Research Article

Genetic Variation of *Coleosporium plumeriae* from Different Provinces in Thailand

Patcharavipa Chaijuckam*, Pattavipha Songkumarn and Onuma Piasai
Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

Sompob Saralamba
Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

Malinee Sriariyanun
Department of Mechanical and Process Engineering, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

Srimek Chowpongpang
National Science and Technology Development Agency, Pathum Thani, Thailand

Jonathan Jaime G. Guerrero
Department of Biology, College of Science, Bicol University, Albay, Philippines

* Corresponding author. E-mail: agrpvp@ku.ac.th DOI: 10.14416/j.asep.2020.01.002
Received: 22 October 2019; Revised: 27 November 2019; Accepted: 29 November 2019; Published online: 17 January 2020

Abstract
Plumeria rust samples were collected from five provinces in Thailand, including Bangkok, Nakhon Pathom, Rayong, Chonburi and Yala. All five isolates produced the uredial stage but only the isolates from Bangkok and Yala also underwent the telial and basidial stages. The morphological characteristics of all three stages present in the life cycle of the isolates were studied under stereo, compound and electron microscopes. Ribosomal DNA (rDNA) sequences at 28S and ITS (internal transcribed spacer) regions were analyzed with those in the GenBank database by Nucleotide BLAST and phylogenetic analyses. *Coleosporium plumeriae* was identified as the causal agent of plumeria rust by structure morphology and rDNA sequences that revealed genetic variation of the fungus as well. In general, there were significant differences in the morphological characteristics of uredospores, teliospores and basidia among the isolates. However, the variation of spore morphology was not related to the sampling locations. According to the phylogenetic analysis of 28S rDNA sequences, the UPGMA tree grouped all *C. plumeriae* from Thailand and foreign countries in the same clade as they shared identical sequences. On the other hand, the UPGMA tree inferred from ITS rDNA sequence data detected genetic variation of the isolate from Chonburi and separated it into the distinct tree branch. In this study, structure morphology and ITS rDNA were suitable genetic markers for both interspecific and intraspecific taxonomy of *C. plumeriae*.

Keywords: Plumeria rust, *Coleosporium plumeriae*, Genetic variation, ITS rDNA, 28S rDNA

Please cite this article as: P. Chaijuckam, P. Songkumarn, O. Piasai, S. Saralamba, M. Sriariyanun, S. Chowpongpang, and J. J. G. Guerrero, "Genetic variation of *Coleosporium plumeriae* from different provinces in Thailand," *Applied Science and Engineering Progress*, vol. 13, no. 1, pp. 38–47, Jan.–Mar. 2020.

1 Introduction

Plumeria (*Plumeria* spp.) is a popular ornamental tree widely planted in tropical and subtropical regions [1]. In Thailand, plumeria is widespread for landscape planting in parks, temples, yards, resorts and many other places due to its colorful and fragrant flowers and being easily cultivated, fast-growing and drought-tolerant plant [2]. Plumeria is used not only for attractive scenery but also medicinal purposes [3]. Moreover, Thailand is an important exporter of ornamental plants in Asia and plumeria is in the top ten of ornamental plants exported to foreign countries [4]. Although plumeria is a tough tree, diseases and insects are still problems in the cultivation [3]. Among them, rust disease caused by *Coleosporium plumeriae* Pat. is ubiquitous on plumeria worldwide. The several small, bright yellow-orange, powdery pustules (uredia) with numerous uredospores on the lower side of leaves are typical symptoms. Severe infection can cause premature defoliation and the tree may die, thus the beauty of plumeria is destroyed. The uredial, telial and basidial stages of *C. plumeriae* on plumeria have been described but the spermagonial and aecial stages are unknown [5], [6]. *C. plumeriae* is considered to be autoecious with no alternate host [7].

Despite the fact that *C. plumeriae* was globally recorded in many locations in Pacific Islands, America, Oceania, Asia and Africa, only the uredial stage was detected from most specimens. The telial or basidial stages were recovered from a few locations, including Canada, Hawaii, Cook Islands, Taiwan, Brazil, Nigeria, India and Australia [1], [8]–[14]. Morphological identification of rust fungi may be problematic when some stages in the life cycle are absent, so another method could be concurrently performed as in the previous studies of plumeria rust in China [15], Louisiana and Malaysia [16]. At present, sequence analysis of ribosomal DNA (rDNA) is prevalent in fungal taxonomic studies because of available large databases. Nucleotide sequences of 18S or 28S rDNA can distinguish distantly related taxa or different kingdom. Besides interspecific taxonomy of closely related species, internal transcribed spacer (ITS) rDNA can reveal intraspecific variation [17]. The occurrence of a pathogen variant possesses the risk to break down the resistance of a plant variety or cause severe symptoms on a variety that it could hardly damage before [18]. Morphological and molecular markers have been implemented to characterize genetic variation in fungal populations [19], therefore these markers were selected to examine genetic variation of the plumeria rust fungus in Thailand.

Plumeria rust in Thailand was first reported by To-anun *et al*. in 2004 [1]. Only the uredial stage was observed, so *C. plumeriae* was identified as the causal agent based on the morphology of uredia and uredospores. Moreover, genetic background of the plumeria rust fungus in Thailand has not been elucidated so far. Hence in this study, the plumeria rust isolates from five provinces in Thailand were examined for all stages in the life cycle to gain knowledge for morphological taxonomy of rust fungi and the fungal species were identified by structure morphology and rDNA sequences to confirm the identity of *C. plumeriae*. Additionally, genetic variation of the isolates was investigated based on structure morphology and genetic analyses of rDNA. Genetic relationship between the plumeria rust isolates from Thailand and those from foreign countries was also determined.

2 Materials and Methods

2.1 *Collection of the plumeria rust isolates*

Rust isolates were collected from plumeria plants (*Plumeria* spp.) showing rust symptoms in 2015 to 2017. The five sampling provinces in Thailand were randomly selected, including Bangkok and Nakhon Pathom in central region, Rayong and Chonburi in east region and Yala in southern region (Figure 1). The mean temperature, mean relative humidity and rainfall of the sampling month and one month before were recorded in accordance with the Thai Meteorological Department, Thailand. Each sample was examined for the uredial, telial and basidial stages in the life cycle. All five isolates were identified based on structure morphology and ribosomal DNA (rDNA) sequences.

2.2 *Identification of the plumeria rust isolates*

2.2.1 Identification based on structure morphology

The morphological characteristics of the isolates were studied and photographed under stereo (SZ-PT Olympus, Japan) and compound (ZEISS Axio Scope.A1, Germany)

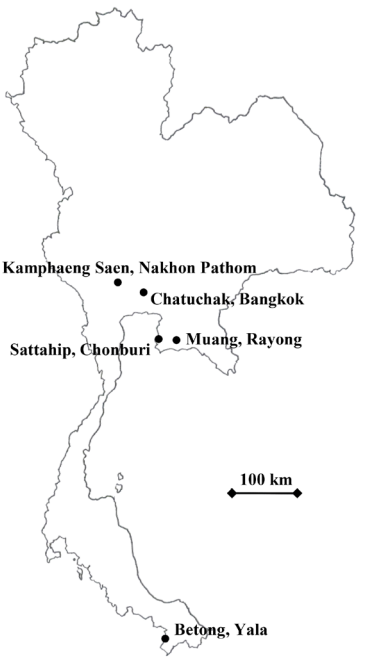

Figure 1: Sampling locations of the plumeria rust isolates collected from five provinces in Thailand.

microscopes. Moreover, the isolate from Bangkok was chosen for camera lucida drawings and submitted for investigating under scanning electron microscope (Hitachi SU8020, Japan) at Scientific Equipment and Research Division, Kasetsart University, Thailand. All isolates were identified according to the previous publications [1], [8], [10]–[16], [20], [21].

2.2.2 Identification based on ribosomal DNA sequences

DNA was extracted based on the modified protocol of Park *et al*. [22]. Uredospores from 10 uredia were transferred into 300 µL of extraction buffer (100 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 1 M KCl) and ground with steel balls using the modified drilling machines for 30 s. Three hundred microliters of chloroform were added and mixed by inverting the tube upside down about 10 times. The suspension was then centrifuged at 13,000 rpm for 10 min at room temperature. Two hundred microliters of supernatant was transferred to a new microcentrifuge tube and 200 µL of isopropanol (–20°C) was added and gently mixed well. The supernatant was discarded after centrifugation at 13,000 rpm for 10 min. DNA pellet was washed in 200 µL of 70% absolute ethanol (4°C) and centrifuged at 12,000 rpm for 15 min at room temperature. The supernatant was discarded and DNA pellet was dried at room temperature. The DNA sample was dissolved in 30 µL of sterile ultrapure water.

Two regions of rDNA were amplified by rust specific primers. Rust2inv and LR6 amplified partial 28S rDNA region and ITS5-u and ITS4rust amplified internal transcribed spacer region (ITS1-5.8S-ITS2) [23]–[26]. The PCR was conducted in a 50-µL volume containing 3.0 µL of template DNA, 9.5 µL of PCR grade water, 1× PCR buffer for KOD FX Neo, 0.4 mM of each dNTP, 0.3 µM of each primer and 1.0 U of KOD FX Neo (Toyobo, Japan). The reaction was performed in a thermal cycler (Major Science, USA) with the following program: 2 min at 94°C; followed by 40 cycles of 15 s at 94°C, 30 s at the annealing temperature and 1 min at 68°C. The annealing temperatures were 57°C and 53°C for primers Rust2inv/LR6 and ITS5-u/ITS4rust, respectively. PCR products were examined by 1% agarose gel electrophoresis and stained with ethidium bromide. The purified PCR products were submitted for DNA sequencing at 1st BASE, Malaysia. The DNA sequences were compared with sequences in the GenBank database using Nucleotide BLAST [27]. Furthermore, multiple sequence alignment of five plumeria rust isolates and *Coleosporium* spp. available in the GenBank database (Table 1) was performed using MUSCLE 3.8 [28]. The phylogenetic trees inferred from 28S and ITS rDNA sequence data were constructed by UPGMA method with a bootstrap of 1000 replicates and the evolutionary distances were computed by the Maximum Composite Likelihood method using MEGA-X [29]. The DNA sequences of *Chrysomyxa arctostaphyli* (accession no. GU049543 and GU049496) were included as an outgroup species because it was a sister taxon of *C. plumeriae* shown in the previous study [30], [31]. All ambiguous positions were removed for each sequence pair. There were totally 336 and 338 positions in the final 28S and ITS rDNA dataset, respectively.

2.3 *Characterization of genetic variation of the plumeria rust isolates*

2.3.1 Morphological characteristics of the structures

Each rust sample was mounted in Shear's mounting agent

after free-hand sectioning. Uredospores, teliospores and basidia were examined with 30 replications for the length, width, ratio (length:width), shape and color. The size of uredospore walls was also measured. The measurements were carried out by using AxioVision SE64 Rel.4.8 software (Carl Zeiss, Germany).

Continuous variables of uredospores were analyzed by analysis of variance (ANOVA) and mean comparison was performed by Tukey's test at $\alpha = 0.05$. Continuous variables of teliospores and basidia were analyzed by t-test. All statistical analyses were computed by Wolfram Engine 12.0 (Wolfram Research, Inc., USA).

Table 1: The DNA sequences from GenBank database included in the phylogenetic analyses

Coleosporium species	Location	Accession Number	
		28S rDNA	ITS rDNA
C. asterum	-	KX386044	KX386012
C. bletiae	-	KX386038	KX386006
C. cacaliae	-	JF273971	KY810462
C. clematidis	-	KX386042	KX386010
C. inulae	-	MG907223	KY783673
C. ipomoeae	-	MF769639	MF769624
C. senecionis	-	KJ716348	KY810472
C. solidaginis	-	MF769650	MF769633
C. tussilaginis	-	MG907228	KY810485
C. zanthoxyli	-	MK530184	MK530182
C. plumeriae (ID)	Bali Is., Indonesia	KX386068	KX386036
C. plumeriae (TW)	Taichung, Taiwan	KX386067	KX386035
C. plumeriae (JP)	Okanawa Is., Japan	KX386066	KX386034
C. plumeriae (MY)	Penang, Malaysia	KX386061	KX386029
C. plumeriae (VN)	Ninb Bins, Vietnam	KX386060	KX386028
C. plumeriae (CK)	Rarotonga, Cook Is.	KX386059	KX386027
C. plumeriae (WS)	Upolu Is., Samoa	KX386057	KX386025
C. plumeriae (CN)	Hainan Is., China	KX386054	KX386022
C. plumeriae (PF)	Tahiti, French Polynesia	KX386053	KX386021
C. plumeriae (GY)	Georgetown, Guyana	MG907225	-
C. plumeriae (IN)	Karnataka, India	-	MH656772

2.3.2 Genetic analyses of ribosomal DNA

Multiple sequence alignment of five plumeria rust isolates from Thailand and *C. plumeriae* from foreign countries available in the GenBank database (Table 1) was performed using MUSCLE 3.8 [28]. The phylogenetic trees inferred from 28S and ITS rDNA sequence data were constructed by UPGMA method with a bootstrap of 1000 replicates and the evolutionary distances were computed by the Maximum Composite Likelihood method using MEGA-X [29]. All ambiguous positions were removed for each sequence pair. There were a total of 336 and 338 positions in the final 28S and ITS rDNA dataset, respectively.

3 Results

3.1 *Collection and identification of the plumeria rust isolates*

All plumeria rust isolates from five provinces in Thailand, including Bangkok and Nakhon Pathom in central region, Rayong and Chonburi in east region and Yala in southern region were identified as *Coleosporium plumeriae* based on structure morphology. All five isolates produced the uredial stage (asexual reproduction) but only the isolates from Bangkok and Yala also underwent the telial and basidial stages (sexual reproduction). However, the climates in both provinces were different, including temperature, relative humidity and rainfall (Table 2). Therefore the conditions favorable to the telial and basidial stages could not be determined from this study. The spermagonial and aecial stages were unknown.

In general, uredia and telia were found on both sides of leaves but chiefly developed on the lower side [Figure 2(a)–(f)]. There were chlorotic lesions on the upper leaf surface correlated with the rust pustules on the lower leaf surface. These lesions progressively enlarged, coalesced, and became necrotic [Figure 2(a) and (c)]. Uredia were erumpent, yellow to orange-yellow and scattered or clustered on the leaf surface [Figure 2(g) and (i), Figure 3(a) and (c)]. Uredospores were single-celled; subglobose, broadly ellipsoid, ellipsoid, obovoid or angular; yellow to orange-yellow and measured 25.49–40.53×16.26–27.45 μm. Germ pores were obscure. Uredial walls were verrucose with capitate annulated tubercles and 1.23–1.98 μm thick

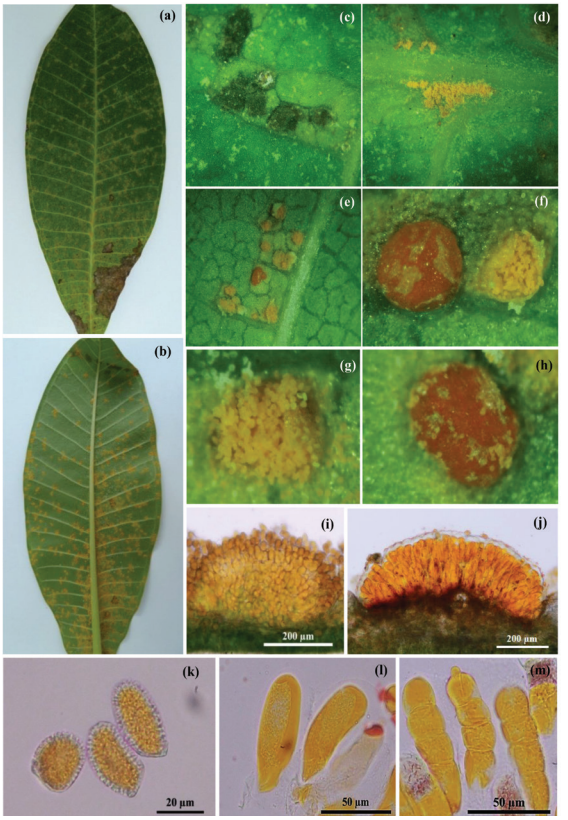

Figure 2: Symptoms on [(a) and (c)] the upper and [(b), (d) and (e)] the lower leaf surfaces and structure morphology of plumeria rust, (f) telium (left) and uredium (right), [(g) and (i)] uredium, [(h) and (j)] telium, (k) uredospores, (l) teliospores and (m) basidia.

[Figure 2(k), Figure 3(e) and Figure 4(a)]. Telia were erumpent, smooth, wax-like, reddish orange to dark orange and scattered or clustered in groups [Figure 2(h) and (j), Figure 3(b) and (d)]. Teliospores were single-celled, smooth, oily, oblong or clavate, yellowish transparent to orange-yellow and 52.09–86.35×16.17–34.83 µm in size [Figure 2(l), Figure 3(f) and Figure 4(b)]. Internally germinating basidia were four-celled, smooth, oblong or clavate, light yellow to orange-yellow and 52.96–103.38×13.79–26.68 µm in size [Figure 2(m) and Figure 4(c)]. Basidiospores were not observed in any isolate. Comparing with the references, the structure morphology of the plumeria rust isolates were corresponding to that of *C. plumeriae*. However, the size of basidia was somewhat larger than that of the previous publications (25.0–93.4×10.0–22.7 µm).

The identity of *C. plumeriae* was confirmed by ribosomal DNA (rDNA) sequences. The 28S and ITS rDNA sequences of the plumeria rust isolates were 99.81–100% and 98.75–99.39% similar to *C. plumeriae* sequences in the GenBank database (accession no. MG907225 and no. MH656772), respectively. Furthermore, all five isolates were in the same clade as *C. plumeriae* in both 28S and ITS rDNA phylogenetic trees (Figures 5 and 6). However, *C. plumeriae* was paraphyletic in the 28S rDNA tree, consisting of *C. ipomoeae* in the same clade (Figure 5) but monophyletic in the ITS rDNA tree, separating *C. ipomoeae* into the sister species with 98.99% identity (Figure 6). Some *Coleosporium* spp. could not be differentiated by the 28S rDNA tree either (Figure 5).

Table 2: The plumeria rust isolates collected from five provinces in Thailand

Isolate	Sampling Location	Month/Year	Temperature (°C)[x]	Relative Humidity (%)[y]	Rainfall (mm)[z]	Stage
TH-BK	Chatuchak, Bangkok	11/2016	29.1 28.7	74.2 80.1	121.7 293.8	Uredial, Telial, Basidial
TH-NP	Kamphaeng Saen, Nakhon Pathom	05/2017	29.7 30.1	82.0 74.7	176.3 13.6	Uredial
TH-RY	Muang, Rayong	09/2015	28.5 29.2	82.0 77.0	407.3 107.9	Uredial
TH-CB	Sattahip, Chonburi	10/2015	27.5 28.5	88.0 84.0	305.2 448.3	Uredial
TH-YL	Betong, Yala	01/2017	25.8 25.9	87.4 88.0	695.7 698.9	Uredial, Telial, Basidial

[x] The mean temperature of the sampling month (upper number) and one month before (lower number).
[y] The mean relative humidity of the sampling month (upper number) and one month before (lower number).
[z] Rainfall of the sampling month (upper number) and one month before (lower number).

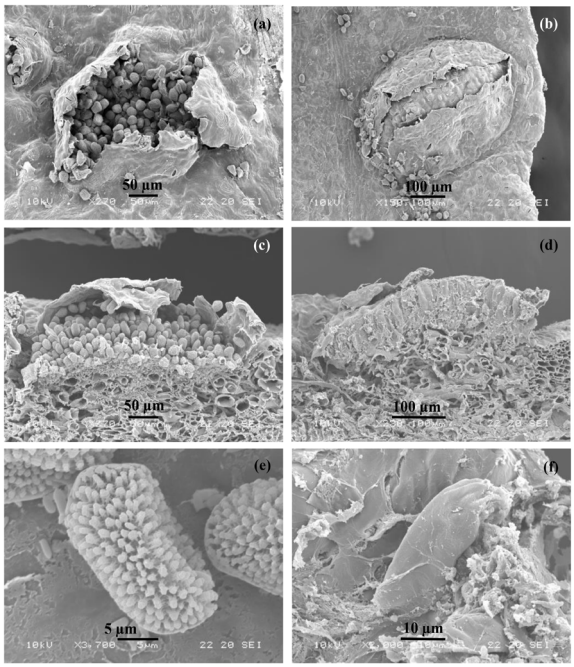

Figure 3: Structure morphology of the plumeria rust fungus under scanning electron microscope; the intact (a) uredium and (b) telium; the cross section of the sorus, (c) uredium and (d) telium; and the spore inside the sorus, (e) uredospore and (f) teliospore, repectively.

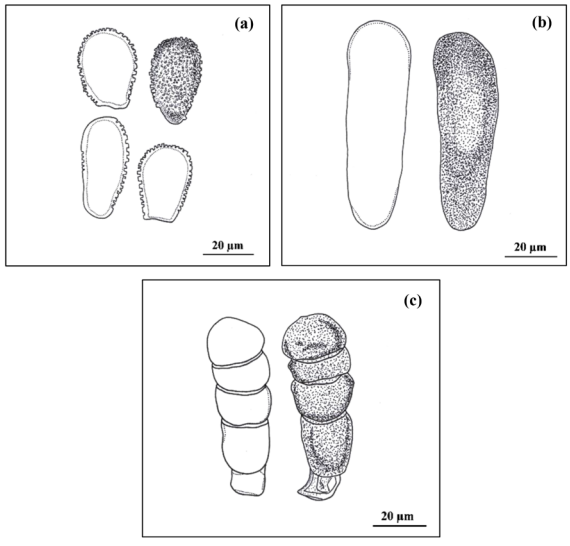

Figure 4: Camera lucida drawings of (a) uredospores, (b) teliospores and (c) basidia of the plumeria rust fungus.

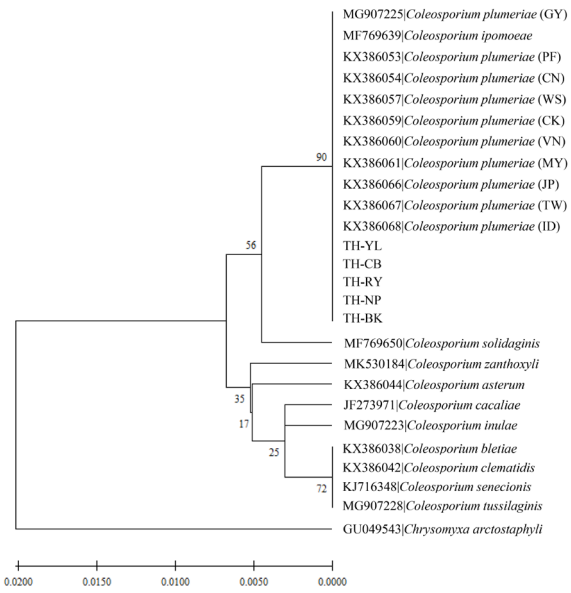

Figure 5: UPGMA phylogenetic tree inferred from 28S rDNA sequence data using MEGA-X. Numbers of the corresponding branches represent bootstrap values from 1000 replicates. The scale bar represents the evolutionary distance. See Table 1 for the identification of taxa.

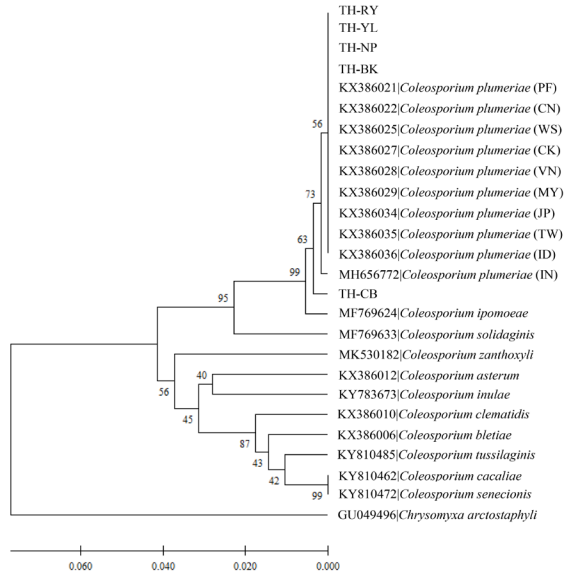

Figure 6: UPGMA phylogenetic tree inferred from ITS rDNA sequence data using MEGA-X. Numbers of the corresponding branches represent bootstrap values from 1000 replicates. The scale bar represents the evolutionary distance. See Table 1 for the identification of taxa.

3.2 *Characterization of genetic variation of the plumeria rust isolates*

All five isolates had the same uredospore color as yellow to orange-yellow. There were significant differences in the length, width, ratio (length:width) and wall of uredospores among the isolates. With respect to the shape of uredospores, the isolate TH-BK had the most for 5 shapes, which the angular shape was unique to this isolate. The isolate TH-RY had the least for 2 shapes, which were present in all isolates. The isolate TH-BK and TH-YL, undergoing sexual reproduction tended to have more uredospore shapes than the other three isolates (Table 3). These two isolates had the same teliospore color as yellowish transparent to orange-yellow and basidium color as light yellow to orange-yellow. The shape of teliospores and basidia of both isolates was similar as oblong or clavate. Almost all characteristics of teliospores and basidia, except the length of teliospores, were significantly different between them (Table 4). Generally, the variation of spore morphology was not related to the sampling locations.

Based on the UPGMA phylogenetic trees, all *C. plumeriae* from Thailand and foreign countries had 100% identity of 28S rDNA, which they were grouped in the same branch (Figure 5). Interestingly, almost all Thai isolates, except the isolate TH-CB shared identical ITS rDNA sequence with most foreign isolates that they were clustered in the main *C. plumeriae* clade. The isolate TH-CB from Thailand and *C. plumeriae* (IN) from India were differentiated from the main clade with 99.33% and 99.66% identity, respectively (Figure 6).

The results from the study of structure morphology and ITS rDNA suggested genetic variation of *C. plumeriae* in Thailand and over the world as well.

Table 3: Uredospore characteristics of the plumeria rust isolates collected from five provinces in Thailand

Isolate	Uredospore				
	Length (µm)[z]	Width (µm)[z]	Ratio (L:W)[z]	Wall (µm)[z]	Shape
TH-BK	30.56 b	22.09 b	1.39 cd	1.63 bc	Subglobose Broadly ellipsoid Ellipsoid Obovoid Angular
TH-NP	32.94 a	21.84 b	1.52 bc	1.65 abc	Broadly ellipsoid Ellipsoid Obovoid
TH-RY	32.54 a	18.79 c	1.75 a	1.72 ab	Ellipsoid Obovoid
TH-CB	30.51 b	18.75 c	1.64 ab	1.56 c	Broadly ellipsoid Ellipsoid Obovoid
TH-YL	32.49 a	23.91 a	1.36 d	1.73 a	Subglobose Broadly ellipsoid Ellipsoid Obovoid

[z] Data were analyzed by analysis of variance (ANOVA); means in columns followed by the same letter are not significantly different according to Tukey's test at $\alpha = 0.05$.

Table 4: Teliospore and basidium characteristics of the plumeria rust isolates collected from Bangkok and Yala provinces in Thailand

Isolate	Teliospore				Basidium			
	Length (µm)[y]	Width (µm)[y]	Ratio (L:W)[y]	Shape	Length (µm)[y]	Width (µm)[y]	Ratio (L:W)[y]	Shape
TH-BK	71.08	26.42	2.73	Oblong Clavate	67.58	22.57	3.02	Oblong Clavate
TH-YL	73.11	24.07	3.14	Oblong Clavate	78.16	20.45	3.96	Oblong Clavate
PValue	0.29	0.02*	0.01*	-	0.00*	0.01*	0.00*	-

[y] Data were analyzed by t-test and means are presented in the table.
[z] * indicates significant at $\alpha = 0.05$.

4 Discussion

Plumeria rust fungus in Thailand produced three stages in the life cycle, including uredial, telial and basidial stages like very few overseas reports [10], [14]. Normally, only the uredial stage is present worldwide. In this study, all five plumeria rust isolates reproduced asexually (uredial stage) but only two isolates also reproduced sexually (telial and basidial stages). Unfortunately, basidiospores were not found, which they were ever described only in Taiwan and Australia [10], [14]. Three isolates had no sexual reproduction at the sampling time because either the sexual mode may occur later in the season or the isolates may only reproduce asexually. There has been no report of the conditions favorable to the telial and basidial stages up to now and they could not be determined from this study either because the climates in the locations discovered these two stages were different.

Coleosporium plumeriae was identified as the causal agent of plumeria rust in Thailand based on structure morphology and rDNA sequences. *C. plumeriae* was paraphyletic that identical 28S rDNA sequence was shared with *C. ipomoeae*, but monophyletic in the ITS rDNA tree that separated both species by three base pairs. These results were consistent with McTaggart and Aime [32]. These two species have different morphology and host range. Combined methods, including host range, morphology and DNA sequence data were recommended for an accurate identification of *Coleosporium* species [32].

Genetic variation of *C. plumeriae* in Thailand, detected by structure morphology and ITS rDNA sequences may be the consequence of genetic recombination through sexual reproduction and mutation through asexual reproduction [18]. Such variation was not associated with the geographic origin of the isolates. Intraspecific variation of structure morphology and ITS rDNA was likewise observed in *Puccinia allii*, the rust pathogen of garlic, leek, chives and onion [33].

In the present study, structure morphology and ITS rDNA could be used as genetic markers for both interspecific and intraspecific taxonomy. On the other hand, 28S rDNA sequences could not resolve the relationship of some *Coleosporium* taxa. However, 28S region has been used for taxonomic study within and between genera of rust fungi in many publications. In addition, 28S rDNA could reveal intraspecific variation of *C. montanum* into two phylogenetic groups [32].

5 Conclusions

Coleosporium plumeriae, the causal agent of plumeria rust in Thailand could reproduce asexually (uredial stage) and sexually (telial and basidial stages). Genetic variation of the fungus, revealed by morphological and ribosomal DNA markers may result from genetic recombination and mutation. More important knowledge of plumeria rust, such as screening resistant genes and testing efficacy of fungicides to manage the disease in plumeria should be studied in further research with various *C. plumeriae* isolates.

Acknowledgments

The authors would like to thank National Science and Technology Development Agency (NSTDA), Thailand under A New Researcher Grants sponsored by Ministry of Science & Technology for financial support of this project. The authors also would like to thank Mr. Ronnakrit Chaithong and Ms. Patchara Kietrungwilaikul, the research assistants of this work.

References

[1] C. To-anun, N. Visarathanonth, J. Engkhaninun, and M. Kakishima, "First report of plumeria rust, caused by *Coleosporium plumeriae*, in Thailand," *The Natural History Journal of Chulalongkorn University*, vol. 4, no. 1, pp. 41–46, Apr. 2004.

[2] V. Prodkornburi, B. Manasmunkong, S. Suthiarom, W. Wongnikong, S. Sahaya, and C. Buamas. (2013, Aug.). Efficacy of some insecticides for controlling important pests of plumaria. Plant Protection Research and Development Office, Bangkok, Thailand [Online]. Available: http://www.doa.go.th/research/attachment.php?aid=404

[3] S. Ngorien. (2009, Mar.). Plumeria, a beautiful ornamental tree with high economic value that should not be neglected. Plant Protection Research and Development Office, Bangkok, Thailand [Online]. Available: http://doa.go.th/pibai/pibai/n12/v_2-mar/rai.html

[4] Department of Agricultural Extension. (2015, Dec.). Ornamental plants. Bureau of Agricultural

Commodities Promotion and Management, Bangkok, Thailand [Online]. Available: http://www.agriman.doae.go.th/home/news/year%202015/025_ornamental.pdf

[5] S. Nelson. (2009, Jan.). Plumeria Rust. University of Hawaii, Hawaii, USA [Online]. Available: http://scholarspace.manoa.hawaii.edu/handle/10125/12415

[6] M. Kakishima, J.-X. Ji, P. Zhao, Q. Wang, Y. Li, and E. H. C. McKenzie, "Geographic expansion of a rust fungus on *Plumeria* in Pacific and Asian countries," *New Zealand Journal of Botany*, vol. 55, pp. 178–186, Feb. 2017.

[7] J. C. Arthur, *Manual of the Rusts in United States and Canada*. New York: Hafner, 1962, pp. 36–39.

[8] J. A. Traquair and E. G. Kokko, "Spore morphology in *Coleosporium plumeriae*," *Canadian Journal of Botany*, vol. 58, pp. 2454–2458, Dec. 1980.

[9] D. Y. Ogata and D. E. Gardner, "First report of plumeria rust, caused by *Coleosporium plumeriae*, in Hawaii," *Plant Disease*, vol. 76, pp. 642, Jun. 1992.

[10] W. H. Chung, J. P. Abe, Y. Yamaoka, J. W. Haung, and M. Kakishima, "First report of plumeria rust disease caused by *Coleosporium plumeriae* in Taiwan," *Plant Pathology*, vol. 55, pp. 306, Apr. 2006.

[11] J. F. Hennen, M. B. Figueiredo, A. A. Jr. Carvalho, and P. G. Hennen. (2005). Catalogue of the species of plant rust fungi (Uredinales) of Brazil. Jardim Botânico do Rio de Janeiro, Brazil [Online]. Available: http://www.jbrj.gov.br/publica/uredinales/Brazil_Catalogue1drevisado.pdf

[12] J. R. Hernández, D. O. Eboh, and A. Y. Rossman, "New reports of rust fungi (Uredinales) from Nigeria," *Caldasia*, vol. 27, pp. 213–221, Dec. 2005.

[13] P. Baiswar, S. Chandra, and R. Kumar, "First report of rust caused by *Coleosporium plumeriae* on Plumeria alba in India," *Plant Pathology*, vol. 57, pp. 787, Aug. 2008.

[14] R. G. Shivas, D. R. Beasley, and A. R. McTaggart. (2014). Rust Fungi of Australia. Department of Agriculture and Fisheries, Australia [Online]. Available: http://collections.daff.qld.gov.au/web/key/rustfungi/Media/Html/coleosporiumplumeriae.html

[15] Q. Y. Yang, B. R. Lin, J. X. Zhang, H. F. Shen, X. M. Pu, Z. W. Wang, and D. Q. Zeng, "First report of rust of *Plumeria rubra* caused by *Coleosporium plumeriae* in Guangdong Province, China," *Plant Disease*, vol. 98, pp. 1154, Aug. 2014.

[16] G. E. Holcomb and M. C. Aime, "First report of *Plumeria* spp. rust caused by *Coleosporium plumeriae* in Louisiana and Malaysia and *Catheranthus roseus*, a new host of this rust," *Plant Disease*, vol. 94, pp. 272, Feb. 2010.

[17] J. Webster and R. W. S. Weber, *Introduction to Fungi*. New York: Cambridge University Press, 2007, pp. 34–37.

[18] G. N Agrios, *Plant Pathology*. Massachusetts: Elsevier Academic Press, 2005, pp. 128–134.

[19] J. H. Burnett, *Fungal Populations and Species*. New York: Oxford University Press, 2003, pp. 29–64.

[20] J. Thomas, "A serious rust disease affecting frangipani (*Plumeria rubra*) in Kerala State, India," *Journal of Pest Science*, vol. 1, pp. 22–26, Aug. 2014.

[21] Q. Wang, P. Q. Thu, and M. Kakishima, "First report of a rust disease of plumeria caused by *Coleosporium plumeriae* in Southern China and Vietnam," *New Disease Reports*, vol. 23, pp. 10, Feb. 2011.

[22] S.-Y. Park, S.-H. Jang, S.-O. Oh, J. A. Kim, and J.-S. Hur, "An easy, rapid, and cost-effective method for DNA extraction from various lichen taxa and specimens suitable for analysis of fungal and algal strains," *Mycobiology*, vol. 42, pp. 311–316, Dec. 2014.

[23] M. C. Aime, "Toward resolving family-level relationships in rust fungi (Uredinales)," *Mycoscience*, vol. 47, pp. 112–122, Jun. 2006.

[24] R. Vilgalys and M. Hester, "Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species," *Journal of Bacteriology*, vol. 172, pp. 4238–4246, Aug. 1990.

[25] M. Pfunder, S. Schürch, and B. A. Roy, "Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi s. lat.*) on *Euphorbia cyparissias*," *Mycological Research*, vol. 105, pp. 57–66, Jan. 2001.

[26] L. Beenken, S. Zoller, and R. Berndt, "Rust fungi on Annonaceae II: The genus *Dasyspora* Berk. &

M.A. Curtis," *Mycologia*, vol. 104, pp. 659–681, June. 2012.

[27] M. Johnson, I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis, and T. L. Madden, "NCBI BLAST: A better web interface," *Nucleic Acids Research*, vol. 36, pp. W5–W9, Apr. 2008.

[28] F. Madeira, Y. M. Park, J. Lee, N. Buso, T. Gur, N. Madhusoodanan, P. Basutkar, A. R. N. Tivey, S. C. Potter, R. D. Finn, and R. Lopez, "The EMBL-EBI search and sequence analysis tools APIs in 2019," *Nucleic Acids Research*, vol. 47, pp. W636–W641, Apr. 2019.

[29] S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, "MEGA X: Molecular evolutionary genetics analysis across computing platforms," *Molecular Biology and Evolution*, vol. 35, pp. 1547–1549, Jun. 2018.

[30] N. Feau, A. Vialle, M. Allaire, W. Maier, and R. C. Hamelin, "DNA barcoding in the rust genus Chrysomyxa and its implications for the phylogeny of the genus," *Mycologia*, vol. 103, pp. 1250–1266, Dec. 2011.

[31] M. C. Aime, C. D. Bell, and A. W. Wilson, "Deconstructing the evolutionary complexity between rust fungi (Pucciniales) and their plant hosts," *Studies in Mycology*, vol. 89, pp. 143–152, Mar. 2018.

[32] A. R. McTaggart and M. C. Aime, "The species of *Coleosporium* (Pucciniales) on Solidago in North America," *Fungal Biology*, vol. 122, pp. 800–809, Aug. 2018.

[33] Y. Anikster, L. J. Szabo, T. Eilam, J. Manisterski, S. T. Koike, and W. R. Bushnell, "Morphology, life cycle biology, and DNA sequence analysis of rust fungi on garlic and chives from California," *Phytopathology*, vol. 94, pp. 569–577, Jun. 2004.