Characterization of *Ulva* (Ulvaceae, Chlorophyta) species cultured in commercial abalone farms in South Africa, and comparison with closely related wild species, using morpho-anatomical and molecular methods

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Contents

Abstract

Seaweeds are among the five marine sub-sectors of species cultured in South Africa, with *Ulva* species cultured at a commercial scale. In South Africa, the annual production of *Ulva* is approximately 2000 tonnes (wet weight), with the majority of *Ulva* being grown in land-based paddle raceway systems receiving abalone effluent water. Cultured *Ulva* is mainly used as abalone feed and for bioremediation of farm effluent water. It is not sold but rather is used either as fresh feed or dried and incorporated into formulated feeds. The main commercial abalone farms growing *Ulva* in paddle raceway systems in South Africa are Irvin & Johnson (I&J) Cape Abalone, Abagold, Buffeljags Abalone and Diamond Coast Aquaculture in the Western Cape province, and Wild Coast Abalone in the Eastern Cape province. The main aim of this study is to precisely identify the cultured *Ulva* species as their identity is not clearly understood. This will provide information on the genetic diversity in the cultured material and could enable farmers to select for a species/strain that has the desired traits such as high nutritional value, rapid growth rate, resistance to diseases, and the ability to grow vegetatively, amongst others, so that the best feed is given to abalone. Next, the cultured *Ulva* species will be compared with closely related seashore *Ulva* species and with *Ulva* specimens from the main farming area in Hermanus to see if they are genetically similar. *Ulva* specimens from these farms, nearby seashores, including the Hermanus abalone farming complex in the New Harbour were identified using morpho-anatomical and molecular methods. The molecular markers employed in this study were the plastid large subunit of Ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L), Internal Transcribed Spacer of nuclear ribosomal DNA (ITS nrDNA) and the elongation factor *tuf*A. The 12 cultured *Ulva* specimens belonged in the *U. lacinulata* clade with weak support value of 0.57 for PP in the *rbc*L tree, high support value of 0.86 for PP in the ITS tree and high support value of 92% and 0.92 for BP and PP, respectively, in the *tuf*A tree. The seashore *U. capensis* and farmed *Ulva* specimens belonged in the same large *U. lacinulata* clade in the *rbc*L tree. However, the *U. capensis* samples and the locally cultivated *Ulva* samples belonged in separate sister clades with a support value of 70% and 0.75 for BP and PP, respectively, in the ITS phylogenetic tree, and 97% and 1 for BP and PP, respectively, in the *tuf*A phylogenetic tree. Therefore, the identity of the cultivated *Ulva* samples is *U. lacinulata* and the clade containing the *U. capensis* samples has now been labelled as *U. uncialis* as it is an older available name than *U. capensis*. Furthermore, the foliose *U. lacinulata* was also found growing attached near the inlets of the Hermanus abalone farming complex in New Harbour. There was no genetic variation within the farmed *Ulva* samples as they were collapsed as a single haplotype by the three molecular markers. The genetic distance between the *U. uncialis* and farmed *U. lacinulata* samples were 0.16%, 0.76% and 0.92% for the markers, *rbc*L, ITS and *tuf*A, respectively. Even though the low sequence divergence between the farmed *U. lacinulata* and *U. uncialis* specimens fits within the range of variability, these two clades are separate species that are closely related. Incongruences between the molecular and morpho-anatomical identification methods were observed, as the morpho-anatomical identification method identified 9 of the 12 farmed *Ulva* specimens as *U. lactuca* and the remaining as *U. rigida sensu* Stegenga *et al.* (1997). Fewer *Ulva* species were resolved morphologically because of the overlap in morphological description within *U. lactuca sensu* Stegenga *et al.* (1997) and *U. rigida sensu* Stegenga *et al.* (1997). Additionally, three new records of *Ulva* species (*U. ohnoi*, *U. australis* and *U. stenophylloides*) for South African seashore specimens were molecularly identified in this study, and foliose *U. compressa* was recorded for the first time in the region. In this study, the molecular marker *tuf*A, was the best marker to delimit species, as its internal clades were better supported compared to the other two markers and it was able to better separate the farmed *U. lacinulata* samples and the seashore *U. uncialis* samples into two different clades.

Keywords: *Ulva lacinulata*, *Ulva capensis*, *Ulva uncialis*, morpho-anatomical, *tuf*A, *rbc*L, ITS

Introduction

Abalone (*Haliotis midae*) are one of the world's most expensive seafood products, and with the rise in demand they have been labelled as 'white gold' (DAFF, 2018). South Africa is the third largest producer of cultured abalone in the world following China and Korea (DAFF, 2018). The farmed abalone, *Haliotis midae*, is one of the five abalone species which are endemic to South Africa and the only species that is commercially exploited. The abalone industry in South Africa is fast-growing with a total production volume of approximately 15324 tons from 2000 to 2016 (DAFF, 2017). Abalone is the most profitable species to the South African aquaculture industry, contributing 75% of the total value of South Africa's aquaculture sector in 2016 (DAFF, 2017). The total production by volume of cultured abalone (*Haliotis midae*) for human consumption was recorded at 1703 tons in 2016 (DAFF, 2017).

There are several factors that affect the quality of seafood products. Processes such as post-harvest storage, handling and preparation have been shown to significantly affect the quality of the final product (Smit *et al.,* 2010). However, pre-harvesting processes such as the diet fed to abalone may also influence the sensory qualities (taste, texture, colour and aroma) of the final product and affect the growth rates of the animals during on-growing, thereby influencing the time taken for products to reach market size (Smit *et al.,* 2010). The cultivation of *Ulva* species as a supplementary feed for farmed abalone in integrated systems has been commercially successful in South Africa for more than 20 years (Rothman *et al.*, 2020). Since *Ulva* is cultured on a large-scale as a supplementary feed and the bio-remediation capacity of this seaweed allows for partial recirculation of water, saving on pumping costs/electricity, it is important to precisely identify the species of *Ulva* grown in the farms and to investigate whether it reveals genetic diversity. Different species may have different nutritional properties and be more suitable to local conditions (improved growth and/or disease resistance). With the knowledge of which *Ulva* species are cultivated in the abalone farms, the culture conditions can be properly maintained, and the cultures can be protected from alien *Ulva* species, hence ensuring their long-term success.

In this study, the traditional morpho-anatomical and molecular identification methods, with the markers *rbc*L, ITS and *tuf*A, will be used to identify *Ulva* species cultured in the five main *Ulva* producing commercial abalone farms. The cultured species will be compared with species from the wild to determine if they are genetically similar. Moreover, the cultured species will be compared with *Ulva* species collected in the vicinity of the most productive abalone aquaculture area in Hermanus, which comprises the farms HIK Abalone, Aqunion (Whalerock) Farm and Abagold Farm, which consists of four grow-out farms namely: Sea view, Amaza, Bergsig and Sulamanzi.

Global and local aquaculture production and the seaweed industry in South Africa

Aquaculture is a flourishing industry worldwide and contributed a total of 82.1 million tonnes, valued at USD 250.1 billion, to global fish production, which was estimated to have reached 179 million tonnes in 2018 with an estimated worth of USD 401 billion (FAO, 2020). In 2018, farmed seaweeds represented 97.1% by volume of the total 32.4 million tonnes of wild-collected and cultured seaweeds combined, valued at USD 13.3 billion, which means that 31.5 million tonnes of cultured seaweeds were produced in 2018 (FAO, 2020; Chopin and Tacon, 2020). The majority of seaweed production/cultivation occurs in China (18.5 million tonnes) and Indonesia (9.3 million tonnes), which combined accounts for nearly 90% of global seaweed production (Hua *et al.*, 2019; FAO, 2020). The top six cultivated seaweed species globally in million tonnes in 2018, which represent 95% of the total production, are *Saccharina japonica* (Kombu) (11.5), *Eucheuma* species (9.2), *Gracilaria* species (3.5), *Undaria pinnatifida* (Wakame) (2.3), *Neoporphyra and Neopyropia* (nori), (previously *Porphyra* species) (2.0) and *Kappaphycus alvarezii* (1.6) (Hua *et al.*, 2019; FAO, 2020). Most seaweed production is directly consumed by humans and the remaining biomass is utilised for other applications, such as feed for farmed abalone (FAO, 2018; FAO, 2020). Moreover, seaweeds are also used in cosmetics, medicines, for the production of fertilizer, paper, food additives, animal feed, wastewater treatment and in integrated aquaculture (McHugh, 2003; Ferdouse *et al.*, 2018).

The major component of African aquaculture is freshwater fish, whereas marine aquaculture remains small. In 2017, African aquaculture production amounted to 2.2 million tonnes, comprising of 93.3% of finfish (mostly freshwater fishes), 0.5% of shellfish and 6.2% of other species (primarily aquatic plants) (Cai *et al.,* 2020). South Africa is one of the top ten aquaculture producing countries in Africa, with the highest aquaculture production value recorded in 2017 (Cai *et al.,* 2020). The aquaculture industry in South Africa is in a developing stage, with an increase in aquaculture production value from 14833 thousand USD in the year 2000 to a value of 44147 thousand USD in 2017 (Cai *et al*., 2020). The aquaculture sector has been prioritised by the government of South Africa as it has considerable potential to develop and expand in the future. The total value of the aquaculture sector was estimated at R 700 million or 0.2% of the South African GDP in 2015 (AgriSETA, 2018). It is estimated that the GDP contribution of aquaculture in South Africa will grow by 6% per annum between 2012 to 2033. Moreover, there would also be a 15% growth in employment per annum, with an increase in jobs from 2227 in 2015 to 15000 jobs by 2033 (AgriSETA, 2018). This sector has the potential to not only create jobs and increase food security, but may also prevent the depletion of natural fish stocks (AgriSETA, 2018). According to a DAFF report, the total value of the aquaculture sector in 2016 was estimated at R 1042 million, with the marine sector contributing R 904 million, which is 87% of the entire aquaculture value (DAFF, 2017). The marine aquaculture sector in South Africa continues to be dominated by the abalone sub-sector, which has an estimated worth of R 780 million in 2016, representing 86% of the marine value and 75% of the entire aquaculture value (DAFF, 2017).

Seaweed is among the five marine sub-sectors of species cultured in South Africa, with *Ulva* species cultured at a commercial scale (DAFF, 2017). Historically, in South Africa, beach-cast kelps (*Ecklonia* and *Laminaria*) were collected for alginate production, but the yields have steadily declined over the years even though this activity has continued (Rothman *et al.*, 2020). The yield has decreased gradually from a maximum of about 5000 tonnes (dry weight) in the 1970's to around 1500 tonnes (dry weight) by 1999 (Anderson *et al.,* 2003a), and just over 1000 tonnes by 2003 (Troell *et al.*, 2006). In the 1970s, Kelpak, a commercial plant-growth stimulant, was extracted from *E. maxima* by a South African company called Kelp Products Ltd. The quantity of annual kelp used to produce Kelpak was reported as approximately 1000 tonnes fresh weight by 2003 and 2942 tonnes (fresh) in 2017 (Troell *et al.*, 2006; Rothman *et al.*, 2020). In the mid-1990s, the use of kelp grew even more with the developing abalone aquaculture industry (Troell *et al.*, 2006). From 2001 to 2003, the annual harvest of fresh kelp for abalone feed reached 6000 tonnes fresh weight (Troell *et al.*, 2006). The demand for kelp along the northern west coast is not as high as compared to the southern parts of South Africa where there are only a few abalone farms in this region and no large industries that are dependent on kelp (Rothman *et al.*, 2020). Furthermore, only 7000 tonnes of kelp are currently harvested although the total MSY (Maximum Sustainable Yield) for kelp is 25 588 tonnes (Rothman *et al.*, 2020). There are other seaweeds that are also exploited in South Africa but in small quantities. In South Africa, there has been a decline in the collection of *Gracilaria* for agar extraction, with a decrease in the annual yields of over 1000 tonnes (fresh) in the 1960s and 1970s to around 300 tonnes (fresh) in the late 1990s, and with a further crash in 2005 (Rothman *et al.*, 2009). With the washups being small and unpredictable, there has been no commercial collection of *Gracilaria* in South Africa since 2008 (Rothman *et al.*, 2020). Since the 1950's, the hand collection of *Gelidium* species for agar extraction on the south coast of South Africa has been a small but stable industry (Rothman *et al.*, 2020). The yields were generally 100-150 tonnes dry weight per annum from 1986 to 2003 and the sustainable collection of *Gelidium* is still continuing in South Africa (annual harvest data of *Gelidium* from 1986 to 2018 provided in Rothman *et al.* (2020) (Troell *et al.*, 2006; Rothman *et al.*, 2020). Furthermore, Gigartinaceae (species of *Gigartina*, *Sarcothalia* and *Mazzaella*) were harvested intermittently between 1956 and 1978, but currently no Gigartinaceae is collected in South Africa (Rothman *et al.*, 2020).

Integrated Multi-Trophic Aquaculture (IMTA): towards a sustainable method of aquaculture

With the global expansion of the aquaculture industry, it is important to find environmentally friendly farming techniques. One of the main environmental problems of intensive fed aquaculture is the direct discharge of considerable amounts of nutrients into coastal waters from open-water systems and effluents from land-based systems (Chopin *et al.*, 2001). A suggested technique of dealing with this issue is integrated multi-trophic aquaculture (IMTA) as it produces environmentally-safe aquaculture habits and allows for proper resource management through a balanced ecosystem method (Chopin *et al.*, 2001). A recent definition of IMTA states that IMTA is the "enhanced production of aquatic organisms (with or without terrestrial organisms) of two or more functional groups, that are trophically connected by demonstrated nutrient flows and whose biomass is fully or partially removed by harvesting to facilitate ecological balance" (Dunbar *et al.,* 2020). IMTA consists of combining the production of fed aquaculture species such as fish, shrimp or abalone with extractive species such as shellfish, seaweeds, polychaetes and others, where the extractive organisms use the particulate and dissolved waste products produced by the fed animals (Chopin *et al.*, 2012; Holdt and Edwards, 2014). Hence, the loss of enormous amounts of dissolved nutrients is prevented when the wastes or nutrients of one species is converted into the resources of another species (Chopin *et al.*, 2001). Besides helping in the bioremediation of nutrients, IMTA can also provide economic diversification by producing other value-added species, it provides mutual benefits to the co-cultured organisms and it also increases profits for the aquaculture industry (Chopin *et al.*, 2001; Chopin *et al.*, 2012; Kleitou *et al.,* 2018). It has been shown that using suitable selected seaweeds as an extractive species in the IMTA system is cost-effective as they are renewable biological nutrient scrubbers, appropriate for bio-filtration (as they have the highest productivity of all plants) and can be economically cultured (Chopin *et al.*, 2001; Neori *et al.*, 2004). Moreover, since seaweeds are species that are found lower in the food chain, they are easier to grow and can be cultivated more efficiently by simple methods (Bolton, 2006). Some species of seaweeds such as kelps, *Ulva* and *Gracilaria* are commercially cultivated in IMTA systems and their selection for IMTA is based on characteristics such as simplicity of cultivation and easily controlled life cycle; high growth rate, high nutrient uptake efficiency and high nutrient uptake rate; resistance to epiphytes and disease-causing organisms; compatibility of their eco-physiological characteristics and their growth condition (Neori *et al.*, 2004; Pereira *et al.,* 2013; Neveux *et al.*, 2018).

In South Africa, the annual production of *Ulva* on abalone farms is approximately 2000 tonnes wet weight, with the majority of *Ulva* grown in abalone effluent (Bolton *et al.*, 2016; Neveux *et al.*, 2018). Since 2002, the commercial cultivation of *Ulva* occurred in large oval paddle raceways (*ca.* 30 m long, 0.5-1 m deep) (Neveux *et al.*, 2018; Rothman *et al.*, 2020)

(**Figure 1)**. The cultured *Ulva* is mainly used as abalone feed and for bioremediation of effluent water (Bolton *et al.,* 2013; Bolton *et al.*, 2016). The cultured *Ulva* is not sold, contrary to many other IMTA grown seaweeds, and is used either fresh or dried and incorporated into formulated feeds (Bolton *et al.*, 2016; DAFF, 2016).

Figure 1: Commercial cultivation of *Ulva* in paddle raceway systems in South Africa.

In the United States, kelp grown in IMTA systems with a variety of shellfish are sold to specialty food markets and restaurants as fresh and freshly frozen seaweed products (Kim *et al.,* 2019). In Portugal, seaweeds species such as *Porphyra dioica*, *Porphyra umbilicalis*, *Agarophyton vermiculophyllum* and *Ulva rigida* are produced in IMTA systems for human nutrition and industrial food processing (Machado *et al.*, 2020). IMTA-cultivated *Ulva* species are either used as a bio-filter to treat aquaculture water and/or used as feed for co-cultured species such as fish, shrimp, sea urchins and abalone. Based on a study in South Africa, it has been demonstrated that the inclusion of *Ulva* in the artificial diet of sea urchin acts as a feed stimulant, increasing the palatability of the diets and boosting protein intake (Cyrus *et al.*, 2015a). However, the use of fresh *Ulva* alone as a feed for the sea urchins, *Tripneustes gratilla*, for the entire production cycle is not recommended as the gonad production is significantly lower than that obtained using a formulated diet (Cyrus *et al.,* 2015b). This formulated diet (20U), consisting of 20% dried *Ulva*, was successfully developed by Cyrus *et al.*, (2014) and was shown to produce gonads of marketable quality in terms of both size and colour. According to Cyrus *et al.* (2015b), fresh *Ulva* should be used for the somatic growth phases of *Tripneustes gratilla*, while formulated feeds, especially the dry formulated feed containing 20% dried *Ulva* (20U), should be used subsequently for gonad development and/or enhancement. However, another study has shown that IMTA-cultivated *Ulva* used as the sole feed for the sea urchin (*Tripneustes gratilla elatensis*) is suitable for both somatic and gonadal growth compared to *Gracilaria conferta* and formulated diets (Shpigel *et al.*, 2018). Additionally, IMTA-cultivated *Ulva* can be used as a partial replacement in fish meal fed to cultured Nile tilapia (*Oreochromis niloticus*) and as a protein supplement in juvenile gilthead seabream (*Sparus aurata*) diets, hence reducing dependency on fish meal (Marinho *et al.*, 2013; Silva *et al.*, 2015; Valente *et al.*, 2016; Shpigel *et al.*, 2017). Another study has shown that IMTA-produced *Ulva lactuca* can replace the commercial diet of shrimp (*Litopenaeus vannamei*) by up to 25% without affecting the overall shrimp production, thus saving costs in feed (Laramore *et al.*, 2018). A recent study by Mangott *et al*. (2020) demonstrated that *Ulva lactuca* can be used both as a feed supplement in the diet of the commercially cultivated pacific white shrimp, *Litopenaeus vannamei*, and for bioremediation of wastewater. *Ulva* can be extremely efficient for nutrient uptake and is particularly beneficial for the removal of ammonium (Neveux *et al.*, 2018). *Ulva*'s high affinity for ammonium uptake makes it particularly suitable in systems that cultivate species such as abalone, fish and shrimp, as they excrete ammonium (Sales and Britz, 2001; Chopin *et al.*, 2001; Copertino *et al.,* 2009). For instance, in Australia *Ulva ohnoi* is commercially cultured at a land-based aquaculture facility integrated with prawn production primarily for bioremediation purposes (Lawton *et al.*, 2013; Magnusson *et al.*, 2019). It has been shown that the large biomass of *Ulva ohnoi* produced from this system can be used to produce high-value and commodity bio-products (e.g. seaweed salt or ulvan, a sulphated polysaccharide) and high-quality protein products by a bio-refinery process (Glasson *et al.*, 2017; Magnusson *et al.*, 2019; Glasson *et al.*, 2019; Kidgell *et al.*, 2019). When *Ulva* assimilates ammonia, it produces high biomass and when nitrate and other dissolved organic nitrogen (DON) are assimilated, high-protein *Ulva* is produced (Shpigel *et al.*, 2019). Hence, it is important to determine which *Ulva* species is the best candidate for the IMTA system depending on the purpose of their cultivation, as the nitrogen assimilation rate and nitrogen uptake efficiency varies among species (Luo *et al.,* 2012; Shpigel *et al.*, 2019; Shahar *et al.*, 2020).

IMTA system in South Africa

The main commercial *Ulva* producing abalone farms that grow *Ulva* in paddle raceway systems in South Africa are Irvin & Johnson (I&J) Cape Abalone, Abagold, Buffeljags Abalone and Diamond Coast Aquaculture in the Western Cape province and Wild Coast Abalone in the Eastern Cape province. In addition to these farms, there are also a few other farms that have smaller cultures of *Ulva* that are used as feed supplement (Bolton *et al.,* 2013). In 2002, Wild Coast Abalone started growing *Ulva* and *Gracilaria* in large paddle-raceway systems receiving abalone effluent water (Bolton *et al.*, 2009; Rothman *et al.*, 2020). *Ulva* proved to be the better candidate for large-scale production than *Gracilaria*, as it is easier to grow and unlike *Gracilaria* there were less problems with clumping of biomass and fouling with epiphytic diatoms and filamentous brown algae, which resulted in the cessation of *Gracilaria* culture in these systems (Bolton *et al.*, 2009; Rothman *et al.*, 2020). Small amounts of *Gracilaria* cultivation in aerated tank systems has started again, especially at Wild Coast Abalone, with its production estimated to be approaching 600 t (wet) per year on South African abalone farms (Rothman *et al.*, 2020).

In 2006, Irvin & Johnson Cape Abalone started growing *Ulva* and abalone in an integrated system, allowing approximately 50% of effluent water to be re-circulated back into the abalone tanks after most of the ammonia was removed by the *Ulva* (Bolton *et al.*, 2009). Based on an experimental system involving the integrated cultivation of *Ulva* and abalone using 25% recirculation rate, it was shown that the mean total ammonia nitrogen (TAN) did not accumulate in recirculation units, with the flow-through units having a significantly higher TAN (3.3±1.3 µmol) than the 25% recirculation units (2.0±1.1 µmol) (Robertson-Andersson *et al.*, 2008). This study demonstrated that *Ulva* could be grown in abalone effluent water to remove ammonia, allowing partial recirculation of water without having any adverse effects on the health of the abalone or the growth rate of the seaweeds (Robertson-Andersson *et al.*, 2008). Recirculation of water has several benefits, such as an *ca.* 40% reduction of farm pumping costs, increased water temperature in abalone tanks which is beneficial to abalone growth, reduced harvesting of natural kelps and the potential for switching off seawater pumping completely for a short amount of time during harmful algal bloom events (Bolton *et al.*, 2009; Bolton *et al.,* 2013; Rothman *et al.*, 2020). It was reported that this integrated system at Irvin & Johnson Cape Abalone increased profits by 1.4-5% (Nobre *et al.*, 2010). Two newer farms are currently operating at 50% water recirculation using *Ulva* as a biofilter (Rothman *et al.*, 2020). While the majority of *Ulva* is grown in large paddle raceways receiving nitrogen-rich abalone effluent, it is also grown on some farms in paddle raceways using fertilised seawater due to biosecurity reasons (Bolton *et al.*, 2009; Neveux *et al.*, 2018).

In South African abalone farms, cultured *Ulva* was first collected from populations that grow naturally unattached in sheltered waters (harbours or bays) that are close to the farms (Troell *et al.*, 2006; Bolton *et al.*, 2009). For example, *Ulva* cultured at Irvin & Johnson Cape Abalone was collected at Gansbaai harbour and from Saldanha Bay for farms on the west coast (Bolton *et al.,* 2009). In the culture systems, the life history of these *Ulva* species did not have to be controlled because they underwent somatic growth and did not become fertile (Troell *et al.*, 2006; Bolton *et al.*, 2009). During sexual reproduction of *Ulva*, their productivity is reduced because the formation of reproductive cells negatively affects the growth of *Ulva* (Gao *et al.,* 2017). Eventually, this leads to discolouration of the thallus and part or complete disintegration as reproductive cells are released (Gao *et al.*, 2017). *Ulva* in IMTA systems is highly successful because of the following reasons: easy to grow; good biofilter; good feed source; does not disintegrate as they remain in a vegetative state (Bolton *et al.*, 2009; Bolton *et al.*, 2016).

One of the many benefits of feeding IMTA-cultivated *Ulva* as a supplementary feed to cultured abalone is improved growth of abalone, as cultured *Ulva* has higher protein levels and is of better quality compared to other natural feed sources such as fresh kelp, *Gracilaria* and others (Robertson-Andersson *et al.,* 2011). Besides being a good feed source for marine species, IMTA-produced *Ulva* is also being sold for human consumption in some European countries. For example, a company in Portugal, ALGAplus, cultivates and trades high-quality IMTA-cultured *Ulva rigida*, grown in the effluent water from an organic fish farm (seabass and seabream) (Ghaderiardakani *et al.*, 2019; Lopes *et al.*, 2019; Califano *et al.*, 2020). With the rise in popularity of using healthy, sustainable and natural products among consumers, the production of farmed *Ulva* is expected to increase rapidly in Europe (Barbier *et al.,* 2019). With a large amount of *Ulva* grown on South African abalone farms, the cultured algae could also be used in human diets by finding domestic markets and by educating the public about the health benefits associated with cultured *Ulva.* For example, omega-3 fatty acids, polysaccharides and polar lipids, which are the main lipid components of macroalgae, have been associated with several biological activities with potential health benefits such as antimicrobial, anti-inflammatory and anti-tumour effects (Yu-Qing *et al.*, 2016; Sakamoto *et al.*, 2019; Freitas and Campos, 2019; Moreira *et al.*, 2020; Roleda *et al.*, 2021).

Taxonomy of *Ulva*, *Ulva* species concepts, identification methods & nomenclature

Ulva is a genus of green algae that was first named by Linnaeus (1753), and belongs to the phylum Chlorophyta, the order Ulvales and family Ulvaceae (Hayden *et al.,* 2003). *Ulva* is found worldwide, occurring in various habitats such as estuaries, in some freshwater environments, exposed rocky shores and sheltered bays, either floating freely or attached to solid substrata (Hayden and Waaland, 2004; Miladi *et al.*, 2018). The taxonomy of *Ulva* is complex, because morphological variation is not always reflected in the evolutionary history of these algae. In the past, the genus *Enteromorpha* and the genus *Ulva* were considered different based on their morphology (Hayden *et al.,* 2003). The genus *Ulva* consisted of taxa with distromatic (two cell layers thick) blades, while the genus *Enteromorpha* consisted of tubular green seaweeds that formed hollow liquid or gas-filled tubes and were monostromatic (one cell layer thick) (Tan *et al.,* 1999; Hayden and Waaland, 2004). However, numerous studies have shown that these two genera are not evolutionarily distinct entities and they have subsequently been synonymized (Tan *et al.,* 1999; Hayden *et al.,* 2003; Hayden and Waaland, 2004). *Ulva* species do not only exist in these two forms (tubular and bladed) in nature, as species with intermediate forms have also been observed (Hayden *et al.*, 2003). For example, some species have intermediate forms such as *Ulva linza* (Hayden *et al.,* 2003), in which portions of the blade are distromatic and others monostromatic. Gayral (1959, 1967) demonstrated that some *Ulva* species in culture developed a tubular blade and Bonneau (1977) observed three different types of blades in *Ulva lactuca*: distromatic, intermediate and monostromatic. In addition, *Ulva linza* and *Ulva atroviridis* are examples of intermediate species in South Africa and were previously grouped in the *Enteromorpha* genus, but they are mostly two cells thick (Stegenga *et al.,* 1997).

Species concepts in *Ulva* are not well-defined because species have been described based on highly variable morpho-anatomical features (Hayden and Waaland, 2004). In the search of a species concept to circumscribe species, an abundance of species concepts have been proposed with each based on different biological properties of a species (Hausdorf, 2011). The chronological order of the species concepts with the oldest to most recent concepts proposed to distinguish algal species were the morphological species concept, biological species concept, ecological species concept, the phylogenetic species concept and genotypic cluster concept. The morphological species concept, which has been abundantly used due to its practicality, defines species based on discontinuities in morphological variation (John & Maggs, 1997; Mann, 1999; Leliaert *et al.*, 2014). However, the shortcoming with this species concept is that many algal species exhibit intraspecific phenotypic plasticity caused by either the environment or genetically controlled polymorphism. Eventually, this can lead to an overestimation of species if this intraspecific morphological variation is misunderstood (Trainor, 1998; Macaya & Zuccarello, 2010; Leliaert *et al.*, 2014). Moreover, the detection of recently diverged species can be missed because the differences in morphology between species often only emerge after enough time has passed since ancestry divergence (Leliaert *et al.*, 2014; Reddy *et al.,* 2018). Next, because of the difficulties related to the morphological species concept, the biological species concept was used to delineate algal species. This widely accepted concept relied on reproductive isolation to delimit species. Culture studies are used to overcome limitations of the combined morphology and molecular-based taxonomy as they help to determine where reproductive barriers exist among genetically identifiable *Ulva* populations and whether such barriers correlate with molecular markers (O'Kelly *et al.,* 2010). For example, the combined use of molecular and culture studies has provided strong evidence that *Ulva* and *Enteromorpha* are not distinct evolutionary entities (Hayden *et al.,* 2003). The biological species concept has been tested by crosses by a few studies such as Hiraoka *et al.* (2004, 2011 and 2017). To be able to say if *Ulva* species are the same biological species or not, their hybridization should be studied as it is possible for different but closely related species to possess an identical ITS sequence if speciation has occurred between them (Hiraoka *et al.,* 2004). Hiraoka *et al.* 2017 have revealed reproductive relationships among closely related *Ulva* species and potentially resolved taxonomic problems using the culturing and hybridization approach (Cui *et al.,* 2018). However, this concept was not deemed satisfactory with algae as there was no clear indication of how strong reproductive isolation between populations had to be to qualify them as different species (Leliaert *et al.*, 2014). The phylogenetic species concept is the latest concept used to define species. It involves gene trees that contain crucial information about the speciation process and can detect recently diverged species. DNA sequences have progressively been used for species identification, species discovery and delimitation, and for testing traditional species-level taxonomies (Wiens, 2007; Leliaert *et al.,* 2014).

Even though several authors have accepted the phylogenetics concept to delimit seaweed species, they have also argued that other concepts such as morphological, breeding, geographic, ecological and so on should also be used as additional lines of evidence (Leliaert *et al.,* 2014). For example, there is an increased number of authors using combined concepts to identity *Ulva* species (Kraft *et al.,* 2010; Wolf *et al.*, 2012; Kirkendale *et al.,* 2013; Hiraoka *et al.*, 2017; Lamb *et al.*, 2019). With the growing acceptance of the phylogenetic species concept among researchers, a large amount of DNA sequence data has been deposited in repositories such as GenBank and BOLD Systems (De Clerck *et al.*, 2013). However, upon the examination of algal sequences deposited in GenBank, it was found that most (~ 80%) of these DNA sequences are not linked to a full and proper scientific name (a binomial: genus and species name) (De Clerck *et al.*, 2013; Bolton, 2019). Furthermore, it is probable that a good number of the algal DNA sequences, 20% or so, which have a full binomial name are not correctly named; as GenBank is not curated and there is no easily available information on the taxonomic credibility of the person providing the name (De Clerck *et al.*, 2013; Bolton, 2019). For example, similar *rbc*L sequences have different names or distinguishable sequences bear the same name (Kirkendale *et al.,* 2013). The increasing number of sequenced specimens that have not been linked to existing species or formally described as new species is due to the incongruence between the morphological and molecular methods (De Clerck *et al.*, 2013). As most recognized seaweed species were described using the morphological species concept, researchers face the problem of linking gene sequences of specimens to existing names based on a concept that is not always reliable for defining species (De Clerck *et al.*, 2013). Hence, to make molecular data properly useable, it is important to sequence nomenclatural type material or at least samples from type locations so that there is a lower chance of misidentification of sequences on GenBank (Hofmann *et al.*, 2010). For example, the *rbc*L gene sequences from type specimens from the lectotype specimen of *U. laetevirens*, the lectotype and two syntype specimens of *U. pertusa*, the lectotype and two syntype specimens of *U. conglobata* f. *conglobata* and two specimens on the holotype sheet of *U. conglobata* f. *densa*, have shown that *U. laetevirens*, *U. pertusa* and *U. spathulata* are synonyms of *U. australis*, and *U. conglobata* f. *conglobata* and *U. conglobata* f. *densa* represent a single separate and distinct species, *U. conglobata* (Hughey *et al.*, 2020). Although, the sequencing of holotype material will ease taxonomic confusion, it is not always possible to sequence the type specimens either because they are unavailable or DNA extraction is difficult, for example, due to it being preserved in formalin, or as microscope slides or as drawings (Hofmann *et al.*, 2010; De Clerck *et al.*, 2013). Due to the difficulty of identifying *Ulva* species based on morphological data, the name *Ulva* *lactuca* is misapplied in many aquaculture studies around the globe; with 65% of the *U. lactuca* entries representing incorrectly labelled specimens of *U. fenestrata* (O'Kelly *et al.,* 2010; Cyrus *et al.*, 2015b; Bolton *et al.,* 2016; Bolton, 2019; Fort *et al.*, 2020). It has been recently revealed that the Linnaeus type specimen of *Ulva lactuca* Linnaeus, on which the species is based, is in fact a sample of *Ulva fasciata* Delile (O'Kelly *et al.*, 2010; Hughey *et al.*, 2019; Bolton, 2019). Thus, *U. fasciata* Delile is a synonym of *U. lactuca* and it has a warm temperate to tropical distribution (Guiry and Guiry, 2020). The oldest available name for what was previously known as the European *U. lactuca* is *U. fenestrata* Postels and Ruprecht and *U. stipitata* is a heterotypic synonym (Hughey *et al.*, 2019; Guiry and Guiry, 2020). Therefore, it is imperative to sequence the type specimens if possible as they contain valuable genetic information that can help answer questions related to algal taxonomy, biogeography, phylogenetics and nomenclature (Hughey *et al.*, 2019; Hughey *et al.*, 2020).

Species identification in the genus *Ulva* is difficult due to its simple morphology and few diagnostic morphological characters that often show intraspecific variation and interspecific overlap (Hofmann *et al.*, 2010). A number of taxonomic criteria were proposed by Bliding (1968) during his general revision of the European Ulvales (Malta *et al.,* 1999). The microscopic features such as thallus thickness, presence or absence of dentations on the blade edge, cell arrangement, number of pyrenoids, chloroplast position, cell size and shape in the blade and basal region viewed in both surface and in section were observed (Bliding, 1968; Koeman and Hoek, 1981; Hoeksema and Van Den Hoek, 1983; Malta *et al.,* 1999; Loughnane *et al.,* 2008). These criteria were largely in agreement with the study on Dutch *Ulva* species conducted by Koeman & van den Hoek (1981), who found the morphology of the basal area and the holdfast of the thallus to be even more crucial (Malta *et al.,* 1999). In addition to microscopic characters, macroscopic traits such as shape, perforations, thallus colour, texture, size of holdfast, and the presence of stipe and its size were also used to identify species (Woolcott and King, 1993; Mantri *et al.*, 2020). However, several authors have shown that these features may be phenotypically variable due to various factors such as the age of the thallus, condition of the environment, whether they are free-floating or attached, as well as the associated bacteria that have also been shown to have a crucial role in defining thallus morphology (Titylanov *et al.,* 1975; Provasoli and Pintner, 1980; Tanner

1979, 1986; Malta *et al.,* 1999; Loughnane *et al.,* 2008; Mantri *et al.*, 2020). For example, variation in thallus thickness is noted throughout the year in different seasons and is probably related to thallus age (Bliding, 1968; Phillips, 1988). Furthermore, different environmental conditions such as salinity, light availability and temperature, are known to cause the cell size to vary and hence cell size is not deemed a good feature for identification (Koeman and Hoek, 1981; Israel *et al.,* 1995; Malta *et al.,* 1999). Pyrenoid number and its distribution as an identification feature is not considered useful by all authors as it was found to be too variable by Tanner (1986) and Phillips (1988), whereas it was found reliable by Bliding (1968); Koeman & van den Hoek (1981); Hoeksema and Van Den Hoek, 1983 and Dion *et al.* (1998). Varying pyrenoid numbers in cells may be due to changing environmental conditions, especially when the algae are exposed to changes in the levels of dissolved inorganic carbon, which was also noticed in some planktonic chlorophyte species (Miyachi *et al.,* 1986; Ramazanov *et al.,* 1996; Malta *et al.,* 1999). Differences in thallus morphology were observed not only among individuals of the same species but also during the growing season (Malta *et al.,* 1999). Bliding (1968) considered the arrangement of cells in surface view to be a trait for species delimitation, but Hoeksema & van den Hoek (1983) found it to be too variable to be used on its own (Loughnane *et al.,* 2008). Also, chloroplast position as a feature was deemed to be useful by many authors (Koeman & van den Hoek 1981; Hoeksema & van den Hoek 1983; Malta *et al.,* 1999), but it has been demonstrated that its position is not fixed and it changes due to photon irradiance levels, circadian rhythm or it alters in recently divided cells (Titlyanov *et al.,* 1975; Britz & Briggs, 1976; Løvlie, 1964; Loughnane *et al.,* 2008). Therefore, the description of *Ulva* species based solely on morpho-anatomical features is difficult as this morphological plasticity may result in misidentification of species (Loughnane *et al.,* 2008).

The use of molecular markers to delineate species of algae started in the 1990s (Friedl, 1997; Proschold and Leliaert, 2007). Several studies showed that molecular techniques can aid in species delineation in the absence of clear-cut morphological characters (Hayden *et al.,* 2003; Hayden and Waaland, 2004; Saunders, 2009; Destombe *et al.,* 2010; O'Kelly *et al.,* 2010). Several markers of different DNA regions such as nuclear, plastid and mitochondrial have been used to improve species identification of seaweeds (Wolf *et al.,* 2012). Different markers are needed for species delimitation in seaweeds, because they form a phylogenetically divergent group (Leliaert *et al.*, 2014). Thus, a single universal marker for species identification is not possible. For example, the plastid large subunit of Ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) is the most used marker in seaweed systematics (Freshwater *et al.,* 1994). The *rbc*L gene has laid the foundation of several taxonomic and phylogenetic studies in marine green macroalgae and seaweeds in general; for instance, the merging of the genera *Enteromorpha* and *Ulva* (Hayden *et al.*, 2003; Saunders and Kucera, 2010). However, the *rbc*L gene is a slow evolving gene that results in low resolution within species populations (Yang *et al.*, 2008). In addition to *rbc*L, the rapidly changing internal transcribed spacers of nuclear ribosomal DNA (ITS nrDNA) is another example of a molecular marker that helps in precisely identifying green seaweeds (Hayden and Waaland, 2004). ITS has been used abundantly in a broad range of taxa including marine green macroalgae to investigate phylogeny, evolution and molecular ecology (Saunders and Kucera, 2010). However, the drawbacks with ITS is that there are sometimes inconsistencies in the alignments due to insertions and deletions, poor amplification success, and double bands, which indicate divergent copies or contamination, and there are also potential problems such as intragenomic variation, potentially blurring the transition between species-level and population-level genetic distance (Yang *et al.*, 2008; Saunders and Kucera, 2010; Leliaert *et al.*, 2014). The elongation factor *tuf*A has also been used for differentiation among green algal species (Famà *et al.,* 2002; Wolf *et al.,* 2012). This chloroplast gene (*tuf*A) has been identified as a viable marker and it is advocated to be the best marker for green macroalgal species differentiation because it has the highest universality, lowest levels of contamination and highest sequence quality compared to other markers (Saunders and Kucera, 2010; Moniz *et al.,* 2014). Moreover, studies have found that the *tuf*A gene has a higher resolution power at the species level compared to other molecular markers, including the *rbc*L gene (Saunders and Kucera, 2010; Wolf *et al.*, 2012). Hence, it is important to use a combined approach of morpho-anatomical data and molecular analyses involving a selection of molecular markers to precisely identify *Ulva* species.

Taxonomy of *Ulva* in South Africa

Along the coastline of South Africa, approximately 850 seaweed species have been identified, showing the richness and diversity of the marine flora (Bolton and Stegenga, 2002; John J. Bolton, personal communication). Of the total seaweed species identified, around 15% of them are green algae with a relatively poorly known diversity around the entire coastline (Bolton and Stegenga, 2002). A few studies on *Ulva* taxonomy have been conducted in South Africa (Joska, 1992; Joska and Bolton, 1992; Stegenga *et al.,* 1997; Kandjengo, 2002; Kandjengo *et al.,* 2009). Joska and Bolton (1992) found *Chloropelta caespitosa* Tanner 1980, an unusual chlorophyte, at Dalebrook in False Bay and Cape Hangklip, which is currently regarded as a synonym of *Ulva tanneri* H.S.Hayden & Waaland 2003 (Guiry and Guiry, 2021). Joska (1992), conducted a morphological taxonomic study of *Ulva* species in the south western Cape province, South Africa to determine the correct name and distribution of the eight *Ulva* species (*Ulva atroviridis* Levring, *Ulva capensis* Areschoug, *Ulva fasciata* Delile, *Ulva insignis* (Areschoug) Papenfuss, *Ulva lactuca* Linnaeus, *Ulva rhacodes* (Holmes) Papenfuss, *Ulva rigida* C. Agardh and *Ulva uncialis* (Kützing) Montagne) that were previously recorded on the South African coasts. The classification of the eight *Ulva* species was based on the morphology of the thallus; thallus colour; form, size and arrangement of cells viewed in surface and in section; the number of pyrenoids; chloroplast; method of reproduction; growth and morphology of sporelings and ecological factors. According to Joska (1992), five species of *Ulva* occur in the south western Cape, namely: *Ulva capensis* Areschoug, *Ulva fasciata* Delile, *Ulva lactuca* Linnaeus, *Ulva rigida* C. Agardh and *Ulva rhacodes* (Holmes) Papenfuss. Furthermore, *Ulva uncialis* (Kützing) Montagne was found to be an invalid species and was included with *Ulva rigida* C. Agardh and *Ulva atroviridis* Levring was assigned to the genus *Enteromorpha*. The first comprehensive documentation of seaweeds of the South African west coast was conducted by Stegenga *et al.* (1997). Within the genus *Enteromorpha*, six species were identified, namely: *E. intestinalis* (Linnaeus) Link, *E. atroviridis*, *E. linza* (Linnaeus) J. Agardh, *E. compressa* (Linnaeus) Greville, *E. prolifera* (O.F. Mueller) J. Agardh and *E. flexuosa* (Wulfen) J. Agardh (Stegenga *et al.,* 1997). Within the genus *Ulva*, five species were identified, namely: *U. fasciata*, *U. capensis*, *U. rigida*, *U. rhacodes* and *U. lactuca* (Stegenga *et al.,* 1997). With the increased use of molecular techniques to study algal taxonomy around the world, this new concept was also applied to South African seaweeds. The study by Kandjengo (2002) was the first in South Africa to use molecular data to elucidate the taxonomic status of South African species of *Ulva* and *Enteromorpha*. The molecular marker, ITS, was employed together with morphology, to identify *Ulva* samples collected from seashores and from one aquaculture farm (Irvin & Johnson Cape Abalone). Based on morpho-anatomical and cytological characters, 10 algal species were identified, namely: *E. compressa*, *E. flexuosa*, *E. intestinalis*, *E. linza*, *U. capensis*, *U. fasciata*, *U. lactuca*, *U. rhacodes*, *U. rigida*, *U. uncialis* and an unidentified *Ulva* species from Irvin & Johnson Abalone Farm (Kandjengo, 2002). Based on the molecular data, it was proposed that *Ulva capensis* and *Ulva rigida* represented a single polymorphic species and the unidentified *Ulva* sample collected from the Irvin & Johnson Cape Abalone was grouped within the *Ulva capensis*/*rigida* clade (Kandjengo, 2002). According to the conference abstract 'Molecular systematics of *Ulva* in commercial aquaculture in South Africa' by Kandjengo *et al.* (2009: conference Abstract, data unpublished), the identity of cultured *Ulva* from the two abalone farms (Irvin & Johnson Abalone Farm and Wild Coast Abalone Farm) were determined using the molecular markers ITS and *rbc*L. According to the study, four species (*Ulva capensis*, *Ulva rigida*, *Ulva lactuca* and another form that closely resembles *Ulva linza* morphologically) were identified from the 37 specimens sequenced for the ITS and *rbc*L molecular markers. Moreover, the specimen identified morphologically as *Ulva linza* belonged in a well-supported clade that contained *U. armoricana* Dion, Reviers & Coat 1998*,* *U. scandinavica* Bliding, nom. inval. 1969 and *U. rigida* C. Agardh 1823. Furthermore, they reported that the South African '*Ulva lactuca*' which was considered the main cultured species in South African abalone farms was different from the European *Ulva lactuca* (the latter itself no longer known as *U. lactuca*, which is a synonym of *U. fasciata* Delile) and it needed to be described as a new species. According to those authors, it is probable that the South African '*Ulva capensis*' is related to *Ulva rigida* from elsewhere and that the South African '*Ulva rigida*' is also an undescribed species (Kandjengo *et al.,* 2009: unpublished). Thus, it is important to precisely identify the *Ulva* species grown in the abalone farms as it will enable farmers to select for a species/strain that have the desired traits such as high nutritional value, rapid growth rate, resistance to diseases, the ability to grow vegetatively, amongst others, so that the best feed is available for the commercially cultivated abalone and/or used for bioremediation. Furthermore, the right culture conditions will be provided to the farmed *Ulva* as different *Ulva* species/strains may have different growth requirements and chemical compositions.

Aims and objectives of this study

The aquaculture industry is a lucrative industry which provides socio-economic benefits to South Africa. The cultivation of *Ulva* in abalone farms has been commercially successful, but the identity of the *Ulva* species cultured in the main commercial abalone farms are not clearly understood. The aim of this study is to identify the *Ulva* species grown in the five main commercial abalone farms producing the majority of the cultured *Ulva*: Irvin & Johnson (I&J) Cape Abalone, Abagold, Buffeljags Abalone, Diamond Coast Aquaculture, and the Wild Coast Abalone. These five abalone farms have a wide geographical range, and it is hypothesized that different *Ulva* species may be growing in the farms due to their different temperature conditions. The farms are located over a coastal range of *ca.* 1925 km and range from the cool temperature northern west coast of South Africa (Diamond Coast Aquaculture) to the warm temperate southeast coast (Wild Coast Abalone). Additionally, the cultured *Ulva* species will be compared to closely related *Ulva* species from South African seashores to determine if they are genetically similar. Furthermore, *Ulva* specimens from the nearby rocky shores of the farms in the Hermanus abalone farming complex in New Harbour will be analysed to determine if they are closely related to the cultured species. The *Ulva* species will be initially identified morphologically using available local literature and these identifications will later be compared with international literature and data. Three molecular markers, *rbc*L, ITS and *tuf*A, will be used as they are the most widely applied markers for *Ulva* species identification. Furthermore, the three molecular markers will also be compared to each other to determine which marker best separates the wild and cultured *Ulva* species. Finally, based on the available molecular data, the identity of *Ulva* species cultivated from elsewhere will be compared with that of the South African *Ulva* species.

Materials and Methods

Collection and storage of samples

Collections of fresh *Ulva* blades were made from the five main abalone farms producing *Ulva* in paddle-raceway systems in South Africa, namely: Irvin & Johnson Cape Abalone, Abagold, Buffeljags Abalone, Diamond Coast Aquaculture and Wild Coast Abalone. Fresh, bladed *Ulva* specimens from nearby seashores (Kommetjie, Sea Point, Eersterivier and Knysna) were also collected; as well as bladed *Ulva* specimens dried in silica gel from Namibia (Swakopmund and Lüderitz), which were made available through the intertidal biodiversity survey conducted by the Benguela Current Commission (BCC) in 2019. Seashore *Ulva* specimens that were closely related to the farmed *Ulva* species were collected based on morphology. Moreover, attached *Ulva* specimens found near the main farming area in Hermanus were also collected (**Figure 2**). The collection details of these specimens are given in **Table 1**, **2** and **3**. Twelve *Ulva* specimens from the abalone farms were selected for morpho-anatomical and molecular analyses (**Table 1**). Fourteen *Ulva* attached samples from the seashores were collected for morpho-anatomical and molecular analyses (**Table 2**). Four *Ulva* samples and three *Ulva* samples were collected near the inlets and outlets, respectively, in the Hermanus abalone farming complex in New Harbour for morpho-anatomical and molecular analyses (**Table 3**). All specimens were pressed and dried on herbarium sheets for deposition in the Bolus Herbarium at the University of Cape Town. The fresh *Ulva* samples were stored in sterile pre-labelled plastic bags and immediately stored in a -80°C freezer without rinsing for DNA analysis. ITS and *rbc*L sequences of *Ulva rigida* and *Ulva capensis* species from a previous unpublished study conducted by Lineekela Kandjengo in 2009 were included to compare with the *U. rigida* and *U. capensis* species collected in this study (**Table 4**). The details of the *Ulva* specimens obtained from GenBank for the three molecular markers are listed in (**Supplementary Table S1**).

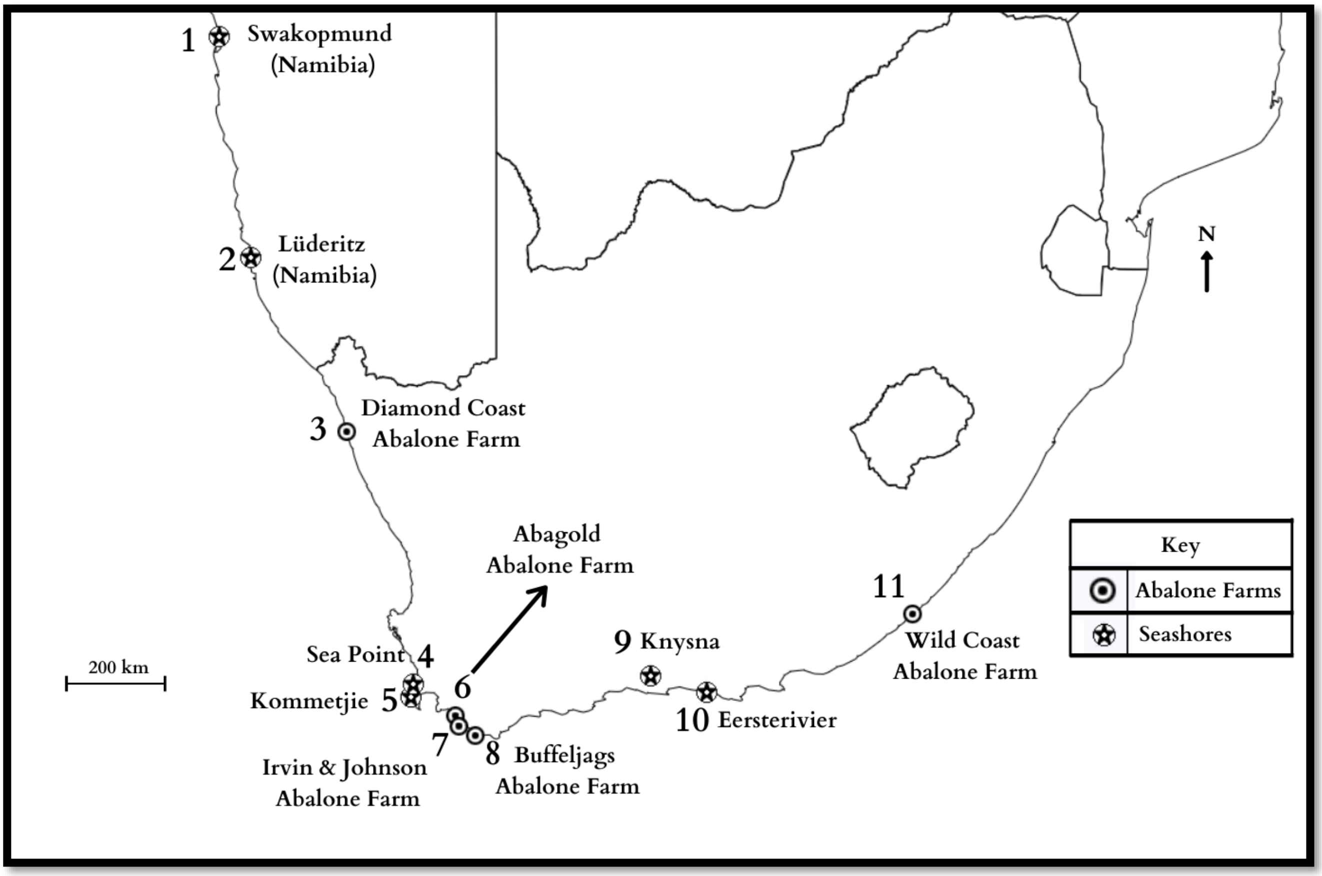

Figure 2: Location of commercial abalone farms and seashore sites where *Ulva* samples were collected from are numbered from west to east. The five main *Ulva* producing commercial abalone farms along the coastline of South Africa are numbered **3** (Diamond Coast Aquaculture), **6** (Abagold Abalone), **7** (Irvin & Johnson Cape Abalone), **8** (Buffeljags Abalone) and **11** (Wild Coast Abalone). The seashore sites are numbered **1** (Swakopmund), **2** (Lüderitz), **4** (Sea Point), **5** (Kommetjie), **9** (Knysna) and **10** (Eersterivier).

Table 1: Location and description of the culture conditions for the various *Ulva* specimens collected from the five *Ulva* producing commercial abalone farms investigated in this study during 2018.

Name of farms and collection date	**Sample number**	**Sample name**	***Ulva* culture conditions**
Buffeljags Abalone, Cape south coast (07/06/2018)	1	BL-S	Seawater: *Ulva* is grown in non-effluent water (hatchery incoming water)
	2	BL-E	Effluent: *Ulva* is grown in abalone grow-out recirculation water.
Abagold, Hermanus (18/06/2018)	3	AG-P1	P1: P1 is a large production *Ulva* paddle raceway. It is the commercial stock grown and fed to the abalone. There is daily fertilisation at night when water flow is switched off and during the day water flow is switched on again. There is one paddle wheel per raceway and the surface area of the ponds are approximately 240 m^2 with a sloping depth from 40 cm to 80 cm and with high irradiances.
	4	AG-M1	M1: They are stock culture housed in smaller 1000 litre tanks and are only fertilised on demand (once/twice a week at most). They receive normal seawater filtered through drum filters and there is a lot of aeration in these tanks to keep them tumbling.
	5	AG-M2	M2: They are stock culture housed in smaller 1000 litre tanks and are only fertilised on demand (once/twice a week at most). They receive normal seawater filtered through drum filters and there is a lot of aeration in these tanks to keep them tumbling.
Diamond Coast Aquaculture, Kleinzee (13/06/2018)	6	KS-Coiled	Effluent: *Ulv*a grown in abalone effluent/grow-out recirculation water. Coiled *Ulva* blades were selected.
	7	KS-Flat	Effluent: *Ulv*a grown in abalone effluent/grow-out recirculation water. Flat *Ulva* blades were selected.
Wild Coast Abalone, Haga-Haga (11/06/2018)	8	HG-1	Sterile Paddle Raceways in which *Ulva* is grown in non-filtered water directly from the sea. It is fertilized by Open Water Fertilizing four times a week.
	9	HG-2	Sterile Holding Tank in which *Ulva* is harvested from paddle raceways above (1) and held for 2-3 days with no fertilizing before it is sent to the hatchery.
	10	HG-3	Grow-Out Paddle Raceways in which *Ulva* is grown in run-off water from the Grow-Out section with animals ranging from 15-150 g. These paddle raceways are fertilized 2-3 times a week in Closed Water Fertilizing.
	11	HG-4	5F Grow-Out Paddle Raceways Grow-Out section in which *Ulva* is grown in run-off water from the 5F Grow-Out section with animals ranging from 90-350 g. These paddle raceways are fertilized.
Irvin & Johnson Cape Abalone, Danger Point (20/06/2018)	12	I&J	*Ulva* is grown in paddle raceways with fertilised seawater.

Table 2: Fresh *Ulva* attached specimens from local seashores, and dried *Ulva* specimens from Namibia.

Seashores and collection date	Sample number	Sample name	Notes on the region where samples were collected
Kommetjie (18/02/2019)	1	D2991-GT	Fresh, attached *Ulva* samples from a green tide area.
	2	D2993-GT	
	3	D2994-GT	
Kommetjie (08/04/2019)	4	D2995-SAK	Fresh, attached *Ulva* sample from a sheltered region.
	5	D2996-MGK	Fresh, attached *Ulva* sample from a mid-shore gully area.
Sea Point (19/05/2019)	6	SPT-UC	Fresh *Ulva* samples from the intertidal zone of the seashore. Samples SPT-UC and SPT-UL were attached, while sample SPT-UR was non-attached.
	7	SPT-UL	
	8	SPT-UR	
Eersterivier (14/06/2019)	9	D3044	Fresh, attached *Ulva* samples from the intertidal zone of the seashore.
Knysna (16/06/2019)	10	D3047	Fresh, attached *Ulva* samples from the intertidal zone of the seashore.
Kommetjie (15/08/2019)	11	D3049	Fresh, attached *Ulva* samples from the intertidal zone of the seashore.
Swakopmund (Namibia) (11/10/2019)	12	NAM19	Silica gel dried foliose *Ulva* from the BCC study.
Lüderitz (Namibia) (11/10/2019)	13	NAM102	Silica gel dried foliose *Ulva* from the BCC study.
	14	NAM103	Silica gel dried foliose *Ulva* from the BCC study.

Table 3: *Ulva* attached specimens collected in the vicinity of the most productive abalone aquaculture area in South Africa, Hermanus. The farms include HIK Abalone Farm, Aqunion (Whalerock) Farm and Abagold, which consists of four grow-out farms, namely: Sea View, Amaza, Bergsig and Sulamanzi.

Hermanus Abalone Farm complex	Sample number	Sample name	Notes on the region where samples were collected from
Inlets (30/10/2019)	1	AG-IN1	All the foliose *Ulva* samples that were found in the main farming area in Hermanus were collected. Attached *Ulva* samples were collected near the inlets (joint water intake of the farms) of the most productive abalone aquaculture area.
	2	AG-IN2	
	3	AG-IN3	
	4	AG-IN4	
Outlets (30/10/2019)	5	AG-OUT1	Attached *Ulva* samples were collected near the outflow of the farms, i.e., where water from the farms is released into the nearby seashore.
	6	AG-OUT3	
	7	AG-OUT4	

Table 4: ITS and *rbc*L sequences from *Ulva* samples collected from seashores and from abalone farms by Lineekela Kandjengo in 2009.

Kandjengo's ITS and *rbc*L sequences	**Sample number**	**Sample name**	**Collection site**
*rbc*L	1	U03 I&J	I&J Cape abalone
	2	U114 I&J P1	I&J Cape abalone
	3	U117 I&J	I&J Cape abalone
	4	U274 LK264	Dalebrook, False Bay
	5	LK14	Yzerfontein
	6	LK255	Abagold Farm
	7	LK256	Abagold Farm
	8	LK258	Abagold Farm
	9	LK280	V&A waterfront, Cape Town
	10	U36	Seashore
	11	U02	Gansbaai harbour
	12	U56	Dalebrook, False Bay
	13	CE0	Long Beach, Namibia
	14	CD6	Swakopmund, Namibia
ITS	15	U104	Abagold Farm
	16	U109	HHPB8B
	17	U113	HHWCA3
	18	U114 I&J P1	I&J Cape abalone
	19	U107	HHPA3C
	20	U104F	Abagold Farm
	21	U113F	Farm
	22	U16	Seashore
	23	U21 I&J	I&J Cape abalone
	24	U21F	Farm
	25	U09	Seashore
	26	U63	Seashore
	27	U134	Seashore
	28	U135	Seashore

Morpho-anatomical characterization

The size, texture and colour of the *Ulva* specimens were assessed visually. The maximum length and width of the blades were measured using a ruler. For the morpho-anatomical analysis, the fresh *Ulva* samples were stored in fresh seawater prior to examination and stored in a cold room adjusted to a temperature of 13-14℃ and under light intensity ranging between 60-100 µmol.m^{-2}s^{-1}. Sections of length and width of approximately 5 mm by 5 mm were cut from the edge and middle region of the thallus as farm specimens do not possess a holdfast, so no basal region could be ascertained. The sections were finely hand-sectioned using a scalpel to obtain a surface and transverse view of the specimens. Specimens were viewed under a Leica Wild M10 light microscope to examine the important anatomical features at 400× magnification. The anatomical characters recorded included the dentations on the blade margin, blade thickness, vegetative cell length and cell width, number of pyrenoids per cell, shape of cells in surface view and the chloroplast arrangement in the cells. Photomicrographs were taken using an Olympus D50 digital camera mounted on the microscope and a scale bar was included. Pictures of the pressed specimens on herbarium sheets with a ruler for scale were taken using a 13 megapixels Samsung Galaxy J7 Prime phone camera. The collages of pressed samples and photomicrographs were carried out on the website collage maker *BeFunky*. Stegenga *et al.* (1997) was used as a morphological reference for identifying the *Ulva* species as was www.algaebase.org (Guiry & Guiry, 2021) to confirm updated taxonomic names.

DNA extraction, PCR amplification and sequencing

Most of the foliose *Ulva* samples were freshly collected from abalone farms and seashores while the three foliose *Ulva* samples collected from Namibia (NAM19, NAM102 and NAM103) were dried in silica gel. DNA was extracted from fresh or silica-dried *Ulva* samples using the QIAamp DNA Micro Kit (50) (Cat# 56304) in accordance with the manufacturer's instructions, with minor modifications. Prior to the extraction of DNA from the frozen *Ulva* samples, the *Ulva* blades were thawed and washed with distilled water to remove any epiphytes. Excess water was removed from the blades using a salad spinner, before being ground in liquid nitrogen to fine powder using a pestle and mortar. For silica gel dried *Ulva* samples, DNA was extracted by grinding them in liquid nitrogen in a 2.0 ml microcentrifuge tube using a sterile pellet-pestle. An aliquot of ≤ 10 mg of each ground *Ulva* sample was added to separate 1.5 ml microcentrifuge tubes for DNA extraction following the manufacturer's instructions. All DNA samples were eluted in 30 µl of the elution buffer provided in the kit. Prior to PCR amplification, the quantity and quality of the extracted DNA was estimated using a Nanodrop spectrophotometer and DNA integrity was assessed on a 1% agarose gel. The three molecular markers, *rbc*L, ITS and *tuf*A were PCR amplified using a Bio-Rad C1000^{TM} thermal cycler. The gene region *rbc*L was amplified using the primers RH1 (5'- ATGTCACCACAAACAGAAACTAAAGC-3') and 1385r (5'-AATTCAAATTTAATTTCTTTCC-3') (Manhart 1994). The ITS spacer was amplified using the primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTT- ATTGATATGC-3') (White *et al.,* 1990). The *tuf*A molecular marker was amplified using the primers *tuf*GF4 (5'-GGNGCNGCNCAAATGGAYGG-3') (Saunders and Kucera, 2010) and *tuf*AR (5' CCTTCNCGAATMGCRAAWCGC-3') (Famà *et al.,* 2002). The PCR reaction mixtures (25 µl volume) were prepared using 2 µl of approximately 50 ng DNA extract, 1× (12.5 µl) PCR-Master Mix (KAPA *Taq* ReadyMix, Kapa Biosystems; Catalog #KK1006), 0.5 µl (400 nM) of each primer and 9.5 µl of sterile water. The PCR amplification thermal profile for each marker is listed in **Table 5**. PCR products were assessed on a 1 % agarose gel to verify reaction specificity and fragment size. The PCR products were sent to the Central Analytical Unit at Stellenbosch University where they were purified using a PCR purification kit (Roche) before being sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and AB13730xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. PCR products were also sent to Macrogen-Europe for sequencing, where they were purified by centrifugation using the Wizard® SV Gel and PCR Clean-Up System. Both forward and reverse primers for each gene were used for cycle sequencing.

Table 5: PCR thermal cycling conditions for each gene region (*rbc*L, ITS and *tuf*A) targeted for the molecular identification of *Ulva* specimens in this study.

Steps	*rbc*L		ITS		*tuf*A	
Initial denaturation	95.0°C for 6 min		95.0°C for 6 min		94.0°C for 4 min	
Denaturation	95.0°C for 1 min	35 cycles	95.0°C for 1 min	35 cycles	94.0°C for 1 min	38 cycles
Annealing	45.0°C for 1 min		58.0°C for 1 min		45.0°C for 30 s	
Extension	72.0°C for 1 min		72.0°C for 1 min		72.0°C for 1 min	
Final extension	72.0°C for 10 min		72.0°C for 10 min		72.0°C for 7 min	

Phylogenetic analyses

A total of 33 *rbc*L, 20 ITS and 33 *tuf*A sequences were obtained from the present study. Homology searches were then carried out using the BLASTN algorithm available on the National Centre for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.,* 1990). Suitable *Ulva* sequences were chosen from the nucleotide collection available in GenBank and relevant GenBank accession numbers from previous publications were chosen for comparison with southern African material (Loughnane *et al.,* 2008; Kirkendale *et al.,* 2013; Melton *et al.,* 2016; Fort *et al.,* 2020). The *rbc*L, ITS and *tuf*A sequences were edited using the software BioEdit Sequence Alignment Editor version 7.0.5.3 (Hall, 1999). Additional sequences from GenBank were included in each alignment. A total of 99 *rbc*L, 96 ITS and 83 *tuf*A sequences downloaded from GenBank were aligned with the sequences obtained in the present study. An additional 14 *rbc*L and 14 ITS sequences were included from a previous unpublished study (with permission) by Lineekela Kandjengo. The *rbc*L dataset included 132 sequences and the alignment consisted of 1501 positions. The ITS dataset included 116 sequences and the alignment consisted of 1316 positions. The *tuf*A dataset included 116 sequences and the alignment consisted of 1224 positions. Both Maximum Likelihood (ML) and Bayesian phylogenetic analyses were used for the *rbc*L, ITS and *tuf*A datasets. Bayesian analyses were performed using MrBayes (Ronquist and Huelsenbeck, 2003). For the Bayesian analysis, two separate concurrent MCMC runs, each composed of four chains, three heated and one cold were performed. Each Markov chain ran for 5,000,000 generations for the *rbc*L, ITS and *tuf*A markers, sampling trees every 1000 generations. The first 25% of the *rbc*L, ITS and *tuf*A trees were discarded as burn-in. The burn-in values used for these gene regions were based on the stationarity obtained from the trace files. Parameter stability and run convergence were inspected using Tracer v1.7.1 (Rambaut *et al.*, 2018). The consensus tree was generated from the remaining 75% of *rbc*L, ITS and *tuf*A trees. For the Maximum Likelihood trees, the best evolutionary model for each of the three alignments was determined based on their AIC (Akaike Information Criterion) score using jModeltest 2 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). The model TIM3, TIM1 and GTR+I+G was deemed the most appropriate for the *rbc*L, ITS and *tuf*A alignments, respectively. Maximum Likelihood trees were obtained using RAxML-NG and the trees were run for 100 bootstrap replicates (Kozlov *et al.*, 2019). The Bayesian Inference phylogeny was presented with bootstrap values from the Maximum Likelihood phylogram in front of the posterior probabilities at each internode. Maximum Likelihood bootstrap values of 70 and above were shown in the tree. All the trees were visualized using Figtree (http://tree.bio.ed.ac.uk/) and edited on Corel Draw. Pairwise distances for the three molecular markers, *rbc*L, ITS and *tuf*A, were calculated based on the *p*-distance model using MEGA version 10.1.8 (Kumar *et al.*, 2018). *Ulva* samples that were resolved into the *U. lacinulata* clade were collapsed into haplotypes using DnaSP v6 (Rozas *et al.*, 2017). The collapsed samples and representatives of *Ulva* species from different countries within the *U. lacinulata* clade were selected to compute the sequence distances between these taxa.

Results

Morpho-anatomical identification

To identify the *Ulva* species cultured on the five commercial abalone farms included in this study from South Africa, a total of 12 *Ulva* samples were collected from the different culture conditions used in the respective abalone farms. The *Ulva* species were initially identified using literature on local species, and these identifications were then tested with international literature and data. Based on the descriptions of *Ulva* and *Enteromorpha* species in Stegenga *et al.* (1997), three of the twelve cultured *Ulva* specimens (samples BL-S, AG-M2 and KS-Coiled) were identified as *Ulva rigida* (**Figure 3, 7, 8**). Samples identified as *U. rigida* fitted the description of this species documented in Stegenga *et al.* (1997): Angular or polygonal cells in surface view, 10-16 µm in diameter; rectangular cells in cross-section with cell height of 25-35 µm and cells 2-4 times higher than broad; thickness of lamina from 75 µm in the apical region to 100 µm in the mid-thallus and with 1-3 pyrenoids. The thalli of samples BL-S, AG-M2 and KS-Coiled, which were identified as *Ulva rigida,* varied in form, texture, colour, and size (**Table 6**). Samples BL-S and AG-M2 had flat thalli, while sample KS-Coiled had coiled thalli and all the samples had a mid-green colour except for KS-Coiled that had a dark green colour. KS-Coiled also had a rough texture compared to the other three samples which had a smooth texture. The pyrenoid number of the aforementioned samples corresponded to the species description in Stegenga *et al.* (1997), which was 1-3 pyrenoids per cell. The cell height of the samples identified as *U. rigida* were 2-4 times higher than broad and all the samples were within the documented range of 25-35 µm. However, the blade KS-Coiled (55-57.5 µm) was thinner than the documented range of *Ulva rigida* (75-100 µm). Moreover, the cell size of BL-S (17.5-20 µm) and AG-M2 (17.5 µm) in surface view was slightly greater than the documented range (10-16 µm in diameter). The angular/polygonal cell shape of all three *U. rigida* specimens matched with the description in Stegenga *et al.* (1997). Based on the description of *Ulva rigida* in Stegenga *et al.* (1997), minute teeth were present along the edge of the thallus, but this was observed only in sample AG-M2.

Samples identified as *U. lactuca* (BL-E, AG-P1, AG-M1, KS-Flat, HG-1, HG-2, HG-3, HG-4 and I&J) (**Figure 4, 5, 6, 9, 10, 11, 12, 13, 14**) fitted the following description: Rounded-angular cells in surface view; isodiametric cells in cross-section, 12-22 µm in diameter; thickness of the lamina varying from 45-60 µm in the apical region, to 55-110 µm in the mid-thallus and with usually one pyrenoid. The thalli of the samples identified as *Ulva lactuca* varied in their form, texture, colour, and size. Most of them had a mid-green colour except for samples HG-1 and HG-2, which had a light green colour, and all the samples had a smooth texture, except for KS-Flat that had a rough texture. The pyrenoid number of the nine samples matched with the documented pyrenoid number of *Ulva lactuca*, which is usually one pyrenoid per cell (**Table 6**). The blade thickness of all the samples identified as *U. lactuca* were within the documented range except for samples AG-P1 (35 µm), HG-1 (32.5 µm), HG-2 (35 µm) and HG-4 (32.5 µm), which were slightly less than the documented range. Moreover, the size of the cells in surface view of all the samples were within the range of 12-22 µm in diameter, except for AG-P1 that had a bigger cell size, 25-30 µm. According to Stegenga *et al.* (1997), the margins of *Ulva lactuca* were edentate and none of the samples examined had dentated margins.

To investigate whether local seashore samples were closely related to the cultured *Ulva* species, fresh specimens of *Ulva* were collected from local seashores (Kommetjie, Sea Point, Eersterivier and Knysna). In addition, other samples of foliose *Ulva* that were available for comparison were sequenced. These samples (NAM19, NAM102 and NAM103) were collected from Lüderitz and Swakopmund in Namibia and were included in this study for comparison with *U. rigida* and *U. capensis* in South Africa. Among the 14 *Ulva* specimens collected in total, four of the samples (D2994-GT, SPT-UR, NAM19 and NAM102) were morphologically identified as *Ulva rigida* and two samples (D3049 and NAM103) were identified as *Ulva capensis*. Samples D2994-GT was collected from a green tide along the seashore at Kommetjie (**Figure 15**). SPT-UR was collected from seashore at Sea Point (**Figure 16**). NAM19 and NAM102 were obtained from the BCC study. Sample D3049 (**Figure 17**) was collected from a seashore at Kommetjie and sample NAM103 was obtained from the BCC study. Most of the morphological features of samples D2994-GT and SPT-UR corresponded with the description of *Ulva rigida* in Stegenga *et al.* (1997), except for the cell size of SPT-

UR in surface view, which is slightly bigger (17.5-20 µm) and did not fit in the range 10-16 µm in diameter documented for *U. rigida*. Samples identified as *U. capensis* fitted the following description: rounded cells in surface view; bullet or spindle-shaped cells in cross-section, 8-15 µm in diameter; thickness of the lamina *ca.* 70 µm in the apical region, increasing to 120-150 µm in the mid-thallus, and up to *ca.* 200 µm in the upper basal region; and with dentate margins. Samples D3049 and NAM103 corresponded with all the traits of *Ulva capensis* described in Stegenga *et al.* (1997), with the distinguishing features dentate margins and bullet-shaped cells in cross-section present.

Ulva samples were also collected in the vicinity of the main abalone aquaculture area in South Africa, namely Hermanus, to investigate whether the cultured *Ulva* species found on these farms were genetically similar to the seashore *Ulva* species growing nearby. Samples AG-IN1 (**Figure 18**), AG-IN2 (**Figure 19**), AG-IN3 (**Figure 20**) and AG-OUT3 (**Figure 22**) were morphologically identified as *Ulva rigida* and sample AG-OUT1 (**Figure 21**) was identified as *Ulva capensis*. Samples AG-IN1, AG-IN2, AG-IN3 and AG-OUT3 matched with the description of *Ulva rigida* in Stegenga *et al.* (1997), except that the cell size in surface view for AG-IN3 (22.5 µm) and AG-OUT3 (17.5 µm) did not fit within the documented range but matched with the other descriptions (**Table 6**). Sample AG-OUT1 matched with the description *of U. capensis sensu* Stegenga *et al.* (1997). Seashore *Ulva* samples that were not morphologically identified as *U. rigida* or *U. capensis* are listed in (**Supplementary Table S2**).

A summarised table of the morphological descriptions of *Ulva rigida* and a list of junior synonyms from several studies around the world was prepared to investigate the morphological variation of this species (**Supplementary Table S3**). According to most of the descriptions of *Ulva rigida*, the thalli are described as stiff and have a mid-green to dark green colour. In most of the studies dentated margins were observed, especially at the basal region of the thallus. The cell shape in surface view varied among the different studies, from isodiametric, polygonal, rectangular to rounded. Moreover, the chloroplast position also varied among the different studies from being parietal to covering the whole cell. Most of the studies reported a pyrenoid number of 2-4 and above. In the studies considered for this comparison, the blade thickness increased from the apical region to the basal region with a range of 40-200 µm. The cell height was 2-4 times broader than the width in most of the studies. The cell size in surface view varied among the different studies with an increasing range of 8-25 µm from the apical region to basal region.

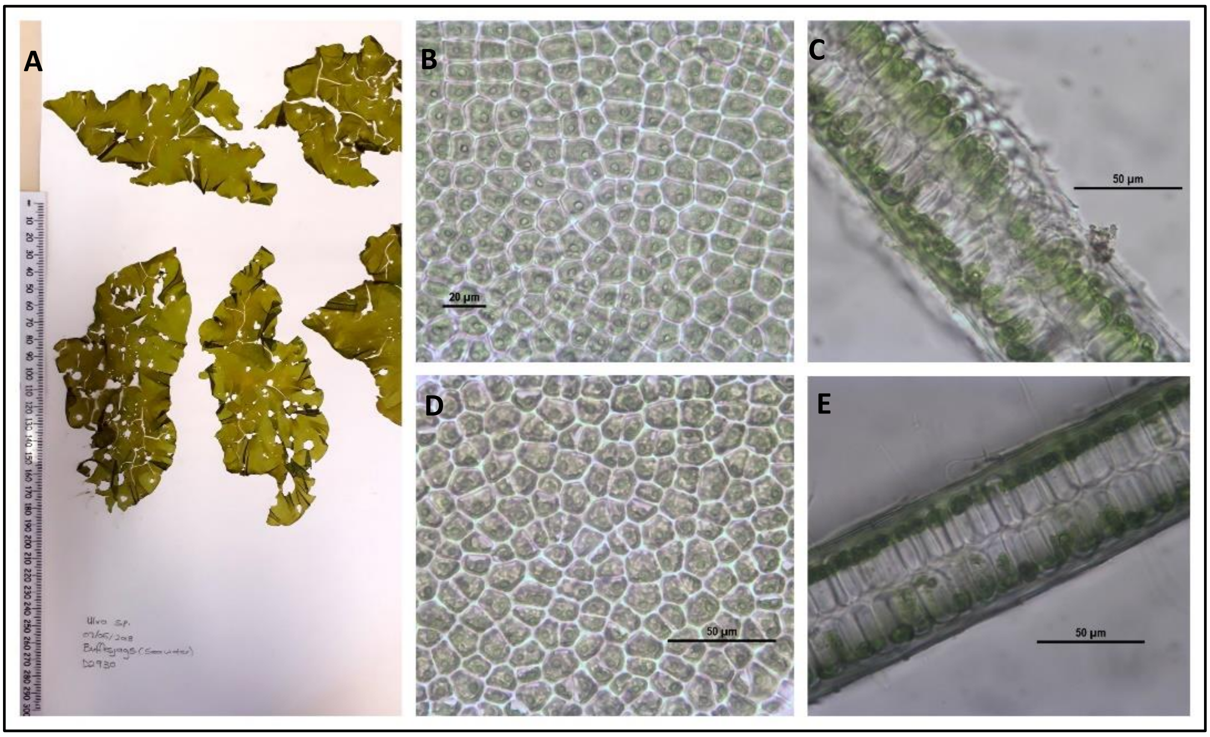

Figure 3: **A**: Morphology of sample BL-S, collected from Buffeljags Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

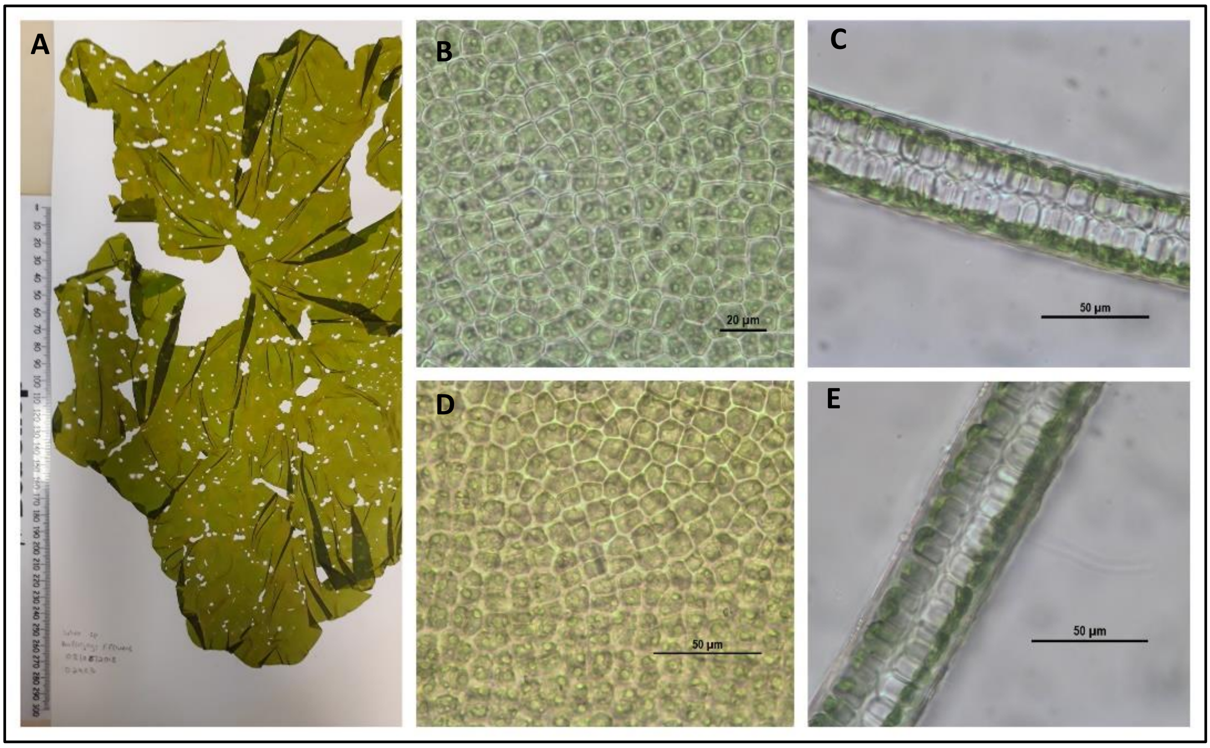

Figure 4: **A**: Morphology of sample BL-E, collected from Buffeljags Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

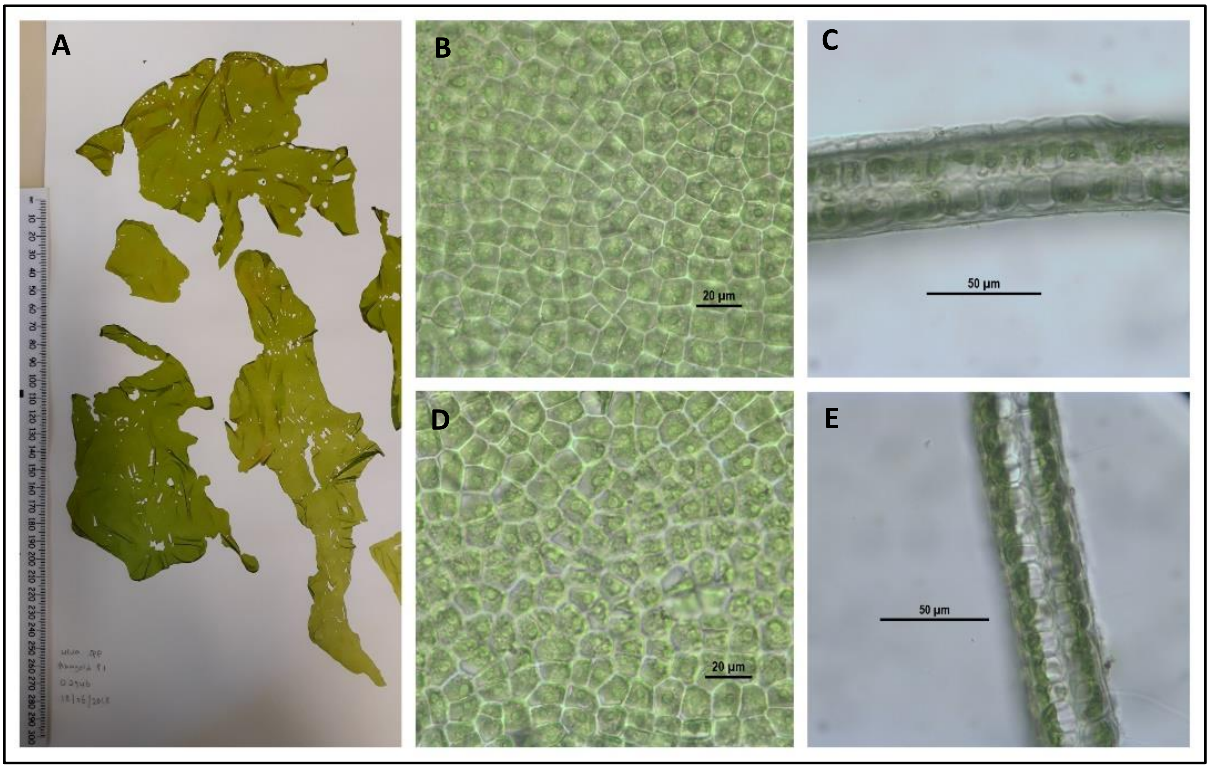

Figure 5: **A**: Morphology of sample AG-P1, collected from Abagold. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

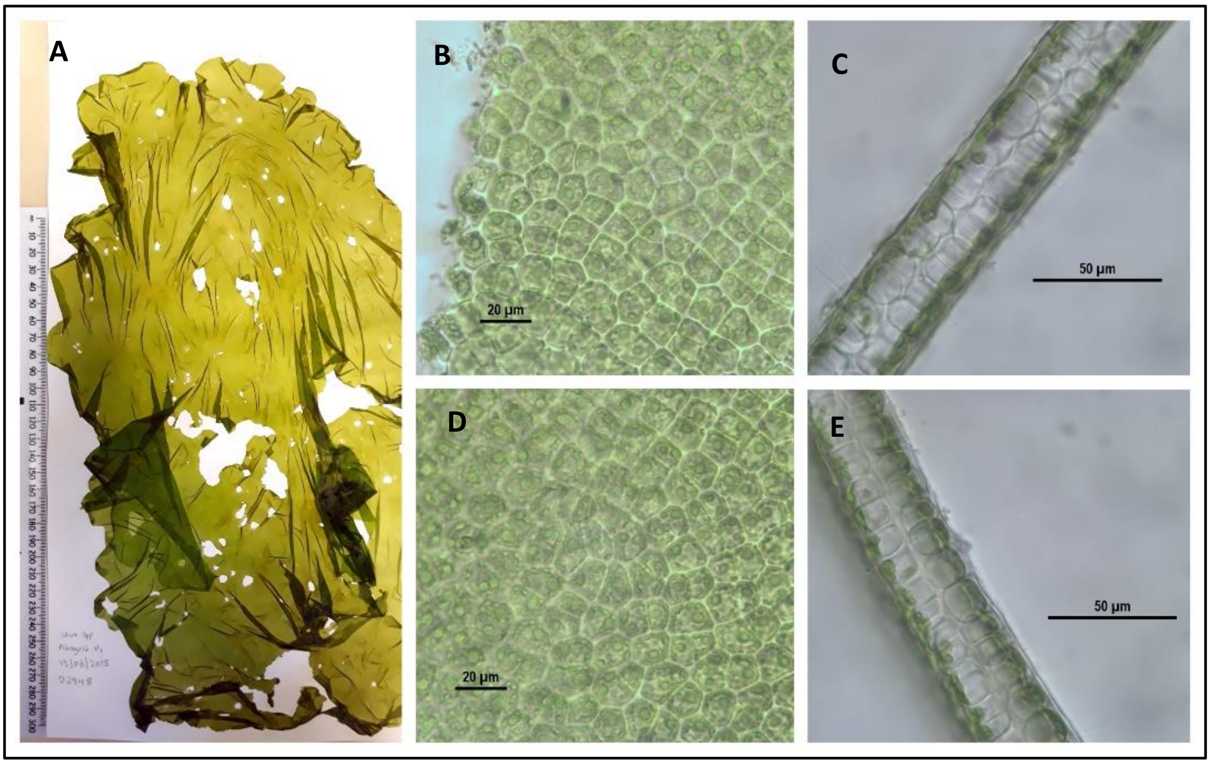

Figure 6: **A**: Morphology of sample AG-M1, collected from Abagold. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

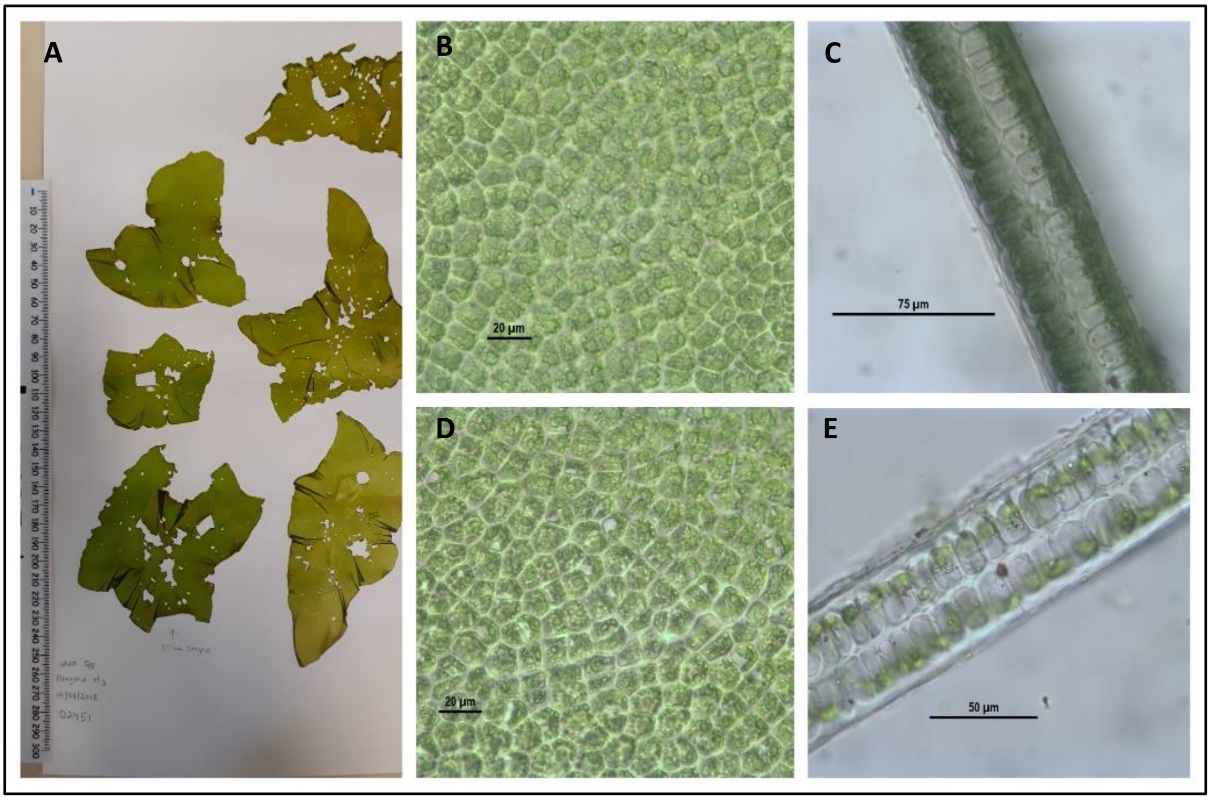

Figure 7: **A**: Morphology of sample AG-M2, collected from Abagold. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

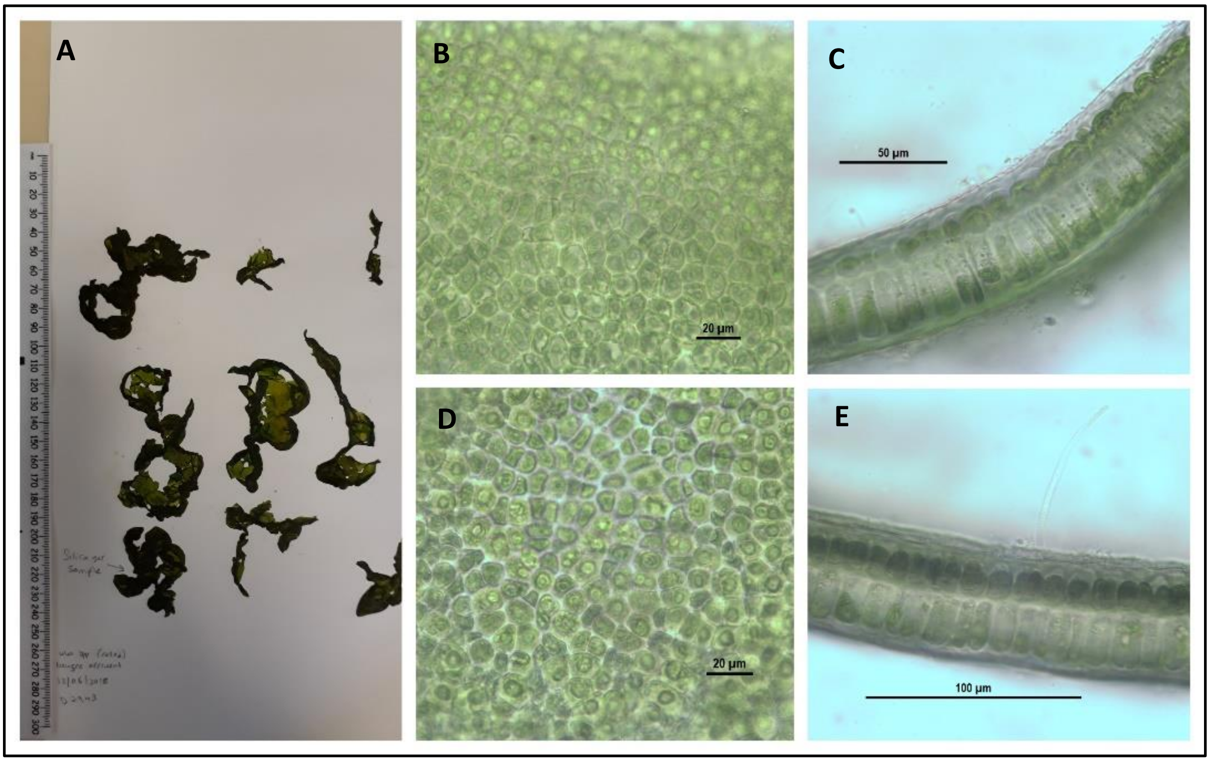

Figure 8: **A**: Morphology of sample KS-Coiled, collected from Kleinzee. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

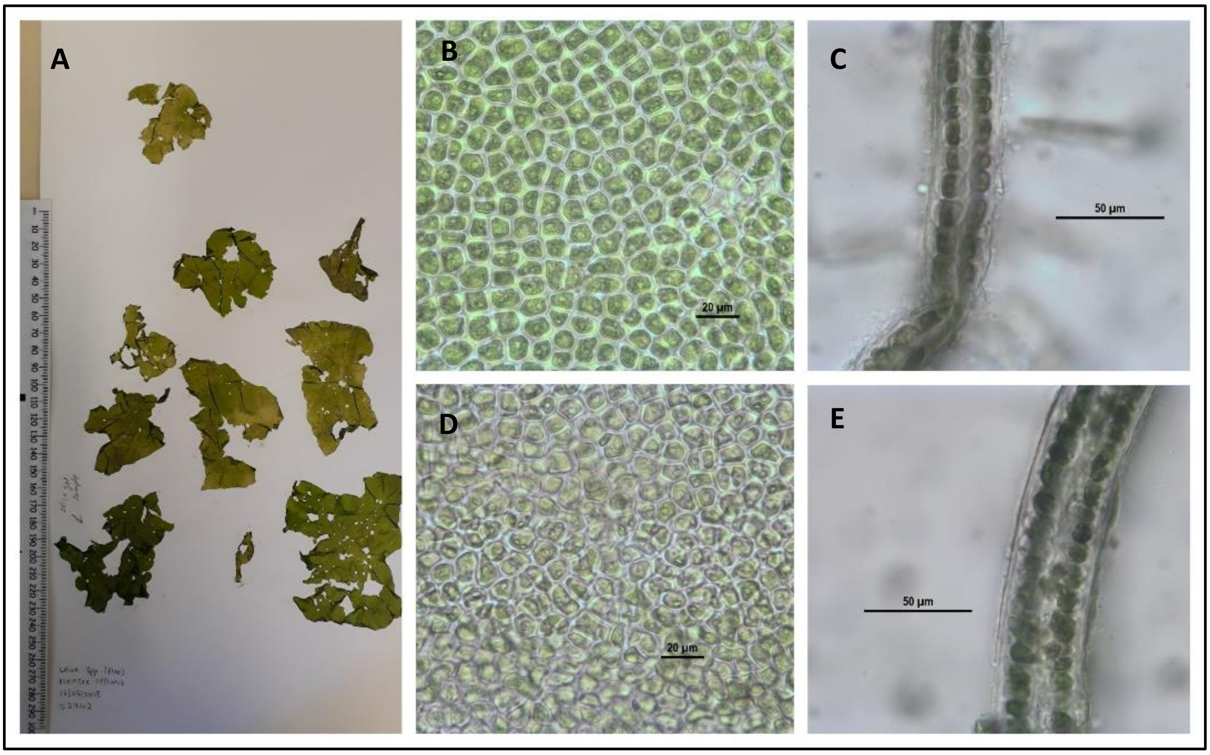

Figure 9: **A**: Morphology of sample KS-Flat, collected from Kleinzee. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

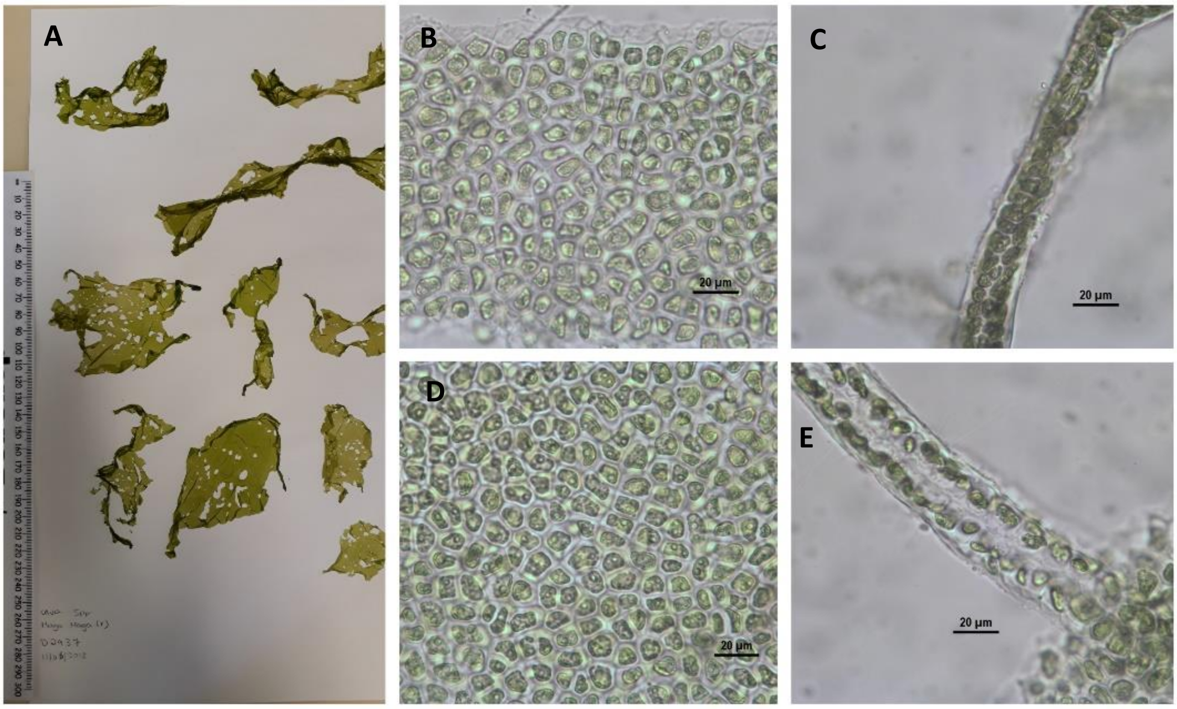

Figure 10: **A**: Morphology of sample HG-1, collected from Wild Coast Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

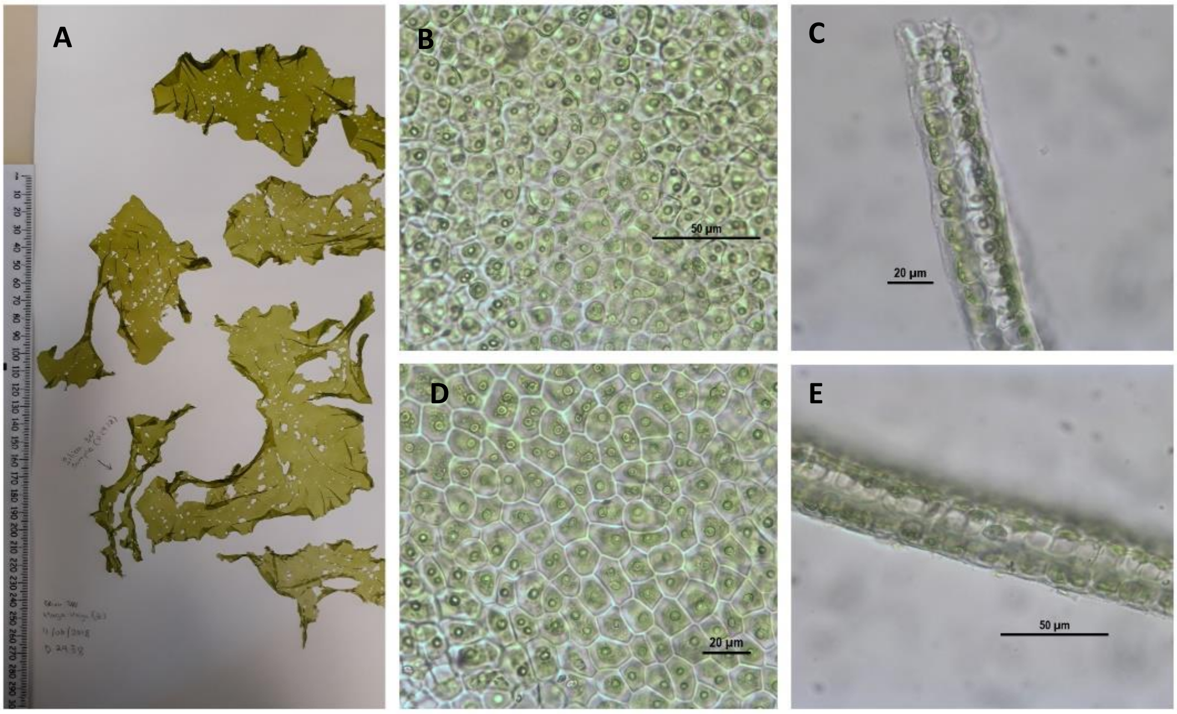

Figure 11: **A**: Morphology of sample HG-2, collected from Wild Coast Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

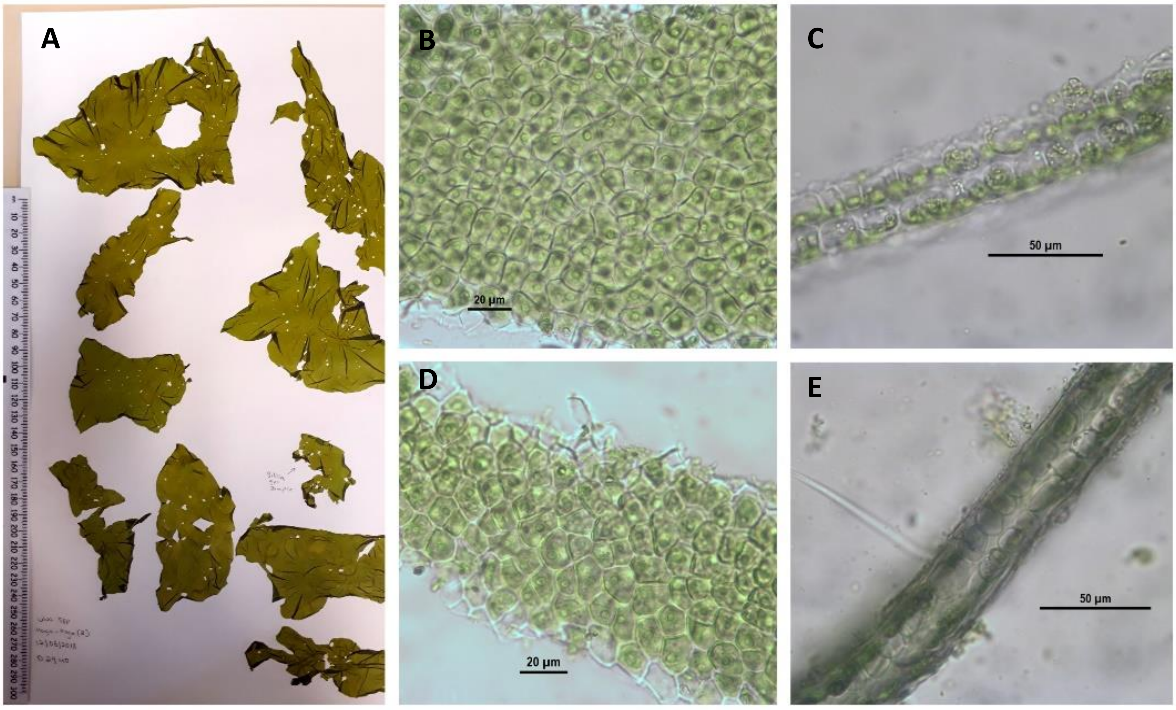

Figure 12: **A**: Morphology of sample HG-3, collected from Wild Coast Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

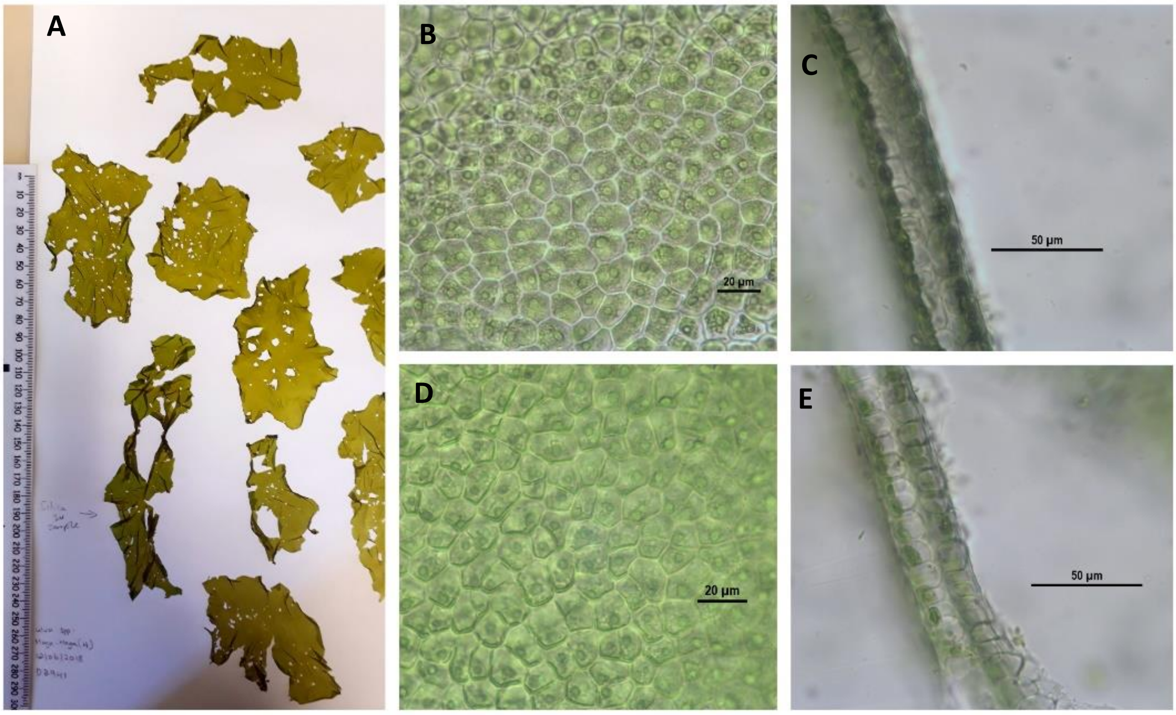

Figure 13: **A**: Morphology of sample HG-4, collected from Wild Coast Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

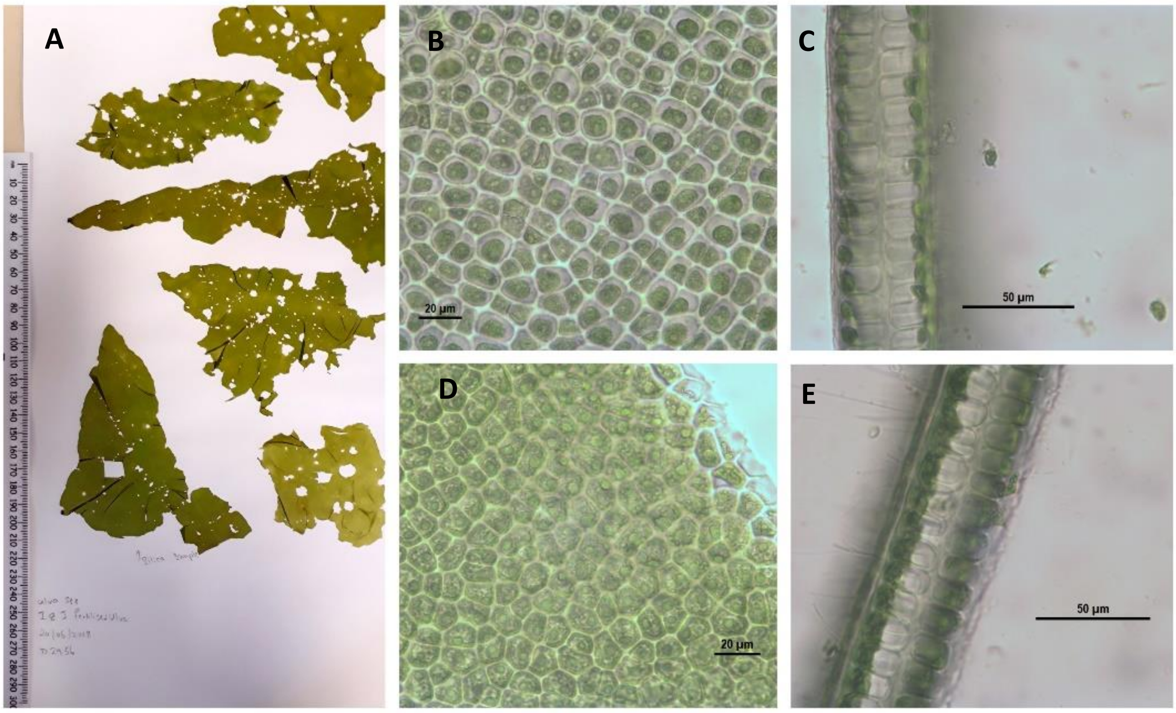

Figure 14: **A**: Morphology of sample I&J, collected from Irvin & Johnson Cape Abalone. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

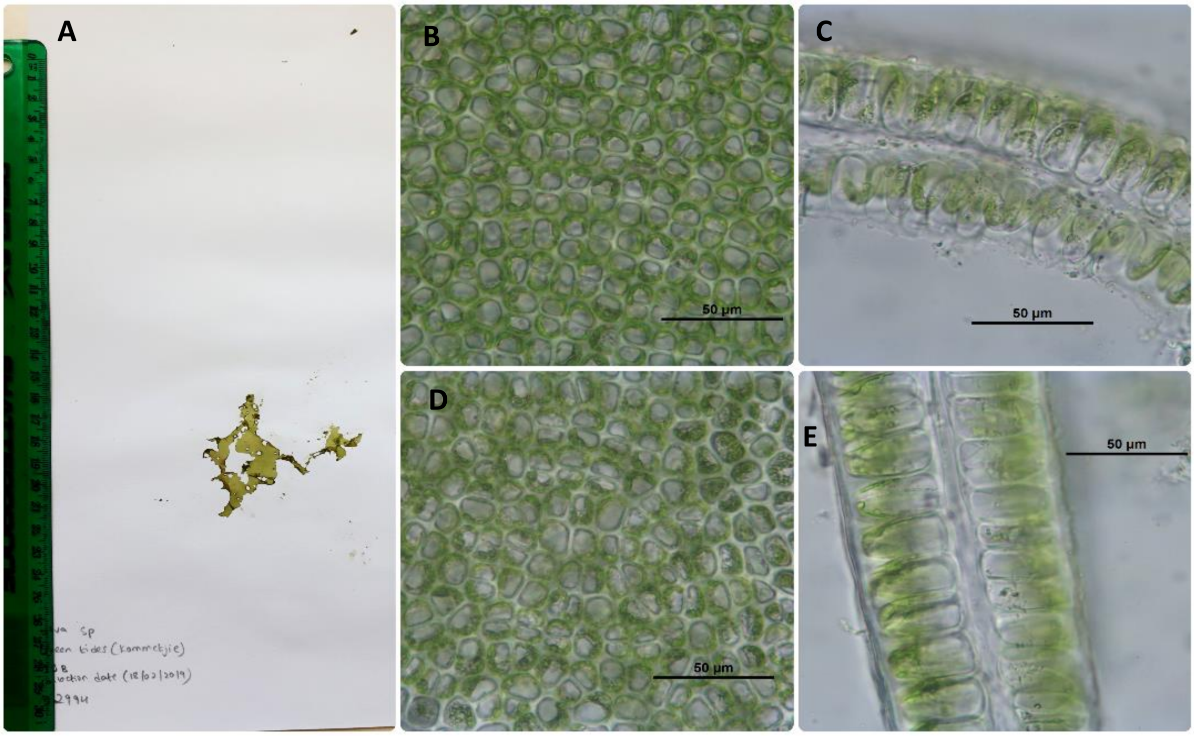

Figure 15: **A**: Morphology of sample D2994-GT, collected from a green tide along the seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

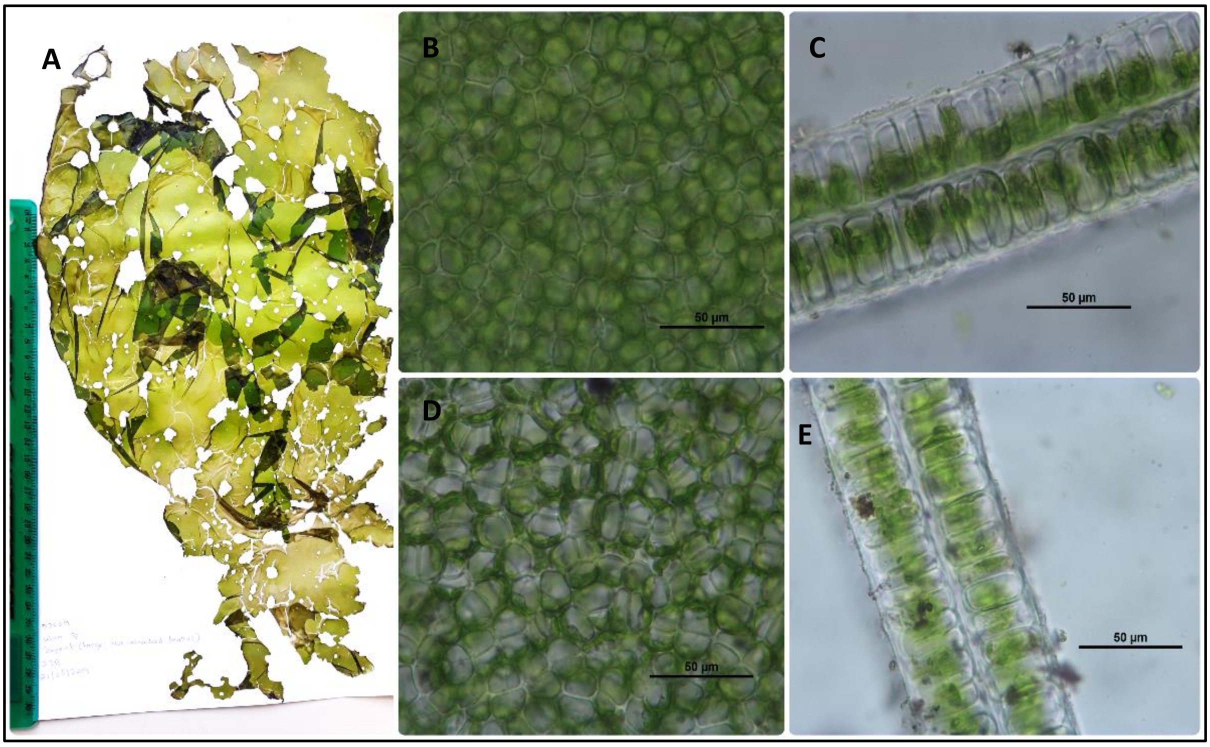

Figure 16: **A**: Morphology of sample SPT-UR, collected from seashore at Sea Point. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

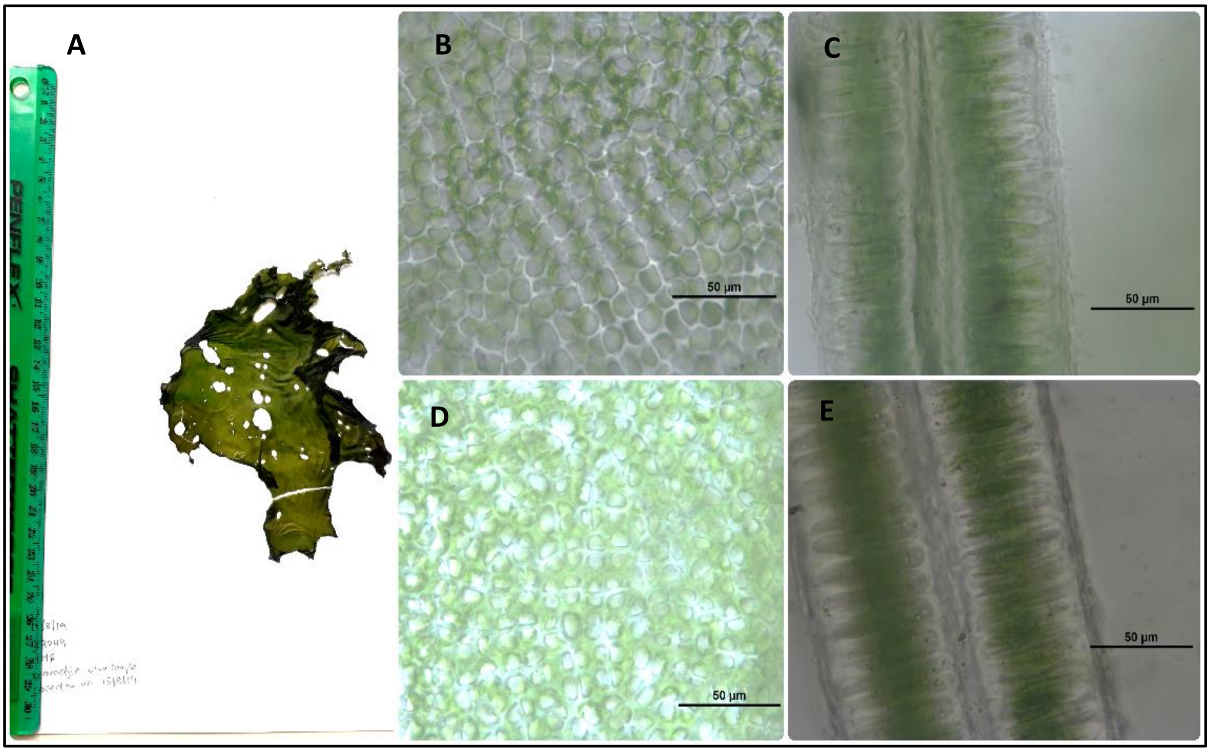

Figure 17: **A**: Morphology of sample D3049, collected from seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

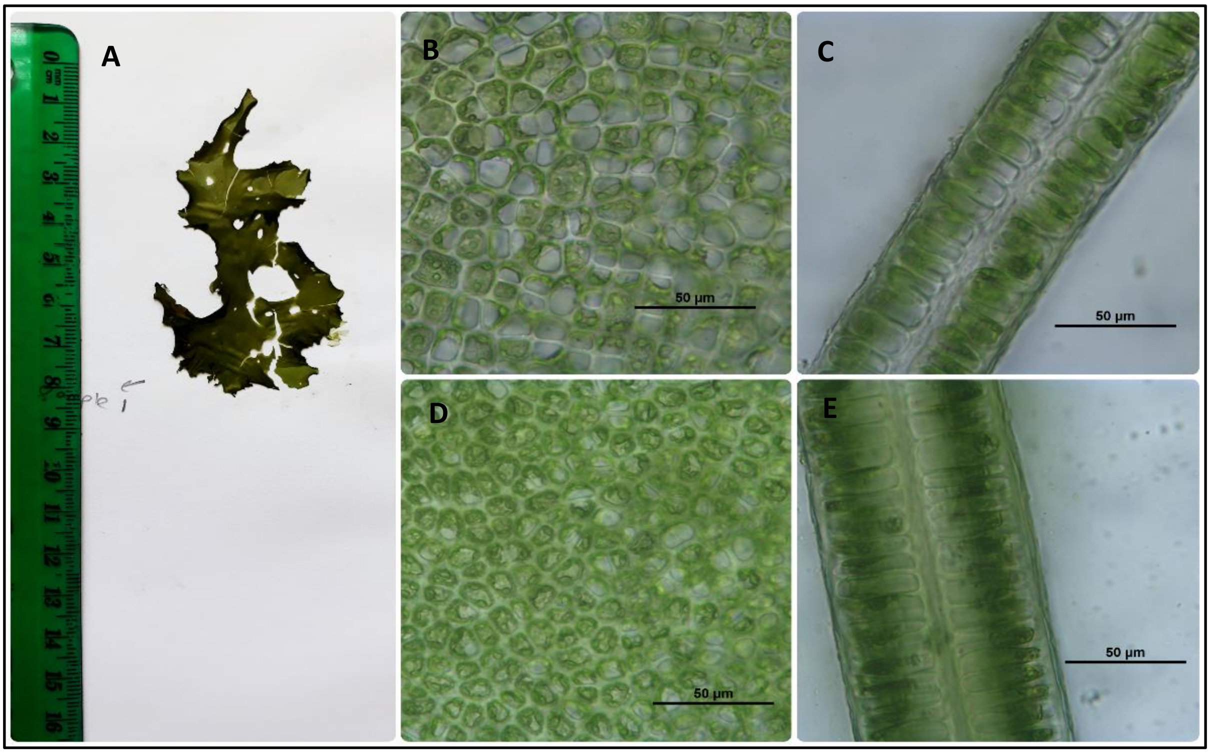

Figure 18: **A**: Morphology of sample AG-IN1, collected near the inlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

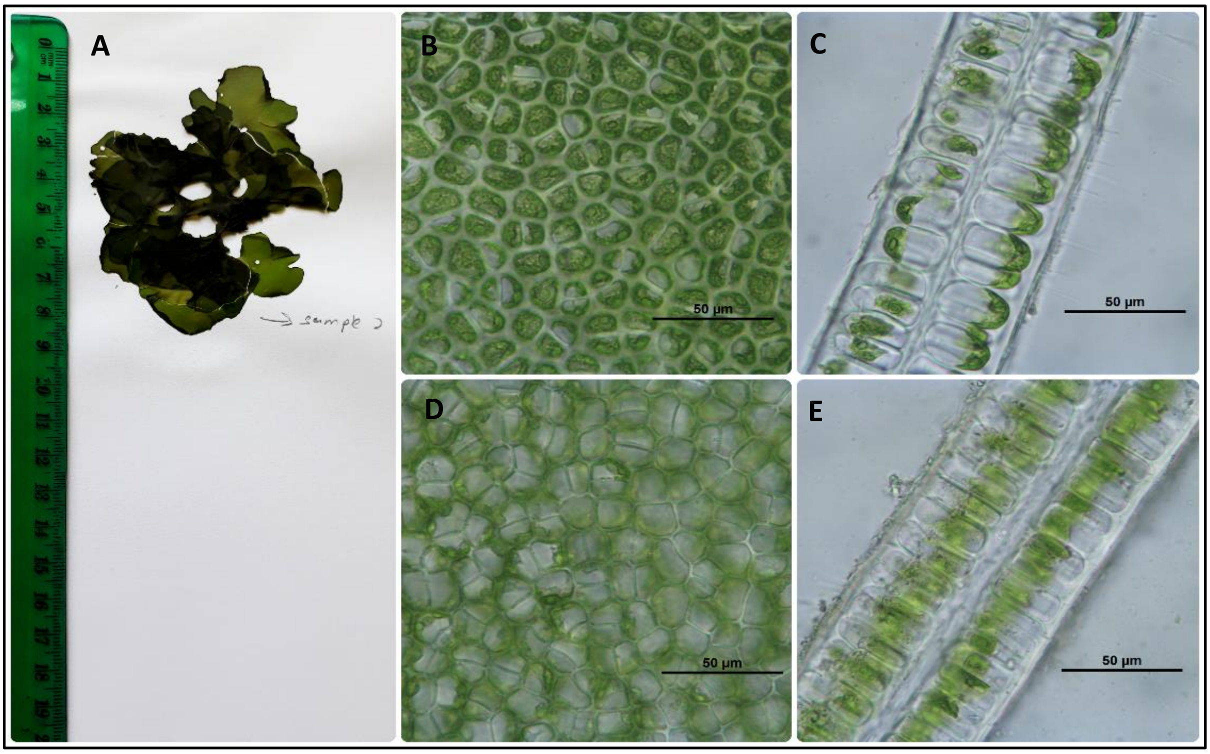

Figure 19: **A**: Morphology of sample AG-IN2, collected near the inlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

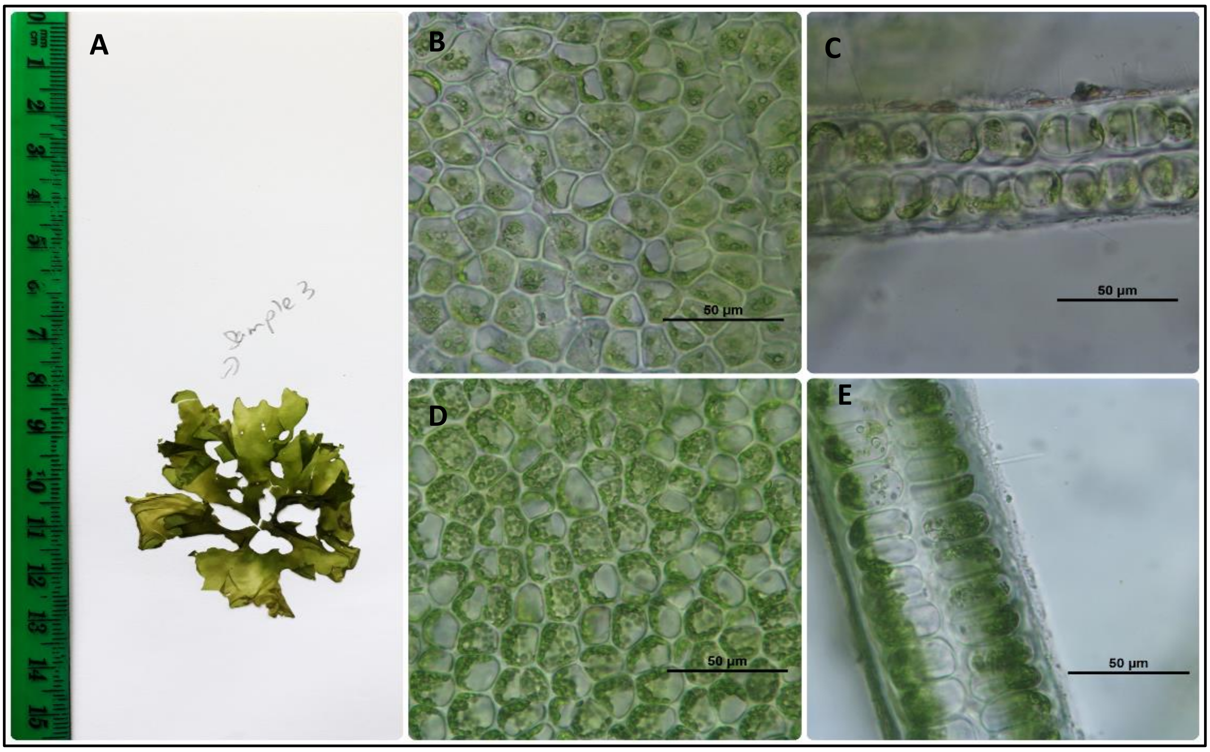

Figure 20: **A**: Morphology of sample AG-IN3, collected near the inlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

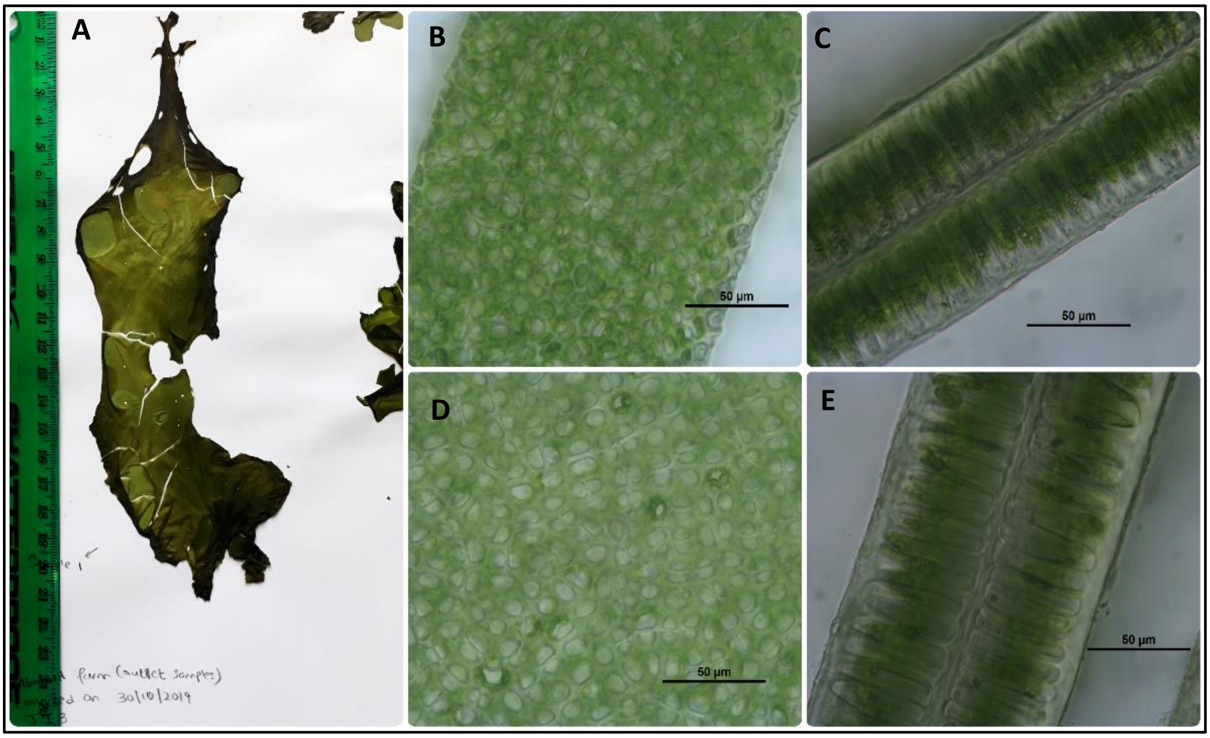

Figure 21: **A**: Morphology of sample AG-OUT1, collected near the outlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

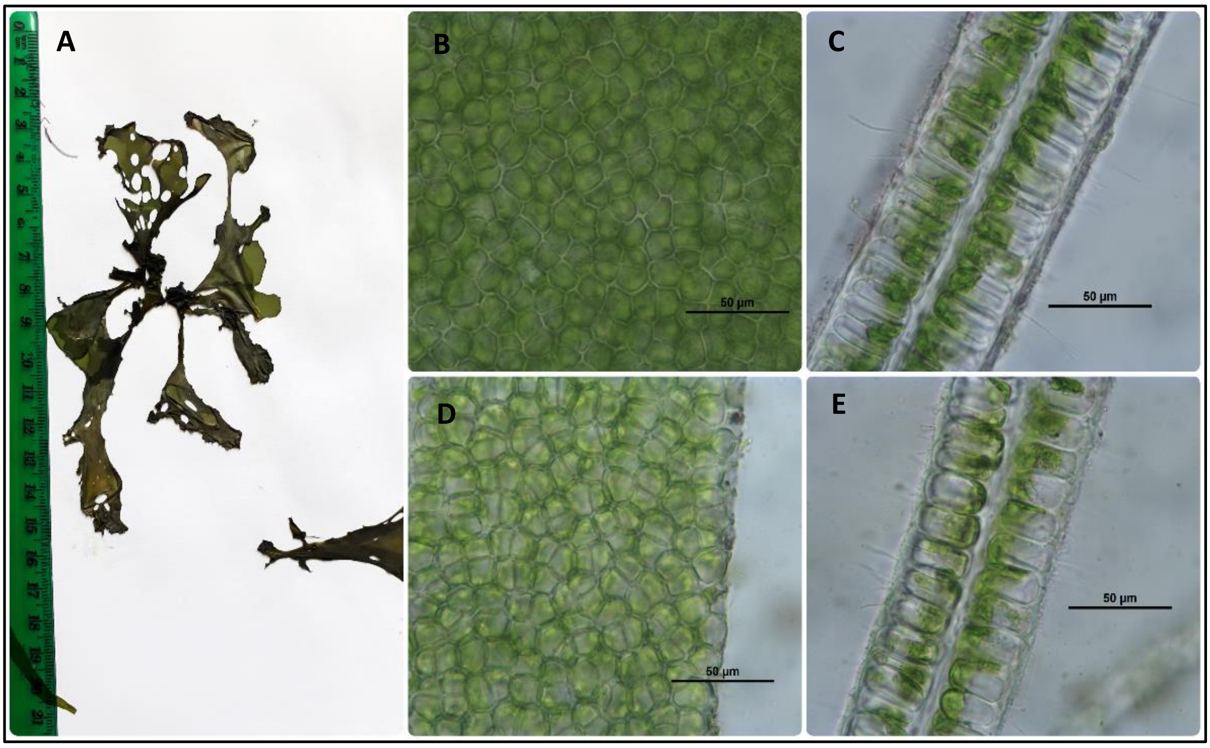

Figure 22: **A**: Morphology of sample AG-OUT3, collected near the outlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

Table 6: Morphological characteristics of the samples identified as *U. rigida*, *U. capensis* and *Ulva lactuca*. Chl = Chloroplast; CS = Cross-section view of cells; SV = Surface view of cells; E = an average size of cells from the edge of *Ulva* blade; M = an average size of cells from the mid-section of *Ulva* blade.

Name	No. of pyrenoids	Dentated Margin	Cell shape & chloroplast arrangement	Blade thickness (µm; CS)	Vegetative cell length (µm; CS)	Vegetative cell width (µm; CS)	Cell size (µm; SV)	Thallus texture and colour	Morphological identification
BL-S	1-2	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Oval cells with rounded ends in CS	E (122.5) M (65)	E (32.5) M (27.5)	E (15) M (12.5-15)	E (20) M (17.5)	Smooth and thick; few perforations in the thallus; Mid-green; Medium-sized thallus	*U. rigida*
BL-E	1-2	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Isodiametric cells in CS.	E (52.5) M (55)	E (17.5) M (20)	E (12.5) M (17.5)	E (17.5) M (15)	Smooth and thin; Lots of holes; Mid-green; Large-sized thallus	*U. lactuca*
AG-P1	Mostly 1 (Occasionally 2-3)	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Isodiametric cells in CS.	E (35) M (32.5)	E (27.5) M (27.5)	E (22.5) M (27.5)	E (30) M (25)	Smooth and thin; Lots of holes; Mid-green; Medium-sized thallus	*U. lactuca*
AG-M1	1-2 (Occasionally 2-3)	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Isodiametric cells in CS.	E (55) M (50)	E (22.5) M (20)	E (20) M (15)	E (20) M (20)	Smooth and thin; Lots of holes; Mid-green; Large-sized thallus	*U. lactuca*
AG-M2	1-2	Present	Angular cells in SV. Chl covers the whole outer cell wall. Rectangular cells with rounded edges in CS.	E (72.5) M (50)	E (30) M (20)	E (17.5) M (17.5)	E (17.5) M (17.5)	Smooth and thin; lots of holes; Mid-green; Small-sized thallus	*U. rigida*
KS-COILED	Mostly 1 (Occasionally 2-3)	Absent	Polygonal cells in SV. Chl covers the whole outer cell wall. Rectangular cells in CS.	E (57.5) M (55)	E (30) M (25)	E (12.5) M (15)	E (15) M (12.5)	Rough and thin; Dark green; Coiled; Small-sized thallus	*U. rigida*
KS-FLAT	Mostly 1 (Occasionally 1-2)	Absent	Rounded-angular cells in SV. Chl covers the whole outer cell wall. Rounded cells in CS	E (40) M (52.5)	E (17.5) M (17.5)	E (15) M (15)	E (12.5) M (12.5)	Rough and thin; Lots of holes; Mid-green; Small-sized thallus	*U. lactuca*
HG-1	Mostly 1 (Occasionally 1-2)	Absent	Rounded-angular cells in SV. Chl covers the whole outer cell wall. Rounded cells in CS.	E (32.5) M (32.5)	E (12.5) M (12.5)	E (12.5) M (10)	E (10) M (15)	Smooth and very thin; Lots of holes; Light green; Small-sized thallus	*U. lactuca*
HG-2	Mostly 1 (Occasionally 1-2)	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Rounded cells in CS.	E (35) M (32.5)	E (12.5) M (12.5)	E (17.5) M (15)	E (15) M (12.5)	Smooth and very thin; Lots of holes; Light green; Small-sized thallus	*U. lactuca*

HG-3	Mostly 1	Absent	Rounded-angular cells in SV. Chl covers the whole outer cell wall. Rounded cells in CS.	E (45) M (42.5)	E (15) M (15)	E (17.5) M (17.5)	E (17.5) M (22.5)	Smooth and thin; Mid-green; Small-sized thallus	*U. lactuca*
HG-4	Mostly 1	Absent	Angular cells in SV. Chl covers the whole outer cell wall. Isodiametric cells in CS.	E (32.5) M (32.5)	E (17.5) M (17.5)	E (20) M (15)	E (22.5) M (22.5)	Smooth and thin; Lots of holes; Mid-green; Small-sized thallus	*U. lactuca*
I&J	Mostly 1	Absent	Angular cells in SV. Orientation of the Chl was to the sidewall. Oval cells with rounded ends in CS.	E (47.5) M (55)	E (22.5) M (20)	E (22.5) M (17.5)	E (17.5) M (12.5)	Smooth and thin; Lots of holes; Mid-green; Small-sized thallus.	*U. lactuca*
D2994-GT	Mostly 1	Present	Rounded cells in SV. Orientation of the Chl was to the sidewall. Oval cells with rounded ends in CS.	E (80) M (102.5)	E (35) M (40)	E (17.5) M (17.5)	E (12.5) M (15)	Rough and thick; Few holes; Light to Mid green; Small-sized thallus	*U. rigida*
SPT-UR	1	Absent	Rounded cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (80) M (75)	E (37.5) M (32.5)	E (17.5) M (17.5)	E (17.5) M (20)	Rough and crumpled; Lots of holes; Mid green; Large-sized thallus	*U. rigida*
D3049	No pyrenoids	Present	Rounded cells in SV. Chl along the upper and lower surface of the blade in CS. Bullet-shaped cells in CS.	E (127.5) M (140)	E (25) M (60)	E (15) M (12.5)	E (15) M (15)	Rough; Lots of holes; Dark green; Medium-sized thallus	*U. capensis*
AG-IN1	1-2 (Occasionally 2-4)	Present	Angular cells in SV. Orientation of the Chl was to the sidewall mainly. Rectangular cells in CS.	E (70) M (95)	E (27.5) M (37.5)	E (15) M (15)	E (17.5) M (15)	Rough; Few holes; Dark green; Small-sized thallus	*U. rigida*
AG-IN2	1-2	Absent	Rounded cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (77.5) M (90)	E (35) M (30)	E (20) M (17.5)	E (17.5) M (20)	Rough; Few holes; Dark green; Small-sized thallus	*U. rigida*

AG-IN3	1-2 (Occasionally 2-4)	Absent	Angular cells in SV. Orientation of the Chl was to the sidewall mainly. Rounded cells in CS.	E (60) M (80)	E (22.5) M (32.5)	E (20) M (17.5)	E (22.5) M (22.5)	Smooth and thin; Few holes; Light to Mid green; Small-sized thallus	*U. rigida*
AG-OUT1	1-2	Present	Rounded cells in SV. Orientation of the Chl was to the sidewall. Bullet-shaped cells in CS.	E (102.5) M (127.5)	E (45) M (52.5)	E (15) M (15)	E (17.5) M (17.5)	Rough; Few holes; Dark green; Medium-sized thallus	*U. capensis*
AG-OUT3	1-2	Absent	Angular cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (87.5) M (77.5)	E (35) M (32.5)	E (20) M (17.5)	E (17.5) M (17.5)	Rough; Lots of holes; Dark green; Small-sized thallus	*U. rigida*

Molecular analyses

*rbc*L sequence analysis

The 12 *Ulva* samples cultivated on the abalone farms clustered together in a single large clade with weak bootstrap support (BP) for the Maximum Likelihood analysis and Bayesian Posterior Probabilities (PP) (0.57) (**Figure 23**). This clade also contained sample AG-IN3, a foliose attached *Ulva* sample collected near the inlet of Abagold in Hermanus, and *U. lacinulata* specimen from Ireland (MT160587), *U. rigida* isolates from different countries, such as South Korea (KP233772), the USA (AY422564), New Zealand (LK022428), Ireland (EU484408), Portugal (MN450427), China (KP975386), France (MT160586) and Italy (HE600163); *U. armoricana* isolates from Japan (AB097632), France (AB097630) and New Zealand (EF110294); Bliding's specimens of *U. scandinavica* from Norway (MW570777 and MW570778), *U. scandinavica* isolates from the UK (EU484412), the USA (AY255870), Ireland (EU484416) and Netherlands (AB097629); and *U. laetevirens* isolates from Australia (EU933943), Canada (HQ603664), and the USA (JQ048945). Moreover, the *Ulva* samples (U114 I&J, U117 I&J, LK14, LK255, LK256 and LK258) collected from a previous unpublished study by Lineekela Kandjengo also belonged in the same clade as the farmed *Ulva* samples analysed in this study. The specimens *U. capensis sensu* Stegenga *et al.* (1997) (U03 I&J, U274 LK264, LK280, D3049, AG-OUT1 and NAM103) and *U. rigida sensu* Stegenga *et al.* (1997) (D2994-GT and AG-IN1) were resolved into a smaller internal clade, with moderate support of 0.71 for PP. Moreover, this smaller internal clade also contained *U. rigida* from the USA (AY422564); New Zealand (LK022428); Ireland (EU484408) and *Ulva* sp. *A* from Portugal (MT160528) and Ireland (MT160573). The *U. lacinulata* (MT160587) and *Ulva* sp. *A* (MT160586) specimens from Fort *et al.* (2020) formed a well-supported small internal clade within the single large clade, with a high support value of 89% and 0.95 for BP and PP, respectively.

Samples SPT-UR, NAM19, NAM102 and AG-OUT3 that were morphologically identified as *U. rigida sensu* Stegenga *et al.* (1997), were resolved within a clade with high support of 90% and 1 for BP and PP, respectively. This clade also contained *Ulva australis/Ulva pertusa* specimens from Australia (EU933957, MT815849, LC331300 and MT815847), the USA

(AY422549), South Korea (KP233769) and France (MT160564). The five samples morphologically identified as *U. rigida* (U36, U02, U56, UR_CE0 and CD6) by Kandjengo also belonged to this clade.

*rbc*L pairwise distance analysis

Based on the *rbc*L phylogeny, there were 27 *Ulva* samples that were resolved into the *U. lacinulata* clade. The 27 *Ulva* samples were collapsed into three haplotypes. Haplotype 1 contained 8 samples (U03, U274 LK264, LK280, D2994-GT, D3049, AG-IN1, NAM103 and AG-OUT1). Haplotype 2 contained 18 samples (U114 I&J P1, LK14, LK255, LK256, LK258, AG-IN3, AG-P1, BL-E, HG-1, HG-2, HG-3, I&J, KS-Coiled, KS-Flat, AG-M1, BL-S, AG-M2 and HG-4). Haplotype 3 contained 1 sample (U117 I&J). A sample representative was chosen from each group of haplotypes. D3049 was selected from haplotype 1, BL-S from haplotype 2 and U117 I&J from haplotype 3. A representative of *Ulva* species from different countries within the *U. lacinulata* clade was selected.

There was no sequence divergence (0%) between sample D3049 and the *U. rigida* isolates from the USA (AY422564), New Zealand (LK022428), Ireland (EU484408), and *Ulva* sp. *A* from France (MT160586) (**Table 7**). The sequence divergence between D3049 and *U. rigida* Italy (HE600163) was the highest (0.29%). There was no sequence divergence (0%) between sample BL-S and *U. rigida* isolates from Portugal (MN450427) and Italy (HE600163); *U. armoricana* isolate from France (AB097630); *U. laetevirens* from Australia (EU933943), *U. lacinulata* from Ireland (MT160587); *U. scandinavica* isolate from Ireland (EU484416) and *U. lacinulata* (MW570778) from Norway. The sequence divergence between sample BL-S and *U. rigida* from New Zealand LK022428 was the highest (0.17%). The sequence divergence between sample U117 I&J and *U. lacinulata* (MW570778) was the lowest (0%). The sequence divergence between sample U117 I&J and *U. rigida* from New Zealand LK022428 was the highest (0.26%). Based on the *rbc*L pairwise distance analysis, sample D3049 that was morphologically identified as *U. capensis*, was found to be genetically identical to *U. rigida* specimens from the USA (AY422564), New Zealand (LK022428), Ireland (EU484408), and *Ulva* sp. *A* from France (MT160586) as the sequence divergence between them was 0%. Next, sample BL-S was genetically identical to *U. rigida* specimens from Portugal (MN450427) and Italy (HE600163); *U. armoricana* isolate from France (AB097630); *U. laetevirens* from Australia (EU933943), *U. lacinulata* from Ireland (MT160587), *U. scandinavica* isolate from Ireland (EU484416) and *U. lacinulata* (MW570778) from Norway as the sequence divergence between these specimens was 0%. Moreover, all the farmed samples were collapsed as a single haplotype, indicating that all the farmed materials collected in this study represented the same taxon and sample AG-IN3 was also recovered in the same haplotype group. Samples D2994-GT and AG-IN1 were recovered in the same haplotype group containing the *U. capensis* samples (D3049, NAM103 and AG-OUT1). The genetic distance between the farmed sample BL-S and sample D3049 was 0.16%. Sample U117 I&J collected by Lineekela Kandjengo in 2009 was very closely related to the farmed sample BL-S with a genetic difference of 0.081%.

According to the pairwise distance analysis of the *rbc*L gene, the local farmed *Ulva* samples are genetically identical to *U. lacinulata* as the sequence divergence between the farmed *Ulva* samples and the *U. lacinulata* (MT160587) specimen from Fort *et al.* (2020) was 0%. The *U. capensis* samples (U03, U274 LK264, LK280, D2994-GT, D3049, AG-IN1, NAM103 and AG-OUT1) are genetically identical to the *Ulva* sp. *A* from Fort *et al.* (2021) as the sequence divergence between the *Ulva* sp. *A* (MT160586) and the *U. capensis* samples was 0%.

Table 7: Pairwise distances (%) among species within the *U. lacinulata* clade and the haplotypes of farmed *Ulva* samples for the *rbc*L gene region. Representatives of *U. rigida, U. armoricana, U. laetevirens and U. scandinavica* from different countries were selected. The 27 *Ulva* samples for the *rbc*L gene were collapsed into three haplotypes.

	U. rigida **AY422564** USA	*U. rigida* **LK022428** New Zealand	*U. rigida* **EU484408** Ireland	*U. rigida* **MN450427** Portugal	*U. rigida* **HE600163** Italy	*Ulva* sp. *A* **MT160586** France	*U. armoricana* **AB097630** France	*U. laetevirens* **EU933943** Australia	*U. lacinulata* **MT160587** Ireland	*U. scandinavica* **EU484416** Ireland	*U. lacinulata* **MW570778** Norway	D3049	BL-S
D3049	0	0	0	0.22	0.29	0	0.16	0.16	0.16	0.16	0.27		
BL-S	0.16	0.17	0.16	0	0	0.16	0	0	0	0	0	0.16	
U117 I&J	0.24	0.26	0.24	0.11	0.15	0.24	0.081	0.081	0.081	0.081	0	0.24	0.081

Figure 23: The Bayesian Inference tree for *Ulva rbc*L sequence data (scale at bottom) is presented and was congruent with RAxML. The tree was rooted with *Blidingia minima* and *Monostroma nitidum*. Numbers near each node refer to the posterior probability values (PP) of the Bayesian Inference tree added in front of the Maximum-Likelihood (ML) bootstrap values (≥70). Numbers accompanying the species names are GenBank accession numbers for the sequences used in the analysis. Fort *et al.* 2020 specimens of *U. lacinulata* (**MT160587**) and *Ulva* sp. *A* (**MT160586**) in bold.

ITS sequence analysis

The 12 *Ulva* samples cultivated on the abalone farms clustered together in a single clade with high support of 0.86 for PP (**Figure 24**). This clade also contained *U. rigida* isolates from Portugal (MN444712), (MN444714-MN444720); *U. scandinavica* from the UK (AJ234317) and *U. laetevirens* specimens from China (KC411870), from Australia (EU933989), from Ireland (MT894611) and an *Ulva* species from South Korea (MN069993). The *Ulva* samples (U104, U109, U113, U107, U104F, U114 I&J P1 and U113F) collected from a previous unpublished study by Lineekela Kandjengo, also belonged in the same clade as the 12 farmed *Ulva* samples analysed in this study. Sample AG-IN3 was also resolved in the same clade containing the farmed *Ulva* samples. The *U. capensis* samples collected in this study (D3049 and AG-OUT1) formed a sister clade to the clade containing the farmed *Ulva* samples with moderate support value of 70% and 0.75 for BP and PP, respectively. This clade also contained the samples U16, U21 I&J and U21F that were collected by Lineekela Kandjengo in 2009. Furthermore, the samples AG-IN1 and NAM103 were resolved into a smaller internal group with a moderate support of 75% and 0.68 for BP and PP, respectively. The farmed *Ulva* samples and the *U. capensis* samples were in separate sister clades but with short branch lengths, indicating low divergence. The sister clades containing the farmed *Ulva* samples and the *U. capensis* samples belonged in a larger well-supported clade with a high support value of 0.92 for PP. The *U. lacinulata* lectotype (MW544060) from Croatia belonged within an internal smaller group with a support of 0.92 for PP containing the *U. rigida* lectotype (MW544059) from Cádiz, Spain and *U. pseudorotundata* (MT894650) from Ireland.

High quality ITS sequences could not be obtained from samples SPT-UR, AG-OUT3, NAM19 and NAM102 that were morphologically identified as *U. rigida*. Moreover, the following *Ulva* samples collected from seashores: SPT-UC, SPT-UL, D2993-GT, D2995-SAK, D2996-MGK, AG-IN2, AG-IN4 and AG-OUT4 were not included in the tree because good quality ITS sequences could not be obtained for them. The branch length in the ITS tree was short compared to the branch length in the other two phylogenetic trees.

ITS pairwise distance analysis

Based on the ITS phylogeny, there were 27 *Ulva* samples that were resolved into the *U. lacinulata* clade. The 27 *Ulva* samples were collapsed into four haplotypes. Haplotype 1 contained 19 samples (HG-4, HG-3, AG-P1, HG-2, BL-E, HG-1, BL-S, KS-Coiled, KS-Flat, AG-M2, I&J, AG-M1, U104, U109, U113, U114 I&J P1, U107, U104F and U113F). Haplotype 2 contained 6 samples (AG-IN1, NAM103, AG-OUT1, U16, U21 I&J and U21F). Haplotype 3 contained 1 sample (AG-IN3). Haplotype 4 contained 1 sample (D3049). A sample representative was chosen from each group of haplotypes. BL-S was selected from haplotype 1, AG-IN3 from haplotype 2, NAM103 from haplotype 3 and D3049 from haplotype 4. Representatives of *Ulva* species from different world regions within the *U. lacinulata* and *U. uncialis* clades were selected.

There was a low sequence divergence (0.19%) between sample BL-S and *U. rigida* isolate from Portugal (MN444720) (**Table 8**). The sequence divergence between sample BL-S and *U. lacinulata* from Croatia (MT078695) was the highest (4.46%). The sequence divergence between sample AG-IN3 and *U. laetevirens* from Australia (EU933989) was the lowest (0.38%) and highest between *U. lacinulata* from Croatia (MW544060) (4.46%). The sequence divergence between sample NAM103 and *U. rigida* from France (MT078965) was the lowest (0.38%) and highest (4.85%) between *U. lacinulata* from Croatia (MW544060). The sequence divergence between sample D3049 and *U. rigida* from France (MT078965) was the lowest (0.38%) and highest (4.85%) between *U. lacinulata* from Croatia (MW544060). Based on the ITS pairwise distance analysis, sample BL-S was closely related to *U. rigida* from Portugal (MN444720) as the sequence divergence between the two was 0.19%. Moreover, all the farmed *Ulva* samples were collapsed as a single haplotype, indicating that all the farmed materials collected in this study represented the same taxon. However, compared to the *rbc*L analysis, sample AG-IN3 was not collapsed with the farmed *Ulva* materials. Furthermore, samples NAM103 and D3049 were not collapsed as a single haplotype by this gene, whereas with the *rbc*L analysis they were recovered as a single haplotype. Sample AG-IN1 was also recovered in this haplotype group containing the *U. capensis* samples (NAM103, AG-OUT1 and U16). The sequence divergence between the farmed sample BL-S and the *U. capensis* samples (NAM103 and D3049) was 0.76%. The sequence divergence between the *U. capensis* sample D3049 and NAM103 was 0.22%. The sequence divergence between the farmed sample BL-S and sample AG-IN3 was 0.45%.

Table 8: Pairwise distances (%) among species within the *U. lacinulata* and *U. uncialis* clades and the haplotypes of farmed *Ulva* samples for the ITS marker. Representatives of *U. rigida, U. armoricana, U. laetevirens* and *U. scandinavica* from different countries were selected. The 27 *Ulva* samples for the ITS molecular marker were collapsed into four haplotypes.

	U. rigida **MT894503** France	*U. rigida* **MT078965** France	*U. rigida* **KP975379** China	*U. rigida* **MN444720** Portugal	*U. armoricana* **AB097660** France	*U. scandinavica* **AJ234317** UK	*U. scandinavica* **AF153484** Netherlands	*U. laetevirens* **EU933989** Australia	*U. laetevirens* **MT894611** Ireland	*U. lacinulata* **MW544060** Croatia	BL-S	AG-IN3	NAM103
BL-S	1.91	1.32	0.81	0.19	0.20	0.17	0.91	0.38	1.15	4.46			
AG-IN3	2.29	1.32	0.81	0.93	0.98	0.52	0.68	0.38	1.53	4.46	0.45		
NAM103	1.15	0.38	1.21	1.50	1.58	1.04	1.36	0.75	1.91	4.85	0.76	0.78	
D3049	1.15	0.38	1.41	1.50	1.58	1.04	1.36	0.75	1.91	4.85	0.76	0.78	0.22

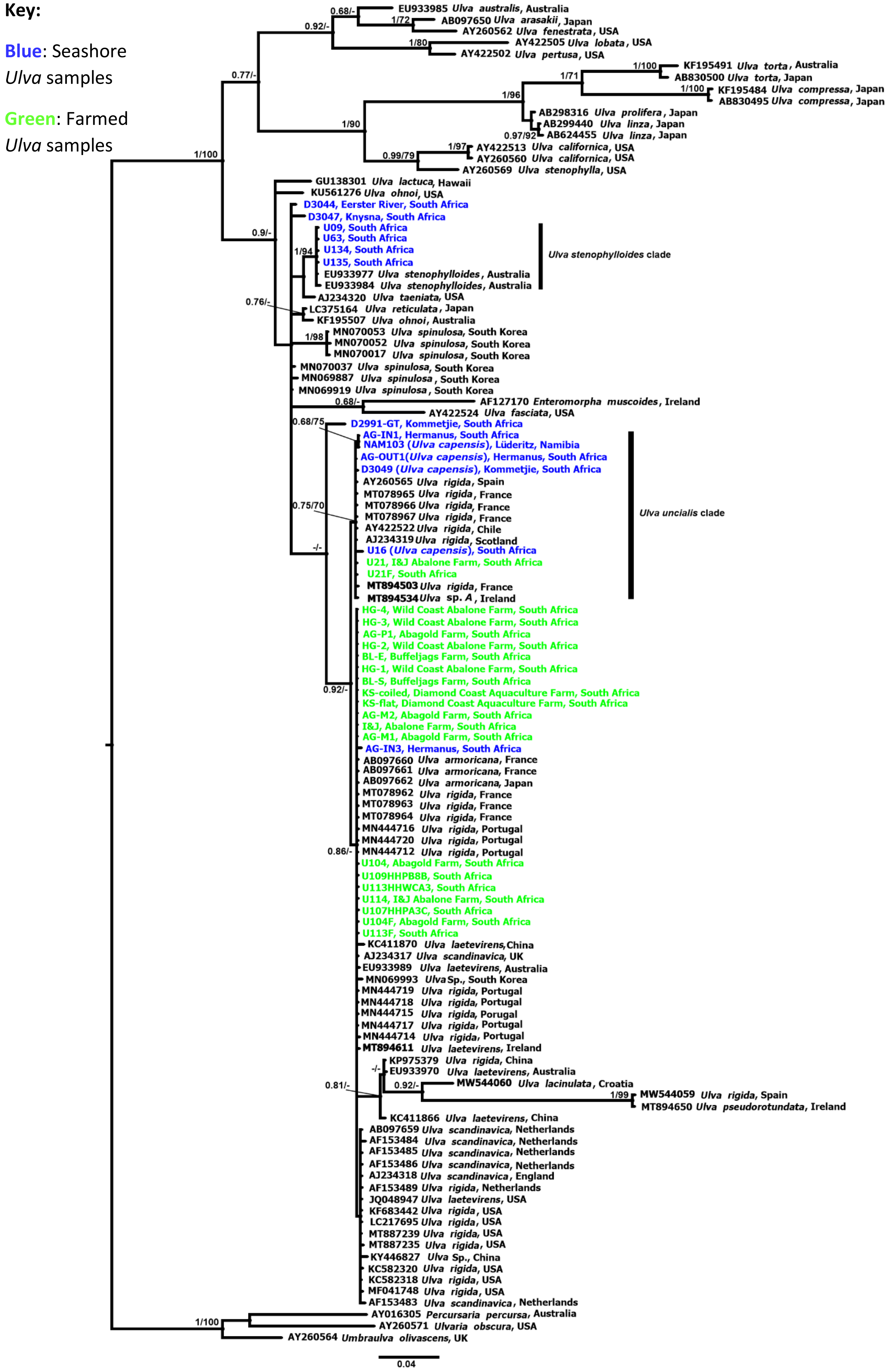

Figure 24: The Bayesian Inference tree for *Ulva* ITS sequence data (scale at bottom) is presented and was congruent with RAxML. The tree was rooted with *Percursaria percursa, Umbraulva olivascens* and *Ulvaria obscura*. Numbers near each node refer to the posterior probability values (PP) of the Bayesian Inference tree added in front of the Maximum-Likelihood (ML) bootstrap values (≥70). Numbers accompanying the species names are GenBank accession numbers for the sequences used in the analysis. Fort *et al.* (2020) specimens of *U. laetevirens* (**MT894611**) and *U. rigida* (**MT894503**) in bold.

*tuf*A sequence analysis

The 12 *Ulva* samples cultivated on the abalone farms clustered together in a single clade containing *Ulva lacinulata*, *Ulva rigida* and *Ulva laetevirens* with high Bayesian support of 0.92 and Maximum-Likelihood bootstrap value (BP) of 92% (**Figure 25**). This large clade contained *U. rigida* specimens from Germany (MH538641 and MH538633); *U. laetevirens* specimens from Australia (JN029325 and JN029327); and *U. lacinulata* from Ireland (MT160697). Sample AG-IN3 collected near the inlets of the Abagold farm in Hermanus also belonged in this clade. The *U. capensis* samples (D3049, AG-OUT1 and NAM103) and the *U. rigida* samples (D2994-GT and AG-IN1) were resolved into a sister clade with a high support value of 97% and 1 for BP and PP, respectively. The clade also contained the specimens *Ulva* sp. *A* from France (MT160696) and from Ireland (MT160683) from Fort et *al.* (2020). The specimens *Ulva* sp. *A* from Fort *et al.* (2020) (MT160696) and the *U. capensis* samples from this study are labelled as *U. uncialis*.

The other samples (SPT-UR, AG-OUT3, NAM19 and NAM102) that were morphologically identified as *U. rigida* were resolved within an internal clade containing *U. australis/U. pertusa* isolates from Canada (HQ610378), Australia (JN029281), Italy (HE600190), South Korea (JN029265), France (MT160674) and the USA (KM255001) and had a high support value of 100% and 1 for BP and PP, respectively.

Based on the topology of the *tuf*A phylogenetic tree, this gene showed better resolution compared to the other two trees and it resolved all the *Ulva* specimens collected in this study, except for sample D2991-GT. The *tuf*A tree formed well-supported internal clades compared to the ITS and *rbc*L phylogenetic trees.

*tuf*A pairwise distance analysis

Based on the *tuf*A phylogeny, there were 18 *Ulva* samples that were resolved into the *U. lacinulata* clade. The 18 *Ulva* samples were collapsed into two haplotypes. Haplotype 1 contained 13 samples (AG-M2, AG-IN3, HG-1, HG-2, HG-3, HG-4, I&J, KS-Coiled, KS-Flat, AG-M1, AG-P1, BL-S and BL-E). Haplotype 2 contained 5 samples (D2994-GT, D3049, AG-IN1, AG-OUT1 and NAM103). A sample representative was chosen from each group of haplotypes. Sample BL-S was selected from haplotype 1 and sample D3049 was selected from haplotype 2. A representative of *Ulva* species from different countries within the *U. lacinulata* clade was selected.

There was no sequence divergence (0%) between sample BL-S and *U. rigida* isolate from Germany (MH538640) and *U. laetevirens* isolates from Australia (JN029325) and *U. lacinulata* from Ireland (MT160697) (**Table 9**). The sequence divergence between sample BL-S and *Ulva* sp. *A* from France (MT160696) was the highest (0.89%). There was no sequence divergence between sample D3049 and *Ulva* sp. *A* from France (MT160696). The sequence divergence between sample D3049 and *U. rigida* from Germany (MH538640) was the highest (1.19%). Based on the *tuf*A pairwise distance analysis, sample BL-S was genetically identical to *U. rigida* isolate from Germany (MH538640) and *U. laetevirens* isolates from Australia (JN029325) and *U. lacinulata* from Ireland (MT160697) as the sequence divergence between them was 0%. Sample D3049 was genetically identical to *Ulva* sp. *A* from France (MT160696) as the sequence divergence between them was 0%. All the farmed samples were recovered as a single haplotype by this marker as well as by *rbc*L and ITS. Furthermore, this marker did not separate the *U. capensis* samples (NAM103 and D3049) into different haplotypes as was observed with the ITS analysis. The sequence divergence between sample BL-S representing the farmed *Ulva* samples and the *U. capensis* sample (D3049) was 0.92%. Moreover, the sequence divergence between the *U. capensis* samples and the *Ulva* sp. *A* (MT160696) from Fort *et al.* (2020) was 0%, implying that the *U. capensis* samples are genetically identical to *Ulva* sp. *A*. The sequence divergence between the local cultured *Ulva* samples and the *U. lacinulata* (MT160697) from Fort *et al*. (2020) was 0%, indicating that the farmed *Ulva* specimens are genetically identical to *U. lacinulata*.

Table 9: Pairwise distances (%) among species within the *U. lacinulata* clade and the haplotypes of farmed *Ulva* samples for the *tuf*A gene region. Representatives of *U. rigida* and *U. laetevirens* from different countries were selected. The 18 *Ulva* samples for the *tuf*A gene were collapsed into 3 haplotypes.

	U. rigida **HE600180** Italy	*U. rigida* **MH538640** Germany	*Ulva* sp. *A* **MT160696** France	*U. laetevirens* **HQ610428** Canada	*U. laetevirens* **JN029325** Australia	*U. laetevirens* **JQ048942** USA	*U. laetevirens* **MF172086** Italy	*U. laetevirens* **MF614792** Tunisia	*U. laetevirens* **MK992229** South Korea	*U. lacinulata* **MT160697** Ireland	BL-S
BL-S	0.13	0	0.89	0.13	0	0.13	0.13	0.38	0.15	0	
D3049	0.79	1.19	0	0.79	0.92	0.79	0.79	1.05	0.74	0.92	0.92

Key:

Blue: Seashore *Ulva* samples

Green: Farmed *Ulva* samples

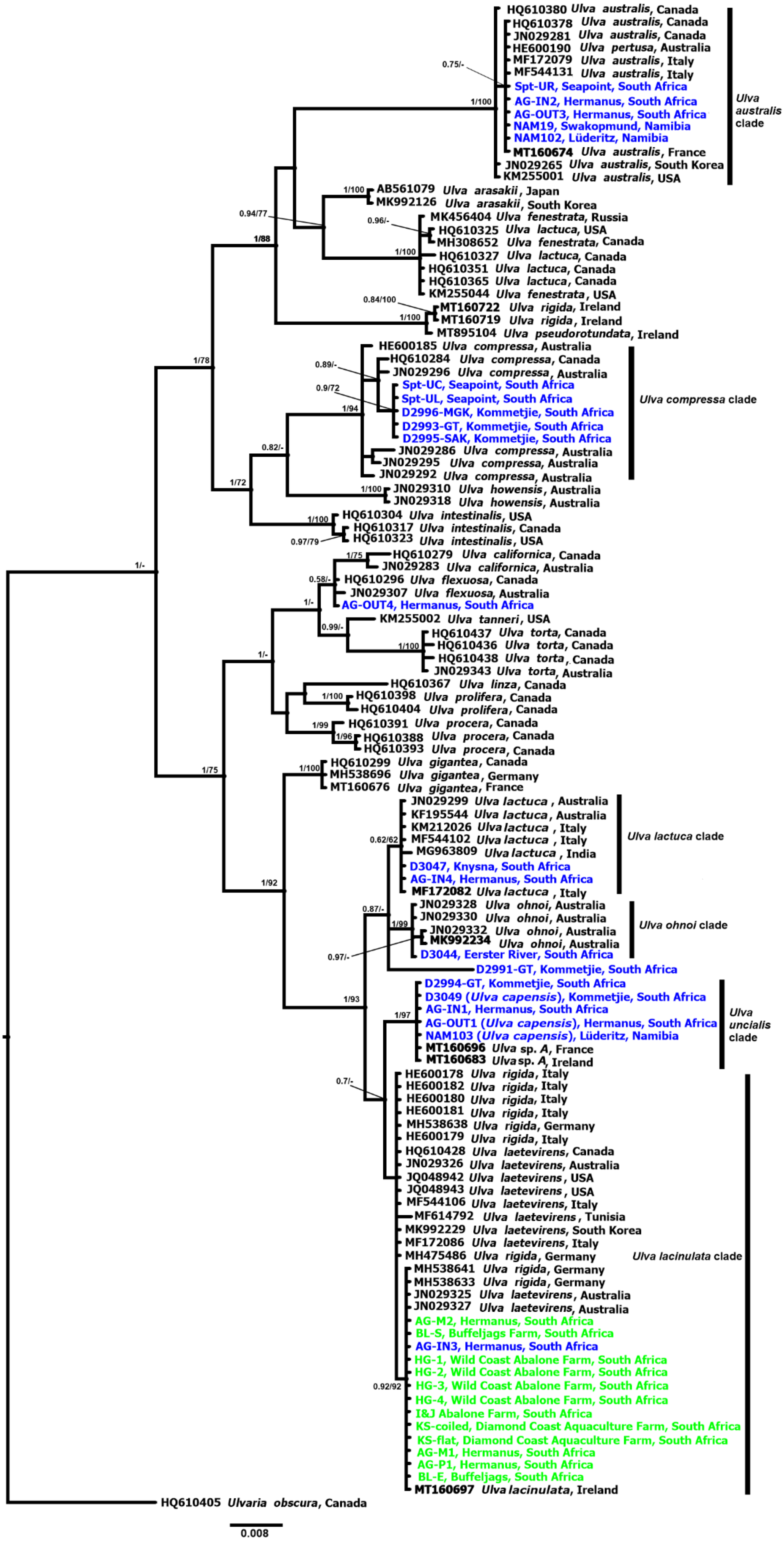

Figure 25: The Bayesian Inference tree for *Ulva tuf*A sequence data (scale at bottom) is presented and was congruent with RAxML. The tree was rooted with *Ulvaria obscura*. Numbers near each node refer to the posterior probability values (PP) of the Bayesian Inference tree added in front of the Maximum-Likelihood (ML) bootstrap values (≥70). Numbers accompanying the species names are GenBank accession numbers for the sequences used in the analysis. Fort *et al.* (2020) specimens of *U. lacinulata* (**MT160697**) and *Ulva* sp. *A* (**MT160696**) in bold.

Table 10: Molecular and morphological identity of the *Ulva* specimens collected from the farms and the seashores in the present study and from Lineekela Kandjego's unpublished study. ITS sequence was not obtained from some samples because of poor sequence quality.

Sample number	ID	Habitat	Morphology	*tuf*A	ITS	*rbc*L
1	BL-S	Farm	*U. rigida*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
2	BL-E	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
3	AG-P1	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
4	AG-M1	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
5	AG-M2	Farm	*U. rigida*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
6	KS-coiled	Farm	*U. rigida*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
7	KS-flat	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
8	HG-1	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
9	HG-2	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
10	HG-3	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
11	HG-4	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
12	I&J	Farm	*U. lactuca*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
13	D2991-GT	Seashore	*U. lactuca*	Unidentified but related to *U. lactuca*	Unidentified	Unidentified but related to *U. clathrata*
14	D2994-GT	Seashore	*U. rigida*	*U. uncialis*	No sequence	*U. lacinulata*
15	D3049	Seashore	*U. capensis*	*U. uncialis*	*U. lacinulata*	*U. lacinulata*
16	AG-IN1	Seashore	*U. rigida*	*U. uncialis*	*U. lacinulata*	*U. lacinulata*
17	AG-IN3	Seashore	*U. rigida*	*U. lacinulata*	*U. lacinulata*	*U. lacinulata*
18	AG-OUT1	Seashore	*U. capensis*	*U. uncialis*	*U. lacinulata*	*U. lacinulata*
19	D2993-GT	Seashore	*U. lactuca*	*U. compressa*	No sequence	*U. compressa*
20	D2995-SAK	Seashore	Unidentified	*U. compressa*	No sequence	*U. compressa*
21	D2996-MGK	Seashore	Unidentified	*U. compressa*	No sequence	*U. compressa*
22	SPT-UR	Seashore	*U. rigida*	*U. australis*	No sequence	*U. australis*
23	SPT-UC	Seashore	Unidentified	*U. compressa*	No sequence	*U. compressa*
24	SPT-UL	Seashore	*U. lactuca*	*U. compressa*	No sequence	*U. compressa*
25	D3044	Seashore	*U. uncialis*	*U. ohnoi*	Unidentified	*U. ohnoi*
26	D3047	Seashore	*U. fasciata*	*U. lactuca*	Unidentified	*U. lactuca*
27	AG-IN2	Seashore	*U. rigida*	*U. australis*	No sequence	*U. australis*
28	AG-IN4	Seashore	*U. fasciata*	*U. lactuca*	No sequence	*U. lactuca*
29	AG-OUT3	Seashore	*U. rigida*	*U. australis*	No sequence	*U. australis*
30	AG-OUT4	Seashore	*U. flexuosa*	*U. flexuosa*	No sequence	*U. flexuosa*
31	NAM103	Seashore	*U. capensis*	*U. uncialis*	*U. lacinulata*	*U. lacinulata*
32	NAM102	Seashore	*U. rigida*	*U. australis*	No sequence	*U. australis*
33	NAM19	Seashore	*U. rigida*	*U. australis*	No sequence	*U. australis*
34	U09	Seashore	*U. lactuca*		*U. stenophylloides*	
35	U63	Seashore	*U. lactuca*		*U. stenophylloides*	
36	U134	Seashore	*U. rigida*		*U. stenophylloides*	
37	U135	Seashore	*U. lactuca*		*U. stenophylloides*	

38	U16	Seashore	*U. capensis*	*U. lacinulata*	
39	U21 I&J	Farm	*U. rigida*	*U. lacinulata*	
40	U21F	Farm	*U. rigida*	*U. lacinulata*	
41	U104	Farm	*U. lactuca*	*U. lacinulata*	
42	U109	HHPB8B	Unknown	*U. lacinulata*	
43	U113	HHWCA3	Unknown	*U. lacinulata*	
44	U113F	Farm	*U. linza*	*U. lacinulata*	
45	U114 I&J P1	Farm	*U. rigida*	*U. lacinulata*	*U. lacinulata*
46	U107	HHPA3C	Unknown	*U. lacinulata*	
47	U104	Abagold Farm	*U. lactuca*	*U. lacinulata*	
48	U104F	Farm	*U. lactuca*		
49	U36	Seashore	*U. unicalis*		*U. australis*
50	U02	Seashore/harbour	*U. rigida*		*U. australis*
51	U56	Seashore	*U. rigida*		*U. australis*
52	CE0	Seashore	*U. rigida*		*U. australis*
53	CD6	Seashore	*U. rigida*		*U. australis*
54	U03 I&J	Farm	*U. capensis*		*U. lacinulata*
55	U274LK264	Seashore	*U. capensis*		*U. lacinulata*
56	LK280	Seashore/harbour	*U. capensis*		*U. lacinulata*
57	U117 I&J	Farm	*U. rigida*		*U. lacinulata*
58	LK14	Seashore	*U. capensis*		*U. lacinulata*
59	LK255	Farm	*U. rigida*		*U. lacinulata*
60	LK256	Farm	*U. rigida*		*U. lacinulata*
61	LK258	Farm	*U. rigida*		*U. lacinulata*

Discussion

Molecular identification of cultured *Ulva* species and seashore *Ulva* species

The abalone farms range from Kleinsee on the extreme northwest coast to Haga-Haga on the southeast coast. South Africa's annual mean coastal water temperature ranges from 12.0 ± 0.9°C (mean ± SD) at its north-western limit near the Namibian border to 24.0 ± 1.9°C on the east coast near the Mozambican border (Smit *et al.*, 2017). Although, the Wild Coast Abalone Farm in Haga-Haga experiences a warmer temperature regime (a monthly mean temperatures of 17.9°C-18.6°C) compared to the Diamond Coast Aquaculture Farm in Kleinsee (monthly mean temperatures of 11.3°C-13.3°C: Smit *et al.*, 2017), these farms are all growing the same species of *Ulva*. Based on the results from the *rbc*L, ITS and *tuf*A phylogenetic analyses, all the locally farmed *Ulva* specimens were resolved in the same single large clade and were found to be genetically identical to *U. laetevirens sensu* Fort *et al.* (2020) as there was no sequence divergence between the cultured *Ulva* samples and the Fort *et al.* (2020) specimen. According to a recent nomenclature study by Hughey *et al.* (2020), the type specimen of *Ulva laetevirens* is synonymous with *U. australis*. Hence, the *U. laetevirens sensu* Fort *et al.* (2020) needs a new name. According to Hughey *et al.* (2021), the lectotype specimen of *U. lacinulata* was resolved within the clade containing *U. laetevirens sensu* Fort *et al.* (2020) (MT160587). Hence, the new name of this clade is *U. lacinulata*. The hypothesis that the abalone farms were cultivating different *Ulva* species due to their wide geographical distribution and the different environmental condition in the different regions is not supported in this study. All the *U. capensis* samples, except for sample LK14, that were collected attached near the farm inlets and outlets, the seashores, and from Lineekela Kandjengo's previous unpublished study, were resolved into an internal small clade within the larger *U. lacinulata* clade in the *rbc*L phylogenetic tree. In the ITS phylogenetic tree, the *U. capensis* samples were resolved into a small internal clade, containing *Ulva* sp. *A* (MT894534) from Fort *et al.* (2021) and is sister to the larger *U. lacinulata* clade. In the *tuf*A phylogenetic tree, the *U. capensis* samples were resolved in a well-supported small sister clade to the *U. lacinulata* clade and it is now labelled as *U. uncialis*. This indicates that the plants ascribed locally to *U. lacinulata* and *U. uncialis* are different species as they are resolved into two different clades in the *tuf*A and ITS phylogenetic trees, but these two different clades are not well-defined in the *rbc*L phylogenetic tree. Moreover, it was found that *Ulva lacinulata* was growing attached near the inlets of the main farming area in Hermanus in all the three phylogenetic trees. New *Ulva* records were also molecularly identified along the seashores of South Africa. These included *Ulva ohnoi*, *Ulva australis* and *Ulva stenophylloides*. Furthermore, foliose *Ulva compressa* was also found at seashores in Kommetjie and Sea Point. The bladed form of *U. compressa* (Steinhagen *et al.,* 2019b) was not previously identified on the seashores of South Africa. *Ulva lactuca* and *Ulva flexuosa* species were also identified using these molecular markers along the seashores of South Africa. Based on the support provided by the Bayesian PP and RAxML bootstrap values, the *tuf*A gene showed better resolution compared to the other two trees and it resolved all the *Ulva* specimens collected in this study, except for sample D2991-GT which was morphologically identified as *U. lactuca*. The *tuf*A tree formed better supported internal clades compared to the ITS and *rbc*L phylogenetic trees.

Using the *rbc*L, ITS and *tuf*A molecular markers, the cultivated *Ulva* species were resolved in a single large clade containing *Ulva rigida*, *Ulva armoricana*, *Ulva scandinavica*, *Ulva laetevirens* and *U. lacinulata* specimens from different countries. Furthermore, *Ulva* samples U117 I&J, U114 I&J, LK14, LK255, LK256 and LK258 were also recovered within this clade in the *rbc*L tree. The *Ulva* samples U104, U109, U113, U114 I&J, U107, U104F and U113F were also recovered in the single large *U. lacinulata* clade in the ITS tree. This indicates that the *U. lacinulata* samples collected from the Irvin & Johnson Cape Abalone and Abagold farm in 2009 by Lineekela Kandjengo are the same species as the farmed *Ulva* samples collected in this study. Thus, the same *Ulva* species is being grown now as more than a decade ago. Some farms collected *Ulva* initially in sheltered bays/harbours, and some have taken *Ulva* from other farms, but it is not clear where these *Ulva* came from (some may have colonised naturally in the culture systems). Finer scale genetic markers such as microsatellite loci, which are amplified short tandem repeats, are particularly used for strain selection in commercial aquaculture and they can also provide information on relationships between materials on the different farms, and potentially the origin of the materials

(Kostamo *et al.,* 2008). According to a new paper by Hughey *et al.* (2021), the lectotype specimen of *Ulva rigida* from Cádiz, Spain was found to be identical or very similar to sequences identified as *U. rotundata* Bliding from Ireland and Portugal. Moreover, high-throughput sequencing of the lectotype of *Ulva lacinulata* (Kützing) Wittrock from Lesina, Croatia was found to be identical or highly similar to sequences of *U. armoricana*, *U. laetevirens*, *U. rigida* and *U. scandinavica* that are on GenBank (Hughey *et al.,* 2021). Since *U. lacinulata* is the oldest valid published name, it is the correct name that should be applied to the worldwide distributed species that was previously but incorrectly known as *U. rigida* (Hughey *et al.,* 2021). Genetic evidence also showed that *U. rigida* is restricted to European waters (Hughey *et al.,* 2021). The sequences previously assigned as *U. rigida* by Fort *et al.* (2020) are provisionally referred to as *Ulva* sp. *A* since they currently do not have an available name with a sequenced type (Fort *et al.,* 2021). The identity of the cultured *Ulva* species in the five main *Ulva* producing abalone farms in South Africa is *U. lacinulata*. The possible reasons of why all the farmed *Ulva* species belong to the single large *U. lacinulata* clade could be because this species survives the best under the cultured conditions; and since it grows in the farms that have a wide geographical range with varying climates, it could be possible that it is resistant to a range of temperatures. Furthermore, the foliose attached sample AG-IN3 was also resolved in the same large single clade containing the farmed *Ulva* samples, indicating that the *Ulva* species grown unattached commercially was collected attached near the farm.

Using the *rbc*L gene, the *U. capensis* samples (U274 LK264, LK280, U16, D3049, NAM103 and AG-OUT1), *U. rigida* samples (D2994-GT and AG-IN1) that were collected from seashores, and sample U03 I&J that was collected from the Irvin & Johnson Cape Abalone farm was resolved within the larger *U. lacinulata* clade. The *U. capensis* samples were genetically identical to *U. rigida* specimens from the USA (AY422564), New Zealand (LK022428), Ireland (EU484408), and *Ulva* sp. *A* from France (MT160586) as there was no sequence divergence between them for the *rbc*L gene. The sample U03 I&J collected by Lineekela Kandjengo in 2009 from Irvin and Johnson Cape Abalone demonstrates that this entity was found in an abalone farm, but it is not known with certainty whether it was being grown commercially unattached or was attached somewhere on the farm as it is the only *U. capensis* specimen collected from the abalone farms. In the recent sampling for this study, *U. capensis* was not found in the abalone farms but only from seashores. *U. laetevirens sensu* Fort *et al.* (2020) (MT160587) and *U. rigida sensu* Fort *et al.* (2020) (MT160586) specimens were both resolved within the larger single *U. lacinulata* clade in the *rbc*L phylogenetic tree. Moreover, the Bliding's specimens of *Ulva scandinavica* (MW570777 and MW570778) were also resolved within the *U. lacinulata* clade and the lectotype of *U. lacinulata* was identical to the *rbc*L and *tuf*A sequence to Bliding's specimens of *U. scandinavica* (Hughey *et al.,* 2021). Therefore, in this study, the *rbc*L analysis suggests that the specimens *U. laetevirens sensu* Fort *et al.* (2020) and *U. rigida sensu* Fort *et al.* (2020) are conspecific as they could not be separated using this marker. In the ITS and *tuf*A phylogenetic trees, the *U. capensis* samples were resolved in a well-supported clade containing *Ulva* sp. *A* from Fort *et al.* 2021. According to Fort *et al.* 2021, the sequence of the *Ulva* sp. *A* type specimen is not currently available in public repositories and this clade will be renamed when a suitable type is found. The clade containing the *U. capensis* specimens has now been labelled as *U. uncialis* because *Phycoseris uncialis* Kützing 1849 is an older name than *Ulva capensis* Areschoug 1851, hence the name *U. capensis* is considered superfluous and illegitimate (Silva *et al.,* 1996). Thus, with respect to the *tuf*A and ITS markers, the local *U. uncialis* and the farmed *U. lacinulata* are different species as they were resolved into two different clades. Fort *et al.* (2020) recognized *U. lacinulata* and *Ulva* sp. *A* as separate species using the *tuf*A and ITS1 markers but not with *rbc*L and this was observed in this study as well. *Tuf*A was able to separate the *U. lacinulata* and *Ulva* sp. *A* species in Fort *et al.* (2020) because in that study a larger dataset was used, and different methods were used where *Ulva* strains were first clustered based on SNPs (Single Nucleotide Polymorphisms) present within their chloroplast genome. Then, a species was determined for the strains within the clusters based on the reads mapping that were extracted onto the *rbc*L and *tuf*A barcodes. According to Fort *et al.* (2021), the hybridization of *U. lacinulata* and *Ulva* sp. *A* species has been observed to occur occasionally in the wild. Fort *et al.* (2020) showed that a strain (U99) represented an F1 hybrid between the species *Ulva* sp. *A* and *U. lacinulata* as half of this strain's 45S sequences possessed *Ulva* sp. *A* variants and the other half of variants were assigned to *U. lacinulata* using the cytoplasmic genomes. However, Hughey *et al.* (2021) considers *U. armoricana*, *U. 'rigida'*, *U. 'laetevirens'* and *U. scandinavica* to represent a single taxon, *U. lacinulata*. This is due to the current lack of morphological and genetic disparity, and the discovery of a hybrid specimen in the *U. lacinulata* clade (Hughey *et al.,* 2021). A paper by Fort *et al.* (2019) showed that a large degree of variation in growth such as tissue expansion or biomass accumulation can be observed in *Ulva* strains. Hence, by creating hybrids from suitable selected strains, the biomass yield of *Ulva* can be increased which would be beneficial in aquaculture. Another possible outcome of the inter-species hybrids may be infertility which is also an important characteristic in aquaculture as sporulation in *Ulva* causes thallus discolouration and disintegration (Fort *et al.*, 2019; Fort *et al.,* 2021).

Kandjengo (2002) suggested that there is no difference between the South African *U. capensis* and *U. rigida* and that they are the same species, based on his ITS data. This hypothesis was not supported in this study as the local *U. uncialis* and farmed *U. lacinulata* species were resolved into two different clades in both the ITS and *tuf*A phylogenetic trees. This indicates that the local *U. uncialis* and farmed *U. lacinulata* species are closely related as they were resolved into separate sister clades in the ITS and *tuf*A trees. Kandjengo *et al.* (2009) hypothesized that the South African *Ulva capensis* is likely related to *U. rigida* from elsewhere in the world and that the South African *Ulva rigida* is an undescribed species based on the *rbc*L and ITS markers used in that study. This hypothesis was not supported in this study by the *rbc*L and ITS markers as the South African *Ulva capensis* is not related at all to *U. rigida* from elsewhere in the world as the lectotype of *U. rigida* has been found to belong in a clade with specimens previously identified as *U. rotundata*, *U. pseudorotundata* and *Ulva* sp. from Ireland and Portugal. Moreover, the hypothesis that the South African *Ulva rigida* is an undescribed species is also not supported as this species was described as *U. lacinulata* in 1882.

Morpho-anatomical identification of cultured *Ulva* species

Based on the morphology and anatomy, out of the 12 *Ulva* specimens collected from the five main *Ulva* producing commercial abalone farms, three of these specimens were identified as *Ulva rigida* (BL-S, AG-M2 and KS-Coiled) and the remaining specimens as *Ulva lactuca* (BL-E, AG-P1, AG-M1, KS-Flat, HG-1, HG-2, HG-3, HG-4, and I&J). The key morphological traits of *U. rigida sensu* Stegenga *et al.* (1997) are that cells in cross-section are rounded to rectangular and the cells are 2-4 times higher than broad. Furthermore, minute teeth along the thallus margins are present. The key morphological traits of *U. lactuca sensu* Stegenga *et al.* (1997) is roundish thallus, without marginal dentation and isodiametric cells in cross section. *U. rigida sensu* Stegenga *et al.* (1997) is a south/east coast species (Cape Peninsula to tropical East Africa) which comprises warm temperate to tropical regions, lower in nutrients. *U. laetevirens sensu* Fort *et al.* (2020) is a widely distributed species which occurs on the European Atlantic coast, the Mediterranean Sea, East Asia, the Americas, Australia and New Zealand. This provides further evidence that the South African cultured *Ulva* is *U. laetevirens sensu* Fort *et al.* (2020) as both groups of specimens occur in warmer areas (e.g., the Mediterranean, East Asia for the Fort *et al.* specimens).

Ulva capensis is considered a Southern African west coast species (Cape Agulhas to Namibia) which is a cool temperate nutrient-rich upwelling region (Stegenga *et al.,* 1997). The key morphological traits for this species are bullet to spindle-shaped cells in cross-section and dentated margins. The *U. rigida sensu* Fort *et al.* (2020) is a more geographically localised species, occurring on the East Atlantic coast and New Zealand. Hence, since this provides further evidence that the South African *U. capensis* species is synonymous with *U. rigida sensu* Fort *et al.* (2020) as both occur only in cooler temperate areas. However, the two samples (D2994-GT and AG-IN1) morphologically identified as *U. rigida sensu* Stegenga *et al.* (1997) were also resolved in the same clade containing the *U. capensis* samples. This implies that *Ulva rigida* species show great variation in morphology which is usually related to environmental conditions (Joska, 1992). According to the South African *Ulva rigida* specimens analysed by Joska (1992), the thalli were short rosette-like with a firm to rigid texture and usually had microscopic dentations often only on the stipe and basal portions of the thallus. Joska (1992) also found that some *U. rigida* samples had no microscopic dentation while others had macroscopically visible dentation. Both samples, D2994-GT and AG-IN1, had dentated margins but did not have bullet-shaped cells which is one of the main diagnostic features of *U. capensis* (now known as *U. uncialis*). This shows that there is phenotypic variation within the smaller internal clade of *U. uncialis* in the ITS and *tuf*A phylogenetic trees as both *U. capensis sensu* Stegenga *et al.* (1997) and *U. rigida sensu* Stegenga *et al.* (1997) belonged in this well-supported clade. Interestingly, similar to samples D2994-GT and AG-IN1, the farmed sample AG-M2 was also morphologically identified as *U. rigida sensu* Stegenga *et al.* (1997), but it was resolved within the larger *U. laetevirens sensu* Fort *et al.* (2020) clade in all the three phylogenetic trees.

According to Fort *et al.* (2020), *U. armoricana* and *U. scandinavica* are synonyms of *U. laetevirens*. The morphological description of the local *U. capensis* is similar to the *U. laetevirens* specimen described in Kraft *et al.* (2010). For example, both the local *U. capensis* and their *U. laetevirens* have teeth along the blade margins, the cells in surface view are quadrangular to polygonal, bullet-shaped cells in cross-section, the cell length and cell width of local *U. capensis* (25-60 µm high by 12.5-15 µm) fits within their range of 35-60 µm high by 5-16 µm wide in cross-section, the blade thickness of the local *U. capensis* (102.5-140 µm) is within their range of blade thickness (100-160 µm) and 1-3 pyrenoids per cell were observed in both specimens. The molecular findings agree with the morphological findings that the description of the local *U. capensis* matched with the description of *U. laetevirens* since the local *U. capensis* was resolved within the large *U. laetevirens sensu* Fort *et al.* (2020) clade in the *rbc*L tree. In the Maximum-likelihood (ML) tree for *rbc*L in Kraft *et al.* (2010), their *U. laetevirens* samples belonged in a clade with moderate support containing *U. scandinavica* specimens (AY255870, EU484412 and EU484416) and *U. rigida* specimens (EU484417 and EU484408). In the *rbc*L phylogenetic tree in the present study, these above-mentioned specimens belonged within the larger *U. lacinulata* clade with a weak support value of 0.57 for PP, implying that the South African *U. capensis* is the same species as the *U. laetevirens* described in Kraft *et al.* (2010). In the Maximum-likelihood (ML) tree for ITS in Kraft *et al.* (2010), their *U. laetevirens* samples were closely related to *U. scandinavica* (AJ234317) with weak bootstrap values of 67% and 60 for ML and Neighbour-Joining (NJ). In the ITS phylogenetic tree in the present study, the specimen *U. scandinavica* (AJ234317) belonged in a much larger clade which was a sister clade to the South African *U. capensis* samples. This implies that the *U. laetevirens* described in Kraft *et al.* (2010) is closely related to the South African *U. capensis* samples based on the ITS marker. According to Fort *et al.* (2020), *U. scandinavica* and *U. armoricana* are synonyms of *U. laetevirens*. The sequencing of the *U. laetevirens* lectotype by Hughey *et al.* (2021) showed that the name *U. laetevirens* was synonymous with *U. australis* (Fort *et al.,* 2021). Since *U. scandinavica* is an invalid name and the clade *U. laetevirens* requires a new name, this clade has been named *U. lacinulata* as it contains the newly sequenced *U. lacinulata* lectotype (Hughey *et al.,* 2021). Furthermore, *U. lacinulata* is morphologically similar to *U. rigida* with macroscopic marginal teeth occurring in both species (Hughey *et al.,* 2021). There is a congruency between the morpho-anatomical and molecular methods used in the current study as both methods were able to distinguish the South African *U. capensis* (*U. uncialis)* from the South African *U. laetevirens* (*U. lacinulata*). Based on the morphological method, the South African *U. capensis* (*U. uncialis)* has spindle-shaped cells while the South African *U. laetevirens* (*U. lacinulata*) has isodiametric cells.

New *Ulva* records: *U. ohnoi*, *U. australis* and *U. stenophylloides* for southern Africa

According to the *rbc*L marker, sample D3044, collected from Eersterivier, False Bay near Cape Town, was resolved in a moderate to high support clade containing *U. ohnoi* specimens from Japan (AB116039), Israel (MG704795) and the USA (GU138284) with support of 0.99 for PP. According to the *tuf*A marker, D3044 was resolved in a clade containing *U. ohnoi* specimens from South Korea (MK992234) and Australia (JN029330, JN029328 and JN029332) with a high support of 99% and 1 for BP and PP, respectively. The sample D3044 was not resolved in the ITS tree as it belonged in single large clade with no support for BP and PP, respectively. Sample D3044 was morphologically identified as *U. uncialis* based on the descriptions provided by Joska (1992) and Kandjengo (2002) but it was resolved as *U. ohnoi* molecularly. According to the description of *U. uncialis* by Joska (1992) and Kandjengo (2002), the key distinguishing features are a small thallus, irregularly lobed and undulate margins, thallus forming rosettes, a blade thickness of 35-40 µm in the apical thallus and *ca.* 100 µm thick in the basal thallus and rectangular cells in cross-section. Silva *et al.* (1996) reported that *Ulva uncialis* is a synonym of *Ulva capensis*, based on comments in unpublished notes of G.F. Papenfuss, and that the former is the older name. The *Ulva uncialis* discussed by Joska (1992) and Kandjengo (2002) is clearly a different species. *Ulva ohnoi* M. Hiraoka & S. Shimada 2004 was described from southern and western Japan (type from Tosa Bay, Tosa, Kochi Prefecture (Hiraoka *et al.*, 2004: 20). The authors found the thalli of *U. ohnoi* could be either attached or free-floating. In comparison with the description of the attached *U. ohnoi* from Japan, there were some morphological differences between their *U. ohnoi* and the South African *U. ohnoi* (sample D3044) and hence, this study extends the morphological circumscription of *U. ohnoi*. For example, the thallus of their *U. ohnoi* was light green in colour and was fragile whereas the thallus of sample D3044 was tough and dark green. Additionally, their thallus was 20-30 cm in height while the local *U. ohnoi* formed small rosettes. Next, the thallus of their *U. ohnoi* had microscopic marginal teeth while the South African *U. ohnoi* did not have dentate margins. According to Hiraoka *et al.* (2003), the presence or absence of marginal teeth reflect the phylogeny of *Ulva* and this trait is considered a useful genetic diagnostic feature. Their *U. ohnoi* had 1-3 pyrenoids per cell while the number of pyrenoids of ours ranged from 2-5. However, there were some similarities between their *U. ohnoi* and the South African *U. ohnoi* such as the cell shape in surface view which was rounded-angular and the cell size of D3044 in surface view was 17.5-25 µm which fitted with their cell size of 14-30 µm long by 12-20 µm broad. In cross-section, the cells were rectangular in both specimens. Furthermore, the blade thickness of D3044 in the edge and in the middle of the thallus was 62.5 µm and 80 µm, respectively, which was within the range of their blade thickness of 30-55 µm in the upper to middle regions and 80-90 µm in the basal region. *U. ohnoi* favours warm temperate waters (Fort *et al.* 2020) and the South African specimen is clearly *U. ohnoi* based on the *rbc*L and *tuf*A genes as the *U. ohnoi sensu* Fort *et al.* (2020) (MK992234) recognised in their trees is within the same well-supported clade as sample D3044.

According to the *rbc*L marker, *Ulva* samples (SPT-UR, AG-IN2, AG-OUT3, NAM19 and NAM102) collected from South Africa and Namibia in the present study were resolved in a well-supported clade containing *U. australis/U. pertusa* specimens from Australia (EU933957, MT815849, LC3331300 and MT815847), South Korea (KP233769), the USA (AY422549) and France (MT160564). The *Ulva* samples (U36, U02, U56, CE0 and CD6) collected by Lineekela Kandjengo in 2009 were also resolved within this clade. According to the *tuf*A marker, samples SPT-UR, AG-IN2, AG-OUT3, NAM19 and NAM102 were resolved in a well-supported clade containing *U. australis/U. pertusa* specimens from Canada (HQ610380 and HQ610378), Australia (JN029281), Italy (HE600190, MF172079 and MF544131), South Korea (JN029265) and the USA (KM255001). Although, the samples SPT-UR, AG-IN2, AG-OUT3, NAM19, NAM102, U36, U02, U56, CE0 and CD6 were molecularly resolved as *U. australis*, these specimens were morphologically identified as *U. rigida sensu* Stegenga *et al.* (1997). Due to the morphological resemblance between *U. rigida* and *U. australis*, these two *Ulva* species were previously synonymized but based on molecular data they were shown to be genetically distinct (Kraft *et al.,* 2010). Furthermore, because of the wide range of phenotypic variation between the morphological description of the species, *U. lactuca* and *U. rigida sensu* Stegenga *et al.* (1997), the identification of *Ulva* species can be difficult. Hence, it is possible that the *U. australis* species collected from Namibia and South Africa were not described for this reason. *Ulva australis* Areschoug 1854 was originally reported as a specimen from Port Adelaide, South Australia and *Ulva pertusa*

Kjellman is a synonym of it (Kraft *et al.* 2010). The morphological features of *U. australis* includes a thallus thickness of up to 300 µm near the base, 1-2 pyrenoids per cell and fronds that are irregularly divided from the base (Lee *et al.,* 2019). According to Fort *et al.* (2020) and Kirkendale *et al.* (2013), *U. australis* is a widely distributed species, occurring in the Atlantic, the Mediterranean Sea, East Asia, the Americas, Australia, and New Zealand. However, it is notably absent from the Irish and British coasts, despite its occurrence in nearby areas such as Brittany and the Netherlands. In comparison with the original description of *U. australis*, the *U. australis* samples (SPT-UR, AG-IN2, AG-OUT3) analysed in this study also displayed 1-2 pyrenoids per cell and had a blade thickness ranging from 75-90 µm in the edge and middle of the thallus. Considering that this species is cosmopolitan, the reason that it was not previously recorded in South Africa could be because of its close resemblance with *U. rigida* and because of the wide morphological description of *U. lactuca* and *U. rigida sensu* Stegenga *et al.* (1997) that has caused the diversity of several *Ulva* species to be concealed.

The ITS spacer resolved the samples (U09, U63, U134 and U135) collected by Lineekela Kandjengo in 2009, as *U. stenophylloides*. This clade had a high support of 94% and 1 with BP and PP, respectively. The holotype locality of *U. stenophylloides* is Victoria, Australia and this species was described by Kraft *et al.* (2010). The morphological description of *U. stenophylloides* in Kraft *et al.* (2010) consists of thalli that are variable in size and ranging from 1-10 cm in length by 4-13.5 cm in breadth. The colour of the thallus is pale to medium green. Inconspicuous to pronounced teeth are present along the blade margins. In surface view, cells are unordered, rectilinear to trapezoidal and cell size is usually 8-25(-40) µm wide by 6.5-20(-35) µm long. Cells contain mostly one pyrenoid per cell, rarely two. In upper regions, the thallus thickness is 80-100 µm and cells are cuboidal in cross-section, with cell length ranging from 25-45 µm and cell width ranging from 15-45 µm. In the mid-to lower thallus, the thallus thickness is usually 140-155 µm and the cells are longer than wide (50-65 µm long by 10-36 µm wide) in cross-section. The blade thickness is 190-220 µm at the transition to rhizoidal region and the cells are either rounded or tapering apically (50-80 µm long by 15-50 µm wide) in cross-sections. The morpho-anatomical description of the Kandjengo specimens was not available in this study hence comparison between the *U.*

stenophylloides from Kraft *et al.* (2010) and with the samples in this study could not be done. Moreover, sequences of the samples were not available for the other two markers, *rbc*L and *tuf*A.

The foliose form of *U. compressa* has not previously been found along the seashores of South Africa. Only the tubular form of *U. compressa* was previously recorded by Stegenga *et al.* (1997). In the *rbc*L tree, samples D2995-SAK, D2996-MGK, SPT-UC, SPT-UL and D2993-GT were resolved in a well-supported clade with a support of 80% and 1 for BP and PP, respectively. This clade contained *U. compressa* specimens from China (HM046603), Australia (EU933942 and AF387106) and the USA (AY255859). In the *tuf*A tree, samples D2995-SAK, D2996-MGK, SPT-UC, SPT-UL and D2993-GT were resolved in a well-supported clade with a support of 94% and 1 for BP and PP, respectively. The clade contained *U. compressa* specimens from Canada (HQ610284) and Australia (HE600185, JN029292, JN029295, JN029286 and JN029296). The samples (D2995-SAK and D2996-MGK) were difficult to morphologically identify as they had a bladed form and were one-cell thick in cross-section. Samples SPT-UL and D2993-GT were morphologically identified as *U. lactuca*. Sample SPT-UC was difficult to identify as it had spindle-shaped cells in cross-section which is a critical morphological character of *U. capensis*. However, it did not have dentate margins which is another key character of *U. capensis*. Because of the wide phenotypic variation within the morphological description of *U. lactuca sensu* Stegenga *et al.* (1997), samples SPT-UL and D2993-GT were incorrectly identified as *U. lactuca*. Moreover, morphological diversity within *U. compressa* was observed as samples SPT-UC, SPT-UL and D2993-GT were two-cells thick unlike samples D2995-SAK and D2996-MGK which were one-cell thick in cross-section. Studies have shown that *U. mutabilis* Föyn 1958 which has been synonymized with *U. compressa* Linnaeus 1753, has a range of morphologies such as tubular, bladed and a mixed morphotype (Steinhagen *et al.,* 2019a; Steinhagen *et al.*, 2019b). In comparison with the description of foliose *U. compressa* by Steinhagen *et al.* (2019b), the foliose *U. compressa* in the current study had some similarities such as perforations in the thallus, the cells in surface view are rounded-angular or polygonal in the apical and middle part of the thallus, the cell size in surface view of the local foliose *U. compressa* is within the range 9-27 µm in Steinhagen *et al.* (2019b). The cell length and the cell width of the local foliose *U. compressa* in cross-section also fitted within the cell size range of 11–45 µm × 4–17 µm in Steinhagen *et al.* (2019b). In cross-section, the blade thickness of all the local foliose *U. compressa* specimens also fitted within their range of 30–50 µm thick, except for sample SPT-UC, which had a thicker blade (60-125 µm). Moreover, similar to their study, mostly one pyrenoid and sometimes 2 or 3 pyrenoids were observed in some cells.

Ulva lactuca was also identified among the seashore *Ulva* specimens. In the *rbc*L gene, samples D3047 and AG-IN4 were resolved in a clade with a high Bayesian support. This clade contained *U. lactuca* specimens from the USA (AY422565), Portugal (EU484418), *Ulva fasciata* epitype from Egypt (MK456397) and *Ulva lactuca* holotype from Indo-Pacific (MK456395). In the *tuf*A gene, samples D3047 and AG-IN4 were resolved in a clade with weak support of 62% and 0.62 for BP and PP, respectively. This clade contained *U. lactuca* specimens from Australia (JN029299 and KF195544), Italy (KM212026, MF544102 and MF172082) and India (MG963809). Based on the recent study involving the sequencing of types by Hughey *et al.* (2019), the type specimen of *Ulva lactuca* has been shown to be what was commonly known as *Ulva fasciata*, including those described in South Africa by Stegenga *et al.* (1997). Therefore, the species *U. fasciata* is now known as *U. lactuca* and the former European *U. lactuca* is now known as *U. fenestrata* (Hughey *et al.* 2019). Thus, samples D3047 and AG-IN4 which were morphologically identified as *U. fasciata* based on the description of *U. fasciata* by Stegenga *et al.* (1997) are *U. lactuca* species according to this recent nomenclatural change. Furthermore, *U. fenestrata* was not found on the South African shores in this study but its absence on the shores of South Africa is not confirmed as a comprehensive study has not been carried out. The absence of *U. fenestrata* on the South African shores aligns with the findings of Hughey *et al.* (2019) that this species occurs mostly in the northern hemisphere.

Comparison of the two identification methods employed in this study

The two identification methods, morpho-anatomical and molecular were not always in agreement with each other in this study. Fewer *Ulva* species were morphologically identified while all species were capable of molecular identification. *U. ohnoi*, *U. stenophylloides*, and *U. australis* were either morphologically resolved as *U. lactuca sensu* Stegenga *et al.* (1997) or as *U. rigida sensu* Stegenga *et al.* (1997). Due to morphological variability in the genus *Ulva*, several of the specimens in this study were morphologically misidentified. Therefore, species identification based solely on the morphological concept is not always reliable. Hence, it is important to use other identification methods together with the morpho-anatomical method such as the molecular method used here. In this study, the molecular marker *tuf*A produced the best phylogenetic tree compared to the *rbc*L and ITS markers. The ITS spacer was not a good marker as it had a low amplification success and because of the deletions and insertions in the gene region, full length sequence data could not be obtained for all the samples. Hence, some *Ulva* samples were not included in the ITS phylogenetic tree. Moreover, the ITS spacer did not resolve all the *Ulva* specimens. The internal clades in the *tuf*A tree were better supported compared to the *rbc*L tree, although both genes could resolve most *Ulva* samples. The molecular marker, *tuf*A, had great amplification success, no contamination and higher resolution power compared to the other two markers. All the *Ulva* samples in this study were resolved in well-supported clades in the *tuf*A tree, except for sample D2991-GT which was morphologically identified as *U. lactuca sensu* Stegenga *et al.,* (1997). This sample could not be resolved by the *tuf*A gene because the sequences of *U. clathrata* and *U. muscoides* were not available on GenBank. In the *tuf*A phylogenetic tree, sample D2991-GT was found to be related to *U. lactuca* with weak support. In the *rbc*L phylogenetic tree, sample D2991-GT was not resolved in a clade but was found to be closely related to *U. clathrata* and *U. muscoides* with high Bayesian support. Finally, several studies have found *tuf*A as a viable molecular marker for species-level identification of *Ulva* species compared to the other markers, *rbc*L and ITS, as it has a stronger amplification success and higher resolution power (Saunders and Kucera, 2010; Kirkendale *et al.,* 2013; Miladi *et al.*, 2018). This study agrees with these authors that *tuf*A is the best marker to separate *Ulva* species compared to *rbc*L and ITS as it showed better resolution based on the support provided by the Bayesian PP and RAxML bootstrap values. These molecular markers have been reported to have low discriminatory power in several closely related groups of *Ulva* (Cui *et al.,* 2018). Therefore, the hybridization of the *Ulva* species should be studied to determine whether they are the same biological species or not (Hiraoka *et al.,* 2004). The combined used of culturing and hybridization have revealed reproductive relationships among closely related *Ulva* species and potentially resolved taxonomic problems (Hiraoka *et al.,* 2017). Furthermore, the 5S rDNA spacer which is a more resolved DNA marker could also be used as it is ten times more variable than the ITS spacer and thus allows a better understanding of the phylogenetic relationships (Shimada *et al.,* 2008). The maximum intraspecific divergences for species delimitations are found in pioneering studies such as Hayden *et al.* (2004) and Kirkendale *et al.* (2013) of 0.4% for *rbc*L, 0.7% (Hayden *et al.,* 2004) to 2.3% (Blomster *et al.,* 1998) for ITS2, and 0.52% (Saunders and Kucera, 2010) to 1.185% (Kirkendale *et al.,* 2013) for *tuf*A. The low sequence divergence between the South African *U. lacinulata* and South African *U. uncialis* specimens demonstrates that *rbc*L was the most conserved of the three molecular markers and it was not able to separate these two *Ulva* species into two different clades. Even though the sequence divergence values between the South African *U. lacinulata* and South African *U. uncialis* specimens for ITS and *tuf*A were within the range of variability, these two species were resolved into two different clades in the ITS and *tuf*A trees. According to the paper by Melton and Lopez-Bautista (2021), high intraclade divergences were observed for the ITS2 marker in the following clades: *U. flexuosa* clade (8.1%), *U. rigida* (3.5%), and *U. torta* (3.2%). In addition, high intraclade divergences were also observed for the *rbc*L marker at 2% and 0.9% for *U. flexuosa* and *U. tepida* clades, respectively (Melton and Lopez-Bautista, 2021). Therefore, this shows that sequence divergence might not be a good indicator for species level differences.

Ulva species in aquaculture farms

In order to decide which *Ulva* species is best for aquaculture depends on the purpose of their cultivation. For example, *Ulva* species aquacultured for bioremediation should have high growth rates, able to grow in different conditions so that they can be cultured throughout the year, have wide environmental tolerances, and occur locally as this minimizes the risk of the cultured material from invading the natural *Ulva* populations and influencing the natural ecosystems (Lawton *et al.,* 2013). *Ulva* species that are cultivated for feed or food must preferably have the potential for a high protein content level, be of high quality if it is for human consumption and able to produce large biomass, among others. In Australia, *U. ohnoi* is cultivated to bioremediate discharge waters from land-based prawn aquaculture and it can be used to produce high-value bio-products (Magnusson *et al.,* 2016; Magnusson *et al.,* 2019). In the *tuf*A phylogenetic tree, sample D3044 was resolved as *U. ohnoi* in a well-supported clade containing *U. ohnoi* specimens from Australia (JN029328 and JN029330) that were collected from New South Wales (southeastern Australia) (Kirkendale *et al.*, 2013). *U. ohnoi* is the most common foliose species identified in natural populations and land-based aquaculture farms in Eastern Australia (Lawton *et al.,* 2013). Sample D3044 is closely related to both the *U. ohnoi* cultured in land-based aquaculture farms and the *U. ohnoi* occurring in the wild in Australia. Since *U. ohnoi* occurs on the South African shores, this species has the potential to be grown for bioremediation in aquaculture farms in South Africa. Moreover, strains of *U. ohnoi* have been shown to have higher growth rates in temperatures that correspond to local conditions, and they can survive and grow under aquacultured conditions (Lawton *et al.,* 2013). In the *rbc*L phylogenetic tree, sample D3044 was resolved in a well-supported containing *U. ohnoi* from Japan (AB116039). According to Hiraoka *et al.* (2004), this species formed green tides mainly in warm-temperate regions of southern and western Japan and it grows in summer when the water temperature is high.

U. rigida is commercially cultivated at an IMTA site in Portugal to produce high-quality *Ulva* biomass that satisfy the demands of the market (Califano *et al.,* 2020). In the *rbc*L phylogenetic tree, the IMTA-cultivated *U. rigida* specimens from Portugal (MN450419, MN450420 and MN450422) were resolved in a single large clade with weak support containing the local farmed *Ulva lacinulata* and there was no sequence divergence between the *Ulva lacinulata* and the *U. rigida* specimens from Portugal. This clade also contained an *U. rigida* specimen from Portugal (MN450427) collected from the lagoon. This implies that the IMTA-cultivated *U. rigida* from Portugal is *U. lacinulata* and that the cultured and attached *Ulva* are the same species as they belonged in the same clade. In the ITS phylogenetic tree, the IMTA-cultivated *U. rigida* specimen from Portugal (MN444712) was resolved in clade with high support for PP, containing the local farmed *Ulva* samples and there was a low sequence divergence between the *U. rigida* from Portugal and the South African cultured *Ulva* samples. Thus, it appears that both South Africa and Portugal are independently cultivating *U. lacinulata*, unattached in land-based integrated systems, in South Africa with abalone and in Portugal with fish.

Conclusion and future studies

The main aim of this study was to identify the *Ulva* species cultured in the five main *Ulva* producing abalone farms in South Africa and to compare them with closely related wild *Ulva* species. Based on the phylogenetic trees and the pairwise distance analysis of ITS and *tuf*A, the locally farmed *Ulva* samples and the *U. capensis* samples are closely related species as they belonged in two separate sister clades. In the *rbc*L phylogenetic tree, the farmed *Ulva* samples and the *U. capensis* samples were resolved in a single large clade with weak support value. The *U. laetevirens sensu* Fort *et al.* (2020) and the *U. rigida sensu* Fort *et al.* (2020) species also belonged in the same large *U. lacinulata* clade containing the locally farmed *Ulva* samples and the *U. capensis* samples in the *rbc*L phylogenetic tree. This demonstrates that the *rbc*L data in this study could not separate the *U. laetevirens sensu* Fort *et al.* (2020) and the *U. rigida sensu* Fort *et al.* (2020) species while with the ITS and *tuf*A data, these two specimens were separate species. The identity of the South African farmed *Ulva* is *U. lacinulata* and the identity of the *U. capensis* specimens is *U. uncialis*. Moreover, all the three molecular markers, *tuf*A, ITS and *rbc*L, showed that there is no genetic variation within the 12 *Ulva* farmed samples and that they also grow attached on the South African seashores. According to Fort *et al.* (2020), *U. laetevirens* is a widely distributed species growing in Australia, East Asia and both sides of the North Atlantic (and South Africa). Hence, it cannot be said with certainty whether the local *U. laetevirens* species is indigenous or was introduced at some time in the past. Additionally, based on the *rbc*L and ITS sequences obtained from Lineekela Kandjengo, the farmed material has not changed since he collected in 2009 as his sequences were also resolved within the same clade containing the farmed *Ulva* species collected in the current study. This study also shows that there was an incongruency between the identification methods, morpho-anatomical and molecular, employed here as fewer *Ulva* species were identified morphologically than molecularly. Therefore, it is important to use other identification methods together with the morpho-anatomical method to better identify species. For future studies, to investigate the genetic differences at the strain level between the South African *Ulva lacinulata* and South African *Ulva uncialis*, microsatellites or organellar genome analysis should be used. The organellar genome analysis would provide the number of

Single Nucleotide Polymorphisms (SNPs) per strain within these sequences and would be able to better separate *Ulva* species. A thorough study of *Ulva* species growing along the seashores of South Africa should be carried out using both the morpho-anatomical method and the molecular markers *rbc*L and *tuf*A as they have been able to resolve all the *Ulva* species collected except for sample D2991-GT.

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Supplementary data

Table S1: List of *Ulva* species with collection details from GenBank for each molecular marker.

Species	**Collection (date and voucher specimen number or reference)**	**Accession number *rbc*L**	**Accession number ITS**	**Accession number *tuf*A**
Umbraulva olivascens	Ireland	EU484405		
Umbraulva olivascens	Strangford Lough, N. Ireland, UK (GBR), Hayden and Waaland (2004)		AY260564	
Ulva rotundata	Ballyvaughan, Co. Clare, Ireland (IRE), Loughnane *et al.* (2008)	EU484406		
Ulva pseudorotundata	Ireland		MT894650	
Ulva pseudorotundata	Ireland			MT895104
Ulva lactuca	Strangford Lough, N. Ireland, UK (GBR), Hayden and Waaland (2004)	AF499669		
Ulva lactuca	USA	AY422546		
Ulva lactuca	Crosshaven, Co. Cork, Ireland (IRE), Loughnane *et al.* (2008)	EU484409		
Ulva lactuca	Egypt	MK456397		
Ulva lactuca	Indo-Pacific (Hughey *et al.* 2019)	MK456395		
Ulva lactuca	Maine, USA			HQ610325
Ulva lactuca	Manitoba, Canada			HQ610327
Ulva lactuca	BC, Canada			HQ610351
Ulva lactuca	BC, Canada			HQ610365
Ulva lactuca	Maui, Hawaii		GU138301	
Ulva lactuca	Italy	MW570779		
Ulva fenestrata	USA	AF499668		
Ulva fenestrata	Kamchatka Peninsula, Siberia, Russia (Hughey *et al.* 2019)	MK456393		
Ulva fenestrata	Siberia, Russia			MK456404
Ulva fenestrata	NB, Canada			MH308652
Ulva fenestrata	CA, USA			KM255044
Ulva fenestrata	WA, USA		AY260562	
Ulva pertusa	South Korea	KP233769		
Ulva pertusa	USA	AY422549		
Ulva pertusa	Italy			HE600190
Ulva pertusa	WA, USA		AY422502	
Ulva australis	VIC, Australia	EU933957		
Ulva australis	Port Phillip, Victoria, Australia	MT815849		
Ulva australis	Port Adelaide, Australia (Hanyuda & Kawai 2018)	LC331300		
Ulva australis	Port Phillip, Victoria, Australia	MT815847		
Ulva australis	BC, Canada			HQ610380
Ulva australis	BC, Canada			HQ610378
Ulva australis	WA, Australia			JN029281
Ulva australis	Italy			MF172079
Ulva australis	Italy			MF544131
Ulva australis	South Korea			JN029265
Ulva australis	CA, USA			KM255001
Ulva australis	VIC, Australia		EU933985	
Ulva intestinalis	Vancouver Island, BC (CAN), Hayden and Waaland (2004)	AF499671		
Ulva intestinalis	Nova Scotia, Canada			HQ610304
Ulva intestinalis	Maine, USA			HQ610317
Ulva intestinalis	Maine, USA			HQ610323
Ulva compressa	Yellow Sea, China	HM046603		
Ulva compressa	VIC, Australia	EU933942		
Ulva compressa	Strangford Lough, N. Ireland, UK (GBR), Hayden and Waaland (2004)	AY255859		
Ulva compressa	Australia	AF387106		
Ulva compressa	Adriatic Sea, Italy			HE600185
Ulva compressa	NB, Canada			HQ610284
Ulva compressa	Tasmania, Australia			JN029296
Ulva compressa	NSW, Australia			JN029286
Ulva compressa	NSW, Australia			JN029295
Ulva compressa	SA, Australia			JN029292
Ulva compressa	Australia		KF195484	
Ulva compressa	Fukui, Mihama, Hiruga, Japan		AB830495	
Ulva pseudocurvata	North Star Beach, Newport, Orange County, California, USA (Hayden & Waaland 2004)	AY422553		
Ulva pseudocurvata	Aberdeenshire, Scotland, UK (GBR), Hayden and Waaland (2004)	AY255869		
Ulva linza	South Korea	KP233764		
Ulva linza	Yellow Sea, China	HM046607		
Ulva linza	New Zealand	EF110016		
Ulva linza	Humbolt Co., California (USA), Hayden and Waaland (2004)	AY255861		
Ulva linza	BC, Canada			HQ610367
Ulva linza	Kochi, Port of Murotsu, Japan		AB299440	
Ulva linza	Kochi, Japan		AB624455	
Ulva prolifera	Yellow Sea, China	HM046602		

Ulva prolifera	NB, Canada			HQ610398
Ulva prolifera	NB, Canada			HQ610404
Ulva prolifera	Iwate, Yamada, Japan		AB298316	
Ulva procera	New Zealand	EF110134		
Ulva procera	BC, Canada			HQ610391
Ulva procera	NB, Canada			HQ610388
Ulva procera	NB, Canada			HQ610393
Ulva laetevirens	SA, Australia	EU933961		
Ulva laetevirens	VIC, Australia	EU933943		
Ulva laetevirens	Canada	HQ603664		
Ulva laetevirens	Connecticut, USA	JQ048945		
Ulva laetevirens	Ireland	MT160587		
Ulva laetevirens	NB, Canada			HQ610428
Ulva laetevirens	Tasmania, Australia			JN029325
Ulva laetevirens	WA, Australia			JN029326
Ulva laetevirens	WA, Australia			JN029327
Ulva laetevirens	Connecticut, USA			JQ048942
Ulva laetevirens	Connecticut, USA			JQ048943
Ulva laetevirens	Torre Faro, Italy			MF544106
Ulva laetevirens	Monastir, Tunisia			MF614792
Ulva laetevirens	South Korea			MK992229
Ulva laetevirens	Venice Lagoon, Italy			MF172086
Ulva laetevirens	Ireland			MT160697
Ulva laetevirens	Connecticut, USA		JQ048947	
Ulva laetevirens	SA, Australia		EU933989	
Ulva laetevirens	China		KC411870	
Ulva laetevirens	VIC, Australia		EU933970	
Ulva laetevirens	China		KC411866	
Ulva laetevirens	Ireland		MT894611	
Ulva armoricana	Yokohama, Kanagawa Prefecture, Japan (14 June 1998; SAP095074)	AB097632		
Ulva armoricana	Brittany, France (11 October 1998; SAP095072)	AB097630		
Ulva armoricana	Brittany, France (11 October 1998; SAP095072)		AB097660	
Ulva armoricana	Miyajima, Hiroshima Prefecture, Japan (10 July 1999; SAP095073)		AB097661	
Ulva armoricana	Yokohama, Kanagawa Prefecture, Japan (14 June 1998; SAP095074)		AB097662	
Ulva scandinavica	Langstone Harbour, Hampshire, UK (GBR), Loughnane *et al.* (2008)	EU484412		
Ulva scandinavica	Portsmouth, England, UK (GBR), Hayden and Waaland (2004)	AY255870		
Ulva scandinavica	Langstone, UK	MG704798		
Ulva scandinavica	NY, USA	MH469629		
Ulva scandinavica	Kilbrittan, Co. Cork, Ireland (IRE), Loughnane *et al.* (2008)	EU484416		
Ulva scandinavica	Wadden Sea, The Netherlands (6 October 1998; SAP095071)	AB097629		
Ulva scandinavica	Wadden Sea, The Netherlands (6 October 1998; SAP095071)		AB097659	
Ulva scandinavica	Veerse Meer, Netherlands		AF153484	
Ulva scandinavica	Veerse Meer, Netherlands		AF153485	
Ulva scandinavica	Veerse Meer, Netherlands		AF153486	
Ulva scandinavica	Langstone Harbour, Portsmouth, England (Tan *et al.* 1999)		AJ234318	
Ulva scandinavica	Veerse Meer, Netherlands		AF153483	
Ulva scandinavica	Portsmouth, England, UK (GBR), Hayden and Waaland (2004)		AJ234317	
Ulva rigida	South Korea	KP233772		
Ulva rigida	Puerto Montt, Chile (CHE), Hayden and Waaland (2004)	AY422564		
Ulva rigida	New Zealand	LK022428		
Ulva rigida	Killary Harbour, Co. Galway, Ireland (IRE), Loughnane *et al.* (2008)	EU484408		
Ulva rigida	Portugal	MT160528		
Ulva rigida	Inis Mor, Aran Islands, Ireland (IRE), Loughnane *et al.* (2008)	EU484395		
Ulva rigida	Adriatic Sea, Italy	HE600163		
Ulva rigida	Murrisk, Ireland	HG940511		
Ulva rigida	Qingdao, China	KP975386		
Ulva rigida	Aveiro, Portugal	MN450419		
Ulva rigida	Aveiro, Portugal	MN450427		
Ulva rigida	Aveiro, Portugal	MN450422		
Ulva rigida	Aveiro, Portugal	MN450420		
Ulva rigida	Ireland	HG940507		
Ulva rigida	France	MT160586		
Ulva rigida	Adriatic Sea, Italy			HE600178
Ulva rigida	Adriatic Sea, Italy			HE600182
Ulva rigida	Heligoland, Germany			MH538641
Ulva rigida	Adriatic Sea, Italy			HE600179
Ulva rigida	Adriatic Sea, Italy			HE600180
Ulva rigida	Adriatic Sea, Italy			HE600181
Ulva rigida	Heligoland, Germany			MH538633
Ulva rigida	Schleswig-Holstein, Germany			MH538638
Ulva rigida	Schleswig-Holstein, Germany			MH538639
Ulva rigida	Schleswig-Holstein, Germany			MH538640
Ulva rigida	Schleswig-Holstein, Germany			MH538695

Ulva rigida	Schleswig-Holstein, Germany			MH475486
Ulva rigida	France			MT160696
Ulva rigida	Cadiz, Spain (ESP), Hayden and Waaland (2004)		AY260565	
Ulva rigida	Roscoff, France		MT078965	
Ulva rigida	Roscoff, France		MT078966	
Ulva rigida	Roscoff, France		MT078967	
Ulva rigida	Puerto Montt, Chile (CHE), Hayden and Waaland (2004)		AY422522	
Ulva rigida	Skara Brae, Orkney, Scotland (Tan *et al*. 1999)		AJ234319	
Ulva rigida	Veerse Meer, Netherlands		AF153489	
Ulva rigida	NY, USA		KF683442	
Ulva rigida	WA, USA		LC217695	
Ulva rigida	VA, USA		MT887239	
Ulva rigida	USA		MT887235	
Ulva rigida	USA		KC582320	
Ulva rigida	USA		KC582318	
Ulva rigida	USA		MF041748	
Ulva rigida	Aveiro, Portugal		MN444716	
Ulva rigida	Aveiro, Portugal		MN444715	
Ulva rigida	Aveiro, Portugal		MN444714	
Ulva rigida	Aveiro, Portugal		MN444720	
Ulva rigida	Aveiro, Portugal		MN444712	
Ulva rigida	Aveiro, Portugal		MN444719	
Ulva rigida	Aveiro, Portugal		MN444718	
Ulva rigida	Aveiro, Portugal		MN444717	
Ulva rigida	Roscoff, France		MT078962	
Ulva rigida	Roscoff, France		MT078963	
Ulva rigida	Plestin-les-Greves, France		MT078964	
Ulva rigida	Qingdao, China		KP975379	
Ulva rigida	France		MT894503	
Ulva rigida	Cádiz, Spain		MW544059	
Ulva rigida	Ireland		MT894534	
Ulva rigida	Portugal	MT160560		
Ulva rigida	Ireland	MT160609		
Ulva rigida	Ireland			MT160722
Ulva rigida	Ireland			MT160719
Ulva stenophylloides	Australia	EU933956		
Ulva stenophylloides	Point Lonsdale, Victoria, Australia (Kraft *et al.* 2010)	EU933949		
Ulva stenophylloides	VIC, Australia		EU933977	
Ulva stenophylloides	VIC, Australia		EU933984	
Ulva taeniata	Point Loma, San Diego, San Diego County, California, USA (Hayden & Waaland 2004)	AY422566		
Ulva taeniata	Seal Rock, Oregon, USA (Tan *et al*. 1999)		AJ234320	
Ulva reticulata	Cebu Island, The Philippines (16 April 1998; SAP095077)	AB097635		
Ulva reticulata	Japan		LC375164	
Ulva spinulosa	Fubenhama, Kochi, Japan (29 April 1999; SAP095078)	AB097636		
Ulva spinulosa	South Korea		MN070037	
Ulva spinulosa	South Korea		MN069887	
Ulva spinulosa	South Korea		MN069919	
Ulva spinulosa	South Korea		MN070053	
Ulva spinulosa	South Korea		MN070052	
Ulva spinulosa	South Korea		MN070017	
Ulva fasciata	USA	AY422565		
Ulva fasciata	Faial, Azores, Portugal (PRT), Loughnane *et al.* (2008)	EU484418		
Ulva fasciata	NSW, Australia			JN029299
Ulva fasciata	Australia			KF195544
Ulva fasciata	Lake Ganzirri, Italy			KM212026
Ulva fasciata	Venice Lagoon, Italy			MF544102
Ulva fasciata	India			MG963809
Ulva fasciata	WA, USA		AY422524	
Ulva ohnoi	USA	GU138284		
Ulva ohnoi	Isreal	MG704795		
Ulva ohnoi	Japan	AB116039		
Ulva ohnoi	NSW, Australia			JN029328
Ulva ohnoi	NSW, Australia			JN029330
Ulva ohnoi	NSW, Australia			JN029332
Ulva ohnoi	South Korea			MK992234
Ulva ohnoi	Australia		KF195507	
Ulva ohnoi	USA		KU561276	
Ulva clathrata	Cadiz, Spain (ESP), Hayden *et al.* (2003)	AY255862		
Ulva muscoides	Los Toruños, Puerto Réal, Cádiz, Spain (Hayden & Waaland 2004)	AY422563		
Ulva gigantea	Port Issac Bay, Cornwall, UK (GBR), Loughnane *et al.* (2008)	EU484414		
Ulva gigantea	Canada	HQ603535		
Ulva gigantea	NB, Canada			HQ610299
Ulva gigantea	Schleswig-Holstein, Germany			MH538696
Ulva stenophylla	Seattle, Washington (USA), Hayden *et al.* (2003)	AY255874		
Ulva stenophylla	Seattle, Washington (USA), Hayden *et al.* (2003)		AY260569	
Ulva curvata	Canada	AF189071		

Ulva californica	San Diego, California (USA), Hayden and Waaland (2004)	AY255866		
Ulva californica	BC, Canada			HQ610279
Ulva californica	VIC, Australia			JN029283
Ulva californica	WA, USA		AY422513	
Ulva californica	San Diego, California (USA), Hayden and Waaland (2004)		AY260560	
Ulva flexuosa	BC, Canada	HQ603532		
Ulva flexuosa	South Korea	KP233758		
Ulva flexuosa	New Zealand	EF110051		
Ulva flexuosa	BC, Canada			HQ610296
Ulva flexuosa	WA, Australia			JN029307
Blidingia minima	Australia	AF387109		
Monostroma nitidum	Australia	AF387110		
Ulva tanneri	CA, USA			KM255002
Ulva torta	BC, Canada			HQ610437
Ulva torta	BC, Canada			HQ610436
Ulva torta	BC, Canada			HQ610438
Ulva torta	Australia		KF195491	
Ulva torta	Fukui, Mihama, Hiruga, Japan		AB830500	
Ulva clathratioides	Tasmania, Australia			JN029343
Ulva howensis	NSW, Australia			JN029310
Ulva howensis	WA, Australia			JN029318
Ulva lobata	BC, Canada			HQ610369
Ulva lobata	BC, Canada			HQ610370
Ulva lobata	WA, USA		AY422505	
Ulva arasakii	Japan			AB561079
Ulva arasakii	Shizugawa, Miyagi Prefecture, Japan (11 May 1999; SAP095062)		AB097650	
Ulvaria obscura	Canada			HQ610405
Ulvaria obscura	Padilla Bay, Washington State (USA), Hayden et al. (2003)		AY260571	
Enteromorpha muscoides	Ireland		AF127170	
Ulva sp.	China		KY446827	
Ulva sp.	South Korea		MN069993	
Ulva sp. *A*	Ireland	MT160573		
Ulva sp. *A*	Ireland			MT160683
Ulva lacinulata	Croatia		MW544060	
Ulva lacinulata	Norway	MW570777		
Ulva lacinulata	Norway	MW570778		
Percursaria percursa	Australia		AY016305	

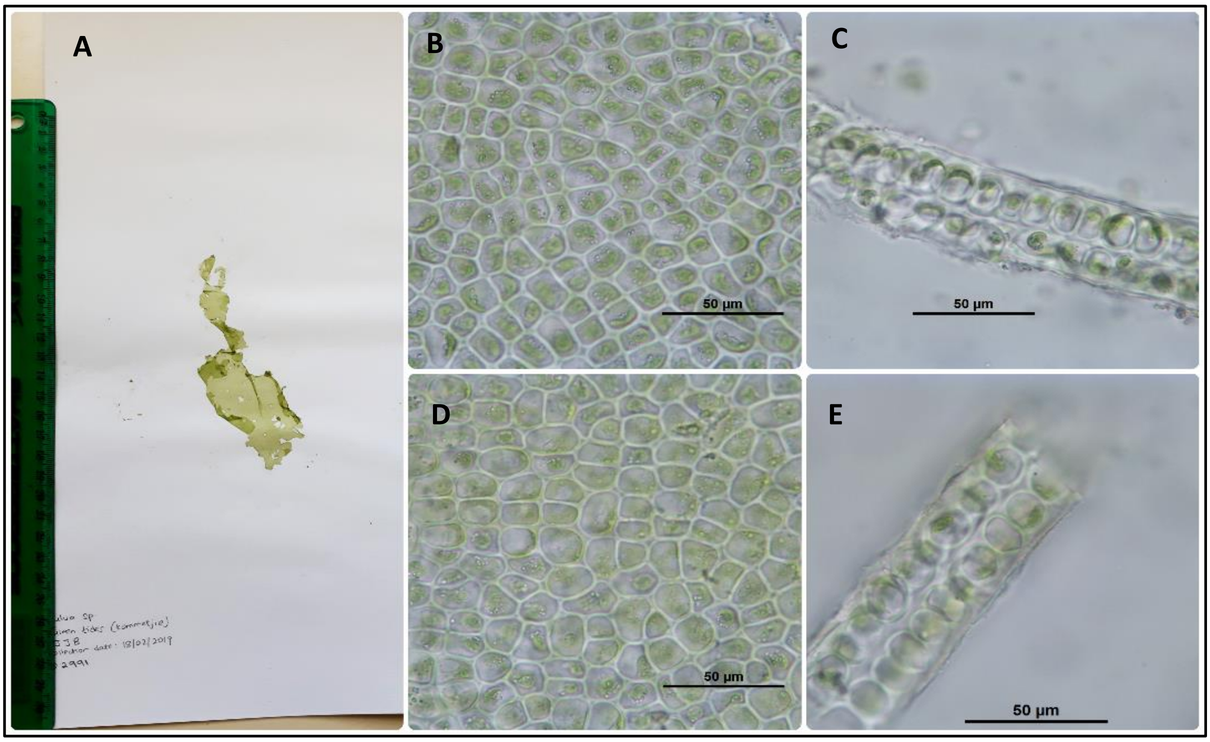

Figure S1: **A**: Morphology of sample D2991-GT, collected from a green tide along the seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

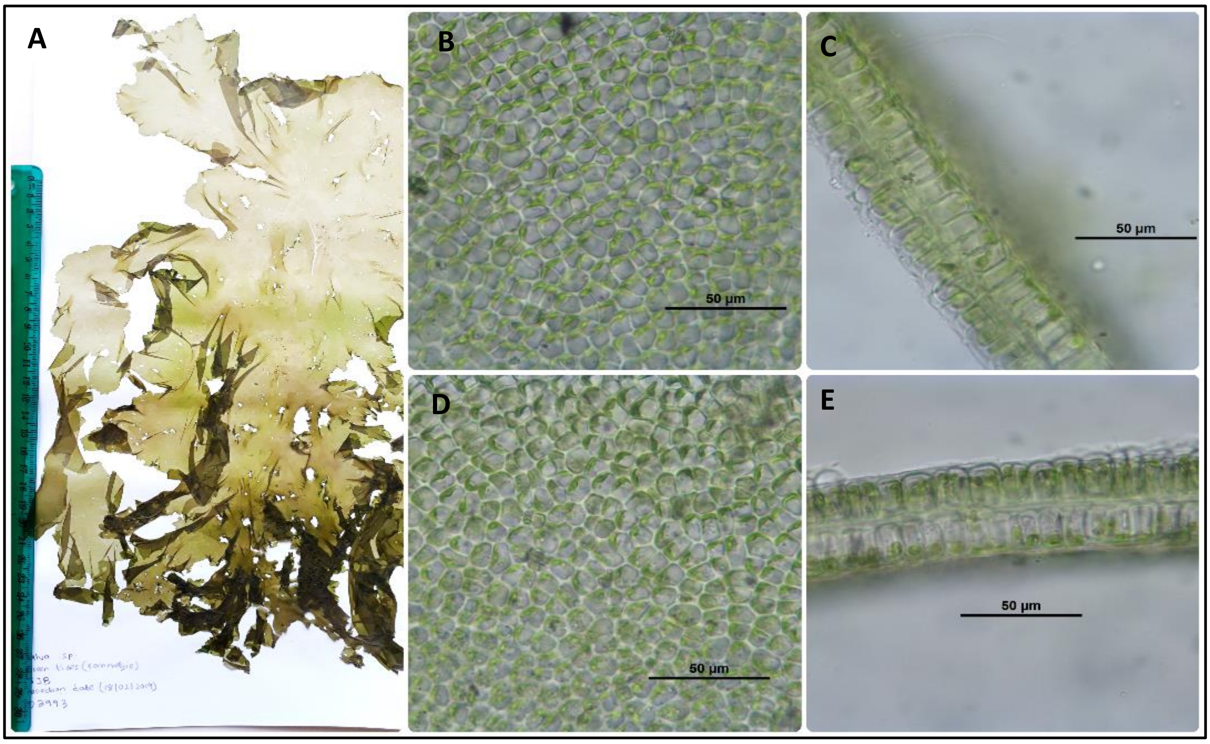

Figure S2: **A**: Morphology of sample D2993-GT, collected from a green tide along the seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

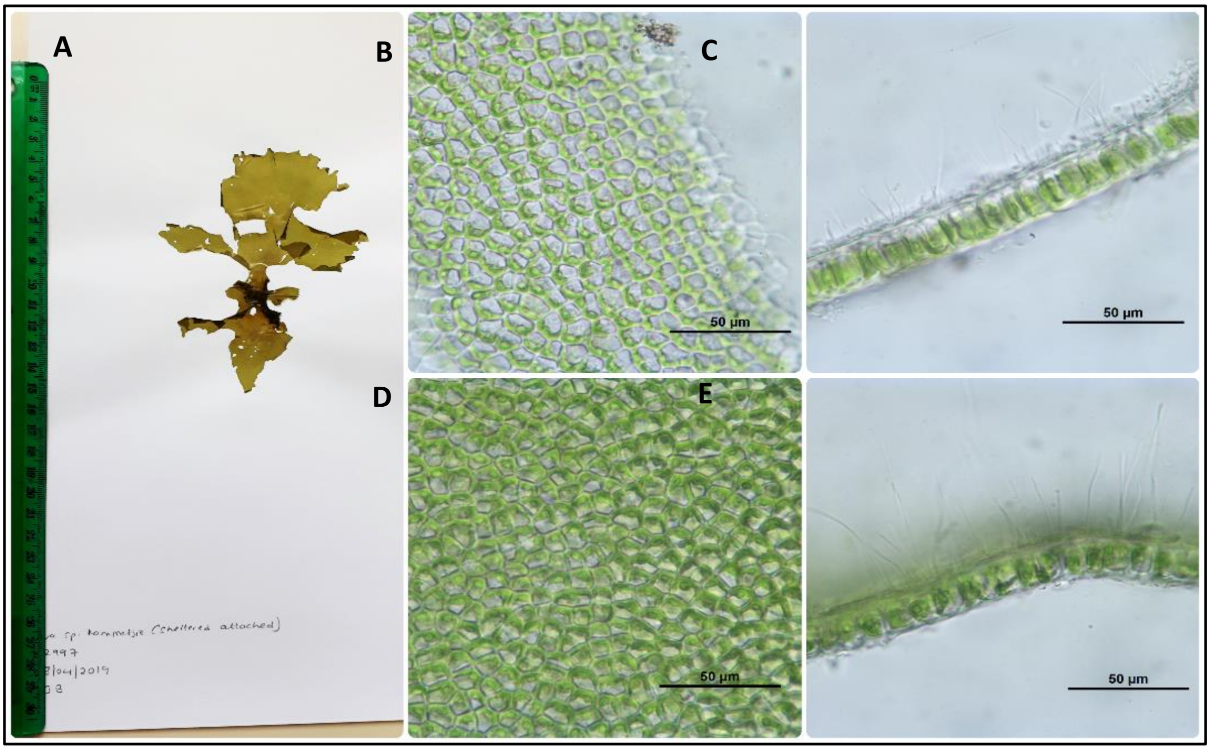

Figure S3: **A**: Morphology of sample D2995-SAK, collected from a sheltered region along the seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

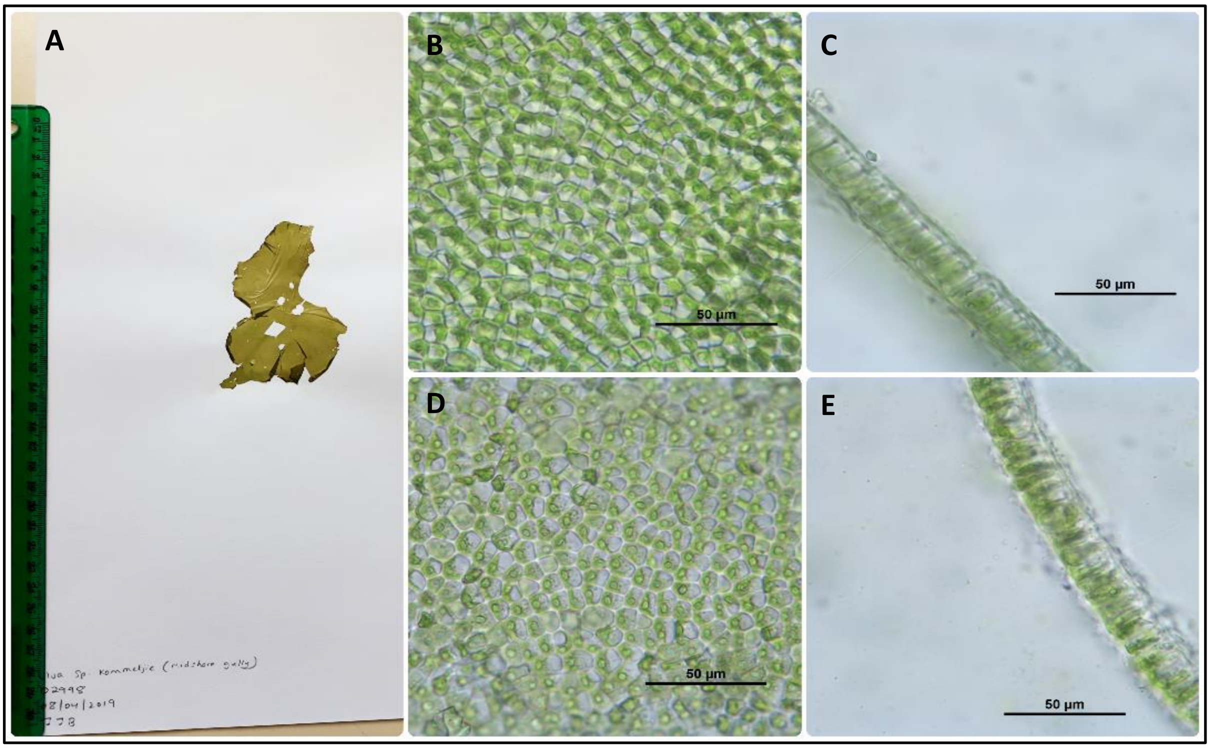

Figure S4: **A**: Morphology of sample D2996-MGK, collected from a mid-shore gully along the seashore at Kommetjie. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

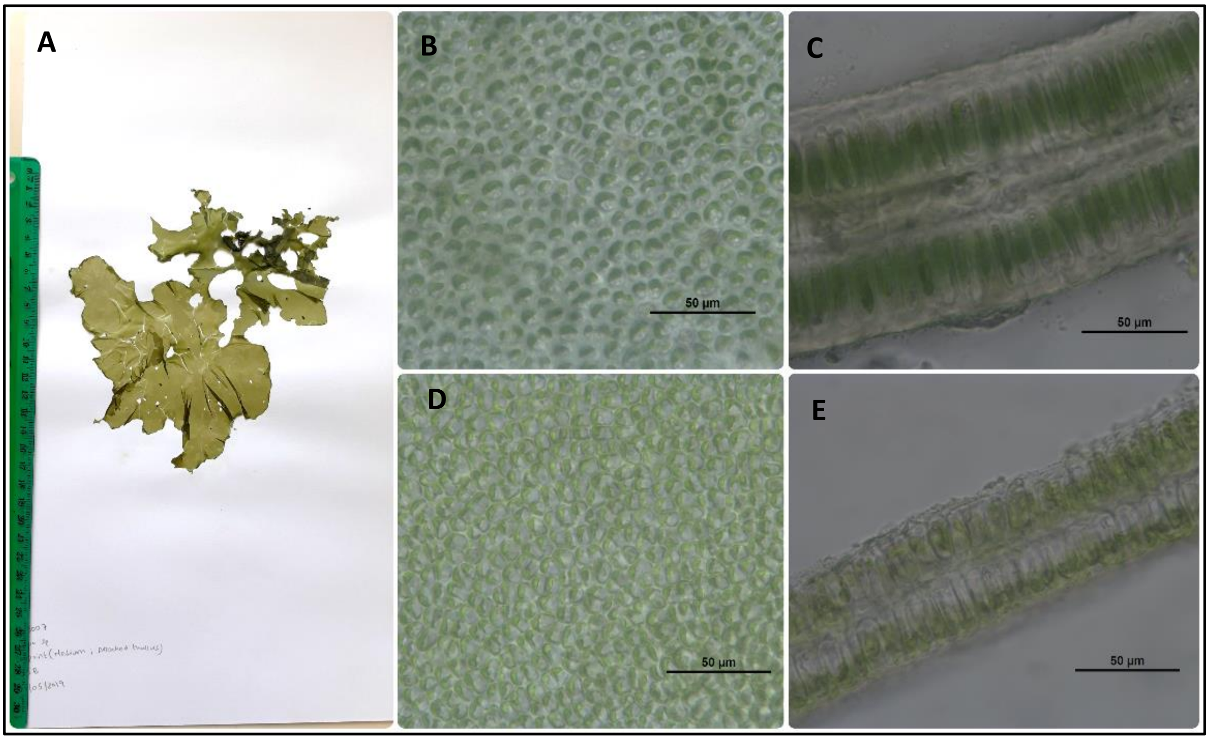

Figure S5: **A**: Morphology of sample SPT-UC, collected from seashore at Sea Point (Cape Town). **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

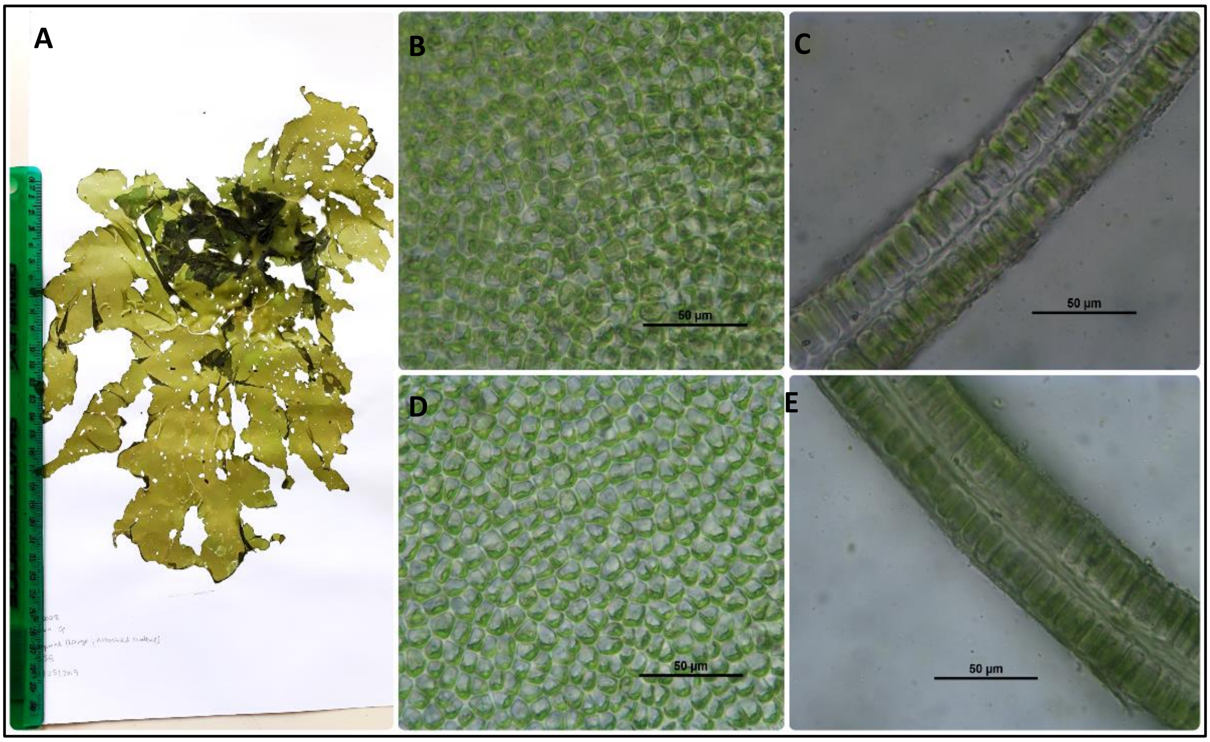

Figure S6: **A**: Morphology of sample SPT-UL, collected from seashore at Sea Point. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

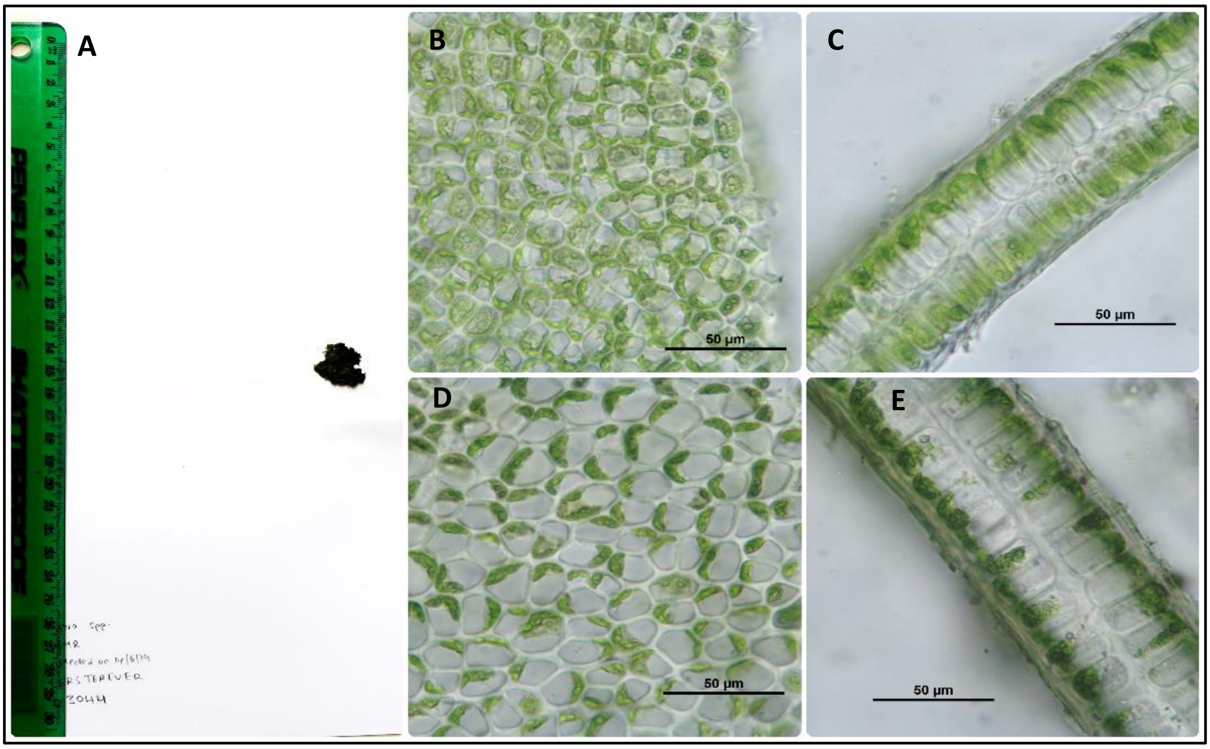

Figure S7: **A**: Morphology of sample D3044, collected from seashore at Eersterivier. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

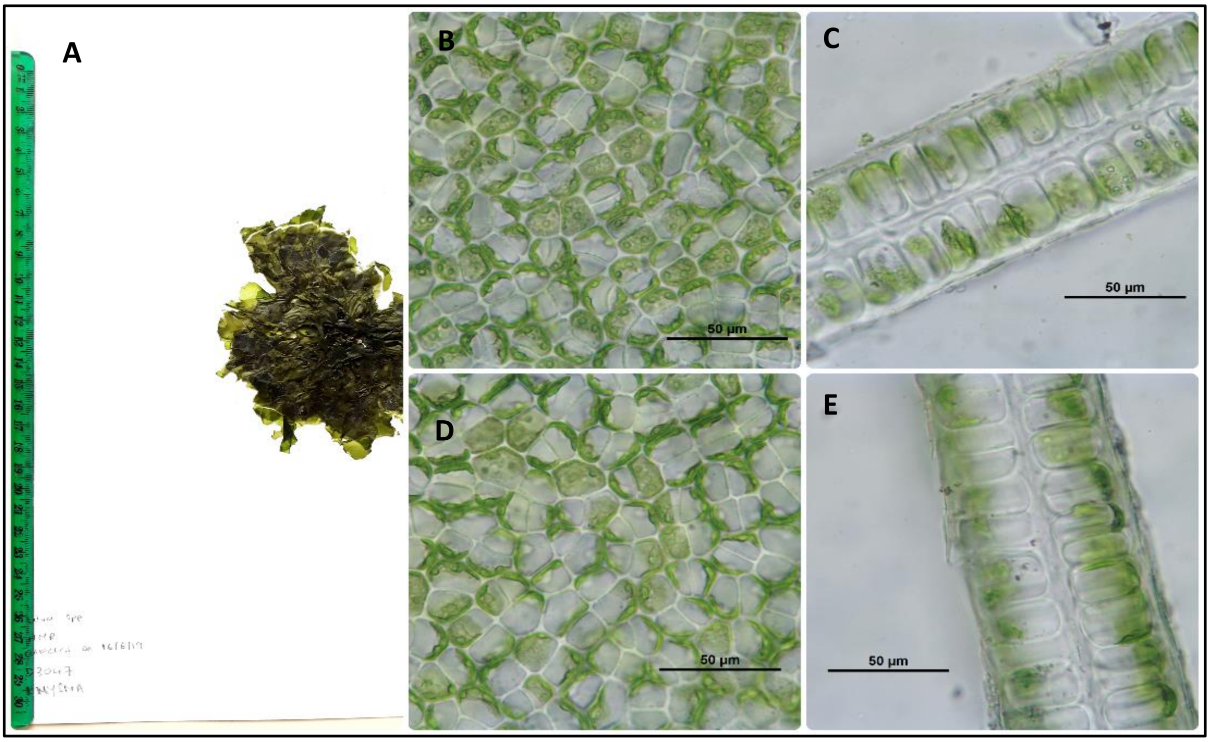

Figure S8: **A**: Morphology of sample D3047, collected from seashore at Knysna. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

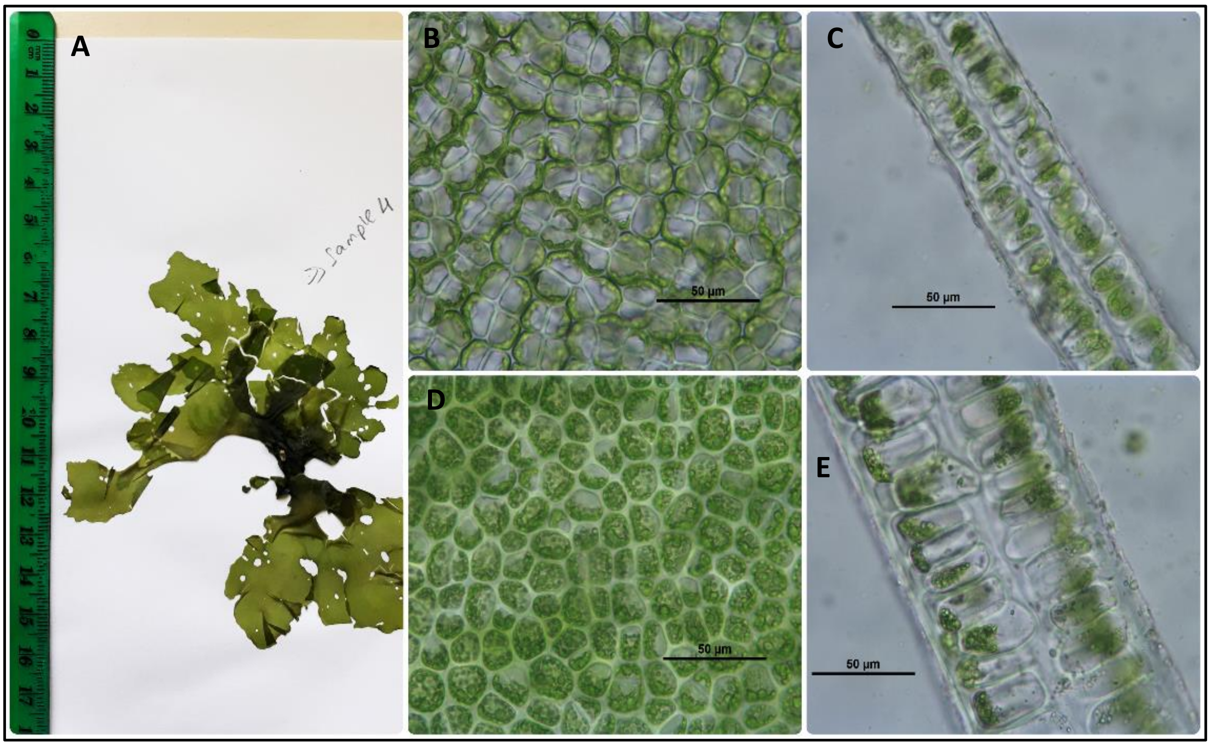

Figure S9: **A**: Morphology of sample AG-IN4, collected near the inlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

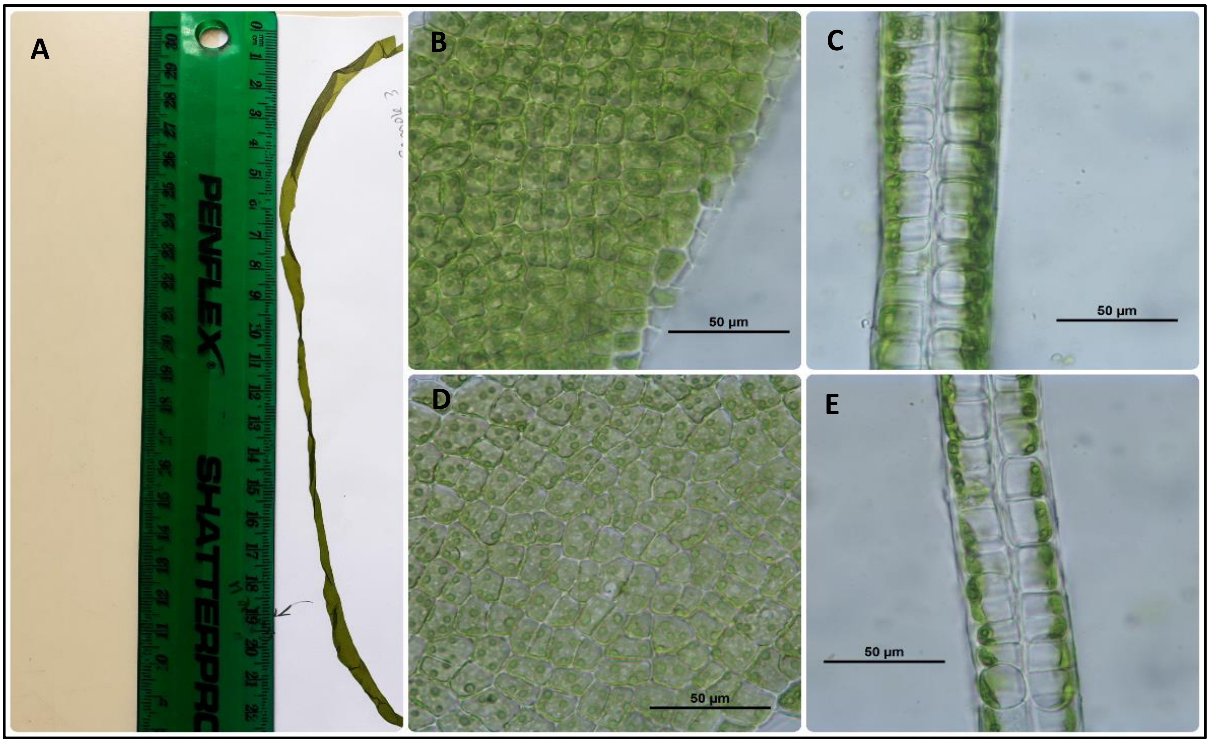

Figure S10: **A**: Morphology of sample AG-OUT4, collected near the outlets of the main farming area in Hermanus. **B & C**: Surface view and transverse section through edge region of the thallus. **D & E**: Surface view and transverse section through middle region of the thallus.

Table S2: Morpho-anatomical characteristics of the *Ulva* samples collected from seashores. Chl = Chloroplast; CS = Cross-section view of cells; SV = Surface view of cells; E = an average size of cells from the edge of the *Ulva* blade; M = an average size of cells from the mid-section of *Ulva* blade.

Name	**Number of pyrenoids**	**Dentated margin**	**Cell shape & chloroplast arrangement**	**Blade thickness (μm; CS)**	**Vegetative cell length (μm; CS)**	**Vegetative cell width (μm; CS)**	**Cell size (μm; SV)**	**Thallus texture and colour**	**Morphological identity**
D2991-GT	Mostly 1 (Occasionally2-4)	Absent	Rounded-angular cells in SV. Orientation of the Chl was to the sidewall. Spherical cells in CS.	E (40) M (42.5)	E (17.5) M (17.5)	E (15) M (12.5)	E (17.5) M (22.5)	Smooth and thin; Few holes; Light green; Small-sized thallus	*U. lactuca*
D2993-GT	Mostly 1	Absent	Rounded cells in SV. Orientation of the Chl was to the sidewall. Isodiametric cells in CS.	E (50) M (45)	E (20) M (17.5)	E (15) M (12.5)	E (15) M (15)	Smooth and thin; Few holes; Light to Mid green; Large-sized thallus	*U. lactuca*
D2995-SAK	1	Absent	Angular cells in SV. One-cell thick. Chl covers the whole outer cell wall. Rectangular cells in CS.	E (27.5) M (22.5)	E (17.5) M (15)	E (12.5) M (12.5)	E (12.5) M (15)	Smooth and thin; Few holes; Mid-green; Medium-sized thallus	Unidentified
D2996-MGK	Mostly 1 (Occasionally 2)	Absent	Angular cells in SV. One-cell thick. Chl covers the whole outer cell wall. Rectangular cells in CS.	E (25) M (25)	E (17.5) M (20)	E (15) M (17.5)	E (15) M (25)	Smooth and thin; Few holes; Mid-green; Small-sized thallus	Unidentified
SPT-UC	Mostly 1 (Occasionally 2-3)	Absent	Rounded cells in SV. Chl covers the whole outer cell wall. In CS, spindle-shaped cells in the edge region of the thallus and rectangular cells in the middle region of the thallus.	E (125) M (60)	E (40) M (22.5)	E (12.5) M (12.5)	E (15) M (15)	Rough and crumpled; Mid green; Medium-sized thallus	Unidentified
SPT-UL	1	Absent	Polygonal cells in SV. Chl covers the whole outer cell wall. Rectangular cells in CS.	E (50) M (55)	E (22.5) M (22.5)	E (15) M (15)	E (15) M (12.5)	Rough and crumpled; Lots of holes; Mid-green; Large-sized thallus	*U. lactuca*

D3044	2-5	Absent	Rounded-angular cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (62.5) M (80)	E (27.5) M (27.5)	E (17.5) M (20)	E (17.5) M (25)	Blades forming small rosettes; tough; Dark green; Very small-sized thallus	*U. uncialis*
D3047	2-4	Absent	Angular cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (75) M (75)	E (30) M (30)	E (17.5) M (17.5)	E (20) M (22.5)	Rough, frilly edge and crumpled; Dark green; Medium-sized thallus	*U. fasciata*
AG-IN4	1-2 (Occasionally 2-3)	Absent	Rounded cells in SV. Orientation of the Chl was to the sidewall. Rectangular cells in CS.	E (52.5) M (107.5)	E (22.5) M (40)	E (20) M (22.5)	E (17.5) M (17.5)	Smooth; Lots of holes; Mid-green to Dark green; Medium-sized thallus	*U. fasciata*
AG-OUT4	2-4	Absent	Polygonal cells in SV. Chl covers the whole outer cell wall. Isodiametric cells in CS.	E (52.5) M (45)	E (25) M (20)	E (20) M (22.5)	E (17.5) M (20)	Filamentous; Mid-green	*U. flexuosa*

Table S3: Morpho-anatomical description of *Ulva rigida* species and its synonymized species from different studies around the world and *Ulva* species that are closely related to it.

Name of species	**Place and Reference paper/book**	**Thallus texture and colour**	**Denticulation**	**Cell shape and arrangement**	**Chloroplast orientation**	**Number of pyrenoids**	**Blade thickness (µm)**	**Vegetative cell length (µm)**	**Vegetative cell width (µm)**	**Average cell size in surface view (µm)**	**Method used (Morphological and/or Molecular)**
U.rigida C. Agardh 1823: 410 Type locality: Cadiz, Spain	Europe **Reference**: A critical survey of European taxa in Ulvales, Part II. *Ulva*, *Ulvaria*, *Monostroma*, *Kornmannia*. *Botaniska Notiser* 121: 535-629 (Bliding, 1968)	Stiff; Light to Mid to Dark green	Present (macro- and microscopic)	In surface view, rectangular or slightly polygonally rounded; Long rows, rarely without order in the apical region. In the basal region, mostly only slightly ordered or without any order.	Chlorophyll chiefly concentrated to the outer walls of the cells but sometimes to the side walls or even to the inner walls. (Cap-like)	2-4+	In the apical region: 38-42; In central part of the thallus: 48-76; At the base: up to 200	In the apical region: 21; At the base: 70 and tapering at the top		18 x 14 for a fully grown cell	Morphology
U. rigida	Ireland **Reference**: Morphology, rbcL phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain (Loughnane *et al*., 2008)	Stiff; Light to dark green; perforated in some	Present (basal region only in some)	Rounded, long rows in the apical and middle region. In the basal region, cells were spindle-shaped with tapered ends. Closely packed groups of rhizoidal cells were evident in the basal region.	Surface	2-4+				15-25 x 9-31	Morphology and molecular
U. rigida	Netherlands **Reference**: The taxonomy of *Ulva* in the Netherlands (Koeman & C. Van den Hoek, 1981)	Medium to light green, mostly irregularly orbicular to lanceolate, more or less lobed and perforated	Entire or with small denticules	Rectangular to irregularly polygonal cells in the middle and apical region. Arranged in mostly long distinct straight to curved rows. In the basal region, cells are distinctly rounded, arranged in two-celled groups. In cross-section, spindle-shaped cells present. In lower basal region, closely packed bundles of rhizoids present.	Cap-like appearance as the chloroplasts is slightly tilted towards one side of the outer cell wall in surface view.	Mostly 2-4. On average 3 per cell	For wild *Ulva*: In the apical region:65-90 (average: 75); In the middle region: 75-95 (average: 85); In the basal region: 95-125 (average: 115) For cultured Ulva: In the apical region: 40-48; In the middle region: 55-60; In the basal region: 90-125.	For wild *Ulva*: In the apical region: 14-28 (average: 21); In the middle region: 30-36 (average 33); In the basal region: 26-40 (average 33)		In the apical region: (7 - 11) 9 x (11-18) 15 In the middle region: (7-10) 9 x (11-13) 12 In the basal region: (8-14) 12 x (12-18) 14	Morphology
U. rigida	Roscoff, France **Reference**: The taxonomy of *Ulva* from coastal region of Roscoff France (Hoeksema & Van den Hoek, 1983)	Mostly stiff; Light to medium to dark green; irregularly orbicular and lobed in sheltered places and lanceolate on exposed shores.	Often clearly denticulate or even doubly denticulate	Mostly rounded or polygonal cells; arranged in long curved or straight rows. In the basal region: closely packed bundles of rhizoids present. Narrowly ovate cells or spindle-shaped cells with tapered ends.	Chloroplasts mostly placed against the sidewall (Cap-like appearance) but may also be situated against the outer cellwall.	Mostly more than 1 pyrenoid per cell.	40-100			In the apical region: (10-23) x (7-18) In the middle region: (8-20) x (7-14) In the basal region: (12-24) x (9-21)	Morphology

U. rigida	British Isles **Reference**: Seaweeds of the British Isles (Burrows, 1991)	Stiff, fluted margins and often deeply divided; Dark green flat lamina	Microscopic tooth-like projections, especially at base	In surface view, marginal cells in the apical region are spherical. In surface view, polygonal with rounded corners cells arranged in longitudinal and transverse rows in the middle to apical region. In surface view: rounded, unordered cells interspersed between dark-coloured rhizoidal cells.	Parietal chloroplast	2-4 per cell, often situated at one side of cell so as to appear somewhat hood-shaped in surface view.	40-120			(11-)15 (-29) x (7-) 9 (-14)	Morphology
U. rigida	Sri Lanka **Reference**: Sri Lankan seaweeds-Methodologies and field guide to the dominant species (Coppejans *et al*., 2009)	Stiff; orbicular, undulated, lobed, with smooth surface; Dark green	Small, pluricellular, marginal teeth	In surface view, polygonal cells; Irregularly arranged; In cross-section, broader isodiametric cells in the apical region; In cross-section, narrower cells in the middle region resulting in a palisade-like appearance.		2-5 per cell				15-40	Morphology
U. rigida	Tropical West Africa Sub-region **Reference**: The Marine Macroalgae of the Tropical West Africa Sub-region (John, Lawson & Ameka; 2003)	Firm; ovate to slightly lance-shaped, occasionally orbicular and deeply lobed, with margins undulate or folded.	Presence of dentations not obvious in West African plants	In cross-section: rectangular cells	Cap-like appearance		50-70 (-110)	30	13-22		Morphology
U. rigida	Southern Australia **Reference**: The marine benthic flora of Southern Australia (Womersley; 1984)	Smooth surface; thallus plane to irregularly divided or lacerate above, often perforate; Grass-green	Slight to prominent microscopic, multicellular spines often near the base, sometimes above, and usually only evident on parts of the thallus.	In surface view, isodiametric to slightly elongate cells; In rows in both directions, more irregular in the basal region. In cross-section, rounded cells in the apical region. In cross-section, cells tapered from their base to their outer end in the middle region. In cross-section, cells tapering outwardly in the basal region.	Cap-like appearance	1-2 per cell	In the apical region: 35-55; In the middle region: 80-100; In the basal region: 100-200.	In the apical region: 12-18; In the middle region: 25-35 (-40); In the basal region: 40-50	In the middle region: L/B 2-3; In the basal region: L/B 2.5-4 (-5)	In both the apical and basal region: (8-) 12 - 18 (-25)	Morphology

U. rigida	Kwazulu-Natal **Reference**: Guide to the seaweeds of Kwazulu-Natal (De Clerck *et al*., 2005)	Stiff bladelets; Orbicular, undulated, lobed, with smooth surface; Dark-green.	Small, multicellular, marginal teeth	In surface view, polygonal cells; Irregularly arranged; In cross-section, broader isodiametric cells in the apical region; In cross-section, narrower cells in the middle region resulting in a palisade-like appearance.		2-5 per cell				15-40	Morphology
U. rigida	Florida **Reference**: The seaweeds of Florida (Dawes & Mathieson; 2008)	Smooth to ruffled; Irregularly lobed blades; Solitary or tufted; Attached by basal rhizoids; Lobed margins; Bright-green	Microscopic projections/ marginal teeth	In surface view, rectangular to rounded cells; In rows in the apical region. In cross-section, cells are taller than broad in the apical region.	Parietal plastid	2 (-8)	In the apical and middle region: 32-40; In the basal region: up to 200.	In the apical region: 29; In the basal region: 70	In the apical region: 16 (-21)	11-18 x 15-22	Morphology
U. rigida	Hawaiian Islands **Reference**: Marine Green and Brown Algae of the Hawaiian Islands (Abbott & Huisman; 2004)	Firm; Small blades planar, not ruffled; Small rounded to orbicular slightly undulate blades; Dark-green. Note: Plants from Australia and California are described as lobed or irregulalrly divided, which is not the case with the Hawaiian specimens.	Few to many stiff, pointed uniseriate teeth or spines	In surface view, polygonal cells; Irregularly arranged cells.	Plastids filling about 1/3 of cell	1-2 per cell	62	15-25	10-15 µm	12	Morphology
U. rigida	Southeastern United States **Reference**: Seaweeds of the Southeastern United States (Schneider & Searles; 1991)	Blades irregularly lobed, often richly perforate	Microscopic marginal teeth	In surface view, rectangular to rounded (to polygonal) cells in ordered rows in the apical region; In cross-section, globose/ spherical cells, slightly taller than broad in the apical region; In cross-section, cells tapering toward the top in the basal region.	Position of plastids variable	(1-) 2 (-8) per cell	In the apical region: 38-42; In central part of the thallus: 48-76; At the base: up to 200	In the apical region: 21-29; In the basal region: 70	In the apical region:16	(11-) 14 (-17) x (15-) 18 (-22)	Morphology

U. rigida	North Carolina **Reference**: An illustrated guide to the benthic marine algae of coastal North Carolina 2. Chlorophyta and Phaeophyta (Kapraun, 1984)	Irregularly lobed thallus, usually less than 10 cm long	Margins bearing microscopic teeth	In surface view, polygonal to rounded cells; Mostly unordered below, in series above. In cross-section, cells slightly taller than broad.	Plastid covering most of the cell face	Mostly 2-3 (-4)	50-60			12-14 x 16-18	Morphology
U. rigida Type locality: Atlantic Ocean	Monterey Peninsula California **Reference**: Marine Algae of the Monterey Peninsula California (Smith; 1944)	Blades at first lanceolate or ovate; later becoming broadly orbicular, ruffled and deeply lobed; Dark-green	n/a	In cross-section, cells are vertically elongated and with a length 1.5-3 times the breadth	Cup-shaped chloroplast, massive, filling the outer two-thirds of a cell.		40-60; Note: Accordiing to the measurements of Setchell and Gardner, 1920; blade thickness is 60-110.				Morphology
U. rigida	South Western Cape, South Africa **Reference**: Taxonomy of *Ulva* species (Chlorophyta) in the South Western Cape, South Africa (Joska, 1992)	Firm to rigid texture; Elongate orbicular or lanceolate; Numerous perforations; Pale green to grass green	Microscopically visible dentation, often only on the stipe and basal portions of the thallus. Occasionally some plants had no microscopic dentations whilsts for others it was macroscopically visible	In surface view, polygonal cells, forming rows but usually irregular in the apical region. In cross-section, well rounded to rectangular cells in the apical region. In surface view, polygonal cells, irregular, sometimes forming short rows in the middle region. In cross-section rounded, rectangular cells in the middle region. In surface view, rounded polygonal cells in the basal region. In cross-section, rounded, rectangular occasionally narrowing at the top in the basal region.	Cap-like	In the apical region: 1-3; In the middle region: 1-2	In the apical region: 50-95; In the middle region: 70-110; In the basal region: 80-190.	In the apical region: 24-30; In the middle region: 35-45	In the apical region: 10-18; In the middle region: 15-20		Morphology
U. rigida	South African West Coast **Reference**: Seaweeds of the South African West Coast (Stegenga *et al*., 1997)	Firm; Irregularly incised or lobed; Pale to grass green	Marginal minute teeth near the base of thallus	In surface view, rounded angular cells; Organised in long curved rows; In cross-section, cells 2-4 times higher than broad, cyclindrical with rounded angles at the outer periclinal wall.	Parietal chloroplast, located against the outer periclinal cell wall or shifted to one of the radial cell walls	1-3 per cell	In the apical region: 75; In the middle region: 100; In the basal region: up to 200	25-35		10-16 µm	Morphology

U. rigida	South Western Cape, South Africa **Reference**: The Molecular Systematics of *Ulva Linnaeus* and *Enteromorpha Link* (Ulvales, Chlorophyta) from the South Western Cape, South Africa (Kandjengo, 2002)	Thick, consistency firm, tufty at the base, incised, entire margins, smooth and shiny, flat sheet, perforated	No dentation	In surface view, rectangular-squared, rounded- polygonal; arranged in short rows to none. In cross-section, rectangular (rounded angular) - slender.		1-3 (-4)	52-247	21-53	6-27 µm	6-21 x 10-29	Morphology and molecular
U. rigida	Southern and Eastern coast of Korea **Reference**: New record of two marine ulvalean species (Chlorophyta) in Korea (Hee Lee *et al*., 2014)	Stiff; Membranous, distromatic, frond irregular in shape, usually undulate at the margin; Light to dark green	Many small macroscopic denticulations along the margin. Note: denticulations in *U. rigida* of this study are numerous and branched.	In surface view, rectangular to polygonal cells with round corners; Cells usually arranged in pairs. In cross-section, length to width ratio of 1.2-2.0	Chloroplasts cap-like, parietal	(1-) 2-3	40-60			5-15 x 5-10	Morphology and molecular
U. rigida	Veerse Meer Lagoon in southwest Netherlands **Reference**: Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison (Malta *et al*., 1999)	Stiff, strong thallus; Dark green	No dentate thallus margin	In surface view, polygonal cells, distinctly rounded corners; Some cells arranged in rows, mostly in indistinct groups	Occasionally cap-like chloroplast in surface view.	2 per cell; Note: Many starch grains in most cells	85-90			15-20	Morphology and molecular
U. rigida	Oosterschelde estuary in southwest Netherlands **Reference**: Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison (Malta *et al*., 1999)	Stiff, strong thallus; Dark green	No dentate thallus margin	In surface view, polygonal cells, distinctly rounded corners; Most of the cells arranged in rows	Mostly cap-like chloroplast in surface view	Mostly 2-3, very rare 4 per cell	95-110			13-15 (-17.5) Note: extremes in brackets	Morphology and molecular
U. rigida	Tanzania **Reference**: Marine plants of Tanzania: a field guide to the seaweeds and seagrasses (Oliveira *et al*., 2005)	Rounded blades form lettuce-like, dark green rosettes, 2-4 cm wide		In cross-section, rounded cells in the apical region. In cross-section of the middle and basal region, vertically elongated cells.			Cross-section of mature thallus shows two rows of elongated, palisade-like cells; 100-200 µm due to growth of rhizoidal filaments between cell layers in the basal portion.				Morphology

U. rigida	San Diego County **Reference**: Marine algae and seagrasses of San Diego County (Stewart, 1991)	Relatively stiff, orbicular, deeply lobed, ruffled, with short solid stipe; Densely tufted, have irregularly lobed thalli; Dark green	Present along the margin			Several pyrenoids per cell					Morphology
U. rigida Note: Type locality: Cape of Good Hope, S. Africa	California **Reference**: Marine algae of California (Abbott and Hollenberg, 1976)	Firm and relatively stiif, broadly orbicular, usually deeply lobed and somewhat ruffled; Dark green	Present along the margin	In surface view, irregularly arranged cells. In cross-section, cells 1.5-3 times as long. The 2 layers of blade separated by pronounced accumulation of mucilaginous material.	Chloroplast filling the outer half of cell	1 or 2(3)				10-15 µm	Morphology
U. rigida C. Agardh (1823), p. 410.	Namibia **Reference**: Marine benthic algae of Namibia (Lluch, 2002)	Blade surface smooth, with numerous perforations irregular in shape and variable in size; the perforations possibly causing the split of the blade.	Exceptionally minutely dentate in some places.	In surface view, polygonal cells with the corners more or less rounded, elongate, triangular or almost isodiametric. In cross-section, cylindrical cells, 1.3-4.3 times higher than broad, anticlinally arranged.	Plastid hood shaped	1-2(-4)	52-100	20-34 µm	8-18 µm	6-20 X 4-14	Morphology
U. laetevirens Areschoug 1854: 370-71 Type locality: Port Phillip, Victoria, Australia	South Australia **Reference**: Investigations into southern Australian *Ulva* (ulvophyceae, chlorophyta) taxonomy and molecular phylogeny indicate both cosmopolitanism and endemic cryptic species (Kraft *et al*., 2010)	Thin and papery in appearance and texture; Irregularly contoured, lobed but unbranched; Medium to light green	Blade margins either smooth or, more commonly, lined with irregularly spaced acute to blunt teeth	In surface view, rectilinear to occasionally quadrangular to polygonal cells, usually twice as long as wide; Unordered or showing some alignment both longitudinally and transversely. In cross-section, most cells pear shaped (pyriform), with the broadly to sharply tapered end directed toward the frond surface in the middle region. In cross-section, cells tapering, often sharply, to acute tips in the basal region.	Parietal, usually taking up two-thirds of the cell	1-3 per cell	In the middle region: 55-65; In the basal region: 100-160	In the middle region: 23-36; In the basal region: 35-60	In the middle region: 4-12; In the basal region: 5-16	8-16 (-20) long x 8-16 (-20) wide	Morphology and molecular

U. laetevirens Also reported in New Zealand, Israel, Cyprus, Greece, Slovenia, Italy and Canada	Northeast Coast of the United States of America **Reference**: The appearance of *Ulva laetevirens* (Ulvohyceae, Chlorophyta) in the Northeast Coast of the United States of America (Mao *et al*., 2014)	Foliose thalli, naturally grow singly or in small clumps fixed to the hard substratum with rhizoidal; Medium green	Marginal teeth along the basal and median regions were not found.	In surface view, polygonal or quadrangular cells in the apical and middle region. In surface view, round cells in the basal region. In cross-section, cylindrical or conical cells in the rhizoidal and basal region and their height is 2-3 times of their diameter. In cross-section, cell length is not distinctive in the apical and middle region, but they have a conical shape as they taper towards the thallus surface.			In the apical region: 30-40; In the rhizoidal region: 100-120			Morphology
U. laetevirens	Jeju Island, Korea **Reference:** Taxonomy of *Ulva* causing blooms from Jeju Island, Korea with new species, *U. pseudo-ohnoi* sp. nov. (Ulvales, Chlorophyta) (Lee *et al.,* 2019)	Irregularly contoured and lobed with small perforations; Medium green to green, and sometimes fairly dark green. Fronds are broadly palmate to irregularly lobed or irregularly furcated and expanded. Thallus ranged from 15-30 cm high. Distromatic thallus.	Fronds have multifid, sharp, long and irregularly protuberant marginal teeth. Marginal denticulation is simple to multiple ordered by growing.	In surface view, cells are unordered or show both longitudinal and transverse alignments, and have cuboidal or sub-rectilinear shapes with roundish outlines. In cross-section, cells are bluntly rounded, with some shapely tapered to acutely pointed apices.			Thallus thickness: 70-85			Morphology and molecular
U. armoricana P.Dion, B.de Reviers & G.Coat 1998: 74 Type locality: Coast of Brittany, France	France: Finistère: Île Verte, Roscoff **Reference**: *Ulva armoricana* sp. nov. (Ulvales, Chlorophyta) from the coasts of Brittany (France). I. Morphological identification (Dion *et al*., 1998)	Crumpled texture, fragile, easily torn and sometimes perforated; Pale green	Presence of tooth-like protuberances at the margin of the blade	In surface view, polygonal and quadrangular cells in the middle region; Arranged without order in small longitudinal rows in rapidly growing parts. In cross-section, rounded cells in the apical and middle region. In cross-section, generally ovate, sometimes longitudinally elongated and with tapered ends in the basal region.	Chloroplasts are often perforated and occur mostly against the outer cell wall.	Mainly 1-2-(3-4) per cell	In the apical region: 30-40 (up to 30-55 in winter); In the middle region: 40-55 (up to 70-80 in winter)		In the apical region: 9-20 x 7-16; In the middle region: 10-23 x 7-14; In the basal region: 15-25 x 10-15	Morphology

U. armoricana	Japan **Reference**: Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva* (Shimada *et al*., 2003)			Polygonal cells and irregularly arranged; Corner of cells not rounded	Chloroplast always filled the cell	1-3 per cell			(length x width): 17.6 ± 3.7 x 13.1 ± 1.8; 18.1 ± 3.1 x 12.0 ± 1.9	Morphology and molecular
U. scandinavica Bliding 1968: 554 Type locality: West Coast, Kristineberg, Sweden	Sweden, West Coast, Kristineberg **Reference**: A critical survey of European taxa in Ulvales, Part II. *Ulva*, *Ulvaria*, *Monostroma*, *Kornmannia*. *Botaniska Notiser* 121: 535-629 (Bliding, 1968) & Morphology, rbcL phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain (Loughnane *et al*., 2008)	Stiff; Light to mid to dark green	Sparse microscopic, tooth-like protuberances, always unramified and smaller than in *U. rigida*	In surface view, polygonal to rounded cells; Mainly unordered cells with unarranged cells alternate with areas where a small number of cells are arranged in rows. In cross-section, the height of the cell is less than the breadth of the cell in the apical region. In cross-section, the cells are not tapering as in *U. rigida* in the basal region.	Surface	2-4+	In the apical region: 50; In the middle region: ~75; In the basal region: ~100	In the apical region: ~21; In the basal region: ~40	21-27 x 15-19; averaging 24 x 17	Morphology
U. scandinavica	Ireland & Britain **Reference**: Morphology, rbcL phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain (Loughnane *et al*., 2008)	Membranous; Light to mid-green	No denticulation; Note: denticulations were found on attached specimens but green-tide plants completely lacked denticulations.	In surface view, angular/ polygonal cells with rounded corners; Arranged without order. In cross-section, oval shaped cell with rounded ends in the lower middle/basal region. No spindle-shaped cells in the basal region.	Cap-like (sidewall)	1-2 or 2-4+			In the middle region: length (14-25) x width (15-24)	Morphology and molecular
U. scandinavica	Netherlands **Reference**: The taxonomy of *Ulva* in the Netherlands (Koeman & C. Van den Hoek, 1981)	Lubricous, firm near the base; Mostly irregularly orbicular, often lobed, sometimes lanceolate and curved, often perforated; Medium to light green	Margins entire or sometimes with small denticules	In surface view, irregularly polygonal with slightly rounded corners in the apical, middle and basal region; arranged without much order, or in indistinct groups whose cells may form short, often curved rows. In cross-section, the L/B is 1.2-3 in the apical and middle region. In cross-section, the L/B is 1.9-3.5.	Against outer cell wall	In the apical and middle region: 2-4 (-6); In the basal region: 3-4	In the apical region: (42-) 50 (-58); In the middle region (45-) 60 (-78); In the basal region: (85-) 95 (-110)	In the apical region: (16-) 18 (-22); In the middle region: (20-) 22 (-24); In the basal region: (28-) 36 (-44)	In the apical region: (14-) 19 (-24) x (11-) 13 (-16); In the middle region: (13-) 17 (-22) x (9-) 13 (-15); In the basal region: (15-) 18 (-24) x (11-) 14 (-17)	Morphology

U. scandinavica	Roscoff, France **Reference**: The taxonomy of *Ulva* from coastal region of Roscoff France (Hoeksema & Van den Hoek, 1983)	Stiff; thallus lobes are teared off along rows of perforations; dark-green to medium to light green	Often has denticulate or doubly denticulate margins	In surface view, polygonal with angular or slightly rounded corners in the apical and middle region; Usually unordered but may also be arranged in small indistinct groups or in short rows. In cross-section, the L/B is 0.7-3 in the apical and midde region. In surface view, polygonal with slightly rounded corners cells in the basal region. In cross-section cells have a cylindrical form with rounded ends and a L/B between 1.8-3.	Against outer cell wall	Mostly 2-4 (rarely up to 6).	40-85	In the apical region (L X B): (11-25) x (7-18); In the middle region: (9-24) x (8-18); In the basal region: (10-24) x (7-21)	Morphology
U. scandinavica	Veerse Meer Lagoon in southwest Netherlands **Reference**: Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison (Malta *et al*., 1999)	Membranous, slimy; Light green	No dentate thallus margin. Note: Many starch grains in most cells.	In surface view, polygonal with corners slightly rounded; Arranged mostly without order	Extremely rare chloroplast cap-like was observed.	2-5 per cell. 1 per cell also was observed	(43-) 48-56 (-65-70)	(10-) 12-19 (-26)	Morphology and molecular
U. scandinavica	Oosterschelde estuary in southwest Netherlands **Reference**: Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison (Malta *et al*., 1999)	Membranous, slimy; Light green	No dentate thallus margin. Note: Many starch grains in most cells.	In surface view, polygonal with corners slightly rounded; Arranged mostly without order	No chloroplast cap-like was observed	Mainly 2-3, rarely 1 per cell	(50-) 52-58 (-60)	14-16	Morphology and molecular

U. thuretti B. Föyn 1955: 267	Föyn suggested the name *U. thuretti* for the southern European populations. This name is invalid, since Föyn failed to provide a Latin description or designate a type specimen. Bliding (1969, p. 554) showed that *U. thuretii* Föyn is conspecific with *U. rigida* C. Agardh. He stated, however, that one of the typical characteristics of *Ulva rigida* is the presence of very small marginal teeth. Since the specimens lack such marginal teeth, it is possible that the eastern Mediterranean and Red Sea plants may represent still another species. **Reference**: Marine algae and seagrasses of the Dahlak Archipelago,southern Red Sea (Lipkin and Silva, 2002)									Morphology
U. spathulata Papenfuss 1960: 309 Note: Synonymized with *Ulva petiolata* (J. Agardh) Womersley 1956: 354	Type locality: Port Phillip, Victoria, Australia **Reference**: The marine benthic flora of southern Australia. Part I. (Womersley, 1984)	Smooth surface; simple or branched with expanded, often lobed; Medium green	Intact margin entire	In surface view, isodiametric to elongate cells in the apical and middle region; Arranged in rows in both directions (more irregular below). In cross-section, rounded to angular cells in the apical region with L/B 1-1.5.	Covers 2/3 of the cell	1-2 per cell	In the apical region: 70-80; In the 'petiolar' region (110-) 150-300	In the apical region: 15-20; In the petiolar region: 30-50 with L/B 2-3	In the apical and middle region: 10-20; In the petiolar region: 10-20	Morphology
U. petiolata J. Agardh Womersley 1956: 354 Original name: Letterstedtia petiolata J. Agardh 1883: 176-177	Type locality: Port Phillip, Victoria, Australia **Reference**: On the genera of the Ulvales and the status of the order (Papenfuss, 1960)	Lacerated fronds, with the proximal parts of the segments are narrow and stalk-like.								Morphology

U. uncialis (Kützing), Montagne 1850: 248 Synonymized with *U. rigida* C. Agardh	Syntype localities: Table Bay and Algoa Bay, South Africa, including Indian Ocean records. Taxonomic synonym: *Ulva capensis* Areschoug, 1851: 15-16, nom illeg. Note: J. Agardh (1883) considered *U. uncialis* as a capensis form of *U. rigida*. **Reference**: Catalogue of the benthic marine algae of the Indian Ocean (Silva *et al*., 1996); Taxonomy of *Ulva* species (Chlorophyta) in the South Western Cape, South Africa (Joska, 1992)	Small thallus, irregularly lobed and with undulate margins	Due to poor condition of the specimen, dentation was not clear	In cross-section, cells approximately 1.5 x higher than broad and with rounded corners in the apical region. In cross-section, rectangular cells in the basal region.			In the apical region: 35-40; In the basal region: 100				Morphology
U. capensis Areschoug 1851: 15-16	South African West Coast **Reference**: Seaweeds of the South African west coast (Stegenga *et al*., 1997)	Firm, ovate to broadly lanceolate, often irregularly laciniate, edges ruffled, sometimes numerous holes; Grass-green to dark green	Dentate margins	In surface view, rounded cells often in pairs, unordered or in curved rows in the middle region. In cross-section, bullet or spindle-shaped cells in the middle and basal region.	Located at the distant cell walls		In the apical region: 70; In the middle region: 120-150; In the basal region: up to 200	In the middle and basal region region: 40-70		In the middle region: 8-15	Morphology
U. capensis Areschoug 1851: 15-16	South Western Cape, South Africa **Reference**: Taxonomy of *Ulva* species (Chlorophyta) in the South Western Cape, South Africa (Joska, 1992)	Firm/thick and tough ot the touch; Thallus may be simple, lanceolate, linear, cuneate, often deeply torn and with numerous perforations; Bright grass green but often dark green	Macroscopically visible single or double dentation on the margin	In surface view, rounded cells and usually arranged in pairs and in short rows in the apical region. In cross-section, narrowly ovate or ellipsoidal, occasionally rounded rectangular cells with a L/B 2.5-1 in the apical region. In surface view, rounded cells, often paired and sometimes form rows in the middle region. In cross-section, spindle-shaped cells in the middle region. In surface view, rounded, not paired cells in the basal region. In cross-section, spindle-shaped sometimes ovate elongate cells, with a L/B 7-2 in the basal region.	In the apical and middle region, chloroplast lie at the top of the cells. In the basal region, chloroplast commonly appeared granular, and often spread throughout the cell length.	In the apical region: usually 1, sometimes 2 per cell. In the middle region: 1-2 per cell.	In the apical region: 60-95; In the middle region: 95-150; In the basal region: 135-265	In the apical region: 25-40; In the middle region: 30-60	In the apical region: 8-12; In the middle region: 10-20		Morphology

U. capensis Areschoug 1851: 15-16	Namibia **Reference**: Marine benthic algae of Namibia (Lluch, 2002)	Papery texture, rigid, orbicular in shape; blade surface smooth, often perforated	Margin of blade and perforations usually dentate, sometimes irregularly two-lipped	In surface view, rectangular or squarish, with rounded corners cells, more or less arranged in groups. In cross-section, conical cells in the middle region, anticlinally arranged, with the narrow tip towards the exterior	Plastid parietal	1-4 pyrenoids	In the middle region: 100-181	In the middle region: 42-62	In the middle region: 6-16	6-18 X 4-14	Morphology
U. capensis	South Western Cape, South Africa **Reference**: The Molecular Systematics of *Ulva Linnaeus* and *Enteromorpha Link* (Ulvales, Chlorophyta) from the South Western Cape, South Africa (Kandjengo, 2002)	Porous, wrinkled around perforations, undulate, thick lamina, branched, tough basally, dull and rough, lanceolate, dark patches	Dentate (double)	In surface view, rectangular-rounded, polygonal or irregular, bean-shaped and well paired; arranged in curved rows partly. In cross-section, rectangular (rounded angular), spindle-shaped.		1-4 (-5)	72-209	27-67	8-17 µm	4-17 x 8-23	Morphology and molecular