

Three new species in the lichen genus *Piccolia* (Biatoraceae, lichenized Ascomycota) from the Palaeotropics

Dries Van den Broeck^{1,*}, André Aptroot² & Damien Ertz¹

¹National Botanic Garden of Belgium, domein van Bouchout, Nieuwelaan 38, BE-1860 Meise, Belgium.

²ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, The Netherlands.

*Author for correspondence: Dries.van.den.Broeck@br.fgov.be

Background – As part of taxonomic revisions of tropical African lichens, two species of *Piccolia* are described as new to science while a third unknown one collected in the Solomon archipelago was kindly sent to us by the second author.

Material and Methods – Morphology was studied using a stereo and light microscope. Chemistry was studied with spot tests and TLC.

Key results – *P. congolensis* Van den Broeck, Aptroot & Ertz sp. nov. was collected twice in the Democratic Republic of the Congo and is characterized notably by a thallus with KC+ orange soralia. The material recorded recently from Reunion Island as *P. elmeri* (Vain.) Hafellner was shown to represent a different species characterized by a completely dark brown excipulum and is described here as *P. kalbii* Van den Broeck & Ertz sp. nov. *P. nivea* Van den Broeck, Aptroot & Ertz sp. nov. is only known from the type location on the Solomon archipelago and is characterized by grey pruinose ascomata and a thallus resembling a leprose crust. A worldwide key to the genus is provided.

Key words – *Piccolia*, Biatoraceae, anthraquinones, Congo basin, Reunion Island, Solomon Islands, tropical forests.

INTRODUCTION

Piccolia A.Massal. is a very small genus of crustose lichens with only six species currently accepted. It is characterized by multispored asci, a chlorococcoid photobiont and the presence of anthraquinone pigments in the apothecia (Hafellner 1995, Smith et al. 2009). Hafellner (1995) removed the genus *Piccolia* A.Massal. from synonymy with *Biatorella* De Not., recognizing four species. Recently Knudsen & Lendemer (2007) transferred *B. nannaria* (Tuck.) Zahlbr. to *P. nannaria* (Tuck.) Lendemer & Beeching and Hafellner (2004) transferred *Strangospora ochrophora* (Nyl.) R.A.Anderson to *P. ochrophora* (Nyl.) Hafellner. No molecular data are available so far, so that the taxonomic position of the genus is uncertain. The current version of Myconet includes *Piccolia* as a genus *incertae sedis* within the Lecanoromycetes (Lumbsch & Huhndorf 2010).

Four of the species, *P. elmeri* (Vain.) Hafellner, *P. haematina* (Müll. Arg.) Hafellner, *P. nannaria* (Tuck.) Lendemer & Beeching and *P. wrightii* (Tuck.) Hafellner, are restricted to tropical and subtropical regions. The widely distributed *P. conspersa* (Fée) Hafellner reaches also warm temperate regions and *P. ochrophora* (Nyl.) Hafellner occurs throughout Europe as well as in North and South America (Hafellner

1995, 2004, Knudsen & Lendemer 2007). During a collection trip in D.R.Congo in 2009, one of us (D.E.) discovered a crustose lichen with conspicuous apothecia. Microscopical investigation yielded multispored asci, a chlorococcoid photobiont and the presence in the epihyemium of an orange substance that reacted K+ purplish. During a new expedition in 2012 (COBIMFO project) a second specimen was discovered by the first author. A comparison with herbarium specimens of *P. elmeri* led us to consider the specimens from Reunion Island (van den Boom et al. 2011) to represent an additional new species. The third species, *P. nivea*, was found on the Solomon Islands and sent to us by the second author.

MATERIALS AND METHODS

External morphology was studied using a Wild M38 stereomicroscope. Hand-cut microscopical preparations were mounted in water or a solution of 5% potassium hydroxide, and for ascus structure in Lugol's iodine solution and studied using an Olympus CHR-TR45 and an Olympus BX51 microscope. Colour reactions of the thallus were studied according to Orange et al. (2001). Secondary substances were identified by TLC. For measurements of ascospores the minimum and maximum values are given, all values rounded

to the nearest multiple of 0.5 μm , followed by the number of measurements (N). Measurements refer to dimensions in water. Additional specimens examined for comparison include six species from four herbaria: *Biatoridium monasteriense* (Ahles s.n., BR), *Piccolia conspersa* (Aptroot 41338, 41495, BR), *P. elmeri* (Sipman 39070, B, as *P. haematina*), *P. elmeri* (type, TUR), *P. haematina* (holotype, G), *P. ochrophora* (Van den Broeck 4973, BR), *Strangospora pinicola* (Van den Broeck 2644, BR).

THE NEW SPECIES

Piccolia congolensis Van den Broeck, Aptroot & Ertz, **sp. nov.**

Mycobank No.: MB 805373

Species similar to *Piccolia haematina*, but differs by a KC+ orange-red reaction of the soralia and slightly larger apothecia. – Type: D.R.Congo, Equator Province, Mbangi (± 20 km upstream of Lisala), left bank of the Congo river, at c. 3 km of the river, old secondary forest, on bark of cf. *Microdesmis* sp., 2°6'44.64"N, 21°44'13.2"E, alt. 345 m, 23 Jun. 2009, D. Ertz 14310 (holo-: BR).

Thallus crustose, continuous, with numerous small, convex granules or verrucae, brownish-grey, without a well-defined margin and without a prothallus. Verrucae or granules 60–100 μm , breaking up into whitish soralia. **Soralia** 0.1–0.5 mm, becoming confluent and up to 1 mm. **Photobiont** chlorococcoid, angular-rounded, individual cells clustering together in packets of 3 to 4 cells, 3–7 \times 2.5–6.5 μm . **Ascomata** apothecia, sessile, rounded, heavily red to orange pruinose, slightly constricted at base, (0.2–)0.4–0.8(–1) mm. Disc plane to concave. Margin distinct, young prominent but slightly excluded with age. Thalline exciple not pruinose, the inner part blackish-brown, the outer part beige to whitish with only a few algae. Proper exciple pruinose, slightly raised. **Excipulum** 40–45 μm thick, in inner parts dark brown (c. 10–15 μm thick), in outer parts grey (c. 25–35 μm thick), basally 20–25 μm thick, with crystals dissolving in K. **Epithymenium** composed of orange granules or crystals, K+ pur-

plish, not dissolving. **Hymenium** hyaline, not interspersed, 80–100 μm , I+ deep blue, KI+ deep blue. **Subhymenium** pale brown, 10–20 μm . **Hamathecium** of paraphyses, 1–1.5 μm , partly entwining the asci, sparsely branched in the epithymenium, the apices not distinctly enlarged. **Asci** clavate, 55–75 \times 10–15 μm , I+ deep blue, KI+ deep blue, multispored (c. 100–200 spores). **Ascospores** globose, simple, thin walled, colourless, (1.5–)1.93(–2.5) μm (N= 80), I-, KI-. **Conidiomata** not observed. **Chemistry**: thallus C-, K-, KC-, P-, UV-. Orange pigments present in the epithymenium, K+ purplish. Soralia C+ slightly orange, K-, KC+ orange, P-, UV- (specimen Van den Broeck 5583) or UV+ white (holotype). TLC (solvent G) of the apothecia revealed two substances: parietin and a UV+ orange spot before heating, becoming reddish-brown after heating, just below the spot of parietin, possibly a second anthraquinone. In the thallus, two other substances, showing a UV+ green-olive spot after heating, were found (R_f 55 and R_f 61). A comparison of these spots with controls for thiophanic, arthothelin, thiophanic and granulosis (others substances known to be responsible for a KC+ orange reaction) did not allow identification of those substances. TLC of the thallus with solvent C revealed two spots with R_f 21 and R_f 32 and in solvent B with R_f 26 and R_f 36. Fig. 1A & B.

Distribution – Known only from forests in the Congo basin of D.R.Congo.

Habitat – On bark of lianas and hardwood tree species in dense and rather well-preserved evergreen and semi-evergreen tropical forests at low altitudes (345–427 m).

Other collections examined – **D.R.Congo**: Oriental Province, Yangambi, biospherical reserve, evergreen forest dominated by *Gilbertiodendron dewevrei*, on liana sp., 0°48'28.62"N, 24°31'42.3"E, alt. c. 427 m., 23 Oct. 2012, Van den Broeck 5583 (BR).

Remark – The species is very close to *Piccolia haematina* (Müll.Arg.) Hafellner (fig. 1C & D) but differs by a KC+ orange-red reaction of the soralia and slightly larger apothecia (Hafellner 1995). It differs from *P. elmeri* by the absence of a purplish pigment in the excipulum and the presence of soralia.

Key to the species of *Piccolia*

1. Apothecia white or grey pruinose, K-.....2
- 1'. Apothecia orange to red or purplish pruinose, K+ or K-.....3
2. Thallus composed of smooth granules or verrucae of 100–150 μm in diam.....*P. wrightii*
- 2'. Thallus sorediate; soredia of 40–100 μm in diam.....*P. nivea*
3. Thallus with soredia.....4
- 3'. Thallus without soredia.....5
4. Soralia KC-; apothecia 0.2–0.5 mm.....*P. haematina*
- 4'. Soralia KC+ orange; apothecia 0.4–0.8 mm.....*P. congolensis*
5. Excipulum with purplish-red pigment.....*P. elmeri*
- 5'. Excipulum without purplish-red pigment.....6
6. Thallus smooth; ascospores 3–4 μm*P. ochrophora*
- 6'. Thallus verrucose; ascospores 1.5–3 μm7
7. Apothecia orange pruinose; thallus not whitish.....8
- 7'. Apothecia purplish pruinose; thallus whitish.....*P. kalbii*
8. Thallus orange; apothecia 0.4–0.8 mm.....*P. conspersa*
- 8'. Thallus yellow-green; apothecia 0.25 mm.....*P. nannaria*

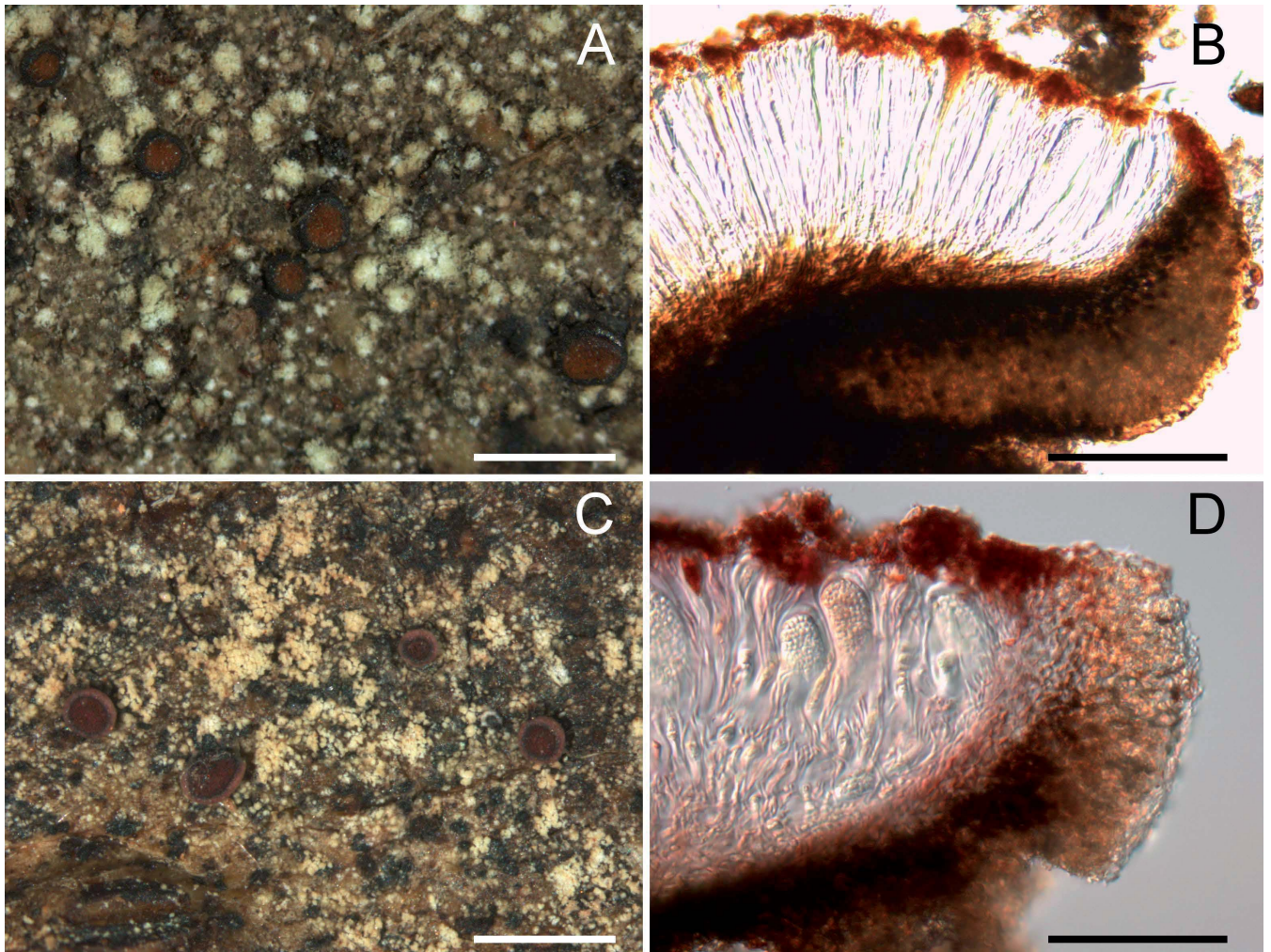


Figure 1 – Thallus with apothecia and section through apothecium of *Piccolia* species: A & B, *Piccolia congolensis*; C & D, *P. haematina*. A & C, thallus with apothecia; B & D, section through apothecium. A & B from Ertz 14310 (BR); C & D from Bailey 600 (holo:- G). Scale bars: A & C = 1 mm; B = 100 μ m; D = 50 μ m.

***Piccolia kalbii* Van den Broeck & Ertz, sp. nov.**

Mycobank No.: MB 805374

Species similar to *Piccolia elmeri*, but differs by an entirely dark brown excipulum and the absence of a purplish pigment in the excipulum. – Type: Maskaranen/ Réunion: wenige km S von le Brûlé (S von St-Denis), in einem Forst aus *Cryptomeria japonica*, 14 Aug. 1991, 20°55'S, 55°26'E, alt. 1000–1050 m, K. & A. Kalb 38915 (holo:- herb. Kalb).

Thallus crustose, continuous, composed of small, not disintegrating, convex granules or verrucae, white-grey, slightly shiny, without a well-defined margin and without a prothallus. Verrucae or granules 90–270 μ m. **Photobiont** chlorococoid, cells rounded, 5.5–7 \times 5.5–6.5 μ m. **Ascomata** apothecia, sessile, rounded, purplish pruinose, slightly constricted at base, (0.4–)0.5–1 mm. Disc plane to concave, becoming convex in old apothecia. Thalline margin absent. Proper exciple purplish pruinose, slightly raised. Margin distinct, young prominent but slightly excluded with age. **Excipulum** dark brown, 80–95 μ m thick, biatorine, composed of brown, branched, non-inflated, anastomosing hyphae of 1 μ m thick, forming more or less a network. **Epihymenium** composed of

orange-brown to reddish granules or crystals, K+ purplish, the red colour dissolving leaving colourless to dark roundish crystals of 1.5–3 \times 1–2.5 μ m. **Hymenium** hyaline, not interspersed, 105–135 μ m, I+ deep blue, KI+ deep blue. **Hypothecium** dark brown, 45–50 μ m. **Hamathecium** of paraphyses, 1.5 μ m, sparsely branched, the apices not distinctly enlarged. **Asci** obclavate, 60–70 \times 14–18 μ m, I+ deep blue, KI+ deep blue, multispored (c. 100–200 spores). **Ascospores** globose, simple, thin walled, colourless, (2–)2.45(–2.5) μ m (N= 60), I-, KI-. **Conidiomata** not observed. **Chemistry**: thallus C-, K-, KC-, UV-. As the available specimens are too small, TLC was not performed by us. The type specimen was analysed by Klaus Kalb who noted “piccoliansäure” (43/66/56, major) and “subpiccoliansäure” (38/53/34, major). Fig. 2D & E.

Distribution – Known only from Reunion Island.

Habitat – On bark in tropical forests at high altitudes (1000–1600 m).

Other collections examined – **Reunion Island**: Le Tampon, 2008, P.P.G. van den Boom 40751 (herb. van den Boom); zwischen le Brûlé (S von St-Denis) und Plaine des Cnicots. Tropischer Regenwald mit *Nastus borbonicus*, *Acacia heterophylla*, *Cyathea borbon-*

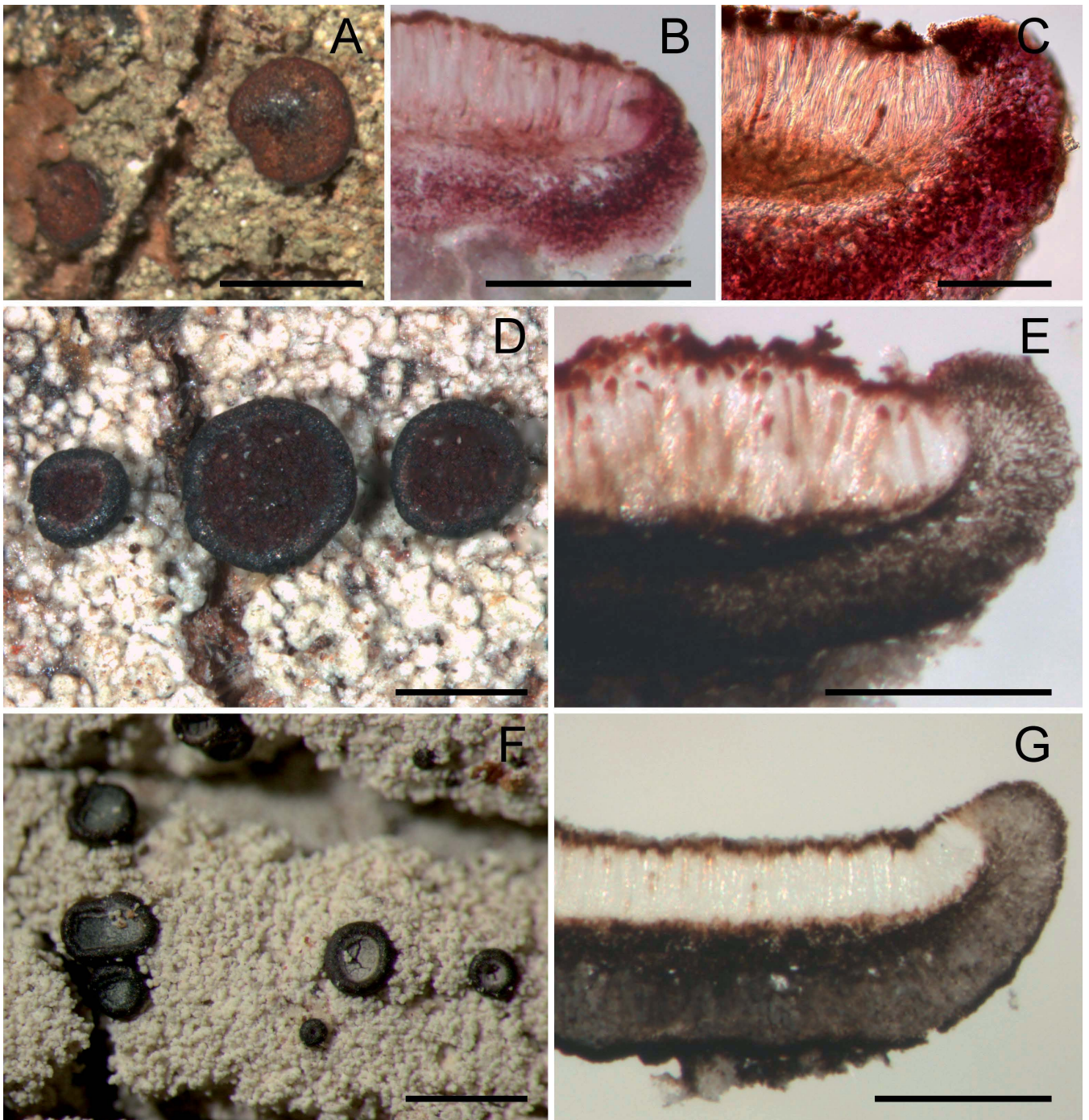


Figure 2 – Thallus with apothecia and section through apothecium of *Piccolia* species: A–C, *P. elmeri*; D–E, *P. kalbii*; F–G, *P. nivea*. A, D & F, thallus with apothecia; B, C, E & G, section through apothecium. A & B from *P. elmeri* 14877 pr. p. (type:- TUR); C, from Sipman 39070 (B); D & E, from Kalb 38915 (herb. Kalb); F & G, from Hill 11144 (ABL). Scale bars: A, D = 0.5 mm; B, E, G = 200 μm; C = 100 μm; F = 1 mm.

ica, *Philippia montana* etc., alt. 1400–1600 m, 20°57'S, 55°27'E, 15 Aug. 1991, K. & A. Kalb 33597 (herb. Kalb).

Etymology – This new species is named in honour of the lichenologist Klaus Kalb who collected the type specimen and in recognition of his many contributions to tropical lichenology.

Remark – The species is very close to *Piccolia elmeri* (fig. 2A–C) but differs by the absence of a purplish pigment in the excipulum that is entirely dark brown (fig. 2E). The material cited here was published as *P. elmeri* in a preliminary checklist for Reunion Island (van den Boom et al. 2011).

Piccolia nivea Van den Broeck, Aptroot & Ertz, **sp. nov.**

MycoBank No.: MB 805375

Species similar to the neotropical *Piccolia wrightii* (Tuck.) Hafellner but differs by the presence of confluent soralia so that the thallus resembles a leprose crust. – Type: Solomon Islands, Santa Isabel Island, between Tatamba and Rego, alt. 0 m, 2 Oct. 1965, *D.J. Hill* 11144 (holo-: BM; iso-: ABL).

Thallus crustose, granulose, corticate, cream-coloured, smooth with soralia, becoming soon confluent so that the thallus resembles a leprose crust, very fragile, with crystals. **Soredia** smooth, without visible individual hyphae, 40–100 µm diam. **Photobiont** chlorococcoid, cells rounded, 4.5–9 × 4.5–8.5 µm. **Ascomata** apothecia, lecideine, sessile, rounded, black, slightly to heavily grey pruinose, 0.4–1 mm. Disc plane to concave. Margin distinct, glossy, young prominent but slightly excluded with age. Thalline exciple absent. Proper exciple not pruinose, mostly blackish but sometimes partly with a brown tinge. **Excipulum** laterally 85–95 µm thick, in inner parts brown (c. 10–15 µm thick), in outer parts dark brown (c. 25–35 µm thick), with cubic colourless crystals up to 4 µm, not dissolving in K or H₂SO₄ (not calcium-oxalate crystals), basally 65–75 µm thick, I-, KI-. **Epihymenium** 10–15 µm thick, brown, composed of paraphyses with dark brown walls near or at the end and then sometimes swollen up to 3.5 µm, with colourless crystals on top, not dissolving in K. **Hymenium** hyaline, not interspersed, I+ dark blue, KI+ dark blue. **Subhymenium** orange-brown, a little bit lighter than the colour of the epihymenium, 10–15 µm thick. **Hypothecium** dark brown, 45–55 µm thick. **Hamathecium** of paraphyses, 1–1.5 µm thick, partly entwining the asci, sparsely branched, I-, KI-. **Asci** clavate, 55–65 × 10–20 µm, I+ deep blue, KI+ deep blue, multispored (c. 100 or less spores) with a K/I+ blue outer layer. Ascospores globose, simple, thin walled, colourless, (4–)4.67(–5) µm (N = 60), I-, KI-. **Conidiomata** not observed. **Chemistry**: thallus and soralia C-, K-, KC-, P-, UV-. Apothecial sections K-. TLC (solvent G) revealed five unidentified lichen substances. Two of them showed a UV+ light blue spot (R_f 50 and R_f 57), one a UV+ dark blue spot (R_f 66) and two a UV+ orange spot (R_f 75 and R_f 79) after heating. Fig. 2F & G.

Distribution – Known only from Santa Isabel Island in the Solomon Islands archipelago.

Habitat – On bark of tree in coastal lowland rainforest.

Remark – The corticolous or muscicolous genera *Biatoridium*, *Maronea*, *Maronella*, *Maronina*, *Piccolia* and *Strangospora* are all characterized by multispored asci (Hafellner & Rogers 1990, Hafellner 2004, Smith et al. 2009). *Maronea*, *Maronella* (Hafellner 2004) and *Maronina* (Hafellner & Rogers 1990) have elliptic to bacilliform ascospores. The ascospores of *Biatoridium* and *Strangospora* are globose but the first genus has asci with a multi-layered K/I+ apical dome and an inner cap K/I+ intense blue while *Strangospora* is characterized by asci with a strongly thickened K/I+ blue wall, particularly at the apex (Smith et al. 2009). We were able to observe those characteristics with a specimen of *Biatoridium monasteriense* and one of *Strangospora pinicola*. *Piccolia* is thought to have a K/I+ gelatinous outer layer and a young I+ blue, later I- apical dome. This characteristic was not observable with the type specimen due to the impossibility

of separating the asci clearly from the hymenial gel and the conglutinated paraphyses of the subhymenium. We decided nevertheless to place the species provisionally in *Piccolia* because of the well-developed true crystalline exciple, the granular epihymenium with crystals not dissolving in K and the dark brown hypothecium. Further studies are necessary to confirm the generic placement of this species.

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