

Halimeda pygmaea and *Halimeda pumila* (Bryopsidales, Chlorophyta): two new dwarf species from fore reef slopes in Fiji and the Bahamas

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Halimeda pygmaea and *Halimeda pumila*, two diminutive calcified green algal species, are described from material collected on Fijian and Bahamian reef slopes, respectively. The species resemble *Halimeda cryptica* in having a single siphon traversing the nodes between subsequent calcified segments and living in sheltered fore-reef slope habitats. They differ from *H. cryptica* by their diminutive size and various anatomical features. Molecular sequence data (*tufA*) underpin the identity of *H. pygmaea* and reveal its phylogenetic position as a sister taxon to *H. cryptica* in the *Halimeda* section *Micronesicae*.

KEY WORDS: Anatomy, Bryopsidales, *Halimeda cryptica*, *Halimeda pumila*, *Halimeda pygmaea*, Molecular phylogeny, Morphology, Taxonomy, *tufA*

INTRODUCTION

Halimeda is one of the most prevalent and ecologically important green algal genera in tropical ecosystems down to depths in excess of 150 m. Its species are important primary producers and structural components of tropical reef ecosystems, harboring an entire range of small animals and epiflora (e.g. Littler *et al.* 1988; Naim 1988; Rossier & Kulbicki 2000). After the algae die, their calcified thalli break up and become part of the lagoonal and fore-reef sediments. *Halimeda* can contribute up to 90% of tropical beach sand and carbonate rock of tropical reefs (e.g. Chapman & Mawson 1906; Drew 1983; Hillis-Colinvaux 1986; Freile *et al.* 1995).

The genus is characterized by a segmented appearance. Thalli are composed of a few to thousands of segments, which often appear whitish-green due to the combination of chlorophyll pigments and inter-siphon calcium carbonate (aragonite) deposits. The segments can take various forms, but are usually flattened and attached to one another like beads on a string. Thalli branch where parent segments bear more than one daughter segment. The uncalcified flexible regions between sequential segments are called nodes.

Like other representatives of the order Bryopsidales, *Halimeda* thalli are composed of a single, giant, multinucleate, tubular cell. This enormous siphonous cell branches and anastomoses to form the characteristic anatomy of *Halimeda*, consisting of a medullary region of sparsely branching, longitudinally oriented siphons and an outer cortical region of siphons that branch more densely and are

oriented perpendicular to the medullary siphons. Cortical siphons are often inflated and, for that reason, are more commonly known as utricles. Segments are connected by the longitudinally oriented medullary siphons.

In most species, medullary siphons fuse just below the nodes and branch out again in the subsequent segment. The pattern of siphon fusion and general thallus habit are the primary characters for distinguishing between groups of species. In the species of section *Micronesicae*, the focus of the current study, there is no siphon fusion. Instead, one or a few siphons run through the nodes without fusing (Verbruggen & Kooistra 2004). To distinguish between species within groups of similar overall appearance, cortical characters such as the number of utricle layers and the shape and size of utricles are important.

The taxonomy of the genus has been studied intensively. Aside from the large body of morpho-taxonomical work, including monographs by Barton (1901), Taylor (1950), Hillis (1959), Hillis-Colinvaux (1980) and Noble (1987), the taxonomy and evolution of the genus have been studied extensively using molecular methods (e.g. Hillis *et al.* 1998; Kooistra *et al.* 2002; Kooistra & Verbruggen 2005; Verbruggen *et al.* 2005a) and combinations of morphometric and molecular work (Verbruggen *et al.* 2005b, c, d, 2006). The main conclusion from these last studies is that species diversity has been severely underestimated by morpho-taxonomic methods alone, primarily because of the existence of pairs of very similar species, which have originated largely independently in the Caribbean and Indo-Pacific basins.

In this study, two new *Halimeda* species are described and their morphologies are contrasted with those of related species. DNA sequence data are presented for one of the

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Table 1. List of specimens in the phylogenetic analysis with species' authors, geographic origins and Genbank accession numbers.

Species	Specimen	Geographic origin	<i>tufA</i>
<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	NA ¹	Philippines: Luzon: Cape Bolinao	AJ512467
<i>Pseudocodium devriesii</i> Weber-van Bosse	KZNB2257	South Africa: KwaZulu Natal: Mission Rocks	EF667068
<i>Halimeda borneensis</i> Taylor	HV183b	French Polynesia: Tahiti: Arue	AM049955
<i>Halimeda copiosa</i> Goreau & Graham	H.0330	Jamaica: Discovery Bay	EF667065
<i>Halimeda cryptica</i> Colinvaux & Graham	H.0237	Jamaica: Discovery Bay	EF667056
	HV483	Jamaica: Priory	EF667057
<i>Halimeda cylindracea</i> Decaisne	SOC364	Yemen: Socotra	AM049956
<i>Halimeda discoidea</i> Decaisne (1)	SOC299	Yemen: Socotra	AY826360
<i>Halimeda discoidea</i> Decaisne (2)	H.0207	Spain: Gran Canaria	AY826361
<i>Halimeda fragilis</i> Taylor	H.0125	Guam: Bile Bay	EF667058
<i>Halimeda gracilis</i> Harvey ex J. Agardh (1)	HV317	French Polynesia: Rangiroa: Avatoru	AY826368
<i>Halimeda gracilis</i> Harvey ex J. Agardh (2)	HV461	Jamaica: St. Ann's Bay	EF667063
<i>Halimeda lacrimosa</i> Howe	H.0308	Bahamas	EF667064
<i>Halimeda micronesica</i> Yamada	WLS184-02	Wallis and Futuna: Wallis Island	EF667059
	WLS420-02	Wallis and Futuna: Wallis Island	AM049964
	H.0014	Australia: Great Barrier Reef	EF667060
	DML40033	Fiji: North Astrolabe Reef	EF667061
<i>Halimeda minima</i> (Taylor) Colinvaux	SOC384	Yemen: Socotra	EF667067
<i>Halimeda monile</i> (Ellis & Solander) Lamouroux	H.0034	Panama: Galeta	AM049962
<i>Halimeda opuntia</i> (Linnaeus) Lamouroux	HV61	French Polynesia: Moorea: Cook Bay	EF667066
<i>Halimeda pygmaea</i> H. Verbruggen, D.S. Littler & M.M. Littler	DML40143	Fiji: Great Astrolabe Reef	EF667062
<i>Halimeda tuna</i> (Ellis & Solander) Lamouroux	H.0113	Italy: Naples	AY826366

¹ NA, not applicable.

new species and its phylogenetic position in the genus is reported. The implications of the discovery of the new species for the sectional subdivision of the genus are discussed.

MATERIAL AND METHODS

Several new collections, especially from *Halimeda* section *Micronesicae*, were studied using standard protocols (Barton 1901; Hillis-Colinvaux 1980; Verbruggen *et al.* 2005b). Measurements were made only on segments from central thallus parts (Verbruggen *et al.* 2005c). Specimens appropriately preserved for DNA analysis were extracted, polymerase chain reaction (PCR) amplified and sequenced as described in Verbruggen *et al.* (2005a). For this study, we focused our efforts on the plastid *tufA* marker. We selected sequences of three species of each section of the genus *Halimeda* as close outgroups and a *Pseudocodium* and a *Caulerpa* species as more distant outgroups (see Table 1 for a complete specimen list, including all species author names). The sequences were all of equal length and could be aligned unambiguously. Maximum likelihood (ML) analysis was carried out in PHYML (Guindon & Gascuel 2003), using the GTR+ Γ +I nucleotide substitution model suggested by the Akaike Information Criterion (MrAIC 1.4; Nylander 2004), BioNJ starting trees and optimization of model parameters during the ML runs. Confidence levels for branches were assessed using the bootstrap method (1000 replicates; Felsenstein 1985). Bayesian inference (BI) of the phylogenetic relationships was carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), using a GTR+ Γ +I model and default priors. Two independent runs of four increasingly heated chains were run for 10⁶

generations, sampled every 1000 generations, and the first 2 10⁵ generations were discarded as burn-in. Uncorrected and GTR+ Γ +I corrected pairwise genetic distances were calculated with PAUP* 4.0b10 (Swofford 2003). For the calculation of GTR+ Γ +I-corrected distances, model parameters were set to the values estimated during the ML run by PHYML.

RESULTS

Halimeda pygmaea H. Verbruggen, D.S. Littler & M.M. Littler, *sp. nov.*

A speciebus congeneribus in siphonibus solitariis vel interdum breviter binatis differt, in siphonibus solitariis vel interdum breviter binatis fissis et tum reunitis in nodis percursis, in siphonibus primariis in mediis segmentorum (55–) 75–100 (–125) μ m latis et in nodis sensim latioribus ad (95–) 110–130 (–150) μ m latis, in segmentis plerumque 3.7 mm angustioribus et 3.8 mm brevioribus, in utriculis peripheralibus (40–) 45–55 (–60) μ m in diametro et (35–) 40–50 (–55) μ m longis.

Differs from its congeners through the combination of the following characters: a single siphon traverses the nodes, although at times this siphon splits just below the node, both parts traverse the node, and join again above; the main siphon is (55–) 75–100 (–125) μ m wide in the center of segments and broadens to (95–) 110–130 (–150) μ m at nodes; the great majority of segments are narrower than 3.7 mm and shorter than 3.8 mm; peripheral utricles are (40–) 45–55 (–60) μ m in diameter and (35–) 40–50 (–55) μ m in height.

ETYMOLOGY: The epithet refers to the Greek mythological word *pygmaio*, meaning "member of a race of dwarfs."

HOLOTYPE: DML40143 (US), collected on the SW side of North Astrolabe Reef, Fiji (S18°40.44', E178°31.13') on 17 February 1996.

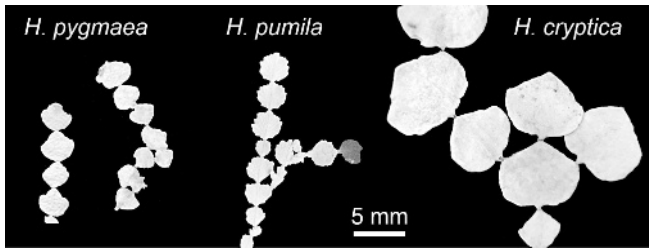


Fig. 1. Thallus fragments of *Halimeda pygmaea*, *H. pumila* and *H. cryptica*, illustrating differences in segment shape and size. Voucher information – *H. pygmaea*: DML40143 (US); *H. pumila*: DML22241 (US); *H. cryptica*: HV945 (GENT).

HABITAT: Hanging from rocks on deep vertical walls or north facing or shaded shallow walls of crevices and caves to 50 m deep.

HABIT AND VEGETATIVE ANATOMY: The exceedingly small thalli (up to 2.5 cm) are pendant from a single point of attachment at the base and consist of a chain of pale green segments. Thalli are sparsely branched in one plane and occasionally have two or three daughter segments originating from the parent segments. Segments are heavily calcified and brittle, (2.3–) 2.5–3.4 (–3.7) mm wide,

(1.8–) 2.2–3.0 (–3.8) mm long and approximately 240 μm thick. Most of the segments have three lobes, are wider than high and are broadest near the middle (Fig. 1). The central lobe is the largest and initiates the next segment. Lateral branches are initiated from the smaller lateral lobes.

The single central siphon that traverses the segments longitudinally is (55–) 75–100 (–125) μm in diameter and widens to (95–) 110–130 (–150) μm diameter at nodes. The central siphon trifurcates several times within each segment; the interval between subsequent trifurcations is short just above the node and gradually lengthens towards the center of the segment (Fig. 2). Except for the first few ramifications of the main siphon, siphons show deep constrictions above the trifurcations, and the two side branches are markedly smaller than the main siphon. In many segments, small siphons branch off the main siphon closely below and/or above the node. These small siphons immediately give rise to the cortical utricles of the nodal zone. In some segments, the first large ramification above the node is unusual in the sense that its side branches bifurcate immediately or after a very short distance, often giving the impression that the main siphon gives rise to five branches, two siphons on each side and the main siphon above (Fig. 2). The central siphon normally goes through

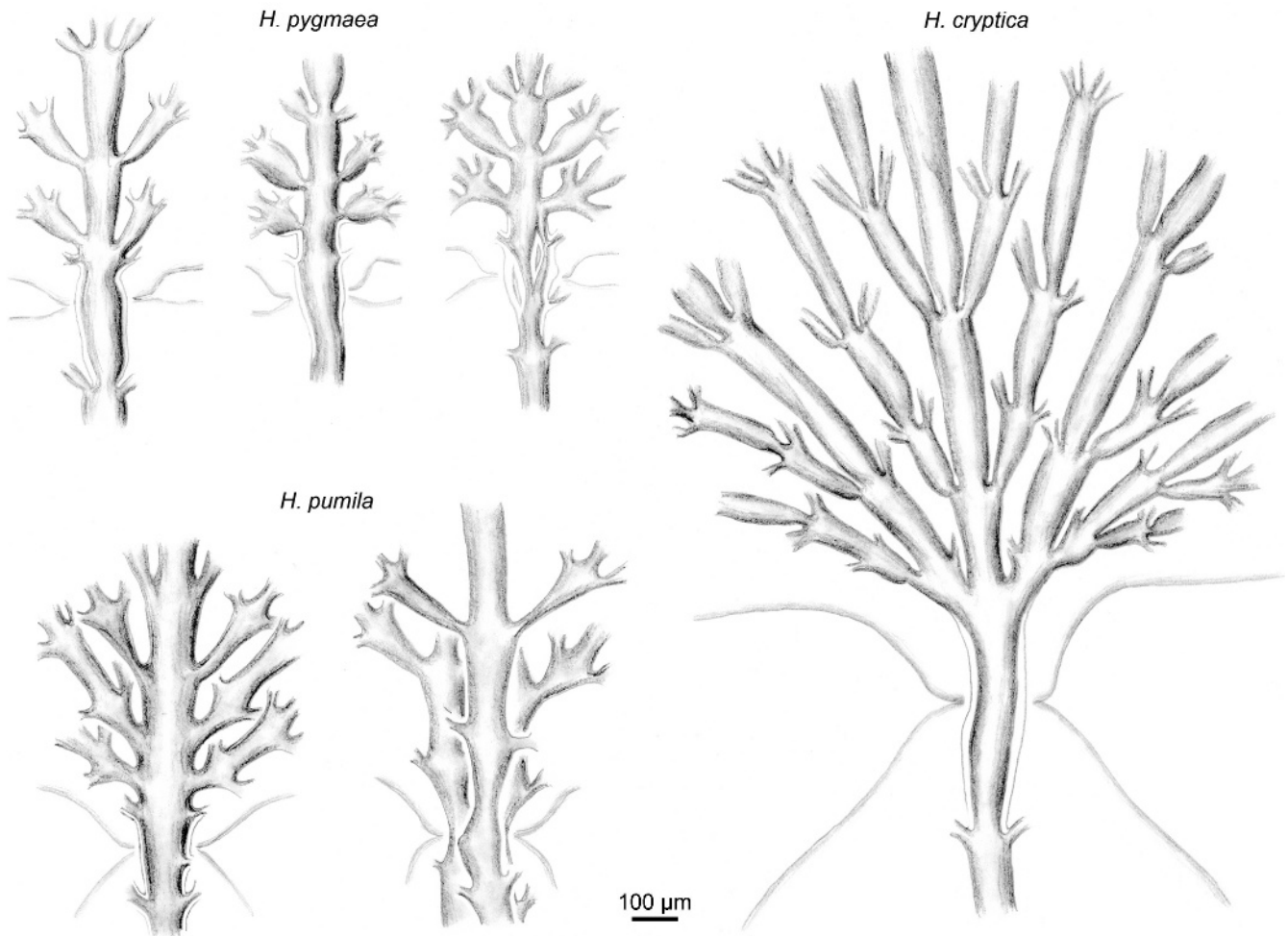


Fig. 2. Structure of the medullar siphons of *Halimeda pygmaea*, *H. pumila* and *H. cryptica*. Voucher information – *H. pygmaea*: DML40143 (US); *H. pumila*: DML22241 (US); *H. cryptica*: HV483 (GENT).

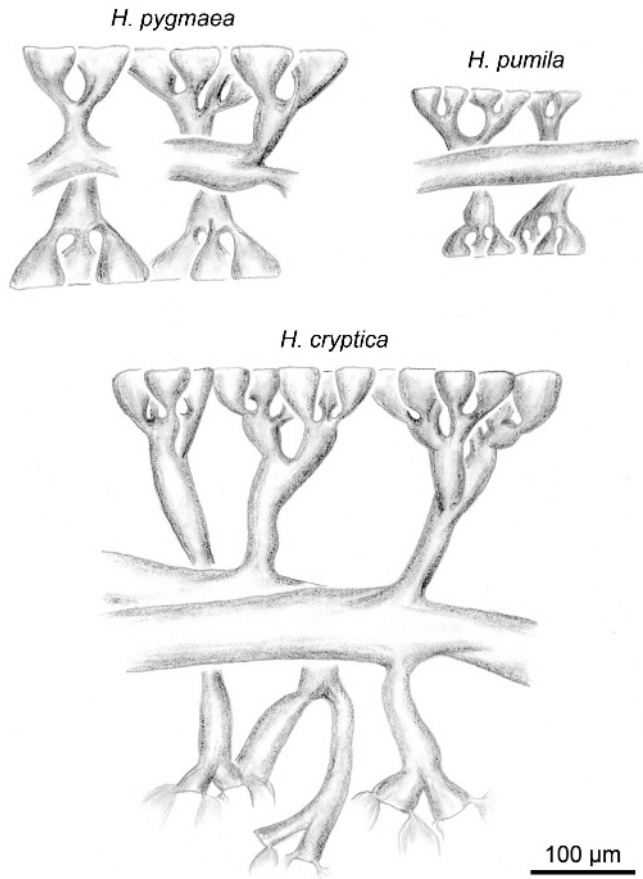


Fig. 3. Structure of the cortical utricles of *Halimeda pygmaea*, *H. pumila* and *H. cryptica*, illustrated as transverse cross-sections through segments. Voucher information – *H. pygmaea*: DML40143 (US); *H. pumila*: DML22241 (US); *H. cryptica*: HV483 (GENT).

the node unchanged (but widened) or splits for a short distance. This split can occur at any point from the last ramification of the central siphon in the parent segment to the first ramification of the daughter segment or occasionally the entire distance (Fig. 2).

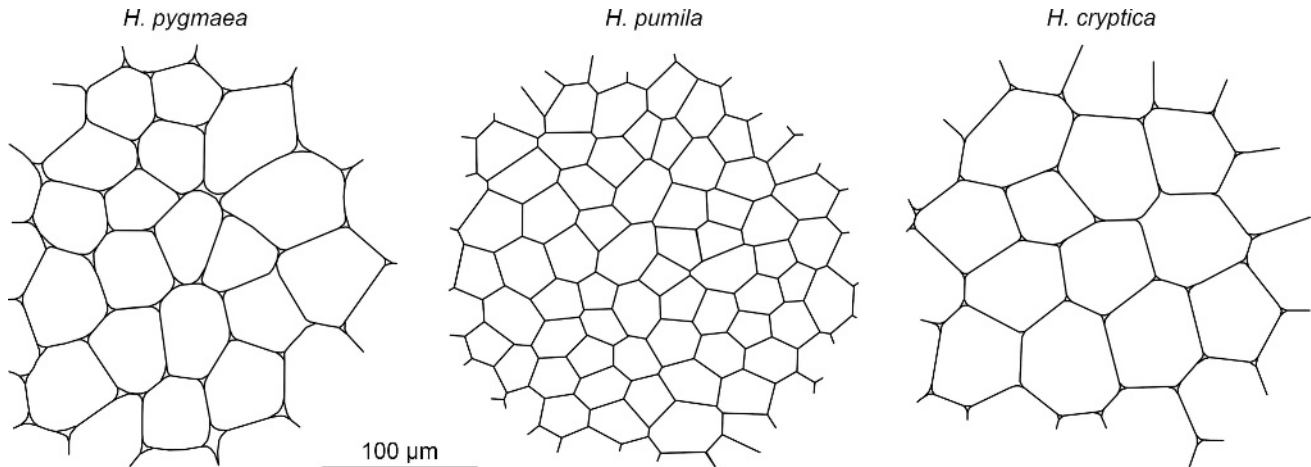


Fig. 4. Surface view of the peripheral utricles of *Halimeda pygmaea*, *H. pumila* and *H. cryptica*. Voucher information – *H. pygmaea*: DML40143 (US); *H. pumila*: DML22241 (US); *H. cryptica*: HV483 (GENT).

The cortex consists of two layers of utricles (Fig. 3). The peripheral utricles are irregularly polygonal in surface view, firmly attached along their sides and rounded at the corners (Fig. 4), (40–) 45–55 (–60) µm in surface diameter and (35–) 40–50 (–55) µm in height. Subperipheral utricles are (20–) 25–50 (–60) µm in diameter and (20–) 25–50 (–60) µm in height supporting 2–3 peripheral utricles.

Halimeda pumila* H. Verbruggen, D.S. Littler & M.M. Littler, *sp. nov.

A species congeneribus in proprietatibus combinatis sequentibus differt, in siphonibus solitariis vel interdum breviter binate fissis et tum reunitis in nodis percursis, in siphonibus primariis in mediis segmentorum (55–) 75–100 (–125) µm latis et in nodis sensim latioribus ad (95–) 110–130 (–150) µm latis, in segmentis plerumque 3.7 mm angustioribus et 3.8 mm brevioribus, in utriculis peripheralibus (40–) 45–55 (–60) µm in diametro et (35–) 40–50 (–55) µm longis.

Differs from its congeners through the combination of the following characters: a single siphon traverses the nodes; the main siphon is (70–) 80–95 (–115) µm wide in the center of segments and narrows to about (50–) 60–90 (–100) µm at nodes; the great majority of segments are narrower and shorter than 3.2 mm; peripheral utricles are (20–) 25–35 (–40) µm in diameter and (20–) 25–35 (–40) µm in height.

ETYMOLOGY: The epithet is Latin for dwarf.

HOLOTYPE: DML22241 (US), collected on the southwest side of San Salvador Island, Bahamas (N24°02.54', W74°32.52') on 15 August 1992. See Littler & Littler (2000, p. 401) for an *in situ* photograph of the holotype (as *H. cryptica* var. *acerifolia* D.L. Ballantine).

HABITAT: Hanging from rocks on deep vertical walls.

HABIT AND VEGETATIVE ANATOMY: The small thalli (up to 2 cm) are sparsely branched in one plane and occasionally have two or three daughter segments originating from the parent segments. Segments are heavily calcified and brittle, (1.6–) 2.4–3.0 (–3.2) mm wide, (1.6–) 2.0–2.9 (–3.2) mm long and approximately 160 µm thick. Most segments are slightly wider than long and broadest just above their middle; lower margins are generally smoothly rounded, whereas the upper and side margins generally have several

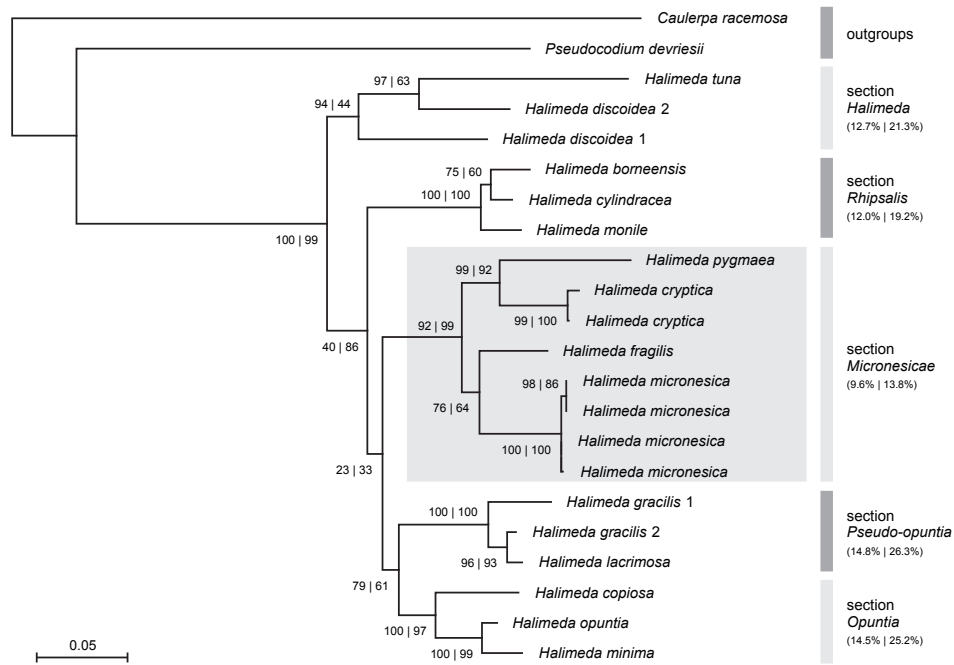


Fig. 5. Maximum likelihood phylogeny of 20 *Halimeda* and two outgroup specimens, showing the distinct nature and affinities of the new species *H. pygmaea*. Node support values are given at each ramification of the tree. Bayesian posterior probabilities are before the bars and maximum likelihood (ML) bootstrap proportions after the bars; both are in percent. The log-likelihood of the tree is -5105.50 . Base frequencies are A = 0.342, C = 0.141, G = 0.218, T = 0.299. The proportion of invariable sites in the alignment is 0.389 and the shape parameter of the gamma distribution of among-site rate heterogeneity is 0.652. The substitution rates are AC = 4.202, AG = 3.180, AT = 1.345, CG = 1.109, CT = 6.932 relative to GT = 1. The percentages beneath section names represent the maximum genetic distance between species belonging to that section, calculated as uncorrected p-distances (before bars) and GTR+ Γ +I corrected distances (after bars).

shallow lobes (Fig. 1). The central lobe is the largest and initiates the next segment. Lateral branches are initiated from the smaller lateral lobes.

A single central siphon, measuring (70–) 80–95 (–115) μm in diameter, traverses the segments longitudinally. At nodes, the siphon is generally narrower than in the center of the segment, measuring (50–) 60–90 (–100) μm in diameter, and traverses the node unchanged. The central siphon trifurcates several times within each segment; the interval between subsequent trifurcations is short just above the node and gradually lengthens towards the center of the segment (Fig. 2). The main siphon does not constrict above the first few ramifications above the node, yet other siphons show deep constrictions above trifurcations, and the two side branches are markedly smaller than the main siphon. In a few segments, small siphons that immediately give rise to cortical utricles branch off the main siphon closely below and/or above the node.

There are two layers of cortical utricles (Fig. 3). The peripheral utricles are irregularly polygonal in surface view, firmly attached along the sides with angular corners (Fig. 4), (20–) 25–35 (–40) μm in surface diameter and (20–) 25–30 (–40) μm in height. Subperipheral utricles are (8–) 10–20 (–25) μm in diameter and (10–) 15–35 (–50) μm in height supporting 2–3 peripheral utricles.

MOLECULAR PHYLOGENETICS: The phylogenetic tree inferred from *tufA* sequence data (Fig. 5) shows the evolutionary relationships of 20 *Halimeda* species. Support values for the genus and its sections are high, except for section *Halimeda*. The relationships among most sections

receive no or meager support. The new species *H. pygmaea* is recovered within section *Micronesicae*. It has a discrete *tufA* signature separating it from all other species of the section. The Caribbean species *H. cryptica* was inferred to be the sister species of *H. pygmaea*. The uncorrected genetic distance between these species is approximately 7.5%.

DISCUSSION

The data we have gathered provide strong evidence of the existence of two new dwarf species in *Halimeda* section *Micronesicae*. *Halimeda pumila* and *H. pygmaea* differ from *Halimeda cryptica* and each other in a variety of morphological characters (Table 2; Figs 1–4). The most obvious difference between *H. cryptica* and the two new species is their size, *H. cryptica* being much larger than the other two. Whereas *H. cryptica* grows to about 10 cm, *H. pumila* and *H. pygmaea* rarely reach more than 3 cm. The segments of *H. cryptica* are much larger than those of the other two species (Table 2) and often are keeled with the two wings slightly facing downwards (towards the substrate), whereas those of *H. pumila* and *H. pygmaea* are flat. *Halimeda cryptica* has multiple dominant siphons radiating from the base of each segment (Fig. 2) and its cortex differs on the upper and lower faces of the segments (Fig. 3; see also Colinvaux & Graham 1964). In contrast, *H. pygmaea* and *H. pumila* have a single, lengthwise dominant siphon with markedly smaller side-branches and the same pattern of cortication on both segment faces. Lastly, the dominant

Table 2. Comparative table of measurements.

Character	<i>H. pygmaea</i>	<i>H. pumila</i>	<i>H. cryptica</i>
Segment width	(2.3–) 2.5–3.4 (–3.7) mm	(1.6–) 2.4–3.0 (–3.2) mm	(6.3–) 7.5–11.0 (–11.6) mm
Segment length	(1.8–) 2.2–3.0 (–3.8) mm	(1.6–) 2.0–2.9 (–3.2) mm	(5.5–) 6.0–8.0 (–9.0) mm
Siphon diameter at node	(95–) 110–130 (–150) μ m	(50–) 60–90 (–100) μ m	(200–) 210–260 (–290) μ m
Siphon diameter in center of segment	(55–) 75–100 (–125) μ m	(70–) 80–95 (–115) μ m	(95–) 110–170 (–180) μ m
Diameter peripheral utricles	(40–) 45–55 (–60) μ m	(20–) 25–35 (–40) μ m	(45–) 50–70 (–75) μ m
Height peripheral utricles	(35–) 40–50 (–55) μ m	(20–) 25–30 (–40) μ m	(40–) 50–65 (–75) μ m
Diameter subperipheral utricles	(20–) 25–50 (–60) μ m	(8–) 10–20 (–25) μ m	(25–) 30–55 (–85) μ m
Height subperipheral utricles	(20–) 25–50 (–60) μ m	(10–) 15–35 (–50) μ m	(35–) 40–75 (–85) μ m

siphon is narrower in *H. pumila* and *H. pygmaea* than in *H. cryptica* (Fig. 2).

Because of the similar-sized thalli and segments, the differences between the two new species are less obvious. A primary difference lies in the shape of the segments, which are generally trilobed in *H. pygmaea*, and rounded with several shallow lobes in *H. pumila*. Anatomical differences are present in the medullary siphons as well as in the cortex. Although the main siphon is about the same diameter in the segments of both species, it widens considerably at the nodes in *H. pygmaea*, whereas in *H. pumila* it is thicker in the center of the segment rather than at the node. In this respect, *H. pygmaea* resembles *H. cryptica*. Secondly, *H. pumila* has considerably smaller peripheral utricles than *H. pygmaea*, and they have angular corners in surface view whereas those of *H. pygmaea* are rounded (Fig. 4).

Ballantine (1982) described *Halimeda cryptica* var. *acerifolia*, a form of *H. cryptica* with maple-leaf shaped segments that show slight resemblance to the segments of *H. pumila*. Segments of *H. cryptica* var. *acerifolia* are slightly smaller than those of typical *H. cryptica* but still outside the range of *H. pumila*. Similarly, the dominant siphon and cortical utricles of variety *acerifolia* are slightly smaller than those of typical *H. cryptica*, but still much larger than those of *H. pumila*. As in typical *H. cryptica*, segments of the *acerifolia* variety are keeled. The cortication of the upper and lower surfaces was not described in detail by Ballantine (1982). Even though the segment size of the *H. cryptica* specimens previously reported from San Salvador Island (Bahamas) are smaller than the specimens we have examined, they represent true *H. cryptica* and are distinct from *H. pumila* in segment shape and anatomical characters (Blair and Norris 1988). Another dwarf species, *H. hummii*, described originally from Puerto Rico (Ballantine 1982) but presumably more widespread in the Caribbean Sea (Wysor & Kooistra 2003), belongs to *Halimeda* section *Halimeda*. It is similar in size to the two new species but differs from both by its obvious nodal fusions.

The two new *Halimeda* species and *H. cryptica* all grow on vertical walls along deep reef slopes. They are attached by a single rhizoidal holdfast at the base of the basal segment and dangle from the vertical walls on which they grow. *Halimeda cryptica*, the best-studied species, forms large populations that, together with *H. copiosa*, drape the rocky slopes between 30 and 50 m deep along the north coast of Jamaica (Colinvaux & Graham 1964; Littler & Littler 2000). It has been reported to occur down to 152 m in the Bahamas, which is the current depth record for the

genus (Littler *et al.* 1986; Blair & Norris 1988). The type specimens of *H. pygmaea* and *H. pumila* were collected from fore reef slope habitats at approximately 50 m depth in Fiji and 40 m depth in the Bahamas, respectively. *Halimeda pygmaea* also grows on shallower shaded reef walls, but we have never observed it shallower than about 10 m. This deep habitat, where the physical forces of the ocean are much attenuated, probably has allowed the evolutionary reduction from multiple siphons to a single siphon traversing the node (Kooistra *et al.* 2002; Verbruggen & Kooistra 2004).

The taxonomy of *Halimeda* has been studied intensively during the past five years, and one of the main conclusions has been that there are extensive gray zones between the 'traditional' species, which are defined using the morphological species concept. In *Halimeda* sections *Halimeda* and *Rhipsalis*, application of molecular and specialized morphometric techniques has been necessary to form a clear image of species boundaries (Verbruggen *et al.* 2005a, d). This stands in sharp contrast with section *Micronesicae*, which is investigated in this paper. Species demarcation in this section is evident, even without application of molecular taxonomic techniques. Species differ from each other in several aspects and, for many of the qualitative diagnostic characters, there is no overlap between the species. In this context, it should be noted that we had a limited number of specimens of the new dwarf species at our disposal, and the morphological range of these species may still expand somewhat with the discovery of additional material.

Morphological affinities between species in section *Micronesicae* are reflected in their molecular phylogenetic relationships. The main diagnostic difference between the *H. pygmaea/cryptica* and the *H. fragilis/micronesica* clades is the number of siphons traversing the node; whereas *H. pygmaea* and *H. cryptica* have a single siphon, *H. fragilis* and *H. micronesica* have multiple intra-nodal siphons. The *H. fragilis/micronesica* clade is further characterized by unique, enlarged basal segments giving rise to multiple branches. In *H. micronesica*, the basal segment is fan-shaped; that of *H. fragilis* is funnel-shaped. Basal segments in the *H. pygmaea/cryptica* clade are minute and bear no more than 1 to 3 daughter segments. Unfortunately, we were unable to generate molecular data for *H. pumila* because there was no material suitably preserved for molecular phylogenetic work. The single siphon going through most of its nodes suggest it is related to *H. cryptica* and *H. pumila*, but the fact that it has significantly smaller utricles sets it apart. The presence of occasional nodes with

2–3 siphons suggests that *H. pumila* may be related to *H. fragilis* and *H. micronesica*. In our opinion, the most probable options are that *H. pumila* is either sister to the *H. pygmaealcryptica* clade or represents a separate clade branching off early in section *Micronesicae*. Former molecular phylogenetic studies based on 18S rDNA and ITS (Kooistra *et al.* 2002; Verbruggen & Kooistra 2004; Verbruggen *et al.* 2005b) recovered *H. cryptica* within the *H. fragilis/micronesica* clade (as sister to *H. fragilis*). However, Kooistra *et al.* (2002) pointed out that having *H. cryptica* sister to *H. micronesica/fragilis* would require only a single extra step. Our reanalysis of the 18S–ITS data of Kooistra *et al.* (2002), including a sequence of *H. pygmaea*, resulted in distinct *H. fragilis/micronesica* and *H. pygmaealcryptica* clades (unpublished data).

The description of two new species related to *Halimeda cryptica* raises the question of whether *Halimeda* section *Crypticae*, created by Hillis-Colinvaux (1980) and subsumed in section *Micronesicae* by Verbruggen & Kooistra (2004), should be reinstated. Hillis-Colinvaux (1980) argued that *H. cryptica* stood apart from other species of the genus by the single siphon traversing the node. Her section *Micronesicae*, which we will call section *Micronesicae sensu stricto*, grouped the species with multiple unfused siphons traversing the node. Phylogenetic studies of the 18S rDNA and ITS regions placed *H. cryptica* within section *Micronesicae* s.s., suggesting that the single nodal siphon in *H. cryptica* is simply a reduction from the situation in section *Micronesicae* s.s. (Kooistra *et al.* 2002). These observations led to the definition of an expanded section *Micronesicae* (*sensu lato*), including *H. cryptica* (Verbruggen & Kooistra 2004). In our phylogenetic analyses of the *tufA* gene, *H. cryptica* and *H. pygmaea* form a distinct lineage with high support, sister to a less well-supported clade containing *H. micronesica* and *H. fragilis*, the two species that fit in the original definition of section *Micronesicae* (Hillis-Colinvaux 1980). With this information at hand, one could argue for reinstatement of section *Crypticae*. However, a number of observations argue against this. First, the clade of section *Micronesicae* s.l. sits on a long branch in our *tufA* phylogeny and receives strong support, whereas both subclades sit on short branches and section *Micronesicae* s.s. receives meager statistical support. Second, divergence levels of the *tufA* marker within section *Micronesicae* s.l. are smaller than those within other sections of the genus (Fig. 5). Third, we have observed nodes with a single siphon as well as three unfused siphons in a single *H. pumila* specimen (Fig. 2), suggesting that there is no strict developmental distinction between these nodal patterns. In conclusion, retaining *H. cryptica*, *H. pygmaea* and *H. pumila* in a broader section *Micronesicae* seems to be the most sensible solution.

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