A New Species of *Graphis* and New Lichen Records from Vietnam, Including a Second Worldwide Report of *Sarcographina cyclospora*

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Abstract *Graphis upretii* is a new lichen species discovered in Vietnam. The species is characterized by a loosely corticate, rough, whitish grey to greyish green thallus, elongate and irregularly branched lirellae with an apically thin complete thalline margin (*negrosina* morph), laterally carbonized, entire proper exciple, clear hymenium, hyaline, 16–20 transversely locular ascospores, and about 50–95 × 10–15 µm in size. In addition, members of the taxon produce norstictic and stictic acids. Currently, the lichen flora of Vietnam include *Arthonia radiata*, *Brigantiaea tricolor*, *Coenogonium implexum*, *Dirina paradoxa*, *Herpothallon sipmanii*, *Pertusaria pertusa*, and *Sarcographina cyclospora*.

Keywords Corticolous, Dak Lak Province, Graphidaceae, Mt. Doi Sao, New species, Norstictic acid, Taxonomy

The tropical rainforests of Vietnam have experienced extensive species migration from China, India-Himalaya, Malaysia-Indonesia, and other neighboring temperate regions because of its varied climate and topographic position. These rainforests are thus currently comprised of a wide variety of corticolous, lichenized ascomycetes [1–6]. During a recent visit to a central highland region of the country, several interesting lichenized fungi were encountered in the relatively shaded side of the vegetation belt and in semi-exposed isolated tree pastures.

A luxuriant diversity of Graphidaceae was noted and was previously recorded from different parts of the country [2, 3]. However, the genus *Graphis* has been less thoroughly studied and until recently was represented by only six species:

Graphis caesiella Vain., *G. dussii* Vain., *G. leptogramma* Nyl., *G. librata* C. Knight, *G. proserpens* Vain., and *G. rimulosa* (Mont.) Trevis. In a more recent study on the Graphidaceae from the northern and central highlands of Vietnam, Joshi *et al.* [4] additionally recorded 11 *Graphis* species, namely *G. caesiocarpa* Redinger, *G. cervinonigra* Zahlbr., *G. cycasicola* A. W. Archer & Elix, *G. epiphloea* Zahlbr., *G. handelii* Zahlbr., *G. japonica* (Müll. Arg.) A. W. Archer & Lücking, *G. renschiana* (Müll. Arg.) Stizenb., *G. schroederi* Zahlbr., *G. subserpentina* Nyl., *G. subvittata* Adaw. & Makhija, and *G. supracola* A. W. Archer. This study describes a new species, *G. upretii*, which was recently discovered and collected from the Dak Lak Province of Vietnam. Superficially, the new taxon more closely resembles *Diorygma* or *Platythecium*, but it is distinguished as *Graphis* in having a whitish grey to greenish grey thallus, irregularly branched lirellae with complete thalline margin, a black and entire labia, a slit-like disc, and laterally carbonized proper exciple, as well as being transversely septate, possessing I+ blue ascospores, and a thallus that produces norstictic acid as the major lichen compound.

MATERIALS AND METHODS

The material collected in April 2012 and 2013 from the northern and central highland areas of Vietnam, was deposited in the herbarium of the Korean Lichen Research Institute, South Korea (KoLRI) and subjected to morpho-anatomical and chemical analyses. A Nikon SMZ-168

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dissecting microscope (Nikon, Tokyo, Japan) was used for observation of morphological features, whereas the anatomical details were investigated under an Olympus BX-50 compound microscope (Olympus, Tokyo, Japan). Ten to fifteen apothecial sections were examined thoroughly for confirmation of the species. Thin, hand-cut sections were observed in water and lactophenol cotton blue solution. Additionally, Lugol's iodine (I) was applied to check the amyloidity of the ascus, ascospores, and interascal filaments. All measurements were made under higher magnifications of 40× and 1,000× prior to the application of KOH. Chemical analysis was conducted by using thin-layer chromatography (solvent systems C and A) and color spot tests as described by Orange *et al.* [7]. The relevant literature describing Graphidaceae [8-11] was consulted for identification of the new taxon.

RESULTS AND DISCUSSION

Taxonomic treatment of the species.

G. upretii S. Joshi & Hur sp. nov. (Fig. 1A–1F)

Mycobank No.: MB 807869.

Similar to *G. longispora*, but differing in having a complete thalline margin, smaller ascospores, and a thallus that produces norstictic and stictic acids.

Type: Vietnam, Dak Lak Province, Buon Ma Thuot City, Mt. Doi Sao, 12°40'31.8" N, 108°05'24.4" E, alt. c. 539 m, on

tree bark, Oh & Thanh VN130043 (holotype KoLRI).

Description: Thallus corticolous, epiperidermal, crustose, continuous, spread in patches up to 15 cm, loosely corticate, rough, rather delicate, crystalline, whitish grey, greenish grey or greyish white, ± dull, 100–200-μm thick in cross-section. Cortex poorly developed, 10–20-μm thick. Photobiont layer densely interspersed with calcium oxalate crystals, 70–90-μm thick. Crystalline layer more distinct below algal cells, 40–70-μm thick. Medulla endoperidermal, indistinct to absent; prothallus whitish.

Ascomata lirelliform, erumpent to prominent. Lirellae completely covered by thalline margin (10–12-μm thick apically), elongate and irregularly branched (*negrosina* morph), up to 3 cm long, with ends mostly acute to sub-acute. Labia entire, black, appearing greyish in the cross-section because of the thin cortex cover being absent from algal cells. Disc slit-like, epruinose, concealed. Thalline margin complete, sometimes flaking off and exposing black labia, laterally 80–95-μm thick. Proper exciple entire, laterally carbonized, convergent, apically 10 μm to laterally 60–90-μm thick. Epithymenium greyish, crystalline, 15–20-μm high. Hymenium hyaline, clear, 100–130-μm high, I–. Paraphyses simple, rather lax, apically granulate, ± conglutinate, 1–2-μm thick. Hypothecium hyaline, indistinct to 20-μm high, I–. Ascus clavate, 2–6-spored, 120–150 × 15–20 μm, I–. Ascospores hyaline, fusiform, clavate to ellipsoidal with rounded to sub-acute ends, transversely 15–19 septate, about 50–95 × 10–15 μm, locules lenticular, halo not seen, I+ violet to blue.

Chemistry: Thallus and medulla K+ yellow turning red, P+ yellow-orange, C–, KC–; norstictic (major) and stictic acids (trace) detected in thin-layer chromatography.

Etymology: The specific epithet is named in honor of an eminent lichenologist, Dr. D. K. Upreti, from the CSIR-National Botanical Research Institute, Lucknow, India.

Distribution and ecology: The type locality is situated in the central (or western) highlands (Tây Nguyên) of Vietnam, more toward neighboring Cambodia, in Mt. Doi Sao of Dak Lak Province. The region has many primitive forests growing on basalt soil and is conserved as a protected area. The new species was widely dispersed in large or small patches on thick tree bark in an evergreen forest and collected at a height of c. 1 m from the tree base in semi-exposed or shaded conditions. The other taxa sharing similar microclimate at an elevation between 400–500 m belonged to *Chiodecton*, *Pyrenula*, and *Diorygma*, in addition to several associated members of foliicolous lichens.

The forest composition of Vietnam shares several characteristics (vegetation, climate, habitat, and distribution) with those of other southeast Asian countries and shows a greater affinity toward the Indo-Burman region. Hence, existence of the new species in lowland areas of Cambodia, Laos, Thailand and the eastern part of India cannot be denied.

Remarks: The new species is well characterized by whitish grey to greenish grey, loosely corticate thallus, lirellae with apically thin, complete thalline margin (*negrosina* morph) and

Fig. 1. *Graphis upretii* (holotype). A, B, Habitat; C, Habit; D, Ascomata (the arrow indicates the complete thalline margin); E, Cross-section of ascomata; F, Ascospores (scale bars: A = 15 cm, B, C = 5 cm, D = 1 cm, E = 100 μm, F = 50 μm).

entire labia, concealed disc, convergent, laterally carbonized proper exciple, transversely septate ascospores of about $50\sim 95 \times 10\sim 15 \mu\text{m}$, 2~6-spored asci, and the presence of norstictic and stictic acids as lichen substances.

G. upretii belongs to a group with taxonomic characteristics that include an entire labia, laterally carbonized excipulum, clear hymenium, and transversely septate ascospores. The organism closely resembles *G. longispora* D. D. Awasthi & S. R. Singh in having $\pm$ erumpent, elongate and irregularly branched lirellae, a thallus containing norstictic acid, and a similar geographical distribution (Indo-Burma region). However, the latter differs in having a thick, but lateral thalline margin, slightly larger ascospores reaching up to c. $130 \mu\text{m}$ in length, saxicolous habitat, and a thallus that produces salazinic acid in place of stictic acid [11, 12]. Another similar species of the group is *G. salacinilongiramea* Adaw. & Makhija, which has elongate and erumpent lirellae and long ascospores that are comparable to *G. upretii*, but is distinct in having a lateral thalline margin and produces salazinic acid together with stictic acid. *G. ajarekarii* Patw. & C. R. Kulk. is closely related to the new taxon in producing norstictic and stictic acids, and has lirellae with a concealed disc and epruinose labia, but differs markedly in having very small ascospores of $15\sim 45 \mu\text{m}$ in size.

G. assamensis Nagarkar & Patw resembles *G. upretii* in having erumpent lirellae, complete thalline margin, concealed disc, non-pruinose labia, the presence of stictic acid in the thallus, and a similar geographical distribution, but differs in having a completely carbonized proper exciple, smaller ascospores of $45\sim 70 \mu\text{m}$, and a thallus that produces salazinic acid in addition to stictic acid.

G. verminosa Müll. Arg. shares some characteristics with *G. upretii* such as irregularly branched, erumpent lirellae covered by an apically thin complete thalline margin, laterally carbonized proper exciple, transversely septate ascospores, and the presence of norstictic acid in the thallus, but differs in having a striate labia and a thallus lacking stictic acid as an associated lichen compound.

The thin and complete thalline margin of the new taxon gives the appearance of a pruina, which differs in composition, being made up of a dead hyphae and crystals. However, in the cross-section of lirellae, a thin layer of cortex lacking algal cells reaching apically can easily be observed. The new taxon can easily be mistaken as the pruinose *Carbacanthographis*, but non-amyloid ascospores and spinulose periphysoids, which are characteristics of the latter, are absent in *G. upretii*.

Additional specimen examined: Vietnam, Dak Lak Province, Buon Ma Thuot City, Mt. Doi Sao, $12^{\circ}40'31.8''$ N, $108^{\circ}05'24.4''$ E, alt. 539 m, on tree bark, 19 Feb 2013, Oh & Thanh, VN130042 (KoLRI).

Arthonia radiata (Pers.) Ach. (Fig. 2A)

K. Vetensk-Acad. Nya Handl. 29: 131 (1808).

Description: The corticolous species is characterized by a lichenized, 55 to $75\text{-}\mu\text{m}$ thick, pale grey to olive, immersed

thallus delimited in patches; linear to aggregated, irregular to substellate ascomata of 1~1.5-mm diameter; flat to slightly convex, black, epruinose disc; brown or olive-brown, hyaline, clear and gelatinous I+ blue hymenium, up to $40\text{-}\mu\text{m}$ high; 8-spored, broadly clavate asci, $35\sim 40 \times 14\sim 16 \mu\text{m}$; hyaline to grey-brown, oblong-ovoid to oblong, transversely 3-septate, gelatinous, I– ascospores, $14\sim 16 \times 4\sim 5 \mu\text{m}$, and the absence of lichen substances. *Arthonia radiata* is widely distributed across Europe, North America, Asia, Africa, and New Zealand [13].

Specimen examined: Vietnam, Dak Lak Province, Buon Ma Thuot City, Museum, $12^{\circ}40'32.2''$ N, $108^{\circ}02'28.9''$ E, alt. 516 m, on tree trunk, 19 Feb 2013, Oh & Thanh, VN130048 (KoLRI).

Brigantiaea tricolor (Mont.) Trevis. (Fig. 2B)

Spighe Paglie: 9 (1853).

Description: The species is characterized by a whitish grey to grey green, effuse, indistinctly sorediate thallus; sessile, round ascomata constricted at the base, 1~1.5 mm; disc brownish, plane, covered by $\pm$ yellow pruina, with prominent, shiny, reddish brown margin reacting K+ blue-violet; biatorine exciple; hyaline, clear to slightly inspersed hymenium, $120\sim 130 \mu\text{m}$; 1-spored asci, $90\sim 100 \times 30\sim 45 \mu\text{m}$; hyaline, ellipsoidal to oblong, muriform ascospores, $65\sim 80 \times 25\sim 30 \mu\text{m}$, and a thallus containing atranorin and zeorin as the major lichen compounds. *B. leucoxantha* R. Sant. & Hafellner differs from *B. tricolor* in having an orange ascomatal margin reacting K+ purple, and a disc containing yellow-orange pruina. *B. tricolor* is widely distributed in Africa, Australia, eastern and southern Asia, the Hawaiian Islands New Caledonia, and Papua New Guinea [14].

Specimens examined: Vietnam, Kon Tum Province, Kon Plong District, Mt. Kon Plong, PAK-SY waterfall, $14^{\circ}35'46.1''$ N, $108^{\circ}15'24.3''$ E, alt. 1,033 m, on dead tree branch, 21 Feb 2013, Oh & Thanh, VN130127 (KoLRI); Dak Lak Province: Yok Don National Park, $12^{\circ}51'20.0''$ N, $107^{\circ}45'58.1''$ E, alt. c. 760 m, on tree trunk, 22 Apr 2012, Hur & Oh, VN120228 (KoLRI).

Coenogonium implexum Nyl. (Fig. 2C)

Ann. Sci. Nat., Bot., Ser. 4. 16: 92 (1862).

Description: The species is characterized by filamentous (very densely woven filaments of *Trentepohlia* cells, $30\sim 35 \times 14\sim 16 \mu\text{m}$), pannose, ecorticate, yellow-green thallus, up to 50 mm; sessile and round ascomata, $0.18\sim 0.2 \times 0.5\sim 1$ mm; flat, yellow-orange; smooth cream-colored disc with a thin margin; hyaline, paraplectenchymatous proper exciple of radiating isodiametric hyphal cell rows, $40\sim 70 \mu\text{m}$; hyaline, clear hymenium reacting I+ blue to sordid green to reddish brown, $60\sim 80 \mu\text{m}$; 8-spored asci, $50\sim 60 \times 6\sim 7 \mu\text{m}$; hyaline, ellipsoid, 1-septate ascospores, $6\sim 8 \times 2\sim 3 \mu\text{m}$, and a lack of lichen compounds in the thallus. *C. implexum* is also distributed across the Neotropics, as well as in the eastern Palaeotropics [15].

Fig. 2. New records of lichens in Vietnam. A, *Arthonia radiata*; B, *Brigantiaea tricolor*; C, *Coenogonium implexum*; D, *Dirina paradoxa*; E, *Herpothallon sipmanii*; F, *Pertusaria pertusa*; G, *Sarcographina cyclospora* (the arrow indicates the stromatoid ascumata) (scale bars: A~F = 1 mm, G = 5 mm).

Specimen examined: Vietnam, Vinh Phuc Province, Tam Dao National Park, 21°27'21.7" N, 105°39'0.8" E, alt. c. 1,092 m, on tree trunk, 25 Apr 2012, Hur & Oh, VN120324 (KoLRI).

***Dirina paradoxa* (Fée) Tehler (Fig. 2D)**

Lichenologist 18: 296 (1986).

Description: The species is characterized by a whitish grey, greenish grey to greyish white, pruinose, rimose cracked, verrucose c. 100-µm thick thallus flaking off the substratum and exposing loose white medulla; solitary to aggregate, pluricarpocentral, discothecia, numerous, sessile, circular to distinctly undulate ascomata constricted at base, 0.5~2.5 mm; dark brown, thinly white pruinose disc; entire to undulating thalline margin, 0.1~0.9 mm, thin to indistinct proper exciple; hyaline and interspersed hymenium, 80~100 µm; 8-spored, clavate asci, 75~90 µm; hyaline, fusiform (one end thick than other), transversely 3-septate ascospores, 20~23 × 4~6 µm, and the presence of erythrin, lecanoric acid, and a yellowish brown unknown substance in the thallus. The species has previously been reported in the coastal regions of the Caribbean, in the Bahamas, Netherland Antilles, Venezuela, and Trinidad [16].

Specimen examined: Vietnam, Dak Lak Province, Buon Ma Thuot City, Museum, 12°40'32.2" N, 108°02'28.9" E, alt. 516 m, on tree trunk, 19 Feb 2013, Oh & Thanh, VN130046 (KoLRI).

***Herpothallon sipmanii* Aptroot, Lücking & Rivas Plata (Fig. 2E)**

Bibl. Lichenol. 99: 64 (2009).

Description: The species is characterized by a distinctly byssoid, pale mineral grey to green or whitish green thallus,

± firmly appressed to the substrate; compact brown hypothallus; a prothallus made up of radiating whitish hyphae; numerous, pale to off-white, flattened to slightly convex, disc-shaped (schizidia) outgrowths (pseudisidia) of round to wavy margin, up to 1 mm in diameter, and the presence of protocetraric and hypoprotocetraric acids. The species is distributed in Papua, New Guinea, the Philippines, and Thailand. For more details, see Aptroot *et al.* [17].

Specimen examined: Vietnam, Kon Tum Province, Sa Thay district, 14°41'48.9" N, 107°52'15.3" E, alt. 768 m, on tree trunk, 22 Feb 2013, Oh & Thanh, VN130040 (KoLRI).

***Pertusaria pertusa* (Weigel) Tuck. (Fig. 2F)**

Enum. N. Am. Lich.: 56 (1845).

Description: The species is characterized by a pale grey to greenish grey, ± shiny, smooth, wrinkled or warted thallus delimited by a brown line; semi-globose, crowded fertile warts constricted at the base, 1~2-mm in diameter; immersed, perithecioid apothecia, 1~5 per wart; punctiform, brownish disc; hyaline and clear hymenium, 300~400 µm; 2~4-spored asci; hyaline and simple ascospores, 100~140 × 35~40 µm, and a thallus containing norstictic and stictic acids. The species has been reported in Europe, Macaronesia, Africa, and Asia [18].

Specimen examined: Vietnam, Dak Lak Province, Yok Don National Park, 12°51'20" N, 107°45'58.1" E, alt. 760 m, on tree trunk, 22 Apr 2012, Hur & Oh, VN120223 (KoLRI).

***Sarcographina cyclospora* Müll. Arg. (Fig. 2G)**

Flora Jena 70: 425 (2887).

Description: The species is characterized by pale fawn, thin, smooth, glossy thallus; lirellate ascomata immersed in white, round to irregular stromata of 2~5 mm in diameter;

thin, black, $\pm$ open lirellae in irregular stellate clusters; black disc covered by white pruina; non-carbonized, pale yellow to brownish proper exciple; hyaline and clear hymenium, 80–100 μm ; 8-spored asci; pale brown to dark-brown, narrowly ellipsoidal, 2×2 -locular, I– ascospores, $9\text{--}12 \times 5\text{--}7 \mu\text{m}$, and the presence of psoromic acid chemosyndrome. This is the second record of the species and has been previously reported only in Australia [8]. The collection of *S. cyclospora* from Vietnam increases the possible distribution of this species in other neighboring countries of tropics.

Specimens examined: Vietnam, Dak Lak Province, Buon Ma Thuot City, Chu Yang Sin National Park (waterfall), $12^{\circ}29'00.1''$ N, $108^{\circ}20'25.6''$ E, alt. 450 m, on dry rock (sandstone), 19 Feb 2013, Oh & Thanh, VN130022 (KoLRI); Chu Yang Sin National Park, $12^{\circ}27'57.0''$ N, $108^{\circ}20'34.9''$ E, alt. c. 780 m, on tree trunk, 21 Apr 2012, Hur & Oh, VN120171 (KoLRI); Vinh Phuc Province, Tay Thien Mt., $21^{\circ}27'53.3''$ N, $105^{\circ}35'06.6''$ E, alt. c. 77 m, on tree trunk, 26 Apr 2012, Hur & Oh, VN120377, VN120381 (KoLRI).

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