

# Phylogeny of *Betaphycus* (Gigartinales, Rhodophyta) as inferred from COI sequences and morphological observations on *B. philippinensis*

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**Abstract** The phylogeny of the three species that comprise the genus *Betaphycus* Doty in Silva, Basson and Moe and their phylogenetic relationships with other eucheumatoids are still unresolved. In this study, the utility of the mitochondrial cytochrome oxidase I (COI) marker in resolving their relationships was evaluated. Analyses of the COI sequences from *Betaphycus philippinensis* Doty and *Betaphycus speciosus* (Sonder) Doty ex Silva specimens collected from their type localities (Sorsogon, Philippines and Western Australia, respectively) revealed that the two species formed a well-supported clade distinct from *Eucheuma* J. Agardh and *Kappaphycus* Doty ex Silva. The genotyped specimens of *B. philippinensis* were observed to exhibit dorsal protuberances, a characteristic which has been regarded as a key diagnostic feature of *Betaphycus gelatinus* (Esper) Doty ex Silva. This observation raised the possibility that these two taxa are conspecific. In addition, *B. philippinensis* specimens were also observed to exhibit morphological features that could be used to distinguish the species from other eucheumatoids, such as the location tetrasporangial nemathecium in the thallus and the presence of apical or lateral pit connections in the tetraspores. The species referred to in the literature as “*B. gelatinus*” (as *Eucheuma gelatinae*) collected from northwestern Philippines was identified as a species of *Eucheuma* based on molecular and morphological

evidence. The phylogenetic relationships of this species with other related eucheumatoid taxa were also determined.

**Keywords** *Betaphycus* · COI · Eucheumatoids · Rhodophyta · Morphology · Phylogeny · Taxonomy

## Introduction

The marine Indo-Pacific rhodophyte genus *Betaphycus* Doty ex P. Silva, Basson and Moe was initially established by Doty (1995) and later validated by Silva et al. (1996) on the basis of *Betaphycus philippinensis* Doty ex Silva from eastern Sorsogon, Philippines as its type species. The genus is distinguished from the two other genera in the tribe Eucheumatoideae (*Eucheuma* and *Kappaphycus*) by having cell walls that produce beta ( $\beta$ )- and kappa ( $\kappa$ )-carrageenan (Doty 1995). The diagnostic morphological features of the genus as proposed by Doty (1995) are generally not unique as they are also found in the four recognized species belonging to *Eucheuma* section *Gelatiformia* Doty and Norris. The report of Santos (1989) on carrageenan types found in various eucheumatoid species motivated Doty (1995) to transfer two members of *Eucheuma* into the section *Gelatiformia* and subsequently into the new genus *Betaphycus* and which was later validated by Silva et al. (1996) as *Betaphycus gelatinus* (Esper) Doty ex Silva and *Betaphycus speciosus* (Sonder) Doty ex Silva.

The recognition of *B. philippinensis* as a new taxon has caused taxonomic confusion considering its potential conspecificity with a recognized *Betaphycus* species, *B. gelatinus* (Tseng and Trono 2001). Doty (1995) stated that *B. philippinensis* differed from other taxa in the new genus by having cystocarps issued from ventral proliferations. He discriminated *B. gelatinus* from *B. philippinensis* by citing Schmitz (1895) who only mentioned that proliferations are located dorsally in the former. However, it has been

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previously noted that proliferations in *B. gelatinus* (as *Eucheuma gelatinae*) have tendencies to develop on either surface especially in mature thalli (Doty 1988). Doty (1995) did not further comment upon other morphological differences between these two species despite the fact that *B. philippinensis* was frequently recognized before as *B. gelatinus* (as *E. gelatinae* in Santos 1989). The possible conspecificity of these two species was addressed by Tseng and Trono (2001) based on Philippine materials but both species continue to be recognized as two separate taxa (Doty 1995; Silva et al. 1996).

Another species frequently confused with *B. gelatinus* is *Eucheuma serra* (J. Agardh) J. Agardh. The application of the name “*E. serra*” as trade name for what is now recognized as *B. gelatinus*/*B. philippinensis* has also been evident in the literature (Kraft et al. 1999). Kraft (1969) also reported that *B. gelatinus* (as *E. gelatinae*) may be identical to and shows a closer affinity with *E. serra*. The illustrations and parts of the description of *E. gelatinae*/*B. gelatinus* from several previous works (e.g., Trono and Ganzon-Fortes 1988; Trono 1997; Tseng and Trono 2001) were originally used to identify what was then known as *E. serra* in the Philippines (Trono 1986). The arbitrary change of name from *E. serra* to *E. gelatinae*/*B. gelatinus* is questionable since these two species differ in terms of both morphology (Yamada 1936; Doty 1988) and *rbcL* sequence (Fredericq et al. 1999). Among Philippine records, it was only Cordero (1977, 1978) who treated these two species as separate. Hitherto, the taxonomic status of Philippine *E. serra* remains unresolved especially when compared with *B. gelatinus*/*B. philippinensis*.

The affinity of *Betaphycus* to other members of the order Solieriaceae has previously been investigated using *rbcL* gene sequences but its phylogenetic position relative to *Kappaphycus* and *Eucheuma* still remains unresolved (Fredericq et al. 1999). Various molecular markers have already been used to infer the phylogeny of *Eucheuma* and *Kappaphycus*, including mitochondrial gene cytochrome oxidase I (COI), *cox2-3* spacer, nuclear-encoded ribosomal internal transcribed spacer, and RuBisCO spacer genes (Zuccarello et al. 2006; Conklin et al. 2009; Zhao and He 2011; Tan et al. 2012, 2013) but none of these markers have been used to specifically investigate the molecular phylogeny of the genus *Betaphycus*.

Previous studies have established that the COI gene can be an informative marker to elucidate phylogenetic relationships among the red algae (Saunders 2005). In this paper, COI sequences are used to infer the phylogenetic relationships of *Betaphycus* and other Philippine species known under the name “*B. gelatinus*” with the other members of the tribe Eucheumatoideae. The vegetative and reproductive morphologies of *B. philippinensis* are also described and for the first time, detailed descriptions on the tetrasporic and female samples are provided.

## Materials and methods

Information on the collection and identification of specimens used in this study is provided in Table 1. Materials of *B. philippinensis* used for morphological observations were collected from Bulusan, eastern Sorsogon Province, Philippines and some herbarium specimens from the Gregorio T. Velasquez Phycological Herbarium in the Marine Science Institute, University of the Philippines. Upon collection from the field, specimens were immediately preserved in 10 % formalin in seawater then air-dried prior to sectioning. One specimen numbered 921001 obtained from the Herbarium of the Institute of Oceanography—Nha Trang, labeled “*Betaphycus gelatinum* Doty” collected from Thai An, Phan Rang, Vietnam, was also examined morphologically.

### Morphological observations

Sections were made by hand with a razor blade, rehydrated in seawater, stained with either 5 % Cotton Blue or 0.1 % Methylene Blue solutions, and mounted on glass slides in 30 % glycerine in distilled water. Photographs of the specimens in situ were taken using Pentax Optio WG-1 GPS while pictures of habits and details of morphological structures were documented using a digital still camera (Sony DSC-F828). Scale marking and final editing (i.e., removal of shadow and other background noise) were done using the program Adobe™ Photoshop CS5 Extended ver. 12 x64 (Adobe Systems Inc.).

### Molecular analyses

A portion (ca. <5 g) of the field-collected thallus was cut and preserved in either silica gel or soaked in absolute ethanol immediately after collection. Most of the materials obtained abroad were preserved in silica gel. DNA samples were extracted from previously powdered materials using liquid nitrogen following the DNA extraction described by Zuccarello et al. (2006) but without the enzymes (i.e., RNase A and Proteinase K). The COI genes were amplified using primers: GazF1 5′ TCAACAAATCATAAAGATATTGG 3′ and GazR1 5′ ACTTCTGGATGTCCAAAAAYCA 3′ designed by Saunders (2005). Each 30 μL PCR reaction contained 1× PCR Titanium buffer (Clontech), 1 mM DNTPs, 0.75 mM of forward and reverse primers, 1 U Taq polymerase, and 1–2 μL template DNA (ca. 30–100 ng μL<sup>-1</sup>). The PCR amplification profile used followed that of Hebert et al. (2003), with adjustments on the annealing temperature (50 to 52 °C). All PCR products were visualized by electrophoresis in 2 % agarose gel to confirm the right size of the amplicons (600–750 bp), purified using QIAquick Gel Extraction Kit (Qiagen) according to manufacturer’s protocol and were then sent to 1st Base (Selangor, Malaysia) for DNA sequencing.

**Table 1** Collection data or published reference and Genbank numbers for all the specimens included in this study

Species	Collection details (voucher, collection site, collector or published reference)	Genbank accession number
<i>Betaphycus philippinensis</i>	AOL 435; Dancalan, Bulusan, Sorsogon, Philippines; R. Dumilag	KC249495
<i>B. philippinensis</i>	AOL 436; Dancalan, Bulusan, Sorsogon, Philippines; R. Dumilag	KC249496
<i>B. philippinensis</i>	AOL 441; Dapdap, Bulusan, Sorsogon, Philippines; R. Dumilag	KC249497
<i>B. philippinensis</i>	AOL 443; Dapdap, Bulusan, Sorsogon, Philippines; R. Dumilag	KC249498
<i>B. speciosus</i>	JMH101; Cape Peron, South of Perth, Western Australia; J. M. Huisman	KC249499
<i>B. speciosus</i>	GWS025572; Port Denison, Western Australia; G. W. Saunders	KC249500
<i>B. speciosus</i>	JH04D10; Long Reef, Eastern Australia; J. M. Huisman	KC249501
“ <i>B. gelatinus</i> ” (= <i>Eucheuma</i> sp.)	AOL530; Caparispisan, Ilocos Norte, Philippines; R. Dumilag & Z. Agngarayngay	KC249502
“ <i>B. gelatinus</i> ” (= <i>Eucheuma</i> sp.)	AOL 531; Caparispisan, Ilocos Norte, Philippines; R. Dumilag & Z. Agngarayngay	KC249503
<i>Eucheuma serra</i>	SML101; Northern Taiwan; Showe Mei-Lin	KC249504
<i>E. denticulatum</i>	Conklin et al. (2009)	FJ554855.1
<i>E. denticulatum</i>	Montes et al. (2008)	EU334419.1
<i>E. denticulatum</i> (“Endong”)	Montes et al. (2008)	EU334418.1
<i>E. denticulatum</i> (“Spagghetti”)	Montes et al. (2008)	EU33420.1
<i>K. alvarezii</i> (Bola-bola)	Montes et al. (2008)	EU334416.1
<i>Kappaphycus</i> sp. (Tambalang)	Montes et al. (2008)	EU334415.1
<i>Kappaphycus</i> sp.	Conklin et al. 2009	FJ554853.1
<i>K. cottonii</i>	Montes et al. (2008)	EU334417.1
<i>Sarcodiotheca furcata</i>	Verbruggen et al. (2010)	GQ497318
<i>Caulacanthus ustulatus</i>	Sherwood et al. (2010)	HQ423094

Electropherograms for each sequence were viewed using the software Bioedit (Hall 1999). The sequences were assembled into contiguous sequences (contigs) using DNA Baser ver. 3.5 (Heracle Biosoft). The 10 COI sequences generated in this study and 13 solieriacean sequences retrieved from GenBank were aligned using Clustal W 1.4 (Thompson et al. 1994) via the software Bioedit (Hall 1999). The COI sequence for *Caulacanthus ustulatus* (Mertens ex Turner) Kützing (Caulacanthaceae, Gigartinales) was included in the alignment as outgroup based on a previous study by Fredericq et al. (1999). The aligned sequences were truncated to the same length. jMODELTEST version 0.1.1 (Guindon and Gascuel 2003; Posada 2008) was used to select the appropriate substitution model (based on the corrected Akaike information criterion value). The general time reversible (GTR+I+G) model of sequence evolution was used for the data set. Maximum likelihood (ML) analysis (Felsenstein 1981) was performed using the program PhyML 3.0 (Guindon et al. 2010) with 100 bootstrap replicates. Parameters used such as transition/transversion ratio, proportion of invariable sites, and gamma shape parameter were set to be optimized by the program. Substitution rate categories were set to 4. For the tree topology search, the nearest neighbor interchanges and subtree pruning and regrafting methods were used. The initial tree was generated by the program using BioNJ (Gascuel 1997). Phylogenetic trees were also inferred using the

Neighbor-joining (NJ) algorithm (Saitou and Nei 1987) under the Tajima-Nei and Maximum Composite Likelihood models using MEGA version 5 (Tamura et al. 2011). The computation of pairwise distance divergence among sequences was carried out using the same computer program. The same data set was analyzed with Bayesian inference (BI) using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). The analysis also used GTR+I+G model, consisted of four independent Markov Chain-Monte Carlo runs and 5,000,000 generations with sampling every 100 generations. The majority-rule consensus tree was inferred after discarding a burn-in sample of 10,000 trees.

## Results

### Molecular phylogeny

Ten new COI sequences were obtained from four specimens of *B. philippinensis*, three specimens of *B. speciosus*, a single specimen of *E. serra* from Taiwan, and from two specimens of unidentified species of *Eucheuma* colloquially referred to as “*B. gelatinus*.” The matrix of 24 aligned COI sequences were 566 nucleotides long, of which 378 sites (66.78 %) were not polymorphic. The average base composition was as follows: A = 0.25, C = 0.17, G = 0.19, and T = 0.39.

The phylogenetic tree inferred by ML analysis is shown in Fig. 1. Posterior probability values derived from BI analysis were also indicated in the same figure. Phylogenetic trees inferred from the same data set using BI analysis and NJ algorithm were similar to the trees inferred from ML analysis with respect to general tree topology and clade support (data not shown).

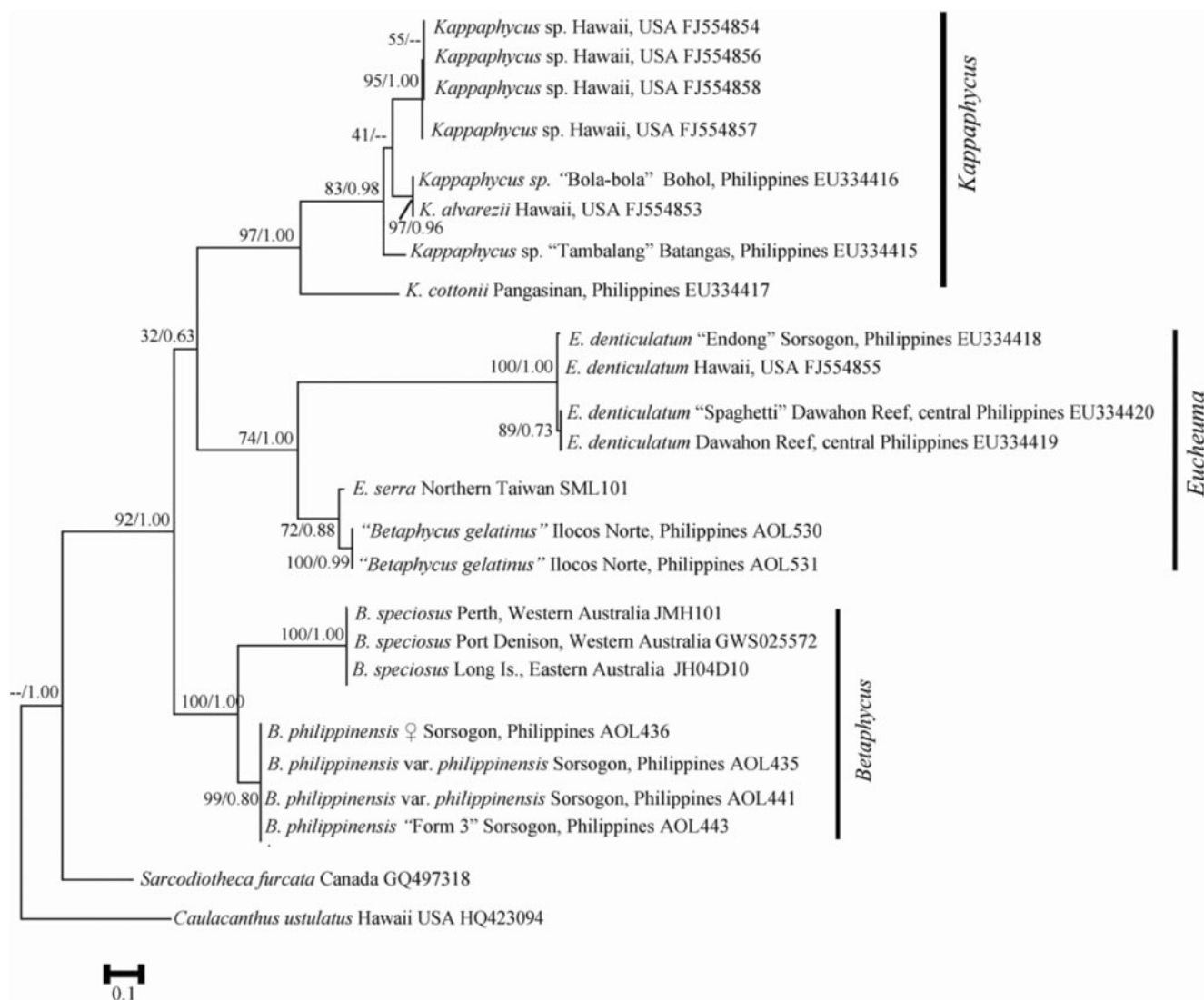
The results of phylogenetic analysis revealed that the three genera that constitute the tribe Eucheumatoideae, i.e., *Betaphycus*, *Eucheuma*, and *Kappaphycus*, formed three major clades, but their intergeneric relationships remain unresolved.

Within the *Betaphycus* clade, all Australian materials identified as *B. speciosus* grouped with *B. philippinensis* with very strong support. The putative “*B. gelatinus*” specimens collected from northwestern Philippines did not group with the

*Betaphycus* species. Instead, the two identical COI sequences clustered with *Eucheuma* species, showing the highest affinity to *E. serra*.

Morphological observations on *B. philippinensis* specimens

*Specimens studied* The voucher specimens included in this study were collected from Sorsogon, Philippines (type locality): Dancalan (as *E. gelatinae* interposed with *Betaphycus gelatinae*, coll. G. C. Trono Jr., R. Savella, R. Conde, H. Gabito and A. Gallose, ix.4.2001, T22411; coll. R. V. Dumilag, 19.iv.2012, AOL434, AOL435, AOL 436, RVD01-RVD8), Dapdap (as *B. gelatinae*, coll. M. Y. Roleda, 14.xii.2000, T24436, coll. G. C. Trono Jr., R. Savella, R. Conde, H. Gabito and A. Gallose, ix.4.2001, T22353, coll. R. V. Dumilag, 20.iv.2012, AOL441, AOL443; RVD09-



**Fig. 1** Phylogeny of *Betaphycus* and other members of the tribe Eucheumatoideae as inferred from COI sequences using maximum likelihood (ML) analysis. Numbers at the nodes indicate ML bootstrap support and BI posterior probabilities values

RVD16), Prieto Diaz (coll. M. Roleda, L. Bande, D. Tan and M. Samson, v.21.2000, T23818), and no specified locality in Sorsogon (as *E. gelatinae* interposed with *B. gelatinae*, coll. G. C. Trono Jr., 11.x.1991, det. E. G. Fortes, T968).

**Habitat** Plants were found growing in mid-intertidal rock pools usually in reef margins with moderate to strong current, thriving among other macrophytes such as *Sargassum* spp., *Caulerpa* spp., *Hypnea* spp., *Halimeda* spp., and others (Fig. 2a).

**Description** Thalli cartilaginous, decumbent, or prostrate with generally irregular to pseudodichotomous branching (Fig. 2b–d), borne from a crustose base that immediately issued a short, erect, cylindrical, basal segment were noted. Fronds were dorsiventral, terete to compressed and measured 0.2–1.0 cm broad and 1–2.5 mm thick. The ventral surface had deep purple red color while on dorsal surface; the color ranged from deep purple red to yellowish green or a mixture thereof. Margin may be smooth but usually dentate on both or one of the either segment margins. Apical tips may be simple and/or forked, acuminate to spinose (Fig. 2e). Numerous tubercles were consistently found on the ventral surface where protuberances were few or undeveloped. Simple, spinose, and lanceolate to spindle-shaped protuberances developed mainly on but not limited to the ventral and marginal surfaces (Fig. 2f). Generally, fronds exhibited smooth dorsal surface; however, as the thalli become more mature, few protrusions may develop dorsally. Young portion of the thalli may not produce any protuberance making both dorsal and ventral surfaces smooth, especially near apical tips.

The most common form included in this study was the *B. philippinensis* var. *philippinensis* (Fig. 2b, c) which was characterized by its rigid thallus and horizontal growth. Doty's "third form" of *B. philippinensis* which possesses slender fronds (with surfaces almost smooth) that branched pseudodichotomously was also observed (Fig. 2d).

A sample bearing the name "*B. gelatinum*" (Thai An, Phan Rang, Vietnam collected by Pham Huu Tri, 12.iv.1998, No. 921001, kind gift from Mr. Nguyen Xuan Hoa) was also examined morphologically. The specimen from Mr. Nguyen had ventral protuberances and other morphological features that were also found in *B. philippinensis*. Unfortunately, the material was preserved in formalin and attempts to PCR-amplify the COI marker were unsuccessful.

**Vegetative features** Transverse sections from within 5 mm below apical tips revealed compact rhizoidal cells (Fig. 2g). Flat rhizoidal core was strictly found in compressed main axes while spherical to somewhat flattened rhizoidal cores were found in cross sections of protuberances and in the terete basal segments (i.e., segment borne immediately from crustose base). Cell walls of rhizoidal cells are 6–27  $\mu\text{m}$  thick, with heavily stained lumens that measured 2–4  $\mu\text{m}$  in diameter.

Two to three pit plugs that were usually found in bigger rhizoidal cells may develop from lumen, passing through the thick cell wall to the lumen of the next rhizoidal cell. Medullary cells were pseudoparenchymatous, six to eight layers, 12–60  $\mu\text{m}$  in diameter, with thin cell walls. The outer cortical cells, which are spherical to ellipsoidal, were observed to be arranged in an apparent Y-shaped pattern. The basal cells of outer cortex were mostly ellipsoidal.

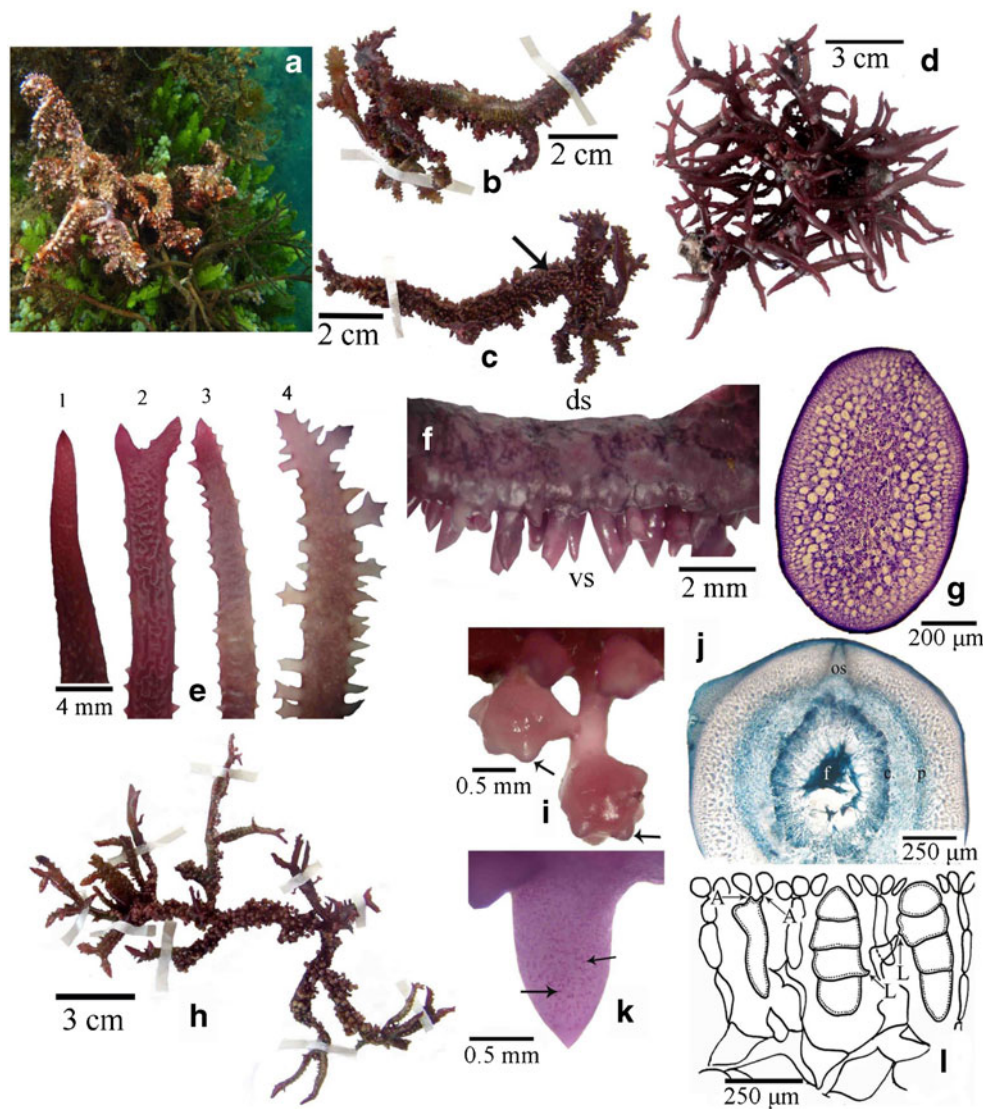
**Reproductive features** Figure 2h shows the habit of a cystocarpic plant. The cystocarps were borne from protuberance on the ventral surface and on margins measuring 0.3–1.4 mm high. Carposporophytes were localized inside cystocarps that were borne by single or branched stalks. The cystocarps were observed to possess blunt spines (Fig. 2i). The stalks measured 0.1–1.0 mm long and 0.3–0.7 mm wide. In median cross section, mature cystocarps were composed of a large central fusion cell that produced radially disposed clusters of carposporangia borne on branching filaments and interspersed with sterile traversing filaments. The traversing filaments were loosely arranged, branching six to eight times, located between the clusters of carposporangia and extended to the encircling pericarp. A cluster of carposporangia was subtended by sterile filaments that branched six to eight times. Each filament was terminated with a darkly stained, uninucleate carposporangium. Mature carposporangia measured 18–20  $\mu\text{m}$  in length by 6–16  $\mu\text{m}$  in diameter. No male thallus was found.

Tetrasporic thalli were easily distinguished by the presence of deep red dots representing tetrasporangia scattered on the ventral surface of the main axes and on the entire surface of the protuberance (Fig. 2k). There were no tetrasporangia on the dorsal surface of the main axes and in branch tips. Tetrasporangia were born on the penultimate superficial cortical cells. Apical pit connections were seen in tetrasporangial initials while lateral pit connections were found in mature tetrasporangia (Fig. 2l). Tetraspores divided zonately. The mature tetrasporangia measured 40–45  $\mu\text{m}$  in length and 10–16  $\mu\text{m}$  in diameter. Each tetraspore had almost uniform size ranging up to 8–10  $\mu\text{m}$  in length.

Table 2 shows a summary of the comparison of *B. philippinensis* and other related taxa with morphologically similar diagnostics.

## Discussion

The results of the molecular analyses indicated that *Betaphycus*, comprising the two species *B. philippinensis* and *B. speciosus*, is a well-supported clade. The COI sequences resolved the separation of the three genera from each other, although the data were not informative enough to establish which of the two genera (*Eucheuma* or *Kappaphycus*) is more closely related to



**Fig. 2** **a** Photograph of *B. philippinensis* in situ (Dapdap, Bulusan, Sorsogon, Philippines; AOL441). Plants were found growing in intertidal rock pools with strong wave actions, thriving among other macrophytes. **b–d** General morphology of *B. philippinensis*. A typical *B. philippinensis* var. *philippinensis* (AOL435) showing smooth dorsal surface (**b**) and with heavy protuberances on its ventral surface (**c**). Arrow indicates protuberances. Plant (AOL443) with consistently smooth dorsal surface with pseudodichotomous branching (**d**). **e** Four different types of segments based on margin and apical tip found in *B. philippinensis*: (1) simple pointed tip on a linear smooth segment, (2) shallow forcipate tip on a compressed dentate segment, (3) simple pointed tip on a flat dentate segment, (4) spinous tip in a compressed dentate segment. **f** A flat main axis of *B. philippinensis* showing its smooth dorsal surface (*ds*) with numerous proliferations on ventral surface (*vs*). **g** Cross section of a less

than 5 mm below apical tip of *B. philippinensis*. **h** Habit of a fertile *B. philippinensis* specimen (AOL436) showing cystocarps located on the ventral surface. **i** A portion of the ventral surface of the fertile thallus showing cystocarps with blunt spines (arrows). **j** A median section through a mature cystocarp of *B. philippinensis* showing an ostiole (*os*) and central fusion cell (*f*) with filaments extending from gonimoblast that bear mature carpospores (*c*) to the pericarp (*p*). **k** A portion of a fertile thallus of *B. philippinensis* (RVD103) showing a typical lanceolate-shaped ventral protuberance bearing deep red dots (tetrasporangial nemathecia) scattered on entire surface of the protuberance (arrows). **l** A schematic diagram of a developing tetrasporangium in *B. philippinensis* borne from cortical cells with apical pit connections (*A*) and pit connection on the lateral side in mature tetrasporangia (*L*)

*Betaphycus*. This can probably be improved by using the full length of COI genes for the inference of the phylogeny of the group (Tan et al. 2012). In the current study, only 566-bp portion of the ~1.6 kb gene was used. The use of additional markers and specimens might also be necessary to resolve intergeneric phylogenetic relationships within the tribe.

The inferred tree also showed that the two putative “*B. gelatinus*” specimens from northwestern Philippines grouped within *Euclima*, with a moderate bootstrap support to *E. serra*, indicating that these are species of *Euclima*. The degree of sequence divergence, as well as some morphological differences (data not shown), between the specimens and

**Table 2** A comparison of *B. philippinensis* and other related taxa with morphologically similar diagnostics

Taxonomic characters	<i>B. philippinensis</i>	<i>B. gelatinum</i>	<i>E. serra</i>
Color	Purple red to yellowish-green <sup>a</sup>	Purple red to yellowish-green <sup>b, c</sup>	Yellow to deep purple <sup>b</sup>
Gross habit	Prostrate/decumbent <sup>d, a</sup>	Prostrate/decumbent <sup>b, c, d, e, f, g, h</sup>	Prostrate/decumbent, erect <sup>e, i, j</sup>
Holdfast	Crustose, discoid rhizoid, immediately issue a short terete segment <sup>a, d</sup>	Crustose, discoid rhizoid, immediately issue a short terete segment <sup>d, f, g</sup>	Discoid rhizoid <sup>e, i</sup>
Fronde shape	Dorsiventrally terete to compressed <sup>a, d</sup>	Dorsiventrally terete to compressed <sup>b, c, d, e, f, g, h</sup>	Pinnately compressed, terete <sup>e, i, j</sup>
Branching pattern	Irregularly dichotomous to pseudodichotomous	Irregularly dichotomous <sup>b, c, d, f, g</sup>	Whorled branching <sup>e, g, i</sup>
Shape of apical tips	Spatulate <sup>k</sup> , acuminate <sup>a, d</sup> , spinous <sup>l</sup>	Acuminate <sup>d</sup>	Pointed <sup>i</sup>
Margin	Smooth to dentate <sup>a, d</sup>	Smooth to dentate <sup>b, d, e, j, g</sup>	Dentate <sup>e, g, i</sup>
Dorsal surface	Generally smooth <sup>a, d</sup> or with protuberances <sup>a</sup>	Smooth <sup>b, d, e, g, j, m</sup> , with protuberances <sup>c, n</sup>	–
Ventral surface	Generally with protuberances or tubercles <sup>a, d</sup> , sometimes bare specially near apical tips <sup>a</sup>	With protuberances or tubercle <sup>b, c, g, h, j</sup> , sometimes bare <sup>b</sup>	–
Type of axial core	Rhizoidal <sup>a, d</sup>	Hyphal <sup>m, o</sup> , rhizoidal <sup>b, d, e, f, g</sup>	Hyphal <sup>m, p</sup> , rhizoidal <sup>e, i, j</sup>
Location of cystocarps	Mostly ventral to marginal <sup>a, d</sup>	Ventral/marginal <sup>g, m</sup> , dorsal <sup>n, d</sup>	Upper portion of fertile spines, mostly marginal <sup>i</sup>
Type of cystocarp	Single and branched spinous stalked <sup>a, d</sup>	Single and branched spinous stalked <sup>g</sup>	Single and branched spinous stalked <sup>a</sup>
Shape of spines on cystocarps	Blunt <sup>a</sup>	Blunt (based only in illustration perhaps from materials of <i>B. philippinense</i> [Doty 1988, p. 190, Fig. 24A])	Pointed <sup>l</sup>
Location of spermatangial spores	Not seen	Upper parts of the thallus <sup>h</sup>	Not seen
Location of tetrasporangia	On surface of ventral axes and on ventral protuberances <sup>a</sup>	On surface of ventral axes and on ventral protuberances <sup>h</sup>	Below surface of spines and branches <sup>l</sup>
Carageenan content	More kappa- type than beta-type <sup>k, d</sup>	More kappa- than beta-type or more beta than kappa-type <sup>h, m, q</sup>	Iota carageenan <sup>k</sup>
Distribution	Bulusan, Sorsogon, Philippines <sup>a, d</sup>	Indian Ocean <sup>m</sup> , Taiwan <sup>e</sup> , China <sup>b</sup> , Vietnam <sup>c</sup> , Philippines <sup>e</sup>	Japan <sup>c</sup> , Taiwan <sup>e</sup> , Indonesia <sup>g</sup> , Philippines <sup>l</sup> , Australia <sup>i</sup> , Mauritius <sup>g</sup>

<sup>a</sup> This study

<sup>b</sup> Tseng (1984)

<sup>c</sup> Nguyen and Huynh (1995)

<sup>d</sup> Doty (1995)

<sup>e</sup> Yamada (1936)

<sup>f</sup> Kraft (1969)

<sup>g</sup> Doty (1988)

<sup>h</sup> Tseng and Trono Jr (2001)

<sup>i</sup> Gabrielson and Kraft (1984)

<sup>j</sup> Cordero (1978)

<sup>k</sup> Inconsistent morphological diagnostics

<sup>l</sup> Schmitz (1895)

<sup>m</sup> Doty and Norris (1985)

<sup>n</sup> Weber-van Bosse (1928)

<sup>o</sup> Santos (1989)

<sup>p</sup> Weber-van Bosse (1928)

<sup>q</sup> Greer and Yaphe (1984)

*E. serra* suggests that the specimens represent a distinct species (manuscript in preparation).

That not all *Euclidean* species contain iota ( $\iota$ )-carrageenan has previously been reported (Santos 1989). Ragasa et al. (2004) noted that specimens of *Euclidean* sp. (initially identified as *B. gelatinus*) contain a mixture of beta ( $\beta$ )- and kappa ( $\kappa$ )-carrageenan. This result suggested that the type of cell wall polysaccharide may not be a reliable character to be used as basis for the beta taxonomy (i.e., above-species level) of the euclideanoids as it is known that hybrid structures of carrageenan exist naturally in algal cell walls (Chopin et al. 1999).

The localization of tetrasporangia and the position of pit connections in the tetrasporangia of *B. philippinensis* may represent an important key feature that could help distinguish *Betaphycus* from other euclideanoid genera. Like in *B. philippinensis*, tetrasporangial nemathecias are also restricted within protuberances in *B. speciosus* (Doty 1988) and in *B. gelatinus* (Tseng and Trono 2001), whereas in *Kappaphycus* and *Euclidean*, the tetrasporangia are not localized in protuberances but rather scattered randomly on their main axes (Doty 1988). Our morphological and molecular analyses suggest that the presence of dorsal protuberances cannot be used as a morphological key to distinguish *B. gelatinus* from *B. philippinensis*.

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