

Morphology and molecular phylogeny of *Chordaria okhotskensis* sp. nov. (Ectocarpales, Phaeophyceae) from the Sea of Okhotsk

Tatyana A. KLOCHKOVA^{a*}, Nina G. KLOCHKOVA^b, Mihail N. BELIJ^c,
Hyung-Seop KIM^{d*} and Gwang Hoon KIM^a

^a Department of Biology, Kongju National University, Kongju 314-701, Korea

^b Kamchatka State Technical University (KamchatGTU),
Petropavlovsk-Kamchatsky, Klyuchevskaya 35, 683003, Russia

^c Magadan Scientific-Research Institute of Fishery and Oceanography
(MagadanNIRO), Magadan, Portovaya 36/10, 685014, Russia

^d Department of Biology, Gangneung-Wonju National University, Gangneung,
Gangwon-do, Korea

Abstract – Three *Chordaria* species were reported from the Russian Far East, viz. *C. chordaeformis*, *C. flagelliformis*, and *C. gracilis*. *Chordaria flagelliformis* and *C. gracilis* are common and widely distributed in the Bering Sea and Kamchatka, Kuril Islands, and the Sea of Okhotsk, while *C. chordaeformis* was only reported from the Avacha Bay (southeast Kamchatka). We describe a new species, *Chordaria okhotskensis*, from the Taujskaya Bay (northern Sea of Okhotsk, Russia). The external morphology of *C. okhotskensis* is similar to *C. chordaeformis* in having unbranched or sparsely branched thalli with branches borne at acute angles. The new species differs from *C. chordaeformis* in having large elongate medullary cells and thalli that are entirely flattened. Phylogenetic analysis based on *rbcL* gene data showed that this new species from the Sea of Okhotsk is distinct from the Atlantic and Pacific types of *C. chordaeformis*. Based on the phylogenetic results, the Kamchatka specimens of *C. chordaeformis* were also attributed to a new taxon, *C. okhotskensis*.

Brown algae / *Chordaria* / flora / Kamchatka / Sea of Okhotsk

Résumé – Morphologie et phylogénie moléculaire de *Chordaria okhotskensis* sp. nov. (Ectocarpales, Phaeophyceae) de la mer d'Okhotsk – Trois espèces de *Chordaria* ont reportées de l'extrême Est de la Russie, i.e. *C. chordaeformis*, *C. flagelliformis* et *C. gracilis*. *Chordaria flagelliformis* et *C. gracilis* sont communes et largement distribuées dans la mer de Bering et le Kamchatka, les Îles Kuril et la mer d'Okhotsk, tandis que *C. chordaeformis* n'est connue que de la Baie Avacha (Kamchatka SE). Nous décrivons une nouvelle espèce, *Chordaria okhotskensis*, de la Baie Taujskaya (au nord de la mer d'Okhotsk, Russie). La morphologie externe de *C. okhotskensis* ressemble à celle de *C. chordaeformis* par les thalles non ou très peu ramifiés avec des rameaux naissant dans les angles aigus. La nouvelle espèce diffère de *C. chordaeformis* par de larges cellules médullaires allongées et des thalles qui sont entièrement aplatis. L'analyse phylogénétique basée sur le gène *rbcL* montre que cette nouvelle espèce de la mer d'Okhotsk est distincte des types atlantiques et pacifiques de *C. chordaeformis*. Sur la base des résultats phylogénétiques les spécimens du Kamchatka *C. chordaeformis* sont aussi attribués à ce nouveau taxon, *C. okhotskensis*.

Algues brunes / *Chordaria* / flore / Kamchatka / Mer d'Okhotsk

* Correspondence and reprints: tatyana1905@rambler.ru, hskim@gwnu.ac.kr
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INTRODUCTION

In the Russian Far East, a renewed interest in macroalgal floristic studies has arisen since many inaccessible coastal areas were previously overlooked (e.g. Klochkova *et al.*, 2010). In particular, the flora of northern continental areas and western coast of the Sea of Okhotsk requires detailed investigation. A recent taxonomic treatment of the Sea of Okhotsk and western Kamchatka reduced the number of species from approximately 300 to 222, excluding non-valid, synonymous and misidentified taxa (Klochkova & Emelyanova, 2005). However, more detailed studies, in taxonomic revisions of particular algal genera, will be required to get a better understanding of macroalgal diversity in the region.

In the Russian Far East, the genus *Chordaria* Agardh is represented by three species, including *C. chordaeformis* (Kjellman) Kawai *et* Kim (Kim & Kawai, 2002), *C. flagelliformis* (Müller) Agardh, and *C. gracilis* Setchell *et* Gardner (Klochkova, 1995, 1998a; Klochkova *et al.*, 2009a). *Chordaria flagelliformis*, the type of the order Chordariales, was described from the North Atlantic. Kjellman (1877) described four morphological forms within the species [f. *typica* (f. *flagelliformis*), f. *chordaeformis*, f. *ramusculifera*, and f. *subsimplix*] based on samples collected during 1872-1873 from Isfjorden (Svalbard, Spitsbergen). However, because of the great morphological plasticity of the species, the taxonomic characters discriminating the forms are not always reliable. All forms were circular in cross-section (terete) with almost indistinguishable anatomy (Kjellman, 1877; Kim & Kawai, 2002).

Chordaria flagelliformis f. *chordaeformis* was elevated to the species rank (*C. chordaeformis*) based on its simple thallus, limited distribution in colder-water regions of the northern hemisphere, and distinctive DNA sequences (Kim & Kawai 2002). In their study, 41 *Chordaria* specimens from various localities in the northern Atlantic (including Spitsbergen, the type locality of *C. flagelliformis* f. *chordaeformis*) and northern Pacific were analyzed phylogenetically. Existence of two clades (a Pacific and Atlantic clade) was revealed for *C. chordaeformis* as well as *C. flagelliformis* (Kim & Kawai, 2002). In addition, the forms *ramusculifera* and *subsimplix* were considered as ecotypes of *C. flagelliformis sensu stricto*.

In Russian phycological references, *C. flagelliformis* has often been misidentified as *C. magellanica* (Hooker *et* Harvey) Cotton (summarized in Klochkova, 1995, 1998a). Also, *C. flagelliformis* was listed as synonym of *C. magellanica* during studies of the marine algal flora of Chile (Héctor Etcheverry, 1986; Levring, 1960). Clayton *et al.* (1997) mentioned that *C. magellanica* is related to *C. linearis* Kylin, while Wienckelm and tom Dieck (1990) stated that it is related to *C. flagelliformis*. Molecular phylogenetic analysis by Kim (2002: 113) based on ribosomal DNA internal transcribed spacer (ITS) sequences attributed Chilean specimen of *C. magellanica* to the *C. flagelliformis* clade (unregistered sequence). Taking these arguments into account, *C. magellanica* seems to be an ambiguous taxon requiring confirmation of taxonomic distinctiveness. In any case, *C. magellanica* has only been recorded from Chile, Antarctica and subantarctic islands and has never been observed in the Russian Far East.

According to Kim and Kawai (2002), the diagnostic characters of *C. gracilis* are not very clear. Later, Klochkova *et al.* (2009a) described morphological and anatomical characteristics of this taxon from Kamchatka, which differed from the other *Chordaria* species.

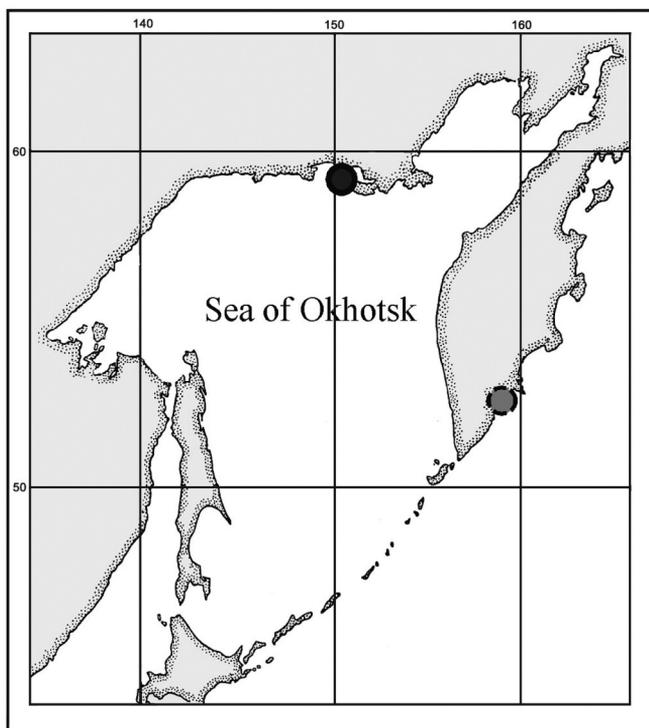
In this paper, we discuss the members of the genus *Chordaria* from the Russian Far Eastern area and describe a new *Chordaria* species collected from the northern continental area in the Sea of Okhotsk.

MATERIALS AND METHODS

Sample collection and observation

Specimens of *Chordaria* were collected from cape Veselij in the Taujskaya Bay, Sea of Okhotsk, Russia (Fig. 1) on 14 July 2008 at depths 3-4 m using SCUBA during a hydrobiological expedition attended by the authors M.N.B. and N.G.K. Collected plants were rinsed in freshwater and air dried. Dried material (not pressed) is kept in KamchatGTU and Kongju National University. Herbarium specimens [Type (NIBR_Cokh001Ru) and paratypes (NIBR_Cokh002Ru, NIBR_Cokh003Ru)] were deposited in the National Institute of Biological Resources (NIBR), Korea.

For microscopic observations, pieces of plant were cut 45 μm thick with a fine razor blade using freezing microtome. Micrographs were taken with Olympus DP50 digital camera affixed to an Olympus BX50 microscope using Viewfinder Lite and Studio Lite computer programs.



- Type locality of *Chordaria okhotskensis* sp. nov. from the Taujskaya Bay (Sea of Okhotsk)
- Collection cite of *C. okhotskensis* from the Avacha Bay (*C. chordaeformis* auct. non (Kjellman) Kawai et Kim, 2002: 333, fig. 4. AB066063, AB066064)

Fig. 1. Currently known distribution of *Chordaria okhotskensis* sp. nov.

Molecular phylogenetic analysis

Voucher specimens of plants used for the DNA extraction are kept in Kongju National University and Gangneung-Wonju National University. Total genomic DNA was extracted from silica gel-preserved material using the Invisorb Spin Plant Mini Kit (Inviteck, Berlin, Germany), following the manufacturer's instructions. Algal material (0.05 g) was ground in liquid nitrogen. Extracted total DNA was monitored on ethidium bromide-stained 0.8% agarose gels.

Plasid *rbcL* regions were amplified using the primer sets, DRL11F-DRL1R, DRL2F-DRL2R, DRL3FN-RU2 (Lee & Bae, 2002; Hwang *et al.*, 2005). PCR amplifications were carried out with a thermal cycler (Biometra, Goettingen, Germany) using an Enzynomics™ *n*Tag DNA Polymerase kit (Daejeon, Korea). The PCR conditions were as follows; an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were monitored on ethidium bromide-stained 0.8% agarose gels. DNA purification with High Pure™ PCR Product Purification Kit (Roche Diagnostics, Indianapolis, IN, USA) was used to clean PCR products. The sequencing reactions of the *rbcL* region were performed using ABI PRISM BigDye™ Terminator Cycle Sequencing Kits and its fluorescent signal was detected by ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, USA). Sequences were edited with the SeqEd DNA sequence Editor (ABI) software package. Sequences were aligned using the SeqPub multiple alignment program (Gilbert, 1996), and were refined visually with previously published NCBI data (see Table 1). The final alignment consisted of 44 sequences, including two new determined sequences. *Ectocarpus siliculosus* (Dillwyn) Lyngbye and *Acinetospora crinita* (Carmichael ex Harvey) Kornmann were used as outgroups. The 1750 aligned nucleotide positions of *rbcL* sequences were edited to remove the 5' and 3' PCR primer regions and *rbcL*-S spacer regions, to yield 1467 bp for phylogenetic inference. Phylogenetic reconstructions were implemented by Maximum Parsimony (MP) and Neighbor-Joining (NJ) with PAUP v. 4.0b 10 (Swofford, 2003) and Bayesian Inference (BI) using MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001). Prior to BI analyses, the Akaike Information Criterion (Akaike, 1974) was applied using the program Modeltest 3.6 (Posada & Crandall, 1998) to determine the best-fit model of sequence evolution. The TrN + I + G model was selected to reconstruct BI phylogenetic tree. BI was inferred running four simultaneous Monte-Carlo Markov chains for 1,000,000 generations, sampling every 100 generations, resulting in 10,000 trees. The first 300 trees generated before the chain reached stationary ('burn in') were discarded, and remaining trees were used to construct a majority-rule consensus tree showing relative occurrence (i.e. posterior probabilities) of all nodes in the tree. MP analyses were performed in a general heuristic search with the TBR branch-swapping algorithm, the MulTrees option, the branches with a maximum length zero collapsed, and 1000 random-additions replicates. The robustness and stability of the resulting MP tree topologies were assessed with bootstrap (BS) analyses (Felsenstein, 1985) using 100 replicates, each with 10 random sequence-addition. Unrooted phylogram of *Chordaria* species is also reconstructed based on MP and BI using same method as above.

Table 1. Species of *Chordaria* used for phylogenetic analysis

<i>Taxon</i>	<i>Collection locality</i>	<i>GenBank accession No.</i>	<i>Reference</i>
<i>Chordaria flagelliformis</i>	Kongsfjorden, Spitsbergen, Norway (KN834. Intertidal, col.: 1 Aug. 2003)	JN599169	Present study
<i>C. flagelliformis</i>	Orafsfjord, Iceland	AB066073	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Elby Point, Isle of Man	AB066074	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Port Erin, Isle of Man	AB066075	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066077	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066076	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Bergen, Norway	AB066078	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Hokkaido, Japan	AB066084	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Hokkaido, Japan	AB066083	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Hokkaido, Japan	AB066086	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Hokkaido, Japan	AB066085	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Avacha Bay, Kamchatka, Russia	AB066079	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Avacha Bay, Kamchatka, Russia	AB066080	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Cambell, St. Lawrence Island, USA	AB066081	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Cambell, St. Lawrence Island, USA	AB066082	Kim & Kawai (2002)
<i>C. flagelliformis</i>	Avacha Bay, Kamchatka, Russia	AY095324	Cho <i>et al.</i> (registered in GenBank as unpublished)
<i>C. flagelliformis</i>	Unspecified	AF207798	Simer & Pederson (registered in GenBank as unpublished)
<i>C. chordaeformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066065	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066068	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066066	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Ny-Ålesund, Spitsbergen, Norway	AB066067	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Hokkaido, Japan	AB066069	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Hokkaido, Japan	AB066071	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Hokkaido, Japan	AB066072	Kim & Kawai (2002)
<i>C. chordaeformis</i>	Gambell, St. Lawrence Island, USA	AB066070	Kim & Kawai (2002)

Table 1. Species of *Chordaria* used for phylogenetic analysis (*continued*)

<i>Taxon</i>	<i>Collection locality</i>	<i>GenBank accession No.</i>	<i>Reference</i>
<i>C. okhotskensis</i> sp. nov.	Taujskaya Bay, Sea of Okhotsk, Russia	JN590042	Present study
<i>C. okhotskensis</i> (<i>C. chordaeformis</i> auct. non (Kjellman) Kawai <i>et</i> Kim: Kim & Kawai, 2002: 333, fig. 4)	Avacha Bay, Kamchatka, Russia	AB066063	Kim & Kawai (2002)
<i>C. okhotskensis</i> (<i>C. chordaeformis</i> auct. non (Kjellman) Kawai <i>et</i> Kim: Kim & Kawai, 2002: 333, fig. 4)	Avacha Bay, Kamchatka, Russia	AB066064	Kim & Kawai (2002)

RESULTS

Chordaria okhotskensis N. G. Klochkova *et* H.-S. Kim sp. nov.

Figs 2-6

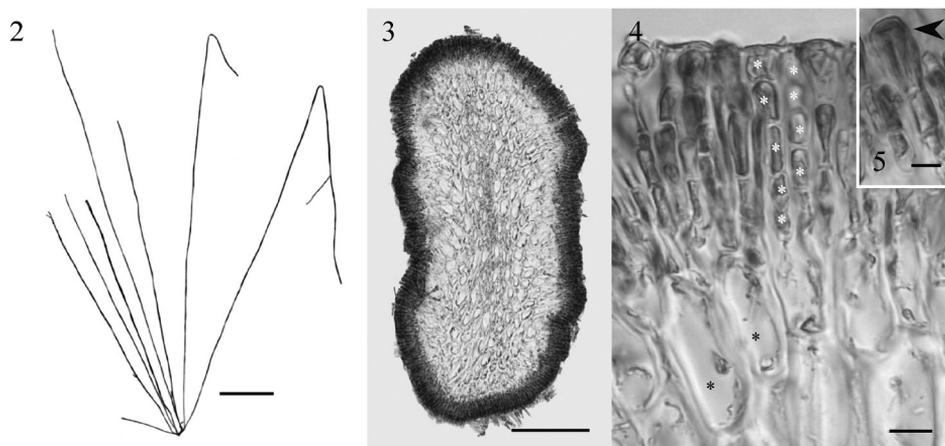
Diagnosis

Thalli erect, 9-30 cm high, flattened overall, elastic, flexible, slightly slimy, medium to dark brown in color, with shiny and smooth plant surface, attached by a small discoid holdfast. Erect axes simple, unbranched or with very few branches, generally issued as long dichotomies of the main axis and borne at acute angle. Primary dichotomous branches unbranched or with very few short branchlets of 1-3 mm at length. Occasionally, each primary dichotomous branch issues secondary dichotomy. Axes composed of medullar filaments and pigmented cortical assimilatory filaments. Medullary cells large (10-26 μm wide and 35-70 μm long), elongate (3-3.5 times longer than wide), with relatively thick cell wall; first row of peripheral medullary cells beneath the assimilatory filaments especially elongate. In cross-section, entire thallus flattened-elliptic, with rounded margins, 0.9-1.4 mm in length and 480-700 μm wide. Assimilatory filaments 4-5 celled, terminating in a slightly enlarged and elongated and often pyriform cell. Phaeo-phycean hairs were not found in our samples. Unilocular sporangia 35-61 μm in length, distributed sparsely (20-25 sporangia in one cross-section).

Holotype: Specimen No. NIBR_Cokh001Ru, Cape Veselij, Taujskaya Bay, Sea of Okhotsk, Russia (Fig. 2).

Etymology: The species was named after the locality where it was found, viz. the Sea of Okhotsk.

Presently, 45 species and infraspecific names of *Chordaria* are listed (Guiry, 2011), of which only five are currently in the genus *Chordaria* (Table 2). Among them, three species with distinct ecologies were reported from the Russian Far East (*C. chordaeformis*, *C. flagelliformis*, *C. gracilis*), and two other species are restricted to Antarctic and subantarctic islands and Chile (*C. linearis*, *C. magellanica*). The morphologies of the latter two are clearly different from our Okhotsk specimens (Table 3).



Figs 2-5. *Chordaria okhotskensis* sp. nov. (Taujskaya Bay, Coll.: Klochkova N. G. and Belij M. N., 07.14.2008). **2.** Type specimen (NIBR_Cokh001Ru). **3.** Cross-section of thallus, showing flattened-elliptic shape with obtuse polar sides. **4.** Enlarged photograph, showing 4-5 celled assimilatory filaments (white asterisks) and elongated medullary cells (black asterisks). **5.** Pyriform terminal cell of assimilatory filament. Fig. 2. Scale = 3 cm. Fig. 3. Scale = 100 μ m. Fig. 4. Scale = 10 μ m. Fig. 5. Scale = 5 μ m.

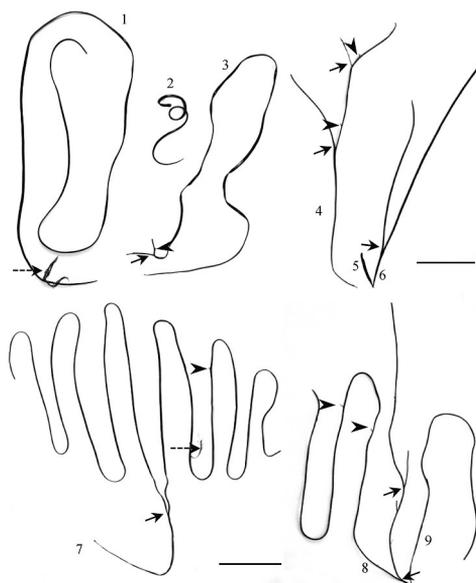


Fig. 6. Morphology of *Chordaria okhotskensis* sp. nov. (Taujskaya Bay, Coll.: Klochkova N. G. and Belij M. N., 07.14.2008). Field materials of nine plants that grew all together in a cluster on the sea bottom. Plants 1, 2, 5, 9 have simple unbranched thallus. Plant 3 has one short dichotomous branching of the top of main axis, whereas plants 6 and 7 have long dichotomous branching. In plants 4 and 8, one of the primary dichotomous branches has another dichotomous branching. Dichotomous branching is shown with arrows. Arrowheads point to very short branches several mm-long. Dashed arrows point to epiphytic algae growing on the surface of *C. okhotskensis*. Scales = 2 cm.

Table 2. *Chordaria* species, which are flagged as currently taxonomically accepted (data from Guiry, 2011). These 7 species were flagged as currently taxonomically accepted in the AlgaeBase. It should be noted, however, that two species (*C. disessa* and *C. nagaii*) currently belong to different genera (see footnotes 3-4 of this table)

Taxon	Type information	General distribution ¹	Distribution on Russian Far East	NCBI nucleotide sequence ²
<i>Chordaria chordaeformis</i>	Lectotype locality: Mosselbay, Spitsbergen (Kim & Kawai, 2002: 334) Lectotype: Kjellman F.R., 10 Jan. 1873; UPS (Kim & Kawai, 2002: 334)	Arctic: Greenland, Spitsbergen North Pacific: Alaska Japan	–	30*
<i>C. disessa</i> Setchell et Gardner ³ (Current name: <i>Sphaerotrichia disessa</i> (Setchell et Gardner) Zinova)	Type: USA, Washington, Orcas Island (Setchell & Gardner, 1924: 8)	Atlantic: USA, Washington, Orcas Island	–	0
<i>C. flagelliformis</i>	Type: 'Oc. Norvegico' (Silva <i>et al.</i> , 1996: 898)	Europe: Britain, Denmark, Greenland, Faroe Islands, France, Helgoland, Iceland, Ireland, Netherlands, Norway, Scandinavia, Spain, Sweden North America: Connecticut, Labrador, Maine, New Hampshire, New Jersey, Oregon, Quebec Arctic: Canada, Spitsbergen North Pacific: Alaska, Russia (Bering Sea, Kamchatka) Sea of Okhotsk, Japan, Korea Antarctic and subantarctic islands: Campbell Island Africa: Namibia, South Africa	Bering Sea and Kamchatka (summarized in Klochkova, 1998a: 389, depicted in Klochkova <i>et al.</i> , 2009a: 100, fig. 42) In flora of the Kuril Islands, this species was listed at least 25 times from 1936 till 1997 In flora of the Sea of Okhotsk, this species was listed at least 20 times from 1851 till 2006 Recorded for the flora of Peter the Great Bay and Tatars Strait	57
<i>C. gracilis</i>	Type: Unalaska, Alaska (Yoshida, 1998: 240)	North Pacific: Alaska, Bering Sea (Kamchatka) Sea of Okhotsk (continental coast), Kuril Islands, Japan, Korea	Bering Sea and Kamchatka (summarized in Klochkova, 1998a: 389, depicted in Klochkova <i>et al.</i> , 2009a: 102, fig. 43)	0

Table 2. *Chordaria* species, which are flagged as currently taxonomically accepted (data from Guiry, 2011). These 7 species were flagged as currently taxonomically accepted in the AlgaeBase. It should be noted, however, that two species (*C. disessa* and *C. nagaii*) currently belong to different genera (see footnotes³⁻⁴ of this table) (*continued*)

Taxon	Type information	General distribution ¹	Distribution on Russian Far East	NCBI nucleotide sequence ²
<i>C. gracilis</i>			<p><i>Kuril Islands</i> (Nagai, 1940: 46 (Islands Urup, Iturup, Kunashir and Small Kuril island chain), Zinova, 1959: 150 (islands Iturup, Shikotan, Kunashir), Mihajlova, 1959: 382 (Shikotan Island), Zinova <i>et al.</i>, 1980: 17, Gussarova & Semkin, 1986: 785 (islands Urup, Iturup, Kunashir), Gussarova <i>et al.</i>, 1993: 146 (islands Urup, Iturup, Kunashir))</p>	
<i>C. linearis</i>	<p>Type: Cape Horn (Peters, 1992a: 678)</p>	<p>Chile <i>Antarctic and the subantarctic islands</i>: Antarctica, Anvers Island, King George Island</p>	<p>In flora of the continental part of the <i>Sea of Okhotsk</i>, this species was listed at least 6 times from 1968 till 1993</p>	5
<i>C. magellanica</i>	<p>Type: "Sandy Point, Strait of Magellan" (Chile: Punta Arenas?) (Index Nominum Algarum database)</p>	<p>Tierra del Fuego, Chile <i>Antarctic and the subantarctic islands</i>: Fuegia</p>	-	0
		<p>Patagonia (Skottsberg l.c.), Concepcion (Bory l.c.) (Levring, 1960: 20)</p>		

Table 2. *Chordaria* species, which are flagged as currently taxonomically accepted (data from Guiry, 2011). These 7 species were flagged as currently taxonomically accepted in the AlgaeBase. It should be noted, however, that two species (*C. disessa* and *C. nagaii*) currently belong to different genera (see footnotes ³⁻⁴ of this table) (*continued*)

<i>Taxon</i>	<i>Type information</i>	<i>General distribution</i> ¹	<i>Distribution on Russian Far East</i>	<i>NCBI nucleotide sequence</i> ²
<i>C. nagaii</i> Tokida ⁴ (Current name: <i>Pseudochorda nagaii</i> (Tokida) Inagaki)	Type: Japan	Russia (southwest coast of Sakhalin Island, Tatars Strait, Sea of Japan) Japan	This species was rare in Tatars Strait, growing as single plants at depths 0-1 m in places with moderate surf (Klochkova, 1995: 88).	5

1 – Data obtained from AlgaeBase (Guiry, 2011).

2 – GenBank (NCBI, 2011).

3 – The name is an unambiguous synonym of *C. disessa*. Currently, *C. disessa* is recognized as a taxonomic synonym of *Sphaerotrichia disessa* (Setchell et Gardner) Zinova.

4 – Currently, *C. nagaii* is recognized as a taxonomic synonym of *Pseudochorda nagaii* (Tokida) Inagaki.

* – Out of 30 sequences registered in the NCBI, 2 sequences should be attributed to a new taxon, *C. okhotskensis* (*C. chordaeformis* auct. non (Kjellman) Kawai et Kim: Kim & Kawai, 2002: 333, fig. 4).

Table 3. Comparative morphology of existing *Chordaria* species. Data summarized from Kim (2002), Kim & Kawai (2002), Kjellman (1877), Klochkova (1995), Klochkova et al. (2009a), Peters (1992a, b)

<i>Taxon</i>	<i>External morphology</i>	<i>Plant size</i>	<i>Branching angle</i>	<i>Shape of thallus on cross-section</i>	<i>Shape of medullary cells on cross section</i>
<i>Chordaria chordaeformis</i> (plants from type locality, Spitsbergen)	Unbranched or with few branches	Up to 10 cm high (lectotype, Kim & Kawai, 2002: 334)	Acute	Terete (Kjellman, 1877)	NI
		Up to 20 cm high (plants from Ny-Ålesund, Svalbard; Kim & Kawai, 2002)			
<i>C. chordaeformis</i> (plants from Hokkaido)	Unbranched or with few branches	Up to 40 cm high (Kim & Kawai, 2002)	Acute	Slightly flattened (1.3 times longer than wide)*	Cylindrical (small cells)
<i>C. flagelliformis</i>	Branched	10-30 or more cm high	Obtuse	Terete	Cylindrical
<i>C. gracilis</i>	Branched	Up to 50 cm high	Acute	Terete	Cylindrical
<i>C. linearis</i>	Much branched, bushy	10-30 cm high	Acute (Peters 1992a: 680, fig. 2) / more or less obtuse (Peters 1992b: 687, fig. 18)	Terete	Cylindrical
<i>C. magellanica</i>	Branched	10-30 cm high	Obtuse	Terete	Data not found
<i>C. okhotskensis</i> sp. nov. (plants from the Sea of Okhotsk)	Unbranched or with few branches	9-30 cm high	Acute	Entirely flattened-elliptic (1.9-2.1 times longer than wide)	Elongate (large cells, 3-3.5 times longer than wide)
<i>C. okhotskensis</i> (plants from the Avacha Bay, Kamchatka: <i>C. chordaeformis</i> auct. non (Kjellman) Kawai et Kim: Kim & Kawai, 2002: 333, fig. 4)	Unbranched or with few branches	Up to 20 cm high (Kim & Kawai, 2002)	Acute	Slightly flattened (1.7 times longer than wide)*	Some cells are elongate (large cells, 1.7-1.8 times longer than wide)*

* - Estimations were made from the micrographs presented by Kim (2002: 75). Only the lower portions near the holdfast were sectioned (Kim, 2002).

NI - Data not indicated.

The three *Chordaria* species currently recorded from Russian Far East, and our new species *C. okhotskensis* are morphologically distinct from each other (Table 3).

Chordaria chordaeformis (formerly *C. flagelliformis* f. *chordaeformis*) is characterized by thalli that are simple and unbranched or with very few branches borne at acute angles. Although the anatomy of only recorded population from Russian Far East (Kim & Kawai, 2002) was not studied by us as we were unable to find plants, it was shown by Kim (2002) and summarized here in Table 3.

Kim (2002) depicted anatomical features of *C. chordaeformis* from Nemuro (Hokkaido) and Avacha Bay (Kamchatka), showing cross-sections of thalli at lower portion near the holdfast because this region showed comparable structures, while middle and upper inner structures were incomparable due to structural complexity. Some differences were clearly noticeable between *C. flagelliformis* and *C. chordaeformis*. While *C. flagelliformis* was terete and had large cylindrical cells (ca 17.5-47.5 μm in diameter) with thick cell wall, *C. chordaeformis* thalli were slightly flattened (Table 3). It is not known, however, if the entire thalli were slightly flattened or only the lower portions near the holdfast. Also, *C. chordaeformis* from Nemuro had smaller cylindrical cells (ca 9.5-16.6 μm in diameter) with thin cell walls. Plants of *C. chordaeformis* from the Avacha Bay had larger and slightly elongate cells (ca 16.6-23.8 μm wide and 28.5-42.8 μm long) with thick cell walls.

In cross-section, our Okhotsk specimens were more flattened than *C. chordaeformis* (1.9-2.1 times longer than wide). Moreover, thalli of our specimens were entirely flattened-elliptic, looking like a ribbon, and the medullary cells were large and elongate throughout the thallus (10-26 μm wide and 35-70 μm long). This morphology was stable in our Okhotsk specimens.

Chordaria gracilis is characterized by terete thalli, up to 50 cm high, light to medium or dark brown in color, sometimes black, with clearly traceable main axis and numerous long branches borne at acute angles (Fig. 7). In young plants, assimilatory filaments are 2-3 celled and in older plants up to 4 celled; medullary cells are cylindrical (Klochkova *et al.*, 2009a).

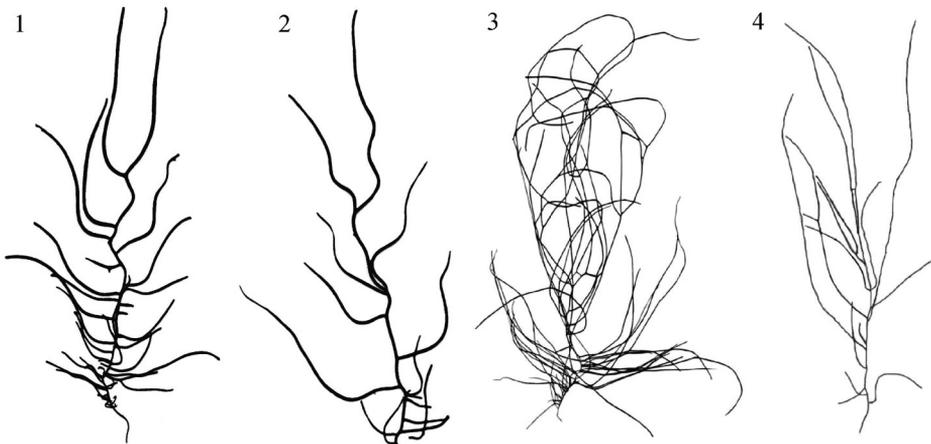


Fig. 7. Morphology of *Chordaria flagelliformis* (plants 1-2) and *C. gracilis* (plants 3-4) from Kamchatka (reproduced with permission from Klochkova *et al.* 2009a, plate 42, figs 2, 4 & plate 43, figs 1, 3). Plants 1 and 3 have numerous branches, while plants 2 and 4 have less branches.

Chordaria flagelliformis is characterized by terete thalli, 10-30 cm high or more, cartilaginous, almost black in color, has discernible main axis with numerous long branches 2.1 mm thick, borne at obtuse angles (Fig. 7). Assimilatory filaments are 6-8 celled (plants from Kamchatka, Klochkova *et al.*, 2009a) and 6-9 celled (plants from Tatars Strait, Klochkova, 1995); medullary cells are cylindrical.

Thus, our new species from the Sea of Okhotsk is most similar to *C. chordaeformis* morphologically, but differed anatomically. *Chordaria okhotskensis* was abundant in the Taujskaya Bay (Sea of Okhotsk, Russia) in July 2008, growing at depths of 3-4 m. Some large plants were fertile; however the number of unilocular sporangia was not large (20-25 sporangia distributed in the cortex in one 100-150 μm thick cross-section of 1.4 mm at length and 700 μm wide).

Molecular phylogeny

The genus *Chordaria* was resolved as monophyletic within the family Chordariaceae *sensu* Peters & Ramirez and composed of three clades with strong support: *C. flagelliformis*, *C. chordaeformis*, and *C. okhotskensis* sp. nov. (Fig. 8). The relationship among the three species was robust and similar to the results of Kim & Kawai (2002, fig. 10a).

Our phylogenetic tree of *Chordaria* provided information on existing geographically isolated sister clades of Atlantic and Pacific populations of *C. flagelliformis* and *C. chordaeformis*, and *C. okhotskensis* formed a separate clade. Sequence variation among *C. flagelliformis* geographical subclades varied from 0-7 bp (0.00-0.48 %) in the Atlantic and 0-14 bp (0.00 -0.95 %) in the Pacific populations. The total variation within *C. flagelliformis* varied from zero to 19 bp (0.00-1.29%). Similarly, variation within *C. chordaeformis* ranged from 1-6 bp (0.07-0.27%) in the Atlantic and 4-8 bp (0.27-0.55 %) in the Pacific populations. The total sequence variation within *C. chordaeformis* ranged from one to 18 bp (0.07-1.23%). *C. okhotskensis* showed somewhat lower levels of intraspecific variation, ranging from 3-6 bp (0.20-0.42%). Interspecific variation was remarkably higher: 29-38 bp differences between *C. flagelliformis* and *C. chordaeformis*, 36-43 bp between *C. flagelliformis* and *C. okhotskensis*, and 22-29 bp between *C. chordaeformis* and *C. okhotskensis*.

DISCUSSION

In the Russian Far East, three *Chordaria* species were reported, of which *C. flagelliformis* and *C. gracilis* are the most common (Klochkova, 1995, 1998a; Klochkova *et al.*, 2009a). The morphology and ecology of these two species from Kamchatka and surrounding waters were described and illustrated by Klochkova *et al.* (2009a). Our new species of *Chordaria* from the Sea of Okhotsk differs in morphology, anatomy, ecology, and distribution. It is morphologically very close to *C. chordaeformis*, but clearly genetically and anatomically distinct, which warrants recognition at the species level.

Chordaria chordaeformis was reported from the Avacha Bay in Kamchatka, Russia (Kim & Kawai, 2002). However, although outer morphology of specimens from the Avacha Bay supported attribution to *C. chordaeformis*,

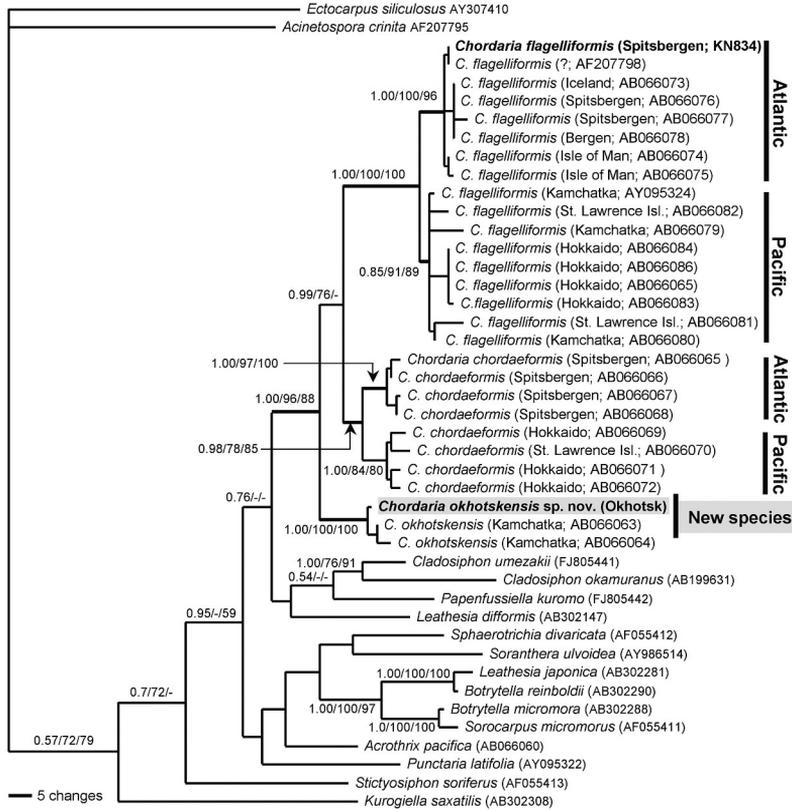


Fig. 8. Bayesian tree for 41 Chordariacean taxa (except two outgroup species) estimated from *rbcL* sequence data (1467 bp) showing three independent genetic lineages of genus *Chordaria* including new species *C. okhotskensis*. Newly sequenced members are represented as boldface. Numbers above the branches indicate Bayesian PP, MP and NJ bootstrap values in respectively (values $\leq 0.50/50\%$ shown only).

phylogenetically they were distinct from the Pacific and Atlantic clades, forming a separate group (Kim & Kawai, 2002). Also, some anatomical differences are noticeable between plants from Nemuro (e.g. true *C. chordaeformis*) and the Avacha Bay (see Kim, 2002). Our molecular phylogenetic results support attribution of the specimens from the Avacha Bay (AB066063, AB066064) to *C. okhotskensis*. The Okhotsk specimens differ from those from the Avacha Bay in some respects, including the thallus that is somewhat more flattened and the medullary cells which are more elongate. Since the current diagnosis of *C. okhotskensis* is based on the observations of the Okhotsk specimens only, additional morphological observations from specimens collected elsewhere (including Kamchatka) will be required to amend its description.

It should be noted that *C. okhotskensis* (*C. chordaeformis* auct. non (Kjellman) Kawai *et al.* Kim, 2002: 333, fig. 4) found by Dr. H. Kawai in the Avacha Bay in July 1998 was not found in this locality before (Klochkova, 1998a) and in recent years (Klochkova *et al.*, 2009b). It was once included in floristic list of Starichkov Island, an area located 30 km from the city Petropavlovsk-Kamchatsky

in the Avacha Gulf, Kamchatka (Selivanova & Zhigadlova, 2009), but without morphology or photographic evidence to support species identification. Moreover, another research group has also worked on Starichkov Island in the same time period and was unable to find plants morphologically referable to this taxon (Klochkova *et al.*, 2009b). From 1970-es, the Avacha Bay has been suffering from an extremely high anthropogenic pollution, and in 1990-es its ecological degradation was especially severe (Klochkova & Klotchkova, 1998; Klochkova & Berezovskaya, 2001; Ocheretyana & Klochkova, 2010). The number of species in that area has reduced dramatically and ephemeral green tide-forming algae with very short life cycles became dominant (Ocheretyana & Klochkova, 2010). We do not know if this species has become extinct in the Avacha Bay due to pollution or we were unable to find it for some other reasons. Whatever the case, data by Kim & Kawai (2002) are the only reliable evidence of this species existing in Kamchatka supported with morphology and phylogeny. To date, this taxon has never been observed in any other coastal area of Kamchatka peninsula, including its eastern and western coasts (N.G.K., personal observations). A possibility of an introduction of this taxon into the Avacha Bay cannot be overruled completely, because the locality where it was collected in 1998 was the cape situated at the entrance to the large seaport located inside the Avacha Bay. Therefore, it is interesting to find *Chordaria* species on the continental coast of the Sea of Okhotsk, which is phylogenetically related to the taxon from the Avacha Bay because the two geographical localities are very far from each other and have very different climates and hydrology.

In both *C. flagelliformis* and *C. chordaeformis*, the simple comparison of heterogeneity values gave a hint that the Pacific population was older than the Atlantic population, because its heterogeneity values were higher. Also, so far the genetic lineage of *C. okhotskensis* occurred only in the North West Pacific. Furthermore, our tree also suggested that *C. okhotskensis*, which is positioned at base as a sister group for the other two *Chordaria* species, can be regarded as a progenitor of the genus *Chordaria*. Of course, it should be noticed that not all existing *Chordaria* species were included in the analysis. The isolation of the Atlantic and Pacific subclades of *C. flagelliformis* and *C. chordaeformis* might have occurred at least before the last Glacial Maximum (during 26,500 - 19,000 years ago).

The unique macroalgal flora of the Sea of Okhotsk (e.g. Perestenko, 1994; Klochkova, 1998b; Klochkova & Emelyanova, 2005; Klochkova *et al.* 2009a) is due to the system of currents in the region, mosaic climatic and hydrological conditions in its different areas, and geological history, which ensured long-term geographic isolation (e.g. Zinova *et al.*, 1980; Perestenko, 1994). This long-term geomorphological situation is also supported with paleogeological data (e.g. Biskae, 1975). The Sea of Okhotsk is the coldest sea in East Asia, with winter temperatures nearly comparable to the Arctic, especially on its north. The ice regime of this sea is especially severe. On the northwest coast, coastal seawater becomes freshened due to the freshwater discharge by the Amur River near northwest of Sakhalin Island, and to some extent this factor contributes to the floristic isolation of the continental part of the Sea of Okhotsk from warmer-water species. In its northern areas, heavy ice inhibits species movement from the continental coast to western Kamchatka. On its eastern border, the sea has weak seawater exchange with the Sea of Japan and Bering Sea, and also the Kuril Islands act like a barrier protecting it from the direct influence of the Pacific Ocean's water mass. Moreover, on southwest of Kamchatka substrates are soft and sandy and not suitable for seaweed attachment, thus inhibiting species exchange with the Bering Sea.

One of the authors of this paper (M.N.B.) has SCUBA-dived in various northern localities of the Sea of Okhotsk for many years and has conducted underwater algal collections in different seasons of the year. Another author (N.G.K.) participated in several hydrobiological expeditions held on the continental coast of the Sea of Okhotsk and on western Kamchatka (Klochkova *et al.*, 2009a). To date, we found this new *Chordaria* species on the north of the Sea of Okhotsk, Taujskaya Bay, where it grew very abundantly in the sub-littoral zone at depths 3-4 m. We presume its main distribution to be on the continental coast of the Sea of Okhotsk; however, more field studies are necessary to verify the distribution area or other possible existing populations of our new *Chordaria* species.

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