Front of

Diversity Research on Kelp

in the North Pacific

AN INTERNATIONAL RESEARCH MEETING

Hokkaido University, Sapporo & Hakodate, May 27-29, 2013

Invited Speakers: **Louis Druehl**, Canada + **Rae Hopkins**, Canada + **Nina Klochkova**, Russia + **Dmitry Galanin**, Russia + **Sung Min Boo**, Korea + **Shao Jun Pang**, China + **Tsuyoshi Abe**, Sapporo + **Shinji Kirihara**, Aomori + **Tadashi Kawai**, Wakkanai , + **Yasunori Kinoshita**, Hakodate

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Organizer & Speaker: **Norishige Yotsukura**, Hokkaido University, Sapporo, Japan

HOKKAIDO UNIVERSITY

An International Research Meeting:

Front of Diversity Research on Kelp in the North Pacific

Hokkaido University, May 27-29, 2013

PROGRAM

Monday May 27

13:00-13:05 Welcome and introduction (*Norishige Yotsukura*)

13:05-13:50 **Present situation and assignment of biodiversity research on kelp in Japan**
Norishige Yotsukura (Field Science Center for Northern Biosphere, Hokkaido University, Japan)

13:50-14:35 **Present situation and assignment of biodiversity research on kelp in North America**
Louis Druehl (Bamfield Marine Science Centre & Canadian Kelp Resources, Canada)

14:35-15:20 **Present situation and assignment of biodiversity research on kelp in Russia**
Nina Klochkova (Department of Ecology and Nature Management, Kamchatka State Technical University, Russia)

15:20-15:35 Coffee and tea break

15:35-16:20 **Present situation and assignment of biodiversity research on kelp in Korea**
Sung Min Boo (Department of Biology, Chungnam National University, Korea)

16:20-17:05 **Present situation and assignment of biodiversity research on kelp in China**
Shao Jun Pang (Institute of Oceanology, Chinese Academy of Science, China)

17:05-17:20 Qs and As

18:00-20:00 Convivial party

Tuesday May 28

9:30-10:00 **Geographical features of the North Pacific for algal distribution**
Tsuyoshi Abe (The Hokkaido University Museum, Hokkaido University, Japan)

10:00-10:30 **Present situation and assignment of ecological diversity research on kelp**
Dmitry Galanin (Sakhalin Research Institute of Fisheries and Oceanography, Russia)

10:30-10:45 Coffee and tea break

10:45-11:15 **Present situation and assignment of diversity conservation research on kelp**
Shinji Kirihara (Fisheries Institute, Aomori Prefectural Industrial Technology Research Center, Japan)

11:15-11:45 **Present situation and assignment of diversity utilization research on kelp**
Tadashi Kawai (Wakkanai Fisheries Experimental Station, Hokkaido Research Organization, Japan)

11:45-12:00 Qs and As

12:00-12:50 Lunch

12:50-13:20 Poster Presentation

No. 1 - Novel uses of kelp in Canada
Rae Hopkins (Canadian Kelp Resources, Canada)

No. 2 - Novel use of kelp in Japan
Yasunori Kinoshita (Hokkaido Industrial Technology Center, Japan)

No. 3 - Long-term variation in distribution of konbu (*Saccharina*) beds along coasts of Hokkaido, Japan
Kentaro Watanabe (Akkeshi Marine Station, Hokkaido University, Japan)

No. 4 - Approach to elucidation of genetic diversity of *Saccharina Japonica* in northern part of Japan based on DNA fingerprinting
Takashi Maeda (Graduate School of Environmental Science, Hokkaido University, Japan)

No. 5 - Spatial estimation of the kelp forests (*Sacchaina* spp.) distributions in coastal waters of Aomori, Japan, using acoustic method
Huamei Shao (Grauate School of Environmental Science, Hokkaido

University, Japan)

No. 6 - A taxonomic re-examination of *Saccharina longipedalis* (Laminariales, Phaeophyceae)

Nobu Nagai (Graduate School of Environmental Science, Hokkaido University, Japan)

13:20-14:20 General discussion (Part I)

14:20-14:30 General overview (*Louis Druehl*)

14:30 Closing

<u>Wednesday May 29</u>

Afternoon General Discussion (Part II)

Venue

- Enyuu Gakusha, Hokkaido University (May 27-28)
  Kita 18 Nishi 7, Kita-ku, Sapporo,
  Hokkaido, 001-0018 Japan
- Usujiri Fisheries Station, Hokkaido University (May 29)
  Usujiri 151, Hakodate, Hokkaido, 041-1613 Japan

Organizer

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Support

Hokkaido University

Introduction

The coasts of the North Pacific Ocean are home to the richest biodiversity of kelps growing in cold seawater, and not a few researchers specialize in the kelp taxonomy in the countries bordering this region. However, because this area has many endemic species that are found only in restricted locations, it is not easy for even the researchers to know about species growing in distant locations. Therefore, it is necessary to identify the species found in different locations and to organize taxonomic problems for future broad discussion on the classification and speciation of kelps.

In general, although kelps show considerable morphological variation, they been classified according to only a few morphological features. Furthermore, the morphological characters used for the classifications are not stable. It is necessary to organize morphological features to compare kelps under common classification characters. Meanwhile, taxonomic reorganization of kelps based on genetic information has been performed. However, the results of genetic analyses do not necessarily support the result of analyses based on morphological information, and hence, taxonomic views vary among researchers. Under circumstances where scientists in different areas have to share information on kelps and also have to promote awareness of the biodiversity and future conservation and utilization, it is important to assemble the latest findings from various fields and identify problems to reach a common understanding.

Researchers who study biodiversity of kelp in cold seawater and who live in countries on the coasts of the North Pacific Ocean are invited to an international workshop to be held at Hokkaido University, which has a long history of kelp research initiated by Dr. Kingo Miyabe.

Norishige YOTSUKURA
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Present situation and assignment of biodiversity research on kelp in Japan

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The laminarialean algae growing at the coast of Japan, which is one of the sea areas richest in species diversity, form kelp forests. They play an important role in the marine ecosystems in various regions and are used in Japanese cuisine. Because of the importance of Japanese kelps to ecology and fisheries, taxonomic investigation was initiated in earnest by Dr. Kingo Miyabe[1)] and Dr. Kintaro Okamura[2)] more than a century ago. Features of the individual species have been described in detail over the years on the basis of a classification system developed by Oltmanns[3)], Setchell and Gardner[4)], and Kawashima[5)].

For Japanese kelps, morphological features of the lamina, stipe, and holdfast of adults have been recognized as taxonomically important in Japan, although features of the micro-sized young sporophyte have been reported in some species. In addition, the presence or absence of mucilage lacuna / mucilage gland in adult tissues has been used as a standard for classification. These features are useful for systematically organizing levels of the hierarchy. However, these classification characters are known to vary widely according to growing environments; thus, object information is required for the recognition of kelp species. Under such circumstances, recently, a classification system for kelps based on morphological information described in lists by Kawashima[6)] and Yoshida *et al.*[7)] has been accepted among researchers in Japan. However, regarding the so-called “advanced kelps,” which have been recognized as Alariaceae or Laminariaceae and for which a difference of opinion exists between researchers in foreign countries and those in Japan. The difference of opinion has often led to confusion during discussions between the groups.

In the past 15 years molecular phylogenetic studies have been performed energetically, yielding new taxonomic implications for Japanese kelps based on quantitative data[8)~15)]. By reorganizing the classification system of Japanese kelps based on these findings, “primitive kelps” are classified into 3 families: Akkesiphycaceae, Chordaceae and Pseudochordaceae, including 3 genera and 6 species, and “advanced kelps" are classified into 4 families: Alariaceae, Costariaceae, Laminariaceae and Lessoniaceae, including 11 genera and 31 species[16)]. As of now, the resulting system is not explained clearly by morphological comparisons. For example, for the species in Laminariaceae growing in Japan, in the accumulated morphological information since Dr. Miyabe's research, the taxon shows a relatively simple morphology, unlike that in other families, and seems unified. However, it is difficult to briefly and clearly describe the characteristics of Laminariaceae that are applicable to all species in the family, including both those growing in Japan and those growing in foreign countries that have complicated morphologies, e.g. *Macrocystis*, *Nereocystis*, *Pelagophycus, Postelsia* and *Pseudolessonia*. The same holds true at the genus level; it is difficult to show morphological characteristics that discriminate between the 2 genera, *Laminaria* and *Saccharina*, which were reorganized from the conventional system when comparing the genera after considering all species, including those growing in foreign countries.

Kelps widely distributed in the world have various classification characters that seem important. Considering that many of the morphological characters of kelps change according to their growing process and environment, the characters that seem apparently unrelated to classification may indeed be important. To create an acceptable classification system for Japanese kelps based on molecular analyses by adding morphological information, it is necessary for researchers from around the world to provide detailed comparisons and discussions of the common characters of kelps and to search for commonly recognized classification characters.

On Japanese kelps, the taxonomical assignment that should be clarified has been organized with accumulation of the objective information. Here, several outcomes of recent biodiversity researches and current taxonomical assignments on Japanese kelp, notably on advanced kelp in cold seawater, are enumerated.

Outcomes

- Each of three traditional saccharinan species: *Saccharina religiosa, S. ochitensis* and *S. diabolica,* is a regional variety of *S. japonica* var. *japonica*[13)].
- *Laminaria sachalinensis* should be changed to *S. sachalinensis*[14)].
- Genetic diversity within *S. japonica* is able to be investigated by DNA fragment analysis[17)].

Assignments

- taxonomic position of *Laminaria japonica* f. *membrance*
- taxonomic position of *Saccharina longipedalis*
- taxonomic relationship between *S. angustata* and *S. longissima*
- taxonomic relationship between *S. cichorioides* and *S. sachalinensis*

- taxonomic relationship between *S. yendoana*, *L. gurjanovae* and *L. sikotanensis*
- taxonomic relationship between *Alaria angusta* and *A. marginata*

References

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Current classification system of Japanese kelp

Akkesiphycaceae コンブモドキ科
Akkesiphycus コンブモドキ属
Akkesiphycus lubricus コンブモドキ
Alariaceae アイヌワカメ科
Alaria アイヌワカメ属
Alaria angusta ホソバワカメ
Alaria crassifolia チガイソ
Alaria praelonga アイヌワカメ
Alaria marginata クシロワカメ
Undaria ワカメ属
Undaria peterseniana アオワカメ
Undaria pinnatifida f. *pinnatifida* ワカメ
(*Undaria pinnatifida* var. *elongata* Suringar)
(*Undaria pinnatifida* var. *vulgaris* Suringar)
Undaria pinnatifida f. *distance* ナンブワカメ
Undaria undarioides ヒロメ
Chordaceae ツルモ科
Chorda ツルモ属
Chorda asiatica ツルモ
Chorda kikonaiensis キコナイツルモ
Chorda rigida カタツルモ
Costariaceae スジメ科
Agarum アナメ属
Agarum clathratum アナメ
[*Agarum cribrosum* f. *cribrosum**]
Agarum cribrosum f. *rishiriense** リシリアナメ
Agarum cribrosum f. *rugosum** ザラアナメ
Agarum cribrosum f. *yakishiriense** テウリアナメ
Agarum oharaense オオノアナメ
Costaria スジメ属
Costaria costata スジメ

Laminariaceae コンブ科
Arthrothamnus ネコアシコンブ属
Arthrothamnus bifidus ネコアシコンブ
Laminaria コンブ属
Laminaria yezoensis ゴヘイコンブ
Saccharina カラフトコンブ属
Saccharina angustata ミツイシコンブ
Saccharina cichorioides チヂミコンブ
Saccharina coriacea ガッカラコンブ
Saccharina gyrata トロロコンブ
Saccharina japonica var. *japonica* マコンブ
Saccharina japonica var. *diabolica* オニコンブ
Saccharina japonica var. *ochotensis* リシリコンブ
Saccharina japonica var. *religiosa* ホソメコンブ
Laminaria japonica f. *membrancea** ドテメ
Saccharina kurilensis アツバスジコンブ
Saccharina latissima カラフトコンブ
Saccharina longipedalis エナガコンブ
Saccharina sachalinensis カラフトトロロコンブ
Saccharina longissima ナガコンブ
Saccharina sculpera ガゴメコンブ
Saccharina yendoana エンドウコンブ
Streptophyllopsis クロシオメ属
Streptophyllopsis kuroshioense クロシオメ

Lessoniaceae レッソニア科
Ecklonia カジメ属
Ecklonia cava カジメ
Ecklonia kurome クロメ
Ecklonia stolonifera ツルアラメ
Eckloniopsis アントクメ属
Eckloniopsis radicosa アントクメ
Eisenia アラメ属
Eisenia arborea サガラメ
Eisenia bicyclis アラメ
Pseudochordaceae ニセツルモ科
Pseudochorda ニセツルモ属
Pseudochorda gracilis ホソツルモ
Pseudochorda nagaii ニセツルモ

Present situation and assignment of biodiversity research on kelp in North America

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Vancouver Island shares with Hokkaido the richest kelp floras in the world. Initially, Setchell and Gardner (1), working with the northeast Pacific kelp, described three kelp families that were defined by gross morphological features. Mostly, their classification worked, but some difficulties existed. For example, *Alaria* (Alariaceae) and *Lessoniopsis* (Lessoniaceae) share features distinctive to the two families (3). Molecular analysis showed these two genera were interfertile (4). A series of molecular studies have shown that the morphologically defined families do not reflect genetic relationships (5,6,7, fig 1). As an example, the molecular family, Laminariaceae, contains all of the Northern Hemispheric morphological Lessoniceae (Fig. 2).

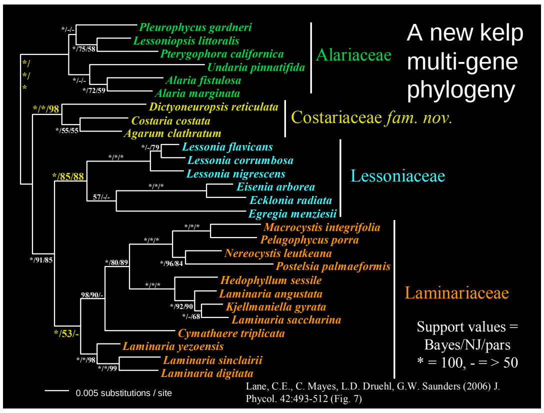

Figure 1. Present status of molecular kelp families.

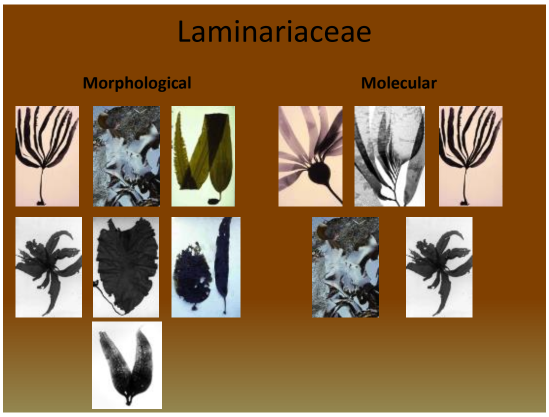

Figure 2. Morphological and Molecular Laminariaceae.

More recently molecular barcoding and AFLP (amplified-fragment length polymorphisms) analyses have defined closely related taxa (8,9). AFLP and similar analyses allow one to track the direction of gene flow and discover areas important to the conservation of wild kelp stock. In the *Alaria* AFLP study, 206 score –able bands were produced (9). With these bands genetic similarity coefficients could be determined within patches and between patches in a stand (distance up to 15 m) (Fig. 3), and between stands (6.5 km—185 km). The results followed an isolation-by-distance model.

DNA barcoding of brown algae is based on sequences in Cytochrome c oxidase 1 (8). This system showed within species divergence of 0.00—0.46%. Classification using this approach involves analyzing several specimens, taking herbarium and DNA samples and combining these with notes on collection, etc. (Fig. 4).

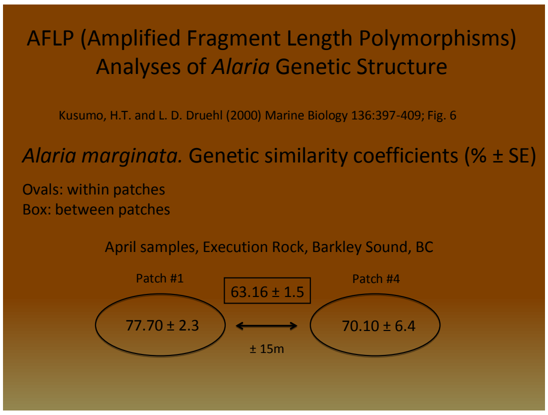

Figure 3. An example of within patch and between patch genetic similarity coefficients for the genus *Alaria*. The patches were separated by 15 m.

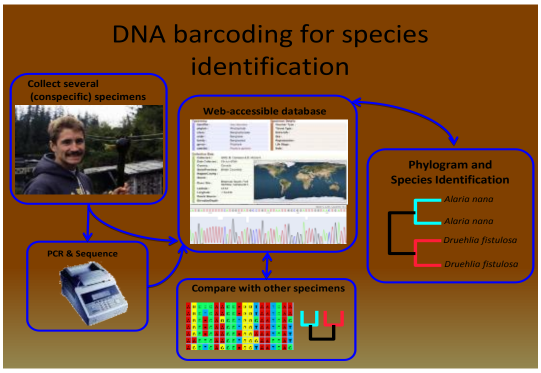

Figure 4. Barcoding-based classification.

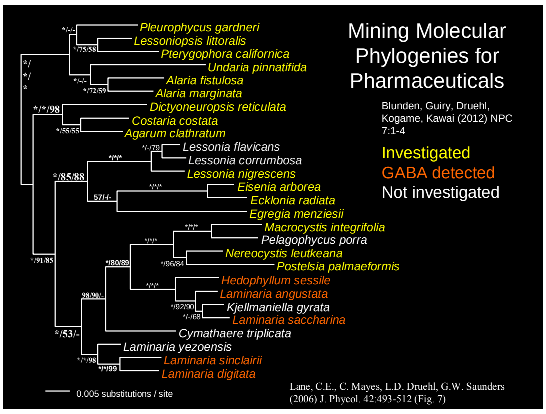

Figure 5.The distribution of GABA (γ-Aminobutyric acid) among kelp species (10).

Molecular phylogenies provide a powerful tool in guiding the researcher in mapping out the potential distribution of fascinating biochemicals. This approach could be used in discovering interesting amino acid combinations in searching out umami (10,12, Fig 6).

Seaweed	Extracted glutamate mg/100g	Extracted aspartate mg/100g	Extracted alaninate mg/100g
Rausu-konbu (farmed, Japanese)	145 ± 5	85 ± 15	20 ±3
Hidaka-konbu (farmed, Japanese)	70 ± 15	40 ± 20	20 ± 10
Sugar kelp (farmed, Danish)	3 ± 3	3 ± 2	7 ± 4
Dulse (farmed, Danish)	40 ± 10	27 ± 8	25 ± 6
Dulse (wild, Icelandic)	10 ± 5	11 ± 2	12 ± 2
Graceful red weed (farmed, Danish)	6	1	4

Figure 6. Umami flavour oriented compounds in selected kelp (12).

An understanding of inheritance in kelp will accelerate the production of superior kelp hybrids. Presently, One should select parents, placing emphasis on the female donor parent. However, the initial findings need to be verified (11, Fig. 7).

Figure 7. Production of apogamous, normal and hybrid kelp sporophytes in seven kelp species (11).

Key Issues To Be Addressed:

- Reconcile molecularly defined kelp species with classic identification.
- Determine the mode of genetic inheritance in kelp and the role of apogamy.
- Make molecular advances in kelp genetics and phylogeny accessible to industry.

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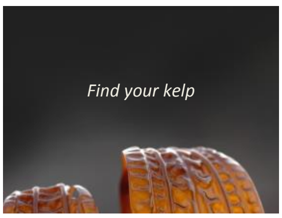

Present situation and assignment of biodiversity research on kelp in Russia

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Brief introduction to the present state of knowledge on Russian Far Eastern kelp

In Russian Far East, the exploration of marine flora and fauna began in 1733 with the Second Kamchatka Expedition led by Vitus Bering in the Sea of Okhotsk. Thereafter, Ivan F. Krusenstern explored the eastern coast of Sakhalin Island in 1805 and Ivan G. Voznesensky explored the Far East and Russian possessions in North America during 1839-1849. The earliest phycological works in the region are associated with F.J. Ruprecht in 19th century and several Russian phycologists in 20th century: T.F. Schapova, Anna D. Zinova, Elena S. Zinova, V.B. Vozzhinskaja, E.I. Blinova, Yu.E. Petrov, M.V. Suchovejeva, K.L. Vinogradova, L.P. Perestenko, and I.S. Gussarova. For several coastal areas, these early phycological works are the only existing records on species composition and structure of algal communities in the intertidal and subtidal zones. More recently, macroalgae of east and west Kamchatka, Commander Islands and North Kurile Islands were illustrated and described by N.G. Klochkova *et al.* (2009).

Investigation of the laminarian flora of Russian Far East has begun in 19th century, and by 1950-es twenty six laminarian species from 10 genera were recorded. A big contribution was made by Yu. Petrov (Komarov Botanical Institute, Rus. Acad. Sci.), who described the following laminarian taxa from this region:

– Four new monotypic genera and their species, such as *Phyllariella ochotensis*, *Costularia kurilensis* (currently: *Costulariella kurilensis*), *Feditia* simuschirensis and *Undariella kurilensis*;

– Three new species of *Laminaria*: *L. appressirhiza*, *L. inclinatorhiza* and *L. multiplicata*;

– A new family, Arthrothamnaceae, which included the genus *Arthrothamnus* comprising 2 species.

Yu. Petrov also excluded the following previously recorded kelp species from the list of Russian Far Eastern flora: *Laminaria agardhii, L. saccharina, L. digitata, L. bullata, L. nigripes, L. ruprechtii*, and Postelsia ***palmaeformis***.

Yu. Petrov's contribution to the knowledge of Russian kelp cannot be underestimated. However, he also made several presumptions, which seem not quite supported by the data from the field observations. Still, his opinion is dominant among Russian phycologists regarding the taxonomy of kelp species.

For example, he synonymized the following taxa with *Laminaria bongardiana* or suggested them to be its forms: ***Hedophyllum subsessile***, ***Hedophyllum spirale***, *Streptophyllum spirale*, *Laminaria taeniata*, *Laminaria groenlandica* and *Laminaria subsimplex*.

He also reported that species *Laminaria saccharina*, which was listed for Russian Far Eastern flora by other researchers, was in fact not distributed in our waters. He suggested that the specimens previously recorded as *L. saccharina* were misidentified. Therefore, he assigned all plants that looked similar and were previously identified as *L. saccharina* to a special form of another *Laminaria*, which was known as an endemic of the northwest Okhotsk flora – *Laminaria gurjanovae* (A. Zinova 1964, 1969). This new form was named by him as *Laminaria gurjanovae* f. *lanciformis*. However, when describing this form, he neither published pictures or photos of the type specimen, nor mentioned how many specimens were examined. In the form's diagnosis, he wrote only one sentence "Blade lanceolate, linear-lanceolate or oval, with cuneate or roundish basis".

After studying kelp flora of South Kurile islands, Yu. Petrov also combined species *L. longissima* with *L. angustata*, reducing the first taxon to a synonym of the second one. Furthermore, he described a new form, *L. angustata* f. *sibirica*, for a particular group of plants from the middle Primorskij region (Sea of Japan). However, when describing this form, he neither published pictures or photos of the type specimen nor mentioned how many specimens were examined.

He also revised the genus *Alaria* in Russian Far East. According to his opinion, species previously reported from this region, such as *Alaria crassifolia*, *A. crispa*, *A. dolichoraches*, *A. esculenta*, *A. lanceolata*, *A. laticosta*, *A.* ***membranacea***, *A. praelonga*, *A. pylaii*, *A. taeniata*, *A. tenuifolia*, and *A. valida*, should be reduced to synonymy or regarded as wrongfully identified. Current list of Far Eastern species of *Alaria* comprises *A. macroptera*, *A. marginata*, *A. angustata*, *A. ochotensis* and *A. fistulosa* (currently: *Eualaria fistulosa*).

Assignment of research problems

From the personal algal floristic and taxonomic investigations we assume that Yu. Petrov's opinion regarding several kelp species was not correct and not properly supported by the experimental data and filed observations. One major problem concerns species *Laminaria gurjanovae* from Okhotsk Sea and *L. bongardiana* (currently: *Saccharina bongardiana*) from Kamchatka.

According to Yu. Petrov, *Laminaria gurjanovae* comprises 2 forms – *L. gurjanovae* f. *gurjanovae* (i.e. forma *typica*) and *Laminaria gurjanovae* f. *lanciformis.* Species *L. gurjanovae* was originally described by A. Zinova (1964, 1969) and is known as an endemic of the Sea of Okhotsk, with type locality in Aniva Inlet on western Sakhalin Island. We found this species in Taujskaya Bay (northern continental coast of the Sea of Okhotsk), at depths of 9–11 m. In our collecting site, *L. gurjanovae* formed communities with a density of 4–7 plants per 1 m^2, covering 40–60% of the sea bottom on average.

We also found the taxon, which Yu. Petrov called as *L. gurjanovae* f. *lanciformis*. In Taujskaya Bay, this species was the most abundant and widely distributed, occurring in the subtidal and low intertidal areas. It grew on various hard substrates and could also grow on silt at depths of 20–25 m if some small pebbles and cobbles were present. Under favorable conditions, it formed mono-species communities with a biomass of 40–50 kg per 1 m^2.

Yu. Petrov did not demonstrate type specimen of *L. gurjanovae* f. *lanciformis* upon publishing this form. We located the type specimen in the Komarov Botanical Institute (herbarium code: LE), as well as the type specimen of *L. gurjanovae* f. *gurjanovae*. These two specimens look very different from each other, strongly presuming that they are different species, rather than different forms of the same species. Our observations of the age and seasonal changes in two forms from the Sea of Okhotsk demonstrated that the typical plants of *L. gurjanovae* (i.e. forma *typica*) had quite unique and very stable morphological, anatomical and ecological characteristics. Same characteristics were not found in the plants, which were attributed to *L. gurjanovae* f. *lanciformis*; however they too had very stable and unique characteristics. We presume that species *L. gurjanovae* does not have forms, such as f. *gurjanovae* and f. *lanciformis*, but these are two separate species.

Regarding the taxon *Laminaria bongardiana* (currently: *Saccharina bongardiana*), we have recorded the live longevity of its form called *S. bongardiana* f. *taeniata*. The species *Laminaria taeniata* was described by A. Postels and F. Ruprecht in 1840; however Yu. Petrov (1972) reduced it to a form of *Laminaria bongardiana* without providing enough amounts of data from the field observations to support his argument.

Our field observations showed that *S. bongardiana* f. *taeniata* have shorter live longevity comparing to the plants of the typical form, *S. bongardiana* f. *bongardiana.* Also, their distribution areas do not coincide and f. *taeniata* occurs in very different habitats on the Far East, and therefore cannot be regarded as a result of ecological variability of *S. bongardiana* f. *bongardiana.* We presume that these are two separate species.

Another form of *S. bongardiana*, f. *bifurcata* (Petrov 1972), also cannot be considered as a taxonomic category because of the following argument. We have observed that on the 3-rd year of life some blades of the typical form (i.e. f. *bongardiana*) develop specific morphology, which is a characteristic key character for f. *bifurcata.* Therefore, this so-called characteristic morphology of f. *bifurcata* seems to result from the morphological changes of the typical plants (i.e. *S. bongardiana* f. *bongardiana*) according to age. If so, separation of the two forms has no ground.

In conclusion, investigation of the kelp species is one of the major research problems in Russian Far East. Especially, Sea of Okhotsk and Arctic Russia were named as regions of the world still not comprehensively studied, where systematic studies remain morphologically based and there has been little recent work (Bolton 2010), i.e. phylogenetically based systematics. It should be emphasized that for many algal taxa distributed in these regions, including endemics and commercially important kelp species, information on morphology and anatomy and differences in the morphogenesis of species and their forms is very limited. These basic studies are essential because incomplete understanding of genus- or species-specific (or species form-specific) morphology and anatomy can lead to a wrongful presumption of the existence or non-existence of a taxon. We briefly discussed current problems with two species, *Laminaria gurjanovae* and *Saccharina bongardiana.* Another example is *Laminaria multiplicata* Petrov *et* Suchovejeva that was often cited in floristic lists of the Sea of Okhotsk based on its first record in 1976 and referred to as rare endemic species. Detailed morphological analysis of the type and paratype specimens and long-term surveys of the type locality and proximate bays and inlets have not confirmed the existence of such entity at all (Klochkova et al. 2010). Therefore, detailed re-evaluation of previous floristic studies based on morphological and anatomical characteristics should be carried out comprehensively for all kelp taxa in Russian Far Eastern region.

Present situation and assignment of biodiversity research on kelp in Korea

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The Korean peninsula has a rich diversity of marine algae due to diverse habitats and the mixing of both warm and cold currents. In the first catalogue of marine algae, Kang (1966) listed 422 taxa, and then the species number increased to 627 taxa by Lee & Kang (1986). It is surprising for approximately 900 marine algae to be listed in Korean waters. Many species are familiar to Korean people because of their use for sea-vegetables. Of these, *Undaria* has been a very popular food material in Korea for a very long time. It has been a strict traditional custom for a woman delivered of a baby to have *Undaria* soup three times a day for three weeks, since they believe that *Undaria* contains a lots of components which clean up body, in particular, the state of blood. *Undaria* soup has also been a menu that should be unmistakeably prepared for every birthday soup. It is therefore considered that *Undaria* is an emotional and cultural species for Koreans.

A total of seven genera and 13 species are recognized in the Laminariales in Korea. All species are easily collected on Korean coasts. With my students, I have studied on molecular systematics of kelp from Korea and surrounding waters. The first molecular systematics was phylogenetic relationships of *Undaria* based on sequences of RuBisCO spacer (Yoon & Boo 1999). This study indicates that *U. pinnatifida, U. petersenia*, and *U. undarioides* are very closely related, hypothesizing a reticulate evolution among the species. In addition, we expanded this work to the whole Laminariales genera using ITS and RuBisCO spacer sequences (Yoon et al. 2001). Our analyses indicate that all taxa in the Alariaceae, Laminariaceae,
and Lessoniaceae form a monophyletic lineage (the Laminariales *sensu stricto*). The phylogenetic
analyses show that the kelps form eight well-supported clades (represented by *Egregia, Laminaria, Hedophyllum,Macrocystis, Alaria, Agarum, Ecklonia,* and *Lessonia*) that conform to the tribes of the current morphological classification system of the "advanced" kelps. Our recent study on *Agasum* showed the low degree of substitutions

between *A. clathratum* and *A. yakishiriense*, suggesting their recent divergence from a common ancestor, and we recognize the latter as a subspecies of the former (Fig. 1., Boo et al. 2011).

To date we have been studying on phylogeography of *Costaria costata* from Korea and surrounding waters. We analyzed the mitochondrial-coding *cob* and *cox*3 genes and the nuclear noncoding internal transcribed spacer (ITS) region from specimens throughout the distributional range of the species. Preliminary results revealed many haplotypes in *cob*, *cox*3, and the ITS region. Analyses of *cob*, *cox*3, and ITS data consistently divided *C. costata* into two divergent clades: a northwest Pacific group and a north-east Pacific group, and these two groups are genetically isolated based on gene flow test. Our results suggest that the northwest Pacific might play an important role in generating diversity in this genus. We discuss evolutionary processes that may have resulted in the current distribution of the two species.

We are also constructing a haplotype map of *Undaria pinnatifida* along all coasts of Korea using DNA barcoding markers. The barcoding map of Korean *Undaria* will provide a further estimate of the effect of oceanographic changes on the biomass and distribution.

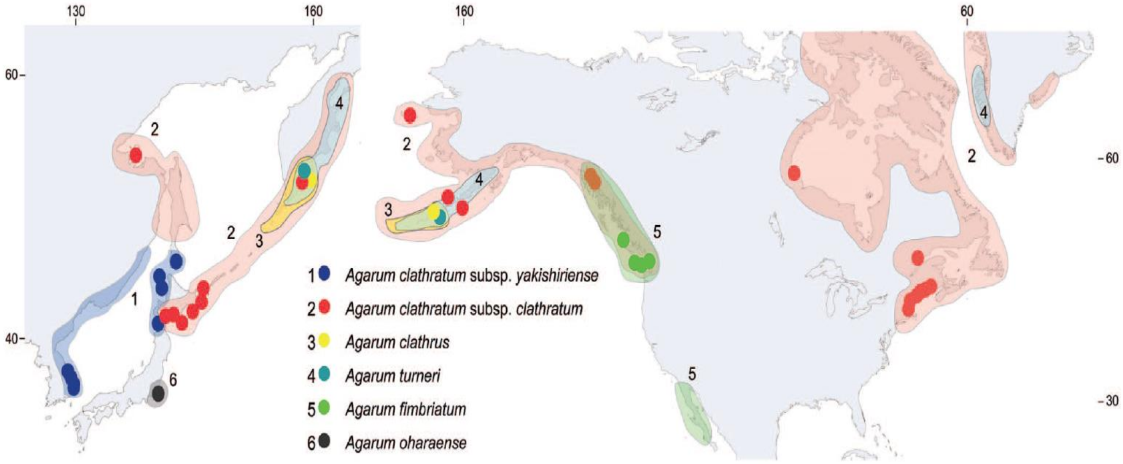

Fig. 1. Distribution of *Agarum* species in the world (Boo et al. 2011).

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Present situation and assignment of biodiversity research on kelp in China

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In the most representative 35 Chinese investigation reports about seaweed species diversity along the Chinese coast from the temperate-cold Liaoning province to the tropical Hainan island from 1980 to 2010, twenty three of them are based on field investigations, the remaining are summary reports. There are only two related with sublittoral seaweed flora out of diving investigation in the 1990s. Results in these investigations, plus the records in two of the authority books "common seaweeds in China" and "Seaweeds in Yellow Sea and Bohai Sea of China", we have three seaweed species that belong to the title of Kelps which is described in Wikipedia as "kelps are large seaweeds belonging to the brown algae (Phaeophyceae) in the order Laminariales". They are *Sacharina japonica, Undaria pinnatifida* and *Ecklonia kurome*. The first one is believed to be introduced in 1920s from Hokkaido and thereafter spread to the Chinese coast down to somewhere between Rizhao and Qingdao city. *U. pinnatifida* is native to China. Its distribution is recorded from Liaoning province down to Qixing Island in between Zhejiang and Fujian province in the Eastern China sea. *E. kurome* is reported to be native to Lvshun, Liaoning province and also was recorded in Zhejiang province. But it is rarely seen now days.

By farming, the Chinese produce about 60-800,000 tons (DW) *S. japonica* each year in Liaoning, Shandong and Fujian provinces, about 400,000 tons (FW) *U. pinnatifida* each year in the above provinces except the last one. Natural harvest of these two species can be neglected in comparison to cultivation. Due to the economic significance, most of the previous diversity studies are centered on these two species, especially in the past ten years in which molecular markers are becoming more convenient to use in elucidating the genetic structure of populations both farmed and in the wildness.

A few groups' work that are related with *S. japonica* diversity studies are, (1) Shi et al. (2006) developed 18 polymorphic microsatellite DNA markers after screening 65 pair of universal primers. The averaged number of alleles at the 18 loci was 4.6 when judged by use of 53 male and female gametophyte clones from different cultivars; (2) Wang et al., (2010) analyzed 4099 ESTs and found 254 SSRs, from which they designed 63 pairs of primers, after testing, 23 pairs were found to be polymorphic in species in Laminariales; (3) Shan et al. (2011) used AFLP markers to analyze samples of *S. japonica* from 12 populations including six cultivars and six wild populations from the Far east of Russia, Korea and China (north an south). They found a strong genetic differentiation among the populations and this differences are positively correlated with geographic distance; (4) Li et al. (2012) screened ESTs of *Laminaria digitata* and found 83 ESTs that contained SSR and accordingly designed 45 pairs of primers. Finally thirteen of them were successfully used to analyze the genetic structure and relationship among cultivars of wild populations of *S. japonica* isolated from Russia, Japan and China. For *S. japonica* in summary, most of the diversity studies are centered on cultivar analyses, with a trend that the more the population is used in hatchery, the more the offspring tend to be identical in genotypes. Wild populations were believed to be the offspring of the early introduced strains or of the farmed populations when they released spores in the sea at the end of harvesting season. In the subtropical province of Fujian province, *S. japonica* could not complete life cycle in nature due to too high water temperature in summer.

There are few diversity studies of *Undaria pinnatifida* in China though it is an important farmed seaweed since 1980s. The morphology of this seaweed is believed to be largely determined by the abiotic environment. For example, difference in water current and space limitation (determined by the density of plants at a fixed benthic area) will lead to completely different blade morphologies of the adults. Very recently, Shibneva et al. (2013) further confirmed that morphological parameters of adult *Undaria pinnatifida* found at different niches are largely due to ontogenetic changes that were caused by the environment. Thus, again molecular markers are indispensable in order to present a clearer picture of population diversity. The most comprehensive and recent study regarding the diversity of farmed and wild populations was done by Li et al., (2012), in which the authors reported how hatchery practice resulted in differentiations among cultivars by use of AFLP markers.

Diversity of Kelps on species level, according to the present records in China, is low. There might be more new species living in the largely unexplored sublittoral zones along the temperate-cold provinces. This remains to be identified in the future. Diversity variance of kelps on population levels in *S. japonica* and *U. pinnatifida* are presently a hot topic among different phycological research groups. Since the results will tell us the specific genetic structure of farmed population (cultivar) and its relation with others.

Hybrid vigor has been more and more often used in kelp breeding practice which involves the use of genetically distant stock materials. It is apparent that such basic investigation will help breeding more new varieties. Inbreeding depression (ID), which mostly is resulted from crossing of closely related parental materials, has started to draw attention for the breeders. This is out of the request by the algal farmers who complained that a new cultivar will become not productive after 3-5 years farming in practice. There has been no data on how depressed a farmed population of either species is. Also no data on the relation of the decrease in fitness to genetic structure (homozygous vs heterozygous among the parental stocks). Considering the scale and importance of kelp farming in China for the farmers, research in ID and ways to prevent is urgently needed for the future.

Key words: Kelps, *Saccharina japonica*, *Undaria pinnatifida*, diversity, cultivation.

Geographical features of the North Pacific for algal distribution

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Present situation and assignment of ecological diversity research on kelp

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Saccharina japonica (Laminariales, Phaeophyta) is an important component of the coastal communities as part of background on one hand and an object for the active commercial and amateur fishery on the other hand. On Sakhalin its habitat areas are located close to the human settlements, so kelp has a great social implication.

The main tasks in studying *Saccharina japonica* along Sakhalin Island are related with its fisheries importance. The first records about the commercial fishery of Laminariales (kelp) along Sakhalin Island occur in the works of Yu.P. Schmidt (1905).

The resource studying of the commercial macrophyte species has been started in the 1940s-1950s under the direction of G.I. Gail. Since that time the problems on ecology and commercial use of kelps began to be posed. In the 1950s-1970s the researcher of TINRO V.F. Sarochan had worked on Sakhalin and studied commercial species of the Laminariales genera. Further the annual expeditions of SakhNIRO, including the algologists Kizevetter I.V., Balkonskaya L.A., Bukhryakova L.K., Shpakova T.A., Repnikova A.R., and some other, have surveyed the coastal zone of Sakhalin Island.

The biological state of *Saccharina japonica* resources along southern Sakhalin has been monitored more than 50 years in order to use them rationally. These surveys were focused on the densest kelp fields and traditional fishery. Annually the scientists precise the boundaries of distribution and density of one-year and two-year kelp assemblages; collect data on the size-weight structure of kelp covers; estimate a reproductive potential of the two-year kelp. As a result of these researches, there are annually prepared recommendations on a rational use of *Saccharina japonica* resources for the nearest two years. Besides, recently a great attention was paid to the following lines of investigations. First – studying the abiotic and biotic conditions that affect the formation of Laminariales resources in places of their active fishery. Second – assessing characteristics of safety that are necessary in food and other processing of raw material.

The surveys are conducted in the basic fishery areas. All the main assemblages of *Saccharina japonica* along southern Sakhalin are located mainly between 46 and 49N. The length of this area along the western coast is about 200 km. Along the eastern coast the area includes Aniva Bay, southwestern part of Terpeniya Bay, and individual sites of the southeastern coast. A total length of the coast with the fields of *Saccharina japonica* here is about 270 km. The great latitudinal length of habitat, different geomorphological peculiarities of coasts and hydrological characteristics of Japan and Okhotsk seas create diversity of biotopes. From the 2012 data the total area covered by *Saccharina japonica* along the southwestern Sakhalin coast is 2.78 km^2, and in Aniva Bay – 15.8 km^2.

The proportion of *Saccharina japonica* among other macrophytes is not equal. In the warm seawater areas it competes with *Costaria costata* and *Phyllospadix iwatensis*, and in the cold seawater areas with *Saccharina cichorioides*, *Alaria marginata* and *Cystoseira crassipes*. On the coastal sites with the high wave activity in the upper stratum, *S. japonica* is often accompanied with *Alaria marginata*. There are significant differences in the by-depth distribution in different areas. Along the eastern coast *Saccharina japonica* occurs between 0 to 10 m, and along the western coast between 0 to 6 m. The commercial covers are concentrated in Aniva Bay up to 5-6 m depth, along southwestern Sakhalin to 3-4 m. In the cold seawater areas the range of *S. japonica* distribution by depths is wider than in the warm seawater areas. More often the width of kelp covers is 5 to 150 m. Its belt is much narrow along the western coast (15 m on average) than along the eastern one (55 m on average).

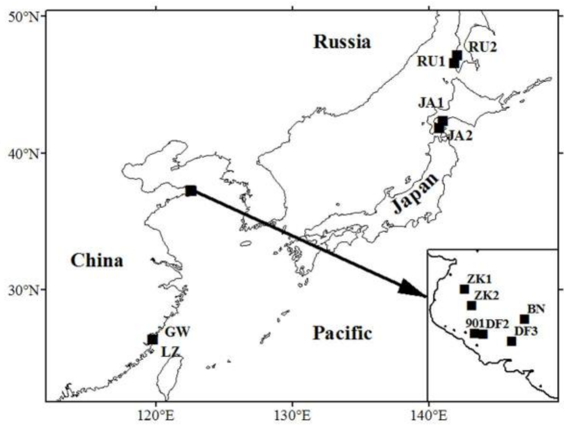
The quantitative characteristics of *Saccharina japonica* abundance vary greatly depending on abiotic and biotic factors. Cycling of abundant and non-abundant generations is observed along the western coast of Sakhalin Island. Sometimes this cycle can be disturbed. In 2012 the mean density of *Saccharina japonica* thalli at the age to 1 year was 15 ind./m^2 (0.5-67 ind./m^2) along southwestern coast, and 20 ind./m^2 (0.7-342 ind./m^2) along the eastern coast. The mean specific density of the two-year thalli of *Saccharina japonica* was 23 ind./m^2 (3-60 ind./m^2) along southwestern coast, and 8 ind./m^2 (0.6-22 ind./m^2) along the eastern coast. The specific biomass of the two-year *Saccharina japonica* in different areas and in different years varied widely; however, in 2012 its mean values along southwestern and eastern coasts were practically equal (3.51 kg/m^2 and 3.54 kg/m^2, respectively).

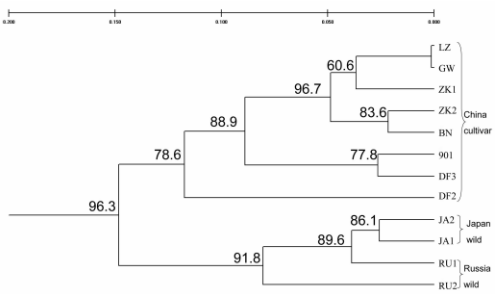
The sizes and weights of *Saccharina japonica* thalli vary greatly more often because of the environmental conditions. Temperature is the most powerful factor. The morphometric investigations along the western Sakhalin coast proved clear differences between “marine” and “lagoon” and also “typical” and “long-stalk” forms of *Saccharina japonica*. At the same time it is revealed that the stalk length in a “typical” form of *Saccharina japonica* within the traditional fishery area along the southwestern Sakhalin coast increases consecutively from north to south. From the 2011 and 2012 data, the sizes and weights of the two-year kelp in Aniva Bay are smaller than along the southwestern coast. The active growth of *Saccharina japonica* in length is observed at the water temperature to 6ºC (Shpakova T.A., 1985) that corresponds to the March-May period along western Sakhalin and April-June in Aniva Bay. There is an interesting fact that some fields, even closely located, are formed by the plants which have serious differences not only on morphological but also on a genetic level. Thus, similarity of *Saccharina japonica* from the two areas of the western Sakhalin coast (Cape Bogdanovich and Cape Tukotan) with that from southern Hokkaido is greater than with each other (Fig. 1).

We continuously study sporogenesis as one of the most important features of reproduction. In mid July, spore-bearing is observed in a two-year *S. japonica* all over the western and southeastern Sakhalin coastlines. However, in July a spore-bearing tissue occurs in more than 60% of plates in the warm seawater area of the western coast, whereas in Aniva Bay only in 25%. Further, by mid September, a total of 99% of plates are observed with the spore-bearing tissue. A distinguishing feature of *Saccharina japonica* from the southwestern coast is its ability to form a spore-bearing tissue on the first-year plates. It appears in different time in different areas and is positively related with the warm-water phenomenon.

A direct consequence of biological state of *Saccharina japonica* in its habitat areas is numbers of the total and commercial biomasses. The data on the current level of reproduction allow us to predict trends of changes in the total biomass in the nearest future. However, there are some other important biotic and abiotic factors which can affect the formation of kelp resources.

Water temperature is a very important regulatory factor of environment. Its fast increase promotes thallom's growing in spring, early spore-bearing and thallom's disturbance in summer. Annually the water temperature makes conditions for size-weight characteristics of thalli, and so the number of the total and commercial biomasses, and also a reproductive potential (Sarochan, 1969, Balkonskaya, Shpakova 1989). A success in reproduction and growth at the early stages of development depend greatly on hydrochemical conditions in the coastal zone. Concentration of nitrates in winter and spring seasons is known to affect significantly the formation of kelp biomass (references to Japanese authors). One of the abiotic factors that can catastrophically affect the commercial part of kelp resources is the ice situation; however, such cases are rare. Along the western Sakhalin coast their frequency is approximately 6-7 years. Many scientists consider the increase in a specific density of sea urchins as one of the important biotic factors that can affect significantly the biomass of kelp covers (references to Japanese authors). The high abundance of sea urchins is related first of all with hydrological conditions (mainly with the high water temperature) that are favorable for their existence and reproduction (references to Japanese authors). A warm-water regime of the area does not fit for growth of kelp biomass. But because of the increased numbers of sea urchins-phytophags the situation may become worse. In our opinion, sea urchins can really affect the abundance and biomass of kelps but only in the depressed two-stratum community where Laminariaceae and Corallinaceae algae dominate (Otaru, Japan). However, in the well-developed three- and four-stratum community where *Phyllospadix iwatensis* and red algae underbrush occur, the sea urchins prefer feeding red algae and grass (Reubun Island, Japan and southwestern Sakhalin, Russia). Thus, in our waters the dynamics of kelp resources depends first of all not on sea urchin abundance in algal fields but on hydrological conditions.





B

A

Fig. 1 Geographic loci (A) and UPGMA dendrogram (B) of four wild populations (RU1, RU2 from Russia; JA1 and JA2 from Japan) and eight cultivars (ZK1, ZK2, BN, 901, DF2, DF3, LZ and GW were cultivated in China) based on Nei's unbiased genetic distances.

Present situation and assignment of diversity conservation research on kelp bed on the coast of northern end of Honshu Island, Japan.

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Fluctuations in distribution of Laminarialean species.

The Cape Ohma region of Shimokita Peninsula, the northernmost point of Honshu Island, Japan, is subject to both the warm Tsugaru Current and the cold Kurile Current. As a result, the Laminarialean flora includes both cold temperature species (*Saccharina japonica* (J.E. Areschoug) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, *Kjellmaniella crassifolia* Miyabe and *Costaria costata* (C. Agardh) Saunders) and warm temperature species (*Undaria peterseniana* (Kjellman) Okamura, *Ecklonia stolonifera* Okamura), as well as *Undaria pinnatifida* (Yendo) Okamura, which is distributed in both waters. The frequency of occurrence (as a measure of distribution) and the biomass of these species were recorded in June 1976 (at 50 points in depths between 8-30 m), July 1988 (192 points, 2.5-25 m) and July 2001 (78 points, 2.5-25 m). Comparison of these data revealed a decrease in cold temperature species and an increase in warm temperature species from 1976 or 1988 to 2001.

Long-term data of seawater temperature measured at 5 m depth near the study site showed that mean temperatures in the middle of winter (late January to February) in 1989-2000 were 0.9-1.1 ^{o}C higher than those in 1980-1988. Higher seawater temperatures in the last decade appear to have affected the frequency of occurrence and biomass of the Laminarialean species along the coasts of Cape Ohma.

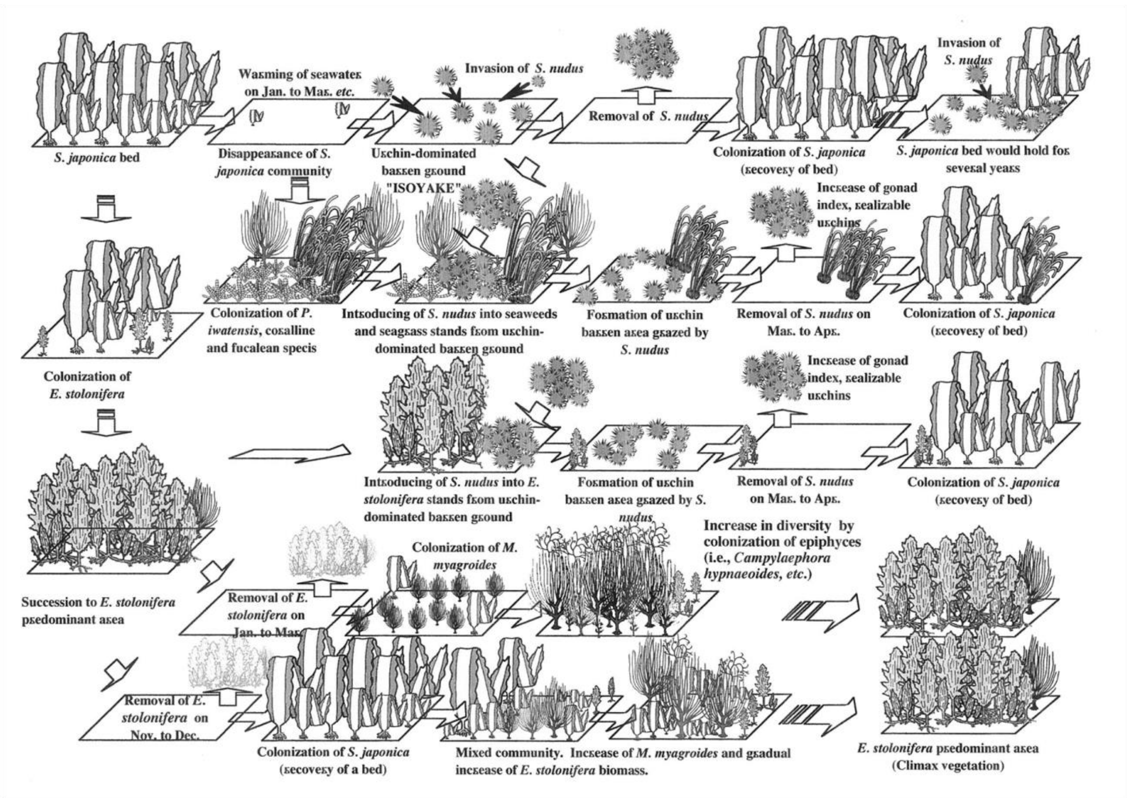
This result supported our previous conclusion that 1 ^{o}C higher mean seawater temperature in late January caused a decrease in the biomass of *S. japonica* (by ca. 64%) along the same coast.

Restoration of kelp bed from "ISOYAKE-BA".

The restoration techniques of kelp bed by *S. japonica* communities from sea urchin-dominated ground "ISOYAKE-BA" and useless perennial algae-dominated area were researched on the coasts of Shimokita Peninsula. The population of

Strongylocentrotus nudus (A. Agassiz) of 60,000–194,000 individuals was removed from the area of 1.2-4.15ha in each year of 1994-2001. The colonies of *S. japonica* occurred on February and dominated after March 1995 on the area where sea urchins were removed on September 1994. The biomass of the *S. japonica* became 10.1kg/m^2 on June 1996, while the natural seabed around the removal area was “ISOYAKE-BA” continuously. The dominant communities of *S. japonica* were also restored on other seven removal areas.

E. stolonifera communities were removed from the area of 1-25 ㎡ on October 1995, November 1996 and each month of November 1997 to March 1998. *S. japonica* communities were re-constructed on the removal areas where *E. stolonifera* communities had been removed on November and December. On the other hand, on the areas where they had been removed from January to March, useless algae of fucalean species occurred. From these results, on the coasts of northern end of Honshu Island, kelp bed by *S. japonica* can be re-constructed by removal of *S. nudus* population from



the “ISOYAKE-BA” or removal of useless perennial algae on December or before, as shown in the following figure.

Present situation and assignment of diversity utilization research on kelp

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1. Kelp forest ensure species diversity

Hokkaido Prefecture, Japan, has some ocean current, Tsushima warm current, and their branch current, Soya warm current Tsugaru warm current, Oyashio cold current. Various sea currents make different type kelp forest, Hokkaido have number of kind kelp forests. Also, diverse water movement makes various shape of kelp. There is longer and narrower kelp grow at mouth of bay, whereas kelp is wider at inner part of bay. Longevity of kelp depend on water temperature, in general kelp live two-year, however, warmer area, southwest coast of Hokkaido, lifetime of kelp is annual.

Kelp forest maintain rich species diversity of sea algae, in fact, we can see many kinds of algae into kelp forest, also we can collect extremely diverse and voluminous invertebrate species from kelp forest, especially their holdfast. Smaller fishes were attracted the kelp forest to eat the invertebrate, then larger fish eater come it the place, finally seals and other small sea mammals nurse their babies in the kelp forest. They knew that kelp forest is good nursery ground

If the kelp forest extends for deeper area, it makes diversity of invertebrate and fish in that area.

2. Diversity of utilization

In Hokkaido, during Edo period, Ainu (native aboriginean in Hokkaido) fish the kelp to export for Honshu, so Edo Shogunate (former government during Edo Era) develop the kelp recourse as important exported goods. Since Edo period, kelp have utilized in Japanese people as traditional and historical foods, but recent year, production volume of kelp sharply decrease and to be less than 20000 ton. Now, we use several kinds of kelp, *Shaccharina japonica, S. longissima, S. coriacea, S. sculpera, Arthrothamnus bifidus*, *S. longissima*, dominate the production of kelp.

Kelp fisherman have two fishing route, the first is catch from natural sea shore using fishing gear, second one is artificial aquaculture. Plus, the aquaculture has one year cycle and two year cycle.We generally fish two-year-old kelp in peak period of each year (generally August), however, some area in eastern Hokkaido, fisherman collect before the highest period of fisheries. They catch younger kelp it was so-called “Saomae”, it means that before fishing gear using. The Saomae is shorter but it more tasty. In fishing ground, various kinds of hook used for collecting kelp, based on depth of kelp forest, density or length of kelp. Most suitable hook is selected in each fishing ground.

Kelp is used for Japanese traditional foods, especially ”Sashi (flavor base of soup)” every moring in Japan, Japanese people take Miso-soup with Dashi, kelp make Japanese traditional foods culture. Recently, new utilization of kelp was discovered, “functional food (fucoidan)”, medical science focused for kelp.

Fisheries maketing need stable providing of kelp, artificial kelp culture help stability of production and making reasonable price. Otherwise, natural kelp made higher expensive kelp bland. Kelp has divers values.

3. Diversity of contribution for primary production

Kelp forest help have higher biomass, it make high primary production. It always use for spawning bed of the herring, also juvenile or young fish swimming into kelp bed. Those fishes attract large fish eaters, sometime small mammals nurse their babies in kelp bed.

However, recently southwestern Hokkaido, kelp forest is limiting at very shallower area, almost kelp bed were disappeared, it so-call “Isoyake”. Recent study reveals that warmer and poor nutrient seawater bring Isoyake phenomenon.

4. Various ecosystem service

Kelp forest can stock voluminous “N” and ”CO_2”, and also peoples in Hokkaido (especially family including children) enjoyed in beach shore along kelp bed during summer season. Every summer, almost junior school or elementary school takes their schoolboy into beach near kelp bed for social education. It make familiar for kelp and need for study of regional nature and industry (kelp fisheries) and culture. Also high school and university students often visit seashore to study biology of marine organism into kelp bed, their species diversity help studying marine biology. Kelp provides various ecosystem services.

Novel uses of kelp in Canada

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Unique elements in the Northeast Pacific kelp flora, particularly *Macrocystis pyrifera* (Giant Kelp) and *Nereocysitis leutkeana* (Bull Kelp), have inspired the creation of new culinary uses (kelp sushi wraps, kelp chips, kelp swizzle sticks to name a few), works of art, wearing apparel, musical inspiration and spa resources. British Columbia long-line farming, employing a continuous seeding method, allows for the efficient production of kelp for industrial extraction (for example, fucoxanthin, fucoidan) and for aquaculture feed for abalone and sea urchins (1). Presently, long-line kelp farming is being explored as an important element in marine polyculture and as a means of de-nutrifying seawater surrounding salmon rearing pens (2,3). Long-ling farming may provide material of biofuel production (4). Continuous long-line kelp farming is achieved by winding 20 m seeded string around a farm rope (Figs.1,2). Numerous kelp species can be farmed in this manner (Figs.3-6). Production on the continuous long-line kelp cultivation can range from 5-45 wet kg kelp per meter cultivation rope (Table 1.).

Figure 1. A spool of 20 m of seeded string.

Figure 2. Seeding a 20 m cultivation rope.

Figure 3. Young farmed *Alaria marginata*.

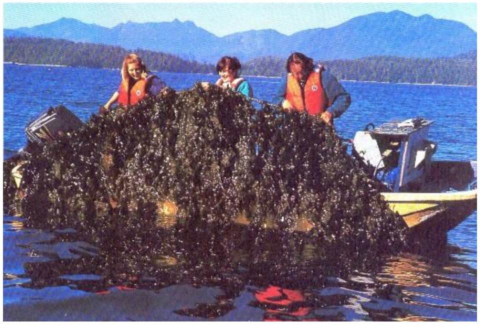

Figure 4. Mature farmed *Saccharina latissima*

Figure 5. Young farmed *Nereocystis leutkeana.*

Figure 6. Young farmed *Macrocystis pyrifera*

Table 1. FARMED KELP BIOMASS PRODUCTION

(wet kg kelp per meter of cultivation rope)

Nereocystis leutkeana (Bull Kelp)	5-22 kg	Merrill & Gillingham
Saccharina latissima (Sugar Kelp)	8-29 kg	Kain, Druehl
Sacchasrina groenlandica (Tangle)	9-24 kg	Druehl
Saccharina japonica (Kombu)	8-24 kg	Kawashima

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Novel use of kelp in Japan

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Kombu (*Saccharina*) is brown seaweeds that have been used for many years in Japan. It is mostly used in the dried form. On the one hand, boiled and salted wakame (*Undaria pinnatifida*) is often used as fresh food in Japan. However, there is very little knowledge of kombu in its fresh form. Consequently, the research on the novel use of kombu as fresh food is now going.

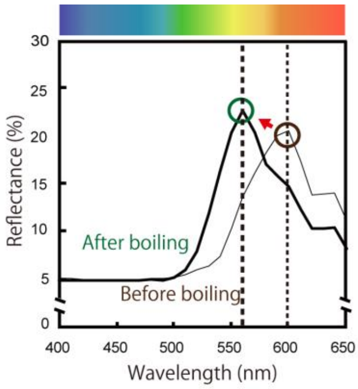

Fig.1 Reflectance spectrum profiles of kombu before and after boiling.

Firstly, we tried to develop a method for objective evaluation of the quality of raw kombu. The brown color of raw kombu changes to brilliant light green upon boiling and this property is lost upon storage. This is a good index for evaluating the quality of raw kombu. Raw kombu (*S. japonica* var. *religiosa*, *S. japonica* var. *japonica*, *S. sculpela*) blades showed maximum reflectance at 600 nm and the peak shifted to 560 nm upon boiling (Fig.1). The 560/600 nm reflectance ratio for the boiled blades decreased gradually upon storage. The changes in this value and the visual color had a similar pattern. These results show that the quality of the raw kombu can be evaluated by measuring the reflectance ratio of the boiled blades (greenish index: GI). For wakame, the blades showed maximum reflectance at 580 nm and the peak shifted to 560 nm upon boiling. These results indicate that it is necessary to use a wavelength specific to the genus for evaluation of raw seaweed.

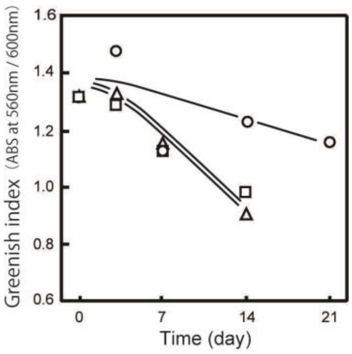

Fig. 2 Change in greenish index of kombu during the storage at 5 ºC under different atmospheric conditions (O_2 (○), Air (Δ), N_2 (□)).

Secondly, to develop quality control technology of raw kombu, the effects of oxygen and temperature on the change in quality of them was studied using the GI. Kombu (*S. japonica* var. *religiosa*) blades were stored in a plastic bag at various concentrations of oxygen at 5ºC with shielding from light. Storage of kombu blades in oxygen maintained a high GI (560/600 nm reflectance ratio) for 21 days (Fig.2), with a bright green color obtained upon boiling, similarly to that before storage. In contrast, the GI for kombu blades stored with nitrogen gas decreased upon storage and the bright color was lost. Respiration activity by kombu blades during storage in air was proved by the consumption of oxygen and production of carbon dioxide. In storage in oxygen, the standard plate count did not increase. Incidentally, storage of wakame blades under the same conditions resulted in changes in GI (560/580 nm reflectance ratio) and color changes that were essentially the same as those for kombu. These results demonstrate that storage of raw kombu in oxygen at low temperature to suppress respiratory activity is effective for maintenance of quality.

Long-term variation in distribution of konbu (*Saccharina*) beds along coasts of Hokkaido, Japan

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Costal ecosystems represented by seaweed beds, seagrass beds and coral reefs provide habitats for various animals and plants. They are also known to exert high ecosystem functions such as biological production and carbon fixation, tourist resources. However, recent deterioration of biodiversity and ecosystem functions in these habitats is serious concern as represented by rocky-shore denudation and coral bleaching. Analyses of long-term variation in costal ecosystems are necessary to evaluate the human-induced impacts and to plan effective conservation protocols.

We analyzed long-term change in biodiversity of konbu (*Saccharina*) and area of konbu beds, using data of five-times interviews conducted by Ministry of the Environment and Hokkaido government between 1970's and 2000's. We analyzed the similarities of species diversity among coastal cities based on records of species occurred. As a result, four groups were distinguished; (1) along the Pacific coast of eastern Hokkaido, (2) along Hidaka to Funkawan Bay, (3) along Oshima to Ishikari, and (4) along Ishikari via Wakkanai to Shiretoko. The group 1 showed highest biodiversity. The observed variation in species diversity probably reflect biogeographic pattern in relation to different ocean currents. We also compared konbu bed area in each coastal local governmental unit, and found that konbu bed area decreased in many regions, especially in southern part of Hokkaido. We also evaluated the effect of water temperature in winter and summer on the variation of konbu bed area in each area between 2000 and 2009, using GLM. However, apparent effect on temperature was not detected. Longer-term data incorporating records of museum specimens may shed light on the factors affecting the decline in kombu beds in future studies. We will also introduce The Biological Information System for Marine Life (BISMaL), which is a web-based data system for biodiversity information, particularly in biogeographic data of marine organisms including kelps.

Approach to elucidation of genetic diversity of *Saccharina japonica* in northern part of Japan based on DNA fingerprinting

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Saccharina japonica is divided to four varieties and, in northern part of Japan that is the center of distribution of this species in the world, any variety is growing at its regional area. This species known to constitute the marine forests in the coastal areas is the primary producer in the marine ecosystem. Furthermore, it has been used as a precious foodstuff, and the commercial value has been decided according to variety that the product belongs to. And not only that, even within a variety, the value between products changes greatly depending on the production area because each populations are ecologically and morphological different. Therefore, to obtain the knowledge of genetic diversity in distribution area must be significant for biodiversity conservation and management of fishery resources. It seems that DNA fingerprinting is effective tool to study genetic differences between local populations. On the other hands, the analysis with fingerprinting needs high quality DNA to obtain reproducible results. Therefore, to extract pure DNA is indispensable. However, since saccharinan kelp contains a large amount of polyphenol and viscous polysaccharides such as alginic acid and fucoidan,the extraction of pure DNA from kelp samples is not easy because of interference by these substances. In a previous study, the effective DNA extraction method was developed. This time, we provide a technique for effective DNA extraction method, and results obtained by Amplified fragment length

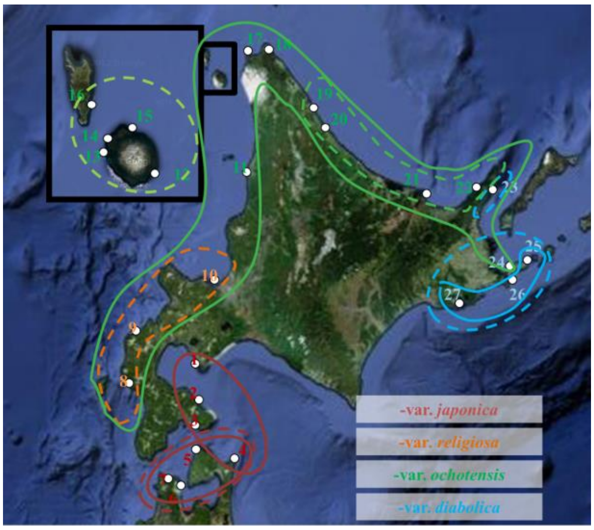

Fig. 1 The relationship of genetic similarity coefficient and Genetic differentiation between populations. Solid line represents sorted population based on *S*. Short-dashes line represents sorted population based on *Gst*.

polymorphism (AFLP) analysis.

As a result of the analysis, genetic difference was detected between local populations by AFLP analysis. Furthermore, less frequently, several unique fragments that might be able to detect the difference of variety were found out in some areas. It is recognized that genetic similarity coefficient (*S*) between local population was correlated with regional continuity. Some local populations (i.e. 8-23, 4-7 in Fig. 1) were grouped by *S*. These populations were further divided according to genetic differentiation coefficient (*Gst*), and each of segmentalized groups has limited distribution area. It seemed that genetic differentiations of these segmentalized groups were caused by current flowing along the coast of Hokkaido.

AFLP is helpful tools for detecting genetic diversity in *S japonica* because of the convenient means and the abundant information. However, because it is impossible to distinguish heterozygous and homozygous, we developed micro-satellite marker (co-dominant marker) to get more information such as genetic exchange between local populations. We continue the study to investigate and elucidate genetic diversity with AFLP and microsatellite marker analysis.

Spatial estimation of the kelp forests (*Sacchaina* spp.) distributions in coastal waters of Aomori, Japan, using acoustic method

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Sea desertification happened in recent years with increasing of seawater temperature and feeding damage by sea urchin and other animals living on seaweed in kelp forests, which has negative influence on coastal kelp fisheries and ecosystem. In coastal waters off Shimokita Peninsula, Aomori, Japan, sea desertification was reported as a serious problem and the solution is being discussed, current situation and change trend of kelp forests (*Sacchaina* spp.) is important. In order to prevent sea desertification expansion and recovering kelp forests, estimation of the spatial distribution and standing crop is necessary. Until now, the data on seaweed distribution were most from visual observation and aerial photography costing a lot of time, effort and observation limit due to depth. The objective of this study is to get spatial distribution and thickness of kelp forests in wide region using acoustic method in coastal waters off Shimokita Peninsula, Aomori, Japan. The surveys were conducted in the coastal waters off Shiriyazaki (8-9th November, 2011 and 5th November, 2012) and Ishimochi (11-14th June, 2012 and 6-8th November, 2012), Aomori, Japan. The survey areas were 0.38km^2 and 6.22km^2, respectively. Data on the presence/absence and thickness of kelp forests were collected via acoustic observation on transects using an on-board small quantitative echosounder (KCE-300, Sonic Co. Tokyo) at 120 kHz by the fishery boat with speed about 3knots. Acoustic data were geostatistically interpolated, and the areas covered by kelp forests were estimated. Kelp forests distribution was analyzed from two different steps using Geographic Information System software (ArcGIS Ver.9 ESRI Inc.). Presence or absence of the forest patches and the variation in thickness among the present forest patches was analyzed. On the same time, identification of kelp forest from other seaweed and sea urchin (*Strongylocentrotus nudus*) inhabitation information was got by visual observation using underwater camera. Marine environments data were collected by Compact-CTD (Alec Electronics Co. Kobe). In the present study, the average thickness of kelp forests from acoustic data in Shiriyazaki was 0.45m in 2011 and 0.44m in 2012, kelp distribution area was 23% and 43% of survey area. While, in Ishimochi, the average thickness and kelp distribution area proportion of survey area was 0.72m, 22% in spring, 0.37m and 0.01% in autumn which is less than in spring. More than 80% kelp forests living in water depth below 15m and sea urchin average size was below 6cm in survey areas. Different kelp distribution feature, substrate and sea urchin inhabitation was certified between Shiriyazaki and Ishimochi. Sea urchin barren is occurring in Shiriyazaki area and sand substrate and turbidity was considered the reasons in Ishomochi. In order to evaluate fisheries and ecosystem, continuous monitoring is necessary for kelp forests and marine environment change tendency.

Key words
Acoustic method, geostatistical analysis, kelp forests, sea desertification

A taxonomic re-examination of *Saccharina longipedalis* (Laminariales, Phaeophyceae)

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Saccharina longipedalis that is an endemic kelp species around Lake Akkeshi that is brackish lake, in eastern Hokkaido has a morphological feature with a long stipe. It is reported that this species is closely related to *S. japonica* by DNA sequencing (ITS and RuBisCo spacer) and the comparison. However, for re-examination of taxonomy on this kelp, more information has been requested. In this study, outward morphology and genetic diversity were investigated using sporophytes collected at seven sites around distribution boundary of *S. longipedalis* and *S. japonica* in Akkeshi (i.e. S. japonica var. diabolica). Furthermore, cross experiment between seedling from the sites was performed.

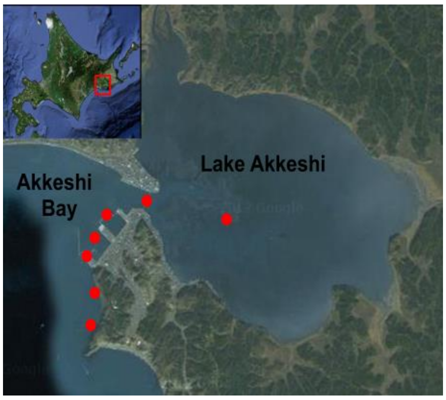

Figure 1 Collecting sites in this study.


Figure 2 Sporophyte collected at Lake Akkeshi.


Figure 3 Sporophyte collected at Akkeshi Bay.

The morphological variation was noticeable among the sporophytes even from one site. Average measuring value of blade thickness and stipe length of samples collected at Lake Akkeshi were different significantly from those from the other sites. Meanwhile, no or a little genetic variation was detected in 5S rDNA spacer and tested microsatellite regions among the samples from the all seven sites. The Dice Similarity Coefficient (S) in AFLP analysis was generally high between samples in this study, and the value including *S. japonica* var. *diabolica* from several populations in Rausu and Nemuro corresponded to that in a variety of *S. japonica*. As the result of cross experiment, fertilization and normal early development of the young sporopyte were confirmed in all the combinations.

This time, a couple of diagnostic morphological characteristics of sporophyte from Lake Akkeshi were confirmed. However, from the little genetic divergence in not only ITS and RuBisCo spacer but also 5S rDNA spacer and several microsatellite regions between *S. longipedalis* and *S. japonica* and the result of cross experiment, it is appropriate that *S. longipedalis* is included in *S. japonica*. The discriminative morphology with long stipe of sporophyte from Lake Akkeshi seems to be influenced by the physical environment there. In consideration of genetic similarity by AFLP analysis, we conclude that *S. longipedalis* should be treated as *S. japonica* var. *diabolica*.

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