

## Standard Paper

# *Aptrootia khaoyaiensis* (Trypetheliaceae), a new corticolous lichen from the dry dipterocarp forest in central Thailand

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## Abstract

*Aptrootia khaoyaiensis* Polyiam & Lücking is described as new to science from dry dipterocarp forest in central Thailand, based on morphological assessment and sequence data of the mitochondrial small subunit (mtSSU). The new species is characterized by a corticolous habit, a corticate thallus, ascomata typically immersed in the thallus, with a brown to blackish ostiolar region, an inspersion hamathecium, and dark brown, muriform ascospores occurring 1–2 per ascus. Phylogenetic analyses support placement of the new species in *Aptrootia*; it is morphologically close to *A. elatior* but differs in the smaller ascospores and the inspersion hamathecium.

**Keywords:** biodiversity; phylogeny; taxonomy; tropical rainforest

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## Introduction

The lichenized genus *Aptrootia* Lücking & Sipman was introduced by Lücking *et al.* (2007). It is mainly characterized by a crustose thallus, immersed to erumpent ascomata and large, dark brown, muriform ascospores (Aptroot & Lücking 2016). *Aptrootia* is a small genus belonging to the family Trypetheliaceae, with only three species known so far: *Aptrootia elatior* (Stirt.) Aptroot, *A. robusta* (P. M. McCarthy & Kantvilas) Aptroot and *A. terricola* (Aptroot) Lücking *et al.* (Lücking *et al.* 2007; Aptroot 2009; Aptroot & Lücking 2016). Members of this genus are corticolous, terricolous or muscicolous and found in tropical areas (Aptroot 1999, 2009; Aptroot & Lücking 2016). Up to now, the genus has not been reported from Thailand (Buaruang *et al.* 2017).

During intensive fieldwork along the gradient of elevational ranges in the dry dipterocarp forest of Khao Yai National Park in central Thailand, an unidentified *Aptrootia* species was discovered which did not correspond to any of the known species. Consequently, we describe this material here as a new species, *Aptrootia khaoyaiensis* sp. nov., based on diagnostic morphological characters and supported by molecular phylogenetic analyses.

## Material and Methods

### Morphology and anatomy

All specimens studied were collected from tree bark in the dry dipterocarp forest of Khao Yai National Park, Thailand, in

2020–2021. Morphology was examined using an Olympus SZ30 dissecting microscope. Sections of thalli and ascoma were hand-cut with a razor blade and studied using an Olympus BX41 compound microscope. All measurements were made on material mounted in water. Images were captured with an Olympus Tough TG-6 digital camera. Lichen substances were analyzed by spot tests using K (10% aqueous solution of potassium hydroxide), ultraviolet light (365 nm), and thin-layer chromatography (Orange *et al.* 2010).

### DNA extraction, PCR and sequencing

Perithecia were carefully cut from the thallus with a razor blade and ground with a pestle tissue grinder. Genomic DNA was extracted from 5–10 perithecia using the Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, The Netherlands), according to the manufacturer's instructions. A portion of mycobiont mitochondrial small subunit (mtSSU) was amplified and sequenced using combinations of the primers mrSSU1-AGCAGTGAGGAATAT TGGTC and mrSSU3R-ATGTGGCACGTCTATAGCCC (Zoller *et al.* 1999). Amplifications were performed in a 25 µl volume and the amplification mixture consisted of 1 µl total DNA, 9.5 µl MasterMix (TaqDNA Polymerase, 25 mM MgCl<sub>2</sub>, 8 mM dNTPs, 10× buffer; Thermo Fisher Scientific Inc., USA), 2.5 µl of each primer (10 µM) and 9.5 µl nuFree H<sub>2</sub>O. The cycle sequencing conditions were as follows: initial denaturation for 5 min at 96 °C, followed by 1 min at 95 °C, 1 min at 55 °C, 1 min 30 s at 72 °C, and a final extension step of 10 min at 72 °C. The amplification products were visualized on a 1% agarose gel and PCR products were purified using the FavorPrep™ GEL/PCR Purification Kit (Favorgen, Ping-Tung, Taiwan). Sanger sequencing of the purified amplicons was

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performed by the Toxicology Center, National Institute of Health, Thailand.

### Sequence alignment and phylogenetic analyses

Alignments were carried out using MUSCLE v. 3.8.31 (Edgar 2004). Ambiguously aligned positions were corrected manually. Additional mtSSU sequences belonging to different genera of *Trypetheliaceae* were downloaded from GenBank (Table 1), their selection based on the present phylogenetic classification of the family (Del Prado *et al.* 2006; Nelsen *et al.* 2009, 2014; Luangsaphabool *et al.* 2016, 2018; Lücking *et al.* 2016). For the final analysis, we used a subset of selected species from the genera *Aptrootia*, *Architrypethelium*, *Astrothelium*, *Bathelium*, *Pseudopyrenula* and *Trypethelium*. Phylogenetic analyses were conducted using maximum likelihood (ML) and maximum parsimony (MP). ML analysis was performed in IQ-TREE (Trifinopoulos *et al.* 2016) using the GTR+I+G model, whereas MP was analyzed in MEGA X (Kumar *et al.* 2018). Branch support obtained from both methods was estimated using 1000 bootstrap pseudoreplicates. Only clades that received bootstrap support  $\geq 70\%$  were considered supported. Phylogenetic trees were visualized using FigTree v. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>).

## Results

### Phylogeny

Both ML and MP analyses showed a congruent tree topology, and therefore only the ML tree (lnL = -2865.74) with bootstrap support (BS) > 70% is shown (Fig. 1). The phylogenetic position of *Aptrootia khaoyaiensis* was resolved as an early diverging lineage within *Aptrootia* (ML-BS = 88%; MP-BS = 73%), forming a sister group with *A. elatior*, *A. robusta* and *A. terricola* (Fig. 2). The

relationships within *Aptrootia* were supported, but the backbone relationships between the various genera were largely not supported.

### The Species

#### *Aptrootia khaoyaiensis* Polyiam & Lücking sp. nov.

MycoBank No.: MB 849988

Similar to *Aptrootia elatior* (Stirt.) Aptroot in growing on bark and producing a corticate thallus, but differing in the smaller ascospores without obvious ornamentation, the fully inspersed hamathecium, and the uneven to verrucose but not bullate thallus.

Type: Thailand, Prachinburi, Prachantakham District, Khao Yai National Park, Klong Peka, dry dipterocarp forest, 14° 11'51"N, 101°29'31"E, c. 253 m a.s.l., on bark of *Gluta usitata*, 19 July 2020, W. Polyiam 33238 (RAMK—holotype). GenBank Accession no.: OR392814.

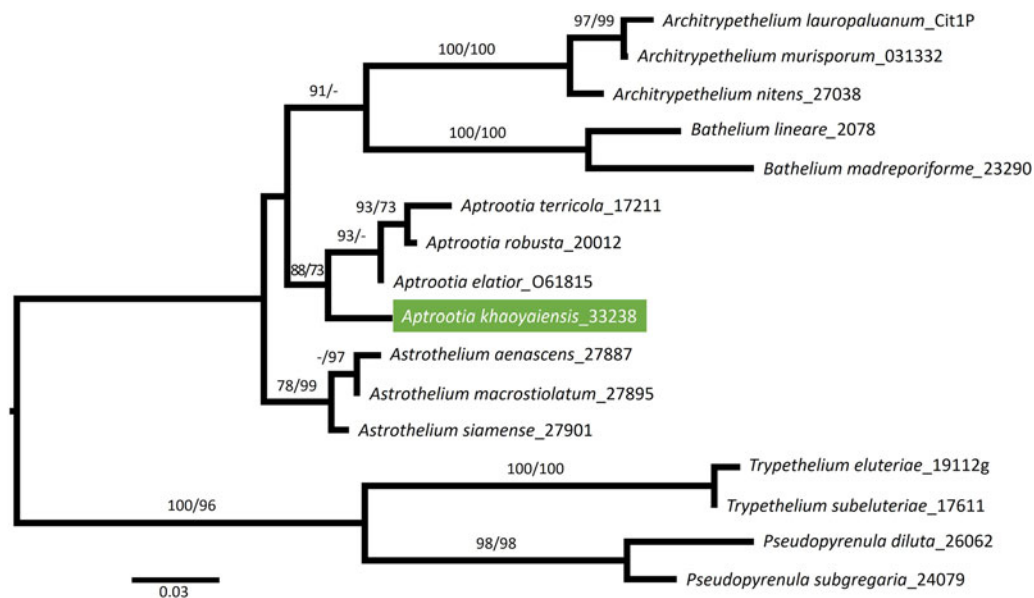
(Fig. 2)

*Thallus* crustose, corticolous, corticate, 35–50  $\mu\text{m}$  thick, pale yellow-brown to green, uneven to irregularly verrucose, without prothallus. *Photobiont* trentepohlioid.

*Ascomata* trypethelioid, solitary or irregularly confluent, with apical ostioles, ampulliform (in section), 0.5–1.2 mm diam., immersed to erumpent, largely covered by thallus, ostiolar area black. *Ascomata* wall black, 40–60  $\mu\text{m}$  thick. *Hamathecium* gelatinous, fully inspersed with irregular, large oil droplets. *Paraphysoids* anastomosing, 400–550  $\mu\text{m}$  long. *Asci* clavate to subcylindrical, with 1–2 ascospores per ascus. *Ascospores* densely muriform, not ornamented, 80–150  $\times$  30–50  $\mu\text{m}$ , ellipsoid, hyaline but becoming dark brown when mature, I– to I+ pale violet when still hyaline.

**Table 1.** GenBank Accession numbers and voucher information for samples of *Trypetheliaceae* used in the phylogenetic analyses (Fig. 1). New sequence generated for the present study is indicated in bold.

Taxon	Country of origin	Specimen voucher	GenBank Accession no.	Reference
<i>Aptrootia elatior</i>	New Zealand	<i>Knight</i> O61815 (OTA)	KM453821	Nelsen <i>et al.</i> 2014
<b><i>A. khaoyaiensis</i></b>	<b>Thailand</b>	<b><i>Polyiam</i> 33238 (RAMK)</b>	<b>OR392814</b>	<b>Present study</b>
<i>A. robusta</i>	Australia	<i>Lumbsch</i> 20012 (F)	KM453822	Nelsen <i>et al.</i> 2014
<i>A. terricola</i>	Costa Rica	<i>Lücking</i> 17211 (F)	DQ328995	Del Prado <i>et al.</i> 2006
<i>Architrypethelium lauropaluanum</i>	Peru	<i>Nelsen</i> Cit1P (F)	KX215566	Lücking <i>et al.</i> 2016
<i>A. murisporum</i>	Thailand	<i>Luangsaphabool</i> 031332 (RAMK)	LC361339	Luangsaphabool <i>et al.</i> 2018
<i>A. nitens</i>	Panama	<i>Lücking</i> 27038 (F)	KM453823	Nelsen <i>et al.</i> 2014
<i>Astrothelium aenascens</i>	Thailand	<i>Luangsaphabool</i> 027887 (RAMK)	LC128018	Luangsaphabool <i>et al.</i> 2016
<i>A. macrostiolum</i>	Thailand	<i>Luangsaphabool</i> 027895 (RAMK)	LC128022	Luangsaphabool <i>et al.</i> 2016
<i>A. siamense</i>	Thailand	<i>Luangsaphabool</i> 027901 (RAMK)	LC128020	Luangsaphabool <i>et al.</i> 2016
<i>Bathelium lineare</i>	Vietnam	<i>Gueidan</i> 2078 (F)	KM453839	Nelsen <i>et al.</i> 2014
<i>B. madreporiforme</i>	Brazil	<i>Lücking</i> 23290 (F)	KM453840	Nelsen <i>et al.</i> 2014
<i>Pseudopyrenula diluta</i>	Venezuela	<i>Lücking</i> 26062 (F)	KM453861	Nelsen <i>et al.</i> 2014
<i>P. subgregaria</i>	Thailand	<i>Lücking</i> 24079 (F)	GU327699	Nelsen <i>et al.</i> 2009
<i>Trypethelium eluteriae</i>	Australia	<i>Lumbsch</i> 19112 g (F)	DQ328990	Del Prado <i>et al.</i> 2006
<i>T. subeluteriae</i>	Costa Rica	<i>Lücking</i> 17611 (F)	DQ329009	Del Prado <i>et al.</i> 2006



**Figure 1.** Maximum likelihood (ML) tree based on mtSSU gene sequences showing the position of *Aptrootia khaoyaiensis* within the *Trypetheliaceae*. The numbers at branch nodes indicate bootstrap percentages resulting from 1000 replications. ML and MP bootstrap values  $\geq 70\%$  are indicated above the branches (ML/MP). Specimen voucher numbers are given after species names. In colour online.

*Conidiomata* not observed.

**Chemistry.** K–, UV–. TLC: no lichen substances detected.

**Etymology.** The specific epithet is a reference to Khao Yai National Park where the holotype was collected.

**Ecology and distribution.** The specimens studied were collected from tree bark between 200 and 400 m altitude in dry dipterocarp forest with open canopy, growing on the bark of *Dipterocarpus obtusifolius* and *Gluta usitata*, indicating that they prefer hard, thick and acid tree barks (Wolseley & Aguirre-Hudson 1997). The topography of the area consists of inclining slopes, sandy soil and sandy rocks on the ground (Fig. 1). The area is frequently affected by forest fires during the dry season.

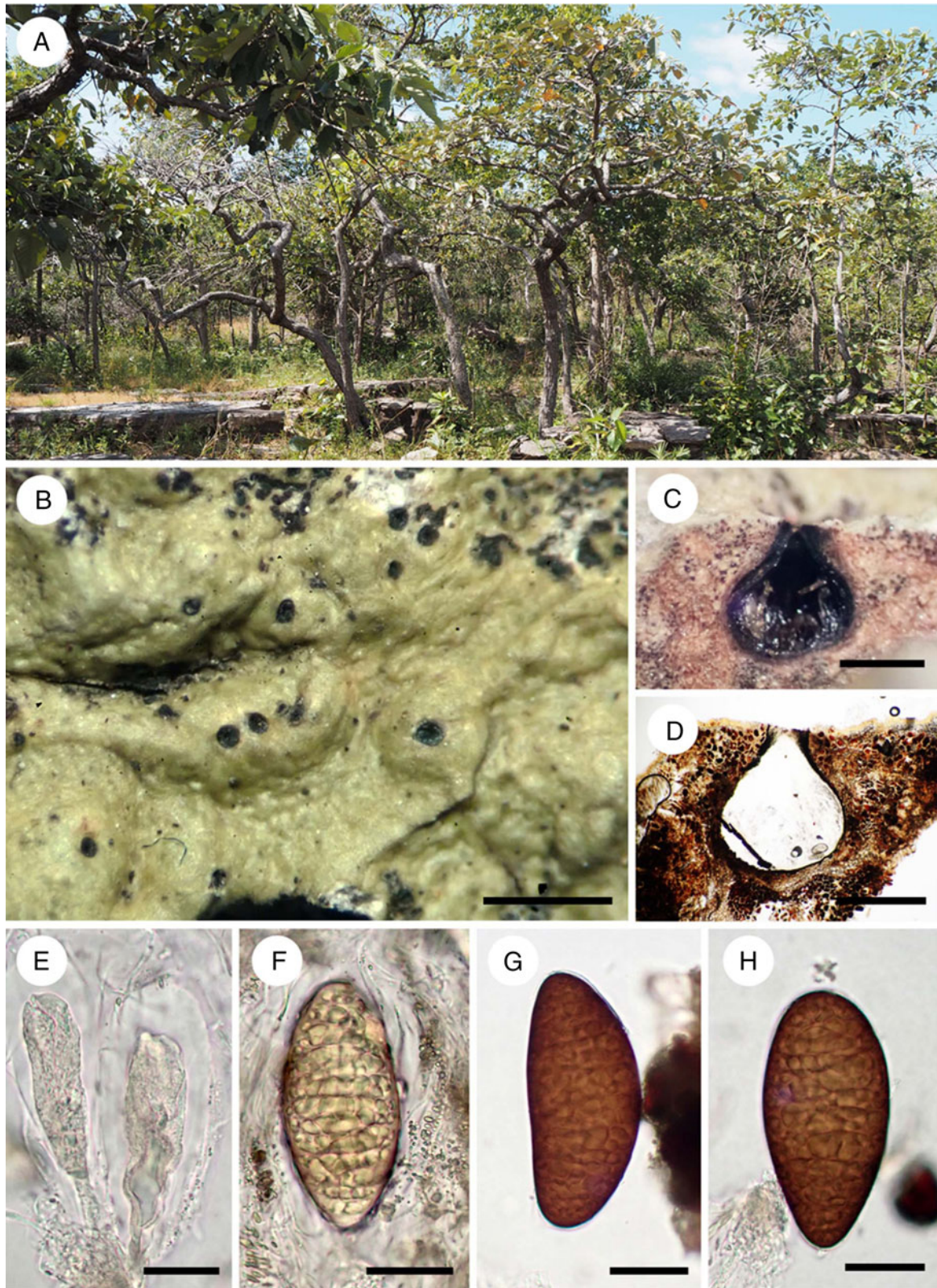
**Remarks.** *Aptrootia khaoyaiensis* is characterized by its corticolous habit, its corticate, smooth to uneven thallus, its inspersed hamathecium and dark brown, densely muriform ascospores. The new species is most similar to *A. elatior* in external morphology and substratum ecology (Aptroot & Lücking 2016). The

latter, however, differs in having ornamented and much larger ascospores ( $200\text{--}330 \times 60\text{--}90 \mu\text{m}$ ), a largely clear hamathecium (except for oil droplets near the ostiole), and the thallus is bullate. *Aptrootia terricola* and *A. robusta* grow on soil or bryophytes, their thallus is smooth to uneven, rather thin and greyish, they have a clear hamathecium and their ascospores, although also with smooth walls, are larger ( $150\text{--}400 \times 40\text{--}140 \mu\text{m}$ ) (Aptroot & Lücking 2016). The new species is also superficially similar to *Anthracothecium macrosporum* (Hepp) Müll. Arg. in the erumpent perithecia covered by thallus tissue, but differs in the conical shape of the perithecia, the unbranched periphysoids, and the clear hamathecium (Awasthi 1991; Aptroot *et al.* 2008).

**Additional specimens examined. Thailand:** Prachinburi Province: Prachantakham District, Khao Yai National Park,  $14^\circ 11'51''\text{N}$ ,  $101^\circ 29'31''\text{E}$ , 253 m a.s.l., dry dipterocarp forest, on bark of *D. obtusifolius* Teijsm. ex Miq., 2021, *W. Polyiam* 33237 (RAMK); *ibid.*,  $14^\circ 11'54''\text{N}$ ,  $101^\circ 29'26''\text{E}$ , 214 m a.s.l., dry dipterocarp forest, on bark of *D. obtusifolius*, 2020, *W. Polyiam* 32239 (RAMK).

**Key to the species of *Aptrootia***

- 1 Thallus corticolous ..... 2
- Thallus terricolous or muscicolous ..... 3
- 2(1) Thallus smooth to uneven; hymenium fully inspersed; ascospores not ornamented,  $80\text{--}150 \times 30\text{--}50 \mu\text{m}$  ..... **A. khaoyaiensis**
- Thallus verrucose to bullate; hymenium inspersed only near the ostiole; ascospores ornamented,  $200\text{--}330 \times 60\text{--}90 \mu\text{m}$  ..... **A. elatior**
- 3(1) Ascospores  $170\text{--}230 \times 40\text{--}70 \mu\text{m}$  ..... **A. terricola**
- Ascospores  $150\text{--}360\text{--}400 \times 65\text{--}140 \mu\text{m}$  ..... **A. robusta**




**Figure 2.** *Aptrothia khaoyaiensis* (RAMK 33238—holotype). A, habitat of the holotype dominated by *Dipterocarpus obtusifolius*. B, perithecia and thallus structure. C & D, sections through perithecia. E, asci. F, pale brown ascospore surrounded by paraphysoids and oil drops. G & H, dark brown ascospores. Scales: B = 1 mm; C & D = 300  $\mu$ m; E–H = 30  $\mu$ m. In colour online.

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