

# *Kuwaitiella rubra* gen. et sp. nov. (Bangiales, Rhodophyta), a new filamentous genus and species from the north-western Indian Ocean

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## SUMMARY

A new filamentous marine red alga, *Kuwaitiella rubra* gen. et sp. nov. (Bangiales, Rhodophyta), is described from Kuwait in the north-western Arabian Gulf (also referred to as the Persian Gulf). It was found on a submerged fishing line. The intensively red upright thallus of up to 1 cm in length consists of cells containing a single stellate plastid. The thallus is initially uniseriate but becomes biseriata in its distal part upon transformation into globular reproductive cells, possibly archaeospores. The biseriata fertile part is the only morphological difference from other filamentous species of the Bangiales, in which this region is parenchymatous. In culture, bipolar asymmetric germination of the spores of *Kuwaitiella* led to a new generation of identical erect thalli, fixed to the substratum by colourless rhizoids. According to phylogenetic analyses of partial small subunit nuclear ribosomal DNA (18S) and of the plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), the new species forms an additional lineage of the Bangiales, genetic similarity with other taxa being limited (maximum 91% in SSU and 90% in *rbcL*). It was no clear member of any known lineage of the Bangiales but was weakly associated with the filamentous species *Minerva* and *Dione* from New Zealand. In a SSU phylogeny, it formed a basal branch in the Bangiales and clustered with *M. aenigmata* and *D. arcuata* as sister clade to all other species of the order. In a *rbcL* phylogeny, it was part of a large polytomy of lineages, its closest relative being *D. arcuata*. *Kuwaitiella* forms the 8<sup>th</sup> lineage of filamentous Bangiales detected so far.

Key words: *Bangia*; small subunit ribosomal DNA (nrSSU); Rhodophyta, Kuwait, plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*).

## INTRODUCTION

The red algal order Bangiales Nägeli (Nägeli 1847), known for the commercial seaweed Nori, was traditionally split into two genera; *Porphyra* C.Agardh (Agardh 1824), comprising the species with a foliose (bladed or sheet-like) gametophyte, was distinguished from *Bangia* Lyngbye (Lyngbye 1819) for species with a filamentous gametophyte morphology (Boedeker

et al. 2008; Zheng & Li 2009). The examination of the diversity of the Bangiales – at the moment a total of 186 described species (Guiry & Guiry 2021) – with karyological and molecular tools has largely changed this concept. While the order Bangiales was supported as a monophyletic lineage, recent studies revealed that there are at least 16 different clades and that both the foliose and the filamentous forms are polyphyletic (Sutherland et al. 2011).

International collaborative research has recently classified the foliose forms in nine clades and corresponding genera with well-identified names and type species, namely *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea*, *Neothemis*, *Porphyra*, *Pyropia* and *Wildemanina* (Sánchez et al. 2014; Yang et al. 2017). However, the nomenclatural situation is dynamic and additional genera of foliose Bangiales, such as *Neoporphyra*, *Neopyropia*, *Phycocalidia* and *Uedaea* have been proposed more recently (Yang et al. 2020).

The filamentous species are at the moment classified in seven different lineages (Lindstrom and Fredericq 2003; Broom et al. 2004; Müller et al. 2005; Nelson et al. 2006; Sutherland et al. 2011; Deng et al. 2015). Apart from the clade of *Bangia*, which includes the freshwater taxon *B. atropurpurea* (Mertens ex Roth) C.Agardh as generic type species, marine *B. fuscopurpurea* (Dillwyn) Lyngbye and other marine filamentous Bangiales appear in three different clades provisionally referred to as “*Bangia*1”, “*Bangia*2” and “*Bangia*3” (Sutherland et al. 2011; Deng et al. 2015). In addition, monotypic genera have been erected for three filamentous species, each one forming a lineage of its own. Morphologically, *Pseudobangia kaycoleae* K.M.Müller & R.G.Sheath is distinguished by several chloroplasts per cell (Müller et al. 2005), *Dione arcuata* W.A.Nelson has very broad filaments (Nelson et al. 2005), whereas *Minerva aenigmata* W.A. Nelson has no distinctive morphological characters except slightly smaller cell sizes (Nelson et al. 2005). In

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phylogenetic analyses, *Bangia*, *Bangia* 1–3 and *Pseudobangia* were interspersed between the lineages of bladed Bangiales, whereas *Dione* and *Minerva* appeared in two basal branches; they formed a monophyletic sister group to the remainder of the Bangiales (Nelson *et al.* 2005; Boedeker *et al.* 2008; Sutherland *et al.* 2011; Deng *et al.* 2015; Reddy 2018).

The aim of the present study is to describe a *Bangia*-like alga that we have collected in Kuwait, Arabian Gulf (also known as the Persian Gulf; henceforth referred to as Gulf), north-western Indian Ocean, and which differs genetically from the other Bangiales. Our DNA sequence analyses, based on the two markers 18S nrDNA and *rbcL*, in combination with an assessment of morphological characters, result in the proposal of a new genus and species, *Kuwaitiella rubra*.

## MATERIALS AND METHODS

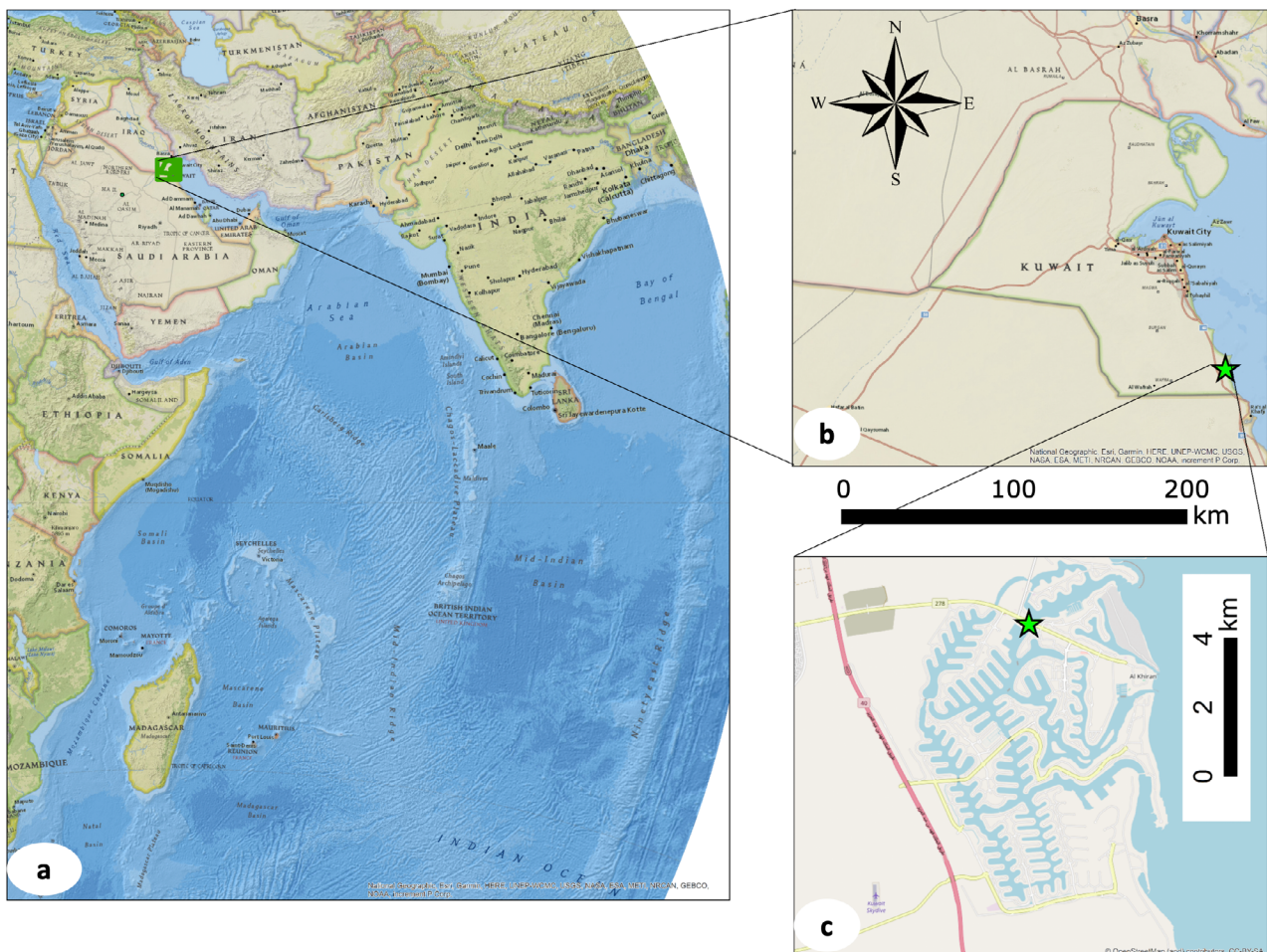
### Field site, specimens and laboratory culture

Multiple individuals of a red, unbranched filamentous alga, about 1cm long, were collected as sample KHI50219-7 on 5th

February 2019 under a bridge over Khiran Canal (Southern Province, Kuwait) at 28°40'32.1"N, 48°21'19.1"E, an inland marine waterway constructed in order to provide sea access for the artificial lagoons of Sabah al Ahmed Marine City, Khiran (Fig. 1, see also a photograph of the site in Appendix S4; Jones *et al.* 2012). The algae were attached to a permanently submerged fishing line. Despite additional searches, further individuals similar to KHI50219-7 were not detected at the same site or elsewhere in Kuwait. The sea water temperature was 16°C, which is common in the winter season in Kuwait (Al-Rashidi *et al.* 2009; Al-Yamani *et al.* 2004; Al-Yamani 2021), and the salinity (measured 4 days after collection) was 43 psu.

Several of the red unbranched filaments were preserved as a single sample in cetyltrimethylammonium bromide (CTAB) buffer (Gachon *et al.* 2009) for further molecular study. Living material was transported to the laboratory of Bezhin Rosko for isolation into culture. The rest of the sample was retained as a herbarium specimen (PC0616708, Fig. 2).

Laboratory culture in Petri dishes of 8 or 35 mL content followed standard methods (Coelho *et al.* 2012), with monthly serial transfer. The culture medium was half-strength Provasoli-enriched autoclaved natural sea water, the temperature oscillated between 17 and 25°C (similar to spring



**Fig. 1.** Collection site of *Kuwaitiella rubra* sp. nov. (a) Kuwait located in the NW part of the Indian Ocean. (b) Location of Khiran in the Southern Province of Kuwait. (c) Collecting site in a water channel connecting artificial lagoons to the sea. A photograph of the site is available as supporting information (see Appendix S4).





**Fig. 2.** Holotype (PC0616708) of *Kuwaitiella rubra* sp. nov.

conditions in Kuwait), and irradiance was by dimmed natural light ( $<40 \mu\text{mol m}^{-2}\text{s}^{-1}$  photon fluence rate) and the natural change of daylengths. Fragments of the field-collected alga were initially incubated in culture medium containing ca. 3 mg  $\text{GeO}_2/\text{L}$  to prevent proliferation of diatoms (Lewin 1966). After 25 days, a newly germinated uniseriate red filament was transferred into a Petri dish containing medium without  $\text{GeO}_2$ . All observations in culture were made with this clone (culture code K118) and its progeny.

### Molecular work and phylogenetic analyses

DNA was extracted both from the CTAB-preserved field sample and from cultured material, using the protocol of Gachon *et al.* (2009). Polymerase chain reaction (PCR) was carried out on the samples to amplify the DNA of two markers (for the primers see Appendix S1). We chose partial nrSSU and entire *rbcl*, which were the most common markers in previous

studies to infer the phylogenetic relationships within the Bangiales (Oliveira *et al.* 1995; Müller *et al.* 1998; Nelson *et al.* 2005; Broom *et al.* 2010; Sutherland *et al.* 2011; Deng *et al.* 2015; Guillemin *et al.* 2016; Abe *et al.* 2021; Sano *et al.* 2021). A total of 25.1  $\mu\text{L}$  PCR reaction volume contained 20 units of Taq DNA Polymerase (in 20  $\mu\text{L}$  GoTaq™ buffer containing 5 mM  $\text{MgCl}_2$ , 1.25 mM dNTPs), 1.7  $\mu\text{L}$  of each primer and 1.7  $\mu\text{L}$  of template DNA. Amplification was under the following conditions: 3-min initial denaturation at 94°C, 35 cycles of 60-s denaturation at 94°C, 30-s annealing temperature (see Appendix S1), 1.5-min extension at 72°C and a final extension of 5 min at 72°C. Purified (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 were reviewed by eye and the complementary sequences aligned using the software BioEdit (Hall 1999). The consensus sequences from plus and minus strands were compared with entries in the GenBank database of the National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) via the online Basic Local Alignment Search Tool (BLAST) algorithm function (Altschul *et al.* 1990; Zhang *et al.* 2000).

The sequences of the two genes determined in the samples KHI50219-7 and KA118 were aligned with published homologous sequences of about 35 taxa of the Bangiales. The sequences included the ones with the highest degree of homology, as indicated by BLAST, as well as others, which were representatives of clades identified by previous authors, in order to cover the diversity of the Bangiales (see Appendix S2). *Erythrotrichia carnea* (Dillwyn) J. Agardh and *Chlidophyllon kaspar* (W.A. Nelson & N.M. Adams) W.A. Nelson (Erythropeltales, Compsopogonophyceae) served as a monophyletic outgroup, following previous works (Nelson *et al.* 2006; Deng *et al.* 2015). Alignments were calculated by using Multiple Sequence Comparison by Log-Expectation statistical method (MUSCLE) in MEGA X v. 10.2.4 (<https://www.megasoftware.net/>) (Edgar 2004; Kumar *et al.* 2018).

Phylogenetic trees were constructed following two complementary approaches, maximum likelihood and Bayesian inference. The maximum likelihood analyses were performed using IQ-TREE (Nguyen *et al.* 2015) implemented within the W-IQ-TREE web interface (Trifinopoulos *et al.* 2016) with the 10 000 ultra-fast bootstrap approximation (Minh *et al.* 2013). IQ-TREE first estimates the best substitution model and then runs the analysis with the selected model. The Bayesian tree was constructed using MrBayes 3.2.6 (Ronquist *et al.* 2012) with parameters set on GTR + G model for 10M generations and 25% burn-in.

## RESULTS

*Kuwaitiella rubra* A.H. Hasan, P. Van der Aa, F.C. Küpper, D. Al-Bader & A.F. Peters gen. et sp. nov.

### Description

Thallus filamentous, characterised by an initially uniseriate, unbranched erect filament, up to 1 cm in length and 20–

25 µm in diameter, which is attached to the substratum by colourless aseptate rhizoids of 10 µm diameter, which are formed from the lowermost filament cells, or by a small holdfast. Cells of the erect filament are 8–20 µm in length, containing a single stellate red plastid. The distal part of the erect filament becomes biserial at maturity, producing two rows of globular reproductive cells, which are 20 µm in diameter. The life history obtained in laboratory culture was direct.

#### Holotype

PC0616708 (Museum National d'Histoire Naturelle, Paris – Cryptogamie), herbarium sheet KHI50219-7 (Fig. 2), collected on 5 February 2019 by A.H. Hasan, F.C. Küpper and A.F. Peters, at Khiran Canal (28°40'32.1"N, 48°21'19.1"E), Khiran, Kuwait (see Appendix S4 for a photograph of the site).

#### Culture from holotype

K118, clonal culture, maintained in the Algal Culture Unit at Kuwait University (KUAC), and at CCAP (Oban, Scotland) under accession 2365/1.

#### Isotypes

PC0616709 (slide of rehydrated field material); Kuwait University Herbarium; ABDUH 10/2 (Herbarium of the University Museum, University of Aberdeen, Scotland, UK).

#### Habitat and geographical distribution

Near seawater surface, attached to permanently submerged abandoned fishing line under a bridge over an artificial sea water canal, water temperature 16°C and salinity 43 psu. Site is sheltered from waves but tidal currents can be strong. Known only from the type locality.

#### Etymology

The genus refers to the collection site, the ending *-ella* describing the small size of the alga. The specific epithet reflects the strongly red coloration of the alga, which attracted our attention in the field.

#### Molecular sequences

5'-partial 18S rRNA, gene accessions OL471388 for the field material (KHI50219-7) and OL471389 for the clonal culture (K118); *rbcL* gene, accessions OL839312 for KHI50219-7 and OL839313 for K118.

### Morphology and life history

Initial microscopic observation indicated that both the field and the culture material were similar to *Bangia*, i.e. the alga was an unbranched erect filament, attached to the substratum by multiple rhizoids and forming globular reproductive cells in the distal portion of the filament. In culture, the released cells elongated during bipolar asymmetric germination (Fig. 3a), with a basal, unpigmented rhizoid being formed at one pole and an erect, monosiphonous, unbranched pigmented filament at the opposite pole (Fig. 3b). Additional rhizoids, which originated as extensions from supra-basal cells, grew initially within the common outer cell wall, before diverging in different directions. Their diameter was 10 µm (Fig. 3c). Occasionally,

attachment was achieved by an extended unpigmented basal cell (holdfast, Fig. 3d). Holdfast-like attachment and rhizoids were observed in the same culture dish. The erect filament consisted of cylindrical cells 20–25 µm in diameter (Fig. 3f) and 8 µm in length proximally to 20 µm distally, containing a single stellate plastid. Maturation of the upright filament was associated with longitudinal cell divisions, resulting in biserial filaments (Figs 3g, h). The cells of the biserial part of the filament enlarged and developed into globular reproductive cells, about 20 µm in diameter (Fig. 3i), which after release, or in situ, germinated into a new generation morphologically similar to the parent thallus. During the 3 years of cultivation, the several subsequent generations have all shown this development.

### Molecular work and phylogenetic analyses

DNA of the nuclear ribosomal small subunit gene and the plastid-encoded Rubisco operon (*rbcL* gene plus partial Rubisco spacer) was amplified and sequenced for both field material and the cultured strain. The sequences from the field sample and the culture were identical. BLAST searches showed for the SSU ≤91.32% identity with other members of the Bangiales, *Minerva aenigmata* having the most similar sequence. In *rbcL*, the highest similarities were 90.43% and 90.26% with *Wildemanian occidentalis* (Setchell & Hus) S.C.Lindstrom and *Wildemanian* sp., respectively (for details see Appendix S3).

In the phylogenetic tree calculated from nrSSU sequences (Fig. 4), *Kuwaitiella* formed the basal branch of the Bangiales together with *Minerva* and *Dione*, and its association with *Minerva* had 90% statistical support in the ML analysis and a posterior probability of 0.98 in the Bayesian tree. *Kuwaitiella* did not cluster with any *Bangia* or with *Pseudobangia*, or with any lineage of bladed Bangiales.

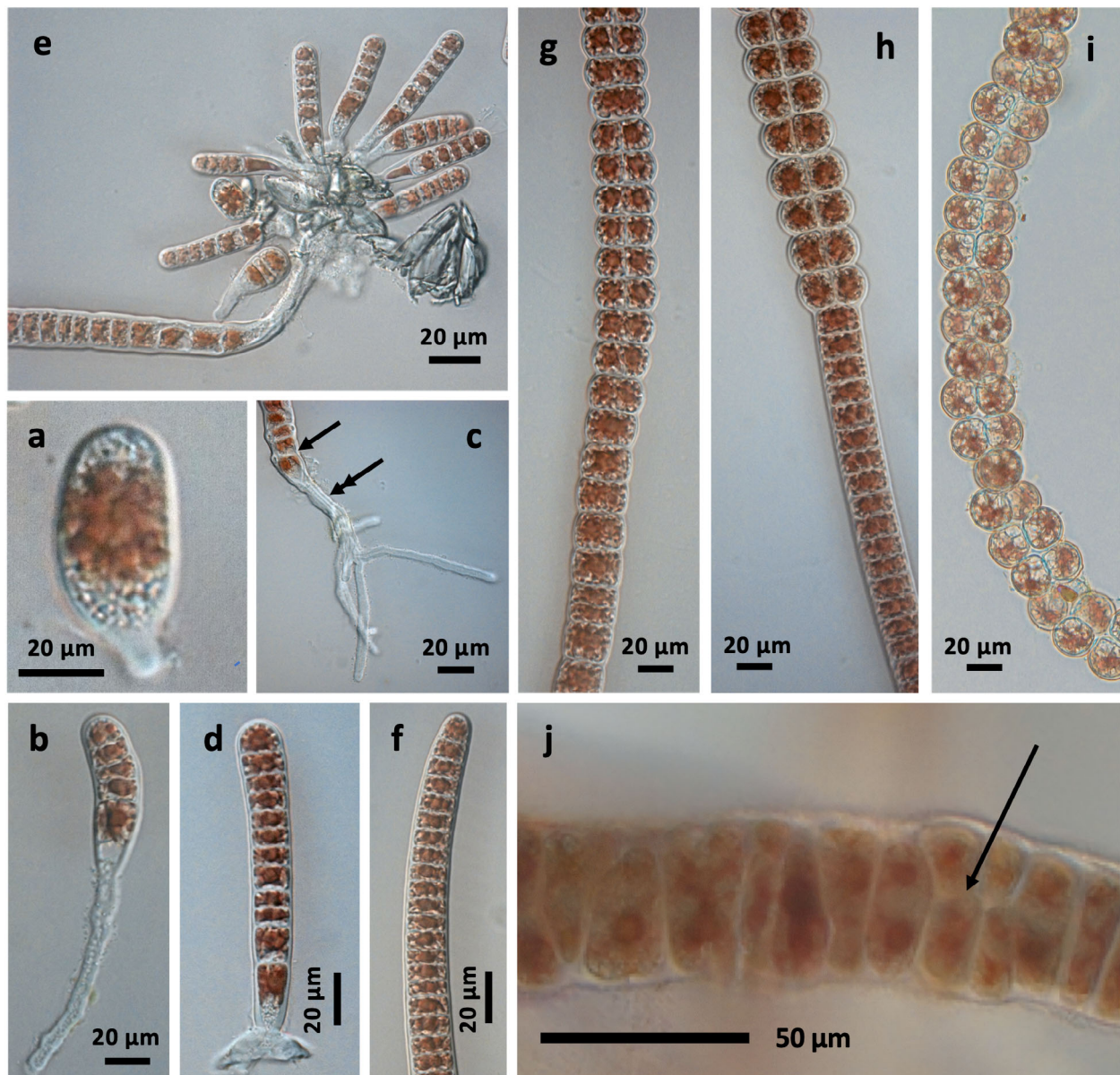
In the phylogenetic tree based on *rbcL* sequences (Fig. 5), *Kuwaitiella* formed a clade with *Dione*, with weaker support (ML: 82%; no support in the Bayesian tree). The deep relationships of the *rbcL* tree were weakly supported and the Bayesian topology presented different deep relationships. The backbone of the *rbcL* tree should rather be seen as a large polytomy, making the placement of *K. rubra* uncertain. As in the SSU tree, *Bangia*1 was paraphyletic. The genus *Pseudobangia* was missing in the *rbcL* phylogeny because there is no published *rbcL* sequence for *P. kaycoleae*. The two *Wildemanian* taxa that gave the best BLAST hits for *rbcL* (see Appendix S3) grouped with the other *Wildemanian* species and not with *Kuwaitiella*. These two taxa were not included in the SSU phylogeny (Fig. 4) because of missing data (*W. occidentalis*) and high sequence similarity of *Wildemanian* sp. HM080 with the other *Wildemanian* sequences.

In the phylogeny calculated from concatenated sequences of the two markers (see Appendix S5), *Minerva*, *Kuwaitiella* and *Dione* were the most basal branches of the Bangiales, as in the SSU tree. However, the clustering of *Minerva* and *Kuwaitiella* was only weakly supported (ML: 45%; no support in the Bayesian tree).

## DISCUSSION

An unknown species of filamentous Bangiales, *Kuwaitiella rubra* gen. et sp. nov., was detected in Kuwait.

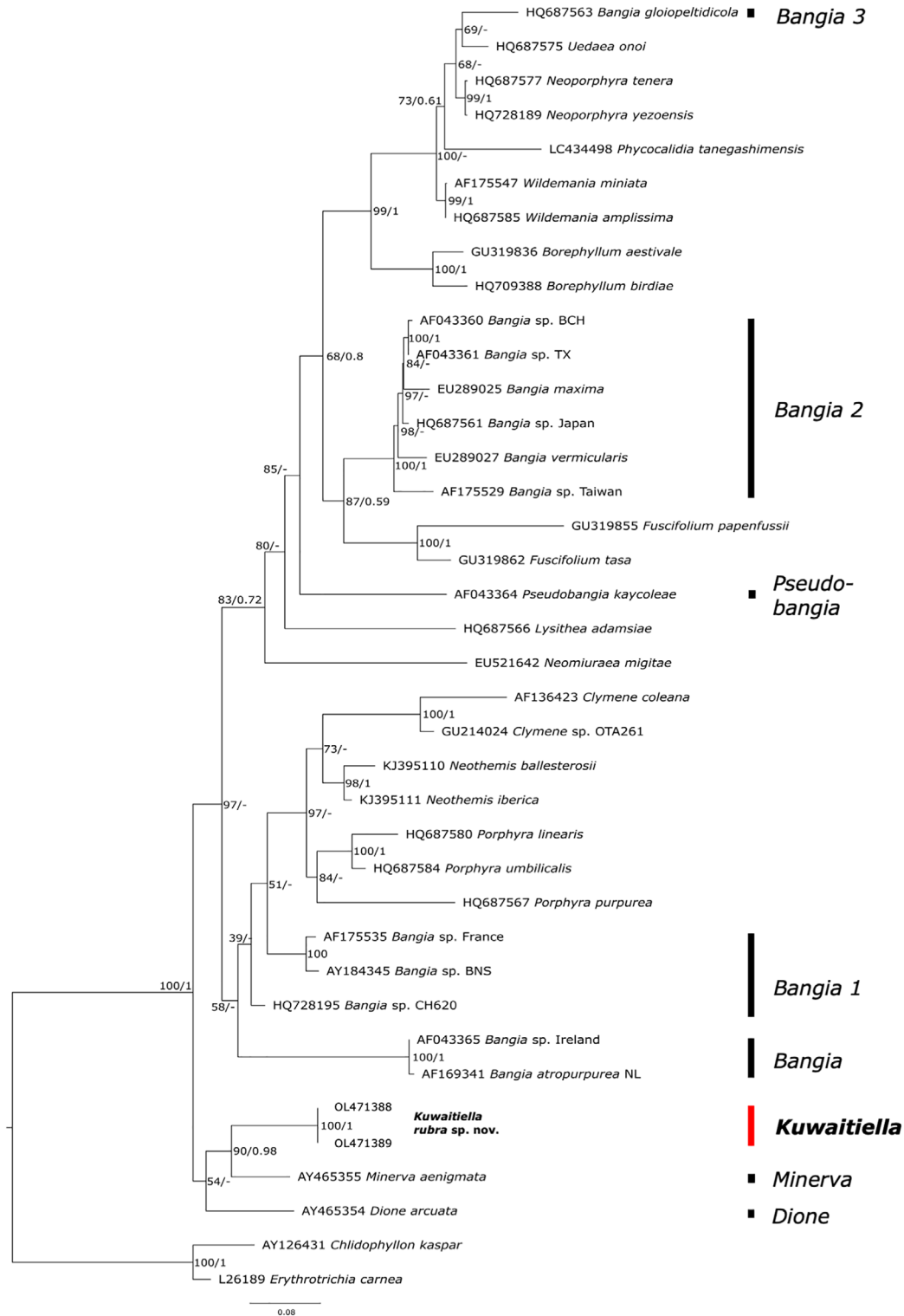




**Fig. 3.** Morphology and development of *Kuwaitiella* sp. nov., a-i culture, j rehydrated herbarium material. (a) Germinating spore. (b) Germling with five cells of upright filament and first rhizoid growing from the basal cell. (c) Base of larger germling, with additional rhizoids extending down within common cell wall from lowermost cells (arrow). The rhizoids initially grow together in one strand (double arrow), before they diverge into different directions. (d) Developing germling, basal cell forming holdfast. (e) Several embryonic germlings germinating on substratum. (f) Distal part of developing immature filament. (g, h) At maturation, the upper part of the filament shows longitudinal divisions and two rows of equally sized cells. The transition from uniseriate to biserial part is gradual (g) or abrupt (h). (i) Cells of the biserial filament enlarge and become globular spores. (j) cross section of rehydrated field material showing biserial portion (arrow).

Morphologically, its overall size, single stellate plastid, the unbranched erect thallus and the attachment by multiple rhizoids do not distinguish the new species from most other *Bangia*-like taxa. However, the distal fertile part of the thallus was only biserial in our culture (Figs 3g-i) and field (Fig. 3h) material, whereas in *Bangia*, *Dione*, *Minerva* and *Pseudobangia* it is regularly parenchymatous (Kornmann & Sahling 1977; Müller et al. 2005; Nelson et al. 2005). A comparison of *Kuwaitiella* with other filamentous Bangiales is provided in Table 1.

The life history of *K. rubra* in laboratory culture was of the direct type, i.e. successive generations were morphologically identical. Spermatangia or a *Conchocelis*-like sporophyte generation, which are present in many species of the Bangiophyceae (e.g. *Minerva aenigmata*, Nelson et al. 2005), were not detected. In *Dione*, in which most spores in culture replicate the parent gametophyte thallus, they occasionally develop into sporophytic *Conchocelis* filaments (Nelson et al. 2006). Such a phenomenon was not seen in *Kuwaitiella*, in spite of examining many *Kuwaitiella* germlings.

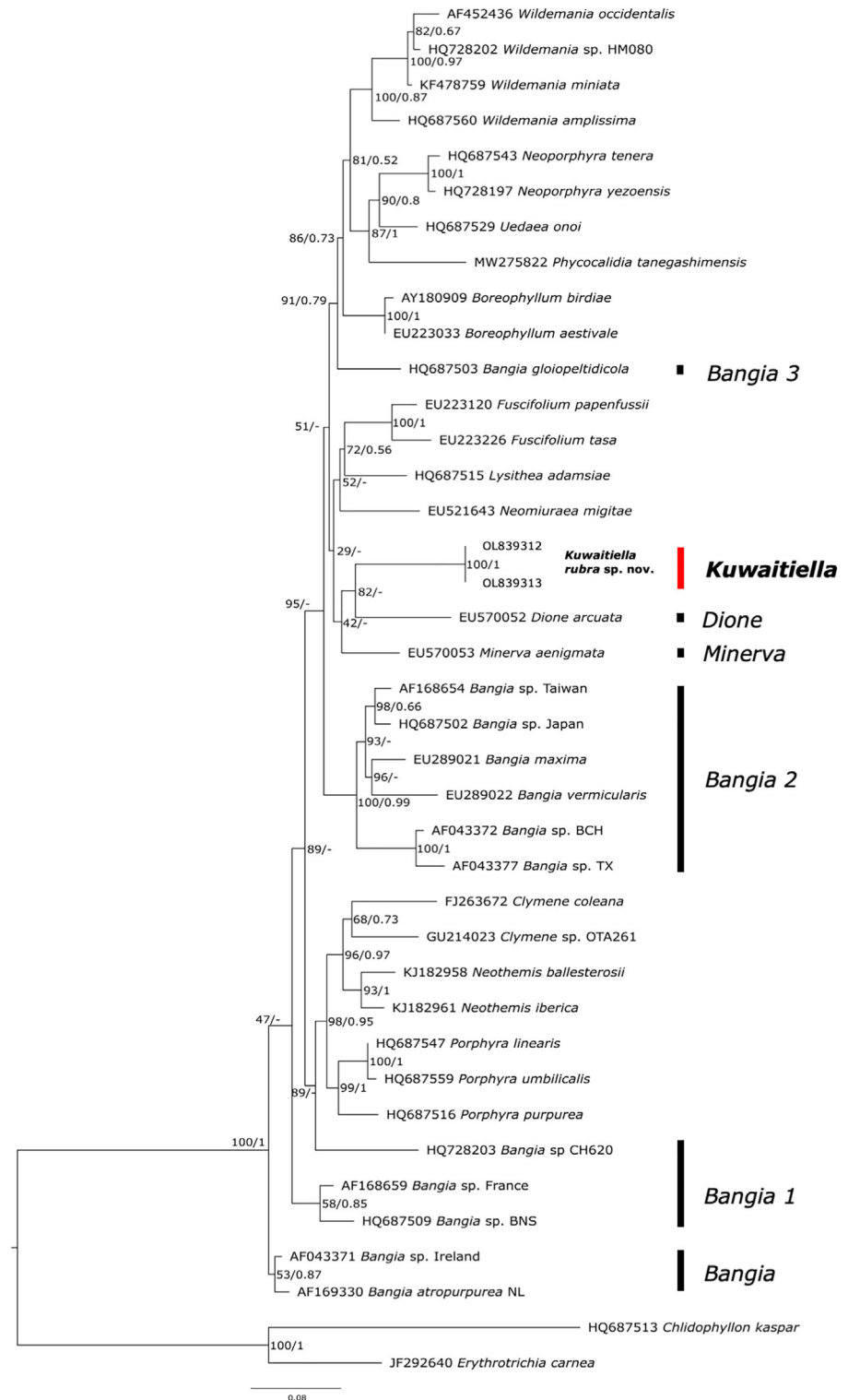


**Fig. 4.** Maximum likelihood phylogeny of nrSSU of 36 sequences of Bangiales and two outgroup species of the Erythrotrichiaceae, *Erythrotrichia carnea* and *Chlidophyllum kaspar*. Bootstrap values (on the left) and posterior probabilities (on the right) are displayed at the nodes. The eight lineages of filamentous Bangiales are indicated by vertical bars.

The reproductive cells observed in *Kuwaitiella* were thus possibly asexual spores (archaeospores), which are well known in Bangiophyceae (e.g., Kornmann & Sahling (1977) for *Bangia*,

Nelson *et al.* (1999) and De Reviers (2002) for *Porphyra*, and Nelson *et al.* (2005) for *Minerva* and *Dione*). However, because we possessed a single strain, it is also possible that

**Fig. 5.** Maximum likelihood phylogeny of *rbcL* of 37 sequences of Bangiales and the same two outgroup species as in the SSU tree. Bootstrap values (on the left) and posterior probabilities (on the right) are displayed at the nodes. Lineages of filamentous Bangiales are indicated by vertical bars. Note that *Pseudobangia* is absent because of unavailable *rbcL* sequence. Replicate *Wildemanina* spp. sequences are included because two of them were most similar to *Kuwaitiella* in BLAST searches (see Appendix S3).



the reproductive cells we observed were female gametes that germinated parthenogenetically. In addition, the field material may show only incipient fertility and our culture conditions may not have allowed spermatangia or a parenchymatous fertile region to be formed.

Because of extensive cryptic diversity in Bangiales (e.g., Guillemin *et al.* 2016), sequence analyses are essential

for reliable species identification and classification. The most striking result of our sequence analyses was the large distance of *Kuwaitiella* to any other taxon of the Bangiales. The highest genetic identities were about 91% in SSU and 90% in *rbcL*, suggesting that our alga did not belong to any lineage so far recognised. In molecular phylogenies, it clustered either with *Minerva* (SSU tree, with 90% bootstrap support in the ML

**Table 1.** *Kuwaitiella rubra* sp. nov. and other filamentous Bangiales

	<i>Kuwaitiella rubra</i> sp. nov.	<i>Bangia</i> spp.	<i>Dione arcuata</i>	<i>Minerva aenigmata</i>	<i>Pseudobangia kaycoleae</i>
Habitat and substratum	Subtidal, on fishing line, shaded	Upper intertidal, on rock	Upper subtidal zone on rock, shaded faces, fresh water influence	High intertidal zone on sand-scoured rock	Vertical rock in the upper intertidal
Wave exposure	Sheltered artificial waterway, strong tidal current	Exposed	Exposed open coast	Exposed to moderately sheltered open coast	Exposed
Distribution	Kuwait	World-wide	New Zealand	New Zealand	British Virgin Islands
Seasonality	Late winter; no data on presence in other seasons	All year but main occurrence from autumn to spring	Early summer	Summer to late winter	Winter
Thallus size	1 cm	1–10 cm	1.5 cm	1.5–3 (–10) cm	1 cm
Plastid number	1	1	1	1	Multiple (up to 9)
Basal region	Uniseriate filament; rhizoids extending down within common cell wall from several lowermost cells	Uniseriate filament; rhizoids extending down within common cell wall from several lowermost cells	Biseriate filament in rhizoidal area, uniseriate above; rhizoids extending down within common outer cell wall from lowermost 10–25 (paired) cells	Uniseriate filament; rhizoids extending down within common cell wall from lowermost 9–25 (–43) cells	Uniseriate filament; rhizoids extending down within common cell wall from several lowermost cells
Distal fertile region	Biseriate	Parenchymatous	Parenchymatous	Parenchymatous	Parenchymatous
Sterile filament width	20–25 µm	8–50 µm	47–80 µm	17–40 µm	29–49 µm
Mature sporangial filaments (width)	50 µm	up to 300 µm	120–210 µm	(42–) 45–68 (–77) µm	34–83 µm
Sexuality	Not found	Principally present but not found in all populations; dioicous	Present, gametophytes monoicous or dioicous	Present, gametophytes monoicous or dioicous	Not found
Conchocelis	Not found	Present in sexual populations	Infrequent development of spores in culture; most spores replicate the gametophyte thallus	Common development of spores in culture	Not found
Archaeospores	Present, produced in upper part of filament	Present, produced in upper part of filament	Present, produced in upper part of filament	Present, produced in upper part of filament	Present, produced in upper part of filament

Based on Kornmann & Sahling (1977), Womersley (1994), Müller *et al.* (2005) and Nelson *et al.* (2006).

analysis; Fig. 4) or with *Dione* (*rbcL* tree, 82% bootstrap support). The topology of the phylogenetic tree was not consistent between the two data sets. With SSU, the new species formed the most basal branch of the Bangiales, together with *Minerva* and *Dione*; Fig. 4). With *rbcL*, it was again associated with *Dione* and *Minerva* but was in an unclear position in the order (Fig. 5). Both markers are not perfect; SSU data may suffer from the uncertain alignment of the more variable loop positions, whereas *rbcL*, albeit perfectly alignable, may be too variable to resolve the deeper taxonomic levels. The phylogeny calculated from concatenated sequences of both markers resembled the SSU tree (Appendix S5). Both genetic distance data and molecular phylogenies suggested that a new genus is required for this alga from Kuwait.

It is tempting to speculate, based on the SSU tree, that the three filamentous Bangiales *Minerva*, *Dione* and *Kuwaitiella*, forming basal branches, show primitive characters, and that the morphologically least developed – because only biseriate – fertile upright of *Kuwaitiella* is similar to the original condition in the common ancestor of all Bangiales. However, the putatively bangiophyte very ancient (1200 Ma) fossil species *Bangiomorpha pubescens* N.J.Butterfield was already multiseriate (Butterfield 2000). The *rbcL* tree, placing other filamentous species in more basal positions, would also rather suggest that the biseriate condition of *Kuwaitiella* is a reduction from parenchymatous forms. It is possible that in Bangiales a deeper understanding of development and evolution will require phylogenomics and more powerful analyses based on multiple markers.



In the phycological literature of the Gulf, *Bangia* has so far only been mentioned for Kuwait and Iraq (Al-Hasan & Jones 1989; Basson 1992; Maulood *et al.* 2013; Al-Saboonchi & Al-Shawi 2014; Al-Yamani *et al.* 2014; John & Al-Thani 2014). These authors recorded the presence of *Bangia atropurpurea* and *B. fuscopurpurea* based on morphological identification. Precisely, Al-Yamani *et al.* (2014) reported *B. atropurpurea* from East Doha (Kuwait Bay) and Kubbar Island (central Kuwait, offshore) as an epiphyte on brown algae in early December 2012. However, in that time there was uncertainty as to the relationship between the freshwater species *B. atropurpurea* and the marine *B. fuscopurpurea* (John & Al-Thani 2014). Molecular-based identification has meanwhile revealed that the freshwater and the marine species are not closely related (Sutherland *et al.* 2011). Without molecular data, we would have identified our material as little developed (and therefore only biseriata) *B. fuscopurpurea*. However, the sequences of *Kuwaitiella* are different from all sequences published for *B. atropurpurea* or *B. fuscopurpurea* (or for any other *Bangia*) and thus leave no doubt that it is a distinct species. Herbarium material of the previous records of *Bangia* in Kuwait was unavailable and micrographs not informative because they only showed vegetative uniseriate filaments (T. Mikhaylova, personal communication).

In the Indo-West Pacific region, filamentous species of the Bangiales have been reported previously, and they were either identified as *B. fuscopurpurea* (e.g., from coastal areas near Karachi (Pakistan); Hayee-Memon & Shameel 1996), or described as additional taxa within *Bangia*. Sequences are available for *B. gloiopeltidicola* Tanaka from Japan, Korea, and China (Guiry & Guiry 2021), which is classified in the *Bangia3* clade (Sutherland *et al.* 2011; our Figs 4-5). A recent checklist of benthic marine macroalgae in the Philippines (Lastimoso and Santiañez 2020) mentioned *B. yamadae* Tanaka, originally described from Taiwan (Tanaka 1944). Similar to *Kuwaitiella*, *B. yamadae* shows a division of filament cells into two (rarely four) cells in the fertile region but it has dioicous gametophytes, very thick cell walls, and a more prolific development of rhizoids formed from the lower end of filament cells. *Kuwaitiella*, in which we have not detected sexuality, appears morphologically different. Another species, *B. halymeniae* M.J.Wynne, was described from the Maldiv Islands in the Indian Ocean (Wynne 1993). However, that species is minute (up to 1.8 mm in length) and the distal part of the filament transformed into packages of small cells (possibly spermatia), whereas our alga reaches a cm in size and is biseriata at fertility. In Bangladesh, *B. discoidea* A.Aziz was recently described from brackish water of the Passur River (Aziz 2016). In contrast, *Kuwaitiella* was found in a fully saline environment. There are no published sequences for *B. yamadae*, *B. halymeniae* or *B. discoidea*. A further taxon, *B. biseriata* Meneghini (Meneghini 1841), attracted our attention because of its specific epithet. However, it was collected in a freshwater site in Tuscany (Italy; Guiry & Guiry 2021) and again, there is no sequence available for this taxon. Because of the different habitat and geographic distance, we regard it as unlikely that our alga is conspecific with *B. biseriata*. In conclusion, we have not found any previously described species matched by our alga from Kuwait and therefore propose it as a species that is new to science.

It is the only representative of the 8<sup>th</sup> lineage of filamentous Bangiales.

*Kuwaitiella rubra* was collected at Khiran (Southern Province of Kuwait) in a channel constructed in 2004 (Jones *et al.* 2012). It was attached to a fishing line and surrounded by anthropogenic substrata, mostly riprap. We have not seen other populations of the species at that site or elsewhere during collections in Kuwait. The country regularly receives large vessels from all over the globe, however the main harbours (Mina Abdulla and Shuaiba) are at 57–63 km distance ([distancecalculator.globefeed.com](http://distancecalculator.globefeed.com)) from Khiran, which has only a small harbour and local marine traffic. Thence, for the moment it is not possible to describe the preferred habitat of the species or to know whether it is a native species or an introduction.

*Kuwaitiella rubra* inhabits a harsh environment; the collection of this species was during the winter season (February 2019), in which sea water temperatures can be as low as 10°C (Al-Rashidi *et al.* 2009). In the summer, in contrast, they commonly rise to around 35°C (Al-Yamani *et al.* 2004; Al-Shammari & Ali 2018; Al-Yamani 2021). Elsewhere in the world, *Bangia*-like marine algae mostly occur on intertidal rocks, where they are exposed to severe abiotic environmental conditions, such as strong wave exposure, desiccation, direct sunlight and extreme fluctuations of temperature and salinity (Müller *et al.* 2003; Wang *et al.* 2008, Sutherland *et al.* 2011; Wang *et al.* 2019). *K. rubra* thus appears to share its probable stress-tolerance with many other members of the order.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** List of oligonucleotide primers used for amplification and sequencing.

**Appendix S2.** List of GenBank/DDBJ/EMBL accessions and species identifiers for bangiophyte sequences used in phylogenetic analyses.

**Appendix S3.** Table of best matches of *Kuwaitiella rubra* sp. nov. SSU and *rbcL* sequences with public sequence data.

**Appendix S4.** Photograph of the type locality of *Kuwaitiella rubra* sp. nov.

**Appendix S5.** Phylogenetic tree (Maximum likelihood) calculated from concatenated sequences of nrSSU and *rbcL* of *Kuwaitiella rubra* sp. nov., 33 taxa of Bangiales and two species of Erythrotrichiaceae serving as outgroup.