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Morphological description and ecology of some rare macroalgae in south-central Spanish rivers (Castilla-La Mancha Region)

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Abstract

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The Castilla-La Mancha Region (south-central Spain) is scarcely studied in terms of freshwater algae. However, both the implementation of the Water Framework Directive (2000/60/CE) and the evaluation of the ecological state of European aquatic ecosystems have increased the intensity and frequency of water body monitoring, including the rivers, lakes and wetlands of this region. Thus, our knowledge on algal biodiversity and the geographical distribution of many species is rapidly increasing. In this study we describe the occurrence, ecological conditions and morphological characteristics of five algal species which are rare at the European level: *Nostochopsis lobata* Wood ex Bornet & Flahault, *Batrachospermum atrum* (Hudson) Harvey, *Chroothece richteriana* Hansg., *Oocardium stratum* Nägeli and *Tetrasporidium javanicum* Möbius. In addition, we include *Hydrurus foetidus* (Vill.) Trev., a more common alga in Spain, since this is the first record for the region. Finally, we compare morphological and ecological characteristics of the studied populations with other European records.

Keywords: Algae, stream, river, Spain, Cyanophyceae, Rodophyceae, Chlorophyceae.

Resumen

Moreno Alcaraz, J.L., Canales Monteagudo, L. & Aboal Sanjurjo, M. 2013. Descripción morfológica y ecología de algunas macroalgas fluviales de la España centromeridional (Castilla-La Mancha). *Anales Jard. Bot. Madrid* 70(1): 81-90 (en inglés).

El conocimiento sobre la biodiversidad algal de la región de Castilla-La Mancha, situada en la zona centro-sur de España, es escaso en comparación con el de otras regiones peninsulares. Sin embargo, la aplicación de la Directiva Marco del Agua (2000/60/CE), y la evaluación del estado ecológico de los ecosistemas acuáticos europeos, ha traído consigo un aumento en la frecuencia e intensidad en el muestreo de ríos, lagos y humedales. De esta forma, durante los últimos años se han producido nuevos hallazgos en la región que han permitido ampliar el conocimiento de la biodiversidad de algas así como de la distribución geográfica de muchas de sus especies. En este trabajo se describen las condiciones ecológicas y las características morfológicas de cinco especies que pueden considerarse raras a nivel europeo: *Nostochopsis lobata* Wood & Bornet & Flahault, *Batrachospermum atrum* (Hudson) Harvey, *Chroothece richteriana* Hansg., *Oocardium stratum* Nägeli y *Tetrasporidium javanicum* Möbius; y de una sexta especie, frecuente en España y Europa, pero que supone la primera cita para esta región, *Hydrurus foetidus* (Vill.) Trev. Finalmente, se comparan las características morfológicas y ecológicas de las poblaciones estudiadas con otras citas Europeas.

Keywords: Algae, arroyo, río, España, Cyanophyceae, Rodophyceae, Chlorophyceae.

INTRODUCTION

The Castilla-La Mancha administrative region (south-central Spain) remains as one of the most unknown of the Iberian Peninsula in relation to river algae diversity. Five main river basins are included in this region: Tajo, Júcar, Guadiana, Guadalquivir and Segura, but only the last has been intensely surveyed with regards to algae (e.g. Aboal & Llimona, 1985; Aboal, 1988a-c, 1989a-c; Sabater & al., 1989; Aboal & al., 1996). The rest of region is scarcely studied (e.g. Aboal, 1996; Álvarez & al., 2007) although a species list of Charophytes in Castilla-La Mancha focusing mainly on wetland areas has been published by Cirujano & Medina (2002). Additionally, a recent review on the status of river aquatic plants in this region (Moreno & al., 2011) provides an up to date regional catalogue of aquatic bryophyte species as well as a list of macroalgae genera.

The implementation of the Water Framework Directive (2000/60/CE) and the evaluation of the ecological state of European aquatic ecosystems have increased the intensity and frequency of monitoring of water bodies, including the rivers, lakes and wetlands of the study area. The assessment of the ecological state of rivers by applying the Water Framework Directive (WFD) implies the monitoring of the biological element "macrophytes and phytobenthos". As a consequence, knowledge on macroalgal biodiversity and the geographical distribution of many species has rapidly increased in recent years.

In this study we describe the occurrence, ecological conditions and morphological characteristics of some uncommon algal taxa. One of them has been the first record for Spain and the third for Europe: *Nostochopsis lobata* Wood ex Bornet & Flahault; four additional species are hardly cited in Europe: *Batrachospermum atrum* (Hudson) Harvey, *Chroothece richteriana* Hansg., *Oocardium stratum* Nägeli and *Tetrasporidium javanicum* Möbius; and finally, *Hydrurus foetidus* (Vill.) Trev., which has been collected in cold streams of some mountains of Spain although our record is the first cite for the study area.

All the taxa were found within the boundaries of the Autonomous Community of Castilla-La Mancha (south-central Spain) (Fig. 1) which occupies an area of 79409 km^2. This region includes the upper and middle reaches of five large river basins: Tajo, Guadiana, Guadalquivir, Júcar and Segura. Land uses are mainly agriculture (46 % of the regional area) and forest (44 %). Regarding geology, three zones can be distinguished: the western zone is rich in Precambrian siliceous rocks (mostly quartzite, slate, shale, granite and gneiss); Meso-

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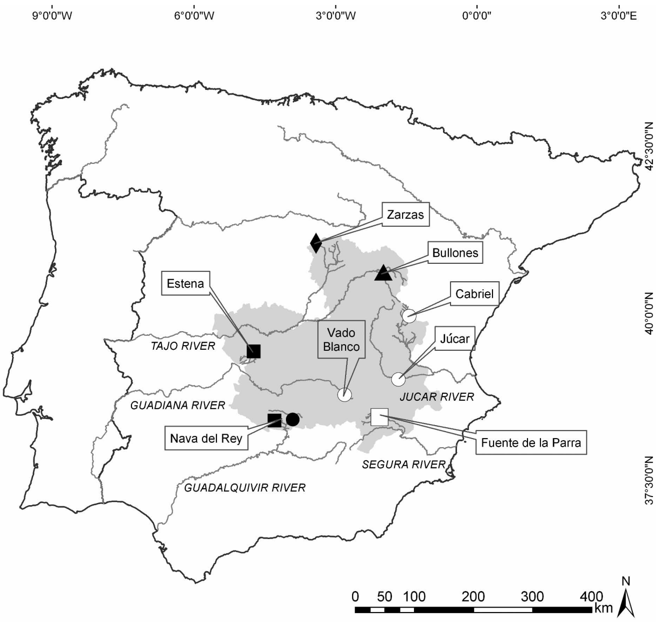

Fig. 1. Map of the Iberian Peninsula showing the limits of the Castilla-La Mancha Region, the main rivers crossing the study area and the location of the species recorded. ♦ *Hydrurus foetidus*; □ *Oocardium stratum*; ● *Nostochopsis lobata*; ▲ *Chroothece richteriana*; ○ *Batrachospermum atrum*; ■ *Tetrasporidium javanicum*; ▭ Castilla-La Mancha Region.

zoic calcareous rocks (limestone, dolomite, sandstone and conglomerates) are dominant in the eastern area; and finally, Tertiary sedimentary fills are accumulated in the great central plateau located at 700 m a.s.l. called "La Mancha", where clays, sandstones, gravels, stones, conglomerates, marls and gypsum are predominant (González & Vázquez, 2000). Mountains are located mainly on the edges of the region and can reach more than 2000 m (2273 m, Pico del Lobo)

MATERIAL AND METHODS

As part of a regional river monitoring program, a stream reach of approximately 100 m long was deeply surveyed for macroalgae, including all microhabitats present, e.g. riffles, pools, runs or stream margins as well as different kinds of substrata (sand, gravel, stones, aquatic vegetation, etc.). Macroalgal samples were collected in the river by hand and taken to the laboratory. One part of the samples was fixed in 3 % formaldehyde in the field whereas the other part was maintained fresh until laboratory observations. Collected material was examined under a Leica M165C stereoscope and a light microscope OLYMPUS BX50. Glycerin-gelatine was used to make permanent slides which were used to take cell measurements. Drawings were made with the help of macroscopic and microscopic images taken by a Leica DFC 420 C camera and also by direct observation of samples. Electric conductivity, pH and dissolved oxygen were measured *in situ* using appropriate sensors (Multiline P4 WTW). Alkalinity was also obtained in the field using the sulphuric acid method (APHA, 1998). In addition, water samples were collected in polyethylene bottles (500 ml) and were kept in the refrigerator at 4 ºC. All physico-chemical parameters were analyzed within 48 h after sampling. The concentration of nutrients (N-NO_3^-, N-NO_2^-, N-NH_4^+, and P-PO_4^{-3}) was determined photometrically with MERCK Kits (Spectroquant®); ion chromatography was used to analyse chloride and sulphate; turbidity with a turbidimeter TN-100 (Eutech Instruments; infrared light); calcium and magnesium by complexometry (volumetry); sodium and potassium by atomic emission. All these parameters were analysed following standard procedures detailed in APHA (1998).

RESULTS

Nostochopsis lobata Wood ex Bornet & Flahault (Fig. 2a-c)

Some specimens of *N. lobata* were found at the Nava del Rey stream (Fig.1), a temperate temporary stream tributary of the Guadalquivir river. The stream was located in the southern part of Castilla-La Mancha (Province of Ciudad Real), and it flows over Palaeozoic siliceous metamorphic rocks (quartzites, slates and shales). The study site was located at medium altitude (590 m) at 9.5 km from the source with an upstream drainage area of 41.76 km^2. *N. lobata* was found when the stream was at base flow condition (June 2009). Two morphological forms were collected: compact globular specimens corresponding to young colonies (Fig. 2a,b) were found attached to a moss stem whereas free floating diffluent fragments coming from senescent colonies were collected entangled with other macrophytes. Young colonies measured 0.5-3 cm in diameter while diffluent fragments were about 0.1-1 cm; and its colour changed from brownish to bluish, respectively. The radial disposition of filaments could be clearly observed in the outer part of the colonies, in transversal sections (Fig. 2b). Cells were isodiametrical to cylindrical, measuring from 2.0 µm up to 6.17 µm in diameter and length up to 7 µm. Heterocytes were predominantly lateral, sessile or pedicellate (Fig. 2c), measuring from 6.7 µm to 8.0 µm in diameter. Intercalary heterocytes were rare. The waters were oligo-mesotrophic, with low conductivity and alkalinity values (Table 1). The specimens were collected at marginal depositional habitats along with other aquatic macroflora such as *Ranunculus peltatus* Schrank, *Cladophora* sp., *Spirogyra* sp., *Oedogonium* sp., *Zygnema* sp. and *Tetrasporidium* sp.

Batrachospermum atrum (Hudson) Harvey (Fig. 2d-f)

B. atrum was found at three study sites: the Vado Blanco stream, Cabriel stream and Júcar river (Fig. 1). The Vado Blanco stream connects two lagoons of the Ruidera lagoon complex made up of 15 karstic lagoons located on a high altitude calcareous plateau (around 900 m a.s.l.) where forest and irrigation land are the main land uses. Nitrate concentration in groundwater and surface water is high due to intensive agriculture practises developed in the last decades (Berzas & al., 2004). The Cabriel Stream is the main tributary of the Júcar River and the study site was located at a high altitude near its source (1156 m a.s.l.) in a mountainous area where forest is predominant. Groundwater inputs from springs and upwellings are important in this stream reach. The last study site was located in a middle reach of the River Júcar at medium altitude (600 m) in a wide valley dedicated to agricultural uses. The influence of a reservoir located 96.65 km upstream is very signi-

Table 1. Geographical and ecological data for the algae species studied.

Species	*Nostochopsis lobata* Wood ex. Bornet & Flahault	*Batrachospermum atrum* (Hudson) Harvey			*Chroothece richteriana* Hansg.	*Oocardium stratum* Nägeli	*Tetrasporidium javanicum* Möbius		*Hydrurus foetidus* (Vill.) Trev.
River/Stream	Nava del Rey	Vado Blanco	Cabriel	Júcar	Bullones	Fte. de la Parra	Estena	Nava del Rey	Zarzas
date (dd/mm/yyyy)	09/06/2009	27/05/2009	30/03/2005	03/11/2009	29/05/2007	27/02/2012	13/05/2002	28/04/2010	26/03/2012
altitude (m)	590	860	1156	607	1032	851	816	590	1564
lithology (predominant)	siliceous	calcareous	calcareous	calcareous	calcareous	calcareous	siliceous	siliceous	siliceous
mean channel width (m)	3.5	2.5	6.9	9.8	2	1	1.5	3.5	2.4
discharge ($m^3·s^{-1}$)	0.03	0.185	0.38	1.8	–	–	0.08	70	0.02
T (ºC)	18.8	19.9	13.5	15.6	12.9	11.5	13.7	24.5	7.8
pH	7.47	8	7.98	8.1	7.34	7.91	7.46	8.3	7.53
dissolved oxygen ($mg·L^{-1}$)	6.84	8.8	15.5	9.2	9.81	9.21	7.85	9.11	9.5
conductivity 25º ($\mu S·cm^{-1}$)	237	870	577	847	2470	547	29	193	12
ammonium ($mg\ L^{-1}$)	0.042	0.045	0.029	0.13	0.137	0.001	0.112	<0.03	0
nitrate ($mg\ L^{-1}$)	0.33	11.103	0.44	0.746	1.14	2.4	0.9	0.33	0.5
ortophosphate ($mg\ L^{-1}$)	0.001	0.02	0.007	0.019	0	0.027	0.08	n.d.	<0.010
alcalinity ($mg\ L^{-1}$)	92	218.1	212	161.3	288	284	30	104.00	8
chloride ($mg\ L^{-1}$)	15.44	48.45	6.71	23.68	383.02	19.47	12.82	15.44	7.48
sulphate ($mg\ L^{-1}$)	41.5	114.19	85.52	167.65	313.66	31.05	8.93	41.50	16.61
calcium ($mg\ L^{-1}$)	23	110.71	67	77.2	126.12	55.17	15.5	23.00	0.92
magnessium ($mg\ L^{-1}$)	10	19.35	41	25.39	53.1	25.19	2.24	10.00	0.41
sodium ($mg\ L^{-1}$)	8.57	24.17	2.85	13.19	174.76	10.73	3.73	8.57	0.98
potassium ($mg\ L^{-1}$)	1.27	1.79	0.64	2.18	4.62	0.91	1.1	1.27	0.1

ficant due to flow regulation for agriculture. The river reach is also influenced by groundwater upwelling and seepages that contribute to feed the river. The specimens were collected submerged in the stream margins, mainly over other macroalgae or entangled with them and exposed to a low current velocity. Thallus size ranged from 2.5-8 cm long and 60-120 µm wide, colour red-brownish, without mucilage (Fig. 2d and 2e), showing a regular cortication (Fig. 2f) along its central axis and branches. The mean distance between nodes was of 250-525 µm and decreasing from the central axis base towards the apical branches (up to 80 µm in branch tips). The whorls were very reduced, 100-200 µm diameter, non-adherent to the main axis, and ring or pearl shaped (obconical). Primary fascicles were composed of 3-6 barrel-shaped cells of 3.3-6.8 µm in diameter. Secondary fascicles were composed of 1-3 cells, abundant and scattered along the internodes or concentrated in nodes, the apical cell of fascicles usually sharp-pointed. Gonimoblasts were hemispherical, 135-220 µm in diameter and 27-80 µm high, attached to the axis in internodes or nodes. Tricogyns shape was clavate, 9.3-16.2 µm long and 6.2-8.3 µm wide, located mainly in internodes but also in nodes and axillas at the base of branches. Tricogyns usually included 1-2 round spermatia of 4.7-6 µm diameter attached to the tip. Regarding environmental conditions, it is important to highlight that the three localities were under groundwater influence. Dissolved nutrients showed low values except for one site (Vado Blanco stream, Lagunas del Ruidera) located at an irrigation area with high levels of nitrate contents (Table 1). Accompanying aquatic macroflora included *Anabaena* sp., *Batrachospermum gelatinosum* (L.), *Chara aspera* C.L. Dethard. ex Willd. and *Chara hispida* L. in Vado Blanco stream; *Apium nodiflorum* (L.) Lag., *B. gelatinosum* and *Bryum* sp. in Júcar river; and *Apium nodiflorum*, *Audouinella* sp. and *B. gelatinosum* in Cabriel stream.

Chroothece richteriana Hansg (Fig. 3a-c)

C. richteriana was found in Bullones stream (Fig. 1), a headwater tributary of the River Tajo situated in a calcareous landscape in contact with marls and limestones. The drainage area of subcatchment was 137.97 km², with 30% of the area occupied by agriculture and 70% by natural vegetation. The immediate vicinity of the site was a semi-natural area mainly occupied by *Juniperus communis* L. and *J. thurifera* L. The site was located at 1032 m a.s.l., 25 km downstream from the source. In general, colonies of this species were bright green, gelatinous and hemispheric in shape. Transversal sections revealed a stratified structure of the hemispherical colony (Fig. 3a): the surface was conformed by a continuous layer of cells on the top of their radially-arranged stalks, with some parallel lines of sediments and carbonate precipitates probably corresponding to growth rings. *C. richteriana* was found growing epilithic, covering around 80% of most submerged rocks. Cells were cylindrical and elongated, measuring from 5 µm to 9 µm in width (6.9 µm average) and from 10 µm to 18 µm in length (15.9 µm average) (Fig. 3b,c). The cells were surrounded by a thick cell wall (from 2 to 6 µm wide) and disposed on the stalks (Fig. 3b). Inside some cells, a star-shaped chromatophore was visible (Fig. 3c). Regarding physico-chemical conditions (Table 1) water was slightly saline due to the presence of marls alterna-

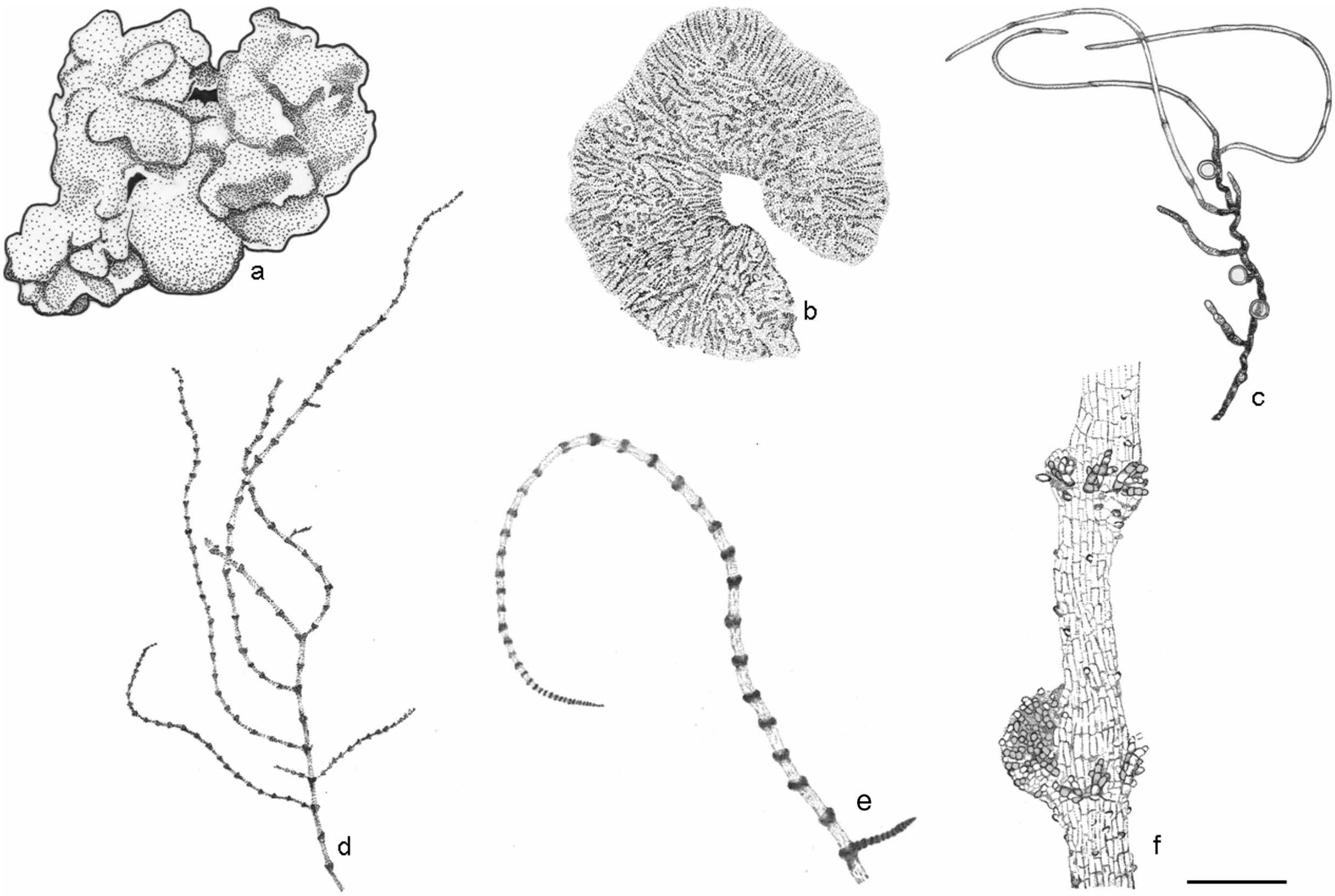

Fig. 2. *Nostochopsis lobata*: **a,** macroscopic view; **b**, transversal section; **c,** mature filament with heterocytes (from Moreno & al., in press). *Batrachospermum atrum*: **d,** macroscopic view; **e,** filament; **f,** detail of gonimoblast and cortication of the thallus. Scale: a, d = 50 mm; b = 1 mm; c, f = 50 µm; e = 500 µm.

ting with limestones (I.T.G.E., 2000), with a high content of chloride and sulphate. An industry of salt extraction is located 8 km upstream (Salinas de Almallá) due to the presence of evaporite outcorps.

Other aquatic taxa detected at the site were: *Apium nodiflorum*, *Veronica anagallis-aquatica* L., *Chara vulgaris* sp., *Cladophora* sp., *Tetraspora* sp., *Vaucheria* sp., *Batrachospermum* sp., *Tolypothrix* sp., and *Phormidium* sp.

Oocardium stratum Nägeli (Fig. 3d-i)

The desmid zygnematal *O. stratum* was found in Fuente de la Parra stream (Fig. 1), a small tributary of the Mundo River (Segura river basin). This stream runs over calcareous rocks in a mountainous area covered by a *Pinus pinaster* Ait. forest. The site was located at 851 m a.s.l. very close to the source (1.73 km downstream). The biodiversity and proliferation of macroalgae was low, due to the dense canopy reducing the entry of light. *O. stratum* appeared on mosses, sticks and wet rocks in a small dam 3 m in height built to retain water from the source, where a travertine formation was extensive across the waterfall. To the naked eye, colonies first formed hemispherical structures like small grains a few millimetres long (Fig. 3d). However, with the growth and the increase of carbonate precipitates, the colonies formed a lobulated layer of calcareous concretions several centimetres thick on twigs and sticks (Fig. 3e). Under the microscope, a lateral vision of a colony (Fig. 3b) showed that this species formed gelatinous branched cylinders surrounded by calcite with bright green cells at the top (Fig. 3g). Cells were heart-shaped, showing a small median constriction as in other desmids such as *Cosmarium*, and measured around 13.5 µm in diameter (11-16 µm) (Fig. 3h).

In some cases, colonies of *Chroothece rupestris* Hangs. were found over colonies of *O. stratum*. After using acetic acid to eliminate carbonate precipitates from *Oocardium* colonies, filaments of *Rivularia sp.* were observed inside them (Fig. 3i). These three algae species, together with the moss *Didymodon tophaceus* (Brid.) Lisa, were revealed to be the main biological builders of the global travertine structure. The accompanying aquatic macroflora included *Callitriche stagnalis* Scop., *Veronica anagallis-aquatica*, *Chara vulgaris*, *Batrachospermum gelatinosum*, *Phormidium* sp., *Oedogonium* sp., *Cladophora glomerata* (L.) and *Microspora* sp.

Tetrasporidium javanicum Möbius (Fig. 4a-e)

In the study area, this clorophyte was found at two sites. The first site was Estena stream (Fig. 1), a small tributary of the Guadiana river running over siliceous rocks. The study site was located in a mountainous area of the Cabañeros National Park,

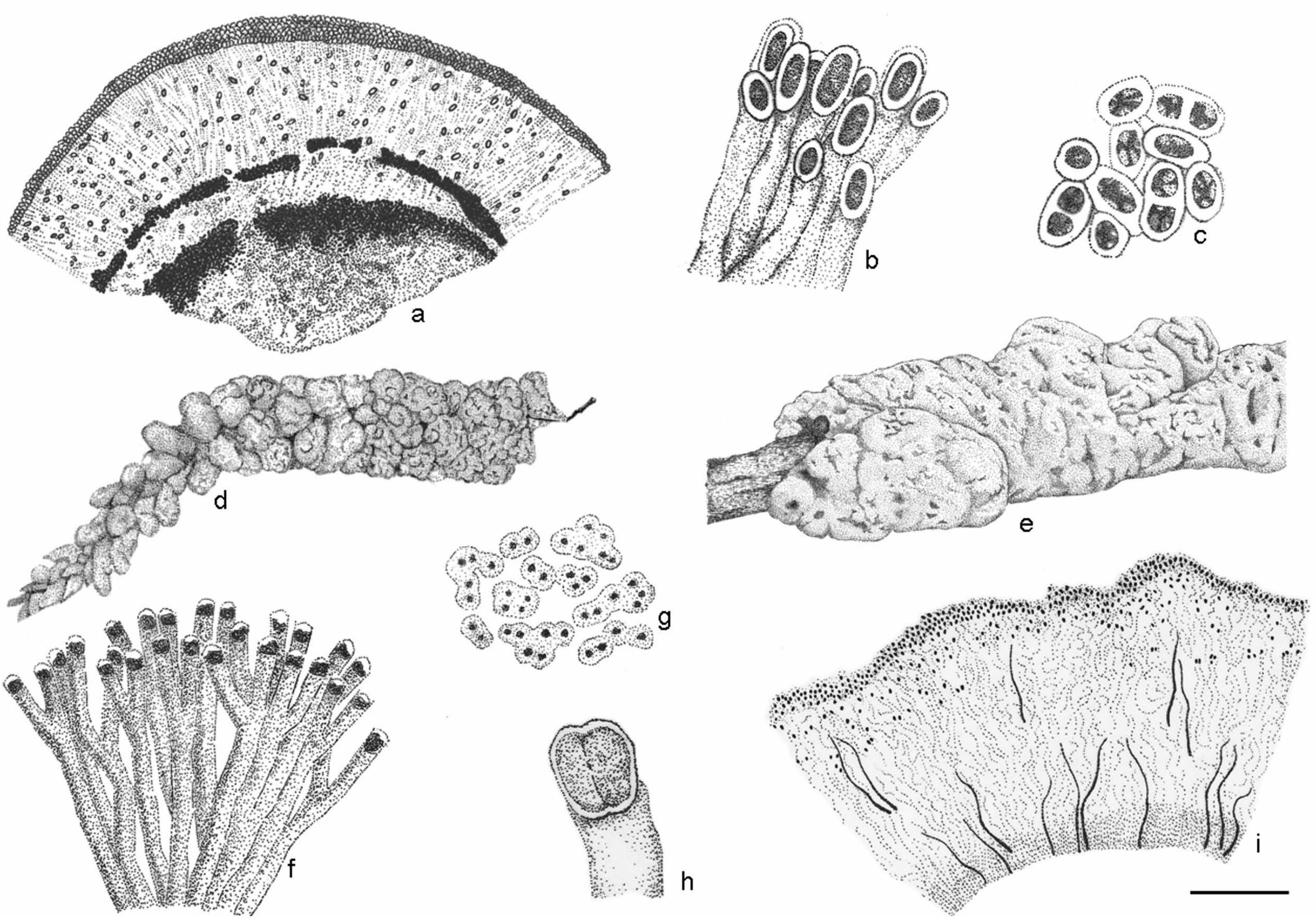

Fig. 3. *Chroothece richteriana*: **a,** transversal section of a colony; **b,** lateral view of pedunculated cells; **c,** cells with star-shaped chromatophores. *Oocardium stratum*: **d,** small colonies covering a moss branch; **e,** aged colony forming a thick lobulated layer; **f,** lateral microscopic view showing the calcite cylinders surrounding cell stalks; **g,** upper view of cells surrounded by calcite; **h,** detail of cell with stalk; **i,** colony of *O. stratum* together with *Rivularia* after carbonate removal. Scale: a, i = 500 µm; b, c, h = 20 µm; d = 5 mm; e = 1cm; f = 100 µm; g = 200 µm.

and 6.1 km downstream from the source. The drainage area was covered by a near natural forest of *Quercus* Mediterranan maquia. *T. javanicum* appeared as small (environ 8 mm in length) free floating colonies forming a light green gelatinous tubular-net thallus (Fig. 4a). These small colonies probably corresponded to fragments of a bigger colony. The 'ribbons' making up the thallus (Fig. 4b) measured 100-300 µm wide and presented revoluted margins. Cells were heterogeneously arranged in the mucilage and were spherical to slightly oval in shape (Fig. 4c). A wide range of cell size was observed: from 5.1-10.7 µm in diameter, with 8.2 µm on average. Chloroplasts were parietal with a single large pyrenoid (around 1.6 µm in diameter). Pseudo-flagella, typical of the genus *Tetraspora*, were absent. Environmental and physico-chemical data recorded for this site indicated that the stream was mesotrophic, showing a very low mineralisation due to the siliceous lithology (Table 1). The waters showed a carbonated character but with a high proportion of chloride. Accompanying aquatic macroflora included *Audouinella* sp., *Draparnaldia* sp., *Zygnema* sp., *Tolypothrix* sp., *Scytonema* sp., *Nostoc* sp., and *Chiloscyphus polyantos* (L.) Corda.

In addition, we collected *Tetrasporidium* in another siliceous oligo-mesotrophic stream, the Nava del Rey stream (described in *Nostochopsis lobata* section), but different morphological characteristics were observed. In this case, colonies were thin perforated sheets (Fig. 4d), the biggest one measuring 1.5 × 2.1 cm. Cells were smaller and surrounded by concentric gelatinous sheaths (Fig. 4e). The smaller cell size (from 3.3 µm to 5.7 µm in diameter) of this specimen matched with the description of *T. fottii* Couté et Traccana included in the key of Ettl & Gärtner (2009), although for other authors *Tetrasporidium* is a monospecific genus (see discussion).

Hydrurus foetidus (Vill.) Trev. (Fig. 4f-h)

H. foetidus was found at Zarzas stream (Fig. 1), a headwater tributary of the Hoz stream which belongs to the largest Spanish river basin, the Tajo river. The Zarzas stream runs through the Natural Park of Sierra Norte of Guadalajara, located in the northernmost part of the region. This area is mountainous and is covered by a forest of *Pinus sylvestris* L. with the presence of *Fagus sylvatica* L. The study site was located at an altitude of 1564 m, in one of the coldest areas of the region, 7.90 km downstream from its source. The drainage area of subcatchment is 26.90 km^2. This alga was found as gold-brown tufts up to 20 cm long attached to boulders and stones (Fig. 4f,g) some-

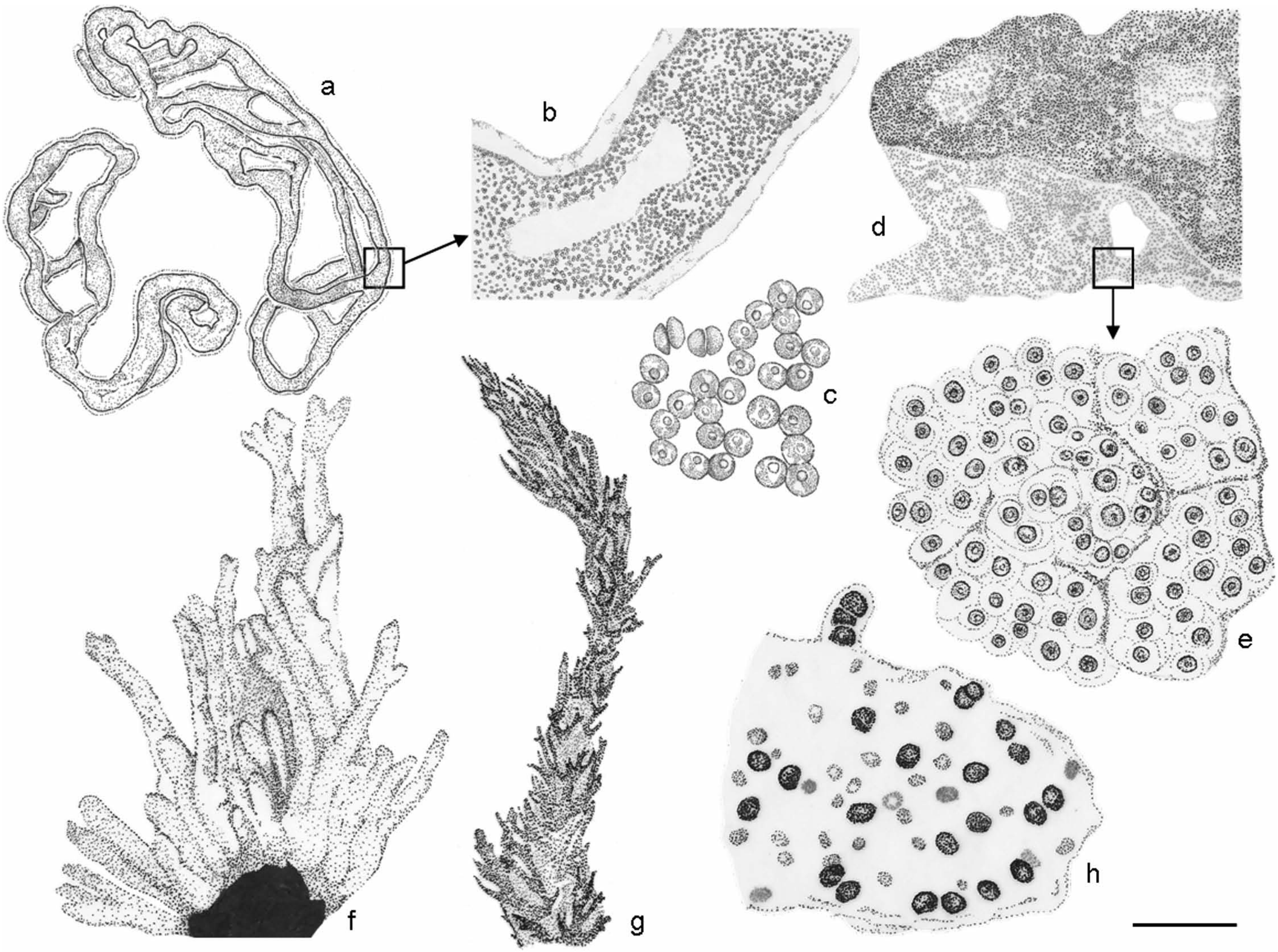

Fig. 4. *Tetrasporidium javanicum*: **a,** macroscopic view of a tubular-net colony ("ribbon-like"); **b,** detail of a part of the colony; **c,** detail of cells; **d,** macroscopic view of a laminar colony; **e,** detail of the colony showing cells surrounded by gelatinous sheaths. *Hydrurus foetidus*: **f,g,** macroscopic view of two thallus shapes; **h,** detail of cell arrangement within the mucilage. Scales: a = 2 mm; b = 100 µm; c, h = 20 µm; d = 500 µm; e = 50 µm; f, g = 1 mm.

times covering 100 % of the substratum. Thalli were branched and gelatinous. Cells embedded in the mucilage were peripherally arranged and oval to ellipsoidal in shape (Fig. 4h). Cell size was 5-8 µm wide (6.5 µm on average) and 7-13.5 µm long (10.5 µm on average) in. Regarding nutrients, waters were oligo-mesotrophic (Table 1). Accompanying aquatic macroflora was made up of the following taxa: *Lemanea* sp., *Hildenbrandria* sp., *Scapania undulata* (Hedw.) P. Beauv., and *Marchantia polymorpha* L.

DISCUSSION

N. lobata is included in the family Nostochopsaceae (Anagnostidis & Komarek, 1990; Komárek & al., 2003). This species is characterized by a globular lobed thallus composed of uniseriate and branched thricomes with intercalar or lateral heterocytes (pedicellate or sessile) (Bornet & Flahault, 1886-1888). *N. lobata* is widely distributed in tropical areas of the world and has been reported in several African countries (Frémy, 1929), North and South America and Australia (Cáceres, 1973; Sarma & Chapman, 1975; Branco & al., 2001) and Asia (Geitler, 1930-1932; Desikachary, 1959; Tiwari, 1978; Peerapornpisal & al., 2006). In Europe there are only third records from flowing warm waters: Banyuls-sur-Mer (France) (Frémy & Feldmann, 1934), Corsica (France) (Hoffmann, 1990), and L'Aquila (Italy) (Del Grosso, 1977). The specimens of *N. lobata* collected in Nava del Rey were the first recorded in Spain (Moreno & al., in press). Morphologically, our material matched fairly well the detailed description of the first specimen reported in Europe by Frémy & Feldmann (1934). In both cases *N. lobata* was found in small temporary streams running over siliceous metamorphic rocks, located at low altitude and living in warm and oligotrophic waters, and it was collected in summer. In terms of habitat, we found *N. lobata* growing epiphytic (young) and free floating (senescent) even though it has been reported in different habitats: lakes (Tiwari, 1978; El Saied, 2007), running waters (Frémy & Feldmann, 1934; Palmer, 1941; Pandey & Pandey, 2008), thermal springs (Yoneda, 1939), epiphytic (Sarma & Chapman, 1975), attached to rocks (Hoffmann, 1990; Peerapornpisal, 2006), free floating (Wood, 1872), on moist soils (Skinner & Entwisle, 2001; Aziz, 2008), and on humid walls (Skinner & Entwisle, 2001) among others.

However, the most extreme habitat where *N. lobata* has been found is probably as cryptoendolithic in an arid climate (Weber & al., 1996). Current data point out that this species is distributed worldwide with an underlying tendency: it grows abundantly in tropical regions where its optimal climatic conditions are stable for several months whereas it grows scarcely in other regions only when conditions allowing its germination and growth are reached, probably for a few weeks in occasional years (Moreno & al., in press). This fact explains the difficulty in detecting this species in Europe, where it has always been recorded only once per site.

B. atrum belongs to subgenus *Batrachospermum,* which is characterized by a carposporophyte multicellular, and to section *Setacea* which is characterized by short carpogonium-bearing branches and whorls/fascicules (Eloranta & Kwandrans, 2007). *B. atrum* (section *Setacea*) is on divergent branches of molecular trees (Kumano, 2002) and constitutes a distinct section, as proposed by Sheath & al. (1993). Gonimoblasts form mamilliform or semispherical swellings on the central axis and the trichogyn is clavate; the thallus is subgelatinous (Starmach, 1977). The habitus shows a compressed and compact thallus due to its short fascicules and whorls, resembling a bamboo stem, similar to the thallus of the red algae *Lemanea*. According to Kumano (2002), *B. atrum* is distributed throughout Europe (UK, France, Belgium, Germany, Poland, Portugal and Sweden), Eastern Asia (China, Korea and Japan), Australia, New Zealand, South America (Brazil) and Africa (Angola), in addition to the USA (California and Texas; Sheath & al, 1993). In Spain there is only one previous record from the Murcia Region (Aboal & al., 1995). The morphological characteristics match with other descriptions provided by other authors (Israelson, 1942; Sheath & al., 1993; Eloranta & Kwandrans, 2007). *B. atrum* is an oligotrophic hard water species (Sládecek, 1973; Rott & al., 1999; Eloranta & Kwandrans, 1996, 2002). In Spain, Aboal & al. (1995) found this species in calcareous springs and small streams with environmental conditions similar to those described in this work. The three sites where *B. atrum* was collected were calcareous and they showed groundwater influence associated to upwelling areas, springs or seepages. The trophic conditions in our sites were oligo-mesotrophic, with one site showing a high concentration of dissolved nitrate due to its location within an intensively irrigated cropland area (Álvarez-Cobelas & al., 2005). According to Eloranta & Kwandrans (2007) the species grows epiphytic or epilithic, but in our sites it was collected as epiphytic, floating among other macrophytes close to river banks and avoiding high current velocity, more limnephilic than rheophilic. According to Israelson (1942) and Aboal & al. (1995) the species seem to occur only in spring, but in the study area the species was recorded both in spring and autumn, similar to the seasonal pattern frequently shown by the rest of the Batrachospermaceae in lotic waters (Aboal & al., 1995). The fact that the species was collected only once per site despite several visits may be due to the rapid development and short life cycle of the species or to the occasional interannual occurrence.

After *C. richteriana* was described by Hansgirg (1884), few publications about this species have been released. *C. richteriana* has been reported in Britain (Sheath & Sherwood, 2002; Eloranta & al., 2011) and Spain (Eloranta & al., 2011). Recently it has been found growing in slightly saline streams of south-eastern Spain (Murcia), mostly on subaerial conditions (unpublished data). This rare Rhodophyte included in the Porphyridiaceae family is characterized by mucilaginous colonies conformed by elliptical cells enclosed in a layered gelatinous matrix (Eloranta & Kwandrans, 2007; Eloranta & al., 2011). Additionally, a star-shaped chromatophore is clearly visible inside the cells. Starmach (1977) and Eloranta & al. (2011) consider cell size as a differential characteristic between the two species of *Chroothece* present in Castilla-La Mancha: *C. richteriana* measuring 6-10 µm wide and 15-18 µm long; *C. rupestris* measuring 5-7 µm wide and 9-15 µm long. Additionally, Sheath & Sherwood (2002) reported a cell size for *C. richteriana* of 8-12 µm wide and 10-21 µm long (British Islands). Both studies match with the cell size of our colonies collected in Bullones stream (5-9 µm wide, 10-18 µm long). However, we noticed that according to our observations, the cell size range of a *Chroothece* population can exceed that one reported from identification keys. On the other hand, the relatively high water salinity of Bullones stream was in accordance with the "more or less saline" environment described for *C. richtheriana* in the identification keys (Eloranta & al., 2011; Starmach, 1977).

Regarding *Chroothece* populations from Fuente de la Parra (see *Oocardium stratum* section), they were identified as *C. rupestris* according to the identification keys criteria: lower cell size, low water mineralisation, and also the environment "wet rocks". In the same way, Margalef (1989) recorded *C. rupestris* from wet rocks of calcareous springs, described by him as "hygropetric environment", a similar environment to that of Fuente de la Parra stream.

Oocardium is a monospecific genus belonging to the family Desmidiaceae, characterized by being mostly unicellular, either solitary or in colonies, with cells divided into two compartments separated by an isthmus. West (1904) described *Oocardium* as the most extraordinary of all the genera of Desmids usually occurring in large colonies. According to West (1904) and Bourrelly (1990), the colonies of *O. stratum* are hemispherical in shape (1-2 mm in diameter), and are formed by cells standing on more or less parallel, radiating strands of mucus which are encrusted in calcite. During the growing period, calcite crystals are formed by continuous deposition by surrounding cells which, following division, calcify into slightly different directions upwards (Sanders & Rott, 2009). The colonies detected in Fuente de la Parra stream matched exactly with the above description and appeared jointed, forming a nodular crust covering substrates, similarly to colonies observed by Pentecost (1991). Regarding the environmental conditions, *O. stratum* seems to have very specific requirements: it is only found in waterfalls and springs with calcareous water (Margalef, 1983; Pentecost, 1991) usually being associated to deposits of travertine and tufa (Pentecost, 2001; Sanders & Rott, 2009; Bellinger & Sigee, 2010). Because of these environmental restrictions, *O. stratum* is often considered as an unusual alga (e.g. Margalef, 1983; Bellinger & Sigee, 2010) even though its presence has been detected worldwide: Switzerland (Nägeli, 1849) the United States of America (Prescott, 1981), Austria (Sanders & Rott, 2009), United Kingdom (West, 1904; Pentecost, 1991), Ireland (Carter, 1923), Belgium (van Oye & Hubert, 1937), France (Kouwets, 1999), Slovenia (Vrhovsek & al., 2006). Particularly in Spain, *O. stratum* has been previously reported in Barcelona (Margalef, 1955), Murcia (Aboal, 1991) and in Castilla-La Mancha (Ruidera Lakes) (Álvarez-Cobelas & al., 2007), although we think that it could be a common species in calcareous mountains and springs.

T. javanicum is a clorophyte classified within the order Tetrasporales, family Palmellopsidaceae (Ettl & Gärtner, 2009). In the mature state the thallus is a thin, flat, more or less circular sheet with numerous irregular perforations with a smooth or lobed margin (Iyengar, 1932). Nevertheless, the specimen collected in the Estena River was a tubular-net thallus, probably as a result of the increase in the size perforation with age. This fact supports the idea that ribbon-like and sheet-like thallus could represent plants of different ages (Entwisle & Skinner, 2001). According to Ettl & Gärtner (2009) cells are oval to spherical in shape ranging from 6 µm to 15 µm in diameter, slightly larger than our material (from 5.1 to 10.7). Regarding distribution, this species is abundant in tropical areas within India (Iyengar, 1932), China (Hu & Wei, 2006), Hawaiian Islands (Sherwood, 2004), Java, the United States, Australia and Bangladesh (Entwisle & Skinner, 2001) being quite common in tropical areas (Entwistle & Skinner, 2000). In Europe, *T. javanicum* has been reported in the Czech Republic, France, Portugal and Spain (Fott & al., 1965; Coute & Tracanna, 1981; Calado & Rino, 1992; Aboal & al., 1994, respectively). In Spain, this species has been found in the Alicante Province (Aboal & al., 1994), Cáceres Province (Marín-Murcia & Aboal, 2007), Galicia (López-Rodríguez & Penalta-Rodríguez, 2007) and, recently, in the Ebro river basin (NE Spain) (Tomás & al., in press). In almost all cases, the species is found growing in conditions of low current velocity, well oxygenated alkaline waters, high turbidity and nutrient enrichment due to agricultural practises. However, the material described in this work was found in low-mountain clear water streams. These conditions are more similar to those described by Calado & Rino (1992) and López-Rodríguez & Penalta-Rodríguez (2007) for specimens collected in Portugal and Galicia, respectively. Despite the fact that the Estena stream is oligotrophic, *T. javanicum* was found coinciding with a relatively high concentration of nitrogen compounds (nitrite, nitrate and ammonium). This fact could be due to the preference of *T. javanicum* for eutrophic waters, as suggested by some authors (Aboal & al., 1994; Marín-Murcia & Aboal, 2007; Tomás & al., in press) pointing to this species as a potential good indicator of eutrophy. Our observations lead us to think that *T. javanicum* is more common than reported up to now in Spain and it will be collected more frequently in upcoming river macrophyte surveys.

Regarding *T. fottii*, the species was described by Couté & Tracanna (1981) from some specimens found in Breton ponds. However, we were able to find only one other report on this species in literature, made by Neag & al. (2005) in the "Alexandru Borza" Botanical Garden, Cluj-Napoca, Romania. Ettl & Gärtner (2009) differenced *T. fottii* from other *Tetrasporidium* species according to the lateral position of the pyrenoid and its cell size (from 6.4 µm to 9.5 µm in diameter). However, the material collected in Nava del Rey was slightly smaller, measuring from 3.3 µm to 5.7 µm in diameter. Unfortunately, an appropriate observation of the ultrastructure of cells was not possible and, like Entwisle & Skinner (2001), we could not conclusively identify our material as *T. fottii*. On the other hand, Calado & Rino (1992) suggest that the specific characteristics of *T. fottii* could be explained by the cytologic variability within *Tetrasporidium javanicum* and, therefore, *T. fottii* should not be considered as an independent species. This idea is contemplated by other authors that classify *Tetrasporidium* as a monospecific genus (e.g. Bourrelly, 1990). Thus, we assigned our material to *T. javanicum* until more studies unraveling the taxonomy of this interesting genus are released.

The chrysophyte *Hydrurus foetidus* is included in the order Chromulinales, family Hydruraceae. The thallus is gelatinous, branched and attached to substrate reaching up to 30 cm long. Cells are ellipsoid to subspherical and they are randomly arranged in the mucilage (Rodriguez & Vergon, 1996). After collecting, it emanates a characteristic smell. This species is a cold water stenotherm also adapted to strong current velocity, being an exclusive inhabitant of cold mountain streams worldwide (2-12 ºC) (Hieber & al., 2001; Wehr & Sheath, 2003; Krizmanic & al., 2008). Under optimal conditions, *H. foetidus* becomes dominant and may entirely cover submerged rocks, making them very slippery. In Europe it is a well-known inhabitant of alpine streams and those draining glaciated landscapes, where it is described as a predominant or very common macroalga by several authors (e.g. Hieber & al., 2001; Cantonati & al., 2006; Krizmanic & al., 2008). In Spain, *H. foetidus* has been reported in the Pyrenees (NE Spain) (Margalef, 1948; Llimona & al.,1985), Cantabrian mountains (N Spain) (Margalef, 1950) and Sierra Nevada (Granada, S Spain) (Sánchez-Castillo, 1984) with this study being the first recordin for the Castilla-La Mancha region. As for habitat conditions, *H. foetidus* is clearly rheophylic and stenothermic, disappearing when water temperature rises above 10 ºC or 15 ºC according to Wehr & Sheath (2003) and Starmach (1985), respectively. A combination of hydrological factors and light conditions controls the seasonal fluctuations of this macroalga (Cantonati & al., 2006). In general, this species is commonly found during winter and spring (Hieber & al., 2001). In Spain, it is a vernal species found occasionally in summer at high altitudes (>2000 m) (Llimona & al., 1985). Our material was collected in early spring at a high altitude (1564 m) when water temperature was 7.8 ºC. Both the cellular size (from 7 µm to 13.5 µm in length) and the thallus length (up to 20 cm) of the specimens coincide with previous literature which describes large specimens (up to 30 cm) with cells measuring from 8 µm to 12 µm in length (Bourrelly, 1957; Rodriguez & Vergon, 1996; Wehr & Sheath, 2003).

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