Research Article

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Assessment of the macroalgal diversity of Kuwait by using the Germling Emergence Method

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Cryptic stages of diverse macroalgae present in natural substrata, "the bank of microscopic forms", were isolated into clonal cultures and identified based on both morphological characteristics and DNA barcoding. Approximately 120 clonal isolates from 308 natural substratum samples were collected from the entire coastline of Kuwait. Amongst these isolates, 77 (64%) were identified through DNA barcoding using the nuclear ribosomal small subunit, RuBisCO spacer (ITS2, *tufa*, *rbc*L, *psa*A, and *psb*A) and sequencing. Twenty-six isolates (34%) were identified in the division Chlorophyta, 18 (23%) as Phaeophyceae, and 33 (43%) as Rhodophyta. For all DNA sequences in this study, species-level cut off applied was ≥98% homology which depend entirely on the markers used. Three putative new records of Chlorophyta new for the Arabian Gulf were made: *Cladophora laetevirens* (Dillwyn) Kützing, *Ulva torta* (Mertens) Trevisan and *Ulvella leptochaete* (Huber) R. Nielsen, C. J. O'Kelly & B. Wysor in Nielsen, while *Cladophora gracilis* Kützing and *Ulva ohnoi* M. Hiraoka & S. Shimada are new records for Kuwait. For Phaeophyceae, *Ectocarpus subulatus* Kützing and *Elachista stellaris* Areschoug were new records for the Gulf and Kuwait. In the Rhodophyta, *Acrochaetium secundatum* (Lyngbye) Nägeli in Nägeli & Cramer, *Ceramium affine* Setchell & N. L. Gardner, *Gelidium pusillum* var. *pakistanicum* Afaq-Husain & Shameel and *Dasya caraibica* Børgesen are new records for the Gulf and Kuwait, while the red alga *Stylonema alsidii* (Zanardini) K. Drew is a new record for Kuwait. Several isolates identified corresponded to genera not previously reported in Kuwait and / or the Arabian Gulf, such as *Porphyrostromium* Trevisan, a new genus from the Bangiales, and two unidentified species for the Planophilaceae Škaloud & Leliaert. The isolates cultivated from substrata enhance understanding of the marine macroalgal diversity in the region and confirmed that the Germling Emergence Method is suitable for determining the actual diversity of a given study area through isolation from cryptic life-history phases.

Keywords: Chlorophyta; cryptic stages; diversity; DNA barcoding; Germling Emergence; Phaeophyceae; Rhodophyta

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INTRODUCTION

Globally, numerous shorelines are yet to be explored for benthic marine algal diversity (Wynne et al. 2020). Marine algae are important shelter or habitats for sessile - micro- and macro-organisms and are the basis of food webs in marine ecosystems (Wolf 2012, Macreadie et al. 2017). However, only approximately 10% of algal diversity have been discovered and formally described, with a lack of knowledge especially in remote regions (De Vargas et al. 2015, Bartolo et al. 2020). Seaweeds produce reproductive cells or multicellular propagules at maturity, many of which will attach to available substrata and, after a period of time, form a "bank of algal microscopic forms" (Hoffmann and Santelices 1991, Peters et al. 2015, Schoenrock et al. 2021). However, these cryptic life forms are difficult to capture and characterize.

In the last 20–30 years, the development of *in vitro* culture techniques for isolates from incubated natural substrata, such as small pebbles and sand grains collected during fieldwork or diving surveys, has helped in the investigation of macroalgal microscopic stages from remote and under sampled areas (Ramírez and Müller 1991, Müller and Ramírez 1994, Peters et al. 2015). The Germling Emergence (GE) technique has the potential to discover cryptic species and new records of macroalgal taxa by a combination of morphological characterization with DNA barcoding. Comparing short DNA barcodes with available reference sequences in public databases can lead to accurate identification of cultured microscopic species or microstages that can be difficult to classify morphologically (Peters et al. 2015, Bartolo et al. 2020). For example, Zuccarello et al. (2011) and West et al. (2012) reviewed the order Erythropeltidales (Rhodophyta) using a combination of the GE method and molecular data that enabled new records and discoveries of macroalgal taxa around the world. These discoveries included, for example, a novel *Desmarestia* species and a number of Ectocarpales species from the Canadian Arctic (Küpper et al. 2016), two novel members of the Pelagophyceae, *Sungminbooa kuepperi* R. A. Andersen & B. Melkonian from the Beagle Channel, Tierra del Fuego, and *Chrysoreinhardia muelleri* M. Melkonian & R. A. Andersen from the Falklands (Han et al. 2018). Peters et al. (2015) used the GE method combined with 5′-cytochrome oxidase (COI) barcoding to detect cryptic diversity (including three putative new species) within Ectocarpales collected from different geographical regions. Additionally, in circalittoral waters (24 m depth) of the Mediterranean Sea, the GE method succeeded in isolating the minute benthic multicellular alga *Schizocladia ischiensis*; thus, this method appears to reveal benthic algae even when small in size (Rizouli et al. 2020). In another study, isolates of the genus *Ectocarpus* and associated bacterial strains from Hopkins River in Australia, obtained using the GE method, provided the possibility to study *Ectocarpus* adaptations to abiotic parameters - especially low salinity and the interactions of the alga with the endogenous microbiome (Dittami et al. 2020*b*). Recently, the GE method enabled the description of a new species of red alga from the Celebes Sea, *Hypoglossum sabahense* (Wynne et al. 2020).

The aim of the work presented here was to identify algal isolates from a collection of microscopic forms from different locations in Kuwait, obtained using the GE technique. A few additional algal cultures were obtained from fragments or reproductive cells of macroscopic algae. Molecular identification and establishment of gene phylogenies were carried out using commonly employed markers, for which there were sufficient reference sequences in public databases. The mitochondrial COI is a useful barcode marker since it discriminates well between species and it has become a preferred locus in DNA barcoding of brown and red macroalgae (Saunders and McDevit 2013). The plastid-encoded large and small subunits of ribulose-1,5-bisphosphate carboxylase (*rbc*L and *rbc*S, respectively) are separated by a spacer, which is variable in length (Destombe and Douglas 1991). *rbc*L is also among the preferred loci for phylogenetic studies and DNA barcoding of brown macroalgae (Siemer et al. 1998, Saunders and McDevit 2013), although it is slightly less discriminatory than COI between species of Phaeophyceae . The RuBisCO spacer is highly variable and cannot serve for phylogenetic analyses because of limited alignability across species and genera. Sequences of the spacer, however, are occasionally published together with *rbc*L sequences and can be used as barcodes for the identification of species (Siemer et al. 1998).

MATERIALS AND METHODS

Field sites, specimens, and laboratory culture

Samples were collected during late winter and spring (February and April 2019), from 38 sampling points (sites) in 12 localities representing the different maritime regions from Northern and Southern Provinces in Kuwait including offshore islands (Fig. 1). Of the 308 total individual samples, 261 (85%) were abiotic (small fragments

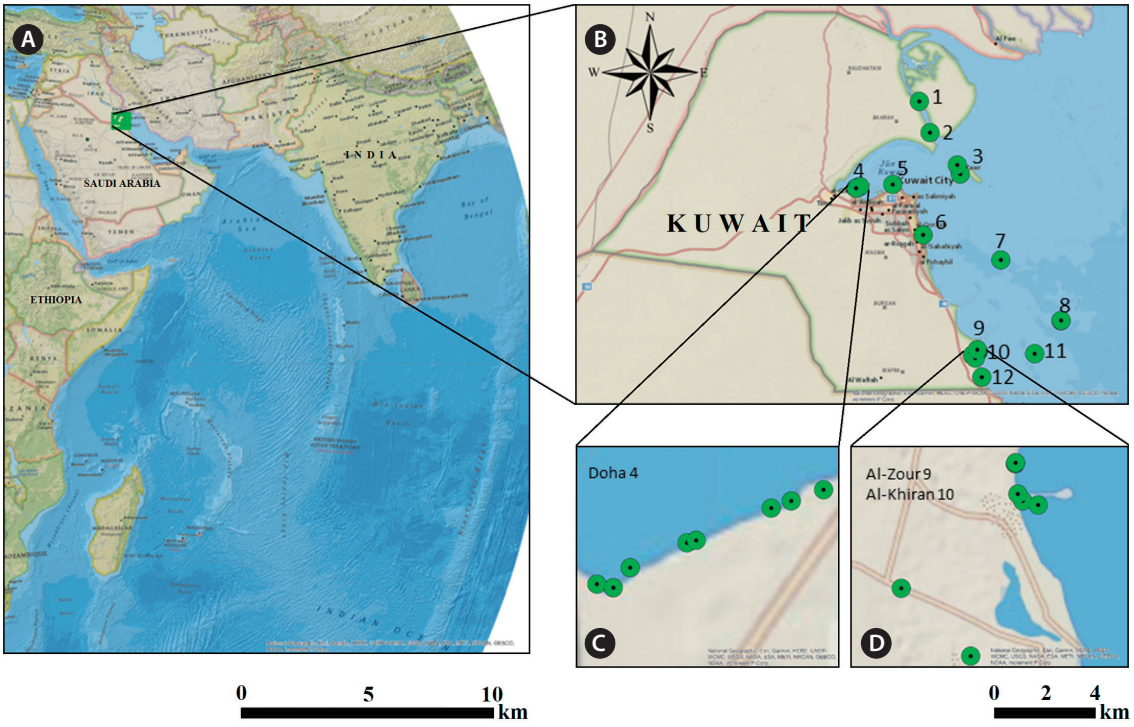

Fig. 1. Localities and sites sampled. (A) General location of Kuwait in the northwestern of the Arabian Gulf. (B) Map of Kuwait represents 1–12 sampling points including shoreline and off shore Islands namely; Bubiyan, Sabiya, Failaka, Doha, Kuwait City Sharq, Fintas, Kubbar, Qaruh, Al-Zour, Al-Khiran, Umm Al-Maradim, and Nuwaiseeb, respectively. Subset of maps showing sample locations at (C) Doha 4 and (D) Al-Zour 9 and Al-Khiran 10 sites (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

of rock, or pebbles) while 47 (15%) were biotic substrata (fragments of subtidal macroflora, such as seagrass leaves and *Sargassum* blades). Generally, samples were collected from each sector at 3 transects; upper (closest to the shore) middle, lower intertidal zone and rocky shore at each location during low tide. Furthermore, substratum samples were collected from several of the islands of Kuwait by SCUBA diving in the shallow subtidal zone around Failaka and Umm al-Maradim (15 m), Qaruh (6 m), and Kubbar (10–35 m) (Supplementary Table S1). Also, the sampling were covered various distances from brine outfalls at typical two desalination plants, namely; Doha East (DE) and Al-Zour South (ZS). Triplicate samples of natural substrata were placed in 15 mL FALCON tubes previously filled with 10 mL heat-sterilized sea water, before transport to the Bezhin Rosko Laboratory of AFP (Santec, France). There, the contents of each tube were transferred to 55 mm diameter Petri dishes containing 8 mL Provasoli-enriched natural autoclaved sea water culture medium (Provasoli 1966, Starr and Zeikus 1993, Tarakhovskaya et al. 2012) and incubated at 15°C in natural daylight at a north-facing window for 2–6 weeks. In order to prevent diatom growth, germanium dioxide (GeO_2, 3–4 mg L^{-1}) was added to the medium (Peters et al. 2015). After three to four weeks, unialgal isolates were obtained from the dishes by cutting accessible parts of germlings with a sharp, freshly snapped, glass Pasteur pipet tip and transferred into fresh culture medium without GeO_2 (Lewin 1966). The laboratory culture followed standard methods (Coelho et al. 2012), with monthly serial transfer. The temperature was maintained at a constant 25 ± 1°C in a CO_2 incubator (240 L MIR-253-CFC FREE; Sanyo, UK), irradiance was by dimmed natural light (less than 40 µmol m^{-2} s^{-1} photon fluence rate) measured with a Skye Instruments SKP 200 light meter (Skye Instruments Ltd., Llandrindod Wells, Wales, UK) and daylengths were based on the natural change. To minimize duplicate isolation of the same species, multiple isolates were made from each dish only when algae exhibited different morphologies. Strains that were not single cultures after a further two months of cultivation were excluded from subsequent analyses. The February and April isolates were deposited in collections at the University of Aberdeen, Scotland, UK, in the Bezhin Rosko Laboratory (France) and in the Algal Culture Unit (KUAC) at Kuwait University, Kuwait.

Molecular work and phylogenetic analyses

DNA was extracted from 5–15 mg wet weight of algal cultures using CTAB, and the GENEJET Plant Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's protocol (Gachon et al. 2009). Extracted DNA from the unialgal isolates was amplified by polymerase chain reaction (PCR) and sequenced. First, the nuclear ribosomal small subunit nrSSU locus was used to confirm that the extracted DNA was suitable for amplification. However, this marker did not discriminate sufficiently between species, and barcode markers with higher resolution were used for final identification (Bartolo et al. 2020). Therefore, several markers were used together with the nrSSU locus to obtain species-level identification. Internal transcribed spacer 1 (ITS1), internal transcribed spacer 2 (ITS2), and plastid elongation factor (*tuf*A) locus used for Chlorophyta, the partial mitochondrial gene regions (5'COI) to examine Phaeophyceae and plastid-encoded *psa*A, *psb*A gene and plastid locus, such as RuBisCO spacer region *rbc*L were used for Rhodophyta (Table 1). Two markers were used for isolates of Chlorophyta, the plastid-encoded DNA barcode marker elongation factor *tufa* and the nuclear ITS2. *tuf*A has been used to discriminate amongst green algal species (Kirkendale et al. 2013) and in an evaluation of barcode markers for marine green macroalgae (Saunders and Kucera 2010), this primer had high levels of discriminatory power between species. The second marker, ITS2, has also been widely used in species-level phylogenetic studies of green algae (Lawton et al. 2013).

PCR products were Sanger sequenced by a commercial service (Source Biosciences, Cambridge, UK). Consensus sequences were aligned using the software BioEdit Editor (Hall et al. 2010) and sequences had a higher homology than 98% identity were compared to published data by using NCBI BLAST searches (http://www.ncbi.nlm.nig.gov) (Altschul et al. 1997). Sequence alignments were done by using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) statistical method with the software MEGA X 11.0.11 (http://www.megasoftware.net) (Edgar 2004, Kumar et al. 2018).

RESULTS

Field collections and algal isolates

We obtained 345 unialgal macroalgal clones from 308 substrate samples (i.e., an average of 1.12 strains per sample). One hundred and twenty clonal isolates were included in this study. Out of these 120 clones, 84 (70%) were obtained using the GE method, while 35 (29%) were isolated directly from macroscopic thalli collected in the field and 1 strain (0.8%) was obtained in culture by fertilization of fertile cells *in vitro* (settled zoids) (Supplementary Table S1). Amongst the 120 isolates, 87 (72%) strains were collected in the vicinity of the DE and ZS desalina-

Table 1. List of oligonucleotide primers of amplification used to amplify different fragments of DNA from genomes of algal isolates

Locus	Target DNA	Primer name	Sequence (5′ to 3′)	Fragment size (bp)	Reference
Nuclear	nrSSU	NS1F	GTAGTCATATGCTTGTCTC	1,100	White et al. (1990)
		NS4R	CTTCCGTCAATTCCTTTAAG		
	ITS1	AFP4LF	CAATTATTGATCTTGAACGAGG	1,056	Peters et al. (2004)
		5.8S1R	TGATGATTCACTGGATTCTG		
	ITS2	KP5F	ACAACGATGAAGAACGCAG	600	Lane et al. (2006), Hodge et al. (2010)
		KG4R	CTTTTCCTCCGCTTAGTTATATG		
Plastid elongation factor	*tuf*A	*tuf*AF	TGAAACAGAAMAWCGTCATTATGC	850	Famà et al. (2002)
		*tuf*AR	CCTTCNCGAATMGCRAAWCGC		
Chloroplast	*psb*A	*psb*A1F	ATGACTGCTACTTTAGAAAGACG	1,000	Yoon et al. (2002)
		*psb*A1R	GCTAAATCTARWGGGAAGTTGTG		
	*psa*A	*psa*A130F	AACWACWACTTGGATTTGGAA	900	Yoon et al. (2002)
		*psa*A970R	GCYTCTARAATYTCTTTCA		
Mitochondrial	5′COI	GazF2	CCAACCAYAAAGATATWGGTAC	~700	Saunders (2005)
		GazR2	GGATGACCAAARAACCAAA		
Plastid	RuBisCO spacer	RH3F	AGCCCCATCACGATGCAGTT	1,000	Peters and Ramírez (2001)
		*rbc*S139R	AGACCCCATAATTCCCAATA		
		*rbc*LP2F	GAWCGRACTCGAWTWAAAAGTG	950	Kawai et al. (2007)
		*rbc*S952R	CATACGCATCCATTTACA		
		*rbc*L1273F	GTGCGACAGCTAACCGTG	~400–600	Peters and Ramírez (2001)
		*rbc*S139R	AGACCCCATAATTCCCAATA		
	*rbc*L	KitoF1	ATGTCTCAATCCGTAGAATCA	1,600	Kunimoto et al. (1999), Broom et al. (2010)
		JrSR	AAGCCCCTTGTGTTAGTCTCAC		

tion plants (April 2019) and the other 33 (28%) were collected from different sites on the Kuwait coast (February 2019).

DNA sequences of isolates obtained by Germling Emergence

Of the final 120 isolates selected for molecular identification, the majority were Rhodophyta (n = 51/120 strains; 42%), with the most commonly identified species belonging to the Erythrotrichiaceae G. M. Smith, especially *Erythrotrichia* Areschoug sp., and Chlorophyta (n = 45/120 strains; 38%) were present in smaller numbers, with many independent replicates of *Ulva tepida* Y. Masakiyo & S. Shimada (KT374006; described from Australia by Masakiyo and Shimada 2014). Phaeophyceae (n = 24/120 strains; 20%) were present in still smaller numbers, the most frequently identified species being a close relative of *Feldmannia mitchelliae* (Harvey) H. -S. Kim sensu lato (s.I) (AB302306; described from Japan by Tanaka et al. 2010). Only for 77 out of the 120 isolates, PCR-friendly DNA could be extracted (64%). Of the remaining 43 strains, 17 showed weak PCR amplification which suggested an imperfect match of PCR primers and 14 strains were only identified morphologically (*Ulva tepida*), but for 12 strains no PCR amplification was achieved.

Based on 130 DNA sequences, using the nuclear ITS2 locus and the chloroplast-encoded *tuf*A primers for Chlorophyta, 26 of the 77 extracted isolates yielding PCR-friendly DNA (corresponding to 34%, representing 17 species) were identified. In the Phaeophyceae, several sequences were obtained of chloroplast *psa*A, mitochondrial COI, *rbc*L and RuBisCO spacer (18 of the 77 extracted isolates yielding PCR-friendly DNA, i.e., 23%, representing 14 species). In the Rhodophyta, *rbc*L and *psb*A sequences are used to identify 33 out of the 77 isolates with PCR-friendly DNA (43% of the total, representing 26 species). Based on the barcoding results (Supplementary Table S2), 20 sequences supported species-level identity (6 green, 7 brown, and 7 red algae, respectively), 21 enabled genus-level identification, and 14 clustered with higher-level taxonomic entities in each phylum. All sequences were deposited in GenBank/NCBI (outlined in Supplementary Table S2).

DISCUSSION

In the present work, the GE method with subsequent DNA barcoding to characterize cryptic macroalgal diversity was applied for the first time to seaweeds sampled in the Arabian Gulf region. While this work revealed a number of novel species for the region, the isolated taxa probably underrepresent the diversity of cryptic stages present in the field, as GE is biased towards rapidly growing and proliferating, early successional species.

Diversity and distribution of GE isolates in Kuwait

In the present work, a total of 57 species of algae (Table 2) were identified from 77 DNA-sequenced isolates sampled on the coast of Kuwait, identified morphologically and underpinned by molecular information (Supplementary Table S2). In addition, genetic analyses revealed that the GE isolates sequences were not uniformly distributed among the 3 major macroalgal phyla. Among 120 unialgal isolates, the results revealed 45 isolates representing 17 species of Chlorophyta including Ulvaceae J. V. Lamouroux ex Dumortier, Ulvellaceae Schmidle, Bryopsidaceae Bory, Cladophoraceae Wille, and Planophilaceae Škaloud & Leliaert. Twenty-four isolates representing 14 species in the Sphacelariaceae Decaisne, Acinetosporaceae G. Hamel ex Feldmann, Chordariaceae Greville, Ectocarpaceae C. Agardh, Scytosiphonaceae Farlow, and Bachelotiaceae T. Silberfeld, M. -F. Racault, R. L. Fletcher, A. F. Peters, F. Rousseau & B. de Reviers for Phaeophyceae. According to Peters et al. (2015), species of Chordariaceae are small, with some lacking a macroscopic stage, making it particularly difficult to distinguish species based solely on morphological features. Thus, DNA barcoding has a key role in facilitating species identification in this family. Finally, 51/120 strains coveting Stylonemataceae K. M. Drew, Acrochaetiaceae Melchior, Erythrotrichiaceae G. M. Smith, Spyridiaceae De Toni, Gelidiaceae Kützing, Delesseriaceae Bory, Ceramiaceae Dumortier, Callithamniaceae Kützing, Rhodomelaceae Horaninow, and Bangiaceae Duby represent 26 species of Rhodophyta (Table 2). Overall, 95/120 (79%) of the strains were collected from the Southern Province, including Fintas, whereas 25 (21%) strains were collected from the Northern Province, including Boubiyan Island.

The proportion of Phaeophyceae (n = 18/77 isolates; 23%) was rather small among the initial cultures. Also, species richness among the brown algae isolated here was lower than for the other identified phyla. Amongst the isolates of filamentous Ectocarpales Bessey in the data obtained, *Feldmannia* Hamel is less well studied than the genus *Ectocarpus* Lyngbye (Peters et al. 2015), with few reference sequences available in GenBank when using the locus RuBisCO spacer / *rbc*L region (amplified

Table 2. Species names and authorities for Chlorophyta, Phaeophyceae, and Rhodophyta isolated from Kuwait (February–April 2019) based on DNA barcoding results

No.	Strains ID	Present study	Synonyms	Comments
Chlorophyta				
Cladophoraceae				
1	K56	*Cladophora laeteviren* (Dillwyn) Kützing	*Conferva laetevirens* Dillwyn	New record for Kuwait & the Gulf based on molecular analysis
2	K57	*Cladophora gracilis* Kützing		Kokabi and Yousefzadi (2015) mentioned this sp. in Gulf, it is a new based on molecular analysis for Kuwait
3	K73, K74	*Chaetomorpha* Kützing sp.		Al-Yamani et al. (2014) mentioned *Ch. crassa* (C. Agardh) Kützing, *Ch. indica* (Kützing) Kützing, *Ch. linum* (O. F. Müller) Kützing, first record underpinned by molecular information in Kuwait
4	K58	*Rhizoclonium* Kützing sp.		Al-Yamani et al. (2014) mentioned *R. riparium* (Roth) Harvey and *R. tortuosum* (Dillwyn) Kützing, first underpinned by molecular analysis in Kuwait
Ulvaceae				
5	K1, 3, 4, 5, 25, 51, 52, 54	*Ulva tepida* Y. Masakiyo & S. Shimada	*Ulva paschima* F. Bast	Recorded based on molecular information by Pirian et al. (2016) for the Gulf and by Al-Adilah et al. (2021) for Kuwait
6	K7	*Ulva torta* (Mertens) Trevisan	*Conferva torta* Mertens	New record for Kuwait & Gulf based on molecular analysis
7	K55	*Ulva ohnoi* M. Hiraoka & S. Shimada		First record for the Gulf, supported by molecular data showing homology described by Pirian et al. (2016)
Ulvellaceae				
8	K45	Ulvellaceae Schmidle sp.		Kokabi and Yousefzadi (2015) mentioned *U. viridis* in Gulf, but our strain is different and a new record for Kuwait, with molecular support
9	K42, K43, K46	Ulvellaceae Schmidle sp.		
10	K50	*Ulvella leptochaete* (Huber) R. Nielsen, C. J. O'Kelly & B. Wysor in Nielsen	*Endoderma leptochaete* Huber	New record for Kuwait & the Gulf, based on molecular information
11	K39, K40, K44	*Ulvella* P. Crouan & H. Crouan sp.		
Planophilaceae				
12	K38, K41	Planophilaceae Škaloud & Leliaert sp.		John and Al-Thani (2014) mentioned *P. dendroides* (P. L. Crouan & H. M. Crouan) Batters in the Gulf; this family is a new record for Kuwait
Bryopsidaceae				
13	K49	*Bryopsis* J. V. Lamouroux sp.		Al-Yamani et al. (2014) mentioned *B. hypnoides* J. V. Lamouroux and *B. plumosa* (Hudson) C. Agardh, first record underpinned by molecular data in Kuwait
Phaeophyceae				
Acinetosporaceae				
14	K19	Acinetosporaceae G. Hamel ex Feldmann sp.		Al-Yamani et al. (2014) mentioned *F. mitchelliae* and *F. irregularis* (Kützing) Hamel in Kuwait; according to DNA sequences, K19 is a different species
15	K47, 48, 198, 216	*Feldmannia mitchelliae* (Harvey) H. -S. Kim	Basionym: *Ectocarpus mitchelliae* Harvey, homotypic synonyms: *Hincksia mitchelliae* (Harvey) P. C. Silva, *Giffordia mitchellae* var. parva W. R. Taylor	First record underpinned by molecular information in Kuwait, Al-Yamani et al. (2014) mentioned this taxon for Kuwait
Ectocarpaceae				
16	K23	*Ectocarpus subulatus* Kützing	*Ectocarpus subulatus* Kützing	John and Al-Thani (2014) mentioned *Ectocarpus siliculosus* (Dillwyn) Lyngbye in the Gulf; *E. subulatus* is a new record for Kuwait and the Gulf, supported by molecular data
Scytosiphonaceae				
17	K87	*Colpomenia sinuosa* Derbes et Solier	*Ulva sinuosus* Mertens ex Roth	Previously reported for Kuwait (Al-Adilah et al. 2020), further molecular information provided here
18	K24	*Iyengaria stellata* (Børgesen) Børgesen	*Rosenvingea stellata* Børgesen	Previously reported for Kuwait (Santiañez et al. 2020), further molecular information provided here
Sphacelariaceae				
19	K17, K71	*Sphacelaria* Lyngbye sp.		
20	K72, K84	*Sphacelaria tribuloides* Meneghin	*Sphacelaria reticulata* Lyngbye	Al-Yamani et al. (2014) mentioned this taxon for Kuwait; this work provides further molecular information
Chordariaceae				
21	K20, 21, 22	Chordariaceae Greville sp.		Al-Yamani et al. (2014) mentioned some sp. from Chordariaceae family under *Cladosiphon*, *Myriactula*, *Nemacystus* genus

Table 2. Continued

No.	Strains ID	Present study	Synonyms	Comments
22	K26	*Elachista stellaris* Areschoug	*Areschougia stellaris* (Areschoug) Meneghini	New record underpinned by molecular information & the Gulf, this genus not mentioned before in Kuwait & the Gulf
23	K69	*Nemacystus decipiens* (Suringar) Kuckuck	Basionym Mesogloia decipiens Suringar	Al-Yamani et al. (2014) mentioned this sp. in Kuwait, first record underpinned by molecular information in Kuwait
Bachelotiaceae				
24	K70	*Bachelotia* (Bornet) Kuckuck ex Hamel sp.		Kokabi and Yousefzadi (2015) mentioned *B. antillarum* (Grunow) Gerloff in the Gulf; this genus was not mentioned before in Kuwait, it is a new record for Kuwait, based on molecular analysis
Rhodophyta				
Acrochaetiales				
25	K34, 35, 109, 110	*Acrochaetiales* Feldmann sp.		In the Gulf, Kokabi and Yousefzadi (2015) mentioned *A. robustum* Børgesen and *A. savianum* (Meneghini) Nägeli
Acrochaetiaceae				
26	K13	*Acrochaetium* Nägeli & Cramer sp.		John and Al-Thani (2014) mentioned *A. robustum* in the Gulf
27	K101, 108	*Acrochaetium secundatum* (Lyngbye) Nägeli in Nägeli & Cramer	*Callithamnion daviesii* var. *secundatum* Lyngbye	New record for Kuwait & the Gulf underpinned by molecular information
Ceramiaceae				
28	K82	*Ceramium affine* Setchell & N. L. Gardner	No synonyms	New record for Kuwait & the Gulf underpinned by molecular information
29	K62	*Ceramium* Roth sp.	*Ceramium virgatum* Roth	Al-Yamani et al. (2014) mentioned *C. luetzelburgii* O. C. Schmidt in Kuwait
Gelidiaceae				
30	K80	*Gelidium pusillum* var. *pakistanicum* Afaq-Husain & Shameel	No synonyms	New record for Kuwait & the Gulf underpinned by molecular information
Rhodomelaceae				
31	K61	*Polysiphonia* Greville sp.		Al-Yamani et al. (2014) mentioned *P. brodiei* (Dillwyn) Sprengel; *P. coacta* C. K. Tseng; *P. denudate* (Dillwyn) Greville ex Harvey; *P. platycarpa* Børgesen in Kuwait
Erythrotrichiaceae				
32	K65	Erythrotrichiaceae G. M. Smith sp.		
33	K14, 29, 32, 33, 67	*Erythrotrichia* Areschoug sp.		John and Al-Thani (2014) mentioned *E. carnea* (Dillwyn) J. Agardh; Kokabi and Yousefzadi (2015) mentioned *E. irregularis* Rosenvinge in the Gulf
34	K36	*Porphyrostromium* Trevisan V. B. A. sp.		New record underpinned by molecular information for Kuwait & the Gulf, this genus not mentioned before in Kuwait & the Gulf
35	K27, 28, 103	*Sahlingia subintegra* (Rosenvinge) Kornmann	*Erythrocladia subintegra* Rosenvinge	First underpinned by molecular analysis in Kuwait and Al-Yamani et al. (2014) mentioned this species in Kuwait
Spyridiaceae				
36	K66, 78	*Spyridia* Harvey sp.		Al-Yamani et al. (2014) mentioned *S.a. filamentosa* (Wulfen) Harvey species in Kuwait
Stylonemataceae				
37	K9	*Chroodactylon* Hansgirg sp.		New record underpinned by molecular information for Kuwait. *C. ornatum* (C. Agardh) Basson has previously been mentioned by Al-Yamani et al. (2014)
38	K12, 30, 32, 64, 104	*Stylonema alsidii* (Zanardini) K. Drew	*Bangia alsidii* Zanardini	John and Al-Thani (2014) mentioned this taxon for the Gulf, but it is a new record underpinned by molecular analysis for Kuwait
Bangiaceae				
39	K118	*Kuwaitiella rubra* gen. et sp. nov.		New record for Kuwait & Gulf underpinned by molecular information (Hasan et al. 2022)
Callithamniaceae				
40	K63	*Crouania* Agardh sp.		John and Al-Thani (2014) mentioned in the Gulf; *C. attenuate* J. Agardh, new record underpinned by molecular information for Kuwait; also, this genus was not reported before in Kuwait
Delesseriaceae				
41	K68	*Dasya caraibica* Børgesen	No synonyms	New record underpinned by molecular analysis for the Gulf and Kuwait
42	K81	*Heterosiphonia* Montagne [J. F.] sp.		Al-Yamani et al. (2014) mentioned *H. dendroidea* Hollenberg; *H. crispella* (C. Agardh) M. J. Wynne

with primers *rbc*LP2F, *rbc*S 952R or *rbc*L RH3F and *rbc*-S139R). The results obtained in this study showed that the strain K23 [K19-4-130-1] using the 3′-*rbc*L plus RuBisCO spacer (using primers *rbc*L1273F, *rbc*S 139R) was identical (100% homology in both *rbc*L and RuBisCO spacer) to a sequence for *Ectocarpus subulatus* from Port Aransas, Texas, USA (accession No. U38750), indicating that this strain belonged to the same species. Originally identified as *E. siliculosus*, the *Ectocarpus* from Port Aransas is now regarded as a different species, *E. subulatus*, which is, in ecophysiological terms, the most low salinity-tolerant species of the genus (Dittami et al. 2020*a*, 2020*b*). Port Aransas harbors the southernmost population of *Ectocarpus* known in North America, with a large temperature amplitude of 17°C between winter (13°) and summer (30°) (Bolton 1983). It is interesting that the same *Ectocarpus* species appears to be present in Kuwait, where the summer temperature is even higher. Further analyses of the *rbc*L gene sequences showed that isolates (K72 [K19-4-123-1] and K84 [K19-2-72-5]) had closest matches (98.70 and 98.16% homology) to sequences of *Sphacelaria tribuloides* Meneghin from the Netherlands (AJ287892) and Okinawa (AJ287891), respectively. The RuBisCO spacer of K84 (262 bp in length) showed 91.6% homology with that of *S. tribuloides* from Okinawa (AJ287947). Taken together, the results from *rbc*L and RuBisCO spacer suggest that the material from Kuwait was a closely related species, possibly, to the morphologically similar *Sphac-*

Table 3. Diversity of algal isolates obtained by Germling Emergence Method based on 90 algal cultures collected from Kuwaiti costal sites

No.	Location sites	Taxa		
		Chlorophyta	Rhodophyta	Phaeophyceae
1	Bubiyan Island	-	-	*Colpomenia sinuosa* (K87)
2	Sabiya	-	-	*Ectocarpus subulatus* (K23)
3	Failaka Island	*Ulva tepida* (K54)	*Spyridia* sp. (K66)	*Sphacelaria* sp. (K71)
4	Doha	*Ulva tepida* (K1, K3), *Ulvella* sp. (K39, K40), Ulvellaceae sp. (K43), *Ulvella* sp. (K44), Ulvellaceae sp. (K45, K46), *Ulvella leptochaete* (K50), *Ulva ohnoi* (K55), *Rhizoclonium* sp. (K58)	*Stylonema alsidii* (K12)	
5	Kuwait City Sharq	-	-	-
6	Fintas	-	*Sahlingia subintegra* (K28), *Stylonema alsidii* (K31), *Erythrotrichia* sp. (K29, K32, K33), *Porphyrostromium* sp. (K36), *Gelidium pusillum* var. *pakistanicum* (K80), *Heterosiphonia* sp. (K81), *Ceramium affine* (K82)	*Iyengaria stellata* (K24), *Feldmannia mitchelliae* s.I (K48)
7	Kubbar Island	-	-	*Feldmannia mitchelliae* s.I (K198, K216)
8	Qaruh Island	*Ulva tepida* (K51, K52)	*Crouania* sp. (K63), *Stylonema alsidii* (K64), Erythrotrichiaceae sp. (K65)	*Sphacelaria tribuloides* (K84)
9	Al-Zour	*Ulva tepida* (K4, K5, K25), Planophilaceae sp. (K38, K41), *Acrochaete* sp. (K42), *Cladophora laetevirens* (K56), *Cladophora gracilis* (K57), *Chaetomorpha* sp. (K73, K74)	*Arcochaetium* sp. (K13), *Erythrotrichia* sp. (K14, K33), *Sahlingia subintegra* (K27, K103), *Stylonema alsidii* (K30, K104), Acrochaetiales sp. (K34, K35, K109, K110), *Acrochaetium secundatum* (K101, K108), *Dasya caraibica* (K68)	Acinetosporaceae sp. (K19), Chordariaceae sp. (K21), Chordariaceae sp. (K20), Chordariaceae sp. (K22), *Feldmannia mitchelliae* s.I (K47), *Nemacystus decipiens* (K69), *Sphacelaria tribuloides* (K72)
10	Khiran	*Bryopsis* sp. (K49)	*Chroodactylon* (K9), *Polysiphonia* sp. (K61), *Spyridia* sp. (K78), *Kuwaitiella rubra* gen. et sp. nov. (K118)	*Sphacelaria* sp. (K17), *Elachista stellaris* (K26)
11	Umm Al-Maradim Island	-	-	*Bachelotia* sp. (K70)
12	Nuwaiseeb	-	*Ceramium* sp. (K62)	-

elaria novae-hollandiae Sonder, for which no published sequences are available. According to Al-Yamani et al. (2014), both *S. tribuloides* and *S. novae-hollandiae* occur in Kuwait. However, for example, *Sargassum* and *Padina* sporophytes were not encountered in cultures starting from substrates despite a clear abundance in the field. These algae have no long-lived microstages (microscopic gametophytes or sporophytes) in their life cycle (Peters et al. 2015).

In Kuwait, at the Northern province sites; Boubiyan, Sabiya, Failaka, and Doha, respectively, had less algal diversity than the Southern provience. Three brown algal isolates, *Ectocarpus subulatus* (K23), *Colpomenia sinuosa* Derbes et Solier (K87) - *Sphacelaria* Lyngbye sp. (K71), plus two red taxa, *Stylonema alsidii* (Zanardini) K. Drew (K12) which has a world-wide geographic distribution (Zuccarello et al. 2011), which had previously been reported from Kuwait (Al-Yamani et al. 2014), and *Spyridia* Harvey sp. (K66) were identified (Table 3). However, these are clearly adapted to unstable substrates. The limited occurrence of Phaeophyceae and Rhodophyta the Northern Province (Doha) can also be correlated with the muddy substrates there (Jones 1986) which suggests that this area may lack the microstages of taxa in these phyla, compared with other species that were obviously present in this area and that were mostly Chlorophyta. The Northern Province including the Doha site (Table 3) is characterized by shallow, extensive intertidal mud flats with turbid water and semi-enclosed bodies of water with very limited exchange (65 days) to the open sea (Pokavanich and Alosairi 2014) which is typical for Kuwait Bay (Al-Mutairi et al. 2014), compounded by a scarcity of solid, rocky substrates (Alghunaim et al. 2019). The soft nature of these substrates can also explain why the Northern Province is mostly unsuitable for the attachment and growth of several macroalgae particularly large brown macroalgae, such as *Sargassum* C. Agardh, except for some taxa with small thalli such as *Feldmannia* sp., which agrees with our observations.

In most temperate coastal waters, macroalgal growth and diversity are directly controlled by nutrient availability (Pedersen et al. 2010, Martínez et al. 2012). The marine environment of Kuwait is also strongly influenced by the discharge of the Shatt Al-Arab estuary with a maximum peak of discharging fresh water from March to July (Al-Said et al. 2017). This freshwater input is associated with a higher concentrations of nutrients, resulting in increased productivity and abundance of green algae in the Doha area. Also, the green algal taxa recorded inhabited calm waters (tidal pools), which were plentiful in the Doha area during winter and early spring where there are many flat, intertidal rock platforms with low current waves, allowing the development of a stable community with higher diversity of green algae (Prathep et al. 2007). Nevertheless, nutrient enrichment and low wave action (Nishihara and Terada 2010) can, therefore, increase the growth of opportunistic seaweeds like green algae characteristically found at Al-Doha, represented in this study by *Ulva* Linnaeus spp. and members of the Ulvellaceae. It seems that the *Ulva* species are among the fastest growing macroalgae under conditions of high nutrient and light availability (Phillips and Hurd 2003). Competition among thalli of green algae for space at Doha is intense, in particular when light levels increase in spring and *Ulva* species generally have high levels of desiccation resistance (Nybakken 1993).

The Chlorophyta recorded in this work (n = 26/77, 34%) mostly have small holdfasts, are smaller and lighter in weight, and are tolerant to the prevailing turbidity and trophic levels compared to Phaeophyceae (Al-Hasan and Jones 1989, Uddin et al. 2011). Furthermore, as *Sargassum* species are very sensitive to environmental perturbations, substrate type and slope (Fatemi et al. 2012), they are not encountered in the Doha area. A limited number of more tolerant species, however, such as *Feldmannia* sp. and *Iyengaria* Børgesen sp., are encountered at Doha. There is some evidence that, over the last two decades, algal diversity on the Doha coast has decreased (Dhia Al-Bader, personal communication / unpublished results).

The Southern Province was the richest area in terms of diversity of algal isolates (95 strains/120; 79%), especially at Fintas and Al-Zour (Table 3). The more benign physical conditions compared to the northern areas of Kuwait probably result in an increase in richness in the “bank” of microscopic stages on this sandy and rocky coast. This finding may be attributed to several factors, such as the lesser anthropogenic impacts on the open sea environment in the south, along with the higher hydrodynamic energy with low turbidity in this region (2 m tidal range) (Al-Yamani et al. 2004). One of the most important factors in species distribution and abundance in tidal areas is exposure to wave action and overall hydrodynamism (Mayakun and Prathep 2005, Nishihara and Terada 2010). According to Al-Yamani et al. (2004), the scattered rocks available on the beaches of this province are suitable for seaweed attachment by holdfasts, resulting in diverse communities of algae. Microscopic stages will inhabit rock surfaces, pebbles and shell fragments, creating a more diverse range of isolates compared to Doha. This

finding was emphasized by Al-Yamani et al. (2014) and Al-Hasan and Jones (1989), who also mention this pattern in their previous publications about the macroalgal flora of Kuwait. This is also consistent with Uddin et al. (2011), who mentioned that the southern waters of Kuwait have coral reefs that can also support rich biodiversity of marine macrophytes. Al-Zour is characterized by a rocky shore and overall is a protected and sheltered area, therefore a high diversity of seaweeds, especially among the Rhodophyta, was not surprising. Overall, in the present study, the Rhodophyta were the phylum with the highest species richness compared to the green and brown phyla among seaweeds isolated and identified in this study, constituting 42% (n = 51/120) of the total algal isolates, representing 26 species. These observations are consistent with several previous reports (Silva et al. 1996, Price et al. 2006, John 2012, John and Al-Thani 2014) who suggested that among a total of approximately 282 benthic marine algal species in the Arabian Gulf, the most diverse were the Rhodophyta (147 spp.).

A further exploration of the macroalgal diversity on the coast of Kuwait will likely reveal greater diversity, especially of smaller epiphytic algal species with short life cycles, as the time period covered in this work encompassed only late winter (February 2019) and spring (April 2019). The GE method was clearly a good tool to investigate cryptic macroalgal floral diversity. Compared to environmental sequencing (metabarcoding) it offers the advantage of providing access to a plethora of biological information such as morphology, life cycle, physiology and biochemistry. This method has substantially helped revealing a diversity in the local Kuwaiti waters that was not previously known. This study has revealed several new records of species and genera, including noteworthy are new records of *Porphyrostromium*, a new genus from the Bangiales namely *Kuwaitiella rubra* gen. et sp. nov., and two unclear species for Planophilaceae. The data obtained in this present study will also help to predict the future of algal diversity and floristic composition under ongoing and predicted climate change effects impacting this already harsh environment.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. Identification, habitat, and collection sites of the 120 algal cultures in Kuwait (https://www.e-algae.org).

Supplementary Table S2. List of isolated algal strains collected from Kuwait, grouped according to the closest sequence match in GenBank (https://www.e-algae.org).

Supplementary Fig. S1. (A–C) The identification of the species was based upon molecular analyses of *in vitro* algal cultures (under laboratory conditions with specific light : dark time period)—mostly in a microscopic stage and not always similar morphologically to growth in the natural environment (https://www.e-algae.org).

REFERENCES

Al-Adilah, H., Al-Bader, D., Elktob, M., Kosma, I., Kumari, P. & Küpper, F. C. 2021. Trace element concentrations in seaweeds of the Arabian Gulf identified by morphology and DNA barcodes. Bot. Mar. 64:327–338.

Al-Adilah, H., Peters, A. F., Al-Bader, D., Raab, A., Akhdhar, A., Feldmann, J. & Küpper, F. C. 2020. Iodine and fluorine concentrations in seaweeds of the Arabian Gulf identified by morphology and DNA barcodes. Bot. Mar. 63:509–519.

Alghunaim, A., Taqi, A., Al-Kandari, M. & Al-Said, T. 2019. Distribution and nature of *Sargassum* species in the Kuwait waters. J. Geosci. Environ. Prot. 7:53–59.

Al-Hasan, R. H. & Jones, W. E. 1989. Marine algal flora and sea grasses of the coast of Kuwait. J. Univ. Kuwait Sci.

16:289–341.

Al-Mutairi, N., Abahussain, A. & Al-Battay, A. 2014. Environmental assessment of water quality in Kuwait Bay. Int. J. Environ. Sci. Dev. 5:527–532.

Al-Said, T., Al-Ghunaim, A., Subba Rao, D. V., Al-Yamani, F., Al-Rifaie, K. & Al-Baz, A. 2017. Salinity-driven decadal changes in phytoplankton community in the NW Arabian Gulf of Kuwait. Environ. Monit. Assess. 189:268.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Al-Yamani, F. Y., Bishop, J., Ramadhan, E., Al-Husain, M. & Al-Ghadban, A. N. 2004. *Oceanographic atlas of Kuwait waters*. Kuwait Institute for Scientfic Research, Kuwait, 203 pp.

Al-Yamani, F. Y., Polikarpov, I., Al-Ghunaim, A. & Mikhaylova, T. 2014. *Field guide of marine macroalgae (Chlorophyta, Rhodophyta, Phaeophyceae) of Kuwait*. Kuwait Institute for Scientific Research, Kuwait, 190 pp.

Bartolo, A. G., Zammit, G., Peters, A. F. & Küpper, F. C. 2020. The current state of DNA barcoding of marcroalgae in the Mediterranean Sea: presently lacking but urgently required. Bot. Mar. 63:253–272.

Bolton, J. J. 1983. Ecoclinal variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to temperature growth optima and survival limits. Mar. Biol. 73:131–138.

Broom, J. E. S., Nelson, W. A., Farr, T. J., Phillips, L. E. & Clayton, M. 2010. Relationships of the *Porphyra* (Bangiales, Rhodophyta) flora of the Falkland Islands: a molecular survey using *rbc*L and nSSU sequence data. Aust. Syst. Bot. 23:27–37.

Coelho, S. M., Scornet, D., Rousvoal, S., Peters, N. T., Dartevelle, L., Peters, A. F. & Cock, J. M. 2012. *Ectocarpus*: a model organism for the brown algae. Cold Spring Harb. Protoc. 2012:193–198.

Destombe, C. & Douglas, S. E. 1991. Rubisco spacer sequence divergence in the rhodophyte alga *Gracilaria verrucosa* and closely related species. Curr. Genet. 19:395–398.

De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Tara Oceans Coordinators, Acinas, S. G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M. E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P. & Karsenti, E. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348:1261605.

Dittami, S. M., Corre, E., Brillet-Guéguen, L., Lipinska, A. P., Pontoizeau, N., Aite, M., Avia, K., Caron, C., Cho, C. H., Collén, J., Cormier, A., Delage, L., Doubleau, S., Frioux, C., Gobet, A., González-Navarrete, I., Groisillier, A., Herv, C., Jollivet, D., KleinJan, H., Leblanc, C., Liu, X., Marie, D., Markov, G V., Minoche, A. E., Monsoor, M., Pericard, P., Perrineau, M. M., Peters, A. F., Siegel, A., Siméon, A., Trottier, C., Yoon, H. S., Himmelbauer, H., Boyen, C. & Tonon, T. 2020*a*. The genome of *Ectocarpus subulatus*: a highly stress-tolerant brown alga. Mar. Genomics 52:100740.

Dittami, S. M., Peters, A. F., West, J. A., Cariou, T., KleinJan, H., Burgunter-Delamare, B., Prechoux, A., Egan, E. & Boyen, C. 2020*b*. Revisiting Australian *Ectocarpus subulatus* (Phaeophyceae) from the Hopkins River: distribution, abiotic environment, and associated microbiota. J. Phycol. 56:719–729.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Famà, P., Wysor, B., Kooistra, W. H. C. F. & Zuccarello, G. C. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. J. Phycol. 38:1040–1050.

Fatemi, S. M. R., Ghavam Mostafavi, P., Rafiee, F. & Saeed Taheri, M. 2012. The study of seaweeds biomass from intertidal rocky shores of Qeshm Island, Persian Gulf. Int. J. Mar. Sci. Environ. 2:101–106.

Gachon, C. M. M., Strittmatter, M., Müller, D. G., Kleinteich, J. & Küpper, F. C. 2009. Detection of differential host susceptibility to the marine oomycete pathogen *Eurychasma dicksonii* by real-time PCR: not all algae are equal. Appl. Environ. Microbiol. 75:322–328.

Hall, J. D., Fučíkov, K., Lo, C., Lewis, L. A. & Karol, K. G. 2010. An assessment of proposed DNA barcodes in freshwater green algae. Cryptogam. Algol. 31:529–555.

Han, K. Y., Graf, L., Reyes, C. P., Melkonian, B., Andersen, R. A., Yoon, H. S. & Melkonian, M. 2018. A re-investigation of *Sarcinochrysis marina* (Sarcinochrysidales, Pelagophyceae) from its type locality and the descriptions of *Arachnochrysis*, *Pelagospilus*, *Sargassococcus* and *Sungminbooa* genera nov. Protist 169:79–106.

Hasan, A. H., Van der Aa, P., Küpper, F. C., Al-Bader, D. & Peters, A. F. 2022. *Kuwaitiella rubra* gen. et sp. nov. (Bangiales, Rhodophyta), a new filamentous genus and species from the north-western Indian Ocean. Phycol. Res. 70:192–202.

Hodge, F. J., Buchanan, J. & Zuccarello, G. C. 2010. Hybridization between the endemic brown algae *Carpophyllum maschalocarpum* and *Carpophyllum angustifolium* (Fucales): genetic and morphological evidence. Phycol. Res. 58:239–247.

Hoffmann, A. J. & Santelices, B. 1991. Banks of algal microscopic forms: hypotheses on their functioning and comparisons with seed banks. Mar. Ecol. Prog. Ser. 79:185–194.

John, D. M. 2012. Marine algae (seaweeds) associated with coral reefs of the Gulf. *In* Riegl, B. M. & Purkis, S. J. (Eds.) *Coral Reefs of the Gulfs: Adaptation to Climate Extremes*. *Coral Reefs of the World, Vol. 3*. Springer, Dordrecht, pp. 170–186.

John, D. M. & Al-Thani, R. F. 2014. Benthic marine algae of the Arabian Gulf: a critical review and analysis of distribution and diversity patterns. Nova Hedwigia 98:341–392.

Jones, D. 1986. *A field guide to the seashores of Kuwait and the Arabian Gulf*. University of Kuwait, Kuwait, 193 pp.

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

Kirkendale, L., Saunders, G. W. & Winberg, P. 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. J. Phycol. 49:69–81.

Kokabi, M. & Yousefzadi, M. 2015. Checklist of the marine macroalgae of Iran. Bot. Mar. 58:307–320.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35:1547–1549.

Kunimoto, M., Kito, H., Kaminishi, Y., Mizukami, Y. & Murase, N. 1999. Molecular divergence of the SSU rRNA gene and internal transcribed spacer 1 in *Porphyra yezoensis* (Rhodophyta). J. Appl. Phycol. 11:211–216.

Küpper, F. C., Peters, A. F., Shewring, D. M., Sayer, M. D. J., Mystikou, A., Brown, H., Azzopardi, E., Dargent, O., Strittmatter, M., Brennan, D., Asensi, A. O., van West, P. & Wilce, R. T. 2016. Arctic marine phytobenthos of northern Baffin Island. J. Phycol. 52:532–549.

Lane, C. E., Mayes, C., Druehl, L. D. & Saunders, G. W. 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. J. Phycol. 42:493–512.

Lawton, R. J., Mata, L., de Nys, R. & Paul, N. A. 2013. Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: selecting target species and strains. PLoS ONE 8:e77344.

Lewin, J. 1966. Silicon metabolism in diatoms. *V. Germanium dioide*, a specific inhibitor of diatom growth. Phycologia 6:1–12.

Macreadie, P. I., Jarvis, J., Trevathan-Tackett, S. M. & Bellgrove, A. 2017. Seagrasses and macroalgae: importance, vulnerability and impacts. *In* Phillips, B. F. & Pérez-Ramírez, M. (Eds.) *Climate Change Impacts on Fisheries and Aquaculture: A Global Analysis*. Wiley-Blackwell, Oxford, pp. 729–770.

Martínez, B., Pato, L. S. & Rico, J. M. 2012. Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquat. Bot. 96:14–22.

Masakiyo, Y. & Shimada, S. 2014. Species diversity of the genus *Ulva* (Ulvophyceae, Chlorophyta) in Japanese waters, with special reference to *Ulva tepida* Masakiyo et S. Shimada sp. nov. Bull. Natl. Mus. Nat. Sci. Ser. B Bot. 40:1–13.

Mayakun, J. & Prathep, A. 2005. Seasonal variations in diversity and abundance of macroalgae at Samui Island, Surat Thani Province, Thailand. Songklanakarin J. Sci. Technol. 27:653–663.

Müller, D. G. & Ramírez, M. E. 1994. Filamentous brown algae from the Juan Fernandez Archipelago (Chile): contribution of laboratory culture techniques to a phytogeographic survey. Bot. Mar. 37:205–211.

Nishihara, G. N. & Terada, R. 2010. Spatial variations in nutrient supply to the red algae *Eucheuma serra* (J. Agardh) J. Agardh. Phycol. Res. 58:29–34.

Nybakken, J. W. 1993. *Marine biology an ecological approach*. Harper Collins College Publishers, NY, 10022 pp.

Pedersen, M. F., Borum, J. & Fotel, F. L. 2010. Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. Mar. Ecol. Prog. Ser. 399:103–115.

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

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

Peters, A. F., Scornet, D., Müller, D. G., Kloareg, B. & Cock, J. M. 2004. Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus* (Phaeophyceae). Eur. J. Phycol. 39:235–242.

Phillips, J. C. & Hurd, C. L. 2003. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, stor-

age and utilisation in relation to shore position and season. Mar. Ecol. Prog. Ser. 264:31–48.

Pirian, K., Piri, K., Sohrabipour, J., Jahromi, S. T. & Blomster, J. 2016. Molecular and morphological characterisation of *Ulva chaugulii*, *U. paschima* and *U. ohnoi* (Ulvophyceae) from the Persian Gulf, Iran. Bot. Mar. 59:147–158.

Pokavanich, T. & Alosairi, Y. 2014. Summer flushing characteristics of Kuwait Bay. J. Coast. Res. 30:1066–1073.

Prathep, A., Wichachucherd, B. & Thongroy, P. 2007. Spatial and temporal variation in density and thallus morphology of *Turbinaria ornata* in Thailand. Aquat. Bot. 86:132–138.

Price, A. R. G., Vincent, L. P. A., Venkatachalam, A. J., Bolton, J. J. & Basson, P. W. 2006. Concordance between different measures of biodiversity in Indian Ocean macroalgae. Mar. Ecol. Prog. Ser. 319:85–91.

Provasoli, L. 1966. Media and prospects for the cultivation of marine algae. *In* Watanabe, A. & Hattori, A. (Eds.) *Cult. Collect. Algae: Proc. US-Japan Conf*. Japan Society of Plant Physiology, Hakone, pp. 63–75.

Ramírez, M. E. & Müller, D. G. 1991. New records of benthic marine algae from Easter Island. Bot. Mar. 34:133–137.

Rizouli, A., Küpper, F. C., Louizidou, P., Mogg, A. O. M., Azzopardi, E., Sayer, M. D. J., Kawai, H., Hanyuda, T. & Peters, A. F. 2020. The minute alga *Schizocladia ischiensis* (Schizocladiophyceaase, Ochrophyta) isolated by germling emergence from 24 m depth of Rhodes (Greece). Diversity 12:102.

Santiañez, W. J. E., Al-Bader, D., West, J. A., Bolton, J. J. & Kogame, K. 2020. Status, morphology, and phylogenetic relationships of *Iyengaria* (Scytosiphonaceae, Phaeophyceae), a brown algal genus with a disjunct distribution in the Indian Ocean. Phycol. Res. 68:323–331.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360:1879–1888.

Saunders, G. W. & Kucera, H. 2010. An evaluation of *rbcL*, *tufA*, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogam. Algol. 31:487–528.

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

Schoenrock, K. M., McHugh, T. A. & Krueger-Hadfield, S. A. 2021. Revisiting the 'bank of microscopic forms' in macroalgal-dominated ecosystems. J. Phycol. 57:14–29.

Siemer, B. L., Stam, W. T., Olsen, T. J. & Pedersen, P. M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and spacer sequences. J. Phycol. 34:1038–1048.

Silva, P. C., Basson, P. W. & Moe, R. L. 1996. *Catalogue of the benthic marine algae of the Indian Ocean. Vol. 79*. University California Press, Berkeley, CA, 1259 pp.

Starr, R. C. & Zeikus, J. A. 1993. UTEX: the culture collection of algae at the University of Texas at Austin. 1993 List of cultures. J. Phycol. 29:1–106.

Tanaka, A., Uwai, S., Nelson, W. & Kawai, H. 2010. *Phaeophysema* gen. nov. and *Vimineoleathesia* gen. nov., new brown algal genera for the minute Japanese members of the genus *Leathesia*. Eur. J. Phycol. 45:107–115.

Tarakhovskaya, E. R., Kang, E. J., Kim, K. Y. & Garbary, D. J. 2012. Effect of GeO_2 on embryo development and photosynthesis in *Fucus vesiculosus* (Phaeophyceae). Algae 27:125–134.

Uddin, S., Al Ghadban, A. N. & Khabbaz, A. 2011. Localized hyper saline waters in Arabian Gulf from desalination activity: an example from South Kuwait. Environ. Monit. Asses. 181:587–594.

West, J. A., Loiseaux-De Goër, S. & Zuccarello, G. C. 2012. Upright Erythropeltidales (Rhodophyta) in Brittany, France and description of a new species, *Erythrotrichia longistipitata*. Cah. Biol. Mar. 53:255–270.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Eds.) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, CA, pp. 315–322.

Wolf, M. 2012. Molecular and morphological investigations on seaweed biodiversity and alien introductions in the Adriatic Sea (Mediterranean, Italy). Ph.D. dissertation, Universit Degli Studi Di padova Dipartimento Di Biologia, Padova, Italy, 183 pp.

Wynne, M. J., Kamiya, M., West, J. A., Loiseaux-de Goër, S., Lim, P.-E., Sade, A., Russell, H. & Küpper, F. C. 2020. Morphological and molecular evidence for the recognition of *Hypoglossum sabahense* sp. nov. (Delesseriaceae, Rhodophyta) from Sabah, Malaysia. Algae 35:157–165.

Yoon, H. S., Hackett, J. D. & Bhattacharya, D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proc. Natl. Acad. Sci. U. S. A. 99:11724–11729.

Zuccarello, G. C., Yoon, H. S., Kim, H., Sun, L., de Gor, S. & West, J. A. 2011. Molecular phylogeny of the upright erythropeltidales (Compsopogonophyceae, Rhodophyta): multiple cryptic lineages of *Erythrotrichia carnea*. J. Phycol. 47:627–637.