

Article

On the Presence and Ubiquity of the Exotic *Batophora* (J. Agardh) in the Mar Menor Lagoon (SE Spain)

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Abstract: We first report the presence of a species of the genus *Batophora* in the Iberian Peninsula, in the Mar Menor lagoon (Murcia, SE Spain). We detected this macroalga in November 2021. However, according to some observations, it could have been present in the lagoon at least since 2016, being described as a “rare” form of *Dasycladus vermicularis*. A further survey made during May 2022 confirmed the presence of this species in much of the perimeter of the Mar Menor. Morphologic analyses are not conclusive regarding the species description, but most traits point to *Batophora occidentalis* or *Batophora occidentalis* var. *largoensis*. A consensus sequence from the rbcL barcode gene was compared by the Blastn tool showing its closeness to other *Batophora* specimens, yet the specific level was unresolved. The recent confirmation of a *Batophora* species in a littoral lagoon from Formentera (Balearic Islands) with similar morphologic characteristics could indicate that both putative introductions are connected. Further morphologic and genetic analyses are required in order to know the origin and expansion of this genus along the Mediterranean coasts.

Keywords: alien species; Mediterranean Sea; Iberian Peninsula; algae; rbcL barcode; global change



Citation: Terradas-Fernández, M.; Valverde-Urrea, M.; López-Moya, F.; Fernández-Torquemada, Y. On the Presence and Ubiquity of the Exotic *Batophora* (J. Agardh) in the Mar Menor Lagoon (SE Spain). *Water* **2022**, *14*, 2909. <https://doi.org/10.3390/w14182909>

Academic Editor: Manel Leira

Received: 20 July 2022

Accepted: 14 September 2022

Published: 17 September 2022

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1. Introduction

The Mar Menor is a hypersaline coastal lagoon located in the southeast of the Iberian Peninsula (Figure 1), considered to be one of the largest coastal lagoons in the Mediterranean, with a surface of 135 km² and a maximum depth of 6.5 m [1]. At present, its salinity ranges from 39 to 46 psu, and its water temperature ranges from 11 °C in winter to 30 °C in summer [1,2]. Its seabed is mainly covered by soft substrates, with few rocky areas, combined with monospecific or mixed-macrophytes meadows of *Caulerpa prolifera* and *Cymodocea nodosa* [3,4].

This lagoon is a singular environment, with an elevated ecological and economical importance [5–7]. It has been protected as a Natural Park, a Ramsar international site, and a Site of Community Importance (SCI) integrated in the Natura 2000 Network, among others, and includes 18 habitats of European interest, according to the EU Habitats Directive [5]. It is the habitat of some emblematic and protected species, such as the macrophytes *Ericaria amaentacea* and *Cymodocea nodosa*; the fishes *Hippocampus guttulatus*, *Syngnathus abaster*, and *Aphanius iberus*; the sponges *Tethya aurantium* and *Tethya citrina*; and the mollusk *Pholas dactylus* [8]. Currently, the Mar Menor acts as a reservoir for the “Critically Endangered” fan mussel *Pinna nobilis*, which colonized the lagoon in the mid-1980s from the Mediterranean Sea [9]. However, it is also important as the habitat of some target species for fisheries, such as *Penaeus kerathurus*, *Sparus aurata*, or *Engraulis encrasicolus*, among others [6].

Despite its importance and protection, the Mar Menor has suffered several impacts related to anthropogenic activities, such as mining, agriculture, and urban development [5,7,10,11]. In fact, in recent years it has suffered some events of high eutrophication (in 2015–2016, 2019, and 2021), some of them being associated with extreme rainfalls, which increased the inflow of water with a high concentration of nutrients, organic matter, and sediments into

the lagoon [2,12]. These eutrophication episodes generated anoxia and massive mortality of marine organisms [9,12].

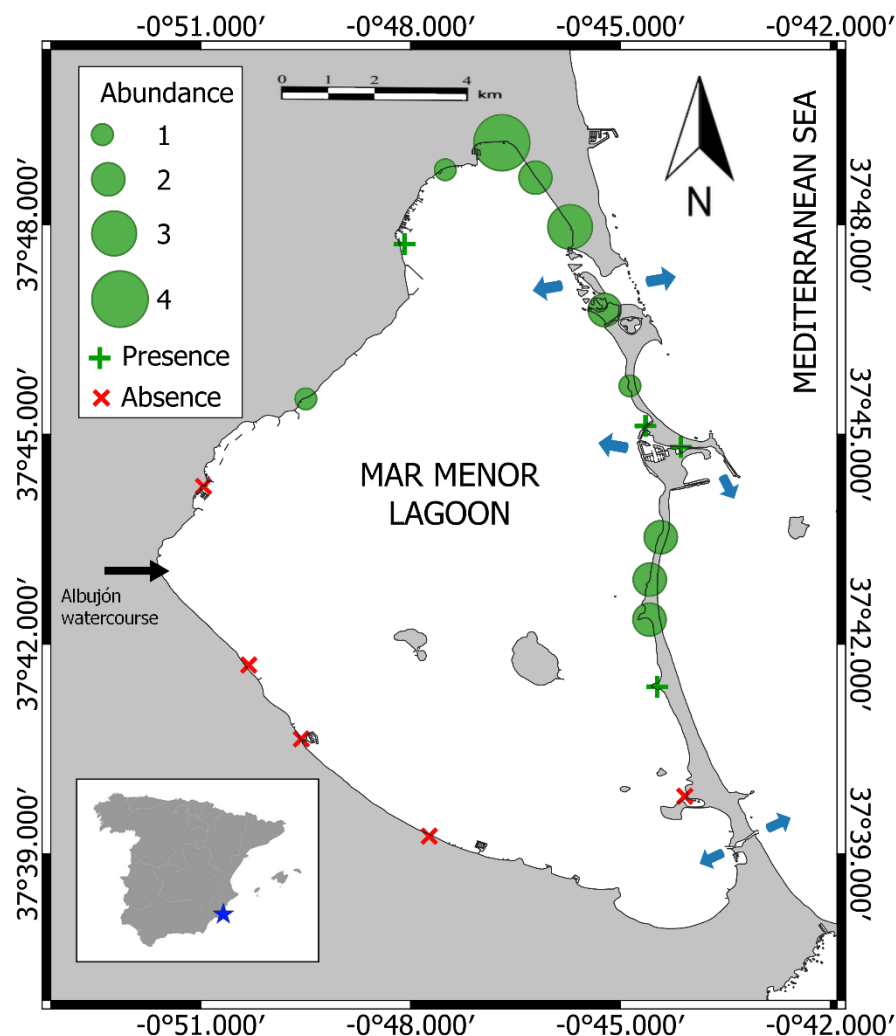


Figure 1. Location of the Mar Menor lagoon and sites where *Batophora* has been observed. Green circles mark sites where *Batophora* was detected in the present study (size indicates abundance of this species, see Material and Methods); green crosses indicate presence, while red crosses mark studied sites where this species was not found. The black arrow marks the Rambla del Albuji3n watercourse outlet, whereas blue arrows show the three inlets connecting the lagoon and the Mediterranean Sea.

Initially, the lagoon was naturally connected to the Mediterranean Sea by three inlets, but in 1973 the widening and enlargement of the main channel increased the connectivity of both water masses, diminishing the Mar Menor salinity and facilitating the introduction and expansion of some Mediterranean species, such as *Caulerpa prolifera*, *Pinna nobilis*, *Cotylorhiza tuberculata*, and *Rhizostoma pulmo* [1,13]. Another cause of regression in this lagoon is the appearance and expansion of alien species. Thus, vessel traffic and aquaculture, among others, have acted as vectors for the introduction of species that are also cited as exotic in the Mediterranean Sea, such as *Mnemiopsis leidyi*, *Bugulina stolonifera*, *Branchiomma boholense*, *Cerithium scabridum*, *Fulvia fragilis*, *Bursatella leachii*, *Callinectes sapidus*, *Styela canopus*, and *Palisada maris-rubri* [13–18].

Some of these exotic species could survive and adapt to this new habitat, colonizing it and negatively affecting its biodiversity, which makes it crucial to detect and monitor any new introduction in the lagoon. In the present work, we first report the detection of a new exotic green macroalga of the genus *Batophora* J. Agardh in the Mar Menor lagoon. We

have morphologically characterized our specimens and sequenced the *rbcL* barcode gene. Through a rapid assessment method, we have also mapped and quantified its abundance along the perimeter of the lagoon.

Further studies which derive from this work will include a complete sampling of the lagoon for estimating the expansion and possible effects of this species in order to recommend control measures for this invasion, if necessary. Moreover, and due to its characteristics, we consider the Mar Menor lagoon as a good case of study for evaluating the presence, development, and consequences of exotic species, which could be incremented due to the effects of climate change.

2. Materials and Methods

We photographed and collected specimens of *Batophora* in Punta del Galán (37.69° N, 0.74° W) and Pueblo Cálido (37.73° N, 0.74° W) in November 2021. A further survey was carried out in May 2022 in order to know the distribution of *Batophora* along the entire perimeter of the Mar Menor, and for taking more samples. A total of 19 localities were studied (Figure 1).

In each of these localities, we sampled in shore strands which were 15 m wide and 200 m long (<1 m depth) (Figure 1). Abundance was calculated according to the density of *Batophora* thalli by using four semiquantitative categories, as follows [19]: 1: Rare and sparse clumps (<15 cm in diameter) separated from each other by more than 5 m. 2: Frequent clumps and decimetric patches separated from each other by less than 5 m. 3: Decimetric patches and clumps separated by less than 1 m. 4: Patches only separated by a few centimeters, resulting in the appearance of a discontinuous belt.

Fresh specimens were immediately transported in plastic bags to the laboratory, where they were described and photographed using a microscope (Olympus BH2, Olympus Corporation, Tokyo, Japan) fitted with a camera (Leica DFC 480, Leica Camera AG, Wetzlar, Germany).

For DNA analysis, samples were washed in distilled autoclaved water in order to remove salts and sand. The epiphytes were removed using sterilized tweezers.

DNA was extracted according to CTAB protocol (0.1 M Tris HCl, 0.05 M EDTA, 1.5 M NaCl, 0.05 DTT and 2% CTAB) [20]. PCR amplifications of samples were carried out using the VWR Red Taq DNA polymerase Master Mix with a final volume of 50 µL per reaction. The *rbcL* (ribulose-1,5-biphosphate carboxylase/oxigenase large subunit) gene was amplified using the pair of primers *rbcL* B (5'-ATGTCACCACAAACAGAACTAAAGCA-3') and *rbcL* Q (5'-GATCTCCTTCCATACTTCAACAAGC-3') [21]. Amplification was conducted using a LifeExpress Classic BIOER thermal cycler (Life Express, Hangzhou Bioer Technology Co., Ltd., Zhejiang, China). PCR reactions were performed following Zechman [21], increasing to 35 cycles in order to obtain a greater DNA quantity. Then, amplification products were assessed on 1.5% TAE 1X agarose gels and were purified for sequencing with GeneJET Gel Extraction Kit (Thermo Scientific) according to the instructions. Finally, PCR products were Sanger sequenced by an external laboratory (Macrogen, Madrid, Spain).

The forward sequence and the reverse complement of the reverse sequence were aligned in order to generate a consensus sequence. The consensus sequence was deposited in the NCBI database with the Accession Number ON971347, and voucher specimens were deposited in the ABH (herbario oficial institucional de la UA). Gaps and sequence discordances were fixed by analyzing the electropherograms. The obtained consensus sequence was compared to the NCBI database by the Blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (accessed on 20 June 2022)). Finally, a phylogenetic tree was constructed through the Fast Minimum Evolution model [22], including representative specimens from the main genera of Dascycladales found in the NCBI database.

3. Results

A *Batophora* species was detected in fourteen localities throughout the perimeter of the lagoon (Figure 1) on multiple substrata such as pebbles, mollusks such as *Hexaples*

trunculus and *Pinna nobilis*, and artificial substrata (plates of PVC, ropes, etc.). Specimens form aggregates of 5–40 cm in diameter found in sandy shallow bottoms along with *Cymodocea nodosa* and *Caulerpa prolifera* meadows. Other species present in the same areas were *Acetabularia acetabulum*, *Caulerpa cylindracea*, *Chaetomorpha linum*, *Chondria capillaris*, *Cladophora* spp., *Ulva* spp., and *Ruppia cirrhosa*.

Batophora aggregates were more abundant in the east of the lagoon, with higher abundances in the northeastern localities forming shallow, discontinuous belts. These belts were distributed right on the edge of the lagoon along the shore, being replaced by *Cymodocea* and *Caulerpa* meadows seawards (Figures 1 and 2).

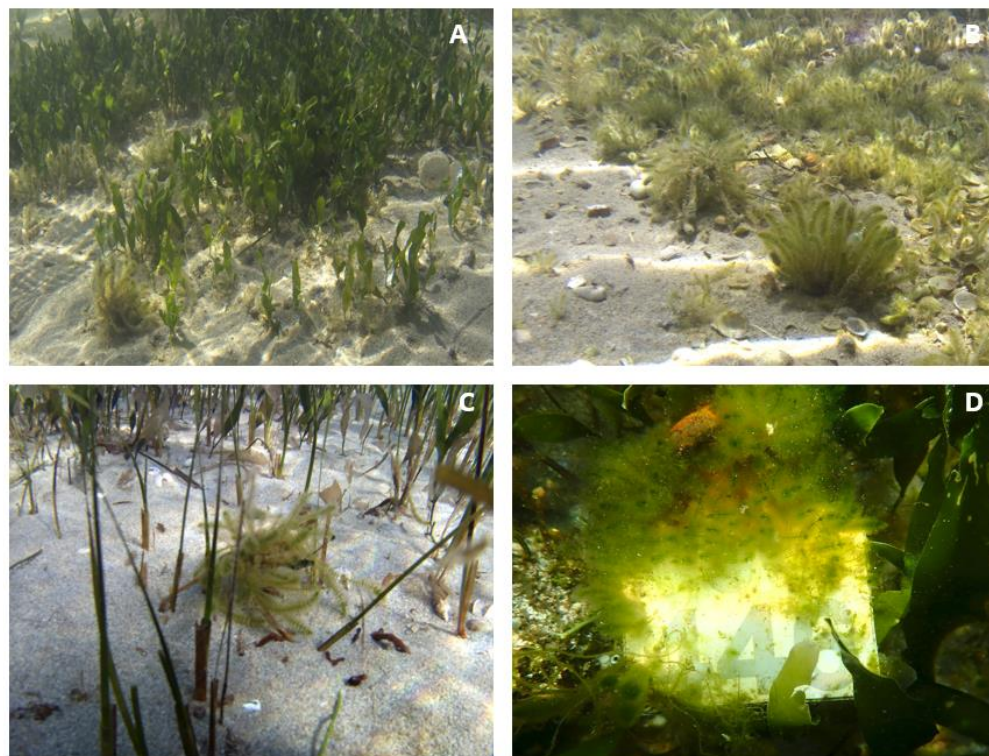


Figure 2. Specimens of *Batophora* in different habitats. Shallow *Caulerpa prolifera* meadow (0.5 m depth) (A), sandy bottom (B), shallow *Cymodocea nodosa* meadow (0.5 m depth) (C), and PVC plates on experimental plots settled on *Caulerpa prolifera* bottoms (D).

In the eastern localities, some clumps of *Batophora* were floating, being carried away by the waves and currents, and some of them were returning to the sea bottom or found as debris accumulated on the beach (Figure 3).

Batophora was not detected in the southwest of the lagoon, where opportunistic algae (*Ceramium deslongchampsii*, *Chaetomorpha linum*, *Chondria capillaris*, and *Cladophora* spp.) thrive.

The thallus of our specimens is greenish, 30–50 mm long with whorled axes, forming a cylinder of 3–5 mm in diameter arising from a holdfast with rhizoids (Figure 4). Each whorl consists of six to nine ditrichotomous branchlets which fork 3–4 times, decreasing in diameter and becoming hyaline from the second or third fork (Figure 5). The main axis where branchlets are inserted is 400–500 μm in diameter. The first internode of the branchlet is 650–900 μm long and 175–250 μm wide. Whorls were separated ca. 1.5 mm from each other in the middle part of the axis. Fertile axes with 3(–2) gametophores in the distal part of the basal internode inserted by means of a very short pedicel. Fertile gametophores are spherical with 400–650 μm in diameter, or oval, being 350–500 μm wide and 550–620 μm long. Gametophores are grouped in the upper (1/5–1/8) part of the main axis (Figures 4–6). Gametangia are spherical and are 59–68 μm in diameter.



Figure 3. Clumps of *Batophora* floating in the sea (A) and *Batophora* debris scattered on the shore (B).



Figure 4. Habit of collected specimens (A) and detail of a fertile (B) and vegetative axis (C). Basal part of an axis showing a holdfast with rhizoids (D).

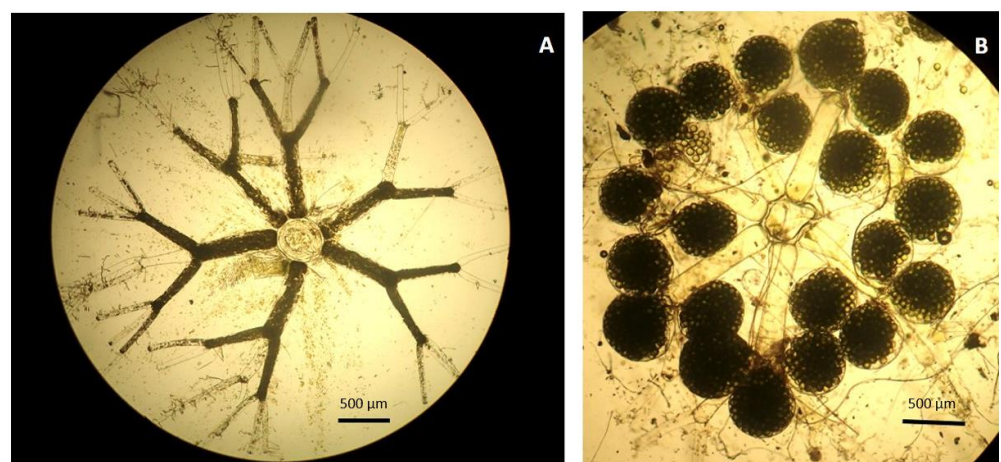


Figure 5. Cross-section of a vegetative axis (A) and a fertile axis (B). Six ditrichotomous branchlets shaping a whorl can be noted (A). Fertile axes with spherical gametophores inserted in the distal part of the basal internode of the whorl branchlets (B).

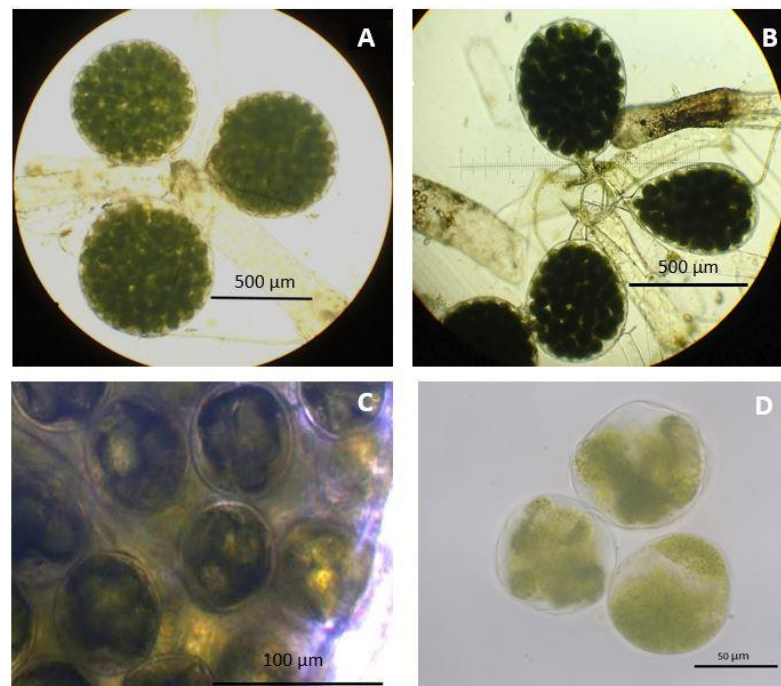


Figure 6. Spherical (A) and oval (B) gametophores. Detail of gametangia in a gametophore (C) and free gametangia (D).

After considering most genera of Dasycladales from the NCBI database, the molecular analysis includes our sequence in the genus *Batophora* (Figure 7). However, our sequence (consensus sequence of 1338 bp) is identical to those from *B. oerstedii* and *B. occidentalis*, preventing any distinction at the species level.

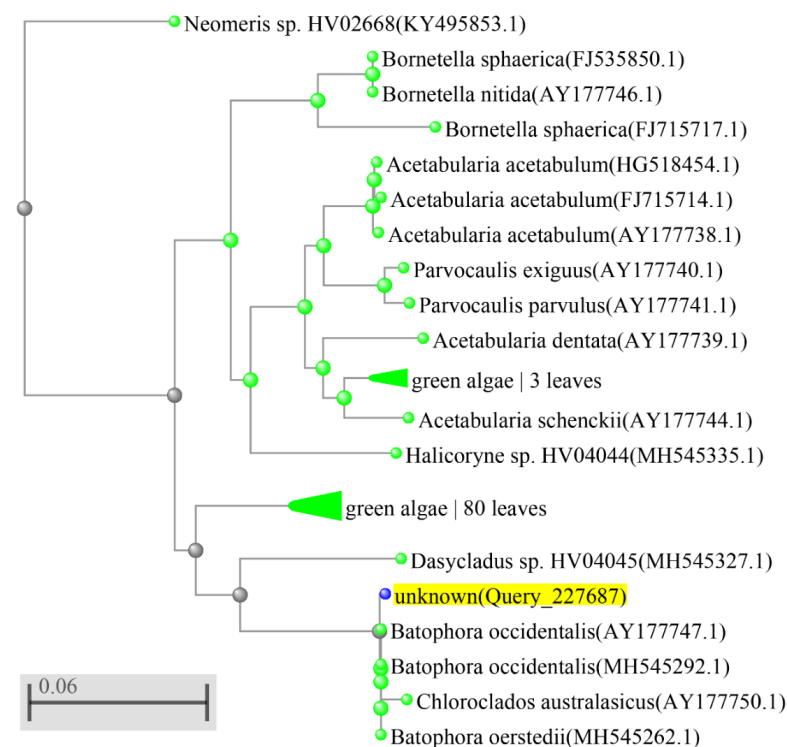


Figure 7. Phylogenetic tree from most genera of Dasycladales found in the NCBI database constructed with the Fast Minimum Evolution model and using the *rbcl* barcode gene. Our sequence is shown in yellow.

4. Discussion

The genus *Batophora* J. Agardh is reported in the western tropical and subtropical Atlantic [23–25], the Canary Islands [26], and the Mexican Pacific [27]. Three species or varieties of *Batophora* are recognized [28]: *B. oerstedii* J. Agardh, *B. occidentalis* (Harvey) S. Berger and Kaever ex M.J. Wynne, and *Batophora occidentalis* var. *largoensis* (J.S. Prince and S. Baker) S. Berger and Kaever ex M.J. Wynne. The size of the algae, the location of the whorls along the main axis, the position and size of the gametophores, as well as the size and shape of the gametangia have been the most widely used characters with a taxonomic value. However, there is no consensus on how these characters define the different species and varieties [28–32].

Our specimens show gametophores with a diameter compatible to that described for any species of *Batophora* [28,32]. On the other hand, the presence of axes with oval as well as spherical gametophores along with spherical gametangia would bring our specimens closer to *B. occidentalis*, whereas the insertion of the gametophores exclusively in the distal part of each first whorl branchlet internode points to *B. occidentalis* var. *largoensis* [28]. Molecular data include our specimens in the genus *Batophora*, but the results are not conclusive at the species and variety level, due to the lack of molecular data for *B. occidentalis* var. *largoensis*. Furthermore, *rbcL* barcode gene sequences are identical for both species (*B. occidentalis* and *B. oerstedii*). All in all, the lack of morphological data consensus regarding the ability to discriminate between species of *Batophora*, along with the few molecular data available, highlight the need for further research on these issues.

As for habitat preferences, according to Prince and Baker [30], *B. occidentalis* var. *largoensis* (as *B. largoensis*) is more typical of exposed environments, while *B. occidentalis* and *B. oerstedii* would be more related to mangrove or lagoon environments [32,33]. Despite this, mixed populations with the three taxa have been found in mangroves and other communities of protected coastal environments [28] where this genus can be very ubiquitous [34]. In our case, this alga is well-established in a large part of the Mar Menor, even forming discontinuous inner belts in the shallowest landside zones, where it is the dominant macrophyte in many cases. However, these “*Batophora* belts” are usually replaced by mixed meadows of *Caulerpa prolifera* and marine angiosperms (*Cymodocea nodosa*, *Ruppia cirrhosa*) in the “seaside” just a few meters beyond (pers. obs.) suggesting some difficulties of *Batophora* to thrive under dense macrophyte canopies. The same pattern has also been observed in the case of other exotic species [35–37]. This alga was not detected in the SW of the lagoon, probably because this area is affected by the Rambla of Albuñón, the main watercourse draining into the lagoon, which discharges large amounts of nutrients and sediments, especially during extreme storm events, such as those which took place in 2019 and 2021 [2,38]. This discharge has affected the S-SW of the Mar Menor, creating extensive eutrophic and hypoxic zones in its vicinity [9]. This could be the reason for us to only find opportunistic ulvophycean and red “turf-like” algae in SW localities, as *Batophora* does not tolerate high levels of eutrophication [33], while it seems to show a high dispersal ability, by means of those “floating clumps”.

A population of this genus has recently been discovered in a coastal lagoon connected to the sea in the island of Formentera (Balearic Islands), where it experienced a rapid bloom [39]. The characters analyzed in our work (habit, gametophore size, gametophore distribution, gametangia size) largely overlap with those reported in the population of Formentera except for the presence, in our case, of some fertile axes with distinctly oval mature gametophores and nondetection of gametophores in the second whorl branchlet internode (Figure 5, Table 1).

Table 1. Comparison between the main characters found in our specimens and those found in Ballesteros [39].

Main Observed Characters	This Study	Ballesteros, 2020
No. of branchlets shaping a whorl	6–9	6–10
Main axis diameter	400–500 μm	450 to 600 μm
First internode diameter	175–250 μm	140–180 μm
Gametophores location	1st internode	1st (2nd) internode
Gametophores shape	Spherical (oval)	Spherical
Gametophores diameter	350 to 650 μm	440 to 650 μm
Gametangia shape	Spherical	Spherical
Gametangia diameter	59–68 μm	60–87 μm

Given the phenotypic plasticity of *Batophora* [28], and the overlapping in morphologic traits, it is likely that both populations could be linked to the same introduction event. This argument would be strengthened because both detections are practically simultaneous. In our case, this species may have been mistaken for “rare” forms of *Dasycladus vermicularis* (Scopoli) Krasser detected since approximately 2016 during a severe anoxic event that affected mixed meadows of *Caulerpa prolifera* and *Cymodocea nodosa* (Giménez-Casaldueiro, F and Rubio, I.; pers. com.) [40].

Dasycladus C. Agardh is frequent both in the tropical and the warm temperate seas, including the Atlantic and the Mediterranean. Just like *Batophora*, it is also reported in shallow protected habitats such as lagoons and mangroves [41–43]. In spite of the high macroscopic resemblance between *Batophora* and *Dasycladus*, some morphological characteristics of *Batophora* (such as the larger distance between whorls and the presence of several gametophores in each whorl branchlet) properly separate this genus from *Dasycladus*. The easy confusion with *Dasycladus vermicularis* and the detection of vegetative thalli attributable to *Batophora* species almost 20 years ago could underestimate the presence of *Batophora* in the Mediterranean Sea [44].

In any case, the ecological similarity of the Mediterranean systems where *Batophora* is detected with those tropical or subtropical systems where this alga probably comes from, including its thermal range [28,45,46], makes it plausible for a future expansion of *Batophora* throughout the Mediterranean Sea, at least in warm and sheltered enclaves. Seasonal monitoring and mapping, together with genetic studies of Mediterranean, Canarian, and tropical populations, are essential to better understand the origin and potential expansion of this “new” introduction.

5. Conclusions

This study shows the first occurrence of *Batophora* species in the Iberian Peninsula (Mar Menor, SE Spain), which had probably been confused with *Dasycladus vermicularis* since 2016. Its ubiquity along much of the perimeter of the Mar Menor, together with the recent expansion of this alga in other Mediterranean lagoons, show its high expansion capability, acting as a potential invader. Further morphological and molecular data are necessary to better know the species identity and its possible origin.

Author Contributions: All authors contributed to each step of the manuscript, including methodology, drafting, and fieldwork. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Deposition of Sequences and of Expression Data: The consensus sequence was deposited in the NCBI database with the Accession Number ON971347.

Acknowledgments: The first *Batophora* specimen was detected by sampling for the project LIFE PIN-NARCA (LIFE20-NAT/ES/001265). We also thank Eleuterio Abellán Gallardo and Francisca Giménez-Casalduero for their help and advice about potential distribution of this alga, and Andrés Izquierdo-Muñoz for provide us Figure 2D. Iván Egea kindly reviewed the English version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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