The Lichenologist **48**(6): 727–737 (2016) doi:10.1017/S0024282916000499

Five new species and one new record of *Astrothelium* (*Trypetheliaceae*, Ascomycota) from Thailand

Theerapat LUANGSUPHABOOL, H. Thorsten LUMBSCH, André APTROOT, Jittra PIAPUKIEW and Ek SANGVICHIEN

Abstract: Five new species and one new record of *Astrothelium* (*Trypetheliaceae*) are reported from Thailand. Phylogenetic analysis of four DNA loci (ITS, nuLSU, mtSSU rDNA and *RPB1*) demonstrated the placement of the new species within *Astrothelium*, as well as their distinction from similar or related taxa. The new species are: *A. flavocoronatum*, with a yellow pigment surrounding the ostiole, and 3-septate ascospores 22–28 × 8·0–9·5 µm; *A. macrostiolatum*, with large whitish ostiolar area, a hamathecium inspersed with small oil droplets, and 9–11-septate ascospores 80–100 × 17–19 µm; *A. neglectum*, with lichexanthone on the thallus and pseudostromata, a non-inspersed hamathecium and 3–5-septate ascospores 21–25 × 7·5–9·5 µm; *A. neovariolosum*, with inspersed hamathecium and 3-septate ascospores 17–23 × 6–7 µm; and *A. siamense*, with inspersed hamathecium and 4–7-septate ascospores 30–50 × 10·5–12·0 µm. *Astrothelium aenascens* is reported from Thailand for the first time.

Key words: lichen-forming fungi, phylogeny, taxonomy, tropical lichens, *Trypetheliales*

Accepted for publication 6 *June* 2016

Introduction

The genus *Astrothelium* Eschw. includes pyrenocarpous lichen-forming fungi within *Trypetheliaceae* (Harris 1984, 1995). Originally restricted to species with lateral, fused ostioles and transversely septate ascospores, in its revised delineation it comprises the majority of species in the *Trypetheliaceae* (Aptroot & Lücking 2016), with variable ascoma arrangement and ascospore septation. In both its traditional and its current circumscription, the genus has a pantropical distribution (Harris 1984; Makhija & Patwardhan 1989; Awasthi 1991; Aptroot *et al*. 2008; Kirk *et al*. 2008; Aptroot 2009; Hyde *et al*. 2013).

Studies on species diversity in taxa with astrothelioid ascomata and ascospores in *Astrothelium* (i.e. *Astrothelium* in its previous sense) have focused mostly on neotropical regions (Malme 1924; Harris 1984; 1995; Aptroot *et al*. 2008; Lücking *et al*. 2011; Lima *et al*. 2013; Córdova-Chávez *et al*. 2014). In contrast, the diversity of this group in the Indomalayan area is far less known, with the exception of the Indian subcontinent (Makhija & Patwardhan 1988, 1989; Awasthi 2000; Singh & Sinha 2010; Weerakoon & Aptroot 2014). Some of the species within this group found in the Indian subcontinent are believed to be endemic to the area (Makhija & Patwardhan 1989; Weerakoon & Aptroot 2014). The diversity of *Astrothelium* species with astrothelioid ascomata and ascospores in Thailand is poorly known, with only four species recorded: *A. cinnamomeum* (Eschw.) Müll. Arg., *A. eustomum* (Mont.) Müll. Arg., *A. galbineum* Kremp., and

T. Luangsuphabool: Program in Biotechnology, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok, 10330 Thailand.
H. T. Lumbsch: Science and Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois 60605, USA.
A. Aptroot: ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, The Netherlands.
J. Piapukiew: Department of Botany, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok, 10330 Thailand.
E. Sangvichien (corresponding author): Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, 10240 Thailand. Email: eks@ru.ac.th

A. variolosum (Ach.) Müll. Arg. (Vongshewarat 2000; Aptroot *et al*. 2007). These taxa are either widely distributed in South-East Asia or are pantropical (Aptroot *et al*. 2008).

The aim of this study was to investigate the diversity of astrothelioid species of *Astrothelium* in Thailand, including taxonomy, chemistry and molecular data. We found the diversity to be higher than previously understood, with five species described here new to science and one new record for Thailand.

Material and Methods

Specimen collection, identification and mycobiont isolation

Specimens were collected from tropical rainforest and submontane evergreen forests in several locations in Thailand (Table 1). Cross-sections were mounted in tap water and investigated using an Olympus SZ11 stereomicroscope and Olympus BX41 compound microscope with differential interference contrast (DIC) (Olympus U-DICT), connected to a Canon EOS650 digital camera. Secondary metabolites were examined by colour spot test (10% KOH, saturated solution NaClO and *p*-phenylenediamine dissolved in ethanol), reaction under long-wave UV light (360 nm), and thin-layer chromatography (TLC) using solvent A (Culberson 1972; Lumbsch 2002).

The lichen mycobionts were isolated from fresh material by the ascospore discharge technique (Sangvichien *et al*. 2011). Ascospore germination and cultivation of the mycobiont was carried out on Malt-Yeast Extract medium incubated at room temperature (32–35 °C). Fifteen mycobionts developed colonies after 4–6 weeks that were used in this study. Lichen specimens and mycobiont cultures were deposited at the Lichen Herbarium of Ramkhamhaeng University (RAMK), Bangkok.

DNA extraction and PCR amplification

Fragments of mycobiont cultures were used for genomic DNA extraction using the CTAB method as modified by Cubero & Crespo (2002). DNA amplification was performed for four nucleotide markers: 1) internal transcribed spacer (ITS), 2) nuclear large subunit ribosomal DNA (nuLSU), 3) mitochondrial small subunit ribosomal DNA (mtSSU), and 4) the largest subunit of RNA polymerase II (*RPB1*) using the following primers: 1) ITS1F (Gardes & Bruns 1993) with ITS4 (White *et al*. 1990), 2) LR0R with LR3 (Vilgalys & Hester 1990), 3) mrSSU1 (Zoller *et al*. 1999) with MSU7 (Zhou & Stanosz 2001), and 4) RPB1-Af with RPB1-Cr (Matheny *et al*. 2002).

The 50 µl PCR reaction consisted of 5 µl 10× *Pfu* Buffer with $MgSO_4$, 2 mM of dNTP mix, 20 µM of each primer, 1·25 U of *Pfu* DNA Polymerase (Thermo Fisher Scientific Inc.) and 5 µl of 1/10 dilution of DNA solution. PCR conditions were as follows: initial denaturation for 1 min at 94 °C and 38 cycles of 94 °C for 1 min, 51 °C for 1 min (ITS1F/ITS4), 52 °C for 45 s (LR0R/LR3), 53 °C for 45 s (mrSSU1/MSU7) and 52 °C for 1·30 min (RPB1-Af/RPB1-Cr), followed by an extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The samples were detected under UV light using agarose gel electrophoresis containing DNA Stain G (SERVA). The Gel/PCR DNA Fragments Extraction Kit (Genaid, Taiwan) was used to clean up the PCR products, according to the manufacturer's instructions, and DNA was sequenced at 1st BASE Laboratories (Malaysia).

Sequence alignments and phylogenetic analyses

DNA sequences were aligned using MUSCLE (Edgar 2004) and manually adjusted using MEGA v.6 software (Tamura *et al*. 2013). Two samples of *Bathelium madreporiforme* were selected as outgroup. Single locus analyses (data not shown) did not show conflict, hence a concatenated dataset from our loci was used (Table 1). The nucleotide substitution model was determined using jModelTest v.2.1.4 (Darriba *et al*. 2012) with the Akaike Information Criterion (AIC). The GTR+I+G model was chosen for phylogenetic tree reconstruction through maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood analyses were carried out using RAxML-HPC2 v.8.2.4 (Stamatakis *et al*. 2008) on the Cipres Web Portal (https://www.phylo.org) and bootstrap values were calculated with 1000 pseudoreplicates. Bayesian analysis was performed using MrBayes v.3.2.1 (Ronquist & Huelsenbeck 2003) for 10 million generations with two independent runs of four chains. Tree samples were saved every 100th trees and the mean standard deviation of split frequencies <0·01. Additionally, maximum parsimony (MP) trees were estimated using PAUP* v.4.0b10 (Swofford 1999) with a heuristic search algorithm and bootstrap values were calculated using 1000 replicates. Phylogenetic trees were visualized using FigTree v.1.4.2 (http:// tree.bio.ed.ac.uk/software/figtree/).

Results and Discussion

Seventy-two new DNA sequences from our loci (ITS, nuLSU, mtSSU, *RPB1*) were generated for this study (Table 1). The concatenated dataset had 3138 nucleotide positions. Molecular data supported the presence of 17 lineages of *Astrothelium* (Fig. 1), including five new species (*A. flavocoronatum*, *A. macrostiolatum*, *A. neglectum*, *A. neovariolosum* and *A. siamense*) and a new record from Thailand (see Taxonomic Treatment).

https://doi.org/10.1017/S0024282916000499 Published online by Cambridge University Press

TABLE 1. *Species and specimens used in this study, with vouchers, location, herbarium (RAMK) and GenBank Accession numbers. Newly generated sequences are in bold.* ⋆ *= sequences from lichen thalli.*

		GenBank Accession numbers			
Taxon	Voucher information	ITS	nuLSU	mtSSU	*RPB1*
Astrothelium aenascens HRK93	Thailand, Phitsanulok, 027887 (RAMK)	**LC127385**	**LC127403**	**LC128018**	**LC128036**
A. aenascens HRK98	Thailand, Phitsanulok, 027888 (RAMK)	**LC127386**	**LC127404**	**LC128019**	**LC128037**
A. cinnamomeum DUKE	Costa Rica, *Lücking* 15322b (F)	DQ782839	AY584652	AY584632	DQ782824
A. crassum MPN98	Peru, *Nelsen* 4004a (F)	–	GU327710	GU327685	–
A. crassum MPN335	Brazil, *Cáceres* 6011 (F)	–	KM453761	KM453827	–
A. flavocoronatum KY859	Thailand, Nakhon Nayok, 027890 (RAMK)	**LC127381**	**LC127398**	**LC128014**	**LC128030**
A. flavocoronatum TSL63	Thailand, Phetchabun, 027889 (RAMK)	**AB758900**	**LC127397**	**AB759874**	**LC128031**
A. laevigatum MPN43	Peru, *Nelsen* s. n. (F)	–	KM453768	KM453833	–
A. leucoconicum MPN42	Peru, *Nelsen* 4000c (F)	–	KM453764	KM453830	–
A. leucosessile MPN258	Panama, *Lücking* 27059 (F)	–	KM453762	KM453828	–
A. macrocarpum MPN260	Panama, *Lücking* 27077 (F)	–	KM453763	KM453829	–
A. macrocarpum NSR6	Thailand, Nakhon Si Thammarat, 027891 (RAMK)	**AB759880**	**LC127402**	**AB759879**	**LC128033**
A. macrocarpum UBN37	Thailand, Ubon Ratchathani, 027892 (RAMK)	**LC127384**	**LC127400**	**LC128015**	**LC128032**
A. macrocarpum UBN43	Thailand, Ubon Ratchathani, 027894 (RAMK)	**LC127383**	**LC127399**	**LC128016**	**LC128034**
A. macrocarpum UBN113	Thailand, Ubon Ratchathani, 027893 (RAMK)	**LC127382**	**LC127401**	**LC128017**	**LC128035**
A. macrostiolatum PHL84⋆	Thailand, Loei, 027895 (RAMK)	**LC127389**	**LC127407**	**LC128022**	**LC128040**
A. neglectum TAK8	Thailand, Tak, 027898 (RAMK)	**LC127392**	**LC127410**	**LC128025**	**LC128043**
A. neglectum TAK12	Thailand, Tak, 027896 (RAMK)	**LC127393**	**LC127411**	**LC128026**	**LC128044**
A. neglectum TAK17	Thailand, Tak, 027897 (RAMK)	**LC127394**	**LC127412**	**LC128027**	**LC128045**
A. neovariolosum KY777	Thailand, Nakhon Nayok, 027899 (RAMK)	**LC127390**	**LC127408**	**LC128023**	**LC128041**
A. neovariolosum KY848	Thailand, Nakhon Nayok, 027900 (RAMK)	**LC127391**	**LC127409**	**LC128024**	**LC128042**
*A. obtectum*MPN422	Brazil, *Lücking* 31242 (F)	–	KM453767	KM453832	–
A. robustum MPN754	Costa Rica, *Mercado–Díaz* 586(F)	–	KM453760	KM453826	–
A. scorioides MPN770	Fiji, *Lumbsch* 20556h (F)	–	KM453766	KM453831	–
A. siamense KRB105	Thailand, Krabi, 027901 (RAMK)	**LC127387**	**LC127405**	**LC128020**	**LC128038**
A. siamense KRB139	Thailand, Krabi, 027902 (RAMK)	**LC127388**	**LC127406**	**LC128021**	**LC128039**
A. versicolor MPN259	Panama, *Lücking* 27045 (F)	–	KM453769	KM453834	–
A. versicolor MPN703	Brazil, *Cáceres & Aptroot* 11137 (F)	–	KM453765	–	–
Bathelium madreporiforme NAN95	Thailand, Nan, 027903 (RAMK)	**LC127396**	**LC127414**	**LC128029**	**LC128047**
B. madreporiforme UBN147	Thailand, Ubon Ratchathani, 027904 (RAMK)	**LC127395**	**LC127413**	**LC128028**	**LC128046**

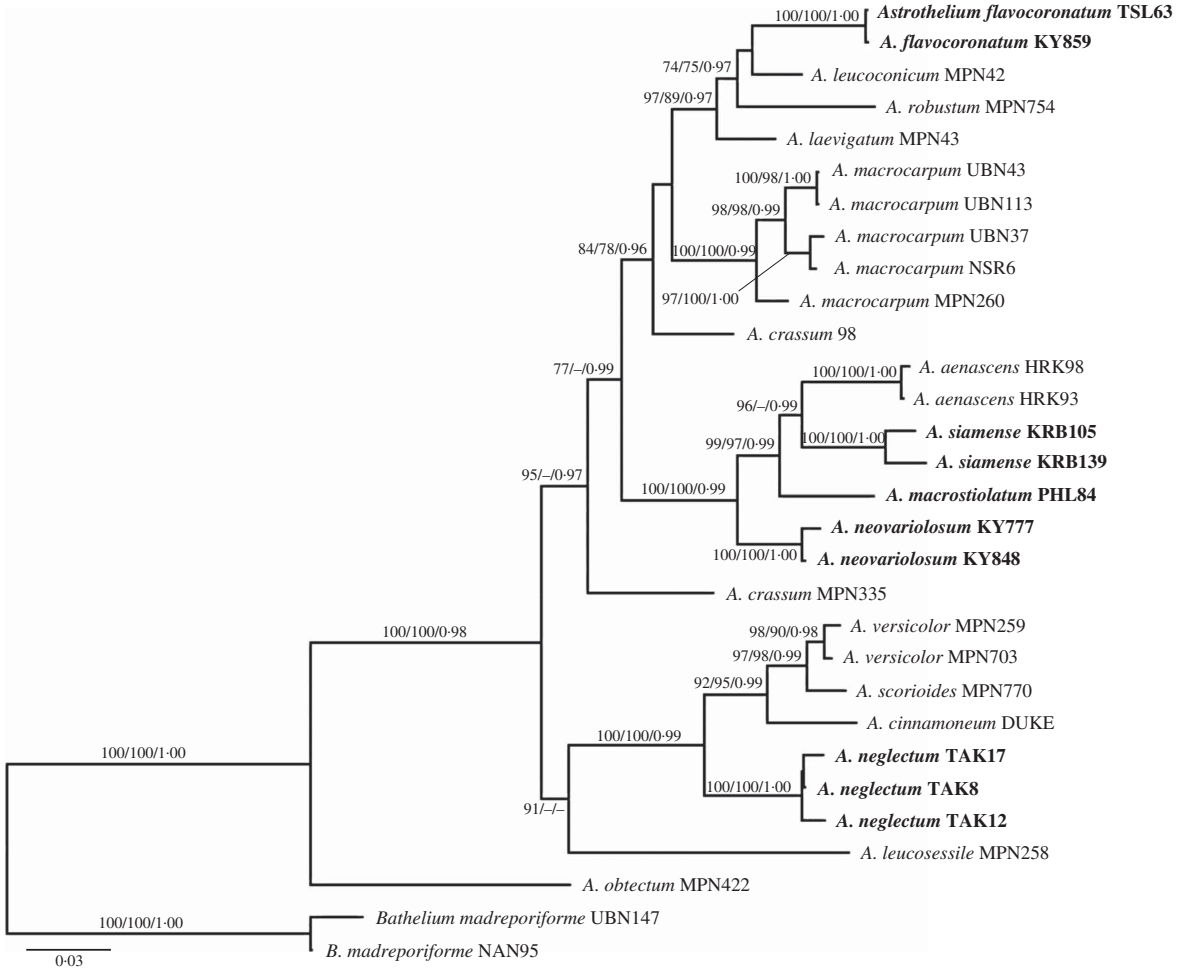

FIG. 1. Phylogenetic relationships of selected astrothelioid species in the genus *Astrothelium* based on maximum likelihood, maximum parsimony and Bayesian inference analyses using four loci (ITS, nuLSU, mtSSU, *RPB1*). The most likely tree obtained using RAxML is shown here. ML and MP bootstrap values ≥70% and posterior probabilities ≥0·95 are given at branches in this sequence.

The new species *A. macrostiolatum*, *A. neovariolosum* and *A. siamense* are closely related and also share certain phenotypic characters, such as a green thallus, white pseudostromata lacking anthraquinones and a hamathecium inspersed with oil droplets. However, they differ in ascospore characters (see below). These three new species form a paraphyletic grade basal to *A. aenascens* Aptroot (Fig. 1), which agrees in the inspersed hamathecium but differs in producing anthraquinones on ascomata. *Astrothelium flavocoronatum* also differs from the other new species in containing anthraquinones on the ascomata. This new species is similar to *A. aenascens* Aptroot and *A. macrocarpum* (Fée) Aptroot & Lücking (syn.: *A. galbineum* Kremp.) (Aptroot & Lücking 2016) in having anthraquinones and 3-septate ascospores of a similar size. However, our molecular data supported the distinction of *A. flavocoronatum* from *A. aenascens* and *A. macrocarpum*. The molecular data also support that *Astrothelium neglectum*, a fifth new species, is distinct from *A. neovariolosum* and *A. siamense*, two species that are similar to the new taxon in having a green thallus, white pseudostromata and containing lichexanthone. However, ascospore characters differ between these taxa and the hamathecium lacks oil droplets in the latter two species.

Astrothelium macrocarpum (Fée) Aptroot & Lücking (syn.: *A. galbineum* Kremp.) has been reported as the most common *Astrothelium* species in Thailand (Vongshewarat 2000). Specimens morphologically consistent with that species were also found in this study. However, although specimens from the Neotropics and Thailand form a monophyletic clade, the molecular data suggest that the Thai material is somewhat distinct from the neotropical material (Fig. 1). The circumscription of *A. macrocarpum* (as *A. galbineum*) has been discussed previously. Harris (1984) reduced *A. ochrothelizum* Müll. Arg. to synonymy with *A. galbineum*, while these two species were separated by Makhija & Patwardhan (1989) based on ascoma characters. In fact, in *A. macrocarpum* the ascomata are totally embedded in the pseudostromata as in the Thai material (Vongshewarat 2000; Aptroot *et al.* 2008; Aptroot 2009).

Taxonomic Treatment

Astrothelium flavocoronatum Luangsuphabool, Aptroot & Sangvichien. sp. nov.

MycoBank No.: MB 816951

Similar to *Astrothelium diplocarpum* in having anthraquinone pigments around the ostiole neck, but differing in having smaller ascospores; thallus yellow to green, perithecial wall carbonized, ostiole with yellow anthraquinone, ascospores 3-septate, 22–28 × 8·0–9·5 µm.

Type: Thailand, Nakhon Nayok Province, Khao Yai National Park, montane evergreen forest, on tree bark, 14°26'N, 101°22'E, alt. 760 m, 2015, *Luangsuphabool* KY859 (RAMK-027890—holotype).

(Fig. 2A–C)

Thallus crustose, corticate, yellow to green, smooth, continuous, prothallus black; cortex distinct, 40–70 µm thick; algal layer continuous, 35–75 µm thick; medulla 120–175 µm thick. *Algae* trentepohlioid.

Ascomata perithecia, pyriform, black, 0·40–0·85 mm diam., semi-immersed to emergent, solitary, usually consisting of two cavities that are joined with a common ostiole. *Wall* carbonized, ≤ *c.* 50 µm thick. *Ostiole* apical, black, surrounded by yellow layer. *Pseudostromata* raised above the thallus, covered with thallus cortex or naked and carbonized. *Hamathecium* hyaline, not inspersed; *paraphyses* anastomosing, 0·85–1·00 µm thick. *Asci* clavate, 105–110 × 18·5–19·0 µm. *Ascospores* 8 per ascus, hyaline, transversely 3-septate, narrowly ellipsoid, 22–28 × 8·0–9·5 µm, lumina diamond-shaped to rounded.

Pycnidia not observed.

Chemistry. Thallus UV−, K+ yellow, C−, KC−, P−. Ascomata: around ostiole UV + orange, K+ red, C + red, P−. TLC: parietin, emodin.

Etymology. The specific epithet refers to the yellow tissue which surrounds the ostiole of the new species.

Notes. This new species is similar to the neotropical *Astrothelium diplocarpum* Nyl. in

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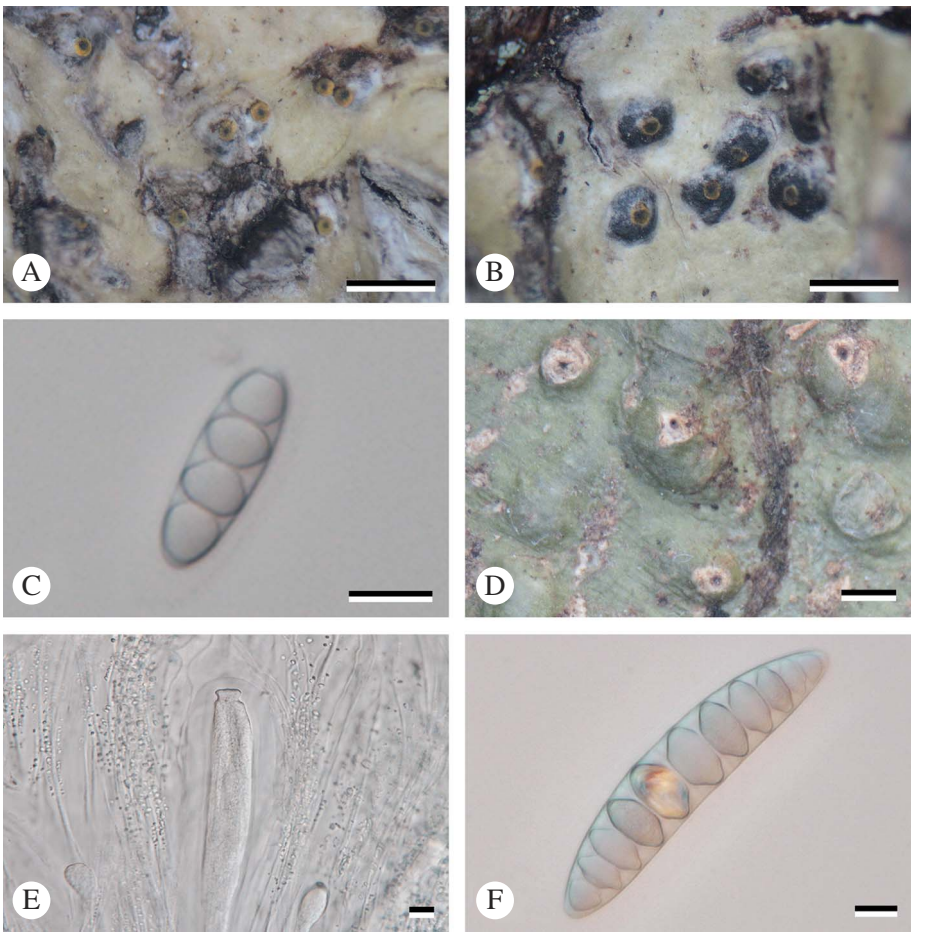

FIG. 2. A–C, *Astrothelium flavocoronatum* (holotype): A & B, thallus with ascomata; C, ascospores. D–F, *Astrothelium macrostiolatum* (holotype): D, thallus with ascomata; E, ascus and hamathecium inspersed; F, ascospores. Scales: A, B & D = 1 mm; C, E & F = 10 µm. In colour online.

having anthraquinone pigments around the ostiole neck, but differs in having smaller ascospores (9-septate, 90–110 × 22–28 µm in *A. diplocarpum*) (Harris 1995; Aptroot *et al.* 2008). Also, *A. macrocarpum* (Fée) Aptroot & Lücking (syn.: *A. galbineum* Kremp.) and *A. aenascens* Aptroot are similar in having ascomata with anthraquinones and in the ascospore characters, but the new species differs in having ascomata with two locules (several locules with one to several ostioles in *A. macrocarpum*) and a non-inspersed hamathecium (inspersed in *A. aenascens*). Molecular evidence supports this distinction.

Additional specimen examined. **Thailand:** *Phetchabun*: Thung Salaeng Luang National Park, montane evergreen forest, on tree bark, 16°35'N, 100°52'E, alt. 740 m, 2008, *Luangsuphabool* TSL63 (RAMK-027889).

Astrothelium macrostiolatum Luangsuphabool, Aptroot & Sangvichien. sp. nov.

MycoBank No.: MB 816952

Similar to *Astrothelium eustomum* in thallus and pseudostroma characters, but differing in having 9–11 septate, 80–100 × 17–19 µm ascospores, an inspersed hamathecium and lacking secondary metabolites; thallus

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olive-green, pseudostroma with whitish ostiolar area, hamathecium inspersed with small oil droplets.

Type: Thailand, Loei Province, Phu Ruea District, Phu Luang Wildlife Sanctuary, montane evergreen forest, on tree bark, 17°16'N, 101°30'E, alt. 1460 m, 2014, *Luangsuphabool* PHL84 (RAMK-027895—holotype).

(Fig. 2D–F)

Thallus crustose, corticate, olive-green, smooth or somewhat warted, shiny, prothallus black; cortex distinct, 15–40 µm thick; algal layer continuous, 20–60 µm thick, medulla 25–90 µm thick. *Alga* trentepohlioid.

Ascomata perithecia, pyriform, black, 0·9–1·1 mm diam., common ostiole with two cavities, solitary or immersed in pseudostroma. *Wall* carbonized, ≤ *c.* 100 µm thick. *Ostiole* apical, black. *Pseudostromata* white, mostly covered by thallus but leaving a large whitish ostiolar area free. *Hamathecium* hyaline, inspersed with small oil droplets usually less than 2 µm diam.; *paraphyses* anastomosing, 0·8–1·0 µm thick. *Asci* clavate, 240–300 × 37–55 µm. *Ascospores* 8 per ascus, hyaline, transversely 9–11 septate, fusiform, 80–100 × 17–19 µm, lumina diamond-shaped to rounded.

Pycnidia not observed

Chemistry. Thallus UV−, K+ yellow, C−, KC−, P−. Pseudostromata UV−, K−, C−, P−. TLC: no substances detected.

Etymology. The specific epithet refers to the large whitish ostiolar area.

Notes. This new species is similar to *Astrothelium eustomum* (Mont.) Müll. Arg. in thallus and pseudostromatal characters, and also to *A. diplocarpoides* Müll. Arg. and *A. diplocarpum* Nyl. in having rather large ascospores. However, it differs from those taxa in having more numerous septa, an inspersed hamathecium and a lack of secondary metabolites; 3–5-septate ascospores, a non-inspersed hamathecium, and lichexanthone are found in *A. eustomum*, 5–7-septate ascospores and lichexanthone are characteristic of *A. diplocarpoides*, and 9-septate ascospores, a non-inspersed hamathecium and anthraquinones are found in *A. diplocarpum* (Harris 1984; Aptroot *et al*. 2008; Lücking *et al*. 2011; Aptroot & Lücking 2016).

Astrothelium neglectum Luangsuphabool, Aptroot & Sangvichien. sp. nov.

MycoBank No.: MB 816953

Similar to *Astrothelium eustomum* in thallus, pseudostroma and ascospore characters, but differing by containing lichexanthone in the thallus; thallus yellow to green, pseudostromata white, hamathecium not inspersed, ascospores 3–5 septate, 21–25 × 7·5–9·5 µm.

Type: Thailand, Tak Province, Umphang District, Palatha Village, on tree bark, 15°49'N, 98°50'E, alt. 500 m, 2010, *Luangsuphabool* TAK17 (RAMK-027897—holotype).

(Fig. 3A–D)

Thallus crustose, corticate, yellow to green, smooth, shiny, prothallus black; cortex distinct, 65–120 µm thick; algal layer continuous, 15–55 µm thick, medulla 60–165 µm thick. *Alga* trentepohlioid.

Ascomata perithecia, pyriform, black, 0·65–1·15 mm diam., 2–5 cavities with common ostiole immersed in pseudostroma. *Wall* carbonized, ≤ *c.* 70 µm thick. *Ostiole* apical, black. *Pseudostromata* white, rounded to irregular, flattened top and raised above the thallus. *Hamathecium* hyaline, not inspersed; *paraphyses* anastomosing, 1·3–2·0 µm thick. *Asci* clavate, 110–140 × 15–20 µm. *Ascospores* 8 per ascus, hyaline, transversely 3–5 septate, fusiform, 21–25 × 7·5–9·5 µm, lumina diamond-shaped to rounded.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow (lichexanthone), K+ yellow, C−, KC−, P−. Pseudostromata UV+ yellow (lichexanthone), K−, C−, P−. TLC: lichexanthone.

Etymology. The specific epithet refers to this species having been previously overlooked.

Notes. The new species is similar to *Astrothelium eustomum* (Mont.) Müll. Arg. in

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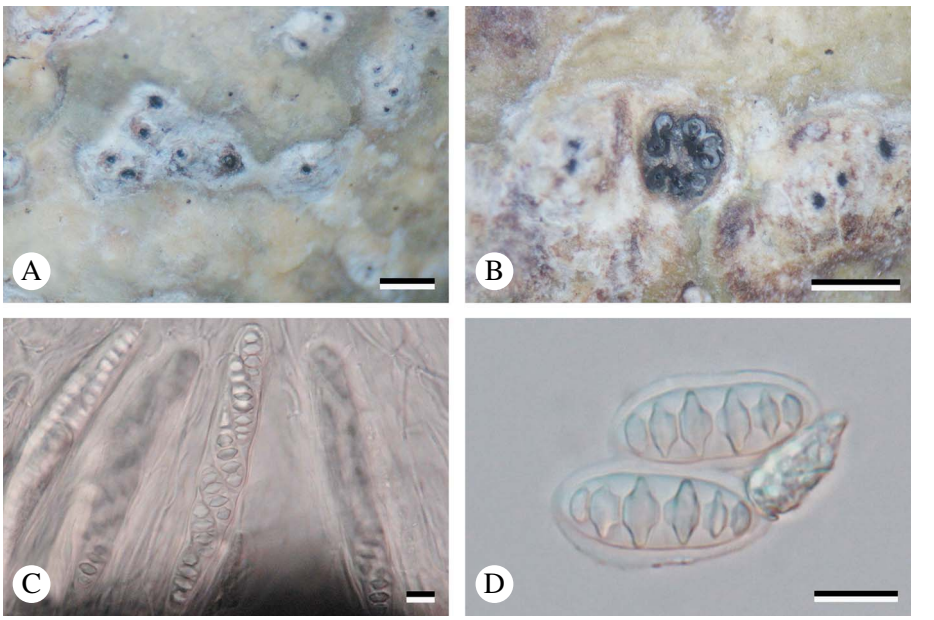

FIG. 3. *Astrothelium neglectum* (holotype). A, thallus with ascomata; B, ascomata; C, ascus with ascospores; D, mature ascospores. Scales: A & B = 1 mm; C & D = 10 µm. In colour online.

thallus, pseudostromatal and ascospore characters, but differs by containing lichexanthone in the thallus, whereas this substance is present only on the ostioles in *A. eustomum* (Harris 1984; Aptroot *et al.* 2008; Aptroot 2009). Also, *A. neovariolosum* and *A. siamense* are similar in having a corticated thallus, white pseudostromata, KOH− and lichexanthone, but the new species differs in ascospore characters and the non-inspersed hamathecium (3-septate ascospores, 17–23 × 6–7 µm, and hamathecium inspersed in *A. neovariolosum*; 4–7-septate, 30–50 × 10·5–12·0 µm, and hamathecium inspersed in *A. siamense*).

Additional specimens examined. **Thailand:** *Tak*: Umphang District, on tree bark, 15°49'N, 98°50'E, alt. 500 m, 2010, *Luangsuphabool* TAK8 (RAMK-027898), TAK12 (RAMK-027896).

Astrothelium neovariolosum Luangsuphabool, Aptroot & Sangvichien. sp. nov.

MycoBank No.: MB 816954

Similar to *Astrothelium variolosum* in pseudostroma and ascospore characters, but differing by an inspersed hamathecium; thallus greenish, pseudostromata grey to yellowish, ascospores 3-septate, 17–23 × 6–7 µm.

Type: Thailand, Nakhon Nayok Province, Khao Yai National Park, montane evergreen forest, on tree bark, 14°25'N, 101°22'E, alt. 750 m, 2013, *Luangsuphabool* KY777 (RAMK-027899—holotype).

(Fig. 4A–C)

Thallus crustose, corticate, greenish, smooth or somewhat warted, shiny, prothallus black; cortex distinct, 16–28 µm thick; algal layer continuous, 18–35 µm thick; medulla 40–85 µm thick. *Alga* trentepohlioid.

Ascomata perithecia, pyriform, black, 0·5–0·8 mm diam., fused ostiole with two cavities, single to 2–8 aggregate groups immersed in pseudostroma. *Wall* carbonized, ≤ *c.* 50 µm thick. *Ostiole* apical, black. *Pseudostromata* grey to yellowish, raised above the thallus, round to irregular. *Hamathecium* hyaline, inspersed with oil droplets; *paraphyses* anastomosing, 0·9–1·0 µm thick. *Asci* clavate, 115–125 × 12·0–13·5 µm. *Ascospores* 8 per ascus, hyaline, transversely

https://doi.org/10.1017/S0024282916000499 Published online by Cambridge University Press

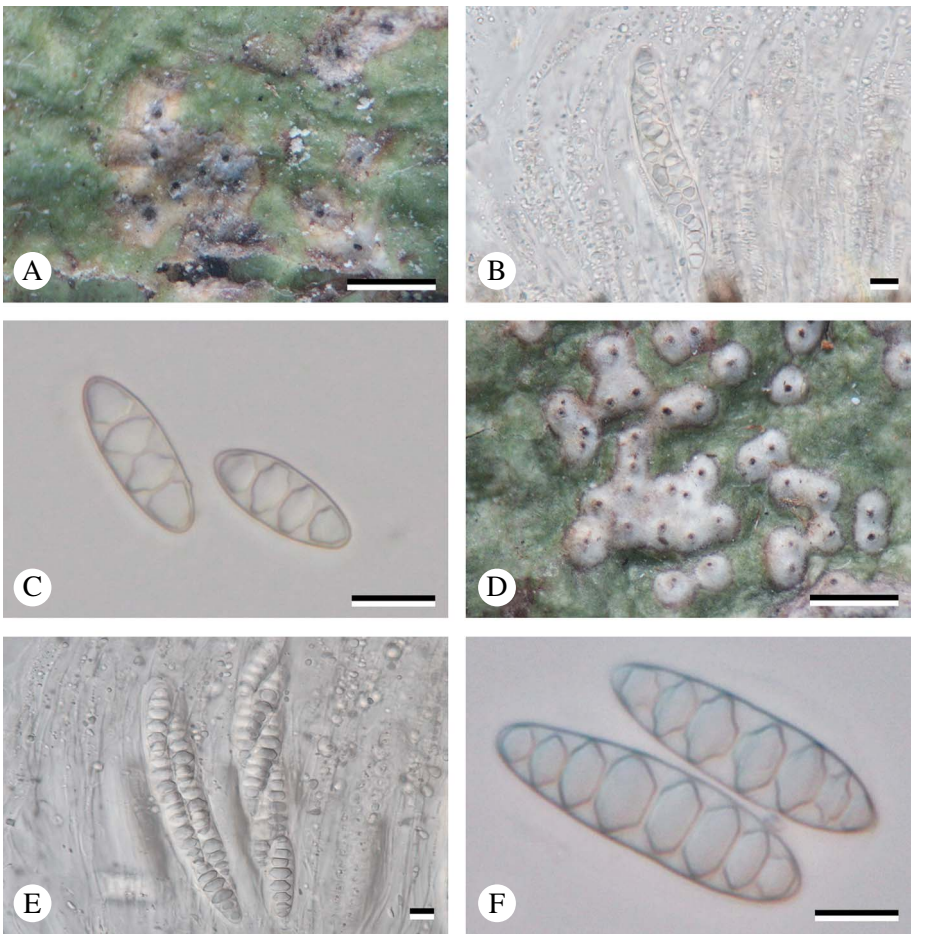

FIG. 4. A–C, *Astrothelium neovariolosum* (holotype); A, thallus with ascomata; B, ascus with ascospores; C, ascospores. D–F, *Astrothelium siamense* (holotype); D, thallus with ascomata; E, ascus with ascospores; F, ascospores. Scales: A & D = 1 mm; B, C, E & F = 10 µm. In colour online.

3-septate, narrowly ellipsoid, 17–23 × 6–7 µm, lumina diamond-shaped to rounded.

Pycnidia not observed.

Chemistry. Thallus UV + yellow (lichexanthone), K+ yellow, C−, KC−, P−. Pseudostromata UV + brown-orange, K−, C−, P−. TLC: lichexanthone.

Etymology. The specific epithet refers to the morphological similarities with *A. variolosum*.

Notes. The new species is most similar to *Astrothelium variolosum* (Ach.) Müll. Arg. in having white to grey pseudostromata and in ascospore characters, but differs by its inspersed hamathecium (hamathecium not inspersed in *A. variolosum*) (Aptroot *et al.* 2008; Aptroot 2009).

Additional specimen examined. **Thailand:** *Nakhon Nayok*: Khao Yai National Park, tree bark, 14°25'N, 101°22' E, alt. 760 m, 2014, *Luangsuphabool* KY848 (RAMK-027900).

Astrothelium siamense Luangsuphabool, Aptroot & Sangvichien. sp. nov.

MycoBank No.: MB 816955

https://doi.org/10.1017/S0024282916000499 Published online by Cambridge University Press

Similar to *Astrothelium variolosum* in thallus and pseudostroma characters, but differing in having larger ascospores and an inspersed hamathecium; thallus olive-green to yellow, pseudostromata white, hamathecium inspersed, ascospores 4–7 septate, 30–50 × 10·5–12·0 µm.

Type: Thailand, Krabi Province, Khlong Thom District, Thung Tieo-Sra Morakot trail, tropical rainforest, on tree bark, 7°55'N, 99°16'E, alt. 70 m, 2012, *Luangsuphabool* KRB139 (RAMK-027902—holotype).

(Fig. 4D–F)

Thallus crustose, corticate, olive-green to yellow, smooth, shiny, prothallus black; cortex distinct, 20–60 µm thick; algal layer continuous, 10–40 µm thick; medulla 10–40 µm thick. *Alga* trentepohlioid.

Ascomata perithecia, pyriform, black, 0·26–0·52 mm diam., common ostiole with two cavities, solitary to aggregated groups immersed in pseudostroma. *Wall* carbonized, ≤ *c.* 30 µm thick. *Ostiole* apical, black. *Pseudostromata* white, raised above the thallus, round to irregular. *Hamathecium* hyaline, inspersed with oil droplets; *paraphyses* anastomosing, 0·7–1·0 µm thick. *Asci* clavate, 125–150 × 20–23 µm. *Ascospores* 8 per ascus, hyaline, transversely 4–7 septate, fusiform, 30–50 × 10·5–12·0 µm, lumina diamond-shaped to rounded.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow (lichexanthone), K+ yellow, C−, KC−, P−. Pseudostromata UV−, K−, C−, P−. TLC: lichexanthone.

Etymology. The specific epithet refers to 'Siam', the traditional name for Thailand, where the species was collected.

Notes. This new species is similar to *Astrothelium variolosum* (Ach.) Müll. Arg. in having a green thallus and white to grey pseudostromata, but differs in having larger ascospores and an inspersed hamathecium (3-septate ascospores, 20–26 × 7–9 µm and hamathecium not inspersed in *A. variolosum*) (Aptroot *et al*. 2008; Aptroot 2009). The new species is also similar to *A. neovariolosum* in thallus and pseudostroma characters and in containing lichexanthone, but differs in having larger ascospores (3-septate, 17–23 × 6–7 µm in *A. neovariolosum*).

Additional specimen examined. **Thailand:** *Krabi*: Khlong Thom District, Hin Phoeng Waterfall, on tree bark, 7°51'N, 99°15'E, alt. 75 m, 2012, *Luangsuphabool* KRB105 (RAMK-027901).

New record for Thailand

Astrothelium aenascens Aptroot

Lichenologist **48:** xx (2016).

Thallus crustose, corticated, greenish to grey, smooth. *Alga* trentepohlioid.

Ascomata perithecia, black, carbonized, aggregated groups immersed in pseudostroma and sharing common ostiole. *Ostiole* apical, black. *Pseudostromata* raised, containing yellow to orange pigment. *Hamathecium* hyaline, inspersed with oil droplets, branched and anastomosing. *Ascospores* 8 per ascus, transversely 3-septate, 24–30 × 9–10 µm, lumina diamond-shaped to rounded.

Chemistry. Thallus UV+ yellow to orange, K+ yellow. Pseudostromata UV+ red-orange, K+ red. TLC: lichexanthone, parietin.

Specimens examined. **Thailand:** *Phitsanulok Province*: Nakhon Thai District, Phu Hin Rong Kla National Park, montane evergreen forest, on tree bark, 16°59'N, 100°59'E, alt. 1310 m, 2009, *Luangsuphabool* HRK93 (RAMK-027887), HRK98 (RAMK-027888).

This study was financially supported by the Royal Thai Government through Ramkhamhaeng University, a CU Graduate School Thesis Grant and a scholarship from the Human Resource Development in Science Project (Science Achievement Scholarship of Thailand). We would like to thank Kansri Boonpragob and Kajonsak Vongshewarat for their helpful suggestions and Anupan Kongbangkerd and Thanakorn Wongsa for their assistance during fieldwork.

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