

Advancing the taxonomy of economically important red seaweeds (Rhodophyta)

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Abstract

The cultivation of red seaweeds for food (nori), agar and carrageenans is the basis of a valuable industry. However, taxonomic knowledge of these cultivated seaweeds and their wild relatives has not kept pace with advances in molecular systematics despite the fundamental importance of being able to identify commercially important species and strains, discover cryptic and endemic taxa and recognize non-native species with potentially damaging diseases and epiphytes. This review focuses on molecular taxonomic advances in the cultivated red algae with the highest commercial value globally: *Euचेuma*, *Kappaphycus*, *Porphyra sensu lato* ~~*Porphyra*/*Pyropia*~~ and *Gracilaria*. All four genera are similarly taxonomically challenging. They are speciose, morphologically plastic, have poorly resolved species boundaries, and a stable taxonomy ~~for each genus~~ is yet to be achieved. *Euचेuma* and *Kappaphycus* are frequently misidentified and the molecular markers *cox2-3* spacer, *cox1* and RuBisCO spacer have helped ~~to~~ ~~in~~ understanding phylogenetic relationships, and identifying new species and haplotypes. In *Porphyra sensu lato* (Bangiales) species identification and phylogenetic relationships were highly problematic until a major taxonomic revision based on a two-gene phylogeny (18S and *rbcL*) resulted in nine genera of bladed species. *Pyropia*, with at least 89 species, three in nori cultivation, has potential for new commercial evaluation. The recently published *Porphyra* genome will aid the exploration of evolutionary relationships in this group. In *Gracilaria sensu lato*, earlier efforts to resolve species-level taxonomy and generic descriptions were superseded by application of molecular tools, including DNA sequences of the RuBisCO spacer, *rbcL* gene, 18S and the ITS region. ~~Relationships between clades are now fairly well established, but much research on species and genera is still needed.~~ Studies of these cultivated red algal genera highlight the need for a robust taxonomy, a more standardized approach to the molecular markers used and a comprehensive dataset for each representative species. Recent Current work on DNA-based species delimitation, the emergence of high throughput sequencing, multi-gene phylogenies and publication of whole genomes (e.g. *Porphyra umbilicalis*) and the large number of genomes in the pipeline (e.g. *Gracilaria*) is increasingly improving our understanding of phylogenomic relationships and hence a better understanding of species relationships. This knowledge, in turn, can then be applied to improving red seaweed aquaculture.

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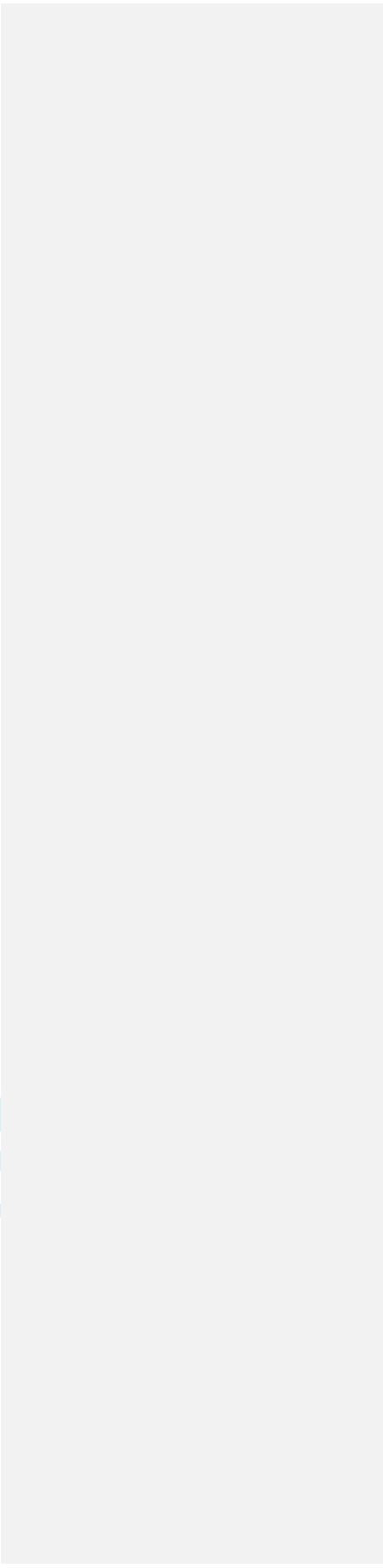
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Keywords: agar, Bangiales, carageenans, commercial value, Gracilariaceae, molecular taxonomy, Solieraceae

For Peer Review Only



Introduction

Red seaweeds have been collected from the wild for food and other products for thousands of years (Tseng, 1935; Brodie & Irvine, 2003; Collén *et al.*, 2014; Ramirez *et al.*, 2014 and references therein). The main uses of red algae, apart from food, have been as a source of the gelling hydrocolloids agar and carrageenan (Craigie, 1990). Until the Second World War (WWII, 1939-1945), seaweeds were mostly harvested from natural populations (Marshall *et al.*, 1949), although *Porphyra sensu lato* *Porphyra* has been cultivated in China and Japan for hundreds of years as food (Blouin *et al.*, 2011; Yang *et al.*, 2017). After WWII, in Asia the need for a more reliable crop after a major failure of the nori harvest in Japan led to the development of the modern nori industry (Yang *et al.*, 2017). Growing demand for products over the second half of the 20th century (e.g. Marshall *et al.*, 1949; Kim, 2012) saw a fundamental shift from wild harvesting in the North Atlantic of e.g. species including *Chondrus crispus* in the North Atlantic to farmed crops, such as *Eucheuma* in warmer tropical areas, particularly in the Pacific (Doty *et al.*, 1987). A more recent drive towards the development and commercialization of functional foods, nutraceuticals, pharmaceuticals and bioactives from seaweeds is pushing up demand and leading to innovative methods of production (e.g. Hafting *et al.*, 2011; Gutierrez Cuesta *et al.*, 2016). Current research indicates that macroalgal proteins contain all essential amino acids for food products and have additional bioactives (Garcia-Vaquero & Hayes, 2016).

~~Despite the fundamental shift in the production and supply of red seaweeds and the range of taxonomic tools now available, relatively little attention has been given to the molecular taxonomy of species under cultivation. In general, the application of molecular techniques in red algal taxonomy has revolutionized species concepts and taxonomic relationships, uncovered cryptic diversity (Robba *et al.*, 2006; Diaz-Tapia *et al.*, 2017) and provided a greater understanding of species distributions in different geographical areas (Brodie *et al.*, 2007), including evidence of much greater endemism than originally thought based on morphological identification (Brodie *et al.*, 2008; Payo *et al.*, 2013). Molecular analysis has also revealed that in many groups of red seaweeds there is considerable genetic diversity that is not reflected in the morphology at the species level (e.g. Sutherland *et al.*, 2011; Saengkaew *et al.*, 2016).~~

Twelve red algal taxa are listed as currently in aquaculture production (FAO, 2015) and/or have been cultivated for consumption between 1990 and 2015 (Table 1), although the number and identity of many of these species are uncertain. The main taxa in cultivation are

species of *Kappaphycus*, *Euचेuma*, *Porphyra sensu lato* *Pyropia* (as *Porphyra*) and *Gracilaria*. Estimates of their wet weight harvest per continent are given in Table 2. However, the reliability of these data is questionable as the figures are based on reported “output from aquaculture activities designated for final harvest for consumption” (FAO, 2015). – (Table 2).– For details of dry tonnage of agarophyte and carrageenophyte seaweeds for 2009 and 2015 see also Porse & Rudolph (2017). The main sources of carrageenan are *Euचेuma denticulatum*, *Kappaphycus alvarezii* and *Kappaphycus striatum* (Ask & Azanza, 2002; Aquaculture Compendium, 2006), with *Euचेuma* “cottonii”, making up 73% of the world consumption; (Porse & Rudolph, 2017), and a Agar from cultivated red seaweeds comes mostly from the genus *Gracilaria* not identified to species level (FAO, 2015; Porse & Rudolph, 2017): *Gelidium* has yields agar of better quality than *Gracilaria* but it is not possible yet to grow it in cultivation and wild stocks have been severely over-exploited (Porse & Rudolph, 2017).

Commercial marine seaweed cultivation is practiced heavily in the Asian Pacific region, with China, Indonesia and the Philippines contributing ~~up to~~ 88.7% (21 million tonnes) of the global farmed algal production in 2012 (FAO, 2014; Valderrama, 2015). The largest producer of agarophytes and carrageenophytes is Indonesia (Porse & Rudolph, 2017). The carrageenan-producing seaweeds *Kappaphycus* and *Euचेuma* make up approximately 33% of total algal production (FAO, 2014). From 1990 to 2012, the farming of these red seaweeds steadily increased in tandem with the rising demand for carrageenan (FAO, 2014; Hehre & Meeuwig, 2016). This is especially evident in Indonesia, currently the largest producer of *K. alvarezii* and *Euचेuma* spp. (FAO, 2014; Porse & Rudolph, 2017). The country produced 6.5 million metric tons of dried seaweed in 2012 (13.6% increase from 2009), of which c. 60% was from *Kappaphycus* and *Euचेuma* (KKP, 2013; Safari & Dardak, 2015). In 2013, Indonesia utilized 45% (343,643 hectares) of its viable coastal areas for seaweed farming (KKP, 2013) and it has vast potential to increase its seaweed production (Hurtado *et al.*, 2016).

Despite the fundamental shift in the production and supply of red seaweeds and the range of taxonomic tools now available, in most genera relatively little attention has been given to the molecular taxonomy of species under cultivation. In general, the application of molecular techniques in red algal taxonomy has revolutionized species concepts and taxonomic relationships, uncovered cryptic diversity (Robba *et al.*, 2006; Leliaert *et al.*, 2014; Filorama & Saunders, 2016; Diaz-Tapia *et al.*, 2017) and provided a greater understanding of species distributions in different geographical areas (Brodie *et al.*, 2007).

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7 including evidence of much greater endemism than originally thought based on
8 morphological identification (Brodie *et al.*, 2008, Payo *et al.*, 2013; Dumilag & Aguinaldo,
9 2017). Molecular analysis has also revealed that in many groups of red seaweeds there is
10 considerable genetic diversity that is not reflected in the morphology at the species level (e.g.
11 Sutherland *et al.*, 2011; Leliaert *et al.*, 2014; Saengkaew *et al.*, 2016).
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14 Determining the correct taxonomic status of species in cultivation is crucial.

15 Confusion in the taxonomy and systematics of cultivated red seaweed species has arisen due
16 to the different names used in farming and commerce and the lack of material for proper
17 identification. The names used by the FAO (2015) provide a general overview and do not
18 take into account recent taxonomic changes. *Euclidean*, *Kappaphycus*, *Gracilaria* and
19 *Porphyra sensu lato* have particularly challenging taxonomies: species are cosmopolitan,
20 often lack reliable morphological characters for identification, and some have been
21 accidentally or deliberately introduced to different parts of the world. For example, *Pyropia*
22 *yezoensis* ~~is has been~~ reported from the Northwest Atlantic ~~and where it~~ was most likely
23 introduced from Japan (West *et al.*, 2005, Mathieson *et al.*, 2008, Neefus *et al.*, 2008).
24 *Kappaphycus* and *Euclidean* species, which have been introduced for aquaculture in many
25 different parts of the world (Table 1), are successful invaders (Williams & Smith, 2007;
26 Sellers *et al.*, 2014). The introduction of *Kappaphycus* spp. into Hawaii, for example, has
27 resulted in negative impacts on coral reef ecosystems (Rodgers & Cox, 1999; Conklin &
28 Smith, 2005) and the spread of *K. alvarezii* outside its cultivation sites in Panama has caused
29 impacts on native biota (Sellers *et al.*, 2014).
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37 Introductions of non-native species for aquaculture can have consequences for the
38 introduced species and for the indigenous flora. For example, cultivars with limited genetic
39 stock are potentially susceptible to disease and epiphyte outbreaks (Cottier-Cook *et al.*,
40 2016). Invasions from cultivated stocks of indigenous species have also been demonstrated in
41 *Pyropia-P. yezoensis* in Japan, where there is evidence of plastid introgression from
42 cultivated crops to wild populations (Niwa *et al.*, 2009). This highlights the importance of, as
43 well as the risks to, the genetic resource of wild species in natural populations for
44 improvement of cultivated strains.
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49 Products from different species and varieties can vary: agar polysaccharides from
50 *Gracilaria* species have been shown to have different gel strengths (e.g. Marinho-Soriano,
51 2001), and different species of *Euclidean* vary in their carrageenans (Phang *et al.*, 2010). In
52 nori cultivation, *Pyropia tenera* is considered to have a better texture than cultivated *P.*
53 *yezoensis* (Niwa *et al.*, 2005). Wild populations remain the source for new stocks for
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7 cultivation. At the same time as the seaweed industry is expanding in size and value (Fig. 1),
8 environmental change due to increasing pressures on coastlines (Yang *et al.*, 2017), loss of
9 habitat due to land reclamation (Niwa *et al.*, 2005) and climate change (Brodie *et al.*, 2014)
10 are all impacting on seaweed populations.
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12 In this review we focus on molecular taxonomic advances in the red algal genera
13 *Eucheuma*, *Kappaphycus*, *Porphyra* *sensu lato* (including *Pyropia*) and *Gracilaria*, which
14 have the greatest harvests globally and/or the highest commercial value globally (Tables 1-2,
15 Fig. 1).
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21 ***Kappaphycus* and *Eucheuma***

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24 The foundations of the modern taxonomy of *Kappaphycus* and *Eucheuma* (Solieraceae,
25 Gigartinales) are M-ax S. Doty's studies based on examination of tetrasporophytic,
26 carposporophytic and gametophytic material (Doty, 1985, 1987, 1988; Doty & Alvarez,
27 1975; Doty & Norris, 1985). *Eucheuma* was originally divided into four sections,
28 Cottoniformia, *Eucheuma*, Gelatiformia and Anaxiferae, of which the section Cottoniformia
29 was later established as the segregate genus *Kappaphycus* (Doty, 1988). *Betaphycus*,
30 proposed by Doty (1995) for *B. philippinensis* based partly on its carrageenan type, was
31 validated by Silva *et al.* (1996), and there are currently three recognized species (Guiry &
32 Guiry, 2017). The morphological characters described by Doty (Table 3) are still in use
33 today. Currently there are six taxonomically accepted *Kappaphycus* species and 30
34 *Eucheuma* species (Guiry & Guiry, 2017). *Kappaphycus alvarezii*, *K. striatus* and *E.*
35 *denticulatum* are among the best known because of their commercial value, ~~but all~~ All six
36 species of *Kappaphycus* are generally well documented in terms of morphology and to a
37 certain extent, genetically (Tan *et al.*, 2014); ~~in~~ in contrast, in *Eucheuma* the lack of
38 specimens and taxonomic research have impeded progress over the years.
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45 In spite of their commercial importance, *Kappaphycus* and *Eucheuma* are often
46 misidentified as a result of morphological plasticity and the widespread and often indifferent
47 use of colloquial, commercial (*cottonii* and *spinosum*) and local names (Doty, 1985;
48 Zuccarello *et al.*, 2006; Tan *et al.*, 2013; Hurtado, 2013). In the Philippines, four varieties of
49 *K. alvarezii* and three varieties of *K. striatus* have been reported (Hurtado, 2013). Likewise,
50 six varieties of *K. alvarezii* were reported from Malaysia, each with its own local name (Tan
51 *et al.*, 2013; Lim *et al.*, 2014b). This phenomenon was also seen in China (Zhao & He, 2011)
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7 and Brazil (de Barros-Barreto *et al.*, 2013) and is likely to be prevalent where these seaweeds
8 are commercially cultivated. The plasticity of *Kappaphycus* and *Eucheuma* often results in
9 the cultivation of mixed populations by local farmers (Tan *et al.*, 2013), which hinders the
10 processing of *kappa*- (from *Kappaphycus*) and *iota*- (from *Eucheuma*) carrageenans,
11 requiring prior separation of these seaweeds (Lim *et al.*, 2014b). Morphological examination
12 is often challenging due to the lack of cystocarpic specimens which exhibit more distinctive
13 characters and aggravated by the fact that upon drying specimens lose some of their form and
14 structure.

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18 Zuccarello *et al.* (2006) ~~employed~~ sequenced the mitochondrial *cox2-3* spacer and
19 plastid RuBisCO spacer ~~genetic markers~~ to better understand the phylogeny and genetic
20 variation of ~~*Betaphycus*~~, *Kappaphycus* and *Eucheuma* worldwide. Their molecular analyses
21 supported the genetic distinction between *K. alvarezii* and *K. striatus*, as well as revealing
22 several distinct genotypes of *K. alvarezii* and *E. denticulatum*, some of which are unique to
23 certain ~~localities~~ regions (e.g. Hawaii, Africa; Zuccarello *et al.*, 2006). The study also
24 demonstrated the feasibility of using molecular markers in species identification, which was
25 corroborated by Tan and co-workers (2013) who applied a combination of markers to verify
26 *Kappaphycus* and *Eucheuma* varieties in Malaysia, leading to the description of *K.*
27 *malesianus* (Tan *et al.*, 2014). Currently genetic data are available (at least one molecular
28 marker in published literature) for 83% and 10% of species of *Kappaphycus* and *Eucheuma*,
29 respectively. Of the DNA markers used ~~for these rhodophytes~~, the *cox2-3* spacer was the
30 preferred one due to its resolution in inter- and intraspecific relationships. Over the years, the
31 ~~mitochondrial~~ *cox2-3* spacer has been used for DNA barcoding (Tan *et al.*, 2012), molecular
32 identification and systematics (Zhao & He, 2011; Araújo *et al.*, 2013; Tan *et al.*, 2013;
33 Dumilag & Lluisma, 2014), species description (Ganzon-Fortes *et al.*, 2012; Tan *et al.*, 2014)
34 and detection of bioinvasions (Conklin *et al.*, 2009). The *cox2-3* spacer was also combined
35 with the mitochondrial *cox1* gene in a collaborative study by the major carrageenan producers
36 of Southeast Asia to document the genetic diversity of *Kappaphycus* and *Eucheuma* within
37 the region (Lim *et al.*, 2014a). Although not exhaustive, the study revealed several new
38 haplotypes or potential species of *Kappaphycus* and *Eucheuma*, some of which were already
39 being farmed commercially. **The establishment of an improved genetic database of these**
40 **carrageenophytes would undoubtedly help in marker-assisted selection or breeding, a**
41 **technique already applied in agriculture and animal breeding.**

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53 The application of molecular markers has provided insight into the taxonomy of
54 *Kappaphycus*, *Eucheuma* and *Betaphycus* (Fig. 2). Apart from allowing the identification of
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7 tiny, dried or deformed specimens, the use of genetic markers has provided an independent
8 approach to phylogenetic reconstruction. Although ~~incapable of confidently~~
9 ~~resolving uninformative for intergeneric levels of~~ phylogenetic relationships among the
10 ~~genera, i.e. between~~ *Kappaphycus*, *Eucheuma* and *Betaphycus* (Fig. 2), the *cox2-3* spacer is
11 remarkably accurate ~~at for~~ inter- and intraspecific delineation within a genus (Zuccarello *et*
12 *al.*, 2006; Tan *et al.*, 2012). Taxonomically, the use of this marker has revealed: (i) three
13 genotypes in commercial strains of *K. alvarezii*, presumably originating from the Philippines
14 (Ask & Azanza, 2002; Ask *et al.*, 2003; Hurtado *et al.*, 2015), as well as other strains unique
15 to Africa and Hawaii; (ii) two potentially cryptic species of *K. striatus* in Southeast Asia; (iii)
16 ~~a genetic differences~~ between *K. malesianus*, *K. inermis* and *K. cottonii*; (iv) three genotypes
17 of *E. denticulatum* – commercially farmed strains, “Endong” strains from Southeast Asia and
18 strains unique to Africa; (v) several genotypes that are to date not assessed; and (vi)
19 potentially misidentified taxa, e.g. *E. isiforme* (Zuccarello *et al.*, 2006; Conklin *et al.*, 2009;
20 Ganson-Fortes *et al.*, 2012; Dumilag & Lluisma, 2014; Lim *et al.*, 2014a; Tan *et al.*, 2012,
21 2013, 2014).

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28 Multiple genetic markers are required to elucidate the phylogeny of *Kappaphycus*,
29 *Eucheuma* and *Betaphycus* at generic and family levels. The degree of genetic variation in
30 different DNA markers (especially from different organelles) would be normalized when
31 analysed together, and would provide a better representation of evolutionary pathways. For
32 instance, the use of a concatenated dataset of *cox1+cox2-3* spacer sequences resulted in a
33 phylogenetic tree (Fig. 3) different to that of the *cox2-3* spacer alone. This dataset is expected
34 to better resolve the relationship between *Kappaphycus* and *Eucheuma* when more genetic
35 data for *K. cottonii* and *E. arnoldii* (seaweeds suspected to be “intermediary” between both
36 genera) becomes available. However, the “multigene” approach will only be possible when
37 sequences are available for each representative species, which in turn requires the
38 standardization of the molecular markers utilized. ~~Nevertheless, this situation is expected to~~
39 ~~improve with the development of simpler and inexpensive DNA sequencing technology.~~

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46 Under-sampling is a major hurdle for the advancement of *Kappaphycus* and
47 *Eucheuma* (and *Betaphycus*) taxonomy. Although specimens have been collected worldwide,
48 ~~of the few specimens that have been sequenced,~~ the majority were either procured from
49 markets or seaweed farms, leading to a general ~~underestimation~~ of biodiversity and genetic
50 diversity as cultivars were typically vegetatively propagated from the same few commercial
51 strains. For example, there is a lack of specimens of *K. procrusteanus* after its first
52 description and attempts to sequence DNA from the type specimen proved futile (Tan *et al.*,
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Comment [CM1]: We have to respond to the referee on this – I don't think there is a conflict.

2014). In contrast, preliminary results on genetic diversity in south-east Asia (Lim *et al.*, 2014) have revealed numerous unidentified genotypes and potential species, suggesting that more genotypes are yet to be discovered. Therefore, future sampling efforts should focus on unsampled areas or places distant from seaweed farms. However, the extensive area involved will require coordination and concerted effort between stakeholders, industry players and academia.

Porphyra sensu lato (including *Pyropia*)

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The Bangiales is a diverse, cosmopolitan order of red algae and a major economic resource in the production of nori (Guilleman-Guillemain *et al.*, 2015). Species of *Porphyra sensu lato* (bladed Bangiales) have been a food source for thousands of years in different parts of the world, for example, in Wales (laver), Chile (luche or luchi), Japan (nori) and China (Tsu-Tsai) (Blouin *et al.*, 2011; Brodie & Irvine, 2003; Brodie *et al.*, 2008; Guillemain *et al.*, 2015; Ramirez *et al.*, 2014; Yang *et al.*, 2017). *Porphyra sensu lato* may have been the first seaweed to be cultivated (Kain, 1991; Blouin *et al.*, 2011) and its cultivation in Tokyo Bay, Japan, can be traced back to 1736 (Okazaki, 1971), 1640 (Miura, 1975), or possibly ~~as far back as~~ even to 1570 (Tseng & Chang, 1954).

Until the application of molecular techniques, the identification and taxonomic placement of taxa within the ~~order~~ bladed Bangiales was highly problematic due to the simple morphology and variation within and between species (Brodie *et al.*, 2008; Gunnarsson *et al.*, 2016). However, a concerted effort by a group of scientists from around the world focussing on the taxonomy of the Bangiales led to a major taxonomic revision of the order based on a two-gene phylogeny (Sutherland *et al.*, 2011). The result was that the bladed Bangiales were split into eight genera: *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea*, *Porphyra*, *Pyropia* and *Wildemania* (Fig. 4). A re-evaluation of the taxonomy of the bladed Bangiales from other parts of the world has led to a ninth bladed genus, *Neothemis*, being described based on a study in the western Mediterranean (Fig. 4; ~~)(~~ Sánchez *et al.*, 2014, 2015).

Comment [JB2]: I've changed this here which I think solves the problem picked up by the referee.

Comment [CM3]: Referee's comment: It is better to specify that you mean within the genus *Porphyra* or *Bangia*. Placement within the order was very simple: Bladed species were considered as *Porphyra* and filamentous species were considered as *Bangia*.

Comment [CM4]: Needs reference in list

Comment [JB5]: Reference added

There are over 160 described species of bladed Bangiales but the ~~actual number is~~ re are thought to be considerably ~~higher more than that~~ species. For example, recently 17 new species of *Porphyra*, *Pyropia* and *Wildemania* were discovered in the southeastern Pacific (Ramirez *et al.*, 2014; Guillemain *et al.*, 2015) and four new species of *Pyropia* were described from the west coast of North America (Lindstrom *et al.*, 2015). A re-evaluation of

the bladed Bangiales along the coast of China indicates that there is a rich flora within the genus *Pyropia* (Yang *et al.* unpublished data).

With 89 species to date (some of which are yet to be described), *Pyropia* (*Py.*) is the most speciose genus of the Bangiales (Brodie & Yang, personal observation). According to Sutherland *et al.* (2011), the *Pyropia* clade is strongly supported as monophyletic, although a number of various clades (at least eight) were resolved with strong support. In a more up to date phylogeny, five ~~clades~~ intrageneric clades are ~~clearly~~ resolved (Fig. 5) (Yang *et al.*, unpublished data) with a strong biogeographical signal. *Pyropia* also contains most of the economically important species. Three species of *Pyropia*, *Py. yezoensis*, *Py. tenera* and *Py. haitanensis* (Figs 5-7), are cultivated in Japan, China, and Korea and the industry is worth about US\$1.3 billion per year (Blouin *et al.*, 2011). *Py. yezoensis* is the main species in cultivation in all three countries (although *Py. haitanensis* is also cultivated in China), and its main products are known as nori (のり) in Japan and Hai-Tai (海苔) in China (Yang *et al.*, 2017). Phylogenetically, *Py. yezoensis* and *Py. tenera* are resolved in one clade and *Py. haitanensis* is in a sister clade (Fig. 5).

In China, different *Pyropia* strains have been developed and used in the nori cultivation industry. Two novel cultivars of *Py. yezoensis* have been certified by the National Certification Committee for Aquatic Varieties (NCCAV) and named Su-Tong Nos 1 and 2 (Yang *et al.*, 2016). These cultivars are extensively used in the industry. Four novel cultivars of *Py. haitanensis* ~~were~~ certified by NCCAV and are named as Shen-Fu Nos 1 and 2 (Song, 2016), Min-Feng No. 1 (Wang *et al.*, 2013) and Zhe-Dong No. 1 (Luo *et al.*, 2015). These cultivars can be distinguished by genetic markers including AFLP (Yang *et al.*, 2016) and ITS-5.8S sequences (Xie *et al.*, 2013). Many other strains are being studied (Cao *et al.*, 2016; Yang *et al.*, 2016). ~~However,~~ but none of these cultivars has been taxonomically formally described ~~as a form or variety~~. Zheng & Li (2009) described ~~Threethree~~ varieties of *Py. haitanensis* ~~have been described~~: var. *culata* Zheng & Li, var. *grandidentata* Zheng & Li and var. *schizophylla* Zheng & Li (Zheng & Li, 2009). ~~While *Porphyra haitanensis* Chang & Zheng~~ was has been transferred into *Pyropia* as *Pyropia haitanensis* (Chang & Zheng) N.Kikuchi & M.Miyata (Sutherland *et al.*, 2011), but the identity and generic relationship position of these three varieties still need to be verified. Whether these varieties are extensively used in the industry remains unknown.

In Japan, *Pyropia tenera* (as *Porphyra tenera* Kjellman) was extensively cultivated before the artificial seeding of conchospores was developed (Ueda, 1932). ~~After that~~

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8 Miura were described and ~~both were~~ extensively cultivated in Japan (Miura, 1984). With the
9 impact of environmental change, *P. tenera* var. *tamatsuensis* ~~become was~~ endangered and *P.*
10 *yezoensis* f. *narawaensis* became the main cultivar in Japan (Niwa *et al.*, 2005). After the
11 transfer of *P. tenera* Kjellman and *P. yezoensis* Ueda to *Pyropia*, these two cultivars in Japan
12 were transferred respectively to *Pyropia tenera* (Kjellman) N. Kikuchi, M. Miyata, M.S.
13 Hwang & H.G. Choi var. *tamatsuensis* (A. Miura) N. Kikuchi, Niwa & Nakada and *Pyropia*
14 *yezoensis* (Ueda) M.S. Hwang & H.G. Choi f. *narawaensis* (A. Miura) N. Kikuchi, Niwa &
15 Nakada (Kikuchi *et al.*, 2015). In Korea, *Py. yezoensis* is the main cultivated species although
16 the form or variety is currently unknown. Hwang *et al.* (2014) sequenced the mitochondrial
17 genome of *Py. yezoensis* cultivated in Korea (KF561997) but the data have not yet been
18 released yet which might enable us to resolve the question.
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24 For those taxa of the bladed Bangiales that are used as food but are not in cultivation,
25 there is some uncertainty as to the species involved. It is probable that several species of
26 *Porphyra* are used as laver in Britain (Brodie & Irvine, 2003). The species used for luche or
27 luchi in Chile has traditionally been called *Porphyra columbina*, although this species does
28 not appear to occur there (Nelson & Broom, 2010). Specimens collected under this name
29 have been shown to belong to three recently diverged haplotypes of *Pyropia orbicularis*
30 (Ramirez *et al.*, 2014; Guillemin *et al.*, 2015).
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34 Given the extent of the diversity both at the species and generic level within the
35 bladed Bangiales, there is potential for new species and/or strains from different parts of the
36 world to be brought into culture. Molecular taxonomic/phylogenetic analysis has been
37 valuable in demonstrating species relationships ~~and illustrating that species currently in~~
38 ~~cultivation belong in different clades within *Pyropia*.~~ Clearly there is a considerable amount
39 of taxonomy still to be undertaken with the aim of determining the full extent of species and
40 genera. However, the evidence so far suggests that although just a very tiny number of
41 species are used in cultivation, there is scope for a new evaluation of this group of red algae.
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45 The publication now of the *Porphyra umbilicalis* genome (Brawley *et al.*, 2017) opens up this
46 quest. We have the potential to find new genetic markers for identification and, coupled with
47 high-throughput sequencing, the possibility that multi-gene phylogenies ~~that will enable us~~
48 to undertake this evaluation.
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54 *Gracilaria* and *Gracilariopsis*

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8 *Gracilaria sensu lato* has been the major world source of food-grade agar for several decades
9 (McHugh, 1991; Hurd *et al.*, 2014). Its high commercial value led to widespread efforts in
10 the 1980s and 1990s to resolve species-level taxonomy and generic circumscriptions. Bird &
11 McLachlan (1982) noted that *Gracilaria* species were poorly defined, due to their notorious
12 plasticity, with over 300 described species including multiple synonyms of the 100
13 recognized species. *Gracilaria sensu lato* was thus an important element of the *Taxonomy of*
14 *Economic Seaweeds* workshops initiated by Isabella Abbott and Jim Norris in 1984, which
15 addressed the difficulties in establishing correct names for commercially important seaweeds.
16 In the proceedings of the first workshop, the economically significant species in Japan and
17 China were considered to be the flat, digitate *G. textorii* (Suringar) De Toni, knobably *G.*
18 *eucheumatoides* Harvey (as *G. eucheumoides*), compressed *G. bursa-pastoris* (S.G.Gmelin)
19 P.C.Silva, and the terete species *Gracilariopsis* (as *Gracilaria*) *lemanieformis* (Bory) Weber
20 van Bosse, *G. "verrucosa"*, *G. tenuistipitata* C.F.Chang & B.M.Xia, *G. vermiculophylla*
21 Ohmi, *G. chorda* Holmes and *G. hainanensis* C.F.Chang & B.M.Xia (Bangmei & Yamamoto,
22 1985). Abbott *et al.* (1985) noted the problems in finding diagnostic morphological features
23 in terete species. In particular, the seaweed known as *G. verrucosa* (Hudson) Papenfuss, and
24 reported to occur almost worldwide, was clearly heterogeneous at both species and genus
25 levels. As *G. verrucosa* was then considered to be the type species of *Gracilaria*, originally
26 described from the British Isles, the nomenclature of these economically important species
27 was conserved by designating *Gracilaria compressa* (C.A.Agardh) Greville (a synonym of
28 *Gracilaria bursa-pastoris*) as the lectotype of the genus (Steenftoft *et al.*, 1995).

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38 The most useful morphological characters were found in the spermatangial structures
39 (Yamamoto, 1978; Bird & McLachlan, 1982) which are distributed in superficial layers or in
40 conceptacles of different types: shallow crypts (*textorii* type), single, deep crypts (*verrucosa*
41 type), or deep, confluent compound crypts (*henriquesiana* type, used to segregate
42 *Polycavernosa* C.F.Chang & B.M.Xia (a synonym of *Hydropuntia* Montagne), in 1963 from
43 *Gracilaria* species). However, morphological overlap ~~between them was observed, casting~~
44 doubt on the diagnostic value of types of spermatangial arrangement (Abbott *et al.*, 1991).
45 Female reproductive characters were employed by Fredericq & Hommersand (1989b) to
46 show that *Gracilariopsis* E.Y.Dawson, which had been regarded for decades as a synonym of
47 *Gracilaria*, was distinct. Fundamental differences in both female and male reproductive
48 morphology were used to separate the Gracilariales from the Gigartinales, which aligned with
49 the formation of agar by the Gracilariales in contrast to the carageenans of the Gigartinales
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(Fredericq & Hommersand, 1989a). The Gracilariales currently contains only the families Gracilariaceae and the parasitic Pterocladophilaceae (Guiry & Guiry, 2017).

The large number of species and the paucity of morphological characters were so challenging that, as soon as molecular tools became available to phycologists, they were applied to define and circumscribe members of the Gracilariales. Rice & Bird (1990) applied RFLP markers to 11 populations of “*G. verrucosa*” from around the world and found that they were markedly heterogeneous (including what was later understood to be *Gracilariopsis* spp.). The first sequence data for the RuBisCO spacer (Destombe & Douglas, 1991), the 18S rDNA gene (Bird *et al.*, 1990, 1992) and the ITS (Goff *et al.*, 1994) all showed high divergences between *Gracilaria* and *Gracilariopsis*. Gurgel & Fredericq (2004) reviewed molecular work to date, which had provided strong evidence supporting the taxonomic distinctiveness of the genera *Curdiea*, *Melanthalia*, *Gracilaria* and *Gracilariopsis*, but had not resolved the position of *Hydropuntia*. Using *rbcL* sequences for a then relatively large taxon set, Gurgel & Fredericq (2004) resurrected *Hydropuntia* (type species: *H. urvillei* Montagne, a synonym of *Gracilaria edulis* (S.G.Gmelin) P.C.Silva; see Guiry & Guiry 2017) for algae including the commercial crop species *Hydropuntia* (formerly *Gracilaria*) *eucheumatoides* and *Hydropuntia* (formerly *Gracilaria*) *edulis*. They also identified a clade with two commercial species, *G. chilensis*, the basis of the modern seaweed aquaculture industry in Chile (Buschmann *et al.*, 2008; Bixler & Porse, 2011) and *G. vermiculophylla* (as “*Gracilaria*G- aff. *tenuistipitata*”) that they felt merited generic status, but which was never formally described. The spermatangial characters used to divide the genus by earlier workers were not diagnostic for lineages – the *textorii* type of spermatangial conceptacle had arisen at least twice in the evolutionary history of *Gracilaria sensu lato* (Gurgel & Fredericq, 2004).

Subsequent phylogenetic analyses using various molecular markers have reported broadly congruent trees to those of Gurgel & Fredericq (2004), but taxonomic and nomenclatural interpretations have differed according to authors. While the relationships between clades have now been established fairly robustly using a three-gene dataset (Fig. 68; Lyra *et al.*, 2015), taxonomic treatment of the genera has not yet stabilized, even for economically significant species. Lyra *et al.* (2015) recovered Gurgel & Fredericq's (2004) *G. chilensis*/*G. vermiculophylla* clade (Fig. 8, clade II). Multiple generic reassignments were required as *Hydropuntia* was again subsumed in *Gracilaria* (Lyra *et al.*, 2015; Fig. 8). Currently, of the six recognized genera in the Gracilariaceae, only *Gracilaria* and *Gracilariopsis* are of major commercial interest.

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At the species level, many new taxa are still being described, recognized or transferred between genera, and there is still a lot of uncertainty concerning the biodiversity and taxonomy of this group. In particular, lack of morphological characters for the terete species has led to ongoing confusion both locally and globally, such that species are being newly discovered even in well-studied areas (e.g. *G. dura* was confused with *G. gracilis* in the British Isles; Destombe *et al.*, 2010). Species circumscriptions are not always resolved by molecular data: hybridization between these two species was revealed by comparing organellar and nuclear DNA sequence markers, and cryptic species are present in the Atlantic and Mediterranean regions (Destombe *et al.*, 2010). *G. dura* is considered to be an economically important species in India with the potential for aquaculture production of agarose (e.g. Veeragurunathan *et al.*, 2015). However, the lack of reference to type materials and the high sequence divergence of purportedly conspecific samples in GenBank (Pareek *et al.*, 2010) indicate that this is another example where further investigation is required for correct identification. Even when type materials are consulted, these may consist of multiple species or even genera due to the lack of diagnostic features (Muangmai *et al.*, 2014).

Aquaculture of *Gracilaria*, with a large part of the production in Chile and Indonesia, has ensured that it remains the main genus used for agar and the price is stable (Bixler & Porse, 2011; FAO, 2015; [Porse & Rudolph, 2017](#)). As Steentoft *et al.* (1995) noted, a revised definition of agar should include the correct name of the species of origin to ensure a uniform product. Molecular markers have been and will continue to be critical in developing a new taxonomy of the Gracilariaceae (Lyra *et al.*, 2015). As an example, a recent *cox1* barcoding study of the family in Australasia found five of the 22 discrete species to be unknown and potentially undescribed (Yang & Kim, 2015).

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The way forward/future perspectives

The three groups considered here share common taxonomic problems despite their commercial value and the importance of having a good taxonomy in underpinning aquaculture. In each group, there have been major changes in generic circumscriptions over the last three decades, yet all are still fluid, with conflicting viewpoints adopted by different workers. All three are highly speciose, morphologically plastic, and boundaries between species are often poorly resolved with evidence of incipient or recent speciation (e.g. Destombe *et al.*, 2010; Gillemain *et al.*, 2015). At the intraspecific level, relationships

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7 between cultivated strains and wild strains are almost unknown. Phylogenetic and
8 phylogeographic approaches could assist in the search for possible sources of additional
9 species to cultivate, and in the search for disease-resistant strains. A clearer view of species
10 boundaries will provide opportunities to better understand the distribution of species and their
11 value as genetic resources, both for conservation and management. A concerted global DNA
12 barcoding approach with common markers (e.g. *cox1*, *cox2-3* spacer, partial *rbcL* sequences)
13 would clarify which species are in cultivation and their distributions, as well as providing
14 information on relationships among populations (e.g. Yow *et al.*, 2013).
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24 Natural History Museum, London.
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Table 1. Red seaweeds with countries where they are or have been cultivated between 1990 and 2015. Source: FAO (2015).

| Countries | Species | | | | | | | | | | | |
|------------------|---------------------|---|-------------------------|--|-------------------------|-----------------------------------|---------------------------|---------------------------------------|--|-----------------------------------|-------------------------------------|------------------------|
| | <i>Asparagopsis</i> | <i>Chondracanthus</i> <i>chamissoi</i> | <i>Eucheuma</i> spp. | <i>Eucheuma</i> <i>denticulatum</i> | <i>Gelidium</i> spp. | <i>Gelidium</i> <i>amansii</i> | <i>Gracilaria</i> spp. | <i>Gracilaria</i> <i>verrucosa</i> | <i>Kappaphycus</i> <i>alvarezii</i> | <i>Palmaria</i> <i>palmata</i> | <i>Porphyra</i> <i>columbina</i> | <i>Pyropia</i> spp. |
| Belize | | | + | | | | | | | | | |
| Brazil | | | | | | | + | | + | | | |
| Chile | | | | | | | + | | | | + | |
| China | | | + | | + | + | + | | | | | + |
| Fiji | | | + | | | | | | | | | |
| France | + | | | | | | | | | | | |
| India | | | | | | | | | + | | | |
| Indonesia | | | + | | | | + | | | | | |
| Ireland | | | | | | | | | | + | | |
| Japan | | | | | | | | | | | | + |
| Kiribati | | | + | | | | | | | | | |
| Malaysia | | | | | | | | | + | | | |
| Madagascar | | | + | | | | | | | | | |
| Myanmar | | | | | | | | | + | | | |
| Namibia | | | | | | | + | | | | | |
| Papua New Guinea | | | | | | | | | + | | | |
| Peru | | + | | | | | | | | | | |
| Philippines | | | | + | | | + | | + | | | |
| Portugal | | | | | | | | | | | | |
| Saint Lucia | | | + | | | | | | | | | |

Table 2. Global aquaculture production by continent for red seaweeds (wet weight). Source: Tonnage and value of 2015 Food and Agriculture Organization of the United Nations (FAO) stats. F = FAO estimate; data estimated from available source of information or calculation based on specific assumptions.

| Continent | Species | Quantity (t) | Value (USD000) |
|-----------|------------------------------|-----------------|----------------|
| Africa | <i>Kappaphycus alvarezii</i> | 5840 | 103 |
| | <i>Eucheuma</i> spp | 22127 | 2841 |
| | <i>Gracilaria</i> spp | 130 F | 62 F |
| | <i>Eucheuma denticulatum</i> | 166650 | 1686 |
| | Total | 194747 | 4691 |
| Americas | <i>Kappaphycus alvarezii</i> | 700 F | 32 F |
| | <i>Eucheuma</i> spp | 5 F | 34 |
| | <i>Gracilaria</i> spp | 11982 | 29284 |
| | Total | 12687 | 29349 |
| Asia | <i>Kappaphycus alvarezii</i> | 1730946 | 211291 |
| | <i>Eucheuma</i> spp | 10163657 | 779436 |
| | <i>Gracilaria</i> spp | 3868636 | 955724 |
| | <i>Gracilaria verrucosa</i> | 634 | 43 |
| | <i>Porphyra tenera</i> | 686784 | 930284 |
| | <i>Porphra</i> spp | 1158750 | 74457 |
| | <i>Eucheuma denticulatum</i> | 106950 | 7925 |
| | Total | 17716357 | 2959160 |
| Europe | Red seaweeds | 0* | 0* |
| Oceania | <i>Kappaphycus alvarezii</i> | 16200 | 926 |
| | <i>Eucheuma</i> spp | 4150 F | 245 F |
| | Total | 20350 | 1171 |

*data not available

Table 3. General differences between *Kappaphycus*, *Eucheuma* and *Betaphycus* (Doty, 1985; 1995)

| <i>Kappaphycus</i> | <i>Eucheuma</i> | <i>Betaphycus</i> |
|---|--|--|
| Fronds variable but commonly cylindrical with blunt or spiny, irregular protuberances | Fronds cylindrical; spines simple | Thalli compressed; spines simple with broadening bases |
| Irregular branching, some irregularly pinnate | Spines in regularly spaced pairs on whorls first, but later others may appear scattered. Branches from whorls; often opposite; pectinate | Spines arranged in rows marginally and later dorsally and ventrally; branching from the margins, pinnate |
| Hyphal axial core usually present; not rhizoidal; cylindrical | Axial core rhizoidal and cylindrical | Axial core tortuous, often flattened, hyphal |
| Produces <i>kappa</i> carrageenan | Produces <i>iota</i> carrageenan | Mixture of <i>beta</i> , <i>iota</i> and <i>kappa</i> -carrageenans |
| Cystocarps on main axes (non-laterals) | Cystocarps on lateral axes | Cystocarps on laterals, often bearing spines |

Comment [CM6] : Has been suggested we provide same for *Gracilaria* and *Pyropia*. Nice idea but may not be feasible.

Comment [U7] : For *Porphyra* sl that is beyond the scope of the paper.

Figure legends (also copied below figures)

Fig. 1. Comparison of production weights and values globally (the great majority in Asia), based on data from FAO (Table 1), arranged by value. *Eucheuma* spp. and *Kappaphycus alvarezii* are high volume, low value crops, whereas *Gracilaria* spp. are produced in much smaller quantities but are high value. *Porphyra/Pyropia* spp. are intermediate in volume and value.

Fig. 2. Simplified phylogeny of *Kappaphycus*, *Eucheuma* and *Betaphycus* based on *cox2-3* spacer datasets from Conklin *et al.*, (2009), Dumilag & Lluisma, (2014), Dumilag *et al.* (2014), Lim *et al.*, (2014a), Tan *et al.*, (2012, 2014), Zuccarello *et al.*, (2016) and relevant GenBank sequences. Supplementary details are summarized in Table S1. ML= Maximum Likelihood bootstrap support; BI= Bayesian posterior probabilities expressed in percentage.

Fig. 2. Simplified phylogeny of *Kappaphycus*, *Eucheuma* and *Betaphycus* based on the *cox2-3* spacer datasets from Dumilag & Lluisma (2014), Dumilag *et al.* (2014), Lim *et al.* (2014a), Tan *et al.* (2012, 2014), Zuccarello *et al.* (2016) and relevant GenBank sequences. ML= Maximum Likelihood bootstrap support; BI= Bayesian posterior probabilities.

Fig. 3. Phylogeny of *Kappaphycus* spp. based on concatenated *cox1-cox2-3* spacer molecular markers. DNA sequences were based on Conklin *et al.*, (2009), Dumilag & Lluisma, (2014), Lim *et al.*, (2014a), Tan *et al.*, (2012, 2014), Zuccarello *et al.*, (2016) and relevant GenBank sequences. Supplementary details are summarized in Table S1. ML= Maximum Likelihood bootstrap support; BI= Bayesian posterior probabilities expressed in percentage. Diagrams not drawn to scale.

Fig. 4. Genera of bladed Bangiales. Triangles represent proportion of species (numbers in brackets) in each genus. Source: Guiry & Guiry (2017), Sanchez *et al.*, Yang & Brodie, personal observations.

The result was that the bladed Bangiales were split into eight genera: *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea*, *Porphyra*, *Pyropia* and *Wildemania*. A re-evaluation of the taxonomy of the bladed Bangiales from other parts of the world has led to a ninth bladed genus, *Neothemis*, being described based on a study in the western Mediterranean (Fig. 4;

Figs 5-7. *Pyropia* species used in aquaculture. Fig. 5. *P. tenera*. Fig. 6. *P. haitanensis*. Fig. 7. *P. yezoensis*.

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Comment [CM8]: This needs a lot more information on Genbank numbers, types of analyses, support, etc. To be placed in supplementary materials?

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Fig. 3. Phylogeny of *Kappaphycus* spp. based on the concatenated *cox1-cox2* 3spacer molecular markers. DNA sequences were based on Dumilag & Lhuisma (2014), Lim *et al.* (2014a), Tan *et al.* (2012, 2014), Zuccarello *et al.* (2016) and relevant GenBank sequences. ML= Maximum Likelihood bootstrap support; BI= Bayesian posterior probabilities. Diagrams not drawn to scale.

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Fig. 4. Representative species of nine genera of *Porphyra* sensu lato. Figures are of herbarium specimens identified by S. Lindstrom as *Wildemania cuneiformis*, *Pyropia californica*, *Fuscifolium tasa* and *Boreophyllum aestivale*, by W.A. Nelson as *Lysithea adamsiae* and *Chymene coleana*, by N. Kikuchi for *Miuraea migiata*, by C.D. Neefus for *Porphyra purpurea* and by N. Sanchez for *Neothemis ballesterosii*.

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Fig. 5. Phylogeny of *Pyropia* based on analysis of *rbcL* and 18S, showing the number of species in each of the five clades resolved (based on Yang *et al.* in prep.) and showing the position of the cultivated species *P. yezoensis* and *P. tenera* in one clade and *P. haitanensis* in a separate clade. *Porphyra purpurea* was outgroup. The values on the node are BPP/ML values and only values above 50 are shown.

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Fig. 6-8? Phylogenetic analysis of some *Gracilaria*, *Gracilariopsis* and *Hydropuntia* species based on three genes (*rbcL*, UPA, and *cox1*), rooted with *Rhodymenia* and *Gelidium*. Values above branches are ML bootstrap values (left) and Bayesian posterior probabilities expressed as percentages (right), with full support indicated by an asterisk. The genera *Melanthalia* and *Curdiea* (not shown) are basal to *Gracilaria* and *Gracilariopsis*. *Gracilaria* has subclades I-V; *Hydropuntia* is paraphyletic, and spermatangia and thallus type are mapped to the right of the phylogeny (from Lyra *et al.*, 2015, with permission (being requested))

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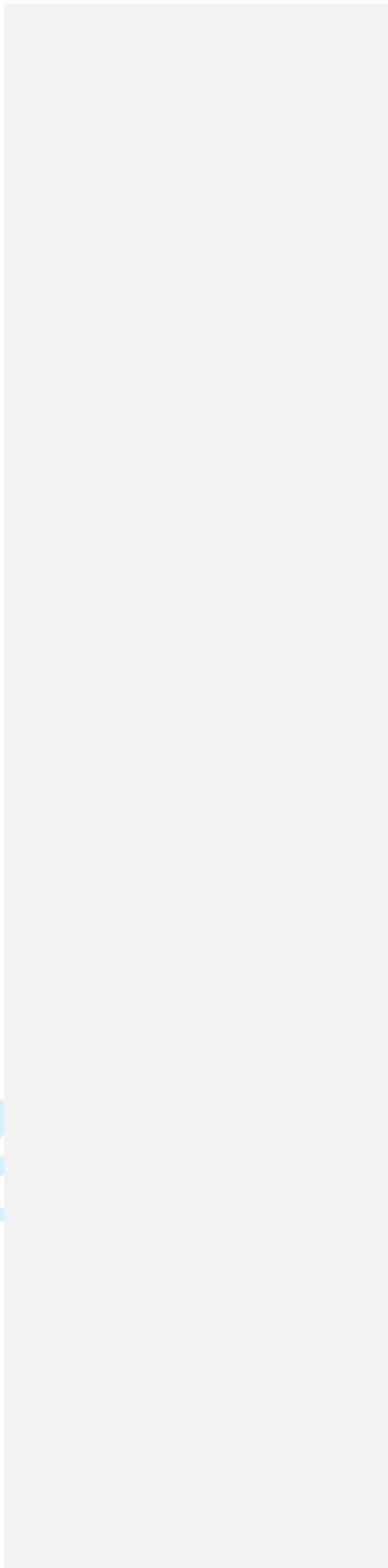


Table S1 Details of *Betaphycus*, *Eucheuma* and *Kappaphycus* specimens used for phylogenetic reconstruction (Figures 2 and 3)

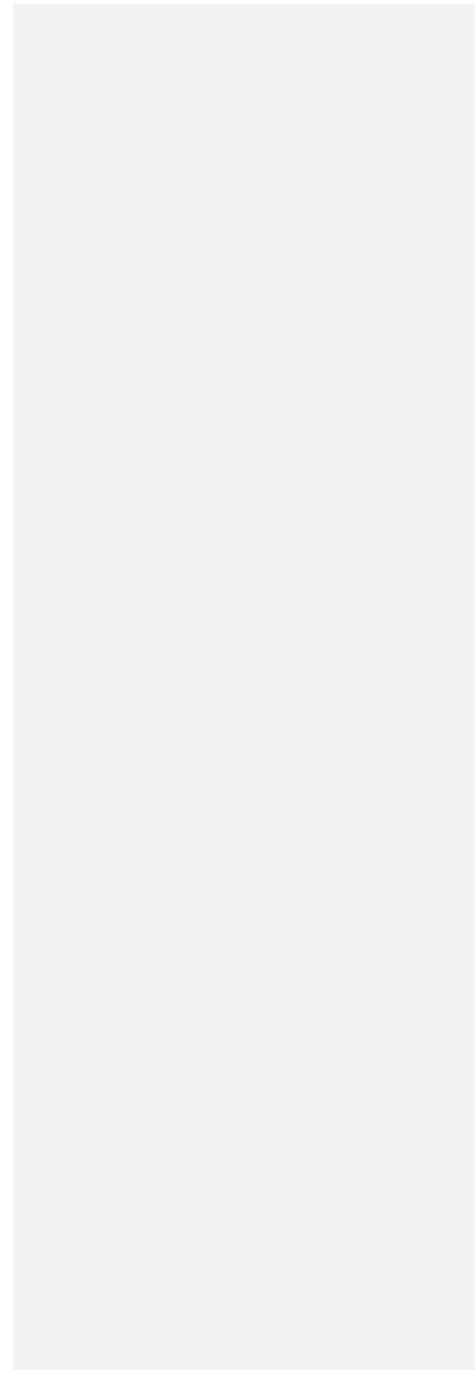
| No. | Name | Taxonomic grouping | Locality | GenBank accession no. | | Reference |
|-----|--------------------------------------|----------------------|-----------------------------|--------------------------|--------------------------|---|
| | | | | <i>cox1</i> | <i>cox2-3 spacer</i> | |
| 1 | K. alvarezii 58 | KA1 | Malaysia | JX624014 | JN663774 | Tan et al., (2012; 2013) |
| 2 | K. alvarezii 89 | KA1 | Malaysia | JX624015 | JN663766 | |
| 3 | K. alvarezii 103 | KA1 | Malaysia | JX624016 | JN663776 | |
| 4 | K. alvarezii 109 | KA1 | Malaysia | - | JN663775 | |
| 5 | K. alvarezii E2614 | KA2 | Hawaii | - | FJ554862 | Conklin et al., (2009) |
| 6 | K. alvarezii 3955 | KA2 | Hawaii | FJ554861 | - | |
| 7 | K. alvarezii E3 | KA3 | Venezuela | - | AY687427 | Zuccarello et al., (2006) |
| 8 | K. alvarezii E16 | KA3 | Madagascar | - | AY687430 | |
| 9 | K. alvarezii E130 | KA3 | Tanzania | - | AY687436 | |
| 10 | K. striatus E48 | KS1 | Indonesia | - | AY687431 | |
| 11 | K. striatus E117 | KS1 | Indonesia | - | AY687435 | Tan et al., (2013) |
| 12 | K. striatus 98 | KS1 | Malaysia | - | JN663782 | |
| 13 | K. striatus 105 | KS1 | Malaysia | - | JN663783 | |
| 14 | K. striatus E89 | KS2 | Philippines | - | AY687434 | |
| 15 | K. striatus 1 | KS2 | Malaysia | JX624021 | JN663779 | Zuccarello et al., (2006) |
| 16 | K. striatus 31 | KS2 | Malaysia | JX624022 | JN663780 | |
| 17 | K. malesianus 14 | KM1 | Malaysia | - | JN663784 | |
| 18 | K. malesianus 49 | KM1 | Malaysia | JX624032 | JN663785 | Tan et al., (2012; 2013) |
| 19 | K. malesianus 93 | KM1 | Malaysia | JX624033 | JN663786 | |
| 20 | K. inermis | KI1 | Philippines | - | KF719020 | Dumilag & Lluisma, 2014 |
| 21 | K. inermis AOL538 | KI1 | Philippines | - | KF687980 | |
| 22 | K. inermis V15 | KI1 | Vietnam | KC905321 | KC905431 | Lim et al., (2014) |
| 23 | K. sp. GU11 | KSP1 | Philippines | KC905320 | KC905430 | |
| 24 | K. cottonii E108 | KC1 | Philippines | - | AY687426 | Zuccarello et al., (2006) |
| 25 | K. cottonii | KC1 | Philippines | EU334417 | - | |
| 26 | E. denticulatum 44 | ED1 | Malaysia | JX624035 | JN663787 | Tan et al., (2013) |
| 27 | E. denticulatum 57 | ED1 | Malaysia | - | JN663791 | |
| 28 | E. denticulatum E13 | ED1 | Indonesia | - | AY687411 | |
| 29 | E. denticulatum E45 | ED2 | Indonesia | - | AY687412 | |
| 30 | E. denticulatum E32 | ED2 | Indonesia | - | AY687437 | Zuccarello et al., (2006) |
| 31 | E. denticulatum 3953 | ED2 | Hawaii | - | FJ561733 | |
| 32 | E. denticulatum 888 | ED2 | Hawaii | - | FJ554859 | Tan et al., (2013) |
| 33 | E. denticulatum 41 | ED2 | Malaysia | JX624040 | JX624083 | |
| 34 | E. denticulatum 97 | ED2 | Malaysia | JX624042 | JX624085 | |

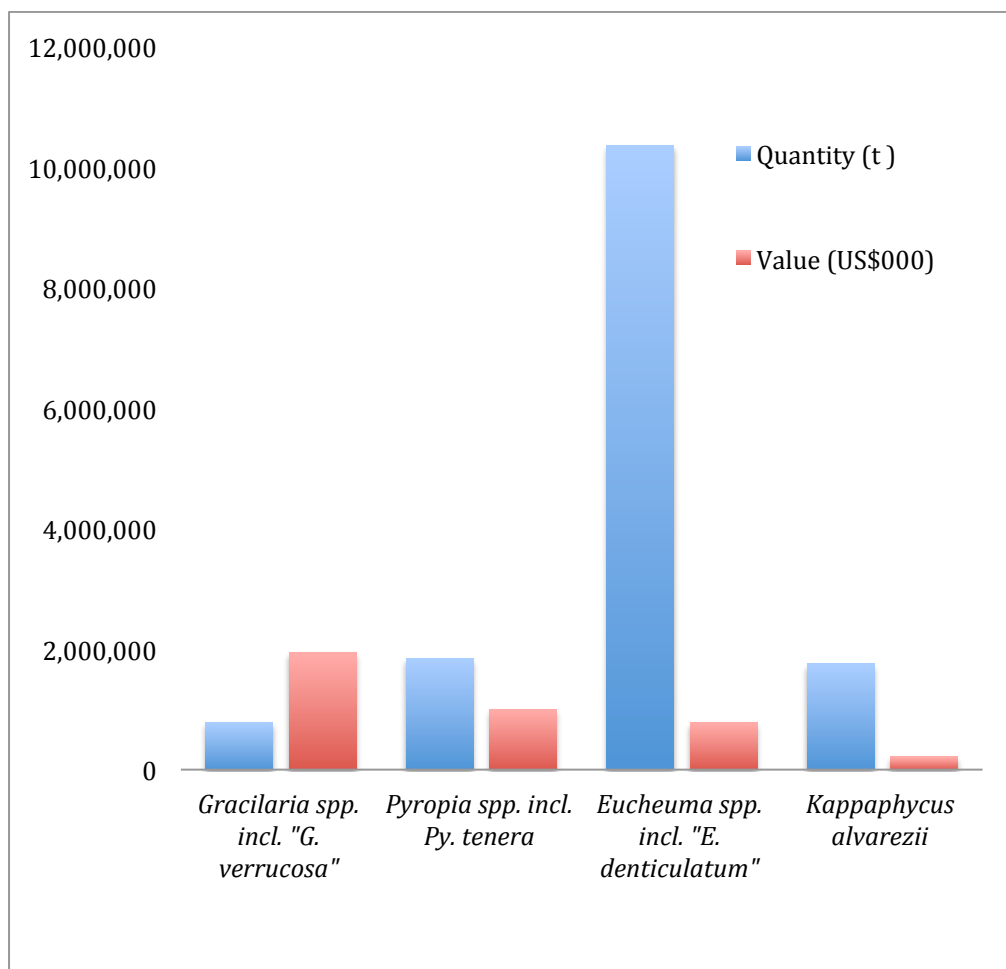
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| No. | Name | Taxonomic Grouping | Locality | GenBank accession no. | | Reference |
|-----|-----------------------------------|--------------------|-------------|-----------------------|----------------------|-----------------------------------|
| | | | | <u>cox1</u> | <u>cox2-3 spacer</u> | |
| 35 | <i>E. denticulatum</i> E46 | ED3 | Tanzania | - | AY687438 | |
| 36 | <i>E. denticulatum</i> E60 | ED3 | Mauritius | - | AY687439 | |
| 37 | <i>E. denticulatum</i> E8 | ED3 | Madagascar | - | AY687428 | |
| 38 | <i>E. platycladum</i> E111 | EP1 | Kenya | - | AY687422 | Zuccarello <i>et al.</i> , (2006) |
| 39 | <i>E. platycladum</i> E65 | EP2 | Tanzania | - | AY687423 | |
| 40 | <i>E. sp.</i> E110 | ESP1 | Tanzania | - | AY687424 | |
| 41 | <i>E. sp.</i> E59 | ESP2 | Hawaii | - | AY687425 | |
| 42 | <i>B. philippinensis</i> E118 | BP1 | Philippines | - | AY687417 | |
| 43 | <i>B. cf. gelatinus</i> | BG1 | China | - | JN854256 | Unpublished |
| 44 | " <i>E.</i> " <i>isiforme</i> E2 | EI1 | Florida | - | AY687421 | |
| 45 | " <i>E.</i> " <i>isiforme</i> E35 | EI1 | Florida | - | AY687420 | Zuccarello <i>et al.</i> , (2006) |
| 46 | " <i>E.</i> " <i>isiforme</i> E37 | EI1 | Florida | - | AY687419 | |
| 47 | <i>Solieria</i> sp. | | Malaysia | - | JN663793 | Tan <i>et al.</i> , (2013) |

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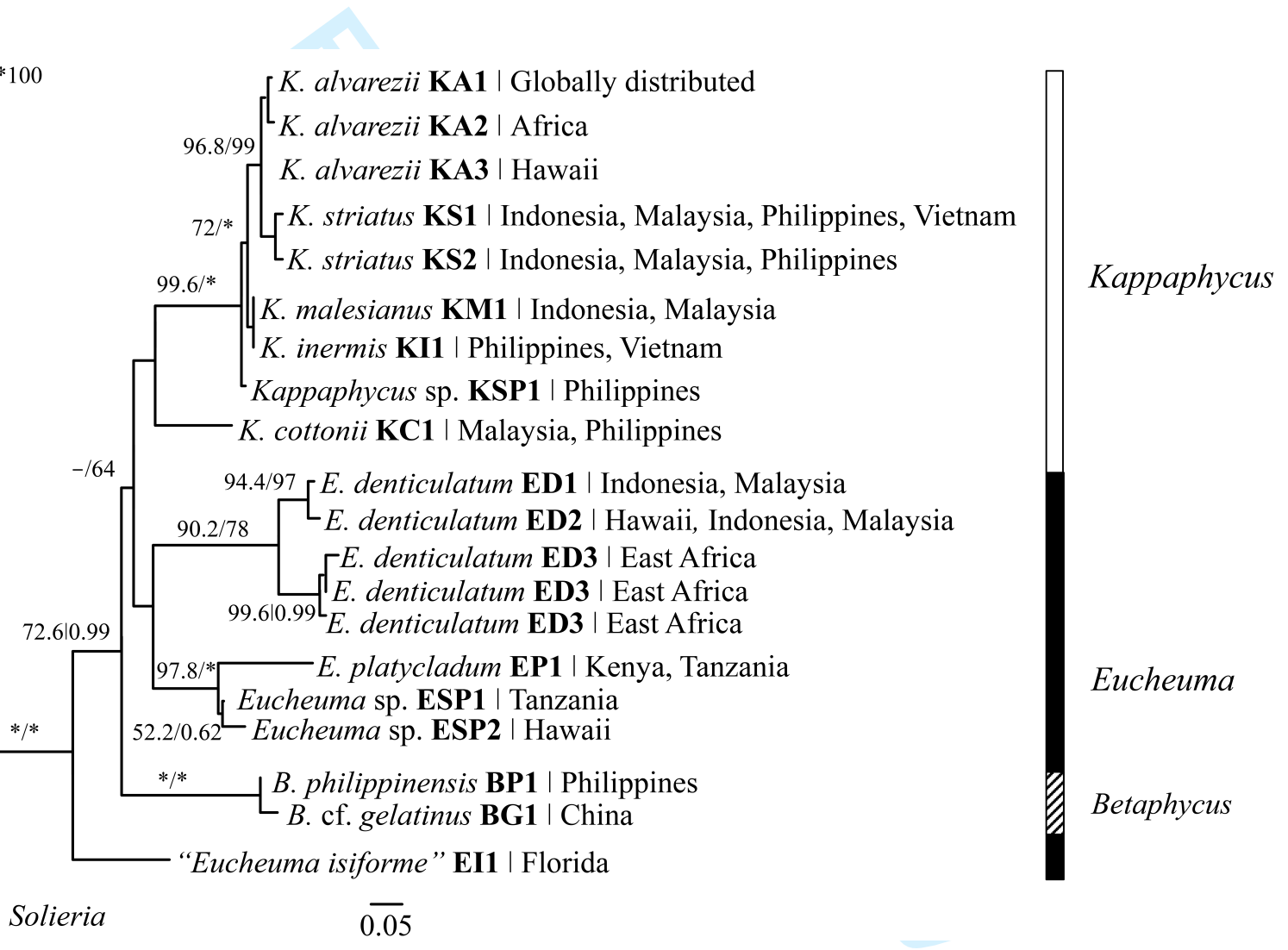
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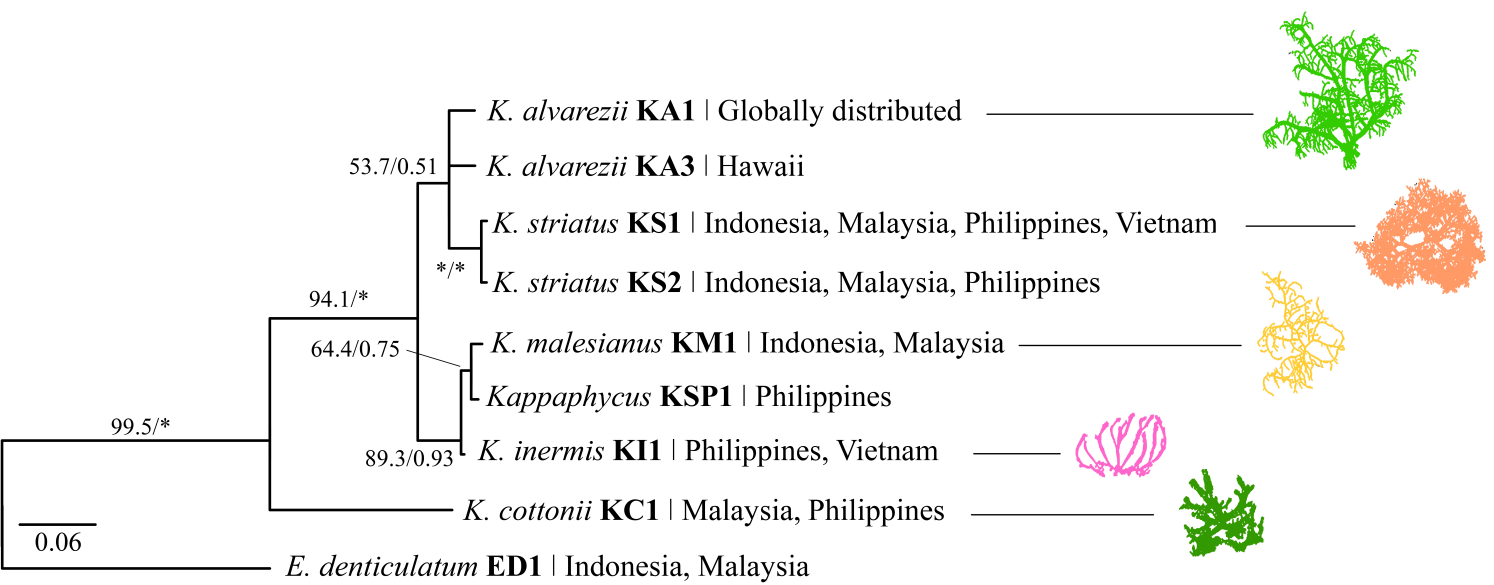
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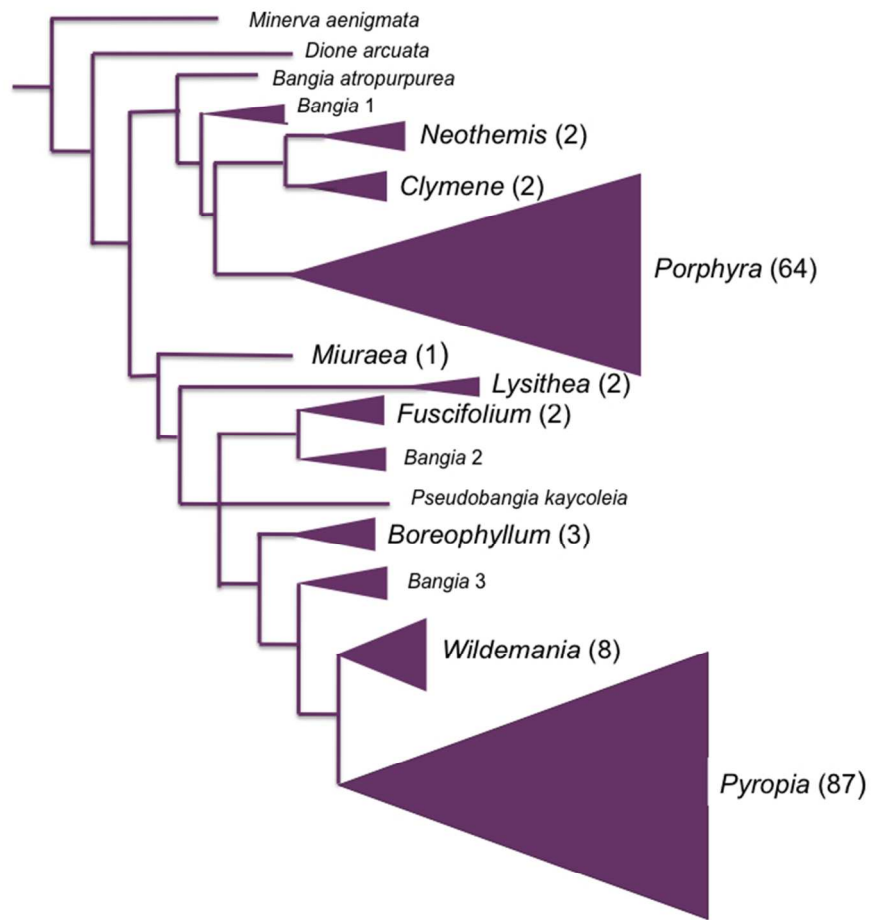
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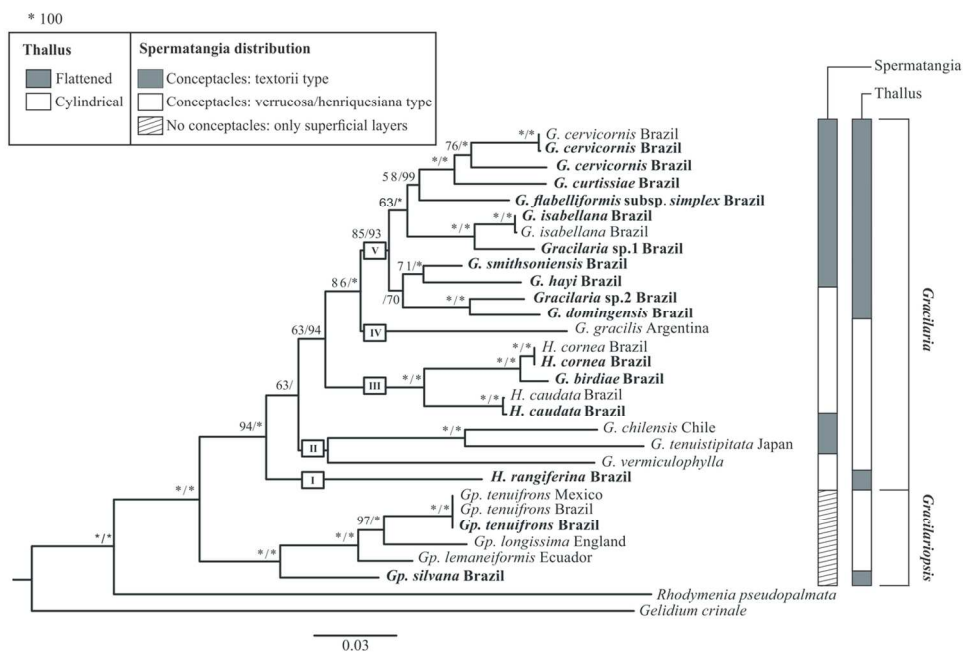
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