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Cephaleuros parasiticus, associated with algal spot disease on *Psidium guajava* in Thailand

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Abstract During 2014–2015, algal spot disease was noticed in the guava orchards of northern and southern Thailand. Symptoms of the disease occurred on the leaves and fruit. Scabs were formed on heavily infected fruits. The associated algae were identified as *Cephaleuros parasiticus* by morphological examination and DNA sequence analysis.

Keywords Green algae · Trentepoliales · Guava · Morphology · Parasite

Cephaleuros is a genus of green algae (Chlorphyta) in the order Trentepohliales, family Trentepohliaceae. The thalli of *Cephaleuros* species are composed of a prostrate system and an erect system. The prostrate system consists of compact filamentous cells that form the thallus and gametangia, whereas sporangiophores and setae are included in the erect system. The filamentous cells of *Cephaleuros* penetrate the cuticle of the plant host and usually colonize the area between the cuticle and epidermal cells. Some *Cephaleuros* species grown intercellularly between plant cells of the epidermis and palisade parenchyma and into the mesophyll. The growth of *Cephaleuros* species causes necrosis in the tissues beneath its thallus. The necrotic tissues on leaf or stem surfaces of a host plant are visible symptoms of the disease referred to as "algal spot".

Guava (*Psidium guajava*) is a medium-to-large-sized perennial fruit tree in the family Myrtaceae. Guava orchards are commonly found in tropical and subtropical areas of Southeast Asia and is cultivated in all parts of Thailand, ranging from North to South. However, the guava plantations are facing several diseases due to the long annual period of high rainfall and high temperatures (Misra 2004). The most severe pathogens that cause devastating damage on the guava are plant parasitic algae in the genus *Cephaleuros* (Marlatt and Campbell 1980).

Algal spot was noticed in guava orchards in different ecological zones of southern Songkhla province and northern Phrae province, Thailand. Small lesions developed on both the upper and the lower leaf surfaces and expand with the growth of the algal thallus. Lesions usually became brownish purple late in their development (Fig.1A: a). Tufts of sporangiophores growing from brown, necrotic tissue were mostly observed on the lower leaf surfaces of guava leaves (Fig.1A: b and c). The dark brown lesions with tufts of sporangiophores were also observed on the fruit, (Fig.1A: d − f), with scabs developing in severe infections (Fig. 1A: g).

Symptomatic leaf samples ($n = 30$) were collected from each guava orchard and brought to the laboratory for diagnosis. Filamentous cells of the algae were highly irregular making size measurement difficult (Fig. 1B: a). Gametangia were beneath the cuticle, solitary or in clusters, ovoid to ellipsoid in shape with 27.5 − 40 × 12.5 − 27.5 μm (mean = 32.58, 21.16 μm) (Fig. 1B: b). Sporangiophores projected through the cuticle of the upper and lower leaf surfaces 4–7 cells, 232.5 − 490 × 10–22.5 μm (mean = 335.63, 12.75 μm) with radiating sporangiate-laterals (crooked suffultory cells

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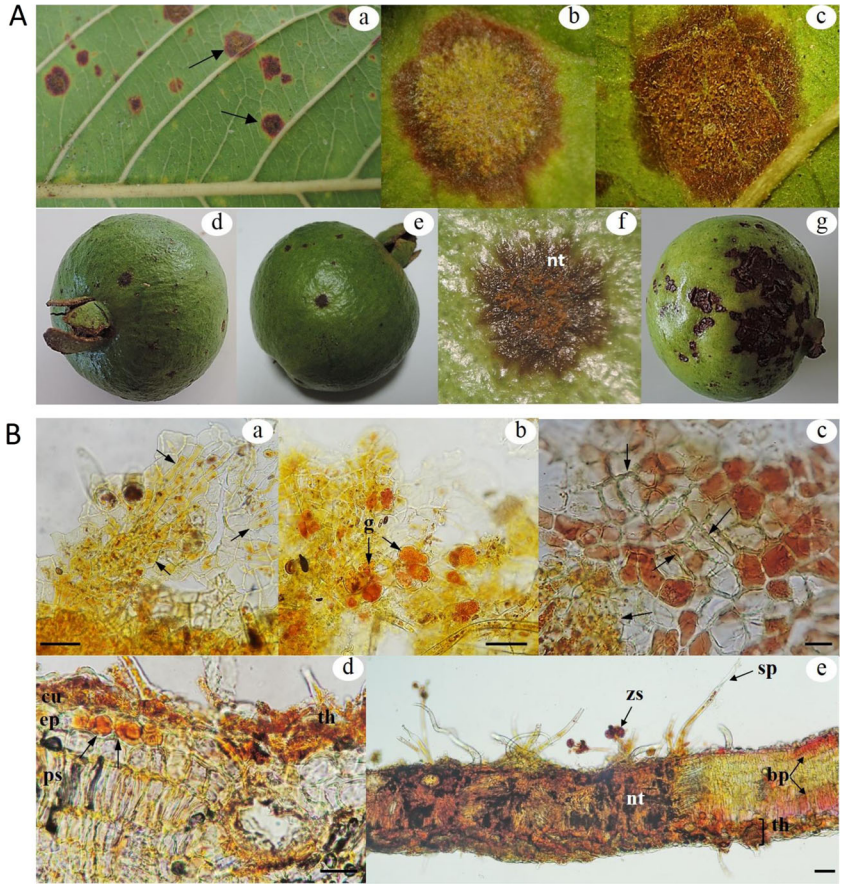

Fig. 1 **a**: Lesions caused by *Cephaleuros parasiticus* on *Psidium guajava*; (*a*) – brownish purple lesions surrounded by yellow halos on a lower leaf surface; (*b*) – tufts of sporangiophores projecting from necrotic tissues of the upper leaf surface; (*c*) – tufts of sporangiophores projecting from necrotic tissue of the lower leaf surface; (*d* & *e*) lesions on fruit; (*f*) – tufts of sporangiophores with necrotic tissue (nt) on fruit; (*g*-) – heavily infected fruit. **b**: Morphological characteristics of *C. parasiticus*. (*a*) – irregular shape filamentous cells, (*arrows*); (*b*) – clusters of gametangia (g); (*c*) – vertical section through a lesion showing algal filaments (*arrows*) growing among host plant cells; (*d*) transverse section of a lesion showing subepidermal growth below the upper leaf surface, and gametangia forming beneath cuticle (*arrows*), cuticle (cu), epidermis (ep), palisade (ps), thallus (th); (*e*) – Transverse section of a guava leaf showing full-thickness necrosis, sporangiophores (sp), zoosporangia (zs), thallus (th), and brownish purple (bp) tissues. The *scale bars* represent: *a* – *c*, 50 μm; *d* – *e*, 100 μm

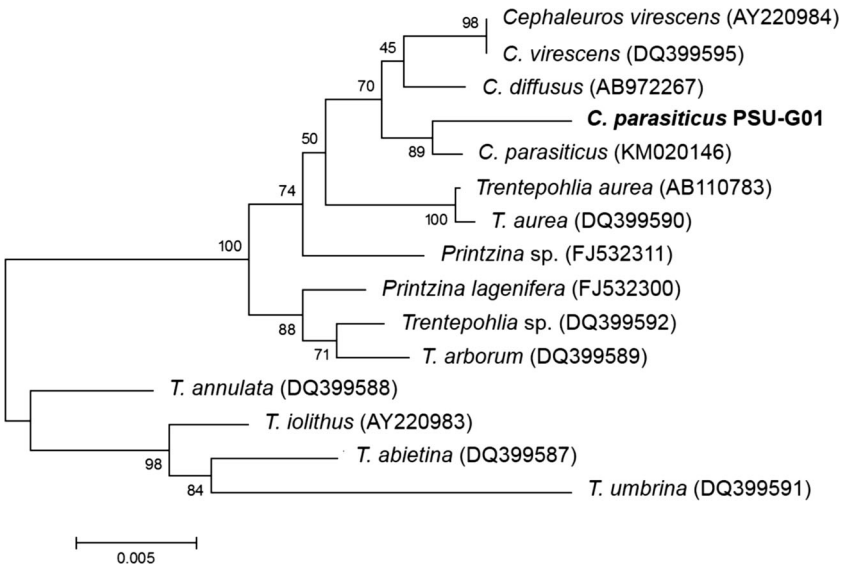

Fig. 2 Neighbour-joining tree generated using sequences of the 18S rDNA sequences of *Cephaleuros parasiticus* PSU-G01 and other green algae (Trentepohliaceae, Chlorophyta). The bootstrap values are shown on the branches and the GenBank accession numbers are shown in parentheses

attached to zoosporangia). Sporangia were globular to ovoid with 20–30 × 15–25 μm (mean = 25.08, 18.83 μm). Erect sterile filaments (setae) were rarely produced, and then apparently predominantly through the upper epidermis. Longitudinal leaf sections showed algal filaments growing among the parenchyma and mesophyll cells (Fig. 1B: c). Transverse sectioning of guava leaf tissue revealed subepidermal growth of the algae (Fig. 1B: d). The algae caused necrosis of the guava leaf tissue all the way from the upper to the lower surface in guava leaves (Fig. 1B: e). Based on the monograph by Thompson and Wujek (1997) the algae were identified as *Cephaleuros parasiticus*.

DNA-based identification of different groups of algae using encoding genes has been very successful. The 18S small subunit ribosomal DNA and chloroplast-encoded ribulose-1, 5-bisphosphate carboxylase/oxygenase large (*rbc*L) subunit are common genes for identification. These genes are considered an excellent tool for phylogenetic inference among the green algae (Chapman and Buchheim 1991; Hamby and Zimmer 1992). To confirm the genus *Cephaleuros* for these algae, PCR amplification and nucleotide sequencing were conducted. The algae were cultured on Bold's basal media (BBM) (Bischoff and Bold 1963; Andersen 2005). Filamentous colonies were harvested and the DNA extracted using the CTAB method (Doyle and Doyle 1987; Doyle and Dickson 1987; Cullings 1992). Portions of 18S rRNA were amplified by PCR using the PNS1 and NS41 primer pair (Hibbet 1996). The partial nucleotide sequence was 1113 bases long. The 18 s rDNA sequence was compared to known *Cephaleuros* spp. and other genera in the NCBI (the National Center for Biotechnology Information) database using the BLAST search function. The sequence for the Thailand *Cephaleuros parasiticus* strain was deposited in GenBank database with accession number LC104282. According to the phylogenetic relationship derived from the neighbor-joining analysis with selected sequences imported from NCBI indicated that the Thailand isolate PSU-G01 clustered with a sequence of *Cephaleuros parasiticus*, well separated from other species (Fig. 2). The phylogenetic relationships of 18 s rDNA has been used to identified *Cephaleuros* species in Thailand (Pitaloka et al. 2015; Sunpapao and Pitaloka 2015; Sunpapao et al. 2015, 2016).

An earlier study of algal spot in Thailand (Visarntanon 2010) stated that *C. virescens* was responsible for most infections. Recently, Pitaloka et al. (2014) identified *C. solutus* as a causal organism of algal spot in Thailand and may have been the first in this country to characterize the algal pathogen by morphology, using the Thompson and Wujek (1997) monograph. Since then six species of *Cephaleuros* have been identified on hosts in southern Thailand: *C. expansa*, *C. diffusus*, *C. karstenii*, *C. pilosa*, *C. solutus* and *C. virescens* (Pitaloka et al. 2014, 2015; Sunpapao and Pitaloka 2015; Sunpapao et al. 2015, 2016).

A prior report by Sunpapao et al. (2015) described algal thalli on guava hosts in southern Thailand expanding radially, and identified the causal agent as *C. expansa*. We collected specimens from both southern and northern Thailand, and the filamentous thallus cells of *C. parasiticus* were different from those in the previous report. The subepidermal growth habit, intercellular spread of the filamentous cells, sporangiophores protruding through lower leaf surfaces, and head cells with radiating sporangiate-laterals (crooked suffultory cells attached to zoosporangia) distinguished *C. parasiticus* from the other species of the genus identified in Thailand. However, the steps to confirm *Cephaleuros* as a pathogen (Koch's postulate) have not been completed because zoospores could not be produced on synthetic media for inoculation (Chapman and Good 1983; Holcomb et al. 1998; Suto and Ohtani 2011). Algal specimens from our study were deposited in the culture collection of the Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Thailand with accession number PSU-PMPG1501. To our knowledge, this is the first report of *C. parasiticus* on guava in Thailand.

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