

## ***Riella cossoniana* Trab. (Riellaceae, Marchantiophyta) new to France**

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**Abstract** – *Riella* (Riellaceae, Sphaerocarpaceae) is a genus of aquatic liverworts with disjunct range in areas of Mediterranean-type climates. *Riella* has been traditionally subdivided into two subgenera, subgenus *Riella*, whose plants show smooth or papillose female involucre and *Trabutiella*, whose plants show winged female involucre. To date only three species of *Riella* are known in France, all belonging to subgenus *Riella*. During the course of a study of the plant diversity of temporary brackish ponds in the Camargue area (Southern France), soil sediments were collected from 10 localities and cultivated in the laboratory. From cultures of five of these localities emerged dioicous plants of *Riella* showing winged female involucre that unambiguously assigned them to subgenus *Trabutiella*. The study of those specimens with Light and Scanning Electron Microscopy allowed us to identify them as *R. cossoniana* var. *cossoniana*. Fresh materials allowed for detailed morphological analyses on these new French materials which were fully described and illustrated. These findings represent the first report of a species belonging to subgenus *Trabutiella* from France and the second European country where *R. cossoniana* is reported. Four nearby populations occur within the perimeter of the Tour du Valat Regional Natural Reserve, and another in the industrial polygon of the Grand Port Maritime de Marseille.

**Aquatic liverworts / bryophytes / disjunct distribution / threatened species / Marchantiophyta**

**Résumé** – *Riella* (Riellaceae, Sphaerocarpaceae) est un genre d'hépatiques aquatiques possédant une aire de distribution disjointe dans le monde entier, dans des régions à climat méditerranéen. *Riella* a été traditionnellement divisé en deux sous-genres : sous-genre *Riella* où les plantes possèdent des involucre femelles lisses ou papilleux, et sous-genre *Trabutiella*, où les plantes sont munies d'involucre femelles ailés. À ce jour, seules trois espèces de *Riella* sont signalées en France, toutes appartenant au sous-genre *Riella*.

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Au cours d'une étude sur la diversité végétale des lagunes et mares saumâtres temporaires de Camargue (sud de la France), des sédiments du sol ont été prélevés dans 10 localités et mis en culture en laboratoire. Des plantes appartenant à un *Riella* dioïque ont émergé des cultures de cinq de ces localités, montrant des involucre femelles ailés qui les rattachent sans ambiguïté au sous-genre *Trabutiella*. L'étude de ces échantillons au microscope optique et microscope électronique à balayage nous a permis de les identifier comme *R. cossoniana* var. *cossoniana*. Des analyses morphologiques détaillées ont été réalisées sur ce nouveau matériel français, qui est pleinement décrit et illustré. Ces résultats représentent la première mention d'une espèce du sous-genre *Trabutiella* pour la France, et le deuxième pays européen où *R. cossoniana* est signalé. Quatre populations se trouvent dans le périmètre de la Réserve Naturelle Régionale de la Tour du Valat et une autre dans la zone industrielle du Grand Port Maritime de Marseille.

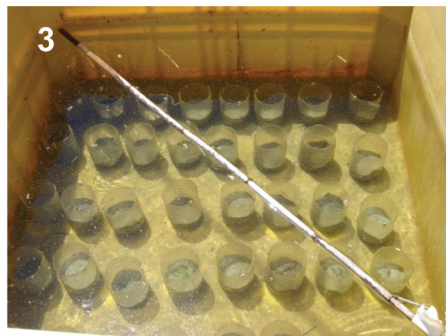
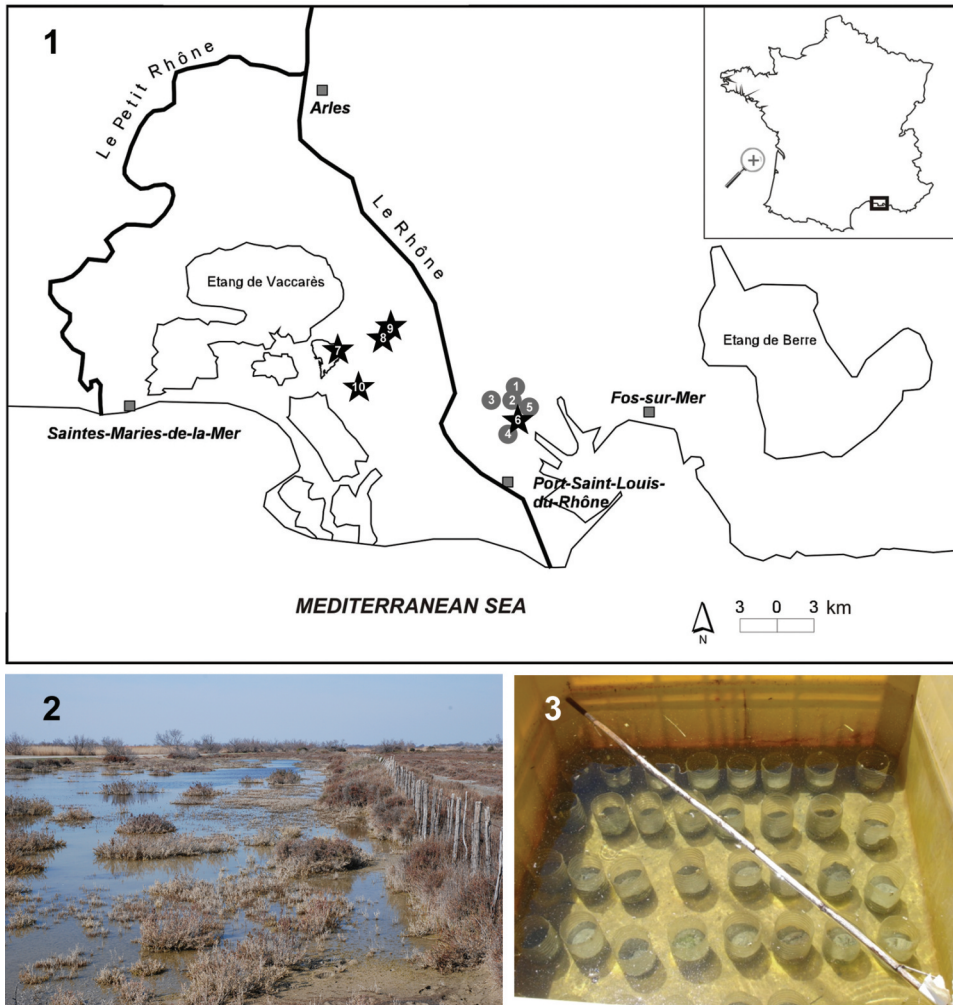
**Bryophytes / disjonction d'aire / espèces menacées / hépatiques aquatiques / Marchantiophyta**

## INTRODUCTION

The genus *Riella* Mont. (Riellaceae, Sphaerocarpaceae) includes liverworts that grow submerged in fresh or brackish water temporary pools, in areas of Mediterranean-type or seasonal climate of all five continents (Geissler, 2001). The diversity centre of the genus is placed in the Mediterranean area where the largest number of species occurs, compared to other parts of the distribution range of the genus. *Riella* is subdivided into two subgenera based on the absence (subgenus *Riella*) or presence (subgenus *Trabutiella*) of wings in the female involucre (Porsild, 1902). Subgenus *Trabutiella* is less diverse and currently includes two monoicous (Segarra-Moragues *et al.*, 2012a) and two to four dioicous taxa, depending on authors (Jelenc, 1957; Hässel de Menéndez, 1972). Of this later group *R. cossoniana* Trab. is the most widespread, but scattered, with a fragmented area extending from the whole Mediterranean basin to western Asia in India (Patel, 1977; Söderström *et al.*, 2002; Ros *et al.*, 2007).

Because of its rarity, *R. cossoniana* has been included in lists of endangered bryophytes (ECCB, 1995; Brugués & González-Mancebo, 2012; Brugués *et al.*, 2012). However, there is no consensus on the category of threat in which it is included, since this varies from Endangered (EN) at European (ECCB, 1995) and Iberian Peninsula (Brugués *et al.*, 2012) scales to Data Deficient (DD) in Spain (Brugués & González-Mancebo, 2012), which altogether point to a lack of knowledge on its actual distribution and abundance.

In coastal brackish temporary pools plant germination may be conditioned by the dates of flooding and drying out which vary widely among years and by salt concentration, as also noted for *Riella* (Marín, 1982). Accordingly, as for many short-lived species growing in temporarily flooded habitats, the non-observation of plants at a particular moment is not a good indicator of its real absence. The culture of sediments from potentially suitable habitats has proved to be a good method to overcome this problem and reveal the presence of *Riella* in areas where the genus or a particular species had not been previously recorded (Porsild, 1902; Cavers, 1903; Marín, 1982; Segarra-Moragues *et al.*, 2012a, b; Puche & Segarra-Moragues, 2013).



Figs.1-3. **1.** Location of the 10 sampled localities in the Camargue area, the five ones that revealed the presence of *Riella cossoniana* in cultures are designated by stars. Localities 1 and 4 include two sites each. **2.** Aspect of the population from Cabane de Barthélemy. **3.** Example of the container used in the first germination experiment.

During the course of a study of the plant diversity of temporary brackish ponds in the Camargue area (Southern France), intended to establish the germination requirements of the rare aquatic species, soil sediments were sampled from ten localities (Figs 1-2). Surprisingly, plants of a dioicous *Riella* showing involucre were obtained from five of these localities.

This paper deals with the morphological study of plants from these five localities and reports on the presence of *R. cossoniana* in France. Besides, details on the habitats, environmental data and conservation status of the populations are reported.

## MATERIALS AND METHODS

### Germination of sediments and establishments of cultures

For the purpose of studying the plant diversity of temporary brackish ponds in the Camargue area (Southern France), intended to establish the germination requirements of the rare aquatic species *Riella helicophylla* (Bory *et Mont.*) Mont., *Althenia filiformis* Petit, and *Tolypella salina* Corillion, ten localities showing a high density of these species in the Camargue area were sampled from 1<sup>st</sup> to 11<sup>th</sup> May 2012 (Figs 1-2). Forty eight samples of sediments (8 cm in diameter, 4 cm depth) were cored in an area of 2 m<sup>2</sup> from each of the 10 localities (totalling 480 core samples), transferred into individual PET vessels and kept in dry conditions for 1.5 months.

Germination experiments were conducted outdoors in 12 open containers (hereafter mesocosms, Fig. 3), each containing 400 L of water supplemented with 0, 5, 10, 15, 20 or 30 grL<sup>-1</sup> of sea salt (two containers per salinity treatment). Water level of containers was controlled once or twice a week depending on the evaporation rate and these were refilled accordingly with fresh water to avoid changes in salt concentration during the experiment. In 25<sup>th</sup> June 2012 four core samples of sediment from each of the ten localities were transferred to each container. Therefore, germination of plants at each locality was tested in eight samples of sediment in each of the six salinity conditions. As sediments contained precipitated salts, concentration of salt in the containers was then measured to assess possible changes in the culture conditions. We noticed a homogeneous increase of < 1 gL<sup>-1</sup> in salt concentration in the containers thus, ruling out an effect on the different conditions tested. Turbidity (in Nephelometric Turbidity Units, NTU) and Chlorophylla content (in µgL<sup>-1</sup>, resulting from algal blooms) of cultures was measured with a fluorimeter (Aquafluor, Turner Designs, Sunnyvale, CA). Culture of sediments lasted for three months ending on 19<sup>th</sup> September 2012. Samples were inspected weekly for plant germination. *Riella cossoniana* unexpectedly appeared firstly in some of these cultures (see Results) but then was noticed in cultures from all 10 localities. As the occurrence of *Riella* in all cultures could be due to the spread of propaguliferous scales within the container (note that the experiments were not specifically designed for *Riella*, as it was unknown in the area, but for other aquatic macrophytes with no such vegetative spread mechanisms), a second germination experiment was run in spring 2013 (March-June). In this case 86 samples of sediments (4 cm in diameter, 2 cm depth) were taken from the sampled localities in Camargue during September 2012. Seventy one cores were sampled from the largest area at Caban (localities 1 to 6, Fig. 1), and three cores each were sampled from 4 localities (localities 7-10, Fig. 1). The sediment cores were transferred into individual vessels and kept in dry conditions during six months. In order to avoid possible contamination from one locality to another (potentially occurring in the first germination experiment), sediment culture was conducted in isolated vessels from one another. On March 2013 the two samples taken at each site were flooded with 10 cm water at 0 gL<sup>-1</sup>, and 10 gL<sup>-1</sup>, respectively. Germination of *Riella* was checked afterwards every two weeks until 10 June.

### Morphological study of *Riella* specimens

Fresh specimens including male and female individuals with mature sporophytes were used for morphological characterization. Ten individuals from

each sex in each locality were measured for each morphological character included in the description.

Measurements of vegetative 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 0.01 $\mu$ m on digital images. All measurements were taken under a Leica DMLB 100S light microscope, except for the size of gametophytes and the width of wing from the thallus that were taken under a Leica M76 stereomicroscope. A Leica DFC425 digital camera was used for producing high resolution images of gametophyte parts and spores. 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 carried out in a Hitachi S-4100 field emission scanning electron microscope (SEM) at the University of Valencia (SCSIE-UV). Terminology for spore characters follows Perold (2000).

## RESULTS

The first individuals of *R. cossoniana* were observed on 23 July 2012, four weeks after starting the culture of sediments. Given the large uniformity of thallus morphology of most species of *Riella*, they could not be ascribed to *R. cossoniana* until 1<sup>st</sup> august 2012, when the reproductive organs started to develop. Ripe spores were first seen during the first week of September. In this first experiment, *R. cossoniana* was initially observed in samples from three different localities (in four sampled cores), all from Tour du Valat Regional Nature Reserve: one sampled core each from Cerisières (locality 8) and Baisse des Montilles de Redon (locality 10), and two cores from Cabane de Barthelemy (locality 7). We assume that in these early observations, individuals of *R. cossoniana* were issued from germination from the corresponding sediment cores as individuals of *Riella* do not have other desiccation-resistant structures apart from spores. Three weeks after the first observation, *R. cossoniana* was found on sediment cores from all localities, possibly resulting from the spread of propaguliferous scales within the container.

The frequency of *R. cossoniana* was contrasted between salinities and mesocosm replicates. It was found only in both duplicates of freshwater cultures (0 gL<sup>-1</sup> salinity) of locality 7 and in one of the duplicates each of localities 8 and 10. Containers at higher concentration of salts (5, 10, 15, 20 or 30 gL<sup>-1</sup>) did not reveal plants of *R. cossoniana*. However, specimens of unidentified plants of *Riella* (only in vegetative state) were found in cultures supplemented with salt at 5 gL<sup>-1</sup>. During this experiment the frequency and abundance of *R. cossoniana* was high in one mesocosm (40/40 vessels; average of 56 individuals/sample) while it remained very low in the other (1/40 vessels, 1 individual in 1 sample). The very low frequency and abundance of *R. cossoniana* could be related to an algal bloom which occurred in the latter mesocosm, resulting in higher turbidity. Turbidity and concentration of Chlorophyll *a* (Chl*a*) were 2.6 NTU and 1.3  $\mu$ g.L<sup>-1</sup> Chl*a* and 9.7 NTU and 69.3  $\mu$ g.L<sup>-1</sup> Chl*a* in the first (clear) and second (turbid) mesocosms, respectively.

The second germination experiment, in 2013, confirmed the presence of *R. cossoniana* in the former three localities (Localities 7, 8 and 10) and revealed its presence in two sampled cores from Caban (locality 6) and Rendez-vous pools (locality 9), and on a new site (a different pool) at Cerisières (locality 8, Fig. 1).



This second germination experiment ruled out possible contamination between sampled localities because of propaguliferous scales and thus provides reliable indication of the presence of *R. cossoniana* in Camargue.

In this second experiment, we noticed the germination of *Riella* plants 1 month after starting the cultures (April 2013). Similarly, the identification was not possible until two months after first germination (June 2013). *Riella cossoniana* was found mostly in freshwater ( $0 \text{ gL}^{-1}$ ), in 5/86 individual vessels (ranging 10-100 plants/vessel) from 4 different localities, and also in 1/86 vessels at salinity of  $10 \text{ gL}^{-1}$  (20 plants).

A description of the species based on materials from the French populations is presented below and illustrated in Figs 4-26. The range of variation of morphometric characters spanned those published for this species (*i.e.* Ros, 1987).

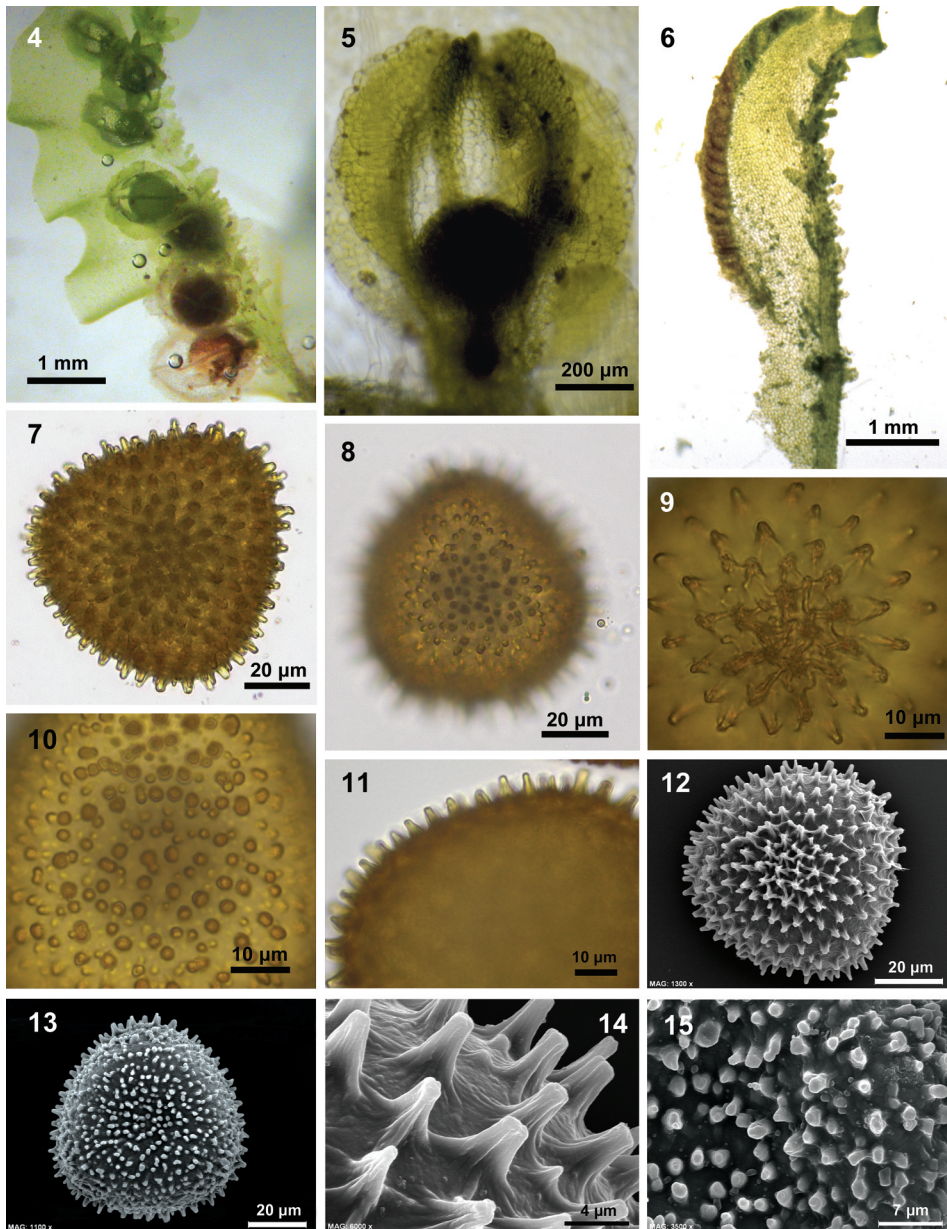
***Riella cossoniana* Trabut var. *cossoniana***, *Revue Bryologique*, 14: 12. 1887. Icon. *Revue Bryologique*, 13, plate I. 1886. — Type. ALGERIA Oran province, El Kreider, Trabut (JE! iso, Krypto-S! iso).

Plants 0.9-3 cm, erect, unbranched or bifurcate from the base. Thallus apex falciform. Axis slight flattened 0.11-0.26 mm wide. Dorsal wing 0.8-2.9 mm wide, flat or undulate, margin entire, marginal cells rectangular, quadrate, chlorophyllose,  $12.5\text{-}42.1 \times 11.2\text{-}24.4 \text{ }\mu\text{m}$ ; cells from middle part of wing  $32\text{-}74 \times 20.5\text{-}42.2 \text{ }\mu\text{m}$ ; cells from wing near axis rectangular or hexagonal  $90.1\text{-}231.6 \times 14.3\text{-}59.3 \text{ }\mu\text{m}$ ; oil cells  $10\text{-}23.5 \times 12.9\text{-}22.3 \text{ }\mu\text{m}$ , oil bodies 9-18  $\mu\text{m}$  globose, opaque and rough. Scales dimorphic: vegetative scales linear-rectangular or lanceolate  $140.6\text{-}702.5 \times 106.1\text{-}855.4 \text{ }\mu\text{m}$ , larger towards apex of thallus; propaguliferous scales panduriform,  $87.3\text{-}411.9 \times 28.6\text{-}195.8 \text{ }\mu\text{m}$ , more abundant towards the apex. Dioicous, male plants smaller than female plants; antheridia numerous, continuous, in a single linear series in pockets along thickened wing margin, antheridial body  $215.1\text{-}257 \times 94.3\text{-}161.2 \text{ }\mu\text{m}$ . Archegonia on axis, produced in acropetal sequence. Archegonial involucre globose, elliptical  $1.04\text{-}1.96 \times 0.82\text{-}1.60 \text{ mm}$ , with 8 lamellae 5-10 cells and  $133.1\text{-}293.1 \text{ }\mu\text{m}$  high, apex blunt. Sporophyte with seta of  $0.16\text{-}0.45 \text{ mm}$ . Capsule globose,  $0.49\text{-}1.01 \times 0.46\text{-}0.98 \text{ mm}$ .

Spores  $70\text{-}87 \times 62\text{-}76 \text{ }\mu\text{m}$  including spines, light brown, subglobose, subround in outline. Distal face densely covered with 16-22 irregular rows of spines across diameter and interspersed smaller papillae, distance between spines  $1.6\text{-}5.9 \text{ }\mu\text{m}$ , and (36-) 39 (-44) projecting spines at periphery at the equatorial plane. Spines (3.4-)  $4.14 \text{ (-}5.1) \text{ }\mu\text{m}$  long (1.9-)  $2.62 \text{ (-}3.3) \text{ }\mu\text{m}$  wide, with truncate, rarely acute apices; basal membranes interconnecting spines restricted to distal pole,  $0.3\text{-}0.8 \text{ }\mu\text{m}$  high, forming imperfect reticulations and not defining areolae, absent from other parts of distal face; basal membranes at the equatorial plane absent. Proximal face concave; triradiate mark indistinct; surface of proximal face smooth or rugose. Spines dense (distance between spines  $0.6\text{-}1.6 \text{ }\mu\text{m}$ ), small,  $1.4\text{-}3.1 \times 1\text{-}3.1 \text{ }\mu\text{m}$ , with blunt apices, not basally interconnected by basal membranes.

#### Materials examined

FRANCE: (6) Bouches-du-Rhône, Port-Saint-Louis-du-Rhône, anciens salins du Caban  $4^{\circ} 48' 18''\text{E}$ ,  $43^{\circ} 26' 12''\text{N}$ , 0 m, *L. Martinez*, 10 Jun 2013. (VAL-Brief. 9.253). (7a) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale du Tour du Valat, Cabane de Barthélemy,  $43^{\circ} 29' 23''\text{N}$ ,  $4^{\circ} 38' 35''\text{E}$ , 0 m, *L. Martinez*, 18 Oct 2012 (VAL-Brief. 9.209). *Ibid.*



Figs 4-15. Light and SEM images of *Riella cossoniana*. **4.** Thallus of a female plant. **5.** Female involucre. **6.** Thallus of a male plant. **7-11.** Optical microscopy images of spores. **7.** Distal view. **8.** Proximal view. **9.** Detail of distal pole. **10.** Detail of proximal pole. **11.** Spines at the equatorial plane. **12-15.** Scanning Electron Microscopy (SEM) images of spores. **12.** Distal view. **13.** Proximal view. **14.** Detail of spines from distal face. **15.** Detail of spines from proximal face. (4-6, 8, 9, 12 and 15 from VAL-Brief. 9.210; 7, 10, 11 and 13 from VAL-Brief. 9.209; 14, VAL-Brief. 9.208)

Herbarium B. Offerhaus 181012-01. (7b) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale de la Tour du Valat, Cabane de Barthélemy, 43° 29' 20"N, 4° 38' 40"E, 0 m, *L. Martinez*, 18 Oct 2012 (VAL-Brief. 9.208). *Ibid.* Herbarium B. Offerhaus 181012-02. (8a) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale de la Tour du Valat, Cerisières, 43° 29' 47"N, 4° 40' 33"E, 0 m, *L. Martinez*, 18 Oct 2012. (VAL-Brief. 9.251). (8b) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale de la Tour du Valat, Cerisières, 43° 29' 37"N, 4° 40' 31"E, 0 m, *L. Martinez*, 10 Jun 2013. (VAL-Brief. 9.252). (9) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale de la Tour du Valat, Mares du Rendez-vous; 43° 30' 1"N, 4° 40' 38"E 0 m, *L. Martinez*, 10 Jun 2013. (VAL-Brief. 9.250). (10) Bouches-du-Rhône, Arles, Réserve Naturelle Régionale de la Tour du Valat, Baisse des Montilles de Redon, 43° 28' 7"N, 4° 39' 13"E, 0 m, *L. Martinez*, 18 Oct 2012. (VAL-Brief. 9.210). *Ibid.* Herbarium B. Offerhaus 181012-04.

### Habitat

Throughout its distribution range *Riella cossoniana* is found in brackish water seasonal ponds in semiarid to arid environments. The French populations were found in sediments from small flooded depressions of less than 1 ha, where competition with other hydrophytes is weak, and alternate long enough periods of winter flooding to allow the plants to complete their reproductive cycle, with total summer drought. These habitats are exclusively maintained by rain water supply and thus, water levels are highly variable from one year to another. The amount of precipitation during the autumn-winter period is critical for the development of the populations of *Riella*. Water levels must be high enough for a sufficient dilution of the salt content in sediments to allow the germination of the spores and growth of the thalli (Marín, 1982). These seasonal aquatic habitats occur at the borders or scattered within halophyte communities dominated by *Arthrocnemum macrostachyum* (Moric.) K. Koch, and *Sarcocornia perennis* (Mill). A.J. Scott (Fig. 2). The associated species are mainly Characeae: *Tolypella hispanica* Nordst. ex T.F. Allen, *T. glomerata* (Desv.) Leonhardi, *Chara canescens* Desv. et Loisel., *Ch. aspera* Dethard. ex Willd. and *Riella helicophylla*.

## DISCUSSION

Five species have been recognised within subgenus *Trabutiella*. Two of these, *Riella affinis* Howe et Underwood and *R. heliospora* Segarra-Moragues, Puche et Sabovljević are monoicous and have longer (> 8 µm) distal spines (Segarra-Moragues *et al.*, 2012a) and thus, rule out any possible misidentification with the French specimens. From the three remaining ones which are dioicous, some taxonomical controversy exists among several of these taxa (Müller, 1953; Lipkin & Proctor, 1975) and thus, a detailed comprehensive revision of the group is needed. *Riella gamundiae* Hässel de Menéndez is so far known from the type locality in Argentina and differs from the French populations in its apiculate involucre with low (< 108 µm), discontinuous wings that do not reach the base of the involucre (Hässel de Menéndez, 1972). *Riella paulsenii* Porsild reported from Uzbekistan (Porsild, 1902) and Kazakhstan (Ladyzhenskaja & Obuchova, 1956; Obuchova, 1961; Ladyzhenskaja & Fedorova-Shaknundes, 1976) was separated from *R. cossoniana* based solely on the acute distal spines compared to the truncate ones in *R. cossoniana* (Porsild, 1902). This character was considered



apparently variable within both species therefore, blurring their taxonomical distinctness. Consequently, *R. paulsenii* was reduced to the synonymy of *R. cossoniana* (Lipkin & Proctor, 1975). Whether *R. cossoniana* and *R. paulsenii* represent different taxa requires further investigation. However, the studied French specimens showed spores with mostly truncate distal spines (Figs 7, 11, 12, 14 and 25) with few interspersed acute distal spines (Fig. 25) that tightly fitted the description of *R. cossoniana* (Trabut, 1886, 1887).

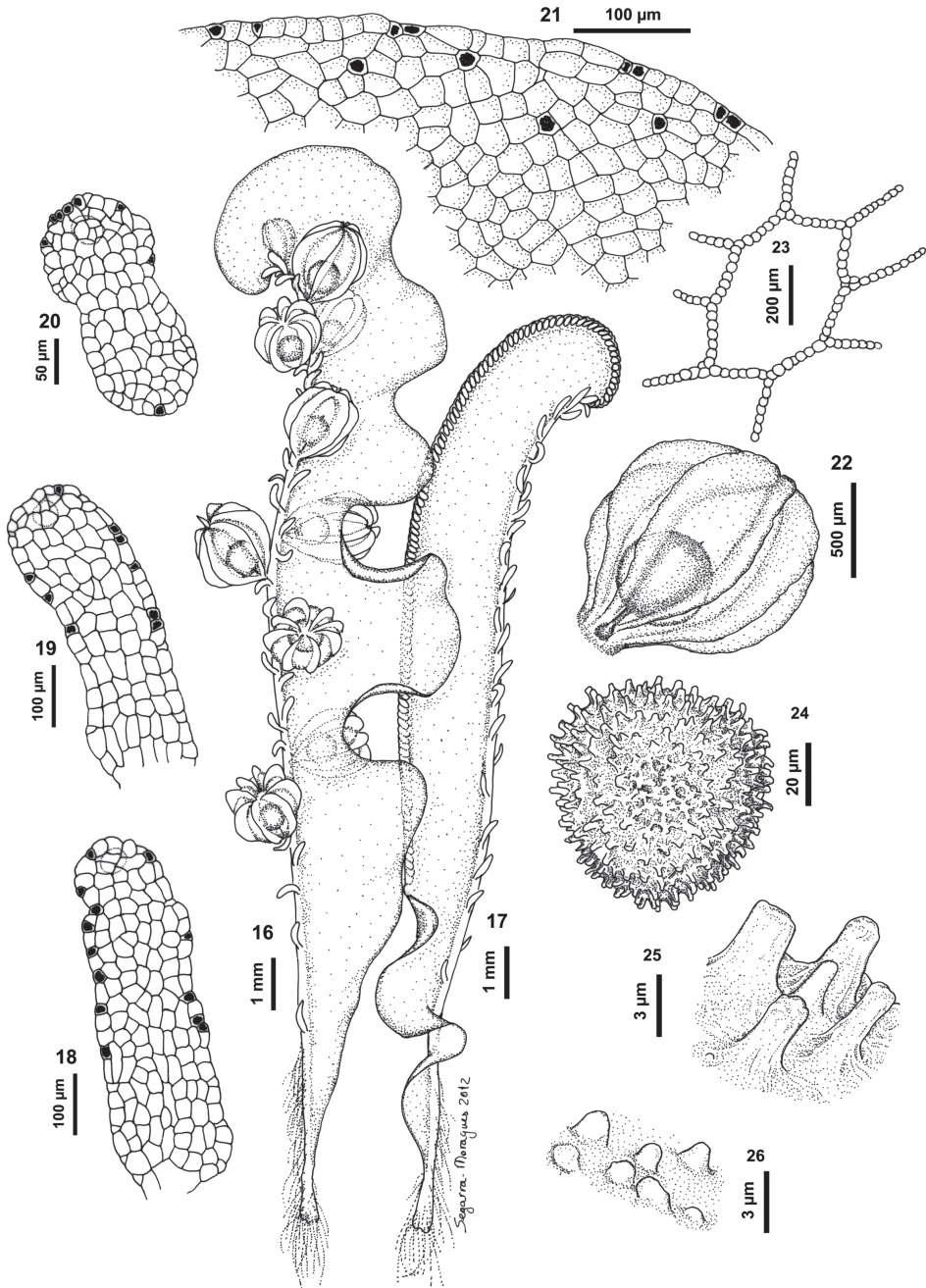
Two varieties have been recognised within *Riella cossoniana*, differing in the length and shape of distal spines (Müller, 1953). French samples also differ from *R. cossoniana* var. *echinata* Müller by their shorter (up to 5.1 µm) truncate distal spines compared to the longer (ca 7 µm), acute distal spines that characterize *R. cossoniana* var. *echinata* (Müller, 1953). The taxonomic identity of *R. cossoniana* var. *echinata* was discussed by Lipkin & Proctor (1975) who reduced this taxon to the synonymy of *R. helicophylla*, a species with smooth involucre and thus, corresponding to subgenus *Riella*. However, this taxonomical controversy needs further study. As mentioned above, the French populations did not match the morphological attributes of *R. cossoniana* var. *echinata*, which doubtless indicate their taxonomical adscription to *R. cossoniana* var. *cossoniana*.

Until now, three species of *Riella* have been reported from France. These include the monoicous *R. notarisii* s.l. (Corbière, 1902; Crozals, 1903) and the dioicous *R. helicophylla* (Dubois & Héban, 1968; Skrzypczak, 2001), and *R. parisii* Gottsche (Skrzypczak, 2001). All these three taxa have smooth involucre and thus correspond to subgenus *Riella*. Accordingly, the newly found populations of *R. cossoniana* not only add a fourth species of *Riella* to France, but also extend the representation of subgenus *Trabutiella* to this country.

Despite the broad distribution of *R. cossoniana*, as for most of the species in the genus, its known populations have been increased by few recent records. To date the presence of *R. cossoniana* in the Mediterranean area is highly disjunct (see below), a characteristic of its global distribution. Current records include the following countries: Algeria (Gauthier-Lièvre, 1931; Trabut, 1887; Maire, 1937, 1940; Jelenc, 1957; Frahm, 1978), France (this study), India (Patel, 1977), Israel (Lipkin & Proctor, 1975), Jordan (Ros *et al.*, 2007), Kazakhstan (Ladyzhenskaja & Obuchova, 1956; Obuchova, 1961; Ladyzhenskaja & Fedorova-Shaknmundes, 1976, as *R. paulsenii*), mainland Spain (Ros, 1987; Cirujano *et al.*, 1988, 1992, 1993; Ros *et al.*, 1996; Ortega-González *et al.*, 2002; Seguí *et al.*, 2005; Seguí & Pérez, 2006), the Canary Islands (Van Dort & Nieuwkoop, 2003), and Uzbekistan, (Porsild, 1902, as *R. paulsenii*). The occurrence of *R. cossoniana* in Tunisia and Morocco was reported by Bischler (2004). Both reports are excluded here as they lack sufficient support, the report for Morocco probably corresponding to the type locality of *R. cossoniana* var. *echinata*. Our reports represent the second European country where this species, and a member of subgenus *Trabutiella*, has been found.

Several authors have assigned the populations of *Riella* to exclusive aquatic communities (Cirujano *et al.*, 1993). From this phytosociological point of view, *R. cossoniana* would be included within *Rielletea helicophyllae* Cirujano, Velayos & García-Murillo 1993, an association containing halophilous species of *Riella* of which *R. helicophylla* is characteristic (Cirujano *et al.*, 1993).

The biotopes containing populations of *Riella* have been designated as priority habitats for conservation by the Habitats Directive 92/43/EEC and encoded in the Natura 2000 network as “1150 Coastal lagoons” and “1420 Mediterranean and thermo-Atlantic halophilous scrubs (*Sarcocornetea fruticosi*)”.



Figs 16-26. Habit of *Riella cossoniana* from the Camargue, France. **16.** General habit of a female plant. **17.** General habit of a male plant. **18-19.** Vegetative scales. **20.** Propagiferous scale. **21.** Detail of cells from margin of thallus wing. **22.** Female involucre. **23.** Cross section of female involucre. **24.** Spore in distal view. **25.** Spines from distal face. **26.** Spines from proximal face. (All from VAL-Brief. 9.210).

Our study confirmed the usefulness of culture of sediments in revealing the previously unnoticed presence of *Riella* populations (Segarra-Moragues *et al.*, 2012a; Puche & Segarra-Moragues, 2013). Our assay on culture of such sediments in different salinity concentrations also revealed that the best results are obtained in the absence of salt supplementation. Despite *R. cossoniana* is considered to be a halophyte species, such as *R. helicophylla*, our first results indicate that germination of spores is strongly inhibited at salt concentrations as low as 5 gL<sup>-1</sup>. Nonetheless, we noticed in the second experiment that some individuals were able to develop to the reproductive stage at higher salinity (10 gL<sup>-1</sup>). This is consistent with the halophilous characteristics of *R. cossoniana* that already have been reported from other populations, with salt concentrations as high as 25 gL<sup>-1</sup> (Ros, 1987), and which are expected to increase as the water evaporates. Such ecological requirement may represent an adaptation to developing at the flooded edges of saline habitats only during exceptionally rainy years, when the water column has sufficient depth (and that would be signalled by the dilution of the salts in the sediments). This would allow completing their life cycle before the habitats become completely dry, as reported for the similarly halophytes *R. helicophylla* (Marín, 1982), *R. alatospora* Wigglesworth (Segarra-Moragues *et al.*, 2012a) and other *Riella* species from fresh water habitats (Segarra-Moragues *et al.*, 2012b; Puche & Segarra-Moragues, 2013). Such particular conditions may have contributed to these and other potentially suitable localities having passed undetected. Our study also suggests that *R. cossoniana* is very sensitive to water transparency as shown for other species (Cirujano *et al.*, 1993; Segarra-Moragues *et al.*, 2012a).

### Conservation Status of French populations

The fact that populations of *R. cossoniana* were not observed in the field during the sampling season precludes a refined assessment of the population extension. This fluctuation of populations is a general rule to populations of *Riella*, that experience cycles of demographic blooms in favorable years and others of complete absence (Proctor, 1972). This peculiar population dynamics depend on the particular ecological conditions required for their development. Thus, if such ecological requirements are fulfilled, populations can rapidly spread first by germination from the highly resistant spore bank and, afterwards by vegetative multiplication of individuals through propaguliferous scales. In that situation the only limitation is that of habitat extension. In the Camargue area this constitutes a considerable limitation as the total area where *R. cossoniana* has been revealed accounts for only 1 ha.

Of the five known localities, the four ones (localities 7-10, Fig. 1) from Tour du Valat are included within the area of the Regional Nature Reserve and thus, have fully ensured protection. This area is also part of the Natura 2000 network and is included in the Site of Community Importance FR9301592 “Camargue”.

Like many aquatic plants, populations of *Riella* are highly sensitive to biotic and abiotic factors acting at the ecosystem level and that lead to habitat loss or habitat degradation. As a species typical of temporarily flooded habitats it depends on fluctuating water levels to suppress more aggressive plant species that are usually favoured by stable water levels. However, most of the abiotic factors affecting populations of *Riella* are derived directly or indirectly from human activity. They include the alteration of water regimes of the populations by

drainage or water extraction for irrigation (especially in the drier years when populations are more sensitive to life-cycle completion), the pollution of water by pesticides, fertilizers, etc., and the changes in the use of the territory for urbanization or industrial development. Some of these human activities cause irreversible damage to the populations.

*Riella cossoniana* is likely more widespread in the area and other populations may be revealed after intensive prospection of other suitable areas and laboratory cultures of sediments. Nonetheless, current geographically closer populations are placed at 300 km linear distance in Spain, and have not been found again since 2004 (Seguí *et al.*, 2005; Seguí & Pérez, 2006). Considering the reasons of the rarity of *R. cossoniana* we should infer that its distribution insufficiently known. Therefore, it seems premature to draw any conclusion on its particular IUCN (2011) status. Future field work will reveal the actual extent of the populations of *R. cossoniana*, and population monitoring will help to identify the particular biological and anthropogenic threats to its populations.

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