DE GRUYTER Botanica Marina

The current state of DNA Barcoding of Macroalgae in the Mediterranean Sea: presently lacking but urgently required

Journal:	*Botanica Marina*
Manuscript ID	BOTMAR.2019.0041.R3
Manuscript Type:	Review
Date Submitted by the Author:	29-Jan-2020
Complete List of Authors:	Bartolo, Angela; University of Aberdeen, School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, ; University of Malta, Laboratory of Applied Phycology, Department of Biology, Fourth Floor, Biomedical Sciences Building, University of Malta, MSD2620 Zammit, Gabrielle; University of Malta, Laboratory of Applied Phycology, Department of Biology, Fourth Floor, Biomedical Sciences Building, University of Malta, MSD2620, Peters, Akira; Bezhin Rosko, 40 rue des pêcheurs, 29250 Santec Kuepper, Frithjof; University of Aberdeen, School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU,
Classifications:	1600 Algal genetics < 1 Algal biochemistry/molecular biology/genetics, 5600 Algal biogeography < 5 Algal systematics/floristics/biogeography
Keywords:	barcoding, Chlorophyta, algae, Phaeophyceae, Rhodophyta

Review

The current state of DNA barcoding of macroalgae in the Mediterranean Sea: presently lacking but urgently required

Angela G. Bartolo[1,2,*], Gabrielle Zammit[2], Akira F. Peters[3] and Frithjof C. Küpper[1]

[1)] School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK

[2)] Laboratory of Applied Phycology, Department of Biology, Fourth Floor, Biomedical Sciences Building, University of Malta, MSD2620, Msida, Malta

[3)] Bezhin Rosko, 40 rue des pêcheurs, 29250 Santec, Brittany, France

*Corresponding author: e-mail: angiebartolo@gmail.com

A.G. Bartolo et al.: The current state of DNA barcoding of macroalgae in the Mediterranean Sea

Received 12 June, 2019; accepted 18 February, 2020

Abstract: This review article explores the state of DNA barcoding of macroalgae in the Mediterranean Sea. Data from the Barcode of Life Data System (BOLD) were utilised in conjunction with a thorough bibliographic review. Our findings indicate that from around 1124 records of algae in the Mediterranean Sea, only 114 species have been barcoded. We thus conclude that there are insufficient macroalgal genetic data from the Mediterranean and that this area would greatly benefit from studies involving DNA barcoding. Such research would contribute to resolving numerous questions about macroalgal systematics in the area and address queries related to biogeography, especially those concerned with non-indigenous species. It could also possibly result in the development and application of better, cost-effective biodiversity monitoring programmes emanating from UN conventions and EU Directives. One possible way of achieving this is to construct DNA libraries via sequencing and barcoding, subsequently enabling better cost-effective biodiversity monitoring through environmental DNA metabarcoding.

Keywords: algae; barcoding; Chlorophyta; Phaeophyceae; Rhodophyta.

Introduction

This review delves into the current state of DNA barcoding of macroalgae with a focus on the Mediterranean Sea. To this end, data from the Barcode of Life Data System (BOLD) were researched and a literature review was conducted. The study concludes that there is a lack of genetic data for these organisms. This article discusses the steps required for improving DNA-based methods in this region.

Algae including macrophytes and phytoplankton are the base of marine food webs, provide oxygen to aquatic environments and humans, can be used as biological indicators, and are a potential food source which is underutilized in most parts of the world (Wolf 2012). However, in direct contrast to their importance in marine ecosystems (Brodie et al. 2017), only 10% of algal species are believed to have been described so far (De Vargas et al. 2015).

Macroalgae include mainly three phylogenetic lineages. Green and red algae originated about 1.5 billion years ago after the endosymbiotic event that resulted in the first photosynthetic eukaryotes (Yoon et al. 2004, Rodríguez-Ezpeleta et al. 2005). On the other hand, the brown algae evolved after a secondary endosymbiotic event that occurred around 1.3 billion years ago, involving a unicellular red alga engulfed by a heterokont protist that was only distantly related to the green and red algae (Yoon et al. 2004, Ševčíková et al. 2015). Complex multicellularity is thought to have evolved independently in the three large groups (Cock et al. 2010).

The term 'seaweed' has traditionally been used to define the macroscopic, multicellular marine algae (Wolf 2012). However, these may also exist as microscopic representatives since all macroalgae are unicellular at some point in their life cycle, as zygotes or spores, which could also be temporarily planktonic (Amsler and Searles 1980).

The term Mediterranean is derived from the Latin *Mare medi terraneum*, meaning 'sea in the middle of the land' (Coll et al. 2010). It is the largest and deepest semi-enclosed sea in the world. The Mediterranean is connected to the Atlantic Ocean through the Strait of Gibraltar, to the Sea of Marmara and the Black Sea through the Dardanelles and to the Red Sea and the Indian Ocean through the Suez Canal (Coll et al. 2010). The Mediterranean has a large area classified as deep sea with high salinity (37.5-39.5) and homothermy from around 300-500 m to the bottom of 12.8°C-13.5°C in the western basin and 13.5°C-15.5°C in the eastern basin (Coll et al. 2010). It also has a geological history that included segregation from the other seas causing the Messinian Salinity Crisis around 5.96 million years ago, which brought about major changes in climate, salinity and sea level (Coll et al. 2010).

Although Mediterranean species evolved from ancestors originating from the Atlantic following the Messinian Salinity Crisis, both temperate and subtropical organisms have managed to survive in the Mediterranean's wide range of hydrology and climate. However, the Mediterranean Sea also has a high level of endemism (Coll et al. 2010, Pascual et al. 2017) and is considered a biodiversity hotspot.

DNA barcoding involves the identification of DNA sequences by checking their identity against a publicly-accessible database. DNA barcoding has revolutionized classical species identification, biogeographical and population studies. The concept of DNA barcoding is based upon sequencing one or a few relatively short loci, which are standardized among a large number of taxa, and which provide an unambiguous identification of the given taxon – similar to barcoding a commercial product (Hebert et al. 2003). It is meant to be rapid, relatively inexpensive and applicable using generic methodology and equipment – thus, at least in principle, empowering non-specialists or even non-scientists to obtain a correct identification of any previously identified taxon (including groups for which very few taxonomic experts exist worldwide).

So far, barcoded taxa span across almost all known eukaryotic phyla, including groups which are morphologically difficult to identify such as the oomycete genus *Pythium* (Robideau et al. 2011), dinoflagellates (Stern et al. 2012) or the ciliate *Tetrahymena* (Kher et al. 2011). However, large gaps in coverage still exist, both geographically and phylogenetically.

DNA barcoding provides many benefits including identification at a faster speed especially for routine monitoring and also for the discovery of new algal species which could be flagged faster (Saunders and Kucera 2010). In addition, it enables the identification of algal species at different life stages and even degraded DNA samples

from museum specimens (Comtet et al. 2015). DNA barcoding has often highlighted the occurrence of cryptic taxa (McIvor et al. 2001, Andreakis et al. 2007) and the detection of morphologically cryptic algae at new locations (Provan et al. 2005, Uwai et al. 2006). This is important since cryptic species are frequently produced by recent speciation or convergent evolution, and these species are impossible to differentiate morphologically; however, they are genetically distinct (Cianciola et al. 2010). Moreover, diverse genetic populations which are indistinguishable morphologically may have a different invasive range (e.g. Voisin et al. 2005). Crypticisms could refer to cryptic size (i.e. microscopic species), cryptic stages that may be unknown microscopic forms of a known macroalgal species, cryptic morphology (i.e. where morphology is hardly discernible) or cryptic species (i.e. different species of similar morphologies) (Peters et al. 2015). Morphological identification is problematic in the case of crypticisms and thus DNA barcoding is necessary to reveal them. For example, as indicated in Zanolla et al. (2018), genetic studies have revealed two cryptic lineages in *Asparagopsis armata* and six in *Asparagopsis taxiformis* (Andreakis et al. 2004, 2016, Dijoux et al. 2014) and, due to their cryptogenic status, their native ranges remain unknown (Dijoux et al. 2014). Cryptogenic species are species with an undefined origin which are often small and/or cryptic, belong to taxonomic groups which have not been studied extensively and are frequently described taxonomically by diverse names in different new areas (Carlton 2009, Mineur et al. 2012).

DNA metabarcoding combines the concepts of DNA barcoding with that of Next Generation Sequencing (NGS) and involves High-Throughput Sequencing (HTS) methods applied directly to an environmental sample without the prerequisite of isolation of individuals (Porter and Hajibabaei 2018). The importance of environmental

DNA (eDNA) metabarcoding is that it simultaneously characterizes the composition of species in environmental DNA, and is thus contributing significantly to taxonomic, ecological and biogeochemical studies (Deagle et al. 2014).

eDNA consists of genetic material which is present in samples such as sediment, air and water, including whole cells, cell debris, different life stages such as propagules, whole microorganisms and/or extracellular DNA (Ficetola et al. 2008). Community DNA also relies on metabarcoding; however, it is distinguished as 'bulk organismal samples'. The difference to eDNA is blurred in this case, especially when whole microorganisms are captured in eDNA samples (Ruppert et al. 2019). eDNA metabarcoding is useful in detecting invasive species rapidly, toxic species (such as species that cause Harmful Algal Blooms), the presence of endemic species which are threatened, rare species or species which are traditionally difficult to detect (Ruppert et al. 2019). Algal metabarcoding could target single cells, planktonic life stages, or cell debris from macrophytes to answer ecological questions or to address policy requirements. The application of eDNA metabarcoding to the marine environment has adopted many collection procedures which include sampling of sediments, water, specimens and biofilms (Ruppert et al. 2019). Koziol et al. (2019) compared the results obtained from different substrates including marine sediment, surface water, settlement-plates and planktonic tows and concluded that there was a significant variation between taxa observed in the different substrates. Thus, they concluded that a single substrate underestimates eukaryotic diversity and that future studies should use more than one substrate, as well as choosing the substrate in conjunction with the taxa of interest carefully. Sampling for eDNA metabarcoding from different substrates is preferably carried out on the same day, as in the study by Koziol et al. (2019), where water was

sampled in sterile bottles, filtered and stored at -20°C. On the other hand, sediment was obtained through a Van-Veen grab and plankton was captured in planktonic tows and concentrated in 50-70 ml sterile containers that were stored at -20°C.

For DNA metabarcoding to be successful, it must be ensured that the barcode locus chosen with given primers is suitable for all target species and that the barcode is sufficiently variable to allow distinction between species (Hebert et al. 2003). The barcode also needs to be flanked by two conserved regions.

On the other hand, the Germling Emergence Method, which involves the incubation of a sample of substratum in a herbivore-free and nutrient-rich environment, facilitates the subsequent isolation of developing germlings (Peters et al. 2015). This, coupled with DNA barcoding, provides opportunities to study morphology and life cycles of macroalgae in parallel with genetic analysis (Fredericq et al. 2014).

With regard to the Mediterranean Sea, the study of genetic diversity is particularly important since geographically enclosed ecosystems are at a higher risk of species loss and genetic erosion in view of dispersal and range shift barriers (Buonomo et al. 2018). In fact, Buonomo et al. (2018) concluded that the coverage of *Cystoseira tamariscifolia*, *Cystoseira amentacea* and *Cystoseira compressa* would substantially decrease under two different climate change models, especially in the Mediterranean region where *Cystoseira amentacea* would be especially at risk of extinction (Buonomo et al. 2018). The genetic data of the aforementioned species showed that the populations were differentiated with low inter-population connectivity; thus, the loss of any population could cause a permanent loss of genetic variability (Buonomo et al. 2018). Since *Cystoseira* species have a particular ecological niche and function in the

Mediterranean, their loss could have wider negative implications (Buonomo et al. 2018).

Traditionally, studies of Mediterranean macroalgae have involved their morphology. A comprehensive review of macroalgal identification through morphology of macroscopic and microscopic features is provided in Cormaci et al. (2012, 2014, 2017) and Rodríguez-Prieto et al. (2013). To date, the major shortcomings of these works is that they are not connected to genetic information and there is no English translation. Moreover, AlgaeBase (Guiry and Guiry 2019) provides a database which is constantly being updated to reflect the latest algal biodiversity-related research.

Established checklists or lists of macroalgae based on morphology exist for many Mediterranean countries such as Algeria (Ould-Ahmed et al. 2013), Cyprus (Taşkın et al. 2013, Tsiamis et al. 2014b), Egypt (Shabaka 2018), France (e.g. Thau Lagoon: Verlaque 2001), Greece (Tsiamis et al. 2013, 2014a), Israel (Einav and Israel 2008), Italy (Furnari et al. 2003, 2010), Malta (Cormaci et al. 1997), Morocco (Benhissoune et al. 2001, 2002a, 2002b, 2003), Spain (eg. Catalonia: Ballesteros 1990), Turkey (Taşkın 2008) and regions such as the eastern Adriatic coast (Antolić et al. 2001, 2010, 2011, 2013). Moreover, a few checklists of the Mediterranean as a whole are available, such as those by Gallardo et al. (1993) for Chlorophyta, Ribera et al. (1992) for Phaeophyceae and Gómez Garreta (2001) for Rhodophyta. However, such checklists need to be updated regularly in view of new discoveries and the introduction of non-indigenous species.

Mediterranean European Union (EU) Member States are legally bound to adhere to the EU Water Framework Directive (WFD) and EU Marine Strategy Framework Directive (MSFD) in order to protect aquatic ecosystems. In addition, the UN

'Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean', also referred to as the "Barcelona Convention", which is one of the Regional Sea Conventions, applies not only to EU Member States, but also includes countries from north Africa and the eastern Mediterranean. The overall aim of the Barcelona Convention is to protect and improve the marine and coastal environment of the Mediterranean.

There have been extensive morphological studies in the Mediterranean in view of ecological indices utilised for the EU WFD, EU MSFD and the UN Barcelona Convention. These monitoring programmes have been time-consuming, as well as costing EU Member States millions of Euros. One of the shortcomings of the MSFD is that it studies specific taxa whereas the choice of taxa should ideally be based on a study of all taxa including 'microbial community interactions', which are currently not considered under the MSFD (Bourlat et al. 2013). Such shortcomings could be overcome through the inclusion of DNA-based methods. The greatest costs of including DNA-based methods in monitoring programmes would be in the setting up of the reference library, however this could be offset by future benefits and savings (Bourlat et al. 2013).

Currently, the MSFD indicators sparsely consider DNA-based methods. Recent literature is pointing towards the integration of DNA barcoding and DNA metabarcoding in MSFD indices which would result in more representative (Bourlat et al. 2013) and less costly (Aylagas et al. 2016) monitoring programmes. Table 1 (adapted from Aylagas et al. 2016) shows which tools could possibly be applied for each MSFD descriptor.

Some Mediterranean macroalgae, such as coralline algae, have received a lot of attention from a conservation point of view, despite sparse DNA sequence data which are still insufficient to define species diversity in detail (Pezzolesi et al. 2019). Pezzolesi et al. (2019) show how this is in contrast to other regions where genetic studies have been undertaken, such as the Pacific Ocean, Indo-Pacific Ocean, western Indian Ocean, Subarctic and Boreal Atlantic, Atlantic and Caribbean, among others. The lack of sequence data for the Mediterranean has also been acknowledged for the genus *Ulva* (Miladi et al. 2018), which on the other hand, has been well-studied morphologically (Cormaci et al. 2014).

The present review article is in agreement with the above inferences by Pezzolesi et al. (2019) and Miladi et al. (2018). In fact, both a thorough bibliographic search and the analysis of BOLD data suggest that DNA barcodes for macroalgae are underrepresented in the Mediterranean Sea.

The reason for the underrepresentation of DNA barcodes in the Mediterranean cannot be attributed to it being a species-poor region. In fact, the Mediterranean is considered species-rich based on morphological data, and it was estimated that there are ca. 277 brown, 657 red and 190 green algae in the Mediterranean Sea, making up 17.3%, 10.6% and 7.6% of the world's taxa respectively (Coll et al. 2010).

DNA barcoding

An ideal DNA barcode contains significant species-level genetic variability, it possesses conserved sites for developing polymerase chain reaction (PCR) primers, and is of an adequate size (approximately 600 to 1000 base pairs) to facilitate DNA sequencing (CBOL Plant Working Group 2009). A 600 base pair (bp) DNA sequence of the 5'-end of

the mitochondrial cytochrome c oxidase subunit 1 gene (*cox*1, COI or COI-5P) in general fits the aforementioned description and was thus accepted as the universal species-level barcode for animals (Kress and Erickson 2012). In fact, the mitochondrial COI together with the plastid large subunit of ribulose-1,5-bisphosphate carboxylase (*rbc*L) are the preferred loci in DNA barcoding of brown and red macroalgae (Saunders and McDevit 2013, Peters et al. 2015) since they discriminate well between species. The preference for the COI biomarker is due to its length which is not too short to hinder detection of species variation, nor is it too long (i.e. when sequencing a longer gene broken into fragments by multiple overlapping primer pairs) to make it expensive and time consuming (Minicante et al. 2014). COI is effective at discriminating even closely related species since it is highly variable, particularly in the third codon position (Hebert et al. 2003). However, COI is not suitable for barcoding green algae due to the presence of introns in this lineage (Pombert et al. 2004, 2006, Saunders and Kucera 2010). As a result, this review is divided into two sections: brown and red algal genes are discussed together, while green algal genes are in a separate section.

COI and *rbc*L barcodes have been used to identify species and to address the taxonomy and classification of the phylum Rhodophyta (Robba et al. 2006, Saunders and McDevit 2013) including for example the genera *Hypnea* (Manghisi et al. 2011, Wolf et al. 2011), *Grateloupia* (Wolf et al. 2014, Yang and Kim 2015), *Pachymeniopsis* (Yang and Kim 2015), *Kintokiocolax* (Yang and Kim 2015), *Kallymenia* (Vergés et al. 2014), *Pyropia* (Minicante et al. 2014), *Gracilaria* (Sfriso et al. 2010, Rueness 2010), *Polysiphonia, Plumaria, Ptilota*, *Antithamnion*, *"Heterosiphonia"* (Rueness 2010), *Gelidium, Gracilariopsis*, *Chondracanthus, Solieria* (Mineur et al. 2012), *Porphyra* (Milstein et al. 2012), *Felicinia* (Manghisi et al. 2014), *Agardhiella* (Manghisi et al. 2010), *Laurencia*

(Machin-Sanchez et al. 2014), *Laurenciella* (Machin-Sanchez et al. 2014), *Sebdenia* (Küpper et al. 2019) and *Nemalion* (Le Gall and Saunders 2010).

The plastid-encoded intergenic RuBisCO spacer is also well suited to be used for red and brown algal barcoding (Brodie et al. 1998, Robba et al. 2006, Ni-Ni-Win et al. 2011). The RuBisCO spacer has been useful in identification of species (Stache-Crain et al. 1997) even though it is less sensitive than COI; it is also sometimes not long enough for phylogenetic analysis (Robba et al. 2006) and may, like the internal transcribed spacer region of the ribosomal cistron ITS, contain indels, which make sequences difficult to align. However, the small size of the non-coding regions of the *rbc*L-*rbc*S spacer region makes them a good marker to amplify from old material (Wolf 2012).

Some other genes which are used in red and brown algae include the D2/D3 variable domains of the nuclear ribosomal large subunit (LSU-D2/D3) which is mostly used as a secondary DNA barcode (Bittner et al. 2008, Saunders and McDevit 2012, Manghisi et al. 2014), the mitochondrial spacer between the cytochrome oxidase subunit 2 and subunit 3 genes (cox 2-3), which has been found useful in red algal population studies since it is more variable (Wolf 2012), and the universal plastid amplicon (UPA), which has less resolution than COI; however, it is useful in separating certain genera such as in the Florideophyceae (Milstein et al. 2012).

The *rbc*L marker is also used in green algal studies, but it is not the best marker in this respect. Saunders and Kucera (2010) studied the applicability of *rbc*L, plastid elongation factor Tu gene (*tuf*A), UPA, LSU-D2/D3 and ITS in many genera to identify the best barcode marker for green algae. They concluded that the plastid elongation factor *tuf*A is applicable as the standard marker for all green algae except for the

identification of Cladophoraceae (Saunders and McDevit 2013). It has been used for genera such as *Caulerpa* (Fama' et al. 2002, Aplikioti et al. 2016) and *Ulva* (Wolf et al. 2012, Lawton et al. 2013, Minicante et al. 2014, Miladi et al. 2018), which are difficult to identify morphologically. However, *rbc*L (Wolf et al. 2012, Saunders and McDevit 2013) and ITS (Lawton et al. 2013) are other options for species-level markers in *Ulva*.

ITS and the nuclear ribosomal 18S subunit (nrSSU) are the preferred genes for *Cladophora* species discrimination (Hayakawa et al. 2012, Taylor et al. 2017) which has been a challenge for DNA barcoding since many biomarkers have not been successful in this genus. ITS is useful in green algal genera and has been used for studying molecular ecology and evolution as well as phylogeny (Hayden and Waaland 2004).

Generally, the choice of barcode locus will depend on which genus is being studied, as well as the question being addressed. This review provides an overview of a list of primers (Table 2) that are currently in use by scientists and that could be applied to regions where DNA barcoding is relatively new.

Metabarcoding

The success of metabarcoding depends on the choice of primers and often involves a trade-off between taxonomic resolution and species amplification (Zhang et al. 2018). Often, however, studies use just one marker in metabarcoding (Hebert et al. 2003, Leray et al. 2013, Schmidt et al. 2013). In contrast, Zhang et al. (2018) used a mock zooplankton community to validate their methods in metabarcoding and concluded that the use of multiplexed markers increased species detection. The use of a single organelle marker could sometimes cause errors in species identification in view of interspecific mitochondrial introgressions (Meyer and Paulay 2005); thus both

uniparentally and biparentally inherited DNA is recommended (Taberlet et al. 2012). Zhang et al. (2018) used the mitochondrial COI gene and nuclear 18S in a single Illumina run since the multiple markers approach can increase detection limits as well as decrease amplification biases. Such studies that test both taxonomic identification accuracy and species detection using multiple primers and markers against a mock community have been scarce so far (Zhang et al. 2018). Oliveira et al. (2018) describe how algal systematics can be advanced through HTS technologies and how species discoveries can be augmented through metabarcoding. Metabarcoding has the potential to help in better estimation of algal biodiversity and distribution of species even if they have not been formally described yet (Oliveira et al. 2018). However, metabarcoding currently cannot be used to characterize new species (Oliveira et al. 2018).

There are two main uses of metabarcoding in ecology and each has its challenges. One involves inferring all species present within an environmental sample and the other comprises checking the absence or presence of a particular set of species of interest (Ficetola et al. 2015). In order to overcome certain shortcomings and challenges of metabarcoding, such as false positives, Ficetola et al. (2015) studied the implications of replicates and how to reduce errors through the number of replicates. However, the challenges associated with metabarcoding can be overcome and, in another study, Ji et al. (2013) compared metabarcoding and standard taxonomy for two conservation issues and they made similar policy decisions. The same study also acknowledged how, in comparison to the classical taxonomical method, metabarcoding was taxonomically more comprehensive, quicker, did not rely on taxonomic experts and was more auditable, which is important in the case of dispute resolution. A possible limitation of

metabarcoding is the lack of data in reference libraries since one can end up with sequences that do not identify with a species name due to a lack of morphological data and information related to life cycles (Danovaro et al. 2016). Moreover, incorrect (and non-updated) identifications in databases may also cause problems.

The benefits of metabarcoding outweigh its shortcomings and thus it is becoming increasingly recognised in biodiversity monitoring programmes (Taberlet et al. 2012, Baird and Hajibabaei 2012, Bohmann et al. 2014, Thomsen and Willerslev 2015, Aylagas et al. 2016). In the microalgal realm, metabarcoding has revealed a high, unexpected diversity of dinoflagellates in aquatic environments (Stern et al. 2010). Moreover, some studies have also started testing the applicability of DNA methods to the EU Marine Strategy Framework Directive (MSFD) with results showing that metabarcoding could provide a more cost-effective and holistic environmental status assessment in European directives (Aylagas et al. 2016, 2017).

Genus and species delimitation in macroalgae

The issue of what comprises a species and what criteria should be used to delineate them has been debated extensively (Leliaert and De Clerck 2017). Identification based solely on morphological features has been problematic for many macroalgae, especially for morphologically simple species and in species that have recently diverged, including complexes of cryptic species (Leliaert and De Clerck 2017). Saunders and McDevit (2012) attribute these challenges in macroalgal identification to simple and convergent morphologies as well as to phenotypic plasticity and variations of heteromorphic generations within life histories in macroalgae. These problems are encountered in the identification of most macroalgae (Saunders 2005, 2008).

Notwithstanding the efforts taken to identify the red algae morphologically and via reproductive structures, many inaccuracies have nonetheless occurred in classification. Likewise, brown algae have been challenging to classify at all levels of taxonomic hierarchy due to their homoplasy (Silberfeld et al. 2014). The same applies for green algae. Genera such as *Ulva* are among the most problematic as they do not have many distinctive features, and as changes in morphology can be induced by environmental factors such as salinity or bacterial metabolites (Provasoli and Pintner 1980, Blomster et al. 1998, Marshall et al. 2006 as cited in Miladi et al. 2018).

Genera of macroalgae with well-known challenges in morphology-based identification include *Ectocarpus* (Leliaert and De Clerck 2017, Montecinos et al. 2017), *Desmarestia* (Yang et al. 2014), *Gracilaria* (Kim et al. 2010), *Ulvella* (Nielsen et al. 2013), *Fucus* (Kucera 2010), *Porphyra/Pyropia* and relatives (Kucera 2010, Koh and Kim 2018), *Ulva* (Kucera 2010, Silva et al. 2013), *Grateloupia* (Gavio and Fredericq 2002) and *Cladophora* (Hayakawa et al. 2012, Taylor et al. 2017) among others. For example, *Ulva* species have been underestimated in the Mediterranean, as a result of cryptic species within this group (Wolf et al. 2012). Additionally, new introductions of *Ulva* species could also go unnoticed because many species are similar morphologically (Melton et al. 2016). Species of *Cystoseira* are also challenging due to their morphological adaptability to different ecological conditions (Ercegović and Herausgeber 1959), so that difficulties arise in distinguishing whether the morphological variation is a different species or whether it is an adaptation (Rožić et al. 2012). Similar issues are also found in the genus *Sargassum* (Mattio and Payri 2011, Amaral-Zettler et al. 2016). *Cladophora* is challenging due to the lack of absolute discontinuities (John and Maggs 1997), and morphological changes associated with algal age and a response to

environmental conditions (van den Hoek 1982). Genetically identical species may also exhibit different morphologies when grown in the same conditions, as with *Acetabularia acetabulum* (Nishimura and Mandoli 1992).

In view of the above challenges in resolving morphological identification of macroalgae, DNA barcoding has become increasingly important in this field. However, despite obvious advantages, criticism of DNA barcoding-based approaches relates especially to species discovery and delimitation, especially those using a single gene (Taylor and Harris 2012). There are evolutionary differences between mitochondrial and nuclear DNA, which means that reliance upon a single gene such as COI will not necessarily reflect nuclear divergence and implies that a genetic divergence taken from only one part of the genome is not an accurate representation of divergence/speciation (Humphries and Winker 2011).

Although single-locus data are effective for a quick identification of species, they are not very effective for species delimitation (Leliaert et al. 2014). In fact, Leliaert and De Clerck (2017) discuss how combining multi-locus data coupled with model-based species delimitation has become increasingly popular.

Metabarcoding suffers the same limitations as DNA barcoding and cannot rely on a single organellar marker since it cannot detect cases of recent hybridisation and organelle introgression (Taberlet et al. 2012). Montecinos et al. (2017), using a nuclear (ITS1) and a mitochondrial (COI) marker, conducted a study on *Ectocarpus* spp. that revealed introgressions among species. Interspecific plastid and mitochondrial introgressions are prevalent in algal genera and species that are evolutionarily young and could result in identification errors (Taberlet et al. 2012, Pawlowski et al. 2018).

Thus, using multiplexed biomarkers of uniparentally (COI) and biparentally inherited DNA (nrRNA genes) is recommended.

DNA barcoding data from the Mediterranean Sea

According to a 'Taxonomy' search conducted in BOLD (12th September 2018), there are 6,182,866 barcoded specimens of animals, plants, fungi and protists from all over the world. BOLD Systems is an online data storage and analysis platform that also mines in public data from GenBank that meet certain criteria. This means that it will mine any data uploaded to an International Nucleotide Sequence Database Collaboration (INSDC) institution which includes GenBank at the National Center for Biotechnology Information (NCBI) together with the DNA DataBank of Japan (DDBJ) and the European Nucleotide Archive (ENA). These three databases exchange data daily (National Center for Biotechnology Information 2011). Presently, NCBI has no reliable way to gather statistics, including terms such as 'Mediterranean', since there is no controlled vocabulary for the collection location (NCBI 2017, personal communication). The advantage of BOLD is that it is a workbench that combines morphological, genetic and distributional data (Ratnasingham and Hebert 2007), even though this is still not comprehensive.

By using the BOLD workbench, the present study focused on the macroalgae of the Mediterranean Sea by creating a polygon around this area (Figure 1) which mined all data referring to GPS coordinates located within the region.

As a result, it was discovered that, in the case of the Rhodophyta, there were only 148 specimens and 117 sequences in the area of interest as compared to a total of 37,316

macroalgal specimens with sequences worldwide.

Results for the Chlorophyta and Phaeophyceae were similar to that for the Rhodophyta and show that, in general, macroalgal DNA barcodes for Mediterranean specimens are underrepresented (Table 3). In fact, there were only 15 specimens and 18 sequences for green macroalgae in the Mediterranean compared to a total of 8,614 specimens with sequences world-wide, and 23 specimens/sequences for brown algae compared to a total of 7,764 worldwide. Another search on the Workbench included a country search, for example, 'Spain' or 'Italy', which also gave sparse results. Therefore, notwithstanding that there are records that lack a GPS reference, it is evident that very little has been published on DNA barcoding of macroalgae in the Mediterranean.

As such, data searches on databases, including BOLD, need to be treated with caution since they do not capture macroalgae that lack GPS or other location data. Taking this disadvantage into consideration, we searched the Taxonomy page on BOLD (Figure 2) and compared other geographical locations, with the result that the above inference was further reinforced. When including other areas outside the Mediterranean, Canada, Australia, the U.S. and France were found to be the leading four countries to report DNA barcodes of Rhodophytes.

DNA barcodes that are not georeferenced are a shortcoming within databases. BOLD requires GPS coordinates for the DNA barcode to gain formal status (Ratnasingham and Hebert 2007). For researchers about to embark on sampling for DNA barcoding, the use of the free smartphone application 'DNA Barcoding Assistant' could facilitate the provision of GPS data (Santschi et al. 2013). In fact, the DNA Barcoding Assistant was created for students in order to streamline, standardize and simplify the data

collection process since it provides an intuitive interface for users to compile records which contain provisional taxonomic identification, digital images, temporal and geospatial data and other collection event details (Santschi et al. 2013). Therefore, using this application also provides a check-list during sampling which ensures that no data are overlooked.

A search of the literature confirmed that the Mediterranean is underrepresented in molecular-systematic papers. Data from 121 papers were analysed, using search criteria which included the term 'Mediterranean' and the names of countries such as 'Italy'. Due to the dearth of Mediterranean sequences, data from papers published up through 2019 were analysed, of which only 65 of the 121 papers contained DNA barcodes of Mediterranean samples. Further analyses show that only 1126 (11.9%) of the 9438 barcodes reported in these papers were Mediterranean (Figure 3). The taxonomic coverage of these barcodes is also relatively small, with only 114 species (45 brown, 42 red, and 27 green) represented.

It was also revealed, from the data analysed, that the majority of DNA barcodes were of algae collected in the USA, Canada, Korea, Australia, Chile and France (Figure 4), which, with the exception of Korea and Chile, was the same result obtained for searches of the BOLD dataset for Rhodophyta.

Another important point concerning Mediterranean data is that the majority (>75%) of DNA barcodes originate from samples collected in Italy, France, Croatia and Spain (Figure 5). This suggests that other Mediterranean countries such as Malta, Greece, Turkey and Cyprus are underrepresented and possibly non-existent when compared to the Mediterranean area in general.

Our conclusion on the situation of sparse DNA data in the Mediterranean also holds true for full-genome sequencing of macroalgae. So far, only a few species of macroalgae have been genome sequenced. These include *Ectocarpus* sp. (Cock et al. 2010), *Saccharina japonica* (Ye et al. 2015), *Cladosiphon okamuranus* (Nishitsuji et al. 2016), *Nemacystus decipiens* (Nishitsuji et al. 2019), *Chondrus crispus* (Collen et al. 2013), *Pyropia yezoensis* (Nakamura et al. 2013), *Gracilariopsis chorda* (Lee et al. 2018), *Porphyra umbilicalis* (Brawley et al. 2017) and *Ulva mutabilis* (De Clerck et al. 2018). However, none of these are from the Mediterranean.

Genetic studies in the Mediterranean have focused on important foundation species such as members of *Cystoseira* (Draisma et al. 2010, Buonomo et al. 2018), some other taxa of unique quality such as the genus *Padina* (Ni-Ni-Win et al. 2011), which is one of the only two genera of calcified Phaeophyceae (Herbert et al. 2016), and on the endangered endemic deep-water kelp *Laminaria rodriguezii* (Žuljević et al. 2016).

Most often, studies from the Mediterranean have been part of larger projects which took into consideration species from other seas, such as the studies on Nemaliales (Le Gall and Saunders 2010) and Ectocarpales (Peters et al. 2015). Yet, rarely, has there been research which focused specifically on the Mediterranean, such as the description of a new red algal genus *Felicinia* which is endemic to the Mediterranean (Manghisi et al. 2014).

DNA barcoding of macroalgae in the Mediterranean has been particularly popular in studies of alien species, such as those shown in Table 4. The reason for this could be that the Mediterranean has been described as being “under siege” by various anthropogenic pressures (Piroddi et al. 2017) and one of the major stressors

associated with the Mediterranean is the infiltration of non-indigenous species. A key contributor to this stressor is the Suez Canal, which is an artificial connection to the Red Sea that was opened in 1869 and further expanded in 2015 (Galil et al. 2015). Many species that entered via the Suez Canal have established themselves and also reached the western Mediterranean, where they compete with local populations. A threat to human health is posed by some species that are venomous (Galil et al. 2015). Moreover, molecular studies have shown that gene flow has occurred in some species between populations of the Red Sea and the Mediterranean (Galil et al. 2015). Tsiamis et al. (2018) provided a synthesis of studies pertaining to non-indigenous species in Europe in relation to species inventories, pathways, gateways, trends, impacts and biological traits, as well as providing the first study on the native distribution range of such species. These authors concluded that the majority of the marine non-indigenous species in Europe (Mediterranean, NE Atlantic Ocean, Black, Baltic Sea), especially molluscs and fish, which are distributed in the Western and Central Indo-Pacific, are associated with the Suez Canal. On the other hand, non-indigenous macroalgal species from the temperate north-west Pacific have been introduced mostly through the importation of oyster spat into the western Mediterranean (Verlaque et al. 2015). Zenetos (2017) showed how the recent expansion of the Suez Canal did not result in the expected major increase in non-indigenous species; so far the reported increases were mostly fish.

Non-indigenous species are of concern in the Mediterranean since it is the most invaded region in the world (Klein and Verlaque 2008, Galil et al. 2018, Zenetos et al. 2017). In fact, some papers based on morphological studies (Balata et al. 2004, Tsiamis et al. 2008, Zenetos et al. 2009, Occhipinti-Ambrogi et al. 2011, Zenetos et al. 2018)

have focused on establishing lists of non-indigenous species or invasive species as well as on their effects on the structure of macroalgal assemblages. Studies on invasive species are important as these play a noticeable role in the receiving ecosystem, including the replacement of keystone species, as well as possible economic impacts (Boudouresque and Verlaque 2002). Macroalgae represent a significant 40% of invasive species documented in the world's oceans (Schaffelke et al. 2006). Excluding foraminifera, phytoplanktonic organisms, cryptogenic and questionable species that could be true aliens, there were 821 established multicellular non-indigenous species reported in the Mediterranean through early 2017 (Zenetos et al. 2017). Moreover, the 100 'Worst Invasives' of non-indigenous species in the Mediterranean have been identified in Streftaris and Zenetos (2006) due to their proliferation as well as their impact on indigenous populations. There are 19 species of macroalgae among these 100 invasive species, including ubiquitous species such as *Caulerpa taxifolia var. distichophylla* (Figure 6).

Conclusion

An extraordinary variation in the physiology, morphology, reproductive systems and genetic content of algae, a polyphyletic group of lineages, is presently recognised. Algae sustain ecosystems as primary producers of energy and also provide many biotechnological and commercial products which are increasingly being studied through 'omics' approaches (Brodie et al. 2017). In this regard, DNA barcoding and metabarcoding are becoming increasingly important and both techniques are promising approaches when it comes to monitoring programmes for algae in the Mediterranean. A reference genetic library of DNA barcodes, coupled with taxonomic

and georeferenced data is required to provide the basis for such mechanisms to be accurate. Additionally, the importance of biotic indices is expected to increase in the coming years due to their applicability in studies on the impacts of climate change (Brodie et al. 2017). In a study that compared the rate of discovery of new species over time, it appears that new species are still being discovered globally (De Clerck et al. 2013). Therefore, sequencing DNA barcodes of currently known species, as well as supporting the discovery and description of new species through DNA barcoding and taxonomy seems to be a good way forward in this field.

Apart from the underrepresentation of DNA barcoding in the Mediterranean, Salonen et al. (2019) also note the prevalence of unexplored environments or sparsely explored habitats such as sediments. The sediments contain eukaryotes that form diverse and complex assemblages (Kim et al. 2016) and DNA-based methods provide the potential to explore these. Therefore, exploring diverse substrates is also required.

This present review has revealed that there are major information gaps for macroalgal studies in the Mediterranean Sea, which need to be addressed for DNA-based methods such as metabarcoding to be successful. Thus, the focus for Mediterranean scientists should be on building an extensive DNA barcode library in order to facilitate easy identification of algae for ecological, legislative and commercial purposes. In this respect, unless the database library is given priority, the Mediterranean will be at a disadvantage with respect to other regions. Research in the Mediterranean area has so far focused on morphological studies, with the genetics of macroalgae being sparsely applied. In this respect, it should be easy to adopt methods applied in other regions as regards DNA extraction protocols together with amplification using well-known primers.

Subsequently, sequencing data must be uploaded to one of the databases which makes part of the International Nucleotide Sequence Database Collaboration (INSDC) institution. This study also reveals that the lack of georeferences of DNA barcodes is a major shortcoming of databases, therefore it is important that scientists provide the correct metadata including provisional taxonomic identification, digital images, temporal and geospatial data and other collection event details (Santschi et al. 2013).

Thus, the collection of algal genetic data from the Mediterranean region, its deposition through public DNA reference libraries and the funding of such research is imperative to help fill in the present knowledge gaps.

Acknowledgments: The research work disclosed in this publication is partially funded by the ENDEAVOUR Scholarship Scheme (Malta)- Group B – National Funds. FCK would also like to thank the UK Natural Environment Research Council (grants NE/D521522/1, NE/J023094/1, *Oceans 2025* / WP 4.5), the TOTAL Foundation (Project 'Diversity of brown algae in the Eastern Mediterranean') and the Marine Alliance for Science and Technology for Scotland (MASTS) pooling initiative, which is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions.

References

Amaral-Zettler, L.A., N.B. Dragone, J. Schell, B. Slikas, L.G. Murphy, C.E. Morrall and E.R. Zettler. 2017. Comparative mitochondrial and chloroplast genomics of a genetically distinct form of *Sargassum* contributing to recent "Golden Tides" in the Western Atlantic. *Ecol. Evol. 7*: 516-525.

Amsler, C.D. and R.B. Searles. 1980. Vertical distribution of seaweed spores in a water column offshore of North Carolina. *J. Phycol. 16*: 617-619.

Andreakis, N., G. Procaccini and W.H. Kooistra. 2004. *Asparagopsis taxiformis* and *Asparagopsis armata* (Bonnemaisoniales, Rhodophyta): genetic and morphological identification of Mediterranean populations. *Eur. J. Phycol. 39*: 273-283.

Andreakis, N., G. Procaccini, C. Maggs and W.H.C.F. Kooistra. 2007. Phylogeography of the invasive seaweed *Asparagopsis* (Bonnemaisoniales, Rhodophyta) reveals cryptic diversity. *Mol. Ecol. 16*: 2285-2299.

Andreakis, N., P. Costello, M. Zanolla, G.W. Saunders and L. Mata. 2016. Endemic or introduced? Phylogeography of *Asparagopsis* (Florideophyceae) in Australia reveals multiple introductions and a new mitochondrial lineage. *J. Phycol.* 52:141-147.

Antolić, B., A. Špan, A. Žuljević and A. Vukovič. 2001. Check list of the benthic marine macroalgae on the eastern Adriatic coast: I. Chlorophyta. *Acta. Adriat. 42*: 43-58.

Antolić, B., A. Špan, V. Nikolić, I. Grubelić, M. Despalatović and I. Cvitković. 2010. A checklist of the benthic marine macroalgae from the eastern Adriatic coast: II. Heterokontophyta: Phaeophyceae. *Acta. Adriat. 51*: 9-33.

Antolić, B., A. Špan, A. Žuljević, V. Nikolić, I. Grubelić, M. Despalatović and I. Cvitković. 2011. A checklist of the benthic marine macroalgae from the eastern Adriatic coast: III. Rhodophyta 1: Ceramiales. *Acta. Adriat. 52*: 67-86.

Antolić, B., A. Špan, A. Žuljević, V. Nikolić, I. Grubelić, M. Despalatović and I. Cvitković. 2013. A checklist of the benthic marine macroalgae from the eastern Adriatic coast: IV. Rhodophyta 2: Ceramiales excluded. *Acta. Adriat. 54*: 41-66.

Aplikioti, M., P. Louizidou, A. Mystikou, M. Marcou, P. Stavrou, S. Kalogirou, K. Tsiamis, P. Panayotidis, et al. 2016. Further expansion of the alien seaweed *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procacini (Ulvophyceae, Bryopsidales) in the Eastern Mediterranean Sea. *Aquat. Invasions 11*: 11-20.

Aylagas, E. 2017. DNA metabarcoding derived biotic indices for marine monitoring and assessment. Universidad del País Vasco-Euskal Herriko Unibertsitatea.

Aylagas, E., Á. Borja, X. Irigoien and N. Rodríguez-Ezpeleta. 2016. Benchmarking DNA Metabarcoding for Biodiversity-Based Monitoring and Assessment. *Frontiers in Marine Science* 3: 96.

Bohmann, K., A. Evans, M.T.P. Gilbert, G.R. Carvalho, S. Creer, M. Knapp, W.Y. Douglas and M. De Bruyn. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* 29: 358-367.

Baird, D.J. and M. Hajibabaei. 2012. Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Mol. Ecol.* 21: 2039-2044.

Balata, D., L. Piazzi and F. Cinelli. 2004. A comparison among assemblages in areas invaded by *Caulerpa taxifolia* and *C. racemosa* on a subtidal Mediterranean rocky bottom. *Mar. Ecol. 25*: 1-13.

Ballesteros E. 1990. Check-list of benthic marine algae from Catalonia (North-West Mediterranean). *Treb. Inst. Bot. Barcelona* 13: 5-52.

Benhissoune, S., C. F. Boudouresque and M. Verlaque. 2001. A check-list of marine seaweeds of the Mediterranean and Atlantic coasts of Morocco. I. Chlorophyceae Wille sl. *Bot. Mar.* 44: 171-182.

Benhissoune, S., C. F. Boudouresque and M. Verlaque. 2002a. A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. II. Phaeophyceae. *Bot. Mar. 45*: 217-230.

Benhissoune, S., C. F. Boudouresque, M. Perret-Boudouresque and M. Verlaque, M. 2002b. A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. III. Rhodophyceae (excluding Ceramiales). *Bot. Mar.* 45: 391-412.

Benhissoune, S., C. F. Boudouresque, M. Perret-Boudouresque and M. Verlaque. 2003. A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. IV. Rhodophyceae-Ceramiales. *Bot. Mar. 46*: 55-68.

Bittner, L., C.E. Payri, A. Couloux, C. Cruaud, B. de Reviers and F. Rousseau. 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol. 49*: 211-226.

Boudouresque, C.F. and M. Verlaque. 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Mar. Pollut. Bull. 44*: 32-38.

Bourlat, S.J., A. Borja, J. Gilbert, M.I. Taylor, N. Davies, S.B. Weisberg, J.F. Griffith, T. Lettieri, et al. 2013. Genomics in marine monitoring: New opportunities for assessing marine health status. *Mar. Pollut. Bull. 74*: 19-31.

Bustamante, D. E., B. Y.Won and T. O. Cho. 2013. *Neosiphonia ramirezii* sp. nov.(Rhodomelaceae, Rhodophyta) from Peru. *Algae 28*(1): 73-82.

Brawley, S.H., N.A. Blouin, E. Ficko-Blean, G.L. Wheeler, M. Lohr, H.V. Goodson, J.W. Jenkins, C.E. Blaby-Haas, et al. 2017. Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *P. Natl. Acad. Sci. U S A. 114*: E6361-E6370.

Brodie, J., P.K. Hayes, G.L. Barker, L.M. Irvine and I. Bartsch. 1998. A reappraisal of *Porphyra* and *Bangia* (Bangiophycidae, Rhodophyta) in the Northeast Atlantic based on the *rbc*L–*rbc*S intergenic spacer. *J. Phycol. 34*: 1069-1074.

Brodie, J., C.X. Chan, O. De Clerck, J.M. Cock, S.M. Coelho, C. Gachon, A.R. Grossman, T. Mock, et al. 2017. The Algal Revolution. *Trends Plant Sci. 22*: 726-738.

Buonomo, R., R.M. Chefaoui, R.B. Lacida, A.H. Engelen, E.A. Serrão and L. Airoldi. 2018. Predicted extinction of unique genetic diversity in marine forests of *Cystoseira* spp. *Mar. Environ. Res. 138*: 119-128.

Carlton, J.T. 2009. Deep invasion ecology and the assembly of communities in historical time. In (Gil Rilov and Jeffrey A. Crooks, eds). *Biological invasions in marine ecosystems,* Springer, Berlin, Heidelberg. pp. 13-56.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *P. Natl. Acad. Sci. U S A. 106*: 12794-12797.

Cianciola, E.N., T.R. Popolizio, C.W. Schneider and C.E. Lane. 2010. Using molecular-assisted alpha taxonomy to better understand red algal biodiversity in Bermuda. *Diversity 2*: 946-958.

Cock, J.M., L. Sterck, P. Rouzé, D. Scornet, A.E. Allen, G. Amoutzias, V. Anthouard, F. Artiguenave, et al. 2010. The Ectocarpus genome and the independent evolution of multicellularity in brown algae. *Nature 465*: 617-621.

Coll, M., C. Piroddi, J. Steenbeek, K. Kaschner, F.B.R. Lasram, J. Aguzzi, E. Ballesteros, C.N. Bianchi, et al. 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLoS* One *5*: e11842.

Collen, J., B. Porcel, W. Carre, S.G. Ball, C. Chaparro, T. Tonon, T. Barbeyron, G. Michel, et al. 2013. Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. *P. Natl. Acad. Sci. U S A. 110*: 5247-5252.

Comtet, T., A. Sandionigi, F. Viard and M. Casiraghi. 2015. DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens. *Biol. Invasions 17*: 905-922.

Cormaci, M., E. Lanfranco, J.A. Borg, S. Buttigieg, G. Furnari, S. Micallef, C. Mifsud, F. Pizzuto, et al. 1997. Contribution to the knowledge of benthic marine algae on rocky substrata of the Maltese Islands (Mediterranean Sea). *Bot. Mar. 40*: 203-216.

Cormaci, M., G. Furnari, M. Catra, G. Alongi and G. Giaccone. 2012. Flora marina bentonica del Mediterraneo: Phaeophyceae. *Bollettino Dell'Accademia Gioenia Di Scienze Naturali Di Catania 45*: 1-508.

Cormaci, M., G. Furnari and G. Alongi. 2014. Flora marina bentonica del Mediterraneo: Chlorophyta. *Bollettino Delle Sedute Della Accademia Gioenia Di Scienze Naturali in Catania 47*: 11-436.

Cormaci, M., G. Furnari and G. Alongi. 2017. Flora marina bentónica del Mediterraneo: Rhodophyta (Rhodymeniophycidae escluse). *Bollettino Accademia Gioenia di Scienze Naturali in Catania*, *50*(380): 1-391.

Danovaro, R., L. Carugati, M. Berzano, A.E. Cahill, S. Carvalho, A. Chenuil, C. Corinaldesi, S. Cristina, et al. 2016. Implementing and innovating marine monitoring approaches for assessing marine environmental status. *Frontiers in Marine Science 3*: 213.

De Clerck, O., M.D. Guiry, F. Leliaert, Y. Samyn and H. Verbruggen. 2013. Algal Taxonomy: A Road to Nowhere? *J. Phycol. 49*: 215-225.

De Clerck, O., S. Kao, K.A. Bogaert, J. Blomme, F. Foflonker, M. Kwantes, E. Vancaester, L. Vanderstraeten, et al. 2018. Insights into the evolution of multicellularity from the sea lettuce genome. *Curr. Biol. 28*: 2921-2933. e5.

De Vargas, C., S. Audic, N. Henry, J. Decelle, F. Mahé, R. Logares, E. Lara, C. Berney, et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science 348*: 1261605.

Deagle, B.E., S.N. Jarman, E. Coissac, F. Pompanon and P. Taberlet. 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biol. Lett. 10*: 20140562.

Díaz-Tapia, P., C. A. Maggs, E. C. Macaya and H. Verbruggen. 2018. Widely distributed red algae often represent hidden introductions, complexes of cryptic species or species with strong phylogeographic structure. *J. Phycol. 54*(6), 829-839.

Dijoux, L., F. Viard and C. Payri. 2014. The more we search, the more we find: discovery of a new lineage and a new species complex in the genus *Asparagopsis*. *PLoS One* 9: e103826.

Draisma, S.G.A., E. Ballesteros, F. Rousseau and T. Thibaut. 2010. DNA sequence data demonstrate the polyphyly of the genus *cystoseira* and other sargassaceae genera (phaeophyceae). *J. Phycol. 46*: 1329-1345.

Durand, C., M. Manuel, C. Boudouresque, A. Meinesz, M. Verlaque and Y. Le Parco. 2002. Molecular data suggest a hybrid origin for the invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta) in the Mediterranean Sea. *J. Evolution. Bio. 15*: 122-133.

Einav, R. and A. Israel. 2008. Checklist of seaweeds from the Israeli Mediterranean: Taxonomical and ecological approaches. *Israel J. Plant Sci. 56*: 127-191.

Ercegović, A. and D. Herausgeber. 1959. Les Facteurs de Sélection et D'Isolement Dans la Genèse de Quelques Espèces D'Algues Adriatiques. *Int. Revue ges. Hydrobiol. Hydrogr. 44*: 473-483.

Fama', P., O. Jousson, L. Zaninetti, A. Meinesz, F. Dini, G. Di Giuseppe, A. Millar and J. Pawlowski. 2002. Genetic polymorphism in *Caulerpa taxifolia* (Ulvophyceae) chloroplast DNA revealed by a PCR-based assay of the invasive Mediterranean strain. *J. Evolution. Bio. 15*: 618-624.

Ficetola, G.F., C. Miaud, F. Pompanon and P. Taberlet. 2008. Species detection using environmental DNA from water samples. *Biol. Lett. 4(4):* 423-5.

Ficetola, G.F., J. Pansu, A. Bonin, E. Coissac, C. Giguet-Covex, M. De Barba, L. Gielly, C.M. Lopes, et al. 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Resour. 15*: 543-556.

Fredericq, S., N. Arakaki, O. Camacho, D. Gabriel, D. Krayesky, S. Self-Krayesky, G. Rees, J. Richards, T. Sauvage, D. Venera-Ponton and W.E. Schmidt. 2014. A dynamic approach to the study of rhodoliths: a case study for the Northwestern Gulf of Mexico. *Cryptogamie, Algol. 35*(1):77-99.

Freshwater, D.W. and J. Rueness. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia 33*:187-94.

Freshwater, D., J. Idol, S. Parham, C. Fernández-García, N. León, P. Gabrielson and B. Wysor. 2017. Molecular assisted identification reveals hidden red algae diversity from the Burica Peninsula, Pacific Panama. *Diversity*, *9*(2), 19.

Furnari, G., G. Giaccone, M. Cormaci, G. Alongi and D. Serio. 2003. Marine Biodiversity of the Italian Coast: Catalogue of the Macrophytobenthos. *Biol. Mar. Mediterr. 10*: 3-482.

Furnari, G., G. Giaccone, M. Cormaci, G. Alongi, M. Catra, A. Nisi and D. Serio. 2010. Macrophytobenthos *Biol. Mar. Mediterr.* 17: 801-828.

Galil, B.S., F. Boero, M.L. Campbell, J.T. Carlton, E. Cook, S. Fraschetti, S. Gollasch, C.L. Hewitt, et al. 2015. 'Double trouble': the expansion of the Suez Canal and marine bio*invasions* in the Mediterranean Sea. *Biol. Invasions 17*: 973-976.

Galil, B.S., A. Marchini and A. Occhipinti-Ambrogi. 2018. East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea. *Estuar. Coast. Shelf S. 201*: 7-16.

Gallardo, T., A.G. Garreta, M. Ribera, M. Cormaci, G. Furnari, G. Giaccone and C.F. Boudouresque. 1993. Check-list of Mediterranean seaweeds. II. Chlorophyceae Wille sl. *Bot. Mar. 36*: 399-422.

Gavio, B. and S. Fredericq. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *Eur. J. Phycol. 37*: 349-359.

Gómez Garreta, A., T. Gallardo, M. Ribera, M. Cormaci, G. Furnari, G. Giaccone and C.F. Boudouresque. 2001. Checklist of Mediterranean Seaweeds. III. Rhodophyceae Rabenh. 1. Ceramiales Oltm. *Bot. Mar. 44*: 425-460.

Guimarães, S. M., L. P. Soares, M. T. Fujii and P. Díaz-Tapia. 2019. Alsidium oliveiranum sp. nov.(Rhodomelaceae, Rhodophyta), an overlooked species from the southwestern Atlantic based on morphology and DNA sequence data. *Algae 34*(3), 187-198.

Guiry, M.D. and G.M. Guiry. 2019. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 27 March 2019.

Hamsher, S.E., K.M. Evans, D.G. Mann, A. Poulícková and G.W. Saunders. 2011. Barcoding diatoms: exploring alternatives to COI-5P. *Protist 162*:405–422

Hanyuda, T., Y. Suzawa, T. Suzawa, S. Arai, H. Sato, K. Ueda and S. Kumano. 2004. Biogeography and taxonomy of Batrachospermum helminthosum (Batrachospermales, Rhodophyta) in Japan inferred from rbcL gene sequences. *J. Phycol. 40*: 581–8.

Hayakawa, Y., T. Ogawa, S. Yoshikawa, K. Ohki and M. Kamiya. 2012. Genetic and ecophysiological diversity of *Cladophora* (Cladophorales, Ulvophyceae) in various salinity regimes. *Phycol. Res. 60*: 86-97.

Hayden, H.S. and J.R. Waaland. 2004. A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia 43*: 364-382.

Hebert, P.D.N., S. Ratnasingham and J.R. DeWaard. 2003. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *P. Roy. Soc. B-Biol Sci. 270*: S96-S99.

Herbert, R.J.H., L. Mal, A. Marston, W.F. Farnham, I. Tittley and R.C. Cornes. 2016. The calcareous brown alga *Padina pavonica* in southern Britain: population change and tenacity over 300 years. *Mar. Biol. 163*: 46.

Hoffman, R., M.J. Wynne, T. Schils, J. Lopez-Bautista and H. Verbruggen. 2017. *Codium pulvinatum* (Bryopsidales, Chlorophyta), a new species from the Arabian Sea, recently introduced into the Mediterranean Sea. *Phycologia 57*: 79-89.

Humphries, E.M. and K. Winker. 2011. Discord reigns among nuclear, mitochondrial and phenotypic estimates of divergence in nine lineages of trans-Beringian birds. *Mol. Ecol. 20*: 573-583.

Iha, C., D. Milstein, S. M. P. Guimarães, D. W. Freshwater and M. C. Oliveira. 2015. DNA barcoding reveals high diversity in the Gelidiales of the Brazilian southeast coast. *Bot. mar. 58*(4), 295-305.

Ji, Y., L. Ashton, S.M. Pedley, D.P. Edwards, Y. Tang, A. Nakamura, R. Kitching, P.M. Dolman, et al. 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecol. Lett. 16*: 1245-1257.

John, D.M. and C.A. Maggs. 1997. Species problems in eukaryotic algae: a modern perspective. *Syst. Assoc. Spec. Vol. 54*: 83-108.

Jongma, D.N., D. Campo, E. Dattolo, D. D'Esposito, A. Duchi, P. Grewe, J. Huisman, M. Verlaque, et al. 2013. Identity and origin of a slender *Caulerpa taxifolia* strain introduced into the Mediterranean Sea. *Bot. Mar. 56*: 27-39.

Jousson, O., J. Pawlowski, L. Zaninetti, A. Meinesz and C. Boudouresque. 1998. Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. *Mar. Ecol. Prog. Ser. 172*: 275-280.

Kawai, H., T. Hanyuda, S.G.A. Draisma and D.G. Müller. 2007. Molecular phylogeny of *Discosporangium mesarthrocarpum* (phaeophyceae) with a reinstatement of the order Discosporangiales. *J. Phycol. 43*: 186-194.

Kher, C.P., F.P. Doerder, J. Cooper, P. Ikonomi, U. Achilles-Day, F.C. Küpper and D.H. Lynn. 2011. Barcoding *Tetrahymena*: Discriminating species and identifying unknowns using the cytochrome c oxidase subunit I (cox-1) barcode. *Protist 162*: 2-13.

Kim, M.S., M.Y. Yang and G.Y. Cho. 2010. Applying DNA barcoding to Korean Gracilariaceae (Rhodophyta). *Cryptogamie Algol. 31*: 387-401.

Kim, E., B. Sprung, S. Duhamel, C. Filardi and M. Kyoon Shin. 2016. Oligotrophic lagoons of the South Pacific Ocean are home to a surprising number of novel eukaryotic microorganisms. *Environ. Microbiol. 18*: 4549-4563.

Klein, J. and M. Verlaque. 2008. The *Caulerpa racemosa* invasion: A critical review. *Mar. Pollut. Bull. 56*: 205-225.

Koh, Y.H. and M.S. Kim. 2018. DNA barcoding reveals cryptic diversity of economic red algae, *Pyropia* (Bangiales, Rhodophyta): description of novel species from Korea. *J. Appl. Phycol.*, *30*: 3425-3434.

Koziol, A., M. Stat, T. Simpson, S. Jarman, J.D. DiBattista, E.S. Harvey, M. Marnane, J. McDonald and M. Bunce. 2019. Environmental DNA metabarcoding studies are critically affected by substrate selection. *Mol. Ecol. Resour. 19(2)*: 366-76.

Kress, W.J. and D.L. Erickson. 2012. DNA barcodes: methods and protocols. In: DNA Barcodes. Humana Press, Totowa, NJ. pp. 3-8.

Kucera, H. 2010. Species identification and discovery in common marine macroalgae: *Fucus*, *Porphyra* and *Ulva* using a DNA barcoding approach. The University of New Brunswick.

Küpper F.C., K. Tsiamis, N. R. Johansson, A. F. Peters, M. Salomidi, L. Manousakis, M. Kallergis, M.H. Graham, B. Kinlan, A. Mystikou, A. Žuljević, V. Nikolić, V. Gerakaris, C. Katsaros, P. Panayiotidis. 2019. New records of the rare deep-water alga *Sebdenia monnardiana* (Rhodophyta) and the alien *Dictyota cyanoloma* (Phaeophyta) and the unresolved case of deep-water kelp in the Ionian and Aegean Seas (Greece). *Bot. Mar. 62*: 577-586

Lane, C.E., S.C. Lindstrom and G.W. Saunders. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Mol. Phylogenet. Evol. 44*: 634-648.

Lawton, R.J., L. Mata, R. de Nys and N.A. Paul. 2013. Algal Bioremediation of Waste Waters from Land-Based Aquaculture Using *Ulva*: Selecting Target Species and Strains. *PLoS One 8*: e77344.

Le Gall, L. and G.W. Saunders. 2010. Establishment of a DNA-barcode library for the Nemaliales (Rhodophyta) from Canada and France uncovers overlooked diversity in the species *Nemalion helminthoides* (Velley) Batters. *Cryptogamie Algol. 31*: 403-421.

Lee, J., E.C. Yang, L. Graf, J.H. Yang, H. Qiu, U. Zelzion, C.X. Chan, T.G. Stephens, et al. 2018. Analysis of the draft genome of the red seaweed *Gracilariopsis chorda* provides insights into genome size evolution in Rhodophyta. *Mol. Biol. and Evol. 35*: 1869-1886.

Leliaert, F. and O. De Clerck. 2017. Refining Species Boundaries in Algae. *J. Phycol. 53*: 12-16.

Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J.M. Lopez-Bautista, G.C. Zuccarello and O. De Clerck. 2014. DNA- based species delimitation in algae. *Eur. J. Phycol. 49*: 179-196.

Leray, M., J.Y. Yang, C.P. Meyer, S.C. Mills, N. Agudelo, V. Ranwez, J.T. Boehm and R.J. Machida. 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front. Zool. 10*: 34.

Machin-Sanchez, M., L. Le Gall, A.I. Neto, F. Rousseau, V. Cassano, A. Senties, M.T. Fujii, J. Diaz-Larrea, et al. 2014. A combined barcode and morphological approach to the systematics and biogeography of *Laurencia pyramidalis* and *Laurenciella marilzae* (Rhodophyta). *Eur. J. Phycol. 49*: 115-127.

Manghisi, A., M. Morabito, C. Bertuccio, L.L.E. Gall, A. Couloux, C. Cruaud and G. Genovese. 2010. Is routine DNA barcoding an efficient tool to reveal introductions of alien macroalgae? A case study of *Agardhiella subulata* (Solieriaceae, Rhodophyta) in Cape Peloro lagoon (Sicily, Italy). *Cryptogamie Algol. 31*: 423-433.

Manghisi, A., C. Bertuccio, S. Armeli Minicante, V. Fiore, L. Le Gall, G. Genovese and M. Morabito. 2011. Identifying alien macroalgae through DNA barcoding: the case of *Hypnea cornuta* (Cystocloniaceae, Rhodophyta). *Transitional Waters Bulletin 5*: 42-49.

Manghisi, A., L.L. Gall, M.A. Ribera, C. Bonillo, G.M. Gargiulo and M. Morabito. 2014. The Mediterranean endemic new genus *Felicinia* (Halymeniales, Rhodophyta) recognized by a morphological and phylogenetic integrative approach. *Cryptogamie Algol. 35*: 221-243.

Mattio, L. and C.E. Payri. 2011. 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *The Botanical Review 77*: 31-70.

McIvor, L., C.A. Maggs, J. Provan and M.J. Stanhope. 2001. *rbc*L sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Mol. Ecol. 10*: 911-919.

Melton J.T.I., L. Collado-Vides and J.M. Lopez-Bautista. 2016. Molecular identification and nutrient analysis of the green tide species *Ulva ohnoi* M. Hiraoka & S. Shimada, 2004 (Ulvophyceae, Chlorophyta), a new report and likely nonnative species in the Gulf of Mexico and Atlantic Florida, USA. *Aquat. invasions 11*: 225–237.

Meyer, C.P. and G. Paulay. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol. 3*: e422.

Miladi, R., A. Manghisi, S. Armeli Minicante, G. Genovese, S. Abdelkafi and M. Morabito. 2018. A DNA barcoding survey of *Ulva* (Chlorophyta) in Tunisia and Italy reveals the presence of the overlooked alien *U. ohnoi*. *Cryptogamie Algol. 39*: 85-107.

Milstein, D., A.S. Medeiros, E.C. Oliveira and M.C. Oliveira. 2012. Will a DNA barcoding approach be useful to identify *Porphyra* species (Bangiales, Rhodophyta)? *J. Appl. Phycol. 24*: 837-845.

Mineur, F., A. Le Roux, H. Stegenga, M. Verlaque and C.A. Maggs. 2012. Four new exotic red seaweeds on European shores. *Biol. Invasions 14*: 1635-1641.

Minicante, S.A., G. Genovese and M. Morabito. 2014. Two new alien macroalgae identified by the DNA barcoding. *Biol. Mar. Mediterr. 21*: 70-72.

Montecinos, A.E., L. Couceiro, A.F. Peters, A. Desrut, M. Valero and M. Guillemin. 2017. Species delimitation and phylogeographic analyses in the *Ectocarpus* subgroup *siliculosi* (Ectocarpales, Phaeophyceae). *J. Phycol. 53*: 17-31.

Nakamura, Y., N. Sasaki, M. Kobayashi, N. Ojima, M. Yasuike, Y. Shigenobu, M. Satomi, Y. Fukuma, K. Shiwaku and A. Tsujimoto. 2013. The first symbiont-free genome sequence of marine red alga, Susabi-nori (*Pyropia yezoensis*). *PLoS one.* 8: e57122.

National Center for Biotechnology Information. 2011. GenBank Overview.

Nielsen, R., G. Petersen, O. Seberg, N. Daugbjerg, C.J. O'Kelly and B. Wysor. 2013. Revision of the genus *Ulvella* (Ulvellaceae, Ulvophyceae) based on morphology and *tuf*A gene sequences of species in culture, with *Acroehaete* and *Pringsheimiella* placed in synonymy. *Phycologia 52*: 37-56.

Ni-Ni-Win, T. Hanyuda, S.G.A. Draisma, G. Furnari, A. Meinesz and H. Kawai. 2011. *Padina ditristromatica* sp nov and *Padina pavonicoides* sp nov (Dictyotales, Phaeophyceae), two new species from the Mediterranean Sea based on morphological and molecular markers. *European J. Phycol. 46*: 327-341.

Nishimura, N. and D. Mandoli. 1992. Population analysis of reproductive cell structures of *Acetabularia acetabulum* (Chlorophyta). *Phycologia 31*: 351-358.

Nishitsuji, K., A. Arimoto, K. Iwai, Y. Sudo, K. Hisata, M. Fujie, N. Arakaki, T. Kushiro, et al. 2016. A draft genome of the brown alga, *Cladosiphon okamuranus*, S-strain: a platform for future studies of 'mozuku' biology. *DNA Res. 23*: 561-570.

Nishitsuji, K., A. Arimoto, Y. Higa, M. Mekaru, M. Kawamitsu, N. Satoh and E. Shoguchi. 2019. Draft genome of the brown alga, *Nemacystus decipiens*, Onna-1 strain: Fusion of genes involved in the sulfated fucan biosynthesis pathway. *Sci. Rep. 9*: 4607.

Nuber, N., O. Gornik, G. Lauc, N. Bauer, A. Zuljevic, D. Papes and V. Zoldos. 2007. Genetic evidence for the identity of *Caulerpa racemosa* (Forsskal) J. Agardh (Caulerpales, Chlorophyta) in the Adriatic Sea. *Eur. J. Phycol. 42*: 113-120.

Occhipinti-Ambrogi, A., A. Marchini, G. Cantone, A. Castelli, C. Chimenz, M. Cormaci, C. Froglia, G. Furnari, et al. 2011. Alien species along the Italian coasts: an overview. *Biol. Invasions 13*: 215-237.

Oliveira, M.C., S.I. Repetti, C. Iha, C.J. Jackson, P. Díaz-Tapia, K.M.F. Lubiana, V. Cassano, J.F. Costa, et al. 2018. High-throughput sequencing for algal systematics. *Eur. J. Phycol. 53*: 256-272.

Ould-Ahmed, N., A.G. Garreta, M.A.R. Siguan and N. Bouguedoura. 2013. Checklist of the benthic marine macroalgae from Algeria. I. Phaeophyceae. *70*: 136-143.

Pascual, M., B. Rives, C. Schunter and E. Macpherson. 2017. Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS One 12*: e0176419.

Pawlowski, J., M. Kelly-Quinn, F. Altermatt, L. Apothéloz-Perret-Gentil, P. Beja, A. Boggero, A. Borja, A. Bouchez, T. Cordier, I. Domaizon, M.J. Feio. 2018. The future of biotic indices in the ecogenomic era: Integrating (e) DNA metabarcoding in biological assessment of aquatic ecosystems. *Sci. Total Environ. 1*: 1295-310.

Peters, A. F., and M. E. Ramírez. 2001. Molecular phylogeny of small brown algae, with special reference to the systematic position of Caepidium antarcticum (Adenocystaceae, Ectocarpales). *Cryptogamie Algol.*, *22*(2), 187-200.

Peters A.F., S.J. Van Wijk, G.Y. Cho, D. Scornet, T. Hanyuda, H. Kawai, D.C. Schroeder, J.M. Cock and S.M. Boo. 2010. Reinstatement of Ectocarpus crouaniorum Thuret in Le Jolis as a third common species of Ectocarpus (Ectocarpales, Phaeophyceae) in Western Europe, and its phenology at Roscoff, Brittany. *Phycol. Res. 58*:157-70.

Peters, A.F., L. Couceiro, K. Tsiamis, F.C. Kuepper and M. Valero. 2015. Barcoding of cryptic stages of marine brown algae isolated from incubated substratum reveals high diversity in Acinetosporaceae (Ectocarpales, Phaeophyceae). *Cryptogamie Algol. 36*: 3-29.

Pezzolesi, L., V. Peña, L. Le Gall, P.W. Gabrielson, S. Kaleb, J.R. Hughey, G. Rodondi, J.J. Hernandez-Kantun, et al. 2019. Mediterranean *Lithophyllum stictiforme* (Corallinales, Rhodophyta) is a genetically diverse species complex: implications for species circumscription, biogeography and conservation of coralligenous habitats. *J. Phycol.*

Piroddi, C., M. Coll, C. Liquete, D. Macias, K. Greer, J. Buszowski, J. Steenbeek, R. Danovaro, et al. 2017. Historical changes of the Mediterranean Sea ecosystem: modelling the role and impact of primary productivity and fisheries changes over time. *Sci. Rep. 7*: 44491.

Pombert, J., C. Otis, C. Lemieux and M. Turmel. 2004. The complete mitochondrial DNA sequence of the green alga *Pseudendoclonium akinetum* (Ulvophyceae) highlights distinctive evolutionary trends in the Chlorophyta and suggests a sister-group relationship between the Ulvophyceae and Chlorophyceae. *Mol. Biol. Evol. 21*: 922-935.

Pombert, J., P. Beauchamp, C. Otis, C. Lemieux and M. Turmel. 2006. The complete mitochondrial DNA sequence of the green alga *Oltmannsiellopsis viridis*: evolutionary trends of the mitochondrial genome in the Ulvophyceae. *Curr. Genet. 50*: 137-147.

Porter, T.M. and M. Hajibabaei. 2018. Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. *Mol. Ecol. 27*: 313-338.

Provan, J., S. Murphy and C.A. Maggs. 2005. Tracking the invasive history of the green alga *Codium fragile* ssp. *tomentosoides. Mol. Ecol. 14*: 189-194.

Ratnasingham, S. and P.D.N. Hebert. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Mol. Ecol. Notes 7*: 355-364.

Ribera, M., A.G. Garreta, T. Gallardo, M. Cormaci, G. Furnari and G. Giaccone. 1992. Check-list of Mediterranean seaweeds. I. Fucophyceae (Warming, 1884). *Bot. Mar. 35*: 109-130.

Robba, L., S.J. Russell, G.L. Barker and J. Brodie. 2006. Assessing the use of the mitochondrial cox1 marker for use in DNA barcoding of red algae (Rhodophyta). *Am. J. Bot. 93*: 1101-1108.

Robideau, G.P., A.W.A.M. De Cock, M.D. Coffey, H. Voglmayr, H. Brouwer, K. Bala, D.W. Chitty, N. Désaulniers, et al. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Mol. Ecol. Resour. 11*: 1002-1011.

Rodríguez-Ezpeleta, N., H. Brinkmann, S.C. Burey, B. Roure, G. Burger, W. Löffelhardt, H.J. Bohnert, H. Philippe, et al. 2005. Monophyly of Primary Photosynthetic Eukaryotes: Green Plants, Red Algae, and Glaucophytes. *Curr. Biol. 15*: 1325-1330.

Rodríguez-Prieto, C., E. Ballesteros, F. Boisset and J. Afonso-Carrillo. 2013. Guía de las macroalgas y fanerógamas marinas del Mediterráneo occidental. *Omega, Barcelona*.

Rožić, S., J. Puizina, I. Šamanić, A. Žuljević and B. Antolić. 2012. Molecular identification of the brown algae, *Cystoseira* spp. (Phaeophycae, Fucales) from the Adriatic Sea–preliminary results. *Acta Adriatica 53*: 447-456.

Rueness, J. 2010. DNA barcoding of select freshwater and marine red algae (Rhodophyta). *Cryptogamie Algol. 31*: 377.

Ruppert, K. M., R. J. Kline and M. S. Rahman. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecol. Conserv.* e00547.

Salonen, I., P. Chronopoulou, E. Leskinen and K. Koho. 2018. Metabarcoding successfully tracks temporal changes in eukaryotic communities in coastal sediments. *FEMS Microbiol. Ecol. 95*: fiy226.

Santschi, L., R.H. Hanner, S. Ratnasingham, M. Riconscente and R. Imondi. 2013. Barcoding Life's Matrix: translating biodiversity genomics into High School settings to enhance life science education. *PloS Biol. 11*: e1001471.

Saunders, G. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philos. T. Roy. Soc. B.* 360: 1879-1888.

Saunders, G.W. 2008. A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity.The foliose *Dilsea-Neodilsea* complex and *Weeksia*. *Botany 86*: 773-789.

Saunders, G.W. and H. Kucera. 2010. An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. *Cryptogamie Algol. 31*: 487-528.

Saunders, G.W. and D.C. McDevit. 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. In: (W. John Kress and David L. Erickson, eds) DNA barcodes. Humana Press, Totowa, NJ. pp. 207-222

Saunders, G.W. and D.C. McDevit. 2013. DNA barcoding unmasks overlooked diversity improving knowledge on the composition and origins of the Churchill algal flora. *BMC Ecol. 13*: 9.

Saunders, G.W. and T.E. Moore. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae*. *28*(1), 31-43.

Schaffelke, B., J.E. Smith and C.L. Hewitt. 2006. Introduced macroalgae - A growing concern. *J. Appl. Phycol. 18*: 529-541.

Schmidt, P., M. Bálint, B. Greshake, C. Bandow, J. Römbke and I. Schmitt. 2013. Illumina metabarcoding of a soil fungal community. *Soil Biol. Biochem.* 65: 128-132.

Ševčíková, T., A. Horák, V. Klimeš, V. Zbránková, E. Demir-Hilton, S. Sudek, J. Jenkins, J. Schmutz, et al. 2015. Updating algal evolutionary relationships through plastid genome sequencing: did alveolate plastids emerge through endosymbiosis of an ochrophyte? *Sci. Rep. 5*: 10134.

Sfriso, A., S. Maistro, C. Andreoli and I. Moro. 2010. First record of *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the Po delta lagoons, Mediterranean Sea (Italy). *J. Phycol. 46*: 1024-1027.

Shabaka, S.H. 2018. Checklist of seaweeds and seagrasses of Egypt (Mediterranean Sea): A review. *The Egyptian Journal of Aquatic Research 44:* 203-212.

Silberfeld, T., F. Rousseau and B.D. Reviers. 2014. An updated classification of brown algae (Ochrophyta, Phaeophyceae). *Cryptogamie Algol.35*: 117-156.

Silva, M., L. Vieira, A.P. Almeida and A. Kijjoa. 2013. The marine macroalgae of the genus *Ulva*: chemistry, biological activities and potential applications. *Journal of Oceanography and Marine Research 1*: 2332-2632.

Stache-Crain, B., D.G. Müller and L.J. Goff. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear-and plastid-encoded DNA sequences. *J. Phycol. 33*: 152-168.

Streftaris, N and A. Zenetos. 2006. Alien marine species in the Mediterranean-the 100 'Worst Invasives' and their impact. *Mediterr. Mar. Sci. 7*:87-118.

Stern, R.F., A. Horak, R.L. Andrew, M.-. Coffroth, R.A. Andersen, F.C. Küpper, I. Jameson, M. Hoppenrath, et al. 2010. Environmental barcoding reveals massive dinoflagellate diversity in marine environments. PLoS *One 5*: e13991.

Stern, R.F., R.A. Andersen, I. Jameson, F.C. Küpper, M.-. Coffroth, D. Vaulot, F. Le Gall, B. Véron, et al. 2012. Evaluating the ribosomal internal transcribed spacer (its) as a candidate dinoflagellate barcode marker. *PLoS One 7*: e42780.

Stuercke, B. and D.W. Freshwater. 2008. Consistency of morphological characters used to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae): analyses of North Carolina, USA specimens. *Phycologia 47*: 541–559.

Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann and E. Willerslev. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol. 21*: 2045-2050.

Taşkin, E. 2008. The check-list of the marine flora of Turkey. Manisa, Turkey.

Taşkın, E., M. Öztürk, O. Kurt and S. Ulcay. 2013. Benthic marine algae in Northern Cyprus (eastern Mediterranean Sea). *Journal of Black Sea/Mediterranean Environment 19*: 143-161.

Taylor, H.R. and W.E. Harris. 2012. An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. *Mol. Ecol. Resour. 12*: 377-388.

Taylor, R.L., J.C. Bailey and D.W. Freshwater. 2017. Systematics of *Cladophora* spp.(Chlorophyta) from North Carolina, USA, based upon morphology and DNA sequence data with a description of *Cladophora subtilissima* sp. nov. *J. Phycol. 53*: 541-556.

Thomsen, P.F. and E. Willerslev. 2015. Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv. 183*: 4-18.

Tsiamis, K., P. Panayotidis and A. Zenetos. 2008. Alien marine macrophytes in Greece: a review. *Bot. Mar. 51*: 237-246.

Tsiamis, K., P. Panayotidis, A. Economou-Amilli and C. Katsaros. 2013. Seaweeds of the Greek coasts. I. Phaeophyceae. *Mediterr. Mar. Sci. 14*: 141-157.

Tsiamis, K., P. Panayotidis, A. Economou-Amilli and C. Katsaros. 2014a. Seaweeds of the Greek coasts. II. Ulvophyceae. *Mediterr. Mar. Sci. 15*: 449-461.

Tsiamis, K., E. Taşkın, S. Orfanidis, P. Stavrou, M. Argyrou, P. Panayotidis, T. Tsioli, B.A. Cicek, et al. 2014b. Checklist of seaweeds of Cyprus (Mediterranean Sea). *Bot. Mar. 57*: 153-166.

Tsiamis, K., A. Zenetos, I. Deriu, E. Gervasini and A.C. Cardoso. 2018. The native distribution range of the European marine non-indigenous species. *Aquat. Invasions.* 13.

Uwai, S., W. Nelson, K. Neill, W.D. Wang, L.E. Aguilar-Rosas, S.M. Boo, T. Kitayama and H. Kawai. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondria genes–origins and succession of introduced populations. *Phycologia.* 45: 687-695.

van den Hoek, C. 1982. The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol. J. Linn. 18*: 81-144.

Vergés, A., D. Gey, J. Utgé, C. Cruaud and L. Le Gall. 2014. Recognition of a new species of *Kallymenia* (Gigartinales, Rhodophyta) from Croatia (Mediterranean Sea) based on morphology and DNA barcode. *Eur. J. Phycol. 49*: 332-344.

Verlaque, M. 2001. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanologica Acta 24*: 29-49.

Verlaque, M., C. Durand, J.M. Huisman, C. Boudouresque and Y. Le Parco. 2003. On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta). Eur. *J. Phycol. 38*: 325-339.

Verlaque, M., S. Ruitton, F. Mineur and C. Boudouresque. 2015. CIESM Atlas of Exotic Species in the *Mediterranean*: Macrophytes.

Voisin, M., C.R. Engel and F. Viard. 2005. Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. *Proc. Natl. Acad. Sci. U. S. A. 102*: 5432-5437.

Wolf, M.A. 2012. Molecular and morphological investigations on seaweed biodiversity and alien introductions in the Adriatic Sea (Mediterranean, Italy). University of Padua.

Wolf, M.A., A. Sfriso, C. Andreoli and I. Moro. 2011. The presence of exotic *Hypnea flexicaulis* (Rhodophyta) in the Mediterranean Sea as indicated by morphology, *rbc*L and *cox*1 analyses. *Aquat. Bot. 95*: 55-58.

Wolf, M.A., K. Sciuto, C. Andreoli and I. Moro. 2012. *Ulva* (Chlorophyta, Ulvales) Biodiversity in the North Adriatic Sea (Mediterranean, Italy): Cryptic species and new introductions. *J. Phycol. 48*: 1510-1521.

Wolf, M.A., A. Sfriso and I. Moro. 2014. Thermal pollution and settlement of new tropical alien species: The case of *Grateloupia yinggehaiensis* (Rhodophyta) in the Venice Lagoon. *Estuar. Coast. Shelf S. 147*: 11-16.

Yang, M.Y. and M.S. Kim. 2015. Taxonomy of *Grateloupia* (Halymeniales, Rhodophyta) by DNA barcode marker analysis and a description of *Pachymeniopsis volvita* sp. nov. *J. Appl. Phycol. 27*: 1373-1384.

Yang, E.C., A.F. Peters, H. Kawai, R. Stern, T. Hanyuda, I. Bárbara, D.G. Müller, M. Strittmatter, et al. 2014. Ligulate *Desmarestia* (Desmarestiales, Phaeophyceae) revisited: *D. japonica* sp. nov. and *D. dudresnayi* differ from *D. ligulata. J. Phycol. 50*: 149-166.

Ye, N., X. Zhang, M. Miao, X. Fan, Y. Zheng, D. Xu, J. Wang, L. Zhou, et al. 2015. *Saccharina* genomes provide novel insight into kelp biology. *Nat. Commun. 6*: 6986.

Yoon, H.S., J.D. Hackett, C. Ciniglia, G. Pinto and D. Bhattacharya. 2004. A Molecular Timeline for the Origin of Photosynthetic Eukaryotes. *Mol. Biol. Evol. 21*: 809-818.

Zanolla, M., M. Altamirano, R. Carmona, J. De la Rosa, V. Souza-Egipsy, A. Sherwood, K. Tsiamis, A.M. Barbosa, A.R. Muñoz and N. Andreakis. 2018. Assessing global range expansion in a cryptic species complex: insights from the red seaweed genus *Asparagopsis* (Florideophyceae). *J. Phycol. 54*: 12-24.

Zenetos, A. 2017. Progress in Mediterranean bioinvasions two years after the Suez Canal enlargement. *Acta Adriatica 58*.

Zenetos, A., M. Pancucci-Papadopoulou, S. Zogaris, E. Papastergiadou, L. Vardakas, K. Aligizaki, A.N. Economou and Aristotle University of Thessaloniki (Greece). 2009. Aquatic alien species in Greece (2009): tracking sources, patterns and effects on the ecosystem. *J. Biol. Res.-Thessalon 12*: 135-172.

Zenetos, A., M.E. Çinar, F. Crocetta, D. Golani, A. Rosso, G. Servello, N. Shenkar, X. Turon, et al. 2017. Uncertainties and validation of alien species catalogues: The Mediterranean as an example. *Estuar. Coast. Shelf S. 191*: 171-187.

Zenetos, A., M. Corsini-Foka, F. Crocetta, V. Gerovasileiou, P.K. Karachle, N. Simboura, K. Tsiamis and M. Pancucci-Papadopoulou. 2018. Deep cleaning of alien and cryptogenic species records in the Greek Seas (2018 update). *Management of Biological Invasions 9*: 209-226.

Zhang, G.K., F.J.J. Chain, C.L. Abbott and M.E. Cristescu. 2018. Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. *Evol. Appl. 11*: 1901-1914.

Zuccarello, G. C., G. Burger, J. A. West and R. J. King. 1999. A mitochondrial marker for red algal intraspecific relationships. *Mol. Ecol. 8*:1443–7.

Žuljević, A., A.F. Peters, V. Nikolić, B. Antolić, M. Despalatović, I. Cvitković, I. Isajlović, H. Mihanović, et al. 2016. The Mediterranean deep-water kelp *Laminaria rodriguezii* is an endangered species in the Adriatic Sea. *Mar. Biol. 163*: 69.

Table 1: Genomic tools and their possible use with respect to Marine Strategy Framework Directive (MSFD) descriptors (source: Aylagas et al. 2016).

Genomic tool	Application to monitoring	MSFD descriptors
Barcoding and Metabarcoding	Community taxonomic characterization	D1, D2, D4, D5, D6
Metagenomics	Community metabolic potential characterization	D1, D2, D4, D5, D6
Metatranscriptomics	Community metabolic activity characterization	D1, D2, D4, D5, D6
Microarrays	Metabolic activity characterization and high-throughput species detection and quantification and gene expression quantification	D2, D5
qPCR	Low-throughput species detection and quantification and gene expression quantification	D2, D5
SNP genotyping	Connectivity assessment and assignment of individuals to populations	D1, D3

Table 2: Some of the primers presently used for barcoding in marine macroalgae.

Gene	Primer name	Sequence	Reference	Length (circa bp)
		Brown algae		
COI	GazF2	CCAACCAYAAAGATATWGGTAC	Lane et al. (2007)	650
	GazR2	GGATGACCAAARAACCAAAA	Lane et al. (2007)	
rbcL + Rubisco spacer	rbcLP2F or rbcL40DF	GAWCGRACTCGAWTWAAAAGTG	Kawai et al. (2007)	>1400
	rbcS139R	AGACCCCATAATTCCCAATA	Peters and Ramirez (2001), Peters et al. (2010)	
rbcL + Rubisco spacer	rbcL1273F	GTGCGACAGCTAACCGTG	Peters et al. (2010)	500
	rbcS139R	As above	Peters et al. (2010)	
Other rbcL primers	rbcL3F	GGCACCGGAGAATCTATATG	Peters and Ramirez (2001)	
	rbcL77F	TGGGNTAYTGGGATGCTGA	Yang et al. (2014)	
	rbcL461F	CTTACTTAAAAACTTTCCAAGG	Peters and Ramirez (2001)	
	rbcLRH3F	TTAAYTCTCARCCDTTYATGCG	Hanyuda et al. (2004)	
	rbcl952R	CATACGCATCCATTTACA	Kawai et al. (2007)	

	P1F	GKGTWATTTGTAARTGGATGCG	Kawai et al. (2007)	
Green algae				
*tuf*A	tufAF	TGAAACAGAAMAWCGTCATTATGC	Fama` et al. (2002), Nielsen et al. (2013)	900
	tufAR	CCTTCNCGAATMGCRAAWCGC	Fama` et al. (2002), Nielsen et al. (2013)	
ITS1+ 5.8S +ITS2	CladoITS-9Fshort	GTCGCTCCTACCGATTGGGTGTG	Hayakawa et al. (2012), Taylor et al. (2017)	945-1100
	CladoITS-7R	TCCCTTTTCGCTCGCCGTTACTA	Hayakawa et al. (2012), Taylor et al. (2017)	
*tuf*A	tufGF4	GGNGCNGCNCAAATGGAYGG	Saunders and Kucera (2010)	800
	tufAR	As above	Saunders and Kucera (2010)	
rbcL-3P	GrbcLFi	TCTCARCCWTTYATGCGTTGG	Saunders and Kucera (2010)	740
	1385R	AATTCAAATTTAATTTCTTTCC	Saunders and Kucera (2010)	
Red algae				
COI	GazF1	TCAACAAATCATAAAGATATTGG	Saunders (2005)	665
	GazR1	ACTTCTGGATGTCCAAAAAAYCA	Saunders (2005)	

COI	GazF2	CCAACCAYAAAGATATWGGTAC (same as above - for browns)	Saunders (2005)	665
	DumR1	AAAAAYCARAATAAATGTTGA	Saunders (2005)	
COI	GWSFn	TCAACAAAYCAYAAAGATATYGG	Le Gall and Saunders (2010), Saunders and McDevit (2012)	665
	GWSRx	ACTTCTGGRTGICCRAARAAYCA	Saunders and McDevit (2012)	
COI	GHalF	TCAACAAATCATAAAGATATYGG	Saunders 2008, Iha et al. 2015	
	GWSFn (could be used instead of GHalF)	As above	Le Gall and Saunders (2010), Guimarães et al. (2019)	665
	Cox1R1	GTATACATATGATGHGCTCAA	Saunders 2008, Guimarães et al. (2019)	
rbcL	F753	GGAAGATATGTATGAAAGAGC	Freshwater and Rueness 1994 as cited in Freshwater et al. (2017)	700
	RrbcSstart	GTTCCTTGTGTTAATCTCAC	Freshwater and Rueness (1994), Freshwater et al. (2017)	
rbcL	F7	AACTCTGTAGAACGNACAAG	Gavio and Fredericq (2002)	F7, R753, F645 and RbcSStart combined

				give 1400bp
	R753	GCTCTTTCATACATATCTTCC	Freshwater and Rueness (1994)	
rbcL	F645	ATGCGTTGGAAAGAAAGATTCT	Lin et al. (2001) as cited in Bustamante et al. (2013)	
	RbcSStart	As above	Lin et al. (2001) as cited in Bustamante et al. (2013)	
rbcL	F2	TGTCTAACTCTGTAGAACAACGGA	Dıaz-Tapia et al. (2018)	1400
	R1452	TGGAGTTTCYA CRAAGTCAGCTGT	Dıaz-Tapia et al. (2018)	
rbcL	F7	As above	Gavio and Fredericq (2002), Dıaz-Tapia et al. (2018)	1400
	RbcSStart	As above	As above	
rbcL	F57	GTAATTCCATATGCTAAAATGGG	Saunders and Moore (2013)	1350
	rbcLrvNEW	ACATTTGCTGTTGGAGTYTC	Saunders and Moore (2013)	
rbcL	F7	As above	As above	800
	R893	GAATAAGTTGARTTWCCIGCAC	Stuercke and Freshwater (2008)	

rbcL	CfD	CCRTTYATGCGTTGGAGAGA	Hamsher et al. (2011), Saunders and McDevit 2012	748 (diatoms)
	DPrbcL7	AARCAACCTTGTGTAAGTCT	Saunders and McDevit (2012)	
Cox2-3	cox2F	GTACCWTCTTTDRGRRKDAAATGTGATGC	Zuccarello et al. (1999)	350-400
	cox3R	GGATCTACWAGATGRAAWGGATGTC	Zuccarello et al. (1999)	
LSU	T01N	GATGACCCGCTGAATTTAAG	Saunders and Moore (2013)	2700
	T15	TGATAGGAAGAGCCGACATCGA	Saunders and Moore (2013)	
LSU	T04	GCAGGACGGTGGCCATGGAAGT	Saunders and Moore (2013)	1500
	T15	As above	Saunders and Moore (2013)	
UPA	p23SrV_f1	GGACAGAAAGACCCTATGAA	Saunders and Moore (2013)	370
	p23SnewR	TCAGCCTGTTATCCCTAGA	Saunders and Moore (2013)	

Table 3: Data obtained from Barcode of Life Data System (BOLD) for macroalgae in the Mediterranean Sea.

Taxon	No. of specimens	No. of sequences	Details	No. of sequences recorded globally
Rhodophyta (Division)	148	117	Florideophyceae (class): 113 Bangiophyceae (class): 35	37,316
Chlorophyta (Division)	15	18	Ulvophyceae (class): 13 Trebouxiophyceae (class): 2	8,614
Phaeophyceae (Class)	23	23	Dictyotales (order): 12 Ectocarpales (order): 5 Sphacelariales (order): 4 Cutleriales (order): 2	7,764

Table 4: List of DNA barcoding studies on non-indigenous macroalgal species in the Mediterranean Sea.

Green algae	**Red algae**
Ulva ohnoi (Minicante et al. 2014, Miladi et al. 2018)	*Pyropia yezoensis* (Minicante et al. 2014)
Caulerpa racemosa var. *cylindracea* (Durand et al. 2002, Verlaque et al. 2003, Nuber et al. 2007, Klein and Verlaque 2008)	*Hypnea cornuta* (Manghisi et al. 2011)
Caulerpa taxifolia (Jousson et al. 1998, Fama' et al. 2002)	*Hypnea flexicaulis* (Wolf et al. 2011)
Caulerpa taxifolia var. *distichophylla* (Jongma et al. 2013, Aplikioti et al. 2016)	*Asparagopsis taxiformis* (Andreakis et al. 2007)
Codium pulvinatum (Hoffman et al. 2017)	*Agardhiella subulata* (Manghisi et al. 2010).

((captions))

Figure 1: Polygon used to delineate the Mediterranean Sea whilst searching for records in the Barcode of Life Data System (BOLD).
Source: http://www.boldsystems.org/

Figure 2: Rhodophyta data by country from Taxonomy page on the Barcode of Life Data System (BOLD).
Source: http://www.boldsystems.org/index.php/TaxBrowser_Home

Figure 3: Total number of DNA barcodes obtained from the literature-based results of 121 papers.

Figure 4: DNA barcoding by country obtained from the literature-based results of 121 papers: USA, Canada, Korea, Australia, Chile and France lead in the number of barcodes world-wide.

Figure 5: DNA barcoding of macroalgae by Mediterranean country: Italy, France, Croatia, Spain and Greece lead in the literature-based results.

Figure 6: Pinnate fronds of *Caulerpa taxifolia* var. *distichophylla* growing among the white flabellate blades of *Padina* sp.
Photo taken in Maltese waters in June 2017.

Figure 1: Polygon used to delineate the Mediterranean Sea whilst searching for records in the Barcode of Life Data System (BOLD). Source: http://www.boldsystems.org/

80x45mm (1200 x 1200 DPI)

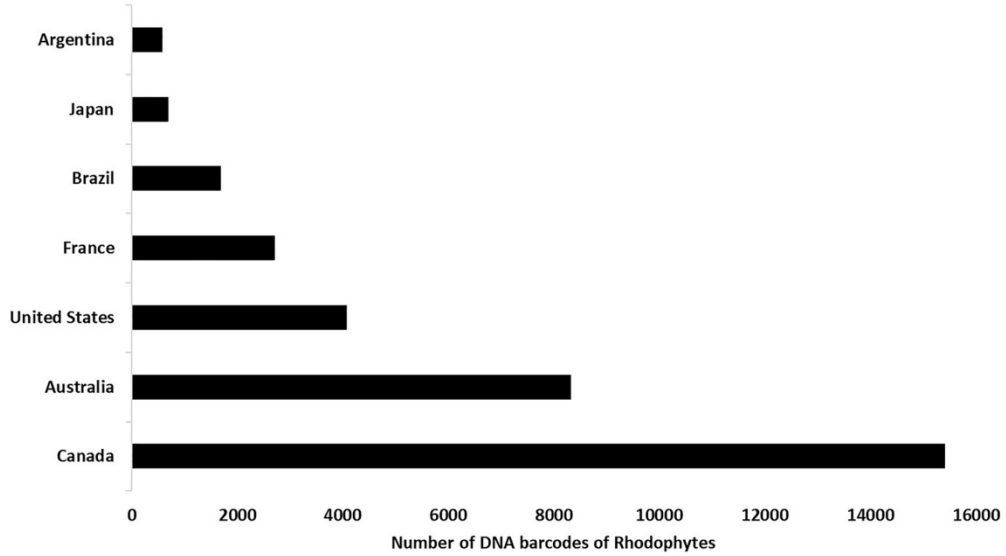

Figure 2: Rhodophyta data by country from Taxonomy page on the Barcode of Life Data System (BOLD). Source: http://www.boldsystems.org/index.php/TaxBrowser_Home

80x46mm (600 x 600 DPI)

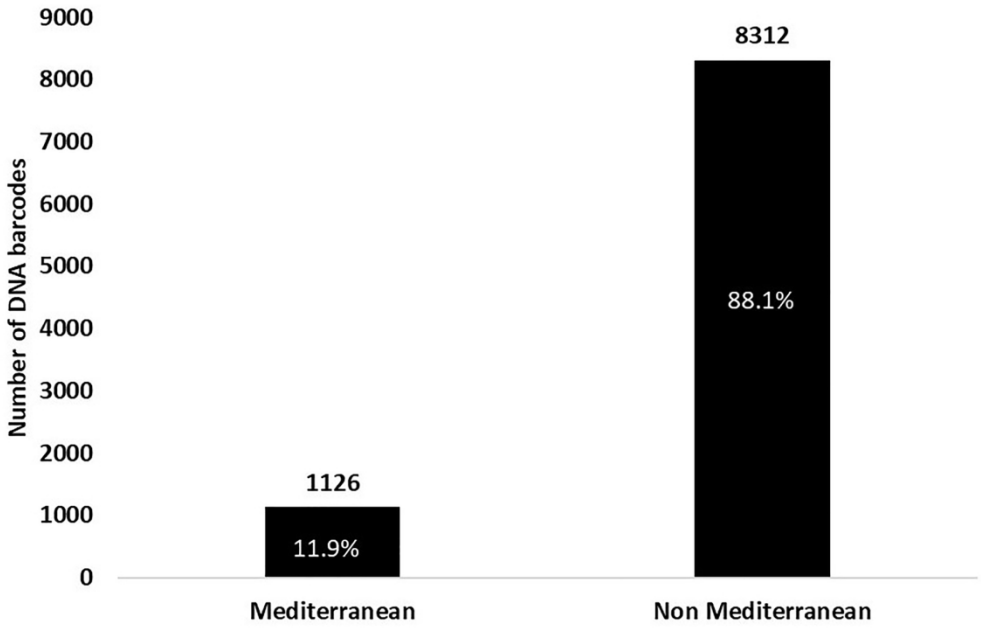

Figure 3: Total number of DNA barcodes obtained from the literature-based results of 121 papers.

79x50mm (600 x 600 DPI)

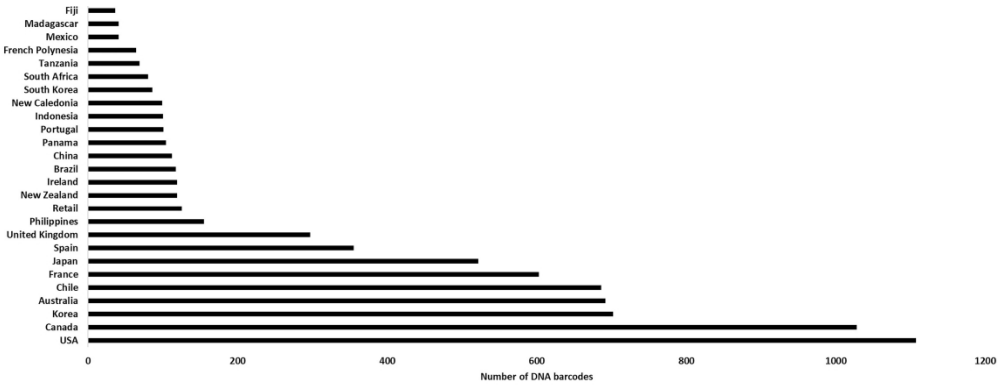

Figure 4: DNA barcoding by country obtained from the literature-based results of 121 papers: USA, Canada, Korea, Australia, Chile and France lead in the number of barcodes world-wide.

164x63mm (600 x 600 DPI)

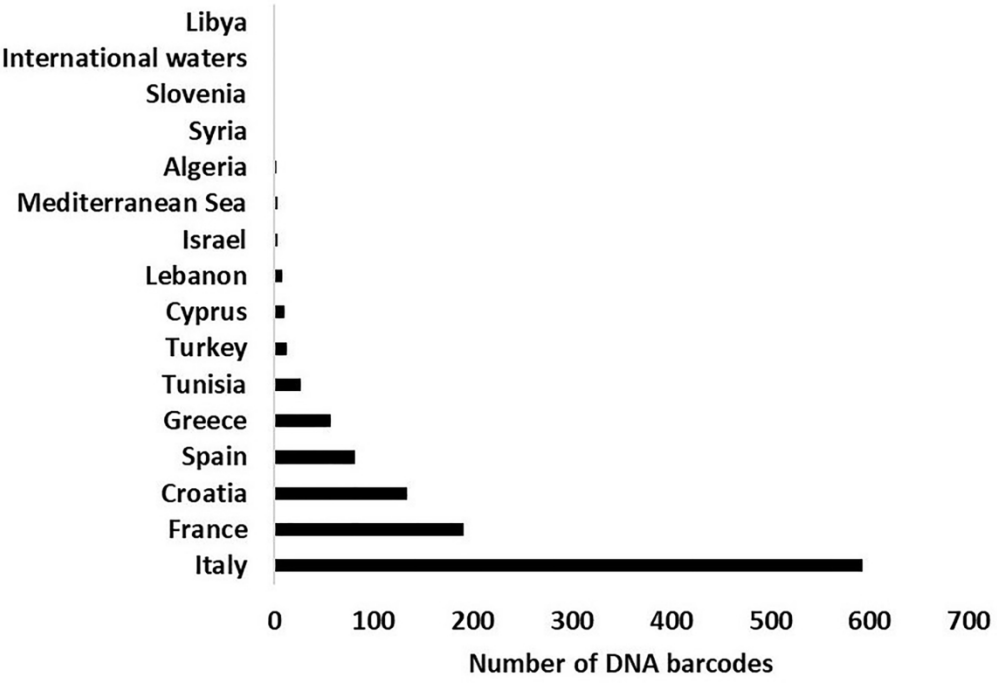

Figure 5: DNA barcoding of macroalgae by Mediterranean country: Italy, France, Croatia, Spain and Greece lead in the literature-based results.

79x57mm (600 x 600 DPI)

Figure 6: Pinnate fronds of Caulerpa taxifolia var. distichophylla growing among the white flabellate blades of Padina sp. Photo taken in Maltese waters in June 2017.

80x90mm (300 x 300 DPI)

The current state of DNA barcoding of macroalgae in the Mediterranean Sea: presently lacking but urgently required

Angela G. Bartolo, Gabrielle Zammit, Akira F. Peters and Frithjof C. Küpper

Review: The Mediterranean Sea is an understudied region as regards the genetic barcoding of macroalgae, with less than 10% of the marine macroalgae of the Mediterranean having been barcoded, with important implications for future DNA metabarcoding efforts in this region.

Keywords: algae; barcoding; Chlorophyta; Phaeophyceae; Rhodophyta.

80x90mm (300 x 300 DPI)

80x79mm (300 x 300 DPI)

78x108mm (300 x 300 DPI)

Frithjof C. Küpper, Chair in Marine Biodiversity at the University of Aberdeen, is studying the biodiversity and biochemistry of marine plants/algae. His research found that iodide serves as an inorganic antioxidant in kelp, the first known from a living system, impacting atmospheric and marine chemistry. A certified scientific diver, Frithjof has worked in the Mediterranean, South Atlantic (Ascension and Falklands), but also in the Antarctic and the Arctic for algal diversity-related projects.

Angela G. Bartolo is a PhD student at the University of Aberdeen in the division of Marine Biology. Her research addresses DNA barcoding in Malta. She holds a dual Master's degree MSc/MS from the University of Malta and James Madison University (Virginia) where she focused her research on sea water quality through the use of ecological indicators. Angela also works on the EU Marine Strategy Framework Directive and Water Framework Directive at the Environment and Resources Authority in Malta.

Gabrielle Zammit is a member of the academic staff of the University of Malta. For the past fifteen years, she has lectured and supervised students enrolled in various undergraduate and postgraduate degrees both in Malta and abroad. She has also carried out research in phycology in a number of European institutions and is especially interested in phototrophic biofilms and the systematics of Maltese cyanobacteria and algae, of which she has described genera and species that are new to science.

Akira F. Peters has a PhD from Konstanz University, Germany, is director of Bezhin Rosko (www.bezhinrosko.com) and lives near Roscoff in Brittany, France. He is honorary research fellow of Aberdeen University, Scotland, UK. Since 1980 he has worked on life histories, taxonomy, phylogenetics, ecology, pathology, genetics, development, cultivation and utilisation of macroalgae, principally on browns. His main techniques are isolation, purification and laboratory cultivation of seaweed microstages.

Frithjof C. Küpper, Chair in Marine Biodiversity at the University of Aberdeen, is studying the biodiversity and biochemistry of marine plants/algae. His research found that iodide serves as an inorganic antioxidant in kelp, the first known from a living system, impacting atmospheric and marine chemistry. A certified scientific diver, Frithjof has worked in the Mediterranean, South Atlantic (Ascension and Falklands), but also in the Antarctic and the Arctic for algal diversity-related projects.