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Riella bialata Trab. (Riellaceae, Marchantiophyta): a new addition to the European liverwort flora

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Résumé – *Riella* (Riellaceae, Sphaerocarpales) est un genre d'hépatiques aquatiques avec une répartition disjointe dans le monde entier dans les domaines de climat méditerranéen. Son centre de diversité se trouve dans le bassin méditerranéen qui concentre environ la moitié des espèces du genre. Au cours d'une révision mondiale du genre *Riella*, des plantes d'une espèce monoïque avec des involucres dépourvus d'ailes, non papilleux, lisses ont été trouvées dans deux localités ibériques lointaines. Ces plantes ont une aile dorsale géminée, contrairement à l'aile isolée qui caractérise les autres espèces du genre. Ce caractère affiche sans ambiguïté ces populations à *R. bialata*, une espèce jusqu'ici connue à partir d'une collection unique de sa localité type algérienne. *Riella bialata* est donc signalée comme nouvelle pour l'Europe. Des matériaux frais ont été utilisés pour des analyses morphologiques détaillées et les matériaux ibériques sont ici amplement décrits et illustrés.

Hépatiques aquatiques / bryophytes / Marchantiophyta / Sphaerocarpales / espèces menacées

Abstract – *Riella* (Riellaceae, Sphaerocarpales) is a genus of aquatic liverworts with a disjunct worldwide distribution in areas of seasonal Mediterranean-type climates. Its centre of diversity is located in the Mediterranean basin, where about half the species number of the genus is concentrated. In the course of a worldwide revision of the genus *Riella*, plants from a monoicous species with wingless, not papillose and smooth involucres were found in two distant Iberian localities. These plants showed a geminate dorsal wing, unlike the single wing occurring in the remaining species of the genus. This character unambiguously assigns these populations to *R. bialata*, a species known to date from a single collection in its Algerian type locality. *Riella bialata* is thus reported as new to Europe. Fresh materials enabled detailed morphological analyses, and the Iberian materials are extensively described and illustrated herein.

Aquatic liverworts / bryophytes / Marchantiophyta / Sphaerocarpales / threatened species

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INTRODUCTION

The genus *Riella* Mont. (Riellaceae, Sphaerocarpales) is unique among liverworts because of its submerged, ephemeral habit in seasonally flooded areas in fresh and brackish waters. Despite the worldwide distribution of *Riella*, its populations are scattered and occur in areas of seasonal climate with temperate winters and dry summers from arid to subdesertic areas (Banwell, 1951; Jelenc, 1957; Proctor, 1972; Hässel de Menéndez, 1987; Cirujano *et al*. 1988; Perold, 2000). Its centre of diversity lies in the Mediterranean basin, where almost half the species in the genus occur (Wigglesworth, 1937). Being typical of Sphaerocarpales, gametangia are produced within sac-like gametophytic structures, usually named involucres. The morphology of female involucres has been used in the systematics of *Riella*. Based on this organ morphology two subgenera have been recognised (Porsild, 1902). Species with smooth or papillose involucres have been included within subgenus *Riella* whereas those with winged involucres have been incorporated into subgenus *Trabutiella* Porsild. Both subgenera include monoicous and dioicous taxa, although monoicous taxa are fewer as compared to the dioicous ones in both subgenera. Within subgenus *Riella* up to eight monoicous taxa have been described at the species rank, namely *Riella battandieri* Trab. (Trabut, 1886), *R. bialata* Trab. (Trabut, 1908, 1909), *R. gallica* Balansa *ex* Trab. (Trabut, 1891), *R. indica* Kashyap (Kashyap, 1917), *R. notarisii* (Mont.) Mont. (Montagne, 1838; 1852), *R. reuteri* Mont. (Montagne, 1852), *R. sersuensis* Trab. (Trabut, 1934), and *R. spiculata* J. Tayl. (Taylor, 1954), depending on the authors. All these taxa are endemic to the Mediterranean region, except for *R. indica* and *R. spiculata* which are endemic to Pakistan (Kashyap, 1917) and Australia (Taylor, 1954), respectively.

The taxonomical status of some of these taxa has long since been a matter of debate (Corbière, 1902; Trabut 1911). For example, *R. battandieri*, *R. gallica*, and *R. reuteri* have been subject to a variety of taxonomical arrangements by means of which some of these species have been recognised at the rank of variety or form of others, or have been placed together in a single taxon (Corbière, 1902; Trabut 1911; Jelenc, 1957). Currently, there is a general consensus that these three taxa are considered within the synonymy of *R. notarisii* (Ros *et al.*, 2007). The remaining taxa in this aggregate have not been included in comparative studies since the time they were described. Nonetheless, Wigglesworth (1937) considered a number of distinctive gametophytic and, more specifically, spore traits, which would allow their identification with artificial keys, but her synthesis has not been based on a systematic study of specimens other than the South African ones. Thus, her overuse of spore size to differentiate some taxa has been criticised by later works (Proskauer, 1955), suggesting the need of a deep taxonomical revision of the genus *Riella*.

Among the species in this complex, the taxonomic recognition of *R. bialata* as a distinct species has remained undisputed since its description (Trabut, 1908; Jelenc, 1957). This is due to its unique thallus bearing a geminate dorsal wing (Trabut, 1908, 1909), whereas the thallus wing in the remaining species is single. This unique character was apparently morphologically stable as laboratory cultures of plants from the original Algerian locality also showed plants with geminate dorsal wings (Trabut, 1908).

To date, *Riella bialata* is known only from a single collection from the type locality, thus it has been considered an Algerian endemic (Jelenc, 1957; Söderström *et al.*, 2002; Ros *et al.*, 2007).

In the course of a worldwide revision of the genus *Riella*, samples of many species have been collected from natural habitats and kept in culture in the laboratory. Furthermore, cultures of plants have been started from soil sediments collected in *Riella* localities, proving the stability of distinctive morphological characters of plants grown from cultures (Segarra-Moragues *et al.*, 2012a, b). One of the cultures started from soil sediments from a previous record of a population of *R. notarisii* in eastern Spain (Gimeno-Colera & Puche-Pinazo, 1999; Valentín *et al.*, 2007) rendered plants with geminate dorsal wings, a trait that unambiguously matched the original description of *R. bialata* (Trabut, 1908, 1909). A further locality with plants showing the same traits was later found in southern Spain. This paper addresses the morphological study of the plants from these two populations and reports the presence of *R. bialata* in Europe more than one century after its description from a single Algerian locality.

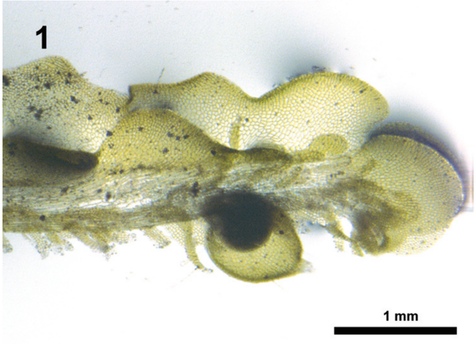
MATERIALS AND METHODS

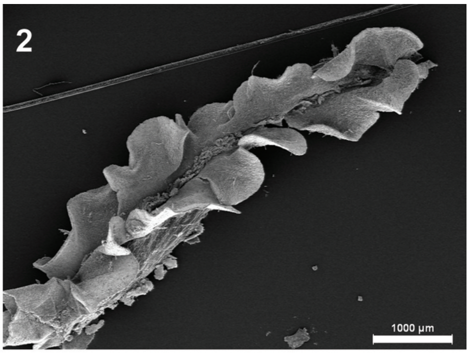
Measurements of vegetative and spore characters were taken using the interactive measurement module of the Leica Application Suite (LAS), v. 3.8 (Leica microsystems, Barcelona, Spain) calibrated to the nearest 0.01 µm on digital images. All the measurements were taken under a Leica DMLB 100S light microscope (LM), except for the size of gametophytes and the width of the wing from the thallus, which were taken under a Leica M76 stereomicroscope. A Leica DFC425 digital camera was used to produce high resolution images. For the scanning electron microscope (SEM), mature gametophytes were fixed with 2% Osmium tetratoxide, dehydrated in increasing ethanol series, critical-point dried using carbon dioxide in an AUTOSAMDRI 840 critical point dryer (Tousimis, Rockville, MD, USA) and were subsequently mounted on stubs for gold/palladium coating. Mature spores were mounted directly on stubs using double-sided adhesive tape and coated with gold/palladium in a BIORAD SC-500 ion sputtering coater. Morphological observations were made in a Hitachi S-4100 field emission SEM at the University of Valencia (SCSIE-UV). The terminology for spore characters follows Perold (2000).

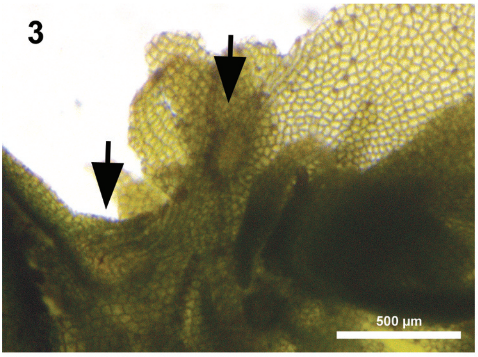
RESULTS

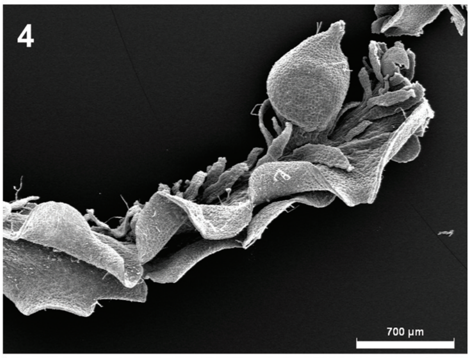
***Riella bialata* Trab.**, *Revue bryologique*, 35(4): 96. 1908 – Type. ALGERIA: Département d'Alger, dans la vallée du Chèlif à Duperré, 15-19 May 1907, terrain inondé en légère pente, touffes émergées en association avec *Chara sp*. et *Juncus bufonius* (holotype: AL; B45319 Krypto-S!) **Figs 1-28**

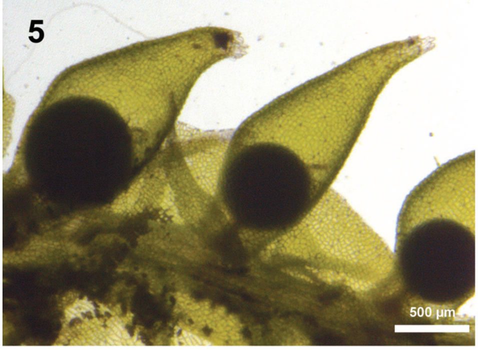
Plants 0.9-2.8 cm long, caespitose, several times bifurcate, rarely unbranched (Figs 1-2, 4, 20). Axis slightly flattened 128.8-259.9 µm wide (Fig. 24). Dorsal wing geminate, sometimes becoming single or interrupted in older plants in the fertile portion, undulate (Figs 1-2, 4, 20). Each wing 0.68-1.77 mm wide,


1
1 mm


2
1000 µm


3
500 µm


4
700 µm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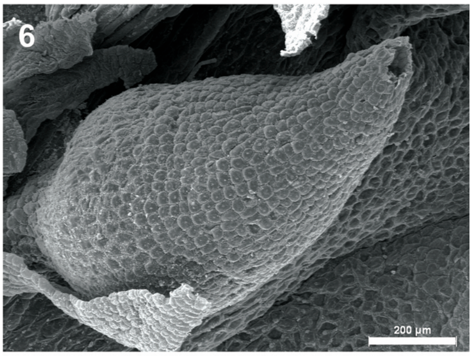
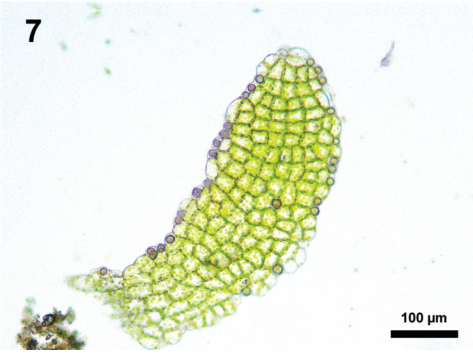
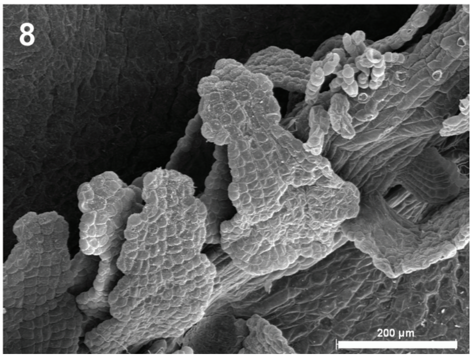

6
200 µm


7
100 µm


8
200 µm

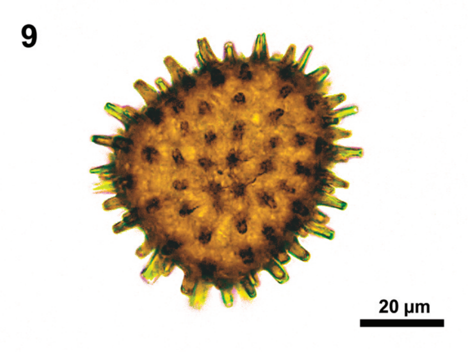
deeply lobed. Marginal cells from wing quadrate-rectangular, chlorophyllose, 22.3-52.3 × 12.01-22.04 µm (Fig. 23); cells from wing near axis, 97.8-167.2 × 18.6-48.8 µm; oil cells 11.2-31 × 10.8-31.6 µm quadrate-rectangular, oil bodies 9.4-21.05 × 9.1-18.8 µm spherical, rough, opaque (Fig. 23). Scales dimorphic; vegetative scales ligulate, linear or lanceolate, 210.6-889.02 × 79.3-262.8 µm (Figs 7, 21-22); propaguliferous scales panduriform, 295.8-339.6 × 179.8-200 µm (Fig. 8). Sexual condition monoicous, protandrous. Antheridia solitary or in small groups of 2-3, above stem axis, between both wings (Figs 18-19), solitary to seriate, with up to six consecutive antheridia; antheridial body 138.5-184.1 × 73.4-115.5 µm (Fig. 3). Archegonial involucre ellipsoid, acuminate, 1203.4-1799.02 × 574.1-792.7 µm, wingless, smooth or with cells slightly bulging, not papillose (Figs 4-6, 25). Apex of female involucre not occluded. Sporophyte with seta of 174.4-300.3 µm long, capsule globose, spherical, 411.1-689.57 × 412.5-647.8 µm (Figs 5, 25).

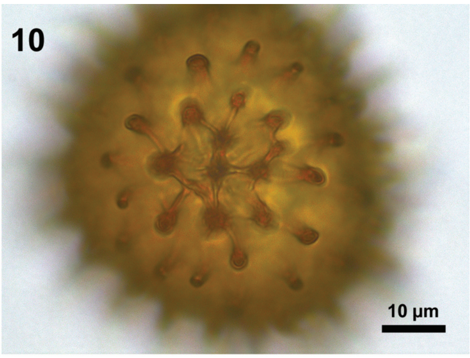
Spores brown, tetrahedral, triangular in outline, 64-82 × 61-80 µm, and 53-66 × 52-63 µm including and excluding spines, respectively (Figs 9-14, 26). Distal face with 12-14 irregular rows of spines across the diameter, and 22-32 spines projecting at the periphery on the equatorial plane (Figs 9, 12, 13). Surface of distal face granulose (Fig. 15). Spines (5.6-) 7.2 (-9.3) µm long, (2.8-) 3.3 (-4) µm wide (Figs 15, 27) with granulose surface, apices truncate, rarely blunt or acute, basal membranes interconnecting spines restricted to distal pole, faint (0.6-) 0.9 (-1.3) µm high (Figs 10, 13), towards equatorial plane becoming interrupted and vestigial, producing short papillae interspersed among spines (Fig. 13); distance between spines, 3.5-8.4 µm. Proximal face with granulose surface (Fig. 16); triradiate mark indistinct or faint, as clusters of spines towards edges of spore (Fig. 14). Spines of proximal face with granulose surface; shorter than those of distal face, (1.9-) 2.92 (-3.9) µm long (1.3-) 2.1 (-2.9) µm wide, generally with acute apices, rarely blunt, not basally interconnected (Figs 16, 28); distance between spines, 1.5-6.7 µm.

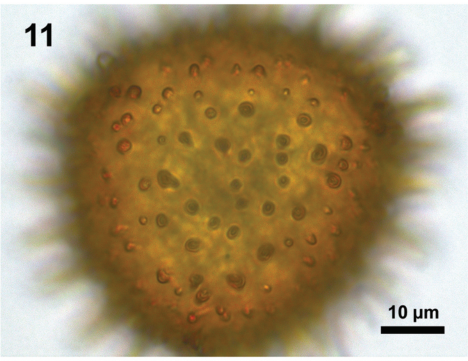
Distribution and ecology. Fresh-water seasonal ponds and temporarily inundated in rice crops. Algeria (Trabut, 1908) and Spain, this study (Fig. 29).

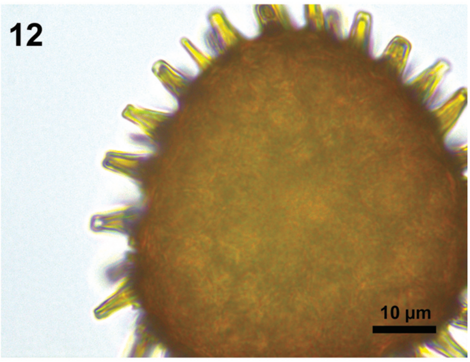
Additional material examined. Spain: Cádiz, Puerto Real, Pinar de La Algaida, laguna de La Vega, 29SQA5046, 3 m, Jun 2010, *S. Paula* (VAL-Briof. 9.199). Valencia, Sueca, margen de arrozal, 30SYJ2945, 22 Jan 1991, *Gimeno* (VAL-Briof. 2.335). *Ibid*. Cultured from sediments started on 29 Feb. 2012 and dried on 21 June 2012, *F. Puche, J.G. Segarra & C. Gimeno* (VAL-Briof. 9.198).

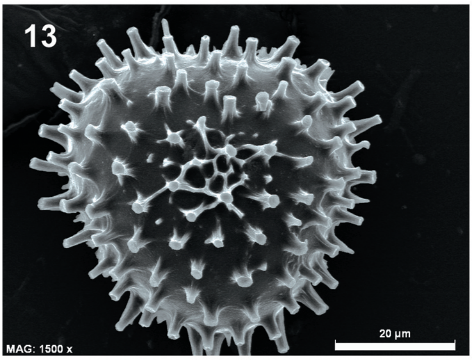
◀ Figs 1-8. LM and SEM images of *Riella bialata*. **1.** Thallus in side view. **2.** Thallus in dorsal view showing the lobulate geminate wing. **3.** Detail of thallus apex in ventral view showing the antheridia located at the base of wings (arrows). **4.** Side view of thallus and a female involucre in ventral position and vegetative scales. **5.** Ventral view of thallus showing seriate female involucres and vegetative scales. **6.** Detail of smooth female involucre. **7.** Vegetative scale. **8.** Panduriform propaguliferous scales. (1-2, 4, 7-8 from VAL-Briof. 9.198; 3, 5-6 from VAL-Briof. 9.199).

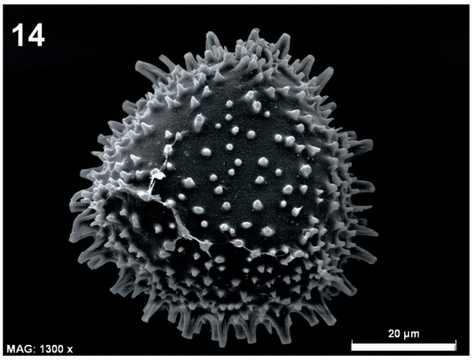

9
20 µm

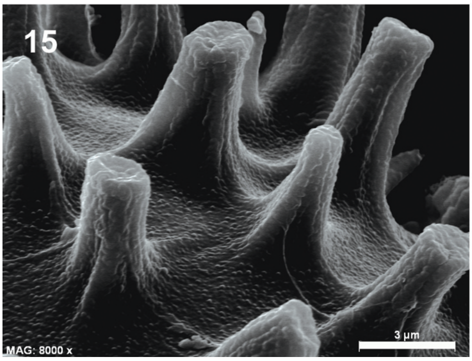

10
10 µm

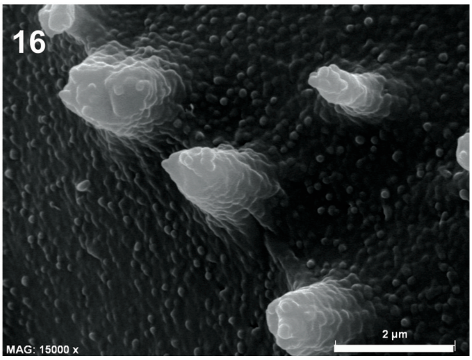

11
10 µm


12
10 µm


13
MAG: 1500 x
20 µm


14
MAG: 1300 x
20 µm


15
MAG: 8000 x
3 µm


16
MAG: 15000 x
2 µm

DISCUSSION

Monoicous species of *Riella* subgenus *Riella* show a high degree of morphological similarity, with most taxonomically informative characters being restricted to the degree of papillosity of female involucres and spore morphology.

The scarcity of data also extends to the establishment of the sexual condition for some taxa and populations, which increases the risk of unreliable identifications. Yet some taxa, such as *R. gallica,* have been described as dioicous, although male individuals or individuals with antheridia were unknown at the time of description (Trabut, 1891). Similarly, *R. notarisii* and *R. reuteri* have been erroneously regarded as dioicous (Trabut, 1891), as later noted (Corbière, 1902; Trabut 1911). The confusion of assigning the sexual condition to taxa stems from the difficulty of observing their antheridia. Contrarily to dioicous *Riella*, where antheridia are produced in large continuous series on the margin of the wing of the thallus of male individuals, in these taxa they are produced individually or in small numbers. They are located on the wing, close to the growing point at the thallus apex, and are generally obscured by the profusion of the vegetative and propaguliferous scales in this area. Besides, the mark left by the antheridia on the wing upon antherozoid discharge is quite inconspicuous, which makes their observation in old plants even more difficult. Thus, the sexual condition is best scored from fresh materials as in herbarium specimens it would generally involve damaging the specimens with few guarantee of success. This has often led to confusing samples of this species complex with the females of dioicous taxa, which results in erroneous identifications.

Altogether, the difficulty of studying the morphological characters in this species complex has led to some of the taxa becoming the centre of taxonomic debate (Corbière, 1902, 1913; Trabut, 1911), and urges a complete taxonomical revision of the group with modern tools for morphological and molecular analyses. These analyses will help to establish not only the taxonomical boundaries of the taxa, but also the taxonomic potential of different sources of morphological and molecular data, in order to resolve the relationships in this species complex.

Nonetheless among the monoicous species of subgenus *Riella*, *R. bialata* has so far been taxonomically undisputed since its description (Trabut, 1908, 1909; Jelenc, 1957; Geissler, 2001), and has been recognised in recent checklists of European, Macaronesian and Mediterranean liverwort flora (Söderstrom *et al.*, 2002; Ros *et al.*, 2007). However, the fact that no further collections other than the type have been known until now has certainly precluded detailed studies contributing to its morphological characterisation.

The most outstanding distinctive feature of *R. bialata* is the presence of a geminate dorsal wing. This trait is unique among the monoicous taxa of subgenus *Riella* and in the whole genus. Thanks to this remarkable trait, Trabut (1908) suggested that it could be separated from *Riella* in an independent genus. However, he refrained from this decision given the overall similarity of all the other traits to the genus *Riella* (Trabut, 1908). The stability of this distinctive morphological trait has been confirmed by laboratory cultures (Trabut, 1908). In

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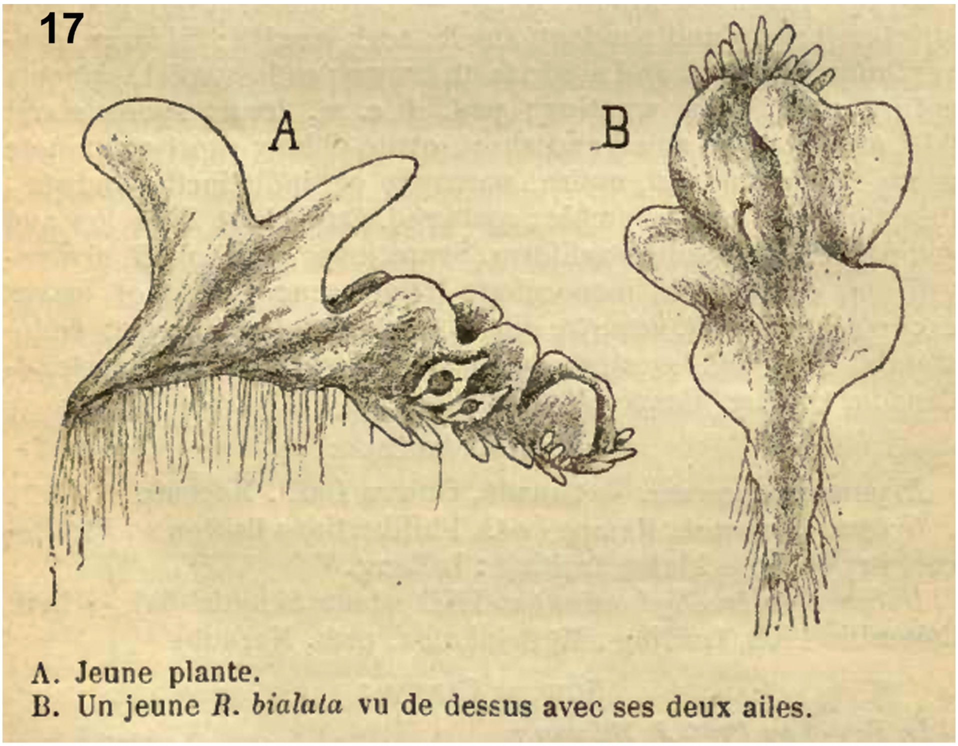
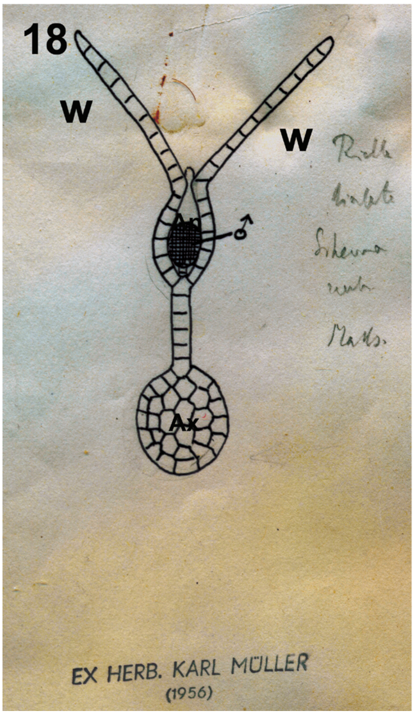
◀ Figs 9-16. LM and SEM images of spores of *Riella bialata*. **9.** Distal view. **10.** Detail of distal pole. **11.** Proximal view. **12.** Equatorial plane. **13.** Distal view. **14.** Proximal view. **15.** Detail of spines from distal face. **16.** Detail of spines and surface of spores from proximal face showing the granulose appearance. (9-16 from VAL-Briof. 9.198).

17


A
B
A. Jeune plante.
B. Un jeune *R. bialata* vu de dessus avec ses deux ailes.

18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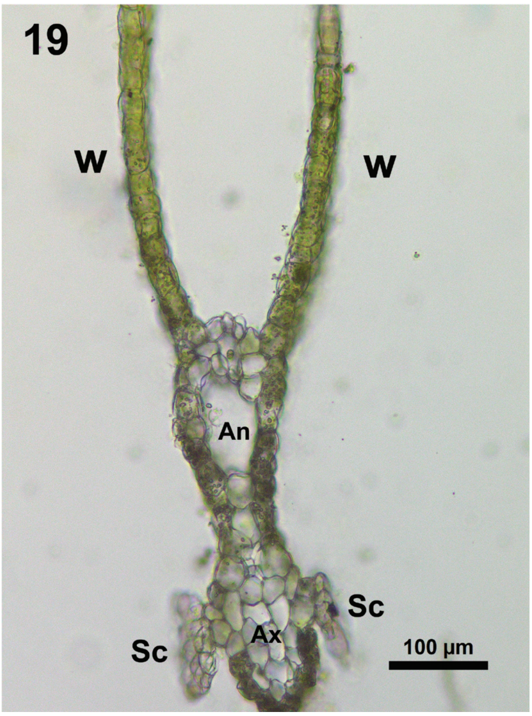
EX HERB. KARL MÜLLER
(1956)

19


W
W
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100 µm

these cultures, plants lost their creeping habit, probably because of constant water levels which do not occur naturally. Plants then became erect, but still retained their geminate dorsal wing (Trabut, 1908). Another outstanding feature of *R. bialata* is the position of the antheridia sunk between both thallus wings (Figs 18-19), which contrasts to the position on the margin of the wing in the other species of the genus.

The study materials from the two Iberian localities have revealed the presence of *R. bialata* with individuals showing its distinctive geminate dorsal wing. Specimens grown from spores cultivated in the laboratory from soil sediments from both Iberian localities have confirmed the stability of this distinctive morphological character. However, in older overgrown individuals, the thallus wing became narrower and discontinuous, and somewhat resembled individuals of *R. notarisii*, an apparently more widespread species in the Iberian Peninsula in similar habitats to those of *R. bialata* (Valentín *et al.*, 2007).

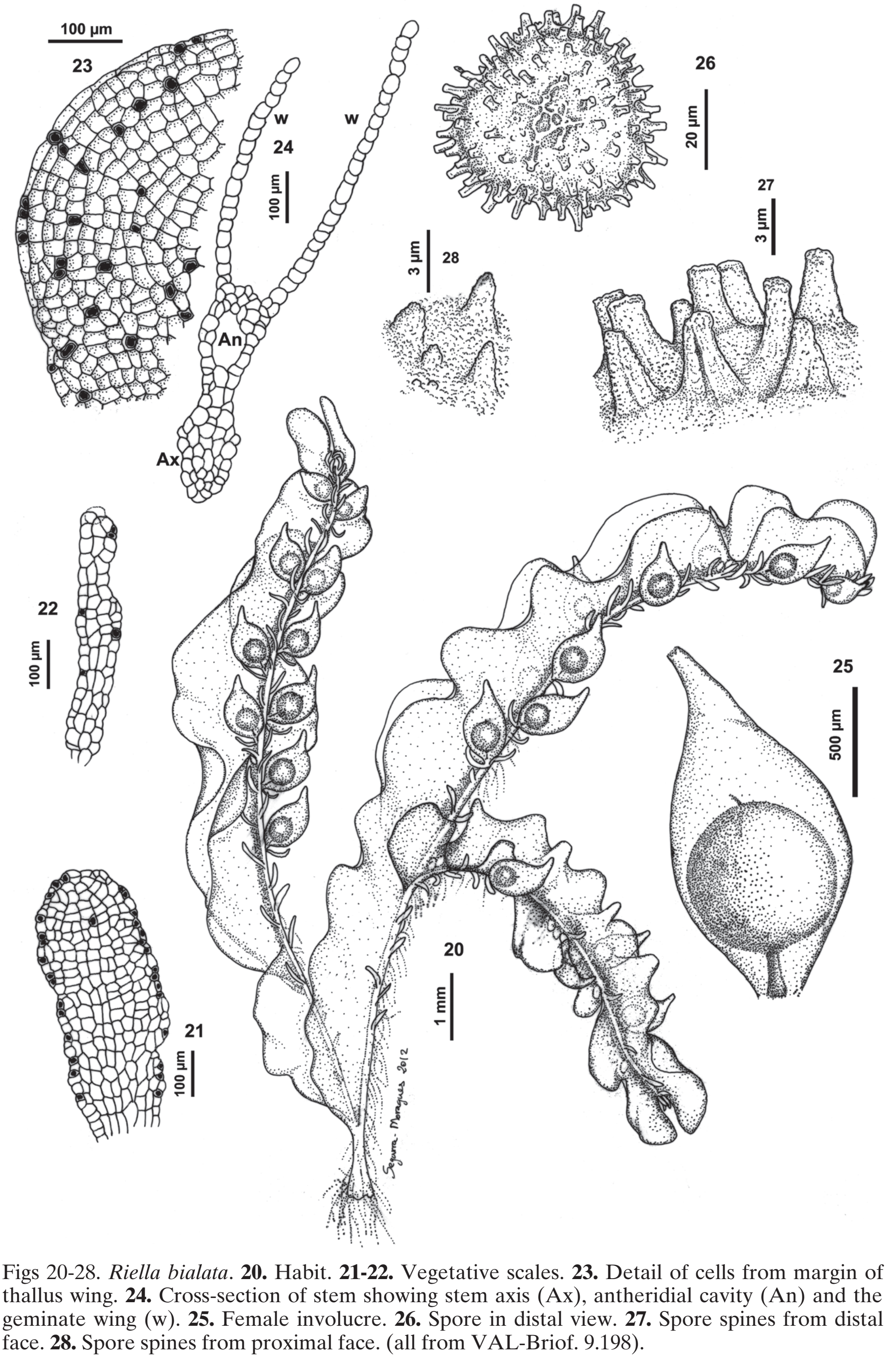
For most *Riella* species, characters from spore morphology have been indicated as the most taxonomically informative. Thus, at the time of collection, a priority is given to specimens with mature spores. In these individuals, the observation of gametophytic traits, especially those deriving from younger parts of the thallus, like the geminate wing of *R. bialata*, is not straightforward task. This fact contributes to the misidentification of many *Riella* specimens which, such as *R. bialata,* need a combination of gametophytic and spore traits for their reliable species assignment. Thus, a trustworthy identification is better achieved with freshly collected young individuals or from cultured materials.

The stability of both, distinctive vegetative characters of gametophytes (e.g. the geminate wing) and reproductive characters (e.g. the position of antheridia) of plants differing for more than one century, in their geographical origin, and collected in the wild and obtained from cultures (Trabut, 1908 and this study), support their taxonomical usefulness for distinguishing *R. bialata* from other monoicous species of *Riella*, and similarly support the recognition of the species rank for *R. bialata*.

Our study reports the occurrence of *R. bialata* in two distant Spanish localities (Fig. 26). The occurrence of *R. bialata* in both these populations represents a new and significant addition to the liverwort flora of Spain and Europe. It is likely that the species is more widespread in the territory as potential sites abound. The species could have been overlooked because of its similarity (especially if identification is based on the revision of herbarium materials) to other taxa of this species complex (e.g. *R. notarisii*), with which it is likely to co-occur.

As this species has not been reported again since the time of its description, over one century ago, and as no further Algerian populations are known for this species apart from the type locality, *R. bialata* could well have been considered extinct (UICN, 2001). Despite the Spanish populations demonstrating that the species cannot be listed in this category, the actual fate of the Algerian population is uncertain, and further searches of potential Algerian sites should be conducted to confirm its current presence and range extension.

◀ Figs 17-19. **17.** Original drawing of *Riella bialata*, reproduced from Trabut (1909). **18.** Karl Müller's scheme of a cross-section of the stem from an isotype of *R. bialata* reproduced from B45319 Krypto-S. **19.** Cross-section of the stem from Iberian populations of *R. bialata* (VAL-Briof. 9.198). Antheridial cavity (An), Axis (Ax), vegetative scales (Sc), and the geminate wing (w).



Figs 20-28. *Riella bialata*. **20.** Habit. **21-22.** Vegetative scales. **23.** Detail of cells from margin of thallus wing. **24.** Cross-section of stem showing stem axis (Ax), antheridial cavity (An) and the geminate wing (w). **25.** Female involucre. **26.** Spore in distal view. **27.** Spore spines from distal face. **28.** Spore spines from proximal face. (all from VAL-Briof. 9.198).

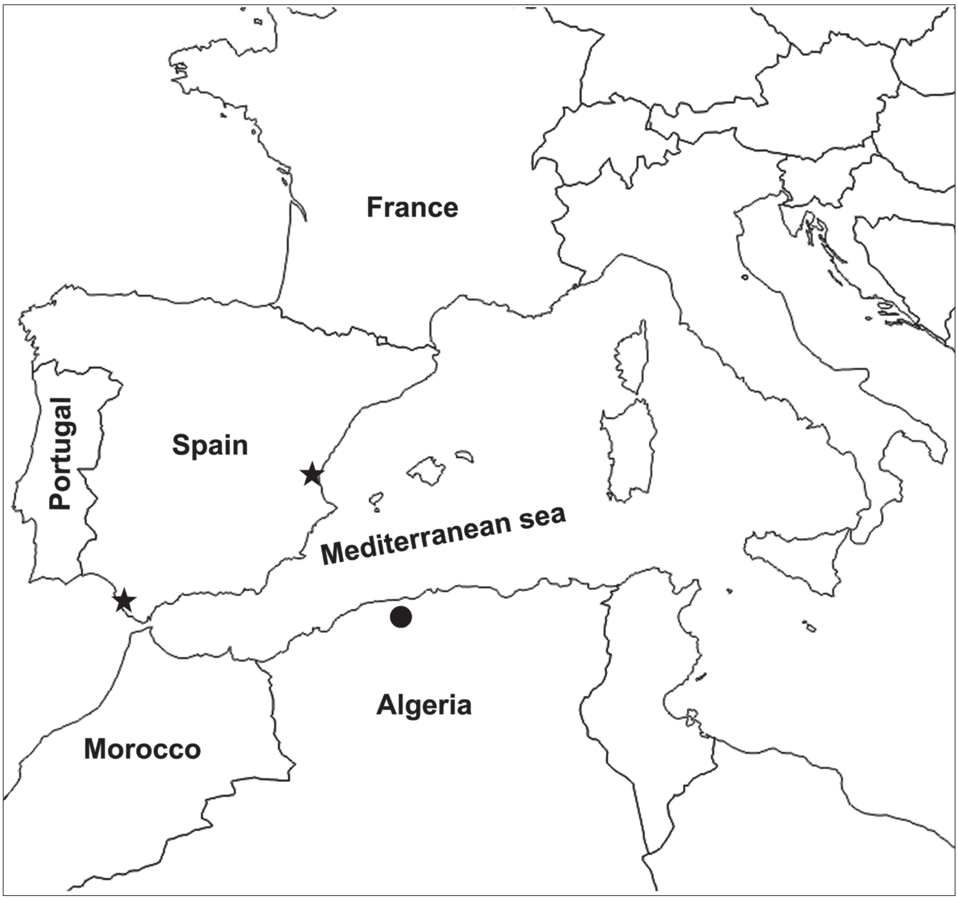


Fig 29. Worldwide distribution of *Riella bialata.* Black dot: type locality; stars: new localities.

Of the two Spanish populations, the Valencian one occurs in privately owned land. Thus, it is outside the legal protection of its habitat. Population persistence and annual occurrence may be subject to land practices during the cultivation of rice. The southern Spain population occurs within the perimeter of the "Pinar de la Algaida" Natural Park. So, habitat protection is not likely compromised. According to the IUCN 2001 criteria, the species should be provisionally considered as Endangered EN [B1ac(ii)+2ac(ii)]. Likewise, it should be included in the corresponding lists of endangered bryophytes from Spain, Europe and the world.

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