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First records of *Ulvella spongophila* (Ulvophyceae: Ulvellaceae) for the Palaearctic region: the discovery of four populations in the SW Iberian Peninsula

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ABSTRACT

First records of *Ulvella spongophila* (Ulvophyceae: Ulvellaceae) for the Palaearctic region in the SW Iberian Peninsula

During extensive Spanish river macrophyte and invertebrate surveys, the freshwater chlorophyte *Ulvella spongophila* was detected growing inside the freshwater sponge *Ephydatia fluviatilis* attached to stones. This finding is relevant from the phycological, biogeographical and ecological points of view, and is the second world record of the species after one single known locality (Lake Manindjau, Sumatra, Malaysia), from which the species was collected and described around 130 years ago. The species was collected under summer base-flow conditions in four Mediterranean temporary/seasonal streams in 2018 and 2019. New information about the species' morphological characteristics and ecological preferences in the Iberian Peninsula is presented. A comparative description of the observed specimens with previously published data, some biogeographical aspects of findings, certain biological facets of the sponge-algal association, and its conservation status, are discussed.

Key words: *Ephydatia fluviatilis*, freshwater algae, freshwater sponges, Palaearctic, Spain, Iberian Peninsula, Spongillidae, symbiosis, temporary streams, *Ulvella spongophila*, Ulvellaceae, Chlorophyta

RESUMEN

Primeros registros de* Ulvella spongophila *(Ulvophyceae: Ulvellaceae) para la región paleártica en el SO de la Península Ibérica

Durante muestreos extensivos de macrófitos e invertebrados en ríos españoles, se detectó la presencia del clorófito de agua dulce Ulvella spongophila *dentro de esponjas de agua dulce (*Ephydatia fluviatilis*) adheridas a piedras. Este hallazgo resulta relevante desde el punto de vista ficológico, biogeográfico y ecológico, siendo la segunda cita mundial de la especie, después de la única localidad conocida (Lago Manindjau, Sumatra, Malasia), donde la especie fue recogida y descrita hace unos 130 años. Los especímenes fueron recogidos en condiciones de caudal base, en verano, en cuatro arroyos mediterráneos temporales o estacionales durante 2018 y 2019. En este trabajo se presentan nuevos datos acerca de las características morfológicas y preferencias ecológicas de la especie en la Península Ibérica. También se discute la adscripción de los especímenes observados a la especie, los aspectos biogeográficos más relevantes del hallazgo, algunas particularidades biológicas de la asociación alga-esponja, así como el estado de conservación de la especie.*

Palabras clave: Ephydatia fluviatilis*, algas dulceacuícolas, esponjas de agua dulce, Paleártico, España, Península Ibérica, Spongillidae, simbiosis, ríos temporales,* Ulvella spongophila*, Ulvellaceae, Chlorophyta*

INTRODUCTION

Monitoring programmes for the evaluation of river ecological status in the Water Framework Directive (WFD) application are being developed in all Spanish river basins, as in the rest of European countries. Consequently, the geographical distribution of many freshwater species in Spain is being extended and new records of species for Spain are becoming more frequent (Moreno et al., 2012, 2013; Tomás et al., 2013).

Ulvella spongophila (Weber-van Bosse) Škaloud & Leliaert 2018 is a chlorophyte that is currently classified in the class Ulvophyceae, order Ulvales Blackman & Tansley 1902, and family Ulvellaceae Schmidle 1899 (Skaloud et al., 2018). It is a freshwater endozoic alga that lives inside the encrusting sponge *Ephydatia fluviatilis* (Linnaeus, 1759). The species is only known from Malaysia (type locality: Manindjau, Malaysia), collected from Lake Manindjau shores by Weber-van Bosse (1890). Existing knowledge about the biology of *U. spongophila* (apart from its original description) is almost absent, and the nature of the association with its host *E. fluviatilis* remains unknown. Additionally, there are no molecular or phylogenetic analyses.

E. fluviatilis is a worldwide distributed species that is very common in European inland waters. Found in both running and standing waters, from coastal brackish waters to inland salt lakes, it is able to tolerate long periods of drought (Pronzato & Manconi, 2001). It is also a common freshwater species in the Iberian Peninsula (Traveset, 1986). Like other freshwater sponges, it can harbour symbiotic algae, usually unicellular green algae that confer the sponge a green color, i.e. zoochlorellae (Wilkinson, 1980; Sand-Jensen & Pedersen, 1994; Hall et al., 2021). The presence of filamentous green algae inside the body of freshwater sponges is less frequent, and *U. spongophila* is a rare example that is scarcely documented.

The taxonomic position of *U. spongophila* has undergone numerous changes and has been described with different names since its first description. We herein address a review of this aspect. Moreover, to date *U. spongophila* has been considered a tropical species, but it might be spreading to more temperate latitudes like Mediterranean streams as other algal species do

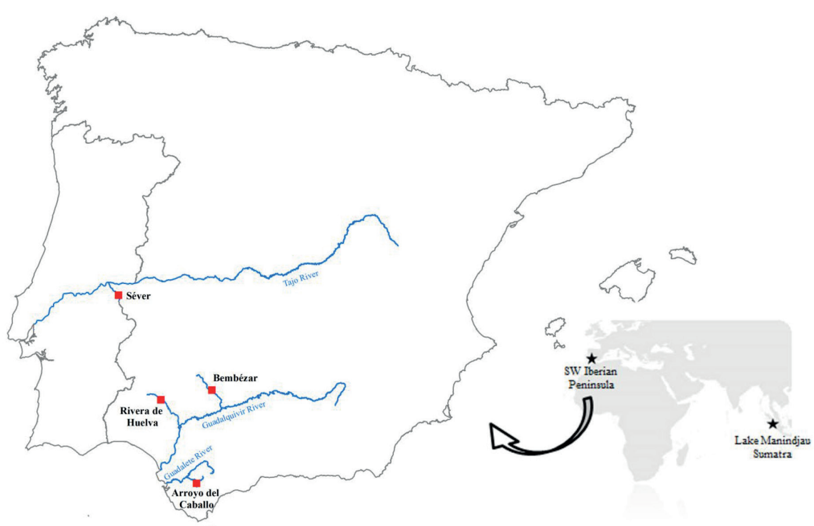

Figure 1. Map showing the new localities for *U. spongophila* (red squares): the Arroyo del Caballo, Rivera de Huelva, Bembézar and Sever streams. The global distribution, with type locality, Lake Manindjau (Sumatra, Malaysia), and the new Iberian localities are depicted in the adjacent box. *Mapa con las nuevas localidades de* U. spongophila *(cuadrados rojos): Arroyo del Caballo, Rivera de Huelva, río Bembézar y río Sever. La distribución global, con la localidad tipo, el lago Manindjau (Sumatra, Malaysia) y las nuevas localidades ibéricas se muestran en el recuadro adjunto.*

Figure 2. Sampling sites. From left to right and from top to bottom: the Arroyo del Caballo, Rivera de Huelva, Bembézar and Sever streams. *Localidades de muestreo. De izquierda a derecha y de arriba abajo: Arroyo del Caballo, Rivera de Huelva, río Bembézar y río Sever.*

(Moreno et al., 2012, 2013).

In this study, we address a taxonomic review of *U. spongophila* that includes new information about morphological characters and its ecological preferences in the Iberian Peninsula. The description of the studied specimens of this species is discussed, along with the biogeographical relevance of the findings, some biological aspects and its conservation status and interest.

MATERIALS AND METHODS

Macrophyte and macroinvertebrate samples were taken during national surveys in the spring-summer of 2018 and 2019 to assess the ecological status of Spanish rivers according to the WFD. The 2018 survey covered 210 sites in the Guadalquivir River basin (south Spain). The 2019 survey covered 217 sites scattered throughout the Iberian Peninsula. The surveyed stream reaches were 100 m long. Aquatic macrophytes were collected by hand and fixed in the field with Kew liquid (65 % ethanol, 30 % water, 5 % glycerol). Benthic macroinvertebrates (including sponges) were preserved in 70 % ethanol.

After the first specimen was detected by chance in 2018 while identifying freshwater sponges from macroinvertebrate samples, we examined all the collected sponges from the 2018 and 2019 surveys to check its presence. Thus, we found the alga only in four streams (positive sites) out of 17 sites where *E. fluviatilis* occurred: Arroyo del Caballo, Rivera de Huelva, Bembézar and Sever (Figs. 1 and 2).

The *U. spongophila* samples were collected under summer base-flow conditions. They were growing inside the freshwater sponge specimens which were, in turn, attached to boulders or cobbles (Fig. 3 A, B). Sponges were abundant in the four stream reaches, with density higher than 1 ind/m^2 in all cases. Several small sponge fragments (2 cm^2 at the most) were collected from each site where the sponge was present. Small aliquots were examined under an inverted microscope Leica DMIRB (Leica Microsystems, Wetzlar GmbH, Germany) from ×10 to ×1000 magnification. Specimens were photographed and measured using the Leica image LAS v4.13 software and preserved in Kew liquid in plastic vials. The sponge specimens were identified by microscopic observations of siliceous macroscleras and gemoscleras (Fig. 3 C, D). The examined material is deposited in the Centro de Estudios Hidrográficos CEDEX, Área de Medio Ambiente Hídrico (Address: Pº Bajo de la Virgen del Puerto 3, 28005, Madrid, Spain).

For the environmental characterisation of sites, physico-chemical parameters (dissolved oxygen, Ph, temperature and conductivity) were determined by a YSI ProPlus multiparameter (YSI Inc., Yellow Springs, OH, USA) during the 2018 survey, and by a Hach HQ40d (Hach Lange, Loveland, CO, USA) during the 2019 survey. The nutrient data (NO_3 and PO_4) for the 2018 survey were retrieved from the Guadalquivir River Basin authority online platform (Analysed by ionic chromatography and available at: https://idechg.chguadalquivir.es/nodo/Redes/index.html). For the2019 survey, water samples were taken, and nutrients were analysed by ionic chromatography. Riparian plant communities were visually recorded in each stream reach.

Finally, an ordination based on environmental data (Principal Components Analyses, PCA) of the 17 sites where sponges were present was performed with the PRIMER v.6 software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK).

RESULTS

Taxonomical changes

The classification and names of the studied species have changed since its first description (Ta-

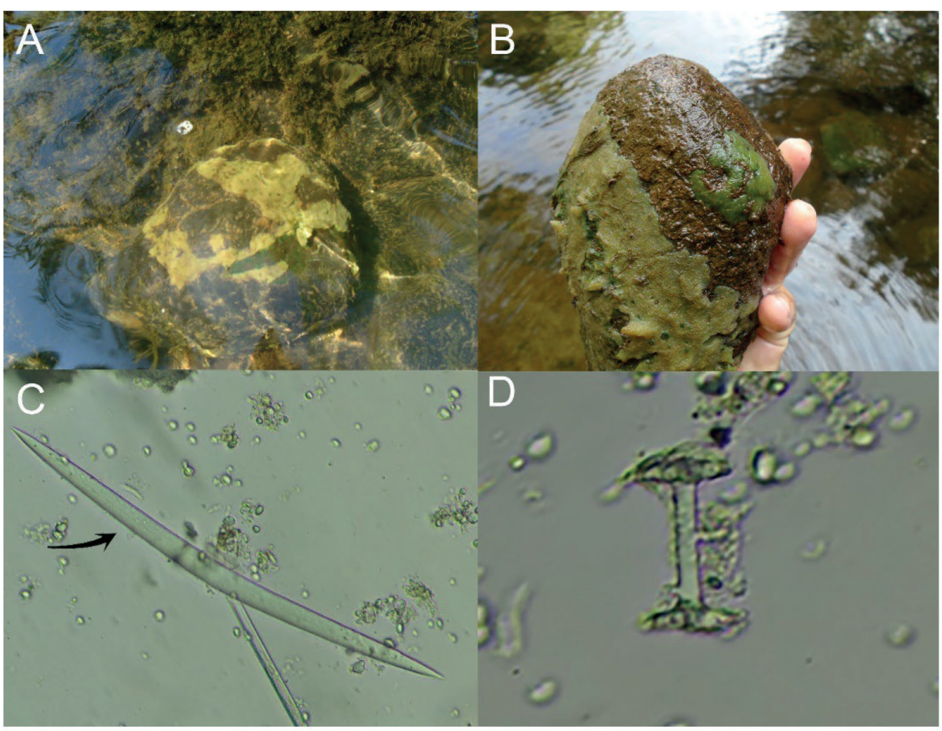

Figure 3. (A, B): *E. fluviatilis* encrusting stones in the Rivera de Huelva stream. Different sponge coloration (light green, bright green) is distinguished. (C, D): macrosclera (arrow) (C) and gemosclera (D) of *E. fluviatilis* from the Bembézar stream. *(A, B):* E. fluviatilis *sobre piedras en el arroyo Rivera de Huelva. Se pueden distinguir diferentes coloraciones de la esponja (verde claro y verde brillante). (C, D): macrosclera (flecha) (C) y gemosclera (D) de* E. fluviatilis *del arroyo Bembézar.*

Table 1. Changes in the taxonomic classification of *U. spongophila*. *Cambios en la clasificación taxonómica de* U. spongophila.

Name	Author	Reference	Synonymised taxa	Location
Trentepohlia spongophila	Weber-van Bosse	Weber-van Bosse (1890)	None	Lake Manindjau, Sumatra
Stereococcus spongophilus	(Weber v. Bosse)	Wille (1911)	*Cladophora spongophila* Koorders*	Java**
Endoderma spongophila	(Web. van Bosse) Printz	Printz (1964)	*Trentepohlia spongophila* Web. van Bosse; *Cladophora spongophila* Web. van Bosse*; *Gongrosira spongophila* (Web. van Bosse) Wille*	Sumatra
Entocladia spongophila	(Web. van Bos.) Starmach	Moshkova & Gollerbach (1986)	*Endoderma spongophila* (Web. van Bos.) Printz; *Cladophora spongophila* Web. van Bos.*; *Gongrosira spongophila* (Web. van Bos.) Wille*	Sumatra
Ulvella spongophila	(Weber-van Bosse) Škaloud & Leliaert	Škaloud et al. (2018)	*Trentepohlia spongophila* Weber-van Bosse	Manindjau, Malaysia

Synonyms marked with * indicate unknown name origin; possible mistakes. **Wille means Sumatra.

Table 2. Environmental and psychoo-chemical characteristics of the studied stream sites with *U. spongophila* in Spain. *Características ambientales y físico-químicas de las localidades estudiadas con presencia de* U. spongophila *en España.*

Stream name	Arroyo del Caballo	Rivera de Huelva	Bembézar	Sever
Coordinates (UTM)	H30 XUTM: 263052 YUTM: 4053482	H30 XUTM: 201123 YUTM: 4197620	H30 XUTM: 289250 YUTM: 4214500	H30 XUTM: 128010 YUTM: 4378453
Collection date	10/07/2019	26/06/2018	27/06/2018	13/06/2019
National WFD river type	R-T07 (Low altitude mineralised Mediterranean streams)	R-T08 (Low mountain siliceous Mediterranean rivers)	R-T08 (Low mountain siliceous Mediterranean rivers)	R-T08 (Low mountain siliceous Mediterranean rivers)
Altitude (m a.s.l.)	107	282	214	283
Flow regime	Permanent, seasonal, slow-flowing	Temporary (summer dry)	Temporary (summer dry)	Permanent, seasonal, slow-flowing
Average depth (cm)	15	30	10	20
Lithology	calcareous	siliceous	siliceous	siliceous
Conductivity (µS/cm)	2807	221	325	141
Oxygen saturation (%)	64	85	97	96
Temperature (ºC)	20	17	25	19
pH	7	7.5	7.8	7.5
NO_3 (mg/l)	<2.5	3.53	0.54	4.14
PO_4 (mg/l)	<0.2	0.21	0.46	<0.2
Dominant substrate	Boulders and sand	Boulders and sand	Boulders and sand	Boulders, cobbles and sand
Anthropogenic impacts	Low. Livestock	High. Hydromorphological (regulated river downstream of a reservoir)	High. Hydromorphological (regulated river downstream of a reservoir)	Low. Riparian clearing (recreational area)

ble 1). *U. spongophila* was originally described as *Trentepholia spongophilla* (Weber-van Bosse, 1890). Later, Wille (1911) included the following comment within the framework of the genus *Stereococcus* Kütz description: "The alga *Trentepohlia spongophila* Web. V. Bosse (= *Cladophora spongophila* Koorders) found in symbiosis on the sponge *E. fluviatilis* in Java could belong to the genus *Stereococcus* Kütz, and it should be called *S. spongophilus* (Weber v. Bosse)". The full name of this taxon is currently listed in Algaebase as *Stereococcus spongophilus* (A. A. Weber Bosse) J. N. F. Wille (Guiry & Guiry, 2021). In 1964, it was redescribed and moved to the genus *Endoderma* (Printz, 1964). The species was renamed as *Endoderma spongophila* (Weber Van Bosse) Printz, among other epi- or endophytic and endozoic green algae. In a more recent publication (Moshkova & Gollerbach, 1986), the species was renamed again as *Entocladia spongophila* (Web. Van Bos.) Starmach. Finally, the species was described in the book Süßwasserflora von Mitteleuropa (Ulvophyceae) (Škaloud et al. 2018) as *U. spongophila* (Weber-Van Bosse) Škaloud & Leliaert.

Table 3. Flora accompanying *U. spongophila* at the study sites, including macroscopic algae and coenobial cyanobacteria, bryophytes and vascular plants. *Flora acompañante de* U. spongophila *en las localidades estudiadas, incluyendo algas macroscópicas, cianobacterias cenobiales, briófitas y plantas vasculares.*

	Arroyo del Caballo stream	Rivera de Huelva stream	Bembézar stream	Sever stream
Riparian flora	*Populus alba* L., *Fraxinus angustifolia* Vahl, *Nerium oleander* L., *Rubus ulmifolius* Schott	*Fraxinus angustifolia, Salix spp.* Host, *Nerium oleander, Flueggea tinctoria* (L.) G.L.Webster	*Fraxinus angustifolia, Salix* sp., *Tamarix* sp. L., *Flueggea tinctoria, Nerium oleander*	*Alnus glutinosa* (L.) Gaertn., *Fraxinus angustifolia, Populus deltoides* W.Bartram ex Marshall, *Ficus carica* L., *Rubus ulmifolius, Sambucus nigra* L.
Aquatic flora	*Cladophora* sp. Kützing, *Spirogyra* sp. Link, *Zygnema* sp. C. Aghard, *Oedogonium* sp. Link ex Hirn, *Audouinella* sp. Bory, *Apium nodiflorum* (L.) *Veronica anagallis-aquatica* L.	*Cladophora* sp., *Stigeoclonium* sp. Kützing, *Spirogyra* sp., *Oedogonium* sp., *Vaucheria* sp. A.P.de Candolle, *Paralemanea* sp. (P.C.Silva) M.L.Vis & Sheath, *Audouinella* sp., *Phormidium* sp. Kützing ex Gomont, *Lyngbya* sp. C.Agardh ex Gomont, *Leptolyngbya* sp. Anagnostidis & Komárek, *Melosira varians* C.Agardh, *Apium nodiflorum, Myriophyllum spicatum* L., *Rorippa nasturtium-aquaticum* (L.), *Veronica anagallis-aquatica, Ranunculus penicillatus* (Dumort.), *Rhynchostegium riparioides* (Hedw.) Cardot., *Leptodictyum riparium* Warnstorf, *Fissidens crassipes* Wilson ex Bruch & W.P. Schimper, *Fissidens fontanus* (Bach.Pyl.) Steud., *Fontinalis hypnoides* C.J. Hartman, *Cinclidotus fontinaloides* (Hedw.) P.Beauv.	*Cladophora* sp., *Rhizoclonium* sp., *Spirogyra* sp., *Paralemanea* sp., *Cylindrospermum* sp. Kützing ex É.Bornet & C.Flahault, *Nostoc* sp. Vaucher ex Bornet & Flahault, *Apium nodiflorum*, *Rorippa nasturtium-aquaticum*, *Veronica anagallis-aquatica*, *Ranunculus peltatus* Moench	*Cladophora* sp., *Spirogyra* sp., *Gongrosira* sp. Kützing, *Heribaudiella fluviatilis* (Areschoug) Svedelius, *Phormidium* sp., *Melosira varians, Apium nodiflorum, Veronica anagallis-aquatica, Ranunculus penicillatus, Myriophyllum spicatum, Rorippa nasturtium-aquaticum, Rhynchostegium riparioides, Leptodictyum riparium, Fissidens fontanus, Fontinalis hypnoides*

Sites and habitat

The *U. spongophila* specimens were found in four stream reaches in south Spain (Figs. 1 and 2) between 2018 and 2019: the Arroyo del Caballo, Rivera de Huelva, Bembézar and Sever streams. The environmental characteristics of the study sites are shown in Table 2, and a list of the accompanying aquatic and riparian flora is provided in Table 3. Streams are small, at low altitude, seasonal or temporal (summer dry), and are located in mountainous areas. Three streams have low conductivity values (siliceous streams), while one is a calcareous stream with high conductivity. The species thrives within a wide range of trophic conditions (0.54-4.14 mg/l NO_3; < 0.2-0.6 mg/l PO_4), mostly at low to moderate nutrient concentrations. Two of the sites are located in downstream reservoirs, whereas the other two support low anthropogenic pressures.

The streams show typical SW Mediterranean riparian forest. They harbour a rich com-

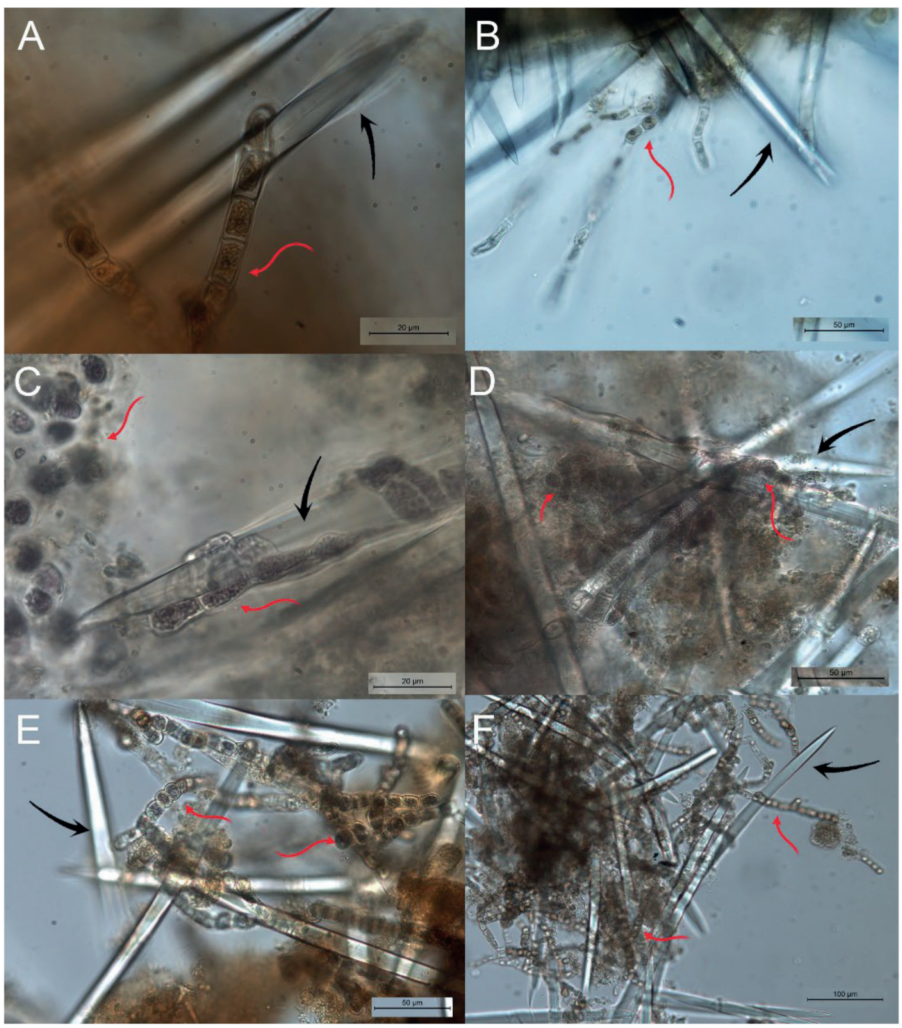

Figure 4. Studied *U. spongophila* specimens on different sampling sites: A) and B) the Rivera de Huelva stream; C) and D) the Arroyo del Caballo stream; E) and F) the Bembézar stream. Black arrows: sponge spicules; red arrows: algae. *Especímenes estudiados de* U. spongophila *de diferentes localidades de muestreo: A) y B) Rivera de Huelva; C) y D) Arroyo del Caballo; E) and F) río Bembézar. Flechas negras: espículas de esponja; flechas rojas: alga.*

munity of aquatic vegetation that accompanies the sponge-algae association, the commonest of which include filamentous green algae, some rhodophytes, diatom masses, coenobial cyanobacteria, as well as bryophytes and phanerogams (Table 3).

Morphological description

The endozoic thallus consisted of small green, irregularly branched filaments, creeping, surrounding or attached to macroscleras, sometimes erected, sometimes forming parenchymatous aggregations (Figs. 4 and 5). Cells were barrel-shaped to cylindrical, or more irregular, sometimes swollen. The length of filaments, number of cells per filament or branches, and shape and size of cells were widely variable. Size ranged from 4.0 µm to 14.6 µm wide, and from 7.3 to 38.4 µm long, ranging from 0.8 to 6-fold longer than wide.

The studied Iberian populations were similar in morphology terms, but with some variability among them: the Sever and Arroyo del Caballo specimens presented longer and narrower cells, with apical ones being the longest (e.g. 38.4 µm long x 3.2 µm wide). In contrast, the Bembézar and Rivera de Huelva specimens had more isodiametric cells that were either wider than longer (e.g. 8.3 µm long x 9.7 µm wide) or longer than wider (e.g. 15.5 µm long x 6.2 µm wide), and with shorter apical cells. Overall, we found wide morphological variability, even within specimens or filaments of the same population. Cells had a single parietal chloroplast, with or without pyrenoids. Akinetes were observed in one specimen (Bembézar stream) to form rows of up to 15, sometimes branched (Fig. 6).

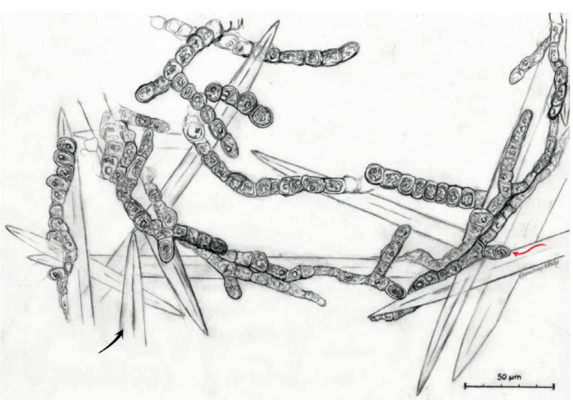

Figure 5. *U. spongophila* growing on spicules inside the sponge *E. fluviatilis* in the studied SW Spanish streams. Black arrow: sponge spicules; red arrow: algae. U. spongophila *creciendo sobre las espículas de la esponja* E. fluviatilis *en los arroyos estudiados del SO de España. Flecha negra: espículas de esponja; flecha roja: alga.*

DISCUSSION

Although we examined all the freshwater sponges collected during each survey in Iberian streams since our first finding in 2018, only four cases were positive, and were always in association with the same sponge species: *E. fluviatilis*. To compare the environmental conditions that could determine the presence of the alga (4 positive sites for *Ulvella*) in relation to the other sites where sponges without the symbiotic alga (13 negative sites for *Ulvella*), we performed a PCA (Fig. 7; Table 5). The first two axes (PC1, PC2) accounted for 57.2 % of variation, with PH and altitude being the main variables to define the PC1 gradient. Dissolved oxygen and the PO4 concentration were the main variables that correlated with PC2. The position of the sites with *Ulvella* present, Bembezar and Caballo streams, were clearly separated on the right side of the ordination (higher PH values and altitude) from the sites without *Ulvella*. The Sever and Huelva streams were located in the centre of ordination and showed similar environmental conditions to the other sites without *Ulvella*. However, the ecological conditions of the four streams where the species was found allowed us to make some generalisations. They were located in SW Spain, 1st to 2nd order (Strahler, 1952), at low altitude (< 300 m), and were high seasonal or temporary Mediterranean streams flowing over siliceous metamorphic rocks, except for one calcareous stream. Thus, they are low-mineralised streams (conductivity ranging from 141 to 325 µS/cm, except for the calcareous stream with > 2000 µS/cm) with relatively low nutrient contents.

Some sponge specimens were bright green despite not being exposed directly to sunlight (they were frequently located on the underside

Figure 6. Branched rows of the *U. spongophila* akinetes from the Bembézar stream. Black arrows: sponge spicules; red arrow: algae. *Filas ramificadas de acinetos de* U. spongophila *en el río Bembézar. Flechas negras: espículas de esponja; flecha roja: alga.*

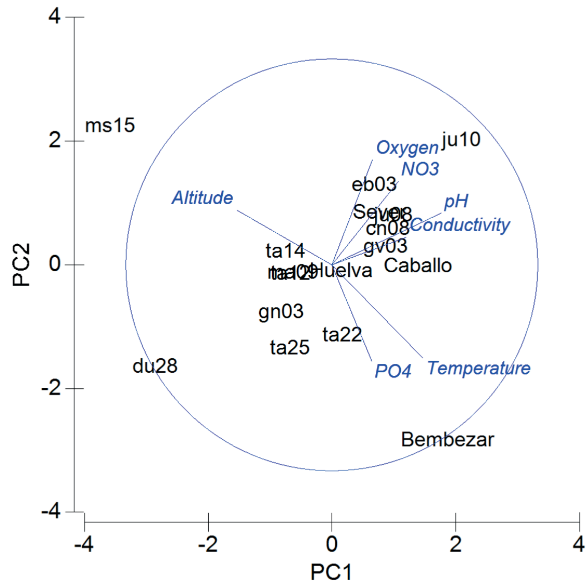

Figure 7. Ordination of sites in the first two axes (PC1, PC2) of the Principal Components Analyses (PCA) based on environmental data. *Ordenación de las estaciones en los dos primeros ejes (PC1, PC2) del Análisis de Componentes Principales (ACP) basado en datos ambientales.*

of boulders or in slightly exposed bedrock cornices). In other cases, sponge colour was brownish. We did not observe any correlation between green sponge pigmentation and the presence or abundance of *U. spongophila*. Thus, colour might be more related to the quantity of endocellular zoochlorellae that green sponges harbour. As Gaino et al. (2003) pointed out, the acquisition of zoochlorellae from the environment increases from spring onwards with the progressive pigmentation of brownish sponges.

A comparative analysis of the available morphological data (Table 4) revealed that the four studied specimens matched not only the original description by Weber-van Bosse (1890), but also the subsequent redescriptions by Printz (1964), Moshkova and Gollerbach (1986) and Škaloud et al. (2018). Our cell size, shape, length and width measurements were in accordance with these previous morphological descriptions, although we found some wider cells. The presence of pyrenoids, clearly observed in some of our filaments and whose absence seemed characteristic in the species' original description, should not be considered strange as their presence is a characteristic of the genus *Ulvella* (Nielsen et al., 2013). Moreover, the species descriptions were always based on the specimen found in Malaysia, where perhaps pyrenoids were either absent or not clearly observed. Although microfilamentous green algae in the Ulvellaceae are not easy to identify (Nielsen et al., 2013), we believe that the species' endozoic character, which has always been associated with the siliceous spicules of *E. fluviatilis*, along with the morphological description, clearly evidence the identity of the observed algae as *U. spongophila*.

As far as we know, and in view of the available descriptions (Table 4), we interpret that no other record of this species has been published since the original description back in 1890, and the four later published descriptions are slight modifications of the detailed original description by A. Weber-van Bosse (1890). There is no evidence that the later published descriptions came from newly collected material (no sample dates, no herbarium references), and the published drawings are the same as the originals. In this case, our records would be the second world record of *U. spongophila* after more than one century since its first collection, and are the first report for Spain,

Table 4. Morphological description of the studied U. spongophila specimens compared to the four previously published descriptions, all based on the specimens of the type locality "Lake Manindjau" (Sumatra, Malaysia). *Descripción morfológica de los especímenes estudiados de U. spongophila, comparada con las cuatro descripciones publicadas de la especie, todas basadas en los especímenes de la localidad tipo "Lago Manindjau" (Sumatra, Malaysia).*

	Growth form	Vegetative cell shape	Vegetative cell size	Intracellular elements	Reproduction
This study, Spain	Endozoic. Filaments irregularly branched, sometimes parenchymatous aggregations	Apical cells: conical, sometimes longer than middle cells Middle cells: isodiametric to cylindrical cells, occasionally swollen	4-14.6 µm wide and 7.3-38 µm long	A single parietal chloroplast with or without one pyrenoid	Intercalary rounded akinetes in rows of up to 15 akinetes, sometimes branched
Weber-van Bosse (1890)	Filaments constructed by isodiametric cells and irregularly branched filaments. Creeping in symbiosis on sponge spicules from *Ephydatia fluviatilis*.	Young cells long and thin, sometimes branched; other isodiametric	5.5 µm wide and up to 85 µmm long	Cells with green content and a big chromatophore, one nucleus	Reproductive cells, 9 µm long and 7.2 µm wide. Biflagellate zoospores formed in sporangia (around 12 per sporangium). Vegetative multiplication by separating cells after cell membrane gelification.
Printz (1964)	Macroscopic green spots in *Ephydatia fluviatilis*. Creeping irregularly branched green threads usually growing along spicules or forming parenchymatous cell clusters.	Cylindrical or slightly swollen	5.5-6.4 µm wide and up to 85 µm long	Chromatophore with no pyrenoid. Cells with one nucleus.	When gametes form, cells divide into almost isodiametric 7-9 µm broad sporangia via transverse walls, where ovoid biflagellate zoospores form (formed in intercalary or terminal sporangia, usually 4 to 6 rows). Vegetative propagation by separating individual cells.
Moshkova, & Gollerbach, (1986)	Over Ephydatia fluviatilis. Filaments randomly branched over substrate; in places they are closely in contact with forming single-layer plates	Cylindrical cells, rarely slightly swollen	5.5 to 6.4 µm wide, the same in length or up to 85 µm long	Chloroplast with no pyrenoid	Sporangia 7-9 µm wide, same length, intercalary or terminal, often 4-6 in a row, with a number of zoospores (or gametes?) released by an opening in the wall cell; the posterior developing ones not tracked. Vegetative propagation by fragmentation of thallus, unicellular or pluricellular fragments.
Škaloud et al. (2018)	Thallus endophytic, formed by irregularly branched filaments, sometimes parenchymatous.	Cylindrical or slightly swollen	5.5-6.4 µm wide and up to 85 µm long	A single parietal chloroplast and no pyrenoids	Observed asexual reproduction include ovoid biflagellate zoospores (formed in intercalary or terminal sporangia, usually in rows) and the formation of akinetes.

Table 5. Principal Components Analyses results (PCA). Eigenvalues, % of explained variation and eigenvectors (correlation coefficients of the environmental variables with the first two axes of the PCA). *Análisis de Componentes Principales (ACP). Autovalores, % de variación explicada y autovectores (coeficientes de correlación de las variables ambientales con los dos primeros ejes del ACP).*

	PC1	PC2
Eigenvalues	2.31	1.69
%Variation	33	24.2
Cum. % Variation	33	57.2
Eigenvectors		
Variable	PC1	PC2
Altitude	-0.462	0.266
Conductivity	0.36	0.134
Oxygen	0.198	0.512
Temperature	0.441	-0.452
pH	0.531	0.251
NO_3	0.323	0.405
PO_4	0.194	-0.467

Portugal (the Sever stream site is located precisely on the Spanish-Portuguese border), Europe, and even for the whole Palaearctic region. Therefore, this species apparently shows a disjunct geographical distribution between SW Europe and SE Asia. Nevertheless, the endozoic growth form of *U. spongophila* could have led the species to be overlooked because of the difficulty in detecting it, in which case, the species would show a more widespread distribution.

Climate change can make the Iberian peninsula more prone to species range expansions from Tropical climate regions, as previously hypothesised in other freshwater cyanobacteria like *Nostochopsis lobata* (Moreno et al., 2012), and as could be the case of the chlorophyte *Tetrasporidium javanicum* (Moreno et al., 2013).

In relation to the species name *Stereococcus spongophilus* proposed by Wille (1911), we noticed that the given geographical location in Java was a mistake (instead of Sumatra) because the author referred to the original Weber-van Bosse 1890 publication (Table 1). However, we think that the presence of *Stereococcus spongophilus* on the *AlgaeBase* platform (therein listed as ‘P’, which indicates a preliminary *AlgaeBase* entry that has yet not been verified), and considered a different species from *U. spongophila*, would appear be another error because *Stereococcus spongophilus* would be another synonym for *U. spongophila*. To our knowledge, up to seven different names for the same species, from the same locality (Sumatra) and from the same original description by Weber-van Bosse (1890) have been proposed (Table 1). They should all be treated as synonyms of *U. spongophila*.

As for species conservation status, the type locality Lake Manindjau (Sumatra) has undergone major alterations since the late 19th century and is currently a severely impacted ecosystem. Anthropogenic pressure, such as intensive *Tilapia* cage-aquaculture, organic waste pollution, heavy metal pollution, hydroelectric power generation, etc. (Nurdin et al., 2020), along with the changes in climate that have occurred in the last 150 years (with a marked change from a warm to a humid climate around 1868; Widyani et al., 2020), could have led to the species’ local extinction. This could have made the newly discovered Iberian *U. spongophila* populations the only remnants of this interesting species. We believe that new sampling efforts are needed to confirm the presence of the species in the type locality, or in other Mediterranean and Tropical freshwater lakes and streams.

On the nature of the association with *E. fluviatilis*, as the existence of endocellular zoochlorellae is widely documented in different freshwater sponges (Wilkinson, 1980; Sand-Jensen & Pedersen, 1994; Hall et al., 2021), we can hypothesise a similar symbiotic relation between *E. fluviatilis* and *U. spongophila*. This species’ sciophilous character, and the fact that it even grows under stones, can be explained if sponge spicules function as optical fibres by transmitting light to algal cells. This adaptation to low-light environments has been proposed for the symbiotic association between the marine sponge *Theytia seichellensis* and the photobiont *Ostrebium* sp. by Gaino and Sara (1994). Later Cattaneo-Vietti et al. (1996), Aizenberg et al. (2004) and Müller et al. (2006) demonstrated the light transmission capacity of the spicules of *Euplectella aspergillum* and *Hyalonema sieboldi*. Nevertheless, we cannot rule out other types of relations, such as pathogenic, which have been observed in some related Ulvaceae and corals in marine ecosystems (Goldberg et al., 1984). Our observations reveal that algal filaments grow using sponge spicules as a mere physical substrate, which does not support the last hypothesis.

CONCLUSIONS

U. spongophila has been recorded in four streams of the Iberian Peninsula in symbiosis with the sponge *E. fluviatilis*. After reviewing the available publications on this species, we found seven synonyms for it, and can conclude that Iberian populations are the second world record after only one previous collection at Lake Maninjau, Malaysia, in 1890 by Webber van Bosse. The species is probably more widespread, but its endozoic nature could have been overlooked for more than one century. We collected *U. spongophila* in lotic ecosystems (streams) whereas the previous collection took place in a lentic ecosystem (a lake). Iberian specimens were morphologically similar to the Malaysian specimens, although we observed other characters not previously mentioned in descriptions, such as chloroplasts with pyrenoids or long rows of branched akinetes.

More sampling effort, along with further molecular studies, will help to confirm the identity of this species and to make more conclusive assertions about its habitat preferences or global rarity. New fresh samples from both sites (Malaysia and Spain) will allow species comparisons to be made from the morphological and genetic points of view. We encourage researchers studying both freshwater sponges and associated stream algae to collaborate and share their findings about the presence of this interesting species.

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