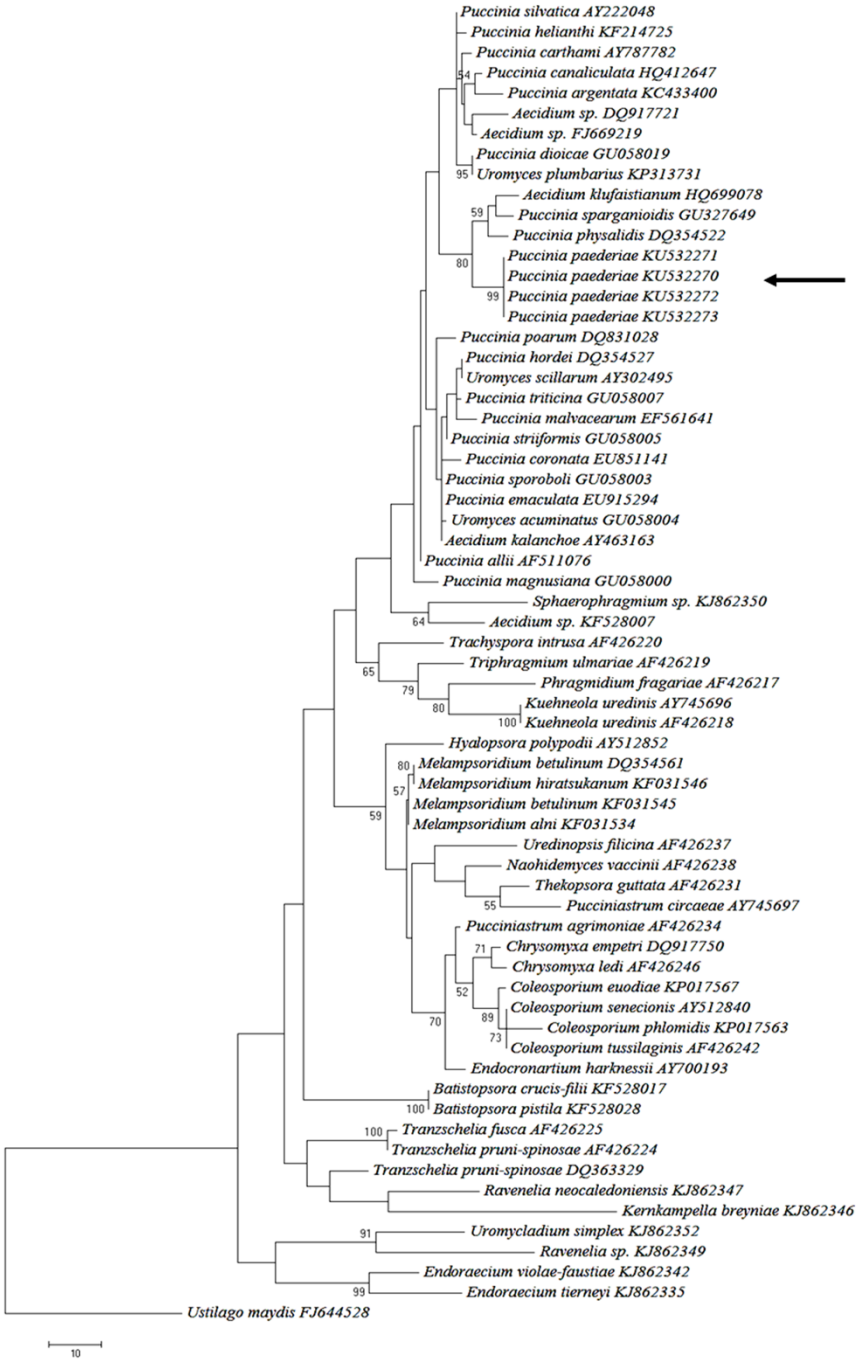

Figure 5 Phylogenetic tree obtained from maximum parsimony using LSU regions of species in Pucciniales indicates *Puccinia paederiae* is clustered in the same clade with Pucciniaceae and *Puccinia physalidis* with bootstrap score at 80 (arrow head). Bootstrap values greater than or equal to 50 are shown at nodes.

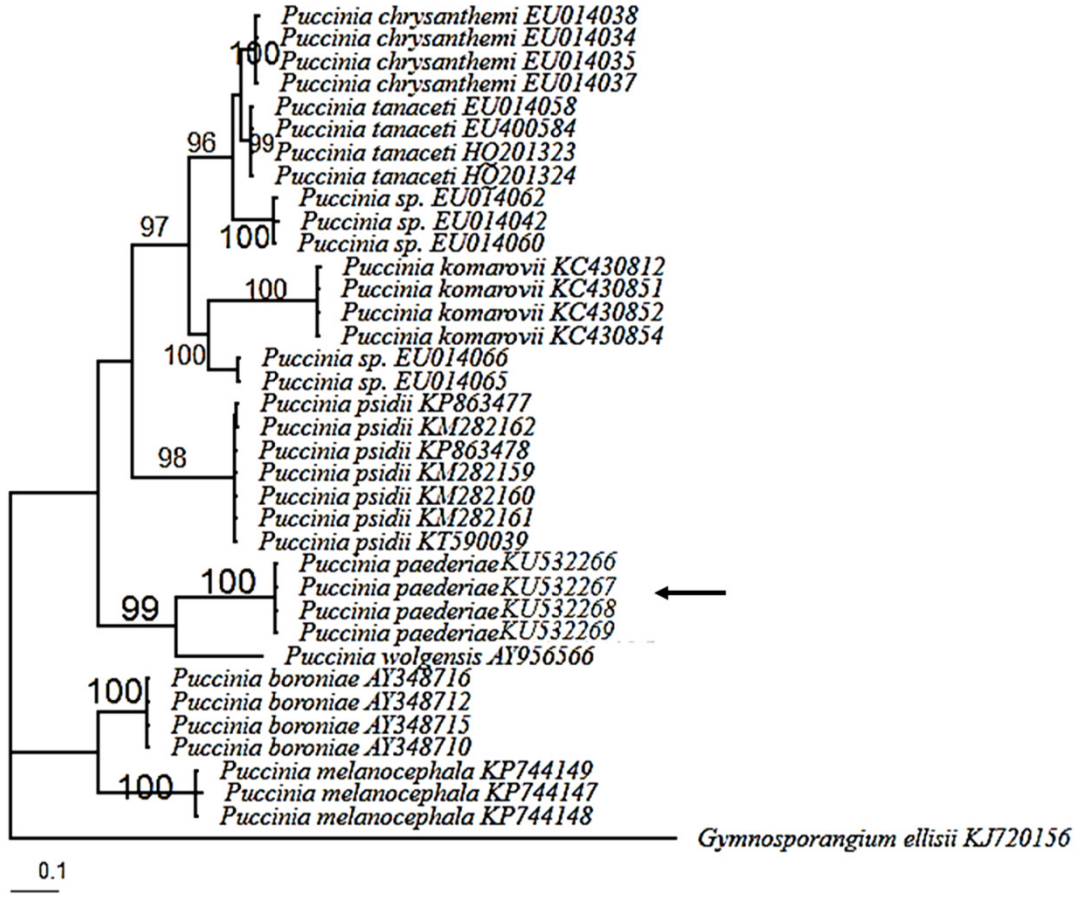

Figure 6 Phylogenetic tree obtained from Bayesian inference using ITS regions of *Puccinia* species indicates *Puccinia paederiae* is situated inside *Puccinia* clades with high posterior probability, 99 in relation to *Puccinia wolgensis* (arrow head). Posterior probability values greater than 90 are shown at nodes.

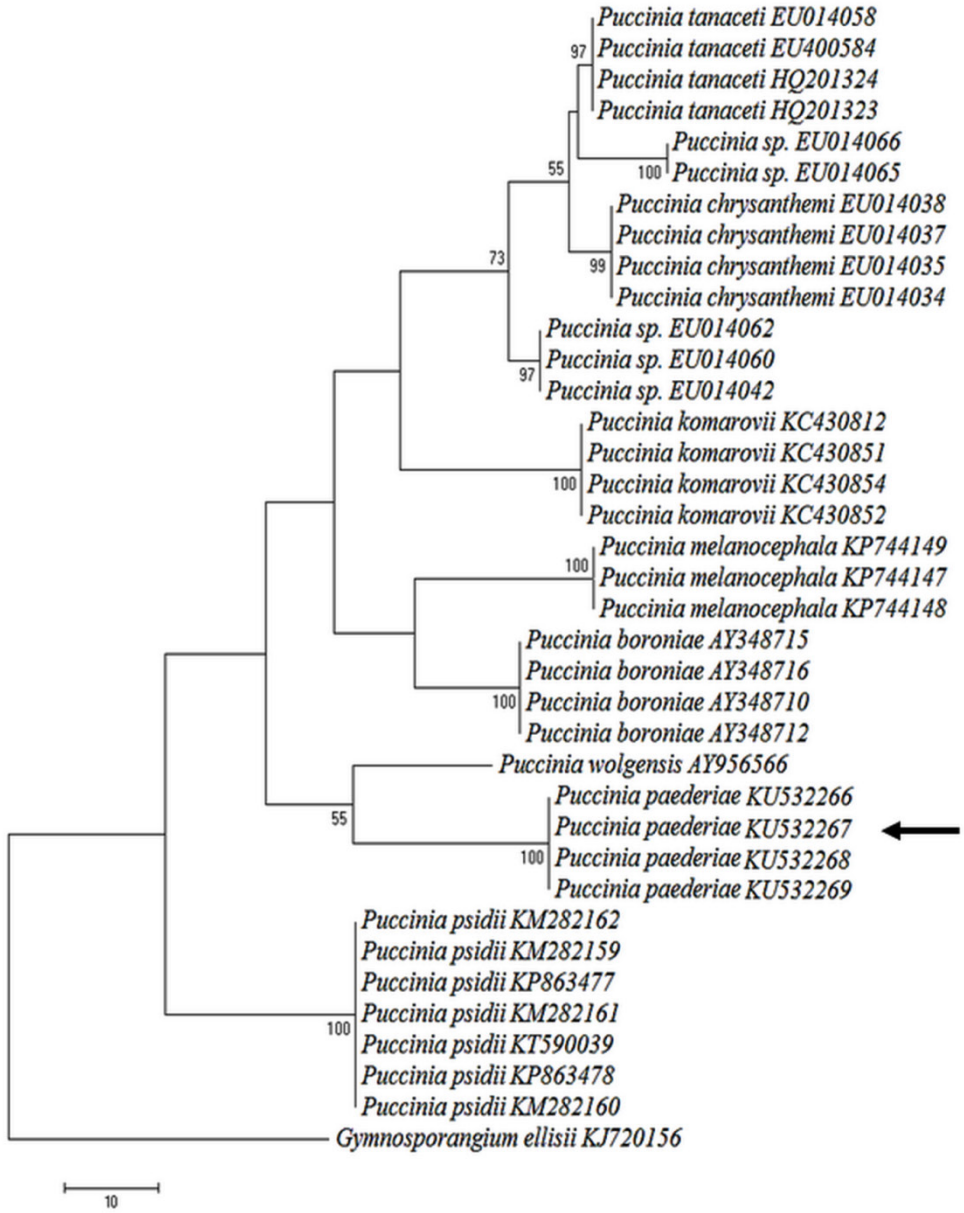

Figure 7 Phylogenetic tree derived from maximum parsimony using ITS regions of *Puccinia* species indicates *Puccinia paederiae* is situated inside *Puccinia* clades with bootstrap score at 55 in relation to *Puccinia wolgensis* (arrow head). Bootstrap values greater than 50 are shown at nodes.

Phylogeny

In dataset 1 which is composed of SSU sequences from representative fungi in Pucciniales with the majority of Pucciniaceae members, the analysis of Baysian inference and maximum parsimony method suggested similar results. The fungus, *P. paederiae* was situated in the same branch with those members in Pucciniaceae with the supportive statistical scores, 92 of posterior probability as shown in **Figure 2** and

50 of bootstrap value illustrated in **Figure 3**. In the parsimonious tree, the tree length was 464 with a consistency index at 0.775, retention index at 0.912 and a composite index at 0.727. The isolates that were composed of the interested fungus were also clustered in the same branch with another rust fungus, *P. physalidis* Peck which confirmed that this fungus was in Pucciniaceae.

Additionally, dataset 2 with the alignment of LSU sequences, retrieved from different remembers in Pucciniales was used to perform further phylogenetic analysis to confirm whether it yielded similar results. As expected, the isolates of the fungus in this study were grouped in the same clade with members in Pucciniaceae related to *P. physalidis* and *P. sparganioidis* with high posterior probability and bootstrap values at 100 and 80, respectively (**Figures 4** and **5**). In the parsimonious tree, the length of the tree was 684. The consistency index was 0.381 with a retention and composite index of 0.679 and 0.308, respectively. It was clear that using LSU sequence was able to maximize the phylogenetic resolution.

Due to the trees obtained from datasets 1 and 2, they indicated that the fungal isolates were closely related to *Puccinia* species which were in Pucciniaceae. Thus, to ascertain if the fungus was one of the *Puccinia* species, the ITS1 region was sequenced and another phylogenetic analysis using ITS sequences of different *Puccinia* species was performed. Both Bayesian and parsimonious trees showed similar results, the fungus of the study was placed in between the representative *Puccinia* species in the same branch with *P. wolgensis* Navashin with supportive scores of posterior probability at 99 and bootstrap at 55 as shown in **Figures 6** and **7**. The parsimonious tree length was 239. The consistency index was 0.632. The retention index was 0.898 and the composite index was 0.601.

Ribosomal rDNA sequences e.g. SSU, LSU and ITS are widely used to infer the phylogeny of organisms because they are informative in evolutionary relationship [18,19]. The phylogenetic trees derived from Bayesian method and maximum parsimony with SSU and LSU sequences suggested that in order level, the fungus of interest, *P. paederiae* fitted in the same clade with others in Pucciniales and also in Family of the Pucciniaceae because it was grouped with the *Puccinia* species. In the trees using SSU sequences, the resolution of the tree branch was not very clear as there were some members from other families grouped in the Pucciniaceae clade. In contrast to the trees with LSU sequences, the phylogenetic analysis yielded the interested fungus and other Pucciniaceae fungi clustered in the same branch. Although there were *Aecidium* species, the family *Incertae sedis*, included in the clade with other members in Pucciniaceae, they could be one of the rusts in this family as no specific family has been assigned for them (**Figures 2 - 5**). Thus, regarding the branch containing *P. paederiae*, *P. physalidis* and *P. sparganioidis* in the trees using SSU and LSU sequences, the fungus causing gall could be put in the family Pucciniaceae and it should be in genus *Puccinia*. Furthermore, to assure that it was one of *Puccinia* species, the trees with ITS sequences were accordingly generated. Among the *Puccinia* species, both Bayesian and parsimonious trees suggested the results that were expected i.e. *P. paederiae* was nested in the branches related to *P. wolgensis* Navashin with supportive scores (**Figures 6** and **7**). *P. wolgensis* is a rust found on a feather grass (*Stipa* sp.) distributed in Morocco, Syria and Central Asia but the aecial stage of this fungus is still unknown [7]. However, in the database, there was no deposited sequence of *P. paederiae* available prior to this study to compare. Thus, due to the phylogenetic indication by these rDNA sequences, the fungus, *P. paederiae* could be claimed.

Pathologically, rust fungi perform different stages in different host plants to complete the life cycle [20]. For this one, it was found on a skunk vine, *P. linearis* and caused the galls on the stem. On the galls, there were a number of aecia with yellow aeciospores covering the surface of the gall which was succulent and unique in its smell similar to the plant odor. Firstly, it was identified as *E. paederiae* and *A. paederiae* on *P. scandens* [8]. It has also been proved that *A. paederiae* on *P. scandens*, a synonym of *P. foetida* [21], is able to infect a lawn grass, *Zoysia sp.* and performs the telial stage. Therefore, the name, *P. zoysia* Diet. is used. The distribution of this rust on the *Zoysia* grass is in Japan, Korea, China and USA [7,22]. However, in Thailand, there is no report on the rust, *P. zoysia* available and the pathological proof of the collected fungus whether it is able to infect *Zoysia* spp. has not been conducted. Further investigation should be done in details on this issue. In addition, there is a report on the control of *P. foetida* by the galling rust, *E. paederiae* derived from *P. pilifera* Hook. f. from the northern region of Thailand but the rust was unsuccessful to infect the plant indicating that the rust is very specific to a certain host range [6].

Regarding the phylogenetic trees of ITS dataset, the collected fungus was closely related to another rust fungus, *P. wolgensis* causing a disease on *Stipa* grass [7]. Thus, *P. paederiae* might be one of the rusts that could infect a grass species. Therefore, in this scenario, the scientific term of this fungus reported in this paper is *P. paederiae* after the host it was found on as the current name [22], until the host plant that allows the fungus to infect and performs the telial stage is discovered.

Conclusions

The galling rust on *P. paederiae* was seen in the aecial stage producing a large number of aeciospores but the other hosts of the rust are still unknown. The collected rust fungus was then subjected to the phylogenetic study using ribosomal DNA sequences. The results suggested that the fungus was in Pucciniales, Pucciniaceae and situated among *Puccinia* species closely related to *P. wolgensis* and the name *P. paederiae* should be assigned according to the current host plant.

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