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Observations on *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr. and its occurrence in Portugal and the Azores

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ABSTRACT

Introduction. Recent bryological research in Portugal has revealed the occurrence of a little-known segregate of the *Riccia sorocarpa* group, representing the first record for mainland Portugal and the Azores of *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr. New data are presented on the taxonomy and phytogeography of this globally rare taxon.

Methods. A revision of approximately 200 samples of *Riccia sorocarpa* s.l. from selected Portuguese herbaria, together with some recent field collections, was carried out, based on the results of light and scanning electron microscopy.

Key results and conclusions. Our findings confirm that *Riccia sorocarpa* subsp. *erythrophora* is a distinct subspecies, and show that morphological differences of the thallus, primarily the violet-red purple bases of the ventral scales, are the most important characters for its delimitation. Scanning electron micrographs of spores of this taxon are presented for the first time. Phytogeographically, our data significantly extend the known distribution of the subspecies.

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Distribution; liverwort flora; Macaronesia; SEM; spore morphology; taxonomy

Introduction

The genus *Riccia* L. is one of the most speciose genera in the Iberian flora, 28 species having been reported to date (Casas et al. 2009). These include 17 in Madeira and 10 in the Azores Islands (Hodgetts and Lockhart 2020).

Riccia sorocarpa Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr., originally described from North America (Schuster 1992a; 1992b), was for a long time known only from California and the Mexican state of Baja California. Konstantinova and Doroshina (2011) then reported the subsp. *erythrophora* as new to Europe, based on the finding of specimens in southern Russia, and reviewed its most important diagnostic features.

Although *Riccia sorocarpa s.s.* is one of the most distinctive and unmistakable species of the genus, the subsp. *erythrophora* has been largely ignored since its inception. This is presumably because of its dubious taxonomic status and particularly because its spores were unknown (Schuster 1992b); furthermore, very few collections are available for critical study. The subspecies is, however, accepted by Hodgetts et al. (2020), although they considered it to have been invalidly published and validated it in their checklist.

According to Schuster (1992a), the subsp. *erythrophora* was known from several localities in California, the plants differing from the typical subspecies mainly by their vinaceous or purplish ventral scales, thicker thallus (the mature thallus segments being 0.9–1.5 times as wide as they are high), and smaller size.

Riccia sorocarpa can be distinguished from other members of the subgenus *Riccia* by its deep median groove, evident hyaline margins and scales, and epidermal and subepidermal cells with thickened walls. The characteristics of the ventral scales, such as the presence or absence of colour, size, shape, position, and margin conditions, are taxonomically important in the genus *Riccia* (Jovet-Ast 1986; Perold 1991, 1995). The absence of colour in the scales of *R. sorocarpa* has long been used as a defining character. However, its taxonomic value in this common and cosmopolitan species is difficult to establish (Jovet-Ast 1994) because morphological variation in different taxonomic characters is often observed and some floras mention the possibility of the species

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having purplish or violet scales (Paton 1999; Özenoğlu-Kiremit et al. 2019; Hugonnot and Chavoutier 2021).

The aims of this study were (i) to describe and illustrate the little-known *Riccia sorocarpa* subsp. *erythrophora* in Europe and to describe its distribution in mainland Portugal and the Azores archipelago; (ii) to authenticate the name based on the description by Schuster (1992a) and the data available for the type specimen (Hodgetts and Lockhart 2020); and (iii) to provide a description and scanning electron microscopy (SEM) micrographs of the spores, which have hitherto not been published.

Materials and methods

Following the initial discovery in Portugal of *Riccia sorocarpa* subsp. *erythrophora*, we carried out a revision of material in the LISU herbarium for this possibly overlooked subspecies to determine its distribution in Portugal. In the LISU herbarium collection, there are about 200 samples of *R. sorocarpa* from continental Portugal and the Madeiran and Azores archipelagos.

Apart from three fertile collections from the Portuguese mainland (Santarém [LISU 266717], Barragem do Xarrama [LISU 265418] and Monchique [Herb. R. D. Porley]), we located only one other specimen, a sterile collection from São Jorge Island in the Azores (LISU 225760), resulting in a total of four specimens available for study.

We provide a detailed description of the Portuguese material of *Riccia sorocarpa* subsp. *erythrophora*, including the micromorphology of the spores of two specimens as observed using SEM, and a comparison with spores of subsp. *sorocarpa*, also from Portugal. We did not find subsp. *erythophora* when reviewing the material from the Madeiran archipelago.

Representative specimens examined

***Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr.**

PORTUGAL. **Ribatejo:** Santarém, Caneiras, in sandy soil, open grassland with natural vegetation, 10 m, associated with *Riccia gougetiana*, *R. bicarinata*, *Corsinia coriandrina*, *Petalophyllum ralfsii* and *Entosthodon schimperi*, 29SND2739, 17 February 2019, *leg.* C. A. Garcia (LISU 266717). **Baixo Alentejo:** Trigo de Morais, pr. Barragem do Xarrama, open grassland in riverbank, 60 m, 29SNC6739, 22 February 2013, *leg.* C. Sérgio et al. (LISU 265418). **Algarve:** Serra de Monchique, Caminho do Convento, 500 m, on open ground by track with *Bryum dichotomum*, 29SNB3930, 21 November 2019, *leg.* R. D. Porley (Herb. R. D.Porley). **Azores:** S. Jorge Island, Ponta da Queimada, near the port, soil between rocks near the sea, 10–20 m, associated with *Exormotheca pustulosa*, *Riccia crozalsii*, *Fossombronia husnotii*, *Trichostomum* sp., 26SLH9681, 21 June 1999, *leg.* C. Sérgio 11572A (LISU 225760).

Riccia sorocarpa* Bisch. subsp. *sorocarpa

PORTUGAL. **Estremadura:** Serra da Arrábida, Portela, solo de caminho, 50 m, 29SMC9760, 10 February 2008, *leg.* C. Sérgio (LISU 266671).

Results and discussion

Taxonomic treatment

The description below is based on examination of four specimens.

***Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr.**

(Figure 1)

Description. Thallus medium-sized, in complete or partial irregular rosettes, up to (1–)2–3 cm in diameter; light green or glaucous green, yellowish with age, median groove narrow and distinct, extending along length of thallus, widening at base. Thalli 1- to 2(3)-furcate, hardly to strongly divided, with subtly to generally divergent lobes, ultimate branches widely spreading, 1–2.5(–3.5) mm long, 0.5–0.8(–1.3) mm wide, 0.5–0.8(–0.9) mm thick; apex slightly narrowed, subacute to rounded. Margins acute, slightly winged, hyaline or cream-coloured at apices and weakly undulate when dry, flanks vertical to slightly inclined, ventral face with violet or dark-red cells. Thallus transverse section of lobes forming an open V, 1–1.5(–1.8) times wider than high in apical part, chlorenchyma 200–350(–400) µm high, assimilatory tissue 100–200 µm thick; dorsal epithelium 2-layered, hyaline, upper layer of cells globose, 22–25(–35) × 25–40 µm, occasionally oblong, not mammillate, soon collapsing, second layer of cells short-rectangular cup-shaped, 15–30 µm wide. Ventral scales conspicuous, mainly in the apical part, rounded, imbricate, persistent, up to 300–400 × 600–800 µm, reaching the lobe margins but not extending above, with hyaline border of 2–6 cell rows at margin, with a violet-red purple base, cells in body of scales oblong-hexagonal to subquadrate, 20–35 × 35–50 µm, marginal smaller and fragile.

Monoicous. Antheridia with prominent violet necks, 70–80(–100) µm long, in 1 or 2 rows along dorsal apical groove. Archegonia with purple-brown necks and hyaline upper parts. Sporangia (capsule) about 300–400 µm in diameter, single or in groups of 2, bulging ventrally, each containing more than 100–150 spores. Spores 75–110(–120) µm in diameter, subglobular, colour brown to dark brown, opaque, black when mature, with triradiate mark and wing apparent.

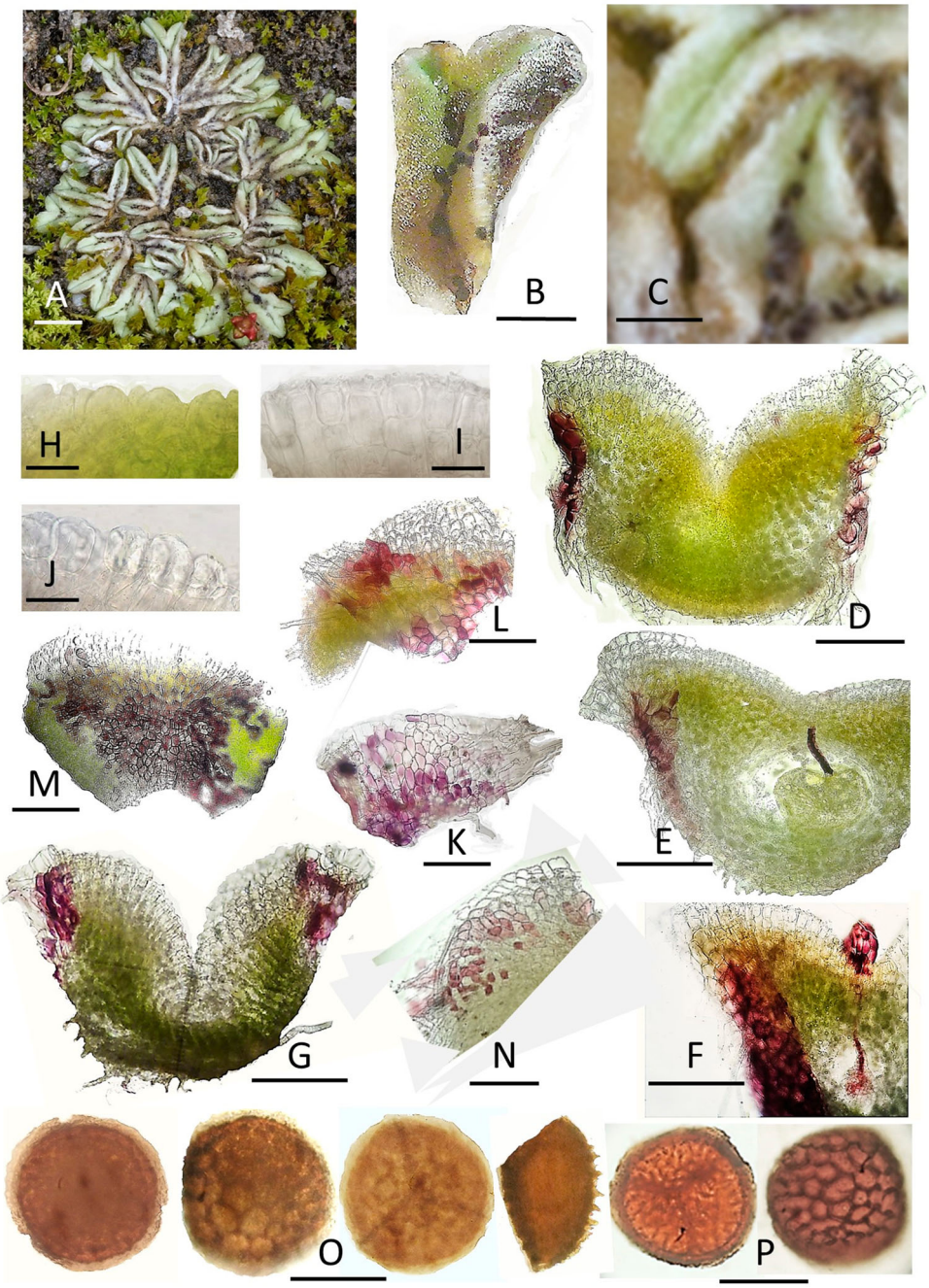

Figure 1. *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr. (A) Hydrated thallus forming rosettes. (B, C) Branches with distinct imbricate rounded purple scales. (D–G) Thallus sections from apex to base, with purple scales, and archegonium and antheridium with purple necks. (H, J) Dorsal epithelium sections, showing upper layer of rounded to oblong cells. (I) Second epithelial cell layer, comprising short-rectangular cup-shaped cells with thick-walled bases. (K–N) Scales with purple bands and a hyaline fragile margin. (O, P) Spores, which are brown, subglobose and anisopolar, with alveolus on distal face and wing crenulate to serrulate. A–M, from Santarém, Caneiras, *leg.* C A. Garcia, 2019 (LISU 266717); G and L, from the Azores, São Jorge, *leg.* C. Sérgio (LISU 225760); N and P, from Serra de Monchique, *leg.* R. Porley (Herb. R. D. Porley). Scale bars: A = 2 mm; B = 0.75 mm; C = 0.5 mm; D–G = 150 µm; H–J = 30 µm; K–N = 200 µm; O = 60 µm; P = 50 µm. Photographs: Cecília Sérgio.

SEM observations

It is evident from SEM observations that the spores of the four fertile specimens of *Riccia sorocarpa* subsp. *erythrophora* studied are morphologically similar to each other, with only some minor differences in dimensions (Figures 2, 3). The largest spores, present in the Santarém specimen, are 85–120 µm in diameter (Figure 2A, B), whereas in the Barragem do Xarrama material they are 90–110 µm in diameter (Figure 2F). Those from Monchique, however, although similar in form and structure, do not reach 100 µm in diameter; they are mostly 80–95 µm in diameter (Figure 2C–E). The spore diameters

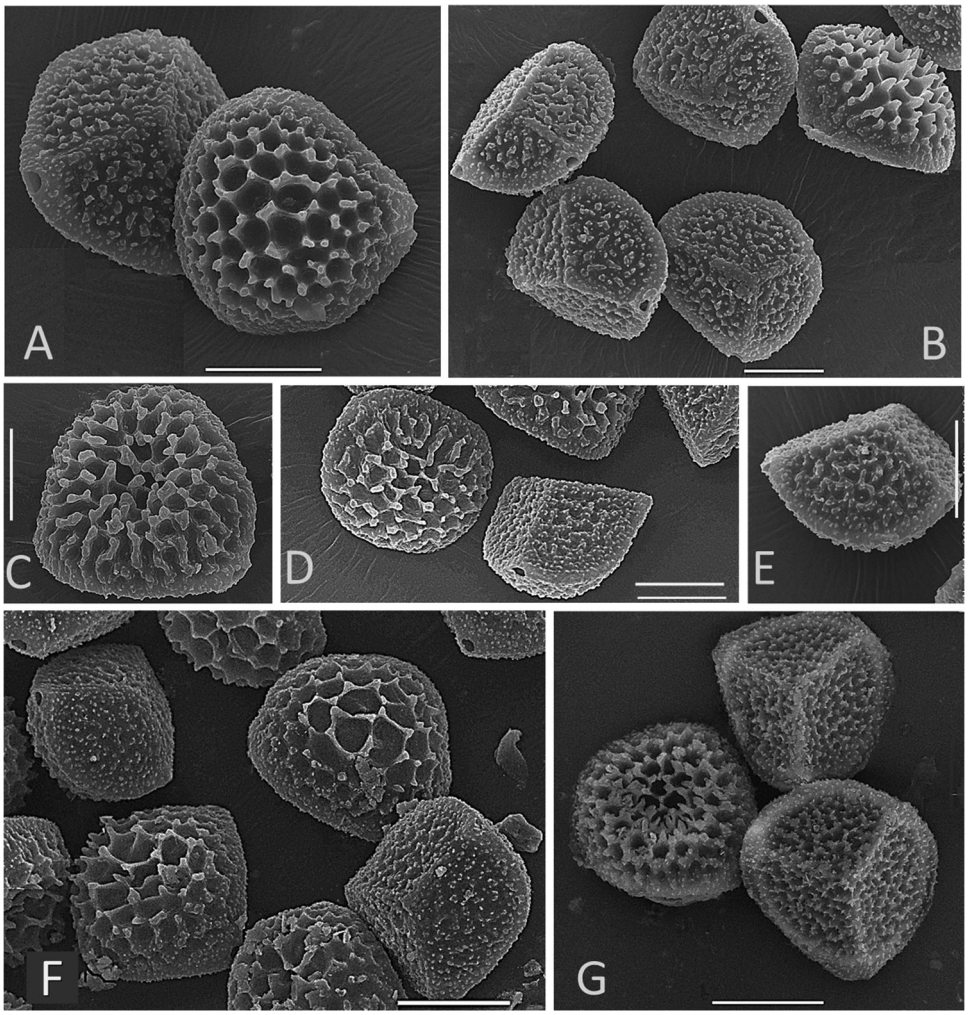

Figure 2. Scanning electrron micrographs of *Riccia* spores. (A–F) *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr.), proximal and distal faces. (G) *Riccia sorocarpa* subsp. *sorocarpa*, proximal and distal faces. Scale bar = 50 µm. A and B, from Santarém, *leg.* C A. Garcia (LISU 266717); C–E, from Serra de Monchique, *leg.* R. Porley (Herb. R. D. Porley); F, Barragem do Xarrama, *leg.* C. Sérgio et al. (LISU 265418); G, Serra da Arrábida, *leg.* C. Sérgio (LISU 266671). Note: images A, C, F and G are at the same scale, emphasising the larger spores of subsp. *erythrophora* compared with those of subsp. *sorocarpa*. Photographs: Cecília Sérgio.

reported for *Riccia sorocarpa* s.l. are somewhat variable (70–96 µm, Jovet-Ast 1986 for Mediterranean material; 80–100 µm, Perold 1989; 64–95 µm, Paton 1999; 61–70 µm, Singh and Singh 2008; 70–90 µm, Casas et al. 2009; 75–98 µm, Özenoğlu-Kiremit et al. 2008, 2019).

The differences in spore size between *Riccia sorocarpa* subsp. *erythrophora* and *R.* subsp. *sorocarpa* do not invalidate their assignment to the same taxon, and indeed signify a close relationship to the *R. sorocarpa* complex. However, as shown in Figure 3, neither the median nor maximum values obtained for the diameter of spores from subsp. *sorocarpa* exceed 100 µm, whereas for subsp. *erythrophora* the maximum spore diameter (120 µm) substantially exceeds this, and the median value exceeds 100 µm for spores from one specimen.

In addition to spore size, it is also clear that the spores of *Riccia sorocarpa* subsp. *sorocarpa* (Figure 2G) cannot be separated from those of subsp. *erythrophora* by the sporoderm structure of the distal and proximal surfaces; in both species, the alveolar walls on the distal faces and on the wings are densely papillose or covered with granules. Micromorphological analysis of *R. sorocarpa* s.l. by other workers revealed a similar ornamentation pattern, indicated by the alveolar type (*sensu* Jovet-Ast 1987; Perold 1989; Özenoğlu-Kiremit et al. 2019).

The thickened dorsal cell walls of the thallus, as seen in transverse section, provide the most useful diagnostic character for separating *Riccia sorocarpa* from other Mediterranean species of *Riccia*. Moreover, three specimens of *R. sorocarpa* from Portugal had vinaceous or purple ventral scales, thallus segments about 1.2–1.5 times wider than tall, and rounded-ovoid or cylindrical (but never mammillae) epidermal cells. This unique combination of characters corresponds to "subsp. *erythrophora*" as described by Schuster (1992a, 1992b), leading us to conclude that this taxon is present in Portugal.

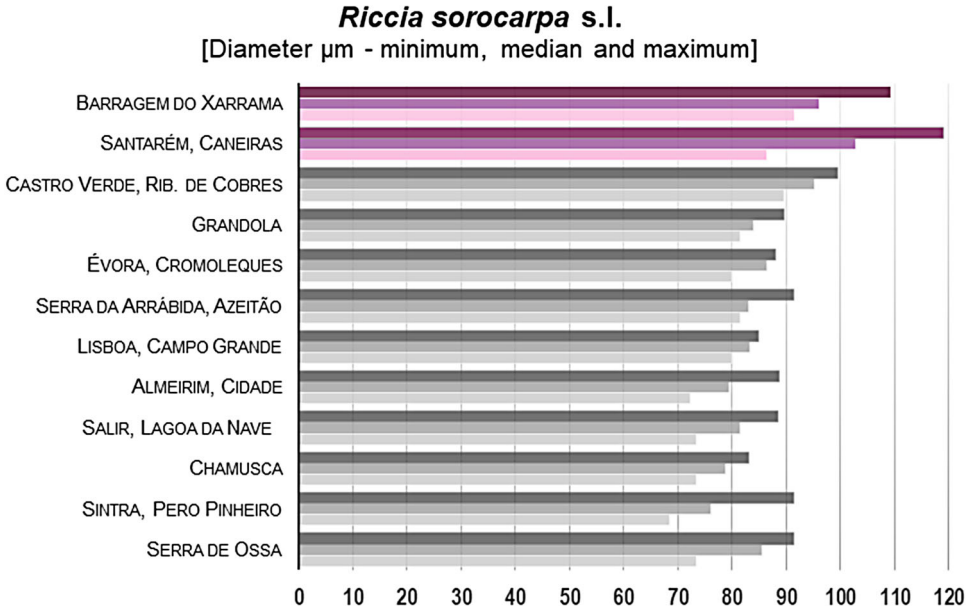

Figure 3. Spore diameter of *Riccia sorocarpa* subsp. *erythrophora* R.M.Schust. ex Konstant. & L.Söderstr. (purple) and *R. sorocarpa* subsp. *sorocarpa* Bisch. (grey), based on data from specimens from 12 localities in the central and southern areas of Portugal and held in the LISU herbarium. For each specimen, the maximum, median and minimum values are represented by the top, middle, and bottom bar, respectively).

Hitherto, *Riccia sorocarpa* subsp. *erythrophora* was previously known only from California, Mexico and southern Russia; it is here reported for the first time from the western Mediterranean and Macaronesia. This significantly extends the known distribution of the taxon, potentially representing an example of anthropogenic long-distance dispersal.

It is hoped that this preliminary work will raise awareness of the *Riccia sorocarpa* complex and encourage workers to collect and study material throughout Europe. At this stage, we cannot determine the taxonomic level at which *R. sorocarpa* subsp. *erythrophora* should be recognised; evidence from the Iberian Peninsula and the Azores, however, strongly suggests that it is a distinct taxon.

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Disclosure statement

No potential conflicts of interest were reported by the authors.

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Anabela Martins is a Ph.D. student at the Faculty of Sciences, University of Lisbon. Her research focuses on sexual expression in and evolution of bryophyte species endemic to oceanic islands.

Ron D. Porley is an independent researcher who has collected bryophytes in many parts of the world and is currently studying the bryoflora of Serra de Monchique in Portugal. His interests mainly focus on floristics, conservation, threatened species and taxonomy.

César Augusto Garcia is a curator of the bryophyte collection and researcher at the National Museum of Natural History and Science, University of Lisbon. He obtained a Ph.D. in Ecology from the University of Lisbon in 2006. His

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