Diversity, Distribution and Variations within Species of Genus *Halimeda* J.V.Lamour. (Chlorophyta) in Peninsular Thailand

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Ecology (International Program)

Prince of Songkla University

2009

Thesis Title Diversity, Distribution and Variations within Species of Genus *Halimeda* J.V.Lamour. (Chlorophyta) in Peninsular Thailand

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ชื่อวิทยานิพนธ์ ความหลากหลาย การแพร่กระจาย และความแปรผันภายในชนิดของสาหร่ายในสกุล *Halimeda* J.V.Lamour. (Chlorophyta) ในคาบสมุทรไทย

ผู้เขียน นางสาวสุภัทรา พงศ์ภราดร

สาขาวิชา นิเวศวิทยา (นานาชาติ)

ปีการศึกษา 2552

บทคัดย่อ

วัตถุประสงค์ของงานวิจัยครั้งนี้เพื่อประเมินความหลากหลาย การแพร่กระจาย และ ความแปรผันภายในชนิดของสาหร่ายในสกุล *Halimeda* J.V.Lamour. ในคาบสมุทรไทย ผลการศึกษาพบความหลากหลายของสาหร่ายสกุล *Halimeda* ทั้งหมด 8 ชนิด คือ *Halimeda macroloba* Decne., *H. discoidea* Decne., *H. opuntia* (L.) J.V.Lamour., *H. heteromorpha* N'Yeurt, *H. tuna* (J. Ellis & Solander) J.V.Lamour., *H. borneensis* W.R. Taylor, *H. gigas* W.R. Taylor และ *Halimeda* sp. โดยเป็นสาหร่ายที่มีการรายงาน เป็นครั้งแรกในประเทศไทย จำนวน 4 ชนิด คือ *H. borneensis*, *H. gigas*, *H. tuna*, และ *H. heteromorpha*. นอกจากนี้ *Halimeda* sp. (S. Pongparadon 17) เป็นชนิดพบในคลองบริเวณป่าชายเลน ซึ่งเป็นถิ่นที่อยู่อาศัย ที่ค่อนข้างเฉพาะและแตกต่างจากชนิดอื่น ลักษณะทางด้านสัณฐานวิทยาและกายวิภาควิทยามีความแตกต่างจาก *Halimeda* ที่มีการรายงานทั่วโลก ซึ่งอยู่ในระหว่างการศึกษาเพิ่มเติม ด้านการแพร่กระจาย พบว่า *H. macroloba* เป็นเพียงชนิดเดียวที่มีการแพร่กระจายได้ทั้งอ่าวไทยและทะเลอันดามัน ในขณะที่ชนิดอื่น ๆ มีการแพร่กระจายเฉพาะฝั่งทะเลอันดามันเท่านั้น ดังนั้นคาบสมุทรไทย กระแสน้ำ และ ชีววิทยาการสืบพันธุ์ อาจจะส่งผลต่อความหลากหลาย และการแพร่กระจายของ *Halimeda* ตลอดแนวชายฝั่งและระหว่างอ่าวไทยและทะเลอันดามัน การศึกษาความแปรผันภายในชนิด พบว่า มีความแปรผันทั้งด้านสัณฐานวิทยาและกายวิภาควิทยาระหว่างกลุ่มประชากร ภายในชนิดของ *H. macroloba* โดยมีความแตกต่างอย่างมีนัยสำคัญ ($P < 0.001$) ของ 3 ลักษณะทางสัณฐานวิทยา และ 5 ลักษณะทางกายวิภาควิทยา นอกจากนี้ ขนาดของใบ และจำนวนชั้นของ Utricle จะเพิ่มขึ้นเมื่อได้รับความเข้มแสงลดลง และจะลดลงเมื่อได้รับความแรงของคลื่นที่เพิ่มขึ้น

Thesis Title	Diversity, Distribution and Variations within Species of Genus *Halimeda* J.V.Lamour. (Chlorophyta) in Peninsular Thailand
Author	Miss Supattra Pongparadon
Major Program	Ecology (International Program)
Academic Year	2009

ABSTRACT

This study is to investigate diversity, distribution and variations within species of genus *Halimeda* J.V.Lamour. (Chlorophyta) in Peninsular Thailand. The results showed that a total of eight species were recorded in this study: *Halimeda macroloba* Decne., *H. discoidea* Decne., *H. opuntia* (L.) J.V.Lamour., *H. heteromorpha* N'Yeurt, *H. tuna* (J. Ellis & Solander) J.V.Lamour., *H. borneensis* W.R. Taylor, *H. gigas* W.R. Taylor and *Halimeda* sp. This study has added four species of new record of marine flora to Thailand, namely *H. borneensis*, *H. gigas*, *H. tuna*, and *H. heteromorpha.* In addition, *Halimeda* sp. (S. Pongparadon 17) found in the canal of mangrove forest, a rather unique habitat with different morphological and anatomical characters differs from other *Halimeda* worldwide. Interestingly, diversity study showed that *H. macroloba* is the only species found in both the Gulf of Thailand and the Andaman Sea while other species are found only in the Andaman Sea. Peninsular Thailand, surface current and the reproductive biology of *Halimeda* might influence diversity and distribution of *Halimeda* along the coast and also between the Gulf of Thailand and the Andaman Sea. In variation study, there are significant differences in 3 morphological and 5 anatomical among populations within species of *H. macroloba* ($P < 0.001$). In addition, segment size and number of utricle layers increased with decreasing of light intensity and decreased with increasing of wave exposure.

ACKNOWLEDGEMENTS

First and foremost, I am truly grateful to Assistant Professor Dr. Anchana Prathep, my advisor, for inspired and guided me throughout my course of research at Prince of Songkla University (PSU), and has supported me throughout this work.

I would like to express my gratitude to Professor Dr. Khanjanapaj Lewmanomont, Assoc. Prof. Dr. Kitichate Sridith to whom I am indebted for their time and valuable guidance.

I am grateful to Professor Eric Coppejans for many valuable suggestions; field collection methods during his visit to Prince of Songkla University (PSU), and Dr. HeroenVerbruggen for molecular data for confirm species and many suggestions during this work.

I would like to thank you to Professor Dr. Khanjanapaj Lewmanomont, Assoc. Prof. Chatcharee Kaewsuralikhit, Ms Thidarat Noiraksar and Ms Pimonrat Thongroy who provided specimens and additional information. Special thanks to the team from the Seaweed and Seagrass Research Unit, Prince of Songkla University, Hat Yai, Songkhla, for helping me in the field work.

The financial support from the TRF/BIOTEC Special program for Biodiversity Research and Training grant BRT T_000519 made this thesis possible. This work was partially funded by Graduate School, Prince of Songkla University, Hat Yai, Songkhla and Research Assistantship 2006, Faculty of Science, Prince of Songkla University.

Finally, my deepest appreciation is to my family for their understanding and support.

Supattra Pongparadon

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CHAPTER 1

INTRODUCTION

The green calcified seaweed genus *Halimeda* J.V.Lamour. (Chlorophyta) are common inhabitant of tropical and warm-temperate marine environments (Hillis-Colinvaux, 1980) in reefs and lagoons (Hillis-Colinvaux, 1988; Littler and Littler, 2000). The roles of *Halimeda* species are primary producer, source of food and habitat (Chittaro, 2004), shelter and nursery grounds for invertebrates (Hillis-Colinvaux, 1980), CO_2 sink and carbonate sand producer (Payri, 1995). Thallus of *Halimeda* is composed of flattened, green calcified segment for photosynthesis and holdfast that attached on the substrate. For anatomically, segment consists of a single giant tubular cell that is ramified siphons forming a medullar and utricles in cortex. All species of *Halimeda* deposit calcium carbonate in aragonite form (Hillis-Colinvaux, 1980).

Most studies of genus *Halimeda* were carried out from Atlantic region; *H. tuna*, *H. incrassata* and *H. opuntia*, but the study in Indo-pacific is limited, especially, *H. macroloba*. There are a few researches in Indo-pacific region; were carried out in Pacific Ocean: Philippine and French Polynesia, Indian Ocean: Australia and Tanzania (Verbruggen and Kooistra, 2004; Verbruggen *et al*., 2005a; Verbruggen *et al*., 2005b).

In Thailand, there are many taxonomic problems of genus *Halimeda*. The first problem, *Halimeda* are very common and distribute throughout Thai waters, but the researches on genus *Halimeda* in Thailand are limited. There is only one checklist in 1995, which Lewmanomont and Ogawa reported five species of *Halimeda* found in Thailand: *H. macroloba* Decne., *H. discoidea* Decne., *H. incrassata* (J.Ellis) J.V.Lamour., *H. opuntia* (L.) J.V.Lamour. and *H. velasquezii* Taylor. The second problem, Verbruggen (2005) reported that *H. incrassata* (J.Ellis) J.V.Lamour. has a distribution range in Atlantic, but some have reported in Indian Ocean and Pacific Ocean (Lewmanomont and Ogawa, 1995; Egerod, 1971; 1974;

1975). This might be because of the taxonomic confusion or less intensive study of *Halimeda* in the region Thailand. The last taxonomic problem of genus *Halimeda*, there are great morphological and anatomical variations within species and these variations could cause taxonomic problem. Especially, *H. macroloba* is the dominant species and commonly found both in the Peninsular Thailand. Preliminary observation showed that there were morphological differences in thallus size, segment shape and size among population of the Gulf of Thailand and the Andaman Sea and there was larger segment in the subtidal than that in the intertidal zone. From these taxonomic problems, the purposes of this research are to investigate diversity and distribution of genus *Halimeda* J.V.Lamour. and variations within species of *H. macroloba* Decne. in Thailand.

Review of Literatures

Classification of Genus *Halimeda* J.V.Lamour.

Division	Chlorophyta
Class	Bryopsidophyceae
Order	Bryopsidales
Family	Halimedaceae
Genus	*Halimeda*

The characteristics of genus *Halimeda* J.V.Lamour.

The green calcified seaweed genus *Halimeda* J.V.Lamour. (Chlorophyta) is common inhabitant of tropical and warm-temperate marine environments (Hillis-Colinvaux, 1980) and in reefs and lagoons (Littler and Littler, 2000). *Halimeda* species are primary producer, source of food and habitat (Chittaro, 2004), shelter and nursery grounds for invertebrates (Hillis-Colinvaux, 1980), CO_2 sink and carbonate sand producer (Payri, 1995).

The genus *Halimeda* J.V.Lamour. belongs to the chlorophyta algae. Thallus of *Halimeda* is composed of flattened, green calcified segments for photosynthesis and holdfast that attached on the several substrates. Each segment consists of a single giant tubular cell that are ramified siphons forming medullar zone, utricles in cortex zone and nodal zone. Thalli consist of a single, multinucleate, tubullar cell. The branches of this tubular cell are siphon, are organized to form of segment and string these segments together to form the thallus of *Halimeda.* This filament without cross wall or coenocytic filament, occurs also in other seaweeds such as the genus *Caulerpa* and genus *Udotea*, all of them are member of green algae in order Bryopsidales (Hillis-Colinvaux, 1980). In the interfilament space of the cortex and medulla siphon, the deposition of calcium carbonate in form aragonite occur (Wilbur *et al*., 1969), excepted interfilament space of node, apical segments younger than about 36 hours old and holdfast (Blaxter, 1980).

Morphological Structures

There are three main morphological characters in *Halimeda* genus: 1) thallus appearance, 2) shape of segments and 3) the type of holdfasts. These characters are useful in field identification.

General Appearances of Thallus

Thallus of *Halimeda* consists of articulated sequences of flattened segment of various shapes, nodes, alternating with segments, and holdfast which provides attachment to or in the several substrates. From its holdfast, *Halimeda* contributed the basal segment and give rise to a new flat segment (Colinvaux *et al*., 1965; Hillis-Colinvaux, 1980; Hay *et al*., 1988). New segment grow from distal edge point of mother segment (Verbruggen and Kooistra, 2004). This progressive growth of new segment on top of thallus gives to a pattern of growth form (Figure 1).

Halimeda has three different growth forms: erect (Figure 2A), pendant (Figure 2B) and sprawling (Figure 2C). The axis of new growth is predominantly vertical for thalli with erect pattern, this form of a member of species of the *Rhipsalis* section such as *H. macroloba* (Figure 3A), *H. discoidea* (Figure 3B), *H. borneensis, H. simulans, H. incrassata,* or pendant pattern**,** form of member of the *Halimeda* section such as *H. tuna* (Figure 3C), *H. gigas*, and horizontal for those sprawling, such as *H. opuntia* (Figure 3D).

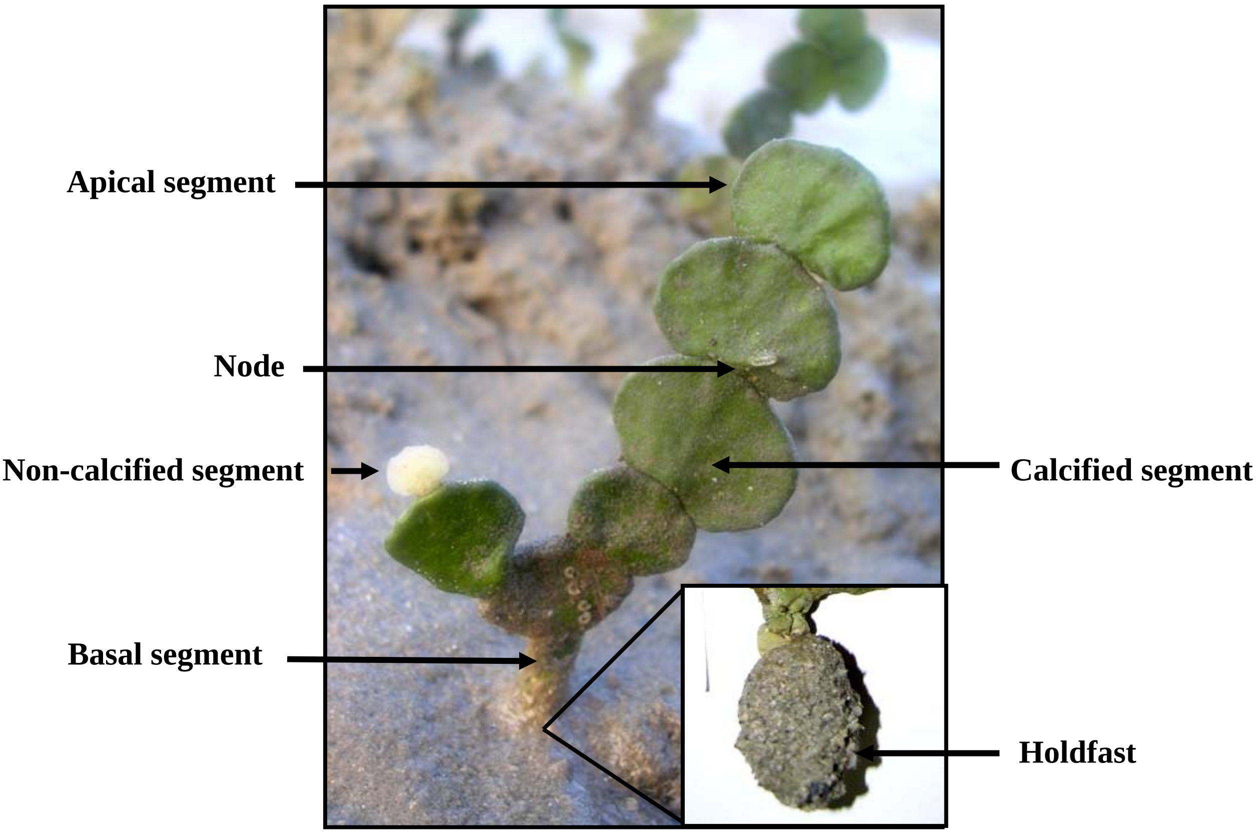


Figure 1 Thallus of *Halimeda* J.V.Lamour. consists of flattened calcified segment, non-calcified segment, apical segment, basal segment, and holdfast.

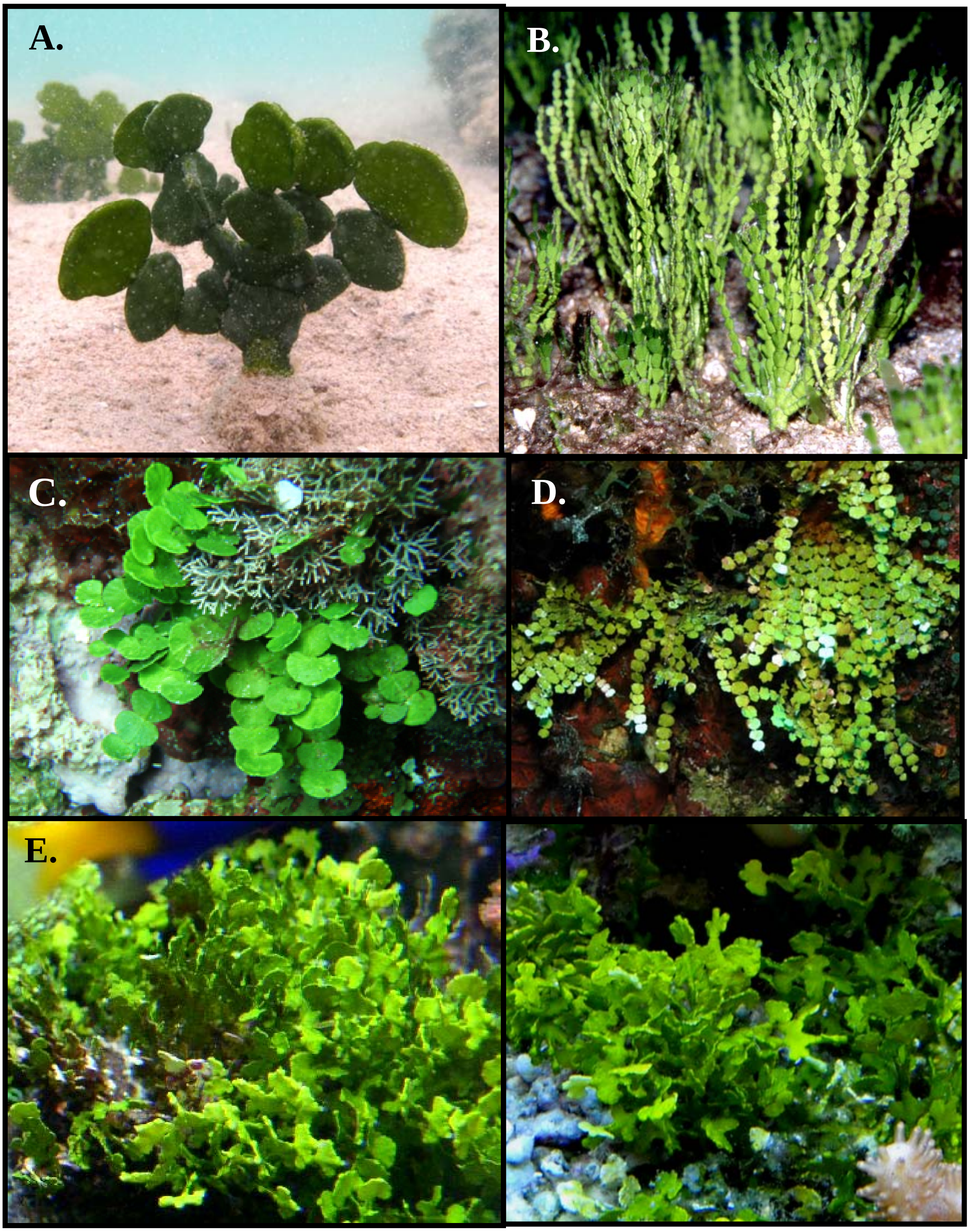


Figure 2 Growth form of *Halimeda*: (A) and (B) erect form: *Halimeda macroloba*, *H. incrassata* (http://www.algaebase.org), (C) and (D) pendant or hanging form: *H. tuna* (http://shokubutuhan.ti-da.net), *H. goreaui* (http://www.wetwebmedia.com), (E) and (F) sprawling form: *H. opuntia* (http://www.sloreef.com and http://www.brettsreef.com)

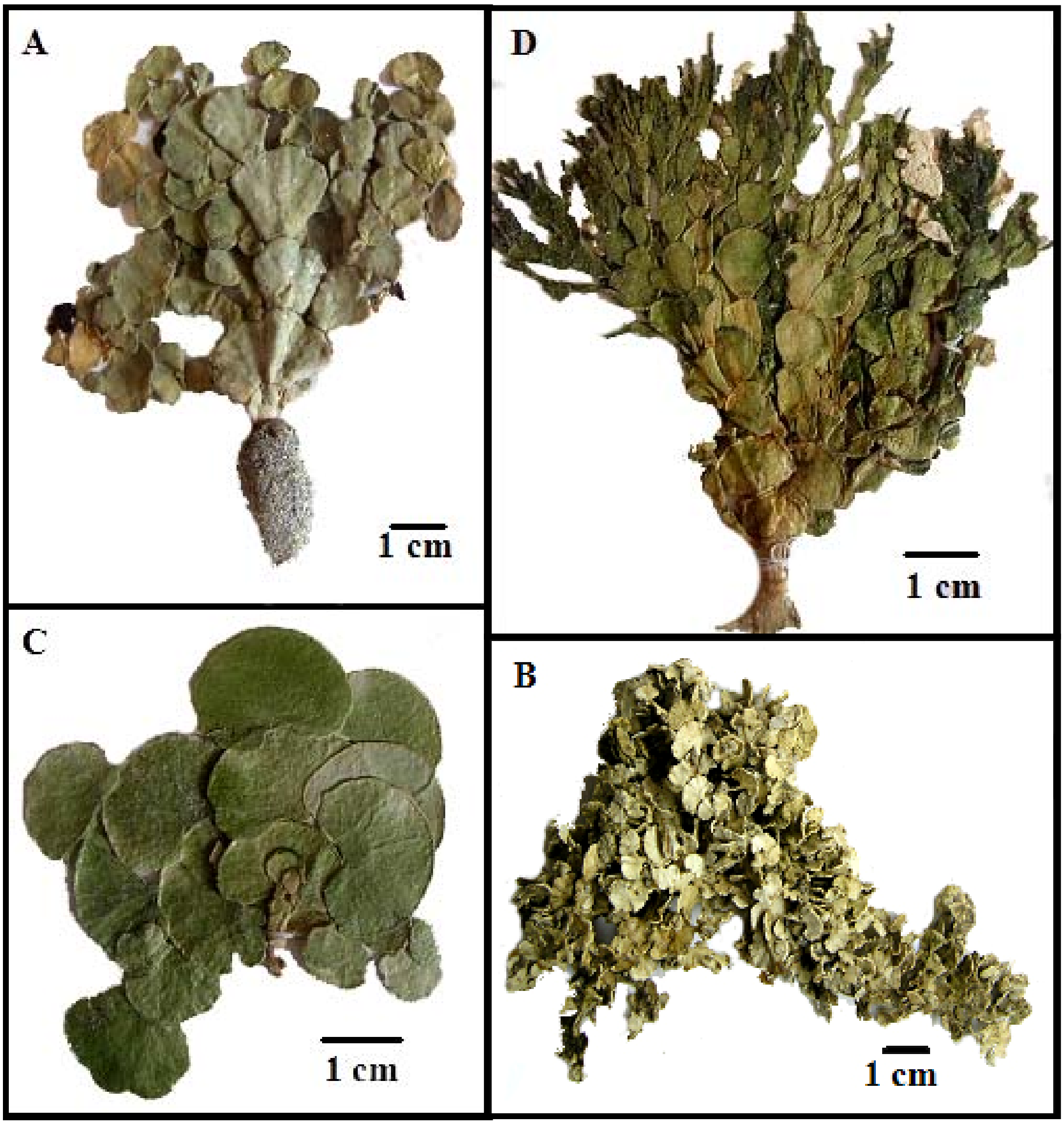


Figure 3 General appearance of *Halimeda* thalli: (A) *Halimeda macroloba*, specimen; S. Pongparadon 35, (B) *H. discoidea*; specimen P. Tuntiprapas 1, (C) *H. gigas*, specimen; P. Tuntiprapas 3, (D) *H. opuntia*; specimen S. Pongparadon 3

Features of Segments

Segment shape also varies considerably. Among the most common shapes are reniform, subreniform, discoid, elliptical-discoid, ovate, broad ovate, obovate, broad obvate, cuneate, cylindrical and subcylindrical. Some species have segments with entire margins or shallow to deep lobes along the margin of the segment are present in a variety of species (Figure 4) (Hillis-Colinvaux, 1980; Noble, 1987; Verbruggen *et al*., 2005a). The shape of the segment base varies from articulate to acute and features a small stalk in some species. The size of segment were different in each species; the range from the small (2-5 mm) of *H. lacrimosa* to the big one of *H. gigas* (42 mm) (Hillis-Colinvaux, 1980). Appearances of the surface of segment were different in size and pattern among different species. There are two pattern of segment surface: polygonal and circle, represents the surface of peripheral utricle.

These segments appearance, shape, size and segment surface are used in species identification. However, the similar shape of segments in many species and variation in segments shape and size cause a difficulty in identification, for examples, group specimens of species *incrassata-melanesica-heteromorpha-kaoloana* (Verbruggen, 2005).

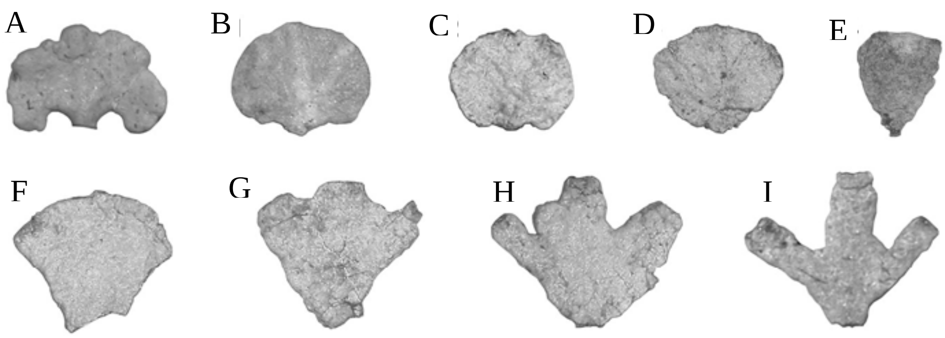


Figure 4 Segment shape: (A) reniform segment: *H. opuntia*, (B) broad ovate segment: *H. distorta*, specimen HV767. (C) elliptical–discoid segment: *H. heteromorpha*, (D) broad obovate segment: *H. heteromorpha*, (E) cuneate segment: *H. discoidea*, (F) segment with entire distal margin: *H. heteromorpha*, (G) shallowly lobed segment:

H. heteromorpha, (H) medium deeply lobed segment: *H. heteromorpha*, (I) deeply lobed segment: *H. heteromorpha*. (Verbruggen, 2005)

Type of Holdfasts

Halimeda holdfast composed of a more or less organized mass of branching rhizoids (Hillis-Conlinvaux, 1980). In contrast to the segment, the filaments of the holdfast are not organizied into a definite pattern of organization such as a segment. There are three types of *Halimeda* holdfast. The first holdfast type is bulbous holdfast. This holdfast consists of a mass of loose filaments, adheres to sand grain or finds particles, is formed to a bulbous structure of sand, look like cemented conglomerate of sand. This holdfast ranges 1 cm – over 13 cm in length (Figure 5A; Hillis-Conlinvaux, 1980); it occurs in unconsolidated substrate; sand or mud; of sandy beach zone, coral reef with sand bottom zone and seagrass bed. The holdfast type presents in the *Rhipsalis* section of the genus as in *H. macroloba, H. borneensis, H. simulans, H. incrassata, H. cylindracea*. The second type of holdfast is a rock-grower holdfast or felt-like holdfast, rhizoids are compacted into a dense mass that look like a cup shape (Figure 5B), this could be found in *H. tuna, H. gigas, H. discoidea.* These species are growing on hard substrate, dead coral or rock surfaces. The third type, the sprawling holdfast occurs in species sprawling; the holdfast consists of a few branched, loose rhizoids attaching to morsels of rock and sand (Hillis-Conlinvaux, 1980; Verburggen and Kooistra, 2004). The bulbous and rock-grower holdfast types have only single holdfast attaches the thallus at its substrate, while the sprawling type of holdfast occurs at multiple points along the sprawling thallus that rhizoid occurring at intervals where the segment contact with substrate (Figure 5C; Hillis-Conlinvaux, 1980).

Figure 5 General appearance of holdfast type: (A) The bulbous holdfast of *Halimeda macroloba* Decne; specimen S. Pongparadon 1, (B) Rock-grower holdfast or felt-like holdfast of *H. discoidea*; specimen P. Tuntiprapas 1, (C) Sprawling holdfast of *H. opuntia*; specimen S. Pongparadon 3

Anatomical Structures

There are three main anatomical characters in *Halimeda* genus: 1) features of medulla siphon, 2) features of cortex siphon (utricles) and 3) features of nodal zone. These characters are useful in species identification.

Features of Medulla and Cortex

For anatomical structures of each segment, there are two zones of different siphons: a central medulla and cortex. In the medulla, siphons are arranged parallel to the thallus axis, proceeding through the nodes and stringing segments together (Figures 6A, 6B). The size of medulla siphon varies considerably between species, within species and within individual segments (Verburggen *et al*., 2005) Ramifications in medulla siphons show, the most species are trichotomous with the central branch continuing upwards and the two side branches proceeding into the cortex zone. However, dichotomous and quadrichotomous ramifications occur. In addition, the ribbed segments consist of thick bundles of medulla siphons surrounded by a cortex (Kooistra and Verburggen, 2005).

Cortex is siphon originates from side branch of medullar siphons; these filaments are called utricles: the external branches are called primary utricles and the inside branches are secondary utricle and tertiary utricle, respectively (Figure 6C). The cortex of segment is absent in the joint between segments.

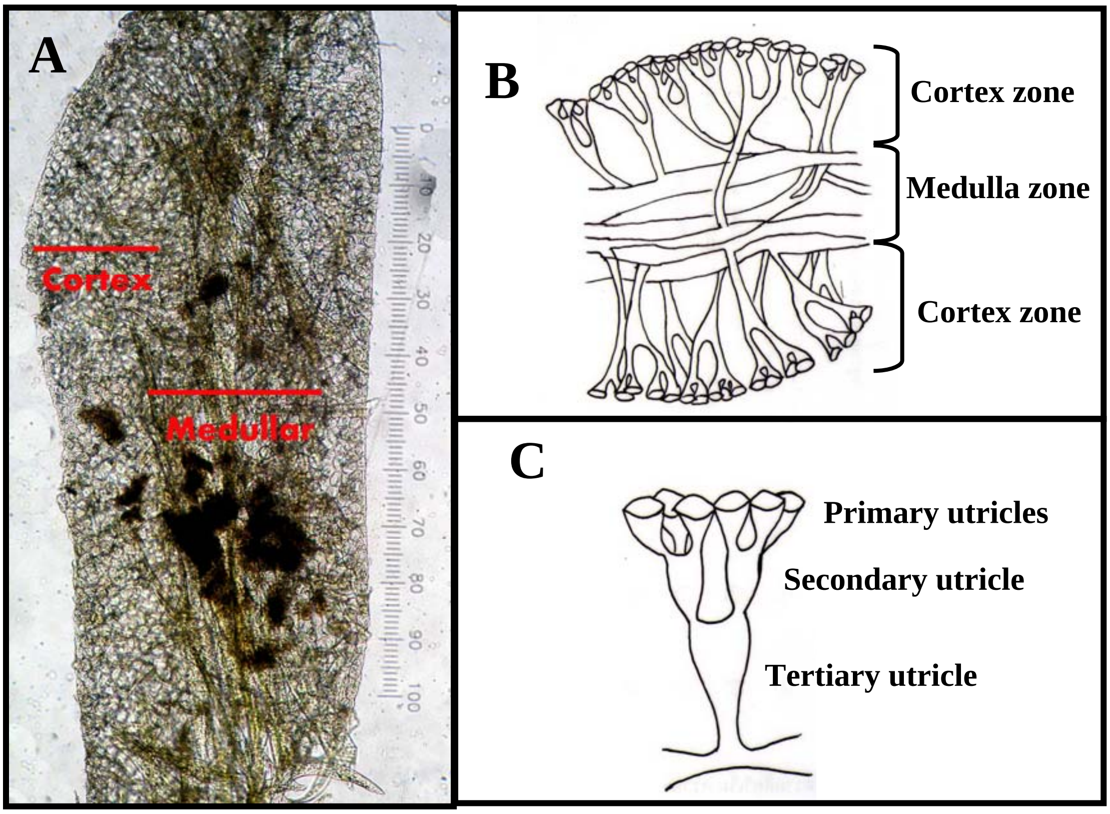


Figure 6 Anatomical structures of *Halimeda*: (A), (B) Longitudinal section through part of a segment showing medullar and cortex, (C) Utricles in cortex layer

Features of Nodal Zone

In a first nodal fusion type, all siphons fused into a single unit. Siphons keep their normal appearance below the node, and, at the node, fuse sidewise with their adjacent neighbors. This results in a pattern of large pores visible in properly prepared slides (Figure 7A). Adaptations of this fusion, pattern the large pores have been reduced to small ones or are absent altogether (Hillis-Colinvaux, 1980). A second common pattern of nodal siphon anatomy is present in many rock-growing species of wave affected habitats. At the node, pairs or triplets of these narrow siphons anastomose (Figure 7B). Anastomosis of several siphons into a single unit that continues in the subsequent segment as a single siphon is called complete fusion. Filaments are complete fusion in pair, (Verbruggen and Kooistra, 2004; Figure 7C). A third pattern is one of incomplete fusion of a small number of siphons (usually 2–4). At the node, siphons fuse with one or two neighbors over a short distance (Figures 7D, 7E). A fourth pattern is siphons proceed through the node without any form of fusion (Figure 7F). The last node pattern is node composed of a single filament.

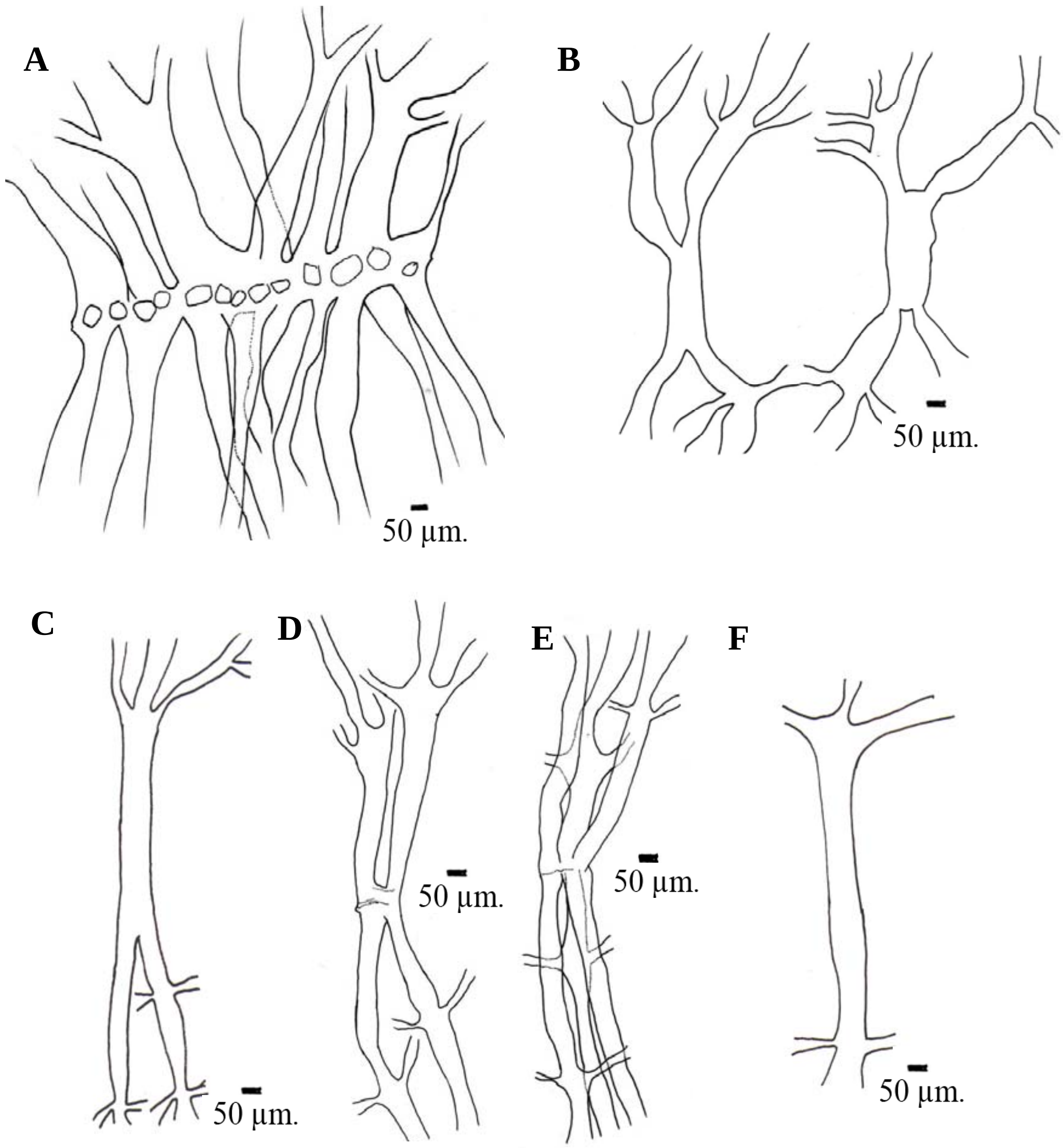


Figure 7 Features of nodal zone: (A) fusion into a single unit with obvious pores connecting adjacent siphons, (B) complete fusion of siphons in pairs or triplets, (C) complete fusion of a siphon pair, (D) and (E) incomplete fusion of siphons in triplets or pairs, (F) siphon going through the node without any kind of fusion

The Reproduction of *Halimeda* J.V.Lamour.

Halimeda is dioecious plant, the male and female gametes are born on different plant. Sexual reproduction is rarely seen in *Halimeda* while asexual reproduction is successful and may contribute substantially to populations. In Thailand, sexual reproduction was observed only in *Halimeda macroloba*.

Sexual Reproduction

In sexual reproduction, reproductive events show seasonal and lunar periodicity (Beth, 1962; Drew and Abel, 1988; Clifton, 1997; Clifton and Clifton, 1999), but certain species are found reproductive in different seasons at different localities (Drew and Abel, 1988). Where observed in detail, gametangial clusters are formed during the night, grow darker during the first day and second night, these green dots that produced in the tip of segment are gametophores and consist of filaments upon which are borne grape-like clusters of globular gametangia (Figure 8) and discharge around dawn of the second day (Hillis-Colinvaux, 1980; Clifton and Clifton, 1999). The gamete is biflagellate. The macrogamete is bigger in size and has eye spot while the microgamete is smaller and no eye spot. We can distinguish between the macrogametangia and microgametangia by different color; macrogametangia is brown to dark green and microgametangia are yellowish green (Clifton and Clifton, 1999). After the gametes have been released the thallus dies and quickly disintegrates. The dead segments are shed until, within two or three days, the plant has gone and the carbonate segments become part of the surface sediments. Released gametes are highly motile and within a few minutes many will fuse with other gametes to form zygotes. There is still conjecture or whether the organism has to pass through another phase before a mature plant is formed. Recent observations have shown that sexual reproduction in *Halimeda* plants is to some extent synchronized. Many individuals in a population may become fertile within a period of only a few days, and sometime on the same day (Figure 9).

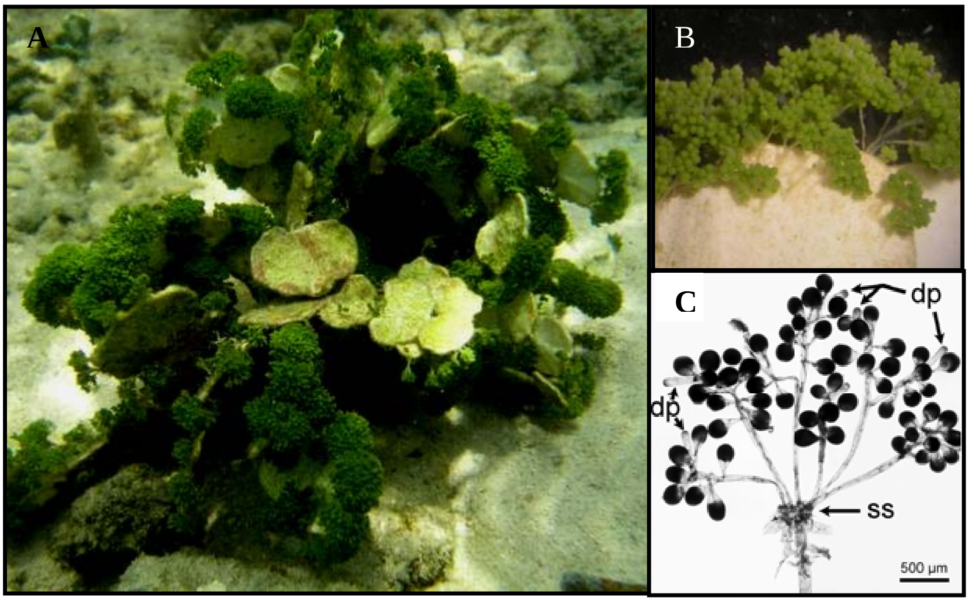


Figure 8 Reproductive structure: (A) and (B) *H. macroloba*; gametangial clusters originating from distal edges of segments, (C) gametophores originating from the main medullary filaments subsequent to nodal fusion at the distal segment edge; dp – discharge pores, ss – segment surface; *H. macroloba* (Verbruggen, 2005)

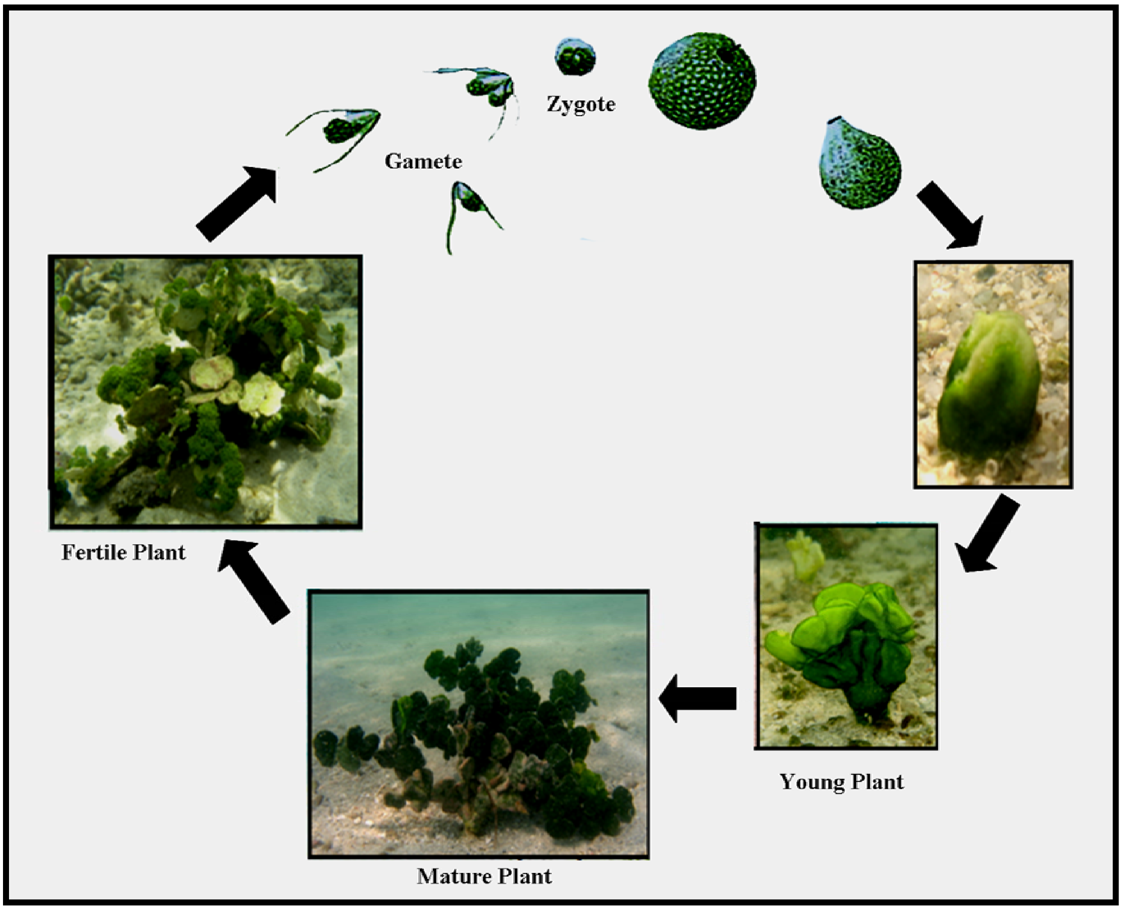


Figure 9 The sexual reproductive cycle of genus *Halimeda* (modified from http://www.aims.gov.au)

Asexual Reproduction

Much of the reproduction of *Halimeda* can propagate very successfully through vegetative cloning (Hillis-Colinvaux, 1980), which enables copies of the same plant to be produced. Many species can produce runners or filaments. These filaments can grow up to 20 cm long, spread laterally through the substrate, then push up to form new segments. These new buds can quickly grow new segments, sometimes at the rate of one a day per growing tip. Eventually the physical connections between the young and parent thallus are lost (Hillis-Colinvaux, 1980).

Other variations of vegetative reproduction have been identified. Sprawling *Halimeda* may produce rhizoidal filaments between segments. These initially provide additional anchorage but they also facilitate division of the alga into separate thalli as the older segments of the branch die and drop off. This method of reproduction appears to be important in maintaining large patches of *Halimeda*. In addition to these more specialized modes: *Halimeda* may also survive and flourish after being fragmented by storms, waves or animals. Healthy branches, which have been separated from the main thallus, can develop holdfast systems. Under favorable conditions they can attach, become established and develop into complete thalli.

The importance and advantage of vegetative fragmentation are increased abundance and distribution of species and colonization of areas. On the other hand, the disadvantage of vegetative fragmentation are reduced in a variety of species and increased in chance of extinction (Walters and Smith, 1994).

The Calcification of *Halimeda* J.V.Lamour.

All species of *Halimeda* deposit calcium carbonate in form of aragonite. In the interfilament space of the cortex and medulla siphon, the deposition of aragonite occur (Wilbur *et al*., 1969; Figure 10). Uncalcified portion are node, apical segments younger than about 36 hours old, and holdfast (Blaxter, 1980). Initial segment growth occurs at night (Hay *et al*., 1988) and calcification, which is dependent upon photosynthesis, starts on the second day of segment development, when the segment is about 36 hours old (Wilbur *et al*., 1969). Segments are fully calcified after about 2–3 days. Flexible thalli allow specimens to grow in habitats characterized by high water movement by aligning the thallus with the current, thus reducing drag. When calcified segments are shed from the thallus after reproduction, they form an important fraction of the sediment and are responsible for much of the sand formation in tropical lagoons (Chapman and Mawson, 1906; Drew, 1983; Freile *et al*., 1995).

In addition, the degree of calcification is an important determinant of color, flexibility and brittleness of *Halimeda* segments. Segment color varies from dark green in barely calcified segments to greenish white in segments of strongly calcified species (Verbruggen, 2005). Then, amount of calcium is the additional character for identification.

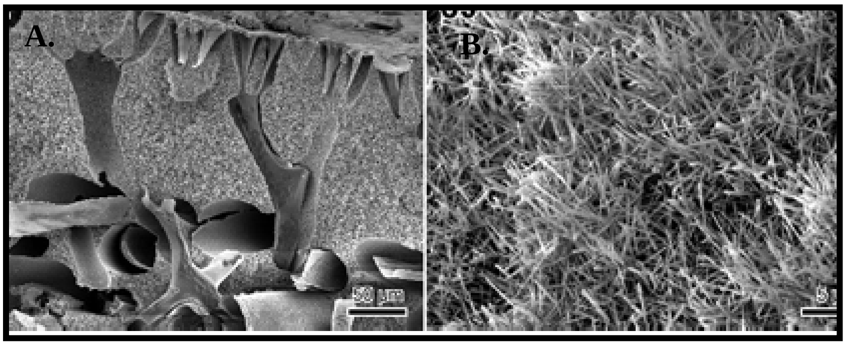


Figure 10 (A) SEM micrograph of cortical zone of fractured segment showing the calcium carbonate depositions in between cortical siphons; *H. gracilis*, (B) SEM micrograph of needle-shaped aragonite crystals; *H. gracilis* (Verbruggen, 2005)

Research questions

1. How many species of genus *Halimeda* J.V.Lamour. are there in Peninsular Thailand?

2. How does *Halimeda* J.V.Lamour. distribute in Peninsular Thailand?

3. Are there any morphological and anatomical variations of *Halimeda* macroloba Decne. in Peninsular Thailand? And how?

Objectives

This study is:

1. To investigate diversity and distribution of genus *Halimeda* J.V.Lamour. in Peninsular Thailand.

2. To investigate morphological and anatomical variations of *Halimeda macroloba* Decne. in Peninsular Thailand.

3. To obtain the details of morphology, anatomy and ecology of these *Halimeda* J.V.Lamour. found in Peninsular Thailand.

CHAPTER 2

METERIALS AND METHODS

Study Site

The study has cover various localities both in the Gulf of Thailand and the Andaman Sea; 1) Chumphon province: Ko Khai, Ao Bomao, Had Thung Vaulan, Ko Pitak, Kabana beach and MuKo Tha Le Chumphon, 2) Nakhon Si Thammarat Province: Ko Tha Rai and Lam Prathub, 3) Surat Thani Province: Ko Samui, Ban Pang-Ka, Bo-Phut Beach, Hue Ha-non, Taling-ngam, Wad Mai, Wat Sila-ngue Ko Tan, Ko Mudsum, Ko Wang Nak, Ko Wang Nai and Ko Rab, 4) Songkhla province: Ko Kham, Samila beach, Ko Meaw and Ko Noo, Kaoseng, 5) Pattani province: Ao Pattani, Ranong province: Ao Khao-Kwai and Ko Payam, 6) Phang-nga province: Similan Marine National Park and Mu Ko Surin Marine National Park, 7) Phuket province: Tang Khen bay, Punwa cape, Makham bay, Ko Pling, Sirinart National Park, Nai Yang beach, Rawai beach, Kata beach, Karon beach, Patong beach, Ko Khai and Ko Ra Cha, 8) Krabi province: Ko Lanta, Kor Kwang beach, Khlong Yang, Seashell Fossil, Ko Sri Bo Ya and Ko Phi Phi, 9) Trang province: Ko Libong, Samran beach, Had Chaomai National Park, Morakot cave, 10) Satun province: Ko Lidee Yai, Ko Lidee Lek, Mu Ko Phetra National Park, Ko Adank, Ko Ravee, Ko Ling (Ao Dong) and Ko Tarutao (Figure 11). This was aimed to collect as much as specimens possible.

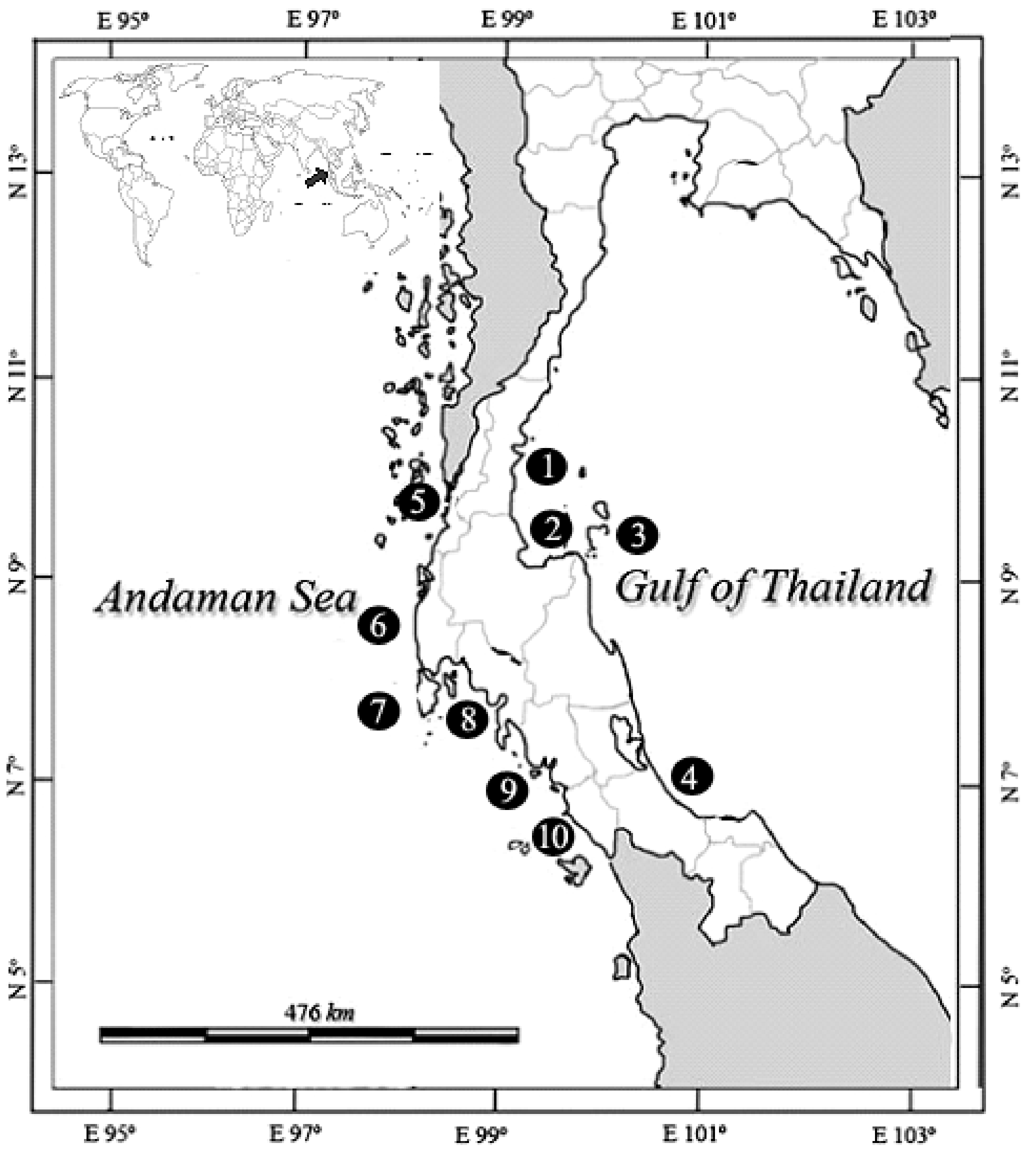


Figure 11 Study site that surveyed the genus *Halimeda* at various localities both in the Gulf of Thailand and the Andaman Sea; (1) Chumphon province, (2) Nakhon Si Thammarat Province, (3) Surat Thani Province, (4) Songkhla province, (5) Pattani province, (6) Phang-nga province, (7) Phuket province, (8) Krabi province, (9) Trang province and (10) Satun province

Specimen Collection and Laboratory Study

The study was conducted from December 2006 to November 2008. Only mature plants were collected from all the surveyed sites both in the Gulf of Thailand and the Andaman Sea. Specimens were preserved in ethanol 95% or formaldehyde 5% for morphometric analyses; silica gel for molecular study if needed and made in the form of herbarium specimen, voucher specimen, to observe and describe their morphology. Ecological data such as depth, wave action, substrate, habitats, habit and some diagnostic characters of each specimen were closely observed noted and taken photo in the field.

The collected specimens were identified using descriptions from available taxonomic literatures (see citations; CHAPTER 3 descriptions of *Halimeda*).

In each specimen, ten segments were randomly chosen after exclusion of deviant segments, apical and non-calcified segments and segments from basal thallus zone (Verbruggen *et al.,* 2005b), to examine characters of morphological and anatomical using morphometric method following Verbruggen *et al.* (2005a). These segments and parts of nodal region were dissected with forceps and needles, after decalcified in 10% HCl, under stereomicroscope. The details of morphology and anatomy were studied and measured under a microscope. Permanent dissections of important characters were made of every specimen. The preparations of permanent slide were mounted in a 50% karosyrups solution.

Surface view of primary utricles, node zone and utricle zone were drawn under Camera Lucida and an Olympus CH2 microscope.

Description of each species was prepared based on specimen collected from the study sites. For the plant name authors and other citations used in this thesis were followed the International Plant Names Index (IPNI) (The Plant Names Project, 1999).

All voucher specimens were deposited at Prince of Songkla University Herbarium (PSU), with at least three duplicate. In addition, I had visited herbarium and loan specimens from Prince of Songkla University Herbarium, Kasetsart

University and Burapa University; this was an attempt to cover all the *Halimeda* found in Thai water.

Morphometrics Method (Verbruggen *et al*., 2005a)

The recent study by Verbruggen *et al*. (2005a) using the morphometric methods allowed us to better understand morphological and anatomical variations within and among species of *Halimeda.* Thus, this study has adopted such techniques to investigate *Halimeda* found in Thailand.

Morphological Variables

Ten randomly chosen segments and nodes per specimen were observed and photographs were taken.

Categorical shape variable

Eight variables of categorical shape segment: segment form, variable describing the proximal stalk zone, the form of the segment base, the form of the segment apical, variable describing the segment's lobed , number of lobes, surface pattern and the diameter of peripheral utricle that attached to the surface were investigated (Table 1).

Conventional measurements

Individual segments were divided into five landmarks. Two landmarks were placed on the right and left sides of the segment's attachment zone, three more were fixed at the left, top and right edge of the segment (Figure 12A). The segment were examined segment length, width, the height where the width is maximal and width of the attachment zone were calculated by following equations (Figure 12B), segment thickness were measured using calipers.

Ratio shape variables

From data of segment size variables (conventional measurements), two ratios segment shape variables were calculated: segment thickness over segment length, and segment thickness over width of attachment zone (Table 1).

Holdfast variables.

The type of holdfast was examined (Figure 13). This was an additional character from Verbruggen.

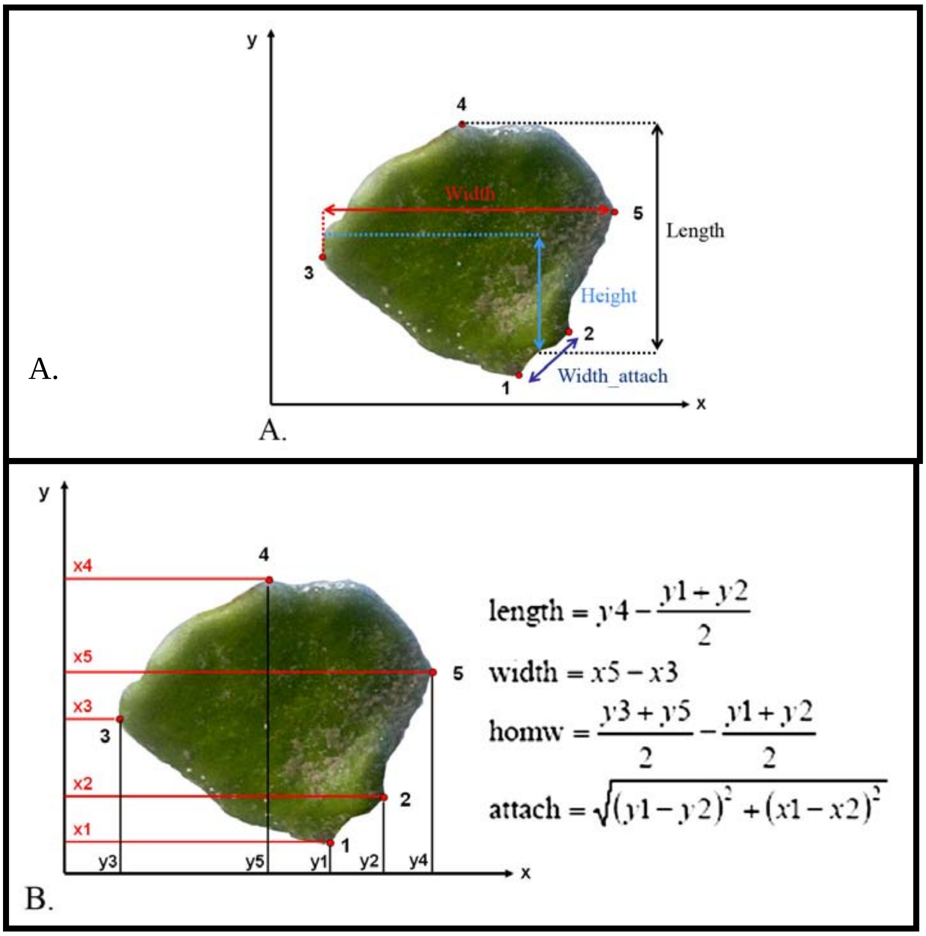


Figure 12 Segment Morphological: (A) conventional measurement, (B) five landmarks of segment and equations for calculated (modified from Verbruggen *et al*., 2005a)

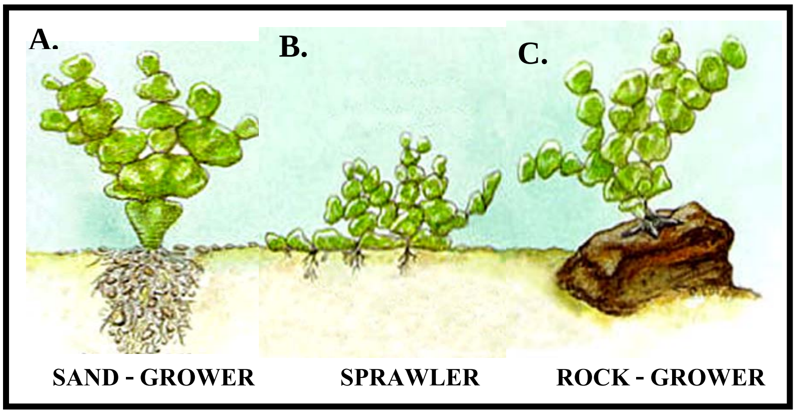


Figure 13 Type of holdfast: (A) sand-grower, bulbous holdfast, (B) sprawler holdfast, (C) rock-grower holdfast (modified from http://www.aims. gov.au)

Table 1 List of morphological variables

Variable	Description
<u>**Categorical shape variables**</u>	
CH 1	**categorical segment form**: 1 = reniform, 2 = subreniform, 3 = discoid, 4 = elliptical-discoid, 5 = ovate, 6 =broad ovate, 7 = obovate, 8 = broad obvate , 9 = cuneate, 10= cylindrical, 11 = subcylindrical
CH 2	**categorical variables describing the proximal stalk zone**: 1 = absent, 2= intermediate, 3 = present
CH 3	**categorical variables for the form of the basal segment:** 1 = reniform, 2 = subreniform, 3 = discoid, 4 = elliptical-discoid, 5 = ovate, 6 =broad ovate, 7 = obovate, 8 = broad obvate , 9 = cuneate, 10= cylindrical, 11 = subcylindrical
CH 4	**categorical variables for the form of the apical segment:** 1 = reniform, 2 = subreniform, 3 = discoid, 4 = elliptical-discoid, 5 = ovate, 6 =broad ovate, 7 = obovate, 8 = broad obvate , 9 = cuneate, 10= cylindrical, 11 = subcylindrical

CH 5	**categorical variables describing the segment's lobedness:** 0 = absent, 1 = shallow, 2 = medium, 3 = deep
CH 6	**numbers of lobes**
CH 7	**categorical variables for the form of surface pattern:** 1 = circle, 2 = polygonal
CH 8	**diameter of peripheral utricle that attached to the surface** (µm)
<u>Conventional measurements</u>	
CH 9	**segment length** (mm)
CH 10	**segment width** (mm)
CH 11	**width of attachment zone** (mm)
CH 12	**height of maximal segment width** (mm)
CH 13	**segment thickness** (mm)
<u>Ratio shape variables</u>	
CH 14	**relative segment thickness over length ratio**
CH 15	**ratio of segment thickness over the width of the attachment zone**
<u>Holdfast variable</u>	
CH 16	**type of holdfast:** 1= bulbous holdfast, 2 = sprawler holdfast, 3 = rock grower holdfast

* **CH** = Character

Anatomical Variables

Ten segments and nodes from previous specimens for morphological study were decalcified in a 10% HCl solution and rinsed in water. All anatomical variables observations were observed under microscope. They were drawn using using Camera Lucida and an Olympus CH2 microscope and taken photo. Permanent slides were prepared for further investigation. In each segment, at least ten replications were randomly measured.

Medulla and node

Seven types of medulla: distance between to subsequent ramifications (µm), medullary siphon diameter (µm), length over diameter ratio of the siphon (µm), and medullar ramification that are the fraction of dichotomous, trichotomous and quadrichotomous ramifications were measured and calculated.

For the node, type of node, height of node belt, pore diameter, distance from below node to supranodal ramification (µm) and thickness of the supranodal interramification (µm) were measured (Table 2 and Figure 14).

Cortex

For peripheral utricles, surface diameter (µm), height (µm), maximal width (µm),width of peripheral utricle at **¾ (**width at **¾** over width ratio)**, ½ (**width at **½** over width ratio) **, ¼ (**width at **¼** over width ratio) of its height and height over width ratio were measured (Figure 15).

For secondary and tertiary utricles were measured length (µm), maximal width (µm), length over maximal width ratio and number of peripheral utricle carried by the secondary utricle for secondary and number of secondary utricle carried by the tertiary utricle for tertiary utricles (Table 2 and Figure 15); and these replicate measurements were averaged to single value for each segment.

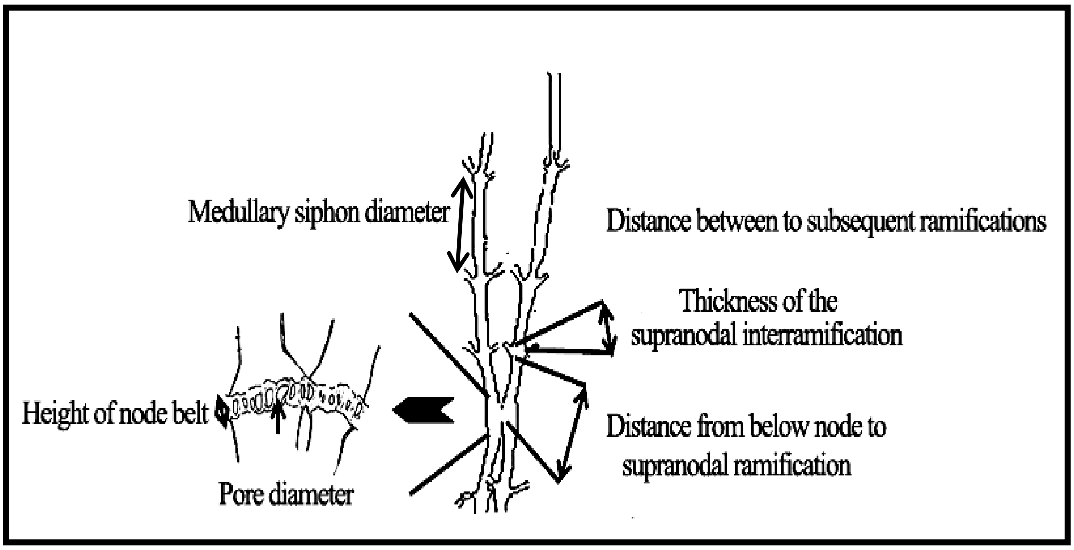


Figure 14 Anatomical variables of node (modified from Verbruggen *et al.*, 2005a)

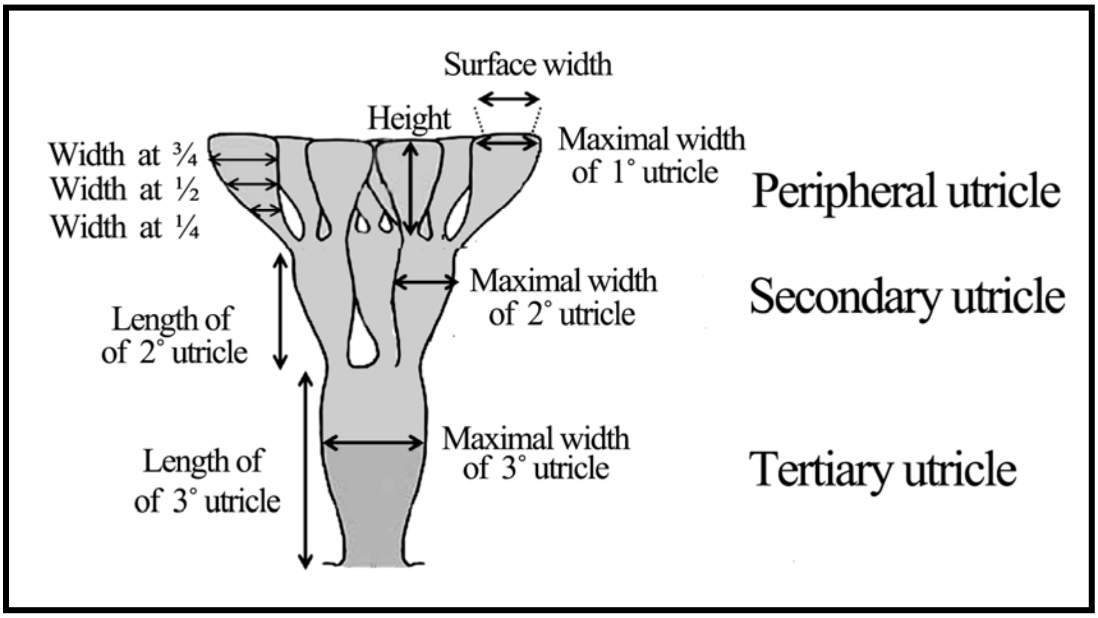


Figure 15 Anatomical variables of utricles (modified from Verbruggen *et al.*, 2005a)

Table 2 List of anatomical variables

Variable	Description
Medullar properties	
CH 17	**distances between two subsequent ramifications**(µm)
CH 18	**medullary siphon diameter** (µm)
CH 19	**lengths over diameter ratio of the siphon**
CH 20	**constriction of main branch diameter** (µm)
CH 21	**fraction dichotomous ramifications:** 0 = absent, 1 = present
CH 22	**fraction trichotomous ramifications:** 0 = absent, 1 = present
CH 23	**fraction quadrichotomous ramifications:** 0 = absent, 1 = present
Nodal properties	
CH 24	**type of node:** 1 = fusion into a single unit, 2 = complete fusion of siphons, 3 = incomplete fusion of siphons, 4 = filament unfusion, 5 = node composed of a single filament
CH 25	**height of node belt** (µm)
CH 26	**pore diameter** (µm)
CH 27	**distances from below node to supranodal ramification** (µm)
CH 28	**thickness of the supranodal interramification** (µm)
Peripheral utricles	
CH 29	**surface diameter peripheral utricle** (µm)
CH 30	**height of peripheral utricle** (µm)
CH 31	**maximal width of peripheral utricle** (µm)
CH 32	**relative width of peripheral utricle at ¾ of its height**

CH 33	**relative width of peripheral utricle at ½ of its height**
CH 34	**relative width of peripheral utricle at ¼ of its height**
CH 35	**relative height of the peripheral utricle: height over width ratio**
<u>Secondary utricles</u>	
CH 36	**heights of the secondary utricle** (µm)
CH 37	**maximal widths of the secondary utricle** (µm)
CH 38	**relative length of secondary utricle: height over width ratio**
CH 39	**number of peripheral utricle carried by the secondary utricle**
<u>Tertiary utricles</u>	
CH 40	**heights of the tertiary utricle** (µm)
CH 41	**maximal widths of the tertiary utricle** (µm)
CH 42	**relative length of utricle: height over width ratio**
CH 43	**number of secondary utricle carried by the tertiary utricle**

* **CH** = Character

Statistical Analysis

Cluster analysis and Principal component analysis (PCA) were conducted using PC-ORD version 3.2, to determine diversity, distribution and variations among populations within species.

Canonical correspondence analysis (CCA) the most widely used direct gradient ordination method was employed to determine the multivariate correlations between distribution and environmental factors; morphological and anatomical variations and environmental factors.

SPSS version 14.0 for Window was used to analyze data; significance level 95% and 99% were used. Because the data were normal distributed, 1-Way ANOVA, was used for test for differences of morphological and anatomical character among groups of *H. macroloba*.

CHAPTER 3

RESULT

Morphometric Data

A total of sixty-eight specimens of genus *Halimeda* (Appendix 1) were examined. A total of 680 segments were dissected; and 6800 utricles, 6800 medullar siphon and 680 nodal structures were closely observed and measured.

Diversity of Genus *Halimeda* J.V.Lamour. in Peninsular Thailand

Principal Component Analysis and Cluster analysis

Ordinations of morphometric data (Figure 16) showsed the biplots of the Principal Component Analysis based on the morphological and anatomical data. Most of the genotypic cluster occupied non-overlapping regions. Some of the genotypic cluster showed complete overlap (e.g. HSP 00001- HSP 00036, HSP 00006- HSP 00020, HSP 00005 – HSP 00007 –HSP 00009 – HSP 00034, HSP 00010 – HSP 00011- HSP 00012; encircled in Figure). Shape variables and segment size were located in the first and second quadrants whereas anatomical variables concentrated in the first, second and third quadrants. There were separated into 17 groups. The first and second quadrant contented nine groups of *Halimeda*.

Cluster analysis of *Halimeda* taxa based on morphological and anatomical data consisted of more than 50 divergent clusters, percent chaining is 2.4 (Figure 17). There were 17 groups separated; similar to PCA analysis.

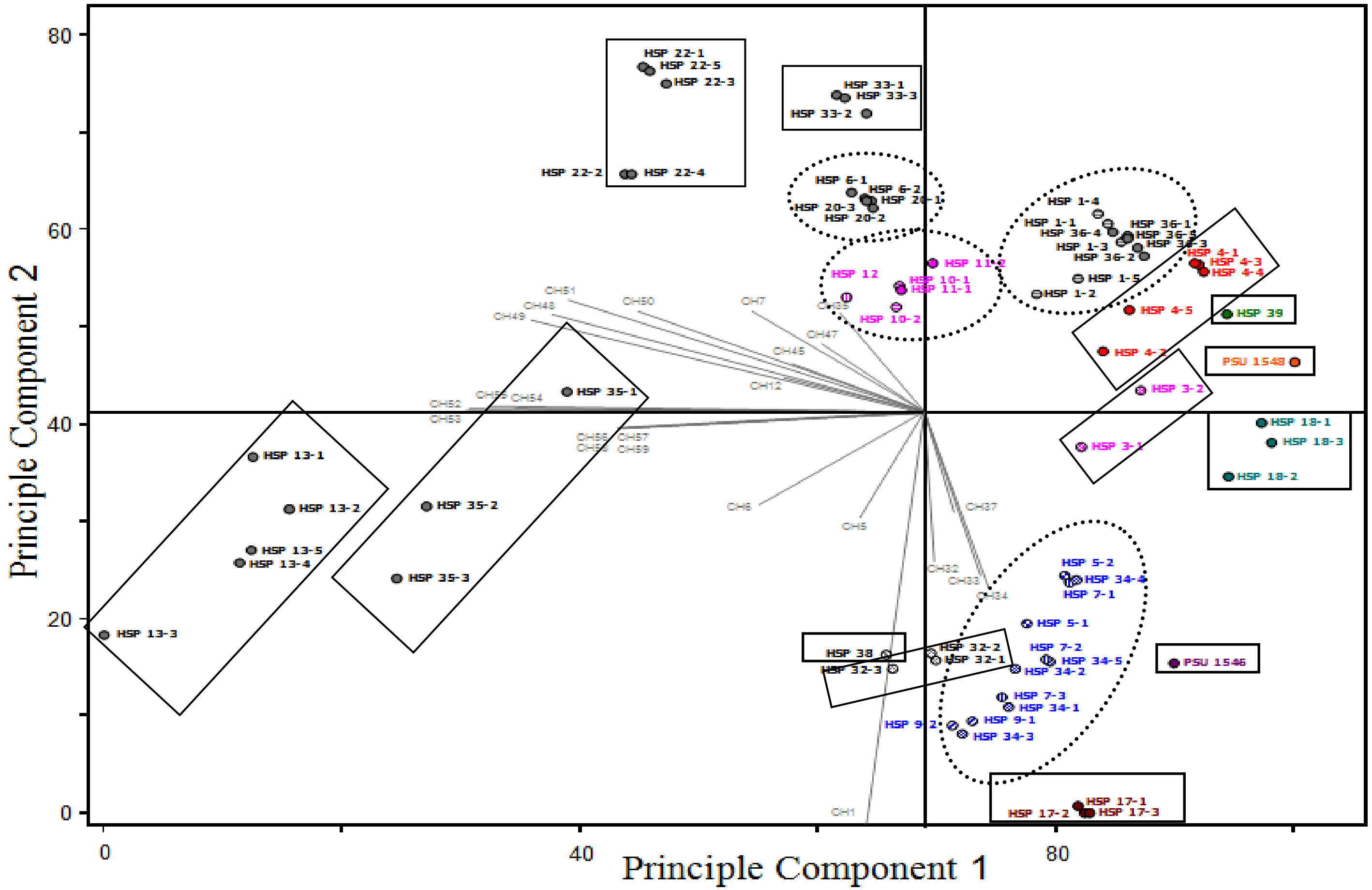


Figure 16 Principal Component Analysis biplots of morphological and anatomical data.

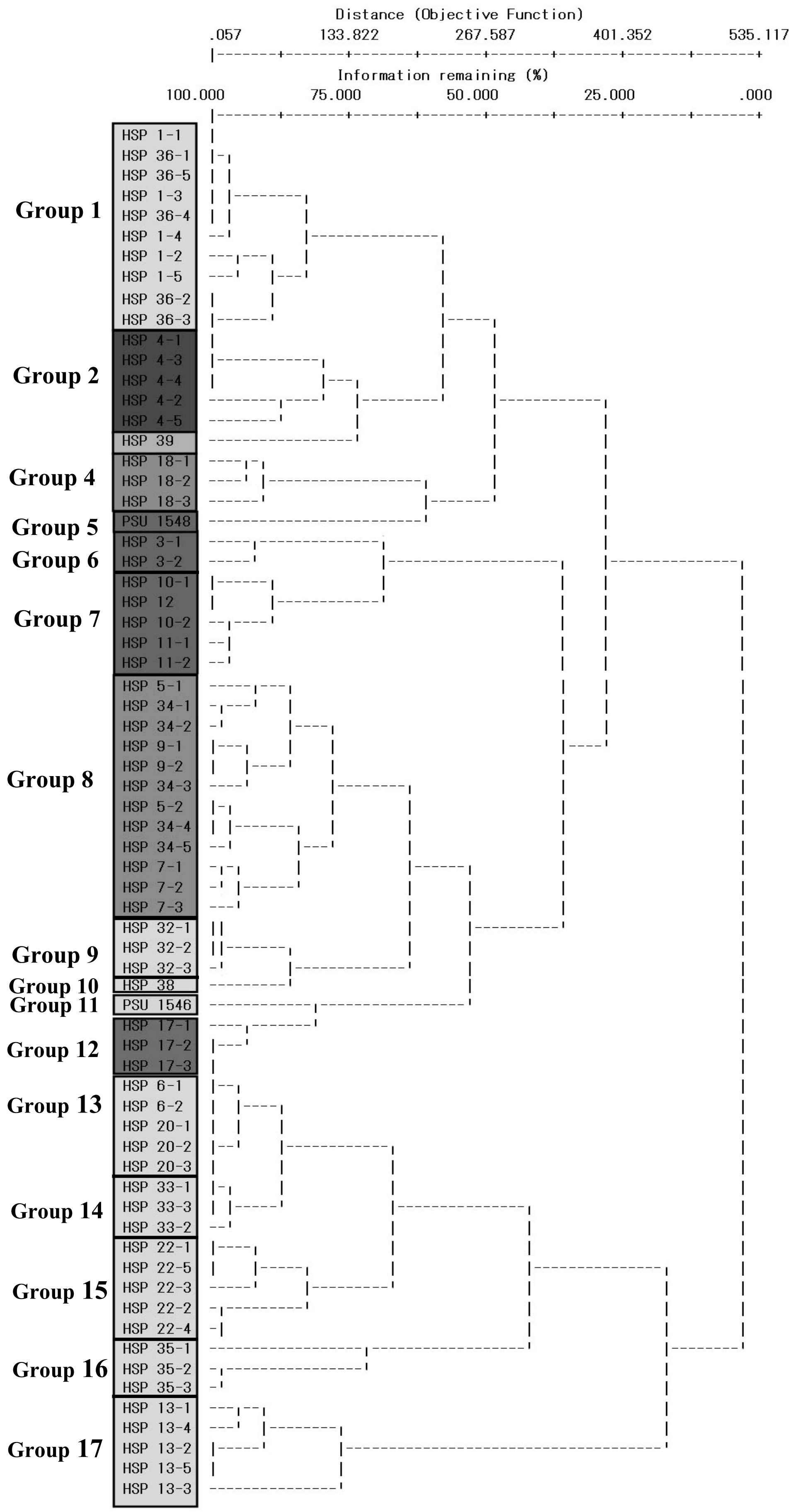


Figure 17 Cluster Analysis of *Halimeda* taxa based on morphological and anatomical data. Percent chaining is 2.4

Taxonomic Diversity

These characters of seventeen groups were classified into 8 species (Table 3) (See additional information in descriptions of *Halimeda* section). **The first species** consists of specimens in group 1, 9, 10, 13, 14, 15, 16 and group 17. The characters used for grouping these together are 1) type of holdfast- bulbous holdfast, 2) thallus appearance- erect, 3) shape of segment- broad cuneate or rounded or broad ovate to reniform or subreniform, 4) surface pattern- circle, 5) type of node- fused in a single units with many pores, 6) number of utricles- 3 to 7 layers. However, there are variations in size of these characters between groups. Then, this species was further investigated in morphological and anatomical variations among populations (See: morphological and anatomical variations section). **The second species** consists of specimens in group 6 and 7. The characters used for grouping these together are 1) type of holdfast- sprawler holdfast, 2) thallus appearance- *sprawling*, 3) shape of segment- reniform, 4) surface pattern- polygonal, 5) type of node- incomplete fusion in small group of siphons, 6) number of utricles- 3-5 layers. **The third species** was specimens in group 11, the characters used for grouping these together are 1) type of holdfast- rock-grower holdfast, 2) thallus appearance- erect, 3) shape of segment- middle segments broadly cuneate, outer segments usually cylindrical or cuneate, 4) surface pattern- polygonal, 5) type of node- complete fusion in pair siphons, 6) number of utricles- 2 layers. **The forth** was specimens in group 4. The characters used for grouping these together are 1) type of holdfast- rock-grower holdfast, 2) thallus appearance- pendent, 3) shape of segment- broad ovate, discoidal or reniform, 4) surface pattern- polygonal, 5) type of node- complete fusion in pair siphons, 6) number of utricles- 3 layers. **The fifth species** was specimens in group 2. The characters used for grouping these together are 1) type of holdfast- bulbous holdfast, 2) thallus appearance- erect, 3) shape of segment- elliptical-discoid to subreniform, sometime cuneate, 4) surface pattern- polygonal, 5) type of node- fused in a single units, 6) number of utricles- 4-5 layers. **The sixth** was specimens in group 5. The characters used for grouping these together are 1) type of holdfast- rock-grower holdfast, 2) thallus appearance- pendent, 3) shape of segment- discoidal to reniform, 4) surface pattern- polygonal, 5) type of node- complete fusion in pair siphons, 6) number of utricles- 3-5 layers. **The seventh species** was specimens in group 8, the characters used for grouping these together are 1) type of holdfast- rock-grower holdfast, 2) thallus appearance- pendent, 3) shape of segment- broad-ovate, elliptical-discoid, broad obovate, broad cuneate to reniform, 4) surface pattern- polygonal, 5) type of node- fused in a single units, 6) number of utricles- 3-4 layers. **The last species** was specimens in group 12, the characters used for grouping these specimens together are 1) type of holdfast- rock-grower holdfast, 2) thallus appearance- erect, 3) shape of segment- cylindrical, cuneate to broad cuneate, broad ovate to discoidal or rarely reniform, 4) surface pattern- polygonal, 5) type of node- incomplete fused in two filaments for a short distance, 6) number of utricles- 3 layers.

Specimens were classified into 8 species in this study following the identification keys and descriptions namely: *Halimeda macroloba* Decne., *H. discoidea* Decne., *H. opuntia* (L.) J.V.Lamour., *H. heteromorpha* N'Yeurt, *H. tuna* (J. Ellis & Solander) J.V.Lamour., *H. borneensis* W.R. Taylor, *H. gigas* W.R. Taylor and *Halimeda* sp. There were 4 new records to Thailand; *Halimeda borneensis, H. gigas*, *H. heteromorpha* and *H. tuna.* Additionally, *Halimeda* sp. was expected to be a new species (Table 4).

Table 3 Morphological and anatomical characteristics of species.

Species	Members	Character																													
		Types of Holdfast			Thallus Appearance			Shapes of segment											Surface Pattern		Types of node					Number of Utricles					
		A 1	A 2	A 3	B 1	B 2	B 3	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	C 11	D 1	D 2	E 1	E 2	E 3	E 4	E 5	F 1	F 2	F 3	F 4	F 5	F 6
1	Group 1	*			*			*	*			*	*			*			*		*						*	*			
	Group 9	*			*			*	*				*			*			*		*						*	*	*		
	Group 10	*			*				*			*							*		*						*	*	*		
	Group 13	*			*			*					*			*			*		*						*	*	*		
	Group 14	*			*			*											*		*						*	*	*		
	Group 15	*			*				*			*				*			*		*							*	*	*	
	Group 16	*			*			*				*				*			*		*							*	*	*	
	Group 17	*			*			*	*				*						*		*								*	*	*
2	Group 6		*				*	*												*			*				*	*	*		
	Group 7		*				*	*												*			*				*	*	*		
3	Group 11			*	*				*	*	*						*			*		*				*					
4	Group 4			*		*		*		*			*							*		*					*				
5	Group 2	*			*				*		*					*				*	*							*	*		
6	Group 5			*	*			*	*											*		*					*	*	*		
7	Group 8			*	*			*			*		*		*					*	*						*	*			
8	Group 12			*	*			*		*		*	*			*	*			*			*				*	*	*		

Note: **Type of Holdfast**; A1 = Bulbous holdfast, A2 = Sprawler holdfast, A3 = Rock-grower holdfast, **Thallus Appearance**; B1 = erect, B2 = pendant, B3 = sprawling, **Shape of Segment**; C1 = reniform, C2 = subreniform, C3 = discoid, C4 = elliptical discoid, C5 = ovate, C6 =broad ovate, C7 = obovate, C8 = broad obvate , C9 = cuneate, C10= cylindrical, C11 = subcylindrical, **Surface pattern**; D1 = Circle, D2 = Polygonal, **Type of node**; E1 = fusion into a single unit, E2 = complete fusion of siphons, E3 = incomplete fusion of siphons, E4 = filament unfusion, E5 = node composed of a single filament, **Number of Utricles**; F1 = 2, F2 = 3, F3 = 4, F4 = 5, F5 = 6, F6 = 7

Species 1: (A1)(B1)(C1,2,5,6,9)(D1)(E1)(F2,3,4,5,6) = *Halimeda macroloba* Decne.

Species 2: (A2)(B3)(C1)(D2)(E3)(F2,3,4) = *H. opuntia* (L.) J.V.Lamour.

Species 3: (A3)(B1)(C2,3,4,10*)(D2)(E2)(F1) = *H. discoidea* Decne. (*= Apical segment)

Species 4: (A3)(B2)(C1,3,6)(D2)(E2)(F2) = *H. tuna* (J. Ellis & Solander) J.V.Lamour.

Species 5: (A1)(B1)(C2,4,9)(D2)(E1)(F3,4) = *H. borneensis* W.R. Taylor

Species 6: (A3)(B1)(C1,2)(D2)(E2)(F2.3.4) = *H. gigas* W.R. Taylor

Species 7: (A3)(B1)(C1,4,6,8)(D2)(E1)(F2,3) = *H. heteromorpha* N'Yeurt

Species 8: (A3)(B1)(C1,3,5,6,9,10)(D2)(E3)(F2,3,4) = *Halimeda* sp.

Table 4 Species lists of genus *Halimeda* found in Peninsular Thailand.

Scientific name	**Collector number**	**Geographical origin**		
		Locality	**Province**	
Halimeda borneensis*	S. Pongparadon 4 KL 5907 PA 0163 C.Kaewsuralikhit	Tang Khen Bay Ko Rawai Ko Ra Ao Khao-Kwai	Phuket Phuket Ranong Ranong	Andaman sea Andaman sea Andaman sea Andaman sea
H. discoidea	P. Tuntiprapas 1 P. Tuntiprapas 2 P. Wanichaphon	Similan Island Similan Island Similan Island	Phang-nga Phang-nga Phang-nga	Andaman sea Andaman sea Andaman sea
***H. gigas* ***	P. Tuntiprapas 3	Similan Island	Phang-nga	Andaman sea
***H. heteromorpha* ***	S. Pongparadon 5 S. Pongparadon 7 S. Pongparadon 9 S. Pongparadon 34	Tang Khen Bay Tang Khen Bay Tang Khen Bay Shell Fossils Beach	Phuket Phuket Phuket Krabi	Andaman sea Andaman sea Andaman sea Andaman Sea
H. macroloba	S. Pongparadon 1 S. Pongparadon 6 PA 0148 S. Pongparadon 36 S. Pongparadon 20 S. Pongparadon 32 S. Pongparadon 33 S. Pongparadon 38 S. Pongparadon 22 S. Pongparadon 13 PA 0121 K. Lewmanomont S. Pongparadon 35	Tang Khen Bay Tang Khen Bay Tang Khen Bay Ko Lidee Lek Ko Lidee Yai Ko Pi-Tuk Had Bo-moa Ko Tan Ko Tan Ko Samui Ko Samui Ko Wang Nai Ko Tha Rai	Phuket Phuket Phuket Satun Satun Chumporn Chumporn Surat Thanii Surat Thanii Surat Thanii Surat Thanii Surat Thanii Nakorn Sri Thummarat	Andaman sea Andaman Sea Andaman sea Andaman sea Andaman Sea Gulf of Thailand Gulf of Thailand Gulf of Thailand Gulf of Thailand Gulf of Thailand Gulf of Thailand Gulf of Thailand Gulf of Thailand
H. opuntia	S. Pongparadon 3 K. Lewmanomont PA 0206 S. Pongparadon 10 S. Pongparadon 11 S. Pongparadon 12 HEC 16129 SR-SW-177 PP-093	Tang Khen Bay Ko Rawai Ko Rawai Ko Ravee Ao Dong Ko Ravee Kor Kwang Cape, Ko Lanta Yai Mu Ko Surin Ko Phi Phi	Phuket Phuket Phuket Satun Satun Satun Krabi Phang-nga Phang-nga	Andaman sea Andaman sea Andaman sea Andaman sea Andaman sea Andaman sea Andaman sea Andaman sea Andaman sea
***H. tuna* ***	S. Pongparadon 18 HEC 16180	Ko Pling Ko Pling	Phuket Phuket	Andaman Sea Andaman Sea
Halimeda* sp. *	S. Pongparadon 17 HEC 16155	Klong Yang Klong Yang	Krabi Krabi	Andaman Sea Andaman Sea

Note: * New record; ** Expected to be a new species

Comparative Morphology and Anatomy Characters

Eight main characters of genus *Halimeda* were compared between species; holdfast type, thallus form, segment size, segment shape, primary utricle in surface view pattern, size of primary utricle in surface view, node type and number of utricle layers.

The most apparent differences among the eight species are the type of holdfast and thallus form. There are three type of holdfast; bulbous holdfast, rock-grower holdfast and sprawler holdfast. *H. macroloba* and *H. borneensis* have bulbous holdfast anchored in sand by bulbing of rhizoid and sand or mud (Table 5, Figures A1, A5), *H. tuna, H. gigas, H. heteromorpha, H. discoidea and Halimeda* sp. have rock-grower holdfast that attached to rocky or dead coral substrata by flat like mat of rhizoid (Table 5, Figures A2, A3, A4, A7, A8); the last species *H. opuntia* has a sprawler holdfast. In additional, *H. macroloba* has a reduced holdfast when growing on sand-covered rock or dead coral (personal observation). There are three thallus form in this study: erect, pendent and sprawling. Thallus were sprawling in *H. opuntia* and pendent or hanging in *H. tuna,* while other species were erect (Table 5, Figures A1- A8).

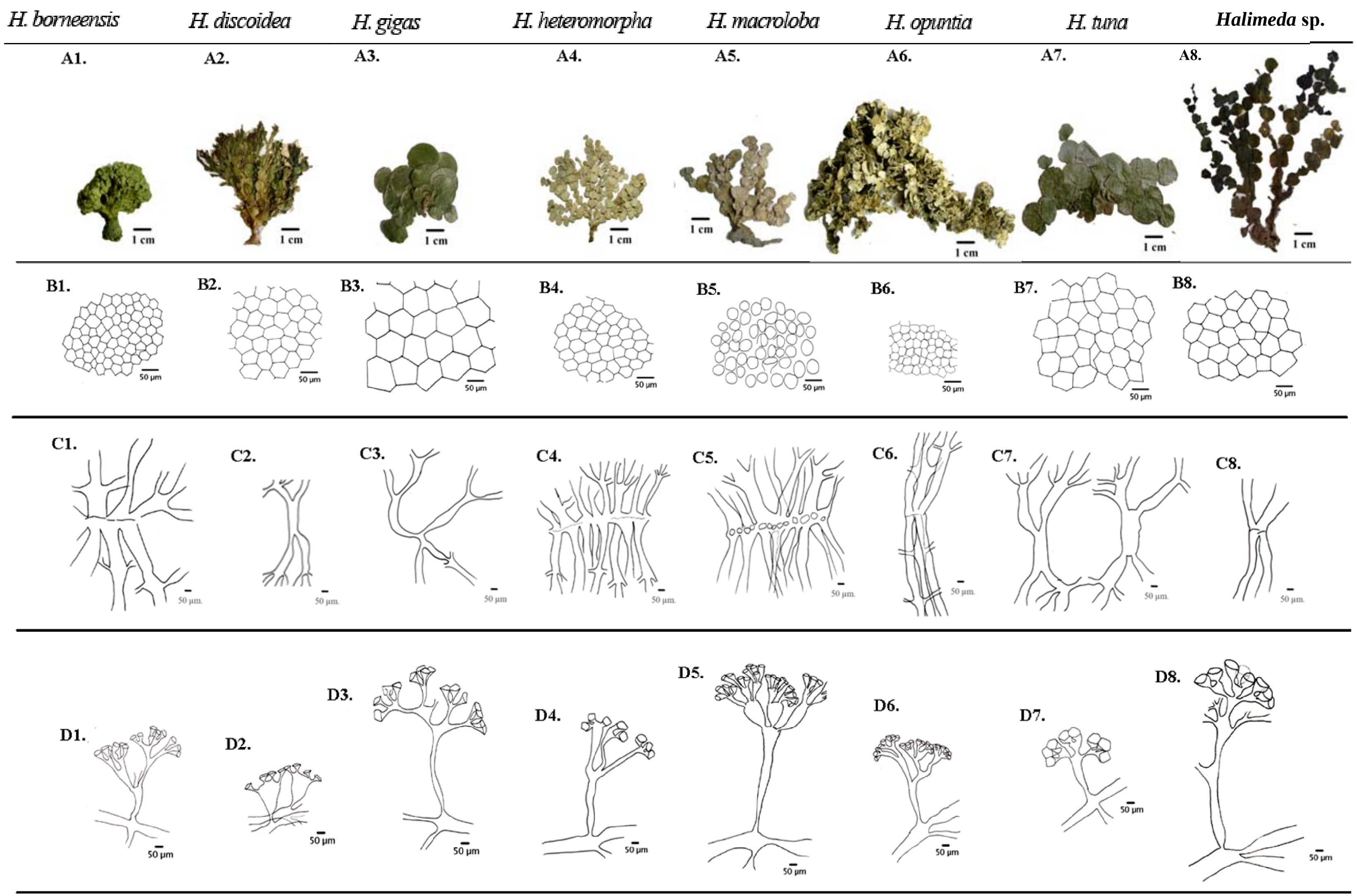
Segments range from 0.1 mm in *H. opuntia* to over 2.5 cm in *H. macroloba*. Segment shape also varies considerably; they are reniform, ovate, elliptical, obovate and cuneate. Segments of *H. discoidea* are normally discoidal but outer margin segments are cylindrical or cuneate (Table 5, Figure A2). Basal segment of *H. macroloba* compressed-cylindrical, rectangular and the majority of segments were subcunate or rounded to subreniform (Table 5, Figure A5).

In surface view, the appearance of peripheral utricles depends mostly on their degree of attachment. When they were fully attached, the surface view is one of polygons (mostly hexagonal) closely fitted together. When the attachment is less strict, polygons become rounded in the corners or get reduced to loose circles when the utricles are not attached at all (Table 5, Figure B5). There are two patterns of the peripheral utricles in surface view: circle pattern and polygonal pattern. Whereas those of other species are polygonal pattern, the surfaces view of primary utricle of *H. macroloba* is irregularly circle pattern (Table 5, Figures B1- B8). Then, *H. macroloba* can distinguish from the other species. Furthermore, size of polygonal pattern can be used to distinguish between *H. gigas* and *H. opuntia*, *H. gigas* has a much larger size than the other species (Table 5, Figure B3) and *H. opuntia* has the smallest size (Table 5, Figure B6).

Nodal type of *Halimeda* in peninsular Thailand consisted of three types. The first, filaments at node are fused into a single unit: *H. borneensis, H. heteromorpha* and *H. macroloba*. Nodal zone of *H. macroloba* has many large pores; while there were no pore at nodal zone in *H. borneensis* and *H. heteromorpha* are not (Table 5, Figures C1, C4, C5). The second type of nodal fusion is incomplete fused in small group of siphons, two or three filaments, for a short distance. Filament at node of *H. opuntia* is fused in two or three (Table 5, Figure C6) whereas *Halimeda* sp. is usually fused in pair (Table 5, Figure C6). The last type of node is complete fusion of siphons in pairs, with chaotic branching of siphons beneath the node, *H. tuna* (Table 5, Figure C7) and without chaotic branching of siphons beneath the node, *H. discoidea, H. gigas* (Table 5, Figures C2, C3).

The cortex zone, considerable size and shape variations were observed in utricles. Utricle size and shape is diagnostic for a few species. In this study, secondary utricles of some species are strongly inflated. Opposite to that, secondary utricles can be non-inflated, cylindrical tubes. For example, secondary utricles of *H. discoidea* are cuneate and bigger than other species (Table 5, Figure D2), whereas other species shown cylindrical secondary utricle. Most species, however, have highly similar cortex features and are usually diagnosed on the basis of size characteristics of the utricles. There is only species that the primary utricle are separating after decalcification, *H. macroloba* while other species are remaining attach. In addition, there is difference in the number of utricle between these *Halimeda.* For instance, there are two layers of utricle in *H. discoidea* (Table 5, Figure D2) and five to seven layers in specimens of *H. macroloba* (Table 5, Figure D5); other species consisted of three- five layers.

Table 5 General morphology, surface views of segments, nodal fusion and shapes of utricles of different species of *Halimeda* J.V.Lamour.



The first characters that divided the genus *Halimeda* in Peninsular Thailand are the habit and number of holdfast per thallus. Both character separated the specimens into two groups; thalli sprawling with more than one holdfast and thalli erect with only one holdfast. First, these characters can distinguish *H. opuntia* from other species, only species in peninsular Thailand is sprawling thalli and there are more than one holdfast regions. The second character that are separating these specimens of group 2 (thalli erect with only one holdfast) is holdfast type. This group is consisted of two type of holdfast. The first holdfast type is bulbous holdfast group: *H. macroloba* and *H. borneensis*; and the second holdfast type is rock-grower holdfast group: *H. tuna, H. gigas, H. heteromorpha, H. discoidea* and *Halimeda* sp. For bulbous holdfast group can be separated using by the pattern of peripheral utricle in surface view. Surface pattern of *H. macroloba* are irregulars circle pattern while *H. borneensis* are irregular polygonal pattern. The forth character is node type. Relying on this character to distinguish specimens in rock-grower holdfast group are consisted of two types of nodal: fusion into small group of siphon, two or three filaments, and fusion in a single unit. This character can distinguish *H. heteromorpha* from other species, *H. tuna, H. gigas, H. discoidea* and *Halimeda* sp. There are several diagnostic features separating these four species: (i) the fusion at node of *H.* sp. is incomplete fusion while other three species are complete fusion; (ii) the median diameter of second utricle is larger in *H. discoidea* than in *H. tuna* and *H. gigas*; (iii) the last character that separated *H. tuna* and *H. gigas* is median surface diameter of peripheral utricle, diameter of primary utricle in *H. tuna* are smaller than in *H. gigas*. In additional, utricle layer of *H. gigas* consisted of 3 or 4 layers whereas there are three layers in *H. tuna*. Complete fusion of siphons in pairs, with chaotic branching of siphons beneath the node shown in *H. tuna* while *H. gigas* are complete fused without chaotic branching of siphons beneath the node (Figure 18). The key to species of genus *Halimeda* in Peninsular Thailand is presented in page 46.

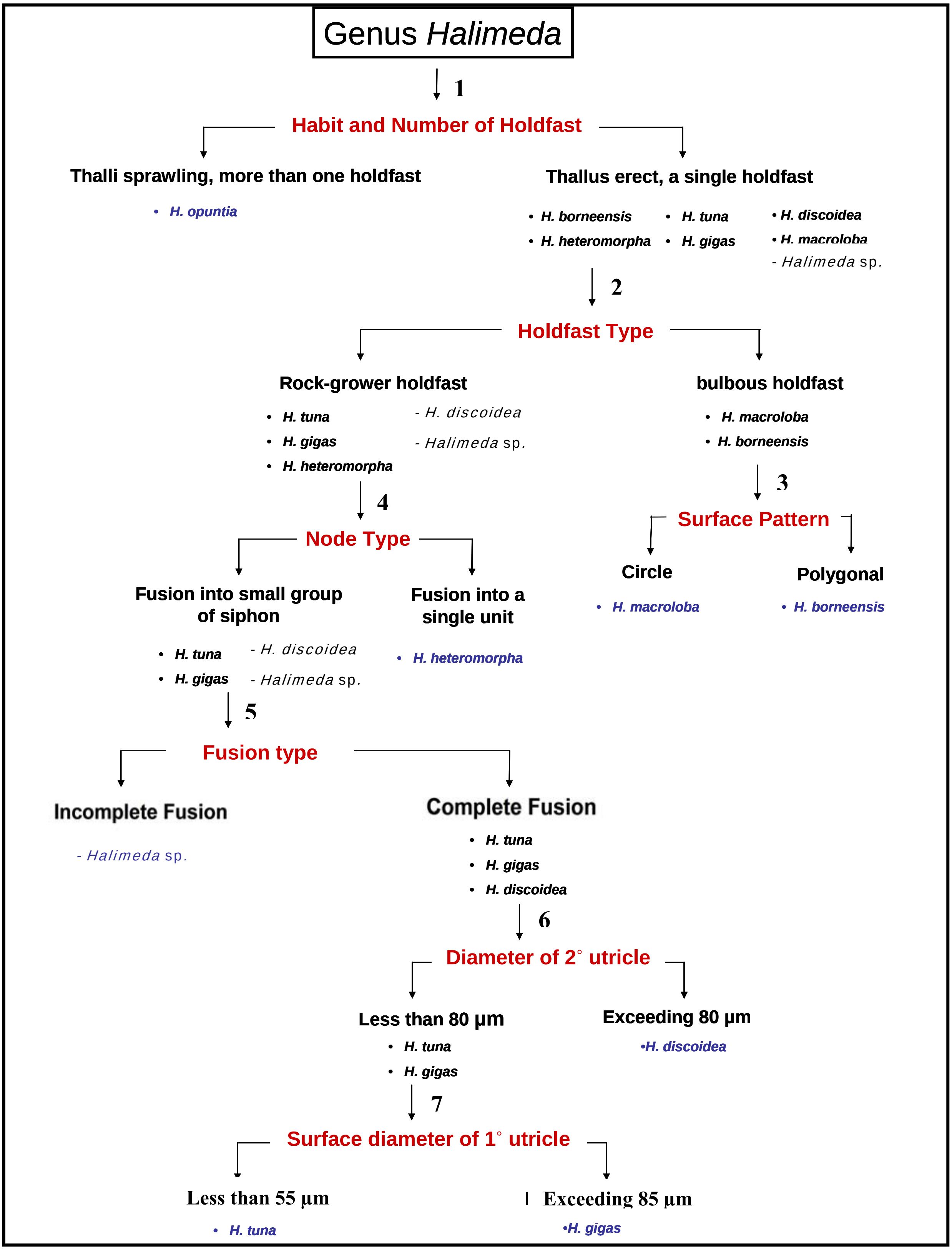


Figure 18 Diagram to distinguish genus *Halimeda* J.V.Lamour. in Peninsular Thailand

Key to species of genus *Halimeda* J.V.Lamour. in Peninsular Thailand

1a. Thalli erect, single holdfast………………………………………………………… 2

1b. Thalli sprawling, more than one holdfast region that occurring where segment contact with substrate………………………………… ***Halimeda opuntia***

2a. Plants anchored in sandy substrata or lose substrate, bulbous holdfast……….... 3

2b. Plants attached to rock and dead coral or hard substrate, felt-like holdfast …….. 4

3a. Surface pattern, circle; peripheral utricle seperated after decalcification...……….. ***H. macroloba***

3b. Surface pattern, polygonal; peripheral utricles remain attachted after decalcification…………………………………..…………..……***H. borneensis***

4a. Fusion into a single unit of siphon at nodal zone……………....***H. heteromorpha***

4b. Fusion in small groups of siphon, triplets or pair, at nodal zone………….….... 5

5a. Incomplete fusion in pair of nodal filament………………………. ***Halimeda* sp.**

5b. Complete fusion in pair of nodal filament …………………………………….. 6

6a. Diameter of secondary utricles inflated, generally exceeding 80 µm……………. ………………..……………………………………………………***H. discoidea***

6b. Diameter of secondary utricles less than 80 µm……………………………….. 7

7a. Surface diameter of peripheral utricles exceeding 85 µm………………..***H. gigas***

7b. Surface diameter of peripheral utricles less than 55 µm………………….***H. tuna***

Descriptions of *Halimeda* J.V.Lamour.

Full descriptions are as follows:

1. ***Halimeda borneensis*** W.R. Taylor, Contr. Univ. Michigan Herb.11: 81-83. 1975; Hillis-Col., Advances in Marine Biology 17: 105-108, Figure. 27. 1980. **Figures 19A-19D**.

Thallus erect, compact, bushy and rather small, 2 to 3 cm high and 4 to 5 broad, anchored in sandy substrates by 0.5 to 1 cm long of bulbous holdfast; Bright green to greenish when fresh, greenish when dried, calcification moderately heavy; branching mainly trichotomous or more than four branching of segments, often polychotomous branching in the basal region; basal segment compressed-rectangular and supporting suprabasal segment fan-shaped; other segments elliptical-discoid to subreniform, sometime cuneate, the upper margin of which is entire or shallowly lobed, 0.9 to 1.1 cm long and 0.9 to 1.0 cm broad (Figure 19A). All nodal medullar siphons fused together into a single unit, an obvious adhesion node belt of 12.50 to 27.50 µm high, the adjacent filaments communicating by many small pores or absent; supranodal siphon 70 to 105 µm long and 17.50 to 32.50 µm in diameters; medullar siphon ramify in dichotomies, 15 to 20 µm in diameters (Figure 19C). Cortex generally composed of 3 to 4 utricles layers of utricles; outermost utricles generally remaining attached after decalcification; peripheral utricles 35 to 62.50 µm in diameters and 55 to 87.50 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of polygonal, 30 to 33 µm in diameters (Figure 19B); Secondary utricles 37.50 to 62.50 µm in diameter and 57.50 to 287.50 µm long; heights over diameters ratio ranging from 3.83 to 13.19 (Figures 19D - 19E).

Habitats.— This species grows in a sandy-bottom habitats in sandy beach, sand patch in coral reef, mud or other unconsolidated substrate, in shallow water or intertidal zone.

Specimens Examined.— S. Pongparadon 4, A. Prathep 0163, KL 5907, C. Kaewsuralikhit, PA 0163

Peninsular Thailand Distributions.— *Andaman Sea*: Ko Ra, Ko Pitak, Plumesuk beach, Rawai Beach and Tang Khen Bay.

Worldwide Distributions.— *Africa*: Tanzania; ***South-east Asia*:** Indonesia, Philippines, Thailand; ***Australia and New Zealand***: Papua New Guinea, Queensland; ***Pacific Islands***: Fiji, French Polynesia, New Caledonia, Tahiti

Remarks.— *Halimeda borneensis* is new record of Thailand. This species may be mistaken with *H. simulans.* The growth form and segment shape of *H. borneensis* is similar to *H. simulans*. There is differences in primary utricles of *H. borneensis* has a larger *H. simulans*. In addition, *H. simulans* is known to be common in and distribution only in the Atlantic where *H. borneensis* distributed in the Indo-Pacific region.

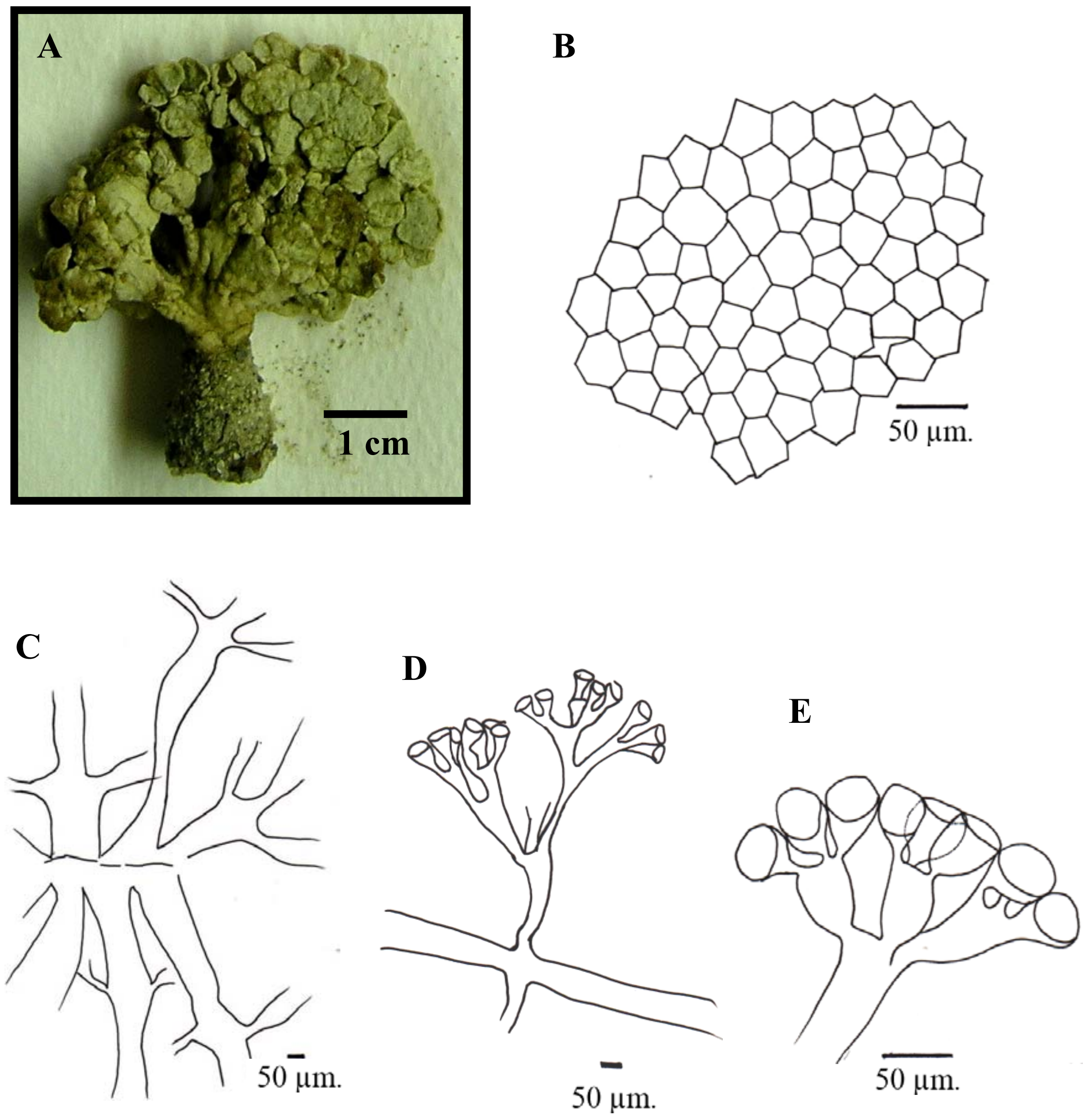


Figure 19 *Halimeda borneensis* W.R. Taylor: (A) thallus of *H. borneensis*, (B) appearance surface view of primary utricles; polygonal pattern, (C) type of node; all filaments fused in a single unit, (D) utricle zone; there are four to five layers of utricle. Voucher information – S. Pongparadon 4

2. *Halimeda discoidea* Decne**.**, Botanique 2: 102: Figure. 18. 1842.; Hillis-Col., Advances in Marine Biology 17: 136-139, Figure. 41. 1980**.**; El-Manawy and Shafik, Am-Euras. J. Agric. & Environ. Sci., 3 (4): 535-536: Figure. 5. 2008. **Figure 20A-20D.**

Thallus erect, 5.5 to 7cm high, attached to rocky or dead coral substrate by a small rhizoidol mat, the holdfast consists of a firm, dense mat of rhizoids; bright green to dark green when fresh, greenish to dark green when dried, calcification light; branching mainly dichotomous; basal segment compressed-rectangular or broadly cuneate, upper basal segment broad ovate or elliptical-discoid to discoid, middle segments broadly cuneate 1.40 to 1.50 cm long and 0.30 to 0.50 cm broad, outer segments usually cylindrical or cuneate; the outer margin entire (Figure 20A). Medullar siphons fused in small groups at node, node belt heights range from 790 to 880.75 µm; supranodal siphon, 840 to 895.50 µm long and 80 to 96.50 µm in diameters; medullar siphon ramify in trichotomies, from 87.50 to 100.50 µm in diameters (Figure 20C). Cortex generally composed of 2 utricles layers; outermost of peripheral utricles remain attached after decalcification; peripheral utricle, ranged from 50 to 58.25 µm in diameters and 160.75 to 192.50 µm long, peripheral utricles appearing in surface view as a pattern of polygonal, 50.50 to 55 µm in diameters (Figure 20B); secondary utricles 85 to 96.50 µm in diameter and 190 to 210 µm long (Figure 20D).

Habitats.— Growing attached on rocks or dead coral or hard substrate in coral reef habitats, subtidal zone.

Specimens Examined.— P. Tuntiprapas 1, 2

Peninsular Thailand Distributions. — ***Andaman Sea***: Similan Island

Worldwide Distributions. — ***Atlantic Islands*****:** Bermuda, Canary Islands, Cape Verde Islands; ***North America*****:** Florida, Gulf of California, Mexico, Texas; ***Central America*****:** Belize, Costa Rica, Islas Revillagigedo, México (Pacific), Panama; ***Caribbean Islands*****:** Bahamas, Caribbean, Cuba, Jamaica, Lesser Antilles, Puerto Rico, Virgin Islands; ***South America*****:** Brazil, Colombia, Venezuela; ***Africa*****:** Djibouti, Eritrea, Kenya, Madagascar, Mauritius, Mozambique, Somalia, Tanzania; ***Indian Ocean Islands*****:** Amirante Islands, Andaman Islands, Chagos Archipelago, Maldives, Nicobar Islands, Réunion*;* ***South-west Asia*****:** Bangladesh, India, Oman, Red Sea, Sri Lanka ***Asia*****:** China, Japan; ***South-east Asia***: Indonesia, Philippines, Singapore, Thailand , Vietnam ; ***Australia and New Zealand*****:** Papua New Guinea, Queensland, Western *Australia ;* ***Pacific Islands*****:** American Samoa, Federated States of Micronesia, Fiji, French Polynesia, Hawaiian Islands, Marshall Islands, Samoan Archipelago, Solomon Islands;

Remarks.— *Halimeda discoidea* can be distinguished from the other species of *Halimeda* in peninsular Thailand by growth form, the most apparent character difference form other species is segments shape are normally broadly cuneate but outer segments are cylindrical or cuneate. In cortex zone, there are 2 layers of utricle. Secondary utricles of *H. discoidea* are strongly inflate and bigger than other species, whereas other species shown cylindrical secondary utricle.

H. discoidea were deep subtidal species, were found only in subtidal zone from slightly below low tide line to 50 meter. In Thailand were found this species only at Similan Island, deep subtidal zone and quite clear water. This *H. discoidea* bed was attached on dead coral, *Acopora* sp.

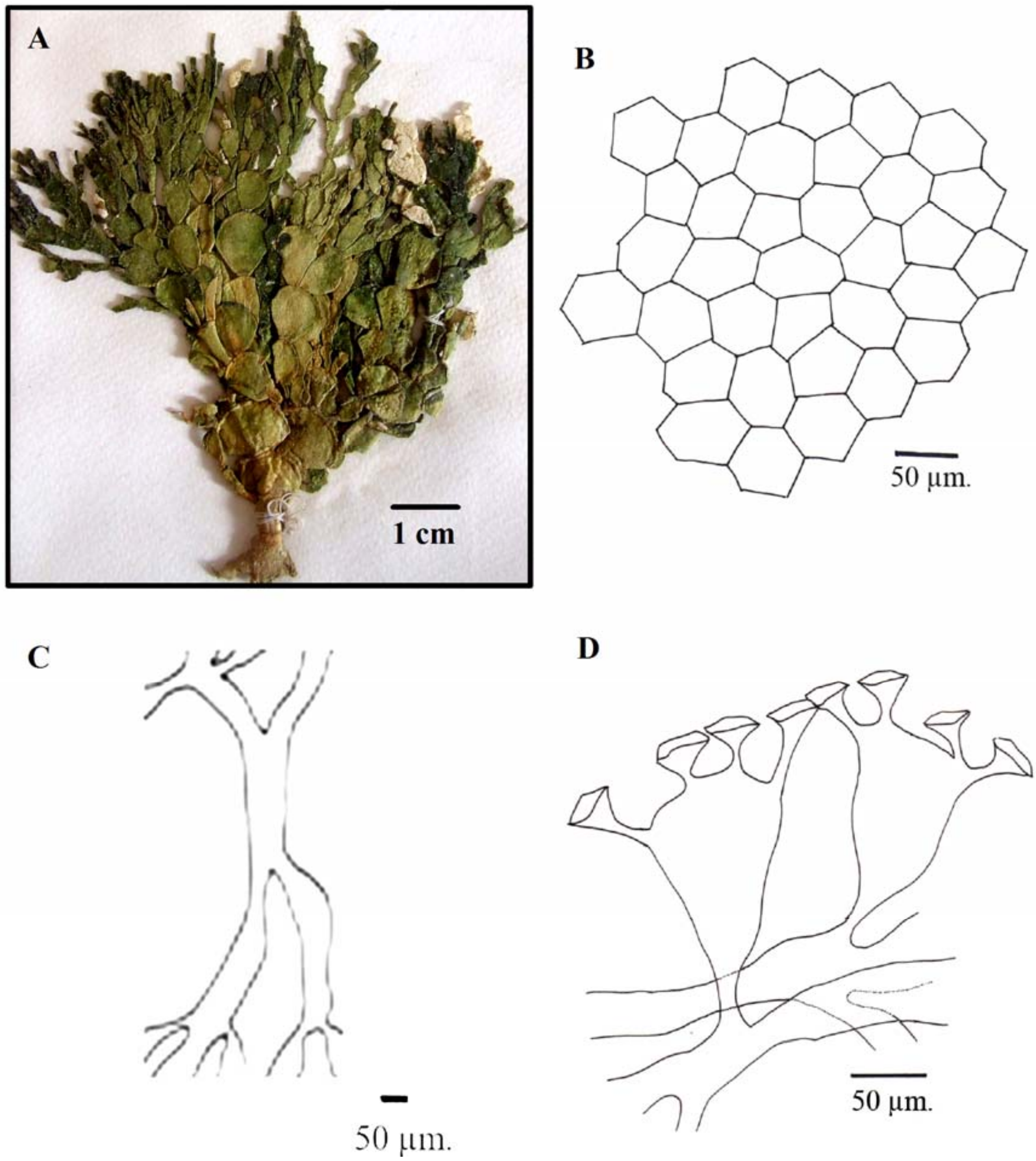


Figure 20 *Halimeda discoidea* Decne**:** (A) thallus of *H. discoidea*, (B) appearance surface view of primary utricles: pattern of polygonal, (C) type of node: filaments fused in small unit, (D) utricle zone; there are two layers of utricle. Voucher information – P. Tuntiprapas 1

3. *Halimeda gigas* W.R. Taylor., Ann. Arbor*:* University of Michigan Press. 84-85, 206, pl. 44: Figures 2. 1950. Hillis-Col., Advances in Marine Biology 17: 132-134, Figure. 39. 1980. **Figures 21A - 21D**.

Thallus erect, loosely organized, 4 to 5 cm high and 5 to 7 broad, anchored a rocky or dead coral substrate by a small rhizoidol mat, the holdfast consists of a firm, dense mat of rhizoids; bright green to greenish when fresh, greenish when dried, the surface slightly glossy, tending to crack when drying, calcification rather light; branching mainly dichotomous or trichotomous, sometime polychotomous from large segment; basal segment compressed-rectangular to cylindrical, majority segments mainly discoidal to reniform 2.30 to 2.60 cm long and 2.50 to 2.80 cm broad, the upper margin of which is entire or shallowly lobed (Figure 21A). Medullar siphons completed fusion in pair at node, node belt heights range from 142.50 to 162.50 µm; supranodal siphon 292.50 to 500 µm in diameters and 95 to 120 µm long; medullar siphon ramify in trichotomies, medullar siphon 52.50 to 75 µm in diameters (Figure 21C). Cortex generally composed of 2-3 utricles layers; outermost of peripheral utricles are remaining attach after decalcification; peripheral utricle 72.50 to 100 µm in diameters and 172.50 to 215 µm long, resulting in surface view, peripheral utricles appear as an irregular pattern of polygonal, 87.50 to 100 µm in diameters (Figure 21B); secondary utricles 95 to 125 µm in diameter and 12.50 to 300 µm long; heights over diameters ratio ranging from 0.3 to 7.31 (Figure 21D).

Habitats.— This species attached on rock or dead coral surface in coral reef habitats, subtidal zone.

Specimens Examined.— P. Tuntiprapas 3

Peninsular Thailand Distributions.— *Andaman Sea*: Similan Island

Worldwide Distributions.— *South-east Asia*: Indonesia, Philippines; ***Australia and New Zealand***: Papua New Guinea, Queensland; ***Pacific Islands***: American Samoa, Federated States of Micronesia, Fiji, Marshall Islands

Remarks.— *Halimeda gigas* is new record of Thailand. Most segment were reniform or subreniform, were low variation within species. This species may be mistaken for a large segmented of *H. tuna.* Microscopic differences with *H. tuna* is the large diameter of the peripheral utricles in surface view, these diameter are more than 85 µm in *H. gigas* as compared to less than 55 µm in *H. tuna.*

H. gigas was found at deep subtidal habitat as same as *H. discoidea*. In Thailand were found only at Similan Island.

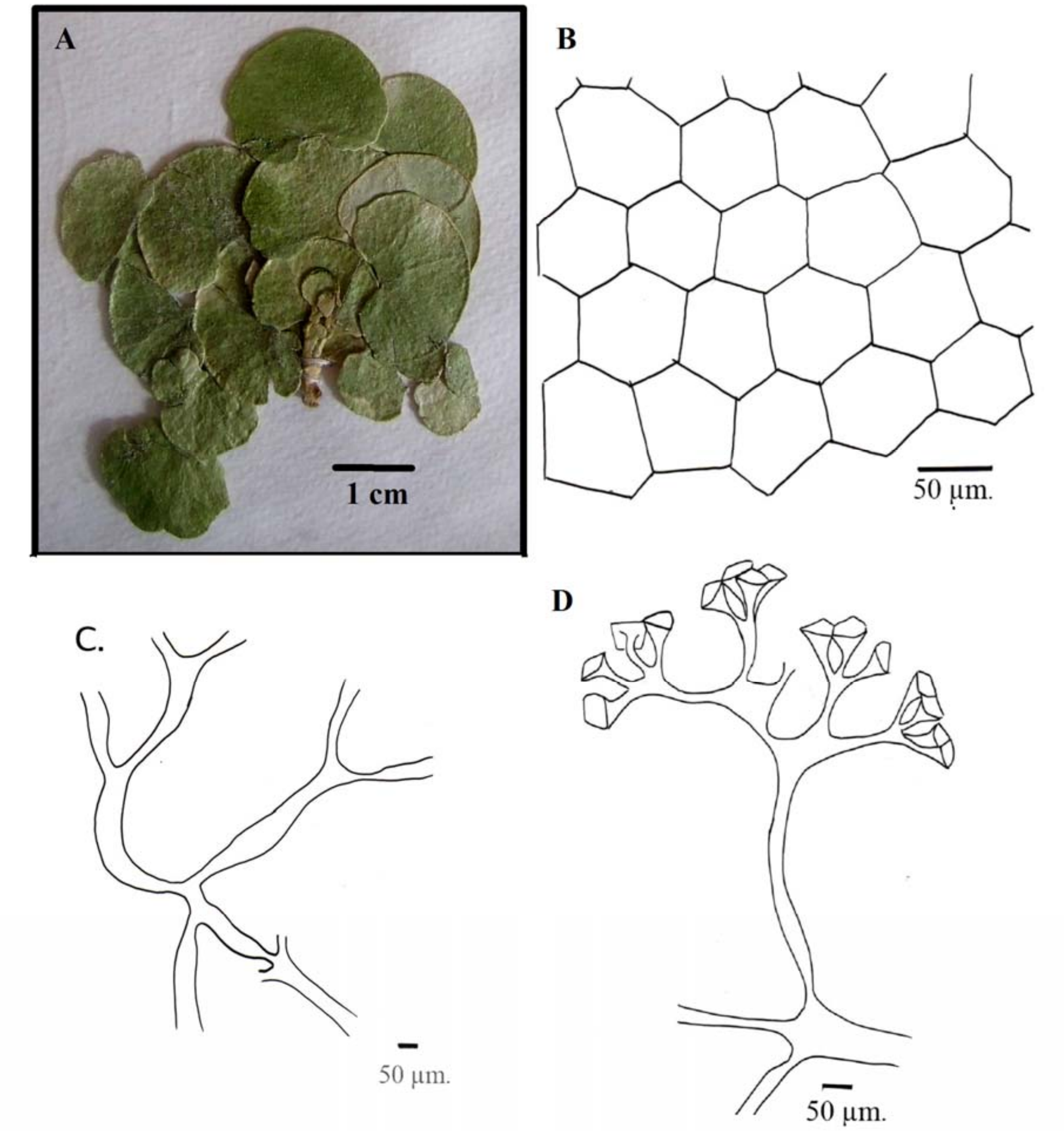


Figure 21 *Halimeda gigas* W.R. Taylor: (A) thallus of *H.* gigas, (B) appearance surface view of primary utricles; polygonal pattern, (C) type of node; complete siphon fusion in pair, (D) utricle zone; there are four layers of utricle. Voucher information – P. Tuntiprapas 3

4. *Halimeda heteromorpha* N'Yeurt, Eur. J. Phycol 41: 351, Figures 14-26, 41-43, 51-54, 63, 64. 2006. **Figures 22A-22E**.

Thallus erect, loosely organized or sometime compact, 5 to 6.50 cm high and 7 to 9 broad, attached to rocky or dead coral substrate by a small rhizoidol mat, the holdfast consists of a firm, dense mat of rhizoids; bright green to greenish when fresh, brownish-green to whitish when dried, calcification moderately heavy; branching mainly dichotomous or rarely trichotomous, often trichotomous or polychotomous branching in the basal region; basal segment compressed-rectangular or cylindrical and supporting suprabasal segment usually broadly cuneate with medium deeply lobed; other segments commonly broad-ovate, elliptical-discoid, broad obovate, broad cuneate to reniform, the upper margin of segments which is shallowly lobed, 0.80 to 1.30 cm long and 0.70 to 1.30 cm broad (Figure 22A). All nodal medullary filaments fused together in a single unit, an obvious adhesion node belt and many small pores,10 to 25 µm; node belt heights range from 37.50 to 87.50 µm; supranodal siphon 180 to 417.50 µm long and 40 to 450 µm in diameters; medullar siphon ramify in trichotomies, 20 to 130 µ in diameters (Figure 22C). Cortex generally composed of 3 to 4 utricles layers; outermost utricles generally remaining attached after decalcification; peripheral utricles 20 to 62.50 µm in diameters and 25 to 80 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of polygonal, 28 to 43.50 µm in diameters (Figure 22B); secondary utricles 12.50 to 70 µm in diameter and 47.50 to 205 µm long; heights over diameters ratio ranging from 3.17 to 14.25 (Figures 22D-22E).

Habitats.— This species grows on rock or dead coral in coral reef, shallow water or intertidal zone.

Specimens Examined.— S. Pongparadon 5, 7, 9, 34

Peninsular Thailand Distributions.— *Andaman Sea*: Shell fossils, Similan Island and Tang Khen Bay

Worldwide Distributions.— *Australia and New Zealand*: Queensland , Between Papetoai and motu Tiahura, Moorea; ***Pacific Islands*:** French Polynesia, Chwaka Bay, Zanzibar, Tanzania, Matemwe, Marquesas, French Polynesia, Tahiti, Moorea, Olango; ***South-east Asia***: Philippines, Tangat, Philippines, Panjang Island, Berau Archipelago, Indonesia.

Remarks.— *H. heteromorpha* is a new record to Thailand. The morphology and anatomy of *H. heteromorpha* are similar with *H. incrassata, H. melanesica* and *H. kanaloana*. The most obvious difference between the four species is type of holdfast. *H. heteromorpha* and *H. melanesica* attach to rock or dead coral substrata by rock-grower holdfast; the felt-like mat of rhizoid, whereas *H. incrassata* and *H. kanaloana* are anchored in sand or mud by bulbous holdfast. Specimens of *H. heteromorpha* have often been mistaken for *H. melanesica* based on similar nodal fusion pattern and type of holdfast. In addition, their segment shape of *H. heteromorpha* and *H. melanesica* are extremely similar; by environments; however, peripheral utricles of *H. melanesica* are smaller and slander.

H. heteromorpha differs from these related species by having matted rhizoid attached to rock or dead coral, nodal siphon fused in a single unit and absence of pore or smallish nodal pores at the segment node.

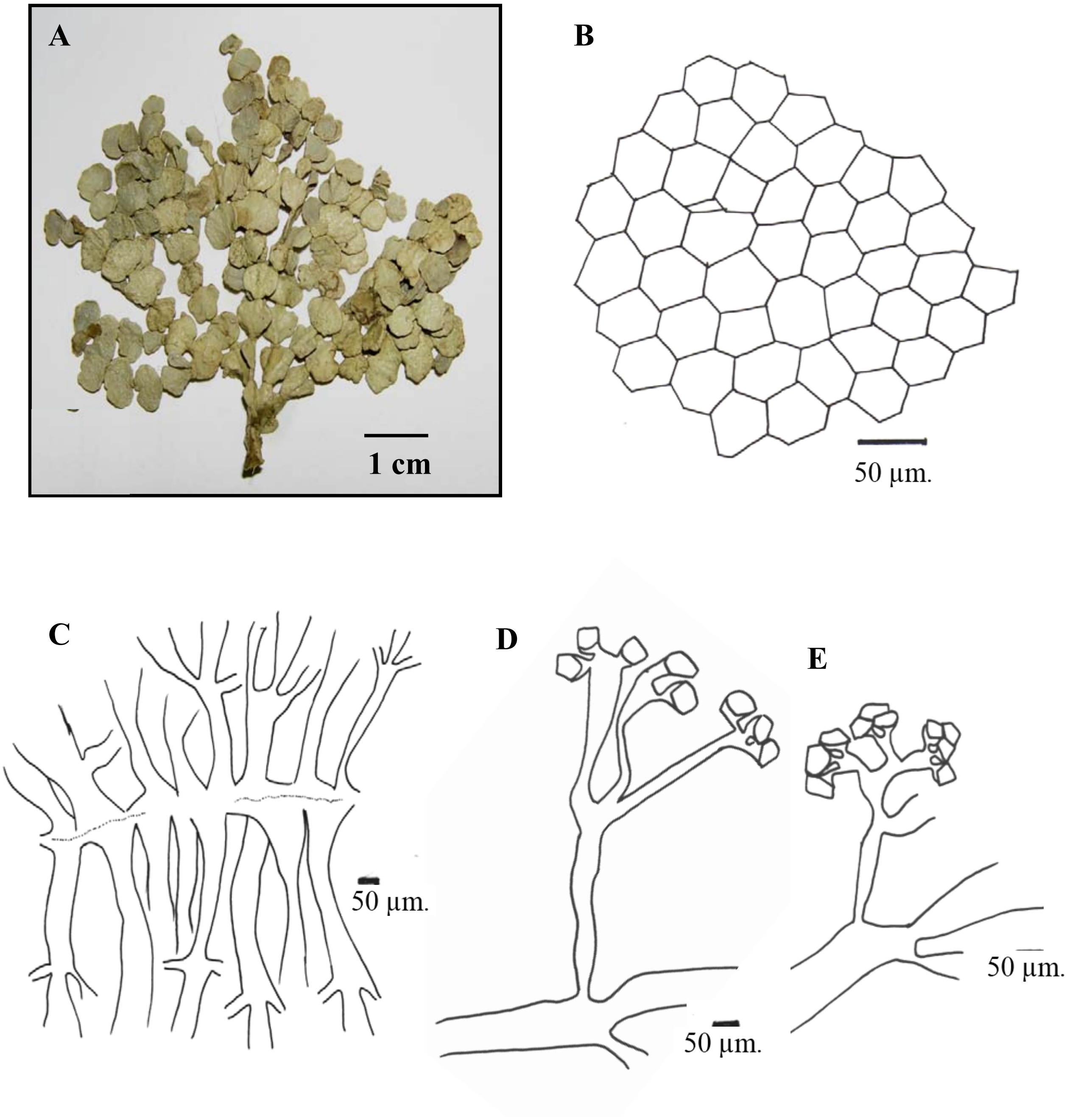


Figure 22 *Halimeda heteromorpha* N'Yeurt: (A) thallus of *H. heteromorpha*, (B) appearance surface view of primary utricles: polygonal pattern, (C) type of node: all filaments fused in a single unit, (D) and (E) cortex zone: there are three to four layers of utricle. Voucher information – S. Pongparadon 5

5. *Halimeda macroloba* Decne., Arch. Mus. Hist. Nat. 2: 118, Plates V-VII. 1841.; E.S.Barton, Siboga-Expeditie LX THE GENUS HALIMEDA: 24-25, pl. III: Figure. 33-38. 1901.; Hillis-Col., Advances in Marine Biology 17: 108-110, Figure. 28. 1980.; Lewmanomont and H. Ogawa, Seaweed and Seagrass in Thailand: 51. 1995.; El-Manawy and Shafik, Am-Euras. J. Agric. & Environ. Sci., 3 (4): 534-535: Figure. 3. 2008. **Figures 23A-23H**.

Thallus erect, mostly expanded in a single plane, fan-shaped appearance, more rarely bushy, up to 6 to 24 cm high and 8 to 12 cm broad, anchored in sandy substrates by 2 to 3 cm long of bulbous holdfast (up to 3 cm long); bright green when fresh, greenish to dark green when dried, moderately calcified; branching mainly dichotomous or trichotomous but often becoming polychotomous in the basal region; basal segment compressed-cylindrical, rectangular to trapezoidal or subcunate and supporting several segments which may remain separate or consolidate laterally; the majority of segments usually broad cuneate or rounded or broad ovate to subreniform, the surface slightly smooth, the upper margin entire or shallowly lobed, sometime medium or deeply lobed, 1.6 to 2.4 cm long and 0.8 to 2.9 cm broad (Figures 23A- 23B). Medullar siphons fused into a single unit at node, an obvious adhesion node belt and many large pores, 30 to 60 µm; node belt 100 to 150 µm high; supranodal siphon 350 to 550 µm long and 120 to 160 µm in diameters; medullar siphon ramify in trichotomies; medullar siphon 15 to 20 µm in diameters (Figure 23D). Cortex generally composed of 5 to 7 layers of utricles; outermost utricles generally remaining attached after decalcification; peripheral utricles 32.50 to 42.50 µm in diameters and 70 to 87.50 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of circle, 30 to 42.50 µm in diameters (Figures 23G-23H); secondary utricles 30 to 62.50 µm in diameter and 57.50 to 162.50 µm long; heights over diameters ratio ranging from 3.47 to 9.23 (Figures 23E-23F).

Habitats.— *Halimeda macroloba* is a common species of sandy beach, sand patch in coral reef and seagrass bed (Figure 23C). Mostly on sandy substrate, but also on coral rubble or sand-covered rock in the shallow intertidal zone in shelter area to subtidal zone; all the fan-shaped plants are frequently growing in the same direction.

Specimens Examined.— S. Pongparadon 1, 6, 13, 20, 22, 32, 35, 36, 38, PA 0148, K. Lewmanomont, PA 0121

Peninsular Thailand Distributions.— *The Gulf of Thailand*: Ban Pang-Ka, Bo-Phut Beach, Hue Tha-non, Samae San, Samui Island, Taling-ngam, Wad Mai, Wat Sila-ngue, Koh Pituk, Had Bo-moa, Koh Tha Rai, Koh Wang Nai; ***The Andaman Sea***: Koh Lidee Yai, Koh Lidee Lek, Lham Pun Wa, Libong Island, Similan Island, Tang Khen Bay.

Worldwide Distributions.— *Africa*: Djibouti, Egypt, Kenya, Madagascar, Somalia, Tanzania; ***Indian Ocean Islands*****:** Aldabra Islands, Andaman, Comoros, Laccadive Islands, Nicobar Islands, Seychelles***; Asia*****:** China, Japan; ***South-east Asia*****:** Indonesia, Malaysia, Philippines, Thailand, Vietnam; ***Australia and New Zealand***: Northern Territory, Papua New Guinea, Queensland, Western Australia; ***Pacific Islands*****:** American Samoa, Federated States of Micronesia, Fiji, French Polynesia, Hawaiian Islands, Samoan Archipelago, Solomon Islands, Tahiti

Remarks.— *Halimeda macroloba* is one of the easiest species of *Halimeda* to recognize in Thai water. It is the only species forming the segment of thallus in a single plane or a fan-shaped appearance and plants are frequently growing in the same direction. The most holdfast type of *H. macroloba* was the bulbous holdfast that anchored in sand substrates, accepted some thallus that grows on the rock or dead coral substrates by a loosed rhizoidol holdfast (Figure 23B). Thallus sizes of this holdfast are smaller than thallus of bulbous holdfast. *H. macroloba* showed morphological and anatomical variability between distantly separated populations. Lengths and widths of segment, diameters of peripheral utricles in surface view, node height and number of utricles layers were high variations. Lengths and widths of segment from deeper species were bigger than lower specimens. Plants were composed of a laxly branched thallus from deeper water (Figure. 23A). In the Peripheral utricles pattern in surface view, these were appeared as a pattern of circle but the diameter of peripheral utricles surface varied greatly among populations. All nodal siphons fused into a single aggregate, but the node height varied among these groups. The number of utricle layers is highly varied among populations that some populations varied into six or seven layers of utricle. However, shape of utricle thallus form, holdfast type, type of nodal filament, surface pattern of primary utricle and shape of primary utricle were similar.

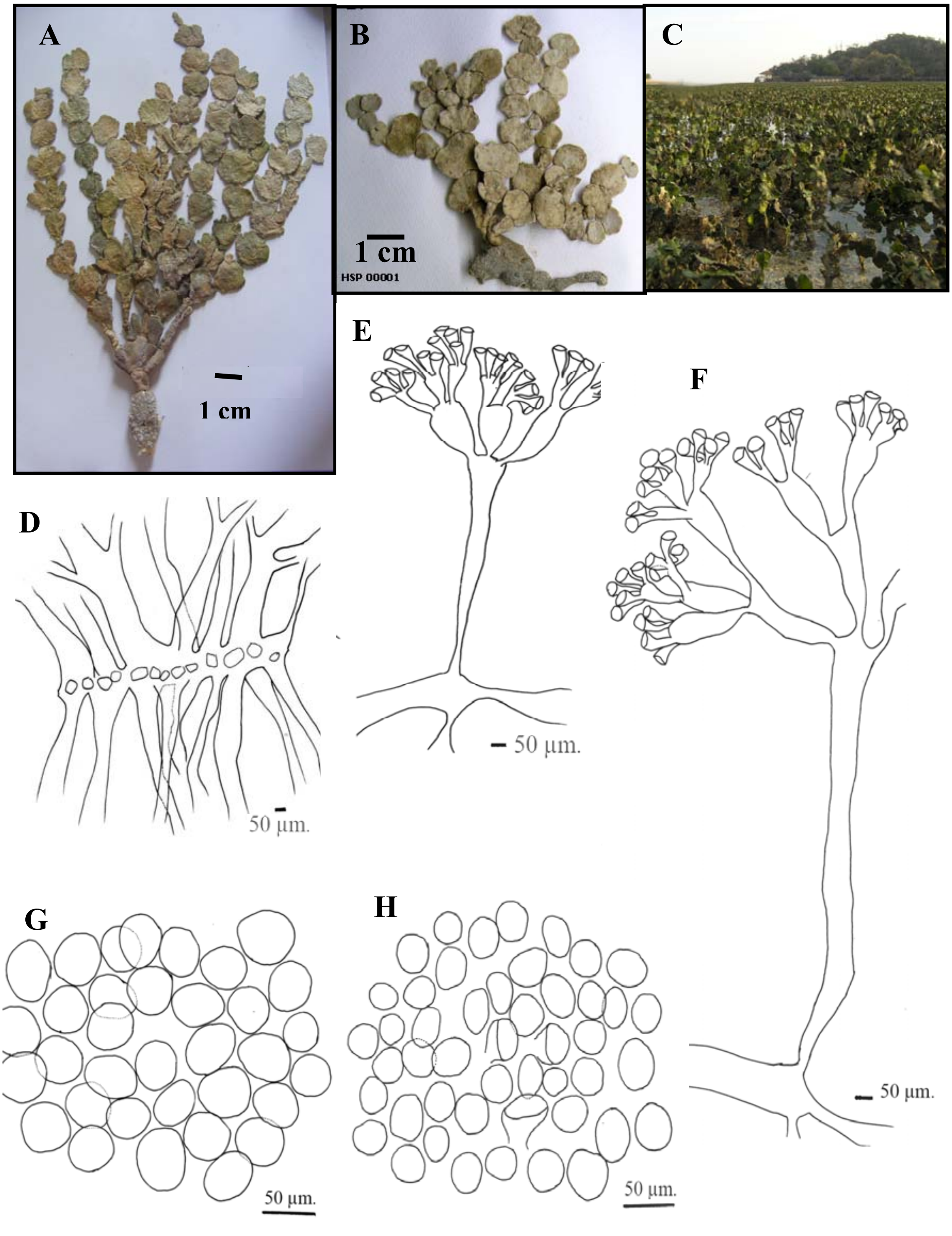


Figure 23 *Halimeda macroloba* Decne.: (A) and (B) thalli of *H. macroloba*, (C) habitat: sandy beach and seagrass bed, (D) type of node; all filaments fused in a single unit with many large pores, (E) and (F) utricle zone: there are three to seven layers of utricle, (G) and (H) appearance surface view of primary utricles: circle pattern. Voucher information – S. Pongparadon 1, 38

***6. Halimeda opuntia* (L.) J.V.Lamour.**, *Histoire des polypiers coralligènes flexibles*, vulgairement nommés zoophytes.**:** pp. [i]-lxxxiv, chart, [1]-560, [560, err], pls I-XIX. 1816; E.S.Barton, Siboga-Expeditie LX THE GENUS HALIMEDA: 18-22, pl. II: Figure. 19-27. 1901; Hillis-Col., Advances in Marine Biology 17: 110-112, Figure. 19, 51, 92. 1980; K. Lewmanomont and H. Ogawa, Seaweed and Seagrass in Thailand: 52. 1995; El-Manawy and Shafik, Am-Euras. J. Agric. & Environ. Sci., 3 (4): 535-536: Figure. 4. 2008. E. Coppejans, Abc Taxa 6: 112, Figure. 97. 2009. **Figures 24A-24G**.

Thallus compact or sprawling, often with both vertical and horizontal system of growth, sprawls over substrate by more than one rhizoidal holdfast, holdfast region not restricted that rhizoids occurring at intervals where the segment contact with substrate, bright green to dark green when fresh, brownish-green to whitish when dried; calcification moderate to heavy; branches often numerous, segment commonly reniform and frequently ribbed, the upper margin of which is shallowly lobed, 0.80 to 1.20 cm long and 0.10 to 1 cm broad (Figure 24A). Nodal medullary filaments fused in two or three, Node belt heights range from 37.50 to 112.50 µm high; supranodal siphon , 242.50 to 700 µm long and 57.50 to 112.50 µm in diameters; medullar siphon ramify in trichotomies, 55 to 80µm in diameter (Figures 21C, 24D). Cortex generally composed of 3 to 5 layers of utricles; outermost utricles generally remaining attached after decalcification; peripheral utricles 17.50 to 30 µm in diameters and 22.50 to 42.50 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of polygonal, 21.50 to 38.25 µm in diameters (Figure 24B); Secondary utricles 12.50 to 32.50 µm in diameter and 5 to 80 µm long; heights over diameters ratio ranging from 0.50 to 8.33 (Figures 24E, 24G).

Habitats.— Specimens of this group are occurs on rocky and dead coral substrate in coral reef, from intertidal to subtidal zone, 6-7 m

Specimens examined.— S. Pongparadon 3, 10, 11, 12, K. Lewmanomont, PA 0206, HEC 16129, SR-SW-177, PP-093

Thailand distributions. —***Andaman Sea***: Ko Ravee, Ko Ling, Kor Kwang beach, Ko Lanta, Ko Pling, Ko Ra Cha, Phi Phi Le, Rawai beach, Similan Island and Tang Khen Bay.

Worldwide Distributions. — ***North America*****:** Florida; ***Europe*****:** Greece **:** ***North America*****:** Florida, Isla Guadalupe, Mexico; ***Central America***: Belize, Costa Rica, Honduras, Islas Revillagigedo, Panama; ***Caribbean Islands*****:** Bahamas, Barbados, Caicos Islands, Caribbean, Cayman Islands, Cuba, Hispaniola, Jamaica, Lesser Antilles, Netherlands Antilles, Puerto Rico, Trinidad & Tobago, Virgin Islands; ***South America***: Aves, Brazil, Colombia, Venezuela; ***Africa*****:** Djibouti, Eritrea, Kenya, Madagascar, Mauritius, Mozambique, Somalia, Tanzania; ***Indian Ocean Islands***: Aldabra Islands, Amirante Islands, Andaman Islands, Cargados Carajos, Chagos Archipelago, Comoros, Diego Garcia Atoll, Laccadive Islands, Maldives, Nicobar Islands, Réunion, Rodrigues Island, Saya de Malha Bank, Seychelles; ***South-west Asia*****:** Bangladesh, India, Sri Lanka; ***Asia*****:** Japan; ***South-east Asia***: Burma, Indonesia, Malaysia, Philippines, Singapore, Thailand, Vietnam; ***Australia and New Zealand*****:** Papua New Guinea, Queensland; ***Pacific Islands*****:** American Samoa, Federated States of Micronesia, Fiji, French Polynesia, Guam, Hawaiian Islands, Marshall Islands, Samoan Archipelago, Solomon Islands, Wallis & Futuna Island.

Remarks.— *H. opuntia* is well characterized by the sprawling growth form whereas other species in Peninsular Thailand are erect thalli. In additional, there are more than one sprawler holdfast regions whereas there is only one holdfast per thallus in other species.

This species sometime provides about 70-80 percentage cover of rock surfaces or dead coral and it may be associated with *H. macroloba, H. heteromorpha, Enhalus acoroides and Thallusia hemprichii.*

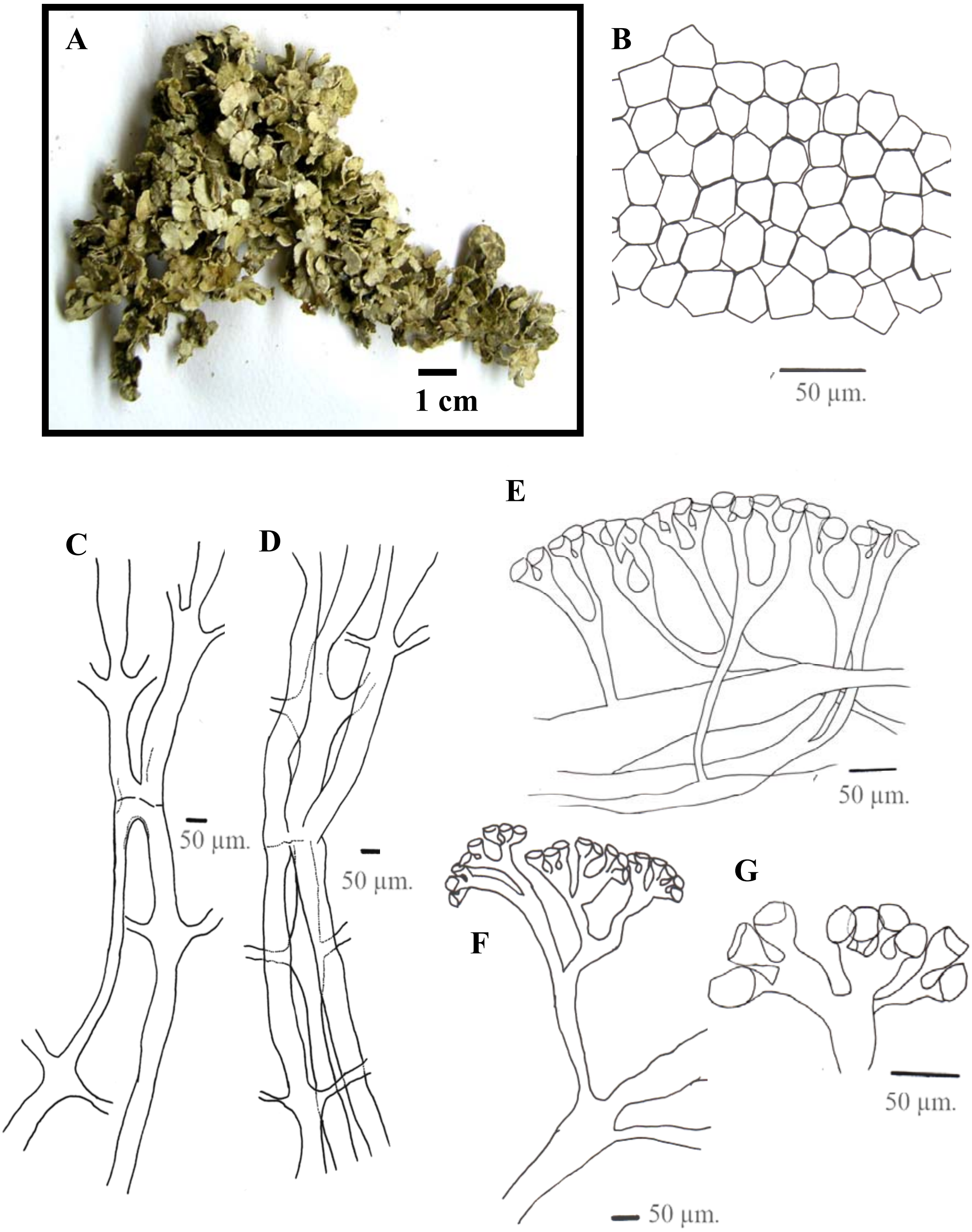


Figure 24 *Halimeda opuntia* (L.) J.V. Lamour.: (A) thallus of *H. opuntia*, (B) appearance surface view of primary utricles: irregulars polygonal pattern, (C) and (D) type of node: filaments fused in small group, two or three, (E), (F) and (G) utricle zone: there are five layers of utricle. Voucher information – S. Pongparadon 3, 10

7. ***Halimeda tuna*** (J. Ellis & Solander) J.V.Lamour., vulgairement nommés zoophytes. pp. [i]-lxxxiv, chart, [1]-560, [560, err], pls I-XIX. 1816.; E.S.Barton, Siboga-Expeditie LX THE GENUS HALIMEDA: 11-15, pl. I: Figure. 1-6. 1901.; Hillis-Col., Advances in Marine Biology 17: 122-124, Figure. 35. 1980.; El-Manawy and Shafik, Am-Euras. J. Agric. & Environ. Sci., 3 (4): 535-536, Figure. 6. 2008. **Figures 25A-25D**.

Thallus loosely organized and spreading, 4 to 4.5 cm high and 9 to 13 cm broad, attached to substrate by an inconspicuous small rhizoidol mat, the holdfast consists of a firm, dense mat of rhizoids, bright green when fresh, greenish to whitish when dried, calcification moderate to light, sometime heavier calcified at the basal; branching mainly dichotomous or rarely trichotomous; basal segment often cuneate, the other segment mainly broad ovate, discoidal or reniform, 1.60 to 1.80 cm long and 1.50 to 1.80 cm broad, the surface slightly smooth, the upper margin of which is entire (Figure 25A). Nodal medullary filaments complete fusion in pair or triplet, an obvious adhesion node belt of 325 to 382.50 µm high; supranodal siphon, 550 to 612.50 µm long and 80 to 105 µm in diameters; medullar siphon ramify in dichotomies, 50 to 62.50 µm in diameters (Figure 25C). Cortex generally composed of 3 utricles layers of utricles; outermost utricles generally remaining attached after decalcification; peripheral utricles 47.50 to 60 µm in diameters and 62.50 to 87.50 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of polygonal, 51 to 53.50 µm in diameters (Figure 25B); Secondary utricles 52.50 to 65 µm in diameter and 105 to 150 µm long; heights over diameters ratio ranging from 4.38 to 6.82 (Figure 25D).

Habitats.— They occur in coral reef habitats, subtidal zone.

Specimens Examined.— S. Pongparadon 18, HEC 16180

Peninsular Thailand Distributions.— *Andaman Sea*: Ko Pling.

Worldwide Distributions.— *Europe*: Adriatic, Balearic Islands , Corsica, France, Greece, Italy, Spain, Turkey; ***North America***: Florida, Mexico; ***Central America*: ** Belize, Islas Revillagigedo, Panama; ***Atlantic Islands***: Azores, Bermuda, Canary Islands, Cape Verde Islands; ***Caribbean Islands***: Bahamas, Barbados, Caicos Islands, Caribbean, Cuba, Hispaniola, Jamaica, Lesser Antilles, Puerto Rico, Virgin Islands ***South America***: Brazil, Chile Colombia, Venezuela; ***Africa***: Algeria , Angola, Egypt, Kenya, Libya, Madagascar, Mauritius, Morocco, Mozambique, Somalia, South Africa , Sudan, Tanzania, Tunisia; ***Indian Ocean Islands***: Aldabra Islands, Amirante Islands, Andaman Islands, Cargados Carajos, Chagos Archipelago, Diego Garcia Atoll, Laccadive Islands, Maldives, Nicobar Islands, Réunion, Rodrigues Island, Saya de Malha Bank, Seychelles; ***South-west Asia***: India, Israel, Pakistan, Sri Lanka, Turkey (Asia), Yemen; ***Asia***: Japan; ***South-east Asia***: Indonesia, Malaysia, Philippines, Singapore, Vietnam; ***Australia and New Zealand***: Papua New Guinea, Queensland, Western Australia; ***Pacific Islands***: Easter Island, Federated States of Micronesia, Fiji, Solomon Islands ***South-west Asia***: Levant states, Sri Lanka

Remarks.— *H. tuna* is new record of Thailand. This species grow on vertical wall along deep reef slope. *H. tuna* and *H. gigas* exhibited very similar gross morphology that makes difficulties in distinguishing some specimens. Both of thallus appearance were pendent and attach had substrate by grower-holdfast but segment shape were difference. Most segments of *H. tuna* were discoid and broad ovate while *H. gigas* were reniform and subreniform. In this present study, surface view of peripheral utricle pattern of both species was similar, polygonal pattern, but *H. tuna* can be distinguished by peripheral utricle diameter, smaller than *H. gigas*. In addition, node belt of *H. tuna* were larger than *H. gigas*, 325 to 382.50 µm high in *H. tuna* and 142.50 to 162.50 µm in *H. gigas*.

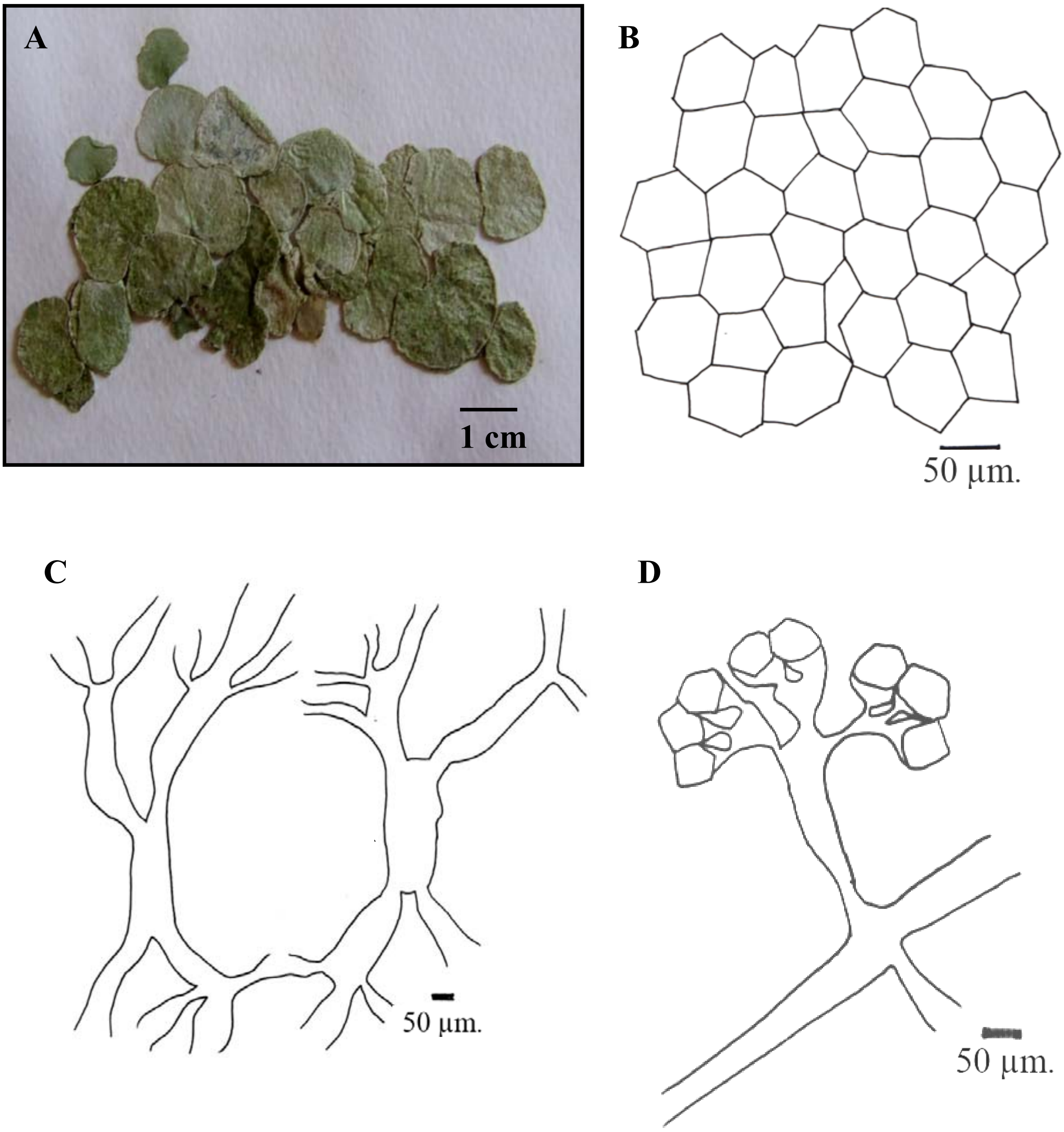


Figure 25 *Halimeda tuna* (J. Ellis & Solander) J.V.Lamour.: (A) thallus of *H. tuna*, (B) appearance surface view of primary utricles: polygonal pattern, (C) type of node: complete fusion of siphon in pair, (D) utricle zone: there are three layers of utricle. Voucher information – S. Pongparadon 18

8. ***Halimeda* sp.**

Figures 26A-26F.

Thallus erect, loosely organized, 12 to 14.5 cm high and10 to 12 cm broad, anchored on sponge substrates by a small rhizoidol mat, the holdfast consists of a firm, dense mat of rhizoids, dark green when fresh, brownish-green to dark green when dried, the surface smooth and calcification light, branching mainly dichotomous or rarely trichotomous, basal segment often cylindrical, the other segment extremely variable, cylindrical, cuneate to broad cuneate, broad ovate to discoidal or rarely reniform, 8 to 2.0 cm long and 1.6 to 2.1 cm broad, mostly segment with stalk; the upper margin of which is entire or shallowly lobed (Figure 26A). Medullar siphons fused in small group at node, an obvious adhesion node belt of 498.25 to 513.75 µm high; supranodal siphon, 2205.25 to 2224.25 µm long and 127.50 to 138.75 µm in diameters; medullar siphon ramify in dichotomies, 119.75 to 128.75µm in diameters (Figures 26C-26D). Cortex generally composed of 3-5 utricles layers of utricles; outermost utricles generally remaining attached after decalcification; peripheral utricles 68.75 to 62.50 µm in diameters and 102.75 to 109 µm long, resulting in a surface view, peripheral utricles appear as an irregular pattern of polygonal, 58.5o to 65.25 µm in diameters (Figure 26B); Secondary utricles 69.50 to 72.25 µm in diameter and 110.75 to 125 µm long; heights over diameters ratio ranging from 1.57 to 1.62 (Figures 26E-26F).

Habitats.— Specimens of this species are attaches on sponge substrate in canal of mangrove habitats, subtidal zone, 2-3 m.

Specimens Examined.— S. Pongparadon 17, HEC 16155

Peninsular Thailand Distributions.— ***Andaman Sea***: Khlong Yang

Remarks.— Specimens of *Halimeda sp.* is found in the canal of mangrove forest, low salinity, and attached on sponge, this is a rather unique habitat and differs from *Halimeda* reported worldwide. The morphological and anatomical characters of this group were difference from the others. The characters have also never seen before in other *Halimeda*, thallus erect, anchored on sponge substrates by a small rhizoidol mat, calcification light, and segment thinner than other species, medullar siphons fused in pair at node, 3-5 utricles layers and surface view of peripheral utricles appear as a pattern of polygonal.

The morphology and anatomy and molecular analyses are now in process, these would allow us to have another additional character to confirm the possible new species to science.

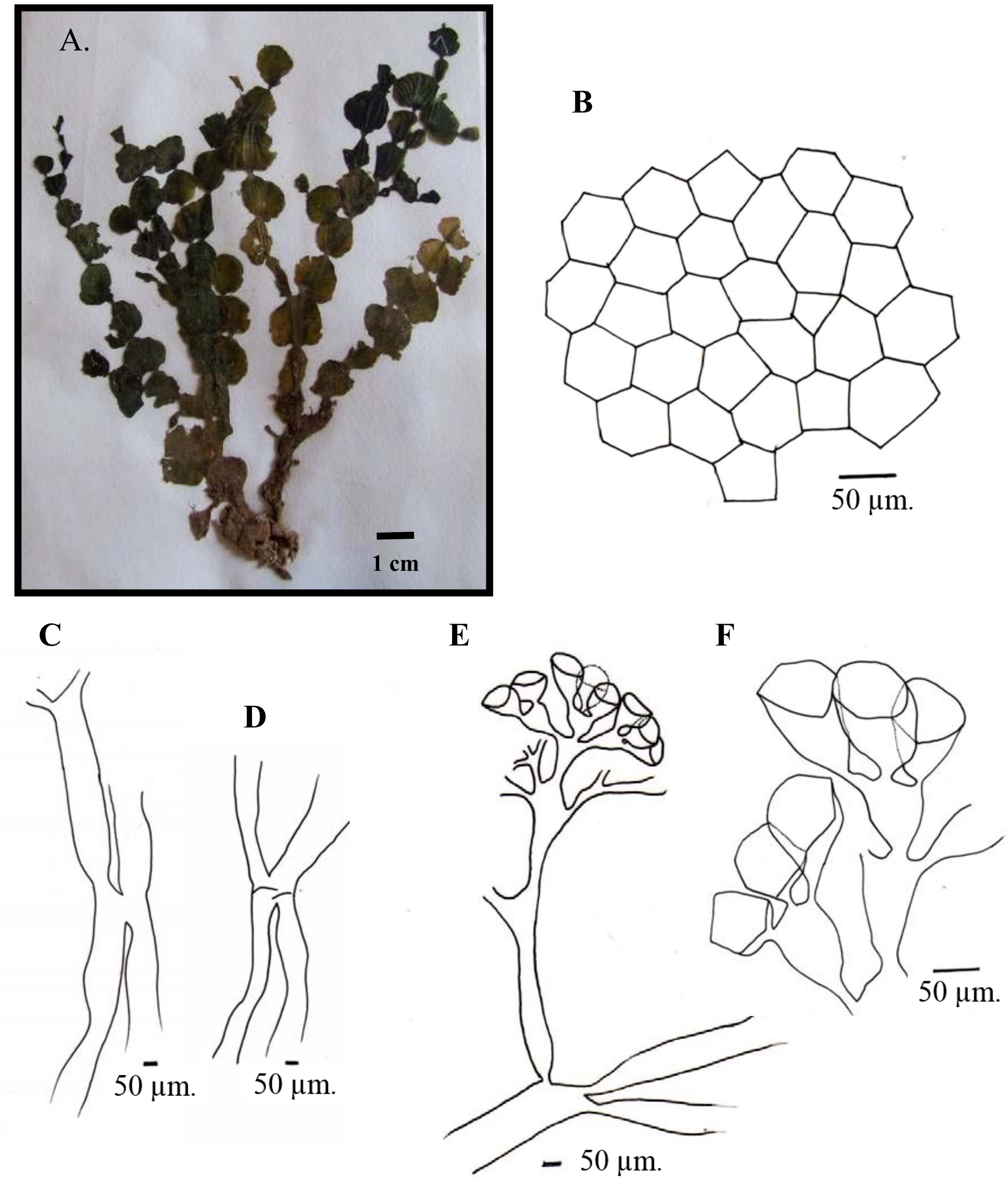


Figure 26 *Halimeda* sp.: (A) thallus of *Halimeda* sp. (B) appearance surface view of primary utricles: polygonal pattern, (C), (D) type of node; filaments fused in pairs for a short distance, (E) and (F) utricle zone: there are five layers of utricle. Voucher information – S. Pongparadon 17

Distribution of genus *Halimeda* J.V.Lamour. in Peninsular Thailand

A total of 8 species of *Halimeda* are reported from the Andaman Sea and Gulf of Thailand (Table 6). There is only species found in the Gulf of Thailand; *H. macroloba* and eight species found in the Andaman Sea; *H. macroloba, H. opuntia, H. discoidea, H. tuna, H. borneensis, H. gigas, H. heteromorpho* and *Halimeda* sp. Of these, Similan Island has the most number of species, 5 species found; Tang Khen Bay has 4 species; Ko Pling, Rawai beach and Ko Ravee have 2 species, and other sites have only one species recorded. *H. macroloba* is the most dominant species, found in both the Gulf of Thailand and the Andaman Sea (Table 6). *H. macroloba* is the only species found in the Gulf of Thailand; and limited into the upper and mid Gulf of Thailand (Figure 27). In addition, there are a few populations of *H. macroloba* producing the reproductive organ, gametophores, at Ko Tan, Ko Wang Nai and Ko Pitak.

Table 6 Distribution of genus *Halimeda* J.V.Lamour. in Peninsular Thailand

Site		*H. macroloba*	*H. opuntia*	*H. discoidea*	*H. tuna*	*H. borneensis*	*H. gigas*	*H. heteromorpha*	*Halimeda* sp.
Gulf of Thailand	Ban Pang-Ka	+							
	Bo-Phut Beach	+							
	Hue Ha-non	+							
	Ko Tan	+							
	Samui Island	+							
	Taling-ngam	+							
	Wad Mai	+							
	Wat Sila-ngue	+							
	Ko Pitak	+							
	Ao Bo-moa	+							
	Ko Tha Rai	+							
	Ko Wang Nai	+							
Andaman Sea	Ao Khao-Kwai					+			
	Ko Ra					+			
	Mo Ko Su-Rin		+						
	Ko Ravee		+						
	Ko Ling (Ao Dong)		+						
	Kor Kwang Beach		+						
	Khlong Yang								+
	Ko Lidee Yai	+							
	Ko Lidee Lek	+							
	Shell fosills							+	
	Ko Lanta		+						
	Ko Pling		+		+				
	Ko Ra Cha		+						
	Punwa cape	+							
	Ko Libong	+							
	Phi-Phi		+						
	Rawai Beach		+			+			
	Similan Marine National Park	+	+	+			+	+	
	Tang Khen Bay 1(Sandy Beach)	+							
	Tang Khen Bay 2 (Coral reef)		+			+		+	

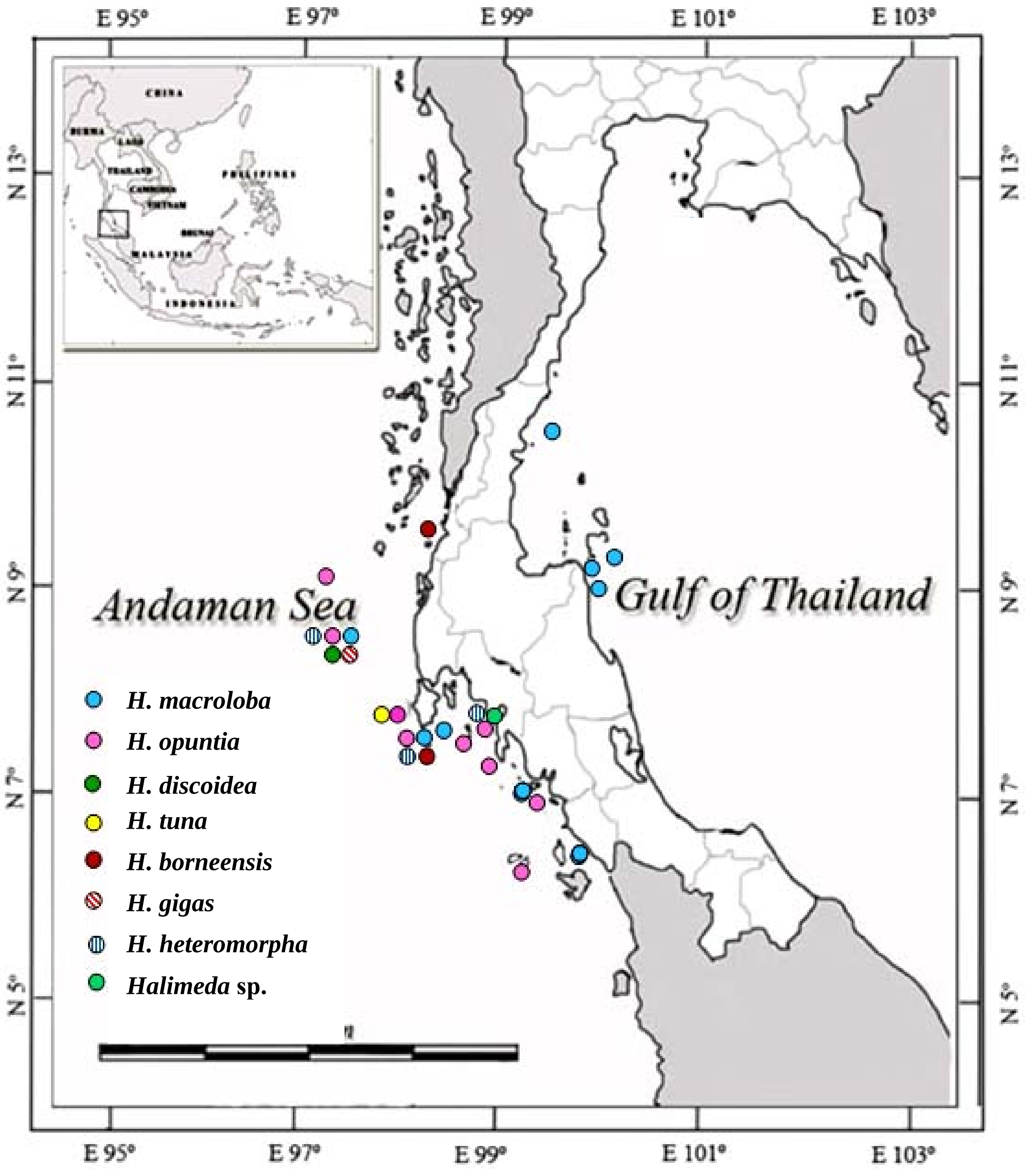


Figure 27 Distribution map of genus *Halimeda* J.V.Lamour. in Peninsular Thailand

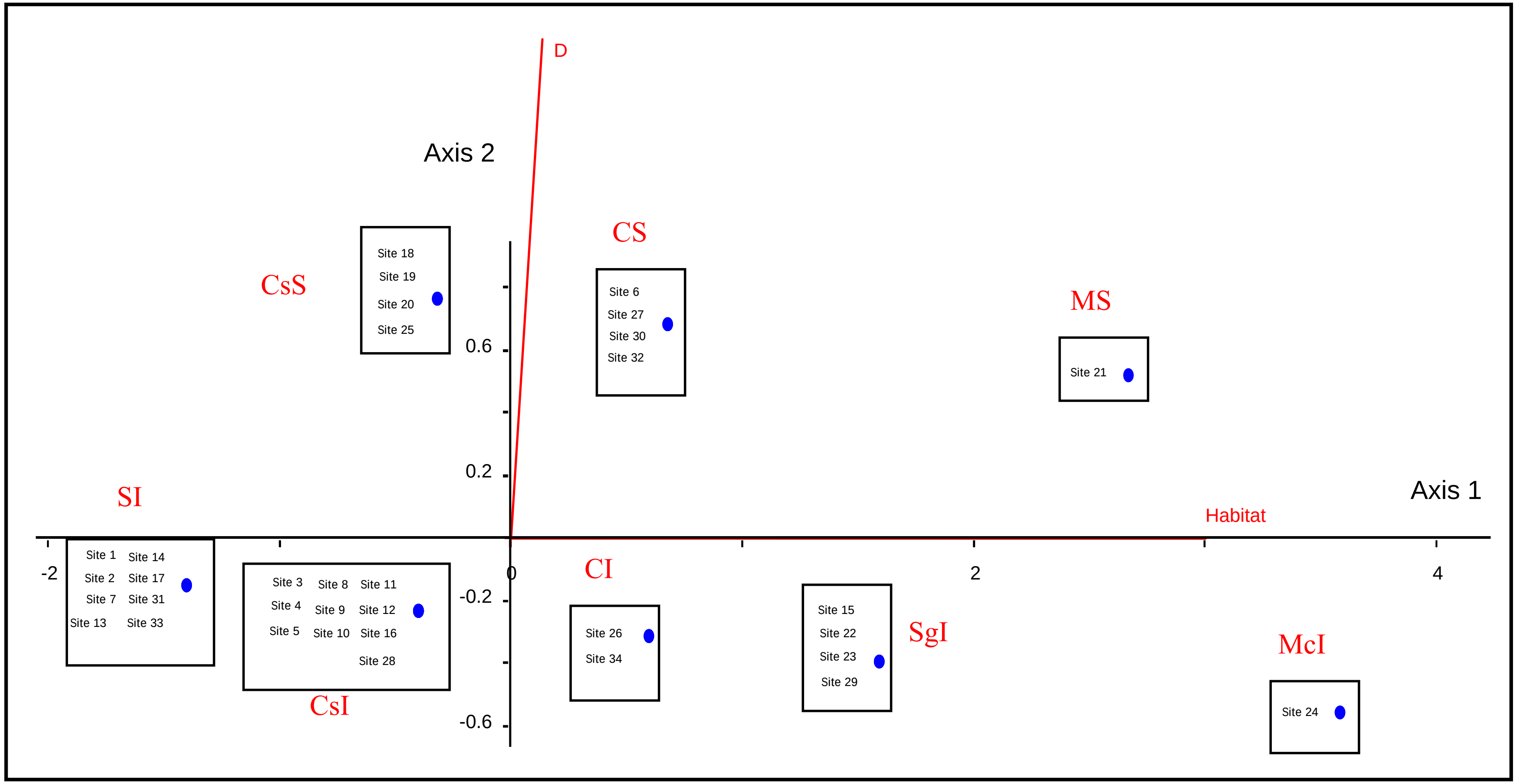


Figure 28 Principal Component Analysis biplots of habitat type

There are 8 types of habitat based on substrate and depth: sandy beach_intertidal (SI), coral reef_intertidal (CI), coral reef_subtidal (CS), coral reef with sand bottom_ intertidal (CsI), coral reef with sand bottom_subtidal (CsS), Mud flat with coral rubber_intertidal (McI), Seagrass_intertidal (SgI) and mangrove_subtidal (MS) (Figure 28).

Principal Component Analysis (Figure 29) showed that most of *H. macroloba* Decne. were restricted in sandy beach and seagrass bed, intertidal zone, but some populations also occurred in coral reef in subtidal zone. *H. opuntia* was associated in the coral reef zone, occurred from intertidal zone to subtidal zone. *H. borneensis* was restricted in sand bottom of sandy beach and coral reef. *H. discoidea* Decne. and *H. gigas* W.R. Taylor were associated in only coral subtidal zone.

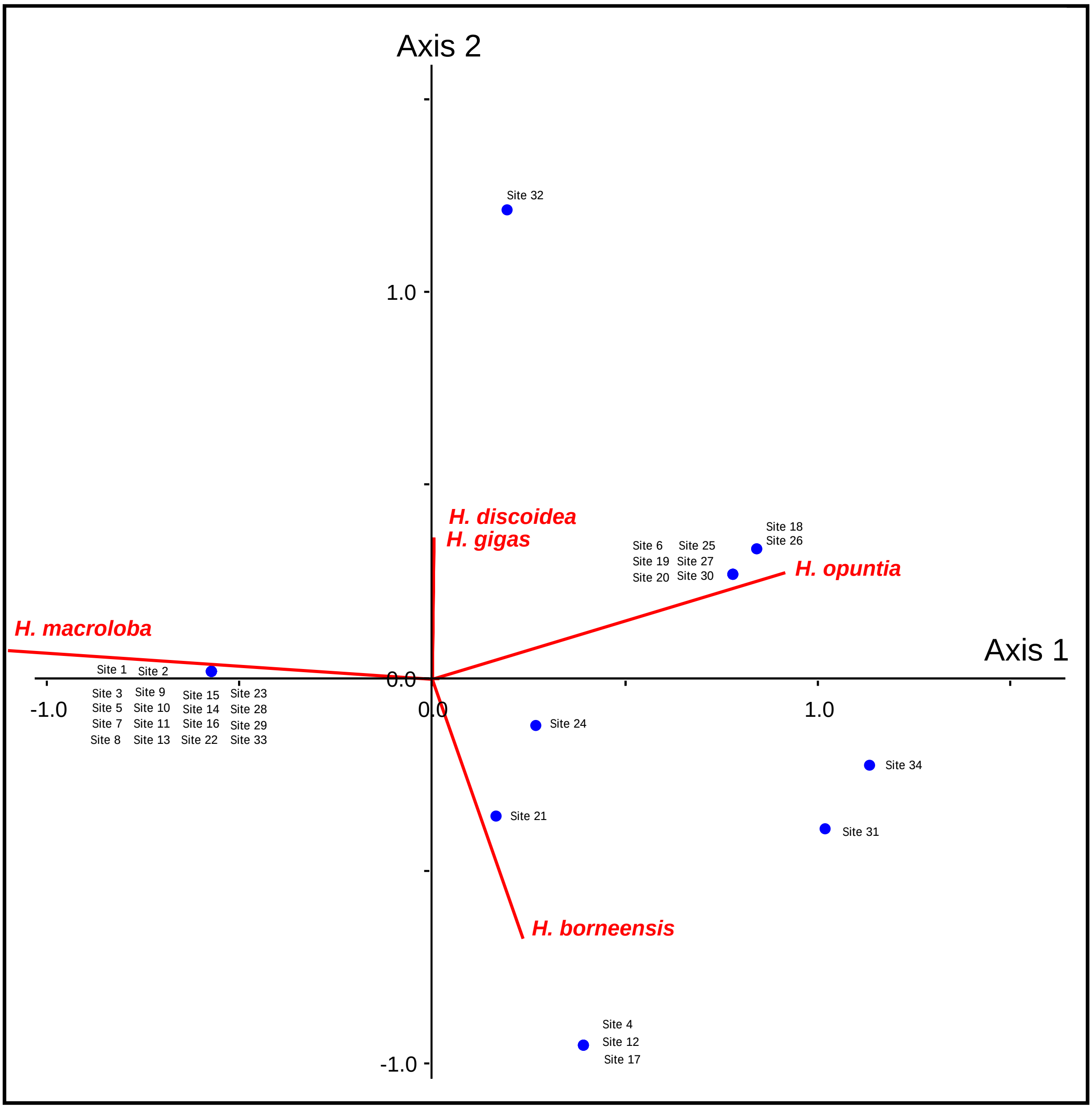


Figure 29 Principal Component Analysis biplots of *Halimeda* J.V.Lamour. distribution and habitat

Morphological and Anatomical Variations among Populations within Species of *Halimeda macroloba* Decne.

Ordinations of morphometric data (Figure 30) revealed the biplots of the Principal Component Analysis based on the morphological and anatomical data. There were nine groups of *H. macroloba*. Of these, most of the cluster occupied non-overlapping regions. Some of the clusters showed overlapping (e.g. *Halimeda macroloba* 6, *H. macroloba* 4) (Figure 30). Ordinations of morphometric data (Figure 31) showed the biplots of the Principal Component Analysis based on the anatomical data of *H. macroloba*. There were ten group of *H. macroloba*. Of these, the entire cluster occupied non-overlapping regions; suggested that anatomical characters would give a good understanding in variation within species.

Halimeda macroloba taxa consisted of nine clusters, percent chaining is 3.79 (Figure 32). There are 3 clades collected in the Andaman Sea and 6 Clades collected in the Gulf of Thailand.

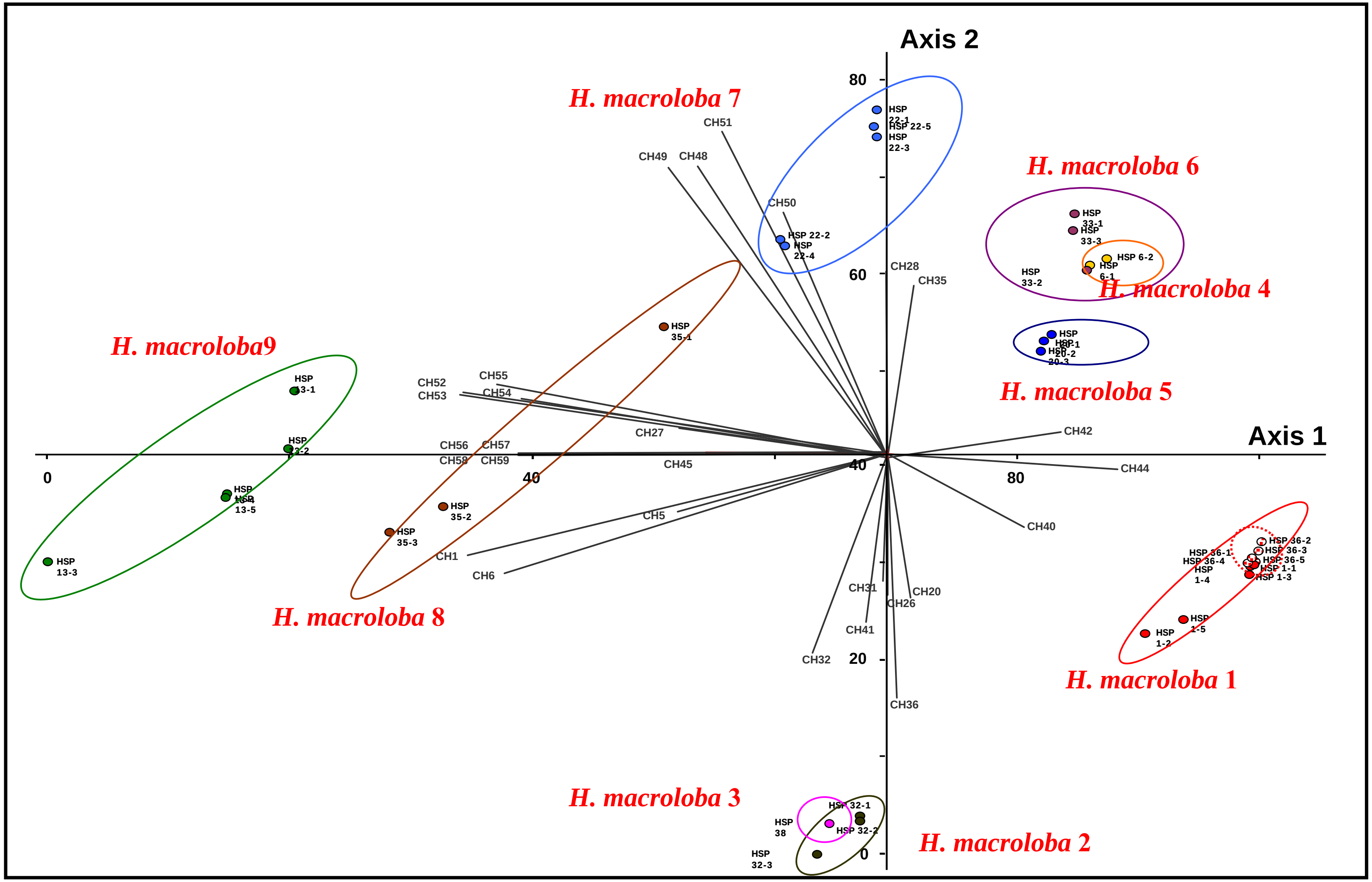


Figure 30 Principal Component Analysis biplots of *Halimeda macroloba* based on morphological and anatomical data

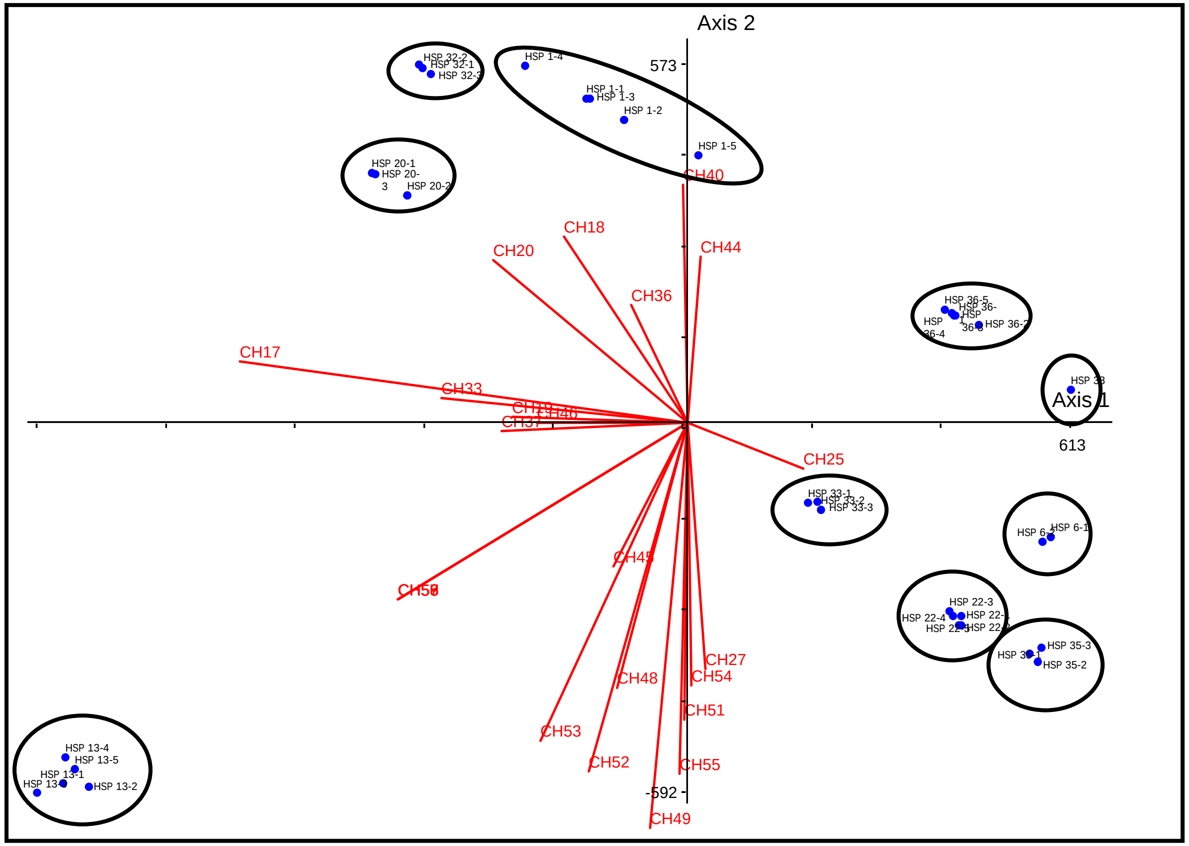


Figure 31 Principal Component Analysis biplots of *Halimeda macroloba* based on anatomical data

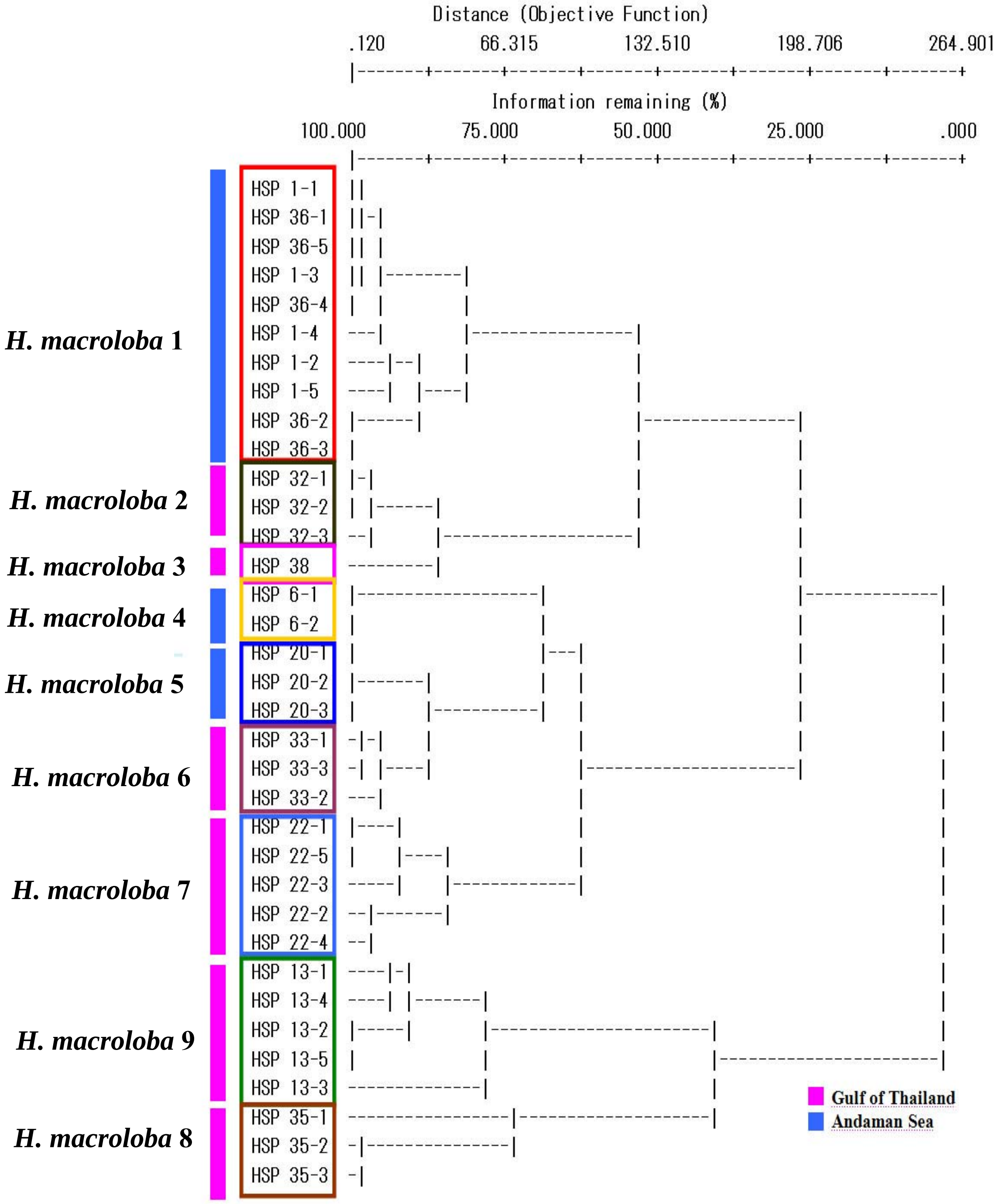


Figure 32 Cluster analysis of *Halimeda macroloba* taxa based on morphological and anatomical data. Percent chaining is 3.79

Comparative morphology and anatomy character of *H. macroloba*

Characters of *H. macroloba* were compared between groups: thallus size, segment size, holdfast type, thallus form, primary utricle in surface view pattern, size of primary utricle in surface view, node type, node height, number of utricle layers, length and width of primary utricle.

The morphological apparent differences among nine groups of *H. macroloba* were thallus size and segment size. The height of thallus varied among these groups (range: 6-24 cm) and the maximum value was found in *H. macroloba* 3 (Ko Tan, subtidal zone), while the minimum belonged to *H. macroloba* 4 (Tang Khen Bay, intertidal zone) (Table 7). In the type of holdfast, the most group had the bulbous holdfast that anchored in sand substrates, except some specimens of *H. macroloba* 1 that grows on the rock or dead coral substrates, covered with thin layer of sand, by a loosed rhizoidol holdfast (Table 7). The maximum length and width of segment also varied in *H. macroloba* 3 (2.40-2.50 cm and 2.40-2.80 cm, respectively, Table 7 and 8). However, thallus forms of all groups formed in a single plane or fan-shape.

The peripheral utricles pattern in surface view of all groups was appeared as a pattern of circle but the diameter of peripheral utricles surface varied greatly among these groups (22.50-30.00 µm for *H. macroloba* 7 and 37.50-50.00 µm for *H. macroloba* 3) (Table 7 and 8).

In these groups where all nodal siphons fused into a single aggregate, but the height varied among these groups and the maximum value was found in *H. macroloba* 5 (470.00-550.00 µm) while the minimum was *H. macroloba* 2 (70.00-130.00 µm)(Table 7 and 8).

The number of utricle layers was very different among these *H. macroloba* groups. This character was separated into 3 main groups. The first consisted of *H. macroloba* 1 – *H. macroloba* 6, there were 3-(4)5 layers. The second was *H. macroloba* 7 and *H. macroloba* 8, 4-6 layers and the last was *H. macroloba* 9, 5-7 layers (Table 7 and 8).

In addition, the length and width of primary utricle were different, the highest in peripheral utricle width was *H. macroloba* 1 (60.00 µm), and the lowest was *H. macroloba* 4 (25.00 µm). Peripheral utricle length of the *H. macroloba* 5 group (140.00 µm) was highest, and *H. macroloba* 1 and 2 (50.00 µm) were the lowest.

Morphological and Anatomical Variations

There were showed significant differences in three morphological character: diameter of peripheral utricles pattern in surface view, segment length, segment width; and five anatomical characters: node height, pore size, length of peripheral utricle, max width of peripheral utricle and number of utricle layers (Table 8).

Morphological Variations

1. Variations in diameter_surface (CH 8)

There were significant difference in diameter of peripheral utricles pattern in surface view of *H. macroloba* groups (df = 8, F = 40.36, P < 0.0001; Figure 33A, Table 8). *H. macroloba* 3 had the widest surface diameter of peripheral utricle, 32.5 – 50.0 µm; and *H. macroloba* 4 and *H. macroloba* 7 had the narrowest peripheral utricle 25.0 – 30.0 µm and 32.5 – 37.5 µm, respectively. The highest in diameters was *H. macroloba* 3 group (50.0 µm) and *H. macroloba* 7 (22.25 µm) was the lowest. Diameter of peripheral utricles in surface view could be used to distinguish between *H. macroloba* 3 group and other groups. There were significantly different among groups (Tukey HSD, P < 0.0001).

2. Variations in length of segment (CH 9)

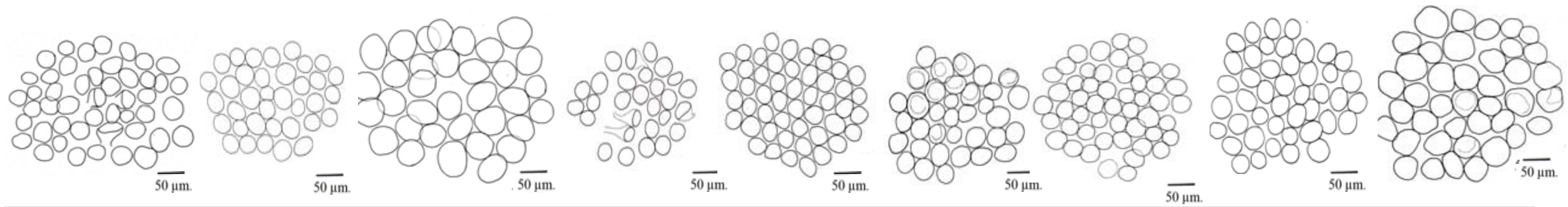
There was significant difference in segment length of *H. macroloba* (df = 8, F = 11.370, P < 0.0001; Figure 33B, Table 8). *H. macroloba* 1 had the widest length, 1.60 – 2.40 cm; and *H. macroloba* 3 and *H. macroloba* 4 had the narrowest

Table 7 General morphology, surface views of segments, nodal fusion and shapes of utricles of different group of *Halimeda macroloba* Decne.

***H. macroloba* 1**	***H. macroloba* 2**	***H. macroloba* 3**	***H. macroloba* 4**	***H. macroloba* 5**	***H. macroloba* 6**	***H. macroloba* 7**	***H. macroloba* 8**	***H. macroloba* 9**

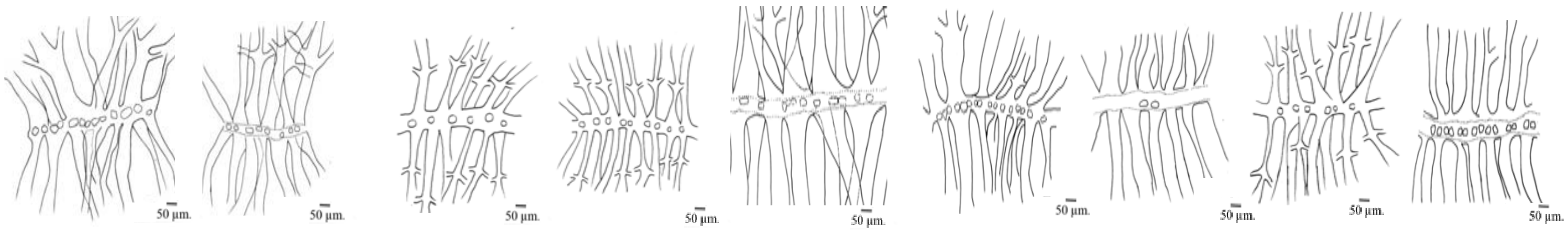
<u>Thallus</u>

<u>Peripheral utricles pattern in surface view</u>



H. macroloba 1	H. macroloba 2	H. macroloba 3	H. macroloba 4	H. macroloba 5	H. macroloba 6	H. macroloba 7	H. macroloba 8	H. macroloba 9

Node and pore



Number of utricle layers

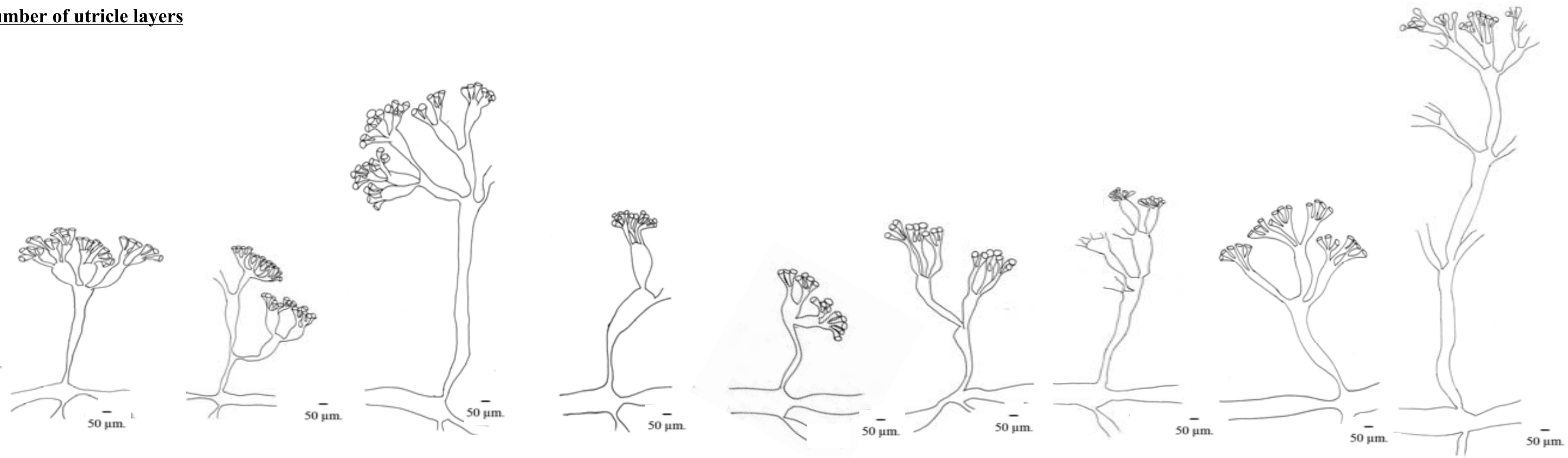


Table 8 Morphological and anatomical characteristics of *Halimeda macroloba* Decne. groups. Show the variability of morphology and anatomy between the groups of specimens.

Characters	*H. macroloba* 1	*H. macroloba* 2	*H. macroloba* 3	*H. macroloba* 4	*H. macroloba* 5	*H. macroloba* 6	*H. macroloba* 7	*H. macroloba* 8	*H. macroloba* 9	p-value
Diameter of peripheral utricles in surface view (µm)	32.5-50.00	25.00-37.50	37.50-50.00	25.00-32.50	25.00-32.00	27.50-35.00	22.50-30.00	32.50-37.50	30.00-42.50	0.000**
Segment Length (cm)	1.60-2.40	1.40-1.80	2.40-2.50	1.50-1.70	1.40-2.00	1.50-1.90	2.20-2.80	1.40-1.70	1.80-2.20	0.000**
Segment Width (cm)	0.80-2.70	1.40-1.70	2.40-2.80	1.70-1.80	1.70-2.00	2.10-2.40	2.50-3.30	1.80-2.30	2.10-2.90	0.002*
Node height (µm)	100.00-160.00	70.00-130.00	320.00-380.00	130.00-170.00	470.00-550.00	95.00-112.50	180-230.00	150.00-200.00	100.00-150.00	0.002*
Pore size (µm)	20.00-70.00	40.00-70.00	20.00-40.00	60.00-80.00	120.00-210.00	25.00-42.50	40.00-60.00	30.00-50.00	30.00-60.00	0.000**
Length of peripheral utricle (µm)	50.00-125.00	50.00-105.00	82.50-105.00	52.50-75.00	112.50-140.00	87.50-112.50	75.00-112.50	72.50-90.00	70.00-87.50	0.000**
Max width of peripheral utricle (µm)	27.50-60.00	27.50-40.00	40.00-52.50	25.00-32.50	27.50-40.00	30.00-35.00	27.50-37.50	32.50-42.50	32.50-42.50	0.000**
Number of utricle	3-4	3-5	3-5	3-5	3-5	3-5	4-6	4-6	5-7	0.000**

peripheral utricle 2.40 – 2.50 cm and 1.60 – 1.70 cm, respectively. The highest was in the *H. macroloba* 7 group (2.80 cm), and *H. macroloba* 5 and *H. macroloba* 8 (1.40 cm) were the lowest. Length of segment could be used to separate between group of *H. macroloba* 3 and other groups, except group 7 and 9. There were significant differences in length of segment between group 3 and 1 (Tukey HSD, P = 0.006); and among groups 3 and 2, 4, 5, 8 (Tukey HSD, P < 0.0001).

3. Variations in width of segment (CH 10)

There was significant difference in segment width of *H. macroloba* (df = 8, F = 4.199, P = 0.002; Figure 33C, Table 8). *H. macroloba* 1 had the widest, 0.80 – 2.90 cm; and *H. macroloba* 4 had the narrowest, 1.70 – 1.80 cm. The highest width was *H. macroloba* 7 group (3.30 cm), and group of *H. macroloba* 1 (0.80 cm) had the lowest. Only four groups of *H. macroloba* 2, 3, 5 and 7 were significantly different in width of segment, between group 2 and 3 (Tukey HSD, P = 0.038), between group 2 and 7 (Tukey HSD, P = 0.003), between group 5 and 7 (Tukey HSD, P = 0.031). Width of segment could be used to separate among groups of *H. macroloba* group 2 and group 3, 7 (Tukey HSD, P = 0.038).

Anatomical Variations

1. Variations in node height (CH 25)

There was significant difference in node heights of *H. macroloba* (df = 8, F = 4.199, P = 0.002; Figure 33D, Table 8). *H. macroloba* 2 and 3 had the widest, 70.00 – 130.00 µm and 320.00 – 380.00 µm, respectively; and *H. macroloba* 6 had the narrowest height, 95.00 – 112.50 µm. The highest in node height was *H. macroloba* 3 group (380.00 µm), and *H. macroloba* 2 (70.00 µm) was the lowest. Node height could be used to distinguish between group of *H. macroloba* 3 and other groups, range of this group were non-overlapping with other groups. There was significant differences in node height among groups (Tukey HSD, P < 0.0001).

2. Variations in pore size (CH 26)

There was significant difference in pore size of *H. macroloba* (df = 8, F = 13.110, P < 0.0001; Figure 33E, Table 8)**.** *H. macroloba* 1 had the widest pore diameter, 20.00 – 70.00 µm and *H. macroloba* 6 had the narrowest diameter, 25.00 – 42.50 µm. The highest in node height pore size was *H. macroloba* 4 group (80.00 µm), and *H. macroloba* 1 and 3 (20.00 µm) were the lowest. Pore size could be used to distinguish between group of *H. macroloba* 4 and other groups, except group 1 and 6. There were significant differences in pore size between group 2 and 7; Tukey HSD, P < 0.0001; and among *H. macroloba* 8, 9 and 5; Tukey HSD, P = 0.005, 0.007 and 0.003, respectively.

3. Variations in length of peripheral utricle (CH 30)

There was significant difference in length of peripheral utricle of *H. macroloba* (df = 8, F = 21.464, P < 0.0001; Figure 33F, Table 8)**.** *H. macroloba* 1 had the widest, 50.00 – 125.00 µm and *H. macroloba* 8 had the narrowest length, 70.00 – 87.50 µm. The highest in peripheral utricle length was *H. macroloba* 5 group (140.00 µm), and *H. macroloba* 1 and 2 (50.00 µm) were the lowest. Length of peripheral utricle could be used to distinguish between group of *H. macroloba* 5 and other group, except group 6. There were significant difference in length of peripheral among group 5 and 1, 2, 4, 8, 9 (Tukey HSD, P < 0.0001) and between group 5 and 3, 7 (Tukey HSD, P = 0.002).

4. Variations in width of peripheral utricle (CH 31)

There was significant difference in width of peripheral utricle of *H. macroloba* (df = 8, F = 15.159, P < 0.0001; Figure 33G, Table 8)**.** *H. macroloba* 1 had the widest width of peripheral utricle, 27.50 – 60.00 µm and *H. macroloba* 6 had the narrowest width 30.00 – 35.00 µm. The highest in peripheral utricle width was *H. macroloba* 1 group (60.00 µm), and *H. macroloba* 4 (25.00 µm) was the lowest.

Width of peripheral utricle could be used to distinguish between group of *H. macroloba* 3 and other group. There was significant difference in width of peripheral utricle among groups (Tukey HSD, P < 0.0001).

5. Variations in number of utricle layers (CH 60)

There was significant difference in number of utricle of *H. macroloba* (df = 8, F = 230.84, P < 0.0001; Table 8). The highest was in the *H. macroloba* 8 group (7 layers), and *H. macroloba* 1, 2 and 3 (4 layers) were the lowest. Layer of utricles could be used to distinguish between group of *H. macroloba* 9 and other group. There were significant differences among groups (Tukey HSD, P < 0.0001).

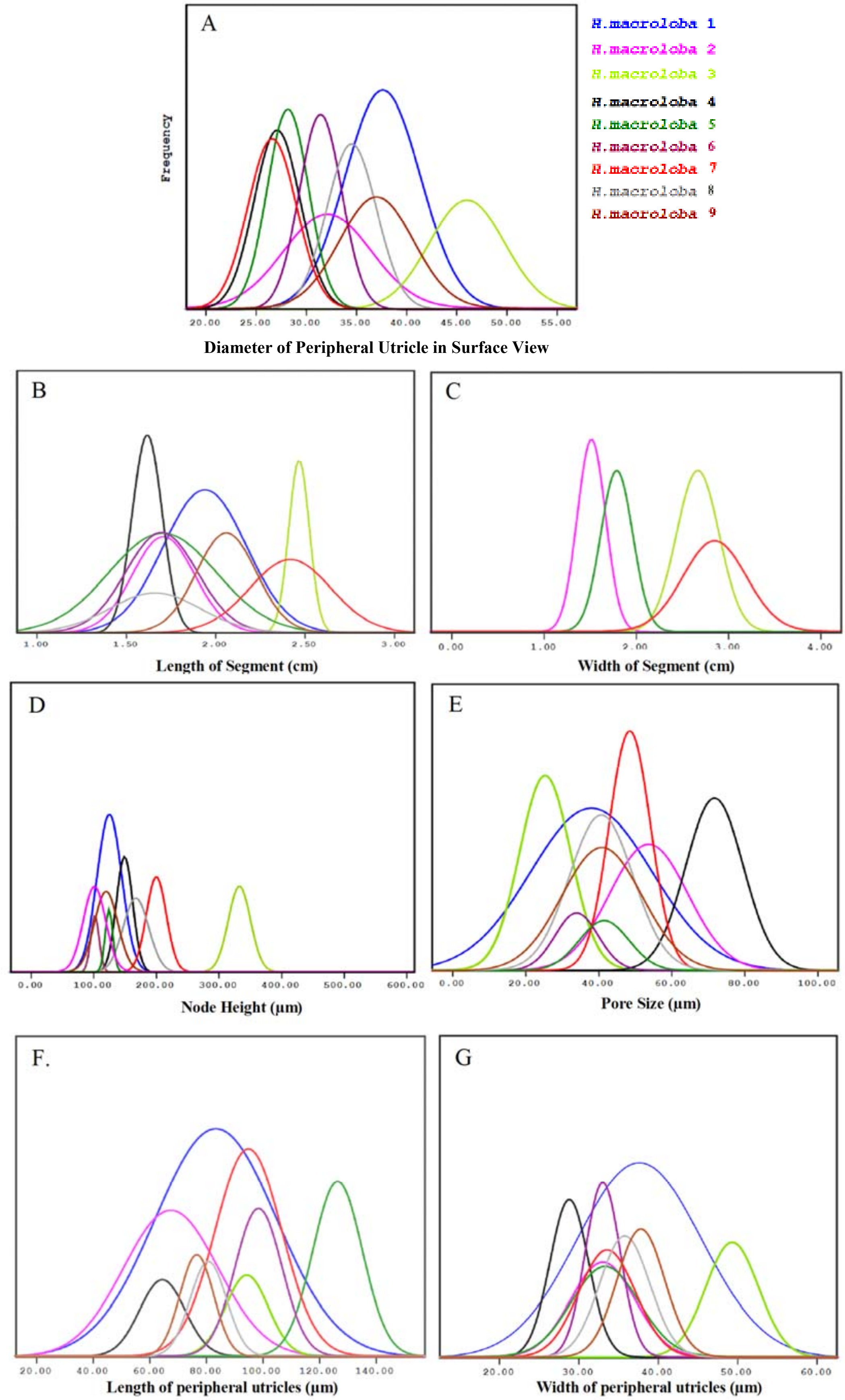


Figure 33 Estimated variation of character of *Halimeda macroloba* Decne. using morphological and anatomical characters.

Lengths of segment, widths of segment, node height and number of utricles layers were strongly influenced by depth and they were negative influenced by wave action. Lengths and widths of segment and number of utricle layers increased with increasing of depth and decreasing of wave. On the other hand, diameters of peripheral utricles in surface view, pore size, length and width of peripheral utricle were not influence by environmental factors (Figure 34).

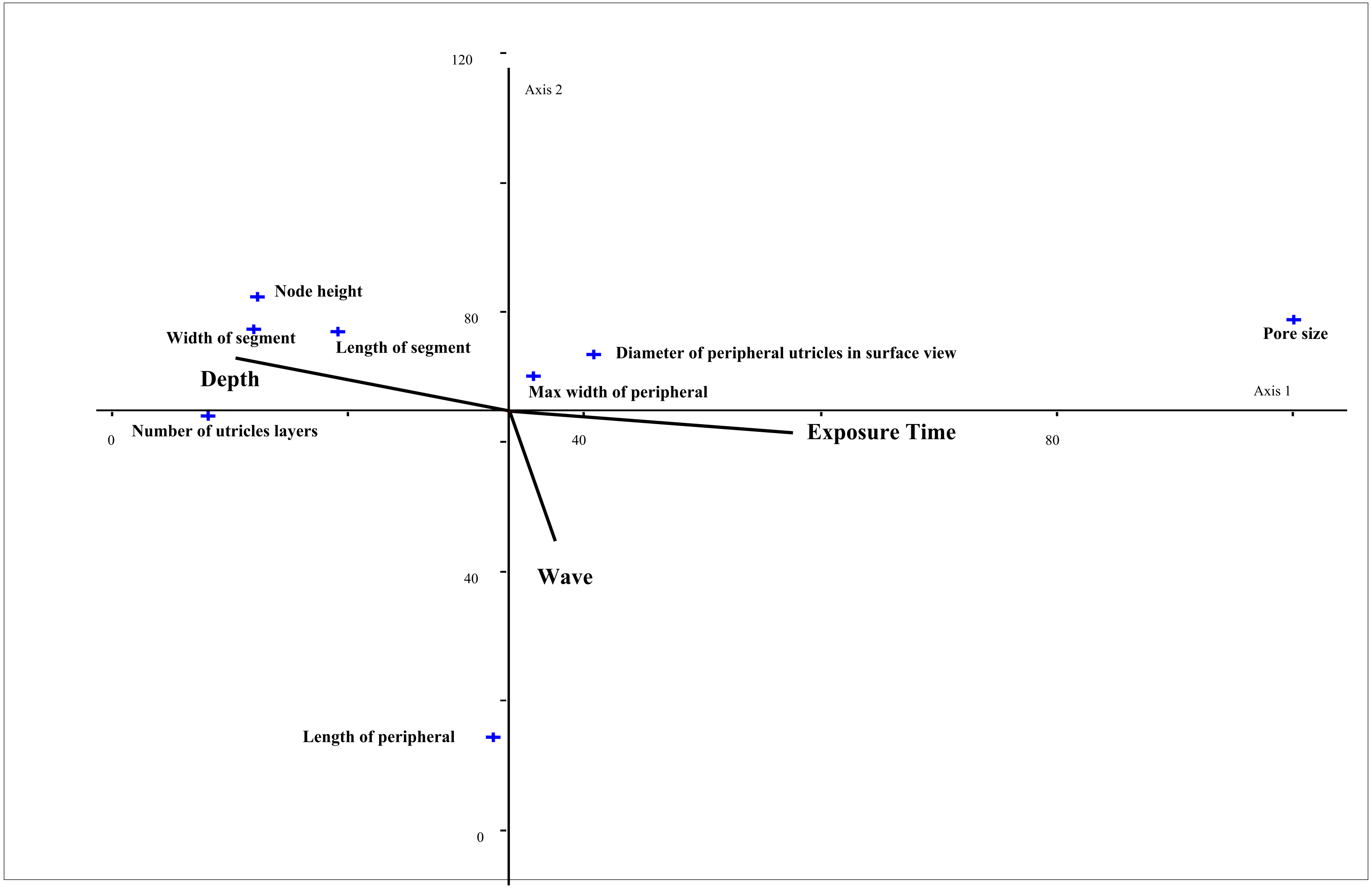


Figure 34 Canonical Correspondence Analysis biplots of *Halimeda macroloba* Decne.

CHAPTER 4

DISCUSSION

Diversity of Genus *Halimeda* J.V.Lamour. in Peninsular Thailand

Taxonomic Diversity

There were eight species of *Halimeda* found in this study namely: *Halimeda macroloba* Decne., *H. discoidea* Decne., *H. opuntia* (L.) J.V.Lamour., *H. heteromorpha* N'Yeurt, *H. tuna* (J. Ellis & Solander) J.V.Lamour., *H. borneensis* W.R. Taylor, *H. gigas* W.R. Taylor and *Halimeda* sp. These accounted for 34.62 % and 15 % of the total number of *Halimeda* species reported in South-East Asia (26 species) and the world (60 species) respectively. Three species of section *Rhipsalis* J. Agardh ex De Toni: *H. macroloba*, *H. heteromorpha*, *H. borneensis*; three species of section *Halimeda*: *H. discoidea*, *H. gigas*, *H. tuna*; and one species of section *Opuntia* J. Agardh ex De Toni: *H. opuntia*. were reported from this study. *H. macroloba* is the only species found in both the Gulf of Thailand and the Andaman Sea whiles other species: *H. opuntia, H. discoidea, H. tuna, H. borneensis, H. gigas, H. heteromorpha and Halimeda* sp. are found only in the Andaman Sea. *Halimeda macroloba* is the dominant species in Thailand, it is commonly found in the Gulf of Thailand and the Andaman Sea. Similan Island, Phang nga province is home to the highest diversity of *Halimeda* in this study.

New Records

This study had added four new records of *Halimeda* to Thai macroalgal flora; they are *Halimeda borneensis, H. gigas, H. tuna,* and *H. heteromorpha.* The studies of taxonomy of seaweed in Thailand are limited, a checklist of algae in Thailand was compiled by Lewmanomont *et al*., (1995) and a list of seaweed and seagrass collection was reported from PMBC reference collection (Aungtonya and Liao, L.M., 2002). During the past 10 years, the taxonomic study has been focused on the economic species such as *Gracilaria* spp. ((Lewmanomont and Kaewsuralikhit, 1993; Lewmanomont, 1994; Lewmanomont 1995; Lewmanomont and Chirapart, 2004; Chirapart, 2008), and recently on *Caulerpa* (Lewmanomont, 2008) and *Ulva* (Pongparadon *et al*., 2008), but not in the genus *Halimeda* even though it is common and important in the coastal and coral reefs. This study has increased the number of *Halimeda* species found in Thailand, and allowed us to understand more of the distribution and variations of this genus. An extensive collection covers a subtidal habitat as well as off-shore islands could also add the knowledge of marine flora in this region.

Unidentified Species

In this study, one species could not be identified to the species lavel, *Halimeda sp.* (S. Pongparadon 17). It is found in the canal of mangrove forest and attached on sponge; this is a rather unique habitat and differs from *Halimeda* reported worldwide (Verbruggen, personal communication). The morphological and anatomical characters of this group were different from the others; and cluster analysis and ordinations showed that this cluster occupied non-overlapping regions with other species. The characters have also never seen before in other *Halimeda,* thallus erect, calcification light, anchored on sponge substrates by a small rhizoidol mat, medullar siphons fused in pair at node, 3-5 utricles layers and surface view of peripheral utricles appear as a pattern of polygonal. The morphology and anatomy and molecular analyses are now in process, these would allow us to have another additional character to confirm the possible new species to science.

Taxonomic Problem of Genus *Halimeda* J.V.Lamour.

Revision of genus *Halimeda* (Verbruggen, 2005) has shown that there were similarities in *Halimeda incrassata* and *H. melanesica*, *H. simulans* and *H. borneesis* causing the identification problems for phychologists. Recent studies using biogeography and molecular technique had solved the problems of *H. incrassata.* They suggested that *H. incrassata* (J.Ellis) J.V.Lamour. belonged to Atlantic Ocean; and there were 3 species, namely *H. kanaloana*, *H. heteromorpha* and *H. melanesica* belonged to the Indo-Pacific basin and Pacific Ocean (Verbruggen *et al*., 2005). Thus, the report of *H. incrassata* in Thailand by Lewmanomont *et al*. (1995) is not currently correct and needed to be revised. This is also true with other species such as *Parvocaulis parvulus* (Solms-Laubach) S. Berger, U. Fettweiss, S. Gleissberg, L. B. Liddle, U. Richter, H. Sawitsky & G.C. Zuccarello, *Rhipidosiphon javensis* Montagne (Berger *et al*., 2003) and *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck (De Clerck *et al*., 2006). An updated checklist of marine macroalgae in Thailand is needed, this would provide a current status of macroalgae in Thailand since it has been last complied in 1995.

I have found the problems with references' specimen since they have not been well preserved and there were no clear labeled of each specimen. This caused such problems when the specimens' comparison needed. This, however, might not be true in terrestrial plants since there are a few good reference collections in Thailand such as The Forest Herbarium and PSU herbarium. Thus, further studies of seaweed should have a better reference collection. They should be preserved in four systems: herbarium specimen, 4% formaldehyde, 70 % or 95% alcohol and silica gel. The specimens from herbarium, 70 % or 95% alcohol and silica gel could be used for the molecular analysis. The herbarium specimen could preserve the color of the specimen, while 4% formaldehyde, 70 % or 95% alcohol could preserve the form of the specimen.

Distribution of Genus *Halimeda* J.V.Lamour. in Peninsular Thailand

Halimeda occurs in various habitats both in the tropical and subtropical marine environments; they are commonly found in sandy beaches, seagrass beds and coral reefs (Goreau and Graham, 1967; Hillis-Colinvaux, 1974; 1977; 1980; Noble, 1987; Littler and Litter, 2000; 2003). In this study, eight species of *Halimeda* has been reported from the Andaman Sea and the Gulf of Thailand (*H. macroloba, H. opuntia, H. discoidea, H. tuna, H. borneesis, H. gigas, H. heteromorpha* and *Halimeda* sp.). Surprisingly, *H. macroloba* was the only species found in the Gulf of Thailand. The Peninsular Thailand might act as a barrier between the Gulf of Thailand, Pacific Ocean and the Andaman Sea, Indian Ocean; limit the dispersal abilities of seaweeds and other marine organisms. In addition, surface current could also influence the diversity and distribution of marine life. On the Andaman coast, organisms are influenced by the surface current from the Indian Ocean, while on the Gulf of Thailand, the surface current is influenced by the South China Sea (Morton and Blackmore, 2001). Together with the Peninsular Thailand, these limited water circulation between both seas, restricted the dispersal abilities of *Halimeda* between the Andaman Sea and the Gulf of Thailand. Furthermore, fertilization of *Halimeda* occurs in minutes that gametes released until 1 hour, gametes still motile but the chance becomes less within 90 minutes after the releasing of gametes (Kenneth *et al*., 1999). The Peninsular Thailand barrier, surface current and the reproductive biology of *Halimeda* could influence diversity and distribution of *Halimeda* along the coast and also between the Gulf of Thailand and the Andaman Sea. Further, studies, however, are needed for a better under standing of such distribution.

The sediment inhibit or prevent the attachment and survival of macroalgae spores (Eriksson and Johnsson, 2003; Isaeus *et al*., 2004). The scouring of sediment from moving water can affect early post-settlement stages (Ekriksson and Johnsson, 2003). Sediment can also affect growth indirectly by shading and inhibiting photosynthesis (Chapman and Fletcher, 2002). There are rather high amount of sediment accumulation in the Gulf of Thailand (Morton and Blackmore, 2001), which could limit the spore settlement of *Halimeda* in the Gulf of Thailand resulted in only a species of *H. macroloba*. The common and dominant of *H. macrolobla* in the Gulf of Thailand might be because of their bulbous holdfast, preferable to soft-bottom substrate; and their high growth rate, 1-2 segments per thallus per day (Sinutok *et al*., 2008).

The habitat reflects in their morphology in a variety of ways (Gilmartin, 1960; Colinvaux *et al*., 1965; Hillis-Conlinvoux, 1980; Kooistra *et al*., 2002; Verburggen *et al*., 2006) and the most apparent adaptation to environmental factors is the holdfast of *Halimeda*, *Udotea* and *Avrainvillea*. The holdfast type could influence their distribution; and they are known to have a specifically substrate. The Andaman Sea provides greater coastal habitats and microhabitats such as coral reef, sandy beach, mangroves and seagrass bed than in the Gulf of Thailand. This could also promote the diversity of *Halimeda* on the Andaman Sea. However, further studies to understand the source and dispersal abilities of seaweeds between the Indian Ocean and the Pacific Ocean would be important and they would clearly allow us to understand the distribution pattern of seaweeds in this region, which is still little known.

Morphological and Anatomical Variations among Populations within Species of *Halimeda macroloba* Decne.

The morphological and anatomical characters of *H. macroloba* in Thailand were highly varied. The quantitative characters of *H. macroloba*; lengths of segment, widths of segment, diameters of peripheral utricles in surface view, node height and number of utricles layers showed highly variations and some characters were influenced by environmental factors. Thus, these characters were not good characters to use for an identification key. The good characters that suitable are the qualitative characters such as thallus form, holdfast type, surface pattern of primary utricle, node type and shape of primary utricle. These qualitative characters of *H. macroloba in* Thai water might be using to identification when were combined the other information such as environmental factor, distribution range of each species. Then, the identification of *Halimeda* at species level is possible only by combination of several criteria including biotopes, gross morphology and anatomy of mature segments: thallus form, holdfast type, surface pattern of primary utricle and shape of primary utricle and type of nodal filament.

Based on the results of this study, it was observed that some of the morphological and anatomical characters of *H. macroloba* varied with the environments. These variations were related to wave action, depth and light intensity in each area. Thallus size and segment size decreased with increasing of wave exposure, the smaller thallus and segment size has decreased risk of thallus broken. This has been shown in *H. macroloba*, causing such variation among population. This phenomenon is also known in *H. heteromorpha*, specimens collected from high wave action on the reef flat exhibited smaller segment than the shelter area (Verbruggen *et al*. 2006). Then, wave action was influence the size of thallus and segment.

Thallus size and segment size of *H. macroloba* increased with increasing of depth and decreasing of light intensity. Their gross morphology of *H. macroloba* showed specimens from the shallow lightened depths were lower thallus and smaller segments whereas specimens from deeper and less lightened areas were higher thallus and larger segments. *H. macroloba* were become taller and bigger to maximize light capture. For instance, *H. tuna*, individuals from deep water had broad segments with more surface area than shallow water (Hillis-Colinvaux, 1980; Vroom et al., 2003) In addition, El-Manawy and Shafik (2008) were reported that *H. opuntia* showed marked difference in branching pattern and segment morphology when grows under different light intensity. In high light intensity, this species forms extensive clumps with entangled branches, while it forms loose tufts with distichously branching in low light intensity.

In addition, this study found there is anatomical variation within species that influenced by light intensity. The number of utricle layers of *H. macroloba* increased with decreasing of light intensity. This phenomenon leads to increasing size of *H. macroloba* segments that increased area to maximize light capture.

In conclusion, wave action, depth and light intensity were influence the morphological and anatomical characters of *H. macroloba*. That lead to morphological and anatomical variations: thallus size, segment size and the number of utricle layers among localities those differences in wave action, depth and light intensity. This suggested that *H. macroloba* has a very high plasticity and high adaptation to differences environmental factors; this might also support the common and dominant of this species in Thai water.

CHAPTER 5

CONCLUSION

1. There are eight species of *Halimeda* found in this study:

   Halimeda macroloba Decne.,

   H. discoidea Decne.,

   H. opuntia (L.) J.V.Lamour.,

   H. heteromorhpa N'Yeurt,

   H. tuna (J. Ellis & Solander) J.V.Lamour.,

   H. borneensis W.R. Taylor,

   H. gigas W.R. Taylor

   Halimeda sp.
2. *Halimeda macroloba* is the dominant species in Thailand and commonly found both in the Peninsular Thailand.
3. This study has added four species of new record of marine flora to Thailand, namely *Halimeda borneensis, H. gigas*, *H. tuna,* and *H. heteromorpha.*
4. *Halimeda* sp. (S. Pongparadon 17) is found in the canal of mangrove forest, a rather unique habitat with different morphological and anatomical characters, differs from other *Halimeda* worldwide. The morphology and anatomy and molecular analyses are now in process, these would allow us to have another additional character to confirm the possible new species to science.
5. *H. macroloba* was the only species found in the Gulf of Thailand, while 8 species were found along the shoreline of Andaman Coast. (*H. macroloba, H. opuntia, H. discoidea, H. tuna, H. borneensis, H. gigas, H. heteromorpha* and *Halimeda* sp.)
6. Peninsular Thailand, surface current and the reproductive biology of *Halimeda* might influence diversity and distribution of *Halimeda* along the coast and also between the Gulf of Thailand and the Andaman Sea.

7. There are morphological and anatomical variations among populations within species of *H. macroloba*; diameter of peripheral utricles pattern in surface view, segment length, segment width, node height, pore size, length of peripheral utricle, max width of peripheral utricle and number of utricle layers
8. These variations of *H. macroloba* influenced by light intensity and wave action: segment size and number of utricle layers increased with decreasing of light intensity and decreased with increasing of wave exposure.

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Lists of Publications and Proceeding

- Pongparadon, S. Thongroy, P. and Prathep, A. 2008. Diversity and Distribution of *Ulva* in Thailand. Taxonomy of Southeast Asian Seaweeds, Phang, Lewmanomont, Lim (eds): pp 15-26.
- Sinutok, S., Pongparadon, S. and Prathep, A. 2008. Seasonal variation in density, growth rate and calcium carbonate accumulation of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand. Malaysian Journal of Science 27 (2):1-8.
- Pongparadon, S. and Prathep, A. Diversity and Distribution of Genus *Halimeda* J.V.Lamour. (Chlorophyta) in Peninsular Thailand. Cryptogamie-Algologie. (In preparation)
- Pongparadon, S. and Prathep, A. Morphological and Anatomical Variations among Populations within Species of *Halimeda macroloba* Decne. in Peninsular Thailand. Botanica Marina. (In preparation)