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Morphological characterization of lineages within the calcified tropical seaweed genus *Halimeda* (Bryopsidales, Chlorophyta)

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Halimeda Lamouroux constitutes a genus of calcified and segmented green seaweeds within the Bryopsidales. Molecular phylogenetic assessments have uncovered five principal monophyletic lineages within the genus. In the present study we define these lineages morphologically. We gathered morphological data from specimens used in the molecular analyses as well as from collections having a similar morphology and originating from the same geographical region. Starting from the lineages and their morphological synapomorphies, we define and illustrate five natural sections within *Halimeda*. All or most medullary siphons traversing the nodes between segments fuse into a single unit in specimens of lineage 1 (section *Rhipsalis*), and segments at the thallus base fuse with one another. Medullary siphons of specimens in lineage 2 (section *Micronesicae*) traverse the node without fusing. Medullary siphons of specimens in lineage 3 (section *Halimeda*) divide frequently below the nodes and become entangled among one another. The segments of specimens in this lineage possess a continuous uncorticated band along the distal perimeter instead of three or more pits as encountered in segments of specimens in all other lineages. Members of lineage 4 (section *Pseudo-opuntia*) possess club-shaped subperipheral utricles in their cortical region. Medullary siphons of specimens in lineage 5 (section *Opuntia*) fuse over only a short distance at the nodes and retain their identity. Apart from these synapomorphies, the lineages can be delimited further by a characteristic combination of symplesiomorphies and homoplasies. In addition we examined the morphology of *H. bikinensis* Taylor, a species not included in the molecular analyses, and discuss its ambiguous position in our sectional system.

Key words: *Halimeda*, morphology, sections, phylogeny, SSU rDNA, taxonomy

Introduction

The green calcified seaweed genus *Halimeda* Lamouroux, (1812) (Bryopsidales, Chlorophyta) occurs in reefs and lagoons across the tropics and subtropics (Barton, 1901; Taylor, 1950; Tsuda & Wray, 1977; Dong & Tseng, 1980; Hillis-Colinvaux, 1980, 1988; Drew & Abel, 1988; Tsuda & Kamura, 1991; Drew, 1995; Littler & Littler, 2000; Bandeira-Pedrosa *et al.*, 2001). The characteristically segmented thalli are composed of ramifying siphons forming a medulla and a surrounding cortex (Barton, 1901; Hillis-Colinvaux, 1980). The siphons in the medulla string segments together and ramify into the cortex. There they rebranch frequently and terminate in a layer of inflated peripheral utricles. The latter adhere to one another and so enclose the segment's intersiphonal spaces (Barton, 1901; Hillis-Colinvaux, 1980). There, calcium carbonate precipitates as aragonite (Borowitzka & Larkum, 1977). Some medullary siphons surface in weakly calcified regions along the segment's distal perimeter where they adhere and may fuse. New segments (Hay *et al.*, 1988), secondary holdfasts (Hillis-Colinvaux, 1980; Walters & Smith, 1994) or gametophores bearing bladder-like gametangia (Gepp, 1904; Kamura, 1966; Graham, 1975; Drew & Abel, 1988) develop from their tips. Thalli propagate clonally by means of 'runner' rhizoids (Hillis-Colinvaux, 1980) or fragmentation (Walters & Smith, 1994; Walters *et al.*, 2002). Sexual reproduction occurs periodically; the gametes are released in concert in species-specific short intervals (Meinesz, 1980; Drew & Abel, 1988; Clifton, 1997; Clifton & Clifton, 1999).

The genus currently comprises 34 described extant species and several fossil taxa (Braga *et al.*, 1996; Schlagintweit & Ebli, 1998; Hillis, 2000). All extant species and their taxonomic authorities are listed in Table I. Hillis-Colinvaux (1980) proposed five sections within the extant diversity based predominantly on patterns of medullary siphon anatomy at nodes between segments (Askenasy,

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Table 1. List of currently recognized *Halimeda* species and their taxonomic authorities. Species indicated with an asterisk were not examined in this study

H. bikinensis	Taylor
H. borneensis	Taylor
H. copiosa	Goreau & Graham
H. cryptica	Colinvaux & Graham
H. cuneata	Hering
H. cylindracea	Decaisne
H. discoidea	Decaisne
H. distorta	(Yamada) Colinvaux
H. favulosa	Howe
H. fragilis	Taylor
H. gigas	Taylor
H. goreauii	Taylor
H. gracilis	Harvey *ex* J. Agardh
H. howensis	Kraft & Noble*
H. hummii	Ballantine
H. incrassata	(Ellis) Lamouroux
H. lacrimosa	Howe
H. lacunalis	Taylor
H. macroloba	Decaisne
H. macrophysa	Askenasy
H. magnidisca	Noble
H. melanesica	Valet
H. micronesica	Yamada
H. minima	(Taylor) Colinvaux
H. monile	(Ellis & Solander) Lamouroux
H. opuntia	(Linnaeus) Lamouroux
H. renschii	Hauck
H. scabra	Howe
H. simulans	Howe
H. stuposa	Taylor
H. taenicola	Taylor
H. tuna	(Ellis & Solander) Lamouroux
H. velasquezii	Taylor
H. xishaensis	Dong & Tseng*

1888; Barton, 1901). These patterns often conflict with distributions of character states associated with utricle morphology and branching modes as well as with thallus habit across the taxa (Kooistra *et al*., 2002). Results of molecular phylogenetic studies in Kooistra *et al*. (2002) indicate that most sections *sensu* Hillis-Colinvaux are not monophyletic.

The principal goals of this study are to demarcate monophyletic sections within *Halimeda* and to uncover their defining morphological traits. A morphological definition of these natural groups not only provides a helpful tool towards accurate identification of species but also allows, at least tentatively, placement of relatively recent fossil specimens in these sections. To achieve our goals, we inferred a maximum likelihood phylogeny from nuclear rDNA sequences of specimens across the taxonomic diversity and demarcated principal lineages therein. We then examined morphology and anatomy of the specimens included in the phylogeny in search of those traits whose states define one or more of these lineages. In addition, we included specimens in the morphological analyses for which no sequences were available but we used the latter specimens only to ascertain their fit into sections, not to redefine the sections.

Materials and methods

A list of specimens, together with their taxonomic identifications, herbarium codes and the GenBank accession numbers for their partial nuclear rDNA sequences is presented in Table 2. Details of preservation, taxonomic identification, DNA extraction, PCR and sequencing protocols can be found in Kooistra *et al*. (2002). The 155 specimens of *Halimeda* used in this study were attributable to 32 of 34 currently recognized species (Table 1). All 49 specimens used for molecular analyses in this study as well as those used in previous publications on *Halimeda* by Kooistra and co-workers (Kooistra *et al*., 2002) are deposited in the GENT herbarium.

Phylogenetic analyses of the alignment were carried out using PAUP* version 4.0.b10 (Swofford, 2002). In all analyses, ambiguities were treated as uncertainties and gaps as missing data. Sequences of *Udotea flabellum* (Ellis & Solander) Howe and *Penicillus capitatus* Lamarck were used as the outgroup (Kooistra, 2002; Kooistra *et al*., 2002).

Hierarchical likelihood ratio tests (hLRT's) were performed using Modeltest v3.06 (Posada & Crandall, 1998). Resulting optimal parameters were then used to constrain maximum likelihood (ML) analysis. The ML analysis was carried out under the heuristic search option and tree bisection/reconnection branch swapping and was constrained using optimal hLRT parameter settings. Weighted (K = 2; Goloboff, 1993) maximum parsimony (MP) analysis was carried out under the heuristic search option and tree bisection/reconnection branch swapping. Bootstrap analyses (1000 replicates) were performed in weighted MP under the same settings.

Morphological analysis was also carried out on specimens used in the molecular analysis unless, in a few cases, not enough material was available. In that case, specimens unambiguously belonging to the same species and coming from the same geographical region were used. Additional specimens, for which no sequences were available, have also been examined (Table 2). Thallus and segment characteristics were noted. Anatomical details were gathered by dissection of segments as described in Hillis-Colinvaux (1980) with the following modifications. The cortex was sectioned following decalcification. The medullary and nodal regions therein were examined after decalcification and removal of the surrounding cortical parts. In those cases where all nodal siphons fused into a single aggregate, nodal structures were also sectioned lengthwise. Scraped-off cortex fragments were used to examine segment surface. Observations on cortical structures were done using a slide with a cavity, allowing a better 3D impression. Camera lucida drawings were made using an Olympus BX51 microscope (Olympus, Tokyo, Japan).

Results

Hierarchical likelihood ratio tests performed on the sequence data set favoured a general-time-reversible base substitution model with estimated values for the following parameters: base frequencies: A = 0.206, C = 0.271, G = 0.305, T = 0.218; substitution rates: A↔C = 1.211, A↔G = 1.890, A↔T = 1.524, C↔G = 0.653, C↔T = 3.525 relative to G↔T = 1.000; proportion of invariable sites = 0.550; gamma shape parameter = 0.455. The tree resulting from our ML analysis constrained with these parameters is presented in Fig. 1. The topology is highly similar to those in Kooistra *et al.* (2002). The tree-topology resulting from weighted MP analysis (not shown) differed only in a single aspect from that in Fig. 1: *H. hummii* and *H. lacunalis* did not form a clade. Five principal lineages marked in Fig. 1 obtained high bootstrap support as did the clade containing lineages 4 and 5. Yet, the basal clades grouping these lineages obtained poor or insufficient support as in Kooistra *et al.* (2002). All sequence pairs belonging to the same morphologically defined species obtained high bootstrap support.

Figs 2 to 42 illustrate the general morphology and anatomical characters of specimens in each of the five lineages.

Lineage 1, Figs 2 – 10.

Western Atlantic (Caribbean region): *H. favulosa, H. incrassata, H. monile, H. simulans*
Indo-Pacific basin: *H. borneensis, H. cylindracea, H. incrassata, H. macroloba, H. melanesica.*

Most specimens were anchored in sandy substrata by means of a bulbous holdfast (Figs 2, 3). Lower segments were large and barrel-shaped and the walls of their cortical siphons were strongly thickened thus giving rise to a stiff, stipe-like structure. In many species, segments on top of this so-called pseudo-stipe were moderately calcified, enlarged and partially fused in a fan- or squat-pillar-like structure (Fig. 2). *Halimeda melanesica* was also recovered in lineage 1, yet it lacked a bulbous holdfast and a pseudo-stipe. Nonetheless, the lowermost segments were also considerably larger than those in the upper region of the thallus. This species was encountered on wave-affected rock and rubble.

Nodes connecting segments in the middle thallus region possessed relatively thick-walled medullary siphons connecting with all their immediate neighbours by means of pores (Figs 4, 5, 6) thus giving rise to a single pack of interconnected medullary siphons. Notably, siphons did not fuse at the nodes in partially fused (basal) segments of *H. borneensis* and *H. macroloba*.

The cortex was dense and, depending on the species and the location of the examined segment in the thallus, consisted of three to many layers of moderately inflated utricles (Figs 9, 10). In general, peripheral utricles were irregularly polygonal in surface view (Figs 7, 8).

Lineage 2, Figs 11 – 17.

Western Atlantic (Caribbean region): *H. cryptica*
Indo-Pacific basin: *H. fragilis, H. micronesica*

The specimens of Indo-Pacific species were found in wave-affected biotopes (mostly *H. micronesica*), on shallow reef slopes and in channels with strong tidal currents (both *H. fragilis* and *H. micronesica*). Our specimens of *H. cryptica* originated from deep (> 25m) cliffs facing the open sea. Segments of lineage 2 specimens appeared strongly calcified and brittle with flexible nodes. The specimens belonging to *H. fragilis* and *H. micronesica* were dull greyish green whereas those of *H. cryptica* were grass green on the segment side facing the light and white on the opposite side.

Specimens from this lineage possessed a single huge nodal siphon (*H. cryptica*) or several smaller ones passing through the nodes without fusion (*H. fragilis* and *H. micronesica*, Figs 13, 14).

The cortex was relatively thin and consisted of a series of cylindrical utricles gradually becoming longer and broader from the periphery inwards (Fig. 17). Both Indo-Pacific species possessed primary utricles separating completely on decalcification of the segment and being round in surface view (Fig 16). In contrast, the peripheral utricles of *H. cryptica* adhered to each other and were irregularly polygonal in surface view (Fig. 15).

Lineage 3, Figs 18 – 27.

Atlantic: *H. discoidea, H. hummii, H. scabra* Mediterranean *H. tuna*, Western Atlantic *H. tuna*
Indo-Pacific basin: *H. discoidea, H. gigas, H. lacunalis, H. macrophysa, H. magnidisca, H. taenicola*
Indo-Pacific and possibly Brazil: *H. cuneata* (Bandeira-Pedrosa *et al.*, 2001)

Specimens of this lineage were found in semi-sheltered to exposed biotopes. In general, the thallus attached to hard substrata by means of a felt-like, discoid holdfast. Two major thallus morphologies were encountered: *Halimeda lacunalis, H. hummii* and *H. cuneata* possessed smooth, small and moderately calcified segments with flexible nodes whereas others such as *H. discoidea,*

Table 2. List of *Halimeda* specimens used in this study

Species	Specimen number	Voucher	Geographical location	GenBank
H. borneensis	HV18		Zanzibar, Tanzania (WI)	
″	HEC12603a		Zanzibar, Tanzania (WI)	
″	HEC12603b	99 – 128	Zanzibar, Tanzania (WI)	AF525559
″	Snellius-II 10101		Maisel Islands, Indonesia (WP)	
″	PH534		Mindanao, Philippines (WP)	
″	H.0267	99 – 138	New Caledonia (WP)	AF525550
H. stuposa	L0238148 (L)		Marshall Islands (CP)	
″	L0238149 (L)		Marshall Islands (CP)	
″	WRT46-591 (MICH)		Marshall Islands (CP)	
H. melanesica	HV22		Zanzibar, Tanzania (WI)	
″	L0238145 (L)	L0238145	Taka Garlarang, Indonesia (WP)	AF407237
H. incrassata IP	PH194		Cebu, Philippines (WP)	
″	PH197	99-073	Cebu, Philippines (WP)	AF407241
″	HV146		Moorea, French Polynesia (CP)	
″	H.0019	98 – 117	Great Barrier Reef, Australia (WP)	
″	H.0035	99 – 001	Tahiti, French Polynesia (CP)	
″	H.0040	99 – 009	Rangiroa, French Polynesia (CP)	
″	H.0045	99 – 021	Rangiroa, French Polynesia (CP)	AF525573
H. macroloba	HV5		Zanzibar, Tanzania (WI)	
″	HEC12583		Zanzibar, Tanzania (WI)	
″	H.0157	98 – 017	Pangasinan, Philippines (WP)	AF525560
″	H.0228	97 – 486	Exmouth, W Australia (EI)	
″	H.0038	99 – 006	Tahiti, French Polynesia (CP)	AF525563
″	HV183		Tahiti, French Polynesia (CP)	
″	HV206		Tahiti, French Polynesia (CP)	
H. cylindracea	HV323		East Sinai, Egypt (RS)	
″	SOC364	99 – 030	Socotra, Yemen (WI)	AF525546
″	HEC7612		Madang, Papua New Guinea (WP)	
″	H.0018	98 – 105	Great Barrier Reef, Australia (WP)	AF525548
H. simulans	H.0032		Galeta, Panama (CAR)	
″	H.0071	97 – 071	Bocas del Toro, Panama (CAR)	
″	H.0367	97 – 089	Panama (CAR)	AF407235
H. incrassata CAR	H.0179	99 – 087	Bahamas (CAR)	AF407233
″	H.0180	99 – 084	Florida, USA (CAR)	
″	H.0181	99 – 083	Florida, USA (CAR)	AF525537
H. favulosa	L0351088 (L)		Bahamas (CAR)	
H. monile	H.0145	98 – 100	Florida, USA (CAR)	
″	HOD-RD2.02-65		Dominican Republic (CAR)	
″	HOD-RD2.02-50		Dominican Republic (CAR)	
″	H.0228	98 – 034	Yucatan, Mexico (CAR)	AF407234
H. cryptica	H.0237	97 – 482	Discovery Bay, Jamaica (CAR)	AF407244
″	HV401		St. Ann's Bay, Jamaica (CAR)	
″	HV483		Priory Bay, Jamaica (CAR)	
H. micronesica	HV4		Zanzibar, Tanzania (WI)	
″	SEY484		Poivre Atoll, Seychelles (CI)	
″	no voucher	99 – 050	Great Barrier Reef, Australia (WP)	AF407243
″	H.0014	98 – 110	Great Barrier Reef, Australia (WP)	
″	WLS184-02		Wallis Island (CP)	
″	WLS420-02		Wallis Island (CP)	
H. fragilis	HEC14230		Mnazi Bay, Tanzania (WI)	
″	HV53		Mnazi Bay, Tanzania (WI)	
″	PH316		Luzon, Philippines (WP)	
″	HEC10230		Motupore, Papua New Guinea (WP)	
″	H.0125	99 – 092	Bile Bay, Guam (WP)	AF407245
″	WRT46-394 (MICH)		Marshall Islands (CP)	
H. hummii	H.0002		Galeta, Panama (CAR)	
″	H.0232	99 – 052	Portobelo, Panama (CAR)	
″	H.0235	99 – 107	Isla Mamey, Panama (CAR)	AF525582
″	H.0251	98 – 164	Portobelo, Panama (CAR)	
″	H.0253	98 – 053	San Blas, Panama (CAR)	AF525581
H. discoidea ATL	H.0138	99 – 187	Isla Grande, Panama (CAR)	
″	H.0144	99 – 105	Florida, USA (CAR)	
″	H.0207	97 – 547	Gran Canaria, Canary Islands (EA)	AF407249

(*continued*)

Table 2. (*continued*)

Species	Specimen number	Voucher	Geographical location	GenBank
″	H.0209	98 – 052	Sao Vicente, Cape Verde (CA)	
H. tuna MED	H.0113	99 – 043	Naples, Italy (MED)	AF407250
″	HV54		Ischia Island, Italy (MED)	
″	HV319		Rosas, Spain (MED)	
H. tuna ATL	H.0074	97 – 069	Panama (CAR)	AF525589
″	H.0140	99 – 189	Panama (CAR)	
″	H.0231	98 – 038	Puerto Morelos, Mexico (CAR)	AF407248
H. scabra	L0351081 (L)		Florida, USA (CAR)	
″	L0351084 (L)		Florida, USA (CAR)	
H. lacunalis	H.0118	99 – 095	Bile Bay, Guam (WP)	
″	H.0121	99 – 101	Agat Bay, Guam (WP)	AF525579
″	WRT46-21 (MICH)		Marshall Islands (CP)	
″	WRT46-424 (MICH)		Marshall Islands (CP)	
H. magnidisca	SOC252	99 – 031	Socotra, Yemen (WI)	AF525595
″	SOC348	99 – 028	Socotra, Yemen (WI)	AF525596
″	SOC385		Socotra, Yemen (WI)	
H. discoidea IP	HV3		Zanzibar, Tanzania (WI)	
″	SOC299	99 – 032	Socotra, Yemen (WI)	AF407254
″	H.0041	99 – 014	Moorea, French Polynesia (CP)	AF525604
″	H.0203	98 – 068	Huatulco, Mexico (EP)	
″	H.0204	98 – 161	Bahia Banderas, Mexico (EP)	
″	HV215		Tahiti, French Polynesia (CP)	
″	HV216		Tahiti, French Polynesia (CP)	
H. taenicola	WRT46-551 (MICH)		Marshall Islands (CP)	
″	H.0037	99 – 004	Tahiti, French Polynesia (CP)	AF407255
″	HV285		Rangiroa, French Polynesia (CP)	
″	HV306-1		Rangiroa, French Polynesia (CP)	
H. cuneata	no voucher	96-AU-3	W Australia (EI)	AF525606
″	WA102		Rottnest Island, W Australia (EI)	
″	WA182		Rottnest Island, W Australia (EI)	
″	WA206		Carnac Island, W Australia (EI)	
″	KZN352		KwaZulu Natal, South Africa (WI)	
″	KZN703		KwaZulu Natal, South Africa (WI)	
″	KZN2048		KwaZulu Natal, South Africa (WI)	
″	HEC15194		Fort Dauphin, Madagascar (WI)	
H. macrophysa	HV8		Zanzibar, Tanzania (WI)	
″	HEC15023		Tuléar, Madagascar (WI)	
″	H.0024	98 – 125	Great Barrier Reef, Australia (WP)	
″	H.0271	99 – 142	New Caledonia (WP)	AF525590
H. gigas	HV48		Mnazi Bay, Tanzania (WI)	
″	H.0122	99 – 102	Cocos Island, Guam (WP)	
″	WRT46-419 (MICH)		Marshall Islands (CP)	
″	L0238136 (L)		Marshall Islands (CP)	
H. gracilis IP	HEC11839	HEC-11839	Beruwala, Sri Lanka (CI)	
″	HEC12045		Zanzibar, Tanzania (WI)	AF407257
″	C&PvR13087B		Madang, Papua New Guinea (WP)	
″	C&PvR13255B		Madang, Papua New Guinea (WP)	
″	C&PvR13346B		Madang, Papua New Guinea (WP)	
″	HV317		Rangiroa, French Polynesia (CP)	
H. lacrimosa	H.0308	95-BA-010	Bahamas (CAR)	AF407258
″	L0351077 (L)		Mariguana, Bahamas (CAR)	
H. gracilis CAR	H.0259	99 – 109	Galeta, Panama (CAR)	AF407259
″	H.0266	99 – 112	Galeta, Panama (CAR)	
″	H.0405	98 – 093	Isla Grande, Panama (CAR)	AF525609
H. minima	SOC251	99 – 025	Socotra, Yemen (WI)	AF407264
″	SOC384	99 – 026	Socotra, Yemen (WI)	AF407263
″	Snellius-II 10184		Tukang Besi, Indonesia (WP)	
″	Snellius-II 10229		Tukang Besi, Indonesia (WP)	
″	no voucher	98 – 031	Tukang Besi, Indonesia (WP)	AF525621
″	PH526	99 – 075	Mindanao, Philippines (WP)	AF525618
″	HOD-PH99-46		Mindanao, Philippines (WP)	
″	H.0380	99 – 093	Bile Bay, Guam (WP)	AF525622
″	H.0382	99 – 098	Apra Harbor, Guam (WP)	AF407265

(*continued*)

Table 2. (*continued*)

Species	Specimen number	Voucher	Geographical location	GenBank
"	WRT46-108 (MICH)		Marshall Islands (CP)	
"	HV67		Moorea, French Polynesia (CP)	
H. renschii	HV7		Zanzibar, Tanzania (WI)	
"	HEC15079		Tuléar, Madagascar (WI)	
"	SOC384		Socotra, Yemen (WI)	
"	Snellius-II 10943		Komodo Island, Indonesia (EI)	
"	C&PvR13855B	99 – 114	Madang, Papua New Guinea (WP)	AF407262
"	no voucher	95 – Guam8A	Double Reef, Guam (WP)	AF525614
H. opuntia ATL	HOD-RD2.02-1		Dominican Republic (CAR)	
"	HOD-RD2.02-41		Dominican Republic (CAR)	
"	H.0263	97 – 083	Bocas del Toro, Panama (CAR)	
"	H.0262	98 – 189	Tamandare, Brazil (EA)	AF525639
H. opuntia IP	HV19		Zanzibar, Tanzania (WI)	
"	HV5		Zanzibar, Tanzania (WI)	
"	HEC12584	99 – 131	Zanzibar, Tanzania (WI)	AF525629
"	HV162		Tahiti, French Polynesia (CP)	
H. distorta	no voucher	98 – 143	Cebu, Philippines (WP)	AF525652
"	H.0280	99 – 151	New Caledonia (WP)	AF525641
"	H.0475	99 – 045	Great Barrier Reef, Australia (WP)	AF407269
"	HV199		Tahiti, French Polynesia (CP)	
H. hederacea IP	HV1		Kunduchi, Tanzania (WI)	
"	HV9		Zanzibar, Tanzania (WI)	
H. copiosa ATL	H.0264	97 – 085	Bocas del Toro, Panama (CAR)	
"	H.0265	97 – 095	Bocas del Toro, Panama (CAR)	
"	H.0330	97 – 481	Discovery Bay, Jamaica (CAR)	AF525612
H. goreauii	A.3336 (MICH)		Jamaica (CAR)	
"	A.3337 (MICH)		Jamaica (CAR)	
"	H.0258	99 – 108	Isla Galeta, Panama (CAR)	AF525610
H. velasquezii	HV28		Zanzibar, Tanzania (WI)	
"	GV2379 (MICH)		Luzon, Philippines (WP)	
H. bikinensis	WRT46-156 (MICH)		Marshall Islands (CP)	
Penicillus capitatus	H.0349	98 – 181	San Blas, Panama (CAR)	AF407271
Udotea flabellum	H.0415	98 – 196	Portobelo, Panama (CAR)	AF407270

The second column lists the accession number of the specimens as they are lodged in the GENT herbarium. Codes in brackets after the specimen number indicate specimens from other herbaria; L denotes Leiden, MICH denotes Michigan. The third column lists the specimen numbers as used in Fig. 1 and in Kooistra *et al.* (2002). Geographical location: CAR: Caribbean Sea; MED: Mediterranean Sea; RS: Red Sea; C: central; E: eastern; W: western; A: Atlantic Ocean; I: Indian Ocean; P: Pacific Ocean

H. gigas and *H. macrophysa* had pliable, weakly calcified and large segments with broad but rather inflexible nodes. Yet, the division is not strict because *H. magnidisca* possessed large, pliable and weakly calcified segments with narrow and flexible nodes and thalli of *H. taenicola* were composed of small, moderately calcified segments with broad, inflexible nodes. These distinct thallus morphologies did not cluster in the phylogenetic tree. Our specimens of *H. magnidisca* deviated from the type material in that their holdfasts, though sand-encrusted, were not perfectly bulbous; the thalli grew on hard substrata covered with a thin layer of sand. On the other hand, we occasionally observed specimens of other lineage 3 species anchoring in unconsolidated substrata by a minute bulbous holdfast.

Medullary siphons branched frequently and entangled strongly below the distal perimeter of the segment to fuse in a single band in the segment's upper rim (Figs 20 – 23). New segments emerged from anywhere along this band (Figs 18, 19). The nodal fusions were complete: the fused units continued into the subsequent segment as single, broad siphons (Figs 20 – 23) until they ramified.

In species with large pliable segments, the cortex consisted of a single or double layer of large and swollen sub-peripheral utricles leaving little space for calcification (*H. discoidea, H. gigas, H. macrophysa, H, magnidisca*) whereas in species with small segments and flexible nodes, the cortex contained one to several layers of variously formed sub-peripheral utricles (*H. cuneata, H. lacunalis*, see Hillis-Colinvaux, 1980, fig. 20; see also *H. hummii* in Ballantine, 1982). In all but one species (*H. macrophysa*), peripheral utricles adhered firmly, did not separate after we decalcified the segment, and

showed an irregularly polygonal surface pattern (Figs 24, 25).

Lineage 4, Figs 28–33.

Atlantic (Caribbean region): *H. gracilis, H. lacrimosa*

Indo-Pacific basin: *H. gracilis*

The specimens of this lineage were collected from relatively deep sites; they sprawled over rocky or partially unconsolidated substrata on reef slopes.

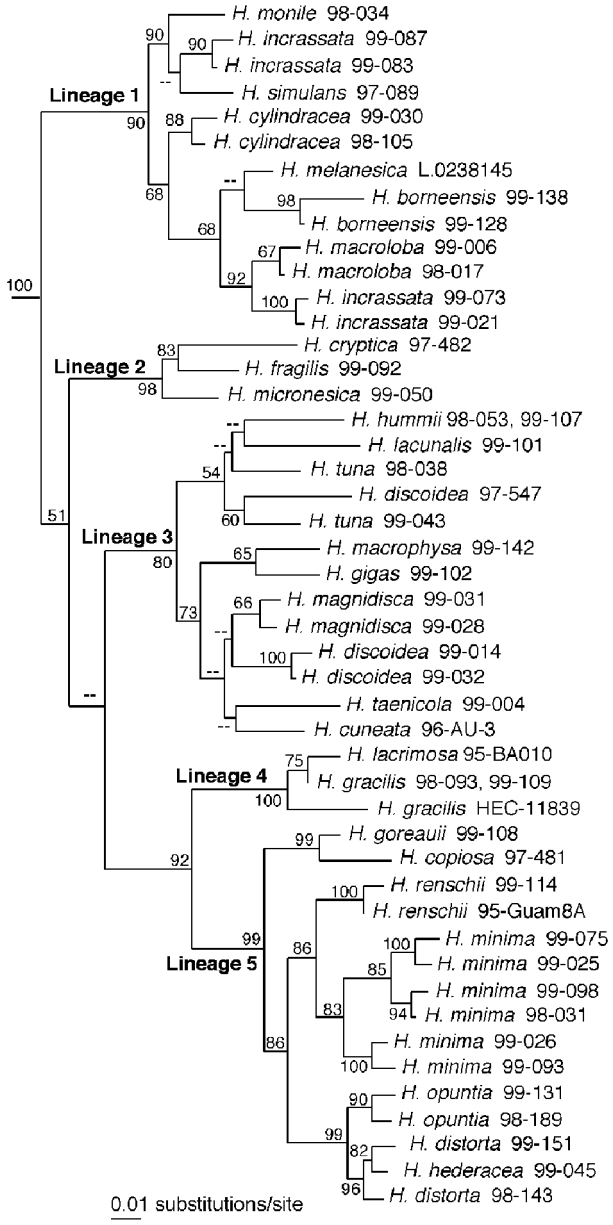


Fig. 1. Maximum likelihood phylogram inferred from partial SSU nrDNA, ITS1, 5.8S rDNA and ITS2 of 47 specimens of *Halimeda* species and two outgroup species (see Table 2). – Ln likelihood = 9895.44751, tree-length = 1390 steps. The phylogram presented here has been redrawn with the outgroup taxa pruned away. MP bootstrap values $\geqslant 50\%$ are indicated below internodes. Lineages 1–5 are explained in text.

Their fairly small segments were strongly calcified. In *H. gracilis*, three to several uncorticated pits were distributed along the segment's apical rim. In *H. lacrimosa* these pits appeared reduced and scattered over the upper part of the segment.

Medullary siphons fused completely at the nodes (Figs 30, 31) though Hillis-Colinvaux (1980) reported occasional occurrence of incomplete fusions in *H. lacrimosa*. The distance between subsequent ramifications in the subnodal region was larger than in other lineages and the siphons did not entangle among one another.

Secondary cortical utricles expanded only at their apex, the expanded areas forming a distinct layer. Numerous peripheral utricles sprouted from the broadened distal end of each secondary utricle (Fig. 33). Similarly, peripheral utricles broadened only slightly at their base and more strongly towards their distal end (Fig. 33). Peripheral utricles were round in cross-section. Around their tips, they formed lateral cell wall extensions that adhered to those of adjacent utricles in a hexagonal pattern. In the resulting surface view, the utricles appeared rounded as well as hexagonal, the prominence of each depending on the focal plane (Fig. 32). The peripheral utricles adhered to each other, although not strongly.

Lineage 5, Figs 34–42.

Atlantic (Caribbean region): *H. copiosa, H. goreauii*

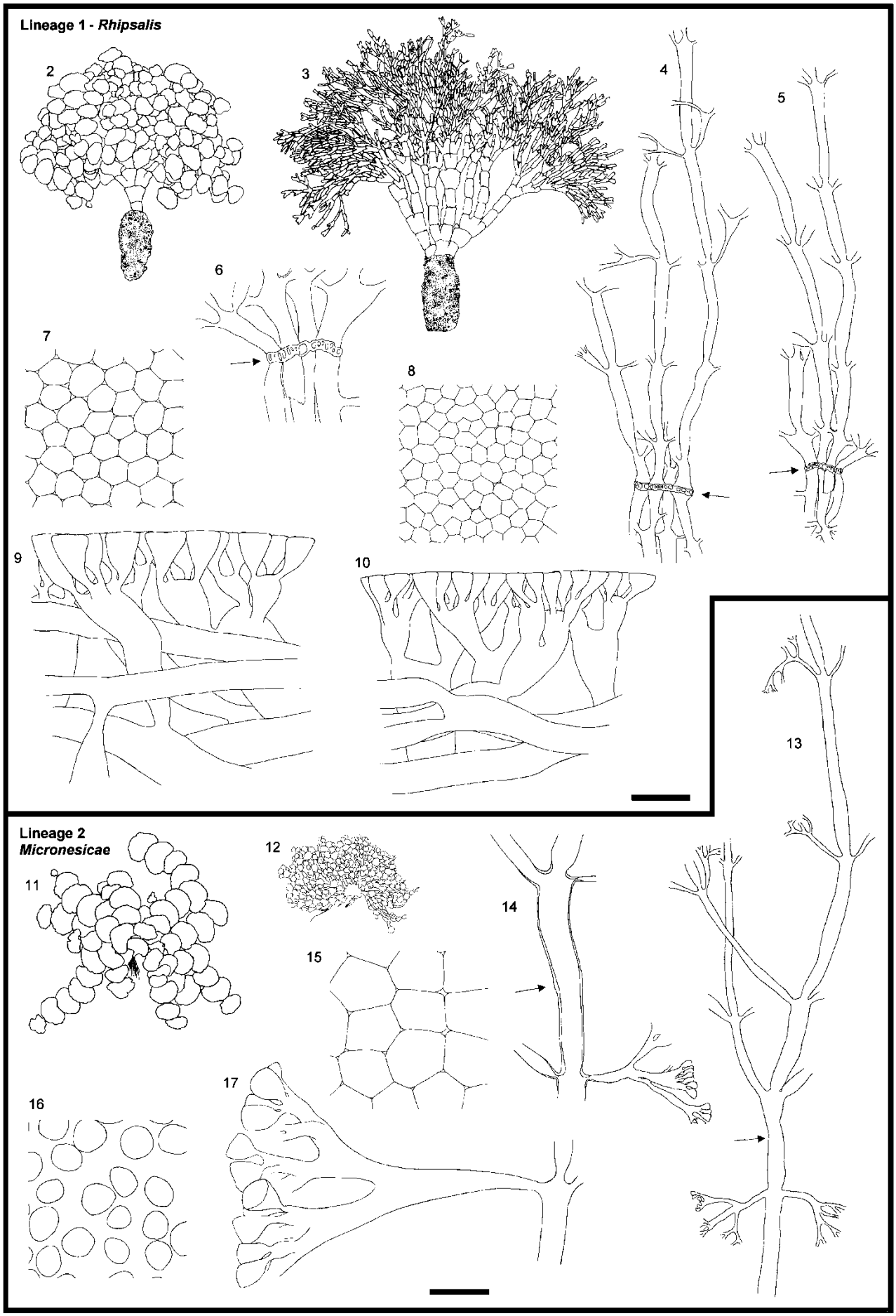
Indo-Pacific basin: *H. distorta-hederacea* species complex, *H. minima, H. renschii, H. velasquesii*

Pan-tropical: *H. opuntia*

The specimens of this lineage were collected from various reef habitats. Most species showed a preference for a single habitat type: our specimens of *H. renschii* were found in moderately wave-exposed localities whereas those of *H. copiosa, H. goreauii, H. minima* and *H. distorta* always came from sheltered localities. *Halimeda opuntia* was ecologically plastic, abounding in a range of habitats from shaded sheltered lagoons and deep fore reefs to moderately exposed reef crests. Thallus shape was also strongly linked with habitat type: *H. renschii* thalli were erect, whereas those of specimens found in more sheltered habitats were pendant or sprawling (Figs 34, 35). The segments of specimens belonging to this lineage were relatively small and heavily calcified.

Nodal medullary siphons fused briefly (in pairs and threes) without losing their identity (Figs 36–38).

The cortex appeared thin: the few siphons emerging from the medulla usually did not


Lineage 1 - Rhipsalis
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Lineage 2
Micronesicae
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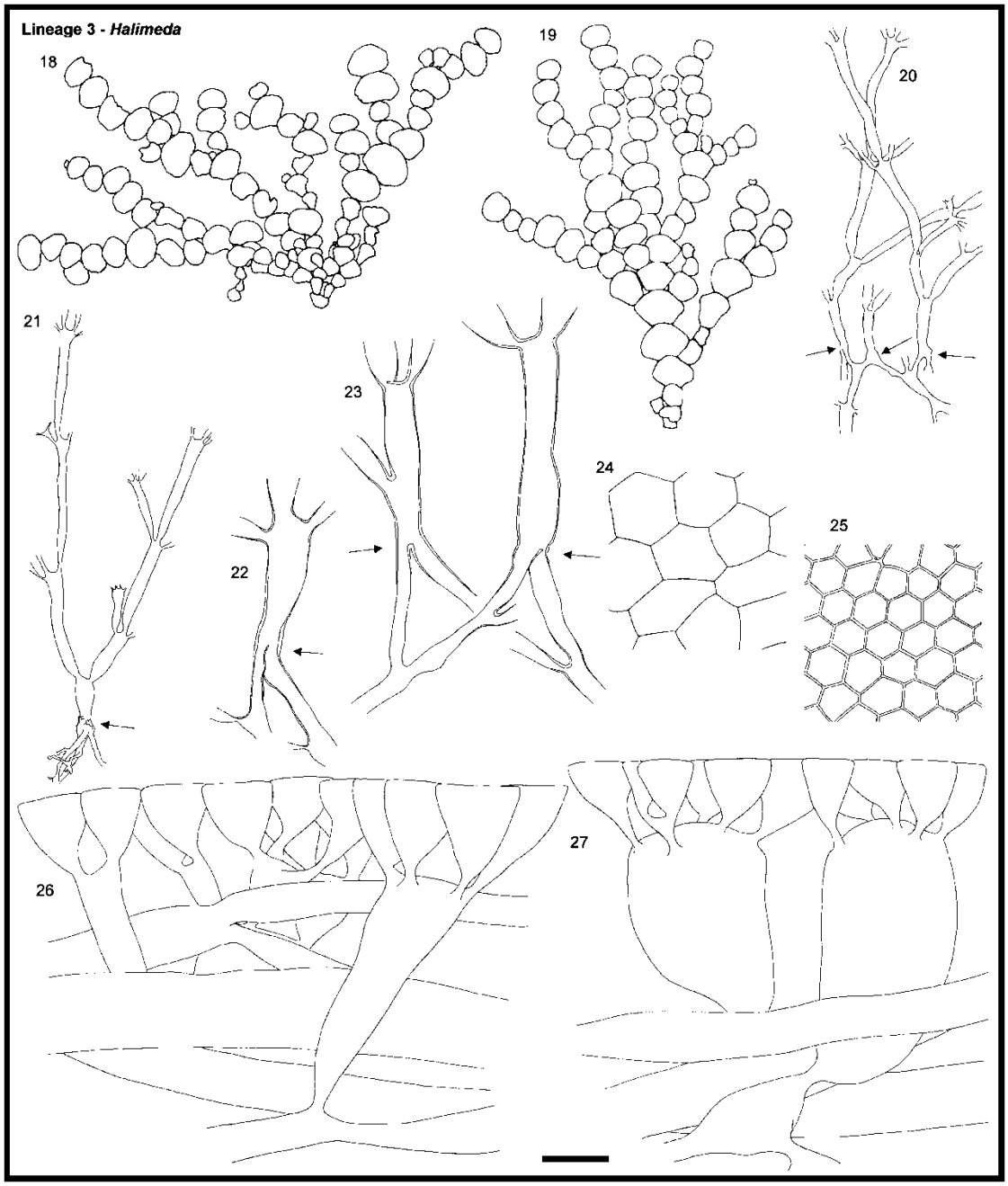
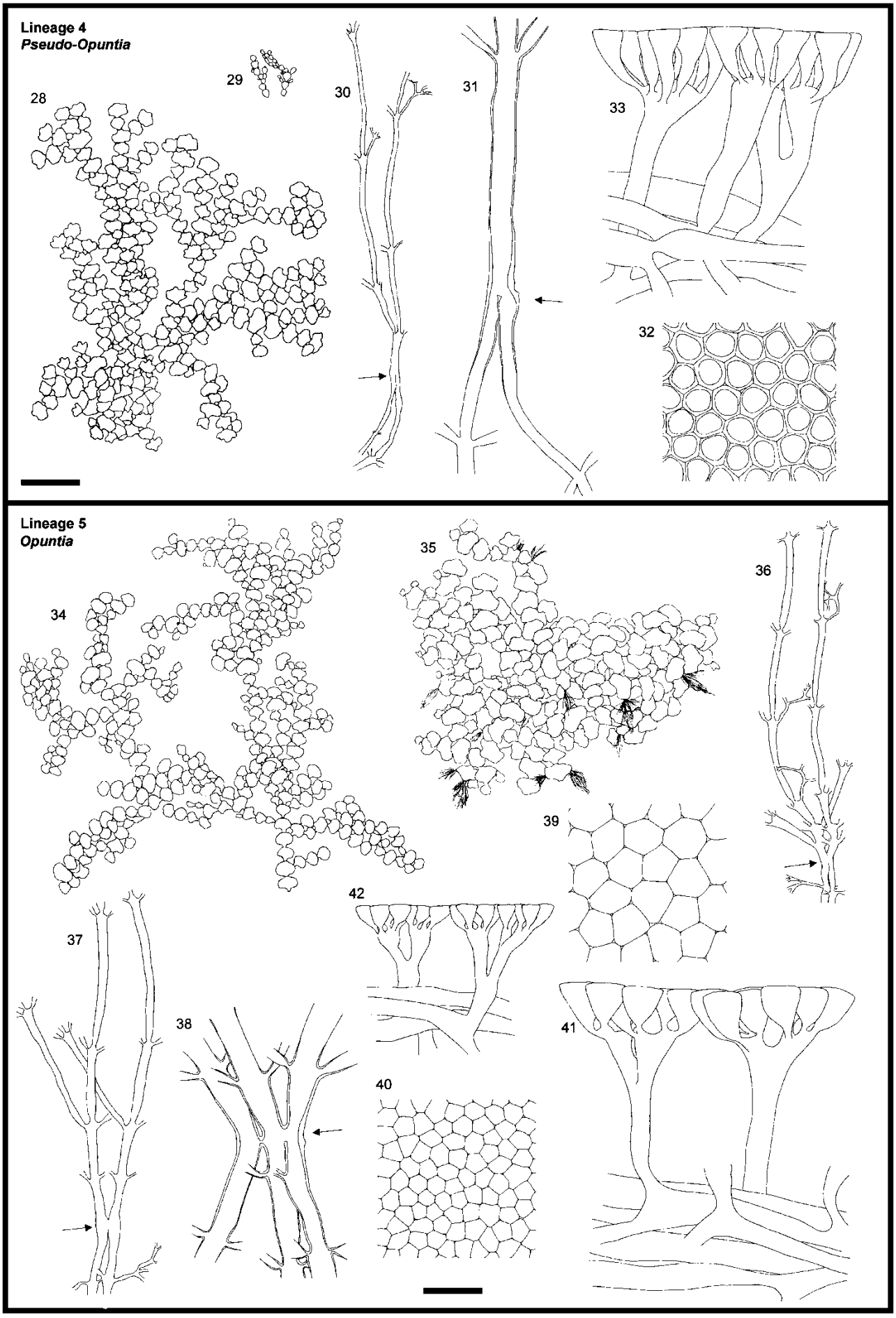


Figs. 18–27. Section *Halimeda*, Lineage 3. Figs. 18, 19. General morphology. Fig. 18. *H. tuna*, HV55. Fig. 19. *H. lacunalis*, HV306. Figs. 20, 21. Medullary and nodal fusion. Fig. 20. *H. tuna*, HV54. 21. *H. lacunalis*, HV306. Figs. 22, 23. Detail of nodal fusion. Fig. 22. *H. lacunalis*, H.0118. Fig. 23. *H. tuna*, H.0113. Figs. 24, 25. Surface view. Fig. 24. *H. tuna*, HV54. Fig. 25. *H. lacunalis*, HV306. Figs. 26, 27. Cortical structures. Fig. 26. *H. tuna*, H.0113. Fig. 27. *H. taenicola*, H.0037. The cortical structures of *H. taenicola* are highly variable between specimens and can be quite different from what is drawn in Fig. 27. Arrows indicate the location of the node. Scale bar represents: 25 mm for thalli, 500 μm for medullary, 250 μm for details of nodal structure, 60 μm for cortical structures and surface view.

Figs 2–17. (facing page) Morphology of *Halimeda* sections. Figs 2–10. Section *Rhipsalis,* Lineage 1. Figs 2, 3. General morphology. Fig. 2. *H. simulans*, H.0032. Fig. 3. *H. cylindracea*, HEC7612. Figs 4, 5. Medullary and nodal fusion. Fig. 4. *H. cylindracea*, H.0018. Fig. 5. *H. simulans*, H.0071. Fig. 6. Detail of nodal fusion. *H. simulans*, H.0071. Figs 7, 8. Surface view. Fig. 7. *H. simulans*, H.0071. Fig. 8. *H. cylindracea*, H.0018. Figs 9, 10. Cortical structures. Fig. 9. *H. simulans*, H.0071. Fig. 10. *H. cylindracea*, H.0018. Arrows indicate the location of the node. Figs 11–17. Section *Micronesicae*, Lineage 2. Figs 11, 12. General morphology. Fig. 11. *H. fragilis*, HEC14230. Fig. 12. *H. micronesica*, WLS184-02. Fig. 13. Medulla going through the nodal region. *H. micronesica*, H.0014. Fig. 14. Detail of a siphon at the node. *H. fragilis*, HV53. Figs 15, 16. Surface view. Fig. 15. *H. cryptica*, H.0237. Fig. 16. *H. micronesica*, WLS184-02. Fig. 17. Cortical structures. *H. micronesica*, WLS184-02. The cortical structures of *H. micronesica* are drawn from a slide prepared differently from those of all other species, because of the total lack of adhesion between utricles. Arrows indicate the location of the node. Scale bars represent: 25 mm for thalli, 500 μm for medulla, 250 μm for details of nodal structure, 60 μm for cortical structures and surface view.


Lineage 4
Pseudo-Opuntia
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Lineage 5
Opuntia
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ramify until close to the segment's periphery (Figs 41, 42).

Opuntioid lineages

The clade with lineages 4 and 5 possessed morphological synapomorphies as well. Specimens abounded in habitats under moderate to high grazing pressure and often revealed a sprawling mode of growth. The primary holdfast of full-grown thalli was often difficult to locate or was altogether absent. In the latter case, numerous secondary holdfasts attached the thallus to the substratum. Specimens of the sister species *H. goreauii* and (Atlantic) *H. copiosa* lacked such holdfasts.

The medullary siphons were generally narrower and smaller than those in the three other lineages.

The cortical siphons emerging from the medulla usually did not ramify until close to the segment's periphery. The large intersiphonal space was filled with aragonite, rendering the segments rigid and generally brittle. As in lineage 2, subperipheral utricles were cylindrical or widened only slightly towards their distal end.

Morphological observations on the type specimen of H. bikinensis

We have re-examined the type material of *H. bikinensis* (WRT46-156, MICH). The cortex was thin, the utricles were club-shaped, and the peripheral utricles possessed lateral cell wall extensions at their tips. In the subnodal medullary, siphon ramifications were widely spaced and trichotomous and did not become entangled with one another. Complete and incomplete fusions occurred together at the node. These character states were all typical for lineage 4 taxa. Furthermore, the segments were heavily calcified and brittle and possessed rhizoid tufts emerging from the uncorticated rim adjacent to the attachment region of daughter segments. These character states are also encountered in *H. gracilis* (lineage 4). What was peculiar, however, is that the type specimen of *H. bikinensis* also possessed an uncorticated rim along the distal segment perimeter, a character state typical for species in lineage 3. We attempted a molecular examination of the type specimen but unfortunately, its DNA was totally degraded.

Discussion

This study reveals that the five natural lineages from phylogenies based on partial nuclear rDNA sequences (Kooistra *et al*., 2002) possess readily recognizable morphological characters. Using the groups' morphological character states, we redefine sections established by Hillis-Colinvaux (1980). Her sections based solely on medullary siphon patterns at the nodes between segments are already surprisingly close to the ones we establish here indicating that Barton (1901) and Hillis-Colinvaux (1980) were right in their notion that these patterns delimited natural groups. We define our sections only through synapomorphies, though we note the symplesiomorphies since each section is defined by a particular combination. Although we provide some ecological information with the sections, we refer to Kooistra *et al*. (2002) for detailed historic ecological patterns in the evolution of the lineages and for habitat descriptions of species to Hillis-Colinvaux (1980) and references therein.

History of subdivisions in Halimeda

De Toni (1889) introduced sections in *Halimeda* taxonomy. He cited the descriptions of Agardh's (1887) subgeneric groupings of implicit hierarchy as diagnoses for his sections *Tunae, Pseudo-opuntiae, Opuntia* and *Rhipsales*. His division is based mainly on thallus appearance, a notoriously unreliable feature (Hillis-Colinvaux, 1980; Kooistra *et al*., 2002). Moreover, several species in his sections are of uncertain status (Hillis-Colinvaux, 1980). Almost a century later, Hillis-Colinvaux (1980) revised the generic subdivision. She redescribed three out of De Toni's four sections (*Tunae, Opuntiae, Rhipsales*) and diagnosed two novel sections (*Micronesicae* and *Crypticae*). She further altered the spelling of De Toni's section names to conform to the ICBN. Section *Tunae* was renamed *Halimeda* because it contains *H. tuna*, the type species of the genus. She based her sectional descriptions solely on nodal fusion patterns,

Figs. 28–42. Morphology of *Halimeda* lineages. Figs. 28–33. Section *Pseudo-opuntia*, Lineage 4. Fig. 28. General morphology. *H. gracilis*, C&PvR13865B. Fig. 29. General morphology. *H. lacrimosa*, redrawn from Hillis-Colinvaux (1980). Fig. 30. Medullary and nodal fusion. *H. gracilis*, HV317. Fig. 31. Detail of nodal fusion. *H. gracilis*, H.0259. Fig. 32. Surface view. *H. gracilis*, HV317. Fig. 33. Cortical structures. *H. gracilis*, HV317. Figs. 34–42. Section *Opuntia,* Lineage 5. Fig. 34. General morphology. *H. hederacea*, HV1 Fig. 35. General morphology. *H. distorta*, HV199. Figs. 36, 37. Medullary and nodal fusion. Fig. 36. *H. opuntia*, HV19. Fig. 37. *H. hederacea*, HV9. Fig. 38. Detail of nodal fusion. *H. copiosa*, H.0265. Figs. 39, 40. Surface view. Fig. 39. *H. distorta*, HV199. Fig. 40. *H. hederacea*, HV9. Figs. 41, 42. Cortical structures. Fig. 41. *H. distorta*, HV199. Fig. 42. *H. hederacea*, HV9. Arrows indicate the location of the node. Scale bars represent: 25 mm for thalli, 500 μm for medullary, 250 μm for details of nodal structure, 60 μm for cortical structures and surface view.

although she noted that other characters accompanied these patterns.

A new sectional division

Section *Rhipsalis* J. Agardh ex De Toni, Lineage 1
Type species: Caribbean *H. incrassata*.

The defining characters of this lineage are the interconnecting pores of the nodal siphons and the segment agglutination in the basal thallus region in *H. melanesica* and in the thallus region above the pseudo-stipe in all other species. The bulbous holdfast and the pseudo-stipe are not diagnostic for this section because *H. melanesica* (lineage 1) lacks these traits whereas *H. magnidisca,* which is a member of lineage 3, does possess a bulbous holdfast and a stipe-like basal zone when growing on sand (Noble, 1986). Both the bulbous holdfast and the pseudo-stipe are adaptations to growth in unconsolidated substrata (Hillis-Colinvaux, 1980; Kooistra *et al*., 2002).

Hillis-Colinvaux (1980) assigned *H. melanesica* to her section *Micronesicae* (our lineage 2) because the description (Valet, 1966) mentions only sparse siphon fusion if any at all. Yet placement in lineage 1 corroborates its morphology because minute pores connect the nodal medullary siphons (Kooistra *et al*., 2002).

Section *Micronesicae* Hillis-Colinvaux, Lineage 2
Type species: *H. micronesica*.

A single character defines this lineage: broadened siphons pass unfused through the nodes. Unfused nodal siphons appear to render nodes flexible minimizing drag in wave-affected environments (*H. micronesica*), and habitats with strong tidal currents (*H. fragilis* and *H. micronesica*). The single nodal medullary siphon observed in *H. cryptica* may result from secondary reduction related to the species' adaptation to deep sites. Yet such environments are not necessarily sheltered. There, thalli are exposed to current, swell from long surface waves and high-amplitude internal waves (Pinkel, 1983; personal observations).

The single siphon traversing the node between segments of *H. cryptica* enticed Hillis-Colinvaux (1980) to propose a monotypic section *Crypticae* because it sets the species apart from all other *Halimeda* species. However, recovery of *H. cryptica* in lineage 2 indicates that section *Crypticae* Hillis-Colinvaux is obsolete.

Section *Halimeda,* Lineage 3

The defining traits of this lineage are the subnodal entanglement of medullary siphons and the presence of an uncorticated band along the distal part of the segment perimeter. New segments emerge from anywhere along this band.

De Toni (1889) validly described this section as *Tunae* based on the description of a grouping of implicit hierarchy by Agardh (1887). Hillis-Colinvaux (1980) renamed the section *Halimeda* because a section containing the type species of the genus must have the same name as the genus (ICBN). However, she retained the original authorities, meaning that the full name of the section was *Halimeda* J. Agardh ex De Toni. The Saint Louis ICBN states that the name of a section containing the type species of the genus should not be followed by an author citation. This is here corrected.

Noble (1986) did not allocate *H. magnidisca* to any of Hillis-Colinvaux's sections because the specimens examined by her possess segments and siphon fusion patterns typical for section *Halimeda* but bulbous holdfasts and stipitate lower segments typical of section *Rhipsalis* sensu Hillis-Colinvaux (1980). Kooistra *et al*. (2002) showed that this species is a member of lineage 3 (section *Halimeda*). Apparently, bulbous holdfasts have been acquired multiple times independently as an adaptation to growth on soft substrata.

Halimeda hummii was placed in section *Opuntia* sensu Hillis-Colinvaux (1980) by Hillis *et al*. (1998), but according to the molecular phylogeny in Kooistra *et al*. (2002) this species belongs within lineage 3 (section *Halimeda*). Apparently, many characters have evolved in this species to states similar to those in lineage 5 (section *Opuntia*). Incidentally, some medullary siphons may fuse incompletely, but if present, they are always accompanied by completely fused siphons. The non-entangling behaviour of siphons in the subnodal region, too, is reminiscent of lineages 4 or 5 rather than of lineage 3. The difference from lineages 4 and 5 lies in the way new segments arise. In *H. hummii*, as in all other members of the *Halimeda* section, segments can emerge anywhere along the uncorticated band in the distal part of the segment perimeter whereas in lineages 4 and 5, new segments emerge only from uncorticated pits.

Section *Pseudo-opuntia* J. Agardh ex De Toni,
Lineage 4
Type species: Indo-Pacific *H. gracilis*.

The defining character of this lineage is encountered in the cortical structure: secondary cortical utricles expand only at their apex and have a large and fairly constant number of peripheral utricles arranged around their distal end (Kooistra *et al*., 2002). The section was first validly described by De

Toni (1889) but later rendered obsolete by Hillis-Colinvaux (1980). She moved *H. gracilis*, the only unambiguous species in it, to her section *Halimeda*. Complete nodal siphon fusion is the defining trait of Hillis-Colinvaux's (1980) section *Halimeda*. Yet, her section is paraphyletic and the trait is a symplesiomorphy shared between specimens in lineages 3 and 4. Therefore we propose to re-establish De Toni's (1889) section *Pseudo-opuntia*, with *H. lacrimosa* and *H. gracilis* as its members. This treatment renders both sections *Halimeda* and *Pseudo-opuntia* natural units.

The *Pseudo-opuntia* lineage shares its main nodal fusion pattern with the *Halimeda* lineage. The distinction lies in the behaviour of medullary siphons just below the nodes and in the way new segments arise. In members of section *Halimeda,* the medullary siphons ramify frequently below the nodes and consequently, become entangled with one another. In members of *Pseudo-opuntia*, the medullary siphons show no sign of entanglement in the subnodal zone because the distance between subsequent ramifications is relatively large. Moreover, in members of section *Halimeda*, segments arise from anywhere in the uncorticated band that spans the distal part of the segment perimeter. In section *Pseudo-opuntia*, segments arise from round to slightly elongated pits.

Section *Opuntia* J. Agardh ex De Toni, Lineage 5
Type species: *H. opuntia*.

This section has only a single defining character: the nodal medullary siphons fuse briefly in pairs or threes (and rarely in small groups) without losing their identity. Yet, *H. lacrimosa* (Hillis-Colinvaux, 1980), *H. hummii* (Ballantine, 1982) and *H. borneensis* can occasionally show similar patterns in the nodes, alongside the typical patterns in these species. Section *Opuntia* was erected by De Toni (1889), and drastically expanded by Hillis-Colinvaux (1980). The species composition of this section as in Hillis-Colinvaux (1980) is maintained unaltered.

Opuntioid lineages

Lineages 4 and 5 could also be merged into a single section. The defining traits would then be a thin subperipheral cortex and heavily calcified segments. All but two species (*H. lacrimosa, H. renschii*) show a sprawling, or pendant, habit and live in sheltered to semi-exposed habitats. Nonetheless, we believe that the dominant patterns of nodal fusion and the shape of the peripheral and secondary utricles differ sufficiently between the two lineages to maintain them as different sections.

Morphological symplesiomorphies and homoplasies

Many characters are present in two or more lineages that are not sister clades. For example, the presence of uncorticated pits from which daughter segments can emerge is an ancestral trait. This character only changed state in the common ancestry of lineage 3. The central uncorticated pit appears to have stretched out laterally to occupy most of the upper segment rim (Kooistra *et al*., 2002). Unlike in all other lineages where new segments emerge exclusively from the uncorticated pits in the segment rim, new segments emerge from anywhere along this band.

Lineages 3 and 4 share patterns of nodal siphon fusion while all other lineages possess their own nodal pattern of siphon behaviour. In Fig. 1, complete fusion is a symplesiomorphy of these lineages.

Lineage 2 and the opuntioid clade (lineages 4 and 5) share generally well-calcified and often brittle segments. In addition, the utricles in the subperipheral cortex are generally not notably swollen. Yet, it should be noted that calcification and utricle shape are related because the more swollen the utricles, the less intersiphonal space remains to be filled with aragonite. Morphologically, it would be more parsimonious to let lineages 2 and 3 switch positions. Such a switch is not improbable because bootstrap support for the clade uniting lineages 3, 4 and 5 is below 50% and Kooistra *et al*. (2002) showed that this alternative topology is not significantly worse using the Kishino-Hasegawa test option in PAUP*. It should be noted however, that even in this alternative topology strong calcification does not become a synapomorphy because several species in lineage 3 also possess strongly calcified segments.

Species not included in the phylogeny

Although we did not have access to specimens of the following taxa for molecular analyses, we expect that *H. favulosa* will be recovered in lineage 1 (section *Rhipsalis*), *H. scabra* and *H. xishaensis* will fall within lineage 3 (section *Halimeda*), and *H. howensis* will be recovered in lineage 5 (section *Opuntia*) because according to their descriptions in Hillis-Colinvaux (1980), Dong & Tseng (1980) and Kraft (2001) these taxa share all synapomorphies and the proper combination of symplesiomorphies with these lineages. However, whether these species constitute genetically and biologically valid taxa or just plastic extremes within other species remains to be resolved.

Placement of *H. bikinensis* in our system remains puzzling. Its original description (Taylor, 1950) and

those in Hillis (1959) and Hillis-Colinvaux (1980) as well as the anatomical characteristics we observed in the type permit placement in either lineage 3 or 4. The anatomy and general morphology of the type specimen indicate that it belongs to section *Pseudo-opuntia* rather than section *Halimeda*. The thin cortex, the club-shaped secondary utricles and the lateral cell wall extensions at the tips of peripheral utricles as well as the widely spaced, trichotomous ramifications of the subnodal medullary and the complete and incomplete nodal fusions occurring side by side are all typical for species in our section *Pseudo-opuntia* (lineage 4). However, the presence of the uncorticated rim along the distal segment perimeter of *H. bikinensis* is a synapomorphy of section *Halimeda* (lineage 3) in our molecular phylogeny. In section *Pseudo-opuntia*, the uncorticated region is limited to a series of round to slightly elongate pits along the perimeter (*H. gracilis*) or a number of reduced pits scattered over the upper part of the segment (*H. lacrimosa*).

If *H. bikinensis* groups within lineage 4 then the uncorticated rim is not a synapomorphy of lineage 3 whereas if it goes with lineage 3 then the apically inflated secondary utricles in the cortex do not define section *Pseudo-opuntia*. If the species forms a lineage on its own behalf, then only the entangling siphons below the nodes remain a synapomorphy of lineages 3, and taxa in lineage 4 do not possess a single synapomorphy.

Given the problems *H. bikinensis* creates in our sectional division, it is understandable that Hillis-Colinvaux (1980) grouped species in lineages 3 and 4 in a single section *Halimeda* defined by complete fusion of medullary siphon in pairs and triplets at the nodes. According to the molecular phylogenies, however, her section is paraphyletic. It should be stressed also that Hillis-Colinvaux's (1980) concept of *H. bikinensis* differs from that of Taylor (1950). The rhizoidal tufts emerging from the uncorticated rim suggest a sprawling habit of the type specimen Taylor collected. This sprawling behaviour goes unnoticed in Hillis-Colinvaux (1980). Instead, she states that thalli are erect. The general morphology of the specimen depicted by her corresponds very well to that of some Indo-Pacific *Halimeda discoidea*. Also, re-examination of specimens from the National History Museum (London) identified by her as *H. bikinensis* revealed only thoroughly swollen, albeit smallish, secondary utricles as encountered in some Indo-Pacific *H. discoidea*.

Key to the sections

In order to facilitate assignment of specimens to our sections, we present a key based on morphological characters. The two most problematic cases of morphological convergence (*H. hummii* and *H. melanesica*) key out even if a misstep occurs. *Halimeda bikinensis* also keys out separately.

(1a) Siphons not fusing at the nodes between segments. 2

(1b) Siphons fusing at the nodes between segments, either over a short or a long distance. 3

(2a) Subperipheral cortex dense, consisting of moderately swollen utricles with a constriction at their base. Nodal siphons adhering in groups and usually communicating with each other through minute pores. Thalli erect. . . . *H. melanesica* of section *Rhipsalis*

(2b) Subperipheral cortex thin, consisting of cylindrical utricles (neither swollen, nor constricted). Diameter and length of subperipheral utricles increasing towards the medulla. Adherence of nodal siphons absent or weak. No pores connecting the neighbouring nodal siphons. Thalli erect or pendant. . . . . section *Micronesicae*

(3a) Complete fusion of siphons at the node: fused siphons continue into the subsequent segment as a single, thicker siphon. 4

(3b) Nodal siphon fusion over a short distance (once to twice the siphon diameter). 8

(4a) Siphons below the node relatively narrow and frequently branching. Subnodal branches numerous, entangling and fusing towards the node, resulting in difficult observation of the fusion pattern. Segments weakly to moderately calcified. Uncorticated rim present along the distal perimeter of the segment. Daughter segments arising from any point along this rim. Thalli erect. . . . . section *Halimeda*

(4b) Siphons not branching more frequently below the node than elsewhere in the medullary. Entanglement of subnodal siphons absent or weak, resulting in easy observation of the fusion pattern. 5

(5a) Uncorticated belt extending along the distal part of the segment perimeter. Daughter segments emerging anywhere along this belt. Thalli erect or partially sprawling. . . . . *H. bikinensis*

(5b) No uncorticated belt along the distal part of the segment perimeter. Daughter segments emerging from isolated pits or from small, slightly elongate uncorticated regions along the segment perimeter. Thalli erect, sprawling or pendant. 6

(6a) Segments flattened, paper-thin, moderately calcified. Segments emerging from anywhere along the distal part of the perimeter of the parent segment. . . . . *H. hummii* of section *Halimeda*

(6b) Segments thicker than 0.6 mm, either flattened or globose to tear-shaped, and strongly calcified. 7

(7a) Segments flattened or globose to tear-shaped. Daughter segments of flattened segments emerging from large pits along the distal segment perimeter; daughter segments of globose to tear-shaped segments arising from (generally three) reduced pits spread over the top region of the segment. . . . . section *Pseudo-opuntia*

(7b) Segments flattened. Daughter segments emerging anywhere along the uncorticated belt along the distal part of the segment perimeter. . . . . *H. bikinensis*

(8a) Nodal siphons generally fusing into a single unit. Nodal siphons adhering to all their neighbours and communicating with them by means of large pores. Occasionally, siphons fusing in large groups (more than five siphons) rather than into a single unit. Thallus erect and often possessing a bulbous holdfast. Segments in the basal zone of the thallus usually agglutinating into stipe- and/or fan-like structures. Segments moderately to strongly calcified. Pores occasionally minute rather than large; in this case nodal siphons adhering in groups and thallus holdfast felt-like. . . . . section *Rhipsalis*

(8b) Siphons fusing in twos, threes, or occasionally in small groups (less than five siphons). Thalli erect, pending or sprawling. Attachment to the substratum by means of rhizoid tufts or a non-bulbous, felty holdfast. Segments in the basal thallus region not agglutinating to a stipe- and/or fan-like structure. 9

(9a) Incomplete (short) fusions and complete fusions co-occur. Mature thalli smaller than 5 cm. . . . . *H. hummii* of section *Halimeda*

(9b) All fusions incomplete. Mature thalli larger than 5 cm. . . . . section *Opuntia*

Perspectives

A revision of *Halimeda* species is needed, given the existence of paraphyletic species, of cryptic diversity hidden within a single perceived species (e.g. *H. minima*) and of cognate pairs, genetically distant species that have converged morphologically (Kooistra *et al*., 2002). Hillis-Colinvaux (1980) had access only to morphological character states; she had no analysis of independent data to determine which morphological features were homologous. For instance, she could not identify all the cognates reported by Kooistra *et al*. (2002). Although she noticed slight morphological differences between what she perceived as the same species in different geographical regions [see Colinvaux (1969) on *H. copiosa* – *H. hederacea* and Hillis-Colinvaux (1980) on Mediterranean and western Atlantic *H. tuna*], she could not evaluate the meaning of these differences because the characters also varied within geographical regions. Many species in her most recent monograph (Hillis-Colinvaux, 1980) are thus biphyletic entities. They may, in fact, differ between one another morphologically, either in as yet unexplored characters or in characters that are now considered variable. A new monograph ought, apart from proper illustrations of anatomical details, to incorporate a thorough evaluation of measurable features associated with medullary, cortical and gametangial siphon anatomy and segment morphology. Such an approach may uncover currently overlooked differences among species and sections; differences that will not only facilitate distinction of cognates and other look-alikes but will also permit more sound comparison with the morphology of fossil *Halimeda*.

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