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A complete description and conservation assessment of *Riella affinis* Howe & Underwood (Riellaceae, Sphaerocarpales) new to continental Europe

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ABSTRACT
Riella affinis Howe & Underwood is a rare species that is thus far known from eight populations worldwide. Only half of these were recorded in the last 50 years and none were recollected since first reported. Study of a Spanish population of *R. cossoniana* Trab., recorded in 2002 from Las Lomas, Malaga province, revealed that it belongs to *R. affinis*. The correct identity of the populations, which had not been available for study, was already suggested by reproductive and ecological characteristics of the plants mentioned in the original publication. The three main lagoons from the Las Lomas endorheic system, where the plants had been recorded, were sampled and living plants of *R. affinis* were found in Viso lagoon whereas culture of sediments yielded plants of this species from Viso and Caja lagoons. *Riella affinis* is new to continental Europe and the new Spanish populations are described and illustrated in detail. *Riella cossoniana* is excluded from Malaga province. *Riella affinis* is classified as critically endangered (CR) globally and for Spain; the new Iberian populations should be targeted for conservation. An identification key to the currently recognised European species of *Riella* Mont. is included.

KEYWORDS
Aquatic liverworts,
floristic studies,
Marchantiophyta,
Mediterranean ponds,
Riellaceae,
threatened species.

RÉSUMÉ

Description complète et évaluation de la conservation de Riella affinis *Howe & Underwood (Riellaceae, Sphaerocarpales), nouveau pour l'Europe continentale.*

Riella affinis Howe & Underwood est une espèce rare, connue à ce jour de huit populations dans le monde. Seulement la moitié d'entre elles ont été enregistrées au cours des 50 dernières années et aucune n'a été rappelée depuis le premier rapport. L'étude d'une population espagnole de *R. cossoniana* Trab., enregistrée en 2002 à Las Lomas, dans la province de Malaga, a révélé qu'elle appartenait à *R. affinis*. L'identité correcte des populations, qui n'étaient pas disponibles pour l'étude, était déjà suggérée par les caractéristiques reproductives et écologiques des plantes mentionnées dans la publication originale. Les trois principales lagunes du système endoréique de Las Lomas, d'où les plantes avaient été rapportées, ont été échantillonnées. Des plantes vivantes de *R. affinis* ont été trouvées dans la lagune de Viso alors que la culture de sédiments a donné des plantes de cette espèce provenant des lagunes de Viso et de Caja. *Riella affinis,* nouvelle pour l'Europe continentale et les nouvelles populations espagnoles, a été décrite et illustrée en détail. *Riella cossoniana* est exclue de la province de Malaga. *Riella affinis* est classée en danger critique d'extinction (CR) à niveau mondial et pour l'Espagne ; les nouvelles populations ibériques devraient être ciblées pour la conservation. Une clé pour l'identification des espèces européennes actuellement reconnues de *Riella* Mont. est incluse.

MOTS CLÉS
Hépatiques aquatiques, études floristiques, Marchantiophyta, Étangs Méditerranéens, Riellaceae, espèces menacées.

INTRODUCTION

Riella Mont. (Riellaceae, Sphaerocarpales) is a genus of aquatic, ephemeral liverworts of great biogeographical, evolutionary, ecological and conservation interest. About half of the 24 currently accepted species grow submerged in seasonally, brackish ponds, representing exceptional ecological rarities among bryophytes, in particular liverworts. The largest diversity is concentrated in countries surrounding the Mediterranean Basin, where 10 species have been reported. On the European side of the Mediterranean, Spain has the highest representation of this genus, both in terms of known species and number of populations. The four known mainland species (Cirujano *et al.* 1988) have now increased to seven due to intensive research in recent years (Puche & Segarra-Moragues 2013; Segarra-Moragues *et al.* 2014).

The species of *Riella* are grouped into two subgenera based on the female involucres being smooth or papillose (subgenus *Riella*) or winged (subgenus *Trabutiella* Porsild). The latter group is the least diversified and includes six species (Segarra-Moragues *et al.* 2014), of which three are dioicous, *R. cossoniana* Trab., *R. echinata* (Müller) Segarra-Moragues, Puche & Sabovljević and *R. mediterranea* Segarra-Moragues, Puche, Sabovljević, Infante & Heras, and are known from continental Europe. A fourth, monoicous species, *R. affinis* Howe & Underwood, has its European distribution restricted to the two largest islands of the Canary archipelago, Gran Canaria and Tenerife (Losada-Lima 1986; Dirkse *et al.* 1993; Segarra-Moragues *et al.* 2014; Fig. 1). The two remaining species have a more restricted distribution, the monoicous *R. heliospora* Segarra-Moragues, Puche & Sabovljević being endemic to California (Segarra-Moragues *et al.* 2012a , 2014), and the dioicous *R. gamundiae* Hässel de Menéndez (Hässel de Menéndez 1972), endemic to Argentina. A recent revision of the subgenus *Trabutiella* (Segarra-Moragues *et al.* 2014) established the taxonomic boundaries of the six species based on the study of abundant, fresh material and herbarium specimens. Many herbarium collections proved misidentified, not only among the species of subg. *Trabutiella*, but also within subg. *Riella*. *Riella cossoniana* and *R. affinis* proved to be the most widely distributed members of subg. *Trabutiella*, with the largest number of records (Segarra-Moragues *et al.* 2014). Both species are readily separated based on the distribution of the sexual organs (monoicous vs dioicous) and spore morphology (Segarra-Moragues *et al.* 2014). Notwithstanding, we wondered if a population of *R. cossoniana* from the southern Spain (Ortega-González *et al.* 2002) could have been misidentified.

As indicated by Ortega-González *et al.* (2002), the southern Iberian plants of *R. cossoniana* were growing submerged in a fresh water lagoon (water conductivity 0.42 mS/cm); only female individuals were found. The habitat contrasted with that of other *R. cossoniana* populations, which usually grow in brackish ponds and lagoons with higher conductivity (3.25-3.10 mS/cm, Ros 1987; 3.3-3.6 mS/cm, Cirujano *et al.* 1988; 2.65 mS/cm, Cirujano *et al.* 1992; 2.7 mS/cm, Cirujano *et al.* 1993). The absence of male individuals was also unusual since in all localities of *R. cossoniana* visited by the first two authors, male and female plants were found (Segarra-Moragues & Puche, pers. obs.). Nonetheless, it could be that male plants had already decayed as the species was apparently collected late in the season (Ortega-González *et al.* 2002). Unfortunately, the specimens supporting the record were not available for study and the identification could thus not be verified in a recent study of the species (Segarra-Moragues *et al.* 2014). For reasons given above, the ecological and reproductive characteristics of the population cast reasonable doubts about its identity, suggesting instead that a monoicous species of *Trabutiella*, such as *R. affinis*, could be at hand. The exact collection site of the population, reported from Laguna de Las Lomas (Antequera, Málaga province) (Ortega-González *et al.* 2002), was unclear as this

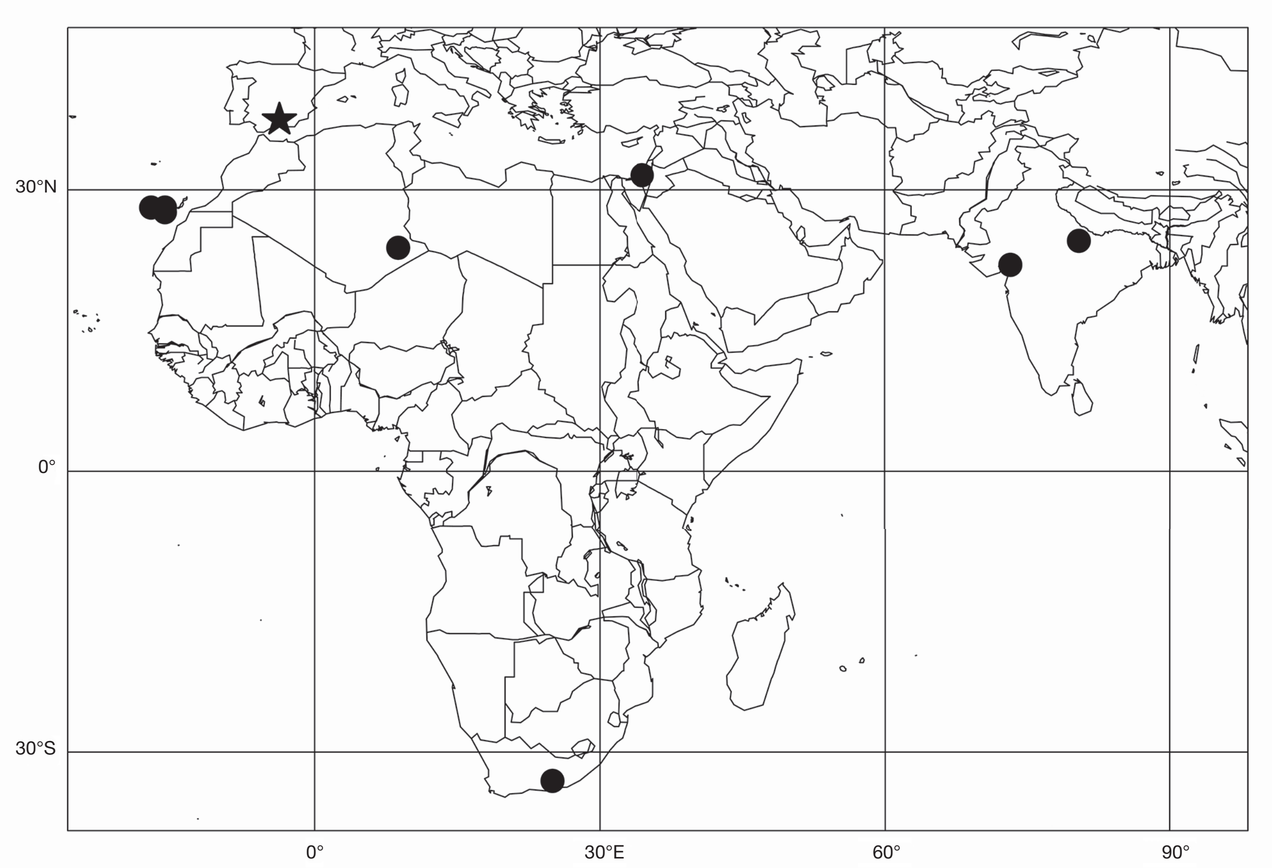

Fig. 1. — World distribution of *Riella affinis* Howe & Underwood. Each symbol represents a population. Known records of *R. affinis* are designated by black dots, new records by a star (populations from Caja and Viso lagoons, Las Lomas, Malaga province, Spain).

site corresponds to an endorheic system composed of three main lagoons, El Chaparral, Caja, and Viso (the latter also known as Jaralón). From details given in the paper, however, it could be assumed that the specimens were from Laguna de Caja, being the only of the three lagoons that had been managed before the time of collecting in order to increase water depth (Ortega-González *et al.* 2002).

During recent field studies aimed at studying *Riella* populations of the Iberian Peninsula, we visited the Las Lomas area making an attempt to recollect specimens of *Riella*, or to obtain plants from cultures of sediments from the lagoons, allowing us to verify the report by Ortega-González *et al.* (2002). Here we present the results of this study.

MATERIAL AND METHODS

Study sites, sediment sampling and culture

The endorheic basin at Las Lomas area in Antequera (Málaga province, southern Spain) includes three neighboring fresh water seasonal lagoons namely, Laguna del Chaparral (37°01'45.43"N, 4°28'11.42"W, 727 m a.s.l.), Laguna de Caja (37°01'19.88"N, 4°28'11.01"W, 729 m a.s.l., Fig. 2A) where the *Riella* specimens were presumably collected by Ortega-González *et al.* (2002), and Laguna de Viso (37°00'52.56"N, 4°28'14.87"W, 724 m a.s.l.). El Chaparral and Caja lagoons were visited in December 2017. At the time of our visit, El Chaparral was almost dry and the lagoon seemed unsuitable for *Riella*. The water level at Caja, however, was adequate for the development of *Riella*. Since living plants could not be found here, about 3 kg of soil sediments were collected at different points along the margin of the lagoon, for culture in the laboratory. This procedure has been proved successful for obtaining populations of *Riella* in previous studies (Proctor 1972; Hässel de Menéndez 1987; Segarra-Moragues *et al.* 2012a, b, 2019; Segarra-Moragues & Puche 2014). The sampled sediments were dried at room temperature prior to initiation of cultures.

The three lagoons were again visited in June 2018. At this time, water levels in all three lagoons were such that plants of *Riella* were collected from Viso lagoon. The plants were brought to the laboratory and grown in culture until plants were fully developed and the spores matured.

Following the procedure described above, soil sediments were collected from El Chaparral and Caja lagoons, and immediately put into culture as described in Segarra-Moragues *et al.* (2019). Cultures were inspected weekly for the presence of *Riella* and, where successful, were maintained to obtain fully developed, fertile plants with sporophytes for identification and morphological study.

Morphological study

Specimens were examined using Light (LM) and Scanning Electron Microscopy (SEM) techniques. Measurements of vegetative and spore characters observed under LM were obtained using the interactive measurement module of Leica Application Suite (LAS) v. 3.8 (Leica microsystems, Barcelona, Spain) calibrated to the nearest 0.01 μm for digital images. For SEM analysis mature gametophytes and spores were prepared following the protocols described in Segarra-Moragues *et al.* (2014, 2019). Morphological observations were carried out using a Hitachi S-4100 field emission SEM at the University of Valencia (SCSIE-UV). Terminology of spore characters followed Segarra-Moragues *et al.* (2014). The gametophytes and spores of the obtained individuals were compared with herbarium material and with LM and SEM images of previously studied herbarium specimens (Segarra-Moragues *et al.* 2014).

Other specimens examined

Specimen. — **Algeria**. Tassili n'Ajjer, Tafilalet canyon, in a seasonal rock pool with a surface of around 10 m^2 on very fine mud from the surface to 1 m depth, 24°36'05"N, 9°37'26"E, 1600 m, 13.I.2007, *F. Boisset* (VAL-Briof. 7.372); *ibid*., cultured in the laboratory, dried 12.IV.2013 (VAL-Briof. 9.231).
South Africa. Dam at Table Hill Farm, Cradock road, 6 miles from Grahamstown, III.1953, *M.A. Pocock* (NMW C46.668.1695); *ibid*., "Proskauer's" laboratory culture from *U. Kutschera* (VAL-Briof. 9.230); *ibid*., culture dried on 29.VIII.2012 (VAL-Briof. 9.332); *ibid*., culture dried on 03.IX.2012 (VAL-Briof. 9.333).
Spain. Canary Islands: Gran Canaria, Tafira, bank of reservoir, VI.1897, *O. F. Cook* 729 (NY00713614), *ibid*., am Rande eines Reservoirs, VI.1897, *O. F. Cooke*, Schiffner Hepat. Europ. Exicc. XXV: 1241 (JE04003557, Krypto-S B45318, NY01076615), *ibid*., VI.1897, fr., *A. Carter Cook*, comm. Smithsonian Institution, Kryptogamae Exsiccatae editae a Mus. Hist. Nat. Vindobon. 2971 (HBG 1/2244, Krypto-S B177457, MA-Hepat. 4097, 4273, NY00713615, YU064009); Barranco de Berriel, W of the presa de Berriel, dry rocky slopes, boulders, small stream, xerophytic vegetation: *Plocama pendula*, *Euphorbia canariensis*, *E. obtusifolia*, *Asphodelus micorcarpus*, *Kickxia scoparia*, *Rubia peregrina*, *Launaea arborescens* and *Pancratium canariense*, UTM 445-3075, 50-100 m, 22.II.1996, *G.M. Dirkse* (Herb. Dirkse 015081); Tenerife, Cruce de los Abrigos, comienzo de la carretera de San Miguel, parcial o totalmente sumergida en pequeña presa, 26.V.1984, *W. Wildpret & M. del Arco* (TFC-Bry. 1375); *ibid*., 13.VI.1984, *W. Wildpret, M. del Arco & C. Gil* (TFC-Bry. 1270, BCB 14098, Herb. Dirkse 015081).

Conservation status assessment

Given that species of *Riella* are globally rare, once identified the specimens to the species level we conducted an assessment of the conservation status. The classification into IUCN categories (IUCN 2012) for the global distribution and for the Spanish distribution was estimated using the Geospatial Conservation Assessment Tool (GeoCAT, available at http://geocat.kew.org/, last accessed 19 June 2019). This interactive tool was used to estimate the extent of occurrence (EOO) and area of occupancy (AOO) applying a conservative criterion considering all known populations and a more strict criterion for reduction analyses based on current knowledge of population status, when available (see Results). For the estimation of area of occupancy a size grid of 0.5 km^2 was applied, since this area was deemed sufficient to cover the whole extension of lagoons and ponds for most of the localities.

RESULTS

Cultures of sediments from the 2017 collection from Caja lagoon commenced on 17 April 2018. Abundant plants of *Riella* were obtained two weeks after initiation of cultures. Sporophyte development was observed by 22 May 2018.

Morphology of Iberian populations

The morphological study of the specimens confirmed that they belonged to a species of subgenus *Trabutiella* due to their winged involucres (Fig. 2B-F). As in the original paper, no male individuals were observed. Unexpectedly for the dioicous condition of *R. cossoniana*, individuals with female involucres and sporophytes also carried antheridia (Fig. 2B, C). These were solitary or clustered in small groups in the sinuses of the dorsal, lobulate wing (Fig. 2C). These morphological characteristics indicated that the plants belonged to *R. affinis*, rather than *R. cossoniana*. The identification was subsequently confirmed based on spore traits (Fig. 3). The characteristics of the cultured plants from Caja lagoon matched those of plants collected *in situ* from Viso lagoon on 18 June 2018.

The two southern Iberian populations constitute a significant northwards extension of the range of *R. affinis* (Fig. 1). Indeed, the nearest record is the type locality of the species at Tafira, Canary Islands, more than 1400 km from the Iberian locality and where the species has not been collected since 1897. A detailed description of the Iberian populations is given below.

Riella affinis Howe & Underwood

Bulletin of the Torrey Botanical Club 30: 221 (1903).

Riella vishwanathai Pandé, Misra & Srivastava, *Revue bryologique et lichénologique* 23: 166 (1954). — Type: **India**. Uttar Pradesh, Lake Latif Shah, in shallow water or along the lake margin, on mud. 1950, *K. C. Misra 3590* (4817 LWU!).

Type. — **Spain**. Canary Islands, Grand Canary, Tafira, on the bank of a reservoir, VI.1897, *O. F. Cooke 729* (holo-, 01093954 NY!; iso- HBG!, JE!, Krypto-S!, MA-Hepat.!, NY!, YU!).

New records. — **Spain**. Málaga, Antequera, Laguna de Caja, 37°01'19.88"N, 4°28'11.01"W, 729 m, *M. J. Gil-López*, cultures started on 17 Apr 2018, plants dried on 18.VII.2018. (VAL-Briof. 11.797); Antequera, Laguna de Viso/Jaralón, 37°00'52.56"N, 4°28'14.87"W, 724 m, *M. J. Gil-López*, plants collected on 18.VI.2018, culture continued in the laboratory and plants dried on 03.IX.2018 (VAL-Briof. 11.798).

Description

Plants

0.9-3.5 cm tall, caespitose, bifurcate from the base, rarely unbranched, shoot apex falciform (Figs 2B, 4A). Axis slightly flattened 0.16-2.4 mm wide. Dorsal wing 2-2.7 mm wide,

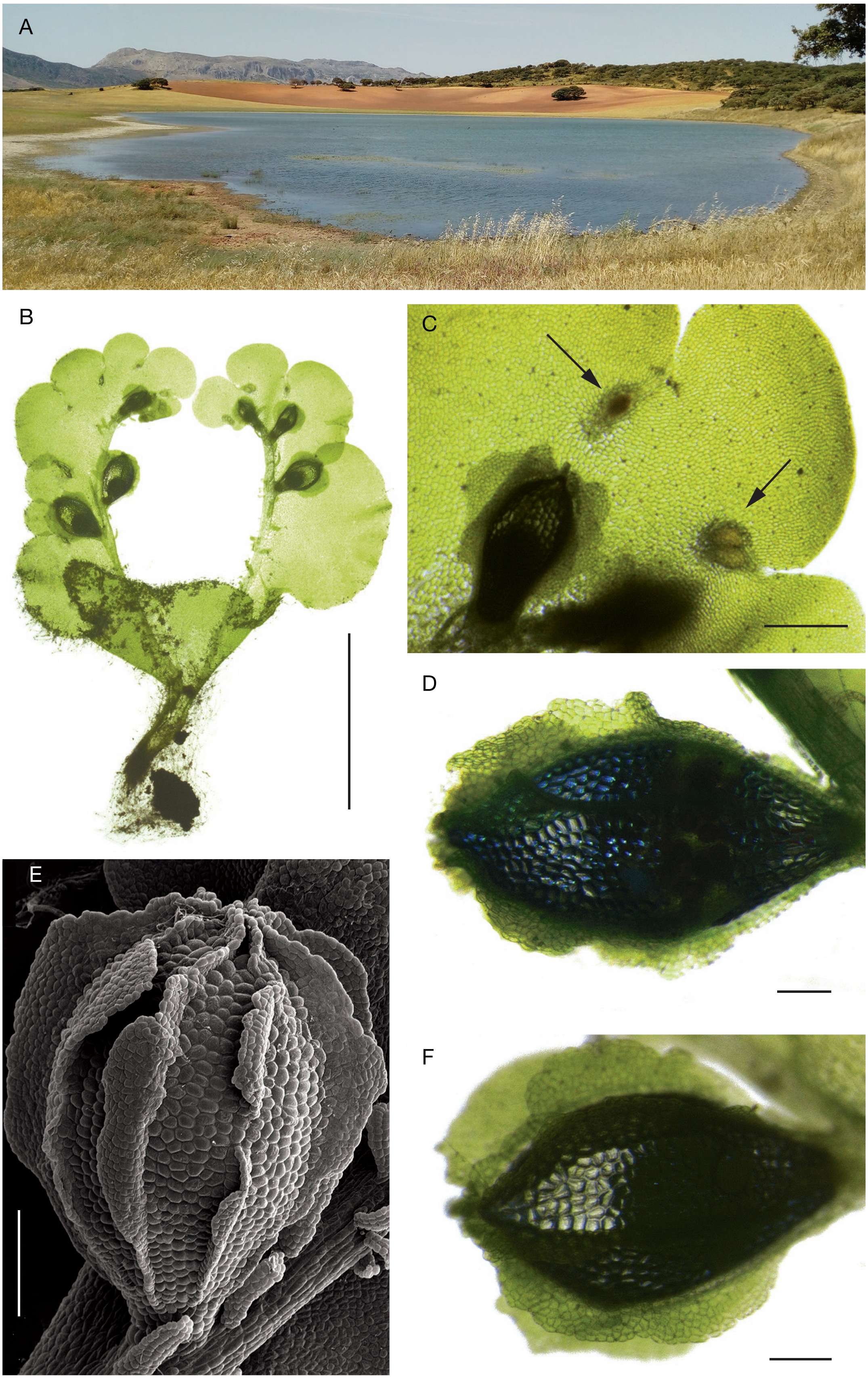

FIG. 2. — Habitat, LM and SEM images of *Riella affinis* Howe & Underwood: **A**, habitat at the Caja lagoon; **B**, one individual showing the lobulate wing; **C**, thallus apex showing a developing winged female involucre with a sporophyte enclosed and antheridia solitary or in small groups in sinuses of the thallus wing (**arrowed**); **D**, **E**, two female involucres showing the variation in involucre wing morphologies; **F**, SEM image of female involucre showing an interrupted wing (all from VAL-Briof. 11.797). Scale bars: B, 5 mm; C, 500 µm; D, F, 200 µm; E, 300 µm.

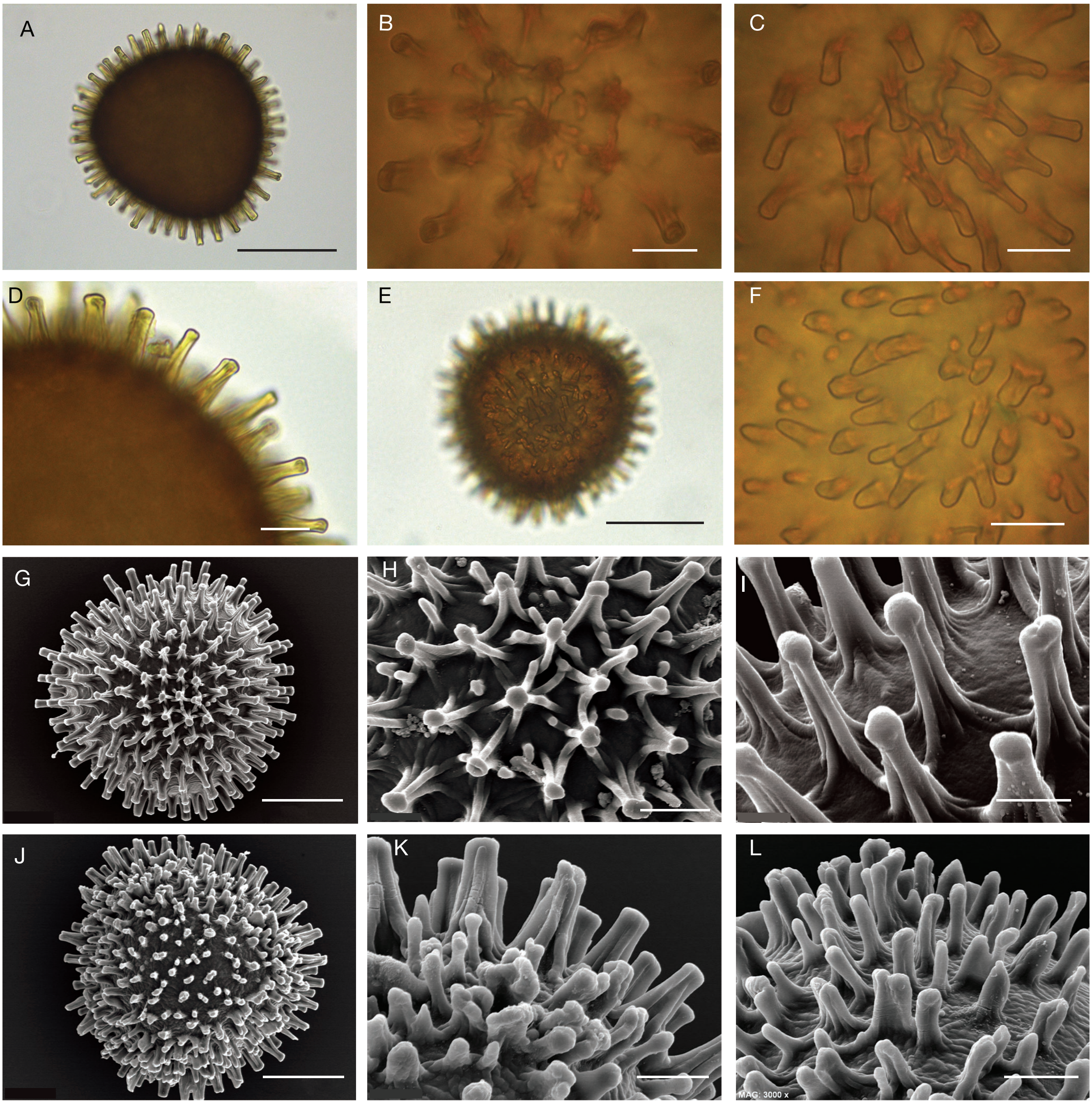

FIG. 3. — LM and SEM images of spores of *Riella affinis* Howe & Underwood: **A**, distal view; **B**, spines and reticulum from distal pole; **C**, spines from distal side; **D**, equatorial row of spines; **E**, proximal view; **F**, spines from proximal side; **G**, distal view; **H**, spines and reticulum of basal membranes at distal pole; **I**, spines from distal side; **J**, proximal view; **K**, transition between distal and proximal side, showing the equatorial row of distal spines; **L**, proximal spines and rugose spore surface. (all from VAL-Briof. 11.797). Scale bars: A, E, 50 µm; B, C, D, F, 10 µm; G, J, 30 µm; H, 9 µm; I, 5 µm; K, L, 8 µm.

deeply lobed (Figs 2B, 4A). Marginal wing cells quadrate-rectangular, chlorophyllose, 17-47 × 13-22 µm (Fig. 4E); wing cells near axis rectangular-polygonal, 107-164 × 23-35 µm; oil cells quadrate, polygonal, 11-31 × 10-27 µm, oil bodies 9-17 × 9-15 µm irregularly spherical, rough, opaque. Scales dimorphic; vegetative scales ligulate to linear-lanceolate, 183-395 × 66-148 µm (Fig. 4B-D); propaguliferous scales panduriform, 389-992 × 220-534 µm. Sexual condition monoicous, protandrous. Antheridia solitary or in groups of 2-12, rarely in larger groups, in sinuses of the dorsal wing (Figs 2B, C; 4A), antheridial body 149-200 × 92-130 µm. Archegonial involucre ellipsoid 1.1-1.9 × 0.9-1.6 mm, blunt to obscurely apiculate, with (7) 8 (10) wings, 217-312 µm high and (4) 7 (11) cells high at their highest part, widest at the middle of involucre, margin entire or undulate-sinuate (Figs 2D-F, 4F); some wings interrupted, not reaching the base or apex of involucre. Sporophyte with seta of 0.25-0.42 mm, capsule more or less spherical, 0.64-0.95 × 0.58-0.99 mm.

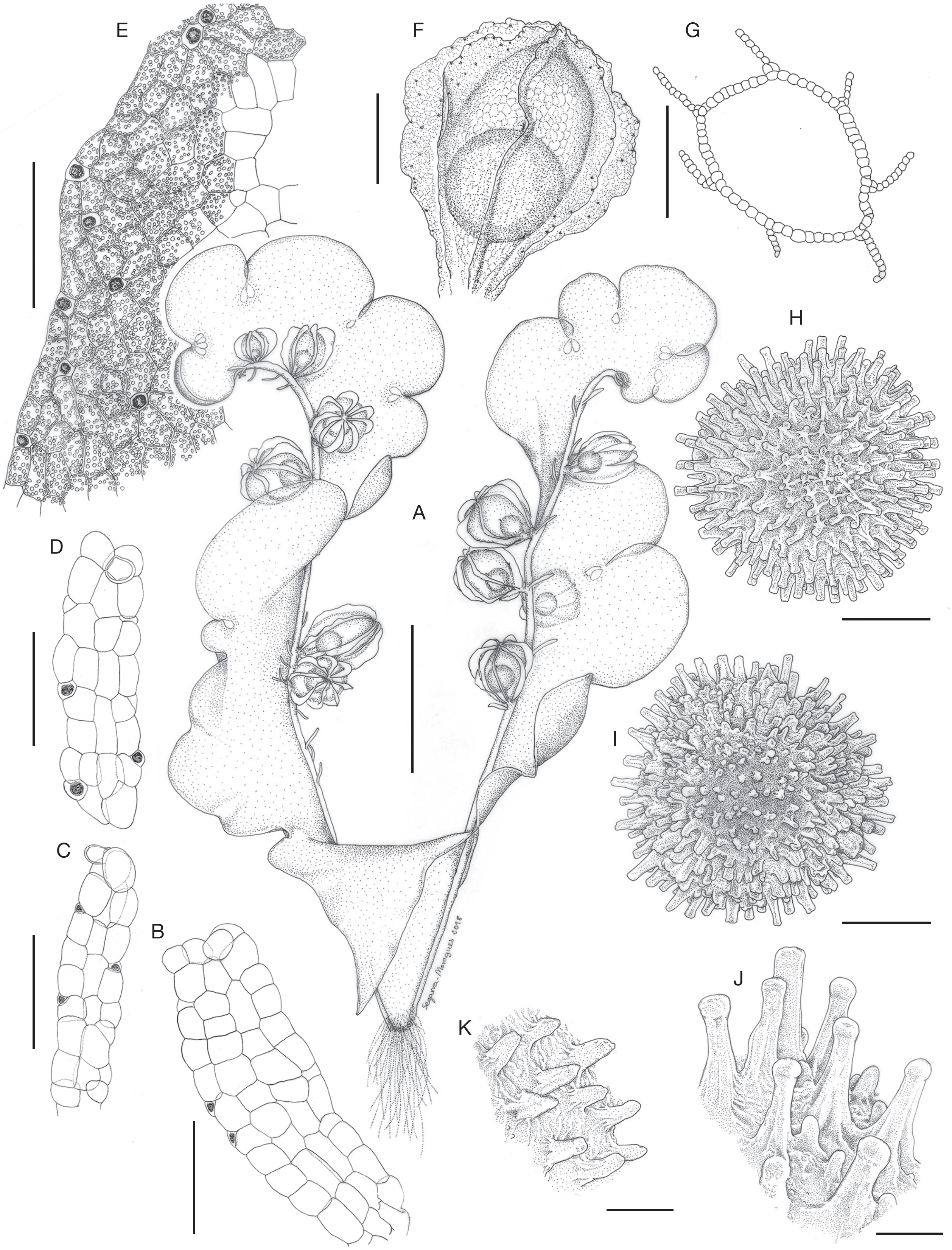

Fig. 4. — *Riella affinis* Howe & Underwood: **A**, habit; **B**-**D**, vegetative scales; **E**, cells from margin of thallus wing; **F**, female involucre; **G**, cross-section of female involucre showing seven wings; **H**, spore in distal view; **I**, spore in proximal view; **J**, spore spines from distal face; **K**, spore spines from proximal face. (all from VAL-Briof. 11.797). Scale bars: A, 5 mm; B, C, D, 100 µm; E, 200 µm; F, G, 500 µm; H, I, 30 µm; J, K, 8 µm.

Spores
106-116 × 100-115 µm in cross diameter including spines, brown, globose, rounded to subtriangular in outline (Figs 3A, E, G, J; 4H-I). Distal face densely covered with 13-15 irregular rows of spines across diameter and interspersed smaller papillae, distance between spines 2.5-5.3 µm, and (30) 32 (34) projecting spines around periphery of equatorial plane (Figs 3A, G-H, J; 4H-I). Spines (10.6) 12.5 (14.3) µm long (4.1) 5.3 (6.7) µm wide, apices blunt or truncate, with rounded or truncate apices, rarely dilated or flaring (Figs 3C-D, I; 4J); basal membranes interconnecting spines below, 0.7-1.3 µm high or absent, sometimes forming imperfect reticulations at the distal pole (Fig. 3H); basal membranes at the equatorial plane, 1-2.6 µm high or absent, not forming conspicuous wing-like marginal webbing (Fig. 3K). Proximal face flat to convex, without triradiate mark, surface of proximal face rugose (Figs. 3J-L; 4I, K); spines dense (distance between spines 1.5-5.0 µm), shorter than those of distal face (4.5-) 6.9 (-9.5) × (2.3-) 4.2 (-5.7) µm, with blunt or acute apices, not basally interconnected but sometimes joined in clusters, especially towards the periphery of the proximal face (Figs 3K, L, 4I, K).

Habitat
Riella affinis occurred in the shallow (10 cm deep) margins of the Viso lagoon, growing in clearings of areas occupied by *Potamogeton gramineus* L., *Eleocharis palustris* (L.) Roem. & Schult. and an unidentified species of Characeae. Water analysis measurements on 18 June 2018 indicated the water was alkaline (pH=8.8) and fresh (0.18 mS/cm, at 35.1°C water temp. at 16:50 h). Based on the results of the culturing of the 2017 soil samples from Caja lagoon, it is likely that *R. affinis* occurs at greater depth (50-100 cm) in this lagoon (Fig. 2A) because the samples were collected when water levels were lower than during the 2018 visit. Here, cultured plants of *R. affinis* co-occurred with *Myriophyllum spicatum* L. and a species of the *Riella notarisii* (Mont.) Mont. complex. Water analysis measurements from Caja lagoon showed that the water was alkaline (pH=9.7), and fresh (0.46 mS/cm, at 33.8°C water temp. at 13:44 h), confirming previous conductivity measurements by Ortega-González *et al.* (2002). Although *R. affinis* was not observed in the Chaparral lagoon nor obtained from culture of sediments of this site, the close proximity of this lagoon to the other two and its similar water characteristics (pH=9.1, 0.29 mS/cm, at 28.8°C water temp. at 11:30 h), suggest that the species could also be present here.

DISCUSSION

Our study has for the first time reported the occurrence of *R. affinis* in continental Europe, based on two populations from southern Spain (Fig. 1). This finding adds an eighth species of *Riella* to mainland Europe and doubles the number of currently accepted *Riella* species here as compared to that known three decades ago (Cirujano *et al.* 1988). Other recent additions to the *Riella* flora of mainland of Europe include *R. bialata* Trab. (Puche & Segarra-Moragues 2013) and two species of subg. *Trabutiella*, *R. echinata* and *R. mediterranea* (Segarra-Moragues *et al.* 2014). The proportion of *Trabutiella* species in the Mediterranean area is larger than in any other part of its range, including four of the six currently accepted species of this subgenus. This could suggest that the Mediterranean basin is the diversification centre of subgenus *Trabutiella*. However, it cannot be discarded that additional species or species records may be found in other suitable areas of the world, as they have been much less explored for *Riella* than the Mediterranean area (see Segarra-Moragues *et al.* 2012a; Cargill & Milne 2013; Segarra-Moragues & Puche 2014).

Our ongoing taxonomical studies of *Riella* have revealed many misidentifications of herbarium specimens (Segarra-Moragues *et al.* 2014), caused in part by inaccurate observation and interpretation of morphological traits, particularly of reproductive ones. This includes the record of *R. cossoniana* from Malaga province (Ortega-González *et al.* 2002), which is now rejected based on our results. Due to misidentification of this population, the occurrence of *R. affinis* in mainland Europe remained undetected during 16 years, precluding a critical assessment of the conservation status of the species in the Spanish (González-Mancebo *et al.* 2011) and European territories and a proposal for an active conservation strategy to protect the southern Iberian population of the species.

World distribution of *Riella affinis*
Riella affinis has a broad, albeit scattered, amphitropical, old world distribution (Segarra-Moragues *et al.* 2014; Gradstein 2017; Fig. 1). In the northern part of its range it extends from Macaronesia (Canary Islands) through the Mediterranean into Asia (India), whereas in the southern part it is represented by a single South African population (Segarra-Moragues *et al.* 2014; Gradstein 2017; Fig. 1). Despite its broad distribution, *R. affinis* is known from only 10 populations, including the two reported here. Six out of these 10 reports date back more than 30 years. Its eastern distribution limit in Asia is based on records from two Indian populations (Pandé *et al.* 1954; Patel 1977). The first of these two reports was considered to belong to a different species, *R. vishwanathai* Pandé, Misra & Srivastava (Pandé *et al.* 1954), which was reduced to synonymy under *R. affinis* by Proskauer (1955) and subsequent authors (Patel 1977; Segarra-Moragues *et al.* 2014). The Indian populations have not been recollected and their status is uncertain. A recent study showed that the habitats of one of the two Indian localities have been extensively transformed, likely providing unsuitable conditions for the survival of *R. affinis* (Shah & Gujar 2016).

In the eastern Mediterranean part of its range *R. affinis* was reported from Israel by Lipkin & Proctor (1975). However, the precise locality is unknown and material supporting the record does not exist. Thus, the occurrence of *R. affinis* in Israel still needs clarification. The western Mediterranean record from Tassili n'Ajjer National Park, South Algeria, represented the most recent account of the species (Puche & Boisset 2009). It was found there in a very small pool of about 10 m². Since such small pools are common in the area, it is likely that *R. affinis* could be more widespread in the region.

Key to the European species of *Riella* Mont.

1. Female involucre winged 2
— Female involucre smooth or papillose 5

2. Monoicous, antheridia in small groups, on sinuses of the thallus wing .... *R. affinis* Howe & Underwood
— Dioicous, antheridia in large continuous series, along the margin of male plants 3

3. Female involucre acuminate, generally with more than 10 low, discontinuous wings, spores densely spinose with distal spines longer than 8 µm .... *R. mediterranea* Segarra-Moragues, Puche, Sabovljević, Infante & Heras
— Female involucre ovoid or ellipsoid with 10 or fewer wings, spore with distal spines generally shorter than 7 µm 4

4. Female involucre ellipsoid, wings of involucre >100 µm extending from the apex to the base, distal face of spores not reticulate .... *R. cossoniana* Trab.
— Female involucre ovoid, wings of involucre < 100 µm, some discontinuous and not reaching the apex or base of involucre, distal face of spores reticulate .... *R. echinata* (Müller) Segarra-Moragues, Puche & Sabovljević

5. Plants monoicous 6
— Plants dioicous 7

6. Thallus wing single, antheridia at wing margin .... *R. notarisii* (Mont.) Mont. s.l.
— Thallus wing geminate, antheridia between thallus wings .... *R. bialata* Trab.

7. Plants generally exceeding 8 cm in length, thallus wing duplicate, one undulate, entire and narrow, the other deeply dissected into ovate lobes larger than the other wing and giving the plants a foliose appearance, distal face of spores reticulate .... *R. parisii* Gottsche
— Plants up to 5 cm long, generally shorter, thallus wing single, entire, plants not foliose in appearance distal face of spores not reticulate .... *R. helicophylla* (Bory & Mont.) Mont. s.l.

The western limit of *R. affinis* is in the Canary Islands, where it has been reported from Gran Canaria (Howe & Underwood 1903) and Tenerife (Losada-Lima 1986). Two populations are known in Gran Canaria, including the type locality in the northern part of the island, where it was only once collected in 1897, and a more recent collection in the southern part, suggesting the continued existence of suitable habitats for the species on the island. A single population is known from Tenerife, dating back more than three decades ago and whose persistence is uncertain (González-Mancebo *et al.* 2011).

The current state of the South African population is similarly uncertain as it has not been recollected since it was found there by M.A. Pocock in 1953 (Proskauer 1955). Attempts to locate the species or obtaining plants from cultures of sediments collected 46 years after its discovery, were not successful (Perold 2000). It is unclear whether this was due to habitat transformation or to collection of sediments at the wrong site.

Like elsewhere, the populations of *R. affinis* from mainland Spain grow submerged in fresh, rather than brackish lagoons and ponds. Since such habitats are abundant throughout the Iberian Peninsula and Mediterranean countries, it could be expected that *R. affinis* is much more widespread in the area. While this may hold for the less intensively explored Mediterranean countries, it is unlikely true for mainland Spain, however. In the latter country, a large number of *Riella* populations have been reported (Cirujano *et al.* 1988; Segarra-Moragues *et al.* 2014), including taxa of the *R. notarisii* complex which typically grow submerged in fresh to hyposaline waters (0.12-19 mS/cm, Cirujano *et al.* 1993; Valentín *et al.* 2007) and were also found at Caja lagoon (see Results). The fact that *R. affinis* has only been found in two lagoons – Caja and Viso – may indicate that its rarity in mainland Spain is not due to incomplete sampling but reflects its infrequency in the country. Its rarity might reflect recent northwards dispersal from Africa into mainland Europe, possibly mediated by birds (Proctor 1961), or air-borne dust blown from the Sahara desert (Middleton & Goudie 2001) or alternatively, fragmentation of an ancient, more continuous distribution. Disentangling between these two scenarios would require molecular data of samples from different geographical sources, which are currently unavailable. However, given that habitat demands of *R. affinis* are apparently not significantly different from those of the more common *R. notarisii*, the hypothesis of a recent colonization of mainland Europe seems the most plausible one.

The newly reported Spanish populations of *R. affinis* have important biogeographical and conservation implications because: 1) they expand the distribution range of the species into mainland Europe (Fig. 1); and 2) they constitute an excellent and currently extant reservoir which could serve for undertaking active conservation management of the species.

Conservation remarks

The poor knowledge of the population biology and largely scattered distribution of *R. affinis*, with few recent collections across a broad geographical range, make it difficult to refine assessment of its conservation status and classification into a particular IUCN category. Only four of the 10 populations known have been recorded in the last 50 years and none were recollected since first reported. Although the continuity of these populations has not been systematically assessed, in part due to their ephemeral and fluctuant dynamics, recent data suggest that some could have disappeared or that the habitat were severely altered by human activity hampering the development of individuals. A tentative classification of Data Deficient (DD) was proposed by Segarra-Moragues *et al.* (2014) for its global range, and for its Indian, Israeli and South African subranges. The species was considered Vulnerable (VU D2) in Algeria and Endangered (EN B1ab(i,ii,iii)+B2ab(i,ii,iii) in its Spanish range in the Canary Islands following González-Mancebo *et al.* (2011). The two new mainland Spanish populations together with the additional population from Gran Canaria (see material examined) which was not included in the species account by González-Mancebo *et al.* (2011), requires a reassessment of the conservation status at the global and Spanish administrative scales. The new data suggest that some of these populations could recently have declined, necessitating a re-evaluation of its conservation status. Habitat loss has been documented for Lake Kanewal, the western Indian locality, likely resulting in unsuitable conditions for growth of *R. affinis* (Shah & Gujar 2016) and thereby in a 50% reduction in the known sites of the species in India. The persistence of the South African population is unclear (see above), the locality of the Israeli record is unknown (Lipkin & Proctor 1975) and within the Canary archipelago the species has not been recollected in the type locality at Tafira since its discovery there more than a century ago (Howe & Underwood 1903). With the exclusion of these 4 populations, EOO and AOO were estimated as 8,897 × 10^6 km^2 and 1.50 km^2 respectively, accounting for a reduction of 81.31% and 40% of EOO and AOO, respectively. In the Spanish range the exclusion of the Tafira population from the Canary Islands reduced the AOO from 1.25 to 1 km^2 (20% reduction), whereas the EOO remained unchanged.

For reasons explained above, the mainland Spanish distribution range of *R. affinis* is unlikely to be significantly larger. Notwithstanding, the fact that one of these populations was recorded in 2001 (Ortega-González *et al.* 2002, as *R. cossoniana*) and in the present study, indicates that populations have been able to endure fluctuations in the ecological conditions and habitat quality during the 17 year period, and maintain a viable spore bank to restore the populations. Thus, no actual population decline can be hypothesised to have occurred in mainland Spain. In conclusion, therefore, a classification of *R. affinis* in the IUCN category Critically Endangered (CR): B2 b(i+ii+iii) c(i+ii+iii) is proposed for both the global and Spanish ranges of the species.

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