

Taxonomy and biogeography of *Agarum* and *Thalassiophyllum* (Laminariales, Phaeophyceae) based on sequences of nuclear, mitochondrial, and plastid markers

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Abstract *Agarum* is a perennial kelp genus that has a broad distribution from the northwest Pacific to the northwest Atlantic Ocean. In order to address questions regarding phylogenetic relationships and present-day distribution patterns of species within this genus, we analyzed the nuclear ITS2, mitochondrial *cox1* and *cox3*, and plastid RuBisCO spacer from a subset of 130 samples of the genus plus *Thalassiophyllum* and *Costaria*. All analyses of the individual markers and the ITS2 + *cox1* + *cox3* dataset consistently produced a clade of *Agarum* that included *Thalassiophyllum*. The clade consisted of two well-resolved subclades: one composed of *A. fimbriatum* and *A. oharaense*, and the other of *A. clathratum*, *A. turneri*, *A. yakishiriense*, and *T. clathrus* (the type of the generic name *Thalassiophyllum*). On the basis of these results, we treat *Thalassiophyllum* as a synonym of *Agarum*, reinstating the combination *A. clathrus* made by Greville in 1830. Despite its wide distribution from Japan through Alaska to the east coast of North America, *A. clathratum* had low genetic diversity, suggesting a relatively recent long-distance dispersal of the species. The low degree of substitutions between *A. clathratum* and *A. yakishiriense* suggests their recent divergence from a common ancestor, and we recognize the latter as a subspecies of the former. Because type material of *A. clathratum* has not been found, we designate as neotype a specimen collected in Kamchatka, one of two syntype localities cited by Gmelin for *Fucus agarum*.

Keywords *Agarum*; biogeography; *cox1*; *cox3*; ITS2; kelp; RuBisCO spacer; taxonomy; *Thalassiophyllum*

Supplementary Material Figures S1–S4 and the Appendix (all in the Electronic Supplement) and the alignment are available under “Supplementary Data” of the online version of this article (<http://www.ingentaconnect.com/content/iapt/tax>).

■ INTRODUCTION

Agarum Dumort. (Dumortier, 1822) is a kelp genus currently comprised of three recognized species: *A. clathratum* Dumort., *A. fimbriatum* Harv. (Harvey, 1862), and *A. oharaense* Yamada (Yamada, 1961). These species are distributed in cold-temperate to arctic waters of the North Pacific through west Greenland down to New England in the northwest Atlantic (Silva, 1991). The genus is a member of the family Costariaceae, which includes three other genera: *Costaria* Grev., *Dictyonium* Rupr., and *Thalassiophyllum* Postels & Rupr. (Lane & al., 2006). *Agarum clathratum* often forms sublittoral meadows in cold-water areas of northeast North America, Alaska, and Japan, and it is considered to be an ecologically important species (Gagnon & al., 2005).

For more than a century, Bory (1826) has been credited with establishing the kelp genus *Agarum*. Silva (1991),

however, pointed out that Bory had been preceded in this action by Dumortier. Both *Agarum* Dumort. (Dumortier, 1822) and *Agarum* Bory (Bory, 1826) take their name from *Fucus agarum* S.G. Gmel. (Gmelin, 1768), an included species and logical type of the generic name. In transferring *Fucus agarum* to *Agarum*, Dumortier and Bory were faced with the creation of a tautonym. Dumortier avoided this by proposing a new epithet, *clathratum*, whereas Bory chose *cribrosum*. To add to the confusion, *Fucus clathrus* S.G. Gmel. (Gmelin, 1768), which was made the type of the generic name *Thalassiophyllum* by Postels & Ruprecht (1840), had previously been placed in *Agarum* by Greville (1830), as *A. clathrus* (S.G. Gmel.) Grev. Moreover, *Fucus cribrosus* K. Mert. (Mertens, 1829), a species based partly on Turner’s concept of *Fucus agarum* (Turner 1809: pl. 75), was placed in *Agarum* by Postels & Ruprecht (1840), who changed the epithet to *turneri* in order to avoid the combination *A. cribrosum*, which would be a later homonym

of *A. cribrosum* Bory. Furthermore, Postels & Ruprecht (1840) adopted the name *Agarum gmelinii* Postels & Rupr. for *Fucus agarum* S.G. Gmel. *Agarum Dumort.* (1822) has been conserved against *Agarum* Link (1809), a genus referred to Phylloporaceae (Rhodophyta).

Assuming that the kelp genus *Agarum* was created by Bory, Setchell & Gardner (1925) placed three previously published species in synonymy with *A. cribrosum*: *A. gmelinii*, an illegitimate name for *A. cribrosum* (itself an illegitimate name for *A. clathratum*), *A. turneri*, and *A. pertusum* (K. Mert.) Postels & Rupr. (based on *Fucus pertusus* K. Mert. 1829). Although *A. turneri* was long considered a distinct species (Klochkova, 1998; Klochkova & al., 2009), it is now listed as a synonym of *A. clathratum* (Guiry & Guiry, 2010), as suggested by Setchell & Gardner (1925) and Silva (1991). *Agarum fimbriatum*, described from Vancouver Island, Canada (Harvey, 1862), occurs from Southeast Alaska to northern Washington and also from southern California to Baja California, Mexico (Scagel & al., 1989; Silva, 1991). *Agarum oharaense* is limited to Ohara, Chiba Prefecture, Japan (Yamada, 1961). *Agarum yakishiriense* Yamada (1962), described from Yakishiri Island, Japan, later was considered a form of *A. clathratum* [as *A. cribrosum*] by Yamada (1974).

Agarum clathratum is one of the best-known species in the genus because it has an amphi-Pacific distribution, occurring in Korea (Kang, 1966), Japan (Yamada, 1974), the Sea of Okhotsk, the Kurile Islands and the Kamchatka Peninsula of Russia (Nagai, 1940; Klochkova, 1998), Alaska, British Columbia and northern Washington (Scagel & al., 1989). It also occurs from the west coast of Greenland to New England (Taylor, 1957; South & Tittley, 1986), and in the central and eastern Canadian Arctic (Lee, 1980). Yamada (1974) described four forms within *A. clathratum* [as *A. cribrosum*] in Japan on the basis of features of the blade, stipe and holdfast: f. *cribrosum*, f. *rishiriense* I. Yamada, f. *rugosum* I. Yamada, and f. *yakishiriense* I. Yamada. *Agarum clathratum* is important in sublittoral habitats because it often dominates communities at a depth of 10 to 30 m, even under heavy grazing pressure by sea urchins (Gagnon & al., 2005).

Thalassiophyllum Postels & Rupr. (1840) has been accepted as a distinct genus (Setchell & Gardner, 1925; Klochkova, 1998; Klochkova & al., 2009). However, it was positioned within *Agarum* in a previous molecular phylogeny (Yoon & al., 2001).

Although many species of cool temperate seaweeds occur on both sides of the North Pacific and North Atlantic Oceans, the identities of the species recorded as occurring on both sides have been based on their morphological similarities (Lindstrom, 2001). Molecular markers to test their identity have been employed for relatively few algal genera. In addition to the commonly used *rbcL* gene (e.g., Cho & al., 2004), spacer regions within the nuclear ribosomal cistron (e.g., Saunders & Druehl, 1993), the mitochondrion (Kogame & al., 2005; Lee & al., 2009) and the plastid RuBisCO cistron (e.g., Yoon & Boo, 1999) have also been used to reveal relationships between closely related species or to detect difference between geographical populations within a single species.

In this study, we elucidate the phylogenetic relationships within the genus *Agarum*, in order to achieve a better understanding of the taxonomy of *A. clathratum*, and to explain the current distribution pattern of the species from the northwest Pacific to the northwest Atlantic. Samples of three forms of *A. clathratum* from their type localities, f. *rugosum* from Muroran, f. *rishiriense* from Rishiri Island, and f. *yakishiriense* from Yakishiri Island, Hokkaido, Japan, are included in this analysis. We analyze sequence data from nuclear ribosomal ITS2 and mitochondrial *cox1* and *cox3* genes. We also include the RuBisCO spacer for specimens showing differences in ITS2 or mitochondrial genes.

■ MATERIALS AND METHODS

Sampling. — Fresh thalli were collected in the field and air-dried or preserved in silica gel. Our sampling encompassed 130 specimens from all currently recognized species of the genus *Agarum* except *A. oharaense*, which we were unable to collect, and therefore we used the published ITS2 and RuBisCO spacer data of Miyata & Yotsukura (2005). These samples also represented the geographic range of the genus. We included *Thalassiophyllum* and *Costaria* as outgroups. Locality information and GenBank accession numbers are listed in the Appendix (see Electronic Supplement). Voucher materials for the DNA extracts are deposited in the Chungnam National University Herbarium, Daejeon, Korea (CNUK), UBC, or LE.

DNA sequencing and phylogenetic analysis. — Genomic DNA was extracted from ca. 5 mg of dried thallus ground in liquid nitrogen using an Invisorb Spin Plant Mini Kit (Invitex, Berlin-Buch, Germany) according to the manufacturer's protocol. Primer pairs for amplification and sequencing of each marker were as follows: for ITS2, YB1 and BC2 (Yoon & al., 2001); for *cox1*, C1F159 (5'-TTC HAC HAA YCA YAA AGA TAT WGG-3') and C1R805 (5'-GGA TCR CCW CCW CCW GCW GGA TCA-3'); for *cox3*, C3F34 (5'-CCA TTT CAT TTA GTN GAY CCH AG-3') and R20 (5'-AAC AAA RTG CCA ATA CCA KG-3'); and for the RuBisCO spacer, RS1 and RS2 (Yoon & Boo, 1999). Polymerase chain reactions (PCRs) and sequencing were performed as detailed in Lee & al. (2009). All sequences of the forward and reverse strands were determined for all taxa, and the electropherograms were edited using the program Sequence Navigator v.1.0.1 (Applied Biosystems, Foster City, California, U.S.A.) and rechecked manually for consistency. Newly determined sequences are deposited in the GenBank databases (details in the Appendix).

Nucleotide sequences were aligned using Se-Al v.2.0a11 (Sequence Alignment Editor Version 2.0 alpha 11; <http://tree.bio.ed.ac.uk/software/seal/>).

Phylogenies of ITS2, *cox1*, *cox3*, RuBisCO spacer, and the combined datasets were reconstructed using maximum likelihood (ML). Model tests show different models for different datasets: HKY+G for ITS2 and *cox3*, K81uf+I for *cox1*, and HKY for RuBisCO spacer. Because GTR is the most common and general model for real-world DNA analysis (Stamatakis, 2006), we also used the GTR+ Γ +I nucleotide model as

implemented in RAxML v.7.0.4. However, because topologies of trees reconstructed by different models are similar to those of the GTR model, we employ the GTR model instead of different models for each gene. The incongruence length tests (Farris & al., 1995) show the ITS2, *cox1*, and *cox3* datasets to be congruent at $P > 0.05$.

We used 300 independent tree inferences, applying options of automatically optimized SPR rearrangement and 25 distinct rate categories in the program to identify the best tree. Statistical support for each branch was obtained by 1000 bootstrap replications with the same substitution model.

Bayesian analyses (BA) were performed for combined and individual datasets with MrBayes v.3.1.1 (Ronquist & Huelsenbeck, 2003) using the Metropolis-coupled Markov chain Monte Carlo (MC³) with the GTR+ Γ +I model. For each matrix, one million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The burn-in period was identified graphically by tracking the likelihoods at each generation to determine whether they reached a plateau. The 14,002 trees sampled at the stationary state were used to infer the Bayesian posterior probability (BPP).

■ RESULTS

Statistics describing the four loci included in the analyses are provided in Table 1. Pairwise divergences within and between species are given in Table 2.

The monophyly of most major clades was well supported by various analyses of ITS2, *cox1*, *cox3*, and RuBisCO spacer datasets (Figs. S1–S4). One exception is that *Agarum clathrus* (= *Thalassiophyllum clathrus*) appeared on a long basal branch clearly separate from the remaining species of *Agarum* in the *cox1* tree (Fig. S2), but on a branch within *Agarum* in the ITS2 and *cox3* trees. Trees of the combined ITS2+*cox1*+*cox3* dataset clearly supported monophyly of *Agarum* and inclusion of *A. clathrus* in the *Agarum* clade (Fig. 1). *Agarum fimbriatum* was sister to the clade including the remaining species of *Agarum* (54% for ML and 0.99 for BA). *Agarum clathrus* formed a clade, although specimens from Kamchatka were not identical with one from Alaska. *Agarum turneri* occurred on separate branches; the specimen from Kamchatka clustered with *A. clathrus*, whereas the specimen from Alaska clustered with the subclade of *A. clathratum* and *A. yakishiriense*. This discrepancy, however, may be due to different genes being

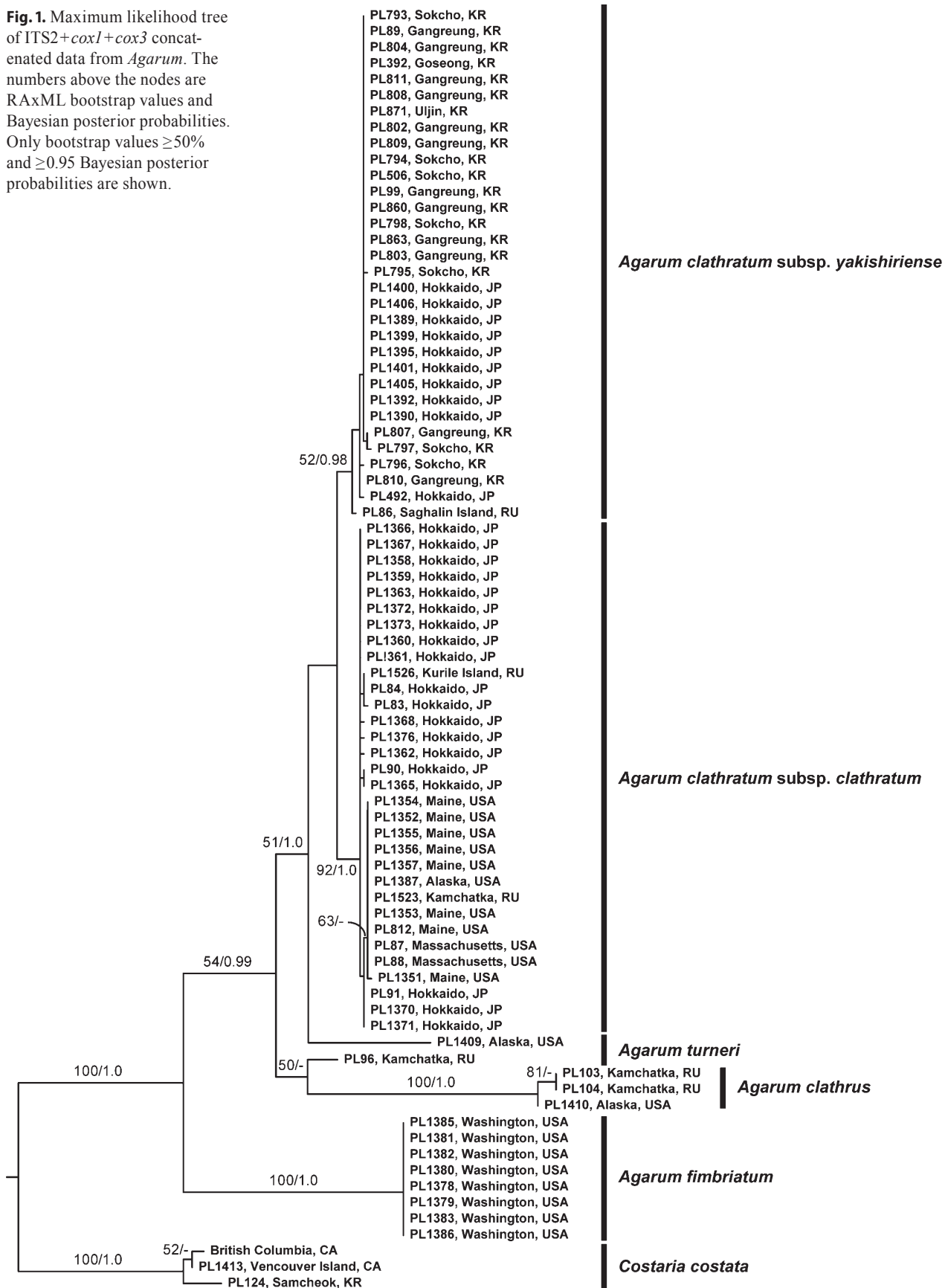
Table 1. Comparison of genetic information from four molecular markers in *Agarum*.

	Nucleus		Mitochondria		Plastid
	ITS2		<i>cox1</i>	<i>cox3</i>	RuBisCO spacer
Number of specimens sequenced	108		107	77	25
Alignment length	295		612	534	283
Number of variable sites (%)	71 (24.07)		82 (13.40)	64 (11.99)	46 (16.25)
Number of informative sites (%)	49 (16.61)		76 (12.41)	43 (8.05)	34 (12.01)
Average nt genetic distance (\pm SD)	2.88 (\pm 3.58)		2.02 (\pm 2.37)	1.59 (\pm 2.12)	3.05 (\pm 3.09)
Base frequency (A/C/G)	0.16/0.35/0.28		0.22/0.18/0.20	0.21/0.14/0.25	0.42/0.09/0.1
–ln L	866.99		1386.06	1141.03	622.41

Table 2. Genetic divergence in ITS2/*cox1*/*cox3*/RuBisCO spacer sequences between *Agarum* species. Each number indicates uncorrected p-distance (below diagonal, %).

	<i>A. clathratum</i> subsp. <i>clathratum</i>	<i>A. clathratum</i> subsp. <i>yakishiriense</i>	<i>A. turneri</i>	<i>A. clathrus</i>	<i>A. fimbriatum</i>	<i>A. oharaense</i>
<i>A. clathratum</i> subsp. <i>clathratum</i>	0.25/0.09/0.09/0.20					
<i>A. clathratum</i> subsp. <i>yakishiriense</i>	1.07/1.00/0.44/0.16	0.16/0.03/0.04/0.00				
<i>A. turneri</i>	1.83/3.27/2.11/2.51	1.70/3.27/1.69/2.35	– / – / – / 0.37			
<i>A. clathrus</i>	1.89/6.78/5.03/4.46	1.61/7.11/4.70/4.30	1.28/7.11/4.31/6.02	1.08/0.16/0.00/–		
<i>A. fimbriatum</i>	4.42/5.40/4.87/7.71	4.68/5.07/4.51/7.55	4.22/5.39/4.87/7.87	4.40/7.76/5.99/11.16	0.00/0.00/0.00/0.00	
<i>A. oharaense</i>	5.18/ – / – / 4.82	5.50/ – / – / 4.66	5.79/ – / – / 4.89	5.35/ – / – / 7.53	6.63/ – / – / 4.67	– / – / – / –

Fig. 1. Maximum likelihood tree of ITS2+*cox1*+*cox3* concatenated data from *Agarum*. The numbers above the nodes are RAxML bootstrap values and Bayesian posterior probabilities. Only bootstrap values $\geq 50\%$ and ≥ 0.95 Bayesian posterior probabilities are shown.



sequenced for these specimens (Table 1; Appendix). The *A. yakishiriense* clade was supported with 52% for ML and 0.98 for BA, and the *A. clathratum* clade was strongly supported with 92% for ML and 1.0 for BA.

The plastid RuBisCO spacer tree (Fig. S4) was similar to those of ITS2, *cox3*, and the combined ITS2+*cox1*+*cox3* dataset. *Agarum fimbriatum* and *A. oharaense* were again on a well-supported clade sister to the remaining species of *Agarum* (including *A. clathrus*), as in the ITS2 tree (Fig. S1). Among the remaining species, *A. turneri* occurred basally, followed by *A. clathrus*, and finally *A. clathratum* and *A. yakishiriense* were intermixed in the terminal group.

The ITS2+*cox1*+*cox3*+RuBisCO spacer tree (not shown) was similar to those of individual and ITS2+*cox1*+*cox3* datasets. However, bootstrap and BPP values on branches of the combined phylogeny were low, probably due to the imbalance in numbers of sequences among the individual datasets (e.g., 108 ITS2, 107 *cox1*, 77 *cox3*, and 25 RuBisCO spacer sequences).

DISCUSSION

Phylogenetic relationships and taxonomic implications.

— *Agarum* is distinguished by a broad, undivided blade with numerous perforations, longitudinal midrib, cylindrical to flattened stipe, and holdfast composed of small branched haptera (Setchell & Gardner 1925; Silva, 1991). The major taxonomic question in our study was whether *Thalassiohyllum* should be considered an independent genus. Since its separation from *Agarum* by Postels & Ruprecht (1840), *Thalassiohyllum* has been accepted as a monotypic genus, diagnosed by stout haptera, an irregularly branched frond with reniform blades provided with perforations but lacking a midrib, and with a scroll-like unfurling of new blades; secondary stipes or branches can arise along its thickened margin (Setchell & Gardner, 1925; Klochkova & al., 2009). Despite these morphological differences, Yoon & al. (2001) suggested that *Thalassiohyllum* might be merged with *Agarum* on the basis of ITS and RuBisCO spacer phylogenies, a suggestion supported by our results.

Our study included more species and specimens of *Agarum* and *Thalassiohyllum* and added *cox1* and *cox3* data. The inclusion of *Thalassiohyllum* within *Agarum* was consistently supported in all datasets read (ITS2, 81% for ML and 1.0 for BA; *cox3*, 66% for ML and 0.99 for BA; ITS2+*cox1*+*cox3*, 54% for ML and 0.99 for BA). Results indicate that perforated blades (Rosenthal, 1890; Setchell, 1893) and the same developmental stages (Setchell, 1905) in *Thalassiohyllum* are important characters that support a close relationship with *Agarum*. In contrast, the scroll-like transition zone and the absence of a midrib, previously used as diagnostic characters for the genus *Thalassiohyllum*, are considered secondarily derived within the genus *Agarum*. The only species of *Thalassiohyllum*, *T. clathrus*, occurs from Kamchatka to Alaska (Klochkova, 1998; Klochkova & al., 2009; Lindeberg & Lindstrom, 2010), within the distribution range of *Agarum*. Accordingly, we propose that *Thalassiohyllum* Postels & Rupr. (Postels &

Ruprecht, 1840) be merged with *Agarum* Dumort. (1822) (see Taxonomic Treatment).

Comparison of all species of *Agarum* is given in Table 3 and morphology of five taxa of *Agarum* is shown in Fig. 2. We are uncertain whether specimens identified as *A. clathrus* represent a single species or several species. The specimen from Akutan Island (PL140), Alaska, showed significant ITS2 sequence differences from two samples (PL103, AB087252) from Avacha Bay, Kamchatka, which also showed significant differences from each other (pairwise divergence 1.08%). There were also differences in the *cox1* gene, the second gene for which specimens of the species from the two areas were sequenced. More sampling across the range of the species is required to determine whether multiple species are included under *A. clathrus*.

Agarum fimbriatum was sister to *A. oharaense* in all analyses of ITS2 (69% for ML and <0.95 for BA) and RuBisCO spacer (95% for ML and 1.0 for BA), although mitochondrial genes for *A. oharaense* were not analyzed, suggesting that the flattened stipe is a synapomorphic character. *Agarum fimbriatum* is distinguished by a bullate blade with numerous fimbriae along the margin of the flattened stipe (Harvey, 1862), and *A. oharaense* by a smooth blade with a twisted stipe and rhizoidal haptera (Yamada, 1961). The distant relationship of *A. oharaense* to *A. clathratum* does not support the view of Yamada (1961) that *A. oharaense* may be a hybrid between *A. fimbriatum* and *A. clathratum*.

Agarum turneri has been considered a synonym of *A. clathratum* under the assumption that the great variety in morphology (broader and narrower midrib and coarser or finer blade perforations) is a result of plasticity (e.g., Setchell & Gardner, 1925; Silva, 1991; Guiry & Guiry, 2010). However, in all four analyses, *A. turneri* was consistently positioned outside *A. clathratum*. This result supports previous studies (Klochkova, 1998; Yoon & al., 2001) that *A. turneri* is a distinct species and not conspecific with *A. clathratum*. *Agarum turneri* is characterized by a flat, smooth blade with large perforations (2–3 cm in diam.), a flattened midrib 2.5–3.8 cm in width, a flattened stipe and branched haptera (Postels & Ruprecht, 1840; Klochkova & Berezovskaya, 1997; Klochkova & al., 2009). Recently, Lindeberg & Lindstrom (2010) also included it as a separate species because of consistency in morphology of recent collections and a distinct biogeography in Alaska, where specimens have been collected only from Cook Inlet westward through the Aleutian Islands, with the species becoming more abundant westward as the occurrence of *A. clathratum* diminishes. When proposing *Fucus cribrosus* (= *Agarum turneri*), Mertens (1829) cited original material from Avacha Bay, Kamchatka, in addition to referring to Turner's concept of *Fucus agarum* (Turner, 1809: pl. 75). The cited material, labeled "Insula Cavagin ad oras Kamtschaticae septentrionales" and "In sinu Awatschae (Kamtsch). Dr. Mertens", is housed in the type collection in LE, and this material clearly represents the type of *A. turneri*. An image of the type can be seen at <http://botany.ubc.ca/sandral/Agaru-tur.jpg>.

A clade including specimens of *Agarum clathratum* from Japan, Alaska, and Maine formed a monophyletic group in

Table 3. Comparison of *Agarum* species.

	<i>A. clathratum</i> (S.G. Gmel.) Grev. (1830)	<i>A. clathrus</i> (S.G. Gmel.) Grev. (1830)	<i>A. fimbriatum</i> Harv. (1862)	<i>A. oharaense</i> Yamada (1961)	<i>A. turneri</i> Postels & Rupr. (1840)	<i>A. clathratum</i> subsp. <i>yakishiriense</i> Yamada ex G.H. Boo & P.C. Silva
Blade size (length × width)	15–80 × 30–40 cm	ca. 50 × 12–15 cm	20–80 × 15–26 cm	ca. 60 × 20–25 cm	50–90 × 15–30 cm	15–30 × 15–20 cm
Blade shape	Thin, often folded, sometimes bullate, margin can be crenate	Thick, fan-shaped, spirally twisted, with many perforations, with pseudo-branches	Thin, bullate	Thin, flat	Thick, flat, with many large perforations	Folded, with scrolls near the base
Stipe	Compressed above, terete below, up to 30 cm but usually <10 cm long	Terete, up to 50 cm long but usually much shorter	Flattened, with branched fimbriae on margins	Flattened, twisted, 5–7 cm long	Terete, up to 30 cm but usually < 10 cm long	Extremely flattened, broader, not terete
Midrib	Thick, ca. 1.5 cm wide	Absent	1–3 cm wide	Unclear, 1.5–2 cm wide	Flat, 2.5–3 cm wide	0.2–0.6 cm wide
Haptera	Short, thick or slender, arising from the lower part	Branched	Profuse, slender	Fibrous, dichotomously branched	Branched	Slender, fibrous, arising from both sides of stipe
Sporangia	Whole surface of the upper to middle parts	Irregular on blade surface	Irregular patches	Both sides of blades, along margins	Blade margins	Unknown
Type locality	Eastern Indian Ocean and Kamchatka Sea (Sea of Okhotsk)	Indian Ocean and Kamchatka Sea (Sea of Okhotsk)	Esquimalt, Victoria, B.C., Canada	Ohara, Chiba, Japan	Awatscha Bay, Russia; Greenland	Yakishiri Island, Hokkaido, Japan
Distribution	Japan, Russia, Alaska to Washington, U.S.A., Arctic Canada to New York, U.S.A.	Kurile Islands, Russia, to Aleutian Islands, Alaska, U.S.A.	Southeast Alaska to northern Washington and Channel Islands, California, to Baja California, Mexico	Japan	Kamchatka Pen., Russia, to Cook Inlet, Alaska, U.S.A.	Korea, Japan, Russia

all analyses of ITS2, *cox1*, *cox3*, and RuBisCO spacer. This result is consistent with morphology: *A. clathratum* from all these areas has a cylindrical stipe and a blade that unfurls from a scroll on either side of the midrib (Setchell & Gardner, 1925). Because the original material of *A. clathratum* has not been found, we here designate as neotype a specimen collected at the type locality of Kamchatka (Fig. 2A), which agrees with the original description of the species (Gmelin, 1768). We note that *A. clathratum* Dumort. is a replacement name for *Fucus agarum* S.G. Gmel. and thus its type is the same as that of *F. agarum*. All of the ITS2, *cox1*, *cox3* and RuBisCO spacer sequences place the neotype specimen (PL1523) in *A. clathratum* subsp. *clathratum* (see Figs. S1–S4).

In a series of studies on local variation in *A. clathratum* (as *A. cribrosum*) in Japan, Yamada (1974, 1977) described four different forms of the species: f. *clathratum* (as f. *cribrosum*) distinguished by flat blades lacking raised edges around perforations, f. *rugosum* with undulate blades with raised edges around perforations and relatively long, terete stipes, f. *rishiriense* with undulate blades and short, compressed stipes, and f. *yakishiriense* with undulate blades and numerous very short, flattened stipes arising from a fibrous holdfast. In our study, samples of f. *rugosum* collected at its type locality were identical to other Hokkaido *A. clathratum* specimens, and samples of f. *rishiriense* from its type locality were identical to *A. yakishiriense* (see below).

Most of our molecular analyses recognize *Agarum yakishiriense* as distinct (ITS2, *cox1*, *cox3*), but it is closely related to *A. clathratum*. However, the RuBisCO spacer data do not support *A. yakishiriense* as distinct from *A. clathratum*. Samples of *A. yakishiriense* come from the east coast of Korea, Sakhalin Island of Russia, and the west coast of Hokkaido, Japan, including samples from Yakishiri Island, the type locality of f. *yakishiriense*. Thalli with flattened stipes, identified as f. *rishiriense*, were collected in Rishiri Island, the type locality of that form. Our data support Y. Yamada's

concept (1962) of *A. yakishiriense*, but not I. Yamada's opinion (1974: 44) that *f. rishiriense* is an intermediate form between *f. yakishiriense* and *f. rugosum*. Subsequently, Nakahara & Yamada (1974) proposed that, because cross-fertilization trials between *A. yakishiriense* and *A. clathratum* produced interspecific hybrids at a rate of 32% to 96%, *A. yakishiriense* belonged to *A. clathratum*. Although application of cross tests in kelps is not a decisive criterion because in the laboratory fertile hybrids commonly arise between different species (Druehl, 1989), the lack of sufficient divergence from *A. clathratum* in the molecular data suggests that *A. yakishiriense* should be recognized

at the subspecies level at this time rather than as a forma or a separate species (see Taxonomic Treatment).

It should be noted that the *cox1* gene produced a different tree topology with respect to *A. clathrus*. Previous studies on kelps have postulated introgression of the *cox1* gene between species. For example, genera of Alariaceae showed different relationships to each other depending on whether the chloroplast *rbcl*, nuclear ITS-5.8S rRNA, or mitochondrial *nad6* gene was being compared (Kawai & al., 2008). Similarly, within the genus *Alaria*, specimens of *Alaria* showed different relationships irrespective of species designations depending on

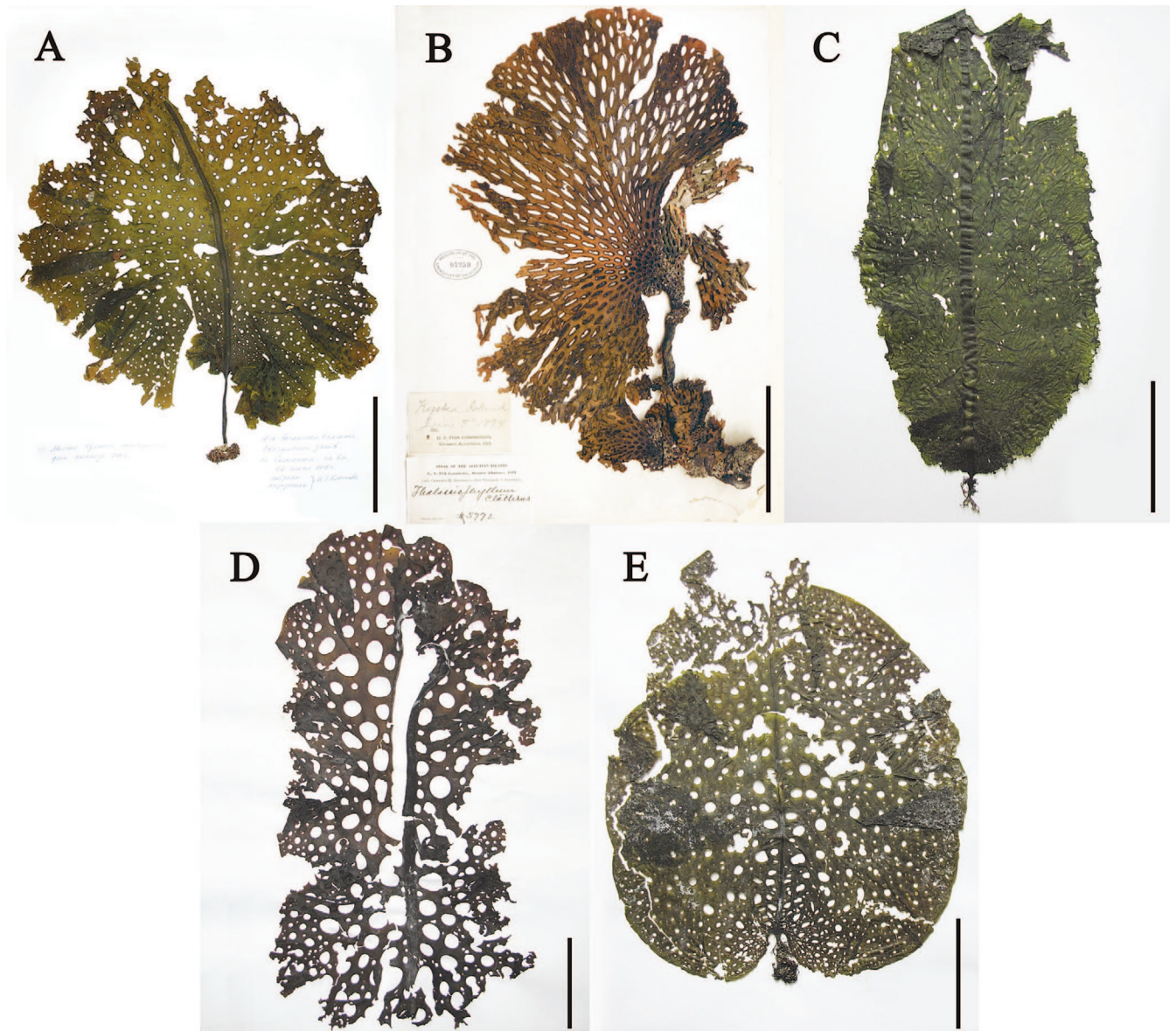


Fig. 2. Representative specimens of the genus *Agarum*. **A**, *Agarum clathratum*, neotype specimen, *N. Klochkova* 001 (LE); at the 6 m depth in Avacha Inlet, Kamchatka, July 26, 2009. **B**, *A. clathrus*, *C.H. Townsend* 5772 (UC 97259); Kiska Island, Alaska, U.S.A., June 8, 1894. **C**, *A. fimbriatum*, *H.G. Kim* 001 (in CNUK, Herbarium of Chungnam National University, Daejeon, Korea); subtidal, San Juan Island, Washington, U.S.A., July 13, 2009. **D**, *A. turneri*, *S.M. Boo* 000025 (in CNUK); Avacha Bay, Kamchatka, Russia, July 28, 1998. **E**, *A. clathratum* subsp. *yakishiriense*, *S.M. Boo* 000026 (in CNUK); subtidal in Sacheon, Gangreung, Korea, May 20, 2009. Scales, 10 cm.

whether the ITS, *cox1* or RuBisCO spacer was being compared (Lane & al., 2007).

Biogeography. — A distribution map of all species of *Agarum* is shown in Fig. 3. Two interesting biogeographic patterns emerge when considering the overall distribution of *Agarum* and its distinct lineages as revealed by phylogenetic analyses. The first is that *A. fimbriatum*, *A. oharaense*, and *A. clathratum* subsp. *yakishiriense* are distributed in more southerly regions than *A. clathratum* subsp. *clathratum*, *A. clathrus*, and *A. turneri*. The sister relationship between *A. fimbriatum* and *A. oharaense* suggests a sister relationship between the northeast and northwest Pacific coasts. *Agarum fimbriatum* occurs in depths of about 20 m from southeastern Alaska south through Puget Sound, Washington and in California from south of Point Conception, including the Channel Islands, to Isla Todos Santos, Baja California, Mexico. Further study is required to determine the genetic relationship between populations from these two isolated regions. All of our sequences were from northern populations. *Agarum oharaense* occurs only in depths of 17 to 50 m in Ohara, Chiba, on the Pacific coast of Japan (Yamada, 1962; Miyata & Yotsukura, 2005), where the Kuroshio Current flows near the coast, keeping the Chiba coast relatively warm in winter and cool in summer compared to temperatures in nearby Tokyo Bay.

Among the more northerly species, all three species overlap in their distributions. All samples of *Agarum clathratum* from Korea in this study belonged to subsp. *yakishiriense*. In Japan, this subspecies occurs on the west coast of Hokkaido. However, its distribution appears parapatric to *A. clathratum* subsp. *clathratum* because these two subspecies probably overlap on Sakhalin Island. *Agarum clathratum* subsp. *clathratum* occurs from Hokkaido, Japan, via Alaska to the west and east coasts of North America. *Agarum turneri* ranges from Kamchatka (Klochkova, 1998) to Greenland (Postels & Ruprecht, 1840), including Alaska (Lindeberg & Lindstrom 2010), and *A. clathrus* occurs from Kamchatka to Alaska (Setchell & Gardner 1925; Klochkova, 1998).

The second interesting biogeographic pattern is that *Agarum clathratum* subsp. *clathratum* is the only taxon of the genus with an interoceanic distribution from Japan via Alaska to the east coast of North America. However, the pair-wise divergences (up to 0.25% for ITS2 and 0.09% for *cox3*) between samples from Japan and Maine are much lower than those of other brown algae (e.g., 4.39%–5.70% for ITS2 and 8.66%–10.31% for *cox3* in *Scytosiphon lomentaria* (Lyngb.) Link; Kogame & al., 2005). Possible explanations for the observed low genetic divergence are a relatively recent long-distance dispersal, slow molecular evolution, and/or frequent gene flow among populations. Based on the occurrence of all six species of *Agarum* from Japan to Alaska, which represents the center of species diversity for the genus, we speculate that *A. clathratum* subsp. *clathratum* moved from the North Pacific to the North Atlantic Ocean. The species might have followed the northward flowing ocean current from the Pacific into the Arctic and thence into the North Atlantic; this current pattern developed after the closing of the Panama Isthmus ca. 3.5 Ma (Marincovich & Gladenkov 1999). This period corresponds to the rapid radiation of kelps in the North Pacific at the middle to late Miocene (3–15 Ma) (Coyer & al., 2001). The very low rate of molecular evolution of nuclear and mitochondrial genes within the species might also come from low selection pressure because *A. clathratum* is a deep-water species (often dominating at 10–30 m) and is chemically defended against sea urchin predation (Gagnon & al., 2005). However, frequent gene flow among populations over its distribution is probably rare, based on present current patterns and geographical distances.

CONCLUSION

Our study is the first to report on the molecular phylogeny of the genus *Agarum*. Of a total of 49 names including infraspecific taxa listed in AlgaeBase (Guiry & Guiry, 2010), only three species have been flagged as currently accepted:

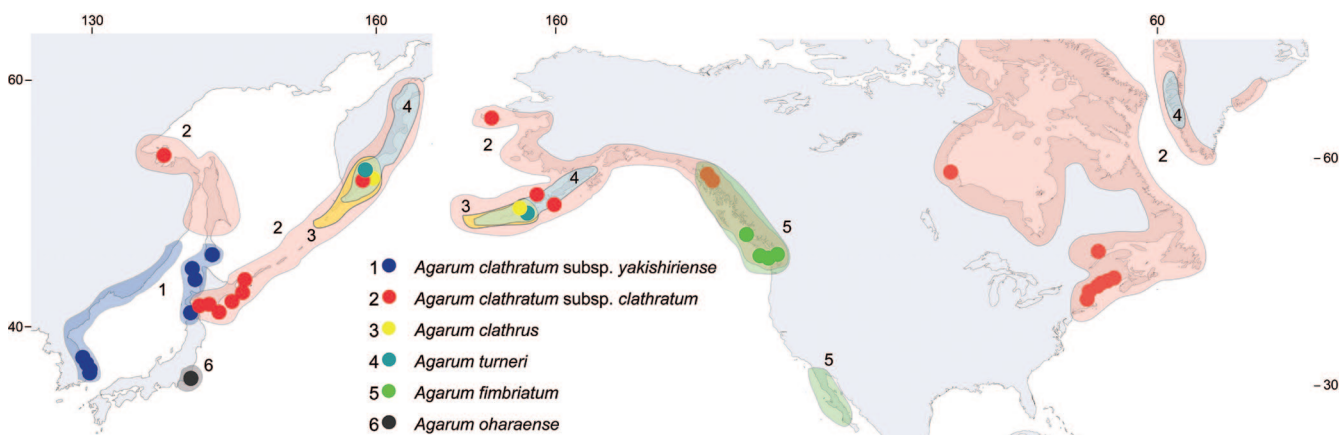


Fig. 3. Map showing current geographic distribution of the species of *Agarum* based on results in the present study and following publications: Taylor (1957), Yamada (1974), Lee (1980), South & Tittley (1986), Scagel & al. (1989), Lüning (1990), Klochkova & al. (2009), and Lindeberg & Lindstrom (2010). Closed circles indicate collection sites of samples used in the present study, including published sequences.

A. clathratum, *A. fimbriatum*, and *A. oharaense*. By sampling members of the genus *Agarum* across its distribution range and sequencing four molecular markers from three genomes, the concept of *Agarum* is extended to include *Thallassiophyllum*. We can now update this taxonomy to recognize five species and two subspecies within the genus *Agarum*: *A. clathratum* subsp. *clathratum*, *A. clathratum* subsp. *yakishiriense*, *A. clathrus*, *A. fimbriatum*, *A. oharaense*, and *A. turneri*.

■ TAXONOMIC TREATMENT

The formal synonymy of *Agarum* and *Thallassiophyllum* is presented below:

Agarum Dumort., *Comment. Bot.*: 102. 1822 (nom. cons.) – Type: *A. clathratum* Dumort. (≡ *Fucus agarum* S.G. Gmel., *Hist. Fuc.*: 210, pl. 32. 1768), Neotype (designated here): Avacha Inlet, Kamchatka, 26 Jul 2009, *N. Klochkova 001* (LE) (Fig. 2A).

= ***Agarum*** Bory, *Dict. Class. Hist. Nat.* 9: 193. 1826, nom. illeg. – Type: *A. cribrosum* Bory (≡ *Fucus agarum* S.G. Gmel.).

= ***Thallassiophyllum*** Postels & Rupr., *III. Alg.*: 11. 1840 – Type: *T. clathrus* (S.G. Gmel.) Postels & Rupr. (≡ *Fucus clathrus* S.G. Gmel., *Hist. Fuc.*: 211, pl. 33. 1768), no type specimen has been designated (it is presumed to be lost; the material on which Postels & Ruprecht based their genus—Mertens, *Oceano. Pacific. Sept.*, Hb. Chamisso in LE—can be seen at <http://botany.ubc.ca/sandral/Fucus-cla.jpg>) ≡ *Ulva clathrus* (S.G. Gmel.) Woodw., *Trans. Linn. Soc.* 3: 50. 1797 ≡ *Laminaria clathrus* (S.G. Gmel.) C. Agardh, *Syst. Alg.*: 269. 1824 ≡ *Agarum clathrus* (S.G. Gmel.) Grev., *Alg. Brit.*: xxxix. 1830.

Because of the lack of a Latin diagnosis, neither “*A. yakishiriense* Yamada” (1962) nor “*A. cribrosum* f. *yakishiriense* I. Yamada” (1974) is a validly published name (*ICBN Art.* 39, McNeill & al., 2006). We rectify the omission below and recognize this taxon at the subspecific rank:

Agarum clathratum subsp. ***yakishiriense*** Yamada ex G.H. Boo & P.C. Silva, **subsp. nov.** (“*A. yakishiriense* Yamada” in *Acta Phytotax. Geobot.* 20: 279, fig. 3. 1962, non rite publ.; “*A. cribrosum* f. *yakishiriense* (Yamada) I. Yamada” in *J. Fac. Sci. Hokkaido Univ.* 10: 40. 1974, non rite publ.).

Stipes maxime complanatus, haptera rhizoidalia tenuia, copiose e omni parte stipites exorientes. Lamina aspera marginis foraminis elevati.

Holotype: *Y. Yamada*, SAP No. 028605, Yagishiri Island, Japan, Aug. 1960.

Validating illustration: Yamada, 1974: pl. 4.

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