

Abstract

Introduction

First “Thalia” referred only to ascidians and they were initially classified as Lamellibranchs because of their sessile lifestyle and soft morphology (Barenbrock, 2005). As soon as the swimming ascidian larva with a true notochord and tube-shaped nervous system was discovered, the Tunicata became classified with chordates. Some molecular studies even hypothesise a closer affinity of Craniota to Tunicata than to Cephalochordata. So Tunicata started to play an important role in the discussion of phylogenetic relationships within chordates (Isaeva& Golubev, 2017). Tunicata includes the classes Ascidiacea (sea squirts), Thaliacea (salps) and Appendicularia (tailed tunicates). While Thaliacea and Appendicularia lead a pelagic lifestyle, Ascidiacea are exclusively sessile organisms, dominating hard substrates in shallow water and soft substrates in the deep sea. Ascidiacea has more than 2,000 species. It is the most numerous and best

Phylogenetic tree of the phylum Mollusca, showing relationships between various classes and orders. The tree is rooted at the bottom left and branches out to the right. Major groups are highlighted in colored boxes: yellow for Stolidobranchiata, Ascidacea, and Aplousobranchiata; green for Thaliacea and Dololida; and blue for Appendicularia. Bootstrap values are indicated at the nodes. A scale bar at the bottom left indicates 0.1 substitutions per site.

Stolidobranchiata

- Molgula citrina
- Molgula menziesii
- Monocerosia clausiana
- Halcyonella costata
- Halcyonella rostrata
- Styela clava
- Styela lacinia
- Styela plicata
- Pelodina constricta
- Dendrobia grossularia
- Belliphaea schrenkii
- Syringopoda australensis
- Kalanmulla borealis
- Acidifolia aspera
- Acidifolia scabra
- Agnostia septentrionalis
- Clava nitidissima
- Acidifolia mentalis
- Acidifolia virgata
- Phialusia mamillata
- Phialusia nigra
- Conella parastyligomma
- Rhodossoma callae
- Perophora japonica
- Perophora viridis
- Diazona violacea
- Eutima obsoletum
- Clovelia apiculatum
- Sycosura sigillifera
- Diaploga stylifera
- Diaploga stelleriana
- Didemnum musciforme
- Liocidium ventral
- Polycidium eximium
- Polycidium constrictum
- Syncaum pulchellum
- Apidium furfuratum
- Murchellia rigida

Ascidacea

- Pyrosomella allentani
- Selpe bathys
- Thalassidroma
- Isois cylindrica
- Dololida notostoma
- Dololida dentostoma
- Oligopelela benthicola
- Oligopelela clava
- Megalocercus huxleyi
- Trielasma borealis

Aplousobranchiata

- Petragastrum maritima
- Lampteroplax
- Lampteroplax
- Branchiostoma lanceolatum
- Branchiostoma floridae

Thaliacea

- Thalassidroma
- Isois cylindrica
- Dololida notostoma
- Dololida dentostoma
- Oligopelela benthicola
- Oligopelela clava
- Megalocercus huxleyi
- Trielasma borealis

Dololida

- Dololida notostoma
- Dololida dentostoma
- Oligopelela benthicola
- Oligopelela clava
- Megalocercus huxleyi
- Trielasma borealis

Appendicularia

- Petragastrum maritima
- Lampteroplax
- Lampteroplax
- Branchiostoma lanceolatum
- Branchiostoma floridae

Fig.1. Phylogenetic tree by Braun et. al. (2020) analyzing 117 morphological characters (113 parsimony-informative) coded for 49 tunicate species and five outgroup species. Quotation marks indicate traditional taxonomic groups found paraphyletic in the present analysis. Note the position of Appendicularia, the monophyly of Ascidiacea, and the paraphyly of “Thaliacea”.

(Fig. 1). These authors suggest that the common ancestor of all “Urochordata” was free-living and not sessile like Ascidiacea.

In a review of invertebrate species of Helgoland (1993) Harms listed 16 species of Tunicata

(Ascidiacea). Later in 2016, Groepler found here already 19 species. In this study, I intend to investigate the current biodiversity of Tunicata with a focus on ascidians and their distribution in the different bays of the island.

Materials and Methods

Sampling

The ascidian species examined in this work were collected during a research trip to Heligoland. The respective collection locations at 4 points on the island are noted in Figure 2. The organisms were removed from the substrate at a depth of less than 5 meters by me and colleagues, deeper by divers from the Alfred Wegener Biological Institute or were discovered during collection at low water tide.

To determine the species of a particular tunicate specimen, it is often necessary to perform dissection.

Preservation

First, anesthesiation was performed to relax the tunicate muscles with menthol crystals for 6 hours in sea water. After that the animals were fixed using a solution of 5 % formalin in sea water for at least 48 hours. Next, the animal was washed in running fresh water to remove the formaldehyde solution.

Dissection.

An external examination of the animals was carried out on both sides (Fig. 4, A). First the ventral - dorsal axis of the animal was determined, and the tunic was completely removed (Fig. 4, B). Then the position of the main organs necessary to establish species identity was determined (digestive tract, gonads, kidneys, renal sac). It is not necessary to proceed to a more advanced dissection, except for the branchial sac to determine the structure of internal papillae, parastigmatic vessels, position of dorsal lamina. After the preparation a sample was stained with Bengal rose for better viewing of small structures such as stigmata (Fig. 4, C) on the branchial sac or dorsal tubercles (Fig. 4, F).

I used identification keys arranged by Millar 1966 and by Monniot 1971.

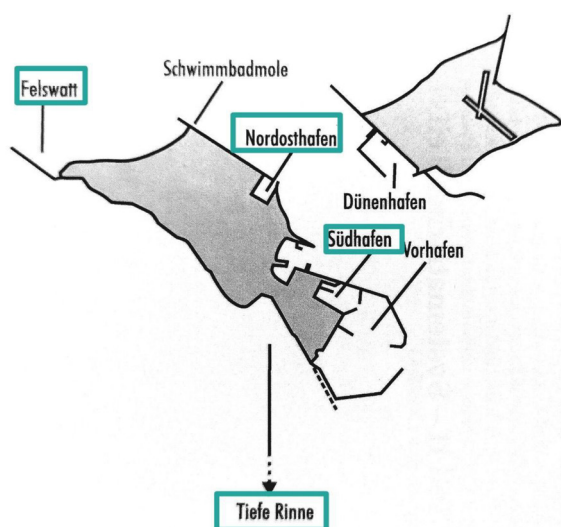


Fig.2. Locations of collections of ascidians from Heligoland according to Groepler W. (2016). The squares enclose the areas for collecting material for this study. Apart from the rock mud flats, the information is based primarily on the evaluation of scraping material that was obtained from diving in the north harbor.

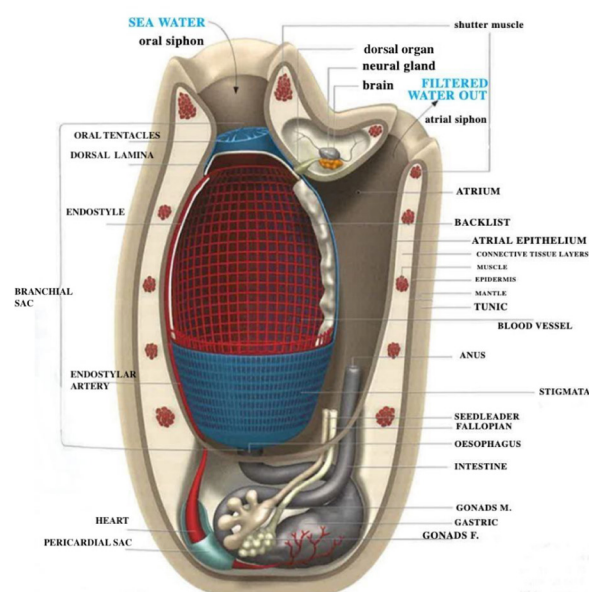


Fig.3. General structure of the ascidian by Kullander et al. (2011) with translation to English by O. Gordetckaia

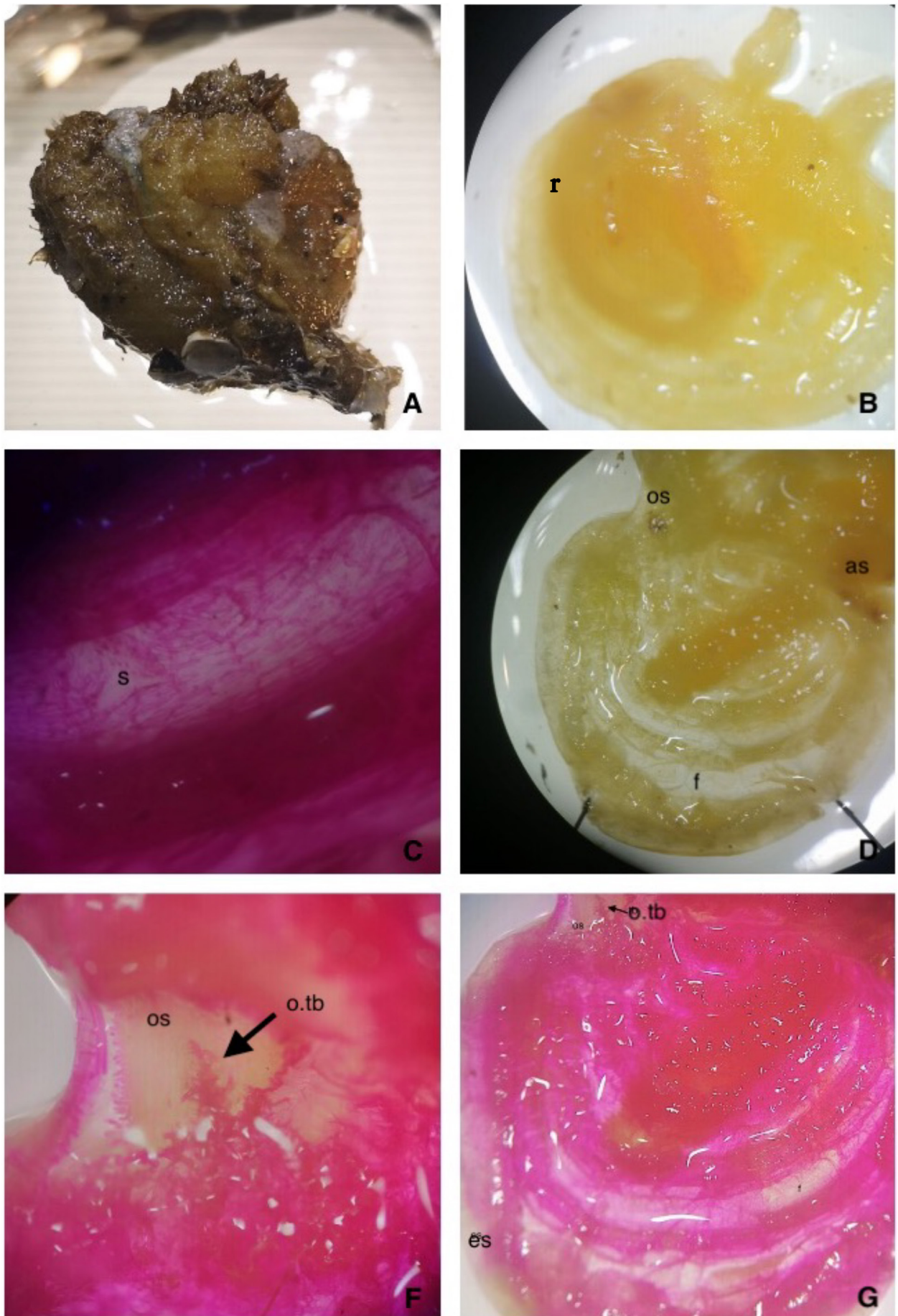


Fig.4. *Molgula manhattensis* dissection process A. General shape. Two individuals. B. An individual after removing the tunic. C. Spiral stigmata on the bronchial sac D. Dissection along the endostyle. F. Oral siphon G. Dissection along the endostyle, colored Bengal rosa. r - large renal-sac attached on the right side of the body wall. os - oral siphon, as - atrial siphon, es - endostyle, s - stigmata, o.tb - oral tentacles

	Family	Species	rocky intertidal	north harbour	south harbour	„Tiefe Rinne“	plankton (250µm)
Asciacea	Asciidae	<i>Asciella</i> sp. ●●		+	+		
Asciacea	Cionidae	<i>Ciona intestinalis</i> ●●		+	+		
Asciacea	Didemnidae	<i>Didemnum maculosum</i> ●●	+				
Asciacea	Didemnidae	<i>Diplosoma listerianum</i> ●●		+	+	+	
Asciacea	Molgulidae	<i>Molgula cf. complanata</i> ●●				+	
Asciacea	Molgulidae	<i>Molgula manhattensis</i>		+			
Appendicularia	Oikopleuridae	<i>Oikopleura dioica</i>					+
Asciacea	Perophoridae	<i>Perophora listeri</i> ●	+	+		+	
Asciacea	Polyclinidae	<i>Aplidium glabrum</i> ●	+				
Asciacea	Polyclinidae	<i>Sidnyum turbinatum</i> ●●	+				
Asciacea	Styelidae	<i>Botrylloides leachii</i> ●●	+	+	+		
Asciacea	Styelidae	<i>Botrylloides violaceus</i> ●			+		
Asciacea	Styelidae	<i>Botryllus schlosseri</i> ●●	+				
Asciacea	Styelidae	<i>Dendrodoa grossularia</i> ●●			+	+	

Fig.5. Species identified during this study and their distribution on Heligoland. The blue dot indicates species also encountered by Groepler W. (2016) and the violet dot indicates species also encountered by Harms (1993).

Results & Discussion

During the present study, I identified 14 species of tunicates on the Heligoland - 8 colonial and 5 solitary ascidians and a planktonic oikopleurid appendicularians. According to the most recent and extensive study of Heligoland Island ascidians (Groepler, 2016), 19 species of ascidians can be found around the island. Due to time restrictions, I cannot sample in all habitats described by Groepler. (Fig. 2), which is probably why I only found 12 ascidians out of the 19 described by Groepler. But I identified one species - *Molgula manhattensis*, that was not detected by Groepler or Harms (Fig. 3 A-F).

Lackschewitz, D (2015) mentioned *Molgula manhattensis* in 2015 near to Heligoland. Due to the difficult taxonomy, several names have been synonymized in the past (cf. WORMS), most of which later turned out to be independent species. Therefore, other authors could describe previously *M. manhattensis* as *M. tubifera* in this region (Jensen, 2010). *M. manhattensis* is native to the East and Gulf Coasts of the United States. It is tolerant of a wide range of temperatures, salinities and pollution levels and affect the structure of native benthic communities (Chen et al., 2021). One possible route for *M. manhattensis* to reach Heligoland is through sea transport or aquaculture of eastern oysters (*Crassostrea virginica*) (Alaska Center for Conservation Science, 2017). This species can attach to the hulls of ships

or be carried in ballast water (Carlton & Geller, 1993). Ship transport is one of the main sources of introduction of alien species to marine and coastal ecosystems (Ruiz et al., 2000).

But the taxonomy of *Molgula* species is very complex, and there is a possibility that the true *M. manhattensis* does not occur in European waters (Haywood & Ryland, 1995). *M. manhattensis* is morphologically not distinguishable from its sister species *M. socialis* in the field (Lackschewitz et al., 2015). Therefore, I consider, molecular studies are needed to confirm or revise our taxonomic identification of *M. manhattensis*.

There are several obvious reasons why I did not find some ascidian species during the study. *Perophora japonica*, *Polyclinium aurantium* both are forming a winter forms from the end of Juli till the end of February. This winter form is hard to detect, because they are often hidden by diatoms (Goepler, 2016). Also, Goepler found *Perophora japonica* on Heligoland until 2010 only at the dune harbor and on the Schwimmbadmole. Therefore, I consider, sampling on these locations during the spring – summer season will be helpful to find them. *Styela clava* was brought to Heligoland by military ships and is only found in dune harbor. *Molgula citrina* is a northern ascidian that is common on Schwimmbadmole together with *M.complanata* (Goepler, 2016). These species inhabit habitats that were

<i>Clavelina lepadiformis</i> ★	X	X	X	X ^H	X	X	
<i>Polyclinum aurantium</i> ★		X	X		X	X	
<i>Sidnium turbinatum</i>	X	X	X		X	X	
<i>Aplidium glabrum</i>		X	X				
<i>Diplosoma migrans</i> ★		X	X	X	X	X	
<i>Diplosoma listerianum</i>		X	X	X ^H	X	X	
<i>Didemnum maculosum</i>	X	X	X	X ^H	X	X	
<i>Ciona intestinalis</i>		X	X	X	X	X	
<i>Perophora listeri</i>			X		X		
<i>Perophora japonica</i> ★		X	X	X	X	X	
<i>Ascidella aspersa</i>		X	X	X	X	X	
<i>Ascidella scabra</i>			X				
<i>Dendrodoa grossularia</i>							X
<i>Styela clava</i> ★					X	X	
<i>Botryllus schlosseri</i>	X	X	X	X	X	X	
<i>Botrylloides leachi</i>	X	X			X	X	
<i>Botrylloides violaceus</i>				X			
<i>Molgula complanata</i>	X ^H	X	X		X	X	
<i>Molgula citrina</i> ★	X ^H	X	X			X	
	Felswatt	Schwimmbadmole	Nordosthafen	Südhafen	Vorhafen	Dünenhafen	Tiefe Rinne

Fig.6. Distribution of ascidians from Heligoland according to Groepler W. (2016). The letter H indicates species not found by Goepler personally but taken from the Harms 1993 study. Green stars indicate species not found during this study.

not considered during the study.

It is surprising that we did not detect *Clavelina lepadiformis*, although it is a widespread ascidian species, and *Diplosoma migrans*, what both authors found practically everywhere on the eulittoral and sublittoral of the island. There is a centuries-old taxonomic debate behind it. Some authors distinguish *D.migrans* as a separate species. Others believe that it is only a variation of *D. listerianum* or *D. gelatinosum* (Groepler, 2016). I do not see any explainable reasons why we did not encounter *Clavelina lepadiformis* in our samples. Therefore, more careful sampling with a larger number of replicates is required.

Botryllus schlosseri is a common tunicate in the rocky shore. But we did not perform statistical studies to determine this thesis. We may have

Tunicata

Ascidella aspersa (Müller, 1776)

Ascidella scabra (Müller, 1776)

Botrylloides leachi (Savigny, 1816)

Botryllus schlosseri (Pallas, 1766)

Ciona intestinalis (Linnaeus, 1767) #

Clavelina lepadiformis (Müller, 1776)

Dendrodoa grossularia (van Beneden, 1846)

Didemnum candidum Savigny, 1816

[*Didemnum maculosum* Berril, 1950]]

possibly *D. helgolandicum* Michaelsen,

1921, only the larvae can be distinguished

Didemnum sp.

Diplosoma listerianum (Milne-Edwards, 1841)

Diplosoma migrans (Menker & Ax, 1970)

Molgula citrina Alder & Hancock, 1848

Molgula complanata Alder & Hancock, 1870

Polyclinum aurantium Milne-Edwards, 1841

Sidnium turbinatum Savigny, 1816

Styela coriacea (Alder & Hancock, 1848)

Styela partita (Stimpson, 1852)

D₂, D₄, G₁, G₂, I₁-I₃, J₁-J₃, K₈

H₁, K₄

A₆-A₈, D₁, D₃, D₄, E₁-E₃, G₂, H₂, I₁, I₄, J₂,

K₁-K₄, K₇

A₂, A₆-A₈, B₁, B₃, D₁-D₄, G₁, G₂, H₁-H₃, I₁-I₃,

J₁-J₃, K₇, K₈

B₃, D₁-D₃, H₁, I₁-I₃, J₁-J₃, K₈

A₇-A₈, B₂, D₁-D₃, G₁, G₂, H₁, I₁, I₃, K₇

G₂, H₁, K₄, K₆

D₁-D₃, E₁-E₃, G₁, G₂, H₁-H₃, I₁-I₃, K₁, K₄, K₇

A₆-A₈

D₄, H₁

H₁, H₂, I₂-I₄

A₆-A₈, D₁, D₂, D₃, G₂, H₁, H₃, I₁, I₃-I₅, J₁-J₃, K₂

H₁-H₃

D₁, D₃, K₂

A₂, A₆-A₈, D₁-D₃, D₅, E₁-E₄, G₁, G₂, H₁-H₃,

K₁, K₂, K₇

H₃

B₁, H₃

Fig.7. Checklist and distribution of tunicate species found in the vicinity of the island of Helgoland (North Sea, German Bight) summarized by Harms (1993) based on previous records about found species. Samples from: A, B, C, H3, L1, O, R - the eulittoral zone; D, E, F, G, H1-2, I, J, L, L2, Q - the subtidal zone; G1 - on *Laminaria digitata*; G2 - on *Laminaria hyperborea*; M - of the deep sea by dredging ("Tiefe Rinne").

seen it more often because it stands out on algae with its white blood vessels of the colonial zooids forming a flower-like shape (Fig. 8).

A pelagic tunicate *Oikopleura dioica* belongs to the class of Appendicularia. Appendicularians are distinguished by their "larval" organization and the unique structure of the filtration apparatus - a house of mucus, which they easily shed and usually only their bodies (Fig. 9) are found in plankton samples (Marine plankton Helgoland). The Alfred Wegener Institute has monitored meso- and macrozooplankton since the 1970s. *O. dioica*'s seasonal distribution depends on its optimal temperature of 14°C (Greve et al., 2004). The median water temperature (18.7°C) in late September 2023 corresponded to the optimum distribution of this species (MarGate-COSYNA Underwater Observatory off Helgoland – Temperature).

Conclusions

Those species we detected are predominantly distributed across the island in agreement with previous Groepler (2016) data. I am confident that additional material collection and molecular methods are needed to better define *M. manhattanensis* and *D. migrans* in this region. The research trip, during which material was collected, was not only focused on tunicates, and the material collected for this group was insufficient for more detailed analysis. For a comprehensive study of the tunicate diversity on Helgoland Island, I sug-



Fig.8. *Botryllus schlosseri* colony on *Fucus* sp., North Sea, Heligoland, 2023.

gest the following recommendations for future research: 1) Consider the biology of each tunicate species already described, including their seasonal behavior, preferred water temperature, depth range, and substrate type. This would help to predict where to find different tunicate species on the island. 2) Examine samples from all known habitats more thoroughly, and possibly propose new sampling sites on the island, for the poten-

tial discovery of new species. 3) Install easily removable underwater platforms in all ports for seasonal observation of tunicate cover on typical substrates. Such a platform is only installed in NO Hafen by the Alfred Wegener Institute. This would enable monitoring of the distribution of existing species and the detection of possible new species of ascidians.



Fig.9. *Oikopleura dioica* (Oikopleuridae) identified by T. Stach at plankton sample (250 μ m).

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MarGate-COSYNA Underwater Observatory off Helgoland – Temperature <https://dashboard.awi.de/?dashboard=10421>