

Research Article

First joint morphological and molecular detection of *Watersipora subatra* in the Mediterranean Sea presented in an updated genus phylogeny to resolve taxonomic confusion

Robin P. M. Gauff^{1,2}, Marc Bouchoucha², Amelia Curd¹, Gabin Droual^{1,3}, Justine Evrard¹, Nicolas Gayet⁴, Flavia Nunes¹

¹ IFREMER, DYNECO, Laboratory of Coastal Benthic Ecology, F-29280 Plouzané, France

² IFREMER, Lab Environm Ressources Provence Azur Corse, CS 20330, F-83507 La Seyne Sur Mer, France

³ IFREMER, INRAE, Institut Agro–Agrocampus Ouest, Ecosystem Dynamics and Sustainability, 44980 Nantes, France

⁴ CNRS, IFREMER, UBO, Biology and Ecology of Deep-Sea Ecosystems, F-29280 Plouzané, France

Corresponding author: Robin P. M. Gauff (gauff.robin@yahoo.de)



Academic editor: Kevin C.K. Ma

Received: 13 March 2023

Accepted: 30 May 2023

Published: 13 September 2023

Citation: Gauff RPM, Bouchoucha M, Curd A, Droual G, Evrard J, Gayet N, Nunes F (2023) First joint morphological and molecular detection of *Watersipora subatra* in the Mediterranean Sea presented in an updated genus phylogeny to resolve taxonomic confusion. *Aquatic Invasions* 18(3): 295–312. <https://doi.org/10.3391/ai.2023.18.3.108128>

Copyright: © Robin P. M. Gauff et al. This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International – CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).

Abstract

Introduced species constitute a critical bio-security issue worldwide and the precise monitoring of their spread is crucial for their management. For species forming cryptic complexes this may remain difficult. Using integrative taxonomy, we formally report for the first time, well-established populations of the cosmopolitan introduced bryozoan *Watersipora subatra* in the French Mediterranean Sea and compile worldwide existing genetic data for *Watersipora* species alongside newly acquired data to establish the most complete phylogeny of the genus to date. This revealed pervasive erroneous identifications in Genbank, which in turn perpetrate further errors in recent studies, primarily misidentifying *W. subatra* as *W. subtorquata*. High abundance and geographic spread of *W. subatra* in our Mediterranean sampling sites suggest that this species has been present for some time but has been misidentified until now. We provide an updated species identification for all current reference sequences in the *Watersipora* genus, which may help future monitoring of *W. subatra* and other *Watersipora* species.

Key words: Bryozoa, integrative taxonomy, introduced species, phylogeny, NIS

Introduction

Marine interconnectivity among nations has risen in recent decades, a trend that will further increase and that is favoring species introductions all over the world (Levine and D’Antonio 2003; Seebens et al. 2016; Carrasco et al. 2017; Sardain et al. 2019). As introduced species lack natural regulators in their new environment (Papacostas et al. 2017), their effects may be unpredictable. Some invaders may completely restructure ecosystems, potentially leading to the loss of biodiversity and ecosystem services (Pejchar and Mooney 2009; Johnston et al. 2015; Walsh

et al. 2016), causing local extinctions (Blackburn et al. 2019), and high economic impacts (Lovell et al. 2006; Olson 2006; Jardine and Sanchirico 2018; Diagne et al. 2021). As an example, direct damages as well as costs generated from combating introduced species accumulated to over \$29 billion in Europe alone (see supplementary material table S1 of Diagne et al. 2021). These damages on ecosystems and the economy do not even require high abundances of an introduced species to be tangible (Blackburn et al. 2011). For these reasons, invasive species (*sensu* Blackburn et al. 2011) are regarded as a crucial global biosecurity issue, and prevention and early management of these species constitutes the best strategy to minimize their impact (Lovell et al. 2006; Olson 2006; Pyšek et al. 2020). This may prove difficult in phyla with few or small morphological identification criteria, that form cryptic species complexes (Mackie et al. 2006; Mackie et al. 2012; Vieira et al. 2014; Mastrototaro et al. 2020; Salonna et al. 2021), or for which taxonomic expertise is rare, such as for many marine invertebrates. Identifying the precise species at a certain location is however important for subsequent evaluation of spread and invasion (Vieira et al. 2014; Golo et al. 2023).

Especially when morphological criteria are lacking to identify a species, genetic methods have been increasingly used to help with the identification of introduced species. DNA barcoding of individual specimens, metabarcoding of communities in bulk (from sediments, scrapings, or other substrates) and more recently environmental DNA (eDNA) are now all regarded as useful techniques for detecting introduced species. However, the efficiency of molecular species identification highly depends on the quality of reference sequences, particularly how well species identification was carried out for the reference sequences before being submitted to public databases (Couton et al. 2022). Incorrect species identification of reference sequences can have important effects on subsequent studies which will base molecular identifications on imprecise species names.

The morphology of the bryozoan genus *Watersipora* is notoriously complicated, and the redescription of the genus by Vieira et al. (2014) has reattributed individuals of introduced populations in multiple localities to *Watersipora subatra* (Ortmann, 1890) (identified as “*Watersipora subtorquata*” (d’Orbigny, 1852) in Mackie et al. 2012; Ryland et al. 2009) and others to *W. subtorquata* (identified as “*W. subvoidea*” (d’Orbigny, 1852) in Mackie et al. 2006, 2012). This has led to confusion regarding the identity of populations of several localities around the world, most notably in Europe. Many species identifications of reference sequences in Genbank have not been updated since the redescription by Vieira et al. (2014). Nevertheless, these sequences continue to be used for e-DNA monitoring. As an example, individuals that were resolved as *W. subatra* are still listed as “*W. subtorquata*” on GenBank (accessed June 2022, NCBI, Benson et al. 2013). This probably induces subsequent errors in the literature, falsely detecting *W. subatra* as “*W. subtorquata*”, hindering the accurate e-DNA detection of several *Watersipora* species.

In the Mediterranean Sea, the presence of *W. subtorquata* has been reliably confirmed (Vieira et al. 2014) and this species is the most frequently described introduced *Watersipora* species in this area (Vieira et al. 2014; Harmelin et al. 2016; Rosso and Di Martino 2016; Tempesti et al. 2020), even though *Watersipora arcuata* Banta, 1969 seems to be spreading rapidly in recent years (Ulman et al. 2017, 2019; Reverter-Gil and Souto 2019). Here however, we identified *W. subatra* as the dominant *Watersipora* species in several French Mediterranean harbors, with only anecdotal presence of *W. subtorquata* (and no observations of *W. arcuata*). This points towards an inherent identification problem, as the high abundance and persistence on many artificial substrates makes it impossible to miss the species (see Fig. 1a) and suggests it has been present some time already.

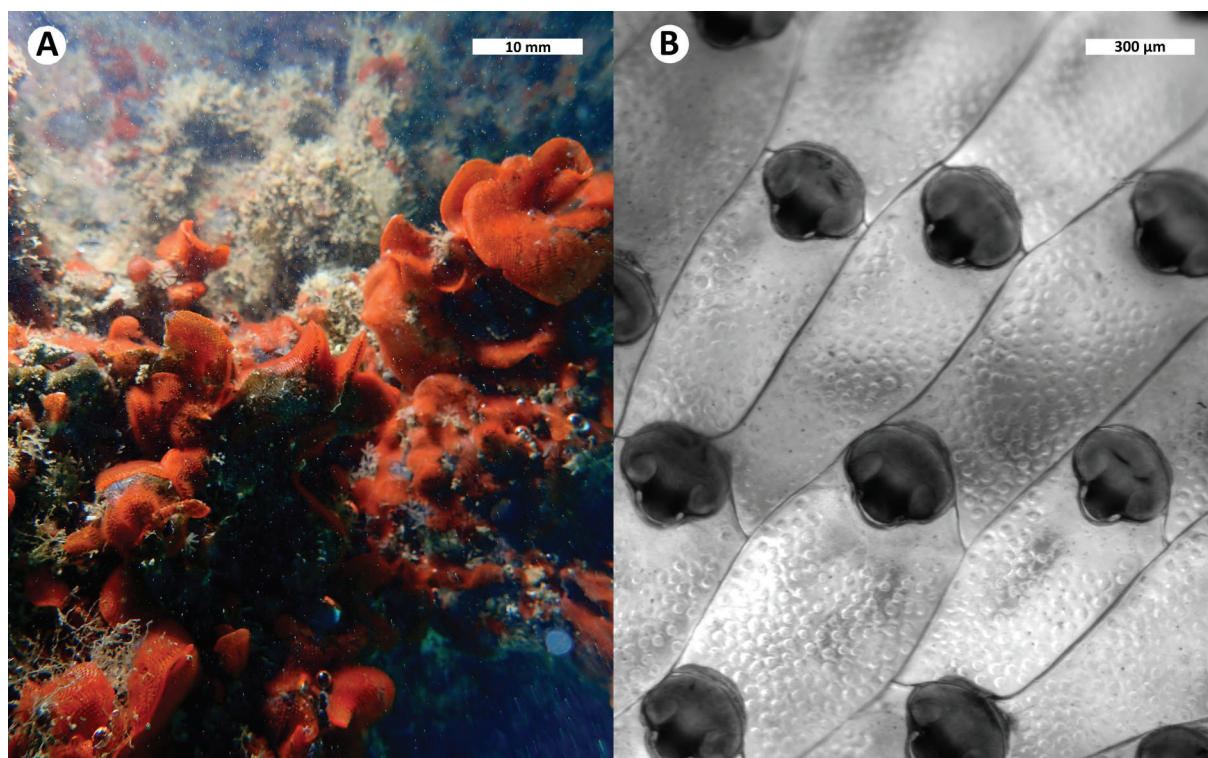


Figure 1. Living *Watersipora subatra* colony from the Toulon Bay *in situ* (A) (Benoist de Vogüé/IFREMER) and under optic microscope (B). Opercula with a dark central band and swirls are visible (Robin Gauff).

The present study has a two-fold objective. Firstly, we wish to declare the first formal record of *W. subatra* as an already well-established introduced species in the French Mediterranean Sea. Secondly, we provide a phylogenetic analysis of existing COI sequences of *Watersipora* from Genbank, including new sequences from individuals that were carefully identified according to morphological criteria, in order to improve molecular identification and detection of non-indigenous *Watersipora* species, particularly the spread of *W. subatra* in the Mediterranean or elsewhere.

Materials and methods

Study area

Specimens for this study were sampled in four different locations along the French Mediterranean coastline. Three sample sites were under pontoons and docks in the Toulon Bay: in front of the Ifremer facilities (43.105415°N, 5.885415°E), in the La Seyne sur Mer marina (43.102007°N, 5.882377°E), and in the Toulon Darse Nord marina (43.114637°N, 5.931267°E), as well as a fourth site in the Old Harbor of Marseilles (43.293622°N, 5.363857°E). The Toulon Bay is a highly urbanized area (Meaille and Wald 1990), with six marinas, several commercial harbors, a large military harbor and ferry activities over an area of approximately 10 km². It is highly impacted by anthropogenic pressures such as habitat modification and loss (Bouchoucha et al. 2016, 2018a, b), chemical contamination (Wafo et al. 2016; Araújo et al. 2019; Mazoyer et al. 2020), and the presence of introduced species (Zibrowius 1991; Ruitton et al. 2005; Gauff et al. 2023a)). The Old Harbor of Marseilles is a recreational marina with approximately 3200 boat moorings, a large hull cleaning area, and commercial activities such as artisanal fisheries and short distance ferry transports. Being the second largest French city and having a large harbor complex 7.5 km², and with

more than 10 km of continuous artificial coast, Marseilles constitutes another key example of massive marine urbanization in the Mediterranean Sea. Tentative identifications have noted *W. subatra* as being present in this area from 2019 on (Gauff et al. 2023b)), however a detailed morphological description and completely reliable analysis was until now lacking. In order to compare Mediterranean individuals with well-known *W. subatra* populations (Ryland et al. 2009), we sampled additional colonies at a fifth site at the Pointe du Diable close to Brest, Brittany, France (48.354768°N, 4.558518°W) where *W. subatra* seems until now to be the only representative species of the genus (Leclerc and Viard 2017; Porter et al. 2017; Gauff et al. 2022).

Morphological analysis

Approximately 200 g of *Watersipora* colonies from the studied locations were sampled for the present study and scanned for different species. Individuals were first identified alive in the laboratory using a ZEISS SteREO Discovery.V12 microscope coupled to a ZEISS Axiocam 506 mono camera and visualized and measured with ZEISS Zen 3.0 software. Operculum structure (see figures 65–68 in Vieira et al. 2014) and general individual characteristics (Zooid Length, Zooid Width, Orifice Length, Orifice Width, Sinus Length, Sinus Width, Pseudopore Diameter, Intrazooidal Septula Presence and Diameter; see tables 1, 3 in Vieira et al. 2014) were used as first identification criteria. A total of 15 high-quality fragments (no epibionts, not epibionts themselves, clean, alive...) were chosen for detailed analysis and description (3 for each of the three areas of the Toulon Bay, 3 for Marseilles and 3 from Brittany). The colonies were then prepared for scanning electron microscopy (SEM). Specimen fragments were bleached for 48 hours, washed in deionized water, then dried at 37 °C overnight. Clean fragments were mounted on stubs with carbon glue, and sputter-coated with 60%Au/40%Pd. Images were taken with 200×, 600× and 2500× magnification with a FEI Quanta 200 SEM. The targeted identification criteria here were the latero-oral intrazooidal septula (IZS) which allow to clearly distinguish *W. subatra* (IZS present) from *W. subtorquata* and *W. souleorum* Vieira, Specer Jones & Taylor, 2014 (IZS absent; Vieira et al. 2014). Measurements of intrazooidal septula and pores were taken with the ImageJ (Rueden et al. 2017) 'Analyse' tool using the scale from SEM images. The remaining colony was immediately preserved in absolute ethanol for genetic sequencing.

DNA extraction, amplification and sequencing

Zooids were removed from their epitheca to avoid contamination with exogenous DNA. Twenty zooids were pooled per colony, and DNA was extracted using the NucleoSpin DNA RapidLyse kit (Macherey-Nagel) following the manufacturer's protocol. Polymerase chain reaction (PCR) of the mitochondrial cytochrome c oxidase I gene was conducted with primers designed specifically for Bryozoa: BryCOIL1548 forward 5'- CAT AAC AGG AAG AGG TTT AAG -3' and BryCOIH2161 reverse 5'- ATY AGG AGC AGG ATT CAG TAT G -3' (Mackey et al 2006). PCR amplifications were performed in a total volume of 25 µl with the DreamTaq DNA polymerase (ThermoFisher), consisting of 2.5 µl DreamTaq PCR Buffer (10×, including 20 mM MgCl₂), 0.5 µl dNTPs (10 mM each), 1 µl of each primer (10 µM each), 0.2 µl of DreamTaq polymerase, 17.8 µl sterile Millipore water, and 2 µl of DNA. The thermal cycling protocol included an initial denaturation step at 94 °C (3 min), followed by 35 cycles including denaturation at 94 °C (30 s), annealing at 50 °C (30 s), and elongation at 72 °C (60 s). The PCR products were run through a 1% agarose gel prepared with Tris-borate EDTA (TBE). Two bands were observed in the

PCR product. The 650 bp fragment was excised from the gel and was purified using the Nucleospin Gel and PCR Clean Up kit (Macherey-Nagel). Sanger sequencing was conducted at Eurofins Genomics in both forward and reverse directions.

Phylogenetic analysis

Sequence chromatograms were trimmed for low quality bases and visually inspected for errors in 'Genious Prime' (v.2020.2.4; Dotmatics). Forward and reverse fragments were aligned to generate a consensus sequence. High quality sequences were obtained for three individuals from Marseilles, two individuals from Toulon and three individuals from Brest. They were deposited on GenBank (NCBI; Benson et al. 2013), under the accession numbers OQ918440–OQ918447. All sequences attributed to the genus *Watersipora* were downloaded from 'GenBank' (accessed on 01/11/2022) by using the search term "*Watersipora*" in the Nucleotide search engine. This returned 264 sequences from which we chose only COI sequences (229). Two sequences of *W. platypora* Seo, 1999 were excluded from our database as they were identical, and one sequence from eDNA detection from Portas et al. (2022) was added. Finally, we added eight sequences acquired for the present study. An alignment was generated for a total of 236 individuals using 'Sequencher' (v.5.3; Gene Codes Corp). The alignment was then trimmed manually to remove sections with high levels of missing data in the 5' and 3' ends. Identical sequences were removed from the dataset using 'DAMBE' (v 7.5.3; Xia 2018), to generate a non-redundant dataset composed of 99 unique sequences. A re-alignment of this final dataset was conducted with 'MAFFT' (v7.490; Katoh and Standley 2013), using the '--localpairs' algorithm and a maximum number of 1000 iterations. Models of sequence evolution were tested with 'modeltest-ng' (v0.1.6; Darriba et al. 2020), and the model with the highest probability score was selected by considering the Bayesian information criterion (BIC), Akaike information criterion (AIC) and the corrected AIC (AICc). Maximum likelihood phylogenetic analysis was conducted with 'iqtree' (v2.0.3; Minh et al. 2020) using the HKY+G4 model and 1000 ultra-fast bootstrap replicates (Minh et al. 2013). Bayesian phylogenetic analysis was conducted with 'BEAST' (v1.10.4; Suchard et al. 2018), using a strict clock and the HKY model of sequence evolution with 4 gamma categories of site heterogeneity. The proportion of invariant sites and base frequencies were estimated, and the Yule process of speciation model was used, using default priors for all estimated operators. Three independent runs were conducted over 10^7 generations sampled every 1000 iterations. The logs for each run were examined to ensure an adequate effective sample size (ESS) had been reached for each estimator. The logs of the three runs were combined using 'logcombiner', and trees were summarized with 'TreeAnnotator' in 'BEAST', using maximum clade credibility and median branch heights, and a burn-in, *i.e.*, the number of samples to be discarded at the start of the run, of 6000 trees (20%). Phylogenetic trees were visualized with 'FigTree' (v1.4.4; Rambaut 2010) and rooted on the *W. arcuata* clade. All datasets, tree files and the code of our analyses can be consulted at <https://gitlab.ifremer.fr/lebco/fnunes/watersipora.git>.

Sequence accession numbers were color coded according to the species identification indicated in the Genbank record. For clarity, in this manuscript identifications from Genbank will appear in double quotation marks (ex: "*W. subtorquata*") as some were already resolved to other species by Vieira et al. (2014). Exceptions to this are proven reliable identifications (ex: Couton et al. 2019; McCann et al. 2019). For redundant sequences, only one accession number was listed per species name on the phylogenetic tree, with the number of individuals having an identical sequence indicated in brackets, however a detailed phylogenetic tree can be accessed in the Suppl. material 1: table S1.

Watersipora distribution map

We used the data obtained from our genetic sequencing and recent records of *Watersipora* spp. (Mackie et al. 2006; Anderson and Haygood 2007; Knight et al. 2011; Mackie et al. 2012; Porter et al. 2015; Ulman et al. 2017; Aleman et al. 2018; McCann et al. 2019; Reverter-Gil and Souto 2019; Ramalho and Caballero-Herrera 2022) to complement the map (figure 72 in Vieira et al. (2014).

Results

Morphological analysis

The characteristics from all 12 sampled individuals, allowing each to be identified as *W. subatra* using Vieira et al. (2014), were as follows: Zooid length $1118 \pm 178 \mu\text{m}$; Zooid width $476 \pm 132 \mu\text{m}$; Orifice skull-shaped with condyles; Orifice length (Zooidal plane) $263 \pm 27 \mu\text{m}$; Orifice width $318 \pm 25 \mu\text{m}$; Sinus U shaped; Sinus length (depth) $72 \pm 13 \mu\text{m}$; Sinus width $155 \pm 14 \mu\text{m}$ (Table 1); Operculum with distinct central band and two clearer swirls (Fig. 1b); Latero-oral intrazoooidal septula present (Fig. 2G–I); Pseudopore diameter $24 \pm 5 \mu\text{m}$; Condyles bar-shaped. Slight differences were observed between individuals from the Toulon Bay and the Marseilles Old Harbor. Toulon individuals were slightly larger, however, their Latero-oral intrazoooidal septula were most often smaller than pseudopores, while they were larger than pseudopores for Marseilles individuals (Table 1). During a 3 h scan of the 200 g of sampled colonies, no other species than *W. subatra* could be identified.

Phylogenetic analysis

The alignment used for phylogenetic analysis contained 99 unique sequences and was 493 base pairs long. Of the 44 models of sequence evolution tested in ‘Modeltest-ng’, the HKY+G4 model had the highest lnLikelihood using BIC and AICc, while the TVM+G4 model had the highest lnLikelihood using AIC. Given the agreement between BIC and AICc, HKY+G4 was selected. Phylogenetic relationships were similar between trees produced with maximum likelihood and Bayesian methods. The phylogenetic tree obtained with the combined results of three independent runs on ‘BEAST’ is shown in Fig. 3. An extended version of this figure as well as all retrieved sequences can be found in the supplementary material (Suppl.

Table 1. Dimensions of the zooids (Mean \pm SD in μm) of *Watersipora subatra* in the sampled areas. ZL: Zooid Length; ZW: Zooid Width; OL: Orifice Length; OW: Orifice Width; SinL: Sinus Length; SinW: Sinus Width; PorD: Pseudopore Diameter; IZSD: Intrazoooidal Septula Diameter.

	Toulon Bay			Marseille			Total Mediterr.			Atlantic		
	Mean	\pm	SD	Mean	\pm	SD	Mean	\pm	SD	Mean	\pm	SD
ZL	1179	\pm	137	935	\pm	166	1118	\pm	178	878	\pm	26
ZW	488	\pm	134	441	\pm	132	476	\pm	132	430	\pm	71
OL	270	\pm	20	242	\pm	35	263	\pm	27	221	\pm	17
OW	323	\pm	21	301	\pm	31	318	\pm	25	298	\pm	57
SinL	70	\pm	14	75	\pm	9	72	\pm	13	46	\pm	10
SinW	153	\pm	13	163	\pm	15	155	\pm	14	138	\pm	25
PorD	24	\pm	5	25	\pm	4	25	\pm	4	23	\pm	4
IZSD	25	\pm	5	36	\pm	6	28	\pm	7	19	\pm	6

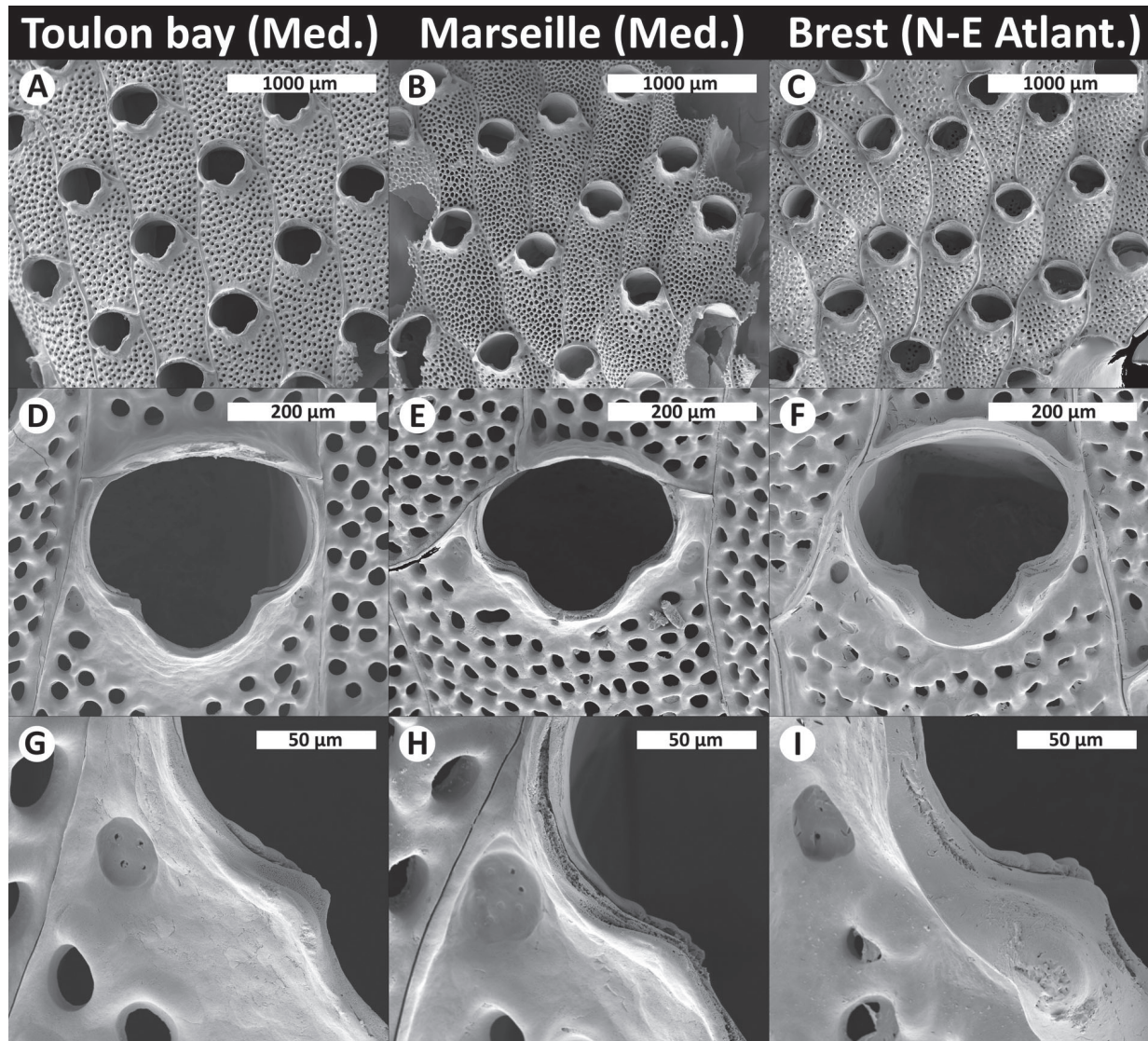


Figure 2. Electron microscope photographs of *Watersipora subatra* colonies from Toulon, Marseilles, and Brest (A–C), their orifices with more detail (D–F) as well as their intra-zooidal septula, orifice margin and condyles (G–I) of the three sampled areas (columns) (Nicolas Gayet).

materials 1, 3: table S1 and fig. S1). Nodes were annotated with posterior probabilities from the ‘BEAST’ analysis followed by the ultra-fast bootstrap values from ‘iqtree’, both expressed as percentages (*i.e.*, 100/100).

Clade 1 formed a monophyletic group with strong support (100/100). All sequences were attributed to *W. arcuata* from three different studies, with samples from Australia, California and Hawaii (Mackie et al. 2006, 2012; Anderson and Haygood 2007) grouped in this clade. Two sequences previously attributed to “*W. subtorquata*” (Mackie et al. 2012) were also clustered within this group. Four additional clades were also found to have strong support values (ranging from 100/97 to 100/100), presumably corresponding to species level distinctions.

Clade 2 included the sequences for *W. subtorquata sensu* Vieira et al. (2014) from Galapagos (McCann et al. 2019), “*W. subovoidea*” from Brazil, Florida and Australia (Mackie et al. 2006; Mackie et al. 2012) and unidentified *Watersipora* sp. from California, Australia (Susick et al. 2020) and Washington (Mackie et al. 2012).

Clade 3 included sequences attributed to a potentially undescribed species of *Watersipora* sp. from California (Mackie et al. 2006, 2012) and “*W. subtorquata*” from Korea (Lee et al. 2011) and California (Anderson and Haygood 2007; Mackie et al. 2014).

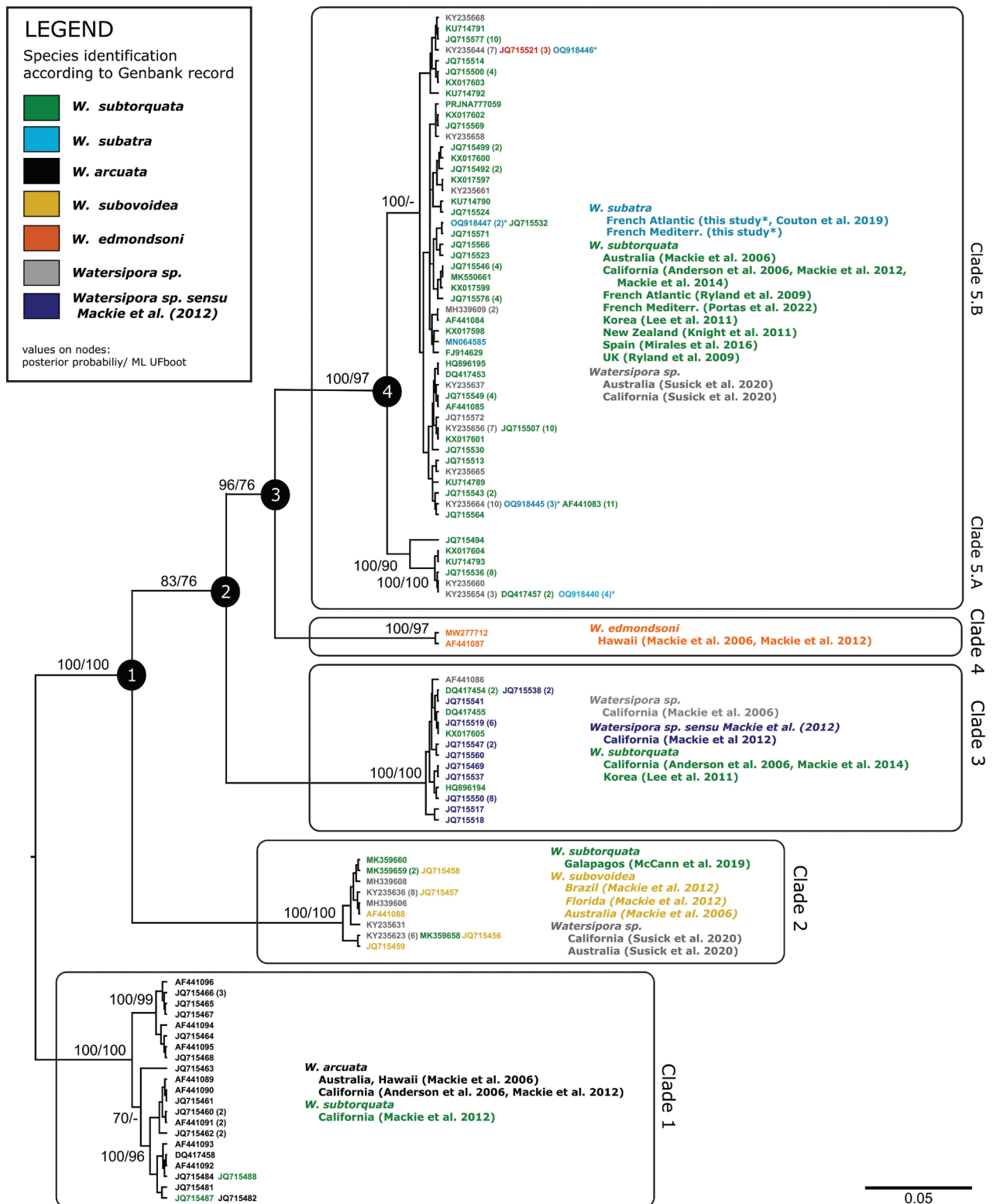


Figure 3. Combined Bayesian (BEAST) and Maximum likelihood (iqtree) phylogenetic tree of all *Watersipora* COI sequences available on Genbank (With accession number and corresponding source references). Numbers at the nodes correspond to the posterior probabilities from the ‘BEAST’ analysis followed by the ultra-fast bootstrap values from ‘iqtree’, both expressed as percentages (*i.e.*, 100/100). Only one accession number was listed per species for redundant sequences (number identical of sequences in parenthesis). New sequences acquired in this study are indicated by *. Color coding refers to species names listed on Genbank. Corrected identification (See Suppl. materials 1, 3: table S1 and fig. S1) are: Clade 1 *W. arcuata*; Clade 2 *W. subtorquata*; Clade 3 “*Watersipora sp. sensu Mackie et al., (2012)*”; Clade 4 “*W. edmondsoni*”; Clade 5 *W. subatra*.

Clade 4 included two sequences attributed to “*W. edmondsoni*” Soule & Soule, 1968 from Hawaii (Mackie et al. 2006; Mackie et al. 2012), a species currently considered invalid and synonymous with *W. subtorquata* (Vieira et al. 2014).

Clade 5 is comprised of sequences attributed to *W. subatra sensu* Vieira et al. (2014) from the Atlantic (Couton et al. 2019), unidentified *Watersipora* sp. from Australia and California (Susick et al. 2020) “*W. subtorquata*” from Australia (Mackie et al. 2006), California (Anderson and Haygood 2007; Mackie et al. 2012, 2014; Suppl. material 1: table S1 [MK550661](#)), Korea (Lee et al. 2011), New Zealand (Knight et al. 2011), Spain (Miralles et al. 2018), the United Kingdom (Ryland et al. 2009) and the French Mediterranean and French Atlantic (Ryland et al. 2009; Portas et al. 2022). This clade also contained our *W. subatra* samples from the French Atlantic and French Mediterranean. Clade 5 can itself be subdivided into two sub-clades (Clade 5.A and Clade 5.B; node 4) which correspond to the two clades previously identified in Mackie et al. (2012; as “*W. subtorquata*”).

Phylogenetic relationships among the clades 2–5 indicate that clades 4 and 5 (“*W. edmondsoni*” and *W. subatra*) are sister taxa grouped into node 3 (96/76). “*Watersipora* sp. *sensu* Mackie et al. (2012)” is grouped with node 3 into node 2, although support values were low for this node (83/76). Finally, *W. subtorquata* was sister to node 2 containing “*W. edmondsoni*”, *W. subatra* and “*Watersipora* sp. *sensu* Mackie et al. (2012)” clade (100/100; node 1).

Watersipora spp. distribution map

The new distribution map of *Watersipora* spp. (Fig. 4; Suppl. material 2: table S2) adds two species identities (“*W. edmondsoni*” and “*Watersipora* sp. *sensu* Mackie et al. (2012)”) and removes one (“*W. complanata*” now *Terwasipora complanata* (Norman, 1864)) compared to Vieira et al. (2014): figure 72). The updated map includes new records of *W. subatra* in Washington state, United States of America, and in the Mediterranean identified through the phylogeny and our samplings. This map extends records of *Watersipora* spp. to regional occurrence scales (*W. subatra* has for instance been shown to occur throughout S-E Australia). New reliable records of *W. subatra* in Norway and the Iberian Peninsula (Porter et al. 2015; Reverter-Gil and Souto 2019; Ramalho and Caballero-Herrera 2022), new records of *W. subtorquata* in Galapagos (McCann et al. 2019), as well as new records of *W. arcuata* throughout the Mediterranean (Ulman et al. 2017) are also included.

Discussion

At least five species of *Watersiporidae* have been reported from the Mediterranean Sea, including *Watersipora cucullata* (Busk, 1854), *W. souleorum*, *Terwasipora complanata* and the introduced *W. subtorquata* and *W. arcuata* (Vieira et al. 2014; Ulman et al. 2017; Reverter-Gil and Souto 2019). *Watersipora subatra*, despite being the most dominant introduced *Watersiporidae* in the north-eastern Atlantic, has only been recorded recently and sporadically in the Mediterranean Sea (Fernández-Romero et al. 2021; Ramalho and Caballero-Herrera 2022; Gauff et al. 2023a, b)). The individuals examined within our study unambiguously correspond to the morphological description of *W. subatra* in Vieira et al. (2014). This is further validated by the genetic analysis that cluster our Mediterranean individuals within the clade regrouping individuals of “*W. subtorquata*” from Mackie et al. (2006, 2012), that were resolved as *W. subatra* (Vieira et al. 2014), individuals identified as *W. subatra* from the French Atlantic (Couton et al. 2019) and our own *W. subatra* from the French Atlantic. We thus can confidently report the presence of this species in

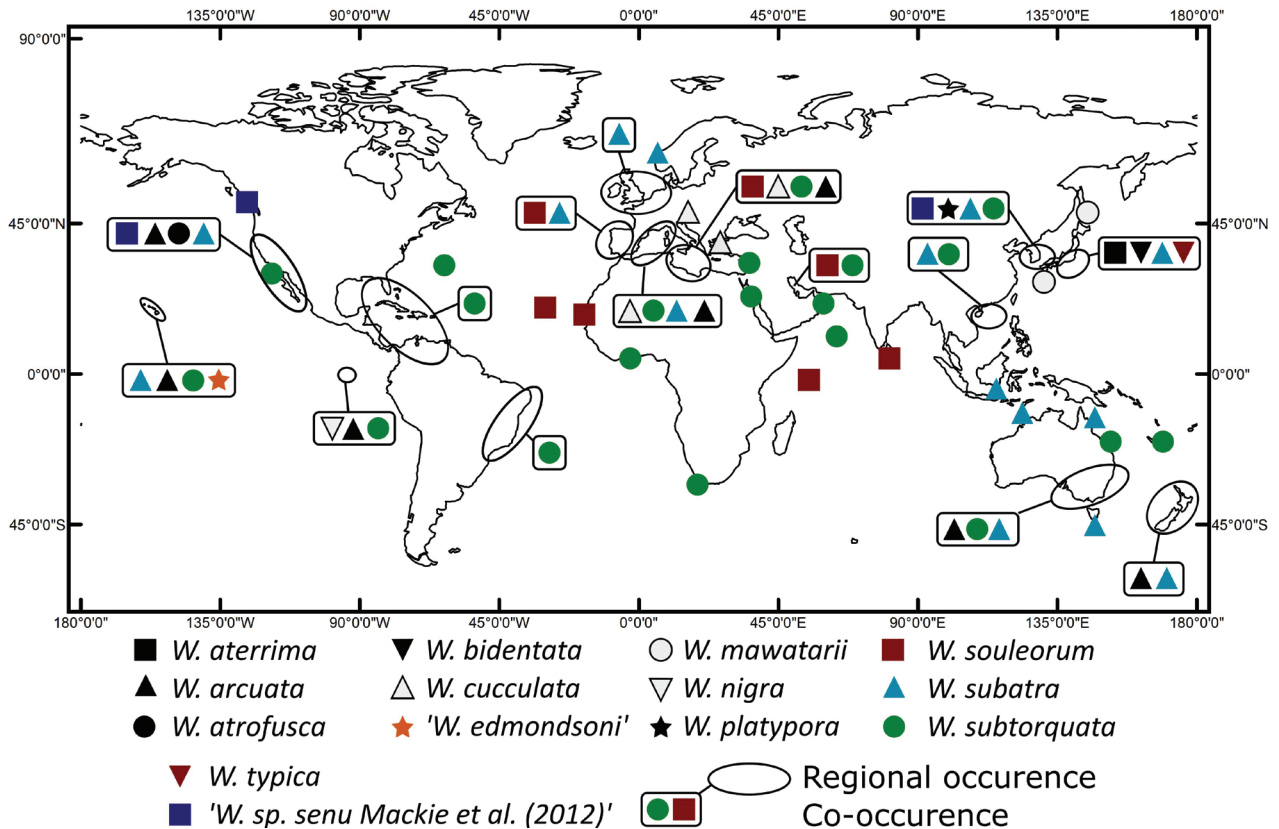


Figure 4. Distribution map of *Watersipora* spp. completing Vieira et al. (2014; fig. 72.) with updated species occurrences and identities based on genetic sequences, and recent reports (Porter et al. 2015; Ulman et al. 2017; McCann et al. 2019; Reverter-Gil and Souto 2019; Ramalho and Caballero-Herrera 2022; See also Suppl. material 2: table S2).

the French Mediterranean Sea. Out of the 13 accepted *Watersipora* spp., all four species that are spreading throughout the world (*W. subtorquata*, *W. souleorum*, *W. arcuata* and *W. subatra*) are thus now present and established in the Mediterranean Sea. *Watersipora subatra* is an invasive fouling species, mostly dispersed by ship traffic, that has recently spread from the north-east Atlantic towards the south of the Iberian Peninsula, suggesting that its introduction into the Mediterranean Sea likely occurred through the straits of Gibraltar (Reverter-Gil and Souto 2019). France possesses an Atlantic and Mediterranean coast, harboring numerous introduced species (Massé et al. 2023). National commerce and exchange (such as shellfish culture) could favor species transfers between those two provinces (Bachelet et al. 2004; Fernández-Rodríguez et al. 2022). Direct ship traffic between the naval bases of Toulon and Brest (Atlantic), where *W. subatra* is common (see Gauff et al. 2022; Rondeau et al. 2022) might further increase this risk of introduction. It is, therefore, not unexpected to find this species in a large Mediterranean harbor like Toulon.

More troubling is the high abundance of this species, suggesting that it is well established and has been present for some time already. This species seems to have been present for at least four years in the Mediterranean, as a previous study in Marseilles has tentatively identified the species in 2019 (Gauff et al. 2023b). *Watersipora subatra* seems to be the most dominant *Watersipora* species in the French Mediterranean as we did not detect any *W. subtorquata* despite this species being identified in the Toulon Darse Nord Marina in past studies (Gauff et al. 2023a)). This might suggest that the species has been misidentified as *W. subtorquata* for several years. The comparatively low number of taxonomic experts still in full activity in this area might explain such misidentifications/absence of identifications (Ferrario et al. 2018). Taxonomic

expertise requires much time and rigor (Caley et al. 2013; Coleman and Radulovici 2020). Currently, there is an increasing tendency to substitute taxonomy with time-efficient genetic tools to either confirm species identifications by barcoding (Liu et al. 2017; Kenworthy et al. 2018) or to detect species in an area *via* metabarcoding (Leray and Knowlton 2015; Miralles et al. 2016; Ardura and Planes 2017; Couton et al. 2019, 2022; Azevedo et al. 2020). These tools can be quite powerful to detect NIS, especially when combined with morphological analyses (Azevedo et al. 2020; Couton et al. 2022), however the efficiency of the method depends on the quality of reference sequences (Viard et al. 2019; Couton et al. 2022). Species identification based on molecular methods still require detailed morphological identifications to be carried out when reference sequences are generated. Integrative taxonomy, where genetics and morphology are both carefully considered, is required in order to ensure accurate species identifications (Dayrat 2005). Monitoring programs that use eDNA or other molecular approaches require reliable databases, with reference sequences generated from specimens that have been carefully identified or updated once errors are detected. The lack of genetic references for some species can result in missing or misidentified NIS and other species using metabarcoding (Couton et al. 2022). Even more problematic however, are reference sequences with misidentifications, as they provide a false sense of certitude to authors without taxonomic expertise (Viard et al. 2019; Cognato et al. 2020). Vieira et al. (2014) first noted that high numbers of *Watersipora* identifications (ex. Mackie et al. 2006; Mackie et al. 2012) were erroneous and our phylogeny has since revealed that 49% of *Watersipora* sequences on Genbank were incorrectly identified, a percentage that rises to 65% when excluding sequences identified only to the genus level. Most misidentifications concern *W. subatra* being listed as “*W. subtorquata*”. This causes subsequent identification errors in publications using these reference sequences (ex: Duncan et al. 2022; Miralles et al. 2016; Portas et al. 2022) This problem with non-updated sequences has already been pointed out in the past, as it prevents clear conclusions on species identity and origin (Miralles et al. 2018). This might explain why *W. subatra* has not been detected for a long time in the Mediterranean Sea. One must note that a recent checklist of NIS in France (Massé et al. 2023) includes *W. subatra* in the Atlantic and Mediterranean as an established species, but the absence of *W. subtorquata* in this list suggests that both species are potentially synonymized. Our updated genetic reference list could be used as a guide by authors having deposited *Watersipora* sp. sequences to update their species identity.

Our phylogeny includes two problematic species identifications: “*Watersipora* sp. *sensu* Mackie et al. (2012)” and “*W. edmondsoni*”. The first can be attributed to the genetic description of a novel species from Mackie et al. (2012). This species however lacks a morphological description and could thus be an already described species that simply lacks a corresponding genetic sequence for now. It may be *Watersipora atrofusca*, as it co-occurs with “*Watersipora* sp. *sensu* Mackie et al. (2012)” in California. However a record of “*Watersipora* sp. *sensu* Mackie et al. (2012)” identified as “*W. subtorquata*” in Korea (HQ896194, Lee et al. 2011) suggests one of three alternatives: either *W. atrofusca* is also present in Korea, another species corresponds to “*Watersipora* sp. *sensu* Mackie et al. (2012)”, or “*Watersipora* sp. *sensu* Mackie et al. (2012)” does indeed constitute an undescribed species. Without morphological identification of the individuals, inference on these three options remains speculative. “*Watersipora edmondsoni*” constitutes a similar problem. The holotype of *W. edmondsoni* Soule & Soule, 1968 was reexamined by Vieira et al. (2014) and has been reattributed as synonymous to *W. subtorquata*. We here however note a genetically distinct clade containing the individuals identified as “*W. edmondsoni*” by two separate studies (Mackie et al. 2006; Suppl. material 1: table S1 MW277712). Vieira et al. (2014) suggested that specimens reported as *W. edmondsoni* in Soule and Soule

(1975) could indeed include one or more species. The identified sequences could thus potentially be attributed to *W. edmondsoni sensu* Soule and Soule (1975) (*non* Soule & Soule, 1968). Further specimens and sequences are required to resolve the species status of specimens reported as “*W. edmondsoni*”.

Due to the high damages of NIS on ecosystems and the economy (Blackburn et al. 2019; Diagne et al. 2021), they constitute a key global biosecurity issue (Lovell et al. 2006; Olson 2006; Pyšek et al. 2020). Proper monitoring of their spread is thus crucial for preventing or mitigating their impact (Pyšek et al. 2020). This however requires correct identification, as the species identity may impact how we evaluate NIS invasion patterns (Vieira et al. 2014; Viard et al. 2019; Golo et al. 2023). Here we show that some authors may have been misled due to misidentifications of *Watersipora* spp. (prior to its’ redescription) in the reference literature and in genetic reference banks. We suggest that authors should maintain genetic references in accordance with new research by including the name originally used on their research and potential changes to their ID after reexamination. However, this would be very time consuming. A new way of genetic database management, similar to the WoMRS database, might compensate for the time-consuming nature of follow-up corrections on sequences. Taxonomists and geneticists dedicated to a family/genus could have a right to modify scientific names associated with sequences following the recommendations of recent peer-reviewed papers. Changes (by whom, references, etc.) should be tracked for transparency. This could help avoid the perpetuation of errors and improve the monitoring of both the spread of NIS and species distributions in general. Our new sequences, as well as the table updating the identity of almost all existing *Watersipora* sequences (Suppl. material 1: table S1) may help with future identification of different *Watersipora* species.

Funding declaration

This study was supported by the Région Bretagne through the CoEcoDigue project. (Ref/Région n° 2017-01, Ref/Ifremer n°21/1001756) and by the Chambre de Commerce et d’Industrie (CCI) du Var. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors’ contribution

Robin P. M. Gauff: research conceptualization, methodology, investigation and data collection, data analysis and interpretation, writing – original draft; Marc Bouchoucha: research conceptualization, investigation and data collection, funding provision, writing – review & editing; Amelia Curd: research conceptualization, funding provision, writing – review & editing; Gabin Droual: research conceptualization, investigation and data collection, data analysis and interpretation, writing – review & editing; Justine Evrard: investigation and data collection, data analysis and interpretation, writing – review & editing; Nicolas Gayet: investigation and data collection; Flavia Nunes: research conceptualization, methodology, investigation and data collection, data analysis and interpretation, funding provision, writing – review & editing.

Acknowledgements

We wish to thank the harbor authorities of the Toulon Darse Nord marina, La Seyne sur Mer marina and Old Harbor of Marseilles for authorizing access for sampling of *Watersipora* colonies. We also wish to thank Jasmine Ferrario (University of Pavia) and Leandro Manzoni Vieira (University of São Paulo) for discussions on *Watersipora* spp. We wish to also thank the reviewers of this article.

References

- Aleman A, Faasse M, Blauwe HDe (2018) The invasive bryozoan *Watersipora subatra* in the Netherlands [In Dutch]. *Concept - Het Zeepaard* 78(4): 50–54.
- Anderson CM, Haygood MG (2007) Alpha-proteobacterial symbionts of marine bryozoans in the genus *Watersipora*. *Applied and environmental microbiology* 73(1): 303–311. <https://doi.org/10.1128/AEM.00604-06>
- Araújo DF, Ponzevera E, Briant N, Knoery J, Bruzac S, Sireau T, Brach-Papa C (2019) Copper, zinc and lead isotope signatures of sediments from a mediterranean coastal bay impacted by naval activities and urban sources. *Applied Geochemistry* 111: 104440. <https://doi.org/10.1016/j.apgeochem.2019.104440>
- Ardura A, Planes S (2017) Rapid assessment of non-indigenous species in the era of the eDNA barcoding: A Mediterranean case study. *Estuarine, Coastal and Shelf Science* 188: 81–87. <https://doi.org/10.1016/j.ecss.2017.02.004>
- Azevedo J, Antunes JT, Machado AM, Vasconcelos V, Leão PN, Froufe E (2020) Monitoring of bio-fouling communities in a Portuguese port using a combined morphological and metabarcoding approach. *Scientific Reports* 10: 13461. <https://doi.org/10.1038/s41598-020-70307-4>
- Bachelet G, Simon-Bouhet B, Desclaux C, Garcia-Meunier P, Mairesse G, de Montaudouin X, Raigné H, Randriambao K, Sauriau PG, Viard F (2004) Invasion of the eastern Bay of Biscay by the nassariid gastropod *Cyclope neritea*: Origin and effects on resident fauna. *Marine Ecology Progress Series* 276(1): 147–159. <https://doi.org/10.3354/meps276147>
- Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. *Nucleic Acids Research* 41(Issue D1): D36–D42. <https://doi.org/10.1093/nar/gks1195>
- Blackburn TM, Bellard C, Ricciardi A (2019) Alien versus native species as drivers of recent extinctions. *Frontiers in Ecology and the Environment* 17(4): 203–207. <https://doi.org/10.1002/fee.2020>
- Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology and Evolution* 26(7): 333–339. <https://doi.org/10.1016/j.tree.2011.03.023>
- Bouchoucha M, Darnaude AM, Gudefin A, Neveu R, Verdoit-Jarraya M, Boissery P, Lenfant P (2016) Potential use of marinas as nursery grounds by rocky fishes: Insights from four *Diplodus* species in the Mediterranean. *Marine Ecology Progress Series* 547: 193–209. <https://doi.org/10.3354/meps11641>
- Bouchoucha M, Brach-Papa C, Gonzalez JL, Lenfant P, Darnaude AM (2018a) Growth, condition and metal concentration in juveniles of two *Diplodus* species in ports. *Marine Pollution Bulletin*, 126: 31–42. <https://doi.org/10.1016/j.marpolbul.2017.10.086>
- Bouchoucha M, Pécheyran C, Gonzalez JL, Lenfant P, Darnaude AM (2018b) Otolith fingerprints as natural tags to identify juvenile fish life in ports. *Estuarine, Coastal and Shelf Science* 212: 210–218. <https://doi.org/10.1016/j.ecss.2018.07.008>
- Caley MJ, O’Leary RA, Fisher R, Low-choy S, Johnson S, Mengersen K (2013) What is an expert? A systems perspective on expertise. *Ecology and Evolution* 4(3): 231–242. <https://doi.org/10.1002/ece3.926>
- Carrasco LR, Chan J, McGrath FL, Nghiem LTP (2017) Biodiversity conservation in a telecoupled world. *Ecology and Society* 22(3): 24. <https://doi.org/10.5751/ES-09448-220324>
- Cognato AI, Sari G, Smith SM, Beaver RA, Li Y, Hulcr J, Jordal BH, Kajimura H, Lin C-S, Pham TH, Singh S, Sittichaya W (2020) The Essential Role of Taxonomic Expertise in the Creation of DNA Databases for the Identification and Delimitation of Southeast Asian Ambrosia Beetle Species (Curculionidae: Scolytinae: Xyleborini). *Frontiers in Ecology and Evolution* 8(27): 1–17. <https://doi.org/10.3389/fevo.2020.00027>
- Coleman CO, Radulovici AE (2020) Challenges for the future of taxonomy: talents, databases and knowledge growth. *Megataxa* 001(1): 28–34. <https://doi.org/10.11646/megataxa.1.1.5>
- Couton M, Comtet T, Le Cam S, Corre E, Viard F (2019) Metabarcoding on planktonic larval stages: An efficient approach for detecting and investigating life cycle dynamics of benthic aliens. *Management of Biological Invasions* 10(4): 657–689. <https://doi.org/10.3391/mbi.2019.10.4.06>

- Couton M, Lévêque L, Daguin-Thiébaud C, Comtet T, Viard F (2022) Water eDNA metabarcoding is effective in detecting non-native species in marinas, but detection errors still hinder its use for passive monitoring. *Biofouling* 38(4): 367–383. <https://doi.org/10.1080/08927014.2022.2075739>
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37(1): 291–294. <https://doi.org/10.1093/molbev/msz189>
- Dayrat B (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85(3): 407–417. <https://doi.org/10.1111/j.1095-8312.2005.00503.x>
- Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić I, Salles JM, A Bradshaw CJ, Courchamp F (2021) High and rising economic costs of biological invasions worldwide. *Nature* 592: 571–576. <https://doi.org/10.1038/s41586-021-03405-6>
- Duncan M, Chow B, Myron K, Stone J, Hubbell M, Schriock E, Hunt C, Khtikian WK, Cohen CS (2022) First report of genetic data from two invasive *Watersipora* (Bryozoa) species in the central California coast rocky intertidal. *Aquatic Invasions* 17(2): 136–152. <https://doi.org/10.3391/ai.2022.17.2.01>
- Fernández-Rodríguez I, López-Alonso R, Sánchez O, Suárez-Turienzo I, Gutiérrez-Martínez R, Arias A (2022) Detection and prevention of biological invasions in marinas and ports: Epibionts and associated fauna of *Mytilus galloprovincialis* revisited. *Estuarine, Coastal and Shelf Science* 274: 107943. <https://doi.org/10.1016/j.ecss.2022.107943>
- Fernández-Romero A, Navarro-Barranco C, Ros M, Arias A, Moreira J, Guerra-García JM (2021) To the Mediterranean and beyond: An integrative approach to evaluate the spreading of *Branchioma luctuosum* (Annelida: Sabellidae). *Estuarine, Coastal and Shelf Science* 254: 107357. <https://doi.org/10.1016/j.ecss.2021.107357>
- Ferrario J, Rosso A, Marchini A, Occhipinti-Ambrogi A (2018) Mediterranean non-indigenous bryozoans: An update and knowledge gaps. *Biodiversity and Conservation* 27(11): 2783–2794. <https://doi.org/10.1007/s10531-018-1566-2>
- Gauff RPM, Davoult D, Greff S, Bohner O, Coudret J, Jacquet S, Loisel S, Rondeau S, Sevin L, Wafo E, Lejeune C (2022) Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment. *Science of The Total Environment* 838: 155911. <https://doi.org/10.1016/j.scitotenv.2022.155911>
- Gauff RPM, Joubert E, Curd A, Carlier A, Chavanon F, Ravel C, Bouchoucha M (2023a) The elephant in the room: Introduced species also profit from refuge creation by artificial fish habitats. *Marine Environmental Research* 185: 105859. <https://doi.org/10.1016/j.marenvres.2022.105859>
- Gauff RPM, Davoult D, Bohner O, Coudret J, Jacquet S, Loisel S, Rondeau S, Wafo E, Lejeune C (2023b) Unexpected biotic homogenization masks the effect of a pollution gradient on local variability of community structure in a marine urban environment. *Journal of Experimental Marine Biology and Ecology* 562: 151882. <https://doi.org/10.1016/j.jembe.2023.151882>
- Golo R, Vergés A, Díaz-Tapia P, Cebrian E (2023) Implications of taxonomic misidentification for future invasion predictions: Evidence from one of the most harmful invasive marine algae. *Marine Pollution Bulletin* 191: 114970. <https://doi.org/10.1016/j.marpolbul.2023.114970>
- Harmelin JG, Bitar G, Zibrowius H (2016) High xenodiversity versus low native diversity in the south-eastern Mediterranean: Bryozoans from the coastal zone of Lebanon. *Mediterranean Marine Science* 17(2): 417–439. <https://doi.org/10.12681/mms.1429>
- Jardine SL, Sanchirico JN (2018) Estimating the cost of invasive species control. *Journal of Environmental Economics and Management* 87: 242–257. <https://doi.org/10.1016/j.jeem.2017.07.004>
- Johnston MW, Purkis SJ, Dodge RE (2015) Measuring Bahamian lionfish impacts to marine ecological services using habitat equivalency analysis. *Marine Biology* 162(12): 2501–2512. <https://doi.org/10.1007/s00227-015-2745-2>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>

- Kenworthy JM, Rolland G, Samadi S, Lejeune C (2018) Local variation within marinas: Effects of pollutants and implications for invasive species. *Marine Pollution Bulletin* 133: 96–106. <https://doi.org/10.1016/j.marpolbul.2018.05.001>
- Knight S, Gordon DP, Lavery SD (2011) A multi-locus analysis of phylogenetic relationships within cheilostome bryozoans supports multiple origins of ascophoran frontal shields. *Molecular Phylogenetics and Evolution* 61(2): 351–362. <https://doi.org/10.1016/j.ympev.2011.07.005>
- Leclerc JC, Viard F (2017) Habitat formation prevails over predation in influencing fouling communities. *Ecology and Evolution* 8: 477–492. <https://doi.org/10.1002/ece3.3654>
- Lee H-J, Kwan Y-S, Kong S-R, Min BS, Seo JE, Won Y-J (2011) DNA Barcode Examination of Bryozoa (Class: Gymnolaemata) in Korean Seawater. *Korean Journal of Systematic Zoology* 27(2): 159–163. <https://doi.org/10.5635/KJSZ.2011.27.2.159>
- Leray M, Knowlton N (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America* 112(7): 2076–2081. <https://doi.org/10.1073/pnas.1424997112>
- Levine JM, D'Antonio CM (2003) Forecasting Biological Invasions with Increasing International Trade. *Conservation Biology* 17(1): 322–326. <https://doi.org/10.1046/j.1523-1739.2003.02038.x>
- Liu J, Jiang J, Song S, Tornabene L, Chabarria R, Naylor GJP, Li C (2017) Multilocus DNA barcoding - Species Identification with Multilocus Data. *Scientific Reports* 7(1): 16601. <https://doi.org/10.1038/s41598-017-16920-2>
- Lovell SJ, Stone SF, Fernandez L (2006) The economic impacts of aquatic invasive species: A review of the literature. *Agricultural and Resource Economics Review* 35(1): 195–208. <https://doi.org/10.1017/S1068280500010157>
- Mackie JA, Keough MJ, Christidis L (2006) Invasion patterns inferred from cytochrome oxidase I sequences in three bryozoans, *Bugula neritina*, *Watersipora subtorquata*, and *Watersipora arcuata*. *Marine Biology* 149: 285–295. <https://doi.org/10.1007/s00227-005-0196-x>
- Mackie JA, Darling JA, Geller JB (2012) Ecology of cryptic invasions: Latitudinal segregation among *Watersipora* (Bryozoa) species. *Scientific Reports* 2: 871. [10 pp.] <https://doi.org/10.1038/srep00871>
- Mackie JA, Wostenberg D, Doan M, Craig SF, Darling JA (2014) High-throughput Illumina sequencing and microsatellite design in *Watersipora* (Bryozoa), a complex of invasive species. *Conservation Genetics Resources* 6: 1053–1055. <https://doi.org/10.1007/s12686-014-0286-5>
- Massé C, Viard F, Humbert S, Antajan E, Auby I, Bachelet G, Bernard G, Bouchet VMP, Burel T, Dauvin J-C, Delegrange A, Derrien-Courtrel S, Droual G, Gouillieux B, Gouilletquer P, Guérin L, Janson AL, Jourde J, Labruno C, Lavesque N, Leclerc JC, Le Duff M, Le Garrec V, Noël P, Nowaczyk A, Pergent-Martini C, Pezy JP, Raoux A, Raybaud V, Ruitton S, Sauriau PG, Spilmont N, Thibault D, Vincent D, Curd A (2023) An overview of marine non-indigenous species found in three contrasting biogeographic metropolitan french regions: Insights on distribution, origins and pathways of introduction. *Diversity* 15(2): 161. <https://doi.org/10.3390/d15020161>
- Mastrototaro F, Montesanto F, Salonna M, Viard F, Chimienti G, Trainito E, Gissi C (2020) An integrative taxonomic framework for the study of the genus *Ciona* (Ascidiacea) and description of a new species, *Ciona intermedia*. *Zoological Journal of the Linnean Society* 190(4): 1193–1216. <https://doi.org/10.1093/zoolinnean/zlaa042>
- Mazoyer C, Vanneste H, Dufresne C, Ourmières Y, Magaldi MG, Molcard A (2020) Impact of wind-driven circulation on contaminant dispersion in a semi-enclosed bay. *Estuarine, Coastal and Shelf Science* 233: 106529. <https://doi.org/10.1016/j.ecss.2019.106529>
- McCann LD, McCuller MI, Carlton JT, Keith I, Geller JB, Ruiz GM (2019) Bryozoa (Cheilostomata, Ctenostomata, and Cyclostomata) in Galapagos Island fouling communities. *Aquatic Invasions* 14(1): 85–131. <https://doi.org/10.3391/ai.2019.14.1.04>
- Meaille R, Wald L (1990) Using geographical information system and satellite imagery within a numerical simulation of regional urban growth. *International Journal of Geographical Information Systems* 4(4): 445–456. <https://doi.org/10.1080/02693799008941558>

- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30(5): 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Miralles L, Ardura A, Arias A, Borrell YJ, Clusa L, Dopico E, de Rojas AH, Lopez B, Muñoz-Colmenero M, Roca A, Valiente AG, Zaiko A, Garcia-Vazquez E (2016) Barcodes of marine invertebrates from north Iberian ports: Native diversity and resistance to biological invasions. *Marine Pollution Bulletin* 112 (1–2): 183–188. <https://doi.org/10.1016/j.marpolbul.2016.08.022>
- Miralles L, Ardura A, Clusa L, Garcia-Vazquez E (2018) DNA barcodes of Antipode marine invertebrates in Bay of Biscay and Gulf of Lion ports suggest new biofouling challenges. *Scientific Reports* 8: 16214. <https://doi.org/10.1038/s41598-018-34447-y>
- Olson LJ (2006) The economics of terrestrial invasive species: A review of the literature. *Agricultural and Resource Economics Review* 35(1): 178–194. <https://doi.org/10.1017/S1068280500010145>
- Papacostas KJ, Rielly-Carroll EW, Georgian SE, Long DJ, Princiotta SD, Quattrini AM, Reuter KE, Freestone AL (2017) Biological mechanisms of marine invasions. *Marine Ecology Progress Series* 565: 251–268. <https://doi.org/10.3354/meps12001>
- Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution* 24(9): 497–504. <https://doi.org/10.1016/j.tree.2009.03.016>
- Portas A, Quillien N, Culioli G, Briand JF (2022) Eukaryotic diversity of marine biofouling from coastal to offshore areas. *Frontiers in Marine Science* 9: 971939. <https://doi.org/10.3389/fmars.2022.971939>
- Porter JS, Jones MES, Kuklinski P, Rouse S (2015) First records of marine invasive non-native Bryozoa in Norwegian coastal waters from Bergen to Trondheim. *BioInvasions Records* 4(3): 157–169. <https://doi.org/10.3391/bir.2015.4.3.02>
- Porter JS, Nunn JD, Ryland JS, Minchin D, Spencer Jones ME (2017) The status of non-native bryozoans on the north coast of Ireland. *BioInvasions Records* 6(4): 321–330. <https://doi.org/10.3391/bir.2017.6.4.04>
- Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM (2020) Scientists' warning on invasive alien species. *Biological Reviews* 95(6): 1511–1534. <https://doi.org/10.1111/brv.12627>
- Ramalho LV, Caballero-Herrera JA (2022) Detection of five non-indigenous species in fishing ports of Málaga Province, Spain (southwestern Mediterranean). *Zootaxa* 5200(2): 196–200. <https://doi.org/10.11646/zootaxa.5200.2.7>
- Rambaut A (2010) FigTree v1.4.4. <http://tree.bio.ed.ac.uk/software/figtree/> [Accessed 9 June 2023]
- Reverter-Gil O, Souto J (2019) Watersiporidae (Bryozoa) in Iberian waters : an update on alien and native species. *Marine Biodiversity* 49: 2735–2752. <https://doi.org/10.1007/s12526-019-01003-4>
- Rondeau S, Davoult D, Lejeune C, Kenworthy JM, Bohner O, Loisel S, Gauff RPM (2022) Persistent dominance of non-indigenous species in the inner part of a marina highlighted by multi-year photographic monitoring. *Marine Ecology Progress Series* 690: 15–30. <https://doi.org/10.3354/meps14052>
- Rosso A, Martino EDi (2016) Bryozoan diversity in the Mediterranean Sea: an update. *Mediterranean Marine Science* 17(2): 567–607. <https://doi.org/10.12681/mms.1474>
- Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18(1): 1–26. <https://doi.org/10.1186/s12859-017-1934-z>

- Ruitton S, Javel F, Culioli JM, Meinesz A, Pergent G, Verlaque M (2005) First assessment of the *Caulerpa racemosa* (Caulerpales, Chlorophyta) invasion along the French Mediterranean coast. *Marine Pollution Bulletin* 50(10): 1061–1068. <https://doi.org/10.1016/j.marpolbul.2005.04.009>
- Ryland JS, Blauwe HDe, Lord R, Mackie JA (2009) Recent discoveries of alien *Watersipora* (Bryozoa) in Western Europe. *Zootaxa* 2093: 43–49. <https://doi.org/10.11646/zootaxa.2093.1.3>
- Salonna M, Gasparini F, Huchon D, Montesanto F, Haddas-Sasson M, Ekins M, McNamara M, Mastrototaro F, Gissi C (2021) An elongated COI fragment to discriminate botryllid species and as an improved ascidian DNA barcode. *Scientific Reports* 11(1): 4078. <https://doi.org/10.1038/s41598-021-83127-x>
- Sardain A, Sardain E, Leung B (2019) Global forecasts of shipping traffic and biological invasions to 2050. *Nature Sustainability* 2(4): 274–282. <https://doi.org/10.1038/s41893-019-0245-y>
- Seebens H, Schwartz N, Schupp PJ, Blasius B (2016) Predicting the spread of marine species introduced by global shipping. *Proceedings of the National Academy of Sciences of the United States of America* 113(20): 5646–5651. <https://doi.org/10.1073/pnas.1524427113>
- Soule DF, Soule JD (1975) Species groups in Watersiporidae. *Bryozoa* 1974. Documents des Laboratoires de Géologie de la Faculté des Sciences de Lyon. Hors série n°3: 299–309.
- Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4(1): 1–5. <https://doi.org/10.1093/ve/vey016>
- Susick K, Scianni C, Mackie JA (2020) Artificial structure density predicts fouling community diversity on settlement panels. *Biological Invasions* 22(2): 271–292. <https://doi.org/10.1007/s10530-019-02088-5>
- Tempesti J, Langeneck J, Maltagliati F, Castelli A (2020) Macrobenthic fouling assemblages and NIS success in a Mediterranean port: The role of use destination. *Marine Pollution Bulletin* 150: 110768. <https://doi.org/10.1016/j.marpolbul.2019.110768>
- Ulman A, Ferrario J, Occhipinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C, Chatzigeorgiou G, Çiçek BA, Deidun A, Ramos-Esplá A, Koçak C, Lorenti M, Martinez-Lai G, Merlo G, Princisgh E, Scribano G, Marchini A (2017) A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ* 5(10): e3954. <https://doi.org/10.7717/peerj.3954>
- Ulman A, Ferrario J, Forcada A, Arvanitidis C, Occhipinti-Ambrogi A, Marchini A (2019) A Hitchhiker's guide to Mediterranean marina travel for alien species. *Journal of Environmental Management* 241: 328–339. <https://doi.org/10.1016/j.jenvman.2019.04.011>
- Viard F, Roby C, Turon X, Bouchemousse S, Bishop J (2019) Cryptic diversity and database errors challenge non-indigenous species surveys: An illustration with *Botrylloides* spp. in the English Channel and Mediterranean Sea. *Frontiers in Marine Science* 6(615): 1–13. <https://doi.org/10.3389/fmars.2019.00615>
- Vieira LM, Jones MS, Taylor PD (2014) The identity of the invasive fouling bryozoan *Watersipora subtorquata* (d'Orbigny) and some other congeneric species. *Zootaxa* 3857(2): 151–182. <https://doi.org/10.11646/zootaxa.3857.2.1>
- Wafo E, Abou L, Nicolay A, Boissery P, Perez T, Ngonu Abondo R, Garnier C, Chacha M, Portugal H (2016) A chronicle of the changes undergone by a maritime territory, the Bay of Toulon (Var Coast, France), and their consequences on PCB contamination. *SpringerPlus* 5: 1230. <https://doi.org/10.1186/s40064-016-2715-2>
- Walsh JR, Carpenter SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences of the United States of America* 113(15): 4081–4085. <https://doi.org/10.1073/pnas.1600366113>
- Xia X (2018) DAMBE7: New and improved tools for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* 35(6): 1550–1552. <https://doi.org/10.1093/molbev/msy073>
- Zibrowius H (1991) Ongoing modification of the Mediterranean marine fauna and flora by the establishment of exotic species. *Mésogée* 51: 83–107.

Supplementary material 1

Watersipora sequences retrieved from Genbank

Authors: Robin P. M. Gauff, Marc Bouchoucha, Amelia Curd, Gabin Droual, Justine Evrard, Nicolas Gayet, Flavia Nunes

Data type: table (Excel spreadsheet)

Explanation note: table S1: *Watersipora* sequences retrieved from Genbank with their original identification, location, and reference as well as their new species attribution according to our phylogenetic tree (See Suppl. material 3: fig. S1).

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3391/ai.2023.18.3.108128.suppl1>

Supplementary material 2

Geographic references for the distribution of various *Watersipora* spp.

Authors: Robin P. M. Gauff, Marc Bouchoucha, Amelia Curd, Gabin Droual, Justine Evrard, Nicolas Gayet, Flavia Nunes

Data type: table (Excel spreadsheet)

Explanation note: table S2: Geographic references for the distribution of various *Watersipora* spp. used for generating Fig. 4 (note that species identity here corresponds to our identification and not necessarily to the original one).

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3391/ai.2023.18.3.108128.suppl2>

Supplementary material 3

Combined Bayesian (BEAST) and Maximum likelihood (iqtree) phylogenetic tree

Authors: Robin P. M. Gauff, Marc Bouchoucha, Amelia Curd, Gabin Droual, Justine Evrard, Nicolas Gayet, Flavia Nunes

Data type: figure (JPG file)

Explanation note: figure S1: Combined Bayesian (BEAST) and Maximum likelihood (iqtree) phylogenetic tree of all *Watersipora* COI sequences available on Genbank (With accession number and corresponding source references). Numbers at the nodes correspond to the posterior probabilities from the 'BEAST' analysis followed by the ultra-fast bootstrap values from 'iqtree', both expressed as percentages (i.e., 100/100). All accession number were listed in this extended version compared to Fig. 3. New sequences acquired in this study are indicated by *. Color coding refers to species names listed on Genbank. Corrected identification (See Suppl. material 1: table S1) are: Clade 1 *W. arcuata*; Clade 2 *W. subtorquata*; Clade 3 "*Watersipora* sp. *sensu* Mackie et al., (2012)"; Clade 4 "*W. edmondsoni*"; Clade 5 *W. subatra*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3391/ai.2023.18.3.108128.suppl3>