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Four new *Pyrenula* species from primary forests in the Guianas, South America, and their ascospore development

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Abstract: Four new species of the genus *Pyrenula* (Pyrenulaceae, Pyrenulales, lichenized Ascomycetes) are presented. They originate from primary forests in the Guianas: *Pyrenula cubica* Sipman, with cubical central lumina in the ascospores; *Pyrenula flavida* Sipman, with a yellow-pigmented thallus; *P. rubromarginata* Sipman, with red pigment on the excipulum and not on the thallus; and *P. submicromma* Sipman, like *P. micromma* but with submuriform instead of bacillar ascospores. In addition, a description for *P. atropurpurea* is provided, and *P. subvariabilis* is reduced to synonymy of *P. infrastroidea*. Special attention was paid to the development of the ascospores. Two different development types are recognized. The phylogenetic significance of these types is unclear.

Keywords: lichen taxonomy, anthraquinones, ascospore ontogeny, primary forest

INTRODUCTION

The lichen family Pyrenulaceae is very common and sometimes dominant in primary tropical lowland forests. However, most species look very similar and in the field the diversity is easily underestimated. In recent decades the understanding of Pyrenulaceae diversity greatly improved by the careful observations of Harris (1973, 1989), who added valuable distinguishing characters. A world key published by Aptroot (2012) for some 170 species, with updates (Aptroot, 2021; Schumm & Aptroot, 2021), presented distinguishing characters for all accepted species and their synonymies. This allowed for much more accurate identifications and the recognition of various still undescribed taxa (e.g., Aptroot et al., 2013). Here results are presented from the identification of herbarium specimens collected during exploratory projects in the Guianas.

MATERIAL AND METHODS

The investigated specimens were collected in primary forest in French Guiana in 1986 and in Guyana in 1992, and are stored in the herbarium of the Botanical Museum in Berlin (B). They were investigated in the usual way by stereomicroscope (dry specimens) and compound microscope (preparations in tap water, without addition of KOH). Ascospores were studied in squash preparations of thick hamathecium sections, so that sufficient spores are available to observe the variation in size, the disposition of the spores inside the ascus, and the spore development. The chemistry was investigated by fluorescence, spot tests or TLC (Orange et al., 2001).

RESULTS

Four undescribed species and a new synonym were discovered, which are presented below. Three of the new species are easily recognizable by traditional characters: *P. flavida* has a pale yellowish-pigmented thallus; *P. rubromarginata* has a conspicuous, red pigment, only on the ascocarp margins; and *P. submicromma* differs from *P. micromma* only because the ascospores are not only transversely septate but show also one or few longitidinal septa, thus being not bacillar but submuriform. The fourth new species, *P. cubica*, deviates mainly by the ascospore development, which receives more attention in the Discussion below.

Added are a description for another *Pyrenula* species, from which the unusual ascospore development was unreported, and a new synonymy.

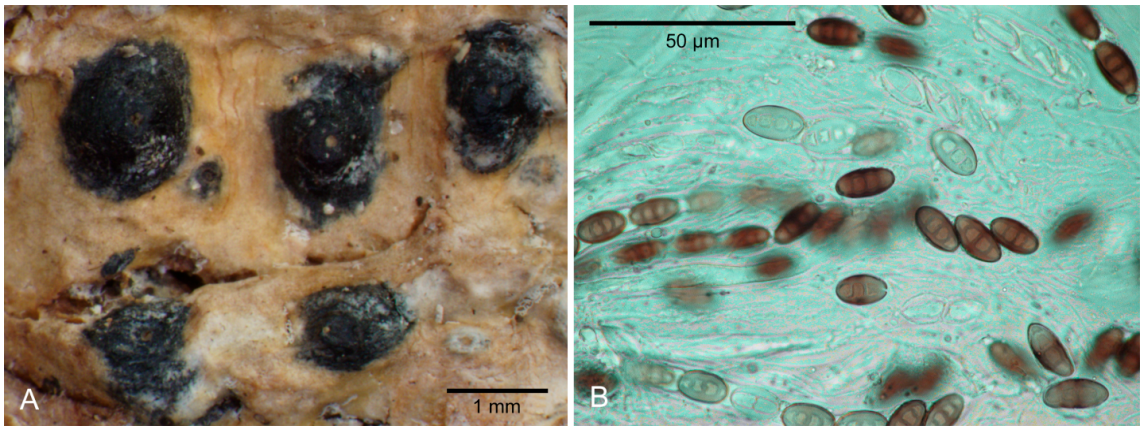

Fig. 1. *Pyrenula cubica*, holotype. A. Habitus. Scale = 1 mm. B. Ascospores in various developmental stages: colourless, unicellular, thick-walled initial stage; first septum-stage; 3-septate, pale grey stage with rectangular lumina; final, pale brown stage with darker bands over the septa. Picture width = 135 µm.

TAXONOMY

PYRENULA CUBICA Sipman spec. nov. (Fig. 1A & B)

MycoBank No.: MB849546

Diagnosis: Resembles *Pyrenula atropurpurea* by the small ascospores with predominantly lateral endospore and differs by the larger ascocarps, to 1.8 mm wide instead of 0.5–1.0 mm wide, and the cubic instead of rounded central ascospore lumina.

Type: GUYANA, Upper Takutu district, c. 40 km S of Aishalton. Kuyuwini Landing, N shore of Kuyuwini river. Elev. c. 230 m. c. 59° 21' W, 2° 06' N. 5–10 m high on 60 cm dbh., 32 m tall *Ormosia flava* tree in c. 30 m tall, seasonally dry, undisturbed forest. 14–15 Oct. 1992. H. Sipman 63890 (BRG holotype; B 60 0203466 isotype).

Thallus corticolous, pale ochraceous brown, UV–, endoperidermal, with smooth surface, without crystal pockets, about 100 µm thick, the upper c. 30 µm with more dense hyphae, and corticoid. Ascomata perithecioid, simple, dispersed, conical to semiglobose, 1–1.8 mm wide, developing inside the periderm of the host and emergent, when young at base thinly covered by thallus, finally completely black and sessile, with c. 200 µm thick, black, carbonized exciple without crystals, with black, apical ostioles. Hamathecium clear. Asci 95–100 × 10–12 µm, cylindrical, without ocular chamber. Ascospores 8/ascus, uniseriate, pale grey when young, brown when full-grown, broadly fusiform, 3-septate, 12–18 × 7–8 µm, with thick septa and cubic central lumina, terminal lumina not or thinly separated from the apical wall by an endospore layer, at age not shriveling (for developmental details see below).

Chemistry: No lichen substances found; all reactions negative.

Etymology. Named after the cubical shape of the ascospore lumina.

All specimens are from the same tree with a thin, leathery, outer bark layer. They were collected at 5–15 m high on the trunk in semideciduous, c. 30 m tall, primary lowland forest. A voucher of the phorophyte is available: M. Jansen-Jacobs et al. 2847 (L).

Pyrenula cubica is unique by its small ascospores with cubic lumina when mature. The cubic lumina are easily overlooked without careful focusing, as Fig. 1B shows. Very similar spores with thick lateral walls but with rounded lumina are shown for *Pyrenula atropurpurea* (Eschw.) Müll. Arg. by Schumm & Aptroot (2021), p. 1413–1414. This species differs by smaller ascocarps, c. 0.8 mm wide, and biseriate ascospores with rounded lumina. A description of this species is given below. Comparable is also *Pyrenula circumfiniens* Vain., described from

the Caribbean, which shares small ascospores with thick lateral walls and rounded lumina, and has the apical locules close to the outer wall. It differs by the reddish brown colour of the ascospores, the lateral ostioles and the thallus with crystal pockets. See also general discussion above.

The slightly darker band over the septa of the ascospores reminds *Pyrenula wetmorei* Harris, which differs clearly by the larger ascospores, 18–24 × 10–14 µm (Harris 1990) instead of 12–18 × 7–8 µm, the lateral instead of apical ostioles and the inspersed instead of clear hamathecium. Banded ascospores are known also from *P. minae* Aptroot & Lücking, which has larger ascospores, 23–28 × 11–13 µm with strong central constriction and an inspersed hamathecium (Aptroot et al. 2008).

The juvenile ascospores are colourless, aseptate with a thick outer wall which is thinner at the apices. In the next stage thin septa appear, first the middle septum, followed by the 2 lateral septa. Then the spores turn grey and the endospore increases, first along the median septum. Finally, 4 locules, the central ones cubic, the apical ones semiglobose, in a straight row are present which keep the same distance to the lateral wall, come close to the apical spore wall, and are rather weakly contrasting with the endospore. Then the colour changes from pale grey to brown, with a slightly more pigmented band around the septa. Examples of the stages are in Fig. 1B. Overaged spores appear to stay unchanged and no shriveled spores are present.

The genus *Lithothelium* at first seemed the most appropriate to accommodate the new species, in view of the endospore predominantly situated along the outer wall and not accumulated in the corners of the septa, and the absence of black lines marking eusepta. However, there is no clear-cut, completely constant character to distinguish between *Lithothelium* and *Pyrenula*. Aptroot (1991) gives as key characters for *Lithothelium* colourless, brown or red brown ascospores with rounded, relatively large lumina; asci often with rounded or sagittiform ocular chamber, and for *Pyrenula* brown or blackish ascospores with relatively small, often angular lumina; asci without ocular chamber. In the description it says further that the ascospores of *Lithothelium* are without a trace of eusepta. In the world key (Aptroot 2012) *Lithothelium* has as key characters: red brown ascospores with locules at least becoming rounded when older, and *Pyrenula* has grey to brown ascospores, rarely red-brown and then locules angular. Aptroot et al. (2008) add that in *Lithothelium* the ascospores may be angular when young, rounded when mature, and the hamathecium is unbranched and often IKI+ blue or partly blue, partly orange. Since the phylogenetic study of Gueidan et al. (2016) found that the included species of *Lithothelium* were polyphyletic, our new species is included here provisionally in *Pyrenula*, awaiting a more natural classification of the *Lithothelium-Pyrenula* complex.

Position in key of Aptroot (2021): B141c Ascospores 12–18 µm long, with cubic central lumina and dark bands over the septa. *Pyrenula cubica* Sipman

Additional specimens examined: from same tree as type, at different altitudes, Sipman 63942 (B 60 0203518), 63892 (B 60 0203468).

PYRENULA FLAVIDA Sipman spec. nov. (Fig. 2A & B)

MycoBank No.: MB849547

Diagnosis: Resembles *Pyrenula cerina*, from which it differs by the greenish-yellow rather than orangish thallus, smaller ascospores, c. 11 µm instead of 25–40 µm long, larger ascocarps, c. 0.6–0.8 mm instead of 0.3 mm wide, and its occurrence in inland forests, not mangroves.

Type: GUYANA, Upper Takutu district, c. 40 km S of Aishalton. Kuyuwini Landing, along trail to Kassikaityu river. Elev. c. 230 m. c. 59° 15' W, 2° 06' N. Small savannah c. 2 km S of Kuyuwini river. On crown branches of 10 m tall, solitary *Agonandra brasiliensis* tree (MJ 3149). 20 Oct. 1992. H. Sipman 58146 (BRG holotype; B 60 0168304 isotype).

Thallus corticolous, pale greenish yellow, UV+ reddish, endoperidermal, with weathered, rough surface, without crystal pockets, c. 50 µm thick. Ascomata perithecioid, simple, dispersed, hemispherical, 0.6–0.8 mm wide, when young immersed in the periderm of the host, soon emergent and finally almost stalked, with completely black and carbonized, c. 150 µm thick exciple without crystals, with apical, black ostioles. Hamathecium clear. Asci c. 80 × 10 µm,

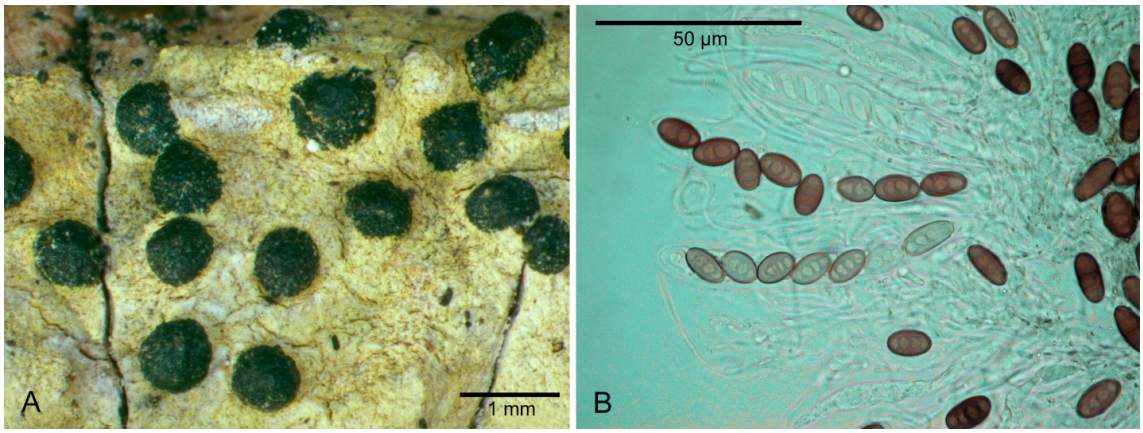

Fig. 2. *Pyrenula flavida*, holotype. A. Habitus. Scale = 1 mm. B. Ascospores in various developmental stages: colourless, unicellular, thick-walled initial stage; 3-septate, pale grey stage with rounded lumina; final, brown, persistently not shriveled stage. Picture width = 135 µm.

cylindrical, without or with small, c. 1.5 µm wide, simple ocular chamber. Ascospores 8/ascus, uniseriate, pale grey when young, brown when full-grown, broadly fusiform, 3-septate, 11 × 6–7 µm, with rounded to lenticular, equal lumina, with thick lateral walls, terminal lumina not separated from the end wall by an endospore layer, at age not shriveling (for further details of the ascospore development see note below).

Chemistry: Unidentified yellow pigment present in thallus; thallus K+ dark red, UV+ reddish; yellow pigment in section K+ orange, dissolving. In TLC a single spot is visible in short-wave UV at c. A50, B50, C50, which is pale brown after charring, perhaps 4−Chloroemodin.

Etymology. Named after the yellow thallus color.

The type and only specimen was collected on canopy branches of an isolated tree in a small lowland savannah expanded by bushfires. A voucher of the phorophyte is available: M. Jansen-Jacobs et al. 3149 (L).

This species is unmistakable by its greenish yellow thallus, unique in the genus, and the small, brown ascospores. The other *Pyrenula* species with pigmented thallus are reddish. The most similar is *P. cerina* Eschw. with an orange thallus and larger ascospores, 26–42 x 12–15 µm.

The juvenile ascospores are initially hyaline and simple, with a thick wall, thinner apically. Next thin septa develop, the median one first, and the spores turn pale grey. Finally, the spores turn brown, with 4 rounded locules, the median ones often somewhat elliptic, the apical ones close to the outer wall. An euseptum may become visible as a black line, mainly in central septa. Old spores remain like this and do not shrivel. See Fig. 2.

Position in key of Aptroot (2021): B77c Ascomata not yellow, thallus yellow. *Pyrenula flavida* Sipman

PYRENULA RUBROMARGINATA Sipman spec. nov. (Fig. 3A & B)

MycoBank No.: MB849548

Diagnosis: *Pyrenula* species with red pigment on the ascocarps, with scattered or slightly aggregated ascocarps, with unpigmented thallus, and with small, 3-septate, uniseriate ascospores c. 13–15 × 6 µm, with lenticular lumina and thickened apical walls.

Type: GUYANA, Upper Takutu district, c. 40 km S of Aishalton, Kuyuwini Landing, S-shore of Kuyuwini river along trail to Kassikaityu river. Elev. c. 230 m. 59° 15' W, 2° 06' N. 20–25 m high on 30 cm tall *Hymenaea courbaril* tree in c. 30 m tall, seasonally dry, undisturbed forest. 10–13 oct. 1992. H. Sipman 64699 (BRG holotype; B 60 0206701 isotype).

Thallus corticolous, pale ochraceous brown, UV–, surface smooth, in part white-weathered,

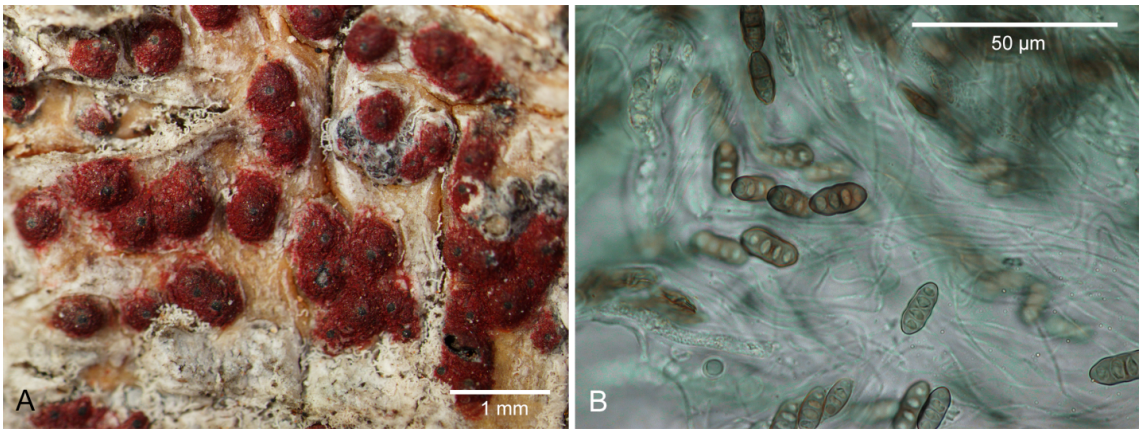

Fig. 3. *Pyrenula rubromarginata*, holotype. A. Habitus. Scale = 1 mm. B. Mature ascospores. Picture width = 135 µm.

UV–, near the ascocarps sometimes with some red pigment, endoperidermal, with smooth surface, without crystal pockets, c. 50 µm thick, the periderm cells of the host containing c. 3 µm wide, dense, contorted and conglutinated hyphae. Ascomata perithecioid, simple, dispersed or 2–5 aggregated, applanate-conical, occasionally almost semiglobose, 0.5–0.6 mm wide, sessile, with black and carbonized, c. 75 µm thick exciple without crystals covered by a c. 20 µm thick, non-carbonized layer containing reddish pigment, dark red-pigmented, with apical, flat, black, c. 0.1 mm wide ostiole. Hamathecium clear. Asci c. 75 × 13 µm, cylindrical, when young with c. 1 µm wide, simple ocular chamber. Ascospores 8/ascus, uniseriate, grey-brown, broadly fusiform, 3-septate, c. 13–15 × 6 µm, with lenticular lumina, with rather thin lateral wall, terminal lumina separated from the end wall by an endospore layer, at age turning brown and shriveling (for further details of ascospore development see note below).

Chemistry: Unidentified, red pigment present on ascocarp margin; excipulum in microscopical preparation with K+ violet solution, pigment completely dissolving into a coloured cloud, which disappears after few minutes. The chemistry of the pigment was not studied by TLC because it is present only in small quantities.

Etymology. Named after the conspicuous, red colour of the ascocarps.

The type and only known specimen was collected in the crown of a mature tree in semideciduous, c. 30 m tall, lowland forest. A voucher for the phorophyte is available: M. Jansen-Jacobs 2828 (L).

Pyrenula rubromarginata differs from other species with red pigment on the perithecia and not on the thallus by the combination of the following characters: dispersed, superficial, conical perithecia, small, 3-septate, uniseriate ascospores with thick apical walls. *P. howeana* Aptroot (Aptroot 2007) is most similar by the red-pigmented, conical, superficial perithecia and non-pigmented thallus with small, irregularly biseriate ascospores and deviates by its slightly larger ascospores, 15–20 × 7–10 µm, with thin terminal walls and a guttulate hamathecium. The only other species with red-pigmented, prominent perithecia and unpigmented thallus is *P. rubroanomala* Aptroot & Lücking (Aptroot et al. 2008), in which the perithecia are fused in distinct pseudostromata, not dispersed or few aggregated, and the spores measure 15–17 × 5–6 µm.

The younger stages of the ascospore development are not well represented and mostly degenerated. The youngest stage is simple, colourless, and thin-walled. Next the median septum appears and becomes thickened by endospore accumulation, mainly in the corners near the outer wall. Afterwards, the lateral septa

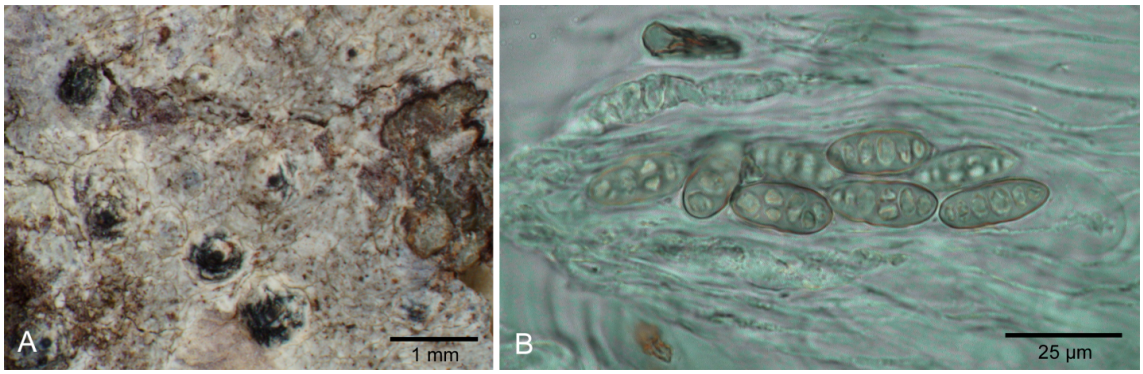

Fig. 4. *Pyrenula submicromma*, holotype. A. Habitus; thalline cover of ascocarps often abraded and showing the black, carbonized excipulum. Scale = 1 mm. B. Mature ascospores. Picture width = 90 µm.

develop, first visible as a ring of endospore. The first frequently available stage is colourless, 3-septate. This soon turns grey and later becomes pale grey-brown. Then the median septa, and to a lesser degree the lateral septa often show a black line in the position of the euseptum. Finally, the spores shrivel and become dark-brown. This type of spore development agrees with type 1, see below.

Position in key of Aptroot (2021): B89c Red coloration on ascocarp wall, not on thallus or in ostiole. *Pyrenula rubromarginata* Sipman

PYRENULA SUBMICROMMA Sipman spec. nov. (Fig. 4A & B)

MycoBank No.: MB849549

Diagnosis: Resembles *Pyrenula micromma* (Mont.) Trevis., from which it differs by larger ascocarps, 0.6-0.8 mm wide instead of 0.4-0.6 mm, and submuriform instead of bacillar ascospores.

Type: FRENCH GUIANA, Saül, 2 km SW of the village, "sentier limonade". Elev. 180–210 m. 03° 32'N, 53° 12'W. Lowland moist forest on lateritic soil. Epiphytic on outermost canopy branches of *Dicoryna guianensis*. 24 July 1986. D. Montfoort & R. C. Ek 224 (B 60 0204029 holotype).

Thallus corticolous, whitish, UV+ yellow, epiperidermal, with smooth surface, without crystal pockets, c. 50 µm thick, with c. 10 µm thick cortex and 40 µm thick, white medulla. Ascomata perithecioid, simple, dispersed, semiglobose, 0.6-0.8 mm wide, prominent, with black and carbonized, c. 75 µm thick exciple without crystals, covered by the thallus except for the apical, protruding, black, c. 0.1 mm wide ostiole; at age thallus cover often abraded and black excipulum exposed. Hamathecium clear. Asci c. 85 × 18 µm, subcylindrical, with small, c. 2 mm wide, simple ocular chamber. Ascospores 8/ascus, biseriate, submuriform, pale grey-brown, broadly fusiform, 4–6 × 1–2 septate, c. 17 × 7 µm, with lenticular lumina and rather thin lateral wall, terminal lumina separated from the end wall by a thin endospore layer, at age turning brown and shriveling (for further details of ascospore development see note below).

Chemistry: lichexanthone present in the thallus, UV+ yellow.

Etymology. Named after the most similar species, *Pyrenula micromma*.

The type and only specimen was collected on outer crown branches of a mature *Dicoryna guianensis* tree in moist lowland tropical forest.

Pyrenula submicromma belongs, with *P. guyanensis* Sipman & Aptroot and *P. micromma* (Mont.) Trevis., to the few species with a whitish, epiperidermal thallus with an often distinct, white medulla. Their whitish, epiperidermal thallus is conspicuously different from the olivaceous to yellowish or brownish, endoperidermal thallus of most *Pyrenula* species. It resembles the pale thalli of the ecorticate species like *P. cocoes* Müll. Arg. or *P.*

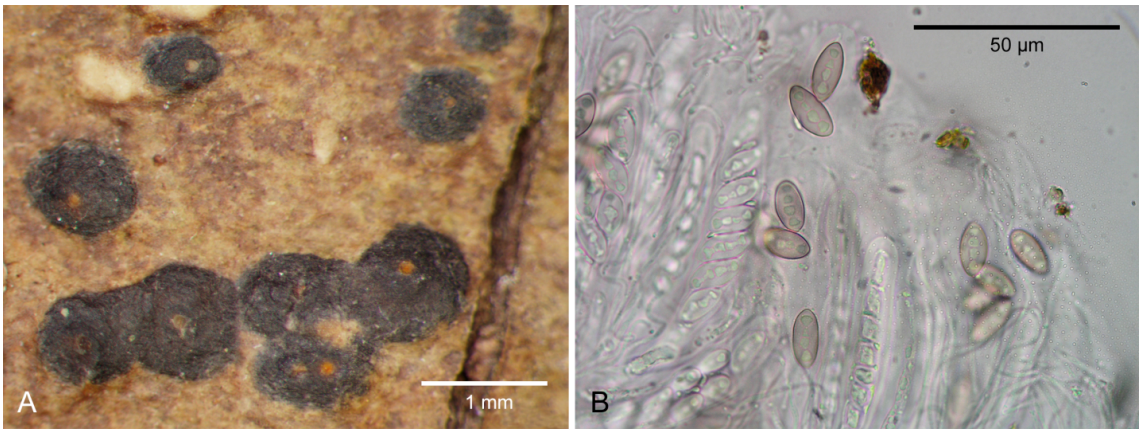

Fig. 5. *Pyrenula atropurpurea* (Sipman 51622). A. Habitus. Scale = 1 mm. B. Ascospores in various developmental stages: colourless, unicellular, thick-walled initial stage; first septum-stage; 3-septate, pale grey stage with rounded to elongate lumina. Picture width = 135 µm.

confinis (Nyl.) R.C. Harris but has a thin, ca 5 µm thick cortex and reminds the genus *Porina*. *P. submicromma* differs from both hitherto known species by the submuriform, not transversely 3-septate ascospores, from *P. guyanensis* by the smaller perithecia, 0.6–0.8 mm instead of 0.8–1.2 mm and from *P. micromma* by the larger perithecia, 0.6–0.8 mm instead of 0.4–0.6 mm (Aptroot et al. 2013). *P. obvoluta* (Nyl.) R. C. Harris & Aptroot is a third species with similar thallus. However, the only known, type specimen is in poor condition and has perithecia of 0.6–0.8 mm lacking hamathecium and ascospores (Aptroot et al. 2013), so that its affinity with *P. submicromma* remains uncertain.

The spore development is incompletely represented in the available specimen. Early stages are scarce and degenerated. They are colourless and thin-walled, and probably develop a muriform septation before endospore production. Stages with a single, median septum and such with three transverse septa were occasionally seen. Spores with fully developed septation are pale grey-brown, with 3 main, mostly transverse septa and 1–3 secondary, longitudinal or oblique septa. No black lines indicating eusepta were visible. Over-aged spores become dark brown and shrivel. Apart from the septation, the ascospore development fits type 1 as described below.

Position in the key of Aptroot (2021): A 20c Ascospores distoseptate; thallus UV+ yellow. *Pyrenula submicromma* Sipman.

Additional species with unusual ascospore development

PYRENULA ATROPURPUREA (Eschw.) Müll. Arg. (Fig. 5A & B)

Thallus corticolous, pale olivaceous brown, UV–, endoperidermal, with smooth surface, without medulla or rarely with up to c. 50 µm thick, white medulla, without crystal pockets, about 50 µm thick, the upper c. 30 µm with more dense hyphae and cortex-like. Ascomata perithecioid, partly simple and dispersed, partly 2–5 fused, low-conical, 0.5–1 mm wide, of variable size, to 1.5 mm wide in aggregates, prominent, when young at base sometimes thinly covered by thallus, otherwise completely black, with c. 200–300 µm thick, black and carbonized exciple without crystals, with often conspicuous, white or yellowish, apical ostioles, with more or less lobed chamber. Hamathecium clear. Asci 65–75(–110) × 11–14 µm, cylindrical, without ocular chamber. Ascospores 8/ascus, uniseriate, pale grey when mature, soon turning brown, broadly fusiform, 3-septate, 12–14 × 6(–7) µm, with thick septa and walls and rounded lumina, the central lumina sometimes somewhat elongated, the terminal lumina sometimes slightly eccentric, and well separated from the apical wall by an endospore layer, at age only slightly shriveling (for further details of ascospore development see note below).

Chemistry: No lichen substances found; all reactions negative.

This species is treated here as another example of type 2 ascospore development and because it is easily mistaken for the widespread *P. aspistea* (Ach.) Ach. (Aptroot 2012) without attention to the ascospore development. In fact, I considered it an undescribed species until I discovered a picture of the ascospores of *P. atropurpurea* in Schumm & Aptroot (2021, p. 1414), from a specimen collected from Argentina, Prov. Misiones (Ferraro 10547), which showed very similar ascospores.

The ascospores are similar with those of *P. circumfiniens* by their thick-walled juvenile stage and the absence of eusepta. This species is probably the closest relative and differs by the always single ascocarps with lateral ostioles instead of often aggregated ascocarps with apical ostioles, the larger ascospores, 18–25 × 7–9(–11) µm instead of 12–17 × 6–7 µm with smaller apical locules, and the presence of crystal pockets ("pseudocyphellae") in the thallus.

Another species known from Costa Rica with type 2 ascospore development, *Pyrenula subgregantula*, deviates because it has multichambered ascocarps and larger ascospores, 15–21 × 7–9(–11) µm.

The frequently aggregated and partly fused ascocarps resemble those of the recently described species *Pyrenula aggregataspistea* and *P. infraleucotrypa* (Aptroot et al. 2013). Both differ by ascospore development type 1 instead of 2.

The numerous specimens from Costa Rica were collected in disturbed primary, lower montane forest at 300 and 1200 m elevation, on smooth, thin bark of saplings in undergrowth.

Additional specimens examined. COSTA RICA, Limón: 40 km S of Limón, Reserva Biológica Hitoy Cerere, forest trail above Biological Station, along Sendero Espavel. Elev. c. 300 m. Co-ord. 83° 02' W, 9° 40' N. Submontane rainforest zone. On trunks in disturbed primary forest. 10 March 2004. H. Sipman 51644 (B 60 0179580), 51621 (B 60 0179579), 51622 (B 60 0179583), 51650 (B 60 0178674); Hitoy Cerere Reserve, near Pandora, 40 km S of Limón (ticolichen site 34a). Elev. 150-500 m. Co-ord. 83° 02' W, 9° 40' N. Submontane rainforest zone. On tree in disturbed primary forest along trail. 10 March 2004. A. Aptroot 60185 (B 60 0166138). Puntarenas: San Vito de Coto Bruz, Estación Biológico Las Cruzes. Elev. c. 1200 m. Co-ord.: 82° 57.6' W, 8° 45.1' N. On ridge beyond Rio Java. On trunks and undergrowth of disturbed primary forest. 14 Oct. 2004. H. Sipman 53256 (B 60 0178675).

New synonym

Pyrenula infrastroidea Aptroot & Sipman, Lichenologist 45(2): 177 (2013)

Syn. Nov. *Pyrenula subvariabilis* Aptroot & Sipman, in Aptroot, Sipman, Mercado Diaz, Mendonça, Feuerstein, Cunga-Dias, Pereira & Cáceres, Lichenologist 50(1): 80 (2018).

A comparison of the descriptions and the two type specimens, the only material known, shows that the only difference is in the presence of crystal pockets. They should be present in *P. infrastroidea* and absent in *P. subvariabilis*. The holotype to be deposited in herbarium BRG has a few spots resembling such "crystal pockets". The isotype in B, which is a fragment of the same thallus, has no such spots, and has an annotation that it is the type of *P. subvariabilis*. Since both types are fragments of the same thallus, the pertinent species must be synonymous. The few white spots suggesting crystal pockets in the type of P. infrastroidea are probably soredia from another lichen or some other impurity incorporated in the thallus. The size of the ascospores in my observations is 14–15(–17) × 8 µm.

DISCUSSION

All new species were found in primary forest. This agrees with the general experience that these forests are important for the biodiversity and that their diversity is still very incompletely known.

While the ascospore morphology is widely used in systematic studies of Pyrenulaceae (e.g., Malme, 1929, Upreti, 1998, Aptroot, 2012, Aptroot et al., 2008), ascospore development is never mentioned, except overmature stages (e.g., Harris 1989). However, it is easily observable in many specimens when thick, squashed sections are studied, and shows considerable differences among the species treated here. Two of the new species, *Pyrenula rubromarginata* and *P. submicromma* (Fig. 3B, 4B), have the usual

ascospore development observable in many tropical *Pyrenula* specimens with 3-septate ascospores (type 1): the youngest stage is simple with thin walls; the first septum is median and starts to accumulate endospore in the corners before the next septa appear; these come simultaneously and accumulate also endospore in the corners; in this stage the first, grey pigment appears; afterwards the endospore increases and fills much of the spores so that the lumina become rounded or lentiform and the outer wall thick; next the spores assume a rather weak, grey-brown colour; finally black lines may become visible in the position of the eusepta; in overaged spores the content dissolves, and the outer wall shrivels. A selection of further species in which this development was observed is given below. The thin-walled initial stages in this group are probably quickly deteriorating, because they are scarce in the preparations. They are not visible in Fig. 3B and 4B, while young stages are common in Fig. 1B, 2B and 5B.

In *Pyrenula cubica*, *P. flavens* and *P. atropurpurea* (Fig. 1B, 2B, 5B) the development is different (type 2): the first, aseptate stage soon gets a thick outer wall; then three thin septa develop, first, the central one, and the spores assume a pale grey tinge; subsequently, the spores assume a more brownish color, endospore is formed and the lumina become rounded; the lumina are less strongly contrasting with the endospore than in the type 1 development and these lumina are less conspicuous under the microscope, without the black surrounding line often seen in type A spores; at age the spores do not develop black lines in the septa, they become more intensely brown, and do not shrivel or get only a few folds.

Species with type 1 ascospore development appear to be widespread in the phylogenetic tree of Gueidan et al. (2016). Such a spore development was found in specimens identified as the following species represented in the tree: *Pyrenula anomala* (Ach.) Vain., *P. aspistea* (Afzel. ex Ach.) Ach., *P. bahiana* Malme, *P. balia* (Kremp.) R.C. Harris, *P. fetivica* (Kremp.) Müll. Arg., *P. mamillana* (Ach.) Trevis., *P. nitida* (Weigel) Ach., *P. nitidula* (Bres.) R.C. Harris, *P. quassiicola* Fée and *P. thelomorpha* Tuck. No species with type 2 spore development seems to be included in the phylogenetic tree, so the phylogenetic significance of this type of spore development remains unclear. Type 2 spore development appears to be less common than type 1 and was found during an examination of about 50 *Pyrenula* species only in *Pyrenula atropurpurea* (Eschw.) Müll. Arg., *P. circumfiniens* Vain., *P. subgregantula* Müll. Arg. and two undescribed species treated above.

The character of non-shriveling, aged ascospores appears to be not restricted to species with spore development type 2 and was observed also in *P. massariospora* (Starbäck) R.C. Harris (one specimen, Sipman 31816) and *P. subpraelucida* Müll. Arg. (several specimens).

The hypothesis that the species with type 2 ascospore development belong to *Lithothelium* rather than *Pyrenula* was rejected because the ascospores do not show a reddish tinge, the key character of the genus (Aptroot, 2012). See also the remarks under *P. cubica*.

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