VENUS 77 (1–4): 1–14, 2019
DOI: http://doi.org/10.18941/venus.77.1-4_1

First Molecular Confirmation of the Presence of *Dendronotus primorjensis* Martynov, Sanamyan & Korshunova, 2015 in Japan and New Distributional Records of *Dendronotus* Species in the North Pacific (Nudibranchia: Dendronotidae)

Tatiana Korshunova[1,5], Rie Nakano[2], Karin Fletcher[3], Nadezhda Sanamyan[4] and Alexander Martynov[5]*

[1]*Koltzov Institute of Developmental Biology RAS, 26 Vavilova Str., 119334 Moscow, Russia*
[2]*Kuroshio Biological Research Foundation, 560-I, Nishidomari, Otsuki, Hata-Gun, Kochi 788-0333, Japan*
[3]*Port Orchard Washington, 98366, USA*
[4]*Kamchatka Branch of Pacific Geographical Institute FEB RAS, Partizanskaya Str. 6, 683000 Petropavlovsk-Kamchatsky Russia*
[5]*Zoological Museum, Moscow State University, Bolshaya Nikitskaya Str. 6, 125009 Moscow, Russia*

Abstract: New data on nudibranch molluscs of the genus *Dendronotus* in the North Pacific are presented. For the first time, the presence of the nudibranch *Dendronotus primorjensis* Martynov, Sanamyam & Korshunova, 2015 in the Japanese fauna is confirmed using molecular data. Recently collected Japanese specimens of *D. primorjensis* from Hokkaido are compared with those from the type locality of this species in the Sea of Japan, Russia, using external and internal morphological data. Furthermore, the presence of the species *Dendronotus robilliardi* Korshunova, Sanamyan, Zimina, Fletcher & Martynov, 2016 is also reported from both the Japanese fauna in the NW Pacific and in the NE North American Pacific. Molecular data for *D. robilliardi* from the NE Pacific (Port Orchard, Washington State, USA) are reported here for the first time, whereas *D. robilliardi* was identified from Japan via photographic records using the previously inferred robust molecular and morphological framework for this species. Finally, the species *Dendronotus kalikal* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan & Martynov, 2015 is reported from the Kuril Islands, which is the first record outside of the type locality in Kamchatka.

Keywords: Japanese fauna, molecular analysis, morphology, North Pacific fauna, nudibranchs, taxonomy

Introduction

Until recently, the majority of representatives of the genus *Dendronotus* with a bushy appearance in a variety of colours were considered to be the single species *D. frondosus* (Ascanius, 1774) whether in the Atlantic or in the north western Pacific (*e.g.*, Robilliard, 1970; Higo & Goto, 1993) as well as in the northern part of Japan (where it was also known by its synonym *D. arborescens*) (Baba, 1957, 1993). Prior to 2018, only a few species of the family Dendronotidae Alder & Hancock, 1864 had been recorded from Japan, *i.e.*, *Dendronotus gracilis* Baba, 1949 and *Pseudobornella orientalis* Baba, 1932 (Baba, 1932, 1949).

Our knowledge about North Pacific *Dendronotus* species composition has considerably improved

* Corresponding author: martynov@zmmu.msu.ru

recently and currently the species *D. kalikal* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan & Martynov, 2015, *D. kamchaticus* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan & Martynov, 2015, *D. primorjensis* Martynov, Sanamyan & Korshunova, 2015, and *D. robilliardi* Korshunova, Sanamyan, Zimina, Fletcher & Martynov, 2016 are also recognised in the North West Pacific (Martynov *et al.*, 2015a, b; Korshunova *et al.*, 2016a, b). None of these species have been reported from Japan until very recently, when *D. primorjensis* and *D. robilliardi*, as well as several unidentified *Dendronotus* species, were included in the colour guide to the Japanese opisthobranch fauna by Nakano (2018). However, no molecular or detailed morphological studies have been conducted on any of these recently described species in order to confirm their presence in Japan.

In this study we present a molecular phylogenetic analysis of the species *D. primorjensis* from Hokkaido (Usujiri), confirming its presence in Japan. A scanning electron microscope (SEM) investigation of the radula was conducted for the first time and is also presented here. The general pattern of geographic distribution of *D. primorjensis* (occurring both in the Russian part of the Sea of Japan and in northern Japan) is thus fundamentally similar to the aeolidacean nudibranch *Trinchesia lenkae* Martynov, 2002, which was recently recorded from the Pacific coast of Hokkaido (Korshunova *et al.*, 2018a). Newly collected specimens of other *Dendronotus* species from the northern Pacific are also included in the molecular phylogeny. As a result, the species *D. kalikal* is recorded for the first time from the Kuril Islands and the presence of *D. robilliardi* is confirmed for the first time in the northeastern Pacific (Port Orchard, Washington State, USA) using molecular data. The molecular and morphological framework for the North Pacific *Dendronotus* species allows us also to confirm the identification of *D. robilliardi* from Japan (Honshu) based on the external morphological data using photographic records. These novel data further contribute to the understanding of biogeographic and taxonomic patterns of the family Dendronotidae, both from the northern Pacific in general and the Japanese fauna in particular.

Materials and Methods

Collecting data and morphological methods

Material for this study was obtained by SCUBA diving at Usujiri (North West Pacific, Hokkaido, Japan), Matua Island (North West Pacific, Middle Kuril Islands, Russia), and Port Orchard (North East Pacific, Washington, USA). The morphology of the molluscs and their egg masses were studied under a stereomicroscope and imaged using Nikon D-90 and D-810 digital cameras. For the description of internal features, both preserved and fresh specimens were dissected under the stereomicroscope. The buccal mass of each specimen was extracted and processed in a weak solution of domestic bleach. The jaws were analysed under the stereomicroscope and scanning electron microscope. The gold and platinum sputter-coated radulae were examined using a scanning electron microscope (JSM 6380).

Molecular methods

Two specimens of *Dendronotus primorjensis* from Japan, *D. robilliardi* and *D. venustus* from the USA, and *D. kalikal* from Russia were successfully sequenced for the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S rRNA, and the nuclear genes 28S rRNA (C1-C2 domain). Additionally two *Dendronotus velifer* G.O. Sars, 1878 from Russia were successfully sequenced for the nuclear genes 28S rRNA (C1-C2 domain). The DNA extraction procedure, PCR amplification options and sequence obtainment are as previously described in detail by Korshunova *et al.* (2017, 2018a, b). COI sequences were translated into amino acids for confirmation of the alignment. All new sequences were deposited in GenBank (Table 1, highlighted in bold). Publicly available sequences of *Dendronotus* species plus several outgroup taxa (*Tritonia*, *Marionia*, and *Notobryon*) were also included in the molecular analysis. Sequences were aligned with the MAFFT

Table 1. List of specimens used for the molecular analyses.

Species name	Voucher	Locality	COI	16S	28S
Dendronotus albus MacFarland, 1966	ZMMU:Op-566	USA: Washington	KX788135	KX788123	KX788114
Dendronotus albus MacFarland, 1966	LACM:2004-2.2	USA: California	KX058081	KX058121	KX058093
Dendronotus arcticus Korshunova *et al.*, 2016	ZMMU:Op-561	Russia: Laptev Sea	KX788140	KX788129	KX788118
Dendronotus arcticus Korshunova *et al.*, 2016	ZMMU:Op-562	Russia: Laptev Sea	KX788141	KX788130	KX788119
Dendronotus dalli Bergh, 1879	ZMMU:Op-330	Russia: Kamchatka	KM396999	KM397081	KM397040
Dendronotus dalli Bergh, 1879	ZMMU:Op-331	Russia: Kamchatka	KM397000	KM397082	KM397041
Dendronotus frondosus (Ascanius, 1774)	ZMMU:Op-380	Norway	KM396976	KM397056	KM397017
Dendronotus frondosus (Ascanius, 1774)	ZMMU:Op-588	Norway	KY391832	KY391852	KY391870
Dendronotus europaeus Korshunova *et al.*, 2017	ZMMU:Op-554	Norway	KY391823	KY391842	KY391860
Dendronotus europaeus Korshunova *et al.*, 2017	ZMMU:Op-581	UK: Irish Sea	KY391826	KY391845	KY391863
Dendronotus iris J. G. Cooper, 1863	CASIZ:174471	USA: Washington	KX058083	HM162631	KX058096
Dendronotus iris J. G. Cooper, 1863	LACM:174194	USA: Washington	KX058082	GU339188	KX058095
Dendronotus kalikal Ekimova *et al.*, 2015	ZMMU:Op-283	Russia: Kamchatka	KC660024	KC611284	KC660012
Dendronotus kalikal Ekimova *et al.*, 2015	ZMMU:Op-285	Russia: Bering Strait	KC660027	KC611287	KC660015
Dendronotus kalikal Ekimova *et al.*, 2015	ZMMU:Op-349	Russia: Kamchatka	KM396987	KM397069	KM397028
Dendronotus kalikal Ekimova *et al.*, 2015	ZMMU:Op-657	Russia: Matua	**MK302458**	**MK302453**	**MK302465**
Dendronotus kamchaticus Ekimova *et al.*, 2015	ZMMU:Op-245	Russia: Kamchatka	KC660032	KC611288	KC660016
Dendronotus kamchaticus Ekimova *et al.*, 2015	ZMMU:Op-565	USA: Washington	KX788144	KX788111	KX788121
Dendronotus lacteus (W. Thompson, 1840)	ZMMU:Op-584	Norway	KY391830	KY391849	KY391867
Dendronotus lacteus (W. Thompson, 1840)	ZMMU:Op-587	Russia: Franz Josef Land	KY391838	KY391851	KY391869
Dendronotus niveus Ekimova *et al.*, 2015	ZMMU:Op-269	Russia: White sea	KM396996	KM397078	KM397037
Dendronotus niveus Ekimova *et al.*, 2015	ZMMU:Op-279	Russia: Barents Sea	KM396995	KM397077	KM397036
Dendronotus patricki Stout *et al.*, 2011	SIO-BIC M12133	USA: California	HQ225828	HQ225829	—
Dendronotus primorjensis Martynov *et al.*, 2015	ZMMU:Op-419	Russia: Sea of Japan	KX672010	KX672008	KX672006
Dendronotus primorjensis Martynov *et al.*, 2015	ZMMU:Op-420	Russia: Sea of Japan	KX672011	KX672009	KX672007
Dendronotus primorjensis Martynov *et al.*, 2015	ZMMU:Op-639	Japan	**MK302456**	**MK302451**	**MK302463**
Dendronotus primorjensis Martynov *et al.*, 2015	ZMMU:Op-640	Japan	**MK302457**	**MK302452**	**MK302464**
Dendronotus regius Pola & Stout, 2008	CASIZ:179492	Philippines	HM162708	HM162629	—
Dendronotus regius Pola & Stout, 2008	CASIZ:179493	Philippines	JN869451	JN869407	—
Dendronotus robilliardi Korshunova *et al.*, 2016	ZMMU:Op-567	Russia: Kamchatka	KX788136	KX788124	KX788115
Dendronotus robilliardi Korshunova *et al.*, 2016	ZMMU:Op-568	Russia: Kamchatka	KX788138	KX788126	KX788116
Dendronotus robilliardi Korshunova *et al.*, 2016	ZMMU:Op-447	Russia: Kamchatka	KX788139	KX788127	KX788117
Dendronotus robilliardi Korshunova *et al.*, 2016	ZMMU:Op-659	USA: Washington	**MK302459**	**MK302454**	**MK302466**
Dendronotus robustus A. E. Verrill, 1870	ZMMU:Op-343	Russia: Barents Sea	KM397002	KM397084	KM397043
Dendronotus robustus A. E. Verrill, 1870	ZMMU:Op-390-5	Russia: Barents Sea	KM396968	KM397051	KM397009
Dendronotus rufus O'Donoghue, 1921	LACM:174861	USA: Alaska	KX058084	GU339191	KX058097
Dendronotus rufus O'Donoghue, 1921	LACM:174863	USA: Alaska	KX058085	KX058122	KX058098
Dendronotus subramosus MacFarland, 1966	LACM:174192	USA: California	—	GU339192	KX058100
Dendronotus subramosus MacFarland, 1966	LACM:174868	USA: California	—	KX058123	KX058099
Dendronotus velifer G. O. Sars, 1878	ZMMU:Op-348	Russia: Kara Sea	MF685027	KY996407	**MK302461**
Dendronotus velifer G. O. Sars, 1878	ZMMU:Op-546	Russia: Laptev Sea	KY996409	KY996405	**MK302462**
Dendronotus venustus MacFarland, 1966	ZMMU:Op-660	USA: Washington	**MK302460**	**MK302455**	**MK302467**
Dendronotus venustus MacFarland, 1966	LACM:174850	USA: California	HM162709	HM162630	—
Marionia arborescens Bergh, 1890	CASIZ:177578	Philippines	HM162722	HM162646	—
Marionia blainvillea (Risso, 1818)	CASIZ:176812	Portugal	HM162721	HM162645	—
Notobryon thompsoni Pola *et al.*, 2012	CASIZ:176362	South Africa	JN869456	JN869413	—
Notobryon wardi Odhner, 1936	CASIZ:177540	Philippines	JN869454	JN869411	—
Tritonia nilsodhneri Marcus, 1983	CASIZ:176219	South Africa	HM162716	HM162641	—
Tritonia plebeia Johnston, 1828	ZMMU:Op-572	Norway	KX788134	KX788122	KX788132

algorithm (Katoh *et al.*, 2002). Separate analyses were conducted for COI (658 bp), 16S (465 bp), 28S (347 bp), and concatenated data (1,470 bp). Evolutionary models for each data set were selected using MrModelTest 2.3 (Nylander *et al.*, 2004) under the Akaike information criterion (Akaike, 1974). Two different phylogenetic methods, Bayesian inference (BI) and Maximum Likelihood (ML), were used to infer evolutionary relationships. Bayesian estimation of posterior probability was performed in MrBayes 3.2 (Ronquist *et al.*, 2012). Four Markov chains were sampled at intervals of 500 generations. Analysis was started with random starting trees and 10^7 generations. Maximum likelihood-based phylogeny inference was performed in GARLI 2.0 (Zwickl, 2006) with bootstrap in 1,000 pseudo-replications. Final phylogenetic tree images were rendered in FigTree 1.4.2. The program Mega7 (Kumar *et al.*, 2016) was used to calculate the intra- and inter- group genetic distances, and uncorrected *p*-distances.

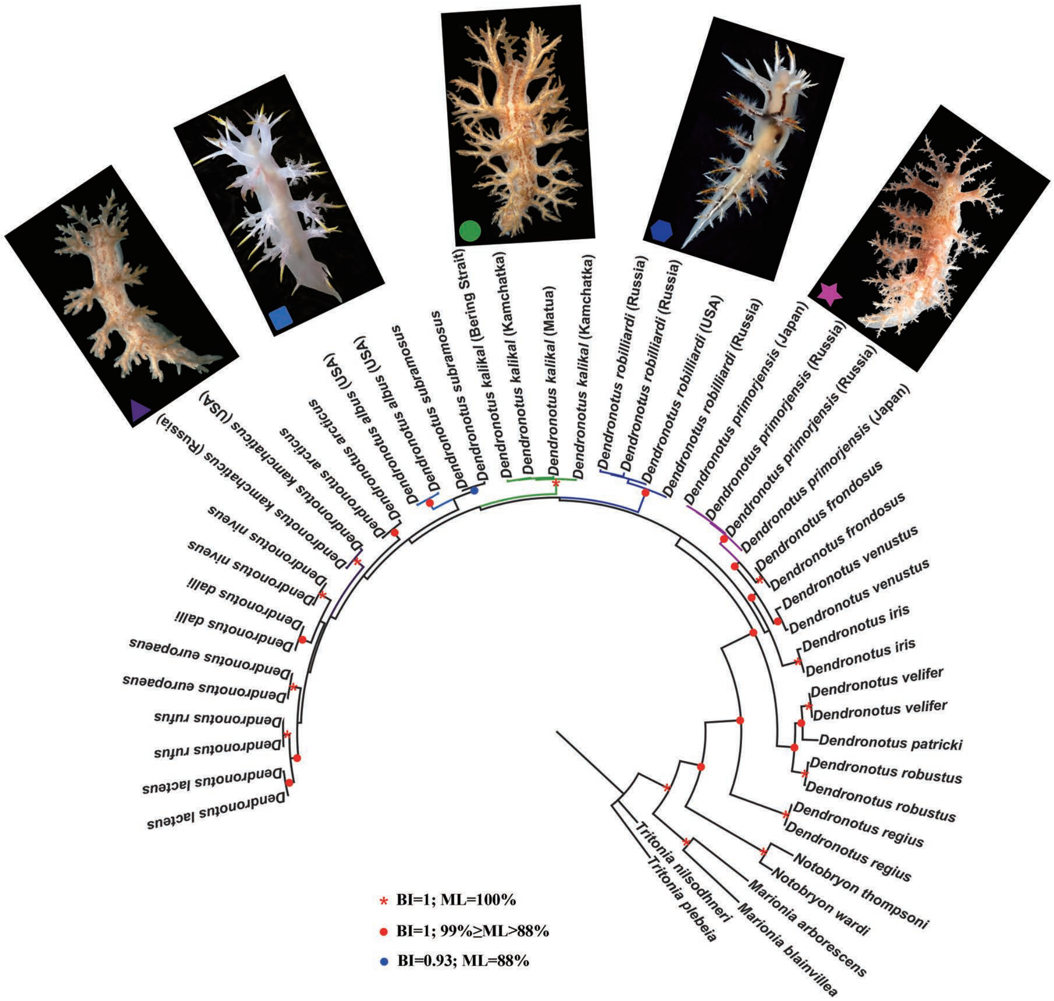


Fig. 1. Phylogenetic tree of representatives of the genus *Dendronotus* based on concatenated molecular data represented by Bayesian Inference (BI). Posterior probabilities from BI and bootstrap values for Maximum Likelihood (ML) indicated by red and blue. External views for type specimens *D. primorjensis*, *D. robilliardi*, *D. kalikal*, *D. kamchaticus*, and for specimen *D. albus* are shown. For colour designation of individual species see legend to Fig. 2.

Results

Molecular analysis

Phylogenetic analysis was performed using nineteen species of the genus *Dendronotus*, and six outgroup specimens. The SYM+G model was chosen for the combined dataset for the mitochondrial COI and 16S and the nuclear 28S genes which yielded a sequence alignment of 1,470 positions. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses based on the combined dataset yielded similar results (Fig. 1).

Two *D. primorjensis* specimens from Japan clustered in a well-supported clade (PP = 1, BS = 98%) together with type specimens of *D. primorjensis* from Russia (Figs 1, 2). Regarding the COI marker, intragroup genetic distances amongst the *D. primorjensis* clade are 0.43%. Maximal *p*-distance is 0.66% between *D. primorjensis* ZMMU: Op-639 (specimen from Usujiri, Japan) and *D. primorjensis* ZMMU: Op-640 (specimen from Usujiri, Japan) or ZMMU: Op-420 (paratype from Russia). In contrast, a minimal *p*-distance of 0.22% was found between *D. primorjensis* ZMMU: Op-640 (from Matua, Russia) and *D. primorjensis* ZMMU: Op-419 (holotype from Russia), as well as *D. primorjensis* ZMMU: Op-419 and *D. primorjensis* ZMMU: Op-420 (paratype). Molecular results indicate that *D. primorjensis* specimens belong to the same species.

Dendronotus kalikal species from Kamchatka, Bering Strait, and Matua Id. (Kuril Islands) clustered in a highly supported clade (PP = 1, BS = 100%; Figs 1, 2). Intragroup genetic distances amongst the *D. kalikal* clade are 1.02% for the COI marker.

For the first time, sequenced *D. robilliardi* from the USA clustered in a well-supported clade (PP = 1, BS = 98%) together with type specimens of *D. robilliardi* from Russia (Figs 1, 2). Intergroup genetic distances for the COI marker between the *D. robilliardi* clade and the *D. albus* clade are 13.17%, and intragroup genetic distances amongst the *D. robilliardi* clade are 1.78% and amongst *D. albus* are 1.09%. Therefore, *D. robilliardi* and *D. albus* are separate species (distinct according to both morphological and molecular data), which sympatrically occur in the USA (Washington), whereas *D. robilliardi* (but not *D. albus*) is an amphi-Pacific species occurring in the North Pacific both in the USA (Washington) and Russia, thus confirming previous conclusions (Korshunova *et al.*, 2016: 37–38).

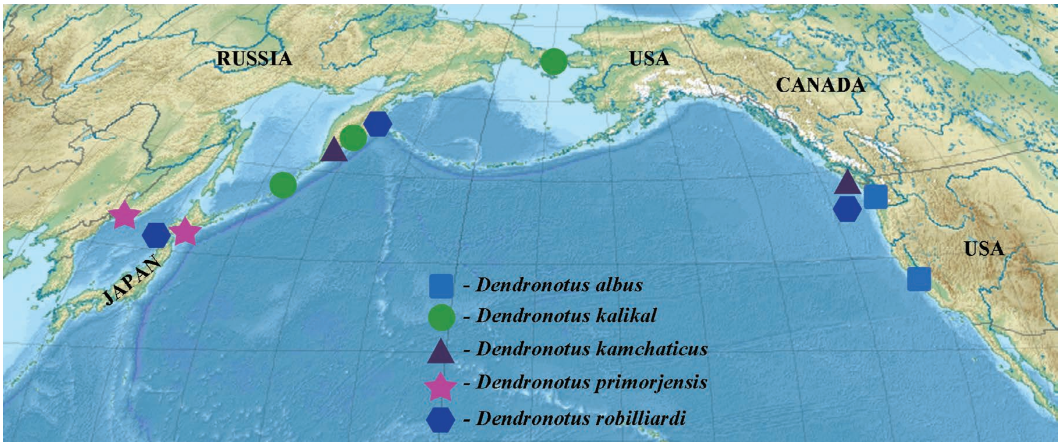


Fig. 2. Map of the North Pacific showing the distribution and new records of *Dendronotus primorjensis* from Japan (purple star), distribution and first record of *D. kalikal* from the Kuril Islands (green circle), distribution and confirmation of *D. robilliardi* from the NE Pacific and a photographic record from Japan (dark blue hexagon). For comparative purposes, the figure also shows the distributions of *D. albus* (light blue square) and *D. kamchaticus* (dark violet triangle).

Taxonomy

Family **Dendronotidae** Allman, 1845
Genus ***Dendronotus*** Alder & Hancock, 1845

Dendronotus primorjensis Martynov, Sanamyan & Korshunova, 2015

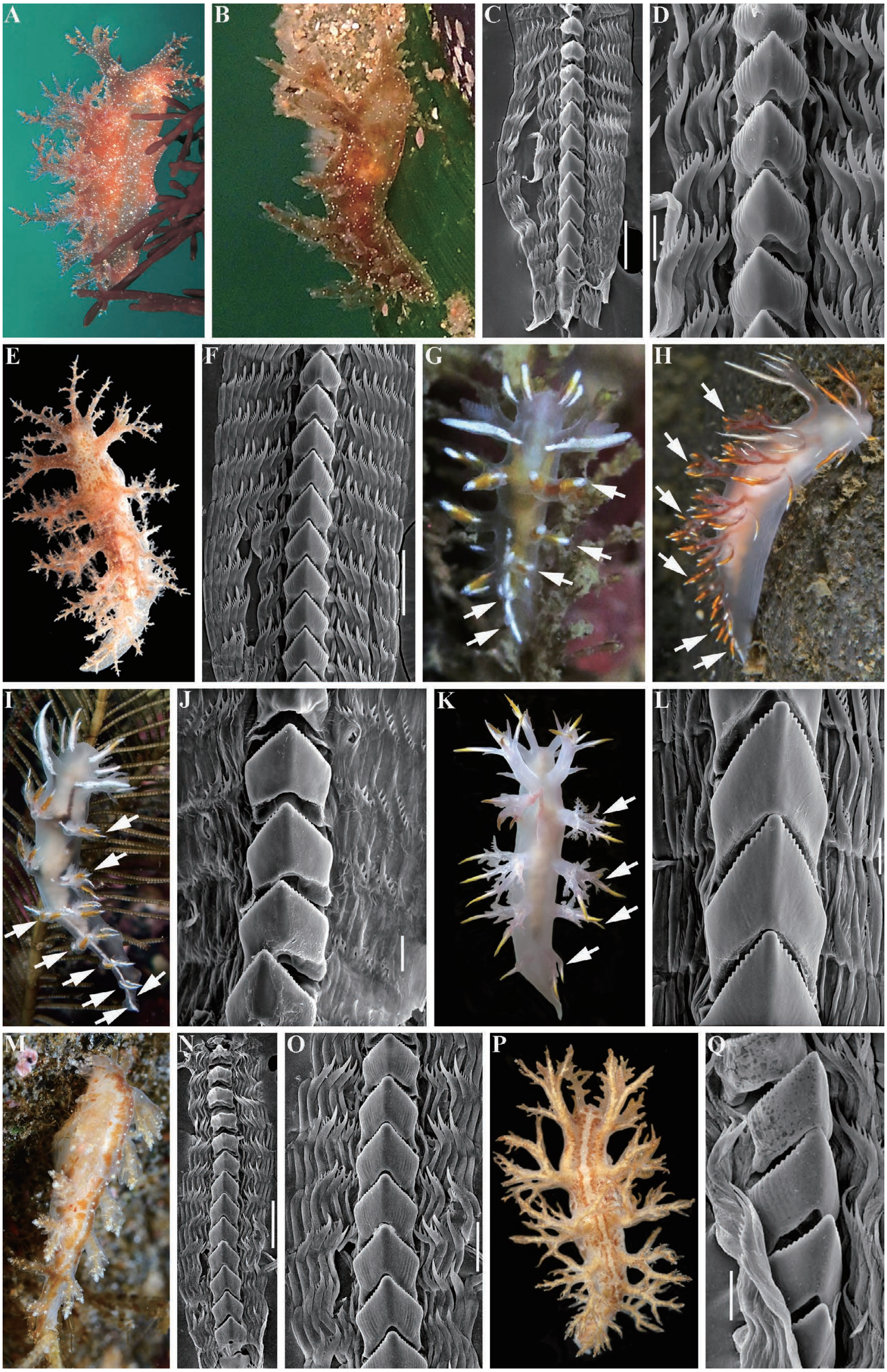
Dendronotus primorjensis Martynov *et al.*, 2015a: 60, fig. 5A–G; Martynov *et al.*, 2015b: 75–76; Korshunova *et al.*, 2016b: 15–28, figs 1, 2; Nakano, 2018: 386.

Material: 1 adult specimen, ZMMU Op-639, 13 August 2017, NW Pacific, Japan, Hokkaido, Usujiri, 10 m depth, on brown algae *Sargassum* spp., leg. and photographs Rie Nakano. 1 adult specimen, ZMMU Op-640, same locality and date. 2 juvenile specimens, ZMMU Op-641, same locality and date. 1 adult individual, not collected, photographic record by Nagaaki Sato, 2003, NW Pacific, Japan, northern Honshu, Onagawa, Miyagi Pref., 9 m depth. Holotype (adult), ZMMU Op-419, 25 September 2014, Russia, the Sea of Japan, 6.5 m depth, on stones, leg. and photographs Tatiana Korshunova, Alexander Martynov.

Description: Based on molecularly investigated specimens from Hokkaido (Usujiri), photographic records from northern Honshu (Onagawa) and the holotype from Russia. The smallest juvenile from Usujiri is 2.5 mm preserved length, the largest adult specimen from Usujiri is 11 mm preserved length (live specimen illustrated in Fig. 3B), the specimen in the photographic record from Onagawa measured 20 mm alive (Fig. 3A), and the holotype from Russia was 35 mm alive (Fig. 3E).

External morphology. There are five branched appendages of the oral veil in the smallest juvenile specimen from Usujiri, *ca.* seven in the largest adult specimen from Usujiri, no fewer than five in the photographic record from Onagawa and 7–8 in the holotype. There are five branched appendages of the rhinophoral stalks in juvenile specimens, five in the adult specimen from Usujiri, no fewer than four in the specimen from Onagawa and five in the holotype. The number of rhinophoral lamellae is not countable in juveniles; there are *ca.* 11–12 in the adult Usujiri specimen, *ca.* 10 in the specimen from Onagawa and up to 11 in the holotype. The branched rhinophoral lateral papilla is present in all specimens studied here. There are five branched pairs

Fig. 3. Morphological data on four species of the genus *Dendronotus* from the North Pacific. **A**–**F**. *Dendronotus primorjensis*; (A) photographic record of specimen from Onagawa, Miyagi Pref., 20 mm length, dorsolateral view of living specimen; (B) specimen ZMMU Op-639 from Usujiri, Hokkaido, Japan utilised in the present molecular phylogeny, 11 mm preserved length, dorsolateral view of living specimen; (C) posterior part of the radula of specimen from Usujiri, Hokkaido; (D) details of the radular teeth of the specimen from Usujiri, Hokkaido; (E) holotype ZMMU Op-419 from Spokoinaya Bay, the Sea of Japan, Russia, 35 mm length, dorsal view of living specimen; (F) posterior part of the radula of the holotype. **G**. *Dendronotus robilliardi*, specimen ZMMU Op-659 from Port Orchard, Washington state, USA, 5 mm length, dorsal view, every dorsolateral appendage is indicated by an arrow. **H**–**J**. *Dendronotus robilliardi*; (H) specimen from Cape Tappi, Aomori Pref., Japan, 40 mm, dorsolateral view, every dorsolateral appendage is indicated by an arrow; (I) holotype ZMMU Op-568, Starichkov Id., Kamchatka, Russia, 35 mm, dorsal view, every dorsolateral appendage is indicated by an arrow; (J) posterior part of the radula of holotype. **K**–**L**. *Dendronotus albus*; (K) specimen ZMMU Op-566 from Port Orchard, Washington state, USA, 70 mm, dorsal view, every dorsolateral appendage is indicated by an arrow (photograph by Karin Fletcher); (L) posterior part of radula of specimen from Port Orchard. **M**–**Q**. *Dendronotus kalikal*; (M) specimen ZMMU Op-657 from Matua Id., middle Kuril Islands, Russia, 6.5. mm preserved length, dorsolateral view; (N) posterior part of radula of specimen from Kuril Islands; (O) close up of posterior part of radula of specimen from Kuril Islands; (P) holotype ZMMU Op-283, Starichkov Id., Kamchatka, Russia, 10 mm, dorsal view; (Q) posterior part of radula of holotype. Scale bars: D, J, L, Q, 30 µm; O, 50 µm; C, F, N, 100 µm.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q

(plus two smaller pairs) of dorsolateral appendages in juvenile specimens, six (plus up to three smaller pairs) in specimens from Usujiri, up to six in the specimen in the photographic record from Onagawa and six (plus up to 2–3 smaller pairs) in the holotype. The dorsolateral appendages have long primary stalks, secondary branches, and elongate tertiary branches and the digestive gland penetrates most of the dorsolateral appendages as well as the rhinophoral sheaths. No lip papillae are evident in the juvenile; there are *ca.* eight in the adult specimens from Usujiri and 7–12 in the holotype. Reproductive and anal openings are placed laterally on the right side.

Colour. The ground colour is non-uniformly reddish brown. The dorsum, dorsolateral appendages and upper sides of foot bear small scattered whitish and yellowish dots and are essentially similar in all studied specimens from Japan (including photographic record) and the holotype from Russia (Figs 3A, B, E).

Anatomy. Digestive system. The jaws are ovoid with strong dorsal processes; denticles are present. The radular formula in the 11 mm (preserved length) specimen from Usujiri is 41 × 7–9.1.7–9 (Fig. 3C). The radular teeth are yellowish. The central tooth is wide, with a broad triangular cusp and up to 14 lateral denticles (Fig. 3D). Lateral teeth bear 2–6 denticles (Fig. 3D). The radular formula in the 35 mm (live) holotype is 37 × 8–9.1.8–9 (Fig. 3F). The central tooth of the holotype is wide, with a broad triangular cusp and up to 14 lateral denticles (Fig. 3F). Lateral teeth of the holotype bear 3–6 denticles (Fig. 3F).

Reproductive system (based on the holotype). The ampulla is twice folded. The prostate is rounded and consists of no fewer than 19 alveolar glands. The distal part of the vas deferens is relatively short, entangled, and expands to an oval penial sheath and a relatively long and curved conical penis. The vagina is moderate in length. The bursa copulatrix is large and rounded. An elongated seminal receptaculum is placed distally.

Habitat: In this study this species was found on a rocky and stony substrate with algae at a depth of 10 m on unidentified brown algae at Usujiri, Hokkaido, Japan.

Distribution (Fig. 2): Northern part of the Sea of Japan, Peter the Great Bay, Russia (Martynov *et al.*, 2015; Korshunova *et al.*, 2016b) and Pacific coast of Hokkaido, Japan (present study). Photographic records include an individual from the Pacific side of northern Honshu at Onagawa in Miyagi Prefecture (Fig. 3A).

Remarks: By molecular p-distances the studied specimens from Hokkaido coincide 0.22% and 0.66% with the COI marker of the holotype of *D. primorjensis* from the Russian part of the Sea of Japan. Small white and yellow spots are present on the dorsal surface and dorsolateral appendages, without large amounts of white pigment. The general reddish-brown colouration is very similar between the holotype of *D. primorjensis* (Fig. 3E) and specimens from Hokkaido (Fig. 3B) which were utilised in the current molecular and morphological study. The individual from Onagawa (Pacific side of northern Honshu), which was not collected but only photographed (Fig. 3A), shares similar colouration both with the holotype and specimens from Hokkaido. The central teeth of the radula in the specimen of *D. primorjensis* from Hokkaido possess up to 14 well-defined lateral denticles (Fig. 3D), which also fully agrees with those of the holotype from the Russian part of the Sea of Japan (Fig. 3F). According to the molecular and morphological study, biogeographic patterns of *D. primorjensis* are fundamentally similar to those of the aeolidacean nudibranch *Trinchesia lenkae*, which was most recently shown to inhabit both the Russian part of the Sea of Japan and the Pacific coast of Hokkaido (Korshunova *et al.*, 2018a). See also Discussion.

Dendronotus kalikal
Ekimova, Korshunova, Schepetov, Neretina, Sanamyan & Martynov, 2015

Dendronotus kalikal Ekimova *et al.*, 2015: 872–874, figs 6F, 8E, 16C, 18B, 19; Korshunova *et al.*, 2016b: 18, 22, 23, 24.

Material: 1 adult specimen, ZMMU Op-657, 20 August 2017, NW Pacific, Russia, Kuril Islands, Matua Island, 14 m depth, stones, hydroids, leg. and photographs Nadezhda Sanamyan. 3 juveniles, ZMMU Op-658, same locality and date. Holotype (adult), ZMMU Op-283, NW Pacific, Russia, Kamchatka, Starichkov Island, 23 August 2009, depth 14–16 m, stones, leg. and photographs Nadezhda Sanamyan.

Description: Based on juvenile and molecularly investigated adult specimen from Matua Id. and holotype from Kamchatka.

External morphology (Fig. 3M). The smallest juvenile from Matua Id. is 2.2 mm long; the adult specimen from Matua is 6.5 mm long (Fig. 3M) and the holotype from Kamchatka is 9 mm long (Fig. 3P). There are four branched appendages of the oral veil in the smallest juvenile specimen, five in the adult specimen from Matua Id. and five in the holotype. There are five branched appendages of the rhinophoral stalks in juveniles, five in the adult specimen from Matua Id. and five in the holotype. There are *ca.* five rhinophoral lamellae in the juvenile specimens, up to eight in the adult Matua specimen and up to ten in the holotype. The branched rhinophoral lateral papilla is present in all specimens studied here. There are four branched pairs (plus two smaller pairs) of dorsolateral appendages in the juvenile specimen, five (plus two smaller pairs) in the specimen from Matua and five (plus up to three smaller pairs) in the holotype. The dorsolateral appendages have a long primary stalk, secondary branches and elongate tertiary branches, and the digestive gland penetrates most dorsolateral appendages as well as the rhinophoral sheaths. There are four lip papillae in the juvenile specimen and *ca.* five in the holotype. The reproductive and anal openings are placed laterally on the right side.

Colour. The ground colour is creamy to whitish. The dorsum, dorsolateral appendages, and upper sides of foot bear brownish orange lines and dots. Both the holotype (Fig. 3P) and specimens from Matua Island (Fig. 3M) possess the subparallel arrangement of dorsal brownish lines that is characteristic of *D. kalikal*.

Anatomy based on the adult specimen from Matua and the holotype. Digestive system. The jaws are ovoid with strong dorsal processes; denticles are present. The radular formula in the 6.5 mm (preserved) specimen from Matua is 31 × 5–8.1.8–5 (Fig. 3N). The radular teeth are yellowish. The central tooth is wide, with a broad triangular cusp and up to 15 lateral denticles (Fig. 3O). The lateral teeth bear 4–8 denticles (Fig. 3O). The radular formula in the 9 mm (preserved) holotype from Kamchatka is 21 × 6–8.1.8–6 (Fig. 3Q). The central tooth of the holotype is wide, with a broad triangular cusp and up to 18 lateral denticles (Fig. 3O). The lateral teeth bear up to eight denticles (Fig. 3O).

Reproductive system. The ampulla is wide and sinuous. The prostate is concentric ring-shaped, consisting of *ca.* 20 oval alveolar glands in the holotype. The distal part of the vas deferens is winding and expands into wide, muscular portion. The penis is slightly curved. The oviduct connects through insemination duct into female gland complex. The vagina is long, convoluted. There are rounded seminal receptaculum and small bursa copulatrix.

Habitat: In this study it was found on a rocky and stony substrate with algae at a depth of 14 m.

Distribution: Northern Pacific, Kamchatka, and Kuril Islands.

Remarks: By molecular p-distances the studied specimen from the Kuril Islands coincides 1.09% with the COI marker of the holotype of *D. kalikal* from Kamchatka. Parallel thin brownish lines are present on the dorsal surface, and the general light yellowish colouration is very similar between the holotype of *D. kalikal* (Fig. 3P) and specimens from the Kuril Islands (Fig. 3M) which were utilised in the present molecular and morphological study. The central teeth of the radula in the specimen of *D. kalikal* from Hokkaido possess up to 12 partly reduced lateral denticles (Fig. 3O), which also fully agrees with the holotype of this species from Kamchatka (Fig. 3Q). See also Discussion.

Dendronotus robilliardi
Korshunova, Zimina, Sanamyan, Fletcher & Martynov, 2016

Dendronotus robilliardi Korshunova *et al.*, 2016a: 28–32, figs 2, 3; Nakano, 2018: 386.

Material: 1 specimen (juvenile), ZMMU Op-659, 2 January 2017, NE Pacific, USA, Washington state, Port Orchard, West of Point Glover, Rich Passage, 9.8 m depth, stones, leg. and photographs Karin Fletcher. Holotype (adult), ZMMU Op-568, NW Pacific, Russia, Kamchatka, Starichkov Island, 17 September 2015, depth 11.5 m, stones, leg. and photographs Nadezhda Sanamyan. 1 individual, adult, not collected, photographic record by Hitoshi Yoshikawa, 29 April 2014, NW Pacific, Japan, northern Honshu, Cape Tappi, Aomori Pref., Japan, 10 m, stones.

Description: Based on a juvenile specimen from Port Orchard (USA), the holotype from Kamchatka (Russia) and an adult specimen from Cape Tappi (Japan, photographic record).

External morphology. The lengths of the examined specimens are 5 mm (USA, Fig. 3G), 40 mm (Japan, Fig. 3H), and 35 mm (Russia, holotype, Fig. 3I). There are four simple appendages of the oral veil in the juvenile specimen from the USA, *ca.* five branched appendages in the specimen from Japan and 4–5 branched appendages in the holotype from Russia. There are four non-branched appendages of the rhinophoral stalks in the juvenile specimen, *ca.* four in the specimen from Japan and 4–6 in the holotype. There are six rhinophoral lamellae in the juvenile specimen, *ca.* 11 in the specimen from Japan and 11–12 in the holotype. Unbranched (or with a few small branches) rhinophoral lateral papilla are present in all specimens studied here. There are three branched pairs (plus two smaller pairs) of dorsolateral appendages in the juvenile specimen, six (plus at least one smaller pair) in the specimen from Japan and six (plus three smaller pairs) in the holotype. The dorsolateral appendages have a moderate primary stalk with secondary branches and pointed tertiary branches; the digestive gland penetrates no fewer than three dorsolateral appendages in the juvenile specimen and no fewer than six in the adult specimen from Japan (photographic record, Fig. 3H), as well as the rhinophoral sheaths. *Ca.* 5–10 lip papillae are present in the holotype. The reproductive and anal openings are placed laterally on the right side.

Colour. The ground colour is uniformly translucent white. Opaque white stripes are present on the oral veil appendages and rhinophoral sheaths, on the posterior part of the dorsum and on the tips of the dorsolateral appendages in the juvenile specimen (Fig. 3G), in the photographic record from Japan (Fig. 3H), and in the holotype from Kamchatka (Fig. 3I). The orange-copper marks may be present in the middle part of the dorsal and oral processes in some specimens of *D. robilliardi* (Korshunova *et al.*, 2016a), but orange lines may also be absent; such variation may be expected in other Japanese specimens as well, although it was not recorded in the present study.

Anatomy (based on the holotype only). Digestive system. The jaws are ovoid with strong dorsal processes. The masticatory borders bear ridge-like denticles. The radula formula is 43 × 3–9.1.9–3. The central tooth bears up to 15 small distinct denticles (Fig. 3J), without furrows. The lateral teeth are slightly curved and bear up to seven distinct long denticles (Fig. 3J). Reproductive system. The ampulla is wide and folded twice. The prostate is concentric ring-shaped, moderate in size, consisting of 19–20 oval alveolar glands. The distal part of the vas deferens is winding and expands into a wide, muscular portion. The penis is slightly curved. The oviduct connects through the insemination duct into the female gland complex. The vagina is narrow, bent, moderate in length and distally expanded. The bursa copulatrix is large. An irregularly small oval seminal receptaculum is placed distally on the vestibulum.

Habitat: In this study this species is reported from a rocky and stony substrate at a depth of 9.8 m from Port Orchard, Washington State, USA. Photographic records include an individual from the Pacific side of northern Honshu at Cape Tappi in Aomori Prefecture (Fig. 3H).

Distribution: Northern Pacific, both NE and NW (Korshunova *et al.*, 2016a; present study).

Remarks: The juvenile specimen from Port Orchard (NE Pacific, USA) coincides 1.09% with the COI marker of the holotype of *D. robilliardi* from Kamchatka (NW Pacific, Russia) by molecular p-distances. Patterns of colouration and the presence of at least five dorsolateral appendages more clearly link the juvenile specimen of *D. robilliardi* from the USA (Fig. 3G) with *D. robilliardi* from Japan (Fig. 3H) than with the holotype from Kamchatka (Fig. 3I). The juvenile specimen from Port Orchard was utilised in the molecular study and confirmed that *D. robilliardi* is present both in the NW and the NE Pacific (Figs 1, 2). According to this study, *D. robilliardi* can be confidently identified from northern Honshu based on external morphological data only (Fig. 3H). See also Discussion for details of the complex taxonomic history of *D. robilliardi*.

Discussion

Morphological and molecular analysis (Fig. 1) of the newly discovered specimens of *D. primorjensis* from the Pacific side of Hokkaido (Fig. 3B, C, D) reveals a high degree of similarity to the specimens from the type locality in the Sea of Japan in Russia (Fig. 3E, F). Diagnostic external and internal features, namely the brownish-reddish colouration with white and yellow spots, 5–6 main pairs of dorsolateral appendages and central radular teeth with up to 14 lateral denticles, are in good agreement between the Japanese and Russian specimens and with the original description (Martynov *et al.*, 2015a, b) and redescription (Korshunova *et al.*, 2016b) of this species.

Korshunova *et al.* (2016a) showed that for many years there was confusion surrounding the species *D. albus*, and separated the new species *D. robilliardi* (Figs 3G–J) from it (Figs 3K, L) using morphological and molecular evidence (Fig. 1). However, despite the existence of *D. robilliardi* having been suspected since Robilliard's *Dendronotus* key (1969) and in his seminal work on *Dendronotus* (1970), this species has been consistently misidentified as *D. albus* so true *D. robilliardi* has not been reported from the NE North American Pacific (Korshunova *et al.*, 2016a). Here we present molecular data (Fig. 1, Table 1) from a specimen of *D. robilliardi* from Port Orchard (Washington state, USA, Fig. 3G) and for the first time confirm its presence on the NE Pacific coast (Fig. 2). This material and novel molecular phylogeny also allow us to identify an individual of *D. robilliardi* which was photographically recorded recently from Cape Tappi in Aomori Prefecture (Fig. 3H) in Japan (Nakano, 2018). *Dendronotus robilliardi* (Figs 3G–J) differs from the superficially similar *D. albus* (Figs 3K, L) not only by considerable molecular distance (these species belong to different clades, see Fig. 1) but also by morphological features, particularly by the digestive gland penetrating into at least three dorsolateral appendages even in a semi-juvenile specimen of *D. robilliardi* 5 mm length from Port Orchard, Washington, USA (Fig. 3G) (the tiny specimen already had five pairs of dorsolateral appendages, see Fig. 3G), whereas in a more than ten-times larger 70 mm specimen of *D. albus* from Port Orchard the digestive gland only penetrated into two dorsolateral appendages (and it had only four dorsolateral appendages in total: Fig. 3K). In turn, the adult holotype of *D. robilliardi* has six pairs of large main dorsolateral appendages plus 2–3 small simple ones (Fig. 3I, arrows). The individual that was photographed at Cape Tappi has five appendages containing branches of the digestive gland and six main pairs of large dorsolateral appendages in total (Fig. 3H, arrows), which fully agrees with the holotype of *D. robilliardi* from Kamchatka (Fig. 3I, arrows). Therefore, using this morphological and molecular framework we can identify the individual from Aomori Prefecture as true *D. robilliardi* (Fig. 2). Further collections of this species in Japan are needed to integrate Japanese specimens of *D. robilliardi* into the molecular phylogeny.

Another species recently separated from the *Dendronotus frondosus* complex is *D. kalikal* (Korshunova *et al.*, 2016a, b). Morphologically, it has two distinct, continuous, fine brownish lines compared to the common colour pattern with no subparallel lines seen in *D. frondosus*, *D. primorjensis*, *D. venustus*, and *D. kamchaticus*. The molecular phylogenetic data places *D.*

kalikal in a different clade to *D. frondosus*, *D. primorjensis*, and *D. venustus* (Korshunova *et al.*, 2016b; present study, Fig. 1). In this study we record *D. kalikal* for the first time from the Kuril Islands (Matua Id.). According to external and internal morphological data (Fig. 3M–O) and the molecular phylogeny (Fig. 1), specimens from the Kuril Islands belong to *D. kalikal*. Particularly, the presence of thin parallel brownish lines on the dorsal surface and the general light yellowish colouration are essentially similar between the holotype of *D. kalikal* (Fig. 3P) and specimens from the Kuril Islands (Fig. 3M), both of which were utilised in the molecular and morphological studies. Furthermore, radular patterns between the holotype of *D. kalikal* (Fig. 3Q) and specimens from the Kuril Islands (Fig. 3N, O) are also fundamentally the same. This finding is very important for the further understanding of taxonomic and biogeographic patterns within the complex of species that were formerly included in *D. frondosus*, which are externally similar but internally and molecularly only distantly related.

One of the important implications of the integrative molecular and morphological taxonomic framework for the genus *Dendronotus* presented here (Figs 1, 2) is the possibility of further predicting biogeographical patterns its species in the North Pacific and particularly within the Japanese fauna. Recently it was shown that another species, *D. kamchaticus*, which was formerly identified as simply a colour variation of *D. frondosus* (Robilliard, 1970), has a very broad amphi-Pacific range (Fig. 2) and occurs both on the NW Pacific Kamchatka coast and along the NE Pacific coast to Port Orchard, Washington State, USA (Korshunova *et al.*, 2016a; present study, Fig. 2). Therefore, judging from this broad distribution in the North Pacific it is highly likely that *D. kamchaticus* will be found in Hokkaido and northern Honshu in the future. Furthermore, in the present study we extend the range of *D. kalikal* from the type locality in Kamchatka to the middle Kuril Islands (Fig. 2). This distribution pattern also implies a high probability of finding *D. kalikal* at least on the Hokkaido coast in the future. Finally, one more species with a similar amphi-Pacific distribution, *D. dalli* Bergh, 1879 (Martynov & Korshunova, 2011; present study, Fig. 1), is also expected to be found in northern Japan.

Acknowledgements

The staff of the Electron Microscopy Laboratory, Moscow State University are gratefully acknowledged for support with electron microscopy. This work was supported by a research grant of MSU Zoological Museum (AAAA-A16-116021660077-3), in frame of Moscow University Project "Noah's Ark" (morphological & molecular study) and RFBR grant No. 17-04-01827a (molecular study).

References

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

Baba, K. 1932. *Pseudobornella orientalis*, nov. gen. et sp. from Japan. *Annotationes Zoologicae Japonenses* 13: 369–376.

Baba, K. 1949. *Opisthobranchia of Sagami Bay Collected by His Majesty the Emperor of Japan*. 194 pp. Iwanami Shoten, Tokyo. (in Japanese and English)

Baba, K. 1957. A revised list of the species of Opisthobranchia from the northern part of Japan, with some additional descriptions. *Journal of the Faculty Science*, *Hokkaido University*, *series 6*, *Zoology* 13: 8–14.

Baba, K. 1993. A northern species of *Dendronotus* (Mollusca: Nudibranchia: Dendronotidae) from Sado Island, Sea of Japan. *Report of the Sado Marine Biological Station* (23): 29–33.

Ekimova, I., Korshunova, T., Schepetov, D., Neretina, T., Sanamyan, N. & Martynov, A. 2015. Integrative systematics of northern and Arctic nudibranchs of the genus *Dendronotus* (Mollusca, Gastropoda), with descriptions of three new species. *Zoological Journal of the Linnean Society* 173: 841–886.

Higo, S. & Goto, Y. 1993. *A Systematic List of Molluscan Shells from the Japanese Is. and the adjacent Area*. 6+ 22(contents)+ 693+ 13(references)+ 148 (index) pp. Elle Corp., Yao, Osaka. (In Japanese).

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Korshunova, T., Sanamyan, N., Zimina, O., Fletcher, K. & Martynov, A. 2016a. Two new species and a remarkable record of the genus *Dendronotus* from the North Pacific and Arctic oceans (Nudibranchia). *ZooKeys* 630: 19–42.

Korshunova, T. A., Sanamyan, N. P. & Martynov, A. V. 2016b. Morphological and molecular evidence indicate *Dendronotus primorjensis* is a valid species that has priority over *D. dudkai* (Nudibranchia). *ZooKeys* 634: 15–28.

Korshunova, T. A., Martynov, A. V., Bakken, T., Evertsen, J., Fletcher, K., Mudianta, I. W., Saito, H., Lundin, K., Schrödl, M. & Picton, B. 2017. Polyphyly of the traditional family Flabellinidae affects a major group of Nudibranchia: aeolidacean taxonomic reassessment with descriptions of several new families, genera, and species (Mollusca, Gastropoda). *ZooKeys* 717: 1–139.

Korshunova, T. A., Fujita, T. & Martynov, A. V. 2018a. The first record of *Trinchesia lenkae* Martynov, 2002 from Japan: morphological and molecular comparison with the material from the type locality in Russia (Nudibranchia: Trinchesiidae). *Venus* 76: 19–28.

Korshunova, T. A., Lundin, K., Malmberg, K., Picton, B. & Martynov, A. V. 2018b. First true brackish-water nudibranch mollusc provides new insights for phylogeny and biogeography and reveals paedomorphosis-driven evolution. *PLoS ONE* 13: e0192177.

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.

Martynov, A. V. & Korshunova, T. A. 2011. *Opisthobranch Molluscs of the Seas of Russia. A Colour Guide to Their Taxonomy and Biology*. 228 pp. Fiton Press, Moscow.

Martynov, A. V., Sanamyan, N. P. & Korshunova, T. A. 2015a. New data on the opisthobranch molluscs (Gastropoda: Opisthobranchia) of waters of Commander Islands and Far-Eastern seas of Russia. *In*: *Conservation of Biodiversity of Kamchatka and Coastal Waters – Proceedings of XV International Scientific Conference Petropavlovsk-Kamchatsky*, pp. 55–69. Kamchat Press, Petropavlovsk-Kamchatsky.

Martynov, A. V., Sanamyan, N. P. & Korshunova, T. A. 2015b. Review of the opisthobranch mollusc fauna of Russian Far Eastern seas: Pleurobranchomorpha, Doridida and Nudibranchia. *Bulletin of Kamchatka State Technical University* 34: 62–87.

Nakano, R., 2018. *Field Guide to Sea Slugs and Nudibranchs of Japan*. 544 pp. Bun-ichi Co., Ltd., Tokyo.

Nylander, J. A., Ronquist, F., Huelsenbeck, J. P. & Nieves-Aldrey, J. L. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53: 47–67.

Robilliard, G. A. 1969. Key to the species of *Dendronotus* of the Puget Sound Region in *Marine Fauna of the San Juan Archipelago. Publication of the Puget Sound Biological Station*. 241 pp. Puget Sound Biological Station, University of Washington, Seattle.

Robilliard, G. A. 1970. The systematics and some aspects of the ecology of the genus *Dendronotus* (Gastropoda: Nudibranchia). *The Veliger* 12: 433–479.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Dissertation, The University of Texas, Austin.

(Received September 18, 2018 / Accepted December 25, 2018)

太平洋北西部産スギノハウミウシ属（裸鰓目・スギノハウミウシ科）の **1** 種 ***Dendronotus primorjensis*** **Martynov, Sanamyan & Korshunova, 2015** の分子系統解析による分布域確認と，***D. robilliardi*** および ***D. kalikal*** の分布記録的報告

Tatiana Korshunova・中野理枝・Karin Fletcher・Nadezhda Sanamyan・Alexander Martynov

要　約

Dendronotus スギノハウミウシ属は裸鰓目スギノハウミウシ科に属するウミウシの仲間で，背面の両側に樹枝状突起が生じるのが特徴である。*Dendronotus primorjensis* Martynov, Sanamyan & Korshunova, 2015 のタイプ産地は日本海のロシア側の沿岸で，中野（2004）が宮城県女川から日本初記録を報告した。しかし写真のみの報告であったため，今回北海道臼尻で得た標本を用いて分子系統解析を行った。その結果，臼尻産の個体は *D. primorjensis* であり，本種は日本東北部以北の沿岸にも分布することが確認された。本種には日本初記録地に因み，和名としてオナガワスギノハウミウシが提唱されている（中野，2018）。次に *Dendronotus robilliardi* Korshunova, Sanamyan, Zimina, Fletcher & Martynov, 2016 が青森県竜飛岬から報告された。本種のタイプ産地は太平洋北西部のカムチャツカであるが，竜飛岬産の個体は特徴的な外部形態から *D. robilliardi* であると考えられる。*D. robilliardi* は太平洋北東部，北アメリカ沿岸からも報告されている。本種には種小名に献名された研究者に因み，和名としてロビラードウミウシが提唱されている（中野，2018）。最後に *Dendronotus kalikal* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan & Martynov, 2015 が千島列島から報告された。本種のタイプ産地もカムチャツカであるが，外部形態と分子系統解析，および歯舌形態から千島列島産の個体は *D. kalikal* であることが確認された。*D. kalikal* のタイプ産地以外からの報告は今回が初めてである。本種には形態的特徴および種小名の意から，ソメワケスギノハウミウシの和名を新唱する。