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ASSESSMENT OF BRYOZOAN XENODIVERSITY IN THE SLOVENIAN COASTAL SEA

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ABSTRACT

The phylum Bryozoa is one of the less studied macroinvertebrate groups in the Slovenian coastal sea. However, these animals play an important role in marine coastal ecosystems, especially as bioconstructors and filter feeders. Non-indigenous species represent five to ten percent of all Mediterranean bryozoan species. In this paper, we present an updated list of non-indigenous or cryptogenic bryozoan species recorded in Slovenia. The list includes six species: Amathia verticillata, Bugula neritina s.l.*,* Celleporaria brunnea, Tricellaria inopinata*,* Watersipora arcuata *and* Watersipora subtorquata *with comments on their morphology, ecology and distribution in the Slovenian coastal sea. Introduction vectors and possible dispersal mechanisms are discussed, as well as methodological approaches and problematic areas as regards the study of non-indigenous bryozoans. Three species reported here, A.* verticillata, C. brunnea *and* W. subtorquata*, represent the first confirmed record from the Slovenian Sea.*

Key words: non-indigenous species, Bryozoa, northern Adriatic Sea, harbour habitats, mussel farms, experimental plates

VALUTAZIONE DELLA XENODIVERSITÀ DI BRIOZOI NEL MARE COSTIERO SLOVENO

SINTESI

Il phylum Bryozoa è uno dei gruppi di macroinvertebrati meno studiati nel mare costiero sloveno. Tuttavia, questi animali svolgono un ruolo importante negli ecosistemi marini costieri, in particolare come biocostruttori e filtratori. Le specie non-indigene rappresentano dal 5 al 10 percento di tutte le specie di briozoi del Mediterraneo. L'articolo presenta un elenco aggiornato delle specie di briozoi non-indigeni o criptogenici ritrovate nel mare sloveno. L'elenco comprende sei specie: Amathia verticillata, Bugula neritina s.l.*,* Celleporaria brunnea, Tricellaria inopinata, Watersipora arcuata e Watersipora subtorquata*, con commenti sulla loro morfologia, ecologia e distribuzione nel mare costiero sloveno. Vengono discussi i vettori di introduzione e i possibili meccanismi di dispersione, nonché gli approcci metodologici e le aree di studio problematiche per i briozoi non-indigeni. Tre specie qui segnalate,* A. verticillata, C. brunnea e W. subtorquata*, rappresentano il primo ritrovamento nel mare sloveno.*

Parole chiave: specie non-indigene, Bryozoa, Adriatico settentrionale, habitat portuali, allevamenti di mitili, piastre sperimentali

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INTRODUCTION

Bryozoans are a phylum of predominantly marine colonial organisms. The phylum contains over 6000 known extant species (Bock & Gordon, 2013), which are found in the seas worldwide, whereas 556 of them are currently known to be present in the Mediterranean Sea (Rosso & Di Martino, 2016). Non-indigenous species (NIS) of bryozoans represent five to ten percent of all Mediterranean species (Rosso & Di Martino, 2016; Ferrario *et al*., 2017).

Although they are common in coastal seas, bryozoans are often overlooked, the primary reasons for that being economic insignificance, their inconspicuous nature and the lack of taxonomic expertise in the scientific community. Nevertheless, bryozoans play an important role in the ecosystems. One of the most important one is acting as bioconstructors, forming habitat and providing shelter and food for other organisms in the community (Cocito *et al*., 2000; Morgado & Tanaka, 2001; Gavira-O'Neill, 2016). Their calcareous, chitinous, membranaceus or gelatinous skeletons create diverse colony forms; from sheets to erect, highly branched morphotypes. In the fouling community, where the feeding mode of animals is predominately suspension feeding, they contribute greatly to the total filtration efficiency of the community (Bullivant, 1968a; Riisgård & Manríquez, 1997; Lisbjerg & Peterson, 2000).

Most bryozoan larvae are short-lived and in most cases cannot swim very far from their maternal colony (Keough, 1989). However, the sessile lifestyle of adult bryozoans enables them to colonise a variety of different man-made substrata and, therefore, allows them to spread easily. Furthermore, long-lived larvae or fragments of the colonies can potentially enter and exit ballast waters (Carlton & Geller, 1993). Thus, at global scale, non-indigenous bryozoans are introduced to new areas mainly through shipping (Ferrario *et al*., 2018). Specifically, the main pathways of introduction for non-indigenous bryozoans in the Mediterranean Sea include maritime transport and corridors (Zenetos *et al*., 2012). Not only are they able to travel great distances on ship hulls, but by harbouring a diverse epifauna, bryozoans can also facilitate the transport and spread of other NIS such as crustaceans (Marchini *et al*., 2015; Gavira-O'Neill *et al*., 2016).

The bryozoan fauna of Slovenia has been poorly investigated. It is one of the taxonomic groups, which was not included in early research of the Slovenian coastal sea (Matjašič & Štirn, 1975). Until 2018, 17 species had been recorded along the Slovenian coast, mostly during marine biodiversity surveys, published as lists of species in scientific reports and other publications (Vrišer, 1978; Frumen *et al*., 2003; Lipej *et al*., 2004, 2012a, 2013). Bryozoan xenodiversity is especially poorly studied. No bryozoan species are listed in the latest study of marine NIS in Slovenia (Lipej *et al*., 2012b). Recently, the status has improved, yielding a list of over 40 bryozoan species (unpublished data), including NIS, such as the record of *Tricellaria inopinata* (Fortič & Mavrič, 2018). In this paper, we present six species of non-indigenous or cryptogenic bryozoans found in Slovenian territorial waters.

MATERIAL AND METHODS

Study area

The Slovenian coastal sea is a southern part of the shallow Gulf of Trieste, the northernmost portion of both the Adriatic and Mediterranean Seas. Salinity in the gulf is typically marine (33-38.5 ‰), but is affected by heavy freshwater inflows. Water temperature normally ranges between 7°C and 26°C (Mozetič & Lipej, 2003). Rivers, mostly Isonzo (Soča), are an important source of detrital material, which originates from the hinterland. This area is influenced by bottom sediment resuspension and increasing pollution (Ogorelec *et al*., 1991). The maximum recorded depth is 33 m. The sea-bed is predominantly soft sedimentary of fluvial origin, while the coastal bottom is mostly rocky, consisting mainly of Eocene Flysch sandstone rock layers, while soft sedimentary bottom prevails in internal bays (Ogorelec *et al*., 1997). In the past decades, the Slovenian coastal area has been subjected to many anthropogenic impacts such as new infrastructure and mariculture, resulting in habitat degradation, as well as intensive fishing and farming and poorly regulated sewage outfalls (Turk, 1999).

Slovenia receives a lot of maritime traffic due to one of the biggest ports in this area, the Port of Koper. Furthermore, there are many additional mooring sites for all sizes of recreational and other vessels, *e.g.* marinas, harbours, piers. In order to obtain the number and size of these mooring sites we performed a simple analysis in QGIS 3.8 Zanzibar, with information obtained from publicly available satellite imagery (Google Earth). We included all municipal mooring sites and other sites where vessels are berthed on a regular basis.

Field and laboratory work

We employed different methods for detecting non-indigenous bryozoans in the marine waters of Slovenia. The first part of the fieldwork consisted in occasional sampling of the fouling community at NIS fouling hotspots, namely, marinas, harbours and mussel farms (Fig. 1). We sampled all three active mussel farms (Sečovlje, Strunjan and Debeli rtič) located in the Slovenian coastal sea. The largest part of the study was carried out in harbours and marinas, as all mooring sites in Slovenia were included. Sampling was performed in the period between November 2016 and September 2019. The fouling community was scraped off various substrata, including vessel hulls, using a scraping net or a paint scraper. The samples were then transported

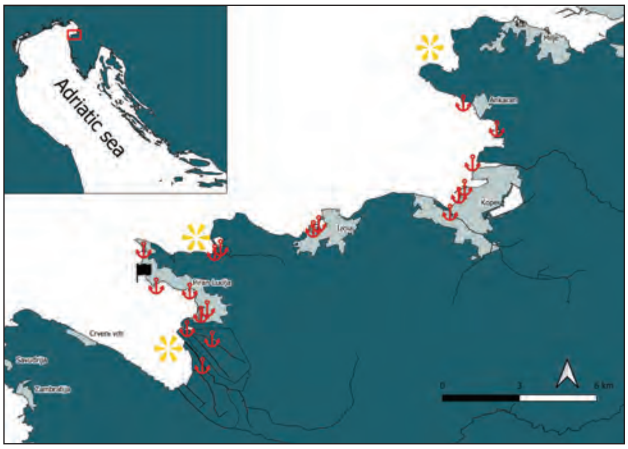

Fig. 1: Map of the Slovenian part of the Gulf of Trieste. Anchors denote the mooring sites; the flag shows the Morgan site of experimental structures and the asterisks the mussel farms.
Sl. 1: Zemljevid slovenskega dela Tržaškega zaliva. Sidra označujejo mesta privezov, zastava lokacijo eksperimentalnih struktur Morgan in zvezdice školjčišča.

to the laboratory and carefully examined for NIS. Some visual inspections were performed at different times of the year for conspicuous and easily identifiable species (*e.g. Amathia verticillata* and *Bugula neritina s.l.*). On few occasions, snorkelling and scuba diving sampling were performed in order to supplement the other work methods. Only presence data was recorded.

The second part of the study included an ongoing experiment on the succession of the fouling community on clay plates, submersed at 8-10 meters of depth. Metal structures carrying 30 vertically positioned tiles each, were gradually deployed every month over a year, starting in March 2018. The structures were set up in the Bay of Piran near the Marine Biology Station, at the locality named Morgan (see Fig. 1) in the Gulf of Trieste. The sea bottom there is muddy, with detritus elements of anthropogenic origin. Each month, a plate was taken from each structure. The plates were then carried to the laboratory, where they were photographed and carefully examined using a stereomicroscope (SZX 16, Olympus) with a camera (DP74, Olympus). This dataset contains presence and absence data on species.

If necessary, sampled species were measured and identified to the lowest possible taxon. They were preserved in an alcohol-based fixative (FineFix) and stored in the bryozoan collection of the Marine Biology Station (National Institute of Biology) in Piran.

RESULTS AND DISCUSSION

In this study, we present an updated list of non-indigenous or cryptogenic bryozoans recorded in Slovenia, with comments on their morphology, ecology and distribution in the Slovenian coastal sea. The introduction vectors and possible dispersal mechanisms are discussed. Three species reported here, *Amathia verticillata, Celleporaria brunnea* and *Watersipora subtorquata*, represent the first record from the Slovenian Sea.

Amathia verticillata (delle Chiaje, 1822)

Amathia verticillata is an erect ctenostomatid bryozoan. It forms large colonies on account of its fast growing stolons, on which clusters of spindle-shaped autozooids are arranged (Fig. 2E). The colonies are semi-transparent but, due to the epiphytes and detritus that are often attached to this bryozoan, they may appear brownish-grey or even green (Marić *et al*., 2017). Because of its large dimensions and structure, this species provides habitat for other macroinvertebrates *(e.g.* Marchini *et al*., 2015) and as such, can be considered as a bioconstructor species.

A. verticillata is regarded as a pseudoindigenous species. It was described in the Mediterranean Sea and was firstly believed to be native to the region, but recent data suggest that it was in fact introduced (Ferrario *et al*., 2014). *A. verticillata* was present in the Gulf of Trieste and other localities in the Mediterranean before 1870 (Reichert, 1870). It has a wide distribution range and it is found in tropical, subtropical and temperate waters, in the Atlantic and the Indo-Pacific region (Winston, 1995; Amat & Tempera, 2009; Bouzon *et al*., 2012; McCann *et al*., 2015; Prince *et al*., 2017; Aslam *et al*., 2019). The Caribbean is probably its native distribution range, mostly because it inhabits natural habitats there, as opposed to the Mediterranean basin and other locations, where it is found entirely in anthropogenically modified habitats. Its occurrence in marinas and harbours suggests an introduction by vessel fouling (Marchini *et al*., 2015). *A. verticillata* is considered as the most common non-indigenous bryozoan in the Mediterranean Sea (Ferrario *et al*., 2018). Nudibranch *Okenia zoobotryon* (Smallwood, 1910), another species native to the Caribbean, is known to live, feed and reproduce on this bryozoan. The close relationship between two species is another indication that *A. verticillata* is not native to the Mediterranean Sea (Winston, 1995; Galil & Gevili, 2014). However, a recent revision of the genus has revealed that several Mediterranean specimens previously believed to belong to *O. zoobotryon* are in fact another species, namely *Okenia longiductis* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli & Crocetta, 2019, and *O. zoobotryon* is restricted to the Western Atlantic Ocean. Further examination of more recent specimens is required to determine their identity (Pola *et al*., 2019).

It is a thermophilic (Bullivant, 1968b) and euryhaline species (Nair *et al*., 1992). Laboratory experiments have shown that it grows and sexually reproduces best at higher temperatures (25-26°C) (Bullivant, 1968b) and survives in a salinity range of 15-35 PSU (Nair *et al*., 1992).

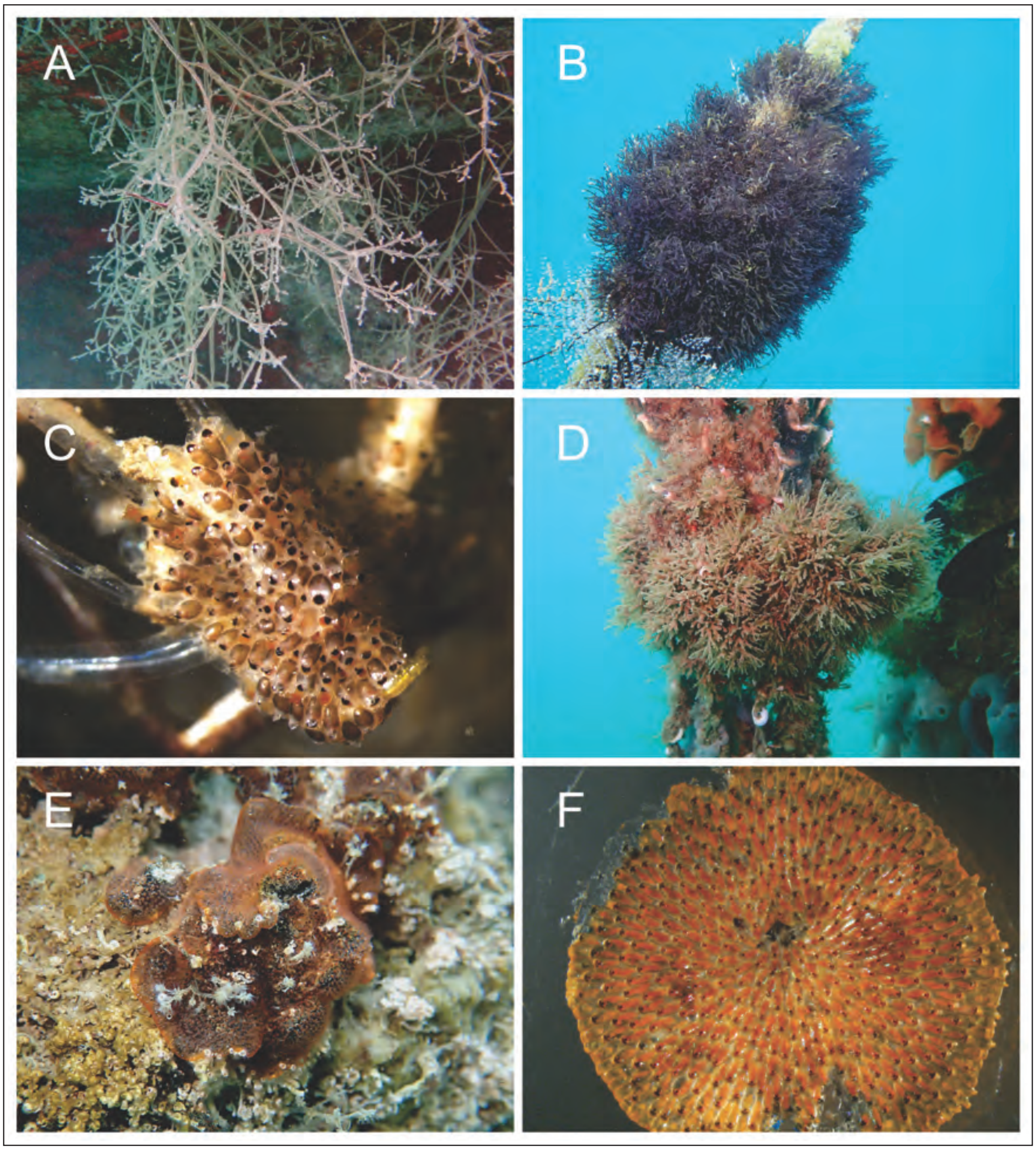

Fig. 2: Non-indigenous bryozoan species in the Slovenian sea: A) *Amathia verticillata****, B)*** *Bugula neritina****, C)*** *Celleporaria brunnea****, D)*** *Tricellaria inopinata****, E)*** *Watersipora arcuata****, F)*** *Watersipora subtorquata****. Photos: A. Fortič and B. Mavrič.***

Sl. 2: Tujerodne vrste mahovnjakov v slovenskem morju: A) *Amathia verticillata****, B)*** *Bugula neritina****, C)*** *Celleporaria brunnea****, D)*** *Tricellaria inopinata****, E)*** *Watersipora arcuata****, F)*** *Watersipora subtorquata****. Slike: A. Fortič in B. Mavrič.***

A. verticillata is one of the bryozoans that are almost exclusively found in the harbours and marinas of Slovenia, where it is well-established. In total, we recorded this species on 43 sampling occasions, which makes it the most common non-indigenous bryozoan in Slovenia (Fig. 3). The large colonies often appear together with *Bugula neritina s.l.* and *Cradoscrupocellaria bertholletii* (Audouin, 1826). The substrata where it was found primarily were vessel hulls and lines. The colonies were observed growing on concrete piers, metal stairs, floating docks and buoys. As expected, we observed that this species was the most abundant in number and size (colonies bigger than 50 cm) in the warmer part of the year (summer and early autumn). In winter, the colonies

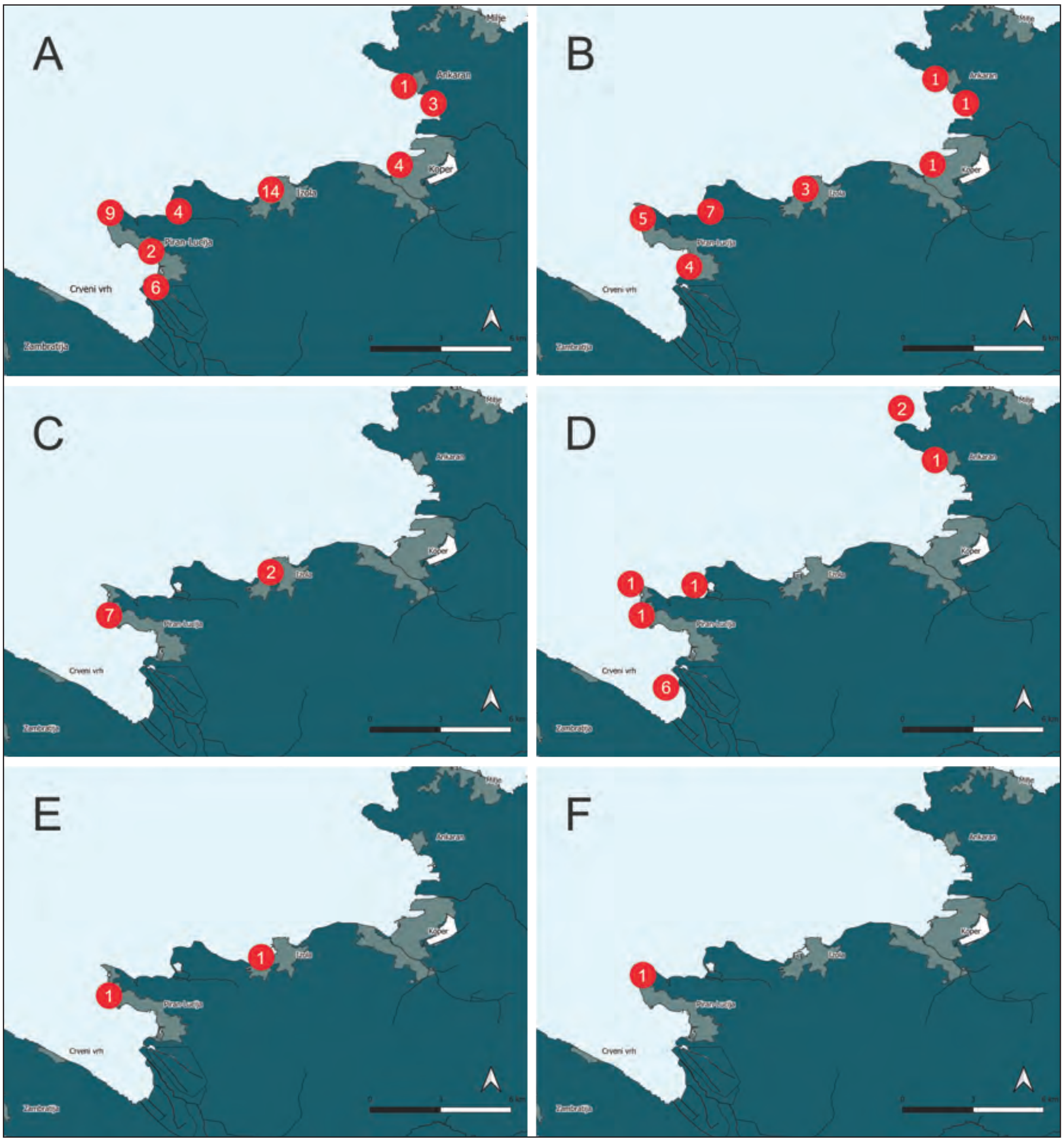

Fig. 3: Map of occurrence of non-indigenous bryozoan species. A circle denotes a macro location and the number inside the circle denotes the number of occurrences inside the macro location where the species was found: A) *Amathia verticillata*, ***B)*** *Bugula neritina*, ***C)*** *Celleporaria brunnea*, ***D)*** *Tricellaria inopinata*, ***E)*** *Watersipora arcuata*, ***F)*** *Watersipora subtorquata*.

Fig. 3: Zemljevid pojavljanja tujerodnih vrst mahovnjakov. Krog označuje število pojavljanj znotraj makrolokacije, kjer je bila vrsta najdena: A) *Amathia verticillata*, ***B)*** *Bugula neritina*, ***C)*** *Celleporaria brunnea*, ***D)*** *Tricellaria inopinata*, ***E)*** *Watersipora arcuata*, ***F)*** *Watersipora subtorquata*.

are probably reduced to fragments, which overwinter (*sensu* Geiger & Zimmer, 2002), as bigger colonies are no longer observed at that time.

***Bugula neritina s.l.* (Linnaeus, 1758)**

Bugula neritina s.l. is an erect cheilostomatid bryozoan. It is purple to brown in colour, and it is characterized by the lack of spines and bird-head avicularia, typical of the family Bugulidae. Round bright ovicells are oriented obliquely to the branch axis (Zabala & Maluquer, 1988) (Fig. 2B).

B. neritina s.l. was described in the Mediterranean Sea and America (Linnaeus, 1758). It is widely distributed in warm-temperate and subtropical coastal waters. Genetic studies have revealed that it is a complex of three cryptic species; one of them (Type S) is widespread in tropical, subtropical and temperate regions, most probably due to anthropogenic transport. Based on genetic diversity, the probable native range of this species is the north-eastern Pacific or the south-western Atlantic (Brazil) (Fehlauer-Ale *et al*., 2013). Only one Mediterranean sample has been included in genetic analysis (from Genoa, Italy) and has been confirmed to be the invasive haplotype (Ryland *et al*., 2011). Some authors consider *B. neritina s.l.* to be a NIS in the eastern Atlantic and the Mediterranean Sea (Ryland *et al*., 2011; Harmelin *et al*., 2016), introduced via shipping (Ryland *et al*., 2011), while others claim that it is cryptogenic (Ferrario *et al*., 2018). However, more tests should be performed in order to determine the origin of the Mediterranean *B. neritina*.

In Mediterranean waters, it is usually present throughout the year, but with a peak in abundance in the warmer part of the year, and it is not very common during the winter (Igić, 2007). In Florida, where this species is found in natural habitats, namely seagrass meadows, its populations increase in the autumn or even dominate during the winter, when the temperatures are lower (Keough & Chernoff, 1987; Winston, 1995). Haplotype-specific differences in temperature-related fitness for *B. neritina* have been observed (Fehlauer-Ale *et al*., 2013). Thus, different haplotypes might be observed in the future even within the Mediterranean populations.

To our knowledge, the first record of its presence in the Slovenian part of the Gulf of Trieste was from the Bay of Piran, in 2003 (Frumen *et al*., 2003). We consider *B. neritina s.l.* an established species and it is almost exclusively found in marinas and harbours. It attaches to vessel hulls, lines, piers, floating piers and to other fouling species, *e.g. A. verticillata*. In total, we recorded this species on 22 sampling occasions. It seems to be more common during the colder part of the year, in autumn and winter, when larvae were also present. Furthermore, it was observed at the Morgan site of experimental structures, in the Bay of Piran.

Celleporaria brunnea (Hincks, 1884)

Celleporaria brunnea is an encrusting white to brown cheilostomatid bryozoan. It is characterized by the dark brown colour of the opercula, sclerites of avicularia mandibles, base of spines and lophophore tentacles. The orifice distal margin is rounded with a moderately raised peristome usually bearing spines, whereas the proximal margin has a rounded border with condyles partially closing the opening (Ferrario *et al*., 2017).

Its native distribution range is the Northern Pacific and tropical Eastern Pacific (Lodola *et al*., 2015). The present distribution of this species extends from British Columbia to Ecuador and the Atlantic Sea (Soule & Soule, 1964; Canning-Clode *et al*., 2013). It was recorded in the Mediterranean in 2004 for the first time in Izmir Bay (Koçak, 2007). The first occurrence in the Adriatic Sea was reported in 2014, from Biograd na Moru, in the eastern Adriatic (Marić *et al*., 2017). The introduction pathway for this species is unknown. As it is commonly found in ports and harbours, as a fouling species on ships and artificial structures, shipping is probably the main pathway of introduction (Canning-Clode *et al*., 2013; Lodola *et al*., 2015).

This species has been known to inhabit tropical as well as warm and cool temperate waters (Soule & Soule, 1964). In the central Mediterranean, this species was mostly recorded during the warmer period of the year, from July to October (Lezzi *et al*., 2015). In Slovenia, however, the species was present sporadically all year round on the experimental plates at Morgan. The colonies were found on structures erected in late summer and autumn, after 5-12 months of exposure. Only a few colonies were observed on each experimental plate.

The colonies found in Slovenia were mostly green-coloured, which might be due to the presence of some microalgae as already observed in some other bryozoans (*e.g. A. verticillata* Marić *et al*., 2017). Besides the experimental plates, the species was found in a marina and was observed exclusively growing on the stolons of *A. verticillata* (Figures 2B and 3B). In total, we recorded this species on 9 sampling occasions.

Tricellaria inopinata d'Hondt & Occhipinti Ambrogi, 1985

Tricellaria inopinata is a light brown to creamy coloured arborescent cheilostomatid bryozoan. It is dichotomously branched, with autozooids arranged in two rows. It has large lateral avicularia and globular, multi-pored ovicells. The scuta partially cover the opesia and are highly diverse in shape, even in a single colony, but they are usually antler-shaped (Dyrynda *et al*., 2000).

Although the native distribution of this species is unknown, it is assumed that the bryozoan originates from the Northern Pacific (Dyrynda *et al*., 2000). *T. inopinata* was first described in the Lagoon of Venice (d'Hondt & Occhipinti Ambrogi, 1985), where it spread rapidly throughout the lagoon. There it has been reported as an invasive NIS as it has replaced most of the native arborescent bryozoans in the area (Occhipinti Ambrogi & d'Hondt, 1994). Since then, it has been found at several locations, from the Eastern and Western Atlantic coasts to the Arctic Sea (De Blauwe & Faasse, 2001; Breton & d'Hondt, 2005; Arenas *et al*., 2006; Marchini *et al*., 2007; Buschbaum *et al*., 2012; Johnson *et al*., 2012; Cook *et al*., 2013; Porter *et al*., 2015). The introduction to Italy has been linked to the import and culture of the Pacific oyster (*Magallana gigas* (Thunberg, 1793)), whereas maritime traffic, specifically hull fouling, is a probable vector for the secondary diffusion of this species (Occhipinti Ambrogi, 2000; De Blauwe & Faasse, 2001; Cook *et al*., 2013).

T. inopinata was first spotted in the Slovenian costal sea at the site of a mussel farm, in Sečovlje in 2018.

Later, it was found at two other Slovenian mussel farming sites, Strunjan and Debeli rtič (Fortič & Mavrič, 2019). In 2019, large colonies, which covered mussel nets, mussels and ascidians, were found down to 8 m depth (Fig. 2D). A few colonies were found on boat hulls in Valdoltra harbour. In total, we recorded this species on 12 sampling occasions, 11 of them were at mussel farms (Fig 3D). Therefore, the exchange of fouled mussels between mariculture sites might be another plausible secondary diffusion vector for this species.

Even though *T. inopinata* has predominately been found on artificial structures, it was also observed attached to macroalgae and other sessile invertebrates (*e.g.* De Blauwe & Faasse, 2001; Cook *et al*., 2013). Recently, we discovered two interesting microhabitats of *T. inopinata*. Small colonies were found attached to a dead seagrass leaf floating near Cape Madona. On another occasion, a single colony of *T. inopinata* was found attached to a leg of a live *Dromia personata* (Linnaeus, 1758) crab at the Morgan experimental structures site in the Bay of Piran. These findings suggest that fouling on natural substrata can be a potential vector for small-scale diffusion of this species. Rafting, in particular, on natural and anthropogenic substrata, is widely used as a dispersal mechanism for both native and NIS species (Watts *et al*., 1998; Thiel & Gutow, 2005; Kuhlenkamp & Kind, 2013; McCuller & Carlton, 2018).

Watersipora arcuata Banta, 1969

Watersipora arcuata is an encrusting cheilostomatid bryozoan belonging to the family Watersiporiidae. The autozooids are orange-red with dark mahogany-coloured opercula and autozooidal borders. The bryozoans of this family are hard to identify due to their lack of typical taxonomical features, namely, spines, avicularia and ovicells. *W. arcuata* was described in San Diego Bay, North-Eastern Pacific (Banta, 1969). There are several key diagnostic features distinguishing this species from other congeneric species. Most importantly, it has an arcuate proximal border of the orifice. Also essential for the identification, are the shape of the orifice and its height and length, the presence and shape of the sinus and condyles and the number of pseudopores (Vieira *et al.,* 2014).

The species is probably native in the tropical Eastern Pacific (Ulman *et al*., 2017). Nowadays, it is also present in San Diego Bay, the Gulf of California, the Galapagos, Australia, New Zealand and Hawaii (Skerman, 1960, Banta, 1969; Gordon & Mawatari, 1992; Coles *et al*., 1999; Mackie *et al*., 2012). This species was first recorded in the Mediterranean Sea from the marina of the Ligurian Sea in 2013 (Ferrario *et al*., 2015). Later, it was reported from the Sardinian Sea (Ferrario *et al*., 2017) and the waters of Sicily, Spain, Malta and Turkey (Ulman *et al*., 2017). Vessel fouling is, as discussed by Ferrario *et al.* (2015), the most probable vector of introduction for this and other watersiporid species.

The specimens found in Slovenia form scale-like encrusting colonies, which are dark red in colour (Fig. 2E). In this study, we report on the findings of this species at two localities (Fig. 3E). Many colonies, forming a belt at 0.5 m depth, were found in a shaded area of a marina, attached to a concrete wall during the summer of 2019. Moreover, this bryozoan was found on the experimental plates placed in the Bay of Piran near the Marine Biology Station (Morgan) (Fig. 3E). The plate, on which we found a single colony, was placed underwater in October 2018 and retrieved in January 2019. The plate was submersed for 89 days during the cold part of the year. Thus, the settlement and growth of organisms on the experimental plate was limited and the community consisted mainly of common pioneer fouling species. The most abundant organisms were the serpulid worm *Spirobranchus triqueter* (Linnaeus, 1758), covering 40% of the surface, and hydrozoans that were found on the edge of the experimental plate. Other native bryozoan species, which were not abundant, recorded on the plate were *Schizoporella dunkeri* (Reuss, 1848), *Terwasipora complanata* (Norman, 1864) and *Chorizopora brogniartii* (Audouin, 1826).

Watersipora subtorquata (d'Orbigny, 1852)

Watersipora subtorquata, similary to *W. arcuata*, forms unilamellar colonies on flat substrata, becoming multilamellar and sometimes erect on irregular substrata. It is orange to brownish-purple or black. It is distinguished from other congeneric species by its distinct U-shaped sinus, an operculum with a parallel-sided dark central band with two proximal lucidae and tooth-like condyles (Vieira *et al*., 2014).

The bryozoans of the genus *Watersipora* have recently received a lot of attention from the scientific community, due to their invasive potential and the confusion regarding their identity (Ryland *et al*., 2009; Vieira *et al*., 2014). Two species were interchangeably misidentified, namely *W. subtorquata* and *W. subovidea* (d'Orbigny, 1852). The species *W. subtorquata* is now confirmed to be present in the Mediterranean Sea, as well as the Atlantic, Red Sea, Arabian Sea and the Pacific (Vieira *et al*., 2014). The first record for the Mediterranean is not reported accurately, due to the aforementioned confusion.

So far, *W. subtorquata* has been recorded at a single sampling location in the Slovenian coastal sea (Fig. 3F). Bright red unilamellar colonies were found attached sailing boat hulls in Piran harbour (Fig 2F), together with barnacle *Amphibalanus amphitrite* (Darwin, 1854) and *Bugula neritina s.l.*. The members of the genus *Watersipora* are known for their resistance to copper and copper-based antifouling paints, the latter can in some cases even facilitate the settlement of larvae (Wisley, 1958; McKenzie *et al*., 2012). Furthermore, studies have shown that watersiporid species can facilitate the settling of other, moderately copper-tolerant species (Dafforn, 2008; Piola & Johnston, 2009). Colonies were known to withstand high-speed cruises, where they are exposed

Tab. 1: Bryozoan species recorded in existing studies with habitat (1- marinas and harbours, a- vessel hulls, b- other substrates, 2- mussel farms, 3- experimental structures Morgan) and depth range for the species found in the Slovenian costal sea.
Tab. 1: Vrste mahovnjakov, ki smo jih zabeležili v raziskavi ter njihov habitat (1- marine in mandrači, a- trupi čolnov, b- druge podlage, 2- školjčišča, 3- eksperimentalne strukture Morgan) in razpon globin, kjer so bili najdeni v slovenskem obalnem morju.

SPECIES	HABITAT	DEPTH (m)
Amathia verticillata	1ab	0 - 3.5
Bugula neritina s.l.	1ab, 3	0 - 10
Celleporaria brunnea	1, 3	0 - 10
Tricellaria inopinata	1a, 2	0 - 9
Watersipora arcuata	1b, 3	0.5 - 9.5
Watersipora subtorquata	1a	0 - 2

to changing temperatures and other abiotic and biotic conditions (Allen, 1953). Therefore, it is not surprising that *W. subtorquata* was discovered for the first time in Slovenia, attached to a vessel hull.

Spatial and temporal distribution of non-indigenous bryozoans

All six species were present in port habitats (*e.g.* marinas and harbours) (Tab. 1). This is not unexpected, as harbours and marinas are one of the hotspots for bryozoan NIS (Ferrario *et al.*, 2017) and the proposed secondary diffusion vector for all of the reported species (Occhipinti Ambrogi, 2000; De Blauwe & Faasse, 2001; Canning-Clode *et al.*, 2013; Cook *et al.*, 2013; Ferrario *et al.*, 2015; Lodola *et al.*, 2015; Marchini *et al.*, 2015). In total, there are 17 mooring sites in Slovenia and, considering that the Slovenian coastline is approximately 46.7 km long, this means a high density of mooring sites, 0.36 per km. The majority of these sites are small local harbours, given that two thirds (10) of the mooring sites are less than 1 km long (Fig. 4). Regardless of their size, they are an important habitat for bryozoan NIS in Slovenia.

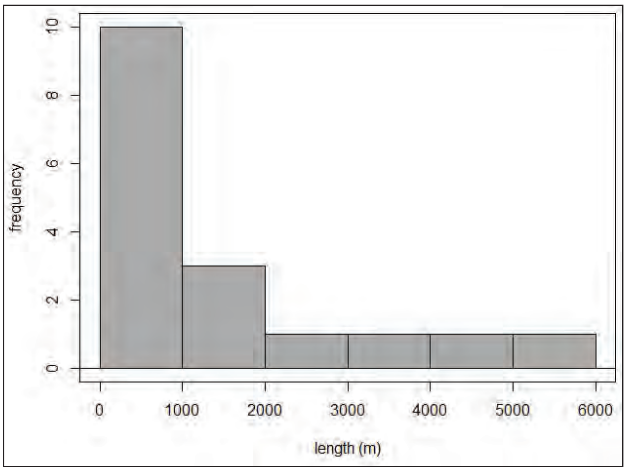

Fig. 4: Frequency distribution of the length of coastal stretch occupied by mooring sites (e.g. marinas, harbours, piers) along the Slovenian coast.
Sl. 4: Frekvenčna porazdelitev dolžine obale, ki jo zasedajo lokacije privezov (npr. marine, mandrači, pomoli) vzdolž slovenske obale.

However, *W. subtorquata* was only found on the hulls of sailing boats. Nevertheless, spreading to other immobile substrata is to be expected. Three species were present on the experimental plates in the Bay of Piran, namely *B. neritina s.l., Celleporaria brunnea* and *W. arcuata*, although none of them was abundant. Moreover, *B. neritina s.l.* and *W. arcuata* were represented only by one colony. Therefore, even though the experimental plates were not a preferential habitat for most species, they proved to be a good indicator of the presence of bryozoan larvae in the water column.

Based on their occurrence on the experimental plates in Piran, more NIS were recorded during the colder part of the year (Tab. 2). *C. brunnea* was present on the experimental plates during all seasons, albeit in low abundances. Occasional *B. neritina s.l.* and *W. arcuata* colonies were present only during the winter. This may seem surprising for these species with assumed subtropical and tropical native range, especially when taking into account that the study area is characterised by the lowest winter temperatures in the Mediterranean Sea (Ogorelec *et al.*, 1991). There are several possible explanations for this observation. One of them might be the lack of competition at the time of settlement, which enabled the sporadic settlement of these opportunistic species. *B. neritina s.l.* was frequently found in harbours and marinas during the colder part of the year when larvae were also observed. Igić (2007) reports this it is

Tab. 2: Seasonal occurrence of non-indigenous bryozoan species on the experimental plates located at Morgan, Bay of Piran (+ = presence, - = absence).
Tab. 2: Sezonsko pojavljanje tujerodnih vrst na eksperimentalnih ploščah na lokaciji Morgan v Piranskem zalivu (+ = prisotna, - = odsotna).

Species	spring	summer	autumn	winter
Bugula neritina s.l.	-	-	-	+
Celleporaria brunnea	+	+	+	+
Watersipora arcuata	-	-	-	+

not a common feature of *B. neritina s.l.* in other parts of the Mediterranean Sea. Haplotype-specific differences in temperature-related fitness have been reported for *B. neritina s.l.* (Fehlauer-Ale *et al.*, 2013). In the future, it would be interesting to know which cryptic species of *B. neritina* is present in the Gulf of Trieste and if indeed it is the invasive one with the wider temperature tolerance.

The distribution data on bryozoan species presented in this study is not yet comprehensive. In order to understand the ecology and temporal as well as spatial distribution of each species better, more work should be done in the future.

Methodological approaches to the study of non-indigenous bryozoans and problematic areas

An important reason for the insufficient study of bryozoan xenodiversity is the lack of expert taxonomists (Ferrario *et al.*, 2018). The taxonomy of these animals is sometimes difficult and often requires expensive equipment such as a scanning electron microscope. Furthermore, bryozoans exhibit high polymorphism and intraspecific plasticity, which has led to many mistaken determinations in the past, followed by extensive revisions of entire genera (*e.g.* Fehlauer-Ale *et al.*, 2013; Vieira *et al.*, 2014). The lack of taxonomic expertise, combined with the small number of studies dealing with bryozoan ecology, compared to other taxonomic groups, can lead to imperfect data on the spatial distribution of bryozoan NIS.

As regards the best methodological approaches for assessing NIS, in our experience, combining several sampling techniques is the best practice. For example, encrusting species are harder to detect, especially if using a scraping net to scrape off the fouling community in marinas, harbours or mussel farms. That is because unilamellar colonies, which are attached directly to the substratum (*e.g.* vessel hulls), cannot be obtained in that way or are broken into small pieces. This is the main reason why underwater sampling techniques, snorkelling or scuba diving, is suggested as a method to supplement above water scraping techniques. Experimental plates or other structures are a good method for sampling non-target, fouling NIS (Tait & Inglis, 2016) and can provide an overview of temporal patterns of species occurrence. Thus, integration of the aforementioned methods is recommended for a more comprehensive overview of bryozoan NIS and other sessile invertebrates.

Finally, we would like to address the problem of determining the status of NIS. There is no consensus regarding the current number and identity of bryozoan NIS. The scientific community lacks a unified model for confirming the status of a NIS. Many criteria have to be met in order to grant this status, namely, ecological, geographical and evolutionary. Currently, the extent of knowledge on some species is unsatisfactory and there is no consensus among researchers regarding the status of those species (Gatto *et al.*, 2013; Katsanevakis *et al.*, 2015; Ferrario *et al.*, 2018). Early detection of new species, together with comprehensive ecological and genetic studies, focusing on clarifying introduction events and determining their natural distribution range, are important for the study of NIS. There is still a lot to be discovered, especially regarding the impact of NIS on the ecosystem (Bonnano & Orlando-Bonaca, 2019).

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OCENA KSENODIVERZITETE MAHOVNJAKOV V SLOVENSKEM OBALNEM MORJU

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POVZETEK

Mahovnjaki imajo pomembno vlogo v morskih obalnih ekosistemih, predvsem kot biokonstruktorji in filtratorji. Kljub temu je deblo Bryozoa ena najslabše preučenih skupin bentoških nevretenčarjev v slovenskem obalnem morju. Tujerodne vrste predstavljajo pet do deset odstotkov vseh vrst mahovnjakov v Sredozemskem morju. V prispevku predstavljamo posodobljen seznam morskih tujerodnih in kriptogenih vrst mahovnjakov Slovenije. Seznam vključuje šest vrst: Amathia verticillata, Bugula neritina s.l.*,* Celleporaria brunnea, Tricellaria inopinata, Watersipora arcuata *in* Watersipora subtorquata *z opisi morfologije, ekologije in razširjenostjo teh vrst v slovenskem morju. Obravnavamo vektorje vnosa in možne mehanizme razširjanja ter metodološke pristope in problematična področja preučevanja tujerodnih mahovnjakov. Za tri obravnavane vrste poročamo tudi o prvem pojavu v Slovenskem morju, in sicer za* A. verticillata, C. brunnea *in* W. subtorquata.

Ključne besede: tujerodne vrste, Bryozoa, severno Jadransko morje, pristaniški habitati, školjčišča, eksperimentalne plošče

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