KEY AND CHECKLIST OF GRAPHIDACEAE LICHENS IN THE KALAHAN FOREST RESERVE, NUEVA VIZCAYA, PHILIPPINES

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ABSTRACT

This study involves a survey of Graphidaceae lichens in the Kalahan Forest Reserve, Imugan, Santa Fe, Nueva Vizcaya. Taxonomic characters of the thallus, ascocarp/lirella, exciple, hypothecium, hymenium, ascospore and lichen acids were used in the identification of the lichens. A key and a checklist of the 52 identified Graphidaceae lichens are presented in this paper. Two new combinations are proposed: *Pallidogramme albida* (Vain.) Tabaquero, Bawingan & Lücking comb. nov. and *Sarcographa dendroides* (Leight.) Tabaquero, Bawingan & Lücking comb. nov.

KEYWORDS**:** Graphidaceae lichens, lichenology, mycology, systematics

INTRODUCTION

Graphidaceae is a family of mostly corticolous lichens in the order Ostropales (Staiger, 2002; Staiger et al. 2006; Rivas Plata et al., 2012). The family Thelotremataceae has recently been subsumed under Graphidaceae (Mangold et al., 2008; Rivas Plata et al., 2012). The emended family comprises probably over 2000 species worldwide (Rivas Plata et al., 2012); it includes more than 40 currently distinguished graphidoid genera with lirelliform ascomata (Aptroot & Sipman, 2007; Lücking & Rivas Plata, 2008; Mangold et al., 2008; Lücking et al., 2008; Lücking, 2009) and about 30 thelotremoid genera with more or less rounded ascomata (Frisch, 2006; Rivas Plata et al., 2010; Rivas Plata et al., 2012). Lücking (2009) described the emended Graphidaceae as having rounded to lirellate or pseudostromatic ascomata; non-amyloid and functionally unitunicate asci with apical wall thickenings; ascospores with mostly thick, often amyloid septa and lens-shaped lumina; and usually having a trentepohlioid photobiont.

Graphidaceae is the dominant element of crustose lichen communities in tropical ecosystems in terms of biodiversity and abundance (Wirth & Hale, 1963, 1978; Hale 1974b, 1978, 1981; Staiger, 2002; Frisch, 2006; Rivas Plata et al.,

2007; Lücking et al., 2008). The family includes species important for both environmental health assessment (Blett et al., 2003; Rivas Plata et al., 2007) and medical concerns (Miyagawa et al., 1994; Tanahashi et al., 1997; Hur et al. 2003; Sanglarpcharonekit & Sangvichien, 2006; Behera et al., 2003, 2006a, 2006b). However, their highly diverse assemblage in tropical forests lacks detailed taxonomic and ecological studies (Rivas Plata et al., 2007). This is especially the case in the Philippines. Many forest trees are richly studded with graphidoid lichens but not sufficient taxonomic studies have been conducted.

The father of Philippine lichenology, Edvard August Vainio, described 118 species classified as Graphidaceae in 1921. A summary of the works of Vainio (1913), of the checklist compiled by Gruezo (1979), of the results of field trips by K. Kalb, H. J. M. Sipman, E. Rivas Plata and R. Lücking in 1983, 1987, 1991, 2007 and 2009 and of the recent work done by A. Linsangan-Tabaquero and P. Bawingan (Parnmen et al., 2012) gives a total of 221 species of Graphidaceae lichens identified in the Philippines. Over 120 species, the second highest number of Graphidaceae species so far recorded for any single site world-wide (Rivas Plata & Lücking 2013), were collected in Mt. Palali in Nueva Vizcaya. With this number in mind, another forest community in Nueva Vizcaya was surveyed for these lichens: the Kalahan Forest Reserve (KFR), a community-initiated reserve managed by the Kalahan Educational Foundation in Imugan, Santa Fe, Nueva Vizcaya. It is a 14,730-ha secondary growth forest reserve (Dolom & Serrano, 2005) with an altitude range of 600-1700 masl and an average annual rainfall of 3000 mm (Rice, 2000). For generations, the indigenous knowledge and environmentally sustainable agricultural practices of the Ikalahans or Kalahans (indigenous people in Imugan) have been crucial in the preservation of the Kalahan Forest Reserve. The community has been relying mainly on hunting, gathering and traditional swidden agriculture to survive (Villamor & Lasco, 2006). The forest is well protected against deforestation and land conversion under a Land Use Plan (LUP) specifying protected areas, watersheds, bird sanctuaries, and agroforest farming lots. The Kalahans have their indigenous system of swidden farming (including fallow for some years) inside the forest but in a limited and fixed area (Synopsis of the BCN Results Dissemination Workshop, 1999). Major forest trees are dipterocarps, pine (*Pinus*), narra (*Pterocarpus*), mahogany (*Swietenia*), alder (*Alnus*), and ipil-ipil *(Leucaena)* species (Dahal & Adhikari, 2008).

MATERIALS AND METHODS

Sampling and collection. Sampling and collection were done on four sampling trails (tourism area, plant nursery/landing area, forested hill, dipterocarp forest) using the quantitative transect sampling method (Caceres et al., 2007; McCarthy, 2004). Each transect trail approximately measured 100 m. The whole sampling site has an altitude range of 1003 – 1046 masl. Lichens were collected from 25 lichen-rich trees per trail, 5 m apart along a 30 m distance parallel to the trail on both sides but approximately 5 m inward from

opposite edges of the trail. Collections were done at the lower trunk (1.5 m from the ground) of the selected tree in a 60 x 20 cm^2 sampling grid (Gradstein et al., 2003). This is considered zone 2a of the vertical zonation in Johansson (1974) and in Ter Steege and Cornelissen (1989). Lichen samples containing necessary taxonomic features were scraped from the tree barks using sharp knives. These were kept in properly labeled specimen paper bags.

Morphoanatomical and chemical characterization. The taxonomic characters determined were as follows: thallus (cortex, texture, color), ascocarp/lirella (morph, form, emergence, branch, color, striation, margin, rim color, disc color, pruina), exciple (carbonization), hypothecium (carbonization), hymenium (inspersion), ascospore (number per ascus, shape, septation, number of locules, length, width, color and color reaction to Iodine solution) and lichen acids.

Gross features of the thallus and lirellae were assessed using a CARTON 6v12w TB-20 stereomicroscope (20 x magnification). Cross sections of the lirellae were prepared to observe detailed characteristics of the exciple, hypothecium, hymenium and ascospores using an OLYMPUS BX50 and N101-B binocular compound microscopes (400 x magnification).

In determining the lichen acids present, the spot color test was used wherein 10% aqueous KOH was dropped on the thallus surface and color reactions were interpreted with reference to the work done by Rivas Plata et al. (2009). For confirmation of lichen acid content, thin layer chromatography (TLC) was performed (Lumbsch, 2002). Identification of chemical constituents was done by computing Rf values and using the Rf classes (Culberson, 1972; Culberson & Kristinsson, 1970). Pure crystals of norstictic, lecanoric and protocetraric acids were used as controls while acetone extracts of the lichens *Parmotrema reticulatum* and *Lobaria isidiophora* were used for salazinic and stictic acids, respectively.

***Lichen identification*.** Morphoanatomical and chemical characteristics were used as bases for identification guided by dichotomous taxonomic keys for tropical Graphidaceae lichens by Staiger (2002), Kalb et al., (2004), Sipman (2005, 2008a, 2008b), Archer (2005, 2009), Frisch (2006), Lücking (2009), Rivas Plata et al., (2009), the pictographic guides of Rivas Plata et al., (2008); Lücking & Rivas Plata (2008); and the lichen images available online (http://www.tropicallichens.net/default.aspx?s=f;http://www.fieldmuseum.org/ticolichen/images.html.) contributed mostly by lichenologists from the Field Museum in Chicago.

RESULTS

Taxonomic identification revealed a total of 52 species distributed in 17 genera. The number of lichen species per genus are as follows: *Graphis* (25), *Sarcographa* (5), *Phaeographis* (4), *Pallidogramme* (3); *Diorygma* (2),

Thalloloma (2), *Fissurina* (1), *Acanthothecis* (1), *Hemithecium* (1), *Glyphis* (1), *Platyggramme* (1), *Platythecium* (1), *Sarcographina* (1), *Thecaria* (1), *Chapsa* (1), *Thelotrema* (1) and *Myriotrema* (1). Most of the species are graphidoids; only a few *Chapsa*, *Thelotrema* and *Myriotrema* species are thelotremoids. Two of the species are proposed as new combinations: *Pallidogramme albida* (Vain.) Tabaquero, Bawingan & Lücking comb. nov. and *Sarcographa dendroides* (Leight.) Tabaquero, Bawingan & Lücking comb. nov. Specimens are deposited at the Father Braeckman Museum of Natural History, Saint Louis University. The checklist below presents the identified Graphidaceae species, with some synonyms.

Graphidoids

***Acanthothecis incondita* (Nyl.) Staiger & Kalb (1999)**

***Diorygma hieroglyphicum* (Pers.) Staiger & Kalb (2004)**
Graphina pallido-ochracea (Kremp.) Zahlbr. (1923)

***Diorygma junghuhnii* (Mont. & v.d. Bosch) Kalb, Staiger & Elix (2004)**
Graphina mendax (Nyl.) Müll. Arg. (1887)
Graphis mendax Nyl. (1859)
Ustalia junghuhnii Mont. & Bosch (1855)

***Fissurina incrustans* (Fee) Müll. Arg. (2002)**
Graphina incrustans (Fee) Müll. Arg. (1887)
Graphis incrustans (Fee) Nyl. (1858)

***Glyphis cicatricosa* Ach. (1814)**
Glyphis verruculosa Zahlbr. (1923)
Glyphis favulosa var. *depauperata* Müll. Arg. (1891)
Glyphis verrucosa C. Knight (1889)
Glyphis cribrosa Fee (1841)
Glyphis confluens Zenker. (1827)

***Graphis brahmanensis* Aptroot (1992)**

***Graphis caesiella* Vain. (1890)**

***Graphis dendrogramma* Nyl. (1875)**

***Graphis dupaxana* Vain. (1921)**

***Graphis duplicata* Ach. (1814)**

***Graphis eburnea* Adaw. & Makhija (2007)**

***Graphis flavens* Müll. Arg. (1882)**

***Graphis glauconigra* Vain. (1921)**

***Graphis immersella* Müll. Arg. (1895)**

***Graphis ingarum* (Vain.) Lücking (2009)**
Graphis angustata var. *ingarum* Vain. (1915)

***Graphis japonica* (Müll. Arg.) A.W. Archer & Lücking (2009)**
Graphina japonica Müll. Arg. (1891)

***Graphis librata* Knight. (1884)**

***Graphis litoralis* Lücking, Sipman & Chaves (2009)**

***Graphis lumbricina* Vain. (1899)**

***Graphis pinicola* Zahlbr. (1930)**

***Graphis polystriata* Makhija & Dube (2006)**

***Graphis proserpens* Vain. (1909)**
Graphis disserpens Vain. (1890)

***Graphis rimulosa* (Mont.) Trevis (1853)**

***Graphis rustica* Kremp. (1875)**
Graphis turgidula Müll. Arg. (1885)

***Graphis* sp.**

***Graphis striatula* (Ach.) Spreng. (1827)**

***Graphis subcelata* A.W. Archer (2009)**

***Graphis subdisserpens* Nyl. (1873)**

***Graphis tenella* Ach. (1814)**

***Graphis vitatta* Müll. Arg. (1882)**

***Hemithecium balbisii* (Fee) Trevis (1853)**

***Pallidogramme albida* (Vain.) Tabaquero, Bawingan & Lücking comb. nov.**
Bas.: *Graphis albida* Vain., J. Bot. 34: 259 (1896).
Syn.: *Phaeographis albida* (Vain.) Zahlbr. *Catal. Lich. Univ* 2:364 (1923).
Holotype: Dominica, *Elliott 528* (BM!)

***Pallidogramme chlorocarpoides* (Nyl.) Staiger, Kalb & Lücking (2008)**
Hemithecium chlorocarpoides (Nyl.) Staiger (2002)
Graphis chlorocarpoides Nyl. (1866)

***Pallidogramme chrysenteron* (Mont.) Staiger, Kalb & Lücking (2008)**
Phaeographina chrysenteron (Mont.) Mull. Arg. (1891)
Hemithecium chrysenteron (Mont.) Trevis. (1853)
Graphis chrysenteron Mont. (1842)

***Phaeographis aff. inconspicua* (Fee) Müll. Arg. (1887)**

***Phaeographis flavescens* Dal-Forno & Eliasaro (2010)**

***Phaeographis intricans* (Nyl.) Staiger (2002)**
Sarcographa intricans (Nyl.) Müll. Arg. (1887)
Graphis intricans Nyl. (1863)

***Phaeographis schizoloma* (Müll. Arg.) Müll. Arg. (1882)**

***Platygramme caesiopruinosa* (Fee) (1874)**
Phaeographina caesiopruinosa (Fee) Müll. Arg. (1877)
Arthonia caesiopruinosa Fee (1837)

***Platythecium* sp.**

***Sarcographa dendroides* (Leight.) Tabaquero, Bawingan & Lücking comb. nov.**
Bas.: *Platygrapha dendroides* Leight., *Trans. Linn. Soc. London.* 27: 179 (1869).
Syn.: *Phaeographis dendroides* (Leight.) Müll. Arg., *Flora.* 65: 336 (1882).
Lectotype: Sri Lanka, *Leighton 165* (BM!).

***Sarcographa difformis* (Vain) Zahlbr. (1923)**
Graphis difformis Vain. (1923)

***Sarcographa kirtoniana* (Müll. Arg.) Müll. Arg. (1887)**

***Sarcographa subtricosa* (Leight.) Müll. Arg. (1887)**
Sarcographa actinota var. *pulverulenta* F. Wilson (1891)

***Sarcographa tricosa* (Ach.) Müll. Arg. (1877)**
Graphis tricosa Ach. (1810)

***Sarcographina glyphiza* (Nyl.) Kr. P. Singh & D. D. Awasthi (1979)**

***Thalloloma anguinaeforme* (Vain.) Staiger (2002)**
Graphis anguinaeformis Vain. (1890)
Graphina anguinaeformis (Vain.) Zahlbr. (1923)

***Thalloloma janeirense* Staiger (2002)**

***Thecaria quassicola* Fee (1824)**
Phaeographina quassicola (Fee) Müll. Arg. (1887)

Thelotremoids

***Chapsa* sp.**

***Myriotrema* sp.**

***Thelotrema porinoides* Mont. & Bosch (1855)**

The species are generally corticate, with smooth and white thallus. Their lirellate to partially rounded ascocarps are mostly labiate, erumpent, irregularly

branched, black, striate, with lateral thalline margin, and non-pruinose. If the lirellae are formed from thalline exciple or exhibit partially opened to fully opened lirellae or in combination with other forms, the labial excipula have mostly a rim that is concolorous with the thallus. If the discs are exposed, these are mostly gray. The exciple carbonization is generally lateral. The hypothecia are generally non-carbonized and the hymenia are mostly clear. Generally, the lichens have hyaline and I+ blue-violet ascospores, with 8 or less ascospores per ascus. Transversely septate ascospores are mostly 8-locular and elongated-ellipsoidal. Muriform ascospores are largely 8 x 2-4 locular. The ascospores are small (length: 8-50 μm; width 1-20 μm) in general. For their chemistry, most species are K- and lack lichen acids. If lichen acids are present, stictic acid is the major lichen acid constituent. The genus *Graphis* has the most number of species identified and mostly conforms to the striatula morph. The surface and cross-section of a typical Graphidaceae is shown in Figure 1. The key to the identification of the lichens is presented below.

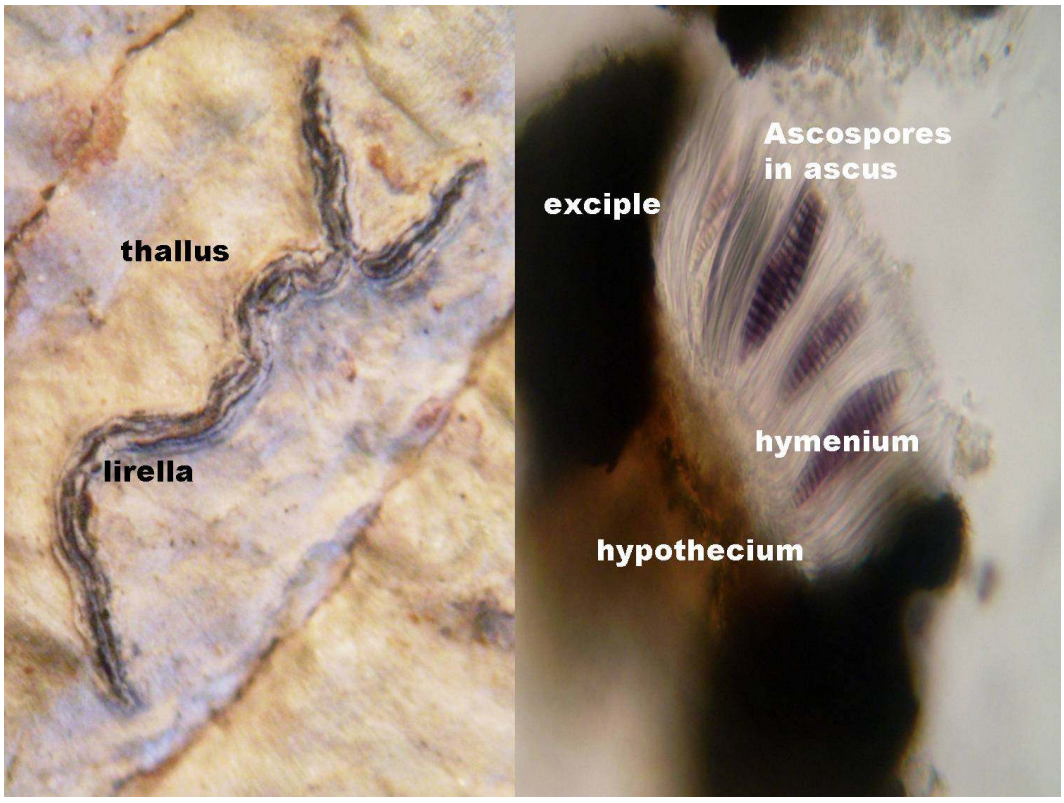

Figure 1. Surface (20x) and cross-section (400x) of a Graphidaceae lichen

Key to the Genera and Species of Graphidaceae Lichens in the Kalahan Forest Reserve

1 Ascocarp elongated/ lirellate ……..…..... 2

1 Ascocarp ±rounded ……...…..... 26

2 Lirellae labiate ……..... 3

2 Lirellae fissurine, ±open to fully open or stromatic/pseudostromati ..……... 7

3 Lirellae black; immersed, erumpent, prominent or sessile……. ***Graphis*** (see separate key for *Graphis* species).

3 Lirellae predominantly white with yellowish, greenish or brownish tones 4

4 Lirellae distinctly white; ascospores transversely septate or muriform, lichen acids absent ……..... 5

4 Lirellae white with yellowish, greenish or brownish tones; ascospores muriform only, stictic acid present ……..…..... 6

5 Ascospores transversely septate, brown ……... ***Pallidogramme albida***

5 Ascospores muriform, hyaline …… ***Hemithecium balbisii***

6 Ascospores 2-4/ascus…… ***Pallidogramme chlorocarpoides***

6 Ascospores 6-8/ascus. ……***Pallidogramme chrysenteron***

7 Lirellae fissurine, ±open to fully open ……..……... 8

7 Lirellae stromatic or pseudostromatic …….……..…… 19

8 Lirellae open (labiate for immature lirellae), disc pruinose enclosed by proper exciple raised from the thallus ….……... 9

8 Lirellae fissurine, ±open to fully open, disc enclosed by thalline exciple …. 10

9 Proper exciple pale brown …… ***Thecaria quassicola***

9 Proper exciple black, wedge-shaped. ……. ***Platygramme caesiopruinosa***

10 Lirellae fissurine with either a thin thalline exciple or a fissure forming a roof-like emergence ……...… 11

10 Lirellae ±open to fully open …..... 12

11 Lirellae fissurine-labiate forming a roof-like emergence……... ***Acanthothecis incondita***

11 Lirellae fissurine-open, disc mostly uncovered and enclosed by thin thalline exciple…***Fissurina incrustans***

12 Disc concolorous with thallus, flesh-colored or brown ...…….…….. 13

12 Disc bluish gray or black …….....16

13 Lichen substances present; ascospores large 14

13 Lichen substances absent; ascospores small to large…….…..… 15

14 Disc concolorous with the thallus or slightly darker; stictic acid present. ***Diorygma hieroglyphicum***

14 Disc flesh-colored or brown (appears gray when dry); norstictic acid present. ***Diorygma junghuhnii***

15 Exciple non-carbonized, stictic acid present…***Thalloloma cf. anguinaeforme***

15 Exciple apically or laterally carbonized, lichen acids absent…… ***Thalloloma janeirense***

16 Exciple formed from thallus forming a white rim surrounding the disc ….. 17

16 Exciple forming a white striate labia surrounding a ±open black disc (Hemithecium-like or Pallidogramme-like)…….. ***Phaeographis schizoloma***

17 Ascospores transversely septate ……..… 18

17 Ascospores murifom …….. ***Platythecium* sp.**

18 Exciple non-carbonized …… ***Phaeographis aff. inconspicua***

18 Exciple non-carbonized in surface view but basally carbonized in cross-section. ***Sarcographa dendroides***

19 Lirellae embedded on white or brown, carbonized stroma …….. 20

19 Lirellae pseudostromatic (embedded in a non-carbonized stroma-like tissue) ……...…….. 25

20 Lirellae open, brown pruinose ……. ***Glyphis cicatricosa***

20 Lirellae fissurine, ±open to fully open, white pruinose …..... 21

21 Ascospores transversely septate ……..…..... 22

21 Ascospores muriform……... ***Sarcographina glyphiza***

22 Hymenium inspersed ……... 23

22 Hymenium clear……..….. ***Sarcographa kirtoniana***

23 Stictic acid present……... ***Sarcographa difformis***

23 Lichen acids absent ……..… 24

24 Exciple and hypothecium non-carbonized…… ***Sarcographa subtricosa***

24 Exciple and hypothecium carbonized…….. ***Sarcographa tricosa***

25 Lirellae irregularly branched and intertwined, stictic acid……. ***Phaeographis flavescens***

25 Lirellae stellately branched, norstictic acid …….. ***Phaeographis intricans***

26 Ascocarp sorediotremoid …… ***Myriotrema* sp.**

26 Ascocarp non-sorediotremoid …….. 27

27 Ascocarp leprocarpoid, immersed . .....***Chapsa* sp.**

27 Ascocarp thelotremoid, prominent, with pore……. ***Thelotrema porinoides***

KEY TO SPECIES OF THE GENUS *GRAPHIS*
(the named morphs refer to Lücking et al. 2009: 374-382)

1 Lirellae striate ……..... 2

1 Lirellae non-striate ..…….. 15

2 Exciple apically to peripherally carbonized ……...…….. 3

2 Exciple laterally to completely carbonized …….. 5

3 Lirellae striatula morph …..……..... 4

3 Lirellae negrosina morph …… ***G. eburnea***

4 Stictic acid present …….. ***G. vitatta***

4 Lichen acids absent ……. ***G. proserpens***

5 Exciple laterally carbonized ……...…… 6

5 Exciple completely carbonized ……..... 12

6 Lirellae striatula morph ……..... 7

6 Lirellae not striatula morph …….. 11

7 Stictic acid present ……. 8

7 Lichen acids absent …… 9

8 With thin complete thalline margin ……...…. ***Graphis* sp.**

8 With basal thalline margin ……...……. ***G. brahmanensis***

9 With basal thalline margin ……..……... ***G. duplicata***

9 Without thalline margin ……...……. 10

10 Lirellae prominent, sparsely branched ……. ***G. striatula***

10 Lirellae erumpent, sparsely to irregularly branched …….. ***G. polystriata***

11 Lirellae tenella morph …….. ***G. tenella***

11 Lirellae acharii morph ……. ***G. ingarum***

12 Lirellae striatula morph ……..…… 13

12 Lirellae not striatula morph, with complete thalline cover …… 14

13 With basal thalline margin ……..…….. ***G. dupaxana***

13 Without thalline margin ……..…….. ***G. rimulosa***

14 Lirellae acharii morph ……. ***G. glauconigra***

14 Lirellae lumbricina morph …… ***G. lumbricina***

15 Exciple laterally carbonized ..…… 16

15 Exciple completely carbonized ……...…… 22

16 Lirellae radiately/ stellately to irregularly branched …….……. 17

16 Lirellae sparsely branched 18

17 Lirellae caesiella morph …… ***G. caesiella***

17 Lirellae dendrogramma morph …….. ***G. dendrogramma***

18 Thallus ecorticate ……... ***G. litoralis***

18 Thallus corticated ……..... 19

19 Lirellae lineola morph …… 20

19 Lirellae not lineola morph ……..… 21

20 Stictic acid present ……..…….. ***G. immersella***

20 Norstictic acid present ……..***G. librata***

21 Lirellae subserpentina morph ……... ***G. japonica***

21 Lirellae desserpens morph ……. ***G. pinicola***

22 Lichen acids absent ……..…… 23

22 Stictic acid present, lirellae marginata-nuda morph …… ***G. rustica***

23 Lirellae erumpent, desserpens morph …….. ***G. subdisserpens***

23 Lirellae prominent ……..…… 24

24 Lirellae marginata morph …….. ***G. flavens***

24 Lirellae illinata morph …… ***G. subcelata***

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