

SP11_011_PF: DIVERSITY OF FOLIICOLOUS LICHENIZED FUNGI IN MANGROVE FOREST FROM CHUMPHON PROVINCE, THAILAND

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Abstract:

Foliicolous lichenized fungi (FLF) in mangrove forests are poorly studied. A few documents have been reported on this group of lichens in Thailand. Thus, this work aimed to study the diversity of FLF in mangrove forests in Chumphon province. A total of 274 specimens were collected from various mangrove tree leaves in February 2018 and identified based on morphological, anatomical and chemical characteristics. In the present study, 10 families, 11 genera and 17 species were recorded including *Arthonia lividula* Vain., *A. trilocularis* Müll. Arg., *Bacidina pallidocarnea* (Müll. Arg.) Vězda, *Byssoloma subdiscordans* (Nyl.) P. James, *Calopadia fusca* (Müll. Arg.) Vězda, *C. puiggarii* (Müll. Arg.) Vězda, *C. subcoerulescens* (Zahlbr.) Vězda, *Coenogonium dilucidum* (Kremp.) Kalb & Lücking, *Dirinaria confluens* (Fr.) DD. *Graphis furcata* Fée., *G. pinicola* Zahlbr., *Mazosia phyllosema* (Nyl.) Zahlbr., *Porina kamerunensis* F. Schill., *P. nitidula* Müll. Arg., *Strigula antillarum* (Fée) Müll. Arg., *S. smaragdula* Fr., *Tricharia demoulini* Sérus. Among them, *S. smaragdula* was commonly found with 72 observed specimens and almost all of them were collected from the leaves of *Xylocarpus granatum* J. Koenig. A key to all species of FLF was provided together with their pictures.

Introduction:

Mangrove forests are among the world's most productive ecosystems which provide habitat and an important source of nutrients for a variety of species.¹ These forests which are distributed throughout tropical and sub-tropical regions encompass 118 countries.² Mangroves in Thailand have covered approximately 1,435,116 Rai along the southern and eastern coasts.³

Lichens are a unique group of fungi living in a symbiotic relationship with an alga or cyanobacterium. They are autotrophs which have an ability to grow on various kinds of substrata. The lichens are classified according to their substrates, including saxicolous, corticolous, terricolous, lignicolous, and foliicolous.

Foliicolous lichenized fungi (FLF) or foliicolous lichens grow entirely on the living leaves of vascular plants and reproduces on surfaces of the living leaves. Most of them are found in tropical areas where high humidity and low light intensity prevail^{4,5} and more than 800 species have been reported worldwide.⁴

The manglicolous lichens are a specific group of lichens which occur in association with mangrove plants. These lichens can adapt to hot, humid and saline breeze environmental conditions prevailing in the mangroves. There are numerous publications on corticolous lichens in mangroves; however, very few of these were on FLF.

FLF in Thailand has been reported by many distinguished lichenologists. In 1998, Boonpragob et al.⁶ listed 34 of FLF from Khao Yai National Park. Buaruang et al.⁷ published a checklist of lichens in Thailand with 1,292 taxa of which about 180 are FLF. Three new to science and 20 new recorded species were added to Thai lichens by Naksuwankul and Lücking in 2019.⁸ However, a few studies of FLF have been done in mangrove forests. Thus, the main purpose of our work was to investigate the diversity of FLF in mangrove forests of Chumphon province.

Methodology:

FLF samples in mangrove forests of Chumphon province were collected from four collection sites; (1) Bang Nam Chud Subdistrict, Lang Suan District (10° 3.468'N 99° 7.601'E) (BN), (2) Haad Sai Ri Subdistrict, Mueang District (10° 21.564'N 99° 13.953'E) (HS), (3) Bang Son Subdistrict, Pathio District (10° 41.494'N 99° 19.870'E) (BS) and (4) Chum Kho Subdistrict Pathio District (10° 41.619'N 99° 19.900'E) (CK) (Figure 1 A-B). All the specimens were air-dried at room temperature for a week before identification. The external morphological characters of thallus and ascomata were examined with an Olympus SZ30 stereomicroscope and images were made by microscope Eye-Piece Camera (Dino-Eye). The anatomical features were investigated by the hand-cut section of thalli and ascoma by razor blade. The iodine reaction of the hymenium and ascospores were studied in Lugol's iodine solution. All sections were mounted in water and observed under light microscope (Olympus CH). Lichen chemistry of thalli and ascomata was characterized by spot tests. The specimens were identified by comparing to own description and using the keys of Lücking 2008,⁴ Naksuwankul and Lücking 2019,⁸ Aptroot et al. 2007,⁹ Ferraro and Lücking 1997,¹⁰ Lücking et al. 2009,¹¹ Rashmil and Rajkumar 2015,¹² Singh and Pinokiyo 2018,¹³ Swinscow and Krog 1988¹⁴ and Santesson 1952.¹⁵

Results and Discussion:

The detailed morphological, anatomical and chemical characters of 274 FLF specimens from the mangrove forests in Chumphon province were identified into 17 species under 11 genera and 10 families (Table 1). The results indicated that FLF in the mangrove forest had the diversity of lichens dominated by crustose growth form with 16 species and *Dirinaria confluens* was the only foliose species found in this study (Figures 2-4). The highest species diversity was found in BN with 15 species followed by BS, CK and HS with 10, 5 and 4 species, respectively.

Members of the families Arthoniaceae, Porinaceae and Strigulaceae were the most common inhabitants of the mangrove forests (Table 1). The family Pilocarpaceae showed the maximum species diversity with 4 species. It was followed by Arthoniaceae, Graphidaceae, Porinaceae and Strigulaceae with 2 species each. Almost all of the species found in this study were also reported from the mangrove forests of Sandarbans Biosphere Reserve, India.¹⁶

The highest number of collections with 72 observed specimens belonged to *Strigula smaragdula*. This species was also reported as a common species from India and as worldwide species.¹³ Similarly, in the family Porinaceae, two species of, *Porina kamerunensis* and *S. antillarum* showed high numbers of collections. In contrast, *Bacidina pallidocarnea*, *C. dilucidum* and *Graphis pinicola* were rarely observed (Table 1).

Observations on substrate preferences of FLF revealed that 13 species were found on various mangrove tree leaves, whereas four species including *B. pallidocarnea*, *Mazosia phyllosema*, *G. pinicola* and *Coenogonium dilucidum* were only found on the leaves of *Heritiera littoralis* Ait. *Porina kamerunensis* was the most generalist lichen which was collected from 4 species of plant. The leaves of *Heritiera littoralis* Ait., were the most preferred substrata which supported 15 species (Figure 1C), followed by *Acrostichum aureum* L. (13 spp.), *Xylocarpus granatum* (2 spp.), *X. moluccensis* (4 spp.), *Bruguiera parviflora* Roxb., (1 sp.) *Hibiscus tiliaceus* L. (1 sp.) and unknown (1 sp.) (Table 2). Interestingly, in Thailand, *D. confluens*, *G. furcata*, *G. pinicola* and *B. subdiscordans* were reported earlier as corticolous forms which were now recorded as foliicolous forms.

Key to FLF in mangrove forests from Chumphon province

1a.	Thallus folios, gray to greenish gray, medulla K+ Yellow.....	<i>Dirinaria confluens</i>
1b.	Thallus crustose.....	2
2a.	Ascomata perithecia; photobiont trentepohlioid (<i>Trentepohlia</i> or <i>Phycopeltis</i>).....	3
2b.	Ascomata apothecia; photobiont Chlorococcoid (<i>Trebouxia</i>) or Trentepohlioid (<i>Trentepohlia</i> , <i>Phycopeltis</i> , or <i>Cephaleuros</i>).....	6
3a.	Ascospores 1-septate; perithecia, immersed-erumpent to adnate, lens-shaped to wart-shaped or conical; pycnidia immersed lacking beak.....	4
3b.	Ascospores 3–5-septate; perithecia immerse, subglobose, yellowish to black.....	5
4a.	Perithecia are comparatively rare; pycnidia aggregate and confluent in center of thallus patches, semi-immersed, wart-shaped.....	<i>Strigula antillarum</i>
4b.	Perithecia are common, covered by thallus tissue, dark green, 0.3–0.5 mm; ascospores usually biseriolate; pycnidia semi-immersed, wart-shaped.....	<i>Strigula smaragdula</i>
5a.	Ascospores 3-septate; perithecia 0.15–0.25 mm, lens-shaped to hemispherical or applanately conical, slightly prominent, base often spreading.....	<i>Porina kamerunensis</i>
5b.	Ascospores 5-septate; perithecia 0.15–0.3 mm, subglobose black but usually with short, white hairs or papillae around ostiole.....	<i>Porina nitidula</i>
6a.	Apothecia rounded to irregular-angular in outline, lobate or lirellate.....	7
6b.	Apothecia rounded or slightly irregular disc-like.....	10
7a.	Apothecial margin dark brown to black, carbonized; ascospore lens-shaped, I+ violet; paraphyses unbranched.....	8
7b.	Apothecial margin pale or brown; ascospores macrocephalic, 2-septate.....	9
8a.	Lirellae erumpent, short to elongate, labia non-pruinose or rarely thinly white-pruinose, lirellae thin 0.1–0.2 mm; ascospores up to 45 µm long.....	<i>Graphis furcata</i>
8b.	Lirellae prominent to sessile, short to very short, with lateral thalline margin, elongate and irregularly branched; ascospores small 9–11 µm broad.....	<i>Graphis pinicola</i>
9a.	Mature ascospores colourless, 9.5–12 × 4–5 µm, apothecia light to dark brown or with a bluish tinge, non-pruinose, K-.....	<i>Arthonia lividula</i>
9b.	Mature ascospores greyish brown, 10–20 × 4–6 µm; apothecia dark brown to black, K or K+ greenish, non-pruinose.....	<i>Arthonia trilocularis</i>
10a.	Apothecia immersed-erumpent.....	11
10b.	Apothecia adnate to sessile.....	12
11a.	Photobiont <i>Phycopeltis</i> ; apothecial disc dark grey to black, immersed-erumpent; ascospores slightly thick-walled, with median cell slightly enlarged.....	<i>Mazosia phyllosema</i>
11b.	Photobiont <i>Trebouxia</i> ; apothecia pale brownish; thallus ecorticate or cortex cartilaginous; with sterile black setae.....	<i>Tricharia demoulinii</i>
12a.	Photobiont <i>Trentepohlia</i> ; apothecia wax-colored to pale yellow, with flat to slightly concave disc; asci entirely thin-walled, unitunicate, I- or I+ bluish-brownish; ascospores 6–12 × 2.5–3.5 µm.....	<i>Coenogonium dilucidum</i>
12b.	Photobiont chlorococcoid (<i>Trebouxia</i>).....	13
13a.	Ascospores transversely septate.....	14
13b.	Ascospores muriform.....	15
14a.	Ascospores fusiform-ellipsoid to narrowly bacillar or filiform and then typically tapering towards proximal end, (3)–7-septate, 40–60 µm long.....	<i>Bacidina pallidocarnea</i>
14b.	Ascospores cylindrical to filiform-acicular, 1–3-septate, 8–15 µm long.....	<i>Byssoloma subdiscordans</i>
15a.	Apothecial disc (at least in young apothecia) greyish black to black; hypothecium aeruginous.....	<i>Calopadia subcoerulescens</i>
15b.	Apothecial disc light to dark brown; hypothecium light to dark brown.....	17
16a.	Apothecial disc light brown to reddish brown; hypothecium light brown.....	<i>Calopadia fusca</i>
16b.	Apothecial disc greyish brown to dark brown; hypothecium dark brown.....	<i>Calopadia puiggarii</i>



Figure 1. A. Map of four collection sites in Chumphon province; (1) Bang Nam Chud Subdistrict, (2) Haad Sai Ri Subdistrict, (3) Bang Son Subdistrict, (4) Chum Kho Subdistrict. B. Mangrove forest at Chumphon province and C. Leaves of *Heritiera littoralis* Ait.

Table 1. The families, species and number of specimens of four studied areas

Family	Lichen species	Number of specimens				Total
		HS	BN	BS	CK	
Arthoniaceae	<i>Arthonia lividula</i>	4	12			16
	<i>Arthonia trilocularis</i>		8		4	12
Caliciaceae	<i>Dirinaria confluens</i>		12		2	14
Coenogoniaceae	<i>Coenogonium dilucidum</i>		2			2
Gomphillaceae	<i>Tricharia demoulinii</i>		7			7
Graphidaceae	<i>Graphis furcata</i>		4	1		5
	<i>Graphis pinicola</i>		1			1
Pilocarpaceae	<i>Byssoloma subdiscordans</i>		15	5	2	22
	<i>Calopadia fusca</i>		6	6		12
	<i>Calopadia puiggarii</i>		8	12	1	21
	<i>Calopadia subcoerulescens</i>			4		4
Porinaceae	<i>Porina kamerunensis</i>	25	5			30
	<i>Porina nitidula</i>		11	10		21
Ramalinaceae	<i>Bacidina pallidocarnea</i>			2		2
Roccellaceae	<i>Mazosia phyllosema</i>		1	2	1	4
Strigulaceae	<i>Strigula antillarum</i>	3	26			29
	<i>Strigula smaragdula</i>	58	1	13		72
Total specimens		90	119	55	10	274

Note: (HS) = Haad Sai Ri Subdistrict, (BN) = Bang Nam Chud Subdistrict, (BS) = Bang Son Subdistrict and (CK) = Chum Kho Subdistrict

Table 2. List of foliicolous lichens and their hosts

Lichen species	Number of specimens							Total
	Aa	Bp	Hl	Ht	Xg	Xm	U	
<i>Arthonia lividula</i>	7		5			4		16
<i>Arthonia trilocularis</i>	2		10					12
<i>Bacidina pallidocarnea</i>			2					2
<i>Byssoloma subdiscordans</i>	8		14					22
<i>Calopadia fusca</i>	4		8					12
<i>Calopadia puiggarii</i>	3		18					21
<i>Calopadia subcoerulescens</i>	1		3					4
<i>Coenogonium dilucidum</i>			2					2
<i>Dirinaria confluens</i>	6		8					14
<i>Graphis furcata</i>	4		1					5
<i>Graphis pinicola</i>			1					1
<i>Mazosia phyllosema</i>			4					4
<i>Porina kamerunensis</i>	4	19	1			6		30
<i>Porina nitidula</i>	8		12				1	21
<i>Strigula antillarum</i>	1			7	16	5		29
<i>Strigula smaragdula</i>	1				9	62		72
<i>Tricharia demoulinii</i>	4		3					7
Total specimens	50	19	95	7	25	77	1	274

Note: Aa = *Acrostichum aureum*, Bp = *Bruguiera parviflora*, Hl = *Heritiera littoralis*, Ht = *Hibiscus tiliaceus*, Xg = *Xylocarpus granatum*, Xm = *Xylocarpus moluccensis* and U = Unknown

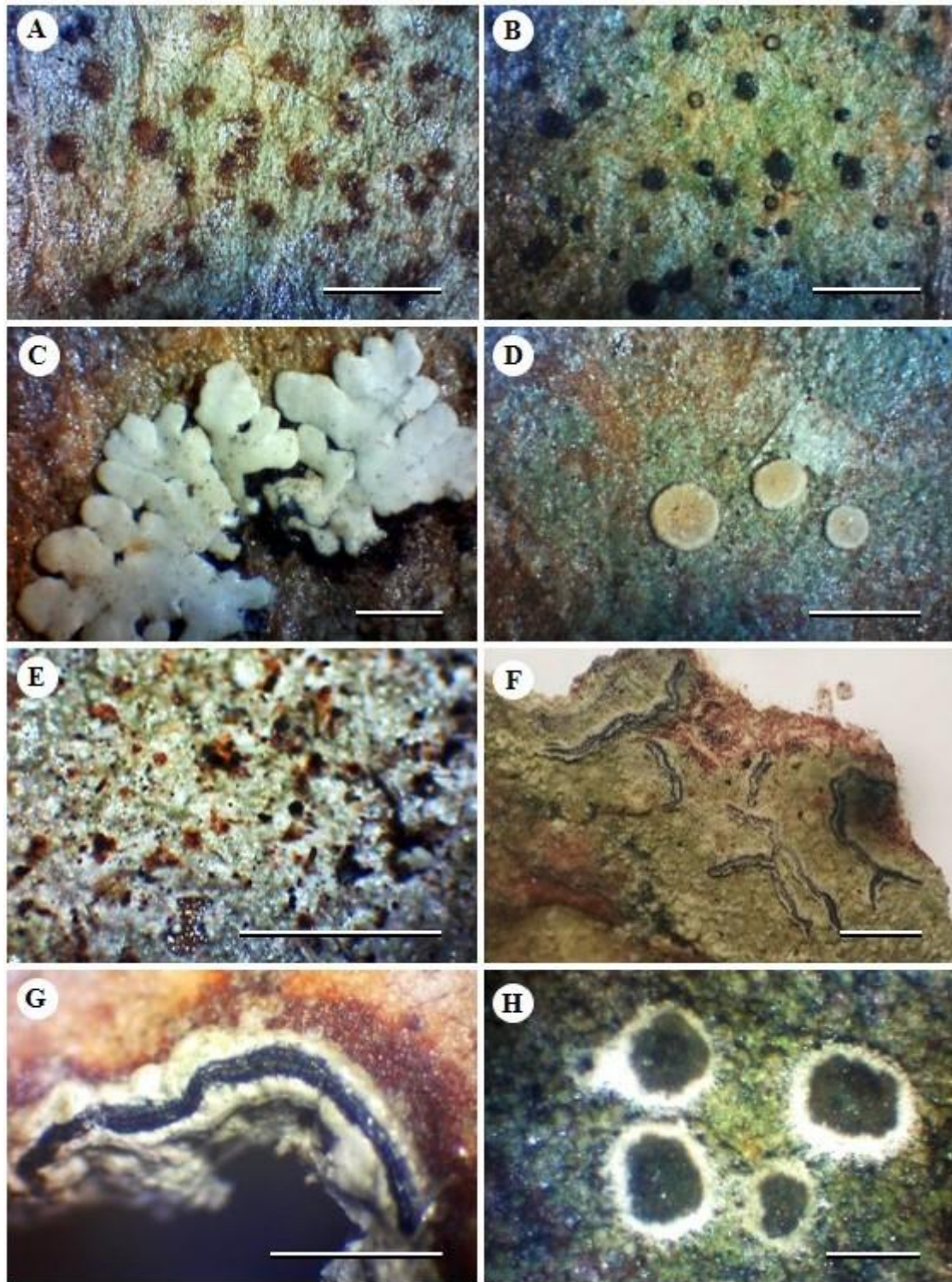


Figure 2.

Thallus with ascomata of FLF. A. *Arthonia lividula*, B. *Arthonia trilocularis*, C. *Dirinaria confluens*, D. *Coenogonium dilucidum*, E. *Tricharia demoulinii*, F. *Graphis furcate*, G. *Graphis pinicola* and H. *Byssoloma subdiscordans*. Scale = 0.5 mm.

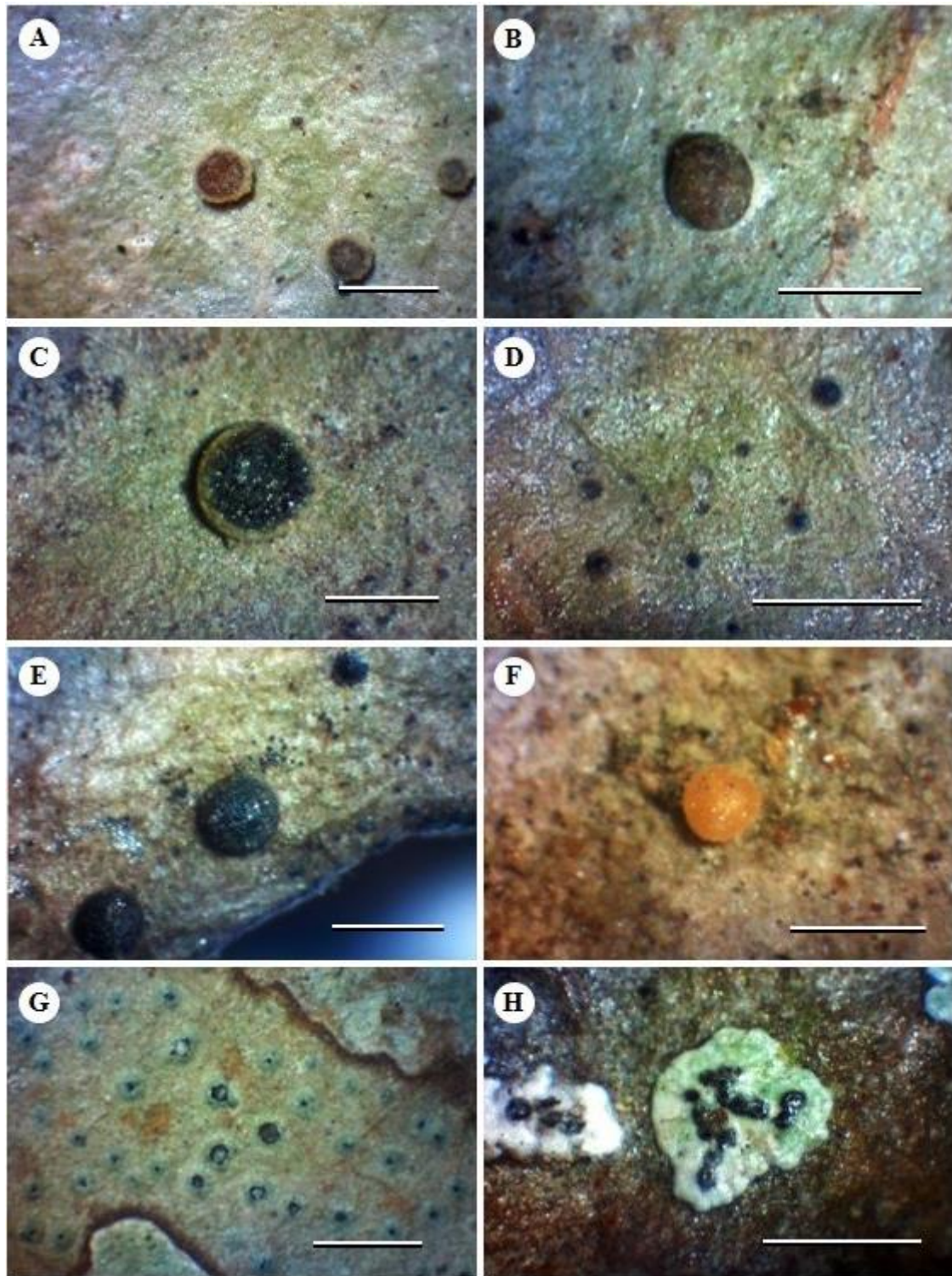


Figure 3.

A-G = Thallus with ascomata of FLF. A = *Calopadia fusca*, B = *Calopadia puiggarii*, C = *Calopadia subcoerulescens*, D = *Porina kamerunensis*, E = *Porina nitidula*, F = *Bacidina pallidocarnea*, G = *Mazosia phyllosema* and H = Thallus and pycnidia of *Strigula antillarum*. Scale = 0.5 mm.

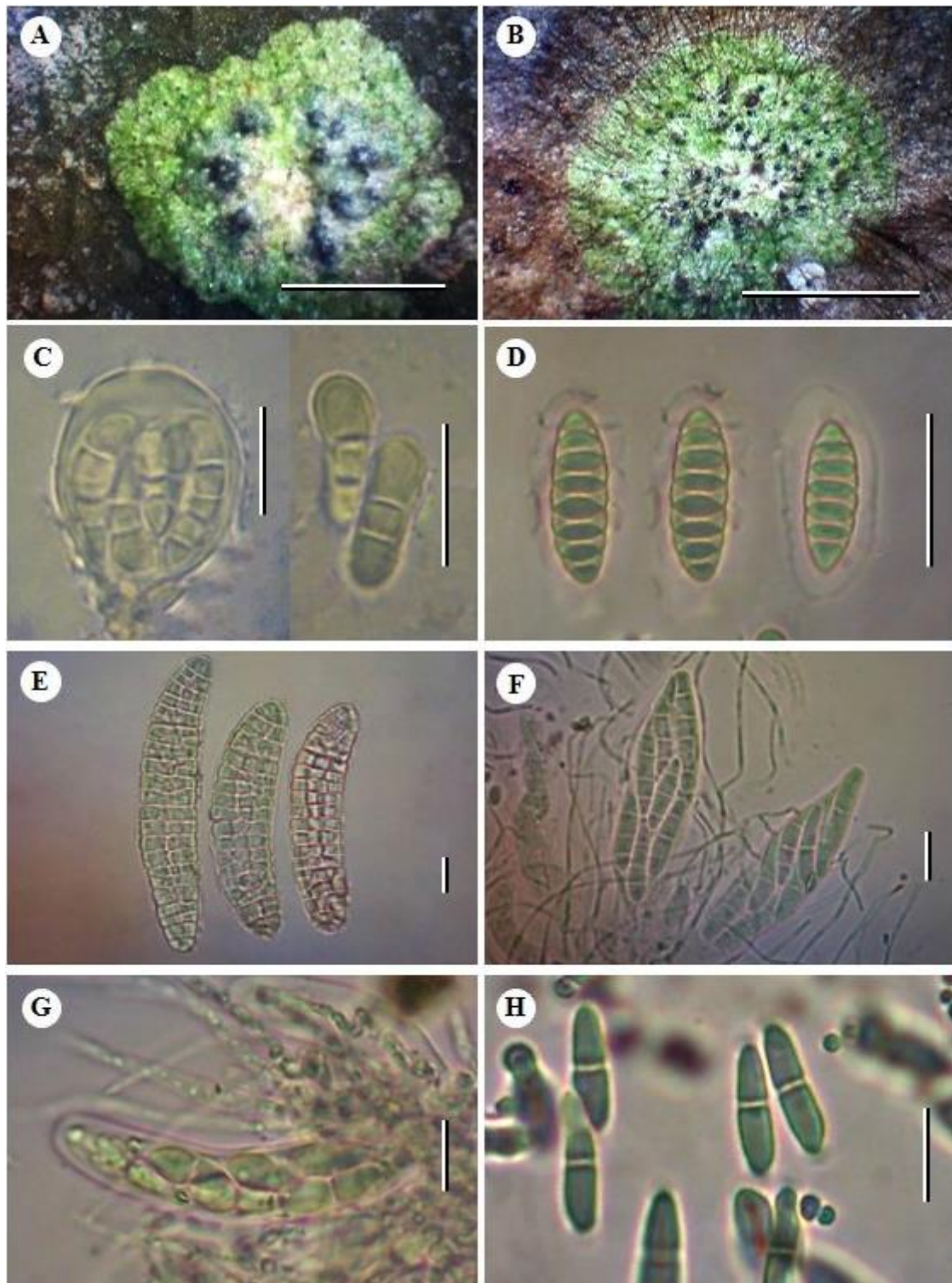


Figure 4.

A-B = *Strigula smaragdula*, A = Thallus with ascumata, B = Thallus with pycnidia. C-H = Ascospores and conidia of FLF. C = Asci and ascospores (*Arthonia lividula*), D = Ascospores (*Graphis pinicola*), E = Ascospores (*Calopadia subcoerulescens*), F = Asci and ascospores (*Porina kamerunensis*), G = Asci and ascospores (*Strigula smaragdula*) and H = Macroconidia (*Strigula antillarum*). Scale for A-B = 0.5 mm; for C-H = 10 μ m.

Conclusion:

The present study was the first-time report on the diversity, distribution and associated host mangrove species of FLF at the mangrove forests of Chumpon province. All 274 samples were classified into 10 families, 11 genera and 17 species. Pilocarpaceae showed the highest diversity with 4 species. *Xylocarpus granatum* was the most preferable mangrove species which housed 15 lichen species.

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