Three new species of *Graphis* (Ascomycota: *Ostropales*: *Graphidaceae*) from Mexico, with updates to taxonomic key entries for 41 species described between 2009 and 2013

Alejandrina BARCENAS PEÑA, Robert LÜCKING, Ricardo MIRANDA-GONZÁLEZ and María de los Angeles HERRERA-CAMPOS

Abstract: Three species of *Graphis (Graphidaceae)* are described from Mexico from two research stations of the Universidad Nacional Autónoma de México (UNAM): Chamela Biological Station in the Cuixmala-Chamela Biosphere Reserve, Jalisco, and Los Tuxtlas Tropical Biological Station in the Los Tuxtlas Biosphere Reserve, Veracruz. The new species *Graphis bungartzii* Barcenas-Peña, Lücking, Herrera-Campos & R. Miranda is characterized by erumpent lirellae with a lateral thalline margin, striate labia and laterally carbonized exciple, transversely septate ascospores, and norstictic and connorstictic acids. *Graphis chamelensis* Barcenas-Peña & Lücking has prominent lirellae with a basal to lateral thalline margin, entire labia and completely carbonized exciple, transversely septate ascospores, and norstictic acid. Finally, *Graphis rosae-emiliae* Barcenas-Peña & Lücking is characterized by erumpent lirellae with lateral thalline margin, striate labia and completely carbonized exciple, transversely septate ascospores, and no substances detected by TLC. We present updates to the world key to *Graphis* for 41 newly described or reinstated species since the world key was published in 2009. The following new combination is proposed: *Pallidogramme parvicarpum* (B. O. Sharma & Khadilkar) Lücking comb. nov., based on *Graphis parvicarpa* B. O. Sharma & Khadilkar.

Key words: diversity, taxonomy, tropical lichens

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Introduction

Mexico's geographical and climatic setting generates a wide variety of diverse ecosystems (Ramamoorthy *et al.* 1993), which make it one of the six megadiverse countries in the world (Mittermeier 1988). The Mexican lichen biota is estimated at between 3000 and 5000 species (Herrera-Campos & Lücking 2003; Lücking *et al.* 2009*a*), of which only about half are reported in the literature (B. D. Ryan, T. H. Nash III & M. A. Herrera-Campos, unpublished data; Sipman & Wolf 1998; Herrera-Campos & Lücking 2002, 2003; Nash *et al.* 2002, 2004, 2007; Herrera-Campos *et al.* 2004*a*, *b*, 2005).

The Lichen Flora of the Greater Sonoran Desert Region, covering one of the best studied regions in the world in terms of its lichen biota (Nash et al. 2002, 2004, 2007), includes c. 1113 micro- and macrolichen species for Mexico. This contrasts with the rest of the country, where the lichenological knowledge is still fragmented or incomplete and lacks floristic and taxonomic treatments, especially for crustose lichens, except for the foliicolous species from its tropical areas (Herrera-Campos et al. 2004b; Lücking 2008).

Currently, an inventory of the crustose lichen biota from different vegetation types in the central and southern portions of Mexico is being undertaken, focusing on tropical rain and seasonally dry forests. An important component of this diversity is *Graphidaceae*, the most speciose family among tropical microlichens (Lücking 2009; Lücking *et al.*

A. Barcenas Peña, R. Miranda-González and M. de los A. Herrera-Campos (corresponding author): Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Apdo. Postal 70-3627, C. P. 04510, México D. F. Email: mahc@ib.unam.mx R. Lücking: Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA.

2009*b*; Rivas Plata *et al.* 2012). Particularly interesting is the genus *Graphis*, with 330 species included in a recent worldwide key (Lücking *et al.* 2008, 2009*b*; Lücking 2009).

Recent molecular studies have produced several taxonomic changes to Graphis s. lat. (Staiger et al. 2006; Mangold et al. 2008; Rivas Plata et al. 2011). We follow the genus concept established by Staiger (2002), Staiger et al. (2006), Lücking (2009), and Lücking et al. (2008, 2009b), characterizing the genus by usually lirelliform, sessile or immersed ascomata with the exciple partially to totally carbonized, hyaline and amyloid (I+ violet), transversely septate to muriform ascospores, as well as the usually corticate, mostly white to greyish thallus. Many species do not have chemical substances, while others produce stictic, norstictic, salazinic, hypostictic, or protocetraric acids, as well as lichexanthone and/or anthraquinones as major compounds. We realize that in the interim, Graphis has been split into two genera (Rivas Plata et al. 2011), but since the correct nomenclature for the second genus has not been clarified, we treat Graphis here in the sense of Staiger (2002) and Lücking (2009).

At the beginning of this project, 54 species of Graphis were known from Mexico: 41 from literature reports (Wirth & Hale 1963; B. D. Ryan, T. H. Nash III & M. A. Herrera-Campos, unpublished data; Staiger & Kalb 2004; Mangold & Lumbsch 2007; Lumbsch et al. 2011) and 13 more represented in MEXU's lichen collection. This figure still represents a low number of species considering the extent of the country's territory and its variety of vegetation types. For example, 115 species have been reported from Costa Rica, a country much smaller than Mexico (Lücking et al. 2008). In the present paper, we describe three new species from tropical rain and dry forest, and provide updates to the world key (Lücking et al. 2009b) for these and an additional 38 species described between 2009 and 2013 (Jagadeesh Ram & Sinha 2009; Jia & Wei 2009; Dal-Forno & Eliasaro 2010; Joshi et al. 2010; Lendemer 2010; Lücking et al. 2010, 2011, 2012; Nakanishi et al. 2010; Chitale et al. 2011; Jia 2011; Lumbsch et al. 2011; Moon et al. 2011, 2012; Neuwirth & Aptroot

2011; Seavey & Seavey 2011; Sharma & Khadilkar 2011; Cáceres *et al.* 2012; Weera-koon *et al.* 2012; Rivas Plata & Lücking 2013).

Materials and Methods

The new species were discovered in Los Tuxtlas Biosphere Reserve, Veracruz and Cuixmala-Chamela Biosphere Reserve, Jalisco. Los Tuxtlas Biosphere Reserve is located in the Sierra de Los Tuxtlas, south of Llanura Costera del Golfo de Mexico (Fig. 1), with an altitudinal range from 200 to 1700 m (Soto & Gama 1997). The climate of the region is classified as warm A and semiwarm A(C), with mean annual temperatures between 22-26°C and a annual average precipitation of 4500 mm (García 1981; Soto & Gama 1997). The dominant vegetation is tropical rainforest (Miranda & Hernández-X 1963; Ibarra-Manríquez et al. 1997). Cuixmala-Chamela Biosphere Reserve is situated on the Pacific coast, north of the Transvolcanic Belt, between sea level and 2400 m (Fig. 1). Its climate is warm sub-humid (Aw_0i), with a mean annual temperature of 24.9°C and a annual average precipitation of 748 mm, 80% of which falls during the summer months (July to November), with the highest values between August and September (García 1981; Bullock 1986; García-Oliva et al. 2002). The main vegetation type corresponds to seasonally dry tropical forest (Miranda & Hernández-X 1963; Durán et al. 2002; García-Oliva et al. 2002).

Collections were made along transects laid for an ecological study (Barcenas Peña *et al.* 2012) and the identification of the specimens followed the keys by Lücking *et al.* (2009*b*) and Staiger (2002). Thallus observations were made with a stereoscope Zeiss Stemi DV4. Microscopic observations were carried out using a compound microscope (Zeiss Axiostar Plus) on thin and semi-thin transverse sections of lirellae and thalli cut with a freezing microtome, Leica CM1850, and mounted in water. Ascospores were stained with Lugol's solution



FIG. 1. Location of study sites. Chamela: Chamela Biological Station in the Cuixmala-Chamela Biosphere Reserve, Jalisco. Los Tuxtlas: Los Tuxtlas Tropical Biological Station in the Los Tuxtlas Biosphere Reserve, Veracruz.

to check the amyloid reaction and measured in water. Secondary metabolites were identified by thin-layer chromatography (TLC) following standard procedures (Culberson & Ammann 1979; Culberson & Johnson 1982; Orange *et al.* 2001); additionally, the usual spot tests (Hale 1979) were performed on the exposed medulla of the thalli. Photographs of thalli were taken using a Macroscope Leica Z16 APO A and Leica Applications Suite. Ascocarp sections and ascospores were photographed with a digital camera Canon PowerShot G6; some sections were observed with a polarized lens and also photographed.

The Species

Graphis bungartzii Barcenas-Peña, Lücking, Herrera-Campos & R. Miranda sp. nov.

MycoBank No.: MB804537

Differing from *Graphis elegans* in the erumpent lirellae with lateral thalline margin and the smaller ascospores, from *G. filiformis* in the labia becoming striate, and from *G. schiffneri* in the laterally carbonized excipulum.

Type: Mexico, Jalisco, Municipio La Huerta, Cuixmala-Chamela Biosphere Reserve, Chamela Biological Station (UNAM), near Hornitos stream, 19°30'56·1"N, 105°02'7·2"W, 59 m, seasonally tropical dry forest, on bark of *Croton* sp., June–July 2011, *Barcenas Peña* 1247 (MEXU—holotype); same locality and date, on bark of *Thouinia* sp., *Barcenas Peña* 1248, 1249 (MEXU paratypes).

(Fig. 2 A-D)

Thallus corticolous, 2-5 cm diam., up to 150 µm thick, continuous; surface smooth, pale greenish grey; prothallus absent. Thallus in section *c*. 30 µm with cartilaginous upper cortex, irregular algal layer, and large clusters of calcium oxalate crystals.

Apothecia lirelliform, flexuose, branched, erumpent, with lateral thalline margin, 1.5-3.5 mm long, 0.1-0.2 mm wide, 0.03-0.05mm high; *disc* concealed; proper margin thin, labia striate, black; thalline margin thick, of the same colour as the thallus. *Excipulum* entire, laterally carbonized, 15–45 µm wide,



FIG. 2. Graphis bungartzii (holotype). A, habit and flexuose, branched, erumpent lirellae; B, concealed disc; striate labia, and thick lateral thalline margin; C, section through a lirella, laterally carbonized excipulum, and not inspersed hymenium; D, mature transversely septate ascospore. Scales: A & B = 1 mm; C = 100 μm; D = 20 μm.

black, basally orange-brown; laterally covered by corticated algiferous thallus including large clusters of calcium oxalate crystals; *hypothecium* prosoplectenchymatous, 20–27 µm high, pale orange-brown; *hymenium* 90–110 µm high, colourless, not inspersed, paraphyses hyaline, unbranched; *epithecium* granulose, $5 \cdot 0 - 7 \cdot 5$ µm high, brown. *Asci* fusiform, $70-90 \times 10-20$ µm. *Ascospores* 8 per ascus, ellipsoid to fusiform, transversely 5– 11-septate, $25-38 \times 5-8$ µm, 3–4 times as long as wide, colourless.

Chemistry (TLC). Norstictic and connorstictic acids (thallus K+ yellow turning red, in sections with yellow outflow with precipitating red needles).

Etymology. This new species is dedicated to Frank Bungartz for his support and encouragement offered to Alejandrina Barcenas-Peña and Ricardo Miranda González, and his interest in Mexican lichens.

Distribution and ecology. The type locality, at an elevation of 59 m, is characterized by a tree mixture of the transition between dry forest and the more humid and taller semideciduous forest at arroyos (Lott et al. 1987). In this locality, three specimens of the new species were found growing on the bark of Croton sp. and Thouinia paucidentata. The additional specimens cited were collected in different sites, between 2 and 13.5 km from the type locality in dry forest at an altitude of 50-300 m. Although morphologically G. bungartzii could be placed in the G. duplicata group, its known ecology contrasts with that of most other species in that group, which have typically been reported from higher elevations (750-1500 m) and from evergreen rainforests with a slight to distinct dry season (Lücking et al. 2008).

Remarks. Currently there is only one other species known with labia becoming striate, a laterally carbonized excipulum, clear hymenium, small, transversely septate ascospores, and the chemical combination of norstictic and connorstictic acids, *viz. Graphis elegans* (Sm.) Ach. (Lücking *et al.* 2009*b*). That species differs from the new one in the erumpent to prominent lirellae lacking a thalline margin and the larger, thick-walled ascospores with 9–13 septa, although it shares the same secondary substances. *Graphis filiformis* Adaw. & Makahija is also similar to *Graphis bungart*zii, but differs in having longer, irregularly to radiately branched lirellae with entire labia, and it lacks connorstictic acid. The eastern paleotropical *Graphis schiffneri* Zahlbr. agrees with the new species in most aspects but has a completely carbonized excipulum, thicker thalline margin, and only norstictic acid as the main secondary substance.

Specimens examined. **Mexico:** Jalisco: Municipio La Huerta, Cuixmala-Chamela Biosphere Reserve, Chamela Biological Station (UNAM), 100 m E from station buildings, between Chachalacas trail and Camino Antiguo Sur dirt road, 19°29′51·1″N, 105°02′30·2″W, 68 m, seasonally tropical dry forest, on bark of *Thouinia paucidentata*, 2009, *Miranda* 1088; 300 m W from Tejón trail at 600 m sign, 19° 30′11·4″N, 105°02′53·8″W, 41 m, seasonally tropical dry forest, on bark of *Thouinia paucidentata*, 2010, *Miranda* 1774; Ejido Gargollo, S of Chamela Biological Station (UNAM), 19°24′17·9″N, 104°58′56·5″W, 57 m, seasonally tropical dry forest, on bark of *Thouinia paucidentata*, 2010, *Miranda* 4266 (all MEXU).

Graphis chamelensis Barcenas-Peña & Lücking sp. nov.

MycoBank No.: MB804538

Differing from *Graphis emersa* in the longer, partly branched lirellae, and from *G. conferta* in the norstictic acid chemistry.

Type: Mexico, Jalisco, Municipio La Huerta, Cuixmala-Chamela Biosphere Reserve, Chamela Biological Station (UNAM), near Hornitos stream, 19°30'52.6"N, 105°02'05.5"W, 59 m, seasonally tropical dry forestsemideciduous arroyo forest transition, on bark of *Forchhammeria pallida*, June–July 2011, *Barcenas Peña* 1239 (MEXU—holotype).

(Fig. 3A-D)

Thallus corticolous, 0.5-1.0 cm diam., 40–80 µm thick, continuous; surface smooth, pale greenish grey; prothallus absent. Thallus in section with thick cartilaginous upper cortex, irregular algal layer, and large clusters of calcium oxalate crystals.

Apothecia lirelliform, flexuose, partly branched, prominent, with basal to lateral thalline margin, 1-3 mm long, 0.2-0.4 mm wide, 0.25-0.30 mm high; *disc* concealed;



FIG. 3. Graphis chamelensis (holotype). A, habit and prominent lirellae; B, concealed disc; labia entire, and basal to lateral thalline margin; C, section through a lirella, entire, completely carbonized excipulum and not inspersed hymenium; D, mature transversely septate ascospore. Scales: A & B = 1 mm; C = 100 μm; D = 20 μm.

proper margin thick, labia entire, black. *Excipulum* entire, completely carbonized, 50–70 μ m wide, black, basal internal parts brown; *hypothecium* prosoplectenchymatous, 20–30 μ m high, colourless to pale yellowish; *hymenium* 80–100 μ m high, colourless, clear, paraphyses unbranched; *epithecium* granulose, 10–25 μ m high, olive-brown. *Asci* fusiform, 70–90 × 15–25 μ m. *Ascospores* 4–8 per ascus, oblong, transversely 7–10-septate, 25–35 × 7–10 μ m, 2–4 times as long as wide, colourless.

Chemistry (TLC). Norstictic acid (thallus K+ yellow turning red, in sections with yellow outflow with precipitating red needles).

Distribution and ecology. The type material of Graphis chamelensis was collected in the same transitional vegetation as G. bungartzii, but on the bark of Forchhammeria pallida. The additional specimens were found growing on Bursera heteresthes and Guettarda ellip*tica* in three different sites in dry forest at 48-95 m, 1-2 km from the type locality. Although *G. chamelensis* was found on a higher number of different phorophytes than *G. bungartzii*, it seems to grow in a more limited area, probably requiring more specific microclimate conditions. Although the species forms part of the *G. nuda* group, its ecological preferences contrast with those of other species in that group, which are more typically found at high altitudes (Lücking *et al.* 2008).

Remarks. Graphis chamelensis is similar to *G. emersa* Müll. Arg., from which it differs mainly by the presence of longer, partly branched prominent lirellae with a sometimes basally developed thalline margin. Both species have the same chemistry (Lücking *et al.* 2009*b*). *Graphis conferta* Zenker is morphologically like *G. chamelensis*; however, it has sessile lirellae without a thalline



FIG. 4. *Graphis rosae-emiliae* (holotype). A, habit and flexuose, unbranched, erumpent lirellae; B, concealed disc; labia striate, lateral thalline margin; C, section through a lirella, completely carbonized excipulum, inspersed hymenium, and calcium oxalate crystals visible with polarized light; D, mature transversely septate ascospore. Scales: A & B = 1 mm; C = 100 μ m; D = 20 μ m.

margin, slightly longer ascospores, and no secondary substances.

Additional specimens examined. **Mexico:** Jalisco: Municipio La Huerta, Cuixmala-Chamela Biosphere Reserve, Chamela Biological Station (UNAM), near Tejón trail, 19°30'25.6"N, 105°02'32.8"W, 48 m, seasonally tropical dry forest, on bark of Forchhammeria pallida, 2011, Barcenas Peña 2000; 19°30'29.3"N, 105°02'29.8"W, 83 m, on bark of Bursera heteresthes, Barcenas Peña 2001; 19°30'34.4"N, 105°02'21"W, 95 m, on bark of Guettarda elliptica, Barcenas Peña 2002 (all MEXU).

Graphis rosae-emiliae Barcenas-Peña & Lücking sp. nov.

MycoBank No.: MB804539

Differing from *Graphis syzygii* in the larger ascospores and the lack of secondary substances, and from *G. aurita* in the inspersed hymenium and larger ascospores.

Type: Mexico, Veracruz, Municipio San Andrés Tuxtla, Los Tuxtlas Biosphere Reserve, Los Tuxtlas Tropical Biology Station, near Laguna Zacatal, 18°35'07·1"N, 95°05'23·4"W, 242 m, lowland rainforest, on bark of *Orthion oblanceolatum*, May 2011, *Barcenas Peña* 1235 (MEXU—holotype); same locality and date, on bark of unidentified phorophyte, *Barcenas Peña* 1254 (MEXU—paratype).

(Fig. 4A-D)

Thallus corticate, 1-3 cm diam., 120-180 µm thick, continuous; surface smooth, greenish grey; prothallus absent. Thallus in section with cartilaginous upper cortex, irregular algal layer, and large clusters of calcium oxalate crystals.

Apothecia lirelliform, flexuose, unbranched, erumpent, with lateral thalline margin, 1–3 mm long, 0.25 mm wide, 0.1 mm high; *disc* concealed; proper margin thick, labia striate, black. *Excipulum* completely carbonized, 70– 90 μ m wide, black; *hypothecium* prosoplectenchymatous, 20–25 μ m high, colourless; *inspersed* (type A according to Lücking 2009), paraphyses unbranched; *epithecium* granulose, 10–15 μ m high, colourless to pale yellowish. *Asci* fusiform, 70–90 × 10–20 μ m. Ascospores 8 per ascus, oblong, transversely 9-13-septate, $45-55 \times 5-10 \ \mu\text{m}$, $5-9 \ \text{times}$ as long as wide, colourless.

Chemistry (TLC). No substances detected (all spot tests negative).

Distribution and ecology. The type locality is characterized by an exuberant tropical rainforest with high humidity, at an elevation of 242 m along the east side of San Martín Tuxtla volcano. The type material of *G. rosaeemiliae* was found on the bark of canopy branches of *Orthion oblanceolatum*. The additional specimen was also found in the canopy, in a different locality and on a different date, 21 km from the type locality, at 345 m, on the northern slope of the Santa Marta Volcano.

Remarks. Graphis rosae-emiliae is characterized by its unbranched, erumpent lirellae with striate labia and lateral thalline margin, the completely carbonized excipulum, and the inspersed hymenium (type A), as well as transversely septate ascospores and lack of secondary metabolites. Other species with a similar lirella type, inspersed hymenium, and transversely septate ascospores are G. leucaenae Aptroot, G. inspersolongula Aptroot, and G. syzygii Aptroot (Lücking et al. 2009b). Both G. leucaenae and G. inspersolongula have much larger ascospores and produce norstictic acid. Graphis syzygii Aptroot differs in its smaller ascospores and the presence of stictic acid. Finally, G. cinerea Fée shares the same chemistry as G. rosae-emiliae but it has very large ascospores (100- $200 \times 18-30$ µm) and a different type of inspersion, among other morphological differences. Morphologically and chemically similar is G. aurita Eschw., but it lacks inspersion and its ascospores are smaller.

Specimen examined. **Mexico:** Veracruz: Municipio Catemaco, Los Tuxtlas Biosphere Reserve, Selva del Marinero, Ejido Adolfo López Mateos, 18°26'0.5"N, 94°57'44.1"W, 345 m, lowland rainforest, on bark of unidentified phorophyte, 2009, *Barcenas Peña* 1170 (MEXU).

Updates to the world key to species of Graphis

The publication by Lücking et al. (2009b) of a first global working key to the genus Graphis, as redefined by Staiger (2002) and Lücking (2009), has boosted the discovery and description of new species. Including the three species introduced here, no less than 41 species have been described or reinstated in the four years following publication of the key, representing an increase of 12.4% over the 330 species treated in the key. Of these, six are from North America (Lendemer 2010; Lücking et al. 2011; Seavey & Seavey 2011), four from Mexico (Lumbsch et al. 2011; this paper), one from Central America (Costa Rica: Lücking et al. 2012), nine from South America (Peru: Rivas Plata & Lücking 2013; Brazil: Dal-Forno & Eliasaro 2010; Lumbsch et al. 2011; Cáceres et al. 2012), three from Europe (Neuwirth & Aptroot 2011), 16 from Asia (India: Jagadeesh Ram & Sinha 2009; Chitale et al. 2011; Sharma & Khadilkar 2011; Sri Lanka: Weerakoon et al. 2012; Cambodia: Nakanishi et al. 2010; Moon et al. 2011; South Korea: Joshi et al. 2010; Moon et al. 2012; China: Jia & Wei 2009; Jia 2011; Indonesia: see key entries below), one from Fiji (Lücking et al. 2010), and one pantropical species (this paper: see key entries below). We expect that this trend will continue in the near future; for the Graphis scripta group alone, Lücking (2012) predicted nearly 50 missing species based on the data from the published key. Some nomenclatural and taxonomic changes were also proposed, the scope of which are summarized below.

Since the publication of a complete, updated key would go beyond this paper and would also be futile in the light of possibly many more species being described in the near future, for each of the newly established species or nomenclatural or taxonomic changes, we provide a detailed entry based on the key published previously (Lücking *et al.* 2009*b*). For the moment, we apply the concept of *Graphis sensu* Staiger (2002) and Lücking (2009), although it has been shown that this encompasses two genera (Rivas Plata *et al.* 2011). However, at present the second genus, which has tentatively been named *Allographa* (Rivas Plata *et al.*

2011), cannot be named formally until the type species of *Hemithecium* has been sequenced. The reason for this is that the name *Allographa* is invalid and must be validated, but the type species of *Hemithecium* potentially belongs in that clade, providing a valid name that would then have priority.

One species, *Graphis parvicarpa* B. O. Sharma & Khadilkar (Sharma & Khadilkar 2011), turned out to belong in the genus *Pallidogramme*. The apical carbonization, as shown in the excellent illustrations in the protologue, is not part of the excipulum but of the epithecium. The type of hymenial inspersion agrees with *Pallidogramme*, and nearly hyaline ascospores are often found in that genus. We therefore propose the following combination:

Pallidogramme parvicarpum (B. O. Sharma & Khadilkar) Lücking comb. nov.

MycoBank No.: MB804862

Graphis parvicarpa B. O. Sharma & Khadilkar, Lichenologist 43: 487 (2011).

Names that were validated or changed in nomenclature or taxonomic concept are:

Graphis arbusculaeformis (Vain.) Lücking (Lücking et al. 2012); validated.

- Graphis betulina (Pers.) Ach. (Neuwirth & Aptroot 2011); replaces G. scripta (L.) Ach. auct. p.p.
- Graphis caribica Lücking (Lumbsch et al. 2011); validated.
- Graphis inustuloides Lücking (Lücking & McCune 2012); replaces G. britannica sensu Staiger auct.
- Graphis macrocarpa (Pers.) Röhl. (Neuwirth & Aptroot 2011); replaces G. scripta (L.) Ach. auct. p.p.
- *Graphis norvestitoides* Sutjaritturakan (Lücking *et al.* 2012); validated and taxonomy corrected (see key below, Group 18, couplet 3 and Group 20, couplet 1).
- Graphis pedunculata Bungartz & Aptroot (Bungartz et al. 2010); validated.
- Graphis pulverulenta (Pers.) Ach. (Neuwirth & Aptroot 2011); replaces G. scripta (L.) Ach. auct. p.p.
- Graphis rongklaensis Sutjaritturakan (Lücking et al. 2012); validated.

The following are updates to the key entries in the world key to *Graphis* (Lücking *et al.* 2009*b*), for 41 newly described or reinstated species from 2009 to 2013:

Group 4, couplet 5: Lirellae prominent to sessile, with thick lateral thalline margin, very short

Group 4, o	couplet 35: Disc exposed, non-pruinose; lirellae with rounded ends; Europe Graphis macrocarpa (Pers.) Röhl. (Neuwirth & Aptroot 2011)
Group 4, c	couplet 35: Disc exposed, pruinose or weakly pruinose; lirellae with acute ends and conspicuously thick lateral thalline margin; Europe Graphis betulina (Pers.) Ach. (Neuwirth & Aptroot 2011)
Group 4, c	couplet 35: Disc exposed, pruinose; lirellae with acute ends and thin lateral thalline margin; Europe
	Graphis pulverulenta (Pers.) Ach. (Neuwirth & Aptroot 2011)
Group 4, c	couplet 41: Graphis scripta (L.) Ach. s. str. would key out under G. pinicola Zahlbr. according to the characters given by Neuwirth & Aptroot (2011); possibly the collections of G. pinicola from subtropical regions (with lateral thalline margin) represent G. scripta s. str., whereas tropical material (with apically thin complete thalline margin) would have to be named G. guineensis C. W. Dodge; this issue needs further study.
Group 6, c	couplet 12: Disc exposed, non-pruinose; South Korea
Group 6,	couplet 12: Disc concealed; labia non-pruinose; lirellae immersed in distinct, raised, pseudostromatic areas; USA
Group 7,	couplet 6: Ascospores $70-100 \times 15-20 \mu m$; lirellae erumpent, with thick lateral thalline margin (<i>subserpentina</i> morph); Fiji Graphis collinsiae Lücking & Lumbsch (Lücking <i>et al.</i> 2010)
Group 8, c	couplet 7: Lirellae prominent to sessile, with thick lateral thalline margin, very short and unbranched (<i>dussii</i> -morph); norstictic and salazinic acids; China
Group 8,	couplet 10: Lirellae prominent, with basal thalline margin, irregularly branched; ascospores 35–65 μm long; USA
Group 8, 6	couplet 15: Lirellae erumpent to prominent, lacking or with basal thalline margin; short and sparsely branched; thallus distinctly verrucose to almost isidiate; China Graphis guangdongensis Z. F. Jia & J. C. Wei (Jia & Wei 2009)
Group 8, o	couplet 17: Lirellae erumpent; thallus verrucose; Brazil
Group 8, o	couplet 18: Lirellae stellately branched and clustered into pseudostromata; thallus isidiate; Brazil
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Group 8, couplet 20: Thallus green; lirellae 1–5 mm long, lacking basal margin, with grey- black labia: Mexico
Group 8, couplet 29: Lichexanthone; disc exposed (<i>handelii</i> morph); South Korea Graphis flavopalmicola Y. Joshi et al. (Joshi et al. 2010)
Group 8, couplet 36: Lirellae prominent, with basal to lateral thalline margin, elongate and irregularly branched; Mexico
Group 8, couplet 42: Lirellae elongate to very long, prominent; thallus sorediate; Brazil Graphis lueckingii Dal-Forno & Eliasaro (Dal-Forno & Eliasaro 2010)
Group 8, couplet 51: Lirellae irregularly branched, with apically thin complete margin; asco- spores regularly 3-septate, 15–20 × 5–6 μm; Peru Graphis pitmanii Rivas Plata & Lücking (Rivas Plata & Lücking 2013)
Group 8, couplet 51: Lirellae irregularly branched but not radiate, with very thin complete margin; ascospores 7–11-septate, 35–55 × 10–12 μm; Brazil
Group 10, couplet 6: Lirellae prominent, with thick lateral thalline margin (<i>marginata</i> morph); ascospores 50–70 μm long; Sri Lanka
Group 10, couplet 13: Lirellae prominent, with basal to lateral thalline margin and exposed disc; Peru Graphis apertoinspersa Rivas Plata & Lücking (Rivas Plata & Lücking 2013)
Group 13, couplet 1: 2-methoxy-psoromic acid; thallus sorediate; ascospores 30–35 × 7– 8 μm; Cambodia Graphis taneina M. Nakan. <i>et al.</i> (Moon <i>et al.</i> 2011)
 Group 13, couplet 7: Ascospores medium-sized to large, 50–95 × 7–9 μm, 8 per ascus; labia non-pruinose; lirellae erumpent to prominent, lacking or with basal thalline margin, very long and irregularly to radiately branched; India
Group 13, couplets 8 and 9: Thallus distinctly verrucose but not isidiate; lirellae with verrucose lateral thalline margin; Sri Lanka
Group 13, couplet 14: Thallus white-grey; lirellae erumpent, labia non-pruinose; ascospores 40–60 μm long; USA
Group 13, couplet 20: Lirellae with lateral thalline margin; thallus white-grey; pantropical
Group 13, couplet 20: Lirellae with basal thalline margin; thallus green-grey; Indonesia

- Group 14, couplet 6: Ascospores medium-sized, 45–65 × 12–16 µm; lirellae erumpent, with lateral thalline margin, elongate and irregularly branched (*tenella* morph); India . . .
 Graphis panhalensis (Patw. & C. R. Kulk.) Chitale *et al.* (Chitale *et al.* 2011) [This species is close to *G. parilis* Kremp. but appears to differ by the narrower ascospores, a feature that needs to be evaluated with more material]
- Group 15, couplet 5: Ascospores small, $25-38 \times 5-8 \mu m$, 5-11-septate, not conspicuously thick-walled; lirellae erumpent, with lateral thalline margin; Mexico Graphis bungartzii Barcenas-Peña *et al.* (this paper)
- Group 16, couplet 1: Norstictic acid; lirellae prominent, with complete thalline margin (*acharii* morph); ascospores 70–95 × 25–35 μm; Cambodia..... Graphis cambodiensis M. Nakan. *et al.* (Nakanishi *et al.* 2010)
- Group 17, couplet 7: Ascospores very large (120–150 μm long); lirellae very short to rounded, prominent, with complete thalline margin; Brazil Graphis brachylirellata M. Cáceres & Lücking (Cáceres *et al.* 2012)
- Group 17, couplet 11: Lirellae erumpent, lacking or with basal thalline margin; Sri Lanka. Graphis dotalugensis Weerakoon *et al.* (Weerakoon *et al.* 2012)
- Group 18, couplet 3: *Graphis norvestitoides* Sutjaritturakan is incorrectly keyed out here since it has an inspersed hymenium (see below, Group 20, couplet 1); the correct species to be keyed out here is; Costa Rica..... Graphis pseudoaquilonia Lücking (Lücking *et al.* 2012)

- Group 19, couplet 2: Stictic acid; ascospores 80–100 × 15–20 μm; lirellae prominent, with complete thalline margin; Brazil Graphis cerradensis Marcelli *et al.* (Lumbsch *et al.* 2011)
- Group 20, couplet 1: Ascospores terminally muriform; norstictic acid; ascospores 80– 100 × 15–20 μm; Thailand Graphis norvestitoides Sutjaritturakan (Lücking *et al.* 2012)
- Group 20, couplet 2: Thallus ecorticate; labia pruinose; Sri Lanka Graphis knucklensis Weerakoon *et al.* (Weerakoon *et al.* 2012)

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