Molecularly assisted taxonomic studies of marine red algae from the north-western Pacific: establishing the ordinal and family positions of the genus Lukinia and the monospecific s…

Article *in* Phycologia · November 2021

DOI: 10.1080/00318884.2021.1988488

CITATION
1

READS
47

3 authors:

Svetlana Y. Shibneva
Institute of Marine Biology
7 PUBLICATIONS **43** CITATIONS
SEE PROFILE

Anna Skriptsova
Russian Academy of Sciences
58 PUBLICATIONS **873** CITATIONS
SEE PROFILE

Alexander Semenchenko
Far Eastern Federal University
27 PUBLICATIONS **74** CITATIONS
SEE PROFILE

Phycologia

Phycologia

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uphy20

Molecularly assisted taxonomic studies of marine red algae from the north-western Pacific: establishing the ordinal and family positions of the genus *Lukinia* and the monospecific status of the genus *Sparlingia* (Rhodymeniales)

Svetlana Yu. Shibneva, Anna V. Skriptsova & Alexander A. Semenchenko

To cite this article: Svetlana Yu. Shibneva, Anna V. Skriptsova & Alexander A. Semenchenko (2021): Molecularly assisted taxonomic studies of marine red algae from the north-western Pacific: establishing the ordinal and family positions of the genus *Lukinia* and the monospecific status of the genus *Sparlingia* (Rhodymeniales), Phycologia, DOI: 10.1080/00318884.2021.1988488

To link to this article: https://doi.org/10.1080/00318884.2021.1988488

PHYCOLOGIA
https://doi.org/10.1080/00318884.2021.1988488

Molecularly assisted taxonomic studies of marine red algae from the north-western Pacific: establishing the ordinal and family positions of the genus *Lukinia* and the monospecific status of the genus *Sparlingia* (Rhodymeniales)

Svetlana Yu. Shibneva[1], Anna V. Skriptsova[1] and Alexander A. Semenchenko[2]

[1]*A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17, Palchevskogo str., Vladivostok 690041, Russia*
[2]*Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms, Institute of the World Ocean, Far Eastern Federal University, Vladivostok, 690022, Russia*

ABSTRACT

The main aim of our study was to determine the taxonomic position of *Lukinia dissecta*, the species of a monotypic genus endemic to the boreal western-Pacific Ocean preliminarily placed with some doubt by several authors in the order Gigartinales. To address its ordinal and family affinities, we sequenced the plastid *rbc*L marker and additionally provided critical cystocarp details. Based both on a phylogenetic analysis using Bayesian inference and a maximum likelihood approach as well as cystocarp structure, we conclude that *Lukinia* belongs to the family Rhodymeniaceae of the order Rhodymeniales. The critical anatomical features that support this conclusion are the carposporophytes, which develop on a stout fusion cell within a large chamber formed by a domed, ostiolate pericarp, with the carposporangia borne in a dense mass on a few distal gonimolobes and most gonimolobe cells becoming carposporangia. To explore a further issue regarding the presence of Rhodymeniaceae in eastern-Russian waters, we also sequenced the *rbc*L of *Sparlingia stipitata* in order to ascertain whether it is an independent taxon or a morphological form of the widespread *S. pertusa*. A comparison of the obtained sequences with those of *S. pertusa* available in GenBank has revealed a low divergence (1 ± 0.1%) from *S. pertusa*, which supports the assignment of *S. stipitata* populations from Russian waters to *S. pertusa*.

ARTICLE HISTORY
Received 31 January 2021
Accepted 30 September 2021
Published online 05 November 2021

KEYWORDS
Boreal west-Pacific; Molecularly assisted alpha taxonomy (MAAT); Rhodymeniaceae

INTRODUCTION

In recent years, due to the extensive use of molecular-phylogenetic methods in phycological studies, the systematics and taxonomy of many marine macroalgae have undergone major changes. These modern methods provide opportunities to resolve long-standing taxonomic questions, which could not be settled previously using traditional morpho-taxonomic methods (Medlin *et al.* 2007; Manoylov 2014). The use of molecular methods for the study of red-algal taxonomy overcomes these limitations and has led to the finding of new taxa and the determination of phylogenetic relationships through the estimation of divergence times of clades (Lee & Kim 2014; Schneider *et al.* 2014). Such approaches are particularly valuable when applied to species with heteromorphic alternations of free-living generations or to species that rarely if ever display critical reproductive features indicative of family relationships (Kurihara *et al.* 2005; Kraft & Saunders 2014). Cryptic species are frequently distinguished based on molecular data (Lee & Kim 2014; Kraft & Saunders 2017; Neiva *et al.* 2017), as are questions about the limits of variability in highly polymorphic species (Conklin *et al.* 2009).

Algal taxa in Russian waters of the Northwest Pacific, especially those inhabiting hard-to-access regions such as the Sea of Okhotsk and the Bering Sea, still remain largely unsubjected to molecularly assisted alpha taxonomic (MAAT) studies (Lopatina *et al.* 2018). In these regions, taxonomic positions of many species require clarification, which is difficult when based solely on morphological and anatomical traits. Significantly among these are *Lukinia dissecta* Perestenko (Perestenko 1994; Schneider & Wynne 2013; Lopatina & Klochkova 2016), *Reingardia laminaricola* Perestenko (Perestenko 1994), *Mazzaella cornucopiae* (Postels & Ruprecht) Hommersand (Hughey *et al.* 2001), *Beringia castanea* Perestenko (Clarkston & Saunders 2012; Selivanova *et al.* 2020) and Russian populations attributed to *Sparlingia stipitata* (Kylin) Klochkova, the latter questioned by Filloramo *et al.* (2017) when they subsumed that species as represented in the eastern Pacific with the type species of the genus, *S. pertusa* (Postels & Ruprecht) G.W. Saunders, I.M. Strachan & Kraft.

Lukinia is a monotypic genus originally described from Medny Island in the Commander Islands of the Bering Sea (Perestenko 1994). Besides the Bering Sea, it is now also recorded from the Sea of Okhotsk and the Sea of Japan (Perestenko 1994; Lopatina & Klochkova 2016). The placement of *Lukinia dissecta* within the Gigartinales has never been confirmed, as Perestenko's (1994) protologue did not

CONTACT Svetlana Yu. Shibneva licht_gestalt@mail.ru
Supplemental data for this article can be accessed on the publisher's website.

assign it to a family for lack of carposporophyte details conforming to those of any gigartinalean family. Schneider & Wynne (2007) placed *L. dissecta* in the family Phyllophoraceae, but they did, however, later maintain that the family affiliation of the genus was still unclear (Schneider & Wynne 2013), as did Lopatina & Klochkova (2016).

A second species in question present in Far-Eastern Russian seas is *Sparlingia stipitata* (Rhodymeniaceae). First named *Rhodymenia stipitata* by Kylin (1925) based on his own collections from Friday Harbour in the San Juan Islands of Washington State, the species as recorded from the type locality and various sites in British Columbia and Alaska is now regarded as a synonym of *Sparlingia pertusa* following MAAT studies by Filloramo *et al.* (2017). These showed that the features that Kylin (1925) regarded as separating it from the generitype, which included stalks capable of reaching half the total length of a frond, thinner blades, fewer blade perforations, and differences in the timing of reproduction (Pisareva & Klochkova 2014), are phenotypical expressions of the one species. Filloramo *et al.* (2017) nevertheless refrained from including putative populations of *S. stipitata* from eastern Russia in *S. pertusa* for lack of molecular data such as those we now provide.

The aim of our study thus has been to not only clarify the taxonomic position of *Lukinia*, but also confirm that *S. stipitata* as found in eastern Russia is, like North American populations, properly regarded as belonging to *S. pertusa*.

MATERIAL AND METHODS

Specimen collection

Specimens identified as *Lukinia dissecta* (6 samples), *Sparlingia* cf. *stipitata* (4 samples) and *S. pertusa* (2 samples) were collected in the Sea of Okhotsk in June–August 2019 during expedition no. 56 to the Sea of Okhotsk and the northwestern Pacific Ocean, by collectors on board the R/V *Akademik Oparin*. Specimens of *L. dissecta* were collected on Simushir Island, Roadstead Vodopadny, from depths of 3–6 m (47°4.2′N, 152°5.4′E) and on Paramushir Island, Krasheninnikova Bay, from depths of 7–13 m (50°10.8′N, 155° 10.8′E). Specimens of *S. pertusa* and *S.* cf. *stipitata* were collected in Ayan Bay (56°15.0′N, 138°1.8′E), Eyreneyskaya Guba (Malaya Molta Bay, 59°13.8′N, 145°29.4′E), at Urup Island (Smugly Bay, Petushkov Island, 46°1.8′N, 149°35.4′E) and at Simushir Island (Milna Bay, 46°30.6′N, 151°28.8′E) from depths of 6–16 m. Samples and collection details are provided in Supplemental Table S1.

Fragments of fresh voucher specimens were removed for molecular analyses and desiccated in silica gel. Vouchers are deposited in the Herbarium of the Museum of A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Vladivostok, Russia) under numbers MIMB40521 and MIMB40522 for *Lukinia*, and MIMB39804, MIMB39807, MIMB40518, MIMB40519, MIMB40523 and MIMB40524 for *Sparlingia*.

Morphological observations of *Lukinia dissecta*

Examination of voucher specimens of *Lukinia dissecta* was made from longitudinal and cross-sections cut by safety razor blades through laminae and cystocarps. Sections were stained with a 1% aqueous solution of aniline blue acidified with a 1% HCl-solution. A Zeiss Axiovert 200M inverted microscope was used for analysis and taking micrographs.

DNA extraction, sequencing and phylogenetic analyses

A total of six *Lukinia* and six *Sparlingia* specimens were analysed, of which four of the latter were identified on habit and anatomical grounds as *S.* cf. *stipitata*, and two as *S. pertusa*.

Genomic DNA was extracted from thallus fragments dried in silica gel by the CTAB-method (Wang *et al.* 2006). A partial chloroplast gene fragment, ribulose-1,5-biphosphate carboxylase/oxygenase (*rbc*L) of 1250–1300 bp in length, was amplified with specific primers F57 (forward) and rbcLrevNEW (reverse) (Saunders & Moore 2013). PCR amplification and preparation of samples for sequencing were carried out as described by Shibneva *et al.* (2020). The PCR products were bidirectionally sequenced on an ABI 3130x sequencer (Applied Biosystems) and aligned in MEGA7 v. 7.0.27 (Kumar *et al.* 2016) using the MUSCLE algorithm (Edgar 2004). MEGA7 was also used for the calculation of inter- and intraspecific *rbc*L distances using the pairwise distance model (p-distance, %). *Gracilaria* spp (Gracilariaceae) were used as outgroup. All sequences were deposited in GenBank under the accession numbers MT783303–MT783308 for *Lukinia* and MW267830–MW267835 for *Sparlingia*. GenBank accession numbers of obtained nucleotides and sequences used in phylogenetic analyses are included in Table S1.

Bayesian phylogenetic analysis was conducted in MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003). Partition Finder 2.1.1 (Lanfear *et al.* 2012) was used to select the best-fit partitioning scheme and models separately for each codon position of *rbc*L gene, using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. The best-fit model for *rbc*L was GTR (Tavaré 1986) + I (a proportion of invariable sites) + G (gamma distribution) for all three positions in the codon. Bayesian inference was carried out using Markov Chain Monte Carlo (MCMC) randomization. Four Markov chains (three heated chains, one cold) were run for 5 million generations, with the first 25% of sampled trees discarded as burn-in. ML analysis was performed in RAxML v. 8.2.4 using bootstrapping with 1000 replications (Stamatakis 2006) and GTR + G model. Moreover, trace files of BI analysis were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018), and phylogenetic trees after analysis were visualized in FigTree v. 1.4.4 (Rambaut 2016).

RESULTS

Molecular analyses

Six specimens of *Lukinia dissecta*, four of *Sparlingia* cf. *stipitata* and two of *S. pertusa* were sequenced. The final *rbc*L alignment yielded 1290 base pairs. The alignment contains 1092 conserved sites and 198 variable sites, most of which were synonymous transitions. The total pairwise

p-distances within *Lukinia dissecta, Sparlingia* cf. *stipitata*, and *S. pertusa* amounted to 0.000%, 0.545%, and 0.104%, respectively. Most substitutions within the species were observed only in the third codon position. The average interspecific p-distance between *S.* cf. *stipitata* and *S. pertusa* was 0.310%. The closest genera to *Lukinia dissecta* were *Botryocladia* and *Rhodymenia* (Fig. 1). The p-distances between *L. dissecta* and the closest members of the genus *Botryocladia* amounted to 12.2%–12.9%. The p-distances between *Sparlingia* and *Rhodymenia*, the closest genus to *Sparlingia*, was 11.4%–12.5%.

The Bayesian phylogenetic and Maximum Likelihood trees had a similar topology, and we here present only the Bayesian tree, including the support for ML (Bootstrap) branches. The order Gigartinales was well-supported (PP = 1; ML = 84) and highly diverse, but *Lukinia dissecta* did not fall into this clade.

The Bayesian phylogeny (Fig. 1) also revealed a well-supported polytomy node (PP = 1; ML = 92) within the family Rhodymeniaceae, which consisted of *Chrysymenia brownii* (Harvey) De Toni, *Sparlingia* cf. *stipitata* + *S. pertusa*, and the remaining taxa including *Lukinia dissecta, Botryocladia* spp and *Rhodymenia* spp. The Russian specimens identified as *Sparlingia* cf. *stipitata* and *S. pertusa* (bold type in Fig. 1) were clustered within a common clade along with the specimens of *S. pertusa* from China and the USA (unbolded type in Fig. 1).

Morphological analysis

An examination of *Lukinia dissecta* cystocarps showed the following features characteristic of the family Rhodymeniaceae: the absence of *tela arachnoidea*;

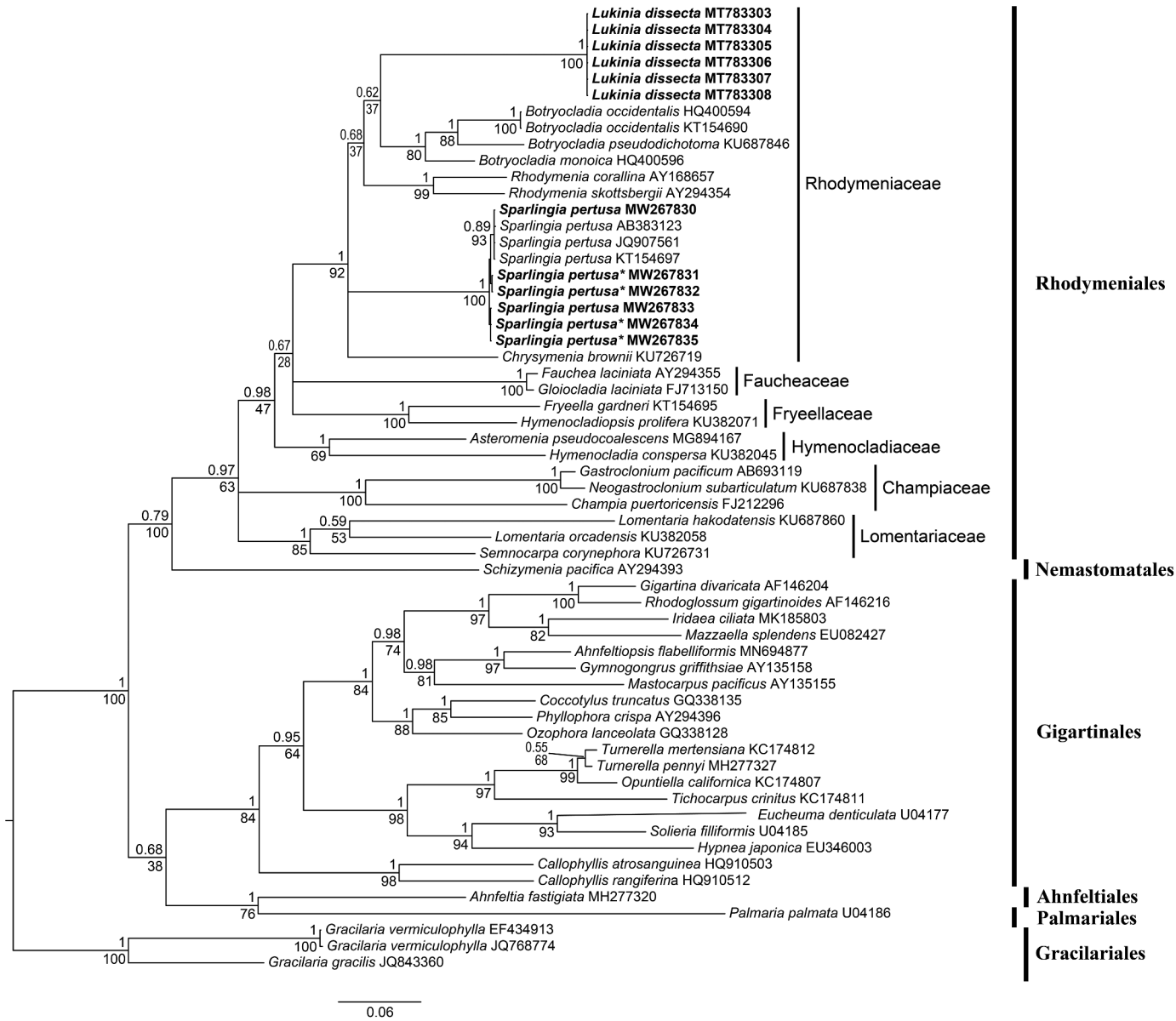
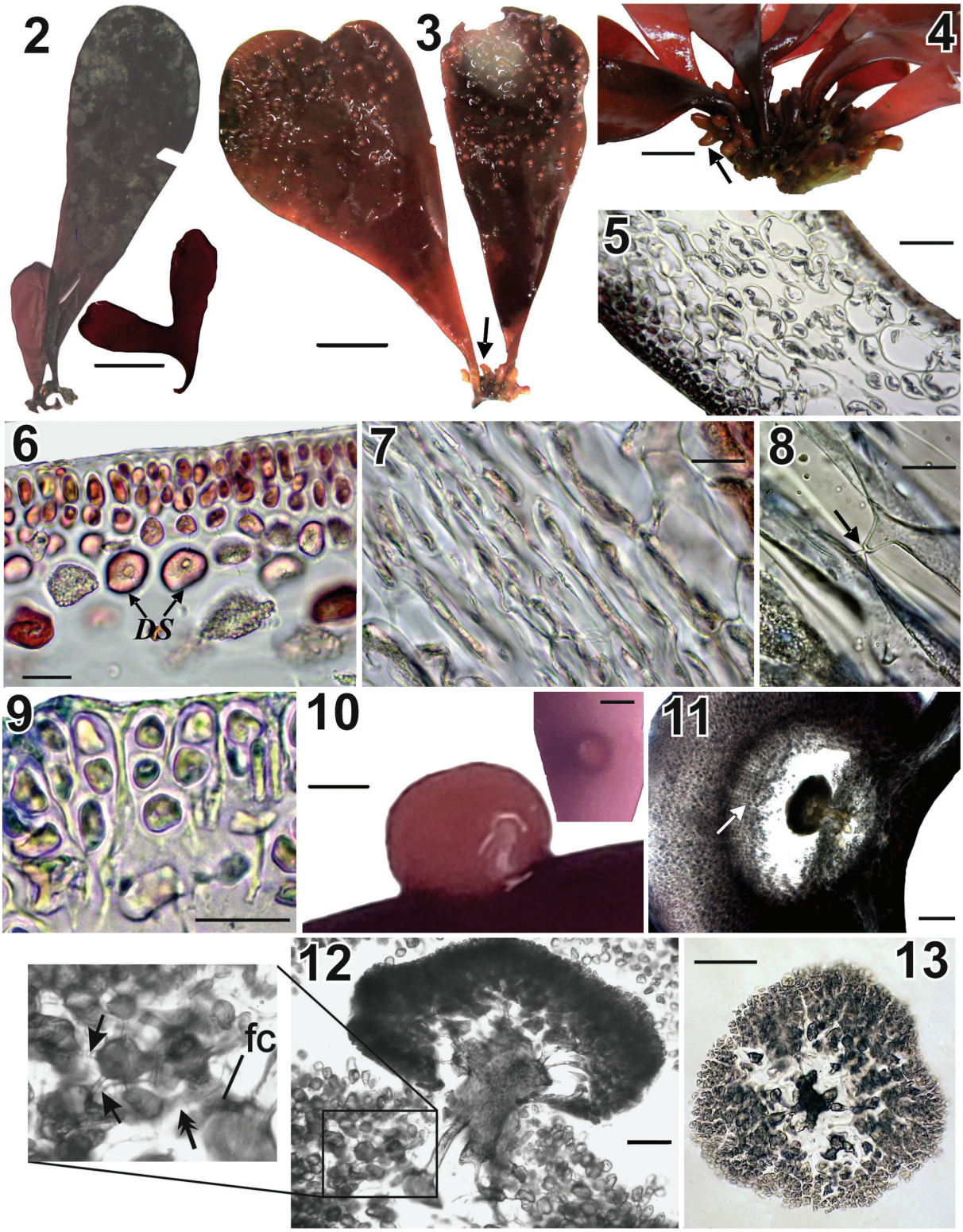


Fig. 1. Bayesian tree for determining the taxonomic positions of *Lukinia dissecta* and *Sparlingia* cf. *stipitata*, inferred from the plastid *rbc*L sequence (1,290 bp). Specimens analysed in this study are highlighted in bold, the specimens preliminary identified as *Sparlingia* cf. *stipitata* are indicated by an asterisk. Numerals above tree nodes are Bayesian posterior probabilities; below nodes, ML bootstrap values.



Figs 2–13. Morphology and anatomy of *Lukinia dissecta* collected from the Kuril Islands, Sea of Okhotsk, Russia.

Fig. 2. External morphology of sterile plants of *Lukinia dissecta* (left, plant from Paramushir Island; right, young plant from Simushir Island). Scale bar = 2 cm.

Fig. 3. External morphology of female gametophyte of *Lukinia dissecta* with mature cystocarps (Paramushir Island). Arrow shows terete stolons growing from holdfast. Scale bar = 2 cm.

Fig. 4. Extensive crustose holdfast with erect stolons (arrow). Scale bar = 1 cm.

Fig. 5. Cross-section of female gametophyte. Scale bar = 20 µm.

Fig. 6. Two differentiated cells (DS) of unknown function in the inner cortical layer. Scale bar = 20 µm.

Fig. 7. Detail of the filamentous medulla in a longitudinal section of female gametophyte. Scale bar = 20 µm.

Fig. 8. Secondary pit-connections between adjacent medullary cells (arrow). Scale bar = 2 µm.

Fig. 9. Details of cortex showing cortical cells enveloped by a common sheath. Scale bar = 20 µm.

Fig. 10. External view of mature cystocarp showing basal constriction. Inset, rear view from the opposite site of the blade showing slight depression in the blade behind the cystocarp. Scale bar = 0.5mm; scale bar in inset = 1 mm.

gonimoblasts consisting of some few synchronously maturing gonimolobes composed almost completely of carposporangia, and a large and thick columnar fusion cell rising from the floor and elevating the carposporangial mass into the cystocarp cavity, most gonimolobe cells becoming carposporangia. We here provide an emended description of the type species based on our observations.

Lukinia dissecta Perestenko 1994, p. 203, pl. X, figs 1, 2; pl. XXXII, fig. 2
Figs 2–13

HOLOTYPE: LE A0000187, female gametophyte collected 13 July 1972 by V.I. Lukin, deposited at the Komarov Botanical Institute, Russian Academy of Sciences (LE).

TYPE LOCALITY: Medny Island, Commander Islands, Bering Sea, Russia, sublittoral, depth 20 m.

REPRESENTATIVE SPECIMENS EXAMINED: MIMB40522, Paramushir Island, Krasheninnikova Bay, 11 August 2019, depth 7–13 m (vegetative and female gametophytes with mature cystocarps); MIMB40521, Simushir Island, Roadstead Vodopadny, 3 July 2019, depth 3–6 m (vegetative); collected by A.V. Skriptsova. Genbank accession numbers of the vouchers examined are MT783303–MT783308.

HABIT AND VEGETATIVE STRUCTURE: Thalli gregarious, flat, purple-red, 7–12 cm long and 3–4.5 cm wide, with cuneate apophyses and short stipe (Figs 2, 3), the fronds adhering to rocky substrata by extensive, often coalesced crustose holdfasts (Fig. 4). Abundant terete stolons also spreading from base of stipe, forming multiple secondary attachments on contact with the substratum, and giving rise to new blades. Laminae obovate or ovoid, with smooth, slightly thickened margins, unbranched or occasionally broadly dichotomous (Figs 2, 3). Blades 250–500 µm thick, varying in thickness within the same thallus, the medulla in cross-section rather loose, sometimes with wide intercellular spaces, consisting of unpigmented rounded or slightly compressed ovoid cells 4–23 × 20–46 µm, with larger cells 25–50 × 50–65 µm developing between them (Fig. 5). Subcortical cells ovoid, angular, 30–75 × 68–178 µm, with length to width ratio 2–3:1. Among them rounded, ovoid or irregular polygonal, 13–22 × 20–30 µm, thick-walled cells with prominent, single pyrenoid-like central inclusions present in some plants (Fig. 6); these cells connect to cortical and subcortical cells via secondary pit-connections. The medulla in longitudinal section composed of axially elongated unpigmented cells 20–60 × 160–550 µm, with length to width ratios of 4–22:1, connecting to adjacent cells through secondary pit-connections (Figs 7, 8). Subcortical cells isodiametric, rounded, 41–102 µm, filled by colourless granules in old plants. Cortex consisting of 2–5 layers of anticlinally arranged pigmented sub-spherical or to ovoid cells, 6–7.5 µm in diameter, enveloped by a common sheath (Fig. 9).

REPRODUCTIVE STRUCTURES: Cystocarps protuberant, spherical, slightly basally constricted (Fig. 10), 0.8 × 1–1.2 mm in diameter, ostiolate, scattered across blades except at bases and margins (Fig. 3). Mature cystocarps with a large central cavity (Fig. 11) enclosing the carposporophyte. Pericarps firm, smooth, *c.* 400 µm thick, without inner stellate cells, the inner layer consisting of regular periclinal rows of rounded cells, the outer layer of anticlinal rows of elongated or rounded small cells (Fig. 11). Floors of cystocarps covered with a cushion-like mass of darkly pigmented nutritive cells, those surrounding the base of the gonimoblast fusion cell connecting to it via secondary pit-connections (Fig. 12). Fusion cells large, thickly trunk-like, anchored in the basal nutritive tissue and elevating the carposporangial mass above the floor into the cystocarp chamber; clusters of carposporangia borne on several confluent gonimolobes at the distal end of the fusion cell (Figs 12, 13). Most gonimolobe cells developing into angular ovoid carposporangia 8–12 µm in diameter. Sporangia and spermatangia were not observed in present study.

DISTRIBUTION: Asia: Commander Islands (Selivanova & Zhigadlova 2013), Kamchatka (Klochkova *et al.* 2009), Kuril Islands (Lopatina & Klochkova 2016), Sakhalin Island (Perestenko 1994; Lopatina & Klochkova 2016).

HABITAT: Thalli grow singly or in clusters (Fig. 4) on rocky substrata from 3 to 20 m depths.

REMARKS: According to Lopatina & Klochkova (2016), spermatangia are spherical, 5–6 µm in diameter, and forming first on one, then on both sides of dioecious blades. Perestenko (1994, p. 129, fig. XI, 2) and Lopatina & Klochkova (2016, p. 76, fig. 2b) have noted that organs of asexual reproduction of *Lukinia* are monosporangia, which are solitairy, undivided, thick-walled, 13–25 × 18–30 µm in diameter, developing intercalary from cells of inner cortex. Our observation of structures with hyaline contents and single central pyrenoid-like bodies positioned intercalary at the junction between the inner cortex and the medulla (Fig. 6), similar to monosporangia illustrated by Lopatina & Klochkova (2016, p. 76, fig. 2b), raised doubts that these cells are either propagules, monosporangia or unseptated tetrasporocytes. It appears that the tetrasporophytes in the life cycle of *Lukinia* remain unknown.

DISCUSSION

The results of the *rbc*L analyses convincingly distance *Lukinia* from the order Gigartinales where Perestenko (1994) first placed it. Cystocarp structures alone show features most characteristic of the Rhodymeniales, to which order molecular data strongly indicate that it belongs. Subordinal affinities lie most convincingly with the largest family, the Rhodymeniaceae, its closest relationship within that cohort, rather unexpectedly, being to the genus *Botryocladia* (Fig. 1).

The Rhodymeniales is one of the most uniform orders of red algae in regard to the structure of procarps, the transfer of zygote nuclei to auxiliary cells, and the subsequent stages of embryo and carposporophyte development. At present, the Rhodymeniales consist of six families that are defined morpho-taxonomically based on differences in the position and division patterns of tetra-/polysporangia, the number of cells in the carpogonial branch and, in some cases (e.g. the Champiaceae), gonimoblast features. Vegetative differences such as whether fronds are centrally hollow, filamentous or pseudoparenchymatous, and whether 'gland' (or 'secretory') cells and *tela arachnoidea* are present or absent, are more important for defining genera than they are for characterizing families (Saunders *et al.* 1999; Le Gall *et al.* 2008).

Based on morphological and anatomical characters alone, it would be difficult to make an accurate conclusion about the family placement of *Lukinia* within the Rhodymeniales.

Fig. 11. Cross-section through a cystocarp showing the extensive inner cavity, basal cushion-like mass of darkly pigmented nutritive cells, and inner layer of pericarp composed of regular periclinal cell rows (arrow). Scale bar = 100 µm.
Fig. 12. A mature carposporophyte showing the distal crown of carposporangia and basal anchorage in, and fusion to, tissue at the floor of the cystocarp chamber. Inset, detail of fusions (double arrow) between fusion cell (fc) and nutritive cells and a pit-connection (arrow) between nutritive cells at the base of the cystocarp. Scale bar = 20 µm.
Fig. 13. Longitudinal section of gonimoblast showing numerous filamentous gonimoblast initials arising from distal part of the stalk. Scale bar = 100 µm.

Although based on molecular data it is placed in Rhodymeniaceae, there is one feature in particular that is displayed by *Lukinia* which does not entirely match that of the majority of rhodymeniaceans. This is the distinctively massive stalk that lifts the gonimolobes above the floor of the cystocarp cavity. The outlines of the components forming the stalk are obscured. This is a configuration most strikingly seen in members of the Lomentariaceae such as *Ceratodictyon* (Price & Kraft 1991, Figs 12, 13) and *Lomentaria* (Price & Kraft 1991, p. 111) although not uniformly throughout that family. In the Rhodymeniaceae stalks are generally much slenderer, the cells that compose them are recognizable, and the several gonimolobes with their synchronously developing carposporangia at varying stages of maturity are more clearly defined (Kim 2013, cf. against Price & Kraft 1991, fig. 12). Nevertheless, the anatomy of the gonimoblast stalk cell and degree of gonimolobe differentiation and compaction do not seem to be anatomical features uniform throughout the Rhodymeniaceae and Lomentariaceae, and the molecular data strongly resolved the issue of *Lukinia*'s family placement in favour of the Rhodymeniaceae. When it comes to weighing factors that determine whether a given alga belongs to one family or another in rhodophytes (as is true of all the algal groups), the deciding factors should default to molecular rather than strictly anatomical features.

In regard to the second element of our eastern-Siberian survey of the order Rhodymeniales, we have addressed the taxonomy of a species originally described from San Juan Island, Washington, as *Rhodymenia stipitata* Kylin. This alga occurs along the western coasts of North America from Vancouver to the Gulf of California (Guiry & Guiry 2021) and in the Sea of Okhotsk mainly along the Kamchatka. It is morphologically close to *Sparlingia pertusa*, which is one of the more distinctive northeastern-Pacific macrophytes due to its leafy blades with their programmed perforations for which the species was named. Described as *Porphyra pertusa* Postels & Ruprecht (1840) and later placed in *Rhodymenia* by J. Agardh (1851), this species, following MAAT studies by Saunders *et al.* (1999), was made the type of the new genus *Sparlingia* into which Klochkova also placed *R. stipitata* as *Sparlingia stipitata* (Kylin) Klochkova (Klochkova *et al.* 2009). Despite clear differences in blade thickness, stipe lengths, size of cystocarps and numbers of perforations between *S. pertusa* and *S. stipitata*, whether *S. stipitata* species is truly distinct from *Sparlingia pertusa* has been questioned (Hawkes & Scagel 1986). Filloramo *et al.* (2017) sequenced topotype and other western-Pacific populations conforming to *S. stipitata* and molecularly confirmed their conspecificity with *S. pertusa*, although they declined to include eastern Russian records for lack of sequenced material from that region. Our analyses of the *rbc*L gene has shown that specimens from the Kuril Islands identified on morphological grounds as *S. stipitata* are almost identical genetically with *S. pertusa* from the eastern Pacific, thus confirming the speculations of Filloramo *et al.* (2017) and formally reducing the former to complete synonymy with *S. pertusa*.

The issues of the taxonomic determination of species and evidence for their standing as independent species are as important today as ever on many levels, and in systematic studies an approach that includes both classical descriptive analyses and modern molecular-genetic methods are equally important to an understanding of the nature and biogeography of marine-algal resources. It has been repeatedly noted that the predominant reliance on phenotypic or genotypic traits often leads to ambiguous and controversial results that can bring confusion to modern taxonomic and systematic studies (Leliaert *et al.* 2014; Klochkova *et al.* 2018). Nevertheless, the modern molecular-genetic approach has played a substantial role in re-evaluations and amendments to red-algal taxonomy and has resulted in the clarification of many taxonomic issues (Saunders *et al.* 1999; Le Gall *et al.* 2008; Costa *et al.* 2016; Selivanova *et al.* 2020; Shibneva *et al.* 2020; Skriptsova & Kalita 2020). If this approach is not adopted, it often is virtually impossible to draw definite forceful conclusions about the taxonomic composition and systematic affiliations of genera or species.

The approach used in the present study, which includes morpho-taxonomic and phylogenetic analyses, has proved its utility in regard to the uncertain alliances of the genus *Lukinia* and the species composition of *Sparlingia*. These methodologies promise many future advances in our knowledge of the macroalgal biodiversity in one of the most remote and inhospitable regions of the world's oceans.

ACKNOWLEDGEMENTS

We are extremely grateful to Dr. Gerald T. Kraft for his assistance in improving the manuscript and to divers A.S. Oskolkov, K.K. Dudka and I.N. Ivanov for their assistance in collecting seaweeds. We also thank an anonymous reviewer for valuable comments and recommendations.

FUNDING

The 56th expedition aboard the R/V Academic Oparin to the Sea of Okhotsk and Northwest Pacific was financially supported by the Ministry of Science and Higher Education of the Russian Federation. This research was carried out within the framework of the project AAAA-A18-118022290003-2 of NSCMB FEB RAS.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

S.Y. Shibneva: analysis of molecular data, original concept, manuscript preparation; A.V. Skriptsova: seaweeds collection, light microscopy, manuscript preparation; A.A. Semenchenko: analysis of molecular data, manuscript preparation.

ORCID

Anna V. Skriptsova http://orcid.org/0000-0001-5943-4522

REFERENCES

Clarkston B.E. & Saunders G.W. 2012. An examination of the red algal genus *Pugetia* (Kallymeniaceae, Gigartinales), with descriptions of *Salishia firma* gen. & comb. nov., *Pugetia cryptica* sp. nov. and *Beringia wynnei* sp. nov. *Phycologia* 51: 33–61. DOI: 10.2216/11-01.1.

Conklin K.Y., Kurihara A. & Sherwood A.R. 2009. A molecular method for identification of the morphologically plastic invasive algal genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) in Hawaii.

Journal of Applied Phycology 21: 691–699. DOI: 10.1007/s10811-009-9404-2.

Costa J.F., Lin S.-M., Macaya E.C., Fernández-García C. & Verbruggen H. 2016. Chloroplast genomes as a tool to resolve red algal phylogenies: a case study in the Nemaliales. *BMC Evolutionary Biology* 16: Article 205. DOI: 10.1186/s12862-016-0772-3.

Edgar R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: Article 113. DOI: 10.1186/1471-2105-5-113.

Filloramo G.V., Savoie A.M., Selivanova O.N., Wynne M.J. & Saunders G.W. 2017. Key Kamchatkan collections provide new taxonomic and distributional insights for reportedly pan–North Pacific species of Rhodymeniophycidae (Rhodophyta). *Phycologia* 56: 296–302. DOI: 10.2216/15-145.1.

Guiry M.D. & Guiry G.M. 2021. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway, Ireland. https://www.algaebase.org; searched on 10 January 2021.

Hawkes M.W. & Scagel R.F. 1986. The marine algae of British Columbia and northern Washington: division Rhodophyta (red algae), class Rhodophyceae, order Rhodymeniales. *Canadian Journal of Botany* 64: 1549–1580. DOI: 10.1139/b86-210.

Hughey J.R., Silva P.C. & Hommersand M.H. 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *Journal of Phycology* 37: 1091–1109. DOI: 10.1046/j.1529-8817.2001.01048.x.

Kim H.-S. 2013. *Algal flora of Korea, vol. 4, no. 8. Rhodophyta: Florideophyceae: Rhodymeniales, Bonnemaisoniales, Sebdeniales, Peyssonneliales*. National Institute of Biological Resources, Incheon, South Korea. 190 pp.

Klochkova N.G., Korolyova T.N. & Kusidi A.E. 2009. *Marine algae of Kamchatka and surrounding areas, vol. 2. Red seaweeds*. KamchatNIRO, Petropavlovsk-Kamchatsky, Russia. 302 pp [in Russian].

Klochkova T.A., Kim G.H., Klimova A.V. & Klochkova N.G. 2018. Taxonomic significance of phenotypic and genotypic characters to describe new genera and species. *The Researches of the Aquatic Biological Resources of Kamchatka and the North-West Part of the Pacific Ocean* 51: 60–72 [in Russian].

Kraft G.T. & Saunders G.W. 2014. *Crebradomus* and *Dissimularia*, new genera in the family Chondrymeniaceae (Gigartinales, Rhodophyta) from the central, southern and western Pacific Ocean. *Phycologia* 53: 146–166. DOI: 10.2216/13-213.1.

Kraft G.T. & Saunders G.W. 2017. *Mychodea* and the Mychodeaceae (Gigartinales, Rhodophyta) revisited: molecular analyses shed light on interspecies relationships in Australia's largest endemic algal genus and family. *Australian Systematic Botany* 30: 230–258. DOI: 10.1071/SB16058.

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. DOI: 10.1093/molbev/msw054.

Kurihara A., Arai S., Shimada S. & Masuda M. 2005. The conspecificity of *Galaxaura apiculata* and *G. hystrix* (Nemaliales, Rhodophyta) inferred from comparative morphology and *rbc*L and ITS1 sequences. *European Journal of Phycology* 40: 39–52. DOI: 10.1080/09670260400005542.

Kylin H. 1925. The marine red algae in the vicinity of the biological station at Friday Harbor, Wash. *Lunds Universitets Årsskrift, n. f.* 21 (9): 1–87.

Lanfear R., Calcott B., Ho S.Y.W. & Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. DOI: 10.1093/molbev/mss020.

Le Gall L., Dalen J.L. & Saunders G.W. 2008. Phylogenetic analyses of the red algal order Rhodymeniales supports recognition of the Hymenocladiaceae fam. nov., Fryeellaceae fam. nov., and *Neogastroclonium* gen. nov. *Journal of Phycology* 44: 1556–1571. DOI: 10.1111/j.1529-8817.2008.00599.x.

Lee H.W. & Kim M.S. 2014. Cryptic species diversity of the red algal genus *Callophyllis* (Kallymeniaceae, Gigartinales) from Korea. *Journal of Ecology and Environment* 37: 395–410. DOI: 10.5141/ecoenv.2014.041.

Leliaert F., Verbruggen H., Vanormelingen P., Steen F., Juan M., López-Bautista J.M., Zuccarello G.C. & De Clerck O. 2014. DNA-based species delimitation in algae. *European Journal of Phycology* 49: 179–196. DOI: 10.1080/09670262.2014.904524.

Lopatina N.A. & Klochkova N.G. 2016. The genus *Lukinia* (Rhodophyta: Gigartinales) in the Far-eastern seas of Russia. *Kamchatka State Technical University Bulletin* 36: 74–78 [in Russian]. DOI: 10.17217/2079-0333-2016-36-74-78.

Lopatina N.A., Sanamyan N.P., Zhigadlova G.G. & Panina E.G. 2018. *Beringia castanea* (Gigartinales, Rhodophyta) from the Russian Far-Eastern seas. In: *Proceedings of the XIV congress of the Russian Botanical Society and the conference "Botany in the modern world" (Makhachkala, June 18–23, 2018), vol. 3: Spore plants. Mycology. Structural botany. Physiology and biochemistry of plants. Plant embryology*, pp 42–43. ALEF, Makhachkala [in Russian].

Manoylov K.M. 2014. Taxonomic identification of algae (morphological and molecular): species concepts, methodologies, and their implications for ecological bioassessment. *Journal of Phycology* 50: 409–424. DOI: 10.1111/jpy.12183.

Medlin L.K., Metfies K., John U. & Olsen J.L. 2007. Algal molecular systematics: a review of the past and prospects for the future. In: *Unravelling the algae: the past, present, and future of algal systematics* (Ed. by J. Brodie & J. Lewis), pp 341–353. CRC Press, Abingdon, UK.

Neiva J., Serrão E.A., Anderson L., Raimondi P.T., Martins N., Gouveia L. & Ladah L.B. 2017. Cryptic diversity, geographical endemism and allopolyploidy in NE Pacific seaweeds. *BMC Evolutionary Biology* 17: Article 30. DOI: 10.1186/s12862-017-0878-2.

Perestenko L.P. 1994. *Krasnye vodorosli Dal'nevostochnyh morey Rossii [Red algae of the Far-Eastern seas of Russia]*. St. Petersburg, Izdatel'stvo OL'GA, Russia. 331 pp [in Russian].

Pisareva N.A. & Klochkova N.G. 2014. Biological variabilities and morphological differences of genus *Sparlingia* (Rhodophyta, Rhodymeniales) in Kamchatka coastal waters. In: *Conservation of biodiversity of Kamchatka and coastal waters. Abstracts of the XV International Scientific Conference*, pp 201–204. Petropavlovsk-Kamchatsky, Kamchatpress, Russia [in Russian].

Price L.R. & Kraft G.T. 1991. Reproductive development and classification of the red algal genus *Ceratodictyon* (Rhodymeniales, Rhodophyta). *Phycologia* 30: 106–116. DOI: 10.2216/i0031-8884-30-1-106.1.

Rambaut A. 2016. FigTree, version 1.4.3. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut A., Drummond A.J., Xie D., Baele G. & Suchard M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904. DOI: 10.1093/sysbio/syy032.

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. DOI: 10.1093/bioinformatics/btg180.

Saunders G.W. & Moore T.E. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae* 28: 31–43. DOI: 10.4490/algae.2013.28.1.031.

Saunders G.W., Strachan I.M. & Kraft G.T. 1999. The families of the order Rhodymeniales (Rhodophyta): a molecular-systematic investigation with a description of Faucheaceae fam. nov. *Phycologia* 38: 23–40. DOI: 10.2216/i0031-8884-38-1-23.1.

Schneider C.W. & Wynne M.J. 2007. A synoptic review of the classification of red algal genera a half century after Kylin's "Die Gattungen der Rhodophyceen". *Botanica Marina* 50: 197–249. DOI: 10.1515/BOT.2007.025.

Schneider C.W. & Wynne M.J. 2013. Second addendum to the synoptic review of red algal genera. *Botanica Marina* 56: 111–118. DOI: 10.1515/bot-2012-0235.

Schneider C.W., Saunders G.W. & Lane C.E. 2014. The monospecific genus *Meredithia* (Kallymeniaceae, Gigartinales) is species rich and geographically widespread with species from temperate Atlantic, Pacific, and Indian Oceans. *Journal of Phycology* 50: 167–186. DOI: 10.1111/jpy.12149.

Selivanova O.N. & Zhigadlova G.G. 2013. Marine benthic algae of the Commander Islands (Pacific coast of Russia) with checklist revised in 2012. *ISRN Oceanography* 2013: 1–13. DOI: 10.5402/2013/470185.

Selivanova O.N., Zhigadlova G.G. & Saunders G.W. 2020. *Commanderella* gen. nov. and new insights into foliose Kallymeniaceae (Rhodophyta) from the Russian Pacific coast based on molecular studies. *Phycologia* 59: 200–207. DOI: 10.1080/00318884.2020.1732150.

Shibneva S.Y., Skriptsova A.V., Semenchenko A.A. & Suzuki M. 2020. Morphological and molecular reassessment of three species of *Besa* (Phyllophoraceae, Rhodophyta) from the Northwest Pacific. *European Journal of Phycology* 56: 72–84. DOI: 10.1080/09670262.2020.1765025.

Skriptsova A.V. & Kalita T.L. 2020. A re-evaluation of *Palmaria* (Palmariaceae, Rhodophyta) in the North-West Pacific. *European Journal of Phycology* 55: 266–274. DOI: 10.1080/09670262.2020.1714081.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. DOI: 10.1093/bioinformatics/btl446.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

Wang D., Wang X.L., Li D.P., Wang F.J. & Duan D.L. 2006. The genetic analysis and germplasm identification of the gametophytes of *Undaria pinnatifida* (Phaeophyceae) with RAPD method. *Journal of Applied Phycology* 18: 801–809. DOI: 10.1007/s10811-006-9099-6.