

論文の内容の要旨

水圏生物科学専攻

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論文題目 Studies on phylogeography of *Sargassum polycystum* C. Agardh in waters of South East Asia and Japan

(東南アジアおよび日本の海域におけるコバモクの系統地理学に関する研究)

The genus *Sargassum* is the most widely distributed brown seaweed, which occurs in temperate and tropical regions, especially in Indo-west Pacific region and Australia. This genus plays an important role in marine ecological system that forms a dense submarine forest as an essential habitat for numerous marine organisms and biosorption for improving environmental conditions. More than 400 species of the genus *Sargassum* have been described based on morphological and anatomical structures; however, this genus shows high levels of morphological variations that may lead to misidentification problem and misunderstanding concept of taxonomic scenarios. In addition, this genus has been used as a model to determine whether the distribution and connectivity of marine population that have been affected by historical events, environmental condition or ocean circulation.

Molecular genetics have been applied as a useful tool for addressing some biological questions regarding to taxonomy and distribution pattern of seaweed. With respect to the genus *Sargassum*, molecular techniques combined with morphological data have been extensively applied to verify the phylogenetic relationship and species boundaries, and also elucidate the perspicuous historical pattern of population movement. Recently, most studies of phylogeny and phylogeography of this genus have been successfully done in temperate region, while very few studies have been carried out in tropical region,

especially in Southeast Asia. As a result, species diversity, population structure and distribution pattern of *Sargassum* in Southeast Asian region are yet to be completely explored.

In order to gain a clear picture of phylogenetic diversity and phylogeographic pattern of the genus *Sargassum* in Southeast Asian region, my PhD researches attempt (1) to investigate the phylogenetic relationship of common species of *Sargassum* from Thailand based on morphological and molecular data and, (2) to elucidate phylogeographic pattern of *Sargassum polycystum* C. Agardh, a widely distributed species in South Asia, in more detail using three different DNA regions: *cox1* and *cox3* from mitochondrial DNA and ITS2 from nuclear DNA and then verify what factors influence such patterns.

Taxonomic research of the genus *Sargassum* in Thailand was investigated mainly on the basis of gross morphology and anatomical structures, and roughly, twelve species were reported to date. Due to a high amount of morphological plasticity in this genus, some closely related species are morphologically indistinguishable that easily lead to misidentification, especially when these species occur in the same area. This study aimed to determine the taxonomic framework and phylogenetic relationship of some common species of *Sargassum* from Thailand using morphological and molecular data. Twenty specimens recently collected from both in the Gulf of Thailand and Andaman Sea were morphologically identifiable to nine species: *Sargassum baccularia* (Mertens) C. Agardh, *S. binderi* Sorder, *S. cinereum* J. Agardh, *S. crassifolium* J. Agardh, *S. cristaefolium* C. Agardh, *S. polycystum* C. Agardh, *S. oligocystum* Montagne, *S. stolonifolium* Phang et Yoshida and *S. swartzii* (Turuner) C. Agardh. Only one species, *S. polycystum*, was found in both sides, while *S. baccularia*, *S. binderi*, *S. cinereum*, *S. crassifolium*, *S. oligocystum* and *S. swartzii* occurred in the Gulf of Thailand and *S. cristaefolium* and *S. stolonifolium* were found in Andaman Sea.

For molecular analysis, internal transcribed spacer 2 (ITS2) was used to investigate the phylogenetic relationship within these nine species. Interspecific genetic variation in *Sargassum* species

found in Thailand was relatively low. Three different methods (MP, ML and BI) of phylogenetic analyses revealed that our specimens formed a monophyletic group of the subgenus *Sargassum* with six distinct phylogenetic clades: *S. oligocystum*/*S. baccularia* clade, *S. cinereum* clade, *S. crassifolium*/*S. crisraefolium* clade, *S. polycystum* clade, *S. stolonifolium* clade and *S. binderi*/*S. swartzii* clade.

In the section level of subgenus *Sargassum*, morphological data and ITS2 sequences presented the controversy classification schemes, which found in section *Binderianae*. Morphologically, the section *Binderianea* consisted of four species found in Thailand: *S. baccularia*, *S. binderi*, *S. oligocystum* and *S. swartzii*, while phylogenetic analyses showed two well-separated clades within this section: *Binderianae* I clade (*S. oligocystum* and *S. baccularia*) and *Binderianae* II clade (*S. binderi* and *S. swartzii*). This result indicated that section *Binderianae* is possibly divided into two distinct sections; however additional samples of all members within this section and further studies in morphology and different molecular markers are required. In the species level, closely related species of *S. binderi* and *S. swartzii* yielded nearly identical sequences of ITS2, indicating possible species complex within these two species.

This study attempted to use a combined morphological and molecular approach for better understanding of taxonomy of Thai *Sargassum*, yet the phylogenetic relationship and species boundaries remained ambiguous. Further studies using multidisciplinary approaches, namely morphometric, multi-markers phylogeny and coalescent strategies of species delimitation, are needed to gain more insight into systematics and evolutionary affinities of this genus.

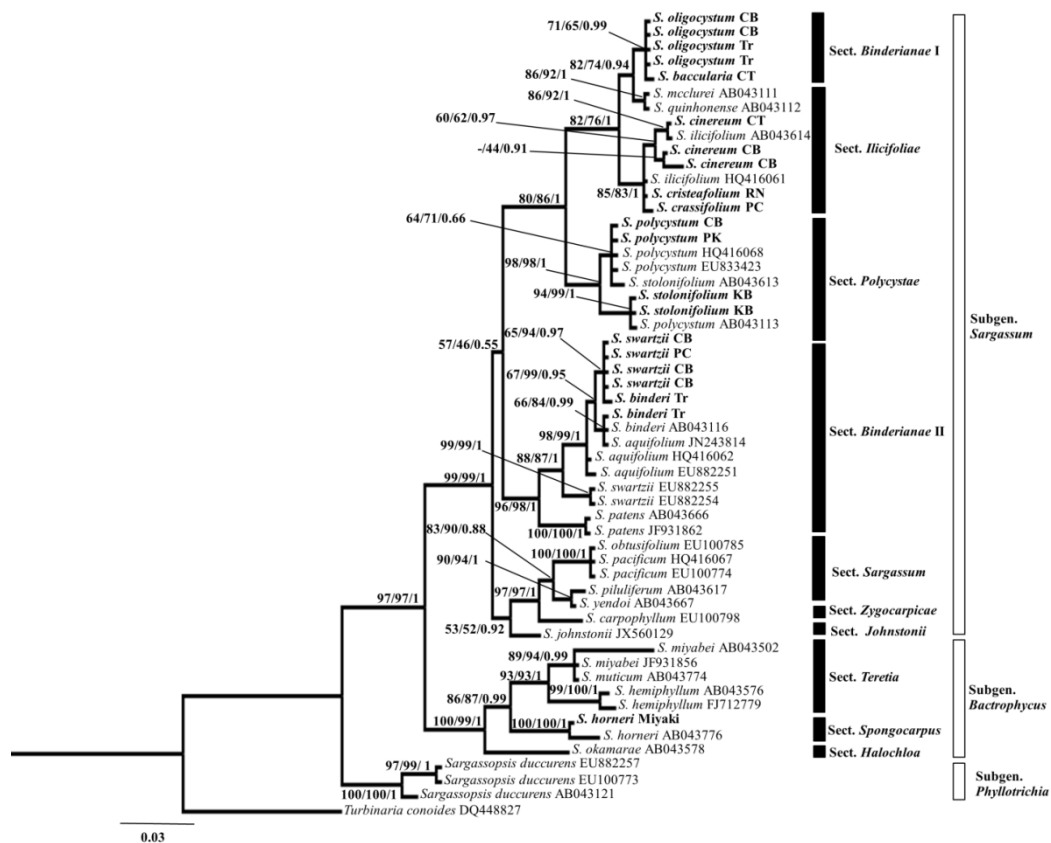


Figure1 Bayesian tree based on ITS2 gene sequences. The bootstrap values shown at each node were MP/ML/Bi (Bayesian analysis). Scale bar = 0.03 substitutions per site.

Climate changes may have affected historical or contemporary geographic distribution, abundance and genetic structure of marine organisms. *Sargassum polycystum* C. Agardh is the most abundant species, which is likely to be an excellent model for studying distribution pattern and population connectivity. In the study, phylogenetic patterns of *S. polycystum* were investigated using different genetic markers of mitochondrial DNA (*cox1* and *cox3*) and nuclear DNA (ITS2). Eleven populations (141 samples) from *cox1*, thirteen populations (141 samples) from *cox3* and ten populations (127 samples) from ITS2 were successfully carried out. All samples were randomly collected from Japan to Indonesia (Two localities in Japan, one locality in Cambodia, four to seven localities in Thailand, one locality in Singapore and one or two localities in Indonesia), emphasizing in the area of the Gulf of Thailand. In mitochondrial DNA, 10 haplotypes (H1-H10) was found in *cox1*, and 6 haplotypes (S1-S6) was found in

cox3, while nuclear DNA ITS2 yielded 12 haplotypes (A1-A12). The results showed that H1, S1 and A1 were likely to be an ancestral haplotype as indicated by consisting these haplotype from almost all populations. Differences in haplotypes diversity and haplotype sharing among population suggested two distinct geographical areas, representing two main groups of population: northern group (Japan, Cambodia and the Gulf of Thailand) and southern group (western of Thailand, Singapore and Indonesia)

High haplotype diversity was found in southern part of South East Asia (Bali, Indonesia and Phuket, Thailand), while low haplotype diversity was encountered in between the Gulf of Thailand and Japan. This suggested that the southern area of Southeast Asia was likely to be an origin of *S. polycystum*, and distribution pattern of this species seemed to expand southward to the northern areas of Southeast Asia.

Distribution pattern and population connectivity of *S. polycystum* corresponded to historical events of the last glacial maximum (LGM). In last glacial period, Southeast Asia region was connected by land bridge between Java Sea and the Gulf of Thailand as called Sundaland. Since the LGM period, sea levels in Sundaland have risen and affected coastal habitats, and this has an important impact on population structure change in *S. polycystum* as found in population connectivity between southern and northern population. Results also indicated that the northern population (the Gulf of Thailand to Japan) has more recently expanded than southern population (Phuket to Bali).

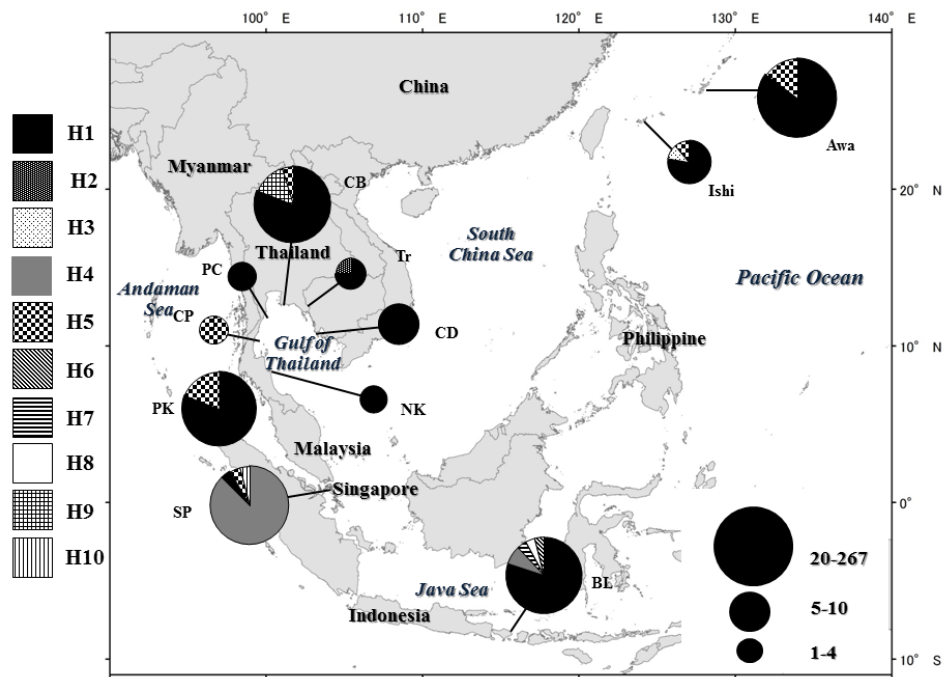


Figure 2 Geographical distribution of haplotypes in *Sargassum polycystum* based on mtDNA *cox1*. Size of the circle is proportional to the sample size of each populations, and each pie-graph shows the frequency of haplotype in the population

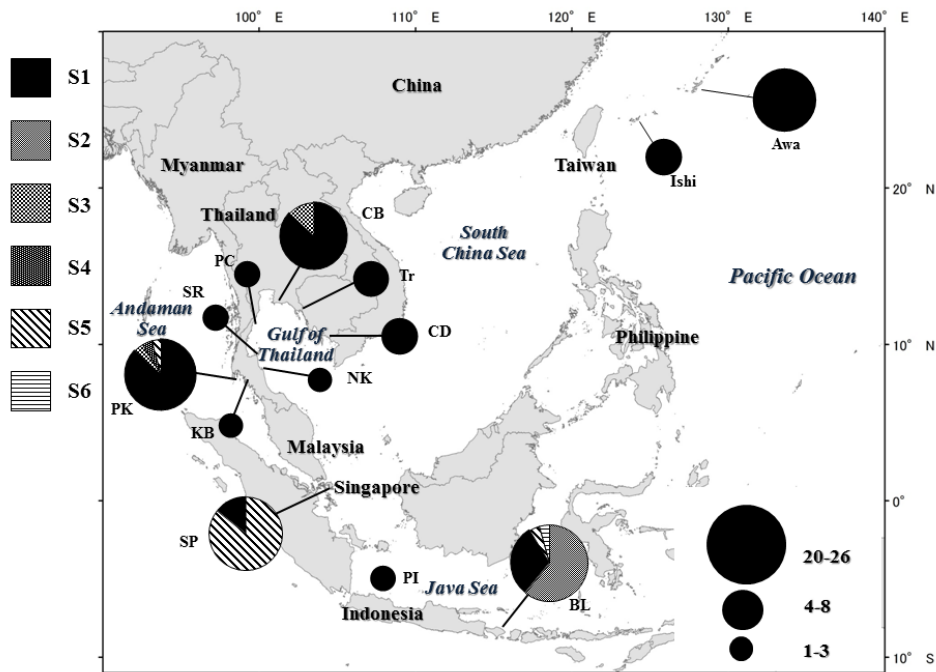


Figure 3 Geographical distribution of haplotypes in *Sargassum polycystum* based on mtDNA *cox3*. Size of the circle is proportional to the sample size of each populations, and each pie-graph shows the frequency of haplotype in the population.

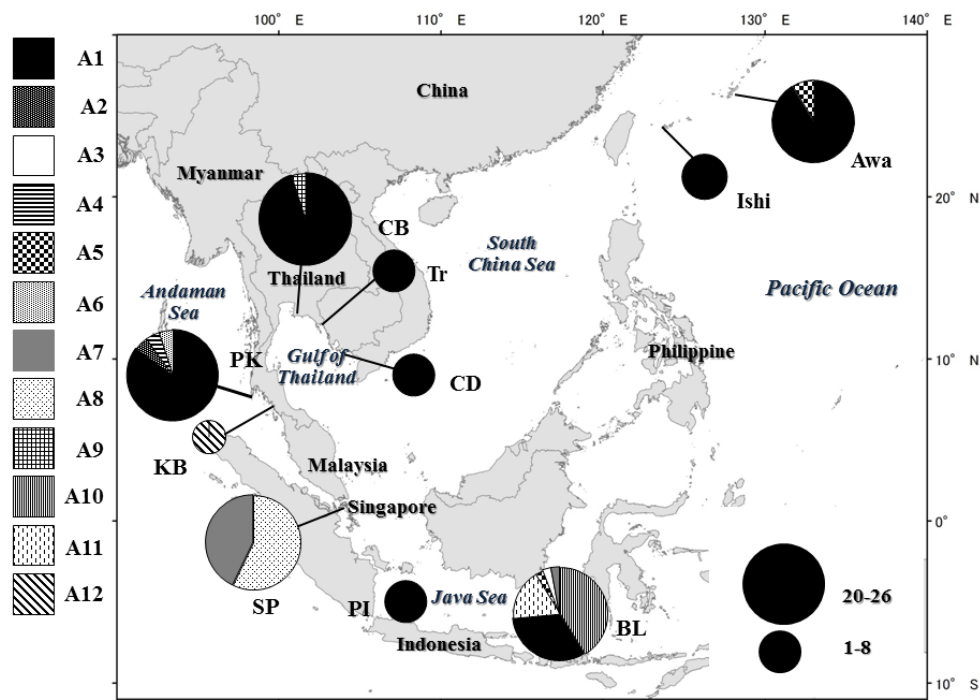


Figure 4 Geographical distribution of haplotypes in *Sargassum polycystum* based on nrDNA ITS2. Size of the circle is proportional to the sample size of each populations, and each pie-graph shows the frequency of haplotype in the population