รายงานวิจัยฉบับสมบูรณ์

องค์ประกอบชนิดและการแพร่กระจายของสาหร่ายปรสิตพืช *Cephaleuros* Kunze ex E.M. Fries ในประเทศไทย

Specie composition and distribution of plant parasitic algae, *Cephaleuros* Kunze ex E.M. Fires in Thailand

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โครงการวิจัยนี้ได้รับทุนสนับสนุนจากเงินรายได้มหาวิทยาลัย
มหาวิทยาลัยสงขลานครินทร์

ประจำปีงบประมาณ 2559 **รหัสโครงการ** NAT590712S

ภาษาไทย องค์ประกอบชนิดและการแพร่กระจายของสาหร่ายปรสิตพืช *Cephaleuros* Kunze ex E.M. Fries ในประเทศไทย

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คณะวิจัยและหน่วยงานต้นสังกัด

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กิตติกรรมประกาศ

ขอขอบคุณมหาวิทยาลัยสงขลานครินทร์ สำหรับทุนวิจัยจากเงินรายได้มหาวิทยาลัย ประเภททั่วไป ที่ได้ให้การสนับสนุนทุนวิจัย และขอขอบคุณ ภาควิชาการจัดการศัตรูพืช คณะทรัพยากรธรรมชาติ มหาวิทยาลัย สงขลานครินทร์ วิทยาเขตหาดใหญ่ จังหวัดสงขลา สำหรับสถานที่ทดลอง อุปกรณ์ เครื่องมือ เพื่อให้ดำเนินการวิจัยได้สำเร็จลุล่วงด้วยดี

อนุรักษ์ สันป่าเป้า

ตุลาคม 2561

บทคัดย่อ

สาหร่ายสีเขียวสกุล *Cephaleuros* Kunze ex E.M. Fries เป็นสาหร่ายเพียงสกุลเดียวที่ดำรงชีวิตเป็นปรสิตก่อให้เกิด “โรคจุดสาหร่าย” บนพืชและแพร่กระจายเซลล์สืบพันธุ์โดยอาศัยน้ำฝน เนื่องจากในประเทศไทยมีความหลากหลายของสิ่งมีชีวิตสูง และมีความผันแปรของสภาพอากาศแต่ละพื้นที่แตกต่างกัน แต่ยังไม่มีการศึกษาด้านองค์ประกอบชนิด และปัจจัยต่าง ๆ ที่มีผลต่อการเจริญของสาหร่ายสกุลนี้ งานวิจัยนี้จึงมีจุดมุ่งหมายเพื่อจำแนกชนิดของสาหร่าย ศึกษาชนิดพืชอาศัย และประเมินความรุนแรงของสาหร่ายสกุลนี้ในประเทศไทย รวมทั้งศึกษาการเปลี่ยนแปลง การเจริญ การสร้างเซลล์สืบพันธุ์ ของสาหร่ายสกุลนี้ตามฤดูกาลในแปลงทดลอง จากการเก็บตัวอย่างสาหร่ายสกุล *Cephaleuros* พบการเจริญของสาหร่ายสกุลนี้บนพืชอาศัย 105 ชนิด เป็นพืชที่ยังไม่มีรายงานการศึกษาการเป็นพืชอาศัยของสาหร่ายสกุลนี้มาก่อน 52 ชนิด เมื่อจำแนกชนิดสาหร่ายโดยลักษณะทางสัณฐานวิทยาสามารถจำแนกได้ 9 ชนิด คือ *C. diffusus*, *C. expansa*, *C. henningsii*, *C. karstenii*, *C. microcellularis*, *C. parasiticus*, *C. solutus*, *C. tumidae-setae* และ *C. virescens* สาหร่ายชนิด *C. henningsii*, *C. microcellularis* และ *C. tumidae-setae* เป็นรายงานการค้นพบครั้งแรกในประเทศไทย เมื่อประเมินระดับความรุนแรงทั้ง 9 ชนิด พบว่าสาหร่ายที่ส่งผลกระทบและสร้างความเสียหายรุนแรงแก่พืชอาศัย คือ *C. henningsii*, *C. microcellularis*, *C. parasiticus* และ *C. solutus* จากการศึกษาการเจริญตามฤดูกาลของสาหร่ายสกุล *Cephaleuros* พบว่า สาหร่ายในสกุลนี้มีการเจริญ การพัฒนาโครงสร้างสืบพันธุ์แบบอาศัยเพศ และไม่อาศัยเพศ ระหว่างฤดูร้อน และฤดูฝน แตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ค่าความเชื่อมั่น 0.05 พบจำนวนทัลลัสของสาหร่ายและโครงสร้างสืบพันธุ์เพิ่มขึ้นเมื่อมีปริมาณน้ำฝนเฉลี่ยมากกว่า 100 มิลลิเมตร และความชื้นสัมพัทธ์มากกว่า 80 เปอร์เซ็นต์ พบการปลดปล่อยเซลล์สืบพันธุ์ในช่วงที่มีปริมาณน้ำฝนเฉลี่ยมากกว่า 200 มิลลิเมตร และมีความชื้นสัมพัทธ์ในอากาศสูงมากกว่า 85 เปอร์เซ็นต์ (เดือนสิงหาคม – เดือนตุลาคม) และจากการศึกษาการปลดปล่อยเซลล์สืบพันธุ์ในห้องปฏิบัติการโดยกระตุ้นการปลดปล่อยเซลล์สืบพันธุ์ด้วยน้ำเย็น พบว่า แกมีทมีพฤติกรรมการเข้าคู่กัน (conjugate) และสลายตัว (burst) ส่วนซูโอสปอร์มีการงอกและสร้างรงควัตถุภายในเซลล์เส้นใย จากผลการศึกษาองค์ประกอบชนิดและการเจริญตามฤดูกาลของสาหร่ายสกุล *Cephaleuros* นี้สามารถใช้เป็นข้อมูลพื้นฐานสำหรับงานวิจัยทางด้านความหลากหลาย และการใช้ประโยชน์จากความหลากหลายในสิ่งมีชีวิตต่อไป

คำสำคัญ: สาหร่ายสีเขียว, ปรสิตพืช, สัณฐานวิทยา, Chlorophyta

Abstract

Cephaleuros Kunze ex E.M. Fries is one of green algal genus, causing “algal spot disease” on plant hosts and distribute by reproductive cells via rainfall. Thailand locates in tropical area which diverse of organisms, and the weather is variable. However, the species composition and seasonal development of *Cephaleuros* is still unknown. This research aimed to identify algal species and plant hosts throughout Thailand, to assess disease severity and to examine the seasonal development of *Cephaleuros* in research field. A total of 103 plant species collected in Thailand, we found 52 plant species represented as new host for *Cephaleuros* spp. Based on morphological characters, 9 species of *Cephaleuros* namely: *C. diffusus, C. expansa, C. henningsii, C. karstenii, C. microcellularis, C. parasiticus, C. solutus, C. tumidae-setae* and *C. virescens* were identified. This is the first report of *C. henningsii, C. microcellularis* and *C. tumidae-setae* in Thailand. A four point necrosis index revealed that *C. henningsii, C. microcellularis, C. parasiticus* and *C. solutus* caused the most severe cases on host plants. The seasonal development of *Cephaleuros* was significant difference between summer and rainy season ($p < 0.05$) in research field. Number of algal spots on leaves and reproductive structures were increased according to rainfall (>100 mm) and relative humidity (>80% RH). Reproductive cells released on August to October as the same manner (>200 mm average rainfall and >85% RH. Furthermore, behavior of reproductive cells was conducted in water drop. Gametes conjugated in water drop and later got burst, whereas zoospores germinated and produced pigment in young filamentous cells. A basic knowledge of species composition and seasonal development of algal genus *Cephaleuros* will be useful for further study in the field of biodiversity.

Keywords: green algae, parasite, morphology, Chlorophyta

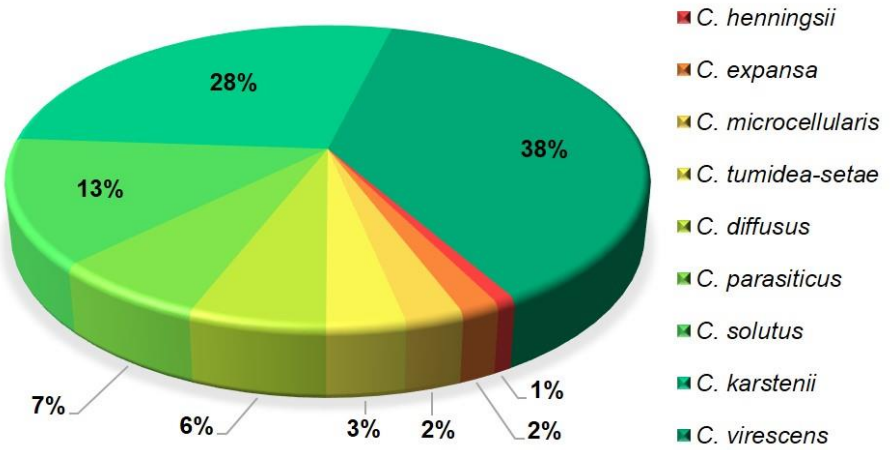
บทสรุปผู้บริหาร (Executive Summary)

สาหร่ายบางชนิดสามารถก่อให้เกิดโรคในพืชได้ โดยเฉพาะสาหร่ายในสกุล *Cephaleuros* Kunze ex E.M. Fries พบได้บนพืชอาศัยหลายชนิด ส่วนใหญ่เจริญอยู่บนใบพืช มองเห็นเป็นจุดฟูคล้ายกำมะหยี่สีเหลืองส้ม บางชนิดมีการเจริญเข้าไประหว่างเซลล์พืช เพื่อรับน้ำและธาตุอาหารจากพืชอาศัยมาใช้ในกระบวนการสังเคราะห์ด้วยแสง การที่สาหร่ายขึ้นปกคลุมใบพืชยังบดบังกระบวนการสังเคราะห์แสงของพืชอาศัย ทำให้พื้นที่รับแสงบนใบพืชลดลง กระบวนการทำงานต่าง ๆ ของเซลล์พืชถูกรบกวน เกิดความเสียหายต่อพืช จึงจัดสาหร่ายในสกุลนี้เป็นสาหร่ายที่ดำรงชีพเป็นปรสิต (parasite) ในประเทศไทยได้มีการรวบรวมโรคจุดสาหร่ายจากพืชอาศัย 74 ชนิด และระบุว่าเกิดจากสาหร่าย *C. virescens* โดยอาศัยเพียงการสังเกตจุดมีลักษณะฟูคล้ายกำมะหยี่ และใช้ลักษณะก้านชูสปอร์ในการจำแนกชนิด ปัจจุบันการใช้ลักษณะดังกล่าว ยังไม่เพียงพอที่จะระบุชนิดของสาหร่ายในสกุล *Cephaleuros* ได้อย่างถูกต้อง การศึกษาและจำแนกชนิดสาหร่ายในสกุล *Cephaleuros* ในปัจจุบันจึงอาศัยลักษณะทางสัณฐานวิทยาตามคีย์ชนิดของสาหร่ายโดย Thompson และ Wujek (1997) ปัจจุบันมีรายงานการพบสาหร่ายสกุลนี้แล้ว 7 ชนิด ได้แก่ *C. expansa, C. diffusus C. karstenii, C. pilosa, C. parasiticus, C. solutus* และ *C. virescens* กระจายอยู่บนพืชอาศัยหลายชนิดในภาคใต้ของประเทศไทย (Sunpapao et.al., 2015) จะเห็นว่าจากรายงานการศึกษาที่ผ่านมาจำกัดอยู่เพียงแค่ภาคใต้ของประเทศไทย และยังขาดองค์ความรู้อีกมาก เช่น การศึกษาปัจจัยต่าง ๆ ที่มีผลต่อการเจริญของสาหร่าย เนื่องด้วยประเทศไทยมีตำแหน่งที่ตั้งในเขตร้อนชื้น มีความหลากหลายของสิ่งมีชีวิตสูงและมีความผันแปรของสภาพอากาศแต่ละพื้นที่แตกต่างกัน

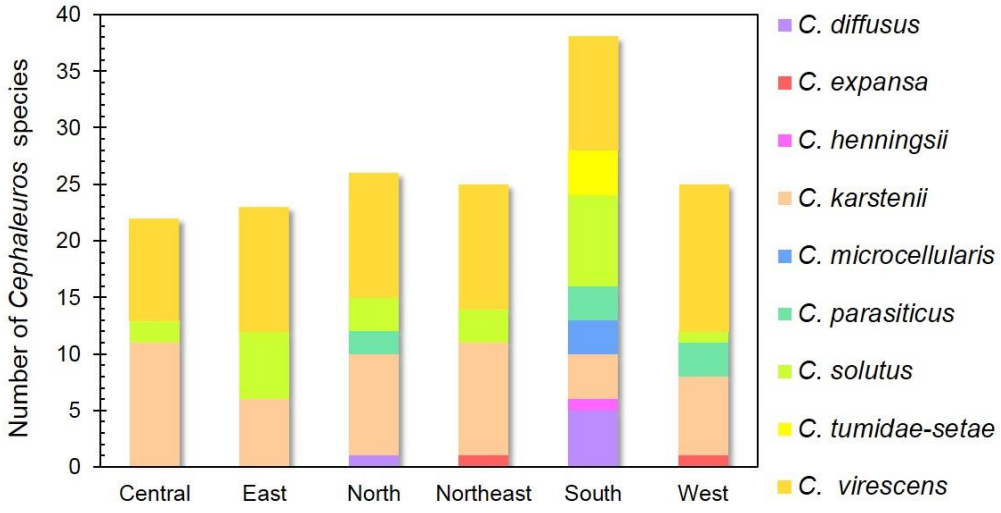
งานวิจัยมีวัตถุประสงค์เพื่อศึกษาชนิดของสาหร่าย ชนิดพืชอาศัยและประเมินความรุนแรงของสาหร่ายสกุลนี้ต่อพืชอาศัย รวมทั้งศึกษาการเปลี่ยนแปลง การเจริญ การสร้างเซลล์สืบพันธุ์ ของสาหร่ายสกุลนี้ตามฤดูกาล เพื่อเพิ่มเติมองค์ความรู้ของสาหร่ายสกุล *Cephaleuros* Kunze ex E.M. Fries ในประเทศไทย

ลักษณะทางสัณฐานวิทยาของสาหร่ายปรสิตพืชสกุล *Cephaleuros* ในประเทศไทย สามารถจำแนกสาหร่ายออกได้เป็น 9 ชนิด จาก 199 ตัวอย่าง ในพืชอาศัย 105 ชนิด (ตารางภาคผนวกที่ 1) จากการพิจารณาชนิดของสาหร่ายทั้ง 9 ชนิด พบว่าสาหร่าย *C. virescens* เป็นสาหร่ายที่พบบนพืชอาศัยมากที่สุด ถึง 38 เปอร์เซ็นต์ หรือ 45 ชนิด จากจำนวนตัวอย่างทั้งหมด รองลงมาคือ *C. karstenii*, *C. solutus*, *C. parasiticus*, *C. diffusus*, *C. tumidae-setae*, *C. expansa, C. microcellularis* และ *C. henningsii* ตามลำดับ (ภาพที่ 1) เมื่อพิจารณาจากจำนวนชนิดของสาหร่ายในการศึกษาครั้งนี้ ภาคใต้มีจำนวนชนิดของสาหร่ายมากที่สุด ทั้งนี้อาจเพราะพื้นที่เก็บตัวอย่างมีสภาพแวดล้อม และชนิดพืชพรรณที่มีความหลากหลายมากกว่าภาคอื่น ๆ (ภาพที่ 2) สาหร่ายที่ส่งผลกระทบและสร้างความเสียหายรุนแรงมี 4 ชนิด คือ *C. henningsii, C. microcellularis, C. parasiticus* และ *C. solutus* ทำให้เนื้อเยื่อพืชเปลี่ยนสีและมีอาการฉ่ำน้ำ ทำให้ใบพืชมีประสิทธิภาพในการสังเคราะห์แสงและสร้างอาหารลดลง ส่งผลให้พืชทิ้งใบ และ

จากการศึกษาการเจริญตามฤดูกาลของสาหร่ายสกุล *Cephaleuros* ในแปลงทดลองพบว่า สาหร่ายในสกุลนี้มีการเจริญ และปลดปล่อยเซลล์สืบพันธุ์ในฤดูฝน ซึ่งมีความแตกต่างกับในฤดูร้อน สาหร่ายในช่วงเดือนมกราคม – เมษายน (ฤดูร้อน) มีลักษณะแห้งและโครงสร้างต่าง ๆ เสียสภาพ แต่ในฤดูฝน (เดือนพฤษภาคม – ตุลาคม) สาหร่ายมีจำนวนเพิ่มขึ้น เนื่องจากมีความชื้นที่เหมาะสมและได้รับธาตุอาหารที่สมบูรณ์จากพืชอาศัย ทำให้มีการพัฒนาโครงสร้างสืบพันธุ์ และปลดปล่อยเซลล์สืบพันธุ์ในช่วงฤดูฝน ปัจจัยปริมาณน้ำฝนและความชื้นจึงเป็นปัจจัยสำคัญต่อการแพร่กระจายเซลล์สืบพันธุ์เข้าสู่พืช และทำให้เกิดโรคจุดสาหร่าย ซึ่งจากที่ตั้งของประเทศไทยที่อยู่ในเขตร้อนชื้น โดยเฉพาะทางภาคใต้ของประเทศไทยที่มีสภาพอากาศร้อนชื้นเกือบตลอดทั้งปี จึงเหมาะสมต่อการอยู่รอด การเจริญ และการแพร่พันธุ์ของสาหร่ายในสกุล *Cephaleuros*



ภาพที่ 1 จำนวนชนิดของพืชอาศัยที่พบสาหร่ายสกุล *Cephaleuros* แต่ละชนิดในประเทศไทย



ภาพที่ 2 จำนวนชนิดของสาหร่ายสกุล *Cephaleuros* ในแต่ละภูมิภาคของประเทศไทย

ข้อเสนอแนะ

จากผลการศึกษาในครั้งนี้ทำให้ทราบถึงองค์ประกอบชนิดของสาหร่ายสกุล *Cephaleuros* ในประเทศไทย ซึ่งสามารถใช้เป็นข้อมูลพื้นฐานในการศึกษาความหลากหลายของสาหร่ายสกุล *Cephaleuros* ได้ในอนาคต นอกจากนั้นการศึกษาการเจริญตามฤดูกาลสาหร่าย ยังเป็นข้อมูลที่มีประโยชน์ในการวางแผนการเก็บตัวอย่าง การศึกษาการสืบพันธุ์ และใช้เป็นข้อมูลพื้นฐานเพื่อให้เกิดความเข้าใจปัจจัยที่เหมาะสมต่อวงจรการเกิดโรคจุดสาหร่ายในธรรมชาติ จากการศึกษาพบว่าสาหร่ายสกุลนี้ไม่ได้ก่อให้เกิดอาการที่รุนแรงต่อพืชอาศัย ในสภาพแวดล้อมที่ชื้นมากและมีแสงแดดส่องถึงจะพบการเจริญของสาหร่ายมากกว่าบริเวณพื้นที่โปร่ง การจัดการโดยตัดแต่งทรงพุ่มให้มีความโปร่ง ลดพื้นที่การสัมผัสกัน มีการระบายอากาศได้ดีช่วยลดจำนวนของทัลลัสสาหร่ายได้ นอกจากนี้การใช้สาร algaecide สามารถใช้ควบคุมโรคของสาหร่ายได้

ภาคผนวก

Wonglom, P., Thithuan, N., Bunjongsiri, P. and **Sunpapao, A.** 2018. Plant-parasitic algae (*Cephaleuros* spp.) in Thailand, including four new records. Pacific Science 72(3): 363–371. Web of Science (IF = 0.822)

Thithuan, N., Bunjongsiri, P. and **Sunpapao, A.** Morphology and Behavior of gametes and zoospores from plant parasitic green algae, *Cephaleuros* (Chlorophyta, Ulvophyceae). Pacific Science. Revised

ตารางผนวกที่ 1 ตารางชนิดของพืชอาศัย และชนิดของสาหร่ายในสกุล *Cephaleuros*

ชนิดพืชอาศัย		ชนิดสาหร่าย
วงค์	**ชื่อวิทยาศาสตร์**	
Acanthacea	*Justicia fragilis*	*C. microcellularis*
Anacardiaceae	*Mangifera indica*	*C. karstenii*
		C. virescens
	Bouea bumanica	*C. karstenii*
	Bouea macrophylla	*C. karstenii*
		C. virescens
	Schinus terebinthifolius	*C. karstenii*
Annonaceae	*Annona muricata*	*C. karstenii*
	Annona squamosa	*C. virescens*
	Artabotrys siamensis	*C. karstenii*
		C. virescens
	Cananga odorata	*C. karstenii*
	Rauwenhoffia siamensis	*C. karstenii*
		C. virescens
Apocynaceae	*Allamanda cathartica*	*Cephaleuros* sp.
	Alstonia scholaris	*C. karstenii*
		C. virescens
	Plumeria obtusa	*C. virescens*
Araceae	*Epipremnum aureum*	*Cephaleuros* sp.
	Philodendron calophyllum	*Cephaleuros* sp.
Asclepiadaceae	*Hoya ovalifolia*	*Cephaleuros* sp.
Bignoniaceae	*Crescentia cujete*	*C. karstenii*
		C. virescens
Bombacaceae	*Durio zibethinus*	*C. solutus*
Boraginaceae	*Carmona retusa*	*C. virescens*
Calophyllaceae	*Mammea siamensis*	*C. karstenii*
		C. parasiticus
		C. solutus
Capparaceae	*Crateva adansonii*	*Cephaleuros* sp.

Chrysobalanaceae	*Parinari anamensis*	*C. virescens*
Clusiacea	*Calophyllum inophyllum*	*C. virescens*
		C. solutus
Combretaceae	*Terminalia bellirica*	*C. virescens*
Costaceae	*Cheilocostus speciosus*	*C. solutus*
Dipterocarpaceae	*Dipterocarpus obtusifolius*	*C. karstenii*
	Shorea obtusa	*C. virescens*
Ebenaceae	*Diospyros malabarica*	*C. solutus*
	Diospyros rhodocalyx	*C. karstenii*
Ericaceae	*Rhododendron ferrugineum*	*C. solutus*
Fabaceae	*Pterocarpus macrocarpus*	*C. virescens*
Flacourtiaceae	*Casearia grewiifolia*	*C. virescens*
	Flacourtia rukam	*C. virescens*
Guttiferae	*Garcinia atroviridis*	*C. diffusus*
	Garcinia mangostana	*Cephaleuros* sp.
		C. solutus
Lauraceae	*Cinnamomum iners*	*C. microcellularis*
	Cinnamomum loureirii	*C. diffusus*
	Cinnamomum porrectum	*C. diffusus*
	Cinnamomum verum	*Cephaleuros* sp.
	Persae americana	*C. parasiticus*
		C. virescens
Lecythidaceae	*Barringtonia acutangula*	*C. diffusus*
Leeaceae	*Leea indica*	*C. microcellularis*
	Leea macrophylla	*C. virescens*
Leguminosae	*Bauhinia aureifolia*	*C. virescens*
	Calliandra haematocephala	*C. virescens*
	Saraca indica	*C. virescens*
	Sindora siamensis	*C. tumidae-setae*
	Tamarindus indica	*C. virescens*
Loranthaceae	*Loranthus sp.*	*C. karstenii*
Lythraceae	*Lagerstroemia floribunda*	*C. karstenii*
		C. solutus

	Lagerstroemia indica	*C. virescens*
	Lagerstroemia speciosa	*C. henningsii*
		C. virescens
Magnoliaceae	*Magnolia figo*	*C. solutus*
	Magnolia sirindhorniae	*C. karstenii*
	Michelia alba	*C. karstenii*
		C. virescens
Marantaceae	*Calathea crotalifera*	*Cephaleuros* sp.
Meliaceae	*Lansium domesticum*	*C. karstenii*
		C. solutus
	Sandoricum koetjape	*C. solutus*
		C. virescens
		C. karstenii
		Cephaleuros sp.
	Swietenia macrophylla	*C. virescens*
	Swietenia mahogany	*C. karstenii*
Moraceae	*Artocarpus heterophyllus*	*Cephaleuros* sp.
		C. karstenii
		C. solutus
		C. virescens
	Artocarpus integer	*C. diffuses*
	Ficus sp.	*C. virescens*
	Ficus altissima	*C. karstenii*
		C. virescens
	Ficus maciellandii	*C. virescens*
	Ficus elastica	*Cephaleuros* sp.
	Streblus asper	*C. karstenii*
		C. solutus
		C. virescens
Myrtaceae	*Callistemon lanceolatus*	*C. solutus*
	Psidium guajava	*C. expansa*
		C. diffuses
		C. karstenii

		C. parasiticus
	Syzygium australe	*C. virescens*
	Syzygium cumini	*C. expensa*
	Syzygium jambos	*C. karstenii*
		C. tumidae-setae
	Syzygium malaccense	*C. karstenii*
		C. virescens
	Syzygium sp	*C. parasiticus*
Ochnaceae	*Ocha kirkii*	*C. virescens*
Oleaceae	*Osmanthus fragrans*	*C. parasiticus*
Opiliaceae	*Melientha suavis*	*C. karstenii*
Phyllanthaceae	*Baccaurea ramiflora*	*C. karstenii*
Piperaceae	*Piper betle*	*C. karstenii*
	Piper nigrum	*C. virescens*
		Cephaleuros sp.
Rhizophoraceae	*Rhizophora apiculata*	*C. tumidae-setae*
	Rhizophora mucronata	*C. tumidae-setae*
Rubiaceae	*Ixora cibdela*	*C. virescens*
	Ixora coccinea	*C. virescens*
	Ixora chinensis	*C. virescens*
	Tamilnadia uliginosa	*C. virescens*
	Tarenna hoaensis	*C. diffuses*
Rutaceae	*Citrus aurantifolia*	*C. karstenii*
		C. virescens
		C. parasiticus
	Citrus hystrix	*Cephaleuros* sp.
	Citrus hystrix	*C. karstenii*
		C. solutus
	Citrus maxima	*Cephaleuros* sp.
	Glycosmis pentaphylla	*Cephaleuros* sp.
	Murraya paniculata	*Cephaleuros* sp.
		C. solutus
Sapindaceae	*Dimocarpus longan*	*C. karstenii*

		C. virescens
	Litchi chinensis	*C. parasiticus*
	Nephelium lappaceum	*C. virescens*
Sapotaceae	*Chrysophyllum cainito*	*C. virescens*
	Manilkara kauki	*Cephaleuros* sp.
	Manilkara zapota	*Cephaleuros* sp.
	Pouteria campechiana	*C. solutus*
Sterculiaceae	*Pterospermum littorale*	*C. virescens*
	Sterculia monosperma	*C. karstenii*
Theaceae	*Camellia japonica*	*C. parasiticus*
	Camellia sinensis	*C. virescens*
		Cephaleuros sp.
Zingiberaceae	*Boesenbergia rotunda*	*Cephaleuros* sp.

Plant-Parasitic Algae (*Cephaleuros* spp.) in Thailand, Including Four New Records[1]

Prisana Wonglom,[2] *Narasinee Thithuan*,[3] *Penpadsorn Bunjongsiri*,[3] *and Anurag Sunpapao*[4,5]

Abstract: Recent work on species composition, taxonomy, and diversity of plant-parasitic algae in the genus *Cephaleuros* in Thailand has provided additional knowledge of the parasitic algae in the country. The seven previously reported species, *Cephaleuros diffusus*, *C. expansa*, *C. karstenii*, *C. parasiticus*, *C. pilosa*, *C. solutus*, and *C. virescens*, are shown to cause algal leaf spot on several host plants in Thailand. Four new records are reported, namely *Cephaleuros druetii*, *C. henningsii*, *C. lagerheimii*, and *C. tumidae-setae*, resulting in a total of 11 *Cephaleuros* species recorded from Thailand.

Keywords: checklist, *Cephaleuros*, diversity, epiphytic, host plant, Ulvophyceae

The filamentous green algae of the genus *Cephaleuros* Kunze ex E. M. Fries (Chlorophyta, Ulvophyceae) are distributed in tropical and subtropical regions worldwide (Alfieri 1969, Guiry and Guiry 2016). The algae cause obvious orange to dark brown velvety lesions on plant leaves, twigs, or fruits (Wolf 1930, Joubert and Rijkenberg 1971, Ogle 1997, Holcomb et al. 1998, Ramya et al. 2013). Most *Cephaleuros* species are parasites that grow subcuticularly or intercellularly and cause necrosis beneath the algal thalli (Chapman 1976, Ogle 1997, Brooks 2004, Suto and Ohtani 2009). The genus *Cephaleuros* has been described as an obligate epiphyte (Thompson and Wujek 1997). The distribution of *Cephaleuros* spp. includes Africa (Rindi et al. 2006), Indonesia (Sarma 1986), Malaysia (Sarma 1986), China (Sarma 1986, Hu and Wei 2006), Taiwan (Nelsen et al. 2011), Japan (Suto and Ohtani 2009), Hawai'i (Brooks 2004, Rindi et al. 2005), Florida (Marlatt and Campbell 1980, Marlatt and Alfieri 1981), and Panama (Rindi et al. 2008). Recently, seven *Cephaleuros* species, namely *C. diffusus*, *C. expansa*, *C. karstenii*, *C. parasiticus*, *C. pilosa*, *C. solutus*, and *C. virescens* have been found on various host plants in southern Thailand (Pitaloka et al. 2014, Pitaloka et al. 2015, Sunpapao and Pitaloka 2015, Sunpapao et al. 2015; Sunpapao, Pitaloka, and Arikit 2016; Sunpapao et al. 2017). However, the species composition of the filamentous green algae in this genus is still not well known. This research describes four new records of *Cephaleuros* species in Thailand, and we also report new observations on host plant associations.

Materials and Methods

Four hundred eighty-five algal specimens were collected from 97 plant taxa (five leaves containing algal thalli were collected per plant taxon) from six regions (northern, central,

[1] Manuscript accepted 9 January 2018. Funded by Prince of Songkla University (grant no. NAT590712S and NAT610511N) and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB) phase 2, Faculty of Natural Resources, Prince of Songkla University.

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Pacific Science (2018), vol. 72, no. 3:363–371
doi:10.2984/72.3.7

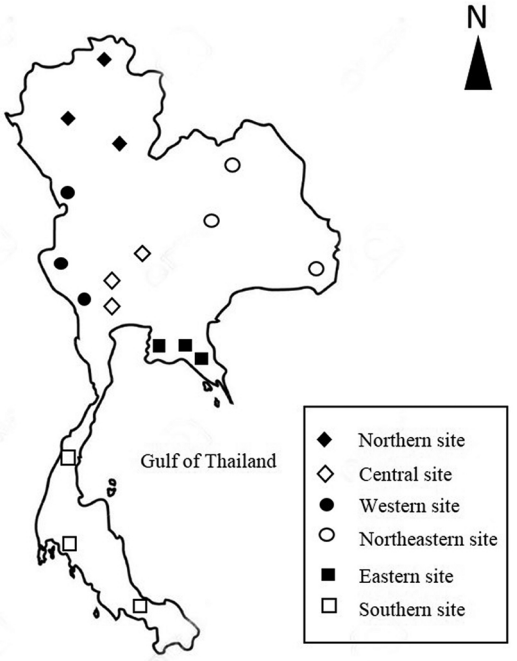

FIGURE 1. Study sites include six regions of Thailand: northern, central, western, northeastern, eastern, and southern Thailand. Each study site contains three provinces (northern site: Chiang Rai, Chiang Mai, Phrae; central site: Nakhon Pathom, Lopburi, Suphan buri; western site: Kanchanaburi, Tak, Ratchaburi; northeastern site: Khon Kaen, Udon Thani, Ubon Ratchathani; eastern site: Chanthaburi, Chonburi, Rayong; southern site: Krabi, Chumphon, Songkhla).

western, northeastern, eastern, and southern sites), each region composed of three provinces (Figure 1). Algal specimens were kept in plastic bags and were brought to a laboratory where identifications were done. Algal thalli were removed from the host leaves that were transversely sectioned with a razor blade. Morphological characteristics of the algae were observed under a light microscope (Olympus CH3, Japan). Dimensions of filamentous cells, gametangia, setae, and sporangiophores were measured ($n = 30$). Species identification was conducted using the identification keys and descriptions provided in Wolf (1930) and in Thompson and Wujek (1997). Voucher specimens for new species records were deposited in the Culture Collection of the Pest Management Department, Faculty of Natural Resources, Prince of Songkla University.

RESULTS

Among the 485 algal specimens, macroscopic symptoms caused by *Cephaleuros* species were found on the leaves of 97 plant species. Based on the keys of Thompson and Wujek (1997), the algal specimens were identified as 11 species: *C. diffusus*, *C. druetii*, *C. expansa*, *C. henningsii*, *C. karstenii*, *C. lagerheimii*, *C. parasiticus*, *C. pilosa*, *C. solutus*, *C. tumidae-setae*, and *C. virescens* (Table 1).

The following checklist gives the name, location, and host of the seven previously recorded *Cephaleuros* spp., along with complete descriptions of the four newly collected species reported in Thailand in this study.

Previously Recorded Cephaleuros *spp.*

Cephaleuros diffusus Thompson & Wujek
Literature: Sunpapao and Pitaloka (2015: 118)
Localities: Songkhla Province
Host: On the leaves of acacia (*Acacia auriculiformis*)

Cephaleuros expansa Thompson & Wujek
Literature: Sunpapao et al. (2015:455)
Localities: Krabi, Phangnga, Patthalung, Songkhla, and Trang Provinces
Hosts: On the leaves of *Piper longum* and *Psidium guajava*

Cephaleuros karstenii Schmidle
Literature: Sunpapao et al. (2015:456)
Localities: Chumphon, Nakhon Si-Thammarat, Phangnga, Ranong, Satun, Songkhla, and Trang Provinces
Hosts: On the leaves of *Citrus aurantiifolia*, *C. maxima*, *Ficus benjamina*, *Syzygium malaccense*, and *Theobroma cacao*

Cephaleuros parasiticus Karsten
Literature: Sunpapao, Thituan, et al. (2016:2)

TABLE 1

A List of *Cephaleuros* Species in This Study Covering Different Regions of Thailand, Each Encompassing Several Provinces

Region	Provinces	*Cephaleuros* Species
Northern	Chiang Rai	*Cephaleuros karstenii*, *C. parasiticus*, *C. solutus*, *C. virescens*, *Cephaleuros* sp.
	Chiang Mai	*C. karstenii*, *C. virescens*
	Phrae	*C. diffusus*, *C. karstenii*, *C. solutus*, *C. virescens*, *Cephaleuros* sp.
Central	Nakhon Pathom	*C. karstenii*, *C. virescens*
	Lopburi	*C. karstenii*, *C. virescens*
	Suphan buri	*C. karstenii*, *C. virescens*
Western	Kanchanaburi	*C. virescens*
	Tak	*C. karstenii*, *C. solutus*, *C. virescens*
	Ratchaburi	*C. karstenii*, *C. virescens*
Eastern	Chanthaburi	*C. solutus*, *C. virescens*
	Chonburi	*C. virescens*
	Rayong	*C. karstenii*, *C. solutus*, *C. virescens*
Northeastern	Khon Kaen	*C. karstenii*, *C. solutus*, *C. virescens*
	Udon Thani	*C. karstenii*, *C. solutus*, *C. virescens*
	Ubon Ratchathani	*C. karstenii*, *C. solutus*, *C. virescens*
Southern	Krabi	*C. druetii**, *C. lagerheimii**, *C. solutus*, *C. parasiticus*, *C. tumidae-setae**, *C. virescens*
	Chumphon	*C. karstenii*, *C. solutus*, *C. virescens*
	Songkhla	*C. diffusus*, *C. henningsii**, *C. karstenii*, *C. pilosa*, *C. solutus*, *C. virescens*

* New records in Thailand of *Cephaleuros* species found in this study.

Localities: Phrae and Songkhla Provinces
Host: On the leaves of *Psidium guajava*

Cephaleuros pilosa Thompson & Wujek
Literature: Sunpapao et al. (2015:457)
Localities: Songkhla Province
Host: On the leaves of *Garcinia mangostana*

Cephaleuros solutus Karsten
Literature: Pitaloka et al. (2014:647), Sunpapao et al. (2015:459)
Localities: Songkhla Province
Host: On the leaves of *Durio zibethinus*

Cephaleuros virescens Kunze *in* Fries
Literature: Sunpapao et al. (2015:459); Pitaloka et al. (2015:2); Sunpapao, Pitaloka, and Arikit (2016:33); Sunpapao et al. (2017: 636)

Localities: Chiang Rai, Chiang Mai, Phrae, Khon Kaen, Udon Thani, Ubon Ratchathani, Nakhon Pathom, Suphan buri, Saraburi, Chanthaburi, Chon Buri, Rayong, Kanchanaburi, Tak, Ratchaburi, Krabi, Chumphon, Trang, Nakhon Si Thammarat, Phangnga, Phatthalung, Phuket, Ranong, Songkhla, Satun, and Surat Thani Provinces

Hosts: On the leaves of *Alstonia scholaris*, *Anacardium occidentale*, *Annona muricata*, *A. squamosa*, *Artabotrys siamensis*, *Artocarpus heterophyllus*, *Baccaurea ramiflora*, *Bauhinia aureifolia*, *Bouea macrophylla*, *Calliandra haematocephala*, *Callistemon lanceolatus*, *Camellia sinensis*, *Carmona retusa*, *Casearia grewiifolia*, *Chrysophyllum cainito*, *Cinnamomum iners*, *Citrus aurantiifolia*, *C. maxima*, *Crescentia cujete*, *Dimocarpus longan*, *Diospyros rhodocalyx*, *Durio zibethinus*, *Ficus maciellandii*, *F. microcarpa*, *Garcinia mangostana*, *Glycosmis pentaphylla*, *Hevea brasiliensis*, *Ixora coccinea*, *Lagerstroemia floribunda*, *L. indica*, *Lansium domesticum*, *L. parasiticum*, *Magnolia champaca*, *Mammea siamensis*, *Mangifera indica*, *Manilkara zapota*, *Michelia alba*, *Nephelium lappacium*, *Parinari anamensis*, *Persea americana*, *Piper nigrum*, *Plumeria obtusa*, *Pterocarpus macrocarpus*, *Pterospermum littorale*, *Rauwenhoffia siamensis*, *Saraca indica*, *Sauropus androgynus*, *Shorea obtusa*, *Spondias mombin*, *Streblus asper*, *Swietenia*

macrophylla, *Syzygium australe*, *S. malaccense*, and *Tamarindus indica*

New Records of Cephaleuros *Kunze ex E. M. Fries from Thailand*

Cephaleuros druetii Thompson & Wujek

Herbarium number: PSU-SD01

Host: *Saraca declinata* (Jack) Miq. (common name: red saraca; local name: sok-khao)

Localities: Krabi Province

Date of collection: 23 October 2016

Collectors: Anurag Sunpapao, Narasinee Thithuan, Penpadsorn Bunjongsiri, and Prisana Wonglom

Description: Circular lesions 5–10 mm diameter on upper leaf surface, with orange algal thalli growing beneath cuticle. Algal thalli have subcuticular growth within the leaf tissue. Thalli divergently ramulate and wide spreading (Figure 2*A*). Ramuli consist of

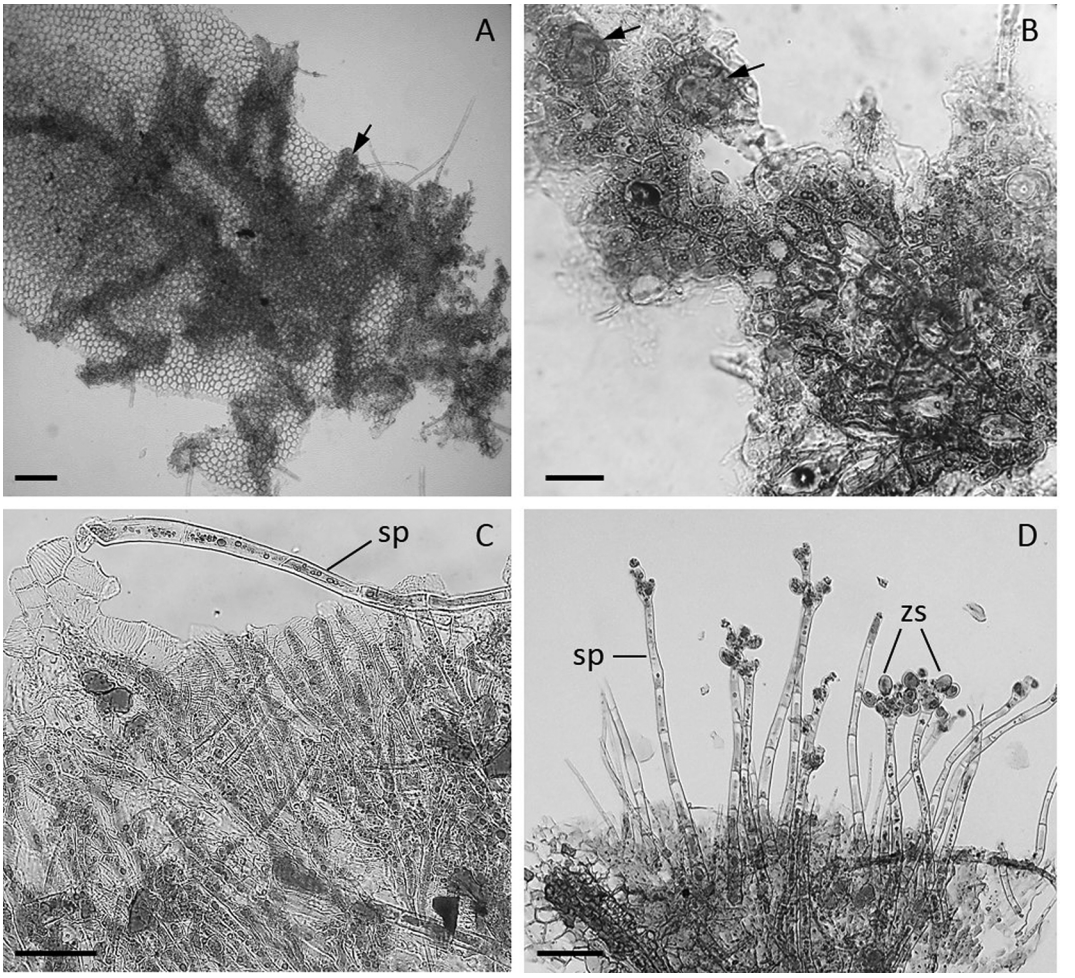

FIGURE 2. Characteristics of *Cephaleuros* species found in this study. *A*, thallus of *C. druetii* divergently ramulate and wide spreading (arrow); *B*, dichotomously or trichotomously lobed filamentous cells of *C. druetii* and gametangia underside of thallus (arrows); *C*, long cylindrical filamentous cells of *C. henningsii;* D, tuft of sporangiophores of *C. henningsii*. Scale bars = 50 μm. sp, sporangiophore; zs, zoosporangia.

single filaments, dichotomously or trichotomously lobed (Figure 2*B*). Filamentous cells too irregular in form for measurement. Setae are long cylindrical, three- or six-celled tapered filaments, 2.5–5 μm wide and 30–70 μm long. Gametangia spherical to elliptical, yellow to orange, 30–35 μm wide and 30–40 μm long and developed beneath thalli. Sporangiophores produced on upper leaf surfaces at apex of ramuli and are cylindrical, four- to six-celled, solitary or in tufts of four or more, 10–12.5 μm wide and 28–52.5 μm long. Head cells develop terminally on sporangiophores bearing three to four sporangiate-laterals, with both sporangia and their suffultory cells. Sporangia elliptical, 20–25 μm wide and 22.5–30 μm long. Gametes and zoospores were not observed in this study. On leaves of *Saraca declinata*, causing necrosis of epidermal cells only.

Remarks: This species is characterized by thallus divergently ramulate and wide spreading in any direction. Algal ramuli are very narrow, consisting of a single filament of dichotomous lobe as described by Thompson and Wujek (1997).

Cephaleuros henningsii Schmidle

Herbarium number: PSU-LS01

Host: *Lagerstroemia speciosa* (L.) Pers. (common name: pride of India; local name: inthanin)

Localities: Songkhla Province

Date of collection: 10 June 2016

Collector: Anurag Sunpapao and Prisana Wonglom

Description: Circular lesions 1–5 mm in diameter on upper leaf surface, with orange algal thalli growing beneath cuticle. Thalli grow beneath host cuticle, are circular or irregularly expanded, and are composed of loosely parallel, radiating filaments with monopodial branching. Filamentous cells cylindrical (Figure 2*C*), 7.5–10 μm wide and 40–72.5 μm long, with a L/W ratio of 1: 5.33–7.25. Sporangiophores sparsely produced on upper leaf surfaces are cylindrical, three to five cells, solitary or in tufts of three or more, 10–12.5 μm wide and 197.5–260 μm long (Figure 2*D*). Head cells develop terminally on sporangiophores and bear two to four sporangiate-laterals, each with a suffultory cell and attached sporangium. Sporangia elliptical, 15–20 μm wide and 17.5–22.5 μm long. Gametangia are elliptical, dark orange, solitary, 20–35 μm wide and 22.5–55 μm long. Gametes and zoospores were not observed in this study. This species causes necrosis of the epidermal cells only.

Remarks: The thalli of *C. henningsii* grow subcuticularly as do those of other *Cephaleuros* species. *Cephaleuros henningsii* is characterized by loosely parallel and radiating filaments, mostly monopodial in development, as described by Thompson and Wujeck (1997).

Cephaleuros lagerheimii Schmidle

Herbarium number: PSU-BA01

Host: *Barringtonia acutangula* (L.) Gaertn (common name: mangopine; local name: cik nam)

Localities: Krabi Province

Date of collection: 23 October 2016

Collectors: Anurag Sunpapao, Narasinee Thithuan, Penpadsorn Bunjongsiri, and Prisana Wonglom

Description: Algal lesions widespread and mostly irregular to fimbriately circular on upper leaf surfaces. Thalli subcuticular, circular to irregular in outline with finely laciniate margins. They are composed of long cylindrical filaments, pinnately branched, with lateral branches growing in various directions (Figure 3*A*). Gametangia spherical to elliptical, dark orange, solitary or in clusters of two to three, 37.5–45 μm wide and 40–50 μm long. Sporangiophores growing perpendicular from upper leaf surface cylindrical, five to eight cells, in tufts of three or more, 10.25–12.5 μm wide and 455–550 μm long (Figure 3*B*). Sporangia spherical, 20–30 μm in diameter. Gametes and zoospores were not observed in this study. This species caused necrosis of epidermal and palisade leaf cells.

Remarks: The thalli of *C. lagerheimii* are open-filamentous circular to very irregular in outline. Margins of thalli are finely laciniate, and the main filamentous cells branch in pinnate fashion as described by Thompson and Wujek (1997).

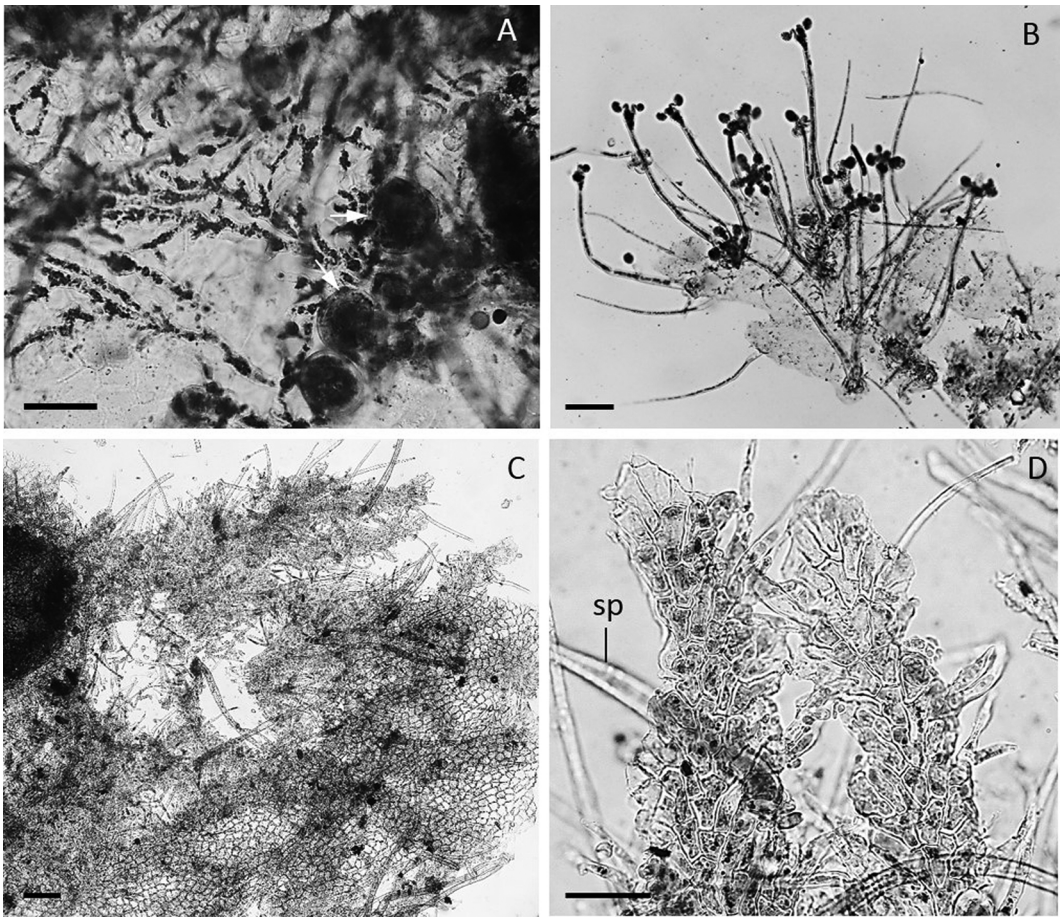

FIGURE 3. Characteristics of *Cephaleuros* species found in this study. *A*, open filaments of *C. lagerheimii; B*, sporangiophores of *C. lagerheimii; C–D*, irregular, loose, or compacted ramuli of *C. tumidae-setae*. Scale bars = 50 μm. sp, sporangiophore.

Cephaleuros tumidae-setae Thompson & Wujek

Herbarium number: PSU-SA01

Host: *Syzygium australe* (H. L. Wendl. ex Link) B. Hyland (common name: rose apple; local name: chom phu)

Localities: Krabi Province

Date of collection: 23 October 2016

Collectors: Anurag Sunpapao, Narasinee Thithuan, and Prisana Wonglom

Description: Orange to brown algal thalli on leaves surrounded by dark brown to dark purple discoloration. Thalli have subcuticular growth within the leaf tissue, causing necrosis beneath algal thalli. Thalli roughly circular with irregular, ragged-appearing margins of loose or compacted ramuli (Figure 3*C*). Margins of thalli are too irregular to measure (Figure 3*D*). Setae short, one- or two-celled filaments. Sporangiophores develop from thalli on upper leaf surfaces. They are cylindrical, two-celled, erect, solitary, 10–12.5 μm wide and 305–367.5 μm long. Head cells develop terminally and produce four sporangiate laterals each. Sporangia spherical to elliptical, 17.5–20 μm wide and 17.5–25 μm long. Gametangia spherical to ellipti-

cal, 22.5–25 μm wide and 22.5–27.5 μm long, and produced beneath the cuticle. Gametes and zoospores were not observed in this study.

Remarks: The thalli of *C. tumidae-setae* grow subcuticularly. *Cephaleuros tumidae-setae* is characterized by thallus with irregular, ragged appearing margin and ramuli mostly developed from a single dichotomizing filament and attenuated, as previously described by Thompson and Wujek (1997). This species is also found on leaves of *Syzygium jambos*, *Rhizophora apiculate*, and *Sindora siamensis*.

DISCUSSION

In this study, 41 plant species in Thailand were found to be hosts for *C. virescens*. Most of the hosts are perennial dicots that have been reported from other tropical and subtropical areas (Marlatt and Alfieri 1981, Holcomb 1986, Thompson and Wujek 1997). Observations of this current study are in agreement with the previous report that *C. virescens* has the broadest host range among *Cephaleuros* species, being found in India (Gokhale and Shaikh 2012) and in Japan (Suto and Ohtani 2009). This suggests that *C. virescens* is a common species and widespread in tropical and temperate zones worldwide. However, the number of algal species identified in Thailand, especially the 11 *Cephaleuros* spp., is higher than the species counts reported in other regions (Table 1). For example, only two species, *C. parasiticus* and *C. virescens*, have been reported in India (Ponmurugan et al. 2010, Ramya et al. 2013, Suto et al. 2014). Suto and Ohtani (2009) reported five *Cephaleuros* species in Japan: *C. aucubae*, *C. biolophus*, *C. japonicus*, *C. microcellularis*, and *C. virescens*.

All *Cephaleuros* species grow subcuticularly and cause damage beneath their thalli, with loss of photosynthetic area due to necrosis of green tissues (Safeeulla and Govindu 1948). Among the 11 species in Thailand, most *Cephaleuros* species in this study have subcuticular growth beneath cuticle, with the exception of *C. parasiticus*. Thompson and Wujek (1997) stated that *C. parasiticus* is an intercellular species growing deeper than the other species. Sporangiophores of *C. parasiticus* were found mostly on the lower leaf surfaces, and the stomata were destroyed. Furthermore, *C. parasiticus* caused cell necrosis from the upper to the lower leaf surface, whereas the other 10 species found in this study caused necrosis of only one or two cell layers beneath the algal thalli. Some *Cephaleuros* species found in this study were common on the lower leaf surfaces of heavily infected leaves. Infected lower leaf surfaces result from the zoospores swimming in a film of water connecting the two sides of a leaf (Chapman and Good 1983). *Cephaleuros* growth on a plant host resulted in depletion of water and mineral nutrients from the host tissues (Wolf 1930), and this is further exacerbated by the secretion of harmful algal metabolites (Jourbert and Rijkenberg 1971).

Prior studies of *Cephaleuros* in Thailand have identified seven species: *C. diffusus*, *C. expansa*, *C. karstenii*, *C. parasiticus*, *C. pilosa*, *C. solutus*, and *C. virescens*. The distribution of plant-parasitic algae in Thailand is now expanded by four new findings: *C. druetii*, *C. henningsii*, *C. lagerheimii*, and *C. tumidae-setae*, with identifications based on morphological characteristics in the current study. Some of the *Cephaleuros* specimens could not be confidently identified and were labeled as *Cephaleuros* spp. (Table 1), because the morphological characteristics in Thompson and Wujek (1997) were not fully met. These specimens will require further identification approaches and have the potential to be identified as species new to Thailand.

The collection of the 11 *Cephaleuros* species in Thailand supports the wide distribution of these foliicolous, parasitic green algae. Tropical and subtropical rain-forest habitats are favorable for the development of diverse subaerial algal flora. The information available on subaerial algae of tropical and subtropical regions is still limited, especially regarding parasitic algae that cause major or minor damage to several host plants. Future work could include updating collections, host range and disease severity studies, and molecular characterization.

ACKNOWLEDGMENTS

The copyediting service of RDO/PSU and the helpful comments of Seppo Karrila are gratefully acknowledged.

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Morphology and Behavior of Gametes and Zoospores from the Plant-Parasitic Green Algae, *Cephaleuro*s (Chlorophyta, Ulvophyceae)[1]

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[1] Funded by Prince of Songkla University (grant no. NAT590712S and NAT610511N) and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB) phase 2, Faculty of Natural Resources, Prince of Songkla University. Manuscript accepted_____.

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Abstract: The plant-parasitic green algae of *Cephaleuros* species infect leaves, twigs and fruits of numerous host plants worldwide. Reproductive structures of *Cephaleuros* are important in the infection process. The goal of this study was to determine the morphology and behavior of gametes and zoospores. The infection cycle of five *Cephaleuros* species, *Cephaleuros karstenii*, *C. pilosa*, *C. solutus*, *C. virescens* and *Cephaleuros* sp. was clarified by investigating the behavior of gametes and zoospores *in vitro*. Microscopic observations revealed that zoospores were ellipsoidal, rod-shaped, or spherical with four flagella. Gametes were spherical in shape with two flagella. Zoospores were released from all five *Cephaleuros* species but gametes were released only by *C. karstenii*, *C. solutus* and *Cephaleuros* sp. After their release from gametangia, gametes swarmed in a water drop in irregular and circular motions until the resting stage; some gametes conjugated and others burst. Zoospores were released from papilla-pores located at the base of zoosporangia and swarmed in a water drop in irregular and circular motions. Some zoospores did germinate and others burst, similar to the gametes. In the present study, germinated zoospores produced orange pigments and later withered in the water drop.

Keywords: Germination, reproductive cells, subaerial algae, Trentepohliaceae, Trentepohliales

INTRODUCTION

Green algae in the genus *Cephaleuros* Kunze ex E.M. Fries are plant parasites belonging to the order Trentepohliales, family Trentepohliaceae (Guiry and Guiry 2017). They are subaerial algae that infect leaves, twigs and fruits of numerous herbaceous and woody plant species worldwide (Printz 1939, Joubert and Rijkenbberg 1971, Thompson and Wujek 1997, Pitaloka et al. 2015, Sunpapao et al. 2015). On some plants the algae cause spot lesions on

leaves, twigs or fruits. Moderate to severe damage is found on tea (*Camelia sinensis* (L.) Kuntze) plantation crops (Marlatt and Alfieri 1981, Thompson and Wujek 1997, Suto and Ohtani 2009) and in guava (*Psidium guajava* Linn.) orchards in northern and southern Thailand (Sunpapao et al. 2016). *Cephaleuros* growth on leaves causes a loss of photosynthetic area due to necrosis of tissues beneath or around the algal thalli (Safeeulla and Govindu 1948). In Thailand, eleven species of *Cephaleuros* have been described recently: *Cephaleuros diffusus, C. druetii, C. expansa, C. henningsii, C. karstenii, C. lagerheimii, C. parasiticus, C. pilosa, C. solutus, C. tumidae-setae* and *C. virescens* (Sunpapao and Pitaloka 2015, Pitaloka et al. 2015, Sunpapao et al. 2015, 2016a, 2016b, 2016c, 2017; Wonglom et al. 2018). However, details of its parasitism are still unknown.

Reproduction of *Cephaleuros* species can be either asexual or sexual. Asexual reproduction produces zoospores with four flagella (quadriflagellate) in zoosporangia. Sexual reproduction produces gametes with two flagella (biflagellate) within gametangia. The gametangia are globular to irregular cells, which develop from terminals of the main filaments of an algal thallus (Thompson and Wujek 1997). The gametes and zoospores are considered the active entities that infect host plants (Mann and Hutchinson 1907, Wolf 1930, Suématu 1962, Chowdary and Jose 1979). Both types of reproductive cells are able to swim in surface moisture and settle on the surface of the host plant, where they can germinate forming new thalli on the epidermis or penetrate into the host tissues (Thompson and Wujek 1997). The algae deplete local water and mineral nutrients (Wolf 1930) and secrete harmful metabolites that damage host tissues (Joubert and Rijkenberg 1971). The reproductive cells of *Cephaleuros* species need water to spread on and within their host and to other host plants. Aspects of the infection process, especially behavior of the reproductive cells, still remain unknown. The goal of this research was to increase our knowledge of the morphology and behavior of the gametes and zoospores of five *Cephaleuros* species in Thailand.

MATERIALS AND METHODS

This study was conducted in the Pest Management Department field, Faculty of Natural Resources, Prince of Songkla University, Hatyai, Thailand. Algal thalli with mature reproductive structures were collected from leaves of *Annona muricata*, *Garcinia mangostana*, *Mangifera indica*, *Murraya paniculata*, *Piper nigrum* and *Sandoricum koetjjape*. The *Cephaeluros* morphospecies were identified using the key to species by Thompson and Wujek (1997). Macroscopic and microscopic features of asexual and sexual reproductive structures were observed under both stereo (Leica S8AP0, Leica, Germany) and light (Leica DM750, Leica, Germany) microscopes. The release of gametes and zoospores from gametangia and zoosporangia was examined once a year from 2015–2016. Algal thalli, 2–3 mm in diameter, were removed with a razor blade and placed on a drop of sterile water on a glass slide, without a cover slip. Reproductive cells (n=30) from 30 thalli of each *Cephaleuros* species were observed immediately under the light microscope. Conjugation of gametes was examined in a water drop immediately after release from gametangia. A glass slide with a water drop containing both gametes and zoospores was incubated in a covered Petri dish with moist paper to maintain humidity at ambient temperature (28±2°C) with 12:12 photoperiod. Germination of gametes and zoospores in the water drop was observed for 2–7 days using the light microscope.

RESULTS

Observation of reproductive structures

We found five *Cephaleuros* species on leaves of the five host taxa examined: *C. karstenii* on *A. muricata*, *C. pilosa* on *G. mangostana*, *C. solutus* on *S. koetjape*, *C. virescens* on *M. indica*, and *Cephaleuros* sp. on *M. paniculata* and *P. nigrum*. Both asexual and sexual structures were observed on the leaves (Fig. 1). Zoospores were observed in the zoosporangia

in amounts differing by species (Table 1). Zoospores of *C. karstenii*, *C. virescens* and *Cephaleuros* sp. (on *M. paniculata*) were ellipsoidal with W × L 5.37±0.21 × 6.76±0.34, 5.62±0.49 × 6.89±0.47, and 5.18±1.13 × 6.04±1.04 µm, respectively. Zoospores of *C. pilosa* and *Cephaleuros* sp. (on *P. nigrum*) were rods with W × L 2.91±0.85 × 9.63±0.78 and 2.88±0.56 × 9.72±0.18 µm, respectively. Zoospores of *C. solutus* were spherical with W × L 9.68±0.52 × 9.59±0.64 µm. Spherical shaped gametes were observed only in gametangia of *C. karstenii*, *C. solutus* and *Cephaleuros* sp. (on *P. nigrum*) with W × L 5.08±0.93 × 5.75±1.07, 4.58±0.52 × 5.03±1.94 and 5.76±1.46 × 5.58±0.81 µm, respectively (Table 2).

Behavior of gametes and zoospores in a water drop

Gametes and zoospores were released 5–10 min after thallus fragments were placed in a cool (10°C) water drop. After release, zoospores of the five *Cephaleuros* species swarmed actively for 1–5 min, performing circular movements for 1–3 rounds. Zoospores of all five species swarmed, then lost their flagella and burst. Zoospores of *C. solutus* and *C. virescens* germinated and formed germ tubes in a water drop 24 h after release from their zoosporangia. After release from gametangia, gametes of the five *Cephaleuros* species swarmed in a water drop for 10–30 min, then some burst whereas others remained active.

Germination of gametes and zoospores

In our experiments, gametes released from gametangia of *C. karstenii* and the *Cephaleuros* sp. from *P. nigrum* conjugated in a water drop (Fig. 4*C*). The zygotes did not germinate, however, and later burst (Fig. 4*C*). Zoospores of *C. solutus* and *C. virescens* germinated following release from their zoosporangia (Fig.5*A*–*G*), and then stopped after swarming. Zoospores of *C. solutus* were swollen, spherical to irregular in shape, 5–9 µm in diameter, with germ tubes 2.91 ± 0.56 µm long (Fig. 5*B*) beginning to develop at their apex (Fig. 5*A*

and *B*). Then the germ tubes formed unbranched filaments 11.43 ± 0.68 μm long after 4 days. Yellow orange carotenoid pigments were produced in the young filaments of *C. solutus* 5 days after zoospore release (Fig. 5*E–F*). For *C. virescens*, the germ tube was 2.31 ± 0.43 μm long 7 days after release from zoosporangia (Fig. 5*C–D*), and then the zoospores died (Fig. 5*G*). The morphological characteristics of the germ tubes did not differ among the *Cephaleuros* species in this study and no germination of gametes was observed.

DISCUSSION

In this study, released gametes were spherical in shape and zoospores were either ellipsoidal, rod-shaped, or spherical. When released, the zoospores appeared to have two flagella because the four flagella were twisted into two pairs. Previous reports described gametes and gametes as ellipsoidal to fusiform, but spherical when they lose their flagella (Suto and Ohtani 2009) and we found one morphology in the gametes (spherical) and three alternative morphologies in the zoospores. From this finding, zoospores of different shape were observed in different species (Fig. 2). This character has been rarely reported in studies on the Trentepohliales and might be useful for distinction at species level.

In this study we investigated the behavior of gametes and zoospores in a water drop. There is evidence of sexual conjugation of the gametes for *C. solutus* and *Cephaleuros* sp. from *P. nigrum*. However, no development of zygotes or dwarf plants was observed in the specimens examined. Similar observations have been reported previously regarding *C. solutus* and *C. virescens* (Suématu 1951, Thompson and Wujek 1997). However, no conjugation of gametes has been observed for *C. virescens* (Chowdary and Jose 1979, Suto and Ohtani 2013). Rindi and Guiry (2002) reported that gametes of the Trentepohlialian algae, *Trentepohlia abietina*, *T. aurea* and *Printzina lagenifera*, germinate and produce plants (sporophytes) without conjugation.

Gametes and zoospores were not released until their gametangia and zoosporangia were placed in water. This suggests that water is needed to release and spread reproductive cells of *Cephaleuros* species within the plant and to other host species. We also noticed that several unconjugated gametes and ungerminated zoospores burst immediately after release and formed masses of spheroidal cells. These are known as aplanospores (Suématu 1951) and do not contribute to the infection process.

Following germination, the young filaments of *C. solutus* and *C. virescens* produced orange pigments, haematochrom (Wolf, 1930). The germination of *Cephaleuros* in a water drop has been reported in five *Cephaleuros* species from Japan: *C. aucubae*, *C. biolophus*, *C. japonicus*, *C. microcellularis* and *C. virescens* (Suto and Ohtani 2013). The authors demonstrated different germination for the five *Cephaleuros* species (Suto and Ohtani 2013). There are no previous reports on germination of zoospore from *C. solutus*. From this current study, the germ tubes of *C. solutus* germinated from zoospores were longer than those of *C. virescens*, in a water drop (Fig. 5). However, both young filaments suffered in a water drop: the young filaments collapsed and desiccated. This finding suggests that although the reproductive cells are able to germinate or conjugate outside the host plants (in a water drop), they may need to penetrate the host tissues to deplete water and nutrients from the host plant for survival.

Here, we have reported an *in vitro* study on the behavior of reproductive cells in a water drop. The infection process in nature by algae in this genus is still unclear. However, Suto and Ohtani (2013) recently clarified that the infection cycles of five *Cephaleuros* species developed from gametes and zoospores during spring to summer (April to July) and the fresh thalli of the algae become evident in summer (August). The infection process by these algae is complicated and environmental factors including seasons may be significant. To assess

these phenomena in nature, the seasonal development of *Cephaleuros* species needs to be carefully documented.

ACKNOWLEDGMENTS

The authors thank Prince of Songkla University (grant no. NAT590712S and NAT610511N) and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB) phase 2, Faculty of Natural Resources, Prince of Songkla University, Thailand for funding and support for this project. The copy-editing service of RDO/PSU and the helpful comments of Dr. Seppo Karrila are gratefully acknowledged.

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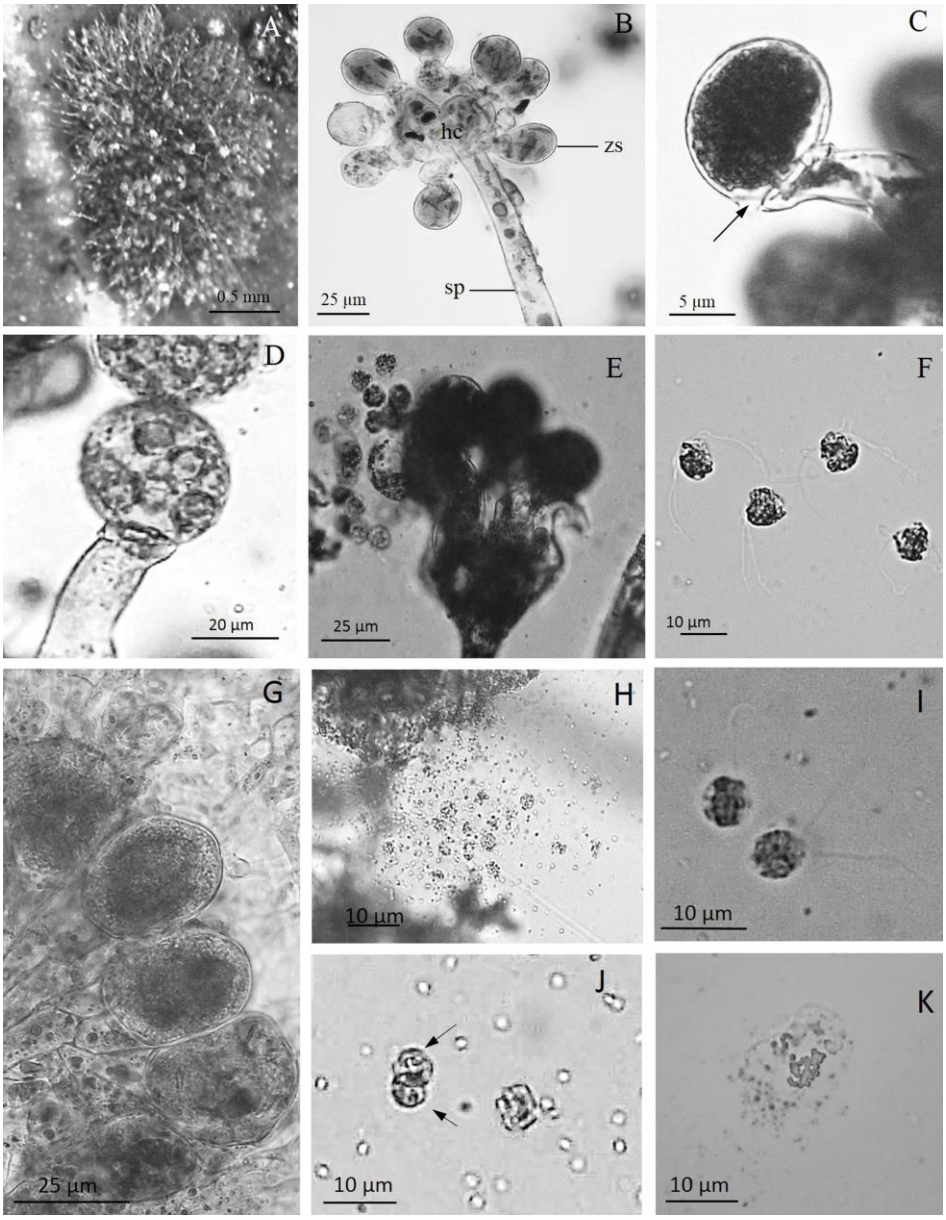

Figure 1. Characterization of *Cephaleuros* reproductive structures. A–F) asexual reproductive structure: A) lesion on host is composed of prostrate and erect system, B) detail of asexual reproductive structure composed of sporangiophores (sp), suffultory cell, head cells (hc) and zoosporangia (zs), C) papilla-pore at the base of sporangia (arrow), D) zoospores in zoosporangia, E) zoosporangia releases zoospores, F) quadriflagellate zoospores in the resting phase. G–K) sexual reproductive structure: G) gametangia, H) release of gametes from gametangia, I) biflagellate gametes, J) conjugation of gametes, and K) burst gametes.

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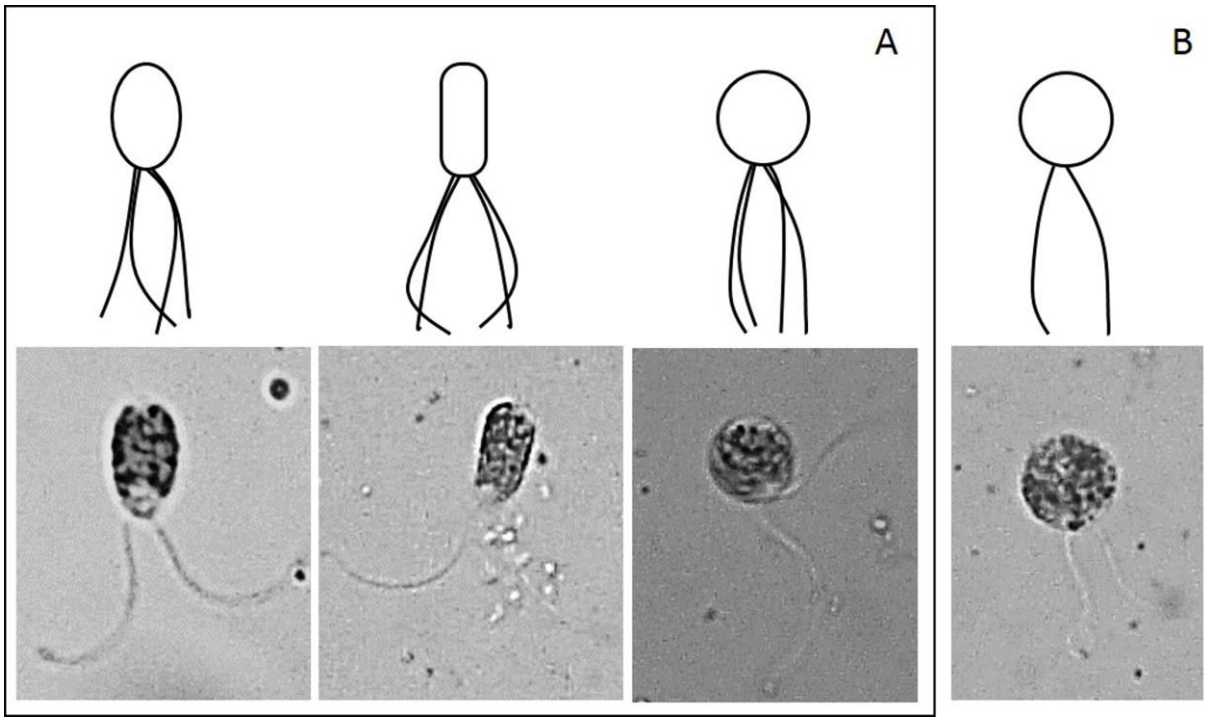

Figure 2. Morphology of asexual reproductive cells (zoospores) and sexual reproductive cells (gametes) of *Cephaleuros* species. A) ellipsoidal, rod- and spherical-shaped zoospores with quadriflagellae, and B) spherical, biflagellate gamete.

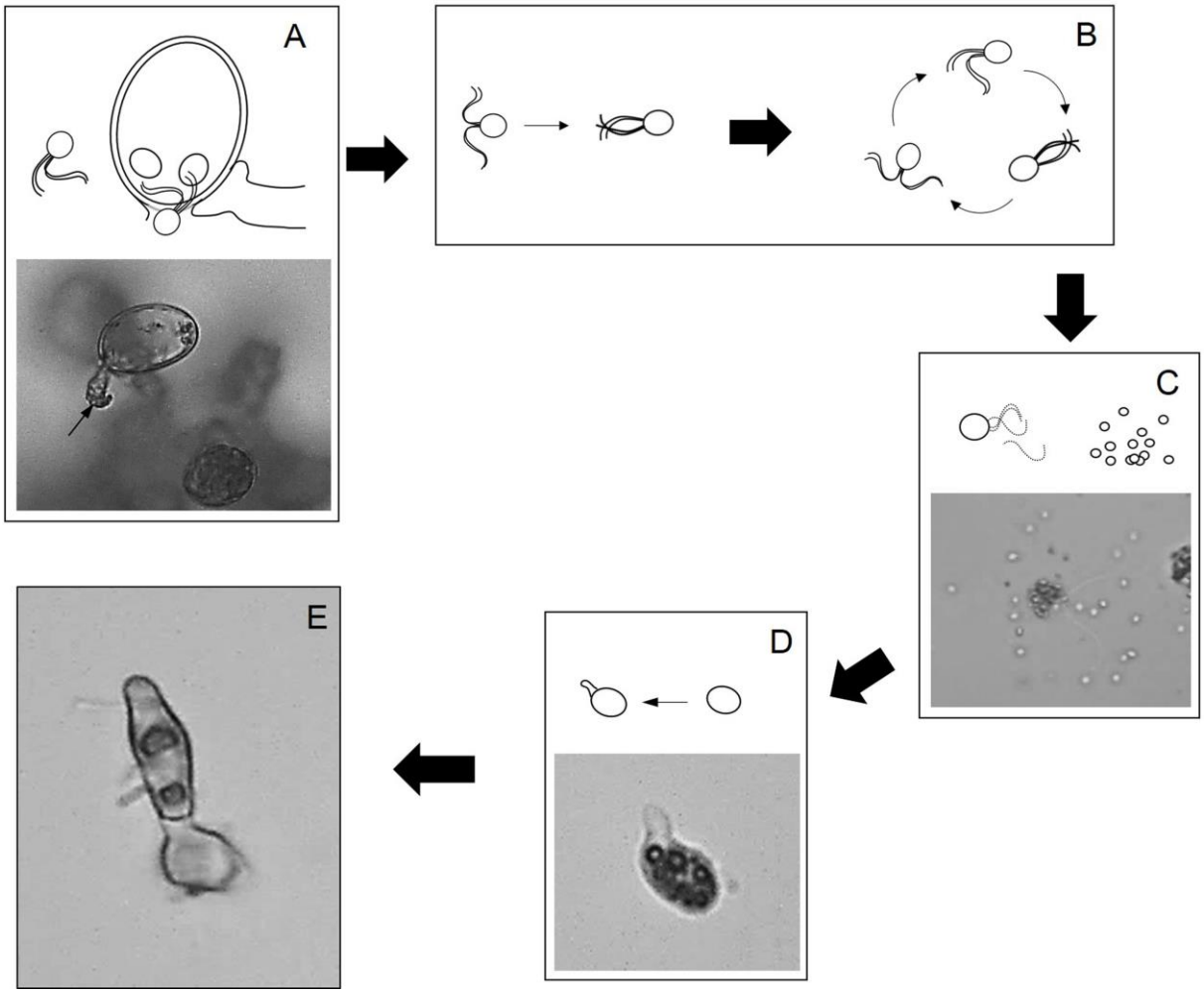

Figure 3. Germination and development of *Cephaleuros* zoospores in water: A) zoospores released from papilla-pore at the base of sporangia, B) zoospores swim in irregular and circular motions, C) zoospores stop movement, release flagella or burst, D) zoospores germinate by germ tube at the apex, and E) the germ tube elongates and produces orange pigments.

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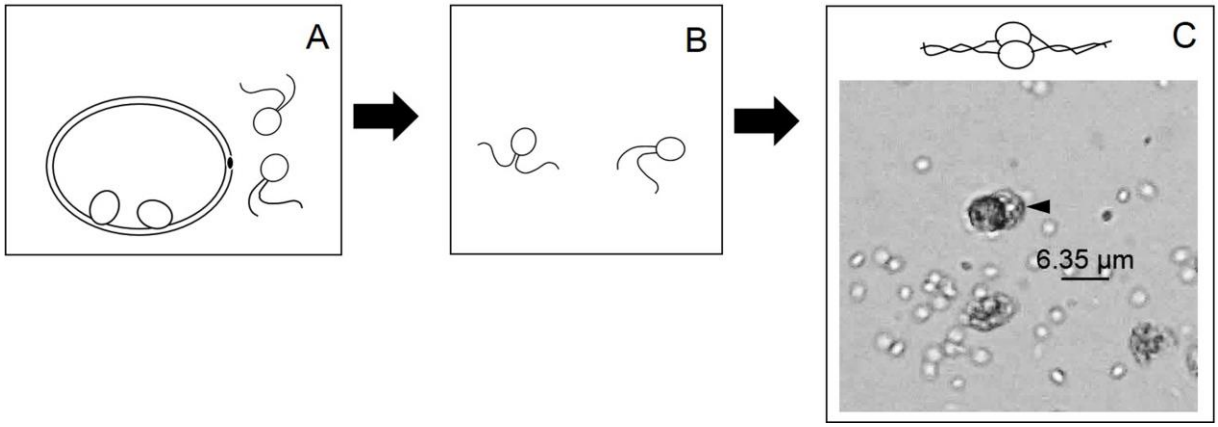

Figure 4. Behavior of *Cephaleuros* gametes in a water: A) gametes released from papilla-pore of gametangia, B) gametes swim in a water drop, C) unconjugated gametes burst while conjugated gametes fuse (arrow).

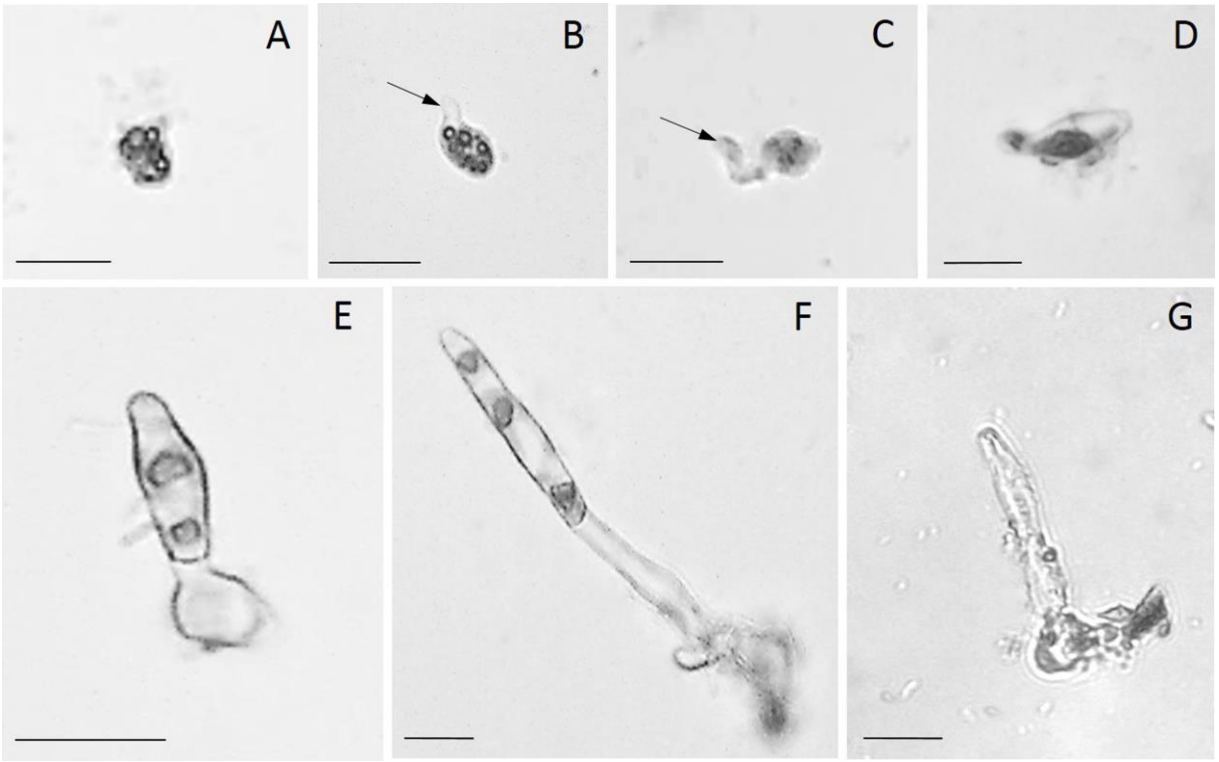

Figure 5. Germination of *Cephaleuros* zoospores in water: A) zoospore 1 day after release, B) zoospore germination, C) zoospore germinates and forms a filament (arrow), D) deformation of zoospore, E) first cell of a young filament, F) a young filament seven days after germination of the sporangia, and G) desiccated filamentous cell. Scale bar = 10 μm.

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Table 1

General Characteristics of *Cephaleuros* Zoospores

Cephaleuros species	host plant	number of zoospores	shape	width[1] (μm)	length[1] (μm)
C. karstenii	*Annona muricata*	24–49	ellipsoidal	5.37±0.21	6.76±0.34
C. pilosa	*Garcinia mangostana*	3–8	rod	2.91±0.85	9.63±0.78
C. solutus	*Sandoricum koetjape*	21–44	spherical	9.68±0.52	9.59±0.64
C. virescens	*Mangifera indica*	21–47	ellipsoidal	5.62±0.49	6.89±0.47
Cephaleuros sp.	*Murraya paniculata*	18–31	rod	2.88±0.56	9.72±0.18
	Piper nigrum	4–7	ellipsoidal	5.18±1.13	6.04±1.04

[1] Average of 30 replicates (n = 30)

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Table 2

General Characters of *Cephaleuros* Gametes

Cephaleuros species	host plants	number of gametes	shape	width[1] (μm)	length[1] (μm)
C. karstenii	*Annona muricata*	27–51	spherical	5.08±0.93	5.75±1.07
C. pilosa	*Garcinia mangostana*	–[2]	–	–	–
C. solutus	*Sandoricum koetjape*	11–34	spherical	4.58±0.52	5.03±1.94
C. virescens	*Mangifera indica*	–	–	–	–
Cephaleuros sp.	*Murraya paniculata*	–	–	–	–
	Piper nigrum	114–155	spherical	5.76±1.46	5.58±0.81

[1] Average of 30 replicates (n = 30)

[2] not detected

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