

Two new species of *Cephaleuros*(Chlorophyta, Ulvophyceae) from southern Thailand

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Abstract

Two new species of *Cephaleuros* are described from the southern Thailand, *C. annonae* and *C. piperis*. *Cephaleuros annonae* sp. nov. grows subcuticularly form thalli in a thin layer with serrate margin or a raised spot showing open-filamentous growth and appressed in marginal portion on *Annona squamosa* host. *Cephaleuros piperis* sp. nov. grows subcuticularly, subepidermally and intramatrically, and forms circular disk with pseudoparenchymatous growth inhabiting on *Piper nigrum* host. Macroscopic and microscopic features of the taxa are described and discussed.

Keywords: *Cephaleuros*, morphology, new species, southern Thailand

Introduction

The study on plant parasitic green algae in genus *Cephaleuros* in Thailand has been conducted in several host plants, and it was stated that most of these algal diseases were caused by only one species, namely *Cephaleuros virescens* (Visarntanon, 2010). Recently, Pitaloka et al., (2014) used the Thompson & Wujek (1997) characterized *Cephaleuros* species on durian host and it was identified as *C. solutus*. Since then, seven species of *Cephaleuros* have been identified in Thailand: *Cephaleurosexpansa*, *C. diffusus*, *C. karstenii*, *C. parasiticus*, *C. pilosa*, *C. solutus* and *C. virescens* (Pitaloka et al., 2014, 2015; Sunpapao & Pitaloka, 2015; Sunpapao et al., 2015, 2016a, 2016b, 2016c, 2017). Here we described two additional new species from southern Thailand based on morphological difference from known taxa.

Materials and Methods

Algal thalli on plant leaf samples were collected from each of the host plants and brought to the laboratory for morphological identification. Macroscopic feature of the algal thalli were observed under a stereoscopic microscope. Algal thalli were removed from plant leaves by razor blade and were placed on glass slide with lactophenol, sealed with cover-glass to observe morphology of the thallus and sporangiophores. Fresh thalli producing gametangia or sporangia were transferred with a drop of sterilized water and placed onto a slide to examine gametes and zoospores. Morphological characteristics were observed under a light microscope (Olympus, Japan). Dimension of filamentous cells, gametangia, sporangia, gametes and zoospores were measured ($n = 30$). Identification of *Cephaleuros* was based on macroscopic and microscopic features, according to the monograph by Thompson and Wujek (1997).

Division Chlorophyta

Class Ulvophyceae

Order Trentepohliales

Family Trentepohliaceae

Genus *Cephaleuros* Kunze, 1827

Cephaleuros annonae Pitaloka & Sunpapao, sp. nov. (Figs 1–8)

Type:—Thailand. Songkhla: Pest Management field, Faculty of Natural Resources, Prince of Songkla University, Hatyai, approximately 7°0'18"N, 100°29'58"E, collected from *Annona squamosa* leaves by Anurag Sunpapao, November, 2016. Figs 1–8

Thallus grows subcuticularly develop on the upper leaf surface, form an irregularly thin layer with serrate margin, 1–6 mm diameter, pale orange color. Filamentous cells long cylindrical to irregular, 12.5–70 µm long and 5–12.5

µm wide, with L/W ratio of 1.25–9.5, branching by monopodium and sometimes irregularly spreading in any direction. Setae develop into slender filament, 52.5–111 µm long and 2.5–6.25 µm wide, two or four cells, pale yellow, solitary or in tuft. Gametangia are produced beneath the cuticle, the gametangia develop from terminal cells of minor branches, enlarging in spherical or elliptical shape, 27.5–77 µm long and 15–70 µm wide, solitary or in cluster of two or three. Sporangioophores project from upper leaf surface, cylindrical, erect, 105–312 µm long and 8–16 µm wide, dark orange, solitary or in tuft of two or four, head cell bear terminally on sporangioophore and produce two to four sporangia laterals, sporangia and the suffultory cells. Sporangia are globose to elliptical, 7.5–30 µm long and 6.5–12.5 µm wide, yellow to orange. The basal cells of sporangia not bulbous, Zoospores ellipsoidal to fusiform 8–12.5 µm long and 5–10 µm wide, with flagella 17–22µm. Gametes are spherical to ellipsoidal, 5–12.5 µm long and 2.5–10 µm wide. Discoloration not observed around the lesion, brown to dark brown necrosis were observed on epidermal cell, no protective corky tissue observed on the leaf tissues.

Comparison of morphological feature is shown in Table 1.

Table 1 Comparative table of features of *Cephaleuros annonae*, *C. piperis* and *C. virescens*.

Character	<i>C. annonae</i>	<i>C. piperis</i>	<i>C. virescens</i> (Suto&Ohtani 2009)
Main Host	<i>Annona squamosa</i>	<i>Piper nigrum</i>	<i>Magnolia grandiflora</i> , <i>Persea thunbergii</i>
Habitat	subcuticular on upper leaf surfaces	intramatrical on upper leaf surface and rarely on lower leaf surface	subcuticular on upper leaf surface
Thalli	Shape	thin layer, serate margin	circular disk without gaps, crenate margin
	Diameter	1–6 mm	1–4 mm
	Growth habit	open-filament	pseudoparenchymatous
Filamentous cell	Shape	long cylindrical to irregular	short cylindrical to irregular
	Length × width (µm)	12.5–70 × 5–12.5	5–36.5 × 2.5–13
	L/W Ratio	1.25–9.5	1.3–4
	Branching manner	monopodial	unequal dichotomy
Setae	Shape	slender filament	rare and slender filament
	Length × width (µm)	52.5–111 × 2.5–6.25	72–155 × 2.5–10
Gametangia	Length × width (µm)	27.5–77 × 15–70	21. 25–71.25 × 17.5–67.5
	Number in clusters	1–3	1–5
Gametes	Length × width (µm)	5–12.5 × 2.5–10	6.25–12.5 × 5–10
	Length of flagella (µm)	15–20	16–20
Sporangiophores	Length × width (µm)	105–312 × 8–16	103–208 × 10–16.5
	Head cell placement	terminally	terminally
	Basal cell	not bulbous	bulbous
Sporangia	Length × Width (µm)	7.5–30 × 6.5–12.5	7.5–15 × 5–12.5
Zoospore	Length × Width (µm)	8–12.5 × 5–10	7.5–15 × 5–12.5
	Length of flagella (µm)	17–22	16–21
Lesion	Discoloration	absent	around thalli
	Necrosis; of epidermal cells	+	+
	of palisade cells	-	+
	of spongy cells	-	+
	of vascular bundle	-	+
	protective corky tissue	-	±

Etymology:—The specific epithet (*annonae*) refers to genus of host plant (*Annona squamosa*)

Observations:—New species was collected on (*Annona squamosa*) from Songkhla province, Thailand. The thalli of *C. annonae* are more or less circular, and composed of open-filamentous growth as those of known species *C. diffusus*, *C. expansa* and *C. henningsii*. The thalli of *C. annonae* form thin layer with serrate margin on upper epidermis. Filamentous cells develop laterally and appressed in marginal portion. Length of filamentous cells of *C. annonae* (means 35.2 µm), however, are seemed to be smaller than those of *C. diffusus* (means 43 µm), *C. expansa* (means=56 µm) and *C. henningsii* (means=55 µm) (Thompson &Wujek, 1997). Sporangia of *C. annonae* is 6.5–12.5 × 7.5–30 µm are smaller than those of *C. diffusus*, *C. expansa* and *C. henningsii*, 18–19 × 25–30 µm, 15–23 × 22–30 µm, and 11–15 × 15.4–17 µm, respectively (Thompson &Wujek, 1997).

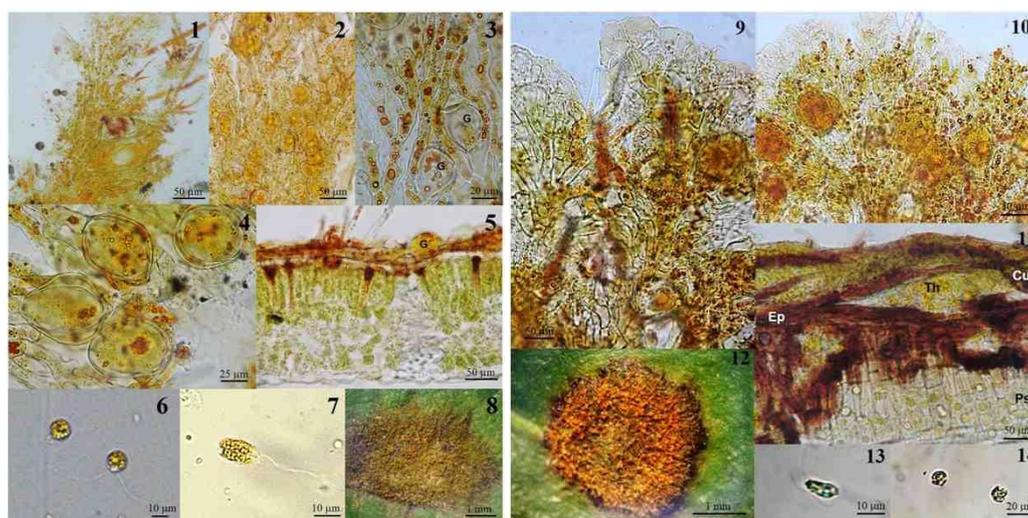


Plate 1 165.6 × 159 mm (300 dpi)

Plate 2 154.8 × 159 mm (300 dpi)

Explanation of Figures 1–8. Plate 1 *Cephaleuros annonae*

Loosely parallel thallus. 2. Surface view of thallus, where long cylindrical cells compacted laterally. 3. Long cylindrical filamentous cells loading with haematochrome pigments. 4. Details of oval shape gametangia. 5. Transverse section of infected *Annona squamosa* showing development of subcuticular thallus with gametangia (G). 6. Details of gametes. 7. Details of zoospore. 8. Development of thallus on *A. squamosa*

Explanation of Figures 9–14. Plate 2 *Cephaleuros piperis*

9. Pseudoparenchymatous thallus. 10. Surface view of pseudoparenchymatous thallus, where short cylindrical to irregular cells compacted. 11. Intramatrical growth habit. 12. Development of thallus on *Piper nigrum*. 13. Details of zoospore. 14. Detail of gametes.

Cephaleuros piperis Pitaloka & Sunpapao sp. nov. (Figs 9–14)

Type:—Thailand. Songkhla: Pest Management field, Faculty of Natural Resources, Prince of Songkla University, Hatyai, approximately 7°0'18"N, 100°29'58"E, collected from *Piper nigrum* leaves by Anurag Sunpapao, December, 2016. Figs 9–14

Thallus subcuticular, subepidermal and intra-dermatological on upper leaf surface and rarely on lower leaf surface, forming circular disc composed of pseudoparenchymatous ramuli without gaps and crenate margin, 1–4 mm diameter, olive to orange. Filamentous cells short cylindrical to irregular, 5–36.5 µm long and 2.5–13 µm wide with L/W ratio of 1.3–4, branching by unequal dichotomy. The filament expand beneath the epidermal cells and invade into intercellular space of the palisade and spongy tissues, branching irregularly and spreading in any direction in the plant tissue. Setae rarely produced and develop into slender filament, 72–155 µm long and 2.5–10 µm wide, two to three cells, hyaline to pale yellow, solitary. Gametangia develop from terminal cells of the tip of branches, enlarging in spherical or elliptical shape, 21. 25–71.25 µm long and 17.5–67.5 µm wide, solitary or in cluster of two or five. Sporangioophores project from upper leaf surfaces, cylindrical and erect, 103–208 µm long and 10–16.5 µm wide, three to five cells, solitary or in tuff of two or three. Head cells bear terminally on the sporangioophores, and produce two or four sporangiate lateral, sporangia and the suffultory cells. Sporangia elliptical, 7.5–15 µm long and 5–12.5 µm wide, yellow to orange. The basal cells of sporangioophores are short and bulbous. Production of sporangioophores rarely observed on the thalli, Zoospores ellipsoidal 7.5–15 µm long and 5–12.5 µm wide, length of flagella 16–21 µm. Gametes spherical to fusiform, 6.25–12.5 µm long and 5–10 µm wide, length of flagella 16–20 µm. A distinct yellow colour observed around the thalli, *Cephaleuros* infection causes brown to red brown necrosis on the whole leaf tissue. The protective corky tissue develops at upper part of palisade cells.

Comparison of morphological feature is shown in Table 1.

Etymology:— Specific epithet (*piperis*) refers to genus of host plant.

Observations:—New species was collected on (*Piper nigrum*) from Songkhla province, Thailand. The thalli of *C. piperis* grow subcuticularly, subepidermally and intramatrically as do those of *C. biolophus*, *C. minimus*, *C. parasiticus* and *C. pilosa*. Length of filamentous cells of *C. piperis* is 2.5–13 μm and smaller than those of *C. minimus* and *C. pilosa*, 25–33 μm , and 20.8–94 μm , respectively (Thompson & Wujek 1997). The thalli of *C. piperis* are circular disk without gap and are composed of pseudo-parenchymatous as those of two known species *C. karstenii* and *C. virescens*. Mean of width and length of filamentous cells of *C. piperis* is 4.75 \times 10.88 μm , and smaller than those of *C. karstenii* and *C. virescens*, 10 \times 36 μm , 14.6 \times 38 μm , respectively (Thompson & Wujek 1997). Sporangia of *C. piperis* is 5–12 \times 7–15 μm and smaller than those of *C. karstenii*, *C. virescens*, 17–26 \times 24–34 μm , and 24–28 \times 32–35 μm , respectively (Thompson & Wujek, 1997). Significant characteristics in *C. piperis* are (i) intramatrical growth habit, (ii) setae are rarely developed, and (iii) filamentous cells are short cylindrical to irregular with unequal dichotomous branching manner. The base of sporangiophores swells bulbously.

Discussion

The genus *Cephaleuros* is distinguished by growth habit and characteristics of sporangiophores from other genera in family Trentepohliaceae (Thompson & Wujek, 1997). They inhabit subcuticularly or deeper within host tissues (leaves, twigs, fruits) of vascular plants (Thompson & Wujek, 1997). *Cephaleuros* is restricted to subaerial with high humidity in tropical and subtropical areas (Printz, 1939; Thompson & Wujek, 1997) as well as in temperate zone (Suto & Ohtani, 2009; Suto & Ohtani, 2013). Species-rich *Cephaleuros* floras are found worldwide, in Florida (Marlatt & Campbell, 1980; Marlatt & Alfieri, 1981, America (Brooks, 2004), Hawaii (Rindiet *al.*, 2005), Africa (Rindiet *al.*, 2006) and Panama (Rindiet *al.*, 2008). In the Asian continent *Cephaleuros* has been recorded in China (Sarma, 1986; Hu & Wei, 2006), Indonesia (Sarma, 1986), Japan (Suto & Ohtani, 2009), Malawi (Sarma, 1986), Malaysia (Sarma, 1986) and Taiwan (Nelsen *et al.*, 2011).

Despite careful assessment, among 500 specimens collected from southern Thailand, some specimens of *Cephaleuros* were unable to identify because the morphological characteristics in Thompson and Wujek monograph (1997) were not fully met. In this study, two new species were *C. annonae* on *A. squamosa* host and *C. piperis* on *P. nigrum*. Although, development of filamentous cells and branching manner of *C. annonae* is as similar as those of *C. diffusus*, *C. expansa* and *C. henningsii*, dimension of filamentous cells and sporangia are differing from those species. For *C. piperis*, they inhabited subcuticular, subepidermal and intramatrical, which distinguished from *C. karstenii* and *C. virescens*. Almost *Cephaleuros* species inhabit on plant host as a parasite, and cause damage below algal thalli, especially intercellular species including *C. biolophus*, *C. minimus*, *C. parasiticus*, *C. parasiticus* var. *nana* and *C. pilosa*. Development of the lesion also differs between species in the transverse section. The alga *C. piperis* invades the host subcuticularly, subepidermally and intramatrically and the tissues necrose from upper to lower leaf surface of plant leaves, whereas *C. annonae* cause necrosis only on epidermis layer.

In addition, *C. virescens*, only one species *Cephaleuros* is recorded in Thailand (Visarntanon, 2010). Recently, study on taxonomy of *Cephaleuros* species has been arisen in several hosts. According to *Cephaleuros* key to species (Thompson & Wujek, 1997), a clear assessment of the thallus growth habit, the manner of bearing head cells, and the kind of lesion produced are the most valuable in determining *Cephaleuros* species. Algal *C. solutus* was firstly identified as causal agent for leaf spot disease on durian (*Duriozibethinus*), using the Thompson and Wujek (1997) monograph (Pitaloka *et al.*, 2014). Since then, seven species of *Cephaleuros* have been identified on several host in southern Thailand: *C. expansa*, *C. diffusus*, *C. karstenii*, *C. parasiticus*, *C. pilosa*, *C. solutus* and *C. virescens* (Pitaloka *et al.*, 2015; Sunpapao & Pitaloka, 2015; Sunpapao *et al.*, 2015, 2016a, 2016b, 2016c, 2017). The two new species distinguish from those species found in Thailand and differ from known species in Thompson & Wujek monograph (1997).

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