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CLASSIFICATION OF THE GENUS *ISHIGE* (ISHIGEALES, PHAEOPHYCEAE) IN THE NORTH PACIFIC OCEAN WITH RECOGNITION OF *ISHIGE FOLIACEA* BASED ON PLASTID *rbc*L AND MITOCHONDRIAL *Cox*3 GENE SEQUENCES[1]

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The taxonomy and biogeography of a genus with species that occur in geographically isolated regions is interesting. The brown algal genus *Ishige* Yendo is a good example, with species that apparently inhabit warm regions of both the northwestern and northeastern Pacific Ocean. We determined the sequences of mitochondrial *cox*3 and plastid *rbc*L genes from specimens of the genus collected over its distributional range. Analyses of the 86 *cox*3 and 97 *rbc*L sequences resulted in congruent trees in which *Ishige sinicola* (Setch. et N. L. Gardner) Chihara consisted of two distinct clades: one comprising samples from Korea and Japan, and the other comprising samples from the Gulf of California. Additional observations of the morphology and anatomy of the specimens agree with the molecular data. On the basis of results, we reinstated *Ishige foliacea* S. Okamura (considered a synonym of *I. sinicola* from the Gulf of California) for plants from the northwest Pacific region and designated a specimen in the Yendo Herbarium (SAP) as the lectotype. *I. foliacea* is distinguished by large (up to 20 cm) and wide (up to 20 mm) thalli, with a cortex of 4–7 cells, and a medulla composed of long, tangled hyphal cells. Both *cox*3 and *rbc*L sequence data strongly support the sister-area relationship between the northwest Pacific region and the Gulf of California. A likely explanation for this pattern would be the presence of a species ancestral to contemporary species of *Ishige* in both regions during the paleogeological period, with descendants later isolated by distance.

Key index words: **amphi-Pacific distribution; biogeography; *cox*3; *I. foliacea*; *I. sinicola*; *Ishige*; Phaeophyceae; *rbc*L; taxonomy**

Abbreviations: **BPP, Bayesian posterior probability; ECC, equatorial counter current; ML, maximum likelihood; MP, maximum parsimony**

The North Pacific Ocean is an ideal place to study the taxonomy and distribution of marine organisms, because it is very large and has a high diversity of species. Since the northwestern region is geographically distinct from the northeast region (Spalding et al. 2007), the occurrence of shared species on both sides presents an interesting question, that is, whether these species are in fact different species, the product of either oceanic dispersal or vicariance. According to Hommersand (1972), warm-temperate species from Japan migrated eastward along the Kuroshio Current toward Vancouver Island, Canada, and then to Ecuador and Peru. With the retreat of the glaciers, some of these species became relics in those areas. Although ~20% of North Pacific seaweeds have amphi-Pacific distributions (Lüning 1990), few studies have explored the taxonomy and distribution of marine algae between Korea and the Gulf of California.

Ishige is a brown algal genus that currently contains two species. Yendo (1907) described *Ishige* based on *Ishige okamurae*, which was described from two different forms (filiform and foliose types) collected on the Pacific coast of Japan. In Yendo's opinion, the foliose morphotype was an abnormally branched variant of the filiform morphotype, *I. okamurae,* because it commonly occurs as an epiphyte on the latter. Okamura (in Segawa 1935), however, described *I. foliacea* as a separate species, based on the foliose morphotype. Dawson (1944) reported the occurrence of *I. foliacea* in the Gulf of California and also mentioned that the species is

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probably the same as *Polyopes sinicola* Setch. et N. L. Gardner (1924), which was described as a red alga. Chihara (1969) made the new combination *I. sinicola* (Setch. et N. L. Gardner) Chihara and synonymized *I. foliacea* with *I. sinicola*. *I. sinicola* occurs in Korea, Japan, and China (Kang 1966, Tseng 1983, Yoshida 1998), and in the Gulf of California (Setchell and Gardner 1924, Pacheco-Ruíz et al. 2008). Despite its occurrence in both Korea/Japan/China and the Gulf of California, regions separated by a distance of ~9,000 km, no modern comparative study on the morphology and anatomy of *I. sinicola* has been conducted.

Recent morphologic and molecular studies on the genus (Lee et al. 2003, Cho et al. 2004) have contributed significantly to its taxonomy and phylogeny, establishing the new order Ishigeales (Cho et al. 2004). However, the extraordinarily high pairwise divergence within *Ishige* (Cho et al. 2004) led us to further work. The *cox*3 is a mitochondrial protein-encoding gene, the utility of which has been recently tested for three brown algae: *Scytosiphon lomentaria* (Kogame et al. 2005), *Sargassum filicinum* (Miller et al. 2007), and *Undaria pinnatifida* (Uwai et al. 2006). The plastid *rbc*L gene is frequently used for elucidation of the taxonomy and phylogeny of many brown algae (e.g., Cho et al. 2004).

The purpose of this study was to reinvestigate species limits in the genus *Ishige* by obtaining a more detailed picture of their phylogenetic and biogeographic relationships, and the origin of their current geographic distributions. Accordingly, we collected samples of *Ishige* over its distributional range and analyzed two molecular markers from different genomes: *cox*3 and *rbc*L. These results, together with additional morphological observations, demonstrate that *I. foliacea* is a species distinct from *I. sinicola*.

MATERIALS AND METHODS

Taxon sampling for molecular analysis. We obtained samples of *Ishige* across its distributional range: Korea, Japan, Taiwan, and the Gulf of California. Information on collection sites for samples used in this study is given in Table S1 (in the supplementary material). Ninety-seven specimens were collected in the field, air-dried, and preserved with silica gel. All vouchers are deposited in the herbarium of the Department of Biology, Chungnam National University, Daejeon, Korea (CNUK), and Herbario Ficológico from Universidad Autónoma de Baja California Sur (FBCS).

Sequencing and phylogenetic analysis. Genomic DNA was extracted from ~5 mg of dried thalli ground in liquid nitrogen with an Invisorb® Spin Plant Mini Kit (Invitek, Berlin-Buch, Germany), according to the manufacturer's instructions. PCR was performed using primers specific for each gene. The mitochondrial *cox*3 region was amplified and sequenced using the primers F21 and R745 (Table 1). The plastid *rbc*L region was amplified using the primers F18–R884 and F630–R1426 and sequenced using the same primers (Table 1). PCR amplification was performed on a total volume of 25 μL, containing 0.2 U of *TaKaRa Ex Taq*™ DNA polymerase (Takara Shuzo, Shiga, Japan), 1.5 mM of each dNTP, 2.5 μL of the 10X *Ex Taq*™ Buffer (Mg^{2+} free), 3 mM $MgCl_2$, 10 pmol of each primer, and 3 ng of template DNA. PCR was carried out with an initial denaturation at 95°C for 4 min, followed by 35 cycles of amplification (95°C for 30 s, 50°C for 30 s, and 72°C for 1 min) with a final extension at 72°C for 6 min. The PCR products were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany). Sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM™ 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The electropherogram output for each specimen was edited using the program Sequence Navigator® v. 1.0.1 (Applied Biosystems). All *cox*3 and *rbc*L sequences were collated using the multisequence editing program SeqPup (Gilbert 1995) and visually aligned. *Dictyota dichotoma* and *Desmarestia viridis* were used as outgroups.

Table 1. Oligonucleotide primers used for amplification of *cox3* and *rbc*L regions (K = G or T; M = A or C; R = A or G; S = C or G; W = A or T; Y = C or T).

Gene	Primer	Direction	Sequences
*cox*3	F21	Forward	5′-TAA TCA AAA ACA YMS TTT TCA TTT AG-3′
	R745	Reverse	5′-CKA CAA AAT GCC AAT ACC AAG C-3′
*rbc*L	F18	Forward	5′-AGA ACG KAC TCG AAT TAA AAG TGA-3′
	F630	Forward	5′-ACT CWC AAC CAT TCA TGC GTT-3′
	R884	Reverse	5′-GAR TTA CCW GCA CGR TGT AAA TG-3′
	R1426	Reverse	5′-GAA ATC AGG WGT ATC CGT AGA-3′

Maximum-parsimony (MP) analyses of the *cox*3 and *rbc*L data were conducted using the PAUP* 4.0b10 program (Swofford 2002). All heuristic searches were performed with 1,000 replicates, employed the random addition of taxa, retained only the best tree, held 10 trees at each step, using tree bisection-reconnection (TBR) branch swapping, collapsed zero-length branches, and using MULTREES. Bootstrap support values were calculated using 1,000 replicates with the following options selected: heuristic search, TBR branch swapping, collapse of zero-length branches, and random-sequence-addition with one replicate.

The maximum-likelihood (ML) phylogenetic analyses were performed using the RAxML program (Stamatakis 2006) with the GTR + Γ + I model. We used 200 independent tree inferences using – # option with default – I (automatically optimized SPR rearrangement) and – c (25 distinct rate categories) options of the program to identify the best tree. Bootstrap values were calculated using 1,000 replicates using the same substitution model.

Bayesian analyses were conducted with MrBayes v.3.1 (Ronquist and Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo (MC^3) with the GTR + Γ + I model for combined and individual data sets. For each matrix, one million generations of two independent runs were performed with four chains, and trees were sampled 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 stationarity were used to infer the Bayesian posterior probability (BPP). Majority-rule consensus trees were calculated using PAUP*.

Morphology of Ishige. Representative specimens of *I. foliacea* and *I. sinicola* were collected in the intertidal zone (Table S1). Tissues were sectioned using a freezing microtome FX–802A

(Coper Electronics Co. Ltd., Kanagawa, Japan), and the sectioned preparations were stained with 1% aqueous aniline blue acidified with a drop of 1% HCl and mounted in 30% glycerin–seawater. Photographs were taken using an FX–35DX camera (Nikon, Tokyo, Japan) attached to a microscope (Vanox AHBT3; Olympus, Tokyo, Japan).

RESULTS

Mitochondrial cox*3 and* rbc*L data.* A total of 611 base pairs (bp) in *cox*3 were aligned for 86 specimens (Table 2). A total of 317 positions were variable (51.9%) and 216 positions (35.4%) were parsimoniously informative (Table 2). *I. foliacea* differed by 111–118 bp (18.2%–19.3%) from *I. sinicola* and by 120–131 bp (19.6%–21.4%) from *I. okamurae*. Between *I. okamurae* and *I. sinicola*, the difference was 86–95 bp (14.0%–15.5%).

The rbc*L alignment.* The sequences determined for the *rbc*L region totaled 1,301 bp from 97 specimens. Of these, 288 bp (22.1%) were variable and 126 (9.7%) were parsimoniously informative (Table 2). *I. foliacea* differed by 83–87 bp (6.4%–6.7%) from *I. sinicola* and by 91–95 bp (7%–7.3%) from *I. okamurae*. Between *I. okamurae* and *I. sinicola,* the difference was 74–78 bp (5.7%–6.0%).

Phylogeny of Ishige. The independent analyses of *cox*3, *rbc*L, and *cox*3 + *rbc*L data sets resulted in congruent phylogenetic reconstructions (Fig. 1). The statistics for the MP analyses were compared among individuals and combined data sets (Table 2). All specimens of *I. foliacea* from Korea and Japan produced a clade (Fig. 1). *I. sinicola* from two locations in Mexico was monophyletic, strongly supported by high bootstrap values. Specimens of *I. okamurae* from Korea, Japan, Taiwan, and Hong Kong produced a strongly supported clade. However, *I. okamurae* was sister to *I. sinicola*, both having *I. foliacea* basal to that clade.

Comparison of I. foliacea *and* I. sinicola. Thalli of *I. foliacea* (Fig. 2) are epilithic or epiphytic on *I. okamurae*, dark brown and almost black on drying. Thalli are dichotomously branched and grow up to 20 cm high, up to 20 mm wide, with width diminishing from the center of the thallus toward apices at each forking. Cortical and medullary layers are haplostichous. Cortical layers are composed of cylindrical cells, 4–7 cells thick, with the terminal cell of each row, or the surface cells, more or less pear shaped. The medulla is composed of very densely compact, long, and tangled filaments merging abruptly on all sides into rows. Phaeophycean hairs are common on the surface of the thallus, colorless, arising from the base of cortical layer, protruding from cryptostomata. Plurilocular sporangia are in uniseriate rows forming sori, less pigmented than vegetative cells. Unilocular sporangia are sausage shaped to elliptical.

In *I. sinicola* (Fig. 3), thalli are complanate, up to 9 cm high, up to 6 mm wide, width diminishing from the center of the frond toward apices at each forking. They are dichotomously branched, brown in color, almost black on drying. Cortical layers are composed of cylindrical cells, 10–13 cells thick, with the terminal cell of each row, or the surface cells, more or less pear shaped. The medulla is composed of very densely compact and intertwined branched filaments merging abruptly on all sides into rows. Phaeophycean hairs are rare, colorless, arising from the base of cortical layer in cryptostomata, deciduous. Thalli with reproductive organs were not collected in the present study.

DISCUSSION

Reinstatement of I. foliacea. Through mitochondrial *cox*3 and plastid *rbc*L gene analyses and morphologic observations, we demonstrated marked differences between *I. foliacea* and *I. sinicola*. Morphologically, *I. foliacea* is distinguished by large (up to 20 cm) and wide (up to 20 mm) thalli, cortical layer of 4–7 cells long, and medulla with long, tangled hyphal cells, and abundant cryptostomata, with protruding hairs (Yendo 1907, Lee et al. 2003, Cho et al. 2004), as seen in the present study (Table 3). The species commonly occurs from spring to summer in the intertidal zone, often inhabits sheltered sites, and is epiphytic on *I. okamurae* or epilithic in the intertidal area in Korea, Japan, and China (Tseng 1983). *I. sinicola* is characterized by small

TABLE 2. Nucleotide composition of the *cox*3 and *rbc*L and statistics from MP analyses of the data sets including outgroups.

	*cox*3	*rbc*L	*cox*3 + *rbc*L
Number of taxa	120	98	86
Length (bp)	611	1,301	1,912
Base frequency (C/G)	0.16850/0.19580	0.17190/0.23004	0.17145/0.21777
Number of variable sites (%)	317 (51.9)	288 (22.1)	600 (31.4)
Number of informative sites (%)	216 (35.4)	126 (9.7)	381 (19.9)
Number of MP trees	72	6	51
MP tree length	507	353	882
Consistency index (CI)	0.866	0.941	0.868
Retention index (RI)	0.913	0.951	0.975

MP, maximum parsimony.

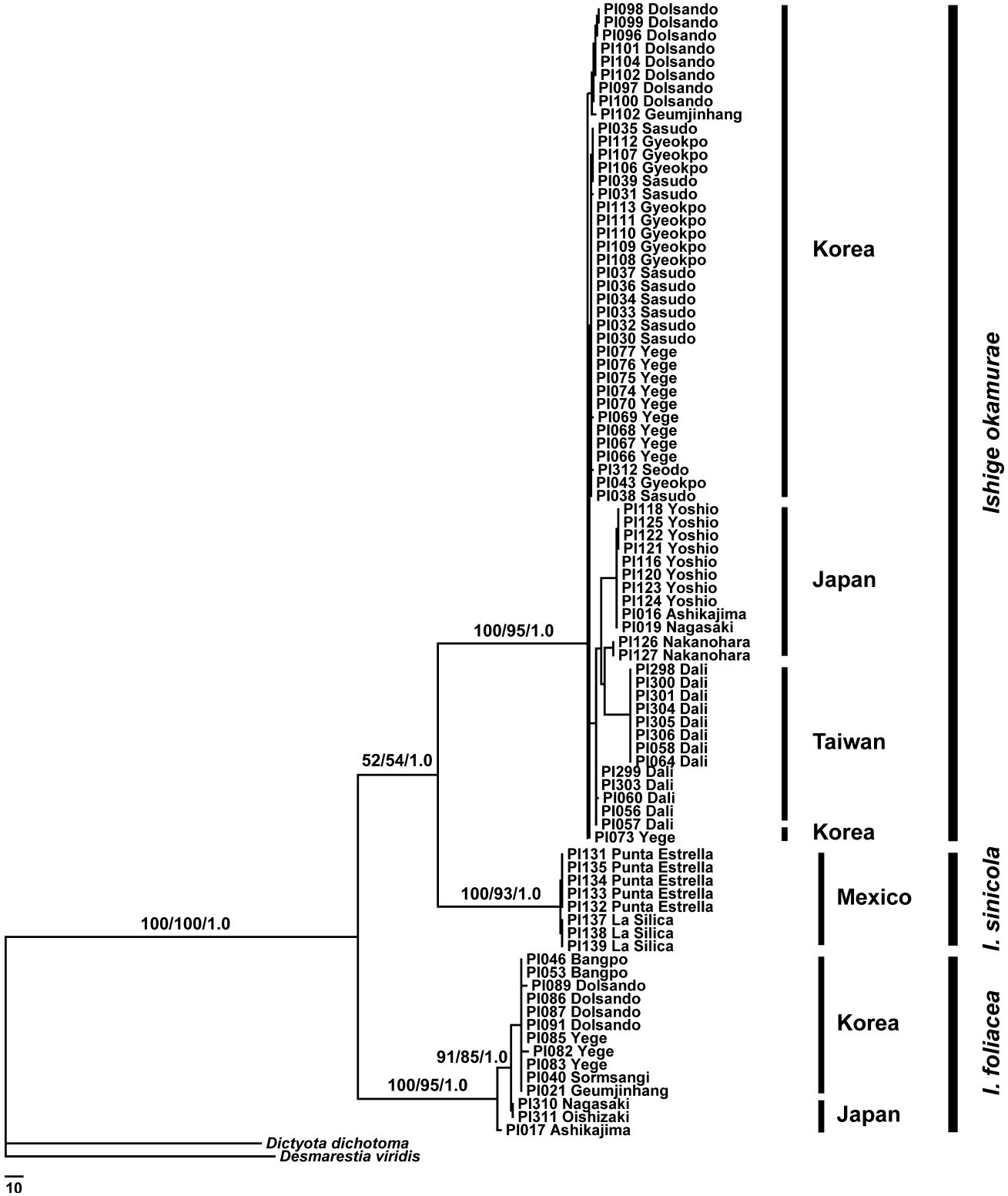

FIG. 1. One of the most parsimonious trees from *cox*3 + *rbc*L combined data of *Ishige*. The bootstrap values shown above the branches are from 1,000 bootstrap resamplings with maximum parsimony, 1,000 bootstrap resamplings with RAxML, and Bayesian inference.

(up to 9 cm) and narrow (up to 6 mm) thalli, cortical layer of 10–13 cells, medulla with short and tangled hyphal cells, and rare cryptostomata, with deciduous hairs (Table 3). Thalli are abundant throughout the year (Dawson 1944). Dawson's report (1944, p. 235) that zoosporangia of *I. sinicola*

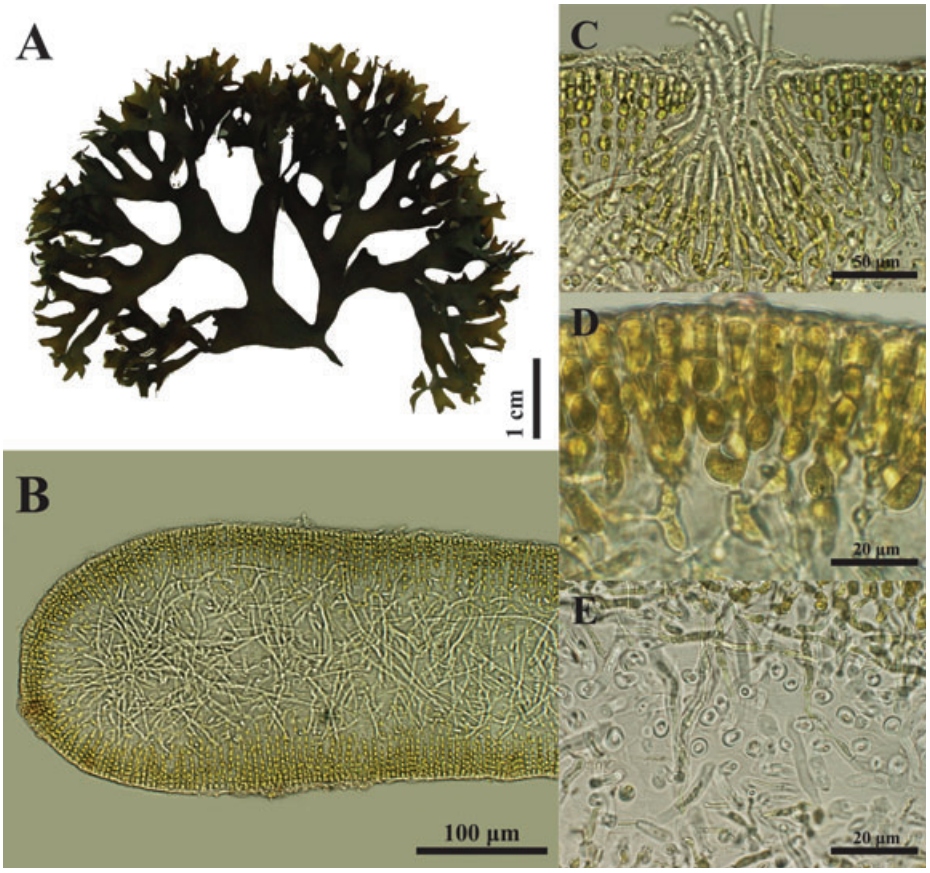

FIG. 2. *Ishige foliacea.* (A) Habit of *I. foliacea,* (B) transverse section of the upper portion of thallus, (C) phaeophycean hairs, (D) cortical cells, and (E) medullary cells.

are very similar to those of *I. foliacea*, as figured by Okamura (1936, fig. 131), is probably based on observations of unilocular sporangia. However, we did not observe sporangia in our collections of *I. sinicola* from Baja California. *I. sinicola* is solely epilithic (not epiphytic) (Setchell and Gardner 1924, this study) and is restricted to the Gulf of California and its surrounding regions (Setchell and Gardner 1924, Dawson 1944, Pacheco-Ruíz et al. 2008).

ML, MP, and Bayesian analyses of both the *cox*3 and *rbc*L sequences analyzed from our collections of the genus produced congruent trees. Each of three species in the genus formed a monophyletic clade with a maximum support of bootstrap and probability (Fig. 1). We found it intriguing that *I. sinicola* was consistently sister to *I. okamurae,* both with *I. foliacea* as the basal taxon. *I. foliacea* is clearly separated from *I. sinicola* in all trees including specimens collected near the type locality of *I. foliacea* and *I. sinicola*: Miura, Kanagawa Prefecture, Japan, for *I. foliacea* (Yendo 1907) and Isla Partida, Gulf of California, for *I. sinicola* (Setchell and Gardner 1924). The results agree well with the morphologic reexamination of both species, as summarized in Table 3. Our molecular and morphologic study does not support the current classification of *I. sinicola,* which includes *I. foliacea* as a synonym (Chihara 1969, Yoshida 1998, Lee et al. 2003, Cho et al. 2004). Thus, we conclude that, based on morphology, *rbc*L and *cox*3 sequences, ecology, and distribution, *I. foliaceae* is different from *I. sinicola* and should be recognized as a distinct species. Although the description and type of *I. sinicola* is well documented by Setchell and Gardner (1924), the type of *I. foliacea* has not been designated elsewhere.

Okamura in Segawa (1935) described *I. foliacea* as the flabellate form of *I. okamurae* Yendo. Both species are described as forms of *I. okamurae* in Yendo's protologue (Yendo 1907). As neither Yendo (1907) nor Okamura in Segawa (1935) designated a type for *I. foliacea* (*I. okamurae* was lectotypified by Lee et al. 2003), we propose the following lectotypification. For *I. foliacea*, we designate the specimen with the annotation '' Fucaceae Pl. II. fig. 1.'' below the label, housed in the Yendo Herbarium in SAP, Hokkaido University, Japan (Fig. 4). According to Kazuhiro Kogame (pers. comm.), the annotation was written by Dr. T. Yoshida ~20 years ago, indicating that he recognized the specimen as the basis for figure 1, plate II in Yendo (1907). The current name of the collection site on the original Yendo label is Jogashima, Miura, Kanagawa Pref., Japan.

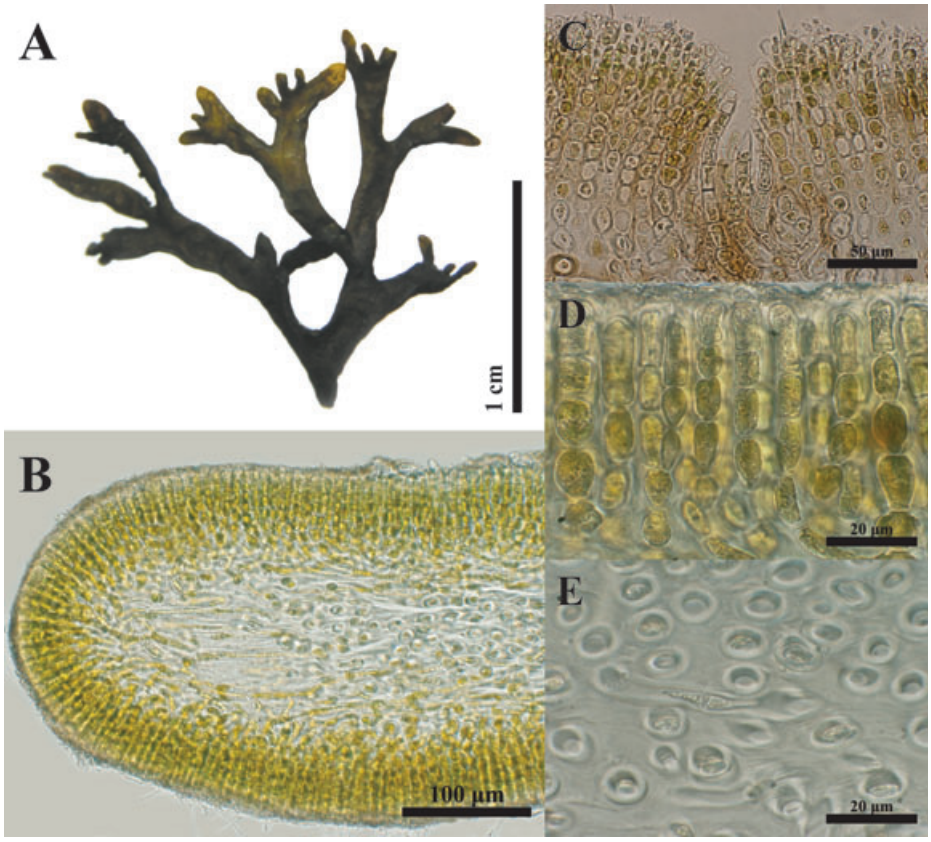

FIG. 3. *Ishige sinicola.* (A) Habit of *I. sinicola,* (B) transverse section of the upper portion of thallus, (C) phaeophycean hairs, (D) cortical cells, and (E) medullary cells.

TABLE 3. Comparative morphology of *Ishige foliacea* and *I. sinicola.*

	I. foliacea	*I. sinicola*
Length	Up to 20 cm	Up to 9 cm
Width	Up to 20 mm	Up to 6 mm
Cortex	Anticlinally arranged cylindrical cells, 4–7 cells thick	Anticlinally arranged cylindrical cells, 10–13 cells thick
Medulla	Tangled to longitudinally arranged, long hyphal cells	Tangled to longitudinally arranged, short hyphal cells
Hair	Frequent, derived from medullary layers, many filaments of hair cells protruded from cryptostomata	Rare, derived from cells between medullar layer, a few filaments of hair cells fallen off from cryptostomata
Plurilocular sporangia	Uniseriate row	Unknown
Unilocular sporangia	Terminally positioned on cortical filaments	Terminally positioned on cortical filaments
Habitat	Epilithic or epiphytic on *I. okamurae*	Epilithic
Distribution	Korea, Japan, China	Gulf of California
References	Okamura 1936, Lee et al. 2003, Cho et al. 2004, this study	Setchell and Gardner 1924, Dawson 1944, this study

Ishige foliacea Okamura in Segawa 1935: 66. Lectotype (designated here, Fig. 4): Without collection number in Yendo Herbarium, probably agreeing with figure 1, plate II in Yendo (1907); collected in Jogashima, Miura, Kanagawa Pref., Japan, in April 1900; Yendo Herbarium in TI (Herbarium, University Museum, University of Tokyo, Tokyo, Japan) on permanent loan to SAP (Herbarium, Hokkaido University, Sapporo, Japan).

Phylogeography of Ishige. Both *rbc*L and *cox*3 sequence data demonstrate the amphi-Pacific distribution of *Ishige*: *I. okamurae* and *I. foliacea* occur in the northwest Pacific region, while *I. sinicola* occurs in the Gulf of California. Our results do not support the existence of *I. sinicola* in Asia, as previously reported by Dawson (1944), Chihara (1969), and Hommersand (1972), but confirm that the distribution of each species is restricted to a single

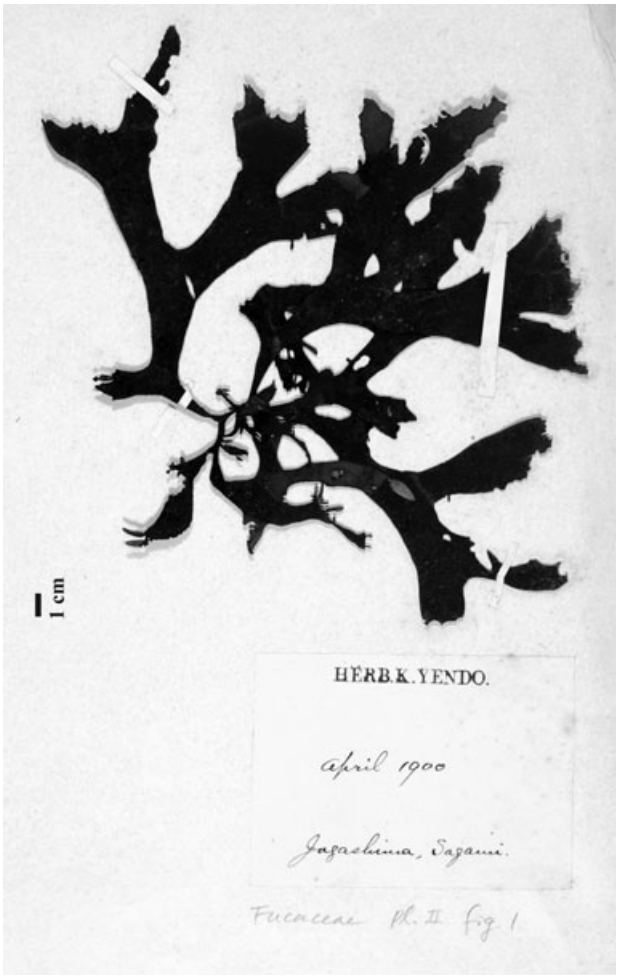

FIG. 4. Lectotype of *Ishige foliacea* collected in Jogashima, Miura, Kanagawa Pref., Japan, in April 1900. Deposited in Yendo Herbarium in TI (Herbarium, University Museum, University of Tokyo, Tokyo) on permanent loan to SAP (Herbarium, Hokkaido University, Sapporo), Japan.

geographic region. Hommersand (1972) suggests a dispersal scenario in which eastern Pacific species are evolutionary relics after their dispersal along the Kuroshio current from Japan to Canada and thence south to Ecuador before the Pleistocene. An alternate agent of dispersal is the Equatorial Counter Current (ECC), which starts near the northern Philippines and flows eastward between ~3° and 10° N. The occurrence of the genus in Hong Kong, Taiwan, and the Gulf of California suggests that the ancestor of *I. sinicola* may have dispersed eastward along the ECC.

On the other hand, the tectonic vicariance hypothesis appears to be a better fit than the dispersal hypothesis for interpreting the present-day distribution of *Ishige*, because each of the three species is distributed in a very limited region. Pairwise divergences within *Ishige* in the present study were very high both in *cox*3 (14.0%–21.4%) and *rbc*L (5.7%–7.3%). These values are consistent with those published by Cho et al. 2004 (6.9%–7.1% for *rbc*L, 8.3%–8.7% for *psa*A, and 6.4%–6.9% for *psb*A). Values for *Ishige* are much higher than those for *Lessonia* (2.7%–2.9% for *rbc*L, 3.5%–3.9% for *psa*A, and 0.8%–1.4% *psb*A; calculated from data of Cho et al. 2006a) and similar to those other brown algae at the family level (e.g., 5.8%–9.6% for *psa*A in the Fucales; Cho et al. 2006b). That *Ishige* occupied a basal position in a brown algal phylogeny (Cho et al. 2004) is consistent with data from studies with a wide taxon sampling (Phillips et al. 2008) and with different markers (Bittner et al. 2008). We therefore conclude that the extraordinarily high divergence of *Ishige* and its basal position in the brown algal tree result from a very long evolutionary history.

The first brown algae are considered to have occurred in the Paleozoic period (Clayton 1984), the Jurassic, which roughly corresponds to 155 mya, based on rDNA SSU sequence divergence (Medlin et al. 1997), or the early Mesozoic (~200 mya), based on 5S rRNA sequences (Lim et al. 1986). During the Paleozoic to Mesozoic, the Pacific Ocean may have been warm to tropical, probably similar to temperatures in the present-day ranges of species in *Ishige*. Our interpretation is consistent with the trans-Pacific zipper theory (see fig. 3 in McCarthy 2003) that suggests that the young age of the ocean crust (<200 mya), the matching Mesozoic circum-Pacific outlines, and a corresponding system of interlocking biogeographical sister areas provide support for a closed Pacific in the Upper Triassic–Lower Jurassic. We hypothesize that the ancestor of *Ishige* was once widely distributed during the Mesozoic period in the northern Pacific Ocean, and following the isolation of the American continent, this ancestor diverged into different species. We also hypothesize that *I. foliacea* may be a descendent of this ancestor because of its basal position relative to *I. sinicola* and *I. okamurae* in our phylogenic trees (Fig. 1).

In conclusion, the genus *Ishige* provides a unique opportunity to study the taxonomy and distribution of marine algae in which species are geographically disjunct. In this study, trees of both *rbc*L and *cox*3 sequences are congruent. Analysis of *cox*3 in brown algae is in its infancy, but it has proved valuable for identification of species and genetic variation within species (Kogame et al. 2005, Uwai et al. 2006, Miller et al. 2007). Historically, the genus *Ishige* comprised two species with amphi-Pacific distributions. We can now update this concept to recognize three species within this genus (*I. foliacea, I. okamurae*, and *I. sinicola*), each with a range restricted to the eastern or western Pacific. Our study is also significant because several patents for pharmaceutical and industrial substances have been published in Korea using the name of *I. sinicola*. We now know that this name is incorrect and should be *I. foliacea*.

Because *Ishige* is considered one of the most basal genera in the phylogeny of the Phaeophyceae, the discovery of the diversity of this genus in the north Pacific Ocean could be an important clue to evolutionary divergence of brown algae in this very old

ocean. Additional studies of species that straddle the Pacific Ocean (see examples in Hommersand 1972 and Santelices 1992) will provide important insights into the phylogeography of Pacific marine algae.

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Bittner, L., Payri, C. E., Couloux, A., Cruaud, C., de Reviers, B. & Rousseau, F. 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* 49:211–26.

Chihara, M. 1969. *Pseudogloiophloea okamurai* (Setchell) comb. nov. and *Ishige sinicola* (Setchell and Gardner) comb. nov. *Bull. Jpn. Soc. Phycol.* 17:1–4.

Cho, G. Y., Klochkova, N., Knupnova, N. & Boo, S. M. 2006a. The reclassification of *Lessonia laminarioides* (Laminariales, Phaeophyceae): *Pseudolessonia* gen. nov. *J. Phycol.* 42:1289.

Cho, G. Y., Lee, S. H. & Boo, S. M. 2004. A new brown algal order, Ishigeales (Phaeophyceae), established on the basis of plastid protein-coding *rbc*L, *psa*A, and *psb*A region comparisons. *J. Phycol.* 40:921–36.

Cho, G. Y., Rousseau, F., de Reviers, B. & Boo, S. M. 2006b. Phylogenetic relationships within the Fucales (Phaeophyceae) assessed by photosystem I coding *psa*A sequences. *Phycologia* 45:512–9.

Clayton, M. N. 1984. Evolution of the Phaeophyta with particular reference to the Fucales. *Prog. Phycol. Res.* 3:11–46.

Dawson, E. Y. 1944. The marine algae of the Gulf of California. *Allan Hancock Pac. Expeditions* 3:189–432.

Gilbert, D. G. 1995. *SeqPup, A Biological Sequence Editor and Analysis Program for Macintosh Computers.* Biology Department, Indiana University, Bloomington.

Hommersand, M. N. 1972. Taxonomic and phytogeographic relationship of warm and temperate marine algae occurring in Pacific North America and Japan. *Int. Seaweeds Symp.* 7:23–45.

Kang, J. W. 1966. On the geographical distribution of marine algae in Korea. *Bull. Pusan Fish. Coll.* 7:1–125.

Kogame, K., Uwai, S., Shimada, S. & Masuda, M. 2005. A study of sexual and asexual populations of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) in Hokkaido, northern Japan, using molecular markers. *Eur. J. Phycol.* 40:313–22.

Lee, E. Y., Lee, I. K. & Choi, H. G. 2003. Morphology and nuclear small-subunit rDNA sequences of *Ishige* (Ishigeaceae, Phaeophyceae) and its phylogenetic relationship among selected brown algal orders. *Bot. Mar.* 46:193–201.

Lim, B. L., Kawai, H., Hori, H. & Ozawa, S. 1986. Molecular evolution of 5S ribosomal RNA from red and brown algae. *Jpn. J. Genet.* 61:169–76.

Lüning, K. 1990. *Seaweeds. Their Environment, Biogeography, and Ecophysiology.* John Wiley & Sons, New York City, New York, 527 pp.

McCarthy, D. 2003. The trans-Pacific zipper effect: disjunct sister taxa and matching geological outlines that link the Pacific margins. *J. Biogeogr.* 30:1545–61.

Medlin, L. K., Kooistra, W. H. C. F., Potter, D., Saunders, G. W. & Andersen, R. A. 1997. Phylogenetic relationships of the 'golden algae' (haptophytes, heterokont chromophytes) and their plastids. *Plant. Syst. Evol.* 11(Suppl.):187–216.

Miller, K. A., Engle, J. M., Uwai, S. & Kawai, H. 2007. First report of the Asiatic seaweed *Sargassum filicinum* Harvey (Fucales) in California, USA. *Biol. Invasions* 9:609–13.

Okamura, K. 1936. *Nippon Kaiso Shi.* Uchida-Rokakuho, Tokyo, 964 pp.

Pacheco-Ruíz, I., Zertuche-González, J. A., Espinoza-Ávalos, J., Riosmena-Rodríguez, R., Galindo-Bect, L., Gálvez-Télles, A., Meling-López, A. E. & Orduña-Rojas, J. 2008. Chapter 7: Macroalgas. *In* Danneman, G. D. & Ezcurra, E. [Eds.] *Bahía de Los Ángeles: Recursos naturales y comunidad, Tercera parte: Aspectos bioecológicos, Línea base 2007*.* INE, Semarnat, Pronatura Noroeste, San Diego Natural History Museum, Instituto Nacional de Ecologia, Mexico D.F., pp. 181–214.

Phillips, N., Burrowes, R., Rousseau, F., de Reviers, B. & Saunders, G. W. 2008. Resolving evolutionary relationships among the brown algae using chloroplast and nuclear genes. *J. Phycol.* 44:394–405.

Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–4.

Santelices, B. 1992. Marine phytogeography of the Juan Fernandez Archipelago: a new assessment. *Pac. Sci.* 46:438–52.

Segawa, S. 1935. On the marine algae of Susaki, Prov. Idzu and its vicinity. *Sci. Pap. Inst. Alg. Res. Fac. Sci. Hokkaido Univ.* 1:59–90.

Setchell, W. A. & Gardner, N. L. 1924. New marine algae from the Gulf of California. *Proc. Calif. Acad. Sci.* 12:695–949.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdana, Z. A., Finlayson, M., Halpern, B. S., et al. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57:573–83.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–90.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods).* Version 4.0b8. Sinauer, Sunderland, Massachusetts.

Tseng, C. K. 1983. *Common Seaweeds of China.* Science Press, Beijing, 316 pp.

Uwai, S., Nelson, W. A., Neill, K., Wang, W. D., Aguilar-Rosas, L. E., Boo, S. M., Kitayama, T. & Kawai, H. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondrial genes – origins and succession of introduced populations. *Phycologia* 45:687–95.

Yendo, K. 1907. The Fucaceae of Japan. *J. Coll. Sci. Tokyo Univ.* 21:1–174.

Yoshida, T. 1998. *Marine Algae of Japan.* Uchida Rokakuho, Tokyo, 1222 pp.

Supplementary Material

The following supplementary material is available for this article:

Table S1. Material used in the present study. Numbers in parentheses indicate number of specimens.

This material is available as part of the online article.

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