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Systematics of Marine Brown Alga *Sargassum* from Thailand: A Preliminary Study Based on Morphological Data and Nuclear Ribosomal Internal Transcribed Spacer 2 (ITS2) Sequences

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Abstract – The marine brown algal genus *Sargassum* has been investigated extensively based on genetic information. In this report, we performed the first comparative study of morphological and molecular data among common species of *Sargassum* found in Thailand and explored the phylogenetic diversity within the genus. Our results revealed an incongruent pattern for species classification in Thai *Sargassum*. Morphologically, our *Sargassum* specimens were distinguishable and represented 8 species, namely, *S. aquifolium* (Turner) C.Agardh, *Sargassum baccularia* (Mertens) C. Agardh, *S. cinereum* J. Agardh, *S. ilicifolium* (Turner) C.Agardh, *S. oligocystum* Montagne, *S. plagiophyllum* C. Agardh, *S. polycystum* C. Agardh and *S. swartzii* (Turuner) C. Agardh. In contrast, using three different methods, phylogenetic analysis of nuclear ribosomal internal transcribed spacer 2 (ITS2) revealed six distinct clades, including *S. baccularia/S. oligosyntum* clade, *S. aquifolium/S. swartzii* clade, *S. cinereum* clade, *S. aquifolium/S. ilicifolium* clade, *S. polycystum* clade, and *S. plagiophyllum* clade, which was suggestive of a phenotypic plasticity species complex. Our molecular data also confirmed the paraphyletic relationship in the section *Binderianae* and suggested that this section requires reassessment. Overall, further studies are required to increase our understanding of the taxonomy, phylogenetic relationships and species boundaries among *Sargassum* species in Thailand.

Key words – *Sargassum*, Thailand, ITS2, phylogeny, taxonomy

1. Introduction

Marine rockweed *Sargassum* C. Agardh is one of the largest genera of Phaeophyceae with 335 species (Guiry and Guiry 2013), and is widely distributed among tropical to temperate basins (Yoshida 1989; Stiger et al. 2000; Oak et al. 2002). The center of diversity for the species is found in the Indo-Malay basin and Australia (Noiraksar and Ajisaka 2008). Furthermore, *Sargassum* beds play important roles in marine ecosystems as spawning areas and nursery grounds for commercial pelagic fish (Komatsu and Kawai 1986; Komatsu and Murakami 1994; Komatsu et al. 1996).

The traditional classification system of *Sargassum* includes four subgenera: *Phyllotrichia*, *Bactrophycus*, *Arthrophycus* and *Sargassum* (Yoshida 1989; Yoshida et al. 2002; Mattio and Payri 2009b), which are mainly based on morphological characteristics of the stem, leaves, vesicles, holdfast and receptacles (Phillips and Frederieq 2000; Noris 2010). In addition, distribution patterns are used as a criterion to distinguish the subgenera of *Sargassum*. For example, temperate subgenera of *Bactrophycus* and *Arthrophycus* are distributed in the northern and southern hemispheres, respectively. Subgenus *Phyllotrichia* is only found in Australia and adjacent areas. On the other hand, the subgenus *Sargassum* is widely distributed in the tropical regions of the northern and southern hemispheres (Mattio and Payri 2009b). Recently, Dixon et al. 2014 revised the subgenera taxonomy and merged subgenus *Arthrophycus* into subgenus *Bactrophycus* section *Halochloa*. Therefore, the traditional classification system of *Sargassum* is presented in 3 subgenera: *Bactrophycus, Phyllotrichia* and *Sargassum*.

Morphological traits and geographical distributions may be used to distinguish among subgenera within the genus *Sargassum*, but the taxonomic framework and classification system of the genus remain unclear due to the high level of

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*Corresponding author. E-mail: brownalgae@gmail.com morphological plasticity caused by differences in their environmental conditions (Kilar et al. 1992; Shimabukuro et al. 2012).

Recently, molecular marker techniques have been applied to resolve taxonomic issues. Some species belonging to the genus *Sargassum* have been revised taxonomically using morphological data combined with molecular data. Molecular markers used to elucidate the phylogenetic relationships and species boundaries within this genus include the partial *rbc* operon, internal transcribed spacer of nuclear ribosomal DNA (ITS), and mitochondrial *cox*3 region (Yoshida et al. 2000; Phillips et al. 2005; Lane et al. 2007; Mattio et al. 2010). Particularly, the ITS sequence is commonly used to analyze phylogenetic relationships among species and populations of *Sargassum* (e.g. Stiger et al. 2000, 2003; Mattio et al. 2008, 2009a, 2010; Cho et al. 2012; Dixon et al. 2012; Dixon et al. 2014).

Thailand is a tropical country located in the Indo-Pacific region, which is considered a species-rich region for *Sargassum*. The total coastline of Thailand is approximately 2,650 km in length, consisting of 1,880 km along the Gulf of Thailand (Pacific Ocean) and 770 km along the Andaman Sea (Indian Ocean), where high levels of seaweed diversity have been reported (Noiraksar et al. 2006). Schemidt first discussed seaweed diversity in Thailand in 1899 and published the first Thai seaweed species list, entitled "Flora of Koh Chang", which included one species of *Sargassum*, namely, *S. polycystum* C Agardh (Schmidt 1900).

At this time, 12 species of *Sargassum* have been reported in Thailand: 10 species found in the Gulf of Thailand, consisting of *S. aquifolium* (Turner) C.Agardh, *S. baccularia* (Mertens) C Agardh, *S. cinereum* J. Agardh, *S. ilicifolium* (Turner) C.Agardh, *S. longifrucum* Tseng et Lu, *S. oligocystum* Montagne, *S. polycystum* C. Agardh, *S. siliquosum* J. Agardh, *S. swartzii* (Turuner) C. Agardh, and *Sargassum* sp., as well as six species found in the Andaman sea, consisting of *S. aqulifoium* (Turner) C. Agardh, *S. ilicifolium* (Turner) C. Agardh, *S. polycystum* C. Agardh, *S. granuliferum* C. Agardh, *S. siliquosum* J. Agardh and *S. plagiophyllum* C. Agardh (Lewmanomont and Ogawa 1995; Aungtonya and Liao 2002; Ajisaka and Lewmanomont 2004; Noiraksar and Ajisaka 2008). However, the majority of studies on species diversity and taxonomy in the genus *Sargassum* have been performed mainly based on gross morphology and the development of thalli structure, and this has resulted in species misidentification or underestimation of the true diversity among the genus *Sargassum* in Thailand.

This study reassesses the current diversity and phylogenetic relationship among common *Sargassum* species found in Thailand using molecular markers of nuclear DNA internal transcribed spacer 2 (ITS2) combined with characteristic morphological features.

2. Materials and Methods

Sampling

A total of 20 *Sargassum* specimens were collected along the coastline of the Gulf of Thailand and Andaman Sea (Fig. 1 and Table 1). Sampling was performed by snorkeling or SCUBA diving. All samples were fixed and stored in 4% formalin/seawater or pressed onto herbarium sheets for morphological observation. In addition, partial tissues from specimens were preserved for DNA analysis using silica gel desiccation. Voucher specimens were deposited in the Marine Science Institute, Burapha University.

DNA extraction, PCR and sequencing

Each small dried tissue section maintained in silica gel was cleaned with distilled water to eliminate contamination by epi- and endophytic algae. Genomic DNA was then extracted using a DNeasy plant mini kit (Qiagen, Hilden, Germany), following the manufacturer's protocol, and was further purified using a GENECLEAN$^{®}$II kit (Bio 101).

The complete sequence of ITS2 was obtained and amplified using primers 5.8S BF (5'-CGATGAAGAACGCAGCGAAA-TGCGAT-3') (Yoshida et al. 2000) and 25BR2 (5'-TCCTCCGCTTAGTATATGCTTAA) (Yoshida et al. 2000). PCR amplifications were performed according to Yoshida et al. (2000) using the following conditions: 35 cycles of denaturing at 94°C for 30s, annealing at 50°C for 30s and extension at 72°C for 45s. PCR products were purified as described by Uwai et al. (2009). The purified PCR products were sequenced directly using an autosequencer ABI PRISM, 3010xl Genetic Analyser (Applied Biosystems, USA) and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 with PCR primers.

Data analyses

All new sequences derived from this study and the published sequences retrieved from GenBank are shown in Table 1. Sequences were aligned manually using the MEGA ver. 5 software (Tamura et al. 2011) and further edited using CLUSTAL-W. The results of the alignment were confirmed

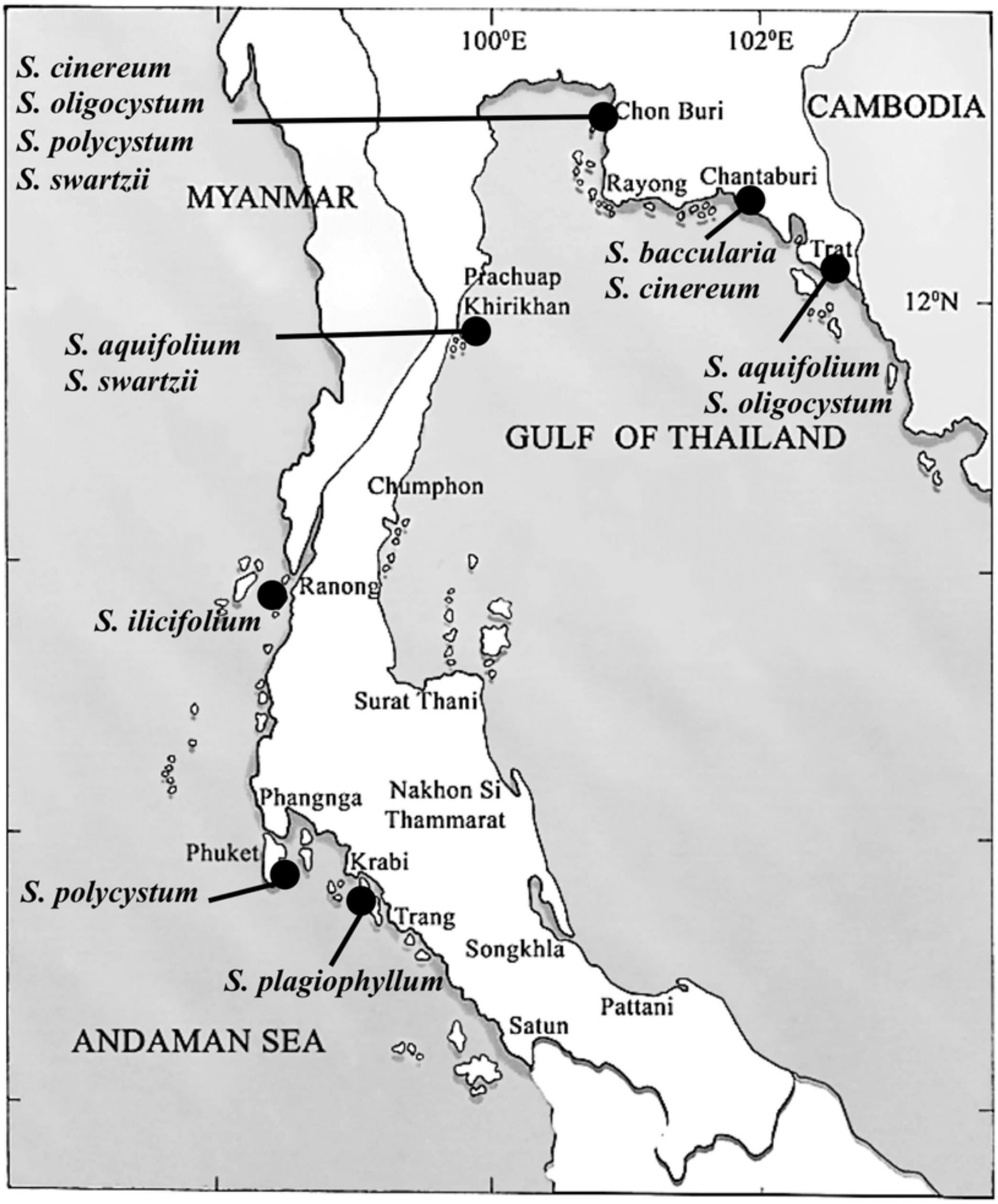


Fig. 1. Map of Thai *Sargassum* collection sites along the coastline of Thailand

manually. Phylogenetic trees were constructed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inferences (BI). MP was performed using PAUP version 4.0b.1 (Swofford, 2002) under the Fitch criterion of equal weights for all substitutions and heuristic search options with 100 random sequence additions and tree bisection reconnection (TBR) swapping. ML trees were generated using RAxML (Stamatakis 2006) with the GTR + I model of evolution. Statistical support for each branch in the MP and ML tree were obtained from 1,000 bootstrap replications. BI analysis was performed using MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). Prior to BI and ML analyses, the best-fit model of nucleotide substitution was selected using JModeltest ver.2.0 (Darriba et al. 2012; Guindon and Gascuel 2003). BI analysis with a random starting tree and four chains of Markov chain Monte Carlo iterations was run for 2,000,000 generations with tree sampling at every 1,000th generation, and the first 25% of trees are removed as burn-in.

Table 1. Sample information of nrDNA ITS2 sequences for this study (bold alphabet) and their GenBank accession numbers.

Subgenus	Species	Collection Data	Code	Date	Accession No.
Sargassum	***Sargassum aquifolium* (Turner) C. Agardh**	Australia		16 October 2009	JN243814
		New Caledonia			HQ416062
		Kermadec Island, New Zealand			EU882251
		Cape Rachado, Port Dickson, Malaysia			AB043116
		Koh Chag, Trat, Thailand	**Tr**	**2 April 2011**	**KP219189**
		Ao Tong Lang, Prachuap Khiri Khan, Thailand	**PC**	**3 May 2010**	**KP271151**
	***Sargassum baccularia* (Mertens) C. Agardh**	**Kung Wiman, Chantaburi, Thailand**	**CT**	**25 December 2009**	**KP219190**
	Sargassum carpophyllum J.Agardh	New Caledonia			EU100798
	***Sargassum cinereum* J. Agardh**	**Kung Wiman, Chantaburi, Thailand**	**CT**	**25 December 2009**	**KP219191**
		Koh Rad, Chon Buri, Thailand	**CB**	**11 February 2011**	**KP271154**
	Sargassum duplicatum (J. Agardh) J. Agardh	Hii-zaki, Wakayama, Japan			AB043614
	***Sargassum ilicifolium* (Turner) C. Agardh**	Tanzania			HQ416061
		Similan Island, Ranong, Thailand	**RN**	**5 April 2011**	**KP219192**
	Sargassum johnstonii Setchell & N.L.Gardner	Mexico			JX560129
	Sargassum mcclurei Setchell	Nhatrang, Vietnam			AB043111
	Sargassum obtusifolium J.Agardh	French Polynesia			EU100785
	***Sargassum oligocystum* Montagne**	**Koh Prow, Trat, Thailand**	**Tr**	**20 April 2011**	**KP219193**
		Koh Rad, Chon Buri, Thailand	**CB**	**18 February 2011**	**KP271152**
	Sargassum pacificum Bory de Saint-Vincent	French Polynesia			EU100774
		French Polynesia			HQ416067
	Sargassum patens C. Agardh	Takahama, Nagasaki, Japan			AB043666
		Seosan, Munseom, South Korea		March 2010	JF931862
	***Sargassum plagiophyllum* C. Agardh**	**Lanta Island, Krabi, Thailand**	**KB**	**16 April 2012**	**KP219196**
		Plau Jerenak, Malaysia			AB043613
	***Sargassum polycystum* C. Agardh**	**Koh Rad, Chon Buri**	**CB**	**18 February 2011**	**KP219194**
		Nai Yang Beach, Phuket, Thailand	**PK**	**11 November 2009**	**KP271153**
		Tanzania			HQ416068
		Solomon Islands			EU833423
		Zanpa-misaki, Okinawa, Japan			AB043113

Table 1. Continued

Subgenus	Species	Collection Data	Code	Date	Accession No.
	Sargassum quinhonense Nguyen Huu Dai	Quy Nhon, Ganh Rang, Vietnam			AB043112
	***Sargassum swartzii* (Turner) C. Agardh**	**Koh Rat, Chon Buri, Thailand**	**CB**	**8 March 2011**	**KP219195**
		Ao Manow, Prachuap Kiri Khan, Thailand	**PC**	**18 March 2011**	**KR676562**
		New Caledonia			EU882254
		New Caledonia			EU882255
	Sargassum yendoi Okamura & Yamada	Tateyama, Chiba, Japan			AB043667
Bactrophycus	*Sargassum hemiphyllum* (Turner) C.Agardh	Tateyama, Chiba, Japan			AB043576
		Nagasaki, Japan			FJ712779
	Sargassum horneri (Turner) C.Agardh	Miyagi, Japan			AB430579
		Katsu-ura, Chiba, Japan			AB043776
	Sargassum miyabei Yendo	Oshoro, Hokkaido, Japan			AB043502
		Pohang, Homigot, South Korea			JF931856
	Sargassum muticum (Yendo) Fensholt	Mangoku-ura, Miyagi, Japan			AB043774
	Sargassum piluliferum (Turner) C. Agardh	Katsu-ura, Chiba, Japan			AB043617
	Sargassum okamurae Yoshida & T. Konno	Chiba, Japan			AB043578
Phyllotricha	*Sargassopsis decurens* (R. Brown ex Turner) C. Agardh	Laregniere, New Caledonia			AB043121
	Sargassopsis decurens (R. Brown ex Turner) C.A gardh	New Caledonia			EU882257
		New Caledonia			EU100773
	Genus *Turbinaria*				
	Turbinaria conoides (J.Agardh) Kützing	French Polynesia			DQ448827

Table 2. Comparison of species of *Sargassum* in Thailand

Feature	*S. aquifolium*	*S. baccularia*	*S. cinereum*	*S. ilicifolium*
Holdfast	Discoid	Discoid	Discoid	Discoid
Stem	Terete, smooth to warty	Terete, warty	Terete, smooth	Terete, Smooth
Primary branch	Flattened to compressed, smooth, up to 46 cm long, smooth	Terete, smooth , up to 200 cm long	Terete to subterete, smooth, up to 125 cm long	Compressed to terete, smooth, up to 29 cm long
Secondary branch	Slightly compressed, smooth	Terete, smooth	Terete, smooth	Terete, smooth
Primary leaves	Lanceolate to slender lanceolate	Lanceolate to linear	Membranous, lanceolate to linear lanceolate	Thick, elliptical to oblong
Secondary leaves	Lanceolate to linear	Lanceolate to linear	Lanceolate to linear	Elliptical-oval, lanceolate to oblong
Vesicles	Spherical to elliptical	Spherical to elliptical	Spherical, obovoid to elliptical	Spherical, elliptical, ovate to obovoid
Receptacle	Monoecious, Flattened often twisted	Dioecious, Male: long, terete simples to once to twice furcate, Female: Triquetrous, simple to furcate	Dioecious, Male: long terete, Female: Short compressed and triquetrous	Monoecious, Terete to slightly compressed
Holdfast	Discoid	Discoid Holdfast trans formed cauline leaves	Discoid	Discoid
Stem	Terete, Smooth	Cylindrical to terete	Terete, warty	Terete, smooth
Primary branch	Flattened to compressed, smooth	Slender, terete, smooth	Terete, muricate with prolifically branched spines, transformed into stolon and secondary holdfast	Compressed, smooth
Secondary branch	Terete to slightly compressed, smooth		Terete, crowded with spine	Compressed, smooth
Primary leaves	Lanceolate to spatulate	Elliptical to lanceolate	Elliptical, lanceolate to linear	Elongated lanceolate to linear-lanceolate
Secondary leaves	Lanceolate to spatulate	Elliptical	Elliptical, lanceolate to linear	Linear lanceolate
Vesicles	Spherical to elliptical	Spherical to obovoid	Spherical to obovate	Elliptical
Receptacle	Monoecious, Slightly compressed, warty to few spines at the margin, simple to furcate two or three times	Dioecious Male: Fusiforim or cylindrical, warty surface	Dioecious, Male: long terete, warty surface, simple to once furcate, Female: terete to slightly compressed, warty surface, simple to once furcate	Monoecious, Slighty terete, small spines at the apices and margins

Sargassopsis decurrens (R. Brown ex Turner) and *Turbinaria conoides* (J. Agardh) Kützing were selected as outgroups because of their close relationship with the genus *Sargassum* (Stiger et al. 2003).

3. Results

Morphology observations

Noiraksar and Ajisaka (2008) observed the morphology of Thai species of the genus *Sargassum* following descriptions reported by previous studies. A total of 20 specimens recently collected from both the Gulf of Thailand and Andaman Sea in this study were classified into eight species according to Matio et al. (2010): *S. aquifolium* (3 specimens), *S. baccularia*, *S. cinereum* (3 specimens), *S. ilicifolium*, *S. oligocystum* (4 specimens), *S. polycystum* (2 specimens), S. *plagiophyllum* (2 specimens) and *S. swartzii* (4 specimens) (Table 1). Only *S. polycystum* was found at both sites, while *S. aquifolium*, *S. baccularia*, *S. cinereum*, *S. oligocystum* and *S. swartzii* were distributed in the Gulf of Thailand, and *S. ilicifolium* and *S. plagiophyllum* were found in the Andaman Sea. Morphological features of these species are detailed in Table 2.

Genetic analyses

The ITS2 sequences of each specimen used for morphological observations were obtained successfully. Our data set included 53 sequences of the ITS2 region, of which 20 sequences were newly identified in this study (Table 1). A total of 33 sequences from 17 species of *Sargassum* were obtained from GenBank. Alignment of the ITS2 sequences revealed 497 base pairs, including gaps.

The results of ML, MP and BI analyses of the ends of the 5.8S gene ITS2 nrDNA sequences from 27 species of *Sargassum* are shown in Fig. 3. Trees were investigated using three different phylogenetic analyses, which showed the same topology with well-resolved clades (95 - 100 for MP and ML, 1.0 for BI). Phylogenetic trees were divided into three main clades corresponding to subgenera *Bactrophycus*, *Sargassum* and *Phyllotrichia*.

Specimens belonging to subgenus *Sargassum* were divided into six sections, namely, *Binderianae*, *Ilicifoliae*, *Polycystae*, *Sargassum*, *Zygocarpicae* and *Johnstonii* (Fig. 3). The clades corresponded to each section, which was strongly supported by high posteriori probability values (BI > 95). The sequences of the specimens collected in Thailand were categorized into three separate sections: *Binderianae* (*S. aquifolium*, *S. baccularia*, *S. ilicifolium*, *S. oligocystum* and *S. swartzii*), *Ilicifoliae* (*S. aquifolium, S. cinereum* and *S. ilicifolium*) and *Polycystae* (*S. polycystum* and *S. plagiophyllum*).

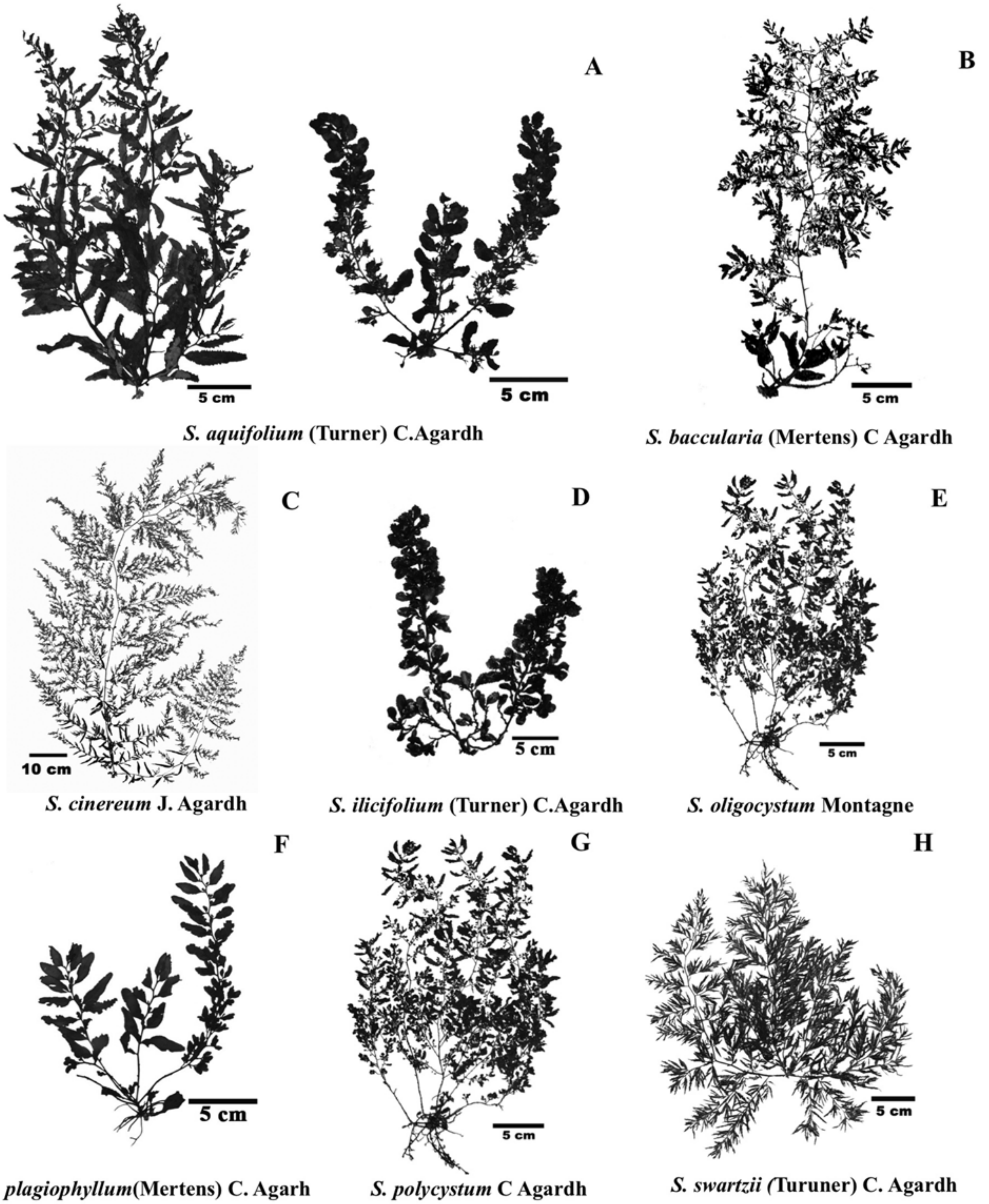
Section *Binderianae* was divided into two independent clades representing sections *Binderianae* I and II. The sequences of *S. baccularia* and *S. oligocystum* were grouped into section *Binderianae* I together with *S. mcclurei* and *S. quinhonense* with moderate support (MP = 71, ML = 65, BI = 0.91). Section *Binderianae* II, consisting of both *S. aquifolium* and *S. swartzii*, formed a well-supported clade as a closely related sister group with *S. patens*. The pairwise distance between *S. baccularia* and *S. oligocystum* was relatively low (<0.02%), suggestive of a close relationship. Genetic divergence between *S. aquilifoium* and *S. swartzii* was 0.4%.

ITS2 phylogenetic analyses did not provide sufficient resolution to confirm a species relationship within the section *Ilicifoliae*. The clade of section *Ilicifoliae* contained *S. aquifolium*, *S. cinereum* and *S. ilicifolium* sequences with moderate support (MP = 85, ML = 83, BI = 1.0). All sequences of *S. cinereum* were clustered with those of *S. ilicifolium* and were supported by low bootstrap values, whereas *S. aquifolium* was grouped with *S. ilicifolium* (Fig. 3). Pairwise differences between *S. cinereum* and other species within a section ranged from 0 - 1.4%, while differences between *S. aquifolium* and *S. ilicifolium* were less than 0.5%.

All sequences of *S. polycystum* and *S. plagiophyllum* formed a well-supported clade (MP = 98, ML = 98, BI = 1.0) within the section *Polycystae* (Fig. 3). Phylogenetic trees were also indicative of the close relationship between *S. polycystum* and *S. plagiophyllum*. Genetic divergence between these two species ranged from 0 to 1%. All sequences of *S. polycystum* samples were identical, whereas the genetic divergence among *S. plagiophyllum* samples was 1%.

4. Discussion

The genus *Sargassum* is one of the most difficult genera with which to perform species-level taxonomic classification due to the large amount of morphological variation and the high-level of adaptation in specific environments. *Sargassum* consists of at least three subgenera: *Bactrophycus*, *Phyllotrichia* and *Sargassum* (Dixon et al. 2014). In tropical regions, members of this genus typically belong to subgenus *Sargassum* (Phillips and Fredericq 2000; Phillips et al. 2005; Mattio et al. 2009a; Mattio et al. 2010; Cho et al. 2012). Our study clearly showed that all Thai *Sargassum* species are of the subgenus *Sargassum*



S. aquifolium **(Turner) C.Agardh**

S. baccularia **(Mertens) C Agardh**

S. cinereum **J. Agardh**

S. ilicifolium **(Turner) C.Agardh**

S. oligocystum **Montagne**

S. plagiophyllum**(Mertens) C. Agarh**

S. polycystum **C Agardh**

S. swartzii **(Turuner) C. Agardh**

Fig. 2. Habit of Thai *Sargassum* species: (**A**) *S. aquifolium* (Turner) C. Agardh, (**B**) *S. baccularia* (Mertens) C. Agardh, (**C**) *S. cinereum* J. Agardh, (**D**) *S. ilicifolium* (Turner) C. Agardh, (**E**) *S. oligocystum* Montagne, (**F**) *S. plagiophyllum* C. Agardh, (**G**) *S. polycystum* C Agardh and (**H**) *S. swartzii* (Turuner) C. Agardh

and could be distinguished morphologically into eight species: *S. aquifolium*, *S. baccularia, S. cinereum, S. ilicifolium, S. polycystum*, *S. oligocystum*, *S. plagiophyllum* and *S. swartzii*. This result is similar to those of morphology-based taxonomic studies of the genus located in Thailand (Lewmanomont and Ogawa 1995; Aungtonya and Liao 2002; Noiraksar and

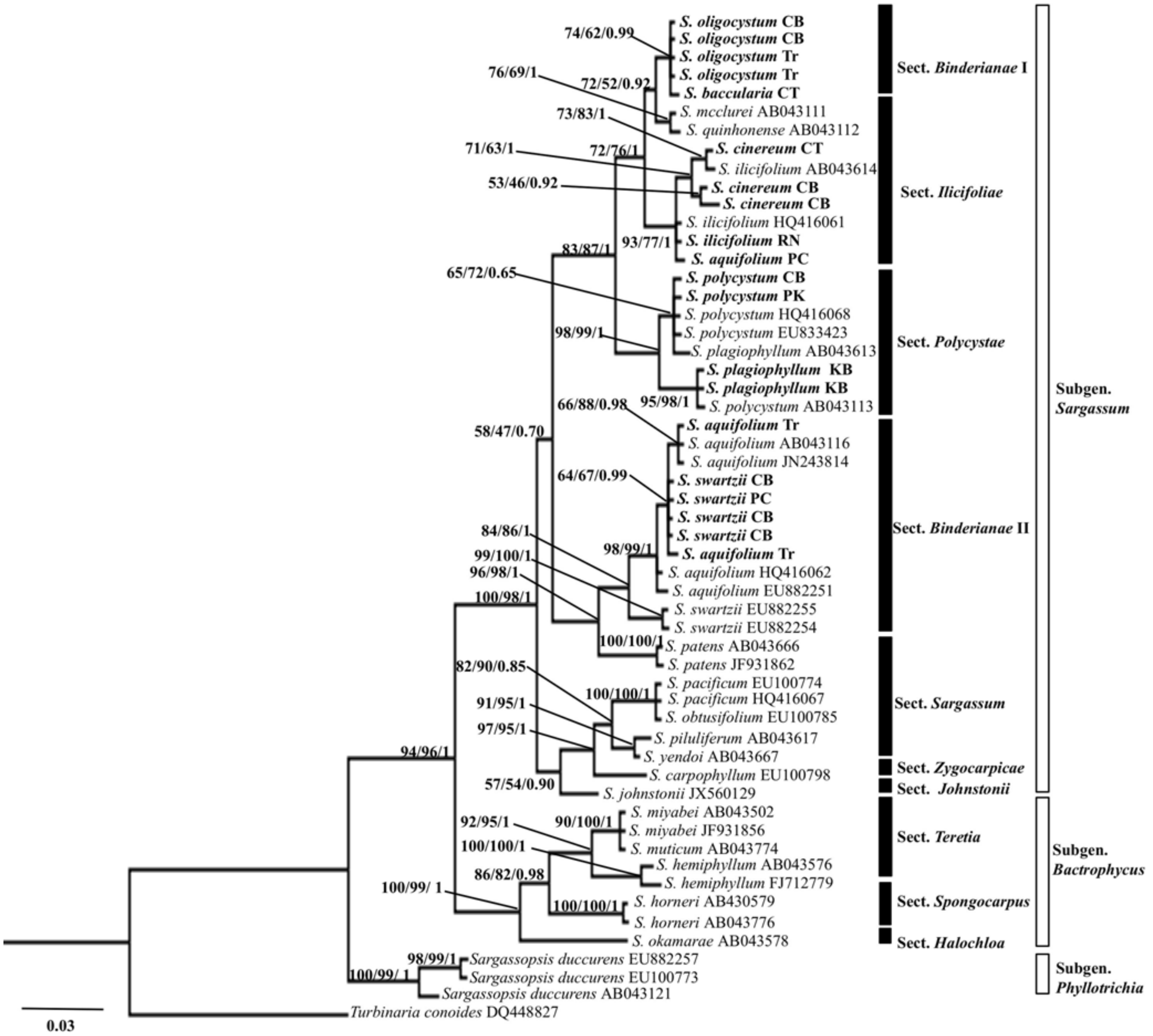


Fig. 3. Bayesian tree based on ITS2 gene sequences. The bootstrap values shown at each node were MP/ML/BI (Bayesian analysis). Scale bar = 0.03 substitutions per site

Ajisaka 2008; Mattio et al. 2008; Mattio et al. 2009a; Mattio and Payri 2009b: Mattio et al. 2010).

Striger et al. (2003) and Cho et al. (2012) reported that ITS2 gene is a suitable marker and has more power in resolving differences in species among subgenera and section within the genus *Sargassum* due to gene mutation rate. Han et al. (2013) stated that the ITS2 can be used to effectively identify species in a wide variety of specimens. On the other hand, it is noted that ITS genes have multiple copies (Draisma et al. 2012). Bailey et al. (2003) suggested that pseudogene sequence might supply important data relating to DNA sequence diversification and interspecific hybridization. In this case, the multiple copies could be important information source. However, other makers will help alleviate the influence of multiple copies on understanding the systematics of *Sargassum* species. For example, *cox*3 is one of the possible genes for this study. *Cox*3 is usually used for Haplotype analysis of *Sargassum* species such as *Sargassum horneri* (Hu et al. 2012) because its mutation rate is higher than ITS2 gene. This might cause another problem for the analysis of the systematics of *Sargassum* species. To resolve the problem of multiple copies of ITS2, there is a need to analyze more genes.

In this study, our genetic analysis was based on ITS2 and showed that the morphology-based taxonomy of Thai *Sargassum* species is not congruent with the phylogenetic tree derived from the ITS2 data set. A total of 20 sequences derived from the samples collected in this study corresponded to eight species (Table 2). Three different methods of phylogenetic analysis based on the ITS2 sequence produced the same topology, indicative of 15 distinct clades, three of which were composed of more than one currently recognized species (Fig. 3, see *S. baccularia/ S. oligocystum* clade, *S. aquifolium/S. ilicifolium* clade and *S. ilicifolium/S. swartzii* clade). Based on this result, there may be species complexes in Thai *Sargassum*. These results were presented as four species complexes (*S. aquifolium/S. ilicifolium, S. baccularia/ S. oligocystum*, *S. polycystum/S. plagiophyllum* and *S. ilicifolium/ S. swartzii*), likely identified because species of the genus *Sargassum* are very difficult to distinguish based on morphology (Kilar et al. 1992; Phillips and Fredericq 2000; Ajisaka 2006). Similarly, species complexes have been reported in *S. horneri/S. filicinum* (Uwai et al. 2009) based on the morphological characteristics between *S. horneri* and *S. filicinum*, which may not be accurate. Furthermore, genetic analysis has revealed high statistical support based on phylogenetic trees.

Our molecular studies found that the clades *S. baccularia* and *S. oligocystum* shared morphological characteristics between the two species. The key for classification of the two species is based on the receptacles, which indicate whether a plant is monoecious or dioecious. However, the majority of plants of *S. oligocystum* in Thailand and Malaysia are monoecious, whereas those in China and the Philippines are dioecious. Moreover, plants of *S. baccularia* are dioecious (Trono 1992; Noiraksar and Ajisaka 2008; Wong et al. 2008). It is not possible to identify *S. baccularia* and *S. oligocystum* using this guideline. Similarly, molecular analysis demonstrated that *S. ilicifolium* and *S. swartzii* belong to sister clades in the phylogenetic tree. Morphological studies also revealed a resemblance in morphological characteristics between the two species (excluding slender leaves and smaller vesicles in specify species), and that the receptacles were arranged cymosely in *S. swartzii* (Table 2) (Noiraksar and Ajisaka 2008; Wong et al. 2008).

The clades *S. polycystum/S. plagiophyllum* (Fig. 3) could be distinguished based on morphological differences in secondary holdfasts, in which cauline leaves are formed in *S. plagiophyllum* and primary branches in *S. polycystum* (Chiang et al. 1992; Lewmanomont and Ogawa 1995; Wong et al. 2008; Mattio et al. 2009a). Molecular analysis between these two species was strongly supported based on the phylogenetic tree. Similar to the revisions of *S. quinhonense* and *S. mcclurei* using molecular analyses (Stiger et al. 2000), sequences of *S. polycystum* and *S. plagiophyllum* were similar but can be distinguished clearly by morphology.

At the section level, traditional taxonomy of the subgenus *Sargassum* comprises three sections: *Acanthocarpicae, Zygocarpcae* and *Malacocarpicae* (Agardh 1820). Mattio et al. (2010) revised section *Acanthocapicae,* based on morphological characteristics and combined data of different genetic markers, and subsequently divided this section into three new sections, namely, *Binderianae, Ilicifoliae* and *Polycystae*. All Thai *Sargassum* were mainly classified genetically into three sections: *Binderianae*, *Ilicifoliae* and *Polycystae*. Two distinct clades of S. *baccularia/ S. oligocystum* and *S. ilicifolium/S. swartzii* are members of sect. *Binderianae. S. cinereum* and *S. aquifolium/S. ilicifolium* clades belong to sect. *Ilicifoliae*, while *S. polycystum* and *S. plagiophyllum* belong to sect. *Polycystae* (Fig. 3).

Our phylogenetic analyses indicated that the members of *Sargassum* sect. *Binderianae* did not form a monophyletic group (see Fig. 3, *Binderianae* I and II). *S. ilicifolium/S. swartzii* (*Binderianae* II) is the sister clade to *S. aquifolium*, while these three species have previously been reported as part of sect. *Binderianae* by Mattio et al. (2010). In contrast, the *S. baccularia/S. oligocystum* clade (*Binderianae* I) is weakly clustered with *S. mcclurei* and *S. quinhonense*, members of the section *Ilicifoliae* (Mattio and Payri 2011). In addition, the section *Binderianae* I is relatively distant in position in the phylogenetic tree from the section *Binderianae* II (Fig. 3). As a result, the clade *S. baccularia/ S. oligocystum* may be considered a novel section in the subgenus *Sargassum.* Based on these results, further studies on the phylogenetic relationships and species boundaries of *Sargassum* species from Thailand using morphological characteristics and different types of DNA markers are required.

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