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SHORT COMMUNICATION

Occurrence and Morphospecies Study of Plant-Parasitic Algae *Cephaleuros* (Trentepohlia, Ulvophyceae) on Betel Vine (*Piper betle*) in Southern Thailand

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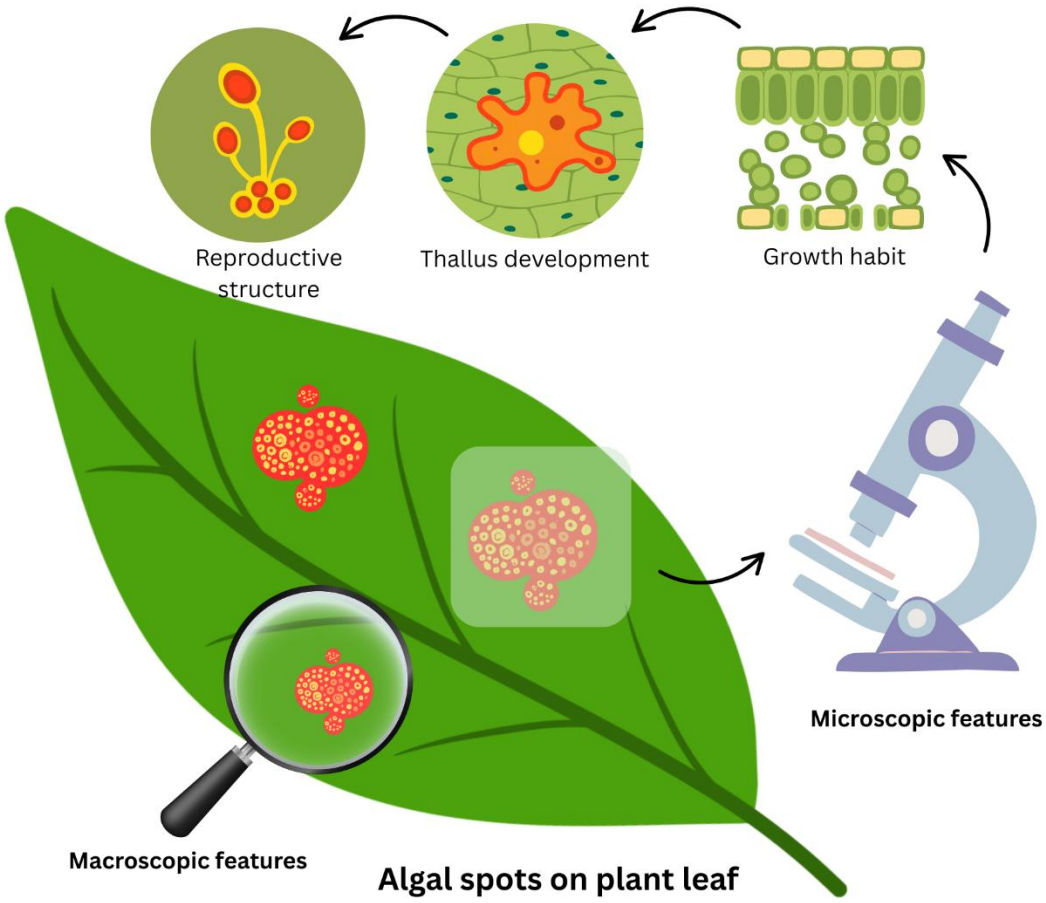
GRAPHICAL ABSTRACT



ABSTRACT

Algal leaf spot caused by *Cephaleuros virescens* was observed on *Piper betle* in Southern Thailand for the first time. Infected leaves exhibited characteristic orange, velvety lesions on the upper surface. Morphological examination under light microscopy revealed typical taxonomic features, including pseudoparenchymatous thalli, sporangiophores, zoosporangia, and gametangia, confirming the identity of the alga. Species identification was based solely on morphology, given limited molecular data and challenges in culturing the alga. This report expands the known host range of *C. virescens* and highlights the importance of algal pathogens in tropical crop systems.
Keywords: Betel vine, morphology, subaerial algae

Algal leaf spots, commonly caused by members of the subaerial algae in the genus *Cephaleuros* (Ulvophyceae, Trentepohliales), are a common disease affecting a wide range of tropical and subtropical plant species (Wolf, 1930). These algae are known as plant-parasitic algae and are found in tropical and subtropical climates (Thithuan *et al*., 2049). These algae colonize the leaves, petioles, and sometimes stems of host plants, resulting in reduced photosynthetic area and commercial value of the crop (Jourbert and Rijkenberg, 1971). The structure of algae in the genus *Cephaleuros* comprises filamentous green algae that reproduce both sexually via gametangia and asexually through zoosporangia (Suto and Ohtani 2009; Thithuan *et al*., 2019). Among these, *Cephaleuros virescens* is the most widely reported species, causing conspicuous orange to reddish leaf spots that are often mistaken for fungal infections (Marlat and Alfieri, 1981). Despite being relatively well-documented in other tropical regions, reports of algal diseases in Thailand remain limited, potentially due to misdiagnosis or lack of awareness.

Piper betle L. (betel vine) is a culturally and economically important plant in Southeast Asia, including Thailand, where its leaves are used in traditional medicine and chewing practices. Diseases affecting betel vines can significantly reduce leaf quality and yield. Previous research has mainly focused on fungal and bacterial pathogens, providing insights into their diversity and management. However, reports on algal pathogens in *P. betle* are extremely limited, with only sporadic cases documented and little information on their biology, epidemiology, or impact. This lack of knowledge hampers the development of effective disease management strategies. Given the increasing recognition of algal diseases in various crops under changing environmental conditions, it is crucial to investigate algal pathogens in betel vine now.

During the rainy season, when rain was frequent and the weather was warm, an algal spot was noticed in betel (*Piper betle*) in Thaksin University, southern Thailand. Algal symptoms exhibited velvety orange spots on the upper leaf surface of betel (**Fig. 1A & B**). Betel leaf samples naturally infected with algal thalli were collected from cultivated fields. From each plant, three symptomatic leaves were sampled, resulting in a total of 30 leaves from 10 plants. All collected leaves exhibited similar morphological symptoms, characterized by spots appearing as orange to rust-colored, raised lesions that can be orange-brown, reddish-brown, or brownish. They often have a velvety or fuzzy texture. The symptomatic leaves were carefully placed in sterile plastic bags, transported on ice, and brought to the Plant Pathology Laboratory, Faculty of Technology and Community Development, Thaksin University, for further examination and identification.

Algal thalli were removed from betel leaves with a razor blade and placed on glass slides. Sterile distilled water was used as a mounting medium. Morphological characteristics of the algae were observed under a light microscope (Olympus CH3, Japan) at magnifications of 40× to 100×. Taxonomic characters, including filamentous cells, sporangiophores, sporangia, and gametangia, were measured (n = 20). Species identification was conducted using a species identification key for Trentepohliales: *Cephaleuros*, *Phycopeltis,* and *Stomatochroon* (Thompson and Wujek, 1997).

The thalli of algae were as pseudoparenchymatous and predominantly ramulose (**Fig.1C**) and subcuticular habitat (**Fig. 1D**). Filamentous cells of the algae were short cylindrical, 5 – 12 µm wide × 22.5 – 42.5 µm long, with width/length ratio (W/L) 1: 2.5 – 6 (**Fig. 1B**). Sporangiophores projected from algal thalli on the upper leaf surface, 15.85 – 39 µm wide × 212.5 – 512.5 µm long. Head cell developed terminally and produced zoosporangia (**Fig. 1E**). Zoosporangia were elliptical, 15 – 22.5 µm wide × 20 – 32.5 µm long (**Fig. 1F**). Gametangia were spherical to elliptical, 22.5 – 37.5 µm wide × 25 – 45 µm long. Morphological characteristics of *Cephaleuros* sp. from this study were compared with other known morphospecies as previously published (**Table 1**). Based on key species of *Cephaleuros*, these characteristics indicate that the algae found on *Piper betle* in Phatthalung province, Thailand, correspond to *Cephaleuros virescens*. Specimens of algae have been deposited at the culture collection of the Faculty of Technology and Community Development, Thaksin University, with accession number TSU-PB01.

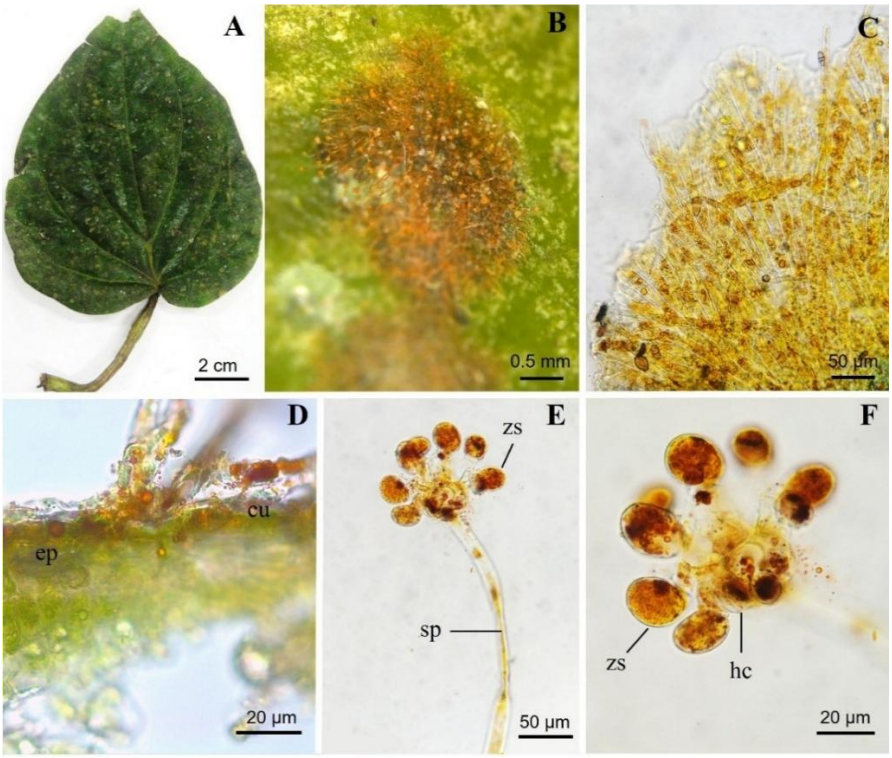


Figure 1 Characteristic of *Cephaleuros virescens* on *Piper betle*. (A) Lesions on host, (B) zoom view of spot, (C) detail of filamentous cells, (D) subcuticular habit, (E) SP – sporangiophore, zs – zoosporangia, (F) hc – head cell, zs – zoosporangia. Cu = cuticle, ep = epidermis

The present study provides the first confirmed report of *Cephaleuros virescens* infecting *Piper betle* in Southern Thailand. The identification was based on morphological characteristics, including the structure of filamentous cells, sporangiophores, zoosporangia, and gametangia, which are consistent with previous descriptions of *C. virescens* (Suto *et al*., 2014). The characteristic velvety, orange-colored lesions observed on the adaxial surface of betel leaves are typical symptoms of algal leaf spot and were associated with reduced aesthetic and commercial value of the foliage. *Cephaleuros virescens* is a well-known plant parasitic alga that has been reported in Thailand to infect a wide range of tropical plant species, including rambutan (*Nephelium lappaceum*), Para rubber (*Hevea brasiliensis*), and sapodilla (*Manilkara zapota*) (Pitaloka *et al*., 2015; Sunpapao *et al*., 2016; Sunpapao *et al*., 2017). The host expansion to *Piper betle* reflects the adaptability and potential threat of this algal pathogen in tropical agricultural systems. The presence of reproductive structures, including gametangia and sporangiophores, suggests that the organism completes both sexual and asexual life cycles on the host, contributing to its persistence and dissemination under favorable environmental conditions.

Table 1 Comparative table of features of *Cephaleuros virescens* and *C. parasiticus*

Characters	***Cephaleuros* species**		
	C. virescens	***C. virescens***	***C. parasiticus***
Host	*Piper betle*	*Hevea brasiliensis*	*Syzygium aromaticum*
Habitat	Subcuticular	Subcuticular	Subepidermal & intramatrical
Thalli	Irregular without a gap	Irregular without a gap	–
Filamentous cells	Pseudoparenchymatous with short filamentous	Pseudoparenchymatous with short filamentous	develop vertically beneath the epidermal cells
Gametangia	Elliptical	Globular to elliptical	–
Sporangia	Spherical to elliptical	Globular to elliptical	elliptical
References	This study	Pitaloka et al. (2015)	Suto et al. (2014)

Recent molecular studies have also advanced our understanding of *Cephaleuros* sp., with phylogenetic and taxonomic clarification (Fang *et al*., 2021) and chloroplast genome analyses revealing adaptive evolution (Huang *et al*., 2023). Although molecular tools are widely used in the identification of plant pathogens, the taxonomy of green algal pathogens, particularly in the genus *Cephaleuros*, still largely depends on morphological features.

This is due to the difficulty in obtaining pure cultures necessary for DNA extraction and sequencing, as *Cephaleuros* species often grow slowly, have specific growth requirements, and are difficult to isolate without contamination (Bunjongsiri and Sunpapao, 2018). Furthermore, molecular phylogenetic studies are limited by the lack of available DNA sequences from ex-type or type specimens in public databases, making species-level comparisons problematic.

In Thailand, reports on plant parasitic algae remain rare and often underrepresented in plant pathology surveillance, possibly due to the challenge of diagnosing algal infections that visually resemble fungal diseases. Identifying *C. virescens* on betel vine emphasizes the importance of incorporating algal pathogens into routine disease diagnostics, especially in crops of cultural and economic significance.

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