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New Graphidaceae from South and Central Brazil

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Abstract: Nine species of Graphidaceae are described as new to science from South and Central Brazil, in 7 different genera: *Acanthothecis normuralis, A. psoromica, Acanthotrema minus, Aggregatorygma submuriforme, Allographa medioinspersa, Diorygma isidiolichexanthonicum, Fissurina excavatisorediosa, Graphis norsorediata, and Graphis tricolor.*

Introduction

Graphidaceae is the second largest family of lichenized fungi, with 2161 species, after Parmeliaceae, with 2765 species (Lücking et al. 2017). Collecting in Brazil started in the 1820s, during expeditions by Martius (Martius 1827). However, the first records of Graphidaceae from South and Central Brazil were published by Müller Argoviensis (1895) and Redinger (1933), respectively, from the states of Santa Catarina, Mato Grosso, Mato Grosso do Sul, and Rio Grande do Sul. Since then, references to the family in these regions can been found in regional surveys, species listings, and new species publications, and ecological papers (e.g. Redinger 1934, 1935; Osorio & Fleig 1982; Kalb 1983; Osorio 1985; Spielmann 2006; Dal-Forno & Eliasaro 2010; Käffer et al. 2014, 2015; Martins & Marcelli 2011; Feuerstein & Eliasaro 2015; Feuerstein et al. 2016; Koch et al. 2016; Menezes et al. 2018). In this paper we provide descriptions of new species found during field expeditions in South and Central Brazil. The collections were made in three different biomes: Cerrado, Pantanal and Atlantic forest.

Material and methods

Specimens were collected by the first author, mostly during the yearly botanical excursion from UFMS, using knife, examined by $10\times$ hand lens (Leuchtlupe with UV) and air-dried. Specimens were often selected in the field as representative of a known species or a characteristic morphology; in addition, a selection of species that cannot be recognized in the field was collected. All specimens are preserved in herbarium CGMS, with some duplicates in ABL.

Specimens were observed with an Olympus SZX7 and pictures taken with Nikon Coolpix 995. Hand-made sections of ascomata and thallus were studied in water, 5% KOH (K) and/or Lugol's reagent (1% I₂) after pre-treatment with KOH (IKI). Microscopic photographs were prepared using an Olympus BX50 with Nomarski interference contrast and Nikon Coolpix 995. Chemical spot reactions are abbreviated as K (5% KOH), C (commercial bleach), KC (K followed by C), P (paraphenylenediamine), and UV refers to fluorescence at 366 nm. Thin-layer chromatography (Orange et al. 2001) has been undertaken by A. Aptroot in solvent A.

New species

Acanthothecis normuralis Aptroot, sp. nov.

Fig. 1A

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Corticolous *Acanthothecis* with thallus with norstictic acid, hamathecium not inspersed, ascospores hyaline, densely muriform, 2/ascus, $40-55 \times 17-25 \mu m$.

TYPE: BRAZIL. MATO GROSSO DO SUL: Serra da Bodoquena, Bonito, Parque Nacional da Serra da Bodoquena, Rio Perdido, alt. 510 m, 21°07′S, 56°43′W, on tree bark in Atlantic rain forest, 3 November 2018, A.Aptroot 77578 (holotype: CGMS; isotype: ABL); Bonito, Fazenda La Harmonia, alt. 460 m, 21°15′S, 56°43′W, on tree bark in Atlantic rain forest, 1 November 2018, A.Aptroot 77344 (paratype: CGMS).

Description. Thallus corticolous, continuous, corticate, dull, pale ochraceous, up to 0.1 mm thick, without prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata erumpent, elongate, rather regularly branched, 0.5–2.5 mm long, c. 0.2 mm wide, c. 0.1 mm high; disc brownish, white pruinose; margin of thallus color. Hamathecium not inspersed. Paraphyse tips spinulose. Ascospores 2/ascus, hyaline, densely muriform, 40–55 × 17–25 μ m, IKI–. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K+ yellow>red. TLC: norstictic acid.

Etymology. Named after the cortical secondary compound norstictic acid.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. The genus *Acanthothecis* comprises 63 species (Feuerstein et al. 2020). Few species contain norstictic acid, and not one of them has densely muriform ascospores of around 50 µm.

Acanthothecis psoromica Aptroot & Feuerstein, sp. nov.

Fig. 1B, 2H

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Corticolous *Acanthothecis* with thallus with psoromic acid, hamathecium not inspersed, ascospores hyaline, muriform, 2-4/ascus, $75-85 \times 20-25 \mu m$.

TYPE: BRAZIL. MATO GROSSO DO SUL: Campo Grande, Campus UFMS, alt. 550 m, 20°30′S, 54°37′W, on tree bark in cerradinho forest, 23 October 2019, A.Aptroot 80435 (holotype: CGMS).

Description. Thallus corticolous, continuous, corticate, dull, pale mineral gray, up to 0.1 mm thick, surrounded by a thin gray prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata erumpent, elongate, regularly branched one time, 0.5–2.5 mm long, c. 0.4 mm wide, c. 0.1 mm high; almost white at the slit, disc concealed; margin of thallus color. Hamathecium not inspersed. Paraphyse tips spinulose. Ascospores 2–4/ascus, hyaline, muriform, 75–85 \times 20–25 μm , IKI–. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P+ yellow, K-. TLC: psoromic acid.

Etymology. Named after the cortical secondary compound psoromic acid.

Ecology and distribution. On tree bark in cerrado forest; only known from Brazil.

Discussion. The genus *Acanthothecis* comprises 63 species (Feuerstein et al. 2020). Few species contain psoromic acid, and not one of them has densely muriform ascospores of around 80 μm.

Acanthotrema minus Aptroot, sp. nov.

Fig. 1C

MYCOBANK MB 836300

Corticolous *Acanthotrema* with thallus without secondary substances, whitish gray, not corticate, apothecia irregular in outline, disc exposed, white pruinose, hamathecium not inspersed, paraphyses tips spinulose, periphysoids copious, spinulose over their whole length , ascospores 8/ascus, 7-septate, $16-18 \times 5-5.5 \mu m$, surrounded by a c. 3 μm thick gelatinous sheath.

TYPE: BRAZIL. MATO GROSSO DO SUL: Pantanal 43 km SE of Corumbá, area 39, alt. 85 m, 19°32′S, 57°24′W, on tree bark, 2 March 2019, A.Aptroot 78104 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, not corticate, dull, whitish gray, up to 0.1 mm thick, surrounded by a thin black prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata immersed to erumpent, solitary, irregular in outline, 0.2–0.4 mm diam.; disc pale, white pruinose, margin brown, irregularly dentate, incurved, c. 0.05 mm wide. Hamathecium not inspersed. Paraphyse tips spinulose. Periphysoids copious, spinulose over their whole length. Ascospores 8/ascus, hyaline, 7-septate, $16-18\times5-5.5~\mu m$, surrounded by a c. 1 μm wide gelatinous sheath. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K-. TLC: no secondary substances.

Etymology. Named after the small size.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. In the genus *Acanthotrema*, currently 5 species are known, usually rather large thalli with green color and long isidia. This species differs by the very small size and the whitish, ecorticate, thallus. It probably only shares the key characters for the genus with the type of the genus, not its parentage.

Aggregatorygma submuriforme Aptroot, sp. nov.

Fig. 1D-E

MYCOBANK MB 836301

Corticolous Aggregatorygma with hamathecium not inspersed, hypothecium pale brown, ascospores submuriform, 3×0 –1-septate, 12– 13.5×6.5 –7 µm, with 2 µm wide gelatinous sheath.

TYPE: BRAZIL. MATO GROSSO DO SUL: Serra da Bodoquena, Fazenda Monte Negro, alt. 530 m, 20°54′25″S, 56°48′04″W, on tree bark in Atlantic rain forest, 8 November 2018, A.Aptroot 77881 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, not corticate, dull, pulverulent, pale greenish white, up to 0.3 mm thick, surrounded by a thin black prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata remaining immersed, elongate, rather irregularly branched, in groups, 0.5–2.0 mm long, 0.2–0.3 mm wide; disc gray, not pruinose. Hamathecium not inspersed, hypothecium pale brown. Paraphyse tips not spinulose. Ascospores 8/ascus, hyaline, submurifom, 3×0 –1-septate, 12– 13.5×6.5 –7 μm , surrounded by a 2 μm wide gelatinous sheath. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K-. TLC: zeorin and 2 fatty acids.

Etymology. Named after the submuriform ascospores.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. Morphologically this species resembles the type and only species of *Aggregatorygma*, *A. triseptatum* M. Cáceres et al. (Cáceres et al. 2014). We sequenced ITS of the new species. It clusters inside the Graphidaceae, but not close to any species. The type of *Aggregatorygma* has also been sequenced, but different gene regions were sequenced. Therefore the generic position of the new species was not confirmed by sequence.

Allographa medioinspersa Feuerstein & Aptroot, sp. nov.

Fig. 1F, 2A, 2G

MYCOBANK MB 836302

Corticolous *Allographa* with thallus mineral gray, without secondary substances, apothecia higher than wide, striate, with complete carbonization, with apically thin complete thalline margin, hamathecium inspersed in upper half, ascospores muriform, 2/ascus, $21-25 \times 1-2$ -septate, $75-87 \times 11-15$ µm, with terminal gelatinous caps of c 4 µm thick.

TYPE: BRAZIL. RIO GRANDE DO SUL: Agudo, Cascata Raddatz, alt. 250 m, 29°35′12″S, 53°10′49″W, on tree bark in Atlantic rain forest, 15 July 2019, A.Aptroot 79191 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, corticate, somewhat shiny, mineral gray, up to 0.1 mm thick, surrounded by a c. 2 mm wide, white, glossy prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata sessile, higher than wide, striate, elongate, rather regularly branched, distinctly wavy in outline, 2.2–12 mm long, c. 0.5 mm wide, c. 0.7 mm high, apically

thin complete thalline margin; disc concealed. Excipulum completely carbonized. Hamathecium inspersed in upper half. Paraphyse tips not spinulose. Ascospores 2/ascus, hyaline, muriform, 21– 25×1 –2-septate, 75–87 \times 11–15 μm , IKI+ blue, with terminal gelatinous caps of c. 4 μm thick. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K-. TLC: no secondary substances.

Etymology. Named after the inspersion that is only present in the upper half of the hamathecium.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. Only one other species of *Allographa* is known to us to have a distinct inspersion only in the upper half of the hamathecium, viz. *A. suprainspersa* Feuerstein & Lücking ined.

Diorygma isidiolichexanthonicum Aptroot, sp. nov.

Fig. 2B

MYCOBANK MB 836303

Corticolous *Diorygma* with thallus isidiate, UV+ yellow, with lichexanthone, hypothallus dense, black.

TYPE: BRAZIL. SANTA CATARINA: Joinville, Recanto das Nascentes Divina, alt. 225 m, 26°12′43″S, 49°00′11″W, on tree bark in Atlantic rain forest, 29 March 2019, A.Aptroot 78472 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, not corticate, dull, pale ochraceous, up to 0.2~mm thick, surrounded by a thin black prothallus. Hypothallus dense, black, brittle, c. 0.1~mm thick. Soredia absent. Isidia numerous, covering most of the central part of the thallus, cylindrical, simple to irregularly branched, 0.1-0.7~mm high, c. 0.1~mm wide. Photobiont trentepohlioid. Ascomata and pycnidia not observed.

Chemistry. Thallus and apothecia UV+ yellow, C-, P-, K-. TLC: lichexanthone.

Etymology. Named after the isidia and the lichexanthone.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil. Already observed in other states, but specimens not reported here.

Discussion. There are only a two *Diorygma* species with isidia, viz. *D. antillarum* (Vainio) Nelsen et al. and *D. australasicum* (Elix) Lücking et al. Although we have not observed fruiting bodies, the morphology of this species is very similar to the first of these species, which is the only other whitish crust with a dense black hypothallus. We are confident that our new species is a *Diorygma*, also because relatively many sterile crusts that we sequenced turn out to be *Diorygma* species (unpublished information).

Fissurina excavatisorediosa Aptroot, sp. nov.

Fig. 2C

MYCOBANK MB 836304

Corticolous *Fissurina* with thallus with excavate soredia, apothecia of dumastii-type, hamathecium not inspersed, ascospores 3-7-septate, $10-13 \times 5 \mu m$.

TYPE: BRAZIL. MATO GROSSO DO SUL: Serra da Bodoquena, Bonito, Parque Nacional da Serra da Bodoquena, Rio Perdido, alt. 510 m, 21°07′S, 56°43′W, on tree bark in Atlantic rain forest, 3 November 2018, A.Aptroot 77502 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, not corticate, dull, pale ochraceous, immersed in the bark except for the soralia, surrounded by a thin black prothallus. Soredia granular, whitish, in regular, rounded, often excavate soralia of 0.2-0.5 mm diam. Isidia absent. Photobiont trentepohlioid. Ascomata in small groups, erumpent, elongate to strongly lobate, regularly branched, 0.3-1.0 mm long, c. 0.4 mm wide, c. 0.1 mm high; disc exposed, pink to gray; margin incurved, much higher than the disc, of thallus color. Hamathecium not inspersed. Paraphyse tips not spinulose. Ascospores 8/ascus, hyaline, 3-7-septate, $10-13 \times 5$ µm. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P+ yellow, K-. TLC: psoromic acid.

Etymology. Named after the excavate soralia.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. *Fissurina* is a genus with c. 145 described species. There are no other *Fissurina* species known with soredia, and psoromic acid is a rare substance in the group.

Graphis norsorediata Aptroot, sp. nov.

Fig. 2D

MYCOBANK MB 836305

Corticolous *Graphis* with thallus sorediate, with norstictic acid, apothecia not striate, with apical carbonization, hamathecium not inspersed, ascospores 11-13-septate, $50-60 \times 9-11 \mu m$.

TYPE: BRAZIL. MATO GROSSO DO SUL: Serra da Bodoquena, Fazenda Monte Negro, alt. 530 m, 20°54′25″S, 56°48′04″W, on tree bark in Atlantic rain forest, 8 November 2018, A.Aptroot 77892 (holotype: CGMS; isotype: ABL); Same details, 77963 (paratype CGMS).

Description. Thallus corticolous, continuous, not corticate, dull, very pale ochraceous white, up to 0.1 mm thick, surrounded by a thin black prothallus. Soredia farinose, whitish, initially originating in regular, rounded soralia of 0.2–0.5 mm diam., but soon covering much of the thallus. Isidia absent. Photobiont trentepohlioid. Ascomata solitary, not striate, immersed to erumpent, elongate, rather wavy, regularly branched, 0.3–5.0 mm long, c. 0.3 mm wide, up to c. 0.1 mm high; disc black, white pruinose; labia black, mostly concealed by thalline margin; margin of thallus color and structure, including soredia. Excipulum apically carbonized. Hamathecium not inspersed. Paraphyse tips not spinulose. Ascospores 8/ascus, hyaline, 11–13-septate, $50–60\times9–11~\mu\text{m}$, IKI+blue, without gelatinous sheath. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K+ yellow>red. TLC: norstictic acid.

Etymology. Named after the cortical secondary compound norstictic acid and the presence of soredia.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. There is only one other *Graphis* species known with soredia, viz. *G. sorediosa* Nagarkar & Patw. (Lücking et al. 2009). It differs e.g. by striate apothecia with lateral carbonization, stictic acid and very long, branched apothecia.

Graphis tricolor Aptroot & Feuerstein, sp. nov.

Fig. 2E-F

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Corticolous *Graphis* with thallus without secondary substances, apothecia erumpent, striate, with lateral carbonization, disc exposed, white pruinose, hamathecium not inspersed, ascospores 7–9-septate, $29–38\times6-7.5~\mu m$.

TYPE: BRAZIL. MATO GROSSO DO SUL: Pantanal 43 km SE of Corumbá, area 39, alt. 85 m, 19°32′S, 57°24′W, on tree bark, 2 March 2019, A.Aptroot 78159 (holotype: CGMS; isotype: ABL).

Description. Thallus corticolous, continuous, corticate, weakly shiny, pale ochraceous, up to 0.2 mm thick, surrounded by a thin black prothallus. Soredia and isidia absent. Photobiont trentepohlioid. Ascomata densely covering the thallus, erumpent, striate, elongate, rather wavy, regularly branched, 0.6–3.0 mm long, c. 0.4 mm wide, up to c. 0.2 mm high; disc black, strongly concave, white pruinose, often separated from the labia by a slit; labia black, usually fully concealed by thalline margin; margin distinctly of a paler color than the thallus, almost white, dull, indistinctly corticate. Excipulum laterally carbonized. Hamathecium not inspersed. Paraphyse tips not spinulose. Ascospores 8/ascus, hyaline, 7–9-septate, 29–38 \times 6–7.5 μ m, IKI+ blue, surrounded by a c. 4 μ m wide gelatinous sheath. Pycnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K+ yellow>red. TLC: norstictic acid.

Etymology. Named after the three colors that alternate, the white from the disc pruina, the bluish gray from the labia and the off-white thallus.

Ecology and distribution. On tree bark in Atlantic rain forest; only known from Brazil.

Discussion. This species would key out in Group 4, couplet 35 in Lücking et al. (2009), at the *Graphis scripta* aggregate. However, it differs from all species in this group by the much smaller ascospores.

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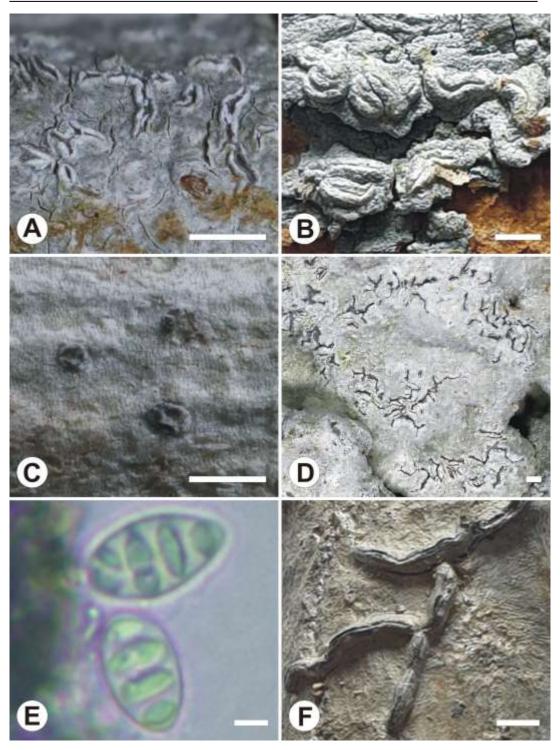


Fig. 1. (A) Acanthothecis normuralis, (B) A. psoromica, (C) Acanthotrema minus, (D–E) Aggregatorygma submuriforme, E, ascospore, (F) Allographa medioinspersa. Scales: 1 mm, except in E = 3 μ m.

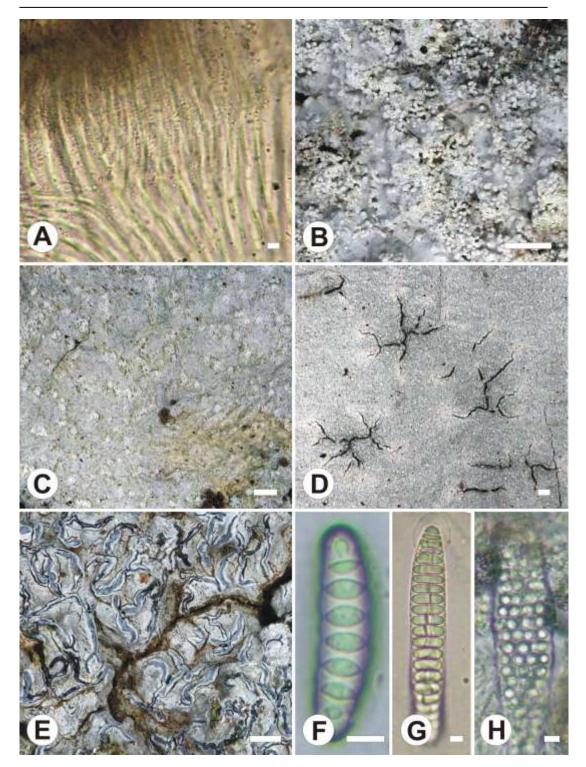


Fig. 2. (A) Allographa medioinspersa, hymenium, (B) Diorygma isidiolichexanthonicum, (C) Fissurina excavatisorediosa, (D) Graphis norsorediata, (E–F) Graphis tricolor, F, ascospore (G) Allographa medioinspersa, ascospore, (H) Acanthothecis psoromica, ascospore. Scales: 1 mm, except in $A=3~\mu m$ and $F-H=5~\mu m$.

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