cryptogamie
Algologie
2023 • 44 • 5

A taxonomic assessment of *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. (Bangiaceae, Bangiophyceae)

Elena VARELA-ÁLVAREZ, Michael D. GUIRY & Ester A. SERRÃO

art. 44 (5) — Published on 30 August 2023
www.cryptogamie.com/algologie

PUBLICATIONS SCIENTIFIQUES
MUSÉUM NAL HIST NATURELLE

Couverture / *Cover*:
Extraits d'éléments de la Figure 2 / Extracts of the Figure 2

Cryptogamie, Algologie est indexé dans / Cryptogamie, Algologie *is indexed in*:
– Aquatic Sciences & Fisheries Abstracts Part I.
– Biological Abstracts
– Chemical Abstracts
– Current Contents
– Marine Science Contents Tables (FAO)
– Science Citation Index
– Publications bibliographiques du CNRS (Pascal)

Cryptogamie, Algologie est distribué en version électronique par / Cryptogamie, Algologie *is distributed electronically by*:
– BioOne® (http://www.bioone.org/loi/crya)

Cryptogamie, Algologie est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris
Cryptogamie, Algologie *is a fast track journal published by the Museum Science Press, Paris*

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publish*: ***Adansonia, Geodiversitas, Zoosystema, Anthropozoologica, European Journal of Taxonomy, Naturae, Comptes Rendus Palévol, Cryptogamie* sous-sections *Bryologie, Mycologie*.**

Diffusion – Publications scientifiques Muséum national d'Histoire naturelle
CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél. : 33 (0)1 40 79 48 05 / Fax : 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / http://sciencepress.mnhn.fr

ISSN (imprimé / *print*) : 0181-1568 / ISSN (électronique / *electronic*) : 1776-0984

A taxonomic assessment of *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. (Bangiaceae, Bangiophyceae)

Elena VARELA-ÁLVAREZ
Centro de Ciências do Mar (CCMAR), CIMAR, Universidade do Algarve, Gambelas, Faro (Portugal)
evarela@ualg.pt (corresponding author)

Michael D. GUIRY
AlgaeBase, Ryan Institute, University of Galway, University Road, Galway, H91 TK33 (Ireland)

Ester A. SERRÃO
Centro de Ciências do Mar (CCMAR), CIMAR, Universidade do Algarve, Gambelas, Faro (Portugal)

Submitted on 26 October 2022 | Accepted on 12 April 2023 | Published on 30 August 2023

Varela-Álvarez E., Guiry M. D. & Serrão E. A. 2023. — A taxonomic assessment of *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. (Bangiaceae, Bangiophyceae). *Cryptogamie, Algologie* 44 (5): 101-109. https://doi.org/10.5252/cryptogamie-algologie2023v44a5. http://cryptogamie.com/algologie/44/5

ABSTRACT
Until recently, all the bladed members of the Bangiaceae Duby were assigned to the genus *Porphyra* C.Agardh, nom. cons.; however, in the last twenty years, major molecular taxonomic revisions of this family have resulted in several new and reinstated genera. *Porphyra linearis* Greville is one of the species retained in *Porphyra* and its growth and reproduction is confined to the upper intertidal in the colder months of the year. *Porphyra hiemalis* Kylin was described for specimens collected from the south-west coast of Sweden but was later referred to the synonymy of *P. linearis* based upon its winter seasonality and linear form. We here compare the morphology and reproduction of isolates of both taxa from various locations in the NE Atlantic, and we sequence the intergenetic RUBISCO spacer and adjacent coding regions in these isolates to verify their phylogenetic relationships with other members of bladed Bangiaceae. Results show that both entities are not only distinct species despite almost identical external morphology, but they belong to different genera. A lectotype and epitype (of sequenced material) is designated for *P. hiemalis* and a transfer to the reinstated genus *Pyropia* J.Agardh is proposed as *Pyropia hiemalis* (Klyin) Varela-Álvarez, Guiry & Serrão, comb. nov.

KEY WORDS
Bangiaceae, *rbc*L-*rbc*S, lectotypification, epitypification, new combination.

RÉSUMÉ

Évaluation taxonomique de Pyropia hiemalis *(Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. (Bangiaceae, Bangiophyceae).*

Jusqu'à récemment, tous les membres à lames des Bangiaceae Duby étaient assignés au genre *Porphyra* C.Agardh, nom. cons. ; cependant, au cours des vingt dernières années, d'importantes révisions taxonomiques moléculaires de cette famille ont donné lieu à plusieurs genres nouveaux ou rétablis. *Porphyra linearis* Greville est l'une des espèces retenues dans *Porphyra* ; sa croissance et sa reproduction sont confinées à la zone intertidale supérieure pendant les mois les plus froids de l'année. *Porphyra hiemalis* Kylin a été décrit selon des spécimens collectés sur la côte sud-ouest de la Suède, mais il a ensuite été mis en synonymie de *P. linearis* sur la base de sa saisonnalité hivernale et de sa forme linéaire. Nous comparons ici la morphologie et la reproduction d'isolats des deux taxons provenant de divers endroits de l'Atlantique Nord-Est, et nous séquençons l'espaceur intergénétique RUBISCO et les régions codantes adjacentes dans ces isolats afin de vérifier leurs relations phylogénétiques avec d'autres membres des Bangiaceae à feuilles. Les résultats montrent que les deux entités ne sont pas seulement des espèces distinctes malgré une morphologie externe presque identique, mais qu'elles appartiennent à des genres différents. Un lectotype et un épitype (du matériel séquencé) sont désignés pour *P. hiemalis* et un transfert au genre rétabli *Pyropia* J.Agardh est proposé sous le nom de *Pyropia hiemalis* (Klyin) Varela-Álvarez, Guiry & Serrão, comb. nov.

MOTS CLÉS
Bangiaceae,
*rbc*L-*rbc*S,
lectotypification,
épitypification,
combinaison nouvelle.

INTRODUCTION

Bladed (foliose) members of the family Bangiaceae Duby (Bangiophyceae) were for a long time assigned to a single genus, *Porphyra* C.Agardh, nom. cons. In the last 20 years, numerous molecular studies (e.g. Sutherland *et al.* 2011; Kucera & Saunders 2012; Mols-Mortensen *et al.* 2012, 2014; Sánchez *et al.* 2014; Guillemin *et al.* 2016; Dumilag & Monotilla 2018; Reddy *et al.* 2018) have revealed an unexpectedly high diversity within the family, and a major taxonomic revision has resulted in many new or reinstated segregate genera including: *Boreophyllum* S.C.Lindstrom, N.Kikuchi, M.Miyata & Neefus, *Clymene* W.A.Nelson, *Fuscifolium* S.C.Lindstrom, *Lysithea* W.A.Nelson, *Neomiuraea* N.Kikuchi, S.Arai, G.Yoshida, J.A.Shin & Miyata, *Pyropia* J.Agardh, *Neothemis* A.Vergés & N.Sánchez, and *Wildemania* De Toni. Species identification of these taxa was and is very difficult due to their simple morphologies. Currently, several of these genetic entities have been pooled and renamed (Zuccarello *et al.* 2022). DNA-based methods are now necessary to correctly identify bladed Bangiaceae, and an update of the nomenclature and taxonomy of all the previous species included in the genus *Porphyra sensu lato* is required. This is especially significant since some of these species are of economic and ecological importance. The aquaculture industry of bladed Bangiaceae (nori) is the single most valuable marine product in the Far East (Japan, China, Korea) with a retail value of more than $1.3 billion per year (e.g. Blouin *et al.* 2011).

Porphyra linearis Greville is one of the species currently placed in the genus *Porphyra sensu stricto*. It was first described by Greville (1830: 170, pl. XVIII) from Sidmouth, Devon, on the south coast of England. Its growth and reproduction in the NE Atlantic are limited to the upper intertidal in the colder months of the year (Fig. 1; Table 1). Currently it has been considered a species complex, where different blade bangialean species display the same morphology (Varela-Álvarez 2002; Lindstrom & Fredericq 2003; Varela-Álvarez *et al.* 2005, 2007, 2018, 2021, 2022). Blades are red-brown, with a linear to ovate outline, and gametangial sori are marginal and mainly dioecious, but is occasionally monoecious. The spermatangial sorus (pale yellow) and female/zygotosporangial sorus (red) appear in sectors at the margins of the blade (Varela-Álvarez *et al.* 2007). Kylin (1907: 111) reported *P. linearis* from the south-west coast of Sweden but also described (Kylin 1907: 112, pl. 3: fig. 2) a new species, *Porphyra hiemalis* Kylin based upon Areschoug's exsiccata *Algae Scandinaviae Exsiccatae* no. 211, despite saying that he had never seen the species himself. Kylin (1907), based on herbarium material, found that *Porphyra hiemalis* was typically dioecious and in monoecious individuals the spermatia and carpogonia seemed to occur in separate parts of the thallus. Rosenvinge (1909: 60) included *Porphyra hiemalis* in *Porphyra umbilicalis* f. *linearis* (Greville) Rosenvinge (as "*Porphyra umbilicalis* f. *linearis* (Grev.) Harvey"). Kylin (1944: 10) subsequently placed this entity in synonymy with *P. linearis* because of his winter seasonality and linear form, and this was followed by Athanasiadis (1996: 13; 2016: 45).

As the bladed Bangiaceae present few morphological features upon which to distinguish species, the objective of the present study was to establish the phylogenetic relationship of the species *P. linearis* and *P. hiemalis*, and to establish if Kylin was correct in describing *P. hiemalis* as a distinct species, or if as claimed by later authors, the two taxa are synonymous. For that we compared the morphology of isolates of former *P. hiemalis* in relation to *P. linearis*, and the original descriptions for both species (Table 1; Fig. 1A, B). We also sequenced the RUBISCO spacer and adjacent coding regions – which has been proved useful for identification of species of *Porphyra* (e.g. Brodie *et al.* 1996, 1998), but also for other red algal species (e.g. Destombe & Doug-

las 1991; Maggs *et al.* 1992; Goff *et al.* 1994; Zuccarello *et al.* 1999) – in several isolates of both species in Europe. Herein, we provide an update of the nomenclature status for former *P. hiemalis*.

MATERIAL AND METHODS

Sample collection and morphological studies

Morphological measurements were made on herbarium specimens of *P. linearis* from Sweden, Germany, Ireland and Portugal, and former *P. hiemalis* from SW Sweden (Fig. 2). Size (length and width), colour, shape attachment, cell layers, reproductive structures and habitat characteristics were recorded. Comparisons were made in relation to the first descriptions for *P. linearis* and former *P. hiemalis* by Greville and Kylin (Table 1). Material was also dried at room temperature after collection and placed in silica gel for DNA analyses. In addition, blades from *Pyropia leucosticta* (Thuret) Neefus & J.Brodie from Ireland were collected for further phylogenetic comparison with other bladed Bangiaceae. Material of each sample was deposited (Table 1) in the GALW herbarium, which is now conserved at the National Botanical Gardens Dublin (DBN) (Table 2).

DNA extraction, PCR amplification, and sequencing

DNA was extracted using a modification of the LiCl extraction protocol described by Hong *et al.* (1992) and modified by Van Oppen *et al.* (1995). A region of approximately 330-350 bp encompassing the 3' region of the *rbc*L gene, the RuBiScO spacer and 5' region of the *rbc*S gene were PCR amplified using primers complementary to the 3' end of *rbc*L (5' TGTGGACCTCTACAAACAGC3') and 5' end of *rbc*S (5'CCCCATAGTTCCCAAT3') (Maggs *et al.* 1992). Reactions were incubated in a thermal cycler (PCR Hybid Omn –E) and incubated as follows: one cycle of 95°C for three minutes (denaturation); 30 cycles of 96°C for one minute (denaturation), 50°C for two minutes (annealing) and 74°C for two minutes (extension). Each reaction mixture contained 22.5 µl 10 × buffer, 22.9 µl 10 × dNTP (Sigma label), 13.5 µl $MgCl_2$, and 2.25 µl of each oligonucleotide primer. The template DNA and Taq polymerase (Sigma, 1.12 µl per reaction), were always added. Double-stranded PCR products were custom sequenced using 4- COR 4200 system using a nested amplification technique (MWG Biotech Milton Keynes, United Kingdom).

Phylogenetic analyses

In addition to the new sequences from the seven isolates obtained in this study, a further 17 available sequences of the same *cp*DNA region (*rbc*S-*rbc*L spacer and adjacent regions) from *P. linearis* and other representative taxa in the blade Bangiaceae group were retrieved from GenBank for further analyses (Table 2), including species of the genera *Porphyra* and *Pyropia*. Phylogenetic analyses were performed constructing two types of phylograms each (Maximum likelihood [ML] and Neighbour joining [NJ]) with the PhyML software (Guindon & Gascuel 2003) through the PHYLO-WIN graphical interphase (Galtier *et al.* 1996) and MEGA software including a sequence of *Bangia fuscopurpurea* (Dillwyn) Lyngbye (KP714733) as an outgroup. We also performed a second type of analysis where we used only the *rbc*S-*rbc*L spacer and a shorter partial *rbc*L regions of the sequences (146 positions) adding two sequences from a further two species related to the *P. linearis* complex (*Pyropia njordii* Mols-Mortensen, J.Brodie & Neefus and *Pyropia elongata* (Kylin) Neefus & J.Brodie) for which the entire *rbc*S-*rbc*L region previously used was not available in GenBank. Bootstrap values were calculated for 10 000 replicates in all analyses.

Moreover, sequence divergences were calculated among taxa using the 333 positions sequence alignment and as well for the 146 positions sequence alignment used in the phylogenetic analyses.

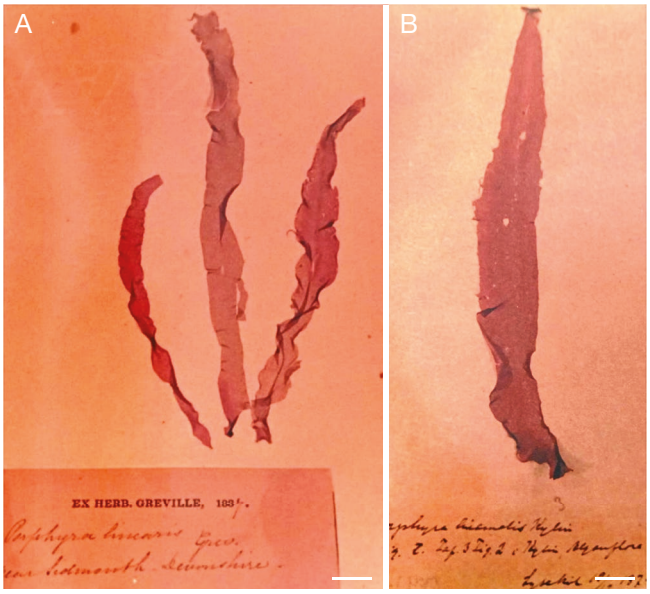

Fig. 1. — *Porphyra linearis* Greville and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov.: **A**, lectotype of *Porphyra linearis* (Sidmouth, E!) which is the most similar specimen to the illustration (pl. XVIII: fig. 1, right) that Greville presented in 1830 in his *Algae Britannicae* chosen from the exicata of *Porphyra linearis* in Greville's collection at the Royal Botanical Garden, Edinburgh (E!); **B**, lectotype of *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov., here designated, *Algae Scandinavicae Exsiccatae* (Areschoug) kept at UPS in Sweden. Scale bars: 1 cm.

RESULTS

Morphological analyses

No morphological (regarding to size, colour, frond thickness) or ecological differences (position and seasonality on the shore) were found between isolates of *P. linearis* regardless of the geographical location (Ireland, Germany, Sweden, Portugal), nor with the former *P. hiemalis* (SW Sweden). Reproductive characteristics were also similar. Reproduction was marginal with the spermatangial sorus as a pale-yellow edge

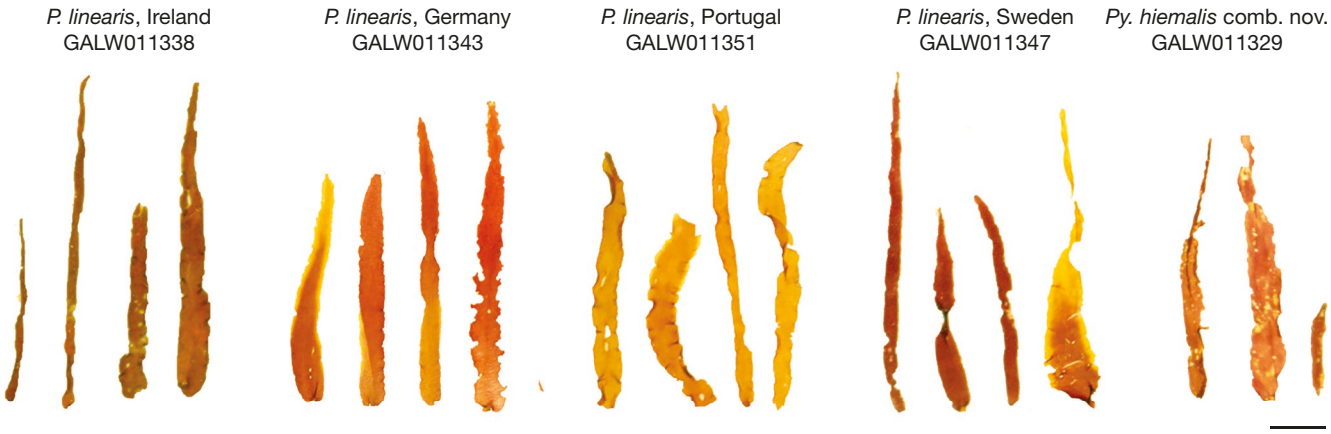

Fig. 2. — Herbarium specimens of the isolates of *Porphyra linearis* Greville and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov.: *Porphyra linearis* from Ireland (Waterford, GALW01138), Germany (Helgoland, GALW011343), Portugal (Viana do Castelo, GALW011351) and Sweden (Lyckeby, GALW011347); *Py. hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. from Sweden (Amundon, GALW011329, epitype of the species). Scale bar: 1 cm.

Table 1. — Morphological characters of *Porphyra linearis* Greville and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov., including the description in the original type.

	Porphyra linearis **Greville**	***P. linearis*** **Greville**	***Pyropia hiemalis*** **comb. nov.**	***Py. hiemalis*** **comb. nov.**
Reference(s)	Greville 1830	Varela-Álvarez 2002; this study	Kylin 1907	This study
Average length (cm)	3-5 inch (7-12 cm)	2-15 cm	6-25 cm	1-6 cm
Average width (cm)	½ inch (1.5 cm)	0.5-3 cm	1-6 cm	0.1-0.7 cm
Colour	Reddish-purple	Dark red to reddish-brown	Red purple or deep red, very shiny	Red/brown
Shape	Linear or linear-lanceolate. Margin slightly waved	Linear to pear shaped on the base	Thallus narrow, extended longitudinally	Linear to pear shaped on the base
Attachment	Very minute disk	Holdfast clearly separated from the thallus. Short stipe of 1 mm length and 1 mm Ø	–	Holdfast clearly separated from the thallus
Cell layer	–	1	–	1
Reproduction	Oval granules, not arranged in a quaternate manner	Mainly monoecious but sometime dioecious	Dioecious, occasionally monoecious. Marginal, similar to *P. laciniata*	Only female plants
Reproduction bodies	Partly scattered, partly in lines	Marginal. Male sorus pale yellow edge. Female sorus: red edge on the base	Antheridia and sporocarps longitudinally extended	Female gametes and zygotospores
Habitat (Exposure)	On rocks and stones, high water mark	On rocks, high littoral, very wave exposed area	Zone-forming Nemalion formation	Half exposed, 1 m
Seasonality	April to May	October to May	October to February	Winter
Locality	England	Sweden, Ireland, Britain, Germany, Portugal	Sweden	Sweden
Zygotosporangial arrangement	–	16 (4*2*2)	–	–
Spermatangial arrangement	–	64 (2*4*8)	–	–
Frond thickness (µm)	–	36-74	55-65 (mid part) 65-75 (lower part)	–
Zygotospore diameter (µm)	–	12.2 +–2.3	–	–
Spermatia diameter (µm)	–	4.56 +–0.8	–	–

and carpogonial and zygotosporangial sorus as a red edge at the base. Algae were generally monoecious and occasionally dioecious (Fig. 2). In the specimens of *P. hiemalis* collected for this study, only female gametes and zygotosporangia were observed.

Molecular analyses

PCR amplification of the *rbc*L-*rbc*S spacer and flanking coding regions was obtained and yielded a product of 333 nt for the seven isolates used in this study and an alignment of also 333 characters. The total data set was composed by

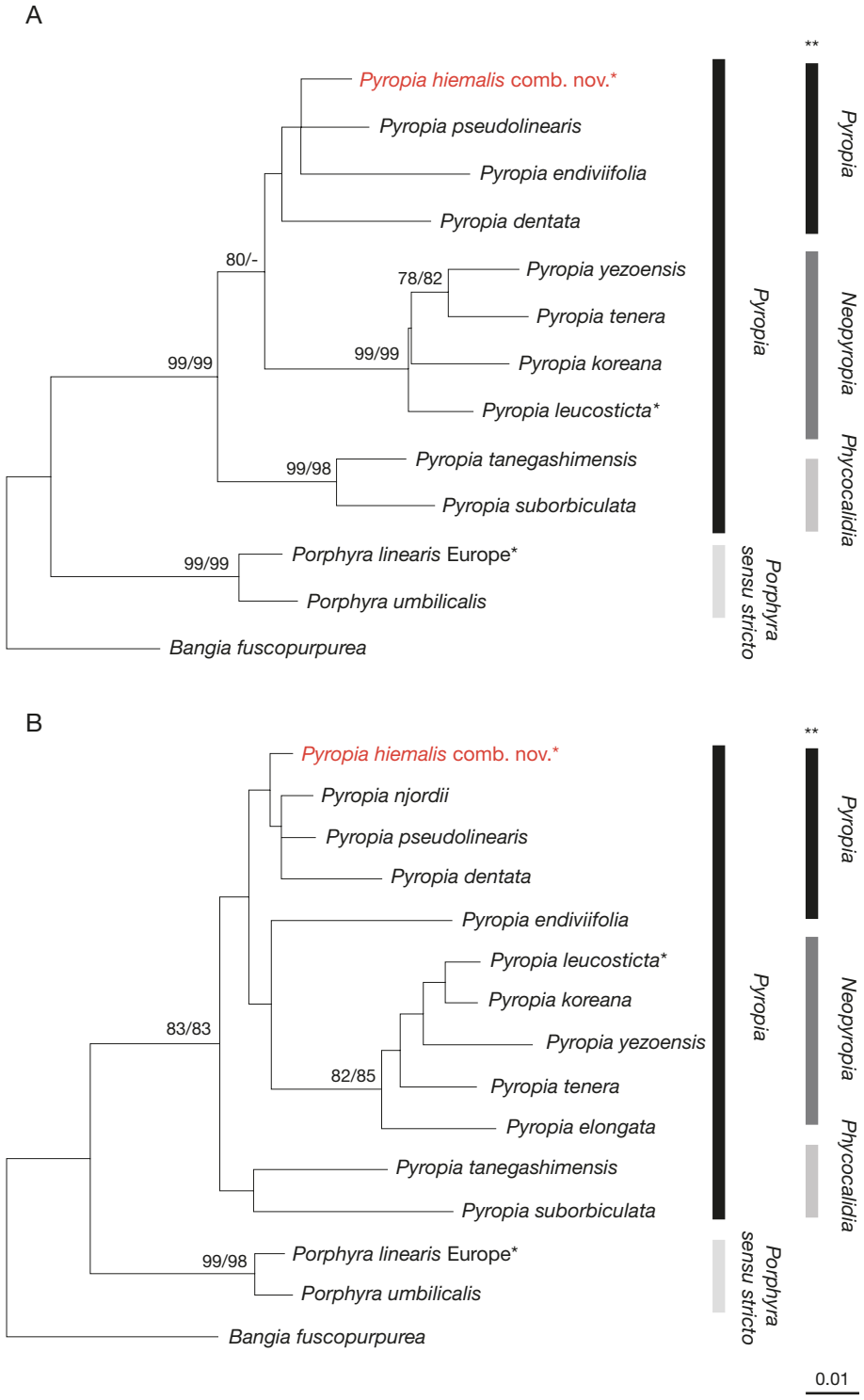

FIG. 3. — Neighbour-joining phylograms: **A**, NJ Phylogram based on the *rbc*S-*rbc*L spacer and adjacent regions (333 nt) from 12 bladed Bangiales; **B**, NJ Phylogram based on a shorter *rbc*S-*rbc*L spacer and partial *rbc*L region (146 nt) of 14 blade Bangiales taxa. Both analyses include isolates of *Porphyra linearis* Greville and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. For each node, 10 000 replicates bootstrap values for NJ and also ML analyses are given using the Jukes-Cantor model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Symbols: *, distinct sequences produced in this study; **, species nomenclature according to Santiañez & Wynne 2020, Yang *et al.* 2020 and *Zuccarello et al.* 2022.

23 bladed Bangiaceae *rbc*S-*rbc*L sequences where 12 were distinct sequences, and each represented a unique species (Table 2). All the sequences identified as *P. linearis* in this study (Ireland, Sweden, Germany, and Portugal) and also the sequences of *P. linearis* retrieved from GenBank were identical. However, the sequence of former *P. hiemalis*, was unique and distinct, and 9-10% divergent in relation to other *Porphyra* species (*P. linearis* and *P. umbilicalis* Kütz-

TABLE 2. — Localities and accession numbers of isolates used in this study. Species named according to Zuccarello *et al.* 2022. Symbol: *, former *Porphyra hiemalis* Kylin.

Taxon	Location	Herbarium	Accession number	Published source
Porphyra linearis	Ireland, Galway	GALW011327	DQ837593	Varela-Álvarez *et al.* 2007
Porphyra linearis	Ireland, Galway	GALW011328	DQ837594	Varela-Álvarez *et al.* 2007
Porphyra linearis	Ireland, Galway	GALW011331	DQ837595	Varela-Álvarez *et al.* 2007
Porphyra linearis	Ireland, Galway	GALW011333	DQ837596	Varela-Álvarez *et al.* 2007
Porphyra linearis	Ireland, Waterford	GALW011338	OP718623	This study
Porphyra linearis	Germany, Helgoland	GALW011343	OP718624	This study
Porphyra linearis	Sweden, Lyckeby	GALW011347	OP718625	This study
Porphyra linearis	Portugal, Viana Castelo	GALW011351	OP718626	This study
Porphyra umbilicalis	United States, Maine	–	MF385003	Brawley *et al.* 2017
Pyropia hiemalis comb. nov*	Sweden, Amundon	GALW011329	OP718627	This study
Pyropia endiviifolia	–	–	KT716756	–
Pyropia pseudolinearis	Japan, Tohaku Tottori	–	AB118581	–
Pyropia pseudolinearis	Japan, Aomori	–	AB287929	–
Pyropia leucosticta	Ireland, Galway	–	OP718628	This study
Pyropia leucosticta	Ireland, Finavarra	–	OP718629	This study
Pyropia koreana	Japan, Aomori	–	LC327005	–
Pyropia tenera	Japan, Yamaguchi	–	AB287949	–
Pyropia yezoensis	–	–	KC517072	Wang *et al.* 2013
Pyropia dentata	South Korea, Songji	–	LC521919	Sung-Je *et al.* 2020
Pyropia tanegashimensis	Japan, Kagoshima	–	AB671541	Tamaki *et al.* 2012
Pyropia suborbiculata	Japan, Kanagawa	–	AB287948	–
Pyropia suborbiculata	Japan, Okinawa	–	LC434502	–
Pyropia suborbiculata	Japan, Yamaguchi	–	AB287943	–
Pyropia elongata	Sweden, Koster	–	FJ817088	Neefus & Brodie 2009
Pyropia njordii	Greenland, Aasiaa	–	JN847258	Mols-Mortensen *et al.* 2012

TABLE 3. — Sequences divergences among *Porphyra linearis* Greville, *P. umbilicalis* Kützing and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. using the 333 nt position alignment of the *rbc*S-*rbc*L region.

	P. linearis	***P. umbilicalis***	***Py. hiemalis* comb. nov.**
Porphyra linearis	–	–	–
Porphyra umbilicalis	0.018	–	–
Pyropia hiemalis comb. nov.	0.093	0.099	–
Pyropia pseudolinearis	0.116	0.106	0.059

ing), whereas it was only 2-7% divergent from other species included in the *Pyropia* genus. When using the shorter *rbc*S-*rbc*L sequences alignment (146 positions, 25 sequences), species divergences between species were 1.4% either within the *Porphyra* genus (e.g. *P. linearis* vs *P. umbilicalis*), or within the *Pyropia* genus (e.g. *Py. pseudolinearis* (Ueda) N.Kikuchi, M.Miyata, M.S.Hwang & H.G.Choi vs *Py. njordii*; *Py. pseudolinearis* vs *Py. hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov.; or *Py. hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. vs *Py. njordii*). When comparing the *P. hiemalis* sequence to *P. linearis* the divergences were of 12% (Tables 3; 4).

Phylograms for both analyses (ML and NJ) displayed congruent and similar tree topologies and shared comparable bootstrap support values for major nodes for the taxa examined, where former *Porphyra hiemalis* was part of the *Pyropia* clade and not part of the *Porphyra* clade, in which they would have been expected to belong if they were a synonym or closely related species regardless of using a longer or shorter *rbc*S-*rbc*L and adjacent sequences.

TAXONOMIC PROPOSAL

Family BANGIACEAE Duby
Genus *Pyropia* J.Agardh

Pyropia hiemalis
(Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov.

Porphyra hiemalis Kylin, *Algenfl. Schwed. Westk*: 112, pl. 3, fig. 2 (Kylin 1907).

LECTOTYPE. — Alg. Scan. Exsicc. no. 211 as "*Porphyra laciniata* Ag. Var. *Porphyra vulgaris* Harv. Phyc. Brit.? Ad saxa in mari Bahusiae – Oct." [leg.] S. Åkermark (lecto-, designated here, UPS).

ISOLECTOYTPES. — Widely distributed with the original exsiccata.

LECTOTYPE LOCALITY. — **Sweden**. Stora Varholmen, leg. S. Åkermark.

EPITYPE. — **Sweden**. Amundon, 11.IV.98, Athanasios Athanasiadis, *GALW 011329* (epi-, designated here, DBN!; GenBank[OP718627]).

DISTRIBUTION. — Currently known only from SW Sweden.

TABLE 4. — Sequences divergences among *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. and related taxa using the 146 nt position alignment of the *rbc*S-*rbc*L region.

	P. linearis	***P. umbilicalis***	***Py. hiemalis* comb. nov.**	***Py. pseudolinearis***	***Py. njordii***
P. linearis	–	–	–	–	–
P. umbilicalis	0.014	–	–	–	–
Py. hiemalis comb. nov.	0.127	0.087	–	–	–
Py. pseudolinearis	0.118	0.095	0.014	–	–
Py. njordii	0.095	0.095	0.014	0.014	–
Py. elongata	0.135	0.135	0.064	0.064	0.064

EMENDED DESCRIPTION

Gametophyte blades linear, 10-60 mm high and 3-20 mm wide, with an irregular undulate margin, with linear to pear shaped on the base. Holdfast clearly separated from the thallus; colour red to brown on outer edges. Dioecious, occasionally monoecious; reproductive structures marginal; dark red zygotosporangia and female sorus on the base of the blade, winter seasonality, on rocks, high littoral, very wave exposed area, and distinct *rbc*S-*rbc*L spacer sequences.

NOTE

Designation of a lectotype for *Porphyra linearis* Greville from a specimen from Sidmouth at E by Brodie & Irvine (2003: 124) was not valid as the phrase "designated here" or an equivalent was not used as required after 1 January 2001 (Art. 7.11; Turland *et al.* 2018). We here designate formally the same specimen ('Sidmouth, Spring', E!) as lectotype.

DISCUSSION

As noted by Greville (1830: 170) in his original description, the "uniformly constant" linear form and April to May occurrence of blades are the main characteristics of *P. linearis*, and all the isolates identified as *P. linearis* in this study (Figs 1; 2) are regarded as authentic populations of *P. linearis*, in the sense of the original description. However, the genetic data (Fig. 3) confirm that Kylin was correct in originally describing *Porphyra hiemalis* as a species separate from *P. linearis*, and this separation is supported by molecular differences, but not morphological differences, and should be referred to the reinstated genus *Pyropia* J. Agardh. This species may be geographically restricted to SW Sweden, since we did not find it in any other location, and it has not been recorded outside Sweden (Kylin 1907; Athanasiadis 2016).

The evolutionary origins of both *Porphyra linearis* and *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov., as well as other related species such as *Pyropia njordii* (a north Atlantic linear species with a wider distribution, present in the top North Atlantic in both sides) or *Pyropia pseudolinearis* (a linear Pacific species), are yet to be discovered, but we can hypothesize that each species arose from different ancestors within the bladed Bangiaceae but morphological convergence occurred. An alternative hypothesis would be that this is the ancestral morphological state in their most recent common ancestor, and it was the other species that evolved differently. The causes of the very restricted distribution of *Pyropia hiemalis* (Kylin) Varela-Álvarez, Guiry & Serrão, comb. nov. are unclear but it can be hypothesized that it was an ancient introduction from the Pacific into SW Sweden. More recent species introductions have happened in other nearby areas such as the Baltic Sea (Leppäkoski 1994).

Acknowledgements

We are very grateful to those who collected plants or helped with sampling: Andreas Wagner, Athanasios Athanasiadis, Mats Kuylenstierna, Isabel Sousa Pinto, David Fewer and Liam Cronin. We would like to thank the two anonymous reviewers whose suggestions and comments improved this manuscript.

Funding

This work was supported by FCT (Fundação para a Ciência e a Tecnologia, Portugal) through SFRH/BPD/109452/2015 in transitional norm DL 57/2016/CP1361/CT0037, both attributed to EVÁ, EU-BiodivERsA BiodivRestore-253 – FCT DivRestore/0013/2020 to ES, and UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 to CCMAR.

REFERENCES

ATHANASIADIS A. 1996. — *Taxonomisk litteratur och biogeografi av Skandinaviska rödalger och brunalger*. Algologia, Göteborg, 280 p.

ATHANASIADIS A. 2016. — *Phycologia Europaea Rhodophyta*. Vol. 1. Published by the author, Thessaloniki, 762 p.

BRODIE J. & IRVINE L. M. 2003. — *Seaweeds of the British Isles*. Vol. 1: *Rhodophyta*. Part 3B: *Bangiophycidae*. Intercept, Andover, 167 p.

BRODIE J., HAYES P. K., BARKER G. L. & IRVINE L. M. 1996. — Molecular and morphological characters distinguishing two *Porphyra* species. *European Journal of Phycology* 31: 303-308. https://doi.org/10.1080/09670269600651521

BRODIE J., HAYES P. K., BARKER G. L. & BARTSCH I. 1998. — A reappraisal of *Porphyra* and *Bangia* (Bangiophycidae, Rhodophyta) in the Northeast Atlantic based on the *rbc*L-*rbc*S intergenic spacer. *Journal of Phycology* 34: 1069-1074. https://doi.org/10.1046/j.1529-8817.1998.341069.x

BLOUIN N. A., BRODIE J. A., GROSSMAN A. C., XU P. & BRAWLEY S. H. 2011. — *Porphyra*: a marine crop shaped by stress. *Trends in Plant Science* 16: 29-37. https://doi.org/10.1016/j.tplants.2010.10.004

Brawley S. H., Blouin N. A., Ficko-Blean E., Wheeler G. L., Lohr M., Goodson H. V., Jenkinsj. W., Blaby-Haas C. E., Helliwell K. E., Chanc. X., Marriage T. N., Bhattacharya D., Klein A. S., Badis Y., Brodie J., Cao Y., Collén J., Dittami S. M., Gachon C. M. M., Green B. R., Karpowicz S. J., Kim J. W., Kudahl U. J., Lin S., Michel G., Mittag M., Olson B. J. S. C., Pangilinan J. L., Peng Y., Qiu H., Shu S., Singer J. T., Smith A. G., Sprecher B. N., Wagner V., Wang W., Wang Z. Y., Yan J., Yarish C., Zäuner-Riek S., Zhuang Y., Zou Y., Lindquist E. A., Grimwood J., Barry K. W., Rokhsar D. S., Schmutz J., Stiller J. W., Grossman A. R. & Prochnik S. E. 2017. — Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *Proceedings of the National Academy of Sciences* 114 (31): 6361-6370. https://doi.org/10.1073/pnas.1703088114

Destombe C. & Douglas S. E. 1991. — Rubisco spacer sequence divergence in the Rhodophyta alga *Gracilaria verrucosa* and closely related species. *Current Genetic*s 19: 395-398. https://doi.org/10.1007/BF00309601

Dumilag R. V. & Monotilla W. D. 2018. — Molecular diversity and biogeography of Philippine foliose Bangiales (Rhodophyta). *Journal of Applied Phycology* 30: 173-186. https://doi.org/10.1007/s10811-017-1201-8

Galtier N., Gouy M. & Gautier C. 1996. — SeaView and Phylo_win, two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences* 12: 543-548.

Guindon S. & Gascuel O. 2003. — A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704. https://doi.org/10.1080/10635150390235520

Greville R. K. 1830. — *Algae britannicae, or descriptions of the marine and other inarticulated plants of the British Islands, belonging to the order Algae; with plates illustrative of the genera*. McLachlan and Stewart, Baldwin and Cradock, Edinburgh, 218 p.

Goff L. J., Moon D. A. & Coleman A. W. 1994. — Molecular delineation of species and species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). *Journal of Phycology* 30: 521-537. https://doi.org/10.1111/j.0022-3646.1994.00521.x

Guillemin M. L., Contreras-Porcia L., Ramirez M., Macaya E. C., Contador C. B., Woods H., Wyatt C. & Brodie J. 2016. — The bladed Bangiales (Rhodophyta) of the South Eastern Pacific: molecular species delimitation reveals extensive diversity. *Molecular Phylogenetics and Evolution* 94: 814-826. https://doi.org/10.1016/j.ympev.2015.09.027

Hong Y. K., Coury D. A., Polne-Fuller M. & Bibor A. 1992. — Lithium chloride extraction of DNA from the seaweed *Porphyra perforata* (Rhodophyta). *Journal of Phycology* 28: 217-220. https://doi.org/10.1111/j.0022-3646.1992.00717.x

Kucera H. & Saunders G. W. 2012. — A survey of Bangiales (Rhodophyta) based on multiple molecular markers reveals cryptic diversity. *Journal of Phycology* 48: 69-82. https://doi.org/10.1111/j.1529-8817.2012.01193.x

Kylin H. 1907. — *Studien über die Algenflora der schwedischen Westküste*. Akademische Abhandlung, K.W. Appelbergs Buchdruckeri, Upsala, 287 p.

Kylin H. 1944. — Die Rhodophyceen der Schwedischen westküste. *Acta Universitatis Lundensis* 40 (2): 1-104.

Leppäkoski E. 1994. — Non-indigenous species in the Baltic Sea, *in* Boudouresque C. F., Briand F. & Nolan C. (eds), *Introduced species in European Coastal waters. Report of an international workshop*. Vol. 8. European Commission, Luxembourg: 67-75.

Lindstrom S. C. & Fredericq S. 2003. — *rbc*L gene sequences reveal relationships among north-east Pacific species of *Porphyra* (Bangiales, Rhodophyta) and a new species, *P. aestivalis*. *Phycological Research* 51: 211-224.

Maggs C. A., Douglas S. E., Fenety J. & Bird C. J. 1992. — A molecular and morphological analysis of the *Gymnogongrus devoniensis* (Rhodophyta) complex in the North Atlantic. *Journal of Phycology* 28: 214-232. https://doi.org/10.1111/j.0022-3646.1992.00214.x

Mols-Mortensen A., Neefus C. D., Nielsen R., Gunnarsson K., Egildotttir S., Perdensen P. M. & Brodie J. 2012. — New insights into the biodiversity and generic relationships of foliose Bangiales (Rhodophyta) in Iceland and the Faroe Islands. *European Journal of Phycology* 47 (2): 146-159. https://doi.org/10.1080/09670262.2012.666678

Mols-Mortensen A., Neefus C. D., Poul Moller P. & Brodie J. 2014. — Diversity and distribution of foliose Bangiales (Rhodophyta) in West Greenland: a link between the North Atlantic and North Pacific. *European Journal of Phycology* 49: 1-10. https://doi.org/10.1080/09670262.2013.871062

Neefus C. D. & Brodie J. 2009. — Lectotypification of *Porphyra elongata* Kylin (Bangiales, Rhodophyta) and proposed synonymy of *Porphyra rosengurttii* Coll et Cox. *Cryptogamie, Algologie* 30 (3): 187-192.

Reddy M. M., De Clerck O., Leliaert F., Anderson R. J. & Bolton J. 2018. — A rosette by any other name: species diversity in the Bangiales (Rhodophyta) along the South African coast. *European Journal of Phycology* 53: 67-82. https://doi.org/10.1080/09670262.2017.1376256

Rosenvinge L. K. 1909. — The marine algae of Denmark. Contributions to their natural history. Part I: Introduction. Rhodophyceae I: Bangiales and Nemalionales. *Kongelige Danske Videnskabernes Selskabs Skrifter, 7. Række, Naturvidenskabelig og Mathematisk Afdeling* 7: 1-151.

Sánchez N., Vergés A., Peteiro C., Sutherland J. E. & Brodie J. 2014. — Diversity of bladed Bangiales (Rhodophyta) in western Mediterranean: recognition of the genus *Themis* and descriptions of *T. ballesterosii* sp. nov., *T. iberica* sp. nov., and *Pyropia parva* sp. nov. *Journal of Phycology* 50: 908-929. https://doi.org/10.1111/jpy.12223

Santiañez W. J. E. & Wynne M. J. 2020. — Proposal of *Phycocalidia* Santiañez & M.J.Wynne nom. nov. to replace *Calidia* L.-E. Yang & J.Brodie nom. illeg. (Bangiales, Rhodophyta). *Notulae Algarum* 140: 1-3.

Sung-Je C., Yonguk K., Jawon S., Dong-Wook K., Hak-Sung L. & Chulyung C. 2020. — Complete chloroplast genome sequences of *Pyropia dentata* (Bangiales, Rhodophyta). *Mitochondrial DNA Part B* 5 (2): 1785-1786.

Sutherland J. E., Lindstrom S. C., Nelson W. A., Brodie J., Lynch M. D., Hwang M. S., Choi H. G., Miyata M., Kikuchi N., Oliveira M. C., Farr T., Neefus C., Mols-Mortensen A., Milstein D. & Müller K. M. 2011. — A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). *Journal of Phycology* 47: 1131-1151. https://doi.org/10.1111/j.1529-8817.2011.01052.x

Tamaki M., Fujita Y., Fujiyoshi E., Kobayashi M., Abe M., Kikuchi N. & Sudo Y. 2012. — Morphological and molecular analysis of *Porphyra yamadae* collected at Ginowan, Okinawajima Island. *Biology Magazine Okinawa* 50: 1-15.

Turland N. J., Wiersema J. H., Barrie F. R., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Kusber W.-H., Li D.-Z., Marhold K., May T. W., Mcneill J., Monro A. M., Prado J., Price M. J. & Smith G. F. 2018. — *International code of nomenclature for algae, fungi, and plants (Shenzhen Code)* adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile*. Vol. 159. Koeltz Botanical Books, Glashütten, 253 p.

Van Oppen M. J., Draisma S. G., Olsen J. L. & Stam W. T. 1995. — Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. *Marine Biology* 123: 179-188. https://doi.org/10.1007/BF00350338

Varela-Álvarez E. 2002. — *Phenology, Life History and Genetics of* Porphyra linearis *Greville, a Candidate for Nori Mariculture*

in Europe. Ph.D thesis (unpublished dissertation). National University of Ireland, Galway, 219 p.

VARELA-ÁLVAREZ E., STENGEL D. B., RINDI F. & GUIRY M. D. 2005. — Alternation of nuclear phases and chromosome numbers in *Porphyra linearis* (Bangiales, Rhodophyta) from western Ireland and Maine, USA. *Phycologia* 44 (1): 61-65. https://doi.org/10.2216/0031-8884(2005)44[61:AONPAC]2.0.CO;2

VARELA-ÁLVAREZ E., STENGEL D. B. & GUIRY M. D. 2007. — Seasonal growth and phenotypic variation in *Porphyra linearis* (Rhodophyta) populations on the west coast of Ireland. *Journal of Phycology* 43: 90-100. https://doi.org/10.1111/j.1529-8817.2006.00300.x

VARELA-ÁLVAREZ E., LOUREIRO J., PAULINO C. & SERRÃO E. 2018. — Polyploid lineages in the *Porphyra* genus. *Scientific Reports* 8: 8696. https://doi.org/10.1038/s41598-018-26796-5

VARELA-ÁLVAREZ E., LOUREIRO J., MEIRMANS P. G., CASTRO M. & SERRÃO E. A. 2021. — Genomes vary in size and spatial patterns within chimeric blades of *Porphyra* spp. *Frontiers in Marine Science* 8: 227. https://doi.org/10.3389/fmars.2021.628183

VARELA-ÁLVAREZ E., MEIRMANS P. G., GUIRY M. D. & SERRÃO E. A. 2022. — Biogeographic Population Structure of Chimeric Blades of *Porphyra* in the Northeast Atlantic Reveals Southern Rich Gene Pools, Introgression and Cryptic Plasticity. *Frontiers in Plant Science* 13: 818368. https://doi.org/10.3389/fpls.2022.818368

WANG L., MAO Y., KONG F., LI G., MA F., ZHANG B., SUN P., BI G., ZHANG F., XUE H. & CAO M. 2013. — Complete Sequence and Analysis of Plastid Genomes of Two Economically Important Red Algae: *Pyropia haitanensis* and *Pyropia yezoensis*. *PLOS ONE* 8 (5): E65902. https://doi.org/10.1371/journal.pone.0065902

YANG L. E., DENG Y. Y., XU G. P., RUSSELL S. L. Q. & BRODIE J. 2020. — Redefining *Pyropia* (Bangiales, Rhodophyta): four new genera, resurrection of *Porphyrella* and description of *Calidia pseudolobata* sp. nov. from China. *Journal of Phycology* 56: 862-879. https://doi.org/10.1111/jpy.12992

ZUCCARELLO G. C., WEST J. A. & KIING R. J. 1999. — Evolutionary divergence in the *Bostrychia moritziana*/*B. radicans* complex (Rhodomelaceae, Rhodophyta): molecular and hybridisation data. *Phycologia* 38: 234-244. https://doi.org/10.2216/i0031-8884-38-3-234.1

ZUCCARELLO G. C., WEN X. & KIM G. H. 2022. — Splitting blades: why genera need to be more carefully defined; the case for *Pyropia* (Bangiales, Rhodophyta). *Algae* 37 (3): 205-211. https://doi.org/10.4490/algae.2022.37.9.11

Submitted on 26 October 2022;
accepted on 12 April 2023;
published on 30 August 2023.