

## Distribution, Diversity, and Specificity of a Parasitic Algal Genus *Cephaleuros* in Thailand

(Taburan, Kepelbagaian dan Kekhususan dalam Genus Alga parasit *Cephaleuros* di Thailand)

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### ABSTRACT

*Cephaleuros* has been recognized as a terrestrial parasitic green algal genus causing algal red rust diseases which have violently damaged economic crops worldwide. To obtain information of the algal distribution in Thailand and to understand the specificity of the algal parasite and their hosts, we reviewed the reports on this algal genus in Thailand and performed comparative analyses of the algal-host specificity. Results indicated that more than 10 species of *Cephaleuros* have been reported across Thailand. Their presence varies from regions to regions with the highest numbers of reports from the southern part of Thailand. The leaf area of infection also varied due to the different algal infecting mechanisms. We found that *C. annonae*, *C. drouetii*, *C. henningsii*, *C. lagerheimii*, *C. parasiticus*, *C. pilosa*, *C. piperis* and *C. solutus* appeared to be specific to plant family while *C. diffusus*, *C. expansa*, *C. karstenii*, *C. tumidae-setae* and *C. virescens* infected wider range of plant species. Among these, *C. virescens* had the highest number of reports as it was present in all the regions of Thailand where it infected various plant species belonging to more than 20 plant families. Analyses of *C. virescens* suggested the presence of putative sequence of gene encoding a virulence protein in this alga. The information on the distribution and algal-host specificity might help in understanding the spread of this red rust plant disease due to the algal genus *Cephaleuros* in Thai economic plants.

**Keywords:** Algal red rust disease; *Cephaleuros*; green algae; parasitic algae; specificity

### ABSTRAK

*Cephaleuros* telah diiktiraf sebagai genus alga hijau parasit daratan yang menyebabkan penyakit karat merah alga yang telah merosakkan tanaman ekonomi di seluruh dunia. Untuk mendapatkan maklumat tentang sebaran alga ini di Thailand dan untuk memahami kekhususan parasit alga dan hos, kami mengkaji laporan mengenai genus alga ini di Thailand dan melakukan analisis komparatif tentang kekhususan algal-hos. Hasil kajian menunjukkan bahawa lebih daripada 10 spesies *Cephaleuros* telah dilaporkan di seluruh Thailand. Kehadiran spesies tersebut berbeza di kawasan berbeza dengan bilangan tertinggi laporan dari bahagian selatan Thailand. Kawasan jangkitan daun juga berubah disebabkan oleh mekanisme jangkitan alga yang berbeza. Kami mendapati *C. annonae*, *C. drouetii*, *C. henningsii*, *C. lagerheimii*, *C. parasiticus*, *C. pilosa*, *C. piperis* dan *C. solutus* didapati khusus untuk famili tumbuhan tertentu manakala *C. diffusus*, *C. expansa*, *C. karstenii*, *C. tumidae-setae* dan *C. virescens* menjangkiti pelbagai spesies tumbuhan. Antaranya, *C. virescens* mempunyai bilangan laporan tertinggi yang terdapat di semua wilayah Thailand dan menjangkiti pelbagai spesies tumbuhan melebihi daripada 20 famili tumbuhan. Analisis *C. virescens* mencadangkan kehadiran jujukan gen putatif yang mengkodkan protein kevirulenan dalam alga ini. Maklumat tentang sebaran dan kekhususan alga-hos mungkin membantu dalam memahami penyebaran penyakit tumbuhan karat merah yang disebabkan oleh genus *Cephaleuros* dalam tumbuhan ekonomi Thai.

**Kata kunci:** Alga hijau; alga parasit; *Cephaleuros*; kekhususan; penyakit karat merah alga

### INTRODUCTION

*Cephaleuros* is a genus of terrestrial filamentous ulvophyceean algae in the family Trentepohliaceae, the only family of order Trentepohliales. This algal genus is aerophilic and terrestrial. It has been observed as phycobiont lichenised by fungus (Suto & Ohtani 2009). However, more often, these algae have been observed as parasitic algae due to their ability to invade subcuticular, subepidermal, and intramatrix regions of plant leaves.

The disease caused by *Cephaleuros* can be easily spotted by presence of almost circular algal colony in red

or orangish color caused by accumulation of carotenoid pigments in the algal cells giving this symptom the names 'algal spot' and 'red rust'. The algal colony is made of filamentous body with only one layer of cells at the edge of the colony and multi-layer of cells in the middle. Symptoms of the disease are rarely considered severe on host plants. These symptoms include loss of water, minerals, and nutrients of host plants (Wolf 1930) and loss of photosynthetic area due to necrosis of the plant green tissues in the epidermal and palisade cells (Lopez-Bautista et al. 2006; Muthukumar et al. 2014).

Though these symptoms are normally considered minor, the effects can overall affect the growth of plants. More importantly, they can dramatically affect the economic value of the plant hosts if the disease is spotted on the high economical value fruits or plant leaves.

*Cephaleuros* has been reported worldwide in economically cultivated plantation crops. It has been reported in Africa, Asia, Australia, and North America in a very wide host range. These hosts included avocado (*Persea americana*), breadfruit (*Artocarpus altilis*), cacao (*Theobroma cacao*), coconut (*Cocos nucifera*), coffee (*Coffea arabica*), guava (*Psidium guajava*), kava (*Piper methysticum*), magnolia (*Magnolia grandiflora*), mango (*Mangifera indica*), oil palm (*Elaeis guineensis*), pepper (*Piper nigrum*), tea (*Camellia sinensis*), vanilla (*Vanilla planifolia*) and hundreds of other plant species (Jose & Chowdary 1980; Lopez-Bautista et al. 2002; Muthukumar et al. 2014; Nelson 2008; Sunpapao 2016; Sunpapao & Pitaloka 2015).

The susceptibility of the plant host has not yet been well evaluated as it varies greatly and depends on the *Cephaleuros* species, host species and its environmental condition (Nelson 2008). However, factors involved in algal dispersal have been suggested. It has been suggested that the distribution range of this alga is limited to tropical and subtropical regions (Chapman & Good 1983). The alga could also colonise various types of substrate surfaces such as rocks, tree barks, leaves, stems, fruits or even some artificial substrates. Most of the time, the dispersal of this disease could not be prevented at an early stage as these algae could colonise extensive host range with the presence of a few minuscule thalli which often caused a minute or unnoticed injury on plant surface (Sunpapao 2016; Thompson & Wujek 1997). In addition, they were sometimes misidentified for some other pathogenic diseases due to the similarity in morphology causing a complication in plant treatments (Marlatt & Alfieri 1981).

Due to these reasons, defining the host range and specificity between *Cephaleuros* and plant host is crucial and is a fundamental requirement in preventing the spread of this disease. To obtain this information, we revisited the diversity, distribution and specificity of *Cephaleuros* and its host in Thailand. Our results suggested that *Cephaleuros* species were found more frequently in the southern part of the country. *C. annonae*, *C. drouetii*, *C. henningsii*, *C. lagerheimii*, *C. parasiticus*, *C. pilosa*, *C. piperis* and *C. solutus* appeared to be specific to a few plant families while *C. diffusus*, *C. expansa*, *C. karstenii*, *C. tumidae-setae* and *C. virescens* infected a wider range of plant families. Among these, *C. virescens* had the highest number of reports and was present in all the regions of Thailand, where it infected various plant species belonging to more than 20 plant families. Our transcriptome analyses of *C. virescens* suggested that *C. virescens* had a gene homologous to gene encoding polyketide synthase/peptide synthetase, a known avirulence protein in parasitic fungi.

## MATERIALS AND METHODS

### DATA COLLECTION

We obtained records of presence, diversity, distribution and pathogenicity of *Cephaleuros* species in Thailand from databases. These included Global Biodiversity Information Facility (GBIF; [www.gbif.org](http://www.gbif.org)), the Distribution Maps of Plant Diseases (DMPD; [www.cabi.org/dmpd](http://www.cabi.org/dmpd)), the Distribution Maps of Plant Pest (DMPP; [www.cabi.org/dmpp](http://www.cabi.org/dmpp)), the Algaebase (Guiry & Guiry 2019; <http://www.algaebase.org>), other online documents, related books, and reports from Chulalongkorn University Library ([library.car.chula.ac.th](http://library.car.chula.ac.th)), Thammasat University Library ([library.tu.ac.th](http://library.tu.ac.th)), Kasetsart University Library ([www.lib.ku.ac.th/web](http://www.lib.ku.ac.th/web)) and Mahidol University Library ([www.li.mahidol.ac.th](http://www.li.mahidol.ac.th)). We reviewed all selected documents and retained only the non-redundant information. Then, we performed comparative analyses to find the distribution range and the algal-host specificity across *Cephaleuros* species and their hosts.

### TRANSCRIPTOME ANALYSES

The algal transcriptomic data of *C. virescens* were obtained from the 1,000 Plants (1KP) project (Matasci et al. 2014). We used Basic Local Alignment Search Tool (BLAST) to obtain the putative *Cephaleuros* sequence that might be coding for protein involved in parasitism. We obtained queries for avirulence (*avr*) genes from the Pathogen Receptor Genes database version 3.0 (Osuna-Cruz et al. 2017; [www.prgdb.org](http://www.prgdb.org); accessed on March 4, 2019) and queries for RXLR effectors from plant pathogens (GenBank; accessed on March 6, 2019). Then, we used a localised BLAST (Camacho et al. 2009) to search for the queries in the database constructed from the obtained *C. virescens* transcriptome using e-value equaled to 1e-10. Then we used the returned *C. virescens* transcripts to perform confirmational blastx to search against the NCBI non-redundant protein database (accessed on March 6, 2019).

## RESULTS AND DISCUSSION

### *Cephaleuros* DIVERSITY, DISTRIBUTION, AND PLANT HOSTS

In this study, we reviewed the diversity, distribution and hosts of *Cephaleuros* species reported in Thailand. The results showed that 14 *Cephaleuros* species have been reported across the country (Figure 1). *C. virescens*, *C. karstenii*, *C. diffusus*, *C. parasiticus*, *C. solutus* and *Cephaleuros* sp. were reported from the North (N), *C. virescens*, *C. karstenii*, and *C. parasiticus* were reported from the central basin (C), *C. virescens* and *C. karstenii* were reported from the West (W), *C. virescens*, *C. karstenii*, *C. solutus* and *Cephaleuros* sp. were reported from the East (E), *C. virescens*, *C. karstenii* and *C. solutus* were reported from the Northeast (NE), and all 14 *Cephaleuros* species present in Thailand were reported from the South

(S). Among these, *C. virescens* and *C. karstenii* were reported from all regions of the country with the presence of *C. virescens* in most of the locales where *Cephaleuros* species were reported. *C. tumidae-setae*, *C. expansa*, *C. annonae*, *C. druetii*, *C. henningsii*, *C. largerheimii*, *C. pilosa* and *C. piperis* were reported only from the southern part of the country. *C. virescens*, *C. karstenii*, *C. diffusus*, *C. parasiticus*, *C. solutus* and *Cephaleuros* sp. were reported from a few different parts of the country.

A total of 129 plant species in Thailand have been reported as the hosts of *Cephaleuros*. These plant hosts belong to 45 different plant families from pteridophyte, gymnosperm and angiosperm in all three major clades namely magnoliids, monocots and eudicots. When the frequencies of the presence of *Cephaleuros* across all reported plant hosts in Thailand were compared, we found that the frequencies of the presence of *Cephaleuros* in its host ranged from 1 to 294 records. The 12 hosts with the highest numbers of records included *Durio zibethinus* (14 records), *Elaeis guineensis* (14 records), *Psidium guajava* (12 records), *Citrus maxima* (11 records), *Citrus aurantifolia* (9 records), *Syzygium malaccense* (6 records), *Nephelium lappaceum* (5 records), *Lansium domesticum* (4 records), *Garcinia mangostana* (4 records), *Hevea brasiliensis* (4 records), *Acacia auriculiformis* (4 records) and *Annona squamosa* (4 records) (Table 1).

#### *Cephaleuros*-PLANT HOST SPECIFICITY VS SUSCEPTIBILITY OF PLANT HOSTS

*Cephaleuros* appeared as parasite of a wide range of plant hosts, ranging from seedless plants to angiosperms (Tables 1 and 2). In this study, we found that *C. virescens* infected 129 plant species belonging to 44 plant families, *C. karstenii* infected six plant species belonging to five plant families, *C. tumidae-setae* infected four plant species belonging to three plant families, *C. expansa* infected four plant species belonging to four plant families, *C. parasiticus* infected four plant species belonging to three plant families, *C. diffusus* infected three plant species belonging to three plant families. *C. annonae*, *C. druetii*, *C. henningsii*, *C. largerheimii*, *C. pilosa*, *C. piperis* and *C. solutus* infected one plant species. *Cephaleuros* sp. infected six plant species belonging to five plant families. Among these, *C. virescens*, *C. diffusus* and unidentifiable *Cephaleuros* species infected gymnosperm (*Gnetum gnemon*) and only *C. virescens* infected a pteridophyte (*Platyserium holtumii*) (Table 1).

*Cephaleuros* plant hosts in Thailand also displayed a different degree of susceptibility where different plant species were infected by different number of *Cephaleuros* species. We found that, at the level of plant species, the four hosts that were infected by the highest numbers of *Cephaleuros* species included *Psidium guajava* (five *Cephaleuros* species), *Gnetum gnemon* (three *Cephaleuros* species), *Citrus aurantifolia* (three *Cephaleuros* species), *Lansium domesticum* (three *Cephaleuros* species), and *Durio zibethinus* (three *Cephaleuros* species). In addition,

at the level of plant family, results showed that the eight most susceptible plant families were Myrtaceae which was infected by seven *Cephaleuros* species namely *C. virescens*, *C. karstenii*, *C. tumidae-setae*, *C. expansa*, *C. diffusus*, *C. parasiticus* and *Cephaleuros* sp., Fabaceae which was infected by four *Cephaleuros* species that were *C. virescens*, *C. tumidae-setae*, *C. diffusus* and *C. druetii*, Rutaceae which was infected by four *Cephaleuros* species namely *C. virescens*, *C. karstenii*, *C. expansa* and *Cephaleuros* sp., Malvaceae which was infected by four *Cephaleuros* species specifically *C. virescens*, *C. karstenii*, *C. expansa* and *C. solutus*, Gnetaceae which was infected by three *Cephaleuros* species namely *C. virescens*, *C. diffusus* and *Cephaleuros* sp., Piperaceae which was infected by three *Cephaleuros* species namely *C. virescens*, *C. expansa*, and *C. piperis*, Annonaceae which was infected by three *Cephaleuros* species namely *C. virescens*, *C. annonae* and *C. parasiticus*, and Meliaceae which was infected by three *Cephaleuros* species namely *C. virescens*, *C. parasiticus* and *Cephaleuros* sp. In contrast, many plant families were only infected by one species of *Cephaleuros*. These included Polypodiaceae, Gnetaceae, Piperaceae, Magnoliaceae, Annonaceae, Lauraceae, Araceae, Arecaceae, Marantaceae, Dilleniaceae, Fabaceae, Rhamnaceae, Elaeocarpaceae, Hypericaceae, Ochnaceae, Malpighiaceae, Chrysobalanaceae, Salicaceae, Euphorbiaceae, Phyllanthaceae, Anacardiaceae, Dipterocarpaceae, Loranthaceae, Nyctaginaceae, Sapotaceae, Ebenaceae, Theaceae, Rubiaceae, Gentianaceae, Apocynaceae, Boraginaceae, Acanthaceae, Bignoniaceae, Verbenaceae and Araliaceae (Table 1).

When the number of plant species in each family that was infected by the alga was observed, results showed that nine plant families had comparatively higher number of species infected by *Cephaleuros* namely Myrtaceae (28 plant species), Rutaceae (24 plant species), Fabaceae (21 plant species), Malvaceae (21 plant species), Arecaceae (16 plant species), Annonaceae (14 plant species), Moraceae (13 plant species), Anacardiaceae (10 plant species) and Meliaceae (10 plant species) (Table 2).

#### TRANSCRIPTOME ANALYSES

In this study, we searched for putative *Cephaleuros* gene sequence that might be coding for protein involved in parasitism. The results suggested that a transcript of *C. virescens* (scaffold-YDCQ-2005743-Cephaleuros\_virescens) might be homologous to a known avirulence gene in a parasitic sordariomycetes, *Pyricularia grisea* (e-value = 4e-11; GenBank accession number CAG28798.1). However, we did not find sequences encoding RXLR effectors in the transcriptome.

#### DISCUSSION

Our results showed that 13 *Cephaleuros* species and one unidentifiable *Cephaleuros* species have been reported in Thailand. This accounted for almost 50 percent of

TABLE 1. Frequencies of *Cephaluros* species present in plant host. The colors in the heatmap represent frequencies of records of each *Cephaluros* species present in each plant host

				Algal parasites														References				
		Host order	Host family	Host species	<i>C. virescens</i>	<i>C. karstenii</i>	<i>C. numidiae-vetae</i>	<i>C. expansa</i>	<i>C. difflusus</i>	<i>C. annonae</i>	<i>C. druetii</i>	<i>C. hemingsii</i>	<i>C. lagerheimii</i>	<i>C. parasiticus</i>	<i>C. pilosa</i>	<i>C. piperis</i>	<i>C. solitans</i>	<i>Cephaluros</i> sp.				
Pteridophyte		Polypodiales	Polypodiaceae	<i>Platyterium holtunii</i>															Bunjongsiri & Sunpapao (2018); Ratanapaiboonkit & Brocklehurst (2016); Wisantanon (2010)			
Gymnosperm		Gnetales	Gnetaceae	<i>Gnetum gnemon</i>																		
Angiosperm	Magnoliids	Piperales	Piperaceae	<i>Piper betel</i>															Sunpapao, Pitaloka & Arikrit (2015); Sunpapao & Pitaloka (2017); Sunpapao, Pitaloka & Petcharat (2015); Wisantanon (2010); Wonglom et al. (2018)			
				<i>Piper longum</i>																		
				<i>Piper nigrum</i>																		
				<i>Piper retrofractum</i>																		
		Magnoliales	Magnoliaceae	<i>Magnolia champaca</i>																	Sunpapao, Pitaloka & Arikrit (2015); Sunpapao, Pitaloka & Petcharat (2015); Thithuan & Sunpapao (2016); Wisantanon (2010)	
				<i>Magnolia sirindhorniae</i>																		
				<i>Michelia alba</i>																		
		Magnoliales	Annonaceae	<i>Annona muricata</i>																	Bunjongsiri & Sunpapao (2018); Sunpapao & Pitaloka (2017); Sunpapao, Pitaloka & Petcharat (2015); Ratanapaiboonkit & Brocklehurst (2016); Wisantanon (2010); Wonglom et al. (2018)	
				<i>Annona squamosa</i>																		
				<i>Artabotrys siamensis</i>																		
	<i>Cananga odorata</i>																					
	<i>Melodorum siamensis</i>																					
	Laurales	Lauraceae	<i>Polyathia longifolia</i>																			
			<i>Cinnamomum iners</i>																		Wisantanon (2010); Wonglom et al. (2018)	
	Monocot	Alismatales	Araceae	<i>Philodendron</i> sp.																		
		Arecales	Arecaceae	<i>Cocos nucifera</i>																	Athipanyakhom et al. (2011); Pornsuriya et al. (2013); Wisantanon (2010); Wonglom et al. (2018)	
	<i>Elaeis guineensis</i>																					
	Angiosperm	Zingiberales	Marantaceae	<i>Donax grandis</i>																		
		Dilleniales	Dilleniaceae	<i>Dillenia indica</i>																Wisantanon (2010)		
		Fabales	Fabaceae	<i>Acacia aulacocarpa</i>																	Pongpanich (1997); Sunpapao (2016); Sunpapao & Pitaloka (2015); Sunpapao, Pitaloka & Petcharat (2015); Wisantanon (2010)	
				<i>Acacia auriculiformis</i>																		
				<i>Acacia crassicarpa</i>																		
				<i>Bauhinia aureifolia</i>																		
				<i>Bauhinia purpurea</i>																		
				<i>Butea monosperma</i>																		
				<i>Calliandra haematocephala</i>																		
				<i>Dialium cochinchinense</i>																		
				<i>Erythrina fusa</i>																		
<i>Pterocarpus macrocarpus</i>																						
<i>Saraca declinata</i>																						
<i>Saraca indica</i>																						
<i>Senna occidentalis</i>																						
<i>Sindora siamensis</i>																						
<i>Tamarindus indica</i>																						
Rosales		Rhamnaceae	<i>Ziziphus jujuba</i>																	Athipanyakhom et al. (2010)		
		Moraceae	<i>Artocarpus altilis</i>																		Sunpapao, Pitaloka & Petcharat (2015); Wisantanon (2010); Wonglom et al. (2018)	
<i>Artocarpus heterophyllus</i>																						
<i>Ficus benjamina</i>																						
<i>Ficus carica</i>																						
<i>Ficus maciellandii</i>																						
<i>Ficus microcarpa</i>																						
<i>Ficus religiosa</i>																						
<i>Streblus asper</i>																						
Oxalidales		Elaeocarpaceae	<i>Elaeocarpus hygrophilus</i>																Wisantanon (2010)			
Malpighiales		Rhizophoraceae	<i>Rhizophora apiculata</i>																	Wonglom et al. (2018)		
	Clusiaceae	<i>Calophyllum inophyllum</i>																	Bunjongsiri, Pitaloka & Sunpapao (2016); Bunjongsiri & Sunpapao (2018); Sunpapao (2016); Wisantanon (2010); Wonglom et al. (2018)			
		<i>Garcinia mangostana</i>																				
		<i>Garcinia schomburgkiana</i>																				
		<i>Mammea siamensis</i>																				
	Hypericaceae	<i>Cratogeomys formosum</i>																	Wisantanon (2010)			
	Ochnaceae	<i>Ochna integerrima</i>																		Wisantanon (2010)		
		<i>Ochna kirkii</i>																				
	Malpighiaceae	<i>Malpighia glabra</i>																		Wisantanon (2010)		
	Chrysobalanaceae	<i>Parinari anamensis</i>																		Wonglom et al. (2018)		
	Salicaceae	<i>Casearia grewiaefolia</i>																		Wonglom et al. (2018)		
		<i>Antidesma thwaitesianum</i>																		Pitaloka et al. (2015); Sunpapao, Pitaloka & Petcharat (2015); Wisantanon (2010); Wonglom et al. (2018)		
	Euphorbiaceae	<i>Euphoria longana</i>																				
		<i>Hevea brasiliensis</i>																				
Phyllanthaceae	<i>Jatropha integerrima</i>																		Sunpapao, Pitaloka & Arikrit (2015); Sunpapao, Pitaloka & Petcharat (2015); Wisantanon (2010); Wonglom et al. (2018)			
	<i>Baccaurea ramiflora</i>																					
	<i>Glochidion wallichianum</i>																					
				<i>Sauropus androgynus</i>															Wisantanon (2010); Wonglom et al. (2018)			



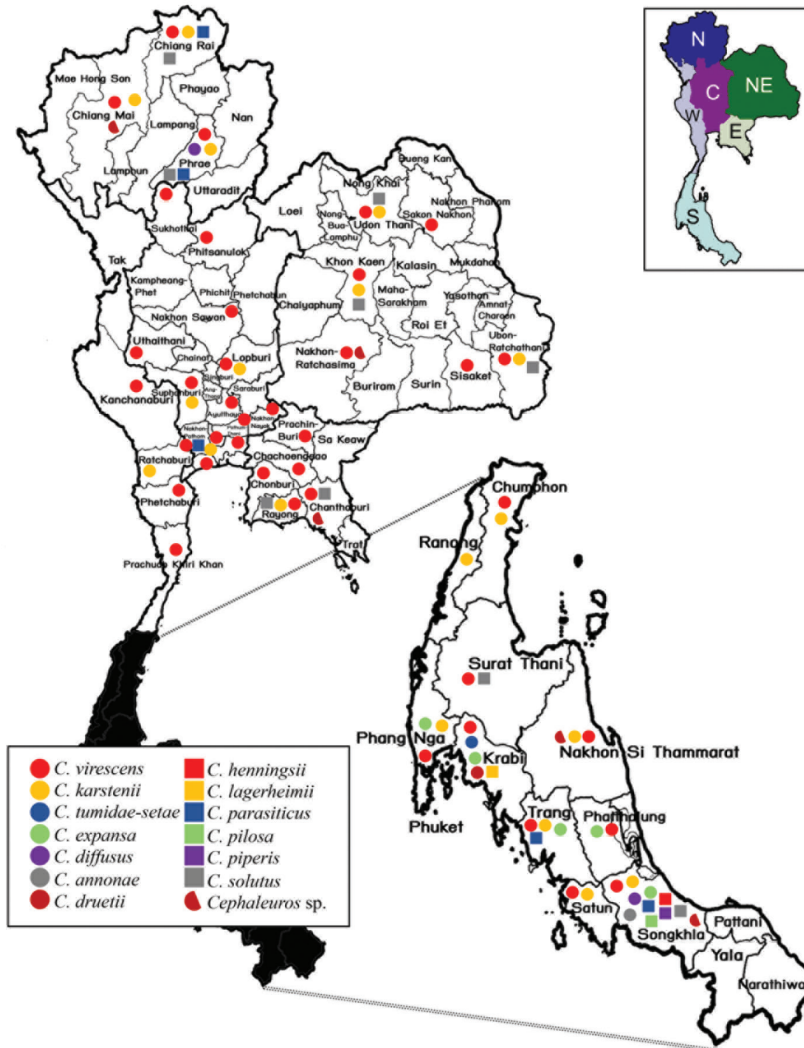


FIGURE 1. Distribution of *Cephaleuros* species reported from Thailand from 1997 to 2018. Color dots in the map represent corresponding *Cephaleuros* species

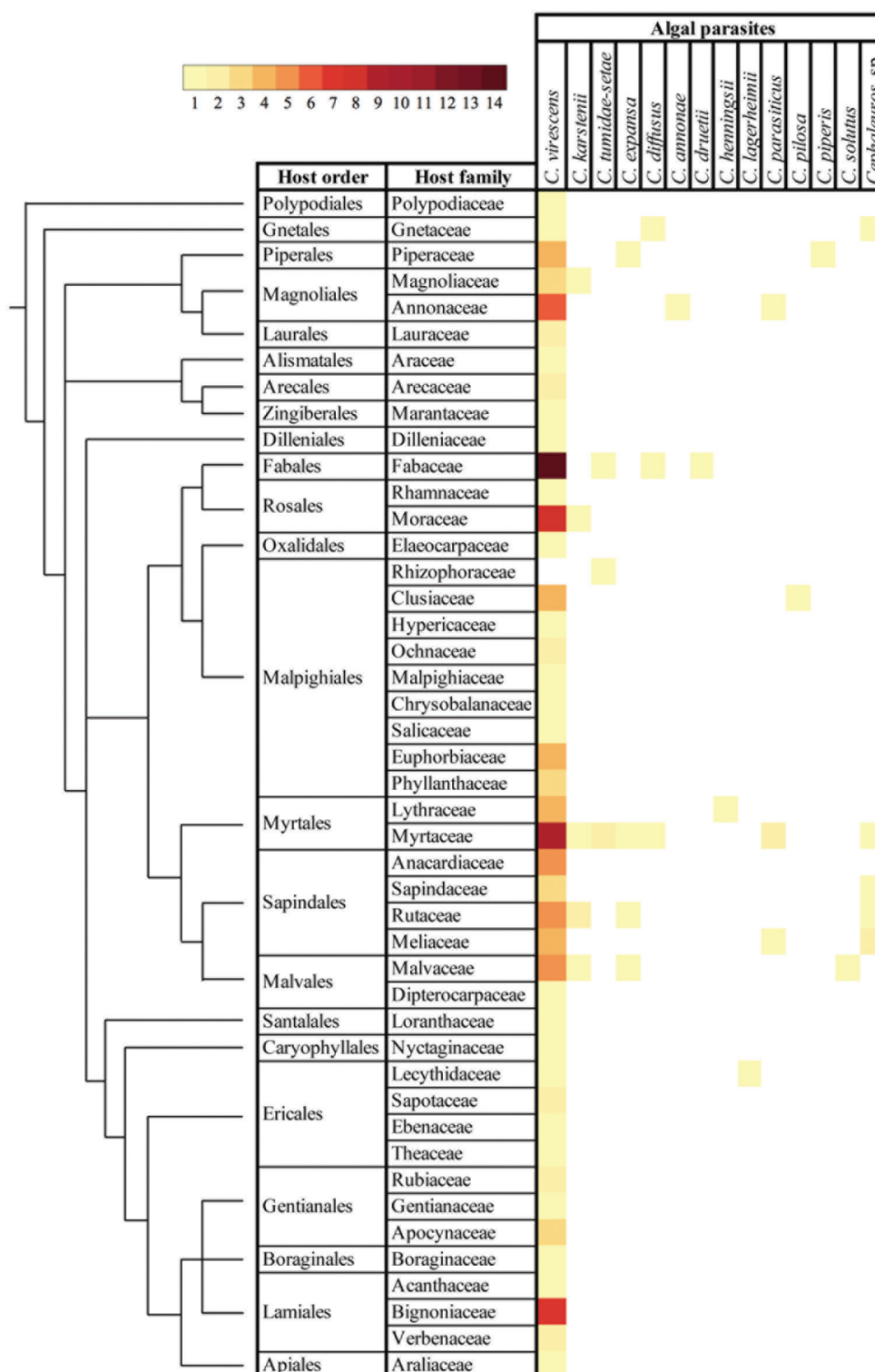
worldwide *Cephaleuros* diversity as 29 *Cephaleuros* species have been reported in AlgaeBase (<http://www.algaebase.org>; accessed on March 7, 2019). These *Cephaleuros* species were present in various locales across Thailand. That was congruent with other reports that suggested this algal genus distributed across temperate and tropical regions (about 33°N and 33°S of the equator), where the temperature and humidity were suitable for the algal growth and reproduction (Chapman & Good 1983; Nelson 2008; Thompson & Wujek 1992).

The *Cephaleuros* species infected as many as 129 plant species belonging to 44 plant families. Different *Cephaleuros* species exhibited different degree of dispersal and frequencies of infection in plant hosts. While a few *Cephaleuros* species were reported only once from a single species of plant host, such as *C. annonae*, *C. henningsii* and *C. pipiperis*, *C. virescens* exhibited the highest distribution range and the highest diversity of plant hosts as it was present in 95 plant species in all the regions across the country. This is congruent with the reports from other

reports from other countries, where they also found a big range of distribution and high diversity of plant hosts of *C. virescens* (Brooks 2004; Chapman & Good 1983; Holcomb 1986; Joubert & Rijkenberg 1971; Marlatt & Alfieri 1981; Thompson & Wujek 1997). For example, this algal species infected at least 287 plant species and cultivars on the U.S. Gulf Coast (Nelson 2007).

One reason why *C. virescens* displayed a big range of distribution and could infect higher number of hosts might due its infecting area on host leaves and the position of its reproductive structure. For example, the thalli of *C. virescens* develop just under the cuticle and its zoosporangia developed on the upper surface of hosts leaves, which gives the algal gametes more ability to disperse when compared to that of another *Cephaleuros* species (*C. parasiticus*) where the zoosporangia developed on the lower surface of the plant leaves (Suto et al. 2014). Local environmental conditions at the time of survey might also play roles in *Cephaleuros* distribution as the algae display higher zoospore dispersal in rainy season and

TABLE 2. Numbers of plant species belonging to plant families that were found as hosts of *Cephaleuros*. The colors in the heatmap represent the numbers of plant species belonging to each plant family



regions with high humidity. For example, it was evident that *Cephaleuros* had significant higher rate of infection during the rainy season (Muthukumar et al. 2014).

Other biological factors might also affect the ability of *C. virescens* infection. For example, presence of other pests in host plants might increase the potential of *C. virescens*

to infect the host as it was suggested that the presence of a mite, *Aceria litchi*, increased the presence of *C. virescens* on litchi leaves in the northern part of Thailand (Schulte et al. 2007).

The mechanisms of how *Cephaleuros* species infect plant hosts have not yet been elucidated. However, it was

evident that the mutation of *Cephaleuros* genetic materials was rapid as *Cephaleuros* species display high variation in chromosome number. In 2011, Suto and Ohtani observed different chromosome numbers from five different *Cephaleuros* species namely *C. aucubae* (n=22), *C. biolophus* (n=34), *C. japonicus* (n=18), *C. microcellularis* (n=12) and *C. virescens* (n=24). The presence of this variation in chromosome number suggested the presence of plasticity of the algal DNA, which is known to be an adaptive character of parasites as they need to have faster parasite-host coevolution to keep up with the plant immune systems (Raffaele & Kamoun 2012; Tyler et al. 2006).

In this study, we investigated *C. virescens* transcriptomes to search for genes that might be involved in its pathogenesis. We found one putative *avr* gene in the transcriptome of *C. virescens*, however, with low similarity value. We did not find any sequence encoding RXLR effectors in *C. virescens*. This is not surprising as it is known that the mutation rate of these effectors is rapid and is host-specific in order to keep up with the host immune systems (Hadwiger 2008; Jiang et al. 2008; Morgan & Kamoun 2007). Therefore, we believe that the availability of genome sequences of *C. virescens* and its plant hosts would certainly improve the understanding of the mechanisms of *C. virescens* infection.

While *C. virescens* was cosmopolitan, in contrast, some *Cephaleuros* species such as *C. annonae*, *C. druetii*, *C. henningii*, *C. largerheimii*, *C. pilosa*, *C. piperis* and *C. solutus* appeared to infect only single plant host species representing a high degree of algal-host specificity. This algal-host specificity suggested the presence of coevolution in these interactions. Comparative analyses of algal and host genomes might help us in understanding this type of algal-host coevolution.

From the reciprocal direction, we observed that different plant hosts exhibited different degree of susceptibility. Some plant species were only infected by a single *Cephaleuros* species while some plant species were infected by more than one *Cephaleuros* species. In addition, some plant families had a higher number of their plant species infected by *Cephaleuros*. This suggested that some plant families were more likely to be infected by *Cephaleuros*. However, we cannot rule out the fact that all the *Cephaleuros* surveys were from economic crops. Therefore, the higher species number of infected plants might be from the fact that some plant families had higher number of its family as crop plants. Furthermore, we cannot deny that the results from this study were heavily influenced by the cultivation preference of Thai people.

#### CONCLUSION

In this study, we surveyed the distribution, diversity and host specificity of *Cephaleuros* in Thailand. We additionally analysed the transcriptome of *C. virescens* in order to find its putative genes encoding proteins known to be involved in plant pathogenesis. Our results

showed that different *Cephaleuros* spp. inhabited different locales and exhibited different degree of host specificity. We found one putative gene sequence that might be involved in *C. virescens* pathogenesis, however, with a low similarity value. We believe that this study has provided preliminary information on the distribution, diversity and host specificity of *Cephaleuros* in Thailand. In addition, the results of the algal and host specificity provide the preliminary results as a gateway for the study of compatibility between *Cephaleuros* species and their hosts at the genomic and transcriptomic levels.

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