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Study of a new population of the Argentinian endemic species *Riella choconensis* Hässel (Riellaceae, Marchantiophyta) reveals a novel anatomical structure of the female involucre in *Riella*

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ABSTRACT
A new population of the Argentinian endemic *Riella choconensis* Hässel (Riellaceae, Marchantiophyta) was discovered from a culture of sediments from the Laguna de los Juncos (Río Negro province, Argentina). This species was known previously from two nearby collections, made in 1984, in the type locality at the Ramos Mexía reservoir (Neuquén province) and was not recorded since. Fresh material from the new population enabled detailed morphological analyses, which are extensively described here, illustrated and compared to type material of the species. Based on the smooth female involucre, the species belongs to subgenus *Riella* Mont. However, cross-sections of this structure showed that *R. choconensis* has bistratose involucre walls, contrasting with the unistratose female involucres of all other species in the genus. The updated morphological data are integrated into a new key to the identification of the American species of *Riella*.

KEYWORDS
aquatic liverworts,
bryophytes,
chorology,
floristic studies,
South America,
threatened species.

RÉSUMÉ

Une étude d'une nouvelle population de l'espèce endémique argentine Riella choconensis *Hässel (Riellaceae, Marchantiophyta) révèle une structure anatomique de l'involucre femelle de* Riella *encore inconnue.* Une nouvelle population de l'espèce endémique argentine *Riella choconensis* Hässel (Riellaceae, Sphaerocarpales) a été découverte à partir de la culture de sédiments de la Laguna de los Juncos (province de Río Negro, Argentine). Cette espèce était auparavant connue de la localité type du réservoir Ramos Mexía (province de Neuquén) et n'a pas été enregistrée depuis ses collections originales en 1984. Les matériaux frais de la nouvelle population ont permis des analyses morphologiques détaillées et sont largement décrites ici, illustrées et comparées au matériau type de l'espèce. Sur la base de l'involucre femelle lisse, l'espèce appartient au sous-genre *Riella* Mont. Les coupes transversales de cette structure ont montré que *R. choconensis* a des involucres à parois bistratifiés, contrastant avec les involucres femelles unistratifiés de toutes les autres espèces du genre. Les nouvelles données morphologiques sont intégrées dans une nouvelle clé d'identification des espèces américaines de *Riella*.

MOTS CLÉS
hépatiques aquatiques,
bryophytes,
chorologie,
études floristiques,
Amérique du Sud,
espèces menacées.

INTRODUCTION

Species of *Riella* Mont. (Riellaceae, Sphaerocarpales) are unique amongst the liverworts because of their ephemeral habit, colonising temporary aquatic ecosystems in arid or semiarid environments of Mediterranean-type seasonal climates on all five continents (Geissler 2001). The distribution is poorly known both for the genus and for most of the species because of their ephemeral and sporadic populations and their development being strongly dependent on climatic parameters. They manifest specific ecological demands throughout their life cycle, including fluctuant water levels, with drought periods that trigger spore germination after reflooding of their habitats (Studhalter 1931). At the same time, drought periods contribute to the elimination of populations of other annual aquatic macrophytes and algae that would outcompete populations of *Riella* during the warmer months (Cirujano *et al*. 1993). The combination of sufficient water depth during the three to five cooler months, and the complete drought, or at least a high fluctuation of the shoreline, are seldom predictable and are typical characteristics of vernal pools in areas of seasonal climates (Grillas 2004). These extreme environmental conditions probably explain the evolution of the high longevity of *Riella* spores, which can endure long periods of drought (Studhalter 1931; Proctor 1972), germinating only during suitable years and thus, strongly reducing the chances of their populations being discovered.

Out of the 20 undisputed species (Söderström *et al*. 2016), to date only five species of *Riella* have been reported from South America (Fig. 1), these being *R. americana* M. Howe & Underw., *R. choconensis* Hässel, *R. pampae* Hässel, and *R. undulata* Hässel, belonging to subgenus *Riella* and *R. gamundiae* Hässel, belonging to subgenus *Trabutiella* Porsild (Hässel de Menéndez 1987a). All these species are endemic to Argentina except for *R. americana*, in which the Argentinian population represents a strong disjunction from the North American (United States and Mexico) core distribution range of the species (Gradstein 2017). *Riella americana*, *R. gamundiae* and *R. undulata* are known each from a single locality in Argentina (Buenos Aires and Río Negro provinces), whereas *R. choconensis* is known from two nearby collections in the Ramos Mexía reservoir in Neuquén province, and *R. pampae* from one locality each in La Pampa and Buenos Aires provinces (Hässel de Menéndez 1987a). Thus, with the only exception of *R. pampae*, the reported populations of the Argentinian endemic species correspond solely to type localities, and have not been further reported since their first description (Hässel de Menéndez 1987a).

As has occurred in other areas in the range of this genus (Porsild 1902; Cavers 1903; Segarra-Moragues *et al*. 2012a, b), the discovery of some of the Argentinian populations of *Riella* has been largely haphazard and the result of chance encounters. Culturing of sediments collected at potentially suitable habitats for *Riella* have driven the discovery of many species and populations (Proctor 1972; Hässel de Menéndez 1979, 1987a; Segarra-Moragues & Puche 2014), including the Argentinian *R. pampae* and *R. undulata*, which have not been observed in the wild (Hässel de Menéndez 1979, 1987a, b). Their relatively easy laboratory culture has allowed a significant advance in the knowledge of these ephemeral, elusive liverworts.

This study aims at providing a detailed morphological description of a new population of *R. choconensis* obtained from culture of sediments from the Laguna de los Juncos, an Argentinian seasonal lagoon.

MATERIAL AND METHODS

Study site

Soil sediment samples were collected from the Laguna de los Juncos (Fig. 2A), an endorheic pond occurring in the arid Northwestern Patagonian steppe (41°03'34" S, 71°00'29" W). This pond is situated inside a private protected area named "Wildlife Refuge Laguna Los Juncos", at an elevation of 910 m.a.s.l., and covers around 7 ha, with a maximum depth of 1.8 m (Perotti *et al*. 2005). The climate in the area is Mediterranean-type, with precipitation occurring mainly in winter, mostly as snow (Pérez *et al*. 2005). The mean annual precipitation is 580 mm; the mean monthly temperature

Fig. 1. — Distribution of the five Argentinian species of *Riella* Mont. The inset map shows the geographical location of records of each species designated by a different symbol across the different provinces in Central Argentina. Previously known records of *Riella choconensis* Hässel are designated by a diamond (type locality) and new record by a star. The map indicates names and administrative boundaries of Argentinian provinces (**grey lines**) which are at some instances coincident with rivers (**blue lines**).

ranges between 15°C in January (summer) and 3°C in July (winter; San Ramón Ranch Meteorological Station 1929-2013, unpublished data). The pond can freeze in winter, while complete desiccation is common in summer. Strong dry winds occur between May and August, predominantly from the west (Pérez *et al.* 2005). Soils are derived from glacial and volcanic materials, and are susceptible to wind erosion particularly during the summer drought (Pérez *et al.* 2005). Water pH in the pond has been reported to range between 8.4 and 10.6, water electric conductivity between 345 and 1100 µS cm-1 and dissolved oxygen levels between 7 and 14 mg L-1 (Perotti *et al.* 2005). The vegetation surrounding the pond is shrubby-herbaceous and occurs in low densities. The most abundant plant species at the edge of the pond are *Schoenoplectus californicus* (C.A. Mey.) Steud. (Cyperaceae), *Trifolium repens* L. (Fabaceae), *Juncus* spp. (Juncaceae), *Plantago lanceolata* L. (Plantaginaceae), *Bromus tectorum* L., *Hordeum murinum* L., and *Poa* spp. (Poaceae), and the aquatic watermilfoil *Myriophyllum* sp. (Haloragaceae) (Galende *et al.* 2013). The pond is within a fenced area; however, the area is used for livestock grazing occasionally (Pérez *et al.* 2005). The high diversity of bird species feeding and nesting in this wetland makes this location especially important for wildlife conservation (Pérez *et al.* 2005; Galende *et al.* 2013). In addition, birds may act as dispersal vectors for *Riella* spores (Proctor 1961).

Sediment sampling and culture

In September 2016, about 0.5 kg of dried sediments were collected each from 12 points around the perimeter of the pond and included the first 5 cm depth of soil. Collection points were randomly distributed to span from the shoreline to a few meters into the flooded area, as this is the area most frequently occupied by populations of *Riella*. Sediments were dried and kept on zip plastic bags in the laboratory at room temperature. Sediment cultures were established into transparent plastic containers. In October 2016, collected sediments were spread in the containers to form a 2-2.5 cm layer and were flooded with at least 10 cm of distilled water and kept at 20°C and natural light in the laboratory. Cultures were inspected weekly for the presence of *Riella* and kept indefinitely to provide fully developed, fertile plants with sporophytes for their identification and detailed study.

Morphological study of the plants

Morphological characterization of the specimens was conducted using Light (LM) and Scanning Electron Microscopy (SEM) techniques following procedures described elsewhere (Segarra-Moragues *et al.* 2014). Measurements of vegetative parts and spore characters were taken using the interactive measurement module of Leica Application Suite (LAS) v. 3.8 (Leica microsystems, Barcelona, Spain) calibrated to the nearest 0.01µm on digital images. For SEM analysis, mature gametophytes were fixed with 2% Osmium tetratoxide, dehydrated in increasing ethanol series and critical-point dried using carbon dioxide in an Autosamdri 840 (Tousimis, Rockville, MD, USA) and then 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 Bio-Rad SC-500 ion sputtering coater. Mor-

phological observations were carried out in a Hitachi S-4100 field emission scanning electron microscope at the University of Valencia (SCSIE-UV). Terminology for spore characters followed Segarra-Moragues *et al*. (2014).

Additional specimens studied

The gametophytes and spores of these individuals were compared to those of other Argentinian species of *Riella* (Hässel de Menéndez 1959, 1972, 1979, 1987b) obtained from BA herbarium in order to assess whether they could be assigned to any of the five species previously reported in Argentina. The following materials were studied:

Riella americana: Argentina, Prov. Buenos Aires, Laguna Brava, *Hässel de Menéndez 378*, 5.IV.1958 (BA9946).

Riella choconensis: Argentina, Prov. Neuquén, embalse Ramos-Mexía, *F. Kaisin*, II.1984 (BA33609, paratype).

Riella pampae: Argentina, Prov. Buenos Aires, salinas chicas, *Hässel de Menéndez 4862*, I.1985, material sembrado (BA33611).

Riella undulata: Argentina, Prov. Buenos Aires, Cochicó, *Hässel de Menéndez 4863*, III.1984 (BA33612, holotype).

RESULTS

Culture of sediments

All culture sediments attempted were successful in rendering plants of *Riella*. In early December 2016, the first germlings appeared in the cultures. By the end of January 2017, plants started to produce abundant propaguliferous scales, which rapidly increased the number of individuals in the cultures. Gametophyte development continued during the following months; however, the first sporophytes were not observed until June 2017; these sporophytes rendered adequate material for detailed morphological study of the specimens.

Morphology, anatomy and updated species description

Morphological observations of the mature specimens described above confirmed that the cultured individuals corresponded to a dioicous species, as both males and females were present in the cultures. Female plants were more abundant in cultures and produced smooth, acuminate involucres with occluded apices, indicating a species of subgenus *Riella*. However, cross-section of the female involucres revealed they were composed of two cell layers (i.e. bistratose involucres), unlike all other species in the genus. The study of the micromorphological characters of spores of the cultured specimens showed spore distal faces covered with 15-17 irregular rows of long, 9.5-15.2 µm spines, with mostly acute apices, and proximal faces with long, 4.1-7.7 µm, conical, acute spines matched those of *R. choconensis*.

Here we supplement the original description of *R. choconensis* (Hässel de Menéndez 1987a, b) with original measurements of gametophyte traits from this new population, and with descriptions of spore traits both from BA herbarium materials and from the new population (Figs 3, 4).

Riella choconensis Hässel

In Symposia Biologica Hungarica 35: 341 1987

Type. — **Argentina**, Prov. Neuquén, Embalse Mexía, estación de muestreo A10, bentos, *F. Kaysin*, 7.I.1984 (holo-, BA[BA 33608]; para-, BA[BA33609]!).

New record. — **Argentina**, Prov. Río Negro, Laguna de Los Juncos, 19GCQ3152, 910 m, sediments collected on 15.IX.2016, *G. Gleiser s.n.*, cultures started on 15.X.2016, plants dried 23.II.2018 (VAL-Briof. 11724); plants dried 16.IV.2018 (VAL-Briof. 11725); plants dried 6.VI.2018 (VAL-Briof. 11726); plants dried 4.VII.2018 (VAL-Briof. 11727).

Plants

2.5-5.7 cm high, unbranched or bifurcate (Fig. 4A, B).

Axis

Slightly flattened 209-336 µm wide. Dorsal wing single, undulate or helicoid (Fig. 4A-B), 1.3-2.3 mm wide.

Marginal cells

From wing rectangular, chlorophyllose, 28.5-58.6 × 14.4-30.2 µm (Fig. 4F).

Cells

From mid wing polygonal, 43.28-77.44 × 23.35-41.89 µm (Fig. 2C); cells from wing near axis, 71.0-172.1 × 18.0-33.1 µm.

Oil cells

11.7-33.1 × 13.0-28.8 µm quadrate-rectangular, oil bodies 7.4-24.1 × 8.8-24.4 µm spherical, rough, opaque (Fig. 2C).

Scales

Dimorphic; vegetative scales ligulate, linear or lanceolate, 220.2-493.1 × 87.9-238.3 µm (Fig. 4C, D).

Propaguliferous scales

Panduriform, 171.8-487.3 × 107.8-269.1 µm (Fig. 4E).

Sexual condition

Dioicous; male plants similar in size to female plants.

Antheridia

In large continuous series or in small groups (Figs 2B, 3A); antheridial body 263.5-415.3 × 128.9-190.2 µm.

Female involucre

Ellipsoid, acuminate, 999.8-1721.4 × 941.1-2200.5 µm, smooth or with cells slightly bulging (Figs 2D-E, 4G), with base partially sheathed by a larger vegetative scale (Figs 2D-E, 4B). Apex of female involucre occluded, with bulging cells (Fig. 2F, H-I); involucre wall bistratose in cross-section (Figs 2G; 4H).

Sporophyte

With seta of 227.7-366.5 µm long, capsule globose, spherical, 838.4-1186.1 × 830.9-1213.0 µm in diameter (Figs 2E, 4G).

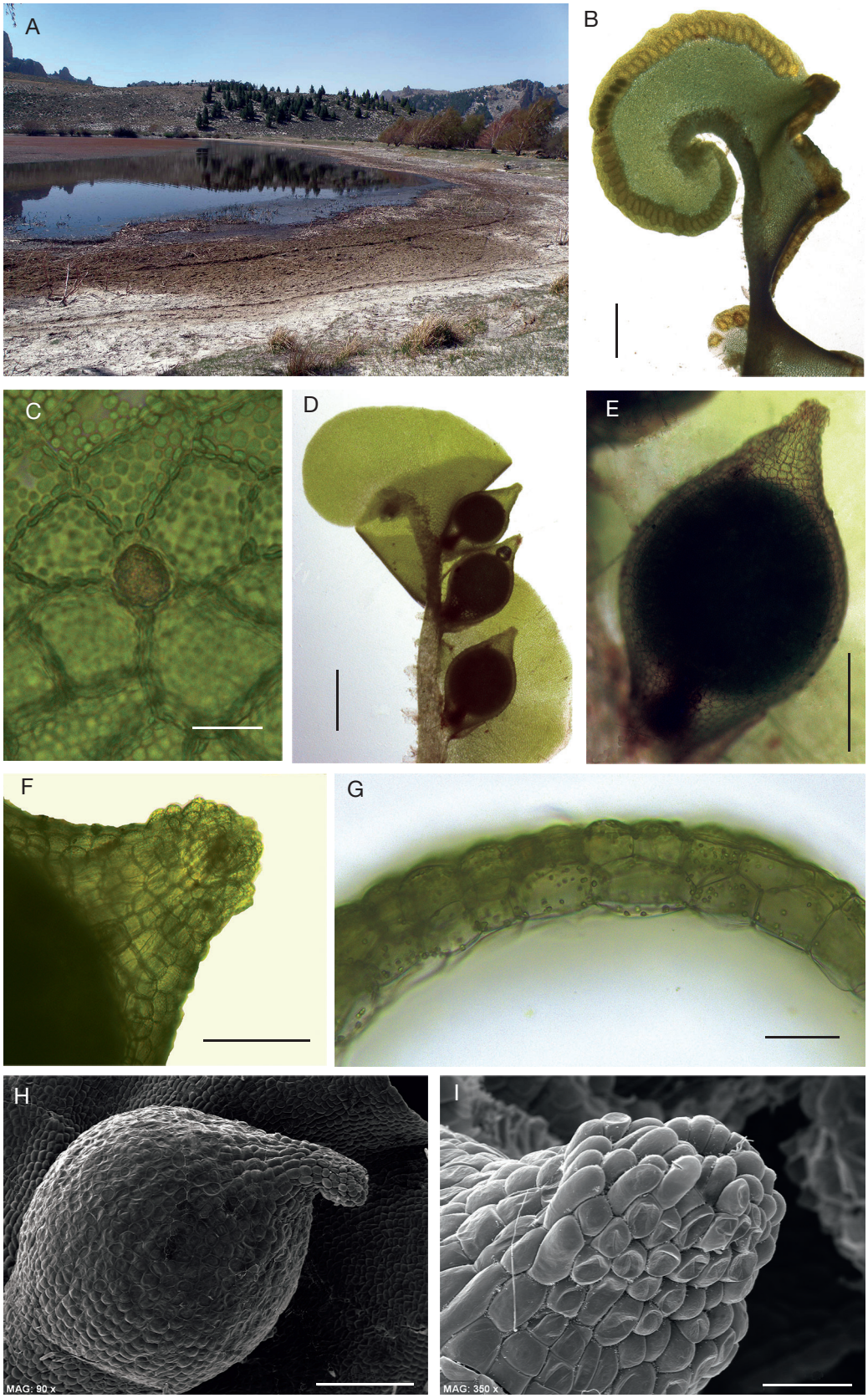

Fig. 2. — Habitat, LM and SEM images of *Riella choconensis* Hässel **A**, view of the Laguna de los Juncos; **B**, circinate apex of a male individual thallus showing a continuous row of antheridia; **C**, cells from thallus wing showing an oil cell with a single, rough oil body; **D**, apex of a female individual thallus showing three developing sporophytes; **E**, female involucre enclosing a sporophyte; **F**, apex of female involucre occluded by inflated cells; **G**, cross-section of female involucre showing the bistratose wall; **H**, female involucre; **I**, Apex of female involucre (**B**-**G** made with LM from VAL-Briof. 11724; **H**,**I** made with SEM from VAL-Briof. 11725), Scale bars: B, D, 1 mm; C, 20 µm; E, 500 µm; F, 200 µm; G, 50 µm; H, 300 µm; I, 70 µm.

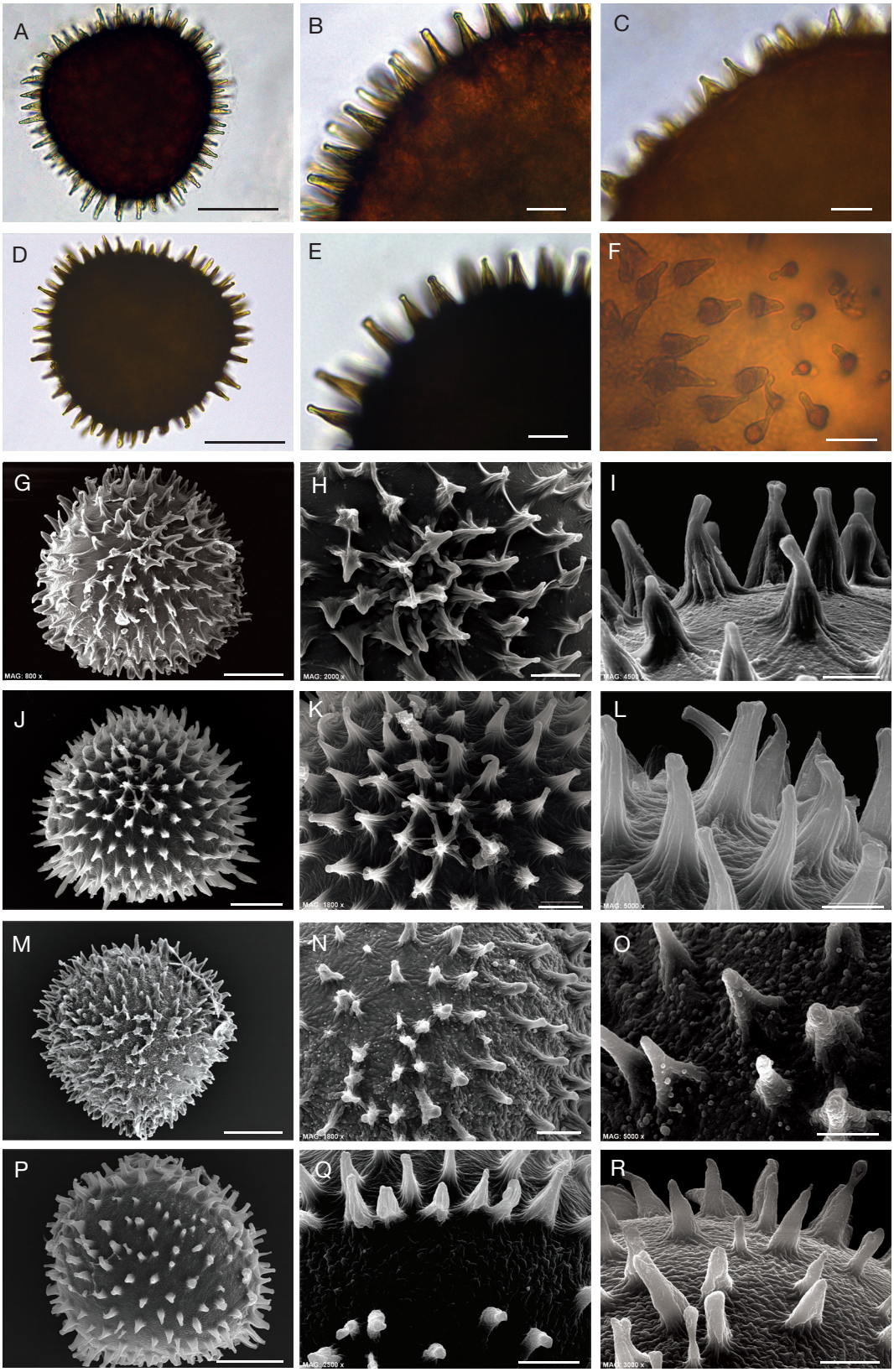

Fig. 3. — LM and SEM images of spores of *Riella choconensis* Hässel. **A**, distal view; **B**, spines from distal side; **C**, spines from proximal side; **D**, distal view; **E**, spines from distal side; **F**, spines from proximal side; **G**, distal view; **H**, Spines and reticulum from distal pole; **I**, spines from distal side and rugose spore surface; **J**, distal view; **K**, spines and reticulum from distal pole; **L**, spines from distal side and rugose spore surface; **M**, proximal view; **N**, proximal spore surface and spines; **O**, proximal spines and rugose-granulose spore surface; **P**, Proximal view; **Q**, transition between distal and proximal side, showing the equatorial row of distal spines; **R**, Proximal spines and rugose spore surface (**A**-**F** made with LM; **G**-**R** made with SEM; **A**-**C**, **I**, from VAL-Briof. 11724; **G**-**H**, **M**-**O**, from VAL-Briof. 11725; **D**-**F**, **J**-**L**, **P**-**R**, from BA 33609). Scale bars: A, D, 50 µm; B, C, E, F, H, K, N, Q, 10 µm; G, J, M, P, 30 µm; I, L, O, 5 µm; R, 8 µm.

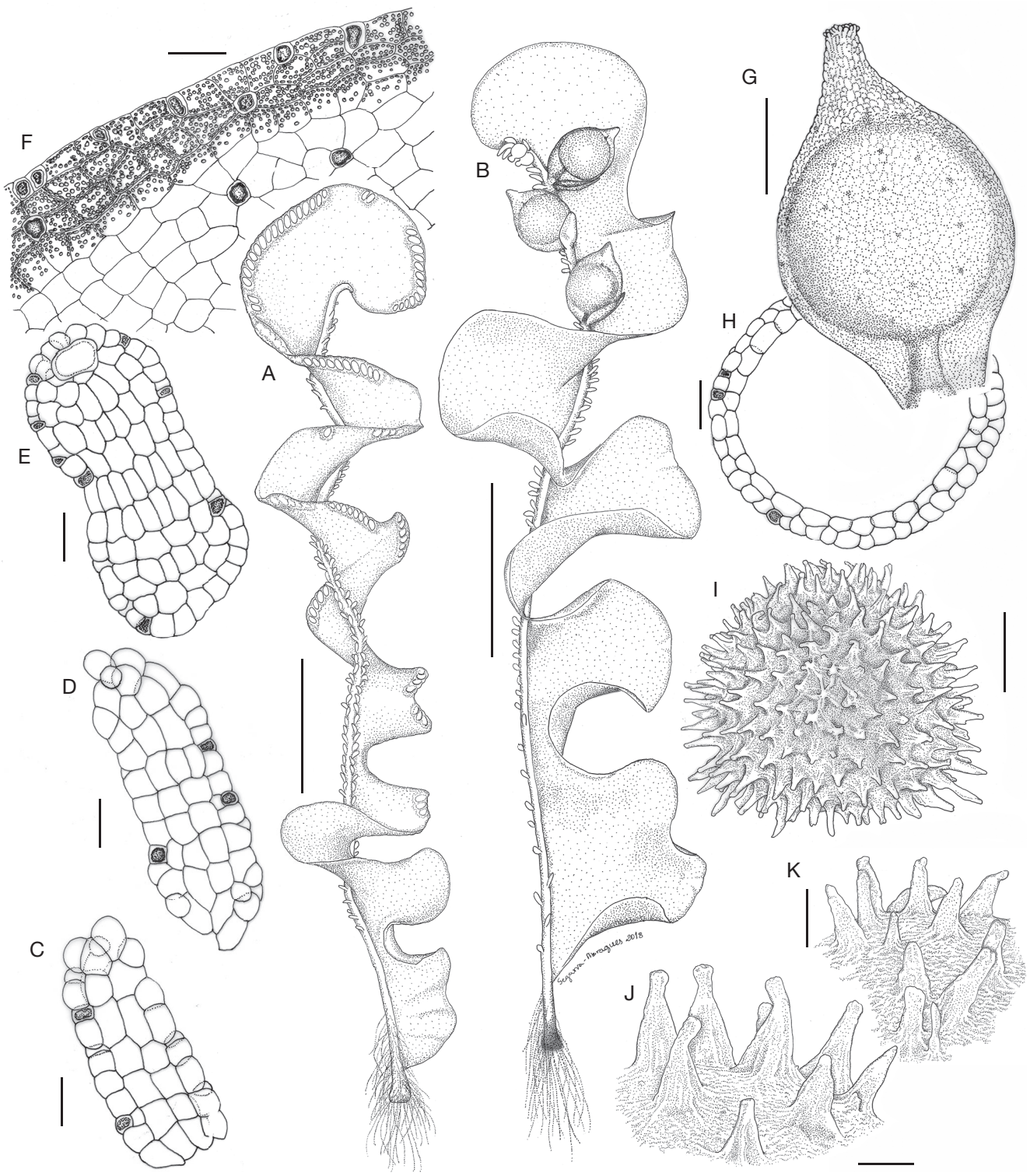

FIG. 4. — *Riella choconensis* Hässel. **A**, **B**, Habit of male and female plants, respectively; **C**, **D**, Vegetative scales; **E**, Propaguliferous scale; **F**, Detail of cells from margin of thallus wing; **G**, Female involucre; **H**, Cross-section of female involucre; **I**, Spore in distal view; **J**, Spore spines from distal face; **K**, Spore spines from proximal face (**A**-**H**, **J**, from VAL-Briof. 11724, **I**, **K**, from BA 33609). Scale bars: A, B, 3 mm; C-F, 50 µm; G, 500 µm; H, 100 µm; I, 30 µm; J, 5 µm; K, 8 µm.

Spores

(121.6-)132.8(-141.3) × (117.2-)127.1(-139.8) µm, and (102.1-)107.8(-116.3) × (99.0-)104.3(-116.7) µm including and excluding spines, respectively, dark brown, subspherical, tetrahedral, circular to subtriangular in outline (Fig. 3A, D, G, J, M, P).

Distal face

With 14-17 irregular rows of spines across diameter, and 32-39 spines projecting at periphery at the equatorial plane (Fig. 3A, D, G, J), surface of distal face rugose (Fig. 3I, L).

Spines
(9.5-)12.3(-15.2) µm long, (4.5-)6.0(-7.6) µm wide (Fig. 3I, L), with blunt or acute apices (Figs 3B, E, I, L; 4I-J), basal membranes interconnecting spines restricted to distal pole, faint (Fig. 3G-H, J-K), distance between spines shorter than the length of the spine (3.7-)6.2(-9.13) µm.

Proximal face
With rugose, sometimes granulose surface (Fig. 3N-O, Q-R), triradiate mark indistinct, spines of proximal face conical, with smooth surface; shorter than those of distal face, (4.1-)6.5 (-8.9) µm long, (3.8-)4.8,(-6.2) µm wide, generally with acute apices, rarely blunt or truncate, not basally interconnected (Figs. 3N-O, Q-R; 4K), distance between spines (2.2-)6.1 (-12.7) µm.

Habitat
In the new locality, it is likely that *R. choconensis* occurs submerged in areas of shallow water as plants were obtained from sediments collected at the margins of the pond. Species simultaneously obtained from these cultures and thus, presumably co-occurring with *R. choconensis* were: *Limosella australis* R.Br. (Plantaginaceae), *Myriophyllum quitense* Kunth (Haloragaceae), *Zannichellia palustris* L. (Potamogetonaceae) and species of Characeae.

DISCUSSION

New morphological characterization of *Riella choconensis*
Our detailed study of the morphological traits of this species supplements the original description of the species by Hassel de Menéndez (1987a). The fact that gametophytes are unisexual (i.e. a dioicous species) and that the female gametophytes carry sporophytes contained into smooth involucres distinguishes this species from the Californian *R. heliospora* Segarra, Puche & Sabovlj., and the Argentinian *R. gamundiae*, the only American species of subgenus *Trabutiella* (Hässel de Menéndez 1972; Segarra-Moragues *et al*. 2014). The four American species of subgenus *Riella* are gametophytically very similar, requiring spore characters for reliable identification. Notwithstanding, here we have revealed for the first time the occurrence of bistratose female involucre walls in *Riella*. This trait has been rarely observed in the Sphaerocarpales, with a single exception in the mostly southern hemisphere *Sphaerocarpos stipitatus* Bertero (Perold 2000a). Moreover, the female involucres of *R. choconensis* showed occluded involucre apex, covered by inflated, bulging cells. While this occurs in all six species of subgenus *Trabutiella* (Segarra-Moragues *et al*. 2014), it has not been observed previously in species of subgenus *Riella* (Puche & Segarra-Moragues 2013; Segarra-Moragues & Puche 2014). Thus, despite the simplicity of gametophytes, the morphology of female involucres provides a set of highly informative gametophytic traits, which aid to establish taxonomic boundaries between species of *Riella*.

The spores of the cultured specimens, densely covered in their distal face with long (9.5-15.2 µm) spines, with low or absent basal membranes, undefined areolae and with acute or blunt apices, unambiguously allowed us to identify the specimens as *R. choconensis*. Other Argentinian species have spores with shorter (4.7-9.4 µm) distal spines (*R. undulata*, Hässel de Menéndez 1987b), have distal spore faces covered with fewer (8-10) rows of longer spines with granulose surfaces (*R. pampae*, Hässel de Menéndez 1979) or have spores with distal spines with flaring, truncate apices and basal membranes defining areolae (*R. americana*, Hässel de Menéndez 1959). Based on new morphological data an updated identification key including all species known to date in the American continent is presented.

Key to the American species of *Riella*

1. Female involucres winged 2
— Female involucres smooth 3

2. Plants monoicous; distal spore face covered with spines 15.8-21 µm long, with basal membranes forming areolae and a marginal webbing at the equatorial row, California ..... *R. heliospora* Segarra, Puche & Sabovlj.
— Plants dioicous, distal spore face covered with spines 3.4-4.5 µm long, with basal membranes not forming areolae or marginal webbing at the equatorial row, Argentina ..... *R. gamundiae* Hässel

3. Spines from the spore equatorial row shorter than 10 µm ..... *R. undulata* Hässel
— Spines from the spore equatorial row longer than 10 µm 4

4. Distal spore face covered with spines of coarsely granulose surface at LM; proximal spore face covered with spines 9-12 µm long, cylindrical .....*R. pampae* Hässel
— Distal spore face covered with spines of smooth surface or with scarce granules only perceivable at SEM; proximal spore face covered with spines shorter than 6.5 µm, conical 5

5. Female involucre bistratose; distal spore face covered with spines with low or inconspicuous basal membranes, not defining areolae and with acute or blunt apices ..... *R. choconensis* Hässel
— Female involucre unistratose; distal spore face covered with spines with basal membranes defining areolae, with truncate apices ..... *R. americana* M. Howe & Underw.

Distribution, ecology and conservation

Previously, *Riella choconensis* was known only from specimens collected in 1984 at the Ramos Mexía reservoir. This artificial lake was formed after the construction of a dam on the Limay river for the establishment of a hydroelectric plant in 1972 (Hässel de Menéndez 1987a). *Riella* specimens were discovered at depths of 0.5-5.8 m, growing among Characeae, while sampling on the benthos. No other populations were discovered for more than 30 years after this, although studies were conducted on cultures of sediments from 12 Argentinian, brackish water seasonal lakes (Hässel de Menéndez 1987a). Nonetheless, as stated by the author, it is likely that many potential habitats were not sampled at that time. This was confirmed in this study, in which we discovered a second population of *R. choconensis* from a culture of sediments. Indeed, cultivation trials have rendered positive results in other parts of the world's distribution range of *Riella* (Proctor 1972; Segarra-Moragues & Puche 2014; Segarra-Moragues *et al*. 2014). This procedure is useful to overcome the difficulties associated with finding plants of *Riella* in the wild, given their sensitivity to water and salinity levels, and to the presence of bare habitats for their development. Thus, culture of sediments may continue to contribute effectively to the knowledge of distribution, ecology and biogeography of this rare genus.

The finding of this new population of *R. choconensis* has further implications for knowledge of the areas of distribution not only of the species *R. choconensis,* but also of the genus *Riella*. The geographical separation by more than 230 km from the two known populations of *R. choconensis* significantly expands the known range of the species (Hässel de Menéndez 1987a, b). This geographical distance implies the separation of the two populations into different Argentinian phytogeographic provinces, the type locality corresponding to the province of del Monte, and the new locality to the boundary between the Patagonian and Subantarctic provinces (Cabrera 1976). Furthermore, it also extends the southern limit of the global distribution range of the genus. All other American populations of *Riella* occur at lower latitudes (Hässel de Menéndez 1987a; Segarra-Moragues *et al*. 2014), including those in African (Perold 2000b; Segarra-Moragues & Puche 2014) and Australian (Banwell 1951; Taylor 1954; Cargill & Milne 2013) ranges. Thus, the new population reported here constitutes the southernmost population reported in the genus.

To our knowledge, the new *R. choconensis* population reported here constitutes the only confirmed record of the present day occurrence of the species in Argentina. Despite the species persistence at the original locality has not been assessed by further collections, it seems unlikely that the actual habitat conditions at the original locality are suitable for the species occurrence, as the lake does not undergo cycles of flooding and desiccation. Instead, it is likely that at the depths where the species was originally found, the habitat is now permanently occupied by aquatic macrophytes. However, shore margins of the reservoir could be inspected for its occurrence as these are likely places to show some fluctuation in water levels.

In the new locality, *R. choconensis* is likely to form abundant populations given the number of plants obtained from a small sample of sediment. However, as plants were not observed in the field, it is uncertain whether the current ecological conditions at the Laguna de los Juncos are suitable for the regular development of the plants, or whether the spore bank is the result of sporadic population blooms. The same applies to its actual distribution range. A large number of ephemeral and fluctuant lagoons occur throughout the Pampean and Patagonian areas in Argentina which have not been investigated for the presence of *Riella,* and which could harbor populations of this and other species. Thus, more surveys are needed in order to properly assess the conservation status of this endemic species. In the current state of knowledge, its conservation status should be considered as Data Deficient (DD).

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