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Standard Paper

A molecular phylogeny of *Pilocarpaceae* Zahlbr., including a new species of *Tapellaria* Müll. Arg. and new records of foliicolous lichenized fungi from Thailand

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Abstract

The phylogeny of foliicolous taxa in *Pilocarpaceae* was reconstructed using Bayesian and maximum likelihood analyses of concatenated ITS and mtSSU sequences. Sixty-six new partial sequences representing 36 taxa were generated and 29 sequences were downloaded from GenBank. Our results indicate that *Lasioloma* R. Sant. is nested within a paraphyletic *Calopadia* Vězda, whereas *Fellhanera* Vězda is polyphyletic and the phylogenetic relationships of *Eugeniella* Lücking *et al.* and *Sporopodium* Mont. with *Fellhanera* require further investigation. In addition, *Tapellaria parvimuriformis* W. C. Wang & J. C. Wei is described as a new species from Khao Yai National Park, Thailand, and is characterized by its small muriform ascospores, grey campylidia with a pale base, and short conidia. Eight new records of foliicolous lichens for Thailand are also listed.

Key words: Asia, Bayesian analysis, foliicolous lichens, ITS, mtSSU

(Accepted 18 April 2020)

Introduction

Foliicolous lichens are one of the most widespread groups of lichenized fungi in tropical South-East Asia, but studies on these lichens in this region are comparatively few (Krempelhuber 1874; Müller 1890*a*, *b*; Nylander 1891; Vainio 1920, 1923; Santesson 1952; Vězda 1977; Aptroot & Sipman 1993; Lücking & Sérusiaux 1997; Boonpragob *et al.* 1998; Papong *et al.* 2007, 2009; Sérusiaux *et al.* 2008; Nguyen *et al.* 2010, 2011; Neuwirth *et al.* 2014; Naksuwankul & Lücking 2019), and no modern systematic inventory has yet been undertaken.

Thailand has a great diversity of suitable biotopes for the growth of foliicolous lichens. However, its foliicolous taxa remain underexplored, although recent studies have recorded a total of 132 foliicolous species (Boonpragob *et al.* 1998; Papong *et al.* 2007, 2009; Neuwirth *et al.* 2014; Naksuwankul & Lücking 2019).

Pilocarpaceae in its broad sense is a crustose, cosmopolitan family comprising 29 genera, with 424 currently recognized species (Lücking *et al.* 2017). Species of *Pilocarpaceae* are characterized by adnate to sessile, biatorine or lecideine apothecia, non-septate to muriform, colourless ascospores, and its conidiomata are pycnidia or campylidia. It is widely distributed in tropical regions around the world, where most species grow on leaves (Lücking 2008).

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Until recently, morphological characters have been the main tool for the delimitation of clades within *Pilocarpaceae*, with DNA-based phylogenies still scarce. Andersen & Ekman (2005) studied the phylogeny of *Micareaceae* and suggested that this family should be reduced to synonymy with *Pilocarpaceae*; *Badimia*, previously classified in *Pilocarpaceae*, has recently been included in *Ramalinaceae* following molecular work (Kistenich *et al.* 2018). In the present study, we aimed to produce new DNA sequence data for tropical *Pilocarpaceae* and to study their morphology in order to increase knowledge of the phylogenetic relationships of these lichens.

We generated new sequence data from specimens primarily collected in China and Thailand for a phylogenetic reconstruction of taxa in *Pilocarpaceae*. We also report some additional results of taxonomic studies of Thai foliicolous lichens. A main finding is the discovery of a new species of the genus *Tapellaria*, with a further eight new species records for Thailand from families *Arthoniaceae*, *Gomphillaceae*, *Pilocarpaceae* and *Ramalinaceae*.

Materials and Methods

Morphology and anatomy

The study is based on the extensive collections of the first author from Thailand and China during fieldwork and lichenological research in 2017–2019. The material is deposited in the herbaria HMAS-L and RAMK, with the exception of one sample from Surinam which is in the private herbarium of Pieter van den Boom. Morphology and anatomy were examined using a Motic dissecting microscope and an Olympus CX21 compound

Table 1. Details of specimens (species name, locality, herbarium, GenBank numbers) of *Pilocarpaceae* used in the phylogenetic analysis in this study. Newly generated sequences are in bold. * = outgroup (*Byssolomataceae*).

Taxon	Locality	Voucher specimens	ITS-GenBank No.	mtSSU-GenBank No.
Byssolecania hymenocarpa	Thailand	*W. C. Wang* KYW0286 (RAMK-31639)	**MK946957**	**MK957152**
B. hymenocarpa	Thailand	*W. C. Wang* KYW0254 (RAMK-31633)	**MK946965**	**MK957159**
Byssolecania sp.	China, Yunnan	*W. C. Wang* 20180247 (HMAS-L 144266)	**MK946973**	**MK957170**
B. variabilis	Costa Rica	*Lücking* 16033b	AY756458	AY567780
Byssoloma leucoblepharum	Thailand	*W. C. Wang* KYW0405 (RAMK-31929)	**MK946966**	**MK957160**
B. leucoblepharum	China, Yunnan	*W. C. Wang* 20180153 (HMAS-L 140621)	**MK946970**	**MK957165**
B. leucoblepharum	China, Yunnan	*W. C. Wang* 20180145 (HMAS-L 140613)	**MK946971**	**MK957166**
B. leucoblepharum	China, Hainan	*W. C. Wang* HN20170357 (HMAS-L 139782)	**MK946977**	**MK957174**
B. leucoblepharum	Portugal	*Ekman* 3502	AY756459	AY567778
B. meadii	USA	*Ekman* L1130 (LD)		AY567776
B. subdiscordans	USA	*Tønsberg* 25968	AY756461	AY567779
Calopadia foliicola	Thailand	*W. C. Wang* KYW0068 (RAMK-31790)	**MK946951**	**MK957146**
C. foliicola	Thailand	*W. C. Wang* KYW0035 (RAMK-31537)	**MK946953**	**MK957148**
C. foliicola	Thailand	*W. C. Wang* KYW0251 (RAMK-31625)	**MK946958**	**MK957153**
C. foliicola	Thailand	*W. C. Wang* KYW0036-2 (RAMK-31538)	**MK946962**	**MK957157**
C. foliicola	Costa Rica	*Lücking* 16011	AY756462	AY567782
C. phyllogena				KJ766365
C. puiggarii	Thailand	*W. C. Wang* KYW0036-1 (RAMK-31764)	**MK946961**	**MK957156**
C. puiggarii	China, Yunnan	*W. C. Wang* 20180158 (HMAS-L 140626)	**MK946972**	**MK957167**
C. puiggarii	China, Hainan	*W. C. Wang* HN20170381 (HMAS-L 139789)	**MK946975**	**MK957172**
Calopadia sp.	Thailand	*W. C. Wang* KYW0365 (RAMK-31869)	**MK946968**	**MK957163**
Eugeniella micrommata	Thailand	*W. C. Wang* KYW0046 (RAMK-31779)		**MK957161**
Fellhanera bouteillei	Thailand	*W. C. Wang* KYW0558 (RAMK-31760)		**MK957177**
F. bouteillei	Sweden	*Ekman* 3417	AY756463	AY567787
F. bouteillei				KJ766392
F. bouteillei		CH-Lucerne LC-118	KX132990	
F. bouteillei			AF414858	
F. fuscatula	Thailand	*W. C. Wang* KYW0462 (RAMK-31976)	**MK946954**	**MK957149**
F. fuscatula	Thailand	*W. C. Wang* KYW0392 (RAMK-31669)	**MK946956**	**MK957151**
F. fuscatula	Thailand	*W. C. Wang* KYW0336 (RAMK-31862)	**MK946959**	**MK957154**
F. fuscatula	Thailand	*W. C. Wang* KYW0264 (RAMK-31630)	**MK946967**	**MK957162**
F. microdiscus	China, Hainan	*W. C. Wang* HN20170313 (HMAS-L 139758)	**MK946978**	**MK957175**
Fellhanera sp.	China, Guizhou	*W. C. Wang* 20180711 (HMAS-L 144462)	**MK946950**	**MK957145**
Fellhanera sp.	China, Yunnan	*W. C. Wang* 20180146 (HMAS-L 140614)	**MK946969**	**MK957164**
Fellhanera sp.	China, Yunnan	*W. C. Wang* 20180166 (HMAS-L 140633)	**MK946980**	**MK957168**
Fellhanera sp.	China, Yunnan	*W. C. Wang* 20180167 (HMAS-L 140634)	**MK946981**	**MK957169**
Fellhaneropsis vezdae	Sweden	*Knutsson* 97-229 (hb. Knutsson)		AY567744
Lasioloma arachnoideum	Thailand	*W. C. Wang* KYW0595 (RAMK-31616)	**MK946960**	**MK957155**
L. arachnoideum	Thailand	*W. C. Wang* KYW0646 (RAMK-31913)	**MK946979**	**MK957158**
L. arachnoideum	Costa Rica	*Lücking* 16005	AY756467	AY567783
*Micarea byssacea**	Finland	*Launis* 289103 (H)	MG521562	MG707768
*M. byssacea**	Finland	*Launis* 289102 (H)	MG521563	MG707769

(*Continued*)

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Table 1. (*Continued*)

Taxon	Locality	Voucher specimens	ITS-GenBank No.	mtSSU-GenBank No.
*M. micrococca**	Finland	*Launis* 299101 (H)	MG521552	MG707753
Sporopodium antoninianum	Costa Rica	*Lücking* 16002d	AY756498	AY567785
S. argillaceum	Thailand	*W. C. Wang* KYW0087 (RAMK-31565)	**MK946963**	
S. argillaceum	Thailand	*W. C. Wang* KYW0090 (RAMK-31566)	**MK946964**	
S. argillaceum	China, Hainan	*W. C. Wang* HN20170001 (HMAS-L 139419)	**MK946974**	**MK957171**
S. argillaceum	China, Hainan	*W. C. Wang* HN20170022 (HMAS-L 139454)	**MK946976**	**MK957173**
Szczawinskia leucopoda				AY756455
S. tsugae	USA	*Tønsberg* 30044	AY756499	AY567746
Tapellaria nana	Surinam	*P. v. d. Boom* 50677		**MK957178**
T. nigrata	Thailand	*W. C. Wang* KYW0539 (RAMK-31592)		**MK957176**
T. nigrata	Thailand	*W. C. Wang* KYW0602 (RAMK-31814)	**MK946952**	**MK957147**
T. parvimuriformis	Thailand	*W. C. Wang* KYW0181 (RAMK-31581)	**MK946955**	**MK957150**

microscope. Micrographs were taken with a Leica M125 dissecting microscope equipped with a Leica DFC450 camera. Anatomical mounts were photographed with a Zeiss Imager A2 compound microscope equipped with a Zeiss AxioCam MRc5 camera. Colour reactions of apothecium sections were tested with KOH (a 10% aqueous solution of potassium hydroxide) and I (a 10% aqueous solution of potassium iodide). Paraphyses were investigated in 10% KOH when they were indistinct due to strong gelatinization.

DNA extraction, PCR amplification and sequencing

DNA was extracted from 8–30 apothecia per specimen using the DNAsecure Plant Kit DP320-03 (200preps; Tiangen, Beijing, China). Apothecia were cut from the thallus with a razor blade, thoroughly cleaned and then ground with a tissue grinding instrument. The instructions of the kit were closely followed and finally the DNA was stored in 1.5 ml microcentrifuge tubes and kept at −20 °C. The PCR amplification of ITS rDNA was performed with the primers ITS1F (Gardes & Bruns 1993), ITS1 and ITS4 (White *et al.* 1990), and mtSSU rDNA with the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). Amplifications were performed in a 25 µl volume: 12.5 µl 2 MasterMix (TaqDNA Polymerase (0.1 units/µl), 4 mM $MgCl_2$, 0.4 nM dNTPs; Aidlab Biotechnologies Co., Ltd, Beijing, China), 1 µl of each primer, 8.5 µl ddH_2O and 2 µl of DNA. Thermal cycling parameters were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min 30 s, and a final extension at 72 °C for 8 min. PCR products were purified (magnetic bead method) and Sanger sequenced by the Beijing Ruibio Biotech Co., Ltd (China).

Sequence alignment and phylogenetic analysis

The program Geneious v.6.1.2 (Biomatters Ltd., Auckland, NZ) was used to assemble and edit primary sequences. A total of 66 newly generated sequences were aligned together with 29 sequences retrieved from GenBank (Table 1). Two species of *Micarea* were chosen as outgroup based on previous phylogenetic analyses (Andersen & Ekman 2005). The assembled sequences were aligned using online MAFFT v.7 (Katoh *et al.* 2009). The program Gblocks v.0.91b (Castresana 2000) was used to delimit ambiguous regions, implementing all the options for a less stringent selection (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), which yielded final alignments of 489 bp (ITS) and 789 bp (mtSSU), respectively. The two alignments were concatenated in Geneious v6.1.2 for the multilocus phylogenetic analysis.

Maximum likelihood (ML) analysis and Bayesian inference (BI) were used to infer phylogenetic trees based on the concatenated ITS and mtSSU data sets. ML analysis was performed using RaxML-HPC v8.2.6 (Stamatakis 2014) on the Cipres Science Gateway (http://www.phylo.org) and support values were based on 1000 non-parametric bootstrap pseudoreplicates. Bayesian analysis was performed using MrBayes v3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). For the Bayesian analysis, the best substitution models were estimated using jModelTest 2.1.4 (Darriba *et al.* 2012). Based on the results, we used the GTR + I + G model for ITS and the TVM + I + G model for mtSSU. Markov chain Monte Carlo (MCMC) was initiated from a random tree using 5 million generations and sampling every 1000 steps, with the first 25% of trees discarded as burn-in. Stationarity of analysis was determined by examining the standard deviation of split frequencies (< 0.01). A majority-rule consensus tree was constructed from the remaining trees to estimate posterior probability (PP), with values ≥ 0.95 considered as significantly supported. ML and BI trees were visualized with the program FigTree v.1.4.3.

Results

The final alignment consisted of 32 ITS sequences and 34 mtSSU sequences newly generated from 36 global *Pilocarpaceae* specimens, and 13 ITS sequences and 16 mtSSU sequences downloaded from NCBI (Table 1).

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference analysis (BI) exhibited the same topology; we therefore present only the Bayesian tree, with bootstrap support ≥ 75% for the ML analysis and posterior probabilities ≥ 0.95 for the Bayesian analysis (Fig. 1).

The phylogenetic tree shows five distinct and well-supported clades: 1) *Fellhaneropsis* and *Szczawinskia*, 2) *Byssoloma*, 3) *Byssolecania* and *Tapellaria*, 4) *Calopadia* and *Lasioloma*, 5)

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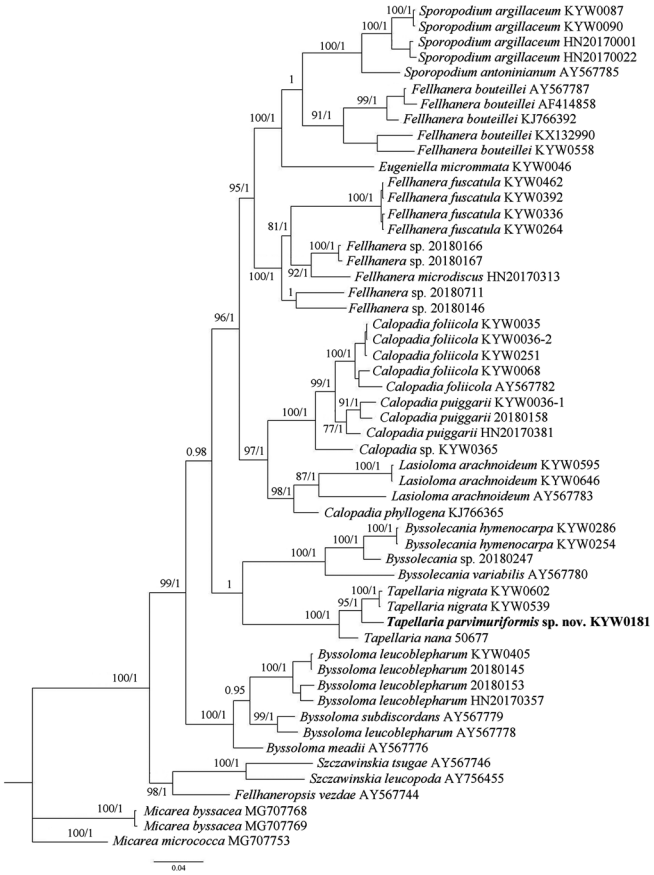

Fig. 1. Phylogram produced in MrBayes after the analysis of a combined ITS +mtSSU-alignment of species in the family *Pilocarpaceae*. Bootstrap supports ≥ 75 for maximum likelihood (ML) and posterior probabilities ≥ 0.95 (second value) for Bayesian methods are indicated above or below the branches. Newly described species are marked in bold. Scale = 0.04 substitution per site.

Fellhanera, *Eugeniella* and *Sporopodium* (Fig. 1). The genus *Fellhaneropsis* is strongly supported as sister to *Szczawinskia* and the sister relationship of the monophyletic genera *Byssolecania* and *Tapellaria* also receives high support. The new species *Tapellaria parvimuriformis*, described and discussed below, is supported (BS = 95, PP = 1) as sister to *T. nigrata*. The phylogenetic distinctness, being placed on a rather long branch compared to the short branches in *T. nigrata*, supports its description as a separate species.

Three accessions of *Lasioloma arachnoideum* form a monophyletic clade sister to most *Calopadia* samples, but due to a sample of *Calopadia phyllogena* falling at the base of this clade, *Lasioloma* appears to be nested within *Calopadia*. *Calopadia* s. lat. (incl. *Lasioloma*) forms a well-supported sister group to a clade containing the genera *Fellhanera*, *Sporopodium* and *Eugeniella*. However, *Fellhanera* is polyphyletic, forming two groups: *Fellhanera bouteillei* is strongly supported as sister to the *Sporopodium* clade, whereas *Fellhanera* s. str. (*F. fuscatula* and related species) is sister to a clade including *Eugeniella*, *Fellhanera bouteillei* and *Sporopodium*.

Discussion

Our analysis shows that most of the genera with a distinct, paraplectenchymatous excipulum (*Fellhanera*, *Eugeniella*, *Sporopodium*, *Calopadia*, *Lasioloma*) form a monophyletic group, whereas other genera (*Byssolecania*, *Byssoloma*, *Szczawinskia*, *Fellhaneropsis*), as well as the outgroup *Micarea*, have a prosoplectenchymatous excipulum or excipulum composed of branched and anastomosing hyphae. This indicates that the excipulum type is phylogenetically informative, although the genus *Tapellaria*, with a paraplectenchymatous excipulum, represents an exception. Lücking (2008) suggested that the prosoplectenchymatous excipulum type is an ancestral character within the family and our preliminary results are congruent with such an interpretation. However, a larger taxon sampling and ancestral character reconstruction analyses will be necessary to test this hypothesis.

In our molecular analysis, *Lasioloma arachnoideum* (type species of the genus) is nested within *Calopadia*, indicating that the distinction of the two genera requires further investigation. Both genera share a dispersed thallus, mostly muriform ascospores, and campylidia with large, hood-shaped lobes. However, they differ in several characters: *Lasioloma* has a woolly prothallus, filiform conidia with 3–5 branches originating from one single point, and an apothecial margin with excipular hairs, whereas *Calopadia* has a glabrous developed prothallus, unbranched filiform conidia with clavate apex and apothecia with glabrous margins. Since the nested topology of *Lasioloma* within *Calopadia* is caused by a single sample, *C. phyllogena* (Netherland Antilles, *Sipman* 54818), it might be prudent to revise the identification of that species, as the hairs typical of *Lasioloma* are sometimes difficult to discern. Given the small-scale diversity of foliicolous lichens on single leaves, it is also possible that a specimen of *Lasioloma* present in a mixed sample of *C. phyllogena* was accidentally sequenced.

Another genus that appears related to *Fellhanera* is *Eugeniella*, even though only one species of that genus was included in the current study. *Eugeniella* shares with *Fellhanera* the usually brown apothecia and the pycnidial anamorph, but differs in the excipulum strongly encrusted with crystals and the indistinct, mostly unbranched paraphyses (Lücking 2008).

The genus *Fellhaneropsis* was established to accommodate some species resembling *Fellhanera* (Sérusiaux 1996) but the genus is not closely related to *Fellhanera*. Morphologically, *Fellhaneropsis* has emarginate apothecia, a feature that is also present in *Micarea*. Its pycnidia and conidia are also different.

The identity of the accession of *Byssoloma leucoblepharum* (AY567778) downloaded from GenBank is questionable; it should not form a clade with *B. subdiscordans* because the two *Byssoloma* species are quite distinct from each other.

The New Species

Tapellaria parvimuriformis W. C. Wang & J. C. Wei sp. nov.

Fungal Names: FN570656

Similar to *Tapellaria floridensis* but differs by its green thallus, grey-white campylidia, shorter conidia (6–9-septate, 45–50 × 2–2.5 µm) and foliicolous growth.

Type: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Lam Ta Khong camping ground, 14°23′N, 101°22′E, 800 m alt., on leaves, 4 May 2018, *W. C. Wang* 31581 (holotype!—RAMK).

(Fig. 2)

Thallus crustose, epiphyllous, pale green, surface uneven, 4–8 mm diam., dispersed in patches, patches slightly convex; *hypothallus*

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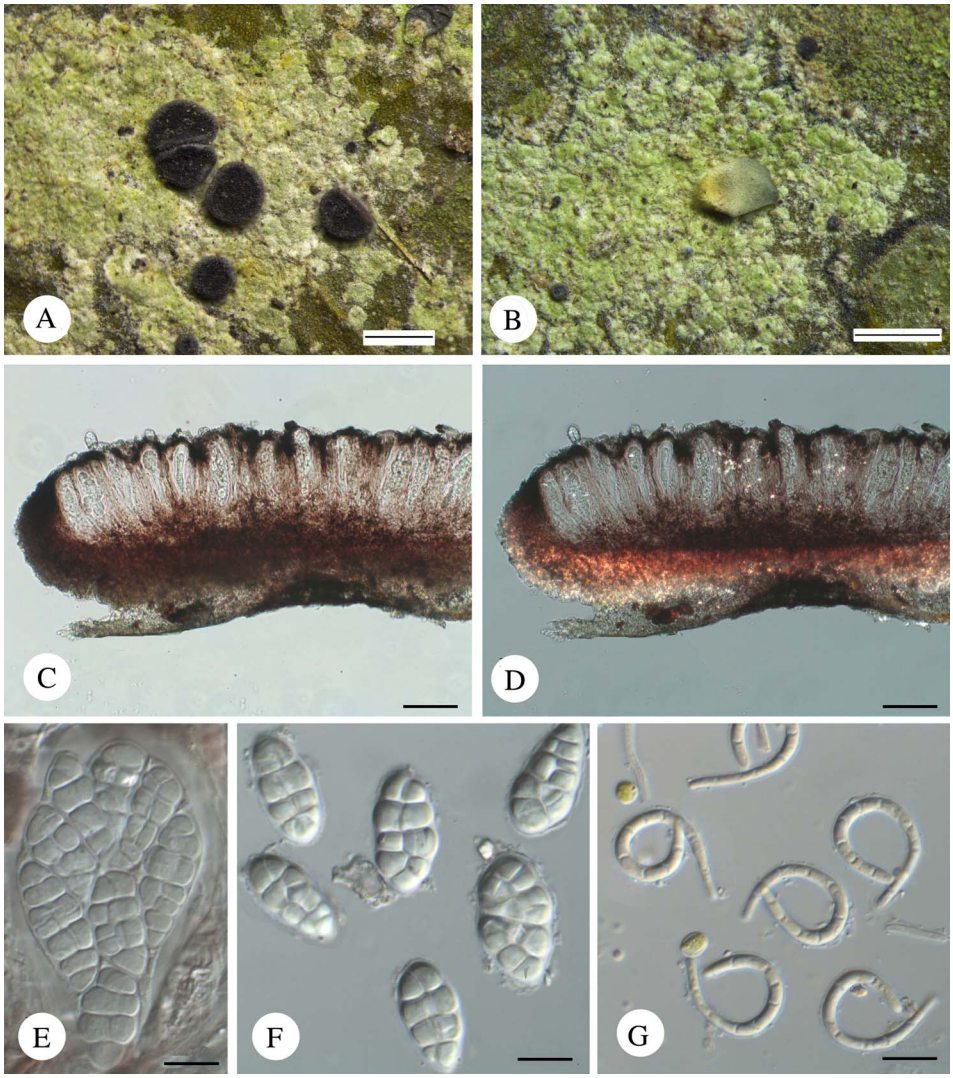

Fig. 2. *Tapellaria parvimuriformis* (holotype, *Wang* 31581, RAMK). A, thallus with apothecia. B, thallus with a campylidium. C & D, section of apothecium showing pigmentation in normal light (C) and crystals in exciple and apothecial base in polarized light (D). E, ascus with ascospores. F, muriform ascospores. G, curved filiform conidia, 6–9-septate. (E–G observed in differential interference mode). Scales: A = 0.5 mm; B = 200 µm; C & D = 50 µm; E–G = 10 µm.

indistinct. *Photobiont* chlorococcoid with globose green cells, 5–7 µm diam.

Apothecia rounded, sessile, basally constricted, 0.3–0.6 mm diam., 180–200 µm high; *disc* flat, black, dull, epruinose; margin thick, prominent and persistent, covered with a thin grey pruina, *c.* 60 µm wide, never irregular in outline; *excipulum* well developed, paraplectenchymatous (cells angular-rounded), inner parts red-brown (K+ red) but with blackish tinge in outer parts, 40–65 µm wide, encrusted with a few crystals which dissolve in K; *epithecium* dark brown or blackish brown, *c.* 12.5 µm high, K+ red; *hymenium* hyaline, mixed with red-brown, 50–65 µm high; *hypothecium* dark red-brown, 25–40 µm high, K+ red; *apothecial base* red-brown, encrusted with a few crystals which dissolve in K, but usually tight because of the existence of crystals, 50–65 µm high, K+ strongly red; *paraphyses* indistinct due to strong gelatinization, branched and anastomosing; *asci* clavate, 60–65 × 9–12 µm, ascus apex I+ dark blue. *Ascospores* 8 per ascus, ellipsoid, hyaline, muriform, with 3–5 transverse septa and 0–1 longitudinal septum per segment, 7–10 cells, without gelatinous perispore, 20–23 × 10–14 µm.

Campylidia sessile, 0.3–0.4 mm wide, 0.4–0.6 mm long; lobe well developed, hood-shaped, dark grey with white-grey base, base not thickened; *conidia* filiform with slightly clavate apex, curved, 6–9-septate, 45–50 × 2–2.5 µm.

Chemistry. Not tested.

Etymology. The epithet of the new species '*parvimuriformis*' is a Latin compound consisting of '*parvus*' (small) and '*muriformis*' (muriform), referring to the small muriform ascospores of the new species.

Habitat and distribution. So far this species is known only from Khao Yai National Park, Thailand, growing on the leaf surface, not abundant, in the shady understory of lowland rainforest near the river.

Notes. Tapellaria parvimuriformis is characterized by its small muriform ascospores, grey campylidia with a white-grey base, and short conidia (45–50 µm long). *Tapellaria floridensis* Common & Lücking is morphologically similar but can be distinguished by the white to pale grey thallus, black campylidia and longer conidia (80–90 µm) (Lücking *et al.* 2011). *Tapellaria parvimuriformis* is also close to *T. schindleri* Kalb & Vězda, which differs by its distorted apothecial margin with blue pruina, its smaller ascospores (16–20 × 9–11 µm) and longer conidia. The new species is also similar to *T. granulosa* Lücking & Rivas Plata, which differs by its granulose thallus and pure black

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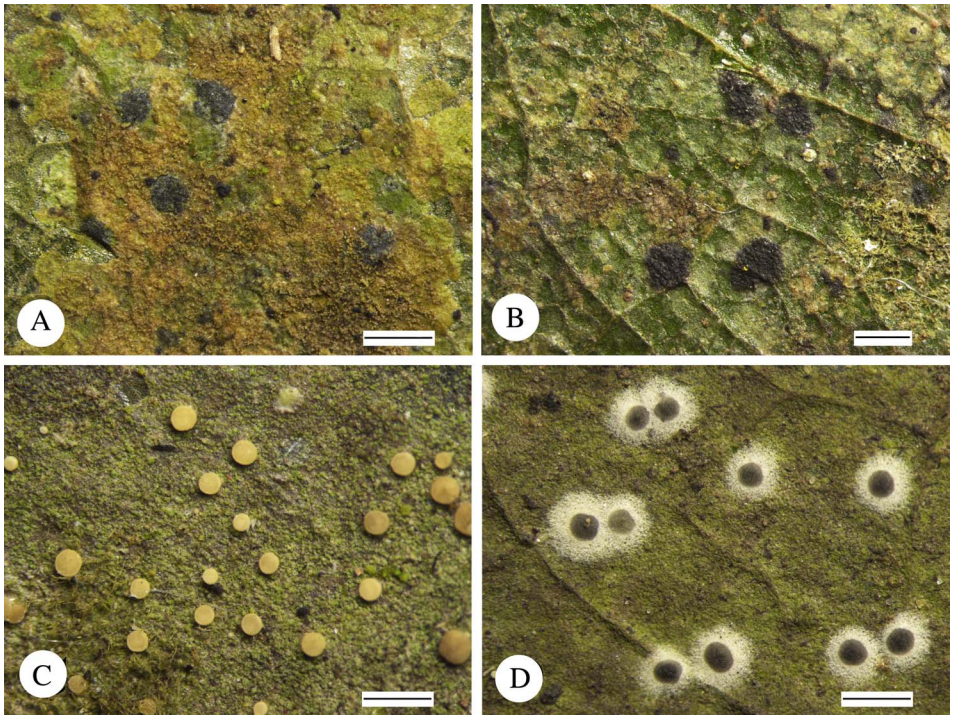

Fig. 3. New records of foliicolous lichenized fungi for Thailand. A, *Arthonia lecythidicola* (*Wang* 31573, RAMK). B, *Arthonia palmulacea* (*Wang* 31849, RAMK). C, *Bacidina apiahica* (*Wang* 31675, RAMK). D, *Byssoloma vanderystii* (*Wang* 31547, RAMK). Scales: A–D = 0.5 mm. In colour online.

apothecia. Furthermore, all three similar species are corticolous and their distribution is restricted to the Neotropics.

Two species of *Tapellaria*, *T. nigrata* and *T. parvimuriformis*, were found in Khao Yai National Park. The former is much more common than the new taxon and differs by having 5-septate ascospores, and smaller apothecia (up to 0.4 mm diam.) with an epruinose margin and usually irregular in outline.

The new species can be inserted into the key by Neuwirth & Stocker-Wörgötter (2017) as follows:

19(18) Campylidia black, conidia 7–13-septate, 80–90 µm long . . . . . **T. floridensis** Common & Lücking
Campylidia grey to dark grey with white-grey base, conidia 5–9-septate, 40–50 µm long 20

20(19) Apothecia with grey margins; thallus smooth; foliicolous, South-East Asia . . . . . **T. parvimuriformis** W. C. Wang & J. C. Wei
Apothecia pure black; thallus granulose; corticolous; Florida . . . . . **T. granulosa** Lücking & Rivas Plata

New Records of Foliicolous Lichens for Thailand

Arthonia lecythidicola (Bat. & H. Maia) Lücking & Sérus.

Lichenologist **30**, 135 (1998).

(Fig. 3A)

Notes. This species is most similar to *A. palmulacea*, with which it shares brown-black apothecia without pruina and macrocephalic ascospores, but it differs in having 3-septate ascospores (*A. lecythidicola*) versus 4–5-septate ascospores (*A. palmulacea*).

Distribution. Neotropics (Lücking 2008). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Lam Ta Khong camping ground, 14° 23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31573 (RAMK).

Arthonia palmulacea (Müll. Arg.) R. Sant.

Symb. Bot. Upsal. **12**(1), 87 (1952).

(Fig. 3B)

Distribution. Pantropical (Lücking 2008), China (Wang & Wei 2018). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31849, KYW0235, KYW0242, KYW0559, KYW0643 (RAMK).

Bacidina apiahica (Müll. Arg.) Vězda

Folia Geobot. Phytotax. **25**(4), 432 (1991).

(Fig. 3C)

Notes. This species is distinct in the pale yellow, translucent apothecia and in the 3-septate, narrowly clavate ascospores.

Distribution. Pantropical (Lücking 2008). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31988, 31675 (RAMK).

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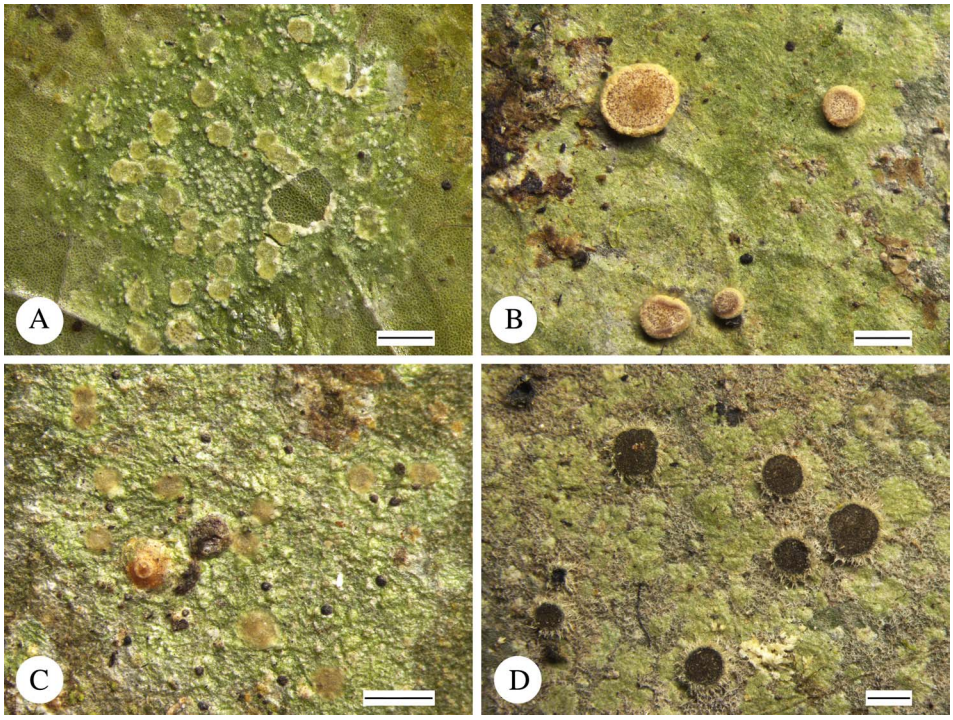

Fig. 4. New records of foliicolous lichenized fungi for Thailand. A, *Calenia lueckingii* (*Wang* 31563, RAMK). B, *Calopadia foliicola* (*Wang* 31537, RAMK). C, *Echinoplaca melanothrix* (*Wang* 31607, RAMK). D, *Lasioloma arachnoideum* (*Wang* 31626, RAMK). Scales: A–D = 0.5 mm. In colour online.

Byssoloma vanderystii Sérus.

Lichenologist **11**, 181 (1979).

(Fig. 3D)

Notes. The main morphological and anatomical features distinguishing *B. vanderystii* are the strongly convex apothecia with a well-developed byssoid margin and the 7-septate ascospores.

Distribution. Pantropical (Lücking 2008), Vietnam (Nguyen *et al.* 2010). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Haew Narok Waterfall, 14°23′N, 101° 22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31547, 31548, 31549, 31550, 31783, 31552, 31553, 31554, 31555, 31556, 31557, 31791, 31559 (RAMK); Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31657, 31880, 31659, 31884, 31666, 31898 (RAMK).

Calenia lueckingii C. Hartmann

Mycotaxon **59**, 484 (1996).

(Fig. 4A)

Notes. This species can be recognized by its verrucose thallus, the green apothecial disc that contains a layer of epithecial algae, the entire apothecial margin and the muriform ascospores, 1 per ascus.

Distribution. Neotropics (Lücking 2008), China (Wang & Wei 2018). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Haew Narok Waterfall, 14°23′N, 101° 22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31563 (RAMK); Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* KYW0570, KYW0572 (RAMK).

Calopadia foliicola (Fée) Vězda

Folia Geobot. Phytotax. **21**(2), 215 (1986).

(Fig. 4B)

Notes. Because of the small number of apothecia, it is an inconspicuous species and easily overlooked if not observed closely. Despite this, the species is unmistakable due to its yellow-brown apothecia with pale yellow pruina and narrowly muriform ascospores, 2 per ascus. *Calopadia puiggarii* may be the species that is most likely to be confused with *C. foliicola* in the field due to their identical habitat and thallus, but it is distinguished by grey-brown apothecia without pruina and shorter ascospores, 1 per ascus.

Distribution. Pantropical (Lücking 2008). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Haew Narok Waterfall, 14°23′N, 101° 22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31536, 31537, 31538, 31769, 31790, 31552, 31553, 31554 (RAMK); Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31902, 31594, 31818, 31997 (RAMK).

Echinoplaca melanothrix Lücking

Biblioth. Lichenol. **65**, 58 (1997).

(Fig. 4C)

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Notes. This species is unique in the genus in having an uneven-verrucose thallus, pale yellow-brown apothecia, and medium-sized (30–50 µm long), muriform ascospores, 1 per ascus.

Distribution. Neotropics (Lücking 2008). New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Haew Narok Waterfall, 14°23′N, 101° 22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31798 (RAMK); Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31607 (RAMK).

Lasioloma arachnoideum (Kremp.) R. Sant.

Symb. Bot. Upsal. **12**(1), 547 (1952).

(Fig. 4D)

Distribution. Pantropical (Lücking 2008), China. New to Thailand.

Specimens examined. **Thailand:** *Nakhon Ratchasima Province*: Khao Yai National Park, Pha Kluay Mai Waterfall to Haew Suwat Waterfall, 14°23′N, 101°22′E, 800 m alt., on leaves, 2018, *W. C. Wang* 31624, 31625, 31626, 31627, 31852, 31942, 31593, 31825, 31905, 31911, 31913, 31940 (RAMK).

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