

Note

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Taxonomic notes on the genus *Alsidium* C. Agardh, including the merging of *Bryothamnion* Kützing (Rhodomelaceae)

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In this study, the phylogenetic relationships among the genera *Alsidium* C. Agardh and *Bryothamnion* Kützing were investigated. Phylogenetic analyses using the plastid-encoded markers *rbcl*, *psbA*, and the mitochondrial barcode region (COI-5P) resolved a well-supported clade that included the species *Alsidium corallinum*, *Bryothamnion seaforthii*, and *B. triquetrum*. Our results indicated that taxonomic recognition of the genus *Bryothamnion* is not supported and two species of *Bryothamnion* are reallocated to *Alsidium*. A reexamination of the morphological definition of *Alsidium* is provided with an updated diagnosis of the genus and a morphology-based comparison of species that are currently circumscribed under this generic name. Furthermore, we reviewed morphological differences and similarities between *Alsidium* and the genus *Digenea*, both belonging to the tribe Alsidieae, discussing the most relevant morphological characters.

Key Words: *Alsidium*; *Bryothamnion*; *Digenea*; phylogenetic inference; *psbA*; *rbcl*; taxonomy

INTRODUCTION

The genus *Alsidium* was first formally described by C. A. Agardh (1827) based on *A. corallinum*, species that serves as the generitype. A total of 25 species have been described under this generic name, yet six remain as currently accepted (Guiry and Guiry 2018). The Mediterranean Sea harbors four of the currently recognized species. However except for two species that are well-represented, *A. corallinum* C. Agardh and *A. helmithochorton* (Schwendimann) Kützing, other species from the Mediterranean Sea, such as *A. notarisium* Bompard and *A. vagum* (Zanardini) Zanardini, remain taxonomically poorly known considering they have not been reported since their original descriptions. Two minute species of *Alsidium* are known to occur in the Pacific Ocean. Dawson described two species based on vegetative mate-

rial, *A. pacificum* (Dawson 1959) from Palmyra Atoll and *A. pusillum* (Dawson 1963) from the Galapagos Islands. A third pacific species, *Alsidium cymatophilum* R. E. Norris, described by Norris (1994) based on Hawaiian collections, has been transferred to *Digenea* based on molecular evidence (Díaz-Tapia et al. 2017).

The genus *Bryothamnion* was originally described by Kützing (1843) without a generitype designation. Two species were included, *B. seaforthii* based on *Fucus seaforthii* Turner and *B. lindenbergi* based on *Thamnophora disticha* Lindenberg. Additionally, Kützing described a second genus, *Physcophora*, based on *Fucus triangularis* Turner. However, in *Sp. Alg.*, Kützing (1849) merged *Physcophora* into *Bryothamnion* and recognized five species, three of them with a complanate habit, *B. seaforthii* Küt-



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zing, *B. ramellosum* Sonder, and *B. linderbengii* Kützing and two species with a triangular habit, *B. triangulare* and *B. hypnoides*. Agardh (1863) described three forms of *B. seaforthii*, f. *fastigiata*, f. *disticha*, and f. *imbricata*, based on characteristics related to the branching pattern. *Bryothamnion seaforthii* (Turner) Kützing was later designated by Schmitz (1889) as the type species of the genus. A sixth species morphologically similar to *B. triquetrum* was described in the 20th century as *B. pacificum* by Taylor (1945). Only three of the six species that have been described remain as currently recognized. Two of them, *B. triquetrum* and *B. seaforthii*, are commonly reported in tropical and subtropical regions of the Atlantic and Pacific coasts of America and several Caribbean islands (Guiry and Guiry 2018), the last species reaching South-West Asia with a record in Sri Lanka (Silva et al. 1996). *B. seaforthii* f. *imbricatum* J. Agardh remains as the only form that has been reported since the original description by Agardh (1863), and it seems to be common on limestone in the Florida Keys (Dawes and Mathieson 2008) and Cuba (Suárez 2005).

The systematic position of *Alsidium* and *Bryothamnion* within the family Rhodomelaceae was recently elucidated by Díaz-Tapia et al. (2017) providing an updated tribal classification of the family based on molecular phylogenomic evidence; these two genera and a third, *Digenea* C. Agardh, were assigned to the resurrected tribe Alsidieae. Furthermore, the apparent non-monophyly of *Alsidium* and *Bryothamnion* was noted by Díaz-Tapia et al. (2017); however, no formal changes to the classification were proposed at the time. Likewise, this new phylogenetic understanding of the tribal classification of Rhodomelaceae calls for the review of the morphological features between the genera of the tribe, *Alsidium* and *Digenea*. Hence, the aim of this study is to clarify the phylogenetic and taxonomic status of the genera *Alsidium* and *Bryothamnion* using molecular and morphological data. The non-monophyly problem is resolved herein by merging *Bryothamnion* into *Alsidium*. In addition, we provide an updated diagnosis of the genus *Alsidium* and establish a morphological comparison between this genus and *Digenea*.

MATERIALS AND METHODS

Collections and vouchers

Samples were either collected from several geographic locations or kindly provided dried in silica gel and / or

pressed on herbarium paper. Voucher specimens are located at The University of Alabama. Collection information and GenBank accession numbers of new sequences produced in this study and sequences retrieved from the GenBank are provided in Supplementary Tables S1 & 2.

Morphological studies

Specimens were rehydrated and thalli portions were fixed through increasing concentrations of ethyl alcohol (from 10 to 100%). Sections of vegetative and reproductive portions of thalli were made using a Sorvall (RMC) MT-5000 Microtome, stained with 1% v/v aqueous aniline blue acidified with 1% diluted HCl and mounted on slides with Karo Corn syrup solution (Tsuda and Abbott 1986). Prepared slides were observed and studied under an Axio Imager 2 Research Microscope (Carl Zeiss AG, Oberkochen, Germany). Image capture was carried out using an integrated camera model EOS 5D Mark III (Canon Inc., Tokyo, Japan). Photographs were edited and assembled on plates using Adobe Photoshop CC (Adobe Systems Inc., San Jose, CA, USA).

Genomic DNA extraction, gene amplification, and sequencing

Genomic DNA was extracted from dried material ground in liquid nitrogen using the cetyl trimethylammonium bromide protocol modified from Doyle and Doyle (1987). After resuspension of total DNA, viscous extractions (probably due to the presence of polysaccharides) were further purified using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to manufacturer's instructions. Full or partial sequences of *rbcL* and *psbA* were obtained following amplification protocols in Saunders and Moore (2013) using the primers F57, R753, F577, R1150, F753, and *rbcLrevNEW* (Freshwater and Rueness 1994, Kucera and Saunders 2012) and *psbAF1*, *psbA600R*, and *psbAR2* (Yoon et al. 2002), respectively. The COI-5P fragment was amplified with a single primer combination, GAZF1 / GAZR1 (Saunders 2009) following amplification profiles in Saunders and Moore (2013). Polymerase chain reaction (PCR) reactions were prepared using either Mango Taq DNA polymerase kit (Bioline, London, UK) or the AccuStart II PCR SuperMix (Quanta Biosciences, Inc., Gaithersburg, MD, USA) following manufacturer's instructions. Amplified products were purified and sent for Sanger sequencing to Advanced Genetics Technology Center and UK Healthcare Ge-

nomics Center (University of Kentucky, Lexington, KY, USA). Contigs were edited and assembled using Geneious R7 (Kearse et al. 2012).

Alignment and phylogenetic analysis

rbcL, *psbA*, and COI sequences were individually aligned using CLUSTALW in MEGA 6 (Tamura et al. 2013) under default conditions. Selection of the nucleotide substitution models for *rbcL* and *psbA* was carried out using MEGA 6. Independent maximum likelihood *rbcL* and *psbA* phylogenies for the family Rhodomelaceae were estimated in RAxML (Stamatakis 2014) under the General Time Reversible model (GTR) + gamma (G) with 1,000 bootstrap replicates. A concatenated alignment of the *rbcL*, *psbA*, and COI-5P was generated in Sequence Matrix (Vaidya et al. 2011). A phylogenetic tree with the concatenated data was built in RAxML under the GTR + G with 1,000 bootstrap replicates. All final trees were visualized and edited using FigTree v.1.4.2 (Rambaut 2012) and Adobe Illustrator (Adobe Systems Inc.).

RESULTS AND DISCUSSION

Phylogenetic analyses

We sampled and analyzed several specimens of the two most common species of *Bryothamnion*, *B. seaforthii* and *B. triquetrum*, and included in our analyses the type species of *Alsidium*, *A. corallinum*. The *rbcL* phylogeny (Fig. 1) resolved a well-supported clade containing the two species of *Bryothamnion* and *A. corallinum*. This clade was sister to the *Digenea* clade, both encompassing the tribe Alsidieae, in agreement with previous results in Díaz-Tapia et al. (2017). In the *psbA* phylogeny (not shown), there was no support for the tribe Alsidieae and the *Bryothamnion* / *Alsidium* clade obtained low support (0.65). Likewise, the phylogenetic tree inferred from the concatenated matrix (Fig. 2) resolved the tribe Alsidieae with moderate support (0.83) and the *Bryothamnion* / *Alsidium* clade with high support (1). However, *Bryothamnion* appears non-monophyletic because either *B. seaforthii* (Fig. 1) or *B. triquetrum* (Fig. 2) clustered with *A. corallinum*, which calls into question their assignment to two different genera.

Morphological analysis

Morphological characterization of species of *Alsid-*

ium. Morphological observations were made on specimens of *Alsidium corallinum*, *B. seaforthii*, and *B. triquetrum* and whole-mount and sectioned material was observed by light microscope. These species are characterized by having an erect, relatively large cartilaginous thalli (Figs 3A & 4A), radially organized (Figs 3B & 5A). However, the flattened portion of *B. seaforthii* becomes bilaterally organized with distichously arranged branchlets (Fig. 4B) while they have a spiral alternating arrangement in the angular thallus of *B. triquetrum*. The main axes and laterals are clothed with short determinate branches in an irregular pattern in *A. corallinum* (Fig. 3B) and regularly in *Bryothamnion* species (Fig. 4A). A conspicuous axial filament is evident in cross and longitudinal sections of the three species and barrel-shaped cells with thick cell walls made up the axial filament (Figs 3D & 5C). This filament is surrounded by six to eight pericentral cells (Figs 3C, 4C & 5B), sometimes up to nine in *B. seaforthii*. An extensive cortication can be found throughout the thalli and in main axes and laterals, cells of outermost cortical layer are usually minute, loosely (Fig. 3E) and irregularly arranged (Figs 4D & 5E). Trichoblasts in these species are early deciduous, strongly incurved, usually poorly developed, with subspherical basal and suprabasal cells tapering sharply upwards (Figs 3G & 5D). Tetrasporophytic specimens have stichidium-like branchlets that are arranged axillary or spirally, with one sporangium bulging in each short fertile segment (Figs 3F, 4E & 5F).

At present six species make up the genus *Alsidium*. While *B. seaforthii*, *B. triquetrum* and *A. corallinum* can be objectively compared in light of the molecular and morphological evidence, the rest remain morphologically-defined species. Provided that most species are currently comparable only in a morphological context, we reviewed all relevant phycological literature and a summary of the differences and similarities between species of *Alsidium* is given in Table 1. *Alsidium vagum* (Zanardini) Zanardini from the Red Sea and *A. notarisium* Bompard from the Mediterranean Sea, were originally described in the mid-1800s and have only been studied once, each probably from a single collection event. Because they were poorly described, any further morphological comparison is unfeasible and their identity remains ambiguous.

Since Dawson's original descriptions of the pacific species *A. pusillum* and *A. pacificum*, these have been mentioned in several floristic accounts (Silva et al. 1987, Tsuda et al. 2012, Tsuda and Walsh 2013, Ang et al. 2014, Phang et al. 2016). Norris (1994) examined the holotype

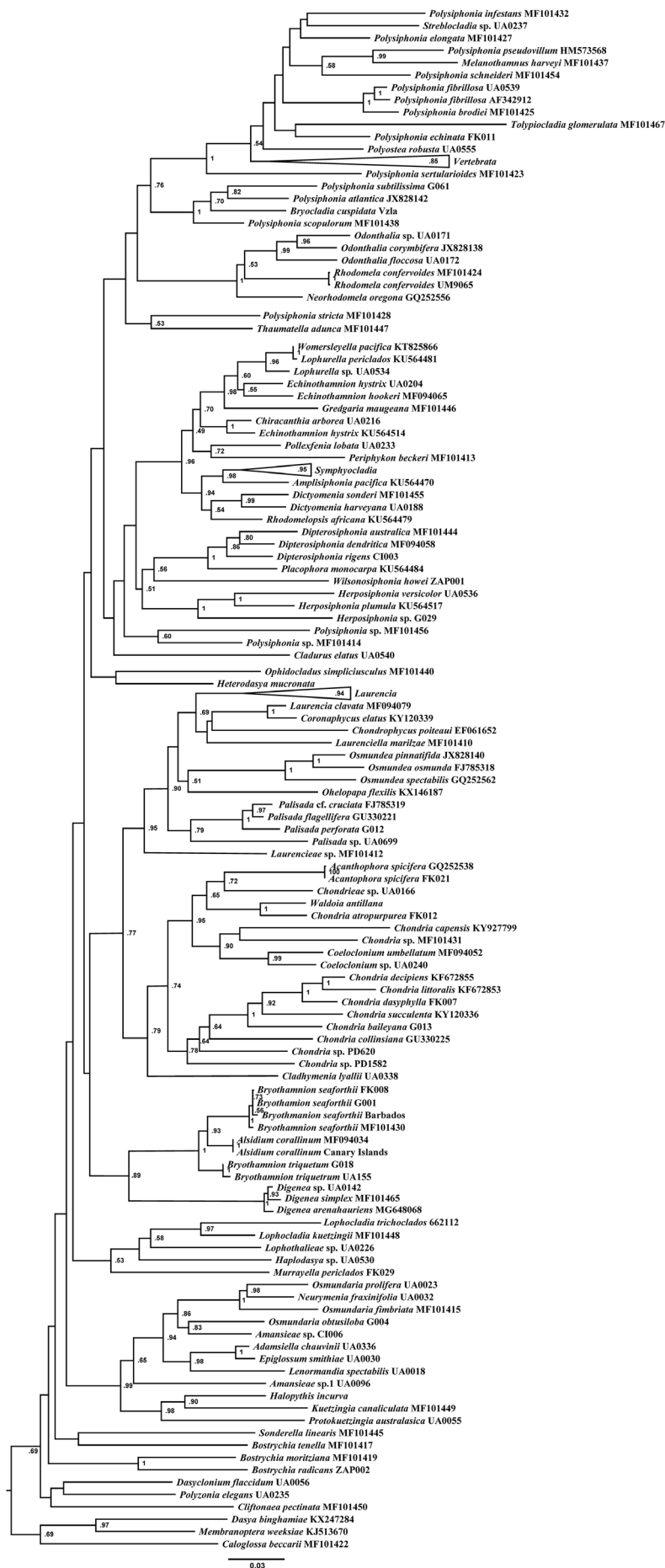


Fig. 1. Maximum likelihood phylogenetic tree estimated from the *rbcL* gene. Values at nodes represent bootstrap support (not shown if node receive <50).

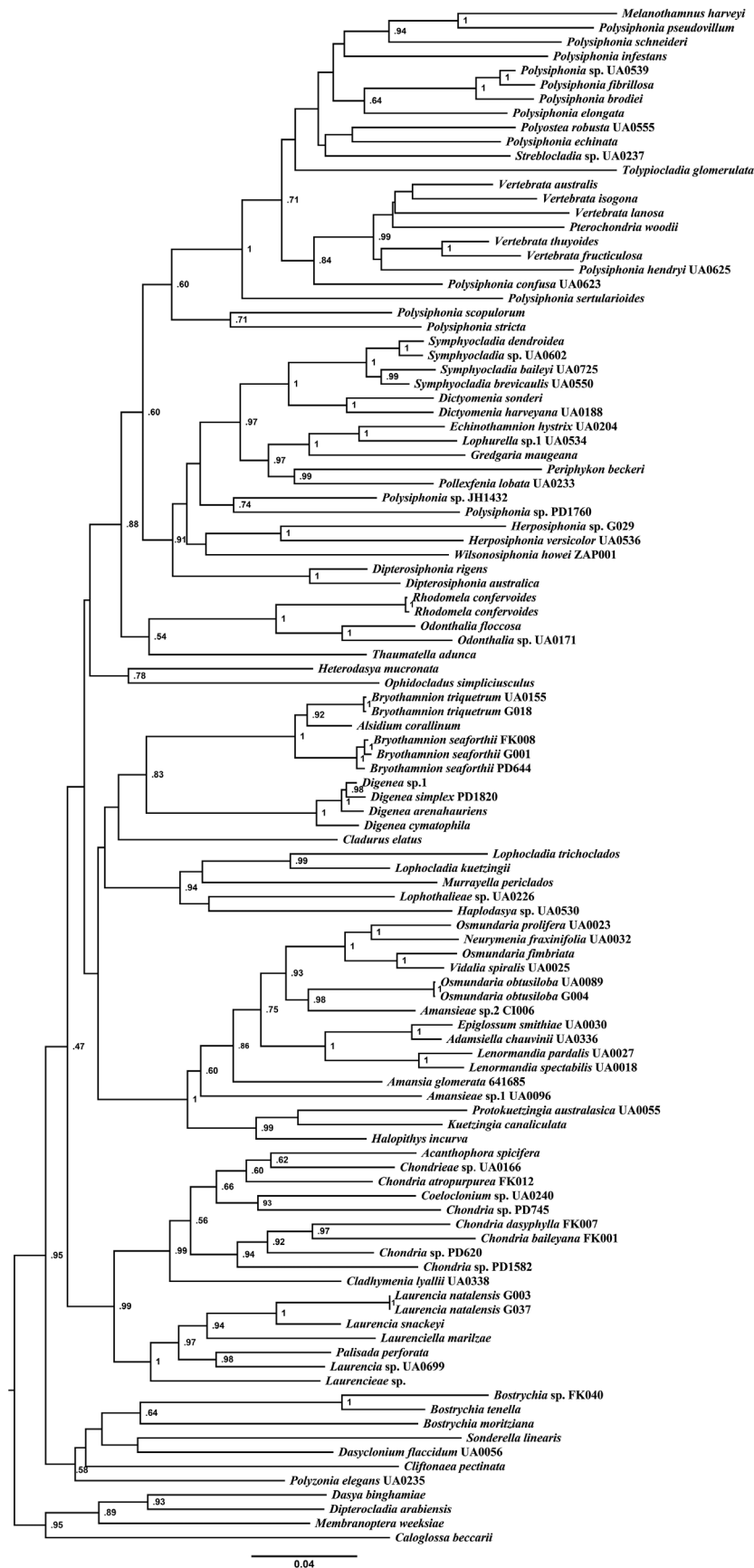


Fig. 2. Maximum likelihood phylogenetic tree of concatenated data of *rbcL* + *psbA* + *COI-5P*. Values at nodes represent bootstrap support (not shown if node received <50).

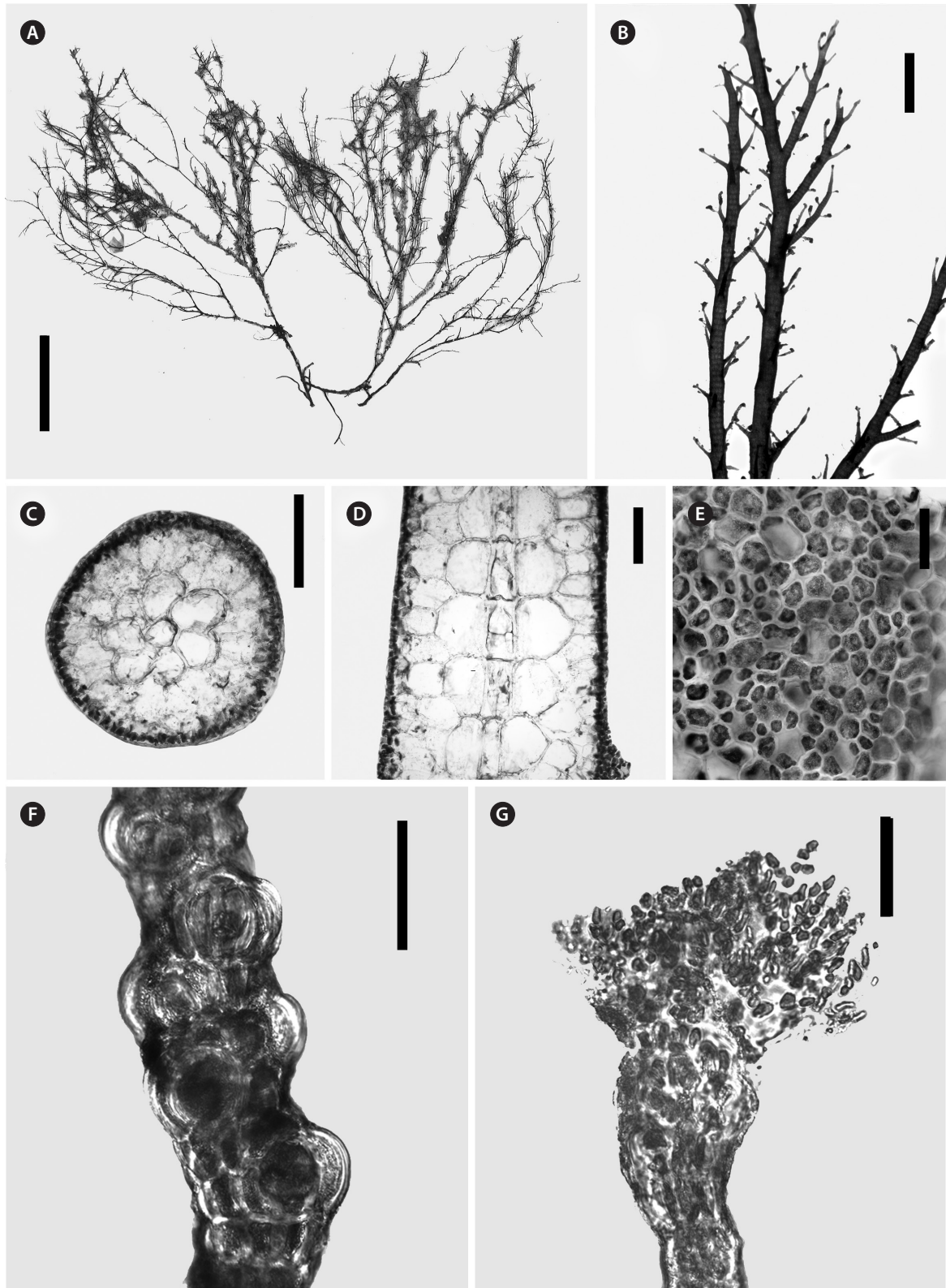


Fig. 3. Morphological features of *Alsidium corallinum*. (A) Habit of the thallus (dried). (B) Detail of laterals showing spirally arranged short branchlets. (C) Cross section through main axes showing axial cell surrounded by six pericentral cells. (D) Longitudinal section through main axes showing the axial filament composed of barrel-shaped cells. (E) Superficial view of thallus cortication. (F) Tetrasporangial shoot. (G) Trichoblasts. Scale bars represent: A, 2 cm; B, 2 mm; C, 500 μ M; D, 370 μ M; E, 100 μ M; F & G, 50 μ M.

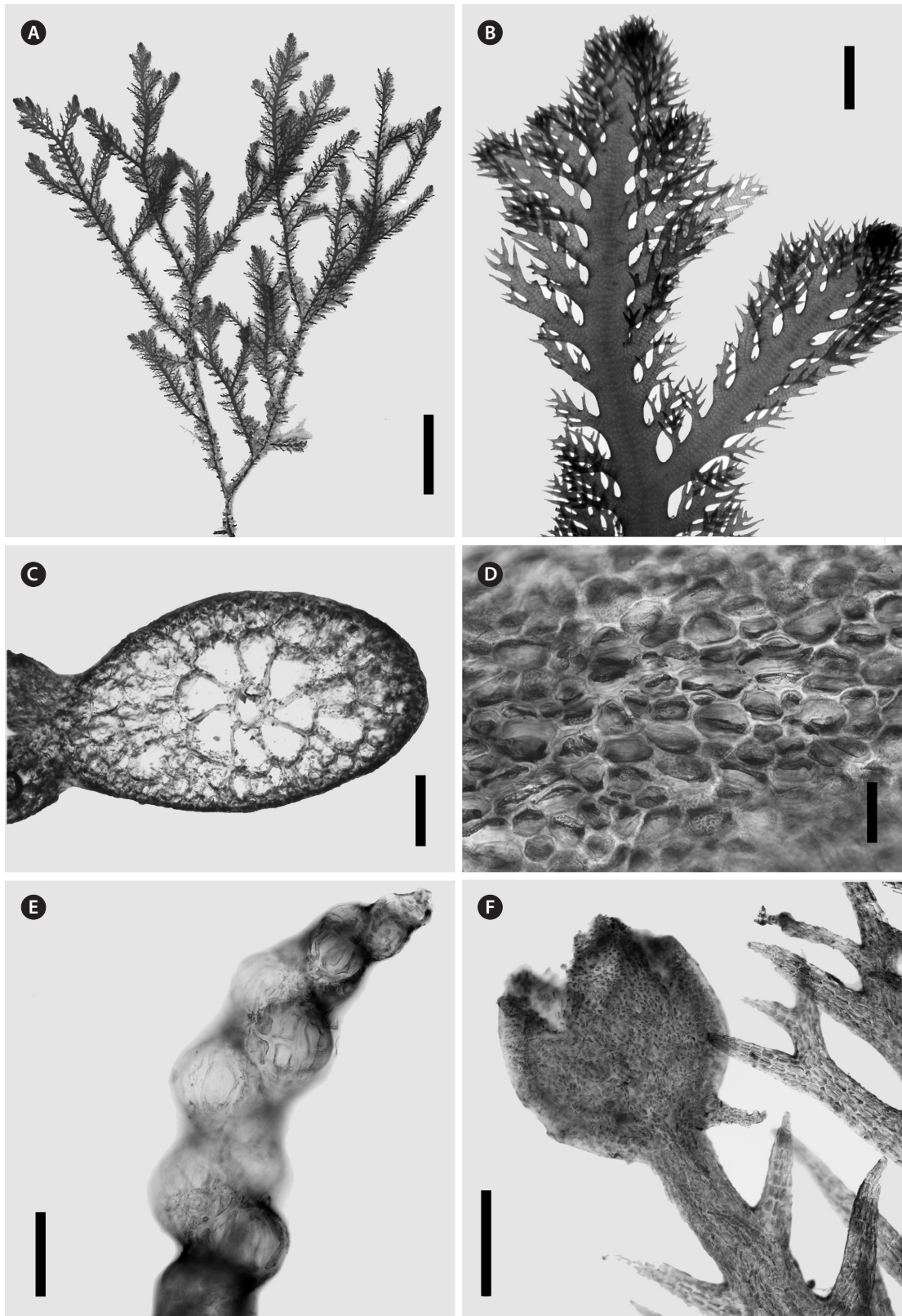


Fig. 4. Morphological features of *Alsidium seaforthii*. (A) Habit of the thallus (dried). (B) Detail of main laterals showing distichously arranged laterals and branchlets. (C) Cross section through main axes showing axial cell surrounded by eight pericentral cells. (D) Superficial view of thallus cortication. (E) Tetrasporangial shoot. (F) Subterminal cystocarp. Scale bars represent: A, 2 cm; B, 2 mm; C, 500 μ M; D, 100 μ M; E, 150 μ M; F, 300 μ M.

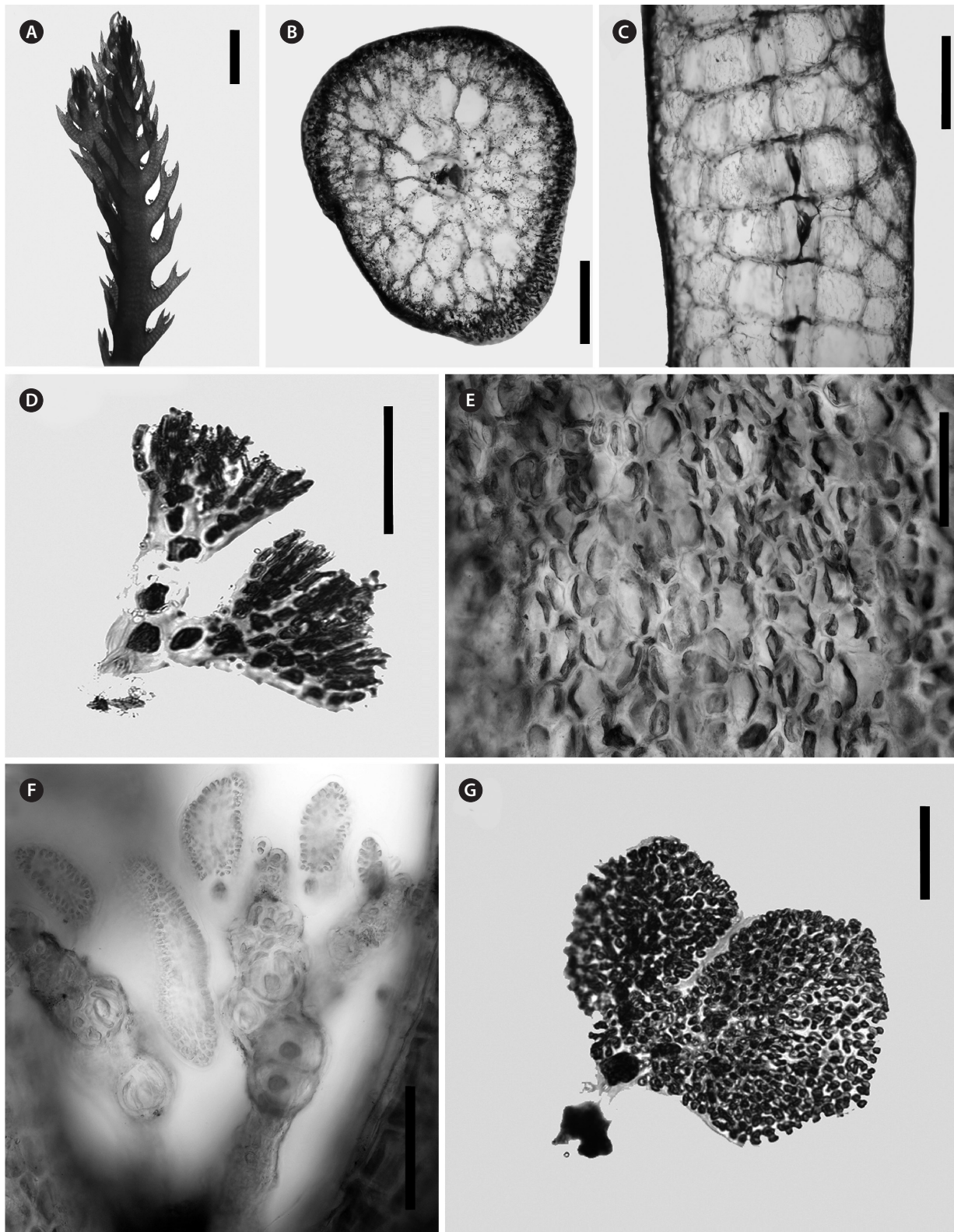


Fig. 5. Morphological features of *Alsidium triquetrum*. (A) Detail of laterals showing spirally arranged short branchlets. (B) Cross section through main axes showing axial cell surrounded by eight pericentral cells. (C) Longitudinal section through main axes showing the axial filament composed by barrel-shaped cells. (D) Trichoblasts. (E) Superficial view of thallus cortication. (F) Dwarf axillary shoots producing tetrasporangia and spermatangial plates. (G) Spermatangial plate. Scale bars represent: A, 2 mm; B & C, 500 μ M; D & G, 50 μ M; E & F, 100 μ M.

Table 1. Morphological comparison among species of *Alsidium*, *Digenea simplex*, *D. cymatophila*, and *Bryothamnion pacificum*

	<i>Alsidium corallinum</i>	<i>Alsidium triquetrum</i>	<i>Alsidium seaforthii</i>	<i>Alsidium helminthochorton</i>	<i>Alsidium pusillum</i>	<i>Digenea simplex</i>	<i>Digenea cymatophila</i>	<i>Bryothamnion pacificum</i>	<i>Alsidium pacificum</i>
Habitat	Semi-exposed parts of the upper sublittoral	Intertidal on rock outcrops, and somewhat exposed areas or subtidal	Intertidal on rock outcrops, or subtidal	Intertidal in sheltered areas, entangled with other species and caespitose, forming sandbinding turfs	Intertidal turf	Common in exposed or sheltered areas of the intertidal, also subtidal, caespitose forming dense turfs	Adapted to strong surf habitat and some subtidal	Subtidal	Subtidal
Distribution	Widely distributed in the Mediterranean Sea, Canary Islands, and Madeira	Tropical and subtropical regions of the Atlantic and Pacific coasts of America, Caribbean islands, the Atlantic coast of Africa	Tropical and subtropical regions of the Atlantic and Pacific coasts of America, Caribbean islands, the Atlantic coast of Africa and Sri Lanka	Widely distributed in the Mediterranean Sea	Isla Santa Cruz, Galapagos Islands ^a	Widely distributed in tropical and warm temperate coastal regions and islands in Atlantic, Pacific, Indian Oceans and Mediterranean Sea	Hawaiian islands ^b	Restricted off the coast of Nayarit, Mexico, near Maria Magdalena Island (Las Tres Marias)	Palmyra Atoll (Line Islands) in the Pacific Oceania
Thallus habit	Erect	Erect	Erect	Erect / prostrate	Erect / prostrate	Erect	Erect / prostrate	Erect	Erect / prostrate
Height	Up to 15 cm	Up to 25 cm	Up to 20 cm	Up to 5 cm	6-10 mm	Up to 25 cm	Aprox. 3 cm tall	Above 11 cm	10-15 mm
Thallus (terete / compressed)	Terete, radially organized	Terete, angular, radially organized, generally triangular in transverse section	Terete, radially organized proximally becoming bilateral	Terete, radially organized	Terete, radially organized	Terete, radially organized	Terete, radially organized	Terete, radially organized	Terete, radially organized
Anchoring system	Basal crust	Basal disc	Rhizomatous holdfast	-	Ventral multicellular discs	Basal disc / basal crust	Basal (prostrate) crust anchored by ventral rhizoids	Unknown	Multicellular haptera
Cortication	Extensively corticated	Extensively corticated, usually heavily corticated proximally	Extensively corticated, usually heavily corticated proximally	Extensively corticated	Extensively corticated	Extensively corticated, very heavy in main axes and laterals	Extensively corticated, secondary cortication occurs proximally	Corticated, 2 layers of medullary outer cells and 1 layer cortical cells	Extensively corticated
Main axes (diameter)	1-2 mm	1-2 mm	1-1.3 mm	0.5-1 mm	100-160 µM	2-3 mm	0.5 mm	2 mm	Prostrated axes about 150-200 µM and erect axes 100 µM
Branching pattern (main or primary axial filament)	Regularly or irregularly alternate	Irregularly alternate	Pinnate, sparingly below, subfastigiately above	Sometimes dichotomous, unilateral, irregular	Infrequently branched	Dichotomous to irregularly branched	Unbranched or branched once to a few times	Alternate	Indeterminate branches sparse, tending to be unilateral

Table 1. Continued

	<i>Alsidium corallinum</i>	<i>Alsidium triquetrum</i>	<i>Alsidium seaforthii</i>	<i>Alsidium helminthochorton</i>	<i>Alsidium pusillum</i>	<i>Digenea simplex</i>	<i>Digenea cymatophila</i>	<i>Bryothammon pacificum</i>	<i>Alsidium pacificum</i>
Branchlets	Spirally arranged, sparse, irregular, with 7 pericentral cells and a layer of cortical cells	Three rows spirally twisted, in spiral succession in the axils of trichoblasts, at most once forked	Distichously arranged, marginal or sometimes in 3-4 rows, from 1 to 4 forked, applied to every other segment	-	-	Spirally arranged, significantly crowded in mid and upper portions, with 6-8 pericentral cells and a thin layer of cortical cells	Spirally arranged, in distal regions of erect axes	Spirally arranged becoming corticated at the base, with 4-10 alternately placed acute uncorticated branchlets, simple or bearing 1-4 subsidiary branchlets	-
No. of pericentral cells	6, 8	7, 9	8, 9	7, 8	5	6, 8, nondiscernible at older portions	10, 12, discernible at older portions	7	7
Trichoblasts	Spirally, present on every segment but soon deciduous	In a 1/3 spiral, present on every second segment, poorly developed, incipient, but soon deciduous	In a 1/2 divergence, present on every second segment, poorly developed, incipient, but soon deciduous	In a 1/4 spiral divergence, present on every segment but soon deciduous, small and markedly inwardly curved	Infrequent and short	In a 1/3 spiral, present on every segment, soon deciduous, prominently developed and markedly inwardly curved	Frequent, usually large with many alternate pinnate branches	In a 1/4 spiral, present on every second segment, often prominently developed, early deciduous	
Tetrasporangia	Formed in slightly thickened upper parts of the branchlets, single or clustered	Distinct axillary shoots on ultimate adventitious branchlets, singly or sometimes clustered	Distinct axillary shoots on ultimate adventitious branchlets, singly or sometimes clustered	Formed in the upper fertile segments of branchlets, in large numbers in consecutive segments	Unknown	Formed in the upper thick fertile segments branchlets, assuming a clavate appearance	Formed in the distal parts of erect branchlets and also from short determinate laterals in distal regions of erect axes	Unknown	Unknown
Male capitula	Replacing whole trichoblasts, sometimes with sterile tips	Replacing whole trichoblast, in axillary shoots at branchlets, ovate, foliose, lobed, sac-like without sterile cell margin	Replacing whole trichoblasts, in axillary shoots at branchlets, ovate, foliose, with sterile marginal cells	Replacing whole trichoblast, small ovate, foliose with a 1-3 sterile cells monosiphonous filament that persist on the top and a crenulated narrow sterile margin	Unknown	Replacing whole trichoblast, in distal region of branchlets; flat, oval, probably with sterile margins	Replacing whole trichoblast, occur in clusters on the distal ends of upright axes, flat, ovoid, plate-like	Unknown	Unknown
Cystocarp	Globose to urceolate, lateral on ultimate branchlets	Ovate, often appearing to terminate short branchlets, subterminal	Globose, often appearing to terminate short branchlets subterminal	Ovate, sessile, lateral on ultimate branchlets	Unknown	Ovate, terminal or lateral on branchlets.	Urceolate, on the second segment of short lateral branches; only one cystocarp occurs on each fertile branch	Unknown	Unknown

References: Ardissonne (1883), Hauck (1885), Schmitz and Falkenberg (1897), Falkenberg (1901), De Toni (1903), Schiffner (1916), Børgensen (1920), Taylor (1945), Kylin (1956), Dawson (1959, 1963), Barcelo and Seoane-Camba (1989), Dreckmann and Santies (1994), Norris (1994), Athanasiadis (2016), Schneider et al. (2018).
^aReported for Luzon, Cavite (Philippines) by Vannajan and Trono (1978) as *A. pusillum* prox. (species proxima).
^bReported by Gomez et al. (2013) as a subtidal species in Aragua and Vargas (Venezuela).

of *A. pacificum* and considered it cons-specific with *A. pusillum* pointing out that *A. pacificum* resembled his newly described species *Alsidium cymatophilum* R. E. Norris (now *Digenea cymatophila*) but it was ultimately different because of the lack of an encrusting base and the number of pericentral cells which are fewer in *A. pacificum*. The morphological resemblance of these species with *D. cymatophila* cast doubts on their current taxonomic placement as members of the genus *Alsidium* (Table 1).

Comparison of the genera *Alsidium* and *Digenea*.

Vegetative structure: *Alsidium* and *Digenea* are for the most part represented by coarse cartilaginous species that reach several centimeters high. Species are extensively corticated and frequently clothed with short determinate laterals. However, when internal structure is compared between the two genera, differences are evident. In *Alsidium* (including *Bryothamnion*), the axial filament is clearly evident and pericentral cells (from 6 to 9) are surrounded by 2-3 layers of large medullary cells that transition into few outermost layers of small and highly pigmented cortical cells. In contrast, in the main axes and indeterminate laterals of species of *Digenea* (with the exception of *D. cymatophila*) the axial filament is only evident right off below the apical region while remains indiscernible along the rest of the thallus. Likewise, an apical cell is not evident in longitudinal view. Several cortical layers of isodiametric cells of similar size build up the thick axes in *Digenea* and most species become heavily corticated throughout. The branchlets (short determinate laterals) in *Digenea* sharply differentiate from the main axes and are corticated by a thin layer of pigmented cells that causes the polysiphonous construction to be discernible. In contrast, determinate branches in *Alsidium* clearly taper sharply upwards, frequently becoming uncorticated distally. Shedding of determinate laterals is a frequently observed phenomenon. Branchlets are early deciduous in *Digenea* while more or less strong but also deciduous in *Alsidium* (Schmitz and Falkenberg 1897, Falkenberg 1901). This shedding could be related to seasonal changes (Barcelo and Seoane-Camba 1989, Cecere et al. 2002) and herbivory, being particularly evident in species of *Digenea* (Vidal Marques et al. 2006, Schneider et al. 2018).

Reproduction: *Male capitulum:* The discoid to reniform spermatangial plates is the main unifying character of the tribe Alsidieae (Fig. 5G). Flat plate-like male capitula were illustrated by Kützing (1865) for *Digenea simplex* and described later by Børgesen (1920) as dis-

coid bodies of oblique reniform shape confirming Kützing's illustrations. Falkenberg (1901) also found analog flat antheridia in *B. seaforthii*. The formation of male capitula in *Digenea simplex* is similar to the formation in *B. seaforthii*. Børgesen (1920) described the process of spermatangial plates formation as follows: two cells are cut off alternately at both sides of the apical cell, these cells produce segments that remain together increasing gradually in length while dividing into smaller cells until the flat bodies are formed. We found also plates in *Bryothamnion triquetrum*. In our observations, aggregations of small axillary dwarf shoots produce at the same time tetrasporangia and spermatangial plates (Fig. 5F). Plates develop from the whole trichoblasts and they are discoid to slightly lobed, sometimes sac-like, with an inner network with no apparent row of sterile marginal cells and a thick outer membrane. Reproductive structures pairing in the Rhodomelaceae has been reported in several species, such as *Chondria baileyana* (Montagne) Harvey (Edelstein et al. 1974), *Laurencia pinnatifida* (Hernández-González and Gil-Rodríguez 1994) and many others from the tribes Strebloladieae and Polysiphonieae (Aguilar-Rosas and Aguilar-Rosas 1984, 2010, Cheung et al. 1984, Aguilar-Rosas et al. 2006, Díaz-Tapia and Bárbara 2013).

Spermatangial plates of *A. helminthochorton* (Schwendemann) Kützing were compared to spermatangial plates of *Chondria tenuissima* (Withering) C. Agardh by Schiffner (1916). In *Alsidium*, the spermatangial plates are small egg-shaped leaflets with a 1-3 sterile cells monosiphonous filament that persist on the top of the flatten structures and a crenulated and narrow sterile margin. Through the bilateral layer of spermatia, in *Alsidium*, the network branches in a regular alternate fashion, while *Chondria*, the network is regularly dichotomous. The spermatangial plates of the latter are more rounded in outline, with a non-crenulated 2-3-cell wide sterile margin.

Tetrasporangial branches: In Agardh's definition of the tribe Alsidieae, genera were separated by the morphological variation of tetrasporangia. In *Alsidium* and *Digenea*, tetrasporangia were described as being produced in simple branches, one per segment, spirally twisted, while *Bryothamnion* was characterized by having sporangia in axillary positioned stichidia. Kylin (1956) considered that in *Bryothamnion*, the sporangia were not produced in stichidia but in upper, thickened short segments. Falkenberg (1901) described *Digenea* as having vegetative segments about as high as wide while the fertile segments reached only one-fifth of this width. Thus, the length

of fertile segments in *Digenea* is evidently reduced and some authors have described the production of sporangia in stichidia for *Digenea simplex* from Mexico (Dreckmann and Sentiés 1994) and *Digenea cymatophila* from Hawaii (Norris 1994). However, a stichidium in the strict sense is a tetrasporangial branch in which the sporangia are produced in whorls from the pericentral cells, like the ones occurring on ordinary branches in *Bostrychia* and the ones produced on trichoblasts in some genera of the Lophothalieae (Hommersand 1963). Agardh (1892) mentioned that *Alsidium* and *Digenea* had “stichidiis siliquaeformibus”. The siliquose stichidia term is more likely referring to a very long sporangial branch, since sporangia are sometimes produced in several consecutive segments and these segments are very thickened and short. In *Alsidium* and *Digenea*, tetrasporangia are arranged in spirally twisted longitudinal rows, individually in very short fertile segments that distort and swell. However, in *Bryothamnion seaforthii* and *B. triquetrum*, fertile dwarf branches that resemble stichidia produce sporangia. These branches are axillary positioned and can be found single or clustered (Figs 4E & 5F).

The production of kainoids: In addition to spermatangial plates, *Digenea* and *Alsidium* from the Alsidieae seem to share a chemical trait with members of the Chondrieae. Naturally derived amino acids of the kainoid type have been known to occur for several decades in some members of both tribes. Norris (1994) suggested that *Alsidium* and the tribe Chondrieae were phylogenetically related not only by the presence of spermatangial plates, but also by the production of domoic acid (DOM). DOM is an excitatory amino acid that exerts a neurotoxic effect (Jiang et al. 2014). It was originally isolated from *Chondria armata* (Takemoto and Daigo 1958) and later from *Alsidium corallinum* (Impellizzeri et al. 1975) and *C. baileyana* (Novaczek et al. 1987). Interestingly, DOM is a structural analog of kainic acid (Ramsdell and Zabka 2008), which has been isolated from *Digenea simplex* (Murakami et al. 1953) and *Alsidium helminthocorton* (Calaf et al. 1989). The latter species has been used for more than a thousand years in some parts of Asia as an anthelmintic due to the presence of this acid (Nitta et al. 1958), which produces a similar neurotoxic effect to DOM (Doucette et al. 2000).

Diagnosis of the genus *Alsidium*. The evidence provided by phylogenetic and morphological analyses in this study does not warrant the taxonomic recognition of the genus *Bryothamnion* and consequently we reallocate *B. seaforthii* and *B. triquetrum* under the generic name *Alsidium* C. Agardh following the Principle of Pri-

ority. The merging of *Bryothamnion* into *Alsidium* calls for a new morphological interpretation of the genus and an updated morphological diagnosis of *Alsidium* is provided as follows:

Alsidium C. Agardh, 1827

Diagnosis: fleshy to cartilaginous erect, radially organized thalli, terete or compressed, sometimes becoming bilateral, fixed to the substrata by a basal crust or rhizomatous holdfast. Branching is exogenous, but endogenous laterals may occur. Thalli are frequently clothed with short determinate laterals, usually simple or branched once. Trichoblasts are typically small and early deciduous. Polysiphonous axis with 6 to 9 pericentral cells, cortication present throughout the thallus. Apical cell protruding. Tetrasporangia are produced 1 per segments of fertile branchlets that originate spirally or axillary. Procarps are produced on the second segment of trichoblast. Male capitulum replaces the whole trichoblast and spermatia are produced in discoid to sac-like flat plates with or without sterile margins.

Type species: *Alsidium corallinum* C. Agardh

New species combinations:

Alsidium seaforthii (Turner) Garcia-Soto & Lopez-Bautista.

Basionym: *Fucus seaforthii* Turner.

Taxonomic synonym: *Bryothamnion seaforthii* (Turner) Kutzinger.

Alsidium triquetrum (S. G. Gmelin) Garcia-Soto & Lopez-Bautista.

Basionym: *Fucus triqueter* S. G. Gmelin.

Taxonomic synonym: *Bryothamnion triquetrum* (S. G. Gmelin) M. Howe 1915.

CONCLUSION

In this study, phylogenetic analyses have provided evidence that the genus *Bryothamnion* is non-monophyletic. Two species of *Bryothamnion*, *B. seaforthii* (genotype) and *B. triquetrum*, are reallocated to the genus *Alsidium*. Successful genomic DNA extractions were limited to these two species while material of the third species of the genus, *B. pacificum*, failed to yield amplifiable DNA, possibly due to age-related DNA degradation. *B. pacificum* was described by Taylor (1945) based on sterile collections from Pacific Mexico and the striking structural resemblance with *B. triquetrum*. Since its original publication, *B. pacificum* remains poorly known and future studies may indicate that these two species

are conspecific.

Most species of *Alsidium* have not been studied since they were originally described and many of the early-described species are insufficiently characterized with regard to present-day requirements in species taxonomy. Consequently, many of them remain without nomenclatural verification, pointing to the possibility that some are more likely to be taxonomic synonyms with known widely distributed species while others may not even belong to the genus *Alsidium*. In light of this uncertainty, the challenge still remains to properly assess the taxonomic placement of these species and their phylogenetic relationships to other taxa in the Rhodomelaceae using molecular techniques.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Collection and voucher information / source of species included in the concatenated analysis including GenBank accession numbers of *rbcL*, *psbA*, and COI-5P sequences (<http://e-algae.org>).

Supplementary Table S2. Additional species included in the phylogenetic analysis of *rbcL* gene, collection information, references and accession numbers (<http://e-algae.org>).

REFERENCES

- Agardh, C. A. 1827. Aufzählung einiger in den österreichischen Ländern gefundenen neuen Gattungen und Arten von Algen, nebst ihrer Diagnostik und beigefügten Bemerkungen. *Flora* 10:625-640.
- Agardh, J. G. 1863. *Species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen secundum: algas florideas complectens*. C.W.K. Gleerup, Lund, pp. 787-1138.
- Agardh, J. G. 1892. *Analecta algologica*. Acta Univ. Lund. 28:1-182.
- Aguilar-Rosas, R. & Aguilar-Rosas, L. E. 2010. Mixed phases tetrasporangial and carposporangial on the same thallus in *Polysiphonia scopulorum* var. *villum* (Rhodomelaceae, Rhodophyta). *Rev. Mex. Biodivers.* 81:187-189.
- Aguilar-Rosas, R., Aguilar-Rosas, L. E. & Pedroche, F. F. 2006. Description of spermatangial thalli and mixed-phase in *Polysiphonia confusa* (Rhodomelaceae, Rhodophyta). *Rev. Mex. Biodivers.* 77:1-6.
- Aguilar-Rosas, R. & Aguilar-Rosas, M. A. 1984. Presencia de las fases carposporofita y tetrasporofita sobre el mismo talo en *Polysiphonia paniculada* Mont. *Cienc. Mar.* 10:181-183.
- Ang, P. O. Jr., Leung, S. M. & Choi, M. M. 2014. A verification of reports of marine algal species from the Philippines. *Philipp. J. Sci.* 142:5-49.
- Ardissone, F. 1883. *Phycologia mediterranea. Parte prima, Floridee*. Mem. Soc. Crittog. Ital. 1:1-516.
- Athanasiadis, A. 2016. *Phycologia Europaea Rhodophyta Vol. II*. Published and distributed by the author, Thessaloniki, pp. 763-1504.
- Barceló, M. C. & Seoane-Camba, J. A. 1989. Variació fenològica d'*Alsidium corallinum* C. Agardh (Rhodomelaceae: Rhodophyta) a les costes de castelló de la Plana I Alacant. *Bull. Inst. Cat. Hist. Nat.* 57:35-39.
- Børgesen, F. 1920. The marine algae of the Danish West Indies. Part 3. Rhodophyceae (6). *Dansk Bot. Ark.* 3:369-504.
- Calaf, R., Barlatier, A., Garçon, D., Balansard, G., Pellegrini, M. & Reynaud, J. 1989. Isolation of an unknown kainic peptide from the red alga *Alsidium helminthocorton*. *J. Appl. Phycol.* 1:257-266.
- Cecere, E., Saracino, O. D. & Petrocelli, A. 2002. Propagules of *Alsidium corallinum* (Rhodomelaceae, Rhodophyta). *Bot. Mar.* 45:580-585.
- Cheung, K. W., Lee, K. Y. & Hodgkiss, I. J. 1984. The occurrence of tetrasporangia and cystocarps on the same thalli in *Polysiphonia harlandii* (Rhodophyta: Ceramiales). *Bot. Mar.* 27:571-572.
- Dawes, C. J. & Mathieson, A. C. 2008. *The seaweeds of Florida*. University Press of Florida, Gainesville, FL, 656 pp.
- Dawson, E. Y. 1959. Changes in Palmyra Atoll and its vegetation through the activities of man, 1913-1958. *Pac. Nat.* 1:3-51.
- Dawson, E. Y. 1963. New records of marine algae from the Galapagos Islands. *Pac. Nat.* 4:3-23.
- De Toni, G. B. 1903. *Sylloge algarum omnium hucusque cognitarum. Vol. IV. Florideae. Sectio III*. Patavii, 775-1525.
- Díaz-Tapia, P. & Bárbara, I. 2013. Seaweeds from sand-covered rocks of the Atlantic Iberian Peninsula. Part 1. The Rhodomelaceae (Ceramiales, Rhodophyta). *Cryptogam. Algol.* 34:325-422.
- Díaz-Tapia, P., Maggs, C. A., West, J. A. & Verbruggen, H. 2017. Analysis of chloroplast genomes and a supermatrix inform reclassification of the Rhodomelaceae (Rhodophyta). *J. Phycol.* 53:920-937.
- Doucette, T. A., Strain, S. M., Allen, G. V., Ryan, C. L. & Tasker, R. A. R. 2000. Comparative behavioural toxicity of domoic acid and kainic acid in neonatal rats. *Neurotoxicol. Teratol.* 22:863-869.

- Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-15.
- Dreckmann, K. M. & Sentiés, G. A. 1994. El alga *Digenea simplex* (Ceramiaceae: Rhodomelaceae) en México: variación biogeográfica. *Rev. Biol. Trop.* 42:443-453.
- Edelstein, T., Bird, C. J. & McLachlan, J. 1974. Tetrasporangia and gametangia on the same thallus in the red alga *Cystoclonium purpureum* (Huds.) Batt. and *Chondria baileyana* (Mont.) Harv. *Br. Phycol. J.* 9:247-250.
- Falkenberg, P. 1901. *Die Rhodomelaceen des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Fauna und Flora des Golfes von Neapel, Monographie* 26. R. Friedländer & Sohn, Berlin, 754 pp.
- Freshwater, D. W. & Rueness, J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33:187-194.
- Gomez, S., Garcia, M. & Gil, N. 2013. Adiciones a la flora marina de Venezuela. I. Rhodomelaceae (Rhodophyta). *Acta Bot. Venez.* 36:183-195.
- Guiry, M. D. & Guiry, G. M. 2018. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: <http://www.algaebase.org>. Accessed Apr 26, 2018.
- Hauck, F. 1885. Die Meeresalgen Deutschlands und Österreichs. In Rabenhorst, L. (Ed.) *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Zweite Auflage. Vol. 2.* Eduard Kummer, Leipzig, pp. 513-575.
- Hernández-González, M. C. & Gil-Rodríguez, M. C. 1994. Existence of plants bearing tetrasporangia and spermatangial receptacles (mixed phases) in the genus *Laurencia* Lamouroux (Rhodomelaceae). *Nova Hedwigia* 59:189-194.
- Hommersand, M. H. 1963. *The morphology and classification of some Ceramiaceae and Rhodomelaceae. University of California Publications in Botany, Vol. 35.* University of California Press, Berkeley, CA, 366 pp.
- Impellizzeri, G., Mangiafico, S., Oriente, G., Piattelli, M. & Sciuto, S. 1975. Amino-acids and low-molecular-weight carbohydrates of some marine red algae. *Phytochemistry* 14:1549-1557.
- Jiang, S., Kuwano, K., Ishikawa, N., Yano, M., Takatani, T. & Arakawa, O. 2014. Production of domoic acid by laboratory culture of the red alga *Chondria armata*. *Toxicon* 92:1-5.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649.
- Kucera, H. & Saunders, G. W. 2012. A survey of Bangiales (Rhodophyta) based on multiple molecular markers reveals cryptic diversity. *J. Phycol.* 48:869-882.
- Kützing, F. T. 1843. *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange.* FA Brockhaus, Leipzig, 458 pp.
- Kützing, F. T. 1849. *Species algarum.* FA Brockhaus, Leipzig, 922 pp.
- Kützing, F. T. 1865. *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. XV.* Gedruckt auf Kosten des Verfassers, Nordhausen, pp. 1-36.
- Kylin, H. 1956. *Die Gattungen der Rhodophyceen.* CWK Gleerups Forlag, Lund, 673 pp.
- Murakami, S., Takemoto, T. & Shimizu, Z. 1953. Studies on the effective principles of *Digenea simplex* Ag. I. Separation of the effective fraction by liquid chromatography. *J. Pharm. Soc. Jpn.* 73:1026-1029.
- Nitta, I., Watase, H. & Tomiie, Y. 1958. Structure of kainic acid and its isomer, allokainic acid. *Nature* 181:761-762.
- Norris, R. E. 1994. Some cumophytic Rhodomelaceae (Rhodophyta) occurring in Hawaiian surf. *Phycologia* 33:434-443.
- Novaczek, I., Bird, C. J. & McLachlan, J. 1987. Phenology and temperature tolerance of the red algae *Chondria baileyana*, *Lomentaria baileyana*, *Griffithsia globifera*, and *Dasya baillouwiana* in Nova Scotia. *Can. J. Bot.* 65:57-62.
- Phang, S. -M., Yeong, H. -Y., Ganzon-Fortes, E. T., Lewmanomont, K., Prathep, A., Hau, L. N., Gerung, G. S. & Tan, K. S. 2016. Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. *Raffles Bull. Zool.* 34:13-59.
- Rambaut, A. 2012. FigTree: Tree Figure Drawing Tool, Version 1.4.2. Available from: <http://tree.bio.ed.ac.uk/>. Accessed Apr 26, 2018.
- Ramsdell, J. S. & Zabka, T. S. 2008. *In utero* domoic acid toxicity: a fetal basis to adult disease in the California sea lion (*Zalophus californianus*). *Mar. Drugs* 6:262-290.
- Saunders, G. W. 2009. Routine DNA barcoding of Canadian Gracilariales (Rhodophyta) reveals the invasive species *Gracilaria vermiculophylla* in British Columbia. *Mol. Ecol. Resour.* 9(Suppl.):140-150.
- Saunders, G. W. & Moore, T. E. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae* 28:31-43.

- Schiffner, V. 1916. Studien über algen des adriatischen Meeres. Helgol. Wiss. Meeresunters. 11:127-198.
- Schmitz, F. & Falkenberg, P. 1897. Rhodomelaceae. In Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen unter Mitwirkung zahlreicher hervorragender Fachgelehrten, Teil 1, Abteilung 2*. Verlag von Wilhelm Engelmann, Leipzig, pp. 421-480.
- Schmitz, F. K. J. 1889. Systematische Übersicht der Bisher Bekannten Gattungen der Florideen. Flora Allg. Bot. Ztg. 72:433-456.
- Schneider, C. W., Hamzeh, B. F., Lane, C. E. & Saunders, G. W. 2018. A new species of *Digenea* (Rhodomelaceae, Ceramiales) based upon a molecular assessment and morphological observations of plants historically known as *D. simplex* in Bermuda. *Phytotaxa* 338:90-98.
- Silva, P. C., Basson, P. W. & Moe, R. L. 1996. *Catalogue of the benthic marine algae of the Indian Ocean*. University of California Publications in Botany. Vol. 79. University of California Press, Berkeley, 1259 pp.
- Silva, P. C., Meñez, E. G. & Moe, R. L. 1987. Catalog of the benthic marine algae of the Philippines. *Smithson. Contrib. Mar. Sci.* 27:1-179.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312-1313.
- Suárez, A. M. 2005. Lista de las macroalgas marinas Cubanas. *Rev. Invest. Mar.* 26:93-148.
- Takemoto, T. & Daigo, K. 1958. Constituents of *Chondria armata* and their pharmacological effects. *Chem. Pharm. Bull.* 6:578-580.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725-2729.
- Taylor, W. R. 1945. *Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands*. Allan Hancock Pacific Expeditions. Vol. 12. University of Southern California Press, Los Angeles, CA, 528 pp.
- Tsuda, R. T. & Abbott, I. A. 1986. Collections, handling, preservation, and logistics. In Littler, M. M. & Littler, D. S. (Eds.) *Handbook of Phycological Methods, Ecological Field Methods: Macroalgae*. Cambridge University Press, Cambridge, pp. 68-85.
- Tsuda, R. T., Fisher, J. R. & Vroom, P. S. 2012. Floristic account of the marine benthic algae from Jarvis Island and Kingman Reef, Line Islands, Central Pacific. *Micronesica* 43:14-50.
- Tsuda, R. T. & Walsh, S. K. 2013. Bibliographic checklist of the marine benthic algae of Central Polynesia in the Pacific Ocean (excluding Hawai'i and French Polynesia). *Micronesica* 2:1-91.
- Vaidya, G., Lohman, D. J. & Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27:171-180.
- Vannajan, S. & Trono, G. C. Jr. 1978. The marine benthic algae of Manila Bay. II. Phaeophyta and Rhodophyta. *Kalikasan, Philip. J. Biol.* 7:7-30.
- Vidal Marques, L., Villaça, R. & Crespo Pereira, R. 2006. Susceptibility of macroalgae to herbivorous fishes at Rocas Atoll, Brazil. *Bot. Mar.* 49:379-385.
- Yoon, H. S., Hackett, J. D. & Bhattacharya, D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 99:11724-11729.