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Callophyllis cornu-cervi (Perestenko) comb. nov. (Gigartinales, Rhodophyta): re-evaluation of the endemic monotypic genus *Ionia* from the Sea of Okhotsk based on morphology and molecular evidence

Abstract: The monotypic kallymeniacean genus *Ionia* established as *Iona cornu-cervi* is endemic to the Sea of Okhotsk. We observed the type material of the species deposited in the Komarov Botanical Institute of the Russian Academy of Sciences (LE, St. Petersburg, Russia) and compared it with fresh collections near the type locality. Analysis of the *rbcL* dataset revealed that *I. cornu-cervi* was not distinct from other genera of the Kallymeniaceae but consistently nested within the genus *Callophyllis* and formed a sister relationship with *Callophyllis variegata*. Here, we propose the new combination *Callophyllis cornu-cervi* based on *I. cornu-cervi* and treat the genus *Ionia* as a synonym of *Callophyllis*. *Callophyllis cornu-cervi* is distinguished by a thallus with many slightly convoluted segments positioned at an angle to each other, forming an erect and more or less rigid plant, and a medulla composed of intermingled large and small cells.

Keywords: *Callophyllis cornu-cervi*; *Ionia*; Kallymeniaceae; *rbcL*; Sea of Okhotsk; systematics; taxonomy.

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Introduction

Morphologically described monotypic genera of red algae require molecular analysis for a better understanding of their phylogenetic relationships, including those occurring in the Sea of Okhotsk, a large seawater basin surrounded by the Kamchatka Peninsula on the east, the Kurile Islands to the southeast, Hokkaido Island to the south and the Siberian coast and Sakhalin Island to the north and west. This is the coldest sea in East Asia, with winter temperatures closely comparable to those of the Arctic, especially on its northern shore, and the ice regime in this sea is especially severe. Recently, interest in these waters has been increasing because of the diversity of seaweed, although accessibility for collecting trips is restricted (e.g., Klochkova et al. 2009, 2010, 2012a,b). Many rare and endemic species contribute to the diversity and uniqueness of the marine algal flora. In the Sea of Okhotsk, representatives of the red algal endemics are *Ionia cornu-cervi* Perestenko, *Palmaria moniliformis* (Blinova et Zinova) Perestenko, *Scagelia breviariculata* Perestenko and *S. subnuda* (Ruprecht) Perestenko.

Information on *Ionia cornu-cervi* described from Iony Island, a small unpopulated island in the Sea of Okhotsk, is extremely limited. It belongs to a monotypic genus assigned to the family Crossocarpaceae (Cryptonemiales, Rhodophyta) established by Perestenko (1975) on the basis of vegetative structure and morphology of the post-fertilization fusion cell. Perestenko also assigned the genera *Cirrularia* Tokida et Masaki, *Crossocarpus* Ruprecht, *Erythrophylum* J. Agardh and *Kallymeniopsis* Perestenko to the Crossocarpaceae, but the family was later rejected by Hansen and Lindstrom (1984), who considered it to be a subgroup within the Kallymeniaceae based on female reproductive anatomy. Nevertheless, Perestenko (1996 [1994]) maintained her earlier taxonomy and added four additional taxa

to the Crossocarpaceae, including *Beringia* Perestenko, *Ionia*, *Velatocarpus* Perestenko and *Hommersandia* Hansen et Lindstrom. Although the Crossocarpaceae was not broadly recognized outside Russia, Schneider and Wynne (2007) included this family in their classification of red algal genera, excluding only one genus, *Erythrophyllum*, from Perestenko's original list. However, the family Crossocarpaceae has lost genera for which molecular sequence data indicate they should be transferred to the Kallymeniaceae (Harper and Saunders 2002, Clarkston and Saunders 2010, 2012, D'Archino et al. 2012).

The family Kallymeniaceae is one of the largest in the Gigartinales, including approximately 28 genera and 155 species (Guiry 2012). *Callophyllis* Kützinger is the most diverse genus in the family, including approximately 57 species that occur in cold and temperate waters of both hemispheres (Guiry 2012). However, the simple morphology of the species has led taxonomists to over- and underestimate the species diversity of the genus. Recently, Arakaki et al. (2011) revised *Callophyllis* from Chile based on morphology and *rbcL* analysis.

The purpose of the present study was to address phylogenetic relationships of *I. cornu-cervi* from the Sea of Okhotsk and determine its relationships to other Kallymeniaceae. We studied in detail the morphology and molecular phylogeny of fresh collections from near the type locality in the Sea of Okhotsk as well as the morphology of the type material. Both morphological and molecular data supported the placement of *I. cornu-cervi* in the genus *Callophyllis*.

Materials and methods

Collection of specimens and observation

Our specimens of *Ionia cornu-cervi* were collected from Cape Veselij in Taujskaya Bay, Sea of Okhotsk, Russia on July 17, 2008 at depths of 6–7 m using SCUBA during a hydrobiological expedition attended by the authors M.N.B. and N.G.K. Taujskaya Bay (59°18'N, 150°24'E) and the type locality of this species, Iony Island (56°24'N, 143°23'E), are located at a distance of approximately 300 km from one another, and the distance from Iony Island to the northern continental coast is about 230 km. Plants collected were briefly rinsed in freshwater, pressed on herbarium sheets and dried. Herbarium specimens used for the observations in this study are held in KamchatGTU, Kongju National University and Chungnam National University. For microscopic observations, a dry piece of

thallus was re-hydrated in sterilized seawater, cut with a fine razor blade and observed under a microscope. Micrographs were taken with an Olympus DP50 digital camera attached to an Olympus BX50 microscope (Tokyo, Japan) using Viewfinder Lite and Studio Lite software (Better Light, Placerville, CA, USA).

The type specimen (Figure 1A) and illustrations of *I. cornu-cervi* (Figure 1B–D), which are currently kept in the Komarov Botanical Institute of the Russian Academy of Sciences (LE, St. Petersburg, Russia), were observed and compared with our samples.

Molecular phylogenetic analysis

Voucher specimens of plants used for the DNA extraction are kept in Chungnam National University (coll.: Cape

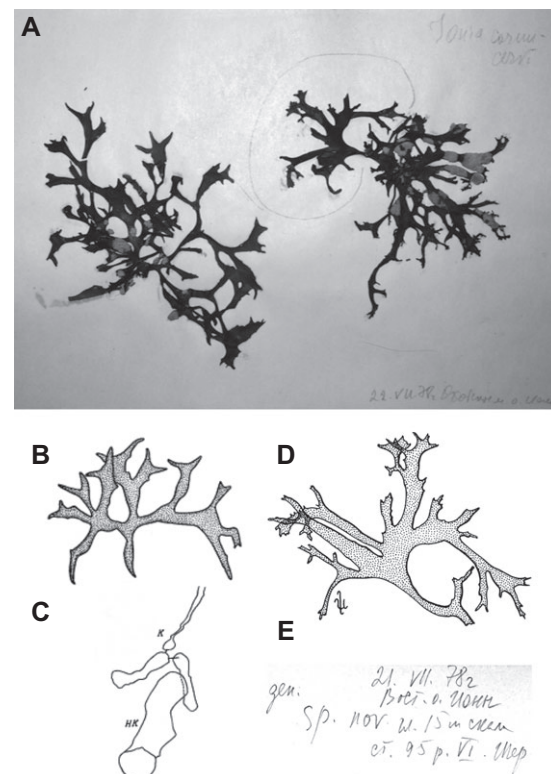


Figure 1 *Callophyllis cornu-cervi*: type and original illustrations (known previously as *Ionia cornu-cervi*), photographed in June 2012. (A) Type specimen preserved in the Komarov Botanical Institute (LE, St. Petersburg, Russia). (B–C). Sketch of a small part of thallus (B) and a carpogonial branch with supporting cells (C) depicted in Perestenko's monograph upon first description of the genus and species (reproduced from Perestenko (1996[1994]), tables IV, 13 and XXXVI, 5). (D) An unpublished drawing of the type specimen by Perestenko preserved in LE. (E) The date on the drawing label does not match the date on the type specimen (21.VII.1978 vs. 22.VII.1978, respectively).

Veselij, Taujskaya Bay, Sea of Okhotsk, Russia. 17.VII.2008, depths 6–7 m, by the authors M.N.B. and N.G.K.).

Genomic DNA was extracted from approximately 5 mg of dried thallus ground in liquid nitrogen using an Invisorb Spin Plant Mini Kit (Invitex, Berlin-Buch, Germany) according to the manufacturer's protocol. Primer pairs for amplification and sequencing of *rbcl* gene were F145–R898 (CAACCAGGWTAGATCCAGTAGAAGC – GACGAGAATAAGTTGARTTACC, Kim et al. 2010) and F645–RrbclS (ATGCGTTGAAAAGAAAGATTCT – GTTCTTTGTGTTAATCTCAC, Freshwater and Rueness 1994, Lin et al. 2001). Polymerase chain reactions (PCR) and sequencing were performed as detailed in Geraldino et al. (2010). All sequences of the forward and reverse strands were determined, and the electropherograms were edited using Chromas, version 1.45 software (McCarthy 1998). The sequences determined in this study have been deposited in GenBank under accession numbers KC408802 and KC408803. Nucleotide sequences (Table 1) were aligned using Se-Al, version 2.0a11 (Rambaut 2002).

rbcl phylogeny was reconstructed using maximum likelihood (ML). ML analyses were performed with RAxML v.7.2.8 software (Stamatakis 2006) using the GTRGAMMA model. We used 300 independent tree inferences, applying options of automatically optimized SPR rearrangement and 25 distinct rate categories in the program to identify the best tree. Statistical support for each branch was obtained by 1,000 bootstrap replications using the same substitution model.

Bayesian analyses (BA) were performed with MrBayes, version 3.2.1 software (Ronquist et al. 2012) using the Metropolis-coupled Markov chain Monte Carlo (MC³) with the GTR+G+I model. For each matrix, 2,000,000 generations of two independent runs were performed with four chains and sampling of trees every 100 generations. The burn-in period was identified graphically by tracking the likelihoods at each generation to determine whether they had reached a plateau. The 34,802 trees sampled at the stationary state were used to infer the Bayesian posterior probability (BPP).

Results

The original description of *Ionia cornu-cervi* by Perestenko (1996 [1994]) contained two ink drawings showing a small part of the thallus and a carpogonial branch with supporting cells (Figure 1B–C). However, no photograph of the herbarium voucher designated by Perestenko as a type specimen was provided. There were also no comments on

the number of plants examined to describe the genus and species.

A single herbarium sheet with two pressed plants labeled in handwriting as *Ionia cornu-cervi* is preserved in the Komarov Botanical Institute (herbarium code: LE, Thiers 2012) in St. Petersburg, Russia (Figure 1A) and is believed to be the type specimen (T.A. Mihailova, personal communication). There is no indication as to whether these two plants are different individuals or pieces separated from the same thallus. We believe that the drawing of the type specimen by Perestenko (1996) was made using these pressed plants as a template, as one encircled branch coincides with the second unpublished drawing, which is deposited together with the type specimen in LE (Figure 1D). However, the type specimen is labeled as collected on 22.VII.1978, whereas the drawing is labeled with a different day, 21.VII.1978 (Figure 1E). As indicated by Perestenko (1996) in the Latin diagnosis of *I. cornu-cervi*, the type was collected on 21.VII.1978 by A.M. Scheremetevsky. As no other herbarium voucher specimen of *I. cornu-cervi* is available in the collection of type specimens in LE, we believe that a mistake was made, and the collection date should be 22.VII.1978, as written in Perestenko's handwriting on her type specimen. This specimen should be recognized as the type (Figure 1A).

Morphological observations

The following description is mainly based on observations made on our samples collected from Taujskaya Bay (Figure 2). The original description by Perestenko (1996) was also taken into account and combined with our observations.

Thalli are erect, bushy, up to 10–12 cm tall, more or less rigid, bright-red when fresh (Figure 3A–B) and dark crimson (Figure 3C–D) or dark burgundy (Figure 3E) when dried, and attached by a tiny disc. Blades are divided into segments; the central axes are not distinct. Axes are di- or trichotomously to oppositely arranged, or randomly divided, fan-shaped and 200–310 µm in thickness, with lighter apices on the segments. Each blade divides in one surface level; however, because the individual segments are slightly convoluted they appear at different angles to each other, thus forming a bush-like, more or less rigid plant. Lateral branches are 2–4 mm broad and linear-shaped or widening where branching occurs (Figure 3C–E). The medulla is composed of intermingled large and small cells with very thick walls. Cortices are one to two cells thick (Figure 3I–J). Plants usually grow in clusters, forming tufts.

Thalli collected in Taujskaya Bay were sterile. According to Perestenko (1996), reproductive structures

Table 1 List of species of the Kallymeniaceae used in this study and accession numbers in GenBank.

Species	Collection site	GenBank accession number	Reference
<i>Callophyllis atrosanguinea</i> (Hooker et Harvey) Hariot	Punta Dungeness, Chile	HQ910504	Arakaki et al. (2011)
<i>C. atrosanguinea</i>	Chatham Is., New Zealand	GQ376537	D'Archino et al. (2010)
<i>C. conceptionensis</i> Arakaki, Alveal et Ramírez	Isla Santa María, Concepción, Chile	HQ910496	Arakaki et al. (2011)
<i>C. cornu-cervi</i> (Perestenko) T.A. Klochkova, N.G. Klochkova et S.M. Boo	Cape Veselij, Taujskaya Bay, Sea of Okhotsk, Russia	KC408802, KC408803	This study
<i>C. crispata</i> Okamura	Tokawa, Choshi, Chiba Prefecture, Japan	UO4190	Freshwater et al. (1994)
<i>C. decumbens</i> Agardh	Northland, Doubtless Bay, Cable Bay, New Zealand	GQ376536	D'Archino et al. (2010)
<i>C. edentata</i> Kylin	Cast Ashore, Hopkins Marine Station, Pacific Grove, California, USA	HQ910499	Arakaki et al. (2011)
<i>C. firma</i> (Kylin) R.E. Norris	Mar Vista Resort, W. San Juan I., WA, USA	HQ910506	Arakaki et al. (2011)
<i>C. hombroniana</i> (Montagne) Kützing	Dunedin, New Zealand	HM587175	D'Archino et al. (2011)
<i>C. japonica</i> Okamura	Nemoto, Awa Co., Chiba Pref., Japan	HQ910507	Arakaki et al. (2011)
<i>C. laciniata</i> Kützing	Penmarch, Brittany, France	HQ910508	Arakaki et al. (2011)
<i>C. lambertii</i> (Turner) Agardh	Port MacDonnell, Australia	HQ910509	Arakaki et al. (2011)
<i>Callophyllis linguata</i> Kylin	Pta. Adley, Bahía Fildes, King George I., Antarctic Peninsula	HQ910510	Arakaki et al. (2011)
<i>C. macrostiolata</i> Arakaki, Alveal et Ramírez	Isla Santa María, Concepción, Chile	HQ910501	Arakaki et al. (2011)
<i>C. obtusifolia</i> J. Agardh	Baja California, México	HQ910511	Arakaki et al. (2011)
<i>C. ornata</i> (Montagne) Kützing	Stewart Is., New Zealand	HM587180	D'Archino et al. (2011)
<i>C. pinnata</i> Setchell et Swezy	Central Beach of Moss Beach, California, USA	HQ910498	Arakaki et al. (2011)
<i>C. rangiferinus</i> (Turner) Womersley	Port MacDonnell, S. Australia	HQ910512	Arakaki et al. (2011)
<i>C. variegata</i> (Bory) Kützing	Isla Santa María, Concepción, Chile	HQ910494	Arakaki et al. (2011)
<i>C. variegata</i>	Playa Mendieta, Ica, Perú	HQ910495	Arakaki et al. (2011)
<i>C. variegata</i>	Stewart Is., New Zealand	HM587184	D'Archino et al. (2011)
<i>C. violacea</i> (Agardh) Kylin	Pigeon Point, San Mateo Co., CA, USA	UO4191	Freshwater et al. (1994)
<i>Callophyllis</i> sp.	Bahía Collins, King George I., Antarctic Peninsula	U21802	Fredericq et al. (1996)
<i>Cirrulicarpus gmelinii</i> (Grunow) Tokida et Masaki	Nosoppu, Hokkaido, Japan	GQ376539	D'Archino et al. (2010)
<i>Ectophora depressa</i> J. Agardh	Chatham Island, New Zealand	GQ376535	D'Archino et al. (2010)
<i>E. marginata</i> D'Archino et W.A. Nelson	Northland, New Zealand	HM587178	D'Archino et al. (2011)
<i>Erythrophyllum delesserioides</i> J. Agardh	Washington, USA	AF212186	Fredericq et al. (1996)
<i>Kallymenia cribrosa</i> Harvey	Tarcoola Beach, Australia	EU349216	Krayesky et al. (2009)
<i>K. feldmannii</i> Codomier	Italy	EU543487	D'Archino et al. (2010)
<i>K. reniformis</i> (Turner) J. Agardh	Piguet, Brittany, France	AY294377	Fredericq et al. (1996)
<i>K. reniformis</i>	France	U26827	Fredericq et al. (1996)
<i>K. rosea</i> Womersley et R.E. Norris	Jervis Bay, Australia	AF212190	Fredericq et al. (1996)
<i>Stauromenia australis</i> D'Archino et W.A. Nelson	Stewart Is., New Zealand	HM587190	D'Archino et al. (2012)
<i>Meredithia microphylla</i> (J. Agardh) J. Agardh	Skomer Is., Wales, United Kingdom	GQ376538	D'Archino et al. (2010)
<i>Psaromenia berggrenii</i> (J. Agardh) D'Archino, W.A. Nelson et Zuccarello	Bay of Islands, New Zealand	GQ376532	D'Archino et al. (2010)
<i>Pugetia delicatissima</i> R.E. Norris	New Zealand	HQ910513	Arakaki et al. (2011)
<i>P. delicatissima</i>	Marsfell Beach, New Zealand	HM587192	D'Archino et al. (2011)
<i>Pugetia</i> sp.	Dunedin, New Zealand	HM587194	D'Archino et al. (2011)
<i>Pugetia</i> sp.	Dunedin, New Zealand	HM587199	D'Archino et al. (2011)
<i>Pugetia</i> sp.	Wellington, New Zealand	HM587195	D'Archino et al. (2011)
<i>Rhizopogonia asperata</i> (Harvey) Kylin	Wellington, New Zealand	HM587196	D'Archino et al. (2011)

(Table 1 Continued)

Species	Collection site	GenBank accession number	Reference
<i>Thamnophyllis laingii</i> (J. Agardh) R.E. Norris	Brighton, New Zealand	HM587197	D'Archino et al. (2011)
<i>Dumontia contorta</i> (Gmelin) Ruprecht	USA	U26823	Fredericq et al. (1996)
<i>Rhodopeltis borealis</i> Yamada	Taiwan	U26824	Fredericq et al. (1996)

are borne at the apices of segments. Carpogonia are $6.5 \times 8.5 \mu\text{m}$; the first and second cells of the carpogonial branch are $9.8 \times 42 \mu\text{m}$ ($14 \mu\text{m}$ wide at the distal end). Supporting cells are $28 \times 72\text{--}75 \mu\text{m}$, and auxiliary cells are $20\text{--}28 \times 48\text{--}70 \mu\text{m}$.

In Taujskaya Bay, this species grew preferentially on rocks in subtidal areas and was restricted to depths of 6–7 m, where it formed a seaweed assemblage with other deep-water red algae, including *Hideophyllum yezoense* (Yamada *et* Tokida) A. Zinova, *Neoptilota asplenioides* (Esper) Kylin, *Odonthalia setacea* (Ruprecht) Perestenko and coralline algae. It also occurred under the canopy of laminarian algae when present in the area. In all plants of *Ionia cornu-cervi* we investigated, both young and old thalli were covered with numerous colonies of bryozoans

and tubes of sphaeodoridaen polychaetes (Figure 3F–G). These organisms abundantly cover the type specimen (Figure 3H).

Phylogenetic relationships

The two *rbcl* sequences of *Ionia cornu-cervi* from Taujskaya Bay were identical. This species was always nested within *Callophyllis* of the Kallymeniaceae (Figure 4). It formed a sister relationship to *C. variegata* (Bory de Saint-Vincent) Kützing from Chile and Peru (HQ910494, HQ910495). It was distantly related to the Japanese species *C. crispata* Okamura and *C. japonica* Okamura.

Our results suggest that the genus *Ionia* should not be maintained and that the genus and species should be incorporated into *Callophyllis*. The following taxonomic combination is proposed: *Callophyllis cornu-cervi* (Perestenko) T.A. Klochkova, N.G. Klochkova *et* S.M. Boo comb. nov.

Basionym *Ionia cornu-cervi* Perestenko (1996 [1994]), p. 106, tables IV, 13 and XXXVI, 5).

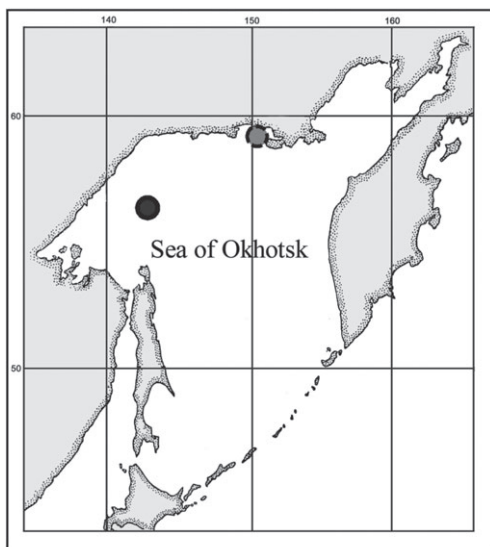
Russian name Иония олени рога ('olenii roga').

Type locality Iony Island, Sea of Okhotsk, Russia.

Type specimen From a depth of 15 m, rocky substratum, Iony Island, Sea of Okhotsk, Russia. Coll.: 22.VII.1978 by A.M. Scheremetevsky. The specimen is kept in the Komarov Botanical Institute of the Russian Academy of Science, St. Petersburg, Russia (herbarium code: LE).

Discussion

Accurate identification of many red algae to the species level from only morphological characters can be difficult (Clarkston and Saunders 2010). We collected plants morphologically attributable to *Callophyllis cornu-cervi*. Although it would be desirable to study plants from the type locality, this area is very difficult to access, as this small, remote and unpopulated island, which appears as



● Type locality of *Callophyllis cornu-cervi* (Perestenko) T.A. Klochkova, N.G. Klochkova *et* S.M. Boo from Iony Island (Sea of Okhotsk).

● Taujskaya Bay, the site at which we collected *C. cornu-cervi*.

Figure 2 *Callophyllis cornu-cervi*: map of currently known distribution.

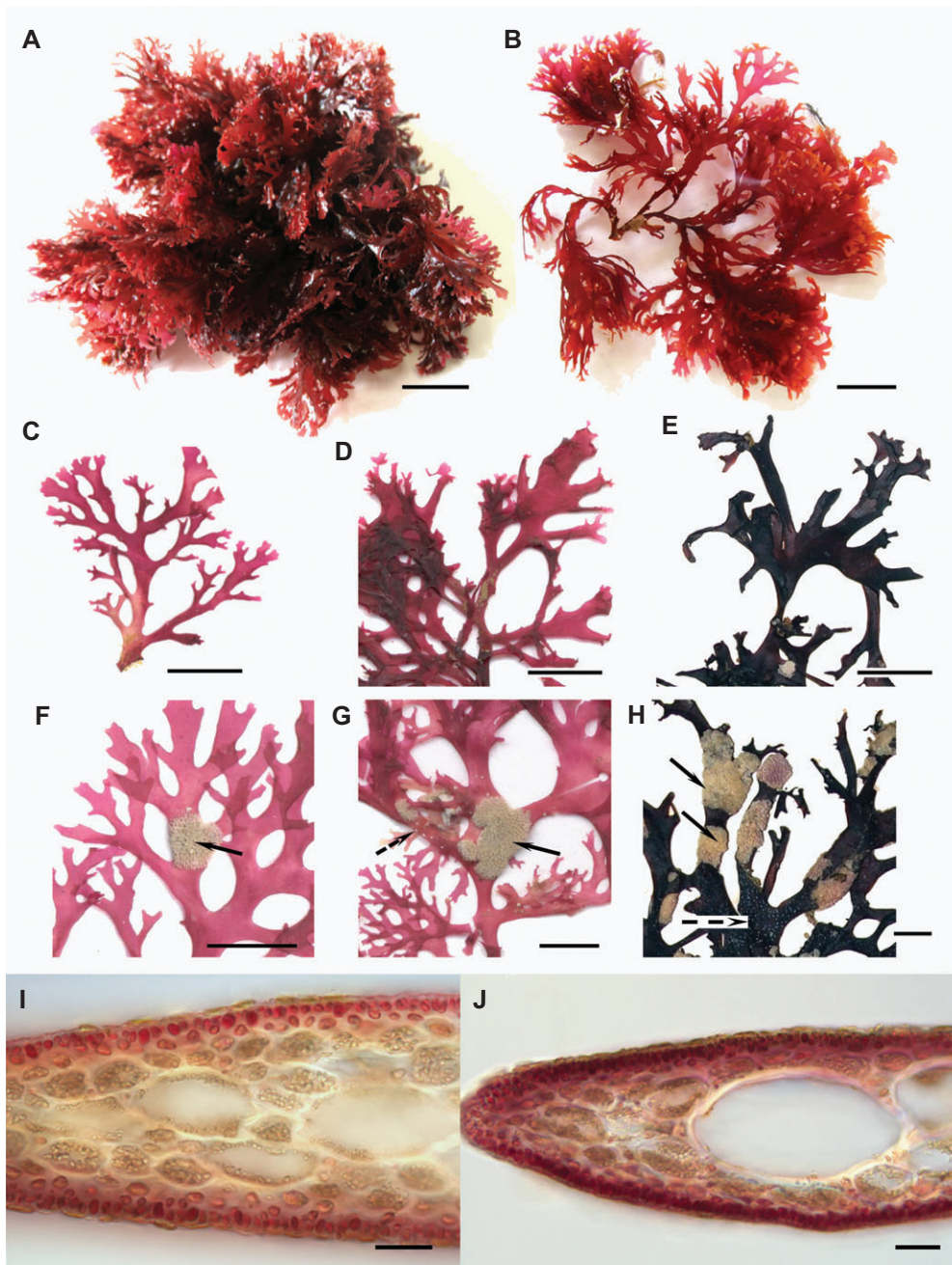


Figure 3 *Callophyllis cornu-cervi*: morphology and anatomy of vegetative plants.

(A–B) Fresh plants from Taujskaya Bay after collection from seawater. (A) Several plants growing in a tuft. (B) Individual plant showing typical *Callophyllis* morphology. (C) Small piece of the young thallus. The color of dried herbarium specimens changes to dark crimson or dark burgundy. (D–E) Enlarged fragments of the type specimen (E) and our material (D) showing similar branching patterns. (F–H). Encrusting bryozoans (solid arrows) and tubes of sphaeodoridaen polychaetes (dashed arrows) on our samples (F, young branch; G, old branch) and on the type material (H). (I) Cross-section of the blade, showing intermingled large and small cells. (J) Occasionally, one large cell occupies the whole medullar layer. Scale bars: A–E, 1 cm; F–H, 5 mm; I, 20 μ m; J, 50 μ m.

a rock in the sea, is located in the open sea in a region of harsh climate and difficult navigation. To our knowledge, only a few biological expeditions have ever surveyed this island, and no data on its seaweed flora exist to date, except for the collection of *C. cornu-cervi*. The harsh

climate of Taujskaya Bay, located 300 km away from the type locality, is similar to that around Iony Island.

The original description of *C. cornu-cervi* was limited and not supported by significantly informative illustrations; however, several distinct characteristics

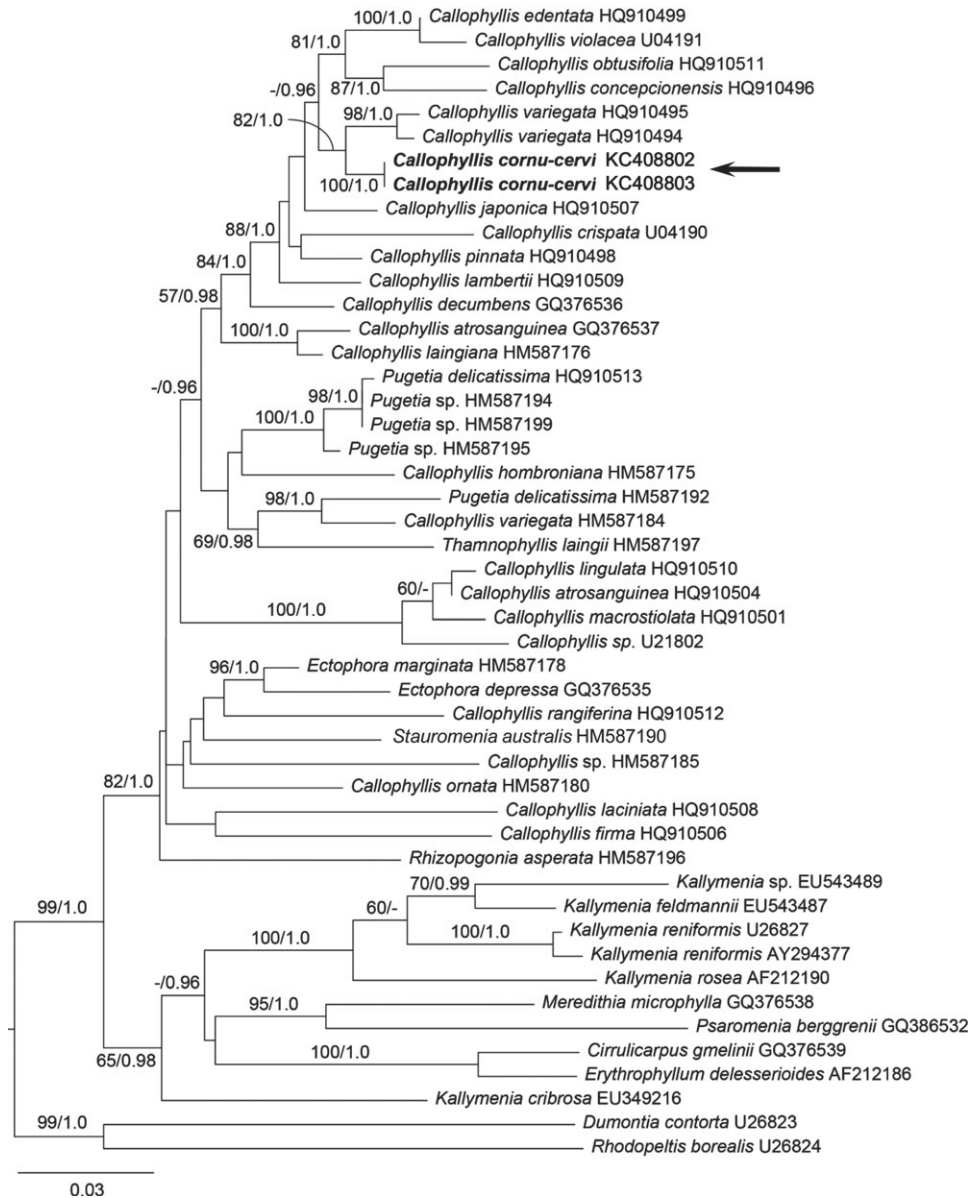


Figure 4 Maximum likelihood tree of *rbcL* data from members of the Kallymeniaceae.

The numbers above and below the nodes are RAxML bootstrap values and Bayesian posterior probabilities. Only bootstrap values $\geq 50\%$ and Bayesian posterior probabilities ≥ 0.95 are shown on the tree.

i.e., the bushy appearance of plants with branches positioned at varying angles to each other (referred to as ‘*multiaxial, branched, bushy thalli*’ by Perestenko (1996 [1994]) match the appearance of our specimens. Also, Perestenko (1996, p. 106) called her species in Russian “Иония оленье рога” (“olenii roga”) because it was an erect tuft resembling the lichen *Ramalina* (Perestenko, personal communication). We searched the northern continental coast of the Sea of Okhotsk (Klochkova et al. 2010, 2012a,b) and several localities on western Kamchatka (Klochkova et al. 2009) and did not find any red alga that

fell under Perestenko’s description, except for our material from Taujskaya Bay.

Phylogenetic analysis supported its taxonomic position within *Callophyllis*; therefore, we propose synonymizing *Ionia* under *C. cornu-cervi*. So far, it has the closest genetic affinity to *C. variegata*, which is the type species of the genus. The syntype locality of *C. variegata* is Concepción, Chile (Silva et al. 1996). It is noteworthy that specimens of *C. variegata* collected from areas geographically proximate to the syntype locality (GenBank No. HQ910494, HQ910495) are closer to our *C. cornu-cervi*,

Table 2 *Callophyllis* species from Russian Far Eastern seas.

Taxon	Type locality	Distribution on Russian Far East	Habitat characteristics
<i>Callophyllis beringensis</i> Perestenko	Cape Chaplino, St. Paul Island, Bering Sea	Northwestern coast of the Bering Sea (Kamchatka). Endemic, very rare	Subtidal, solitary or in tufts, on stony substratum and holdfasts of <i>Laminaria</i>
<i>C. laciniata</i> (Hudson) Kützing (reported as <i>C. flabellata</i> P.L. Crouan et H.M. Crouan)	Yorkshire, UK	Peter the Great Bay (Sea of Japan), southwest coast of Sakhalin Island, Small Kurile Islands (Kunashir)	Subtidal, at depths of 10–30 (40) m
<i>C. papulosa</i> Perestenko	South Kurile Islands	Southwestern coast of Sakhalin Island (Aniva Inlet), South Kurile Islands	Subtidal
<i>C. platyna</i> Perestenko	Bering Sea, St. Lavrentij Bay	Bering Sea, St. Lavrentij Bay	Subtidal, on stony substrata
<i>C. radula</i> Perestenko	Kamchatka, Avacha Bay	Restricted distribution on southeastern sector of Kamchatka Peninsula	Subtidal, at depths of 2–6 m, solitary or in tufts, on stony substrata and holdfasts of <i>Laminaria</i>
<i>C. rhynchocarpa</i> Ruprecht	Ajan Bay, Sea of Okhotsk, Russia	Most common among all other Far Eastern <i>Callophyllis</i> . Distributed in the Bering Sea (Kamchatka), Sea of Okhotsk, Kurile Islands, Sakhalin Island, and Sea of Japan	Subtidal, at depths of 0–42 m (usually at depths below 10–12 m), solitary or in tufts, on stony substrata, small shells, and holdfasts of <i>Laminaria</i>
<i>Callophyllis cornu-cervi</i> (Perestenko) T.A. Klochkova, N.G. Klochkova et S.M. Boo	Iony Island, Sea of Okhotsk, Russia	Sea of Okhotsk (Taujskaya Bay and Iony Island)	Subtidal, at depths of 6–7 m (in Taujskaya Bay) and 15 m (in Iony Island), in tufts, on stony substrata

Data are summarized from Perestenko (1978, 1980, 1996), Klochkova (1996) and Klochkova et al. (2009). Newly collected data from the current authors are also included.

whereas a specimen of *C. variegata* from New Zealand was phylogenetically very distant (GenBank No. HM587184).

In the Russian Far Eastern seas, *Callophyllis* is represented by six species; *C. beringensis* Perestenko, *C. laciniata* (Hudson) Kützing (reported as *C. flabellata* P.L. Crouan et H.M. Crouan), *C. papulosa* Perestenko, *C. platyna* Perestenko, *C. radula* Perestenko and *C. rhynchocarpa* Ruprecht (e.g., Perestenko 1978, 1980, 1996, Klochkova 1996, 1998, Klochkova and Emelyanova 2005, Klochkova et al. 2009). None of these species is morphologically similar to *C. cornu-cervi* (Tables 2 and 3). It would be interesting to address relationships of *Callophyllis* from the Russian Far East using molecular markers, but that is beyond the present study.

In our opinion, the establishment of the new genus *Ionia* belonging to a separate family, Crossocarpaceae, is not strongly supported by the distinctiveness of morphology or anatomy. The original drawing by Perestenko (1996) depicted an entity remarkably similar to *Callophyllis*, and her diagnostic anatomical features of the genus *Ionia* were also very similar to *Callophyllis*, as this genus was said to have ‘medulla composed of large cells and filaments spreading from it, composed of uncolored small round and long rod-shaped cells’ (Perestenko 1996, p. 25). The type specimen is also remarkably similar to the *Callophyllis* species.

Some differences were observed between Perestenko’s diagnosis and our specimens. Perestenko (1996) described plants as chestnut-colored and soft. This seems to be a mistake, perhaps because the diagnosis was based on the observations of old dry specimens. As obviously seen from her type specimen, the plants were not chestnut-colored, but they were very dark burgundy. The plants from Taujskaya Bay were bright-red when fresh and turned dark crimson or dark burgundy upon drying, depending on the age of the branches. The texture of the plants is not soft, but more or less rigid; otherwise, it would not be able to stand upright as a tuft, as depicted in Perestenko’s diagnosis and in the etymology of her species. Our specimens were lighter-colored and taller, which could be because they grew at depths of 6–7 m, whereas plants from the type locality grew at a depth of 15 m. The type material collected from Iony Island on July 22, 1978 was fertile, whereas all plants from Taujskaya Bay collected by us on July 17, 2008 were sterile. The latitude of our collecting site is approximately 230 km northward from that of Iony Island, and the formation of reproductive structures in plants from more northern localities may be delayed. A distinct feature of *C. cornu-cervi* is that, although each fan-like blade divides in one plane, the segments are slightly convoluted and have

Table 3 Comparative characteristics of *Callophyllis* species from Russian Far Eastern seas.

Taxon	Plant dimensions	Lateral branches	Plant texture / blade surface	Plant color	Attachment of microorganisms
<i>Callophyllis beringensis</i>	Up to 15 cm tall. Flattened arrangement of branches	Broad-linear, up to 13–20 mm wide	Soft, flaccid / smooth, filmy	Carmine-violet in fresh plants; red-pink in color in dry plants	Micro-epiphytes (<i>Callocollax aricularis</i> Wynne <i>et</i> Heine)
<i>C. laciniata</i> (reported as <i>C. flabellata</i>)	10–23 cm tall. Flattened arrangement of branches	Linear or becoming sphenoid to the tips, 4–10 mm wide	Soft, flaccid / membranous	Red or chestnut-red	None
<i>C. papulosa</i>	10–18 cm tall. Flattened arrangement of branches	Becoming broader to the upper portion (up to 25 mm)	Soft-cartilaginous in lower portion of thallus / smooth in young plants, warty in old plants	Carmine-violet	None
<i>C. platyna</i>	Up to 4.5 cm tall and 4 cm wide. Flattened arrangement of branches	Broad	Soft, flaccid / smooth, filmy	Carmine-violet	None
<i>C. radula</i>	8–10 cm tall. Flattened arrangement of branches	Broad-linear, 5–10 mm wide	Soft, flaccid / smooth in sterile plants, rough in reproductive plants	Milky-pink in fresh plants; burgundy or brown-burgundy in dry plants	Macro-epiphytes (on old blades)
<i>C. rhynchocarpa</i>	5–12 cm tall. Flattened arrangement of branches	Linear or becoming sphenoid, 5–7 mm wide	Soft, flaccid / smooth, filmy	Carmine-red, pinkish-red or bleached to yellowish-red	Micro-epiphytes (<i>C. aricularis</i>), recorded once. Encrusting bryozoans (occasional, in low portions of thalli near the base)
<i>C. cornu-cervi</i>	3–10(12) cm tall, bushy. Individual segments slightly convoluted and deployed at varying angles to each other	Linear or broadening in the place of branching, 2–4 mm wide	More or less rigid, erect / smooth, leathery	Bright-red in fresh plants; dark crimson or dark burgundy in dry plants	Encrusting bryozoans, tubes of polychaetes (in young and old portions of thalli)

Data are summarized from Perestenko (1978, 1980, 1996), Klochkova (1996) and Klochkova et al. (2009). Newly collected data from the current authors are also included.

varied spatial positionings, appearing at an angle to each other and forming a bush-like plant. In other *Callophyllis* species, the segments are positioned on the blade in one surface plane, and the thalli are flat. Our material and the type specimen were abundantly covered with numerous colonies of bryozoans and tubes of the sphaeodoriden polychaetes. This implies a specific biochemical property that is similar in both specimens (e.g., they might excrete some substance allowing attachment of the same epibionts), and it also indicates similar physical qualities of blades in both specimens, including older and younger parts of thalli. Other *Callophyllis* species observed by us did not bear encrusting bryozoans or tubes of polychaetes on their soft and flaccid surfaces, but, occasionally, bryozoans were able to encrust the lower parts of thalli near the bases in some plants, as those parts were older and denser than the remaining parts of the thalli.

This species is currently considered as a northern Okhotsk endemic that grows preferentially on rocks in the subtidal. More exhaustive field surveys in the Sea of Okhotsk are necessary, as this is a deep-water species restricted to certain depths, and it might have been overlooked in the other areas of this sea. Many coastal areas in the Russian Far East are not easily accessed and are unpopulated and thus poorly investigated. To date, 222 species have been listed for the continental coast of the Sea of Okhotsk and western Kamchatka (Klochkova and Emelyanova 2005). In the northernmost areas of this waterbody, ca. 102–132 species are listed in works published through 2000. This is the smallest number of reported macroalgal taxa for such a large area of the Russian Far East, which indicates that the region is largely understudied. For comparison, the macroalgal flora of the Shantar Islands (Sea of Okhotsk) has been reported to contain 161 species, and

floras of other Russian Far Eastern regions contain the following numbers of species: southeastern Kamchatka – 178, Commander Islands – 204, North Kurile Islands – 169, Middle Kurile Islands – 169, South Kurile Islands – 276, Tatars Strait (Sea of Japan) – 272 and Peter the Great Bay (Sea of Japan) – 316 taxa (N.G. Klochkova, personal unpublished data).

Less than 10% of all macroalgal taxa from the Russian Far East have been confirmed by gene sequencing. Our study clarifies one red algal endemic species from the Sea of Okhotsk and highlights the need for further studies on other macroalgal species in this area.

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