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New species in the family Graphidaceae (Ascomycota: Ostropales) from the Philippines

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Abstract

In the present paper three new species *viz. Thalloloma nitidum*, *Thelotrema isidiosum* and *T. megasporum* belonging to lichen family Graphidaceae are described from the Philippines and compared with the closely related taxa.

Key words: Ifugao, Ilocos Norte, Luzón Island, taxonomy

Introduction

In 2015, one of the authors (Prof. Paulina A. Bawingan) had an opportunity to travel to the northern part of the Philippines (Luzón Island), and during that visit she collected many species of lichens together with some interesting elements, particularly of the family Graphidaceae Dumortier (1822: 69). This cosmopolitan family which is quite diverse in the tropics contains more than 2000 species worldwide (Lücking *et al.* 2014), and is represented by 270 species in the Philippines, with many more taxa likely to be discovered from the undisturbed and well conserved forests (Parnmen *et al.* 2012; Rivas Plata *et al.* 2015).

The present paper reports three new species belonging to this family *viz. Thalloloma nitidum*, *Thelotrema isidiosum* and *T. megasporum* from Luzón Island, which are described below.

Materials and Methods

The specimens were collected from two provinces, *viz.* Ifugao and Ilocos Norte, located in the northern part of Luzón Island in the Philippines and are deposited in the herbarium of the Korean Lichen Research Institute (KoLRI), Sunchon National University, South Korea. The specimens were examined under MSZ-TR dissecting microscope and a Leica DM 500 compound microscope at Lichenology laboratory, CSIR-National Botanical Research Institute, Lucknow, India. All the measurements were taken under higher magnifications of 40× and 1000× in water prior to application of KOH. Spot tests and thin layer chromatography (in solvent system A) were performed following Orange *et al.* (2010), and Lugol's solution was used to check the amyloidity (I+ blue-violet or I–) of the hymenium, asci and ascospores. Asci and ascospores measurements are indicated as (minimum–){X−SD}−{X+SD}(–maximum), where X is the arithmetic mean, and SD the corresponding standard deviation, followed by the number of measurements (*n*). Pictures were taken by Leica EC3 camera using Leica S8APO stereo-zoom microscope, and plates were prepared in Corel Draw (version 12).

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Taxonomic Treatment

Thalloloma nitidum S. Joshi, Upreti & Hur *spec. nov.* (Fig 1A–C)

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Differs from *Thalloloma rubromarginatum* in lacking isohypocrelline along lirellae.

Type:—THE PHILIPPINES. Ifugao province: Luzón Island, Banaue, Aparngao, Banaue ethnic village and pine forest resort, alt. 1453 m, 16°56'52.6'' N, 121°3'18.9'' E, on bark, January 2015, *Paulina A. Bawingan PH150184* (holotype: KoLRI).

Thallus corticolous, crustose, ecorticate, glossy, tightly adpressed to the substratum, off-white to creamish-white, 100–120 µm thick; photobiont trentepohlioid; medulla white crystalline, partially endoperidermal.

Apothecia lirellate, short to long, stellately or irregularly branched, 1–1.2 × 0.08–0.1 mm; disc exposed, brown to dark brown, sometimes thinly white pruinose; thalline margin, thin, persistent at the base in mature apothecia, up to 100 µm thick; proper exciple indistinct to absent; epihymenium brown, granular, 10–15 µm high; hymenium hyaline, clear (lacking oil-droplets), 100–160 µm high, with thick walled and gelatinous, 4–6 µm thick paraphyses, usually amyloid below epihymenium; subhymenium hyaline, 30–60 µm high; asci clavate, 8-spored, (100–)101−120.4−139.8(–150) × (25−)24.8−27.9–31(–32) µm (*n*=10), non-amyloid; ascospores ellipsoidal, hyaline, muriform (10–15 transverse septa and 1–2 vertical septa), (30–)37−37.3−37.8(–42) × (10−)10.8−12.6–14.4(–15) µm (*n*=66), with gelatinous sheath (halonate), amyloid.

Chemistry:—K+ yellow turning red, PD+ yellow, C–; norstictic acid associated with traces of stictic, cryptostictic and constictic acids detected by TLC.

Etymology:—The specific epithet reflects the bright and glossy thallus of the new taxon.

Distribution and Ecology:—The new taxon is known only from the type locality at higher elevation in association with *Diorygma hieroglyphicum* (Pers.) Staiger & Kalb, *Graphis dracaenae* Vain., *Fissurina insidiosa* C. Knight & Mitt., *Myriotrema microporum* (Mont.) Hale and *Pliariona montagnei* (v. d. Bosch) Massal.

Remarks:—The new species is characterized by an ecorticate, shiny, thin, off-white to creamish-white thallus, stellately branched lirellae with wide exposed brown disc, mostly covered by a very thin layer of white pruina, 8-spored asci, distinctly halonate, 30–42 × 10–15 µm, muriform ascospores and the presence of norstictic acid with traces of stictic acid and relatives. Norstictic acid is rare in the genus and hitherto found in two species: *Thalloloma rubromarginatum* Mercado-Díaz, Lücking & Parnmen and *T. ochroleucum* Z-F. Jia & Kalb. *Thalloloma rubromarginatum* resembles new taxon in producing a smooth, shiny off-white thallus, stellately branched lirellae, 8-spored asci producing (sub-)muriform ascospores, but differs in having the dark red pigment isohypocrelline along the lirellae, epruinose disc, and the absence of stictic acid. Further, the ascospores of the former species are comparatively smaller (15–20 × 6–8 µm vs. 30–42 × 10–15 µm in *Thalloloma nitidum*) in size (Mercado-Díaz *et al.* 2014: 200). *Thalloloma ochroleucum* also has a white tinged adpressed thallus resembling the new species, but differs in containing transversely septate ascospores (Jia & Kalb 2014: 114). *Thalloloma pontalense* Dal-Forno & Eliasaro and *T. halonatum* Staiger are the two other species which are close to the new species in thallus color and ascospores size, but differ chemically in producing stictic acid and protocetraric acid, respectively (Staiger 2002: 435; Dal-Forno & Eliasaro 2010: 18).

Additional specimen examined:—THE PHILIPPINES. Ifugao province: Luzón Island, Banaue, Aparngao, Banaue Ethnic village and pine forest resort, alt. 1507 m, 16°56'52.4'' N, 121°3'19.1'' E, on bark, January 2015, *Paulina A. Bawingan PH150178* (KoLRI).

Thelotrema isidiosum S. Joshi, Upreti & Hur *spec. nov.* (Fig 2A–C)

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Differing from *Thelotrema isidiophorum* in that the thallus produces transversely septate ascospores and salazinic acid as a secondary compound.

Type:—THE PHILIPPINES. IIocos Norte: Luzón Island, Adams, Mt. Mabolo, 18°26'26.3'' N, 120°53'27.1'' E, alt. 473 m, on bark, January 2015, *Paulina A. Bawingan PH150039* (holotype: KoLRI).

Thallus corticolous, crustose, green to greenish-grey, continuous, glossy, isidiate, 50–90(–100) µm thick; cortex loose, 10–15 µm thick; algal layer trentipohlioid, 10–25 µm thick; medulla white crystalline, 30–50 µm; isidia cylindrical, branched, coralloid, 0.4–0.6 × 0.09–0.1 mm; prothallus dark brown.

Apothecia porinoid, sessile, barrel-shaped, dispersed, 1–2 mm diam.; pore widely open, 0.2–0.4 mm in diam.; disc flesh colored, epruinose, partly covered by proper exciple, deeply immersed; thalline margin distinct, entire, embedded

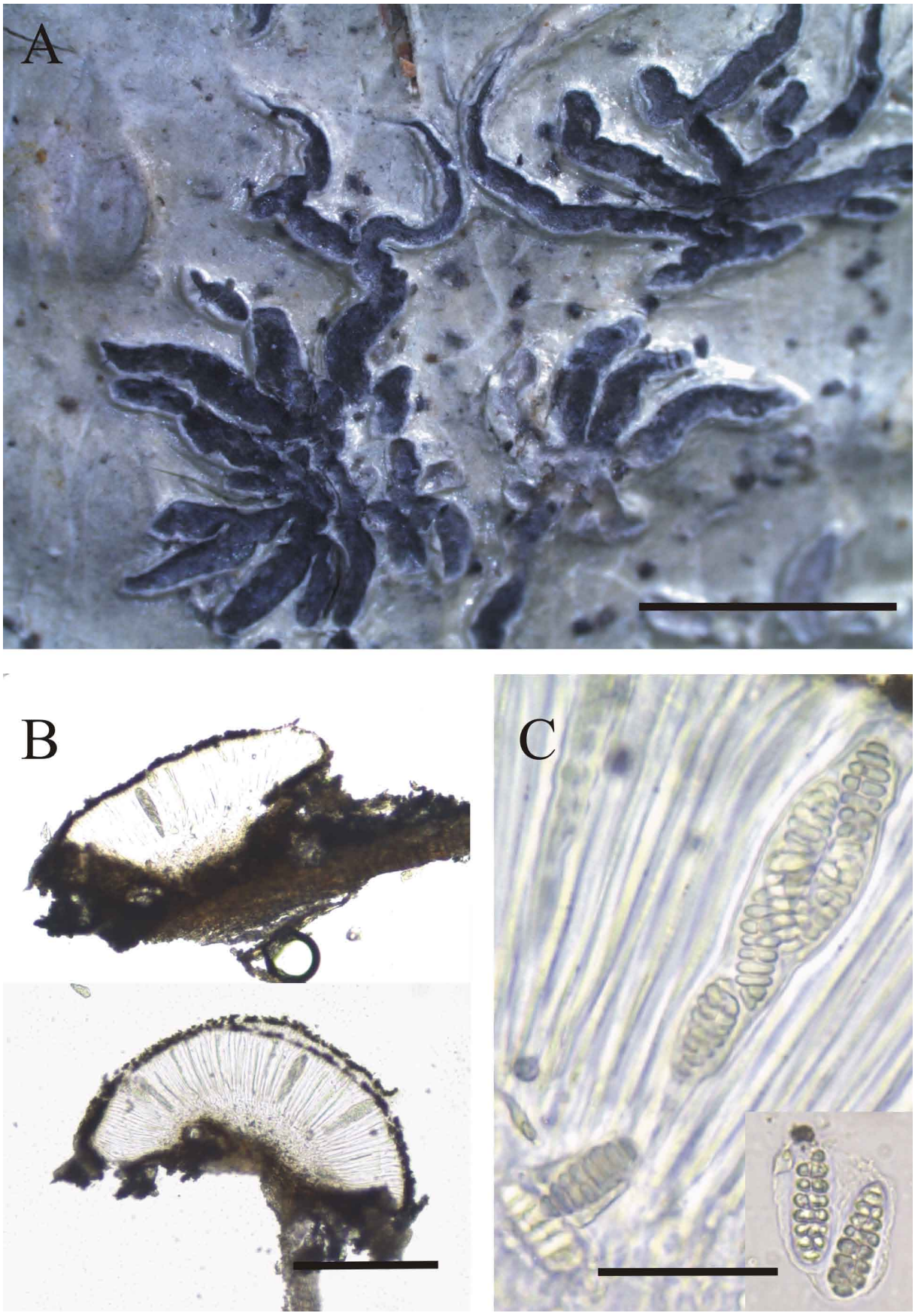

FIGURE 1. *Thalloloma nitidum* (holotype). A. Habit. B. Section through apothecia. C. Ascus and ascospores. Scales: A = 0.5 mm, B = 100 µm, C = 50 µm.

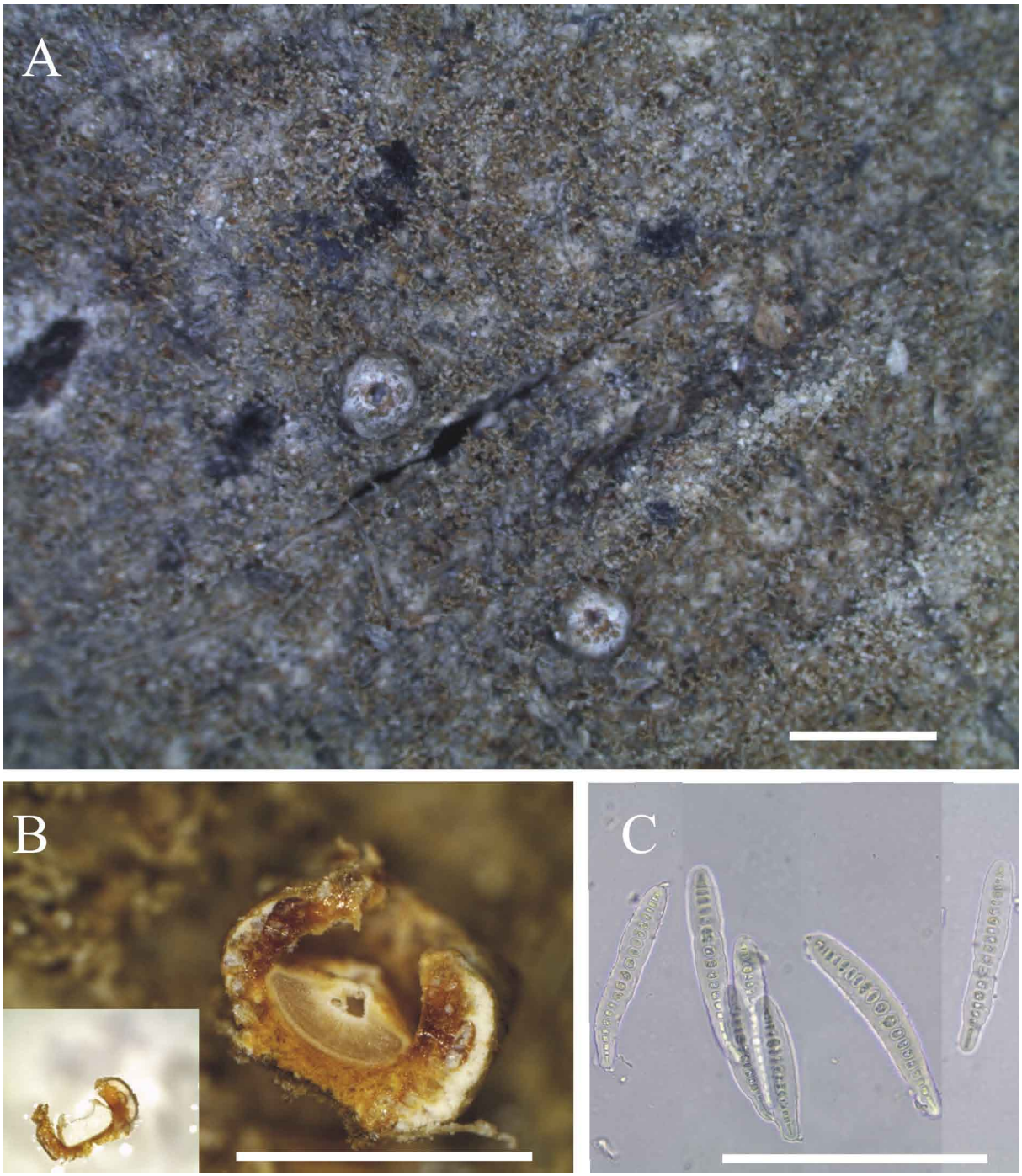

FIGURE 2. *Thelotrema isidiosum* (holotype). A. Habit. B. Section through apothecia showing free and hyaline excipulum. C. Ascospores. Scales: A = 2 mm, B = 1 mm, C = 100 µm.

with crystals, 120–220 µm thick; proper exciple pale brown, reddish-brown to brown, free, denticulate apically; 120–160 µm thick, embedded with crystals, separated from the thalline margin by a narrow split (double margin); epihymenium greyish, crystalline, 15–25 µm high; hymenium hyaline, clear (lacking oil-droplets), conglutinate, resting deep inside proper exciple, 160–260 µm high; paraphyses unbranched, parallel, 1–2 µm thick; subhymenium hyaline, indistinct; asci clavate, 8-spored, (210–)213.9−224−234(–236) × (26.5−)27.3−30–33.3(–34) µm (*n*=10), non-amyloid; ascospores hyaline, fusiform, transversely septate, 15–22-locular with ± wavy spore wall, endospore 5–6 µm thick, (95–)98.2−101.4−104.6(–105.8) × (15−)15.6−16.7–17.8(–18) µm (*n*=40), amyloid.

Chemistry:—K+ red, PD+ yellow-orange, C–; salazinic acid detected by TLC.

Etymology:—The epithet refers the isidiate thallus of the new taxon.

Distribution and Ecology:—The new taxon is described only from the type locality in the Philippines, where it was growing in association with *Leiorreuma exaltatum* (Mont. & Bosch) Staiger, *Pallidogramme chrysenteron*

(Mont.) Staiger, Kalb & Lücking, *Platythecium pyrrhochroum* (Mont. & Bosch) Z. F. Jia & Lücking, *Sarcographa labyrinthica* (Ach.) Müll. Arg., and species of *Pertusaria* DC. and *Porina* Ach. on thin barked trees.

Remarks:—The new taxon is distinguished in having coralloid isidiate, loosely corticate thallus producing salazinic acid, sessile, barrel-shaped apothecia with wide pores and double margin, hyaline to pale brown proper exciple internally lined by peripysoids, 8-spored asci and transversely septate ascospores with thick endospore. The new species differs from and does not seem close to any of the species in *Thelotrema* Acharius (1803: 130). The isidiate thallus in the genus is rare, and found only in *T. isidiophorum* (Kremp.) Zahlbr, which also has sessile apothecia, but is clearly distinguished in having a verrucose thallus containing stictic acid and large, muriform ascospores, 180–300 × 15–25 µm (Rivas Plata *et al.* 2010: 175). *Thelotrema isidiosum* shares some taxonomic characteristics with *Thelotrema porinoides* Mont. & Bosch., *T. heladivense* Weerakoon, Jayalal & Lücking, *T. weberi* Hale and *T. subweberi* Sipman. *Thelotrema porinoides* with transversely 13–25(–27)-septate, amyloid ascospores of 40–150(–170) × 15–22 µm is close, but differs from the new taxon in having a non-isidiate thallus containing the stictic acid chemosyndrome (Mangold *et al.* 2009: 393; Rivas Plata *et al.* 2010: 173). *Thelotrema heladivense* also produces transversely, up to 15-septate ascospores measuring 80–100 × 12–15 µm, but is clearly separated in that the thallus lacks isidia and contains norstictic acid with unknown compounds, as well as in having weakly amyloid ascospores (Weerakoon *et al.* 2015: 84). *Thelotrema weberi* and *T. subweberi* with barrel-shaped apothecia are comparable to the new taxon in having strongly amyloid ascospores, but both the former species are distinct in producing muriform ascospores and norstictic acid in the thallus (Rivas Plata *et al.* 2010: 175).

Additional specimen examined:—THE PHILIPPINES. Ilocos Norte: Luzón Island, Adams, Mt. Mabolo, 18°26’49.5’’ N, 120° 54’ 58.6’’ E, alt. 393 m, on bark, January 2015, *Paulina A. Bawingan PH150064* (KoLRI).

Thelotrema megasporum S. Joshi, Upreti & Hur *spec. nov.* (Fig 3A–D)

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Resembles *Thelotrema thesaurum*, but differs in having 1-spored asci and much larger ascospores (145.8–330 × 35–44.5 µm vs 60–120(–130) × 20–30 µm in *Thelotrema thesaurum*).

Type:—The Philippines. Ilocos Norte: Luzón Island, Adams, Mt. Mabolo, 18°27’13.4’’ N, 120°54’45.9’’ E, alt. 361 m, on bark, January 2015, *Paulina A. Bawingan PH150051* (holotype: KoLRI).

Thallus corticolous, crustose, brownish to yellowish-brown, waxy, glossy, continuous, verruculose, undulate (due to bark texture), hard, 300–400 µm thick; cortex distinct 50–60 µm thick; photobiont trentepohlioid, algal layer embedded with large crystals at the base, 50–90 µm thick; medulla white, mostly embedded with large crystals and periderm cells, up to 250 µm thick; prothallus dark brown.

Apothecia porinoid, sessile, barrel-shaped, numerous, dispersed, 0.5–1 mm diam. and 0.6–0.8 mm high; pore widely open, 0.2–0.3 mm diam.; disc flesh colored, mostly covered by proper exciple, white pruinose; thalline exciple distinct, entire, eroded, whitish apically, embedded with periderm cells, 140–170 µm thick; proper exciple hyaline, free, separated from the thalline exciple by a narrow split (double margin), 30–70 µm thick, internally lined by 50–70 µm long distinct periphysoids; epihymenium granular, crystalline, ± greyish, indistinct to 15–20 µm high; hymenium hyaline, clear (lacking oil-droplets), 300–500 µm high; paraphyses unbranched, parallel, 1–2 µm thick; subhymenium indistinct; asci clavate, 1-spored, (290.2–)295.5−321.7−347.9(–350) × (40−)40.7−42.7–44.7(–45) µm (*n*=32), non-amyloid; ascospores hyaline, fusiform, muticelled, muriform, (145.8–)221.1−273.4−325.7(–330) × (35−)36.6−39.5–42.4(–44.5) µm (*n*=32) µm, non-amyloid.

Chemistry:—K+ yellow, PD+ yellow, C–; stictic acid associated with traces of cryptostictic and constictic acids detected by TLC.

Etymology:—The epithet is named after the large ascospores of the new taxon.

Distribution and Ecology:—The new species was found only at the type locality in the Philippines, where it was growing luxuriantly with *Leucodecton compunctum* (Ach.) A. Massal., *Phyllopsora albicans* Müll. Arg., *Glaucotrema glaucophaenum* (Kremp.) Rivas Plata & Lumbsch, *Myriotrema microporum* (Mont.) Hale, *Ocellularia massalongoi* (Mont.) Hale, and a number of unidentified *Graphis* species.

Remarks:—The new species is characterized by a thick, brownish colored thallus containing stictic acid, sessile, barrel-shaped apothecia with double margins, hyaline proper exciple internally lined by periphysoids, 1-spored asci and hyaline, muriform, large, non-amyloid ascospores. *Thelotrema megasporum* is most similar to *Thelotrema thesaurum* Mangold in having a verrucose, thick, waxy thallus that is pale olivaceous to yellowish-brown, containing the stictic acid chemosyndrome, sessile apothecia and muriform, hyaline, non-amyloid ascospores. However, the latter species

produces (4–)6–8-spored asci with smaller ascospores measuring 60–120(–130) × 20–30 µm (Mangold *et al.* 2009: 404).

Thelotrema conferendum Hale is close to the new taxon in having hyaline and muriform ascospores, and the presence of stictic acid in the thallus, but differs in containing distinctly amyloid ascospores, which are smaller (100–150 µm long) (Rivas Plata *et al.* 2010: 175). *Thelotrema rugulatum* Nyl. is similar in having a verrucose thallus and muriform, hyaline, non-amyloid ascospores (220 × 45 µm), but clearly separated from the new species in lacking secondary compounds (Rivas Plata *et al.* 2010: 176).

The presence of a dense cortex and stictic acid in the new taxon resembles species accommodated in the recently revived genus *Asteristion* Leighton (1870: 163), which however, differs in having small ascospores (up to 60 µm long) (Medeiros *et al.* 2017: 5). The new species is comparable to *Asteristion cupulare* (Müll. Arg.) I. Medeiros, Lücking & Lumbsch and *A. leucopthalmum* (Müll. Arg.) I. Medeiros, Lücking & Lumbsch in producing apothecia with a double margin, as well as hyaline muriform, non-amyloid ascospores, but the latter two species differ in having small ascospores measuring 15–30 × 5–10 µm and 30–60 × 10–15 µm, respectively (Medeiros *et al.* 2017: 10).

Additional specimen examined:—The Philippines. Ilocos Norte: Luzón Island, Adams, Mt. Mabolo, 18°27’13.6” N, 120°54’45.1” E, alt. 353 m, on bark, January 2015, *Paulina A. Bawingan PH150056* (KoLRI).

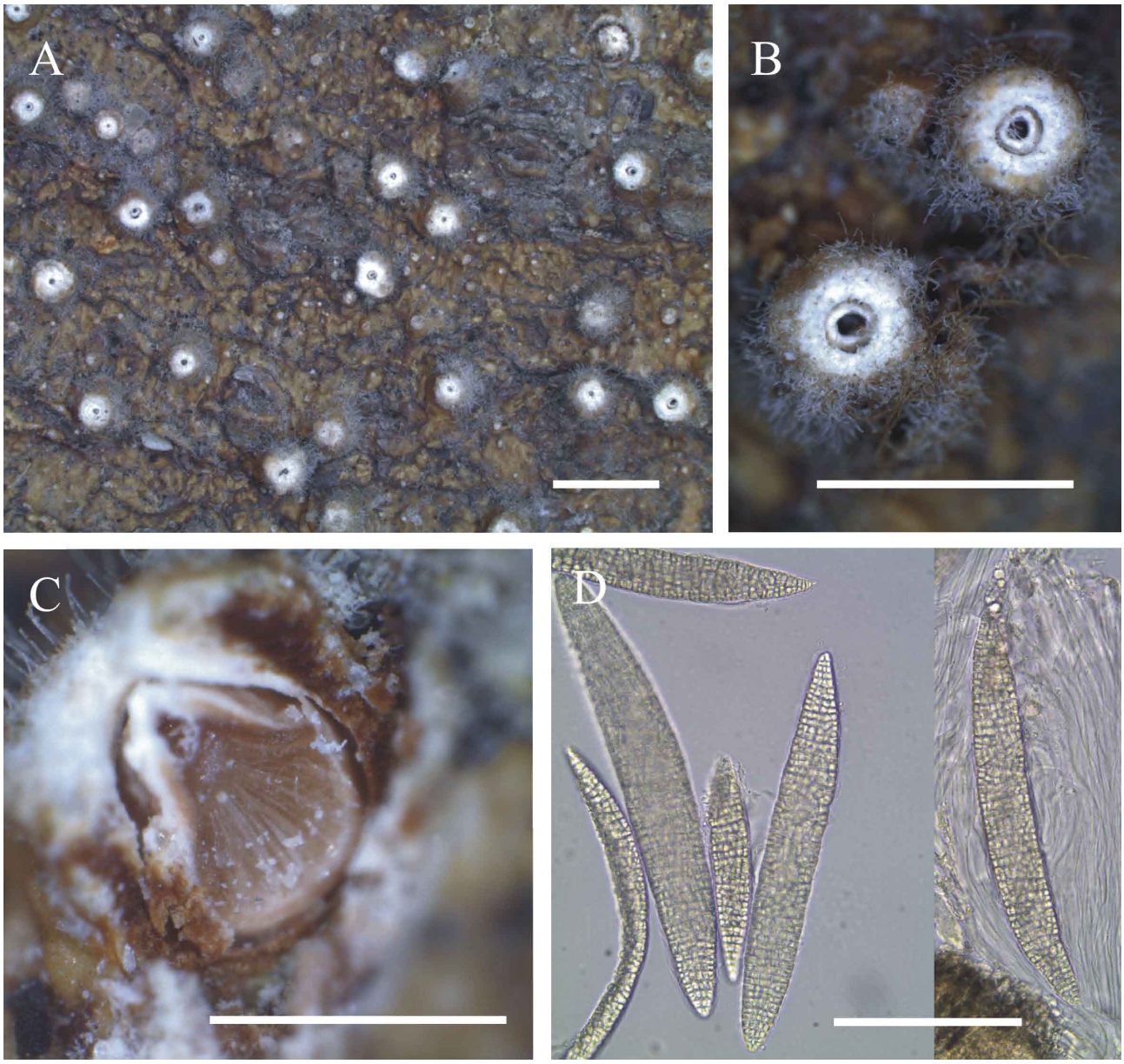

FIGURE 3. *Thelotrema megasporum* (holotype). A. Habit. B. Apothecia. C. Section through apothecia showing free and hyaline to reddish brown excipulum. D. Ascospores. Scales: A–B = 1 mm, C = 500 µm, D = 100 µm.

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