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RAPID COMMUNICATION

Note on important and novel findings

First report of the invasive freshwater sponge *Heterorotula multidentata* (Weltner, 1895) in Europa: a latent threat for aquatic ecosystems?

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Abstract
Freshwater invaders threaten both natural ecosystems and human activities. An invasive freshwater sponge *Heterorotula multidentata* (Weltner, 1895) has been found for the first time in continental waters of Europe (Spain). It is a species native to Australia and New Zealand, from which it spread to Japan, being considered invasive. The species has been found in water intake grids of irrigation and drinking water systems in the basins of the Guadalquivir and Tajo rivers, affecting their hydrological functions. It has also been found growing on the invasive mussel *Dreissena polymorpha* in the Guadalquivir River. To assess the risks associated with invasive alien organisms, a detailed knowledge of their taxonomic status and distribution is necessary. A morphological and molecular evaluation confirmed that the specimens found in Spain belong to the Japanese type. A full morphological description and the fouling problems caused by the species are also provided. It is not yet clear how *H. multidentata* arrived at Spain, but indirect transport by other invasive freshwater species cannot be ruled out either, as *H. multidentata* has been found fouling the invasive species zebra mussel.

Keywords Freshwater sponge · Zebra mussel · Invasive · Biofouling · Dispersal

Introduction

Biological invasions are a global issue arising from progressively interconnected world and increasing human activity (Pyšek et al. 2020). Invasive species alter habitats and affect ecosystem's function representing a significant risk for the environment and socio-economic activities (Katsanevakis et al. 2014). They are considered as the second most important cause of global biodiversity change after direct habitat destruction (CBD 2000).

Sponges are not considered important invaders on a global scale, and only a few invasive species have been reported up to now (Avila and Carballo 2009; Calcinai et al. 2004; Henkel and Janussen 2011; Matsuoka 2011; Pérez et al. 2006; Torii et al. 2023; Van Soest et al. 2007), including two that have moved between the Pacific and Atlantic basins (Carballo et al. 2013). Some of these species have been accidentally introduced likely due to human-mediated activities, such as aquaculture, trawling, and hull-fouling (Carballo et al. 2013). For example, the marine sponge *Hymeniacidon perlevis* (originally

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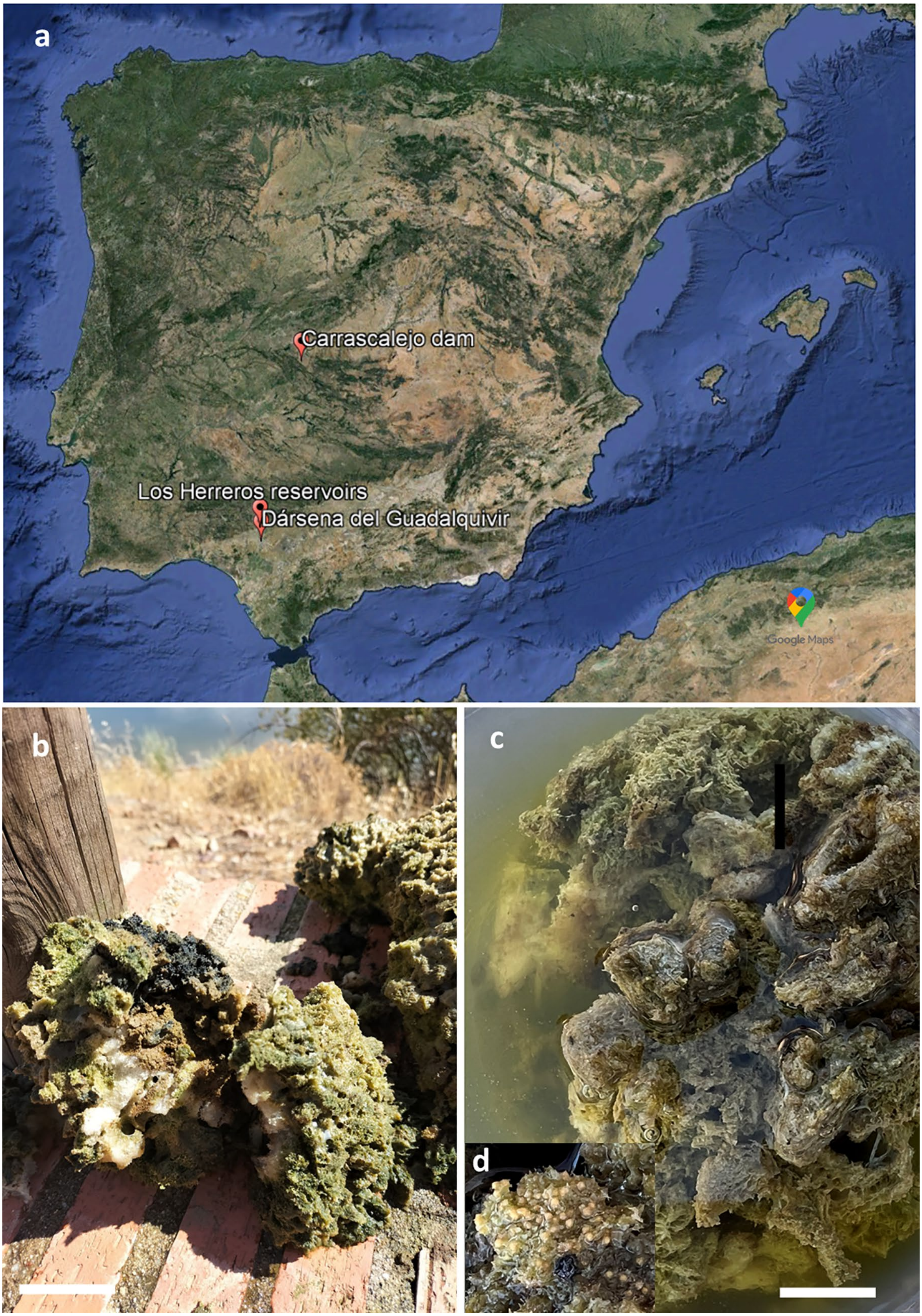
a
Carrascalejo dam
Los Herreros reservoirs
Dársena del Guadalquivir
Google Maps
b
c
d

◂**Fig. 1** **a** Location of the sampling stations: Carrascalejo dam, Herreros Pond and dársena del Guadalquivir. **b** Some of the sponges removed in the clean-up campaign on 17 August 2022 in Carrascalejo which were left on the shore of the reservoir. The sponges were found on 12 October 2022, completely dry and full of gemmules (scale bar 6 cm). **c** Sponges freshly removed in the clean-up campaign of 17 August 2022 (scale bar 3 cm). **d** The sponges show a high density of gemmules

described in England), is a globally distributed exotic species distributed by the East tropical Pacific, Asia, South America, Africa, and Europe (Fuller and Hughey 2013). Limited larval dispersal, historically documented range expansion, and low genetic variation all support the hypothesis that this species has achieved its extraordinary range via unintentional human intervention (Turner 2020). The calcareous sponge *Paraleucilla magna* was first described from Rio de Janeiro (Klautau et al. 2004), and after that, it has proliferated in the Atlantic and Mediterranean (Guardiola et al. 2016; Longo et al. 2007) mainly around shellfish farms such as *Mytilus* cultures (Guardiola et al. 2016), which points to shellfish fouling as the most likely introduction pathway (Henkel and Janussen 2011; Longo et al. 2007).

An increasing global awareness of the economic importance of invasive species has resulted in a recent effort to better understand the biology behind the problem. Therefore, taxonomic identification of introduced species is a pre-requisite of any meaningful study of their biology and ecology, including correct ecosystem management. Taxonomic uncertainties hinder a proper interpretation of biogeographical patterns and inference on the origin of the studied species (Geller et al. 1997).

Heterorotula multidentata (Weltner, 1895) is one of the most common spongillid species of eastern Australia (Racek 1969) and New Zealand (Rützler 1968). It was also reported in Japan as an alien species introduced by human activity (Ishijima et al. 2008; Masuda 2006; Matsuoka 2011). This species and other freshwater invasive species such as *Trochospongilla pennsylvanica* (Masuda 1998, 2006) have conspicuously increased their geographical range in Japan after the World War II (Sasaki 1939, 1941, 1969).

Here, we use morphological, ecological, and genetic data to show that *H. multidentata* is an invasive species from Asia, which is causing problems in hydraulic infrastructures related to irrigation and drinking water distribution in the basins of two of Spain's main rivers: the Guadalquivir and the Tajo.

A complete description of the species, and the fouling problems are provided. Hypotheses about possible dispersal mechanisms are also discussed.

Methods

Study sites and sample collection

Herreros Pond (Alcala de Río, Seville) located on the right bank of the Guadalquivir is one of the various artificial water bodies used by the Irrigation Community of the Viar to store water (Fig. 1a). The ponds have a surface of up to 20 ha and are connected by a network of channels to the irrigation areas. The water enters these reservoirs from the Guadalquivir basin by gravity through the Viar Irrigation Canal and leaves them by pumping. The infrastructure has a network of irrigation pipes with a total length of 950 km to supply water to some 2000 associated farmers, with an irrigable area of 12,000 ha. The water is sucked from the ponds, with a water intake of approximately 80 cm in diameter, at the end of which there is a perforated metal cylindrical filter, approximately 1.5 m in diameter and approximately 2 m long, with perforations of approximately 2 cm in diameter. This structure is suspended in mid-water supported by a floating platform.

Carrascalejo reservoir (Carrascalejo, Cáceres) is used to supply drinking water to several villages in their vicinity (Fig. 1a). It is fed by the Arroyo Recuerda stream which belongs to the Tajo River basin. It has a surface area of some 16,000 ha, and a water storage capacity of 46,000 m^3. The water is distributed to the villages by pumping through a general water intake of approximately 90 cm in diameter, at the end of which there is a cylindrical metal filter of similar characteristics to the one described in the previous case, but smaller, which is also suspended by a floating structure.

The Guadalquivir Canal is a branch of the river that was built to prevent the floods that traditionally inundated the city (Fig. 1a). It is about 13.5 km long, with a branch of the port of about 800 m. The port of Seville was left inside this canal, with the waterway through a lock located to the south that allows maritime traffic.

As a result of routine cleaning filter operations, we were informed by the company in charge of the cleaning of the presence of large masses of sponges. Therefore, we moved to the different areas, and the sponges were collected by scuba diving directly from the filters, photographed in situ if the visibility of the water allowed it, and preserved in absolute ethanol for their subsequent morphological and molecular study. All the material collected has been deposited in the Phylum Porifera collection of the Zoology Department in Seville University (CE-DZ-US).

Morphological analysis

We treated with spicular elements of *H. multidentata*, following to Carballo et al. (2018). Briefly: a small piece of sponge (or complete gemmule) was cut and placed into glass test tubes and nitric acid (70%) was dispensed into each one. The sample was boiled until the fragment was dissolved, and later, the acid was pipetted out of the tube and distilled water was added. Finally, the water was also pipetted out, and pure ethanol was added. For scanning electron microscopy (SEM), spicules isolated with the above method were mounted on the SEM stub with the help of double-sided carbon tape. Samples were sputter-coated with Au/Pd, and scanned and photographed using Analytical SEM (ZEISS EVO, Germany). Measurements for each type of spicule were made under SEM and are given in minimum-(average)-maximum length.

Molecular analyses

Total of metagenomic DNA was isolated from two ethanol preserved fragments of *H. multidentata*, following the steps described in Cruz-Barraza et al. (2017). For molecular analysis, we chose the rDNA ITS's region (Internal Transcribed Spacer) (ITS1-5.8-ITS2), owing to its high-evolving nature and its common use in freshwater sponge systematics (e.g., Carballo et al. 2021; Itskovich et al. 2006). For PCR experiments, purification, and sequencing, we followed the procedures described in Carballo et al. (2021). The obtained sequences were ensembled and edited in Codon Code Aligner 7.1.2 (Codon Code Corporation), confirming the species' identity through the BLAST tool (Search National Centre for Biotechnology Information/Blast). We aligned our sequences and some homologous sequences (downloaded from GenBank) using the CLUSTALW alignment in MEGA 10.2.6 (Kumar et al. 2018) under the default gap opening–gap extension parameters. As our intention was to establish the taxonomic identity of our samples and the relationship between individuals of *H. multidentata* in a molecular taxonomical approximation, we aligned our sequences with available sequences of this species and a few outgroup sequences from Malawispongidae, *Racekiela*, and *Ephydatia* (downloaded from GenBank), by their close phylogenetic relationship to *H. multidentata* evidenced in previously published phylogenies (Carballo et al. 2021; Gómez et al. 2019). We reconstructed a Neighbor-Joining (NJ) tree using the best-fitting DNA substitution model obtained by the same program: Kimura 2-parameter plus gamma distribution (K2+G). For the analysis, non-parametric bootstrap (1000 pseudo-replicates) was used to assess branch support. The sequences of the rDNA ITS's region of *H. multidentata* will be sent to Genk Bank once the manuscript is accepted.

Results

Systematic account of heterorotula multidentata

Phylum Porifera Grant, 1836.

Class Demospongiae Sollas, 1888.

Order Spongillida Manconi & Pronzato 2002

Family Spongillidae Gray, 1867.

Genus *Heterorotula* Penney & Racek 1968

Type species: *Heterorotula capewelli* (Bowerbank 1863).

Heterorotula multidentata (Weltner, 1895).

Material examined: Herreros Pond (37° 34′ 30.9″ N 6° 00′ 12.0″ W), September 30, 2021; three specimens (CE-DZ-US: 4; CE-DZ-US: 5; CE-DZ-US: 6). Carrascalejo reservoir (39° 38′ 47.3″ N 5° 11′ 27.3″ W), October 12, 2022; three specimens (CE-DZ-US: 7; CE-DZ-US: 8; CE-DZ-US: 9). Guadalquivir canal (37° 23′ 45.3″ N 6° 00′ 14.2″ W), July 13, 2023; three specimens (CE-DZ-US: 10; CE-DZ-US: 11; CE-DZ-US: 12).

Description: The species ranged from cushion-shaped patches of several cm in diameter and up to 1.2 cm thick, on the surfaces that keep the water intake afloat, to extensions of several square meter around the metallic filters, even covering them completely (Fig. 2a,b). It can even grow inside the filter. They can also be massive, often lobular, reaching sizes of up to 25 cm in diameter (Fig. 1b,c). It also has been found growing on the zebra mussel *Dreissena polymorpha* (Fig. 2c).

The oscula are simple, minute, dispersed in massive specimens. In cushion-shaped sponges, it is possible to see a system of exhalant canals, which are arranged radially around the oscula. Consistency varying from soft and fragile to rather hard but brittle. In the Carrascalejo reservoir, sponges have also been found on the stones that protect the reservoir walls from erosion. Usually dark yellow to tan in color (Fig. 1b,c), but also green in some encrusting specimens exposed to light.

Skeleton and spicules: The gemmuloscleres are birotulas of conspicuously unequal length 27-(37)-49×2.3-(3.0)-3.4 µm with rather slender shafts, sometimes bears stout conical teeth, with wide and flat rotules of unequal diameter; the inner larger 16.7-(18.08)-23.0 µm than the outer 15.3-(16.7)-17.9 µm (Fig. 3a–c). Faces of rotules granular; arrangement of granules radial; margin of rotules irregularly crenulated or incised.

Skeleton is an irregular meshwork of ascending tracts and ill-defined transverse fibers. Little amounts of spongin. The skeleton is formed by megascleres, which are curved slender fusiform oxea, ranging from entirely smooth to microspined; spines small and inconspicuous (Fig. 3d). Oxeas dimensions: 188-(250)-323×9-(10.7)-13.6 µm microscleres are absent.

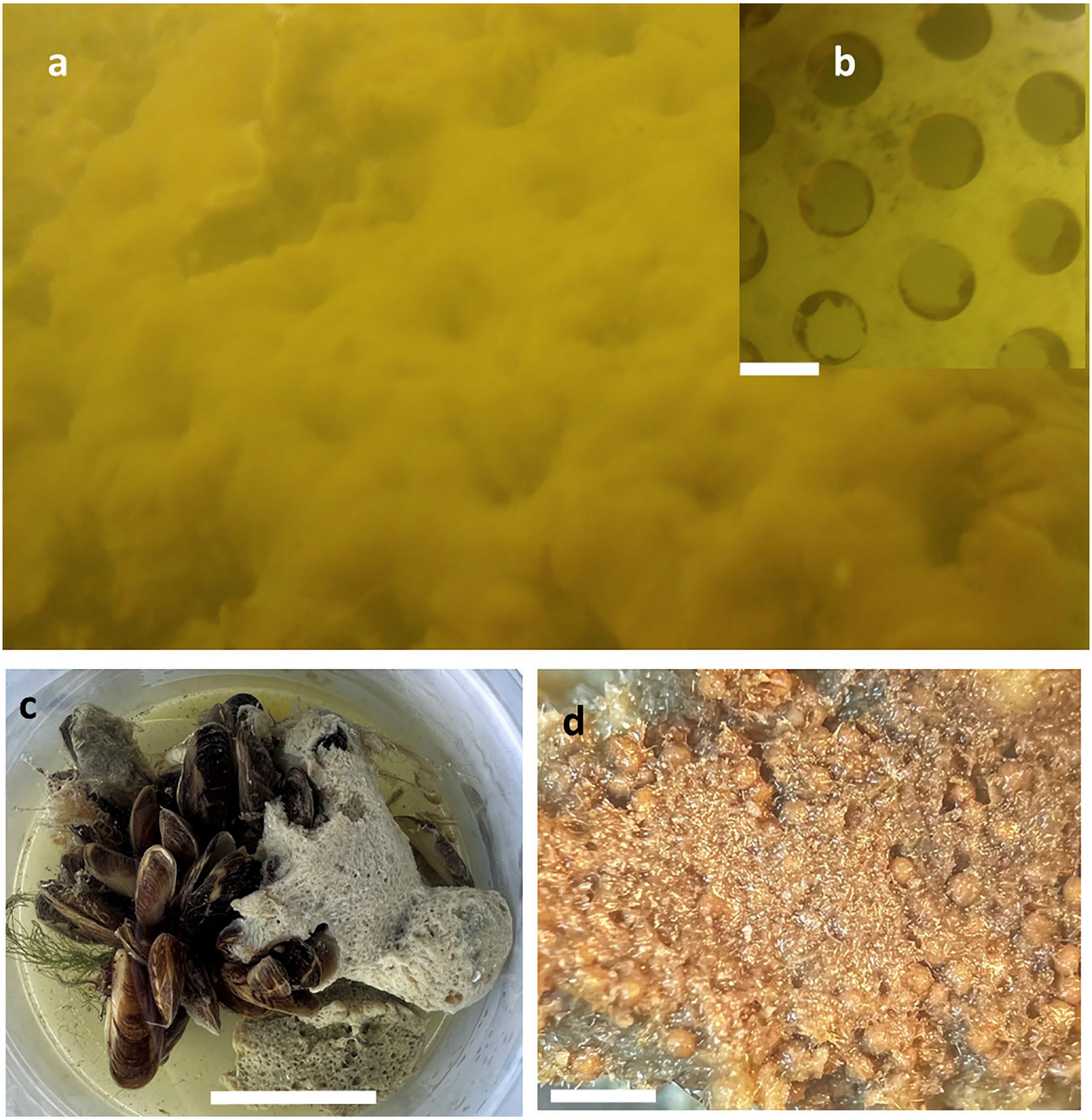

Fig. 2 **a** Filter of the water intake system of the Herreros Pond. It is completely covered by the sponge, which covers several m^2 of continuous surface (30 September 2021). **b** Detail of the filter once it has been cleaned (scale bar 1.5 cm). In the case of Herreros Pond, due to the size of the filter and the support structure, cleaning is carried out underwater by diving. **c** *H. multidentata* growing on zebra mussels in the dársena of Guadalquivir River (scale bar 2 cm). **d** Basal part of another specimen, where the enormous density of gemmules can be seen (scale bar 1.5 mm)

Gemmules: All specimens collected had many spherical gemmules, both scattered throughout skeletal meshwork in massive sponges (Fig. 4A), and at the base attached to the substrate in the encrusting (Fig. 2d). Foramen simple or bearing a very shallow peripheral collar, and slightly elevated (Fig. 4B). Diameter: 410-(493)-629 µm. The gemmuloscleres are radially embedded in the pneumatic layer (Fig. 4C,D). The inner larger rotules often interlocked with each other to form a close covering the inner gemmular membrane.

Molecular identification: The ribosomal region analyzed from our two sequenced samples had an identical nucleotide

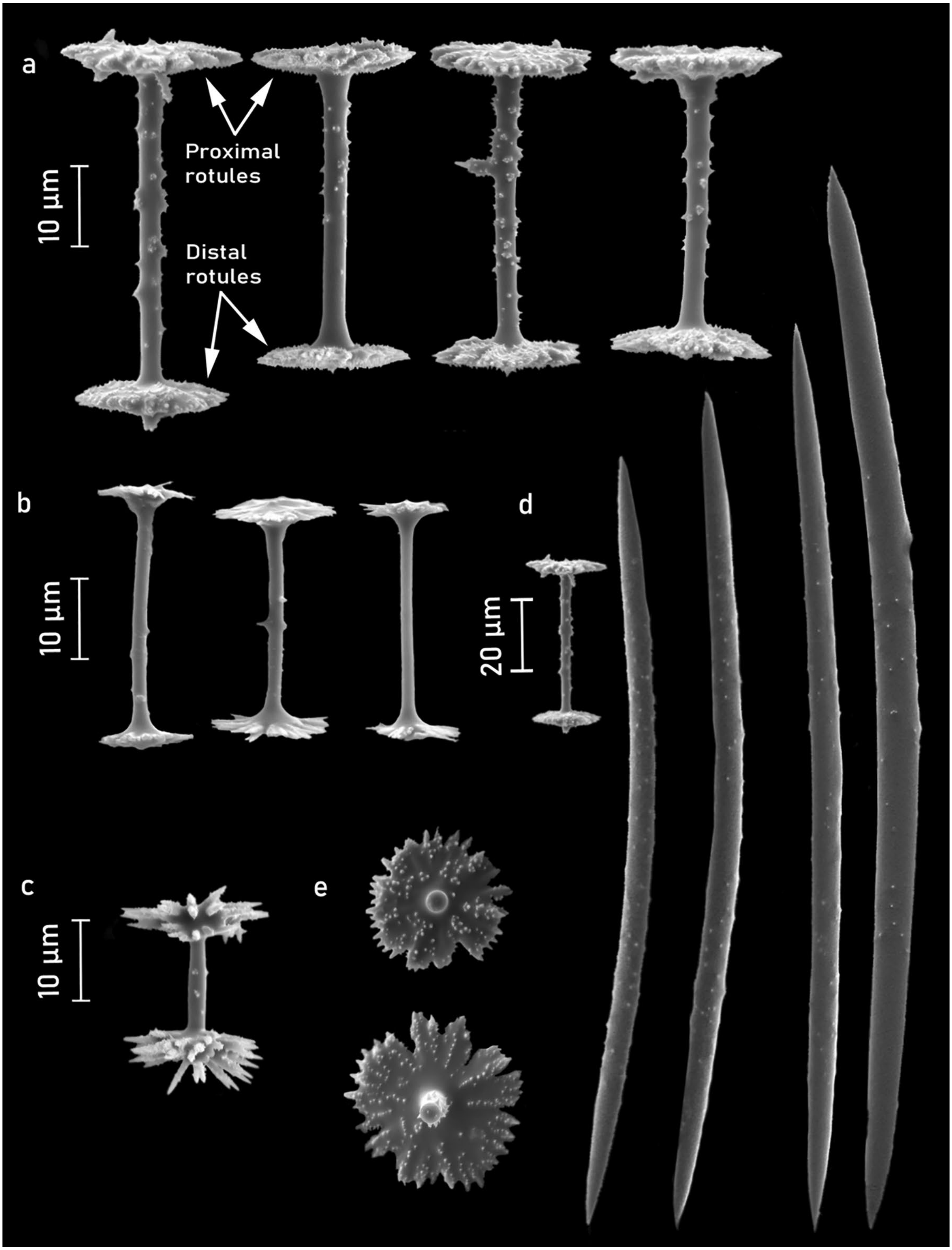

Fig. 3 Scanning electron microscopy images of spicules of *H. multidentata*. **a**, **b** Various shapes of birotule gemmuloscleres. **c** Unusual birotule. **d** Megascleres: acanthoxeas with few scattered small spines. **e** Detail of the rotules of gemmuloscleres

sequence, confirming that they belong to the same species. After Blast search (NCBI/Blast), our sequences were like *H. multidentata* (GenBank accession: EF151933) from Japan, confirming the molecular taxonomy identity of our samples. They were also quite like other sequences of *H. multidentata* (EF151941=99.85%, and AY662498=99.45%) of the same

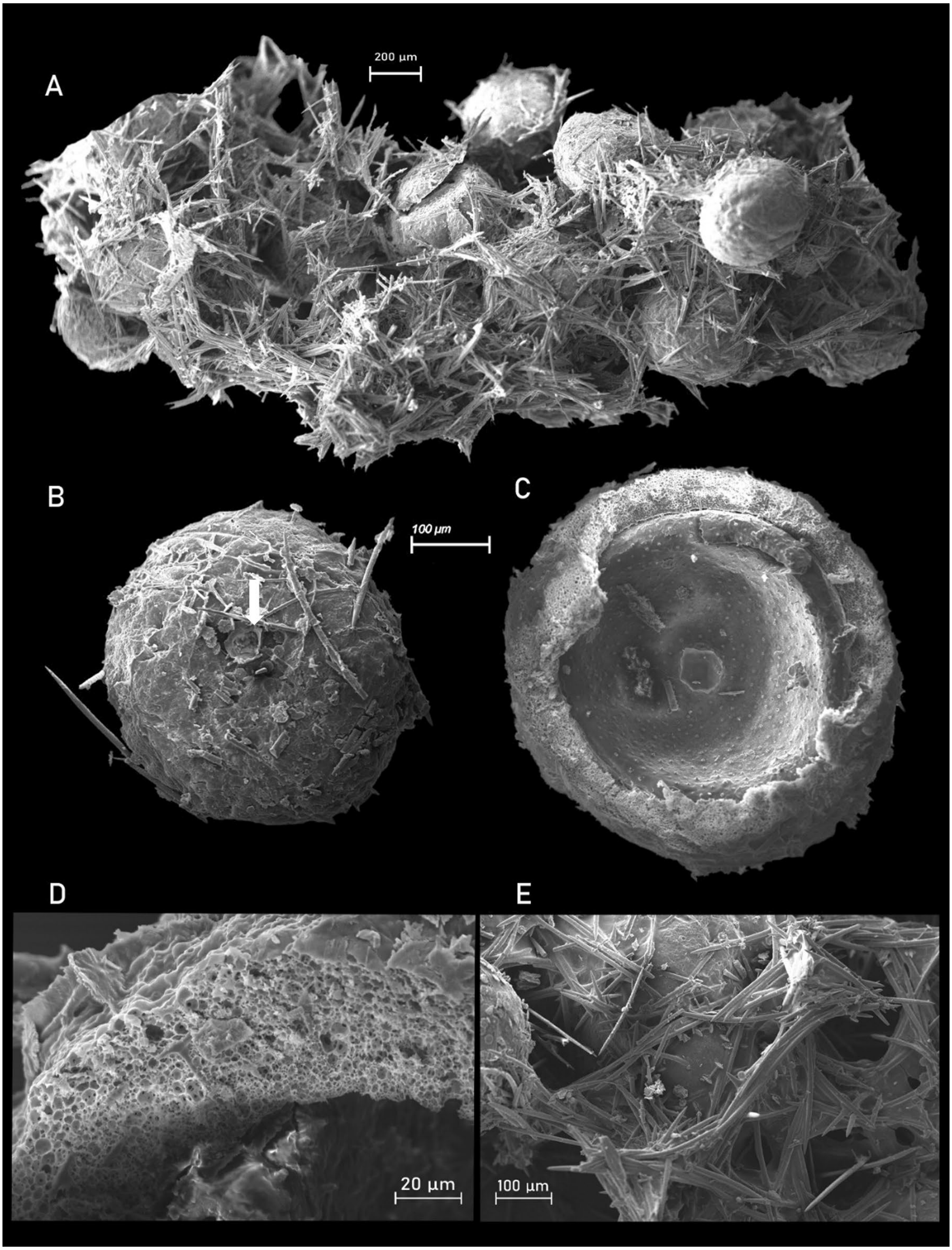

Fig. 4 SEM images of **A** Irregular skeletal network with abundant gemmules. **B** Gemmule. Outer layer of the theca surface armed by dense distal rotules of gemmuloscleres, with a few emerging rotules and surrounded by megascleres. An arrow shows a slightly elevated foramen closed by a membrane. **C** Cross section of a gemmule with trilayered theca armed by radial gemmuloscleres. **D** Close-up of the theca. **E** Image of skeletal network with scanty spongin and some gemmules

locality. DNA of *H. multidentata* specimens from Australian were not available in GenBank, so that molecular comparison was only possible with the specimen from Japan.

From the molecular tree (Fig. 5), our sequences were clustered together with sequences of *H. multidentata*, in a well-supported clade (bootstrap value 100%) and forming a sister group of sequences of the genus *Racekiela*, with a high bootstrap value (99%).

Distribution: Inland waters from eastern Australia (Racek 1969), New Zealand (Rützler 1968), and Japan (Ishijima et al. 2008; Masuda 2006; Matsuoka 2011). On rocks, metallic filters, submerged wood, and metallic structures for water intake. Also fouling the zebra mussel *D. polymorpha*. This paper presents the first record in European inland waters.

Discussion

There are several invasive freshwater sponges that often form part of the fouling on aquatic infrastructures affecting important economic activities. *Eunapius fragilis* is a cosmopolitan species in a wide range of lentic and lotic habitats in Europe (de Voogd et al. 2023), including Iberian Peninsula (Mathes 1952; Oscoz et al. 2009). *Eunapius carteri* is another of the most known freshwater invasive sponges in Europe (Bowerbank 1863), which is very common in South Asian countries from where it has been spreading throughout Europe, including Spain (Carballeira 2018 as *E. carteri* var. *hispanica* Ferrer-Hernández 1934; Gollasch and Nehringn 2006). Colonies of *E. carteri* usually form patches up to several centimeters thick, but as an invasive, it develops massively on submerged structures such as bridge piers, cooling ponds of hydroelectric and power stations (Sylaieva et al. 2010; Trylis et al. 2009). The situation gets worse in summer when the sponges cover the 100% of the surface available, and the biomass can be tripled (Sylaieva et al. 2010). *Spongilla lacustris, Ephydatia fluviatilis,* and *E. fragilis* have been also found colonizing pipes and channel walls, decreasing the flow capacity of conduits, and clogging the screen and pipelines in water treatment and power plants (Beger 1966; Gerasimova et al. 2021; Kocková et al. 2001; Kučera and Opravilová 2003, 2006; Roch 1924/25).

Heterorotula multidentata is one of the most common spongillid species of Eastern Australia and New Zealand (Racek 1969; Rützler 1968), which increased its geographical range to Japan where is an exotic species introduced by human activity (Ishijima et al. 2008; Masuda 2006). Here, we use both morphological and genetic data to show the presence of *H. multidentata* in Europe, because a correct identification of *Hererotula* is not easy. The main morphological trait of the genus is the presence of birotules of varying length, and the unequal development of gemmulosclere rotules (Penney and Racek 1968). The difficulty in identifying species of this genus, and the scarce taxonomical studies of freshwater sponges in the Iberian Peninsula may explain why this species had not been recorded so far.

Sponges are highly specialized filter-feeding metazoans, pumping and filtering water through a network of canals and chambers (i.e., the aquiferous system), and can use different adaptations to achieve a highly effective pumping and filtration of water (Weissenfels 1992). *H. multidentata* settled in intake screens benefits from the active and constant passage of water through its body, getting food and oxygen with minimal effort.

Problems with the mass growth of freshwater sponges in the drinking water reservoirs had been described for first time in the second half of the nineteenth century (Liebmann 1960). In Australia, huge overgrowing of the main outlet pipelines from the Chichester and Monong Reservoirs by *H. multidentata* was first described by Gee (1935). Later, Beger (1966) reported the problems caused by the sponge *Spongilla lacustris* growths at the 53 km length intake piping from water reservoirs in Cardiff (Hastings 1937; Parker 1913). Another freshwater sponge that also caused big problems was *Trochospongilla leidii* (Jones and Rützler 1975), which invaded the raw–water transmission lines between

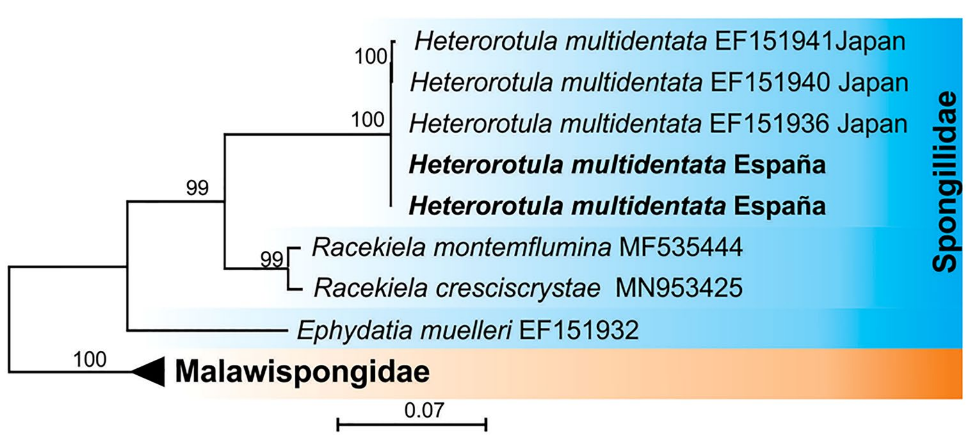

Fig. 5 Neighbor-Joining (NJ) tree topology of rDNA region (ITS1-5.8-ITS2) of *H. multidentata*, including some Spongillidae and Malawispongidae sequences rooting the tree. Numbers associated with each branch represent NJ bootstrap support values

the Meramec River and the St. Louis County Water Company's treatment plant (North America). The inner surfaces of two concrete intake lines with 2286 m long and 76.2 cm in diameter were almost completely covered by a continuous crust of the sponge 2 cm thick. The sponge increased the resistance to flow within the pipes and resulted in a decrease of 25% in delivery capacity. Attempts were made to remove the biofouls with massive doses of chlorine, but even a 24-h chlorine application to chlorine residuals as high as 10 mg/l, followed by back flushing at rates from 5 to 19 mg/l did not remove the sponge (King et al. 1969). It seems that some sponge species have a certain resistance to mild chlorination. A survey in several water treatment companies from UK revealed that bryozoans and sponges occluded underfloor nozzles and tailpipes in gravity filter beds. They were capable of penetrating to the entrance of the granular activated carbon filter, but only sponge was found in abundance. Sponges even penetrated to the entrance of the final chlorination point (Mant et al. 2011). While the most apparent problem of biofouling was the occlusion of nozzles, biofoulers also create three-dimensional structure which can provide habitat for pathogenic bacteria and other organisms, putting workers in the water treatment companies at risk (Okamura and Hatton-Ellis 1995). It was reported that physical contact with the sponge caused skin irritation for the workers, which must bear the expense to wear protective clothing (Mant et al. 2011). Dermatological irritation and eye pathologies caused by siliceous spicules from freshwater sponges was also reported in Amazonian rivers (Magalhães et al. 2006; Volkmer-Ribeiro et al. 2006).

Although it is not limited to the case in the sponges, another side effect of biofoulers on the water supply systems is the increase of corrosion by altering concentrations of reactive chemicals (e.g., oxygen or ammonia) at the metal surface (Nakano and Strayer 2014).

The wide adaptive radiation of freshwater sponges has resulted in the colonization of continental waters at all latitudes from cold deserts to equatorial rainforests and hot deserts; from the high plains to coastline, even has been found in alpine lakes and subterranean environments (Hermann et al. 2019). Ephemeral pools and man-made basins, such as pools in gardens, reservoirs, water tanks, and pipelines, are also suitable habitats (Manconi and Pronzato 2002; Pronzato and Manconi 2002). How does the dispersion of these sponges occur? The continental water provides extremely discontinuous habitats for most aquatic alien species, however, over the past century, the potential for aquatic species to expand their ranges in Europe has been enhanced by the construction of new canals and ship traffic (de Vaate et al. 2002; Panov et al. 2009). It has also been hypothesized that climate change facilitates natural range expansion (Stachowicz et al. 2002). In recent years, winters have generally become milder and growing seasons longer (Bradshaw and Holzapfel 2006), which may have enabled invaders to persist between growing seasons, especially when they inhabit intake screens in hydroelectric power stations (Polman et al. 2012), and thus attain a great biomass enough to cause any problems (Trylis et al. 2009).

In the case of freshwater sponges, it is important to highlight that most species belonging to Spongillida produce gemmules. The gemmules are asexual reproductive structures that contain and protect stem cells surrounded by a layer of spicules, which allow the sponge to survive harsh periods in a dormant state, since gemmules are resistant to desiccation, freezing, and anoxia for long periods of time (Reiswig and Miller 1998). When the environment becomes less hostile, stem cells migrate through an opening in the process of germination and start to differentiate to form a sponge. Therefore, the functional role of gemmules is double as resistant and passive dispersal bodies (Pronzato and Manconi 1994). They are characterized by having a well-developed pneumatic layer which allows it to float and disperse downstream. Also, fishes act as a biological vector (zoochory). Fragments of the freshwater sponge *Oncosclera navicella* with gemmules have been found in the stomach contents of the fish *Hypostomus regani* (armored catfish) and *Megalancistrus aculeatus* (pineapple catfish) (Volkmer-Ribeiro and Hatanaka 1991). Physical vector such as wind (anemochory) and biological vectors such as mammals and birds cannot be ruled out to disperse gemmules, since the spiny spicules radially arranged in the gemmular theca can hook efficiently onto animals. However, in both cases, it is necessary first that sponge's populations are subjected to desiccation of water bodies. In the case of *H. multidentata*, we have observed that the filter cleaning work contributes to the expansion of the species. In the case of the Herreros Pond, the filters are cleaned underwater, and the fragments of sponge with gemmules are detached and remain floating on the surface. In the case of Carrascalejo reservoir, the filter together with the water conduction is extracted to the shore of the reservoir where they are cleaned manually. The sponges are left out of the water, where they disintegrate over time, leaving the gemmules exposed, which can be dispersed by the wind and animals.

Indirect transport by other invasive freshwater species cannot be also ruled out. In the Guadalquivir River, *H. multidentata*, which is a part of the biofouling, has been found on rocks and fouling another invasive species; the zebra mussel *Dreissena polymorpha*, native to the Caspian, Aral, and Black Seas (Fig. 2). This species has spread over Europe in the last two centuries, including Britain and Ireland, and North America (Jernelöv 2017) mainly due to river navigation which could in turn spread *H. multidentata* or more probably its gemmules. *Heterorotula multidentata* in Japan is also found in irrigation canals and drinking water supply systems, where it grows on gravel and wood and, interestingly, fouls the invasive mollusk *Limnoperna fortunei* (Matsuoka 2011).

The introduction of species is a constant in many reservoirs and the mass growth of freshwater sponges in cooling ponds, canals, and pipelines used for hydroelectric power generation has intensified in recent years in Europa (Kučera and Opravilová 2006; Sylaieva et al. 2010; Záková et al. 2004). Increased awareness about the problems associated with invasive species and biofouling doubtlessly has contributed to the greater reporting, but there is good reason to believe that the increase is a real phenomenon.

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Declarations

Ethical approval Authors confirm that this study complies with the current laws of the country in which it was performed.

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