

## A remarkable new *Ramonia* (*Gyalectaceae*) from Brazil, with a key to the species

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**Abstract:** A new corticolous lichen species *Ramonia variospora* is described from Brazil. It has a verrucose thallus, concave, initially almost closed apothecia and variably 8 or *c.* 32–64, 1-septate ascospores per ascus; when 8 per ascus, ellipsoid and  $16\text{--}22 \times 6\text{--}10 \mu\text{m}$ ; when *c.* 32–64 per ascus, broadly ellipsoid and  $10\text{--}14 \times 6\text{--}8 \mu\text{m}$ . A revised world key is given to all currently known species of *Ramonia*.

**Key words:** Brejo de Altitude, lichen, multisporous, *Ostropales*, Pernambuco

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### Introduction

*Ramonia* is a widespread genus of the *Gyalectaceae*, species of which occur equally in tropical and temperate regions, often on soft bark or decaying wood, rarely on rock or terricolous (Coppins *et al.* 1994; Lendemer *et al.* 2009). Species of this genus have tiny, round apothecia with concave discs, and a hymenium with paraphyses and periphyses.

So far 27 species are known in *Ramonia*, only eight of which were treated in the revision of the genus (Vězda 1966), the others being described in subsequent papers (Vězda 1967, 1973; Sherwood 1977; Thor & Vězda 1984; Coppins 1987; Canals & Gómez-Bolea 1992; Coppins *et al.* 1994; Øvstedal & Gremmen 2001; Lendemer & Knudsen 2008; Lendemer *et al.* 2009; Gagarina & Stepanchikora 2013).

During studies by the authors on lichen ecology and diversity in mountain forests in the state of Pernambuco in the north-eastern

region of Brazil, an undescribed species was encountered, which is described below. An updated artificial world key to all currently known species of *Ramonia* is given, with their currently known distribution ranges. The key is based on Lendemer & Knudsen (2008), but includes the additional species *R. subantarctica* Øvstedal, which was already described but not included in that key. The key leaves out the species that have been transferred to the *Graphidaceae* [in the genera *Pseudoramonia* and *Topeliopsis*; see Rivas Plata *et al.* (2010) for references and keys to these species], as well as *Ramonia athallina* Sherwood, which was referred to *Odontura raphidospora* (Rehm) Clem. by Coppins (1987). Vězda (1966) recognized three different formal sections within the genus *Ramonia*. The type species and the other tropical species are all corticolous, not especially short-lived, and have a relatively well-developed thallus and polysporous asci. Examples are *Ramonia valenzueliana* (Mont.) Stizenb. (Fig. 3B) with a verruculose thallus, and *R. malmei* Vězda (Fig. 3C) and *R. kandleri* Kalb (Fig. 3D) with a granulose thallus. The new species is close to the type species. The genus also, however, contains many temperate (and even one subantarctic) species which are generally short-lived, occur on various substrata including wood, soil and rock, all with a much

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less developed thallus or are even not lichenized at all, as is the case in many specimens of *R. interjecta* Coppins (Fig. 3E, which illustrates a non-lichenized specimen with an apothecium protruding through bark covered with free-living algae), and the asci are less often polysporous. The species now united in *Ramonia* may even ultimately prove to belong to different genera, but separating them falls beyond the scope of this study.

The new species was found in Triunfo, one of the few places with mountain forest in the lowland state of Pernambuco. The lichens of these mountain forests, called Brejo de Altitude and part of the former more extensive Atlantic Rainforest biome (Thomas & Barbosa 2008) are not yet well studied. The only lichen records from Brejo de Altitude are those by Cáceres (2007).

### Material and Methods

Identification and descriptive work was carried out in Itabaiana, Universidade Federal de Sergipe, using a Leica EZ4 stereomicroscope and a Leica DM500 compound microscope, and also in Soest 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, in which all measurements were also taken. Ascospore measurements are given as average (in italics) +/- the standard deviation, with extremes between brackets. The chemistry of the type specimen was investigated by thin-layer chromatography (TLC) using solvent C (Orange *et al.* 2001).

The following is a selection of specimens studied.

*Ramonia interjecta*: **The Netherlands**: Gelderland: Ermelo, Groevenbeekse heide, 1999, *A. Aptroot* (BR).

*Ramonia kandleri*: **Brazil**: Minas Geraes: Catas Altas, Parque Natural do Caraça, 1997, *A. Aptroot* 40827 (ABL, SP).

*Ramonia malmei*: **Argentina**: Misiones: Puerto Iguazú, near Hotel Selvático Don Horacio, 2013, *L. I. Ferraro*, *A. Aptroot* & *M. E. S. Cáceres* 10526 (ABL, CTES).

*Ramonia micrococca*: **Kenya**: Nyanza: Kisumu-Londiani, Tinderet Forest Reserve, 1949, *R. A. Maas Geesteranus* 5135 (BR, L).

*Ramonia microspora*: **Indonesia**: Java: Jogjakarta, University Garden, 14 vi 1995, *T. Gandjar* (BR).

*Ramonia valenzueliana*: **Argentina**: Misiones: Puerto Iguazú, near Hotel Selvático Don Horacio, 2013, *L. I. Ferraro*, *A. Aptroot* & *M. E. S. Cáceres* 10626 (ABL, CTES).

*Ramonia variospora*: type (URM).

### The New Species

#### *Ramonia variospora* Sobreira, Aptroot & M. Cáceres sp. nov.

MycoBank No.: MB 809917

Similar to *Ramonia valenzueliana* but with a verrucose thallus, concave, initially almost closed apothecia and variably 8 or *c.* 32–64, 1-septate ascospores per ascus; when 8 per ascus ellipsoid and 16–22 × 6–10 µm; when *c.* 32–64 per ascus broadly ellipsoid and 10–14 × 6–8 µm.

Type: Brazil, Pernambuco, Triunfo, Carro Quebrado trail, 7°52'S, 38°06'W, on bark of tree, *c.* 877 m alt., 1 August 2013, *P. N. B. Sobreira* 46 (URM—holotype).

(Figs 1, 2 & 3A)

*Thallus* crustose, corticate, slightly shiny, grey, cracked into angular areoles of *c.* 0.1–0.3 mm diam. which soon become irregular hemispherical warts, at least towards the centre of the thallus where the thallus is *c.* 0.2 mm thick, not surrounded by prothallus; cortical cells almost globose, *c.* 3–6 µm diam. *Photobiont* trentepohlioid, cells *c.* 10–16 × 8–12 µm.

*Apothecia* numerous, dispersed, sessile, lentiform, on the outside covered with several thallus warts, round, 0.3–0.5 mm diam.; *disc* concave, pinkish, margin covered by thalline warts, *c.* 0.1 mm wide, almost closing inward over the disc leaving a hollow space over the hymenium. *Wall* almost surrounding the whole ascoma, reddish brown on the outside, hyaline inside; lumina elongated, in the central part *c.* 6–10 × 1.5–2.5 µm; near the ostiole almost globose. *Ostiole* almost closed when apothecia young, reddish brown when viewed from above and where not covered by thallus, granular. *Excipulum* a thin brown cupular layer, *c.* 50 µm thick. *Hymenium* not interspersed, after pretreatment with KOH with Lugol + blue, 120–170 µm high. *Paraphyses* unbranched, 2.0–2.5 µm wide, apices not swollen but filaments toward the tip slightly moniliform and up to 3.5 µm wide. *Periphyses* unbranched, numerous, hyaline, 13–29(–58) × 4.5–5.5 µm, the longer ones visible from the outside as glassy hairs in the empty chamber below the ostiole. *Asci* soon disintegrating (no mature ascus tips observed), 90–129 × 10–35 µm, containing either 8 or

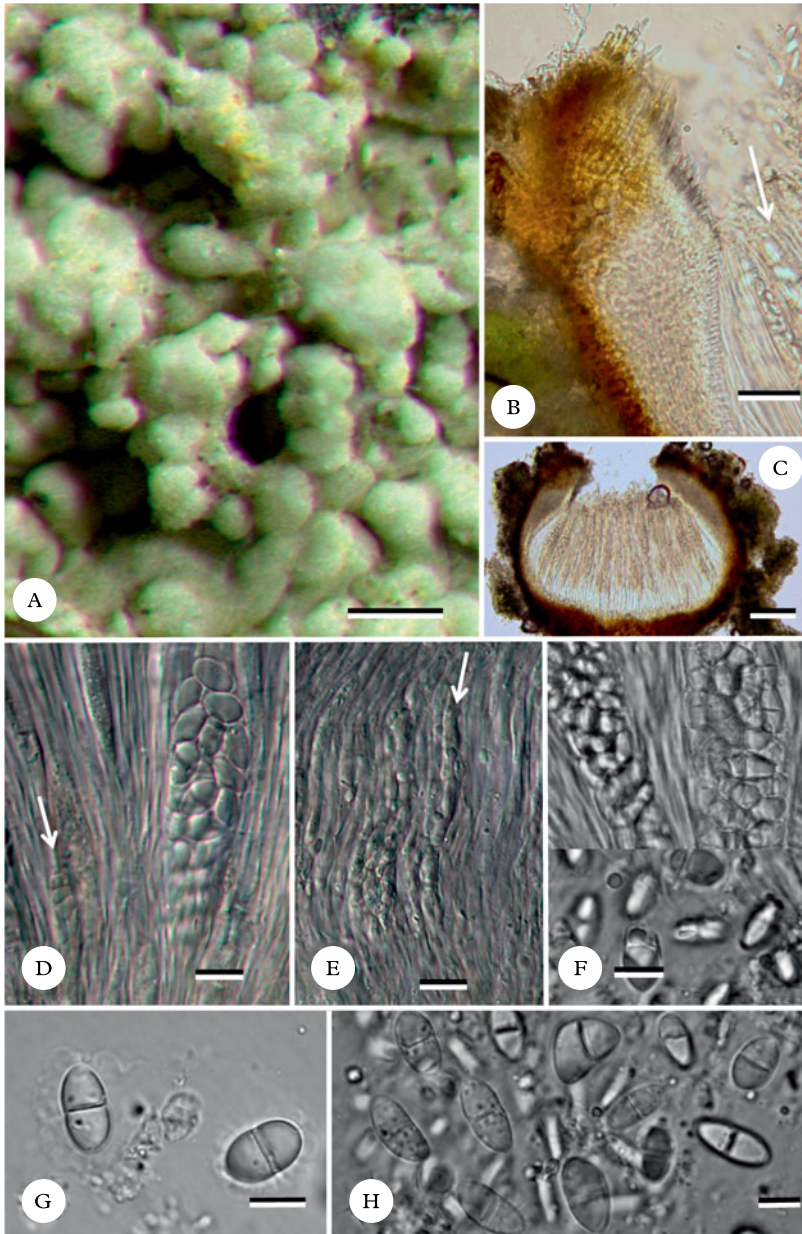


FIG. 1. *Ramonia variospora*, holotype. A, habitus; B, ascoma margin showing periphyses, a polysporous ascus and the tip of an 8-spored ascus (at white arrow), this is a different ascoma than D and E; C, section through ascoma; D–F, parts of the hamathecium of three different ascomata; D, section through part of young ascoma showing octosporous ascus (lower left, at white arrow) and polysporous ascus (right) with young, still aseptate ascospores; E, section through part of old ascoma showing octosporous ascus (right, at white arrow) and polysporous ascus (left); F, section through part of ascoma showing young polysporous ascus (left) and mature polysporous ascus (right), this is the same ascoma as B which also shows octosporous asci; G, ascospores with gelatinous sheaths; H, released ascospores of varying size and shape. Scales: A = 0.1 mm; B & E = 25  $\mu$ m; C = 50  $\mu$ m; D, F, G & H = 10  $\mu$ m. In colour online.

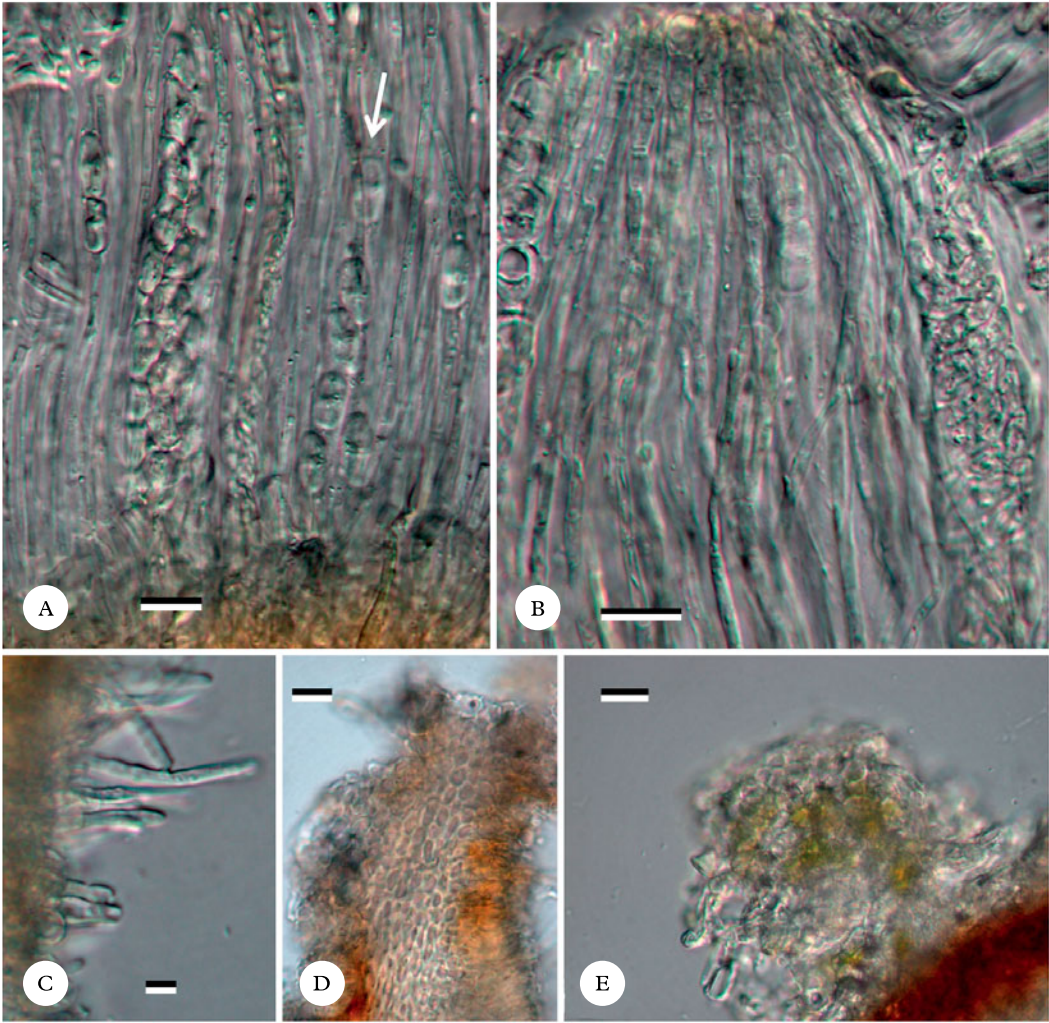


FIG. 2. *Ramonia variospora*, holotype. A, hamathecium showing paraphyses and polysporous ascus and octosporous ascus (at white arrow); B, hamathecium showing somewhat moniliform paraphyse tips; C, periphyses; D, section through ascoma rim showing cell lumina inside ascoma wall; E, thallus wart on ascoma, showing trentepohlioid algal and cortical cells. Scales: A–E = 10  $\mu$ m. In colour online.

*c.* 32–64 ascospores, variable within a hymenium; 8-spored asci cylindrical and slender; multisporous asci fusiform and wide. *Ascospores* hyaline, young simple, 1-septate when mature; when 8 per ascus ellipsoid and (16–) 16.4–18.3–20.2(–22)  $\times$  (6–)6.8–7.4–8.0(–10)  $\mu$ m ( $n = 15$ ); when *c.* 32–64 per ascus broadly ellipsoid and (10–)11.8–12.7–13.6(–14)  $\times$  (6–)6.7–7.2–7.7(–8)  $\mu$ m ( $n = 15$ ),

smooth, but surrounded by a 3–5  $\mu$ m thick gelatinous sheath.

*Pycnidia* not observed.

*Chemistry.* TLC: no substances.

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

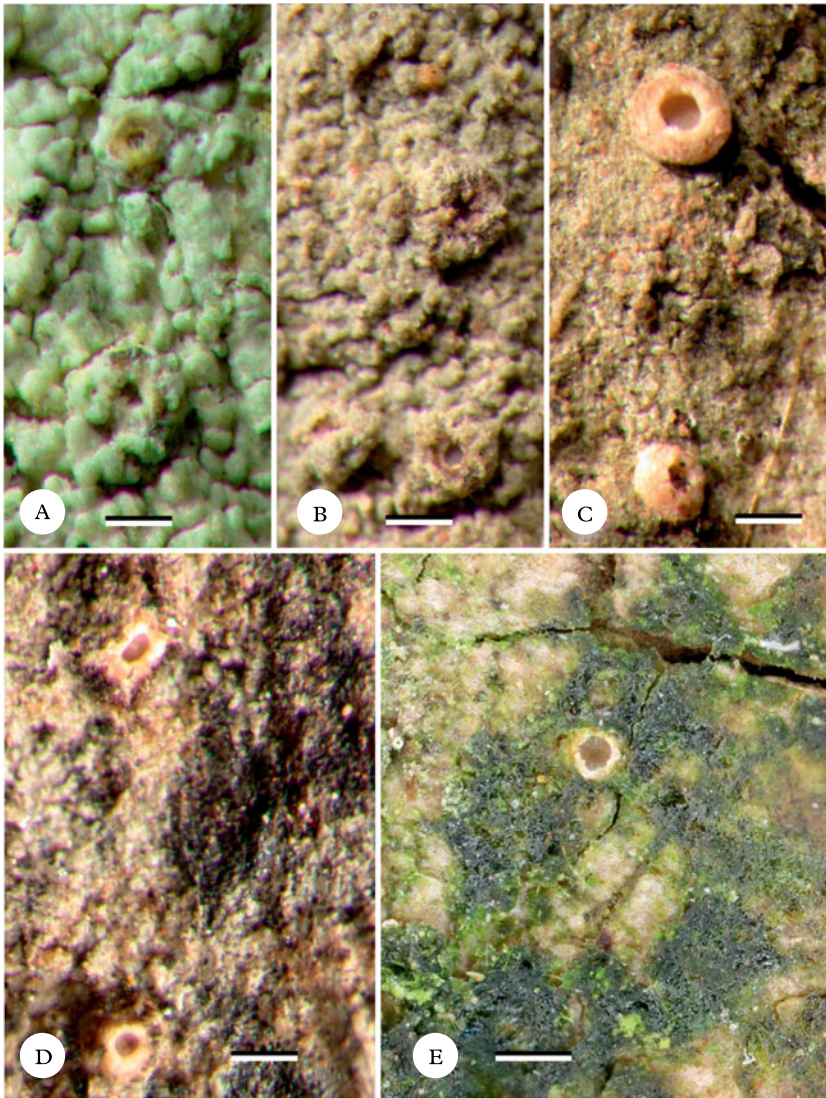


FIG. 3. Habitus of *Ramonia* species. A, *R. variospora*, holotype; B, *R. valenzueliana*, Ferraro et al. 10626; C, *R. malmei*, Ferraro et al. 10526; D, *R. kandleri*, Aptroot 40827; E, *R. interjecta*, leg. D. G. Zimmermann, 2011, Germany, Nordrhein-Westphalen, Solingen, picture by N. Stapper. Scales: A–E = 0.3 mm. In colour online.

*Discussion.* The new species is superficially similar to a tiny *Ocellularia*, but differs by the sessile apothecia. The similarity with *Ramonia valenzueliana* (Fig. 3B) is clear, although that species differs by the stellar slits around the ostiole, the constantly polysporous asci and the less verrucose thallus. The new species has the area around the ostiole obscured by thallus warts.

The new species is surprising because the asci within one apothecium vary as to the number of ascospores, either 8 or 32–64, and the ascospores are ellipsoid in 8-spored asci, while they are broadly ellipsoid in multi-spored asci; they are similar in width, but much longer in 8-spored asci. This is not an occasional aberration, as it was observed in all sectioned ascomata (3 out of *c.* 50

ascomata present on the type specimen were sectioned), and illustrations from three different ascomata are represented in the figure. The difference is especially striking in the youngest sectioned ascoma, where the ascospores are still aseptate: it can be seen that the 8-spored asci are much smaller than the multispored asci.

The number of ascospores is the result of multiple divisions of the ascospore-mother cell. During mitosis, ascospores divide simultaneously and (usually) repeatedly (Webster & Weber 2007). Therefore, the number of ascospores is often a factor of 2; 1, 2, 4, 8, 16, 32, 64 and 128 are the usual numbers. Multispored asci occur in various monophyletic lichen groups, the majority in three families (*Acarosporaceae*, *Biatorrellaceae* and *Theleocarpaceae*) with species predominantly with multispored asci, as well as in 16 complete genera (Aptroot & Schumm 2012), but also in isolated species or species groups within 40 other genera (such as *Ramonia*). See Aptroot & Schumm (2012) for a listing of these groups, to which *Amandinea* and *Relicina* should still be added. Seemingly multispored asci also occasionally originate in a different way by means of the formation of ascoconidia (Hafellner & Bellemère 1983; Ertz & Diederich 2004; Lücking 2008). This is the formation by larger ascospores of tiny conidia, while the ascospores are still within the

ascus. In lichenized fungi, this is occasionally seen, but only in species with large muriform ascospores, viz. so far in *Brigantiaea* (Hafellner & Bellemère 1983), *Calopadia* (Santesson 1952), *Gyalidea* (personal observation), *Oevstedalia* (Ertz & Diederich 2004), *Topeliopsis* (Frisch & Kalb 2006), and *Tricharia* (Ferraro 2000). Although the final developmental stage of ascoconidia can seem similar to those of multispored asci with mitotic ascospores, it is usually possible to distinguish the two phenomena by the observation of intermediate stages. So the ascoconidia of *Oevstedalia* are clustered in 8 balls representing the parent ascospores (Ertz & Diederich 2004). There is no such indication that the spores in the multispored asci in the new *Ramonia* are formed by ascoconidiogenesis.

Reports of species that have asci either 8-spored or multispored are scarce. The only cases seem to be two species of *Cryptolechia* (which is also in the *Gyalectaceae*), viz. *C. nana* (Tuck.) D. Hawksw. & Dibben with ascospores (8–)12–16 per ascus and *C. myriadella* (Nyl.) D. Hawksw. & Dibben also with ascospores 8–12(–16) per ascus (Kalb 2007). This is, however, a much less spectacular case than the dimorphism of the new species described here, which combines within each studied hymenium asci with 8 and with 32–64 ascospores, without intermediates.

### World key to the species of *Ramonia*

This key also mentions the world distribution and the substratum if not tree bark.

- |      |   |   |
|------|---|---|
| 1    | Asci within one apothecium with variable number of ascospores, either 8 or 32–64; ascospores 16–22 × 6–10 µm when 8 per ascus, 10–14 × 6–8 µm when asci polysporous; Brazil . . . . . | <b>R. variospora</b> Sobreira <i>et al.</i> |
|      | Asci either polysporous or not, not variable within one apothecium . . . . .  | 2   |
| 2(1) | Asci polysporous (> 8 spores per ascus) . . . . .   | 3   |
|      | Asci with 8 (or fewer) spores per ascus . . . . .   | 10  |
| 3(2) | Ascospores simple . . . . .   | 4   |
|      | Ascospores septate . . . . .  | 7   |
| 4(3) | Ascospores globose, 2·2–3·5 µm diam.; Kenya . . . . .   | <b>R. micrococca</b> Vězda                  |
|      | Ascospores ellipsoid . . . . .  | 5   |

- 5(4) Ascospores  $< 3 \mu\text{m}$  wide, narrowly ellipsoid; tropical America, Papua New Guinea & Indonesia. . . . . **R. microspora** Vězda  
Ascospores  $> 3 \mu\text{m}$  wide; ellipsoid. . . . . 6
- 6(5) Ascospores  $4\text{--}6 \times 3 \mu\text{m}$ ; apothecia  $0.2\text{--}0.4 \text{ mm}$  diam.; Brazil . . . . .  
. . . . . **R. intermedia** Kalb  
Ascospores  $8\text{--}9 \times 4.0\text{--}4.5 \mu\text{m}$ ; apothecia  $0.2\text{--}0.6 \text{ mm}$  diam.; Brazil . . . . .  
. . . . . **R. kandleri** Kalb
- 7(3) Ascospores 16–24 per ascus, 1–3-septate to submuriform,  $9\text{--}14 \times 4\text{--}5 \mu\text{m}$ ; Nepal. .  
. . . . . **R. nepalensis** Thor & Vězda  
Ascospores  $> 24$  per ascus, 1–3-septate . . . . . 8
- 8(7) Ascospores 1-septate,  $10\text{--}14 \times 5\text{--}6 \mu\text{m}$ ; tropical America . . . . .  
. . . . . **R. valenzueliana** (Mont.) Stiz.  
Ascospores more than 1-septate . . . . . 9
- 9(8) Apothecia  $0.4 \text{ mm}$  diam.; ascospores 3-septate,  $13\text{--}20 \times 4\text{--}6 \mu\text{m}$ ; subtropical North  
America. . . . . **R. absconsa** (Tuck.) Vězda  
Apothecia  $0.3\text{--}0.6 \text{ mm}$  diam.; ascospores 1–3-septate,  $8\text{--}14(16) \times 4\text{--}6 \mu\text{m}$ ;  
Tanzania. . . . . **R. elixii** Kalb
- 10(2) Ascospores vermiform to acicular (length: breadth ratio  $> 10:1$ ) . . . . . 11  
Ascospores ellipsoid to obovoid or fusiform (length: breadth ratio  $< 10:1$ ) . . . . . 13
- 11(10) Thallus corticolous; ascospores  $> 8$ -septate . . . . . 12  
Thallus saxicolous; ascospores 3-septate,  $45\text{--}60 \times 3\text{--}4 \mu\text{m}$ ; Southern California . . .  
. . . . . **R. vermispورا** Lendemer & K. Knudsen
- 12(11) Apothecia  $0.2\text{--}0.4 \text{ mm}$  diam.; ascospores 8–10-septate,  $50\text{--}60 \times 3\text{--}5 \mu\text{m}$ ; Western  
Europe . . . . . **R. subsphaeroides** (Tav.) Vězda  
Apothecia  $0.4\text{--}0.7 \text{ mm}$  diam.; ascospores 8–14-septate;  $45\text{--}75 \times 3.5\text{--}4.0 \mu\text{m}$ ;  
Western Europe. . . . . **R. chrysophaea** (Pers.) Vězda
- 13(10) Ascospores muriform. . . . . 14  
Ascospores transversely septate. . . . . 16
- 14(13) Apothecia pale; ascospores  $21\text{--}38(45) \times 9\text{--}14 \mu\text{m}$ ; British Isles . . . . .  
. . . . . **R. dictyospora** Coppins  
Apothecia black. . . . . 15
- 15(14) Ascospores  $28\text{--}45(50) \times 8\text{--}13 \mu\text{m}$ ; British Isles . . . . . **R. nigra** Coppins  
Ascospores  $18\text{--}22 \times 12\text{--}15 \mu\text{m}$ ; on *Andreaea* moss on rock, Marion Island. . . . .  
. . . . . **R. subantarctica** Øvstedal
- 16(13) Thallus saxicolous or terricolous; ascospores 3-septate . . . . . 17  
Thallus corticolous; septation various . . . . . 20
- 17(16) Apothecia pale; on calcareous rocks; ascospores fusiform,  $18\text{--}23 \times 4\text{--}6 \mu\text{m}$ ; on lime-  
stone, Western Europe. . . . . **R. calcicola** Canals & Gómez-Bolea  
Apothecia black; on weakly or non-calcareous rocks or soil . . . . . 18
- 18(17) Ascospores obovate,  $23\text{--}27 \times 7\text{--}8 \mu\text{m}$ ; on weakly calcareous soils, California. . . . .  
. . . . . **R. ablephora** (Nyl. ex Hasse) R. C. Harris  
Ascospores fusiform; on non-calcareous rocks . . . . . 19
- 19(18) Ascospores  $17\text{--}25 \mu\text{m}$  long; on granite and decomposed granite, California . . . . .  
. . . . . **R. gyalectiformis** (Zahlbr.) Vězda  
Ascospores  $25\text{--}31 \mu\text{m}$  long; on serpentinite, California . . . . .  
. . . . . **R. extensa** Lendemer *et al.*  
Ascospores 3–4 septate,  $12.5\text{--}20.0 \times 3.5\text{--}5.0 \mu\text{m}$  . . . . **R. himelbrantii** Gagarina

- 20(16) Ascospores >3-septate, >20 µm long . . . . . 21  
 Ascospores 1–3-septate, <20 µm long . . . . . 22
- 21(20) Ascospores with perispore, 20–30 × 4–6 µm; apothecia 0.25–0.40 mm diam.;  
 Europe . . . . . **R. luteola** Vězda  
 Ascospores without perispore, 24–43 × 4.5–7.0 µm; apothecia 0.3–0.4 mm diam.;  
 not lichenized; immersed in bark or in soft wood, Europe . . . . .  
 . . . . . **R. interjecta** Coppins
- 22(20) Ascospores 1-septate, 9–12 × 4–5 µm; tropical America . . . . . **R. malmei** Vězda  
 Ascospores 3-septate . . . . . 23
- 23(22) Apothecia 0.1–0.2 mm diam.; ascospores narrowly ellipsoid; Australia. . . . .  
 . . . . . **R. leptospora** (Müll. Arg.) Vězda  
 Apothecia >0.2 mm diam.; ascospores ovoid-ellipsoid . . . . . 24
- 24(23) Ascospores >15 µm long; Australia . . . . . **R. eungellae** Kalb  
 Ascospores <15 µm long . . . . . 25
- 25(24) Apothecia 0.5–0.7 diam.; Indonesia, Brazil, Australia . . . . . **R. cupellina** Vězda  
 Apothecia 0.3–0.4 diam.; Florida . . . . . **R. rappii** Vězda

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