A molecular survey of *Ulva sensu lato* on German Baltic- and North Sea coasts:

A revision of the species inventory reveals their distribution and contributes new taxonomic insights

Dissertation

For the academic degree Dr. rer. nat. at the Faculty of Mathematics and Nature Sciences of the Christian-Albrechts-University Kiel, GEOMAR Helmholtz Centre for Ocean Research

Submitted by

Sophie Steinhagen

Kiel, 2018

The cover photo was taken at the experimental site Wulfen (Fehmarn, Germany) which is located in the Baltic Sea. It provides an underwater view of a typical macroalgae community, while the focus is on the notorious fouling species *Ulva linza*.

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Content

Abstract

In this doctoral project, I investigated the recent inventory, distribution and phylogenetic relationships of *Ulva sensu lato* in northern Germany, including sampling sites at the Baltic Sea, Wadden Sea and on Helgoland. Furthermore, I compared the recent results with historic findings. Therfore, this thesis constitutes a complete revision of the species inventory of *Ulva sensu lato* in northern Germany.

Assessments of biodiversity were based on both the analysis of classical morphological characters and DNA barcoding. Phylogenetic analysis of more than 370 sequences of the *tuf*A marker gene revealed the presence of 20 different species in German waters. Thereof, three were evaluated as native but cryptic (*Ulva compressa*, *Ulva intestinalis*, *Ulva linza*) and five probably new introduced taxa (*Ulva australis*, *Ulva californica*, *Ulva gigantea*, *Ulva sp.*, *Umbraulva dangeardii*) that are here reported for the first time from this area could be identified. Comparative analyses of recently collected specimens with historic vouchers and respective reference literature evidently confirmed that several species that were considered to occur in the investigated area, were rare or even absent whereas others expanded their distribution.

A distinct range expansion was encountered for the circumarctic boreal species *Kornmannia leptoderma*. While the species reestablished at its former southernmost distribution limit in the North Sea - at the German off-shore island Helgoland - it additionally expanded its distribution at the German coastline of the Wadden Sea and along 220 km length into the Baltic Sea. The implementation of culturing studies supported that *K. leptoderma* can conduct and complete its life cycle at temperatures of 15 °C, which was in contrast with previous studies which reported an inhibition of gametogenesis above temperatures of 12 °C. Furthermore, phenological shifts and thus, possibly an adaption to warmer conditions, were observed.

Molecular analyses of recent specimens and historical herbarium material revealed that certain morphotypes within some species were not differentiable by morphological characters. Such peculiarities became especially obvious in ecosystems with relatively extreme environmental conditions, as found in the Kiel Canal.

Contrary to the expectation the evaluated number of invasive species within the Kiel Canal was low, however, divergent morphologies not corresponding with the protologue of *Ulva* *intestinalis* and *Ulva linza* were observed. Species distributions and in the case of *U. intestinalis* also branching patterns, were affected by salinity, while the eutrophication status of sites within the canal appeared to be an indicator for thallus sizes. I was also able to verify the presence of the invasive, perennial seaweed *Fucus evanescens* and its respective distribution patterns inside the canal. By comparisons with previous findings, I found more evidence supporting that *F. evanescens* has partially substituted the native species *Fucus vesiculosus*.

Historical identification problems of *Ulva sensu lato* lead to flawed inventories and certain ecotypes of distinct nuisance algae were not detected or confused with other species. A suitable example for the enormous phenotypic plasticity of species within the genus *Ulva*, is *Ulva compressa*. Different gross morphologies were observed. Whereas one of its morphotypes - that was only observed in the North Sea - was in agreement with the tubular and branched type material of *U. compressa,* specimens encountered in the Baltic Sea indiscriminately exhibited a distinct morphology of distromatic, sheet-like thalli that were predominantly found drifting. The drifting bladed morphotype was also observed in the Wadden Sea but was not found on Helgoland. Investigations of *tuf*A sequencing data of both morphotypes proved their identity and sequence divergence values were extremely low (≤ 0.9%). However, the tubular and sheet-like forms differed in their ecology and only sheet-like forms tended to have a strong nuisance potential, forming massive accumulations of biomass that were associated with oxygen depletion. Such "green tides" that are formed by proliferating specimens of *U. compressa*, were observed to cause severe damages and lead to increased mortality of habitat forming species of the genera *Zostera* and *Ruppia*.

My dissertation additionally highlights the conspecificity of the model organism *Ulva mutabilis* with one of the first described green algae, *U. compressa*. This finding is supported by multiple lines of evidence. Investigations on cultured material and thus comparisons of vegetative and reproductive features revealed a shared morphological mutation pattern. Further, their conspecifity also meets the criteria of the biological species concept as their ability to crossbreed was confirmed. Gametes of *U. compressa* and *U. mutabilis* were successfully crossed and developed into fertile sporophytic offspring. By applying the General Mixed Yule Coalescent model, I was able to show that the strains of *U. mutabilis* belonged to the same Molecular Taxonomic Unit as samples of *U. compressa* from Germany. Thus *U. mutabilis* was suggested to be reduced to synonymy with *U. compressa*.

Zusammenfassung

In der vorliegenden Doktorarbeit wurde nicht nur das heutige Artinventar von *Ulva sensu lato* in Deutschland untersucht, sondern ebenfalls die geografische Verbreitung von Arten und deren phylogenetische Zusammenhänge aufgeklärt. Hierfür wurden beide Küstengebiete, Nordsee und Ostsee, sowie die Hochseeinsel Helgoland berücksichtigt. Weiterhin wurden die von mir in dieser Doktorarbeit erhobenen Daten mit vorherigen Untersuchungen und historischem Material verglichen. Somit stellt diese These eine komplette Überarbeitung des Artinventars von *Ulva sensu lato* in Norddeutschland dar.

Erhebungen der Biodiversität basierten auf Analysen klassisch- morphologischer Charakteristika in Kombination mit der Methode des DNA barcodings, welches kurze Genabschnitte vergleichenden Identitätsüberprüfung eines Organismus nutzt. Durch phylogenetische Analysen von mehr als 370 Gensequenzen des *tuf*A-Markergens wurde die Existenz von 20 verschiedenen Arten nachgewiesen. Hiervon wurden drei als heimische aber kryptische Arten identifiziert (*Ulva compressa, Ulva intestinalis, Ulva linza*). Auch die Existenz von fünf, vermutlich neu eingewanderten Taxa, welche in dem Beprobungsgebiet zuvor noch nicht beobachtet wurden, konnte festgestellt werden (*Ulva australis, Ulva californica, Ulva gigantea,Ulva sp., Umbraulva dangeardii*). Vergleichende Analysen des heutigen Artinventars mit historischem Herbarmaterial und Referenzliteratur konnten bestätigen, dass verschiedene Arten, welche als sehr häufig betrachtet wurden, in dem untersuchten Gebiet sehr selten oder gar nicht vorhanden waren, wohingegen für andere Arten eine deutlich ausgeweitete Verbreitung ermittelt wurde.

Eine Gebietsausbreitung konnte für die circumarktische, boreale Art *Kornmannia leptoderma* nachgewiesen werden. Während die Art sich am ehemals am südlichsten bekannten Standort, der Nordseeinsel Helgoland, reetablierte konnte zusätzlich eine Verbreitung entlang der Küste des Wattenmeeres und entlang 220 km der Ostseeküste nachgewiesen werden. Kulturstudien belegten, dass *K. leptoderma* einen vollständigen Lebenszyklus bei Temperaturen von 15 °C durchführen kann. Diese Ergebnisse stehen in starkem Kontrast zu vorherigen Arbeiten, welche eine Inhibition der Gametogenese durch Temperaturen über 12 °C beschrieben. Weiterhin wurden in meiner Arbeit Veränderungen in der Phänologie *K. leptodermas* beschrieben, was die Adaption an wärmere Bedingungen zu bestätigen scheint.

Molekulare Untersuchungen der rezenten Arten und historischen Materials zeigten, dass bestimmte Morphotypen von verschiedenen Arten nicht anhand deren morpholoischer Bestimmungsmerkmale unterschieden werden konnten. Solch phänotypische Auffälligkeiten waren vor allem in Ökosystemen mit verhältnismäßig extremen Umweltbedingungen vorzufinden, wie beispielsweise im Nord-Ostsee-Kanal.

Den Erwartungen entgegengesetzt wurde nur eine geringe Anzahl von invasiven Arten innerhalb des Nord-Ostsee-Kanals gefunden. Allerdings konnten verschiedene Morphotypen der Arten *Ulva intestinalis* und *Ulva linza* nachgewiesen werden, welche nicht mit deren morphologischen Artkonzepten übereinstimmten. Die Artverbreitung, und im Falle von *U. intestinalis* ebenfalls das Verzweigungsmuster, waren nachweislich abhängig von der Salinität, wohingegen der Zustand der Eutrophierung Einfluss auf die Thallusgröße hatte. Weiterhin wurde die invasive, mehrjährige Braunalgenart *Fucus evanescens* nachgewiesen und deren Verbreitung innerhalb des Kanals aufgenommen. Vergleiche mit vorherigen Studien bestätigen die Vermutung, dass *F. evanescens* die einheimische Art *Fucus vesiculosus* im Kanal ersetzt hat.

Die erheblichen Identifikationsschwierigkeiten von Arten der Gruppe führten in der Vergangenheit nicht nur zur Erstellung fehlerafter Inventarlisten innerhalb der Ordnungen Ulvales und Ulotrichales, sondern vielmehr wurden auch wichtige Ökotypen einiger Arten, welche ein erhebliches Problem darstellen können, nicht als solche erkannt.

Ein besonders eindrucksvolles Beispiel für die enorme phänotypische Plastizität innerhalb der Gattung *Ulva* ist beispielsweise *Ulva compressa*. Sehr verschiedene Morphologien konnten eindeutig dieser Art zugewiesen werden. Während der dem Typmaterial von *U. compressa* entsprechende tubulär verzweigte Morphotyp ausschließlich in der Nordsee vorgefunden wurde, hatten Individuen aus der Ostsee eine obligat blattförmige Morphologie und wurden meistens driftend beobachtet. Der driftende, blattförmige Morphotyp wurde ebenfalls im Wattenmeer vorgefunden, wohingegen er auf Helgoland gänzlich fehlte. Untersuchungen der *tuf*A-Sequenzen beider Morphotypen zeigten, dass diese identisch waren und Sequenzdivergenzen waren mit $\leq$ 0.9% sehr gering. Trotz der genetischen Ähnlichkeit unterschieden sich die beiden Phänotypen deutlich in ihrer Ökologie und lediglich der blattförmige Morphotyp bildete unter geeigneten Bedingungen ausgedehnte Akkumulationen von Biomasse aus. Diese gingen mit der Entstehung von Sauerstoff-Mangelzonen einher. Diese, durch die erhöhte Proliferation von Einzelindividuen ausgelösten „green tides“, führten zu einer erhöhten Sterblichkeit von Arten der Gattungen *Zostera* und *Ruppia*, welche als Habitatbildner wichtig sind.

Ein weiteres wichtiges Ergebnis meiner Doktorarbeit ist der Nachweis der Konspezifität des Modelorganismus *Ulva mutabilis* mit einer der ersten beschriebenen Grünalgenarten, *Ulva compressa*. Verschiedene experimentelle Ansätze wurden durchgrführt, um diese Konspezifität eindeutig nachzuweisen. Untersuchungen von Kulturmaterial lieferten Erkenntnisse zur Übereinstimmung vegetativer und reproduktiver Eigenschaften der beiden Algenlinien. Ich konnte zeigen, dass beide Arten das gleiche morphologische Mutationsmuster teilten. Zusätzlich wurde durch Kreuzungsexperimente das biologische Artkonzept überprüft. Gameten von *U. compressa* und *U. mutabilis* konnten erfolgreich gekreuzt werden und entwickelten sich zu fertilen Sporophyten. Um auch das genetische Artkonzept zu überprüfen, wurde die „General Mixed Yule Coalescent Methode“ genutzt. Hierbei konnte ich schlussendlich zeigen, dass *U. mutabilis* und *U. compressa* der gleichen „Molecular Taxonomic Unit“ (molekular taxonomischen Einheit) angehören und als ein und dieselbe Art angesehen werden können. Somit schlage ich vor, *U. mutabilis* als Synonym von *U. compressa* zu führen.

1. General introduction

1.1 Introduction into the orders Ulvales and Ulotrichales

Even though only a relatively small number of green macroalgae inhabits saline water environments and the vast majority occurs in fresh waters, several representatives of green algae constitute a major component of macrophytobenthic communities in the marine and brackwater environments. Especially species from the orders Ulvales and Ulotrichales are globally abundant within the sublittoral and the upper supralittoral zone.

Both orders are closely related and include diverse groups of uni- and multicellular species, which comprise various morphologies and life styles (Leliaert et al., 2012). Macroscopic species can be filamentous, or constituted of distromatic or monostromatic blades and also tubular branched or unbranched shapes can be observed. Although most entities grow attached to a substrate, a drifting life strategy is common, as well (Figure 1).

The criteria separating Ulvales and Ulotrichales were based on certain life history traits.

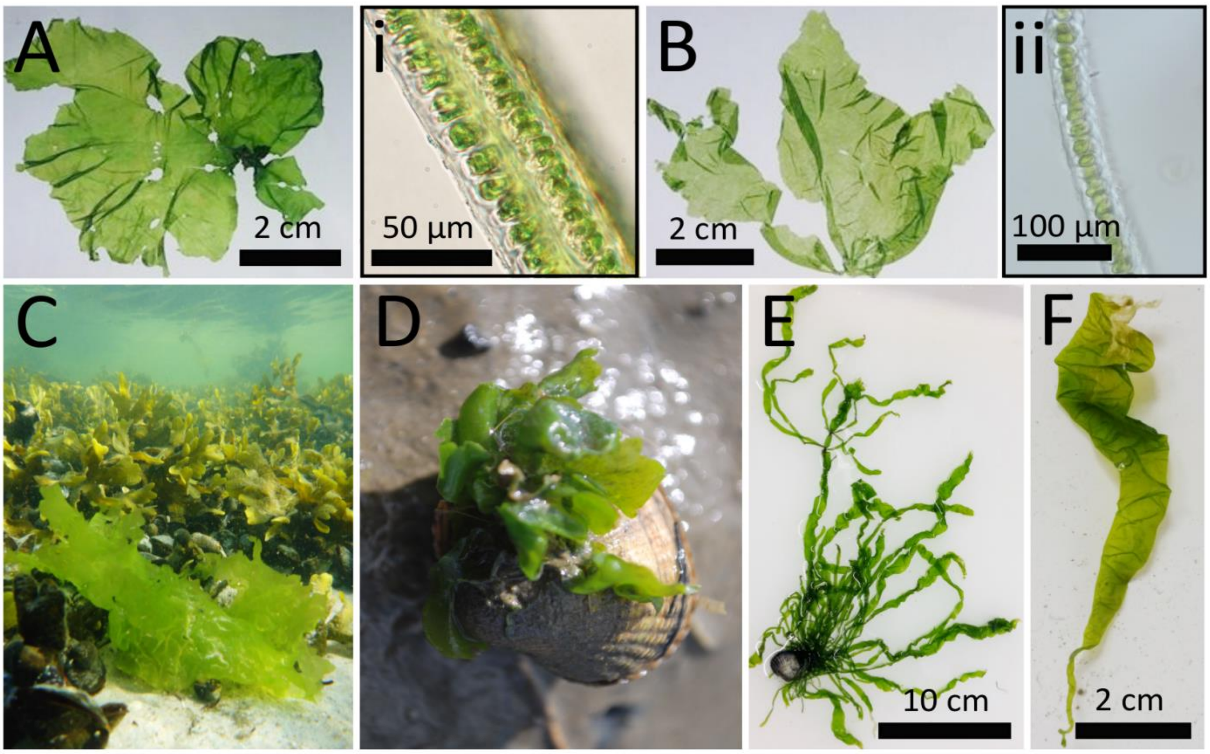

Figure 1: Overview of the different morphologies of macroscopic representatives of the Ulvales and Ulotrichales. (A) Sheet-like, lobed thallus of *Ulva rigida* D. Agardh with several holes. The transection (i) displays the distromatic cell arrangement. (B) Mature specimen of *Monostroma grevillei* (Thuret) Wittrock. Its respective transection (ii) reveals a monostromatic structure. (C) Sheet-like specimens of *Ulva compressa* L. were mostly found drifting, but some were attached to substratum (D). (E) Tubular, branched individual of *U. compressa*. (F) Tubular unbranched specimen of *Ulva linza* L..

Whereas the Ulvales exhibit a life cycle of isomorphic alterations of multicellular stages, in the Ulotrichales the spore-releasing, diploid stage differs strongly from the haploid stage in being a small, thick-walled and petiolated unicell (Leliaert et al., 2012). Notably, molecular phylogenetic studies question the separation of the two orders and provide only poor or no support of their respective monophyly (O'Kelly et al., 2004). Additionally, single species identification based on conventional morphological characters is notoriously difficult and in many cases also incorrect, due to their high morphological plasticity. A noteworthy example is the genus *Ulva*, (Ulvales).

1.2 Characteristics and traits of the genus *Ulva*

The genus *Ulva* is morphologically highly diverse, which has historically hampered the development of stable taxonomic concepts. Cryptic and alien species are often not recognized, while different phenotypes of the same species have in many cases been regarded as distinct taxa. Not surprisingly, the genus underwent many taxonomic rearrangements. The combination of the genus *Enteromorpha* with the presently accepted genus *Ulva* is a typical example.

Previously, *Enteromorpha* (obligate tubular morphology) and *Ulva* (distromatic blades) were treated as separate genera due to their distinct differences in gross morphology. That this segregation was of artificial nature and not underlying phylogenetic boundaries was first unreveled by Hayden et al. (2003). Consequently, species placed in the generic genus construct *Enteromorpha* have been synonymized and are now placed within the genus *Ulva* (Hayden et al., 2003). Since there were no unique morphological synapomorphies identifiable, Tan et al. (1999) suggested a morphogenetic switch that might be able to explain shifts between the gross morphologies of tubes and blades.

However, also ecophenotypic variations have challenged exact species identification and lead to confusions regarding inter- and intraspecific rank. It was for example reported, that specimens of *U. intestinalis* L., a species that is described as unbranched and tubular (Linnaeus, 1753), can exhibit aberrant morphologies under variable and fluctuating environmental conditions. Whereas monostromatic thalli were observed in waterbodies of highly eutrophicated state (Blomster et al., 2002), proliferating, so called ´´bottle-brush´´ morphotypes of *U. intestinalis* were encountered under laboratory conditions after salinity changes and also in media with extreme salinities (Reed & Russell, 1978). Such branched and proliferous morphologies of *U. intestinalis* that strongly differed from the type material (Linnaeus, 1753) were also observed under natural conditions and were predominantly recorded in brackish habitats, like the Baltic Sea (Bliding, 1963). Due to this degree in phenotypic plasticity the different phenotypes present in the Baltic Sea were regarded as different taxa and it required genetic information for exact species determination and analysis of species distribution (Leskinen et al., 2004). Similar morphological variability was also observed in other species, such as in *U. linza* L., which comprises sheet-like unbranched thalli, as well as variously branched tubular morphologies that were formerly described as distinct species (e.g., *Enteromorpha ahlneriana* Bliding, *Enteromorpha procera* K.Ahlner, (Brodie et al., 2007, Guiry & Guiry, 2018)).

Over the last twenty years, more systematic studies have been carried out that were based on both, morphology and DNA sequence data. These studies have revealed many incorrect identifications and lead to new introductions and descriptions of aberrant morphotypes (Blomster et al., 1998, Tan et al., 1999, Blomster et al., 2002, Hayden & Waaland, 2002, Leskinen et al., 2004, Heesch et al., 2009, Kirkendale et al., 2013). Molecular tools have proven to be essential for the systematics in *Ulva*, for which at present different marker genes have been selected and a growing amount of reference sequence data has been generated. The first genetic investigations targeted the internal transcribed spacer region of the ribosomal cistron (ITS) (Blomster et al., 1998, Tan et al., 1999, Blomster et al., 2002, Leskinen et al., 2004), while more recent studies also examined the gene coding for the rubisco large subunit (*rbc*L) (Heesch et al., 2009, Kraft et al., 2010). However, in a comparison of amplification success, the occurrence of double bands was shown for the ITS marker to occur widely among green algae lineages, indicating divergent copies (Saunders & Kucera, 2010). Within the *rbc*L gene low levels of genetic diversity were made responsible to hamper the delimitation of certain species (Heesch et al., 2009). Although the use of molecular data has helped to clarify the systematics in *Ulva*, a curated reference database for Chlorophyta is presently not existent. Consequently, the analysis of DNA sequence data relies solely on the use of GenBank, where in fact high numbers of sequences are assigned to the wrong species names. Distinct sequences with clearly different nucleotide calls often share the same name, while identical sequences are labelled differently. This further complicates the identification by an approach that is highly dependent on correctly annotated reference libraries.

The plastid encoded marker *tuf*A, which is coding for the elongation factor *Tu* has been evaluated as a viable marker for species identification and delimitation within the Ulvales (Saunders & Kucera, 2010). Several studies targeted this marker and even though the database for this marker is relatively small compared to the above mentioned marker genes, uploaded sequences were mostly peer reviewed and carefully annotated. Nevertheless, selecting a species name and thus a species affiliation by references from a databank service has to be done carefully.

As already stated by Kirkendale et al. (2013): „Taxonomy is fundamental to all biological research, however, it is challenging and time-consuming work, especially for difficult taxa such as those in the genus *Ulva"*. That there is an increasing importance for exact species identification is emphasized by the capacity of several *Ulva* species to tolerate a wide range of physical and chemical conditions (nutrients, salinity, temperature etc.). Their opportunistic life-strategy and abundance as fouling organisms on ship hulls (Schaffelke et al., 2006), makes them special candidates for human-mediated dispersal. A closer look into ship traffic analysis has shown a fourfold increase between 1992 and 2012 (Tournadre, 2014). Since most introductions of seaweeds and other organisms into new habitats are related to ship traffic (Simberloff & Rejmanek, 2011), this immense increase puts intense pressure to marine ecosystems.

This background, in combination with the ability of some *Ulva* species for rapid and strong proliferation, underlines the importance of permanently monitoring of this group of green algae.

1.3 The economic and ecological impact of *Ulva* mediated "green tides"

Worldwide, coastal areas are annually affected by accumulations of tons of *Ulva* biomass, so called "green tides". These accumulations can be single- or multi-species blooms and their occurrences are most probably related to the mounting eutrophication of coastal waters (Smetacek & Zingone, 2013). The formation of extensive blooms is facilitated by the large surface area to volume ratios of *Ulva* and their capacity of highly efficient nutrient uptake (Rosenberg & Ramus, 1984). Besides extreme economic losses, relating to tourism and subsequently the disposal of the beached biomass, important ecosystem services get hampered, as the accumulated biomass modifies biogeochemical processes and biodiversity

(Dion & Le Bozec, 1996, Norkko & Bonsdorff, 1996a, Norkko & Bonsdorff, 1996b, Valiela et al., 1997, Charlier et al., 2007, Charlier et al., 2008, Smetacek & Zingone, 2013). It has been shown that particularly at beaches of Brittany, the magnitude of green tides has been increasing since the 1970s (Charlier et al., 2008).

The drastic influence of such accumulations of *Ulva* attained international attention in 2009, as the emission of hydrogen sulfide from decaying biomass from an extensive green tide caused the death of a horse (Source BBC News: http://news.bbc.co.uk/2/hi/8242649.stm.). Only a few years later the news reported about 30 wild boars that were killed by the emitted gaseous substances of rotting *Ulva*, while in 2017 a tragic dead of a jogger resulted in the headlines: “Toxic ´sea lettuce´ fumes may have caused French jogger´s death in Brittany” (Source: International Business Times https://www.ibtimes.co.uk/toxic-sea-lettuce-fumes-may-have-caused-french-joggers-death-brittany-1601012). Besides to the enormous costs (US$10–150 per 1000 kg) of removing the thousands of tons of washed up algae, a strong decrease of tourism was observed in the following years at Brittany´s beaches (Charlier et al., 2007, Charlier et al., 2008).

These recurring phenomena are not restricted to Europe but are observed worldwide. In 2008 vast masses of *Ulva* beached a few weeks before the Olympic Games in Quingdao, China. It took over 10.000 people and about $ 30 million to clean the millions of tons of beached biomass (Liu et al., 2013). High nitrate concentrations in the Yellow Sea were regarded as the most likely cause for growth rates of 21.9 % per day of the algae, which floated in patches that spread over 84.000 km^2 (Keesing et al., 2011, Liu et al., 2013, Liu et al., 2016). The species *Ulva prolifera* O.F: Müller was found to be responsible for these massive green tides in China and it was shown that its rapid proliferation can be induced by the sporulation of cells of fragmented thalli (Gao et al., 2010).

Besides the depicted horrendous economic loss and potential hazard to vertebrates and invertebrates, these phenomena can additionally have severe effects on ecosystems by harming key stone species and habitat formers, such as perannial seaweeds or seagrasses.

Seagrass ecosystems contribute largely to habitat provision by providing sheltered nursery grounds. Furthermore, they play an important role in protection of coastal erosion (Duarte, 2000, Marbà et al., 2006) and they are pivotal organisms, involved in nutrient cycling (Smith et al., 1984, Lee et al., 2007). Considering that green tides are on the rise (Smetacek & Zingone, 2013), it can be expected that seagrass meadows which act as precious ecosystem engineers will increase to suffer population declines caused by macroalgal-blooms (Hartog den, 1994, Ansell et al., 1998). Due to the above mentioned traits, the genus *Ulva* is widely negatively connoted, however not all *Ulva* species tend to proliferate and accumulate to the extent of causing green tides. The genus includes rarely observed species as well. Thus, it is of utmost importance to validate species inventories and monitor threatened areas to maintain an overview of potential new invasions or invasive ecotypes of certain species.

1.4 *Ulva*, a multifaceted and promising model organism

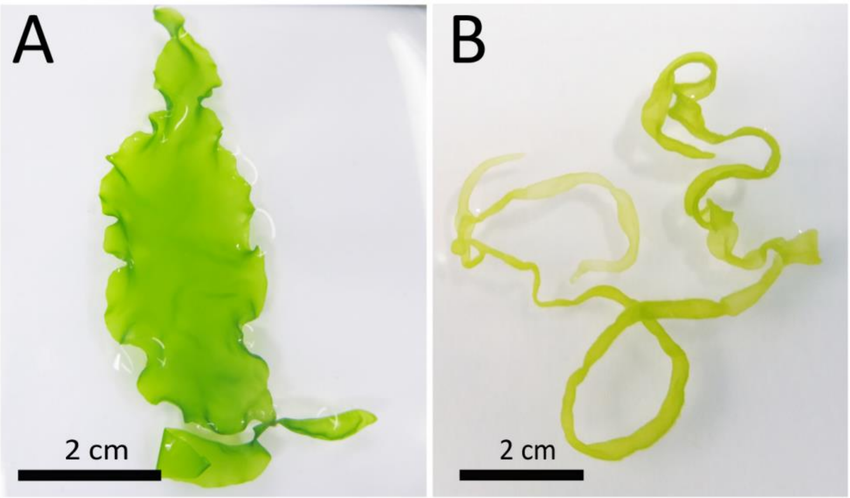

Figure 2: Typical morphotypes of the model organism *Ulva mutabilis*. (A) Leaf-like wildtype morphologies differ strongly from (B) the elongated thalli of the developmental mutant named slender.

Despite – or perhaps because of - their ecological traits and being placed among the most difficult macroalgae to identify, species of the genus *Ulva* provide valuable model organisms due to their high degree of morphological plasticity and their ecological importance (Wichard et al., 2015). As an emerging model system, *Ulva mutabilis* Føyn, constitutes an organism to study reproduction and life cycles of macroalgae, cross-kingdom talk and symbiotic effects, as well as morphogenetic issues (Wichard & Oertel, 2010, Spoerner et al., 2012, Oertel et al., 2015, Wichard, 2015, Wichard et al., 2015, Grueneberg et al., 2016).

Specimens of *U. mutabilis* were first observed by Føyn in 1952 at the south Atlantic coast from Portugal in Faro and Olhão (Føyn, 1958). By cultivating the collected material many spontaneously occurring and induced developmental mutants have been observed and some of them maintained in culture until today. Subsequently, various morphological mutants that descent from gametes of the same parental generation have been characterized. Based on the phenotypic variations Føyn encountered, he named the allegedly new species *U. mutabilis* (Føyn, 1958).

A developmental mutant of *U. mutabilis* that received highest attention and strongly differed from the wildtype material is the elongated phenotype, named slender (Figure 2). Whereas wildtype thalli became fertile within approximately five weeks, individuals of the slender shape required only three weeks for maturation and thus stood out by shorter generation time (Føyn, 1958, Føyn, 1959, Løvlie & Bråten, 1970, Bråten, 1971). Another difference slender thalli express in comparison to the wildtype morphology is the simpler thallus organization and the complete absence, or the presence of only a very reduced rhizoidal zone. This causes thalli to detach after a few days and being encountered drifting from that time onward (Føyn, 1958, Løvlie, 1964, Wichard, 2015). By classical crossing experiments performed with the gemetes of *U. mutabilis*, Føyn was able to divide the isomorphic and morphologically indistinguishable gametes of *U. mutabilis* into sex positive and sex negative strains, and thus established a mating type scheme that was not based on gene loci (Føyn, 1958). The two above mentioned strains of *U. mutabilis* cultures have been determined as mating type positive (mt[+]) for the slender type, whereas the wild-type was evaluated as mating type negative (mt[-]) (Føyn, 1958, Føyn, 1959, Oertel et al., 2015).

That the initial cultures of Føyn are still available today and thus maintained in permanent cultivation, is facilitated by the life cycle of *Ulva* (Figure 3). Both strains are haploid gametophytes and can be propagated parthenogenetically from their unmated biflagellate gametes (Føyn, 1958, Føyn, 1959, Wichard & Oertel, 2010, Oertel et al., 2015, Wichard, 2015).

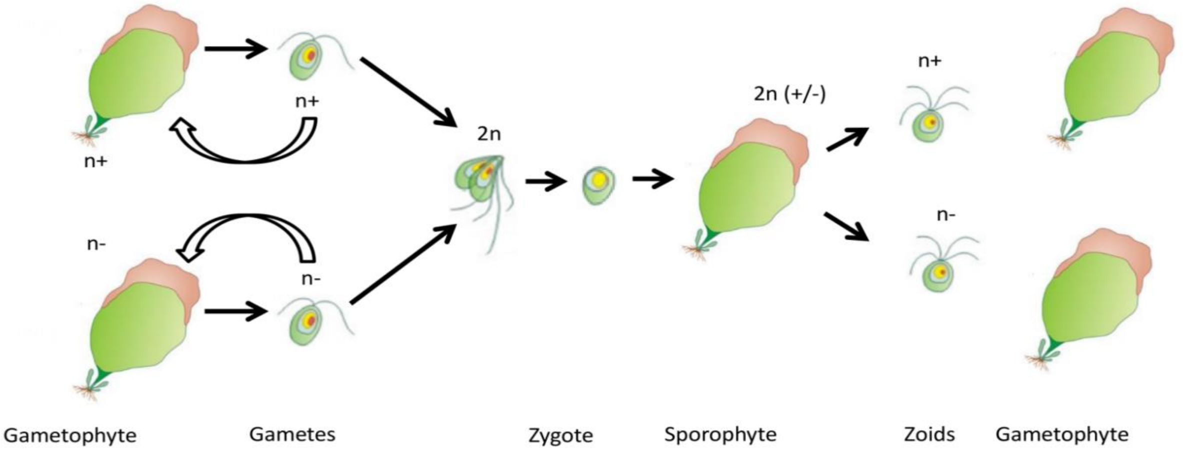

Figure 3: Overview about the isomorphic life cycle of *Ulva*. The life cycle of *Ulva* is based on an alternation between gametophytic and sporophytic life stages of indiscernible morphologies. Whereas the Gametophyte is haploid (n) and propagates by biflagellate haplontic gametes, the sporophyte is diploid and after maturation quadriflagellate, diplontic zoids get released. The respective mating type is indicated by + and - unfilled arrows indicate the parthenogenetic development of unmated gametes (The figure was adapted from Wichard (2015)).

Another advantage of *Ulva* - further contributing to its potential as a model organism - is the fact that gametogenesis can be induced artificially by the removal of certain inhibition factors (Wichard & Oertel, 2010, Wichard, 2015, Wichard et al., 2015). For *U. mutabilis* sporulation inhibitors and a swarming inhibitor have been identified to be involved in controlling the gametogenesis and subsequent gamete release, and acting independent of photoperiod (Wichard & Oertel, 2010, Wichard, 2015, Wichard et al., 2015). The artificial induction of sporulation and subsequently the release of the swarmers is based on the wash off of the respective inhibitors and underlies a special procedure (Wichard & Oertel, 2010, Charrier et al., 2018).

The development and morphology of *Ulva* appears to be dependend on the presence of certain bacteria. From the cultivation of *U. mutabilis* it was shown that these bacteria control growth and development in *U. mutabilis* (Wichard, 2015, Grueneberg et al., 2016). Whereas axenic gametes developed into callus-like thalli with undifferentiated cells comprising malformed cell walls, complete morphogenesis was observed by adding the two bacterial strains *Roseobacter* sp. and *Cytophaga* sp. to the *Ulva*-Culure-Medium (Spoerner et al., 2012, Wichard, 2015, Grueneberg et al., 2016). A symbiotic tripartite community is formed by the three organisms which seem to be able to chemically communicate. It was revealed that the different bacteria species fulfill a complementary task (Spoerner et al., 2012, Wichard, 2015).While *Roseobacter* sp. seems to induce cell division (similar to the effect of cytokinin), *Cytophaga* sp. induces growth of the basal stem cell and primary rhizoidal cells (similar to the effect of auxin) (Spoerner et al., 2012, Wichard, 2015). Furthermore, it was shown that the factor released by *Roseobacter* sp. can be replaced by other Alphaproteobacteria. The factors of *Cytophaga* sp., however, seem to be unique and mandatory which suggests, that specific genes might drive the community of *Ulva* and its associated bacteria (Spoerner et al., 2012, Wichard, 2015).

1.5 The study sites: marine and brackish environments in northern Germany

Northern Germany is bordered by the Baltic Sea in the east and the North Sea in the west, two ecosystems, which are not only geographically separated but also exhibit different ecological conditions. Whereas the total length of the German mainland coastline is expected to be about 1200 km (not considering the islands), the coastline of the state of Schleswig-Holstein represents nearly half of it with 328 km of Baltic Sea coastline (402 when including Fehmarn) and 202 km that stretch along the North Sea (468 when including the islands and Halligen) (Statistisches Amt Schleswig-Holstein, 2008/2009). The North Sea coastline stretches along the unique and protected Wadden Sea that has been declared in 2009 as a UNESCO World Heritage – natural site. The Wadden Sea is a temperate, tidal and fully marine ecosystem, including salt marshes that are protected by groynes (used for land reclamation), mussel- and sand-banks but primarily it is shaped by soft-sediment, which is provided by its extensive mudflats. Its precious character gets underlined by the UNESCO: "The Wadden Sea is one of the last remaining large-scale, intertidal ecosystems where natural processes continue to function largely undisturbed" (UNESCO; https://whc.unesco.org/en/list/1314).

The island of Helgoland, which is located in the German Bight about 40 km off the mainland coast and largely lacks protection from waves, is an exception to the soft bottom environment typical to the Wadden Sea. It is Germany´s only off-shore island, which provides a unique environment by its solid rock pedestal (Reinke, 1889). Thus Helgoland exhibits the only hard substrate formation in fully marine condition throughout whole Germany (Reinke, 1889). Based on its geography and geology, Helgoland is most suitable for macroalgae to grow. In the mid of the 19th century, amateur phycologists started with first phycological research on Helgoland and towards the end of the century professional scientists continued with their work (Bartsch & Kuhlenkamp, 2000). Until today, intensive research on micro- but especially on macroalgae is conducted on Helgoland.

The Baltic Sea, a large postglacial brackish-water basin that is a marginal sea of the Atlantic Ocean, hosts a lower number of marine species, compared to the North Sea. The Baltic Sea is mainly lacking tides, but is being subject to irregular sea level change that is driven by wind and air pressure dynamics (Rönnbäck et al., 2007). Its coast is shaped by substrata of stones, gravel and sand (Rönnbäck et al., 2007) and thus strongly differs from the substrates available at the Wadden Sea coast. Additionally, a strong salinity gradient can be observed in the Baltic Sea. At the entry region of the Danish straits in the Skagerrak, the salinity concentration varies from 25-30 PSU while fresh water inflows reduce the salinity to near fresh water conditions of 1-2 PSU in the northern region at the Gulf of Bothnia and the eastern Gulf of Finland (HELCOM). Along the SW Baltic coastline of Schleswig-Holstein, the salinity drops from approximately 18 to 12 PSU (Gräwe et al., 2014) but steeper gradients can be observed depending on the respective fresh water inflow and season. An additional environment driving and thus important factor is the eutrophicated state of the Baltic Sea. It is considered to be one of the most contaminated seas in the world as a result of anthropogenic pollution (Fonselius, 1972). Eutrophication is defined as the enhanced inputs of nutrients, such as dissolved inorganic nitrogen and phosphorous in the water (Ærtebjerg et al., 2003). Especially anthropogenic activities such as agriculture cause extensive nutrient inflows into marine waters. Thus the degree of pollution and nutrient input within the Baltic Sea increases towards the east, due to the high catchment area of 14 countries (HELCOM, 2011). Consequent scenarios include prolonged algal blooms which, as afore mentioned, benefit from high nutrient levels (HELCOM, 2007-2011).

However, both sea areas, the Baltic Sea in the east and the North Sea in the west, are connected by the world´s most frequented, artificial waterway, the Kiel Canal. With about 100 km length it is Europe´s longest man-made canal for ocean-going vessels and it provides a more rapid and sheltered transit than the passage through the Skagerrak, which is about 400 nautical miles longer (Gollasch & Rosenthal, 2006). The Kiel Canal is bordered by sluice gates in both directions at Kiel, Holtenau, where it starts, and in Brunsbüttel, where it enters the Elbe estuary and subsequently meets the North Sea.

On its complete length, many fresh water inflows discharge in the canal. These inputs, together with the overall direction of water flow result in a seasonal and inter-annual decrease in salinity from east to west, and a net outflow towards the North Sea (Gollasch & Rosenthal, 2006).

Since the Kiel Canal is the most frequently used artificial waterway (Gollasch & Rosenthal, 2006) and ship traffic represents the most common vector for marine invasions (Tournadre, 2014), it can potentially be regarded as an important migratory pathway for biological invasions of non-native species. Even though, some studies recorded growth of macroalgae in the Kiel Canal (Arndt, 1931/32, Aleem & Schulz, 1952, Gollasch & Rosenthal, 2006), a comprehensive overview, which is also covering non-native entities is lacking.

References cited in the general introduction are listed below the general discussion.

1.6 Thesis outline and aim of the examined research

When I began my research during the summer of 2014, most available studies on *Ulva sensu lato* in northern Germany had primarily been focused on their morphological identification. Regarding the notorious identification problems with *Ulva sensu lato* and their risk potential of forming extensive, harmful blooms, in combination with their ability to cope with various biotic and abiotic factors, the special importance of a genetically validated inventory list for this species group in northern Germany became not only mandatory for future ecological monitoring but also focus of political questions in regard of the European Water Framework Directive.

Therefore, the aim of the study was to characterize the genetic diversity of *Ulva sensu lato* over the range of distinct, geographically separated ecosystems of the German state of Schleswig-Holstein, including sampling sites on the Baltic Sea, Wadden Sea and on Helgoland. To this purpose I combined classical morphological methods with genetic barcoding techniques. To assess possible cryptic or newly introduced species and to chart the geographic range of certain species of *Ulva sensu lato* comparisons with previous studies and validations of the different phenotypes within species were carried out. It was also part of my research to investigate abiotic factors that can predict the phenotypic appearance of certain *Ulva* species under natural conditions.

A central research question of this thesis was the assessment of certain species, or ecotypes of species, that caused macroalgae blooms assumed to be involved in the wide spread sediment anoxia of the Wadden Sea during the 80s to 90s of the last century, the so called “Schwarze-Flecken” (Black-patches). Relatively early during the work for this thesis I realized that *U. compressa* may cause such patches and the species therefore became an important focus of my project.

Even though, former studies found evidence for the close relation of the model organism *U. mutabilis* with the “*compressa-pseudocurvata*” complex, a verified validation of their relationship was lacking. One of the main projects of my research therefore focused on the conspecificity of *U. mutabilis* and *U. compressa*. A multidisciplinary approach, combining classical cross-breeding and cultivation methods with up to date phylogenetic techniques, was chosen for the verification of the biological, the morphological and the genetic species concept.

1. 7 Hypotheses

(1) Former and historic species inventories of Ulvales and Ulotrichales of northern Germany do not coincide with a species list based on genetically delimited entities.

  - Morphological species concepts applied to the allegedly identified representatives of *Ulva sensu lato* in northern Germany do not represent phylogenetic species boundaries, due to morphological plasticity of certain species.
  - The occurrence of invasive species is underestimated, due to a lack of suitable identification techniques.
  - The previously specified species composition is overestimated, due to the occurrence of species exhibiting various different phenotypes.

(2) The Kiel Canal, which is one of the most trafficked artificial waterways, functions as a migratory pathway for non-native macroalgae species.

  - A higher number of algal bioinvaders can be found inside or in direct vicinity of the Kiel Canal.
  - Due to its strong salinity gradient the environmental conditions hinder the identification of native and invasive species and enhance the potential of aberrant phenotypes of certain green algal species.
  - Variant morphological forms can be induced by changing abiotic factors.

(3) The circumarctic boreal green alga *Kornmannia leptoderma* (Kjellman) Bliding expanded its distribution from its former extreme southern distribution limit Helgoland, North Sea, into the Baltic Sea.

  - There is genetic evidence for an established *Kornmannia leptoderma* population in the Baltic Sea that is phylogenetically not delimited from populations from Helgoland.
  - *Kornmannia leptoderma* fulfills a complete life cycle and subsequently produces fertile offspring at much higher temperatures than previously examined.

(4) The ubiquitous species *Ulva compressa* expresses a high phenotypic plasticity and has been confused with other species in the past, which resulted in incorrect assessments of its distribution and additionally a potentially invasive ecotype remained unnoticed.

  - Different gross morphologies that strongly deviate from the type material can be frequently observed in northern Germany.
  - Previously examined distributions of *U. compressa* and thus former determined thresholds of its occurrence contained errors due to its high crypticity.
  - *U. compressa* is able to form massive tides under suitable conditions and was involved in the phenomena of extensive anaerobic areas in the Wadden Sea during the last century.

(5) The emerging model organism *Ulva mutabilis* is conspecific with one of the first described green algal species, *Ulva compressa*.

  - Molecular species delimitation methods unequivocally support the monophylie of the two entities.
  - Applying the biological species concept, *U. mutabilis* and *U. compressa* can reproduce into fertile offspring.

2. Chapters and contributions of authors

Parts of this doctoral thesis have been published or submitted:

Chapter 1:

Steinhagen S., Weinberger F., Karez R. **(in preparation for submission to European Journal of Phycology)** Molecular survey on the diversity of *Ulva sensu lato* on German North- and Baltic Sea coasts: About cryptic, alien and lost species.

Statement of authorship: Steinhagen S., Weinberger F., Karez R., experimental design. Steinhagen S., Weinberger F., Karez R., field work and algae collection. Steinhagen S., laboratory work. S. Steinhagen, phylogenetic analysis. S. Steinhagen wrote the manuscript and F. Weinberger commented and corrected the manuscript.

Chapter 2:

Steinhagen S., Karez R., Weinberger F. **(published in Botanica Marina; https://doi.org/10.1515/bot-2018-0020)** Surveying Ulvales and Fucoid seaweeds in the world´s most frequently used artificial waterway, the Kiel Canal.

Statement of authorship: Steinhagen S., Weinberger F., Karez R., experimental design. Steinhagen S., Weinberger F., field work and algae collection. Steinhagen S., Weinberger F., statistical analysis and modeling. Steinhagen S., laboratory work and phylogenetic analysis. S. Steinhagen wrote the manuscript and all co-authors commented and corrected the manuscript.

Chapter 3:

Weinberger F., **Steinhagen S.**, Afanasyev D., Karez R., **(published in Botanica Marina; DOI: https://doi.org/10.1515/bot-2018-0019)** New records from the southern North Sea and first records from the Baltic Sea of *Kornmannia leptoderma*.

Statement of authorship: Weinberger F. and Steinhagen S. contributed equally to the publication. Weinberger F., Steinhagen S., Karez R. experimental design. Steinhagen S., Weinberger F., Afanasyev D., field work and algae collection. Steinhagen S., Weinberger F. cultivation and culture observation. Steinhagen S., laboratory work and phylogenetic *analysis. F. Weinberger wrote the manuscript and all co-authors commented and corrected the manuscript.*

Chapter 4:

Steinhagen S., Karez R., Weinberger F. **(accepted for publication by European Journal of Phycology; DOI:10.1080/09670262.2018.1513167)** Molecular analysis of *Ulva compressa* (Chlorophyta, Ulvales) reveals its morphological plasticity, distribution and potential invasiveness on German North Sea and Baltic Sea coasts.

Statement of authorship: Steinhagen S., Weinberger F., Karez R., experimental design. Steinhagen S., Weinberger F., field work and algae collection. Steinhagen S., laboratory work and phylogenetic analysis. S. Steinhagen wrote the manuscript and all co-authors commented and corrected the manuscript.

Chapter 5:

Steinhagen S., Barco A., Wichard T., Weinberger F. **(submitted to Journal of Phycology, current status: resubmitted after minor revision)** Conspecificity of the model organism *Ulva mutabilis* and one of the first described green algal species, *Ulva compressa* (Ulvophyceae, Chlorophyta).

Statement of authorship: Steinhagen S., Weinberger F., Wichard T., experimental design. Steinhagen S., Weinberger F., field work and algae collection. Steinhagen S., Wichardt T., cultivation and interbreeding experiments. Steinhagen S., Barco A., phylogenetic analysis. Steinhagen S., laboratory work. S. Steinhagen wrote the manuscript and all co-authors commented and corrected the manuscript.

Chapter I **(in preparation for submission to European Journal of Phycology)**

Molecular survey on the diversity of *Ulva sensu lato* on German North- and Baltic Sea coasts: About cryptic, alien and lost species

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Abstract

Barcoding of the *tuf*A marker gene revealed considerable differences between the expected and the current species inventory of *Ulva sensu lato* in the Baltic and North Sea areas of the German state of Schleswig-Holstein. Of 20 discovered genetic entities at least four (*Ulva australis*, *Ulva californica*, *Ulva gigantea* and *Umbraulva dangeardii*) were recently introduced. Three other entities (one of the genus *Ulva*, two of the genus *Blidingia*) could not be identified on species level and might also represent newly introduced species. *Kornmannia leptoderma* and *Ulva rigida* are today much more widely distributed than indicated by historical records, while *Blidingia minima* and *Gayralia oxysperma* appear as absent or much more rare than expected. *U. lactuca* is restricted to Helgoland and analysis of the type material of *Ulva tenera* and of historical vouchers of *Ulva pseudocurvata* from this North Sea island revealed their genetic identity with *U. lactuca*. Past morphological descriptions of *Ulva intestinalis* and *Ulva compressa* have been too restrictive, which also resulted in numerous misidentifications of these abundant taxa. The same is true for *Ulva linza*, which clusters in our area into two closely related genetic entities that are morphologically indistinguishable. One of these entities is only present on Helgoland, while the second is only present on North Sea and Baltic Sea main land coasts.

Key words: *Blidingia*, *Gayralia*, *Kornmannia*, *Monostroma*, *Protomonostroma*, *Ulva*, *Umbraulva*, *tufA*.

Introduction

Macroalgae of the order Ulvales and Ulotrichales are ubiquitous inhabitants of fully marine and brackish coastal waters and in some cases also of freshwater. Several species within this group are on the rise, due to an opportunistic lifestyle and a capacity to benefit from eutrophication and other anthropogenic impact, and the importance to identify these taxa directly increases with their abundance (Charlier *et al.*, 2007; Charlier *et al.*, 2008; Smetacek & Zingone, 2013). However, the identification of some representatives is known to be notoriously difficult. A striking example is the genus *Ulva*. Various studies focused on the morphological instability of different *Ulva* species and disclosed salinity (Reed & Russell, 1978; Steinhagen *et al.*, 2018b [seealso Chapter II]), nutrient concentration (Blomster *et al.*, 2002; Steinhagen *et al.*, 2018b [seealso Chapter II]), associated bacteria (Spoerner *et al.*, 2012; Wichard, 2015), but also an elevated tendency for mutagenesis (Wichard, 2015) as factors being responsible for gross morphological change. As a consequence, morphological plasticity and the appearance of different morphotypes within the same species or the existence of different species exhibiting the same morphology may lead to misidentification and taxonomic confusion. This was repeatedly confirmed when DNA barcoding approaches for species distinction were introduced into the field (Blomster *et al.*, 1998; Tan *et al.*, 1999; Blomster *et al.*, 2002; Hayden & Waaland, 2002; Hayden *et al.*, 2003; Shimada *et al.*, 2003; Brodie *et al.*, 2007; Heesch *et al.*, 2009; Wolf *et al.*, 2012; Kirkendale *et al.*, 2013). They revealed that even the historical separation of the genera *Enteromorpha*, for tubular ``species``, and *Ulva*, for sheet-like representatives, is artificial and not reflecting phylogenetic relationships, as correctly predicted by Linnaeus (1753) (Hayden *et al.*, 2003). The genera *Enteromorpha* and *Ulva* consequently got synonymized and the nowadays accepted genus *Ulva* combines species of different tubular, sheet-like and mixed morphologies, reflecting their phylogenetic affiliation. Thus, allegedly unique morphological characteristics that were indicated in past species descriptions and thus used in identification keys are often not informative, while molecular methods allow for reliable species differentiation (Blomster *et al.*, 1998; Blomster *et al.*, 2002; Hayden *et al.*, 2003; Brodie *et al.*, 2007). In particular the marker gene *tuf*A was found suitable for identification of green algae (Saunders & Kucera, 2010). However, species identification exclusively based upon DNA barcoding remains ambiguous when reference sequences of type material are missing, which is the case for most Ulvales and Ulotrichales. DNA in historical vouchers is often insufficiently conserved, which hampers sequencing (Staats *et al.*, 2011). As a consequence a combination of molecular and morphological methods is still required if ancient concepts of taxa are to be linked with genetic traits (Hillis, 1987).

In northern Germany recordings of seaweeds were carried out since the mid-19th century. Especially the small island of Helgoland – located offshore from main land coasts in the SE North Sea - received much attention by marine botanists and phycologists, which made it the best studied seaweed habitat in Germany (Bartsch & Kuhlenkamp, 2000). With its solid rock pedestal Helgoland provides a natural substrate for macrophytobenthos in a fully marine environment and is in this respect unique in Germany (Reinke, 1889; Bartsch & Kuhlenkamp, 2000). The most recent comprehensive in-depth descriptions of the macroalgal vegetation on Helgoland (Kornmann & Sahling, 1977; Kornmann & Sahling, 1983; Kornmann & Sahling, 1994) were still exclusively based upon morphological identification criteria, whereas an annotated list compiled by Bartsch and Kuhlenkamp (2000) gives an overview of rare and doubtful species and it additionally summarizes taxonomic changes.

The knowledge of macroalgal species diversity gained on Helgoland is only transferable to a limited extend to Germany’s mainland coasts, which largely differ in their ecological conditions. The tidal Wadden Sea also provides a fully marine ecosystem (30-33 PSU) within the North Sea, but it mainly consists of extended sand and mud flats, while hard substrate is relatively scarce. Contrasting both with Helgoland and the Wadden Sea, the brackish German Baltic Sea coast mainly lacks tides and is widely shaped by substrates like stones, gravel and sand (Rönnbäck *et al.*, 2007). Besides of general identification keys for these areas (Rothmaler, 1984; Pankow, 1990) information on macroalgal species and their abundances in the German Wadden and Baltic Sea areas is rather sparse. Based on a summary of literature records, Schories *et al.* (2009) provided a species inventory for macroalgae on the different coastal sections of Germany. However, the taxonomic concepts underlying historical records are often unclear and records based upon molecular species identification are still sparse for the area.

We here present a reassessment of the diversity of *Ulva sensu lato* at geographically separated coasts of the German state of Schleswig-Holstein, including sampling sites on Baltic Sea and North Sea coasts and on Helgoland. Our survey is based upon a combined approach of DNA barcoding and observation of classical morphological identification criteria. Mostly field collected specimens, but also some herbarium vouchers were examined, which allowed us to detect several cryptic or newly introduced species and to unravel some historical misinterpretations.

Material and methods

Collection information

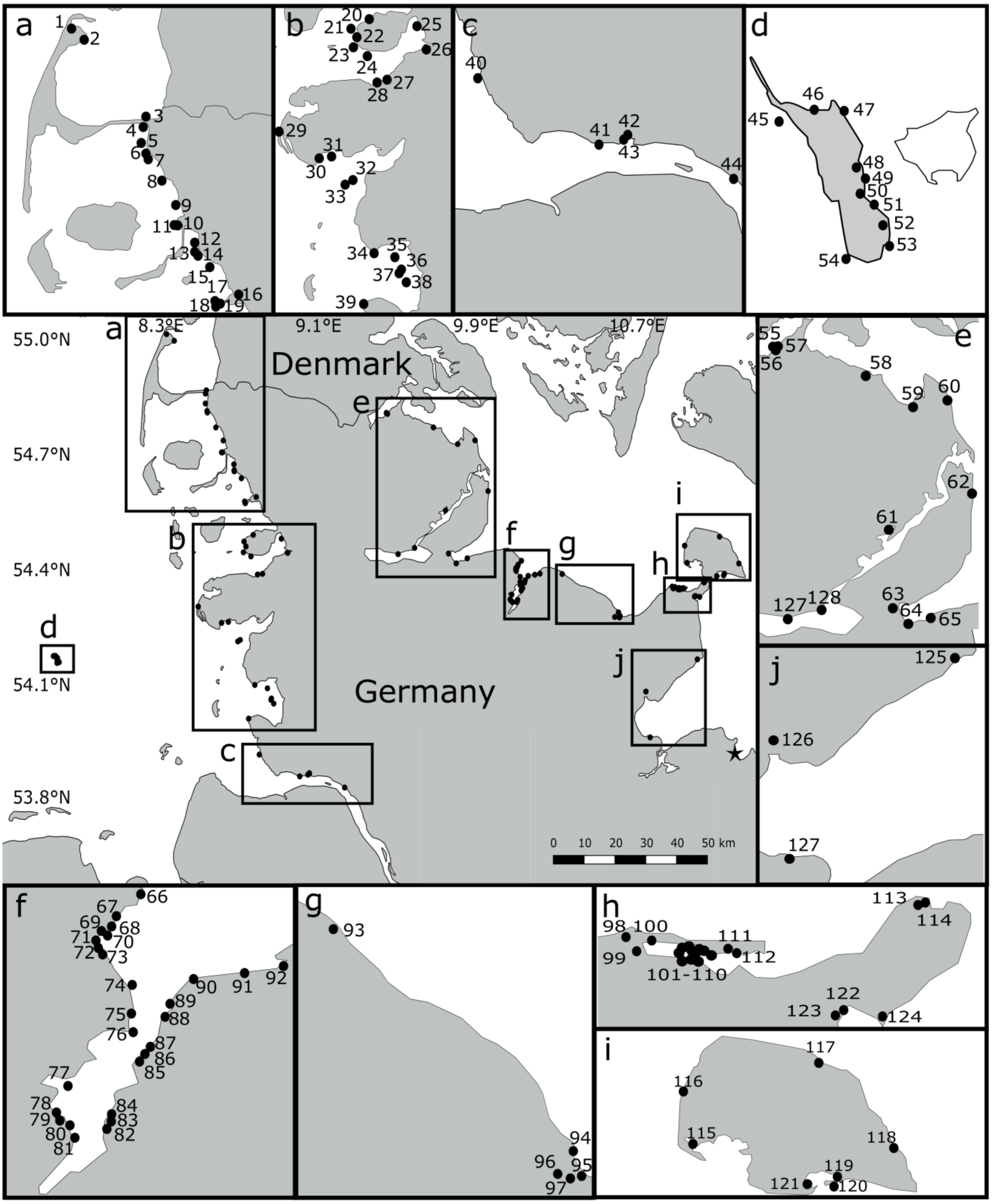

Figure 1: Map of northern Germany indicating the distribution of collection sites visited in this study. Insets a-j provide higher resolution. Numbers 1-126 cross-reference to Table 2 and supplementary Table S 1, number 127 indicates the sampling site Winning and 128 Brodersby, both were additionally sampled in 2017 and 2018. The asterisk marks the site Wohlenberg, investigated in a previous survey.

Samples of *Ulva sensu lato* were collected throughout the northern German region of the state Schleswig-Holstein (Figure 1). We visited altogether 127 collection sites, which were located at the Wadden Sea (44 sites), at the Baltic Sea (73 sites) and at Helgoland (10 sites) and represented diverse habitats within these different ecosystems, such as estuaries, overflow basins and drain channels. The sampling sites spread over 536 km coastal length at the Baltic Sea coast and 466 km coastal length at the North Sea coast. The maximum distance between two sites was below 25 km. Full data on collection sites are available in the supplementary table S 1. To cover species that were only present in certain seasons collections were carried out in summer (July-August 2014 and August-September 2015) and spring (April 2015 and March 2016). Single locations were also visited in 2017 and 2018. Only a limited number of sites was visited in winter (November 2014 – early March 2015), due to reduced green algal growth during this season. Sites at North Sea coasts (mainly groynes, bulwarks, rocks and mudflats) were visited during low tide. Collections at the Baltic Sea were performed when water levels were low by using waders and an aquascope, which allowed for sampling down to a depth of 1.2-1.5 m below mean sea level. On Helgoland three samplings were conducted by divers in August 2014. Of each morphotype that was present in a visited location representative specimens were collected. Epiphytes were also sampled from host specimens. Collected thalli were stored in a cooling box at ~ 10 °C and transported to the laboratory. Pre-identification was based upon morphological characters suggested by valuable identification keys (Koeman & Van den Hoek, 1981; Koeman & Van den Hoek, 1982a; Koeman & van den Hoek, 1982b; Hoeksema & van den Hoek, 1983; Koeman & van den Hoek, 1984) and recorded separately at basal, middle and apical - thallus parts by using light microscopy. For staining starch containing compartments such as pyrenoids Lugol´s solution (Iodine-Potassium Iodide) was used. For further molecular processing epiphyte-free pieces of thallus tissue (1x1 cm) or complete smaller thalli were either frozen and lyophilized or dried in silica gel.

Molecular biology

Total DNA was extracted from dried samples, using the Invisorb Spin Plant Mini Kit (*Stratec, Birkenfeld, Germany*) and following the company’s protocol. For PCR amplification of the plastid encoded barcoding marker *tuf*A the primers tufGF4 (Saunders & Kucera, 2010) and tufAR (Famà *et al.*, 2002) were used . The reaction took place under the following profile: initial denaturation at 94°C for 4 min, then 38 cycles of 94 °C for 1 min, 55°C for 30 sec, 72°C for 1 min, finally 72 °C of final extension for 7 min. Purified amplicons were directly sequenced by GATC biotech (*Konstanz, Germany*). Both strands were sequenced and assemblages of forward and reverse sequences as well as reciprocal editing was done with Sequencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, MI). Obtained sequences were uploaded to GenBank and are publicly available (for complete set of accession numbers see supplementary Table 1). Sequence alignment was carried out using MAFFT (Katoh *et al.*, 2002) while editing was done by eye. The partial alignment reflects a portion of the *tuf*A gene of 777 bp length. For determining the optimal substitution model, the MrModeltest software version v. 2.2. (Nylander, 2004) was used and found to be GTR+G+I. Subsequent phylogenetic analyses were performed in RAxML version 8 (Stamatakis, 2014) for a maximum likelihood (ML) approach applying 1000 bootstrap iterations and the suggested substitution model whereas Bayesian inference (BI) analyses were computed with MrBayes version 3.2.2 (Ronquist *et al.*, 2012) with four simultaneously running Markov Chain Monte Carlo chains for $5 \cdot 10^6$ generations. The run was ended automatically when standart deviation of split frequencies dropped below 0.01. Reference sequences downloaded from GenBank were included in the analyses, however they were chosen with caution. Annotated sequences published in peer-reviewed articels were selected with preference.

Comparison of recent species richness with historical findings

After the genetic survey of recent species richness the variability of micro- and macromorphological characters within each species was assessed, using the above mentioned criteria. To assess potential misidentifications of historically found species several macroalgae collections and herbaria (Herbarium of the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany [BRM], Herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany [GEO]; Herbarium of the Natural History Museum of Denmark, Copenhagen, Denmark [C]) were visited and historical vouchers of Ulvales from our study area and neighboring regions were morphologically compared with the recent findings. If possible, small thallus pieces of vouchers were sampled for molecular species verification. Molecular verification followed the procedure described in Steinhagen *et al.* (2018a) (see also Chapter IV).

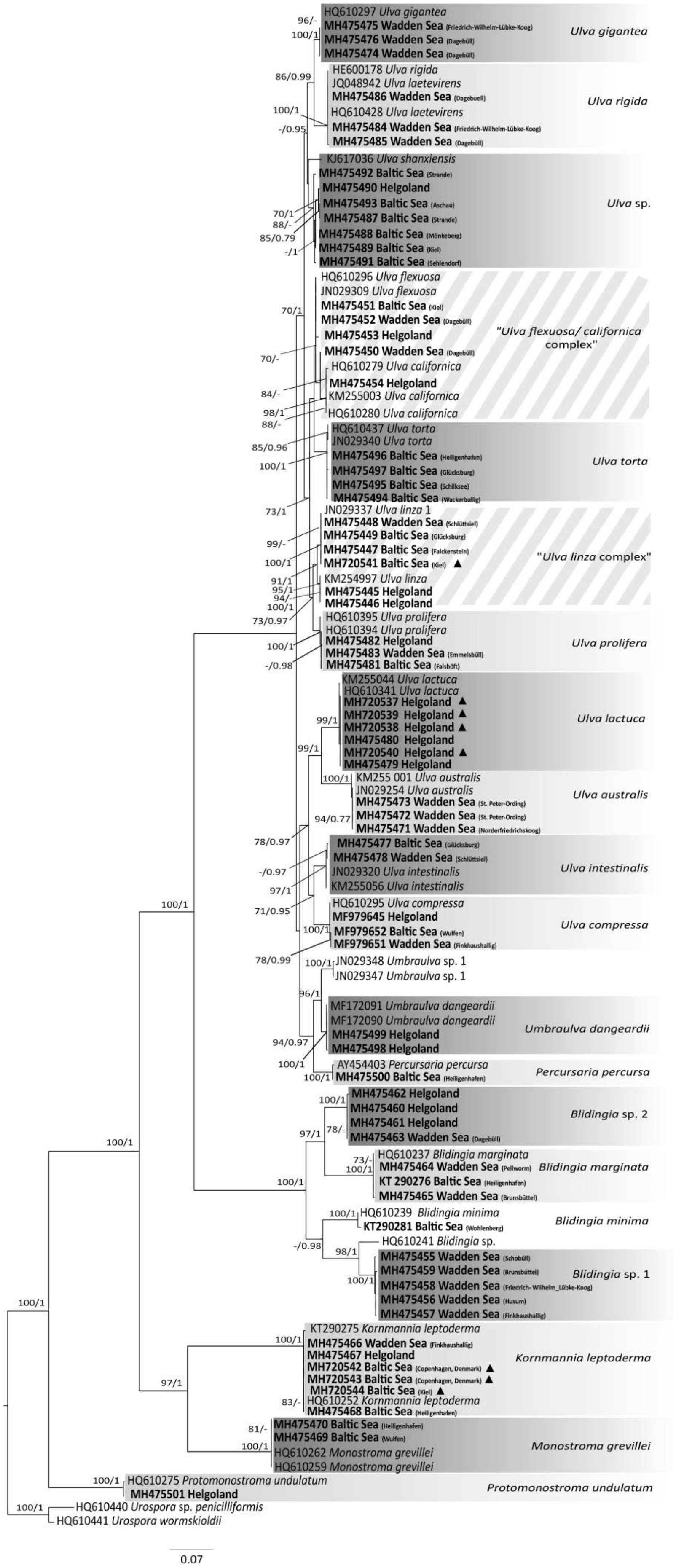

Figure 2: Maximum likelihood phylogram inferred from *tuf*A sequences, displaying entities of *Ulva sensu lato* from northern Germany identified in this study. Herbarium vouchers are marked with a filled triangle (see also Table 2). Two tones of grey indicate clades which are present in the area, hatched boxes indicate species complexes and thus entities that could not clearly be resolved phylogenetically. Numbers at nodes refer to bootstrap values (left) and Bayesian posterior probabilities (1,000 replicates; right). Nodes with < 70% bootstraps and < 0.70 Bayesian support are not labeled. Branch lengths are drawn proportional to the amount of sequence change.

Results

Molecular data and phylogenetic analyses of recent samples

Altogether 370 samples of *Ulva sensu lato* were processed genetically for species distinction and identification on the basis of *tuf*A sequence data. The full dataset was subject to phylogenetic analyses (see Supplement Table S1). In addition an analysis with selected representative sequences was also realized (Figure 2). ML and BI resulted in a congruent consensus tree rooted by an outgroup (*Urospora penicilliformis* HQ610440 and *Urospora wormskioldii* HQ610441).

The different species encountered are shortly addressed below, while a detailed description of their distribution and morphology is given in the supplement. Only a few particularly conspicuous species will be discussed in detail in the following:

The phylogenetic analysis separated the investigated specimens into 20 entities. Based upon peer reviewed reference sequences provided by GenBank nearly all of them could be resolved. They belonged to the genera *Ulva*, *Umbraulva*, *Percursaria*, *Blidingia*, *Kornmannia*, *Monostroma* and *Protomonostroma*.

One major branch within the consensus tree included all members of the first three of these genera and it thus represented the family Ulvaceae. Notably, this phylogenetic lineage split into two subgroups. One of them contains only entities allocated to the genus *Ulva*, whereas the separated smaller subgroup contains representatives of the genus *Ulva* (*U. lactuca, U. australis*, *U. intestinalis, U. compressa*) and of the genera *Umbraulva* (*Umbraulva dangeardii*) and *Percursaria* (*Percursaria percursa*). This particular topology was only observed when sequences of the species *Percursaria percursa* were included. When sequences of *Percursaria* are disregarded *Umbraulva* clusters as a sister group to *Ulva* (supplement, Figure S1).

Most of the species clades obtained full bootstrap and posterior probability support. All sequences of *U. gigantea* were identical to a sequence from New Brunswick, Canada (Figure 2, Table 2). Specimens belonging to this clade were always distromatic blades and they were only found in a limited area in the Wadden Sea and once in the Baltic Sea (For details about morphology and distribution see online supplement).

Table 1: List of green algal samples collected in 2014-2016 in northern Germany and used in the displayed phylogenetic tree (A sequence of *Blidingia minima* from a former survey at an adjacent site in Mecklenburg-Vorpommern is included as reference).

Accession-No [a]	**Species**	**Voucher No.** [b]	**collection_date**	**Region**	**Location**	**Site-No.** [c]	**Lat**	**Lon**
MH475471	*Ulva australis*	TD_10	24-Jul-2014	Wadden Sea	Norderfriedrichskoog	28	N 54,4136	E 8,8789
MH475472	*Ulva australis*	TD_34	15-Aug-2014	Wadden Sea	St. Peter-Ording	30	N 54,2857	E 8,7032
MH475473	*Ulva australis*	TD_36	16-Aug-2014	Wadden Sea	St. Peter-Ording	29	N 54,3267	E 8,5851
MH475450	*Ulva californica*	S_106	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475454	*Ulva californica*	S_791	23-Sep-2015	Helgoland	Helgoland	48	N 54,1837	E 7,8886
MF979651	*Ulva compressa*	S_672	21-Apr-2015	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MF979652	*Ulva compressa*	S_514_B	19-Sep-2014	Baltic Sea	Wulfen	121	N 54,4089	E 11,1731
MF979645	*Ulva compressa*	S_14_B	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475451	*Ulva flexuosa*	S_257	18-Aug-2014	Baltic Sea	Kiel	78	N 54,3538	E 10,1413
MH475452	*Ulva flexuosa*	S_769	16-Aug-2015	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475453	*Ulva flexuosa*	S_794	23-Sep-2015	Helgoland	Helgoland	51	N 54,1780	E 7,8887
MH475474	*Ulva gigantea*	S_775	16-Aug-2015	Wadden Sea	Dagebuell	10	N 54,7304	E 8,6939
MH475475	*Ulva gigantea*	S_564	9-Apr-2015	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	7	N 54,8333	E 8,6142
MH475476	*Ulva gigantea*	S_632	17-Apr-2015	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475477	*Ulva intestinalis*	S_72	24-Jul-2014	Baltic Sea	Gluecksburg	55	N 54,8392	E 9,5176
MH475478	*Ulva intestinalis*	S_133	31-Jul-2014	Wadden Sea	Schluettsiel	13	N 54,6844	E 8,7539
MH475479	*Ulva lactuca*	S_729	24-Apr-2015	Helgoland	Helgoland	47	N 54,1882	E 7,8801

MH475480	*Ulva lactuca*	S_696	23-Apr-2015	Helgoland	Helgoland	50	N 54,1797	E 7,8896
MH475447	*Ulva linza 1*	S_241_U.linza 1	18-Aug-2014	Baltic Sea	Falckenstein	76	N 54,3904	E 10,1922
MH475448	*Ulva linza 1*	S_504_U.linza_1	16-Sep-2014	Wadden Sea	Hamburger Hallig	18	N 54,5990	E 8,8122
MH475449	*Ulva linza 1*	S_64_U.linza_1	24-Jul-2014	Baltic Sea	Gluecksburg	55	N54,8392	E 9,5176
MH475445	*Ulva linza 2*	S_727_U.linza_2	24-Apr-2015	Helgoland	Helgoland	52	N 54,1772	E 7,8930
MH475446	*Ulva linza 2*	S_8_U.linza_2	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475481	*Ulva prolifera*	S_196	12-Aug-2014	Baltic Sea	Falshoeft	60	N 54,7685	E 9,9653
MH475482	*Ulva prolifera*	S_9	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475483	*Ulva prolifera*	S_466	10-Sep-2014	Wadden Sea	Emmelsbuell	8	N 54,7949	E 8,6581
MH475484	*Ulva rigida*	S_449	9-Sep-2014	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	7	N 54,8333	E 8,6142
MH475485	*Ulva rigida*	S_123	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475486	*Ulva rigida*	S_111	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475487	*Ulva shanxiensis*	S_228	13-Aug-2014	Baltic Sea	Strande	71	N 54,4350	E 10,1702
MH475488	*Ulva shanxiensis*	S_269	18-Aug-2014	Baltic Sea	Moenkeberg	82	N 54,3465	E 10,1742
MH475489	*Ulva shanxiensis*	S_256	18-Aug-2014	Baltic Sea	Kiel	78	N 54,3538	E 10,1413
MH475490	*Ulva shanxiensis*	S_2_A	22-Jul-2014	Helgoland	Helgoland	53	N 54,1698	E 7,8894
MH475491	*Ulva shanxiensis*	S_317	22-Aug-2014	Baltic Sea	Sehlendorfer lake	95	N 54,3088	E 10,6886
MH475492	*Ulva shanxiensis*	S_221	13-Aug-2014	Baltic Sea	Strande	70	N 54,4362	E 10,1750
MH475493	*Ulva shanxiensis*	S_92	24-Jul-2014	Baltic Sea	Aschau	65	N 54,4608	E 9,9267
MH475494	*Ulva torta*	S_81	24-Jul-2014	Baltic Sea	Wackerballig	59	N 54,7586	E 9,8778
MH475495	*Ulva torta*	S_231	13-Aug-2014	Baltic Sea	Schilksee	72	N 54,4313	E 10,1693
MH475496	*Ulva torta*	S_350	25-Aug-2014	Baltic Sea	Heiligenhafen	99	N 54,3787	E 10,9555
MH475497	*Ulva torta*	S_73	24-Jul-2014	Baltic Sea	Gluecksburg	56	N 54,8368	E 9,5231
MH475498	*Umbraulva dangeardii*	R_1	8-Aug-2014	Helgoland	Helgoland	45	N 54,1874	E 7,8703

MH475499	*Umbraulva dangeardii*	R_2	8-Aug-2014	Helgoland	Helgoland	45	N 54,1874	E 7,8703
MH475464	*Blidingia marginata*	S_147_A	31-Jul-2014	Wadden Sea	Pellworm	21	N 54,4988	E 8,8087
MH475465	*Blidingia marginata*	S_577	14-Apr-2015	Wadden Sea	Brunsbuettel estuary	41	N 53,8890	E 9,1011
KT290281	*Blidingia minima*	DA_12	18-Jul-2013	Baltic Sea	Wohlenberg	asterisk	N 53,9446	E 11,2444
MH475455	*Blidingia* sp. 1	S_828	24-Jul-2014	Wadden Sea	Schobuell	25	N 54,5079	E 8,9956
MH475456	*Blidingia* sp. 1	S_818	24-Jul-2017	Wadden Sea	Husum	26	N 54,4712	E 9,0280
MH475457	*Blidingia* sp. 1	S_815	24-Jul-2017	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MH475458	*Blidingia* sp. 1	S_813	24-Jul-2017	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog	6	N 54,8374	E 8,6122
MH475459	*Blidingia* sp. 1	S_179	6-Aug-2014	Wadden Sea	Brunsbuettel estuary	41	N 53,8890	E 9,1011
MH475460	*Blidingia* sp. 2	S_34	23-Jul-2014	Helgoland	Helgoland	53	N 54,1720	E 7,8993
MH475461	*Blidingia* sp. 2	S_1	22-Jul-2014	Helgoland	Helgoland	48	N 54,1837	E 7,8886
MH475462	*Blidingia* sp. 2	S_39	23-Jul-2014	Helgoland	Helgoland	49	N 54,1825	E 7,8906
MH475463	*Blidingia* sp. 2	S_124	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6891
MH475466	*Kornmannia leptoderma*	S_154	5-Aug-2014	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MH475467	*Kornmannia leptoderma*	S_698	23-Apr-2015	Helgoland	Helgoland	50	N 54,1797	E 7,8896
MH475468	*Kornmannia leptoderma*	S_337	22-Aug-2014	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9823
MH475469	*Monostroma grevillei*	S_548	8-Apr-2015	Baltic Sea	Wulfen	121	N 54,4089	E 11,1731
MH475470	*Monostroma grevillei*	S_617	16-Apr-2015	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9824
MH475500	*Percursaria percursa*	S_360	25-Aug-2014	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9824
MH475501	*Protomonostroma undulatum*	S_733	24-Apr-2015	Helgoland	Helgoland	49	N 54,1825	E 7,8907

[a] Voucher no. = Accession no. = GenBank accession number for *tuf*A gene. [b] number of the voucher specimen deposited in the herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel. [c] Site no. = Referring to number in Figure 1

Also *U. rigida* was obligately distromatic. Attached specimens were found in all three investigated regions, while dense mats of drifting specimens occurred locally in the Wadden Sea (For details about morphology and distribution see online supplement). The cluster representing *U. rigida* in our phylogenetic tree (Figure 2) contained reference sequences of *Ulva laetevirens* Areschoug 1854 from Conneticut (JN048942) and New Brunswick (HQ610428), as well as a reference of *U. rigida* from the Italian Adriatic Sea (HE600178). All sequences were nearly identical, exhibited divergences from the references of 0 to 0.26 % and fell in a well delimited cluster.

In contrast, a cluster including type sequences of *U. shanxiensis* as the closest reference was more genetically diverse. Its inner sequence distance ranged from 0-2.8 % and thus was higher in this cluster than in other taxa (Figure 2). At the same time the dissimilarity of German specimens with the type sequence of *U. shanxiensis* was always in the range of 2.4-2.8 %, and thus particularly high. Specimens belonging to this cluster were present in all of the three investigation areas and most of the time, in particular in regions with intense anthropogenic impact. They were generally tubular, but nonetheless of variable morphology (For details see online supplement).

The clusters delimiting the species *U. flexuosa* and *U. californica* segregated only with low to medium support values. Both species were found in all three main study areas. The more fragile and relatively rare *U. flexuosa* exhibited only tubular morphologies, which were mostly encountered unattached. Specimens belonging to this clade exhibited a sequence dissimilarity with two different reference sequences originating from Canada (HQ610296) and South Korea (JN029309) of 0 to 0.17 %. In contrast, the more robust *U. californica* developed more variable morphologies ranging from tubular to lanceolate or amorphous forms and it preferentially settled on artificial substrates (For more details about morphologies and habitats see online supplement). At the same time *Tuf*A sequence divergences from three different reference sequences originating from California (KM255003) and Canada (HQ610279 & HQ610280) ranged from 0 to 1.82 % (Fig. 2, Table 2).

Specimens exhibiting the relatively characteristic morphology of *Ulva torta* (long, narrow and entangled unbranched tubular thalli with a central lumen of only 3-11 µm, for details see online supplement) were infrequently found in the Baltic Sea and Wadden Sea, but not found at Helgoland. They clustered together with reference sequences from southern Australia (JN029340) and British Columbia, Canada (HQ610437). These, however, clustered with moderate bootstrap and posterior probability support (85/0.96) outside of our samples (Figure 2), although the genetic divergence was small (0.12 – 0.3 %).

The clade separating specimens of *U. linza* showed a strong genetic divergence (see Figure 2) and segregated into two different subgroups, *U. linza* 1 (clade support: 100/1) and *U. linza* 2 (clade support: 95/1). Specimens of *U. linza* 1 were abundantly found in the whole area except Helgoland and they clustered with a genotype from Tasmania (GenBank accession number JN029337) with very low sequence divergencies ranging from 0 to 0.0014 %. In contrast, specimens of *U. linza* 2 were exclusively found on Helgoland and they clustered with a reference sequence originating from the Northeast Pacific (GenBank accession number KM254997) with sequence divergencies ranging from 0 to 0.49 %. A historical herbarium voucher - originally identified as *Enteromorpha ahlneriana* Bliding nom. illeg. - could be genetically assigned to *Ulva linza* sp.1 (Figure 2, Table 2). However, the phylogenetic differentiation was not reflected morphologically and both groups exhibited a wide spectrum of tubular to lanceolate and partly distromatic morphologies (for details see online supplement).

A *tuf*A reference sequence of *U. prolifera* from Manitoba, Canada (HQ610395) clustered together with sequences of specimens from our three main study areas. However, our samples clearly clustered together (-/98) (Figure 2), even though the genetic divergence was between 0 and 1.23 % and thus variable. *U. prolifera* was always attached and relatively abundant, exhibiting various tubular morphologies and frequently - but not always – a characteristically twisted stipe-like basal part (for details see online supplement).

Specimens that clustered with high bootstrap and posterior probability support (99/1) together with reference specimens of *U. lactuca* from New Brunswick (HQ610341, genetic dissimilarity: 0-0.31 %) and California (KM255044, genetic dissimilarity: 0.12-0.47 %) were exclusively found on Helgoland (Figure 2). They exhibited distromatic thalli of variable shape and were characterized by relatively strong attachment. The specimens were abundantly found within the intertidal zone and grew attached to hard substrate, such as naturally occurring rocks (Figure 3 A), stones and mussel beds or artificial breakwaters and piers. Only few specimens were found drifting, and these drifting thalli exhibited clear indications of recent ruptures of the rhizoidal zone, suggesting that a drifting lifestyle is not tolerated for long time periods. Specimens of this clade were never found in rockpools that could be subject to desiccation or influence of rainwater.

Table 2: Sample list of genetically processed herbarium material

Region	**Location**	**Collection date**	**Herbarium** [a]	**Herbar ID** [b]	**Collector**	**Accession no.** [c]	**Morphological identity** [d]	**Genetic identity *tuf*A** [e]
Helgoland	Helgoland	10.04.1991	BRM001700	BRM	Kornmann and Sahling	MH720537	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	10.04.1991	BRM001703	BRM	Kornmann and Sahling	MH720538	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	22.10.1988	BRM007947	BRM	Kornmann and Sahling	MH720539	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	17.07.1978	BRM007806	BRM	Kornmann and Sahling	MH720540	*Ulva tenera*	*Ulva lactuca*
Baltic Sea (Öresund)	Copenhagen	27.11.2007	73544	C	Ruth Nielsen and Peer Corfixen	MH720542	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea (Öresund)	Copenhagen	17.03.2004	40539	C	Ruth Nielsen and Peer Corfixen	MH720543	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea	Kiel, Friedrichsort	19.09.1962		GEO	Elfriede Kaminski	MH720544	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea	Kiel, Friedrichsort	30.9.1976	95	GEO	Elfriede Kaminski	MH720541	*Enteromorpha ahlneriana*	*Ulva linza*

Herbarium = Abbreviation of the respective Herbarium samples were taken of (Herbarium of the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany [BRM]; Herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany [GEO]; Herbarium of the Natural History Museum of Denmark, Copenhagen, Denmark [C]). [b] Herbar ID = Barcode of respective Herbarium. [c] Accession no. = GenBank accession number for *tuf*A gene. [d] Morphological identity = Name that historically was assigned to specimen by morphological characters. [e] Genetic identity = Refers to the respective genetic identity obtained by analyses of *tuf*A.

All thalli were distromatic throughout and their shape varied from rounded to lobed (Figure 3 B) or laciniate morphologies that could be straight, pediculated-like (Figure 3 C-D) but also strongly bent (Figure 3 E). Filled disc-like rhizoidal zones (Figure 3 i, ii) were frequently observed. The margins of thalli were never toothed (feeding traces can clearly be distinguished) and mostly smooth, whereas rounded individuals sometimes had ruffled margins. Holes were observed infrequently and had a size of 2-6 mm. Thalli reached a length of up to 40 cm and 35 cm width but smaller individuals of maximum 5 cm length and 2 cm width were also observed. Longitudinal ridges were observed in the basal region of most of the investigated specimens, but they were not present in all specimens. Especially young thalli lacked this feature. The thalli were attached by an obconically shaped stipe-like structure that ends in a broad rhizoidal zone (Figure 3 E i, ii). The cells of the middle and apical thalli were arranged in curved rows or short rows, whereas cells of laciniate thalli were sometimes arranged in longitudinal rows. The shape of cells was polygonal with rounded corners, 12-24 x 14-31 µm in surface view, and a parietal chloroplast (rarely filling the whole cell) and 1-2 (rarely up to 4) pyrenoids were observed per cell (Figure 3 F-G). Cells of the rhizoidal zone had up to 7 pyrenoids and the stipe-like region and also the rhizoidal zone itself were filled by the elongated tails of the cell bodies, which became visible in microscopic transections. Thalli that fully corresponded with the above mentioned morphologies were not only observed on Helgoland, but also frequently encountered at the mainland coasts. Here, however, they always corresponded genetically with *U. compressa*.

Also one of the over 100 syntypes of *Ulva tenera* Kornmann & Sahling - originating from Helgoland and stored at the BRM on Helgoland (Figure 3 H-I, Table 2)– clearly clustered with *U. lactuca* (Figure 2). Further, historical voucher specimens of *Ulva pseudocurvata* (BRM: BRM001703 and BRM001700) (Figure 3 J), which provided the first evidence for presence of this species on Helgoland and were displayed in Kornmann and Sahling (1994), also clustered with full bootstrap support in the clade representing *Ulva lactuca* and were identical with sequences of recent *Ulva lactuca* from Helgoland (Fig. 2).

A few specimens detected at the west coast of Helgoland and in an area around the Wadden Sea peninsula of Eiderstedt –and thereby always found in fully marine environments - clustered together with a reference sample of *Ulva australis* from Australia (JN029254, genetic dissimilarity 0 – 0.33 %, Fig. 2, Table 2). Since we were only provided with dried material of the species morphological observations were impossible.

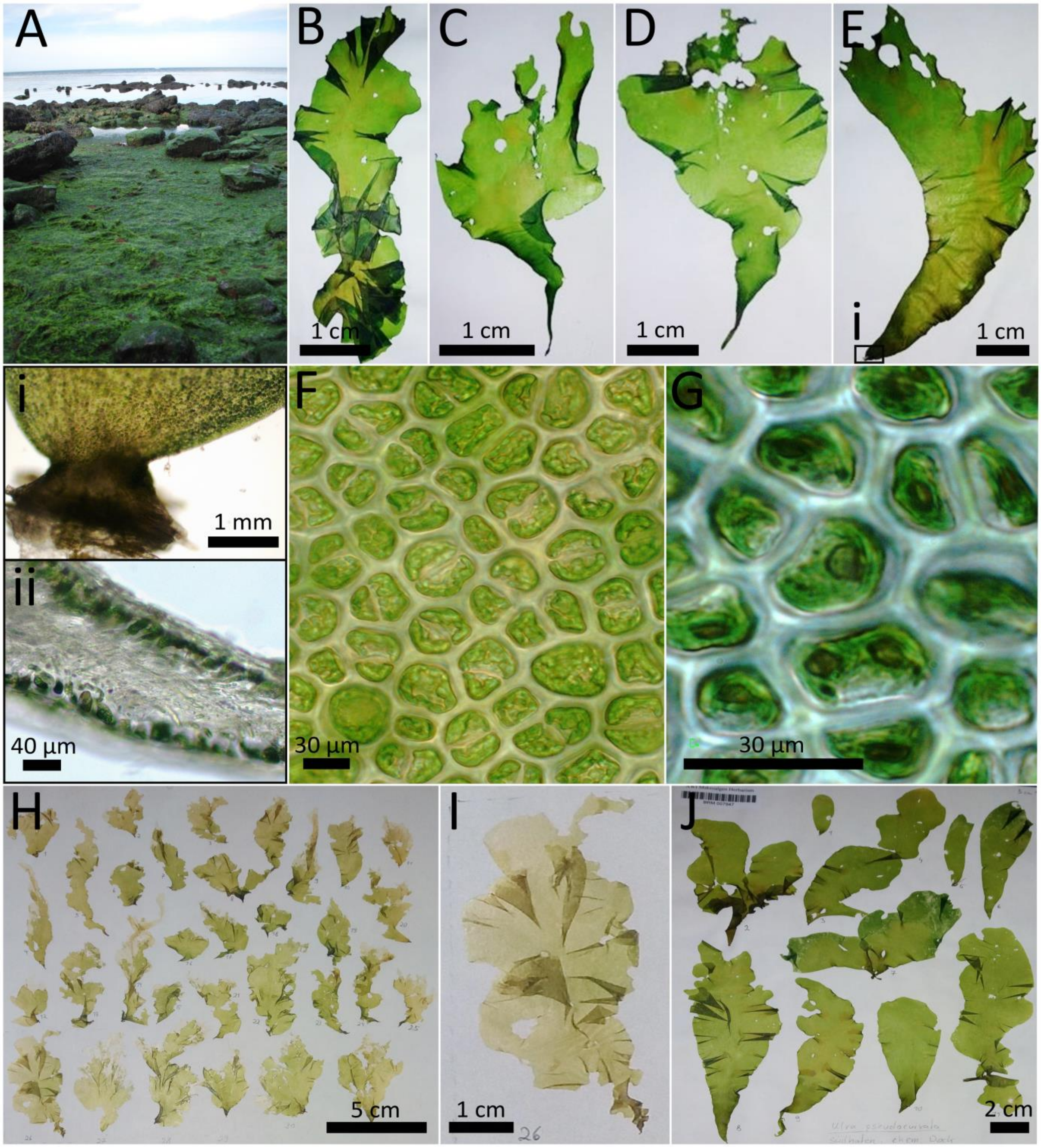

Figure 3: Morphology of *Ulva lactuca* specimens from Helgoland. (A) showing an *U. lactuca* population growing on the northeast rocky tidal flats. Different morphotypes were encountered on recent specimens, representing (B) the typical lobular type, (C-D) petiolate-like and also (E) strongly curved morphologies were observed with a (i) disclike rhizoidal zone that is in cross-section (ii) showing elongated club-shaped cells that extend with their elongated cell bodies to the center of the rhizoidal disc. (F) cells of the apical and middle thallus parts showing a hood-shaped chloroplast and one (sometimes two) central or marginal pyrenoid (G). (H) displays some of the syntypes of *Ulva tenera* sampled on Helgoland in 1978 (locality: Herbarium of the Alfred Wegener Institute, Bremerhaven; ID BRM007806), however by sequencing one individual (I) (see also Table 2 and Figure 2) its genetic affiliation to *U. lactuca* was confirmed. (J) Specimens of *Ulva pseudocurvata* sampled on Helgoland in 1988 (locality: Herbarium of the Alfred Wegener Institute, Bremerhaven; ID BRM007947) of which one specimens (arrowhead) was genetically identified as *U. lactuca* (see also Table 2 and Figure 2).

Together with reference specimens from Australia (JN029320) and California (KM255056) our sequences representing *U. intestinalis* form a distinct clade that has full bootstrap and posterior probability support (100/1) (Figure 2), but consistently exhibits a slight genetic dissimilarity with both reference sequences (0.12-0.45 %). The species was abundantly present in all main study areas and at salinities ranging from freshwater to fully marine conditions. All investigated specimens exhibited a tubular morphology, and most individuals were inflated and unbranched, corresponding with the typical morphology of *U. intestinalis* (for more details about morphology see online supplement). Branched and unbranched morphologies could not be distinguished genetically.

Also *U. compressa* was abundantly present in all three study areas. Interestingly, only tubular and mostly branched individuals were observed on Helgoland, while only distromatic sheet-like specimens were found along the Baltic Sea cost. In the Wadden Sea both morphologies were encountered, and they sometimes occurred together in the same location. However, individuals exhibiting transition forms between the two morphologies were only rarely observed. The different morphotypes did not separate during our phylogenetic analysis of *tuf*A sequences and they clustered together with a reference sample of *U. compressa* originating from Canada, New Brunswick (HQ610295, genetic dissimilarity 0-0.77 %).

*Tuf*A sequences identical to reference sequences of *Umbraulva dangeardii* from southern Italy (MF172091, genetic dissimilarity: 0-0.13 %) were only found in specimens collected at Helgoland at a depth of 8 m. They were distromatic sheets that stood out due to their dark olive color and their thin and soft texture, which corresponds with descriptions of specimens from the British Isles (Brodie *et al.*, 2007).

Percursaria percursa was only encountered once in a macroscopically visible state at Heiligenhafen (Baltic Sea), where it grew unattached in dense mats in the supralittoral zone. Microscopic examination revealed the typical morphology of unbranched biseriate filaments (Brodie *et al.*, 2007). Our sequence clustered with full bootstrap and posterior probability support with a reference sequence of *P. percursa* (AY454403, genetic dissimilarity: 0.13 %).

A well delimited cluster that included reference sequences representing *Blidingia marginata*, *Blidingia minima* and *Blidingia* sp. formed a sister clade to *Ulva*, *Umbraulva* and *Percursaria*. This cluster - representing the genus *Blidingia* - was grouped into four different entities (Figure 2). Two of them could not be resolved, as they did not match with any references in GenBank. Nevertheless, genetically, and also on the basis of overall morphology and growth habit both unresolved entities most probably also belonged to the genus *Blidingia*.

Specimens that exhibited low genetic variability and clustered together with a reference sequence of *Blidingia marginata* from New Brunswick, Canada (HQ610237, genetic dissimilarity: 0-0.28 %) were abundant in all three main study areas. They formed dense populations of variable tubular morphology in the upper intertidal and supralitoral and they were often encountered as the only macroalgal settlers in micro-habitats that are influenced by freshwater and that may fall dry for longer periods (for more details on morphology and distribution see online supplement).

Specimens belonging to the unresolved entity *Blidingia* sp.2 were morphologically indistinguishable from *Blidingia marginata*, but formed a separate cluster with full bootstrap and posterior probability support (genetic dissimilarity from reference sequence HQ610237 of *Blidingia marginata*: 8-8.2 %. They were found on Helgoland and at one collection site in the Wadden Sea (Dagebüll).

We detected no genetic evidence of the presence of *Blidingia minima* in our study area. However, in a previous project *B. minima* was found at Wohlenberg (Figure 1, site marked by an asterisk in general map), only 30 km to the East in the neighboring German state of Mecklenburg-Vorpommern. A sample from this population was included in the phylogenetic tree (KT290281, Figure 2) and clustered with full bootstrap and posterior probability support with a reference sequence of *B. minima* from Canada (HQ610239).

Also the cluster representing *Blidingia* sp. 1 was clearly delimited from other *Blidingia* species with full bootstrap and posterior probability support (genetic dissimilarity from reference sample of *B. minima* HQ610239: 4.5-4.8 %). Specimens belonging to this cluster had a broad distribution and were found in all three main study areas at remote and protected sites, as well as in highly trafficked waters (see also Steinhagen *et al.* [2018b] and Chapter II). They grew as mats on various substrates in the supralittoral zone and were often found in close vicinity of freshwater inflows, such as drainage pipes (Figure 4 A). *Blidingia* sp. 1 exhibited a relatively characteristic morphology. Most specimens were small tubular thalli with smooth and characteristic antler-like branches that were only visible under the microscope (Figure 4 B-E). Macroscopically visible branches were rarely observed and appeared as screwed or inflated. *Blidingia* sp.1 was generally smaller than our specimens of *B. marginata* and *Blidingia* sp.2 and also smaller than *B. minima* according to published descriptions (Kornmann & Sahling, 1978; Brodie *et al.*, 2007). Thalli had a length of 0.2-1.5 cm and a width ranging from 80 to 500 µm (rarely specimens exceeded a width of 1mm). Cells were of rectangular to polygonal shape and had sizes of 2-6 x 3-7 µm in surface view. They were arranged in distinct longitudinal rows and exhibited a centrally located pyrenoid, whereas the chloroplast was either cap-like or filling the whole cell (Figure 4 F-H). The cells of the middle thallus parts fluently merge with the rhizoidal cells and no distinct transition zone was observed nor do they form a particular structure.

The remaining three clades shown in Fig. 2 only include specimens that exhibited monostromatic blades. They clustered with full bootstrap and posterior probability support together with reference sequences representing *Kornmannia leptoderma*, *Monostroma grevillei* and *Protomonostroma undulatum*, respectively. *K. leptoderma* was found on Baltic Sea and Wadden Sea coasts, as well as on Helgoland. Specimens from all three areas clustered together with reference sequences from Canada (HQ610252, dissimilarity: 0-0.38 %) and an additional sequence from northern Germany (KT290275) and showed only small to no genetic intraspecific variability. Individuals belonging to this cluster grew in the middle and lower intertidal zone and were mostly attached to various substrates. They avoided exposition to direct sunlight and were frequently found at the shaded side of stones (Figure 5 A) or jetties or under piers. The 1-5 cm (rarely up to 8 cm) large thalli of *K. leptoderma* were nearly unrecognizable when the substrate fell dry during low tide. The membranous and very soft thalli appeared funnel-shaped (Figure 5 C), lanceolate or rosette-like (Figure 5 B). Older thalli that had sporulated were of amorphous shape and deeply cut. The rhizoidal zone was not defined by a disc-like structure and cells proceeded without tapering in a stipe-like region. Cells in apical and middle thallus parts differed in shape from those in basal thallus parts. Cells of upper and middle thallus regions were either polygonal to round or they had sharp and clearly defined angular edges. Their size was 9-15 x 11-16 µm in surface view and they exhibited one centrally located pyrenoid, whereas the chloroplast was either marginal or filling the whole cell (Figure 5 D-E). Cells of the apical and middle thallus regions were thick-walled (Figure 5 E). Toward the basal region the cell-walls got clearly thinner, the cell size increased and the number of pyrenoids per cell varied between 1 and 3 (rarely 4). In the lower mid-thallus parts cells had a size of 11-21 x 11-27 (32) µm in surface view and some cells appeared grainy, while others already had the character of rhizoidal cells, exhibiting long-drawn-out tips (Figure 5 F-G).

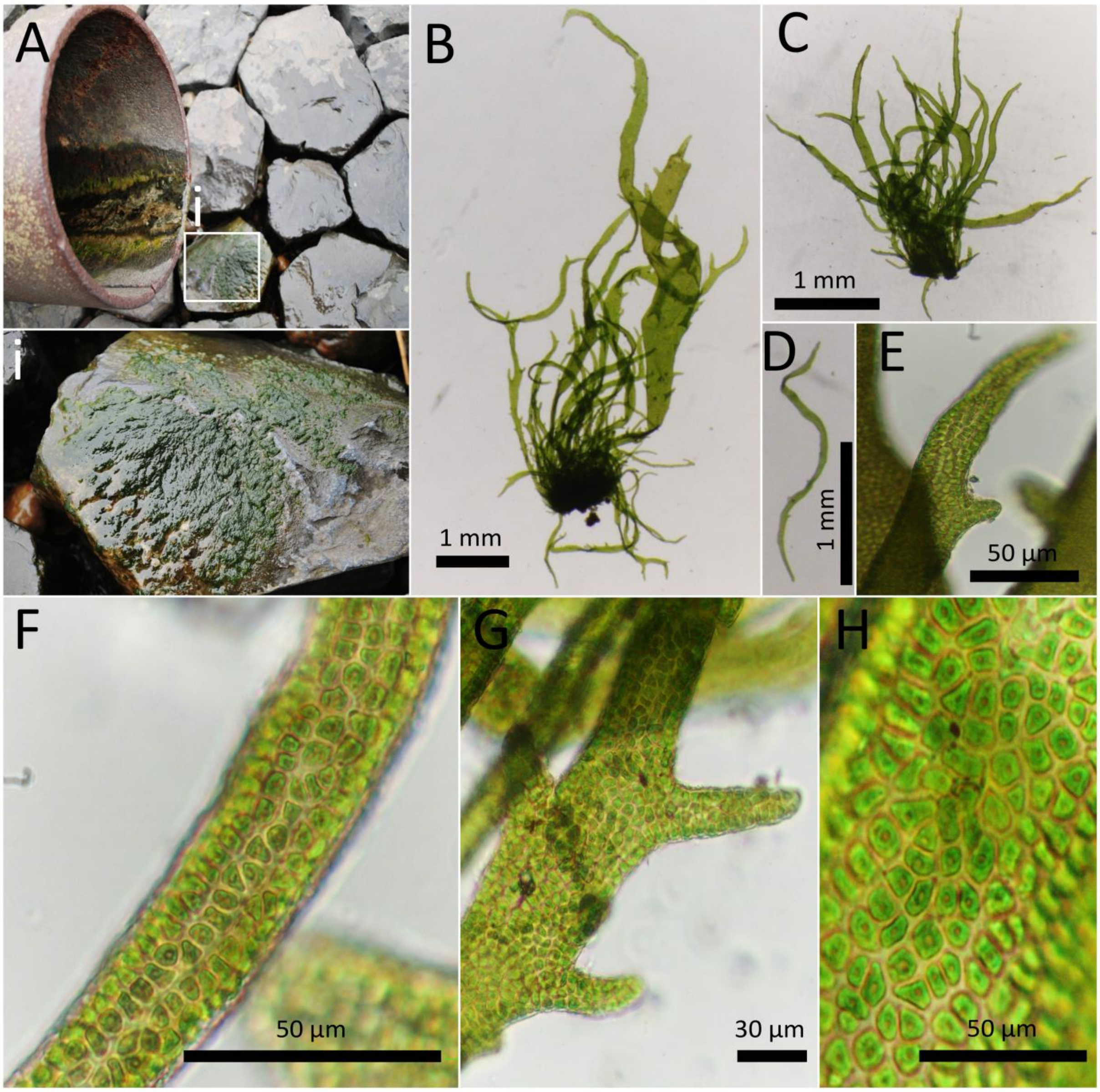

Figure 4: Morphology of *Blidingia* sp. 1 specimens collected at a fresh water drainage in Brunsbüttel, Germany. (A) Showing the drainage with a magnification (i) of the *Blidingia* population that is kept moist by freshwater leakage. (B-C) Overview about the typically branched thallus morphology, but infrequently also unbranched individuals were observed (D). (E) Close-up of an upwards directed branch. (F) Cells are of rectangular to polygonal shape and allocated in distinct longitudinal rows which can be also observed in short branches (G) and usually contain one central pyrenoid (H) (dyed with Iodine-Potassium Iodide).

Cells of the rhizoidal zone were always grainy and from their main body extended rhizoidal tips. These rhizoidal cells were up to 50 µm long and they usually had 1-3 pyrenoids (rarely more) (Figure 5 G).

All investigated specimens of *M. grevillei* clustered together and their sequences were often identical to reference sequences from Maine, US (HQ610262, dissimilarity: 0-0.51 %) and New Brunswick, Canada (HQ610259, dissimilarity: 0-0.39 %). This species was abundant at the Baltic Sea and it occurred also on Helgoland. It was only observed in spring (March to May) and toward the end of this vegetation period drifting mats of *M. grevillei* frequently developed in sheltered bays, harbors and lagoons. Alltogether, cells of *M. grevillei* were more visibly arranged in rows than cells of *K. leptoderma* (for further morphological details see online supplement).

One specimen clustering with a reference sequence from *P. undulatum* (dissimilarity: 0.13 %) was detected in the lower intertidal zone on Helgoland. Similar as with *M. grevillei* cells were often arranged in rows. Instead of a smooth transition from basal cells to rhizoidal cells abrupt changes in cell shape were observed and rhizoidal cells were longer than in *K. leptoderma* (60-90 µm, in some individuals up to 110 µm, for further morphological details see online supplement and the supplementary Figure S 2).

Surprisingly, an additional monostromatic entity that could represent *Gayralia oxysperma* was not detected in our study area, although Kützing (1843) originally described its basionym *Ulva oxysperma* on the basis of material collected in Schleswig-Holstein at Winning (located at the inner Schlei, a narrow inlet of the Baltic Sea, see site 127 in Fig. 1). Unfortunately the type material of *Ulva oxysperma* appears to be lost. However, historical herbarium vouchers of *G. oxysperma* from Friedrichsort, Kiel, Germany sampled in 1962 (GenBank accession numbers: MH720544) and also from Copenhagen, Denmark, sampled in 2004 and 2007 (GenBank accession numbers: MH720542 and MH720543) were available and could be sequenced. Interestingly, all three clustered with *K. leptoderma* within our phylogenetic tree (Figure 2, Table 2). During repeated visits (additional collections took place in 2017 and 2018) at Winning (see site 2127 in Fig. 1, salinity 1 PSU) thalli exhibiting the described morphology of *G. oxysperma* were not detected. However, such specimens could be collected at the inner Schlei in a distance of 10 km from Winning at Brodersby (site 128 in Fig. 1, salinity 7 PSU) and in a distance of 30 km at Lindaunis (site 61 in Fig. 1, see also table S1 in online supplement). Yet, their *tuf*A sequences clustered with *K. leptoderma* in our phylogenetic analysis (GenBank accession numbers: MH720545- MH720547; see also supplementary Table S 1).

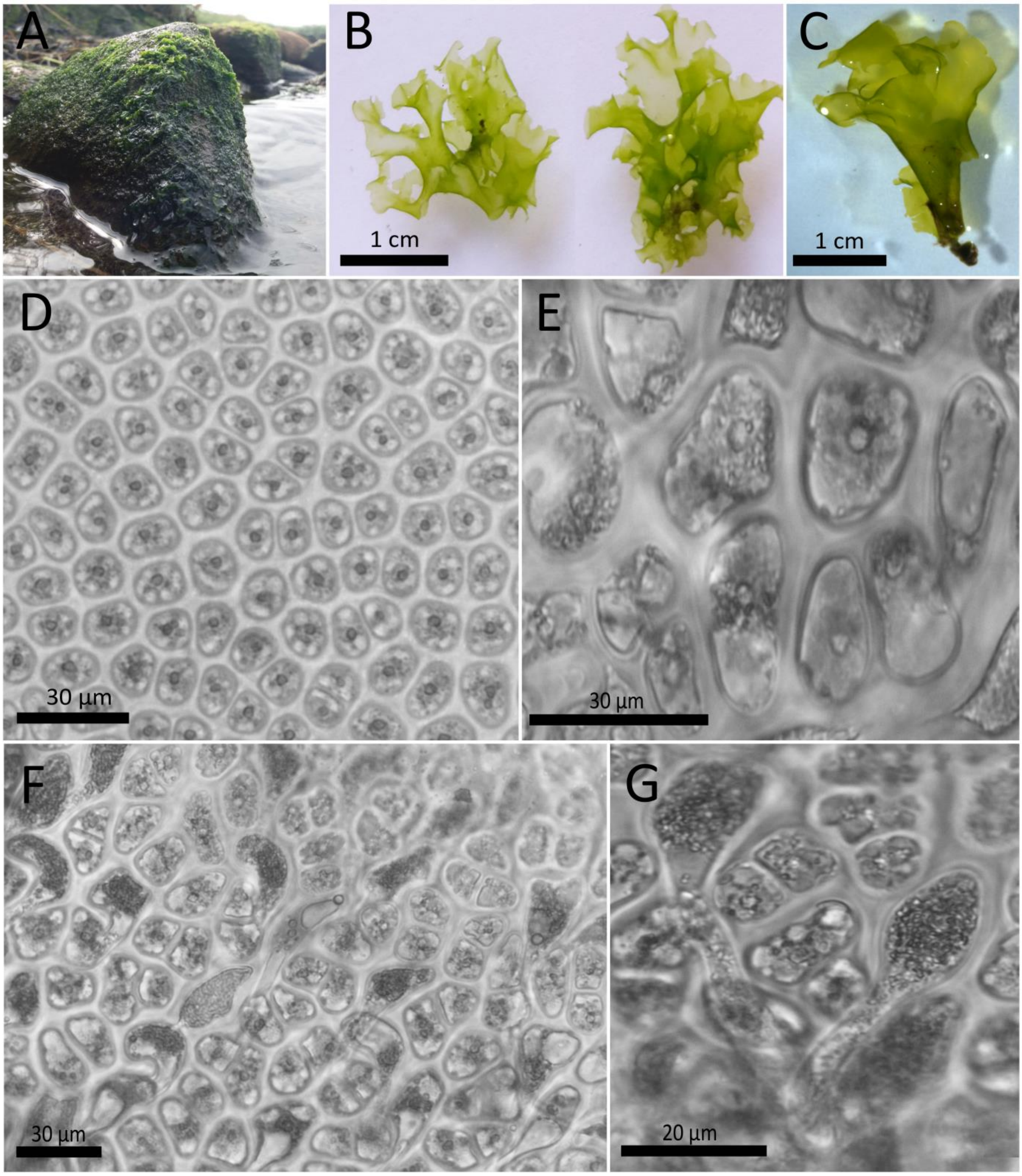

Figure 5: Morphology of *Kornmannia leptoderma* specimens of Northern Germany. (A) Showing a typical sampling site in the Baltic Sea (Aschau lagoon) where *K. leprtoderma* is growing at the shaded side of a rock stone. Specimens of rosette-like shape (B) sampled in the Baltic Sea (Aschau lagoon) and funnel-shaped (C) individual from the coastal inlet of the Baltic Sea, Schlei, Lindaunis. Apical and middle thallus regions showing short cell rows and often cell pairs can be observed (D), while each cell contains a marginal chloroplast and one (rarely two) central pyrenoid (E). Cells of the rhizoidal zone (F) are club-shaped and can extend in their length up to 50 µm (G).

Discussion

The 20 entities of green algae that were detected in our study area (Figure 2) can be identified with variable degrees of certainty. Only one entity included a reference sequence originating from type material, as we were able to generate such a sequence for *Ulva tenera* (Kornmann & Sahling 1994). However, *U. tenera* was relatively recently described and the corresponding cluster in our phylogenetic analysis also encompasses several reference sequences originating from specimens that were recognized elsewhere as *Ulva lactuca* L.. Although we were only able to examine one of the over 100 syntypes of *U. tenera* and although the current concept of *U. lactuca* has been challenged (Butler 2007), our observation strongly suggests that *U. tenera* may be a junior synonym of *U. lactuca*. This view is further supported by the circumstance that recent young material of *U. lactuca* from Helgoland exhibits the described morphological characters of *U. tenera* (Kornmann and Sahling 1994). Other described characteristics of *U. tenera* are its restriction to the uppermost eulittoral and its exclusively vegetative propagation (with biflagellated spores) (Kornmann and Sahling 1994): Apparently the authors observed dwarfish forms of *U. lactuca* that were adapted to extended air exposure.

In several of the remaining entities the species identity indicated by comparison with reference *tufA* sequences was largely in accordance with characteristic morphological traits: This was the case with *U. torta* (which usually formed massively intertwined tubular thalli of small diameter), *U. prolifera* (which mostly exhibited the characteristic twisted stipe) and with *Umbraulva dangeardii* (which is characterized by its olive green pigmentation). Also the genetic identification of the three monostromatic entities was in accordance with phenetic traits: As expected, *Monostroma grevillei* was only found in spring, which was not the case with any other entity, and as reported elsewhere (Weinberger et al. 2018; see also Chapter III) *Kornmannia leptoderma* from our area exhibits the very characteristic heteromorphic lifecycle. *Protomonostroma undulatum* was only observed once, but the found specimen exhibited the typical morphology (see supplement). Also specimens clustering with *Ulva intestinalis* mostly exhibited the tubular and unbranched morphology that is considered as characteristic for this species (Kornmann and Sahling 1977; Pankow 1990; Rothmaler 1984). However – and as also observed by others (Reed & Russell, 1978; Blomster *et al.*, 1998) - tubular branched individuals occasionally occur in our study area and this divergent morphology is promoted by low salinities (Steinhagen *et al.*, 2018b; see also Chapter II).

In some entities the species identity indicated by comparison with reference *tufA* sequences corresponded sometimes, but not in the majority of cases, with characteristic morphological traits. For example, specimens exhibiting the characteristic lanceolate and partly distromatic type morphology of *U. linza* (Kornmann and Sahling 1977; Pankow 1990; Rothmaler 1984) clustered without exception together with reference sequences of this species. However, the cluster also comprised numerous specimens exhibiting tubular branched morphologies. These phenotypes were in accordance with descriptions of *Ulva procera* and *Ulva ahlneriana* (Kornmann and Sahling 1977; Pankow 1990; Rothmaler 1984) and in our study a historical voucher of *U. ahlneriana* also clustered together with *U. linza* (Fig. 2). This observation thus supports previous suggestions that *U. procera* (Brodie et al. 2007) and *U. ahlneriana* (Guiry and Guiry 2018) may be synonyms of *U. linza*. Interestingly, the branch representing *U. linza* in our phylogenetic tree comprised two lineages that are genetically closely related. These can not be distinguished morphologically, as characteristic and non-characteristic morphologies of *U. linza* were present in both of them. One of the lineages was only detected on Helgoland, while the second was only present on mainland coasts, and it is currently not possible to decide whether they represent distinct species or simply two different genotypes within *U. linza* that perhaps developed due to geographic separation.

Also *U. compressa* exhibited multiple gross morphologies. One of them was only found on North Sea coasts and is in full agreement with the tubular and branched type material (Linnaeus, 1753). However - and as discussed elsewhere in detail (Steinhagen *et al.*, 2018a; see also Chapter IV) - all Baltic Sea and many Wadden Sea specimens of this entity exhibited a completely different morphology that was always distromatic and sheet-like. Yet, both morphologies are genetically indistinguishable. Evidently, the distromatic morphology of *U. compressa* strongly overlaps with the allegedly unique morphology of *U. lactuca*, which has caused considerable taxonomic confusion in the past (Steinhagen *et al.*, 2018a; see also Chapter IV). In our area *U. lactuca* is only present on Helgoland (Table 2, supplementary Tabe 2) and historical records from the Baltic Sea (Schories *et al.*, 2009) are often due to the fact that the distromatic morphology of *U. compressa* has been misidentified (Steinhagen *et al.*, 2018a; see also Chapter IV). Interestingly, historical vouchers from Helgoland (Kornmann & Sahling, 1994) exhibiting the described curved morphology of *U. pseudocurvata* (Hoeksema & van den Hoek, 1983) clustered in our study together with *U. lactuca*, while recent specimens exhibiting the same morphology on main land coasts of Northern Germany (Steinhagen *et al.*, 2018a; see also Chapter IV) and elsewhere (Hayden & Waaland, 2004) clustered with *U. compressa*. This not only challenges *U. pseudocurvata* as a taxonomic entity (because it was described based upon morphological traits that are clearly not specific), but it also confirms the strong morphological conversion of *U. lactuca* on Helgoland and *U. compressa* in its distromatic form on the main land coasts of our study area.

Three additional entities that always exhibited distromatic blades were locally present in our study area besides of *U. lactuca* and the distromatic form of *U. compressa*. They clustered together with reference sequences of *U. gigantea, U. australis, U. rigida* and *U. laetevirens* (Fig. 2). In these cases the morphologies were generally in accordance with the corresponding type morphologies, but at the same time they could not be reliably distinguished from each other, from *U. lactuca* and from the distromatic form of *U. compressa*. As recently also demonstrated in a phylogenetic study that was based upon analysis of ITS and rbcL marker genes (Horta *et al.*, 2018) *U. rigida* and *U. laetevirens* can not be genetically distinguished based upon *tufA* gene sequences. In this light *U. laetevirens* Areschoug 1854 should be regarded as a junior synonym of *U. rigida* C. Agardh 1823.

Some clades with tubular morphologies could not be clearly resolved. As in various other studies (Heesch et al. 2009; Kirkendale et al. 2013; Kraft et al. 2010; Saunders and Kucera 2010), also in ours the species boundary of *Ulva flexuosa* and *Ulva californica* blurs (Figure 2). That the species boundary of *U. flexuosa* is ambiguous and that this group was often merged with the closely related group representing *U. californica* was already discussed by Hiraoka et al. (2017), who were nontheless able to demonstrate in hybridization experiments the existance of a species boundary between members of both groups that originated from Japan. Cross-breeding was not possible in our study and we have chosen to indicate the lack of genetic resolution of the two species with the term "*Ulva flexuosa*/ *californica* complex".

A reference sequence originating from type material of the tubular species *Ulva shanxiensis,* recently described from a freshwater stream in northern China (Chen et al. 2015), clustered next to a clade also comprising tubular specimens within our phylogenetic analysis (Figure 2). Nevertheless, the clade encompassing *U. shanxiensis* and our samples together obtained only low bootstrap and posterior probability support values, indicating that the sequence divergence within this cluster was higher than in others (Figure 2, branch length drawn proportionally to sequence change). In this light the identity of our samples with *U. shanxiensis* appears as unlikely and the clade so far remains unidentified.

Table 3: Comparison of the literature based inventory list of Schories et al. (2009) with recent results based upon investigations of the *tuf*A marker. Listed are the predicted species by Schories et al. (2009) and detected species for the three sampling areas Baltic Sea, Wadden Sea and Helgoland. Annotations by other authors are marked respectively. Expected and found species are marked by a cross (X), expected but absent species are indicated by a circle (O), unexpectedly found species are indicated by a check mark, while empty fields marks sampling areas where species were not expected and not found. Agreement between Schories et al. (2009) and our study is indicated by green shading, while disagreement is indicated by red shading. Absence of shading indicates cases where both studies could not be compared, for example because reference sequences of *tufa* were missing.

Order	**Family**	**Genus**	**Species**	**Baltic Sea**	**Wadden Sea**	**Helgoland**	**Taxonomic notes**
Ulvales	Kornmanniaceae	*Blidinigia*	*Blidingia marginata* (J.Agardh) P.J.L.Dangeard ex Bliding 1963	**X**	**X**	**X**	
			Blidingia minima (Nägeli ex Kützing) Kylin 1947	**O**	**O**	**O**	
			Blidingia chadefaudii (Feldmann) Bliding 1963			**O**	On Helgoland, *B. chadefaudii* was taxonomically separated from *B. minima* on the basis of developmental differences, habit and zonation. However, a thickened inner cell wall, distinctive for *B. chadefaudii*, was absent from Helgoland´s populations (Kornmann and Sahling 1978)
			Blidingia subsalsa (Kjellmann) Kornmann & Sahling ex Scagel et al. 1989			O	First listed as subspecies of *B. marginata* (*Blidingia marginata* subsp. *subsalsa* [Kjellmann] Bliding), *B. subsalsa* was given the rank as a species (Kornmann and Sahling 1978) on the basis of developmental differences observed on specimens from Helgoland.
		Kornmannia	*Kornmannia leptoderma* (Kjellmann) Bliding 1969	✓	✓	**X**	Indicated as rare species and absent from Helgoland since 1975 (Kornmann and Sahling 1977)
	Ulvaceae	*Ulva*	*Ulva compressa* Linnaeus 1753	X	**X**	**X**	Highly variable in overall morphology (Blomster *et al.* 1998; Brodie *et al.* 2007; Steinhagen *et al.* 2018a [see also Chapter IV]; Tan *et al.* 1999); Observation of different

				🟩	🟩	🟩	morphotypes in northern Germany (Steinhagen *et al.* 2018a)
			Ulva pseudocurvata Koeman & Hoek 1981			**O**	Different studies highlight conspecificity *of U. pseudocurvata* and *U. compressa* (Tan *et al.* 1999, Loughnane *et al.* 2008; Steinhagen *et al.* 2018a [see also Chapter IV]), but material from Helgoland is shown in this study to be conspecific with *U. lactuca*.
			Ulva curvata (Kützing) De Toni 1889		**O**		Specimens formerly identified as *U. curvata* on Helgoland, were later assigned to the species *U. pseudocurvata*, due to morphological reconsiderations (Kornmann and Sahling 1977; Kornmann and Sahling 1994)
			Ulva flexuosa Wulfen 1803	🟩 **X**	🟩 **X**	🟩 **X**	"Clearly multiple closely related species" (Kirkendale *et al.* 2013). ITS based species boundaries are ambiguous. Observation of life history and hybridization experiments clarified species boundaries of the "flexuosa-complex" (Hiraoka *et al.* 2017)
			Enteromorpha flexuosa subsp. *linziformis* (Bliding) Bliding 1963		**O**		Regarded as synonym of *Ulva flexuosa var. linziformis* Guiry & Guiry, 2018)
			Ulva flexuosa subsp. *paradoxa* (C. Agardh) M.J. Wynne 2005		**O**	**O**	Regarded as synonym of *U. paradoxa* C. Agardh1817
			Ulva californica Wille 1899	🟥 ✓	🟥 ✓	🟥 ✓	Closely related to *U. flexuosa.* complex that is "clearly composed of multiple closely related species"(Kirkendale *et al.* 2013).
			Ulva intestinalis Linnaeus 1753	🟩 **X**	🟩 **X**	🟩 **X**	Aberrant morphologies induced by various factors (Bliding 1963; Blomster *et al.* 2002; Reed and Russell 1978; Steinhagen *et al.* 2018b [see also Chapter II])
			Ulva lactuca Linnaeus 1753	🟥 **O**	🟥 **O**	🟩 **X**	Based on missing morphological characters

				🟥	🟥	🟩	Kornmann and Sahling (1994) conclude *that U. lactuca* is absent from Helgoland. Later, its presence was verified by Bartsch and Kuhlenkamp (2000).
			Ulva tenera Kornmann & Sahling 1994		**O**	**O**	Shown in the present work to be conspecific with *U. lactuca*
			Ulva linza Linnaeus 1753	🟩 **X**	🟩 **X**	🟩 **X**	Gross morphology highly variable (from sheets to proliferous tubes). Several studies stated different closely related genetic groups in the "linza-ahlneriana-procera" cluster (Kirkendale *et al.* 2013; Kraft *et al.* 2010), others support the conspecificty of *U. linza* and *U. procera* (Brodie *et al.* 2007).
			Enteromorpha jugoslavica Bliding	**O**			Records from the area are conspecific with *U. linza* (Gesche Bock, pers. comm)
			Ulva prolifera O. F. Müller 1778	🟩 **X**	🟩 **X**	🟩 **X**	
			Ulva torta (Mertens) Trevisan 1841	🟩 **X**	🟩 **X**	🟩	
			Ulva lobata (Kützing) Harvey 1855	🟩	🟩	🟥 **O**	
			Ulva radiata (J.Agardh) H.S.Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland 2003		**O**		
			Ulva ralfsii (Harvey) Le Jolis 1863		**O**		
			Ulva simplex (K.L.Vinogradova) H.S.Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland 2003	**O**	**O**		
			Ulva clathrata (Roth) C. Agardh 1811	**O**	**O**		Type locality: Fehmarn, SW Baltic (original material missing) (Guiry and Guiry 2018)
			Ulva rigida C. Agardh 1823 and *Ulva scandinavica* Bliding 1968	🟥 ✓	🟩 **X**	🟥 ✓	*U. rigida*, *U. scandinavica* and *U. armoricana* are considered as conspecific (Loughnane *et al.* 2008)
			Ulva gigantea (Kützing) Bliding 1969	🟥 ✓	🟥 ✓	🟩	"*U. gigantea* is notoriously difficult to separate from *U. pseudocurvata* on

				🟥	🟥	🟩	morphological grounds alone"(Loughnane *et al.* 2008). First Observations in Ireland and Britain byLoughnane *et al.* (2008).
			Ulva australis Areschoug 1854	🟩	🟥 ✓	🟥 ✓	*U. australis* and *U. pertusa* can be regarded as conspecific (Kirkendale et al. 2013). Likely to be a NIS in Australia (Kirkendale *et al.* 2013).
		Umbraulva	*Umbraulva dangeardii* M.J.Wynne & G.Furnari 2014	🟩	🟩	🟥 ✓	The name *Umbraulva dangeardii* was proposed to replace the invalid names *Ulva olivascens* J.P.L.Dangeard nom. inval. and *Umbraulva olivascens* (P.J.L.Dangeard) G. Furnari nom. inval.
		Ulvaria	*Ulvaria fusca* (Postels & Ruprecht) Vinogradova 1967	🟥 O	🟩	🟩	
		Percursaria	*Percursaria percursa* (C. Argardh)Rosenvinge 1893	🟩 **X**	🟥 **O**	🟥 **O**	
Ulotrichales	Monostromaceae	*Monostroma*	*Monostroma grevillei* (Thoret) Wittrock 1866	🟩 **X**	🟥 **O**	🟩 **X**	Samples identified as *M. grevillei* segregated into two clusters in phylogenetic analyses of (Saunders and Kucera 2010), suggesting crypticity.
			Monostroma arcticum Wittrock1866			**O**	Regarded as a synonym of *Monostroma grevillei* var. *arcticum* (Guiry and Guiry 2018)
	Ulotrichaceae	*Protomonostroma*	*Protomonostroma undulatum* (Wittrock) Vinogradova 1969	🟩	🟩	🟩 **X**	
	Gayraliaceae	*Gayralia*	*Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel et al. 1989	🟥 **O**	🟩	🟩	Type locality: Winning, SW Baltic (original material missing)

Also within the genus *Blidingia* two genetic entities could not be resolved, as they did not match with any available reference sequences. Specimens of the clade *Blidingia* sp. 2 showed strong morphological overlap with a second clade encompassing a reference sequence of *Blidingia marginata*, and could only be distinguished genetically. Both clades would be morphologically in agreement with *B. marginata*, but perhaps also with descriptions of *B. ramifera* (Garbary & Barkhouse, 1987), a species that was not yet reported from the area and is for formal reasons invalid (Cormaci *et al.*, 2014) and currently regarded as a synonym of *B. marginata* (Guiry & Guiry 2018). In contrast, specimens of the relatively abundant clade *Blidingia* sp. 1 could not only be separated by phylogenetic methods but they also clearly differed morphologically from other entities within *Blidingia* that were discovered in our study area, being smaller and more branched (Figure 3). In addition to *B. marginata* and *B. minima*, which was only found outside of our main sampling area, two other *Blidingia* species were also reported to occur on German North Sea coasts (Kornmann & Sahling, 1978; Bartsch & Kuhlenkamp, 2000; Schories *et al.*, 2009). However, no genetic reference data were available for *Blidingia chadefaudii* and *Blidingia subsalsa* and morphological identification criteria remain ambiguous and blurry. Hence, to identify *Blidingia* sp. 1 and *Blidingia* sp. 2 and to confirm the identity of *B. marginata* and *B. minima* genetic comparisons with the type material of *different Blidingia species* and lifecycle observation on cultivated material have to be carried out. The same strategy might also allow for an identification of *Ulva sp.* in the future.

Notably, our phylogenetic analysis does not support the monophyly of the genus *Ulva* (Figure 2). The species clades of *U. lactuca*, *U. australis*, *U. intestinalis* and *U. compressa* clustered with high bootstrap and posterior probability support as a sister clade of *Umbraulva dangeardii*, *Umbraulva* sp. and *Percursaria percursa*. Thus, based on their *tuf*A sequences the named *Ulva* species show a higher genetic relation to species of other genera than to other representatives of the genus *Ulva* (Figure 2), which contrasts with other studies (Hayden *et al.*, 2003; Heesch *et al.*, 2009; Kirkendale *et al.*, 2013). Alterations on our phylogenetic analysis show that the given topology developed only when *Percursaria percursa* was included (compare supplementary Figure S1without inclusion of *P. percursa*). However, only the inclusion of more rather than less taxa may clarify the true phylogenetic relationships. Recent genus affiliations have to be reconsidered if even broader samplings in the future still support the relationships among species of the genera *Ulva, Umbraulva* and *Percursaria* observed here.

The species inventory of *Ulva sensu latu* in our study area diverges considerably from the expected inventory (Schories *et al.*, 2009). Four species (*U. australis, U. californica, U. gigantea, Umbraulva dangeardii*) were observed for the first time within the area (Table 3). *U. australis* was first introduced to the South of France and very recently reported from the Dutch Oosterschelde estuary (Fort *et al.*, 2018). Obviously the species is nowadays also present in the North Friesian Wadden Sea. The same is true for *U. gigantea*, which was in Europe so far only recorded in Britain or in more westerly locations (Brodie *et al.*, 2007). Single individuals of *U. californica* were first observed in Germany in 2008 on the Wadden Sea island Wangerooge in Lower Saxony (Lackschewitz *et al.*, 2015). Either from there or from elsewhere the species has reached the SW Baltic Sea within six years. *Umbraulva dangeardii* only appeared at one single location on Helgoland (Table 2, supplementary Table 1). Helgoland is a phycological hotspot within Germany since the mid-18th century, but *Umbraulva dangeardii* was never listed in its inventories before (Kornmann & Sahling, 1977; Kornmann & Sahling, 1983; Kornmann & Sahling, 1994; Bartsch & Kuhlenkamp, 2000), which makes a recent introduction probable. Yet, the presence of *Umbraulva dangeardii* within Germany may have been ignored for some time, due to the preference of the species for subtidal habitats. In addition to these newly introduced species three (*Blidingia* sp.1, *Blidingia* sp. 2, *Ulva* sp.,) or even four (if one of the two genetic entities within *U. linza* is included) additional taxa that were observed in our study probably represent cryptic and perhaps undescribed species that have so far not been recognized. Some other species that are already known from parts of our study area were found to have a broader distribution range than expected. For instance, *U. rigida* was so far only recorded in the Wadden Sea and on Helgoland, but is now also present in the Baltic Sea (Table 2, supplementary Table S 1). *K. leptoderma* was only known from Helgoland and the Baltic Sea (Weinberger *et al.*, 2018; see also Chapter III), but is also present at different locations in the Wadden Sea (Table 2, supplementary Table S 2).

Despite of these new records the species inventories of all three main study areas were expected to be larger than the genetically validated ones (Table 3). Alltogether 14 species within the genera *Ulva*, *Blidingia*, *Monostroma*, *Gayralia* and *Ulvaria* that are listed in Schories *et al.* (2009) and are currently accepted taxonomically (Guiry & Guiry, 2018) could not be encountered genetically in the recent survey. This lack of detection could indicate their absence from the area. However, the lack of detection could also be due to other reasons, in particular low abundance or lack of genetic reference material. Indeed, no *tufA* reference sequences are available for 11 of the above mentioned 14 missing species. Moreover, numerous historical records in the area may result from misidentifications and taxonomic confusion.

As already discussed above records of *U. pseudocurvata* from our area are often - and perhaps always - due to misidentification of either *U. compressa* or *U. lactuca*. Also the only record of *Ulva splitiana* from our area (as *Enteromorpha jugoslavica*, [Kaminski, 1980]) was due to misidentification of *U. linza*, which could be demonstrated by barcoding of the ITS marker gene in the corresponding herbarium voucher (Gesche Bock, pers. comm.). Further, we were able to sequence some historical vouchers of *Gayralia oxysperma* from our area and adjacent locations, which were all genetically identical with *Kornmannia leptoderma*. *K. leptoderma* was up to now considered as a relatively rare species that has in the past only been observed on Helgoland (Kornmann & Sahling, 1983) and is not included in identification keys that cover other parts of our area (Rothmaler, 1984; Pankow, 1990) or adjacent areas (Brodie *et al.*, 2007). However, today the species is present in all main parts of our study area (see also Weinberger *et al.* [2018]; see also Chapter III). In striking contrast, *Gayralia oxysperma* was not discovered in our area, not even at the type locality of its basionym *Ulva oxysperma* Kützing (see the supplement for a description of the relatively complicated nomenclatural history of *Gayralia oxysperma*). This apparent absence or rarity of *G. oxysperma* surprises because the species should be present along the whole Baltic Sea area (Schories *et al.*, 2009). Descriptions of *Gayralia oxysperma* (Rothmaler, 1984; Pankow, 1990; Brodie *et al.*, 2007) are in complete agreement with the morphology of *Kornmannia leptoderma* in our area (Fig. 5, see also Weinberger *et al.* [2018] and Chapter III). Both species have very different life cycles (Vinogradova, 1969), but ontogenetic observations are time consuming and for this reason most historical records of *G. oxysperma* are probably only based upon morphological traits of field collected material. As a consequence records of *G. oxysperma* in our area are probably in most cases due to misidentification of *K. leptoderma*. Similarly, specimens from the northwest Atlantic that corresponded with the habitus of *G. oxysperma* segregated into two clusters of *Monostroma grevillei* when their *tuf*A genes were examined (Saunders & Kucera, 2010). In this light a thorough taxonomic reassessment of *G. oxysperma* and its different populations would be urgently needed, but this was not the aim of our study.

Some species that were reported to occur only in certain of the investigated areas were found to have a broader range expansion than expected. For instance, *U. rigida*, which was expected to occur in the Wadden Sea and on Helgoland only, was also found to be present in the Baltic Sea (Table 2, supplementary Table S 1). As described before, *K. leptoderma*, which was only reported from Helgoland before, was found at different locations in the Baltic- and the Wadden Sea and was genetically validated for these areas (Table 2, supplementary Table S 2).

In summary the morphological concepts that are used for the identification of *Ulva* and related species in the studied region are currently neither in agreement with the species inventory in this area, nor with the actual morphology of species that are present. Past morphological descriptions of *U. linza*, *U. intestinalis* and *U. compressa* have been too restrictive, which resulted in numerous misidentifications of these abundant taxa. Further, several cryptic and/or newly introduced species are currently present in our area. These are *K. leptoderma, U. australis*, *U. californica*, *U. gigantea* and *Protomonostroma undulatum*, while *Ulva* sp., *Blidingia* sp. 1 and *Blidingia* sp. 2 could not yet be identified, but at least morphologically characterized. At the same time species such as *Blidingia minima* and *Gayralia oxysperma* appeared as absent or much more rare than expected. Certain other taxa that are expected in the area – namely, *U. tenera* – are synonyms. Our observations provide a basis for the future development of more correct identification keys, but presumably a morphological distinction of all species that are present in our area will probably not be possible, due to considerable overlap of traits. The DNA barcoding approach used in the present study clearly provides a better resolution. However, *U. californica* and *U. flexuosa* can not be clearly distinguished by *tufA* sequence analysis alone and more sequences of type material will be needed for a definite identification of species in the future. Further, more genetic markers have to be investigated and cultivation studies have to be performed to resolve remaining questions, such as the taxonomic affiliation of the newly found *Blidingia* species or relations among the genera *Ulva*, *Umbraulva* and *Percursaria*.

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Supplement

Distribution and specific characteristics of found species

In the following the distribution of recently found species within the examined area and their respective ecological and morphological characteristics is presented in detail:

Ulva gigantea

Specimens of *U. gigantea* were only found in the Wadden Sea around the ferry terminal of Dagebüll and an adjacent area and in the Baltic Sea only one individual of the species was found. Its distromatic, sheet-like thalli were mostly lobed or rosette-like while only few lanceolate individuals were observed. Blades were attached by a small rhizoidal zone during the beginning of the growth season (June-July) and mature thalli were mostly found drifting (August) and comprised thalli with small holes (0.5-3 mm). At the end of the vegetation phase the size of the holes increased up to 3 cm, however for mature thalli in some cases, the decision if the holes were naturally occurring or feeding traces were not obvious. The margins of the lobed or rosette-like thalli had no microscopic teeth (sometimes macroscopic teeth, probably due to feeding) and were mostly found frilled or ruffled. Thalli were rather rigid and 12-42 cm (few individuals up to 50 cm) in length. The polygonal (rarely round) cells of the middle and apical regions had rounded corners and were arranged in short rows, sometimes curved, or even unordered, and 19-23 x 20-22 µm in surface view, with a marginal chloroplast and one (sometimes 2) pyrenoids. Instead, the elongated cells of the rhizoidal zone contained various pyrenoids (1-4).

Ulva rigida

U. rigida was most prominently found in the northern parts of the Wadden Sea but also occurred at one location in the Baltic Sea (Heiligenhafen) and at different spots on Helgoland. Whereas in the Wadden Sea dense mats of drifting *U. rigida* but also attached specimens were observed, on Helgoland and in the Baltic Sea only attached single individuals were encountered. Individuals inhabited remote sites, strongly trafficked harbors and touristic used areas. Mature individuals were mostly of lanciniate, lobed or rounded and deeply folded shape, whereas young thalli often grew as rosettes, all were obligate distromatic. Some specimens exhibited small holes of 1-3 mm, while most thalli displayed no holes. Most specimens presented microscopic teeth at the outer thallus margins, which were often invisible in matured or drifting thalli. Cells were of various shape, either arranged in short, curved rows or irregular and of polygonal shape with rounded corners and of 8-26 x 8-30 µm in surface view. The chloroplast was arranged marginal or cap-like and most cells contained 2 sometimes 3 (rarely one or more than three) pyrenoids. The vegetative thallus parts were either passing straight into the rhizoidal zone and no clear "stipe-like-region" was discernible, or the thalli were slowly tapering until cells of the rhizoidal zone were formed. A distinct disc-like structure resembling a holdfast was not observed in the examined specimens.

Ulva sp.

Specimens belonging to this clade were present at all of the three main areas and most of the time, individuals were found in regions of intensive anthropogenic use, such as harbors, marinas or touristic trafficked waters. The morphology was highly variable. The tubular thalli were either smooth or wrinkled, 5-13 cm in length, and some individuals showed highest similarities to unbranched thalli of *U. intestinalis*. Branched specimens showed first- level, uniseriate branches that overlapped morphologically with young *U. flexuosa* thalli. However the main-axis was always apparent. Cells of the middle and apical thallus region were rectangular, quadratic or of polygonal shape and 9-18 x 12-29 µm in surface view. The chloroplast is either cap-like or filling the whole cell and each cell is containing 1-3 (rarely 4) pyrenoids, while cell of the rhizoidal zone can contain up to six pyrenoids per cell.

Ulva flexuosa

The species was found in all three observation areas - Baltic Sea, Wadden Sea and Helgoland - at rather low abundance. However, the rare detection of *U. flexuosa* may in part be due to a sampling bias that could result from its delicate texture and from the circumstance that drifting individuals are easily overlooked. Specimens were found in brackish and fully marine habitats, both at remote and highly trafficked sites, either attached to substrates but more dominantly as drifting mats. Due to the scarcity the following descriptions are based on observations of three individuals only: Specimens of *U. flexuosa* were always found to be tubular, either as delicate, narrow cords (1mm thick), or as broader tubes (8 mm thick). Thalli can reach a length of up to 40 cm. First-level branchlets can already be observed within the rhizoidal zone, while first- and secondary-level branches often cover the middle and apical thallus parts. Cells forming multiseriate rows in broader thallus parts, those rows can extend through the complete thallus (mostly including the rhizoidal zone), while uniseriate branchlets having a broader base and end in filigree tips. Due to strong proliferations in some specimens the main axis can not be identified. Rectangular cells of the middle and apical thallus region were 7-26 x 7-32 µm in surface view, contained a cap-like chloroplast and 1-3 (rarely up to 5) pyrenoids, while cells of the small rhizoidal zone contained 2-6 pyrenoids.

Ulva californica

Individuals of this species were first observed on the German North Sea island Wangerooge in 2008 (Lackschewitz et al. 2015), six years later the species was found in all investigated main areas, Baltic- (> 180 km distance from first introduction [through Kiel Canal], > 1000 km distance [through Skagerrak]), Wadden Sea (> 110 km distance) and on the remote deep-sea island Helgoland (> 40 km distance). Thus *U. californica* was found in a broad range of salinities. The morphology varied from branched to unbranched tubes up to lanceolate thalli or thalli of amorphous shape and were mostly found attached to artificial substrate, such as concrete, groynes or jetties. *Tuf*A Sequences of *U. californica* were identical to reference sequences from the US, California (KM255003) (Fig. 2, Table 2), however even the cluster representing *U. californica* delimits from other clusters, support for the separation from the closely related species *U. flexuosa* is small (70/-).

Ulva torta

Specimens were infrequently found in the Baltic Sea and at one site in the Wadden Sea (Nordstrand), but during our survey we were not able to verify *U. torta* for Helgoland. They appeared mostly drifting in shallow waters or disused areas of harbors, but they also formed mats of entangled thalli in the upper intertidal zone. The delicate, filigree thalli were less than 1mm (35-80 µm) in width while the total length was 12-30 cm. However, measuring the total length of one individual was hard to determine since specimens found in entangled mats often lack a rhizoidal zone, probably due to rupture, and were also found fragmented in some cases. Thalli were unbranched and straight and cells were arranged in multiple longitudinal rows, with 8-27 x 4-17 µm in surface view. Cells were of rectangular to quadratic shape with rounded corners and a parietal chloroplast, mostly averted from the lumen, as well as one pyrenoid, located marginal and embedded in the chloroplast, was observed. Characteristic for its identification and differentiation of young thalli of other species from *U. torta* is a central lumen, which was of 3-11 µm in surface view, and was running through the complete length of the thallus.

Ulva linza

Specimens were found at all main investigated sampling sites in a broad range of habitats, including remote and strongly trafficked sites. Specimens grew on various substrates including hard substrates such as stones, mussel shells or artificial structures but were also found as epiphytes on other phytobenthic species (on *Fucus* spp., *Gracilaria vermiculophylla*, Laminariales etc.) or on the vegetation of the intertidal zone, such as on *Phragmites* spp.. Even though, specimens of *U. linza* were mostly found attached, especially in harbors with low wave movement or shallow lagoons drifting, inflated individuals were observed. Strongest morphological variability among the investigated samples was encountered in specimens of *U. linza*. Their phenotypic appearance varied from unbranched tubes over branched individuals to mixed morphologies where the basal part was found to be tubular and apical thallus parts appeared distromatic and sheet-like. Tubular thalli may appear ruffled with hollow margins (typical “*linza*-morphology”), or tapering progressively from apical to basal thallus parts, some specimens only exhibit branches at the basal thallus region, whereas besides compressed unbranched individuals also “bottle-brush-morphologies” with highly branched thalli were found (similar to “*procera-ahlneriana*-morphology”). Also the overall thallus size was found to be very variable ranging from small epiphytes of 1-8 cm length and 0.5-1 cm width to thalli exceeding 160 cm in length and 10 cm in width that were predominantly found in sheltered bays, calm inlets and ditches. Cells are arranged in longitudinal and transverse rows throughout whereas apical cells are mostly quadrangular or rectangular but also polygonal cell shapes were observed, in the middle thallus section cells were either rectangular or quadrangular ranging between 13-19 x 13-25 µm in surface view. Cells of the apical and middle thallus section contain one pyrenoid (rarely 2) and a marginal choroplast that does not necessarily has to be orientated in same direction in all cells. Also rhizoidal cells possess one pyrenoid and cells containing two were less frequently observed. The phylogeny of U. linza is discussed in the main document.

Ulva prolifera

Individuals of *U. prolifera* were abundantly collected in the intertidal zone at the Baltic Sea, Wadden Sea and on Helgoland. Specimens were always found attached, never drifting. Thalli were mostly compressed and frequently branched, while exhibiting uniseriate but also multiseriate branches of varying length. Unbranched individuals were observed less frequently while in some thalli constrictions were observed. Specimens varied in size from 1 cm length up to 25 cm and 0.5 cm width up to 2 cm. Even though, in some individuals the stipe-like region was characteristically twisted and “cork-screw-like” some specimens had a straight basal region that proceeds in a not clearly distinguishable stipe-like region which in some individuals was more, in some less tapered and ends in a small (sometimes very reduced) rhizoidal zone. Cells were often found in pairs of two, of rectangular to polygonal shape, 8-22 x 8-26 µm in surface view, and arranged in distinct longitudinal and transverse rows (rarely short rows). A prominent central pyrenoid (rarely two) and a parietal or the cell filling chloroplast was observed. Most samples possessed only few rhizoidal cells, and those hardly differed in their size and number of pyrenoids from cells in the apical or middle thallus region. Cells with elongated tails, as frequently observed in other species, were nearly absent.

Ulva lactuca

Please see main document.

Ulva intestinalis

U. intestinalis was abundantly present in all main study areas and it inhabited various ecosystems, including fully marine brackish- but also freshwater ecosystems and it was also often found as being epiphytic on perennial seaweeds (e.g. *Fucus* spp.). Especially in overflow-basins, rock pools or water bodies with salinities < 5 PSU, *U. intestinalis* was a dominating species and in areas of fresh water inflow to the sea (small streams or drainage ditches) dense *U. intestinalis* communities were encountered. Notably, the thallus texture of specimens collected in low salinities was rigid and inelastic. It thus corresponded with descriptions of var. *crispa* Roth(Greville) (which is listed in the species inventory by Schories et al. (2009) for the Baltic Sea coast of our area), while it strongly differed from the rather soft and fragile specimens of *U. intestinialis* at fully marine sites. All examined specimens of the investigated area were obligate tubular. The thalli were mostly inflated, sometimes compressed, and the tubes appeared to be corrugated with ruffled margins or were smooth with pale margins, while some specimens exhibited multiple constrictions. Whereas the majority of investigated individuals was unbranched, Several specimens of *U. intestinalis* were found to be highly branched and thus differed from the unbranched type material of this species. Even though, branched specimens were more often found in low salinity environments they appeared also randomly in fully marine habitats.

Thalli had a length of 4-35 cm in unsheltered or wave exposed habitats, whereas certain individuals of up to 140 cm length were found in sheltered lagoons or overflow-basins. Cells of the apical and middle thallus were rectangular, quadratic or polygonal and either arranged in short, longitudinal rows or unordered and 12-21 x 14-28 cm in surface view. The chloroplast was parietal and arranged in the same direction in every cell or was filling the cell completely and one pyrenoid, often located marginal, was observed. The apical thallus part often ends in a tapered section of stipe-like shape that passes into the rhizoidal zone. Cells of the rhizoidal zone were cone-shaped and possessed 1-3 pyrenoids.

Ulva compressa

The species was abundantly present at the Baltic Sea, Wadden Sea and on Helgoland, inhabiting various habitats ranging from sheltered and remote locations to strongly trafficked harbors, drain channels and overflow basins. *U. compressa* was found at various salinities ranging from brackish (9 PSU) to fully marine conditions (32 PSU). The thalli are highly variable in their overall morphology. On Helgoland only tubular, mostly branched and generally attached individuals were observed. In sharp contrast, only distromatic and sheet-like specimens were found along the Baltic Sea cost, and these were – with the exception of one location at Wulfen on Fehmarn - always unattached. In the Wadden-Sea both morphologies were present and sheets could be encountered drifting or attached. The different morphotypes did not separate during our phylogenetic analysis of *tuf*A sequences. For further details on the morphology see (Steinhagen et al. 2018a; see also Chapter IV). Notably, during the summer months (July-August) this species tend to strongly proliferate in sheltered lagoons of the Baltic, as well as the Wadden Sea and “green-tide-like” states were observed (See also Steinhagen et al. [2018a] and Chapter IV).

Umbraulva dangeardii

Specimens of *U. dangeardii* were only found on Helgoland at a depth of 8 m, where they grew attached to the solid rock pedestal in communities with other annual and perennial algae. Individuals stood out with their dark olive color and species typical thin and soft texture and met the already published morphological identification criteria (Brodie et al. 2007). *Tuf*A sequences of *Umbraulva dangeardii* were identical to sequences from southern Italy (MF172091) (Fig. 2, Table 2).

Percursaria percursa (Morphological data was recorded at one population only)

The species was only found at one location in the Baltic Sea (Heiligenhafen) where it grew unattached in dense mats of entangled specimens in the supralittoral zone. The length of single individuals was hard to determine but disentangled single thalli were 3-6 cm in length. Rhizoidal zones were not observed. Thalli were mostly biseriate with opposing, quadratic cells that were 5-14 µm in surface view and a chloroplast that was located at the interior cell wall. Cells contained 1-3 pyrenoids. More populations have to be studied to investigate a possible morphological variability.

Blidingia marginata

Specimens were observed at all main study areas (Baltic-, Wadden Sea and Helgoland) and grew as dense populations in the upper intertidal zone and also colonized the supralittoral, where they were often found as moist or desiccated mats. Especially in freshwater influenced micro-habitats that were not directly connected to the sea and that fell dry for longer periods (in the immediate vicinity of beach showers or rain water drainages), only specimens of *B. marginata* (sometimes also *U. intestinalis*) were found. *B. marginata* grew on various substrates, ranging from stones, concrete and plastic sheathings of pillars to wood and at some sites it grew epiphytic on beach vegetation such as on roots of higher plants or on *Phragmites*.

The species was abundantly present at remote sites and protected areas as well as in harbors or highly frequented marinas. Specimens were either growing as single individuals or in tufts. Thalli are either corrugated with slightly to strongly ruffled margins or straight and compressed. Various sizes of thalli were observed, which can vary between thin compressed tubes of no more than 200 µm width and 1-3 cm height up to broad, inflated thalli of 0.5-1 cm width and 5-18 cm length. Different forms were observed in the same population. Besides predominantly found unbranched specimens, also individuals with small microscopic branchlets, or those with macroscopic branches were observed. The cells were arranged in distinct longitudinal rows, sometimes short, curved rows, were of rectangular to polygonal shape with rounded corners of 3-8 µm in diameter and exhibited a central, the cell filling chloroplast and one centrally located pyrenoid. Notably, randomly occurring, bigger (5-9 µm) rounded cells were also observed.

Monostroma grevillei (Morphology gametophyte)

This species was abundant at the Baltic Sea and it occurred also on Helgoland. It was only observed in spring (March to May) and toward the end of this vegetation period drifting mats of *M. grevillei* frequently developed in sheltered bays, harbors and lagoons. Thalli are 2-13 cm (rarely over 20 cm) in length, monostromatic and sac-like, lanceolate or of armorphous shape and especially in Mai, individuals began to rupture and appeared deeply cut. Individuals lacked a stipe-region, but the microscopic rhizoidal zone clearly separated from the basal thallus region. Cell shape and size varied with the respective thallus position. Apical cells were quadrangular to round with rounded corners, having a size of 5-20 x 5-26 µm in surface view and were arranged in distinct cell rows, often forming pairs of four, while exhibiting one central pyrenoid and the chloroplast mostly filled the compete cell. Thickened call walls were more prominent in cells of the distal thallus regions but were also found in cells of the middle thallus region. In the mid-region cells were of rectangular to quadrangular shape, some polygonal, with distinct angular corners, arranged in clear rows, while exhibiting one pyrenoid (rarely 2) and a cell filling chloroplast. Basal cells were distinct angular and elongated to drawn-out rectangles of 5-12 x 15-32 (46) µm which were arranged in small but distinct cell-rows. Rhizoidal, club-shaped cells were very limited to the rhizoidal zone and were directed proximal.

Protomonostroma undulatum

Specimens of *P. undulatum* were genetically only validated for Helgoland. Individuals grew predominantly on stone but were sometimes also found on piers in the lower and middle intertidal zone. The monostromatic, lobed, rosette-like or round thalli had a size of 2-18 cm length and 4-13 cm width. Cells of the apical and middle thallus parts were angular, polygonal, of 6-16 x 6-24 µm in surface view and arranged in packs of 2-8 in distinct rows. In some individuals the cell walls were thickened, similar to individuals of *K. leptoderma*. Cells contained 1-3 pyrenoids and a marginal to cell filling chloroplast. Instead of a smooth transition zone from basal cells to rhizoidal cells, abrupt changes in cell shape were observed. Cells of the rhizoidal zone were 60-90 µm, in some individuals up to 110 µm, long and extended basally as rhizoids and contained 1-3 pyrenoids.

The nomenclatural history of *Gayralia oxysperma*

The basionym of *Gayralia oxysperma* is *Ulva oxysperma*, which was described by Kützing (1843) on the basis of material from the Schlei, (Winning [Schaalby], Schleswig-Holstein, Germany, see also site 127 in Fig. 1). Today this type locality is a freshwater habitat (Salinity: 1) that is characterised by extended stands of *Phalaris arundinacea* and presence of *Ulva intestinalis* var. *crispa*. Similar environmental conditions certainly also existed 150 years ago, as no important geological changes occurred since then. Kützing characterized the morphology of *Ulva oxysperma* as follows: "*U. late expansa, plicato-crispa, foraminibus irregularibus perforata; cellularum interaneis monogonimicis, homogeneis, turgidis, primo globosis, demum in spermatia elongate basi acuminata transeuntibus. Ostsee: Schleybusen bei Winning: Frölich (als Ulva Lactuca)*" [interpreted translation: A wide spread *Ulva*, being multiply folded, perforated by irregularly appearing holes, cells in rows, of uniform shape, then turgid (probably referring to thickened cell walls), initially round (unclear, perhaps referring to early crust like thallus stages?), then changing into an elongated fertile thallus with a tapering, elongated base. Baltic Sea; Schlei near Winning: Frölich (as *Ulva lactuca*)]. Two years later, Kützing (1845) synonymized the validly published species *Ulva oxysperma* with *Ulva oxycocca* Kützing nom. illeg.. This was not in agreement with the taxonomic rules, which required to choose the first legitimate epithet of an entity, in this case *Ulva oxysperma*. More than one century later, Doty (1947) changed the affiliation to *Monostroma oxyspermum*, while Bliding (1968) placed *M. oxyspermum* in the genus *Ulvaria* on the basis of its ontogenetic stage of a single-row filament that reveals similarities to representatives of the family Ulvaceae. However, Vinogradova (1968) introduced the nomen novum *Gayralia oxysperma* and placed the species in the newly introduced family of Gayraliaceae within the independent genus *Gayralia*. As stated by Vinogradova (1969), this taxonomic replacement was widely based on the morphologic observations made by Gayral (1965). Vinogradova (1969) also considered Bliding's transfer of the species to the family of Ulvaceae as incorrect due to major differences in spore release. Probably because the original type material of *Ulva oxysperma* was lost a lectotype originating from the coast of Calvados in France was established (Womersley, 1984). Since the annotations made by Scagel et al. (1989) the species *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel et al. is validly established.

Seasonal species variation

The recently investigated species composition was found to vary seasonally (supplementary table 1). During the summer months (June-beginning of September), representatives of *Ulva sensu lato* were found at mostly all of the visited sites and formed conspicuous intertidal and/or subtidal communities. During winter time (November-beginning March) only reduced algal growth was observed and, if any, only small thallus remainders or fragments were found, while maximum vegetation is only observed in the summer months (June-August). With the beginning of April blooms of the typical spring alga *Monostroma grevillei* were observed in high abundances in the Baltic Sea as well as on Helgoland. *Monostroma grevillei* was not found at North Sea mainland coasts. The bloom of the monostromatic thalli of this species lasted for 3-4 weeks and individuals were only recorded until the end of Mai (supplementary Table 1), which makes the species lifecycle strictly restricted to a short time frame.

Table S1: Sample list with collection sites of *Ulva sensu lato* (Accession No.= GenBank accession number; Voucher = respective number of specimens voucher; Station map = used in figure 1).

Accession No.	**Species**	**Voucher**	**Date**	**Region**	**Country**	**Lat**	**Lon**	**Station map**
MH538542	*Blidingia marginata*	S_129	30. Jul 2014	Wadden Sea	Germany: Schleswig-Holstein, Schluettsiel	N 54,6813333	E 8,7544167	14
MH538543	*Blidingia marginata*	S_147_B	31. Jul 2014	Wadden Sea	Germany: Schleswig-Holstein, Schobuell	N 54,5078167	E 8,9955667	25
MH538544	*Blidingia marginata*	S_661	21. Apr 2015	Wadden Sea	Germany: Schleswig-Holstein, Nordstrand	N 54,4707167	E 8,8068333	23
MH538545	*Blidingia marginata*	S_708	23. Apr 2015	Helgoland	Germany: Heligoland	N 54,1780333	E 7,8887167	51
MH538546	*Blidingia marginata*	S_474	12. Sep 2014	Wadden Sea	Germany: Schleswig-Holstein, Schluettsiel	N 54,68435	E 8,75385	13
MH538547	*Blidingia marginata*	S_737	24. Apr 2015	Helgoland	Germany: Heligoland	N 54,1825	E 7,8906167	49
MH538548	*Blidingia marginata*	S_156	05. Aug 2014	Wadden Sea	Germany: Schleswig-Holstein, Woehrden	N 54,1173167	E 8,9359333	35
MH538549	*Blidingia marginata*	S_327	25. Aug 2014	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3765333	E 10,9800667	103
MH475464	*Blidingia marginata*	S_147_A	31. Jul 2014	Wadden Sea	Germany: Schleswig-Holstein, Pellworm	N 54,49882	E 8,8087	21
MH475465	*Blidingia marginata*	S_577	14. Apr 2015	Wadden Sea	Germany: Schleswig-Holstein, Brunsbuettel estuary	N 53,889	E 9,101133	41
MH538691	*Blidingia sp.* 1	S_93	24. Jul 2014	Wadden Sea	Germany:Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538692	*Blidingia sp.* 1	S_622	16. Apr 2015	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3787167	E 10,95545	99
MH538693	*Blidingia sp.* 1	S_21	23. Jul 2014	Helgoland	Germany: Heligoland	N 54,1825	E 7,890617	49
MH475455	*Blidingia sp.* 1	S_828	24. Jul 2014	Wadden Sea	Germany: Schleswig-Holstein, Schobuell	N 54,50782	E 8,995567	25
MH475456	*Blidingia sp.* 1	S_818	24. Jul 2017	Wadden Sea	Germany: Schleswig-Holstein, Husum	N 54,47113	E 9,027917	26
MH475457	*Blidingia sp.* 1	S_815	24. Jul 2017	Wadden Sea	Germany: Schleswig-Holstein, Finkhaushallig	N 54,41558	E 8,903633	27
MH475458	*Blidingia sp.* 1	S_813	24. Jul 2017	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,83735	E 8,6122	6
MH47	*Blidingia sp.*	S_179	06.	Wadden	Germany: Schleswig-Holstein,	N	E	41

5459	1		Aug 14	Sea	Brunsbuettel estuary	53,889	9,101133	
MH475460	*Blidingia sp.* 2	S_34	23. Jul 2014	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH475461	*Blidingia sp.* 2	S_1	22. Jul 2014	Helgoland	Germany: Heligoland	N 54,18367	E 7,888633	48
MH475462	*Blidingia sp.* 2	S_39	23. Jul 2014	Helgoland	Germany: Heligoland	N 54,1825	E 7,890617	49
MH475463	*Blidingia sp.* 2	S_124	30. Jul 2014	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MH720546	*Kornmannia leptoderma*	Lin_1	08. Jul 2018	Baltic Sea	Germany:Schleswig-Holstein, Lindaunis	N 54,5852667	E 9,8173833	61
MH720547	*Kornmannia leptoderma*	Lin_2	08. Jul 2018	Baltic Sea	Germany:Schleswig-Holstein, Lindaunis	N 54,5852667	E 9,8173833	61
MH720545	*Kornmannia leptoderma*	Lin_3	08. Jul 2018	Baltic Sea	Germany:Schleswig-Holstein, Lindaunis	N 54,5852667	E 9,8173833	61
MH538551	*Kornmannia leptoderma*	S_204	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538552	*Kornmannia leptoderma*	S_223	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4369167	E 10,1734667	69
MH538553	*Kornmannia leptoderma*	S_248	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Falckenstein	N 54,3903667	E 10,1922	76
MH538554	*Kornmannia leptoderma*	S_334	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,9823833	101
MH538555	*Kornmannia leptoderma*	S_337	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,382131	E 10,96375	100
MH538556	*Kornmannia leptoderma*	S_342	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3758	E 10,9874167	110
MH538557	*Kornmannia leptoderma*	S_376	27. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3566167	E 11,0610167	123
MH538558	*Kornmannia leptoderma*	S_382	27. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MH538559	*Kornmannia leptoderma*	S_525	17. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3581	E 11,0655	122
MH538560	*Kornmannia leptoderma*	S_535	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Brodten	N 54,9911667	E 10,8322833	127
MH538561	*Kornmannia leptoderma*	S_698	23. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538562	*Kornmannia leptoderma*	S_705	23. Apr	Helgoland	Germany: Heligoland	N 54,1780	E 7,8887	51

			15			333	167	
MH538563	*Kornmannia leptoderma*	S_715	23. Apr 15	Helgoland	Germany: Heligoland	N 54,177167	E 7,892944	52
MH538564	*Kornmannia leptoderma*	S_716	23. Apr 15	Helgoland	Germany: Heligoland	N 54,177167	E 7,892944	52
MH475466	*Kornmannia leptoderma*	S_154	05. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Finkhaushallig	N 54,41558	E 8,903633	27
MH475467	*Kornmannia leptoderma*	S_698	23. Apr 15	Helgoland	Germany: Heligoland	N 54,17967	E 7,889583	50
MH475468	*Kornmannia leptoderma*	S_337	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,98238	101
MH538580	*Monostroma grevillei*	S_529	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538581	*Monostroma grevillei*	S_530	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538582	*Monostroma grevillei*	S_533	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538583	*Monostroma grevillei*	S_537	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Brodten	N 54,9911667	E 10,8322833	127
MH538584	*Monostroma grevillei*	S_541	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3566167	E 11,0610167	123
MH538585	*Monostroma grevillei*	S_543	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3554667	E 11,0863667	124
MH538586	*Monostroma grevillei*	S_545	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3554667	E 11,0863667	124
MH538587	*Monostroma grevillei*	S_548	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MH538588	*Monostroma grevillei*	S_550	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MH538589	*Monostroma grevillei*	S_554	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Hohwacht	N 54,31815	E 10,6807333	94
MH538590	*Monostroma grevillei*	S_585	15. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Moenkeberg	N 54,3526667	E 10,1779	84
MH538591	*Monostroma grevillei*	S_587	15. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Moenkeberg	N 54,3526667	E 10,1779	84
MH538592	*Monostroma grevillei*	S_593	15. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538593	*Monostroma grevillei*	S_599	15. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Eckernförde	N 54,4732167	E 9,8330833	63

MH538594	*Monostroma grevillei*	S_604	15. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5230333	56
MH538595	*Monostroma grevillei*	S_605	15. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Gluecksburg	N 54,839204	E 9,517577	55
MH538596	*Monostroma grevillei*	S_616	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,9823833	101
MH538597	*Monostroma grevillei*	S_614	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3765333	E 10,9800667	103
MH538598	*Monostroma grevillei*	S_623	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3759333	E 10,9797333	104
MH538599	*Monostroma grevillei*	S_624	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3787167	E 10,95545	99
MH538600	*Monostroma grevillei*	S_627	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3787167	E 10,95545	99
MH538688	*Monostroma grevillei*	FM 6	23. Apr 15	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH538689	*Monostroma grevillei*	FM 1	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1796667	E 7,8895833	50
MH538690	*Monostroma grevillei*	FM 5	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH475469	*Monostroma grevillei*	S_548	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,40892	E 11,17313	121
MH475470	*Monostroma grevillei*	S_617	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,98238	101
MH475500	*Percursaria percursa*	S_360	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,98238	101
MH475501	*Protomonostroma undulatum*	S_733	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1825	E 7,890617	49
MH538642	*Ulva australis*	R_9	13. Aug 14	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH538643	*Ulva australis*	R_10	13. Aug 14	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH538644	*Ulva australis*	TD_11	24. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Wesselburenerkoog	N 54,23823661	E 8,78568942	33
MH475471	*Ulva australis*	TD_10	24. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Norderfriedrichskoog	N 54,41362	E 8,878922	28
MH475472	*Ulva australis*	TD_34	15. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, St. Peter-Ording	N 54,2857	E 8,703204	30
MH47	*Ulva*	TD_36	16.	Wadden	Germany: Schleswig-Holstein,	N	E	29

5473	*australis*		Aug 14	Sea	St. Peter-Ording	54,32695	8,585063	
MH538645	*Ulva californica*	S_233	13. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Schilksee	N 54,4278	E 10,1717167	73
MH538646	*Ulva californica*	TD_67	23. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Büsum	N 54,1253667	E 8,87305	34
MH475454	*Ulva californica*	S_791	23. Sep 15	Helgoland	Germany: Heligoland	N 54,18367	E 7,888633	48
MH475450	*Ulva californica*	S_106	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MF979360	*Ulva compressa*	S_112	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MF979645	*Ulva compressa*	S_14_B	22. Jul 14	Helgoland	Germany:Helgoland	N 54,1881667	E 7,8742333	46
MF979646	*Ulva compressa*	S_29	23. Jul 14	Helgoland	Germany: Helgoland	N 54,1698167	E 7,8894167	53
MF979647	*Ulva compressa*	S_115	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MF979648	*Ulva compressa*	S_254	18. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Kiel	N 54,3538	E 10,14125	78
MF979649	*Ulva compressa*	S_459	16-Mar-2016	Wadden Sea	Germany:Schleswig-Holstein, Emmelsbuell	N 54,7948667	E 8,6580667	8
MF979650	*Ulva compressa*	S_ 305	20. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Stein	N 54,41775	E 10,2645	91
MF979651	*Ulva compressa*	S_672	21. Apr 15	Wadden Sea	Germany:Schleswig-Holstein, Finkhaushallig	N 54,415583	E 8,90363	27
MF979652	*Ulva compressa*	S_514_B	19. Sep 14	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MF979653	*Ulva compressa*	S_ 356	25. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,3787167	E 10,95545	99
MF979654	*Ulva compressa*	S_361	25. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	N 54,37925	E 11,0050167	111
MF979655	*Ulva compressa*	S_381	27. Sep 14	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MF979656	*Ulva compressa*	S_383	27. Sep 14	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MF979657	*Ulva compressa*	S_113	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MF979658	*Ulva compressa*	S_6	22. Jul	Helgoland	Germany: Helgoland	N 54,1698	E 7,8894	53

			14			167	167	
MF979659	*Ulva compressa*	S_563	09. Apr 15	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MF979661	*Ulva compressa*	S_ 79	24. Jul 16	Baltic Sea	Germany: Schleswig-Holstein, Wackerballig	N 54,75863	E 9,87783	59
MG575234	*Ulva compressa*	S_107	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575235	*Ulva compressa*	S_120	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575236	*Ulva compressa*	S_128_plu	31. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Schluettsiel	N 54,6813	E 8,7544167	14
MG575237	*Ulva compressa*	S_151	31. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Schobuell	N 54,5078167	E 8,9955667	25
MG575238	*Ulva compressa*	S_155	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Woehrden	N 54,1173167	E 8,93593	35
MG575239	*Ulva compressa*	S_157	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Woehrden	N 54,1173167	E 8,93593	35
MG575240	*Ulva compressa*	S_166	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Meldorf	N 54,077633	E 8,968117	38
MG575241	*Ulva compressa*	S_171_A	06. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	N 54,03743	E 8,84485	39
MG575242	*Ulva compressa*	S_182	11. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Lübke-Koog	N 54,882483	E 8,6031	4
MG575243	*Ulva compressa*	S_183	11. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Lübke-Koog	N 54,882483	E 8,6031	4
MG575244	*Ulva compressa*	S_188	12. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Rickelsbüllerkoog, Hindenburgdamm	N 54,89113	E 8,60683	3
MG575245	*Ulva compressa*	S_443	08. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8567	E 8,60343	5
MG575246	*Ulva compressa*	S_447	09. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MG575247	*Ulva compressa*	S_452	09. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MG575248	*Ulva compressa*	S_468	10. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Galmsbuellkoog	N 54,76115	E 8,6967	9
MG575249	*Ulva compressa*	S_473	10. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Galmsbuellkoog	N 54,76115	E 8,6967	9
MG575250	*Ulva compressa*	S_506	16. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,598983	E 8,812167	18

MG575251	*Ulva compressa*	S_514_C	18. Sep 14	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MG575252	*Ulva compressa*	S_631	17. Apr 15	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575253	*Ulva compressa*	S_652	21. Apr 16	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4707167	E 8,80683	23
MG575254	*Ulva compressa*	S_713	23. Apr 15	Helgoland	Germany: Helgoland	N 54,177156	E 7,892952	52
MG575255	*Ulva compressa*	S_742	01. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575256	*Ulva compressa*	S_743_A	01. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575257	*Ulva compressa*	S_744	02. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575258	*Ulva compressa*	S_748_A	04. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,460417	E 8,844567	24
MG575259	*Ulva compressa*	S_749	05. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,460417	E 8,844567	24
MG575260	*Ulva compressa*	S_803	27. Sep 15	Helgoland	Germany: Helgoland	N 54,18817	E 7,874233	46
MG575261	*Ulva compressa*	CL_1_A	17. Jul 15	Wadden Sea	Germany:Schleswig-Holstein, Husum	N 54,471133	E 9,027917	26
MG575262	*Ulva compressa*	CL_1_B	17. Jul 15	Wadden Sea	Germany:Schleswig-Holstein, Husum	N 54,471133	E 9,027917	26
MG575263	*Ulva compressa*	FLS_1_A	16. Jun 15	Wadden Sea	Germany:Schleswig-Holstein, Sylt	55.035205	E 8,40016	1
MG575264	*Ulva compressa*	FLS_1_B	16. Jun 15	Wadden Sea	Germany:Schleswig-Holstein, Sylt	55.035205	E 8,40016	1
MG575265	*Ulva compressa*	FLS_2(1)	16. Jun 15	Wadden Sea	Germany:Schleswig-Holstein, Sylt	55.035205	E 8,40016	1
MG575266	*Ulva compressa*	FLS_3(3)	16. Jun 15	Wadden Sea	Germany:Schleswig-Holstein, Sylt	55.01832	E 8,43958	2
MG575267	*Ulva compressa*	S_171_B	06. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	N 54,03743	E 8,84485	39
MG575268	*Ulva compressa*	S_104	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575269	*Ulva compressa*	S_125	31. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 2	N 54,7304	E 8,6939	10
MG57	*Ulva*	S_126	31.	Wadden	Germany:Schleswig-Holstein,	N	E	10

5270	*compressa*		Jul 14	Sea	Dagebuell 2	54,7304	8,6939	
MG575271	*Ulva compressa*	S_12	22. Jul 14	Helgoland	Germany: Helgoland	N 54,188167	E 7,87423	46
MG575272	*Ulva compressa*	S_134	30. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Amsinck Haus	N 54,615367	E 8,866883	16
MG575273	*Ulva compressa*	S_135	31. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Amsinck Haus	N 54,615367	E 8,866883	16
MG575274	*Ulva compressa*	S_137	01. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,51663	E 8,854367	20
MG575275	*Ulva compressa*	S_141	02. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Pellworm	N 54,4988167	E 8,8087	21
MG575276	*Ulva compressa*	S_143	02. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Pellworm	N 54,4988167	E 8,8087	21
MG575277	*Ulva compressa*	S_144_A	02. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Pellworm	N 54,4988167	E 8,8087	21
MG575278	*Ulva compressa*	S_158	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Woehrden	N 54,1173167	E 8,93593	35
MG575279	*Ulva compressa*	S_159	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Woehrden	N 54,1173167	E 8,93593	35
MG575280	*Ulva compressa*	S_160	05. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Woehrden	N 54,1173167	E 8,93593	35
MG575281	*Ulva compressa*	S_172	06. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	N 54,03743	E 8,84485	39
MG575282	*Ulva compressa*	S_174_A	07. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	N 54,03743	E 8,84485	39
MG575283	*Ulva compressa*	S_174_C	08. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	N 54,03743	E 8,84485	39
MG575284	*Ulva compressa*	S_177	08. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Kaiser-Wilhelm-Koog	N 53,936033	E 8,90525	40
MG575285	*Ulva compressa*	S_185	11. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Rickelsbüllerkoog, Hindenburgdamm	N 54,89113	E 8,60683	3
MG575286	*Ulva compressa*	S_190	13. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	N 54,83735	E 8,6122	6
MG575287	*Ulva compressa*	S_192	13. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	N 54,83735	E 8,6122	6
MG575288	*Ulva compressa*	S_193	13. Aug 14	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	N 54,83735	E 8,6122	6
MG575289	*Ulva compressa*	S_335	25. Aug	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,3794	E 10,982	101

			14			5	383	
MG575290	*Ulva compressa*	S_340	22. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	N 54,378983	E 10,9872167	105
MG575291	*Ulva compressa*	S_346	25. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,383367	E 10,9502167	98
MG575292	*Ulva compressa*	S_348	25. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,383367	E 10,9502167	98
MG575293	*Ulva compressa*	S_353	25. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,383367	E 10,9502167	98
MG575294	*Ulva compressa*	S_362	26. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	N 54,378	E 11,008583	112
MG575295	*Ulva compressa*	S_368	26. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,1825	E 7,8906167	49
MG575296	*Ulva compressa*	S_37_plus	23. Jul 14	Helgoland	Germany: Helgoland	N 54,1825	E 7,8906167	49
MG575297	*Ulva compressa*	S_4_plus	22. Jul 14	Helgoland	Germany: Helgoland	N 54,1698167	E 7,8894167	53
MG575298	*Ulva compressa*	S_450	25. Aug 17	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MG575299	*Ulva compressa*	S_478	12. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Schluettsiel	N 54,68435	E 8,75385	13
MG575300	*Ulva compressa*	S_507	16. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,598983	E 8,812167	18
MG575301	*Ulva compressa*	S_511	17. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,598983	E 8,8122	18
MG575302	*Ulva compressa*	S_512	18. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,598983	E 8,8122	18
MG575303	*Ulva compressa*	S_513	19. Sep 14	Wadden Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,598983	E 8,8122	18
MG575304	*Ulva compressa*	S_549	08. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MG575305	*Ulva compressa*	S_651	21. Apr 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4707167	E 8,80683	23
MG575306	*Ulva compressa*	S_540	08. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Großenbrode	N 54,39303	E 11,1102	114
MG575307	*Ulva compressa*	S_626	16. Apr 15	Baltic Sea	Germany:Schleswig-Holstein, Heiligenhafen inland lake	N 54,3787167	E 10,95545	99
MG575308	*Ulva compressa*	S_670	21. Apr 15	Wadden Sea	Germany:Schleswig-Holstein, Finkhaushallig	N 54,415583	E 8,90363	27

MG575309	*Ulva compressa*	S_707	23. Apr 15	Helgoland	Germany: Helgoland	N 54,1780333	E 7,8887167	51
MG575310	*Ulva compressa*	S_739_A	31. Aug 15	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MG575311	*Ulva compressa*	S_741	31. Aug 15	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MG575312	*Ulva compressa*	S_740	31. Aug 15	Baltic Sea	Germany:Schleswig-Holstein, Wullfen	N 54,4089167	E 11,17313	121
MG575313	*Ulva compressa*	S_743_B	01. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575314	*Ulva compressa*	S_743_C	01. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575315	*Ulva compressa*	S_745	02. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575316	*Ulva compressa*	S_746	03. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575317	*Ulva compressa*	S_752	06. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG575318	*Ulva compressa*	S_754	07. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG575319	*Ulva compressa*	S_756_A	08. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG575320	*Ulva compressa*	S_756_B	09. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG575321	*Ulva compressa*	S_760_A	16. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Lübke-Koog	N 54,882483	E 8,6031	4
MG575322	*Ulva compressa*	S_770	17. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575323	*Ulva compressa*	S_771	18. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575324	*Ulva compressa*	S_778	19. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575325	*Ulva compressa*	S_779	20. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Dagebuell 1	N 54,730067	E 8,6891667	11
MG575326	*Ulva compressa*	S_780A	09. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG575327	*Ulva compressa*	S_782	10. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,4604167	E 8,844567	24
MG57	*Ulva*	S_788	23.	Helgoland	Germany: Helgoland	N	E	49

5328	*compressa*		Sep 15			54,183667	7,888639	
MG575329	*Ulva compressa*	S_790	24. Sep 15	Helgoland	Germany: Helgoland	N 54,75863	E 9,87783	59
MG575330	*Ulva compressa*	S_795	25. Sep 15	Helgoland	Germany:Helgoland	N 54,183667	E 7,888639	48
MG575331	*Ulva compressa*	S_797	26. Sep 15	Helgoland	Germany: Helgoland	N 54,17803	E 7,8887167	51
MG575332	*Ulva compressa*	S_747	01. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Nordstrand	N 54,486052	E 8,818574	22
MG575333	*Ulva compressa*	S_805	27. Sep 15	Helgoland	Germany: Helgoland	N 54,18817	E 7,874233	46
MG575334	*Ulva compressa*	S_799	28. Sep 15	Helgoland	Germany: Helgoland	N 54,17803	E 7,8887167	51
MG575335	*Ulva compressa*	S_146_B	31. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Schobuell	N 54,5078167	E 8,995567	25
MG575336	*Ulva compressa*	S_454	09. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MG575337	*Ulva compressa*	S_479	12. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Schluettsiel	N 54,68435	E 8,75385	13
MG575338	*Ulva compressa*	S_1003	16-Mar-2016	Wadden Sea	Germany:Schleswig-Holstein, Emmelsbuell	N 54,7948667	E 8,6580667	8
MG575340	*Ulva compressa*	S_1001	05. Jul 16	Baltic Sea	Germany:Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MG575341	*Ulva compressa*	S_1000	17-Mar-2016	Wadden Sea	Germany:Schleswig-Holstein, Emmelsbuell	N 54,7948667	E 8,6580667	8
MG575342	*Ulva compressa*	S_441	08. Sep 15	Wadden Sea	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8567	E 8,6034333	5
MG575339	*Ulva compressa*	S_1002	16. Nov 15	Baltic Sea	Germany:Schleswig-Holstein, Moenkeberg	N 54,3526667	E 10,1779	84
MH475451	*Ulva flexuosa*	S_257	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel	N 54,3538	E 10,14125	78
MH475452	*Ulva flexuosa*	S_769	16. Aug 15	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MH475453	*Ulva flexuosa*	S_794	23. Sep 15	Helgoland	Germany: Heligoland	N 54,17803	E 7,888717	51
MH538550	*Ulva gigantea*	S_102	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,7300667	E 8,6891667	11
MH538696	*Ulva gigantea*	S_87_A	24. Jul	Baltic Sea	Germany:Schleswig-Holstein, Eckernförde	N 54,4732	E 9,8330	63

			14			167	833	
MH475474	*Ulva gigantea*	S_775	16. Aug 15	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,7304	E 8,6939	10
MH475475	*Ulva gigantea*	S_564	09. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	N 54,8333	E 8,6142	7
MH475476	*Ulva gigantea*	S_632	17. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MH538514	*Ulva intestinalis*	S_200	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538515	*Ulva intestinalis*	S_38_A	23. Jul 14	Helgoland	Germany: Heligoland	N 54,1825	E 7,8906167	49
MH538516	*Ulva intestinalis*	S_294	19. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Laboe	N 54,39725	E 10,2126333	88
MH538517	*Ulva intestinalis*	S_195	12. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Falshöft	N 54,76845	E 9,9653333	60
MH538518	*Ulva intestinalis*	S_94	24. Jul 14	Baltic Sea	Germany:Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538519	*Ulva intestinalis*	S_95	24. Jul 14	Baltic Sea	Germany:Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538520	*Ulva intestinalis*	S_569	14. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Brokdorf	N 53,8611667	E 9,3231333	44
MH538521	*Ulva intestinalis*	S_642	21. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Schobuell	N 54,5078167	E 8,9955667	25
MH538522	*Ulva intestinalis*	S_181	06. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Brunsbüttel estuary	N 53,889	E 9,1011333	41
MH538523	*Ulva intestinalis*	S_191	11. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Neukirchen	N 54,8018333	E 9,7554833	58
MH538524	*Ulva intestinalis*	S_214	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4442	E 10,18125	67
MH538525	*Ulva intestinalis*	S_251	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel-Wik	N 54,3662	E 10,1488333	77
MH538526	*Ulva intestinalis*	S_271	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Moenkeberg	N 54,3464667	E 10,1742	82
MH538527	*Ulva intestinalis*	S_303_B	20. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Stein	N 54,41775	E 10,2645	91
MH538528	*Ulva intestinalis*	S_307	20. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Marina Wendtorf	N 54,42055	E 10,2899	92
MH538529	*Ulva intestinalis*	S_316	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Hohwacht	N 54,31815	E 10,6807333	94

MH538530	*Ulva intestinalis*	S_322	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3759333	E 10,9797333	104
MH538531	*Ulva intestinalis*	S_325	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3759333	E 10,9797333	104
MH538532	*Ulva intestinalis*	S_343	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3783167	E 10,99125	107
MH538533	*Ulva intestinalis*	S_359	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3783167	E 10,99125	107
MH538534	*Ulva intestinalis*	S_381	27. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MH538535	*Ulva intestinalis*	S_415	02. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3773	E 10,9948667	108
MH538536	*Ulva intestinalis*	S_426	03. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Kellenhusen	N 54,19275	E 11,0708167	125
MH538537	*Ulva intestinalis*	S_430	03. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538538	*Ulva intestinalis*	S_539	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Grossenbrode	N 54,3566167	E 11,0610167	123
MH538539	*Ulva intestinalis*	S_607	15. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5230333	56
MH538540	*Ulva intestinalis*	S_662	21. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Finkhaushallig	N 54,4155833	E 8,9036333	27
MH538541	*Ulva intestinalis*	S_700	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1796667	E 7,8895833	50
MH475477	*Ulva intestinalis*	S_72	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,8392	E 9,517577	55
MH475478	*Ulva intestinalis*	S_133	31. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Schluettsiel	N 54,68435	E 8,75385	13
MH538565	*Ulva lactuca*	S_5	22. Jul 14	Helgoland	Germany: Heligoland	N 54,183667	E 7,888633	48
MH538566	*Ulva lactuca*	S_10	22. Jul 14	Helgoland	Germany: Heligoland	N 54,188172	E 7,880024	47
MH538567	*Ulva lactuca*	S_11	22. Jul 14	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538568	*Ulva lactuca*	S_20	22. Jul 14	Helgoland	Germany: Heligoland	N 54,183667	E 7,888633	48
MH538569	*Ulva lactuca*	S_673	23. Apr 15	Helgoland	Germany: Heligoland	N 54,188172	E 7,880024	47
MH53	*Ulva lactuca*	S_678	23.	Helgoland	Germany: Heligoland	N	E	47

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MH538571	*Ulva lactuca*	S_679	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538572	*Ulva lactuca*	S_681	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538573	*Ulva lactuca*	S_696	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1796667	E 7,8895833	50
MH538574	*Ulva lactuca*	S_721	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538575	*Ulva lactuca*	S_725	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538576	*Ulva lactuca*	S_728	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538577	*Ulva lactuca*	S_729	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1825	E 7,8906167	49
MH538578	*Ulva lactuca*	S_734	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1825	E 7,8906167	49
MH538579	*Ulva lactuca*	S_735	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1796667	E 7,8895833	50
MH475479	*Ulva lactuca*	S_729	24. Apr 15	Helgoland	Germany: Heligoland	N 54,18817	E 7,880024	47
MH475480	*Ulva lactuca*	S_696	23. Apr 15	Helgoland	Germany: Heligoland	N 54,17967	E 7,889583	50
MH538647	*Ulva linza* 1	S_63	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5230333	56
MH538648	*Ulva linza* 1	S_64	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5230333	56
MH538649	*Ulva linza* 1	S_65	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5230333	56
MH538650	*Ulva linza* 1	S_132	30. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Schluettsiel	N 54,6813333	E 8,7544167	14
MH538651	*Ulva linza* 1	S_192	12. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,83735	E 8,6122	6
MH538652	*Ulva linza* 1	S_197_B	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Schoenhagen	N 54,6361167	E 10,03125	62
MH538653	*Ulva linza* 1	S_198	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Schoenhagen	N 54,6361167	E 10,03125	62
MH538654	*Ulva linza* 1	S_199	12. Aug	Baltic Sea	Germany: Schleswig-Holstein, Schoenhagen	N 54,6361	E 10,031	62

			14			167	25	
MH538655	*Ulva linza* 1	S_201	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538656	*Ulva linza* 1	S_203	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538657	*Ulva linza* 1	S_206	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538658	*Ulva linza* 1	S_207	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiekut	N 54,4476	E 9,8716833	64
MH538659	*Ulva linza* 1	S_209	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4442	E 10,18125	67
MH538660	*Ulva linza* 1	S_211	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4442	E 10,18125	67
MH538661	*Ulva linza* 1	S_212	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4362333	E 10,1750167	70
MH538662	*Ulva linza* 1	S_217	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4362333	E 10,1750167	70
MH538663	*Ulva linza* 1	S_220	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4362333	E 10,1750167	70
MH538664	*Ulva linza* 1	S_222	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4362333	E 10,1750167	70
MH538665	*Ulva linza* 1	S_224	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4369167	E 10,1734667	69
MH538666	*Ulva linza* 1	S_226	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4349833	E 10,17015	71
MH538667	*Ulva linza* 1	S_229	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,4349833	E 10,17015	71
MH538668	*Ulva linza* 1	S_237	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Falckenstein	N 54,3903667	E 10,1922	76
MH538669	*Ulva linza* 1	S_240	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Falckenstein	N 54,399039	E 10,190814	75
MH538670	*Ulva linza* 1	S_243	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Falckenstein	N 54,399039	E 10,190814	75
MH538671	*Ulva linza* 1	S_250	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel-Wik	N 54,3662	E 10,1488333	77
MH538672	*Ulva linza* 1	S_255	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel	N 54,3538	E 10,14125	78
MH538673	*Ulva linza* 1	S_260	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel-Wik	N 54,3514667	E 10,1432	79

MH538674	*Ulva linza* 1	S_262	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel	N 54,3478833	E 10,1503	80
MH538675	*Ulva linza* 1	S_267	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel	N 54,34635	E 10,1526333	81
MH538676	*Ulva linza* 1	S_279	19. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heikendorf	N 54,3766833	E 10,1958667	85
MH538677	*Ulva linza* 1	S_284	19. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heikendorf	N 54,3827	E 10,2025833	86
MH538678	*Ulva linza* 1	S_288	19. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heikendorf	N 54,38285	E 10,2028	87
MH538679	*Ulva linza* 1	S_290	19. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Laboe	N 54,4035333	E 10,2159167	89
MH538680	*Ulva linza* 1	S_301	20. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Stein	N 54,41775	E 10,2645	91
MH538681	*Ulva linza* 1	S_314	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Hohwacht	N 54,31815	E 10,6807333	94
MH538682	*Ulva linza* 1	S_341	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3783833	E 10,9879667	106
MH538683	*Ulva linza* 1	S_364	26. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37925	E 11,0050167	111
MH538684	*Ulva linza* 1	S_390	01. Sep 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3783833	E 10,9879667	106
MH475447	*Ulva linza* 1	S_241_U. linza 1	18. Aug 14	Helgoland	Germany:Schleswig-Holstein, Falckenstein	N 54,39037	E 10,1922	76
MH475448	*Ulva linza* 1	S_504_U. linza_1	16. Sep 14	Baltic Sea	Germany:Schleswig-Holstein, Hamburger Hallig	N 54,59898	E 8,8122	18
MH475449	*Ulva linza* 1	S_64_U.linza_1	24. Jul 14	Wadden Sea	Germany:Schleswig-Holstein, Gluecksburg	N54,8392	E 9,517577	55
MH538685	*Ulva linza* 2	S_726	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1780333	E 7,8887167	51
MH538686	*Ulva linza* 2	S_12	22. Jul 14	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH538687	*Ulva linza* 2	S_719	24. Apr 15	Helgoland	Germany: Heligoland	N 54,1881667	E 7,8742333	46
MH475445	*Ulva linza* 2	S_727_U. linza_2	24. Apr 15	Helgoland	Germany: Heligoland	N 54,17717	E 7,892944	52
MH475446	*Ulva linza* 2	S_8_U.linza_2	22. Jul 14	Helgoland	Germany: Heligoland	N 54,18817	E 7,874233	46
MH53	*Ulva*	S_9	22.	Helgoland	Germany: Heligoland	N	E	47

8601	*prolifera*		Jul 14			54,188172	7,880024	
MH538602	*Ulva prolifera*	S_78	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Wackerballig	N 54,7586333	E 9,8778333	59
MH538603	*Ulva prolifera*	S_114	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,7300667	E 8,6891667	11
MH538604	*Ulva prolifera*	S_136	31. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Nordstrand	N 54,5166333	E 8,8543667	20
MH538605	*Ulva prolifera*	S_139	31. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Nordstrand	N 54,5166333	E 8,8543667	20
MH538606	*Ulva prolifera*	S_142	31. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Pellworm	N 54,4988167	E 8,8087	21
MH538607	*Ulva prolifera*	S_168_A	05. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Meldorf	N 54,0776333	E 8,9681167	38
MH538608	*Ulva prolifera*	S_196	12. Aug 14	Baltic Sea	Germany:Schleswig-Holstein, Falshöft	N 54,76845	E 9,9653333	60
MH538609	*Ulva prolifera*	S_358	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,3787167	E 10,95545	99
MH538610	*Ulva prolifera*	S_424_B	02. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Burg	N 54,4146333	E 11,21145	119
MH538611	*Ulva prolifera*	S_440	08. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8567	E 8,6034333	5
MH538612	*Ulva prolifera*	S_444	08. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538613	*Ulva prolifera*	S_446	08. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538614	*Ulva prolifera*	S_461	10. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Emmelsbüll	N 54,7948667	E 8,6580667	8
MH538615	*Ulva prolifera*	S_464	10. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Emmelsbüll	N 54,7948667	E 8,6580667	8
MH538616	*Ulva prolifera*	S_466	10. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Emmelsbüll	N 54,7948667	E 8,6580667	8
MH538617	*Ulva prolifera*	S_470	10. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Galmsbüllkoog	N 54,76115	E 8,6967	9
MH538618	*Ulva prolifera*	S_488	12. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Großengaarde	N 54,6636833	E 8,7909667	15
MH538619	*Ulva prolifera*	S_508	16. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Hamburger Hallig	N 54,5989833	E 8,8121667	18
MH538620	*Ulva prolifera*	S_509	16. Sep	Wadden Sea	Germany: Schleswig-Holstein, Hamburger Hallig	N 54,5989	E 8,8121	18

			14			833	667	
MH538621	*Ulva prolifera*	S_532	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538622	*Ulva prolifera*	S_532_B	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Neustadt	N 54,1107167	E 10,8135333	126
MH538623	*Ulva prolifera*	S_537_A	08. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Brodten	N 54,9911667	E 10,8322833	127
MH538624	*Ulva prolifera*	S_546	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Wulfen	N 54,4089167	E 11,1731333	121
MH538625	*Ulva prolifera*	S_552	08. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Hohwacht	N 54,31815	E 10,6807333	94
MH538626	*Ulva prolifera*	S_555	09. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538627	*Ulva prolifera*	S_619	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37945	E 10,9823833	101
MH538628	*Ulva prolifera*	S_639	17. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Schluettsiel	N 54,68435	E 8,75385	13
MH538629	*Ulva prolifera*	S_680	23. Apr 15	Helgoland	Germany: Heligoland	N 54,188172	E 7,880024	47
MH538630	*Ulva prolifera*	S_709	23. Apr 15	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH538631	*Ulva prolifera*	TD_43	18. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, St. Peter Ording	N 54,28570206	E 8,703204001	30
MH538632	*Ulva prolifera*	TD_65	23. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Grothusenkoog	N 54,28858739	E 8,73684072	31
MH475481	*Ulva prolifera*	S_196	12. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Falshoeft	N 54,76845	E 9,965333	60
MH475482	*Ulva prolifera*	S_9	22. Jul 14	Helgoland	Germany: Heligoland	N 54,18817	E 7,874233	46
MH475483	*Ulva prolifera*	S_466	10. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Emmelsbuell	N 54,79487	E 8,658067	8
MH538633	*Ulva rigida*	R_13	13. Aug 14	Helgoland	Germany: Heligoland	N 54,17195	E 7,8993	53
MH538634	*Ulva rigida*	S_116	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,7300667	E 8,6891667	11
MH538635	*Ulva rigida*	S_121	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,7300667	E 8,6891667	11
MH538636	*Ulva rigida*	S_189	11. Aug 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,83735	E 8,6122	6

MH538637	*Ulva rigida*	S_442	08. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8567	E 8,6034333	5
MH538638	*Ulva rigida*	S_453_A	09. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538639	*Ulva rigida*	S_558	09. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538640	*Ulva rigida*	S_560	09. Apr 15	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,8333	E 8,6142	7
MH538641	*Ulva rigida*	S_701	23. Apr 15	Helgoland	Germany: Heligoland	N 54,1796667	E 7,8895833	50
MH538695	*Ulva rigida*	S_613	16. Apr 15	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37925	E 11,0050167	111
MH475484	*Ulva rigida*	S_449	09. Sep 14	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	N 54,8333	E 8,6142	7
MH475485	*Ulva rigida*	S_123	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MH475486	*Ulva rigida*	S_111	30. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Dagebuell	N 54,73007	E 8,689167	11
MH538697	*Ulva* sp.	S_773	16. Sep 15	Wadden Sea	Germany: Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	N 54,83735	E 8,6122	6
MH475487	*Ulva* sp.	S_228	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,43498	E 10,17015	71
MH475488	*Ulva* sp.	S_269	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Moenkeberg	N 54,34647	E 10,1742	82
MH475489	*Ulva* sp.	S_256	18. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Kiel	N 54,3538	E 10,14125	78
MH475490	*Ulva* sp.	S_2_A	22. Jul 14	Helgoland	Germany: Heligoland	N 54,16982	E 7,889417	53
MH475491	*Ulva* sp.	S_317	22. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Sehlendorfer lake	N 54,30882	E 10,68863	95
MH475492	*Ulva* sp.	S_221	13. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Strande	N 54,43623	E 10,17502	70
MH475493	*Ulva* sp.	S_92	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Aschau	N 54,4608	E 9,92665	65
MH538694	*Ulva torta*	S_138	31. Jul 14	Wadden Sea	Germany: Schleswig-Holstein, Nordstrand	N 54,4707167	E 8,8068333	23
MH475494	*Ulva torta*	S_81	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Wackerballig	N 54,75863	E 9,877833	59
MH47	*Ulva torta*	S_231	13.	Baltic Sea	Germany: Schleswig-Holstein,	N	E	72

5495			Aug 14		Schilksee	54,43132	10,16933	
MH475496	*Ulva torta*	S_350	25. Aug 14	Baltic Sea	Germany: Schleswig-Holstein, Heiligenhafen	N 54,37872	E 10,95545	99
MH475497	*Ulva torta*	S_73	24. Jul 14	Baltic Sea	Germany: Schleswig-Holstein, Gluecksburg	N 54,83675	E 9,5231	56
MH475498	*Umbraulva dangeardii*	R_1	08. Aug 14	Helgoland	Germany: Heligoland	N 54,18743	E 7,8703	45
MH475499	*Umbraulva dangeardii*	R_2	08. Aug 14	Helgoland	Germany: Heligoland	N 54,18743	E 7,8703	45

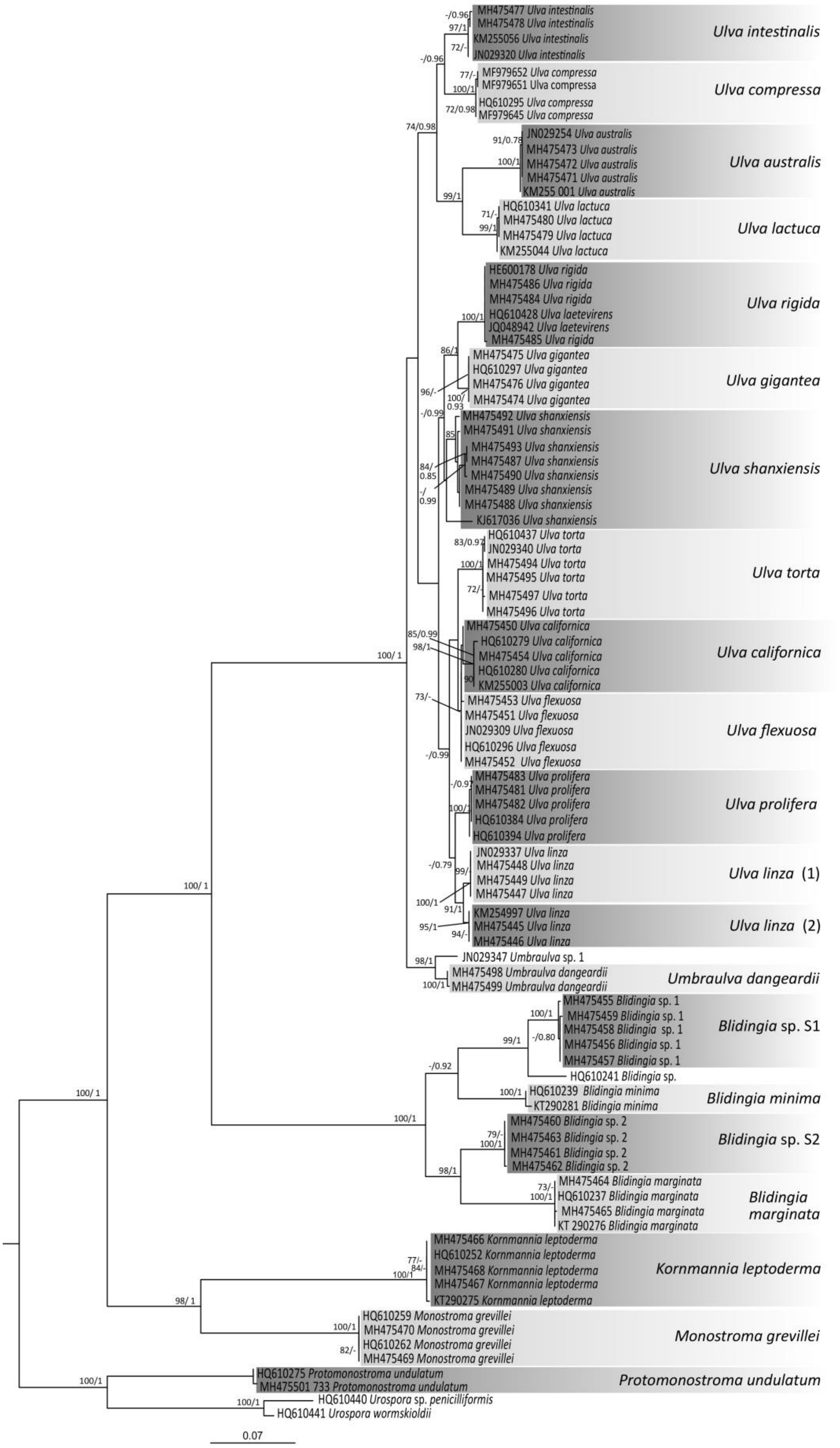

Figure S1: Maximum likelihood phylogram inferred from *tuf*A sequences, displaying entities of *Ulva sensu lato* from northern Germany identified in this study. Herbarium vouchers are marked with a filled triangle (see also Table 2). Two tones of grey indicate clades which are present in the area, hatched boxes indicate species complexes and thus entities that could not clearly be resolved phylogenetically. Numbers at nodes refer to bootstrap values (left) and Bayesian posterior probabilities (1,000 replicates; right). Nodes with < 70% bootstraps and < 0.70 Bayesian support are not labeled. Branch lengths are drawn proportional to the amount of sequence change.

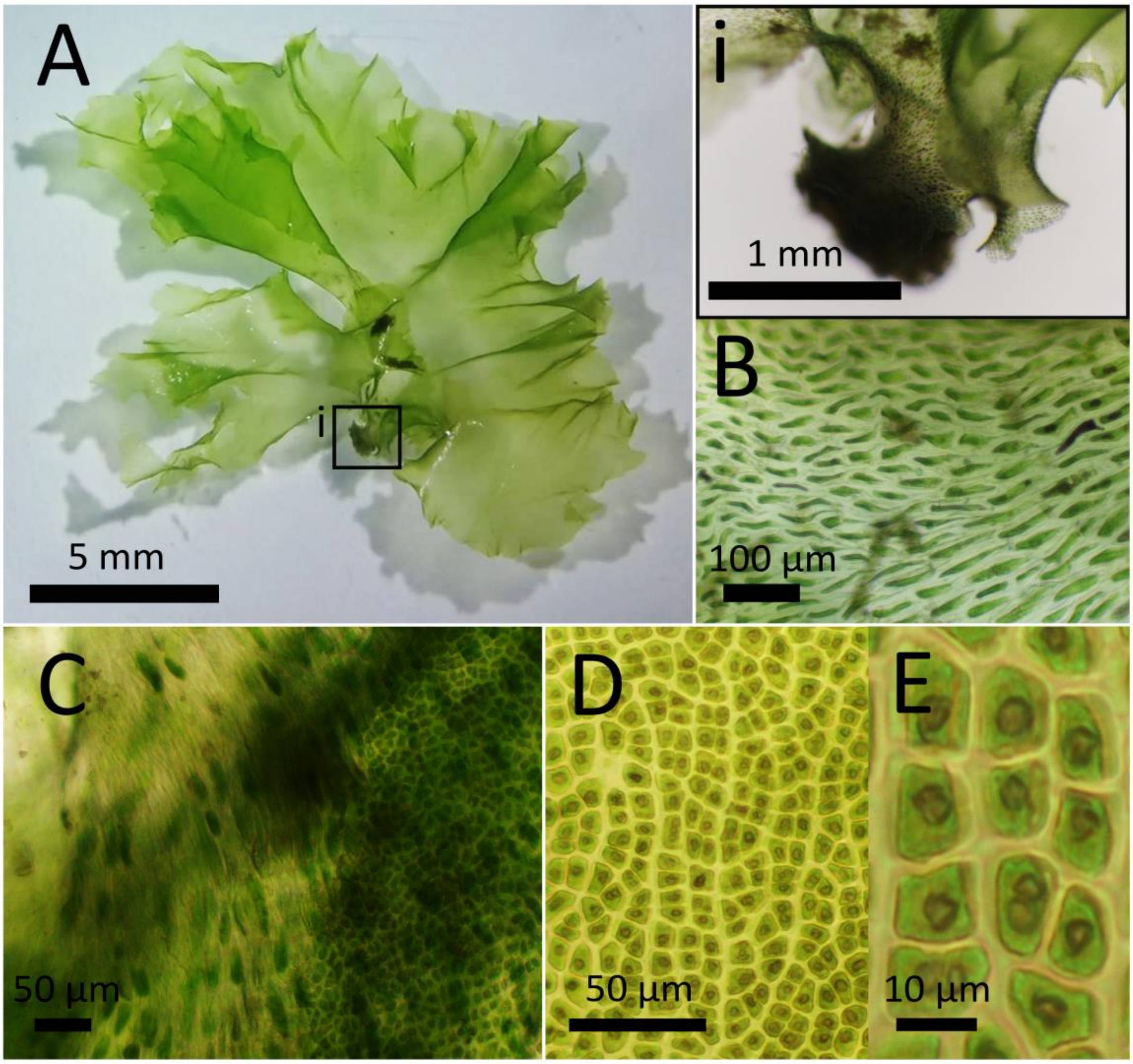

Figure S 2: Morphology of *Protomonostroma undulatum*, collected on Helgoland. (A) Overview about the monostromatic thallus with (i) close-up of the rhizoidal zone. (B) Cells of the rhizoidal zone are of elongated shape with thickened cell walls. Cells of the rhizoidal zone and the basal thallus part are separated be a distinct transition zone (C). (D) Cells of the middle and apical thallus region are arranged in packs of 2-8 and form distinct rows, whereas the thickened cell-walls appear as veins. (E) The cell-shape is rectangular to polygonal and one to three, mostly centrally arranged, pyrenoids are located within each cell.

Chapter II **(Accepted for publication by Botanica Marina [https://doi.org/10.1515/bot-2018-0020])**

Surveying seaweeds from the Ulvales and Fucales in the world´s most frequently used artificial waterway, the Kiel Canal

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Abstract

The Kiel Canal is one of the world's most frequently used inland waterways and connects the SW Baltic Sea with the Wadden Sea. At the same time, the canal is a highly eutrophicated environment that is characterized by salinities that range from 3 to 16. This brackish character could make the Kiel Canal an important stepping stone for the introductions of species into the inner Baltic Sea. It could also hinder the identification of native and introduced species, given the fact that salinity sometimes severely affects algal morphology. Here we report on a survey of introduced and native seaweed species in the canal, focusing on the dominant groups, which are Fucales and Ulvales. Of the Fucales, the introduced species *Fucus evanescens* was detected nearly exclusively inside the canal, while *Fucus vesiculosus* dominated rockweed communities directly outside the sluice gates. Morphological analysis and genetic barcoding distinguished three species of Ulvales, *Ulva linza*, *Ulva intestinalis* and an unknown and possibly introduced species of the genus *Blidingia*. Species distributions and - in the case of *U. intestinalis* - branching patterns were clearly affected by salinity, while thallus sizes appeared to be affected by the specific eutrophication status of sites within the canal.

Key words: Kiel Canal, invasive seaweeds, morphology, green algae, *Fucus*

Introduction

Marine biological invasions are globally on the rise (Seebens et al. 2013, Tournadre 2014) and most introductions of seaweeds and other organisms into new habitats are related to ship traffic (Simberloff and Rejmanek 2011). Ports are also often hot spots of species introductions (Pollumaee et al. 2006) and, in particular, canals for seagoing vessels are important gateways for species introductions and genetic exchange (Minchin et al. 2006). The Kiel Canal is one of the two inland waterways that are most traveled by seagoing vessels worldwide (Gollasch and Rosenthal 2006) and it constitutes a bridge between two different ecozones - the brackish and non-tidal Baltic Sea in the East and the tidal and fully marine North Sea in the West. This waterway of about 100 km provides a more sheltered and shorter (by 400 nautical miles) transit between the North Sea and the Baltic Sea than the alternative route through the Skagerrak.

As a brackish inland sea that only developed after the last ice age, the Baltic Sea received most of its species inventory from the North Sea and few – if any – cases so far exist of documented genetic exchange through the Kiel Canal instead of the Skagerrak. The canal must nonetheless be expected to be a corridor for species invasions into the Baltic Sea. Salinity tolerant alien species could not only be passively transported through the canal as fouling organisms on ship hulls, but also be actively spread by colonizing the canal itself. To a water depth of approximately 2 m, both shores of the canal are stabilized by packing of natural stones, which provides a substratum for algae and other sessile organisms.

As a habitat the Kiel Canal is characterized by a salinity gradient. The waterway is closed at both ends by sluice gates at Kiel-Holtenau (Baltic Sea) and Brunsbüttel (Elbe river estuary, which meets the North Sea) and, due to numerous inflows of surface and ground water into the canal and because this water is mainly released into the North Sea, salinity in the canal drops from approximately 16 at Kiel-Holtenau to about 1-3 at Brunsbüttel (Gollasch and Rosenthal 2006). Thus, the Kiel Canal offers approximately the same range of salinities as the whole Baltic Sea from the Kiel Bight to its innermost parts in the Gulf of Finland and the Bothnian Sea (Rönnbäck et al. 2007) - a circumstance that could increase the canal’s potential as a stepping stone for species introductions into the inner Baltic Sea. Nevertheless, potential invaders have to be able to manage and tolerate the strong salinity fluctuations present, at least until they are able to settle in their preferred range.

Only few studies so far have recorded algae within the Kiel Canal (Brandt 1896, Hinkelmann and Schulz 1899, Arndt 1931/32, Aleem and Schulz 1952). After building operations finished in 1895, the first algae to be observed were representatives of the former genus *Ulva* and several weeks later the former genus *Enteromorpha* was also established (Brandt 1896). Perennial algae like *Fucus vesiculosus* were first observed in 1899 (Hinkelmann and Schulz 1899). Approximately 15 years after the first enlargement of the canal in 1914, Arndt (1931/32) also observed *Fucus vesiculosus* Linnaeus at different locations in the eastern section of the canal, but dense populations were only present near the sluice gates in Kiel-Holtenau. Some decades later, Aleem and Schulz (1952) found no *Fucus*, as did Schütz (1963), who observed mostly species formerly placed in the genus *Enteromorpha* throughout the canal, in addition to some other brown and red algal taxa that became less frequent with decreasing salinity. Since the second enlargement of the canal in 1965, there have been no documented observations of its algal flora.

In the present study we aimed to detect algal bioinvaders within the Kiel Canal and to update the species inventory. We focused on dominant taxa and, in particular, on representatives of the orders Ulvales and Fucales. Members of the genus *Ulva* - that nowadays combines the former genera *Enteromorpha* (tubular species) and *Ulva* (sheet-like species) - can be found throughout the canal with many different morphologies, which could hint at the presence of cryptic introduced species. *Ulva* occurs in a wide range of environments worldwide and many of its members are characterized by an opportunistic life history strategy that may facilitate their introduction into new ecosystems (Baamonde et al. 2007, Mineur et al. 2008, Wolf et al. 2012). At the same time, the taxonomy of *Ulva* and related species is notoriously difficult. An identification solely based on morphological traits often leads to the misinterpretation of different *Ulva* morphotypes as different species (Tan et al. 1999, Hayden et al. 2003). Aberrant morphotypes of certain *Ulva* species were commonly recorded in eutrophicated habitats or in environments with strongly fluctuating abiotic factors like salinity (Dangeard 1957, Burrows 1959, Reed and Russell 1978, Blomster et al. 2002). A remarkable example is *Ulva intestinalis* Linnaeus. It characteristically exhibits unbranched, tubular thalli (Linnaeus 1753) but monostromatic thalli of this species were found in highly eutrophicated waterbodies (Blomster et al. 2002) and so called ´´bottle-brush´´ morphotypes of *Ulva intestinalis* have been observed under controlled laboratory conditions in relation to salinity change and also in media with extreme salinities (Reed and Russell 1978). Under natural conditions, branched, proliferous specimens of *Ulva intestinalis* have been predominantly recorded in brackish habitats, such as the Baltic Sea (Bliding 1963) but, due to the overlap in morphology with other species, the exact identification and analysis of the morphotype distribution in different environments was largely hampered, as it required genetic information (Leskinen et al. 2004). Similar morphological variability can also be observed in *Ulva linza* Linnaeus, which comprises sheet-like unbranched thalli, as well as variously branched tubular morphologies that were formerly described as distinct species (e.g., *Enteromorpha ahlneriana* Bliding, Brodie et al. 2007). However, the spatial distribution patterns of morphologies concomitant with genetic species validation have rarely been investigated along natural gradients of salinity or other environmental factors and the above described findings were based on single observations.

Using a combined approach of genetic identification via DNA barcoding and classical morphological identification, we were able to assess the taxonomic and morphological diversity of Ulvales within the Kiel Canal. We could also link the distribution of different morphological forms to salinity and nutrient gradients that are present in the canal. Moreover, we analyzed the current distribution of native and non-native *Fucus* species.

Material and methods

Field observations and sample preparation. In total, 16 sites, mostly separated by no more than 10 km, were visited to obtain samples of Ulvales in all parts of the canal (Figure 1). Sampling took place in May 2016 and in winter 2017-2018. Specimens representing all morphologies found were collected at each sampling site and preserved in a freezer. For species identification, representative epiphyte-free small thalli or thallus sections of 1 x 1 cm were dried in silica gel for subsequent DNA extraction. After DNA barcoding, ten specimens of each species from each sampling site were defrosted and examined for their branching pattern and thallus height. Examination of branching was carried out carefully to discriminate between true branches and possible epiphytism. During the May sampling, salinity was measured at each site. In February 2018 – when algal and plant biomass in the canal was at its minimum – 20-ml water samples were collected in duplicate at all sites, including three control sites outside the canal (two in the Kiel Fjord and one in the Elbe estuary) for an estimation of the eutrophication status. Water samples were filtered through cellulose acetate filters with a pore size of 0.2 µm and immediately preserved at -20°C. Dissolved inorganic nitrogen (DIN [$NH_{4,}$ $NO_{2,}$ NO_3]) and phosphate (PO_4), were measured, using an automated wet chemistry analyzer (San^{++}, Skalar Analytical B.V., Breda/Netherlands). For full collection data for all sites see supplementary Table S1. The distribution of *Fucus* species along the canal was repeatedly monitored at different seasons between 2016 and 2018, in order to observe reproductive structures and the development of other morphological features that are relevant for species identification (Schueller and Peters 1994).

Molecular biology. DNA was extracted using the Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany), following the manufacturer´s protocol. For DNA barcoding the plastid-encoded elongation factor tufA was amplified by PCR, using the primers *tuf*GF4 (Saunders and Kucera 2010) and tufAR (Famà et al. 2002) and the following temperature profile: an initial denaturation step (94°C, 4 min), followed by 38 cycles of denaturation (94°C, 1 min), annealing (55°C, 30 s), and extension (72°C, 1 min), with a final extension phase of 7 min at 72°C. Composition and concentration of the single ingredients of the Master Mix were according to the manufacturer's protocol for Phusion High-Fidelity DNA polymerase (Thermo Fosher Scientific, Massachusetts, USA). Subsequent Sanger sequencing was provided by GATC biotech (Konstanz, Germany). Merging of forward and reverse sequences and further editing was done in *S*equencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, MI). Obtained sequences were stored in GenBank (accession numbers: MG797644-MG797657). Reference sequences retrieved from GenBank were included in this alignment and two sequences of *Urospora* species were chosen as an outgroup (HQ610440 and HQ610441). For phylogenetic analysis of the dataset a maximum likelihood approach was chosen. The optimal substitution model was calculated with the MrModeltest version 2.2. software (Nylander 2004) and was found to be GTR+G+I. Maximum *likelihood* analyses were carried out *in* RAxML version 8 (Stamatakis 2014) and the calculated best fit substitution model was applied with 1000 bootstrap iterations. On the basis of the genetic data, species affiliations and their respective distribution within Kiel Canal were determined.

Regression analysis. To determine the potential effect of environmental variables (salinity, dissolved inorganic nitrogen and phosphate) and their interactions on the abundance of branching and thallus height in *Ulva* species, regression models were stepwise developed, using the STEP procedure implemented in the R software package (R Development Core Team 2014). Briefly, this procedure successively eliminates interactions and variables from an original full model and uses the Akaike Information Criterion (AIC) to identify the least complex submodel that still has comparable explanatory power as the full model.

Results

At 16 sites within and 3 additional control sites outside the Kiel Canal (Figure 1A-C), measurements of salinity revealed a successive decrease from 15.8 outside the sluice gates of Kiel-Holtenau to 1.2 outside the sluice gates of Brunsbüttel. In contrast, DIN and phosphate tended to increase from East to West (Figure 1 B, Table S1). With the exception of one site (15), concentrations of DIN were between those observed at the control sites in the Baltic Sea and the Elbe river estuary. Already at the sluice gate in Kiel-Holtenau, the concentration of DIN was almost twice as high (124.41 µmol l^{-1}) as at the adjacent control site in the Kiel fjord (65.76 µmol l^{-1}). A similar increase from the fjord (0.84 µmol l^{-1}) to the canal (1.22 µmol l^{-1}) was also observed for phosphate, which reached its maximum at some inland sites in the Western section of the canal.

As indicated in Figure 1 C, the largest algal species diversity was found in the eastern part of the Kiel Canal, where it borders the Baltic Sea. Only *U. intestinalis* was present

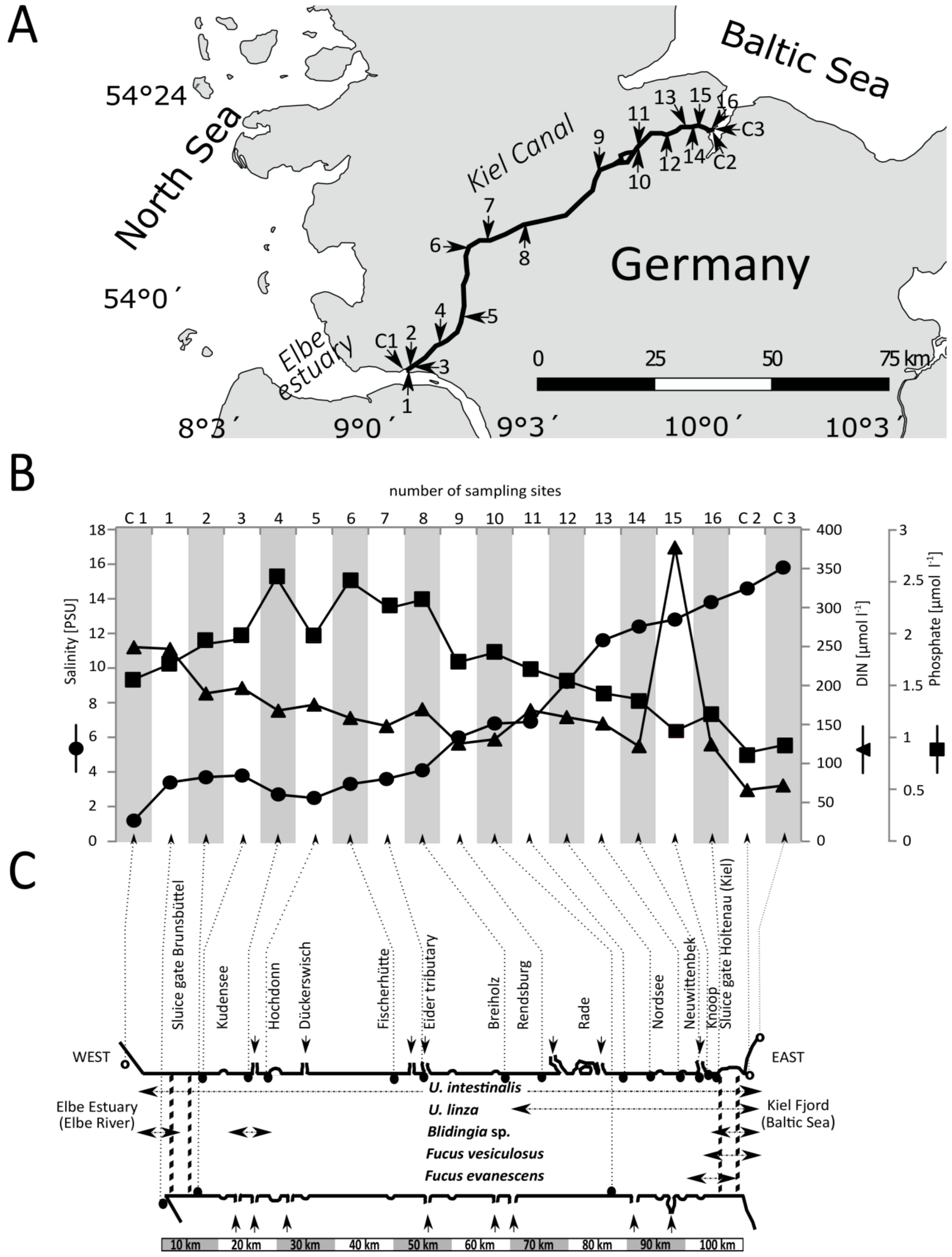

Figure 1: Map of the study area and distribution of chemical parameters and detected species. **A** Map of the Kiel Canal (black line) in Northern Germany with location of sampling sites (arrowheads). **B** Salinity, dissolved inorganic nitrogen (DIN) and phosphate at the sampling sites 1-16 and the reference sites C1-C3 outside the canal, where only water parameters were measured. **C** Spatial distribution of Ulvales and *Fucus* species within the Kiel Canal. Arrows indicate major inflows of freshwater, names refer to larger towns or regions to facilitate orientation.

throughout the whole length of the canal. *Ulva linza* was restricted in its distribution to sites with a minimum salinity of 6 and was not found below this threshold. In addition, a *Blidingia* species was found at three locations (sites 1, 4, 16) through the length of the canal and no distinct distribution pattern could be observed. All three of these species were found attached and never drifting. Both *Ulva* species grew in the upper sublittoral and lower supralittoral zone, directly below and at the water surface. In contrast, the *Blidingia* species was restricted to the upper supralittoral, where it was only reached by waves generated by vessels. Two different *Fucus* species were detected in the canal. As in many other sites at the Baltic Sea, *F. vesiculosus* is a main component of the macrophytobenthos communities in the Kiel Fjord and it also forms dense stands directly outside of the sluice gates at Kiel-Holtenau. However, we found no evidence

that *F. vesiculosus* penetrates more than 500-800 m into the canal. The distribution of *F. evanescens* C. Agardh, in contrast, extends about 8 km from Kiel-Holtenau until the salinity drops below 12.5 around Neuwittenbek (Figure 1 B-C). Interestingly, *F. evanescens* was not present directly outside the sluice gates and its closest known stand in the Kiel Fjord is at a distance of 2.9 km.

All of the examined samples of *Ulva sensu lato* were processed genetically to ensure exact species identification and to test the genetic separation of morphologically distinct specimens. The partial alignment of the *tuf*A sequences comprised 777 base pairs. The maximum likelihood tree obtained from the analysis of representative specimens (Figure 2) supports the genus *Ulva* as a monophyletic clade and the genus *Blidingia* as a sister group to it. Whereas the two *Ulva* species could be resolved with sequence references from GenBank in the phylogenetic analysis and showed the highest similarities with *U. linza* and *U. intestinalis,* the sequences of *Blidingia* fell unequivocally in a well-supported cluster that did not match with any sequences that have been published. Within the phylogenetic analysis, both clades of *U. intestinalis* and *U. linza* contain specimens of branched and unbranched morphology. However, these morphological differences are not reflected in a genetic separation and the monophyletic origin of both clades receives support from high bootstrap support values (Figure 2). Interestingly, our phylogenetic analysis revealed two subclades of *U. linza.* One of these included all samples from the Kiel Canal and a sequence from Tasmania

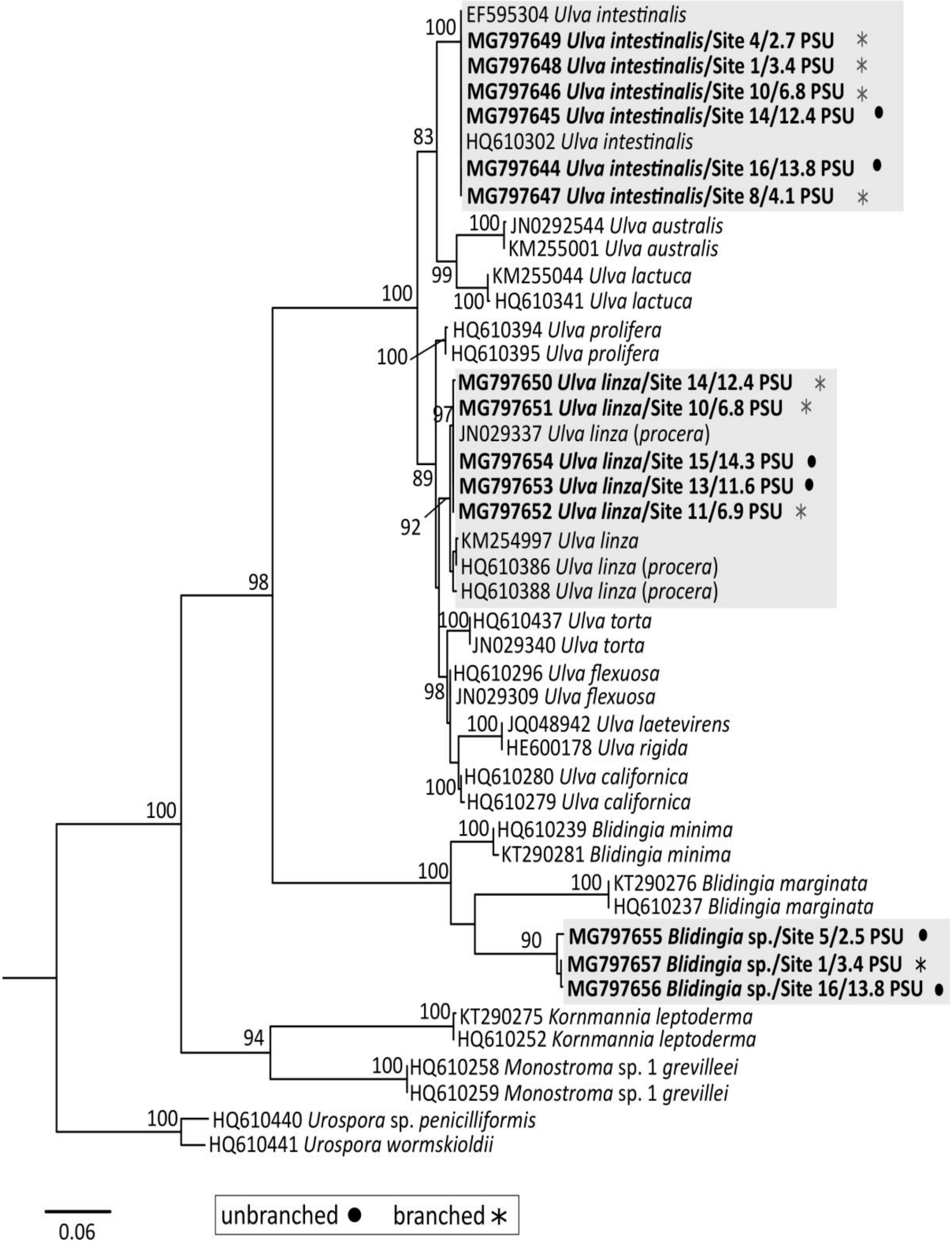

Figure 2: Maximum likelihood tree inferred from *tuf*A sequences, representing Ulvales species and their respective morphotypes, present in the Kiel Canal. Numbers at nodes refer to bootstrap values > 70. Branch lengths are drawn proportionally to the amount of sequence change and GenBank accession numbers are given for all included samples. Clades containing specimens investigated within this study are highlighted in grey. Sample sites and their recorded salinity within the Kiel Canal are indicated. Samples marked with a solid circle are of unbranched morphology, those labeled with an asterisk are branched.

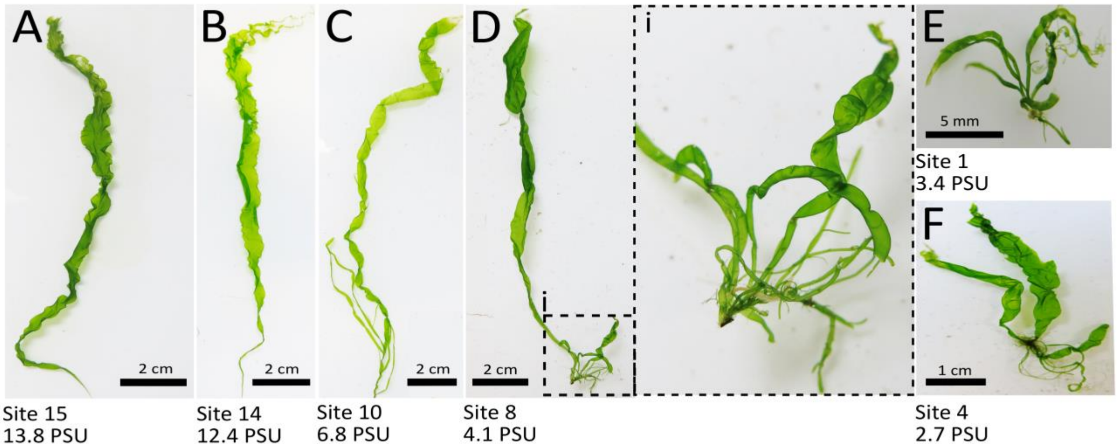

Figure 3: Morphology of material genetically identified as *Ulva intestinalis* collected from the Kiel Canal. Sampling sites with salinity recorded during collection are indicated. **A-B** display the typical unbranched morphotype of *U. intestinalis*, whereas some specimens only exhibited branches at the thallus base (**C-D**); **i** is a close-up of thallus base of D. **E-F** display branched forms of *U. intestinalis* with reduced thallus size encountered at low salinity sampling sites.

(JN029337), while the other comprised samples from different locations in the North Atlantic (KM254997, HQ610386, HQ610388).

Specimens of *U. intestinalis* growing in the eastern part of the Kiel Canal clearly corresponded with the typical unbranched form of *U. intestinalis* (Figure 3 A-B). In very few specimens branching was observed within the rhizoidal zone or at the very base of a thallus. From East to West, the occurrence of branched specimens gradually increased (Figure 3 C-D). Below a salinity threshold of 4.1 branched specimens of *U. intestinalis* were dominantly found whereas unbranched specimens were rare (Figure 3 D-F). Thalli of this branched morphotype were commonly of compact growth and their maximal thallus length was between 0.5 and 4 cm. In contrast, specimens found in higher salinities exhibited thallus lengths of 8 to 19 cm. Altogether, the gradual increase in branching of U. intestinalis correlated with a decrease in thallus size, and the number of highly branched specimens with reduced thallus size visibly increased from Holtenau to Brunsbüttel (Figure 4 A).

Highly branched forms of *U. linza* were also observed (Figure 5 A, C). However, even though the unbranched phenotype prevailed, the branched phenotype was also observed at all sites where *U. linza* was present (Figure 5 B, D). Correspondingly, no clear correlation of branching and thallus size or a gradual change in the distribution of morphotypes along the canal was observed for this species (Figure 4 B).

Table 1: Details of stepwise regression analyses used to model the number of branches and overall thallus height of *Ulva intestinalis* and *U. linza* specimens collected in the Kiel Canal. In each model all measured predictors (salinity, phosphate, dissolved inorganic nitrogen [DIN]) and their combinations were included and the model with the lowest Akaike Information Criterion (AIC) after stepwise reduction was selected. Adjusted r² and p-value of the selected model and p-values of factors and interactions included in the selected model are given. Significant values are indicated in grey.

	Effect	**Adjusted r^2**	**p**	**Salinity**	**PO_4**	**DIN**	**Salinity : PO_4**	**Salinity : DIN**	**PO_4 : DIN**
U. intestinalis	Branch number	0.2613	< 0.0001	< 0.0001	-	-	-	-	-
	Thallus height	0.4354	< 0.0001	0.46363	0.27128	-	0.00198	-	-
U. linza	Branch number	0.04257	0.189						
	Thallus height	0.2914	< 0.0004	0.04127	0.34603	0.00517	0.17324	-	0.00132

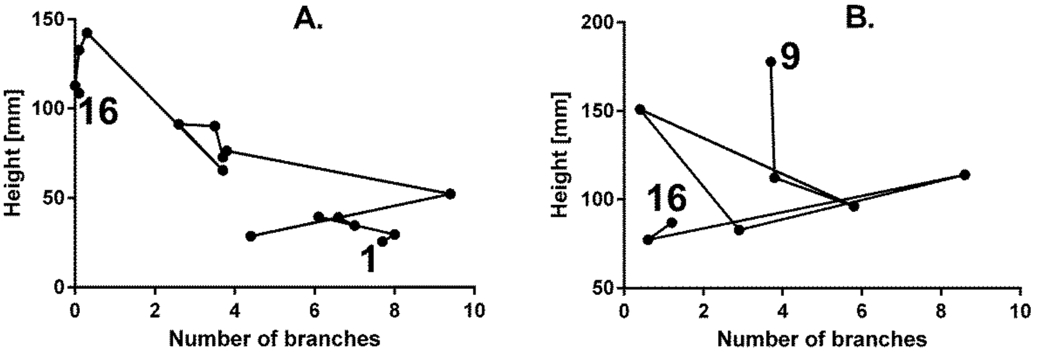

Figure 4: Number of branches versus thallus height for *Ulva intestinalis* (A) and *Ulva linza* (B) collected in the Kiel Canal. Numbers indicate sampling sites and lines connect data in the sequence of sites along the canal (sites 1 to 16 for *U. intestinalis* and 9 to 16 for *U. linza*; compare Figure 1C).

Stepwise regression model selection confirmed the impact of salinity on the morphology of *U. intestinalis.* The selected model for the number of branches of *U. intestinalis* retained only salinity as the best predictor of the observed variability, while concentrations of environmental DIN and phosphate were not selected (Table 1). Also the best fitting model for prediction of the overall thallus height of *U. intestinalis* contained salinity, as well as phosphate concentration and the interaction of salinity and phosphate concentration. Only the interaction of both variables had significant influences on the thallus height. Particularly short thalli were observed at sites with relatively low salinity in combination with relatively high phosphate concentration. Nutrient supply was also identified as a relevant predictor of thallus height in *U. linza.* The best explanatory model selected salinity, DIN concentration and the interaction of phosphate concentration and DIN concentration as significant predictors (Table 1). Particularly long thalli were observed when phosphate concentrations were relatively high, but DIN concentrations were low. In addition, thallus length of *U. linza* was significantly suppressed by low salinity. In contrast, no significant regression model was detected that could predict the number of branches in *U. linza.* Due to the scattered appearance of *Blidingia* sp. at only 3 sampling sites, its morphological variability could not be analysed further.

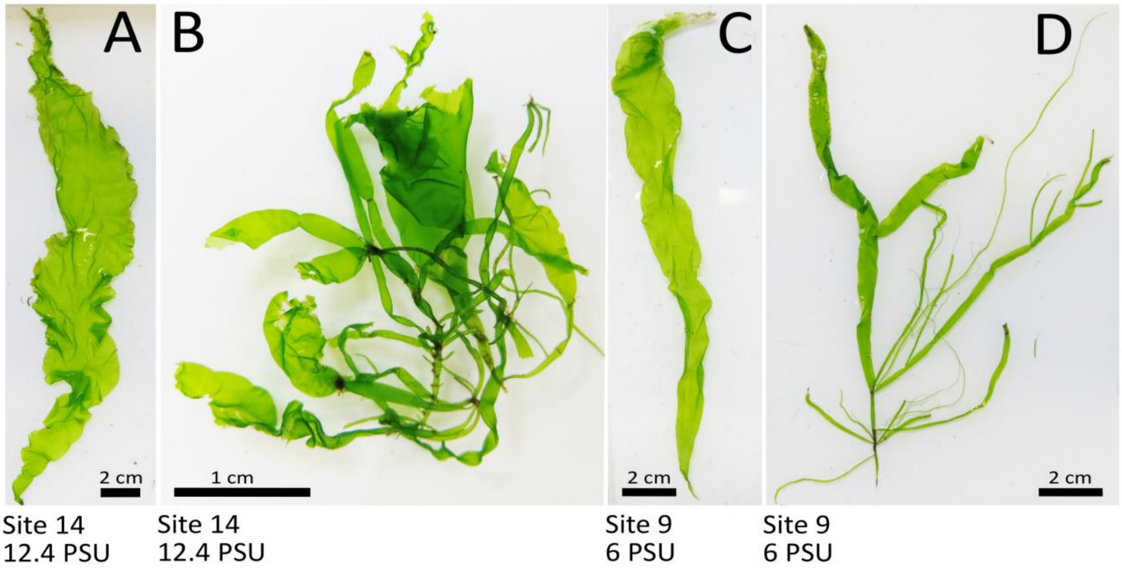

Figure 5: Morphology of material genetically identified as *Ulva linza* collected from the Kiel Canal. Sampling sites with salinity recorded during collection are indicated. Branched and unbranched morphotypes of *U. linza* observed at two sampling sites with relatively high (**A-B**) and low (**C-D**) salinity are shown.

Discussion

Altogether, our expectation of a relatively large number of cryptic Ulvales within the Kiel Canal was not confirmed. In comparison to surrounding ecosystems like the German Wadden Sea and the Baltic Sea (Bartsch and Kuhlenkamp 2000, Schories et al. 2009), the diversity of Ulvales inside the Kiel Canal is rather small. Based on barcoding of the tufA gene region, only three species were delineated. Most of the samples examined could be unambiguously identified as U. intestinalis and *U. linza*, while the third species appears within the *Blidingia* cluster of our phylogenetic analysis and does not match any existing GenBank entries. The phylogenetic analysis separates this clade with bootstrap support values of 100 from *B. minima* (Nägli ex Kützing) Kylin and B. marginata (J. Agardh) P.J.L. Dangeard ex Bliding, the only two species of the genus that were so far recorded in the Baltic Sea (Nielsen et al. 1995). Two other *Blidingia* species have been reported in the North Sea (Bartsch and Kuhlenkamp 2000, Schories et al. 2009). *Blidingia chadefaudii* (Feldmann) Bliding was observed on many European Atlantic coasts and also on the German North Sea island Helgoland, whereas *B. subsalsa* (Kjellman) Kornmann et Sahling ex Scagel was reported from Helgoland and the North Frisian Wadden Sea (Bartsch and Kuhlenkamp 2000, Schories et al. 2009). For both species no genetic reference data are available and an unambiguous identification based upon morphological criteria alone seems problematic. A thickened inner wall that is present in transverse sections has been suggested as a morphological identification criterion for *B. chadefaudii*, but has not been observed in North Sea populations from Helgoland (Kornmann and Sahling 1978). *Blidingia subsalsa* was distinguished from other *Blidingia* species in that it is branched (Burrows 1991) but, of the specimens observed by us in the Kiel Canal, some were branched and most were unbranched. Available descriptions of *B. chadefaudii* and *B. subsalsa* are for material collected in fully marine environments. However, our samples were detected at low (14 to 16) or very low (3 to 4) salinities, which could cause divergent morphologies, as with *U. intestinalis* in the present study. Further, the capacity of the newly discovered *Blidingia* sp. to grow at low salinities should allow it to spread to most parts of the inner Baltic Sea and currently confusion with *B. minima* - which was reported from the Gulf of Finland and the southern Bothnian Sea - cannot be excluded. To achieve nomenclatural certainty, lifecycle observations and genetic comparisons of a larger sample set need to be conducted, but this was not the objective of our study.

Considerable morphological plasticity was observed in both *Ulva* species that are present in the Kiel Canal. However, as did Aleem and Schulz (1952) and Schütz (1963), we only found tubular representatives and no blade-shaped thalli like those observed by Brandt (1896) shortly after the construction of the canal. This variability appeared to be affected by salinity especially *in U. intestinalis*. A certain plasticity of *U. intestinalis* has been observed before and the impact of salinity on the morphology of this species has been discussed (Bliding 1963, Reed and Russell 1978, Blomster et al. 1998). Reed and Russell (1978) observed that the typical unbranched morphology of *U. intestinalis* (Linnaeus 1753) developed from cut thallus pieces even at salinities below 8. The authors therefore suggested that extreme changes of salinity, rather than a specific salinity, induce branching in *U. intestinalis*. In contrast, our study of thalli that developed under natural conditions confirms that highly branched specimens of *U. intestinalis* dominate at low salinities. We observed a salinity gradient within the Kiel Canal that corresponds very well with previous observations (Gollasch and Rosenthal 2006), which strongly suggests that salinities at different sampling sites must be relatively stable. A gradual increase of branching was observed with decreasing salinity and microscopic observation confirmed that the branches were true and not a result of epiphytism of multiple individuals upon each other (data not shown). Branched individuals largely dominated at salinities below 4.1. This morphological variation was not reflected by genetic variation within the *tuf*A marker gene. These findings agree with previous studies that compared the ITS barcode marker of specimens of different morphologies (De Silva and Burrows 1973, Blomster et al. 1998). Strong morphological plasticity was also observed in *U. linza*. However, our observations do not support an effect of salinity or the overall eutrophication status of the environment on branching patterns in this species.

In contrast, the maximal thallus length of both *Ulva* species was affected interactively by salinity and the eutrophication status. Because the Kiel Canal is an artificial environment, its eutrophication status is not regularly monitored and long-term data are lacking. However, our measurements of phosphate and DIN in winter are similar to winter conditions in the surrounding lakes, which are also highly eutrophicated (LLUR 2014). Likewise, our finding of lower nutrient loads in the Kiel Fjord and even higher nutrient loads in the Elbe estuary corresponds with long-term measurements (LLUR 2014). These suggest that gradients of phosphate and nitrogen availability exist within the canal (Figure 1) that probably result from the fact that nutrient-dense freshwater input is mostly discharged in a westerly direction (Gollasch and Rosenthal 2006). Although salinity affected the thallus height in both *U. linza* and *U. intestinalis*, nutrient concentrations had a comparable or even stronger impact (Table 1). Given the relatively limited database, the impact of single nutrients should be interpreted with caution. However, we suggest that salinity alone can predict branching patterns in *U. intestinalis*, while indicators of the eutrophication status improve the prediction of thallus size in both species considerably.

Notably, all specimens genetically identified as *U. linza* were more closely related with a sequence which originates from Tasmania (JN029337) than with other samples collected in the North Atlantic, for example in New Brunswick, Canada. This could indicate that the specimens found of *U. linza* represent an invasive genotype, though it remains unclear where its region of origin is.

We observed *Fucus* in the eastern parts of the canal at salinities of 12.5 or more. These observations were in agreement with the previous studies of Arndt (1931/32) and

Hinkelmann and Schulz (1899), but contrast with the findings of Aleem and Schulz (1952) and Schütz (1963). However, nowadays only the introduced species *F. evanescens* occurs abundantly within the canal, while dense stands of the native *F. vesiculosus* dominate directly outside the sluice gates at Kiel-Holtenau. Under controlled conditions the reproduction of *F. evanescens* fails at salinities lower than 10 (Wikström et al. 2002), which approximately corresponds with the distribution boundary of the species in the canal that was observed within this study. In contrast, *F. vesiculosus* is generally able to settle at salinities as low as 4 (Rönnbäck et al. 2007). For this reason, its absence from the canal cannot be explained by unsuitable salinity. Interestingly, Bokn et al. (1992) observed a decline of native *Fucus* species after the severe eutrophication of the inner Oslo Fjord in the 1970s, while *F. evanescens* flourished after this increased nutrient supply. Reduction of nutrient inputs resulted in a reversal of these developments in the late 1980s, i.e. a recovery of native *Fucus* species and a decline of *F. evanescens* occurred (Bokn et al. 1992). The rapid spread of *F. evanescens* within Kiel Bight after its introduction was also assumed to be facilitated by the decline of *F. vesiculosus* and *F. serratus* in earlier years (Schueller and Peters 1994), which was primarily due to eutrophication (Vogt and Schramm 1991). Hence, the evident decline and the final absence of *F.* vesiculosus from the canal during the middle of the 20th century (Aleem and Schulz 1952) possibly resulted from the onset of eutrophication in the Kiel Canal, which is still at a much higher level than in most coastal environments of the Baltic Sea.

In conclusion, the Kiel Canal currently appears to be a suitable habitat for *Fucus evanescens* and it harbours an unidentified species of Blidingia that may spread into the inner Baltic Sea, due to its capacity for growth at low salinities. The canal also harbours a genotype of *Uva linza* that was so far only detected in Tasmania, which could suggest a certain invasion potential. Nonetheless, the current abundance of non-native seaweeds in the canal appears to be moderate. Species such as *Gracilaria vermiculophylla* (Ohmi) Papenfuss and *Dasya baillouviana* (S.G. Gmelin) Montagne, which were recently introduced into the SW Baltic and are tolerant of low salinities, have not been detected in the canal so far (G. Bock, pers. comm.). Moreover, the high variability of *Ulva* morphologies in the canal is not due to a presence of multiple and possibly introduced cryptic species, but results from an adaptation of only two species to different salinity and nutrient regimes. Because gross morphological characters like branching are only partly diagnostic for certain *Ulva* species, and environmental factors may influence morphology, exact species identification within the genus based upon morphological patterns is still difficult. Obviously, the identification method of choice, especially in environments with variable salinity, should be a combined approach of molecular and morphological identification criteria.

Acknowledgements

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Supplement

Table S 1: Measured values of salinity (May 2016), mean SiO_4, mean PO_4, Mean DIN (February 2018) for all sampling sites in the Kiel Canal (1-16) and reference sites outside the canal (17-19) examined in this study

Sampling site	**Salinity [PSU]**	**geographic coordinates [N]**	**geographic coordinates [E]**	**Mean SiO_4 [µmol l^{-1}]**	**Mean PO_4 [µmol l^{-1}]**	**Mean DIN [µmol l^{-1}]**
C 1 Brunsbüttel Elbe estuary	1.2	53°53.3267	9°06.994	181.57	1.54	249.04
1 Brunsbüttel sluice gate outside	3.4	53°53.321	9°08.669	190.11	1.68	246.80
2 Brunsbüttel sluice gate inside (S)	3.7	53°53.841	9°09.394	155.78	1.89	189.58
3 Brunsbüttel sluice gate (N)	3.8	53°53.875	9°08.915	171.10	1.93	196.69
4 Weiche Kudensee	2.7	53°56.665	9°13.475	144.63	2.53	167.72
5 Weiche Dückersbusch	2.5	54°01.916	9°18.010	142.34	1.95	175.35
6 Weiche Fischerhütte	3.3	54°09.017	9°21.712	123.92	2.50	158.12
7 Weiche Olderbüttel	3.6	54°10.422	9°26.515	110.16	2.25	147.82
8 Weiche Breiholz	4.1	54°12.033	9°33.138	126.05	2.32	169.54
14 Weiche Schwartenbek	12.4	54°21.675	10°03.533	103.37	1.36	122.00
12 Weiche Nordsee	9.2	54°20.583	9°57.482	106.49	1.54	159.33
10 Weiche Rade (S)	6.8	54°20.708	9°45.666	145.06	1.81	130.71
9 WSA Rendsburg	6	54°17.874	9°41.601	142.51	1.72	125.28
11 Weiche Rade (N)	6.9	54°21.107	9°45.893	152.86	1.65	168.19
13 Warleberg	11.6	54°21.569	10°01.024	136.83	1.42	151.28
15 Knoop	12, 8	54°22.358	10°05.615	138.50	1.05	376.75
16 Holtenau	13.8	54°22.146	10°07.495	122.61	1.22	124.41
C 2 Holtenau ferry	14.6	54°22.118	10°08.0424	68.84	0.84	65.76
C 3 Holtenau sluice gate outside	15.8	54° 22.1325	10°08.98177	92.29	0.92	71.52

Chapter III (Accepted for publication at Botanica Marina [DOI: 10.1515/bot-2018-0019])

New records from the southern North Sea and first records from the Baltic Sea of *Kornmannia leptoderma*

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Abstract

Combined genetic, morphological and ontogenetic observations show that the circumarctic boreal green algal macrophyte *Kornmannia leptoderma* has expanded its distribution range into the Baltic Sea, on a German coastal section of 220 km length. The species is also again (or still) established at its former extreme southern distribution limit in the North Sea, the German island of Helgoland, where it has not been detected during the last four decades. Macroscopic visible sporophytes of *K. leptoderma* are nowadays present in the Baltic Sea and at Helgoland from February to September, while they were in the past only detected from February to May at Helgoland. This capacity for formation of sporophytes in summer correlates with the circumstance that *K. leptoderma* from the Baltic Sea can complete its life cycle at 15 °C while several studies conducted decades ago with material from Helgoland and from Pacific coasts consistently reported an inhibition of the algal gametogenesis at temperatures that exceed 12 °C. Possibly *K. leptoderma* has undergone adaptations that facilitate its spread into warmer environments, unless the *Kornmannia* present in the Baltic Sea and on Helgoland today represents a newly introduced cryptic species.

Key words: Baltic Sea, *Kornmannia*, Marine invader, Ulvales, Range expansion

Introduction

The SW Baltic Sea is an atidal brackish water environment that offers similar temperature conditions to the SE North Sea, although seasonal minimum and maximum temperatures are more extreme (Lennartz et al. 2014). Between the Danish Straits and the Darss Sill at the German island of Rügen, its mean salinity decreases over a distance of approximately 300 km from more than 20 to 8 (Meier and Kauker 2003). However, the surface salinities along this relatively steep gradient vary considerably both in time and space, due to changes in river runoff, periodic seawater inflow from the North Sea, stratification and upwelling. Many marine species reach their distribution limit within this salinity gradient, which is not only the reason for a decreased diversity (Schubert et al. 2011), but often also for significantly reduced growth (Russell 1988) and - especially in certain groups of macrophytes - for other morphological changes (Russell 1994, Ruuskanen and Kiirikki 2000).

Some green algal groups within the order Ulvales are notorious for their morphological variability. Salinity has repeatedly been reported to affect the morphology of Ulvales (Burrows 1959, Reed and Russell 1978, Sanders 1979) and members of this order exhibiting “unusual” morphologies have occasionally been reported from the Baltic Sea. For example, in Finland green tides of the usually tubular species *Ulva intestinalis* were observed that exhibited a sheet-like monostromatic morphology (Blomster et al. 2002). The variability of Ulvales often hampers their identification based upon morphological characteristics, which became apparent with the introduction of DNA barcoding techniques into the field (Hayden and Waaland 2002, Hayden et al. 2003). As a consequence, the potential for cryptic introductions and hidden extinctions of Ulvales appears relatively high.

We here report that our recently conducted revision of the species inventory of Ulvales along the German Baltic Sea coast resulted in the discovery of *Kornmannia leptoderma* (Kjellman) Bliding which was so far not known from this ecoregion. *Kornmannia leptoderma* was first found on Novaja Zemlja, and originally described as *Monostroma leptodermum* Kjellman (1877), due to its monostromatic sheet-like thallus that is composed of only one cell layer. Later studies revealed major differences among the life cycles of members of *Monostroma* (Kornmann and Sahling 1962, Tatewaki 1969) and, based on its heteromorphic life cycle with a monostromatic sporophyte and a disk-like gametophyte, *M. leptodermum* was redescribed as *Kornmannia leptoderma* (Bliding 1968). Specimens of the genus *Kornmannia* from the North Pacific (Washington) that lived primarily as epiphytes on seagrass and other macrophytes and exhibited slightly divergent morphologies were described as *K. zostericola* (Tilden) Bliding (Bliding 1968). A later comparative study concluded that the two species were indistinguishable with respect to morphology and ontogeny (Golden and Cole 1986). Since then *K. leptoderma* is regarded as the only taxon within the genus *Kornmannia*, but molecular comparisons of Atlantic and Pacific populations and type specimens are still missing.

Kornmannia leptoderma has a circumarctic-boreal distribution (Golden and Cole 1986), and in Europe its documented southern distribution limits are in Norway (Rueness et al. 2001) and at the Faroe Islands (Nielsen and Gunnarsson 2001), with the remarkable exception of the German North Sea island of Helgoland, at least 500 km to the South (Figure 1). At Helgoland *K. leptoderma* was probably observed for the first time in 1934 (Schmidt 1938) and thereafter each year from 1960 (Kornmann and Sahling 1962) to 1966 and three more times in the 1970s (Kornmann and Sahling 1983). The species disappeared after 1977 and has been considered as extinct in Germany since 1996 (Ludwig and Schnittler 1996).

Materials and methods

Sheet-like monostromatic Ulvales and Ulothrichales were collected during repeated samplings between February 2013 and September 2015 at 110 sites on the German Baltic Sea coast between Flensburg and Rerik and at the North Sea island Helgoland (Table 1). They underwent microscopic examination in the laboratory and parts were conserved for DNA barcoding. At sixteen sites material was observed that could not be clearly assigned to *Monostroma grevillei* - an abundant monostromatic species in the area – and was investigated further (Table 1, Figure 1). To observe the algal life cycle, pieces of approximately 1 cm² of the material collected at Mönkeberg were transferred into glass Petri dishes (diameter 9 cm) containing 40 ml of ¼ strength Provasoli Enriched Seawater (salinity 17). The Petri dishes were maintained for 15 months at 15 °C, with a light regime of 40 µmol photons m^{-2} s^{-1} ("cool white", 12L:12D) and replacement of the medium every 3 months. The same approach was repeated with identical cultivation conditions and material collected at Heiligenhafen Binnensee on 30th August 2017.

Genomic DNA was extracted from material dried in silica gel or from frozen fresh material, using the Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany) and following the manufacturer´s instruction protocol. DNA-barcode fragments of the plastid encoded elongation factor *Tu* (*tuf*A) were amplified by polymerase chain reaction (PCR), using the primers *tuf*GF4

Table 1: List of samples of *Kornmannia leptoderma* identified by DNA barcoding with information on sampling sites. Collector = SST: Sophie Steinhagen, FW: Florian Weinberger, DA: Dmitry Afanasyev. Site no. = Number in Figure 1. Temperature = Temperature at collection time. Salinity = Salinity at collection time. Accession no. = GenBank accession number for *tuf*A gene sequence included in Figure 3. Two lines printed in bold indicate samples used for life cycle studies.

Date	Collector	Site No.	Site name	Environment	Coordinates	Temperature [°C]	Salinity	Exposure	Accession no.
12.08.2014	SST	1	Kiekut	Beach	N 54°26.856‘ ;E 09°52.301‘	18.4	16.1	Semi-exposed	MF441477
15.04.2015	SST	2	Aschau	Lagoon	N 54°27.648‘ ;E 09°55.599‘	10.3	14.2	Protected	MG944397
13.08.2014	SST	3	Strande	Harbor	N 54°26.215‘ ;E 10°10.408‘	20.2	17.0	Protected	
18.08.2014	SST	4	Kiel-Falkenstein	Beach	N 54°24.556‘ ;E 10°11.380‘	17.4	16.1	Protected	
10.02.2013	**FW**	**5**	**Mönkeberg**	**Harbor**	**N 54°21.160' ;E 10°10.673'**	**2.4**	**17.0**	**Protected**	**MF441478**
16.04.2015	SST	6	Heiligenhafen, Binnensee	Lagoon	N 54°22.556‘ ;E 10°58.784‘	8.6	13.8	Protected	
20.07.2013	DA	7	Marina Heiligenhafen	Marina	N 54°22.708' ;E 10°58.846'	22.8	11.5	Protected	
22.08.2014	SST	8	Heiligenhafen, Binnensee	Marina	N 54°22.767‘ ;E 10°58.943‘	16.9	14.0	Protected	MG944400
25.08.2014	SST	8	Heiligenhafen, Binnensee	Marina	N 54°22.767‘ ;E 10°58.943‘	16.9	14.0	Protected	
30.08.2017	**SST**	**8**	**Heiligenhafen, Binnensee**	Marina	**N 54°22.767‘ ;E 10°58.943‘**	**17.4**	**18.1**	**Protected**	

22.08.2014	SST	9	Heiligenhafen, Graswarder	Lagoon	N 54°22.548‘ ;E 10°59.245‘	16.8	16.9	Protected	
02.09.2014	SST	9	Heiligenhafen, Graswarder	Lagoon	N 54°22.548‘ ;E 10°59.245‘	16.8	16.9	Protected	
17.09.2014	SST	19	Großenbroder Fähre	Lagoon	N 54°23.582‘ ;E 11°06.612‘	15.1	16.1	Protected	
27.09.2014	SST	11	Wulfen	Lagoon	N 54°24.535‘ ;E 11°10.388‘	20.1	15.3	Semi-exposed	
27.09.2014	SST	12	Marina Großenbrode	Marina	N 54°21.397‘ ;E 11°03.661‘	18.3	16.6	Protected	
08.04.2015	SST	13	Brodtener Ufer	Beach	N 53°59.470‘ ;E 10°49.937‘	9.6	13.8	Exposed	
18.07.2013	FW	14	Hohen-Wieschendorf	Beach	N 53°56.807' ;E 11°20.760'	21.3	11.0	Semi-exposed	MF441479
19.07.2013	DA	15	Redentin	Beach	N 53°55.918' ;E 11°28.787'	22.7	13.0	Protected	
19.07.2013	DA	16	Gollwitz	Beach	N 54°01.383' ;E 11°29.109'	20.7	10.0	Semi-Exposed	
23.04.2015	SST	17	Helgoland, Südstrand	Beach	N 54°10.780‘ ;E 07°53.375‘	8.0	32.5	Semi-Exposed	MG944398
23.04.2015	SST	18	Helgoland, Binnenhafen	Harbor	N 54°10.682‘ ;E 07°53.323‘	8.0	32.5	Protected	MG944399
23.09.2015	SST	18	Helgoland, Binnenhafen	Harbor	N 54°10.682‘;E 07°53.323‘	14.3	32.0	Protected	

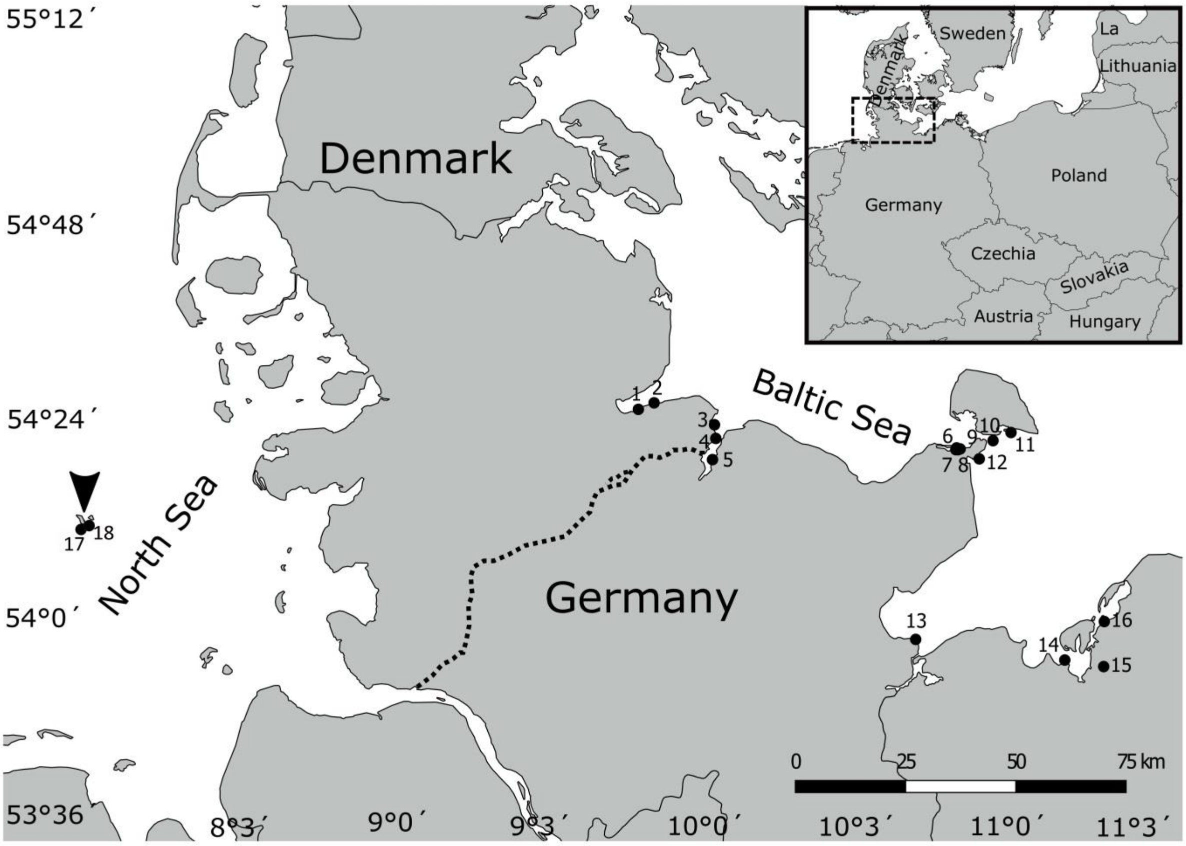

Figure 1: Sites in Northern Germany where *Kornmannia leptoderma* has been collected. Numbers 1 to 16 indicate the location of collection sites along the Baltic Sea shore that were visited since 2013 in the present study, as listed in Table 1. Arrow indicates location of Helgoland in the North Sea, where additional samples were obtained at two sites in close proximity. Dotted line represents the Kiel Canal.

(Saunders and Kucera 2010) and *tuf*AR (Famà et al. 2002). For amplification the following temperature profile was used: initial denaturation 4 min at 94 °C, 38 cycles of 94 °C for 1 min, 55 °C for 30 s, 72 °C for 1 min, final extension for 7 min at 72 °C. Sequencing in both directions was provided by GATC biotech (Konstanz, Germany). Sequences were assembled, reciprocally edited with Sequencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, MI) and aligned, using the multiple sequence alignment program MAFFT v. 7.311 (Katoh and Standley 2013).

Phylogenetic analysis - We analysed the dataset using the Maximum likelihood (ML) approach. For a robust analysis we included several reference sequences downloaded from GenBank indicated by their accession numbers. Reference sequences of *Bryopsis corticulans* (accession number: HQ610243) and *Prasiola stipitata* (accession number: GWS004831) were used as outgroups. We constructed ML phylogenies with RAxML v. 8 (Stamatakis 2014). As nucleotide substitution model, GTR+GAMMA was used. To test the robustness of the tree an iteration of

1,000 pseudoreplicates was performed. DNA sequences of the *tuf*A gene of *K. leptoderma* are available from GenBank (for accession numbers see Table 1).

Results

The first specimen was discovered in late winter (February) 2013. It drifted in very sheltered and shallow water at Mönkeberg in the Kiel Fjord. Cultivation of this specimen allowed for observation of the heteromorphic life cycle of *Kornmannia*. Within three months spores were released that formed monostromatic disks of characteristic morphology (Figures 2A and 2B). During seven more months the crusts increased in size until they reached a diameter of up to 1.5 mm (Figure 2C). At the same time pseudoparenchymatous growth also increased the thickness of the disks, in particular toward their center. A release of propagules was not directly observed, but in December 2014 – ten months after the launch of the experiment – spores or gametes had been released that germinated into small filaments (Figure 2D). Some of these formed a new generation of disks, which gave rise to minute thalli with an erect tubular morphology (Figures 2C and 2D), that were closed at the tip. Within three more months these germlings increased in size to a length of approximately 5 mm before they liberated their content as spores. Only the cell wall structures remained (Figure 2E). Attached material from Heiligenhafen Binnensee that had been collected in late summer (August 2017) developed considerably faster. Large numbers of tetraflagellate swarmers were immediately released from the thallus. As previously observed with the material from Mönkeberg, germination of attached swarmers resulted in a formation of disks, which increased in size and thickness. After 5-6 months some of the primary disks formed saccate thalli (usually one, in one case two, Figure 2F and 2G) that increased in length as previously observed on secondary disks with the material from Mönkeberg. At the same time biflagellate swarmers were also released by primary disks (Figure 2H) and after attachment they germinated into a second generation of filaments (Figure 2F-H) that eventually formed secondary disks.

DNA sequencing revealed that the *tuf*A marker gene of the first parental sample from Mönkeberg had more than 99 % identity with a reference sample of *Kornmannia leptoderma* from the Canadian west coast (Saunders and Kucera 2010). Twenty additional specimens were collected until April 2015 (Table 1) at 16 different locations along a Baltic Sea coastal section approximately 220 km long and at Helgoland (Figure 1). They were all - based upon fully or

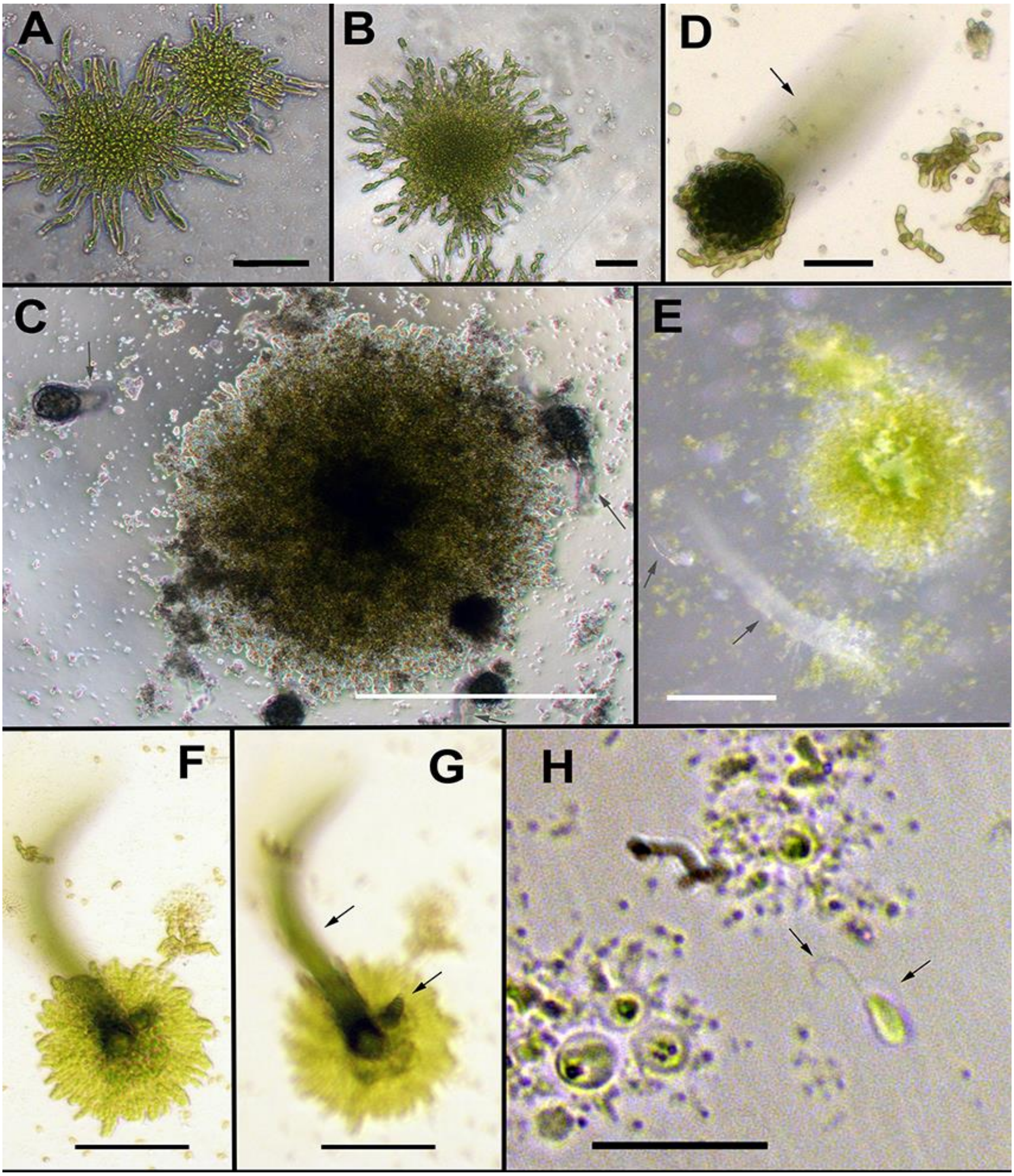

Figure 2: Life cycle stages of *Kornmannia leptoderma*, raised from material collected in Mönkeberg (A-E) and Heiligenhafen (F-H). Primary thallus disks after (A) 3 months, (B) 4 months and (C) 10 months, in (C) surrounded by five secondary thallus disks. (D) secondary basal disk bearing a young erect tubular thallus on the left and newly germinated filaments on the right (after 12 months). (E) primary disk and two dead tubular thalli (after 15 months). (F) and (G) primary basal disk bearing two erect tubular thallus branches of different size and surrounded by early filamentous stages of secondary disks (5.5 months). (H) biflagellate swarmer. Arrows indicate flagella in (H) and tubular sporophytes in other images, the latter appear often blurred because images were taken with an inverted microscope through the bottom of the culture vessel. Length of scale bars: 50 µm in (A), (B), (D), (F) and (G), 1 mm in (C) and (E), 20 µm in (H).

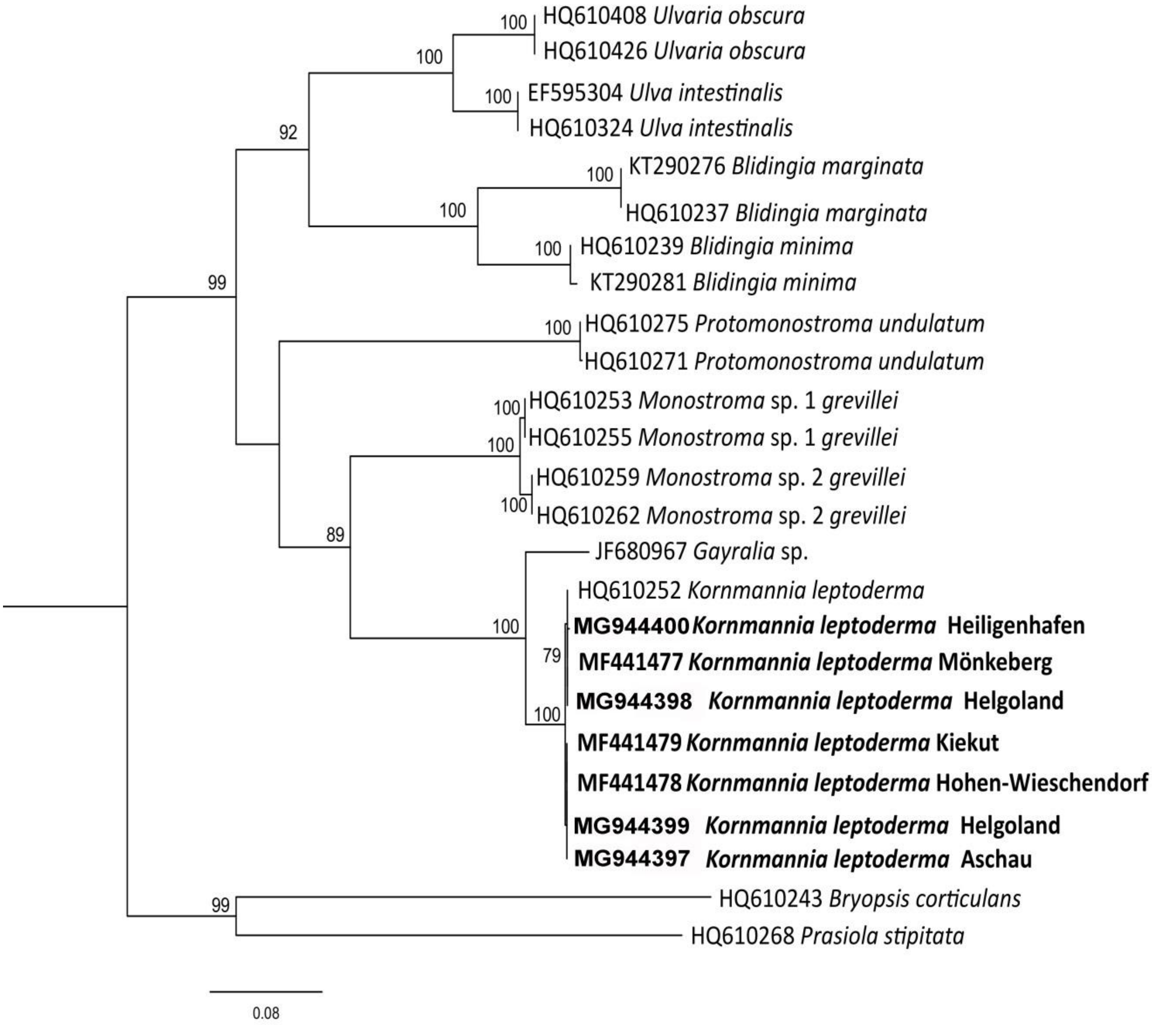

Figure 3: Phylogenetic tree of Ulvales and Ulothrichales exhibiting monostromatic morphologies. Maximum likelihood (ML) phylogenetic tree based on analysis of plastid *tuf*A gene DNA partial sequences. ML bootstrap support values ≥ 90 are shown at each node. Branch lengths are drawn proportional to the amount of sequence change. GenBank accession numbers are indicated before species names. Names of target samples from the Baltic Sea are in bold. *Prasiola stipitata* and *Bryopsis corticulans* were used as outgroups.

partially sequenced *tuf*A marker genes - closely related and most probably conspecific with the specimen collected in Mönkeberg and with the reference sample (see Figure 3 for a phylogenetic tree with selected samples). All specimens were light green monostromatic sheets with central or basal attachment, in most cases with strongly ruffled margins (Figure 4). Occasionally multiple thalli arose from the same base. Thallus lengths of 5 cm were rarely - if ever - exceeded. Cells in the basal parts were always stretched and between 1.5 and 4.5 times longer than wide. Cells in the middle parts of thalli were much less stretched. They exhibited diameters of 14-26 x 8-19 µm and they typically clustered in groups of two or four.

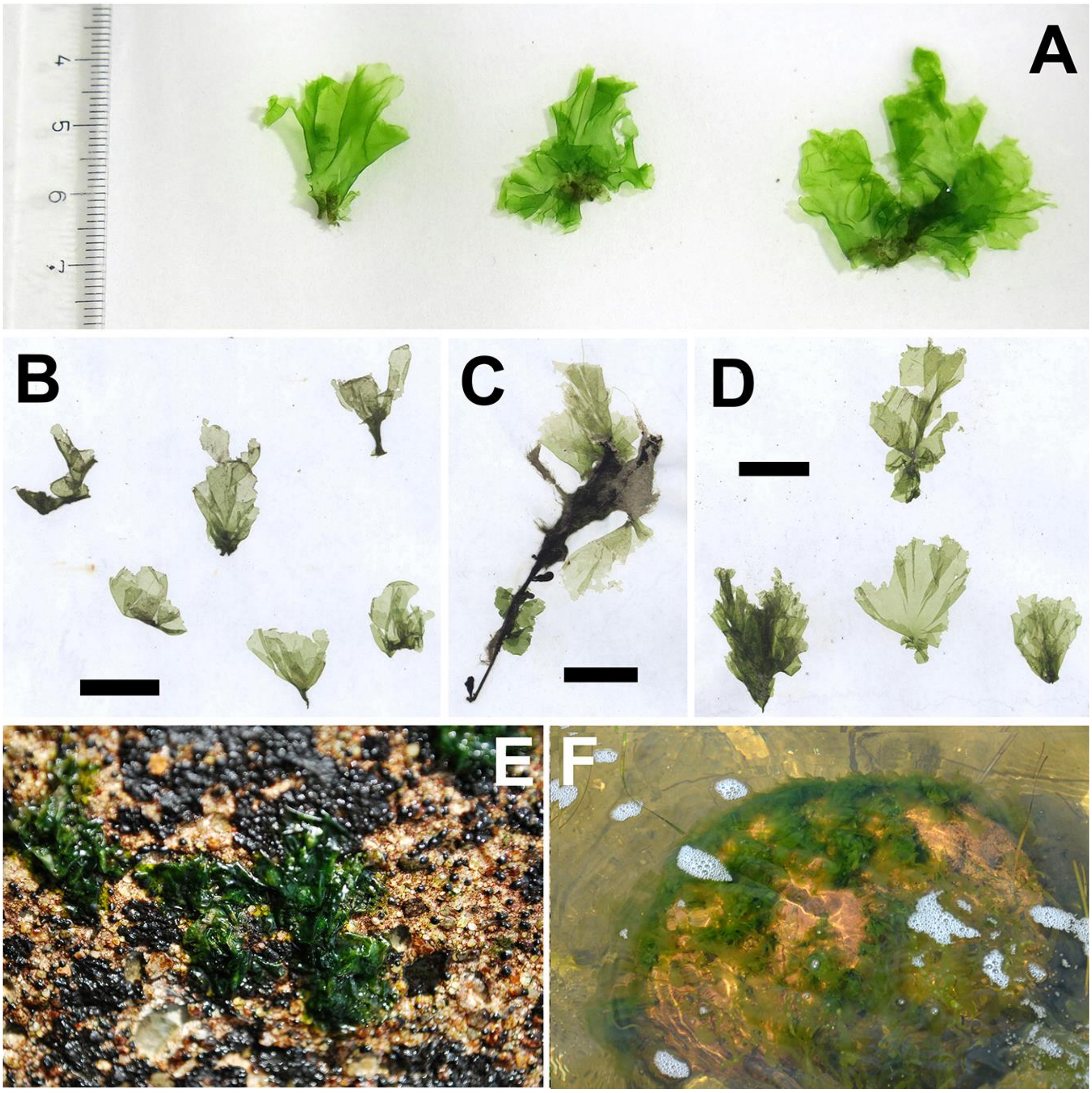

Figure 4: Sporophytes of *Kornmannia leptoderma*. (A) Living specimens from Heiligenhafen-Graswarder (22.8.2014); herbarium specimens from (B) Redentin (19.7.2013), (C) Heiligenhafen (20.7.2013, epiphytic on *Fucus vesiculosus*) and (D) Gollwitz (19.7.2013); natural assemblages at (E) Wulfen (27.9.2014) and (F) Redentin (19.7.2013). Scale bars in B-D = 2 cm.

Discussion

Our combination of genetic, ontogenetic and morphological observations allows for a relatively unambiguous identification of the examined material as *Kornmannia*. A unique character of the genus is its heteromorphic life cycle that combines a discoidal or sometimes filamentous gametophyte and an erect, first tubular, then saccate and finally monostromatic sporophyte that emerges from an initial discoidal stage (“Disk-sac ontogeny”; Golden and Cole 1986). This life cycle has been described in detail for *Kornmannia leptoderma* from Helgoland (Kornmann and

Sahling 1962) and Norway (Bliding 1968) and for *Kornmannia zostericola* from Japan (Yamada and Kanda 1941, Tatewaki 1969) and British Columbia (Golden and Cole 1986). Despite small variations, the life cycle traits of both taxa appeared similar, and this was an important argument in support of the view that the two taxa are synonyms (Golden and Cole 1986). In our study tetraflagellate spores released by monostromatic thalli from the Baltic Sea gave rise to a primary discoidal life stage that appeared morphologically identical to the discoidal gametophytes described by the authors mentioned above. We also observed a release of biflagellate swarmers from these disks, which is again in agreement with earlier observations of biflagellate gametes in *Kornmannia*. We could not directly observe a fusion of these swarmers as described by Tatewaki (1969), but such formation of zygotes probably happened, since the subsequent development of our cultures was again in accordance with earlier reports (Kornmann and Sahling 1962, Bliding 1968, Tatewaki 1969, Golden and Cole 1986): The propagules that had been released germinated into short filaments. These filaments formed secondary disks, which gave rise to tubular erect sporophytes that were closed at the apex, as previously described for *K. leptoderma* (Kornmann and Sahling 1962, Bliding 1968, Tatewaki 1969, Golden and Cole 1986). We were unable to observe the final development into monostromatic thalli because the sporophytes released zoospores and thereby emptied their cells before the necessary size had been reached. Nonetheless, the observed ontogeny – including the morphological traits of both generations – was fully in agreement with that of *K. leptoderma*. Tubular germlings were observed in culture, while monostromatic sheets were observed in nature. We did not observe the intermediate saccate morphologies in the Baltic Sea, but absence of saccate forms has also been observed elsewhere (Golden and Cole 1986). In our second experiment tubular erect sporophytes were already formed by primary disks. Such asexual reproduction of *K. leptoderma* sporophytes has also previously been reported, for example by Kornmann and Sahling (1962) with material from Helgoland and by Yamada and Kanda (1941) with material from Japan.

Further evidence for the identity of our material with *Kornmannia* comes from the DNA barcoding approach. The *tuf*A marker gene indicated that our material is genetically more close to the only reference sample of the genus *Kornmannia* that has so far been published than to any other green algal genus that forms monostromatic blades. The reference sample in question represents a specimen of *K. leptoderma* from British Columbia (Saunders and Kucera 2010). Unfortunately the authors did not mention how their specimen was recognized as *K. leptoderma*, possibly it was found in the characteristic epiphytic association with *Zostera* or *Phyllospadix* that is often observed on the North American Pacific coast and allows for a relatively reliable identification based upon morphological traits (Golden and Cole 1986). Together with our samples from the Baltic Sea and Helgoland, the reference sample of *K. leptoderma* clearly formed one distinct cluster (Figure 3). The identities between pairs of sequences within this branch were always larger than 99.2 % and often complete. For example, no base pair divergence was detected between the reference sequence from British Columbia, two samples from Helgoland and two of the sequences from the Baltic Sea (sites Aschau and Hohen-Wieschendorf). All these specimens apparently belonged not only to the same genus, but to the same species. Thus, our data confirm that the same *Kornmannia* species is present at Atlantic and Pacific coasts, as previously suggested (Golden and Cole 1986). We were unable to compare our data to DNA sequences obtained from type material, but most probably the species is *K. leptoderma*, given that the only other species described within the genus (*K. zostericola*) is considered as a synonym. An in-depth comparison of the genetic structure of different Atlantic and Pacific populations of *Kornmannia* might provide an answer to the question whether the genus harbors more than one species or not. However, this was beyond the scope of the present work. Not only our DNA barcoding data and our observations of the ontogenetic development, but also our morphological observations of field-collected sporophytes largely correspond with descriptions of *K. leptoderma* (Bliding 1968, Kornmann and Sahling 1983). We therefore conclude that *K. leptoderma* is currently present not only at Helgoland – where the species has not been observed for four decades – but also in the SW Baltic Sea, where it has not previously been recorded (Nielsen et al. 1995, Schories et al. 2009).

Interestingly, *K. leptoderma* from the Baltic Sea differs from populations from Helgoland and Hokkaido that were investigated half a century ago by its capacity for life cycle completion at a temperature of 15 °C. The optimal water temperature for life cycle completion of material from Hokkaido was approximately 5 °C and gametophytes could only become fertile at temperatures below 10-12 °C (Tatewaki 1969). Also in *K. leptoderma* from Helgoland a temperature of 15 °C inhibited the formation of sporophytes completely and caused parthenogenetic multiplication and malformations of gametophytes (Kornmann and Sahling 1962). In contrast, a continuous temperature of 15°C could not prevent the formation of sporophytes in our experiments and also no malformation of gametophytes was observed. Instead, the full life cycle was completed within 15 months and a parthenogenetic reproduction of sporophytes was observed within 5.5 months, which clearly contrasts with the above mentioned studies.

In addition to temperature, daylength often affects algal life cycles and this was also reported for *K. leptoderma*. At temperatures of 10 °C or less and a daylength of 16 h, Golden and Cole (1986) observed formation of branched filaments instead of gametophytic disks and no reproduction of sporophytes. However, a development of gametophytic disks and reproduction of sporophytes already at an early stage was observed by the same authors at a daylength of 8 h. This behavior in short day conditions was very similar to the development of *K. leptoderma* from the Baltic Sea at a daylength of 12 h. We did not test the effect of long day conditions on our material, therefore we cannot exclude that such conditions would also inhibit the formation of sporophytes in specimens from the Baltic Sea. However, we frequently detected *K. leptoderma* sporophytes in summer between July and August (Table 1), when water temperatures may easily reach 20°C (Table 1, see also Lennartz et al. 2014) and days are longer than 12 h. This is in agreement with our observations of life cycle completion at temperatures above 10 °C, but it contrasts with past observations by Kornmann and Sahling (1983), who explicitly described *K. leptoderma* from Helgoland as a “spring alga”. Also at Helgoland we recently discovered sporophytes not only in spring, but also in September, although mean sea surface temperatures of 12°C or less occur at Helgoland only from November to May (Table 1, see also Wiltshire et al. 2009). Clearly, the formation of sporophytes on Helgoland and in the SW Baltic is not restricted to spring or to the cool season.

These field observations – together with our ontogenetic observations, that were obtained under temperature controlled conditions – strongly suggest that the Baltic Sea and Helgoland have been reached by an ecotype of *K. leptoderma* that is adapted to elevated temperatures. Our attempts to isolate DNA from existing historical samples of *K. leptoderma* from Helgoland so far failed. It is for this reason currently not possible to examine the genetic similarity between recent specimens and those that were present in Germany half a century ago. Thus, we are unable to decide whether recent German populations are descendants of the Helgoland population that existed five decades ago. Possibly this population never really became extinct, adapted to warm summer conditions and expanded its distribution range into the Baltic Sea, which is at a distance of less than 200 km from Helgoland through the Kiel Canal (Figure 1).

Alternatively, all recent populations in Germany could result from introductions of more resistant individuals. For example, two different populations of *K. leptoderma* with overlapping geographical distribution have been distinguished in the NE Pacific (Golden and Cole 1986). One of these (“KZ”) had a more southerly distribution limit than the second and was not only discovered in British Columbia, but also in California. This more southern population was reportedly sometimes sexual when adjacent stands of the second population were asexual. The authors compared the ontogenetic development of both populations under controlled conditions at 5 °C and 10 °C (but not at higher temperatures) and found no important differences. The authors therefore concluded that both populations probably belong to the same species, but they nonetheless suggested that *K. leptoderma* as a species might currently undergo radiation (Golden and Cole 1986). In this light, the question obviously arises whether recent southern North Sea and Baltic Sea populations in Germany are derived from southern Pacific populations of *K. leptoderma*. Also a range expansion into the Baltic Sea of populations in Northern Europe cannot be excluded. Such a scenario would be reminiscent of the southward range expansion by *Fucus evanescens* into the Baltic Sea that happened approximately 25 years ago (Schueller and Peters 1994) and it would perhaps mirror the development of southern and northern populations of *K. leptoderma* in the Pacific that was proposed by Golden and Cole (1986). However, the genetic similarities or dissimilarities between Northern and Southern populations on Pacific and Atlantic coasts – as well as between the putative synonyms *K. leptoderma* and *K. zostericola* - have so far not been explored, and it is for this reason not possible to decide with certainty whether the *Kornmannia* populations that are present in our study area today and that were also reported from British Columbia by Saunders and Kucera (2010) represent the same species as *Kornmannia* populations that were present in our study area 40 years ago.

Interestingly, *K. leptoderma* was not found on a section of nearly 100 km between the Danish border and Kiekut (Figure 1). This observation could suggest that *K. leptoderma* did not reach the German Baltic Sea area by continuous southward migration from the Kattegat area through the Danish Belt, but rather by long distance transport, followed by a point introduction. At the same time, the apparent absence of *K. leptoderma* from the northern coastal section could indicate that its spread into the area is incomplete and perhaps relatively recent.

Conclusion

The increased performance of *K. leptoderma* at elevated temperature in the Baltic Sea suggests that its establishment may be less transient than that of *K. leptoderma* on Helgoland 50 years ago. A synopsis of the environmental conditions at all 16 confirmed collection sites of *K. leptoderma* in the SW Baltic Sea (Table 1) suggests that the species mostly occurs in locations that are relatively protected from waves and very shallow (< 50 cm below mean sea surface level). In such environments the species typically grows on stones or epiphytically on the bladder wrack *Fucus vesiculosus*. Salinities down to at least 10 are tolerated. Given the general adaptation of *K. leptoderma* to boreal environments, its spread northward and eastward into the Baltic Sea seems very probable, if the species can tolerate salinities below 10 that predominate east of the Darss Sill. On the other hand, a further spread in the southern North Sea may also be expected, given the tolerance of this species to corresponding temperature conditions.

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Molecular analysis of *Ulva compressa* (Chlorophyta, Ulvales) reveals its morphological plasticity, distribution and potential invasiveness on German North Sea and Baltic Sea coasts

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Abstract

To resolve historical misinterpretations of species descriptions and to comprehend the morphological diversity together with the distribution of *Ulva compressa* Linnaeus in Northern Germany, a morphological and molecular study was undertaken of recently collected specimens and herbarium vouchers. Phylogenetic analyses from sequences of the plastid encoded *tuf*A gene confirmed that *U. compressa* is abundant along the German Baltic Sea and North Sea coasts. We were able to genetically confirm the presence of *U. compressa* in the Baltic Sea below salinities of 15 PSU. However, we detected morphologies agreeing with the attached and branched tubular type material only in the North Sea, while *U. compressa* on Baltic Sea coasts indiscriminately exhibited a very distinct morphology of sheet-like thalli that were always unattached, with the exception of one collection site. Drifting forms were also frequently detected in the Wadden Sea, but not on the island of Helgoland. The *tuf*A sequences of attached and tubular forms of *U. compressa* from the German Wadden Sea were identical to the drifting sheets found in the Wadden and Baltic Sea and the sequence divergence was extremely small at ≤ 0.9%. The proliferating, blade-like thalli of *U. compressa* appear as a nuisance ecotype that is able to form massive accumulations that were associated with oxygen depletion. Mass accumulations were observed to cause severe damages and increased mortality of habitat forming *Zostera* and *Ruppia* populations.

Key words: Baltic Sea, barcoding, green tide, Helgoland, morphology, phylogeography, Ulvales, *tuf*A, *Ulva compressa*, Wadden Sea

Introduction

The order Ulvales encompasses a variety of conspicuous green seaweeds distributed throughout marine, brackish and fresh water environments around the world. Their capacity to tolerate changing biotic and abiotic conditions and highly polluted and nutrient enriched environments ranks them among the best known species for potential anthropogenic dispersal (Schaffelke et al., 2006). These opportunistic traits, as well as a potential for strong proliferation under suitable nutrient supply (Teichberg et al., 2010), increase the nuisance potential of some representatives. Several *Ulva* species have demonstrated the capacity to form large blooms, so called ´green tides´ (Smetacek & Zingone, 2013). Such mass accumulations of drifting algal biomass in sheltered bays and shorelines have increased substantially in number and extension during the last century (Fletcher, 1996), mainly due to nutrient supply from agriculture and livestock (Fletcher, 1996; Charlier et al., 2008; Smetacek & Zingone, 2013).

Green tides often cause severe economic losses in tourism and problems related with the removal and disposal of beached biomass (Dion & Le Bozec, 1996; Charlier et al., 2008; Smetacek & Zingone, 2013). Anoxic decomposition of *Ulva* produces and releases gaseous hydrogen sulphide, which can be a severe health hazard (Reiffenstein et al., 1992). In addition, green tides also hamper ecosystem services provided by coastal environments, as they modify biogeochemistry and biodiversity (Norkko & Bonsdorff, 1996a; Norkko & Bonsdorff, 1996b; Valiela et al., 1997; Raffaelli et al., 1998; Charlier et al., 2008; Arroyo et al., 2012). Highly productive Seagrass meadows are often impacted by such mass accumulations of organic biomass. Seagrass ecosystems, as represented for example by dense populations of the eelgrass *Zostera marina* L. and *Ruppia cirrhosa* (Petagna) Grande play a pivotal role in protection of coastal erosion and provide nurseries for many organisms (Duarte, 2000; Marbá et al., 2006). Additionally, they play a key role in coastal carbon and nutrient cycling and thus offer important services for a healthy ecosystem (Smith et al., 1984; Lee et al., 2007). Even though, seagrass species were abundant and widely distributed in northern Europe, a strong decline in these important primary producers has been observed over recent decades (Borum et al., 2006; Waycott et al., 2009). It has also been observed that “macroalgal-tides” noticeably change the abundance of seagrasses and lead to a decline in their populations (Hartog den, 1994; Ansell, 1998; McGlathery, 2001).

However, not every *Ulva* species necessarily has the same potential to cause such negative effects. Some taxa within the genus *Ulva* are seemingly rarely found (Bliding, 1963; Kornmann & Sahling, 1977; Brodie et al., 2007). Rare recordings of *Ulva* species can also be due to the high intraspecific morphological plasticity and interspecific similarity within the genus, which makes precise species identification, based solely on morphological characters, extremely difficult (Bliding, 1963; Koeman & Van den Hoek, 1982). As an alternative, molecular approaches have been developed during the last two decades, which have resolved many taxonomic problems. One of the most significant changes was the transfer of the former genus *Enteromorpha* – that contained tubular species only – to the genus *Ulva* that now comprises tubular and sheet-like species (Tan et al., 1999; Hayden et al., 2003).

It is generally agreed that due to high plasticity many species within the genus *Ulva* have been wrongly recorded and listed in inventories. Worldwide more than 402 historic names and synonyms exist for approximately 132 species that are currently recognized (Guiry & Guiry, 2018). A striking example of frequent taxonomic changes due to a highly variable morphology is *Ulva compressa* Linnaeus. Having been first described as a highly branched species and named *Tremella marina tenuissima, compressa* by Dillenius (1742), Linnaeus (1753) integrated this species in his binomial system and named it *Ulva compressa*. For a detailed history of the taxonomic changes and phylogenetic regroupings of *U. compressa* see Blomster et al. (1998).

A characteristic that has been subject of debate is the allegedly unique tubular form and the branching pattern of *U. compressa*. Several studies have stressed that branching of *Ulva* species may depend on extrinsic factors such as salinity, which excludes thallus branching as a suitable character for taxonomic identification (De Silva & Burrows, 1973; Reed & Russell, 1978; Blomster et al., 1998; Blomster et al., 2002). The flexibility of the gross morphology of *U. compressa* was first observed and genetically underlined by Tan et al. (1999) in the British Isles. Also, Blomster et al. (2002) reported that DNA sequences obtained from specimens of *U. compressa* clustered with a sheet-like individual that, based on morphology, was assigned to the species *Ulva pseudocurvata* Koeman & Hoek.

Together, with such taxonomic observations, the phylogeographical distribution of *U. compressa* also received some attention. For example, the tubular and highly branched form of *U. compressa* has originally been reported by several independent authors to occur in inner and outer parts of the Baltic Sea (Bliding, 1963; Nielsen et al., 1995; Tolstoy & Willén, 1997). In a more recent survey, Leskinen *et al.* (2004) systematically investigated its distribution along the Baltic Sea salinity gradient and concluded that *U. compressa* is not present in areas where the salinity drops below 15 PSU. However, the authors exclusively focused on specimens that exhibited the branched tubular morphology of the holotype.

The objective of the present study was to assess the morphological diversity and to re-evaluate the distribution of *U. compressa* over a range of distinct, geographically separated habitats in Northern Germany, that offer different ecological conditions. Sampling sites in the Baltic Sea, Wadden Sea and on the North Sea island of Helgoland were included to examine the nuisance potential of different morphological forms within these areas.

Lacking tides, but being subject to irregular sea level change driven by wind and air pressure dynamics, the SW Baltic Sea is a brackish water body that is mainly shaped by substrata of stones, gravel and sand (Rönnbäck et al., 2007). Along approximately 260 km of coastline in the northern German state of Schleswig-Holstein the annual mean salinity of the Baltic drops from approximately 18 to 12 PSU (Gräwe et al., 2014), but freshwater inflow and seasonal fluctuations locally generate considerably steeper gradients. In contrast, the Wadden Sea is a fully marine and tidal ecosystem in the SE North Sea. It is protected in part by barren sands and small islands and characterized by extended sand and mud flats. Hard substrata for algal attachment are scarce and mostly provided by molluscs or artificial structures. Whereas the Wadden Sea mainly provides soft bottoms, the North Sea offshore island Helgoland represents a unique environment in northern Germany. The island largely lacks protection from waves and with its rock pedestal it provides the only hard substratum formation at fully marine conditions in Germany (Reinke, 1889).

All three areas were surveyed for the presence of *U. compressa* during two consecutive years at different seasons. For species identification a genetic approach was used, based upon use of the plastid encoded genetic marker *tuf*A. Preceding studies mostly used the ITS or rbc*L* gene markers (Blomster et al., 1998; Tan et al., 1999; Blomster et al., 2002; Hayden et al., 2003; Leskinen et al., 2004; Heesch et al., 2009; Kraft et al., 2010, Hoffmann et al., 2010). However, a more recent study that compared different markers clearly indicated that *tuf*A was a more suitable barcode marker for green algae (Saunders & Kucera, 2010). The genetic approach was combined with morphological observations. Observations of mass accumulations of *U. compressa* were also documented during the survey. In order to reveal potential historical confusions of *U. compressa* with other species in the study area several herbarium specimens were also examined and genetically characterized.

Materials and methods

Field collection and sample preparation. Samples of *Ulva compressa* were collected at 121 locations along the northern German state of Schleswig-Holstein. These were distributed along 537 km of Baltic Sea coast and 644 km of North Sea coast (Statistisches Jahrbuch Schleswig-Holstein 2016/2017) in such a way that distances between adjacent locations never exceeded 25 km. Seasonal collections were carried out in summer (July-August 2014 and August-September 2015) and spring (April 2015 and March 2016). A limited number of sites were also visited during winter (November 2014/2015-beginning of March 2015), but no substantial green algal growth was observed. In the Wadden Sea and at Helgoland samples were collected at low tide. Also interior waterbodies directly adjacent to the coast (overflow basins, drain channels, reservoirs) were checked. On the Baltic Sea coast samples were collected when water levels were low, using waders and an aquascope and penetrating the depth gradient on open coasts, in shallow lagoons and in estuaries to approximately 120 cm below mean water level.

Representative *Ulva* specimens of all morphologies that were present at each site were collected. The specimens were pre-identified on the basis of morphological characters such as cell size and form, number of pyrenoids and location of the chloroplast at three thallus parts: basal, middle and apical. Morphological examinations followed the identification schemes of Koeman and Van den Hoek (1982), taking into account comments by Tan et al. (1999) and Maggs et al. (2007) regarding the variable overall morphology. Afore mentioned characters were studied using light microscopy, whereas Lugol´s solution (Iodine-Potassium Iodide) was used to dye pyrenoids. In addition, epiphyte-free material of each specimen was conserved either by usage of silica gel or by lyophilization for DNA barcoding (see below). Residual thalli were preserved as herbarium vouchers and voucher specimen got deposited at GEOMAR herbarium. Salinity was measured using a WTW portable conductivity meter (Xylem Analytics, Weilheim, Germany).

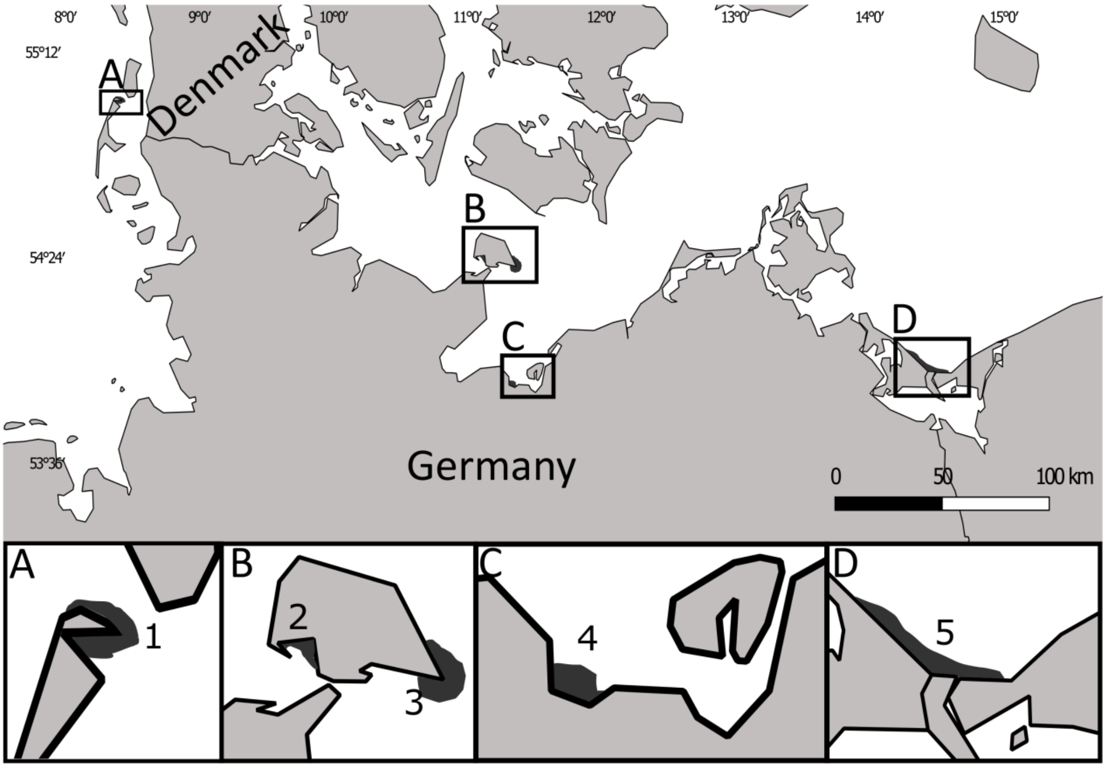

Figure 1: Sites of historic herbarium samples in northern Germany processed in this study. Numbers 1-5 refer to the location of historic collection sites, as listed in Table 1. Dark grey areas correspond to the potential range of the respective sample. Insets A-D provide higher resolution.

Some *U. compressa* specimens from herbarium collections (Haussknecht Herbar Friedrich-Schiller-University Jena [JE], Herbarium of the Alfred Wegener Institute at List, Sylt and Herbarium of the GEOMAR Helmholtz Centre for Ocean Research, Kiel) were also included into the survey, in order to investigate past distributions and to reveal potential historical misidentifications. For additional information about the herbarium samples used in this study see Fig. 1 and Table 1.

DNA extraction, amplification and sequencing. Genomic DNA was isolated from the collected algal tissue with an Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany) following the manufacturer´s protocol. Herbarium material, not older than 35 years and accessible in sufficient amount (at least 1 x 1 cm) was extracted using the same method. For herbarium material that was collected earlier a direct PCR method was used using the Phire Plant Direct PCR kit (Thermo Fisher Scientific). For DNA barcoding the elongation factor *tuf*A gene was amplified by polymerase chain reaction (PCR), using the primers *tuf*GF4 (Saunders & Kucera, 2010) and tufAR (Famà et al., 2002) and the following temperature profile: initial denaturation at 94°C for 4 min, then 38 cycles of (a) 94°C for 1 min, (b) 55°C for 30 sec, (c) 72°C for 1 min, finally 72°C of final extension for 7 min. The PCR-amplified products were directly sequenced by GATC biotech (Konstanz, Germany). Assemblage of forward and reverse sequences and reciprocal editing was done with Sequencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, MI). Sequences obtained in this *way* were uploaded in GenBank (accession numbers MF979636-MF979643 for herbarium material and MF979644-MF979661 for fresh material).

Phylogenetic analyses. The sequences obtained were aligned with reference sequences downloaded from GenBank and used for further phylogenetic analysis. Alignments were prepared with MAFFT (Katoh et al., 2002) and sequence divergence values were calculated with the uncorrected p distance in MEGA v. 5.0 (Tamura et al., 2011). By using MrModeltest software version v. 2.2. (Nylander, 2004) the optimal substitution model was determined and found to be GTR+G+I. *P*hylogenetic analyses were then carried out using the maximum likelihood method (ML) implemented in RAxML version 8 (Stamatakis, 2014) and the chosen substitution model with 1000 bootstrap iterations. Bayesian inference (BI) analyses were computed with MrBayes version 3.2.2

Table 1: Sample list of genetically processed herbarium material

Region	**Location (Site No.)** [a]	**Collection date**	**Herbarium** [b]	**Herbar ID** [c]	**Collector**	**Accession no.** [d]	**Morphological identity** [e]	**Gross morphology** [f]	**Genetic identity** ***tuf*****A** [g]
Wadden Sea	Sylt, Ellenbogen (1)	14.05.1971	AWI	n.a.	n.a.	MF979639	*Ulva lactuca*	blade	*Ulva compressa*
Wadden Sea	Sylt, Ellenbogen (1)	07.07.1978	AWI	n.a.	n.a.	MF979640	*Ulva lactuca*	blade	*Ulva compressa*
Baltic Sea	Fehmarn, Lemkenhafen (2)	04.09.1984	GEO	n.a.	E. Kaminski	MF979636	*Ulva lactuca*	blade	*Ulva compressa*
Baltic Sea	Klütz, Wohlenberg (4)	10.09.1983	JE	JE8003000	A. Krumbiegel	MF979637	*Ulva lactuca*	blade	*Ulva compressa*
Baltic Sea	Fehmarn, Lemkenhafen (2)	07.08.1984	GEO	n.a.	E. Kaminski	MF979638	*Ulva lactuca*	blade	*Ulva compressa*
Baltic Sea	Fehmarn, Staberhuk (3)	08.09.1957	GEO	n.a.	E. Kaminski	MF979641	*Ulva* (*Enteromorpha*) *compressa*	tube	*Ulva linza*
Baltic Sea	Klütz, Wohlenberg (4)	10.09.1983	JE	JE8002999	A. Krumbiegel	MF979642	*Ulva* (*Enteromorpha*) *compressa*	tube	*Ulva linza*
Baltic Sea	Usedom, Heringsdorf (5)	05.07.1961	JE	JE8002998	n.a.	MF979643	*Ulva* (*Enteromorpha*) *compressa*	tube	*Ulva intestinalis*

[a] Site no. = Number in figure 1. [b] Herbarium = Abbreviation of the respective Herbarium samples were taken of (GEO = Herbarium of GEOMAR Helmholtz Centre for Ocean Research Kiel; JE = Haussknecht Herbar Jena; AWI = Herbarium of the Wadden Sea Station Sylt of the Alfred Wegener Institute). [c] Herbar ID = Barcode of respective Herbarium. [d] Accession no. = GenBank accession number for *tuf*A gene. [e] Morphological identity = Name that historically was assigned to specimen by morphological characters. [f] Gross morphology = Refering to the overall morphology of the examined specimen. [g] Genetic identity = Refers to the respective genetic identity obtained by analyses of *tuf*A.

(Ronquist et al., 2012). Four Monte Carlo Markov chains were run for $5 \cdot 10^6$ generations and the sampling frequency was set to 1000. The Bayesian analysis ran 1,015,000 generations and was stopped automatically when the average standard deviation for split frequencies dropped below 0.01. With default settings the first 25% of generations got discarded and 1,524 trees remained. Hereof 50% consensus trees and posterior probabilities were calculated.

Evaluation of Ulva mass accumulations. Surface area coverage and depth of *U. compressa* drifting or beached accumulations were quantified with a tape measure. With the afore mentioned conductivity meter the state of oxygen depletion within the algal bloom was also recorded at 15 cm intervals to a water depth of 150 cm and a beach distance of 400 cm in six replicates. For the evaluation of anoxic zones within the bloom, sediment cores were punched out by using a 45 x 15 cm transparent PVC cylinder. Unaffected areas of the same site served as controls. Thalli of bloom-forming algae were collected from the study sites and underwent molecular identification by barcoding as described before. Within randomly selected plots in the affected areas (1 x 1 m; n = 6) we evaluated the damage and mortality of *Zostera* and *Ruppia* species. Dead specimens and those exhibiting damaged leaves (measured by brown spots and rotted leaves) were counted and correlated with unaffected areas of the same sampling site which functioned as control sites.

Results

Altogether, 128 samples of *U. compressa* were detected by barcoding in 55 out of 121 different locations, thereof 18 out of 73 locations at the Baltic Sea coast, 30 out of 38 at the Wadden Sea coast and 7 out of 10 on the island of Helgoland (Fig. 2, Supplementary Table 1). At the collection time salinities at the Baltic Sea locations ranged from 9.0 to 17.4 PSU, while those at the North Sea locations ranged from 30 to 32.5 PSU (Supplementary Table 1). Thus, *U. compressa* was detected over a wide geographic range and a broad range of habitats and salinities.

Within the sample set a variation of morphological characters was observed throughout northern Germany (Fig. 2, Fig. 3) and only 34% of all specimens corresponded to the tubular and highly branched morphology (Fig. 3A) of the holotype of *U. compressa*

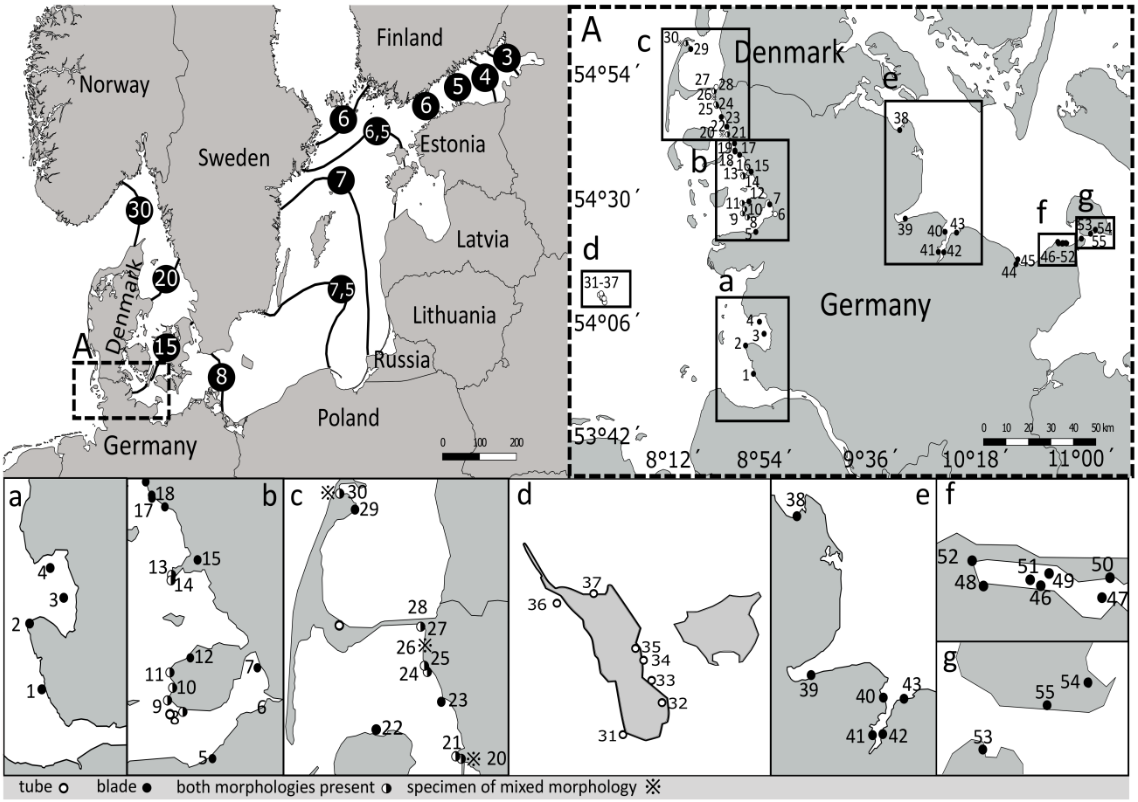

Figure 2: Sites in northern Germany where Ulva compressa was collected. Overview map about the Baltic Sea with respective sea surface salinity. Visualization of the salinity gradient within the Baltic Sea by isohalines with particular salinity values (PSU) in circles (HELCOM-data) dropping with increasing distance from the North Sea. Dashed box (A) represents sampling area for specimen used in this study. (A) Map of northern Germany with numbered sampling sites and indication of found morphologies (legend) at the Wadden Sea (no. 1-27), on Helgoland (no. 28-33) and in the Baltic Sea (no. 35-47). See insets a-g for a better resolution.

(Linnaeus, 1753). The remaining specimens mostly exhibited morphologies with extended blades, but mixed forms combining both morphologies within a single specimen were also found (Fig. 3B-D). A gradient of gross morphologies was observed throughout the sampling area. On Helgoland we only found tubular specimens of *U. compressa.* The specimens grew attached and resemble with the holotype described by Linnaeus (1753). In the Wadden Sea both morphologies were present, sometimes occurring together in the same location. Tubular forms in the Wadden Sea were mostly attached, while sheet-like thalli were found drifting and only few individuals grew attached. In contrast, in the Baltic Sea only specimens with bladed thalli were observed (Fig. 2, Fig. 3). With the exception of one single population detected at Wulfen (N 54°24.535, E 011°10.388), these were all unattached. Notably, at Wulfen, attachment was only observed on artificial substrata (rubber mat, bricks or concrete), despite high abundance of natural stones. In spring (March - April) bladed *U. compressa* were

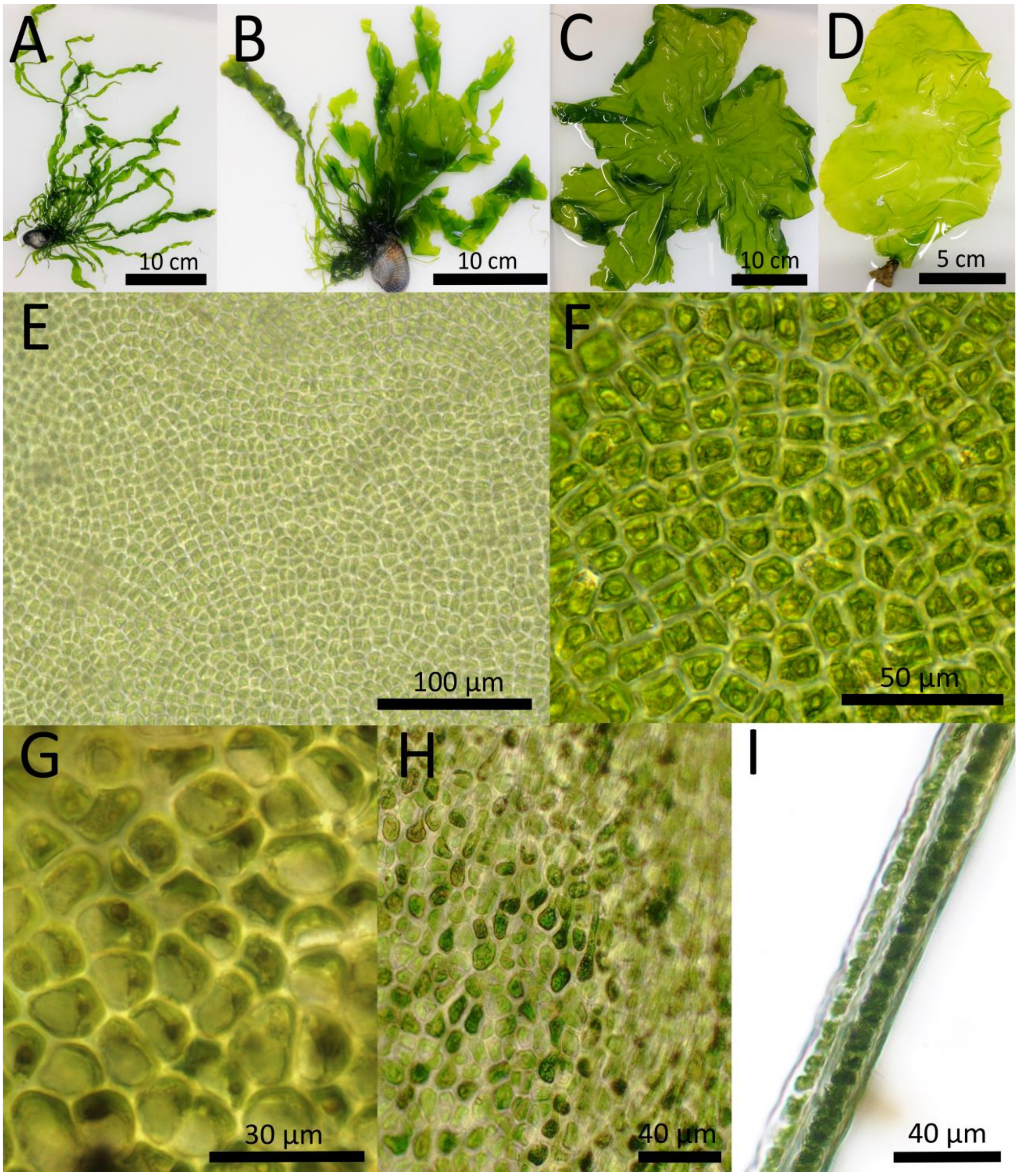

Figure 3: Morphotypes of *Ulva compressa* found in northern Germany and micromorphology of bladed thalli (A) Tubular, branched form of *U. compressa* sampled in the Wadden Sea, Nordstrand (September 2015), referring to the morphology of the holotype. (B) Specimen exhibiting mixed morphologies of the tubular and blade-like form of *U. compressa* from the Wadden Sea, Dagebüll (September 2015). (C) Drifting *U. compressa* blade without rhizoidal zone and (D) blade attached to concrete, collected in the Baltic Sea, Wulfen, Fehmarn (August 2015). (E-I) display the micromorphology of blade-like *U. compressa* collected at Wulfen, Fehmarn in the Baltic Sea (August 2015). (E) Cells of driftinAg *U. compressa* are arranged in indistinct slightly curved rows and (F) of rectangular or irregularly polygonal shape with rounded corners. The single lobed chloroplast is either parietal and cells containing 1-2(3) central pyrenoids or (G) chloroplasts appear cap-like and pyrenoids are marginal. Attached thalli provide the same micromorphology in the middle and apical thallus parts and additionally they exhibit strongly elongated cells of a club-shaped form in the rhizoidal area (H), if present, which contain a variable number of pyrenoids (1-4). (I) Transections show the distromatic thallus structure of *U. compressa*.

Table 2: Comparison of micromorphological features of the middle and apical thallus parts of the different gross morphologies represented by *Ulva compressa*.

	Tubular (after Brodie *et al.* (2007) and own observations)	Sheet-like
Shape of cells	Polygonal or irregularly rounded	Rectangular or irregularly polygonal
Number of pyrenoids	1	1-2 (3)
Position of pyrenoids	Marginal	Central or marginal
Position of chloroplast	Hood-shaped chloroplast at the apical cell end	Parietal or cap-like
Cell arrangement	Mostly irregular, sometimes short rows or rosettes, seldomly clear longitudinal rows	Mostly curved rows
Shape of cell corners	Rounded	Rounded
Cell size	15-30 µm	10-30 µm

typically 3 to maximally 9 cm in diameter. Individuals then increased in size until July-August. Drifting thalli could become larger than attached individuals and some specimens in very sheltered locations in the Baltic Sea reached diameters of up to 150 cm. Drifting thallus sheets appeared round, lobed or with an amorphous shape and they were often interspersed by holes of various sizes. Attached bladed thalli in the Wadden Sea were often rosette-shaped, while those at Wulfen differed from the drifting representatives by having a more lanceolate form with either no or only sparse holes.

Cells of the apical and middle part of the bladed morphotype were arranged in indistinct slightly curved rows and of rectangular or irregularly polygonal shape with rounded corners 9-27 µm in surface view and 11-45 µm x 4-17 µm in transverse sections (Fig. 3E-F). Most cells of the middle and apical thalli contained a single, lobed, parietal chloroplast and in most cases, one central pyrenoid, while some cells exhibited 2 or rarely 3 pyrenoids (Fig. 3F). Thalli with cap-like chloroplasts and 1 or 2 (rarely 3) marginal pyrenoids were less common (Fig. 3G). In attached specimens cells of the

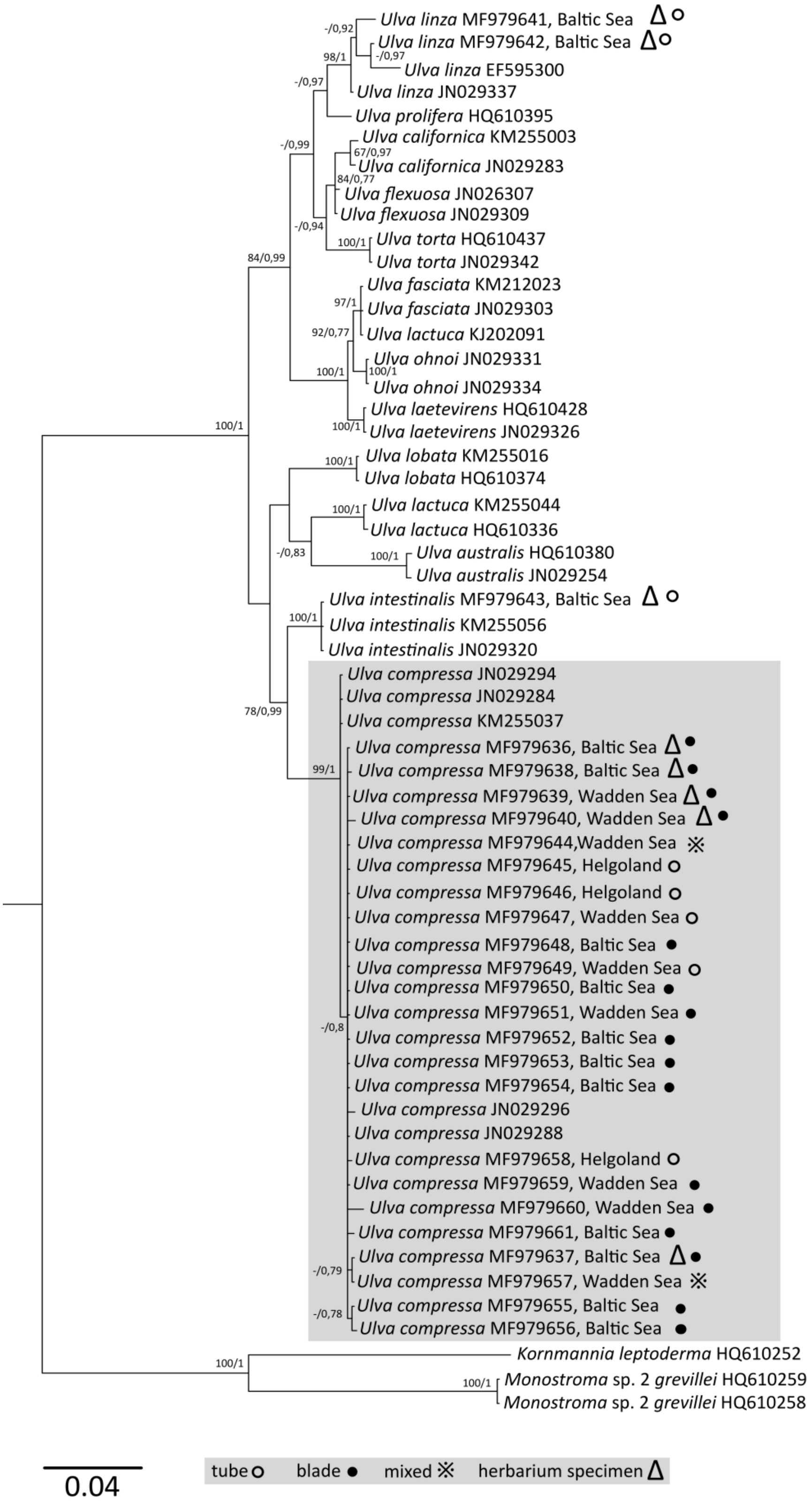

Figure 4: Maximum likelihood tree inferred from *tuf*A sequences. Numbers at nodes refer to bootstrap values left and Bayesian posterior probabilities (1,000 replicates). Nodes with < 50% bootstraps and < 0.70 Bayesian support are not labeled. Branch lengths are drawn proportional to the amount of sequence change. Collection areas (Baltic Sea, Wadden Sea, Helgoland) are indicated. Samples marked with an unfilled circle are of tubular morphology, those labeled with a black circle are thalli with a blade shape and thalli exhibiting a mixed morphology are labeled with an asterisk. Triangles were used to mark sequences of herbarium material. Specimen of *Ulva compressa*, regardless their origin or morphology, appearing in a monophyletic clade.

exhibiting pyrenoids in variable number, ranging from 1 to 4 per cell (Fig. 3H). The main body of these rhizoidal cells was 3-15 µm in surface view, while the tail was up to 40 µm. Transverse sections confirmed that blade-like thalli of *U. compressa* were distromatic and 30-50 µm thick (Fig. 3I).There were no micro- or macromorphological differences observed in sheet-like specimens originating from the Baltic Sea or Wadden Sea, except for some specimens exhibiting a rosette shape in the Wadden Sea. Tubular specimens of the Wadden Sea resembled the characters already described for this morphotype of *U. compressa* (Linnaeus, 1753; Blomster et al., 1998; Maggs et al., 2007). For a comparison of micromorphological characters from tubular and sheet-like specimens of *U. compressa*, see Table 2.

For a selected set of 18 sequences (Table 3), representing all the detected morphotypes, ML and BI analyses were conducted using 773 aligned characters of *tuf*A. Eight sequences of herbarium material (Table 1) and 29 reference sequences of *Ulva* specimens were additionally included in the alignment and as outgroup sequences of *Kornmannia leptoderma* (Kjelman) Bliding (HQ610252) and *Monostroma grevillei* (Thuret) Wittrock (HQ610259 & HQ610258) were added to the analyses. The topologies of the BI and ML trees were similar and the ML tree was used to visualize the topology (Fig. 4). The node separating the genus *Ulva* from outgroup taxa received strong bootstrap and posterior probability support (100/1). Bootstrap support and Bayesian probability values were higher at the terminal nodes than for internally located ones. The analysis confirmed several well-supported clusters of downloaded *Ulva* reference sequences that correspond to morphologically distinguishable species and have been described elsewhere (e.g. Saunders & Kucera, 2010; Rinkel et al., 2012; Kirkendale et al., 2013).

For *U. compressa* the analyses revealed a well-supported clade (99/1) including all of our samples, regardless of their morphological habit, and a number of reference sequences referring to the tubular morphology of the holotype of *U. compressa*. Additionally some of the examined herbarium specimens also clustered within this clade. Sequence divergence between recent tubular, blade-like individuals and specimens exhibiting a mix of both morphologies was extremely low within this clade and ranged from 0-0.9% (Fig. 4, branch length drawn proportionally to sequence

Table 3: Sample list of *Ulva compressa* samples collected in 2014-2016 in northern Germany and used for phylogenetic analyses.

Region	**Location**	**Geographic coordinates**	**Date**	**Voucher No.** [a]	**Accession-No.** [b]	**Site-No.** [c]	**Gross morphology** [d]
Wadden Sea	Friedrich-Wilhelm-Lübke-Koog	N 54°49.998 E 008°36.852	09.08.2014	S_456	MF979644	24	mixed morphology tubuar and blade
Wadden Sea	Dagebüll	N 54°43.804 E 008°41.35	30.07.2014	S_115	MF979647	20	tubular
Wadden Sea	Emmelsbüll	N 54°47.692 E 008°39.484	16.03.2016	S_459	MF979649	23	tubular
Wadden Sea	Finkhaushallig	N 54°24.935 E 008°54.218	21.04.2015	S_672	MF979651	5	blade
Wadden Sea	Dagebüll	N 54°43.804 E 008°41.35	30.07.2014	S_113	MF979657	20	tubular
Wadden Sea	Friedrich-Wilhelm-Lübke-Koog	N 54°49.998 E 008°36.852	09.04.2015	S_563	MF979659	24	blade
Wadden Sea	Dagebüll	N 54°43.804 E 008°41.35	30.07.2014	S_112	MF979660	20	sheet
Helgoland	Helgoland	N 54°11.290 E 007°52.454	22.07.2014	S_14B	MF979645	37	tubular
Helgoland	Helgoland	N 54°10.189 E 007°53.365	23.07.2014	S_29	MF979646	31	tubular

Helgoland	Helgoland	N 54°10.189 E 007°53.365	22.07.2014	S_6	MF979658	31	tubular
Baltic Sea	Kiel	N 54°21.228 E 010°08.475	18.08.2014	S_254	MF979648	41	blade
Baltic Sea	Stein	54°25.065 E 010°15.870	20.08.2014	S_305	MF979650	43	blade
Baltic Sea	Wulfen	N 54°24.535 E 011°10.388	19.09.2014	S_514B	MF979652	55	blade
Baltic Sea	Heiligenhafen	N 54°22.723 E 010°57.327	25.08.2014	S_356	MF979653	48	blade
Baltic Sea	Heiligenhafen	N 54°22.755 E 011°00.301	25.08.2014	S_361	MF979654	50	blade
Baltic Sea	Wulfen	N 54°24.535 E 011°10.388	27.04.2017	S_381	MF979655	55	blade
Baltic Sea	Wulfen	N 54°24.535 E 011°10.388	27.04.2017	S_383	MF979656	55	blade
Baltic Sea	Wackerballig	N 54°45.518 E 009°52.670	24.07.2014	S_79	MF979661	38	blade

[a] Voucher no. = number of the voucher specimen deposited in the herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel; [b] Accession no. = GenBank accession number for *tuf*A gene. [c] Site no. = Referring to number in Figure 2.[d] Gross morphology = Referring to the overall morphology of the examined specimen.

change). The gross morphology of a sample, tubular or blade-like, was not correlated with its position within the phylogenetic tree nor with a morphological species concept. Both BI and ML supported this morphologically mixed clade and thus confirms *U. compressa* as a good example of phenotypic plasticity within the genus *Ulva*.

It was not an objective of our study to identify the minimum salinity in which tubular forms of *U. compressa* can be found. However, the apparent absence of such forms at the German Baltic Sea coast supports the idea that their distribution must be restricted to salinities above 17 PSU (Fig. 2, Supplementary Table 1). Three different historical samples that had been collected between 1957 and 1983 at the German Baltic Sea cost as Enteromorpha compressa and exhibited the holotype morphology of *U. compressa* consistently belonged to other species, either *U. linza* L. or *U. intestinalis* L. (Table 1). In contrast, *U. compressa* exhibiting blade-like morphologies was found at salinities as low as 9 PSU. We also detected five different historical *Ulva* specimens that had been collected in northern Germany between 1971 and 1984 which all exhibited the blade-shaped morphology of *U. compressa* and also corresponded genetically with *U. compressa*. Notably, all of them had been wrongly assigned to *U. lactuca* Linnaeus (Table 1), suggesting that both species have been confused in the area for decades.

Both on Baltic Sea and Wadden Sea coasts the blade-like and drifting morphotype of *U. compressa* occasionally formed local mass accumulations. For example, this was observed in 2016 at a shallow and wave protected beach at Wulfen on the Baltic Sea coast (Fig. 5). *U. compressa* thalli formed a loose drifting mat of 100-150 cm thickness, covering 23 x 12 m of a *Zostera marina* L. and *Ruppia cirrhosa* (Petagna) Grande bed in a water depth of 150-200 cm below mean sea surface (Fig. 5). The lowest 5 to 30 cm of these aggregations consisted of decaying biomass. Oxygen concentrations dropped from 9.8 mg l^{-1} at the sea surface to 0 mg l^{-1} directly above the seafloor beneath the drifting biomass. An intense odour of H_2S was emitted from the surface of sediment cores, which exhibited an anoxic, dark coloured top layer to a depth of 4-16 cm. Concomitantly, increased mortality of *Z. marina* and *R. cirrhosa* was observed and approximately 70% of the covered seagrass appeared seriously damaged or dead. No anoxic water or sediment and no damaged seagrass were observed in reference areas in the close vicinity that were free of drifting *U. compressa* (Fig. 5). Very similar effects of *U. compressa* aggregations, i.e. drifting blades closely entangled with filaments of Chaetomorpha melagonium (Weber & Mohr) Kützing, were also seen at Nordstrand (N54°29.163, E 008°49.114) in the Wadden Sea, in summer 2016.

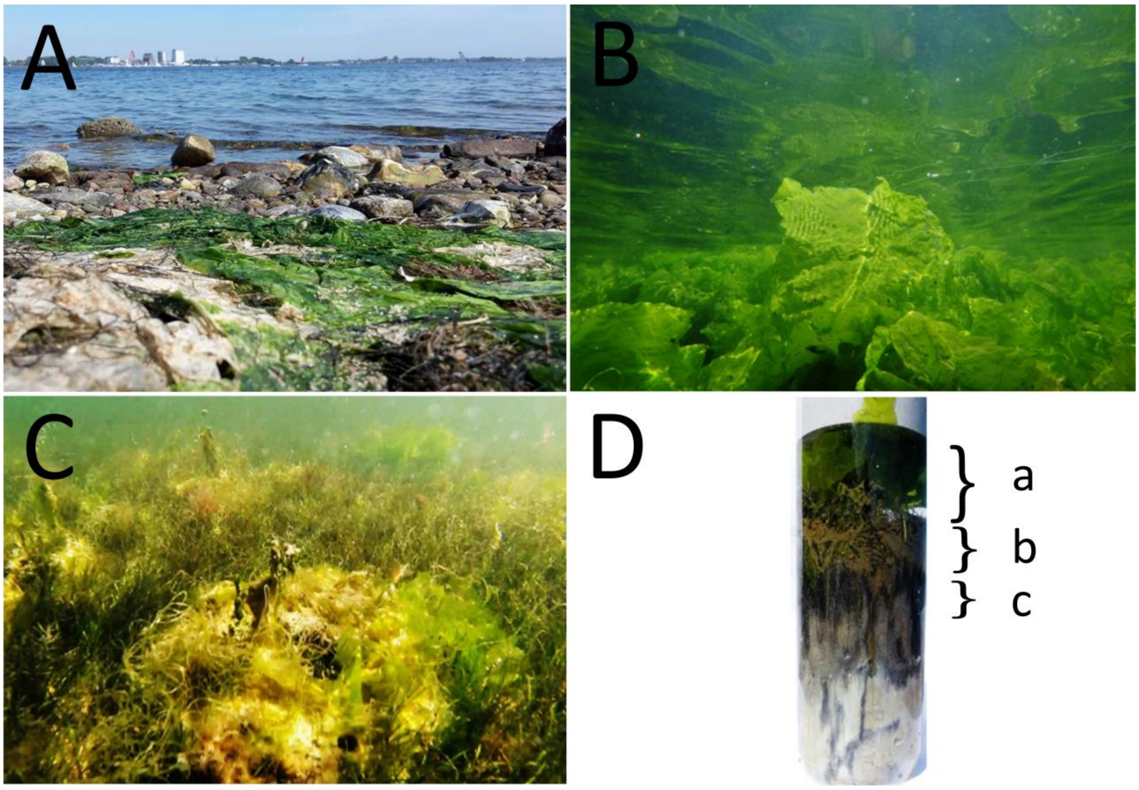

Figure 5: Mass accumulation of blade-like *Ulva compressa* in August 2016 inducing hypoxic conditions at a shallow bay (Wulfen) at the German Baltic Sea island Fehmarn. Top (A) and underwater (B) view of the massive drifting mats of blade-like *Ulva compressa*. Thalli forming mats of 100-120 cm thickness. (C) Close-up of strongly affected *Zostera marina* and *Ruppia cirrhosa* populations after removing drifting biomass. Sediment cores (D) show a clear zonation of (a) dense drifting algae material, (b) decomposition zone and (d) black, anoxic zone, emitting a foul odour.

Discussion

Here, we provide a completely revised picture of the distribution and morphological variability of *Ulva compressa* in Schleswig-Holstein, a study area of limited size that nonetheless includes coastal sections of two important sea areas in Northern Europe, namely the North Sea and the Baltic Sea. The clade obtained by phylogenetic analysis of *tuf*A sequences clearly encompasses specimens both of tubular and blade-like morphologies and occasionally the occurrences of mixed morphotypes. This morphological diversity is not in agreement with the taxonomic concepts proposed for *U. compressa* in identification keys for the studied region and adjacent areas (Kornmann & Sahling, 1977; Rothmaler, 1984; Pankow, 1990; Kornmann & Sahling, 1994). As a consequence, it is strongly suggested, that virtually all specimens of *U. compressa* in the German Baltic and many of those in the Wadden Sea have so far been misidentified and this may also be true for adjacent areas. Our observations are at the same time in contradiction and in agreement with the findings of Leskinen et al. (2004). Focusing on the phylogeographical structure and distribution of *U. compressa* in the Baltic Sea, the authors stated that its distribution was very restricted and that the species was absent at salinities lower than 15 ppt. These observations seem to be in stark contrast with our findings of a relatively wide distribution and even of occasional mass developments of *U. compressa* at salinities in the range between 9 and 15 PSU. However, the study of Leskinen et al. (2004) was exclusively focused upon branched tubular algal specimens that corresponded morphologically with the holotype of *U. compressa*. It addressed the issues of branching patterns (Burrows, 1959) and the relationship of *U. compressa* with typically unbranched tubular species like *Ulva intestinalis* (Linnaeus, 1753). The possibility that exclusively sheet-like and nearly exclusively unattached individuals might be present at lower salinities was not considered. Indeed, specimens of *U. compressa* that resembled the holotype were also in the present study never found at salinities of 17 PSU or less, which confirms the findings of Leskinen et al. (2004).

U. compressa with blade-like morphology was first discovered in brackish water in the Ythan Estuary in Scotland (Tan et al., 1999), but it is not limited to low salinities. In the present study specimens with extended distromatic blades were found in high abundance along the coastline of the Wadden Sea, where salinity usually ranges from 30 to 33.5 PSU. Here, *U. compressa* blades were particularly frequent in environments like overflow basins, drain channels and reservoirs, where water temperature can rise temporarily to over 40°C and salinity varies strongly due to desiccation and flooding. In contrast, tubular individuals were relatively rare in such relatively stressful locations. Apparently, the bladed morphology of *U. compressa* is less limited in its distribution than the allegedly characteristic tubular form, suggesting that it may be able to survive under a broader range of environmental conditions.

Both the wide distribution of blade-like morphotypes and the absence of tubular forms at low salinities are also supported by our investigation of herbarium material from Northern Germany. Five different historical samples with blade-like morphology that had been collected in the Wadden and Baltic Sea in the 1970s and 1980s were identified as *U. compressa*, which confirms that the presence of this morphotype in the whole area is not new. Likewise, our findings with historical tubular specimens, that had all been wrongly assigned to *U. compressa* (Table 1), are in accordance with an absence of this morphotype from the Baltic Sea area in the past. Given this and the circumstance that blade-like forms have so far not been recognized as *U. compressa* we predict that all historical records of this species from the German and more easterly Baltic Sea areas were probably based upon confusion with other tubular species, such as *Ulva linza* Linnaeus 1753 (MF979641 & MF979642) or *U. intestinalis* (MF979643), despite the circumstance that *U. compressa* is in the area and has also been there in the past. Further, all historical records of *U. compressa* with blade-like morphology that we discovered were originally wrongly assigned to *U. lactuca*. Most historical and more recent identification keys for Germany (Fraude, 1882; Rothmaler et al., 1984; Pankow, 1990) and species lists (Nielsen et al., 1995; Schories et al., 2009) include *U. lctuca*, although we have been unable to detect any genetic evidence for its presence in our sampling sites in the Baltic Sea and Wadden Sea. We therefore speculate that many other historical records of *U. lactuca* in Schleswig-Holstein and its adjacent areas may have also been incorrect, due to confusion with the blade-like morphotype of *U. compressa*.

The presence of foliose *Ulva compressa* in the North Sea has been described before. On the basis of molecular investigation, Tan et al. (1999) revealed a close relationship between tubular *U. compressa* and foliose *Ulva* specimens from the Scottish East coast that exhibited the typical morphology of *U. pseudocurvata* Koeman and Van den Hoek (1981). ITS gene sequences indicated conspecificity of both forms and Tan et al. (1999) suggested that *U. pseudocurvata* might just be a morphological variation of *U. compressa*. Based upon specimens collected in the Netherlands, *U. pseudocurvata* was originally described as a species with distinctly curved, sometimes straight and more or less symmetrical thalli that were missing a hollow stipe (Koeman & Van den Hoek, 1981). According to these authors, the latter character was one of the main traits that distinguished *U. pseudocurvata* from a highly similar taxon, *U. curvata* (Kützing) De Toni. Due to the absence of the above mentioned morphological features, specimens collected on Helgoland and previously identified as *U. curvata* (Kornmann & Sahling, 1977) were revised and allocated to *U. pseudocurvata* (Kornmann & Sahling, 1994). Since then, *U. pseudocurvata* is listed in the species inventories of Helgoland (Bartsch & Kuhlenkamp, 2000; Schories et al., 2009), while the distribution of *U. curvata* is assumed to extend over the Wadden Sea coasts of Schleswig Holstein and the Baltic Sea coastline of eastern Germany (Schories et al., 2009). Thalli with strongly curved habit that resembled *U. pseudocurvata* or *U. curvata* were occasionally sampled within our field survey on Helgoland and at the Wadden Sea coast. However, material from Helgoland was genetically most similar with *U. lactuca* (GenBank accession no.: HQ610341, data not shown), while material from the mainland coasts always clustered within *U. compressa.* We were not able to detect genetic evidence of a delimited clade representing specimens resembling the descriptions and characteristics of *U. pseudocurvata* or *U. curvata*. Thus *U. pseudocurvata* and *U. curvata* are either very rare species or, and perhaps more probable, simply morphological variations within *U. compressa* and *U. lactuca.* To clarify the taxonomic status of *U. pseudocurvata* and *U. curvata* further genetic investigation of the type material of both species has to be carried out.

Although the sheet-like morphotype of *U. compressa* has so far not been correctly recognized taxonomically, it appears as one of the more problematic nuisance seaweeds in Germany. In its free-floating form, it clearly has the potential to form green tides and on a local scale we repeatedly observed sediment anoxia and damaged seagrass meadows in the direct vicinity of biomass accumulations. The southern North Sea and in particular, the Baltic Sea, are still among the most eutrophic Sea areas worldwide, although nutrient input has been reduced since its peak in the early 1980s (Wernand et al., 2013). Given that the bladed morphotype of *U. compressa* has already been recorded in the area at that time, it may certainly have significantly contributed to the formation of sediment anoxia in the Wadden Sea, which was more abundantly observed some decades ago than today (Reise et al. 2015). Extensive green tides formed by foliose *U. compressa* were observed by Tan et al. (1999) in Aberdeenshire (Scotland), and by Guidone et al. (2013) and Hofmann et al. (2010) in New England. It remains as an open question why the bladed morphotype of *U. compressa* has a stronger capacity to form green tides, a wider tolerance of different environmental conditions and thereby probably a more invasive character than the tubular morphotype.

We are also currently unable to explain why *U. compressa* was exclusively detected unattached in most of the Baltic Sea sites that we surveyed. In *Ulva mutabilis* Føyn 1958 the formation of rhizoids is controlled by specific bacteria (Wichard et al., 2015) and a similar mechanism may act in *U. compressa*. Under these conditions a lack of specific microorganisms at reduced salinity may lead to a reduced formation of rhizoids. However, attached specimens of *U. compressa* were repeatedly detected at one site in the Baltic and in this light a genetic basis for the formation or non-formation of rhizoids appears as more likely.

We can conclude that two extremely different morphologies exist within the species *U. compressa* that rarely exhibit transition forms in our study area. Consequently they have so far been misinterpreted as different species. Our findings highlight once again that the genus *Ulva* comprises a complex of species with morphological varieties and forms that are still poorly understood. The number of morphologies a single species can possess, is in most cases unknown and different morphotypes of the same species are still regarded as distinct taxa. This underlines the utmost importance of applying molecular methods for species identification within this morphologically highly variable genus and it once more points out that species knowledge still is and should be an important focus in phycological research.

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Supplement

Table S 1: Sample list provides overview of sampling sites at which *Ulva compressa* was found. Site-No. = used in Figure 1. Salinity = Sea surface salinity at collection time (summer 2015) in PSU.

Region	Location	Habitat	Latitude	Longitude	Site-No.	Salinity [PSU]	Morphology		
							tubular	sheet	mixed
Wadden Sea	Kaiser-Wilhelm-Koog	Salt meadows	N 53°56.643	E 008°54.018	1	31.2		X	
Wadden Sea	Friedrichskoog-Spitze	Stone groynes in mudflats	N 54°02.246	E 008°50.691	2	32.1		X	
Wadden Sea	Meldorf	mudflats	N 54°04.658	E 008°58.087	3	21.2		X	
Wadden Sea	Wöhrden	mudflats	N 54°07.039	E 008°56.156	4	31.2		X	
Wadden Sea	Finkhaushallig	mudflats	N 54°24.935	E 008°54.218	5	31.8		X	
Wadden Sea	Husum	harbour	N 54°28.268	E 009°01.676	6	30.7	X		
Wadden Sea	Schobüll	mudflats	N 54°30.469	E 008°59.734	7	31.3		X	
Wadden Sea	Nordstrand	mudflats	N54° 27.625	E 008°50.674	8	32.3	X	X	
Wadden Sea	Nordstrand	mudflats	N54°28.243	E 008°48.410	9	32.3	X	X	
Wadden Sea	Nordstrand	mudflats	N54°29.163	E 008°49.114	10	32.3	X	X	
Wadden Sea	Pellworm	Ferry terminal	N 54°29.929	E 008°48.522	11	31.3	X	X	
Wadden Sea	Nordstrand	mudflats	N 54°30.998	E 008°51.262	12	32.3		X	
Wadden Sea	Hamburger Hallig	mudflats	N 54°35.939	E 008°48.730	13	31.5	X	X	
Wadden Sea		mudflats					X	X	
	Hamburger Hallig		N 54°35.939	E 008°48.732	14	31.5			
Wadden Sea	Amsinck Haus	drain channel from salt meadows	N 54°36.922	E 008°52.013	15	n.a.		X	
Wadden Sea	Großengaarde	mudflats	N54° 39.821	E8° 47.458	16	31.1		X	
Wadden Sea		Ferry terminal (south)						X	
	Schlüttsiel		N 54°40.880	E 008°45.265	17	31.3			
Wadden Sea		ferry terminal (north)						X	
	Schlüttsiel		N 54°41.061	E 008°45.231	18	31.1			

Wadden Sea	Janneswarft	mudflats	N54°41.977	E8° 45.12	19	31.1		X	
Wadden Sea	Dagebüll 1	Ferry terminal	N 54°43.804	E 008°41.35	20	31.2	X	X	X
Wadden Sea	Dagebüll 2	Ferry terminal	N 54°43.824	E 008°41.634	21	31.2	X	X	
Wadden Sea	Galmsbüllkoog	Drain channel within salt meadows	N54° 45.669	E8° 41.802	22	31.5		X	
Wadden Sea	Emmelsbüll	mudflats	N 54°47.692	E 008°39.484	23	31.5		X	
Wadden Sea	Friedrich-Wilhelm-Lübke-Koog	mudflats	N 54°49.998	E 008°36.852	24	32.1	X	X	
Wadden Sea	Friedrich-Wilhem-Lübke-Koog	mudflats	N 54°50.241	E 008°36.732	25	32.1	X	X	
Wadden Sea	Friedrich-Wilhelm-Lübke-Koog	mudflats	N54° 51.402	E 008°36.206	26	32.1			X
Wadden Sea	Hindenburgdamm	Salt meadows (south)	N 54°52.949	E 008°36.186	27	29.1	X	X	
Wadden Sea	Hindenburgdamm	Salt meadows (north)	N 54°53.468	E 008°36.410	28	29.1	X		
Wadden Sea	Sylt	Ferry terminal	N 55°1.1	E 008°26.375	29	32.5		X	
Wadden Sea	Sylt	Sandy lagoon	N 55°2.1116	E 008°24.01	30	32.5	X	X	X
Helgoland	Helgoland	Outer breakwater „Westmole“	N 54°10.189	E 007°53.365	31	33.8	X		
Helgoland	Helgoland	Inner harbour „Südhafen“	N 54°10.630	E007° 53.577	32	34.0	X		
Helgoland	Helgoland	Inner harbour „Binnenhafen“	N 54°10.682	E 007°53.323	33	33.8	X		
Helgoland	Helgoland	Outer stone groyne	N 54°10.950	E 007°53.437	34	34.0	X		
Helgoland	Helgoland	Harbour „Nord-Ost Hafen“	N 54° 11.02	E 007° 53.318	35	34.0	X		
Helgoland	Helgoland	Rocky tidal flats (north-west)	N54° 11.246	E007° 52.218	36	33.8	X		
Helgoland	Helgoland	Rocky tidal flats (north-east)	N 54°11.290	E 007°52.454	37	33.8	X		

Baltic Sea	Wackerballig	Sandy beach	N 54°45.518	E 009°52.670	38	16.9	X
Baltic Sea	Aschau	Sheltered sandy lagoon	N54° 27.648	E9° 55.599	39	15.7	X
Baltic Sea	Falkenstein	Stony beach	N54° 24.743	E10° 11.487	40	16.1	X
Baltic Sea	Kiel	Naval base	N 54°21.228	E 010°08.475	41	16.2	X
Baltic Sea	Mönkeberg	Sandy beach	N 54°21.160	E 010°10.674	42	16	X
Baltic Sea	Stein	Sandy beach	N 54°25.065	E 010°15.870	43	17.4	X
Baltic Sea	Sehlendorfer lake	Inland lake	N54° 18.287	E10° 41.159	44	9.1	X
Baltic Sea	Sehlendorfer lake	Inland lake	N54° 18.367	E10° 39.74868	45	9	X
Baltic Sea	Heiligenhafen inland lake	Marina	N 54°22.664	E 010°58.823	46	14.6	X
Baltic Sea	Heiligenhafen natural reserve ´´Graswader´´	Salt meadows in natural reserve	N 54°22.680	E 011°00.515	47	16.8	X
Baltic Sea	Heiligenhafen inland lake	Inland lake	N 54°22.723	E 010°57.327	48	15.3	X
Baltic Sea	Heiligenhafen natural reserve ´´Graswader´´	Salt meadows in natural reserve	N 54°22.739	E 010°59.233	49	16.8	X
Baltic Sea	Heiligenhafen natural reserve ´´Graswader´´	Salt meadows in natural reserve	N 54°22.755	E 011°00.301	50	16.8	X
Baltic Sea	Heiligenhafen inland lake	Marina	N 54°22.767	E 010°58.943	51	15.9	X
Baltic Sea	Heiligenhafen inland lake	Inland lake	N 54°23.002	E 010°57.013	52	15.9	X

Baltic Sea	Großenbrode	Sandy lagoon	N 54°23.582	E 011°06.612	53	15.5		X	
Baltic Sea	Burg, Fehmarn	Stony lagoon	N54° 24.878	E11° 12.687	54	15.2		X	
Baltic Sea	Wulfen	Stony lagoon	N 54°24.535	E 011°10.388	55	14.3		X	

Chapter V (Submitted to Journal of Phycology [26.04.2018]; current status: resubmitted after minor revision)

Conspecificity of the model organism *Ulva mutabilis* and one of the first described green algal species, *Ulva compressa* (Ulvophyceae, Chlorophyta)

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Abstract

As one of the most abundant and ubiquitous representatives of marine and brackish coastal macrophytobenthos communities, the genus *Ulva* is not only an important primary producer but also of ecological and morphogenetic interest to many scientists. *Ulva mutabilis* became an important model organism to study morphogenesis and mutualistic interactions of macroalgae and microorganisms. Here we report that our collections of *Ulva compressa* Linnaeus (1753) from Germany are conspecific with the type strains of the model organism *Ulva mutabilis* Føyn (1958), which were originally collected at Olhão on the south coast of Portugal and have from that time on been maintained in culture as gametophytic and parthenogenetic lab strains. Different approaches were used to test conspecificity: **(1)** comparisons of vegetative and reproductive features of cultured material of *U. mutabilis* and German *U. compressa* demonstrated a shared morphological pattern; **(2)** gametes of *U. compressa* and *U. mutabilis* were successfully mated and developed into fertile sporophytic first-generation offspring; **(3)** molecular phylogenetics and species delimitation analyses based on the Generalized Mixed Yule-Coalescent method showed that *U. mutabilis* isolates (sl-G[mt+]) and (wt-G[mt-]) and *U. compressa* belong to a unique Molecular Operational Taxonomic Unit. According to these findings, there is sufficient evidence that *U. mutabilis* and *U. compressa* should be regarded as conspecific.

Key index words: conspecificity, model organism, morphology, mutation, species delimitation, taxonomy, *tuf*A, *Ulva*

List of abbreviations: BLAST, Basic Local Alignment Search Tool; GMYC, General Mixed Yule Coalescent; MOTU, Molecular operational taxonomic units; UCM, *Ulva* culture medium

Introduction

Although green macroalgae of the order Ulvales are abundant and often dominant in marine and brackish shallow water environments and thus ecologically important, their taxonomy has not been completely clarified. In fact, some of their representatives like the genus *Ulva* have undergone a frequent taxonomic revision. Remaining obscurities have dominated a large part of the taxonomic history of *Ulva*, although it was already described in 1753 by the father of nomenclature, Linnaeus, as one of the first algal genera. Many studies indicated morphologically highly variable characters of some *Ulva* species, which largely interferes with their taxonomic identification (Koeman and van den Hoek 1981, Koeman and van den Hoek 1982a, Koeman and van den Hoek 1982b, Blomster et al. 1998, Hayden and Waaland 2002, Hayden et al. 2003, Brodie et al. 2007). Already in 1797 T.J Woodward described in his synopsis the multiple morphotypes of the plants belonging to the genus *Ulva*: "*The frons* [of Ulva] *is either membranaceous or gelatinous; the latter is either plane or tubulous, and usually diaphanous; the former either filiform or compressed, solid or fistulous diaphanous or opake*" (Woodward, 1797).

Apparently, application of a morphological species concept to the genus *Ulva* is highly problematic. After about 200 years of discussion, Hayden et al. (2003) proved with molecular techniques that the separation of tubular and blade-like morphologies into different genera (*Enteromorpha* and *Ulva*, respectively) was indeed an artificial classification because those morphological differences are not reflected in phylogenetic relationships. The combination of tubular and blade-like species to the taxonomically accepted and by Linnaeus established genus *Ulva* resulted in a first big revision of this species complex. Since then, genetic studies within the group became more and more relevant and scientists in many places started to investigate the correct species composition of Ulvales, adopting molecular techniques (Shimada et al. 2003, Brodie et al. 2007, Charlier et al. 2007, Heesch et al. 2009, Saunders and Kucera 2010, Wolf et al. 2012). Most studies used ITS and *rbc*L as markers, nevertheless, in more recent studies *tuf*A was found as a more reliable barcode marker for inter- and intra-specific species delimitation (Saunders and Kucera 2010). However, an exclusive application of the genetic species concept can still be misleading, because only relatively short DNA barcoding sequences are usually compared to deduce phylogenetic relationships.

Although they remain among the taxonomically most difficult macroalgal groups, *Ulva* species represent remarkable model organisms due to their large degree of morphological plasticity and their ecological importance (Wichard 2015). In 1952 Føyn isolated *Ulva mutabilis* from material collected in Faro and Olhão (Portugal) (Føyn, 1958). Since then *U. mutabilis* became the focus of many studies aiming to investigate the reproduction and life cycles of macroalgae, cross-kingdom talk and symbiotic effects, as well as morphogenetic issues (Wichard and Oertel 2010, Spoerner et al. 2012, Oertel et al. 2015, Wichard et al. 2015, Grueneberg et al. 2016). The species ’*Ulva mutabilis*’ was established based on a wide range of spontaneous developmental mutants that appeared at an unusually high frequency in the offspring of the collected source material (Føyn 1958, Føyn 1959, Fjeld and Borresen 1975, Borresen and Fjeld 1977). One noteworthy form was the ribbon-shaped mutant named ’slender‘, which strongly differed from the typical and genetically stable wildtype's lettuce-like form (Føyn 1958, Løvlie 1964).

Thalli of the slender morphology stood out with a faster growth rate, shorter generation time and in most cases with no or a much reduced form of rhizoid that consisted of only a few primary rhizoidal cells (Føyn 1958, Løvlie 1964, Wichard 2015). The loss of a rhizoid causes mature slender thalli to be mostly encountered drifting.

The strains of *U. mutabilis* generated by Føyn subsequently drove the advancement of reasearch on cell differentiation and growth promoting factors. Studies discovered gowth factors released by symbiotic bacteria (*Roseovarius* sp.= producer of factor MS2 and *Maribacter* sp. = producer of factor MS6) which substantially influenced the development of young algae (Spoerner et al. 2012, Grueneberg et al. 2016, Ghaderiardakani et al. 2017). Additionally, sporulation and swarming inhibitors were detected that control gametogenesis, sporogenesis and release of swarmers in *U. mutabilis* (Stratmann et al. 1996, Wichard and Oertel 2010).

Ulva is a haplo-diplontic, isomorphic and isogamous alga with two genetically determined mating types, mt- and mt+. It splits into the haploid gametophytic and diploid sporophytic life stages that exhibit identical morphologies (Løvlie 1964, Lovlie and Bryhni 1978, Phillips 1990, Wichard 2015). Mature gametophytes release biflagellate, haploid and phototactic positive gametes through mitosis, while sporophytes produce quadriflagellate haploid and phototactic negative zoospores through meiosis. Gametophytes either originate through generation change from zoospores released by a sporophyte, or they emerge from unfertilised gametes through parthenogenesis (Lovlie and Bryhni 1978, Phillips 1990, Wichard and Oertel 2010). Propagation through parthenogenesis allows for the permanent cultivation and preservation of Føyn´s original cultures and for genetic manipulations of haploid specimens (Oertel et al. 2015), while Sexual reproduction requires the mating of gametes of different sexes. Føyn was able to assign a sex to *U. mutabilis* cultures, dividing the isomorphic and consequently morphologically not distinguishable gametes in breeding experiments into sex-positive and sex-negative ones (Føyn 1958). Today´s remaining *U. mutabilis* cultures are of two different mating types. The strain of the slender type is of mating type positive (mt[+]), while cultures of the wildtype are of mating type negative (mt[-]) (Oertel et al., 2015).

Several studies already revealed similarities between *U. mutabilis* and *U. compressa*.

As in *U. mutabilis* also in *U. compressa* different shapes and morphologies have been observed (Tan et al. 1999). These led to suggestions that various forms of environmental stressors or the absence or presence of certain bacteria may influence the morphogenetic switch between so-called ’leafy‘ and ’cylindrical‘ morphologies of *U. compressa* (Tan et al. 1999, Wichard 2015). Additionally, the conspecificity of *U. compressa* and *Ulva pseudocurvata* – two species with quite distinct type morphologies that are nontheless genetically similar - has been suggested in previous studies (Tan et al. 1999, Loughnane et al. 2008). Similarities in the *rbc*L marker gene sequence, germ cell production, gamete size and the regulation of sporulation were quoted as evidence for the close relationship between both species (Bråten 1971, Stratmann et al. 1996, Tan et al. 1999, Wichard and Oertel 2010, Alsufyani et al. 2014).

It was the goal of the present work to verify that the overall similarities of *U. mutabilis* and the ‘*Ulva-compressa-pseudocurvata*’ clade regarding life cycle, morphogenesis and the potential to switch between various morphotypes are due to conspecificity. We, therefore, collected *U. compressa* specimens between 2014 and 2016 at Baltic and North Sea areas of the German state of Schleswig-Holstein and adjacent areas including the island of Helgoland and compared their genetic and morphological characteristics with *U. mutabilis*. Since there is no genetic data of the type material of *U. compressa* available, we compared our data to different reference sequences of *U. compressa* from various locations. Some of the added and peer reviewed reference organisms corresponded with the morphological species concept of

Linnaeus (Linnaus 1753) and represented the authentic *U. compressa* morphotype. Thus, we can be very certain that despite the strong morphological variability of the recent samples, our species concept of *U. compressa* is following the one proposed by Linnaeus (1753). Due to the strong morphological variability of *U. compressa* certain morphotypes were only recently revealed by molecular techniques in more recent studies.

However, until today the definition of a species depends on the respective species concept chosen. One of the most widely accepted species concepts is based on the idea that different parents that can interbreed successfully and produce fertile offspring are closely related and conspecific (biological species concept, Mayr 1942). We therefore also conducted classical interbreeding experiments to test whether the laboratory model organism *U. mutabilis* and the field-observed organism *U. compressa* are conspecific and whether the scattered knowledge collected from various laboratories can be unified for future research lines.

Material and methods

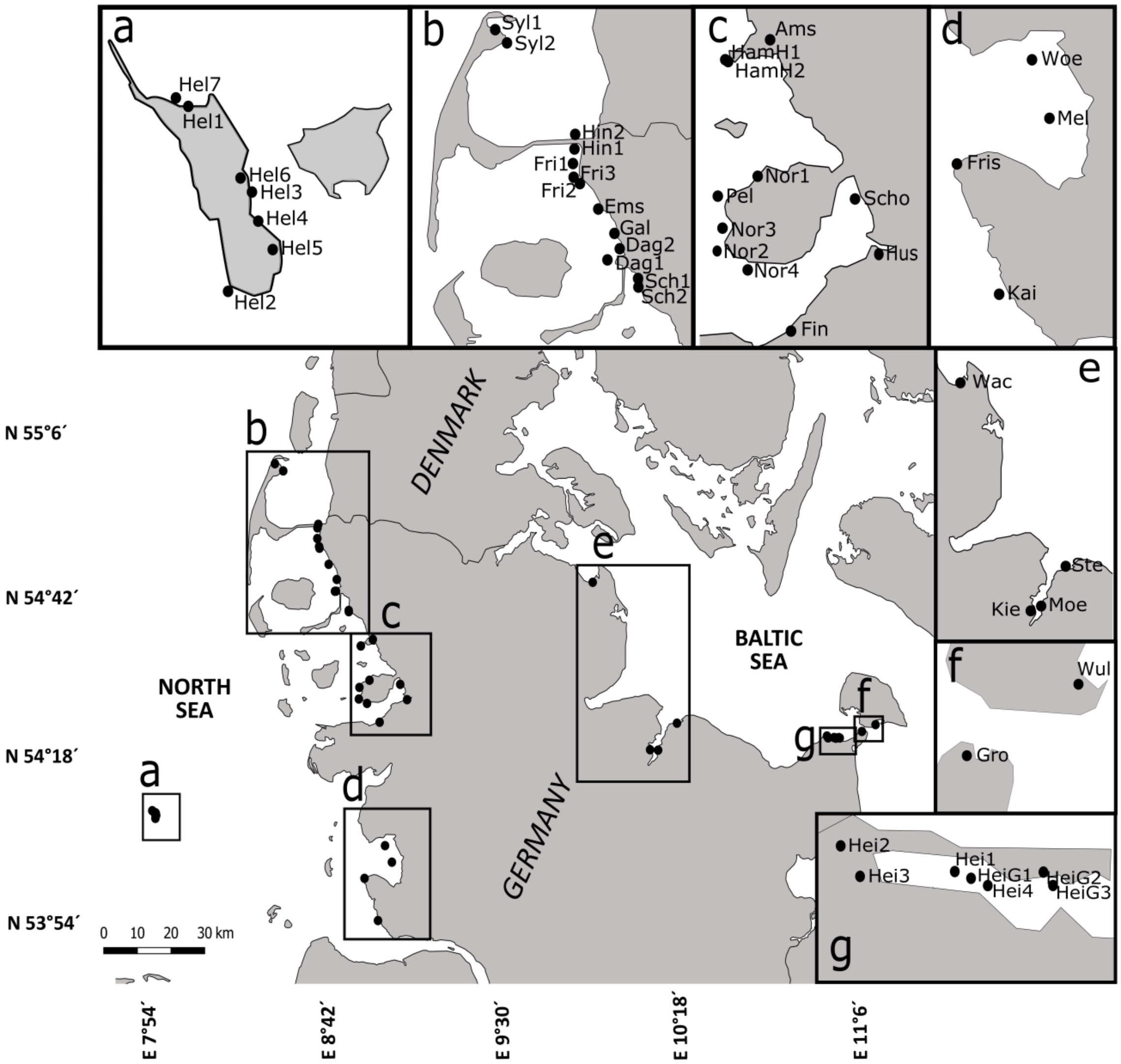

Figure1: Sites in Northern Germany where *Ulva compressa* has been collected. Black dots indicate sampling sites. Inserts a-g provide better resolution and respective abbreviations for the sites (Ams= Amsinck Haus, Dagebuell 1-2= Dag 1-2, Emmelsbuell= Ems, Finkhaushallig= Fin, Friedrich-Wilhelm-Luebke-Koog 1-3= Fri 1-3, Friedrichskoo-Spitze= Fris, Galmsbuellkoog= Gal, Großenbrode= Gro, Hamburger Hallig 1-2= Ham 1-2, Heiligenhafen inland lake 1-4= Hei 1-4, Heiligenhafen natural reserve ´´Graswarder´´ 1-3= HeiG 1-3, Helgoland 1-7= Hel 1-7, Hindenburgdamm 1-2= Hin 1-2, Hus= Husum, Kaiser-Wilhelm-Koog= Kai, Kiel= Kie, Meldorf= Mel, Moenkeberg= Moe, Nordstrand 1-4= Nor 1-4, Pellworm= Pel, Schluettsiel 1-2= Sch1-2, Schobüll= Scho, Stein= Ste, Sylt 1-2= Syl 1-2, Wackerballig= Wac, Woerden= Woe, Wulfen= Wul).

Field collections, sample preparation and culturing of Ulva. Samples of *U. compressa* were collected along the coastline of the German state of Schleswig-Holstein and adjacent areas, including sites located at the Baltic Sea, the Wadden Sea and on Helgoland in summer 2014 (July-August), spring 2015 (April) and summer 2015 (August-September) (Fig. 1, Table S1 in online supplement). Stations located at the Baltic Sea were shallow lagoons and estuaries, which are typical habitats of the populations of *Ulva* in this sea area. They were sampled on the intertidal zone (~ 30-100 cm), and drifting material was collected by hand or with a scoop net. In the Wadden Sea, the intertidal zone with its extensive mudflats was sampled during low tide, while overflow basins and drain channels within salt marshes were sampled during high tide.

Each sample was preserved as a herbarium voucher, while a subsample was kept for morphological and microscopic analysis. Additionally, a small thallus piece was dried on silica gel or frozen for genomic DNA extraction. Voucher specimens were deposited in the GEOMAR herbarium. Photos of specimens were taken during macro- and micromorphological analysis of the sampled material.

Algal cultures preparation. Small thallus parts of selected distromatic, fertile thalli of *U. compressa* were washed thoroughly and repeatedly with artificial *Ulva* culture medium (UCM) (Stratmann et al. 1996) to remove dirt and adhering impurities and were isolated into cultures. A total of 20 unialgal cultures were generated from freshly sampled material and cultivated in UCM at 18°C in polystyrene culture flasks (Sarstedt) under 40-70 µmol/m^2 s and a 17/7 hrs light/dark photo regime (Wichard and Oertel 2010). The wildtype *U. mutabilis* Føyn strain wt-G (mt-) (gametophyte); (mt(-); G/PS-swi$^+$; mut$^-$; RS140$^+$; RS180$^+$); and its "slender" mutant sl-G(mt+)5 (gametophyte) (mt(+); Sl$^+$; G/PS-swi$^-$; mut$^-$; RS140$^+$; RS180$^-$) are direct descendants of the original isolates of B. Føyn from the South Atlantic coast of Portugal near Olhão (Føyn 1958). The abbreviations are defined in the following: mt = mating type; G/PS-swi = capability to switch between the gametophyte and parthenosporophyte modes of parthenogenetic propagation pathways (Hoxmark 1975); mut = mutational instability (i.d."mutabilis trait") (Fjeld and Børresen 1975, Børresen and Fjeld 1977); RS140 and RS180 (see GenBank acc. no.: EU256378, EU263358, EU263359) = chromosomal repetitive sequences (Oertel et al. 2015); Sl = semidominant "slender" mutation (Føyn 1959). The *U. mutabilis* strains, which were used for the mating experiments and comparative studies with *U. compressa,* do not switch between various morphotypes anymore (mut$^-$). The original strains of *U. mutabilis* are kept at the Friedrich Schiller University Jena at the Institute for Inorganic and Analytical Chemistry (Dr. Thomas Wichard).

Induction of sporulation and purification of gametes. We first assessed the life stage and sex type of each of the cultivated isolates. Gametogenesis and sporogenesis of matured thalli of the cultured isolates of *U. compressa* were induced by chopping and washing the cultivated material, which results in the removal of sporulation inhibitors (Stratmann et al. 1996, Kessler et al. 2018). Swarming inhibitors were removed after three days by rewashing the material as described by Wichard and Oertel (2010). Micromorphological observations and additional phototaxis tests determined the life stage of released swarmers (gametophytic or sporophytic). Swarmers showing positive phototactic behaviour were assumed to indicate gametophytic cultures and were selected. Gametes released by the cultures were freed of bacteria under a laminar flow hood and concentrated (Spoerner et al. 2012, Califano and Wichard 2018).

Mating experiments. A working solution containing 40 gametes / µL was prepared by diluting the stock solutions of the purified gametes. Gametogenesis in both *U. mutabilis* strains (slender (sl-G[mt+]) and wildtype (wt-G[mt-]) was induced simultaneously with the release of swarmers of the *U. compressa* material. Mating tests between *U. mutabilis* slender (sl-G[mt+]) and *U. mutabilis* wildtype (wt-G[mt-]) gametes were performed to assess the sex of each of the *U. compressa* cultures previously determined as gametophytes. Two cultures showed positive interactions with *U. mutabilis* (wt-G[mt-]) gametes and formed zygotes. These *U. compressa* cultures were given the sex type *U. compressa* (wt-G[mt+]) following the classification by Føyn (1958) - and used in the crossing experiment.

To assess the potential of *U. compressa* to generally hybridize with other *Ulva* species, gametes of *U. compressa* were also crossed with gametes of *U. linza* and *U. rigida*.
Crossings of *U. mutabilis* (wt-G[mt-]) and *U. compressa* (wt-G[mt+]) were performed in three replicates on a glass slide, observed continuously under a light microscope and photographically documented. The crossings *U. mutabilis* (sl-G[mt+]) × *U. mutabilis* (wt-G[mt-]) and *U. mutabilis* (sl-G[mt+]) × *U. compressa* (wt-G[mt+]) were used as positive and negative controls, respectively, and also conducted in three replicates. Drops of gametes (200 µL each) with equal stoichiometric ratios of the two mating partners were pipetted onto opposite ends of a microscopic slide, with the light cone of the condenser focused on the centre of both drops. In gamete fusion experiments, the concentrated suspensions of gametes with known mating type and the test sample of gametes with unknown mating type were manually fused with a glass stick. Mating or rejection reactions of the gametes could then be observed and documented. In case of gamete pairing, the pairs were tumbling and settling within a few seconds after mixing, while the gamete type outnumbering the other sex showed positive photo accumulation. In this way, settling or backward swimming gamete pairs could be separated from zygotes which accumulated at the dark side of the mixed drop (Føyn 1934, Nordby 1976). However, as the separation was not complete, zygotes and non-mated gametes

from each crossing experiment were pipetted into sterile cell culture flasks (Sarstedt, Germany) filled with 80 mL UCM and oriented with the flat side of the culture dish towards a light source. To support the complete development of thalli, we added 10 µL of the bacterial strains MS2 (*Roseovarius* sp.) and MS6 (*Maribacter* sp.) at a density of ~ 10^6 cells / mL. Active, swimming gametes migrated towards the light source, while zygotes sank to the bottom of the culture dish. After 24 h of incubation under the permanent light, the settlement was allowed to take place during 24 h incubation in darkness. Overall morphology, rhizoid formation and homogeneity of developing thalli were observed with an inverted microscope (Leica, Wetzlar, Germany). The culture medium was replaced every two weeks with sterile UCM under a laminar flow hood, and morphotypes expressed in the cultures were controlled after 4 weeks. The potential of each pair of mating partners to form fertile, diploid sporophytes was verified by observation of the life stage of the first-generation offspring after three months. The release of swarmers from this first-generation offspring was induced, using the same afore mentioned methods as in the crossing experiment and the controls. To draw inferences about the life stage, the number of flagella of the released swarmers was observed on harvested thalli at the bottleneck of the culture dishes.

DNA extraction, amplification and sequencing. Genomic DNA was isolated from 5-10 mm² of freeze-dried or silica gel-dried epiphyte-free algal tissue with an Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany) following the manufacturer's protocol. Extracted DNA was stored at -80°C. The polymerase chain reaction (PCR) was used to amplify the plastid-encoded marker *tuf*A. PCR amplifications were realised using Phusion High Fidelity Polymerase (Thermo Fisher Scientific, Massachusetts, USA), following the manufacturer's instructions with a final volume of 40 µL per reaction. The primers *tuf*GF4 (Saunders and Kucera 2010) and *tuf*AR (Famà et al. 2002) were employed to amplify the *tuf*A gene. PCR reaction of *tuf*A was conducted with the following profile: initial 4 min denaturation at 94°C, 38 cycles of 94 °C for 1 min, 55°C for 30 sec, 72°C for 1 min, followed by 72 °C final extension for 7 min. *PCR* products were purified and sequenced in both directions by GATC Biotech (Konstanz, Germany). Forward and reverse sequences of each sample were assembled, reciprocally edited with Sequencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, MI) and were deposited in GenBank (MG575232-MG575342). BLAST searches were performed to confirm that amplicons were from sampled species and not epibionts. The consensus sequences of 126 *U. compressa* samples were aligned with those of the two clonal lab strains of *U. mutabilis*. Sequences were aligned manually using BioEdit v. 7.2.5 (Hall

1999). Sequences divergence values were calculated with the uncorrected p distance in MEGA v. 5.0 (Tamura et al. 2011).

Species delimitation We tested morphological identifications by estimating the number of Molecular Operational Taxonomic Units (MOTUs) (sensu Blaxter 2004) in the dataset using *tuf*A sequences. We applied the Generalized Mixed Yule-Coalescent (GMYC) method (Fujisawa and Barraclough 2013), a likelihood-based technique that uses a time-calibrated (ultrametric) tree to detect at which point the branching pattern of the tree changes from interspecific to intraspecific (Pons et al. 2006). Recent analyses showed that adding lineages outside the group of interest may improve the performance of the GMYC model (Ahrens et al. 2016). Therefore 59 sequences of Ulvales were added to the dataset. An ultrametric tree was created with the software package BEAST (Drummond and Rambaut 2007) using a strict clock model, a substitution rate fixed to one and a constant coalescent prior. The best-fitting substitution model was calculated using jModelTest (Posada and Crandall 1998). Four BEAST runs with identical prior settings were conducted, each with 20^8 generations and sampling every 20^4. The convergence of each analysis was checked with Tracer (Rambaut and Drummond 2003) to examine that the adequate sample size (ESS) values were > 200. Of the four runs performed, each reached ESS values of >200, and the ´burnin was set for one-half of the generations. Trees from each run were combined and summarised to a maximum credibility tree using TreeAnnotator. The consensus tree was analysed using the single-threshold GMYC model as implemented in the package SPLITS (Ezard et al. 2009) in R (http://www.R-project.org). The single-threshold model ($L_{GMYCsingle} = 1602.444$) in GMYC was better than the null model ($L_0 = 1588.738$, $P\text{-value} = < 0.001$) and had higher likelihood values.

Results

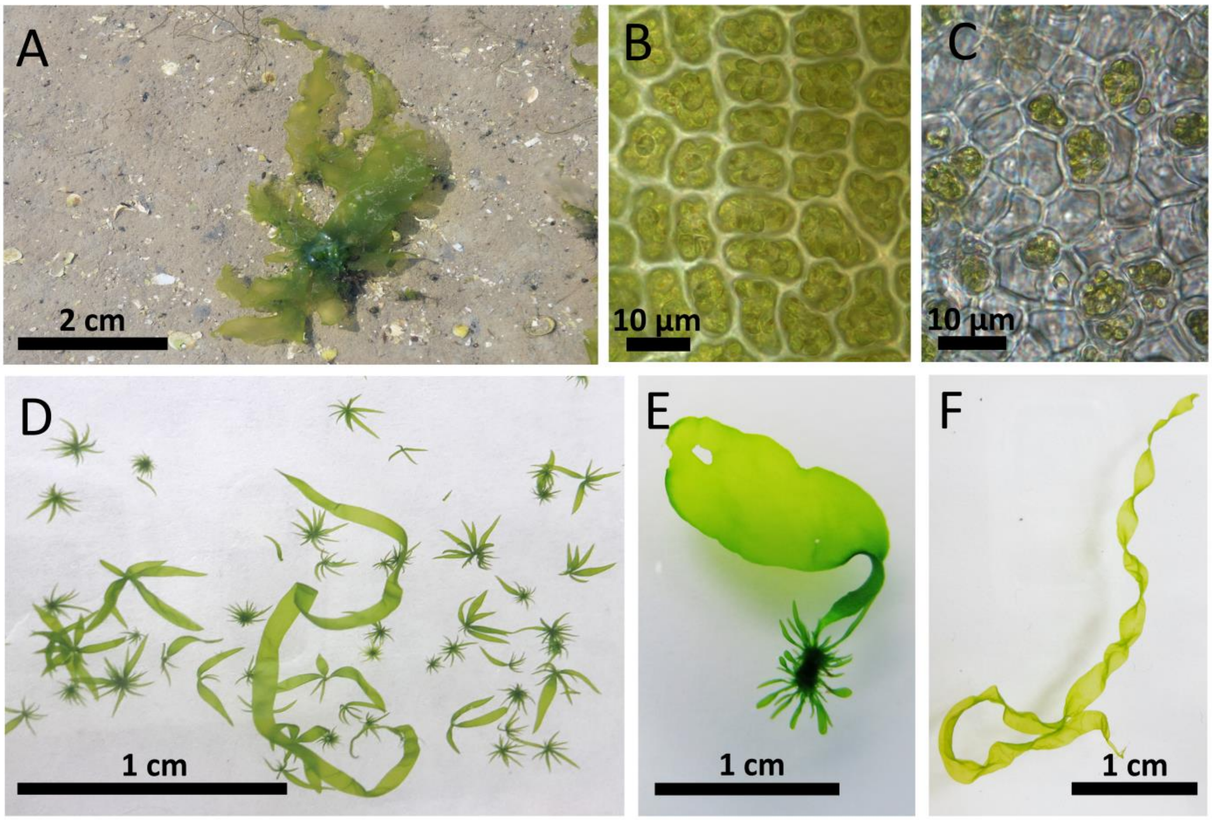

Figure 2: *Ulva compressa* source material and subsequently generated cultures used in this study. (A) Wildtype source material of *U. compressa* from Friedrich-Wilhelm-Lübke-Koog used for gamete release. (B) Overview of mature gametangia and (C) empty gametangia after gamete release. (D) A three weeks old culture generated from gametes released by the source material. Within the wildtype dominated culture, spontaneous slender-like developmental mutants were observed. (E) Typical specimen exhibiting the wildtype morphology and (F) specimen showing similarity to the fast growing developmental mutant slender of *Ulva mutabilis* after 2 months.

Identification and cultivation. *Ulva compressa* can be stably cultivated under laboratory conditions by using purified, mature source material (Fig. 2A). Isolated strains were identified as *U. compressa* by molecular methods based upon analysis of the barcode marker *tuf*A. Importantly, sporulation of the isolated strains could be induced by mincing and washing thallus fragments, which removed sporulation inhibitors in a similar way as in *U. mutabilis.* During the second day after induction, the cells further differentiated into gametangia containing about 16 progametes. The progametes matured during the following night into fully developed gametes that were ready for swarming (Fig 2B-C). In this way, clonal strains for comparative studies could be generated by breeding unmated parthenogametes. A morphological instability was also observed within the *U. compressa* cultures, similar to the initial observation in *U. mutabilis* (Føyn 1958, Borresen and Fjeld 1977) (Fig. 2D). While more than 99% of the offspring exhibited a morphology similar to the wildtype source material (Fig. 2E), < 1 % of the thalli showed a fast growing, elongated form (Fig. 2F) in the descendant generations that developed within two months of cultivation. These elongated, slender-like forms of *U. compressa* exhibited only a few traces of the wildtype morphotype. A distinct rhizoidal zone – as present in thalli exhibiting the wildtype morphology – was reduced or even completely missing in these mutants, even though all isolates obtained the same amount of the rhizoid promoting factor excreted by *Maribacter* sp. (Spoerner et al. 2012, Vesty et al. 2015). After being sessile for about two weeks, the slender-like specimens detached and remained drifting.

In contrast, specimens exhibiting wildtype, morphology had a well-marked rhizoidal zone and generally remained attached to the bottom of culture dishes. The growth of cultured *U. compressa* was observed until the spontaneous induction of gametes under quasi-natural conditions. The spontaneous gametogenesis and release of swarmers were observed in wildtype-like thalli after at least four weeks or even later, whereas some thalli are exhibiting the elongated slender-like morphology matured about 3-5 days earlier indicating a faster life cycle as in *U. mutabilis.*

Interbreeding. One out of 20 *U. compressa* cultures that had been raised from sampled fresh material was gametophytic, as it released biflagellate swarmers. All other cultures either remained sterile or were sporophytic, releasing quadriflagellate swarmers. Phototaxis tests on the released biflagellate swarmers revealed them to be positive phototactic. In contrast, the quadriflagellate swarmers were either not affected by light or negative phototactic. The average size of the released biflagellate gametes was 6.2 µm and that of quadriflagellate zoids 6.4 µm. *U. compressa* gametes were evidently attracted by gametes of *U. mutabilis* (wt-G[mt-]) (parental generation). A formation of zoids was subsequently found in the filial generation F1, while no attraction nor any zoid formation in generation F1 was found when gametes of *U. mutabilis* (sl-G[mt+]) were paired with those of *U. compressa* in the parental generation.

Thus we determined the gametophytic culture of *U. compressa* as sex-positive - *indicated as U. compressa* (wt-G[mt+]) - following the mating type classification defined by Føyn (1958) for *Ulva mutabilis.* This mating type classification requires no analysis of the genetic loci of sex determination - which remain unknown for *Ulva* - as it is solely based on an assessment of compatibilities among algal isolates.

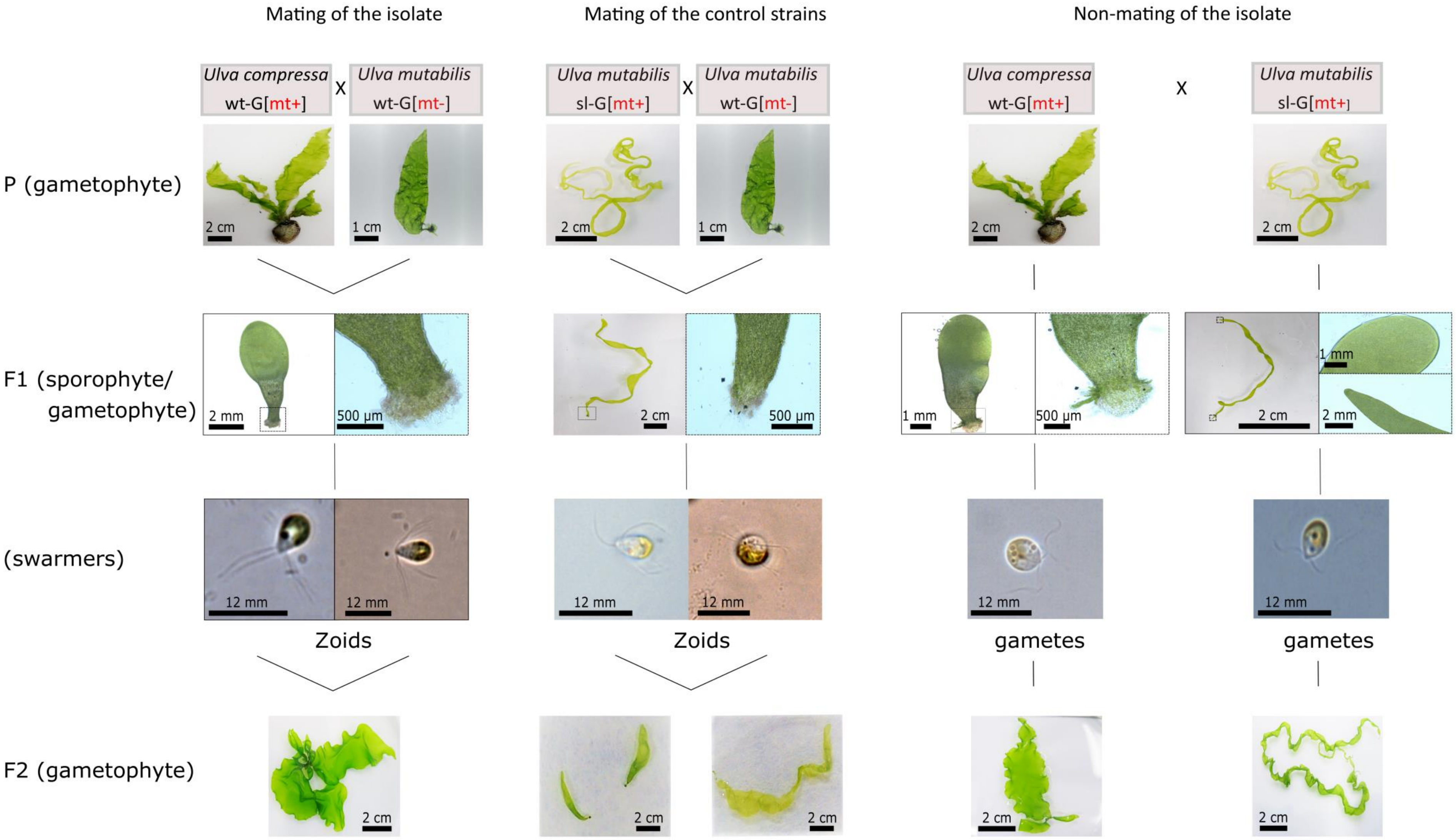

Figure 1: Overview of crossing experiments. Parents and first and second generation offspring of the crossing *Ulva mutabilis* (wt-G[mt-]) × *Ulva compressa* (wt-G[mt+]) and of positive (*Ulva mutabilis* (sl-G[mt+]) × *Ulva mutabilis* (wt-G[mt-])) and negative control (*Ulva mutabilis* (sl-G[mt+]) × *Ulva compressa* (wt-G[mt+])) crossings. The parental gametophytic mating partners, and their respective filial 1 and filial 2 generations and their released swarmers are shown (image of P gametophyte (wt-G[mt-])) is a reprint from Wichard (2017) with permission). The experiment was repeated three times showing identical results.

A crossing experiment (mating partners: *U. mutabilis* (wt-G[mt-]) × *U. compressa* (wt-G[mt+])) was performed after the assessment of life stage and sex, involving positive (mating partners: *U. mutabilis* (sl-G[mt+]) × *U. mutabilis* (wt-G[mt-])) and negative controls (mating partners: *U. mutabilis* (sl-G[mt+]) × *U. compressa* (wt-G[mt+])) (Fig. 3).

Gametes of *U. compressa* (wt-G[mt+]) and *U. mutabilis* (wt-G[mt-]) directly started to revolve around each other, indicating a strong attraction to their mating partners. Transitional, heart-shaped-like stages appeared and indicated the fusion of gametes and the development of zygotes. The same patterns were also observed in the positive control pairing of compatible mating partners (*U. mutabilis* (sl-G[mt+]) × *U. mutabilis* (wt-G[mt-])). In contrast, no copulation was observed in the negative control with mating partners of the same sex type (*U. mutabilis* (sl-G[mt+]) × *U. compressa* (wt-G[mt+]).

Variable differences between different pairings of gametes became visible after cultivation for three weeks under standard conditions (Fig. 3). In negative controls, specimens of the first generation offspring could be separated into two different morphotypes (Fig. 3). A fast-growing drifting phase of thalli - resembling the slender form of parent *U. mutabilis* (sl-G[mt+]) with no or reduced rhizoid - could be distinguished from an attached phase of wildtype-like thalli that exhibited a well-developed rhizoidal zone, similar as parent *U. compressa* (wt-G[mt-]). In contrast, thalli in positive controls were more homogeneous and exhibited traits of both parents, as they had a slender-like elongated form (as parent *U. mutabilis* (sl-G[mt+]) but were attached with a well-developed rhizoid (as parent *U. mutabilis* (wt-G[mt-]). Thalli resulting from the crossing of *U. mutabilis* (wt-G[mt-]) × *Ulva compressa* (wt-G[mt+]) grew uniformly and were attached to the culture dish and had a well-developed rhizoid but a wildtype-like shape (Fig. 3). The observed patterns were identical in each of the three replicates.

After three months of cultivation, sporogenesis was induced, and swarmers were discharged from sporangia, creating the F2 generation (Fig. 3). In case of the negative controls, swarming was separately induced in both morphotypes. Swarmers liberated by wildtype-shaped attached thalli and slender drifting thalli all showed a positive phototaxis and two flagella.

In contrast, swarmers released by the F1 offspring in the two compatible pairings *U. mutabilis* (sl-G[mt+]) × *U. mutabilis* (wt-G[mt-]) and *U. mutabilis* (wt-G[mt-]) × *U. compressa* (wt-G[mt+]) exhibited four flagella (Fig. 3), and phototaxis tests were negative. The observation confirms the existence of sporophytes in the F1 offspring in contrast of the identified gametophytes in the negative control (Fig. 3).

A test of the general hybridizing behavior of *U. compressa* gametes by their ability to mate with *U. linza* or *U. rigida* gametes was negative, and no attraction nor intermediate states of paired gametes were observed. For this purpose, sporogenesis of specimens of *U. linza* and *U. rigida* that were sampled in August 2014 in the Wadden Sea (Friedrich-Wilhelm-Lübke-Koog 1, for geographic coordinates see supplementary table S 1) and subsequently taken into culture was induced by the above mentioned method.

Sequence analysis and species delimitation We obtained a total of 126 *tuf*A sequences of *U. compressa* specimens from Northern Germany, and two additional *tuf*A sequences of the original lab strain slender (sl-G[mt+]) and of the wildtype material (wt-G[mt-]) of *U. mutabilis.* Accession numbers for all sequences are available in Table S1 in the online supplement. All *tuf*A amplicons were 773 bp long and were aligned without gaps. No stop codons were found after translation into amino acids. The alignment contained 443 conserved nucleotides and 340 variable ones. The best-fit model selected in jModelTest according to the AIC was the HKY+I +G. Uncorrected p distances ranged from 0 to 0.08 among sequences of *U. compressa*, and the lowest value of the interspecific distance of 0.07 showed a slight overlap with other *Ulva* species and the out-group.

The GMYC model determined 18 MOTUs (confidence interval, 16-21). All sequences selected as outgroups were clustered according to their taxonomic assignment (Fig. 4A), except *U. lactuca*, which clustered within the *U. fasciata* group. All sequences of *U. compressa* and *U. mutabilis* were found in a single well supported clade (posterior probability value 0.99) with no evidence of distinction based on phylogenetic relationships. The only structural particularity was a clade of *U. compressa* from the Pacific that was found in a distinct well supported clade, sister to the rest of *U. compressa* sequences. The threshold obtained in the GMYC analysis confirmed the inclusion of all *U. compressa* and *U. mutabilis* in a single MOTU. The phylogenetic tree (Fig. 4A), the lineage-through-time plot (Fig. 4B) and the likelihood function (Fig. 4C) were congruent.

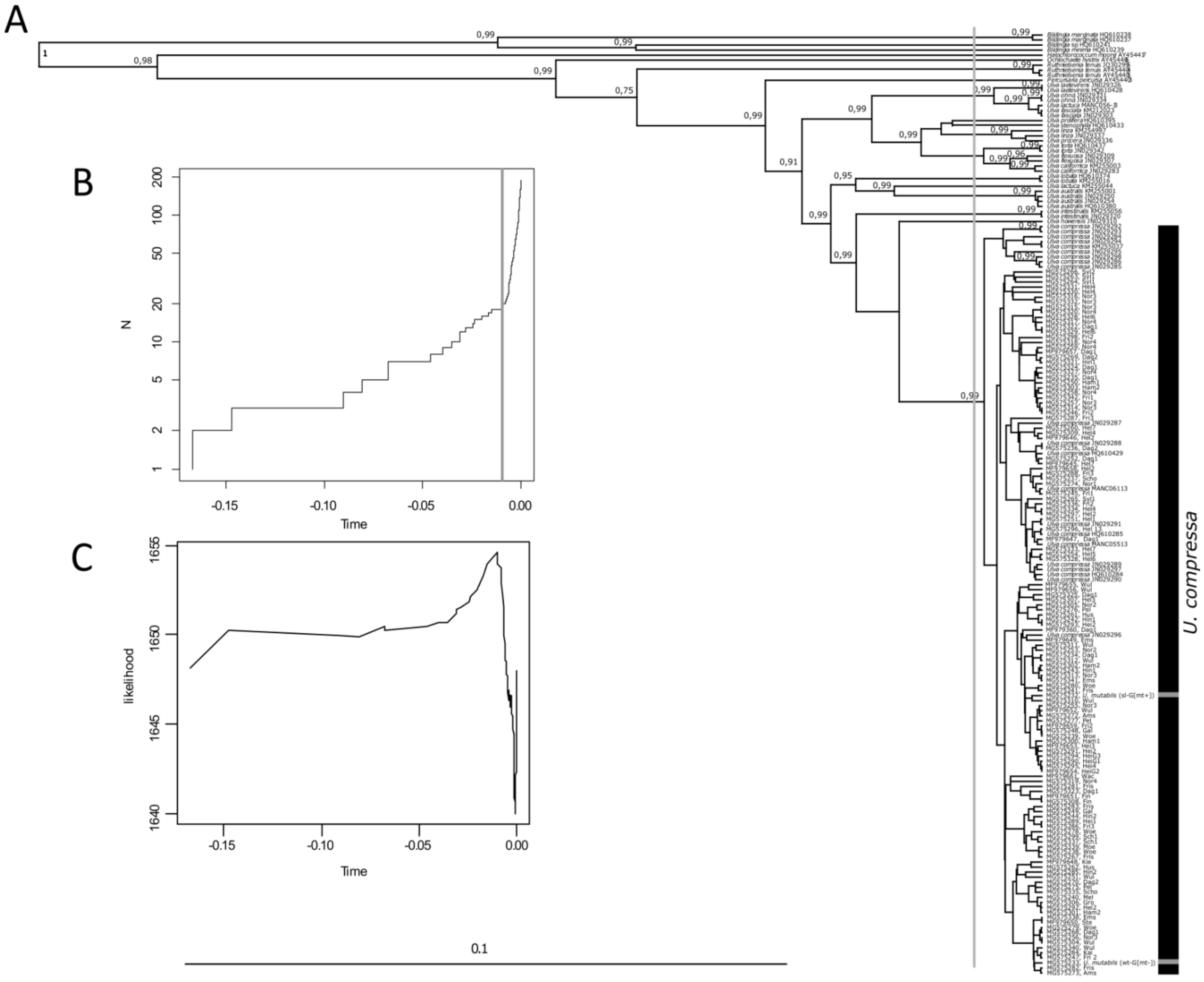

Figure 4: Result of the Generalized Mixed Yule-Coalescent (GMYC) analysis for the *tuf*A dataset. In the phylogenetic tree (A) posterior probability values > 0.70 are provided at each branch. A vertical grey line visualizes the threshold between intra- vs. inter-species variation. The cluster of *Ulva compressa* samples is indicated by a bold black bar besides the tree while grey horizontal bars mark the position of *Ulva mutabilis* (sl-G[mt+]) and *Ulva* mutabilis (wt-G[mt-]) within the *Ulva* compressa cluster. Abbreviations behind the accession numbers refer to sampling sites as used in Figure 1. (B) Lineage-through-time plot based on the ultrametric tree obtained from all tufA sequences included in this analysis. The increase in branching rate corresponds to the transition from interspecific to intraspecific branching events, is indicated by a grey vertical line. (C) Likelihood function produced by GMYC to estimate the peak of transition between cladogenesis and allele intraspecific coalescence along the branches (a high resolution version of the GMYC tree is shown in the online supplement Figure S1).

Discussion

We used the GMYC model to test the presence of species boundaries between *U. compressa* and *U. mutabilis*. *Tuf*A sequences generated from specimens sampled throughout Northern Germany, including sampling sites at the German Baltic- and the Wadden Sea, were used in the phylogenetic analysis (Fig.1). By extensive sampling, we were able to ensure inclusion of *U. compressa* samples from different populations in varying ecosystems and geographically separated habitats (Fig.1).

Despite the broad sampling, we found no particular structure in the phylogenetic analysis (Fig.4), with the exeption of a separated clade containing *U. compressa* from the Pacific. This phylogenetic structure may result from the more distant geographic origin of the Pacific samples, however the confidence interval of the GMYC analysis does not exclude that these specimens may represent a distinct MOTU. Further analyses are beyond the scope of this work and may require further sampling from areas of distribution of *U. compressa* that were not covered in this study.

Culturing *Ulva* species often helps to understand their morphological plasticity and in particular any differences and similarities between cultivated material and natural populations (Bliding 1960, Bliding 1963, Koeman and van den Hoek 1982a, Koeman and van den Hoek 1982b, Koeman and van den Hoek 1984, Hiraoka et al. 2017). In our culture studies, thalli were raised from gametes of *U. compressa* exhibited two different gross morphologies similar to the wildtype and the slender form displayed by *U. mutabilis*. These were equally treated as gametes of *U. mutabilis*. The predominantly blade-like wildtype thalli developed a clear rhizoidal zone and remained attached (Fig. 2). In contrast, slender-like thalli developed a strongly elongated thallus that grew rapidly and quickly switched from an attached phase into a drifting phase, due to the lack of real rhizoidal cells. These slender-like forms appeared spontaneously and corresponded extremely well to spontaneous mutations previously described in isolates of *U. mutabilis* (Føyn 1958, Bryhni 1974, Fjeld and Borresen 1975). *S*pontaneous switches between the wildtype and the slender morphologies of *U. mutabilis* were originally observed with a frequence of 4×10^{-4} (Fjeld and Borresen 1975, Borresen and Fjeld 1977), but this genetic instability is variable and can be about 10 times higher in certain isolates (Fjeld and Lovlie 1976). We found a similar spontaneous mutability of the *compressa* strain, but further studies are necessary to evaluate the potential spontaneous reversion of those developmental mutant strains.

In any case, wild populations with similar morphological variations of *Ulva compressa* have been described along the Scottish east coast by Tan et al. (1999), who suggested that they might be caused by different salinities. However, the distribution and abundance of *U. compressa* phenotypes within Schleswig-Holstein was verified by us in an additional study, and this survey indicated that salinity has probably no influence on the overall morphology in *U. compressa*, since specimens of different morphology were occasionally found together at the same collection sites (data not shown).

In conclusion, our culturing approach revealed numerous developmental similarities between *U. compressa* and *U. mutabilis,* and it thereby complemented the findings of previous studies which focused on other aspects. Shared by both species is a distromatic sheet-like morphotype, that corresponds in *U. mutabilis* with the wildtype (Føyn 1958, Tan et al. 1999, Brodie et al. 2007) and differs from the holotype of *U. compressa* (Linnaeus 1753, Blomster et al. 1998). The distromatic morphotype is characterized by cells that are arranged irregularly, in short rows or rosettes and usually contain one pyrenoid and one parietal, hood-shaped chloroplast, which is mostly located at the apical end of the cell (Føyn 1958, Tan et al. 1999, Brodie et al. 2007). However, the cell layers of the distromatic sheets are not neccessarily fixed to each other and tubular fronds can occur (Bonneau 1977), linking the sheet-like morphotype to the tubular branched morphology of the holotype.

Similar gamete size and regulation of germ cell formation are prerequisites for con-specificity and measurements of the cell size of gametes of *U. compressa* conducted in our study coincide with those made by Bråten (1971) for gametes of *U. mutabilis*. We also observed that sporulation and swarming inhibitors controlled the release of swarmers from mature thalli of *U. compressa* in the same way as it was described for *U. mutabilis* (Stratmann et al. 1996, Wichard and Oertel 2010). Further, metabolic processes such as the wound-induced production of oxylipins – which typically differ among algal taxa - were also observed to be highly similar in *U. mutabilis* and *U. compressa* (Alsufyani et al. 2014). A significant difference between both taxa is in the length of the life cycle (Stratmann et al. 1996, Spoerner et al. 2012), however, this did not result in a species separation.

Given the considerable genetic and phenotypic similarity between *U. compressa* and the clonal descendants of original *U. mutabilis* strains collected and cultivated by Føyn (1958), we hypothesized that gametes of both species should also be able to mate and produce fertile offspring. So far only a few studies tested the biological species concept by crosses and hybridization experiments within the genus *Ulva* (Føyn 1958, Nordby 1976, Larsen 1981, Hiraoka et al. 2004, Hiraoka et al. 2011, Hiraoka et al. 2017). To conduct crossings, it is mandatory to have the gametophytic life stage, which generates biflagellate gametes and not quadriflagellate zoids. We were able to demonstrate that the induction of swarmer release can be used to sort mature thalli of *U. compressa* into gametophytes and sporophytes. Similar as in *U. mutabilis*, gametes and zoids of *U. compressa* exhibited opposing phototactic behavior. To assign the respective sex-type to *U. compressa* cultures, we followed the mating type classification system developed by Føyn (1958) for *U. mutabilis* and identified our gametophytic *U. compressa* culture as sex positive.

Our mating experiments finally revealed that no reproductive boundary between *U. compressa* and *U. mutabilis* is present, as fertile sporophytic offspring was obtained from the gametophytic source material (Fig.3). Positive and negative controls with pairings of compatible and incompatible mating types strengthened the robustness of our experiment. Thalli that developed after the pairing of incompatible gametes exhibited the two distinct morphologies of either the *U. compressa* or the *U. mutabilis* parental isolate. Also, both forms released biflagellate swarmers, which confirmed that the gametes released by the parental material were of the same sex and not copulating, resulting in the formation of gametophytic clones from gametes (Fig. 3). In contrast, a sporophytic first-generation-offspring with a uniform phenotype that released quadriflagellate swarmers developed when compatible gametes of *U. mutabilis* were paired as a positive control (Fig.3). Likewise, a pairing of gametes released by *U. mutabilis* (wt-G[mt-]) and *U. compressa* (wt-G[mt+]) resulted in sporophytic first-generation-descendants that released quadriflagellate swarmers and exhibited conformity in the phenotypic appearance (Fig. 3), which clearly confirmed their mating ability.

Following the biological species concept introduced by Mayr (1942) *U. compressa* and *U. mutabilis* are not distinct biological species. Although this concept is widely accepted, its application becomes challenging in cases of hybridisation. However, *U. compressa* gametes were in our experiments not generally able to hybridize with closely related species, such as distromatic *U. rigida* and tubular *U. linza.*

Nevertheless, the lines of a species definition still blur in certain cases, and under such conditions, combined approaches that focus on multiple delimitation methods become more important. Based on our investigation - that unequivocally confirms a very close genetic and phenotypic relationship of *U. compressa* and *U. mutabilis* and additionally confirms no reproductive boundaries - *U. compressa* and *U. mutabilis* can be treated as conspecific. This supports former considerations that *U. mutabilis* is very closely related to *U. compressa* despite considerable morphological differences (Wichard and Oertel 2010) and that the *Ulva* *compressa*/*mutabilis*/*pseudocurvata* clade is globally distributed (Wichard et al. 2015). We suggest that *U. mutabilis* is reduced to synonymy with *U. compressa*. Other homotypic synonyms of *U. compressa* L. are according to Guiry and Guiry (2018):

Ulva compressa Linnaeus, C. 1753. *Species plantarum*, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Vol. 2 pp. [i], 561-1200, [1-30, index], [i, err.]. Holmiae [Stockholm]: Impensis Laurentii Salvii.

Homotypic synonyms:

Conferva compressa (Linnaeus) Roth 1797

Tubularia compressa (Linnaeus) Roussel 1806

Scytosiphon compressus (Linnaeus) Lyngbye 1819

Enteromorpha compressa (Linnaeus) Nees 1820

Fistularia compressa (Linnaeus) Greville 1824

Solenia compressa (Linnaeus) C.Agardh 1824

Ilea compressa (Linnaeus) Gaillon 1828

Fistularia intestinalis var. *compressa* (Linnaeus) J.P.Jones and Kingston 1829

Hydrosolen compressus (Linnaeus) C.Martius 1833

Enteronia compressa (Linnaeus) Chevallier 1836

Enteromorpha vulgaris var. *compressa* (Linnaeus) Edmondston 1845

Ulva enteromorpha var. *compressa* (Linnaeus) Le Jolis 1863

Enteromorpha intestinalis var. *compressa* (Linnaeus) Rosenvinge 1893

Enteromorpha intestinalis subsp. *compressa* (Linnaeus) M.W.R.N.de Silva and E.M.Burrows 1973

Ulva mutabilis (Linnaeus) Føyn 1958

Although we suggest *U. mutabilis* as a junior synonym of *U. compressa*, we highly recommend indicating the lab strains as cultivars of *U. compressa*, following the International Code of Nomenclature for cultivated Plants (ICNCP), as *U. compressa ´mutabilis´*. We advise to retain this nomenclatural indication exclusively for the genetically different material of *U. mutabilis* isolated by Føyn (1958) for several reasons: Due to parthenogenesis, clones of the gametophytic source material were cultivated until today, and a high number of valuable studies was conducted on this clonal material (e.g., summarized in several studies and reviews; e,g, Løvlie (1964) and Løvlie and Bryhni (1978); Oertel et al. (2015) (genetics) or Wichard et al. (2015) (morphogenesis)). A selection of this well characterized morpho- and genotypes - such as *slender, wildtype, long, lumpy* and *bubble* - is still available, and after decades of repeated asexual clonal propagation, these strains may now largely differ from the original natural material.

Finally, the first successful, stable transformations of macroalgae were achieved by PEG-mediated direct DNA transfer into germ cells of *U. mutabilis* (Oertel et al. 2015). Our finding of conspecificity of this emerging model system and the globally distributed species *U.* compressa facilitates future studies and will allow expanding the model system to local strains worldwide and intriguing studies to identify quantitative trait loci, which might correlate with the variation of *Ulva´s* morphtypes.

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Supplement

Table S 1: Sample list with collection sites of *Ulva compressa*. All samples listed were used in the GMYC analysis. Accession No.= GenBank accession number. Abbreviation = used in figure 1.

Accession No.	Specimen voucher	Collected by	Collection date	Country	Abbreviation	Lat	Lon
MG575234	S_107	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575235	S_120	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575236	S_128_plu	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Schluettsiel	Sch 2	N 54°40.880	E 008°45.265
MG575237	S_151	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Schobuell	Scho	N 54°30.469	E 008°59.734
MG575238	S_155	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Woehrden	Woe	N 54°07.039	E 008°56.156
MG575239	S_157	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Woehrden	Woe	N 54°07.039	E 008°56.156
MG575240	S_166	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Meldorf	Mel	N 54°04.658	E 008°58.087
MG575241	S_171_A	S. Steinhagen	06.08.2014	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	Fris	N 54°02.246	E 008°50.691
MG575242	S_182	S. Steinhagen	11.08.2014	Germany:Schleswig-Holstein, Hindenburgdamm	Hin1	N 54°52.949	E 008°36.186
MG575243	S_183	S. Steinhagen	11.08.2014	Germany:Schleswig-Holstein, Hindenburgdamm	Hin1	N 54°52.949	E 008°36.186
MG575244	S_188	S. Steinhagen	12.08.2014	Germany:Schleswig-Holstein, Hindenburgdamm	Hin 2	N 54°53.468	E 008°36.410
MG575245	S_443	S. Steinhagen	08.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 1	N 54°51.402	E 008°36.206
MG575246	S_447	S. Steinhagen	09.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 2	N 54°49.998	E 008°36.852
MG575247	S_452	S. Steinhagen	09.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 2	N 54°49.998	E 008°36.852
MG575248	S_468	S. Steinhagen	10.09.2014	Germany:Schleswig-Holstein, Galmsbuellkoog	Gal	N 54°45.669	E 008°41.802
MG575249	S_473	S. Steinhagen	10.09.2014	Germany:Schleswig-Holstein, Galmsbuellkoog	Gal	N 54°45.669	E 008°41.802
MG575250	S_506	S. Steinhagen	16.09.2014	Germany:Schleswig-Holstein, Hamburger Hallig	Ham 1	N 54°35.939	E 008°48.730
MG575251	S_514_C	S. Steinhagen	18.09.2014	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575252	S_631	S. Steinhagen	17.04.2015	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575253	S_652	S. Steinhagen	21.04.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 2	N54°28.243	E 008°48.410
MG575254	S_713	S. Steinhagen	23.04.2015	Germany: Helgoland	Hel 5	N54° 10.62937	E007°53.57712

MG575255	S_742	S. Steinhagen	01.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.16312	E 008° 49.11443
MG575256	S_743_A	S. Steinhagen	01.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.16312	E 008° 49.11443
MG575257	S_744	S. Steinhagen	02.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.16312	E 008° 49.11443
MG575258	S_748_A	S. Steinhagen	04.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.62502	E 008° 50.674
MG575259	S_749	S. Steinhagen	05.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.62502	E 008° 50.674
MG575260	S_803	S. Steinhagen	27.09.2015	Germany: Helgoland	Hel 7	N54° 11.29002	E7° 52.45398
MG575261	CL_1_A	C. Lieberum	17.07.2015	Germany:Schleswig-Holstein, Husum	Hus	N 54°28.268	E 009°01.675
MG575262	CL_1_B	C. Lieberum	17.07.2015	Germany:Schleswig-Holstein, Husum	Hus	N 54°28.268	E 009°01.676
MG575263	FLS_1_A	F. Weinberger	16.06.2015	Germany:Schleswig-Holstein, Sylt	Syl 1	N 55° 2.112	E 008° 24.01
MG575264	FLS_1_B	F. Weinberger	16.06.2015	Germany:Schleswig-Holstein, Sylt	Syl 1	N 55° 2.1112	E 008° 24.01
MG575265	FLS_2(1)	F. Weinberger	16.06.2015	Germany:Schleswig-Holstein, Sylt	Syl 1	N 55° 2.1112	E 008° 24.01
MG575266	FLS_3(3)	F. Weinberger	16.06.2015	Germany:Schleswig-Holstein, Sylt	Syl 2	N 55° 1.1	E 008° 26.375
MG575267	S_171_B	S. Steinhagen	06.08.2014	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	Fris	N 54°02.246	E 008°50.691
MG575268	S_104	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575269	S_125	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Dagebuell 2	Dag 2	N 54°43.824	E 008°41.634
MG575270	S_126	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Dagebuell 2	Dag 2	N 54°43.824	E 008°41.634
MG575271	S_12	S. Steinhagen	22.07.2014	Germany: Helgoland	Hel 1	N 54°11.290	E 007°52.454
MG575272	S_134	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Amsinck Haus	Ams	N 54°36.922	E 008°52.013
MG575273	S_135	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Amsinck Haus	Ams	N 54°36.922	E 008°52.013
MG575274	S_137	S. Steinhagen	01.08.2014	Germany:Schleswig-Holstein, Nordstrand	Nor 1	N 54°30.998	E 008°51.262
MG575275	S_141	S. Steinhagen	02.08.2014	Germany:Schleswig-Holstein, Pellworm	Pel	N 54°29.929	E 008°48.522
MG575276	S_143	S. Steinhagen	02.08.2014	Germany:Schleswig-Holstein, Pellworm	Pel	N 54°29.929	E 008°48.522
MG575277	S_144_A	S. Steinhagen	02.08.2014	Germany:Schleswig-Holstein, Pellworm	Pel	N 54°29.929	E 008°48.522

MG575278	S_158	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Woehrden	Woe	N 54°07.039	E 008°56.156
MG575279	S_159	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Woehrden	Woe	N 54°07.039	E 008°56.156
MG575280	S_160	S. Steinhagen	05.08.2014	Germany:Schleswig-Holstein, Woehrden	Woe	N 54°07.039	E 008°56.156
MG575281	S_172	S. Steinhagen	06.08.2014	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	Fris	N 54°02.246	E 008°50.691
MG575282	S_174_A	S. Steinhagen	07.08.2014	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	Fris	N 54°02.246	E 008°50.691
MG575283	S_174_C	S. Steinhagen	08.08.2014	Germany:Schleswig-Holstein, Friedrichskoog-Spitze	Fris	N 54°02.246	E 008°50.691
MG575284	S_177	S. Steinhagen	08.08.2014	Germany:Schleswig-Holstein, Kaiser-Wilhelm-Koog	Kai	N 54°56.162	E 008°54.315
MG575285	S_185	S. Steinhagen	11.08.2014	Germany:Schleswig-Holstein, Hindenburgdamm	Hin 2	N 54°53.468	E 008°36.410
MG575286	S_190	S. Steinhagen	13.08.2014	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	Fri 3	N 54°50.241	E 008°36.732
MG575287	S_192	S. Steinhagen	13.08.2014	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	Fri 3	N 54°50.241	E 008°36.732
MG575288	S_193	S. Steinhagen	13.08.2014	Germany:Schleswig-Holstein, Friedrich-Wilhem-Luebke-Koog	Fri 3	N 54°50.241	E 008°36.732
MG575289	S_335	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 1	N 54°22.767	E 010°58.943
MG575290	S_340	S. Steinhagen	22.08.2014	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	HeiG 1	N 54°22.739	E 010°59.233
MG575291	S_346	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 2	N 54°23.002	E 010°57.013
MG575292	S_348	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 2	N 54°23.002	E 010°57.013
MG575293	S_353	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 2	N 54°23.002	E 010°57.013
MG575294	S_362	S. Steinhagen	26.08.2014	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	HeiG 3	N 54°22.680	E 011°00.515
MG575295	S_368	S. Steinhagen	26.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 4	N 54°22. 664	E 010°58.823
MG575296	S_37_plus	S. Steinhagen	23.07.2014	Germany: Helgoland	Hel l3	N 54°10.950	E 007°53.437
MG575297	S_4_plus	S. Steinhagen	22.07.2014	Germany: Helgoland	Hel 2	N 54°10.189	E 007°53.365
MG575298	S_450	S. Steinhagen	09.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 2	N 54°49.998	E 008°36.852

MG575299	S_478	S. Steinhagen	12.09.2014	Germany:Schleswig-Holstein, Schluettsiel	Sch 1	N 54°41.061	E 008°45.231
MG575300	S_507	S. Steinhagen	16.09.2014	Germany:Schleswig-Holstein, Hamburger Hallig	Ham 1	N 54°35.939	E 008°48.730
MG575301	S_511	S. Steinhagen	17.09.2014	Germany:Schleswig-Holstein, Hamburger Hallig	Ham 2	N 54°35.939	E 008°48.732
MG575302	S_512	S. Steinhagen	18.09.2014	Germany:Schleswig-Holstein, Hamburger Hallig	Ham 2	N 54°35.939	E 008°48.732
MG575303	S_513	S. Steinhagen	19.09.2014	Germany:Schleswig-Holstein, Hamburger Hallig	Ham 2	N 54°35.939	E 008°48.732
MG575304	S_549	S. Steinhagen	08.04.2015	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575305	S_651	S. Steinhagen	21.04.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 2	N54°28.243	E 008°.48.410
MG575306	S_540	S. Steinhagen	08.04.2015	Germany:Schleswig-Holstein, Großenbrode	Gro	N 54°23.582	E 011°06.612
MG575307	S_626	S. Steinhagen	16.04.2015	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 3	N 54°22.723	E 010°57.327
MG575308	S_670	S. Steinhagen	21.04.2015	Germany:Schleswig-Holstein, Finkhaushallig	Fin	N 54°24.935	E 008°54.218
MG575309	S_707	S. Steinhagen	23.04.2015	Germany: Helgoland	Hel 4	N 54°10.682	E 007°53.323
MG575310	S_739_A	S. Steinhagen	31.08.2015	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575311	S_741	S. Steinhagen	31.08.2015	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575312	S_740	S. Steinhagen	31.08.2015	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575313	S_743_B	S. Steinhagen	01.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.163	E 008° 49.11445
MG575314	S_743_C	S. Steinhagen	01.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.163	E 008° 49.11445
MG575315	S_745	S. Steinhagen	02.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.163	E 008° 49.11445
MG575316	S_746	S. Steinhagen	03.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.163	E 008° 49.11445
MG575317	S_752	S. Steinhagen	06.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575318	S_754	S. Steinhagen	07.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575319	S_756_A	S. Steinhagen	08.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575320	S_756_B	S. Steinhagen	09.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575321	S_760_A	S. Steinhagen	16.09.2015	Germany:Schleswig-Holstein, Hindenburgdamm	Hin 1	N 54°52.949	E 008°36.186
MG575322	S_770	S. Steinhagen	17.09.2015	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35

MG575323	S_771	S. Steinhagen	18.09.2015	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575324	S_778	S. Steinhagen	19.09.2015	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575325	S_779	S. Steinhagen	20.09.2015	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MG575326	S_780A	S. Steinhagen	09.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575327	S_782	S. Steinhagen	10.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 4	N54° 27.625	E 008° 50.674
MG575328	S_788	S. Steinhagen	23.09.2015	Germany: Helgoland	Hel 6	N 54° 11.02	E 007° 53.31833
MG575329	S_790	S. Steinhagen	24.09.2015	Germany: Helgoland	Hel 6	N 54° 11.02	E 007° 53.31833
MG575330	S_795	S. Steinhagen	25.09.2015	Germany:Helgoland	Hel 4	N 54°10.682	E 007°53.323
MG575331	S_797	S. Steinhagen	26.09.2015	Germany: Helgoland	Hel 4	N 54°10.682	E 007°53.323
MG575332	S_747	S. Steinhagen	01.09.2015	Germany:Schleswig-Holstein, Nordstrand	Nor 3	N54° 29.163	E 008° 49.114
MG575333	S_805	S. Steinhagen	27.09.2015	Germany: Helgoland	Hel 7	N54° 11.290	E7° 52.45398
MG575334	S_799	S. Steinhagen	28.09.2015	Germany: Helgoland	Hel 4	N 54°10.682	E 007°53.324
MG575335	S_146_B	S. Steinhagen	31.07.2014	Germany:Schleswig-Holstein, Schobuell	Scho	N 54°30.469	E 008°59.734
MG575336	S_454	S. Steinhagen	09.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 2	N 54°49.998	E 008°36.852
MG575337	S_479	S. Steinhagen	12.09.2014	Germany:Schleswig-Holstein, Schluettsiel	Sch 1	N 54°41.061	E 008°45.231
MG575338	S_1003	S. Steinhagen	16.03.2016	Germany:Schleswig-Holstein, Emmelsbuell	Ems	N 54°47.692	E 008°39.484
MG575339	S_1002	S. Steinhagen	16.11.2015	Germany:Schleswig-Holstein, Moenkeberg	Moe	N 54°21.160	E 010°10.674
MG575340	S_1001	S. Steinhagen	05.07.2016	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MG575341	S_1000	S. Steinhagen	17.03.2016	Germany:Schleswig-Holstein, Emmelsbuell	Ems	N 54°47.692	E 008°39.485
MG575342	S_441	S. Steinhagen	08.09.2014	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 1	N 54°51.402	E 008°36.206
MF979650	S_ 305	S. Steinhagen	20.08.2014	Germany: Schleswig-Holstein, Stein	Ste	N 54°25.065	E 010°15.870
MF979661	S_ 79	S. Steinhagen	24.07.2014	Germany: Schleswig-Holstein, Wackerballig	Wac	N 54°45.518	E 009°52.670
MF979653	S_ 356	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen inland lake	Hei 3	N 54°22.723	N 54°22.723
MF979658	S_6	S. Steinhagen	22.07.2014	Germany: Helgoland	Hel 2	N 54°10.189	E 007°53.365
MF979648	S_254	S. Steinhagen	18.08.2014	Germany:Schleswig-Holstein, Kiel	Kie	N 54°21.228	E 010°08.475

MF979645	S_14_B	S. Steinhagen	22.07.2014	Germany:Helgoland	Hel 7	N54° 11.290	E7° 52.45398
MF979646	S_29	S. Steinhagen	23.07.2014	Germany: Helgoland	Hel 2	N 54°10.189	E 007°53.365
MF979647	S_115	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MF979649	S_459	S. Steinhagen	16.03.2016	Germany:Schleswig-Holstein, Emmelsbuell	Ems	N 54°47.692	E 008°39.484
MF979651	S_672	S. Steinhagen	21.04.2015	Germany:Schleswig-Holstein, Finkhaushallig	Fin	N 54°24.935	E 008°54.218
MF979652	S_514_B	S. Steinhagen	19.09.2014	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MF979655	S_381	S. Steinhagen	27.09.2017	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MF979656	S_383	S. Steinhagen	27.09.2017	Germany:Schleswig-Holstein, Wullfen	Wul	N 54°24.535	E 011°10.389
MF979657	S_113	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MF979659	S_563	S. Steinhagen	09.04.2015	Germany:Schleswig-Holstein, Friedrich-Wilhelm-Luebke-Koog	Fri 2	N 54°49.998	E 008°36.852
MF979360	S_112	S. Steinhagen	30.07.2014	Germany:Schleswig-Holstein, Dagebuell 1	Dag 1	N 54°43.804	E 008°41.35
MF979654	S_361	S. Steinhagen	25.08.2014	Germany:Schleswig-Holstein, Heiligenhafen natural reserve ´´Graswader´´	HeiG 2	N54° 22.755	E11° 00.30102
MG575232	*U.mutabilis* (sl-G[mt+])	Føyn	1952	Portugal: Olhão	sl-5.	n.a.	n.a.
MG575233	*U.mutabilis* (wt-G[mt-])	Føyn	1952	Portugal: Olhão	wt-1	n.a.	n.a.

3. General Discussion

This doctoral thesis demonstrates that the taxonomy, species distribution and morphological variability of certain groups of the orders Ulvales and Ulotrichales in northern Germany is challenging to characterize and has been incorrect so far in several cases. The results confirm that previous treatments of species were unreliable due to the difficulty to ascertain species boundaries by morphological methods. Therefore, the identification of commonly occurring taxa in former surveys was in many cases not congruent with phylogenetic boundaries. Consequently, distributions of certain species or their phenotypic variations have been incorrect.

I was able to determine the genetic diversity of *Ulva sensu lato* in Schleswig-Holstein and adjacent areas and demonstrated that former species inventories listed too many taxa (Chapter I). The phenotypic variability single species can exhibit was determined by combining classical morphological methods with molecular barcoding techniques (Chapter I, II, IV and V). Comparisons of recent specimens with historic voucher material demonstrated in several cases that wrong morphological concepts of species have led to misinterpretations in the past (Chapter I and IV). I have shown that native *Ulva compressa* L. bears the potential to form extensive blooms that consist of a potentially invasive ecotype which has probably been involved in past phenomena of anoxic conditions (Chapter IV). For some species a range expansion was ascertained (Chapter I and III) whereas for other species that were expected in the investigated area, their existence could not be genetically validated (Chapter I). Finally, I presented evidence showing that the model organism *Ulva mutabilis* Føyn which originates from the south of Portugal is conspecific with one of the first described green algae lineages, *Ulva compressa* (Chapter V). My cultivation experiments have shown that phenotypic mutants of *Ulva* are not only restricted to the laboratory strain of the model organism *U. mutabilis* but that they also occur with the same characteristics in native strains of *U. compressa* from Germany (Chapter V). These results in combination with genetic evidence and successful cross-breeding experiments evidently confirm the conspecificity of *U. mutabilis* and *U. compressa* (Chapter V).

An overview about the verified hypotheses displayed in the introduction is given in table 1, whereas the most notable details of my research are discussed in the following:

Table 1: Main hypothesis examined within this thesis (see Thesis outline) and their respectively received answers.

Hypothesis	**True/False**	**Result**	**Reference**
Former and historic species inventories of Ulvales and Ulotrichales of northern Germany do not coincide with a species list based on genetically delimited entities	True	a) Generally too much species were listed in inventories. b) Allegedly suitable morphological identification criteria were commonly shared by genetically different species. b) Historic vouchers confirmed incorrect species identifications due to crypticity. c) Cryptic and invasive species were not recognized since they did not resemble morphological species concepts.	Chapter I, II, IV
The Kiel Canal, which is one of the most trafficked artificial waterways, functions as a migratory pathway for non-native macroalgae species.	False/ True	a) A higher number of bioinvaders was expected inside the canal, however that proved not to be true. b) The presence of different morphotypes of certain species could clearly be linked to abiotic factors. c) The high variability of morphotypes hindered morphological identification of green algal species.	Chapter II
The circumarctic boreal green alga *Kornmannia leptoderma* (Kjellman) Bliding expanded its distribution from its former extreme southern distribution limit Helgoland, North Sea into the Baltic Sea	True	a) Specimens of *K. leptoderma* were frequently found in the Baltic Sea, Wadden Sea and on Helgoland, whereas the different geographically separated strains, phylogenetically showed no separation. b) Cultivation experiments evidently confirmed that a complete life cycle can be performed above temperatures of thresholds cited in literature.	Chapter I and III
The ubiquitous species *Ulva compressa* expresses a high phenotypic plasticity and has been confused with other species in the past, which resulted in incorrect assessments of its distribution and additionally a potentially invasive ecotype remained unnoticed.	True	a) Within the examined area different gross morphologies of *U. compressa* were observed, which did not correspond with its protologue. b) Confusion with other species in the past due to cryptic morphotypes caused wrong distribution thresholds of *U. compressa* in northern Germany. c) A potentially invasive ecotype of *U. compressa* caused mass accumulations.	Chapter IV
The emerging model organism *Ulva mutabilis* is conspecific with one of the first described green algal species, *Ulva compressa*.	True	Conspecificity was verified and confirmed by different approaches, including molecular, cultivation and crossbreeding experiments.	Chapter V

3.1 Comparison of recent and historic findings

By defining molecular criteria, this work provides a basis for a genetically validated inventory list and concurrently a reference library for facilitate genetic identification of *Ulva sensu lato* (Chapter I). In total 20 entities could be distinguished along the whole coastline of the German state of Schleswig-Holstein (Chapter I). 12 lineages could be allocated to the genus *Ulva* (*U. gigantea* (Kützing) Bliding, *U. rigida* C. Agardh, *Ulva* sp., *U. flexuosa* Wulfen, *U. californica* Wille, *U. torta* [Mertens] Trevisan, *U. linza* L., *U. prolifera* O.F. Müller, *U. lactuca* L., *U. australis* Areschoug, *U. intestinalis* L., *U. compressa*), respectively one representative of the three genera *Umbraulva* (*Um. Dangeardii* M.J. Wynne & G. Furnari), *Percursaria* (*P. percursa* C. Agarh [Rosenvinge] and *Kornmannia* (*K. leptoderma* [Kjellman] Bliding) was encountered and three entities belonging to the genus *Blidingia* (*B. marginata* J. [J. Agardh] P.J.L. Dangeard ex Bliding, *Blidingia* sp. 1, *Blidingia* sp. 2) (Chapter I). Additionally, the two monostromatic species *Monostroma grevillei* (Thuret) Wittrock and *Protomonostroma undulatum* (Wittrock) K.L. Vinogradova were found.

When comparing the genetically assessed species inventory with respective species keys for the investigated area (Kornmann & Sahling, 1977, Kornmann & Sahling, 1983, Rothmaler, 1984, Pankow, 1990, Kornmann & Sahling, 1994) or with former inventory lists (Bartsch & Kuhlenkamp, 2000, Schories et al., 2009) an overestimation of recognized species in former surveys and reviews became obvious (Chapter I).

The first comprehensive inventory list for macroalgae was compiled by Schories et al. (2009). This valuable study intended to list all literature citing of macroalgae in whole northern Germany and it thus represents a list of entries that were recorded based on literature searches. The inventory of Schories et al. (2009) lists 28 entities within the above mentioned genera, whereas altogether only 20 could be genetically verified. Of these 20 genetically identified lineages, seven were most probably new to the area and did not appear in earlier studies. Concluding, only 13 of 28 expected green algal lineages could be ascertained (disregarding accepted synonyms, see special annotations in Chapter I and Guiry & Guiry [2018]), whereas seven entities were not detected in former studies (Chapter I). The greatest divergence was found within the genus *Ulva*. 17 *Ulva* species were listed, while only for eight genetic evidence was detected. Additional to those I recorded four species (*U. australis*, *U. californica*, *U. gigantea*, *Ulva* sp. [closest relative *Ulva shanxiensis* L. Chen, J. Feng & S. L. Xie]) that were not reported in the northern German region before and were probably newly introduced species (Chapter I).

This underlines once more that especially within the genus *Ulva* single species are notoriously difficult to identify. Assigning names to specimens only based upon morphological characters has evidently lead to confusions among species and thus also to invalid species lists in northern Germany (Chapter I).

This thesis constitutes an inventory list that was based on single specimen collections. With the identification of a specimen´s genetic identity (Chapter I) the distribution of the encountered species (Chapter I-V) and thus also possible range expansions (Chapter I and III) could be determined. Furthermore, morphological variability within species was investigated (Chapter I, II, IV and V) and, finally, a revised picture of *Ulva sensu lato* was revealed. Additionally, important contributions on the phylogeny within the orders Ulvales and Ulotrichales (Chapter I and V) and on the taxonomy of species of different green algae genera (Chapter I-V) contribute to a better understanding of the diversity and systematics in these groups. Thus, based on the inventory list of the first Chapter of this thesis which constituted a solid base for follow-up studies, it was once more underlined, that taxonomy and correct species lists are fundamental to all biological research.

3.2 Phylogenetic conspicuousness

Phylogenetic observations are nowadays important for the foundation of every taxonomic study. Within this doctoral thesis, species and species groups were classified by application and the combination of different phylogenetic techniques (Chapter I-IV). However, whereas some findings were in agreement with previous observations, also notable differences were observed.

One of the main peculiarities that is presented here is the lack of support (Chapter I) for monophyletic organisation of the genus *Ulva* and even though the analysis resolved species clades in accordance with previous results, the topology of our phylogenetic tree (Chapter I, figure 2) was in certain parts not in agreement with those of other studies. The results presented in this thesis clearly emphasize the segregation of two subclades among the entities representing the genus *Ulva* (Chapter I, figure 2) and thus do not indicate its monophyletic origin. Although, also other studies noted the separation of two subclades within the genus *Ulva* (Hayden et al., 2003, Heesch et al., 2009, Kirkendale et al., 2013), they mostly endorse its monophyly. While the larger subclade (Chapter I, figure 2), comprised exclusively species cluster that can be allocated to *Ulva* species, the smaller subclade contained clusters representing species of the genera *Ulva*, *Umbraulva* and *Percursaria*. Notably, when not including *Percursaria* samples in the dataset (Chapter I), the topology was in accordance with several other studies (Hayden & Waaland, 2002, Hayden et al., 2003, Heesch et al., 2009, Kirkendale et al., 2013). The findings made within this thesis shed some new light on the taxonomic relationships of the different green algal lineages and as already proposed by Heesch et al. (2009) for the genus *Umbraulva*, analysing a larger quantity of species could also clarify phylogenetic affiliations within the two subclades represented within this thesis. A larger data set would help to define the boundaries between the two orders Ulvales and Ulotrichales. O'Kelly et al. (2004) already discussed that the segregation of the two orders is unclear.

A phylogenetic analysis aims to depict the evolutionary relationship among groups of organisms, while its nodes indicate when new species diverged from a common ancestor. However, different specifics, on inter- but also intraspecific rank were revealed and should be discussed below.

Within this thesis, as well as in several other studies (Heesch et al., 2009, Kraft et al., 2010, Saunders & Kucera, 2010, Kirkendale et al., 2013), the species boundaries between *U. californica* and *U. flexuosa* were not identifiable (Chapter I). In a study of Hiraoka et al. (2017) the ambiguity of *U. flexuosa* as well as its frequent merging with the closely related *U. californica* clade was discussed in depth. It was suggested that they can be segregated by implementing culturing and hybridization experiments. However, these identification methods were not object of this thesis.

The species *U. linza* was a central organism in several chapters of this thesis (Chapter I, II, IV). As shown in Chapter I and II, even though specimens of different morphology were frequently observed at a genetic level, these differences were not reflected within the analysis of the *tuf*A marker. Although a genetic separation could not be linked to morphological traits the investigated individuals segregated into two groups (Chapter I and II). It became obvious from two independent phylogenetic analyses (Chapters I and II) that specimens collected at the mainland coasts and in the Kiel Canal clustered more closely related to a genotype from Tasmania (GenBank accession number JN029337), while specimens collected at Helgoland (Chapter I) had the closest relation with a genotype originating from the Northeast Pacific (GenBank accession number KM254997). It remains an open question, whether one of the genotypes of *U. linza* encountered in northern Germany represents an invasive ecotype of *U. linza*.

The here reported specimens of *Ulva* that were most closely related to *Ulva shanxiensis*, which was just recently described on material from Shentou Spring, Shanxi Province in northern China (Chen et al., 2015), were reported for the northern German region for the first time (Chapter I). However, the interspecific sequence divergence values of the corresponding cluster, which also contained *U. shanxiensis*, was rather high compared to other species clusters and the respective clade received only low bootstrap support (Chapter I). Due to the high sequence divergence values of up to 2.8 %, the cluster was labeled *Ulva* sp. instead of *U. shanxiensis*, since species boundaries could not be clearly identified on this basis. Even though the marker *tuf*A was evaluated as the most suitable marker for species delimitation in green algae (Saunders & Kucera, 2010), additional markers should be applied in future studies to delimit *U. shanxiensis*. Although within the species description several markers were applied (Chen et al., 2015), the sampling size of specimens was rather small.

3.3 Species occurrence and distribution

3.3.1 New introduced and first recorded species

The occurrence of seven species that were not detected in the area before was verified (*Blidingia* sp. 1, *Blidingia* sp. 2, *U. gigantea, U. californica, U. australis*, *Ulva* sp. [next relative *Ulva shanxiensis*], *Umbraulva dangeardii* M.J. Wynne & G. Furnari) (Chapter I).

Whereas most of the detected species could be resolved with reference sequences from GenBank, including also most of the new introductions, two entities of the genus *Blidingia* remained unresolved and did not match any entry of the online database. However, in the phylogenetic analyses they were genetically well delimited (Chapter I and II). *Blidingia* sp. 1 was a frequent species of the mainland coastline of Schleswig-Holstein (Chapter I) and was also observed within the Kiel Canal (Chapter II). Its morphology is clearly distinct from other *Blidingia* species, such as *B. minima* (Nägeli ex Kützing) Kylin or *B. marginata*, which also formed well delimited clusters in the phylogenetic analyses (Chapter I and II). Although two other species have been recorded in the area, *B. chadefaudii* (Feldmann) Bliding and *B. subsalsa* (Kjellman) Kornmann & Sahling ex Scagel et al., no genetic data for those species is presently available. Nevertheless, their morphological characters (Kornmann & Sahling, 1978, Burrows, 1991) differ strongly from the observations that were made for the antler-like, branched thalli of *Blidingia* sp. 1 (Chapter 1). Specimens that fell in the clade of *Blidingia* sp. 2 originated from Helgoland and showed closest similarities with specimens genetically identified as *B. marginata*. Thus morphologically this species could not be distinguished from other *Blidingia* species by its phenotypic characters (Chapter I). Since the morphological identification characters for the species of the genera *Blidingia* are in general rather ambiguous and blurry (Kornmann & Sahling, 1978, Burrows, 1991), and for at least half of the described species no genetic data is available, a broader survey with a combination of lifecycle observations on cultivated material as well as the application of different molecular markers has to be conducted to clearly identify the taxonomic status of species within this genus. However, both complementary studies presented here in Chapter I and II provide the first evidence for a higher diversity within the genus *Blidingia* than previously expected.

The results within this work additionally revealed five other species (*U. australis*, *U. californica*, *U. gigantea*, *Ulva* sp., *Umbraulva dangeardii*) that were genetically determined in the area for the first time. Furthermore the results support their potential introduction into northern Germany (Chapter I).

The native or non-native character of species has been evaluated by the examination of a number of different molecular and ecological traits, proposed by Heesch et al. (2009) and Kirkendale et al. (2013) (Chapter I). In my study the genetic diversity was an insufficient criterion for the assessment, since the interspecific species divergence values were in general found to be rather small within the sample set. Moreover, as already proposed by (Kirkendale et al., 2013) such evaluation of non-nativity might be biased. The reason for this bias is the larger sample size of global reference sequences, compared to that of specimens collected only at a specific area, which in general provides a smaller gene pool for local samples (Chapter I). An assessment of nativity or non-nativity only based on molecular evaluations becomes even more problematic when no close homology for the respective sequences can be found in genetic reference databanks, as was the case for *Blidingia* sp. 1 and *Blidingia* sp. 2 (Chapter I and II). The evaluation of the respective species distribution and the validation if the species occurred predominantly in anthropogenic habitats proved to be more useful (Chapter I). Another factor that substantiated the assessment of non-nativity within this thesis was the investigation of several herbarium specimens. Absence of species evaluated as non-native from historic herbaria and voucher collections obviously makes an introduction in more recent times more likely.

Besides of the investigations along the mainland coastline of Schleswig-Holstein and on Helgoland an additional assessment of non-native species was conducted in the Kiel Canal (Chapter II). Even though a high number of introduced species was expected to occur in the highly trafficked waters of the canal, only *Fucus evanescens* C. Agardh and the already above mentioned entity, *Blidingia* sp. 1, were observed as potential bioinvaders (Chapter II).

While Hinkelmann and Schulz (1899) and Arndt (1931/32) observed communities of *Fucus vesiculosus* L. in the Kiel Canal Aleem and Schulz (1952) and Schütz (1963) did not mention the perennial macrophyte anymore. Notably, the results of this thesis show that the invasive species *F. evanescens* established in the westerly parts of the Kiel Canal and dense beds of this species were observed up to a minimum salinity of 12.5 PSU. It could be hypothesized that after the declines in *F. vesiculosus* populations due to eutrophication that were widely observed across the Baltic Sea during the last century (Schueller & Peters, 1994), *F. evanescens* settled in the opened niches. This hypothesis is supported by investigations of Bokn et al. (1992), who observed a decline in native *Fucus* populations after severe eutrophication of the inner Oslo Fjord in the 1970s, while the communities of *F. evanescens* benefitted from the increased nutrient supply.

However, contrary to expectations the diversity of bioinvaders in the Kiel Canal was found to be rather small. This does not necessarily mean that the Kiel Canal is not an important migration pathway for the propagation of non-native species. Species could be transported as fouling organisms on ship-hulls and settle in their respective preferred range, which would explain their absence from the canal that has a strong salinity and nutrient gradient (Chapter II), and furthermore individuals as well as their swarmers could be transported in ballast water, thereby finding their way into the Baltic Sea.

3.3.2 Geographical distribution

Whereas some species were distributed along a wide range and present at most of the investigated sites, others showed clear distribution patterns, were restricted to certain areas or their recent distribution deviated from previous studies (Chapter I, III and IV).

One of the most striking differences beween recent and previously determined distributions was that of the species *Ulva lactuca*. Based on the results shown here (Chapter I and IV) the taxon´s distribution is restricted to the island Helgoland, whereas different inventories and identification keys record it to be abundantly present also at all the mainland coasts

(Rothmaler, 1984, Pankow, 1990, Schories et al., 2009). Comparisons of recent specimens with historical vouchers that were identified as *U. lactuca* and originated from mainland coasts demonstrated their misidentification and confusion with morphotypes of other species (Chapter I and IV). This phenomenon of misidentification and thus assessments of incorrect distributions of *U. lactuca* is not only restricted to northern Germany, nor is it a relic of past studies. It was shown that 30 % of peer-reviewed literature on *Ulva* worldwide reports the occurrence of *U. lactuca* (Kirkendale et al., 2013). It is very questionable, wether this broad and global application of the name *U. lactuca* is valid. More probable - and supported by the results of this doctoral thesis - is widespread past and recent misinterpretation and confusion of *U. lactuca* with undetected morphotypes of other species. This hypothesis is further strengthened by the possible ecophenotypic or developmentally mediated variation of *U. lactuca* that additionally caused the misidentification of distromatic specimens from Helgoland. Even though *Ulva pseudocurvata* Koeman & Hoek and *Ulva tenera* Kornmann & Sahling appear in species lists of Helgoland (Kornmann & Sahling, 1977, Kornmann & Sahling, 1983, Kornmann & Sahling, 1994, Bartsch & Kuhlenkamp, 2000) their recent or past presence could not be verified and specimens allocated to these species were genetically validated as phenotypic variations of *U. lactuca* (Chapter I and IV). An essential point is, that the type material of *U. tenera*, which originated from Helgoland (Kornmann & Sahling, 1994), was genetically verified as *U. lactuca* (Chapter I), providing a robust proof for the synonymization of *U. tenera* with *U. lactuca*.

Besides misidentified species resulting in assessments of species distributions, also species for which a wider distribution was ascertained were encountered (Chapter I). This applied to the genetically validated species *U. rigida*. This taxon was previously recorded from the Wadden Sea and from Helgoland, while our results verified an expansion of its distribution into the Baltic Sea (Chapter I).

Whereas the first Chapter discusses the distribution of all recently found species, proceeding from the results obtained in this chapter certain species and their respective traits became the focus of follow-up studies. That was the case for the more detailed investigations on the range expansion of *Kornmannia leptoderma* (Kjellmann) Bliding (Chapter III). This thesis provides the first evidence, that the circumarctic boreal green macrophyte *K. leptoderma* has expanded its distribution range into the Baltic Sea. On a length of 220 km several specimens were genetically identified by their *tuf*A sequence as *K. leptoderma* (Chapter III). Additionally, the taxon was for the first time and abundantly observed at the mainland coast of the Wadden Sea

(Chapter I), and it has reestablished at its former extreme southern distribution limit, Helgoland, where it has not been observed for the last four decades (Kornmann & Sahling, 1977, Kornmann & Sahling, 1983, Kornmann & Sahling, 1994). Phylogenetic analyses of the *tuf*A gene found no segregation between specimens of the Baltic Sea, Wadden Sea or from Helgoland and the interspecific sequence divergence was extremely low (Chapter I and III). After genetic verification of this species, also its unique life cycle was investigated and whereas different studies reported an inhibition of the gametogenesis and thus incomplete life cycles above temperatures of 12 °C the results within this thesis clearly support the view that the species can complete its life cycle at 15 °C (Chapter III). A potential adaptation to warmer conditions might explain the presence of sporophytes of *K. leptoderma* from February to September, while former studies reported its presence only in the months from February to May, when water temperatures are distinctly below 12 °C (Chapter III). That a newly introduced cryptic species invaded the German waters is rather unlikely, since historic specimens of *K. leptoderma* show no sequence divergence to the samples of today (Chapter I).

A notable and unique distribution was also observed for specimens of *U. compressa* (Chapter I and IV). However, its distribution will be discussed in a special paragraph below that is dedicated to the research conducted on this remarkable green algal species.

Additionally, some species that were expected to occur in the investigated area were not encountered genetically (*B. minima*, *B. chadefaudii*, *B. subsalsa*, *U. pseudocurvata*, *U. curvata*, *U. paradoxa*, *U. lobata*, *U. radiata*, *U. ralfsii*, *U. simplex*, *U. clathrata*, *Monostroma balticum*, *Gayralia oxysperma*) (Chapter I and IV). Even though, there was no recent genetic evidence for several species, it can not necessarily be inferred that they were absent or equally rare in the past as well. Nonetheless, processed herbarium material provided no evidence for their presence and some noteworthy species are discussed in the following.

As explained above, for a clear validation of the species diversity within the genus *Blidingia* and thus also of their distribution, additional investigations on the life cycle of the recently found entities as well as genetic investigations of the type material could help to solve the remaining questions. Nevertheless, no genetic evidence for *B. minima* was found in Schleswig-Holstein. However, at an adjacent site in Mecklenburg-Western Pomerania *B. minima* was ascertained within a former survey (Chapter I). The question whether its distribution is limited to more easterly parts of the Baltic Sea, or if this species was also found in the SW Baltic in former surveys remains unanswered. Also here, future analyses of herbarium vouchers could help to understand the distribution of *B. minima*.

Despite explicit searches for the monostromatic species *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel et al. no entity that could represent this species was detected. Notably, Kützing (1843) described its basionym *Ulva oxysperma* on the basis of material that originated from Winning situated at the Schlei, an arm of the Baltic Sea located in Schleswig Holstein (see Chapter I, Figure 1). Unfortunately, this original type material of *G. oxysperma* was undetectable and thus appears to be lost. Therefore historical herbarium vouchers of *G. oxysperma* from close proximities and adjacent areas, including specimens from Denmark, were processed. All herbarium specimens, identified as *G. oxysperma* were genetically allocated to *Kornmannia leptoderma*. For this reason repeated collections at the inner parts of the Schlei, directly at the type locality and in direct vicinity of the type locality were performed. Whereas specimens resembling *G. oxysperma* could be observed at the type locality in Winning, specimens that were in accordance with the morphology of *G. oxysperma* were observed at Brodersby (6 km from Winning) and Lindaunis (30 km from Winning). Their *tuf*A sequences, however, again clustered with *K. leptoderma* (Chapter I). Thus, even though *G. oxysperma* is reported as an ubiquitously present species (Brodie et al., 2007) we were not able to verify its presence in northern Germany (Chapter I).

3.4 Phenotypic plasticity of *Ulva sensu lato*

The application of classical identification methods by investigating a specimens morphological characters at the three thallus regions - basal, middle and apical -, as suggested by (Koeman & Van den Hoek, 1981, Koeman & Van den Hoek, 1982a, Koeman & van den Hoek, 1982b, Koeman & van den Hoek, 1984), in combination with a molecular barcoding approach, revealed the phenotypic plasticity of different species within the examined area (Chapter I, II, IV, V).

Several studies indicated strong phenotypic plasticity for several species of the genus *Ulva* before (Burrows, 1959, Bliding, 1963, Bliding, 1968, Koeman & Van den Hoek, 1981, Koeman & Van den Hoek, 1982a, Blomster et al., 1998, Tan et al., 1999, Blomster et al., 2002, Brodie et al., 2007). However, a combined approach considering multiple species and investigating their morphological variability along geographic range including North Sea and

Baltic Sea coasts and their different habitats and additionally considering their genetic affiliation, has so far not been conducted in this extend.

Even though no phenotypic variation was found and the morphology was regarded as being more or less stable in some species (e.g. *U. torta*), the vast majority of investigated specimens was observed to change micro- and macromorphological features (Chapter I, II and IV). Within this doctoral thesis, some species stood out with their morphological appearance and those will be discussed in more detail.

A certain degree of phenotypic plasticity was observed in the allegedly tubular and unbranched species *U. intestinalis* (Linnaeus, 1753), which was found in all main investigation areas (Baltic Sea, Wadden Sea, Helgoland and in the Kiel Canal) (Chapter I and II). Besides the typical unbranched thalli that resembled the type material of *U. intestinalis* (Linnaeus, 1753), specimens exhibiting various branches were also observed (Chapter I and II). Even though, branched specimens were predominantly observed in brackish waters or in the direct vicinity of fresh water inflows, deviating morphotypes were encountered in fully marine environments as well (Chapter 1). Corroborating the presence of different morphotypes in *U. intestinalis*, a further survey conducted in the definite waters of the Kiel Canal, revealed that branching patterns were clearly affected by salinity, whereas thallus sizes appeared to be affected by the respective eutrophication status of a site (Chapter II). Radical differences in gross morphology under eutrophicated conditions in a low salinity environment were previously reported for *U. intestinalis* (Bliding, 1963, Blomster et al., 2002). However, Blomster et al. (2002) reported on monostromatic, drifting forms of *U. intestinalis* in the Baltic Sea around Finland, while only tubular (branched and unbranched) specimens were observed within the here conducted studies (Chapter I and II). The overlapping morphology of branched *U. intestinalis* - which is based on the recent findings simply a morphological variation rather than an aberrant morphotype – with other species sharing this morphology hampered the identification and thus also the assessment of the distribution of certain species in different environments (Leskinen et al., 2004). Based on the findings from recent material of *U. intestinalis* (Chapter I and III), historical voucher organisms were genetically examined and their designated identification based on the morphological species concept was compared to their genetic species affiliation (Chapter I and IV). My thesis thereby revealed that the morphological concept to discriminate *U. intestinalis* in the study area was only partly applicable and for branched specimens it proved invalid in most of the cases (Chapter IV).

An even higher phenotypic plasticity was found in *U. linza* (Chapter I and II). Different gross morphological differences of specimens were observed. Besides the typical unbranched, tubular thalli, also highly branched individuals were frequently detected (Chapter I and II). Additionally, mixed forms which exhibited a tubular basal part and a distromatic apical part were infrequently observed (Chapter I). Similar to *U. intestinalis*, different morphologies of *U. linza* were observed in the Kiel Canal (Chapter II). However, as demonstrated in this thesis, salinity or the eutrophication status were no predictors for the thallus morphology in *U. linza* (Chapter II). That *U. linza* has a large variety of morphotypes was already emphasized when the allegedly branched species *Enteromorpha ahlneriana* Bliding nom. illeg. and *Ulva procera* (K.Ahlner) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland were synonymized with *U. linza* (Brodie et al., 2007, Guiry & Guiry, 2018). However, most of the identification keys and species inventories do not list the distinct morphotypes *U. linza* can possess, which still leads to wrong identifications.

A conspicuous variety of different phenotypes that led to misinterpretations with several other species was observed in *Ulva compressa* (Chapter I, IV, V). *U. compressa* is one of the first described green algal lineages and was described by the father of nomenclature, Linnaeus as a tubular and branched species from the intertidal zone (Linnaeus, 1753). Since its description scientists mostly refer to its “characteristic” branched morphotype when identifying this species. However, the morphological concept designating *U. compressa* as an obligatory branched species was shown to be too narrow by the survey of Tan et al. (1999). By the use of molecular markers, specimens with a tubular thallus, morphologically allocated to *U. compressa* clustered together with specimens exhibiting a clear distromatic and bladed morphology, strongly resembling the description of *Ulva pseudocurvata* (Tan et al., 1999). However, a systematic evaluation of the different phenotypes of *U. compressa* was never conducted. The here presented results show that under natural conditions the typical tubular phenotype - but also a distromatic, sheet-like phenotype - was frequent along the mainland coasts, whereas the different morphotypes were restricted to certain areas (see below) (Chapter IV).

This implies that the morphology of *U. compressa* is much more variable than its description considers and that these different phenotypes are driven by different conditions. Since studies performed with *U. compressa* represent a central point of this doctoral thesis and bear several innovations and high potential for future research the next paragraph disccusses this green algal lineage in more detail.

3.5 The many faces of *Ulva compressa*

Even though identification keys and inventories of macroalgae of northern Germany correctly recorded *U. compressa* to be present in the Baltic Sea, Wadden Sea and on Helgoland (Kornmann & Sahling, 1977, Rothmaler, 1984, Pankow, 1990, Bartsch & Kuhlenkamp, 2000, Schories et al., 2009) this thesis shows that the morphology at the sampling sites of *U. compressa* was broader than the allegedly “unique” tubular and branched forms on which the original description is based (Chapter I and IV). Furthermore, tubular and branched thalli encountered in the Baltic Sea in previous surveys of *U. compressa* were evidently incorrectly identified morphotypes of other species (Chapter IV, details discussed below). Thus, previous studies lead to a false-positive result when assessing the distribution of *U. compressa*, since the frequently occurring and wide spread leaf-like, distromatic ecotype of *U. compressa* remained undetected until its genetic validation within this dissertation (Chapter I and IV).

Thalli of *U. compressa* collected at Baltic Sea coasts within this project, were indiscriminately exhibiting distromatic, sheet-like morphologies that were (with the exeption of one collection site at Fehmarn, Wulfen) always found unattached. However, on Helgoland only the tubular, branched morphotype was observed, while in the Wadden Sea both morphotypes - drifting forms as well as tubular thalli - were present. The two different morphotypes in the Wadden Sea were often found at the same sampling sites (Chapter I and IV). Especially their co-occurrence at the same sampling sites indicates that salinity is unlikely an important driver of the morphology of *U. compressa*. However, it remains as an open question, whether intrinsic or external factors, or a combination of both, cause the different morphotypes.

Within this dissertation the question, whether the different phenotypes can be delimited genetically was addressed (Chapter I and IV). The clade representing *U. compressa* in the phylogenetic analysis of the *tuf*A gene encompasses sequences of both morphologies. This was supported by a phylogenetic analysis including several closely related species (Chapter I) but also in analyses that focused on a higher number of intraspecific sequences (Chapter IV and V). Additionally, *tuf*A sequence divergence values (≤ 0.9%) were small and no separation of tubular and sheet-like thalli could be found (Chapter IV). These results strongly inditate that different morphotypes belonged to the same species (Chapter I, IV and V).

Whereas in a study focusing on the phylogeographical structure of *U. compressa* in the SW and NW Baltic Sea, its distribution was examined to be restricted to salinities above 15 ppt (Leskinen et al., 2004), this dissertation presents contrasting data (Chapter IV). Specimens of

U. compressa were observed in all main areas and also at salinities of 9 PSU. However the previous survey conducted by Leskinen et al. (2004) only focused on tubular specimens, that were in agreement with the description of *U. compressa* (Linnaeus, 1753), and did not consider potentially variable morphologies.

Discovering the absence of tubular specimens of *U. compressa* from the Baltic Sea within the compiled study was contrary to species lists and identification keys for the Baltic region (Rothmaler, 1984, Pankow, 1990, Schories et al., 2009). This raised the suspiscion on the accuracy of the historically defined distribution of specimens of *U. compressa* and possible misidentification of species within past surveys. Subsequently, different herbaria and macroalgae voucher collections were searched for historic voucher material from northern Germany that had been identified as *U. compressa*.

Thee materials revealed that *U. compressa* has been confused with other tubular species, such as *U. linza* and *U. intestinalis* (Chapter I and IV). Due to the fact that foliose forms of *U. compressa* have not been recognized in northern Germany so far, we predict that historical records of *U. compressa* in the German Baltic Sea and also at more easterly parts were most probably based on confusions with other tubular species (Chapter IV).

Furthermore, historical vouchers from the mainland coasts of Germany (including Baltic Sea and Wadden Sea coasts) identified as *U. lactuca* were genetically re-identified as *U. compressa* (Chapter IV). Even though species keys and inventories list *U. lactuca* as a frequent species of mainland coasts, within this thesis the occurence of *U. lactuca* was genetically only verified on Helgoland (Chapter I). It can be speculated that many other historical records of *U. lactuca* in Schleswig-Holstein and its adjacent areas may have also been incorrect due to confusions with the bladed forms of *U. compressa* (Chapter IV).

By looking more closely on the two completely different morphotypes of *U. compressa* the question of differences in their ecology arose and indeed, essential and utmost important differences between the tubular and blade-like thalli were recognized (Chapter I and IV). Whereas the tubular specimens were mostly found attached and exhibited a well-developed rhizoidal zone, the sheet-like forms were mostly encountered drifting in the Baltic Sea and only at one specific site they were attached to artificial hard substrate. Likewise, sheet-like thalli observed at the coastline of the Wadden Sea were mostly found drifting, although attached forms occurred at some sites as well (Chapter I and IV).

Even though the sheet-like ecotype of *U. compressa* has taxonomically never been identified correctly it clearly is a nuisance seaweed in Germany (Chapter IV). Its capacity to form extensive mass accumulations was revealed by investigating a “green-tide-like” phenomenon that occurred in 2016 in the Baltic Sea, but similar observations were also made at different sites in the Wadden Sea (Chapter IV). On a local scale, sediment anoxia and strongly damaged seagrass meadows have been observed repeatedly in the direct vicinity of such mass accumulations which constituted of the free-floating, sheet-like thalli of *U. compressa*.

The observations made in this doctoral thesis on both coasts were in agreement with other observations from the North Sea. Extensive green tides formed by foliose *U. compressa* were first observed by Tan et al. (1999) in Scotland and by Guidone et al. (2013) and Hofmann et al. (2010) in New England. Therefore it is very likely that *U. compressa* was also involved in the formation of sediment anoxia that occurred frequently during the 1980s and 1990s along the coastline of the German Wadden Sea (Reise et al., 2015). Herbarium material unequivocally confirmed that the bladed morphotype of *U. compressa* was already in the area at that time (Chapter IV).

These findings lay a basis to future studies to determine why the bladed morphotype of *U. compressa* seems to have a strong capacity to form green tides, a wide tolerance to different environmental conditions and thus most probably a more invasive character. The tubular morphotype of *U. compressa,* in contrast, was never encountered to proliferate to such extend.

The investigations conducted on *U. compressa* within this thesis revealed another interesting fact: *U. compressa* as one of the first described green algal species is conspecific with the model organism *U. mutabilis* (Chapter V).

The model organism *U. mutabilis* Føyn, which originated from southern Portugal (Olhão and Faro, Portugal) (Føyn, 1958) constitutes an important system to study algal growth, development and morphogenesis and it largely contributed to our understanding of the importance of mutualistic interactions and cross-kingdom talk (Wichard & Oertel, 2010, Spoerner et al., 2012, Oertel et al., 2015, Wichard, 2015, Wichard et al., 2015, Grueneberg et al., 2016).

An outstanding observation of the variability of morphological appearance within the genus *Ulva* was made on the morphogenetic mutant slender of *U. mutabilis*. Within cultures that were raised from gametes of the same parental source material of *U. mutabilis*, thalli with a spontaneous mutation pattern appeared (Føyn, 1958, Føyn, 1959, Wichard et al., 2015). Those thalli stood out with their elongated shape and thus were named slender. Further, they exhibited no or only a reduced rhizoidal zone, which caused them to be mostly encountered drifting. Thus, the slender type strongly differed from the leaf-shaped wildtype thalli which exhibited a well developed rhizoidal zone and were encountered nearly exclusively attached (Føyn, 1958, Føyn, 1959, Wichard et al., 2015).

In culture studies performed within this thesis on *U. compressa*, similar patterns were observed. Developmental mutants appeared in less than 1% of the *U. compressa* cultures. Also in our studies, gametes of *U. compressa* were raised from the same source material (Chapter V). Thus, comparisons of vegetative and reproductive features of cultured material of *U. mutabilis* and German *U. compressa* demonstrated a shared morphological mutation pattern (Chapter V). By unreveling the phenotypic similarities between *U. compressa* and *U. mutabilis* further tests supporting the conspecificity of both species were initiated.

According to the biological species concept (Mayr, 1942), gametes of both species should be able to mate and produce fertile offspring. I therefore conducted crossing experiments between gametes of both species to test the hypothesis that *U. mutabilis* and *U. compressa* are conspecific, I (Chapter V).

To provide a successful basis for crossing experiments, the crossing partners have to be synchronized in maturity and life stage and the availability of potential mating types is required. These mating experiments revealed, that there is no reproductive boundary between *U. compressa* and *U. mutabilis* (Chapter V). Thus, following the biological species concept (Mayr, 1942), *U. compressa* and *U. mutabilis* are no distinct species.

Even though the biological species concept is widely accepted, its application is challenging in cases of hybridization. Hence, to additionally reveal genetic species boundaries the General Mixed Yule Coalescent method was used, which constitutes a powerful approach for the assessment of species delimitation (Chapter V). *Tuf*A sequence data of *U. compressa* that were provided by the formerly conducted surveys (Chapter I and IV) and comprised sequences of specimens sampled throughout Germany, were aligned with the respective sequences of *U. mutabilis*. The applied phylogenetic analysis clearly demonstrated that the *U. mutabilis* isolates (sl-G[mt+]) and (wt-G[mt-]) and *U. compressa* belonged to a unique Molecular Operational Taxonomic Unit. According to these findings, there is sufficient evidence that *U. mutabilis* and *U. compressa* should be regarded as conspecific.

In summary, by the implementation of different complementary experiments, the conspecificity of *U. mutabilis* and *U. compressa* was evidently verified within this dissertation and suggestions on the taxonomic renaming of the different strains according to the International Code of Nomenclature for cultivated plants (ICNCP) were made (Chapter V).

3.6 Conclusion and future research perspectives

This doctoral thesis shows that the genetically evaluated species diversity of *Ulva sensu lato* in northern Germany strongly differs from previous surveys which relied only on the identification of species by morphological characters. By combining molecular and morphological identifications, and concomitantly recording the species phenotypic plasticity, this dissertation constitutes the first genetically validated inventory list of *Ulva sensu lato* in northern Germany. Thus, this dissertation provides a valuable contribution to future monitoring of this algal group and constitutes a basis that facilitates easy detection of future invasive species. Furthermore, the data set provides an important reference library for metabarcoding studies, which aim to simplify the monitoring by focusing on waterborne DNA as detective particles for species abundances at certain sites.

As revealed by the displayed results, the species *Ulva compressa* exhibits an enormous morphological plasticity. However, certain ecological traits, such as its capability to form extensive green tides remained undetected until recently.

Finally, I have shown that *U. compressa*, a ubiquitously present species, is conspecific with the model organism *Ulva mutabilis*. These results enable new perspectives for the identification of quantitative trait loci, which might correlate with the variation of *Ulva´s* morphtypes. Additionally, it paves the way for future studies that could facilitate expanding the model system to local strains worldwide.

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Eidesstattliche Erklärung

Hiermit bestätige ich, dass die vorliegende Arbeit von mir selbstständig angefertigt wurde. Die Arbeit wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Ich habe keine als die angegebenen Hilfsmittel und Quellen verwendet und die Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft erstellt.

Teile dieser Arbeit sind bereits veröffentlicht, wurden zur Veröffentlichung in wissenschaftlichen Fachzeitschriften eingereicht oder sind in Vorbereitung eingereicht zu werden (siehe S. 19/20).

Kiel den, 5.10.2018

Sophie Steinhagen