

THE RECLASSIFICATION OF *LESSONIA LAMINARIOIDES* (LAMINARIALES, PHAEOPHYCEAE): *PSEUDOLESSONIA* GEN. NOV.¹

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A new genus, *Pseudolessonia*, is proposed for the kelp *Lessonia laminarioides* Postels et Ruprecht (Laminariales, Phaeophyceae), which occurs on the northwest side of the Sea of Okhotsk, in the northwest Pacific Ocean. *Pseudolessonia* is monotypic and differs from *Lessonia* in its short primary stipes and its corrugated, unilaterally arranged blades with entire margins. This species is transferred on the basis of morphology and plastid gene sequence comparisons. We determined *psaA* and *rbcL* gene sequences from 17 taxa of *Pseudolessonia*, *Lessonia*, and putative relatives. Analyses of individual and combined data sets resulted in congruent trees showing a clear separation of *Pseudolessonia laminarioides* from *Lessonia*, but suggesting its sister relationships with the clade of *Nereocystis*, *Macrocyctis*, *Pelagophycus*, and *Postelsia* in the North Pacific Ocean. On the other hand, *Lessonia* species from the South Pacific Ocean formed a strongly supported clade. The results indicate that the basal splitting of the blade, which has been considered a diagnostic character for the family Lessoniaceae, is a result of convergent evolution.

Key index words: kelp; Laminariales; Phaeophyceae; phylogeny; *psaA*; *Pseudolessonia* gen. nov.; *rbcL*; taxonomy

Abbreviations: AIC, Akaike Information criterion; BV, bootstrap values; GTR, general time reversible; ML, maximum likelihood; MP, maximum parsimony; TBR, tree bisection-reconnection; TIM, transitional model

Lessonia Bory is one of the most conspicuous brown algal genera occupying the littoral to sublittoral zone in cold temperate waters of the South Pacific Ocean. The thalli produce large quantities of biomass and provide extensive cover on rocky shores and also contain high percentages of alginates and other primary materials of high commercial value in global seaweed markets (Edding et al. 1994). The genus is distinguished by massive holdfasts of branched to fused haptera, terete to complanate stipes, blades with splits originating at the basal part of the blade, and unilocular sporangial sori on ordinary blades (Womersley 1987, Edding et al. 1994). Although *Lessonia fuscescens* Bory de Saint-Vincent (1826) has been considered the first name for the genus (Womersley 1987, Edding et al. 1994), *L. flavicans* Bory de Saint-Vincent in Dumont d'Urville (1825) is a type of the genus (Searles 1978). To date, 12 species of *Lessonia* have been reported in the North and South Pacific Ocean (Postels and Ruprecht 1840; Ruprecht 1850; Searles 1978; Villouta and Santelices 1986; Hay 1987, 1989). The most recent authority on the taxonomy of *Lessonia* accepted nine species, which occur along the coasts of southern Peru, Chile, southern Argentina, New Zealand, Tasmania, and circumpolar islands (Hay 1987, 1989).

Three species of *Lessonia* from the Sea of Okhotsk have been described (i.e. *L. ciliata* Postels and Ruprecht, *L. laminarioides* Postels and Ruprecht, and *L. repens* Ruprecht; Postels and Ruprecht 1840, Ruprecht 1850). Since these original descriptions, there have been no reports on the occurrence of *L. ciliata* and *L. repens* in the North Pacific Ocean. During our study of the Laminariales, we encountered thalli of *L. laminarioides* in the Sea of Okhotsk. The species was classified as *Lessonia* because of a holdfast of branched haptera, one to several terete to complanate stipes, and blades

¹Received 2 February 2006. Accepted 15 August 2006.

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with a split originating at the basal part of the blade (Postels and Ruprecht 1840). It occurs abundantly near the Shantar Islands in the Sea of Okhotsk (Klochkova and Berezovskaya 1997), and its density decreases to the north (Sinova 1954, Blinova 1968, Petrov 1973). Despite its ecological and economic importance in the Sea of Okhotsk (Kizevetter et al. 1981, Klochkova and Berezovskaya 1997), there have been no phylogenetic studies of this species.

Molecular data have been widely used for the taxonomy and phylogeny of brown algae (Kawai and Sasaki 2000, Draisma et al. 2001, Rousseau et al. 2001, Cho et al. 2004, Lane et al. 2006). Some *Lessonia* and putative relatives have been analyzed with sequences of nuclear small subunit (SSU; Saunders and Druehl 1992), internal transcribed spacer (ITS; Saunders and Druehl 1993a,b, Druehl et al. 1997, Yoon et al. 2001; Lane et al. 2006), and RUBISCO spacer regions (Yoon and Boo 1999, Kraan et al. 2001, Yoon et al. 2001); *rbcL* (Draisma et al. 2001, Cho et al. 2004, Lane et al. 2006); and mitochondrial NADH dehydrogenase subunit 6 (Lane et al. 2006). Of these, the *rbcL* has often been used for brown algae at a variety of taxonomic levels (Draisma et al. 2001, Cho et al. 2004, Lane et al. 2006). The *psaA* gene, encoding a photosystem I protein, is more variable than *rbcL* in brown algae (Cho et al. 2004).

The taxonomy of *L. laminarioides* is particularly interesting because it is the only *Lessonia* species from the North Pacific Ocean; all other *Lessonia* species predominate in the South Pacific Ocean (Hay 1987, Edding et al. 1994). To determine whether *L. laminarioides* should remain in the genus *Lessonia* or be placed in a different genus, we studied the morphology of this species based on our collections and type materials and analyzed both the *rbcL* and *psaA* genes. Together with *Lessonia*, we included *Macrocystis*, *Nereocystis*, *Pelagophycus*, and *Postelsia* of the family Lessoniaceae. Both the morphological and molecular data were consistent and supported placing *L. laminarioides* into a distantly related genus from *Lessonia*, which we have designated *Pseudolessonia*.

MATERIALS AND METHODS

Morphological observations. Field observations and collections were performed in the Shantar Islands in September 2000, as well as in other locations in the Sea of Okhotsk (Fig. 1). Herbarium specimens collected before 2000 and deposited in the Laboratory of Phycology, Kamchatka Division of Pacific Institute of Geography of Russian Academy of Sciences, Russia, were also used for the morphological study. Representative specimens from the Sea of Okhotsk, Russia, were collected from the Shantar Islands (1.7 m depth, June 1974; 4.5 m depth, September 1986), Ayan Bay (littoral zone, August 1982), Nagaev Bay (3 m, September 2000), Nedorazumeniya Island (12 m, September 2000), and Pichij Island (littoral zone, August 2004). Scanned images of *Pseudolessonia laminarioides* that were collected in the Sea of Okhotsk in 1839 and are now in the Herbarium of the Botanical Institute of the Russian Academy of Science (St. Petersburg, LE) were also compared (Fig. 2).

Tissues were sectioned by hand, and the slide preparations were stained with 1% aqueous aniline blue acidified with a drop of 1% hydrochloric acid and mounted in 30% glycerine-seawater. Formalin-preserved and dry vouchers are housed at the herbarium of the Laboratory of Phycology, Kamchatka Division of Pacific Institute of Geography of the Russian Academy of Sciences (Petropavlovsk-Kamchatsky), Russia.

Samples for DNA extraction. In total, 17 taxa were available for our molecular study: *P. laminarioides*, eight taxa of *Lessonia*, and eight species of other kelps (Table 1). Three *rbcL* sequences from *Lessonia* and three *rbcL* and *psaA* sequences from relatives were downloaded from GenBank. *Alaria crassifolia* and *Lessoniopsis littoralis* were used as out-groups because of their position in the Alariaceae, a family distinct from the remaining Laminariaceae and Lessoniaceae (Setchell and Gardner 1925). Voucher specimens used for the molecular systematics were deposited in the herbaria of Chungnam National University (CNUK), Daejeon, Korea.

Analyses of *rbcL* and *psaA* regions. Total DNA was extracted from approximately 5 mg of dried thalli ground in liquid nitrogen using a DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions, and then dissolved in 150 μ L of distilled water. Extracted DNA was stored at -20° C and used to amplify *rbcL* and *psaA*. To check for contamination, two specimens per species were analyzed when possible.

The *rbcL* gene was amplified and sequenced as overlapping fragments using primer sets PRB-F0/R1A, F2/R2, F3/R3A, and RS1/RS2 (Kogame et al. 1999, Yoon and Boo 1999). All PCR amplifications were performed in 25 μ L containing 0.5 U DNA polymerase (TaKaRa Shuzo Co., Tokyo, Japan), 0.2 mM for each dNTP, $1 \times$ *Ex Taq*TM buffer (Mg²⁺ free), 2.5 mM MgCl₂, 10 pmole of each primer, and 1–10 ng of template DNA.

The *psaA* region was amplified and sequenced as overlapping fragments using primer sets *psaA130F/970R* and *psaA870F/1760R* (Yoon et al. 2002). The DNA aliquot used for amplification of the *rbcL* was also used for *psaA*, and the PCR solution of amplification for the *psaA* gene was the same as that used for the *rbcL*. The PCR was run with an initial denaturation at 95° C for 4 min, followed by 30 cycles (33 cycles for *psaA*) of amplification [denaturation at 95° C for 30 s (1 min for *psaA*), annealing at 47 or 50° C for 30 s (1 min for *psaA*), extension at 72° C for 1 min (2 min for *psaA*), and a final extension at 72° C for 6 min].

The PCR products were purified using a High PureTM PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all taxa using an ABI PRISMTM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at the Center for Research, CNUK. Both electropherogram outputs for each sample were edited using the program Sequence Navigator v. 1.0.1 (Applied Biosystems).

All *rbcL* sequences from 26 taxa (15 new and 11 published) of *Pseudolessonia*, *Lessonia*, and putative relatives were collated using the multisequence editing program SeqPup (Gilbert 1995) and aligned by eye to compare the present sequences with those previously published (Cho et al. 2004). The *psaA* sequences from 19 taxa (17 new and 2 published) were also aligned by eye. None of the alignments posed any problems, as there were no gaps in the alignments.

Phylogenetic analyses. Three data sets were used for the phylogenetic analyses: 22 taxa for *rbcL*, 19 for *psaA*, and 19 for the combined *rbcL* + *psaA*. We skipped the combinability test because the combined data of the *rbcL* and *psaA* showed more resolving power and clade support for most nodes (Cho et al. 2004). Maximum parsimony (MP) trees were constructed for each data set with PAUP* 4.0b.10 (Swofford 2001) using a heuristic search algorithm with the following

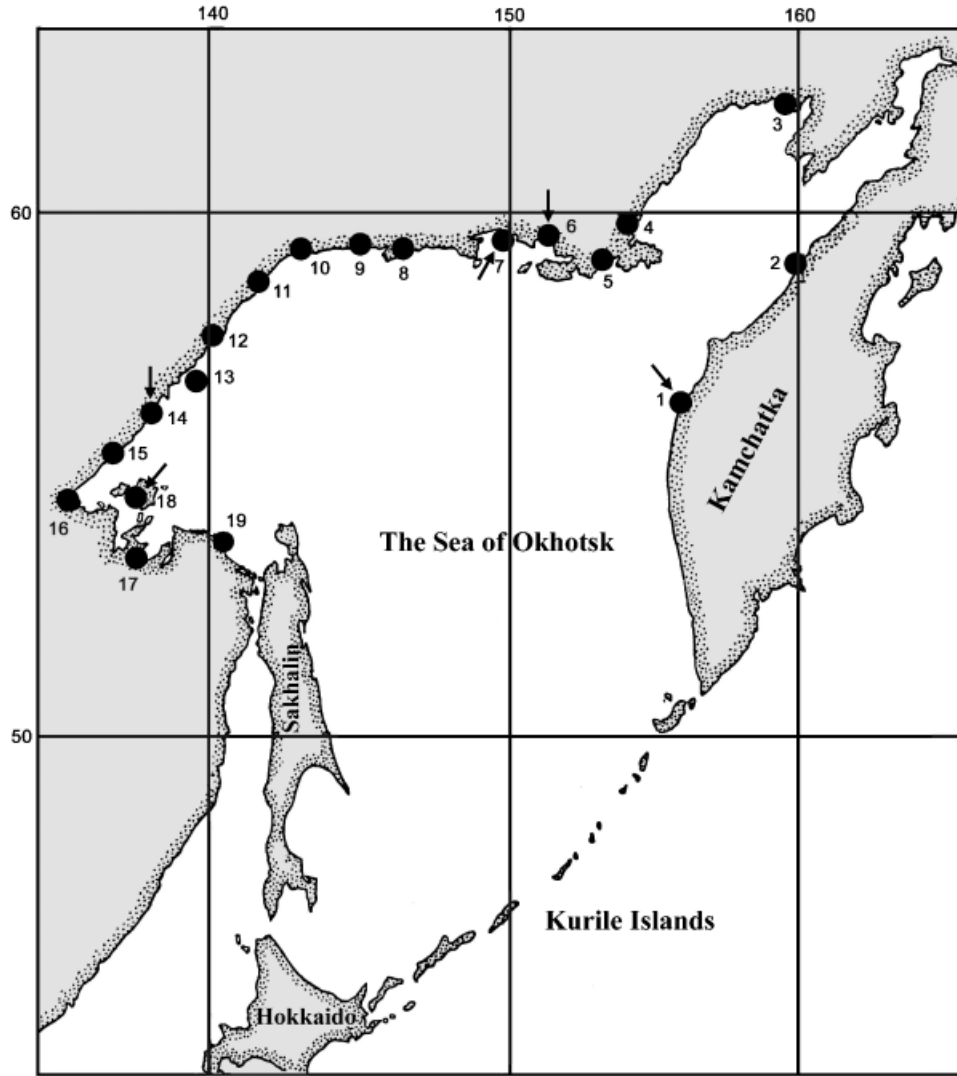


FIG. 1. Distribution map of *Pseudolessonia laminarioides* in the Sea of Okhotsk, northwest Pacific Ocean. Sites—1: Ptichii Island. 2: Pyatibratsky Cape. 3: Nayakhan Cape. 4: Udachi Inlet. 5: Zabayaka Inlet. 6: Nagaevo Bay. 7: Nedorazumenia Island. 8: Lesyanskogo Peninsula. 9: Inya Cape. 10: Okhotsk. 11: Uliya Bay. 12: Enkan Cape. 13: Nanzikan Island. 14: Ayan Bay. 15: Ukoï Bay. 16: Udskeya Bay. 17: Tugur Bay. 18: Shantar Islands. 19: Litke Cape. Localities with arrows were visited in this study; other locations were taken from the literature (Blinova 1968, Petrov 1973, Kizevter et al. 1981, Klochkova and Berezovskaya 1997).

settings: 100 random sequence addition, tree bisection-reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies. The bootstrap values (BV) for the resulting nodes were assessed using 1000 bootstrapping replicates with 10 random sequence additions.

For maximum likelihood (ML) and Bayesian analyses, the best models for individual and concatenated data sets were determined under ModelTest 3.08b (Posada and Crandall 1998): Kimura 3-parameter model unequal frequencies (K3Puf) + proportion of invariable sites (I) + γ shape parameter (G) for *rbcL*, transitional model (TIM) + I + G for *psaA*, and general time-reversible (GTR) + I + G for combined *rbcL* + *psaA*. For ModelTest, model scores were estimated on the basis of the model block for PAUP* (in the ModelTest package). We selected the best model from the Akaike Information criterion (AIC) as recommended by Posada and Buckley (2004) (Table 2). ML analyses were performed by heuristic searches with 100 random sequence addition, TBR branch

swapping, and MulTrees options. Bootstrap analysis was conducted by performing replicate maximum likelihood searches, with two random sequence-addition replicates, using the same search conditions as described above. One thousand bootstrap replicates were conducted for each of the individual and combined data sets using two processors. To run simultaneous bootstrap replicates in different processors, two batch files were prepared that differed in their starting random seed number (seed number = 0), and each was specified to run 500 bootstrap replicates and to save the resulting bootstrap trees into files. A 50% majority rule consensus bootstrap tree was estimated by aggregating and weighting trees according to the number of trees found in each bootstrap replicate, so that the bootstrap replicates had equal weight.

Bayesian analyses were performed for the GTR + I + G model for all data sets using MrBayes (v. 3.1.2, Huelsenbeck and Ronquist 2001), because the program can be set as a six-parameter model only. Each analysis was conducted from a random starting tree, and the program was set to perform two



FIG. 2. Type specimen of *Pseudolessonia laminarioides* deposited in the Herbarium of the Botanical Institute of the Russian Academy of Sciences (LE).

independent runs with four chains of Markov chain Monte Carlo iterations simultaneously for 2 million generations with trees sampled every 100th generation, respectively. We harvested trees after the average standard deviation of split frequencies reached or went below 0.01. These values were <0.01 after 878,000 generations for *rbcL*, 202,000 generations for *psaA*, and 313,000 generations for *rbcL* + *psaA*. The remaining trees for each data set were combined to produce a 50% majority rule tree.

RESULTS

Pseudolessonia G. Y. Cho, N. G. Klochkova, T. N. Krupnova et Boo, gen. nov.

Diagnosis. Genus novum Laminariacearum. Rhizoidea fibrosa fibris numerosis dichotome ramosis discretis. Stipes e basi dichotomus teretis rugulosus brevis. Laminae tenues rugosae margine integro, e basi sursum fissae, unilaterialis. Substantia laminarum chartacea, stipitis ramorumque cornea fragilis. Sporangia sori in utraque facie laminae, sphaerici elongati vel irregulares.

Holdfast hapteroid; fibers numerous, dichotomously branched, discrete. Stipe dichotomous from base, terete, rugulose, short. Blades thin, wrinkled on sur-

face, margin entire, split from base upward, unilaterally arranged. Substance of blades chartaceous; that of stipe and branches horny, brittle. Sporangia sori on both sides of blade, spherical, elongated to irregularly shaped.

Etymology. The genus name refers to a resemblance to the genus *Lessonia*. The specific epithet, given as “*laminariaeoides*” in the original description (Postels and Ruprecht 1840), should be “*laminarioides*” according to the rules of Latin grammar.

Type species: *Pseudolessonia laminarioides* (Postels et Ruprecht) G. Y. Cho, N. G. Klochkova, T. N. Krupnova et Boo, comb. nov.

Basionym: *Lessonia laminariaeoides* Postels et Ruprecht (Illustrationes Algarum, St. Petersburg, p. 22. 1840).

Type: Herbarium of the Botanical Institute of the Russian Academy of Sciences (St. Petersburg, LE, number not given; Fig. 2).

Morphology. Sporophytic thalli are up to 3 m tall and composed of a rhizoidal cluster, abundantly branched stipes (up to 10 times), and numerous blades arising from stipes (Figs. 3, A–C and 4A). Rhizoidal clusters are fibrous, densely and dichotomously branched, dilated into a small disc at the peripheral ends, and 2–3 cm long. Primary stipes are terete to compressed, short, up to 7 cm long, and 0.5–2 cm wide. A primary branch originates from a single rhizoidal branch, and then several branches often arise from the rhizoidal cluster (Fig. 3A).

On the stipe, dichotomous branches occur at intervals of 1.5–30 cm (Fig. 3B). Blades arise from the last branches of the stipe. A juvenile plant has a single blade, while a mature thallus includes up to 50 blades. Blades range from 0.2 to 2.8 m long and from 6 to 33 cm wide, depending on age, growth depth, and exposure. Young blades are 460–650 μm thick, while mature blades are 780–850 μm thick. The base of the blades is thin, narrow, and sphenoid. The blade surface is abundantly wrinkled. The wet weight of a single drained thallus can reach up to 1 kg.

The development of new blades and dichotomous branching of the stipe is shown in Fig. 3A. Vertical cleavage occurs in the area between the stipe and the blade. Initially, the blade is divided and the stipe shows a partial split; thereafter, this cleavage moves up to the apex until it cuts the blade into two parts. The bifurcated part of the base extends, thereby forming a dichotomous crotch with a separate blade on each branch (Fig. 3, A and B). Blades are often unilaterally arranged in mature thalli (Fig. 3B).

Anatomically, the thallus (Fig. 4, C and D) is very similar to the genus *Laminaria*. The cortex is composed of a single row of small cells about 9 μm in diameter in young thalli, and the cortical cells increase up to 14 μm in diameter in mature thalli. The subcortex is formed by two or more rows of large, spherical cells, 25–30 μm in diameter. The cells become much extended with increased age. The width of the medullary layer decreases significantly toward the center. In the apical

TABLE 1. List of species investigated in this study.

Taxon	Type locality	Collection site, date, and voucher number or reference	GenBank accession number	
			<i>rbcL</i>	<i>psaA</i>
<i>Lessonia corrugata</i> Lucas	Tasmania, Australia	Gov. Island Reserve, Tasmania, Australia (Lane et al. 2006)	AY851545	–
<i>L. flavicans</i> Dumont-d'Urville	Falkland Islands, Chile	Rookery Bay, Falkland Islands, Chile (Lane et al. 2006)	AY851543	–
<i>L. nigrescens</i> Bory de Saint-Vincent	Cape Horn, in the Chilean part of Fuegia, Chile	Las Cruces, El Tabo, Chile, ii 2003, PL200	DQ372551	DQ372535
<i>L. tholiformis</i> C. H. Hay	Chatham Island, New Zealand	Las Cruces, Chile (Lane et al. 2006)	AY851544	–
<i>L. trabeculata</i> Villouta et Santelices	La Herradura de Guayacan, Coquimbo, Chile	Owenga, Chatham Island, New Zealand, x 2000, PL472	DQ372552	DQ372536^a
<i>L. vadosa</i> Searles	Punta Conway, Isla de los Estados, Argentina	Waitangi, Chatham Island, New Zealand, iii 2000, PL204	DQ372553^b	DQ372537
		Ilo, Moquegua, Peru, ix 1998, PL208	DQ372554	DQ372538
		Pisco, Ica Province, Peru, x 1998, PL214	DQ372555	DQ372539
		Fuerte Bulnes, Punta Arenas, Chile, i 2003, PL202	DQ372556	DQ372540
		Tierra del Fuego, Bahía Golondrina, Argentina, xi 1998, PL218	DQ372557	DQ372541
<i>Lessonia</i> sp.	–	Ghabunco, Punta Arenas, Chile, i 2003, PL216	DQ372558	DQ372542
<i>Alaria crassifolia</i> Kjellman	–	Cho et al. (2004)	AY372982	AY372962
<i>Costaria costata</i> (C. Agardh) Saunders	–	Oshoro, Hokkaido, Japan, iv 2002, PL124	DQ372559	DQ372543
<i>Cymathæra triplicata</i> Postels et Ruprecht	–	Lane et al. (2006)	AY851562	–
<i>Ecklonia radiata</i> (C. Agardh) J. Agardh	–	Boulders Beach, False Bay, South Africa, i 2001, PL286	DQ372560	DQ372544
<i>Hedophyllum sessile</i> (C. Agardh) Setchell	–	Boiler Bay, Oregon, USA, vii 1998, PL239	DQ372562	DQ372546
<i>Laminaria digitata</i> (Linnaeus) Lamouroux	–	Cho et al. (2004)	AY372984	AY372964
<i>L. sinclairii</i> (J. D. Hooker et Harvey) Farlow, Anderson et Eaton	–	Lane et al. (2006)	AY851558	–
<i>Lessoniopsis littoralis</i> (Tilden) Reinke	–	Boiler Bay, Oregon, USA, vii 1998, PL169	DQ372563	DQ372547
<i>Macrocystis pyrifera</i> (Linnaeus) C. Agardh	–	Carmel Beach, Monterey, California, USA, viii 1998, PL172	DQ372564	DQ372548
<i>Nereocystis luelkeana</i> (Mertens) Postels et Ruprecht	–	Boiler Bay, Oregon, USA, vii 1998, PL192	DQ372565	DQ372549
<i>Pelagophycus porra</i> (Leman) Setchell	–	Lane et al. (2006)/Santa Catalina, California, USA, ix 1999, PL184	AY851547	DQ473542
<i>Postelsia palmaeformis</i> Ruprecht	–	Lane et al. (2006)/Yachats, Oregon, USA, vii 1998, PL187	AY851549	DQ473543
<i>Pseudolessonia laminarioides</i> (Postels et Ruprecht) G. Y. Cho, N. G. Klochkova, T. N. Krupnova et Boo	The Sea of Okhotski, Russia	Babushkina near Nagaev Bay, The Sea of Okhotsk, Russia, ix 2000, PL323	DQ372550	DQ372534
<i>Saccharina latissima</i> (Linnaeus) C. E. Lane, C. Mayes, Druehl et G. W. Saunders	–	Lane et al. (2006)	AY851561	–
<i>S. gyralta</i> (Kjellman) C. E. Lane, C. Mayes, Druehl et G. W. Saunders	–	Lane et al. (2006)	AY851560	–

Bold numbers indicate the sequences that were analyzed in this study.

^aOnly 793 bp.

^bPosition 556–950 is missing.

TABLE 2. Nucleotide composition of *psaA* and *rbcL* sequences, statistics from MP analyses, and selected model parameter values of the individual and combined data sets including the out-group.

	<i>rbcL</i>	<i>psaA</i>	<i>rbcL</i> + <i>psaA</i>
Number of taxa	26	19	19
Number of nucleotides (bp)	1467	1488	2955
Base frequency (A/C/G)	0.288/0.174/0.218	0.297/0.152/0.190	—
MP tree number	2	4	1
MP tree length (steps)	240	323	525
Selected model (methods)	K3Puf+I+G	TIM+I+G	GTR+I+G
Model-based base frequency (A/C/G)	0.284/0.182/0.213	0.295/0.154/0.187	0.293/0.165/0.201
R _{AC}	1	1	1.00586
R _{AG}	3.39604	3.02422	3.34238
R _{AT}	0.63723	0.19944	0.34251
R _{CG}	0.63723	0.18844	0.93262
R _{CT}	3.39604	4.83622	4.78644
Proportion of invariable sites (I)	0.78249	0.68522	0.73788
γ-shape parameter (G)	0.75867	0.61840	0.74353

part of the blades, the medulla is composed of short cylindrical cells of 14–50 × 6–9 μm and occupies about one-third of the blade thickness. In the mature blade, the medulla consists of thinner, long-cylindrical cells

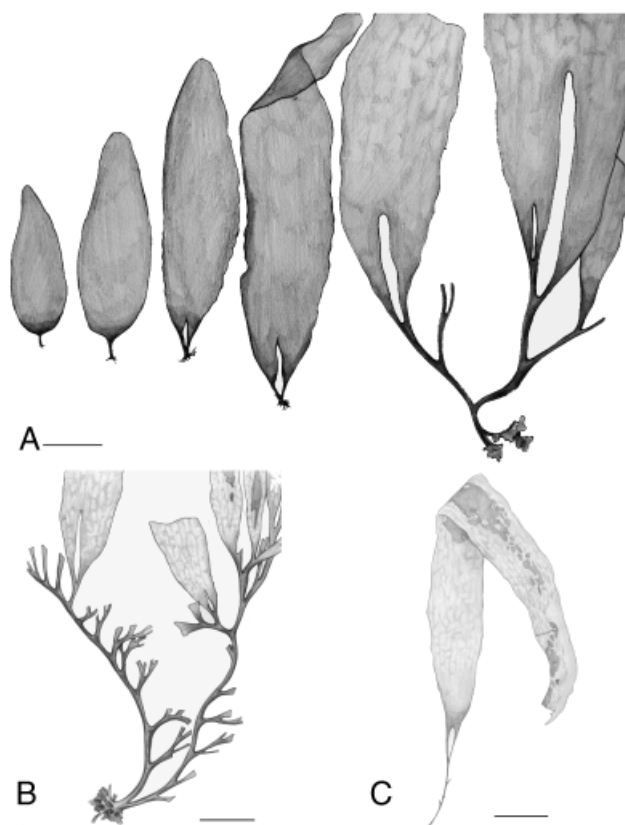


FIG. 3. Illustration of *Pseudolessonia laminarioides* thalli based on specimens collected from the Shantar Islands in September 1986 at a depth of about 4.5 m. (A) A single blade bearing sporangial sori with a split in the transitional zone (scale bar, 10 cm). (B) A perennial thallus having a holdfast with unfused haptera and multiple stipes with many branched blades with splits in the transitional zone (scale bar, 10 cm). (C) Process of split formation in the transitional zone from the young thallus to the mature and unilaterally arranged additional branches and blades (scale bar, 5 cm).

(hyphae) about 50 μm long and 2–4.5 μm wide and occupies over four-fifths of the blade thickness. In the middle part of the blades, medullary cells become twisted. In the holdfast, a cluster of tightly adherent, long-cylindrical cells is present. The cells range from 6 to 21 μm from the periphery toward the central part of the cluster. Mucilage ducts are absent in the blade and stipe.

Sporangial sori (Figs. 3C and 4, B–D) are formed on both sides of the blade and are spherical, longitudinally extended, or irregularly shaped. When mature, the sori develop almost on the whole surface of the blade (Fig. 4B). Sporangia arise by the division into two cells of the outermost cortical cell. The lower cell functions as a support cell, whereas the upper cell extends further and leads to the formation of paraphyses or unilocular sporangia. Mature unilocular sporangia are 19–25 × 7–9 μm in size (Fig. 4D). Paraphyses are narrower at the distal end and are 27–42 μm long and 4–6 μm wide. Sporangial sori were found in September.

Ecology. *P. laminarioides* is epilithic on rocky substrates and large boulders at a depth of 0–12 m in the Sea of Okhotsk, Russia. The thalli produce a dense cover, with a biomass of 0.2–6.2 kg · m⁻² and as many as 210 plants · m⁻². The thickest cover occurs in the low littoral zone or in the edge of the sublittoral zone. Common accompanying species are *Laminaria gurjanovae* A. Zinova, several species of *Alaria*, *Cystoseira crassipes* (Mertens ex Turner) C. Ag., *Leptophytum laeve* (Foslie) Adey, *Phymatolithon polymorphum* (L.) Foslie, and *Lithothamnion sonderi* Hauck. Occasionally, foliaceous algae are found such as *Dilsea socialis* (Postels et Ruprecht) Perestenko, *Tichocarpus crinitus* (Gmel.) Ruprecht, *Crossocarpus lamuticus* Ruprecht, *Hommersandia palmatifolia* (Tokida) Perestenko, *Turnerella mertensiana* (Postels et Ruprecht) Schm., *Palmaria moniliformis* (Blinova et A. D. Zinova) Perestenko, *Rhodymenia pertusa* (Postels et Ruprecht) J. Agardh, *Neoptilota asplenioides* (Esper) Kylin, and some delesseriacean algae.

P. laminarioides occurs rarely toward the north along the continental coast of the Sea of Okhotsk and is very

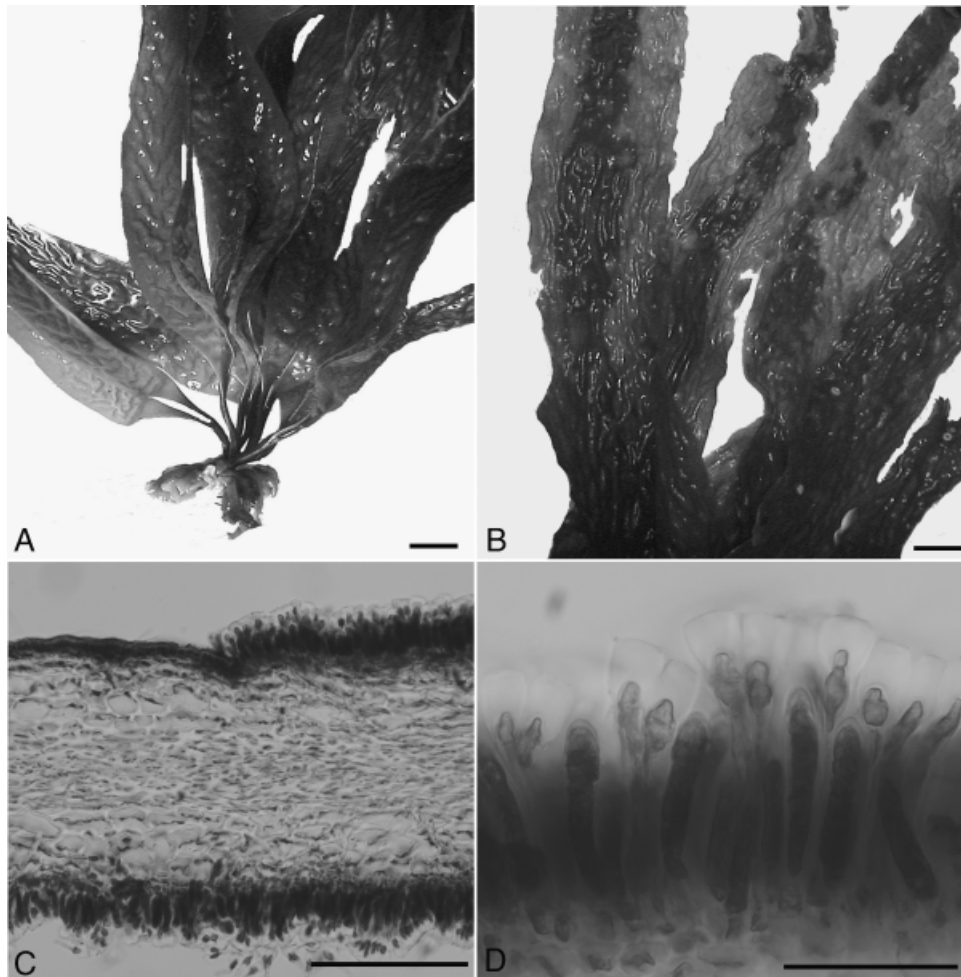


FIG. 4. *Pseudolessonia laminarioides*. (A) Adult sporophyte (50 cm in length) with unfused haptera and multidivided, terete stipe (scale bar, 2.5 cm). (B) Thallus bearing sporophylls (scale bar, 5 cm). (C) Cross-section of a blade showing a medullar and cortical layer (scale bar, 250 μ m). (D) Cross-section of a blade showing zoosporangia and paraphyses with mucilaginous caps (scale bar, 50 μ m).

rarely found on the western coast of Kamchatka Peninsula. We did not observe this species on the southwest coast of the peninsula.

Phylogenies of rbcL and psaA genes. We determined 1467 nucleotides of *rbcL* sequence in this study. The 26 aligned *rbcL* sequences had 142 (9.7%) variable bases and 70 (4.8%) parsimoniously informative sites. The sequences determined for the *psaA* region were 1488 nucleotides long, 195 (13.1%) bases were variable, and 106 (7.1%) were parsimoniously informative. The *rbcL* tree (Fig. 5) showed that *Pseudolessonia* was clearly separated from *Lessonia*, and all *Lessonia* species were strongly monophyletic. Although not supported by BV and Bayesian posterior probabilities, *Pseudolessonia* was a sister group to the clade of *Macrocystis*, *Pelagophycus*, *Postelsia*, and *Nereocystis*. The *psaA* tree (Fig. 6) was similar to the *rbcL* tree in that *Pseudolessonia* was separated from *Lessonia*, while all the *Lessonia* species were monophyletic. However, *Pseudolessonia* was positioned between *Nereocystis/Postelsia* and *Macrocystis/Pelagophycus* clades. Although

the trees of the concatenated data set (2955 bp) were more similar to the *rbcL* tree than the *psaA* trees, all of these trees were congruent in showing a distant relationship of *Pseudolessonia* from *Lessonia*. The monophyly of *Pseudolessonia/Macrocystis/Pelagophycus/Postelsia/Nereocystis* is strongly supported (100 for ML, 98 for MP, and 1.0 for Bayes), but the sister relationship is only marginally supported (69 for ML, 70 for MP, and 0.9 for Bayes; Fig. 7).

DISCUSSION

L. laminarioides described by Postels and Ruprecht (1840) is reclassified to the new genus *Pseudolessonia* based on *rbcL* and *psaA* sequences as well as a reinvestigation of its morphology. Analyses of individual and combined data sets of both genes clearly separated *Pseudolessonia* from *Lessonia*. Although *L. adamsiae* C. H. Hay, *L. brevipes* J. Agardh, and *L. variegata* J. Agardh, which occur only in the South Pacific Ocean, were not examined in this study, an inclusion of these taxa

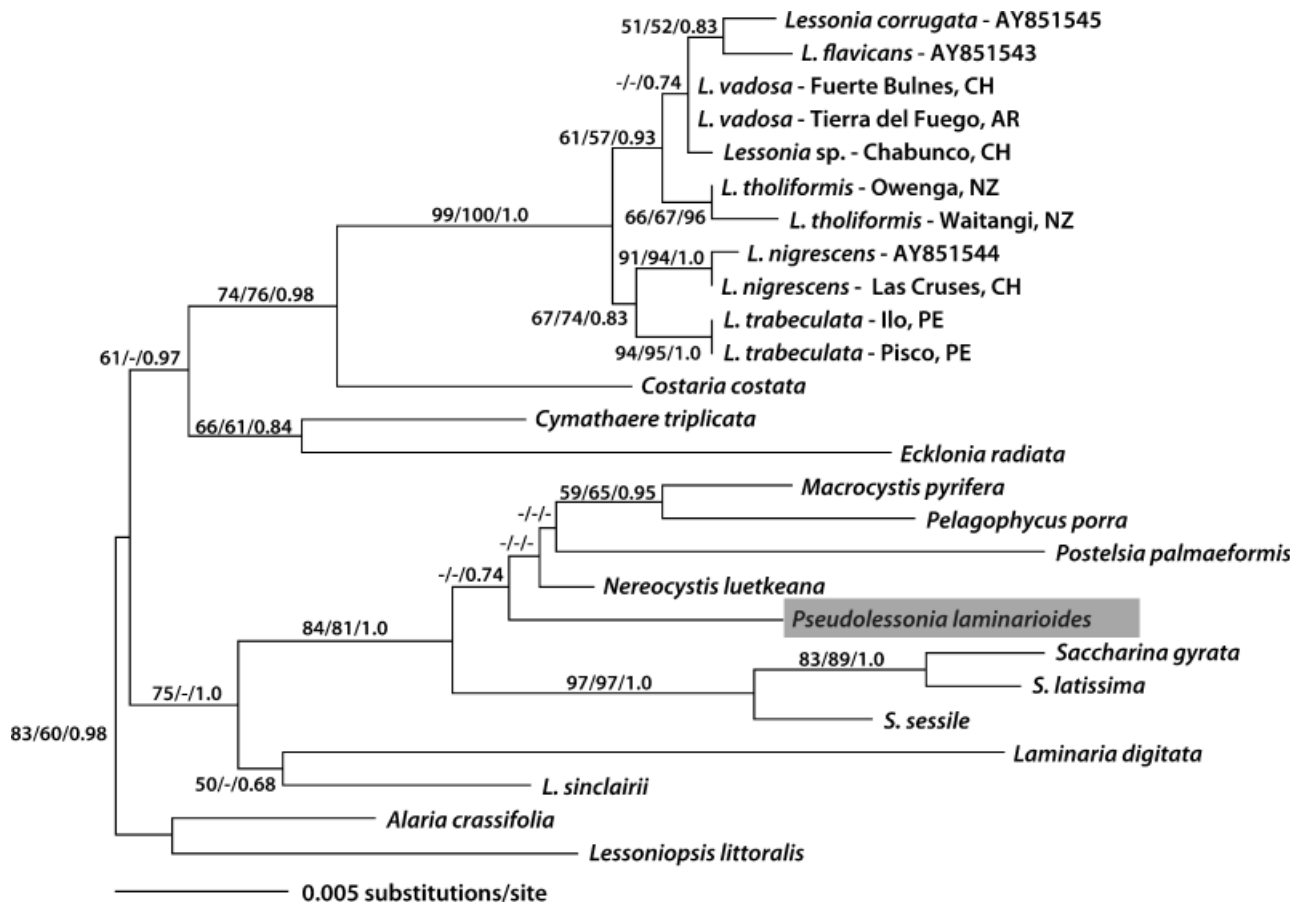


FIG. 5. Maximum likelihood tree for *Pseudolessonia laminarioides* and putative relatives estimated from *rbcL* ($-\ln L = 3515.54361$) sequence data. The supporting values shown above the branches are from ML/MP bootstrap/Bayesian posterior probability analysis. Dashes indicate bootstrap values of <50% and Bayes <0.5.

would not change the finding that *Pseudolessonia laminarioides* is a unique taxon clearly separated from *Lessonia* because *L. flavicans*, the type of the genus, is included in the present study. Morphologically, *Pseudolessonia* is distinguished by short primary stipes; blades with an entire margin and unilaterally arranged; spherical cortical cells with plastids and thin; and cylindrical to twisted threads of the medullary layer. However, in *Lessonia*, the primary stipe lengthens progressively to form a main trunk on which numerous secondary blades are borne. Blades are terminally arranged, and blade margins are dentate to glandulate and have a lacunate cortex, except *L. flavicans* and *L. nigrescens* (Fritsch 1945, Villouta and Santelices 1986, Hay 1987, 1989, Edding et al. 1994).

The occurrence of the basal splits has been regarded as a key character for the family Lessoniaceae (Setchell and Gardner 1925). However, *Lessoniopsis*, with basal splits, previously in the Lessoniaceae, is now classified in the Alariaceae (Saunders and Druehl 1993a, b, Yoon et al. 2001, Lane et al. 2006). *Macrocystis*, *Nereocystis*, *Pelagophycus*, and *Postelsia* have splits at the basal part of the blade (Setchell and Gardner 1925) and were distantly related to *Lessonia* in our study, as also

shown by Yoon et al. (2001) and Lane et al. (2006). These results suggest that basal splits, occurring in *Lessoniopsis*; *Lessonia*; and the group of *Macrocystis*, *Nereocystis*, *Pelagophycus*, *Postelsia*, and *Pseudolessonia*, can no longer be used as a diagnostic character for the Lessoniaceae. This family was not recognized by Yoon et al. (2001) or recognized but in a radically different form from its original circumscription by Lane et al. (2006). Basal splitting may be a character gained at least two to three times in the Laminariales.

It is not surprising to note that *Pseudolessonia* was a sister taxon to the clade of *Macrocystis*, *Nereocystis*, *Pelagophycus*, and *Postelsia* in all analyses of individual and combined data sets used in this study. These five genera have splits in the base of the blades, as mentioned above. However, *Macrocystis*, *Nereocystis*, and *Pelagophycus* are clearly different from *Pseudolessonia* in having air-bladder structures, and *Postelsia* has numerous terminated blades arising from a stipe (Setchell and Gardner 1925). All of these genera are similar in distribution, occurring in the North Pacific Ocean, although *Macrocystis* extends its distribution to the southern Pacific Ocean (Chin et al. 1991). The present molecular trees strongly suggest that

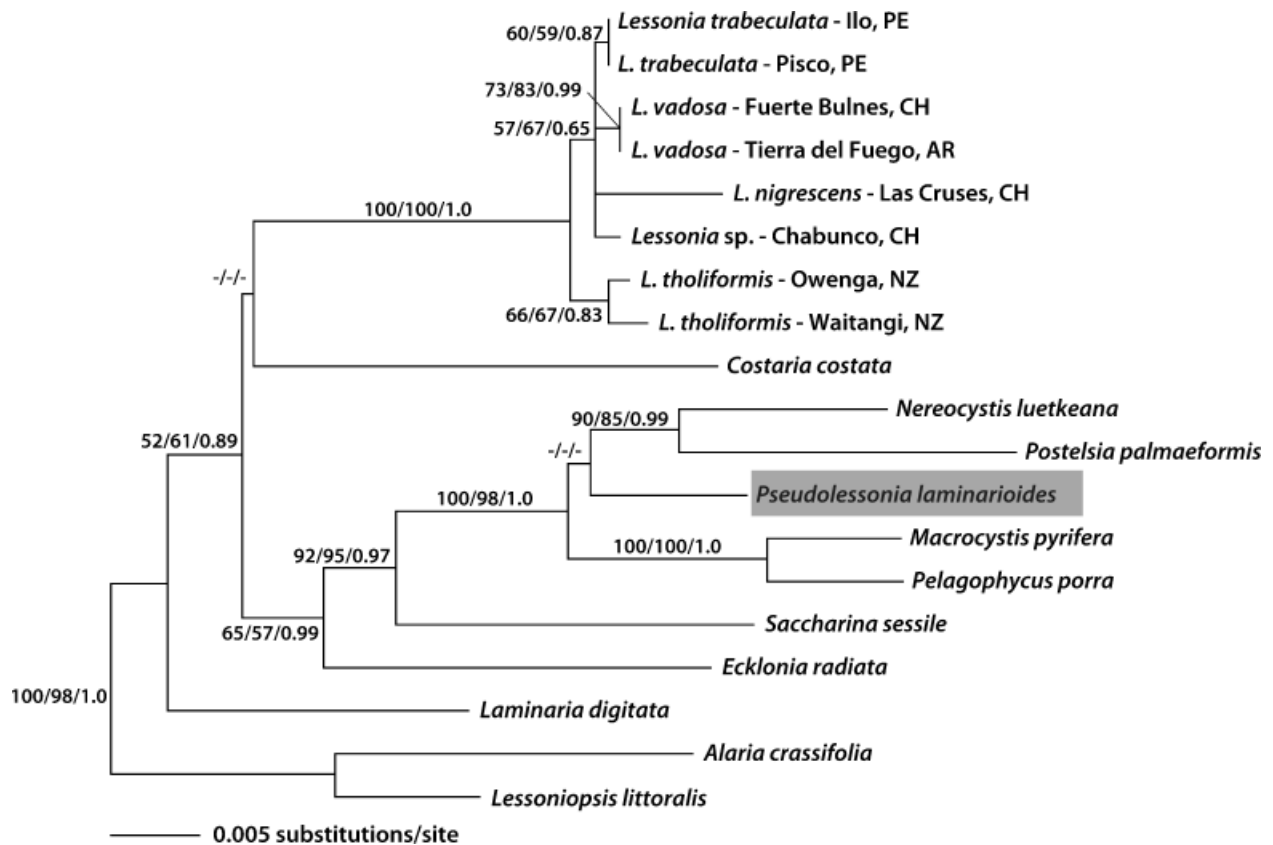


FIG. 6. Maximum likelihood tree for *Pseudolessonia laminarioides* and putative relatives estimated from *psaA* ($-\ln L = 3837.74325$) sequence data. The supporting values shown above the branches are from ML/MP bootstrap/Bayesian posterior probability analysis. Dashes indicate bootstrap values of <50% and Bayes <0.5.

Pseudolessonia might have a common ancestor with contemporary kelps from the North Pacific Ocean. On the other hand, *Macrocystis* and *Pelagophycus* formed a sister relationship in all data sets, and *Nereocystis* and *Postelsia* formed a sister relationship in *psaA* and combined data sets. Of these, *Pseudolessonia* is similar to *Macrocystis* and *Pelagophycus* in having unilaterally arranged blades; a relationship between *Pseudolessonia* and *Macrocystis*/*Pelagophycus* was not supported. *Pseudolessonia* also appears similar to *Julescraneia*, a fossil kelp genus with basal splits, deposited in the late Miocene in Southern California, but the latter had air-bladders (Parker and Dawson 1969).

The Sea of Okhotsk is a huge marine reservoir bounded by the Kurile Archipelago and Hokkaido islands, which are considered as barriers to dispersal of marine organisms between the Sea of Okhotsk and the North Pacific Ocean. Indeed, the Sea of Okhotsk has a high endemism of kelps, including such genera as *Costularia* Petrov et Gusarova, *Feditia* Petrov et Gusarova, *Phyllariella* Petrov et Vozzhinskaya, and *Tauya* Klochkova et Krupnova (Klochkova and Krupnova 2003). Formation of the Kurile Archipelago began in the late Cretaceous (approximately 90 million years ago), and the islands emerged above the sea surface from the early Miocene (Kimura and Tamaki

1985) to the early Pleistocene (Bulgakov 1996). This suggests that *Pseudolessonia* likely originated at least before the Miocene, the period during which the Kurile Islands began to emerge. This is approximately consistent with Jacobs et al. (2004), who proposed that kelps likely diverged during the Neogene in the North Pacific Ocean.

The genus *Lessonia* is now limited to the South Pacific Ocean, southern Peru, Chile, Australia, New Zealand, and the circumpolar islands (Searles 1978, Womersley 1987, Hay 1989, Edding et al. 1994). These results suggest that *Lessonia* species probably originated and diversified in the South Pacific Ocean. Contrary to our expectation that *Lessonia* may be related to the genus *Ecklonia* or *Macrocystis*, both of which occur in the South Pacific Ocean, neither *Ecklonia* nor *Macrocystis* appeared to have sister relationships with *Lessonia* in our *psaA* and *rbcL* data sets. Relationships between *Lessonia* and other kelps from the Southern Hemisphere such as *Macrocystis* and *Ecklonia* were not supported in previous ITS+RUBISCO data (Yoon et al. 2001) or not well resolved in recent molecular study by Lane et al. (2006). Previous reports indicated that *Lessonia* evolved from some North Pacific Ocean ancestor that probably migrated to the South Pacific Ocean during the Miocene cooling (Lüning 1990,

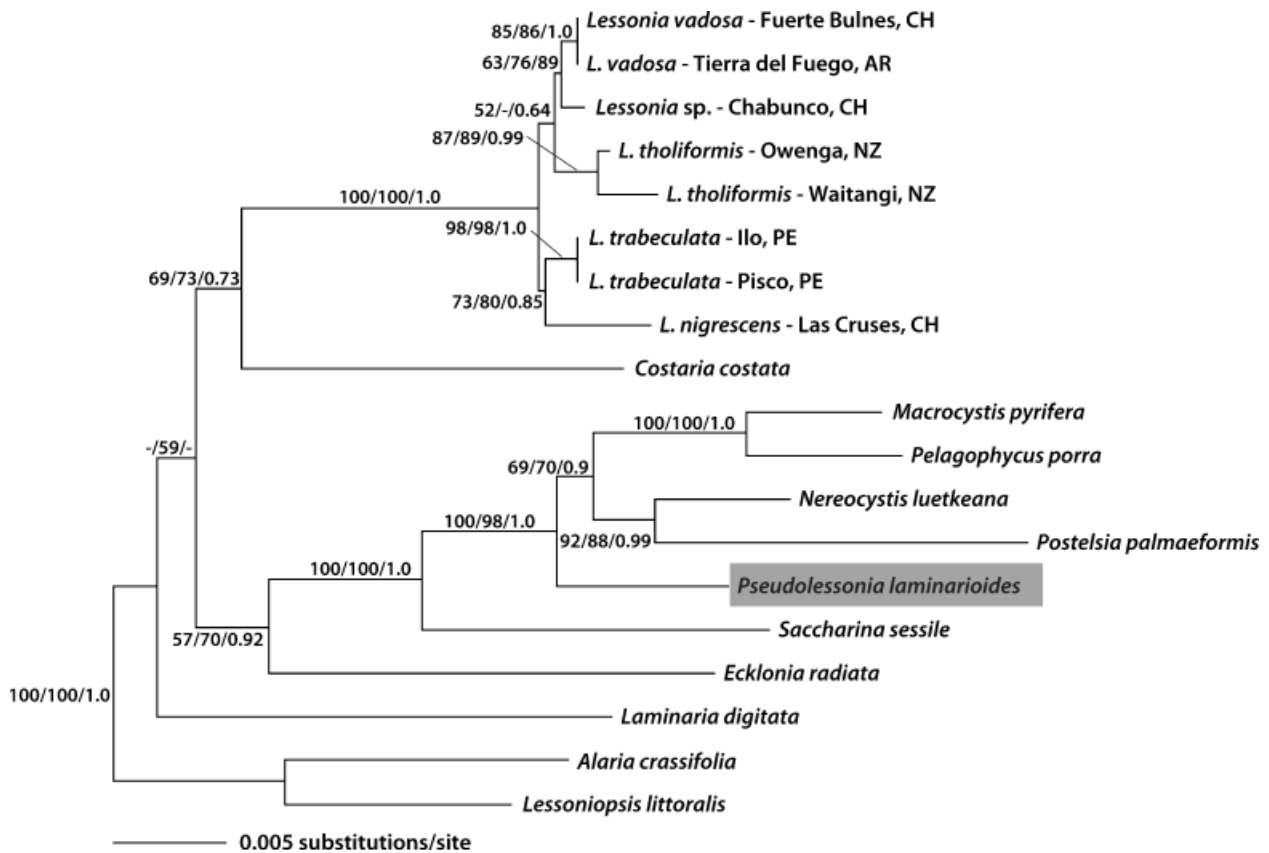


FIG. 7. Maximum likelihood tree for *Pseudolessonia laminarioides* and putative relatives estimated from *rbcL*+*psaA* ($-\ln L = 7137.19675$) sequence data. The supporting values shown above the branches are from ML/MP bootstrap/Bayesian posterior probability analysis. Dashes indicate bootstrap values of <50% and Bayes <0.5.

Chin et al. 1991); alternatively, its ancestor from the North Pacific Ocean probably diversified in the South Pacific Ocean before the introduction of *Macrocystis* and *Eisenia* (*Ecklonia*) to the South Pacific Ocean (Saunders and Druehl 1993b). However, this issue is beyond the scope of the present study.

The authors are indebted to W. Nelson and A. Mansila for help with the collection trip in New Zealand and Chile, respectively, and G. Kraft for supplying a valuable specimen of *Lessonia*. The authors much appreciate L. P. Perestenko for sending scanned images of the type, M. Gerald for the Latin diagnosis, B. de Reviere for an unpublished manuscript on *Lessonia*, and C. E. Lane for galley proof. G. C. Zuccarello and two reviewers improved the manuscript. This work was supported by the Marine and Extreme Genome Research Center Program to S. M. Boo (program leader: Dr. S. J. Kim), Ministry of Maritime Affairs and Fisheries, Korea.

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