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Seaweed diversity in Vietnam, with an emphasis on the brown algal genus *Sargassum*

by
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Vietnamese names

Vietnamese names generally consist of three parts: a family name, a middle name, and a given name, used in that order. Due to the ubiquity of the major family names such as Nguyễn, Trần, and Lê, people are often referred to by their middle name along with their given name in the Vietnamese media. This system conflicts with traditional scientific author citations which are biased toward Western-style names. Thereto, in this thesis Vietnamese authors are cited by their family name followed by the initials of their middle and first names. For example, Pham Hoang Ho becomes Pham H.H. Diacritics, of which the Vietnamese language uses nine marks to create additional sounds or to indicate the tone of each word, are left out. If the combination of family name and initials of both middle and first name are non-discriminatory, names are cited by family name followed by the initial of the middle name and the entire first name. For example, Nguyen Huu Dai and Nguyen Huu Dinh, are cited as Nguyen H. Dai and Nguyen H. Dinh, respectively.

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Chapter 1

General introduction and
thesis outline

1.1 Vietnam: geography and climate

Vietnam is located in the Indo-West Pacific on the eastern margin of Indochinese Peninsula. The mainland of Vietnam, extending from 8°27' to 23°23' N, is bordered by the East Sea and the Gulf of Tonkin in the East and the gulf of Thailand in the South. The coastline of Vietnam extends for 3260 km (excluding islands) and is characterized by a subtropical climate in the North which gradually becomes fully tropical in the South. The coastline is dotted by numerous islands, islets, atolls and reefs in ca. ~1.000.000 km² of sea area (Le B.T. 1998). Because of its predominant North-South orientation, the coastal environment of Vietnam is categorized by distinct zones based on meteorological, hydrological and geological characteristics (Fig. 1.1). The physical and climatological characteristics of these zones may differ significantly (Fig. 1.2). For example the North coast is dominated by river mouths and deltas (Red River delta) interspersed by sandy beaches and only a limited amount of rocky shores, while the Central coast is predominantly rocky. The South Central coast has many atolls and islets of coralline origin. The South coast, however, is dominated by the Mekong delta. Both the Red River in the North which discharges in the Gulf of Tonkin and the Mekong in the South have a strong influence on the coastal topography (Vu T.L. 1978, Le B.T. 1998). The influx of fresh water as well as the sediment carried by these rivers, renders these environments largely unfavourable for the growth of marine macroalgae.

Vietnam lies in the East Asian monsoon zone. Two wind patterns dominate the coastline. The winter monsoon coming from the northeast between October and March makes winters relatively cold on the North, North Central and the Central coast, but dry and warm on the South coast. From April-May to October the Southwest monsoon brings warm, humid weather to the whole country. Between July and November, violent and unpredictable typhoons often develop over the Pacific Ocean and often hit the Central and Northern regions with devastating results. In southern Vietnam a wet and a dry season alternate. The wet season lasts from May to November during which heavy but short-lived downpours are a daily reality. The dry season usually runs from December to April. Much of the precipitation in the coastal region is brought by the northeastern monsoon between December and February (Pham N.T. and Phan T.D. 1993).

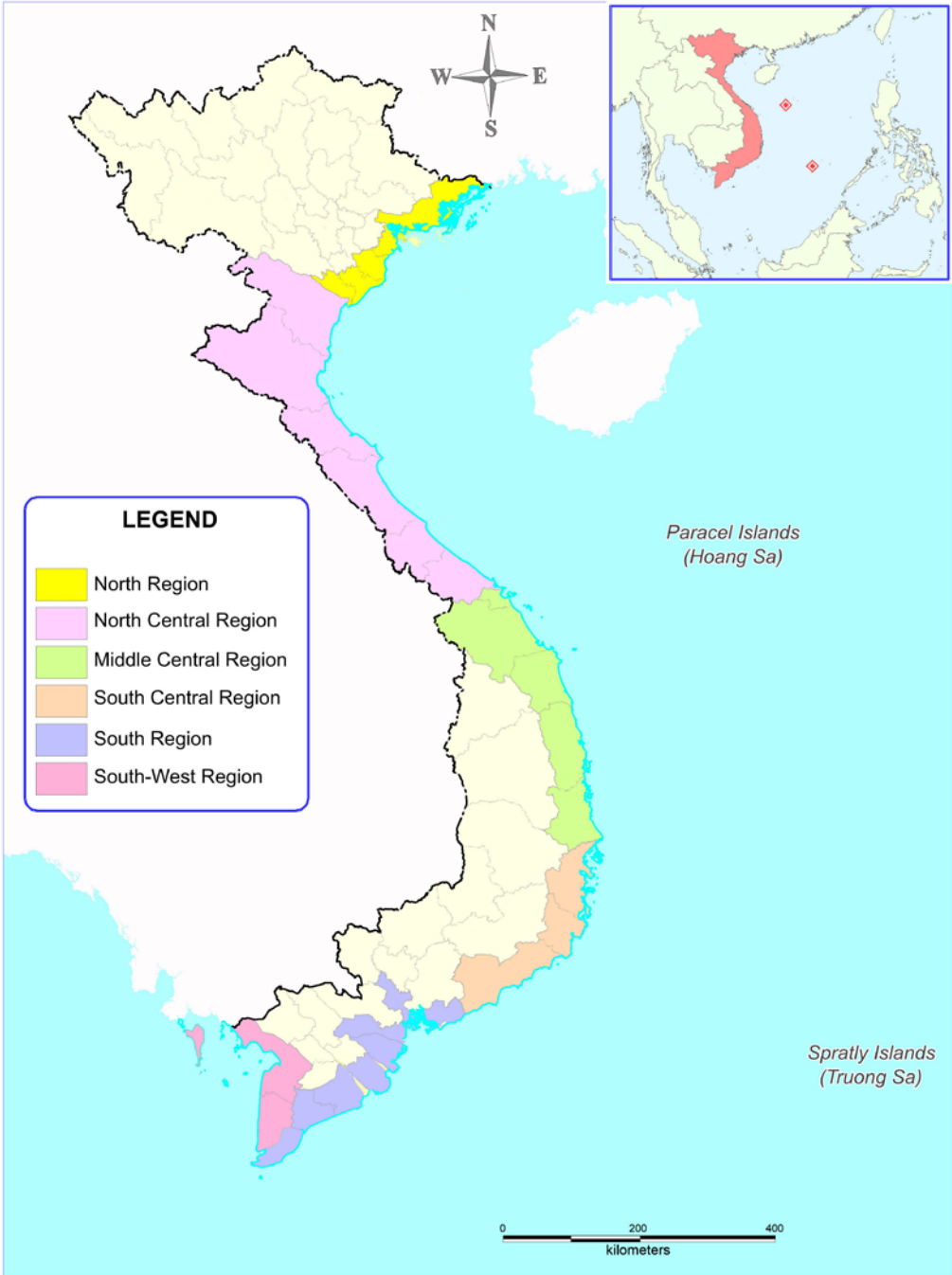


Fig. 1.1: Geographical location of Vietnam with indication of the various coastal zones.

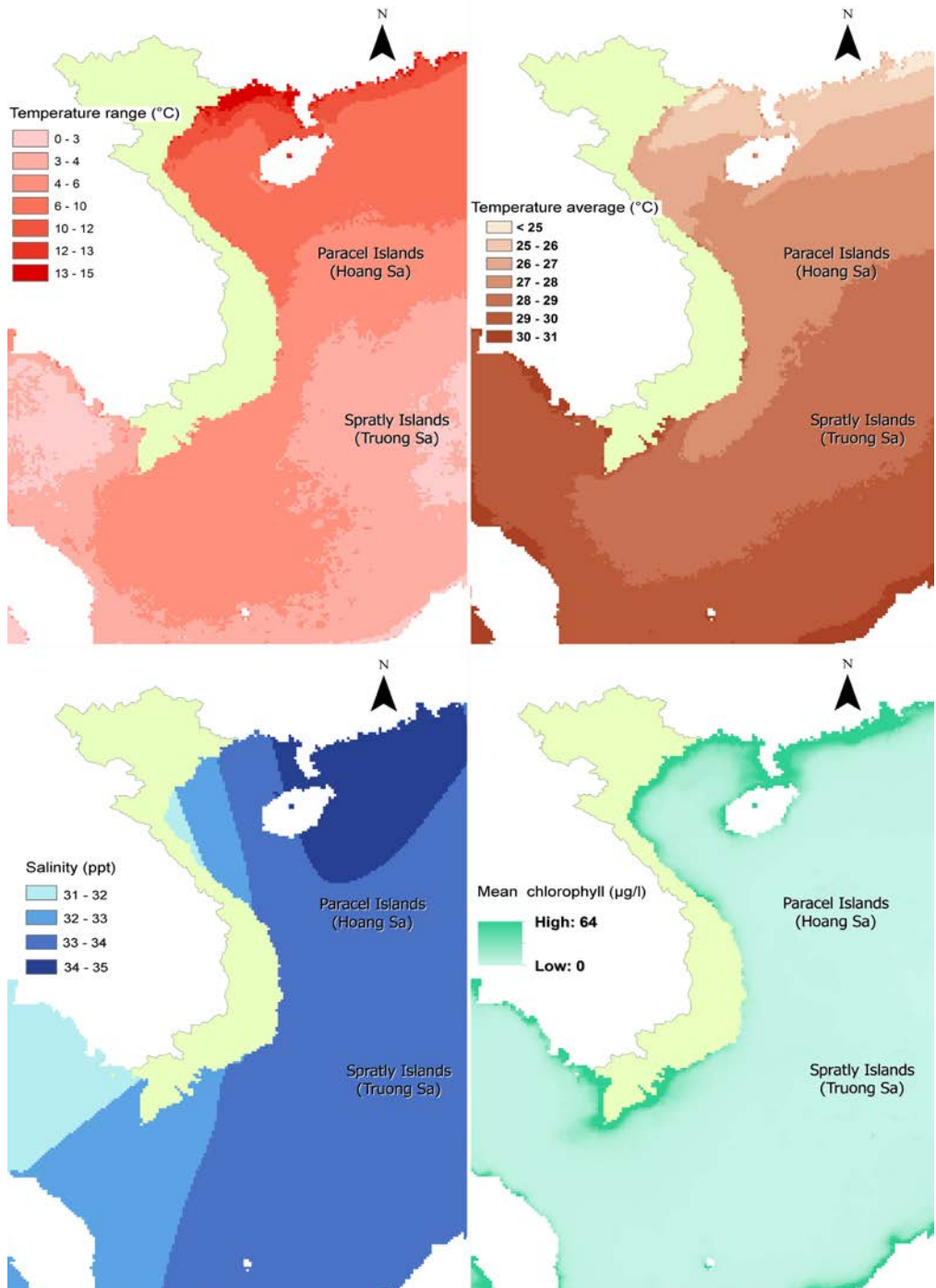


Fig. 1.2: Variation in average temperature, temperature range (seasonality), chlorophyll and salinity along the Vietnamese coastline.

1.2 Seaweed Resources of Vietnam

Global aquaculture has grown dramatically over the past 50 years to around 68.3 million tonnes in 2008 worth US\$ 106 billion (Bostock et al. 2010). In 2011 aquaculture contributed 40.1% to the world total fish production and almost all the seaweed production. Seaweeds are indeed an important component of global aquaculture, amounting to 21 million tonnes in 2011 with an estimated value of US\$ 5.7 billion. Furthermore the amount of farmed seaweed increased at average annual rates by 7.4 % in the years 2000 according to FAO statistics (FAO 2013).

A few species dominate seaweed culture, with 98.9 percent of the world production in 2010 coming from Japanese kelp (*Saccharina japonica*) *Euचेuma* seaweeds (a mixture of *Kappaphycus alvarezii*, formerly known as *Euचेuma cottonii*, and *Euचेuma* spp.), *Gracilaria* spp., nori (*Porphyra* or *Pyropia* spp.), wakame (*Undaria pinnatifida*) and unidentified marine macroalgae species amounting to 3.1 million tonnes, mostly from China (Ohno and Critchley 1998).

In 2010, 99.6 percent of the global cultivated algae production comes from just eight countries: China (58.4 percent, 11.1 million tonnes), Indonesia (20.6 percent, 3.9 million tonnes), the Philippines (9.5 percent, 1.8 million tonnes), the Republic of Korea (4.7 percent, 901,700 tonnes), Democratic People's Republic of Korea (2.3 percent, 444,300 tonnes), Japan (2.3 percent, 432,800 tonnes), Malaysia (1.1 percent, 207,900 tonnes) and the United Republic of Tanzania (0.7 percent, 132,000 tonnes) (FAO 2013). Notably absent in this list is Vietnam, which, as will be demonstrated below, does not support an extensive seaweed aquaculture economy, despite the significant value of seaweed in daily life of Vietnamese people.

1.2.1 Seaweeds use and utilization in Vietnam

Similar to most Southeast Asian countries, the Vietnamese people historically valued marine macroalgae as an important natural resource. The history of seaweed use in Vietnam is not as clearly documented compared to for example neighbouring China, but it can be traced back to the 10th – 13th century. Several sources mention the use of seaweeds as traditional medicine, material to make sweet soups and cakes and eventually as a vegetable resource.

In traditional oriental medicine seaweeds are used to treat various disorders and illnesses. Four seaweeds are commonly used in oriental medicine: *Ecklonia*, *Laminaria*, *Saccharina* and *Sargassum*. *Saccharina* and *Sargassum* have been used in China for the treatment of cancer. Dry *Saccharina* stipes have long been used in obstetrics to dilate the cervix and were known as "*Laminaria* tents" (Dharmananda 2002). According to Chinese medicine, seaweeds have a salty taste that is an indication that the material can disperse phlegm accumulation, particularly as it forms soft masses, include goiter, the thyroid swelling that indicates severe iodine deficiency. *Gracilaria* species are used raw or in the form of agar extract and used as laxatives. *Kappaphycus* and *Euclima* are used to reduce the incidence of tumors, ulcers and headaches. *Sargassum* spp. is used for treating goiter or ailments due to iodine shortage (Huynh Q.N. & Nguyen H.Dinh 1998). Furthermore, polysaccharide extracts of Vietnamese *Sargassum* are used in combination with radiation therapy in the treatment of various cancers. *Sargassum mcclurei* contains fucoidan with anticarcinogenic activities (Tran T.T.V. et al. 2007b). *Sargassum swartzii* and *Ulva reticulata* are effective in analgesic and anti-inflammatory treatments (Dang D.H. et al. 2011). *Caloglossa leprieurii*, *Codium* spp., *Dermonema* spp., *Gymnogongrus* spp. and *Hypnea* spp. are used as vermifuge treatments (Huynh Q.N. and Nguyen H.Dinh 1998). *Ulva lactuca* and *Ulva reticulata* are also sold in traditional medicine (Dang D.H. et al. 2007). *Saccharina* is used as an ingredient for a special sweet soup that people eat to purify, heal and strengthen the human body. *Gracilaria* species are regarded as valuable sources of polyunsaturated fatty acid (Imbs et al. 2012).

Similar to other Asian cultures, Vietnamese people also used several seaweeds as a food crop (Dang D.H. et al. 2007). Vietnamese people use seaweeds on a nearly daily basis as food resources. The habit of consuming seaweeds is widely spread in the communities, in coastal as well as urban areas. Seaweeds are both cultivated (aquaculture) or harvest from natural populations. The most commonly used genera include *Ulva* (incl. *Enteromorpha*), *Gracilaria*, *Caulerpa*, *Porphyra*, *Chnoospora* and *Sargassum*. Applications include the use of seaweeds in salads, jams, jellies, cakes, beverage and soups. (Huynh Q.N. and Nguyen H.Dinh 1998, Le N.H. and Nguyen H.Dai 2010, Titlyanov et al. 2012).

Nowadays, seaweeds are recognized as an important group of organisms for future supplies of food for humans and feed for animals. In addition a number of seaweed species are also used in dairy industry, traditional medicine, bio-fertilizers, biofuels and bioremediation

(Wong and Cheung 2000, McHugh 2003, Smit 2004, Kumar et al. 2008, GBIF 2009, Phang 2010).

1.2.2 Economic species and their distribution

A checklist of marine seaweeds of Vietnam (Nguyen V.T. et al. 2013) lists 827 currently accepted species (412 Rhodophyta, 180 Chlorophyta, 147 Ochrophyta-Phaeophyceae and 88 Cyanophyta). In term of biodiversity, South Central Vietnam is the most diverse zone, with 75 percent of species recorded from that area. In this region, the natural seaweed populations are harvested year-round and some economically important species, especially *Kappaphycus* and *Eucheuma* species, are cultivated.

Cultivation of seaweeds in Vietnam took a start some 50 years ago as a collaborative effort of Vietnamese and German researchers/farmers. Cultivation was entirely focused on *Gracilaria*, an important agarophyte. In a later stage, with support from the former Soviet Union, *Gracilaria* was farmed in the Tam Giang - Cau Hai Lagoon in North-Central Vietnam (Do V.K. 1990, DANIDA & SUMA 2002). Since those early days, both the choice of taxa as well as the number of locations where seaweeds are farmed has expanded considerably.

The economical dimension of marine macroalgal cultivation in Vietnam was summarized in several reports. In an FAO report, Do V.K. (1990) reported 78 economically marine macroalgae in Vietnam: 16 *Gracilaria*, 7 *Gelidium*, 4 *Gigartina*, 2 *Pterocladia*, 44 *Sargassum*, 1 *Porphyra*, 1 *Ulva*, 1 *Caloglossa*, 1 *Codium*, and 1 *Hydroclathrus* species. Ten years later, Huynh Q.N. et al. (1999) listed 85 species which were apparently cultivated: 13 *Gracilaria*, 43 *Sargassum*, 2 *Eucheuma*, 2 *Kappaphycus*, 1 *Betaphycus*, 2 *Acanthophora*, 10 *Hypnea*, 1 *Gigartina*, 8 *Gymnogongrus*, 1 *Porphyra*, and 2 *Ulva* species. Interestingly there is a significant discrepancy between the genera and species listed in both reports.

Most economically relevant species belong to the Rhodophyta and Phaeophyceae. However, the economical value of the Chlorophyta and Cyanophyta is also increasingly considered. Most species are simply harvested from natural populations while some are cultured (e.g. *G. heteroclada*, *G. tenuistipitata*, *K. alvarezii* and *K. striatum*) (Table 1.1).

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Table 1.1. Distribution of economically important seaweed species

(Notes: *** = most common, ** = common, * = found)

| Vietnamese's name | Science's name | North | North Central | Central | South Central | South-west |
|--------------------|-----------------------------------|-------|---------------|---------|---------------|------------|
| Rhodophyta | | | | | | |
| Rong gai râu | <i>Acanthophora muscoides</i> | * | * | ** | ** | * |
| Rong gai | <i>Acanthophora spicifera</i> | * | ** | ** | ** | ** |
| Rong hỏa lư | <i>Ahnfeltiopsis</i> sp. | | | * | * | |
| Rong hồng vân | <i>Betaphycus gelatinus</i> | | | * | * | |
| | <i>Eucheuma arnoldii</i> | | | | * | |
| | <i>Eucheuma denticulatum</i> | | | | * | |
| Rong câu rẽ tre | <i>Gelidiella acerosa</i> | * | * | ** | *** | ** |
| Rong thạch giả | <i>Gelidiopsis intricata</i> | | | | * | * |
| Rong thạch giả mịn | <i>Gelidiopsis gracilis</i> | ** | | | ** | |
| Rong thạch sợi | <i>Gelidium crinale</i> | * | * | * | * | |
| Rong thạch tiên | <i>Gelidium pulchellum</i> | * | * | ** | * | |
| Rong thạch nhỏ | <i>Gelidium pusillum</i> | * | * | * | * | |
| Rong cơm chạc | <i>Gloiopeltis furcata</i> | | * | | | |
| Rong câu cong | <i>Gracilaria arcuata</i> | * | * | *** | ** | * |
| Rong câu chụm | <i>Gracilaria bangmeiana</i> | | | | ** | |
| Rong câu gậy | <i>Gracilaria blodgettii</i> | | | | ** | |
| Rong câu thắt | <i>Gracilaria firma</i> | *** | | | * | ** |
| Rong câu đốt | <i>Gracilaria salicornia</i> | * | * | ** | *** | * |
| Rong câu chỉ | <i>Gracilaria tenuistipitata</i> | *** | *** | *** | *** | *** |
| Rong câu cước | <i>Gracilariopsis heteroclada</i> | | | *** | *** | |
| | <i>Grateloupia</i> sp. | | * | ** | | |
| Rong hồng mạc đốm | <i>Halymenia maculata</i> | | ** | ** | | |
| Rong câu gốc | <i>Hydropuntia changii</i> | | | | | *** |
| Rong câu đá | <i>Hydropuntia edulis</i> | ** | * | *** | ** | * |
| Rong câu chân vịt | <i>H. eucheumatoides</i> | | | ** | *** | |
| Rong câu thái | <i>Hydropuntia fisheri</i> | | | | | ** |
| Rong đồng gai dày | <i>Hypnea boergesenii</i> | | ** | ** | ** | * |
| Rong đồng sừng | <i>Hypnea cervicornis</i> | | | * | ** | |
| Rong đồng nhỏ | <i>Hypnea esperi</i> | * | * | ** | * | * |
| Rong đồng móc câu | <i>Hypnea japonica</i> | * | * | ** | | |
| Rong đồng | <i>Hypnea pannosa</i> | | * | ** | ** | * |
| Rong sụn | <i>Kappaphycus alvarezii</i> | | | | *** | |
| Rong cơm cháy | <i>Kappaphycus cottonii</i> | | | ** | *** | |
| Rong sụn | <i>Kappaphycus inermis</i> | | | * | ** | |
| Rong sú | <i>Kappaphycus striatus</i> | | | | *** | |

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| | | | | | | |
|---------------------------|--------------------------------|-----|-----|-----|-----|-----|
| Rong mào gà | <i>Laurencia similis</i> | | | | * | |
| Rong mào gà | <i>Pallisada perforata</i> | | | | * | |
| Rong mút | <i>Porphyra suborbiculata</i> | ** | | *** | *** | |
| Rong mút | <i>Porphyra vietnamensis</i> | | | | *** | |
| Ochrophyta – Phaeophyceae | | | | | | |
| Rong mơ | <i>Sargassum aquifolium</i> | | *** | *** | *** | * |
| Rong mơ lá hẹp | <i>Sargassum angustifolium</i> | ** | | | | |
| Rong mơ trại | <i>Sargassum assimile</i> | | | ** | | |
| Rong mơ trong | <i>Sargassum baccularia</i> | * | | ** | ** | |
| Rong mơ 2 sừng | <i>Sargassum bicornis</i> | | | ** | *** | |
| Rong mơ lá ngắn | <i>Sargassum brevifolium</i> | | | ** | ** | |
| Rong mơ chùm | <i>Sargassum carpophyllum</i> | * | | ** | *** | |
| Rong mơ tro gai | <i>Sargassum cinereum</i> | ** | ** | *** | ** | * |
| Rong mơ Cô tô | <i>Sargassum cotoense</i> | ** | | | | |
| Rong mơ nhại | <i>Sargassum distichum</i> | | | ** | ** | |
| Rong mơ Feldman | <i>Sargassum feldmannii</i> | | | *** | *** | |
| Rong mơ vàng | <i>Sargassum flavicans</i> | | | | ** | |
| Rong mơ mốc | <i>Sargassum glaucescens</i> | * | *** | ** | ** | |
| Rong mơ mảnh | <i>Sargassum gracillimum</i> | ** | ** | ** | | |
| Rong mơ Henslow | <i>Sargassum henslowianum</i> | ** | * | ** | ** | |
| Rong mơ Herklot | <i>Sargassum herklotsii</i> | ** | *** | *** | * | |
| Rong mơ ô rô | <i>Sargassum ilicifolium</i> | | | *** | *** | |
| Rong mơ Kuetzing | <i>Sargassum kuetzingii</i> | | ** | | ** | |
| Rong mơ McClure | <i>Sargassum mcclurei</i> | *** | *** | *** | *** | |
| Rong mơ phao nhỏ | <i>Sargassum microcystum</i> | | | *** | ** | ** |
| Rong mơ | <i>Sargassum oligocystum</i> | | ** | *** | *** | ** |
| Rong mơ để tán | <i>Sargassum paniculatum</i> | ** | * | | | |
| R. mơ nhiều phao | <i>Sargassum polycystum</i> | ** | *** | *** | *** | *** |
| Rong mơ Lòng chim | <i>Sargassum pyluliferum</i> | ** | ** | *** | *** | |
| Rong mơ | <i>Sargassum serratum</i> | | | *** | ** | |
| Rong mơ | <i>Sargassum siliquosum</i> | ** | ** | ** | ** | ** |
| Rong mơ Swart | <i>Sargassum swartzii</i> | ** | ** | *** | *** | * |
| R. mơ Việt Nam | <i>Sargassum vietnamense</i> | ** | | ** | | |
| Rong mơ Vachel | <i>Sargassum vachellianum</i> | ** | | | | |
| Rong cùi bắp | <i>Turbinaria ornate</i> | ** | ** | *** | *** | ** |
| Rong cùi bắp | <i>Turbinaria decuren</i> | | | ** | ** | ** |
| Rong cùi bắp | <i>Turbinaria conoides</i> | | | ** | ** | |
| Chlorophyta | | | | | | |
| Rong nhỏ | <i>Caulerpa lentillifera</i> | | | | ** | * |
| Rong guột chùm | <i>Caulerpa racemosa</i> | * | ** | *** | *** | ** |
| Rong guột chùm | <i>Caulerpa microphysa</i> | ** | | | ** | |

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|------------------|---------------------------|-----------|-----------|-----------|-----------|-----------|
| Rong nhung gepp | <i>Codium geppiorum</i> | | | ** | ** | ** |
| Rong nhung repen | <i>Codium repens</i> | ** | ** | | ** | |
| Rong giầy | <i>Gayralia oxysperma</i> | ** | | | | |
| Rong cải biển lỗ | <i>Ulva lactuca</i> | * | * | ** | *** | |
| Rong cải biển | <i>Ulva prolifera</i> | ** | * | * | * | |
| Rong cải biển | <i>Ulva reticulata</i> | | | *** | *** | |
| Rong cải biển | <i>Ulva papenfussii</i> | | | ** | ** | |
| Rong bún | <i>Ulva intestinalis</i> | ** | ** | ** | ** | *** |
| Total | 82 | 40 | 37 | 57 | 68 | 27 |

1.2.3 Naturally harvested seaweeds

Seaweeds are harvested from natural populations on a year-round basis from marine environments, river mouths, lagoons and ponds (Figs 1.3 – 1.9). Below I provide an overview of the seaweed farming and harvesting activities per coastal region.

The North coast. Gracilarioids present the bulk of seaweed harvesting activities: *Gracilaria tenuistipitata* 95 wet tons/year, *G. firma* 28 wet tons/year, *Hydropuntia edulis* 2 wet tons/years (data from 2004)(Le N.H. and Nguyen H.Dai 2007). *Sargassum* is harvested mainly in the Quang Ninh province, approximately 70 wet tons/year. In the southern part of the North coast, which receives a significant amount of freshwater input from the Red River (Song Hong), several green algae as well as *Gracilaria* grow very well. The species are used as a source of food for humans and animal feed. Economically less important algae include *Acanthophora*, *Gelidiella*, *Gelidiopsis*, *Gelidium*, *Hypnea*, *Porphyra*, *Turbinaria*, *Caulerpa*, *Codium*, *Gayralia* and *Ulva*.

The North Central coast: The coastal topography of this area is less suited for algal growth and seaweed harvesting is therefore restricted to a few localities: Sam Son, Nghi Son (Thanh Hoa), Quynh Tien, Nghi Tien, (Nghe An), Nghi Xuan, Vung Ang (Ha Tinh), Bo Trach (Quang Binh), Vinh Linh (Quang Tri), Tam Giang - Cau Hai, Lang Co (Hue). The most important species in terms of biomass is *Gracilaria tenuistipitata* which grows in the Lam River and Ron River estuaries and the Tam Giang - Cau Hai lagoon. *Sargassum* is only harvested in Quang Trach (Quang Binh) and Vinh Linh (Quang Tri). Approximately 3,000 wet tons are harvested from April to early July each year. Economically less important algae include *Acanthophora*, *Gelidiella*, *Gelidium*, *Gloiopeltis*, *Halymenia*, *Hypnea*, *Turbinaria*, *Caulerpa*, *Codium* and *Ulva*.

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The Central coast: Many species are harvested in significant quantities from natural populations, e.g. *Sargassum* spp., *Gracilaria* spp., *Euclima* spp., *Gelidiella acerosa*, *Hydropuntia euclimatoides*, *Porphyra*. Among these, *Sargassum* is by far the most important alga (25,000 wet tons/year), and eclipses the other species by two orders of magnitude [*Gelidiella acerosa* 155 wet tons/year, *Gracilaria tenuistipitata* 70 wet tons/year, *Hydropuntia euclimatoides* 37 wet tons/year, *Gracilariopsis heteroclada* 40 wet tons/year, *Gracilaria arcuata* 16.5 wet tons/year, *Gracilaria edulis* 21.5 wet tons/year, *Gracilaria salicornia* 8 wet tons/year, *Hypnea japonica* 1.5 wet tons/year; (Le N.H. and Nguyen H.Dai 2007)]. Economically less important algae include *Acanthophora*, *Ahnfeltiopsis*, *Betaphycus*, *Gelidium*, *Halymenia*, *Hypnea*, *Kappaphycus*, *Turbinaria*, *Caulerpa*, *Codium* and *Ulva*.

The South Central coast: This region is known as the best zone for seaweed growth in Vietnam. The number of seaweed species harvested from natural populations is the highest for the country and a large number of coastal people earn a living collecting and or processing seaweeds. The natural productivity of *Sargassum* is estimated at 20,000 wet tons/year, which again is much higher than all other species combined [*Gelidiella acerosa* 110 wet tons/year, *Gracilaria tenuistipitata* 46 wet tons/year, *Hydropuntia euclimatoides* 20.5 wet tons/year, *Gracilariopsis heteroclada* 56 wet tons/year, *Gracilaria arcuata* 15 wet tons/year, *Gracilaria edulis* 8 wet tons/year, *Gracilaria salicornia* 12 wet tons/year, *Hydropuntia ramulosa* 1.2 wet tons/year; (Le N.H. and Nguyen H.Dai 2007)]. Economically less important algae include *Acanthophora*, *Ahnfeltiopsis*, *Betaphycus*, *Euclima*, *Gelidiopsis*, *Gelidium*, *Hypnea*, *Kappaphycus*, *Laurencia*, *Pallisada*, *Turbinaria*, *Porphyra*, *Caulerpa*, *Codium* and *Ulva*.



Fig. 1.3. *Sargassum* harvested at Quang Tri (Photo: May 31, 2012)



Fig. 1.4. *Sargassum* harvested at Quang Nam (Photo: June 07, 2012)



Fig. 1.5. *Sargassum* harvested at Quang Nam (Photo: June 07, 2012)



Fig. 1.6. *Sargassum* at Nui Thanh – Quang Nam (Photo: June 07, 2012)



Fig. 1.7. *Sargassum* at the reseller house, Quang Ngai (Photo: May 05, 2012)



Fig. 1.8. *Sargassum* drying at Nha Trang (Photo: Sept. 06, 2012)



Fig. 1.9. *Sargassum* being sold at the beach, Ninh Hoa – Khanh Hoa (Photo: April 09, 2012)

The South-West coast: Seaweed harvesting is concentrated in the Ha Tien- Kien Giang province and Phu Quoc islands. Gracilarioids form the most important component: *Gracilaria firma* 33 wet tons/year, *Hydropuntia edulis* 7 wet tons/year, *H. changii* 6.1 wet tons/year, *H. fisheri* 3 wet tons/year. Economically less important algae include *Acanthophora*, *Gelidiella*, *Gelidiopsis*, *Sargassum*, *Turbinaria*, *Caulerpa*, *Codium* and *Ulva*.

Le N.H. et al. (2011) estimated the total biomass of harvested algae to 130,000 wet tons of Rhodophyta per year (data averaged from 2008 and 2009). Annual yields of Phaeophyceae (*Sargassum*) appear to decline, likely due to overexploitation of natural populations (Le N.H. et al. 2011). The numbers reported here are from mainland Vietnam. Harvesting at the offshore islands is less well characterized but should not be entirely neglected. For example standing stocks at the Son Ca and Song Tu Tay (Spratly Islands) of *Kappaphycus cottonii* were estimated to be 6 wet tons and *Caulerpa* 1 wet ton (Pham H.T. 1999). In addition, 20 economically marine macroalgae have reported from the Ly Son Islands with a standing stock estimated to be over 1000 wet tons (Nguyen H.Dai and Pham H.T. 2001).

1.2.4 Cultivated seaweeds

Since demand of seaweeds exceeds the biomass that can be harvested from natural populations, Vietnam started programs to enhance the seaweed production some 40 years ago. At first, and in collaboration with Germany (Do V.K. 1990), efforts to cultivate seaweeds focused on *Gracilaria verrucosa*. Since those early days, trials to cultivate other species have been promoted, e.g. *Betaphycus gelatinus* in Central Vietnam (Nguyen H.Dai 1993, Nguyen X.L. 1995, Nguyen H.Dai et al. 1997), *Acanthophora spicifera* in Southern Vietnam (Huynh Q.N. et al.1999) and *Monostroma nitidum* (Vo D.T. 1999).

At present, 7 species are cultivated at a commercial scale: *Gracilaria firma*, *Gracilaria tenuistipitata*, *Gracilariopsis heteroclada*, *Kappaphycus alvarezii*, *Kappaphycus striatum*, and *Eucheuma denticulatum*, these all being red algae (Nguyen X.L. 1995, Ohno et al. 1996, Huynh Q.N & Isao 2006, Tran M.D. et al. 2007, Le N.H. and Nguyen H.Dai 2010) and the green alga *Caulerpa lentillifera*. The latter is in an early stage for large-scale commercial production. Farming is currently limited to the Cam Ranh and Van Ninh districts (Khanh Hoa province).

Huynh Q.N. et al. (2005) estimated that annually 40.000 – 50.000 wet tons/year of *Gracilaria* were farmed. More recent numbers (Le N.H. 2010) exceed 220,000 wet tons per year.

1.2.4.1 *Gracilaria*

Gracilaria spp. has been cultivated since the 1970s in the Northern provinces and from the 1980s in the Central and Southern provinces (Figs 1.10 – 1.13). Three species have been cultivated in Vietnam: *Gracilaria firma*, *Gracilaria tenuistipitata*, *Gracilariopsis heteroclada* (*syn. G. bailinae*). Cultivation of the *Gracilaria* species is most popular in the Northern provinces (Quang Ninh, Hai Phong, Thai Binh) and in central Vietnam (Ha Tinh, Hue; Phu Yen; Baria-Vungtau). The total cultivation area in 2004 was estimated to be approximately 9,800 hectares, which yielded around 40-50.000 wet tons/year (Le N.H. and Nguyen H.Dai 2010). Under optimum conditions, for example in the Hai Phong province, one hectare can yield as much as 20 wet tons of *Gracilaria* per year (Dinh N.C. and Ho H.N. 1986). In the Northern areas the productivity of *Gracilaria* decreases to 8-10 wet tons/ha/year and also in the South Central provinces only 3-5 wet tons/ha/year can be harvested. *Gracilaria* is cultured mainly in brackish water, semi-closed lagoons, ponds, and river mouths.

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Cultivation typically involves semi-intensive, extensive or modified extensive farming (Huynh Q.N. and Nguyen H.Dinh 1998). In the Northern regions, the main harvest of *Gracilaria* is obtained from November to May. In the Central and Southern regions *Gracilaria* is harvested from January to September and October to April, respectively.



Fig. 1.10. *Gracilariopsis heteroclada* in ponds, Baria-Vungtau (Photo: April 25, 2010)



Fig. 1.11. *Gracilariopsis heteroclada* at Sa Huynh Quang Ngai (Photo: May 11, 2010)



Fig. 1.12. *Gracilariopsis heteroclada* drying at Sa Huynh (Photo: May 11, 2010)



Fig. 1.13. *Gracilaria tenuistipitata* in Quy Nhon (Photo: May 12, 2010)

1.2.4.2 *Kappaphycus*

The genus *Kappaphycus* is represented by 4 species in Vietnam: *K. alvarezii*, *K. cottonii*, *K. inermis*, *K. striatus* (Nguyen H.Dai 1993, Dam D.T. 2003, Huynh Q.N. and Isao 2006). They occur naturally on offshore islands of the Central and South Central provinces. However, the species being cultivated are *K. alvarezii* and *K. striatus* only. The cultivated strains have been imported from the Philippines.

In 1993, in collaboration with the Usa Marine Biological Institute (Kochi University, Japan) the Nha Trang Institute of Technology and Application Research (referred to the Institute of Materials Science, Nha Trang Branch) has investigated the putative cultivation of *K. alvarezii* (the original seedstock from the Philippines) in Southern Vietnam (Huynh Q.N. and Nguyen H.Dinh 1998). After some initial trials the cultivation of *K. alvarezii* expanded to the Phu Yen, Khanh Hoa, Ninh Thuan provinces (Huynh Q.N. and Isao 2006). The species is grown in artificial ponds, semi-closed and closed lagoons as well as small inlets on fixed bottom monolines, fixed bottom rafts and floating rafts (Huynh Q.N. 2005) (Figs 1.14 – 1.17). From May to June the daily growth rate of *K. alvarezii* grown in lagoons is up to 9-11% wet weight per day and 7-9% wet weight per day when grown in inlets. The species has a carrageenan yield of 18 - 25% dry weight and a gel strength of 1566-1712 g/cm². These values are similar to the ones obtained from *K. alvarezii* when cultivated in the Philippines and Indonesia (Ohno et al. 1996). An initial trial to cultivate *K. alvarezii* at Phu Quoc island (Southwestern Vietnam) was carried out in 2000. Although the results were promising the project was not endorsed by the local community and the site was abandoned (DANIDA and SUMA 2002, Huynh Q.N and Isao 2006). *Kappaphycus* production averages 20-30 dried tons/ha/year depending on the locality and cultivation method (Huynh Q.N. and Isao 2006). *K. alvarezii* has become an important aquaculture species in several provinces in southern Vietnam (DANIDA and SUMA 2002).

Kappaphycus striatus was introduced in Vietnam for aquaculture in 2005 from Cebu, Philippines. This species can be cultivated during the warm season. In some localities the cultivation of *K. alvarezii* and *K. striatus* is alternated seasonally while in other areas people cultivate *K. alvarezii* and *K. striatus* simultaneously (e.g. Son Hai – Ninh Thuan, Cam Ranh and Van Phong – Khanh Hoa). *K. striatus* reaches its highest average growth rate in the cool season (October to March) and lowest growth rate in the hot season (April to September). The highest average growth rate was 4.5 – 5.5% wet weight per day in water temperature of 26.5 ± 1°C in November. The lowest growth of *K. striatum* is 1.5 – 3.5% wet weight per day in May with water temperature at 30.7 ± 1.2°C (Tran M.D. et al. 2007, Tran K. et al. 2007). The estimated carrageenan yield varies between 23.9 and 28.3% dry weight with a gel strength from 960 – 1350 g/cm² (Tran K. et al. 2007). *K. striatum* is cultivated mainly in Central and Southern Vietnam even during the dry season, when other species such as *K. alvarezii* do not grow in the shallow waters.



Fig. 1.14. *Kappaphycus* cultivation at Cam Ranh - Khanh Hoa (Photo: April 07, 2010)



Fig. 1.15. *Kappaphycus alvarezii* at Xung ke - Khanh Hoa (Photo: May 14, 2010)



Fig. 1.16. *Kappaphacus striatum* in Van Phong Bay - Khanh Hoa (Photo: May 14, 2010)



Fig. 1.17. *Kappaphycus* cultivation at Son Hai - Ninh Thuan (Photo: June 03, 2010)

1.2.4.3 *Eucheuma*

The species *Eucheuma denticulatum* is being cultivated in South Central Vietnam, mainly in Cam Ranh (Khanh Hoa) and Son Hai (Ninh Thuan). Research data on the evaluation, quality and productivity of this seaweed are almost non-existent however. To my knowledge, only one report evaluated the cultivation of *E. denticulatum* (Nguyen H.Dai 1997). Further research was not pursued. Culture conditions and methods appear similar to those used to culture *Kappaphycus alvarezii*.

1.2.4.4 *Caulerpa* and *Ulva*

Caulerpa lentillifera was introduced as a cash crop in Vietnam less than 10 year ago. The cultivation of the species has gradually expanded in the Khanh Hoa province (Nguyen H.Dai et al. 2002). At present, the productivity of this species does not meet the demand of the local market, since most of the crop is exported to Japan. There are no up-to-date figures on yield and quality of *Caulerpa lentillifera* in Vietnam (Figs 1.18, 1.19).

Recently some *Ulva* species were screened as a source of biomass, potentially used for future energy demands. Cultivation of *Ulva* spp. is still in a trial phase but there are positive prospects, especially when integrated with shrimp farming in the Mekong delta (Fig 1.20, 1.21).



Fig. 1.18. *Caulerpa lentillifera* farm at Ninh Hoa - Khanh Hoa (Photo: May 14, 2010)



Fig. 1.19. *C. lentillifera* farm at Cam Ranh - Khanh Hoa (<http://www.tinmoitruong.vn>)



Fig. 1.20. Harvesting *Ulva* sp. in a shrimp pond, Bac Lieu (Photo: March 19, 2012)



Fig. 1.21. *Ulva* sp. under extensive cultivation in an artificial pond, Ben Tre (Photo: Oct. 22, 2009)

1.2.5 Utilization of seaweeds in Vietnam

Vietnamese people have harvested and utilized seaweed for a long time. Seaweeds are a normal component of the everyday life of Vietnamese people as a food source, feed for animals, folk medicine, pharmacological or industrial applications and more recently as a potential source for biofuels production (Isao et al. 2005, Titlyanov et al. 2012). Unfortunately, almost all use of seaweeds involves the raw material or simple treatments only. Recently, several laboratories started extracting bioactive substances from Vietnamese seaweed resources (Pham H.Hai et al. 2007, Dang D.H. et al. 2007). So far, however, these represent small scale initiatives only. Below I detail the use of harvested Vietnamese seaweeds.

1.2.5.1 Human consumption

We recorded 45 species which are used for human consumption (Table 1.2). About 11 species, 10 of which belong to the Rhodophyta and 1 to the Chlorophyta, present an economic value. Those are *Gracilaria tenuistipitata*, *Gracilaria firma*, *Gracilariopsis heteroclada*, *Gelidiella acerosa*, *Hydropuntia echeumatoides*, *Kappaphycus alvarezii*, *Kappaphycus striatus*, *Euचेuma denticulatum*, *Porphyra suborbiculata*, *Porphyra vietnamensis* and *Caulerpa lentillifera*. Seaweed is mainly used to make relish or sweetened jellies, cakes. Alternatively, they are used as raw vegetables in salads, cooked in vegetable or sweetened soups (Fig 1.22, 1.23). The nutritional value of Vietnamese seaweed has been the subject of several papers (Dang D.H. and Hoang T.M.H. 2004, Le D.H. et al. 2004, Tran T.T.V. et al. 2006).

Table 1.2. Seaweeds used as Food sources (Ck = Cakes, Je = Jellies, Sa = Salads, So = Soups)

| Species names | Food | Species names | Food |
|------------------------------|------------|-------------------------------|------------|
| <i>Betaphycus gelatinus</i> | Ck, Je | <i>Hypnea japonica</i> | Je |
| <i>Euचेuma denticulatum</i> | Ck, Je, So | <i>Hypnea panosa</i> | Je |
| <i>Euचेuma arnoldii</i> | So | <i>Kappaphycus alvarezii</i> | Ck, Je, So |
| <i>Gelidiella acerosa</i> | Je, So | <i>Kappaphycus cottonii</i> | Ck, Je |
| <i>Gelidiopsis intricata</i> | Je | <i>Kappaphycus inermis</i> | Ck, Je |
| <i>Gelidiopsis gracilis</i> | Je | <i>Kappaphycus striatus</i> | Ck, Je |
| <i>Gelidium crinale</i> | Je | <i>Porphyra suborbiculata</i> | So |
| <i>Gelidium pulchellum</i> | Je | <i>Porphyra vietnamensis</i> | So |

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| | | | |
|-----------------------------------|----------------|------------------------------|--------|
| <i>Gelidium pusillum</i> | Je | <i>Sargassum</i> spp. | Sa, So |
| <i>Gracilaria arcuata</i> | Je, So | <i>Sargassum glaucescens</i> | Sa |
| <i>Gracilaria blodgettii</i> | Je, Sa, | <i>Sargassum herklotsii</i> | Sa |
| <i>Gracilaria firma</i> | Sa, So | <i>Sargassum swartzii</i> | Sa |
| <i>Gracilaria salicornia</i> | Ck, Je, Sa, | <i>Caulerpa lentillifera</i> | Sa |
| <i>Gracilaria tenuistipitata</i> | Ck, Je, So | <i>Caulerpa racemosa</i> | Sa |
| <i>Gracilariopsis heteroclada</i> | Ck, Je, Sa, So | <i>Caulerpa microphysa</i> | Sa |
| <i>Hydropuntia changii</i> | Je | <i>Codium geppiorum</i> | Sa |
| <i>Hydropuntia edulis</i> | Je, Sa | <i>Gayralia oxysperma</i> | So |
| <i>Hydropuntia eucheumatoides</i> | Ck, Je, So | <i>Ulva lactuca</i> | Sa, So |
| <i>Hydropuntia fisheri</i> | Ck, Je, So | <i>Ulva prolifera</i> | So |
| <i>Gracilaria bangmeiana</i> | Je | <i>Ulva reticulata</i> | So |
| <i>Hypnea boergesenii</i> | Je | <i>Ulva papenfussii</i> | Sa, So |
| <i>Hypnea cervicornis</i> | Je | <i>Ulva intestinalis</i> | Sa, So |
| <i>Hypnea esperi</i> | Je | | |



Fig. 1.22. *Ulva* sp. and blue crab soup
(Photo: May 30, 2012)



Fig. 1.23. Seaweed – green bean sweet
soup (Source: <http://mebetin.com>)

1.2.5.2 Medicinal and Pharmacological use

The use of seaweed in traditional medicine is very common in Asia. *Sargassum polycystum* is useful against goiter, even though seldomly prescribed (Isao et al. 2005). *Sargassum* species are also used in Vietnam to prepare teas that should have a beneficial effect on one’s health, as well as food additives (Isao et al. 2005) (Fig 1.24). Other uses include traditional cosmetics, treatments for cough, asthma, haemorrhoids, stomach ailments, urinary diseases, treatments for tumors, ulcers and headaches (Dang D.H. and Hoang T.M.H. 2004).

Sulfated polysaccharides, fucoidans, of *Sargassum* species (*S. polycystum*, *S. oligocystum*, *S. mcclurei*, *S. swartzii* and *S. denticarpum*) are investigated as bioactive compounds (Bui M.L. et al. 2005). Fucoidans extracted from *Sargassum mcclurei* proved anticarcinogenic against several cancer cell lines (Tran T.T.V. et al. 2005) (Table 1.3).



Fig. 1.24. Seaweed tea (Sources: <http://www.zenaustralia.com.au>)

Table 1.3. List of Seaweeds used for Medicinal and Pharmacological purposes (Bio = Bioactive, Ff = Functional food, Fm = Folk medicine, Fu = Fucoidan, Na = Nutrition additive). (Lindsey Zemke-White and Ohno 1999, Titlyanov et al. 2012)

| Species | Medicinal & Pharmacological |
|-----------------------------------|-----------------------------|
| <i>Acanthophora muscoides</i> | Bio |
| <i>Acanthophora spicifera</i> | Bio |
| <i>Ahnfeltiopsis</i> sp. | Na |
| <i>Gloiopeltis furcata</i> | Na |
| <i>Halymenia maculata</i> | Ff |
| <i>Hydropuntia eucheumatoides</i> | Fm, Bio |

| | |
|------------------------------|-------------|
| <i>Hypnea boergesenii</i> | Bio |
| <i>Kappaphycus alvarezii</i> | Bio |
| <i>Kappaphycus cottonii</i> | Bio |
| <i>Pallisada perforata</i> | Bio |
| <i>Sargassum</i> spp. | Fm |
| <i>Sargassum feldmannii</i> | Fm |
| <i>Sargassum mcclurei</i> | Fm, Bio, Fu |
| <i>Sargassum oligocystum</i> | Fu |
| <i>Sargassum polycystum</i> | Fu |
| <i>Sargassum swartzii</i> | Fu |
| <i>Turbinaria ornata</i> | Fm |
| <i>Turbinaria decurrens</i> | Fm |
| <i>Turbinaria conoides</i> | Fm |
| <i>Caulerpa lentillifera</i> | Ff |
| <i>Codium repens</i> | Fm |
| <i>Ulva lactuca</i> | Fm |
| <i>Ulva reticulata</i> | Fm |

1.2.5.3 Phycocolloid extraction

The cell walls of many seaweeds contain phycocolloids (algal colloids) that can be extracted by hot water or an alkaline solution. The three major phycocolloids are alginates, agars, and carrageenans. Alginates are extracted primarily from brown seaweeds, while agar and carrageenan are extracted from red seaweeds. These phycocolloids are polymers of chemically modified sugar molecules. Agars and carrageenan are made up of repeating galactose units and 3,6 anhydrogalactose (3,6-AG), both sulfated and nonsulfated. Alginates are chain-forming heteropolysaccharides made up of blocks of mannuronic and guluronic acid (McHugh 2003).

Compared to many other Asian countries, such as China, Japan, Korea, the Philippines and Indonesia, the production of phycocolloids in Vietnam is insignificant. This can be attributed due to the relatively small stocks of commercially important algae, the fact that most aquaculture activities are rather extensive and have not been lifted to industrial levels, and the absence of high-technology processing plants (Titlyanov et al. 2012). The algae most

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commonly used for phycocolloid extraction include carrageenophytes such as *Eucheuma*, *Betaphycus*, *Kappaphycus*, *Hypnea*, agarophytes *Gracilaria*, *Gracilariopsis*, *Hydropuntia*, *Gelidiella* and *Sargassum* for alginates (Table 1.4).

Agar

Agar production took a start in 1973 with the establishment of the Halong Cannery Company, constructed with assistance from the former Democratic Republic of Germany (DDR). Detailed statistics on the production of agar, agar yield of the crops and assessment of economic efficiency of the seaweed processing plant do not exist, but the plant was estimated to process between 4,000 and 5,000 tons dried seaweed per year, yielding 300 - 350 tons of agar per year (DANIDA and SUMA 2002). Until the early 1990's, a good part of the Vietnamese agarophytes were exported to the former Soviet Union and other communist countries. With the collapse of the Soviet Union, however, the export ceased. It should also be noted that many agarophytes (e.g. *Gelidiella acerosa*) are sold on markets to make jelly, locally known as Vietnamese "che" (Isao et al. 2005).

Carrageenan

Kappaphycus and *Eucheuma* are the main seaweeds for carrageenan production, although the list of potential carrageenophytes in Vietnam is much more extensive (Table 1.4). Despite a high demand for purified carrageenan, the local industrial production of carrageenan remains largely undeveloped. Carrageenan quality has been addressed in several papers (Ohno et al. 1996, Kha et al. 2007, Bui M.L. et al. 2007, Le D.H. et al. 2009).

Alginate

Despite the abundance of *Sargassum* along the Vietnamese coastline and the fact that *Sargassum* is harvested at a large scale, there is no industrial processing capacity available for alginate extraction. Some small plants are located in Nha Trang and Ho Chi Minh city (Huynh Q.N and Nguyen H.Dinh 1998), but these do not come close to meeting the needs e.g. for the textile industry *Sargassum*. While most harvested *Sargassum* is exported to China, Vietnam imports alginates from China as well as India.

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Table 1.4. Potential seaweeds for phycocolloid extraction in Vietnam (Ag = Agar, Al = Alginate, Ca = Carrageenan). (Lindsey Zemke-White and Ohno 1999, Titlyanov et al. 2012)

| Species | Phycocolloid | Species | Phycocolloid |
|-----------------------------------|---------------------|------------------------------|---------------------|
| <i>Acanthophora muscoides</i> | Ca | <i>H. eucheumatoides</i> | Ag |
| <i>Acanthophora spicifera</i> | Ca | <i>Hypnea boergesenii</i> | Ca |
| <i>Betaphycus gelatinus</i> | Ca | <i>Hypnea cervicornis</i> | Ca |
| <i>Eucheuma denticulatum</i> | Ca | <i>Hypnea japonica</i> | Ca |
| <i>Eucheuma arnoldii</i> | Ca | <i>Kappaphycus alvarezii</i> | Ca |
| <i>Gelidiella acerosa</i> | Ag | <i>Kappaphycus cottonii</i> | Ca |
| <i>Gracilaria arcuata</i> | Ag | <i>Kappaphycus inermis</i> | Ca |
| <i>Gracilaria blodgettii</i> | Ag | <i>Kappaphycus striatus</i> | Ca |
| <i>Gracilaria firma</i> | Ag | <i>Sargassum</i> spp. | Al |
| <i>Gracilaria tenuistipitata</i> | Ag | <i>Turbinaria ornata</i> | Al |
| <i>Gracilariopsis heteroclada</i> | Ag | <i>Turbinaria decurrens</i> | Al |
| <i>Hydropuntia changii</i> | Ag | <i>Turbinaria conoides</i> | Al |
| <i>Hydropuntia edulis</i> | Ag | | |

1.2.5.4 Seaweeds as fertilisers or biofuels

Green algae are locally used as organic fertilizers for different plant crops (e.g. onion, garlic, sweet potatoes) in the Quang Ngai and Quang Nam provinces. Some brown algae (e.g. *Sargassum*) are processed by pressure-boiling them to make liquid fertilizers. The principle use for these liquid fertilizers is for foliar feeding of orchards, rice fields, and pepper, coffee and tea plantations (Isao et al. 2005). More recently, the potential of seaweeds for biofuel production is being investigated (Le N.H. et al. 2010, Le N.H. et al. 2011) (Table 1.5).

Table 1.5. Potential seaweeds used for fertilisers and biofuel production (BF = Biofuels, Fer = Fertilisers).

| Species | Use |
|----------------------------------|------------|
| <i>Gracilaria</i> spp. | BF, Fer |
| <i>Gracilaria tenuistipitata</i> | BF, Fer |
| <i>Sargassum</i> spp. | Fer |
| <i>Ulva lactuca</i> | Fer |
| <i>Ulva prolifera</i> | Fer, BF |
| <i>Ulva intestinalis</i> | Fer, BF |

1.3 Aims and Thesis Outline

Despite the fact that seaweeds represent a significant part of the everyday life of Vietnamese people, there are several problems regarding our knowledge of the Vietnamese marine macroalgae and potentially also the sustainability of harvesting natural populations.

First, the knowledge on marine macroalgal diversity in Vietnam is scattered over tens of publications, most of them in local journals and written in Vietnamese and therefore largely inaccessible to the international phycological community. For example major uncertainty exists on basic knowledge as to how many seaweed species have been reported from Vietnam. An FAO publication (Do V.K. 1990) mentioned 638 species (taxa). The 'Report on Seaweed Resources of Vietnam' by Huynh Q.N and Nguyen H.Dinh (1998) mentioned 1000 species and this number was used in several later publications (DANIDA and SUMA 2002, Truong N.H. 2007, Dang D.H. et al. 2007). Le N.H. (2010) on the other hand mentioned 800 species.

Therefore, a first aim of my thesis is to summarize the existing knowledge on Vietnamese seaweeds in a comprehensive checklist, compiled by means of an exhaustive bibliographical search and revision of taxon names (**Chapter 2**).

Besides uncertainty on the number of species reported, virtually no critical monographs exist on species-rich or economically important seaweed genera. Hence, the question as to how many species actually are present as opposed to how many species are reported, is even more elusive. An exception is presented by "The *Gracilaria* of Vietnam" (Le N.H. and Nguyen H.Dai 2010), which offers a modern and taxonomically up-to-date treatise of the

diversity of *Gracilaria* in Vietnam. The problem of taxonomic uncertainty is exemplified in the genus *Sargassum*. In the “The *Sargassaceae* of Vietnam” (Nguyen H.Dai 2007) described and illustrated no less than 56 taxa (49 species and 7 intraspecific taxa). These numbers, however, are entirely based on morphological analyses. In recent years a consensus emerged among phycologists that morphology alone is largely inadequate to define species boundaries (reviewed in De Clerck et al. 2013, Leliaert et al. 2014). The reasons for this inadequacy are largely situated in the fact that in morphologically predominantly simple organisms such as algae the number of characters used to differentiate species is a limiting factor and that in addition several of these characters may display considerable plasticity (Verbruggen 2014). Since *Sargassum* is both an important habitat engineer of shallow coastal habitats along the entire Vietnamese coast and a commercially important species the natural vegetations of which are harvested for commercial purposes, we aim to reassess its diversity in Vietnam. The underlying goal is to provide a scientifically sound estimate of species diversity which can serve as a baseline study for future management aimed at sustainable exploiting *Sargassum*-dominated vegetations. In **Chapter 3**, we detail the morphology of the genus, its taxonomy, and provide an overview of the *Sargassum* diversity in Vietnam. In **Chapter 4** we explore the use of gene sequence data (ITS rDNA) to delineate *Sargassum* species. In total we sequenced 126 individuals, belonging to 28 morphospecies, for which we provide descriptions and illustrate the principal morphological characters.

Given the limited resolution of the traditional molecular markers (e.g. ITS rDNA) to delineate species in *Sargassum*, we explore the use of Restriction site Associate DNA markers (RAD-Seq) for *Sargassum* systematics in **Chapter 5**. RAD-sequencing is a Next Generation Sequencing-based technique that generates thousands to hundreds of thousands DNA fragments that can be scored for Single Nucleotide Polymorphisms (SNPs) which in turn can be compared between individuals. RAD markers can be used to pinpoint loci under selection, or are used as neutral markers to reconstruct phylogeographical patterns or elucidate phylogenetic relationships among closely related species. The introduction of RAD Sequencing is entirely novel in phycology.

REFERENCES

- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. & Corner, R. 2010. Aquaculture: global status and trends. *Phil. Trans. R. Soc. Ser. B* **365**: 2897-912.
- Bui, M.L., Q.B. Ngo, D.N. Nguyen, D.T. Pham and T.T.V. Tran. 2005. Studies on fucoidan and its production from Vietnamese brown seaweeds. *Asian Journal of Science and Technology Development* **22**: 371-380.
- DANIDA and SUMA. 2002. Seaweed farming and production in Vietnam, present situation and possibilities. *MARD-VIETNAM*. pp. 57
- Dang, D.H., M.H Hoang, and T. L. A. Hoang. 2011. Studies on the analgesic and anti-inflammatory activities of *Sargassum swartzii* (Turner) C. Agardh (Phaeophyta) and *Ulva reticulata* Forsskal (Chlorophyta) in experiment animal models. *African Journal of Biotechnology* **10**: 2308-2313.
- Dang, D.H., M.H. Hoang and N.S. Pham 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology* **19**: 817-826.
- Dang, D.H. and M.H. Hoang 2004. Nutritional analysis of Vietnamese seaweeds for food and medicine. *Biofactors* **22**: 323-325.
- Dam, D.T. 2003. Thành phần loài và phân bố của rong đỏ (Rhodophyta) tại một số đảo của quần đảo Trường sa. *Journal of Marine Science and Technology* **3**:54-63.
- Do, V.K. 1990. Status of production and utilization of seaweed in Vietnam. *Proceedings of the Regional Workshop on the Culture & Utilization of Seaweeds, Philippines*. FAO.
- De Clerck, O., M. D. Guiry, F. Leliaert, Y. Samyn, and H. Verbruggen. 2013. Algal taxonomy: a road to nowhere? *Journal of Phycology* **49**: 215-225.
- Dinh, N.C and Ho H.N. 1986. Rong câu chỉ vàng (The *Gracilaria tenuistipitata*). Agriculture Publishing House, Hanoi. pp. 112
- Dharmananda, S. 2002. The nutritional and medical value of seaweeds used in chinese medicine. Institute for Traditional Medicine, Portland, Oregon.
- FAO Fisheries and Aquaculture Department . 2013. Global Aquaculture Production Statistics for the year 2011 [online]. <ftp://ftp.fao.org/FI/news/GlobalAquacultureProductionStatistics2011.pdf>
- GBEF. 2009. Algae bases biofuels: A review of challenges and oppotunities for developing countries. FAO.
- Huynh, Q.N. 2005. Kết quả nghiên cứu, hiện trạng nuôi trồng rong biển và định hướng thời gian tới. Technical report, NITRA. pp. 10
- Huynh, Q.N. and H.Dinh Nguyen. 1998. The Seaweed Resources of Vietnam. *In: (Critchley, A.T. and M. Ohno Eds) Seaweed Resources of the World*. Kanagawa International Fisheries Training Centre, JICA. pp. 62-69.
- Huynh, Q.N., V.H. Pham, , T. Kha, N.H. Le, and D.H. Le 1999. Hiện trạng và nguồn lợi rong biển kinh tế ở ven biển phía nam Việt nam. Technical report, NITRA. pp. 77.
- Huynh, Q.N. and T. Isao. 2006. Kappaphycus cultivation and utilization in Vietnam. *In: (Critchley A.T. and Phang S.M., eds) Advances in Seaweed Cultivation and Utilization in Asia*. University of Malaya Maritime Research Centre, Penang. pp. 171-177.
- Imbs, A. B., N. A. Latyshev, V. I. Svetashev, A. V. Skriptsova, T.T. Le, M.Q. Pham, V.S. Nguyen, and L.Q. Pham. 2012. Distribution of polyunsaturated fatty acids in red algae of the genus *Gracilaria*, a promising source of prostaglandins. *Russian Journal of Marine Biology* **38**: 339-345.

- Isao, T., Q.N. Huynh, H.D. Nguyen, A. Shogo, and Y. Tadao. 2005. *The common marine plants of southern Vietnam*. Usa: Japan Seaweed Association. pp. 250.
- Kumar, C. S., P. Ganesan, P. V. Suresh, and N. Bhaskar. 2008. Seaweeds as a source of nutritionally beneficial compounds - A review. *Journal of Food Science and Technology-Mysore* **45**: 1-13.
- Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. Lopez-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA- based species delimitation in algae. *European Journal of Phycology* **49**: 179-196.
- Le B.T. 1998. *Việt Nam: Lãnh thổ và các vùng địa lý (Vietnam: Teritorial and Geography)*. World Publishing House, Hanoi. pp. 607
- Le, D.H., Q.N. Huynh, M.L. Bui, Q.B. Ngo and T.T.V. Tran 2004. The biochemical compositions of some economically red seaweed of the Vietnam coast. *Vietnamese Journal of Chemistry* **42**: 159-162.
- Le, D.H., K. Hori, Q.N.Huynh, K. Tran, and T.H. Le 2009. Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga *Kappaphycus alvarezii* cultivated in Camranh Bay, Vietnam. *Journal of Applied Phycology* **21**: 265-272.
- Le, N.H. and H.D. Nguyen 2010. *Rong câu Việt nam nguồn lợi và sử dụng (The Vietnamese Gracilaria, resources and utilization)*. Vietnamese Natural Science and Technology Publishing House, Hanoi. pp. 241
- Le, N.H. and H.D. Nguyen 2007. Agarophytes species, resources, utility and their potential for culture. Proceeding of National Scientific Conference Proceeding of National Scientific Conference "Biển Đông - 2007". Agriculture Publishing House, HoChiMinh. pp. 109-120.
- Le, N.H., D.T. Vo, B.K. Nguyen, T.T. Vo, T.T. Nguyen, Q.T. Tran, X.M. Vo, M.D Tran, V.S Nguyen and T.N. Ly. 2010. Potential of Vietnamese seaweed for materials of bioethanol production. Proceeding of Scientific Conference VAST-2010. Hanoi. pp. 260-265.
- Le, N.H., P.C. Nguyen, D.K. Dang and M.L. Bui 2011. Potential of seaweed biomass as the raw materials for bioethanol production in Vietnam. 8th Biomass - Asia Workshop, Hanoi.
- Lindsey Zemke-White, W. and M. Ohno. 1999. World seaweed utilisation: An end-of-century summary. *Journal of Applied Phycology* **11**: 369-376.
- McHugh, D. J. 2003. A guide to the seaweed industry. FAO Fisheries Technical Paper No. 441. FAO.
- Nguyen, X.L. 1995. *Nghiên cứu kỹ thuật sản xuất giống và chế biến một số loài rong biển có giá trị xuất khẩu*. Institute of Aquaculture. pp. 128.
- Nguyen, H.Dai 1993. Về chi *Eucheuma* (Rhodophyta) ven biển các tỉnh miền trung Việt Nam. *Vietnamese Journal of Biology* **15**: 9-15.
- Nguyen, H.Dai. 2007. Bộ Rong Mơ (Fucales Kylin) *In: Thực vật chí Việt Nam (Flora of Vietnam)* **11**, Science and Technology Publishing House, Hanoi. pp. 117.
- Nguyen, H.Dai, X.H. Nguyen, H.T. Pham and T.L. Nguyen 1997. Study on the transplantation, adaptation and the ability for development if the resources of the red alga *Echeuma gelatinae* (Eps.) J. Ag. in Nha Trang. Proceeding of the 1st National Symposium on Marine Biology **1**: 209-216.
- Nguyen H.Dai and H.T. Pham 2001. The seaweed resources of Ly son islands. *Vietnamese Collection of Marine Research Works* **11**: 121-134.

- Nguyen, H.Dai, X.V. Nguyen, X.H. Nguyen and H.T. Pham 2002. Transplanting and planting the sea grape (*Caulerpa lentillifera*) in Khanh Hoa province. in Proceedings of 3rd National Scientific Conference on Ecology and Biological Resources IEBC, Hanoi. pp. 942-949.
- Nguyen, V.T., N.H. Le, S.M. Lin, F. Steen, and O. De Clerck. 2013. Checklist of the marine macroalgae of Vietnam. *Botanica Marina* **56**: 207-227.
- Ohno, M. and A. T. Critchley. 1998. Seaweed Resources of the World. Japan International Cooperative Agency. pp 431
- Ohno, M., H.Q. Nang, and S. Hirase. 1996. Cultivation and carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam. *Journal of Applied Phycology* **8**: 431-437.
- Phang, S.-M. 2010. Potential products from tropical algae and seaweeds, especially with reference to Malaysia. *Malaysian Journal of Science* **29**: 160-166.
- Pham, H.Hai, X.N Nguyen, B.T. Nguyen and D.T. Tran 2007. Một số ứng dụng của carrageenan và khả năng sử dụng K-Carrageenan từ rong biển Việt nam trong bảo quản, chế biến thực phẩm. *Journal Science and Technology - Hanoi National University* **45**: 87-93.
- Pham, H.T 1999. Nguồn lợi rong biển hai đảo Sơn ca và Song tử tây (Quần đảo Trường sa) - The resources of marine algae at Son Ca and Song Tu Tay islands (Spratly Archipelago). *Collection of Marine Research Works* **9**: 205-215.
- Pham N.T. and Phan T.D. 1993. *Khí hậu Việt Nam (Climate of Vietnam)*. Science and Technology Publishing House, Ha Noi. pp. 312
- Smit, A. J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology* **16**: 245-262.
- Titlyanov, E. A., T. V. Titlyanova, and V. H. Pham. 2012. Stocks and the use of economic marine macrophytes of Vietnam. *Russian Journal of Marine Biology* **38**: 285-298.
- Thanh T.T.T., M.L. Bui, T.T.V. Tran, and D.T. Pham 2008. Study structure of carrageenan from *Eucheuma denticulatum*. *Proceeding of National Scientific Conference "Biển Đông-2007"*. Agriculture Publishing House, HoChiMinh. pp. 187-190.
- Tran K., Q.N. Huynh, D.T. Vo and N.H. Le. 2008. The results on experimental cultivation of *Eucheuma denticulatum* (Burman) Collins et Harvey and *Kappaphycus striatum* (schmitz) Doty in the seawaters of Khanh Hoa province, Vietnam. *Proceeding of National Scientific Conference "Biển Đông - 2007"*. Agriculture Publishing House, HoChiMinh. pp. 343-352.
- Tran, M.D., Q.N. Huynh, K. Tran And Q.T. Tran 2008. The results on transplantation of *Kappaphycus striatum* (Schmitz) Doty into the waters of Vietnam. *Proceeding of National Scientific Conference "Biển Đông - 2007"*. Agriculture Publishing House, HoChiMinh. pp. 115-121
- Tran, T.T.V., M.L. Bui, D.N. Nguyen and L. J. Joon. 2005. Anticancer activity of fucoidan from the Vietnamese brown seaweed *Sargassum mcclurei*. *Proceeding of the second international Conference "Marine coastal ecosystems seaweeds, invertebrates and products of their processing"*, Archangelsk, Rusia, VNIRO publishing, Moscow. pp. 355-358.
- Tran, T.T.V., N.H. Le, V.M.N. Hieu, L.P. An, and M.L. Bui. 2006. Chemical characteristic and gelling properties of agar extracted from red algae growing at Vietnam coast. *Journal of Marine Science and Technology* **6**: 52-59

- Truong, N.H. 2007. Seaweed: Potential biomass for ethanol production *in* Fourth Biomass-Asia Workshop, Malaysia.
- Verbruggen, H. 2014. Morphological complexity, plasticity, and species diagnosability in the application of old species names in DNA-based taxonomies. *Journal of Phycology* **50**: 26-31.
- Vo, D.T. 1999. Artificial method to generate *Monostroma nitidum*. *Collection of Marine Research Works* **9**: 307-312.
- Vu T.L. 1978. *Địa lý tự nhiên Việt Nam (Physical geography of Vietnam)*. Education Publishing House, Hanoi. pp. 236.
- Wong, K. H. and P. C. K. Cheung. 2000. Nutritional evaluation of some subtropical red and green seaweeds: Part I: proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry* **71**: 475-482.

Chapter 2

Checklist of the marine macroalgae of Vietnam

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Abstract:

Despite a rich seaweed flora, information about Vietnamese seaweeds is scattered in a large number of often regional publications and hence difficult to access. This paper presents an up to date checklist of the marine macroalgae, compiled by means of an exhaustive bibliographical search and revision of taxon names. A total of 827 species are reported of which the Rhodophyta show the highest species number (412 species), followed by the Chlorophyta (180 species), Phaeophyceae (147 species) and Cyanobacteria (88 species). This species richness is comparable to that of the Philippines and considerably higher than Taiwan, Thailand or Malaysia, indicating that Vietnam possibly represents a diversity hotspot for macroalgae. A comparison of the species composition with neighbouring countries yielded surprisingly low similarities. Rather than an indication of a biogeographical pattern, we are of the opinion that the low similarity with neighbouring countries is primarily an artifact resulting from taxonomic inconsistencies. The checklist presented here, could serve as a valuable tool, to reveal the seaweed diversity in Vietnam and to stimulate intraregional comparative research.

Keywords: Chlorophyta, Cyanobacteria, Marine floristics, Phaeophyceae, Rhodophyta, East Sea, Vietnam, Western Pacific Ocean

2.1 Introduction

Vietnam has a coastline ca. 3260 km in length, dotted by numerous islands, islets, atolls and reefs in ca. ~1,000,000 km² of sea area. The primarily north-south orientation of the coastline spans two climatic zones with a subtropical climate at higher latitudes and a tropical climate in the South. A diverse variety of ecosystems, ranging from extensive lagoons and mangroves to rocky shores and coral reefs, provide suitable habitats for luxuriant seaweed growth. Marine macroalgae play an important role in the everyday lives of the people of Vietnam. Several species are used as food (humans and livestock), for the extraction of agar and carrageenan, in traditional medicine or as biofertilizer (Huynh Q.N. and Nguyen H.Dinh 1998, Dang D.H et al. 2007). Yet, the knowledge of the marine seaweed diversity of Vietnam is scattered in the literature and largely inaccessible for phycologists, as most data are published in regional papers, book chapters or reports often published in Vietnamese.

The history of seaweed exploration in Vietnam dates back to 1790 when the Portuguese Jesuit missionary and botanist João de Loureiro reported the names of 11 marine macroalgal species from Vietnam in his "*Flora Cochinchinensis*". In the 19th century, several researchers such as J. Agardh (1848, 1851-1863) and De Toni (1895, 1900, 1905) included Loureiro's records in their publications, but the Vietnamese macroalgal diversity remained largely unexplored. This changed in 1954, when Dawson (1954) published the very first checklist on the Vietnamese marine flora of Nha Trang Bay in the Khanh Hoa province. Dawson reported a total of 204 species, nearly all of which were new species records for Vietnam (16 Cyanobacteria, 118 Rhodophyta, 22 Phaeophyceae and 48 Chlorophyta). His checklist represents the first report in English on the marine flora of an Asian locality. Since then, Vietnamese phycologists have developed a clearer picture of the Vietnamese seaweed flora (Pham H.H. 1969, 1985; Nguyen H.Dinh 1980; Nguyen H.Dinh et al. 1993; Nguyen H.Dai 1997, 1999, 2007; Nguyen H.Dai et al. 2000; Nguyen H.Dai and Pham H.T. 2001, 2002, 2003; Nguyen H.Dai and Le N.H. 2005; Le N.H. 2000, 2001, 2004; Le N.H. and Nguyen H.Dai 2006; Dam and Nguyen V.Tien 1996; Dam D.T. 2003, 2007; Nguyen V.Tien 1996, 2007; Pham H.T. 1996, 1999). Especially the reports of Pham H.H. (1969) and Nguyen H.Dinh et al. (1993) have contributed greatly to the knowledge of the macroalgal flora along the north and the south coasts of Vietnam, respectively. Unfortunately, these lists were

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published in Vietnamese so that the information remained largely inaccessible to the international community.

Funding provided by the California Sea Grant College Program and several expeditions to offshore islands of the Spratly (Truong Sa) archipelago (Dam D.T. and Nguyen V.Tien 1996; Nguyen H.Dai 1999; Pham H.T. 1999; Nguyen H.Dai and Pham H.T. 2001; Nguyen H.Dai et al. 2009) boosted diversity-oriented research on Vietnamese seaweeds in the 1990s. Even though the diversity of some macroalgal genera was explored in depth in a few publications, e.g. *Gracilaria* and *Gracilaropsis* (Nguyen H.Dinh 1992, Ohno et al. 1999, Le N.H. and Nguyen H.Dai 2010), *Eucheuma* and *Kappaphycus* (Nguyen H.Dinh and Huynh 1995), Sargassaceae (Nguyen H.Dai 1997, 2007), Chlorophyta (Nguyen V.Tien 2007), no new attempt was made to bundle all data on Vietnamese macroalgal diversity into a comprehensive list accessible to the world scientific community. The aim of this publication is to compile all of this information into a checklist of the marine algae of Vietnam.

2.2 Materials and Methods

Geographical scope. The Vietnamese coastline (excluding islands) is approximately 3,260 km long, stretching from Mong Cai in the north to Ha Tien in the south. The coastline is bordered in the northeast by the Gulf of Tonkin, eastward by the East Sea and the Gulf of Thailand in the southwest. The coast is physically very heterogeneous, harbouring extensive lagoons and estuaries (e.g. the Mekong Delta), as well as rocky shores with extensive coral reefs and seagrass beds. In addition to the mainland coastline, the East Sea contains thousands of islets scattered midway between the Philippines and the Vietnamese mainland coast. Although claimed by several countries, the checklist includes algal records of the Paracel (Vietnamese: Hoang Sa) as well as the Spratly Islands (Vietnamese: Truong Sa).

The checklist is based on an exhaustive bibliographical search. Both local reports and scientific publications were screened for species records and recorded in a database. Species are ordered alphabetically grouped as follows: Cyanobacteria, Rhodophyta, Chlorophyta and Phaeophyceae. All taxon names were revised to employ currently accepted species names following Algaebase (Guiry and Guiry 2014). References to the original publications are indicated in square brackets, while taxonomic synonyms and their references are included within round brackets. Data are presented as follows: *Current name*

Author [Reference #(Reference of synonym, *Synonym* Author)]. The raw data are available upon request from the corresponding author.

The distribution of the marine algal flora was assessed by grouping the 28 coastal provinces into six coastal regions, based on their meteorological and environmental conditions. The adjacent provinces of Thai Binh, Nam Dinh and Ninh Binh are pooled since species records were too scarce within these provinces. No records were available for the following provinces: Tien Giang, Ben Tre, Tra Vinh, Soc Trang, Bac Lieu and Ca Mau.

The Vietnamese seaweed flora was compared with those of neighbouring countries using Sørensen's similarity index (Magurran 1988). The floristic data of the neighbouring countries were derived from Algaebase and complemented for Taiwan by species records from Orchid Island (Lin 2012).

2.3 Results

2.3.1 Cyanobacteria

Aphanocapsa litoralis Hansgirg [9, 40, 44, 52, 58]

Aphanocapsa marina Hansgirg [40, 58]

Blennothrix cantharidosma (Gomont ex Gomont) Anagnostidis & Komárek [(58 as *Hydrocoleum cantharidosmum* (Montagne) Gormont)]

Blennothrix lyngbyacea (Kützing ex Gomont) Anagnostidis & Komárek [(11, 35, 44, 50, 52, 58 as *Hydrocoleum lyngbyaceum* Kützing)]

Brachytrichia lloydii (P.L.Crouan & H.M.Crouan) P.C.Silva [(50, 52 as *B. balani* Bornet & Flahault)]

Brachytrichia maculans Gomont [11, 40, 58, 59]

Brachytrichia quoyi Bornet & Flahault [11, 40, 44, 58]

Calothrix aeruginea (Kützing) Thuret [11]

Calothrix aeruginosa Woronichin [58, 59]

Calothrix confervicola C.Agardh ex Bornet & Flahault [35, 58, 59]

Calothrix contarenii (Zanardini) Bornet & Flahault [11, 50, 58]

Calothrix crustacea Schousboe ex Thuret [40, 44, 58]

Calothrix nidulans Setchell & N.L.Gardner [50]

Calothrix parietina (Nägeli) Thuret [40, 44, 58]

Calothrix pulvinata (Mertens) C.Agardh [50]

Calothrix scopulorum (Weber & Mohr) C.Agardh [58]

Chamaecalyx swirenkoi (Sirsov) Komárek & Anagnostidis [(58 as *Dermocarpella clavata* (Geitler) J.Feldmann & G.Feldmann)]

Chlorogloea endophytica M.Howe [58]

Chroococcus minor (Kützing) Nägeli [58]

Coleofasciculus chthonoplastes (Gomont) M.Siegesmund, J.R.Johansen & T.Friedl [(40, 50, 58 as *Microcoleus chthonoplastes* (Mertens) Zanardini)]

Cyanothrix primaria N.L.Gardner [52]

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Dermocarpa acervata (Setchell & N.L. Gardner) Pham H.H. [40, 44, 58]
Dermocarpella hemisphaerica Lemmermann [58, 59]
Dermocarpella prasina (Reinsch) Komárek & Anagnostidis [50, 58]
Entophysalis conferta (Kützing) F.E.Drouet & W.A.Daily [11, 50, 58, 59]
Entophysalis granulosa Kützing [50]
Gloeocapsopsis crepidinum (Thuret) Geitler ex Komárek [50]
Gloeotrichia intermedia (Lemmermann) Geitler [50]
Gomphosphaeria aponina Kützing [52]
Heteroleibleinia infixa (Frémy) Anagnostidis & Komárek [(50, 52 as *Lynqbya infixa* Frémy)]
Hormothamnion enteromorphoides Grunow [11, 35, 58]
Hormothamnion solutum Bornet & Grunow [11, 35, 40, 58, 60]
Hydrococcus rivularis Kützing [58]
Hyella caespitosa Bornet & Flahault [58]
Leibleinia agardhii (P.L.Crouan & H.M.Crouan) Anagnostidis & Komárek [(58 as *Lynqbya agardhii* (P.L.Crouan & H.M.Crouan) Gomont)]
Leibleinia epiphytica (Hieronymus) Compère [(58 as *Lynqbya epiphytica* Hieronymus)]
Leptolyngbya rivulariarum (Gomont) Anagnostidis & Komárek [(58, 59 as *Lynqbya rivulariarum* Gomont)]
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O.Komárek & Zapomlová [(58 as *Chroococcus limneticus* Lemmermann)]
Lynqbya aestuarii Liebman ex Gomont [11, 27, 35, 40, 44, 50, 58, 59, 60]
Lynqbya confervoides C.Agardh ex Gomont [11, 35, 40, 50, 58, 59]
Lynqbya infixa Frémy [50, 52]
Lynqbya majuscula Harvey ex Gomont [11, 35, 40, 50, 52, 58, 60, 61]
Lynqbya martensiana f. *tenuivaginata* Gomont ex Forti [50]
Lynqbya martensiana Meneghini ex Gomont [9, 44, 50, 52, 58, 59]
Lynqbya meneghiniana (Kützing) Falkenberg ex Gomont [58]
Lynqbya semiplena J.Agardh ex Gomont [35, 58]
Lynqbya sordida Gomont [58, 61]
Mastigocoleus testarum Lagerheim ex Bornet & Falhault [58]
Merismopedia glauca (Ehrenberg) Kützing [50]
Microchaete tapahiensis Setchell [50]
Microchaete vitiensis Askenasy ex Bornet & Flahault [50]
Microcystis reinboldii (Richter) Forti [58]
Nostoc commune Vaucher ex Bornet & Flahault [58]
Oscillatoria bonnemaisonii (P.L.Crouan & H.M.Crouan) P.L.Crouan & H.M.Crouan ex Gomont [35, 58]
Oscillatoria indica P.C.Silva [(9, 52, 58 as *O. salina* Biswas)]
Oscillatoria limosa C.Agardh ex Gomont [9, 35, 50, 52, 58, 59, 60]
Oscillatoria margaritifera Kützing ex Gomont [11, 35, 50, 52, 58]
Oscillatoria miniata (Zanardini) Hauck ex Gomont [35, 40, 58, 60]
Oscillatoria princeps Vaucher ex Gomont [58]
Oscillatoria tenuis C.Agardh ex Gomont [35, 50, 58, 59]
Phormidesmis molle (Gomont) Turicchia, Ventura, Komárková & Komárek [(35, 43, 60 as *Phormidium molle* Gomont)]

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- Phormidium corallinae* (Gomont ex Gomont) Anagnostidis & Komárek [(50, 52 as *Oscillatoria corallinae* (Kützing) Gomont)]
- hormidium corium* (C.Agardh) Kützing ex Gomont [11, 40, 44, 52, 58]
- Phormidium feldmannii* Frémy [50]
- Phormidium gracile* (Rabenhorst ex Gomont) Anagnostidis [(58 as *Lynqbya gracilis* (Meneghini) Rabenhorst)]
- Phormidium jadinianum* Gomont [50]
- Phormidium nigroviride* (Thwaites ex Gomont) Anagnostidis & Komárek [(11, 50, 58 as *Oscillatoria nigroviridis* Thwaites ex Gomont)]
- Phormidium nigrum* (Vaucher ex Gomont) Anagnostidis & Komárek [(35, 44, 52 as *Oscillatoria nigra* Vaucher)]
- Phormidium simplicissimum* (Gomont) Anagnostidis & Komárek [(40, 52, 58 as *Oscillatoria simplicissima* Gomont)]
- Planktolyngbya limnetica* (Lemmermann) J.Komárková-Legnerová & G.Cronberg [(35, 58 as *Lynqbya limnetica* Lemmermann)]
- Planktothrix isothrix* (Skuja) Komárek & Komárková [(58 as *Oscillatoria agardhii* Gomont)]
- Porphyrosiphon luteus* (Gomont) Anagnostidis & Komárek [(35, 40, 44, 50, 58 as *Lynqbya lutea* (C.Agardh) Areschoug)]
- Pseudanabaena limnetica* (Lemmermann) Komárek [(9, 52, 58 as *Oscillatoria limnetica* Lemmermann)]
- Richelia intracellularis* J.Schmidt [58]
- Rivularia atra* f. *hemisphaerica* (Kützing) Kossinskaja [58]
- Rivularia atra* var. *confluens* Bornet [58]
- Rivularia australis* (Harvey) Bornet & Flahault [58]
- Scytonema ocellatum* Bornet & Flahault [58, 61]
- Scytonematopsis pilosa* (Harvey ex Bornet & Flahault) I.Umezaki & M.Watanabe [(11, 40, 44, 58, 59 as *Calothrix pilosa* Harvey)]
- Spirulina major* Kützing ex Gomont [58]
- Spirulina subsalsa* Oerstedt ex Gomont [58]
- Spirulina subtilissima* Kützing ex Gomont [58]
- Spirulina tenerrima* Kützing ex Gomont [58]
- Stanieria sphaerica* (Setchell & N.L.Gardner) Anagnostidis & Pantazidou [(40, 44, 58 as *Dermocarpa sphaerica* Setchell & N.L.Gardner)]
- Symploca hydroides* Gomont [11, 44, 58, 60]
- Symploca hydroides* var. *fasciculata* (Kützing) Gomont [50]
- Trichocoleus tenerrimus* (Gomont) Anagnostidis [(44, 50, 58 as *Microcoleus tenerrimus* Gomont)]
- Trypanema endolithicum* Ercegovci [52]

2.3.2 Rhodophyta

- Acanthophora muscoides* (Linnaeus) Bory [8, 9, 27, 50, 69]
- Acanthophora spicifera* (Vahl) Børgesen [2, 8, 11, 20, 27, 35, 40, 44, 52, 58, 59, 60, 61, 69, 76, (20, 50 as *A. orientalis* J.Agardh)]
- Acrochaetium barbadense* (Vickers) Børgesen [2, (11, 58 as *A. occidentale* Børgesen)]
- Acrochaetium catenulatum* M.Howe [2, 58]

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- Acrochaetium chaetomorphae* (Tak.Tanaka & Pham H.H.) Heerebout [(58, 67 as *Erythrocladia chaetomorphae* Tak.Tanaka & Pham H.H.)]
Acrochaetium colaconemoides Pham H.H. [58]
Acrochaetium erectum Børgesen [59]
Acrochaetium gracile var. *vietnamense* Pham H.H. [58, 59]
Acrochaetium liagorae Børgesen [2]
Acrochaetium microscopicum (Nägeli ex Kützing) Nägeli [(58 as *A. crassipes* (Børgesen) Børgesen)]
Acrochaetium phuquocensis Pham H.H. [58, 59]
Acrochaetium polysporum M.Howe [(50 as *Rhodochorton polysporum* (M. Howe) Drew 1928)]
Acrochaetium pseudoerectum Pham H.H. [35, 58]
Acrochaetium pulchellum Børgesen [58]
Acrochaetium robustum Børgesen [11, 58, (40, 44 as *Audouinella robusta* (Børgesen) Garbary)]
Acrochaetium sanctaemariae (Darbishire) G.Hamel [11]
Acrochaetium sancti-thomae Børgesen [50, 58]
Acrochaetium subseriatum Børgesen [11, 35, 40, 44, 58]
Acrochaetium virgatulum (Harvey) Batters [58]
Acrochaetium yamadae (Garbary) Y.Lee & I.K.Lee [(2 as *Liaqorophila endophytica* Yamada)]
Acrocystis nana Zanardini [8, 11, 40, 58, 61]
Acrosorium polyneurum Okamura [50]
Actinotrichia fragilis (Forsskål) Børgesen [2, 8, 10, 11, 40, 44, 58, 59, 60, 61, 76]
Aglaothamnion cordatum (Børgesen) Feldmann-Mazoyer [(40, 58 as *A. neglectum* Feldmann-Mazoyer)]
Ahnfeltia plicata (Hudson) E.M.Fries [(58 as *Gymnogongrus plicatus* (Hudson) Kützing)]
Ahnfeltiopsis chnoosporoides (Tak. Tanaka & Pham H.H.) Masuda [(58, 67 as *Gymnogongrus chnoosporoides* Tak. Tanaka & Pham H.H.)]
Ahnfeltiopsis densa (J.Agardh) P.C.Silva & DeCew [(50 as *Gymnogongrus densus* J.Agardh)]
Ahnfeltiopsis divaricata (Holmes) Masuda [(27, 50 as *Gymnogongrus divaricatus* Holmes)]
Ahnfeltiopsis flabelliformis (Harvey) Masuda [76, (11, 27, 50, 58 as *Gymnogongrus japonicus* Suringar), (11, 50, 51, 58 as *Gymnogongrus flabelliformis* Harvey)]
Ahnfeltiopsis pygmaea (J.Agardh) P.C.Silva & DeCew [76, (9, 11, 50, 58 as *Gymnogongrus pygmaeus* J.Agardh)]
Ahnfeltiopsis quinhonensis (Pham H.H.) Masuda [(58, 76 as *Gymnogongrus quinhonensis* Pham H.H.)]
Ahnfeltiopsis serenei (E.Y.Dawson) Masuda [(11, 50, 58 as *Gymnogongrus serenei* E.Y.Dawson)]
Akalaphycus setchelliae (Yamada) Huisman, I.A.Abbott & A.R.Sherwood [(38, 76 as *Stenopeltis setchelliae* (Yamada) Itono & Yoshizaki)]
Amansia rhodantha (Harvey) J.Agardh [76]
Amphiroa anceps (Lamarck) Decaisne [76, (8, 40, 44, 58, 59, 60, 61 as *Amphiroa dilatata* J.V.Lamouroux)]
Amphiroa beauvoisii J.V.Lamouroux [2, 43, (27, 50 as *Amphiroa zonata* Yendo)]
Amphiroa echigoensis Yendo [52]
Amphiroa foliacea J.V.Lamouroux [11, 40, 44, 58]

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- Amphiroa fragilissima* (Linnaeus) J.V.Lamouroux [8, 11, 40, 44, 58, 59, 60, 69, 76]
Amphiroa valonioides Yendo [2]
Anotrichium barbatum (C.Agardh) Nägeli [(50, 52 as *Griffithsia barbata* C.Agardh)]
Anotrichium tenue (C.Agardh) Nägeli [2]
Anotrichium tenue var. *thyrsigerum* (Thwaites ex Harvey) H.S.Kim & I.K.Lee [(11, 58, 59 as *Griffithsia tenuis* C.Agardh)]
Antithamnion erucacladellum R.E.Norris [2]
Antithamnionella basispora (Tokida & Inaba) Cormaci & G.Furnari [(40, 58 as *Antithamnion basisporum* Tokida & Inaba)]
Antithamnionella graeffei (Grunow) Athanasiadis [2]
Antithamnionella spirographidis (Schiffner) E.M.Wollaston [(35, 58 as *Antithamnion spirographidis* Schiffner)]
Antrocentrum nigrescens (Harvey) Kraft & Min-Thein [(50, 58 as *Solieria mollis* (Harvey) Kylin)]
Asparagopsis taxiformis (Delile) Trevisan [2, 8, 9, 10, 20, 27, 40, 44, 50, 51, 54, 58, 60, 61, 76, (2, 8, 11, 51, 58, 60 as *Falkenbergia hillebrandii* (Bornet) Falkenberg)]
Asteromenia anastomosans (W.Bosse) G.W.Saunders, C.E.Lane, C.W.Schneider & Kraft [(8, 40, 44, 58 as *Rhodymenia anastomosans* W.Bosse)]
Asteromenia peltata (W.R.Taylor) Huisman & A.J.K.Millar [42]
Bangia fuscopurpurea (Dillwyn) Lyngbye [8, 10, 58]
Bangia tanakai Pham H.H. [58]
Bangiopsis dumontioides (P.L.Crouan & H.M.Crouan) V.Krishnamurthy [(11, 50, 52, 58 as *Bangiopsis humphreyi* (Collins) G.Hamel)]
Betaphycus gelatinus (Esper) Doty ex P.C.Silva [44, 76, (33, 47 as *Eucheuma gelatinae* (Esper) J.Agardh)]
Bostrychia radicans (Montagne) Montagne [11, 58]
Bostrychia tenella (J.V.Lamouroux) J.Agardh [8, 40, 44, 50, 58, 59, 60, (50, 69, 76 as *Eucheuma gelatinae* (Esper) J.Agardh)]
Botryocladia leptopoda (J.Agardh) Kylin [58]
Botryocladia skottsbergii (Børgesen) Levring [8]
Branchioglossum prostratum C.W.Schneider [2]
Bryocladia cervicornis (Kützinger) F.Schmitz [40, 44, 58]
Callithamnion ramosissimum N.L.Gardner [27, 50, 52]
Caloglossa beccarii (Zanardini) De Toni [15]
Caloglossa bengalensis (G.Martens) R.J.King & Puttock [15, (11, 50, 58 as *Caloglossa adnata* (Zanardini) De Toni)]
Caloglossa continua (Okamura) R.J.King & Puttock [15]
Caloglossa leprieurii (Montagne) G.Martens [50, 58]
Caloglossa ogasawaraensis Okamura [50, 58]
Caloglossa saigonensis Tak. Tanaka & Pham H.H. [15, 58, 67]
Caloglossa stipitata E.Post [58]
Carpopeltis maillardii (Montagne & Millardet) Chiang [21, 76]
Catenella impudica (Montagne) J.Agardh [58]
Catenella nipae Zanardini [11, 50, 52, 58, 69]
Catenella subumbellata C.K.Tseng [50]
Centroceras clavulatum (C.Agardh) Montagne [2, 8, 9, 11, 40, 44, 35, 50, 58, 59, 60, 69, 76]
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- Centroceras gasparrinii* (Meneghini) Kützing [(8, 58, 60 as *C. inerme* Kützing)]
- Ceramium aduncum* Nakamura [2]
- Ceramium cimbricum* H.E.Petersen [8, (8, 10, 35, 58, 59, 60 as *C. fastigiatum* Harvey)]
- Ceramium cingulatum* W.Bosse [40, 44, 50, 58]
- Ceramium clarionense* Setchell & N.L.Gardner [11, 40, 44, 52, 58]
- Ceramium codii* (H.Richards) Mazoyer [50]
- Ceramium deslongchampsii* Chauvin ex Duby [(59 as *C. diaphanum* var. *strictum* (Kützing) Feldmann-Mazoyer)]
- Ceramium diaphanum* (Lightfoot) Roth [(50 as *C. tenuissimum* (Roth) Areschoug), (40, 44, 58, 59, 60, 61 as *C. gracillimum* (Kützing) Zanardini)]
- Ceramium gracillimum* var. *byssoides* Mazoyer [11]
- Ceramium macilentum* J.Agardh [2 (11, 35, 40, 44, 50, 52, 58, 59 as *C. mazatlanense* E.Y.Dawson)]
- Ceramium maryae* W.Bosse [11, 50, 58]
- Ceramium procumbens* Setchell & N.L.Gardner [11, 35, 50, 58]
- Ceramium tenerrimum* (G.Martens) Okamura [50]
- Ceramium vagans* P.C.Silva [2]
- Ceramium vietnamense* Pham H.H. [35, 58]
- Ceramium zacaе* Setchell & N.L.Gardner [50]
- Ceratodictyon intricatum* (C.Agardh) R.E.Norris [(8, 11, 58, 69, 76 as *Gelidiopsis intricata* (C.Agardh) Vickers)]
- Ceratodictyon repens* (Kützing) R.E.Norris [(40, 50, 58 as *Gelidiopsis repens* (Kützing) W.Bosse)]
- Ceratodictyon scoparium* (Montagne & Millardet) R.E.Norris [(58, 59, 76 as *Gelidiopsis scoparia* (Montagne & Millardet) De Toni)]
- Ceratodictyon spongiosum* Zanardini [2, 8, 9, 10, 11, 27, 40, 44, 50, 52, 58, 59, 60, 61, 76]
- Ceratodictyon variabile* (J.Agardh) R.E.Norris [(40, 50, 58, 61 as *Gelidiopsis gracilis* (Kützing) Feldmann), (8, 40, 44, 58 as *Gelidiopsis variabilis* (Greville ex J.Agardh) F.Schmitz)]
- Champia parvula* (C.Agardh) Harvey [2, 8, 10, 11, 40, 44, 58, 59, 61, 76]
- Champia salicornioides* Harvey [40, 44, 58]
- Champia vieillardii* Kützing [2, 11, 40, 58]
- Chondracanthus acicularis* (Roth) Fredericq [(50, 52 as *Gigartina acicularis* (Roth) J.V.Lamouroux)]
- Chondracanthus intermedius* (Suringar) Hommersand [76, (11, 27, 50, 58 as *Gigartina intermedia* Suringar)]
- Chondracanthus tenellus* (Harvey) Hommersand [(50 as *Gigartina tenella* Harvey)]
- Chondria armata* (Kützing) Okamura [40, 43, 44, 76]
- Chondria baileyana* (Montagne) Harvey [11, 58]
- Chondria dangeardii* E.Y.Dawson [2, 11, 58]
- Chondria repens* Børgesen [8, 10, 11, 58]
- Chondria ryukyuensis* Yamada [76]
- Chondria simpliciuscula* W.Bosse [2]
- Chondrophyucus articulatus* (C.K.Tseng) K.W.Nam [(8, 9, 11, 50, 58, 60, 69, 81 as *Laurencia articulata* C.K.Tseng)]
- Chondrophyucus cartilagineus* (Yamada) Garbary & J.T.Harper [44, (8, 10, 20, 40, 61, 81 as *Laurencia cartilaginea* Yamada)]
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- Chondrophyucus verticillatus* (J.Zhang & B.M.Xia) K.W.Nam [(81 as *Laurencia verticillata* J.Zhang & B.M.Xia)]
- Chondrophyucus undulatus* (Yamada) Garbary & Harper [(9, 50 as *Laurencia undulata* Yamada)]
- Chroodactylon ornatum* (C.Agardh) Basson [(2, 9, 11, 27, 40, 50, 58, 59 as *Asterocytis ornata* (C.Agardh) G.Hamel), (52 as *Asterocytis ramosa* (Thwaites) Gobi ex F.Schmitz)]
- Claudea batanensis* Tak. Tanaka [8, 10, 43, 60, 61]
- Colaconema gracile* (Børgesen) Ateweberhan & Prud'homme [(11, 35, 40, 44, 58 as *Acrochaetium gracile* Børgesen)]
- Colaconema hallandicum* (Kylin) Afonso-Carillo, Sanson, Sangil & Diaz-Villa [(35, 43 as *Acrochaetium sargassi* Børgesen)]
- Colaconema hypneae* (Børgesen) A.A. Santos & C.W.N Moura [(2, 40 as *Acrochaetium hypneae* (Børgesen) Børgesen); (44, 58 as *Audouinella seriata* (Børgesen) Garbary)]
- Colaconema thuretii* (Bornet) P.W.Gabrielson [(2, 50, 52 as *Acrochaetium thuretii* (Bornet) F.S.Collins & Hervey)]
- Compsopogon caeruleus* (Balbis ex C.Agardh) Montagne [(50 as *Compsopogon oishii* Okamura)]
- Corallina officinalis* Linnaeus [9, 27, 50, 52]
- Corallina pilulifera* Postels & Ruprecht [50]
- Corallophila bella* (Setchell & N.L.Gardner) R.E.Norris [2]
- Corallophila howei* (W.Bosse) R.E.Norris [(50, 58, 67 as *Ceramium howei* W.Bosse)]
- Corallophila huysmansii* (W.Bosse) R.E.Norris [2, (11, 58 as *Ceramium huysmansii* W.Bosse)]
- Corallophila kleiwegii* W.Bosse [(2 as *C. apiculata* (Yamada) R.E.Norris)]
- Cottoniella filamentosa* (M.Howe) Børgesen [50]
- Crouania attenuata* (C.Agardh) J.Agardh [(2 as *C. minutissima* Yamada)]
- Cryptonemia undulata* Sonder [50]
- Dasya anastomosans* (W.Bosse) M.J.Wynne [(8, 11, 58, 60 as *Dasyopsis pilosa* W.Bosse)]
- Dasya baillouviana* (S.G.Gmelin) Montagne [(11, 58 as *D. pedicellata* (C.Agardh) C.Agardh)]
- Dasya crouaniana* J.Agardh [8, 22]
- Dasya scoparia* Harvey [8]
- Dermonema pulvinatum* (Grunow ex Holmes) Fan [2, 50]
- Dermonema virens* (J.Agardh) Pedroche & Ávila Ortíz [(11, 58, 76 as *D. frappieri* (Montagne & Millardet) Børgesen), (50 as *D. dichotomum* Harvey ex Heydrich)]
- Dermonema zinovae* Nguyen H.Dinh [50]
- Dichotomaria marginata* (J.Ellis & Solander) Lamarck [(40 as *Galaxaura marginata* (J.Ellis & Solander) J.V.Lamouroux), (58 as *Galaxaura clavigera* Kjellman)]
- Dichotomaria obtusata* (J.Ellis & Solander) Lamarck [(8, 9, 40, 44, 51, 58 as *Galaxaura obtusata* (J.Ellis & Solander) J.V.Lamouroux), (10, 27 as *Galaxaura robusta* Kjellman)]
- Dichotomaria papillata* (Kjellman) Kurihara & Masuda [(8 as *Galaxaura papillata* Kjellman)]
- Dictyurus occidentalis* J.Agardh [22]
- Diplothamnion jolyi* C.Hoek [2]
- Erythrocladia irregularis* Rosenvinge [58]
- Erythrotrichia carnea* (Dillwyn) J.Agardh [2, 40, 44, 58]
- Erythrotrichia parietalis* Tanaka [11, 52, 58]
- Erythrotrichia parietalis* var. *majuscula* Tak. Tanaka & Pham H.H. [52, 58, 59, 67]
- Euclidean arnoldii* W.Bosse [8, 39, 47, 60, 61]

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- Eucheuma edule* (Kützing) W.Bosse [7]
Exophyllum wentii W.Bosse [58]
Galaxaura divaricata (Linnaeus) Huisman & R.A.Townsend [(8, 10, 11, 58 as *G. fasciculata* Kjellman)]
Galaxaura filamentosa R.C.Y.Chou [8, 11, 44, 51, 58, 60]
Galaxaura rugosa (J.Ellis & Solander) J.V.Lamouroux [8, 10, 27, 58, 76, (8, 10, 41 as *G. pacifica* Tanaka), (8, 27, 58 as *G. rudis* Kjellman), (58 as *G. glabriuscula* Kjellman), (8 as *G. subverticillata*), (76 as *G. lapidescens* (J.Ellis & Solander) J.V.Lamouroux)]
Ganonema farinosum (J.V.Lamouroux) K.C.Fan & Y.C.Wang [2, 40, 44, 76, (8, 11, 58, 60, 61 as *Liagora farinosa* J.V.Lamouroux)]
Ganonema pinnatum (Harvey) Huisman [(58 as *Liagora pinnata* Harvey)]
Ganonema samaense (C.K.Tseng) Huisman [(2 as *Liagora samaensis* C.K.Tseng)]
Gayliella fimbriata (Setchell & N.L.Gardner) T.O.Cho & S.M.Boo [(11, 58, 59 as *Ceramium fimbriatum* Setchell & N.L.Gardner)]
Gayliella flaccida (Harvey ex Kützing) T.O.Cho & L.J.McIvor [(2 as *Ceramium flaccidum* (Harvey ex Kützing) Ardissoni)]
Gayliella taylorii (E.Y.Dawson) T.O.Cho & S.M.Boo [(11, 50, 58, 59 as *Ceramium taylorii* E.Y.Dawson)]
Gelidiella acerosa (Forsskål) Feldmann & G.Hamel [2, 8, 9, 11, 20, 27, 40, 44, 50, 58, 59, 60, 61, 69, 76]
Gelidiella lubrica (Kützing) Feldmann & G.Hamel [8, 40, 44, 58, 60]
Gelidiella myrioclada (Børgesen) Feldmann & G.Hamel [8, 10, 11, 44, 50, 52, 58]
Gelidium corneum (Hudson) J.V.Lamouroux [9, 50]
Gelidium crinale (Hare ex Turner) Gaillon [9, 11, 27, 40, 44, 50, 52, 58]
Gelidium crinale var. *perpusillum* Piccone & Grunow [11]
Gelidium divaricatum G.Martens [9, 11, 40, 44, 50, 52, 58]
Gelidium fasciculatum G.Hamel [50]
Gelidium pulchellum (Turner) Kützing [11, 44, 50, 52, 58]
Gelidium pusillum (Stackhouse) Le Jolis [8, 9, 11, 27, 40, 44, 50, 58, 59, 69]
Gelidium pusillum var. *minusculum* W.Bosse [50]
Gelidium samoëense Reinbold [50]
Gelidium spathulatum (Kützing) Bornet [40, 44, 58]
Gelidium vietnamense Pham H.H. [58]
Gibsmithia hawaiiensis Doty [22]
Gloiopeltis furcata (Postels & Ruprecht) J.Agardh [50, (58 as *G. minuta* Kylin)]
Gloiopeltis tenax (Turner) Decaisne [50]
Gracilaria arcuata Zanardini [8, 20, 21, 24, 27, 40, 44, 46, 50, 57, 58, 59, 74, 76, 77]
Gracilaria articulata C.F.Chang & B.M.Xia [46, 74]
Gracilaria bangmeiana J.Zhang & I.A.Abbott [(21, 24, 44 as *Hydropuntia ramulosa* (C.F.Chang & B.M.Xia) M.J.Wynne), (50 as *Polycavernosa ramulosa* C.F.Chang & B.M.Xia)]
Gracilaria blodgettii Harvey [21, 24, 46, 50]
Gracilaria bursa-pastoris (S.G.Gmelin) P.C.Silva [46, 50, 52]
Gracilaria canaliculata Sonder [(8, 11, 20, 27, 46, 50, 58, 61, 69 as *G. crassa* Harvey ex J.Agardh)]
Gracilaria changii (B.M.Xia & I.A.Abbott) I.A.Abbott, J.Zhang & B.M.Xia [57, 69]

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- Gracilaria chondracantha* (Kützting) Millar [(40, 46, 74 as *G. bangmeiana* J.Zhang & I.A.Abbott)]
- Gracilaria confervoides* f. *ecorticata* Valerie [46, 58]
- Gracilaria coronopifolia* J.Agardh [8, 58, 61]
- Gracilaria cuneifolia* (Okamura) I.K.Lee & Kurogi [21, 23, 24]
- Gracilaria firma* C.F.Chang & B.M.Xia [8, 21, 24, 46, 57, 69, 74]
- Gracilaria foliifera* (Forsskål) Børgesen [50]
- Gracilaria gigas* Harvey [46, 50, 74]
- Gracilaria hainanensis* C.F.Chang & B.M.Xia [46, 50, 74]
- Gracilaria heteroclada* J.Zhang & B.M.Xia [46, 52, 57]
- Gracilaria longirostris* J.Zhang & Y.C.Wang [21, 23, 24]
- Gracilaria mammillaris* (Montagne)M.Howe [21, 24, 59]
- Gracilaria punctata* (Okamura) Yamada [27, 46, 50, 74]
- Gracilaria rubra* C.F.Chang & B.M.Xia [21, 23, 24]
- Gracilaria salicornia* (C.Agardh) E.Y.Dawson [2, 8, 21, 24, 27, 40, 44, 46, 50, 57, 69, 74, 76, (46, 50 as *G. cacalia* (J.Agardh) E.Y.Dawson)]
- Gracilaria spinulosa* (Okamura) C.F.Chang & B.M.Xia [21, 24, 46]
- Gracilaria stellata* I.A.Abbott, J.Zhang & B.M.Xia [23]
- Gracilaria tenuistipitata* C.F.Chang & B.M.Xia [21, 24, 27, 46, 69, 74, 76]
- Gracilaria tenuistipitata* var. *liui* J.Zhang & B.M.Xia [2, 21, 57, 76]
- Gracilaria textorii* (Suringar) De Toni [21, 24, 46, 74]
- Gracilaria vermiculophylla* (Ohmi) Papenfuss [74, (8, 27, 46 as *G. asiatica* J.Zhang & B.M.Xia)]
- Gracilaria vieillardii* P.C.Silva [74]
- Gracilaria yamamotoi* J.Zhang & B.M.Xia [21, 23, 24]
- Gracilariopsis bailiniae* J.Zhang & B.M.Xia [21, 24, 74, 76]
- Gracilariopsis chorda* (Holmes) Ohmi [(50 as *Gracilaria chorda* Holmes)]
- Gracilariopsis longissima* (S.G.Gmelin) M.Steentoft, L.M.Irvine & W.F.Farnham [(11, 20, 46, 51, 58, 59 as *Gracilaria verrucosa* (Hudson) Papenfuss)]
- Gracilariopsis nganii* Pham H.H. [58]
- Gracilariopsis nhatrangensis* Le N.H. & S.-M. Lin [21, 26]
- Gracilariopsis phanthietensis* Pham H.H. [58]
- Gracilariopsis rhodotricha* E.Y.Dawson [11, 20, 46, 58]
- Grateloupia asiatica* Kawaguchi & H.W.Wang [76]
- Grateloupia dichotoma* J.Agardh [9, 50]
- Grateloupia divaricata* Okamura [9, 50, 58, 59]
- Grateloupia filicina* (J.V.Lamouroux) C.Agardh [11, 20, 27, 50, 58, 69]
- Grateloupia lithophila* Børgesen [9, 27, 50]
- Grateloupia livida* (Harvey) Yamada [50]
- Grateloupia phuquocensis* Tanaka & Pham H.H. [58, 59, 67]
- Grateloupia porracea* Kützting [(9, 50 as *G. filicina* var. *porracea* (Kützting) M.Howe)]
- Grateloupia prolongata* J.Agardh [(50 as *G. filicina* f. *prolongata* (J. Agardh) C.K.Tseng)]
- Grateloupia ramosissima* Okamura [11, 50, 58, 76]
- Griffithsia heteromorpha* Kützting [2]
- Griffithsia japonica* Okamura [58]
- Griffithsia metcalfii* C.K.Tseng [11, 58]

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- Gymnogongrus griffithsiae* (Turner) Martius [9, 27, 50, 58]
Gymnogongrus johnstonii (Setchell & N.L.Gardner) E.Y.Dawson [50]
Gymnothamnion elegans (Schousboe ex C.Agardh) J.Agardh [58]
Halichrysis micans (Hauptfleisch) P.Huvé & H.Huvé [(58 as *Weberella micans* Hauptfleisch)]
Haloplegma duperreyi Montagne [8]
Halymenia dilatata Zanardini [2, 8, 11, 17, 40, 44, 58, 60, 69, 76]
Halymenia floresii subsp. *harveyana* (J.Agardh) Womersley & Lewis [(21 as *H. harveyana* J.Agardh)]
Halymenia floresii var. *ulvoidea* Codomier [(58 as *H. ulvoidea* Zanardini)]
Halymenia maculata J.Agardh [1, 2, 8, 10, 11, 18, 40, 58]
Helminthocladia australis Harvey [38, 76]
Herposiphonia caespitosa C.K.Tseng [9, 50]
Herposiphonia crassa Hollenberg [2]
Herposiphonia delicatula Hollenberg [2]
Herposiphonia insidiosa (Greville ex J.Agardh) Falkenberg [11, 58, 59]
Herposiphonia parca Setchell [2]
Herposiphonia secunda f. *tenella* (C.Agardh) M.J.Wynne [(8, 10, 11, 35, 40, 44, 58, 61 as *H. tenella* (C.Agardh) Ambronn)]
Herposiphonia vietnamica Pham H.H. [35, 58, 59]
Hildenbrandia rubra (Sommerfelt) Meneghini [(11, 58, 59 as *H. prototypus* Nardo)]
Hydrolithon farinosum (J.V.Lamouroux) D.Penrose & Y.M.Chamberlain [40, (11 as *Fosliella farinosa* (J.V.Lamouroux) M.Howe), (35, 58, 59 as *Melobesia farinosa* J.V.Lamouroux)]
Hydrolithon reinboldii (W.Bosse & Foslie) Foslie [11, 40, 58]
Hydrolithon samoëense (Foslie) Keats & Y.M.Chamberlain [76, (11, 58 as *Lithophyllum samoëense* Foslie)]
Hydropuntia changii (B.M.Xia & I.A.Abbott) M.J.Wynne [21, 24]
Hydropuntia divergens (B.M.Xia & I.A.Abbott) M.J.Wynne [21, 24]
Hydropuntia edulis (S.G.Gmelin) Gurgel & Fredericq [21, 24, 44, 76 (2, 8, 9, 27, 40, 46, 50, 57, 69, 74 as *Gracilaria edulis* (S.G.Gmelin) P.C.Silva)]
Hydropuntia eucheumatoides (Harvey) Gurgel & Fredericq [21, 24, 44, (2, 8, 9, 11, 40, 46, 57, 58, 68, 74, 76, 77 as *Gracilaria eucheumatoides* Harvey)]
Hydropuntia fisheri (B.M.Xia & I.A.Abbott) M.J.Wynne [21, 24, (57, 69 as *Gracilaria fisheri* (B.M.Xia & I.A.Abbott) I.A.Abbott, J.Zhang & B.M.Xia)]
Hypnea alopecuroides Kützing [59]
Hypnea boergesenii Tak. Tanaka [9, 11, 20, 27, 40, 44, 50, 51, 58, 69]
Hypnea cenomyce J.Agardh [58]
Hypnea charoides J.V.Lamouroux [8, 27, 40, 44, 50]
Hypnea charoides var. *indica* W.Bosse [50]
Hypnea cornuta (Kützing) J.Agardh [8, 11, 27, 40, 58]
Hypnea esperi Bory [2, 8, 11, 27, 40, 44, 50, 52, 58, 59, 60]
Hypnea flagelliformis Greville ex J.Agardh [9, 27, 50]
Hypnea hamulosa (Esper) J.V.Lamouroux [9, 50, 52]
Hypnea japonica Tak. Tanaka [9, 27, 50]
Hypnea nidulans Setchell [8, 11, 58, 59, 60, 69]
Hypnea pannosa J.Agardh [2, 8, 27, 40, 44, 50, 58, 59, 67, 76]

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- Hypnea spinella* (C.Agardh) Kützing [2, 8, 58, 61, (8, 9, 11, 50, 58, 67, 69, as *H. cervicornis* J.Agardh)]
- Hypnea valentiae* (Turner) Montagne [9, 11, 58, 59, 60, 69, 76]
- Hypoglossum attenuatum* N.L.Gardner [11, 58]
- Hypoglossum barbatum* Okamura [76]
- Izziella orientalis* (J.Agardh) Huisman & Schils [(11, 40, 44, 58 as *Liagora orientalis* J.Agardh)]
- Jania acutiloba* (Decaisne) J.H.Kim, Guiry & H.G.Choi [(59, 76 as *Cheilosporum acutilobum* (Decaisne) Piccone)]
- Jania adhaerens* J.V.Lamouroux [2, 8, 10, 35, 40, 44, 58, 76]
- Jania capillacea* Harvey [9, 11, 35, 40, 58, 59]
- Jania cultrata* (Harvey) J.H.Kim, Guiry & H.G.Choi [(59 as *Cheilosporum cultratum* (Harvey) Areschoug)]
- Jania decussatodichotoma* (Yendo) Yendo [11, 52, 58, 61]
- Jania longiarthra* E.Y.Dawson [9, 11, 40, 44, 58]
- Jania micrarthrodia* J.V.Lamouroux [2]
- Jania pumila* J.V.Lamouroux [2, 27, 40, 58]
- Jania rubens* (Linnaeus) J.V.Lamouroux [8, 9, 35, 40, 44, 58, 60]
- Jania spectabile* (Harvey ex Grunow) J.H.Kim, Guiry & H.G.Choi [(2, 8, 40, 44, 58, 59, 76 as *Cheilosporum spectabile* Harvey ex Grunow)]
- Jania squamata* (Linnaeus) J.H.Kim, Guiry & H.G.Choi [(50, 52 as *Corallina squamata* Linnaeus)]
- Jania ungulata* f. *brevior* (Yendo) Yendo [8, 11, 40, 44, 58, 59, 60]
- Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva [76]
- Kappaphycus cottonii* (W.Bosse) Doty ex P.C.Silva [8, 40, 47, 60, 61, 62, (7 as *Eucheuma okamurae* Yamada)]
- Kappaphycus inermis* (F.Schmitz) Doty ex Nguyen H.Dinh & Huynh Q.N. [8, 47]
- Kappaphycus striatus* (F.Schmitz) Doty ex P.C.Silva [8, 39, 61]
- Laurencia brachyclados* Pilger [9, 11, 27, 50, 58, 69]
- Laurencia caduciramulosa* Masuda & Kawaguchi [30]
- Laurencia calliclada* Masuda [29]
- Laurencia corymbosa* J.Agardh [8, 11, 20, 40, 44, 58]
- Laurencia decumbens* Kützing [81, (11 as *L. pygmaea* W.Bosse)]
- Laurencia fasciculata* C.F.Zhang & B.M.Xia [80]
- Laurencia filiformis* (C.Agardh) Montagne [8]
- Laurencia flexilis* Setchell [21]
- Laurencia galtsoffii* M.Howe [81]
- Laurencia heteroclada* Harvey [58, 60]
- Laurencia intricata* J.V.Lamouroux [8, 27]
- Laurencia lageniformis* Masuda & Suzuki [31]
- Laurencia majuscula* (Harvey) A.H.S.Lucas [2, 81]
- Laurencia mariannensis* Yamada [81]
- Laurencia microcladia* Kützing [8, 27, 58]
- Laurencia nangii* Masuda [76]
- Laurencia nidifica* J.Agardh [40, 58, 60, 61]
- Laurencia obtusa* (Hudson) J.V.Lamouroux [9, 10, 27, 35, 44, 58, 60, 61]
- Laurencia obtusa* var. *densa* Yamada [11]

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- Laurencia pinnata* Yamada [81]
Laurencia silvae J.Zhang & B.M.Xia [81]
Laurencia similis K.W.Nam & Saito [76]
Laurencia tenera C.K.Tseng [11, 58]
Laurencia tropica Yamada [8, 10, 27, 51, 58, 76]
Leveillea jungermannioides (Hering & G.Martens) Harvey [2, 8, 10, 11, 27, 50, 58, 59, 60, 76]
Liagora ceranoides J.V.Lamouroux [2, 8, 10, 11, 58, 60, 61]
Liagora filiformis K.C.Fan & W.H.Li [8, 10, 38]
Liagora hawaiiiana Butters [8]
Liagora japonica Yamada [8, 10]
Lithophyllum okamurae Foslie [8, 9, 10, 11, 40, 44, 58]
Lithophyllum pustulatum (J.V.Lamouroux) Foslie [58]
Lithophyllum pygmaeum (Heydrich) Heydrich [40, 44, (58 as *L. moluccense* (Foslie) Foslie)]
Lithothamnion erubescens f. *subflabellatum* Foslie [11]
Lomentaria hakodatensis Yendo [2]
Lophosiphonia obscura (C.Agardh) Falkenberg [11]
Lophosiphonia prostrata (Harvey) Falkenberg [2]
Lophosiphonia reptabunda (Suhr) Kylin [58]
Martensia flabelliformis Harvey ex J.Agardh [(22 as *Neomartensia flabelliformis* (Harvey ex J.Agardh) Yoshida & Mikami)]
Martensia fragilis Harvey [8]
Mastophora pacifica (Heydrich) Foslie [40, (11, 52, 58 as *Lithoporella pacifica* (Heyrich) Foslie)]
Mastophora rosea (C.Agardh) Setchell [2, 8, 10, 40, 44, 58, 60, 76]
Melanamansia glomerata (C.Agardh) R.E.Norris [40, 44, 56, (8, 58 as *Amansia glomerata* C.Agardh)]
Meristotheca papulosa (Montagne) J.Agardh [8, 42, 44]
Mesophyllum erubescens (Foslie) M.Lemoine [40, 44, 76, (58 as *Lithothamnion erubescens* f. *madagascarense* Foslie)]
Mesophyllum simulans (Foslie) M.Lemoine [(76 as *Lithothamnion simulans* (Foslie) Foslie)]
Metagoniolithon stelliferum (Lamarck) Ducker [(58 as *M. stelligerum* W.Bosse)]
Monostroma nitidum Wittrock [(11, 50, 58 as *Porphyra crispata* Kjellman)]
Montemaria horridula (Montagne) A.B.Joly & Alveal [(58 as *Caulacanthus horridulus* Montagne)]
Neoizziella divaricata (C.K. Tseng) S.-M.Lin, S.-Y.Yang & Huisman [(9, 11, 42, 45, 53, 59, 61 as *Liagora divaricata* C.K.Tseng)]
Neogoniolithon oblimans (Heydrich) P.C.Silva [(11, 58 as *N. myriocarpum* (Foslie) Setchell & L.R.Mason)]
Neogoniolithon trichotomum (Heydrich) Setchell & L.R.Mason [(8, 9, 11, 58 as *Lithophyllum trichotomum* (Heydrich) M.Lemoine)]
Neomonospora pedicellata var. *tenuis* Feldmann-Mazoyer [11]
Neosiphonia ferulacea (Suhr ex J.Agardh) S.M.Guimarães & M.T.Fujii [(8 as *Polysiphonia ferulacea* Suhr ex J.Agardh)]
Neosiphonia harlandii (Harvey) M.S.Kim & I.K.Lee [(40, 44, 50, 52, 58 as *Polysiphonia harlandii* Harvey)]
Neosiphonia poko (Hollenberg) I.A.Abbott [2]

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- Neosiphonia sparsa* (Setchell) I.A.Abbott [2]
Neosiphonia sphaerocarpa (Børgesen) M.S.Kim & I.K.Lee [2, (9 as *Polysiphonia sphaerocarpa* Børgesen)]
Neosiphonia subtilissima (Montagne) M.S.Kim & I.K.Lee [2]
Neosiphonia tongatensis (Harvey ex Kützing) M.S.Kim & I.K.Lee [(11, 58, 59 as *Polysiphonia tongatensis* Harvey ex Kützing)]
Neosiphonia upolensis (Grunow) M.S.Kim & S.M.Boo [8]
Neurymenia fraxinifolia (Mertens ex Turner) J.Agardh [58]
Nitophyllum adhaerens M.J.Wynne [2]
Odonthalia corymbifera (S.G.Gmelin) Greville [50]
Palisada concreta (Cribb) K.W.Nam [(28, 76 as *Laurencia concreta* Cribb)]
Palisada intermedia (Yamada) K.W.Nam [(50 as *Laurencia intermedia* Yamada)]
Palisada parvipapillata (C.K.Tseng) K.W.Nam [(2, 8, 11, 40, 50, 52, 58, 61, 81 as *Laurencia parvipapillata* C.K.Tseng)]
Palisada perforata (Bory) K.W.Nam [(8, 9, 11, 20, 27, 40, 50, 58, 60, 61, 69, 76 as *Laurencia papillosa* (C.Agardh) Greville), (44 as *Chondrophycus papillosus* (C.Agardh) D.J.Garbaray & J.T.Harper), (8, 58 as *Laurencia perforata* (Bory) Montagne)]
Palisada thuyoides (Kützing) Cassano, Senties, Gil-Rodríguez & M.T.Fujii [(11, 58 as *Laurencia paniculata* (C.Agardh) J.Agardh)]
Palisada yamadana (M.Howe) K.W.Nam [(2 as *Laurencia yamadana* M.Howe)]
Parviphycus adnatus (E.Y.Dawson) Santelices [(11, 50, 58, 64 as *Gelidiella adnata* E.Y.Dawson)]
Parviphycus pannosus (Feldmann) G.Furnari [(11, 40, 50, 58 as *Gelidiella tenuissima* Feldmann & G.Hamel)]
Peyssonnelia calcea Heydrich [8, 9, 11, 52, 58]
Peyssonnelia caulifera Okamura [8, 10, 52]
Peyssonnelia conchicola Piccone & Grunow [76]
Peyssonnelia inamoena Pilger [2]
Peyssonnelia rubra (Greville) J.Agardh [8, 9, 58]
Peyssonnelia rubra f. *orientalis* W.Bosse [11]
Pleonosporium borrieri (Smith) Nägeli [58]
Pneophyllum confervicola (Kützing) Y.M.Chamberlain [(35, 58 as *Heteroderma minutulum* (Foslie) Foslie), (58 as *Melobesia confervicola* (Kützing) Foslie)]
Polyopes ligulatus (Harvey ex Kützing) De Toni [58]
Polysiphonia coacta C.K.Tseng [9, 11, 58, 59, 61, 69]
Polysiphonia fragilis Suringar [8, 11, 58]
Polysiphonia herpa Hollenberg [8]
Polysiphonia infestans Harvey [8, 10]
Polysiphonia kampsaxii Børgesen [50, 52]
Polysiphonia nhatrangense Pham H.H. [9, 27, 58]
Polysiphonia scopulorum Harvey [2, 35, 40, 44, 58]
Polysiphonia scopulorum var. *villum* (J.Agardh) Hollenberg [(11, 40, 58, 59 as *Lophosiphonia villum* (J.Agardh) Setchell & N.L.Gardner)]
Polysiphonia sertularioides (Grateloup) J.Agardh [9, 27, 50, 52]
Polysiphonia subtilissima Montagne [9, 11, 27, 35, 40, 44, 50, 52, 58, 59]
Polysiphonia tapinocarpa Suringar [50]

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- Porphyra suborbiculata* Kjellman [9, 50, 76]
Porphyra tanaka Pham H.H. [59]
Porphyra vietnamensis Tak. Tanaka & Pham H.H. [50, 58, 67, 76]
Portieria hornemannii (Lyngbye) P.C.Silva [8, 10, 44, 60, 61, 76, (58 as *Desmia hornemannii* Lyngbye)]
Portieria japonica (Harvey) P.C.Silva [8, 76]
Prionitis vietnamensis Pham H.H. [58]
Pterocladia heteroplatos (Børgesen) Umamaheswara & Kaliaperumal [(50 as *Gelidium heteroplatos* Børgesen)]
Pterocradiella caerulescens (Kützing) Santelices & Hommersand [2]
Pterocradiella caloglossoides (M.Howe) Santelices [(11, 40, 58, 76 as *Pterocladia parva* E.Y.Dawson)]
Pterocradiella capillacea (S.G.Gmelin) Santelices & Hommersand [(58 as *Pterocladia capillacea* (S.G.Gmelin) Bornet in Bornet & Thuret), (8, 58 as *Pterocladia pinnata* (Hudson) Papenfuss)]
Pterocradiella tenuis (Okamura) Shimada, Horiguchi & Masuda [(27, 50 as *Pterocladia tenuis* Okamura)]
Reinboldiella warburgii (Heydrich) Yoshida & Mikami [(50 as *Holmesia neurymenioides* (Okamura) Okamura)]
Rhodogorgon ramosissima J.N.Norris & Bucher [(43 as *Rhodogorgon carriehowensis* J.N.Norris & Bucher)]
Rhodymenia coacta Okamura & Segawa [8]
Rhodymenia intricata (Okamura) Okamura [8, 50]
Rhodymenia liniformis Okamura [50]
Rodriguezella hongngai Pham H.H. [58]
Sahlingia subintegra (Rosenvinge) Kornmann [(50, 58 as *Erythrocladia subintegra* Rosenvinge)]
Schmitzia japonica (Okamura) P.C.Silva [(58 as *Bertholdia japonica* (Okamura) Segawa)]
Scinaia boergesenii C.K.Tseng [40, 43]
Solieria robusta (Greville) Kylin [50]
Sonderophycus capensis (Montagne) M.J.Wynne [(8, 9, 11, 27, 58 as *Peyssonnelia qunniana* J.Agardh)]
Spongoclonium caribaeum (Børgesen) M.J.Wynne [(11 as *Mesothamnion caribaeum* Børgesen)]
Spyridia filamentosa (Wulfen) Harvey [8, 11, 44, 50, 58, 59, 60, 76]
Spyridia hypnoides (Bory) Papenfuss [76]
Stylonema alsidii (Zanardini) K.M.Drew [2, 40, (58 as *Goniotrichum alsidii* (Zanardini) M.Howe)]
Symphyclocladia marchantioides (Harvey) Falkenberg [50]
Taenioma perpusillum (J.Agardh) J.Agardh [2, 11, 40, 44, 58, 59]
Tayloriella dictyurus (J.Agardh) Kylin [50]
Titanophora weberae Børgesen [(8, 11, 44, 58, 60, 61, 76 as *T. pulchra* E.Y.Dawson)]
Titanophycus validus (Harvey) Huisman, G.W.Saunders & A.R.Sherwood [(2 as *Liagora valida* Harvey)]
Tolyptocladia calodictyon (Harvey ex Kützing) P.C.Silva [58]
Tolyptocladia glomerulata (C.Agardh) F.Schmitz [2, 8, 11, 35, 40, 44, 58, 59, 61, 69, 76]

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Tricleocarpa cylindrica (J.Ellis & Solander) Huisman & Borowitzka [40, 44, 76, (8, 9, 11, 27, 52, 58, 60, 61 as *Galaxaura fastigiata* Decaisne)]
Tricleocarpa fragilis (Linnaeus) Huisman & R.A.Townsend [2, 40, 44, (8, 10, 11, 58 as *Galaxaura vietnamensis* E.Y.Dawson), (8, 58, 60, 61 as *Galaxaura oblongata* (J.Ellis & Solander) J.V.Lamouroux)]
Wrangelia argus (Montagne) Montagne [11, 40, 58, 61]
Wrangelia dumontii (E.Y.Dawson) I.A.Abbott [2]
Wrangelia tanegana Harvey [38]
Wurdemannia miniata (Sprengel) Feldmann & G.Hamel [8, 11, 40, 44, 58, 59, 76]
Yamadaella caenomyce (Decaisne) I.A.Abbott [2, (27 as *Liaqora caeomyce* Decaisne)]
Yonagunia formosana (Okamura) Kawaguchi & Masuda [19, 76, (11, 44, 58, 61 as *Carpopeltis formosana* Okamura), (16 as *Priionitis formosana* (Okamura) Kawaguchi & Nguyen H.Dinh)]

2.3.3 Ochrophyta-Phaeophyceae

Acrothrix pacifica Okamura & Yamada [50]
Asteronema breviarticulatum (J.Agardh) Ouriques & Bouzon [(2 as *Hincksia breviarticulata* (J.Agardh) P.C.Silva), (11, 52 as *Ectocarpus breviarticulatus* J.Agardh), (58, 60 as *Feldmannia breviarticulata* (J.Agardh) Pham H.H.), (76 as *Asteronema breviarticulatum* (J.Agardh) Ouriques & Bouzon)]
Canistrocarpus cervicornis (Kützing) De Paula & De Clerck [(9, 50 as *Asteronema breviarticulatum* (J.Agardh) Ouriques & Bouzon)]
Canistrocarpus crispatus (J.V.Lamouroux) De Paula & De Clerck [(2, 42 as *Dictyota crispata* J.V.Lamouroux), (58 as *Dictyota indica* Sonder ex Kützing)]
Chilionema ocellatum (Kützing) Kornmann [35, 58]
Chnoospora implexa J.Agardh [9, 27, 40, 44, 50, 51, 58, 76]
Chnoospora minima (Hering) Papenfuss [9, 27, 50, 58, 59, 76]
Colpomenia bullosa (D.A.Saunders) Yamada [58]
Colpomenia sinuosa (Mertens ex Roth) Derbès & Solier [2, 9, 11, 27, 40, 44, 50, 52, 58, 59, 76]
Dictyopteris delicatula J.V.Lamouroux [44, 50, 58]
Dictyopteris plagiogramma (Montagne) Vickers [59]
Dictyopteris polypodioides (De Candolle) J.V.Lamouroux [(50, 58 as *D. membranacea* (Stackhouse) Batters)]
Dictyopteris woodwardia (R.Brown ex Turner) C.Agardh [58]
Dictyota adnata Zanardini [(27, 58, 67 as *D. submaritima* Tak. Tanaka & Pham H.H.)]
Dictyota bartayresiana J.V.Lamouroux [40, 58, 59, (9, 11, 27, 50, 52, 58 as *D. patens* J.Agardh)]
Dictyota ceylanica var. *anastomosans* Yamada [9, 27, 58, 69]
Dictyota ceylanica var. *rotundata* W.Bosse [60]
Dictyota ciliolata Sonder ex Kützing [42, (9, 50 as *D. ciliata* J.V.Lamouroux), (58, 69 as *D. beccariana* Zanardini)]
Dictyota dichotoma (Hudson) J.V.Lamouroux [11, 40, 44, 50, 58, 59, 69, 76]
Dictyota friabilis Setchell [2, 9, 11, 44, 50, 52, 58, 76]
Dictyota implexa (Desfontaines) J.V.Lamouroux [(35, 50, 76 as *D. linearis* (C.Agardh) Greville), (9, 10, 27, 40, 44, 58, 59, 60, 61 as *D. divaricata* J.V.Lamouroux)]

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- Dictyota mertensii* (Martius) Kützing [(9, 50, 76 as *D. dentata* J.V.Lamouroux)]
Dictyota pinnatifida Kützing [9, 50]
Dictyota polyclada Sonder ex Kützing [(50 as *Pachydictyon polycladum* (Sonder ex Kützing) Womersley)]
Dictyota spinulosa J.D.Hooker & Arnott [76]
Distromium decumbens (Okamura) Levring [(58 as *Chlanidophora repens* (Okamura) Okamura)]
Ectocarpus siliculosus f. *sporangioramosus* A.D. Zinova & Nguyen H.Dinh [50]
Ectocarpus siliculosus var. *dasycarpus* (Kuckuck) Gallardo [(50, 52 as *E. siliculosus* (Dillwyn) Lyngbye)]
Ectocarpus vungtauensis Pham H.H. [58]
Feldmannia enhali Børgesen [58]
Feldmannia filifera (Børgesen) Pham H.H. [58]
Feldmannia indica (Sonder) Womersley & A.Bailey [(2 as *Hincksia indica* (Sonder) J.Tanaka), (50, 52 as *Giffordia indica* (Sonder) Papenfuss & Chihara)]
Feldmannia irregularis (Kützing) G.Hamel [35, 40, 44, 58, 59, 60, (11 as *Ectocarpus irregularis* Kützing)]
Feldmannia zeylanica (Børgesen) P.C.Silva [(59 as *Ectocarpus zeylanicus* Børgesen)]
Hapalospongidion schmidtii (W.Bosse) P.C.Silva [(11, 58 as *Mesospora schmidtii* W.Bosse)]
Hincksia mitchelliae (Harvey) P.C.Silva [2, (11 as *Ectocarpus mitchelliae* Harvey), (44, 50, 52, 58 as *Giffordia mitchelliae* (Harvey) G.Hamel)]
Hormophysa cuneiformis (J.F.Gmelin) P.C.Silva [76, (34, 37, 40, 58, 59, 69 as *H. articulata* Kützing)]
Hydroclathrus clathratus (C.Agardh) M.Howe [2, 11, 27, 40, 44, 50, 52, 58, 60, 76]
Hydroclathrus tenuis C.K.Tseng & Lu B.R. [38, 70]
Kuetzingiella elachistaeformis (Heydrich) M.Balakrishnan & Kinkar [2, (35, 58 as *Feldmannia elachistaeformis* (Heydrich) Pham H.H.)]
Lobophora variegata (J.V.Lamouroux) Womersley ex E.C.Oliveira [2, 9, 10, 44, 50, 52, 58, 59, 76, (11 as *Pocockiella variegata* (J.V.Lamouroux) Papenfuss)]
Myrionema strangulans Greville [58]
Nemacystus decipiens (Suringar) Kuckuck [9, 50]
Neoralfsia expansa (J.Agardh) P.E.Lim & H.Kawai ex Kraft [(35, 58, 59 as *Ralfsia expansa* (J.Agardh) J.Agardh)]
Padina antillarum (Kützing) Piccone [(27, 50, 69 as *P. tetrastromatica* Hauck)]
Padina australis Hauck [2, 9, 27, 40, 44, 50, 51, 52, 58, 59, 60, 61, 76]
Padina australis var. *cuneata* Tak. Tanaka & K.Nozawa [59]
Padina boryana Thivy [9, 40, 44, 58, 69, 76, (11 as *P. commersonii* Bory), (50 as *Dilophus radicans* Okamura)]
Padina gymnospora (Kützing) Sonder [27, 58, (9, 50, 52 as *P. crassa* Yamada)]
Padina minor Yamada [76]
Petalonia fascia (O.F.Müller) Kuntze [50, 52]
Petroderma vietnamensis Pham H.H. [58]
Pylaiella littoralis (Linnaeus) Kjellman [58]
Ralfsia fungiformis (Gunnerus) Setchell & N.L.Gardner [50]
Ralfsia verrucosa (Areschoug) Areschoug [27]
Roseningea fastigiata (Zanardini) Børgesen [11, 50]

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- Rosenvingea intricata* (J.Agardh) Børgesen [50, 58]
Rosenvingea nhatrangensis E.Y.Dawson [11, 58, 76]
Rosenvingea orientalis (J.Agardh) Børgesen [11, 58, 76]
Sargassum aemulum var. *carpophylloides* Grunow [34, 37]
Sargassum aemulum var. *jouanii* Grunow [34, 37]
Sargassum angustifolium C.Agardh [34, 37, 50, 63]
Sargassum aquifolium (Turner) C.Agardh [(2, 11, 34, 37, 40, 44, 58, 63, 65, 76, 79 as *S. crassifolium* J.Agardh), (9, 34, 37, 58 as *S. binderi* Sonder ex J.Agardh), (34, 37, 58 as *S. heterocystum* Montagne), (27, 34, 37, 59 as *S. echinocarpum* J.Agardh)]
Sargassum armatum J.Agardh [58]
Sargassum assimile Harvey [34, 37, 51, 58]
Sargassum baccharia (Mertens) C.Agardh [34, 37, 52, 58, 63]
Sargassum bangmeiana Nguyen H.Dinh & Huynh Q.N. [49]
Sargassum baoreni Nguyen H.Dinh & Huynh Q.N. [49]
Sargassum bicornis J.Agardh [34, 37, 58]
Sargassum brevifolium var. *pergracilis* Greville [58]
Sargassum bulbiferum Yoshida [37, 43]
Sargassum buui Nguyen H.Dinh & Huynh Q.N. [49]
Sargassum capillare Kützinger [(34, 37, 58 as *S. gracile* Greville)]
Sargassum carpophyllum J.Agardh [9, 34, 37, 58]
Sargassum carpophyllum var. *honomense* Nguyen H.Dinh & Huynh Q.N. [48]
Sargassum carpophyllum var. *nhatrangense* (Pham H.H.) Ajisaka [4, 37, (34, 58 as *S. piluliferum* var. *nhatrangense* Pham H.H.)]
Sargassum cinereum J.Agardh [34, 37, 50, 59, 63]
Sargassum confusum C.Agardh [34, 58]
Sargassum congkinhii Pham H.H. [32, 34, 37, 58]
Sargassum cornutifrutum Nguyen H.Dinh & Huynh Q.N. [48]
Sargassum cotoense Nguyen H.Dai [34, 36, 37]
Sargassum cymosum C.Agardh [34, 37, 50, 63]
Sargassum denticarpum Ajisaka [3, 76]
Sargassum distichum Sonder [(34, 37, 58 as *S. aemulum* Sonder)]
Sargassum emarginatum C.K.Tsang & Lu B.R. [75]
Sargassum feldmannii Pham H.H. [34, 37, 58, 63]
Sargassum flavicans (Mertens) C.Agardh [34, 37, 44, 51, 58]
Sargassum glaucescens J.Agardh [27, 34, 37, 50, 58, 63, 71]
Sargassum gracillimum Reinbold [9, 27, 34, 37, 50, 52, 63]
Sargassum graminifolium C.Agardh [27, 34, 37, 50, 63]
Sargassum hemiphyllum (Turner) C.Agardh [34, 50, 58, 63]
Sargassum hemiphyllum var. *chinense* J.Agardh [5, 37, 78]
Sargassum henslowianum C.Agardh [34, 37, 40, 44, 50, 52, 55, 58, 63]
Sargassum henslowianum var. *bellonae* Grunow [34, 37]
Sargassum herklotsii Setchell [9, 34, 37, 50, 63]
Sargassum hieuui Nguyen H.Dinh & Huynh Q.N. [49]
Sargassum ilicifolium (Turner) C.Agardh [34, 37, 58], (34, 37, 51, 58 as *S. cristaefolium* C.Agardh), (11, 34, 37, 58, 73 as *S. sandei* Reinbold), (9, 34, 37, 40, 44, 50, 52, 58, 63, 76 as

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- S. duplicatum* Bory), (34, 37, 40 as *S. berberifolium* J.Agardh), (37 as *S. turbinatifolium* C.K.Tseng & Lu B.R.)]
- Sargassum ilicifolioides* C.K.Tseng & Lu B.R. [72]
- Sargassum incanum* Grunow [34, 37, 50, 63]
- Sargassum kuetzingii* Setchell [27, 34, 37, 58]
- Sargassum longifructum* C.K.Tseng & Lu B.R. [3, 34, 37, 69]
- Sargassum mcclurei* f. *duplicatum* A.D.Zinova & Nguyen H.Dinh [50, 52, 76]
- Sargassum mcclurei* Setchell [2, 11, 32, 34, 44, 52, 58, 63, 65, 66, 76, 79]
- Sargassum microcystum* J.Agardh [34, 37, 58, 69]
- Sargassum miyabei* Yendo [(32, 34, 58 as *S. kjellmanianum* Yendo)]
- Sargassum namoense* Nguyen H.Dai [37, 42]
- Sargassum natans* (Linnaeus) Gaillon [(34, 58 as *S. bacciferum* (Turner) C.Agardh)]
- Sargassum oligocystum* Montagne [2, 27, 37, 40, 44, 69, 76]
- Sargassum paniculatum* J.Agardh [27, 34, 37, 50, 63]
- Sargassum parvifolium* (Turner) C.Agardh [34, 37, 58]
- Sargassum parvivesiculosum* C.K.Tseng & Lu B.R. [71]
- Sargassum phamhoangii* Nguyen H.Dai [34, 36, 37]
- Sargassum phyllocystum* C.K.Tseng & Lu B.R. [75]
- Sargassum piluliferum* (Turner) C.Agardh [50, 63, 73, 78]
- Sargassum piluliferum* var. *serratifolium* (Turner) C.Agardh [5, 37]
- Sargassum polycystum* C.Agardh [11, 14, 27, 32, 34, 37, 40, 44, 50, 58, 59, 63, 65, 69, 76, 79]
- Sargassum polycystum* var. *onustum* J.Agardh [34, 37]
- Sargassum polyporum* Montagne [27, 34, 50, 63]
- Sargassum quinhonense* Nguyen H.Dai [34, 36, 37, 65, 66, 76, 79]
- Sargassum segii* Yoshida [(52 as *S. racemosum* Yamada & Segi)]
- Sargassum serratum* Nguyen H.Dai [37, 41, 44]
- Sargassum siliquosum* J.Agardh [27, 34, 37, 50, 52, 59, 63,]
- Sargassum subtilissimum* C.K.Tseng & Lu B.R. [71]
- Sargassum swartzii* C.Agardh [9, 34, 37, 50, 58, 59, 63, 76]
- Sargassum tenerimum* J.Agardh [34, 37, 50, 58, 63]
- Sargassum tsengii* Nguyen H.Dinh & Huynh Q.N. [49]
- Sargassum turbinarioides* Grunow [10, 34, 58, 60]
- Sargassum vachellianum* Greville [34, 37, 50, 63]
- Sargassum vietnamense* A.D.Zinova & Nguyen H.Dinh [9, 34, 37, 50, 52, 63]
- Sargassum virgatum* C.Agardh [34, 37, 50, 63]
- Scytosiphon lomentaria* (Lyngbye) Link [50]
- Spatoglossum stipitatum* (Tak. Tanaka & K.Nozaawa) Bittner *et al.* [(2, 58 as *Zonaria stipitata* Tak. Tanaka & K.Nozaawa)]
- Spatoglossum vietnamense* Pham H.H. [40, 44, 58, 76]
- Sphacelaria carolinensis* Trono [14]
- Sphacelaria ceylanica* Sauvageau [58]
- Sphacelaria novae-hollandiae* Sonder [2, 11, 14, 58]
- Sphacelaria rigidula* Kützing [2, 14, (50 as *S. variabilis* Sauvageau), (9, 11, 27, 50, 52, 58, 59 as *S. furciqera* Kützing)]
- Sphacelaria solitaria* (Pringsheim) Kylin [(40, 44 as *S. divaricata* Montagne)]
- Sphacelaria tribuloides* Meneghini [2, 11, 14, 40, 44, 58, 59]

Styopodium zonale (J.V.Lamouroux) Papenfuss [58]
Turbinaria conoides (J.Agardh) Kützing [34, 37, 40, 44, 58, 76]
Turbinaria decurrens Bory [34, 37, 40, 44, 58, 59, 66, 69]
Turbinaria gracilis Sonder [34, 37, 58]
Turbinaria ornata (Turner) J.Agardh [2, 9, 10, 11, 27, 34, 37, 40, 44, 50, 58, 60, 61, 69, 76]
Turbinaria ornata var. *prolifera* Pham H.H. [34, 37, 58]
Turbinaria parvifolia C.K.Tseng & Lu B.R. [70]

2.3.4 Chlorophyta

Acetabularia caliculus J.V.Lamouroux [11, 50, 53, 58, 76]
Acetabularia major G.Martens [53, 59]
Anadyomene plicata C.Agardh [40, 44, 53, 58, 76]
Anadyomene wrightii Harvey ex J.E.Gray [2, 11, 27, 40, 53, 58, 76]
Avrainvillea amadelpa (Montagne) A.Gepp & E.Gepp [76]
Avrainvillea erecta (Berkeley) A.Gepp & E.Gepp [11, 44, 53, 58, 69, 76]
Avrainvillea lacerata Harvey ex J.Agardh [53, 58]
Avrainvillea obscura (C.Agardh) J.Agardh [76, (21 as *A. capituliformis* T.Tanaka)]
Boergesenia forbesii (Harvey) Feldmann [10, 11, 40, 44, 53, 58, 60, 61, 76]
Boodlea coacta (Dickie) G.Murray & De Toni [53, 76]
Boodlea composita (Harvey) F.Brand [2, 10, 11, 40, 44, 50, 60, 61, 76, (27, 53, 58, 59 as *B. siamensis* Reinbold)]
Boodlea struveoides M.Howe [53, 58]
Bornetella nitida Munier-Chalmas ex Sonder [76]
Bornetella oligospora Solms-Laubach [11, 44, 53, 58]
Bornetella sphaerica (Zanardini) Solms-Laubach [11, 40, 44, 53, 58, 76]
Bryopsis hypnoides J.V.Lamouroux [10, 50, 53, 60]
Bryopsis indica A.Gepp & E.S.Gepp [44, 50, 53, 58]
Bryopsis pennata J.V.Lamouroux [11, 40, 44, 51, 53, 58]
Bryopsis pennata var. *secunda* (Harvey) Collins & Hervey [(10, 76 as *B. harveyana* J.Agardh)]
Bryopsis plumosa (Hudson) C.Agardh [50, 53, 58, 76]
Bryopsis pseudoplumosa V.J.Chapman [50, 53]
Caulerpa ashmeadii Harvey [50, 53]
Caulerpa brachypus Harvey [44, 53, 58, 61]
Caulerpa corynephora Montagne [(58 as *C. racemosa* var. *corynephora* (Montagne) W.Bosse)]
Caulerpa cupressoides (Vahl) C.Agardh [10, 44, 53, 60, 61, 69, 76]
Caulerpa cupressoides var. *lycopodium* W.Bosse [41]
Caulerpa cupressoides var. *mamillosa* (Montagne) W.Bosse [41]
Caulerpa cupressoides var. *urvilleana* (Montagne) L.J.Hodgson, Pham H.T., Lewmanomont & McDermid [(22 as *C. urvilleana* Montagne)]
Caulerpa fastigiata Montagne [11, 53, 58, 76]
Caulerpa freycinetii C.Agardh [(53, 60 as *C. freycinetii* var. *typica* W.Bosse)]
Caulerpa lentillifera J.Agardh [40, 44, 53, 58, 76]
Caulerpa macrophysa (Sonder ex Kützing) G.Murray [(2, 11, 53 as *C. racemosa* var. *macrophysa* (Sonder ex Kützing) W.R.Taylor)]
Caulerpa mexicana f. *vietnamica* Pham H.H. [53, 58]

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- Caulerpa mexicana* Sonder ex Kützing [44, 53, (43 as *C. crassifolia* (C.Agardh) J.Agardh)]
- Caulerpa microphysa* (W.Bosse) Feldmann [10, 53, 58]
- Caulerpa nummularia* Harvey ex J.Agardh (22)
- Caulerpa peltata* J.V.Lamouroux [27, 50, 53, 60 (58, 76 as *C. racemosa* var. *peltata* (J.V.Lamouroux) Eubank), (76 *C. racemosa* var. *laetevirens* (Montagne) W.Bosse)]
- Caulerpa peltata* var. *macrodisca* (Decaisne) W.Bosse [(10, 44, 53, 58, 60, 61, 69 as *C. macrodisca* Decaisne)]
- Caulerpa racemosa* (Forsskål) J.Agardh [9, 27, 40, 44, 50, 53, 58, 60, 61]
- Caulerpa racemosa* f. *vietnamensis* A.D.Zinova & Nguyen H.Dinh [27, 50, 53]
- Caulerpa racemosa* var. *macrophysa* (Sonder ex Kützing) W.R.Taylor [(76 as *C. racemosa* f. *macrophysa* (Sonder ex Kützing) W.Bosse)]
- Caulerpa lamourouxii* (Turner) C.Agardh [(76 as *Caulerpa racemosa* var. *lamourouxii* (Turner) W.Bosse)]
- Caulerpa scalpelliformis* (R.Brown ex Turner) C.Agardh [50, 53]
- Caulerpa schemnitzia* (Esper) J.V.Lamouroux [(53, 58, 59 as *Caulerpa racemosa* var. *occidentalis* (J.Agardh) Børgesen)]
- Caulerpa serrulata* (Forsskål) J.Agardh [2, 11, 27, 40, 44, 53, 58, 69, (10 as *C. freycinetii* C.Agardh)]
- Caulerpa serrulata* f. *lata* (W.Bosse) C.K.Tseng [41, 76]
- Caulerpa serrulata* var. *boryana* (J.Agardh) Gilbert [76]
- Caulerpa sertularioides* (S.G.Gmelin) M.Howe [44, 53, 58, 61]
- Caulerpa sertularioides* f. *longipes* (J.Agardh) Collins [76]
- Caulerpa taxifolia* (Vahl) C.Agardh [27, 40, 44, 50, 53, 58, 59, 60, 61, 69, 76]
- Caulerpa verticillata* J.Agardh [2, 11, 50, 53, 58, 59]
- Caulerpa verticillata* f. *charoides* W.Bosse [76]
- Caulerpa webbiana* Montagne [44, 53, 60]
- Caulerpa webbiana* f. *tomentella* (Harvey ex J.Agardh) W.Bosse [10, 41]
- Caulerpella ambigua* (Okamura) Prud'homme & Lokhorst [11, 53, 58, (53, 58 as *C. vickersiae* Børgesen)]
- Chaetomorpha aerea* (Dillwyn) Kützing [50, 53, 58]
- Chaetomorpha antennina* (Bory) Kützing [9, 11, 27, 50, 51, 58, 69, 76, (53, 59 as *C. media* (C.Agardh) Kützing)]
- Chaetomorpha capillaris* (Kützing) Børgesen [50, 52, 53, 58]
- Chaetomorpha gracilis* Kützing [53, 58]
- Chaetomorpha indica* (Kützing) Kützing [11, 53, 58]
- Chaetomorpha javanica* Kützing [11, 53, 58]
- Chaetomorpha linum* (O.F.Müller) Kützing [35, 50, 53, 58 (2, 9, 10, 11, 27, 40, 44, 69, 76 as *C. crassa* (C.Agardh) Kützing)]
- Chaetomorpha pachynema* (Montagne) Kützing [53]
- Chaetomorpha spiralis* Okamura [9, 50, 53]
- Chlorodesmis hildebrandtii* A.Gepp & E.Gepp [11, 27, 52, 53, 58, 59, 61]
- Cladophora adhaerens* Harvey [10]
- Cladophora albida* (Nees) Kützing [11, 40, 44, 50, 53, 58]
- Cladophora catenata* (Linnaeus) Kützing [(35, 50, 58 as *C. fuliginosa* Kützing)]
- Cladophora coelothrix* Kützing [(53, 58 as *Cladophoropsis modonensis* (Kützing) Reinbold), (53, 58 as *Cladophoropsis modonensis* (Kützing) Reinbold)]

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- Cladophora crispula* Vickers [10, 35, 50, 52, 53, 58]
Cladophora flexuosa (O.F.Müller) Kützing [(53, 58 as *C. gracilis* (Griffiths) Kützing)]
Cladophora glomerata (Linnaeus) Kützing [52, 53, 58]
Cladophora herpestica (Montagne) Kützing [(11, 53, 58, 76 as *Cladophoropsis herpestica* (Montagne) M.Howe)]
Cladophora laetevirens (Dillwyn) Kützing [35, 50, 52, 53, 58]
Cladophora papenfussii Pham H.H. [53, 58]
Cladophora patentiramea (Montagne) Kützing [10, 35, 50, 53, 58]
Cladophora pellucida (Hudson) Kützing [50, 53]
Cladophora perpusilla Skottsberg & Levring [11, 53]
Cladophora prolifera (Roth) Kützing [(40, 44, 50, 53, 58, 69 as *C. rugulosa* G.Martens)]
Cladophora ryukyuensis Sakai & Yoshida [(50, 53 as *C. fastigiata* Harvey)]
Cladophora sakaii I.A.Abbott [(50, 53 as *C. densa* Harvey)]
Cladophora sericea (Hudson) Kützing [(53, 58 as *C. glaucescens* (Griffiths ex Harvey) Harvey)]
Cladophora socialis Kützing [2, 35, 53, 58 (11 as *C. patentiramea* f. *longiarticulata* Reinbold)]
Cladophora stimpsonii Harvey [53]
Cladophora vagabunda (Linnaeus) C.Hoek [2, (11 as *C. inserta* f. *ungulata* (Brand) Setchell), (50, 53, 58 as *C. inserta* f. *ungulata* (Brand) Setchell), (50, 53 as *C. uncinella* Harvey), (40, 44, 53, 58, 59 as *C. inserta* Dickie)]
Cladophoropsis adhaerens Pham H.H. [53, 58]
Cladophoropsis fasciculata (Kjellman) Wille [(10, 52, 53, 58 as *C. sundanensis* Reinbold)]
Cladophoropsis membranacea (Hofman ex C.Agarth) Børgesen [2, 9, 11, 50, 53, 58]
Cladophoropsis vaucheriiformis (Areschoug) Papenfuss [53]
Codium adhaerens C.Agarth [51, 52, 53, 58, 69]
Codium arabicum Kützing [9, 10, 27, 40, 44, 50, 53, 58, 60, 61, 76]
Codium cylindricum Holmes [53, 58]
Codium duthieae P.C.Silva [50, 53]
Codium formosanum Yamada [53, 58, 59]
Codium geppiorum O.C.Schmidt [11, 44, 58, 69, 76, (53, 60, 61 as *C. geppii* O.C. Schmidt)]
Codium isthmocladum Vickers [(59 as *C. pilgerii* O.C.Schmidt)]
Codium repens P.L.Crouan & H.M.Crouan [27, 50, 52, 53]
Codium tenue (Kützing) Kützing [9, 10, 27, 53, 59]
Codium tomentosum Stackhouse [53, 58, 59]
Codium tunue Kützing [58]
Derbesia attenuata E.Y.Dawson [2, 11, 53, 58]
Derbesia marina (Lyngbye) Solier [52, 53]
Dictyosphaeria cavernosa (Forsskål) Børgesen [2, 10, 11, 44, 53, 58, 60, 61, 76]
Dictyosphaeria spinifera C.K.Tseng & C.F.Chang [50, 53]
Dictyosphaeria versluisii W.Bosse [2, 11, 40, 44, 60, 61, 76, (53, 58 as *D. setchellii* Børgesen)]
Gayralia oxysperma (Kützing) K.L.Vinogradova ex Scagel et al. [(50, 52, 53 as *Monostroma oxyspermum* (Kützing) Doty)]
Geppella prolifera C.K.Tseng & M.L.Dong [13]
Gomontia arrhiza Hariot [53, 58]
Halicystis pyriformis Levring [11, 53, 58]
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- Halimeda cuneata* f. *digitata* E.S.Barton [58]
Halimeda cuneata Hering [10, 40, 44, 53]
Halimeda cylindracea Decaisne [60, 61]
Halimeda discoidea Decaisne [2, 10, 44, 51, 53, 58, 61, 76]
Halimeda gracilis Harvey ex J.Agardh [11, 53]
Halimeda incrassata (J.Ellis) J.V.Lamouroux [10, 53, 58, 60, 61]
Halimeda macroloba Decaisne [12, 53, 76]
Halimeda micronesica Yamada [41, 53, 60]
Halimeda opuntia (Linnaeus) J.V.Lamouroux [2, 10, 11, 12, 40, 44, 53, 58, 60, 61, 76]
Halimeda taenicola W.R.Taylor [12]
Halimeda tuna (J.Ellis & Solander) J.V.Lamouroux [10, 44, 53, 58, 60]
Halimeda velasquezii W.R.Taylor [12, 76]
Halimeda xishaensis C.K.Tseng & M.L.Dong [12]
Microdictyon japonicum Setchell [2, 76]
Microdictyon nigrescens (Yamada) Setchell [50, 53]
Microdictyon okamurae Setchell [53, 58]
Microdictyon vanbosseae Setchell [53]
Monostroma nitidum Wittrock [53, 58]
Neomeris annulata Dickie [2, 10, 11, 40, 44, 53, 58, 60, 61, 76]
Neomeris bilimbata J.T.Koster [53, 58]
Neomeris vanbosseae M.Howe [2, 9, 44, 53, 58, 69]
Ostreobium quekettii Bornet & Flahault [(11 as *O. reineckei* Bornet)]
Parvocaulis clavatus (Yamada) S.Berger et al. [(53, 58, 76 as *Acetabularia clavata* Yamada)]
Parvocaulis parvulus (Solms-Laubach) S.Berger et al. [40, 44, 76, (11, 53, 58 as *Acetabularia moebii* Solms-Laubach)]
Parvocaulis pusillus (M.Howe) S.Berger et al. [(53, 58 as *Acetabularia pusilla* (M.Howe) Collins)]
Penicillus sibogae A.Gepp & E.Gepp [53, 58]
Phyllodictyon anastomosans (Harvey) Kraft & M.J.Wynne [11, 40, 44, 50, 53, 58, 76, (50, 53 as *Struvea tenuis* Zanardini), (53, 58 as *Struvea delicatula* Kützing)]
Pseudobryopsis hainanensis C.K.Tseng [(21 as *Trichosolen hainanensis* (C.K.Tseng) W.R.Taylor)]
Pseudochlorodesmis furcellata (Zanardini) Børgesen (11, 53, 58)
Rhipidosiphon javensis Montagne [76, (9, 11, 53, 58 as *Udotea javensis* (Montagne) A.Gepp & E.Gepp)]
Rhipiliopsis echinocaulos (A.B.Cribb) Farghaly [13]
Rhizoclonium grande Børgesen [50, 53, 58]
Rhizoclonium riparium (Roth) Harvey [9, 27, 50, 53, 58, (9, 11, 35, 50, 53, 58, 59 as *R. kernerii* Stockmayer)]
Rhizoclonium riparium var. *implexum* (Dillwyn) Rosenvinge [(35, 40, 44, 50, 52, 53, 58, 59 as *R. kochianum* Kützing)]
Rhizoclonium tortuosum (Dillwyn) Kützing [50, 53]
Trichosolen mucronatus (Børgesen) W.R.Taylor [(11, 53, 58 as *Pseudobryopsis mucronata* Børgesen)]
Trichosolen parvus (E.Y.Dawson) W.R.Taylor [(11, 53, 58 as *Pseudobryopsis parva* E.Y.Dawson)]

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- Tydemania expeditionis* W.Bosse [7, 10, 43, 53, 60]
Udotea argentea Zanardini [53, 58, 61]
Udotea flabellum (J.Ellis & Solander) M.Howe [7, 41, 53]
Udotea orientalis A.Gepp & E.Gepp [53, 76]
Udotea velutina C.K.Tseng & M.L.Dong [7, 53]
Ulothrix flacca (Dillwyn) Thuret [53]
Ulothrix subflaccida Wille [58]
Ulva chaetomorphoides (Børgesen) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland [(50, 52, 53, 58 as *Enteromorpha chaetomorphoides* Børgesen)]
Ulva clathrata (Roth) C.Agardh [(11, 27, 35, 40, 44, 50, 53, 58, 59, 60 as *Enteromorpha clathrata* (Roth) Greville), including f. *pumilla*]
Ulva compressa Linnaeus [(50, 53, 76 as *Enteromorpha compressa* (Linnaeus) Nees)]
Ulva globata Kjellman [9, 44, 50, 52, 53]
Ulva flexuosa subsp. *pilifera* (Kützing) M.J.Wynne [50, 53]
Ulva flexuosa Wulfen [(11, 27, 52, 53, 58, 59, as *Enteromorpha tubulosa* (Kützing) Kützing), (27, 50, 52, 53, 58 as *Enteromorpha flexuosa* (Wulfen) J.Agardh)]
Ulva intestinalis Linnaeus [(11, 27, 44, 53, 58, 59 as *Enteromorpha intestinalis* (Linnaeus) Nees)]
Ulva kylinii (Bliding) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland [(11, 35, 40, 44, 50, 53, 58, 69 as *Enteromorpha kylinii* Bliding)]
Ulva lactuca Linnaeus [2, 40, 44, 50, 53, 58, 76, (9, 50, 53 as *Ulva fenestrata* Postels & Ruprecht), (53, 58 as *Ulva fasciata* Delile)]
Ulva papenfussii Pham H.H. [40, 44, 53, 58]
Ulva prolifera O.F.Müller [(27, 50, 52, 53, 76 as *Enteromorpha prolifera* (O.F.Müller) J.Agardh)]
Ulva ralfsii (Harvey) Le Jolis [(10, 35, 53, 58 as *Enteromorpha ralfsii* Harvey)]
Ulva reticulata Forsskål [2, 40, 44, 53, 58, 76]
Ulva spinulosa Okamura & Segawa [50, 53]
Ulva stipitata Areschoug [(50, 53, as *Enteromorpha stipitata* var. *catbaensis* A.D.Zinova & Nguyen H.Dinh, a variety not formally transferred yet to *Ulva*)]
Ulva torta (Mertens) Trevisan [(50, 52, 53 as *Enteromorpha torta* (Mertens) Reinbold)]
Ulvella lens P.L.Crouan & H.M.Crouan [53, 58]
Ulvella viridis (Reinke) R. Nielsen et al. [(11, 53, 58 as *Entocladia viridis* Reinke)]
Valonia aegagropila C.Agardh [9, 10, 11, 44, 50, 53, 58, 76]
Valonia fastigiata Harvey ex J.Agardh [40, 44, 53, 58, 76]
Valonia macrophysa Kützing [50, 52, 53]
Valonia utricularis (Roth) C.Agardh [10, 53, 58, 60, 61, 76]
Valonia ventricosa J.Agardh [11, 44, 53, 58, 60, (76 as *Ventricaria ventricosa* (J.Agardh) J.L.Olsen & J.A.West)]
Valoniopsis pachynema (G.Martens) Børgesen [50, 53, 58, 59, 69]
Vaucheria piloboloides Thuret [50]

Notes:

- *Sargassum nigrifolium* Yendo, *S. nipponicum* Yendo, *S. patens* var. *vietnamese* nom. nud., *S. tortile* C. Agardh, *S. tosaense* Yendo were removed from the Vietnamese flora on the authority of Yoshida (2002a). *Sargassum polycystum* var.

longicaule is removed on the authority of Nguyen H.Dai (1997, 2007). The following species are not included in the checklist because they have not been formally described: *Ceramium phuquocense* nom. nud. [59], *Gigartina vietnamensis* nom. nud. [50], *Solieria fastigiata* nom. nud. [45, 69], *Caulerpa minuta* nom. nud. [21], *Chaetomorpha patentiramea* nom. nud. [9]

- Taxonomic changes postdating the publication of the checklist (Nguyen V.T. et al. 2013): *Caulerpa racemosa* var. *lamourouxii* (Turner) W.Bosse has been elevated to species rank and *Caulerpa racemosa* var. *occidentalis* (J.Agardh) Børgesen is now known as *Caulerpa schemnitzia* (Esper) J.V.Lamouroux. See Belton et al. (2014).

2.4 Diversity of marine macroalgae in Vietnam

The present checklist contains a total of 827 species (180 Chlorophyta, 147 Phaeophyceae, 412 Rhodophyta and 88 Cyanobacteria) compiled from all earlier species records published in 81 publications. This work represents the most inclusive list of the Vietnamese flora to date, including 623 species more than in Dawson's first list (Dawson 1954), and 157 species more than reported on Algaebase (Guiry & Guiry 2012). This checklist offers an up to date overview of the Vietnamese algal flora and considerably improves its accessibility to the international community. Importantly, the checklist provides currently accepted species names for Vietnamese seaweeds. Continuous taxonomic refinement means that a considerable proportion (>1/3) of taxon names listed in earlier publications such as Dawson (1954), Pham H.H. (1969) and Nguyen H.Dinh et al. (1993) are no longer considered currently accepted.

The bulk of new records of seaweeds from Vietnam were published in a few publications only (e.g. Dawson 1954, Pham H.H. 1969 and Nguyen H.Dinh et al. 1993). Despite an increase in the number of algal publication from the 1990s onwards, the discovery rate of new records seems to diminish (Figs 2.1, 2.2). From this, the question arises whether we are getting close to documenting most of the Vietnamese seaweed diversity. Previous diversity estimates resulted in a figure of nearly a thousand seaweed species for the Vietnamese coast (Huynh Q.N. & Nguyen H.Dinh 1998; Dang D.H. et al. 2007). In contrast, Algaebase reports only 670 taxa (Guiry & Guiry 2012), but this figure is only indicative since a number of publications had not been included yet (e.g. Pham H.H. 1985, Nguyen H.Dinh et al. 1993; Nguyen H.Dai 1997; Nguyen H.Dai et al. 2000; Nguyen H.Dai and Pham H.T. 2003; Le N.H. 2000, 2001, 2004; Le N.H. and Nguyen H.Dai 2006; Dam 2003; Nguyen V.Tien 2007).

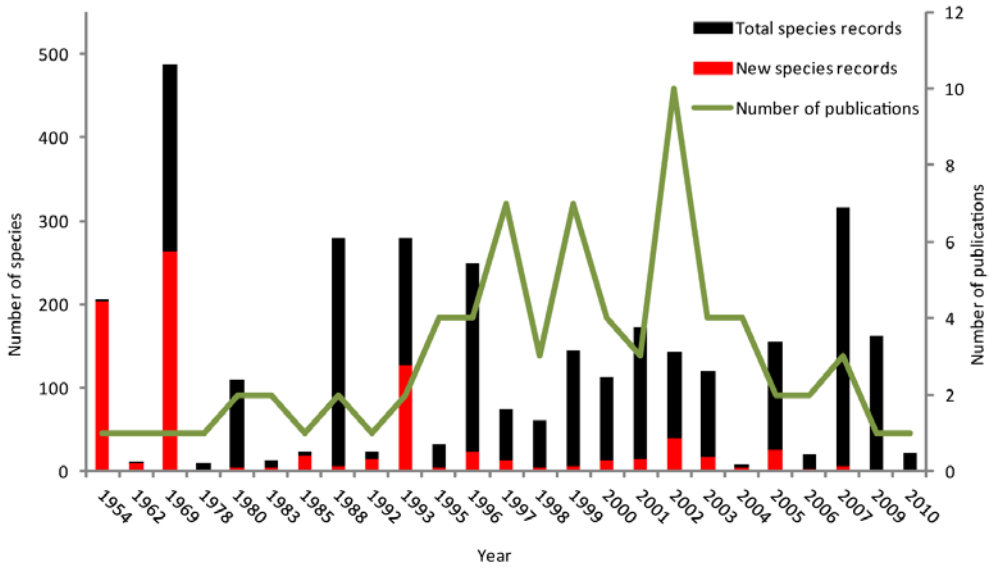


Fig. 2.1. Numbers of publications on Vietnamese seaweeds (green line, right axis; total = 81) and numbers of new algal records reported (bar chart, left axis) by year between 1954 and 2010.

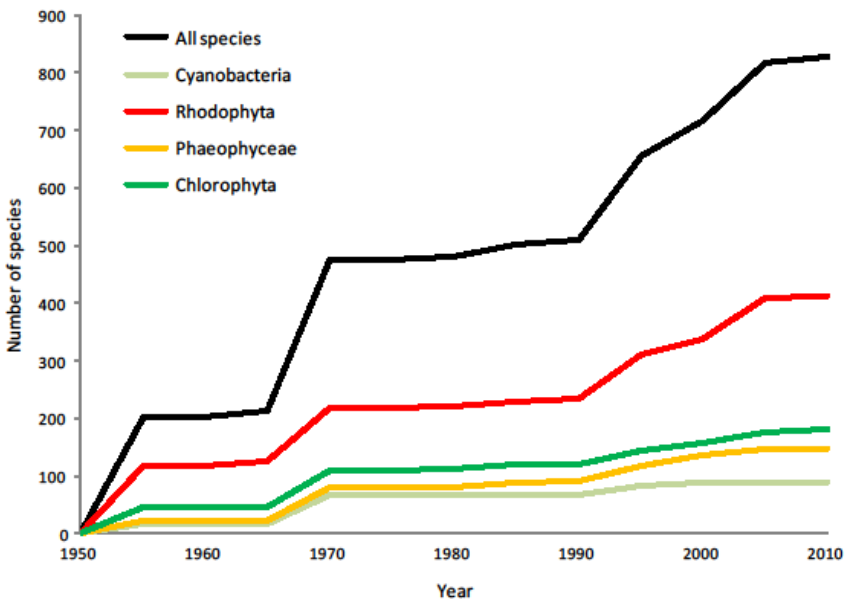


Fig. 2.2. Cumulative curve of new species records from Vietnam.

2.5 Distribution of seaweeds along the Vietnamese coast

Locality data have been included in the database of Vietnamese seaweeds, enabling us, to some extent, to analyse biogeographic and richness patterns (Fig. 2.3). Of the 22 studied provinces, 11 have over 100 species, of which two are northern provinces (Quang Ninh, Hai Phong), seven are central provinces (Thanh Hoa, Quang Tri, Da Nang, Quang Ngai, Khanh Hoa, Ninh Thuan, Binh Thuan) and two are southern provinces (Ba Ria - Vung Tau, Kien Giang). Totals of 60 and 197 species are reported from the offshore Paracel and Spratly islands, respectively. The greatest species number (418) has been reported for the Khanh Hoa province, where half of the total number of species in Vietnam has been found. In general the highest marine macroalgal diversity has been recorded for the South Central Coast region. The difference in species diversity among the provinces and regions is most likely influenced by differences in research effort. The South Central region has traditionally been the most extensively investigated region. At least one third of the publications on Vietnam's seaweed flora are focusing on the Khanh Hoa province or its vicinity. This region was popular with visiting international phycologists (e.g. Dawson 1954, Abbott et al. 2002, Tsutsui et al. 2005). On the other hand, the high diversity might be sustained by the habitat heterogeneity of this region, or by increased primary productivity caused by local upwelling.

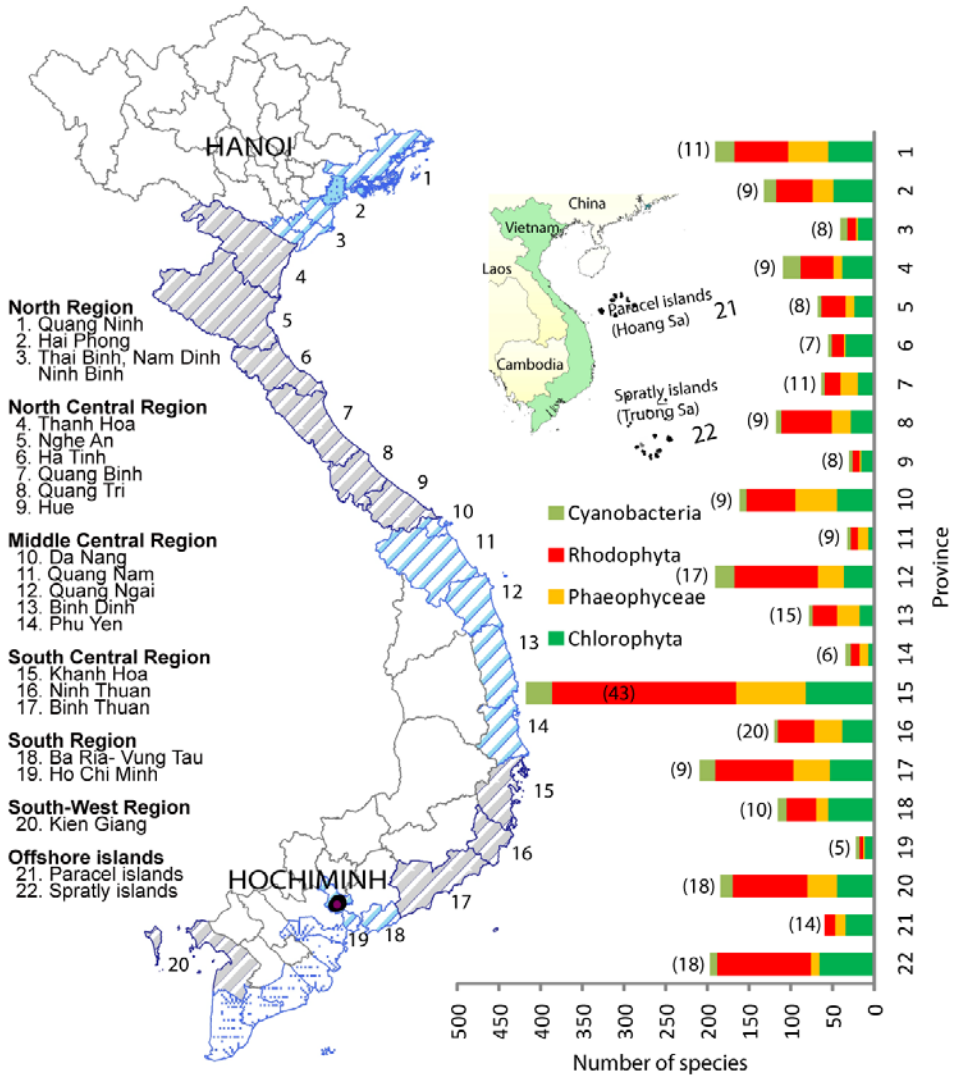


Fig. 2.3. Distribution of seaweeds by province along the coast of Vietnam. The number of publication per province is indicated in brackets next to each bar.

2.6 Diversity of marine macroalgae in Vietnam compared to neighbouring countries

The diversity of the Vietnamese seaweeds was compared with those of a number of neighbouring countries (Malaysia, Philippines, Taiwan and Thailand). The Philippines appear to be the most diverse country with a staggering total of 1011 species, while Vietnam harbours a comparable 827 species (Fig. 2.4). Sørensen similarity indexes are low ($C_s < 0.5$; Table 1). These low values are counterintuitive. Within similar climatic zones and in the absence of major dispersal barriers, marine biodiversity is expected to be much more homogenous (see Spalding et al. 2007). Even though we cannot rule out the existence of a biogeographical pattern making the Vietnamese seaweed flora highly distinct, we are of the opinion that the low similarity with neighbouring countries is primarily an artifact resulting from taxonomic inconsistencies. The checklist presented here, could serve as a valuable tool, listing the Vietnamese seaweed diversity and stimulating intraregional comparative research. It may then become clear that many local endemics have a much wider distribution.

Table 2.1. Comparison of the Vietnamese seaweed flora with neighbouring countries. N_b = number of individuals in the neighbouring country; and N_{a+b} = the number of taxa shared with Vietnam; Sørensen similarity index: $C_s = (2 \cdot N_{a+b}) / (N_a + N_b)$.

| Neighbouring country | Vietnam ($N_a = 827$) | | |
|----------------------|-------------------------|-----------|-------|
| | N_b | N_{a+b} | C_s |
| Philippines | 1011 | 295 | 0.319 |
| Malaysia | 241 | 107 | 0.200 |
| Thailand | 182 | 93 | 0.184 |
| Taiwan | 288 | 112 | 0.201 |

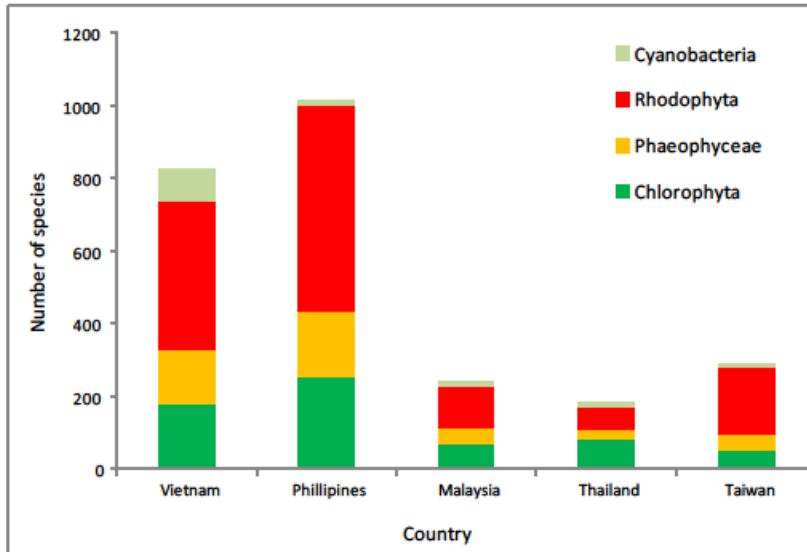


Fig. 2.4. Numbers of seaweed species in Vietnam and neighbouring countries.

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REFERENCES

- Abbott, I.A. 1999. Note on some species of *Halymenia* in the southwestern Pacific In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **7**, California Sea Grant College Program, La Jolla, CA. pp. 163-172. (1)
- Abbott, I.A., J. Fisher and K.J. McDermid 2002. Newly reported and revised marine algae from the vicinity of Nha Trang, Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **8**, California Sea Grant College Program, La Jolla, CA. pp. 291-321. (2)
- Agardh, J.G. 1848. *Species genera et ordines algarum ... Volumen primum: algas fucoideas complectens*. Berling, Lundae [Lund]. viii + 363 pp.
- Agardh, J.G. 1851--1863. *Species genera et ordines algarum ... Volumen secundum: algas florideas complectens*. Berling, Lundae [Lund]. xii + 1291 pp.
- Ajisaka, T., Q.N. Huynh and H.Dinh Nguyen. 1995. Studies of two zygoecarpic species of *Sargassum* (subgenus *Sargassum*) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 45-55 (3)
- Ajisaka, T., Q.N. Huynh, H.Dinh Nguyen and T. Yoshida. 1997a. *S. carpophyllum* J. Agardh var. *nhatrangense* (Pham) Ajisaka comb. Nov. and *S. piluliferum* (Turner) C. Agardh var. *serratifolium* Yamada from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species*. Vol.6, California Sea Grant College Program, La Jolla, CA. pp. 51-60. (4)
- Ajisaka, T., Q.N. Huynh, H.Dinh Nguyen, B.R. Lu, Y.-M. Chiang and T. Yoshida. 1997b. *Sargassum hemiphyllum* (Turnner) C. Agardh var. *chinensis* J.Agardh from Vietnam, the Chinese mainland, and Taiwan. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **6**, California Sea Grant College Program, La Jolla, CA. pp. 51-60. (5)
- Belton, G.S., Prud'homme van Reine, W.F., Huisman, J.M., Draisma, S.G.A. & Gurgel, C.F.D. (2014). Resolving phenotypic plasticity and species designation in the morphology challenging *Caulerpa racemosa-peltata* complex (Caulerpaceae, Chlorophyta). *Journal of Phycology* **50**: 32-54.
- Chiang, Y.-M., T. Yoshida, T. Ajisaka, G. Trono, C.K. Tseng, and B.R. Lu. 1992. Distribution and variation in *Sargassum polycystum* C.A. Agardh (Fucales, Phaeophyta). In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific and Western Atlantic species* **3**, California Sea Grant College Program, La Jolla, CA. pp. 35-42. (6)
- Dam, D.T. 2001. Contribution some new species (Chlorophyta) to Vietnam Seaweed flora. *Vietnamese Journal of Biology* **23**: 31-34. (7)
- Dam, D.T. 2003. Thành phần loài và phân bố của rong đỏ (Rhodophyta) tại một số đảo của quần đảo Trường sa. *Journal of Marine science and Technology* **3**: 54-63. (8)
- Dam, D.T. 2007. Hiện trạng nguồn lợi rong biển vùng Hải vân - Sơn trà, *Proc. 2nd National scientific conference on ecology and biological resources*. Agriculture Publishing House, Hanoi. pp. 133-140. (9)
- Dam, D.T. and V.T. Nguyen. 1996. Species composition and distribution of seaweeds in Truong Sa island - Spratly islands. *Vietnamese Marine environmental Research* **3**: 243-252. (10)
- Dang D.H., M.H. Hoang and N.S. Pham. 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology* **19**: 817-826.

- Dawson, E.Y. 1954. Marine plants in the Vicinity of the Institute Oceanography of Nha Trang. Pacific science **8**: 373-471. Univ. of Hawaii Press. **(11)**
- De Toni, G.B. 1895. *Sylloge algarum ... Vol. III. Fucoideae*. Patavii [Padova]. xvi + 638 pp.
- De Toni, G.B. 1900. *Sylloge algarum ... Vol. IV. Florideae. Sectio II*. Patavii [Padova]. 387-774 + 775-776 pp.
- De Toni, G.B. 1905. *Sylloge algarum ... Vol. IV. Florideae. Sectio IV*. Patavii [Padova]. 1523-1973 pp
- Dong, M.L. and C.K. Tseng. 1980. Studies on some marine green algae from the Xisha Islands, Guangdong Province, China. II. *Studia Marina Sinica* **17**: 1-10 **(12)**
- Dong, M.L. and C.K. Tseng. 1983. Studies on the *Geppella* (Chlorophyta) of Xisha Islands, South China Sea. *Chin. J. Ocean. Limn.* **1**: 190-197. **(13)**
- Draisma, S.G.A., Y.-S. Keum, W.F. Prud'homme van Reine and G.M. Lokhorst. 1998. The species of *Sphacelaria* (Sphacelariales, Phaeophyceae) in China with a description of a new species. *Botanica Marina* **41**: 181-190 **(14)**
- Guiry, M.D. and G.M. Guiry. 2012. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>
- Huynh, Q.N. and H.Dinh Nguyen. 1998. The seaweed resources of Vietnam. In: (Critchley, A.T. and M. Ohno Eds) *Seaweed resources of the World*. Kanagawa International Fisheries Training Centre, JICA. pp. 62-69.
- Kamiya, M., G.C. Zuccarello and J.A. West. 2003. Evolutionary relationships of the genus *Caloglossa* (Delesseriaceae, Rhodophyta) inferred from large-subunit ribosomal RNA gene sequences, morphological evidence and reproductive compatibility, with description of a new species from Guatemala. *Phycologia* **42**: 478-497. **(15)**
- Kawaguchi, S. and K. Lewmanomont. 1999. Morphology and culture study of a red alga, *Halymenia dilatata* Zandardini, from Vietnam and Japan. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 7*, California Sea Grant College Program, La Jolla, CA. pp. 147-161. **(16)**
- Kawaguchi, S. and H.Dinh Nguyen. 1998. Taxonomic studies of the Halymeniaceae (Halymeniales, Rhodophyta) from Vietnam, I. A transfer of *Carpopeltis formosana* Okamura to *Prionitis*. *Botanica Marina* **41**: 391-397. **(17)**
- Kawaguchi, S., K. Lewmanomont and K.J. McDermid. 2002. Morphology of *Halymenia maculata* J. Agardh from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 259-266. **(18)**
- Kawaguchi, S., S. Shimada, H.W. Wang and M. Masuda. 2004. The new genus *Yonagunia* Kawaguchi & Masuda (Halymeniaceae, Rhodophyta), based on *Y. tenuifolia* Kawaguchi & Masuda sp. nov. from southern Japan and including *Y. formosana* (Okamura) Kawaguchi & Masuda comb. nov. from southeast Asia. *Journal of Phycology* **40**: 180-192. **(19)**
- Lam, N.T., K.D. Nguyen, K. Tran and T.H. Luu. 1978. The Agar-Agar content in some species of Rhodophyta along the coasts of Nha Trang - Vung Tau. *Collection of Marine Research Works* **1**: 33-41. **(20)**
- Le, N.H. 2000. Notes on some new species of marine algae from Ninh Thuan province (South Vietnam). *Collection of Marine Research Works* **10**: 141-148. **(21)**
- Le, N.H. 2001. Một số loài rong biển mới ở vùng biển Quần đảo Trường sa. *Collection of Marine Research Works* **11**: 115-120. **(22)**

- Le, N.H. 2004. Some new recorded species for the genus *Gracilaria* of Vietnam. Collection of Marine Research Works **14**. pp. 81-88. **(23)**
- Le, N.H. and H.Dai Nguyen. 2006. Contribution to the study of *Gracilaria* and relative general (Gracilariales - Rhodophyta) from Vietnam. Coastal Marine Science **30**(1): 214-221. **(24)**
- Le, N.H. and H.D. Nguyen 2010. *Rong câu Việt nam nguồn lợi và sử dụng (The Vietnamese Gracilaria, resources and utilization)*. Vietnamese Natural Science and Technology Publishing House, Hanoi. pp. 241 **(25)**
- Le, N.H. and S.-M. Lin. 2006. *Gracilariopsis nhatrangensis* (Gracilariaceae, Rhodophyta) a new marine red alga from Nhatrang, southern Vietnam. Botanical Studies **47**: 329-337. **(26)**
- Le, T.T. 1996. Thành phần loài rong biển ở vùng triều tỉnh Quảng Trị. Collection of Marine Research Works **7**. pp. 182-191. **(27)**
- Lin, S.M. 2012. Marine macro-algal flora of Orchid Island, Taiwan. Kuroshio Science **6**: 129-144.
- Magurran, A.E. 1988. *Ecological diversity and its measurement*. Princeton University Press, Princeton, NJ. pp. 179
- Masuda, M. and K. Kogame. 1998. A taxonomic study of the genus *Laurencia* (Ceramiales, Rhodophyta) from Vietnam. V. *Laurencia concreta* Cribb and *L. dinhii* sp. nov., Cryptogamie Algologie **19**: 201-212. **(28)**
- Masuda, M. and M. Suzuki. 1997. A taxonomic study of the genus *Laurencia* (Ceramiales, Rhodophyta) from Vietnam. III. *Laurencia calliclada* sp. nov. Cryptogamie Algologie **18**: 273-282. **(29)**
- Masuda, M., S. Kawaguchi, Y. Takahashi, Y. Matsuo, and M. Suzuki, 1997a. A taxonomic study of the genus *Laurencia* (Ceramiales, Rhodophyta) from Vietnam. I. *Laurencia caduciramulosa* Masuda et. Kawaguchi sp. nov., Cryptogamie Algologie **18**: 1-10. **(30)**
- Masuda, M., Y. Takahashi, Y. Matsuo, and M. Suzuki. 1997b. A taxonomic study of the genus *Laurencia* (Ceramiales, Rhodophyta) from Vietnam. II. *Laurencia lageniformis* sp. nov., Cryptogamie Algologie **18**: 163-174. **(31)**
- Nguyen, H.Dai. 1980. The *Sargassum* in Hon Chong - Nha Trang. Collection of Marine Research Works **2**: 53-71. **(32)**
- Nguyen, H.Dai. 1993. Về chi *Eucheuma* (Rhodophyta) ven biển các tỉnh miền trung Việt Nam. Vietnamese Journal of Biology. **15**(4): 9-15. **(33)**
- Nguyen, H.Dai. 1997. *Sargassaceae in Vietnam resources and utility (Rong mơ Việt nam nguồn lợi và sử dụng)*, Agriculture Publishing House, Hanoi. pp. 199. **(34)**
- Nguyen, H.Dai. 1999. Rong biển phụ sinh (epiphytes) trên cỏ biển ở Khánh hòa. Collection of Marine Research Works **9**: 196-204. **(35)**
- Nguyen, H.Dai. 2002. Three new species of *Sargassum* (Sargassaceae, Fucales) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **8**, California Sea Grant College Program, La Jolla, CA. pp. 103-112. **(36)**
- Nguyen, H.Dai. 2007. Bộ Rong Mơ (Fucales Kylin) In: *Thực vật chí Việt Nam (Flora of Vietnam)*. **11**, Science and Technology Publishing House, Hanoi. pp. 117. **(37)**
- Nguyen, H.Dai and N.H. Le. 2005. Some new record marine from vietnam - part III. Journal of Marine Science and Technology **4**: 120-128. **(38)**

- Nguyen, H.Dai and H.T. Pham. 1997. Some species of the genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) recently found from Vietnam. *Proc. 1st National symposium on marine biology*. pp. 91-102. (39)
- Nguyen, H.Dai and H.T. Pham. 2001. Nguồn lợi rong biển đảo Lý Sơn. *Collection of Marine Research Works* **11**: 121-134. (40)
- Nguyen, H.Dai and H.T. Pham. 2002. Some new records of marine algae from Vietnam -1. *Collection of Marine Research Works* **12**: 149-158. (41)
- Nguyen, H.Dai and H.T. Pham. 2003. Some new records of marine algae from Vietnam-2. *Collection of Marine Research Works* **13**: 95-104. (42)
- Nguyen, H.Dai, H.T. Pham and X.V. Nguyen. 2000. New records of marine algae from Vietnam. *Collection of Marine Research Works* **10**: 127-140. (43)
- Nguyen, H.Dai, H.T. Pham and X.V. Nguyen. 2009. Thành phần loài và nguồn lợi rong biển, cỏ biển đảo Phú quý (Cù lao Thu) Bình thuận. *Collection of Marine Research Works* **16**: 225-243. (44)
- Nguyen, H.Dinh. 1980. The new species of algae in *Solieria* J. Ag genus. *Collection of Marine Research Works* **2**: 3-5. (45)
- Nguyen, H.Dinh 1992. Vietnamese species of *Gracilaria* and *Gracilariopsis*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **3**, California Sea Grant College Program, La Jolla, CA. pp. 207-210. (46)
- Nguyen, H.Dinh and Q.N. Huynh. 1995. Species of *Eucheuma* and *Kappaphycus* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 229-235. (47)
- Nguyen, H.Dinh and Q.N. Huynh. 1999. Some new taxa of *Sargassum* (Phaeophyta) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **7**, California Sea Grant College Program, La Jolla, CA. pp. 43-51. (48)
- Nguyen, H.Dinh and Q.N. Huynh. 2001. Five new species of *Sargassum* from Vietnam (Năm loài mới thuộc chi rong mơ - *Sargassum* ở ven biển Việt nam). *Vietnamese Journal of Biology* **23**: 1-10. (49)
- Nguyen, H.Dinh, Q.N. Huynh, N.B. Tran and V.T. Nguyen. 1993. *Marine algae of north Vietnam (Rong biển phía bắc Việt Nam)* Science and Technical Publishing House, Hanoi. pp. 344 (50)
- Nguyen, T.Phat 1996. Nghiên cứu một số đặc điểm sinh học và chỉ tiêu hóa sinh của rong biển có ý nghĩa kinh tế ở đảo Xanh, Bình định. *Sci. Rep. Hanoi Univ. Edu.* **1**: 65-70. (51)
- Nguyen, V.Tien 1996. Rong biển vịnh Hạ Long. *Collection of Marine Research Works* **7**: 184-193. (52)
- Nguyen, V.Tien 2007. Ngành rong lục (Chlorhophyta). In: *Thực vật chí Việt Nam (Flora of Vietnam)*. **10**, Science and Technology Publishing House, Hanoi. pp. 279. (53)
- Ní Chualáin, F., C.A. Maggs, G.W. Saunders, and M.D. Guiry 2004. The invasive genus *Asparagopsis* (Bonnemaisoniaceae, Rhodophyta): molecular systematics, morphology, and ecophysiology of *Falkenbergia* isolates. *Journal of Phycology* **40**: 1112-1126. (54)
- Noro, T., Ajisaka, and T. Yoshida. 1995. *Sargassum henslowwanum* var. *condensatum* Yamada in Japan: A synonym of *Sargassum yendoi* Okamura et Yamada. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**. California Sea Grant College Program, La Jolla, CA. pp. 3-9. (55)

- N'Yeurt, A.D.R. 2002. A revision of *Amansia glomerata* C. Agardh, *Amansia rhodantha* (Harvey) J. Agardh and *Melamansia glomerata* (C. Agardh) R.E. Norris (Rhodophyta, Rhodomelaceae). *Botanica Marina* **45**: 231-242. (56)
- Ohno, M., R. Terada and H. Yamamoto. 1999. The species of *Gracilaria* from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **7**, California Sea Grant College Program, La Jolla, CA. pp. 99-111. (57)
- Pham, H.H. 1969. Marine algae of south Vietnam (Rong biển miền nam Việt nam). Sai Gon study Center. Ministry of Education and Youth. pp.558. (58)
- Pham, H.H. 1985. *The plants of Phu Quoc islands (Thực vật ở đảo Phú Quốc)*, Hanoi Publishing House, Hanoi. pp. 183. (59)
- Pham, H.T. 1996. Góp phần nghiên cứu rong biển quần đảo Trường sa. *Collection of Marine Research Works* **7**: 147-162. (60)
- Pham, H.T. 1999. Marine macroalgae resources at Son ca and Song Tu Tay island – Spratly islands (Nguồn lợi rong biển hai đảo Sơn ca và Song tử tây) *Collection of Marine Research Works* **9**: 205-215. (61)
- Pham, H.T. 2002. Morphological variability of *Kappaphycus cottonii* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **8**, California Sea Grant College Program, La Jolla, CA. pp. 247-255. (62)
- Phillips, N. 1995. Biogeography of *Sargassum* (Phaeophyta) in the Pacific basin. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 107-145. (63)
- Santelices, B. 2004. *Parviphycus*, a new genus in the Gelidiellaceae (Gelidiales, Rhodophyta). *Cryptogamie Algologie* **25**: 313-326. (64)
- Spalding, M.D., H.E. Fox, B.S. Halpern, M.A. Mcmanus, J. Molnar, G.R. Allen, N. Davidson, Z.A. Jorge, A.L. Lombana, S.A. Lourie, K.D. Martin, E. Mcmanus, J. Molnar, C.A. Recchia, and J. Robertson. 2007. Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *Bioscience* **57**: 573-583.
- Stiger, V., T. Horiguchi, T. Yoshida, A.W. Coleman and M. Masuda. 2000. Phylogenetic relationships of *Sargassum* (Sargassaceae, Phaeophyceae) with reference to a taxonomic revision of the section *Phyllocystae* based on ITS-2 nrDNA sequences. *Phycological research* **48**: 251-260. (65)
- Stiger, V., T. Horiguchi, T. Yoshida, A.W. Coleman, and M. Masuda. 2003. Phylogenetic relationships within the genus *Sargassum* (Fucales, Phaeophyceae), inferred from its ITS nrDNA, with an emphasis on the taxonomic revision of the genus. *Phycological Research* **51**: 1-10. (66)
- Tanaka, T. and H.H. Pham 1962. Notes of some marine algae from Viet-Nam - I. *Memoirs of the Faculty of Fisheries, Kagoshima University* **11**: 24-40. (67)
- Terada, R., H. Yamamoto 2002. Additional notes on *Gracilaria eucheumatoides* Harvey from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **8**, California Sea Grant College Program, La Jolla, CA. pp. 225-230. (68)
- Thieu, L. 1998. Điều tra nguồn lợi rong biển kinh tế ở Hà Tiên - Kiên Giang. Master Thesis, Nhatrang Univ. pp. 1-76. (69)
- Tseng, C.K. and B.R. Lu. 1983. Two new brown algae from the Xisha Islands, south China Sea. *Chinese Journal of Oceanology and Limnology* **1**: 185-189. (70)

- Tseng, C.K. and B.R. Lu. 1988. Studies on Chinese species of zygotocarpic *Sargassum*. In: (Abbott, I.A. Eds) *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 23-54. (71)
- Tseng, C.K. and B.R. Lu. 2002a. Some new species and records of *Sargassum*(Sargassaceae, Rhodophyta) from the China sea In: (Abbott, I.A. Eds) *Taxonomy of economic seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 135-148. (72)
- Tseng, C.K. and B.R. Lu. 2002b. Study on the Biserrulic *Sargassum* of China: The Series llicifoliae (J. Agardh). In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 35-76. (73)
- Tseng, C.K. and B.M. Xia. 1999. On the *Gracilaria* in the western Pacific and southeastern Asia region. *Botanica Marina* 42: 209-218. (74)
- Tseng, C.K., T. Yoshida and Y.-M. Chiang. 1985. East Asiatic species of *Sargassum* subgenus *Bactrophycus* J.Agardh (Sargassaceae, Fucales), with keys to the sections and species. In: (Abbott, I.A. & Norris, J.N. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific and Caribbean species 1*, California Sea Grant College Program, La Jolla, CA. pp. 1-14. (75)
- Tsutsui, I., Q.N. Huynh, H.Dinh Nguyen, S. Arai and T. Yoshida. 2005. *The common marine plants of southern Vietnam*. Japan Seaweed Association, Japan. pp. 250. (76)
- Xia, B.M. and H. Yamamoto. 1985. *Gracilaria* species from both China and Japan: key, list and distribution of common and economically important species. In: (Abbott, I.A. & Norris, J.N. Eds) *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species 1*, California Sea Grant College Program, La Jolla, CA. pp. 69-70. (77)
- Yoshida, T., Nguyen H.Dai, T. Ajisaka, and T. Noro. 2002a. Verification of *Sargassum* species identified with subgenera *Bactrophycus*, *Phyllotrichia* and *Schizophycus* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 95-102. (78)
- Yoshida, T., V. Stiger, T. Ajisaka and T. Noro. 2002b. A molecular study of section-level classification of *Sargassum* subgenus *Bactrophycus* (Sargassaceae, Phaeophyta). In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 89-94. (79)
- Zhang J.F. and B.M. Xia. 1980. Two new species of *Laurencia* from Xisha islands Guangdong province, Chinese Journal of Oceanologia and Limnologia Sinica. **11**(3): 267-274. (80)
- Zhang J.F. and B.M. Xia. 1988. *Laurencia* from China: key list and distribution of the species. In: (Abbott, I.A. Eds) *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 249-252. (81)

Chapter 3

Taxonomy and morphology
of the genus *Sargassum*

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The genus *Sargassum* is probably the most ubiquitous and ecologically important algal genus of tropical coastal ecosystems, including Vietnam. There are more species described or reported of *Sargassum* than any other algal genus (Silva et al. 1996, Nguyen et al. 2013). Morphological plasticity which apparently characterizes many of its species (de Paula and de Oliveira 1982, Kilar et al. 1992, Stiger and Payri 1999), makes species identification particularly cumbersome and greatly confuses the taxonomy of the genus. Therefore, a major part of my PhD thesis is devoted to diversity and species delineation of *Sargassum*. In this chapter, I first detail the history and taxonomy of the genus from a general point of view, before concentrating on the *Sargassum* diversity encountered in Vietnam.

3. 1. Taxonomy of the genus *Sargassum*

3.1.1. History

The origin of the genus *Sargassum* traces back to Linnaeus (1753), who described the first *Sargassum* species in the genus *Fucus* (*Fucus acinarium* L., *F. natans* L., *F. lendigerus* L.). In the years following, and largely adhering to the algal generic classification proposed by Linnaeus, Gmelin (1768), Forsskål (1775), Turner (1807, 1808, 1809, 1811, 1819) and Mertens (1819) described 48 more species as *Fucus*.

The genus *Sargassum* was established by C. Agardh (1820) as one of five genera of the ‘Fucoideae’. It contained 59 or 62 species, depending on the interpretation, with several species containing numerous intraspecific taxa which according to modern nomenclatural practices are regarded as varieties. The genus was divided into seven groups of unnamed rank. The intrageneric classification was primarily based on the placement of reproductive structures (receptacles), morphology of the leaves, and vesicles. Two of these groups were soon elevated to separate genera, namely *Turbinaria* Lamouroux (1824) and *Carpophyllum* Greville (1830). (Table 3.1)

Table 3.1. Classification of the genus *Sargassum* as proposed by C. Agardh (1820). Names between square brackets refer to currently accepted names.

Group I

S. vulgare C. Agardh

S. bacciferum (Turner) C. Agardh [*S. natans* (L.) Gaillon]

S. dentifolium (Turner) C. Agardh

S. subrepandum (Forsskål) C. Agardh

S. esperi C. Agardh

S. lendigerum (Linnaeus) C. Agardh

S. virgatum C. Agardh

S. swartzii C. Agardh

S. ilicifolium (Turner) C. Agardh

S. aquifolium (Turner) C. Agardh [*S. natans* (L.) Gaillon]

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- S. cristaefolium* C. Agardh [*S. ilicifolium* (Turner) C. Agardh]
S. latifolium (Turner) C. Agardh
S. telephifolium (Turner) C. Agardh
S. incisifolium (Turner) C. Agardh
S. lacerifolium (Turner) C. Agardh [*S. natans* (L.) Gaillon]
S. tortile (C. Agardh) C. Agardh [*S. siliquastrum* (Turner) C. Agardh]
S. serratifolium (C. Agardh) C. Agardh
S. longifolium (Turner) C. Agardh [*Anthophycus longifolius* (Turner) Kützing]
S. enerve C. Agardh [*S. fulvellum* (Turner) C. Agardh]
S. graminifolium C. Agardh
S. flavicans (Mertens) C. Agardh
S. linifolium C. Agardh [*S. acinarium* (Linnaeus) Setchell]
S. cymosum C. Agardh
S. saltii (Turner) C. Agardh
S. heterophyllum C. Agardh [*S. incisifolium* (Turner) C. Agardh]
S. siliquastrum (Turner) C. Agardh
S. acinaria C. Agardh
S. linearifolium (Turner) C. Agardh
S. vestitum (R. Brown ex Turner) C. Agardh
S. myagroides C. Agardh [*Myagropsis myagroides* (Mertens ex Turner) Fensholt]

Group II

- S. desfontainesii* (Turner) C. Agardh
S. verruculosum C. Agardh [*Phyllotricha verruculosa* (C. Agardh) Dixon & Huisman]
S. scoparium (Mertens ex Turner) C. Agardh [*S. siliquastrum* (Turner) C. Agardh]
S. piluliferum (Turner) C. Agardh
S. patens C. Agardh
S. pinnatifolium (C. Agardh) C. Agardh [*S. piluliferum* (Turner) C. Agardh]
S. diversifolium (Turner) C. Agardh

Group III

- S. spinifex* C. Agardh
S. parvifolium (Turner) C. Agardh
S. granuliferum C. Agardh
S. desvauxii (Mertens) C. Agardh
S. angustifolium C. Agardh
S. cystocarpum C. Agardh
S. onustum (Mertens) C. Agardh [*Sirophysalis trinodis* (Forsskal) Kützing]
S. microphyllum C. Agardh

Group IV

- S. fulvellum* (Turner) C. Agardh
S. microceratium (Mertens ex Turner) C. Agardh
S. macrocarpum C. Agardh
S. sisymbrioides C. Agardh [unknown]
S. horneri (Turner) C. Agardh
S. pallidum (Turner) C. Agardh
S. hemiphyllum (Turner) C. Agardh
S. hornschurchii C. Agardh

Group V [Turbinaria]

- S. turbinatum* (Linnaeus) C. Agardh [*Turbinaria turbinata* (Linnaeus) Kuntze]

Group VI

- S. decurrens* (R. Brown ex Turner) C. Agardh [*Sargassopsis decurrens* (R. Brown ex Turner) Trevisan]
S. peronii C. Agardh [*Sargassopsis decurrens* (R. Brown ex Turner) Trevisan]
S. platylobium C. Agardh [*Cystophora platylobium* (Mertens) J. Agardh]

Group VII [Carpophyllum]

- S. phyllanthum* C. Agardh [*Carpophyllum flexuosum* (Esper) Greville]
S. maschalocarpum C. Agardh [*Carpophyllum maschalocarpum* (Turner) Greville]

C. Agardh's (1820) classification can be regarded as the foundations of our current classification system for algae. In this system, *Sargassum* represents the first genus listed in C. Agardh's order *Fucoideae*. In 1824, C. Agardh described 5 more species (*S. confusum*, *S. filipendula*, *S. plagiophyllum*, *S. polycystum*) and several additional species were described by various phycologists: Bory in 1828 (1 species), Harvey in 1834 (1 species), Montagne in 1837, 1842, 1944, 1945 (7 species), Kützing in 1943 (2 species), Sonder in 1845 (5 species) and Greville in 1848, 1849 (5 species).

The taxonomy of *Sargassum* was altered profoundly by Kützing (1843), who split the genus into six genera (*Carpocanthus*, *Halochloa*, *Pterocaulon*, *Sargassum*, *Spongocarpus* and *Stichophora*) and later by J. Agardh (1848) who, contrary to Kützing, adopted a broad definition of *Sargassum*. J. Agardh grouped species in three sections, several of them subdivided into 'tribus'.

In 1889, J. Agardh, described 18 more species. In total 143 species were recognized by that time, rendering *Sargassum* among the largest algal genera at the time. However, it was Grunow (1915, 1916) who single-handedly made *Sargassum* the large genus as we know it today. Grunow described no less than 156 new taxa. Other authors who have since then contributed to our knowledge of *Sargassum* diversity include, Setchell (1924, 1931, 1933) describing some thirty species from the Gulf of California and China (Hong Kong, Canton and Macao), Yamada (1944), Børgesen (1941), Taylor (1945), Pham H.H. (1967, 1969) and Earle (1968) (Fig. 3.1).

At present 615 *Sargassum* taxa have been accepted taxonomically, 349 of which at the species level, 217 varieties and 49 formas (Guiry and Guiry 2014). Parallel to the incessant description of new taxa, the uncertainty on morphology-based species concepts in *Sargassum* also inflated. Are there really 349 *Sargassum* species or is this high number of species the result of a misinterpretation of morphological plasticity whereby ecological growth forms or different developmental stadia are interpreted as different species?

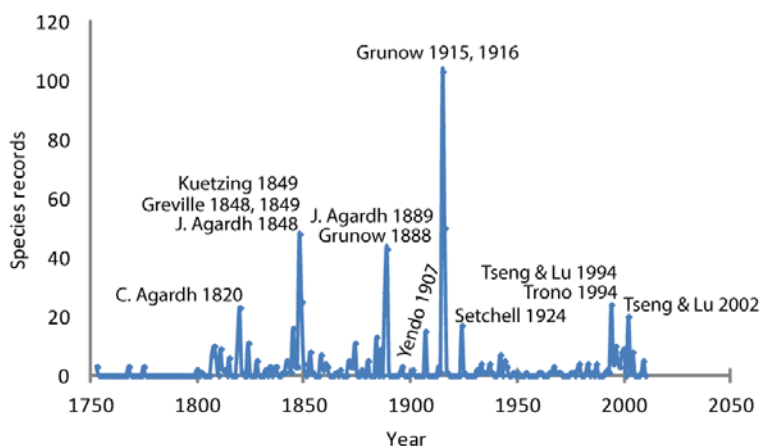


Fig. 3.1. *Sargassum* taxa described over time

Current taxonomic practices in phycology rely greatly on the use of gene sequence data to delimit species and to determine interspecific relationships. Phylogenetic techniques offer a mean to test morphology-based classifications and estimate species diversity (Leliaert et al. 2014). Ho et al. (1995) pioneered the use of molecular data to delimit *Sargassum* species using RAPD-PCR.

Since then, phylogenetic studies based on DNA sequences have been carried out to answer questions ranging from species (Cheang et al. 2008, Mattio et al. 2008, Mattio and Payri 2009, Andrade-Sorcia et al. 2014, Noormohammadi 2013), to subgenus and section (Stiger et al. 2000, Oak et al. 2002, Yoshida et al. 2002b, Stiger et al. 2003, Oak and Lee 2005, 2006, Mattio et al. 2009, Mattio et al. 2010, Cho et al. 2012, Dixon et al. 2012) and generic level diversity (Phillips et al. 2005, Dixon et al. 2012). Alongside, the phylogeography of certain species has been addressed in several studies (Phillips 1995, Uwai et al. 2009, Cheang et al. 2010).

3.1.2. Taxonomy – classification

C. Agardh (1820) described the genus *Sargassum* and placed it alongside four other brown algal genera (*Macrocystis*, *Cystoseira*, *Fucus* and *Furcellaria*) in the family “*Fucoidea*”. *Sargassum* was subdivided into seven unnamed groups. The family “*Fucoidea*” was subsequently elevated to ordinal level and became known as the Fucales Bory de Saint-Vincent (1827). The Fucales included the genera *Turbinaria*, *Cystoseira*, *Moniliformia* and *Himanthalia* next to *Sargassum*.

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A first comprehensive taxonomic system of *Sargassum* was presented by J. Agardh in 1848 in his "Species genera et ordines algarum". The latter divided *Sargassum* into 3 sections (*Pterophycus* J. Agardh, *Arthrophyucus* J. Agardh, *Eusargassum* J. Agardh) and 12 tribus (*Pterocaulon* (Kützing) J. Agardh, *Schizophylla* J. Agardh, *Holophylla* J. Agardh, *Heterophylla* J. Agardh, *Carpophylla* J. Agardh, *Glandularia* J. Agardh, *Siliquosae* J. Agardh, *Biserulae* J. Agardh, *Acanthocarpa* J. Agardh, *Acinaria* J. Agardh, *Ligularia* J. Agardh, *Cymosae* J. Agardh). An update of the original classification was presented in 1889 by J. Agardh. In the latter taxonomic system, the genus *Sargassum* was divided into five subgenera, 3 sections, 10 tribus and many subtribus or informal species group (Fig. 3.2).

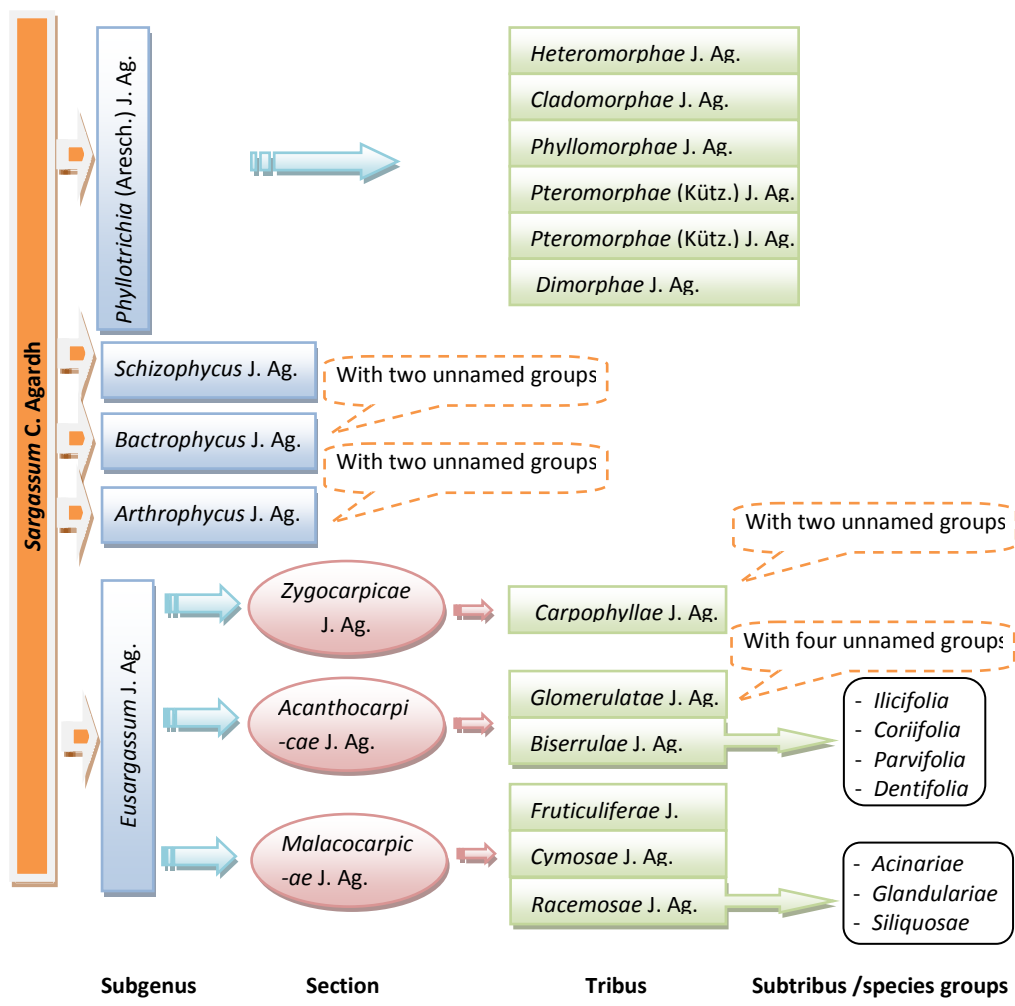


Fig. 3.2. *Sargassum* taxonomic system of J. Agardh 1889

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J. Agardh's (1889) classification became the standard of many subsequent studies focusing on *Sargassum* and was only slightly modified until recently. The most significant changes were proposed by Grunow (1913-1916) and Setchell (1931, 1933).

Molecular phylogenies have altered the traditional classification system considerably. Stiger et al. (2000, 2003) transferred *Phyllocystae* (S. sect.) from S. subg. *Bactrophycus* to S. subg. *Sargassum* and the genus *Hizikia* Okamura became a section within subg. *Bactrophycus*. Yoshida et al. (2004) merged S. subg. *Schizophycus* into S. subg. *Sargassum*. Mattio et al. (2010) adopted the synonymization of S. sect. *Acanthocarpicae* and S. sect. *Malacocarpicae* with S. sect. *Sargassum* and transferred S. ser. *Ilicifoliae* and S. ser. *Binderianae* to S. sect. *Ilicifoliae* and S. sect. *Binderianae*. Dixon et al. (2012) resurrected S. subg. *Phyllotricha* J. Agardh to genus rank *Phyllotricha* Areschoug and S. subg. *Athrophycus* was merged in S. subg. *Bactrophycus* by Dixon et al. (2014). The current and much simplified classification of *Sargassum* as proposed by Mattio & Payri (2011) is shown in Fig. 3.3.

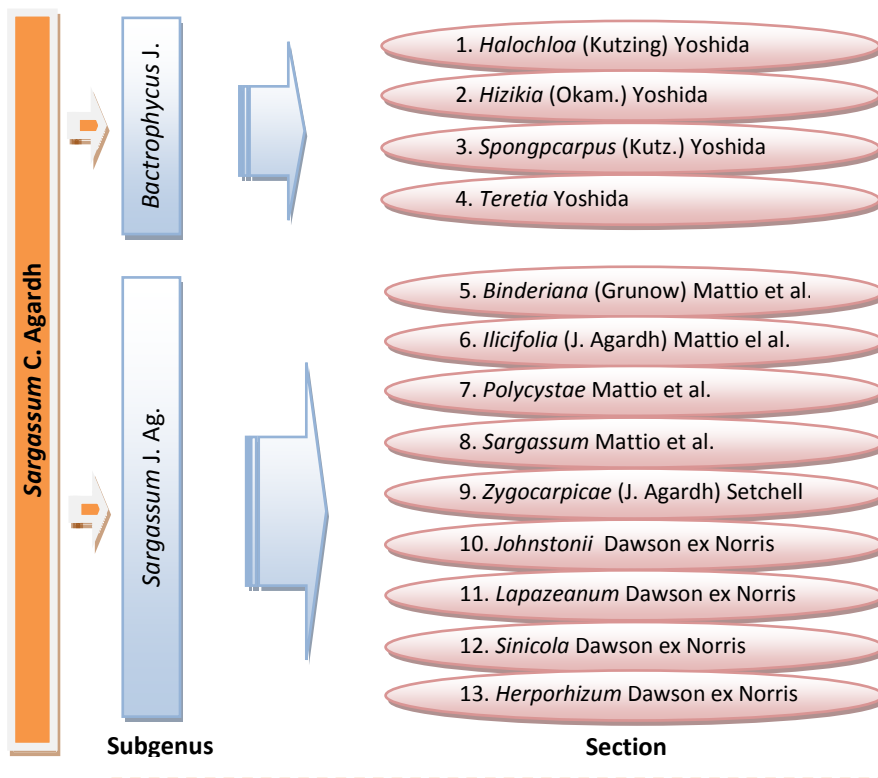


Fig. 3.3. Current *Sargassum* taxonomy system [after Mattio & Payri 2011]

3.1.3. *Sargassum* morphology characteristics

The morphology of the *Sargassum* thallus bears several similarities to vascular plants. The thallus is composed of a holdfast, a primary axis from which secondary axes arise, leaves, vesicles and receptacles (Kilar and Hanisak 1988, Kilar 1992). The shape, dimensions and arrangement of these structures from the basis of the morphology-based classification system.

3.1.3.1 Holdfast

The holdfast plays an important role to anchor the thallus to the substrate. The holdfast normally attaches to rocks, (dead) coral, shells, or any hard substrate. It does however not penetrate the substrate. The shape and size of several typical holdfasts are depicted in Fig. 3.4.

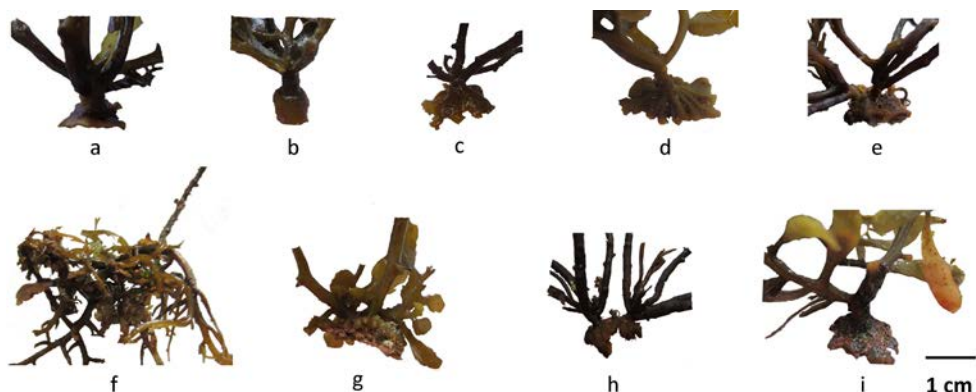


Fig. 3.4. Holdfast morphology of *Sargassum*: discoidal (d, g, h, i), discoidal to conical (a), conical (b, c), discoidal or conical with rhizoids (f), lobed (c, d); the holdfast surfaces smooth (a, b) or warty (c, g); in some species the holdfast of several individuals may coalesce (e, h).

3.1.3.2 Primary axis

The primary axis is perennial and small in most species. From the primary axis deciduous secondary axis arise.

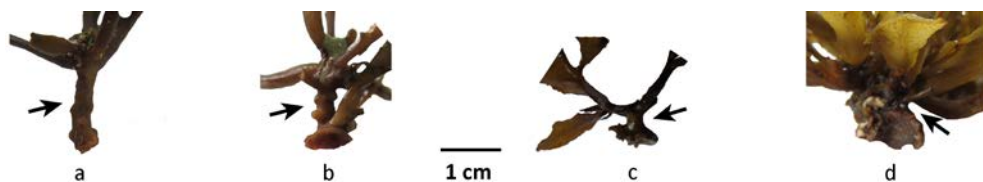


Fig. 3.5. Primary axis morphology of *Sargassum*: cylindrical (a, b), somewhat compressed (c); very short (d) or long (a); the surface smooth (c), warty (a) or with protuberances (b)

3.1.3.3 Secondary axis

Secondary axes are cylindrical or flattened in section, with a smooth or spiny surface. Most commonly secondary axes branch repeatedly. Branches may be distichous or spirally arranged. In some species (e.g. *S. polycystum*) some secondary branches are reflexed and form stoloniferous axes bearing secondary holdfasts.



Fig 3.6. Secondary axes morphology of *Sargassum*: axis may be cylindrical (*S. polycystum*, *S. vietnamense*) or compressed (*S. swartzii*); surfaces smooth (*S. vietnamense*, *S. swartzii*), warty or spiny (*S. polycystum*)

3.1.3.4 Leaf

The shape of the leaves is highly diversified. Leaves can be simple or divided once to several times. The basis of the leaves is rounded or attenuate, symmetrical or not. A pedicel is present or absent, cylindrical or flattened, smooth or bearing spines. The leaf margin may be simple or double at the apex, and smooth to deeply dentate or any intermediate aspect. The midrib may be short and thick or thinner and reaching the apex, or any intermediate length. The apex may be acute, rounded or truncated, simple or showing a cup-like shaped depression. Cryptostomata, of variable number and size, are either randomly distributed over the leaves' surface or aligned on each side of the midrib in one to several rows (Mattio & Payri 2011).



Fig. 3.7. Leaf morphology of *Sargassum*. Leaves simple (a-y, z1) or branched (z); the general shape may be round (f), linear (i, q, v, x), lanceolate (p, u, y), spatulate (j), ovate (e), obovate (a, c, d, g, m), oblong (r, h), or cylindrical (s, t); leaves undulate (i, n, r, y) or strait in lateral view (a, j, l, m, v) or cup-shape (c, d); the apex may be acute (k, s, t, u, w) or obtuse (f, j, m); leaf margin entire (j, r, u, z), wavy (z1), sparse serrate (m), irregular serrate (a, b, f, i, o, p, q, i), finely serrate (g, e, h), profoundly dentate (w, z); midrib percurrent (r, p, z) some part of leaves (h, q, x), unobvious (a, j, k, m, u, z1) or disappear (s, t). Cryptostomata irregularly arranged (b, g, i, o, q, v) or two rows symmetrical with midrib (w), obvious (g, m) or unobvious (r, z, z1).

It should be understood, however, that the morphological shapes described in Fig. 3.7 are not discrete states, but form a morphological continuum where one may intergrade into another. Leaf morphology displays variation at the intra-individual, intraspecific as well as interspecific level.

3.1.3.5 Vesicles

Vesicles or aerocysts are small structures filled with air which help the *Sargassum* to float or remain upright in the water column. Vesicles can be spherical, ovoid or of any intermediate shape, subtended by a pedicel or not, bearing an apical mucron or various spines on the surface not. In some species, the vesicle may develop in the middle of leaf and are then named phyllocyst.

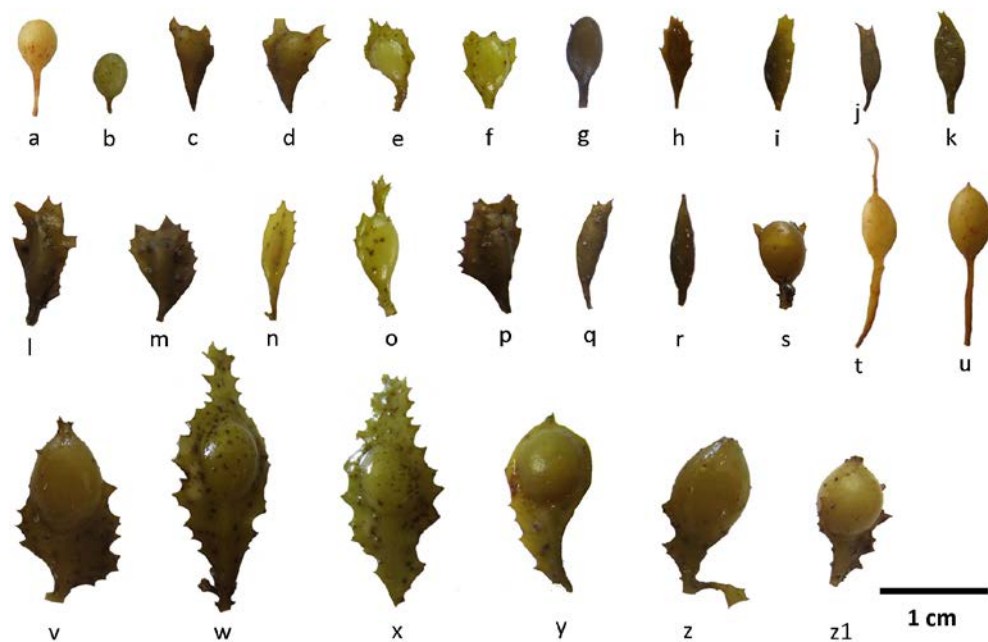


Fig. 3.8. Vesicle morphology of genus *Sargassum*. Vesicle simple (a, b, g, i, j, k, q, r, s, t, u, z1), leaf-like form (m, n, p, v, w, x, y) or ear-like form (c, d, f, l); general shape may be spherical (a, w, x, y) ovate (b, v, u), ovoid (f, s, z), lanceolate (r), linear (n, i, z, k); vesicle may be smooth (a, b) bearing a spine at the apex (t, u, y), ear-like or crown spines (c, d, e, f, h, l, m, n, p, s); petioles cylindrical (a, g, k, u) somewhat compressed (c, d, h, n) or foliose (v, w, x, y).

3.1.3.6 Receptacles

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Receptacles are either solitary or grouped in clusters. Sometimes clusters of receptacles are mixed with small vesicles or leaves, so-called zygoecarptic receptacles. In case of dioecious species, a male/female dimorphism may be observed with male receptacles often more slender and female receptacle shorter and more robust.

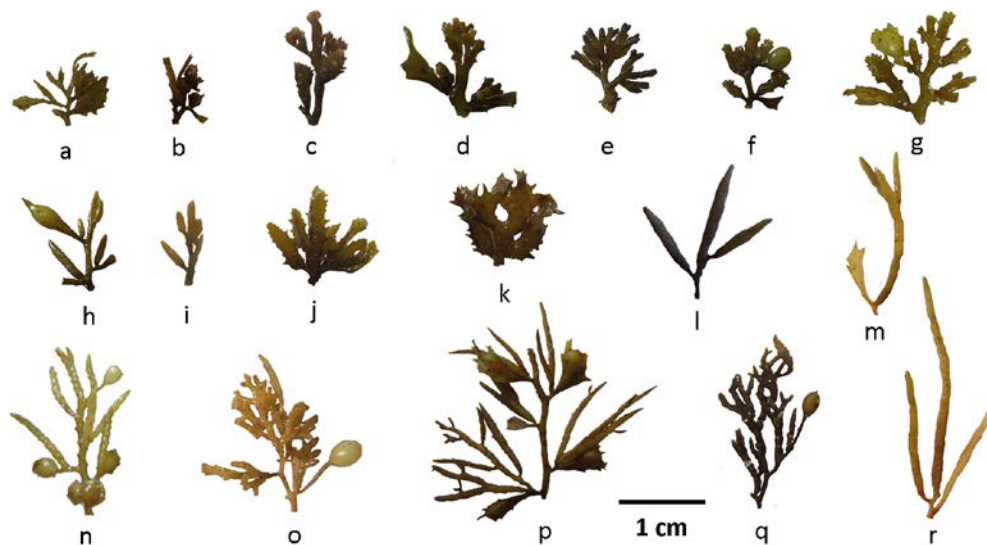


Fig. 3.9. Receptacle morphology in the genus *Sargassum*: cylindrical (h, i, l, m, n, r), compressed (c) or triquetrous (b, f, j, k); vesicle mixed with leaves (a), mixed with vesicle (g, h, n, q) or mixed with leaves and vesicle (o, p); receptacle surface smooth (h, l), warty (n, i, o, q) or bearing spines (a, j, k).

3.1.4 *Sargassum* life cycle

The plant body of *Sargassum* is a diploid sporophyte. In *Sargassum*, the mature plant normally form reproductive structures in late April or May (at least in tropical regions). Gametangia are formed in so-called receptacles. Male and female receptacles are either formed on a single individual (monoecy) or on different individuals (dioecy). Reproduction is oogamous and involves the fusion of a male motile gamete with a female egg cell. Male and female gametes are formed in antheridia and oogonia, respectively (Fig. 3.10). Upon fertilization zygotes initially develop on the female receptacles after which young germlings are shed and grew into new diploid individuals.

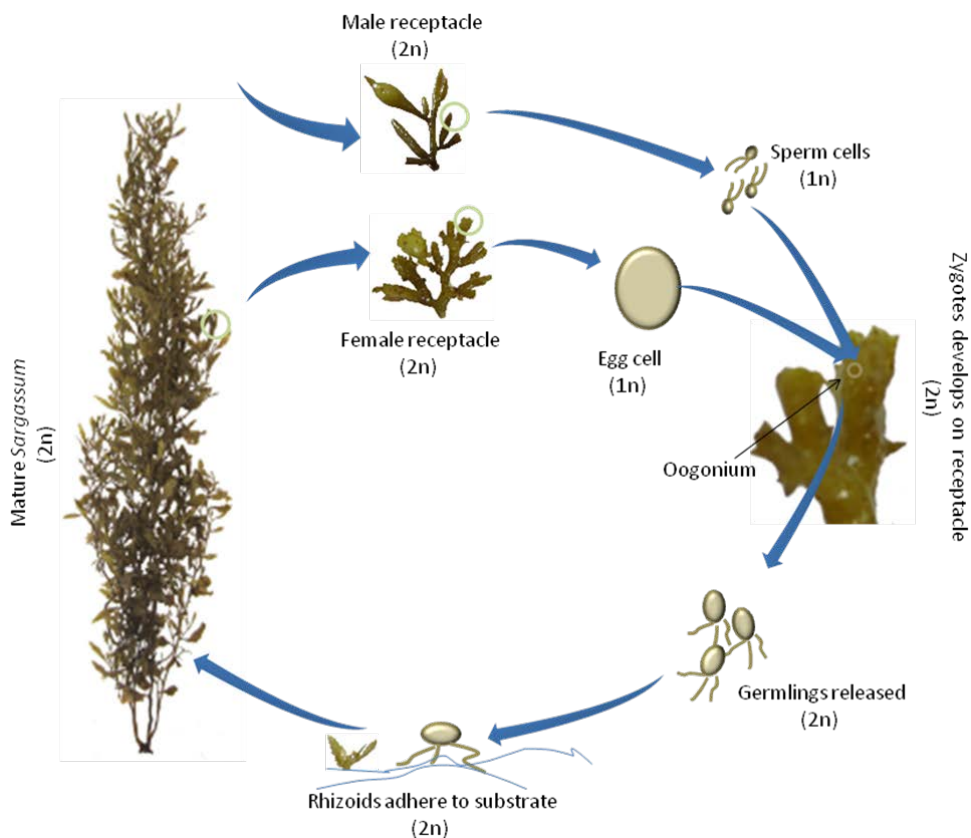


Fig. 3.10. Life cycle of *Sargassum*

3.2. Geography and distributions

Sargassum generally inhabits shallow waters, attached to rock or coral substrate but some Atlantic species are 'planktonic' - never attaching to the seafloor during their lifecycle. Examples are *S. natans* and *S. fluitans* (Dawes and Mathieson 2008). The Sargasso Sea, the area of the North Atlantic Ocean bounded by the Gulf Stream on the west, the North Atlantic Current on the north, the Canary Current on the east, and the North Equatorial Current on the south, is renowned for its high biomass of these rafting *Sargassum* packs.

In the Pacific Ocean *Sargassum* can be found in high abundance on rocky shores and coral reefs down to approximately 15 meter depths (Womersley 1954, Tseng 1985, Tseng et al. 1985, Magruder 1988, Yoshida 1988, Nguyen H.Dai 2007, Mattio et al. 2009). The Pacific Ocean also harbours the highest species diversity, with two-third of the species reported

from that area (Guiry and Guiry 2014). To date however we have little understanding of the distribution of the individual species, although at the subgenus level biogeographical patterns are more obvious, with several subgenera being restricted to temperate regions in the Northern (e.g. subg. *Bactrophyucus*) as well as Southern hemisphere (e.g. subg. *Phyllotrichia* – which was recently elevated to genus-level by Dixon et al. (2012)).

3.3. *Sargassum* in Vietnam

3.3.1 Status of *Sargassum* research in Vietnam

The earliest record of *Sargassum* from Vietnam can be attributed to Loureiro (1790) who in his “*Flora Cochichinensis*” reported the presence of *Fucus natans* Linnaeus which is currently known as *Sargassum natans* (Linnaeus) Gaillon. The expedition of Gaudichaud on the “*La Bonite*” (1836-1837) has contributed four more species of *Sargassum* (*S. armatum* J. Agardh, *S. tortile* J. Agardh (now known as *S. siliquastrum* (Mertens ex Turner) C. Agardh), *S. heterocystum* Montagne (now known as *S. aquifolium* (Turner) C. Agardh) and *S. horneri* J. Agardh. The early stage of *Sargassum* research in Vietnam is also contributed partly to Busseuil (1866), W.B. Hemsley (1874) and Serene (1936).

Modern knowledge on *Sargassum* in Vietnam started with Dawson (1954), who lists eight species of *Sargassum* which he collected in the vicinity of Nha Trang. Four of these were identified to species level (*S. crassifolium* J. Agardh, now known as *S. aquifolium* (Turner) C. Agardh), *S. mcclurei* Setchell, *S. polycystum* C. Agardh, and *S. sandei* Reinbold now known as *S. ilicifolium* (Turner) C. Agardh)). The list of *Sargassum* species recorded for Vietnam grew significantly by contributions of Vietnamese phycologists, most importantly Pham H.H. (1961, 1967, 1969) who reported no less than 36 taxa.

During the 1970s and 1980s research on seaweeds in the North Vietnam was supported by various government initiatives, which resulted in the book “*Seaweed in the North – Viet Nam*” (Nguyen H.Dinh et al. 1993). The latter reports 22 *Sargassum* species (21) and intraspecific taxon (1), but only two species were described *de novo*. In subsequent years collections made under the abovementioned government sponsored seaweed census, however, resulted in the description of several *Sargassum* species: *S. cotoense* Nguyen H.Dai, *S. phamhoangii* Nguyen H.Dai, *S. quinhonense* Nguyen H.Dai.

Research on Vietnamese *Sargassum* flourished again during the 1990's due to the California Sea Grant College Program, a network of mainly Pacific phycologists focused on taxonomy

of economic seaweeds in the region. This forum has given the opportunity for Vietnamese phycologists to study the Vietnamese *Sargassum* collections in collaboration with their colleagues from neighbouring Pacific countries. The efforts of the California Sea Grant College Program resulted in numerous papers describing new taxa (e.g. *S. cornutifractum* Nguyen H.Dinh & Huynh Q.N; *S. carpophyllum* var *honommense* Nguyen H.Dinh & Huynh Q.N.), reporting new species for Vietnam (e.g. *S. henslowianum*), correcting misidentifications and making taxonomic changes (Noro et al. 1995, Ajisaka et al. 1997a, Ajisaka et al. 1997b, Nguyen H.Dinh & Huynh Q.N. 2001). Furthermore, Nguyen H.Dai (1997) published a comprehensive book on Sargassaceae, providing descriptions and an identification key to the species.

More recently, Nguyen H.Dai (2007) recompiled the knowledge on *Sargassum* in his publication “Flora of Vietnam - Fucales”. This book is regarded as the most comprehensive publication on *Sargassum* genus to date. Since most of the documents Vietnamese *Sargassum* are published in Vietnamese and hence little or not accessible to phycologists worldwide, I summarize the current knowledge below.

The earliest Vietnamese *Sargassum* classification has been proposed by Pham H.H. (1967). It included 35 taxa classified into subgenera and sections following the classification of J. Agardh (1889). Although many species were added since, until recently the subgeneric classification of Pham H.H.’s classification still held up. Nguyen H.Dai (1997) classifies *Sargassum* in five subgenera: *Phyllotrichia* J. Agardh, *Schizophycus* J. Agardh, *Bactrophycus* J. Agardh, *Arthrophycus* J. Agardh, *Sargassum* J. Agardh. The subg. *Sargassum* J. Agardh is divided in several sections: *Zygocarpicae* J. Agardh, *Acanthocarpicae* J. Agardh (with two subsection: *Glomerulatae* J. Agardh, *Biserrulae* J. Agardh) and *Malacocarpicae* J. Agardh (with three subsection: *Fructiculiferae* J. Agardh, *Cymosae* J. Agardh, *Racemosae* J. Agardh). However, the presence of *Bactrophycus* and *Schizophycus*, two subgenera with a distinctly temperate distribution in the NW Pacific, in Vietnam is now disputed (Yoshida et al. 2002a).

This classification, however, predates the numerous changes in the classification of the genus *Sargassum* resulting from molecular phylogenetic studies (Stiger et al. 2000, Oak et al. 2002, Yoshida et al. 2002b, Stiger et al. 2003, Oak and Lee 2006, Dixon et al. 2014). Therefore, below we present an update of the classification of Vietnamese *Sargassum* according to the latest insights Table 3.2.

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Table 3.2. New update for *Sargassum* section of Vietnam (Stiger et al. 2000, Oak et al. 2002, Stiger et al. 2003, Mattio et al. 2010, Mattio and Payri 2011)

| Genus <i>Sargassum</i> | | | Nguyen H.Dai 1997 | Nguyen H.Dai 2007 |
|--|--|---|-------------------|-------------------|
| Subgenus | Classification according to Dai (1997; 2007) | Current classification | Species | |
| <i>Phyllotrichia</i> (Aresch.) J. Agardh | | Species belong to subg. <i>Sargassum</i> | 1 | 1 |
| <i>Schizophycus</i> J. Agardh | | Species described are removed from the Vietnamese flora | 2 | 0 |
| <i>Bactrophycus</i> J. Agardh | <i>Phyllocystae</i> Tseng | <i>Illicifolia</i> (J. Agardh) Mattio et al. 2010 | 7 | 4 |
| | <i>Teretia</i> Yoshida | <i>Teretia</i> Yoshida | | |
| <i>Arthrophycus</i> J. Agardh | | Species described belong to subg. <i>Sargassum</i> | 3 | 0 |
| <i>Sargassum</i> J. Agardh | <i>Acanthocarpicae</i> (J. Agardh) Tseng et Lu | <i>Binderiana</i> (Grunow) Mattio et al.2010 | 22 | 17 |
| | | <i>Sargassum</i> Mattio et al. 2010 | | |
| | <i>Malacocarpicae</i> (J. Agardh) Tseng et Lu | <i>Sargassum</i> Mattio et al. 2010 | 11 | 12 |
| | <i>Zygocarpicae</i> (J. Agardh) Setchell | <i>Zygocarpicae</i> (J. Agardh) Setchell | 16 | 22 |
| <i>Polycystae</i> Mattio et al. 2010 | | | | |

Pham H.H. (1967, 1969) reported 36 species of *Sargassum* from Vietnam, of which 26 species are currently accepted taxonomically, and 10 species are synonym of 5 others species. Of 22 *Sargassum* species described for the north of Vietnam only 1 species need to update species name (Nguyen H.Dinh et al. 1993). Nguyen H.Dai (1997) added more species to the Vietnamese flora, 60 taxa were reported but only 43 of these are currently accepted taxonomically (4 were removed in later publications due to reassessment by Yoshida (2002a), 13 are considered synonyms). Even through the “Flora of Vietnam” is the most recent publication for *Sargassum* of Vietnam (Nguyen H.Dai 2007) there is still uncertainty on the status of 10 out of 56 taxa.

70 *Sargassum* taxa have been accepted for the Vietnamese flora and several have originally been described from Vietnam: *Sargassum bangmeiana* Nguyen H.Dinh & Huynh Q.N., *S. baorenia* Nguyen H.Dinh & Huynh Q.N., *S. buui* Nguyen H.Dinh & Huynh Q.N., *S. congkinhii* Pham H.H., *S. cornutifrutum* Nguyen H.Dinh & Huynh Q.N., *S. cotoense* Nguyen H.Dai, *S. feldmannii* Pham H.H., *S. hieuii* Nguyen H.Dinh & Huynh Q.N., *S. namoense* Nguyen H.Dai, *S. quinhonense* Nguyen H.Dai, *S. serratum* Nguyen H.Dai., *S. tsengii* Nguyen H.Dinh & Huynh Q.N., *S. vietnamense* A.D.Zinova & Nguyen H.Dinh.

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A comprehensive list of *Sargassum* species from Vietnam is presented in Table 3.3. The taxa are listed under their currently accepted name.

Table 3.3. List of *Sargassum* taxa currently accepted taxonomically for the Vietnamese flora

| No | Taxon | No | Taxon |
|----|---|----|--|
| 1 | <i>Sargassum aemulum</i> var. <i>carpophylloides</i> Grunow | 36 | <i>Sargassum herklotsii</i> Setchell |
| 2 | <i>Sargassum aemulum</i> var. <i>jouanii</i> Grunow | 37 | <i>Sargassum hieuii</i> Nguyen H.Dinh & Huynh Q.N. |
| 3 | <i>Sargassum angustifolium</i> C. Agardh | 38 | <i>Sargassum ilicifolium</i> (Turner) C. Agardh (Syn.: <i>S. cristaefolium</i> C. Agardh; <i>S. sandei</i> Reinbold; <i>S. duplicatum</i> Bory; <i>S. berberifolium</i> J.Agardh; <i>S. turbinatifolium</i> C.K.Tseng & Lu B.R.) |
| 4 | <i>Sargassum aquifolium</i> (Turner) C. Agardh (Syn.: <i>S. crassifolium</i> J.Agardh; <i>S. binderi</i> Sonder ex J.Agardh; <i>S. echinocarpum</i> J.Agardh; <i>S. heterocystum</i> Montagne | 39 | <i>Sargassum ilicifolioides</i> C.K.Tseng & Lu B.R. |
| 5 | <i>Sargassum armatum</i> J.Agardh | 40 | <i>Sargassum incanum</i> Grunow |
| 6 | <i>Sargassum assimile</i> Harvey | 41 | <i>Sargassum kuetzingii</i> Setchell |
| 7 | <i>Sargassum baccharia</i> (Mertens) C. Agardh | 42 | <i>Sargassum longifructum</i> C.K.Tseng & Lu B.R. |
| 8 | <i>Sargassum bangmeiana</i> Nguyen H.Dinh & Huynh Q.N. | 43 | <i>Sargassum mcclurei</i> f. <i>duplicatum</i> A.D.Zinova & Nguyen H.Dinh |
| 9 | <i>Sargassum baoreni</i> Nguyen H.Dinh & Huynh Q.N. | 44 | <i>Sargassum mcclurei</i> Setchell |
| 10 | <i>Sargassum bicorne</i> J.Agardh | 45 | <i>Sargassum microcystum</i> J.Agardh |
| 11 | <i>Sargassum brevifolium</i> var. <i>pergracilis</i> Greville | 46 | <i>Sargassum miyabei</i> Yendo (Syn.: <i>S. kjellmanianum</i> Yendo) |
| 12 | <i>Sargassum bulbiferum</i> Yoshida | 47 | <i>Sargassum namoense</i> Nguyen H.Dai |
| 13 | <i>Sargassum buuii</i> Nguyen H.Dinh & Huynh Q.N. | 48 | <i>Sargassum natans</i> (Linnaeus) Gaillon (Syn.: <i>S. bacciferum</i> (Turner) C. Agardh)) |
| 14 | <i>Sargassum capillare</i> Kützing (Syn.: <i>S. gracile</i> Greville) | 49 | <i>Sargassum oligocystum</i> Montagne |
| 15 | <i>Sargassum carpophyllum</i> J.Agardh | 50 | <i>Sargassum paniculatum</i> J.Agardh |
| 16 | <i>Sargassum carpophyllum</i> var. <i>honomense</i> Nguyen H.Dinh & Huynh Q.N. | 51 | <i>Sargassum parvifolium</i> (Turner) C. Agardh |
| 17 | <i>Sargassum carpophyllum</i> var. <i>nhatrangense</i> (Pham H.H.) Ajisaka (Syn.: <i>S. piluliferum</i> var. <i>nhatrangense</i> Pham H.H.) | 52 | <i>Sargassum parvivesiculosum</i> C.K.Tseng & Lu B.R. |
| 18 | <i>Sargassum cinereum</i> J.Agardh | 53 | <i>Sargassum phamhoangii</i> Nguyen H.Dai |
| 19 | <i>Sargassum confusum</i> C. Agardh | 54 | <i>Sargassum phyllocystum</i> C.K.Tseng & Lu B.R. |
| 20 | <i>Sargassum congkinhii</i> Pham H.H. | 55 | <i>Sargassum piluliferum</i> (Turner) C. Agardh |

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|----|--|----|---|
| 21 | <i>Sargassum cornutifractum</i> Nguyen H.Dinh & Huynh Q.N. | 56 | <i>Sargassum piluliferum</i> var. <i>serratifolium</i> (Turner) C. Agardh |
| 22 | <i>Sargassum cotoense</i> Nguyen H.Dai | 57 | <i>Sargassum polycystum</i> C. Agardh (Syn.: <i>S. polycystum</i> var. <i>onustum</i>) |
| 23 | <i>Sargassum cymosum</i> C. Agardh | 58 | <i>Sargassum polyporum</i> Montagne |
| 24 | <i>Sargassum denticarpum</i> Ajisaka | 59 | <i>Sargassum quinhonense</i> Nguyen H.Dai |
| 25 | <i>Sargassum distichum</i> Sonder (Syn.: <i>S. aemulum</i> Sonder) | 60 | <i>Sargassum segii</i> Yoshida (Syn.: <i>S. racemosum</i> Yamada & Segi) |
| 26 | <i>Sargassum emarginatum</i> Tseng & Lu | 61 | <i>Sargassum serratum</i> Nguyen H.Dai |
| 27 | <i>Sargassum feldmannii</i> Pham H.H. | 62 | <i>Sargassum siliquosum</i> J. Agardh |
| 28 | <i>Sargassum flavicans</i> (Mertens) C. Agardh | 63 | <i>Sargassum subtilissimum</i> Tseng & Lu |
| 29 | <i>Sargassum glaucescens</i> J. Agardh | 64 | <i>Sargassum swartzii</i> C. Agardh |
| 30 | <i>Sargassum gracillimum</i> Reinbold | 65 | <i>Sargassum tenerrimum</i> J. Agardh |
| 31 | <i>Sargassum graminifolium</i> C. Agardh | 66 | <i>Sargassum tsengii</i> Nguyen H.Dinh & Huynh Q.N. |
| 32 | <i>Sargassum hemiphyllum</i> (Turner) C. Agardh | 67 | <i>Sargassum turbinarioides</i> Grunow |
| 33 | <i>Sargassum hemiphyllum</i> var. <i>chinense</i> J. Agardh | 68 | <i>Sargassum vachellianum</i> Greville |
| 34 | <i>Sargassum henslowianum</i> C. Agardh | 69 | <i>Sargassum vietnamense</i> A.D.Zinova & Nguyen H.Dinh |
| 35 | <i>Sargassum henslowianum</i> var. <i>bellonae</i> Grunow | 70 | <i>Sargassum virgatum</i> C. Agardh |

Because of its importance for the study of the genus *Sargassum* at a regional scale we provide a translation of the identification key provided by Nguyen H.Dai in the “Fucales - Flora of Vietnam” (Nguyen H.Dai 2007). One should bear in mind, however that the key is not comprehensive, i.e. a number of species reported from the area are not included. For convenience we have adapted names of the taxa. Up-to-date names can be found in Table 3.3.

Key to Species of genus *Sargassum*

| | |
|---|--------------------|
| 1a. Main axis without spines | 2 |
| 1b. Main axis with spines..... | 45 |
| 2a. Main axis compressed | 3 |
| 2b. Main axis cylindrical | 9 |
| 3a. Leaf margin entire, without serration | 4 |
| 3b. Leaf margin serrate..... | 6 |
| 4a. Vesicles few, leaf margin entire..... | <i>S. swartzii</i> |
| 4b. Vesicles numerous, apical leaves with some small serrations | 5 |

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|---|------------------------------------|
| 5a. Leaf not ramified, leaf apex obtuse, receptacles forming a dense cluster with edges and spines..... | <i>S. oligocystum</i> |
| 5b. Leaf at base ramified, receptacles cylindrical and without spines | <i>S. vachellianum</i> |
| 6a. Leaves ovate | 7 |
| 6b. Leaves lanceolate, elongate and large | 8 |
| 7a. Leaves 1-2 cm long, leaf margin with sharp serrations, dense and intermixed or duplicate..... | <i>S. ilicifolium</i> |
| 7b. Leaves more than 2 cm long, leaf margin with sharp serrations, scattered and vertically oriented | <i>S. aquifolium</i> |
| 8a. Vesicle in the middle of the leaf, receptacles flattened, less than 1 cm long and forming dense clusters..... | <i>S. feldmannii</i> |
| 8b. Vesicle not in middle of the leaf, receptacles cylindrical or flattened, more than 1 cm long | <i>S. congkinhii</i> |
| 9a. Leaf margin entire without serrations..... | 10 |
| 9b. Leaf margin with sharp serrations..... | 21 |
| 10a. Leaves more than 2cm long..... | 11 |
| 10b. Leaves less than 2 cm long | 16 |
| 11a. Leaves very narrow..... | 12 |
| 11b. Leaves large, more than 0.5 cm wide..... | 13 |
| 12a. Leaves thin, narrow, 0.1-0.2 cm wide..... | <i>S. kuetzingii</i> |
| 12b. Leaves cylindrical, 0.1 cm wide, resembling pine-tree needles | <i>S. cotoense</i> |
| 13a. Leaves 4-20 cm long, without vesicles..... | 14 |
| 13b. Leaves always with a vesicle in the distal part | <i>S. quinhonense</i> |
| 14a. Leaves 10 – 20 cm long, receptacles solitary or forming sparse clusters . | <i>S. phamhoangii</i> |
| 14b. Leaves 4-10 cm long | 15 |
| 15a. Receptacles dense, forming cymes-shaped clusters | <i>S. cymosum</i> |
| 15b. Receptacles spare..... | <i>S. namoense</i> |
| 16a. Leaves ovate | 17 |
| 16b. Leaves hemiphyllous | <i>S. hemiphyllum var chinense</i> |
| 17a. Leaves without midrib | 18 |

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|--|---|
| 17b. Leaves with a conspicuous midrib, leaves near the primary axis with entire margins or some blunt serrations, apical leaves without serrations | 19 |
| 18a. Leaves with acute apices | <i>S. virgatum</i> |
| 18b. Leaves obtuse or with rounded apices, pedicel slender | <i>S. gracillimum</i> |
| 19a. Receptacles repeatedly branched, male receptacles more than 2 cm long | <i>S. longifructum</i> |
| 19b. Receptacles less branched, male receptacles less than 1cm long..... | 20 |
| 20a. Female receptacles short, with big and acute spines | <i>S. cinereum</i> |
| 20b. Female receptacles with small, short and blunt spines | <i>S. incanum</i> |
| 21a. Vesicles always with lateral wings or spines..... | 22 |
| 21b. Vesicles without wings | 26 |
| 22a. Vesicles with large wings, resembling a leaf..... | 23 |
| 22b. Vesicles with small wings or spines | 25 |
| 23a. Leaves tough, dense and thick, more than 1 cm long | <i>S. mcclurei</i> |
| 23b. Leaves supple and thin, up to 1 cm long | 24 |
| 24a. Leaves thin, with small serrations | <i>S. bicorne</i> |
| 24b. Leaves thick, with big serrations | <i>S. serratum</i> |
| 25a. Vesicles with a long tip, leaves narrow 0.1-0.2 cm wide | <i>S. herklotsii</i> |
| 25b. Vesicles with spines or small lateral wings, leaves more than 0.4 cm wide, receptacles flattened or cylindrical with spines | <i>S. vietnamense</i> |
| 26a. Vesicles spherical | 27 |
| 26b. Vesicles ovate | 39 |
| 27a. Apices of the leaves commonly with cup-shaped depression | <i>S. turbinatifolium</i> |
| 27b. Apices of the leaves without cup-shaped depression | 28 |
| 28a. Leaves ramified | 29 |
| 28b. Leaves not ramified | 30 |
| 29a. Leaves only ramified at the base of the male plant..... | <i>S. carpophyllum</i> var. <i>nhtrangense</i> |
| 29b. Leaves generally plant, plants dioecious | <i>S. piluliferum</i> var. <i>serratifolium</i> |
| 30a. Receptacles forming dense clusters with vesicles and leaves | 31 |
| 30b. Receptacles forming cluster without vesicles or leaves | 34 |

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|--|--|
| 31a. Receptacles irregular ramified | <i>S. carpophyllum</i> |
| 31b. Receptacles sparsely branched | 32 |
| 32a. Leaves narrow, 0.2-0.3 cm wide | <i>S. angustifolium</i> |
| 32b. Leaves more than 0.3-1 cm wide..... | 33 |
| 33a. Main axis forming short and thick bulbous structures | <i>S. bulbiferum</i> |
| 33b. Main axis lacking short and thick bulbous structures..... | <i>S. flavicans</i> |
| 34a. Leaves narrow, with acute apices..... | 35 |
| 34b. Leaves wide, with rounded apices..... | <i>S. glaucescens</i> |
| 35a. Receptacles cylindrical, solitary | <i>S. bacularia</i> |
| 35b. Receptacles triquetrous | 36 |
| 36a. Leaves thin, small serrations | <i>S. tenerrimum</i> |
| 36b. Leaves with large serrations | 37 |
| 37a. Leaves more than 0.5 cm wide and 8 cm long | <i>S. aemulum var. carpophylloides</i> |
| 37b. Leaves 1-3 cm wide | 38 |
| 38a. Leaves very narrow, about 0.1 cm wide | <i>S. aemulum var jouanii</i> |
| 38b. Leaves 0.2-0.3 cm wide | <i>distichum</i> |
| 39a. Leaves thick, margin with double serration, receptacles solitary..... | <i>S. assimile</i> |
| 39b. Leaves thick, margin with double serration, receptacles forming dense clusters..... | 40 |
| 40a. Receptacles flattened or triquetrous with numerous spine | <i>S. microcystum</i> |
| 40b. Receptacles cylindrical without spines | 41 |
| 41a. Leaves ovate, up to 1 cm wide | <i>S. siliquosum</i> |
| 41b. Leaves lanceolate | 42 |
| 42a. Leaves stem large, leaves surface covered an ask-like layer | <i>S. graminifolium</i> |
| 42b. Leaves stem slender, without ask-like layer | 43 |
| 43a. Receptacles forming dense clusters, repeatedly branched | <i>S. paniculatum</i> |
| 43b. Receptacles slender, ramified from a single axis | 44 |
| 44a. Leaves more than 0.5 cm wide | <i>S. henslowianum</i> |
| 44b. Leaves narrow, 2-3 cm wide | <i>S. henslowianum var bellonae</i> |
| 45a. Leaves more than 1 cm long..... | 46 |

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- 45b. Leaves narrow, less than 1cm long *S. parvifolium*
- 46a. Base well-defined, not creeping 47
- 46b. Base creeping, vesicles spherical *S. polycystum*
- 47a. Leaf margin undulate, without serrations *S. polyporum*
- 47b. Leaf margin serrate *S. capillare*

The following species, reported from Vietnam, are not included in the key provided by Dai (2007):

- ✓ *Sargassum armatum* J. Agardh, *S. brevifolium* var. *pergracilis* Greville, *S. confusum* C. Agardh, *S. hemiphyllum* (Turner) C. Agardh, *S. natans* (Linnaeus) Gaillon, *S. miyabei* Yendo, *S. turbinarioides* Grunow reported by Pham H.H. (1969) and *S. mcclurei* f. *duplicatum* A.D.Zinova & Nguyen H.Dinh, *S. piluliferum* (Turner) C. Agardh in Nguyen H.Dinh et al. (1993), are not included because their presence in Vietnam is uncertain.
- ✓ *Sargassum bangmeianae* Nguyen H.Dinh & Huynh Q.N., *S. baorenia* Nguyen H.Dinh & Huynh Q.N., *S. buuii* Nguyen H.Dinh & Huynh Q.N., *S. tsengii* Nguyen H.Dinh & Huynh Q.N., *S. hieuii* Nguyen H.Dinh & Huynh Q.N. in Nguyen H.Dinh (2001) and *S. carpophyllum* var. *honomense* Nguyen H.Dinh & Huynh Q.N., *S. cornutifractum* Nguyen H.Dinh & Huynh Q.N. in Nguyen H.Dinh & Huynh Q.N.(1999) were not included in the key because Nguyen H.Dai doubted the validity of the species.
- ✓ *Sargassum emarginatum* Tseng & Lu, *S. ilicifolioides* Tseng & Lu, *S. phyllocystum* Tseng & Lu, *S. parvivesiculosum* Tseng & Lu, *S. subtilissimum* Tseng & Lu have only been reported by Tseng & Lu from the Paracel Islands. The species have not been reported from the Vietnamese mainland coast (1985, 1988, 2002) . Information on their morphology is very scant.

3.3.2 Distribution of *Sargassum* in Vietnam

In order to gain a better insight in the geographical distribution of the various *Sargassum* species in Vietnam, we analysed the distribution of *Sargassum* species according to their latitudinal range (Fig. 3.11). Approximately one third of the species has a range smaller than 1 degree latitude, and half of the species has a range smaller than 4 degrees latitude. Only 10 species are apparently widely distributed, with ranges exceeding more than 10 degrees latitude (*Sargassum baccharia*, *S. cinereum*, *S. glaucescens*, *S. henslowianum*, *S. herklotsii*, *S. ilicifolium*, *S. mcclurei*, *S. polycistum*, *S. siliquosum*, *S. swartzii*).

The pattern emerging could be interpreted as bimodal, with a large of range-restricted species (ranges <1° latitude) and a modest peak of species with ranges centring around 10° latitude. If this distribution of species ranges is correct, i.e. the distribution is not an artefact caused by incomplete sampling and or identification errors, this would imply that a regional loss of biodiversity may well result in extinction of the several species.

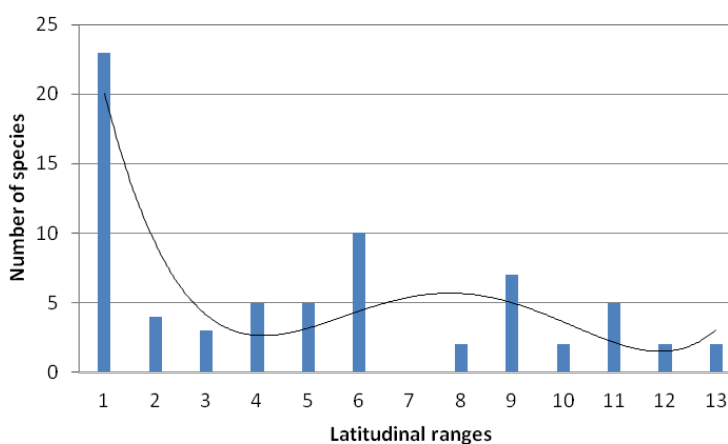


Fig. 3.11. Latitudinal ranges of *Sargassum* species along the Vietnamese coast.

The distribution of *Sargassum* species by province (Fig. 3.12) mimics the distribution of marine macroalgae along the Vietnamese coast (Nguyen V.T. 2013). This pattern confirms ideas by Abbott (2002) that South Central Vietnam is the most diverse area. The Khanh Hoa (37 species) and Da Nang (32 species) provinces have the highest diversity of *Sargassum* species. Followed by Ninh Thuan, Binh Thuan, Quang Ninh with 21-25 species recorded; Binh Dinh has 19 species; Quang Nam, Quang Ngai, Hai Phong have 11-15 species; Quang

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Binh, Quang Tri, Phu Yen, Kien Giang 6-10 species and Thanh Hoa, Nghe An, Ha Tinh less than six species.

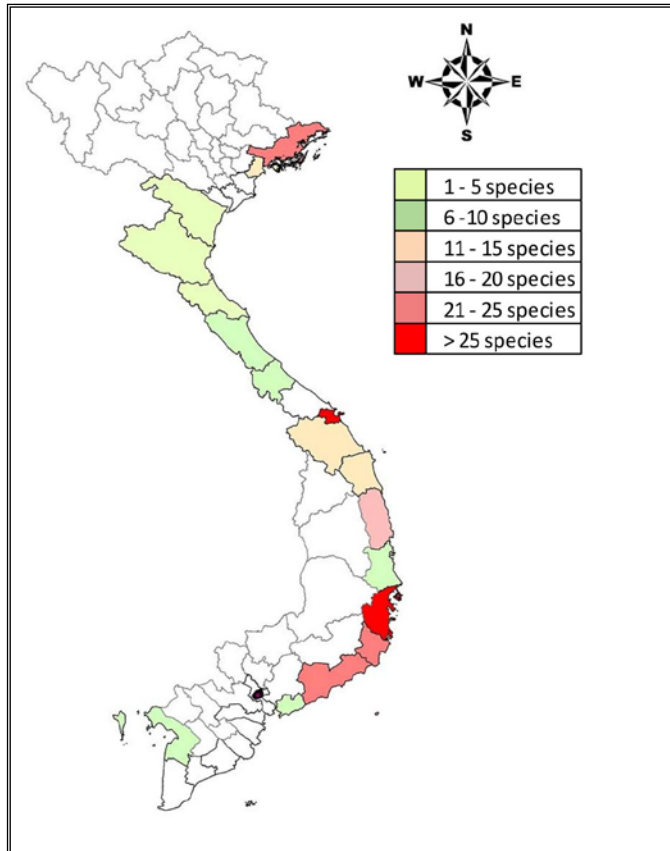


Fig. 3.12. Richness pattern of *Sargassum* in Vietnam

REFERENCE

- Abbott, I. A., Fisher, J. & McDermid, K.J. 2002. Newly reported and revised marine algae from the vicinity of Nha Trang, Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 291-321.
- Agardh, C. A. 1820. Species algarum ... Vol. 1, part 1. Lundae [Lund]. Pp. [I IV +] [1] 168. .
- Agardh, C. A. 1824. Systema Algarum. Lundae [Lund]. XXXVIII Literis Berlingianis [Berling]: 312 pp.
- Agardh, J. G. 1848. Species genera et ordines algarum ... Volumen primum: algas fucoideas complectens. Lundae [Lund]. VIII + 363 pp. .
- Agardh, J. G. 1889. Species Sargassorum Australiae descriptae et dispositae. Kongl. Svenska Vetenskaps Akademiens Handlingar, [ser. 4], 23(3). 133 pp., XXXI pls. .
- Ajisaka, T., Q.N. Huynh and H.D. Nguyen 1995. Studies of two zygotocarpic species of *Sargassum* (subg. *Sargassum*) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 5*, California Sea Grant College Program, La Jolla, CA. pp. 45-53.
- Ajisaka, T., Q.N. Huynh, H.D. Nguyen, L. Baoren, P. O. Ang Jr, P. S. Moi, T. Noro, and T. Yoshida. 1997a. Taxonomic and nomenclature study of *Sargassum duplicatum* Bory and related species. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 6*, California Sea Grant College Program, La Jolla, CA. pp.27-36.
- Ajisaka, T., Q.N. Huynh, H.D. Nguyen, B. R. Lu, C. Y. M., and T. Yoshida. 1997b. *Sargassum hemiphylum* (Turnner) C. Agardh var. *chinensis* J.Agardh from Vietnam, the Chinese mainland, and Taiwan. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 6*, California Sea Grant College Program, La Jolla, CA. pp. 37-49.
- Andrade-Sorcia, G., R.Riosmena-Rodríguez, R.Muñiz-Salazar, J.M.López-Vivas, G.H.Boo, K.M.Lee, and S.M.Boo. 2014. Morphological reassessment and molecular assessment of *Sargassum* (Fucales: Phaeophyceae) species from the Gulf of California, México. *Phytotaxa* **183**: 201-223.
- Børgesen, F. 1941. Some marine algae from Mauritius. II. Phaeophyceae. Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser **16**(3): 1-46.
- Bory de St. Vincent, J. B. 1828. Botanique, Cryptogamie. In: Voyage autour du monde, exécuté par ordre du Roi, sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 1825. (Duperrey, L.I. Eds), pp. 97-200. Paris: Bertrand.
- Cheang, C. C., K. H. Chu, and P. O. Ang. 2008. Morphological and genetic variation in the populations of *Sargassum hemiphylum* (Phaeophyceae) in the North-Western Pacific. *Journal of Phycology* **44**: 855-865.
- Cheang, C. C., K. H. Chu, and P. O. Ang. 2010. Phylogeography of the marine macroalga *Sargassum hemiphylum* (Phaeophyceae, Heterokontophyta) in northwestern Pacific. *Molecular Ecology* **19**: 2933-2948.
- Cho, S. M., S. M. Lee, Y. D. Ko, L. Mattio, and S. M. Boo. 2012. Molecular systematic reassessment of *Sargassum* (Fucales, Phaeophyceae) in Korea using four gene regions. *Botanica Marina* **55**: 473-484.
- Dawes, C. J. and A. C. Mathieson. 2008. The seaweeds of Florida. University Press of Florida. pp. 656.

- Dawson, E. Y. 1954. Marine plants in the Vicinity of the Institute Oceanography of Nha Trang. *Pacific science* **8**: 373-471.
- de Paula, E. J. and E. C. de Oliveira 1982. Wave exposure and ecotypical differentiation in *Sargassum cymosum* (Phaeophyta, Fucales). *Phycologia* **21**: 145-153.
- Dixon, R. R. M., J. M. Huisman, J. Buchanan, C. F. D. Gurgel, and P. Spencer. 2012. A morphological and molecular study of Austral *Sargassum* (Fucales, Phaeophyceae) supports the recognition of *Phyllotricha* at genus level, with further additions to the genus *Sargassopsis*. *Journal of Phycology* **48**: 1119-1129.
- Dixon, R. R. M., L. Mattio, J. M. Huisman, C. E. Payri, J. J. Bolton, and C. F. D. Gurgel. 2014. North meets south - Taxonomic and biogeographic implications of a phylogenetic assessment of *Sargassum* subgenera *Arthrophyucus* and *Bactrophyucus* (Fucales, Phaeophyceae). *Phycologia* **53**:15-22.
- Earle, S. A. 1968. Phaeophyta of the eastern gulf of Mexico. *Phycologia* **7**: 71-254.
- Forsskål, P. 1775. *Flora aegyptiaco arabica Post mortem auctoris edidit Carsten Niebuhr. Havniae [Copenhagen]. 32 + CXXVI + 219 [220] pp., frontispiece [map].*
- Gmelin, S. G. 1768. *Historia fucorum. Petropoli [St. Petersburg]. [xii +] 239 + 6 pp., 35 pls. [IA, IB, IIA, IIB, III XXXIII].*
- Greville, R. K. 1848. *Algae orientales: Descriptions of new species belonging to the genus Sargassum. Annals and Magazine of Natural History* **2**(2): 203–206, 274–277, 431–434.
- Greville, R. K. 1849. *Algae orientales: Descriptions of new species belonging to the genus Sargassum. Annals and Magazine of Natural History* **2**(3): 106–109, 216–219, 254–257, 503–506.
- Grunow, A. 1915. *Additamenta ad cognitionem Sargassorum. Verhandlungen der Kaiserlich Königlichen Zoologisch Botanischen Gesellschaft in Wien* **65**: 329 - 448
- Grunow, A. 1916. *Additamenta ad cognitionem Sargassorum. Verhandlungen der Kaiserlich Königlichen Zoologisch Botanischen Gesellschaft in ibid.* **66**: 1 - 48, 136 - 185.
- Guiry, M. D. and G. M. Guiry. 2014. *AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on May 2014.*
- Ho, C. L., S. M. Phang, and T. Pang. 1995. Application of polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers in the molecular identification of selected *Sargassum* species (Phaeophyta, Fucales). *European Journal of Phycology* **30**: 273-280.
- Kilar, J. A. 1992. Seasonal and between plant variability in the morphology of *Sargassum mathiesonii* Sp-Nov (Phaeophyta) from the gulf of Mexico. *Journal of Phycology* **28**: 114-126.
- Kilar, J. A. and M. D. Hanisak. 1988. Seasonal Patterns of Morphological Variability in *Sargassumpolyceratium* (Phaeophyta). *Journal of Phycology* **24**: 467-473.
- Kilar, J. A., M. D. Hanisak, and T. Yoshida. 1992. On The expression of phenotypic variability: Why is *Sargassum* so Taxonomically difficult? *Taxonomy of Economic Seaweed with reference to some Pacific and Western Atlantic species* **3**:95-118.
- Kützing, F. T. 1843. *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange. Brockhaus, F. A., Leipzig.*
- Kützing, F. T. 1849. *Species algarum. Brockhaus, F. A., Leipzig.*
- Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. Lopez-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA- based species delimitation in algae. *European Journal of Phycology* **49**:179-196.

- Loureiro, J. o. d. 1790. *Flora Cochinchinensis* (Vol. 1). Typis, et Expensis Academicis.
- Magruder, W. H. 1988. *Sargassum* (Phaeophyta, Fucales, Sargassaceae) in the Hawaiian Islands. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 2*, California Sea Grant College Program, La Jolla, CA. pp. 59-63.
- Mattio, L. and C. E. Payri. 2009. Taxonomic revision of *Sargassum* species (Fucales, Phaeophyceae) from New Caledonia based on morphological and molecular analyses. *Journal of Phycology* **45**: 1374-1388.
- Mattio, L. and C. Payri. 2010. Assessment of five markers as potential barcodes for identifying *Sargassum* subgenus *Sargassum* species (Phaeophyceae, Fucales). *Cryptogamie Algologie* **31**: 467-485.
- Mattio, L. and C. E. Payri. 2011. 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *Botanical Review* **77**: 31-70.
- Mattio, L., C. E. Payri, and V. Stiger-Pouvreau. 2008. Taxonomic revision of *Sargassum* (Fucales, Phaeophyceae) from French Polynesia based on morphological and molecular analyses. *Journal of Phycology* **44**: 1541-1555.
- Mattio, L., C. E. Payri, and M. Verlaque. 2009. Taxonomic revision and geographic distribution of the subgenus *Sargassum* (Fucales, Phaeophyceae) in the Western and Central Pacific Islands based on morphological and molecular analyses. *Journal of Phycology* **45**: 1213-1227.
- Mattio, L., C. E. Payri, M. Verlaque, and B. de Reviere. 2010. Taxonomic revision of *Sargassum* sect. *Acanthocarpicae* (Fucales, Phaeophyceae). *Taxon* **59**: 896-904.
- Mertens, H. 1819. Mémoire sur plusieurs espèces de Fucus, nouvelles ou peu connues, observées dans la collection du Muséum. *Mémoires du Muséum d'Histoire Naturelle [Paris]* **5**: 172-190.
- Montagne, C. 1845. Plantes cellulaires. In J.B. Hombron & H. Jacquinot, Voyage au Pôle Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée ... pendant les années 1837 1838 1839 1840, sous le commandement de M.J. Dumont d'Urville. Botanique. Paris. Vol. 1. XIV + 349 pp. Atlas: Botanique: Cryptogamie.
- Nguyen, H.Dai 1997. *Sargassaceae* of Vietnam, resources and utility. Agriculture Publishing House, Hanoi. pp. 199
- Nguyen, H.Dai. 2007. Bộ Rong Mơ (Fucales Kytlin) In: *Thực vật chí Việt Nam (Flora of Vietnam)*. **11**, Science and Technology Publishing House, Hanoi. pp. 117.
- Nguyen, H.Dinh and Huynh Q.N. 1999. Some new taxa of *Sargassum* (Phaeophyta) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 7*, California Sea Grant College Program, La Jolla, CA. pp. 43-51.
- Nguyen, H.Dinh and Huynh Q.N. 2001. Five new species of the genus *Sargassum* from Vietnam. *Vietnamese Journal of Biology* **23**: 1-10.
- Nguyen, H.Dinh, Huynh Q.N.Dinh, Tran N.B. and Nguyen V.T. 1993. *Marine algae of north Vietnam*. Science and Technology Publishing House. pp.344.
- Nguyen, V.Tien 1996. Seaweed of Halong Bay (Rong biển vịnh Hạ Long). *Collection of Marine Research Works* **7**: 184-193.
- Nguyen, V.T., N.H Le, S.M. Lin, F. Steen, and O. De Clerck. 2013. Checklist of the marine macroalgae of Vietnam. *Botanica Marina* **56**: 207-227.
- Noormohammadi, Z. 2013. Bayesian analysis of species relationship in genus *Sargassum*: Oman sea and Persian gulf. *International Journal Plant, Animal and Environmental Science* **3**: 268-278.

- Noro, T., T. Ajisaka, and T. Yoshida. 1995. *Sargassum henslowianum* var. *condensatum* Yamada in Japan: A synonym of *Sargassum yendoii* Okamura et Yamada. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 3-9.
- Oak, J. H. and I. K. Lee. 2005. Taxonomy of the genus *Sargassum* (Fucales, Phaeophyceae) from Korea I. subgenus *Bactrophyucus* section *Teretia*. *Algae* **20**: 77-90.
- Oak, J. H. and I. K. Lee. 2006. Taxonomy of the genus *Sargassum* (Fucales, Phaeophyceae) from Korea II. subgenus *Bactrophyucus* section *Halochloa* and *Repentia*. *Algae* **21**: 393-405.
- Oak, J. H., Y. Suh, and I. K. Lee. 2002. Phylogenetic Relationships of *Sargassum* subgenus *Bactrophyucus* (Sargassaceae, Phaeophyceae) inferred from rDNA ITS Sequences. *Algae* **17**: 235 - 247.
- Pham, H.H. 1967. Contribution à l'étude des algues littorales du Vietnam I: Le genre *Sargassum*. *Ann. Fac. Sci. Saigon*. pp. 259-332.
- Pham, H.H. 1969. Marine algae of south Vietnam (Rong biển miền nam Việt nam). Sai Gon study Center. Ministry of Education and Youth. pp.558.
- Phillips, N. 1995. Biogeography of *Sargassum* (Phaeophyta) in the Pacific Basin. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 107-144.
- Phillips, N. E., C. M. Smith, and C. W. Morden. 2005. Testing systematic concepts of *Sargassum* (Fucales, Phaeophyceae) using portions of the rbcL5 operon. *Phycological Research* **53**: 1-10.
- Setchell, W. A. 1931. Hong Kong seaweeds, II. *Hong Kong Naturalist* **2**:237-253.
- Setchell, W. A. 1933. Hong Kong Seaweeds III: *Sargassaceae*. *Hong Kong Naturalist* **2**:33-49.
- Silva, P. C., P. W. Basson, and R. L. Moe. 1996. Catalogue of the benthic marine algae of the Indian Ocean. University of California Publications in Botany **79**:1-1259.
- Sonder, O. G. 1845. Nova algarum genera et species, quas in itinere ad oras occidentales Novae Hollandiae, collegit L. Preiss, Ph. Dr. *Botanische Zeitung* **3**: 49 57.
- Stiger, V., T. Horiguchi, T. Yoshida, A. W. Coleman, and M. Masuda. 2000. Phylogenetic relationships of *Sargassum* (Sargassaceae, Phaeophyceae) with reference to a taxonomic revision of the section *Phyllocystae* based on ITS-2 nrDNA sequences. *Phycological Research* **48**: 251-260.
- Stiger, V., T. Horiguchi, T. Yoshida, A. W. Coleman, and M. Masuda. 2003. Phylogenetic relationships within the genus *Sargassum* (Fucales, Phaeophyceae), inferred from ITS-2 nrDNA, with an emphasis on the taxonomic subdivision of the genus. *Phycological Research* **51**: 1-10.
- Stiger, V. and C. E. Payri. 1999. Spatial and seasonal variations in the biological characteristics of two invasive brown algae, *Turbinaria ornata* (Turner) J.Agardh and *Sargassum mangarevense* (Grunow) Setchell (Sargassaceae, Fucales) spreading on the reefs of Tahiti (French Polynesia). *Botanica Marina* **42**: 295-306.
- Taylor, W. R. 1945. Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands. *Allan Hancock Pacific Expeditions* 12. iv. pp. 528
- Tseng, C. K. 1985. *Sargassum* sect. *Phyllocystae* sect. nov., A new section of *Sargassum* subgenus *Bactrophyucus*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species* **1**, California Sea Grant College Program, La Jolla, CA. p. 15.

- Tseng, C. K. and B. R. Lu. 1988. Studies on Chinese species of Zygozarpic *Sargassum*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 23-54.
- Tseng, C. K. and B. R. Lu. 2002. Studies on the biserrulic *Sargassum* of China: V. The series *Illicifoliae* (J. Agardh) Tseng et Lu. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 35-76.
- Tseng, C. K., T. Yoshida, and Y. M. Chiang. 1985. East Asiatic species of *Sargassum* subgenus *Bactrophycus* J. Agardh (Sargassaceae, Fucales), with keys to the sections and species. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species 1*, California Sea Grant College Program, La Jolla, CA. pp. 1-14.
- Turner, D. 1808. Fuci sive plantarum fucorum generi a botanicis ascriptarum icones descriptiones et historia. Fuci, or coloured figures and descriptions of the plants referred by botanists to the genus *Fucus*, vol. 1. J. & A. Arch Cornhill, London, England.
- Turner, D. 1809. Fuci sive plantarum fucorum generi a botanicis ascriptarum icones descriptiones et historia. Fuci, or coloured figures and descriptions of the plants referred by botanists to the genus *Fucus*, vol. 2. J. & A. Arch Cornhill, London, England.
- Turner, D. 1811. Fuci sive plantarum fucorum generi a botanicis ascriptarum icones descriptiones et historia. Fuci, or coloured figures and descriptions of the plants referred by botanists to the genus *Fucus*, vol. 3. J. & A. Arch Cornhill, London, England.
- Uwai, S., K. Kogame, G. Yoshida, H. Kawai, and T. Ajisaka. 2009. Geographical genetic structure and phylogeography of the *Sargassum horneri/filicinum* complex in Japan, based on the mitochondrial *cox3* haplotype. *Marine Biology* **156**: 901-911.
- Womersley, H. B. S. 1954. Australian species of *Sargassum*, subgenus *Phyllotrichia*. *Australian Journal of Botany* **2**: 337-354.
- Yamada, Y. 1944. Diagnoses of New *Sargassums* from Japan. *Hokkaido Imperial University*, **3**: 1-10.
- Yoshida, T. 1988. Japanese and Taiwanese species of *Sargassum* subgenus *Sargassum*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 5-21.
- Yoshida, T., H.D. Nguyen, T. Ajisaka, and T. Noro. 2002a. Verification of *Sargassum* species identified with subgenera *Bactrophycus*, *Phyllotrichia* and *Schizophycus* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 95-102.
- Yoshida, T., T. Noro, and T. Horigushi. 2004. Species of the genus *Sargassum* subgenus *Schizophycus*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 9*, California Sea Grant College Program, La Jolla, CA. pp. 93-106.
- Yoshida, T., V. Stiger, T. Ajisaka, and T. Noro. 2002b. A molecular study of section-level classification of *Sargassum* subgenus *Bactrophycus* (Sargassaceae, Phaeophyta). In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 89-94.

Chapter 4

Sargassum diversity in Vietnam: a
molecular phylogenetic approach

4.1. Introduction

The brown algal genus *Sargassum* is one of the most widely distributed and locally abundant genera of brown algae. The genus is especially prominent in tropical regions (Yoshida 1989). For example, Silva et al. (1996) list 239 species and intraspecific taxa of *Sargassum* for the Indian Ocean. This represents 40% of the brown algal diversity of that region. Also in the Pacific Ocean *Sargassum* often dominates tropical ecosystems. In the Philippines 73 species out of 154 brown algae belong to *Sargassum* (Ganzon-Fortes 2012). The genus, however, is also widespread and diverse in some temperate regions (Segawa 1977).

In Vietnam, *Sargassum* is very common on rocky shores, often dominating reefs, shallow coastal lagoons and offshore islands. Generally, specimens grow and reproduce from March to July. In some areas, however, the growth season is either earlier or later in the year, depending on local climate and ecological conditions (Nguyen H.Dai 1997, Huynh Q.N. & Nguyen H.Dinh 1998). Although present along the entire Vietnamese coastline, *Sargassum* is most common along the central coast and offshore islands. This region also appears to be the most diverse, with ca. 75% of the species reported. *Sargassum* is also an important cash crop in central Vietnam with natural populations being harvested for alginates extraction, herbal medicine and human consumption (Le N.H 2009, Nguyen et al. 2013).

The taxonomy of the genus has been addressed by many phycologists in Vietnam (Pham H.H. 1967, 1969, Nguyen H.Dai 1980, 1997, 2007, Nguyen H. Dinh et al. 1993, Pham H.Tri 1996, 1999, Ajisaka et al. 1997b, Nguyen H.Dinh and Huynh Q.N 2001). Pham H.H. (1967) and Nguyen H.Dai (2007) proposed a detailed, but presently outdated, subgeneric classification. This classification was primarily based on morphological characteristics of the holdfast, branches, leaves, vesicle and receptacles. Nevertheless, great uncertainty remains on the species-level diversity, taxonomy and relationships of the various species (Ajisaka et al. 1997c, Yoshida et al. 2002, Nguyen et al. 2013).

The use of molecular markers forms a possible solution to clarify the taxonomy of the genus. Sequence data have been widely applied in recent years addressing *Sargassum* diversity and taxonomy in the South Pacific (Mattio et al. 2008a, 2008b, Mattio and Payri 2009), the Indian Ocean (Mattio and Bolton 2011, Mattio et al. 2013), Persian gulf (Shams and Clerck 2013), Mexico (Andrade-Sorcía et al. 2014), Korea (Oak et al. 2002, Cho et al.

2012) and Japan (Stiger et al. 2000, Shimabukuro et al. 2012). To date no molecular markers have been applied to the Vietnamese *Sargassum* species.

In this study we examine the diversity and phylogenetic relationships of Vietnamese *Sargassum* based on the Internal Transcribed Spacer 2 of nuclear encoded ribosomal cistron (ITS 2 rDNA). Thereto, we sampled along coast of Vietnam from South to North, including several offshore islands.

4.2. Materials and Methods

4.2.1. Field survey and sample collection

The study area covers nearly the entire Vietnamese coastline from Quang Ninh province near the Chinese border to Phu Quoc Island in the Gulf of Thailand near the border with Cambodia. In addition several islands in the East Sea (Con Dao, Phu Quy and Ly Son) were sampled. Sampling was carried out from March to June in 2011, 2012 and 2013, which coincides with spring and early summer in Vietnam. During this period of the year it is possible to collect mature *Sargassum* (Nguyen H.Dai 1997, Huynh Q.N. and Nguyen H.Dinh 1998, Le N.H et al. 2009). Approximately 500 samples were collected during these fieldtrips. Samples were collected using scuba diving, snorkeling or walking at low tide in intertidal regions. Field collected algae were photographed and subsequently pressed as herbarium specimens (deposited in the Herbarium of the Institute of Tropical Biology, Ho Chi Minh City). Pictures of the thallus include the habit of the plant, details of the holdfast, axes, leaves, vesicles and receptacles, which are the main characteristics to identify *Sargassum* species. Fragments of the thallus were desiccated in silica gel (particle size 0.1 – 1 mm and mixed 10% blue silica gel 2 – 5 mm) for DNA extraction and preserved in a 5% formaldehyde-seawater solution for anatomical observations. Morphological identification are based on authoritative references, namely Yoshida (1983) and Nguyen H.Dai (2007) which provide the most comprehensive and up-to-date treatments of *Sargassum* in Japan and Vietnam, respectively. Our morphological identifications were confirmed by Dr. Nguyen H.Dai.

4.2.2. Morphological analysis

Immediately following collection, morphological structures of fresh specimens were photographed using Canon Ixus 220 digital camera. Measurements were made using ImageJ image analysis software (Schneider et al. 2012). Plates illustrating the morphology of the

species were made using Adobe Photoshop CS6. Synonyms for each species are those cited in AlgaeBase (Guiry and Guiry 2014).

4.2.3. DNA extraction, PCR amplification and Sequencing

DNA was extracted using a modified CTAB-method (Box 4.1). A fragment of silica gel-dried *Sargassum* tissue was ground to powder in liquid nitrogen using a micropestle; after adding 500 µl of preheated CTAB isolation buffer the sample was incubated at 60°C for 40 minutes, inverted every 5-10 minutes; after a centrifugation step (13.200 rpm, 10 minutes) the aqueous phase was transferred to a clean tube and an equal volume of 25:24:1 Phenol:Chloroform:Isoamyl alcohol was added and mixed to emulsify it; after a centrifugation step (13.200 rpm, 10 minutes) the aqueous phase was transferred to a clean tube and the cleaning step repeated with 24:1 Chloroform:Isoamyl alcohol; the DNA purified using a Wizard DNA Cleanup System (Promega – USA) and stored at -20°C.

The ITS2 region of the nuclear encoded ribosomal cistron was amplified using forward primer 5.8S-BF (5'-CGATGAAGAACGCGAGAAATGCGAT-3') and reverse primer 25BR-2 (5'-TCCTCCGCTTAGTATATGCTTAA-3'), originally designed by Yoshida et al. (2000) PCR amplifications were performed with an initial denaturation at 94°C for 3 min followed by 35 cycles as follow involving a denaturation step at 94°C for 1 min, annealing at 46°C for 1 min, elongation at 72°C for 2 min, and a final elongation step at 72°C for 10 min. All PCR products were purified and sequenced by Beckman-Coulter Genomics (France).

Box 4.1: Modified CTAB extraction method used for DNA extraction Preparation.

- Heat the bath to 60°C
- Make a mixture of 500 µl CTAB isolation buffer and 5 µl of Proteinase-K (20mg/ml) for each sample and heat in the bath
- Set up 1,5 ml tubes with tissue and grind after putting in liquid nitrogen
- Add 500 µl of the preheated CTAB isolation buffer to each tube or add buffer directly to the tissue and grind
- Incubate at 60°C for 30 - 40 minutes, inverse every 5 – 10 minutes
- Spin at full speed for 10 minutes
- Transfer aqueous (upper) layer to a clean tube and add an equal volume of 25:24:1 Phenol:chloroform:isoamyl alcohol (don't take liquid from the upper layer) and mix (don't vortex) to emulsify
- Spin at full speed for 10 minutes
- Transfer aqueous (upper) layer to a clean tube and add an equal volume of 24:1 Chloroform:isoamyl alcohol and mix to emulsify
- Spin for 5 minutes (12000G)

4.2.4. Phylogenetic analysis

ITS2 sequences were aligned using Muscle in Mega 6 (Tamura et al. 2013). 126 sequences of this studies and 38 sequences from Genbank, representing all subgenera and sections (Stiger et al. 2000, Oak and Lee 2005, Mattio et al. 2008b, 2009a, 2010, Mattio and Payri 2009, Cheang et al. 2010, Cho et al. 2012, Andrade-Sorcía et al. 2014) were analyzed. The alignment contained no ambiguous regions. *Sargassopsis decurrens* and *Turbinaria ornata* were used as outgroup (Mattio et al., 2008). Phylogenies were inferred with Bayesian analysis in MrBayes3.2 (Ronquist et al. 2012). The analysis was initiated with a random starting tree and ran four chains of MCMC iterations simultaneously for 10 million generations, saving every 100th tree. The first 25% of trees sampled were discarded as burn-in, based on the stationarity of lnL as assessed using Tracer version 1.4 (Rambaut and Drummond 2007). A consensus topology and posterior probability values were calculated from the remaining trees. Maximum likelihood (ML) analyses were conducted in RAxML (Stamatakis 2014) and neighbor joining (NJ) in MEGA6 (Tamura et al. 2013). We used a GTR+CAT model for the RAxML analyses and a GTR+I+G+ model for the NJ analysis. The latter was selected using a AICc criterion in MEGA6. Node support was assessed by nonparametric bootstrapping (Felsenstein 1985) using 1000 replications. Nodes were considered as weak below 70%, moderately supported between 71% and 84%, and strongly supported above 85%.

4.3. Results

4.3.1. Morphology description

Our morphological studies identified 26 morphospecies and 2 specimens which could only tentatively be assigned to existing species. These morphospecies are described and illustrated below.

***Sargassum angustifolium* C. Agardh (1820: 32)**

Type locality: East Indies

Type specimen: LD?

Classification: Subgenus *Sargassum*, section *Illicifolia*

Basionym: *Fucus angustifolius* Turner

Synonym: *S. flexile* Greville

Sargassum diversity in Vietnam: a molecular phylogenetic approach

References for Vietnam: Nguyen H.Dinh et al. (1997: 175, Fig. 123); Nguyen H.Dai (1997: 91, Fig. 33); Nguyen H.Dai (2007: 44, Fig. 22)

Other references: C. Agardh (1820: 32); J. Agardh (1889: 81); Silva et al. (1996: 657)

Geographical distribution: Pacific Ocean, Indian Ocean

Habitat: on rocks in the lower intertidal and shallow subtidal

Representative material: NVT1304

Morphology: Thallus bushy, 40 to 70 cm high; holdfast conical and lobed, up to 1.2 cm in diameter; main axis very short, producing 2 – 3 secondary axes; secondary axes cylindrical, smooth, slender, up to 0.2 cm in diameter; lateral branch 10 to 20 cm long; leaves narrow, linear to lanceolate, leaves up to 2.3 cm long and 0.3 cm wide, margins entire to slightly irregularly serrate, petioles short, midribs conspicuous; cryptostomata small and scattered on both sides of the midribs; vesicles spherical or slightly oval, 1 to 2 cm long, petioles cylindrical and smooth; receptacles cylindrical, simple or branched, bearing few spines, probably mixed with vesicles, up to 1.5 cm long.

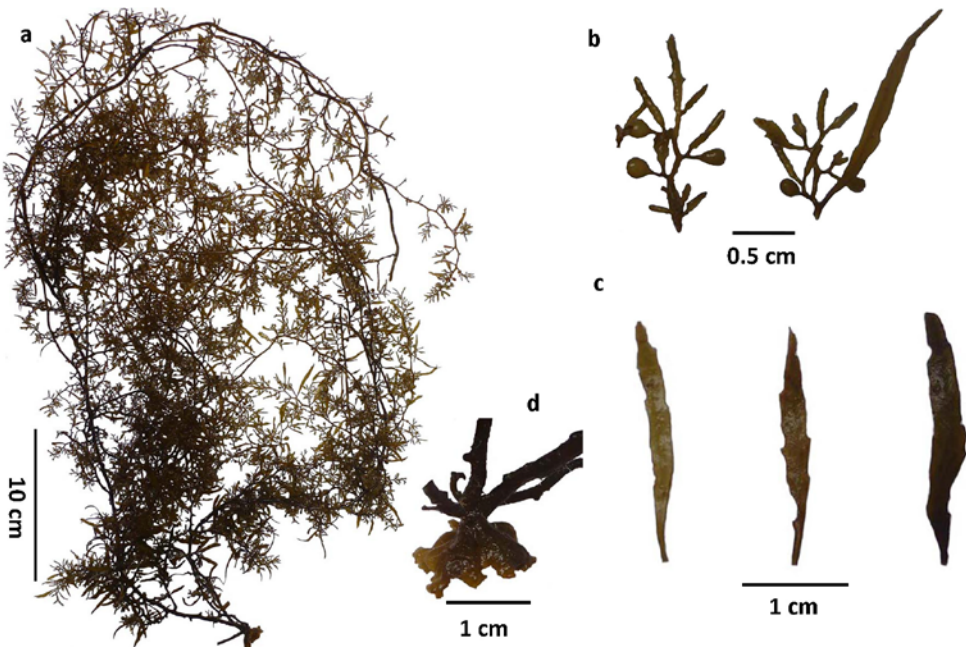


Fig. 4.9. *Sargassum angustifolium* C. Agardh: thallus (a), receptacle and vesicles (b), leaves (c), holdfast (d). Scale bars: detailed in the figure.

***Sargassum aquifolium* (Turner) C. Agardh (1820: 12–13)**

Type locality: Strait of Sunda, Indonesia.

Lectotype: BM563434.

Classification: Subgenus *Sargassum*, section *Binderiana*

Synonym: *S. anapense* Setchell & N.L.Gardner, *S. binderi* Sonder ex J.Agardh, *S. binderi* var. *incisifolium* Sonder ex Grunow, *S. biserrula* J.Agardh, *S. cinctum* var. *echinocarpum* Grunow, *S. crassifolium* J.Agardh, *S. crassifolium* var. *samoensis* Grunow, *S. echinocarpum* J.Agardh, *S. echinocarpum* Greville, *S. echinocarpum* var. *vitiense* Grunow, *S. fonanonense* Setchell & N.L.Gardner, *S. heterocystum* Montagne, *S. oocyste* J.Agardh, *S. odontocarpum* Sonder.

References for Vietnam: Pham H.H. (1969: 373, 377); Nguyen H.Dai (1997: 100, 103, 103, 117); Nguyen H.Dai (2007: 85, 88, 91)

Other references: J. Agardh (1848, 1889); Grunow (1915, 1916), Setchell (1935), Womersley and Bailey (1970), Garrigue and Tsuda (1988), Abbott and Huisman (2004)

Geographical distribution: Probably throughout the Indo – Pacific region (tropical and subtropical)

Habitat: Exposed reef flats.

Representative material: NVT1044, NVT1092, NVT1115, NVT1201, NVT1248, NVT1265, NVT1266, NVT1268, NVT1506.

Morphology: Thallus shape irregular, normally 30 to 50 cm high; holdfast discoid or conical, up to 1.2 cm in diameter; main axis cylindrical, up to 0.2 – 0.5 cm long, producing 2 – 4 secondary axes; secondary axes slightly flattened to flattened and smooth, up to 0.5 cm wide; leaves thick and leathery, obovate to elliptical, oblong or linear, petioles short or absent, base cuneate often asymmetrical, apex obtuse rounded or acute, margins serrate often with two rows of serrations in exposed habitats; midrib thick, short or running to $\frac{3}{4}$ of the length of the leaf, cryptostomata relatively large, prominent, slightly raised above leaf surface, arranged in one or more rows on each side of the midrib but also scattered in wide leaves; Vesicle's shape very variable, obovoid or spherical, smooth, with short spines, petioles flattened or leaf-like, mostly longer but also shorter than the remain part of vesicles. Receptacle in dense clusters, up to 1cm long, female receptacle branched and bearing coarse spine-like protuberances at the apex, male receptacle slightly cylindrical, branched and bearing warty.

Taxonomic note: This species is very variable in morphology. Magruder (1988) as well as Mattio el al. (2009a) suggested to merge *S. binderi* Sonder ex J.Agardh, *S. crassifolium* J.Agardh and *S. echinocarpum* Greville and *S. aquifolium* (Turner) J.Agardh. The latter name has priority.

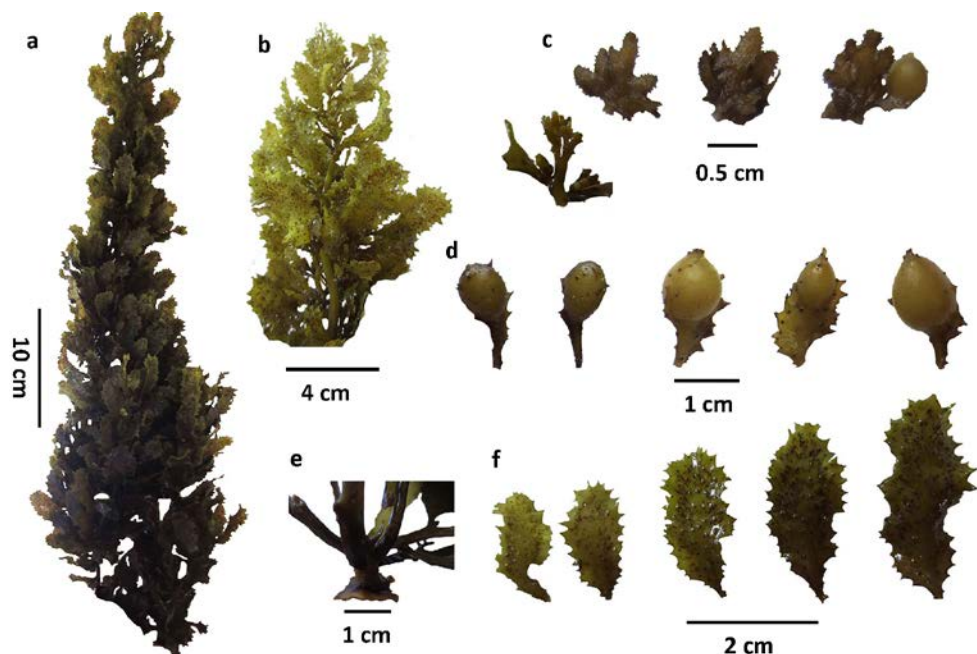


Fig. 4.10. *Sargassum aquifolium* J. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum bicorne* J. Agardh (1848: 306)**

Type locality: Central Vietnam

Type specimen: LD (specimen number not known).

Classification: Subgenus *Sargassum*, section *Illicifolia*

References for Vietnam: Pham H.H. (1969: 391, Fig. 3.86); Nguyen H.Dai (1997: 130, Fig. 53); Nguyen H.Dai (2007: 74, Fig. 45)

Other references: J. Agardh (1848: 306); Agardh, J., (1889: 118); Grunow (1915: 429)

Geographical distribution: only reported from Vietnam.

Habitat: on rocky shores down to -2 meters

Representative material: NVT1210, NVT1211, NVT1212, NVT1245

Morphology: Thallus sparingly branched, 40 to 80 cm high; holdfast discoid, thin, up to 1 cm in diameter; main axis cylindrical, up to 0.2 cm long, producing 2 – 3 secondary axes; secondary axes cylindrical, smooth; cauline leaves wavy, margins entire to slightly irregularly serrate; leaves slender, linear to lanceolate, leaves up to 1.5 cm long and 0.4 cm wide, margins irregular serrate, petioles short, midribs absent or not clear; cryptostomata small and scattered over leaf surface; vesicles in leaf, margins with sharp teeth near the

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apex, up to 1 cm long, 0.4 cm wide and 0.2 cm thick, petioles flattened and short; receptacles branched, cylindrical, up to 1.2 cm long and 0.2 cm wide.

Taxonomic note: This species bears similarity to *S. mcclurei*, the main characters separating both species include: the smaller size of the leaves and vesicles in *S. bicorne*, and the vesicle petioles tapering more abruptly than in *S. mcclurei*.

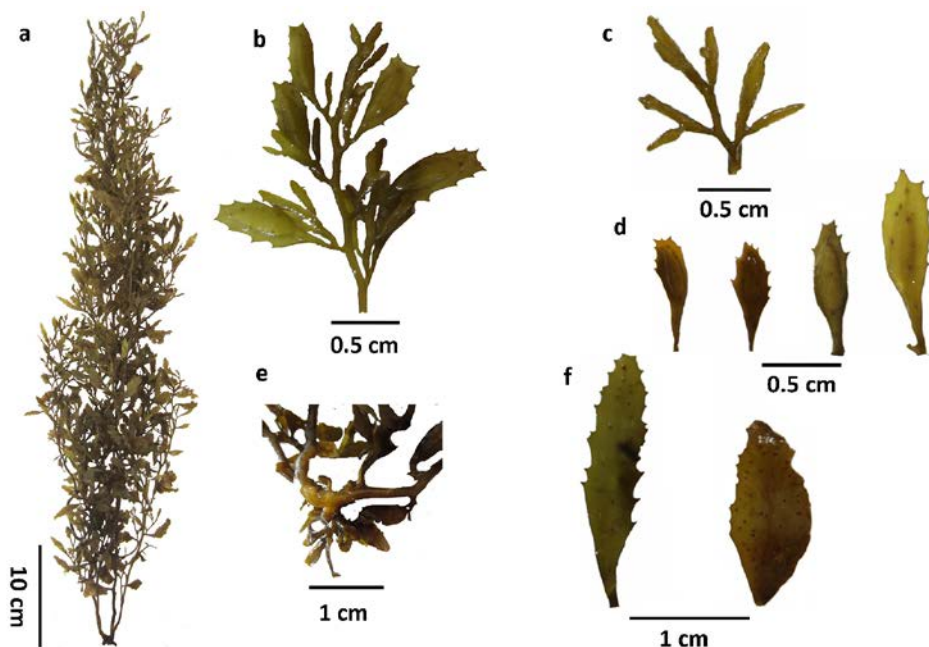


Fig. 4.11. *Sargassum bicorne* J. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), root (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum bulbiferum* Yoshida (1994: 48)**

Type locality: Oburi Island, Japan

Holotype: SAP 059011

Classification: Subgenus *Sargassum*, section *Illicifolia*

References for Vietnam: Nguyen H.Dai (2007: 59-60, Fig. 34),

Other references: Yoshida (1994: 48), Tseng & Lu (2002: 138-140)

Geographical distribution: Vietnam and East Asia

Habitat: on subtidal rocks.

Representative material: NVT1335.

Sargassum diversity in Vietnam: a molecular phylogenetic approach

Morphology: Thallus 50 cm or more long; holdfast discoid, up to 2 cm in diameter; main axis short, surface warty with vestiges of deciduous branches, producing several secondary axes; secondary axes cylindrical or slightly flattened at the base, smooth, up to 0.2 cm wide; leaves on the lower of secondary axis linear to lanceolate up to 7 cm long, 0.7 to 1 cm wide, margin entire or dentate with sparse and small teeth; leaves of the upper part of secondary axis or branches linear – lanceolate, thinner, narrower and shorter, margin sparse and sharp serrations, up to 5 cm long; midrib percurrent, cryptostomata arranged on two rows; vesicle spherical to round–obovate, usually with an apical spine, up to 0.3 cm in diameter, petioles cylindrical up to 0.3 cm long; receptacle androgynous, slender, cylindrical, up to 0.7 cm long.

Taxonomic note: The species is characterized by the formation of a bulbous structure due to the stunted primary axis. The upper part of secondary axes is somewhat similar to the upper parts of *S. carpophyllum* and *S. tenerimum* with thin and narrow leaves, but leaves on the lower part of *S. bulbiferum* are larger.

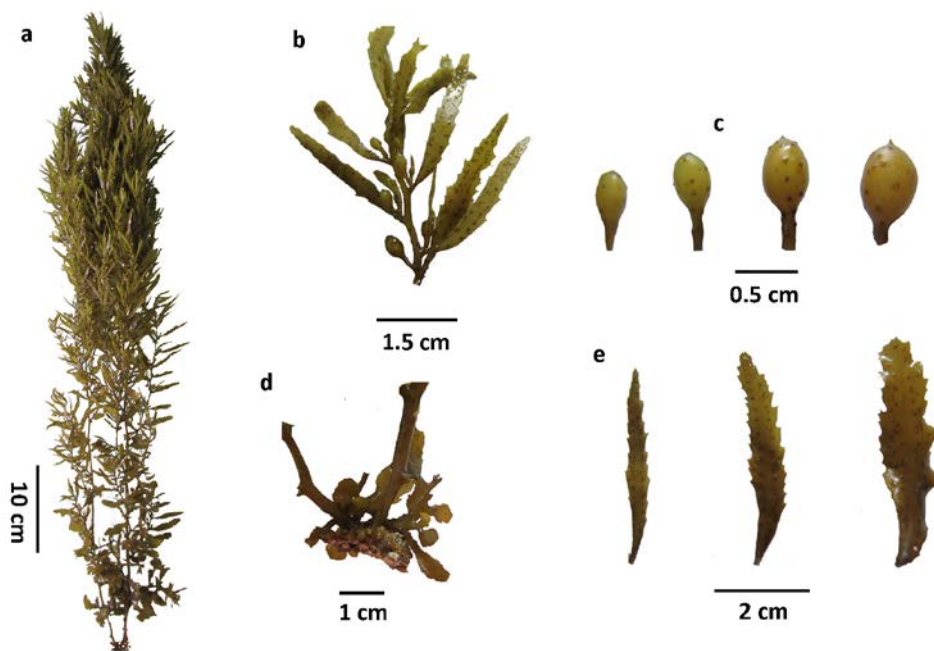


Fig. 4.12. *Sargassum bulbiferum* Yoshida morphology: thallus (a), apex branch (b), receptacles (c), holdfast (d), leaves (e). Scale bars: detailed in the figure.

***Sargassum carpophyllum* J. Agardh (1848: 304)**

Type locality: Sri Lanka (fide Setchell 1935: 7)

Type specimen: LD ?

Classification: Subgenus *Sargassum*, section *Zygocarpicae*

Synonym: *S. flavicans* var. *moretonense* Grunow, *S. flavicans* var. *moretonense* f. *glaucophylla* Grunow.

References for Vietnam: Pham H.H. (1969: 366, Fig. 3.62); Nguyen H.Dai (2007: 46, Fig. 24)

Other references: J. Agardh (1848: 304); Grunow (1915: 366); Tseng (1983: 224), Yoshida (1998: 380)

Geographical distribution: Pacific and Indian Ocean.

Habitat: on coral reef or rocky shores down to -4 meters

Representative material: NVT1193, NVT1195, NVT1196, NVT1180, NVT1181

Morphology: Thallus up to 100 cm high or more; holdfast discoid and warty, up to 1 cm in diameter; main axis cylindrical, smooth, up to 1.2 cm long, up to 0.3 cm wide; secondary axes cylindrical, smooth; leaves narrowly linear to lanceolate, leaves up to 6 cm long and 0.4 – 0.7 cm wide, margins with irregular and small serrations, petioles short and warty, midribs vanishing near the apex; cryptostomata scattered over the leaf surface; vesicles obovoid to elliptic, rounded at the apices, bearing some spines near the apex, up to 0.3 – 0.5 cm in diameter, petioles cylindrical and shorter than the vesicles; receptacle androgynous, cylindrical to fusiform, branched two or three times, racemose, mixed with vesicles, up to 0.4 cm long and 0.1 cm wide.

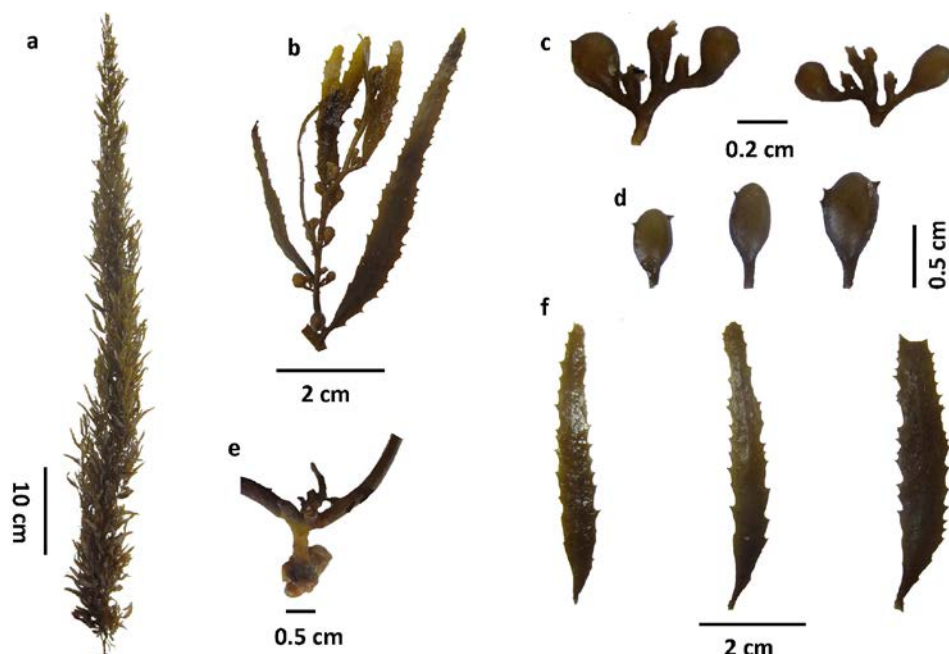


Fig. 4.13. *Sargassum carpophyllum* J. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum carpophyllum* var. *nhatrangense* (Pham H.H.) Ajisaka**

Type locality: Nha Trang, Vietnam

Type specimen: Pham H.H 1081 (Herbarium of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*

References for Vietnam: Pham H.H (1969: 357-358, Fig.354), Ajisaka et al. (1997: 51-57, Figs. 6-13), Nguyen H.Dai (2007: 48-49, Fig. 25).

Geographical distribution: only reported from Vietnam

Habitat: growing on dead coral or rocky shore up to -2 m depths

Representative material: NVT1309, NVT1310, NVT1311, NVT1315, NVT1318

Morphology: Thallus up to 45 cm high, pale yellow; holdfast discoidal, up to 0.7 cm in diameter; main axis cylindrical, warty, up to 1 cm long and 0.25 cm wide; secondary axes cylindrical, up to 0.2 cm in diameter; lateral branches cylindrical, spirally arranged at interval of 3 – 4 cm; cauline leaves simple to furcated, branched once or twice, some leaves branched up to four times, each lobe lanceolate or linear – lanceolate, up to 5.5 cm long and 0.6 – 0.8 cm wide, margin irregularly serrate, midrib conspicuous and percurrent; leaves linear to lanceolate, acute at the apex, margins irregularly serrate, midrib

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conspicuous and percurrent, 2 to 4 cm long and up to 0.5 cm wide, petioles cylindrical, cryptostomata scattered or arranged in two rows; vesicles elliptical, rounded or with a fine spine at the apex, up to 0.7 cm long and 0.35 cm in diameter, petioles cylindrical or somewhat compressed, longer or equal to the vesicle; receptacle branched, bearing spine-like protuberances.



Fig. 4.14. *Sargassum carpophyllum* var. *nhatrangense* (Pham H.H.) Ajisaka: thallus (a), apex branch (b), receptacle (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum cotoense* Nguyen H.Dai (2002: 105-107)**

Type locality: Co To island, Vietnam

Type specimen: Nguyen H.Dinh 68550 (Herbarium of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *Polycystae*

References for Vietnam: Nguyen H.Dai (2002: 105-107, Fig. 2-4); Nguyen H.Dai (2007: 69, Fig. 41)

Geographical distribution: only reported from Vietnam.

Habitat: on rocky shores down to -2 meters

Representative material: NVT1284, NVT1285, NVT1289, NVT1290, NVT1291, NVT1292

Sargassum diversity in Vietnam: a molecular phylogenetic approach

Morphology: Thallus sparingly branched, up to 30 to 40 cm high; holdfast discoid, up to 1 cm in diameter; main axis cylindrical, up to 0.2 cm long, producing 2 – 4 secondary axes; secondary axes cylindrical, smooth, up to 1.5 cm in diameter; leaves thick and very narrow, nearly cylindrical, up to 3.5 cm long and 0.1 cm wide, margin entire, midrib absent; cryptostomata conspicuous; vesicles obovate, up to 0.2 cm in diameter, obtuse or with a long spine at apex, petioles cylindrical shorter than 1/3 of the vesicle; receptacles branched or unbranched, cylindrical and warty, up to 0.8 cm long and 0.1 cm wide, sometimes mixed with vesicles and leaves.

Taxonomic note: This species is endemic to Vietnam. The receptacles of our *S. cotoense* specimens are longer (0.8 cm) than those reported in the original description (0.2 cm, Nguyen H.Dai 2002).

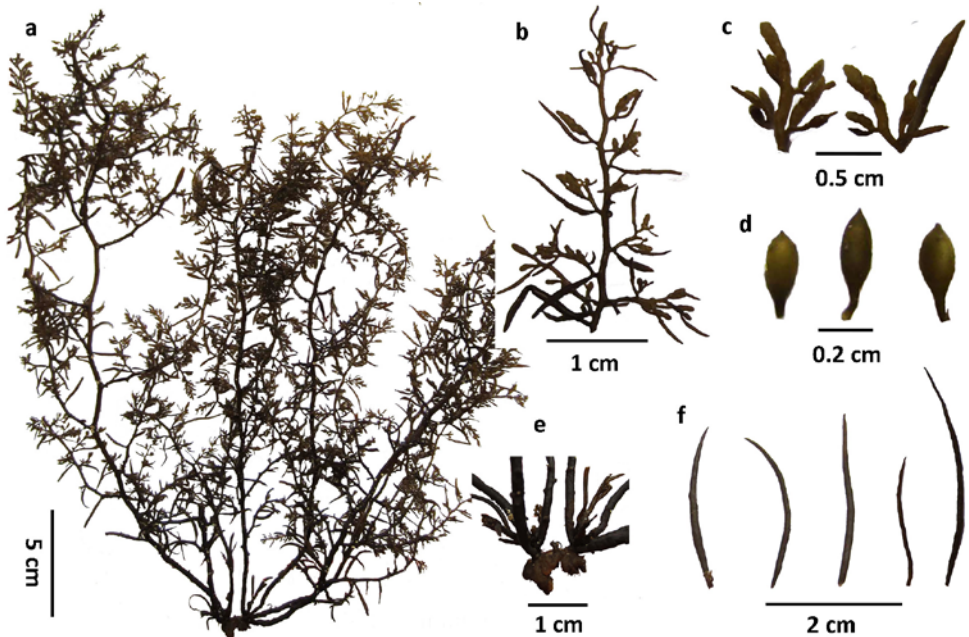


Fig. 4.15. *Sargassum cotoense* Nguyen H.Dai: thallus (a), apex branch (b), receptacles (c), vesicles (d), root (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum distichum* Sonder (1845: 51)**

Type locality: Western Australia

Holotype: MEL 1550992

Classification: Subgenus *Sargassum*, section uncertain

Synonym: *Carpacanthus distichus* (Sonder) Kuetzing, *S. aemulum* Sonder, *S. cristatum* J. Agardh

References for Vietnam: Pham H.H. (1969: 369, Fig. 65); Nguyen H.Dai (2007: 41-43, Fig. 21)

Other references: De Toni (1895: 119), Womersley (1987: 481), Silva et al. (1996: 670)

Geographical distribution: South–East Asia, Australia, New Zealand

Habitat: Subtidal rocky shore

Representative material: NVT1366

Morphology: Thallus 40 cm high or more; holdfast discoidal, up to 0.5 cm in diameter; main axis cylindrical, smooth, up to 0.5 cm long and 0.2 – 0.3 cm wide; secondary axes cylindrical, up to 0.2 cm in diameter; lateral branches cylindrical, less than 0.1 cm wide; cauline leaves thick, margin warty or with stunted serrations, midrib conspicuous and percurrent, up to 7 cm long and 0.7 cm wide; leaves thin, linear to lanceolate, acute at the apex, margins irregularly serrate, 2.5 to 4 cm long and up to 0.3 cm wide, petioles cylindrical, midrib conspicuous and percurrent, cryptostomata arranged in two rows; vesicles spherical to obtuse, up to 0.5 cm in diameter; petioles cylindrical, less than 0.2 cm long; receptacle stocky oblong, bearing spine-like protuberances.

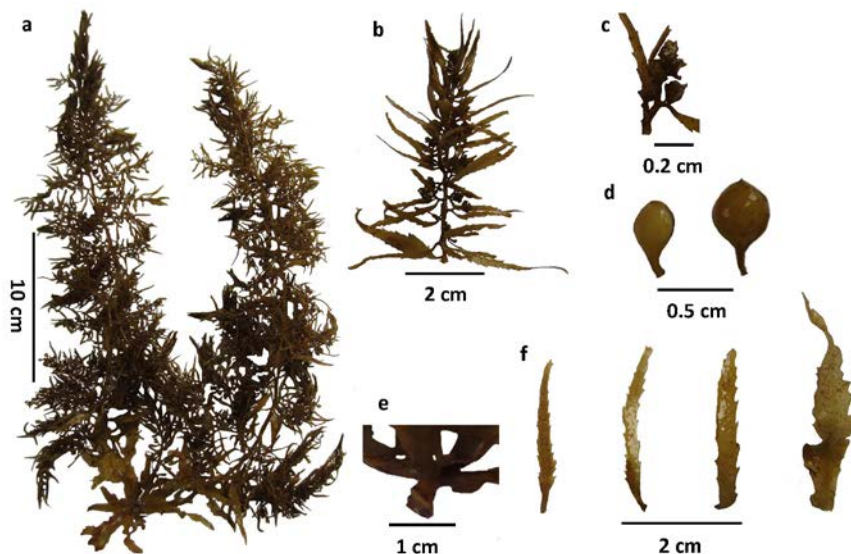


Fig. 4.16. *Sargassum distichum* Sonder: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum feldmannii* Pham H.H. (1967: 297)**

Type locality: Nha Trang, Vietnam

Type specimen: Phamh. 4944 (Herb. of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *Binderiana*

Synonym: *S. echinocarpum* var. *phyllocysta* Grunow

References for Vietnam: Pham H.H. (1967: 297, Fig. 15); Trono (1992: 53-56, Fig. 16-22), Nguyen H.Dai (2007: 93, Fig. 59)

Geographical distribution: Vietnam, Philippines.

Habitat: on coral reef or rocky shores down to -4 meters

Representative material: NVT1148, NVT1202, NVT1269, NVT1494, NVT1509.

Morphology: Thallus up to 60 cm high; holdfast discoid, up to 0.6 cm in diameter; main axis cylindrical, smooth, up to 0.4 cm long, up to 0.3 cm wide, producing 2 – 4 secondary axes; secondary axes compressed, smooth, up to 0.5 cm in diameter; leaves thick, obovate to linear, leaves 2 – 3 cm long and up to 1 cm wide, margins with irregular sharp serrations, obtuse or rounded at the apex, sometimes double serrated or forming a cup-shape, petioles short cylindrical, bearing some spines; midrib vanishing near the apex; cryptostomata numerous, irregular scattered over leaf surface; vesicles in leaf, spherical to elliptic, normally arranged at the central or distal part of the vesicle-leaves, margins sharp dentate, vesicles 0.6 – 0.8 cm wide, vesicle-leaves up to 2 cm long; receptacles forming dense cymes, branched, cylindrical and warty, generally ending with forcipate tips, up to 0.7 cm long.



Fig. 4.17. *Sargassum feldmannii* Pham H.H.: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum gracillimum* Reinbold (1913: 172)**

Classification: Subgenus *Sargassum*, section *Illicifolia* / *Polycystae*

Synonym: None

References for Vietnam: Nguyen H.Dinh et al. (1993: 163-164, Fig. 110), Nguyen H.Dai (2007: 50, Fig. 27)

Other references: Okamura (1936: 360), Trono (1992: 56, Fig. 23-27)

Geographical distribution: Vietnam, Indonesia, Philippines, Japan.

Habitat: on subtidal rocks

Representative material: NVT1363, NVT1365

Morphology: Thallus 30 – 80 cm high; holdfast discoid, up to 1 cm in diameter; main axis cylindrical, smooth, up to 0.35 cm long and 0.25 cm wide, producing 2 – 3 secondary axes; secondary axes cylindrical, smooth, up to 0.15 cm in diameter; leaves thick, oblong to narrow linear, warty, margins entire, 1 – 2.5 cm long and 0.2 – 0.3 cm wide, midrib absent; cryptostomata irregularly scattered over the leaf surface; vesicles elliptical, obtuse, with a spine at the apex, up to 0.3 cm long and 0.15 cm in diameter, petioles cylindrical; receptacle racemose to paniculate, wavy, up to 0.3 cm long.

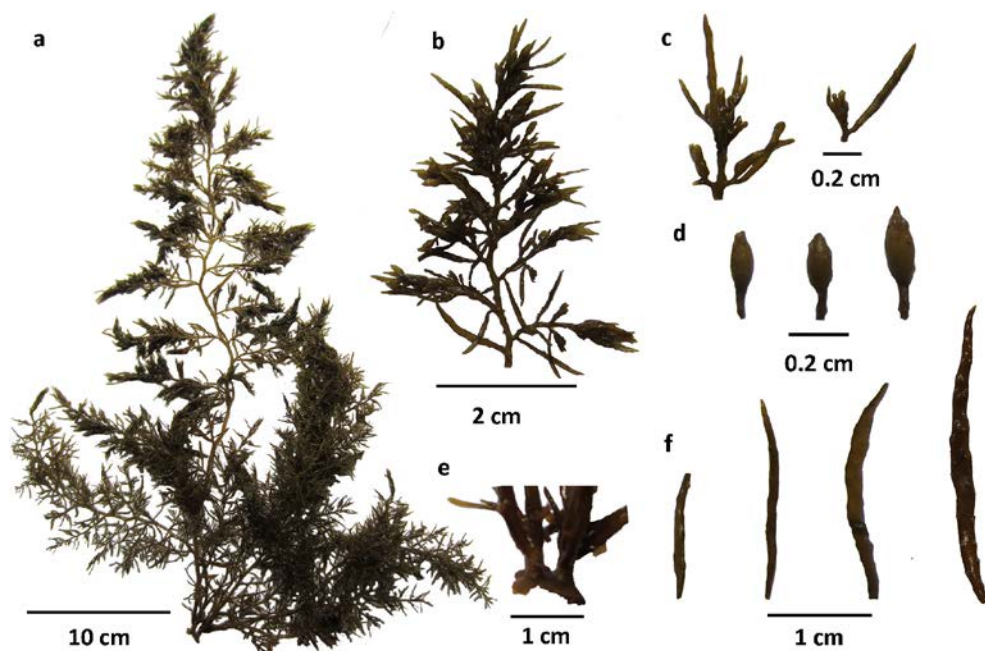


Fig. 4.18. *Sargassum gracillimum* Reinbold: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum graminifolium* C. Agardh (1820: 18)**

Type specimen: LD ?

Classification: Subgenus *Sargassum*, section *Sargassum*

Basinonym: *F. graminifolius* Turner

References for Vietnam: Nguyen H.Dinh et al. (1993: 171-172, Fig. 118), Nguyen H.Dai (2007: 65-66, Fig. 38)

Other references: De Toni (1889: 38), Yendo (1907: 129), Grunow (1915: 364), Okamura (1936: 346), Tseng & Lu (2000: 95).

Geographical distribution: Vietnam, Japan, China.

Habitat: on subtidal rocks

Representative material: NVT1182, NVT1183, NVT1192.

Morphology: Thallus 60 – 80 cm high, dark brown ; holdfast discoid or conical; main axis cylindrical, smooth, up to 0.8 cm long and 0.2 cm wide, producing 2 – 4 secondary axes; secondary axes cylindrical, smooth, up to 0.16 cm in diameter; lateral branch up to 15 cm long; leaves narrow linear, obtuse at the apex, margins thick, serrate, midribs conspicuous and percurrent, 4 – 7 cm long and up to 0.6 cm wide, petioles cylindrical 0.2 – 0.5 cm long; cryptostomata small and inconspicuous; vesicles elliptical, with a fine spine at the apex, up to 1 cm long and 0.3 cm in diameter, petioles cylindrical, longer than the remaining part of vesicle; receptacle branched, cylindrical and wavy, sometimes associated with small vesicles, up to 1.5 cm long.

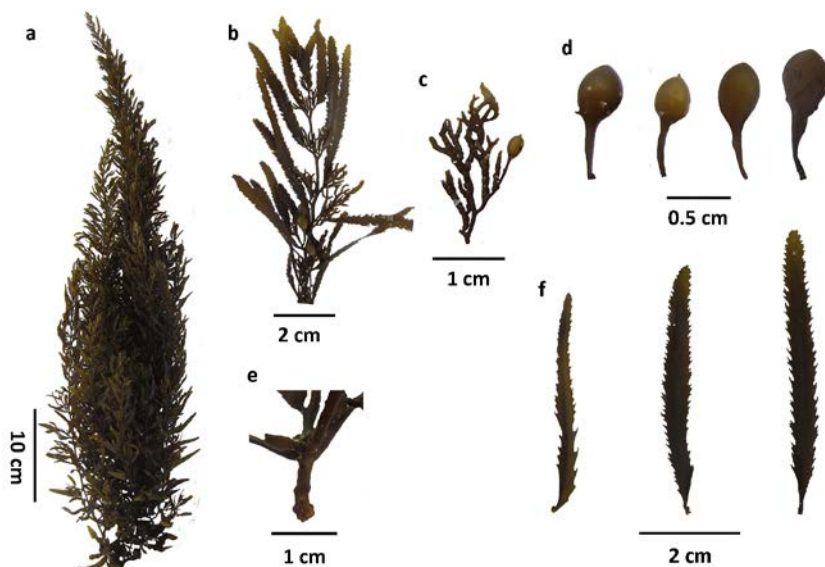


Fig 4.19. *Sargassum graminifolium* C. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum henslowianum* C. Agardh (1848: 315)**

Type locality: Hong Kong, China.

Type specimen: LD ?

Classification: Subgenus *Sargassum*, section *Ilicifolia*

References for Vietnam: Nguyen H.Dinh et al. (1993: 167-168, Fig. 114), Nguyen H.Dai (2007: 78-79, Fig. 48)

Other references: J. Agardh (1848: 315), Grunow (1915: 227), Tseng & Lu (1992: 11-34).

Geographical distribution: Vietnam, China, Taiwan.

Habitat: growing on subtidal rocks

Representative material: NVT1160.

Morphology: Thallus sparsely branched, pale brown, up to 100 cm high; holdfast discoid and lobed, 1 – 1.5 cm in diameter; main axis cylindrical, smooth, short and 0.2 – 0.3 cm in diameter, giving rise to one or two secondary axes; secondary axes cylindrical, smooth, up to 0.2 cm in diameter; cauline leaves lanceolate, obtuse, with an oblique cuneate base, up to 1 cm wide, margins finely serrate, midribs percurrent; leaves narrow, lanceolate, acute, margins sparse and finely serrate, up to 2.5 cm long and 0.3 – 0.6 cm wide; cryptostomata large, irregular arranged on the surface of the leaves, petioles tiny; vesicles spherical to obovate, bearing a spine at the apex or not, petioles cylindrical $\frac{1}{2}$ shorter than vesicles body; receptacle cylindrical, simple or branched, sometimes with small vesicles, up to 1 cm long.

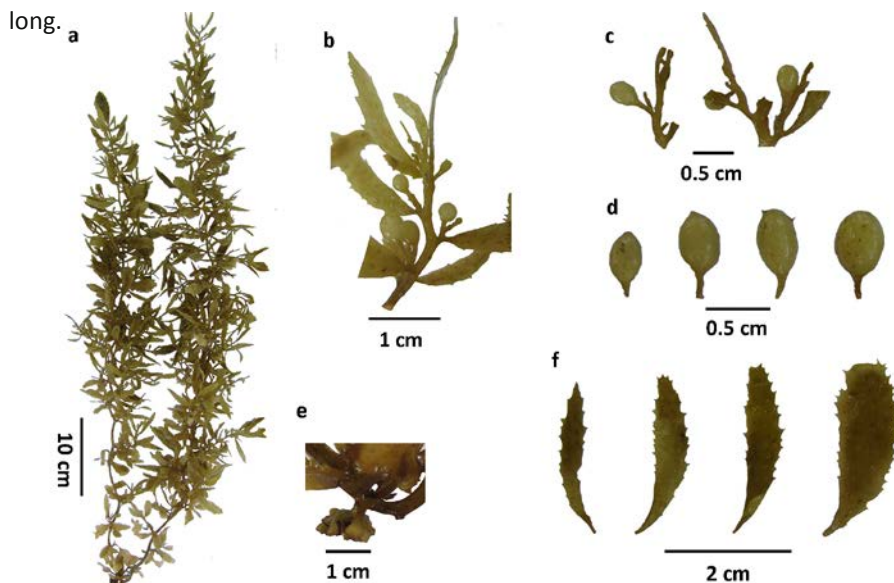


Fig 4.20. *Sargassum henslowianum* C. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

Sargassum herklotsii Setchell (1933: 45)

Type locality: Repulse Bay, Hong Kong, China

Type specimen: UC 484313(female), 484316(male)

Classification: Subgenus *Sargassum*, section *Illicifolia*

References for Vietnam: Nguyen H.Dinh et al. (1993: 168), Nguyen H.Dai (2007: 37, Fig. 18)

Other references: Setchell (1933: 45), Tseng & Lu (2000: 59, Fig. 22).

Geographical distribution: Vietnam, China.

Habitat: intertidal and subtidal rocky shore and coral reef

Representative material: NVT1243, NVT1328, NVT1330, NVT1386, NVT1387, NVT1389, NVT1392, NVT1396, NVT1420, NVT1421, NVT1422, NVT1423, NVT1429.

Morphology: Thallus up to 70 cm high; holdfast discoidal, up to 1.2 cm in diameter, sometimes the holdfast of several plants coalesce; main axis cylindrical or somewhat compressed, smooth, 0.3 – 0.5 cm long and 0.2 – 0.4 cm in diameter, producing 3 – 5 secondary branches; secondary branches cylindrical and somewhat compressed, smooth, up to 0.3 cm in diameter, densely beset with short lateral branches; lateral branches up to 15 cm long; leaves thick in exposed habitat, shape ranging from obovate to spatulate; margins of young leaves coarsely serrate; margins of older leaves with less pronounced dentation; leaves up to 1.5 – 2.5 cm long and 0.5 – 0.9 cm wide; midrib conspicuous, running 2/3 of the length of the leaves; cryptostomata few and scattered over the leaf surface; vesicle linear to ovate-oblong, 0.5 – 0.8 cm long and 0.2 – 0.3 cm in diameter, the apex ending with a tip or several spines; reproductive branches bearing numerous receptacles; female receptacles racemosely arranged, triquetrous, up to 0.8 cm long and with small marginal spines, male receptacle cylindrical, simple or branched, warty and up to 1.2 cm long.

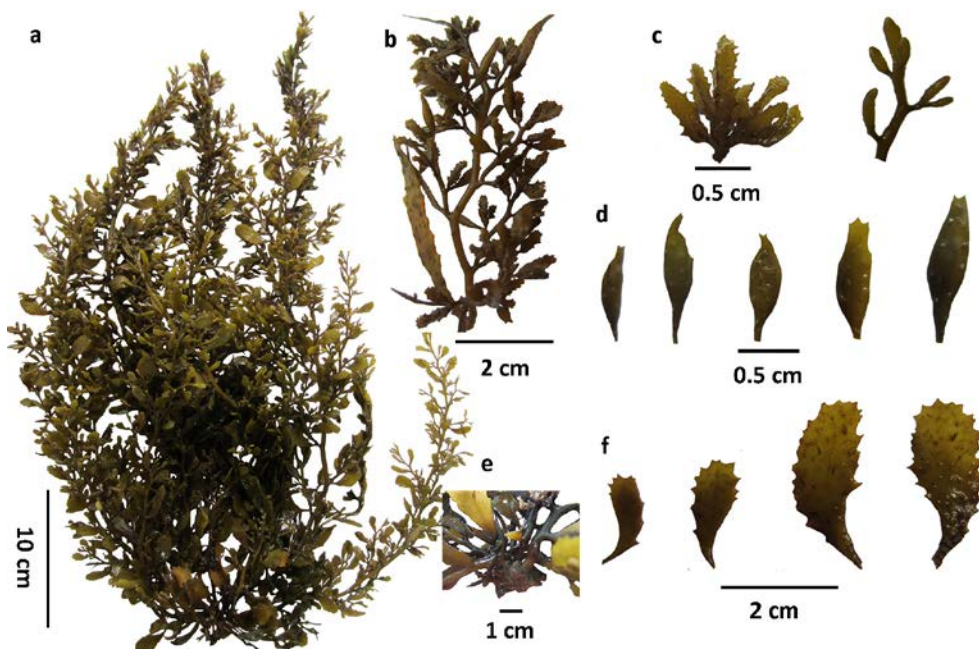


Fig. 4.21. *Sargassum herklotsii* Setchell: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum heterocystum* Montagne (1842: 250)**

Type locality:

Type specimen: Holotype: PC MA9192

Classification: Subgenus *Sargassum*, section *Ilicifolia*

Synonym: *Carpacanthus heterocystus* (Montagne) Endlicher

References for Vietnam: Pham H.H. (1969: 380-381, Fig.76), Nguyen H.Dai (2007: 97, Fig. 62)

Other references: Montagne (1842: 241-282), Yendo (1907: 35), Grunow (1915 : 409), Okamura (1936). Tseng & Lu (2002: 24-25)

Geographical distribution: Vietnam, Philippines, Japan, China

Habitat: subtidal coral flats or rocky shore

Representative material: NVT1215, NVT1486.

Morphology: Thallus 50 – 70 cm high; holdfast discoid, less than 1cm; main axis cylindrical, warty, less than 0.5 cm long, producing 2 – 3 secondary axes; secondary axes cylindrical, smooth, up to 0.2 cm in diameter; lateral branch spirally arranged, 7 – 10 cm long; leaves elliptic, up to 2.4 cm long and 0.8 cm wide, margins sparse dentate, petioles short, midrib vanishing at ½ of leaves long; cryptostomata few, scattered irregular on the surface of

leaves; vesicle spherical to elliptic, up to 0.7 cm long, petioles cylindrical up to ½ vesicle long; female receptacle triquetrous, male receptacle cylindrical.



Fig. 4.22. *Sargassum heterocystum* Montagne: thallus (a), apex branch (b), vesicles (c), main axis (d), leaves (e). Scale bars: detailed in the figure.

***Sargassum ilicifolium* (Turner) C. Agardh (1820: 11)**

Type locality: Strait of Sunda, Indonesia.

Holotype: BM562953

Classification: Subgenus *Sargassum*, section *Illicifolia*

Synonyms: *C. ilicifolius* (Turner) Kützinger, *F. ilicifolius* Turner, *F. latifolius* Poiret, *S. berberifolium* J.Agardh, *S. cristaefolium* C.Agardh, *S. duplicatum* Bory, *S. duplicatum* (J.Agardh) J.Agardh, *S. droserifolium* Bory, *S. ilicifolium* var. *duplicatum* J.Agardh, *S. ilicifolium* var. *oocystoides* Grunow, *S. ilicifolium* var. *venustum* Grunow, *S. sandei* Reinbold, *S. turbinatifolium* C.K.Tseng & Lu.

References for Vietnam: Pham H.H. (1969: 375, 376, 392); Nguyen H.Dinh et al. (1993: 172); Nguyen H.Dai (2007: 83, 89, 90, 104)

Other references: J. Agardh (1848, 1889); Grunow (1915), Womersley and Bailey (1970), Tsuda (1988), Ajisaka et al. (1997a).

Geographical distribution: Pacific and Indian Ocean

Habitat: Shallow reef flats and rocky bottom

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Representative material: NVT1058, NVT1063, NVT1083, NVT1094, NVT1110, NVT1161, NVT1162, NVT1203, NVT1204, NVT1205, NVT1206, NVT1208, NVT1209, NVT1323, NVT1491, NVT1492

Morphology: Thallus shape irregular, normally 50 – 80 cm high or more; holdfast discoid or conical, up to 1.5 cm in diameter; main axis cylindrical and warty, up to 0.2 – 0.5 cm long, producing 3 – 5 secondary axes; secondary axes cylindrical to slightly flattened and smooth, up to 0.4 cm wide; leaves thick and leathery in exposed habitat to thinner and crisp in more sheltered habitats, obovate to spatulate, ovate or orbicular, petioles short or absent, base rounded or oblique, apex obtuse, rounded or acute, often with a cup-shaped depression, margins denticulate, erose or biserrate, midrib thin, running 2/3 of the length of the leaves or percurrent, cryptostomata thin and scattered over leaf surface; vesicles very variable in shape, obovoid or spherical, ovate to pyriform, smooth, with a short spine or ear-like mucron, petioles cylindrical to flattened; receptacle mostly unisexual but some time bisexual; female receptacle flattened and stocky oblong to spatulate, simple or bifid with a deeply dentate margins; Male receptacle cylindrical, slender, simple or bifid bearing thin spine-like protuberances on the margins; bisexual receptacles in dense clusters, warty, branched, cylindrical to slightly flattened, and bearing coarse spine-like protuberances at the margins or near the apex, up to 1.5 cm high.

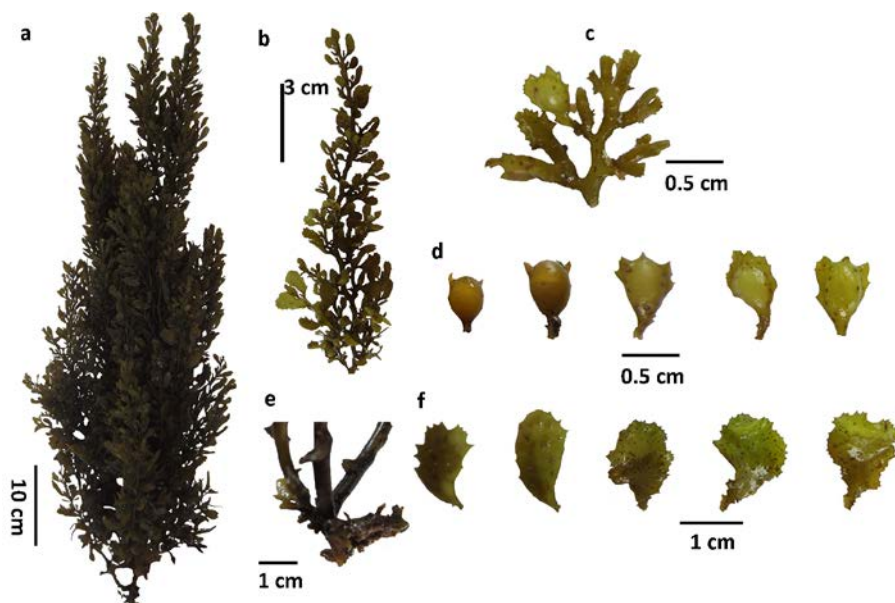


Fig. 4.23. *Sargassum ilicifolium* (Turner) C. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

Sargassum mcclurei Setchell (1933: 45)

Type locality: Repulse Bay, Hong Kong, China

Type specimen: US 2222-2224

Classification: Subgenus *Sargassum*, section *Illicifolia*

References for Vietnam: Dawson (1954: 406), Pham H.H. (1969: 365, Fig. 261), Nguyen H.Dinh et al. (1993: 178, Fig. 127), Nguyen H.Dai (2007: 38-39, Fig. 19), Tsutsui (2005: 110)

Other references: Setchell (1933: 45), Tseng (1983: 202), Silva et al (1996: 685), Tseng & Lu (2000: 60)

Geographical distribution: Vietnam, Hong Kong and Indian Ocean

Habitat: rocky shores, up to - 5 m deep

Representative material: NVT1060, NVT1084, NVT1086, NVT1090, NVT1099, NVT1122, NVT1161, NVT1130, NVT1137, NVT1249, NVT1283, NVT1431, NVT1441, NVT1445, NVT1447, NVT1448, NVT1456, NVT1469, NVT1470, NVT1500.

Morphology: Thallus shape irregular, coarse, 50 – 100 cm high or more; holdfast discoid and slightly lobed, up to 1 cm in diameter, normally several trees link the holdfast together; main axis cylindrical, up to 0.5 cm long, producing 3 – 5 secondary axes; secondary axes cylindrical, smooth, 0.15 – 0.2 cm in diameter; lateral branches dominant, up to 25 cm long; leaves thick and leathery in exposed habitats, spatulate to obovate, petioles short or absent, base rounded or oblique, apex obtuse, rounded or acute, margins irregular denticulate, midrib inconspicuous, 1 – 1.5 cm long and up to 0.8 cm wide; cryptostomata numerous, scattered over leaf surface; vesicle in leaf, up to 1 cm long and 0.5 cm in diameter, margins sparse but larger serrate, petioles cylindrical to flattened; receptacle branched, up to 1.5 cm long, male receptacle cylindrical, warty; female receptacle somewhat compressed and bearing spines.

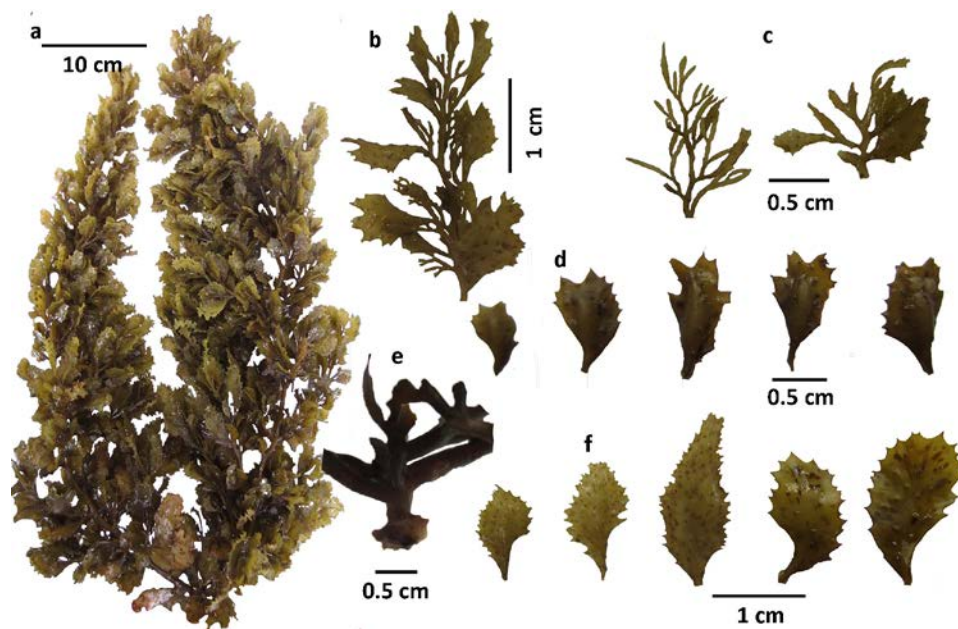


Fig. 4.24. *Sargassum mcclurei* Setchell: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum microcystum* J. Agardh (1848: 323)**

Type locality: Moluccas, Indonesia

Type specimen: LD?

Classification: Subgenus *Sargassum*, section *Ilicifolia*

Synonym: *Carpacanthus microcystus* (J. Agardh) G. Martens

References for Vietnam: Pham H.H. (1967: 212, Fig. 23), Nguyen H.Dai (2007: 101, Fig. 64)

Other references: J. Agardh (1848: 323), Silva et al. (1996: 685)

Geographical distribution: Indian and Pacific Ocean

Habitat: on rocky shores -2 deep

Representative material: NVT1066, NVT1068, NVT1088, NVT1104, NVT1139, NVT1499.

Morphology: Thallus 30 – 80 cm high or more; holdfast conical, up to 1 cm in diameter, warty; main axis cylindrical, warty, up to 0.4 cm long, producing 3 – 5 secondary axes; secondary axes cylindrical or somewhat compressed at lower part, smooth, 0.15 – 0.2 cm in diameter; lateral branches coarse; leaves thick and crisp, ovate to obovate, petioles short or absent, apex obtuse, rounded, margins irregularly denticulate, midrib inconspicuous, vanishing to the apex, 2 – 3.5 cm long and up to 1.4 cm wide; cryptostomata conspicuous, scattered over leaf surface; vesicle numerous, 0.15 – 0.4 cm long, spherical to obovate,

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bearing a fine spine or an ear-like dentate wing; petioles cylindrical, short; receptacles compressed or triquetrous, cymosely branched, up to 1 cm long.

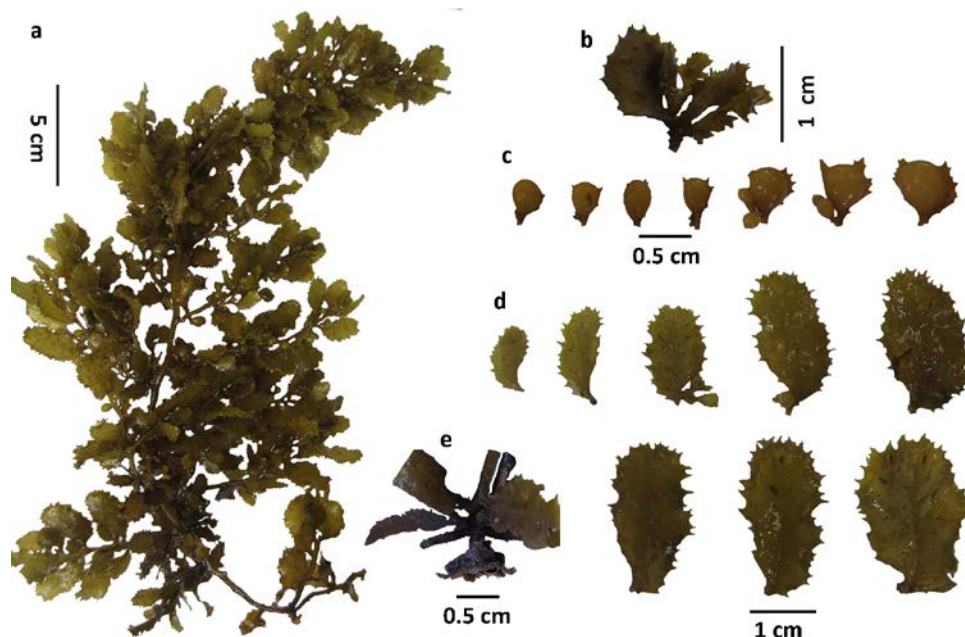


Fig. 4.25. *Sargassum microcystum* J. Agardh: thallus (a), receptacles (b), vesicles (c), leaves (d), holdfast (e). Scale bars: detailed in the figure.

***Sargassum oligocystum* Montagne (1845: 67)**

Type locality: Lampung bay, Indonesia

Type specimen: PC ?

Classification: Subgenus *Sargassum*, section *Binderiana*

Synonym: *Carpacanthus oligocytus* (Montagne) Kuetzing

References for Vietnam: Tsutsui (2005: 114, Fig. 92), Nguyen H.Dai (2007: 86-87, Fig. 54)

Other references: Montagne (1845: 67), Grunow (1915: 385), Silva et al. (1996: 690),

Yoshida (1998: 397), Tseng & Lu (2000: 141)

Geographical distribution: Indian Ocean, Pacific Ocean

Habitat: dead coral and rocks, -2 to -3 meter depths

Representative material: NVT1062, NVT1067, NVT1069, NVT1118, NVT1154, NVT1226, NVT1454, NVT1487, NVT1498.

Morphology: Thallus 40 – 60 cm high; holdfast conical, up to 0.8 cm in diameter, smooth; main axis cylindrical, 0.2 – 0.5 cm long, producing 3 – 4 secondary axes; secondary axes compressed, smooth, 0.3 – 0.7 cm wide; lateral branches coarse, 5 – 10 cm long, arranged

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interval 1- 3 cm, compressed; leaves elliptic to linear, thick and crisp, petioles short, apex obtuse, rounded, margins sparsely denticulate, midrib percurrent, 3 – 5.5 cm long and 0.7 – 1 cm wide; cryptostomata conspicuous, scattered over the leaf surface; vesicles elliptical, 0.3 – 0.4 cm wide, apex ending with a spine or not; petioles cylindrical, short, sometime compressed; receptacle cymosely branched, cylindrical or compressed, warty, bearing spine-like, up to 0.5 cm long.



Fig. 4.26. *Sargassum oligocystum* Montagne: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum phamhoangii* Nguyen H.Dai (2002: 105-109)**

Type locality: Da Nang, Vietnam

Holotype, Nguyen H.Dai 82107 (Herb. of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *polycystae*

References for Vietnam: Nguyen H.Dai (2002: 105-109, Figs. 5,6); Nguyen H.Dai (2007: 53, Fig. 29)

Geographical distribution: only reported from Vietnam.

Habitat: on subtidal rocks

Representative material: NVT1185, NVT1187, NVT1191

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Morphology: Thallus dark brown, up to 100 cm high; holdfast conical, up to 1 cm in diameter; main axis cylindrical, smooth secondary axes irregularly arranged on the primary axis, up to 0.15 cm in diameter; leaves linear to lanceolate, relatively thick, up to 10 (-20) cm long and 0.5 cm wide, petioles slender, margins entire or with few obtuse spines; midrib and cyptostomata not clearly observed; vesicle obovate to ovate-oblong, 0.2 to 0.3 cm in diameter, sometimes with along apical spine, petioles cylindrical and short; receptacle fusiform, warty, solitary at the base of small leaves, sometimes forked, 0.3 – 0.5 cm long.

Taxonomic note: This species is characterized by very long and slender leaves, which distinguish it from *S. nigrifolium* Yendo.

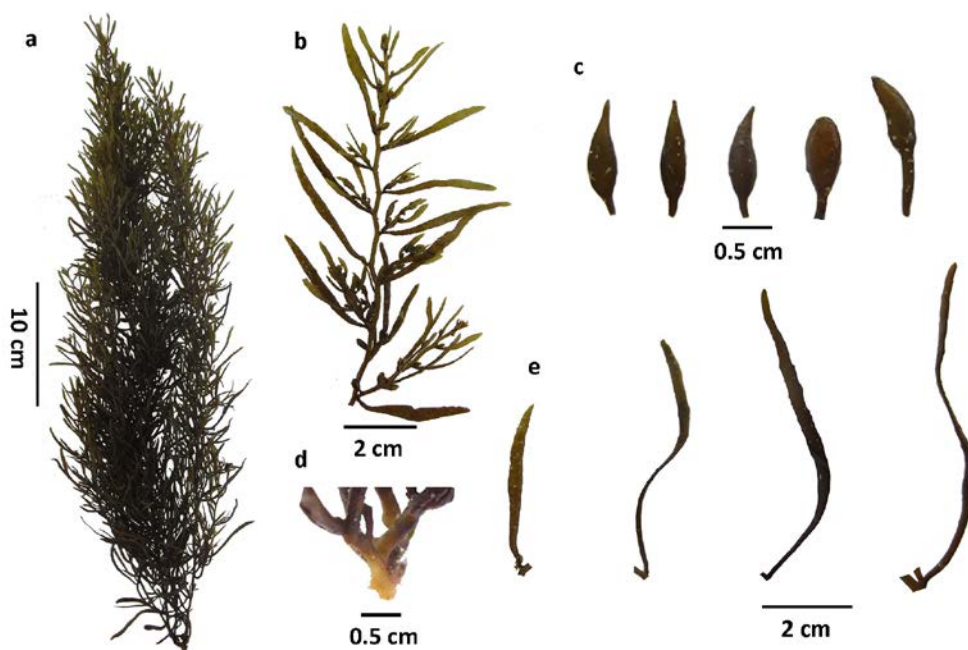


Fig. 4.27. *Sargassum phamhoangii* Nguyen H.Dai: thallus (a), apex branch (b), vesicles (c), primary axis (d), leaves (e). Scare bars: detailed in the figure.

***Sargassum piluliferum* var. *serratifolium* Yamada (1944)**

Type locality: Utinoura – Usumi, Japan

Type specimen: SAP?

Classification: Subgenus *Sargassum*, section *Sargassum*/uncertain

Ref. for Vietnam: Ajisaka et al. (1997: 57-59, Figs. 18-23), Nguyen H.Dai (2007: 35-36, Fig. 16)

Other references: Yamada (1944)

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Geographical distribution: Vietnam, Japan

Habitat: Growing on subtidal rocky shore

Representative material: NVT1349, NVT1367

Morphology: Thallus up to 40 cm high; holdfast discoid to conical, warty, up to 1.1 cm in diameter; main axis short, warty; secondary axes cylindrical, up to 0.15 cm in diameter; lateral branches cylindrical, up to 10 cm long; cauline leaves simple to furcate, some leaves branched up to three times near the base, up to 4 – 5 cm long and up to 0.7 cm wide, margins irregularly dentate, midrib conspicuous and percurrent; leaves narrow lanceolate, margins irregularly serrate, midrib conspicuous and percurrent, 2 to 4 cm long and up to 0.3 cm wide, petioles short, cryptostomata scattered or arranged in two rows; vesicles spherical, rounded or with a fine spine at the apex, up to 0.6 cm long and 0.3 cm in diameter, petioles cylindrical and bearing some spine-like protuberances, longer or equal to the vesicle; receptacle simple, bearing spines.

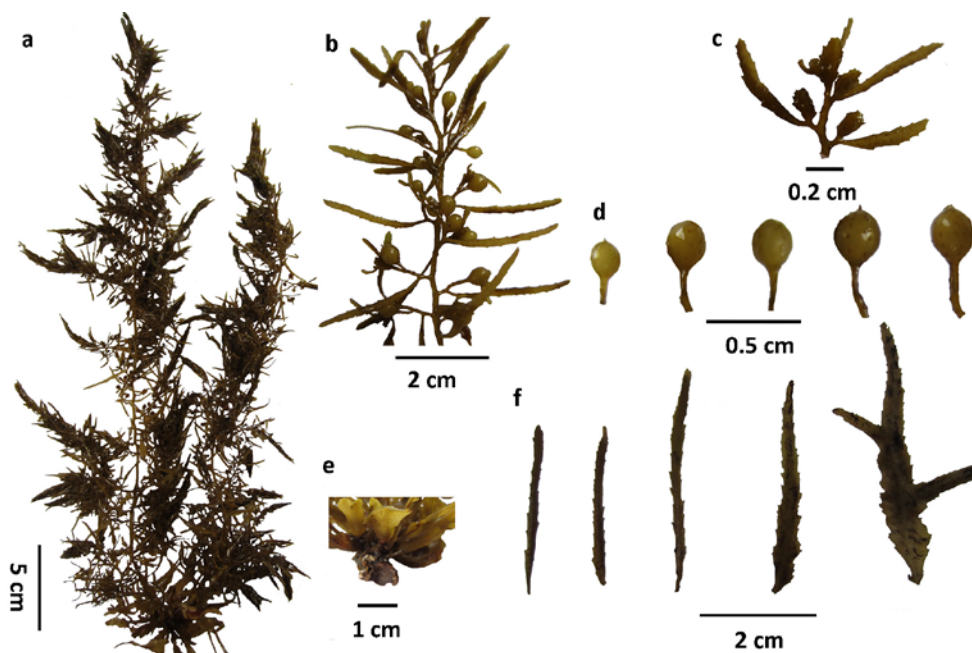


Fig. 4.28. *Sargassum piluliferum* var. *serratifolium*: thallus (a), apex branch (b), receptacle (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

***Sargassum polycystum* C. Agardh (1824: 304)**

Type locality: Sunda Strait, Indonesia.

Type specimen: TCD 1108, 1109 (syntypes).

Classification: Subgenus *Sargassum*, section *Polycystae*

Synonym: *S. ambiguum* Sonder, *S. brevifolium* Greville, *S. cornigerum* Sonder ex Grunow, *S. elegans* Greville, *S. gaudichaudii* Montagne, *S. myriocystum* J.Agardh, *S. myriocystum* f. *horridula* Grunow, *S. myriocystum* var. *elegans* Grunow, *S. opacum* J.Agardh, *S. spinuligerum* var. *fissifolia* f. *humilis littoralis* Grunow, *S. polycystum* var. *onustum* J.Agardh, *S. polycystum* var. *horridulum* Grunow, *S. pygmaeum* Kützing,

References for Vietnam: Dawson (1954: 406), Pham H.H. (1969: 183-184, Fig. 3.79), Nguyen H.Dinh et al. (1993: 175, Fig. 124); Nguyen H.Dai (2007: 54-56, Fig. 30)

Other references: C. Agardh (1824: 304), J. Agardh (1848: 310), Grunow (1915: 444) Yoshida (1998: 400)

Geographical distribution: Worldwide, especially Indo–Pacific region.

Habitat: Shallow reef flats and rocky bottom

Representative material: NVT1043, NVT1064, NVT1071, NVT1119, NVT1123, NVT1124, NVT1125, NVT1127, NVT1231, NVT1241, NVT1334, NVT1381

Morphology: Thallus of variable size from 10 cm in shallow exposed habitats to 1.5 m in deeper areas; holdfast discoidal or conical; main axis cylindrical and warty, bearing up to 10 stolon-like branches, 0.5 – 1 cm long; stolon-like branches cylindrical or flattened, smooth or with few spines, exhibiting sometimes short leaf-like lateral or secondary haptera; secondary axes cylindrical, distichous and bearing numerous “y” shaped spine-like protuberances, up to 0.2 cm in diameter; leaves linear to lanceolate or oblong and straight in lateral view, apex acute or obtuse, margins irregularly serrate, petioles short, base cuneate, midribs percurrent; cryptostomata small, abundant and scattered irregularly on the leaf surface, up to 3 cm long and 0.8 cm wide; vesicles spherical or slightly oval, smooth or bearing prominent cryptostomata and a short spine-like mucron, up to 0.25 cm in diameter, petioles cylindrical and shorter than the vesicles; receptacles unisexual; female receptacles simple or branched, cylindrical or slightly flattened, lanceolate, warty, bearing coarse spines; male receptacles simple or bifid, cylindrical, lanceolate, smooth or bearing a few fine spines, possibly mixed with vesicles.

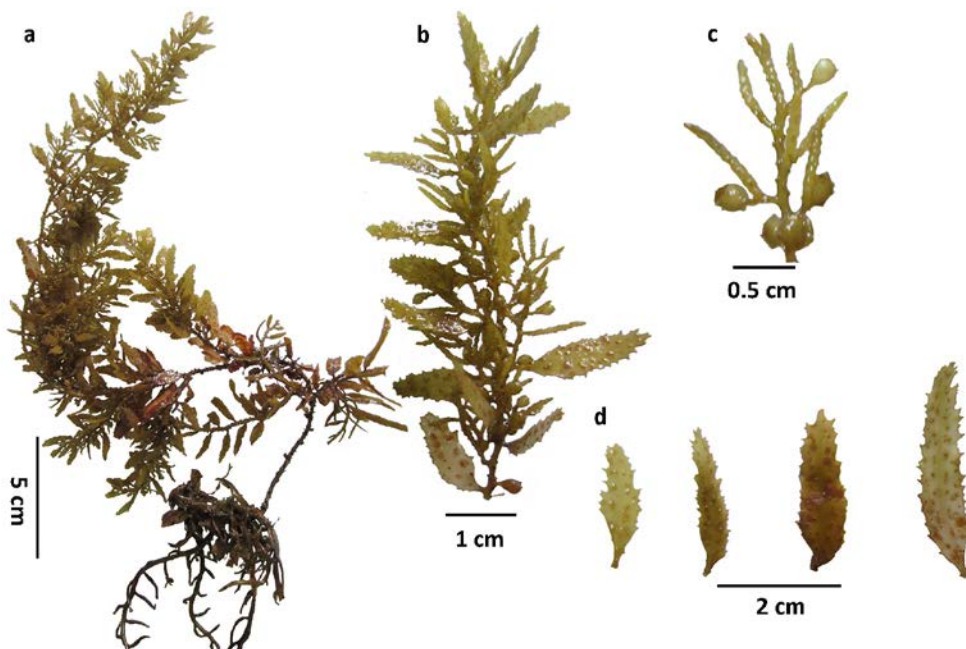


Fig. 4.29. *Sargassum polycystum* C. Agardh: thallus (a), apex branch (b), receptacles and vesicles (c), leaves (d). Scale bars: detailed in the figure.

***Sargassum quinhonense* Nguyen H.Dai (2002: 105-109)**

Type locality: Quynhon, Vietnam

Holotype Nguyen H.Dai 83243 (Herb. of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *Illicifolia*

References for Vietnam: Nguyen H.Dai (2002: 109-111, Fig. 7, 8, 9); Nguyen H.Dai (2007: 40-41, Fig. 20)

Other references: Tsutsui (2005: 116)

Geographical distribution: only reported from Vietnam.

Habitat: on subtidal rocks

Representative material: NVT1418, NVT1472, NVT1473, NVT1474, NVT1475, NVT1479

Morphology: Thallus brown to dark brown, up to 80 cm high; holdfast discoid, attached strongly to substratum, up to 1.3 cm in diameter; main axis cylindrical, 0.2 – 0.3 cm long, up to 0.2 cm in diameter, producing 2 – 4 secondary axes; secondary axes cylindrical, smooth, up to 0.15 cm in diameter; lateral branches up to 20 cm long; cauline leaves lanceolate, 4 – 7 cm long and 0.5 – 0.8 cm in diameter; leaves lanceolate, obtuse at the apex, margins entire to irregularly serrate, 1.5 – 4 cm long and 0.3 – 0.5 cm wide, petioles slender and up to 0.4 cm long, midrib apparent; cryptostomata irregularly scattered over leaf surface;

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vesicles obovate to ovate-oblong, 0.2 – 0.5 cm in diameter, phyllocysts expanded into earlike appendages, often located at the apex of leaves, petioles cylindrical, up to 0.3 cm long; receptacles simple or 2 – 3 racemously arranged, female receptacle triquetrous 0.2 – 0.5 cm long, male receptacle cylindrical, warty and up to 1 cm long.

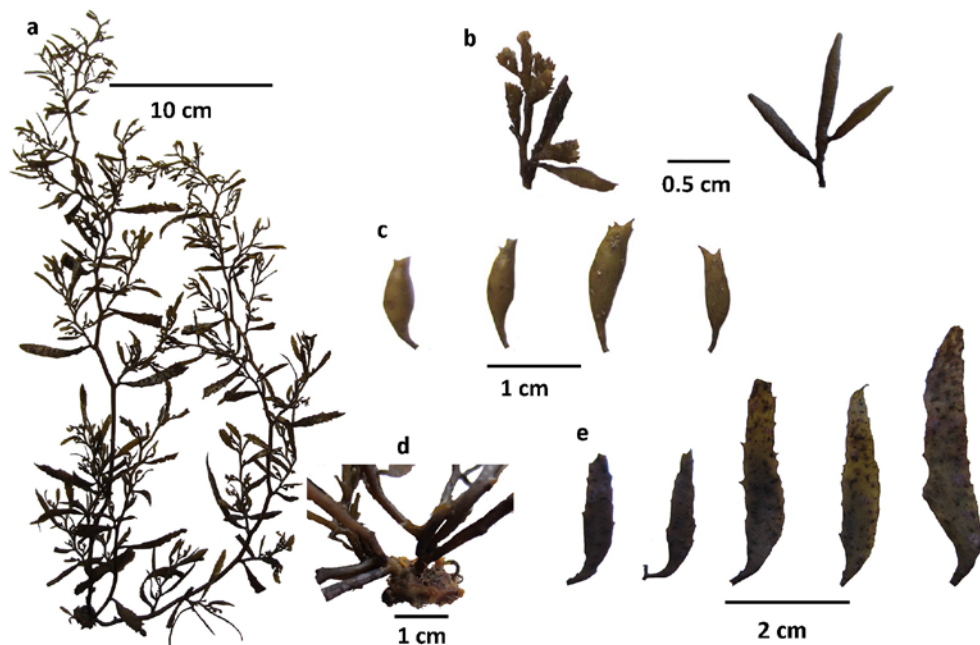


Fig. 4.30. *Sargassum quinhonense* Nguyen H.Dai: thallus (a), receptacles (b), vesicles (c), holdfast (d), leaves (e). Scale bars: detailed in the figure.

***Sargassum serratum* Nguyen H.Dai (2004: 76-79)**

Type locality: Nha Trang, Vietnam

Type specimen: Nguyen H.Dai 86086 (Herbarium of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *Polycystae*

Synonym: None

References for Vietnam: Nguyen H.Dai (2007: 66-67, Fig. 39),

Other references: None

Geographical distribution: only reported from Vietnam

Habitat: dead coral and subtidal rocks.

Representative material: NVT1065, NVT1072, NVT1089, NVT1091, NVT1095, NVT1096, NVT1097, NVT1098, NVT1120, NVT1121, NVT1129, NVT1270, NVT1502.

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Morphology: Thallus 40 to 80 cm high; holdfast conical, 0.5 – 0.7 cm in diameter; main axis cylindrical, smooth, 0.1 – 0.3 cm long, producing 2 – 4 secondary axes; secondary axes cylindrical, 0.1 – 0.2 cm in diameter; cauline leaf 0.7 – 1.5 cm long, obovate to oblong, margins large and serrate, petioles short cylindrical, midrib inconspicuous, cryptostomata inconspicuous; leaves 5 – 10 cm long and 0.2 – 0.4 cm wide, obovate and linear or/ or elliptic, margin serrate, petioles short and compressed, midrib inconspicuous, cryptostomata conspicuous scattered on the surface of leaves; vesicles numerous, ovate, 0.4 – 0.7 cm long and 0.2 – 0.35 cm wide, bearing spine or ear-like appendages; male receptacles cylindrical, warty, 0.5 – 2 cm long; female receptacles compressed, 0.2 – 0.5 cm long, bearing marginal spines.

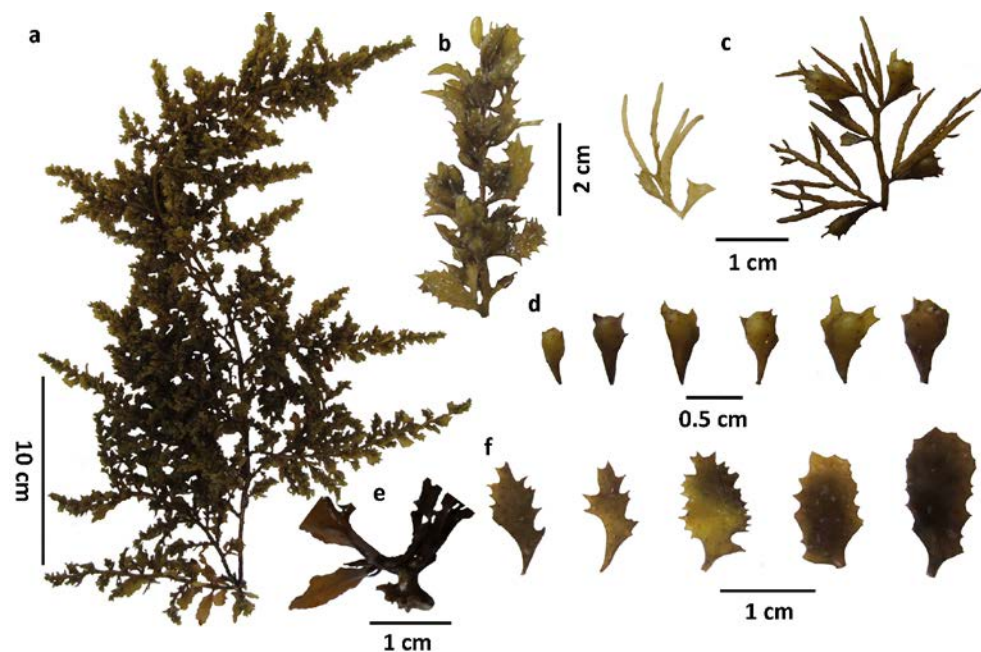


Fig. 4.31. *Sargassum serratum* Nguyen H.Dai: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum swartzii* C. Agardh (1820: 11)**

Type locality: India

Type specimen: Lectotype BM563461

Classification: Subgenus *Sargassum*, section *Binderiana*

Synonym: *S. spathulifolium* J. Agardh, *S. wightii* Grecille ex J. Agardh, *S. acutifolium* Greville

References for Vietnam: Pham H.H. (1969: 371, Fig. 368); Nguyen H.Dinh et al. (1993: 176, Fig.125), Tsutsui (2005: 117, Fig. 95), Nguyen H.Dai (2007: 94-95, Fig. 60).

Other references: C. Agardh (1824: 11), J. Agardh (1848: 328), Grunow (1915: 381), Setchell (1936: 54), Yoshida (1988: 19), Silva et al. (1996: 7000), Tseng & Lu (2000: 130).

Geographical distribution: Indian and Pacific Ocean

Habitat: growing on dead coral flat and subtidal rocky shores

Representative material: NVT1221, NVT1222, NVT1223, NVT1224, NVT1225, NVT1155, NVT1156, NVT1157, NVT1158, NVT1159, NVT1297, NVT1383.

Morphology: Thallus 50 to 80 cm high; holdfast conical, up to 1cm in diameter, sometimes several holdfast are linked together; main axis cylindrical, smooth, 0.4 – 0.5 cm long, producing 2 – 4 secondary axes; secondary axes compressed, smooth, 0.2 – 0.35 cm wide; lateral branch 10 – 20 cm long, arranged at interval 2 – 3 cm; leaves thick, lanceolate to linear – lanceolate; apex acute, 4 – 6 cm long and 0.5 – 0.7 cm wide, petioles compressed and up to 0.3 cm long, midrib percurrent, cryptostomata forming two rows on both side of the midrib; vesicles elliptical, 1.4 cm long and 0.45 cm wide, rounded apex or with a sharp point, petioles compressed, normally longer than vesicle; receptacle cylindrical or compressed, forming bunch, bearing spine or warty, 0.2 – 0.4 cm long.

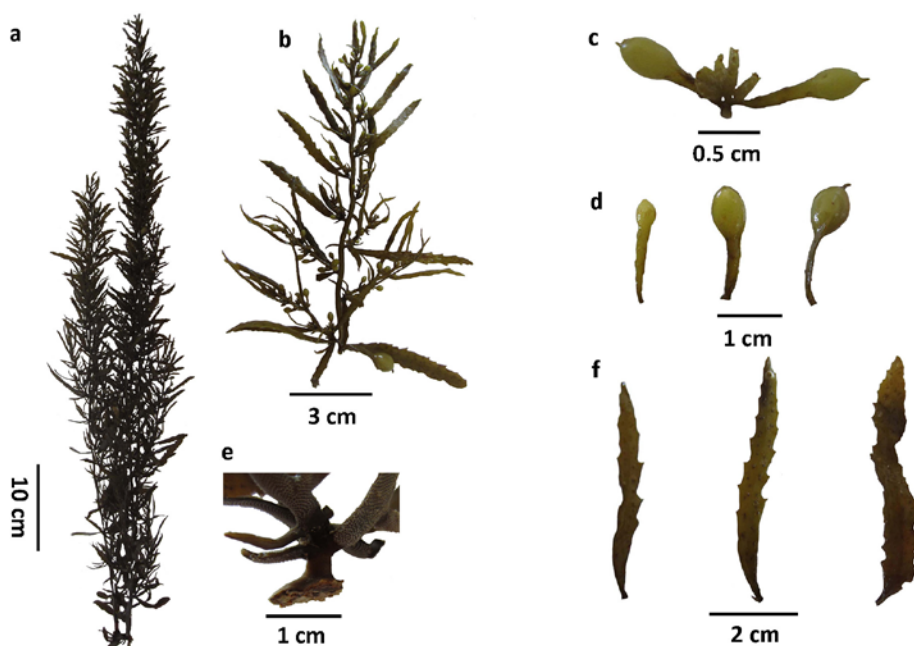


Fig. 4.32. *Sargassum swartzii* C. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum tenerimum* J. Agardh (1848: 305)**

Type locality: Bombay, India

Type specimen: LD ?

Classification: Subgenus *Sargassum*, section uncertain

Synonym: *Carpacanthus tenerrimus* (J. Agardh) G.Marten

References for Vietnam: Pham H.H. (1969: 370, Fig. 3.66); Nguyen H.Dinh (1993: 174, Fig.122), Nguyen H.Dai (2007: 57-58, Fig. 32).

Other references: J. Agardh (1848: 305), Grunow (1915: 370), Setchell (1935: 8), Tseng & Lu (1988: 48-49).

Geographical distribution: South–East Asia, Australia, New Zealand

Habitat: Subtidal rocky shore

Representative material: NVT1351

Morphology: Thallus slender and soft, 30 – 50 cm high; holdfast conical, 0.5 – 0.8 cm in diameter; main axis cylindrical very short; secondary axes compressed, thin, 0.2 – 0.3 cm wide; cauline leaves oblong – lanceolate, about 4 – 6 cm long and 0.8 – 1.2 cm wide, thin; leaves oblong – lanceolate thin, soft, 3 – 4 cm long and 0.3 – 0.7 cm wide, margins irregularly serrate, midrib short and inconspicuous, cryptostomata small and arranged

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irregular on the leaf surface; vesicles spherical to elliptic, 0.2 – 0.3 cm in diameter, petioles cylindrical, about half the length of the vesicle, apex pointed or sometimes rounded; receptacles somewhat compressed, forming a dense cluster, bearing teeth near the apex, 0.2 – 0.3 cm long.

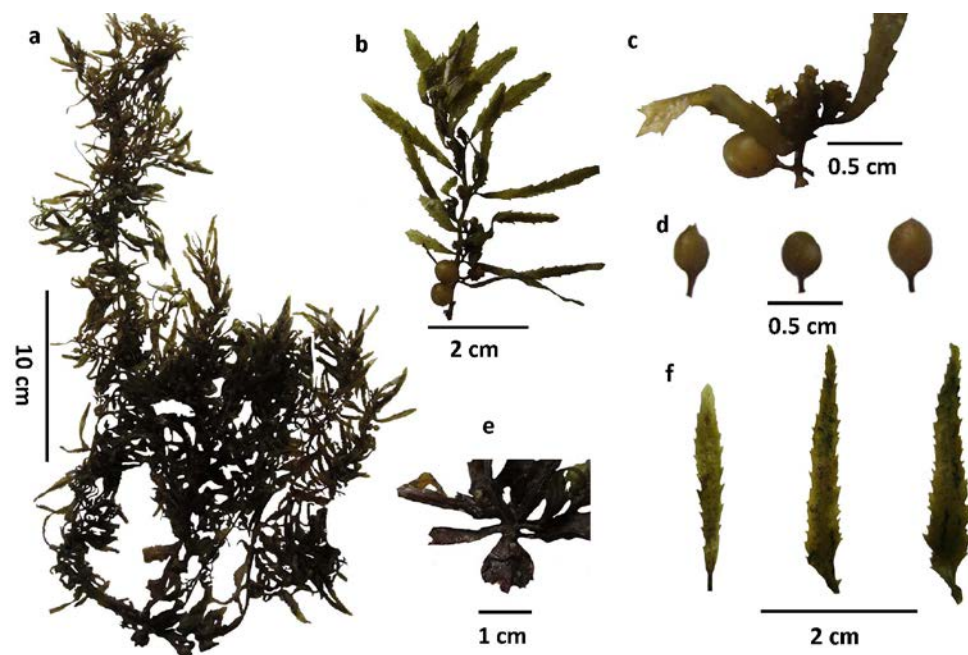


Fig. 4.33. *Sargassum tenerimum* C. Agardh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

***Sargassum vietnamense* Zinova & Nguyen H.Dinh**

Type locality: Tien Yen, Vietnam

Type specimen: Nguyen H.Dinh 69529 (Herbarium of the Institute of Oceanography, Nha Trang, Vietnam)

Classification: Subgenus *Sargassum*, section *Illicifolia*

Synonym:

References for Vietnam: Nguyen H.Dinh (1993: 179-180, Fig.128), Nguyen H.Dai (2007: 58-59, Fig. 33).

Geographical distribution: only describe from Vietnam

Habitat: growing on subtidal rocky shores

Representative material: NVT1057, NVT1483

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Morphology: Thallus 50 – 100 cm high; holdfast thin, discoid, lobed, up to 1 cm in diameter; main axis cylindrical, warty, 0.6 – 0.9 cm long and up to 0.2 cm in diameter; secondary axes cylindrical, smooth, 0.1 – 0.15 cm in diameter; lower leaves obovate or obtuse, apex rounded, upper leaf linear, leaf 2 – 4 cm long and 0.6 – 1.5 cm wide, margins irregularly serrate, midrib percurrent, cryptostomata conspicuous and scattered over the leaf surface; vesicles spherical to elliptic, 0.2 – 0.3 cm in diameter, bearing some spines, petioles cylindrical or compressed; receptacles cylindrical, branched, mixed with vesicles, up to 1.4 cm long.

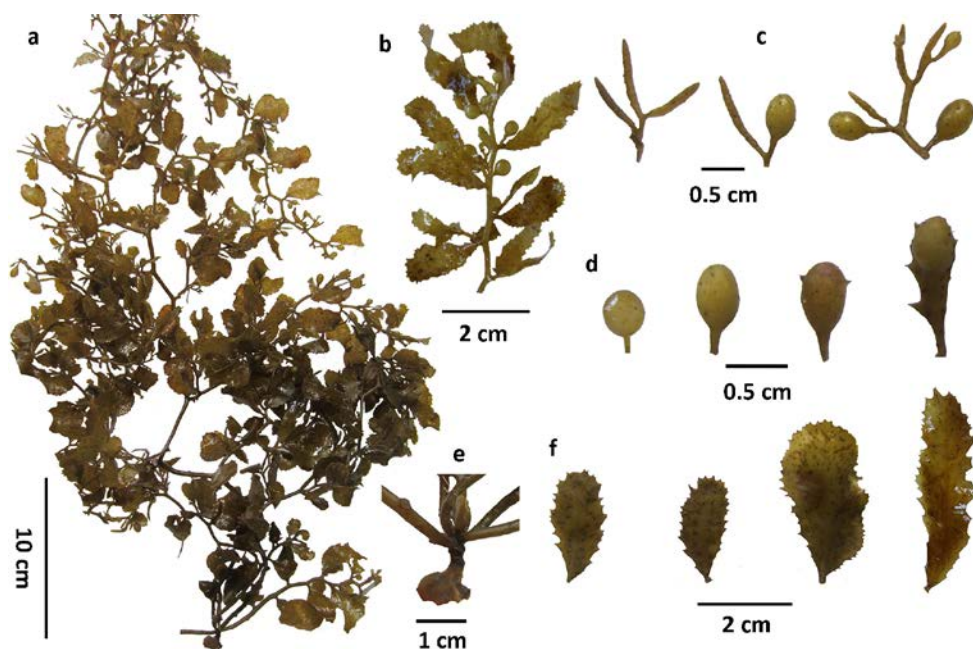


Fig. 4.34. *Sargassum vietnamense* Zinova & Nguyen H.Dinh: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scare bars: detailed in the figure.

Specimen tentatively be assigned to existing species:

***Sargassum cf. kuetzingii* Setchell**

Type locality: Macao, China

Type specimen: UC ?

Classification: Subgenus *Sargassum*, section *Binderiana*

References for Vietnam: Pham H.H. (1969: 392-393, Fig.387), Nguyen H.Dai (2007: 80-81, Fig. 49)

Other references: Setchell (1931: 249), Tseng & Lu (1992: 27-29), Tseng & Lu (2000: 120)

Geographical distribution: Vietnam, China, Taiwan

Habitat: on subtidal rocky shores

Representative material: NVT1281

Morphology: Thallus 20 – 30 cm high; holdfast discoid, up to 1 cm in diameter; main axis cylindrical, smooth, short, producing 5 secondary axes; secondary axes cylindrical, warty, up to 0.15 cm in diameter; leaves thick, narrow linear to cylindrical, bearing spine-like protuberance, 2.5 – 4 cm long and up to 0.2 cm wide, margins absent, midrib absent; cryptostomata inconspicuous; vesicles few, obovate, 0.3 – 0.4 cm in diameter, petioles cylindrical and warty, petioles of the same length as the vesicles; receptacles not observed.

Taxonomic note: Our specimen has no receptacles and the leaves are very narrow, nearly cylindrical. Although bearing a lot of resemblance, we are not entirely confident to assign the specimen to *S. kuetzingii* Setchell.



Fig. 4.35. *Sargassum cf. kuetzingii* sSetchell: thallus (a), apex branch (b), vesicles (c), leaves (d), holdfast (e). Scare bars: detailed in the figure.

***Sargassum cf. longifructum* Tseng & Lu (1987: 516)**

Type locality: China

Type specimen: 551767(AST)

Classification: Subgenus *Sargassum*, section *Sargassum*

References for Vietnam: Nguyen H.Dai (2007: 51-53, Fig. 28), Ajisaka et al. (1995: 45-53)

Other references: Tseng & Lu (1988: 43-44), Ajisaka et al. (1995: 23-30), Yoshida (1998: 389)

Geographical distribution: Vietnam, China

Habitat: subtidal rocky shores.

Representative material: NVT1314.

Morphology: Thallus up to 55 cm high; holdfast discoid, up to 1.3 cm in diameter, primary axis rough, 1.2 – 1.5 cm long, bearing up to 5 secondary axes; secondary axes somewhat compressed, smooth, 0.15 – 0.2 cm wide; cauline leaves linear, obtuse at the apex, up to 6.5 cm long and 0.4 cm wide, margins entire or wavy dentate; leaves linear, obtuse or acute at the apex, 2 – 4 cm long 0.2 – 0.3 cm wide, margins sparse serrate to irregularly serration, midrib conspicuous and percurrent, cryptostomata scattered over leaves surfaces; vesicle spherical to elliptical, up to 0.65 cm long and 0.25 – 0.35 cm wide, petioles cylindrical equal ½ vesicle length; receptacle cylindrical, branched several times, up to 2 cm long and 0.1 cm in diameter.

Taxonomic note: This specimen differs from the description of *S. longifructum* by Tseng & Lu (1987) in the primary axis and the width of the leaves. The primary axis of this specimen is not “terete” and up to 2.7 cm long as described by Tseng & Lu.

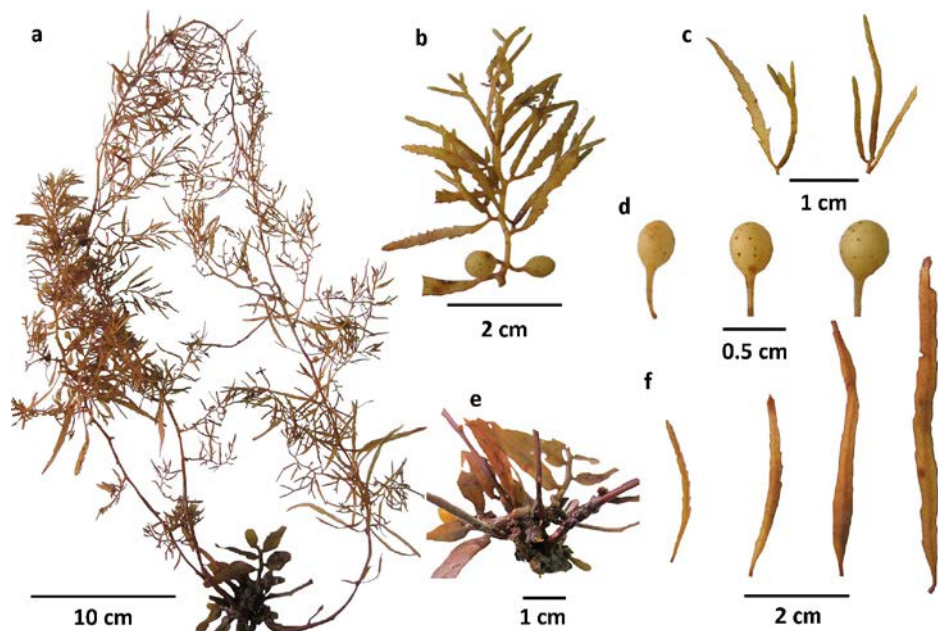


Fig. 4.36. *Sargassum longifructum* Tseng et Lu: thallus (a), apex branch (b), receptacles (c), vesicles (d), holdfast (e), leaves (f). Scale bars: detailed in the figure.

4.3.2 Molecular systematic of Vietnamese *Sargassum* (at section levels)

A total of 164 ITS-2 sequences were aligned. The alignment contained 756 positions, including gaps. NJ, ML and BI analyses produced tree topologies which only differed in unsupported relationships. Fig. 4.1 displays the consensus tree resulting from the Bayesian analysis with support values indicated for each method (BI/ML/NJ). The tree only displays the topology up to section level. Results showed three subgenera *Arthrophyucus*, *Bactrophyucus* and *Sargassum* as strongly supported for only two groups due to the intermixed of *Sargassum* species between *Arthrophyucus* (*S. sinclairii*) and *Bactrophyucus* (*S. nigrifolium*, *S. siliquastrum*) subgenera. The subg. *Sargassum* always formed a monophyletic group subdivided into nine strongly supported clades, representing the named sections *Binderiana*, *Ilicifolia*, *Johnstonii*, *Lapazeanum*, *Polycystae*, *Sargassum*, *Sinicola* and *Zygocarpicae* and one presumably unnamed section (Fig. 4.1). Sequences for the Vietnamese specimens were distributed among section *Binderiana*, *Ilicifolia*, *Polycystae*, *Sargassum* and one unnamed section. Detail on diversity within the various sections is provided section by section in Figs. 4.2 – 4.6.

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Subg. *Bactrophyucus* and *Athrophyucus* contained no Vietnamese specimens (Fig. 4.1). All Vietnamese specimens clustered in the subg. *Sargassum*. In section *Binderiana* we find specimens identified as *S. aquifolium*, *S. feldmanii*, *S. oligocystum*, *S. cf. kuetzingii*, *S. swartzii* and this section included additional sequences from Japan, Malaysia, Philippines, Vietnam, New Zealand, New Caledonia and Hawaii (Fig. 4.2). The section *Illicifolia* contained specimens identified as *S. aquifolium*, *S. angustifolium*, *S. bicorne*, *S. bulbiferum*, *S. carpophyllum*, *S. gracillimum*, *S. ilicifolium*, *S. henslowianum*, *S. herklotsii*, *S. heterocystum*, *S. microcystum*, *S. mcclurei*, *S. quinhonense*, *S. serratum*, *S. vietnamense* and *S. cf. turbinarioides* (Fig. 4.3). Section *Polycystae* contained specimens identified as *S. cotoense*, *S. gracillimum*, *S. polycystum*, *S. phamhoangii* and *S. serratum* (Fig. 4.4). Section *Sargassum* contained specimens identified as *S. carpophyllum* var. *nhatrangense*, *S. graminifolium*, *S. piluliferum* var. *serratifolium*, *S. cf. longifructum*, *S. cf. marginatum* (Fig. 4.5). Three species identified as *S. piluliferum* var. *serratifolium*, *S. distichum* and *S. tenerrimum* cluster in a separate clade, indicative of a new section (Fig. 4.6). ITS2 sequences of Vietnamese specimens are not present in the sections *Zygocarpicae*, *Johnstonii*, *Lapazeanum* and *Sinicola*.

Resolution within each section differed among morphospecies. In most cases all specimens were resolved as a single clade showing no variation among the specimens (e.g. *Sargassum* sect. *Binderiana*). In other instances, morphospecies were resolved as clusters well-separated from other such clusters (e.g. *Sargassum* sect. *Zygocarpicae*).

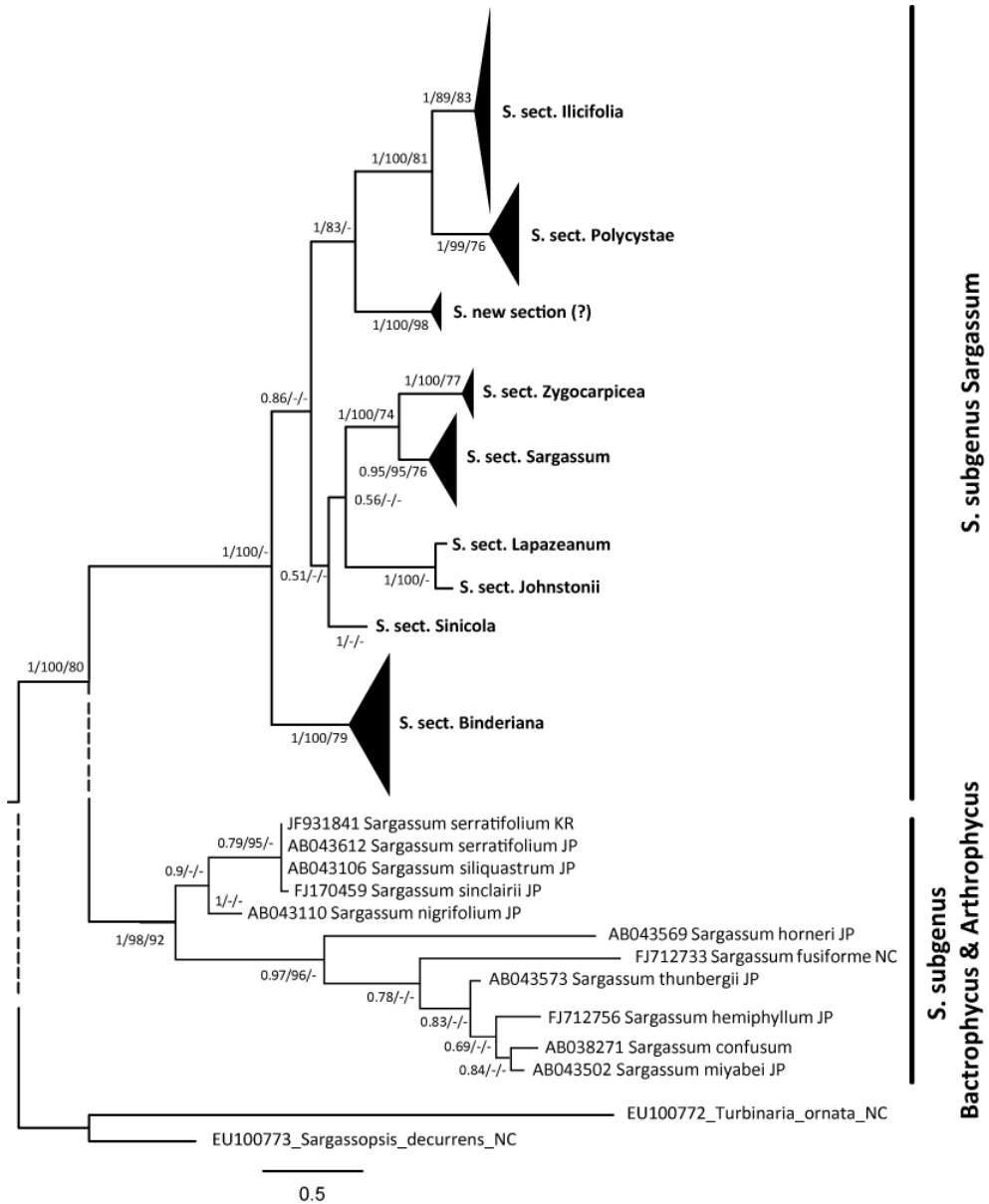


Fig. 4.1. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of *Sargassum* subg. *Bactrophyucus* and *Arthrophyucus*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%). Country origin of sequences from Genbank: JP, Japan; KR, Korea; NC, New Caledonia.

Sargassum sect. *Binderiana*

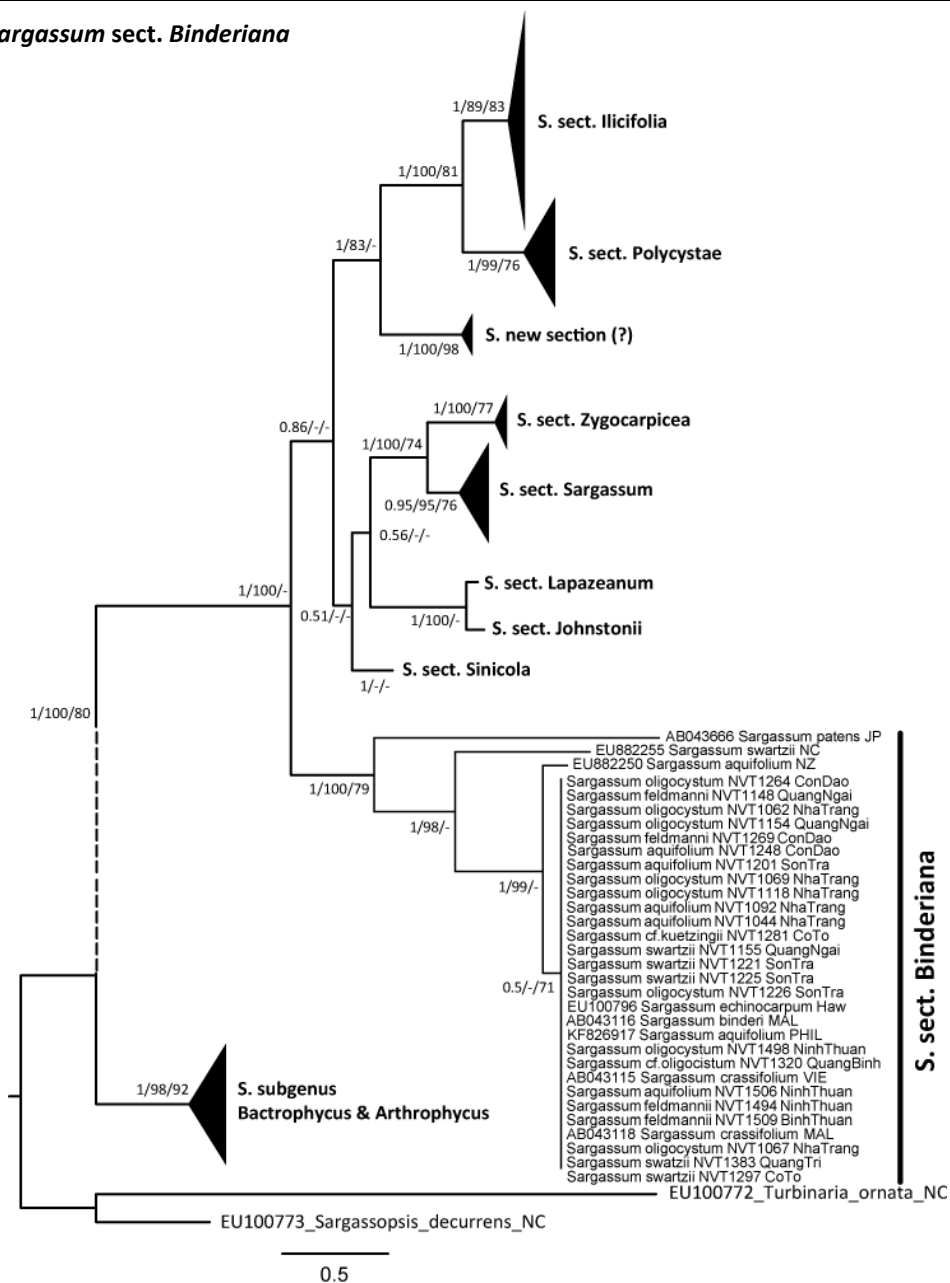


Fig. 4.2. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of *Sargassum* subg. *Sargassum* sect. *Binderiana*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%). Country origin of sequences from Genbank: JP, Japan; Haw, Hawaii; MAL, Malaysia; NC, New Caledonia; NZ, New Zealand; PHIL, Philippines; VIE, Vietnam.

Sargassum sect. *Ilicifolia*

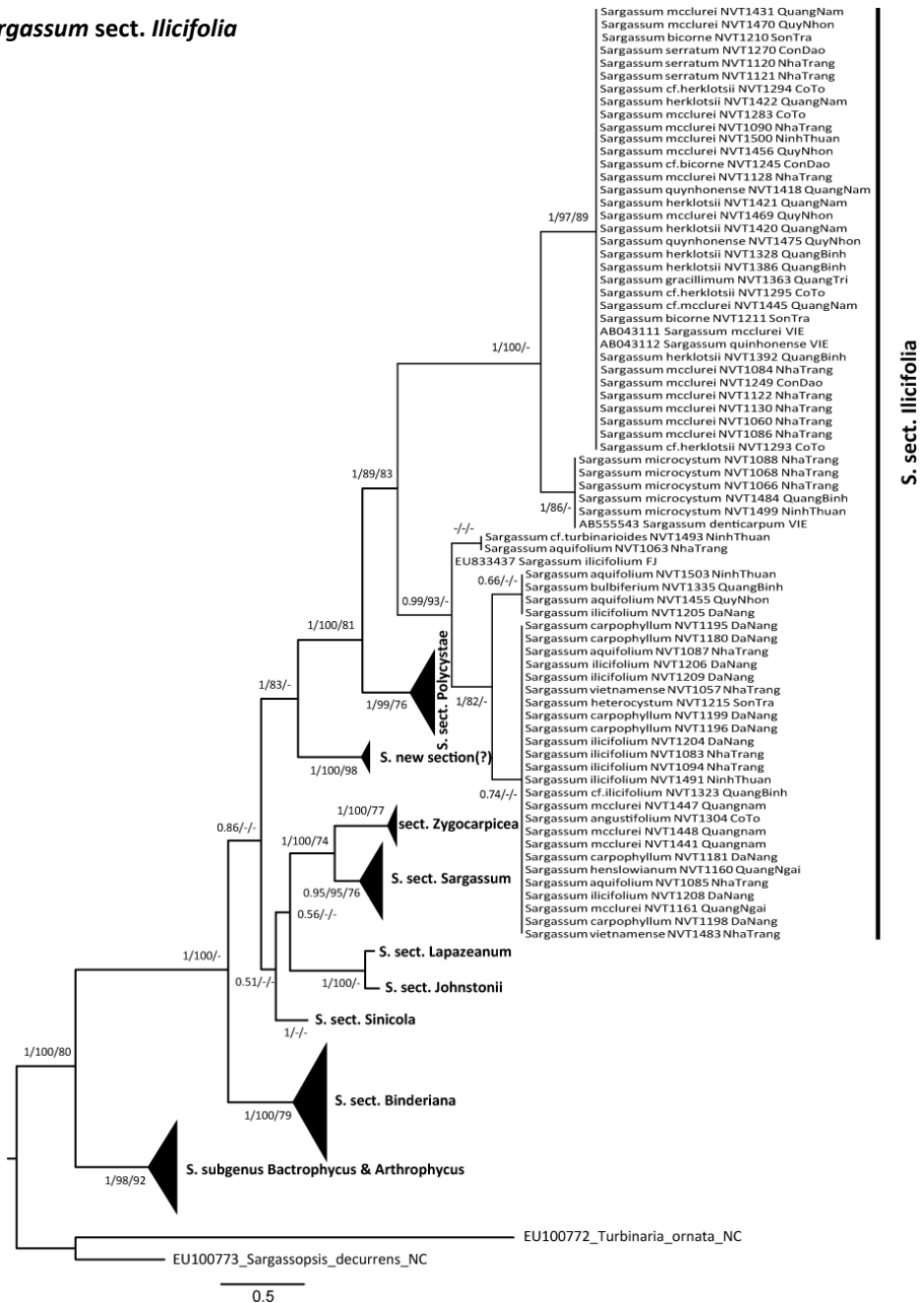


Fig. 4.3. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of *Sargassum* subgenus *Sargassum* sect. *Ilicifolia*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%). Country origin of sequences from Genbank: FJ, Fiji; VIE, Vietnam.

Sargassum sect. *polycystae*

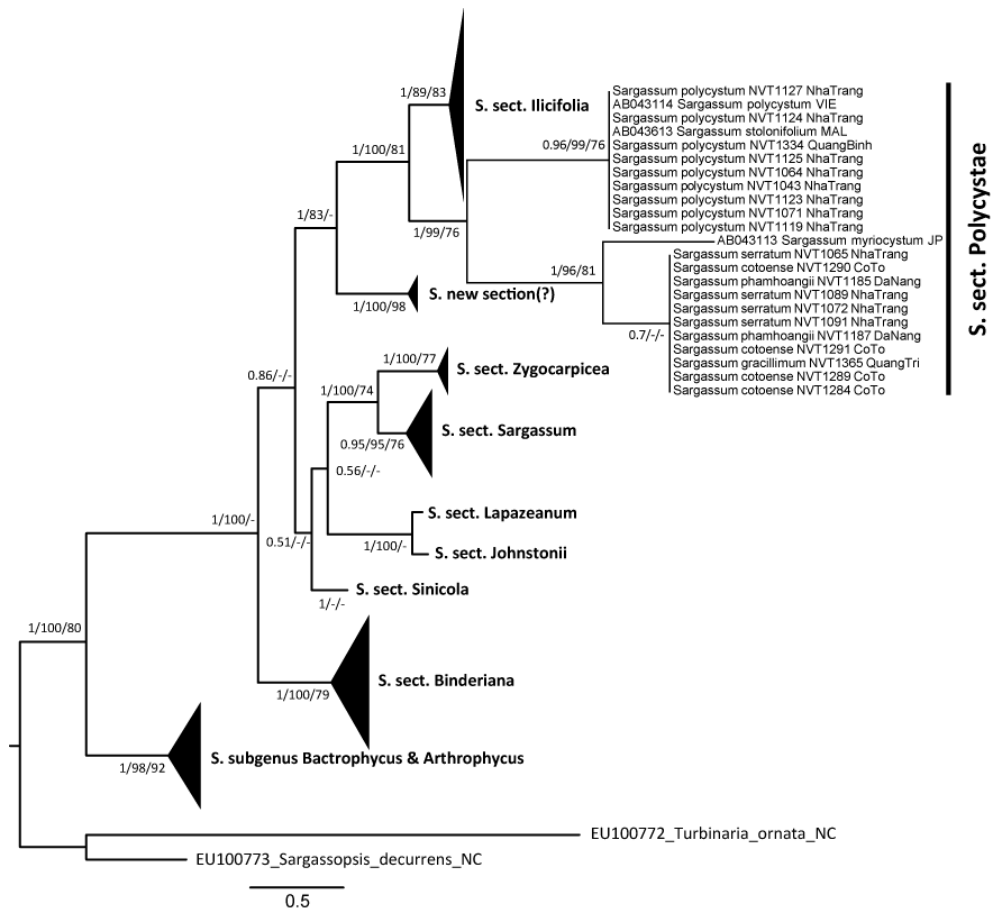


Fig. 4.4. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of *Sargassum* subg. *Sargassum* sect. *Polycystae*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%). Country origin of sequences from Genbank: JP, Japan; MAL, Malaysia; VIE, Vietnam.

Sargassum sect. *Sargassum*

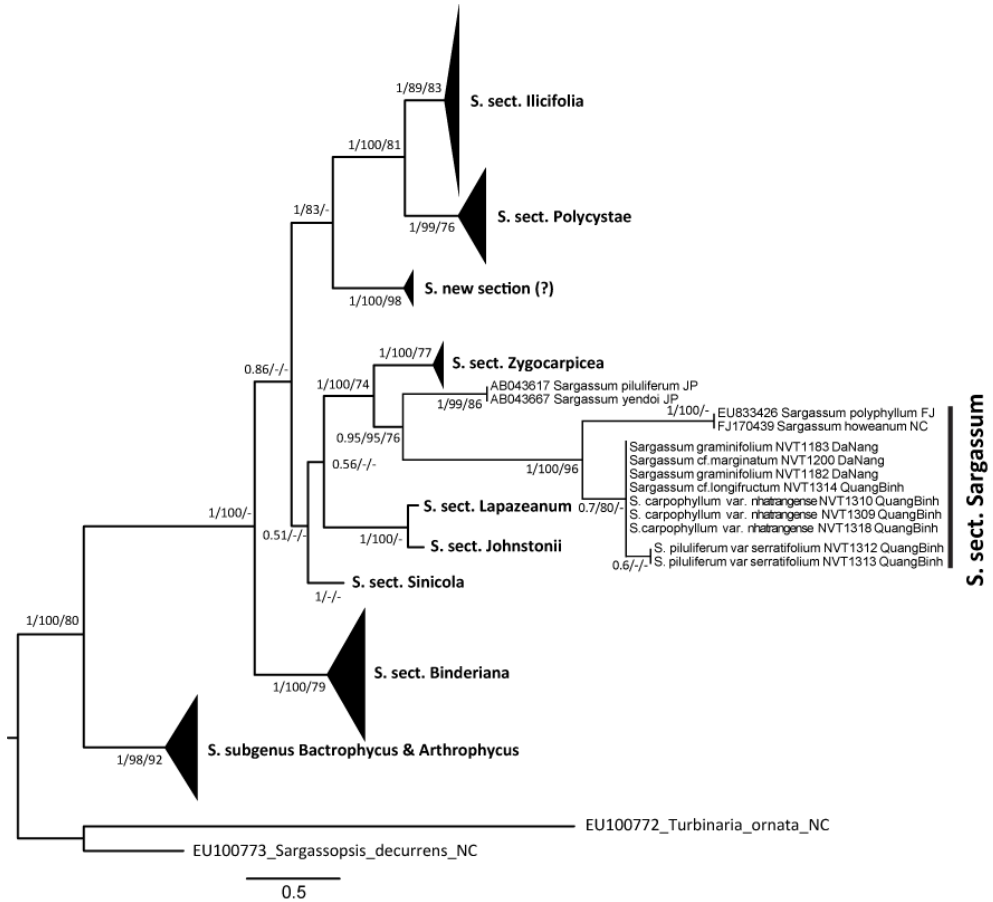


Fig. 4.5. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of *Sargassum* subg. *Sargassum* sect. *Sargassum*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%). Country origin of sequences from Genbank: JP, Japan; NC, New Caledonia.

Sargassum new section (?)

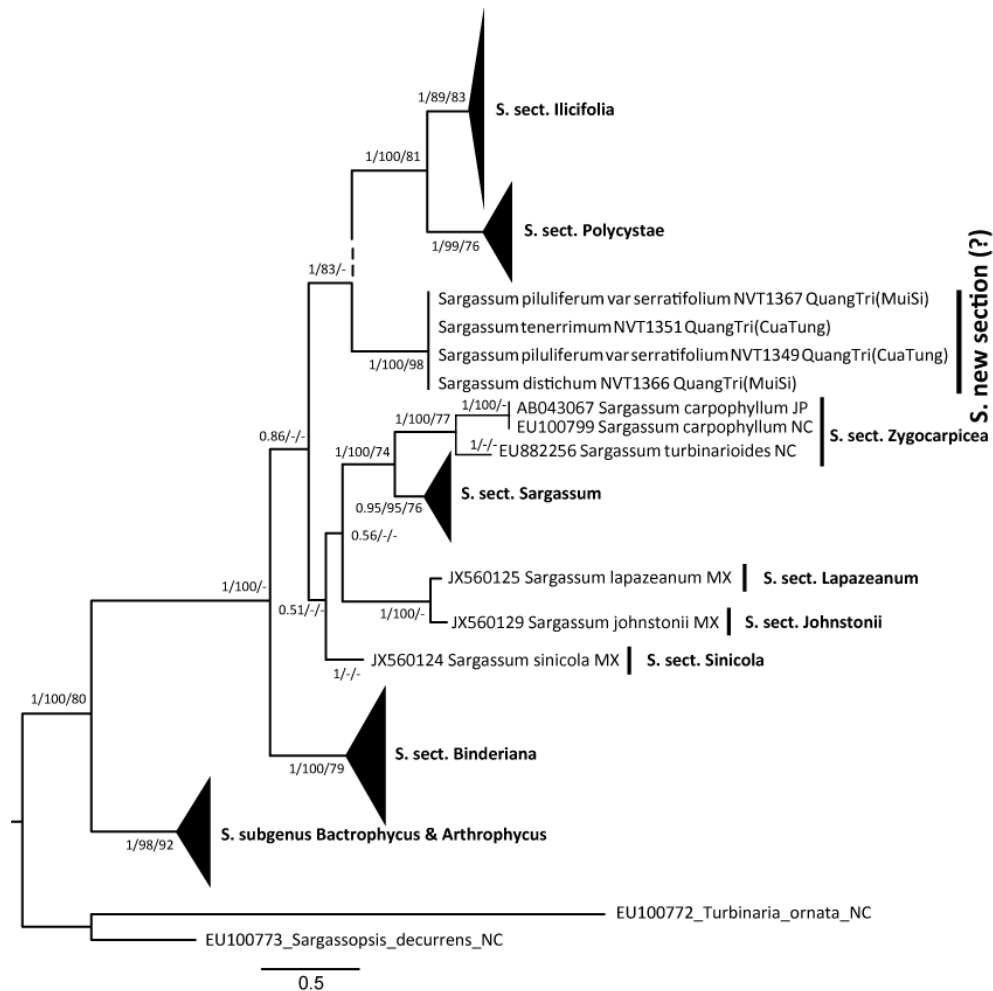


Fig. 4.6. Bayesian tree resulting from ITS rDNA gene sequence analyses, detailing the sampling of the unnamed section in *Sargassum* subg. *Sargassum*. Numbers below the branches represent BI posterior probabilities (left, indicated when >0.5), ML bootstrap values (centre, indicated when >50%), NJ bootstrap values (right, indicated when >50%).

4.5 Discussion

Molecular sequence data are increasingly becoming the standard for species delineation and algal diversity assessment (Leliaert et al. 2014). A series of papers have applied molecular methodologies combined with morphological observations to the *Sargassaceae* (Mattio et al. 2008a, 2008b, 2009a, 2009b, 2010, 2013, Mattio and Payri 2009, 2010, Mattio and Bolton 2011, Dixon et al. 2012).

ITS2, *cox3* and *rbcl-S* are the most frequently used markers for species delineation in *Sargassum*. The ITS2 rDNA marker was the locus with the highest amplification success and proved to be best for species delineation according to Mattio and Payri (2010) and Dixon (2014). Hence our reliance on this marker in the present study, which is the first to attempt an assessment of *Sargassum* diversity from Vietnam. Several studies have addressed the diversity of *Sargassum* from Vietnam in the past, but only using purely morphological observations (Pham H.H. 1969, Nguyen H.Dai 1997, Nguyen H.Dinh and Huynh Q.N. 1999, 2001, Nguyen H.Dai 2007). Below we discuss the outcome of our analyses.

At the subgenus levels it is evident that the subgenera *Arthrophyucus* and *Bactrophyucus* are not present in our dataset. These two subgenera had previously been reported for Vietnam by Pham H.H (1969) and Nguyen H.Dai (1997). The following species, belonging to subg. *Bactrophyucus*, were previously reported from Vietnam: *Sargassum confusum* C. Agardh, *S. hemiphyllum* (Turner) C. Agardh, *S. horneri* (Turner) C. Agardh, *S. kjellmanianum* Yendo, *S. nigrifolium* Yendo, *S. nipponicum* Yendo, *S. tortile* (C. Agardh) C. Agardh. Their presence however had already been doubted by Yoshida et al. (2002), who re-examined several of the specimens on which these reports were based. Likewise, our analysis did not result in a confirmed record of any species belonging to subg. *Bactrophyucus*, thereby confirming the suspicion of Yoshida et al. (2002). It is of course possible that these particular species are present along the Vietnamese coastline, but were not collected in the present study. However, since subg. *Bactrophyucus* is confined to temperate regions in Japan and Korea (Yoshida et al. 2002), its presence on the tropical shores of Vietnam would entail a significant range extension. With respect to the subg. *Arthrophyucus*, Nguyen H.Dai (1997) listed *S. herklotsii*, *S. mcclurei* and *S. quinhonense* as present in Vietnam. Although we identified several specimens as belonging to those three species from Vietnam and the morphological identifications were confirmed by Nguyen H.Dai, our phylogeny does not support the placement of these specimens in the subg. *Arthrophyucus*. Instead specimens

were resolved in the subg. *Sargassum* section *Illicifolia*. Previously, Nguyen H.Dai (2007) had classified these same three species in the subg. *Bactrophycus* section *Phyllocystae*. Again our results would contest this taxonomic placement. Moreover, our results are in agreement with Mattio et al. (2010) who classified *S. mcclurei* and *S. quinhonense* in *S.* subg. *Sargassum* section *Illicifolia*. Our dataset included four specimens which morphologically conform to *S. piluliferum* var. *serratifolium*. The latter was once included in *Sargassum* subg. *Phyllotricha* which has recently been elevated to genus level by Dixon et al. (2012). Previous studies have shown that the *S. piluliferum* var. *serratifolium* doesn't belong to *Phyllotricha* since molecular sequences resolved it within *Sargassum* sub. *Sargassum* (Stiger et al. 2003, Yoshida et al. 2004, Mattio and Payri 2009). Our analyses confirm these results and point toward the absence of *Phyllotricha* from Vietnam. From biogeographical point of view also this makes sense, since all species of *Phyllotricha* are endemic to Australia and New Zealand.

Given our sampling, only *Sargassum* subg. *Sargassum* appears to be present in Vietnam. At section level, our morphospecies clustered in five sections of subg. *Sargassum*. However, four morphospecies appear in more than one section: *S. aquifolium* is present in sect. *Illicifolia* and *Binderiana*, *S. serratum* and *S. gracillimum* are present in the sect. *Illicifolia* and *Polycistae*, *S. piluliferum* var. *serratifolium* is present in the sect. *Sargassum* and a hitherto unnamed section. At this stage we can explain such counterintuitive results either by natural hybridization or cryptic speciation. Hybridization and introgression between closely related species has been described for many algae (e.g. *Gracilaria* (Destombe et al. 2010), *Porphyra* (Lindstrom 2009), *Fucus* (Coyer et al. 2002a, 2002b, Coyer et al. 2004) and may result in discordance between gene trees. Usually however hybrids are formed between closely related species. In the abovementioned cases putative hybrids would have resulted from crossing of specimens belonging to different sections. In addition, ITS2 sequences were always readily obtained without the need for cloning, which points toward the absence of recently formed hybrids. Under the latter scenario multiple divergent copies of the ITS2 are expected. The combination of both factors makes the hybridization hypothesis less likely. As alternative, the species pairs that were resolved in different sections could represent cryptic species. The presence of species that are well-differentiated but morphologically very difficult to tell apart is very common among algae (reviewed in De Clerck et al. 2013). Likely, the genus *Sargassum* contains many cryptic species. In addition uncertainty regarding the taxonomic value of certain morphological characters is likely to

confound species delineation and morphological identifications (Kilar et al. 1992). Molecular phylogenies resulted in trees with well-supported sections with posterior probabilities higher than 0.95 and bootstrap values over 70%. Three morphospecies *S. distichum*, *S. tenerimum* and *S. piluliferum* var. *serratifolium* (NVT1349, NVT1367) clustered in a separate clade for which appears an unnamed section. Detailed comparative morphological analyses and sequencing of complementary genes should make clear if this clade can be considered a new section of the *Sargassum* subg. *Sargassum* (Fig. 4.8).

For the case of *S. carpophyllum*, this species has been classified in the sect. *Zygocarpicea*, but according to our molecular analysis this species belongs in sect. *Illicifolia*. Interestingly, *S. carpophyllum* var. *nhatrangense* is resolved in sect. *Sargassum*. The latter was first described as variety of *S. piluliferum*, but Yoshida (1997) transferred it to a variety of *S. carpophyllum*. Our results show that this transfer is unwarranted and *S. carpophyllum/piluliferum* var. *nhatrangense* should be classified in sect. *Sargassum* (Fig. 4.5).

On the species level, it is evident that our morphospecies exhibit very low molecular polymorphism. Many ITS2 sequences from different morphospecies are virtually identical. Very similar observations were made by Mattio (Mattio et al. 2008b, 2010, Mattio and Payri 2009) and Cho et al. (2012) for morphospecies in subg. *Sargassum* and Dixon et al. (2012) for the related genus *Phyllotricha*. The fact that ITS2 sequences are virtually identical can point toward two facts. First, species diversity in the genus *Sargassum* has been highly overestimated and the ITS2 cluster correspond the good biological species which are highly polymorphic. Alternatively, *Sargassum* presents an example of a recent radiation, whereby many species complex are young and ITS2 does not provide resolution to distinguish the various species. In that sense *Sargassum* would resemble *Fucus*, for which traditional genes used in phylogenetic studies (e.g. ITS rDNA, *cox3*, *rbcl*, etc.) provide little or no resolution either. Only by using highly polymorphic nuclear encoded microsatellite markers have species boundaries satisfactorily been defined in the latter genus. At this stage, for *Sargassum* we cannot outrule one of both scenarios. Development of nuclear markers is a prerequisite to address this question.

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REFERENCES

- Abbott, I. A. and J. M. Huisman. 2004. Marine Green and Brown Algae of the Hawaiian Islands. Bishop Museum Bulletins in Botany. pp. 255
- Agardh, C. A. 1820. Species algarum ... Vol. 1, part 1. Lundae [Lund]. pp. 168.
- Agardh, C. A. 1824. Systema Algarum. Lundae [Lund]. XXXVIII Literis Berlingianis [Berling]. pp. 312.
- Agardh, J. G. 1848. Species genera et ordines algarum ... Volumen primum: algas fucoideas complectens. Lundae [Lund], **8**. pp. 363.
- Agardh, J. G. 1889. Species Sargassorum Australiae descriptae et dispositae. Kongl. Svenska Vetenskaps Akademiens Handlingar, [ser. 4], **23**(3). pp. 133
- Ajisaka, T., H. Q. Nang, N. H. Dinh, L. Baoren, P. O. Ang Jr, P. S. Moi, T. Noro, and T. Yoshida. 1997a. Taxonomic and nomenclature study of *Sargassum duplicatum* Bory and related species. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **6**, California Sea Grant College Program, La Jolla, CA. pp. 27-36.
- Ajisaka, T., Q.N. Huynh, H.D. Nguyen, B. R. Lu, C. Y. M., and T. Yoshida. 1997b. *Sargassum hemiphyllum*(Turnner) C. Agardh var. *chinensis* J.Agardh from Vietnam, the Chinese mainland, and Taiwan. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **6**, California Sea Grant College Program, La Jolla, CA. pp. 37-49.
- Ajisaka, T., H. Q. Nang, N. H. Dinh, and T. Yoshida. 1997c. *S. carpophyllum* J. Agardh var. *nhatrangense* (Pham) Ajisaka comb. Nov. and *S. piluliferum* (Turner) C. Agardh var.*serratifolium* Yamada from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **6**, California Sea Grant College Program, La Jolla, CA. pp. 51-60.
- Ajisaka, T., T. Noro, and T. Yoshida. 1995. Zygozarpic *Sargassum* species (Subgenus *Sargassum*) from Japan. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **5**, California Sea Grant College Program, La Jolla, CA. pp. 11-44.
- Andrade-Sorcía, G., R.Riosmena-Rodríguez, R.Muñiz-Salazar, J.M.López-Vivas, G.H.Boo, K.M.Lee, and S.M.Boo. 2014. Morphological reassessment and molecular assessment of *Sargassum* (Fucales: Phaeophyceae) species from the Gulf of California, México. *Phytotaxa* **183**: 201-223.
- Chan, S. W., C. C. Cheang, C. W. Yeung, A. Chirapart, G. Gerung, and P. Ang. 2014. Recent expansion led to the lack of genetic structure of *Sargassum aquifolium* populations in Southeast Asia. *Marine Biology* **161**: 785-795.

- Cheang, C. C., K. H. Chu, and P. O. Ang. 2010. Phylogeography of the marine macroalga *Sargassum hemiphyllum* (Phaeophyceae, Heterokontophyta) in northwestern Pacific. *Molecular Ecology* **19**: 2933-2948.
- Cho, S. M., S. M. Lee, Y. D. Ko, L. Mattio, and S. M. Boo. 2012. Molecular systematic reassessment of *Sargassum* (Fucales, Phaeophyceae) in Korea using four gene regions. *Botanica Marina* **55**: 473-484.
- Coyer, J. A., G. Hoarau, W. T. Stam, and J. L. Olsen. 2004. Geographically specific heteroplasmy of mitochondrial DNA in the seaweed, *Fucus serratus* (Heterokontophyta : Phaeophyceae, Fucales). *Molecular Ecology* **13**: 1323-1326.
- Coyer, J. A., A. F. Peters, G. Hoarau, W. T. Stam, and J. L. Olsen. 2002a. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta : Phaeophyceae) in a 100-year-old zone of secondary contact. *Proceedings of the Royal Society B-Biological Sciences* **269**: 1829-1834.
- Coyer, J. A., A. F. Peters, G. Hoarau, W. T. Stam, and J. L. Olsen. 2002b. Inheritance patterns of ITS1, chloroplasts and mitochondria in artificial hybrids of the seaweeds *Fucus serratus* and *F. evanescens* (Phaeophyceae). *European Journal of Phycology* **37**: 173-178.
- Dawson, E. Y. 1954. Marine plants in the Vincinity of the Institute Oceanography of Nha Trang. *Pacific science* **8**: 373-471.
- De Clerck, O., M. D. Guiry, F. Leliaert, Y. Samyn, and H. Verbruggen. 2013. Algal taxonomy: a road to nowhere? *Journal of Phycology* **49**: 215-225.
- De Clerck, O., F. Leliaert, H. Verbruggen, C. E. Lane, J. C. De Paula, D. A. Payo, and E. Coppejans. 2006. A revised classification of the *Dictyoteae* (*Dictyotales*, Phaeophyceae) based on *rbcl* and 26S ribosomal DNA sequence analyses. *Journal of Phycology* **42**: 1271-1288.
- De Toni, G. B. 1895. *Sylloge algarum ... Vol. III. Fucoideae*. Patavii [Padova]. 8. pp. 638
- Destombe, C., M. Valero, and M. L. Guillemain. 2010. Delineation of two sibling red algal species, *Gracilaria gracilis* and *Gracilaria dura* (*Gracilariales*, Rhodophyta), using multiple DNA markers: Resurrection of the species *G. dura* previously described in the Northern Atlantic 200 years ago. *Journal of Phycology* **46**: 720-727.
- Dixon, R. R. M., J. M. Huisman, J. Buchanan, C. F. D. Gurgel, and P. Spencer. 2012. A morphological and molecular study of Austral *Sargassum* (Fucales, Phaeophyceae) supports the recognition of *Phyllotricha* at genus level, with further additions to the genus *Sargassopsis*. *Journal of Phycology* **48**: 1119-1129.
- Felsenstein, J. 1985. Confidence-Limits on Phylogenies - an Approach Using the Bootstrap. *Evolution* **39**: 783-791.
- Ganzon-Fortes, E. T. 2012. A historical account of biodiversity studies on Philippines seaweeds(1800-1999). *Coastal Marine Science* **35**: 182-201.
- Garrigue, C. and R. T. Tsuda. 1988. Catalog of the benthic algae from New Caledonia. *Micronesica* **268**: 53-70.
- Gavino C. Trono, J. 1992. The genus *Sargassum* in the Philippines. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific and Western Atlantic species* **3**, California Sea Grant College Program, La Jolla, CA. pp. 43-94.
- Grunow, A. 1915. Additamenta ad cognitionem Sargassorum. *Verhandlungen der Kaiserlich Königlichen Zoologisch Botanischen Gesellschaft in Wien* **65**: 329 - 448

- Grunow, A. 1916. Additamenta ad cognitionem Sargassorum. Verhandlungen der Kaiserlich Königlich Zoologisch Botanischen Gesellschaft in *ibid.* 66: 1 - 48, 136 - 185.
- Guiry, M. D. and G. M. Guiry. 2014. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on May 2014.
- Huynh, Q.N. and H.Dinh Nguyen. 1998. The Seaweed Resources of Vietnam. In: (Critchley, A.T. and M. Ohno Eds) *Seaweed Resources of the World*. Kanagawa International Fisheries Training Centre, JICA. pp. 62-69.
- Isao, T., Q.N. Huynh, H.D. Nguyen, A. Shogo, and Y. Tadao. 2005. *The common marine plants of southern Vietnam*. Usa: Japan Seaweed Association. pp. 250
- Kilar, J. A., M. D. Hanisak, and T. Yoshida. 1992. On The expression of phenotypic variability: Why is *Sargassum* so Taxonomically difficult? In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific and Western Atlantic species 3*, California Sea Grant College Program, La Jolla, CA. pp. 95-118.
- Le N.H., M.L. Bui, D.T. Vo, B.K. Nguyen, X.M. Vo, Q.T. Truong and H.Dai Nguyen. 2009. Mùa vụ rong mơ ở tỉnh Khánh hòa-Việt Nam (*Sargassum* season of Khanh Hoa Province - Vietnam). Proceeding of the 3rd National Scientific Conference on Ecology and Biological Resources: 958 - 961.
- Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. Lopez-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA- based species delimitation in algae. *European Journal of Phycology* **49**: 179-196.
- Lindstrom, S. 2009. Cryptic diversity, biogeography and genetic variation in Northeast Pacific species of *Porphyra sensu lato* (Bangiales, Rhodophyta). In (M. Borowitzka, A. Critchley, S. Kraan, A. Peters, K. Sjøtun, and M. Notoya, Eds) *Nineteenth International Seaweed Symposium*. Springer Netherlands. pp. 501-512
- Magruder, W. H. 1988. *Sargassum* (Phaeophyta, Fucales, *Sargassaceae*) in the Hawaiian Islands. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 59-63.
- Mattio, L. and J. Bolton. 2011. Reappraisal of the *Sargassum* (Fucales) Diversity and distribution in the Southwest Indian Ocean. *European Journal of Phycology* **46**: 65-66.
- Mattio, L., G. Dirberg, C. Payri, and S. Andrefouet. 2008a. Diversity, biomass and distribution pattern of *Sargassum* beds in the South West lagoon of New Caledonia (South Pacific). *Journal of Applied Phycology* **20**: 811-823.
- Mattio, L. and C. Payri. 2010. Assessment of five markers as potential barcodes for identifying *Sargassum* subgenus *Sargassum* species (Phaeophyceae, Fucales). *Cryptogamie Algologie* **31**: 467-485.
- Mattio, L. and C. E. Payri. 2009. Taxonomic revision of *Sargassum* species (Fucales, Phaeophyceae) from New Caledonia based on morphological and molecular analyses. *Journal of Phycology* **45**: 1374-1388.
- Mattio, L., C. E. Payri, and V. Stiger-Pouvreau. 2008b. Taxonomic revision of *Sargassum* (Fucales, Phaeophyceae) from French Polynesia based on morphological and molecular analyses. *Journal of Phycology* **44**: 1541-1555.
- Mattio, L., C. E. Payri, and M. Verlaque. 2009a. Taxonomic revision and geographic distribution of the subgenus *Sargassum* (Fucales, Phaeophyceae) in the Western and Central Pacific islands based on morphological and molecular analyses. *Journal of Phycology* **45**: 1213-1227.

- Mattio, L., C. E. Payri, M. Verlaque, and B. de Reviere. 2009b. New insights on the classification of the *Sargassum* subgenus *Sargassum* (Phaeophyceae, Fucales) from a three-DNA markers phylogeny and morphological analyses. *Phycologia* **48**: 83-83.
- Mattio, L., C. E. Payri, M. Verlaque, and B. de Reviere. 2010. Taxonomic revision of *Sargassum* sect. *Acanthocarpicae* (Fucales, Phaeophyceae). *Taxon* **59**: 896-904.
- Mattio, L., M. Zubia, B. Loveday, E. Crochelet, N. Duong, C. E. Payri, R. Bhagooli, and J. J. Bolton. 2013. *Sargassum* (Fucales, Phaeophyceae) in Mauritius and Réunion, western Indian Ocean: taxonomic revision and biogeography using hydrodynamic dispersal models. *Phycologia* **52**: 578-594.
- Montagne, C. 1842. Troisième centurie de plantes cellulaires exotiques nouvelles. Décades V, VI, VII et VIII. *Annales des Sciences Naturelles, Botanique, Seconde Série* **18**: 241-282.
- Nguyen, H.Dai 1980. The *Sargassum* in Hon Chong - Nha Trang. *Collection of Marine Research Works* **2**: 53-71.
- Nguyen, H.Dai 1997. *Sargassaceae of Vietnam, resources and utility*. Agriculture Publishing House, Hanoi. pp. 199
- Nguyen, H.Dai 2004. Two new species of the subgenus *Sargassum* (Fucales, *Sargassaceae*) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **9**, California Sea Grant College Program, La Jolla, CA. pp. 73-80.
- Nguyen, H.Dai. 2007. Bộ Rong Mơ (Fucales Kylin) In: *Thực vật chí Việt Nam (Flora of Vietnam)*. **11**, Science and Technology Publishing House, Hanoi. pp. 117.
- Nguyen, H.Dinh and Huynh Q.N. 1999. Some new taxa of *Sargassum* (Phaeophyta) from Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **7**, California Sea Grant College Program, La Jolla, CA. pp. 43-51.
- Nguyen, H.Dinh and Huynh Q.N. 2001. Five new species of the genus *Sargassum* from Vietnam. *Vietnamese Journal of Biology* **23**: 1-10.
- Nguyen, H.Dinh, Q.N. Huynh, N.B. Tran, and V.T. Nguyen 1993. *Marine algae of North Vietnam*. Science and Technology Publishing House. pp. 344.
- Nguyen, V.T., N.H. Le, S. M. Lin, F. Steen, and O. De Clerck. 2013. Checklist of the marine macroalgae of Vietnam. *Botanica Marina* **56**: 207-227.
- Oak, J. H. and I. K. Lee. 2005. Taxonomy of the genus *Sargassum* (Fucales, Phaeophyceae) from Korea I. Subgenus *Bactrophyucus* Section *Teretia*. *Algae* **20**: 77-90.
- Oak, J. H., Y. Suh, and I. K. Lee. 2002. Phylogenetic relationships of *Sargassum* subgenus *Bactrophyucus* (*Sargassaceae*, Phaeophyceae) inferred from rDNA ITS Sequences. *Algae* **17**: 235 - 247.
- Okamura, K. 1936. *Nippon kaisô shi [Descriptions of Japanese algae]*.Tokyo: Uchida Rokakuho. pp. 964
- Pham, H.H. 1967. Contribution à l'étude des algues littorales du Vietnam I: Le genre *Sargassum*. *Ann. Fac. Sci. Saigon*. pp. 259-332.
- Pham, H.H. 1969. *Marine Algae of South Vietnam (Rong biển miền nam Việt nam)*. Sai Gon study Center. Ministry of Education and Youth. pp.558.
- Pham, H.T. 1996. Góp phần nghiên cứu rong biển quần đảo Trường sa (Contribution to study on the seaweed of Truongsa achipelagos). *Collection of Marine Research Works* **7**: 147-162.
- Rambaut, A. and A. J. Drummond. 2007. Tracer ver. 1.4. Program available at <http://beast.bio.ed.ac.uk/Tracer>.

- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* **61**: 539-542.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671-675.
- Segawa, S. 1977. *The seaweed of Japan*. Hoikusa Publishing. pp. 195.
- Setchell, W. A. 1931. *Hong Kong seaweeds* II. *Hong Kong Naturalist* **2**: 237-253.
- Setchell, W. A. 1933. *Hong Kong Seaweeds* III. *Sargassaceae*. *Hong Kong Naturalist* **2**: 33-49.
- Setchell, W. A. 1935. The templeton crocker expedition to western Polynesian and Melanesian islands, 1933. No. 21. Some marine plants of southeastern Melanesia. *Proc. Calif. Acad. Sci. Ser. 4* **21**: 259 - 275.
- Shams, M. and O. D. Clerck. 2013. Evaluation of molecular and morphological in the *Sargassum binderi* and *S. oligocystum* (Fucales, *Sargassaceae*) in Iranian southern coasts. *International Journal of Research in Biological Sciences* **3**: 165-170
- Shimabukuro, H., M. Kawane, and M. Hamaguchi. 2012. New record of *Sargassum denticarpum* Ajisaka (Fucales, Phaeophyceae) from Iriomote Island (Ryukyu Archipelago, Japan). *Botanica Marina* **55**: 209-215.
- Silva, P. C., P. W. Basson, and R. L. Moe. 1996. Catalogue of the benthic marine algae of the Indian Ocean. University of California Publications in Botany **79**: 1-1259.
- Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*.
- Stiger, V., T. Horiguchi, T. Yoshida, A. W. Coleman, and M. Masuda. 2000. Phylogenetic relationships of *Sargassum* (*Sargassaceae*, Phaeophyceae) with reference to a taxonomic revision of the section *Phyllocystae* based on ITS-2 nrDNA sequences. *Phycological Research* **48**: 251-260.
- Stiger, V., T. Horiguchi, T. Yoshida, A. W. Coleman, and M. Masuda. 2003. Phylogenetic relationships within the genus *Sargassum* (Fucales, Phaeophyceae), inferred from ITS-2 nrDNA, with an emphasis on the taxonomic subdivision of the genus. *Phycological Research* **51**: 1-10.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.
- Tseng, C. K. 1983. *Common Seaweed of China*. Science Press, Beijing. pp. 316.
- Tseng, C. K. and L. Baoren. 1992. Studies on the Malacocarpic *Sargassum* of China: II. Racemosae J. Agardh. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Western Atlantic species* **3**, California Sea Grant College Program, La Jolla, CA. pp. 11-34.
- Tseng, C. K. and B. Lu. 2000. Flora algarum marinarum sinicarum Tomus III Phaeophyta No. II Fucales. Science Press, Beijing. pp.237
- Tseng, C. K. and B. R. Lu. 1988. Studies on Chinese species of Zygo carpic *Sargassum*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species* **2**, California Sea Grant College Program, La Jolla, CA. pp. 23-54.
- Tseng, C. K. and B. R. Lu. 2002. Studies on the Biserrulic *Sargassum* of China: IV. The series *Plagiophyllae* Tseng et Lu. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with*

- reference to some Pacific species **8**, California Sea Grant College Program, La Jolla, CA. pp. 11-32.
- Tseng, C. K. L. B. 2002. Some new species and records of *Sargassum* (*Sargassaceae*, Phaeophyta) from the China Sea. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 135-148.
- Tsuda, R. T. 1988. *Sargassum* from Micronesia. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific and Caribbean species 2*, California Sea Grant College Program, La Jolla, CA. pp. 59-63.
- Womersley, H. B. S. 1987. The Marine Benthic Flora of Southern Australia. Part II. South Australian Government Printing Division, Adelaide, South Australia. pp. 481.
- Womersley, H. B. S. and A. Bailey. 1970. Marine algae of the Solomon Islands. Phil. Trans. R. Soc. Lond. B Biol. Sci. **259**: 257-352.
- Yamada, Y. 1944. Diagnoses of new *Sargassums* from Japan. Hokkaido Imperial University, **3**: 1-10.
- Yendo, K. 1907. *The Fucaceae of Japan*. Journal of the College of Science, Imperior University of Tokyo **21**: 1-74.
- Yoshida, T. 1983. Japanese species of *Sargassum* subgenus. *Bactrophyucus* (Phaeophyta, Fucales). Journal of the Faculty of Science, Hokkaido University **13**: 99-246.
- Yoshida, T. 1989. Taxonomy of *Sargassum*. *Algae* **4**: 107-110.
- Yoshida, T. 1994. Three new species of *Sargassum* (*Sargassaceae*, Phaeophyta) from Japan. Japanese Journal of Phycology **42**: 43-51.
- Yoshida, T. 1998. *Marine Algae of Japan*. Uchida Rokakuho Publishing, Tokyo. Japan. pp. 1222.
- Yoshida, T., N. H. Dai, T. Ajisaka, and T. Noro. 2002. Verification of *Sargassum* species identified with subgenera *Bactrophyucus*, *Phyllotrichia* and *Schizophycus* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 8*, California Sea Grant College Program, La Jolla, CA. pp. 95-102.
- Yoshida, T., T. Noro, and T. Horigushi. 2004. Species of the genus *Sargassum* subgenus *Schizophycus*. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweed with reference to some Pacific species 9*, California Sea Grant College Program, La Jolla, CA. pp. 93-106.
- Yoshida, T., V. Stiger, and T. Horiguchi. 2000. *Sargassum boreale* sp. nov. (Fucales, Phaeophyceae) from Hokkaido, Japan. Phycological Research **48**: 125-131.

Chapter 4

Annex 4.1. Species and sampling localities

| Species | Localities | Accession nr | Source |
|--------------------------------|---|--------------|-------------------------|
| <i>Sargassum angustifolium</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1304) | | This study |
| <i>Sargassum aquifolium</i> | New Zealand (NZ) (Sar_sect. <i>Binderiana</i>) | EU882250 | Mattio et al. (2010) |
| <i>Sargassum aquifolium</i> | Philippines (PHIL) | KF826917 | Chan et al. (2014) |
| <i>Sargassum aquifolium</i> | HonYen, NhaTrang (Nguyen, Le & De Clerck, 1 May 2012, NVT1044) | | This study |
| <i>Sargassum aquifolium</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck, 2 May 2012, NVT1063) | | This study |
| <i>Sargassum aquifolium</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1085) | | This study |
| <i>Sargassum aquifolium</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1087) | | This study |
| <i>Sargassum aquifolium</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1092) | | This study |
| <i>Sargassum aquifolium</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1201) | | This study |
| <i>Sargassum aquifolium</i> | HonKho, QuyNhon (Nguyen, 10 June 2012, NVT1455) | | This study |
| <i>Sargassum aquifolium</i> | BinhTien, NinhThuan (Nguyen & Le, 22 June 2012, NVT1503) | | This study |
| <i>Sargassum aquifolium</i> | BinhTien, NinhThuan (Nguyen & Le, 22 June 2012, NVT1506) | | This study |
| <i>Sargassum bicorne</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1210) | | This study |
| <i>Sargassum bicorne</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1211) | | This study |
| <i>Sargassum binderi</i> | Malaysia (MAL) | AB043116 | Stiger et al. (2000) |
| <i>Sargassum binderi</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck, 10 May 2012, NVT1248) | | This study |
| <i>Sargassum bulbiferum</i> | VungChua, QuangBinh (Nguyen, 28 May 2012, NVT1335) | | This study |
| <i>Sargassum carpophyllum</i> | Japan (JP) | AB043067 | Stiger et al. (2000) |
| <i>Sargassum carpophyllum</i> | New Caledonia (NC) (Sar_sect. <i>Zygocarpicae</i>) | EU100799 | Mattio and Payri (2009) |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1180) | | This study |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1181) | | This study |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1195) | | This study |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1196) | | This study |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1198) | | This study |
| <i>Sargassum carpophyllum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1199) | | This study |
| <i>Sargassum cf. bicorne</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck, 10 May 2012, NVT1245) | | This study |

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|-------------------------------------|---|----------|---------------------------|
| <i>Sargassum cf. herklotsii</i> | CoTo Island, QuangNinh (Nguyen, 24 May 2012, NVT1293) | | This study |
| <i>Sargassum cf. herklotsii</i> | CoTo Island, QuangNinh (Nguyen, 24 May 2012, NVT1294) | | This study |
| <i>Sargassum cf. herklotsii</i> | CoTo Island, QuangNinh (Nguyen, 24 May 2012, NVT1295) | | This study |
| <i>Sargassum cf. kuetzingii</i> | CoTo Island, QuangNinh (Nguyen, 24 May 2012, NVT1281) | | This study |
| <i>Sargassum cf. longifructum</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1314) | | This study |
| <i>Sargassum cf. marginatum</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1200) | | This study |
| <i>Sargassum cf. mcclurei</i> | HonDua, QuangNam (Nguyen, 8 June 2012, NVT1445) | | This study |
| <i>Sargassum cf. oligocistum</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1320) | | This study |
| <i>Sargassum cf. sandei</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1323) | | This study |
| <i>Sargassum cf. turbinarioides</i> | MyHoa, NinhThuan (Nguyen & Le, 21 June 2012, NVT1493) | | This study |
| <i>Sargassum confusum</i> | Japan (JP) (<i>Bac. sect. Teretia</i>) | AB038271 | Yoshida et al. (2000) |
| <i>Sargassum cotoense</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1284) | | This study |
| <i>Sargassum cotoense</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1289) | | This study |
| <i>Sargassum cotoense</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1290) | | This study |
| <i>Sargassum cotoense</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1291) | | This study |
| <i>Sargassum crassifolium</i> | Vietnam (VIE) | AB043115 | Stiger et al. (2000) |
| <i>Sargassum crassifolium</i> | Malaysia (MAL) | AB043118 | Stiger et al. (2000) |
| <i>Sargassum denticarpum</i> | Bai Tien, Vietnam (VIE) | AB555543 | Shimabokuro et al. (2012) |
| <i>Sargassum distichum</i> | MuiSi, QuangTri (Nguyen, 30 May 2012, NVT1366) | | |
| <i>Sargassum echinocarpum</i> | Hawaii – US (Haw) (<i>Sar. sect. Binderiana</i>) | EU100796 | Matio et al. (2008b) |
| <i>Sargassum feldmanni</i> | BalangAn, QuangNgai (Nguyen, Le & De Clerck, 5 May 2012, NVT1148) | | This study |
| <i>Sargassum feldmanni</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck, 11 May 2012, NVT1269) | | This study |
| <i>Sargassum feldmannii</i> | MyHoa, NinhThuan (Nguyen & Le, 21 Jun 2012, NVT1494) | | This study |
| <i>Sargassum feldmannii</i> | PhuQuy Island, BinhThuan (Nguyen, 6 July 2012, NVT1509) | | This study |
| <i>Sargassum fusiforme</i> | CN (<i>Bac. sect. Hizikia</i>) | FJ712733 | Cheang et al. (2009) |
| <i>Sargassum gracillimum</i> | CuaTung, QuangTri (Nguyen, 30 May 2012, NVT1363) | | This study |
| <i>Sargassum gracillimum</i> | MuiSi, QuangTri (Nguyen, 30 May 2012, NVT1365) | | This study |
| <i>Sargassum graminifolium</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1182) | | This study |

Chapter 4

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|--------------------------------|---|----------|------------------------------|
| <i>Sargassum graminifolium</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1183) | | This study |
| <i>Sargassum hemiphyllosum</i> | Japan (JP) | FJ712756 | Cheang et al. (2010) |
| <i>Sargassum henslowianum</i> | BaLangAn, QuangNgai (Nguyen, Le & De Clerck, 5 May 2012, NVT1160) | | This study |
| <i>Sargassum herklotsii</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1328) | | This study |
| <i>Sargassum herklotsii</i> | HonNom, QuangBinh (Nguyen, 1 June 2012, NVT1386) | | This study |
| <i>Sargassum herklotsii</i> | HonNom, QuangBinh (Nguyen, 1 June 2012, NVT1392) | | This study |
| <i>Sargassum herklotsii</i> | BanThan, QuangNam (Nguyen, 7 June 2012, NVT1420) | | This study |
| <i>Sargassum herklotsii</i> | BanThan, QuangNam (Nguyen, 7 June 2012, NVT1421) | | This study |
| <i>Sargassum herklotsii</i> | BanThan, QuangNam (Nguyen, 7 June 2012, NVT1422) | | This study |
| <i>Sargassum heterocystum</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1215) | | This study |
| <i>Sargassum horneri</i> | Japan (JP) (<i>Bac</i> _sect. <i>Spongocarpus</i>) | AB043569 | Stiger et al. (2000) |
| <i>Sargassum howeanum</i> | New Caledonia (NC) (<i>Sar</i> . sect. <i>Sargassum</i>) | FJ170439 | Mattio and Payri (2009) |
| <i>Sargassum ilicifolium</i> | Fiji (FJ) (<i>Sar</i> . sect. <i>Ilicifolia</i>) | EU833437 | Mattio et al. (2009a) |
| <i>Sargassum ilicifolium</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1083) | | This study |
| <i>Sargassum ilicifolium</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1094) | | This study |
| <i>Sargassum ilicifolium</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1204) | | This study |
| <i>Sargassum ilicifolium</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1205) | | This study |
| <i>Sargassum ilicifolium</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1208) | | This study |
| <i>Sargassum ilicifolium</i> | SonTra, DaNang (Nguyen, Le & De Clerck, 7 May 2012, NVT1209) | | This study |
| <i>Sargassum ilicifolium</i> | MyHoa, NinhThuan (Nguyen & Le, 21 June 2012, NVT1491) | | This study |
| <i>Sargassum johnstonii</i> | Mexico (MX) (<i>Sar</i> . sect. <i>Johnstonii</i>) | JX560129 | Andrade-Sorcia et al. (2014) |
| <i>Sargassum lapazeanum</i> | Mexico (MX) (<i>Sar</i> . sect. <i>Lapazeanum</i>) | JX560125 | Andrade-Sorcia et al. (2014) |
| <i>Sargassum marginifolium</i> | NamO, DaNang (Nguyen, Le & De Clerck, 6 May 2012, NVT1200) | | This study |
| <i>Sargassum mcclurei</i> | Vietnam (VIE) (<i>Sar</i> . sect. <i>Ilicifolia</i>) | AB043111 | Stiger et al. (2000) |
| <i>Sargassum mcclurei</i> | Hon Rua, NhaTrang (Nguyen, Le & De Clerck, 2 May 2012, NVT1060) | | This study |
| <i>Sargassum mcclurei</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1084) | | This study |
| <i>Sargassum mcclurei</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1086) | | This study |
| <i>Sargassum mcclurei</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1090) | | This study |

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| <i>Sargassum mcclurei</i> | Diamonbay NhaTrang (Nguyen, Le & De Clerck, 4 May 2012, NVT1122) | | This study |
| <i>Sargassum mcclurei</i> | Diamonbay NhaTrang (Nguyen, Le & De Clerck, 4 May 2012, NVT1128) | | This study |
| <i>Sargassum mcclurei</i> | Diamonbay NhaTrang (Nguyen, Le & De Clerck, 4 May 2012, NVT1130) | | This study |
| <i>Sargassum mcclurei</i> | BaLangAn, QuangNgai (Nguyen, Le & De Clerck, 6 May 2012, NVT1161) | | This study |
| <i>Sargassum mcclurei</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck, 10 May 2012, NVT1149) | | This study |
| <i>Sargassum mcclurei</i> | CoTo Island, QuangNinh (Nguyen, 28 May 2012, NVT1283) | | This study |
| <i>Sargassum mcclurei</i> | TamQuang, QuangNam (Nguyen, 8 June 2012, NVT1431) | | This study |
| <i>Sargassum mcclurei</i> | HonDua, Quangnam (Nguyen, 8 June 2012, NVT1441) | | This study |
| <i>Sargassum mcclurei</i> | HonDua, Quangnam (Nguyen, 8 June 2012, NVT1447) | | This study |
| <i>Sargassum mcclurei</i> | HonDua, Quangnam (Nguyen, 8 June 2012, NVT1448) | | This study |
| <i>Sargassum mcclurei</i> | HonKho, QuyNhon (Nguyen, 10 June 2012, NVT1456) | | This study |
| <i>Sargassum mcclurei</i> | HonKho, QuyNhon (Nguyen, 10 June 2012, NVT1469) | | This study |
| <i>Sargassum mcclurei</i> | HonKho, QuyNhon (Nguyen, 8 June 2012, NVT1470) | | This study |
| <i>Sargassum mcclurei</i> | BinhTien, NinhThuan (Nguyen, 22 June 2012, NVT1500) | | This study |
| <i>Sargassum microcystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck, 2 May 2012, NVT1066) | | This study |
| <i>Sargassum microcystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck, 2 May 2012, NVT1068) | | This study |
| <i>Sargassum microcystum</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck, 3 May 2012, NVT1088) | | This study |
| <i>Sargassum microcystum</i> | HonNom, QuangBinh (Nguyen, 1 June 2012, NVT1484) | | This study |
| <i>Sargassum microcystum</i> | BinhTien, NinhThuan (Nguyen, 22 June 2012, NVT1499) | | This study |
| <i>Sargassum miyabei</i> | Japan (JP) (<i>Bac. sect. Teretia</i>) | AB043502 | Stiger et al. (2000) |
| <i>Sargassum myriocystum</i> | Japan (JP) (<i>Sar. sect. Polycystae</i>) | AB043113 | Stiger et al. (2000) |
| <i>Sargassum nigrifolium</i> | Japan (JP) (<i>Bac. sect. Halochloa/Repentia</i>) | AB043110 | Stiger et al. (2000) |
| <i>Sargassum oligocystum</i> | HonYen, NhaTrang (Nguyen, Le & De Clerck 1 May 2012, NVT1043) | | This study |
| <i>Sargassum oligocystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1062) | | This study |
| <i>Sargassum oligocystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1067) | | This study |
| <i>Sargassum oligocystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1069) | | This study |
| <i>Sargassum oligocystum</i> | DiamonBay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1118) | | This study |
| <i>Sargassum oligocystum</i> | BaLangAn, QuangNgai (Nguyen, Le & De Clerck 5 May 2012, NVT1154) | | This study |

Chapter 4

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|--|--|----------|-----------------------|
| <i>Sargassum oligocystum</i> | SonTra, Da Nang (Nguyen, Le & De Clerck 7 May 2012, NVT1226) | | This study |
| <i>Sargassum oligocystum</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck 11 May 2012, NVT1264) | | This study |
| <i>Sargassum oligocystum</i> | BinhTien, NinhThuan (Nguyen & Le 22 June 2012, NVT1498) | | This study |
| <i>Sargassum patens</i> | Japan (JP) (Sar_ sect. Binderiana) | AB043666 | Stiger et al. (2000) |
| <i>Sargassum phamhoangii</i> | NamO, DaNang (Nguyen, Le & De Clerck 6 May 2012, NVT1185) | | This study |
| <i>Sargassum phamhoangii</i> | NamO, DaNang (Nguyen, Le & De Clerck 1 May 2012, NVT1187) | | This study |
| <i>Sargassum piluliferum</i> | Japan (JP) | AB043617 | Stiger et al. (2000) |
| <i>Sargassum piluliferum_var</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1309) | | This study |
| <i>Sargassum piluliferum_var</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1310) | | This study |
| <i>Sargassum piluliferum_var</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1318) | | This study |
| <i>Sargassum piluliferum_var_serratifolium</i> | HonLa, QuangBinh (Nguyen, 26 May 2012, NVT1312) | | This study |
| <i>Sargassum piluliferum_var_serratifolium</i> | HonLa, QuangBinh (Nguyen, 1 May 2012, NVT1313) | | This study |
| <i>Sargassum piluliferum_var_serratifolium</i> | CuaTung, QuangTri (Nguyen, 30 May 2012, NVT1349) | | This study |
| <i>Sargassum piluliferum_var_serratifolium</i> | MuiSi, QuangTri (Nguyen, 30 May 2012, NVT1367) | | This study |
| <i>Sargassum polycystum</i> | Vietnam (VIE) (Sar. sect. Polycystae) | AB043114 | Stiger et al. (2000) |
| <i>Sargassum polycystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1064) | | This study |
| <i>Sargassum polycystum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 1 May 2012, NVT1071) | | This study |
| <i>Sargassum polycystum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1119) | | This study |
| <i>Sargassum polycystum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1123) | | This study |
| <i>Sargassum polycystum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1124) | | This study |
| <i>Sargassum polycystum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1125) | | This study |
| <i>Sargassum polycystum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1127) | | This study |
| <i>Sargassum polycystum</i> | VungChua, QuangBinh (Nguyen, 28 May 2012, NVT1334) | | This study |
| <i>Sargassum polyphyllum</i> | Fiji (FJ) (Sar. sect. Sargassum) | EU833426 | Mattio et al. (2009a) |
| <i>Sargassum quinhonense</i> | Vietnam (VIE) (Sar. sect. Illicifolia) | AB043112 | Stiger et al. (2000) |
| <i>Sargassum quinhonense</i> | BanThan, QuangNam (Nguyen 7 Jun 2012, NVT1418) | | This study |
| <i>Sargassum quinhonense</i> | GhenhRang, QuyNhon (Nguyen 11 June 2012, NVT1475) | | This study |
| <i>Sargassum sandei(bicorne)</i> | SonTra, DaNang (Nguyen, Le & De Clerck 7 May 2012, NVT1206) | | This study |

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| <i>Sargassum serratifolium</i> | Japan (JP) | AB043612 | Stiger et al. (2000) |
| <i>Sargassum serratifolium</i> | Korea (KR) | JF931841 | Cho et al. (2012) |
| <i>Sargassum serratum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1065) | | This study |
| <i>Sargassum serratum</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1072) | | This study |
| <i>Sargassum serratum</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck 3 May 2012, NVT1089) | | This study |
| <i>Sargassum serratum</i> | BaiTien, NhaTrang (Nguyen, Le & De Clerck 3 May 2012, NVT1091) | | This study |
| <i>Sargassum serratum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1120) | | This study |
| <i>Sargassum serratum</i> | Diamonbay, NhaTrang (Nguyen, Le & De Clerck 4 May 2012, NVT1121) | | This study |
| <i>Sargassum serratum</i> | ConDao Island, Baria-Vungtau (Nguyen & De Clerck 11 May 2012, NVT1270) | | This study |
| <i>Sargassum siliquastrum</i> | JP(Bac_Sect.Halocholoa/repentia) | AB043106 | Stiger et al. (2000) |
| <i>Sargassum sinclairii</i> | JP(Arthophycus) | FJ170459 | Mattio and Payri (2009) |
| <i>Sargassum sinicola</i> | Mexico (MX) (Sar. Sect. sinicola) | JX560124 | Andrade-Sorcia (2014) |
| <i>Sargassum stolonifolium</i> | Malaysia (MAL) | AB043613 | Stiger et al. (2000) |
| <i>Sargassum swartzii</i> | New Caledonia (NC) (Sar. sect. Binderiana) | EU882255 | Mattio et al. (2010) |
| <i>Sargassum swartzii</i> | BalAngAn, QuangNgai (Nguyen, Le & De Clerck 5 May 2012, NVT1155) | | This study |
| <i>Sargassum swartzii</i> | SonTra, DaNang (Nguyen, Le & De Clerck 7 May 2012, NVT1221) | | This study |
| <i>Sargassum swartzii</i> | SonTra, DaNang (Nguyen, Le & De Clerck 1 May 2012, NVT1225) | | This study |
| <i>Sargassum swartzii</i> | CoTo Island, QuangNinh (Nguyen, 24 May 2012, NVT1297) | | This study |
| <i>Sargassum swartzii</i> | MuiSi, QuangTri (Nguyen, 30 May 2012, NVT1383) | | This study |
| <i>Sargassum tenerrimum</i> | CuaTung, QuangTri (Nguyen, 30 May 2012, NVT1351) | | This study |
| <i>Sargassum thunbergii</i> | Japan (JP) (Bac. sect. teretia) | AB043573 | Stiger et al. (2000) |
| <i>Sargassum turbinarioides</i> | New Caledonia (NC) (Sar. sect. Zygocharpicae) | EU882256 | Mattio et al. (2010) |
| <i>Sargassum vietnamense</i> | HonRua, NhaTrang (Nguyen, Le & De Clerck 2 May 2012, NVT1057) | | This study |
| <i>Sargassum vietnamense</i> | CauDa, NhaTrang (Nguyen & Le 19 June 2012, NVT1483) | | This study |
| <i>Sargassum yendoii</i> | Japan (JP) (Sar_sect.nov) | AB043667 | Stiger et al. (2000) |
| <i>Sargassopsis decurrens</i> | NC | EU100773 | Mattio et al. (2008b) |
| <i>Turbinaria ornate</i> | NC | EU100772 | Mattio et al. (2008b) |

Chapter 5

Marker development for species
delineation in the genus *Sargassum*:
a RAD-Sequencing approach

5.1 Introduction

The understanding that species-level diversity is only inadequately reflected in the morphology of most algal species, rapidly drove a majority of phycologists toward DNA-based species delineation methods. Delineating species based on gene sequences, especially if only a single marker is used, is however not without flaws itself as outlined by Leliaert et al. (2014). Confounding processes like incomplete lineage sorting, trans-species polymorphism, hybridization and introgression may result in gene tree – species tree incongruence and hence mislead researchers regarding species boundaries as well as the relationships between species. Apart from processes which typically play at the species-population level boundary, species delimitation may also be difficult because of the lack of resolution in phylogenetic markers. Different markers in the DNA usually evolve at different rates, because of selective constraints that act upon them. For example, protein coding genes are evolving in most groups at a lower rate compared to noncoding markers. Likewise, third codon positions in protein coding genes evolve faster than first and second codon positions because of the degenerate nature of the genetic code. Silberfeld et al. (2013) determined that the chloroplast encoded *rbcL* and *psaA* genes evolved approximately 2 to 4 times slower than the mitochondrial *cox3* gene in the genus *Padina*. Consequently, algorithmic species delineation based on the *cox3* gene resulted in more separately evolving lineages compared to the chloroplast markers.

It is expected that evolutionary rates of markers may confound species delineation especially when divergence was a recent phenomenon. The best known example in algal systematics is offered by the genus *Fucus*. Canovas et al. (2011) concluded that diversification of *Fucus* was initiated in the Pliocene, ca. 3.8 million years ago, while the most recent and rapid species radiation in the *Fucus vesiculosus-spiralis* clade took place during Pleistocene glacial cycles. Not surprisingly traditional mitochondrial and chloroplast markers, or even a variable marker such as the nuclear rDNA ITS, do not show enough variation to resolve relationships and pinpoint species boundaries. A full resolution of the *Fucus* radiation was only realized by applying a set of 13 nuclear encoded protein markers. Next to increased resolution, this set of nuclear genes also allowed to circumvent problems related to incomplete lineage sorting, hybridization and introgression which characterize the evolution of this young algal lineage (Coyer et al. 2002, Billard et al. 2005, Engel et al. 2005, Pereyra et al. 2009, Neiva et al. 2010, Coyer et al. 2011). It is widely acknowledged that disentangling species-level evolutionary processes from population-level processes

cannot be established using single-locus approaches but requires unlinked loci that are preferentially analysed under a multi-species coalescent model (Kubatko and Degnan 2007, Degnan and Rosenberg 2009, Knowles and Kubatko 2010).

Traditionally, marker development and data generation of multiple nuclear loci is, however, a laborious process and prone to problems (McCormack et al. 2012). For example marker development in *Fucus* was based on existing EST libraries, for which primers were designed and then amplified for the respective set of taxa. Apart from the fact that for most non-model organisms cDNA libraries are not available, a traditional PCR-based approach requires a lengthy process of screening loci for variability, amplifying, often followed by cloning, and sequencing DNA for each sample at each locus. More often than not, primer mismatches make that not all samples can be screened for each locus. This results in incomplete datasets which may reflect on the outcome of the analyses. Understandably next-generation sequencing (NGS) was met with great interest as a potential means to condense the many steps of multilocus data generation for non-model organisms into a more time-efficient and cost-effective process (Carstens et al. 2012, McCormack et al. 2012). Next generation sequencing platforms typically generate millions of relatively short reads that can then be used as markers for phylogenetic, phylogeographic or population genetic analyses. The use of NGS technologies for such analyses usually involve a genome reduction step which can either be obtained by amplicon sequencing in which PCR products are tagged and pooled for NGS, restriction-digest based methods or target enrichment, in which probes bind to genomic DNA, which is then pooled for NGS (Davey et al. 2011, McCormack et al. 2012).

Here we explore the use of RAD-Sequencing, a restriction-digest based method, for marker development in the genus *Sargassum*. *Sargassum* is probably the most important brown algal genus in tropical to warm temperate regions. From the Indian Ocean alone 239 species and intraspecific taxa have been recorded (Silva et al. 1996). This would represent 40% of the brown algal diversity of that region. There is however great uncertainty on the species level diversity in the genus. Despite hundreds of taxa being described over the past 200 years, molecular phylogenies based on ITS rDNA, mitochondrial *cox3* and the chloroplast RuBisCo genes seem to indicate that far fewer species are actually present (Mattio et al. 2008, 2010, 2013, Mattio and Payri 2009). For example in a recent paper Mattio et al. (2013) reduced the number of *Sargassum* species from Mauritius and Réunion from 44 to 6 based on morphological and molecular analyses. It remains however to be

determined whether such a large species concept results from a lack of resolution in the currently used molecular markers or whether *Sargassum* diversity has indeed been gravely overestimated, most likely because the individual species display so much morphological variation. Species delineation based on multiple unlinked nuclear loci could potentially bring a solution to this question.

The Rad-Seq protocol involves a restriction digest of total DNA, followed by ligation of a P1-adaptor and P2-adaptor, a size selection step and a PCR reaction which selects only those fragments which had been labeled by both a P1 and P2 adaptor (Fig. 5.1)

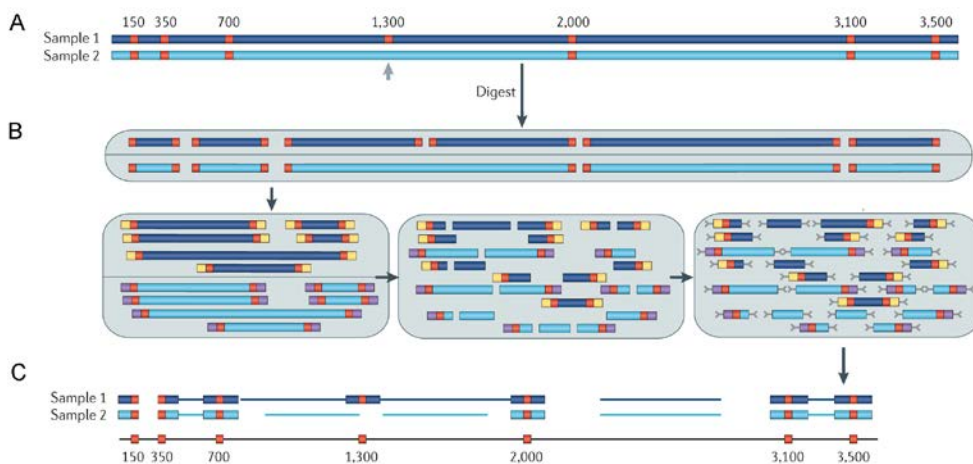


Fig. 5.1: Restriction-site-associated DNA sequencing (RAD-seq) protocol: A. An example of a genomic region containing restriction sites (red). A sample of DNA from each of two individuals (sample 1 is dark blue and sample 2 is light blue) is to be sequenced. Sample 2 has a variation in the cut site at 1,300 bases (grey arrow) and so this site will not be cut. B. Following digestion by a restriction enzyme fragments are ligated to P1 adaptors (yellow for sample 1, purple for sample 2), pooled, randomly sheared and size selected to 300–700 bp. P2 adaptors with divergent ends (grey, Y-shaped) are ligated to the fragments with and without P1 adaptors. The fragments are PCR amplified with P1- and P2-specific primers. This means that only fragments with P1 and P2 adaptors (the fragments containing restriction sites) are amplified. C. RAD-seq fragment. Downstream regions of all fragments above 300 bases are sequenced, but not the fragment between 150 and 350 bases. Thin lines indicate the sequence that are covered using paired-end sequencing. [adapted from Davey et al. 2011]

5.2 Material & Methods

In order to develop RAD markers we selected 18 specimens, 16 belonging to the genus *Sargassum* and 2 outgroups representing the closely related genus *Myagropsis* (Table 5.1). The sampling represented 9 or 10 morphospecies, selected as to present the phylodiversity of the genus *Sargassum* as good as possible. Between 0.775 and 28.75 µg DNA of each specimen was extracted using a modified CTAB method (see De Clerck et al. 2006) whereby DNA was bound to magnetic beads rather than being precipitated. The 260/280 nm absorption ratios ranged from 1.47 to 2.03 with an average of 1.81. Similarly, the 260/230 nm absorption ratio ranged from 0.53 to 1.89 with an average of 1.16. Preparation of the Rad-Seq libraries was based on Etter et al. (2011). The *Sbf*I restriction enzyme was used to digest the DNA. Quality of the pooled libraries was checked on an Illumina BioAnalyzer prior to sequencing on an Illumina MiSeq. Libraries with an insert size of 500-700 bp were sequenced pair-end 250+250bp at the Edinburgh Genomics Ashworth Laboratories (University of Edinburgh). Next to the Rad-Sequencing we generated a *cox3* sequence of each individual, as outlined by Mattio & Payri (2010). A phylogeny based on the mitochondrial *cox3* gene was reconstructed using maximum likelihood. Analyses were performed with RAxML v.7.2.8 (Stamatakis 2006) using GTR+G+I model. We used 200 independent tree inferences, applying options of automatically optimized subtree pruning regrafting (SPR) rearrangement and 25 distinct rate categories to identify the best tree. Statistical support for each branch was obtained from 1,000 bootstrap replications with the same substitution model.

Table 5.1. Specimen and RAD makers

| Nr | Species | Sample ID | Collection site | Collection date | Collector | RAD barcode |
|----|---------------------------------|-----------|---|-----------------|--------------|-------------|
| 1 | <i>Sargassum</i> sp. | ODC2356 | Playa de Las Arenas, Buenavista, Tenerife, Canary Islands | 3. Feb. 2014 | O. De Clerck | ATGCT |
| 2 | <i>Sargassum</i> sp. | ODC2357 | Playa de Las Arenas, Buenavista, Tenerife, Canary Islands | 3. Feb. 2014 | O. De Clerck | ATTAG |
| 3 | <i>Sargassum cymosum</i> | ODC2373 | Punta del Hidalgo, Tenerife, Canary Islands | 3. Feb. 2014 | O. De Clerck | CAACT |
| 4 | <i>Sargassum furcatum</i> | ODC2374 | Punta del Hidalgo, Tenerife, Canary Islands | 3. Feb. 2014 | O. De Clerck | CACAG |
| 5 | <i>Sargassum desfontainesii</i> | ODC2375 | Punta del Hidalgo, Tenerife, Canary Islands | 3. Feb. 2014 | O. De Clerck | CAGTC |
| 6 | <i>Sargassum fusiforme</i> | KML10 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CATGA |
| 7 | <i>Sargassum fusiforme</i> | KML20 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CCAAC |
| 8 | <i>Sargassum horneri</i> | KLM27 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CCCCA |

Chapter 5

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|----|------------------------------|-------|--------------------------|--------------|----------|-----------|
| 9 | <i>Sargassum horneri</i> | KML36 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CCGGT |
| 10 | <i>Sargassum muticum</i> | KML46 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CCTTG |
| 11 | <i>Sargassum muticum</i> | KML47 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CGATA |
| 12 | <i>Sargassum muticum</i> | KML48 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CGCGC |
| 13 | <i>Sargassum thunbergii</i> | KML62 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CGGC G |
| 14 | <i>Sargassum thunbergii</i> | KML64 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CGTAT |
| 15 | <i>Sargassum yezoense</i> | KML86 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CTACC |
| 16 | <i>Sargassum yezoense</i> | KML87 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CTCTT |
| 17 | <i>Myagropsis myagroides</i> | KML94 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | CTTCC |
| 18 | <i>Myagropsis myagroides</i> | KML95 | Mangseokri, Wando, Korea | 1. Jan. 2014 | K.M. Lee | GAAG C |

Following a first quality check using the CLC Bio Genomic Workbench, sequences were analysed using the Stacks package (Catchen et al. 2013), followed by clustering of similar stacks across species in Silix (Miele et al. 2011). We built and genotyped loci *de novo* from the single end of the paired-end sequencing using STACKS (Catchen et al. 2013). In the pipeline, two reads were required as minimum depth of coverage to create a stack. The maximum distance (in nucleotides) allowed between stacks was set at 13, and the maximum distance to align secondary reads to primary stacks as nine. Highly-repetitive stacks were removed by the Removal algorithm. We extracted the consensus sequences out of the tag files, produced by ustacks, and created a fasta file with the name of the each MID tag. Alignments were automatically aligned with MAFFT using the FFT-NS-i iterative refinement method (Kato et al. 2002). Fasta files were then joined into one file combining all RAD tags of a single individual. The SiLiX program was used to cluster homologous sequences, based on single transitive links (single linkage) with alignment coverage constraints (Miele et al. 2011). We analyzed filtering and clustering algorithms of all-against-all BLAST search in tabular format. Gene trees of selected stacks were constructed in MEGA6 after visually checking the alignments using a neighbor joining algorithm.

5. 3 Results

Analysis of the *cox3* dataset resulted in a *Sargassum* phylogeny (Fig. 5.2) in which subgenera and sections are well supported, but within sections support values are low and branch length separating closely related species are extremely short or close to zero. This is best illustrated in section *Halochloa* and section *Sargassum* in which many sequences are characterized by virtually identical *cox3* sequences.

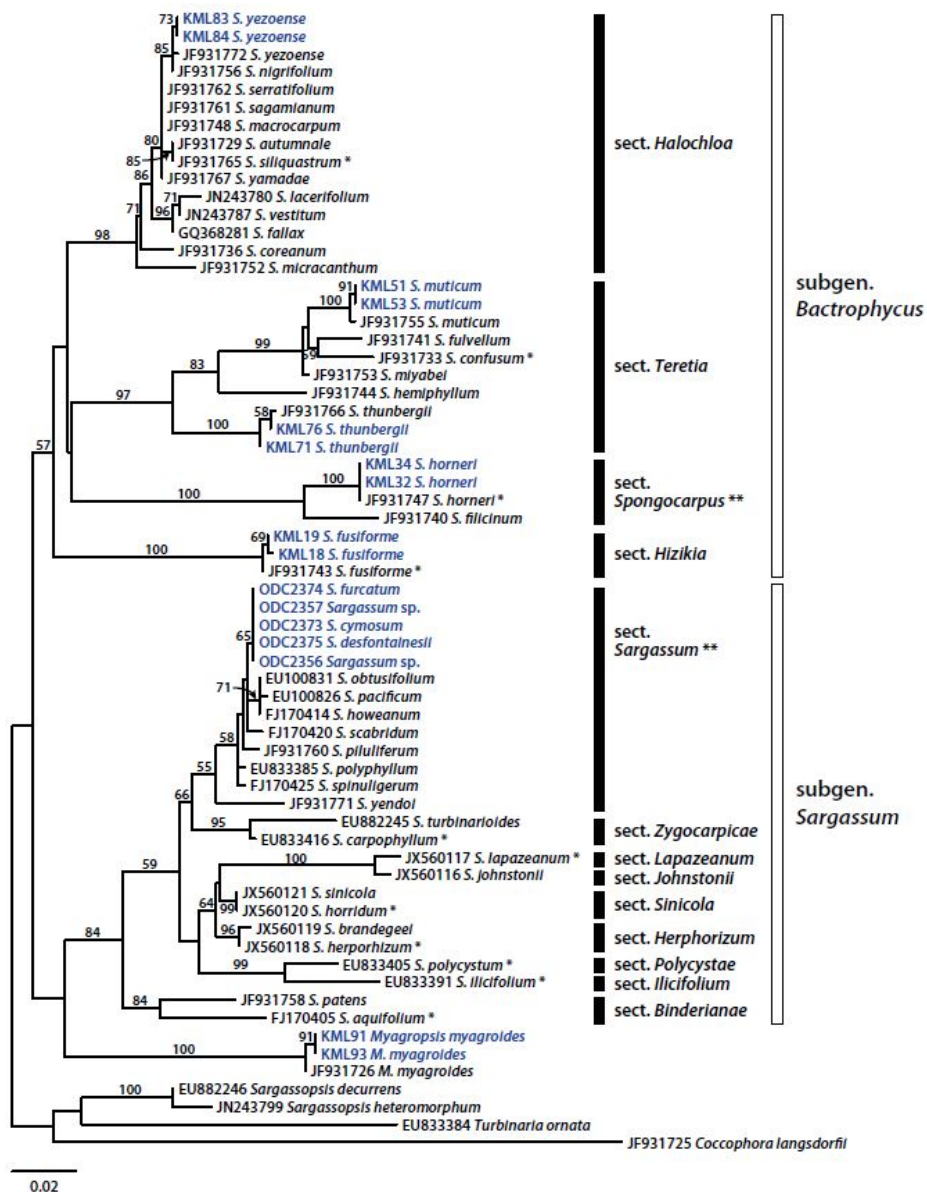


Fig. 5.2. Maximum likelihood tree based on an alignment of 64 *cox3* sequences, representing 13 section and two subgenera of *Sargassum*. Sequencing used for the Rad-Sequencing are indicated in blue. Bootstrap values are indicated when above 50% and were based on 1000 replicates. The tree is rooted with a clade containing *Turbinaria*, *Coccophora* and *Sargassopsis*.

Table 5.1. Results from the RAD-Sequencing and Stacks analysis grouped per specimen.

| Species | <i>Sargassum</i> sp. | | | | <i>S. gymnosum</i> | | | <i>S. furcatum</i> | | | <i>S. desfontainesii</i> | | | <i>S. fusiforme</i> | | | <i>S. horneri</i> | | | |
|--|-----------------------|--------------------------|-----------------------|-----------------------|--------------------------|-----------------------------|--------------------|--------------------------|-------------------------|---------------------------|--------------------------|-------|-------|-------------------------------------|-------|-------|-------------------|-------|-------|-------|
| | Voucher code | ODC2356 | ODC2357 | ODC2373 | ODC2373 | ODC2374 | ODC2375 | KML10 | KML20 | KML27 | KML36 | ATGCT | ATTAG | CAACT | CACAG | CAGTC | CATGA | CCAAC | CCCA | CCGT |
| barcodes | | | | | | | | | | | | | | | | | | | | |
| id | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
| loaded RAD-Tags | 61,309 | 73,566 | 150,326 | 339,663 | 212,866 | 4,136 | 6,030 | 4,136 | 6,030 | 1,975 | 975 | | | | | | | | | |
| inserted elements into RAD-Tags hash map | 9,870 | 12,445 | 24,204 | 49,704 | 34,610 | 641 | 1,104 | 641 | 1,104 | 409 | 112 | | | | | | | | | |
| mean coverage depth | 33 | 32 | 34 | 37 | 33 | 32 | 30 | 32 | 30 | 38 | 46 | | | | | | | | | |
| max merged coverage | 1,652 | 1,946 | 1,796 | 2,771 | 2,440 | 1,330 | 1,268 | 1,330 | 1,268 | 727 | 597 | | | | | | | | | |
| removed stacks | 1,020 | 1,138 | 2,618 | 5,504 | 2,940 | 68 | 61 | 68 | 61 | 31 | 13 | | | | | | | | | |
| stacks remain for merging | 625 | 857 | 1,376 | 2,876 | 2,762 | 45 | 112 | 45 | 112 | 13 | 7 | | | | | | | | | |
| merged stacks | 625 into 205 | 857 into 228 | 1,376 into 482 | 2,876 into 962 | 2,762 into 943 | 45 into 15 | 112 into 46 | 45 into 15 | 112 into 46 | 13 into 9 | 7 into 3 | | | | | | | | | |
| mean merged coverage depth | 259 | 277 | 270 | 309.9 | 194.9 | 240.4 | 110.8 | 240.4 | 110.8 | 178.7 | 294 | | | | | | | | | |
| max merged coverage | 2,149 | 2,156 | 2,040 | 3,281 | 2,805 | 1,460 | 1,607 | 1,460 | 1,607 | 865 | 658 | | | | | | | | | |
| remainder sequences | 8,273 | 10,492 | 20,330 | 41,555 | 39 | 530 | 934 | 530 | 934 | 367 | 93 | | | | | | | | | |
| match remainder reads | 2,316 | 3,433 | 7,697 | 16,809 | 9,639 | 39 | 252 | 39 | 252 | 159 | 9 | | | | | | | | | |
| unable to match reads | 5,957 | 7,059 | 12,633 | 24,746 | 19,423 | 491 | 682 | 491 | 682 | 208 | 84 | | | | | | | | | |
| no. of utilized reads | 55,412 | 66,507 | 137,693 | 314,917 | 193,443 | 3,645 | 5,348 | 3,645 | 5,348 | 1,767 | 891 | | | | | | | | | |
| Species | | | | | | | | | | | | | | | | | | | | |
| Voucher code | KML46 | <i>S. muticum</i> | | | | <i>S. thunbergii</i> | | | | <i>S. yezoense</i> | | | | <i>Myagropsis myagroides</i> | | | | | | |
| barcodes | CCTG | CGATA | CGGC | CGGG | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT | CGGAT |
| id | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| loaded RAD-Tags | 65,305 | 134,083 | 115,598 | 167,366 | 931,219 | 1,645 | 3,632 | 1,570,080 | 420,451 | | | | | | | | | | | |
| inserted elements into RAD-Tags hash map | 12,063 | 22,528 | 19,045 | 28,977 | 144,930 | 1,177 | 588 | 224,721 | 55,908 | | | | | | | | | | | |
| mean coverage depth | 29 | 32 | 33 | 31 | 35 | 4 | 48 | 39 | 45 | | | | | | | | | | | |
| max coverage | 1,978 | 2,054 | 2,207 | 1,990 | 2,880 | 73 | 1,315 | 8,693 | 4,509 | | | | | | | | | | | |
| removed stacks | 904 | 1,991 | 1,810 | 2,490 | 13,436 | 7 | 47 | 19,851 | 4,086 | | | | | | | | | | | |
| stacks remain for merging | 1,022 | 1,682 | 1,282 | 2,168 | 10,312 | 166 | 20 | 16,137 | 4,463 | | | | | | | | | | | |
| merged stacks | 1,022 into 385 | 1,682 into 568 | 1,282 into 464 | 2,168 into 782 | 10,312 into 3,493 | 166 into 150 | 20 into 8 | 16,137 into 4,170 | 4,463 into 1,547 | | | | | | | | | | | |
| mean merged coverage depth | 143.2 | 202.7 | 214.6 | 182.8 | 231.7 | 4.3 | 388.6 | 331 | 241 | | | | | | | | | | | |
| max merged coverage | 2,277 | 2,334 | 2,423 | 2,401 | 3,811 | 80 | 1,438 | 16,310 | 7,345 | | | | | | | | | | | |
| remainder sequences | 10,184 | 18,951 | 16,032 | 24,439 | 121,867 | 1,006 | 523 | 189,726 | 47,665 | | | | | | | | | | | |
| match remainder reads | 3,364 | 6,230 | 6,423 | 10,072 | 38,781 | 124 | 170 | 48,591 | 13,938 | | | | | | | | | | | |
| unable to match reads | 6,820 | 12,721 | 9,609 | 14,367 | 83,086 | 882 | 353 | 141,135 | 33,727 | | | | | | | | | | | |
| no. of utilized reads | 58,485 | 121,362 | 105,989 | 152,999 | 848,133 | 763 | 3,279 | 1,428,945 | 386,724 | | | | | | | | | | | |

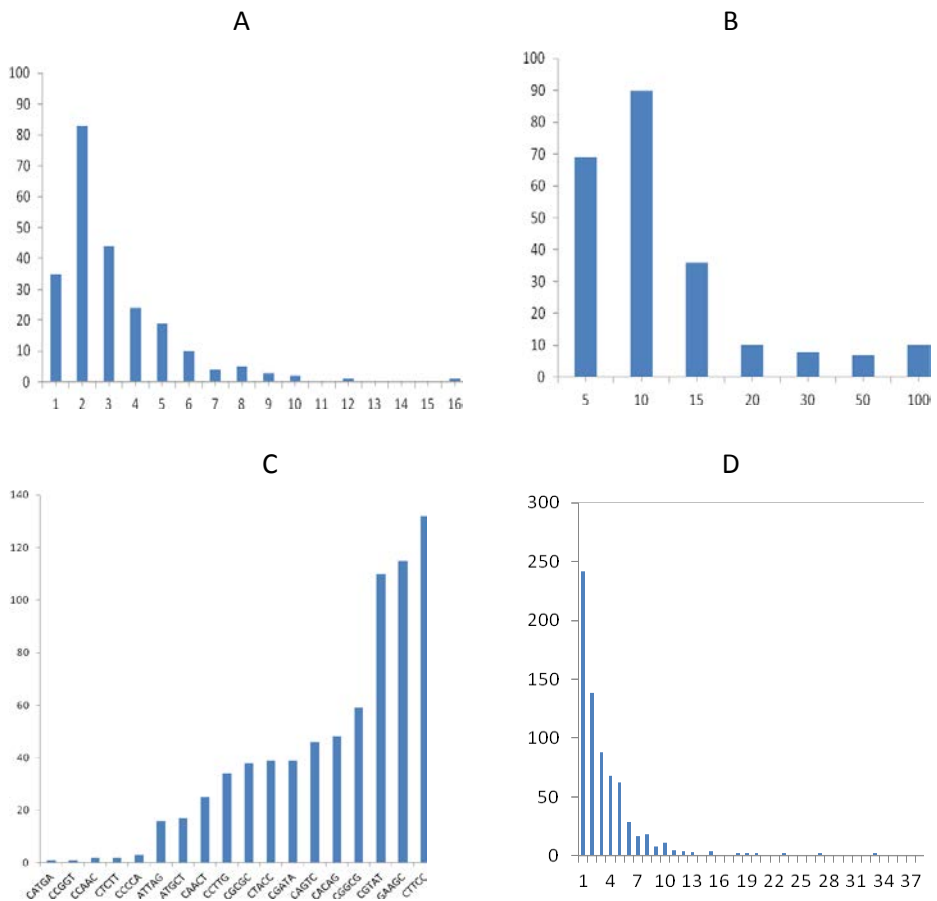


Fig 5.3: Number of specimens per stack (A); Number of sequences per stack (B); Number of stacks that contain sequences of a certain specimen (C); number of alleles per specimen in each stack (D).

Individual stacks were easily aligned in the large majority of cases. After manual inspection we made gene trees of 18 selected stacks, which include 2 to 10 specimens and 5 to 24 sequences. The number of alleles per specimen ranged from 1 to 7 (Fig 5.3).

Marker development for species delineation in the genus *Sargassum*: a RAD-Sequencing approach

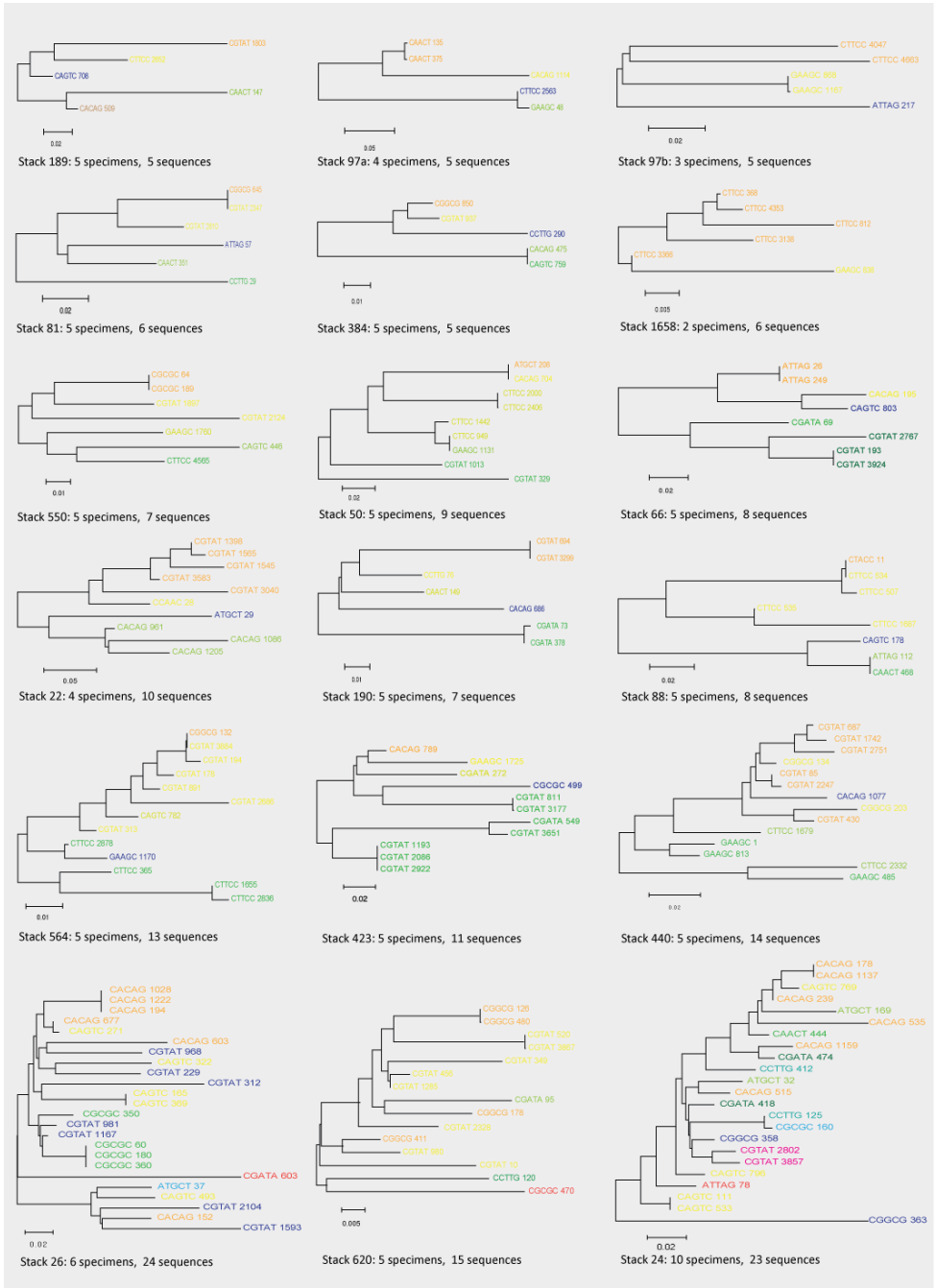


Fig. 5.4: Gene trees of 18 stacks with varying numbers of sequences, specimens and alleles per species.

5.4 Discussion

It has long been recognized that species delineation based on multiple unlinked loci will undoubtedly result in more robust and biologically realistic species boundaries (Dettman et al. 2003). However, development of such markers has been a slow and labor intensive process in most groups of nonmodel organisms. Next Generation Sequencing offers a relatively new technology which has the potential to speed up marker development considerably (Harismendy et al. 2009, Holsinger 2010, McCormack et al. 2013). One of the most promising approaches is offered by RAD Sequencing which reduces genome complexity by limiting marker selection to restriction site associated DNA tags.

Our study represents the first attempt to develop RAD Seq markers for algae. Below we discuss the outcome of the marker development and its potential use in future species addressing species delineation in *Sargassum* and algae in general. During the experiment we generated more than 21 million sequences which after quality control and analyses in Stacks and SiLiX yielded 231 alignments which could potentially be used to address species boundaries in the genus *Sargassum*. The development of more than 200 markers presents a more than significant increase over markers used in traditional studies which generally combine one or two mitochondrial and/or chloroplast markers with sequences from the ribosomal cistron. It also boosts the number of potential markers by a factor 10 to 20 compared to microsatellite-based studies.

A second major improvement is to be found in the resolution of the markers. One of the principal reasons to select a RAD-Seq approach to address species delineation in *Sargassum* was that traditional markers (e.g. *cox3*, see Fig. 5.2) yield little to no variation among entities which are morphologically well-differentiated. As can be deduced from the gene trees (Fig. 5.4), the RAD markers contain considerable variation, while the process of assembling stacks ensures that stacks only contain markers which are readily aligned. Gene trees often show multiple alleles which are sometimes shared among individuals of different morphospecies, completely in agreement with coalescent theory (Hein et al. 2004). Sequences can be analysed in a multispecies coalescent framework (e.g. STEM or *Beast) which allows species tree gene tree incongruence. Therefore we think that Rad markers offer the potential of being very important in future studies addressing species boundaries in algae.

This being said, there are issues that need be addressed in future studies. First, the number of RAD tags obtained per specimen varied considerably, ranging from more than 1.5 million (CTTCC: *Myagropsis*) to as low as 975 (CCGGT: *Sargassum horneri*). In our opinion these differences are related to differences in DNA quality. RAD Seq marker development requires a relatively high concentration of pure DNA that can be fragmented using restriction enzymes. This poses problems for many brown algae. Extracting DNA from brown algae of good quality has always been a challenge (Mayes et al. 1992, Hoarau et al. 2007, Snirc et al. 2010, Holloway et al. 2013) due to co-precipitation of polysaccharides and polyphenolics which have been shown to have an inhibitory effect on *Taq* polymerases. This constraint was, however, relaxed by the use of polymerase chain reaction based techniques (e.g. Sanger sequencing, DGGE, PCR-RFLP) which only require little amounts of DNA which are subsequently amplified. By isolating minimal amounts of DNA one also minimizes the amount of contaminating polysaccharides and polyphenolic substances. In RAD-Seq protocol, PCR is only used to select those fragments (16 cycles) that are labeled by both the P1 and P2 adaptors. There is no significant amplification of the fragments taking place. On the contrary increasing the number of PCR cycles results in overamplification of the library and significantly reduces the quality of the library. The absence of a typical amplification step is compensated by extraction of relatively large amounts of DNA (0.5-1 µg/µl). If contaminating substances inhibit the restriction digest in the first step of the protocol this will result in a lower number of fragments being labeled by the P1 adaptor. Therefore, varying DNA quality is likely to influence the number of RAD tags that are recovered from each specimen. We think it might be worth for brown algae to invest more effort in obtaining pure DNA by for example using gradient CsCl centrifugation.

A second issue posed, relates to the representation of the specimens and the number of alleles per stack. Ideally every stack contains two alleles from each individual. We see however that there are no stacks that contain sequences of all specimens. The specimens from which most sequences are obtained (CTTCC, GAAGC: *Myagropsis*; CGTAT: *Sargassum thunbergii*) are best represented in the stacks (Fig. 5.3). Therefore, the low representation of certain stacks might at least be partially attributed to the lower number of RAD tags resulting from insufficient DNA quality. Apart from representation per specimen, we also observe a varying number of alleles per alignment, which is more difficult to explain. A higher number of alleles per specimen might result from small sequencing errors, but it also possible that certain RAD tags represent duplicated elements (e.g. transposable elements)

in the genome or that *Sargassum* species might have been subject to polyploidization in the past. At present we cannot be more conclusive regarding the origin of multiple alleles per specimen in certain alignments. Apart from often observing more than 2 alleles, there are also numerous cases whereby we only observe one allele per specimen per alignment (Fig. 5.4). As a matter of fact, the majority of specimens is represented by one allele only per alignment. Increasing sequencing depth may reduce this number, but it is also possible that certain RAD tags have their origin in the mitochondrial or chloroplast genome.

Despite the issues posed, I'm quite convinced that RAD Sequencing has a great potential for population based molecular studies in algae. A selection of the current stacks for primer design can then be used to score markers by PCR followed by traditional Sanger sequencing. This approach can easily increase the number of nuclear nuclei is phylogeographic or species delimitation studies by a factor ten.

REFERENCES

- Billard, E., C. Daguin, G. Pearson, E. Serrao, C. Engel, and M. Valero. 2005. Genetic isolation between three closely related taxa: *Fucus vesiculosus*, *F. spiralis*, and *F. ceranoides* (Phaeophyceae). *Journal of Phycology* **41**: 900-905.
- Canovas, F. G., C. F. Mota, E. A. Serrao, and G. A. Pearson. 2011. Driving south: a multi-gene phylogeny of the brown algal family *Fucaceae* reveals relationships and recent drivers of a marine radiation. *Bmc Evolutionary Biology* **11**: 371.
- Carstens, B., A. R. Lemmon, and E. M. Lemmon. 2012. The promises and pitfalls of next-generation sequencing data in phylogeography. *Systematic Biology* **61**: 713-715.
- Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. *Molecular Ecology* **22**: 3124-3140.
- Coyer, J. A., G. Hoarau, J. F. Costa, B. Hogerdijk, E. A. Serrao, E. Billard, M. Valero, G. A. Pearson, and J. L. Olsen. 2011. Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis* and *F. vesiculosus* species complex in the North Atlantic. *Molecular Phylogenetics and Evolution* **58**: 283-296.
- Coyer, J. A., A. F. Peters, G. Hoarau, W. T. Stam, and J. L. Olsen. 2002. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (*Heterokontophyta* : Phaeophyceae) in a 100-year-old zone of secondary contact. *Proceedings of the Royal Society B-Biological Sciences* **269**: 1829-1834.
- Davey, J. W., P. A. Hohenlohe, P. D. Etter, J. Q. Boone, J. M. Catchen, and M. L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* **12**: 499-510.
- De Clerck, O., F. Leliaert, H. Verbruggen, C. E. Lane, J. C. De Paula, D. A. Payo, and E. Coppejans. 2006. A revised classification of the *Dictyoteae* (Dictyotales, Phaeophyceae) based on *rbcl* and 26S ribosomal DNA sequence analyses. *Journal of Phycology* **42**: 1271-1288.
- Degnan, J. H. and N. A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* **24**: 332-340.
- Dettman, J. R., D. J. Jacobson, and J. W. Taylor. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* **57**: 2703-2720.
- Engel, C. R., C. Daguin, and E. A. Serrao. 2005. Genetic entities and mating system in hermaphroditic *Fucus spiralis* and its close dioecious relative *F. vesiculosus* (*Fucaceae*, Phaeophyceae). *Molecular Ecology* **14**: 2033-2046.
- Etter, P. D., S. Bassham, P. A. Hohenlohe, E. A. Johnson, and W. A. Cresko. 2011. SNP discovery and genotyping for evolutionary genetics using RAD sequencing. *Methods in molecular biology* **772**: 157-178.
- Harismendy, O., P. C. Ng, R. L. Strausberg, X. Y. Wang, T. B. Stockwell, K. Y. Beeson, N. J. Schork, S. S. Murray, E. J. Topol, S. Levy, and K. A. Frazer. 2009. Evaluation of next generation sequencing platforms for population targeted sequencing studies. *Genome Biology* **10**: R32
- Hein, J., M. Schierup, and C. Wiuf. 2004. *Gene genealogies, variation and evolution: a primer in coalescent theory*. Oxford university press. pp. 276.
- Hoarau, G., J. A. Coyer, W. T. Stam, and J. L. Olsen. 2007. A fast and inexpensive DNA extraction/purification protocol for brown macroalgae. *Molecular Ecology Notes* **7**: 191-193.

- Holloway, B. A., M. R. Tarver, and T. E. Rinderer. 2013. An economical and effective high-throughput DNA extraction protocol for molecular marker analysis in honeybees. *Entomologia Experimentalis Et Applicata* **148**: 196-200.
- Holsinger, K. E. 2010. Next generation population genetics and phylogeography. *Molecular Ecology* **19**: 2361-2363.
- Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059-3066.
- Knowles, L. L. and L. S. Kubatko. 2010. *Estimating species trees: Practical and theoretical aspects*. Wiley-Blackwell, Hoboken. pp. 215.
- Kubatko, L. S. and J. H. Degnan. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology* **56**: 17-24.
- Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. Lopez-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA- based species delimitation in algae. *European Journal of Phycology* **49**: 179-196.
- Mattio, L. and C. Payri. 2010. Assessment of five markers as potential barcodes for identifying *Sargassum* subgenus *Sargassum* species (Phaeophyceae, Fucales). *Cryptogamie Algologie* **31**: 467-485.
- Mattio, L. and C. E. Payri. 2009. Taxonomic revision of *Sargassum* species (Fucales, Phaeophyceae) from New Caledonia based on morphological and molecular analyses. *Journal of Phycology* **45**: 1374-1388.
- Mattio, L., C. E. Payri, and V. Stiger-Pouvreau. 2008. Taxonomic revision of *Sargassum* (Fucales, Phaeophyceae) from French Polynesia based on morphological and molecular analyses. *Journal of Phycology* **44**: 1541-1555.
- Mattio, L., C. E. Payri, M. Verlaque, and B. de Reviere. 2010. Taxonomic revision of *Sargassum* sect. *Acanthocarpicae* (Fucales, Phaeophyceae). *Taxon* **59**: 896-904.
- Mattio, L., M. Zubia, B. Loveday, E. Crochelet, N. Duong, C. E. Payri, R. Bhagooli, and J. J. Bolton. 2013. *Sargassum* (Fucales, Phaeophyceae) in Mauritius and Réunion, western Indian Ocean: taxonomic revision and biogeography using hydrodynamic dispersal models. *Phycologia* **52**: 578-594.
- Mayes, C., G. W. Saunders, I. H. Tan, and L. D. Druehl. 1992. DNA extraction methods for Kelp (*Laminariales*) tissue. *Journal of Phycology* **28**: 712-716.
- McCormack, J. E., S. M. Hird, A. J. Zellmer, B. C. Carstens, and R. T. Brumfield. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution* **66**: 526-538.
- McCormack, J. E., J. M. Maley, S. M. Hird, E. P. Derryberry, G. R. Graves, and R. T. Brumfield. 2012. Next-generation sequencing reveals phylogeographic structure and a species tree for recent bird divergences. *Molecular Phylogenetics and Evolution* **62**: 397-406.
- Miele, V., S. Penel, and L. Duret. 2011. Ultra-fast sequence clustering from similarity networks with SiLiX. *Bmc Bioinformatics* **12**: 116.
- Neiva, J., G. A. Pearson, M. Valero, and E. A. Serrao. 2010. Surfing the wave on a borrowed board: range expansion and spread of introgressed organellar genomes in the seaweed *Fucus ceranoides* L. *Molecular Ecology* **19**: 4812-4822.
- Pereyra, R. T., L. Bergstrom, L. Kautsky, and K. Johannesson. 2009. Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *Bmc Evolutionary Biology* **9**: 70.

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- Silberfeld, T., L. Bittner, C. Fernandez-Garcia, C. Cruaud, F. Rousseau, B. de Reviere, F. Leliaert, C. E. Payri, and O. De Clerck. 2013. Species diversity, phylogeny and large scale biogeographic patterns of the genus *Padina* (Phaeophyceae, Dictyotales). *Journal of Phycology* **49**: 130-142.
- Silva, P. C., P. W. Basson, and R. L. Moe. 1996. Catalogue of the benthic marine algae of the Indian Ocean. University of California Publications in Botany **79**: 1-1259.
- Snirc, A., T. Silberfeld, J. Bonnet, A. Tillier, S. Tuffet, and J. S. Sun. 2010. Optimization of DNA extraction from Brown Algae (Phaeophyceae) based on a commercial Kit. *Journal of Phycology* **46**: 616-621.

Chapter 6

GENERAL DISCUSSION

Seaweeds are ecologically important primary producers and ecosystem engineers that play a central role in structuring coastal habitats (Harley et al. 2012). Similar to corals and trees, many seaweeds provide a physical substrate and habitat for fish species, crustaceans and other invertebrates in shallow marine environments (Graham 2004, Norderhaug et al. 2005). This role of habitat engineer is often, but not exclusively, fulfilled by brown algae such *Cystoseira*, *Dictyota*, *Padina*, *Sargassum* and *Turbinaria* in warm temperate to tropical regions and *Ecklonia*, *Laminaria* and *Macrocystis* (kelp) in cold temperate seas. Apart from fulfilling a structural role, algae are also important in oceanic food webs as primary producers and to a lesser extent as carbon sinks. The ability of seaweeds to strip the environment of nutrients which result for example from mariculture practices also forms an important contribution of seaweed nowadays (Chung et al. 2002, Kraemer et al. 2004, Al-Hafedh et al. 2014).

Apart from their ecological function, seaweeds especially in Asia, also present an important economic function. Several species are part of the everyday diet of Asian people or are used as traditional medicine, while other species are harvested for colloid extraction, feed for live stock or natural fertilizers (Do V.K. 1990, Huynh Q.N. and Nguyen H.Dinh 1998, Pacheco-Ruiz et al. 1998, Wong and Phang 2004, Pellizzari et al. 2007). The use and importance of seaweeds in Vietnam has been detailed in **Chapter 1**.

Exploitation of seaweed resources by coastal communities may however contribute to increased anthropogenic pressure on coastal habitats. For example, 48,000 wet tons of the brown algal genus *Sargassum*, an important habitat engineer of rocky shores, are harvested on an annual basis. Although harvesting of natural *Sargassum* populations is banned from some areas, in general little or no restrictions are enforced on the amount of biomass harvested as well as the timing of the harvest, which may lead to potential overexploitation, habit degradation and a loss of ecosystem services.

Below I review the results of my thesis focussing on this tension field between ecosystem function and potential overexploitation of biodiversity resources. I first discuss the current status of algal cultivation as a means to reduce the pressure imposed by harvesting natural algal populations in Vietnam. I then review the knowledge or knowledge gap on diversity of Vietnamese seaweeds and their distribution. Finally, I focus on diversity of the genus

Sargassum, undoubtedly the most important algal genus in terms of biomass, ecological function as well as economic value.

6.1 Seaweed mariculture and harvesting natural populations

Despite the fact that the geomorphological characteristics of the Vietnamese coastline are similar to those of the neighbouring countries, cultivation of seaweeds is largely underdeveloped. Only 7 species are now being cultivated from a commercial point of view. Furthermore, productivity is still unstable, with pilot projects often being abandoned after the start-up phase (Huynh Q.N. and Nguyen H.Dinh 1998, Huynh Q.N. and Tsutsui 2006, Le N.H. and Nguyen H.Dai 2010, Le N.H. et al. 2011). The fact that seaweed mariculture is lagging in comparison with neighbouring countries, can at least be partly explained by the absence of seaweed processing facilities in Vietnam. Also the fact that currently no high value products are being extracted from Vietnamese seaweeds, makes farming seaweeds probably less attractive. This however leads to a circle reasoning, whereby seaweed farming remains underdeveloped because of a lack of infrastructure and vice versa. The fact that raw seaweeds (dried biomass) are often unofficially exported to China at a low price, while Vietnam then imports the purified products (e.g. alginates) at a high price (DANIDA and SUMA 2002, Le N.H. and Nguyen H.Dai 2010) illustrates that at least from an economical point of view the need for a true seaweed industry in Vietnam needs to be considered.

The absence of a well-established seaweed mariculture, makes that coastal communities continue to harvest natural populations to meet domestic needs (e.g. seaweeds sold on local markets as vegetables). In most cases the ecological impact on coastal ecosystems is most likely limited, because the amount of seaweed harvested is limited and the species do not clearly fulfil a role as habitat engineer in coastal ecosystems. However, in the case of *Sargassum* the impact of overharvesting may lead to habitat degradation. *Sargassum* species in Vietnam attain heights of up to 3 meters and often form dense canopies structuring shallow subtidal environment, providing food and shelter to numerous invertebrates and fish species. A *Sargassum* plant typically lives for several years, with a short perennial primary axis from which long secondary axes develop. The latter are shed annually. The plants exhibit a clear seasonality which may differ among species and among populations of a single species. Harvesting *Sargassum* usually entails the cutting of annual

secondary axes and therefore does not necessarily result in the immediate removal of individuals in a population. However, if populations are harvested too early in the season, i.e. before the plants become reproductive, natural recruitment is imperilled. Several cases of habitat degradation due to overharvesting *Sargassum* have been reported (Gavino C. Trono and Tolentino 1993, Le N.H. 2009, Scientific Working Group 2011). Even, though the harvest of *Sargassum* might be regulated by local administrations in one or two provinces, these restrictions are rarely based on scientific studies addressing the phenology and population dynamics of the *Sargassum* populations in these areas.

Cultivating *Sargassum* species could offer a means to alleviate the pressure that harvesting natural populations imposes on coastal ecosystems. Contrary to many red and brown algae, cultivation of *Sargassum* is a relatively new phenomenon and definitely not as widespread. A series of papers on cultivation (Hwang et al. 2006, Xie et al. 2013), seedling production (Zhang et al. 2012), early development of germlings (Zhao et al. 2008) and photoperiodic response (Yoshikawa et al. 2014) indicate however that a lot of effort is being devoted to *Sargassum* mariculture. In China five species are currently cultivated: *S. fusiforme*, *S. thunbergii*, *S. fulvellum*, *S. muticum*, and *S. horneri* (Xie et al. 2013), and the cultivation of other species is under development (Zhao et al. 2008). A recent document (Redmond et al. 2014) even investigates the potential of *Sargassum* cultivation in the U.S.A. It should be noted however that most *Sargassum* cultivation refers to cold water adapted species and that transfer of technology to predominantly tropical *Sargassum* species may entail considerable research and development. For example, significant differences could be expected regarding the effect of photoperiod on reproduction. In *Sargassum horneri* and *S. muticum*, both cold water species, reproductive regulation is a response to long-day day lengths (Yoshikawa et al. 2014). For tropical species, which encounter much less variation in day length in their natural environment, the effects of photoperiod on reproduction still need to be examined.

Considering the long tradition of shrimp and fish farming in Vietnam, it may be considered to integrate seaweed farming into these practices. Integrated Multi-Trophic Aquaculture (IMTA), the intensive and synergistic cultivation of species occupying different trophic levels such as fish, invertebrates and seaweeds, opens up an effective and more sustainable way to treat wastewater before the latter leaks into the environment. IMTA has the potential

to create a wealth of ecological and economic advantages for sustainable seafood production. However, in order to make IMTA feasible additional research needs to be done with respect to the fundamental biology, e.g. reproduction, growth and nutrient uptake of the algae.

6.2 Seaweed diversity in Vietnam

Vietnam has a long history with respect to taxonomy and floristics of algae. We pointed out that the earliest records of Vietnamese seaweeds can be traced back to the late 18th century when the Portuguese missionary Juan de Loureiro reported 11 seaweeds in the *Flora Cochinchinensis* (1790). More than two centuries of botanical and marine exploration have gradually led to a more or less complete inventory of the Vietnamese seaweed flora. Yet knowledge of the marine seaweed diversity of Vietnam was scattered in the literature and largely inaccessible to phycologists as most data are published in regional papers, book chapters or reports and often in Vietnamese. As a result, estimates of species diversity ranged from 638 to 1000 (Pham H.H. 1969, Huynh Q.N. and Nguyen H.Dinh 1998, Dang D.H. et al. 2007). In **Chapter 2** I made a compilation of the Vietnamese macroalgal diversity and conclude that 827 species have been reported, of which the Rhodophyta show the highest species number (412 species), followed by the Chlorophyta (180 species), Phaeophyceae (147 species) and Cyanobacteria (88 species). The diversity of the Vietnamese seaweeds was subsequently compared with those of a number of neighbouring countries (Malaysia, Philippines, Taiwan and Thailand) and although the number of reported species is at a comparable level with that of the Philippines, the country with the highest species diversity in the area (>1100 species), the similarity at the species level between both floras appears minimal. A Sørensen similarity index of 0.32 indicated that less than 16% of the floras are shared between both countries. These low values are counterintuitive. Within similar climatic zones and in the absence of major dispersal barriers, marine biodiversity is expected to be much more homogenous (see Spalding et al. 2007). I therefore concluded that the low similarity with neighbouring countries is primarily an artefact resulting from poor taxonomic practices. The isolated nature of the Vietnamese scientific community may have partly contributed to this artefact. The fact that Vietnamese phycologists traditionally published in local journals, in the Vietnamese language and rarely interacted with colleagues from neighbouring countries almost certainly contributed to this situation.

Indications for this can be found in several papers published in Taxonomy of Economic Seaweeds series. These papers emerged from local workshops held between 1992 and 2004, sponsored by the California Sea Grant College, which united experts in specific taxonomic groups. As a result several questionable seaweed records were re-examined and corrected. Most notably Japanese *Sargassum* experts re-examined a list of questionable *Sargassum* records from Vietnam and nearly invariably concluded that the original reports were erroneous (Yoshida et al. 2002). The Taxonomy of Economic Seaweed workshops, however, largely predate the use of molecular sequence data in algal taxonomy and consequently considerable uncertainty remains on the validity of those identifications.

Distribution patterns of seaweed along the coast of Vietnam shows counterintuitive results in comparison to species diversity by province (Fig 2.3 – Chapter 2). Some provinces display a very high species richness (e.g. Khanh Hoa, Binh Thuan, Quang Ninh, Kien Giang, Quang Ngai, Da Nang) while being situated next to provinces that boast only few species (e.g. Binh Dinh, Phu Yen, Quang Nam, Hue). If not an artifact caused by unequal sampling effort, this pattern may highlight the importance of habitat in determining diversity patterns of benthic marine algae (Kerswell, 2006). Understanding species diversity patterns therefore may become more meaningful if metrics such as similarity or diversity indices (Whittaker 1972, Magguran 2004) are integrated with species traits that capture the ecological niche (e.g. substrate preference) of the species.

Identifying seaweed using morphological criteria only is indeed a difficult task. De Clerck et al. (2013) pointed out that the introduction of molecular techniques in algal systematics has profoundly altered our view on every taxonomic level. New classes were discovered, the classification at the ordinal and family level was completely restructured in many cases, but the profound changes were perhaps to be found on the species level, molecular markers unveiled massive cryptic diversity. Although in a few cases the use of molecular techniques resulted in taxonomic deflation, e.g. *Membranella nitens* which was reduced to a taxonomic synonym of *Smithora naiadum* (West and Zuccarello 2009), in most cases it appeared that traditional systematics had underestimated species diversity. Extreme cases include the genus red algal *Portieria* which was generally regarded a species-poor genus with a couple of species at the most, but which may in the end may contain as much as 100 or more species (Payo et al. 2013). Given the realization that morphology is a poor predictor of

species diversity in algae, the results of most taxonomic papers published in international journals is now based at least partly molecular data combined with morphology, ecology or (eco-)physiology of the organisms. It seems that, at least in the peer reviewed literature, it has become rare for extant species to be defined solely by morphology (Leliaert et al. 2014).

6.3 *Sargassum* diversity in Vietnam, a molecular approach

In Vietnam the use of molecular markers to define species boundaries is still in its infancy, at least with respect to algal species. Thereto, I aimed to address the diversity of the genus *Sargassum* using a combination of morphological analyses and gene sequences data (ITS rDNA). *Sargassum* is one of the most important algal genera worldwide. 615 species and intraspecific taxa are currently accepted (Guiry and Guiry 2014). However in the light of recent molecular findings there is major uncertainty regarding the actual number of species. At least Mattio et al. (2013) seem to be of the opinion that traditional practices severely overestimated the number *Sargassum* species. For example in a recent study focusing on the diversity of *Sargassum* in Mauritius and Réunion the authors reduced the number of *Sargassum* species from 44 to 6.

In Vietnam, *Sargassum* is very common on rocky shores, often dominating reefs, shallow coastal lagoons and offshore islands. In total 70 currently accepted species and intraspecific taxa have been reported (**Chapter 3**). I used a classic phylogenetic approach which included 126 sequences generated from samples collected in this study to which 38 sequences from GenBank were added (**Chapter 4**). The sequences represented 28 morphospecies (26 identified and 2 tentative species), which segregated into 5 large clades which correspond to *subgenera* and *sections*. Within those sections however there was little or no resolution, the sequences being virtually identical.

Some morphospecies appear in more than one clade. For example, specimens identified as *Sargassum ilicifolium* and *S. mcclurei* were resolved in several clades, despite very characteristic morphologies. At least two distinct mechanisms can account for such observations. On the one hand, introgression through natural hybridization can cause gene tree – species tree incongruence. We have not been able to demonstrate this, but when we compare *Sargassum* to related genera in the Fucales it is clear that hybridization between closely related species is commonly observed. The lack of resolution in the ITS marker,

however, complicates the interpretation of gene tree – species tree incongruence. Closely related species share virtually identical ITS sequences, making it impossible to detect introgression between closely related species. It seems less likely that hybridization takes place between distantly related species (e.g. species belonging to different sections), making the introgression hypothesis less likely. In addition the ribosomal cistron typically occurs as tandem repeats, which would result in multiple divergent copies of the ITS region and hence impede direct sequencing. This was not the case in our dataset. The ITS2 region amplifies readily and sequences show no traces of multiple copies. An alternative explanation is formed by cryptic speciation. Cryptic diversity is common in brown (e.g. *Fucus*, *Dictyota*), green (e.g. *Caulerpa*, *Chlorodesmis*, *Cladophoropsis*) and red alga (e.g. *Portieria*, *Spyridia*, *Porphyra*) (Coyer 2002, Lindstrom 2009, Conklin 2012, Payo 2013). Therefore, the possibility of cryptic diversity in *Sargassum* does not come entirely as a surprise. More studies, sampling multiple loci (e.g. RAD-Seq) are needed however to confirm these findings and rule out introgression by natural hybridization.

Given our molecular results we could be reasonably conclusive regarding the absence of certain subgenera from Vietnam, thereby confirming previous doubts as to whether subgenera *Arthropycus* and *Bactrophycus* were present despite being commonly reported in the past. Unfortunately our analyses were not conclusive regarding species-level diversity. The fact that ITS sequences revealed little or no differences within a section could point towards the fact that species diversity is indeed grossly overestimated and that what has been traditionally named as different species represent nothing but growth forms of a few species. This would confirm the idea of Mattio et al. (2013). On the other, it could be that *Sargassum* only diverged recently and that therefore the ITS region is largely uninformative. To address this question I attempted a Restriction site Associated DNA-marker approach (**Chapter 5**). The results of these analyses, although still preliminary are very promising. We generated a panel of more than 200 putative nuclear markers which show ample variation at the nucleotide level. In addition, further improvements in the RAD Seq protocol, e.g. with respect DNA extraction, might even increase the number of putative markers. Further experiments need to be conducted to test the applicability of RAD Seq markers for species delineation. The amount of variation encountered is suggestive of a rich

species-level diversity in *Sargassum*, contradicting recent papers which apply a much broader species concept and tend to merge a huge number of species.

Next-generation sequencing technologies are revolutionizing the field of evolutionary biology, opening the possibility for genetic analyses at scales not previously possible. This included RAD-Seq approaches as well (Davey & Blaxter 2010; Etter 2011). However, this versatile method is at present still in its infancy. The RAD-Seq approach to study systematics of *Sargassum* or other algae will eventually refine species boundaries in these genera and therefore be more accurate than the single marker approaches which are currently used. However, before doing so, some technical issues need to be solved first.

REFERENCES

- Al-Hafedh, Y. S., A. Alam, and A. H. Buschmann. 2014. Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. *Reviews in Aquaculture* **6**:1-11.
- Chung, I., K. YH, Y. C, K. GP, and L. JA. 2002. Application of seaweed cultivation to the bioremediation of nutrient-rich effluent. *Algae* **17**:1-10.
- Coyer, J. A., A. F. Peters, G. Hoarau, W. T. Stam, and J. L. Olsen. 2002a. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta : Phaeophyceae) in a 100-year-old zone of secondary contact. *Proceedings of the Royal Society B-Biological Sciences* **269**: 1829-1834.
- Conklin, K. Y. and A. R. Sherwood. 2012. Molecular and morphological variation of the red alga *Spyridia filamentosa* (Ceramiales, Rhodophyta) in the Hawaiian Archipelago. *Phycologia* **51**: 347-357.
- Dang, D.H., M.H. Hoang and N.S. Pham 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology* **19**: 817-826.
- DANIDA and SUMA. 2002. Seaweed farming and production in Vietnam, present situation and possibilities. MARD-VIETNAM. pp. 57
- Davey, J. L. and M. W. Blaxter. 2010. RADSeq: next-generation population genetics. *Briefings in Functional Genomics* **9**: 416-423.
- De Clerck, O., M. D. Guiry, F. Leliaert, Y. Samyn, and H. Verbruggen. 2013. Algal taxonomy: a road to nowhere? *Journal of Phycology* **49**: 215-225.
- Do, V.K. 1990. Status of production and utilization of seaweed in Vietnam. *Proceedings of the Regional Workshop on the Culture & Utilization of Seaweeds, Philippines*. FAO.

- Etter, P. D., S. Bassham, P. A. Hohenlohe, E. A. Johnson, and W. A. Cresko. 2011. SNP discovery and genotyping for evolutionary genetics using RAD sequencing. *Methods in molecular biology* **772**: 157-178.
- Gavino C. Trono, J. and G. L. Tolentino. 1993. Studies on the management of *Sargassum* (Fucales, Phaeophyta) bed in Bolinao, Pangasinan, Phillipines. *The Korean Journal of Phycology* **8**: 249-257.
- Graham, M. H. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. *Ecosystems* **7**: 341-357.
- Group, S. W. 2011. The vulnerability of coastal and marine habitats in South Australia. Marine Parks, Department of Environment, Water and Natural Resources South Australia.
- Guiry, M. D. and G. M. Guiry. 2014. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on May 2014.
- Harley, C. D. G., K. M. Anderson, K. W. Demes, J. P. Jorve, R. L. Kordas, T. A. Coyle, and M. H. Graham. 2012. Effects of climate change on global seaweed communities. *Journal of Phycology* **48**: 1064-1078.
- Huynh, Q.N. and H.Dinh Nguyen. 1998. The seaweed resources of Vietnam. *In: (Critchley, A.T. and M. Ohno Eds) Seaweed Resources of the World*. Kanagawa International Fisheries Training Centre, JICA. pp. 62-69.
- Huynh, Q.N. and T. Isao. 2006. Kappaphycus cultivation and utilization in Vietnam. *In: (Critchley A.T. and Phang S.M., eds) Advances in Seaweed Cultivation and Utilization in Asia*. University of Malaya Maritime Research Centre, Penang. pp. 171-177.
- Hwang, E. K., C. S. Park, and J. M. Baek. 2006. Artificial seed production and cultivation of the edible brown alga, *Sargassum fulvellum* (Turner) C. Agardh: Developing a new species for seaweed cultivation in Korea. *Journal of Applied Phycology* **18**: 251-257.
- Kerswell, A. P. 2006. Global biodiversity patterns of benthic marine algae. *Ecology* **87**: 2479-2488.
- Kilar, J. A., M. D. Hanisak, and T. Yoshida. 1992. On The expression of phenotypic variability: Why is *Sargassum* so Taxonomically difficult? *In: (Abbott, I.A. Eds) Taxonomy of Economic Seaweeds with reference to some Pacific and Western Atlantic species 3*, California Sea Grant College Program, La Jolla, CA. pp. 95-118.
- Kraemer, G. P., R. Carmona, C. Neefus, T. Chopin, S. Miller, X. Tang, and C. Yarish. 2004. Preliminary examination of the bioremediation and mariculture potential of a Northeast U.S.A. and an Asian species of *Porphyra*. *Bull. Fish. Res. Agen. Supplement No. 1*: 77-82.
- Le, N.H., P.C. Nguyen, D.K. Dang and M.L. Bui 2011. Potential of seaweed biomass as the raw materials for bioethanol production in Vietnam. 8th Biomass - Asia Workshop, Hanoi - VietNam.
- Le, N.H. and H.D. Nguyen 2010. *Rong câu Việt nam nguồn lợi và sử dụng (The Vietnamese Gracilaria, resources and utilization)*. Vietnamese Natural Science and Technology Publishing House, Hanoi. pp. 241
- Le, N.H., M.L. Bui, D.T. Vo, B.K. Nguyen, X.M. Vo, Q.T. Truong and H.D. Nguyen 2009. Mùa vụ rong mơ ở tỉnh Khánh hòa-Việt Nam (*Sargassum* season of Khanh Hoa Province -

- Vietnam). Proceeding of the 3rd National Scientific Conference on Ecology and Biological Resources: 958 - 961.
- Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. Lopez-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA- based species delimitation in algae. *European Journal of Phycology* **49**: 179-196.
- Lindstrom, S. 2009. Cryptic diversity, biogeography and genetic variation in Northeast Pacific species of *Porphyra sensu lato* (Bangiales, Rhodophyta). In (M. Borowitzka, A. Critchley, S. Kraan, A. Peters, K. Sjøtun, and M. Notoya, Eds) *Nineteenth International Seaweed Symposium*. Springer Netherlands. pp. 501-512. Loureiro, J. o. d. 1790. *Flora Cochinchinensis* (Vol. 1). Typis, et Expensis Academicis.
- Magurran, A. E. 2004. *Measuring Biological Diversity*. Wiley-Blackwell. pp. 264
- Mattio, L. and C. E. Payri. 2011. 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *Botanical Review* **77**: 31-70.
- Mattio, L., M. Zubia, B. Loveday, E. Crochelet, N. Duong, C. E. Payri, R. Bhagooli, and J. J. Bolton. 2013. *Sargassum* (Fucales, Phaeophyceae) in Mauritius and Réunion, western Indian Ocean: taxonomic revision and biogeography using hydrodynamic dispersal models. *Phycologia* **52**: 578-594.
- Norderhaug, K. N., H. Christie, J. H. Fossa, and S. Fredriksen. 2005. Fish-macrofauna interactions in a kelp (*Laminaria hyperborea*) forest. *Journal of the Marine Biological Association of the United Kingdom* **85**: 1279-1286.
- Pacheco-Ruiz, I., J. A. Zertuche-Gonzalez, A. Chee-Barragan, and R. Blanco-Betancourt. 1998. Distribution and quantification of *Sargassum* beds along the west coast of the Gulf of California, Mexico. *Botanica Marina* **41**: 203-208.
- Pham, H.H. 1969. Marine Algae of south Vietnam (Rong biển miền nam Việt nam). Sai Gon study Center - Ministry of Education and Youth. pp.558.
- Payo, D. A., F. Leliaert, H. Verbruggen, S. D'hondt, H. P. Calumpong, and O. De Clerck. 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proceedings of the Royal Society B-Biological Sciences* **280**: 1753.
- Pellizzari, F. M., T. Absher, N. S. Yokoya, and E. C. Oliveira. 2007. Cultivation of the edible green seaweed *Gayralia* (Chlorophyta) in southern Brazil. *Journal of Applied Phycology* **19**: 63-69.
- Redmond, S., J. K. Kim, C. Yarish, M. Pietrak, and I. Bricknell. 2014. Culture of *Sargassum* in Korea: techniques and potential for culture in the U.S. Orono, M. E: Maine Sea Grant Programme. pp. 16.
- West, J. A. and G. C. Zuccarello. 2009. Taxonomic deflation: molecular evidence places *Membranella nitens* in taxonomic synonymy with *Smithora naiadum* (Erythropeltidales, Compsopogonophyceae, Rhodophyta). *Phycologia* **48**: 543-546.
- Whittaker, R. H. 1972. Evolution and measurement of species diversity. *Taxon* **21**: 213–251.
- Wong, C. L. and S. M. Phang. 2004. Biomass production of two *Sargassum* species at Cape Rachado, Malaysia. *Hydrobiologia* **512**: 79-88.
- Xie, E. Y., D. C. Liu, C. Jia, X. L. Chen, and B. Yang. 2013. Artificial seed production and cultivation of the edible brown alga *Sargassum naozhouense* Tseng et Lu. *Journal of Applied Phycology* **25**: 513-522.

General discussion

- Yoshida, T., N. H. Dai, T. Ajisaka, and T. Noro. 2002. Verification of *Sargassum* species identified with subgenera *Bactrophyucus*, *Phyllotrichia* and *Schizophycus* in Vietnam. In: (Abbott, I.A. Eds) *Taxonomy of Economic Seaweeds with reference to some Pacific species* **8**, California Sea Grant College Program, La Jolla, CA. pp. 95-102.
- Yoshikawa, S., M. Kamiya, and K. Ohki. 2014. Photoperiodic regulation of receptacle induction in *Sargassum horneri* (Phaeophyceae) using clonal thalli. *Phycological Research* **62**: 206-213.
- Zhang, Q. S., Y. Z. Tang, S. K. Liu, S. B. Zhang, Z. C. Lu, S. H. Cu, and Y. Q. Yu. 2012. Zygote-derived seedling production of *Sargassum thunbergii*: focus on two frequently experienced constraints in tank culture of seaweed. *Journal of Applied Phycology* **24**: 707-714.
- Zhao, Z. G., F. J. Zhao, J. T. Yao, J. M. Lu, P. O. Ang, and D. L. Duan. 2008. Early development of germlings of *Sargassum thunbergii* (Fucales, Phaeophyta) under laboratory conditions. *Journal of Applied Phycology* **20**: 925-931.

SUMMARY

Vietnam, a country in South-East Asia, is characterized by a coastline nearly 3300 km long. The coast; stretching from Gulf of Tonkin in the North to the gulf of Thailand in the South, has a predominant North-South orientation resulting in a rich and varied coastal environment. The people in Vietnam have traditionally relied on the rich coastal resources, first by harvesting natural populations and more recently by implementing aquaculture practices. Next to fish and shellfish, marine seaweeds (macroalgae) have traditionally been harvested among most of the Vietnamese coast. A survey of the uses of Vietnamese seaweeds results in 82 species which are considered economically valuable (**Chapter 1**). Many of these seaweeds are used for human consumption, while others have a role in traditional medicine or are used for colloid extraction (e.g. agar, carrageenan) or as feed for animals. Contrary however to neighboring countries such as China, the Philippines and Indonesia where seaweed mariculture has been seriously developed over the last decades, culturing seaweeds remains a largely marginal activity in Vietnam. Reasons as to why seaweed mariculture has not taken off as compared to the neighboring countries are at least partly to be found in the absence of a phycocolloid industry in Vietnam. Instead Vietnam exports large amounts of raw seaweeds, harvested from natural populations, at a low price, and then imports the purified phycocolloids at a much higher price. A steadily growing demand for seaweed-derived products results in an increasing pressure on coastal ecosystems which risk overharvesting of natural populations. This risk is especially eminent for the brown algal genus *Sargassum* of which approximately 50.000 wet tons is harvested every year. *Sargassum* represents an important ecosystem engineering species of subtidal coastal habitats. The plants can grow easily up to 3-4 m in length and form dense vegetations with a major nursery function for countless juvenile vertebrate and invertebrate species. If plants, the uprights at least are annual, are harvested before becoming fertile, this may reduce the rejuvenation of natural populations and eventually lead to habitat degradation.

Despite the value of seaweeds for humans as well as their role in coastal ecosystems in Vietnam, relatively little is known about them. Basic information on for example the number of species present and their distributions is not easily available. Most information on seaweeds is scattered in local literatures, written in Vietnamese. Thereto, we compiled a

checklist of Vietnamese seaweeds (**Chapter 2**). A total of 827 species are reported (412 Rhodophyta, 180 Chlorophyta, 147 Phaeophyceae, 88 Cyanobacteria). This species richness is comparable to that of the Philippines and considerably higher than Taiwan, Thailand or Malaysia. A comparison of the species composition with neighbouring countries yielded surprisingly low similarities. Rather than an indication of a biogeographical pattern, we are of the opinion that the low similarity with neighboring countries is primarily an artifact resulting from taxonomic inconsistencies. The checklist could serve as a valuable tool, to reveal the seaweed diversity in Vietnam and to stimulate intraregional comparative research.

Sargassum with 70 species and intraspecific taxa is undoubtedly the most species-rich seaweed genus in Vietnam (**Chapter 3**). The estimate of *Sargassum* diversity is however entirely based on interpretations of the morphological characters such as the base, secondary axes, the leaves, vesicles and receptacles. Therefore we reassessed *Sargassum* diversity based on molecular gene sequence data of the internal spaces of the ribosomal cistron (ITS rDNA) (**Chapter 4**). Although successful at higher taxonomic levels, subgenera and sections, the resolution of the ITS marker was not sufficient to point species boundaries with confidence. Many morphologically distinct ‘species’ were characterized by virtually identical ITS sequences. Conversely, specimens identified on morphological criteria as one ‘species’ were resolved in different clades. Results could be interpreted in two possible ways. First, traditional species concepts in *Sargassum* have been too broadly defined and the genus contains far less species. Alternatively, species in *Sargassum* result from a recent radiation and even the highly variable ITS region does not display enough variation to resolve the relationship among them. To address these outstanding questions we explored to use of Restriction site Associated DNA Sequences (RAD-Seq) to generate a set of highly variable nuclear markers. RAD-sequencing is a Next Generation Sequencing-based technique that generates thousands to hundreds of thousands DNA fragments that can be scored for Single Nucleotide Polymorphisms (SNPs) which in turn can be compared between individuals. RAD markers can be used to pinpoint loci under selection, or are used as neutral markers to reconstruct phylogeographical patterns or elucidate phylogenetic relationships among closely related species. During the experiment we generated more than 21 million sequences which after quality control and analyses in Stacks and SiLiX yielded 231

alignments which could potentially be used to address species boundaries in the genus *Sargassum*. The development of more than 200 markers presents a more than significant increase over markers used in traditional studies which generally combine one or two mitochondrial and/or chloroplast markers with sequences from the ribosomal cistron. It also boosts the number of potential markers by a factor 10 to 20 compared to microsatellite-based studies. Despite these results, there are issues that remain to be solved. For example, the libraries of the individual specimens are highly unequal in size. Some specimens are represented by 1.5 million sequences while others only have barely 800 sequences. We believe the main reason why some specimens are underrepresented is due to insufficient DNA quality. Therefore, future studies should focus on obtaining high quality DNA from *Sargassum*. A second issue is posed by the presence of multiple alleles in the final alignments. Normally one would expect 2 alleles per marker for a diploid organism. Our final alignments often contain more than 2 alleles per specimen. It is unclear at present if this is a technical issue which could eventually be solved by fine-tuning the parameters of the Stacks analyses or whether the multiple alleles issue has an underlying biological cause (polyploidisation). Despite these issues, RAD Sequencing has a great potential for population based molecular studies in algae. A selection of the current stacks can be used for primer design which can then be used to score markers by PCR followed by traditional Sanger sequencing. This approach can easily increase the number of nuclear markers for phylogeographic or species delimitation studies by a factor ten.

Modest as it may be, I hope that by providing a compilation of the seaweed diversity in Vietnam and their economical importance, and by introducing DNA-based methodologies to assess species diversity in the ecologically and economically important genus *Sargassum*, I have contributed to the foundation for a sustainable exploitation of natural resources and continuous integrity of marine habitats.