New Records of Lichen Genus *Thelotrema* Ach. (Thelotremoid *Graphidaceae*) from South Korea

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This study illustrates the status of thelotremoid *Graphidaceae* in South Korea. The taxonomy and systematics of thelotremoid lichens in the country have recently advanced greatly. Following the earlier known taxa from the group, four new records of the genus *Thelotrema* are reported and described briefly. An artificial key for all known species under thelotremoid group of *Graphidaceae* from South Korea is also provided.

KEYWORDS : Jeju Island, Mt. Halla, Taxonomy, Thelotremoid

Introduction

Ostropalean fungi are an important component of tropical lichen communities. Recent molecular studies have suggested polyphyly among the two families of *Ostropales*, *Thelotremataceae* and *Graphidaceae*; therefore, thelotremoid lichens are considered to be members of the large family *Graphidaceae* [1-3]. Exhaustive work on thelotremoid *Graphidaceae* has been conducted by different lichenologists that resulted in c. 21 genera presently being associated with the group [2-4]. Despite their wide distribution in the tropics, the group has not been thoroughly studied in Korea. Indeed, until recently, there have been no reports of Korean thelotremoid lichens since the origin of Korean lichen taxonomy more than 50 years ago. This may be a result of their being little interest in this difficult to recognize and easily overlooked growth form or to the scarcity of old growth secondary forest, as the bark of mature trees are usually preferred by members of the thelotremoid group [5]. In recent years, the taxonomy and systematics of thelotremoid lichens in South Korea has increased greatly. Following the recent identification of *Diploschistes scruposus*, *D. actinostomous*, *Leucodecton glaucauscens* and *Thelotrema subtile* from different provinces in the country [6-8], some other interesting species have been identified and additional ones are awaiting recognition and placement within the group. In the present study, a taxonomic approach was employed to enrich the Korean lichen diversity, which may further clarify the status of thelotremoid lichens in South Korea.

This study includes four new records of *Thelotrema* Ach. reported from Jeju Island, Korea. *Thelotrema* was treated several times to resolve the heterogeneity within it, and the generic concept of the genus has now been completely changed from the traditional circumscription based on muriform and hyaline ascospores [9-12]. Frisch *et al.* [2] included species of *Thelotrema* with non-carbonized excipulum lined with lateral paraphyses, lepadinoid apothecia with a more or less entire margin and free proper excipulum (double margin), as well as an ecorticate to weakly corticate thallus producing stictic acid and related compounds. Following Hale [13-15], Frisch *et al.* [2] and Mangold *et al.* [3], Rivas Plata *et al.* [4] provided a world-wide key for c. 70 species of the genus and segregated them broadly based on ascospores septation, colour and chemistry.

The genus varies greatly with respect to the presence of thallus compounds. Many species lack secondary metabolites, while others produce stictic, hypostictic, norstictic, protocetraric, salazinic acids and several unknown compounds [4]. All four new records described here are devoid of secondary compounds and show sub-similarities in their morpho-taxonomical characteristics such as thallus colour, texture, and apothecial morphology. Nevertheless, differences in ascospores size and amyloidity, number of ascospores per ascus and excipulum color clearly distinguished the species and provided proper placement. An artificial key for species classification and identification within thelotremoid *Graphidaceae* of South Korea is also provided.

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Materials and Methods

This account is based on the examination of specimens recently collected from Jeju Island of South Korea and preserved in the lichen herbarium of the Korean Lichen Research Institute, South Korea (KoLRI). This volcanic island, dominated by Mt. Halla, is situated in the south of the country and surround by the South Korea Sea. The mountain is approximately 73 km from east to west and 41 km from north to south, with an altitude of 1,950 m. The mountain provides a suitable climate for both tropical and temperate plant species due to its vertical ecosystem; accordingly, it offers ideal conditions for the growth of different crustose lichens.

Fresh samples of lichens collected from Mt. Halla were observed under an SMZ 168 and NIKON C-PS 1068908 dissecting microscope (Nikon, Tokyo, Japan) to identify morpho-taxonomical characters. The anatomy of the thallus and fruiting bodies were investigated using an Olympus BX50 compound microscope (Olympus, Tokyo, Japan). Color spot reaction tests and thin layer chromatography were conducted as described by Orange *et al.* [16]. Thin layer chromatography was performed using a solvent system C with a suitable control [*Lethariella cladonioides* (Nyl.) Krog.]. All morpho-anatomical measurements were based on the studied material, available in less amount. The range of measurements mentioned below was taken from a few apothecia and their anatomy. The lowest and highest measured values reflect the minimum and the maximum range.

Results and Discussion

Artificial key to thelotremoid *Graphidaceae* from South Korea

1. Photobiont *Trebouxia*, lateral paraphyses present 2
 1a. Photobiont *Trentipohlia*, lateral paraphyses present or absent 3

2. Ascomata perithecioid, thallus epruinose, pale to whitish gray, ascospores $16\sim22 \times 10\sim12 \mu\text{m}$ *Diploschistes actinostomus*

2a. Ascomata urceolate, thallus slightly purinose, gray to greenish gray, ascospores $22\sim36 \times 10\sim16 \mu\text{m}$ *D. scruposus*

3. Lateral paraphyses absent, proper exciple cupular, partly to entirely free, thin, hyaline to pale brown, slightly dark or carbonized on apices, ascospores muriform, brown, $19\sim23.3 \times 9.2\sim13.5 \mu\text{m}$

..... *Leucodecton desquamescens*

3a. Lateral paraphyses present, proper exciple free, thin to thick, hyaline to pale brown, ascospores transversely septate to muriform, hyaline or brownish on decaying,

$30 \mu\text{m}$ 4

4. Ascospores transversely septate, apothecia immersed to erumpent 5

4a. Ascospores muriform, apothecia erumpent 6

5. Ascospores $30\sim50 \times 7\sim10 \mu\text{m}$, with thin outer walls in early stages, becoming partially gray brown in older stages, strongly amyloid, proper exciple colorless

..... *T. subtile*

5a. Ascospores mostly over $50 \mu\text{m}$ long, hyaline, with thick outer wall, at least weakly amyloid, proper exciple pale brown *T. diplotrema*

6. Ascospores usually 1 per ascus, proper exciple brown *T. similans*

6a. Ascospores 1~4 per ascus, exciple hyaline 7

7. Ascospores thick walled, remaining hyaline; apothecia prominent, up to 1.5 mm diam *T. lepadinum*

7a. Ascospores thin walled, becoming gray brown when old, apothecia mostly immersed, up to 0.6 mm diam

..... *T. lepademersum*

Taxonomic description of the species.

Thelotrema diplotrema Nyl. (Fig. 1A and 1B)

Ann. Sci. Nat., Bot., Sér. 4 11: 258 (1859).

Description: Thallus corticolous, endophloeodal to epiphloeodal, loosely corticated or ecorticate, thin to c.

Fig. 1. *Thelotrema diplotrema* Nyl. A, Habit; B, Ascospores (scale bars: A = 3 mm, B = 35 μm).

120 µm, whitish gray to gray, greenish gray, usually dull to slightly glossy, smooth to uneven, continuous to cracked. Algal layer well developed, continuous or discontinuous, densely integrated with crystals. Calcium oxalate crystals abundant, small to large, mostly aggregated to dispersed. Medulla indistinct to endophloeodal. Vegetative propagules not seen.

Ascomata conspicuous, up to c. 0.7 (~0.9) mm diam., mostly rounded, apothecioid, erumpent, solitary to completely fused. Disc grayish to blackish or flesh colored, whitish pruinose, becoming partly visible, 0.11~0.26 mm diam. Pores variable, small to moderate to c. 0.25~0.42 mm diam., rounded to irregular, proper exciple apically to completely visible from above, usually free only in the upper parts, off-white, often somewhat shrunk, entire, incurved to straight. Thalline margin rounded, entire, incurved to straight, concolorous with the thallus, 0.02~0.09 mm thick. Proper exciple free, mostly hyaline to slightly pale or brownish, sometimes covered with grayish to dark grey granules on tips, 15~25 µm thick, I-. Lateral paraphyses conspicuous, to c. 18 µm long. Epihymenium, hyaline to dark brown or grayish due to inspersion of crystals, 10~17 µm high. Hymenium hyaline, clear, moderately conglutinated, 50~100 µm high. Paraphyses parallel or slightly interwoven, unbranched, tips slightly thickened, c. 1~1.5 µm thick. Subhymenium hyaline, indistinct. Asci clavate, 8-spored, 60~95 × 12~18 µm, I-. Ascospores hyaline, transversely septate, fusiform, with rounded to sub-acute ends, occasionally pale yellowish when old or decayed, 50~77 × 6~12 µm, with 17~24 locules. Locules lenticular; ascospore wall and endospore thick; wall often with a thin to thick halo. I+ blue to faint blue.

Chemistry: Thallus K-, C-, PD- (- indicates no color reaction); no secondary compounds detected upon thin layer chromatography.

Distribution and ecology: The species is most common to tropical and subtropical regions of Africa, Australia and Asia [2, 3]. In South Korea it is reported from evergreen forests of Mt. Halla in Jeju Island at an altitude between 600~900 m.

Remarks: This species closely resembles *Thelotrema subtile* and *T. pseudosubtile* Mangold in having trans-septate, hyaline ascospores and a thallus lacking secondary metabolites. However, the latter species differ in having ascospores of smaller range (25~60 × 6~10 µm). Further, *T. pseudosubtile* has a less noticeable free proper exciple. Another similar species, *Thelotrema nurelium* Hale, has a thick, verrucose thallus with comparatively large ascomata (> 1 mm) and ascospores of 60~220 × 10~20 µm.

Specimens examined: Jeju Island, Mt. Halla, Gwanum Temple Trail, 33°25'04.08" N, 126°32'52.01" E, alt. 619 m, on tree bark, 1 Jun 2012, Hur *et al.*, KoLRI 120957, KoLRI 120962. Seongpanak Trail, 33°22'76.3" N,

126°36'80.1" E, alt. 834 m, on tree bark, 6 Jul 2012, Hur *et al.*, KoLRI 121515.

***Thelotrema lepademersum* Nagarkar, Sethy & Patw. (Fig. 2A and 2B)**

Kavaka 13: 59 (1987) [1985].

Description: Thallus corticolous, endophloeodal to epiphloeodal, irregularly corticate to c. 150 µm thick, whitish gray to greenish gray to gray green, dull to slightly glossy, usually smooth, continuous. Algal layer usually well developed, continuous or discontinuous, densely integrated with crystals. Calcium oxalate crystals abundant, small to large, clustered. Medulla indistinct to endophloeodal. Vegetative propagules not seen.

Ascomata conspicuous to c. 0.31~0.56 mm diam., somewhat rounded, apothecioid, immersed to scarcely erumpent, solitary. Disc grayish to blackish, whitish pruinose, becoming partly visible, up to 0.15 mm diam. Pores variable, small to c. 0.11~0.25 mm diam., rounded to irregular. Proper exciple apically to completely visible from above, usually free only in the upper parts, off-white, often somewhat shrunk, usually entire to slightly split, incurved. Thalline margin appears rounded, entire, incurved, concolorous with the thallus, 0.09~0.15 mm thick. Proper exciple almost free, mostly hyaline to slightly pale at apices, sometimes covered with grayish to dark gray granules on tips, 16~36 µm thick, I- to rarely amyloid at base corners. Lateral paraphyses often inconspicuous to conspicuous apically to c. 16 µm long. Epihymenium grayish, crystalline, 12~14 µm high. Hymenium hyaline, clear,

Fig. 2. *Thelotrema lepademersum* Nagarkar, Sethy & Patw. A, Habit; B, Ascospores (scale bars: A = 1 mm, B = 50 µm).

moderately conglutinated, 160~200 μm high. Paraphyses parallel or slightly interwoven, unbranched, tips slightly thickened, c. 1~1.5 μm thick. Subhymenium hyaline, indistinct to c. 15 μm high. Asci clavate, 1~4-spored, 150~170 $\times$ 25~30 μm , I-. Ascospores hyaline, muriform, cylindrical to oblong-ellipsoidal or broadly fusiform, occasionally pale yellowish when old or decayed, 75~125 $\times$ 14~26 μm , with numerous locules. Locules rounded to angular, subglobular. Ascospore wall and endospore thin. Thin to thick gelatinous sheath sometimes present, I+ faint blue.

Chemistry: Thallus K-, C-, PD-; no secondary compounds detected upon thin layer chromatography.

Distribution and ecology: The species is most common among the palaeotropical regions of Asia [17]. In South Korea it was collected at an altitude between 800~900 m from evergreen forests of Mt. Halla of Jeju Island.

Remarks: *Thelotrema lepademersum* resembles *T. lepadinum* and *T. monosporum* Nyl. with respect to the size of ascospores (up to 120 $\times$ 60 μm) and number of ascospores (1~4) per ascus, but other characteristics differ slightly. Specifically, *T. lepadinum* has thick walled, persistently hyaline ascospores, and comparatively larger apothecia, whereas *T. monosporum* lacks cortical structures, has indistinctly lepadinoid apothecia and non-amyloid ascospores that soon become brown. The halonate ascospores and almost colorless exciple of the examined specimen show close proximity to *T. rugatulum* Nyl., but the smooth thallus, mostly immersed and smaller apothecia and ascospore size lead the material to be provisionally placed in *Thelotrema lepademersum*. Accordingly, collection of additional specimens and their characterization is required to confirm the position of the specimen.

Specimen examined: Jeju Island, Mt. Halla, Seongpanak Trail, 33°22'76.3" N, 126°36'80.1" E, alt. 834 m, on tree bark, 6 Jul 2012, Hur *et al.*, KoLRI 121517.

***Thelotrema lepadinum* (Ach.) Ach. (Fig. 3A and 3B)**
Methodus 132 (1803).

Description: Thallus corticolous, epiphloeodal to endophloeodal, corticate, thin, up to c. 135 μm , gray green to pale grayish green, dull to slightly glossy, usually smooth, continuous. Algal layer well developed, continuous or discontinuous due to integration of crystals. Calcium oxalate crystals abundant, small to large, clustered; medulla indistinct, endophloeodal. Vegetative propagules not seen.

Ascomata conspicuous, up to c. 1.5 mm diam., somewhat rounded, apothecioid, erumpent, solitary, appears hemispherical. Disc not visible from above, rarely becoming partly visible, grayish, pruinose. Pores variable, small to moderately large, to c. 0.19~0.58 mm diam., almost rounded, proper exciple apically to rarely completely visible from above, more or less free, off-white, often somewhat shrunken, usually entirely to slightly split, incurved.

Fig. 3. *Thelotrema lepadinum* (Ach.) Ach. A, Habit; B, Ascospores (scale bars: A = 3 mm, B = 40 μm).

Thalline margin rounded, entire, thin to thick, incurved, concolorous with the thallus, 0.24~0.42 mm. Proper exciple apically to completely free, thin to thick, hyaline to pale brown, mostly higher than hymenium, forming a roof like structure, 30~35 (~68) μm , I-. Lateral paraphyses conspicuous, c. 25 μm , reaching 57 μm above hymenium. Epihymenium, dark, crystalline, 12~13 μm high. Hymenium hyaline, clear to c. 180 μm high, moderately conglutinated. Paraphyses parallel or slightly interwoven, unbranched, tips slightly thickened, 1~1.5 μm thick. Asci clavate, 1~4-spored, 140~145 $\times$ 20~25 μm , I-. Ascospores hyaline, muriform, oblong~ellipsoidal or broadly fusiform, with sub-acute ends, occasionally pale yellowish when old or decayed, 40~90 $\times$ 10~20 μm , with numerous locules. Locules rounded to angular, subglobular to irregular. Ascospore wall and endospore thick, halo not seen, I- or I+ (+ indicates color reaction) faintly bluish.

Chemistry: Thallus K-, C-, PD-; no secondary compounds detected upon thin layer chromatography.

Distribution and ecology: The taxon is subcosmopolitan and widely distributed in temperate to subtropical regions in both hemispheres and in the tropical mountains [2, 3]. In South Korea it is collected from rather exposed trees of evergreen forests in Mt. Halla of Jeju Island at an altitude of 600~700 m.

Remarks: *Thelotrema lepadinum* shows morphological resemblance to several species of *Thelotrema* s. str., but most closely resembles *T. crassisporum* Mangold, *T. subtile* and *T. adjectum* Nyl. *T. crassisporum* contains stictic acid as the thallus compound and has small ascospores, whereas *T. adjectum* has a layered exiple, flesh colored apothecial disc and 4~8-spored asci. *T. subtile* has smaller ascomata and trans-septate ascospores.

Specimen examined: Jeju Island, Mt. Halla, Gwanum Temple Trail, 33°25'28.5" N, 126°33'68.7" E, alt. c. 619 m, on tree bark, 7 Jul 2012, Hur *et al.*, KoLRI 121536.

***Thelotrema similans* Nyl. (Fig. 4A and 4B)**

Lich. Japon.: 58 (1890).

Description: Thallus corticolous, epiphloeodal, loosely corticate, thin, up to c. 100 µm, gray green to whitish gray or greenish gray, dull, speckled with crystals, continuous. Algal layer well developed, continuous or discontinuous due to integration of crystals. Calcium oxalate crystals abundant, small to large, clustered; medulla indistinct to endophloeodal. Vegetative propagules not seen.

Ascomata conspicuous to c. 0.4~0.6 mm diam., somewhat rounded, apothecioid, erumpent, mostly solitary to rarely fused, appears hemispherical. Disc partly to completely visible, flesh colored, pruinose, 0.1~0.19 mm. Pores variable, small to c. 0.15~0.32 mm diam., rounded, proper exciple apically to completely visible from above, free, off-white, often somewhat shrunk, incurved. Thalline margin rounded

to irregular, entire, thin to thick, incurved, concolorous with the thallus, 0.12~0.19 mm. Proper exciple, apically to completely free, thin to thick, hyaline to pale brown or marginally brown, 30~95 µm, I-. Lateral paraphyses conspicuous, c. 25 µm. Epihymenium, hyaline, crystalline, 12~15 µm high. Hymenium hyaline, clear to c. 185~250 µm high, moderately conglutinated. Paraphyses parallel or slightly interwoven, unbranched, tips slightly thickened, 2~2.5 µm thick. Asci clavate, 1-spored, 185~190 × 35~40 µm, I-. Ascospores hyaline, muriform, oblong-ellipsoidal or broadly fusiform, with rounded to sub-acute ends, 94~150 × 14~30 µm, with numerous locules. Locules rounded to angular, ascospore wall and endospore thick, halo not seen, I+ bluish or I+ faintly bluish.

Chemistry: Thallus K-, C-, PD-; no secondary compounds detected upon thin layer chromatography.

Distribution and ecology: The species is distributed in the eastern palaeotropics and has been reported in Japan [18]. In South Korea it was collected from tree barks in evergreen forest of Mt. Halla of Jeju Island at an altitude around 1,000 m.

Remarks: *Thelotrema similans* exhibits similarity to *T. lepadinum* in having a loosely corticated thallus, pruinose apothecial disc, muriform, hyaline ascospores and lack of secondary metabolites. However, 1~4-spored asci, thick walled small ascospores and an almost hyaline proper exciple of *Thelotrema lepadinum* clearly separate it from *T. similans*. The observed specimen can be confused with *Thelotrema capense* Zahlbr., but the latter species has non-amyloid ascospores and is restricted to Africa [2].

Specimen examined: Jeju Island, Mt. Halla, Seongpanak Trail, 33°22'38.8" N, 126°34'16.4" E, alt. 1,181 m, on tree bark, 8 Aug 2012, Hur *et al.*, KoLRI 121657.

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Fig. 4. *Thelotrema similans* Nyl. A, Habit; B, Ascospores (scale bars: A = 1 mm, B = 50 µm).

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