

This is an 'Accepted Version' of an article published by Taylor & Francis Group in Journal of Bryology on 31 August 2022, © British Bryological Society 2022; available online: <https://doi.org/10.1080/03736687.2022.2106008>

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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Riccia sorocarpa Bisch. subsp. *erythrophora* R.M.Schust. ex Konstantinova & 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, in southern Russia, and reviewed its most important diagnostic features. The genus *Riccia* L. is one of the most speciose genera in the Iberian flora, with 28 species reported so far (Casas et al. 2009), 17 in Madeira and 10 in the Azores Islands (Hodgetts and Lockhart 2020).

Riccia sorocarpa s.str. is one of the most distinctive and unmistakable species of the genus, but 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), and very few collections are available for critical study. The subspecies is, however, accepted in Hodgetts et al. (2020), although the authors considered it to be invalidly published. This situation is remedied in this note.

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 (mature thallus segments 0.9-1.5 as wide as high), and smaller size.

Riccia sorocarpa can be distinguished from other members of the subgenus *Riccia* by a deep median groove, evident hyaline margins and scales, and epidermal and sub-epidermal 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 characters in the genus *Riccia* (Jovet-Ast 1986; Perold 1991, 1995). The absence of colour in the scales of *Riccia sorocarpa* is a defining character long-used, but its taxonomic value in this common and cosmopolitan species is difficult to establish (Jovet-Ast 1994), as morphological variation in different taxonomic characters is often observed and some floras mention the possibility of it having purplish or violet scales (Paton 1999; Özenoğlu-Kiremit et al. 2019; Hugonnot & Chavoutier 2021).

The aims of this study were 1) to describe and illustrate the little-known *Riccia sorocarpa* subsp. *erythrophora* in Europe and to present its distribution in mainland Portugal and the Azores archipelago; 2) to authenticate the name based on the description of Schuster (1992a) and the data available for the type specimen (Hodgetts and Lockhart 2020); and 3) to provide a description and Scanning Electron Microscopy (SEM) micrographs of the spores, which have hitherto not been described.

Following the initial discovery in Portugal of *R. sorocarpa* subsp. *erythrophora* we commenced a revision of material in the LISU herbarium for this possibly over-looked subspecies to determine its distribution in Portugal. In the LISU herbarium collection, there are about 200 samples of *Riccia 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 detected 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. Below we provide a detailed description of the Portuguese material of subsp. *erythrophora*, including the micromorphology of the spores of two samples as observed with SEM and a comparison with spores of subsp. *sorocarpa*, also from Portugal. We did not find subsp. *erythrophora* when reviewing the material from the Madeiran archipelago (Selection of specimens examined in Appendix).

Description (Figure 1, based on four specimens)

Thallus medium-sized, in complete or partial irregular rosettes, up to (1-) 2-3 cm across in diameter; light green or glaucous green, yellowish with age, median groove narrow and distinct, extending along length of thallus, widening at base. Thalli 1-2 (-3) furcate, hardly to strongly divided, subtly to generally with 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 (TS) of lobes making 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) \times 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 \times 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 sub-quadrate, 20-35 \times 35-50 μm , marginal smaller and fragile. Monoicous, antheridia with prominent violet necks, 70-80 (-100) μm long, in 1-2 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, sub globular, colour brown to dark brown, opaque, black when mature, with triradiate mark and wing apparent.

Discussion

It is evident after SEM observation that the spores of the four studied fertile specimens of subsp. *erythrophora* are morphologically similar, with only some minor differences in dimensions. The largest spores, present in the Santarém specimen, are 85 to 120 μm diameter, while in the Barragem do Xarrama material they are 90 to 110 μm diameter (Figures 1 O, P; 2 A, B, 3). Those from Monchique, however, whilst comparable in form and structure (Figures 2 C to E), do not reach 100 μm (they are mostly 80-95 μm). This size variation does not invalidate their assignment to the same taxon, and indeed signifies a close relationship to the *R. sorocarpa* complex.

However, as can be seen from Figure 3, neither the average nor maximum values obtained for spore measurements of samples from subsp. *sorocarpa* exceed 100 μm , while in the two ex-

amples of subsp. *erythrophora* the maximum value substantially exceeds this (up to 120 μm), while the average value exceeds 100 μm in one sample.

The values of spore diameters reported in *R. 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 & Singh 2008; 70-90 μm , Casas et al. 2009; 75-98 μm , Özenoğlu-Kiremit et al. 2008, 2019). It is also clear that spores of *R. sorocarpa* subsp. *sorocarpa* (Figure 2G) cannot be separated from those of subsp. *erythrophora* by the sporoderm structure of the distal and proximal surfaces; the alveoli walls on the distal faces and on the wings in both taxa are densely papillose or covered with granules. Micromorphological analysis of *R. sorocarpa* s.l. by other workers revealed a comparable 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 transversal section, provide the most useful diagnostic character for separating *Riccia sorocarpa* from other Mediterranean species of *Riccia*. Moreover, three specimens 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; b), leading us to conclude that this taxon is present in Portugal.

Hitherto, *Riccia sorocarpa* subsp. *erythrophora* was 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 *Riccia sorocarpa* subsp. *erythrophora* should be recognized; evidence from the Iberian Peninsula and the Azores, however, strongly suggests that it is a distinct taxon.

Acknowledgments

This research was co-funded by Portuguese National Funds, through FCT ‘Fundação para a Ciência e a Tecnologia’, within the thematic line **TL1**- Integrated ecological assessment of environmental change on biodiversity of the project UIDB/00329/2020. We are grateful to Telmo Nunes for assistance with SEM. The authors wish to express their gratitude to all reviewers and to the editor for their valuable suggestions.

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Appendix. Representative specimens examined

***Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. et 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 schimperii*, 29SND2739, 17.02.2019, *leg.* C.A. Garcia (LISU 266717);

Baixo Alentejo, Trigo de Morais, pr. Barragem do Xarrama, open grassland in riverbank, 60 m, 29SNC6739, 22.02.2013, *leg.* C. Sérgio et al. (LISU 265418); **Algarve**, Serra de Monchique, Caminho do Convento, 500 m, on open ground by track with *Bryum dichotumum*, 29SNB3930, 21.11.2019, *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, ass. *Exormotheca pustulosa*, *Riccia crozalsii*, *Fossombronia husnotii*, *Trichostomum* sp., 26SLH9681, 21.06.1999, *leg.* C. Sérgio 11572A (LISU 225760).

Riccia sorocarpa* Bisch. subsp. *sorocarpa

Portugal: Estremadura, Serra da Arrábida, Portela, solo de caminho, 50 m, 29SMC9760, 10.02.2008, *leg.* C. Sérgio (LISU 266671).

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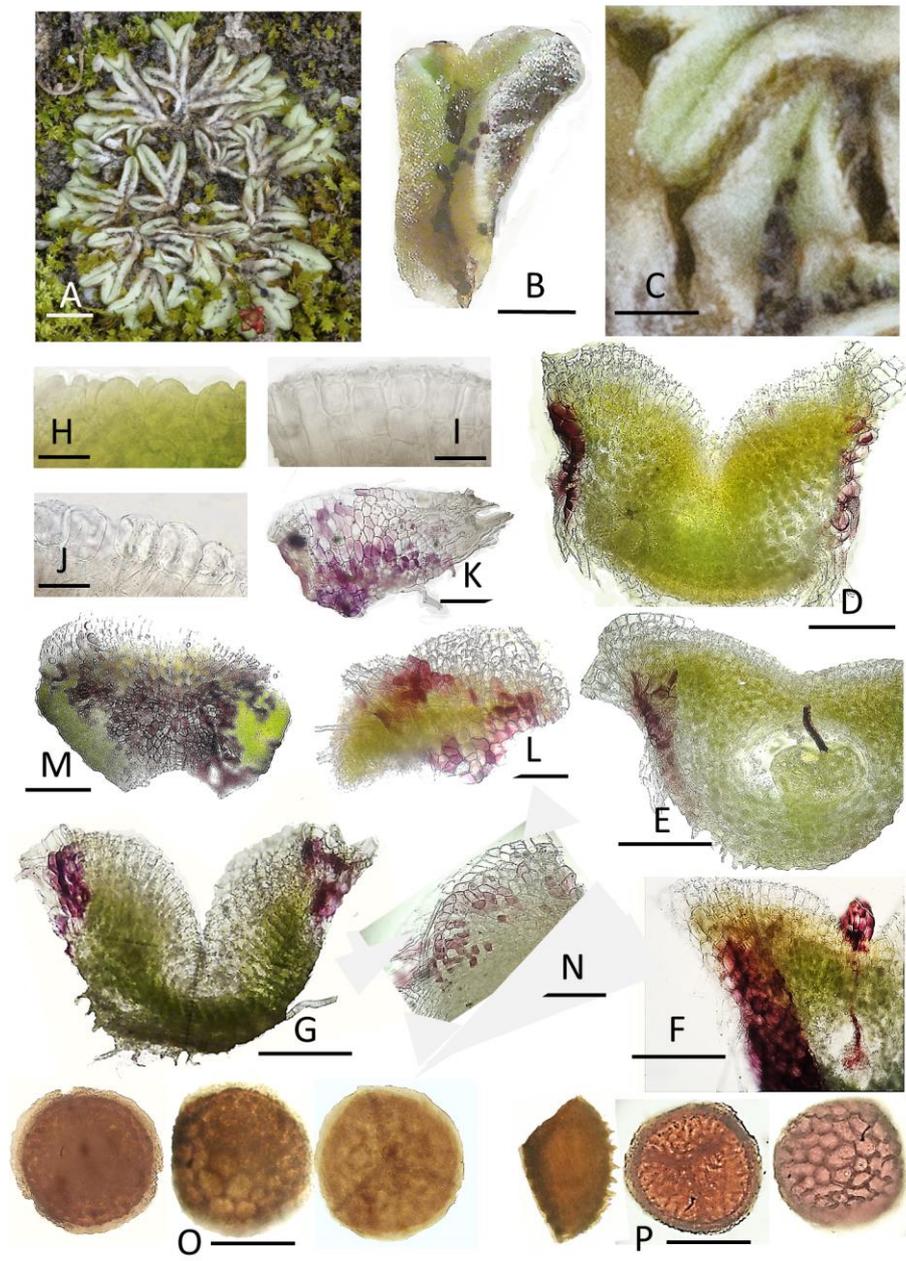


Figure 1

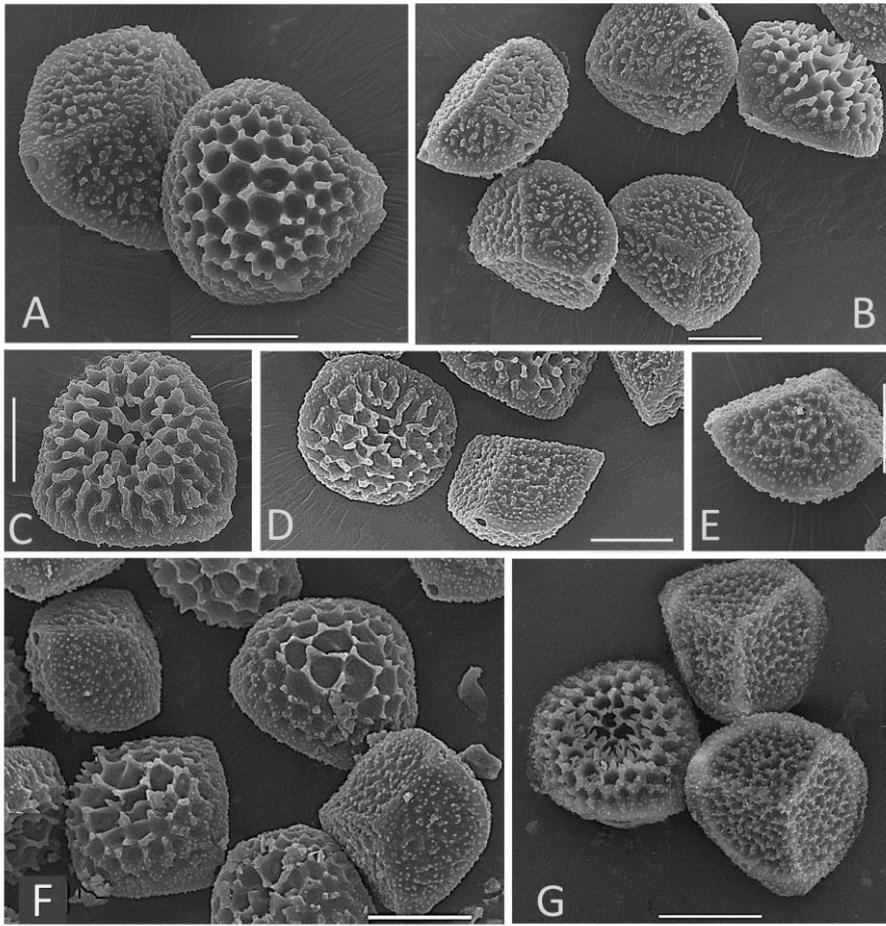


Figure 2

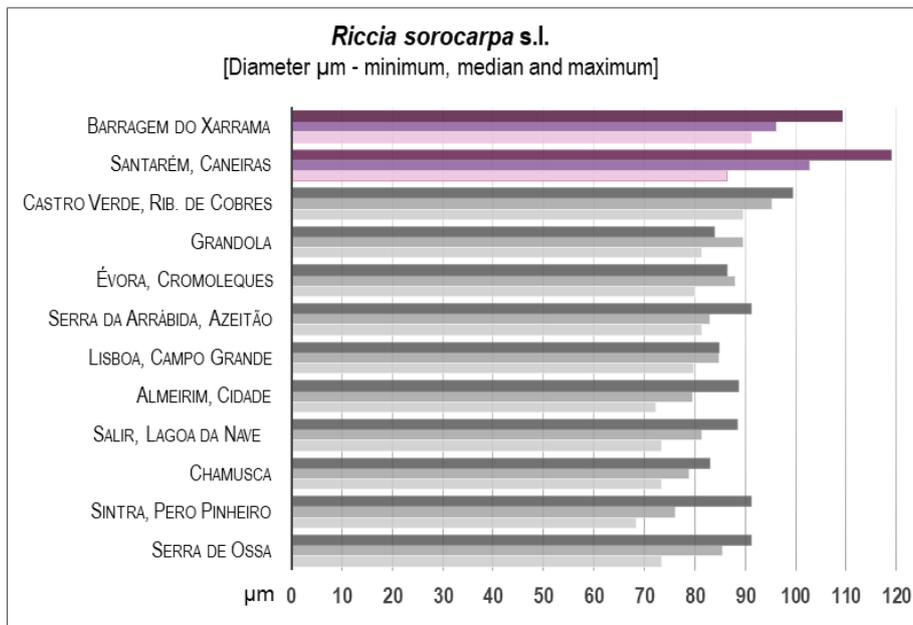


Figure 3

Figures captions

Figure 1. *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. et 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, archegonium and antheridium with purple necks. (H, J) Dorsal epithelium sections, upper layer rounded to oblong cells. (I) Second epithelial cell layer, short-rectangular cup-shaped with thick-walled bases. (K-N) Scales with purple bands and a hyaline fragile margin. (O-P) Spores, brown, subglobose, anisopolar, with alveolus in distal face and wing crenulate to serrulate. A-M, from Santarém, Caneiras, *leg.* CA. 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 (H R.Porley). Scale bars: A= 2 mm; B= 0.75 mm; C= 0.5 mm; D-G =150 μ m; H-J = 30 μ m; K-N = 200 μ m; O = 60 μ m; P = 50 μ m.

Figure 2. SEM micrographs of spores. (A - F) *Riccia sorocarpa* Bisch. subsp. *erythrophora* R.M.Schust. ex Konstant. et L.Söderstr.), proximal and distal faces. (G) *R. sorocarpa* subsp. *sorocarpa*, proximal and distal faces. (Scale bar = 50 μ m). (A, B) from Santarém, *leg.* CA. Garcia (LISU 266717); (C to E) from Serra de Monchique, *leg.* R. Porley (H R. Porley); (F) Ribeira do Xarrama, *leg.* C. Sérgio et al. (LISU 265418). (G). Serra da Arrábida, *leg.* C. Sérgio (LISU 266671) Note: The images A, C, F and G are at the same scale, emphasising the larger spores of subsp. *erythrophora* compared with those of subsp. *sorocarpa* (G).

Figure 3. Spores from 12 localities, all in central/southern areas of Portugal, based on samples from the LISU herbarium. In each sample, the maximum, median and minimum values (μ m) are indicated. Gray color: *Riccia sorocarpa* subsp. *sorocarpa* Bisch. Purple color: *R. sorocarpa* subsp. *erythrophora* R.M.Schust. ex Konstant. et L.Söderstr.