

Cephaleuros virescens, the cause of an algal leaf spot on Para rubber in Thailand

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Abstract In this study *Cephaleuros virescens* was found to be the causal organism of a leaf spot disease on Para rubber (*Hevea brasiliensis*). The algae caused circular orange to dark brown lesions on both leaf surfaces. The morphology of the algae (thallus, filamentous cells, sporangiophore, sporangia, gametangia and zoospore) were examined and found to consistently match the species *Cephaleuros virescens*.

Keywords Algae · *Cephaleuros* · *Hevea brasiliensis* · Leaf spot · Morphology

Para rubber (*Hevea brasiliensis*) is a tree belonging to the family Euphorbiaceae. It is commonly cultivated in tropical and sub-tropical areas and is an economically important source of natural latex rubber. Para rubber trees need long periods of high rainfall to produce a high yield of latex and are therefore cultivated in southern Thailand where suitable weather occurs. Like other plants, Para rubber is attacked by several pathogens and one of the most frequently found is a leaf spot caused by a phytoparasitic algae.

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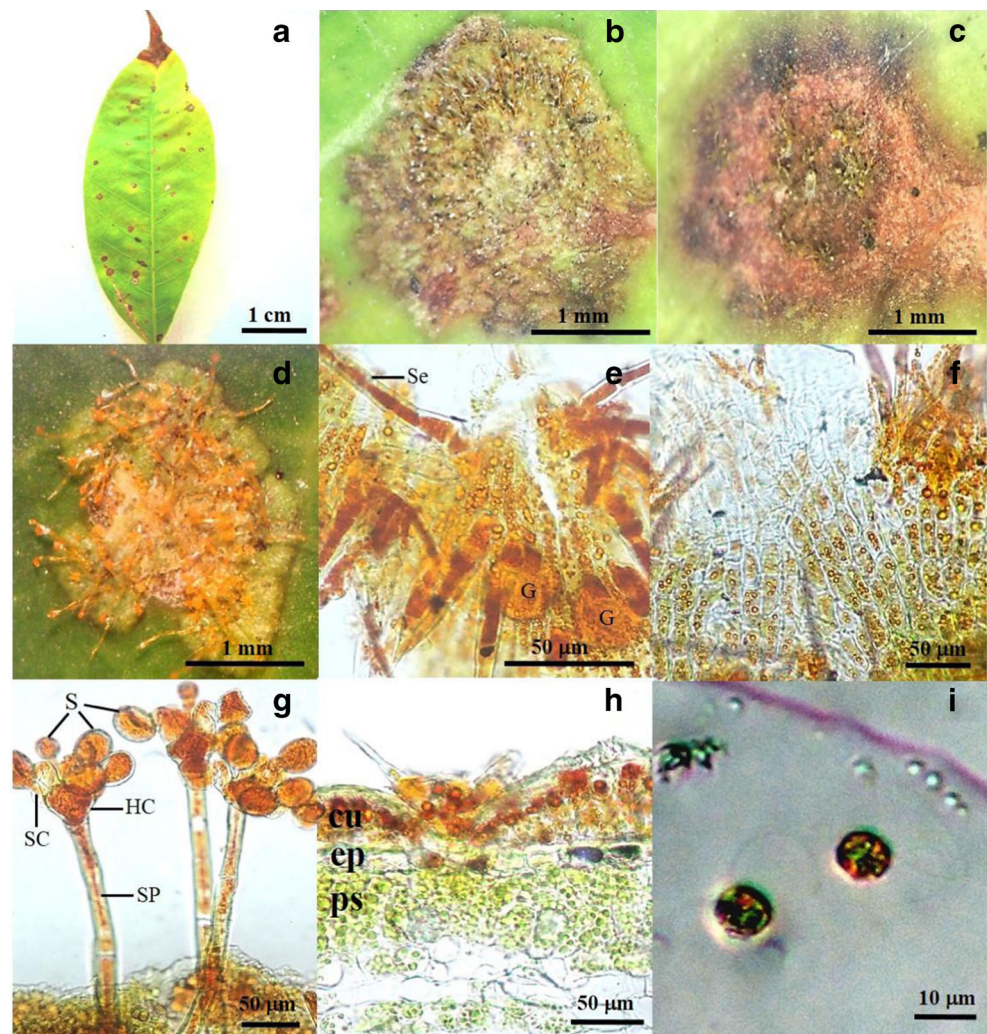
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Green algae in the genus *Cephaleuros* are known to be parasitic on several woody plants. Signs of the disease are often found on the leaf surface in the form of burnt-orange to brown spots (Nelson 2008). Leaf tissues are colonized beneath the epidermis by algal filaments, but host cells are not penetrated (Chapman and Henk 1985). Stems and fruits may also be infected. *Cephaleuros* spp. produce a thallus on the leaf surface, with filaments bearing sporangiophores and sporangia (Thompson and Wujek 1997). *Cephaleuros* spp. predominantly occur in tropical and subtropical regions. Recently, *Cephaleuros solutus* was reported causing a leaf spot on durian in Thailand (Pitaloka et al. 2014). The record of *Cephaleuros solutus* on durian was the first record of *Cephaleuros* sp. causing disease in Thailand. The purpose of our study was to identify and characterize *Cephaleuros* species present on Para rubber in Thailand.

Fifteen specimens of algal leaf spot from Para rubber leaves were collected from Songkhla, Trang and Phattalung provinces, southern Thailand. Algal thalii were selected from fresh leaves and initially observed under a stereomicroscope. The symptoms on leaf tissues were then photographed. Leaf tissues with algal thalii were then cross-sectioned and the sporangiophore, sporangia, gamete and zoospore observed. Morphological characteristics of the *Cephaleuros* spp. on Para rubber were compared to descriptions described in the monograph by Thompson and Wujek (1997).

Based on the leaf symptom and morphological characteristics of the algae as described in Fig. 1, and comparison in Table 1, 15 alga samples were identified as

Fig. 1 *a*) Leaf spot on *Hevea brasiliensis* leaf, *b*) lesion caused by *Cephaleuros* on upper leaf, *c*) lesion on lower leaf surface, *d*) young thallus on upper leaf surface with sporangiophores, *e*) thallus with gametangia (G) and setae (Se), *f*) pseudoparenchymatous thalli of *C. virescens*, *g*) sporangiophores (SP) with the head cell (HC) and suffultory cell (SC) bearing sporangia (S) on the top, *h*) transverse section of leaf tissue show subcuticular and subepidermal growth of the thallus, cuticle (cu), epidermis (ep), palisade cells (ps) and *i*) gametes



Cephaleuros virescens. To confirm the result from morphological characteristics, thallus of *Cephaleuros* was cultured on Bold's basal medium (Bischoff and Bold 1963; Andersen 2005) and subjected to DNA extraction and PCR amplification. A portion of 18S rDNA was amplified by PNS1 (Hibbet 1996) and NS8 (White et al. 1990) primer pair. The PCR performed in 50 μ l reaction volume containing 10 pmol of each primer, 2x DreamTaq Green PCR Master Mix (Thermo Scientific), and 50 ng of template DNA. An initial denaturation step for 3 min at 95 $^{\circ}$ C was followed by 35 cycles of denaturation for 30 s at 95 $^{\circ}$ C, annealing for 30 s at 50 $^{\circ}$ C, and extension for 1 min at 72 $^{\circ}$ C, with a final extension step of 10 min at 72 $^{\circ}$ C. The PCR products were visualized by agarose gel electrophoresis. A PCR product band about 700 bases was observed from *Cephaleuros* sample. The partial 18S rDNA gene region then was bidirectionally sequenced at the Scientific Equipment Center Prince of Songkla University,

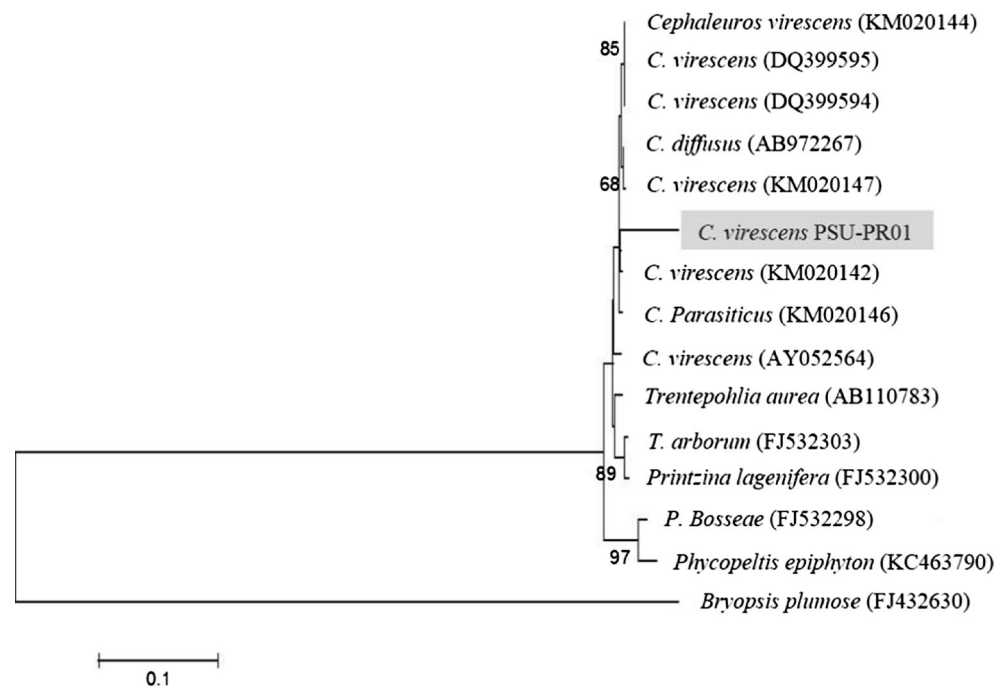
Songkhla, Thailand. The nucleotide sequence analysis of the *Cephaleuros* 18S rRNA using BLAST search revealed that our partial sequence was 707 bases long. This nucleotide sequence was deposited in GenBank with accession number (AB984776) and compared to known *Cephaleuros* and other algal genus on the NCBI (the National Center for Biotechnology Information) databases. A 94 % sequence identity confirmed it to be a member of the *Cephaleuros* group. Neighbor joining tree showing the genetic relationships of Thai *Cephaleuros* (PSU-PR01) was in the same clade with *Cephaleuros* genus, while well separated from the other genus (Fig. 2).

It has been previously reported that algal leaf spot disease on rubber plants in Sri Lanka is caused by *Cephaleuros parasiticus* (Karst) (Munasinghe 1961; Jayasinghe 2001). Furthermore, *C. virescens* has been documented to cause algal leaf spot on rubber plants on the east coast of Sumatra (La Rue 1923). Specimens of

Table 1 Comparative table of features of *Cephaloleuros virescens*, *C. solutus*, *C. parasiticus* and *C. diffusus*

Characters	<i>C. virescens</i> (PSU-PR01)	<i>C. virescens</i> (Suto and Ohtani 2009)	<i>C. solutus</i> (Pitaloka et al. 2014)	<i>C. parasiticus</i> (Suto et al. 2014)	<i>C. diffusus</i> (Thompson & Wujek 1997)
Hosts	<i>Hevea brasiliensis</i>	<i>Magnolia grandiflora</i> & <i>Persea thunbergii</i>	<i>Durio zibethinus</i>	<i>Syzygium aromaticum</i>	<i>Calophyllum viride</i> & <i>Magnolia grandiflora</i>
Habitat	subcuticular on upper, rarely lower leaf surface	subcuticular on upper leaf surface	subcuticular and subepidermal	subepidermal on upper and lower leaf surface and intramatrical	–
Thalli	irregular disk, without gaps	circular disk, without gaps, crenate or entire margin	irregular in outline	–	circular disk, with radial expansion
diameter (mm)	1–2	1–8	1–7	1–5	up to 1
growth habit	pseudoparenchymatous	pseudoparenchymatous	pseudoparenchymatous	–	open filamentous
Filamentous cells	short cylindrical	long cylindrical	long cylindrical to irregular	not observed because develop vertically beneath the epidermal cells	–
length × width (µm)	15–22.5 × 7.5–12.5	22–79 × 7–24	22–27.5 × 15–25	–	26–59 × 8–12
L/W ratio	1.4–2.33	2.7–4.4	1–1.8	–	1 × 2.7–6
branching manner	equal dichotomy	equal dichotomy	unequal dichotomy or irregularly	–	monopodial and unequal dichotomy
Setae	slender filament, one to five cells	1) slender filament; 2) short bunt – tipped filament	slender, two to four cells	no setae project on the lesions	–
Gametangia	globular to elliptical	spherical to elliptical	spherical to elliptical	–	spherical
number in clusters	2–4	2–5	1–2	–	–
Sporangio phores	terminally	terminally	terminally	terminally	terminally
length × width (µm)	25–108 × 15–30	17–27 × 4.5–6.5	152–510 × 7.5–15	300–640 × 13–19	–
Sporangia	globular to elliptical	elliptical	spherical	elliptical	–
Zoospore	ellipsoidal to broad fusiform	ellipsoidal to broad fusiform	obboconic to globose	–	–
length × width (µm)	7–12.5 × 5–7.5	7–11 × 4.5–6.5	4.5–10 × 2.5–7.5	–	15–19 × 9–10
Lesion	absent	absent	absent	–	absent

Fig. 2 Phylogenetic analysis of Thai *Cephaleuros* (*C. virescens* PSU-PR01) and other green alga (Trentepohliaceae, Chlorophyta) based on 18S rDNA sequences. Bootstrap values are shown on the branches and the GenBank accession number are shown in parentheses. *Bryopsis plumose* represents the lack of similarity outside this group



algae have also been deposited at the Culture Collection of the Pest Management Department, Faculty of Natural Resources, Prince of Songkla University, Thailand, for further studies.

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