# Eight new species of *Pyrenulaceae* from the Neotropics, with a key to 3-septate *Pyrgillus* species

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Abstract: Eight new species of Pyrenulaceae are described as new to science from Brazil, Guyana and Puerto Rico. Pyrenula sanguineomeandrata Aptroot & Mercado Diaz (with a thallus with red, KOH+ purple pigmentation of lines or a reticulum, simple ascomata with vertical ostioles, a deep red inspersed, KOH+ orange hamathecium, and dark brown 3-septate ascospores 25-29 × 10-12 µm) and P. sanguineostiolata Aptroot & Mercado Diaz (with a thallus with deeply immersed simple ascomata with vertical ostioles, which are superficial and bright red, and 3-septate ascospores 25-28 × 9-12 μm) are described from submontane evergreen forests in Puerto Rico. Pyrenula biseptata Aptroot & M. Cáceres (with simple ascomata with vertical ostioles, an inspersed hamathecium and 2-septate ascospores 11–12 × 4·5–5·0 μm) and P. xanthinspersa Aptroot & M. Cáceres (with an ecorticate thallus containing lichexanthone, simple ascomata with vertical ostioles, not inspersed hamathecium and 3-septate ascospores 14–17 × 6·0–7·5 μm) are described from rainforest in Amazonian Brazil. Pyrenula subvariabilis Aptroot & Sipman (with fused ascomata with lateral ostioles and submuriform ascospores 17-20(-25) × 6-9 µm) and Sulcopyrenula biseriata Aptroot & Sipman (with a thallus containing lichexanthone, simple ascomata with lateral ostioles and lozenge-shaped ascospores with 8 locules,  $(13-)15-17(-20) \times 8-10$  (width)  $\times 6-7$  (thickness)  $\mu$ m) are described from savannahs in Guyana. Special attention is paid to the genus Pyrgillus: two new species from the 3-septate core group of this small genus are described from Brazil, viz. P. aurantiacus Aptroot & M. Cáceres (with a corticate thallus containing lichexanthone, mazaedium with orange, KOH+ violet, UV+ red pruina and ascospores of 13-16×6·0-7·5 μm) and P. rufus Aptroot & M. Cáceres (with a corticate thallus containing lichexanthone, mazaedium with dark red, KOH+ orange, UV+ red pruina and ascospores of  $15.0-17.5 \times 5.0-6.5 \mu m$ ). An updated key to the 3-septate species of *Pyrgillus* is provided.

Key words: Brazil, Guyana, lichen, Puerto Rico, Pyrenula, Sulcopyrenula

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#### Introduction

The taxonomy of tropical lichens is still incompletely known, but in recent years there has been much progress and a few attempts have even been made to predict the number of species in some predominantly tropical lichen families, based on a detailed analysis of the known species distribution. So far, this has

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been done for the Graphidaceae (Lücking et al. 2014) and the Trypetheliaceae (Aptroot et al. 2016). In both instances, a complete data matrix was published online with known accepted species and distributions across the globe. For the Graphidaceae this is still the only existing complete enumeration of currently accepted species; for the Trypetheliaceae it is in accordance with the revision published simultaneously (Aptroot & Lücking 2016). For the equally predominantly tropical family of the Pyrenulaceae, a similar paper is in preparation (C. O. Mendonça, A. Aptroot, R. Lücking & M. E. S. Cáceres, unpublished data). Several undescribed species are mentioned there and this provided a good incentive to formally describe them.

The family Pyrenulaceae is a mostly tropical family comprising 11 genera, including Pyrenula Ach., Pyrgillus Nyl. and Sulcopyrenula H. Harada (Gueidan et al. 2016). In a recent key to the species of the genus Pyrenula, Aptroot (2012) accepted 169 species out of the c. 745 named taxa in the genus. Today the genus has c. 225 accepted species, including the 169 accepted in Aptroot (2012) plus the many additional species that have been described since (Aptroot et al. 2012, 2013, 2014, 2015; Wijeyaratne et al. 2012; Cáceres et al. 2013; Lima et al. 2013; Gueidan et al. 2016; Mendonça et al. 2016). From the genus Pyrgillus, only seven species are currently known (Aptroot 1991; Kashiwadani et al. 2012; Singh & Singh 2012a, b, 2017), and in Sulcopyrenula only four species (Harada 1999; Aptroot 2002, 2012).

During recent fieldwork and herbarium studies by the authors, several undescribed species in the above genera were discovered. Some of these (the red *Pyrgillus* and the two *Pyrenula* species with red parts) were so characteristic that they were already recognized in the field as undescribed species. The purpose of this paper is to formally describe them.

#### Material and Methods

Descriptive work was carried out using an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water (unless otherwise indicated), in which all measurements were also taken. The chemistry

of the type specimens was investigated by TLC (Orange et al. 2001) using solvent A.

#### The Species

#### Pyrenula biseptata Aptroot & M. Cáceres sp. nov.

MycoBank No.: MB 821612

*Pyrenula* with simple ascomata with vertical ostioles, an inspersed hamathecium and 2-septate ascospores  $11-12 \times 4.5-5.0 \,\mu\text{m}$ .

Type: Brazil, Pará, Fazenda Pantera, 85 km N of Dom Eliseu, 3°49'54"S, 48°03'37"W, on bark of tree, c. 120 m alt., 29 October 2016, M. E. S. Cáceres & A. Aptroot ISE 40367 (ISE—holotype; ABL—isotype).

(Fig. 1A & B)

Thallus corticate, smooth, continuous, thin, dark brown, without pseudocyphellae or pockets of crystals; algal cells trentepohlioid.

Ascomata perithecioid, simple, dispersed, flat conical, superficial, often elongated, 0·2–0·4 mm diam., black, without thallus covering. Wall more or less equally carbonized, without crystals, c. 50 μm thick. Ostioles apical, brown. Hamathecium hyaline, heavily inspersed with oil droplets. Asci cylindricoclavate, with 8 irregularly arranged ascospores. Ascospores brown, 2-septate, long ellipsoid, without constrictions, 11–12 × 4·5–5·0 μm, ends rounded; lumina rounded to diamond-shaped, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. No secondary lichen substances detected.

*Ecology and distribution*. On smooth bark of trees in primary tropical rainforest. Known only from Brazil.

Discussion. This is only the second species in the genus with consistently 2-septate ascospores, a character that is rare throughout lichenized, and even non-lichenized, fungi because ascospore septation is a result of repeated, simultaneous division that affects all ascospore cells, often leading to 1-septate or 3-septate ascospores. The other *Pyrenula* species that has 2-septate ascospores, viz. *P. lineatostroma* 

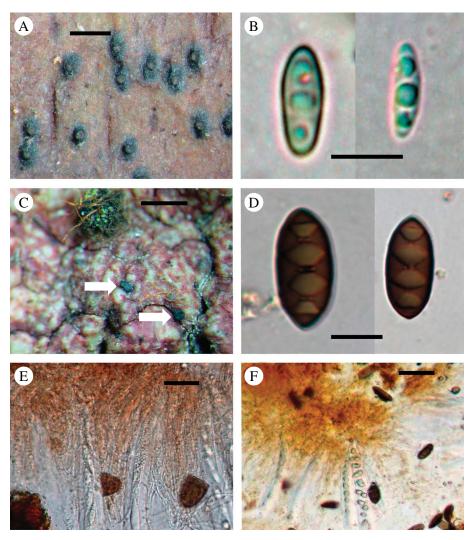


Fig. 1. A & B, *Pyrenula biseptata* (isotype): A, habitus; B, ascospores. C–F, *Pyrenula sanguineomeandrata* (isotype): C, habitus, arrows pointing to ostioles; D, ascospores; E, red hamathecium inspersion in H<sub>2</sub>O; F, orange hamathecium inspersion in KOH. Scales: A & C=0.5 mm; B & D=10 µm; E & F=50 µm. In colour online.

Aptroot (Aptroot *et al.* 1997), differs in the ascomata that are not separate but joined together sideways to form pseudostromata, and by the absence of hamathecium inspersion.

# Pyrenula sanguineomeandrata Aptroot & Mercado Diaz sp. nov.

MycoBank No.: MB 821613

Pyrenula with a thallus with red, KOH+ purple pigmentation of lines or a reticulum, simple ascomata with

vertical ostioles, a deep red inspersed, KOH+ orange hamathecium, and dark brown 3-septate ascospores  $25-29 \times 10-12 \,\mu m$ .

Type: Puerto Rico, Maricao, Bosque Estatal de Maricao, Vereda Los Viveros, 18°09'N, 66°59'W, on bark of tree, c. 850 m alt., 18 February 2014, A. Aptroot 72206 (UPR—holotype; ABL, F—isotypes).

(Fig. 1C-F)

Thallus corticate, smooth, continuous, up to 0·1 mm thick, ochraceous, with lines or a reticulum of dark red pigmentation, without

pseudocyphellae or pockets of crystals; algal cells trentepohlioid.

Ascomata perithecioid, simple, dispersed, pyriform, deeply immersed in the bark and only visible from above by the ostiole, 0·2–0·4 mm diam., black. Wall more or less equally carbonized, without crystals, c. 30 μm thick. Ostioles apical, dark to reddish brown. Hamathecium inspersed with dark red oil droplets. Asci cylindrico-clavate, with 8 irregularly arranged ascospores. Ascospores dark brown, 3-septate, broadly fusiform, without constrictions, 25–29 × 10–12 μm, ends rather pointed; lumina mostly diamond-shaped, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. Red anthraquinone on thallus reacting UV+ red and KOH+ purple; dark red hamathecium inspersion (anthraquinone?) reacting K+ orange.

*Ecology and distribution.* On bark of tree in submontane evergreen wet forest. Known only from Puerto Rico.

Discussion. This is only the second Pyrenula species with a red inspersion in the hamathecium. Pyrenula rubroinspersa Aptroot & Sipman also has a red inspersion in the hamathecium, but that reacts KOH+ green, suggesting the presence of isohypocrellinic acid. The red pigment that reacts KOH+ orange is unusual but is, for example, also present on the outside of the mazaedia of Pyrgillus rufus (see below).

### Pyrenula sanguineostiolata Aptroot & Mercado Diaz sp. nov.

MycoBank No.: MB 821614

*Pyrenula* with a thallus with deeply immersed simple ascomata with vertical ostioles, which are superficial and bright red, and 3-septate ascospores  $25–28\times9-12\,\mu m$ .

Type: Puerto Rico, Maricao, Bosque Estatal de Maricao, Vereda Los Viveros, 18°09'N, 66°59'W, on bark of tree, *c.* 850 m alt., 18 February 2014, *A. Aptroot* 72180 (UPR—holotype; ABL, F—isotypes).

(Fig. 2A & B)

Thallus corticate, smooth, continuous, up to 0·1 mm thick, olivaceous green, without pseudocyphellae or pockets of crystals; algal cells trentepohlioid.

Ascomata perithecioid, simple, dispersed, pyriform, deeply immersed in the bark and only visible from above by the ostiole, 0.3-0.5 mm diam., black. Wall more or less equally carbonized, without crystals, c. 40 µm thick. Ostioles apical, superficial, c. 0.1 mm diam., bright red. Hamathecium hyaline, not inspersed with oil droplets. Asci cylindrico-clavate, with 8 irregularly arranged ascospores. Ascospores brown, 3-septate, broadly fusiform, without constrictions,  $25-28 \times 9-12 \,\mu\text{m}$ , ends rather pointed; lumina mostly rounded to diamond-shaped, somewhat irregularly aligned, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. Red anthraquinone of ostiole reacting UV+ red and KOH+ dark purple (almost black).

*Ecology and distribution*. On bark of tree in submontane evergreen wet forest. Known only from Puerto Rico.

Discussion. This is one of the few species of *Pyrenula* with a bright red ostiole and the first one of these with deeply immersed ascomata.

## Pyrenula subvariabilis Aptroot & Sipman sp. nov.

MycoBank No: MB 821615

*Pyrenula* with fused ascomata with lateral ostioles and submuriform ascospores  $17-20(-25) \times 6-9 \mu m$ .

Type: Guyana, Potaro-Siparuni Region, surroundings of Pakaraima Village, along trail to Kawatipu, 4°42'N, 59°43'W, on bark of canopy branches of tree, *c.* 800 m alt., 22 February 1996, *H. J. M. Sipman* 41155 (B—holotype).

(Fig. 2C–G)

Thallus corticate, smooth, continuous, thin, ochraceous, without pseudocyphellae or pockets of crystals; algal cells trentepohlioid.

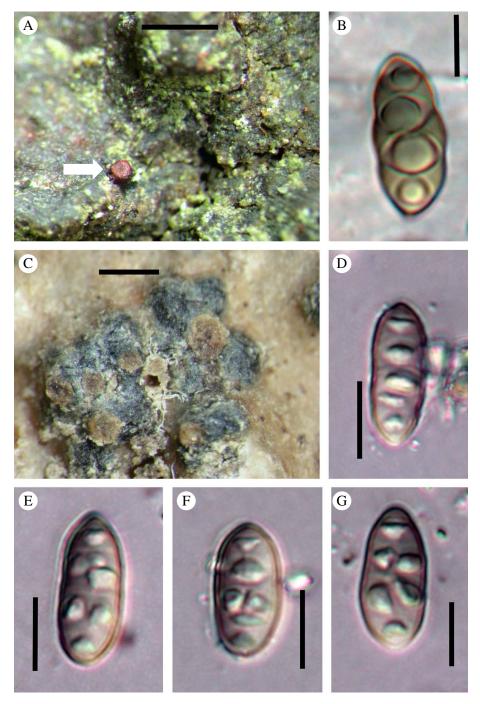


Fig. 2. A & B, *Pyrenula sanguineostiolata* (isotype): A, habitus, arrow pointing to ostiole; B, ascospore. C–G, *Pyrenula subvariabilis* (holotype): C, habitus; D–G, ascospores. Scales: A & C = 0.5 mm; B, D– $G = 10 \mu \text{m}$ . In colour online.

Ascomata perithecioid, in groups of 2–8, pyriform, emergent, 0·4–0·7 mm diam., black, mostly with thallus covering. Wall more or less equally carbonized, without crystals, c. 50 μm thick. Ostioles eccentric, fused, pale brown, c. 0·2 mm wide. Hamathecium hyaline, not inspersed with oil droplets. Asci cylindricoclavate, with 8 irregularly arranged ascospores. Ascospores brown, irregularly submuriform, 3–5 × 0–2-septate, with total number of lumina 6–12, fusiform, without constrictions, 17–20 (–25) × 6–9 μm, ends rather pointed; lumina mostly triangular to rounded, irregular, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

*Chemistry*. No secondary lichen substances detected.

*Ecology and distribution*. On smooth bark of canopy branches in savannah. Known only from Guyana.

Discussion. There are only a small number of species of Pyrenula known with submuriform ascospores (Aptroot 2012), and none with fused ostioles and ascospores of this size; all species with fused ostioles have much larger ascospores.

### Pyrenula xanthinspersa Aptroot & M. Cáceres sp. nov.

MycoBank No.: MB 821616

*Pyrenula* with an ecorticate thallus containing lichexanthone, simple ascomata with vertical ostioles, not inspersed hamathecium and 3-septate ascospores of  $14-17 \times 6.0-7.5 \, \mu m$ .

Type: Brazil, Amazonas, Manaus, Reserva Florestal Ducke, along trails in vicinity of field station, alt. 80 m, 2°56'S, 59°57'W, on tree bark in primary rainforest, 3–8 June 2016, *M. E. S. Cáceres & A. Aptroot* ISE 28435 (ISE—holotype; ABL—isotype).

(Fig. 3A & B)

Thallus not corticate, dull, continuous, thin, pale creamish, without pseudocyphellae or pockets of crystals; algae trentepohlioid.

Ascomata perithecioid, simple, dispersed, low hemispherical, emergent to superficial, 0·3–0·5 mm diam., black, without thallus

covering. Wall more or less equally carbonized, without crystals, c. 40  $\mu$ m thick. Ostioles apical, black. Hamathecium hyaline, not inspersed with oil droplets. Asci cylindricoclavate, with 8 irregularly arranged ascospores. Ascospores brown, 3-septate, broadly fusiform, without constrictions,  $14-17 \times 6.0-7.5 \,\mu$ m, ends rather pointed; lumina mostly diamond-shaped, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow, with lichexanthone.

*Ecology and distribution*. On smooth bark of trees in primary tropical rainforest. Known only from Brazil.

Discussion. This species is close only to Pyrenula cocoes Müll. Arg. (Aptroot 2012) which differs by the larger ascospores  $(18-21 \times 10-13 \,\mu\text{m})$ . In addition, that species is mostly coastal while the new species was collected very far from any coast.

## Pyrgillus aurantiacus Aptroot & M. Cáceres sp. nov.

MycoBank No.: MB 821617

*Pyrgillus* with corticate thallus containing lichexanthone, mazaedium with orange, KOH+ violet, UV+ red pruina and ascospores  $13-16 \times 6.0-7.5 \,\mu m$ .

Type: Brazil, Tocantins, near Itaguatins, alt. 150 m, 5°44'48"S, 47°33'46"W, on tree bark in cerrado remnant on farm, 23 October 2016, *M. E. S. Cáceres & A. Aptroot* ISE 28857 (ISE—holotype; ABL—isotype).

(Fig. 3C–E)

Thallus corticate, fissured, rather smooth and flat, c. 0·1 mm thick, olivaceous green, without pseudocyphellae or pockets of crystals; algal cells trentepohlioid.

Ascomata mazaedioid, sessile, simple or a few together, dispersed, cylindrico-pyriform, 0.5-0.8 mm diam., 0.5-1.2 mm high, black, lower half mostly with thallus covering. Wall more or less equally carbonized, without crystals, c.  $100\,\mu\text{m}$  thick. Ostioles apical, covered with black mazaedium which is mostly covered by a bright orange pruina.

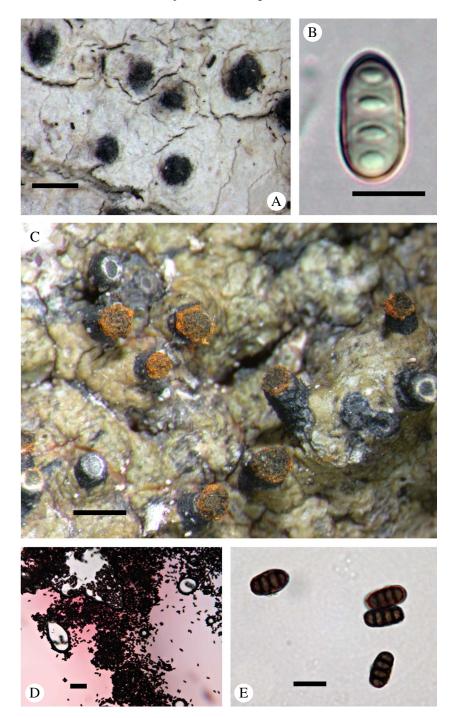


Fig. 3. A & B, *Pyrenula xanthinspersa* (isotype): A, habitus: B, ascospore. C–E, *Pyrgillus aurantiacus* (isotype): C, habitus; D, ascospores in KOH showing reaction of pruina; E, ascospores. Scales: A &  $C = 0.5 \, \text{mm}$ ; B &  $E = 10 \, \mu \text{m}$ ;  $D = 100 \, \mu \text{m}$ . In colour online.

Hamathecium not observed. Ascospores dark brown, 3-septate, long ellipsoid, usually with faint median constriction,  $13-16 \times 6.0-7.5 \,\mu\text{m}$ , c. 2 times as long as wide, ends rounded; lumina mostly rounded, septa thickened and darkened, terminal lumina not separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow, with lichexanthone; orange anthraquinone pruina on ascomata reacting UV+ red and KOH+ violet.

*Ecology and distribution*. On smooth bark of trees in cerrado forest. Known only from Brazil.

Discussion. There is so far only one species known in the genus Pyrgillus with a corticate thallus containing lichexanthone, viz. P. tibellii Kr. P. Singh & Pushpi Singh. That species differs by the dark red pruina on the mazaedium that reacts KOH+ purple and the larger ascospores of  $15-20(-23) \times 7.5-10.0 \,\mu\text{m}$ . See also the next species, which differs in the red, generally lower ascomata with complete thallus cover at the sides, and especially by the dark red-brown pruina that reacts KOH+ orange, and the somewhat longer but thinner ascospores. The two new Pyrgillus species are described here from different states but could almost be regarded as occurring in the same region as their collecting localities are only 200 km apart. Both species belong to the core group of *Pyrgillus*, having 3-septate ascospores with thickened septa.

### Pyrgillus rufus Aptroot & M. Cáceres sp. nov.

MycoBank No.: MB 821618

*Pyrgillus* with a corticate thallus containing lichexanthone, mazaedium with dark red, KOH+ orange, UV+ red pruina and ascospores  $15.0-17.5 \times 5.0-6.5 \,\mu m$ .

Type: Brazil, Pará, Villa Nazaré, 85 km N of Dom Eliseu, 3°53'56"S, 48°05'44"W, on bark of tree, c. 120 m alt., 29 October 2016, M. E. S. Cáceres & A. Aptroot ISE 40256 (ISE—holotype; ABL—isotype).

(Fig. 4A–D)

Thallus corticate, fissured, rather uneven, c. 0·1 mm thick, olivaceous green, without

pseudocyphellae or pockets of crystals; algae trentepohlioid.

Ascomata mazaedioid, erumpent to sessile, simple, dispersed, cylindrico-pyriform,  $0.5-0.8 \,\mathrm{mm}$  diam.,  $0.5-0.8 \,\mathrm{mm}$  high, black, sides almost completely with thallus covering. Wall more or less equally carbonized, without crystals, c. 100 µm thick. Ostioles apical, covered with black mazaedium which is mostly covered by a dark red pruina. Hamathecium not observed. Ascospores dark brown, 3-septate, long ellipsoid, usually with faint median constriction,  $15.0-17.5 \times 5.0 6.5 \,\mu\text{m}$ , c. 2.5-3 times as long as wide, ends rounded; lumina mostly rounded, septa thickened and darkened, terminal lumina not separated from the end wall by an endospore laver.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow, with lichexanthone; red pigment (anthraquinone?) pruina on ascomata reacting UV+ red and KOH+ orange.

*Ecology and distribution*. On smooth bark of trees in primary tropical rainforest. Known only from Brazil.

Discussion. There is so far only one species known in the genus Pyrgillus with a corticate thallus containing lichexanthone, viz. P. tibellii Kr. P. Singh & Pushpi Singh, for which the new species was mistaken in the field. That species differs by the pruina on the mazaedium that reacts KOH+ purple and the larger and especially wider ascospores of  $15-20(-23) \times 7.5-10.0 \,\mu\text{m}$ . See also the previous species. The red pigment that reacts KOH+ orange is unusual but is, for example, also present in the hamathecium of Pyrenula sanguineomeandrata (see above).

## Sulcopyrenula biseriata Aptroot & Sipman sp. nov.

MycoBank No.: MB 821619

Sulcopyrenula with a thallus containing lichexanthone, simple ascomata with lateral ostioles and lozenge-shaped ascospores with 8 locules,  $(13-)15-17(-20) \times 8-10$  (width)  $\times 6-7$  (thickness) µm.

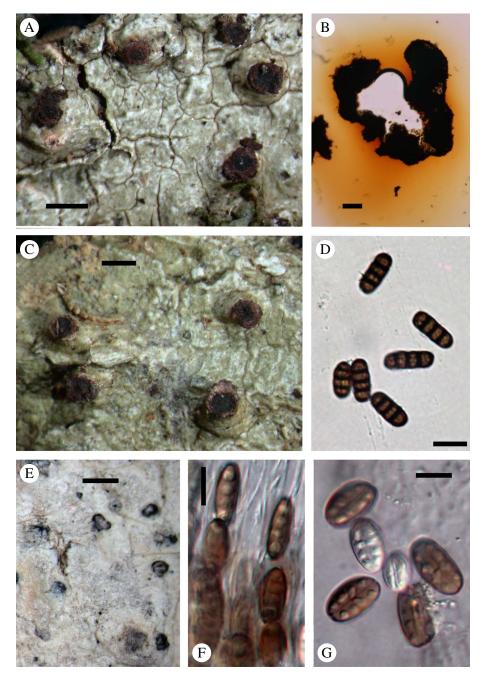


Fig. 4. A–D, *Pyrgillus rufus* (isotype): A & C, habitus; B, ascospores in KOH showing reaction of pruina; D, ascospores. E–G, *Sulcopyrenula biseriata* (holotype): E, habitus; F, ascospores in ascus, upper ones in side view; G, discharged ascospores. Scales: A, C & E =  $0.5 \, \text{mm}$ ; B =  $100 \, \mu \text{m}$ ; D, F & G =  $10 \, \mu \text{m}$ . In colour online.

Type: Guyana, Upper Takutu District, Rupununi Savannah, Kusad Mountain, SE-side, 2°47'N, 59°51'W, on bark of *Aspidosperma ulei* tree, *c.* 450 m alt., 29 September 1992, *H. J. M. Sipman* 57895 (B—holotype).

(Fig. 4E-G)

Thallus indistinctly corticate, smooth, continuous, very thin, pale ochraceous, without pseudocyphellae or pockets of crystals, surrounded by a thin black hypothallus line; alga trentepohlioid.

Ascomata perithecioid, simple, dispersed, pyriform, immersed to emergent, 0·3–0·4 mm diam., black, mostly with thallus covering. Wall more or less equally carbonized, without crystals, c. 40 μm thick. Ostioles lateral, black. Hamathecium hyaline, not inspersed with oil droplets. Asci cylindrico-clavate, IKI-, with 8 almost uniseriately arranged ascospores. Ascospores dark brown, lozenge-shaped with one longitudinal euseptum that is formed first and is paralleled by fissures at upper and lower surfaces, and 3 distosepta in each ascosporehalf giving 8 locules, ellipsoid, without

constrictions,  $(13-)15-17(-20) \times 8-10$  (width)  $\times 6-7$  (thickness) µm, ends rounded; lumina mostly triangular but rounded, terminal lumina separated from the end wall by an endospore layer.

Pycnidia not observed.

Chemistry. Thallus UV+ yellow, with lichexanthone.

Ecology and distribution. On smooth bark of tree in savannah. Known only from Guyana. Growing together with *Pseudopyrenula subgregaria* Müll. Arg. and *Astrothelium* cf. pulcherrimum (Fée) Aptroot & Lücking.

Discussion. This is only the fifth species of this genus. All species in the genus share the lozenge-shaped, flat, submuriform ascospores with either 4 or 8 locules; the two species known with lichexanthone in the thallus, viz. S. cruciata Aptroot (Aptroot 2002) and S. subglobosa (Riddle) Aptroot (Aptroot 2012), have subglobose to ellipsoid ascospores with only 4 locules.

#### World key to the 3-septate species of Pyrgillus

1 Thallus UV-; mazaedium pruina orange, KOH+ purple; ascospores  $10-14 \times 5-7 \,\mu m$ ; Mazaedium pruina white, KOH-; ascospores  $10-13 \times 6.0-8.5 \,\mu\text{m}$ ; pantropical . . . . 2(1)..... P. javanicus (Mont. & Bosch) Nyl. Mazaedium pruina orange or red, KOH+ orange, reddish violet or violet......... 3 3(2) Mazaedium pruina red, KOH+ reddish violet, UV-; ascospores  $15-20(-23) \times 7.5-$ Mazaedium pruina red but KOH+ violet or orange, UV+ red; ascospores 13-0-4(3) Mazaedium pruina orange, KOH+ violet; ascospores  $13-16 \times 6.0-7.5 \,\mu\text{m}$ , c. 2 times as Mazaedium pruina red, KOH+ orange; ascospores  $15.0-17.5 \times 5.0-6.5 \mu m$ , c. 2.5-3

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