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RESEARCH ARTICLE

## Revision of the family *Sargassaceae* (*Fucales*, *Phaeophyceae*) from Kuwait (Arabian / Persian Gulf, NW Indian Ocean) based on analysis of morphology and ITS2 sequences

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**Abstract.** This article investigates the species of the family *Sargassaceae* present in Kuwait, analyzing nuclear ribosomal internal transcribed spacer 2 (ITS2) sequences of 67 individuals collected in 2018–2020. Morphological characterization and DNA barcoding confirmed the presence of eight taxa that belong to *Sargassaceae*. Five species belong to the genus *Sargassum*, while three other taxa, namely *Sirophysis trinodis*, *Hormophysa cuneiformis*, and *Polycladia myrica*, belong to other genera. For the latter species, there was no ITS2 sequence available so far. As for *Sargassum* species, previous records based on phenotypic identifications were only confirmed for a minority of species. Based on 100% sequence identity with records from other regions, we recognize *S. aquifolium*, *S. ilicifolium* var. *acaraeocarpum*, *S. yinggehaiense*, and one still unclear species (*Sargassum* sp.). According to the sequences, the fifth and most common *Sargassum* species in Kuwait, previously identified as *S. asperifolium*, is *S. carpophyllum*. However, the ITS2 sequence of this entity did not show a perfect match with any *S. carpophyllum* specimen so far sequenced. The comparatively limited diversity of *Sargassum* in Kuwait may be due to the prevailing extreme environmental conditions, in particular large temperature amplitude between +14 °C in winter and +37 °C in summer.

**Keywords:** genetic analysis, Kuwait, nuclear ribosomal internal transcribed spacer 2 (ITS2), *Phaeophyceae*, phenotypic, phylogeny, *Sargassum*, taxonomy

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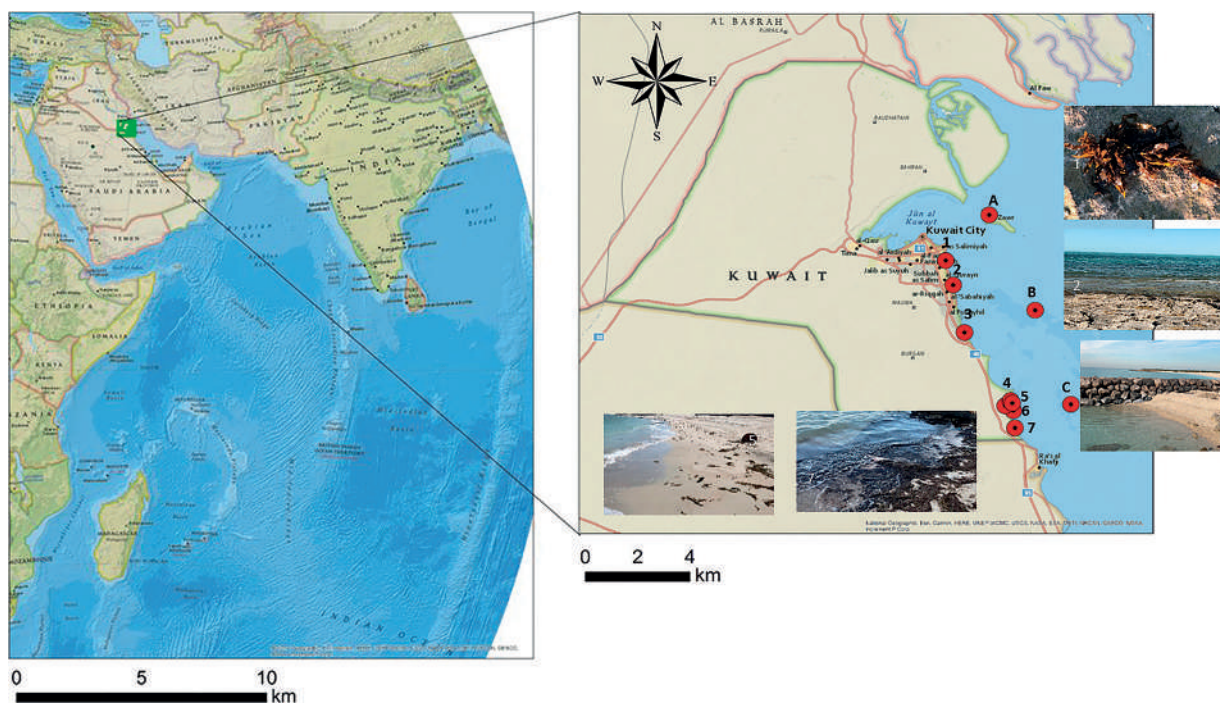
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## Introduction

Around 570 species are currently recognized in the order *Fucales* (*Ochrophyta*, brown algae), many of which are structuring elements of benthic habitats by providing shelter and nursery for a variety of invertebrates, micro- and macro-epiphytes, and other marine organisms (Guiry, Guiry, 2023). Only the family *Sargassaceae* Kützing (526 taxa) is of worldwide distribution and one of the most species-rich families in the class *Phaeophyceae* (Phillips, 1995; Blunt et al., 2008; Yip et al., 2020; Guiry, Guiry, 2023). They occur especially in tropical and sub-tropical regions, where they are the most diverse groups of canopy-forming species (Draisma et al., 2010; Mattio, Payri, 2011). This family is absent in waters of Antarctica and southern South America (Draisma et al., 2010). Their diversity is highest in the Indo-West Pacific (Noiraksar, Ajisaka, 2008; Amaral-Zettler et al., 2017), which is close to

the putative region of origin of the order *Fucales* (Cho et al., 2006), with two species of *Sirophysalis* Kützing (tropical Indo-West-Pacific), one species of *Hormophysa* Kützing, three species of *Polycladia* Montagne (eastern Indian Ocean), and 360 described species of *Sargassum* C.Agardh (1820). However, currently, less than 40% of the *Sargassum* species are well characterized and recognized (Mattio, Payri, 2009; Mattio et al., 2010; Low, Chou, 2013). Even though morphological features of blades, receptacles, pneumatocysts, axes and the holdfast are potentially available to support species identification, *Sargassum* species are notoriously difficult to identify morphologically (Stiger et al., 2000, 2003; Cheang et al., 2008).

Recent approaches to investigate the *Sargassaceae* of the Indo-West Pacific region have therefore employed molecular markers in combination with morphology (Bruno de Sousa et al., 2019). Such studies included a revision of the family by



**Fig. 1.** Sites in Kuwait where specimens of taxa belonging to the family *Sargassaceae* were collected for this study. SubFigure represents: seven shoreline locations (Anjafa, Fintas, Mina Abdulla, Al-Zour (1), Al-Zour (2), Al-Khiran, and Al-Nuwiseeb, shown as 1–7, respectively) and three offshore sampling points in each of Failaka, Kubbar, and Umm Al-Maradim islands (as A, B, C, respectively). GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA

investigating evolutionary relationships (Draisma et al., 2010), in Madagascar (Mattio, Payri, 2011), Australia and New Zealand (Dixon et al., 2012), China (Huang et al., 2017), and Singapore (Yip et al., 2018), leading to the recognition of sub-clades, including segregate genera recognized as different from *Sargassum*, such as *Carpophyllum* Greville, *Phyllotricha* Areschoug, *Sargassopsis* Trevisan, and *Sirophysalis* Kützing. Recently, among the markers studied to elucidate the phylogenetic relationships and species boundaries within this family, particularly the nuclear ribosomal transcribed spacer 2 (ITS2) is suitable for resolving differences among species, subgenera and genera within the *Sargassaceae* due to its mutation rate (Stiger et al., 2000, 2003; Mattio et al., 2008, 2009, 2010; Cho et al., 2012; Dixon et al., 2012, 2014; Kantachumpoo et al., 2015). This marker has therefore been widely used in the *Sargassaceae* and numerous sequences are available from public databases, providing good reference sequences for this kind of study. Names attached to sequences in GenBank may, however, not be correct and have to be treated with caution. To date, full infrageneric resolution of the genus and its position among related genera within the *Sargassaceae* has not been established (Bruno de Sousa et al., 2019).

The present paper adds to the revision of the *Sargassaceae* in the Indian Ocean by studying this family in the Arabian Gulf (also referred to as the Persian Gulf), which is a sub-sea of the NW Indian Ocean (Fig. 1). A total of twelve papers dealing with taxonomic aspects of the marine algal flora of the Gulf have been published, which listed the genera *Hormophysa*, *Polycladia*, *Turbinaria* J.V. Lamour., and *Sirophysalis* along with 25 *Sargassum* species (e.g., Basson, 1992; De Clerck, Coppejans, 1996; Sohrabipour, Rabii, 1999; Gharanjik, 2005; Abdel-Kareem, 2009; Noormohammadi et al., 2011; Shams et al., 2013, 2015; Kokabi, Yousefzadi, 2015). However, in Kuwait only eight publications mention members of the *Sargassaceae*: along members of the genera *Hormophysa*, *Polycladia*, and *Sirophysalis*, ten species of *Sargassum* have been reported (Jones, 1986; Al-Hasan, Jones, 1989; John, Al-Thani, 2014; Al-Yamani et al., 2014; Alghunaim et al., 2019a,

b, 2020). Most recently, two papers have explored concentrations of iodine and fluorine (Al-Adilah et al., 2020) and other trace elements (Al-Adilah et al., 2021) in *Sargassum* species from Kuwait. These papers were the first to report any nucleotide sequence data for *Sargassum* species from the Gulf. Therefore, a study to understand the *Sargassaceae* diversity in Kuwait can be a good start for a general revision of this group in the tropical regions of the Indian Ocean, specifically in the Arabian Gulf. In the present study, evidence from ITS2 sequences is employed to aid this endeavor combined with characteristic morphological features on new collections of the *Fucales* made in Kuwait.

## Materials and methods

### Algal collections and preservation

Algal samples were collected during the period from October 2018 to January 2020. A total of 67 thalli of *Sargassaceae* taxa were collected from 6 locations, covering the northern (Anjafa, Fintas) and southern areas (Mina Abdulla, Al-Khiran, Al-Zour, Al-Nuwiseeb) of Kuwait's coastline and also from three offshore islands, Failaka, Kubbar, Umm Al-Muradin, respectively (Fig. 1; see Table S1 in Supplementary Information). During low tide, samples were collected by hand from intertidal to upper subtidal zones, while around the islands the specimens floating offshore were collected from a speed boat in open waters with a dip net. All samples were maintained in net bags and tagged with all necessary information. Clean young portions of the freshly collected thalli were preserved for subsequent DNA analysis by immersion in cetyltrimethylammonium bromide (CTAB) solution (Gachon et al., 2009). The remaining thalli were then pressed onto herbarium sheets for morphological observation and documentation (voucher specimens). Herbarium specimens were deposited in the Kuwait University Herbarium (KTUH) and in the University of Aberdeen Herbarium (ABDUK).

## Morphological analysis

The morphological examination used for taxonomic analysis covers thallus length, holdfast and stem shape, presence of primary and secondary branches, general shape of blades, blade margins especially at the apex and base, vesicle shape, as well as receptacle shape and length, as diagnostic features. Specimens were studied using a stereomicroscope (Leica S6E with 10x/23 eyepieces; Morrisville, US). Identification of phenotypical criteria was carried out based on established taxonomic characteristics for this group (Womersley, 1987; Yoshida, 1989; De Clerck, Coppejans, 1996; Abbott, McDermid, 2002; Mattio et al., 2008; Cheang et al., 2008; Mattio, Payri, 2010; Draisma et al., 2010; Al-Yamani et al., 2014; summarized in Table 1). In addition, in this study, the species were compared with available thalli of *Sargassaceae* specimens at Kuwait University Herbarium (KTUH; Al-Bader, personal communications). Finally, species nomenclature was checked with AlgaeBase (Guiry, Guiry, 2023).

## Molecular analysis

Most *Sargassaceae* specimens collected within the framework of this study were strongly covered by epiphytic small animals or other seaweeds. Therefore, young tissues, which tend to have less epiphytic organisms, were chosen and carefully cleaned before DNA extraction. Epiphytic organisms may cause DNA impurities and yield false PCR products or double band profiles (Ling Ho et al., 1995). Approximately 20 mg of algal tissue of each sample were ground for 10 minutes at 30 Hz using a mechanical bead grinder (Qiagen Tissue Lyser II, Germany). Subsequently, DNA was extracted using the GENEJET Plant Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's protocol (Gachon et al., 2009). Polymerase chain reaction (PCR) was carried out on the samples to amplify the DNA of the internal transcribed spacer region II (ITS2) locus and amplified using primers KP5F and KG4R (Table S2; Lane et al., 2006; Hodge et al., 2010; Dixon et al., 2012).

PCR was performed in 25.1  $\mu$ L volume containing 20  $\mu$ L of 1.1x VWR® Taq DNA Polymerase Master Mix containing 2 mM MgCl<sub>2</sub>, 1.7  $\mu$ L of 10  $\mu$ M of both forward and reverse primers, and 1.7  $\mu$ L of extracted DNA. For ITS2, PCR conditions were as follows: an initial denaturation step of 94 °C for 3 minutes, followed by 35 cycles of 94 °C denaturation for 1 minute, 50 °C annealing temperature for 30 seconds, 72 °C extension step for 1 minute; after finishing the 35 cycles a final extension step of 72 °C allowed completion of unfinished fragments for 5 minutes. Purification was carried out using the GENEJET PCR Purification kit (Thermo Fisher Scientific, Vilnius, Lithuania). PCR products were sequenced commercially (Source BioScience, Oxford, UK) using Sanger sequencing and the same primers as for PCR. Chromatograms of ITS2 were manually reviewed and the raw sequences corrected, and the complementary sequences were aligned using the software BioEdit™ (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>; Hall et al., 2010).

The consensus sequences generated from amplicons were compared with entries in the GenBank database of the National Center for Biotechnology Information (NCBI) via the Basic Local Alignment Search Online Tools algorithm (BLAST) function (<http://www.ncbi.nih.gov>; Altschul et al., 1997), in order to identify the specimens investigated here to the closest possible match due to the highest degree of homology. The sequences were deposited in GenBank/NCBI (see Table S3 in Supplementary Information).

Sequence alignments were done by using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) statistical method with the software MEGA X 11.0.11 (available from <https://www.megasoftware.net>; Edgar, 2004; Kumar et al., 2018). Moreover, haplotype identification was carried out using the ITS2 marker and this software. In order to construct phylogenetic trees within the framework of this study, 31 additional sequences of different species of *Sargassaceae* were obtained from GenBank and included in the alignment and subsequent analysis, especially taxa with the highest degree of homology to Kuwaiti samples as indicated by BLAST (see Table S4 in Supplementary

Information). The sequences of the Fucacean species *Ascophyllum nodosum* (L.) Le Jol. (AF102971), *Fucus vesiculosus* L. (AF102932), and *F. serratus* L. (AF102945) served as monophyletic outgroup, following Draisma et al. (2010).

Genetic distance analysis was used to investigate inter- and intraspecific evolutionary divergence between *Sargassaceae* sequences. Phylogenetic trees were constructed following a Maximum Likelihood (ML) approach with bootstrap analysis to support the robustness of the tree (1000 bootstrap replicates) (Tamura et al., 2021). The heuristic search was obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and Kimura 2-parameter model (Tamura, 1992), and then selecting the topology with superior log likelihood value. The highest log likelihood (-1671.82) and a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.1835)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 17.39% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Also, all positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option) (Kumar et al., 2018).

## Results

Morphological characters of *Sargassaceae* taxa identified in this study (Table 1, Fig. 2) from Kuwait concurred with the results of molecular identification. Genetically, our data set included sequences of the ITS2 region belonging to 8 different species obtained from a total of 67 samples collected. Alignment of the ITS2 sequences revealed  $\approx$  500–650 base pairs, including gaps. BLAST searches of sequences of all entities from Kuwait had a higher than 99.5% identity when comparing them with the closest matches in the NCBI database, except for *S. trinodis* (Table S3 in Supplementary Information).

Phylogenetic analysis (Fig. 3) was conducted using an alignment of the concatenated sequences from Kuwait, 31 published sequences of *Sargassaceae*, and three members of the *Fucaceae*. The alignment contained 294 nucleotide positions. Among the 12 sequences stemming from Kuwaiti specimens, five sequences belonged to the species *Sirophysalis trinodis*, *Hormophysa cuneiformis*, *Polycladia myrica* and seven sequences are related to five species of the subgenus *Sargassum*: *S. aquifolium*, *S. carpophyllum*, *S. ilicifolium* var. *acaraeocarpum*, *S. yinggehaiense*, and *Sargassum* sp., respectively.

## Discussion

### Morphological analysis

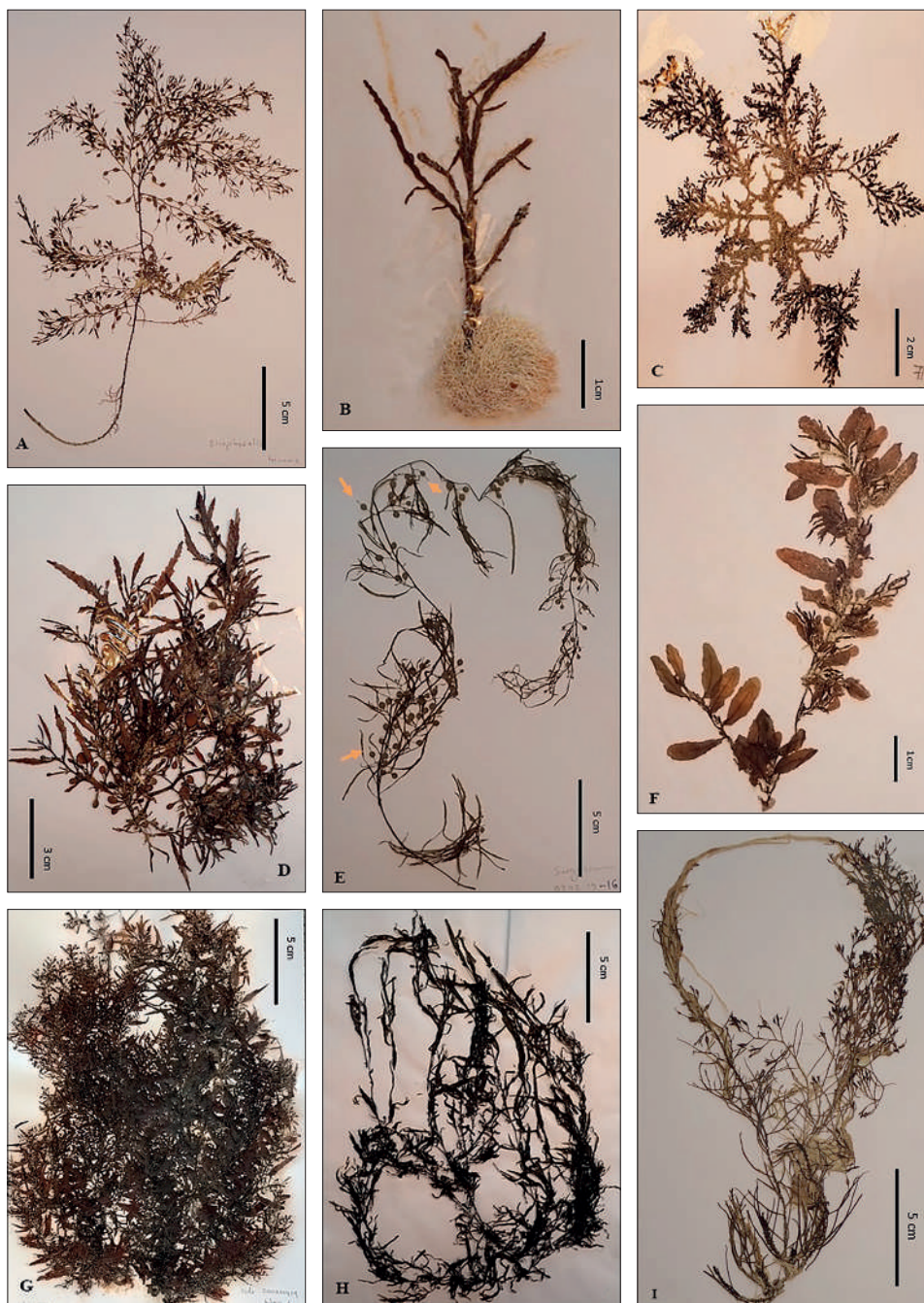
A revision based on morphology and DNA barcoding of *Sargassum* and the related genera *Sirophysalis*, *Turbinaria*, *Landsburgia*, *Carpophyllum*, and *Sargassopsis* for subtropical and temperate regions at the limits of the Indian Ocean was published recently (Dixon et al., 2012), but for much of the remaining tropics, the taxonomy of the family *Sargassaceae* is still ambiguous.

*Cystoseira trinodis* (Forsskål) C. Agardh (Fig. 2A) is currently regarded a synonym of *Sirophysalis trinodis* (Forsskål) Kützing (Guiry, Guiry, 2023). Its distribution ranges from the tropical western Indian Ocean into the west Pacific. The type locality for this taxon name is the Sinai Peninsula, Egypt, from where *Fucus trinodis* Forsskål has been described (Forsskål, 1775: 192). The distinct feature of specimens collected in this work includes spine-like outgrowths, with ultimate branchlets having spindle-shaped air bladders (Table 1). Often the primary branches of such species survive over the summer when frequently carrying heavy epiphytic growth of other filamentous brown algae (see Fig. 2I). *Hormophysa cuneiformis* (J.F. Gmelin) P.C. Silva, albeit in cases reported under its synonym *H. triquetra* (C. Agardh) Kützing (Lipkin, Silva, 2002), is widespread in warm waters in the Indo-West Pacific region and temperate waters (Guiry, Guiry, 2023). The etymology of the species name is from

Table 1. Morphological description of eight *Sargassaceae* morphotaxa identified in the present study

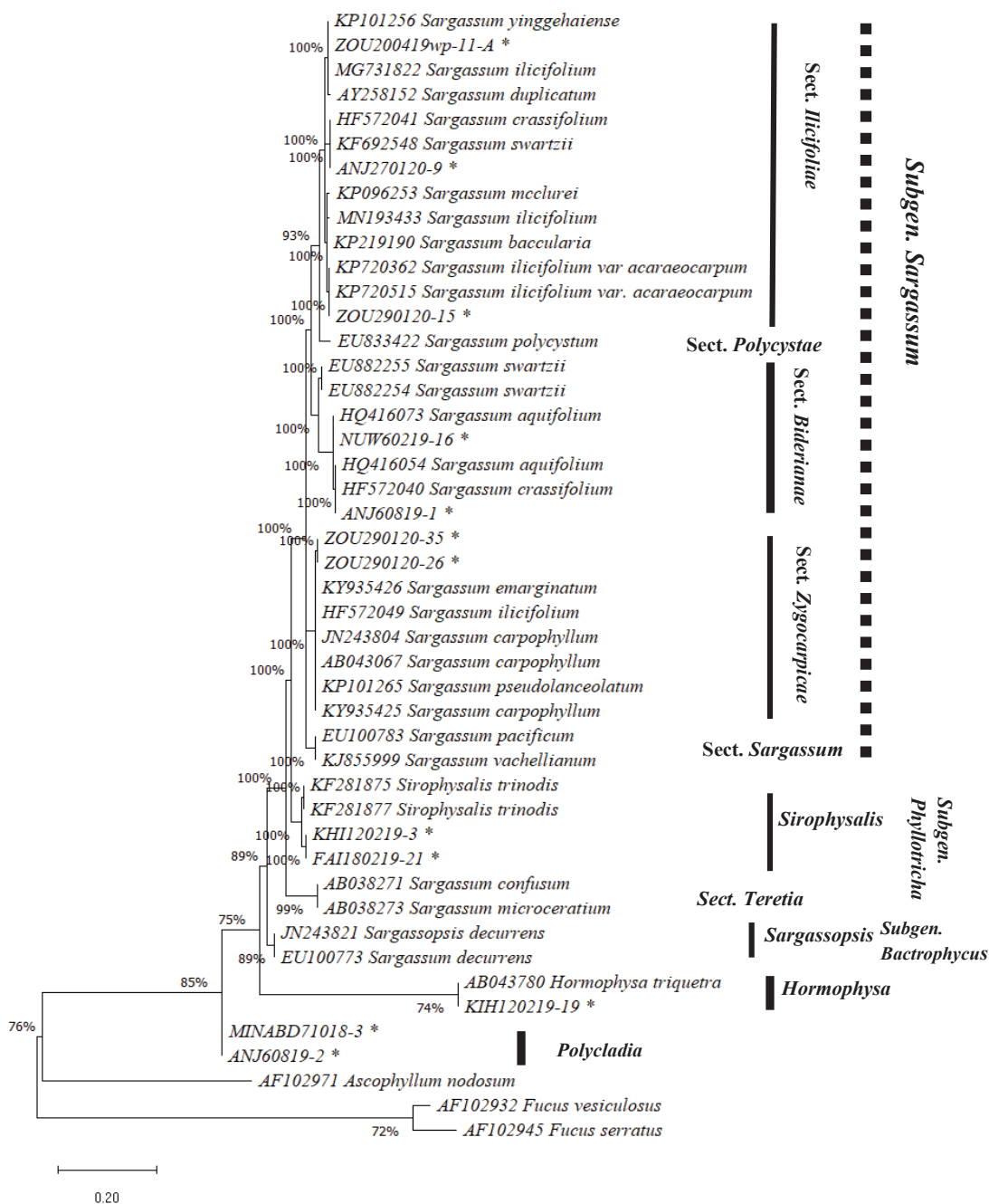
| Feature        | Morphotaxa  |   |  |  |
|----------------|---|---|--|--|
|                | <i>Sirophysalis trinodis</i> (Forsskål) Kützing (syn. <i>Cystoseira trinodis</i> (Forsskål) C. Agardh (Boisset, Ferrer-Gallego, 2015), collected from Failaka island; Fig. 2A | <i>Hormophysa cuneiformis</i> (J.F. Gmelin) P.C. Silva (syn. <i>Hormophysa triquetra</i> (C. Agardh) Kützing), collected from Khiran; Fig. 2B | <i>Polycladia myrica</i> (S.G. Gmelin) Draisma, Ballesteros, F. Rousseau & T. Thibaut (syn. <i>Cystoseira myrica</i> (S.G. Gmelin) C. Agardh, collected from Mina Abdulla; Fig. 2C | <i>Sargassum aquifolium</i> (Turner) C. Agardh (syn. <i>S. crassifolium</i> J. Agardh), collected from Nuwaiseeb; Fig. 2D  |
| Height         | About 35 cm in length   | Thalli approximately 6 cm high  | Up to 15 cm high   | Thalli up from 20 to 30 cm high  |
| Color          | Brown to yellowish brown  | Dark brown to blackish  | Dark brown to blackish-brown   | Light brown to yellowish-brown   |
| Holdfast       | Discoid   | Discoid   | Discoid  | Discoid  |
| Stipe (Axis)   | Terete, smooth, supple, 2 mm wide   | Terete, smooth, 3 mm in diameter  | Terete, many small spiny branchlets (5 mm in diameter) like bear shape   | Terete, smooth, 3–4 mm in diameter   |
| Primary branch | Bear spine-like growth alternately, clavate, densely with radical branched, 14 cm long  | Three winged, rigid, segmented, irregular branching   | Flattened, rigid – alternate to irregularly branched rise each side of axis 3 mm wide, 4 cm, branches fairly rigid and stiff   | Cylindrical, coriaceous, 2 mm wide, giving rise of many branchlets up to 5 cm long   |
| Blades         | Linear phylloid structures, smooth margined with leaf like appendages, 1mm wide, 2cm long   | Margins distinct dental, up to 3 cm long and up to 1 mm wide, narrow  | Small pinnate, alternately pinnate and the pinnae becoming gradually shorter towards apices, thus each branch has a triangular outline   | Lanceolate to oblanceolate, up to 5 cm long and up to 3-6mm width, usually with acute apex, margins dentate, midribs vanishing midway to distinct near apices, asymmetric base |
| Vesicles       | Spindle-ellipsoidal shaped (2 spheres in each brachlets), 1 mm wide, 3 mm long with thin mucro (spine)  | Irregularly pinnate or whorled  | Small spiny ellipsoidal or oval shaped various size from 1–2 mm wide, 2 mm long, resulting in intercalary aerocyst   | Spherical or ovate, smooth, some with thin mucro (spine). Pedicle long, mostly cylindrical, few flattened, variable in size from 1 to 7 mm wide                                |
| Receptacles    | Stalked lanceolate lateral on side branches up to 5 mm long and 1 mm wide   | Not obvious   | Stalked ellipsoidal, 4 mm long   | Receptacles lanceolate and terete in lower part, up to 5–15 mm long and 1 mm wide, arranged in racemes or in cymes   |

| Feature        | Morphotaxa   |   |   |   |
|----------------|--|---|---|---|
|                | <i>Sargassum carpophyllum</i> J. Agardh (syn. <i>S. pseudolanceolatum</i> Tseng & Lu), collected from Umm al-Maradim; Fig. 2E          | <i>Sargassum ilicifolium</i> var. <i>acaraeocarpum</i> Grunow, collected from Al-Zour; Fig. 2F  | <i>Sargassum yinggehaiense</i> Tseng & Lu, collected from Al-Zour; Fig. 2G                      | <i>Sargassum</i> sp., collected from Anjafa; Fig. 2H                                |
| Height         | Thallus approximately 30 cm high   | 30–40 cm high   | Up to 40 cm high and bushy  | Thalli up to 25 cm high   |
| Color          | Yellowish-brown  | Yellowish-brown   | Dark brown  | Dark greenish in color  |
| Holdfast       | Discoid, 1cm wide  | Discoid and conical, 4 mm wide  | Discoid   | Not observed  |
| Stipe (Axis)   | Terete, smooth   | Terete to compressed, smooth  | Terete, smooth  | Terete, compressed to flattened smooth, 2mm in diameter                             |
| Primary branch | Terete, smooth with 1 mm wide  | Asymmetrical margin up to 3 mm wide   | Thick elongated ellipsoidal, mostly rounded, 2 mm width   | Compressed, smooth, 2 mm, giving rise of many branchlets up to 5 cm long            |
| Blades         | Linear-dentate margins and acute apex up to 4 cm long and up to 1 mm wide  | Lanceolate to elliptical, with acute or obtuse apex relatively thick in texture. Margins are smooth, up to 2–3cm long and up to 5mm width | lanceolate, acute tips and irregularly serrulate margins, up to 3–4 cm long and up to 5 mm wide | Elongated lanceolate, up to 3 cm long and up to 2 mm wide, narrow pedicel long base |
| Vesicles       | Spherical or ovate, smooth, some with thin mucro (spine). Pedicle long, mostly cylindrical, few flattened, various in size 2–4 mm wide | Spherical to ovate, smooth. Pedicles cylindrical or foliar, various in size from 2 to 3 mm width  | Ovoid, with thin or foliar mucro. Pedicle cylindrical or foliar, 2 mm wide                      | Elliptical globular or ovate, smooth, various size from 1–2 mm width, 2 mm long     |
| Receptacles    | Lanceolate, up 3 mm long and 1 mm wide, arranged in cymes  | Linear, margins can be smooth or with spine-like protuberances, up to 10 mm long and 2 mm wide. Arranged in cymes or racemes              | Linear, obtuse or acute apex, petiolate or in cymes, up 2 mm long and 1 mm wide                 | Slightly terete, small spines at the apices and margins                             |



**Fig. 2.** A: *Sirophysalis trinodis* (FAI180219-21, Failaka Island, collected by A.F. Peters, F.C. Küpper, A.H. Hasan); B: *Hormophysa cuneiformis* (KHI120219-19, Khiran, collected by A.F. Peters, F.C. Küpper, A.H. Hasan); C: *Polycladia myrica* (MINABD71018-3, Mina Abdulla, collected by A.H. Hasan); D: *Sargassum aquifolium* (NUW60219-16, Nuwaiseeb collected by A.F. Peters, F.C. Küpper, A.H. Hasan); E: *S. carpophyllum* (arrows show thin mucro (spine) (UMM70219-16, Umm Al-Maradim Island, collected by A.F. Peters, F.C. Küpper, A.H. Hasan, D. Al-Bader); F: *S. ilicifolium* var. *acaraeocarpum* (ZOU290120-15, Al-Zour, collected by A.H. Hasan); G: *S. yinggehaiense* (KD14-ZOU200419wP-11-A, Al-Zour, collected by D. Al-Bader); H: *Sargassum* sp. (ANJ270120-9, Anjafa, collected by A.H. Hasan); I: *Sirophysalis trinodis* with filamentous brown epiphytes (KUB210219-12, Kubbar Island, collected by A.F. Peters, F.C. Küpper, D. Al-Bader)





**Fig. 3.** Phylogenetic tree showing positions of 12 sequenced taxa of Sargassaceae from Kuwait based on a dataset of 34 ITS2 nucleotide sequences retrieved from GenBank using Maximum Likelihood and Tamura2-parameter model (Tamura, 1992). Numbers near nodes represent bootstrap support (1000 replicates). Branches with no values have less than 50 % bootstrap support. Sample codes typed with star signs indicate *Sargassum* sp. collected for this framework. Some species were not included in the tree of Fig. 3 because the ITS2 sequence was identical to that of an already included species. *Note:* this tree covers: two specimens of *Sirophysalis trinodis*, one of *Hormophysa cuneiformis*, two of *Polycladia myrica*, two of *Sargassum aquifolium*, two of *S. carpophyllum*, one of *S. ilicifolium* var. *acaraeocarpum*, one of *S. yinggehaiense*, one of unclear *Sargassum* sp.

Latin, meaning 'wedge-shaped' or narrow below and wide above (Silva et al., 1987). This finding concurs with the morphology of our samples – including that branches are three-winged, fairly rigid, more or less segmented, and margins distinctly dentate (Table 1). In addition, some individuals survive over the summer, but several branches are lost in autumn leaving only the basal branches that frequently carry abundant epiphytes (see Fig. 2B). *Polycladia myrica* (S.G. Gmelin) Draisma, Ballesteros, F. Rousseau & T. Thibaut (Fig. 2C) is regarded as a currently accepted name, with a synonym *Cystoseira myrica* (S.G. Gmelin) C. Agardh, including *C. myrica* var. *tenella* Endlicher & Diesing, which is distributed in the Mediterranean Sea, western India to the Arabian Peninsula, and farther extends to the South African east coast (Draisma et al., 2010; Guiry, Guiry, 2023). Other characters to distinguish our samples are erect, rigid branches, covered by numerous, short, rigid spines; branches are alternately pinnate with the pinnae becoming gradually shorter towards the apices; each branch has a triangular outline and spiny oval air bladders are often present (Table 1). The perennial basal portion and main branches bear many small spiny branchlets and air bladders which are cast ashore when lost during autumn. All the aforementioned genera were well documented by several authors in the Gulf and Kuwait as locally abundant in the Gulf region (Jones, 1986; Al-Hasan, Jones, 1989; Basson, 1992; Silva et al., 1996; De Clerck, Coppejans, 1996; John, 2012; John, Al-Thani, 2014; Al-Yamani et al., 2014; Kokabi, Yousefzadi, 2015).

*Sargassum* species are characterized by leaf-like branchlets (hereafter leaves), that distinctly differentiate from a cylindrical or compressed, never foliar, axis (Mattio et al., 2009; Table 1). The first species, *S. aquifolium* (Turner) C. Agardh (Fig. 2D), is well documented by several authors in the Gulf and Kuwait (Jones, 1986; Al-Hasan, Jones, 1989; Basson, 1992; De Clerck, Coppejans, 1996; Silva et al., 1996; Abdel-Kareem, 2009; John, 2012; John, Al-Thani, 2014; Al-Yamani et al., 2014; Shams et al., 2015; Kokabi, Yousefzadi, 2015; Alghunaim et al., 2019a, b, 2020), albeit in cases under its synonym *S. crassifolium* (Turner) J. Agardh (Silva

et al., 1996). Commonly, its main habitat in the Gulf are exposed reef flats and subtidal zones (Shams et al., 2015). The second species identified here, *S. carpophyllum* J. Agardh (Fig. 2E), grows in the lower parts of the intertidal zone on rocky substratum or in the upper subtidal zone in the Gulf (John, 2012). Synonyms are *S. emarginatum* C.K. Tseng & Lu Baroen and *S. pseudolanceolatum* Tseng & Lu (Tseng, Lu, 2002a), respectively, which are both reported from southern China (Abbott, McDermid, 2002). *Sargassum carpophyllum* was the species most frequently encountered in our study with 49 (73%) samples out of 76 in total thalli, found washed ashore or drifting in the lower intertidal zone in the Southern Provinces of Kuwait. Out of several papers mentioning *S. carpophyllum*, none of them are related to the Gulf. Thus, observations of this species in the present study constitute a new record for the Gulf and Kuwait. The third species, *S. ilicifolium* var. *acaraeocarpum* Grunow (originally described "*acaraeocarpa*"; Fig. 2F), is mentioned by only two publications (Silva et al., 1996; Mattio et al., 2015b), in addition to a recent paper from our group related to Kuwait (Al-Adilah et al., 2020). The fourth *Sargassum* species detected in Kuwait is *S. yinggehaiense* Tseng & Lu (Fig. 2G) which was initially described by Tseng and Lu Baoren (2002b) from Yinggehai Beach, Hainan Island (China) as the type locality where it grows on rocks in the lower intertidal and upper subtidal zones (Tseng, Lu, 2002b; Guiry, Guiry, 2023). In Kuwait, this species was collected on a single occasion, cast onto the shore at Al-Zour (Al-Bader et al., unpublished paper). Elsewhere in the world, this taxon has only been mentioned from Yinggehai Beach, Hainan Island, China, by Tseng and Lu (2002b). Thus, *S. yinggehaiense* represents a new record for the Gulf and Kuwait. The fifth species was collected on a single occasion in Anjafa (central coast of Kuwait). Morphologically, it is characterized by a dark green color, and can be separated from other taxa by flattened stems, versus cylindrical to compressed stems. However, identification of this species is still problematic and is therefore only designated *Sargassum* sp. here. It needs further study (see below).

For five species, we obtained identifications differing from prior records (e.g. Al-Yamani et al., 2014) except for *S. aquifolium*. Two species, *S. natans* (L.) Gaillon (Basson, 1992; Silva et al., 1996; John, Al-Thani, 2014) and *S. fluitans* Børgesen (Silva et al., 1996; John, Al-Thani, 2014; Al-Yamani et al., 2014), are Atlantic species which were originally described from the tropical Atlantic Ocean floating in the Sargasso Sea (Louime et al., 2017). Either were not collected in this study, they may have been an ephemeral introduction or were improperly identified. None of our sequences match the reference sequence of *S. fluitans* (KM461674, or KM461675) from USA; North Carolina, Gulf of Mexico, respectively. However, *S. ilicifolium* var. *acaraeocarpum* (our specimens ZOU290120-15 and ZOU200419wP-12-c) agrees morphologically with a thallus of *S. fluitans* in Al-Yamani et al. (2014). There is no ITS2 sequence for *S. angustifolium* C. Agardh nor *S. asperifolium* Hering & G. Martens ex J. Agardh in GenBank. Furthermore, *S. carpophyllum* thalli which were collected in this study have the same morphology as *S. asperifolium* in Al-Yamani et al. (2014). Alternatively, it could be that several species which were covered in previous inventories of Kuwait are synonyms or misidentifications due to the failure to account for the full range of morphological plasticity within each taxon (see Mattio et al., 2013; Low, Chou, 2013; Yip et al., 2018). Since it is clear that none of the targeted collections within the framework of this study belong to any Atlantic species, all the specimens collected here fall within the section *Sargassum*, which is the main group containing the representative of the genus in the Indo-West Pacific region. In this respect, *Sargassum* is one of the most complicated genera with regard to implementing alpha taxonomy, due to the high degree of morphological divergence and high level of adaptation to particular environments (Kantachumpoo et al., 2015).

Our study clearly confirmed the presence of fewer *Sargassaceae* species in Kuwait in comparison with previous reports (Al-Yamani et al., 2014). This could be related to the short coastline of Kuwait,  $\approx$  300 km (Al-Yamani et al., 2004), compared with the total Gulf coastline of about 5930 km (John, Al-Thani, 2014) in combination with extremely harsh

physical conditions (i.e. a large seasonal temperature amplitude 23 °C; Al-Rashidi et al., 2009) resulting in lower diversity of *Sargassaceae* than in other parts of the tropical Indo-West Pacific with more benign conditions and a vastly larger area (Price et al., 2006; Sheppard, 2010; John, Al-Thani, 2014). The most elaborate multi-gene phylogenetic analysis of the *Sargassaceae* is that by Draisma et al. (2010). Mattio et al. (2015b) reported 11 species of *Sargassum* sp., including seven new records for Madagascar, based on multi-gene phylogeny and morphological features. Similarly, Kantachumpoo et al. (2015) investigated the phylogenetic relationships and species boundaries of eight *Sargassum* species from Thailand using morphological characteristics and ITS2 gene sequences. In Singapore, a total of six species were delineated based on morphological characteristics and molecular clades based on phylogenetic analyses of three loci (Yip et al., 2018). Even though the diversity of *Sargassaceae* species in Kuwait varied from one sampling site to another, *Sargassum* species were the key canopy-forming seaweeds in the Southern Province. *Sargassum* species are sensitive to anthropogenic activities (Phillips, Blackshaw, 2011; Yip et al., 2018) which may explain the scarcity of records from Kuwait Bay and the Northern Province within the framework of the present study. Also, it seems that the nature of the Northern Province, i.e. with a coastline scarce of hard rocky substrates and most of the intertidal and sublittoral covered with mud flats, may limit the establishment of *Sargassaceae* members because early stages of these algae need hard substratum for attachment (John, 1986; Al-Hasan, Jones, 1989; Alghunaim et al., 2019a). In contrast, the Southern Province has beaches scattered with rocks that are suitable for holdfast attachment (Al-Yamani et al., 2004). Members of the *Sargassaceae* are prone to remarkable changes in morphology when defoliation occurs following fruiting. John (2012) pointed out that in the Arabian Gulf, only the basal parts of *H. cuneiform* thalli often survive over the summer and new branches rapidly grow from these early in the autumn. For Kuwait, also in autumn, large masses of *S. aquifolium* are dominant and drifting ashore in the entire territory, before it becomes 'defoliated' in late winter (Al-Hasan, Jones,

1989). In line with these observations, the present study was conducted from late autumn (October 2018) until January 2020. It has to be emphasized that the scope of the present study did not address the ecology or seasonality of *Sargassum*.

### Molecular phylogenetic analyses

Overall, the present study demonstrated that the family *Sargassaceae* in Kuwait is represented by four genera: *Sirophysalis*, *Hormophysa*, *Polycladia*, and *Sargassum*, which are considered the "basal taxa" in the family *Sargassaceae* (Draisma et al., 2010). The results presented here are based upon the sequencing results obtained within the framework of this study, which were aligned and used for the molecular phylogenetic analyses described here. *Sargassum* species are clearly polyphyletic (with sister taxa of well-supported clades) – in other words, polyphyletic genera are included under the same clade name (Mattio, Payri, 2011; Kantachumpoo et al., 2015). Based upon sequencing results, an entire ITS2 sequence obtained from our present study (KHI200219-3, FAI180219-21) matches with a published sequence of *S. trinodis* from Australia (KF281875) with 99.28% homology (i.e., five substitutions). This difference can be interpreted as intraspecific variation. With 612 bp, this species had the longest ITS2 among samples from Kuwait. The sequences of the genus *S. trinodis* (KHI200219-3, FAI180219-21) clustered monophyletically in the genus *Sirophysalis*, supporting the notion that *Sirophysalis* is a sister genus to the clade of the subgenus *Sargassum* and that the nearest related neighbors form a monophyletic group sister to the genera *Sargassopsis*, *Polycladia*, *Hormophysa*, respectively. The ITS2 sequence (KHI120219-19) is identical to a published sequence (AB043780), under the name *H. triquetra* from France, except for a 155 bp long insertion of short repetitive motifs. Sequences of additional samples will be required to show if this insertion is common in *H. cuneiformis* from the Gulf (which, interestingly, does not occur in *Polycladia myrica*). Therefore, taking ITS2 sequences in GenBank into account, sequences ANJ60819-2 and MINABD71018-3 correspond to *P. myrica* – a taxon which is solely described

morphologically in several publications from the Gulf as mentioned earlier (which so far does not have an ITS sequence in GenBank), constituting genetically a new record for Kuwait and the Gulf as well. It does not appear to cluster with any branch including those referred to as the "basal taxa" in the family *Sargassaceae*.

In addition, we highlighted the occurrence of 5 distinct *Sargassum* taxa well supported in clades in this tree (Fig. 3). Generally, *Sargassum* is currently subdivided into eight lineages or clades of taxa: *Sargassum* sect. *Sargassum*, *Zygo carpicae*, *Polycystae*, *Ilicifolia*, *Binderiana*, *Johnstonii*, *Lapazaenum*, and *Sinicola* (Mattio, Payri, 2011). The results of the present phylogenetic analysis showed that in Kuwait, *Sargassum* species belong to three of the aforementioned clades, namely *Ilicifolia*, *Binderiana*, and *Zygo carpicae*. Our results suggest that *Sargassum* subgen. *Sargassum* lineages or clades of taxa, which formed and nested sister groups to *Sargassum* subgen. *Arthro phycus* and *Sargassum* subgen. *Bactro phycus*, is sister to those above. Sequences of NUW60219-16 and ANJ60819-1 are identical (100%) to the published sequences for *S. aquifolium* (HQ416054 and HQ416073) from Tanzania, France, respectively, and clustered with *S. aquifolium* in the subgenus *Sargassum* clade of sect. *Binderiana*. The ITS2 of ZOU290120-26 and ZOU290120-35) show 99.61% homology to sequences present under four different species identifications in GenBank: JN243804 from Australia (*S. carpophyllum*), KY935426 from China (*S. emarginatum*), KP101265 from China (*S. pseudolanceolatum*), suggesting that the latter two are synonyms of the former, as well as HF572049 from Iran (*S. ilicifolium*), which was likely a misidentification. Together our data show that this species, which is common in Kuwait, belongs to *S. carpophyllum* or a closely related species. Previously, it may have been treated in Kuwait as *S. angustifolium* or *S. asperifolium* (Al-Yamani et al., 2014). There are no ITS2 sequences for any of these two taxa in GenBank. We adopt a conservative approach and classify this entity in *S. carpophyllum*. Sequences ZOU290120-26 and ZOU290120-35 sequences cluster in the same clade as *S. pseudolanceolatum* and *S. emarginatum*. All our ITS2 sequences cluster in the same clade

as *S. pseudolanceolatum* and *S. emarginatum*. Furthermore, the ITS2 sequences of *S. carpophyllum* and *S. pseudolanceolatum* are identical, but they differed in two positions from the published sequences of *S. carpophyllum*, *S. pseudolanceolatum*, and *S. emarginatum*. Thus, this species belongs to the section *Zygocarpicae*. Overall, three *Sargassum* species investigated here clustered genetically within sect. *Ilicifolia*. The sequence from ZOU290120-15 obtained here is identical (100%) to that of *S. ilicifolium* var. *acaraeocarpum* from Madagascar (KP720362), which suggests that they belong to the same species. The significant genetic distance from *S. ilicifolium* from Indonesia (98.68%) with accession MN193433 suggests that treating this entity as mere variation of *S. ilicifolium* may be incorrect, and that its affiliation to *S. ilicifolium* var. *acaraeocarpum* would be justified. The ITS2 sequence obtained from KD14-ZOU200419wP-11-A shows 100% identity with *S. yinggehaiense* (GenBank entry KP101256 from China), which suggests that the material of this study belongs to this species. The identification of specimen ANJ270120-9 as *S. swartzii* C. Agardh is based on high sequence homology (99.83%) and is nested with sequence KF692548 of *S. swartzii* from India. Although *S. swartzii* was previously identified from the Gulf based on morphology (Basson, 1992; Silva et al., 1996; John, Al-Thani, 2014; Shams et al., 2015), this sequence is important because the type locality of *S. swartzii* is India (Mattio et al., 2010; Guiry, Guiry, 2023). This sequence (KF692548) is of doubtful identity, because none of the 15 other ITS2 sequences of *S. swartzii* available in GenBank from other parts of the world came out as a close match with ANJ270120-9. Additionally, *S. swartzii* is classified under sect. *Binderiana* of the genus *Sargassum* (Mattio et al., 2010, 2013), as do *S. swartzii* sequences EU882254 (Mattio et al., 2008, 2013) and EU882255 (Mattio et al., 2010) from New Caledonia (see Table S4 in Supplementary Information). However, sequence ANJ270120-9 clusters with *S. swartzii* (KF692548) and is under sect. *Ilicifolia*. It seems that the reference sequence of *S. swartzii* (a sample from India) is misidentified and not in a suitable clade (and does not match, e.g. with a sample from New Caledonia). Therefore, ANJ270120-9 may correspond to another species

of *Sargassum* sp., probably most closely related to *Sargassum* sp. from Iran with sequence homology (99.81%; GenBank accession HF572033) rather than *S. swartzii* and will need more study.

However, significantly, the lack of genetic variation observed in Arabian / Persian Gulf members of the family *Sargassaceae*, as well as their overlapping morphologies, suggests that fewer species are actually present than currently recognized. Due to the relatively small sample size and the lack of genetic diversity across one marker (our study) or all three markers tested in others studies, for example, a range of other markers such as the mitochondrial 5'-*Cox1*, *COI*, mt23S and adjacent spacer (Draisma et al., 2010; Mattio et al., 2010) and the chloroplast *rbcl* and *psaA* (Cho et al., 2012) have been tested on closely-related species of *Sargassaceae* but likewise did not provide better resolution than the ITS2 locus. We therefore believe that the present study provides sufficient insights for a first molecular assessment of the diversity of *Sargassaceae* in Kuwait.

In conclusion, the present work confirms that the nuclear ribosomal internal transcriber spacer 2 (ITS2) elucidates phylogenetic relationships of the *Sargassaceae* family including *Sargassum* species boundaries from Kuwait using morphological characteristics, which could provide fundamental data for further studies related to species-level resolution in a poorly studied region like the Arabian Gulf and Kuwait.

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## Ethics Declaration

The authors declare no conflict of interest.

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## Supplementary Information

Table S1. Collection sites and habitat of specimens of the *Sargassaceae* in Kuwait

Table S2. Primer sequences used for amplification of the *Sargassaceae* specimens in this study

Table S3. Specimens of the *Sargassaceae* collected from Kuwait, grouped according to the closest ITS2 sequence match in GenBank. Note that there is no previously published ITS2 sequence of *Polycladia myrica*

Table S4. ITS2 sequences of the *Sargassaceae* retrieved from GenBank for use in phylogenetic analysis

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**Перегляд родини *Sargassaceae* (*Fucales*, *Phaeophyceae*) Кувейту (Аравійська / Перська затока, північний захід Індійського океану) на основі аналізу морфології та послідовностей ITS2**

**Реферат.** У статті наведено результати досліджень водоростей родини *Sargassaceae* Кувейту на основі аналізу послідовностей ядерного спейсера ITS2, виділених із 67 зразків, зібраних у 2018–2020 роках. Морфологічна характеристика та штрихкодування ДНК підтвердили наявність восьми таксонів, які належать до родини *Sargassaceae* – п'яти видів роду *Sargassum* і трьох видів інших родів, а саме *Sirophysalis trinodis*, *Hormophysa cuneiformis* і *Polycladia myrica*. Для останнього виду доступних послідовностей ITS2 ще не було відомо. Що стосується роду *Sargassum*, нами підтверджена меншість із раніше зареєстрованих його видів. На основі 100%-ої ідентичності послідовностей наших зразків і знахідок з інших регіонів ми визнаємо *S. aquifolium*, *S. ilicifolium* var. *acaraeocarpum*, *S. yinggehaiense* та один поки що невизначений вид (*Sargassum* sp.). Відповідно до аналізу послідовностей, п'ятим і найпоширенішим видом роду *Sargassum* у Кувейті є *S. carpophyllum*, раніше ідентифікований як *S. asperifolium*. Проте повного співпадання цієї послідовності ITS2 з жодним із відомих секвенованих зразків *S. carpophyllum* виявлено не було. Порівняно невисоке різноманіття видів роду *Sargassum* у Кувейті може бути наслідком доволі екстремальних умов навколишнього середовища, зокрема значної амплітуди температур від +14 °C взимку до +37 °C влітку.

**Ключові слова:** *Sargassum*, *Phaeophyceae*, генетичний аналіз, Кувейт, таксономія, фенотип, філогенія, ядерний спейсер ITS2

## Supplementary Information

**Table S1. Collection sites and habitat of specimens of the *Sargassaceae* in Kuwait**

| Number on map           | Location collection | Date       | Coordinates                | Habitat                         | Surface sea water temperature |
|-------------------------|---------------------|------------|----------------------------|---------------------------------|-------------------------------|
| <b>Offshore Islands</b> |                     |            |                            |                                 |                               |
| A                       | Failaka             | 18.02.2019 | 29°28'03.8"N 48°17'10.1"E  | Rocky shore and tidal pool      | 16.2 °C                       |
| B                       | Kubbar              | 21.02.2019 | 29°04'18"N, 48°29'30"E     | Rocky shore and intertidal pool | 16.1 °C                       |
| C                       | Umm Al-Muradim      | 7.02. 2019 | 28°40'49.8"N, 48°39'00"E   | Subtidal and intertidal         | 16.5 °C                       |
| <b>Middle zone</b>      |                     |            |                            |                                 |                               |
|                         |                     | 6.08.2019  | 29°16'42.2"N 48°05'21.7"E  | Rocky shore                     | 31.9 °C                       |
| 1                       | Anjafa              | 27.01.2020 | 29°16'42.2"N 48°05'21.7"E  | Rocky shore                     | 16.7 °C                       |
| 2                       | Fintas*             | 17.02.2019 | 29°10'37.9"N 48°07'23.0"E  | Rocky shore near Coastguard     | 19.9 °C                       |
| <b>South zone</b>       |                     |            |                            |                                 |                               |
| 3                       | Mina Abdulla        | 7.10.2018  | 28°58'47.1"N, 48°10'24.9"E | Drifting to shoreline           | 30.1 °C                       |
| 4                       | Al-Khiran           | 12.02.2019 | 28°39'18"N, 48°23'31.4"E   | Drifting to shoreline           | 16.0 °C                       |
|                         |                     | 20.04.2019 | 28°41'49.6"N, 48°22'47.0"E | Drifting to shoreline           | 22.3 °C                       |
| 5                       | Al-Zour (1)         | 29.01.2020 |                            | Drifting at entrance to canal   | 17.4 °C                       |
| 6                       | Al-Zour (2)         | 13.02.2019 | 28°41'09.5"N, 48°23'14.2"E | Drifting to shoreline           | 16.1 °C                       |
| 7                       | Al-Nuwiseb          | 6.02. 2019 | 28°35'00"N, 48°23'56.8"E   | Drifting to shoreline           | 16.1 °C                       |

\* Repeated specimens (e.g., *Sargassum carpophyllum* J. Agardh)

<https://seatemperature.info/kuwait-water-temperature.html>

**Table S2. Primer's sequence (~500-650 bp) used for amplification of *Sargassaceae* specimens in this study**

| Locus   | Marker | Oligo nucleotide primers (F/R) | Annealing DNA | Primer Sequence (5' to 3') | Amplicon Size (bp) | Reference          |
|---------|--------|--------------------------------|---------------|----------------------------|--------------------|--------------------|
| Nuclear | ITS2   | KP5 (F)                        | 5.8S          | ACAACGATGAAGAACGCAG        | >200 to <1000      | Lane et al. (2006) |
|         |        | KG4 (R)                        | LSU           | CTTTTCCTCCGCTTAGTTATATG    |                    |                    |

Table S3. Specimens of *Sargassaceae* collected from Kuwait, grouped according to the closest ITS2 sequence match in GenBank  
 Note that there is no previously published ITS2 sequence of *Polycladia myrica*

| Entity | Taxon identification according to GenBank   | Herbarium vouchers    | Query ID (bp) | Coverage % | Identity % | Accession no. of closest match | GenBank accession no. |
|--------|---|-----------------------|---------------|------------|------------|--------------------------------|-----------------------|
| 1      | <i>Sirophysalis trinodis</i> (Forsskål 1775) Kützing                                  | KHI120219-3           | 699           | 91         | 99.28      | KF281875                       | MZ409024              |
| 2      | <i>Sirophysalis trinodis</i> (Forsskål 1775) Kützing                                  | FAI180219-21          | 699           | 98         | 99.28      | KF281875                       | MZ409025              |
| 3      | <i>Hormophysa cuneiformis</i> (J.F. Gmelin1792) P.C. Silva                            | KHI120219-19          | 636           | 70         | 100        | AB043780                       | MZ409023              |
| 4      | <i>Polycladia myrica</i> (S.G. Gmelin) Draima, Ballesteros, F. Rousseau & T. Thibaut. | ANJ60819-2            | 509           | NA         | NA         | NA                             | MZ409027              |
| 5      | <i>Polycladia myrica</i> (S.G. Gmelin) Draima, Ballesteros, F. Rousseau & T. Thibaut. | MINABD71018-3         | 505           | NA         | NA         | NA                             | MZ409026              |
| 6      | <i>Sargassum aquifolium</i> (Turner) C. Agardh  | ANJ270120-10          | 597           | 74         | 100        | HQ416054                       | MZ409015              |
| 7      | <i>Sargassum aquifolium</i> (Turner) C. Agardh  | NUW60219-16           | 583           | 75         | 100        | HQ416073                       | MZ409013              |
| 8      | <i>Sargassum aquifolium</i> (Turner) C. Agardh  | KHI120219-27          | 635           | 69         | 100        | HQ416054                       | MZ409014              |
| 9      | <i>Sargassum aquifolium</i> (Turner) C. Agardh  | ANJ60819-1            | 636           | 69         | 100        | HQ416054                       | MZ409012              |
| 10     | <i>Sargassum carpophyllum</i> J. Agardh   | NUW60219-17           | 600           | 84         | 99.61      | AB043067                       | MZ409016              |
| 11     | <i>Sargassum carpophyllum</i> J. Agardh   | KHI120219-24          | 600           | 84         | 99.61      | AB043067                       | MZ409017              |
| 12     | <i>Sargassum carpophyllum</i> J. Agardh   | ZOU290120-35          | 561           | 86         | 99.59      | AB043067                       | MZ409021              |
| 13     | <i>Sargassum carpophyllum</i> J. Agardh   | ZOU290120-25          | 576           | 72         | 99.52      | KY935425                       | MZ409022              |
| 14     | <i>Sargassum carpophyllum</i> J. Agardh   | KD10_ZOU200419wP-12-e | 582           | 86         | 99.60      | AB043067                       | MZ447878              |
| 15     | <i>Sargassum carpophyllum</i> J. Agardh <sup>1</sup>                                  | ZOU290120-26          | 553           | 99         | 99.64      | KP101265                       | MZ409019              |
| 16     | <i>Sargassum carpophyllum</i> J. Agardh <sup>1</sup>                                  | ZOU130219-6           | 559           | 100        | 99.64      | KP101265                       | MZ409018              |
| 17     | <i>Sargassum carpophyllum</i> J. Agardh <sup>1</sup>                                  | KUB210219-M           | 589           | 100        | 99.66      | KP101265                       | MZ409020              |
| 18     | <i>Sargassum ilicifolium</i> var. <i>acaraeocarpum</i> Grunow                         | KD8_ZOU200419wP-12-c  | 588           | 99         | 99.49      | KP096253                       | MZ447876              |
| 19     | <i>Sargassum ilicifolium</i> var. <i>acaraeocarpum</i> Grunow                         | ZOU290120-15          | 622           | 73         | 100        | KP720362                       | MZ409028              |
| 20     | <i>Sargassum yinggehaiense</i> Tseng & Lu   | KD14_ZOU200419wP-11-A | 563           | 99         | 100        | KP101256                       | MZ447877              |
| 21     | <i>Sargassum</i> sp.  | ANJ270120-9           | 603           | 98         | 99.83      | KF692548                       | MZ409011              |

<sup>1</sup> Sequence deposited in GenBank as *S. pseudolanceolatum* Tseng & Lu but identical to the sequences of *S. carpophyllum* J. Agardh

Table S4. ITS2 sequences of *Sargassaceae* retrieved from GenBank for use in phylogenetic analysis

| Taxa/entity                                     | GenBank Accessions no. | Collection site          | Collectors                  |
|---|------------------------|--------------------------|-----------------------------|
| <i>Hormophysa triquetra</i>                     | AB043780               | New Caledonia, France    | Stiger and Horiguchi (2000) |
| <i>Sargassopsis decurrens</i>                   | JN243821               | Australia                | Dixon et al. (2012)         |
| <i>Sargassum aquifolium</i>                     | HQ416054               | Tanzania                 | Mattio and Payri (2010)     |
| <i>Sargassum aquifolium</i>                     | HQ416073               | Reunion                  | Mattio and Payri (2010)     |
| <i>Sargassum baccularia</i>                     | KP219190               | Thailand                 | Kantachumpoo et al. (2014)  |
| <i>Sargassum carpophyllum</i>                   | JN243804               | Australia                | Dixon et al. (2011)         |
| <i>Sargassum carpophyllum</i>                   | AB043067               | Nagasaki, Japan          | Stiger et al. (2000)        |
| <i>Sargassum carpophyllum</i>                   | KY935425               | China                    | Huang et al. (2017)         |
| <i>Sargassum crassifolium</i>                   | HF572040               | Iran                     | Shams et al. (2013)         |
| <i>Sargassum crassifolium</i>                   | HF572041               | Iran                     | Shams et al. (2013)         |
| <i>Sargassum confusum</i>                       | AB038271               | Japan                    | Yoshida et al. (2000)       |
| <i>Sargassum decurrens</i>                      | EU100773               | New Caledonia, France    | Mattio et al. (2008)        |
| <i>Sargassum duplicatum</i>                     | AY258152               | Taiwan                   | Chen and Lee (2003)         |
| <i>Sargassum emarginatum</i>                    | KY935426               | China                    | Huang et al. (2017)         |
| <i>Sargassum ilicifolium</i>                    | MG731822               | Singapore                | Yip et al. (2018)           |
| <i>Sargassum ilicifolium</i>                    | MN193433               | Indonesia                | Dharmayanti et al. (2019)   |
| <i>Sargassum ilicifolium</i>                    | HF572049               | Iran                     | Shams et al. (2013)         |
| <i>Sargassum ilicifolium var. acaraeocarpum</i> | KP720362               | Madagascar               | Mattio et al. (2015)        |
| <i>Sargassum ilicifolium var. acaraeocarpum</i> | KP720515               | South Africa             | Mattio et al. (2015)        |
| <i>Sargassum integerrimum</i>                   | KP096254               | China                    | Liu et al. (2014)           |
| <i>Sargassum mcclurei</i>                       | KP096253               | China                    | Liu et al. (2014)           |
| <i>Sargassum pacificum</i>                      | EU100783               | French Polynesia, France | Mattio et al. (2008)        |
| <i>Sargassum polycystum</i>                     | EU833422               | Fiji                     | Mattio et al. (2009)        |
| <i>Sargassum pseudolanceolatum</i>              | KP101265               | China                    | Liu et al. (2014)           |
| <i>Sargassum swartzii</i>                       | KF692548               | India                    | Deshmukhe et al. (2013)     |
| <i>Sargassum swartzii</i>                       | EU882255               | New Caledonia, France    | Mattio et al. (2008)        |
| <i>Sargassum swartzii</i>                       | EU882254               | New Caledonia, France    | Mattio et al. (2008)        |
| <i>Sargassum vachellianum</i>                   | KJ855999               | China                    | Bi et al. (2014)            |
| <i>Sargassum yinggehaiense</i>                  | KP101256               | China                    | Liu et al. (2014)           |
| <i>Sirophysalis trinodis</i>                    | KF281875               | Australia                | Dixon et al. (2014)         |
| <i>Sirophysalis trinodis</i>                    | KF281877               | Australia                | Dixon et al. (2014)         |
| <b>Outgroup</b>                                 |                        |                          |                             |
| <i>Ascophyllum nodosum</i>                      | AF102971               | Isle of Man              | Serrao et al. (1999)        |
| <i>Fucus serratus</i>                           | AF102945               | Nova Scotia, Canada      | Serrao et al. (1999)        |
| <i>Fucus vesiculosus</i>                        | AF102932               | White Sea, Russia        | Serrao et al. (1999)        |