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Research Article

First records of marine invasive non-native Bryozoa in Norwegian coastal waters from Bergen to Trondheim

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Abstract

From 29 June to 25 July 2014, scientists from Heriot Watt University and the Natural History Museum, London, aboard the vessel *MV Halton*, undertook a research cruise along the Norwegian coast. The cruise started in Bergen and over the two-week period surveys were conducted at intervals along the coastline, heading northwards, and including the major ports of Ålesund, Kristiansund, and Trondheim. When the vessel moored up in each harbour, surveys of the local pontoons were conducted to identify fouling species and to ascertain whether any non-native Bryozoa were present. Seven species of fouling Bryozoa were identified. Two of these were the non-native species *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985 and *Schizoporella japonica* Ortmann, 1890. This study represents the first confirmed records for these species from Norwegian coastal waters. It is likely, given the locations of occurrence, that small boat traffic was a vector for the introduction of these species. Recommendations are given for the amendment and addition of species to the Norwegian Black List.

Key words: marine, Bryozoa, non-native, fouling, marina, pontoon, *Schizoporella japonica, Tricellaria inopinata, Bugula simplex, Bugulina simplex, Bugula stolonifera, Bugulina stolonifera, Bugula neritina, Fenestrulina delicia, Watersipora subatra*

Introduction

The introduction of a non-indigenous species into a new geographic area can affect local biodiversity by causing a restructuring of the trophic web (Vitousek 1990) as well as directly affecting society through causing damage to marine infrastructures, such as the fouling of pipes, vessels and other submerged infrastructure, and by having negative effects on fisheries or aquaculture (Occhipinti Ambrogi et al. 2011).

In 2012, a comprehensive assessment of the alien species present in Norway was undertaken by researchers at the Norwegian Biodiversity Information Centre, Trondheim, and was published in a document entitled the Norwegian Black List (Gederaas et al. 2012). At this time, there were no records of non-native or invasive species of marine Bryozoa recorded in Norwegian coastal waters. In Table 10 of the report, four species of bryozoans were listed as 'Doorknockers'; i.e., species that were expected to arrive but had not yet been recorded. These species were: *Watersipora subtorquata* (d'Orbigny, 1852); *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985; *Bugula neritina* (Linnaeus, 1758); and *Bugula stolonifera* Ryland, 1960.

From 29 June to 25 July 2014, scientists from Heriot Watt University and the Natural History Museum, London, undertook a research cruise aboard the vessel *MV Halton*. The cruise covered the region between Bergen (60°23.963'N, 05°18.7 40'E) and Trondheim (63°26.318'N, 10°23.976'E). The aim was to survey the bryozoan communities and to identify whether any non-native species were present, in a range of different habitats. One part of the survey focused specifically on marinas and pontoons, to establish whether any of the 'Doorknocker' species had arrived in Norway.

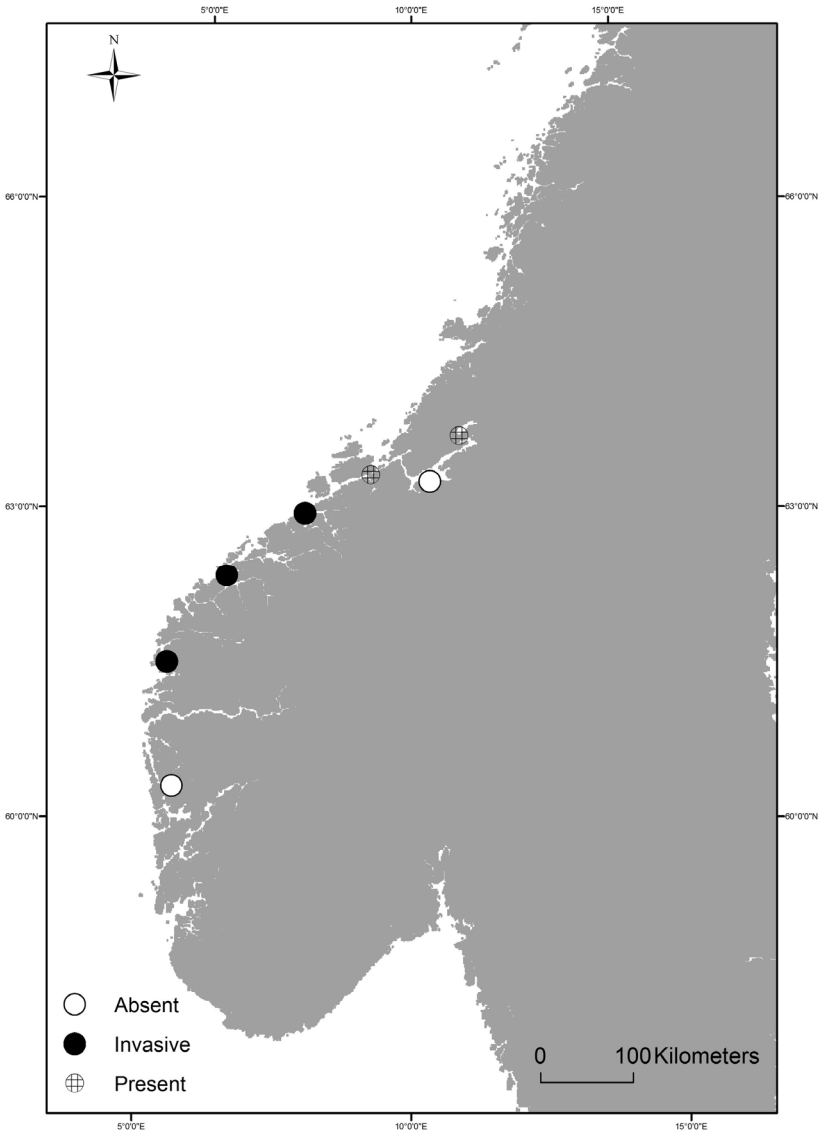

Figure 1. Map of the locations surveyed for fouling and invasive non-native species of Bryozoa along the Norwegian coast between Bergen and Trondheim. An open circle means that no fouling species were found, a closed circle means that invasive non-native species were found and a cross hatched circle means that fouling species were present but not invasive non-native species.

Methods

Visual surveys of pontoons and fenders were conducted at the seven locations (Figure 1). The survey methodology was adapted from that used by Arenas et al. (2006) for rapid assessments of marinas. In this study, two expert bryozoan taxonomists (JSP and MSJ) conducted 30-minute searches at each site. Submerged surfaces of floating plastic pontoons and submerged fenders were inspected for the presence of marine bryozoans. Samples were identified *in situ* where possible; where this was not possible or in order to confirm identification by microscope, samples were collected and subsequently examined under a Wild M5 stereo dissection microscope. Representative material was selected for preparation for imaging on a LEO 1455-VP Scanning Electron Microscope (SEM) at the EMMA unit of the Natural History Museum, London. Selected specimens were deposited with the NHM Bryozoa section and these are listed with location information and accession numbers in Table 1.

Where identifications of fouling and non-native species were confirmed, the significance of the findings was investigated to quantify significant extensions to the known range.

Table 1. Location information, species present and substrates colonised by Bryozoa at the survey sites.

Site	Latitude, N	Longitude, E	Species present	Substrate	Accession No.
Bergen marina	60°23.963'	05°18.740'	Dominated by Barnacles and green filamentous algae	Tyres	NA
Florø marina	61°36.081'	05°02.115'	*Electra pilosa*	Floating plastic pontoons	NHMUK 2014.11.1.3
			Membranipora membranacea	Algal fronds on pontoons	NHMUK 2014.11.1.4
			Cryptosula pallasiana		NHMUK 2014.11.1.1
			Patinella verrucaria		NHMUK 2014.11.1.5
			Tricellaria inopinata		NHMUK 2014.11.1.2
			Celleporella hyalina		NA
			Schizoporella japonica		NHMUK 2014.11.1.6
Ålesund marina, Brunholmkaia	62°28.404'	06°09.162'	*Schizoporella japonica*	Floating plastic pontoons	NHMUK 2014.11.1.8
			Electra pilosa		NHMUK 2014.11.1.9
			Membranipora membranacea	Algal fronds on pontoons	NA
			Celleporella hyalina		NHMUK 2014.11.1.7
Kristiansund harbour, Vaagakaia	63°06.811'	07°43.977'	*Membranipora membranacea*	Algal fronds on pontoons	NA
			Electra pilosa		NA
			Tricellaria inopinata		NHMUK 2014.11.1.10
			Schizoporella japonica	Floating plastic pontoons	NHMUK 2014.11.1.11
Trondheim harbour, Ytre Havna	63°26.318'	10°23.976'	*Green filamentous algae and occasional sponges*	Floating plastic pontoons and tyres	
			Membranipora membranacea		NA
			Electra pilosa		NA
Skarnsundet marina	63°52.570'	11°02.700'	*Electra pilosa*	Floating plastic pontoons	NA
			Membranipora membranacea		NA
Hitratunnellen marina, Hamnskjela	63°29.911'	09°07.729'	*Electra pilosa*	Floating plastic pontoons	NA
			Membranipora membranacea		NA

Results and discussion

In this survey, seven species of bryozoans (Figure 2) were identified from the study locations:

Cyclostomata

Patinella verrucaria (Linnaeus, 1758)

Cheilostomata

Membranipora membranacea (Linnaeus, 1767)
Electra pilosa (Linnaeus, 1761)
Tricellaria inopinata d'Hondt and Occhipinti Ambrogi, 1985
Cryptosula pallasiana (Moll, 1803)
Celleporella hyalina (Linnaeus, 1758)
Schizoporella japonica Ortmann, 1890

The common fouling cheilostome species observed were *Electra pilosa*, *Membranipora membrancea*, *Celleporella hyalina* and *Cryptosula pallasiana*. The first three of these species have been shown, using molecular genetic techniques, to exist as cryptic species complexes (Schwaninger 2008, Gomez et al. 2007; Waeschenbach et al. 2012; Nikulina et al. 2007). The cyclostome *Patinella verrucaria* was growing on a colony of the bryozoan *Tricellaria inopinata*, which was growing attached to the plastic pontoons. The known invasive species observed were *Tricellaria inopinata* and *Schizoporella japonica*. The latter species was not present in the 'Doorknocker' list, whereas the former species was anticipated. These new records represent significant expansions to the known species' distributions and are discussed more fully below.

Tricellaria inopinata (Figure 3)

We found *Tricellaria inopinata* growing on plastic pontoons at Florø and Kristiansund. These are the first known records for this species in Norway and represent a significant northerly extension to the range in North-west Europe based on records in the Orkney Isles, Scotland (Cook et al. 2013; Nall et al. 2015).

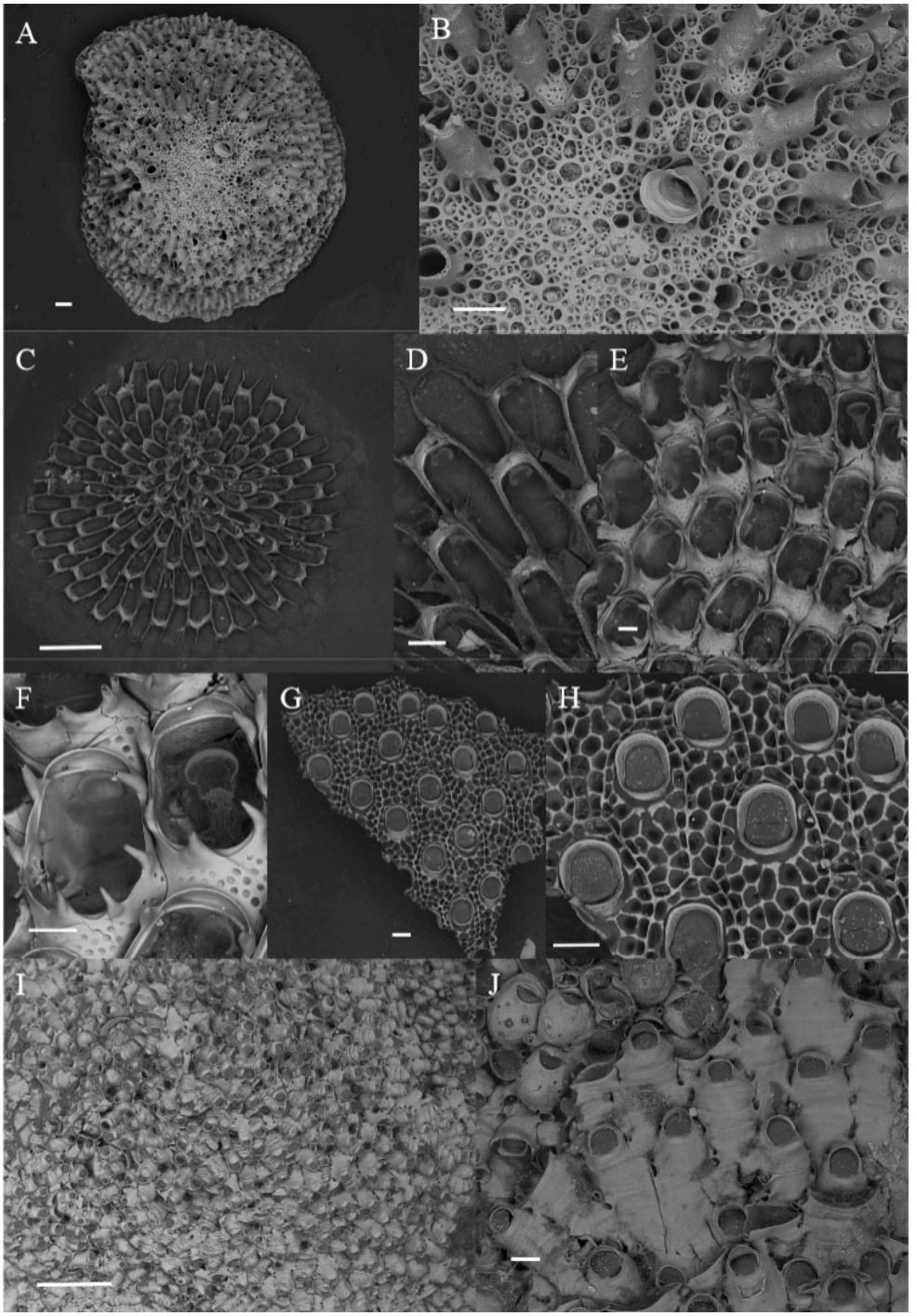

Figure 2. Species of fouling Bryozoa found in Norwegian waters as part of this survey: A) Whole colony of *Patinella verrucaria* (NHMUK 2014.11.1.5) (scale bar = 200µm); B) Ooeciostome in the central region of the colony of *Patinella verrucaria* (scale bar = 200µm); C) Young colony of *Membranipora membranacea* (NHMUK 2014.11.1.4) (scale bar = 1mm); D) Close up of autozooids of *Membranipora membranacea* (scale bar = 200µm); E) Arrangement of the autozooids of *Electra pilosa* (NHMUK 2014.11.1.3) (scale bar = 100µm); F) Close up of autozooids of *Electra pilosa* (scale bar = 100µm); G) Group of autozooids of *Cryptosula pallasiana* with operculae still intact (NHMUK 2014.11.1.1) (scale bar = 200µm); H) Close up of autozooids of *Cryptosula pallasiana* showing the bell shaped orifice (scale bar = 200µm); I) Overview of a colony of *Celleporella hyalina* (NHMUK 2014.11.1.7) (scale bar = 1mm); J) Close up of autozooids of *Celleporella hyalina* (scale bar = 100µm). Photomicrographs by Mary Spencer Jones.

Figure 3. The invasive non-native species *Tricellaria inopinata* (NHMUK 2014.11.1.2): A) Overview of part of a colony showing internode and autozooid arrangement (scale bar = 200μm); B) Close up of autozooids with ovicells attached (scale bar = 100μm); C) Close up of autozooids without ovicells, but with scutum, spines and avicularium (scale bar = 200μm); D) Close up of a single autozooid with scutum, spines and avicularium (scale bar = 20μm). Photomicrographs by Mary Spencer Jones.

Tricellaria inopinata was first described in 1985 (d'Hondt and Occhipinti Ambrogi 1985) from specimens collected in May 1982 from waterways connected to the central region of the Lagoon of Venice. Earlier surveys did not document this species, leading to the hypothesis that it was a recent invader and it subsequently spread rapidly to other parts of the lagoon (Occhipinti Ambrogi 1991; Occhipinti Ambrogi et al. 2011). *Tricellaria inopinata* is thought to have originated in the north Pacific Ocean (Dyrynda et al. 2000; De Blauwe and Faasse 2001) and was later introduced into Australia, New Zealand, Japan, Taiwan, the West Pacific, and Venice (Occhipinti Ambrogi and d'Hondt 1994). Just a few years later, it was reported from locations on the European Atlantic coast, including: France (Breton and d'Hondt 2005); Belgium; the Netherlands (De Blauwe and Faasse 2001); and the northwest of Spain and Portugal (in 2004; Marchini et al. 2007). By 1998,

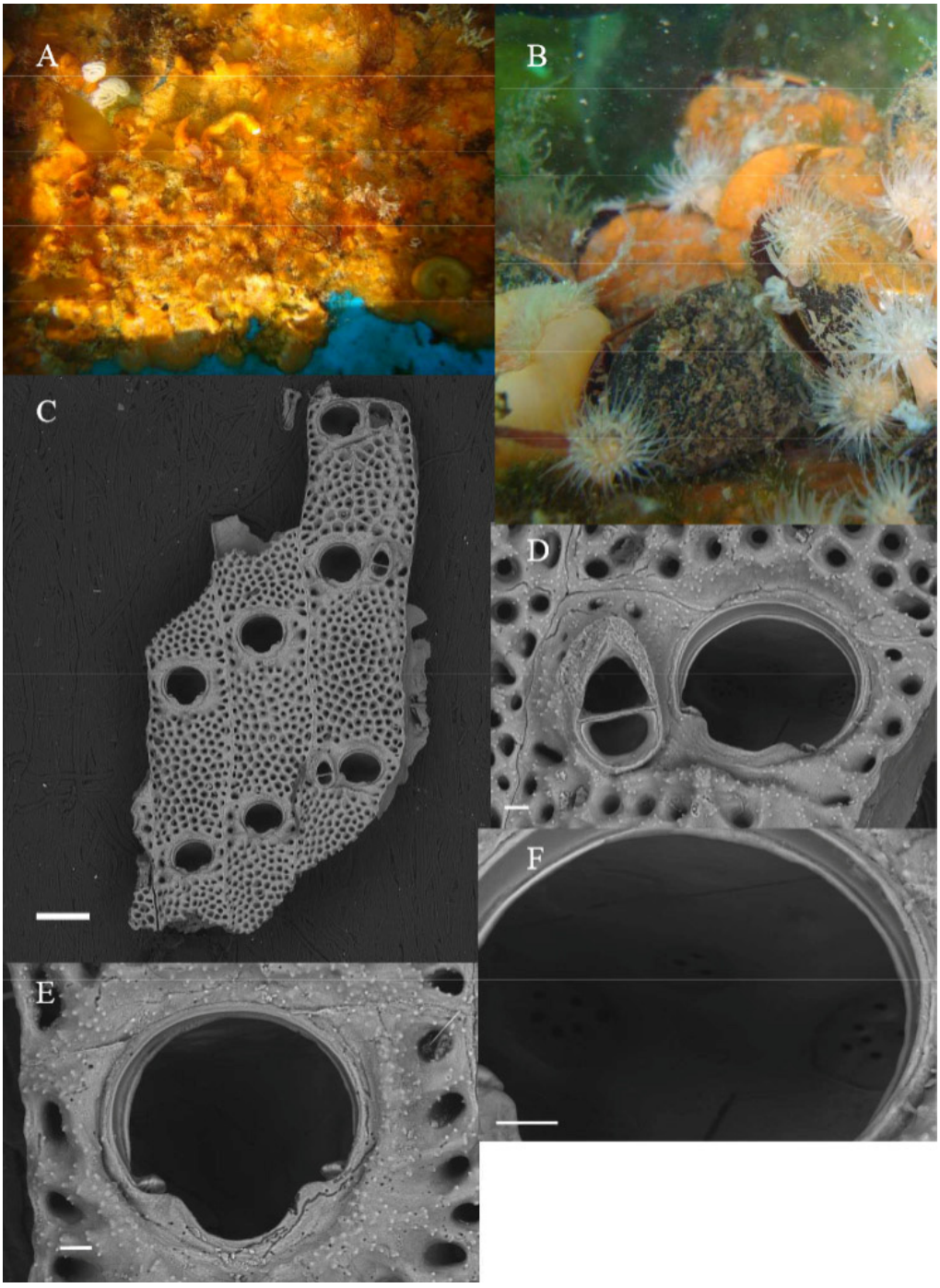

Figure 4. The invasive non-native species *Schizoporella japonica* (NHMUK 2014.11.1.6) : A) In situ image of *Schizoporella* japonica colony growing on the plastic floats of a marina pontoon; B) Colonies of *Schizoporella japonica* growing on the valves of the blue mussel *Mytilus edulis* attached to a marina pontoon; C) A group of autozooids of *Schizoporella japonica* (scale bar = 200µm); D) Close up of an autozooid of *Schizoporella japonica* showing the position of an avicularium to the side of the orifice (scale bar = 20µm); E) Close up image of the orifice of an autozooid of *Schizoporella japonica* showing the detail of the condyles (scale bar = 20µm); F) Close up of the orifice with communication pore plates on the inside surface (scale bar = 20µm). Photomicrographs by Mary Spencer Jones.

this invasive species was present in south-central England (Dyrynda et al. 2000). In the Netherlands it was found to be abundant on: natural surfaces; artificial surfaces such as ropes and buoys; and on other sessile fauna (De Blauwe and Faasse 2001). More recently, it was reported from Woods Hole, Massachusetts, USA, in September 2010 (Johnson et al. 2012). In 2012, it was observed in Dublin Bay, Ireland (Kelso and Wyse Jackson 2012).

Schizoporella japonica (Figure 4)

In our survey, *Schizoporella japonica* was found for the first time in Norwegian coastal waters at the ports of Florø, Ålesund, and Kristiansund. It was growing on the plastic floats of the pontoons and also on the shells of *Mytilus edulis* that also were attached to the pontoons. *Schizoporella japonica* was originally described from Japan, and is known from the Hokkaido area (Grischenko et al. 2007). *S. japonica* was subsequently introduced on Pacific oysters to the Pacific coast of North America, where it is now well established (Powell, 1970; Dick et al. 2005; Blum et al. 2007; Crooks et al. 2011). It was recently recorded in the UK from Holyhead marina, North Wales, the south coast of England, and at several localities around the Scottish coastline including the mainland, Orkney and Shetland Isles (Ryland et al. 2014; Bishop et al. 2015; Nall et al 2015). This species should now be added to the Norwegian Blacklist.

Timing of arrival in Norway of the two invasive species

During this survey, *Tricellaria inopinata* and *Schizoporella japonica* were collected and identifications confirmed by SEM imaging (Figures 2 and 3). Examination of the published literature and museum collections has revealed no previous records of these species in Norway. This suggests that they have been introduced relatively recently. Vectors of transmission could be leisure or commercial craft travelling between ports in Norway, and between Norway, Shetland, the Faroes, and Iceland.

In the case of *Tricellaria inopinata* (a temperate waters species), it is likely that the species has spread northwards from more southerly locations in mainland Europe (De Blauwe and Faasse 2001) or by hopping across the North Sea from Scotland (Cook et al 2013). These are not mutually exclusive hypotheses.

In contrast, *Schizoporella japonica* is an Arctic/ Boreal species that has been documented on the east and west coasts of North America and more recently in the north mainland of Scotland, Orkney, and Shetland (Ryland et al 2014; Nall et al 2015). The species appears to be colonizing into Europe from the Atlantic in a west to east direction and then spreading along coastlines.

The marinas surveyed along the Norwegian coast fell into three categories: i) those with freshwater input and no bryozoans present; ii) fully saline with native or naturalized fouling species; or iii) fully saline with a mixture of native or naturalized fouling species and invasive non-native species (Figure 1).

Update on other 'Doorknocker' invasive Bryozoa for Norway

Bugulina simplex (Hincks, 1886) (Figure 5)

Ryland et al. (2011) provide a comprehensive historical review of the distribution of *Bugulina simplex* in North west Europe, including localities around the British coastline, France, Belgium and the Netherlands. The most northerly record at that time was from Ouddorp in the Netherlands. The species is also present in the Eel Pond at Woods Hole, New England (Ryland 1960). Recently (September 2014), *Bugulina simplex* has been collected by Rachel Shucksmith, in Lerwick Harbour, Shetland (NHMUK 2014.11.1.12). While there are no records of this species as yet in Norway, with regular vessel traffic between Lerwick and Norwegian ports, it seems likely that it is only a matter of time before an introduction is made. This species is not currently listed as a Doorknocker in the Norwegian Blacklist (2012); however, given its current proximity and the rate of spread of the species, we suggest that it be added to the Blacklist.

Bugulina stolonifera (Ryland, 1960) (Figure 5)

The distribution and historical records of *B. stolonifera* are reviewed in Ryland et al. (2011), and the status of the species in Western Europe is unclear. Its distribution appears to be very patchy and one of the more recent records is from the Outer Harbour, Harwich (Ashelby 2005). This species has been recorded from other regions, mainly in warm temperate waters and is stated by Ryland et al (2011) to be manifestly a warm water species. Given its current distribution and its preference for warmer water, we suggest that it

Figure 5. The invasive Non native and or invasive species of *Bugula*: A) Overview of a colony of *Bugulina simplex* showing the arrangement of branches (scale bar = 200µm); B) Close up view of a branches of a *Bugulina simplex* colony showing single spine present on marginal autozooids and position of the bird's head avicularium (scale bar = 200µm); C) Close up of a bird's head avicularium of *Bugulina simplex*, showing a distinct down turned rostrum (scale bar = 20µm); D) image of the reverse side of the zooids of *Bugulina simplex* (scale bar = 200µm); E) The holotype slide of *Bugulina stolonifera* described by Ryland (1960); F) Close up image of the colony on the holotype slide of Ryland (1960) for *Bugulina stolonifera* showing arrangement of spines and the position of birds head avicularia on the autozooids; G) Group of autozooids of *Bugula neritina* colony on a slide from the Johnston collection (NHMUK 1847.9.24.133) showing the arrangement of the autozooids and some crushed ovicells (scale bar = 100µm); H) Slide material from the Johnston collection showing *Bugula neritina* colony from Scarborough collected by William Bean (NHMUK 1847.9.24.133); I) Slide material from the Busk collection with *Bugula neritina* from Scarborough collected by William Bean (NHMUK 1899.7.1.273); J) *Bugula neritina* from Bradwell marina, Blackwater Estuary, Essex (NHMUK 2014.12.11.1) collected September 2014 (scale bar = 1mm); K) Close up of the zooid arrangement of *Bugula neritina* material from Bradwell marina, Blackwater Estuary, Essex (scale bar = 100µm); L) Purple coloured bushy colonies of *Bugula neritina* can be seen growing among seaweed plants on this tyre pulled out of the water at Lowestoft marina, September 2014. Photographs by Mary Spencer Jones.

does not represent a significant threat in terms of its chances of colonizing Norwegian coastal waters. However, with sea water temperatures rising, it could become established in the future and should, therefore, remain on the Norwegian Blacklist.

Bugula neritina (Linnaeus, 1758) (Figure 5)

The original locality of *Bugula neritina* (Linnaeus, 1758) has been seemingly lost in the mists of time, though the first known reference to it in the literature (Ellis 1755) gives the localities as Mediterranean and America in the legend to P1 XIX. In the text of the book Ellis refers to a letter about the specimen from America. Unfortunately there is no indication on what the reference to the Mediterranean locality is based. Linnaeus (1758) gave the Mediterranean as a locality, and Pallas (1766) mentions Bermuda. Today, *Bugula neritina* has a global distribution in temperate, subtropical and tropical waters, which has probably been accomplished by ship fouling or with oyster imports. New molecular genetics studies by Fehlauer-Ale et al. (2013), however, have shown that what was previously thought to have been a complex of three cryptic species of *Bugula neritina* now relates to three distinct biological species; two of which are considered highly invasive.

In the NHM collections, the northernmost historical record of *Bugula neritina* in the British Isles appears to be from Scarborough collected by William Bean (1787–1866) in the collection of Dr George Johnston (NHMUK 1847.9.24.133). In Gray (1848) are five specimens listed for *Bugula neritina* labeled a-e. Four of those (b-e) were slender varieties from Tynemouth and other localities unknown. Specimen 'a' however refers to the material from Scarborough, W Bean Esq figured by Johnston in British Zoophytes plate 60 figures 3,4 (Figure 5, G,H,I). Hincks (1880) did not mention specimen 'a' under the name *Cellularia peachii*, Busk. Johnston (1838) also quoted two other early British records given by Fleming (1828) and Templeton (1836) from the "*coast of Cheshire*". More recently, the species has been recorded from the east coast of the British Isles as far north as Lowestoft as part of Seasearch surveys (September 2014) and as far north as the Netherlands by Hans De Blauwe (Ryland et al 2011). This species is already listed in the Norwegian Blacklist (2012), and as sea temperatures rise, and the likelihood of it reaching Norway increases. We advise that it remains on the Blacklist.

Watersipora subatra (Ortmann, 1890) (Figure 5)

NHMUK 2014.11.1.13. Haslar marina, Portsmouth, September 25th 2014

Previous European records of *Watersipora subtorquata* (d'Orbigny, 1852) have recently been shown to be the species *Watersipora subatra* (Ortmann, 1890) (Vieira et al. 2014). This highly invasive species is not currently known to be present in Norwegian waters; however, it has recently been documented as far north as Helgoland (Kuhlenkamp and Kind 2013). This species is likely to spread further north and, therefore, should remain on the Norwegian Blacklist. The taxon name should, however, be changed from *Watersipora subtorquata* to *Watersipora subatra.*

Horizon scanning for future shifting taxa

The traditional paradigm about 'cosmopolitan' species is currently under strong pressure, as it seems to have been proven wrong in many instances. Molecular studies have shown that many cosmopolitan species are actually complexes of cryptic species. On the other hand, some species recently acquired a widespread distribution pattern because of the increases in marine traffic and stepping stone structures being put into place on the seabed. This has allowed some species to invade into areas that would not have previously been possible. For taxonomists and people working in the field to monitor introductions this mixture of possibilities can cause real problems as was highlighted in Harmelin et al. 2012. In Norway, the Blacklist is used to highlight the Doorknocker species that have not yet arrived, but may be on the verge of doing so.

Fenestrulina delicia Winston, Hayward and Craig, 2000 (Figure 6)

Fenestrulina delicia was first described in 2000 (Winston et al. 2000) from the Damariscotta River, Maine (Holotype AMNH no.713). Colonies form dense white patches encrusting shells or algae. This species was recently recorded in north western Europe by De Blauwe et al. (2014). They report the detecting *Fenestrulina delicia* on the French coast as far south as Pleneuf-Val-Andre (May, 2008). It was also reported as far north as Helgoland, NE Germany (August, 2008) where it was found on floating strands of the brown alga *Himanthalia* that were thought to have originated from the English Channel. Colonies of *F. delicia* were found on offshore natural and artificial

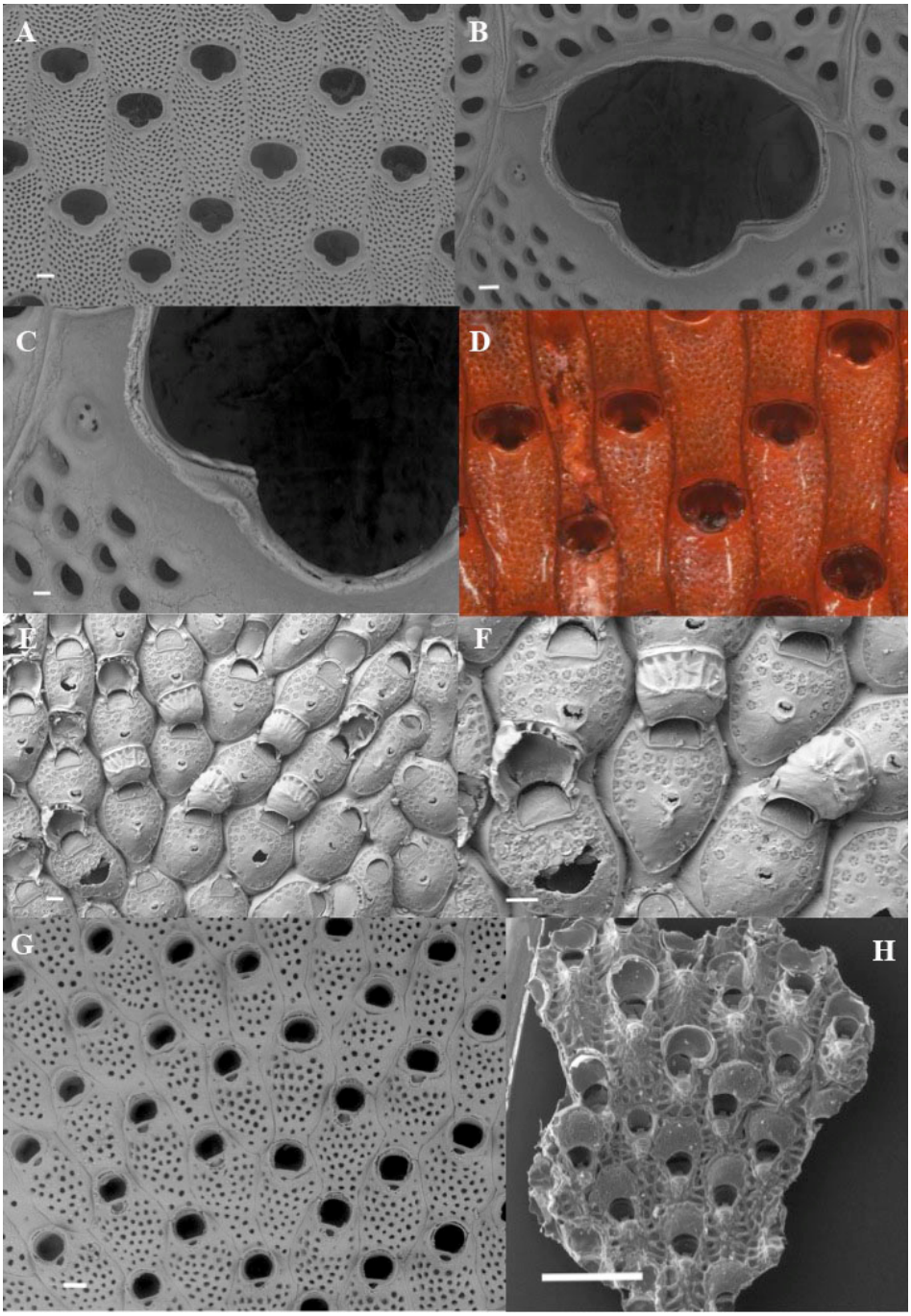

Figure 6. The Doorknocker species: A) Group of autozooids of *Watersipora subatra* from Haslar marina, Plymouth (NHMUK 2014.11.1.3) (scale bar = 100μm); B) Close up of the orifice of a single autozooid of *Watersipora subatra* showing the orifice shape and the position and shape of the condyles (scale bar = 20μm); C) Close up of the condyle and also the pore plate positioned to the side of the orifice of *Watersipora subatra* (scale bar = 10μm); D) Group of autozooids of *Watersipora subatra* live colony material showing the intense deep orange colouration; E) Group of autozooids of *Fenestrulina delicia* (NHMUK 2013.10.28.1) (scale bar = 100μm); F) Close up of autozooids of *Fenestrulina delicia* showing the crescent shaped ascopore, D-shaped orifice and the fluted sculpturing present on the distal portion of the ovicell (scale bar = 100 μm); G) *Pacificincola perforata*, from Japanese material in the NHM collections London (NHMUK 2006.2.27.75-76) (scale bar = 100μm) showing a group of autozooids; H) *Smittoidea prolifica* SEM image from material collected at Goesse Meer, Netherlands and image provided by Hans De Blauwe (scale bar = 500 μm). Photomicrographs by Mary Spencer Jones.

substrates, and the species seems to be spreading along the coastlines rather than hopping from one marina to another. Part of the material collected was encrusting empty bivalve shells at locations known for mariculture of oysters and mussels; therefore, it was suggested that shellfish importation is a primary vector for introduction. Beached plastic waste with colonies of *F. delicia* on it was suggested to be a secondary vector of spread. The bases of offshore wind farm developments form an additional substrate for the species and are providing stepping-stones for the further spread of this exotic species. *Fenestrulina delicia* was reported as far north as Shetland (Wasson and De Blauwe 2014); however, it was not found as part of the harbour surveys during the present study. We suggest it is a likely 'Doorknocker' species because it may reach Norway by means of drifting seaweed and warrants addition to the Norwegian Blacklist.

Pacificincola perforata (Okada and Mawatari, 1937)

This species was described from Japanese waters, and has since been recorded in the Netherlands (De Blauwe and Faase 2004). It is possible that this species could spread northwards into Norwegian waters; therefore, we suggest it be added to the Norwegian Blacklist as a Doorknocker species. We included an SEM image of Japanese material from the NHM collection (NHMUK 2006.2.27.75-76; Figure 6) as an aid to future identification.

Smittoidea prolifica Osburn, 1952 (Figure 6)

De Blauwe and Faase (2004) and more recently Faasse et al. (2013) reported that the Pacific bryozoan *Smittoidea prolifica* Osburn, 1952 has been introduced to the Northeast Atlantic Ocean. Its currently known distribution in the Atlantic is restricted to The Netherlands. They report that all recent records of autochthonous *Smittoidea reticulata* (Macgillivray, 1842) in The Netherlands probably refer to S. *prolifica*. The most likely route of introduction is suggested to be via shellfish imports and the SEM images herein were provided to aid future identification. We recommend that this species be added to the Norwegian Blacklist as a Doorknocker species.

Recommendations

We recommend additional surveys be undertaken to the south of Bergen and to the North of Ålesund to obtain a wider baseline of the distributions of potentially invasive non-native bryozoan species in Norwegian waters. In the future, regular monitoring would provide information useful for understanding the spread of the invasive non-native species along the Norwegian coastline and also to better understand the environmental conditions conducive to their growth and spread. The main vector of transport is highly likely to be leisure craft, as evidenced by the frequent observation of non-native and invasive species in recreational marinas. Some of the doorknocker species may enter Norwegian waters via the aquaculture route; consequently, a different type of monitoring would be required. We suggest that the Norwegian Blacklist 2012 be updated immediately by adding the following species: *Watersipora subatra* (instead of *Watersipora subtorquata*), *Schizoporella japonica, Bugulina simplex, Fenestrulina delicia, Smittoidea prolifica*, and *Pacificincola perforata*.

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