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A reassessment of the foliose Bangiales (Rhodophyta) in the Balearic Islands including the proposed synonymy of *Pyropia olivii* with *Pyropia koreana*

Abstract: A taxonomic reevaluation of the foliose Bangiales placed in the genus *Porphyra* from the Balearic Islands, based on the historical and recent collections, has resulted in a new floristic composition of this group. The molecular, morphological, and karyological analysis reveals that for these islands, there are no *Porphyra* species, and only two members of the genus *Pyropia* are present: *Pyropia elongata* and *Pyropia koreana*. The sequence data of the *rbc*L plastid gene indicate that *Pyropia olivii* and *Pyropia koreana* are conspecific. We, therefore, propose the synonymy of *Pyropia olivii* with *Pyropia koreana*, which is the taxon with nomenclatural priority. The study with the fresh and old material has revealed that neither *Porphyra umbilicalis* nor *Pyropia leucosticta* are present in this area and that most of the old herbarium material that has gone under this name belongs to *Pyropia elongata*.

Keywords: Balearic Islands; Bangiales; mediterranean; *Pyropia elongata*; *Pyropia koreana*.

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Introduction

The order Bangiales has been the subject of much taxonomic change during the last decade due to the several local floristic studies in the different parts of the world (Broom et al. 1999, 2004, Brodie and Irvine 2003, Klein et al. 2003, Lindstrom and Fredericq 2003, Brodie et al. 2007, Mols-Mortensen et al. 2012, Vergés et al. 2013). In addition to the traditional morphological studies, the use of the molecular tools has enabled significant changes to be made in the classification of this group of red seaweeds (Milstein and Oliveira 2005, Sutherland et al. 2011, Kucera and Saunders 2012). A worldwide revision of the Bangiales based on a two-gene phylogeny (Sutherland et al. 2011) has divided the bladed species (*Porphyra sensu lato*) into eight genera: *Boreophyllum* S.C. Lindstrom, N. Kikuchi, M. Miyata, *et* Neefus, *Clymene* W.A. Nelson, *Fuscifolium* S.C. Lindstrom, *Lysithea* W.A. Nelson, *Miuraea* N. Kikuchi, S. Arai, G. Yoshida, J.A. Shin, *et* M. Miyata, *Porphyra* C. Agardh, *Pyropia* J. Agardh, and *Wildemania* De Toni. According to the literature, up to 11 species of the foliose Bangiales have been reported in the Mediterranean of which only three have had their identity confirmed with the molecular tools, and all of them belong to the resurrected genus *Pyropia*: *Pyropia elongata* (Kylin) Neefus and J. Brodie, *Pyropia olivii* (Orfanidis, Neefus, and T. L. Bray) J. Brodie and Neefus, and *Pyropia suborbiculata* (Kjellman) J.E. Sutherland, H.G. Choi, M.S. Hwang, and W.A. Nelson (Brodie et al. 2007, Vergés et al. 2013).

The Balearic Islands are an archipelago of Spain located in the western Mediterranean Sea. The first floristic marine catalogs in this group of islands were produced in the nineteenth century (Colmeiro 1868, Barceló 1881). However, only two members of the genus *Porphyra*, *Porphyra laciniata* C. Agardh (at present considered synonymous in part with *Porphyra purpurea* (Roth) C. Agardh and, in part, with *Porphyra dioica* J. Brodie *et* L. M. Irvine, see Brodie and Irvine 2003) and *Porphyra vulgaris* C. Agardh were included in these surveys. A more recent catalog elaborated by Ribera Siguan and Gómez Garreta (1984) considered these two species (*P. laciniata* and *P. vulgaris*) to be the misidentified samples of *Porphyra umbilicalis* Kützing. In addition, two other species were also reported for the Balearic Islands by these and other authors: *Pyropia leucosticta* (Thuret) Neefus *et* J. Brodie (as *Porphyra leucosticta* Thuret, Rodríguez y Femenías 1889, Ribera Siguán and Gómez Garreta 1984, Cremades 1989) and *Pyropia elongata* (as *Porphyra rosengurttii* J. Coll *et* J. Cox, Brodie et al. 2007).

As a first step in the clarification of the biodiversity of the foliose Bangiales in the Mediterranean Sea, here, we

provide a taxonomic revision of this group in the Balearic Islands using both the molecular and morphological data obtained from the specimens collected in this area by us and the herbarium samples.

Materials and methods

Collections and sampling

The sampling started in December 2009 and was finished in August 2010 in order to establish the seasonality of the bladed Bangiales. The study area was the coast of Majorca Island (Balearic Islands, Spain; 43°27′24″, 36°00′03″) where 14 localities were sampled (Table 1). The specimens were collected from the rocky intertidal zone and the upper sublittoral (Table 1). The vouchers were pressed on the day they were collected, and a subsample of each was stored in the silica gel for the molecular analysis (Table 2). The herbarium sheets of the dried specimens are housed at the Herbarium of the University of Girona (HGI) and at the personal herbarium of Natàlia Comalada. In addition to our collections, all the specimens referred to the genus *Porphyra* located in the 14 herbaria of the Iberian Peninsula [BCN-Phycophyta, BIO-Cryp, FCO, HGI-A, MA-Algae, MACB, MAF-Algae, MGC-Phyc., MUB, PO, SANT, UALG, VAL, and J.J. Rodríguez y Femenías herbarium housed at l'*Ateneu de Maó* (Minorca)] were examined with the aim of detecting any earlier samples of the bladed Bangiales species in the study area. The herbarium abbreviations follow that of Thiers (2012).

Molecular data analysis

The samples for use in the molecular analyses were selected based on the preliminary morphological identification (Tables 1 and 2). The total DNA was extracted using a modified CTAB microextraction protocol (modified from Rogers and Bendich 1994). The standard polymerase chain reaction (PCR) procedures were applied to amplify the *rbc*L fragment using a combination of the four primer pairs: F67 (TAC GCT AAA ATG GGT TAC TG), R502 (TAT CCA TAC GCT CAC GTT CTA CAA), F461 (GTC CTG CAA CTG GAT TGA TTG T) R901 (TAC CAG CTC TAT GTA AAT GTA AAA), F870 (TGA CAT GAT TTT ACA TTT ACA TAG AC) R1312 (3′ GGC CTT CAT TTC TTG CCA TAA C 3′), 5rc (GTG GTA TTC ATG CTG GTC AAA) rbcspc (CAC TAT TCT ATG CTC CTT ATT KTT AT) (Teasdale et al. 2002; A. Mols-Mortensen, personal communication). The PCR protocols used were one initial denaturation cycle at 93°C for 3 min followed by 29 cycles at 93°C for 30 s, 45°C for 1 min and 72°C for 1.5 min, and a final extension cycle at 72°C for 10 min as described in Teasdale et al. (2002). The sequence reactions were run on an ABI 377 DNA sequencer (Perkin Elmer, Waltham, MA, USA).

The sequences were edited and assembled using the Sequencher® version 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) and then aligned using the Mac Clade v4.8 (Maddison and Maddison 2005). The identity of the new sequences was checked by BLAST (Altschul et al. 1990, http://blast.ncbi.nlm.nih.gov). To compare our specimens with the previous identifications, we downloaded representative *rbc*L sequences from Genbank of the most similar species obtained after the extensive BLAST search (*Pyropia elongata*, *Pyropia olivii*, and *Pyropia koreana*) together with the *rbc*L sequences from the traditionally recorded species in this area (*Pyropia leucosticta* and *Porphyra umbilicalis*) and the sequences from the other morphologically similar species that have been found recently from the areas close by including the Iberian Mediterranean coast (*Pyropia suborbiculata*; Vergés et al. 2013). We analyzed 1333 nucleotides for each isolate. The identification of the most appropriate model of the evolution, the number of nucleotide differences, and the pairwise distances between the sequences were calculated using *MEGA* version 5 (MEGA, Tempe, AZ, USA) following Tamura et al. (2011). The pairwise distances were conducted using the Tamura-Nei model, and the rate variation among the sites was modeled with a gamma distribution (shape parameter=0.23) (Tamura and Nei 1993).

Morphological study

The morphological observations were made by rehydrating the selected fragments from the herbarium sheets with seawater. The sections were made by hand with a razor blade, and the morphological characters most frequently used to distinguish the foliose Bangiales (gross morphology, plus anatomical and reproductive structures; Brodie and Irvine 2003, Brodie et al. 2007) were examined. The photomicrographs were taken with a Zeiss AxioCam MRc3 camera attached to an Axioskop 2 plus microscope (Carl Zeiss, Berlin, Germany). The terminology for the reproductive structures follows that of Nelson et al. (1999).

Karyological methods

The chromosome number was obtained using the herbarium specimens that were stained with Wittmann's (1965) aceto-iron-hematoxylin-chloral hydrate technique following the protocol in Holmes and Brodie (2005).

Table 1 The collection information for *Pyropia elongata* and *Pyropia koreana* records in the Balearic Islands.

Species	Collection number	Locality	Data collected	Collected by
Py. elongata	Herb. Rod. y Fem. 0375 (as *Porphyra leucosticta*)	Port de Maó, Minorca	03 March 1886	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0546 (as *P. leucosticta*)	Port de Maó, Minorca	03 April 1886	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0547 (as *P. leucosticta*)	Port de Maó, Minorca	03 April 1887	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0548 (as *P. leucosticta*)	Port de Maó, Minorca	24 March1888	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0549 (as *P. leucosticta*)	Port de Maó, Minorca	14 April 1889	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0550 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0551 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0552 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0553 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0554 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0555 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0556 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0557 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0558 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0559 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0560 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0561 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0562 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0563 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0564 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	Herb. Rod. y Fem. 0565 (as *P. leucosticta*)	Port de Maó, Minorca	05 May 1890	JJ. Rodríguez y Femenías
Py. elongata	SANT-A 1280 (as *P. leucosticta*)	Cala Blava, Mallorca	18 April 1987	J. Cremades
Py. elongata	Herb. N. Comalada PP04	Platja de Ca'n Pere Antoni, Mallorca	6 March 2010	N. Comalada
Py. elongata	Herb. N. Comalada PP13	Platja de Ca'n Pere Antoni, Mallorca	6 March 2010	N. Comalada
Py. elongata	Herb. N. Comalada PP179	Platja de Ca'n Pere Antoni, Mallorca	17 April 2010	N. Comalada
Py. elongata	Herb. N. Comalada PP181	Platja de Ca'n Pere Antoni, Mallorca	17 April 2010	N. Comalada
Py. elongata	HGI-A 9565[b]	Platja de Ca'n Pere Antoni, Mallorca	6 March 2010	N. Comalada
Py. elongata	HGI-A 9566	Platja de Ca'n Pere Antoni, Mallorca	6 March 2010	N. Comalada
Py. elongata	HGI-A 9567	Platja de Ca'n Pere Antoni, Mallorca	6 March 2010	N. Comalada
Py. elongata	HGI-A 9568	Platja de Ca'n Pere Antoni, Mallorca	18 March 2010	N. Comalada
Py. elongata	HGI-A 9569	Cala Gamba, Mallorca	25 March 2010	N. Comalada
Py. elongata	HGI-A 9570	Cala Gamba, Mallorca	25 March 2010	N. Comalada
Py. elongata	HGI-A 9571	Cala Gamba, Mallorca	27 March 2010	N. Comalada
Py. elongata	HGI-A 9572	Cala Gamba, Mallorca	27 March 2010	N. Comalada
Py. elongata	HGI-A 9573	Platja de Ca'n Pere Antoni, Mallorca	4 April 2010	N. Comalada
Py. elongata	HGI-A 9574	Platja de Ca'n Pere Antoni, Mallorca	17 April 2010	N. Comalada
Py. koreana	SANT-A 1279 (as *P. leucosticta*)	Illetes, Mallorca	11 April 1987	J. Cremades
Py. koreana	Herb. N. Comalada ES131	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES132	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES133	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES134	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES135	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES136	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES137	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada ES138	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC139	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC140	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC141	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC142	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC143	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC144	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	Herb. N. Comalada PC145	Porto Colom, Mallorca	05 April 2010	N. Comalada
Py. koreana	HGI-A 9575	Platja deCa'n Pere Antoni, Mallorca	4 April 2010	N. Comalada
Py. koreana	HGI-A 9576	S'Estanyol, Mallorca	05 April 2010	N. Comalada
Py. koreana	HGI-A 9577	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9578	Port d'Andratx, Mallorca	10 April 2010	N. Comalada

(**Table 1** Continued)

Species	Collection number	Locality	Data collected	Collected by
Py. koreana	HGI-A 9579	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9580	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9581	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9582	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9583	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9584	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9585	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9586	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9587[a]	Port d'Andratx, Mallorca	10 April 2010	N. Comalada
Py. koreana	HGI-A 9588	Port d'Andratx, Mallorca	24 April 2010	N. Comalada

[a]The specimens used for the molecular studies.

Table 2 The samples used in calculated genetic distances, voucher or isolate number, locality, Genbank accession number and reference are indicated.

Taxa	Voucher/isolate number	Region/country: locality	GenBank accession number (*rbc*L)	Reference
Pyropia elongata	Lectotype	Sweden: Bohuslan, Koster	FJ817088	Neefus and Brodie (2009)
Py. elongata	"JB6 Specimen 1"	Spain: Málaga	DQ837019	Brodie et al. (2007)
Py. elongata	HGI-A 9565	Spain: Majorca, Balearic Islands	KC347609	This work
Pyropia olivii	BM000806050, holotype	Greece: N. Krini, Thessaloniki Gulf	DQ837007	Brodie et al. (2007)
Py. olivii	NHA78138	United States of America: Dover Point, New Hampshire	DQ813625	Brodie et al. (2007)
Pyropia koreana	strain="HM069"	Korea	HQ728198	Sutherland et al. (2011)
Py. koreana	HGI-A 9587	Spain: Majorca, Balearic Islands	KC347610	This work
Pyropia leucosticta	BM000898751	United Kingdom: Sidmouth	HQ687528	Sutherland et al. (2011)
Pyropia suborbiculata	SVP_TAR547	Spain: Tarifa	JQ327836	Vergés et al. (2013)
Py. suborbiculata	SVP_MA635.1	Spain: Málaga	JQ327835	Vergés et al. (2013)
Pyropia umbilicalis	WELT A024414	United Kingdom: Sidmouth	HQ687559	Sutherland et al. (2011)

Results

Diversity of the genus *Pyropia* in the Balearic Islands

The molecular and morphological data of the representative specimens from the Balearic Islands led us to recognize the two members of the foliose Bangiales occurring in this area: *Pyropia elongata* and *Pyropia koreana*.

Molecular results

The molecular *rbc*L gene characterization based on the calculations of the nucleotide differences and the pairwise distances between the sequences (Table 3) indicated that the closest sequences to that of the specimen HGI-A 9565 collected in Majorca were those identified as *Pyropia elongata* samples (including its lectotype sequence FJ817088). The differences between the sequences ranged from 0.1% to 0.2% (one to three nucleotides). Accordingly, we identified the specimen from which the HGI-A 9565 sequence had been obtained as *Py. elongata*. The analysis for the sequence of the specimen labeled as HGI-A 9587 indicated that it was very similar to *Pyropia olivii* (including its holotype sequence DQ807007) and *Pyropia koreana* (HQ728198) sequences, with the differences between them ranging from 0% to 0.2% (zero to two nucleotides). Furthermore, the sequences from all these specimens (*Py. olivii* and *Py. koreana*), DQ807007 (holotype), DQ813625, HQ728198, and HGI-A 9587 from Greece, USA, Korea, and the Balearic Islands, respectively, indicated that they were strongly related to each other as the differences between their sequences only ranged from 0% to 0.2% (zero to two nucleotides). Given these small molecular differences, together with the very similar morphological, reproductive, and ecological features

Table 3 Genetic differences for *rbc*L gene between *Pyropia elongata*, *Pyropia olivii*, and *Pyropia koreana* in the Balearic Islands and the recently found species in the neighboring areas (Iberian Mediterranean coast).

	Py. elongata[a] FJ817088 (lectotype)-Sweden	*Py. elongata*[b] DQ837019 Iberian Peninsula (Málaga)	*Py. elongata*[b] HGI-A 9565-Balearic Islands	*Py. olivii*[a] DQ807007 (holotype)-Greece	*Py. olivii*[a] DQ813625-USA	*Py. koreana*[c] HQ728198-Korea	*Py. koreana*[b] HGI-A 9587-Balearic Islands	*Pyropia leucosticta*[c] HQ687528-UK	*Pyropia suborbiculata*[d] JQ327835-Iberian Peninsula (Málaga)	*Pyropia suborbiculata*[d] JQ327836-Iberian Peninsula (Tarifa)	[c]*Porphyra umbilicalis* HQ687559-UK
FJ817088		0.001	0.002	0.031	0.032	0.032	0.033	0.051	0.133	0.133	0.199
DQ837019	1		0.002	0.030	0.031	0.031	0.032	0.050	0.132	0.132	0.197
HGI-A 9565	3	2		0.032	0.033	0.033	0.032	0.053	0.127	0.130	0.203
DQ807007	34	33	35		0.001	0.001	0.002	0.042	0.125	0.125	0.190
DQ813625	35	34	36	1		0.000	0.001	0.043	0.128	0.128	0.187
HQ728198	35	34	36	1	0		0.001	0.043	0.128	0.128	0.187
HGI-A 9587	36	35	35	2	1	1		0.044	0.126	0.129	0.189
HQ687528	51	50	52	44	45	45	46		0.141	0.138	0.240
JQ327835	97	96	94	97	98	98	97	104		0.002	0.151
JQ327836	97	96	96	97	98	98	99	102	2		0.151
HQ687559	131	130	132	130	129	129	130	143	110	110	

The absolute number of nucleotide substitutions are below the diagonal and pairwise genetic distance above ([a]Brodie et al. 2007; [b]This work; [c]Sutherland et al. 2011; [d]Vergés et al. 2013).

(Table 4), we concluded that all these taxa are the same species. *Py. koreana* has nomenclatural priority over *Py. olivii*, which is, therefore, considered to be a synonym.

To corroborate these identifications and to be able to provide evidence to confirm their possible misidentification with the other traditionally reported species, we also compared HGI-A 9565 (*Pyropia elongata*) and HGI-A 9587 (*Pyropia koreana*) sequences with *Pyropia leucosticta* and *Porphyra umbilicalis* (Table 3). We observed that the differences between HGI-A 9565 and *P. leucosticta* sequences were approximately 5.3% (52 nucleotides), while the differences between HGI-A 9565 and *P. umbilicalis* were much more marked, at approximately 20.3% (132 nucleotides). The pairwise distances and nucleotide differences with HGI-A 9587 showed divergences of 4.4% (46 nucleotides) with *Py. leucosticta* and of approximately 18.9% (130 nucleotides) with *P. umbilicalis*. A comparison of HGI-A 9565 and HGI-A 9587 sequences with those for *Pyropia suborbiculata*, a species which has recently been reported from the Iberian Mediterranean coast (Vergés et al. 2013) shows differences of approximately 12.7–13.2% (94–96 nucleotides) for HGI-A 9565 and 12.6–12.9% (97–99 nucleotides) for HGI-A 9587.

Overall, these genetic comparisons within the *rbc*L gene between all the analyzed specimens are among the expected variation in the Bangiales. Hence, among our specimens, the intraspecific divergences ranged from 0% to 0.2%, while the divergences between the species ranged from 4.4% to 13.2%, whereas the divergences between the genera ranged from 15.1% to 24% (Klein et al. 2003, Lindstrom and Fredericq, 2003, Brodie et al. 2007, Lindstrom 2008, Kikuchi et al. 2010, Sutherland et al. 2011, Kucera and Saunders 2012).

Taxonomic observations

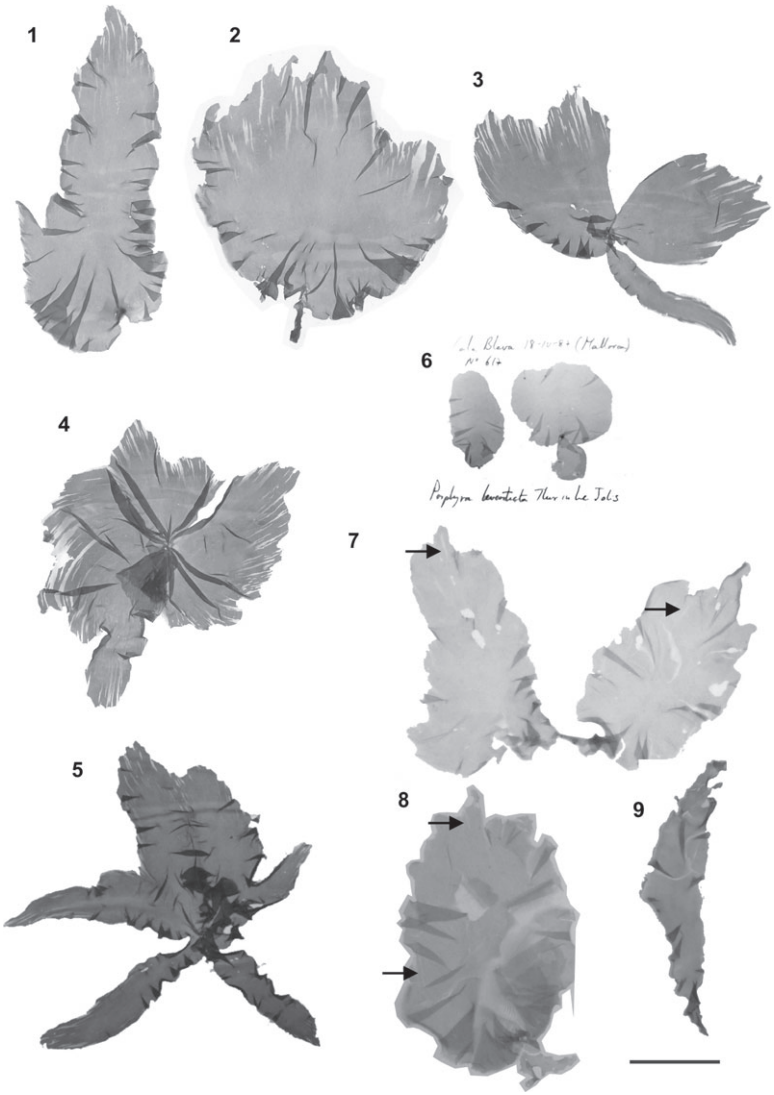
Pyropia elongata (Kylin) Neefus and J. Brodie (Figures 1–22)

The Balearic specimens present foliose thalli, up to 22 cm long and 11 cm broad, with minute discoid holdfasts and sometimes a short stipe that expands into a single or few elongated, rounded, and sometimes lobed or laciniate blades (Figures 1–9); when fresh, the specimens were brown with gray or greenish spots; when dried, they were brownish or slightly blue. The blades were monostromatic, with an entire margin, folded or ruffled. The vegetative cells were easily recognizable as they were polygonal in the surface view (SV) and isodiametric in the TS (38–57 µm). The thalli were monoecious; the male gametangia were

Table 4 A comparison of *Pyropia koreana* from the Mediterranean Iberian Peninsula with the original description from Korea (holotype), the original description of *Pyropia olivii* from Greece (holotype), and the specimens of *P. olivii* from New England (ND=no data).

Data	*Py. koreana* Balearic Islands, Spain	*Py. koreana* (type), Korea	*Py. olivii* (type), Thessaloniki, Greece	*Py. olivii*, New England, USA
Color				
Fresh material	Brown to brownish-red to pink	Bright red or brownish red	Brownish to red with a touch of olive green or brownish red to violet	Brown to greenish beige in vegetative zones; tan to light green around the holdfast
Dried material	Purple or violet to pink	ND	Pink with a hint of blue or grayish with a touch of purple	Light brownish pink, rose pink to pink with touch of blue
Shape	Reniform to elongate to round and sometimes lobulate or laciniate	Elliptical to obovate	Orbicular to broad-ovate or oval, not laciniate	Ovate, obovate to lanceolate, not laciniate
Dimensions (length×breadth cm)	0.4–4×0.5–1	6–10×4–8	2–14×2–13	2.5–18×1–7
Margins	Entire where male sori do not disintegrate; ruffled or folded	Entire, undulate, and cuneate to round at the base	Entire; becoming irregular when male sori disintegrate; slightly to moderate ruffled, especially in the lower part of the frond	Entire; slightly to moderate ruffled; with occasional tears
Base	Pseudoumbilicate with a tiny stipe that is not centered. Several blades growing from the same point	ND	Slightly to deeply cordate; sometimes umbilicate	Minutely stipitate, sometimes very slightly cordate
Fertile areas	Monoecious, spermatangial sori diamond to irregular-shaped; zygotosporangial sori reddish, between the male sori	Monoecious, spermatangia sori small rectangular or rhombic shaped; no data about zygotosporangial sori	Monoecious, spermatangial sori diamond to irregular-shaped or streaky; zygotosporangial sori reddish, between the male sori, and broader at the distal end	Monoecious; spermatangial sori mainly distal in spots or streaks; zygotosporangial sori inconspicuous, marginal to submarginal
Male packet arrangement of gametangia	4 tiers of 8 (16) (32 or 64 male gametes)	4 tiers of 8 (16) (32 or 64 male gametes)	4 tiers of 8 (32 male gametes)	4 tiers of 8 (16) (32 or 64 male gametes)
Female packet arrangement of gametangia	2 tiers of 4 (8 zygotospores)	2 tiers of 4 (8 zygotospores)	2 tiers of 4 (8 zygotospores)	2 tiers of 4 (8 zygotospores)
References	This study	Huang and Lee 1994	Brodie et al. 2007	Brodie et al. 2007

scattered in patches in the upper margin of the blade forming narrow streaks elongated in the direction of the blade expansion and surrounded by a marginal zygotosporangial zone (Figures 1–5, 7–8). In the SV, the male gametangia occurred in 8 packets of 16 each (Figure 10) divided periclinally to give 8 cells in the TS (Figures 10–11), giving 1024 male gametes in each packet, and with n=4 chromosomes in each male gamete and, occasionally, n=3 chromosomes (Figure 12). The female gametangia developed a small protuberance, the trichogyne (Figures 13–18); the cell walls of the blade bulged when the trichogynes protruded beyond them; the cell wall bulge remained even after the zygotospores were formed. The zygotosporangia (Figure 19) occurred in 4 packets of



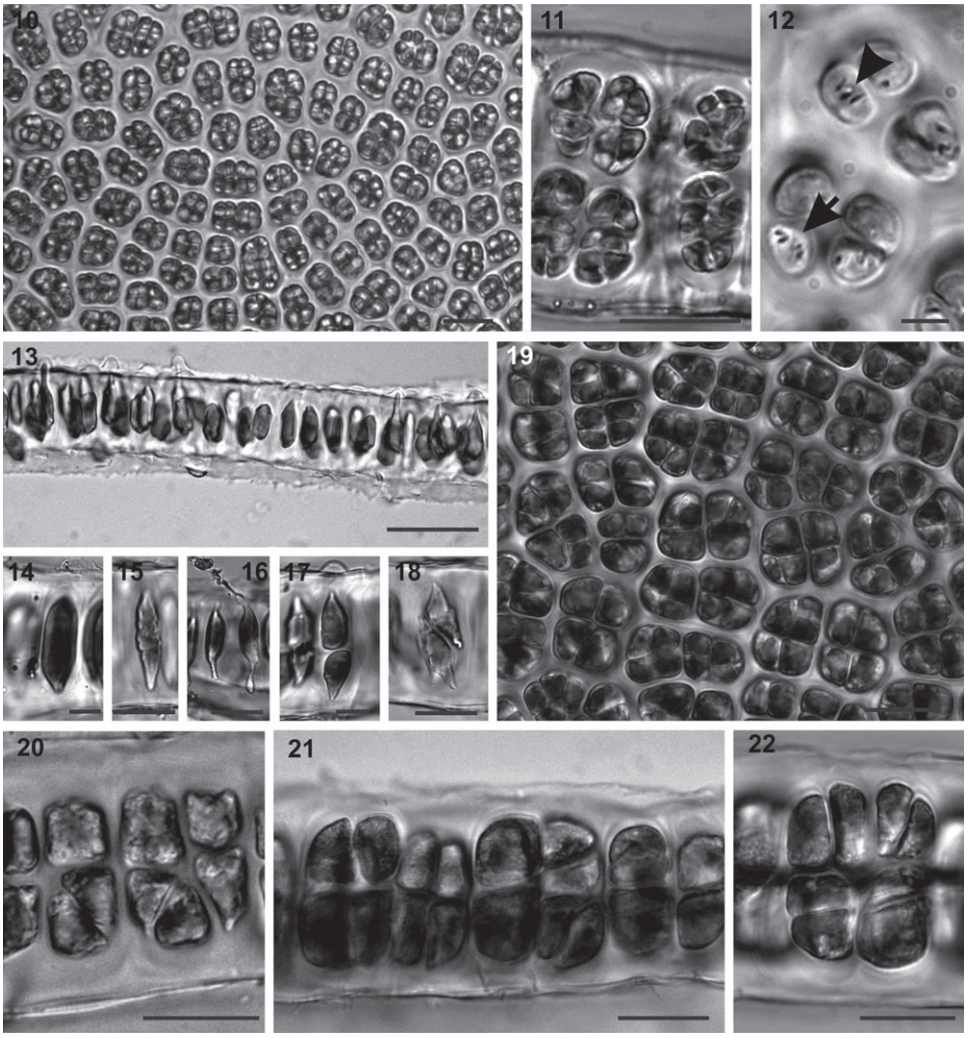
Figures 1–9 *Pyropia elongata*: the habit morphology. Scale bar=3 cm. **(1)** The elongated specimen (N. Comalada Herbarium PP179). **(2)** The rounded specimen (N. Comalada Herbarium PP181). **(3)** The specimen with three different blades growing from the same base (N. Comalada Herbarium PP04). **(4)** The umbilicate thallus (N. Comalada Herbarium PP13). **(5)** The laciniate thallus (HGI-A 9565). **(6)** The specimens from the Sant Herbarium misidentified as *Porphyra leucosticta* (SANT-A 1280). **(7–9)** The specimens from the Herbarium of J.J. Rodríguez-Femenías, misidentified as *Porphyra leucosticta*. The arrows show the radiated male sori (Rodr.-Femenías Herbarium 0550, 0562, 0375).

4 (sometimes eight) each divided periclinally to give 4 cells in the TS (Figures 20–22), giving 64 zygotospores in each packet with each spore 14–16 µm in diameter, which were distributed in scattered streaky areas along the blade margin. The conchocelis phase was not recorded in the field.

The *Pyropia elongata* thalli were found in the intertidal, up to 20 cm above the sea level, growing on the rocks or limpets, and occasionally epiphytic on the other algae or artificial substrata such as the ropes, and formed a clear brown band covering the substrata. They occurred from winter to spring. The individuals appeared in March, after which they began to decline, and they had completely disappeared in May.

Synonymy of *Pyropia olivii* Orfanidis, Neefus, and Bray with *Pyropia koreana* (M.S. Hwang and I.K. Lee) M.S. Hwang, H.G. Choi, Y.S. Oh, and I.K. Lee (Figures 23–48)

The study of the anatomical and reproductive structures characterize this species in the Mediterranean as having a small foliose thallus, 0.4–4 cm long and 0.5–1 cm broad, attached with a rhizoidal holdfast and short stipe, often not located in the center of the blade, which expands into a single or usually several, ovate, elongate, or sometimes reniform blades (Figures 23–38). When fresh, the specimens were brown to brownish-red or pink, translucent and delicate, and when dried, the thalli were purple or pinkish in color. The blades

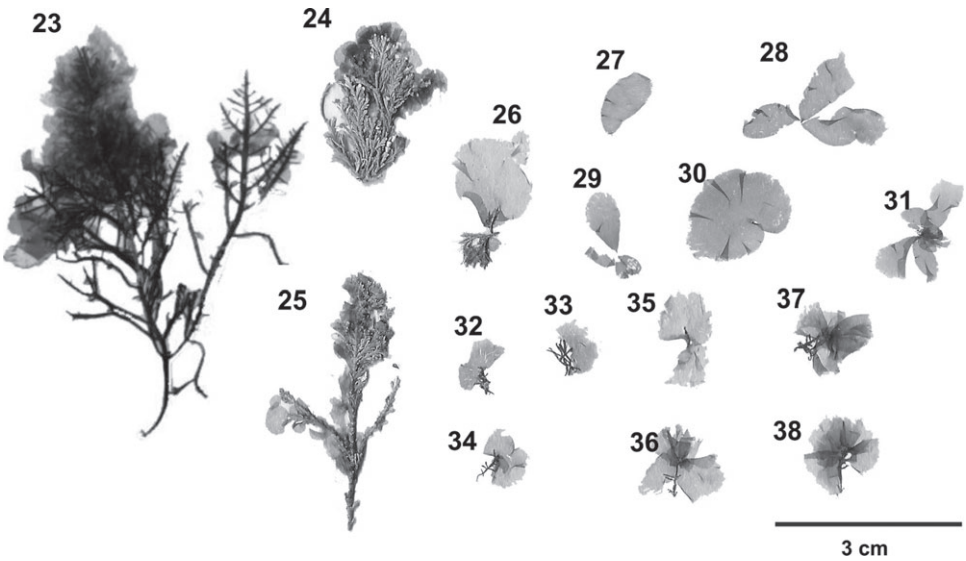


Figures 10–22 *Pyropia elongata*: the anatomical and reproductive structures. Scale bar=20 µm. **(10)** The mature male sorus (HGI-A 9565). **(11)** The TS of mature male gametangia (HGI-A 9565). **(12)** The chromosomes of the male gametes, most of them with n=4 (arrow) and sometimes n=3 (arrowhead) (HGI-A 9570). **(13–18)** The TS of the female gametangia with the trichogynes in the different stages of development and most of them with the bulging cell walls where trichogynes were located (HGI-A 9571). **(19)** The zygotosporangia in the SV (HGI-A 9566). **(20–22)** The zygotosporangia in the TS (HGI-A 9573).

were monostromatic, with an entire margin, sometimes ruffled or slightly folded. The vegetative cells were mixed with rhizoidal filaments at the base of the blade (Figure 39), in the rest of the thallus, they were polygonal in the SV and isodiametric in the TS, up to 16 µm in diameter (Figure 40). The thalli were monoecious; the male gametangia were scattered in small patches (Figure 41) in the middle-upper part of the blade. In the SV, the male gametangia occurred in 4 packets of 8 (sometimes 16; Figures 42 and 43) each divided periclinally to give two cells in the TS (Figure 45), giving 64 male gametes in each packet, with two chromosomes in each male gamete (Figure 44). The female gametangia developed a small protuberance, the trichogyne (Figures 45 and 46); the cell walls of the blade bulged when the trichogynes protruded beyond them; the cell wall bulges and fertilization canals remained even after the zygotospores were formed (Figure 47).

The zygotosporangia consist of 2 packets with one spore each, which are divided periclinally to give 2 cells in the TS (Figure 48), so that each zygotosporangium contains four zygotospores, 10–12 µm in diameter, which were formed adjacent to the male sori. The conchocelis phase was not recorded in the field.

The *Pyropia koreana* thalli were found in the upper subtidal level growing mainly epiphytically on the *Corallina elongata* and also on the other seaweeds of the upper sublittoral (e.g., *Pterocladiella capillacea*, *Gelidium* sp.) or

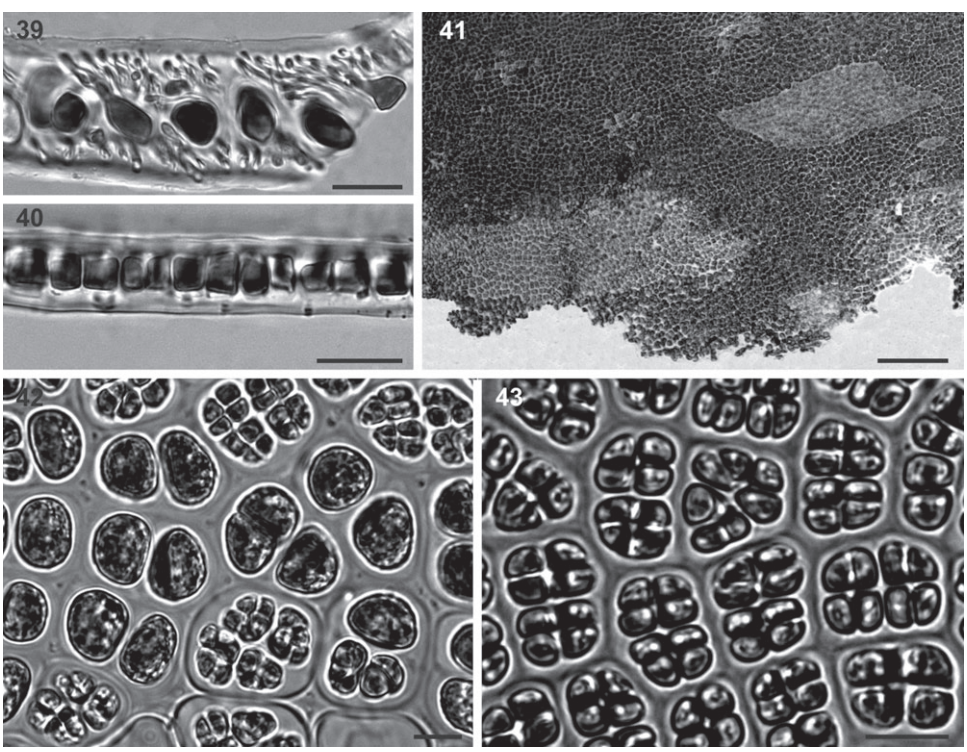


Figures 23–38 *Pyropia koreana*: the habit morphology. Scale bar=3 cm. **(23)** The specimens from the Sant Herbarium misidentified as *P. leucosticta* growing on *Pterocladiella capillacea* (SANT-A 1279). **(24–31)** The epiphytic specimens on *Corallina elongata* (N. Comalada Herbarium PC139-145). **(32–38)** The epiphytic specimens on *Gelidium* sp. (N. Comalada Herbarium ES131-138).

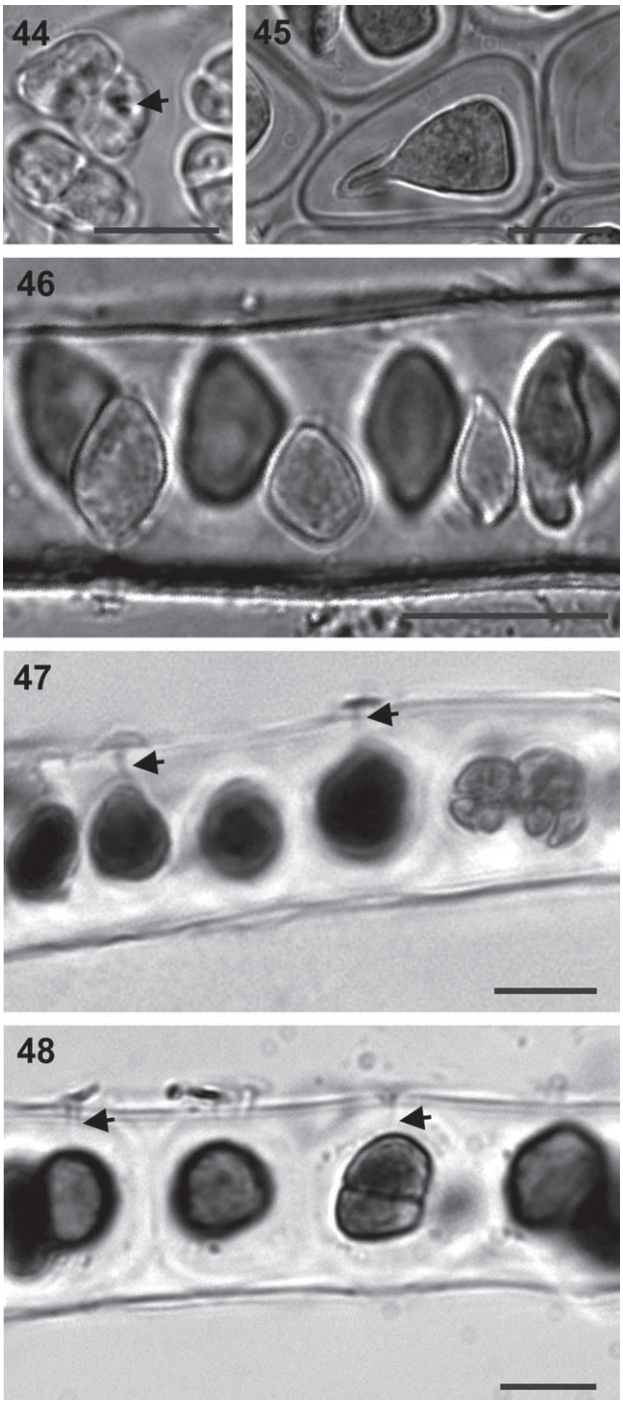
on the artificial substrata such as the ropes. This species was found from winter to spring. The individuals appeared from the end of February until mid-April and completely disappeared in May.

Discussion

The molecular data, morphology, karyology, and ecology of *Pyropia elongata* matched with the other previously



Figures 39–43 *Pyropia koreana*: the anatomical and reproductive structures. Scale bar: 10 μm (Figures 42–43), 20 μm (Figures 39–40), 200 μm (Figure 41). **(39)** The TS of the base showing the rhizoidal filaments (HGI-A 9585). **(40)** The TS of the vegetative cells (HGI-A 9585). **(41)** The sori of the male gametangia in the patches near the margin of the blade (HGI-A9586). **(42)** The packets of the immature and mature male gametangia in the SV (HGI-A 9586). **(43)** The packets of the mature male gametangia in the SV (HGI-A 9586).



Figures 44–48 *Pyropia koreana*: the anatomical and reproductive structures. Scale bar: 10 µm. **(44)** The chromosomes of the male gametes (n=2, arrow) (HGI-A 9578). **(45)** The female gamete with a trichogyne in the SV (HGI-A 9584). **(46)** The female gametes in the TV (HGI-A 9584). **(47)** The young zygotosporangia where fertilization canals (arrows) and bulged trichogynes remain, developing near the male gametangia (HGI-A 9586). **(48)** The zygotosporangia divided in the two zygotospores in the TV with the fertilization canals (arrows) (HGI-A 9586).

published surveys (Brodie et al. 2007, Neefus and Brodie 2009) and allowed us to establish that this species is very common in the Balearic Islands. This report confirms that this species is widespread in the North Atlantic (Brodie et al. 2007, Mols-Mortensen et al. 2012), and its distribution in the Mediterranean has been found to be more widespread as more detailed studies were done. The revision of the old sheets collected since 1886, located in the J.J. Rodríguez y Femenías Herbarium and misidentified as *Porphyra leucosticta* (currently *Pyropia leucosticta*) led us to hypothesize that the identity of *Py. elongata* could have been mistaken for a long time. This highlights the possibility that *Py. leucosticta* was never present in the Balearic Islands and has been confused with *Py. elongata*, as demonstrated in the other parts of the Mediterranean (Brodie et al. 2007).

The other Balearic species of the genus *Pyropia*, *Pyropia koreana*, is easy to differentiate from *Pyropia elongata* as it is much smaller (up to 4 cm), occurs in the sublittoral, and has male sori that are located in very small patches. The molecular analysis demonstrated that the *rbc*L sequences were very similar among the Balearic specimen (HGI-A 9587), *Pyropia olivii* (from Greece, DQ807007, and Atlantic USA, DQ813625) and *Py. koreana* (from Korea, HQ728198), differing by a maximum of two nucleotides (Table 2). This similarity led us to the conclusion that these three entities are not distinct. Moreover, the low level of the genetic distances between them (maximum 0.2%), falls within the limits for intraspecific variation (0% to 1% up to 2% in some taxa, Klein et al. 2003, Lindstrom and Fredericq 2003, Lindstrom 2008, Kucera and Saunders 2012). The anatomical and reproductive structures of *Py. koreana* from the Balearic Islands were compared (Table 4) with the material from the type locality (Korea) and the Mediterranean record that belong to the holotype of *Py. olivii*. No significant differences among these three entities were found (Table 4). As there are molecular and morphological similarities among all these specimens (HGI-A 9587, DQ807007, DQ813625, and HQ728198), we can consider them synonymous, and because *Py. koreana* has nomenclatural priority, we propose the synonymy of *Py. olivii* with *Py. koreana*. However, it is of note that after all the sheets, located at the main herbaria for the bladed Bangiales of the Balearic Islands, have been revised, *Py. koreana* has only been detected in a unique sample (SANT-A 1279, collected in April 1987), indicating that this species is less abundant than *Py. elongata*, at least for the islands (Table 1). Although the existence of *Py. elongata* and *Py. koreana* in the Mediterranean has only been elucidated recently, the

evidence from the herbarium specimens indicates that these species have been present in this area under different names since the nineteenth century (Brodie et al. 2007; this study). As *Py. koreana* had until now only been recorded from Korea (Lee and Kang 2001, Kim and Kim 2011), our results indicate that the known worldwide distribution of this species is significantly greater than previously documented, as this species as *Py. olivii* had been reported from the coasts of Greece and Italy, and in the Atlantic Ocean on the coasts of the United States of America.

It was not possible to check the reports of *Porphyra laciniata* and *Porphyra vulgaris* (Colmeiro 1868) as we were not able to locate those sheets in any herbarium collection. However, considering the abundance of *Pyropia elongata* populations and that it is the only foliose bangiophyte that has been reported in the intertidal of the Balearic islands, so far, we have concluded that these species were almost certainly misidentifications of *Py. elongata* as the gross morphology is very confusing between some members of this group. Our confirmation that the oldest sheets located at the J.J. Rodríguez y Femenías Herbarium were identified as *Porphyra leucosticta* but belong to *Py. elongata*, again demonstrates the confusion between these two entities, and the importance of revising the historical reports with the aim of clarifying the real diversity of this group of algae.

In conclusion, this study provides new data on the foliose Bangiales in the Mediterranean and represents a new contribution to the global knowledge of the biodiversity of the Bangiales. Furthermore, it emphasizes the need to use the molecular tools in conjunction with the old reports and associated collections in order to elucidate the diversity of these organisms.

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