

## Five new species of Graphidaceae (Ascomycota, Ostropales) from Thailand

Khwanyuruan Naksuwankul<sup>1</sup>, Ekaphan Kraichak<sup>2</sup>, Sittiporn Parnmen<sup>3</sup>,  
Robert Lücking<sup>4</sup>, H. Thorsten Lumbsch<sup>5</sup>

**1** Department of Biology and Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham Province, 44150 Thailand **2** Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand **3** Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000 Thailand **4** Botanical Garden and Botanical Museum Berlin, Königin-Luise-Straße 6–8, D-14195 Berlin, Germany **5** Science & Education, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.

Corresponding author: Khwanyuruan Naksuwankul ([khwanruan.p@msu.ac.th](mailto:khwanruan.p@msu.ac.th))

---

Academic editor: P. Divakar | Received 15 September 2016 | Accepted 1 December 2016 | Published 8 December 2016

---

**Citation:** Naksuwankul K, Kraichak E, Parnmen S, Lücking R, Lumbsch TH (2016) Five new species of Graphidaceae (Ascomycota, Ostropales) from Thailand. MycoKeys 17: 47–63. <https://doi.org/10.3897/mycokeys.17.10512>

---

### Abstract

Five new species of Graphidaceae are described from Thailand. Molecular evidence and phenotypical characters support their independent status from related and similar species. *Glaucotrema thailandicum* Naksuwankul, Lücking & Lumbsch is unique within the genus in having submuriform ascospores. *Ocellularia klinhomii* Naksuwankul, Lücking & Lumbsch is characterized by having a whitish gray, rimose thallus with ascomata in verrucae and surrounded by a black ring and lack of secondary metabolites. *Ocellularia phatamensis* Naksuwankul, Parnmen & Lumbsch has a grayish, thick and rimose thallus, differing from *O. klinhomii* in lacking a dark apothecial rim and having ascomata that are not immersed in verrucae. *Ocellularia thailandica* Naksuwankul, Kraichak & Lumbsch differs from *O. albocincta* in lacking a columella. *Ocellularia rotundifumosa* Naksuwankul, Lücking & Lumbsch differs from *O. fumosa* in having ascospores with rounded ends. An epitype for *O. krathingensis* is selected.

### Key words

East Asia, lichens, taxonomy, thelotremoid lichens, tropical diversity

## Introduction

Phenotypical characters, such as morphology of the thallus and ascomata and anatomy of the ascomata as well as secondary chemistry have traditionally guided species delimitation in lichenized ascomycetes. However, especially crustose lichens often exhibit only few traits and without independent markers, such as DNA sequence data, it is often difficult to assess whether variation is due to genetic differences or plasticity. Indeed, recent phylogenetic studies suggest high amounts of homoplasy in phenotypical characters used to delimit taxa in lichenized fungi (Grube et al. 2004; Tehler and Irestedt 2007; Schmitt et al. 2009; Rivas Plata and Lumbsch 2011; Lumbsch et al. 2014a). Hence, molecular data have greatly increased our ability to identify distinct lineages, including the detection of numerous cryptic lineages (Crespo and Lumbsch 2010; Lumbsch and Leavitt 2011; Leavitt et al. 2015). While numerous foliose and fruticose lichen groups have been studied in some detail, especially in the diverse Parmeliaceae, our knowledge on species delimitation in crustose lichens is still in its infancy. However, among predominantly crustose families, Graphidaceae is now relatively well known.

Graphidaceae constitutes the largest family of crustose tropical lichens with about 2100 accepted species (Rivas Plata et al. 2012; Lücking et al. 2013; Cáceres et al. 2014; Van den Broeck et al. 2014; Lumbsch et al. 2014b; Kraichak et al. 2014). The family has its center of distribution in the tropics, but also occurs in temperate regions with a smaller number of species, in some cases even extending towards the Sub-Antarctic region. The family is most common, however, in the tropics where its species occur often on bark, but can also be found on rocks, wood or soil and sometimes on leaves. Recently, the first author started a project on the diversity of thelotremoid Graphidaceae in East Asia (Papong et al. 2014). Thelotremoid Graphidaceae have rounded ascomata (formerly placed in Thelotremataceae), in contrast to species with lirellate ascomata. The group is still relatively poorly known in Thailand and generally in south-east Asia, but preliminary studies have provided important baseline data for the distribution of species and have indicated that numerous additional species can be expected in Thailand (Homchantara and Coppins 2002; Papong et al. 2010; Sutjaritturakan and Kalb 2015). Molecular data have been used to identify distinct lineages in this group of lichenized fungi and subsequent re-analysis of phenotypical characters often allowed identification of morphological or chemical traits to separate those species (Lumbsch et al. 2008; Mangold et al. 2014; Poengsungnoen et al. 2014; Medeiros et al. 2016). This paper employs molecular, morphological and chemical data to identify six distinct lineages of thelotremoid lichens from Thailand and to describe them as species new to science. Based on our limited sampling of thelotremoid Graphidaceae from other regions of southeast Asia, we expect the new species described here from Thailand to occur in other countries of the region.

## Material and methods

This study is mainly based on new collections made by the first two authors deposited in F and MSUT. Sections of thalli and apothecia were cut using a razor blade and examined in water, a solution of KOH, and Lugol's solution using a ZEISS Axioscope 2 plus compound microscope. Chromatography (HPTLC) was performed with standard solvent systems A and C (Culberson 1972; Arup et al. 1993).

We performed two different phylogenetic analyses: 1) sequences of six samples of the genus *Glaucotrema* were aligned with two outgroup taxa (*Leptotrema wightii*, *Reimnitzia santensis*) and 2) sequences of 35 samples of *Ocellularia* s. str. were aligned with *O. cavata* as outgroup. Selection of samples was done using Blast searches and included best hits to ensure that all similar sequences were included. In addition sequences of morphologically similar species were added to the data set. Sequences of mtSSU rDNA, nuLSU rDNA, and the protein-coding RPB2 gene were used for this study. Voucher information and Genbank numbers are listed in Table 1. DNA isolation, PCR, and direct cycle sequencing conditions were described previously (Kraichak et al. 2014).

For the phylogenetic analyses, the alignment of the nucleotide sequences for each dataset was performed separately using Geneious version 8.0.3 (Drummond et al. 2014) and manually inspected for removal of any ambiguous characters. We then performed a maximum likelihood analysis, using RAxML-HPG Blackbox version 8.2.8 (Stamatakis 2006) with the default rapid hill-climbing algorithm and the GTRGAMMA model of nucleotide substitution. The analysis was carried out on the online server CIPRES science Gateway version 3.3 (Miller et al. 2010) with a total of 1,000 pseudoreplicates to assess the rapid bootstrap value support. A bootstrap support value of 70 and above was considered a strong support for a clade. The resulting bipartitioned trees were visualized with the program FigTree version 1.4.2 (Rambaut 2012).

## Results and discussion

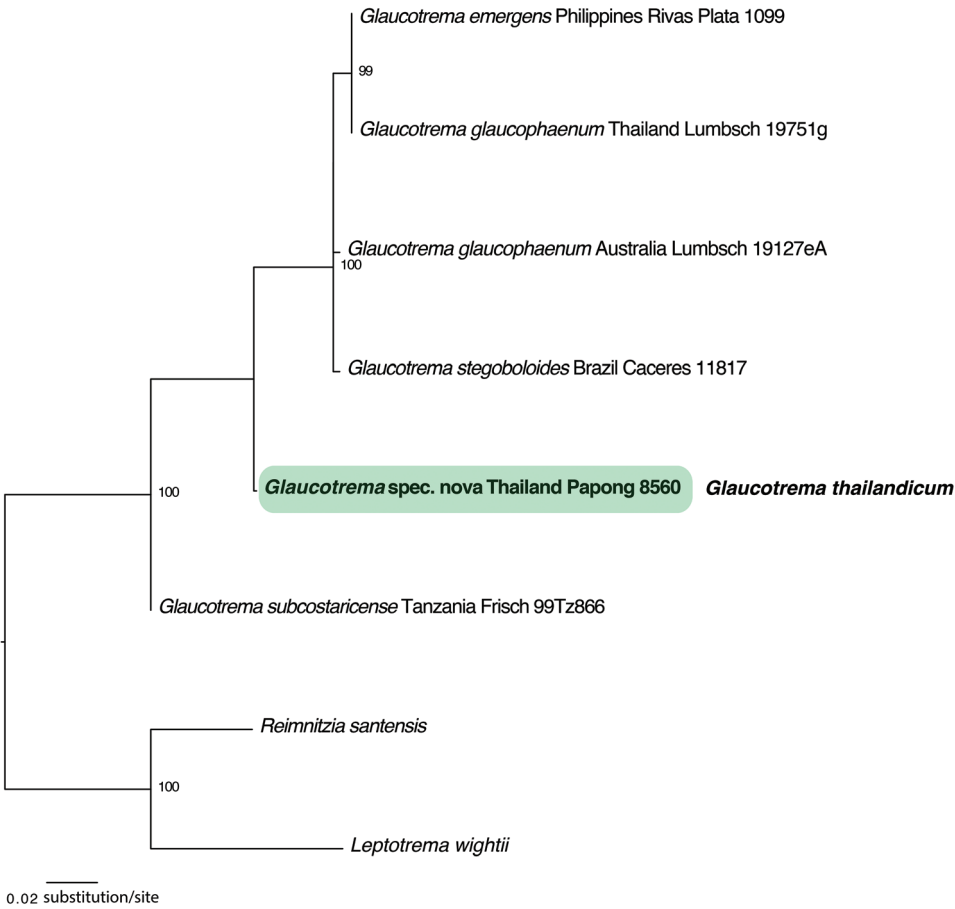
### Phylogenetic analysis

The final alignment of the combined data set for the *Glaucotrema* analysis consisted of 802 unambiguously aligned nucleotide positions for mtSSU, 865 for nuLSU, and for 985 RPB2. The final alignment of the dataset for the *Ocellularia* taxa consisted of 787 unambiguously aligned nucleotide positions for mtSSU, 879 for nuLSU, and for 913 RPB2. As the topologies of the single locus phylogenies for these two datasets did not show any conflicts, they were analyzed in a concatenated matrix.

In the *Glaucotrema* tree (Fig. 1), the Thai material formed an unsupported sister-group relationship with *G. glaucophaenum*, and *G. stegoboloides*. The latter two species were not separated in our analysis but were supported as different species in a broader

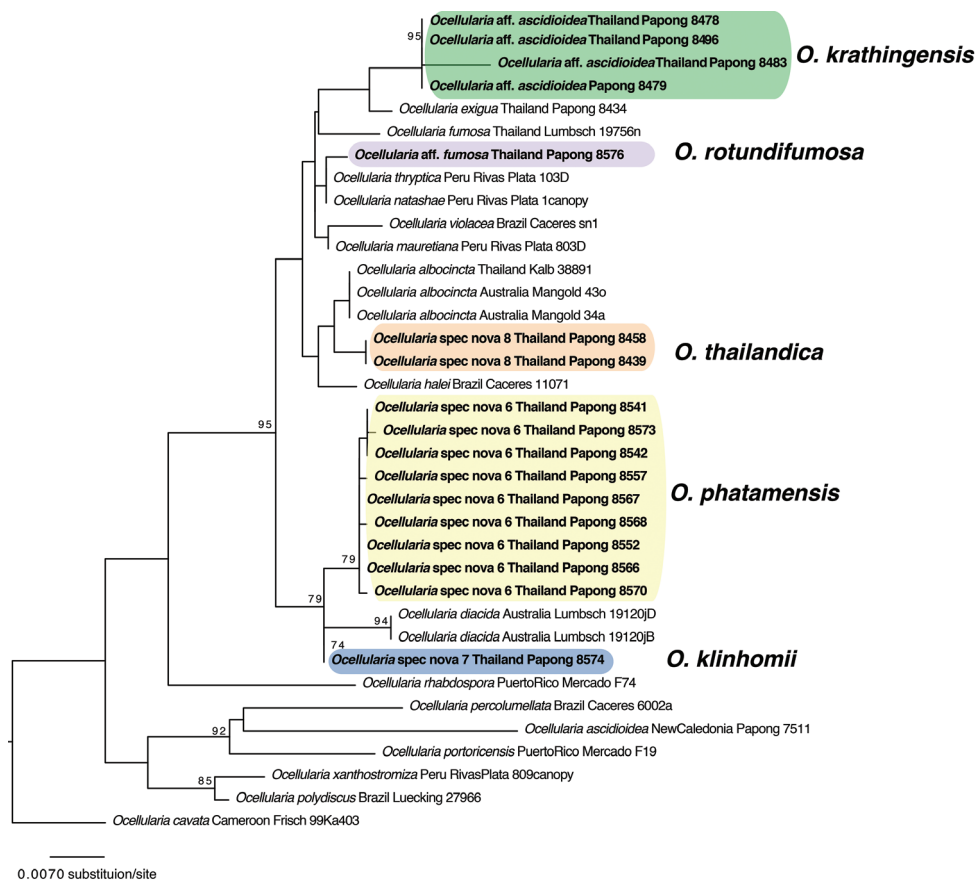
**Table 1.** Genbank numbers and voucher information of specimens used in this study. For author names see *Index Fungorum* (<http://www.indexfungorum.org>). Missing data are indicated by [—].

Species	Country	Collector	Number	mtSSU	nuLSU	RPB2
<i>Glaucotrema glaucophaenum</i>	Philippines	Rivas Plata	1099	JX421061	JX421501	JX420862
<i>Glaucotrema glaucophaenum</i>	Thailand	Lumbsch	19751g	[—]	JX421502	[—]
<i>Glaucotrema glaucophaenum</i>	Australia	Lumbsch	19127eA	JX421060	[—]	[—]
<i>Glaucotrema stegoboloides</i>	Brazil	Cáceres	11817	KJ435228	[—]	[—]
<i>Glaucotrema subcostaricense</i>	Tanzania	Frisch	99Tz866	DQ384899	[—]	[—]
<i>Glaucotrema thailandicum</i>	Thailand	Papong	8560	[—]	<b>KJ435152</b>	[—]
<i>Leptotrema wightii</i>	Costa Rica	Nelsen	2034A	JX421074	EU075622	[—]
<i>Ocellularia albocincta</i>	Thailand	Kalb	38891	JX421114	[—]	[—]
<i>Ocellularia albocincta</i>	Australia	Mangold	43o	EU075585	EU075633	[—]
<i>Ocellularia albocincta</i>	Australia	Mangold	34a	JX421112	[—]	[—]
<i>Ocellularia ascidioidea</i>	New Caledonia	Papong	7511	KJ435201	KJ435125	KJ435267
<i>Ocellularia cavata</i>	Cameroon	Frisch	99Ka403	DQ384879	DQ431935	[—]
<i>Ocellularia diaciada</i>	Australia	Lumbsch	19120jB	EU075583	EU075630	[—]
<i>Ocellularia diaciada</i>	Australia	Lumbsch	19120jD	JF828965	[—]	[—]
<i>Ocellularia exigua</i>	Thailand	Papong	8434	KJ435244	[—]	[—]
<i>Ocellularia fumosa</i>	Thailand	Lumbsch	19756n	[—]	JX421539	[—]
<i>Ocellularia halei</i>	Brazil	Cáceres	11071	KJ435218	[—]	[—]
<i>Ocellularia klinhomii</i>	Thailand	Papong	8574	<b>KJ435252</b>	[—]	[—]
<i>Ocellularia krathingensis</i>	Thailand	Papong	8478	<b>KJ435248</b>	<b>KJ435153</b>	[—]
<i>Ocellularia krathingensis</i>	Thailand	Papong	8479	<b>KJ435246</b>	[—]	[—]
<i>Ocellularia krathingensis</i>	Thailand	Papong	8483	<b>KJ435241</b>	[—]	[—]
<i>Ocellularia krathingensis</i>	Thailand	Papong	8496	<b>KJ435232</b>	<b>KJ435143</b>	[—]
<i>Ocellularia mauritiana</i>	Peru	Rivas Plata	803D	JX421170	[—]	[—]
<i>Ocellularia natashae</i>	Peru	Rivas Plata	1canopy	JX421175	[—]	JX420877
<i>Ocellularia percolumellata</i>	Brazil	Cáceres	6002a	JX421180	[—]	JX420888
<i>Ocellularia polydiscus</i>	Brazil	Lücking	27966	DQ384876	[—]	[—]
<i>Ocellularia portoricensis</i>	Puerto Rico	Mercado	F19	KJ435178	[—]	KJ435256
<i>Ocellularia phatamensis</i>	Thailand	Papong	8541	<b>KJ435239</b>	<b>KJ435150</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8542	<b>KJ435249</b>	<b>KJ435154</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8552	<b>KJ435236</b>	<b>KJ435147</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8557	<b>KJ435238</b>	<b>KJ435149</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8566	<b>KJ435233</b>	<b>KJ435144</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8567	<b>KJ435245</b>	[—]	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8568	<b>KJ435237</b>	<b>KJ435148</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8570	<b>KJ435250</b>	<b>KJ435155</b>	[—]
<i>Ocellularia phatamensis</i>	Thailand	Papong	8573	<b>KJ435251</b>	<b>KJ435156</b>	[—]
<i>Ocellularia rhabdospora</i>	Puerto Rico	Mercado	F74	KJ435172	KJ435108	KJ435254
<i>Ocellularia rotundifumosa</i>	Thailand	Papong	8576	<b>KJ435231</b>	[—]	[—]
<i>Ocellularia thailandica</i>	Thailand	Papong	8439	<b>KJ435235</b>	<b>KJ435146</b>	[—]
<i>Ocellularia thailandica</i>	Thailand	Papong	8458	<b>KJ435247</b>	[—]	[—]
<i>Ocellularia thryptica</i>	Peru	Rivas Plata	103D	JX421222	[—]	[—]
<i>Ocellularia violacea</i>	Brazil	Cáceres	sn	JX421225	[—]	[—]
<i>Ocellularia xanthostromiza</i>	Peru	Rivas Plata	809canopy	JX421171	[—]	[—]
<i>Reimnitzia santensis</i>	El Salvador	Lücking	28015	HQ639622	[—]	JF828952



**Figure 1.** Phenogram depicting phylogenetic relationships of *Glaucotrema* species. Only bootstrap support values above 70 are displayed on the nodes.

analysis in Kraichak et al. (2014) with more samples, in which the Thai material was also included and supported as distinct species. In the *Ocellularia* tree (Fig. 2), *O. aff. ascidioidea* from Thailand did not form a monophyletic group with *O. ascidioidea* from New Caledonia but an unsupported sister-group relationship with Thai material of *O. exigua*, similar to the analysis by Kraichak et al. (2014). Subsequent morphological re-analysis revealed that the Thai samples previously identified as *O. aff. ascidioidea* are identical to *O. krathingensis* described from Thailand (Homchantara and Coppins 2002). As already indicated by Kraichak et al. (2014), *Ocellularia* aff. *fumosa* from Thailand did not cluster with *O. fumosa* but appeared closely related to *O. natashae* and *O. thryptica*. The latter differs in having a clear hymenium and containing protoce-traric acid, whereas *O. natashae* has longer ascospores and contains the hirtifructic acid chemosyndrome (Hale 1973; Rivas Plata and Lücking 2013). The close phylogenetic



**Figure 2.** Phenogram depicting phylogenetic relationships of *Ocellularia* species. Only bootstrap support values above 70 are displayed on the nodes.

relationship of these three taxa, which are not only phenotypically disparate but also have distinct geographic distributions, suggests that the loci here used may be of limited use for species delimitation in recently evolved complexes, which has already been discussed for mtSSU by Kraichak et al. (2014). Two samples, included as spec. nov. 8 in Kraichak et al. (2014), formed an unsupported sister-group relationship with *O. albocincta*, a species that differs morphologically (see below) and so the Thai material is described as a new species (*O. siamensis*) below. Nine samples included as spec. nov. 6 in Kraichak et al. (2014) from Thailand clustered together, related to *O. diacida*, which is readily distinguished by the presence of the hirtifructic acid chemosyndrome. The species is described new to science below as *O. phatamensis*. A single specimen, included as spec. nov. 7 in Kraichak et al. (2014), is also related to *O. diacida* but differs – among other characters – by the absence of secondary metabolites.

## Taxonomic novelties

### *Glaucotrema thailandicum* Naksuwankul, Lücking & Lumbsch, sp. nov.

Mycobank # 818194

Figure 3A–E

**Type.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, Sang Chan waterfall, 15°30'N, 105°35'E, 124 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8560 (holotype: MSUT; isotype: F).

**Diagnosis.** Characterized within the genus by having submuriform ascospores.

**Etymology.** The specific epithet refers to the country where the type specimen was collected.

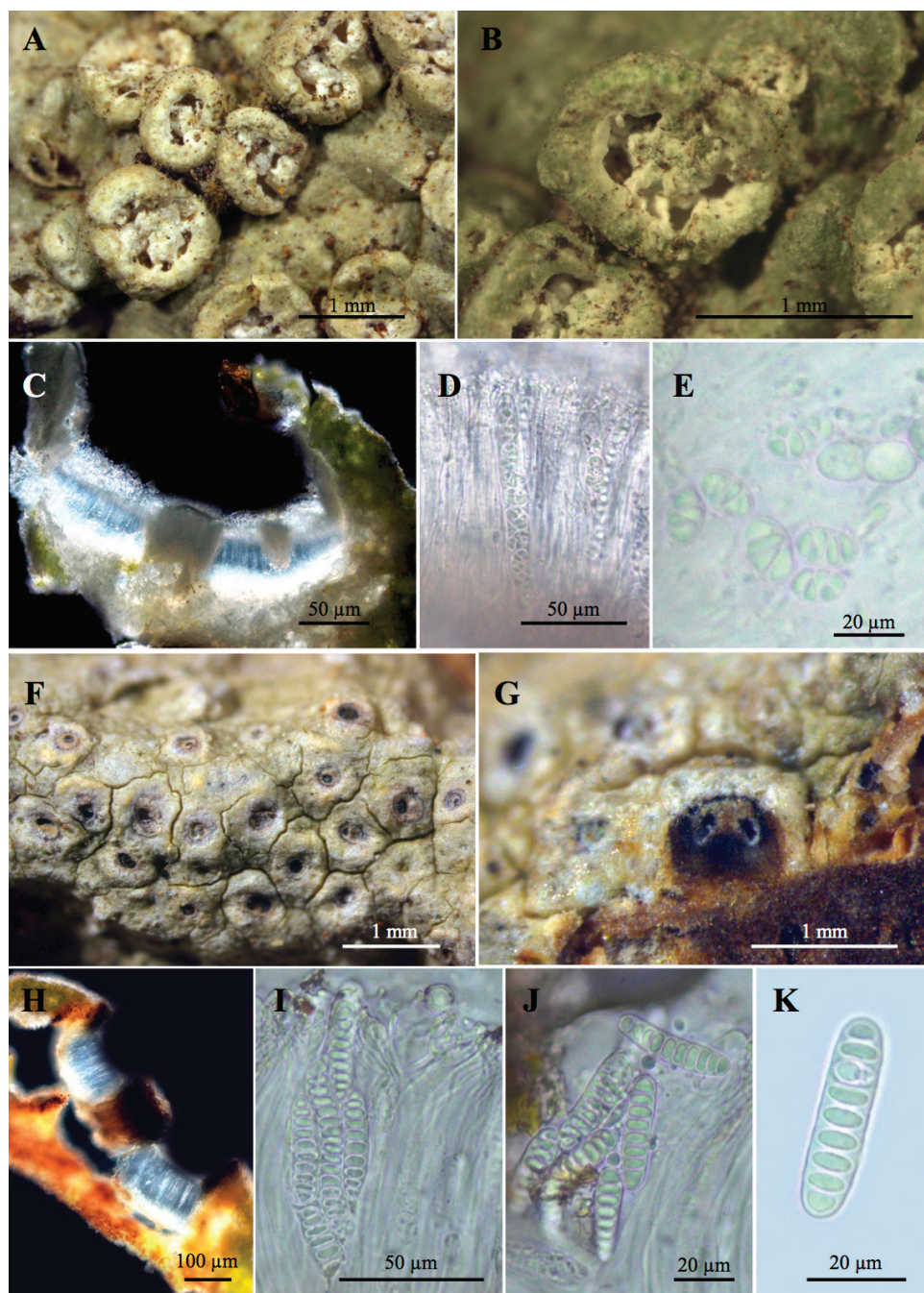
**Description.** Thallus endophloeodal to epiphloeodal, up to c. 120 µm thick, pale green to yellowish green, smooth. True cortex ±continuous, to c. 25 µm thick. Algal layer poorly to well developed, ±continuous; calcium oxalate crystals sparse to abundant, large and clustered; medulla usually distinct. Vegetative propagules not seen. Ascomata conspicuous, to c. 0.8–1.2 mm diam., often larger when fused, ±rounded to irregular, apothecioid to somewhat chroodiscoid, solitary to more often fused, becoming slightly to distinctly emergent, mostly irregularly or regularly urceolate. Disc usually partly visible from above, rarely completely exposed, pale yellowish to whitish green. Pores broad to gaping, to c. 0.6–0.8 mm wide, ±rounded to irregular, entire to slightly ragged; thalline exciple often becoming apically visible, rarely completely visible from above, ±free, whitish. Thalline rim margin broad to gaping, ±rounded, more commonly irregular, thick, entire, concolorous to whitish. Thalline exciple fused to partly or entirely free, thick, hyaline internally, pale yellowish or greenish marginally, with calcium oxalate crystals. Hymenium to c. 120 µm thick, clear, strongly conglutinated; paraphyses thick, irregular and often distoseptate, ±interwoven, with thickened irregular tips; lateral paraphyses absent; columella whitish and reticulate. Epihymenium hyaline, with fine crystals. Asci 8-spored; tholus initially thick, thin when mature, 100–110 × 10–12 µm. Ascospores submuriform with 3 × 0–1 septa, hyaline, slightly amyloid, 15–20 × 7.5 µm. Pycnidia not seen.

**Secondary chemistry.** Thallus K+ yellowish, C–, P+ yellow; containing psoromic acid.

**Distribution and ecology.** The new species was found in northeastern Thailand, growing on bark in a dry evergreen forest. It is known only from the type locality.

**Remarks.** This new species is unique within the genus in having submuriform ascospores, whereas all other described species have transversely septate ascospores. In addition, the ascospores in *G. bahianum*, *G. costaricense* and *G. stegoboloides* are smaller than in the new species. Molecular data support the distinction of the new taxon. In morphology it resembles *G. bahianum* and *G. stegoboloides*.





**Figure 3.** Morphology and anatomy of *Glaucotrema thailandicum* (A–E) A–B habitat of ascoma C cross-section of ascoma show whitish and reticulate columella D asci with spores and E submuriform ascospores (holotype), *Ocellularia klinhomii* (F–K) F–G ascomata immersed in verrucae and surrounded by a black ring H cross-section of ascoma with carbonized columella and apically carbonized exiple I–K ascus and ascospores (holotype). Scale bar A–B, F–G = 1 mm, H = 100 µm, C–D, I = 50 µm, E, J–K = 20 µm.



***Ocellularia klinhomii* Naksuwankul, Lücking & Lumbsch, sp. nov.**

Mycobank # 818195

Figure 3F–K

**Type.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, Sang Chan waterfall, 15°30'N, 105°35'E, 124 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8566 (holotype: MSUT; isotype: F).

**Diagnosis.** Differing from the similar *O. krathingensis* in having a whitish grey, rimose thallus.

**Etymology.** The specific epithet refers to the collector Mr. Winia Klinhom, mycologist from Thailand.

**Description.** Thallus corticolous, epiperidermal, up to c. 5 cm diam., continuous; surface rimose, whitish grey, medulla white; prothallus absent. Thallus in section 30–40 µm thick, with prosoplectenchymatous cortex, 5–10 µm thick, photobiont layer 15–20 µm thick, and medulla 20–25 µm thick, with scattered clusters of calcium oxalate crystals. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, green, 7–9 × 6–8 µm. Ascomata rounded, verrucae and surrounded by a black ring, erumpent to immersed, with complete thalline margin, 0.4–0.7 mm diam., 0.15–0.2 mm high; disc covered by 0.05–0.1 mm wide pore more or less filled with black-tipped columella but columella often immersed; proper margin indistinct, entire to slightly fissured, visible as whitish rim around the pore; thalline margin entire to slightly fissured, smooth, yellowish green. Excipulum entire, prosoplectenchymatous, brown with apically carbonized, 15–20 µm wide, fused with thalline margin and difficult to separate from the bordering periderm; laterally covered by algiferous, corticate thallus containing periderm layers; columella present, finger-like, carbonized, up to 100 µm broad and 120–140 µm high; hypothecium prosoplectenchymatous, 5–10 µm high, light brown; hymenium 125–150 µm high, hyaline, clear; epithecium indistinct, 5–7 µm high, hyaline. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical to narrowly clavate, 110–115 × 12–15 µm. Ascospores 8 per ascus, ellipsoid, 6–9-septate, 25–38 × 7–8 µm, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Pycnidia not seen.

**Secondary chemistry.** No substances detected by TLC.

**Distribution and ecology.** The new species was collected in northeastern Thailand, growing on bark in a dry evergreen forest. It is known only from the type locality.

**Remarks.** Similar in ascospore size, lack of secondary metabolites and only apically carbonized exciple to *O. krathingensis* but differing in having a whitish gray, rimose thallus with ascomata in verrucae and surrounded by a black ring, reminiscent of *O. wirthii* (Mangold et al. 2008). The latter species is readily distinguished by having a broader, carbonized columella and the presence of the psoromic acid chemosyndrome. The species would key out at alternative 60 in the *Ocellularia* key for Thailand (Sutjaritturakan & Kalb 2015).

**Additional specimen examined.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, Sang Chan waterfall, 15°30'N, 105°35'E, 124 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8568, 8552, 8567, 8570, 8542, 8541, 8573, 8574 (MSUT), K. Papong 8557 (RAMK).

***Ocellularia phatamensis* Naksuwankul, Parnmen & Lumbsch, sp. nov.**

Mycobank # 818196

Figure 4A–B

**Type.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, Sang Chan waterfall, 15°30'N, 105°35'E, 124 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8574 (holotype: MSUT; isotype: F).

**Diagnosis.** Differing from the similar *O. krathingensis* in having an a grayish, thick and rimose thallus.

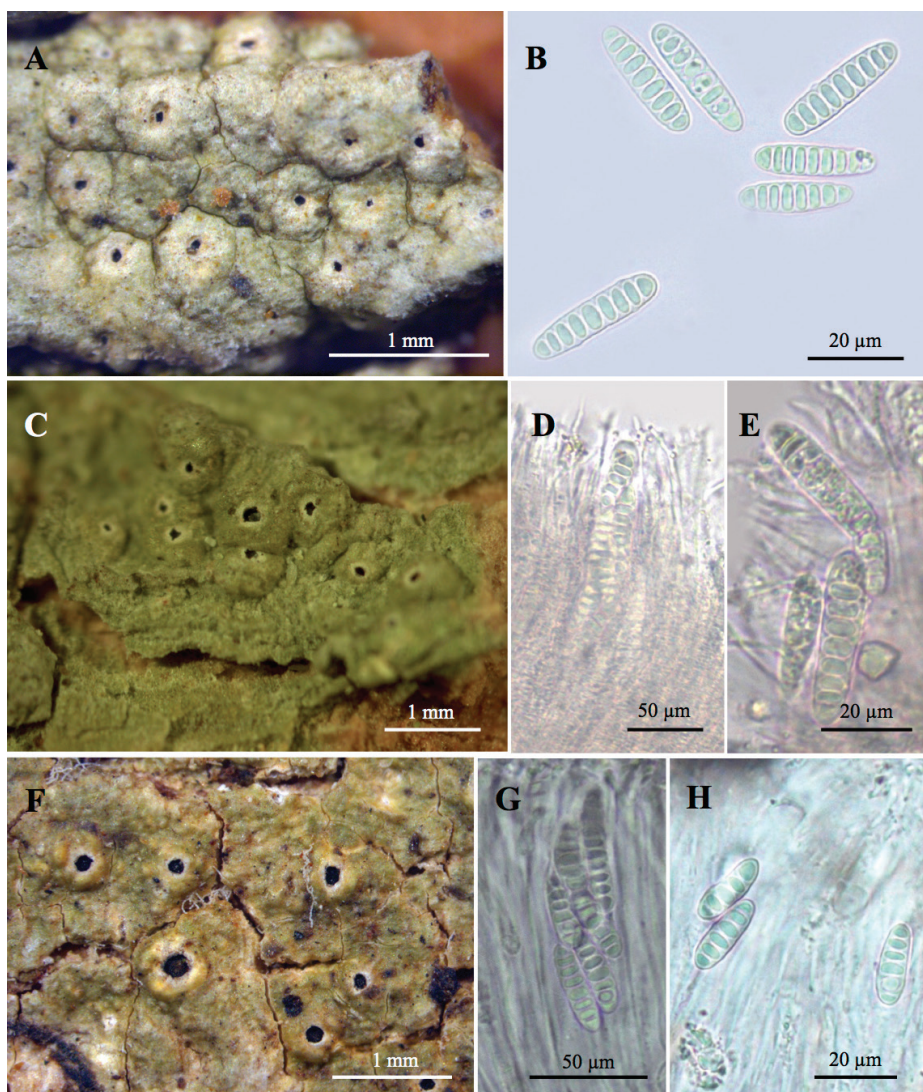
**Etymology.** The specific epithet refers to the name of the Pha Tam National Park in Ubon Ratchathani Province, Thailand.

**Description.** Thallus corticolous, epiperidermal, up to c. 5 cm diam., continuous; surface uneven-verrucose to rimose, grayish, medulla white; prothallus absent. Thallus in section 60–75 µm thick, with prosoplectenchymatous cortex, 5–8 µm thick, photobiont layer 20–25 µm thick, and medulla 35–40 µm thick, with scattered clusters of calcium oxalate crystals. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, green, 8–10 × 6–7 µm. Ascomata rounded, erumpent, with complete thalline margin, 0.4–0.7 mm diam., 0.15–0.2 mm high; disc covered by 0.07–0.1 mm wide pore more or less filled with black-tipped columella but columella often immersed; proper margin indistinct; thalline margin entire to slightly fissured, smooth, light yellowish green. Excipulum entire, prosoplectenchymatous, apically carbonized, 15–20 µm wide, fused with thalline margin and difficult to separate from the bordering periderm; laterally covered by algiferous, corticate thallus containing periderm layers; columella present, finger-like, carbonized, up to 110 µm broad and 120–135 µm high; hypothecium prosoplectenchymatous, 5–10 µm high, light brown; hymenium 120–150 µm high, hyaline, clear; epithecium indistinct, 5–10 µm high, hyaline. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical to narrowly clavate, 100–110 × 12–15 µm. Ascospores 8 per ascus, ellipsoid, 7–8-septate, 25–30 × 7.5–8 µm, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Pycnidia not seen.

**Secondary chemistry.** No substances detected by TLC.

**Distribution and ecology.** The new species was collected in northeastern Thailand, growing on bark in a dry evergreen forest. It is known only from the type locality.

**Remarks.** The new species is similar to *O. krathingensis* in having an apically carbonized exciple and columella, transversely septate, amyloid ascospores, and lacking secondary metabolites, but differs in having a grayish and thicker thallus (Homchantara and Coppins 2002). Another similar species is *O. klinhomii*, but differs in lacking a dark apothecial rim and the ascomata are not immersed in verrucae. Molecular data support the distinction of these two species (Fig. 2). Another similar and related species is *O. diacida*, which is readily distinguished by the presence of the hirtifructic acid chemosyndrome. The species would key out at alternative 60 in the *Ocellularia* key for Thailand (Sutjaritturakan and Kalb 2015).



**Figure 4.** Morphology and anatomy of *Ocellularia phatamensis* (**A–B**) **A** habitat of ascomata **B** ascospores (K. Papong 8574, holotype MSUT!), *O. rotundifumosa* (**C–E**) **C** ascomata **D** hymenium with ascus and **E** ascospores (holotype MSUT), *O. thailandica* (**F–H**) **F** habitat of ascomata **G** ascus with ascospores and **H** ascospores (holotype). Scale bar **A**, **C**, **F** = 1 mm, **D**, **G** = 50  $\mu$ m, **B**, **E**, **H** = 20  $\mu$ m.

***Ocellularia rotundifumosa* Naksukankul, Lücking & Lumbsch, sp. nov.**

Mycobank # 818197

Figure 4C–E

**Type.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, Sang Chan waterfall, 15°30'N, 105°35'E, 124 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8576 (holotype: MSUT; isotype: F).

**Diagnosis.** Differing from *O. fumosa* in having ascospores with rounded ends.

**Etymology.** The specific epithet refers to the ascospore shape with rounded ends and to the similarity with *O. fumosa*.

**Description.** Thallus corticolous, endophloeodal to epiphloeodal, up to c. 200 µm thick, greenish gray to olive, slightly glossy, smooth, rarely continuous to usually ±verrucose. True cortex discontinuous, to c. 15 µm thick, formed by irregular hyphae. Algal layer well developed, continuous; calcium oxalate crystals moderately large, scattered. Photobiont Trentepohlia; cells rounded to irregular in outline, in irregular groups, green, 7–9 × 6–9 µm. Vegetative propagules not seen. Ascomata rounded with complete thalline margin, 0.4–0.9 mm diam., solitary to marginally fused, immersed to rather emergent, then verrucose-hemispherical to urceolate. Disc with the columella visible from above, entire, free, slightly pruinose, dark gray. Pores formed by the thalline rim margin, c. 0.5 mm diam., the apex of the proper exciple becoming visible from above as a brownish to dark gray line, moderately thick, concolorous with the thallus or brighter; thalline rim incurved. Proper exciple fused, dark brown to carbonized marginally and towards the tips, usually distinctly amyloid at the base. Hymenium to c. 150 µm thick, densely inspersed, distinctly conglutinated; paraphyses slightly bent, ±interwoven, unbranched, with moderately thickened tips; columellar structures moderately well developed, to 150 µm wide, entire, the upper parts brownish to carbonized. Epihymenium brownish, with grayish or brownish granules. Asci 8-spored; tholus initially thick, thin when mature. Ascospores 7–9-septate, fusiform to oblong-fusiform, rarely clavate, with rounded ends, 24–35 × 7–10 µm, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Pycnidia not seen.

**Secondary chemistry.** No compounds detectable by TLC.

**Distribution and ecology.** The new species was collected in northeastern Thailand, growing on bark in a dry evergreen forest. It is known only from the type locality.

**Remarks.** Similar to *O. fumosa*, but differing in having rounded ends of the ascospores instead of acute ones in *O. fumosa*. Molecular data support the distinction of the species (Fig. 2). Characters to separate the related *O. natashae* and *O. thryptica* are discussed above. The species would key out at alternative 23 in the *Ocellularia* key for Thailand (Sutjaritturakan and Kalb 2015).

***Ocellularia thailandica* Naksuwankul, Kraichak & Lumbsch, sp. nov.**

Mycobank # 818198

Figure 4F–H

**Type.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, trail to Huai Sanom, 15°27'N, 105°34'E, 245 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8458 (holotype: MSUT; isotype: F).

**Diagnosis.** Differing from the similar *O. viridipallens* in having broader ascospores with up to 7 septa.



**Etymology.** The specific epithet refers to the country where the type specimen was collected.

**Description.** Thallus corticolous, epiperidermal, up to c. 5 cm diam., continuous; surface uneven-verrucose to rimose, light yellowish green, medulla white; prothallus absent. Thallus in section 40–60  $\mu\text{m}$  thick, with prosoplectenchymatous cortex, 5–7  $\mu\text{m}$  thick, photobiont layer 15–25  $\mu\text{m}$  thick, and medulla 20–30  $\mu\text{m}$  thick, with scattered clusters of calcium oxalate crystals. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, green, 7–8  $\times$  5–9  $\mu\text{m}$ . Ascomata rounded, erumpent, with complete thalline margin, 0.3–0.5 mm diam., 0.12–0.2 mm high; disc covered by 0.05–0.1 mm wide pore; proper margin indistinct, entire to slightly fissured, visible as whitish rim around the pore; thalline margin entire to slightly fissured, smooth, light yellowish green. Excipulum entire, prosoplectenchymatous, brown to dark brown, 15–20  $\mu\text{m}$  wide, fused with thalline margin and difficult to separate from the bordering periderm; laterally covered by algiferous, corticate thallus containing periderm layers; columella present, finger-like, carbonized, up to 100  $\mu\text{m}$  broad and 120–135  $\mu\text{m}$  high; hypothecium prosoplectenchymatous, 5–10  $\mu\text{m}$  high, hyaline; hymenium 125–140  $\mu\text{m}$  high, hyaline, clear; epithecium indistinct, 5–10  $\mu\text{m}$  high, hyaline. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical to narrowly clavate, 87–100  $\times$  12–15  $\mu\text{m}$ . Ascospores 8 per ascus, ellipsoid, 5–7-septate, 20–23  $\times$  7–8  $\mu\text{m}$ , hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Pycnidia not seen.

**Secondary chemistry.** No substances detected by TLC.

**Distribution and ecology.** The new species was collected in northeastern Thailand, growing on bark in a dry evergreen forest. It is known only from the type locality.

**Remarks.** This new species is closely related to *O. albocincta* (Fig. 2). However, this species differs in lacking a columella (Papong et al. 2010). Morphologically it resembles *O. viridipallens*, which differs in having narrower ascospores. The species would key out at alternative 60 in the *Ocellularia* key for Thailand (Sutjaritturakan & Kalb 2015).

**Additional specimen examined.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, trail to Huai Sanom, 15°27'N, 105°34'E, 245 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8439 (MSUT).

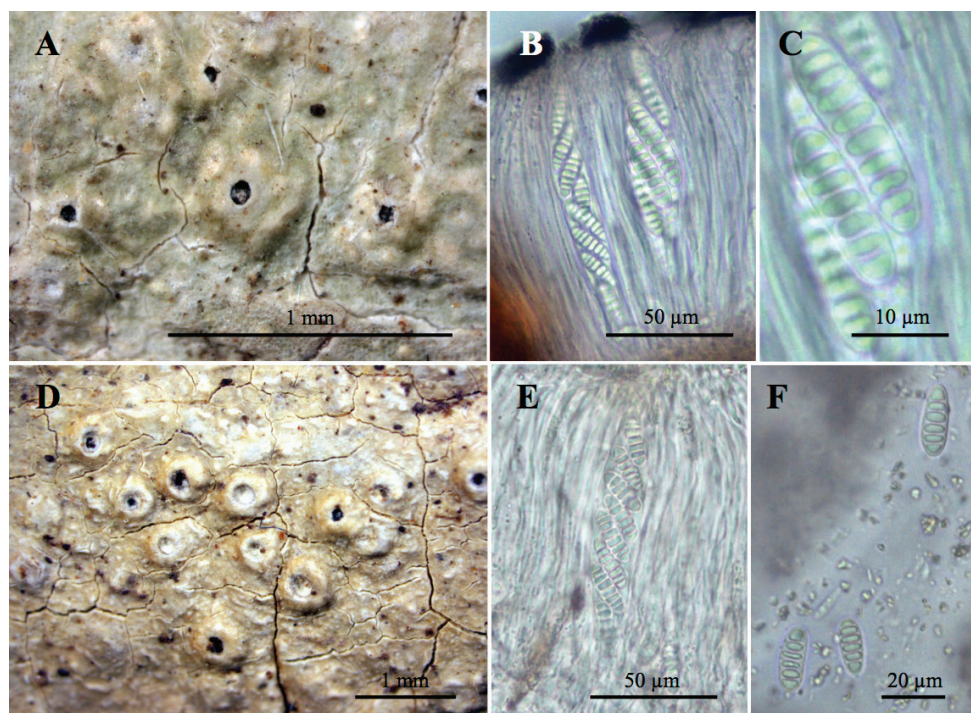
### Epitypification of *Ocellularia krathingensis* Homchant. & Coppins

Figure 5D–F

**Epitype.** THAILAND. Ubon Ratchathani Province: Pha Tam National Park, trail to Huai Sanom, 15° 27' 620" N, 105° 34' 615" E, 245 m, dry evergreen forest, on bark; 12 Apr. 2013, K. Papong 8479 (epitype MSUT!).

In order to clarify the application of the name *Ocellularia krathingensis*, we propose an epitype for this species that agrees morphologically well with the holotype (RAMK!) and has been sequenced.





**Figure 5.** Morphology and anatomy of *O. krathingensis* (A–F); **A** habitat of ascomata **B** hymenium with ascus and **C** ascospores. (D–F) **D** eruptent ascomata **E** ascus and **F** ascospores **A–C** K. Papong 8483 **D–F** K. Papong 8479 (epitype). Scale bar **A**, **D** = 1 mm, **B**, **E** = 50 µm, **C**, **F** = 20 µm.

**Additional specimens examined.** THAILAND, Ubon Ratchathani Province, Pha Tam National Park, trail to Huai Sanom, 15°27'N, 105°34'E, 245 m, dry evergreen forest, on bark; 12 April 2013, K. Papong 8496, 8478, 8483 (F, MSUT) (Figure 5A–C).

## Acknowledgments

This study was financially supported by grants of the Mahasarakham University and Thai Research Fund to the first author (K. Papong RSA 5580045) and the grant *ATM – Assembling a taxonomic monograph: The lichen family Graphidaceae* (DEB-1025861 to The Field Museum; PI T. Lumbsch, CoPI R. Lücking) by the National Science Foundation.

## References

- Arup U, Ekman S, Lindblom L, Mattsson, JE (1993) High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist* 25: 61–71. <https://doi.org/10.1017/S0024282993000076>

- Crespo A, Lumbsch HT (2010) Cryptic species in lichen-forming fungi. *IMA Fungus* 1: 167–170. <https://doi.org/10.5598/imafungus.2010.01.02.09>
- Culberson CF (1972) Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* 72: 113–125. [https://doi.org/10.1016/0021-9673\(72\)80013-X](https://doi.org/10.1016/0021-9673(72)80013-X)
- Cáceres MES, Aptroot A, Parnmen S, Lücking R (2014) Remarkable diversity of the lichen family Graphidaceae in the Amazon rain forest of Rondônia, Brazil. *Phytotaxa* 189: 87–136. <https://doi.org/10.11646/phytotaxa.189.1.8>
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2014) Geneious v. 8.0.3.
- Grube M, Baloch E, Lumbsch HT (2004) The phylogeny of Porinaceae (Ostropomycetidae) suggests a neotenic origin of perithecia in Lecanoromycetes. *Mycological Research* 108: 1111–1118. <https://doi.org/10.1017/S0953756204000826>
- Hale ME (1973) Studies on the lichen family Thelotremaaceae. 1. *Phytologia* 26: 413–420.
- Homchantara N, Coppins BJ (2002) New species of the lichen family Thelotremaaceae in SE Asia. *Lichenologist* 34: 113–140. <https://doi.org/10.1006/lich.2002.0382>
- Kraichak E, Parnmen S, Lücking R, Rivas Plata E, Aptroot A, Cáceres MES, Ertz D, Mangold A, Mercado-Díaz JA, Papong K, Van Den Broeck D, Weerakoon G, Lumbsch HT (2014) Revisiting the phylogeny of Ocellularieae, the second largest tribe within Graphidaceae (lichenized Ascomycota: Ostropales). *Phytotaxa* 189: 52–81. <https://doi.org/10.11646/phytotaxa.189.1.6>
- Leavitt S, Moreau C, Lumbsch HT (2015) The dynamic discipline of species delimitation: progress toward effectively recognizing species boundaries in natural populations. In: Upreti DK, Divakar PK, Shukla V, Bajpai R (Eds) *Recent advances in lichenology*. Springer, India, 11–44. [https://doi.org/10.1007/978-81-322-2235-4\\_2](https://doi.org/10.1007/978-81-322-2235-4_2)
- Lücking R, Tehler A, Bungartz F, Rivas Plata E, Lumbsch HT (2013) Journey from the West: Did tropical Graphidaceae (lichenized Ascomycota: Ostropales) evolve from a saxicolous ancestor along the American Pacific coast? *American Journal of Botany* 100: 844–856. <https://doi.org/10.3732/ajb.1200548>
- Lücking R, Johnston MK, Aptroot A, Kraichak E, Lendemer JC, Boonpragob K, Cáceres MES, Ertz D, Ferraro LI, Jia Z-F, Kalb K, Mangold A, Manoch L, Mercado-Díaz JA, Moncada B, Mongkolsuk P, Papong K, Parnmen S, Peláez RN, Poengsungnoen V, Rivas Plata E, Saipunkaew W, Sipman HJM, Sutjaritturakan J, Van Den Broeck D, Von Konrat M, Weerakoon G, Lumbsch HT (2014) One hundred and seventy five new species of Graphidaceae: closing the gap or a drop in the bucket? *Phytotaxa* 189: 7–38. <https://doi.org/10.11646/phytotaxa.189.1.4>
- Lumbsch HT, Leavitt SD (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* 50: 59–72. <https://doi.org/10.1007/s13225-011-0123-z>
- Lumbsch HT, Mangold A, Martin MP, Elix JA (2008) Species recognition and phylogeny of *Thelotrema* species in Australia (Ostropales, Ascomycota). *Australian Systematic Botany* 21: 217–227. <https://doi.org/10.1071/SB07049>

- Lumbsch HT, Parnmen S, Kraichak E, Papong K, Lücking R (2014a) High frequency of character transformations is phylogenetically structured within the lichenized fungal family Graphidaceae (Ascomycota: Ostropales). *Systematics and Biodiversity* 12: 271–291.
- Lumbsch HT, Kraichak E, Parnmen S, Rivas Plata E, Aptroot A, Cáceres MES, Ertz D, Feuerstein SC, Mercado-Díaz JA, Staiger B, Van den Broeck D, Lücking R (2014b) New higher taxa in the lichen family Graphidaceae (lichenized Ascomycota: Ostropales) based on a three-gene skeleton phylogeny. *Phytotaxa* 189: 39–51. <https://doi.org/10.11646/phytotaxa.189.1.5>
- Mangold A, Elix JA, Lumbsch HT (2008) *Ocellularia wirthii* (Ascomycota, Ostropales), a new species from New South Wales, Australia. *Sauteria* 15: 363–369.
- Mangold A, Lücking R, Lumbsch HT (2014) New species of graphidoid and thelotremoid Graphidaceae from Australia. *Phytotaxa* 189: 180–185. <https://doi.org/10.11646/phytotaxa.189.1.13>
- Medeiros ID, Kraichak E, Lücking R, Lumbsch HT (2016) Assembling a taxonomic monograph of tribe Wirthiotremateae (lichenized Ascomycota: Ostropales: Graphidaceae). *Feldiana, Life & Earth Sci.* 9: in press.
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*, 2010: 1–7. <https://doi.org/10.1109/GCE.2010.5676129>
- Papong K, Boonpragob K, Mangold A, Divakar PK, Lumbsch HT (2010) Thelotremoid lichen species recently described from Thailand: a re-evaluation. *Lichenologist* 42: 131–137. <https://doi.org/10.1017/S0024282909990405>
- Papong K, Boonpragob K, Mangold A, Lumbsch HT (2010) Thelotremoid lichen species recently described from Thailand: a reevaluation. *Lichenologist* 42: 131–137. <https://doi.org/10.1017/S0024282909990405>
- Papong KB, Lücking R, Kraichak E, Parnmen S, Von Konrat M, Lumbsch HT (2014) Preliminary insights into the diversity of the lichen family Graphidaceae in New Caledonia (Ostropales, Ascomycota), with the description of 23 new species. *Phytotaxa* 189: 204–231. <https://doi.org/10.11646/phytotaxa.189.1.15>
- Poengsungnoen V, Manoch L, Mongkolsuk P, Boonpragob K, Parnmen S, Lücking R, Tehler A, Lumbsch HT (2014) Phylogenetic analysis reveals two morphologically unique new species in the genera *Astrochapsa* and *Nitidochapsa* (lichenized Ascomycota: Graphidaceae). *Phytotaxa* 189: 268–281. <https://doi.org/10.11646/phytotaxa.189.1.19>
- Rambaut A (2012) FigTree. Version 1.4.2
- Rivas Plata E, Lücking R (2013) High diversity of Graphidaceae (lichenized Ascomycota: Ostropales) in Amazonian Peru. *Fungal Diversity* 58: 13–32. <https://doi.org/10.1007/s13225-012-0172-y>
- Rivas Plata E, Lumbsch HT (2011) Parallel evolution and phenotypic divergence in lichenized fungi: a case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). *Molecular Phylogenetics and Evolution* 61: 45–63. <https://doi.org/10.1016/j.ympev.2011.04.025>

- Rivas Plata E, Lücking R, Lumbsch HT (2012) A new classification for the family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). *Fungal Diversity* 52: 107–121. <https://doi.org/10.1007/s13225-011-0135-8>
- Schmitt I, del Prado R, Grube M, Lumbsch HT (2009) Repeated evolution of closed fruiting bodies is linked to ascoma development in the largest group of lichenized fungi (Lecanoromycetes, Ascomycota). *Molecular Phylogenetics and Evolution* 52: 34–44. <https://doi.org/10.1016/j.ympev.2009.03.017>
- Stamatakis A (2006) RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Sutjaritturakan J, Kalb K (2015) *Ocellularia* (Ascomycota: Ostropales) – three new species, a new record and a key for all species so far recorded for Thailand. *Herzogia* 28: 545–555. <https://doi.org/10.13158/heia.28.2.2015.545>
- Tehler A, Irestedt M (2007) Parallel evolution of lichen growth forms in the family Roccellaceae (Arthoniales, Ascomycota). *Cladistics* 23: 432–454. <https://doi.org/10.1111/j.1096-0031.2007.00156.x>
- Van Den Broeck D, Lücking R, Ertz D (2014) Three new species of Graphidaceae from tropical Africa. *Phytotaxa* 189: 325–330. <https://doi.org/10.11646/phytotaxa.189.1.23>