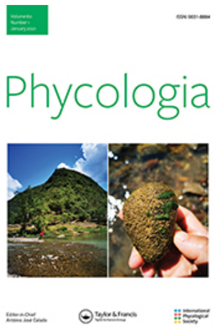

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Devaleraea titlyanoviorum sp. nov. (Palmariaceae, Rhodophyta) from the Russian coast of the northwestern Pacific Ocean

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ABSTRACT
One species of the red algal genus *Devaleraea*, *D. stenogona*, shows remarkable phenotypic plasticity over its geographic range. We assumed this species to be a complex of morphologically close species. To resolve this issue, we sequenced the internal transcribed spacer (ITS) regions, including the 5.8S rDNA, *rbc*L, and 5′ end of the COI gene (COI-5′) of specimens of *D. stenogona sensu lato* from different localities along the Russian coast of the northwestern Pacific Ocean. We also compared their morphology with the holotype and to specimens of *D. stenogona* from the type locality. Morphological and genetic analyses confirmed that *Devaleraea stenogona sensu lato* is a complex of two congeneric species and, therefore, the new species *D. titlyanoviorum sp. nov*. is proposed. This species is characterised by abundantly proliferating thalli, with proliferations being larger than the main blade. First-year non-proliferating plants can be confused with *D. stenogona*, but they differ by a more regular dichotomous branching and by the shape of terminal branches, which are strap-shaped with rounded tips in *D. titlyanoviorum* and narrow-linear or hair-like with acute tips in *D. stenogona*. The species have different geographical distributions with *D. stenogona* occurring further south than *D. titlyanoviorum*.

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INTRODUCTION

Our knowledge of algal species diversity has rapidly expanded with the increasing use of molecular methods in algal research. These methods have provided more accurate knowledge of algal species diversity, highlighting the large number of cryptic species within many morphological species, especially among widespread species or species with high phenotypic plasticity (e.g. *Mastocarpus*, Lindstrom 2008; Lindstrom *et al.* 2011; *Callophyllis*, H.W. Lee & Kim 2014; *Tsengia*, D'Archino & Zuccarello 2020). Applying methods of molecular–phylogenetic analyses in algal research is especially appropriate in groups with simple morphology, such as foliose algae. Much of their identification is based on overall habit, including blade shape, colour, texture, and capacity to proliferate. However, seaweed morphology is highly variable and dependent on environmental conditions or developmental features; for example, light levels, wave exposure and age (Shibneva & Skriptsova 2015). Palmariaceae are a red algal family whose members display simple frond morphologies where identification can be problematic.

Palmariaceae comprise species with solid blade-like or hollow saccate fronds, that are formed by large, loosely coherent medullary cells surrounded by a single-layered or multilayered cortex. In addition, the family is characterised by a heteromorphic life cycle with extreme sexual dimorphism in which the female gametophyte is diminutive; whereas, the male gametophyte and tetrasporophyte are large and isomorphic (Hawkes & Scagel 1986). Palmariaceae include four genera: *Neohalosacciocolax* I.K.Lee & Kurogi (submicroscopic parasitic algae), *Halosaccion* Kützing, *Palmaria* Stackhouse, and *Devaleraea* Guiry (Guiry & Guiry 2020). The last two genera have recently undergone taxonomic revision (Saunders *et al.* 2018; Skriptsova & Kalita 2020).

The genus *Devaleraea* was established when *Devaleraea ramentacea* (Linnaeus) Guiry was segregated from hollow, saccate *Halosaccion* (Guiry 1982). *Devaleraea* was suggested for species having a one- to two-layered cortex composed of small hexagonal cells, a cortex sharply delineated from a medulla consisting of two to three layers of large cells with nonstellate protoplasts. These features clearly distinguish *Devaleraea* from *Halosaccion*, which has a multilayered cortex (three to five rows) formed by rounded cells, not sharply defined from a multilayered medulla (six to eight rows) consisting of cells with stellate protoplasts (Guiry 1982).

When describing the new genus *Devaleraea*, Guiry (1982) noted that hollow *Devaleraea* has anatomical features close to

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Supplemental data for this article can be accessed on the publisher's website.

solid *Palmaria*. Subsequently, Saunders *et al.* (2018) showed that most species of *Palmaria*, based on the generitype *P. palmata* (Linnaeus) F.Weber & D.Mohr, were genetically more closely related to *D. ramentacea*, the generitype of *Devaleraea*. As a result, four *Palmaria* species were transferred to *Devaleraea* as *D. mollis* (Setchell & N.L.Gardner) G.W.Saunders, C.J.Jackson & Salomaki, *D. callophylloides* (M.W.Hawkes & Scagel) G.W.Saunders, Jackson & Salomaki, *D. marginicrassa* (I.K.Lee) Skriptsova & Kalita, and *D. stenogona* (Perestenko) Skriptsova & Kalita (Saunders *et al.* 2018; Skriptsova & Kalita 2020). It has been suggested that *Palmaria* is monotypic, with the single species *P. palmata* from the North Atlantic (Skriptsova & Kalita 2020). Currently eight *Devaleraea* species are recognised worldwide, with seven of them occurring in the northwest Pacific (Guiry & Guiry 2020).

Researchers who studied solid *Devaleraea* (as *Palmaria sensu lato*) noted that *D. stenogona* shows considerable morphological variability (Perestenko 1994), and that it may include several taxa (van der Meer & Bird 1985; Hawkes & Scagel 1986). This species is widely distributed in the northwest Pacific from the Sea of Japan to the Bering Sea (Perestenko 1973, 1994; Guiry & Guiry 2020). The taxonomic question of whether *D. stenogona* is a polymorphic species or species complex of morphologically similar species remains unresolved. Due to the relatively simple morphology of species of the Palmariaceae, with few reliable diagnostic characters, this issue can be resolved only through comparative morpho-anatomical observation and molecular analysis.

The aim of the present study was to investigate the morphological and genetic diversity of *D. stenogona* in the northern Sea of Japan and southern Sea of Okhotsk. Based on combined morphological and molecular evidence, we propose a new species of *Devaleraea* from the Russian Pacific coast, *D. titlyanoviorum sp. nov.*, which is distributed along the mainland coast of the Sea of Japan, at Kunashir Island and Sakhalin Island.

MATERIAL AND METHODS

Seaweed collection

The specimens were collected from the low intertidal and shallow subtidal zones at 0 to 1 m depth at the following sites: in Rudnaya Bay at Cape Brinera (44°20′N, 135°50′E); at Cape Rassypnoy (44°38′N, 136°13′E), Sea of Japan; near Starodubskoe village (47°24′N, 142°50′E) Sakhalin Island, Sea of Okhotsk; at Cape Sukacheva (44°04′N, 145°52′E); and near Cape Remontniy (44°05′N, 145°53′E), Pacific coast of Kunashir Island (Fig. 1, Table S1). The specimens of *Devaleraea* spp. from the northwestern part of Tartar Strait collected by A.A. Dulenin (Khabarovsk branch of Russian Federal Research Institute of Fisheries and Oceanography) were also studied.

Specimens for molecular analysis were dried in silica gel; voucher specimens of the same thalli were made into

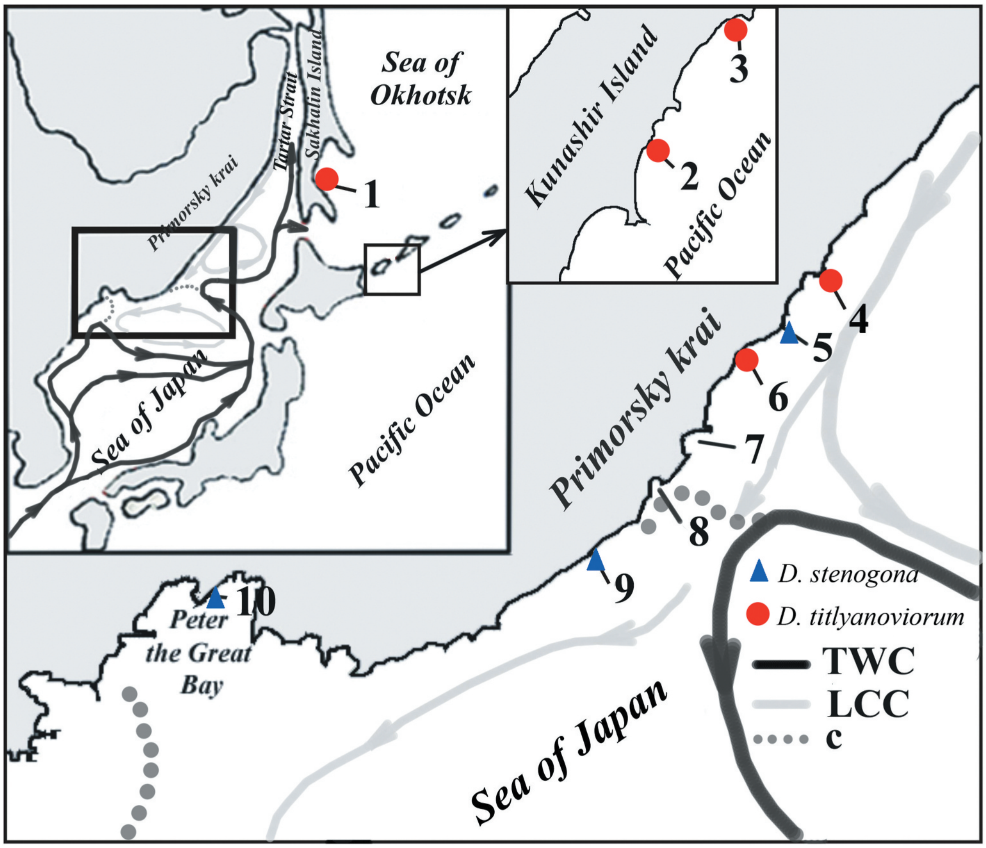


Fig. 1. Sampling localities and the scheme of surface circulation in the Sea of Japan. Sakhalin Island (Sea of Okhotsk): 1, near Starodubskoe village. Kunashir Island (Pacific Ocean): 2, Cape Sukacheva (type locality of *Devaleraea titlyanoviorum sp. nov.*); 3, Cape Remontniy. Continental coast (Sea of Japan): 4, Cape Rassypnoy; 5, Cape Rifoviy; 6, Rudnaya Bay Cape Brinera; 7, Vladimir Bay; 8, Olga Bay; 9, Kit Bay (type locality of *Devaleraea stenogona*); 10, Sobol Bay. Currents: TWC, Tsushima Warm Current; LCC, Liman Cold Current; c, chains of mesoscale anticyclonic eddies carrying warmer waters from the branches of Tsushima Warm Current to continental coast of northern part of the Sea of Japan (adapted from Danchenkov *et al.* 2006; Nikitin *et al.* 2009).

herbarium vouchers and kept in Herbarium (LE) of V.L. Komarov Botanical Institute (St. Petersburg, Russia) and in the Museum of A.V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS (MIMB, Vladivostok, Russia). These vouchers were used for morphological analysis.

Morphological observations

Cross sections were made by hand with a razor blade in the middle and apical portions of thalli and in proliferations. Sections were examined using a Zeiss AxioVert 200M microscope. Thallus length and texture, branch width, branching pattern, and shape of branches were analysed. For analysis of anatomy, we measured blade thickness, dimensions of cortical and medullary cells, number of cell layers in cortex and medulla, and thickness of cell wall and size of tetrasporangia. Specimens were compared with the holotype of *P. stenogona* (LE A0000175) collected from Valentin Bay in 1964 by L.P. Perestenko and housed in LE, with other original material of the species from the type collection (LE A0000176 and LE A0000177) and with specimens of *D. stenogona* collected in Sobol Bay and Kit Bay (Sea of Japan) whose morphology is similar to the holotype. Kit Bay is close to the type locality of *D. stenogona* (Skriptsova & Kalita 2020).

DNA extraction, polymerase chain reaction, sequence editing, and phylogenetic analysis

DNA preparation and polymerase chain reaction amplification is described in detail in Skriptsova & Kalita (2020). A partial fragment (1260 bp corresponding to positions 140–1400 of the complete chloroplast gene ribulose-1,5-biphosphate carboxylase/oxygenase [*rbc*L] gene [1467 bp] of *Palmaria palmata* [KX284726]) was amplified using specific primers, F57 (forward) and rbcLrevNEW (reverse; Saunders & Moore 2013). The ITS1 (forward) and JO6 (reverse) primers (Lindstrom *et al.* 1996) were used to amplify internal transcribed spacers (ITS), including: ITS1, 5.8S rDNA, and a partial sequence of ITS2 with total length of 910 to 927 bp. The COI-5′ (approximately 530 bp), corresponding to positions 177 to 707 of the complete cytochrome oxidase subunit 1 gene of *Palmaria palmata* (KF649305), was amplified using forward primer DevF1 (Bringloe & Saunders 2019), and reverse primer M13Rx (Saunders & Moore 2013), according to the protocol of Saunders & Moore (2013).

Polymerase chain reaction products were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP; ThermoFisher Scientific, Waltham, Massachusetts, USA). DNA bidirectional sequencing was performed using a BigDye 3.1 sequencing kit (ThermoFisher Scientific, California, USA) on an ABI 3130x sequencer (Applied Biosystems; Geospiza Inc., Seattle, Washington, USA). Sequences were processed using FinchTV software (v1.4.0; http://www.geospiza.com/Products/finchtv.shtml). All obtained sequences were aligned in MEGA (vX, Kumar *et al.* 2018) using the MUSCLE algorithm (Edgar 2004); final adjustments were made manually, if necessary. MEGA was also used for calculation of inter- and intraspecific ITS, COI-5′, and *rbc*L distances using the pairwise distance model (*p*-distance).

Phylogenetic relationships in Palmariaceae were estimated using Bayesian inference and maximum likelihood (ML) methods. Bayesian inference tree reconstructions were performed in MrBayes v3.2.7 (Huelsenbeck & Ronquist 2001), and ML was performed using RAxML v8.2.4 (Stamatakis 2006). Bayesian posterior probabilities (BPPs) were estimated using the Metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm, running four chains for 10^7 generations with trees sampled every 100 generations. The trace files generated by Bayesian MCMC runs were analysed in Tracer v1.7.1 (Rambaut *et al.* 2018). The first 250 trees were discarded as burn-in. Consensus topology and posterior probability (BPP) values were calculated from the remaining trees. Nonparametric bootstrap (bs) values were estimated to infer nodal support for ML, with 1000 pseudoreplicates (Felsenstein 1981). PartitionFinder v2.1.1 (Lanfear *et al.* 2012) was used to select the best-fit partitioning scheme and models separately for ITS1, 5.8S, and ITS2 using the greedy algorithm with linked branch lengths for the corrected Bayesian information criterion. FigTree v1.4.4 (Rambaut 2016) was used to visualise and edit trees. The best-fit model for ITS1 was HKY+G (Hasegawa *et al.* 1985), for 5.8S the JC model (Jukes & Cantor 1969), and for ITS2 the K80+G model (Kimura 1980); whereas, HKY+G and T92+G (Tamura 1992) were the best-fit models for COI-5′ and *rbc*L, respectively. *Palmaria palmata* and *P. hecatensis* were used as outgroup species.

RESULTS

Molecular analyses

A total of 18 sequences for ITS, 7 for COI-5′, and 14 for *rbc*L were obtained and deposited in GenBank (Table S2). We analysed a total of 56 ITS, 32 *rbc*L, and 57 COI-5′ sequences of specimens of *Devaleraea* and *Palmaria* from the North Pacific Ocean (Table S2).

In the Bayesian phylogenetic tree of ITS sequences, six well-supported (BPP = 1, ML bs = 97–100) clades corresponding to different species were found within *Devaleraea* (Fig. 2). The specimens of *D. titlyanoviorum sp. nov.* from Rudnaya Bay, Cape Rassypnoy, the eastern coast of Sakhalin Island, and Kunashir Island formed a well-supported clade (BPP = 1, ML bs = 100), sister to *D. mollis.* A clade of *Devaleraea stenogona* included algae from Sobol Bay and Kit Bay (Sea of Japan); the latter location was the closest to the type locality of *D. stenogona*. Specimens of ‘*P. palmata*’ from Japan were sister to *D. stenogona.* From ITS data, *D. titlyanoviorum sp. nov.* differed by 2.6% to 5.3% (*p*-distance) from other *Devaleraea* species (Table S3). These values significantly exceeded interspecific divergence in all analysed *Devaleraea* species (0.14%–0.57%) as well as in *D. titlyanoviorum* (0.57% ± 0.16%).

Although the COI–5′ Bayesian phylogenetic tree was unresolved, it also showed *D. titlyanoviorum* to be distinct (Fig. S1). The specimens of the species from Kunashir Island, Sakhalin Island, and from Rudnaya Bay formed a separate clade. Again, the specimens of ‘*P. palmata*’ from Japan were sister to *D. stenogona*. The *p*-distances between species are listed in Table S3.

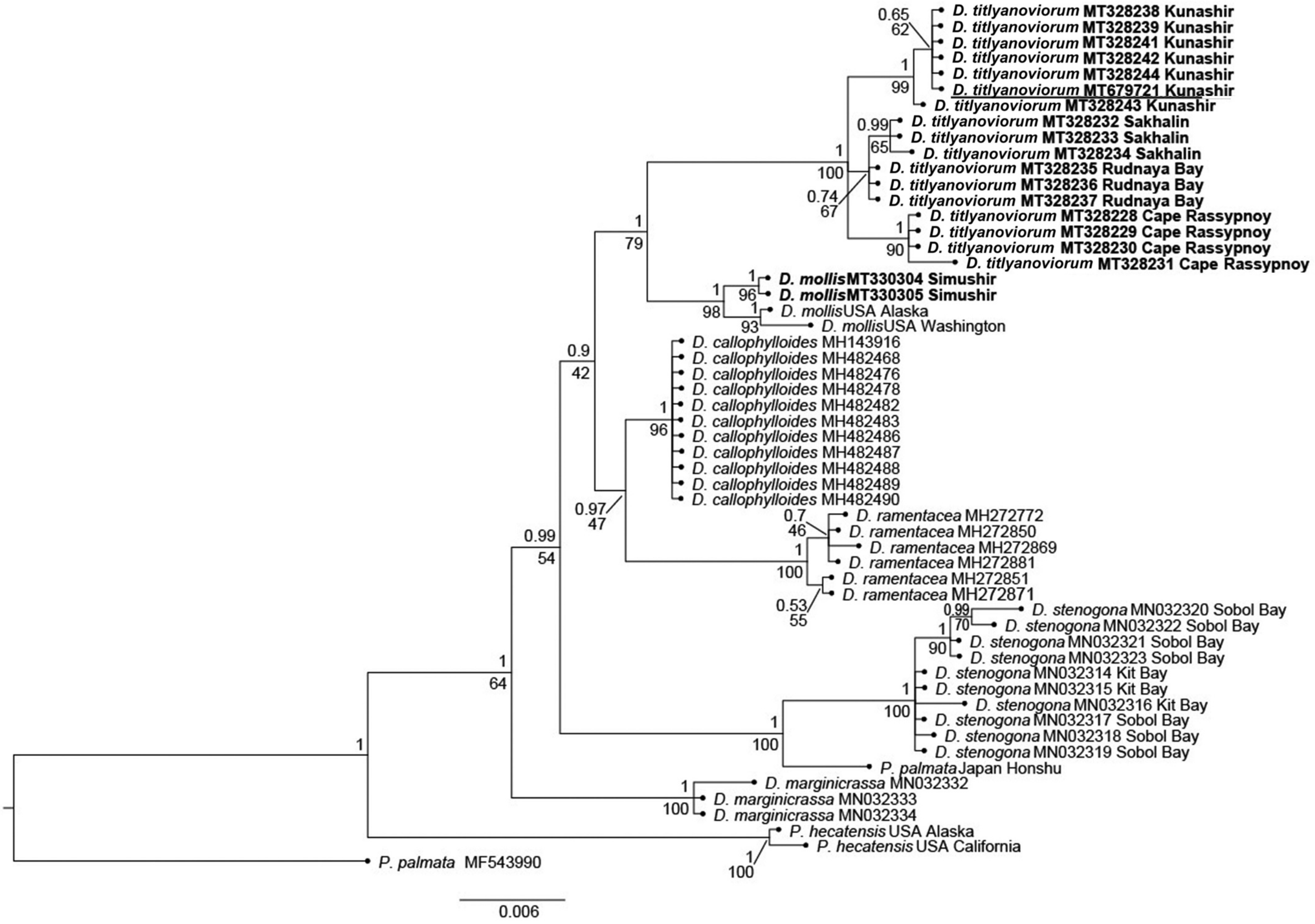


Fig. 2. Bayesian tree inferred from ITS sequences of *Devaleraea*. *Palmaria palmata* and *P. hecatensis* were used as the outgroups. Values of Bayesian posterior probabilities are given above nodes and ML bootstrap values beneath nodes. Specimens obtained in this study are highlighted in bold. The holotype of *Devaleraea titlyanoviorum sp. nov*. is indicated by underlining.

Devaleraea titlyanoviorum sp. nov. and *D. stenogona* formed a single clade only in the ML tree of *rbc*L (Fig. S2). This clade clearly differed from other analysed species.

Morphological observations

A morphological comparison of specimens of *D. titlyanoviorum sp. nov*. from the Pacific coast of Kunashir Island, Sakhalin Island, and the continental coast of the Sea of Japan (Cape Rassypnoy and Rudnaya Bay) with specimens and the holotype (LE A0000175; Fig. 3) of *D. stenogona sensu stricto* as well as description of other *Devaleraea* species (I.K. Lee 1978; Hawkes & Scagel 1986) revealed a number of distinctive features (Table 1). Based on molecular and morphological data, we propose a new species.

Devaleraea titlyanoviorum Skriptsova et Kalita *sp. nov.*

Figs 4–12

DIAGNOSIS: Blades coriaceous, oblanceolate, strap-like or narrow wedge-shaped, dichotomously or palmately branched two to three times, with cuneate bases, wide (> 0.5 cm) terminal branches with rounded or obtuse tips, proliferating on the margin. Medulla consists of one or two layers of large colourless cells surrounded by smaller medullary cells. Cortex one-to three-celled or multilayered. Marginal thickening of the cortex in vegetative thalli absent, but this can occur in old parts of thalli. Tetrasporangial sori formed on both surfaces of lamina or on large proliferations covering entire lamina except margins and basal portion.

HOLOTYPE: LE A0000179, deposited in Herbarium (LE) of Komarov Botanical Institute of the Russian Academy of Sciences (St. Petersburg, Russia), collected on 01 June 2018 by A.V. Skriptsova. GenBank accession: ITS sequence MT679721, *rbc*L sequence MT353898, and COI-5′ sequence MT701560.

ISOTYPES: LE A0000180 and LE A0000181, deposited in Herbarium (LE) of Komarov Botanical Institute of the Russian Academy of Sciences (St. Petersburg, Russia),

TYPE LOCALITY: Southern Kurils, Kunashir Island, Cape Sukacheva (44°04′N, 145°52′E), intertidal zone.

ETYMOLOGY: The specific epithet is dedicated to Prof. Eduard A. Titlyanov and Dr Tamara V. Titlyanova from the National Scientific Center of Marine Biology, Vladivostok, Russia, in recognition of their many contributions to the research of ecology and physiology of marine algae and hermatypic corals.

HABITAT AND DISTRIBUTION: The alga grows in the lower intertidal to upper subtidal zone, forming extensive unialgal beds rarely intermingled with other seaweeds. It is distributed off the continental coast of the Sea of Japan northward up to Rudnaya Bay, off Sakhalin Island (the Sea of Okhotsk coast), and on the Pacific coast of Kunashir Island. It grows in areas usually not subjected to ice scour in winter.

SPECIMENS STUDIES: LE A000184, MIMB39944, Kunashir Island, near Cape Remontniy, 25 August 2016 (sterile); MIMB39942, MIMB39945, Kunashir Island, near Cape Remontniy, 01 June 2018 (with tetrasporangia and sterile); MIMB39946, Kunashir Island, near Cape Remontniy, 09 June 2018 (with tetrasporangia and sterile); LE A000179, LE A0000180, LE A0000181, MIMB39947, Kunashir Island, Cape Sukacheva, 01 June 2018 (sterile and with tetrasporangia); LE A0000183, Sakhalin Island, coast of the Sea of Okhotsk, near Starodubskoe village (offshore), 27 May 2018 (sterile); MIMB39943, Sakhalin Island, coast of the Sea of Okhotsk, near Starodubskoe village, 27 August 2011 (sterile); LE A0000182, MIMB39940, Sea of Japan, Rudnaya Bay, Cape Brinera, 10 May 2018 (sterile and with tetrasporangia); MIMB39941, Sea of Japan, Cape Rassypnoy, 10 May 2018 (with tetrasporangia). All specimens were collected by A.V. Skriptsova.

Habit and vegetative anatomy

Devaleraea titlyanoviorum sp. nov. showed high phenotypic plasticity with plant morphology differing in different areas and ages (Figs 4–7). Blades were 7 to 20 cm long and 0.5 to 4 cm wide, not hollow, with cuneate bases, adhering to substratum by means of discoid crusts up to 2 mm in diameter. Some laminae can grow from a common crust. Laminae strap-like or narrow wedge-shaped, gradually broadening upwards, brownish-red in colour, fading to yellowish in the fertile part (Figs 4–7). Blades dichotomously branched one to three times; terminal branches strap-shaped 0.5 to 1 cm wide, with rounded tips. Blades can perennate and proliferate from the base of the blade (Fig. 6) or from a whole lamina (Fig. 5) on the margin and apex and rarely on blade surface. Proliferations 0.3–1.5 × 1.5–20 cm, narrow lanceolate, cuneiform or strap-shaped, dichotomously divided in the upper part one time (rarely two times), sessile, often consist of two to three orders (Fig. 5). Proliferations with rounded or obtuse tips; often similar to main lamina in size or exceed it (Fig. 5). Proliferations bear tetrasporangia often larger that sterile ones, 2–3.5 × 7–10 cm (Fig. 4). At the end of reproductive season abundant small proliferations can develop on the blade surface.

Blades 150 to 500 µm thick, varying within the same thallus from thin in the proliferations to thick in older blades. Medulla consists of one or rarely two layers of loosely coherent large rounded or oval cells of 58 to 268 µm in diameter, surrounded by one or two layers of smaller cells of 20 to 77 µm in diameter (Fig. 8). These subsurface cells are typically granulated in old plants; their content is brown, grey, or black (not red) in colour (Fig. 9). Cell walls in old plants hyaline, up to 2 to 11 µm thick. Cortex typically composed of two to six rows of densely pigmented cells of 3.8–11.8 × 5–15 µm; in young plants proliferations of one or two rows, and in oldest blades of thallus, up to 10 rows. Cortex at margin frequently not thickened (Fig. 10).

Reproductive morphology

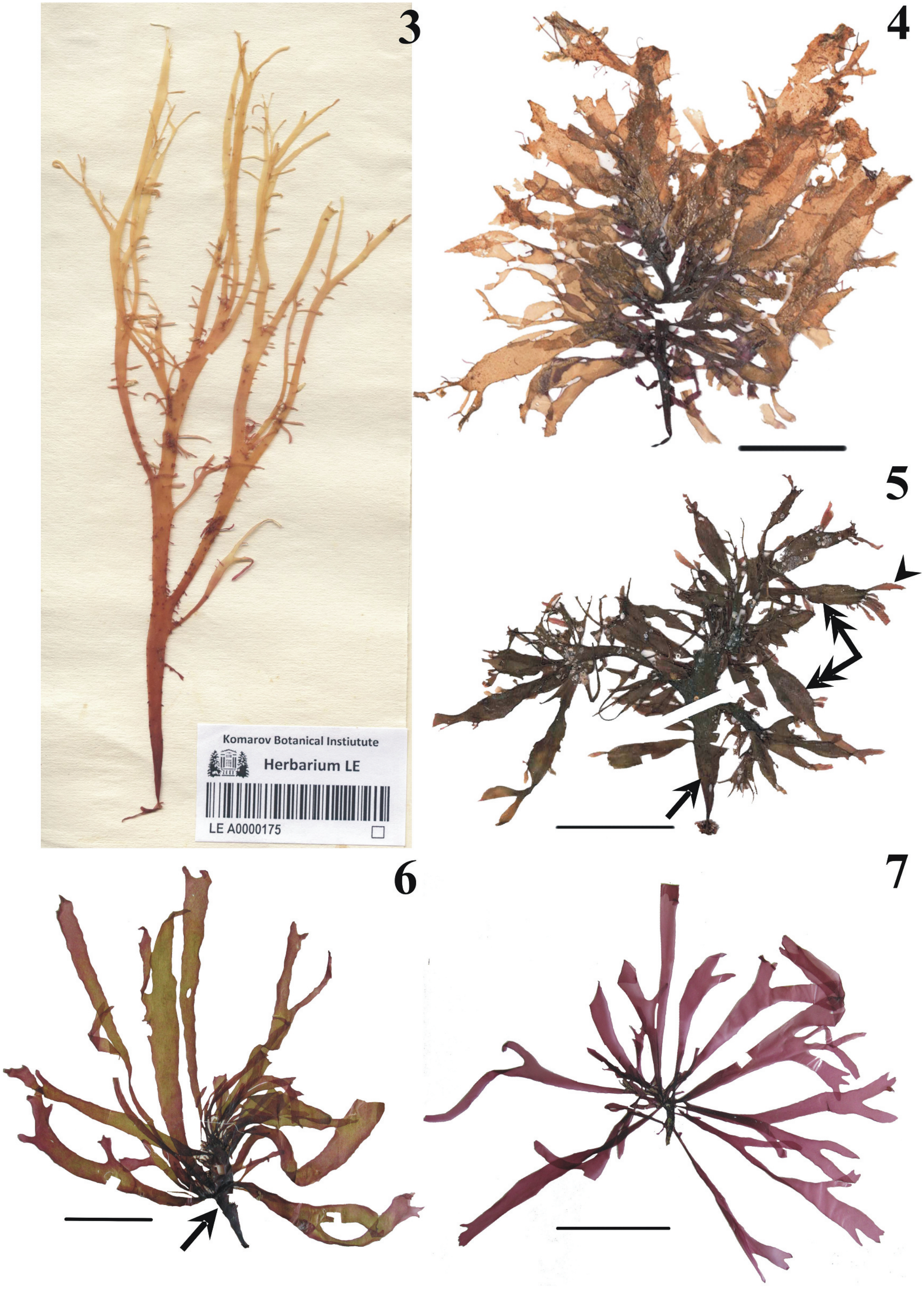
Tetrasporangia formed sori on both blade surfaces in first-year thalli and on proliferations in regenerated plants. Sori began developing as longitudinal darker patches in upper portion of blade/proliferation and then coalesced and spread over entire blade except for margins and lower portions of blades. Parts of blade bearing mature tetrasporangia were yellow or reddish yellow, usually lighter in colour than sterile parts. Boundary between fertile and sterile parts was distinct.

Tetrasporangia were cruciately divided, oval, 26–42 × 26–40 µm with a stalk cell (Fig. 11). When mature, tetrasporangia occurred among two- to four-celled paraphyses up to 35 µm long formed by elongated cells, with the apical cell of paraphyses broadened in the upper part (Fig. 12). In surface view, tetrasporangia were 30 to 43 µm wide. Spermatangial plants were not found.

DISCUSSION

Our phylogenetic and morphological analyses have shown that *D. stenogona sensu lato* is a complex of at least two species and we have segregated *Devaleraea titlyanoviorum sp. nov. Devaleraea stenogona* is now restricted to plants with narrow, wedge-shaped, abundantly and irregularly branched thalli, long (up to 2 cm), narrow-linear (usually less than 2 mm) terminal branches with acute tips as described in Skriptsova & Kalita (2020). Such morphology is similar to the holotype of *D. stenogona* collected from Valentin Bay in 1964 by L.P. Perestenko (LE A0000175) and other original material of the species from the type collection (LE A0000176 and LE A0000177) kept in LE.

To date, four species of solid *Devaleraea* – *D. stenogona*, *D. callophylloides*, *D. mollis*, and *D. marginicrassa* – and three species of *Palmaria* – *P. hecatensis*, *P. integrifolia*, and *P. moniliformis* – whose generic position needs to be verified – have been reported from the northwest Pacific. Among them, only *D. marginicrassa*, *D. mollis*, and *D. stenogona* can be confused with *D. titlyanoviorum*. Young *D. titlyanoviorum* is similar to *D. stenogona*, while the latter species differs from *D. titlyanoviorum* in having irregular ramifications and narrow terminal branches with acute tips (Table 1). Old, abundantly proliferating plants of *D. titlyanoviorum* may look similar to proliferating *D. marginicrassa* or *D. mollis*. However, fronds of *D. marginicrassa*, unlike *D. titlyanoviorum*, are rough, almost firm, and proliferations are simple, widely oblong to ovate (I.K. Lee 1978). Moreover, *D. marginicrassa* clearly differs from other species by its multilayered medulla and especially thick multilayered cortex at the margins. Differentiation of *D. titlyanoviorum* and proliferating *D. mollis* seems difficult. Although blades of *D. mollis*, in general, are simple, lanceolate, or irregularly palmate-lobed (Table 1), they can perennate and regenerate new blades from basal portions (Hawkes & Scagel 1986) similar to *D. titlyanoviorum* (Figs 6–7; for example, see photos of numerous *D. mollis* specimens at Macroalgal Herbarium Portal, 2020). Both species are highly polymorphic and their identification is problematic. Nevertheless, these two species are phylogenetically distinct and differ in tetrasporangial size (Table 1). Comparative morphological and anatomical analysis is necessary to find additional distinguishing features of these species.



Figs 3–7. Holotype of *Devaleraea stenogona* and morphology of *D. titlyanoviorum sp. nov*.

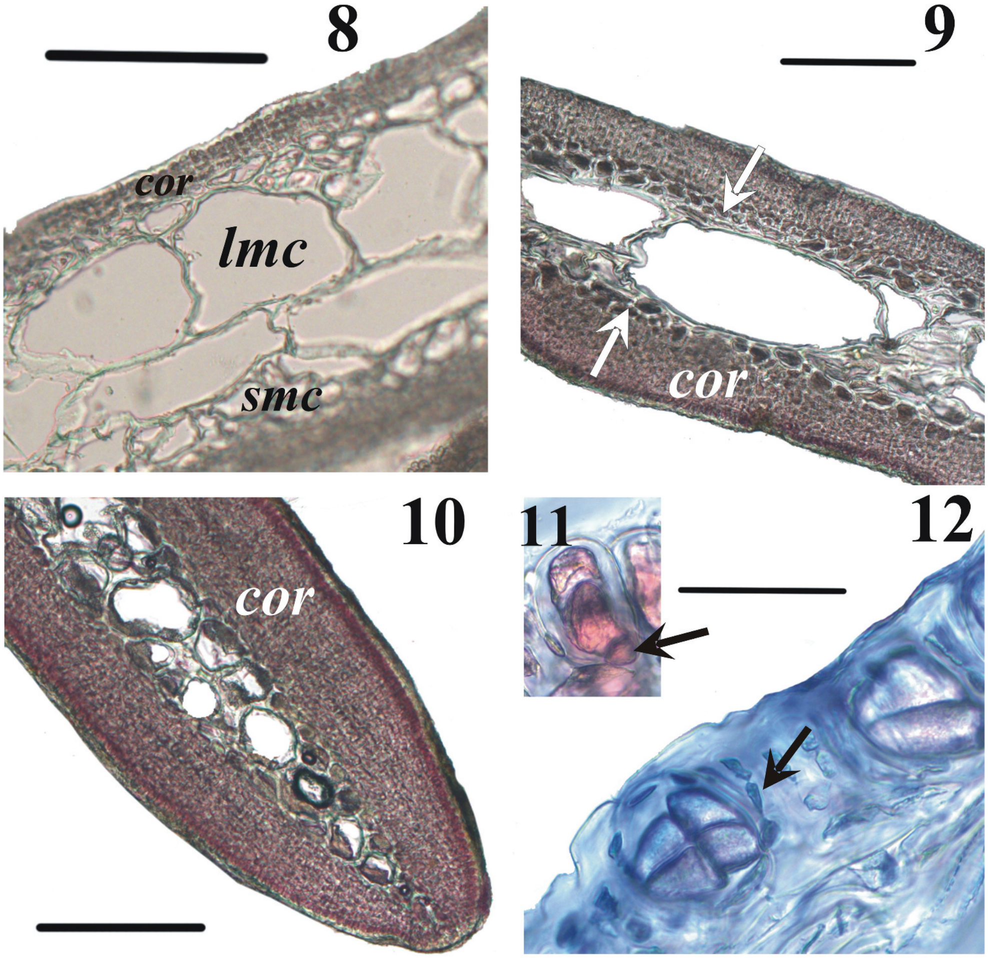
Fig. 3. Holotype of *D. stenogona* (LE A0000175).

Fig. 4. Holotype of *D. titlyanoviorum sp. nov*. (LE A0000179). Plants from Cape Sukacheva, Kunashir Island collected on 01 June 2018. Note large fertile proliferations. Scale bar = 5 cm.

Fig. 5. Plants from Cape Remontniy, Kunashir Island collected on 25 August 2016 (LE A0000184); arrows indicate basal main blade (single arrow); proliferation of first order (double arrow); and proliferations of second order (arrowhead). Scale bar = 5 cm.

Fig. 6. Plants from Rudnaya Bay collected on 10 May 2018 (LE A0000182); arrow indicates basal remnant of main blade. Scale bar = 5 cm.

Fig. 7. Plants from Sakhalin Island collected on 28 May 2018 (LE A0000183). Scale bar = 5 cm.



Figs 8–12. Anatomy of *Devaleraea titlyanoviorum sp. nov*.

Fig. 8. Cross section through vegetative thallus shows medulla with smaller cells (smc) surrounding larger cells (lmc) and three- or four-layered, small-celled cortex (cor). Scale bar = 200 µm.

Fig. 9. Cross section through thallus collected in September with thick medullary cell walls, granulated small medullary cells (white arrows), and thick multilayered cortex. Scale bar = 20 µm.

Fig. 10. Margin of old blade. Scale bar = 20 µm.

Fig. 11. Young tetrasporangia with stalk cell (arrow).

Fig. 12. Mature tetrasporangia surrounded by unbranched paraphyses (arrow). Scale bar = 40 µm.

In solid *Devaleraea*, species boundaries are difficult to characterise in many cases, because environment can influence morphology (light levels, wave exposure, disturbance, grazing). Similar to other species, *D. titlyanoviorum* shows high phenotypic plasticity; that is, plant morphology differs significantly in different areas. Although the species occurs in the low intertidal and in upper subtidal zones, its habitats differ in different areas. On the Pacific coast of Kunashir Island (Cape Sukacheva and Cape Remontniy), *D. titlyanoviorum* grows at wide (up to 150 m), shallow (depth of 0.8–1.2 m), open-coast tidal flats formed by a rocky platform where algae are not directly exposed to waves. Under these conditions the main blades and proliferations are not destroyed after spore discharge and can regenerate new plants (proliferations) from margins of the main blade the following year. In such thalli, proliferations are abundant, simple, short, oval or lanceolate, often of two to three orders (Fig. 5), or, rarely, strap-like, divided on lobes. In contrast, the eastern coast of Sakhalin Island of the Sea of Okhotsk near Starodubskoe village and areas of the continental coast at Cape Rassypnoy and Cape Brinera (Rudnaya Bay) are exposed to waves and are characterised by deep narrow basins between rocks protruding from the water. The strong wave activity destroys the blades, and proliferations develop from the small lower remainders of old thalli. They are long, narrow, and divided on lobes (Figs 6, 7). This agrees with general responses of seaweed morphology to wave exposure (Shibneva & Skriptsova 2015). Thus, habitat seems to influence thallus form of *D. titlyanoviorum*.

Previously, in the Sea of Japan and in the southern part of the Sea of Okhotsk, only two species of solid *Devaleraea*, *D. stenogona* and *D. marginicrassa*, were reported (Perestenko 1994). Although *D. stenogona* was suggested to occur all along the Russian coast of the Pacific from the Sea of Japan to the Bering Sea (Guiry & Guiry 2020), we did not find this species at Sakhalin Island or Kunashir Island, where the alga was reported as common. All collected specimens from these localities were identified as *D. titlyanoviorum*,

Table 1. Comparison of *Devaleraea titlyanoviorum sp. nov*. and morphologically close related species of *Devaleraea* from northwest Pacific.

Characteristic	*D. titlyanoviorum*	*D. stenogona*	*D. mollis*	*D. marginicrassa*
Blade	Oblanceolate, strap-like or wedge-shaped, gradually broaden upwards, dichotomously branched	Cuneate, strap-shaped with narrow terminal branches, dichotomously, trichotomously, irregularly, or palmately branched several times	Simple, proliferous, lacerate to irregularly palmate	Oblanceolate, simple, or rarely branched once dichotomously
Proliferations	Abundant at margin, one to three orders, oblanceolate, strap-shaped, the same size as main blade or larger	Usually non-proliferating; small proliferations can develop at surface at end of reproduction period	Short proliferations near the base of blade or abundant at margins of old blade; proliferations simple or palmate with irregular lobes and dichotomies[a]	At margin and surface of low portion of old blade
Lamina thickness (μm)	150–500	100–390	140–480	350–510
Number of layers of cells in the medulla	One to three layers of large cells (in old parts single layer) surrounded by one to two layers of smaller cells. Subcortical cells are granulated in old parts	One (two) layers of large cells (in old parts typically two to three layers) surrounded by one to two layers of smaller cells; smaller cells fill the spaces between the larger cells. Subcortical cells not granulated	One layer of large cells or two or three layers of smaller ones or smaller cells fill spaces between larger cells	Three to five (eight) layers of thick-walled cells
Number of layers of cells in the cortex	Three to six, up to 10 in basal part, one to two in proliferations of last order	One to two, up to 10 in basal part	One, up to four to five in base of blade	Three to four, up to 15 at margin
Form and position of the sori	Coalescent non-obvious covered entire blade except margin and base	Entire lamina except the basal portion with distinct boundary between sterile and fertile parts	Coalescent non-obvious discrete patches covered entire blade except margin and base	Coalescent hieroglyphic, except margin and base of blade
Paraphyses	Three- to five-celled, branched or unbranched	Three- to five-celled branched or unbranched, 35–60 μm long	Three- to four-celled, branched and unbranched	Four to five cells in common wall with large subspherical uppermost cell
References	This study	Skriptsova & Kalita 2020	Hawkes & Scagel 1986	Lee 1978

[a] These data were obtained from viewing of herbaria at the Macroalgal Herbaria Portal (2020) and from Lindeberg & Lindstrom (2010).

suggesting that *D. stenogona* may have been misidentified here. The distributions of *D. titlyanoviorum* and *D. stenogona* overlap on the continental coast of the Sea of Japan. For example, *D. titlyanoviorum* was found at Cape Rassypnoy and in Rudnaya Bay, and *D. stenogona* was collected in a small bight near Cape Rifoviy, which is located between these two sites (Fig. 1). In addition, four herbarium specimens from the Tartar Strait were identified by us as *D. stenogona*. Our investigation shows that, in comparison with *D. titlyanoviorum* living north and east of Rudnaya Bay, *D. stenogona* occurs farther south to Peter the Great Bay (Sea of Japan). It is unknown whether *D. stenogona* occurs along the continental coast of the Sea of Okhotsk or whether early reports from here are *D. titlyanoviorum* or other species. Additional studies are needed to clarify these issues. However, it is safe to say that *D. titlyanoviorum* does not occur south of Kit Bay. Such species distributions can be related to hydrological conditions determined by surface water circulation in the northwestern part of the Sea of Japan. This part of the sea is influenced mainly by the Liman Cold Current, which is born in the cold brackish water of the Tartar Strait near 50°N and flows to the south near the shelf break along the Russian coast. In the vicinity of Vladimir Bay, the current turns southeastward and deepens, and then flows from the coast (Suprakovich 1976). Nearshore areas south of Vladimir Bay, such as Olga Bay and Peter the Great Bay, are influenced by warm water of the Tsushima Current penetrating to the coast of Primorsky krai through chains of mesoscale anticyclonic eddies that form for 1 to 12 months (Danchenkov *et al.* 2003; Nikitin *et al.* 2009). In summer, environmental conditions here are close to subtropical and average surface water temperature in August is 20 °C to 23 °C, and it can reach 25 °C to 27 °C (Climatic data on hydrometeorological conditions in nearshore zone of the Sea of Japan: water temperature (RU_RIHMI-WDC_1004) 2020). The high water temperature in summer obviously could limit growth of *D. titlyanoviorum* south of Vladimir Bay. The ranges of *D. titlyanoviorum* and *D. stenogona* also may overlap on the coast of the Hokkaido, adjacent to Sakhalin Island and Kunashir Island. The phylogenetic analysis based on COI–5′ showed that algae from southwestern Hokkaido identified as '*P. palmata*' are genetically close to *D. stenogona*, and the proximity of Hokkaido to the type locality of *D. titlyanoviorum* would suggest the distribution of this species here. However, further study is necessary to draw a firm conclusion. Likewise, the distribution of *D. titlyanoviorum* and *D. stenogona* northward from Kunashir Island and along the mainland coast of the Sea of Okhotsk requires additional study.

Thus, despite the family Palmariaceae being well studied (I.K. Lee 1978; Hawkes & Scagel 1986; Selivanova & Zhigadlova 2010; Selivanova 2016), our results extend it by adding another species. The northwest Pacific is a hotspot of biodiversity for Palmariaceae in general, and *Devaleraea* in particular. At least 14 species from three genera occur here. This group shows high endemism, with only six species reported from waters outside the North Pacific. Seven species occur only along the Russian coast and off Hokkaido.

The Russian coast of the northwest Pacific is a geographical region with variable environmental conditions that allow the establishment of different species of red algae. Three centres of speciation exist here (Perestenko 1994), with one of them including waters around the Lesser and southern Kuril Islands, off southern Sakhalin, and Hokkaido. The limited distribution of some *Devaleraea* spp. allows us to speculate that they originated in Russian waters. One such species is obviously *D. titlyanoviorum*. However, this assumption requires confirmation using detailed data on the geographical distribution of the species and thorough comparison of the estimated time of species divergence and the palaeogeography of the region.

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AUTHOR CONTRIBUTIONS

A.V. Skriptsova: seaweed collection, original concept, light microscopy, manuscript preparation. T.L. Kalita: analysis of molecular data and morphological analysis, manuscript preparation. S.Y. Shibneva: sequencing of the specimens, manuscript preparation. A.A. Semenchenko: analysis of molecular data, manuscript preparation.

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