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[Minireview]

Molecular Relationships of Giant Kelp (Phaeophyceae)

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Giant kelp, despite the economic and ecological value, is much confused in taxonomy. To discuss possible changes of the current classification system, we introduce published results and newly analysed sequences of both nuclear ribosomal DNA and plastid-encoded RuBisCo spacer. All members of the Alariaceae, Laminariaceae, and Lessoniaceae are grouped into a single monophyletic clade, while each family is not monophyletic. Eight groups are recognized: i) *Egregia* group, ii) *Alaria* group, iii) *Laminaria* group, iv) *Hedophyllum* group, v) *Ecklonia* group, vi) *Agarum* group, vii) *Lessonia* group, and viii) *Macrocystis* group. This results appear not to support the current familial system of advanced kelp, and rather suggest that a single family or eight varied families should be established covering the kelp genera. Taxonomic revisions of some genera like *Laminaria* and *Kjellmaniella* are necessary because of their paraphyly.

Key Words: ITS, kelp, molecular relationships, RuBisCo spacer

INTRODUCTION

Giant kelp is referred to brown order laminarialean algae and very large with a maximum length of over 40 m. It constitutes marine forest that provides habitats for bacteria, fungi, invertebrates, fishes, and mammals. Kelp is used as daily foods in Korea, Japan, and China; Korean is very familiar with *Undaria* as material of birthday soup. It is also commercially important for alginates production. Recently developed F- and U-Fucoidan from *Laminaria* species has been reported to stimulate apoptosis of cancer cells. However, despite the economic and ecological value of kelp, much of the taxonomy is confused partly due to the morphological simplicity and plasticity, doubts about species, probable interspecific and even intergeneric hybridization. Here in this symposium, we will compare morphologically based phylogeny of kelp with molecular phylogeny. Published results of phylogenetic analyses of gene sequences is introduced. We further present the results of our analyses of both nuclear ribosomal (nr) DNA and plastid-encoded RuBisCo spacer sequences and discuss possible changes of the current classification system.

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TAXONOMY

The taxonomic history of kelp was well reviewed by Setchell in 1893. Until that time, the order was unstable in its circumscription and spelled out as Laminariaceae, that is currently used as the name of the family. The Laminariales is credited to Kylin (1917) and clearly delimited by a strongly heteromorphic, diplohaplontic life cycle, with an alternation between highly differentiated diploid sporophytes, and microscopic haploid gametophytes. The order is classified into six families: Pseudochordaceae, Chordaceae, Phyllariaceae, Alariaceae, Laminariaceae, and Lessoniaceae. The Pseudochordaceae is characterized by diffuse meristematic thalli and stigmata in zoospore. The Chordaceae is characterized by an intercalary meristem and the nature of the sexual pheromones. The Phyllariaceae is characterized by foliose sporophytes and conducting elements called solenocysts and allelocysts. Because of the plesiomorphic features, these three have been regarded as primitive families. The Alariaceae, Laminariaceae, and Lessoniaceae are regarded as advanced in differentiation between blade and stipe, presence of mucilaginous structures, lack of eyespot in zoospores, usually perennial nature of the sporophytes, and lamoxirene as the common sexual pheromone. The Alariaceae is characterized by pinnately compound frond, sporophylls, and midribs.

The Laminariaceae is characterized by simple frond, reproductive sori on the blade, and the absence of midrib. The Lessoniaceae, the tallest of all algae, is characterized by splitting of the blade. Although the primitive families are relatively well circumscribed in most of taxonomic evidences, the classification of advanced members are controversial even in morphology. The taxonomic problems appear inborn, as Setchell and Gardner (1925) mentioned: "The question as to the division of the order into families is not so clear as that of its cleavage from other orders. The best distinctions lie according to the differentiations of the frond arising at the transition place between the blade and the frond and the relation to this and the fertile areas. The alternatives seem to be these: either to retain all the genera under one large and varied family (possibly excepting the Chordaceae) divided into numerous tribes or to divide them between numerous families of unequal value and of often indistinct cleavage."

Following is the current taxonomic scheme of kelp based with the reference of Setchell and Gardner (1925), Papenfuss (1951), and Kawashima (1993).

Order LAMINARIALES Kylin 1917

Family 1. PSEUDOCHORDACEAE Kawai *et* Kurogi 1985

Pseudochorda Kawai *et* Kurogi

Family 2. CHORDACEAE Dumortier 1822

Chorda Stackhouse

Family 3. PHYLLARIACEAE Tilden 1935

Saccorhiza de la Pylaie, *Phyllaria* (Le Jolis) Rostafinski

Phyllariopsis Henry *et* South

Family 4. ALARIACEAE Setchell *et* Gardner 1925

Tribe 1. Alarieae Setchell 1912

Alaria Greville, *Pterygophora* Ruprecht, *Undaria* Suringar

2. Ecklonieae Setchell 1912

Ecklonia Hornemann, *Eckloniopsis* Okamura, *Eisenia* Areschoug

3. Egregieae Setchell 1912

Egregia Areschoug.

Family 5. LAMINARIACEAE Bory 1827

Tribe 1. Laminarieae Bory 1827

Laminaria Lamouroux, *Kjellmaniella* Miyabe in Okamura

2. Agareae Kützing 1843

Agarum Dumortier, *Costaria* Greville, *Thalassiophyllum* Postels *et* Ruprecht

3. Cymathaereae Setchell *et* Gardner 1925

Cymathaere J. Agardh, *Pleurophycus* Setchell *et* Saunders

4. Hedophylleae Setchell 1912

Arthrothamnus Ruprecht, *Hedophyllum* Setchell *Streptophyllopsis* Kajimura

Family 6. LESSONIACEAE Setchell *et* Gardner 1925

Tribe 1. Lessonieae Setchell 1912

Lessonia Bory, *Dictyoneurum* Ruprecht, *Dictyoneuropsis* Schmidt, *Nereocystis* Postels *et* Ruprecht, *Postelsia* Ruprecht

2. Macrocysteae Kützing 1843

Macrocystis Agardh, *Pelagophycus* Areschoug

3. Lessoniopseae Setchell 1912

Lessoniopsis Reinke

MOLECULAR PHYLOGENY

Nuclear ribosomal DNA SSU sequences: Saunders and Druehl (1992) hypothesized, based on sequence divergence of nrDNA SSU. that the most distantly related kelp diverged between 16 and 30 (more probably 16 and 20) million years ago. Tan and Druehl (1996) reported that the Laminariales is paraphyletic based on the SSU sequence. Our analyses of SSU sequences showed that the Pseudochordaceae and Chordaceae were clearly separated from the strongly monophyletic group consisting of the Alariaceae, Laminariaceae, and Lessoniaceae (Boo *et al.* 1999). Considering these molecular data and the reported morphology, life history, and sex pheromones, it appeared likely that the Pseudochordaceae might have branched off first from the laminarialean lineage that leads, through the Chordaceae, to the advanced Laminariales: Alariaceae, Laminariaceae, and Lessoniaceae. However, the limited resolution that results from the close similarities among the SSU sequences gives a need for more informative molecular markers in order to resolve the familial problems.

nrDNA ITS sequences: The molecular relationships based on ITS by Saunders and Druehl (1993) clearly conflicted with laminarialean taxonomy based on traditional interpretations of the stipe-blade transition zone. Both the existing Alariaceae and Lessoniaceae are polyphyletic, whereas the Laminariaceae may be paraphyletic. Druehl *et al.* (1997) reported that the Laminariaceae is not a natural group, and that the separation of the advanced kelp genera into families based on gross morphology, viz. presence of sporophylls in the Alariaceae,

ontogenetic splitting in the Lessoniaceae, and absence of these features for the Laminariaceae, does not yield a phylogenetic system of classification. It is also difficult to solve familial problems based on their ITS and published other data (SSU RFLP and sequence, ITS sequence), that appear to aggravate the taxonomic conundrum of kelp (Druehl *et al.* 1997). On the other hand, Yotsukura *et al.* (1999), finding little divergence in ITS sequence between non-digitate *Laminaria* species, suggested the radical view that there are only two biological species in the non-digitate *Laminaria* species along the coast of Japan.

Plastid-encoded RuBisCo spacer and nrDNA ITS sequences: To get the better results about the phylogeny of the Laminariales, more informative markers should be analyzed. Plastid-encoded RuBisCo spacer region and nrDNA ITS have been proved to be recommendable markers for an improved molecular phylogeny of the Laminariales (Yoon and Boo 1999).

Forty seven species were collected from naturally occurring populations. All currently recognized tribes and genera except *Pleurophycus* and *Streptophyllopsis* of the Laminariaceae and *Dictyoneuropsis* of the Lessoniaceae were sampled. The ITS, RuBisCo spacer, and combined ITS + RuBisCo spacer data sets provide an improved resolution for the advanced kelp families. Phylogenetic trees from the MP (maximum parsimony), NJ (neighbor-joining), and ML (maximum likelihood) analyses show that all members of the Alariaceae, Laminariaceae, and Lessoniaceae are grouped into a single monophyletic clade, while each family is not monophyletic. In further analyses, phylogenetically supported eight groups were recognized: i) *Egregia* group, ii) *Alaria* group, iii) *Laminaria* group, iv) *Hedophyllum* group, v) *Ecklonia* group, vi) *Agarum* group, vii) *Lessonia* group, and viii) *Macrocystis* group. Member of these eight groups are very similar with those of tribes of traditional classification. However, of ten tribes recognized in morphological taxonomy, the tribe Cymathaereae appears to be included in the *Hedophyllum* group and the Lessoniopseae in the *Alaria* group.

Our analyses of ITS, RuBisCo spacer, and combined ITS + RuBisCo spacer data sets appear not to support the current familial system of advanced kelp, and rather suggest that a single family or eight varied families should be established covering the kelp genera. Taxonomic revisions of some genera like *Laminaria* and *Kjellmaniella* are necessary because of their paraphyly. There are, therefore, possibilities that morphological

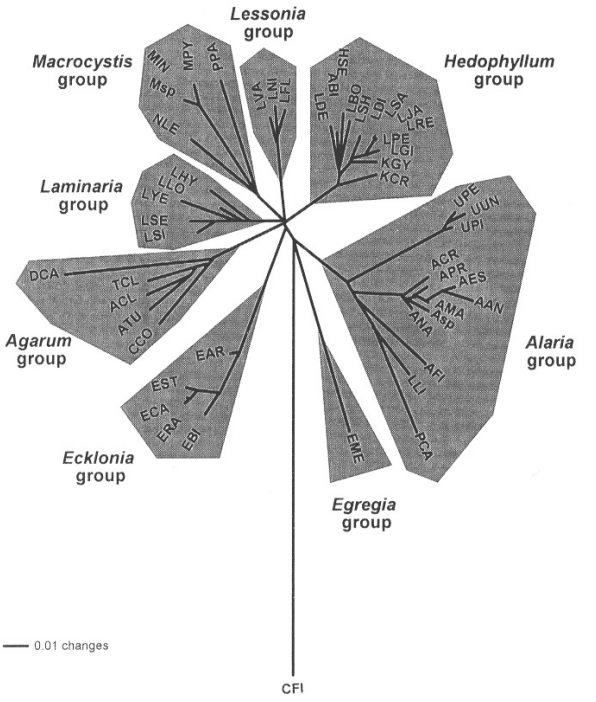

Fig. 1. A neighbor-joining tree of the giant kelp (Alariaceae, Laminariaceae, and Lessoniaceae) based on sequences of ITS, RuBisCo spacer, and combined ITS + RuBisCo spacer.

characters, regarded as diagnostic for separating genera or families, may not reflect their phylogenetic relationships. Our results also indicate the radiated evolution of kelp. Based on biogeography and herbivorous relationships, Estes and Steinberg (1988) proposed that the kelps (Laminariales) radiated in the North Pacific following the onset of late Cenozoic polar cooling. Finally our studies show a general fact that the combined analyses of additional sequences from other genomes improve resolution to provide sweeping answers to phylogenetic questions.

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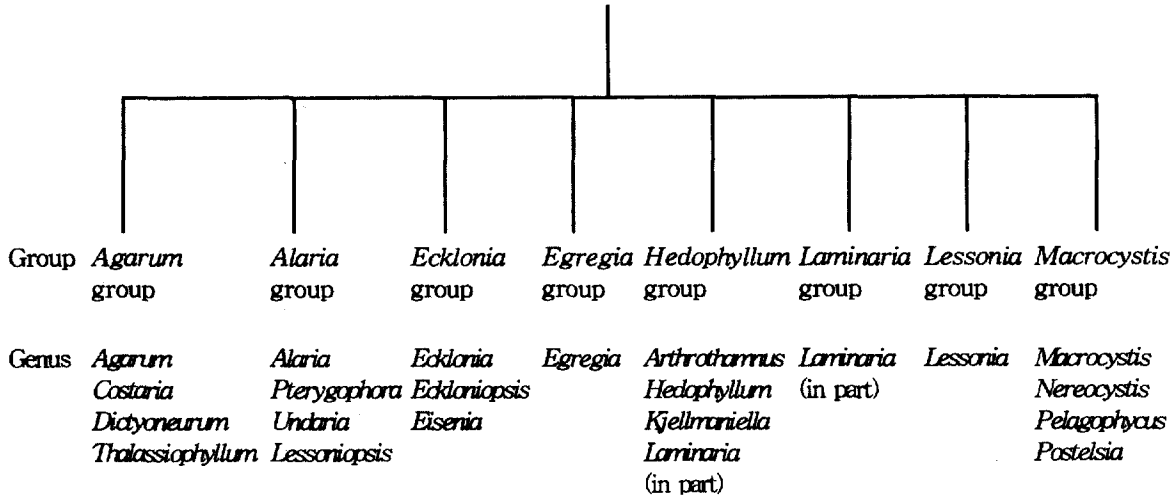

Fig. 2. A systematic scheme of giant kelp (Alariaceae, Laminariaceae, and Lessoniaceae) based on molecular data of Figure 1.

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