

# Nudibranch molluscs of the genus *Dendronotus* Alder et Hancock, 1845 (Heterobranchia: Dendronotina) from Northwestern Sea of Japan with description of a new species

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**ABSTRACT:** Species of the genus *Dendronotus* are among the most common nudibranchs in the northern Hemisphere. However, their distribution and composition in the North-west Pacific remain poorly explored. In the present study, we observed *Dendronotus* composition in northwestern part of the Sea of Japan, using an integrative approach, included morphological and molecular phylogenetic analyses and molecular species delimitation methods. These multiple methods revealed high cryptic diversity within the genus. Two specimens of *Dendronotus frondosus* were found in Amursky Bay and therefore its amphiboreal status was confirmed. In three locations of the Sea of Japan we found specimens, which are very close externally to *D. frondosus*, but show significant distance according to molecular analysis. We show that these specimens belong to a new species *Dendronotus dudkai* sp.n. This species is sister to *D. frondosus* according to morphological and molecular data, therefore the question of sympatric coexistence is discussed. For the first time *Dendronotus kamchaticus* was registered in the Sea of Japan and updated information of some intraspecific variation of this species is provided.

How to cite this article: Ekimova I.A., Schepetov D.M., Chichvarkhina O.V., Chichvarkhin A.Yu. 2016. Nudibranch molluscs of the genus *Dendronotus* Alder et Hancock, 1845 (Heterobranchia: Dendronotina) from Northwestern Sea of Japan with description of a new species // Invert. Zool. Vol.13. No.1. P.15–42. doi: 10.15298/invertzool.13.1.02

**KEY WORDS:** Nudibranchia, northwestern Pacific, species delimitation, molecular phylogeny, integrative taxonomy, Mollusca.

## Голожаберные моллюски рода *Dendronotus* Alder et Hancock, 1845 (Heterobranchia: Dendronotina) Северо-Восточной части Японского моря, с описанием нового вида

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**РЕЗЮМЕ:** Виды рода *Dendronotus* являются одними из наиболее обычных голожаберных моллюсков в северном полушарии. Тем не менее, их распространение и таксономический состав в северо-западной части Тихого океана остается слабо изученным. Нами был исследован таксономический состав рода *Dendronotus* в северо-западной части Японского моря с использованием комплексного подхода, включающего морфологический и молекулярно-филогенетический анализы, а также методы молекулярного разделения видов. Было выявлено высокое криптическое разнообразие видов рода *Dendronotus*. Две особи *Dendronotus frondosus* были обнаружены в Амурском Заливе и, таким образом, было подтверждено его амфибореальное распространение. В трех локациях Японского моря нами также были обнаружены особи, сходные с *D. frondosus* по признакам внешней морфологии, однако резко отличные по молекулярным данным от всех исследованных видов рода *Dendronotus*. Показано, что данные особи относятся к новому для науки виду *Dendronotus dudkai* sp.n. и дано его подробное описание. Ввиду того, что *D. frondosus* и *D. dudkai* sp.n. являются близкими сестринскими видами, обсуждается вопрос о их симпатрическом происхождении и сосуществовании. Впервые вид *D. kamchaticus* был зарегистрирован в Японском море. Приведена обновленная информация о некоторых внутривидовых вариациях данного вида. Как цитировать эту статью: Ekimova I.A., Schepetov D.M., Chichvarkhina O.V., Chichvarkhin A.Yu. 2016. Nudibranch molluscs of the genus *Dendronotus* Alder et Hancock, 1845 (Heterobranchia: Dendronotina) from Northwestern Sea of Japan with description of a new species // *Invert. Zool.* Vol.13. No.1. P.15–42. doi: 10.15298/invertzool.13.1.02

**КЛЮЧЕВЫЕ СЛОВА:** Nudibranchia, северо-западная часть Тихого океана, анализ видовых границ, молекулярная филогения, интегративная таксономия, Mollusca.

## Introduction

Nudibranch genus *Dendronotus* Alder et Hancock, 1845 is distributed mostly in northern Hemisphere and includes approximately 20 extant valid species that are among the most common shallow-water bottom marine invertebrates (Wägele *et al.*, 2013; Ekimova *et al.*, 2015; Gofas, Bouchet, 2015). Until recently, type species of the genus *Dendronotus frondosus* (Ascanius, 1774) was commonly regarded as amphiboreal species, with a very broad range extending from European seas and the Arctic Ocean to California and Chile (Robilliard, 1970, 1975; Behrens, 1980; McDonald, 1983, 2009; Roginskaya, 1987; Behrens, 1991; Schrödl, 2003). In the mid-20th century Frank Mace MacFarland (MacFarland, 1966) described several more North Pacific species of this genus including *Dendronotus albus* MacFarland, 1966, *D. subramosus* MacFarland, 1966 and *D. venustus* MacFarland, 1966. The diversity and distinctness of the North American *Dendronotus* species were studied in a detailed morphological review of this genus (Robilliard, 1970). *D. venustus* was commonly regarded as a synonym of the Atlantic *D. frondosus*, even in the North American literature (McDonald, 2009).

The use of molecular markers caused considerable changes in the taxonomy of northern and Arctic species. The first molecular study of the genus (Stout *et al.*, 2010) showed minor but reliable differences between Pacific and Atlantic populations of *D. frondosus*. The MacFarland's name *D. venustus* was therefore restored. The validity of North Atlantic species *D. lacteus* (Thompson, 1840) was confirmed based on allozyme electrophoresis and morphological data (Thollesson, 1998). Few other North American species e.g. *D. nanus* Marcus et Marcus, 1967 and *D. diversicolor* Robilliard, 1970 were instead shown to be synonyms of *D. iris* Cooper, 1963 and *D. albus*, respectively (Stout *et al.*, 2010). Another North Pacific species *D. patriciki* Stout, Wilson et Valdes, 2011 has been described from the deep sea off California (Stout *et al.*, 2011). *Pseudobornella orientalis* (Baba, 1932) from Japan was transferred from the

genus *Pseudobornella* Baba, 1932 to *Dendronotus* based on concatenated molecular analyses of three genes (Pola, Gosliner, 2010). A recent study conducted by Ekimova *et al.* (2015) revealed three new species of the genus from Arctic and northwestern Pacific: *D. kalikal* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan et Martynov, 2015, *D. kamchaticus* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan et Martynov, 2015, and *D. niveus* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan et Martynov, 2015. In addition, it has been proposed that the highly variable species *D. frondosus* is restricted only to the North Atlantic boreal and subarctic waters, while in the North-West Pacific it could be represented by a complex of several cryptic species.

For a long time *D. frondosus* has been reported from northwestern waters of the Sea of Japan (Roginskaya, 1987; Martynov, 1999, 2006; Martynov, Korshunova, 2011; Chernyshev, 2014) and from the Sado isl near Honshu (Baba, 1993). *D. frondosus* was also discovered at Starichkov isl (the North-West Pacific, Kamchatka peninsula) (Martynov *et al.*, 2010); however this data was reconsidered after the description of *D. kamchaticus* and *D. kalikal* (Ekimova *et al.*, 2015). *Dendronotus* sp. was also reported from all northwestern Pacific seas (Martynov, 1997, 2013). The latest work by Martynov *et al.* (2015) provided a new information on *Dendronotus* species composition in northwestern part of the Sea of Japan. A new species *D. primorjensis* Martynov, Sanamyan et Korshunova, 2015 was described. However, its taxonomic description is too poor and does not contribute any necessary information for species delimitation. Authors also referred to molecular analysis of cytochrome *c* oxidase subunit I (COI) gene, that could prove the *D. primorjensis* validity, but no sequences have been published via GenBank or BOLD and no phylogenetic trees have been provided in the paper. The *D. primorjensis* description is quite similar to the original description of *D. venustus* (MacFarland, 1966) and the latest redescription of *D. frondosus* (Ekimova *et al.*, 2015). Moreover, previous findings of *D. frondosus* or *Dendronotus* sp. in the Sea of Japan have not been

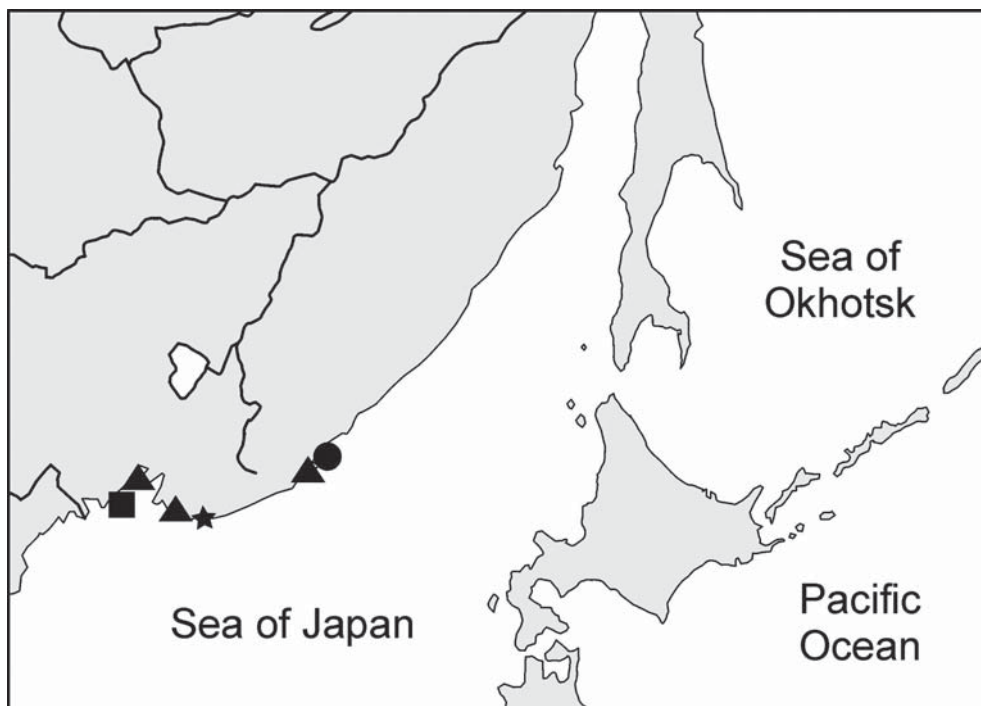


Fig. 1. Map of the north part of the Sea of Japan showing collecting sites of *D. dudkai* sp.n. (triangles), *D. frondosus* (quadrates), *D. kamchaticus* (circle). Star matches the type locality of *D. primorjensis*.

Рис. 1. Карта северной части Японского моря с местами сбора *D. dudkai* sp.n. (треугольники), *D. frondosus* (квадрат), *D. kamchaticus* (круг). Звездочкой отмечено типовое местонахождение *D. primorjensis*.

considered as minor synonyms of *D. primorjensis* (Martynov *et al.*, 2015). In these cases, the composition of “*D. frondosus* species complex” in the Sea of Japan seems to be disordering and requires a major revision. The present paper attempts to provide morphological and molecular data to clarify the taxonomic status of *D. frondosus*, *D. primorjensis* and *Dendronotus* sp. in northwestern Sea of Japan.

## Material and Methods

Due to the difficulties of the genus *Dendronotus* taxonomic studies using traditional methods (e.g. ontogenetic variations, colouration pattern variations and high level of other intraspecific differences), we followed the integrative approach, represented in Ekimova *et al.* (2015). It includes molecular phylogenetic meth-

ods, molecular species delimitation methods and detailed morphological studies with an analysis of the postlarval ontogenetic variations.

## Sampling data

Juvenile and adult specimens probably belonging to species *D. frondosus* with egg masses were obtained for this study by SCUBA diving and snorkeling during May–October 2013–2015 in Amursky, Rudnaya, and Vostok Bays of the Sea of Japan (Fig. 1). Living specimens were photographed with Nikon D300 and D810 cameras with Nikkor 60/f2.8D or Tokina 100/f2.8D lens. Underwater imaging was conducted using Sea&Sea MDX-D810 housing and two Sea&Sea YS-D1 strobes. All specimens were photographed individually and then preserved in 96% or 70% ethanol at ambient temperature before DNA or morphological analyses were performed

respectively. Type material is deposited in Zoological Museum of Lomonosov Moscow State University (ZMMU). Other material is deposited in a subsidiary of ZMMU at the White Sea Biological Station (vouchers and barcode collections — W and WS respectively).

### Molecular analysis

13 specimens of the genus *Dendronotus* from different localities were used for the mo-

lecular analysis. Previously published sequences of different *Dendronotus* species from GenBank (with voucher numbers LACM — Museum of Natural History, Los Angeles, USA, CASIZ — Californian Academy of Science, San Francisco, USA, SIO — Scripps Institute of Oceanology, and ZMMU) were also used to place the new species in a phylogenetic framework (Table 1). Specimens of Scyllaeidae, Dotoidae and Tritoniidae were included as outgroups (according to Ekimova *et al.*, 2015).

Table 1. Specimens used for molecular analysis with GenBank accession numbers.

Markings indicate new sequences obtained for this study.

Таблица 1. Особи, использованные для молекулярного анализа с регистрационными номерами ГенБанка. Выделенные номера обозначают новые сиквенсы, полученные в ходе настоящего исследования.

Species	Locality	Voucher	COI	16S	28S
<i>Dendronotus albopunctatus</i>	Washington	–	GQ292064	–	–
<i>D. albus</i>	California	LACM174845	–	GU339185	–
<i>D. dalli</i>	Kamchatka	ZMMU Op-294	KC660023	KC611294	KC660007
<i>D. dalli</i>	Kamchatka	ZMMU Op-295	KM397001	KM397083	KM397042
<i>D. dalli</i>	Kamchatka	ZMMU Op-330	KM396999	KM397081	KM397040
<i>D. dalli</i>	Kamchatka	ZMMU Op-331	KM397000	KM397082	KM397041
<i>D. dalli</i>	NW Atlantic	–	AF249800	AF249252	AY427450
<i>D. dudkai</i> sp.n.	Sea of Japan	W195	<b>KT031811</b>	<b>KT031824</b>	<b>KT031841</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W196	<b>KT031812</b>	<b>KT031825</b>	<b>KT031842</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W197	<b>KT031813</b>	<b>KT031826</b>	<b>KT031843</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W198	<b>KT031814</b>	<b>KT031827</b>	<b>KT031844</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W199	<b>KT031815</b>	<b>KT031828</b>	<b>KT031845</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W201	<b>KT031816</b>	<b>KT031829</b>	<b>KT031837</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W202	<b>KT031817</b>	<b>KT031830</b>	<b>KT031838</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	W202	<b>KT031818</b>	<b>KT031831</b>	<b>KT031840</b>
<i>D. dudkai</i> sp.n.	Sea of Japan	ZMMU Lc-40366	<b>KT031819</b>	<b>KT031832</b>	<b>KT031839</b>
<i>D. iris</i>	Washington	LACM174471	GQ292062	GU339189	–
<i>D. frondosus</i>	White Sea	ZMMU Op-290	KC660028	KC611280	KC660008
<i>D. frondosus</i>	Norway	ZMMU Op-380	KM396976	KM397056	KM397017
<i>D. frondosus</i>	Barents Sea	ZMMU Op-298	KM396985	KM397067	KM397025
<i>D. frondosus</i>	Barents Sea	ZMMU Op-299	KM396984	KM397066	KM397026
<i>D. frondosus</i>	White Sea	ZMMU Op-317	KM396978	KM397060	KM397019
<i>D. frondosus</i>	Barents Sea	ZMMU Op-291	KC660030	KC611281	KC660009
<i>D. frondosus</i>	Barents Sea	ZMMU Op-292	KC660031	KC611282	KC6600010
<i>D. frondosus</i>	White Sea	ZMMU Op-293	KC660029	KC611283	KC6600011
<i>D. frondosus</i>	Barents Sea	ZMMU Op-324	KM396980	KM397062	KM397021
<i>D. frondosus</i>	Barents Sea	ZMMU Op-325	KM396981	KM397063	KM397022
<i>D. frondosus</i>	Barents Sea	ZMMU Op-326	KM396982	KM397064	KM397023
<i>D. frondosus</i>	Barents Sea	ZMMU Op-327	KM396983	KM397065	KM397024
<i>D. frondosus</i>	Barents Sea	ZMMU Op-359	KM396979	KM397061	KM397020
<i>D. frondosus</i>	Barents Sea	ZMMU Op-382	KM396977	KM397050	KM397018

Table 1 (continued).  
Таблица 1 (продолжение).

Species	Locality	Voucher	COI	16S	28S
<i>Dendronotus frondosus</i>	Sea of Japan	W204	<b>KT031820</b>	<b>KT031833</b>	<b>KT031846</b>
<i>D. frondosus</i>	Sea of Japan	W205	<b>KT031821</b>	<b>KT031834</b>	<b>KT031847</b>
<i>D. kalikal</i>	Kamchatka	ZMMU Op-349	KM396986	KM397068	KM397027
<i>D. kalikal</i>	Kamchatka	ZMMU Op-283	KC660024	KC611284	KC660012
<i>D. kalikal</i>	Kamchatka	ZMMU Op-284	KC660025 KC660026 KM396988	KC611286 KC611285 KM397070	KC660013 KC660014 KM397029
<i>D. kalikal</i>	Bering Strait	ZMMU Op-285	KC660027	KC611287	KC660015
<i>D. kamchaticus</i>	Kamchatka	ZMMU Op-245	KC660032	KC611288	KC660016
<i>D. kamchaticus</i>	Kamchatka	ZMMU Op-246	KC660033 KM396989 KM396990	KC611289 KM397071 KM397072	KC660017 KM397030 KM397031
<i>D. kamchaticus</i>	Kamchatka	ZMMU Op-247	KM396991 KM396992	KM397073 KM397074	KM397032 KM397033
<i>D. kamchaticus</i>	Sea of Japan	W194	<b>KT031822</b>	<b>KT031835</b>	<b>KT031848</b>
<i>D. kamchaticus</i>	Sea of Japan	W194	<b>KT031823</b>	<b>KT031836</b>	<b>KT031849</b>
<i>D. lacteus</i>	Barents Sea	ZMMU Op-286	KC660034	KC611290	KC660018
<i>D. lacteus</i>	Barents Sea	ZMMU Op-287	KC660035	KC611291	KC660019
<i>D. lacteus</i>	Norway	ZMMU Op-383	KM396971 KM396972	KM397054 KM397055	KM397012 KM397013
<i>D. lacteus</i>	Barents Sea	ZMMU Op-288	KM396975	KM397059	KM397016
<i>D. lacteus</i>	Barents Sea	ZMMU Op-333	KM396974	KM397058	KM397015
<i>D. lacteus</i>	Barents Sea	ZMMU Op-335	KM396973	KM397057	KM397014
<i>D. niveus</i>	White Sea	WS1102	KC660036	KC611292	KC660020
<i>D. niveus</i>	White Sea	WS2005	KM396998	KM397080	KM397039
<i>D. niveus</i>	White Sea	ZMMU Op-269	KM396996	KM397078	KM397037
<i>D. niveus</i>	White Sea	ZMMU Op-270	KM396997	KM397079	KM397038
<i>D. niveus</i>	Barents Sea	ZMMU Op-274	KC660037 KM396993 KM396994	KC611293 KM397076 KM397075	KC660021 KM397034 KM397035
<i>D. niveus</i>	Barents Sea	ZMMU Op-279	KM396995	KM397077	KM397036
<i>D. orientalis</i>	China	CASIZ174989	-	HM162628	-
<i>D. patricki</i>	California	SIO-BIC M12134	HQ225828	HQ225829	-
<i>D. regius</i>	Philippines	CASIZ179492	JN869451	HM162629	-
<i>D. robustus</i>	Barents Sea	ZMMU Op-343	KM397002	KM397084	KM397043
<i>D. robustus</i>	Barents Sea	ZMMU Op-344	KM397003	KM397085	KM397044
<i>D. robustus</i>	Barents Sea	ZMMU Op-390	KM396963 KM396964 KM396965 KM396966 KM396968	KM397045 KM397046 KM397047 KM397048 KM397051	KM397004 KM397005 KM397006 KM397007 KM397009
<i>D. robustus</i>	Barents Sea	ZMMU Op-391	KM396970	KM397053	KM397011
<i>D. robustus</i>	Barents Sea	ZMMU Op-392	KM396967	KM397049	KM397008
<i>D. robustus</i>	Barents Sea	ZMMU Op-393	KM396969	KM397052	KM397010
<i>D. robustus</i>	Barents Sea	ZMMU Op-391	KM396970	KM397053	KM397011
<i>D. rufus</i>	Alaska	LACM174861	-	GU339191	-
<i>D. subramosus</i>	Washington	LACM174855	-	GU339197	-
<i>D. venustus</i>	California	LACM174852	HM162709	GU339199	-

Table 1 (continued).  
Таблица 1 (продолжение).

Species	Locality	Voucher	COI	16S	28S
<i>Doto coronata</i>	South Africa	CASIZ 176278	AF249794	HM162657	–
<i>D. koenneckeri</i>	Portugal	CASIZ178247	HM162735	AF249249	–
<i>Notobryon thompsoni</i>	Philippines	CASIZ176362	JM869455	JN869413	–
<i>N. wardi</i>	South Africa	CASIZ177540	HM162714	HM162639	–
<i>Scyella fulva</i>	Philippines	CASIZ182823	JN869458	JN869415	–
<i>Janolus barbarensis</i>	California	CASIZ176833	HM162747	HM162671	–

Table 2. PCR and sequencing primers.  
Таблица 2. Праймеры, использованные для ПЦР и секвенирования.

Name	5'–3'	References
16S arL	CGCCTGTTTAACAAAAACAT	Palumbi <i>et al.</i> 1991
16S R	CCGRITYTGAACCTCAGCTCACG	Puslednik & Serb (2008)
LCO 1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> 1994
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> 1994
28S C1'	ACCCGCTGAATTTAAGCAT	Lê <i>et al.</i> 1993
28S C2	TGAACTCTCTCTTCAAAGTTCTTTTC	Lê <i>et al.</i> 1993

DNA was extracted from small pieces of foot tissue using Diatom™ DNA Prep 100 kit by Isogene according to the manufacturer's protocol. Extracted DNA was used as a template for amplification of cytochrome *c* oxidase subunit I (COI), 16S rRNA and C1–C2 domain of 28S rRNA. The primers that were used for amplification are shown in Table 2. Polymerase chain reaction amplifications were carried out in a 20- $\mu$ L reaction volume, which included 4  $\mu$ L of 5x Screen Mix by Eurogen Lab, 0.5  $\mu$ L of each primer (10  $\mu$ M stock), 1  $\mu$ L of genomic DNA and 14  $\mu$ L of sterile water. The amplification of COI and 28S was performed with an initial denaturation for 1 min at 95 °C followed by 35 cycles of 15 s at 95 °C (denaturation), 15 s at 45 °C (annealment) and 30 s at 72 °C with a final extension of 7 min at 72 °C. The 16S amplification began with an initial denaturation for 1 min at 95 °C followed by 40 cycles of 15 s at 95 °C (denaturation), 15 s at 52 °C (annealment), 30 s at 72 °C with a final extension of 7 min at 72 °C. Sequencing for both strands proceeded with the *Big Dye v3.1* sequencing kit by Applied Biosystems. Sequencing reactions were analyzed using ABI 3500 Genetic Analyser (Ap-

plied Biosystems) in N.K. Koltsov Institute of Developmental Biology (Moscow, Russia). All new sequences were deposited in GenBank. Raw reads for each gene were assembled and checked for improper base-calling using GeneiousPro 4.8.5. Original data and publicly available sequences were aligned with MUSCLE (Edgar, 2004) algorithm in MEGA 6 (Tamura *et al.*, 2013) software. Protein-coding sequences were translated into amino acids for confirmation of the alignment. The resulting alignments were of 641 bp for COI, 471 bp for 16S, 350 bp for 28S. Individual gene analyses, separate mitochondrial and nuclear gene analyses, and a concatenated analysis were performed in this study. The best-fitting nucleotide evolution models were tested in MEGA 6 toolkit using Bayesian information criterion (BIC). The 28S partition scored the lowest BIC for the Hasegawa-Kishino-Yano model. The best-fitting model for COI and 16S partitions was General Time Reversible model with a gamma distribution and a fraction of sites being invariable. Phylogeny reconstructions of individual gene datasets were conducted by maximum likelihood method, implemented in MEGA 6 with 2000 boot-

strap pseudoreplications and in MrBayes 3.2 (Ronquist, Huelsenbeck, 2003). Reconstructions based on combined datasets (mitochondrial, nuclear, and concatenated analyses) were performed applying evolutionary models for partitions separately. The Bayesian estimation of posterior probability was also performed in MrBayes 3.2. Markov chains were sampled at intervals of 500 generations. The analysis was started with random starting trees and  $10^7$  generations. Maximum likelihood-based phylogeny inference for all combined data sets was performed in GARLI 2.0 (Zwickl, 2006) with bootstrap in 1000 pseudoreplications. Bootstrap values were placed on the best tree found with SumTrees 3.3.1 from DendroPy Phylogenetic Computing Library Version 3.12.0 (Sukumaran, Mark, 2010). Final phylogenetic tree images were rendered in FigTree 1.4.0.

## Species delimitation

We used three methods to define species: comparing single- and combined-gene tree topologies, Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule Coalescent method (GMYC). Single- and combined-gene trees were calculated using MEGA 6 and MrBayes 3.2 as explained above. ABGD method (Puillandre *et al.*, 2012) is based on pairwise distances, detecting the breaks in the distribution referred to as the “barcode gap” (Herbert *et al.*, 2003) without any prior species hypothesis. It is commonly used for species delimitation analyses, including the latest works on opisthobran taxa (Jörger *et al.*, 2012; Barco *et al.*, 2013; Jörger, Schrödl, 2013; Krug *et al.*, 2013; Camara *et al.*, 2014; Carmona *et al.*, 2014a, b; Churchill *et al.*, 2014; Ortigosa *et al.*, 2014; Padula *et al.*, 2014; Goodheart *et al.*, 2015; Ekimova *et al.*, 2015 and others). The ABGD program is available at web site <http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>. We analyzed *COI* and *16S* alignments separately (excluding outgroups) using both proposed models: Jukes-Cantor (JK69) and Kimura (K80). In the case of *COI*,  $P_{\max}$  was increased to 0.20 and amount of steps was

increased to 15. Other settings were remained default. In *16S* analysis, the default settings were used. GMYC method proposed by Pons (Pons *et al.*, 2006) and observed by Fujisawa & Barraclough (Fujisawa, Barraclough, 2013), is also rather popular in species delimitation studies (Neusser *et al.*, 2011; Williams *et al.*, 2011; Falniowski, Szarowska, 2012; Jörger *et al.*, 2012; Prévot *et al.*, 2013; Fontaneto, 2014; Fontaneto *et al.*, 2014, and others). Approaches based on the GMYC model rely on the expectation that intraspecific coalescent branching proceeds discernibly quicker than speciation, which is modelled as a Yule process. Therefore, species can be identified in genes tree as clusters of terminals separated by longer internal branches. It requires a fully dichotomous and rooted ultrametric tree without duplicate sequences based on single-gene analyses. The tree was run using BEAST (Drummond, Rambaut, 2007) and then analyzed within the R environment, following instructions represented by Fujisawa & Barraclough (2013).

## Morphological data

The external morphology of molluscs was studied under a stereomicroscope. For the description of internal features preserved specimens were dissected under the stereomicroscope. The buccal mass of each specimen was extracted and soaked in 10% sodium hypochlorite solution for 1–2 minutes to dissolve connective and muscle tissue, leaving only the radula and jaws. The features of the jaws of each species were analyzed under the stereomicroscope and scanning electron microscope and then drawn. The coated radulae were examined and photographed using scanning electron microscopes CamScan, JEOL JSM or EVO-40 Zeiss. Reproductive systems of different species were also examined and drawn using the stereomicroscope.

## Results

### Molecular analysis

We acquired 39 original nucleotide sequences for 13 specimens of the genus *Dendronotus*





Pacific specimens of *D. frondosus* (PP = 0.98, BS = 75%), but *D. venustus* shows more basal placement. Other clades confirm the monophyly of species-level groups. Deepwater *D. patricki* and *D. robustus* turned out to be a sibling species (PP = 0.98, BS = 90%) and show most basal position with *D. regius* and *D. orientalis*. The other branch includes a large group of northern shallow-water species (PP = 0.97, BS = 76%). Within this group, three major clades can be distinguished. The first one includes *D. kalikal*, the second includes *D. iris* and sister to it *D. frondosus* + *D. venustus* + *Dendronotus* sp. (Voucher numbers W195–W203, W206–W207) (PP = 1, BS = 74%). The third clade includes *D. albopunctatus*, *D. albus*, *D. dalli*, *D. kamchaticus* (GenBank specimens and W194), *D. lacteus*, *D. niveus*, *D. rufus* and *D. subramosus* (PP = 0.96, BS = 73%). The relationships within these clades remain unresolved.

### Species delimitation

ABGD analysis of COI dataset, running with two different models, revealed 13 potential species each: *D. robustus*, *D. frondosus* (GenBank specimens and W204, W205), *D. lacteus*, *D. kalikal*, *D. kamchaticus* (GenBank specimens and W194), *D. niveus*, *D. dalli*, *D. venustus*, *D. iris*, *D. albopunctatus*, *D. patricki*, *D. regius* and *Dendronotus* sp. (W195–W203, W206–207). ABGD analysis of 16S dataset revealed 17 potential species each: *Dendronotus* sp. (W195–W203, W206–W207), *D. robustus*, *D. frondosus* (GenBank specimens and W204, W205), *D. lacteus*, *D. kalikal*, *D. kamchaticus* (GenBank specimens and W204, W205), *D. niveus*, *D. dalli*, *D. venustus*, *D. iris*, *D. albus*, *D. orientalis*, *D. patricki*, *D. subramosus*, *D. rufus* and *D. regius*. The prior maximum distance ranged between 0.001 and 0.0116 for COI. In the case of 16S, the prior maximum distance ranged between 0.0012 and 0.0058. Differences in number of potential species in each analysis are determined by lacking of COI data for some NE Pacific species. GMYC analysis of COI marker recovers 13 species units. These independent molecular delimitation ap-

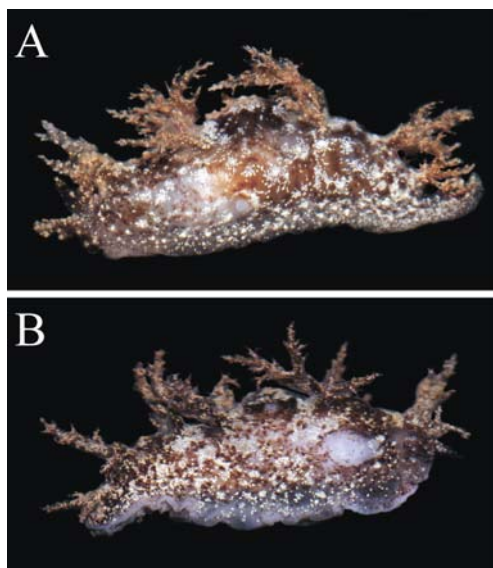


Fig. 3. *Dendronotus frondosus*, living animals. A — W204, specimen 16 mm in length, lateral view from the left. B — W205, specimen 19 mm in length, lateral view from the right. Photos by A. Chichvarkhin. Рис. 3. *Dendronotus frondosus*, фотографии живых особей. А — W204, особь 16 мм длиной, вид с левой латеральной стороны. В — W205, особь 19 мм длиной, вид с правой латеральной стороны. Фотографии А. Чичвархина.

proaches are in congruence with our molecular phylogenetic study and thus, justify a separation status of specimens of *Dendronotus* sp. (W195–W203, W206–W207) and also *D. frondosus* and *D. kamchaticus* findings in the Sea of Japan.

### Morphology

We studied 24 *Dendronotus* specimens from three localities of northwestern Sea of Japan. The colouration patterns in each specimen were nearly similar and corresponded to the common colour of *D. frondosus*: variegated pattern with brown stripes and spots on the dorsal side of the body and upper parts of the foot (Figs. 3, 6, 9). However, 20 specimens also possess clearly visible stripes of white opaque pigment between rows of dorsolateral appendages (Fig. 9). Other external morphological features e.g. branching pattern of dorsolateral processes (Figs. 5A, 8A,

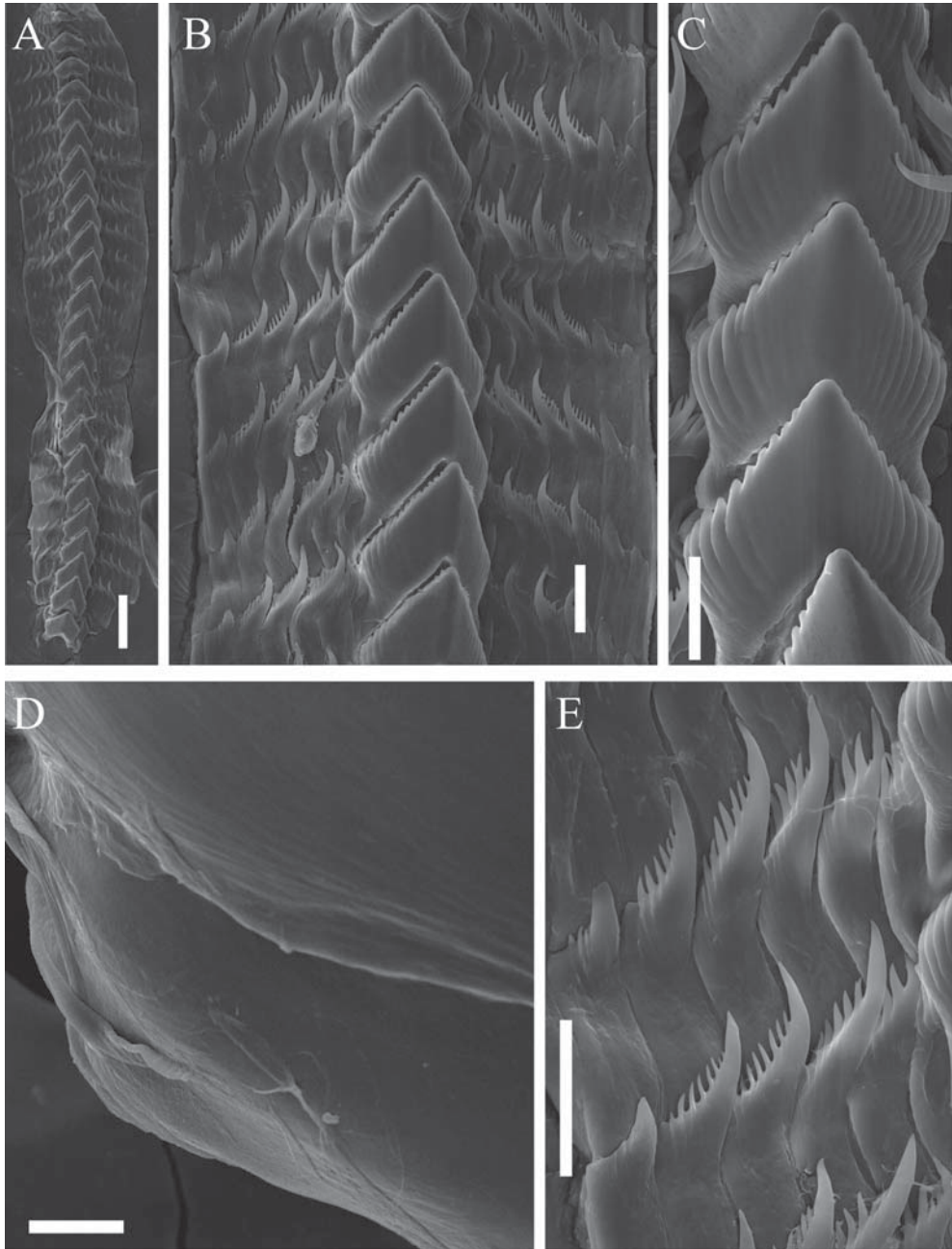


Fig. 4. *Dendronotus frondosus*, scanning electron micrographs of the radula and masticatory border of jaws. W205, specimen 21 mm in length. A — posterior part of the radula, main view; B — rachidian and lateral teeth; C — rachidian teeth; D — masticatory border of jaws, denticles are absent; E — lateral teeth. Scale bars: A — 100  $\mu\text{m}$ ; B, C, E — 20  $\mu\text{m}$ ; D — 30  $\mu\text{m}$ .

Рис. 4. *Dendronotus frondosus*, микрофотографии участков радулы и жевательной поверхности челюстей (СЭМ). W205, особь 21 мм длиной. А — задняя часть радулы, общий вид; В — центральные и латеральные зубы; С — центральные зубы; D — жевательная поверхность челюстей, зубчики отсутствуют; E — латеральные зубы. Масштаб: А — 100 мкм; В, С, E — 20 мкм; D — 30 мкм.

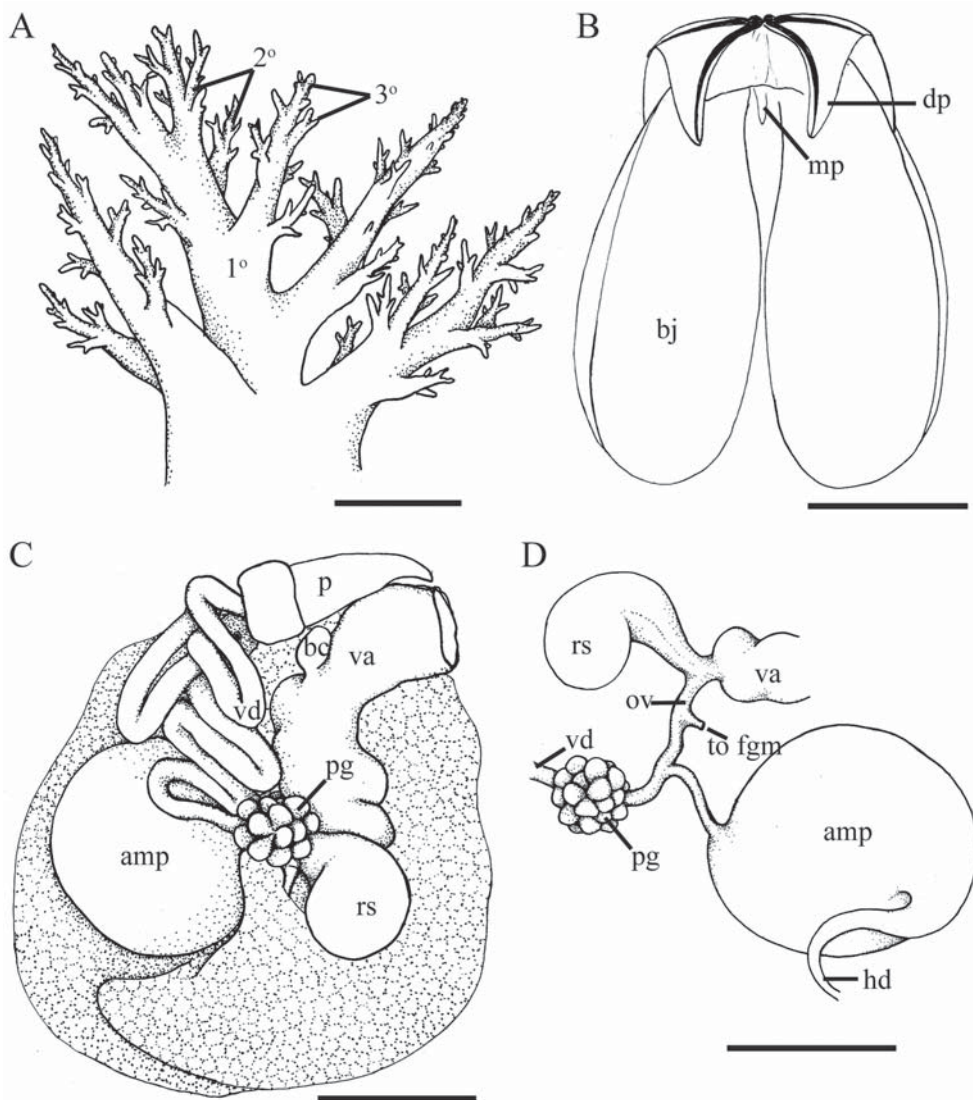


Fig. 5. *Dendronotus frondosus*, drawings of the external morphology and anatomy. A — dorsolateral appendages branching pattern; B — jaws; C — main view of reproductive system; D — reproductive system, detailed.

Abbreviations: 1° — primary stalks; 2° — secondary branches; 3° — tertiary branches; amp — ampulla; bc — bursa copulatrix; bj — jaw body; dp — dorsal process of the jaw; fgm — female gland mass; hd — hermaphroditic duct; mp — masticatory process of the jaw; ov — oviduct; p — penis; pg — prostata; rs — receptaculum seminis; va — vagina; vd — vas deferens. Scale bars 1 mm.

Рис. 5. *Dendronotus frondosus*, особенности внешней и внутренней морфологии. А — особенности ветвления дорсолатеральных отростков; В — челюсти; С — общий вид половой системы; D — область овидукта, детализировано.

Обозначения: 1° — первичные ветви; 2° — вторичные веточки; 3° — третичные веточки; amp — ампулла; bc — копулятивная сумка; bj — тело челюстей; dp — дорсальный отросток челюстей; fgm — женские железы; hd — гермафродитный проток; mp — жевательный отросток челюстей; ov — овидукт; p — пенис; pg — простата; rs — семяриемник; va — вагина; vd — семяпровод. Масштаб 1 мм.

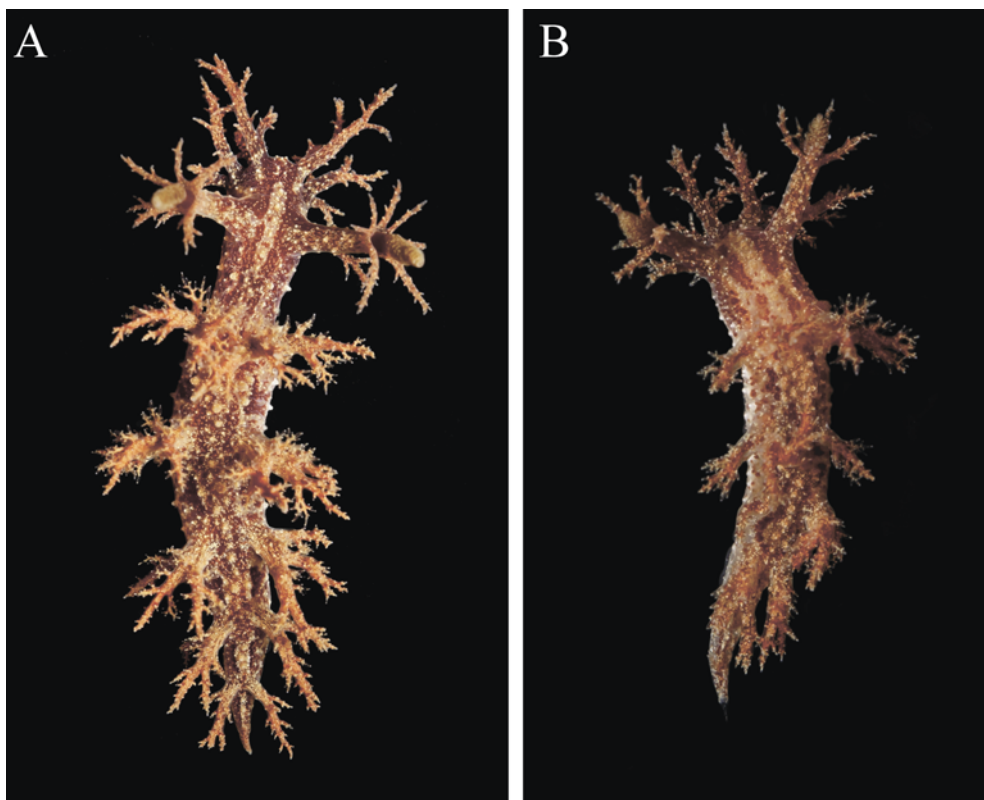


Fig. 6. *Dendronotus kamchaticus*, W194, living animals, dorsal view. A — specimen 18 mm in length; B — specimen 11 mm in length. Photos by T. Antokhina.

Рис. 6. *Dendronotus kamchaticus*, W194, фотографии живых особей, вид с дорсальной стороны. А — особь 18 мм длиной; В — особь 11 мм длиной. Фотографии Т. Антохиной.

11A), the number of veil papillae, the presence of lateral papillae, etc., were identical in all specimens studied. According to internal diagnostic characters, the most important and variable were the denticulation of masticatory border, radular morphology and the reproductive system morphology. Two specimens from Rudnaya Bay (W194) possess a smooth rachidian tooth (Fig. 7), which is a main diagnostic feature of *D. kamchaticus*, combining with variegated colour pattern. Other studied specimens have a highly denticulated rachidian tooth, with deep furrows on its surface (Figs. 4, 10). In two specimens collected in Amursky Bay (W204, W205) the smooth masticatory border of jaws was detected (Fig. 4D). This character is typical for *D. frondosus*. Meanwhile, masticatory border in other specimens possesses a single row of

denticles (Fig. 10D). This feature has been described only for *D. dalli*, *D. iris*, *D. regius*, *D. kalikal* and *D. venustus* (Robilliard, 1970; Pola, Stout, 2008; Stout *et al.*, 2010; Ekimova *et al.*, 2015). The reproductive system morphology also well differs. Two specimens from Rudnaya Bay (W194) have an elongate sinuous ampulla, prostata consists of 7–8 oval alveoli and a narrow, elongate penis (Fig. 8C). These features correspond with original description of *D. kamchaticus* by Ekimova *et al.* (2015). Specimens W204, W205 from Amursky Bay possess very similar morphology of reproductive system to *D. frondosus*: wide semicircular ampulla, elongated penis and discoid prostate, which consists of 16–30 small alveolar glands. Finally, all other specimens show quite similar morphology of the reproductive system: an ampulla is wide

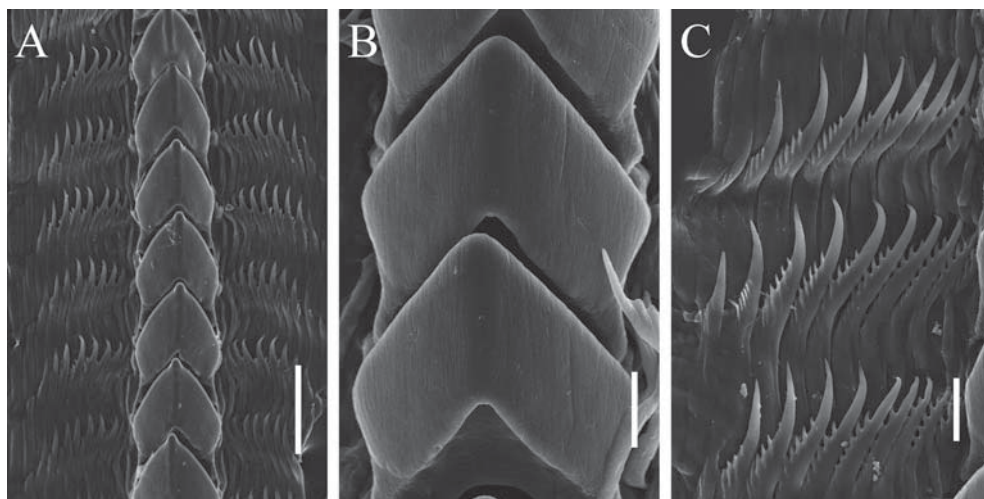


Fig. 7. *Dendronotus kamchaticus*, W194, scanning electron micrographs of the radula. A — rachidian and lateral teeth; B — rachidian teeth; C — lateral teeth. Scale bars: A — 100  $\mu\text{m}$ ; B, C — 30  $\mu\text{m}$ .

Рис. 7. *Dendronotus kamchaticus*, W194, микрофотографии участков радулы (СЭМ). А — центральные и латеральные зубы; В — центральный зуб; С — латеральные зубы. Масштаб: А — 100 мкм; В, С — 30 мкм.

and sinuous, prostate concentric ring-shaped, consists of 12–14 oval alveoli, penis is slightly curved. Such morphology of reproductive system has never been described for any other *Dendronotus* species. Therefore, our morphological data indicate the presence of at least three species in northwestern part of the Sea of Japan: *D. frondosus*, *D. kamchaticus* and *Dendronotus* sp. and thus are congruent with molecular phylogenetic analysis and molecular species delimitation analysis.

### Identification

According to the results of morphological and molecular analyses, we can suggest that studied specimens with Voucher number W194 truly belong to species *D. kamchaticus*. Specimens with Voucher numbers W204 and W205 are highly similar in external and internal morphology with Atlantic and Arctic individuals of *D. frondosus*, this result is strongly supported by molecular analysis. Molecular analysis of *Dendronotus* sp. specimens (Voucher numbers W195–W203; W206–W207) showed their separation from other *Dendronotus* species avail-

able for molecular study (Fig. 2). This species appears in the same clade as *D. frondosus* and *D. venustus*, which corresponds in some ways with diagnosis of *D. primorjensis*, according to its original description (Martynov *et al.*, 2015). However, in this description it was stated that “molecular phylogenetic analysis of COI gene placed *D. primorjensis* at the base of the clade, which includes Atlantic species *D. frondosus* (Ascanius, 1774) and Pacific species *D. venustus* MacFarland, 1966, but as a separate branch, different from these two species” (Martynov *et al.*, 2015). In contrast, in our both concatenated (Fig. 2) and single-gene COI trees, studied specimens do never possess a basal placement to *D. frondosus* and *D. venustus*. Unfortunately, the sequences of *D. primorjensis* type material have not been published by authors. The official request of these sequences was declined by corresponding author of that paper (A.V. Martynov). Type specimens of this species deposited in ZMMU (holotype Op-419 and paratype Op-420) were not allowed for examination. Therefore, due to lack of reliable possibility of these species comparison, we can attribute only indirect evidences e.g. the placement of our speci-

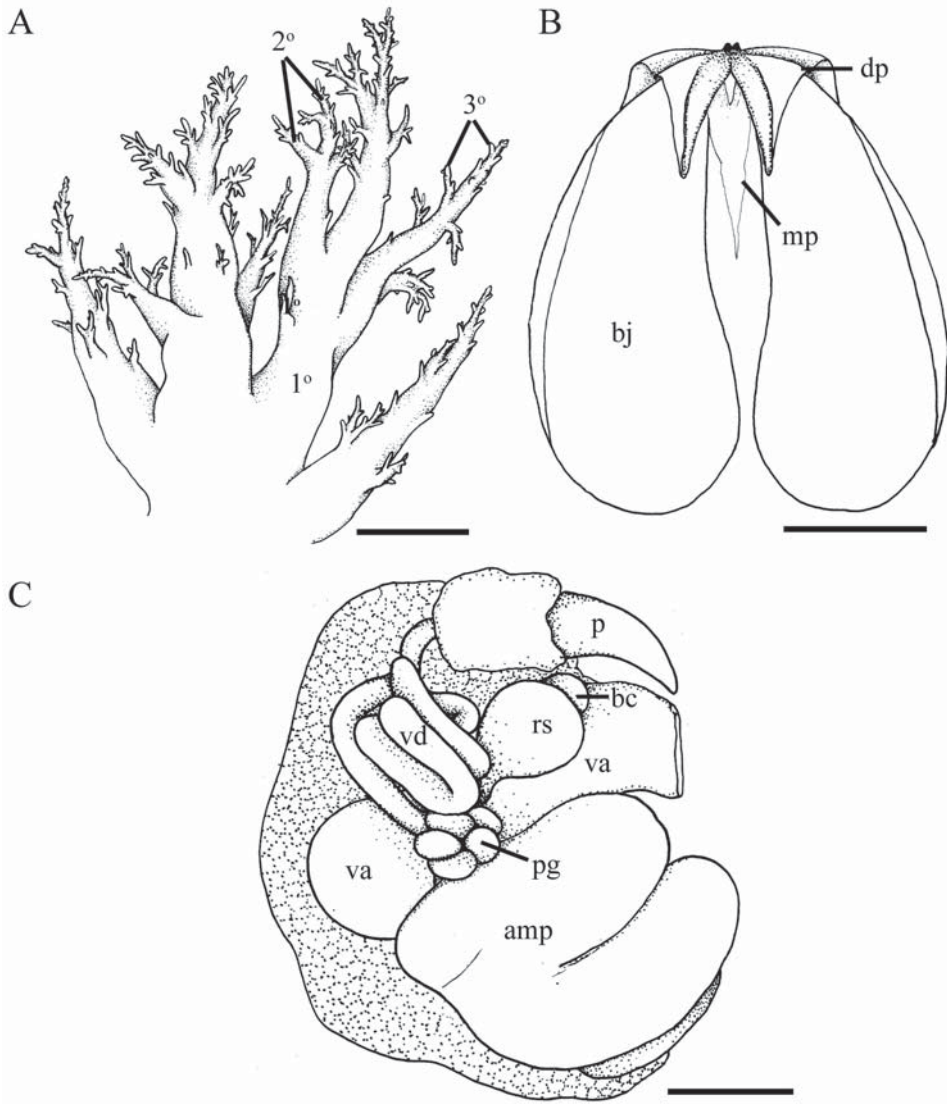


Fig. 8. *Dendronotus kamchaticus*, W194, drawings of the external morphology and anatomy. A — dorsolateral appendages branching pattern; B — jaws; C — main view of reproductive system.

Abbreviations: 1° — primary stalks; 2° — secondary branches; amp — ampulla; bc — bursa copulatrix; bj — jaw body; dp — dorsal process of the jaw; mp — masticatory process of the jaw; p — penis; pg — prostata; rs — receptaculum seminis; va — vagina; vd — vas deferens. Scale bars 1 mm.

Рис. 8. *Dendronotus kamchaticus*, W194, особенности внешней и внутренней морфологии. А — особенности ветвления дорсолатеральных отростков; В — челюсти; С — общий вид половой системы. Обозначения: 1° — первичные ветви; 2° — вторичные веточки; 3° — третичные веточки; amp — ампулла; bc — копулятивная сумка; bj — тело челюстей; dp — дорсальный отросток челюстей; mp — жевательный отросток челюстей; p — пенис; pg — простата; rs — семяприемник; va — вагина; vd — семяпровод. Масштаб 1 мм.

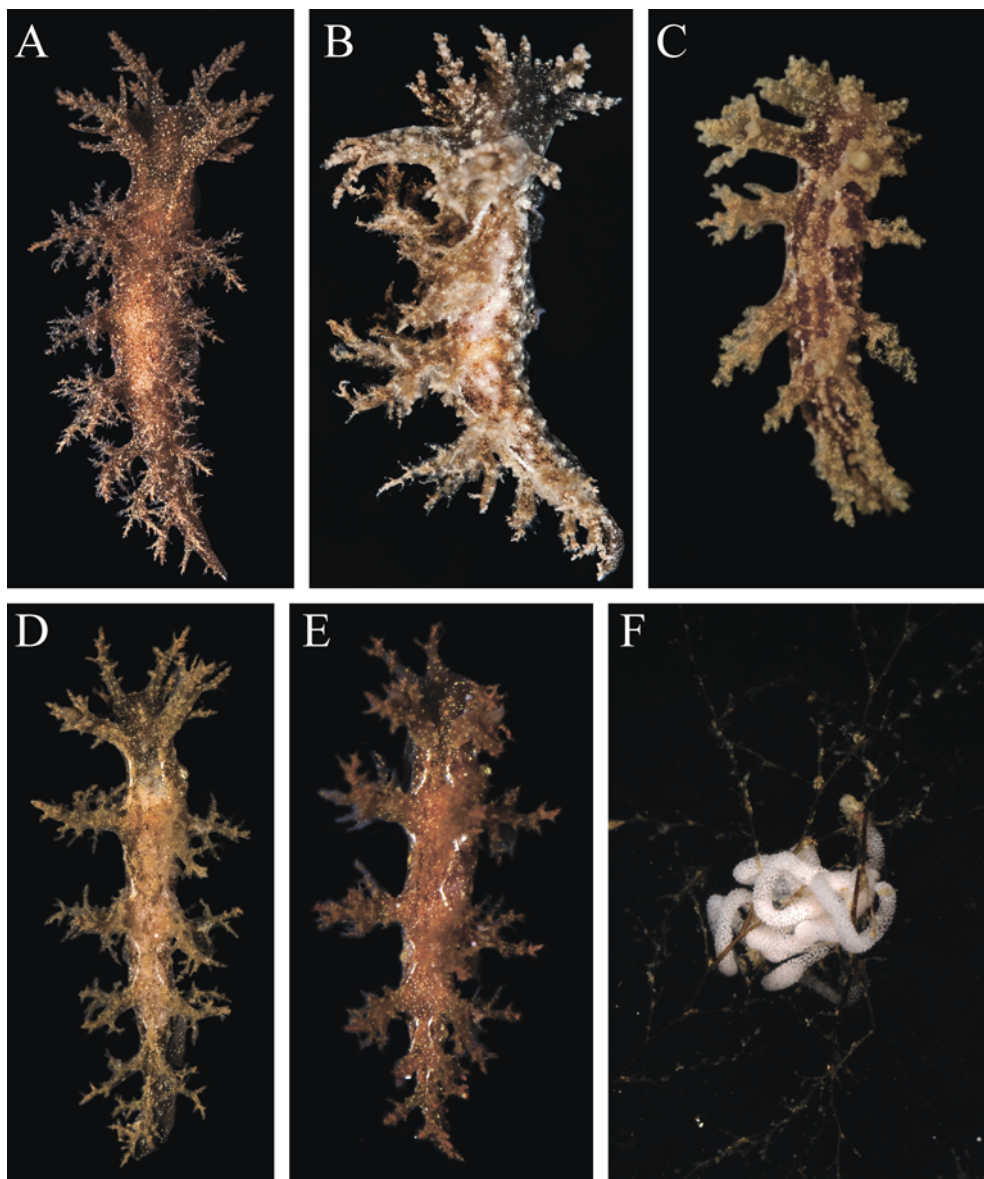


Fig. 9. *Dendronotus dudkai* sp.n., living animals and hatch. A — paratype ZMMU Lc-40366, specimen 21 mm in length; B — W201, specimen 12 mm in length; C — W198, specimen 8 mm in length; D — paratype ZMMU Lc-40367, specimen 28 mm in length; E — WS2894, specimen 24 mm in length; F — hatch on *Obelia* cf. *longissima* colony. Photos by A. Chichvarkhin.

Рис. 9. *Dendronotus dudkai* sp.n., фотографии живых особей и кладки. А — паратип ZMMU Lc-40366, особь 21 мм длиной; В — W201, особь 12 мм длиной; С — W198, особь 8 мм длиной; D — паратип ZMMU Lc-40367, особь 28 мм длиной; E — WS2894, особь 24 мм длиной; F — кладка на колонии *Obelia* cf. *longissima*. Фотографии А. Чичвархина.



mens on the phylogenetic tree, which is contrary to original description of *D. primorjensis*.

Regarding external morphology, our specimens are clearly distinguished from *D. primorjensis* in colour pattern. In *D. primorjensis* description, authors stated that “according to external features, the new species differs from both *D. frondosus* and *D. venustus* by lack of large aggregations of white pigment”. However, our specimens possess well-developed stripes of opaque white pigment on the dorsal side between cerata (Fig. 9). In *D. frondosus*, *D. venustus*, *D. kamchaticus* and *D. kalikal* (species with similar colour pattern) white and golden opaque pigment is placed only in low turbellules (Ekimova *et al.*, 2015; present study). Moreover, in *D. primorjensis* this character is developed even less than in these species, according to the original description. In general, as it was said above, the description of *D. primorjensis* is very poor and provides mostly general information on external and internal morphology. Some important taxonomic morphological characters were not described at all. For example, the description of reproductive system is too general and containing only features that are similar in all *Dendronotus* species (form of the prostate, form of the penis, form of the vas deference and colouration of the hermaphroditic gland). Meanwhile the most important diagnostic characters were not described (form of ampulla, number of prostate lobules and position of bursa copulatrix). The description of the external morphology and radula can also refer to species *D. frondosus*, *D. venustus*, *D. kalikal*.

Based on the foregoing significant discrepancy between *Dendronotus primorjensis* description and our specimens’ characters, and because of unavailability of *D. primorjensis* type material in referred museum collection, and its author’s denial to provide them for detailed molecular and morphological study we suppose these specimens are lost if ever existed. Therefore, we are designating the name *D. primorjensis* as *nomen dubium*, and suggesting that our specimens of *Dendronotus* sp. belongs to a distinct species, new for science, and its description is provided below.

## Systematic descriptions

Due to particular difficulty of distinguishing *Dendronotus* species from the northwestern part of the Sea of Japan we provide an extended systematic descriptions of all three species analyzed.

Nudibranchia Cuvier, 1817  
Dendronotina Odhner, 1934  
Family Dendronotidae Odhner, 1934  
*Dendronotus* Alder et Hancock, 1845

Type species: *Dendronotus frondosus* (Ascanius, 1774), by original designation.

*Dendronotus frondosus* (Ascanius, 1774)  
Figs. 3–5.

*Amphitrite frondosa* Ascanius, 1774: 155–158, pl. 5, fig. 2.

See Robilliard (1970) and Ekimova *et al.* (2015) for a full list of synonyms

Material examined: W204, 1 specimen, Sea of Japan, Amursky Bay, near the Institute of Marine Biology, 2–5 m depth, coll. K. Dudka, 25 May 2014; W205, Sea of Japan, Amursky Bay, near the Institute of Marine Biology, 2–5 m depth, coll. K. Dudka, 25 May 2014.

Description: External morphology (Figs. 3, 5A). Body elongate, laterally compressed. Foot narrow, tail short. Oral veil with 10–14 short lip papillae and 4–5 secondary branched appendages. Rhinophoral sheaths with long stalk and five crown appendages. Lateral papilla moderate in size with small secondary branches. Rhinophores with 6–10 lamellae. 5 pairs of dorsolateral processes. Dorsolateral processes moderately branched by secondary and tertiary branches of similar size (Fig. 5A). Digestive gland diverticula penetrate dorsolateral processes and rhinophoral branched processes. These diverticula in first pair of cerata and rhinophores originate from anterior lobe of digestive gland; others arise from posterior lobe. Anal opening on right side of body about midway between first and second pair of dorsolateral processes. Reproductive openings lateral, below first pair of dorsolateral processes on right side.

Colour (Fig. 3): Background colour usually white or light-pink. Brown spots, lines, stripes and dots placed on dorsal side of body and upper parts of foot. Body covered by opaque golden or white pigment on which placed in small tubercles or gathered in large spots. Digestive gland diverticula can be seen through transparent body wall, digestive gland brown. Rhinophores similar in colour to body.

Internal morphology (Figs. 4, 5B–D): Length of dorsal processes of jaws about one-third length of jaw body. Inclined posteriorly at about 30°. Masticatory process about one-fifth as long as jaw body, slightly curved downwards. Masticatory border without denticles (Fig. 4D). Masticatory process strong, dark at base, transparent and subulate posteriorly. Jaw body yellow, brown towards masticatory border and ligament. Radula formula: 33 × 6–7.1.6–7 (W205). Rachidian tooth strong, triangular, approximately 1.2 times longer than width. Rachidian tooth bears 8–12 well-defined sharp denticles with large furrows on both sides of reduced cusp (Fig. 4B, C). Lateral teeth slightly curved towards midline and possess 5–10 large sharp denticles (Fig. 4E). Outermost lateral teeth flattened with two or three reduced denticles. Innermost laterals thin and highly denticulated. Reproductive system triaulic (Fig. 5C, D). Ootestis consists of numerous white rounded lobules. Long hermaphroditic duct leads to wide semicircular ampulla. Prostate discoid body consists of 16–30 small alveolar glands. Distal part of vas deferens long and winding, expanding to elongate penis. Oviduct connects through insemination duct into female gland complex. Vagina long, gradually narrowing into a rounded seminal receptaculum, and then connecting into oviduct and female gland mass. Bursa copulatrix small and rounded. Female genital aperture opens posterior to penis on right side of body, between first and second pairs of dorso-lateral processes.

Ecology: Depth range does not exceed 20 m (Ekimova *et al.*, 2015; present study). Feeds on several tectophoran hydrozoan species from genera *Obelia*, *Dynamena*, *Gonothyrea*, and *Laomedea*. Egg mass is narrow cord, forms an

irregular, compressed off-white spiral. Reproduction period from June to October; the larva is a planctotrophic veliger with oval shell.

Distribution: North-West and North-East Atlantic, including the White and the Barents seas, north parts of the Sea of Japan

*Dendronotus kamchaticus* Ekimova, Korshunova, Schepetov, Neretina, Sanamyan et Martynov, 2015

Fig. 6–8.

Type material: Holotype ZMMU Op-245, northwest Pacific, Kamchatka, Avacha Bay, 7 m depth, coll. D.B. Semenov, 15 June 2010.

Material examined: W194, 2 specimens, Sea of Japan, Rudnaya Bay, 10–16 m depth, coll. T. Antokhina, A. Chichvarkhin, 8 May 2013.

Description. External morphology (Figs. 6, 8A): Body elongate, laterally compressed. Foot narrow, tail short. Oral veil with 4–6 lip papillae and branched appendages. Primary stalks of veil appendages tall and slender, giving rise to numerous secondary branches with short tertiary branches. Rhinophoral sheath divide into 5–6 crown papillae that about same length. Lateral papillae (about one-third or one-half of sheath length) branches off sheath base and expanded with secondary branches. Rhinophores bear 14–20 lamellae. 5 pairs of dorsolateral appendages. Primary stalks of cerata long, slender and conical (Fig. 8A). Secondary branches of dorsal appendages well-expressed, narrow; tertiary branches of dorsal appendages short, rounded at tip. Digestive gland diverticula penetrate cerata and rhinophoral branched processes. These diverticula in first part of cerata and rhinophores originate from anterior lobe of digestive gland; others arise from posterior lobe. Anal opening on right side of body about midway between first and second pair of dorsal appendages. Reproductive openings lateral near the first pair of cerata on right side.

Colour (Fig. 6): Background colour is transparent white, with complex pattern of light, dark, and red-brown spots and stripes. On dorsal side spots and stripes merge and form char-

acteristic striped pattern. Lateral sides of body devoid of stripes but covered with brown spots. Brown branches of digestive gland clearly visible through thin walls of rhinophoral sheathes, dorsal appendages, and velar papillae. Lamellae of rhinophores brown. All body, including rhinophoral sheathes, cerata, and upper parts of foot covered with white and golden pigment spots. Large dots of pigment are often placed inside tubercles on dorsal and lateral surface of body.

Internal morphology (Figs. 7, 8B, C): Dorsal process of jaws strong; length about one-quarter of jaw body length. Inclined posteriorly at about 15°. Length of dorsal processes of jaws about one-third length of jaw body, strong at base, but becoming transparent and subulate posteriorly. Denticles absent on masticatory border. Radula formula: 24×9–10.1.9–10 (adult specimen), 17×10–11.1.10–11 (subadult specimen). Rachidian tooth large and strong, with quadrangular base and triangular cusp. Rachidian tooth of subadult specimens bear 10–12 small, relatively sharp denticles that continue down dorsal side of cusp as thin furrows. In adult specimens denticles absent, tooth completely smooth, sometimes with faint furrows (Fig. 7A, B). Colour of oldest rachidian teeth is light yellow, others transparent. 9–11 lateral teeth, each consisting of elongate, flattened plate and cusp. Outer border of cusps bear 5–9 sharp and strong denticles. Innermost lateral tooth thin, delicate, and highly denticulated, one or two outermost teeth narrow, thin and flattened and do not bear denticles (Fig. 7C). Reproductive system triaulic (Fig. 8C). Large white ovotestis composed of many rounded lobules, and leading to hermaphrodite duct that expands into elongate sinuous ampulla. Prostata consists of 7–8 oval alveoli, distal part of vas deferens narrow and winding, expanding to narrow, elongate unarmed penis. Oviduct connects through insemination duct into female gland complex. Vagina long, strait, with small bursa copulatrix, gradually narrows into rounded seminal receptaculum. Long insemination duct emerges from seminal receptaculum and connects into oviduct and female gland mass. Female genital

aperture opens posterior to penis on right side of body, between first and second pairs of dorso-lateral processes.

Ecology: Found subtidally at 7–16 m in depth. Inhabits brown and red algae or rocks covered with hydrozoan colonies (Thecophora). Egg masses are unknown.

Distribution: This far, known from Avacha Bay, Kamchatka peninsula (Ekimova et al., 2015) and from Rudnaya Bay, Sea of Japan (present study).

Remarks: *D. kamchaticus* was described from Avacha Bay (North-West Pacific, Kamchatka peninsula) at the depth 7–10 m (Ekimova et al., 2015). Here for the first time this species out of its type locality is described. External and internal morphology of holotype corresponds to our specimens, with a few exceptions. Type specimens of *D. kamchaticus* are defined by small dorsolateral appendages with short and bulbous primary stalks and small secondary branches and lacking tertiary. Probably it can be related with preserving changes of soft tissues. Radula formula described in Ekimova et al. (2015) is 16–20 × 7–10.1.7–10, while in our specimens it is 17–24 × 9–11.1.9–11. However, it can be explained as intraspecific variations of radular teeth number. Morphology of the rachidian and lateral teeth, jaws and reproductive system is quite similar in type specimens and individuals studied.

It can be concluded that our specimens truly belong to *D. kamchaticus* according to both morphological and molecular analyses. The first finding of this species in the Sea of Japan extends its known distribution from the boreal regions of Northwestern Pacific to the northern part of the Sea of Japan. Supposedly, this species can be also found in Sakhalin, Kuril Islands and in the Okhotsk Sea.

*Dendronotus dudkai* sp.n.

Figs. 9–11.

*Dendronotus frondosus* sensu Baba, 1993 non Ascanius, 1774

Type material: Holotype: ZMMULc-40364, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, coll. A. Chich-

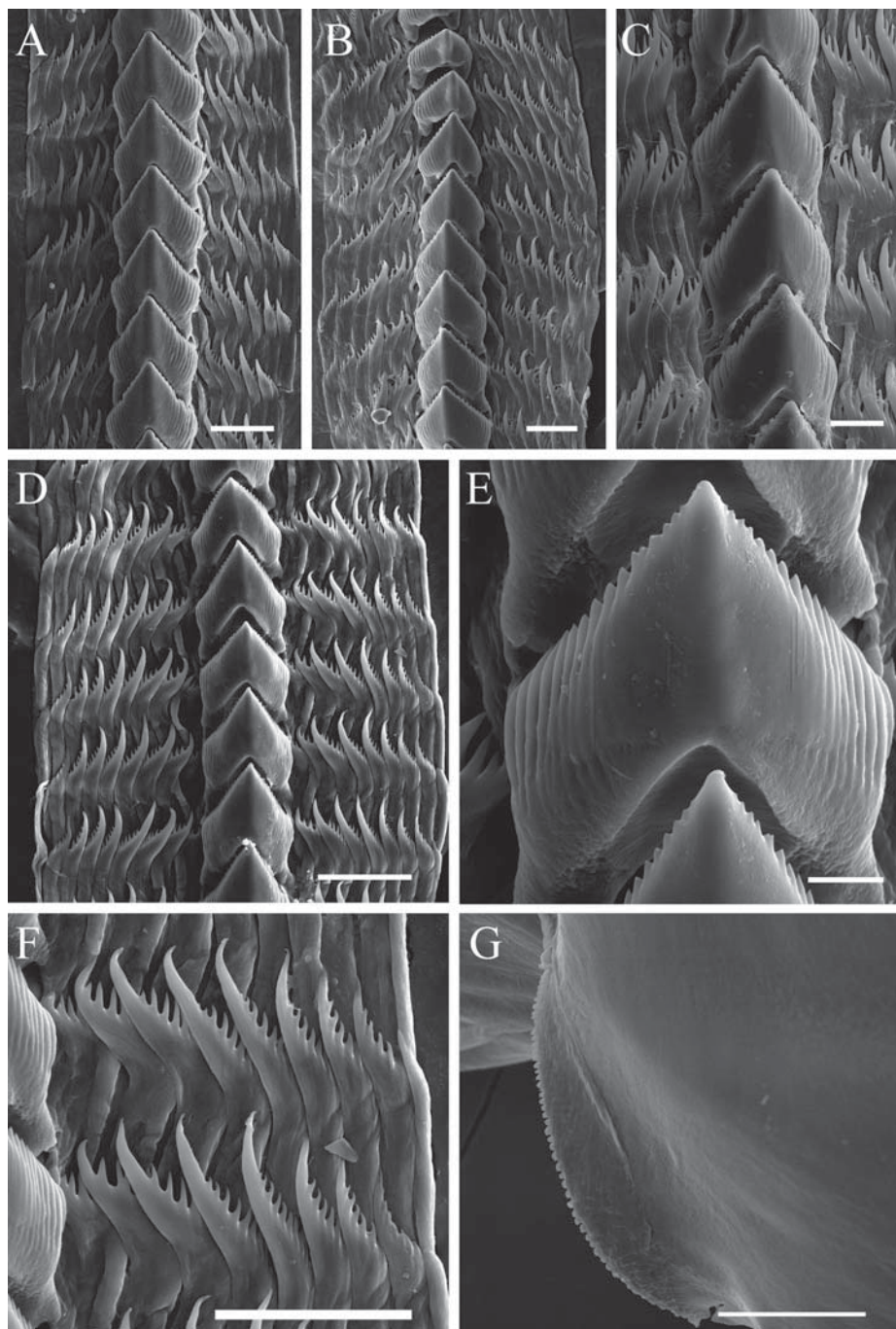


Fig. 10. *Dendronotus dudkai* sp.n., scanning electron micrographs of the radula and masticatory process of jaws. A — W201, rachidian and lateral teeth; B — W203, rachidian and lateral teeth; C — W202, rachidian and innermost lateral teeth, D — W197, rachidian and lateral teeth; E — W197, rachidian tooth; F — W197, lateral teeth; G — masticatory process of jaws, masticatory border with a single row of denticles. Scale bars: A, B, F — 50  $\mu\text{m}$ ; C — 20  $\mu\text{m}$ ; D — 80  $\mu\text{m}$ ; E — 10  $\mu\text{m}$ ; G — 100  $\mu\text{m}$ .

varkhin & A. Goloseyev, 10–15 August 2014. Paratypes: ZMMU Lc-40365, 1 specimen, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, coll. A. Chichvarkhin & A. Goloseyev, 15 August 2014; ZMMU Lc-40366, 1 specimen, Sea of Japan, Amursky Bay, near the Institute of Marine Biology, 2–5 m depth, coll. K. Dudka, 25 May 2014; ZMMU Lc-40367, 2 specimens, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 10–15 m depth, coll. I. Ekimova & A. Chichvarkhin, 10–15 September 2015.

Additional material examined: W195, 1 specimen, Sea of Japan, Rudnaya Bay, 10 m depth, coll. A. Chichvarkhin, 10 June 2012; W196, 1 specimen, Sea of Japan, Rudnaya Bay, 10–12 m depth, coll. A. Chichvarkhin, 8 June 2013; W197–W200, 4 specimens, place, depth, date and collectors are the same as W196; W201, 1 specimen, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, coll. A. Chichvarkhin & A. Goloseyev, 10 August 2014; W202, 2 specimens, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, coll. A. Chichvarkhin & A. Goloseyev, 15 August 2014; W207, 3 specimens, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, coll. K. Dudka, 15 March 2015; WS2894–WS2897, 4 specimens, Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 10–15 m depth, coll. I. Ekimova & A. Chichvarkhin, 10–15 September 2015.

Type locality: Sea of Japan, Vostok Bay, Vostok Marine Biological Station, 6–7 m depth, on *Obelia* cf. *longissima* (Pallas, 1766).

Etymology: Dedicated to our friend and invaluable assistant Mr. Konstantin Dudka, staff diver of Institute of Marine Biology RAS (Vladivostok), one of the first collectors of this species.

Description. External morphology (Figs. 9, 11A): Body elongate, laterally compressed. Foot

narrow, tail short. Oral veil small with 6–12 large, secondary branched cerata. Muscular lips with 5–10 short lip papillae. Rhinophoral sheaths with long stalk and 4–5 crown secondary branched appendages. Lateral papillae moderate in size with small secondary branches. Rhinophores with 8–10 lamellae. 6–8 pairs of highly branched dorsolateral processes, size and degree of branching decrease towards the tail. 2–5 primary stalks on each cerata, branching pattern “rosette” (Fig. 11A). Secondary branches long and rounded, tertiary branches short and sometimes pointed. Digestive gland diverticula penetrate cerata and rhinophoral sheath processes. These diverticula in first pair of cerata and rhinophores originate from anterior lobe of digestive gland; others arise from posterior lobe. Cardiac prominence slightly raised. Body covered with conical papillae that usually tallest on cardiac prominence. Anal opening on right side of body about midway between first and second pair of dorsolateral processes. Reproductive openings lateral, below first pair of dorsolateral processes on right side.

Colour (Fig. 9): General colour pattern varies from beige to dark-brown. Background colour translucent-white or light yellow. A lot of spots, stripes and dots on dorsal side of the body, cerata, rhinophoral sheath processes and upper parts of foot. Their colour varies from yellow to dark-brown. Some specimens covered with dots of golden or white opaque pigment. This pigment locates also in low body papillae and small turbecles. All specimens possess well-visible white stripes between pairs of cerata. Digestive gland diverticula can be seen through transparent body wall. Digestive gland beige or brown. The colour of the rhinophores similar to body colour.

Internal morphology (Figs. 10, 11B–D): Dorsal processes of jaws about 2.5 times shorter

Рис. 10. *Dendronotus dudkai* sp.n., микрофотографии участков радулы и жевательной поверхности челюстей (СЭМ). А — W201, центральные и латеральные зубы; В — W203, центральные и латеральные зубы; С — W202, центральные и внутренние латеральные зубы, D — W197, центральные и латеральные зубы; Е — W197, центральный зуб; F — W197, латеральные зубы; G — жевательный отросток челюстей, жевательная поверхность несет одинарный ряд зубчиков. Масштаб: А, В, F — 50 мкм; С — 20 мкм; D — 80 мкм; Е — 10 мкм; G — 100 мкм.

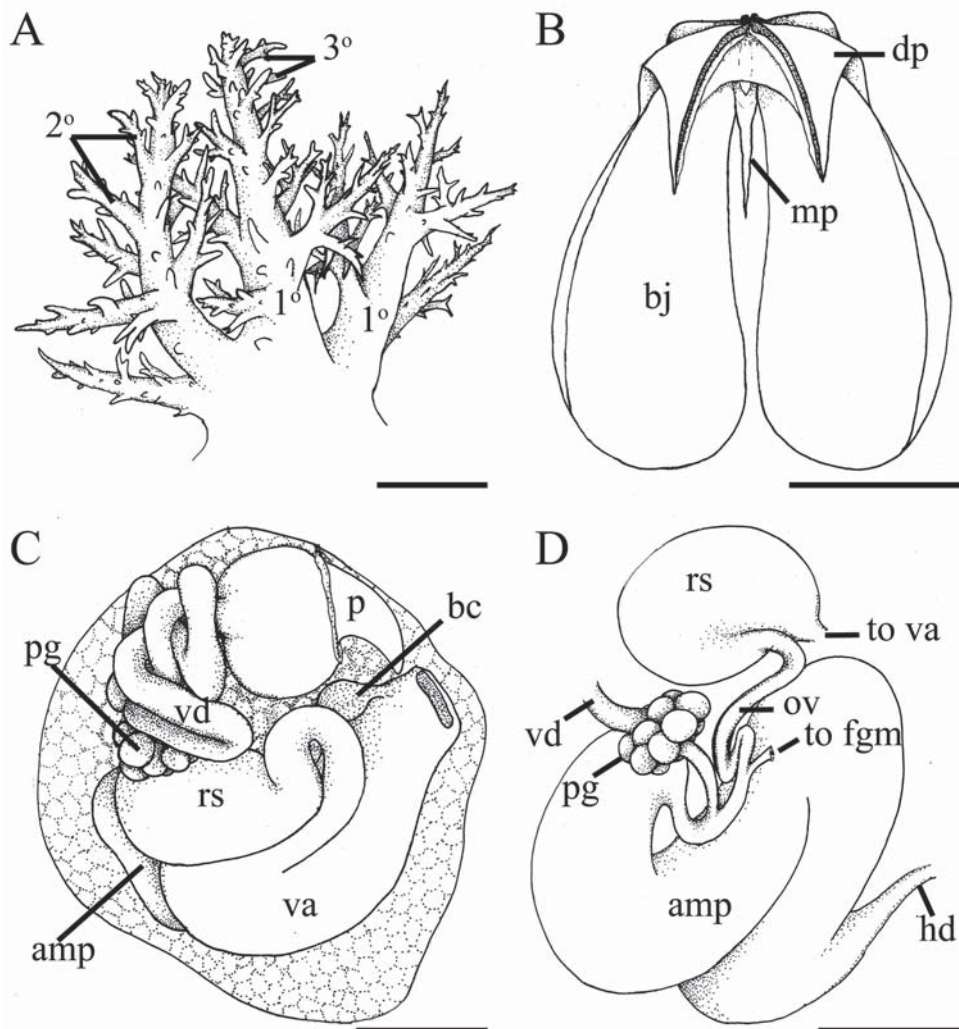


Fig. 11. *Dendronotus dudkai* sp.n., drawings of the external morphology and anatomy. A — dorsolateral appendages branching pattern; B — jaws; C — main view of reproductive system; D — reproductive system, detailed.

Abbreviations: 1° — primary stalks; 2° — secondary branches; 3° — tertiary branches; amp — ampulla; bc — bursa copulatrix; bj — jaw body; dp — dorsal process of the jaw; fgm — female gland mass; hd — hermaphroditic duct; mp — masticatory process of the jaw; ov — oviduct; p — penis; pg — prostata; rs — receptaculum seminis; va — vagina; vd — vas deferens. Scale bars 1 mm.

Рис. 11. *Dendronotus dudkai* sp.n., особенности внешней и внутренней морфологии. А — особенности ветвления дорсолатеральных отростков; В — челюсти; С — общий вид половой системы; D — область овидукта, детализировано.

Обозначения: 1° — первичные ветви; 2° — вторичные веточки; 3° — третичные веточки; amp — ампулла; bc — копулятивная сумка; bj — тело челюстей; dp — дорсальный отросток челюстей; fgm — женские железы; hd — гермафродитный проток; mp — жевательный отросток челюстей; ov — овидукт; p — пенис; pg — простата; rs — семяприемник; va — вагина; vd — семяпровод. Масштаб 1 мм.

than jaw body (Fig. 11B). Inclined posteriorly at about 45°. Masticatory process about one-third as long as jaw body, slightly curved at base and become transparent and subulate posteriorly. Masticatory border with a single row of denticles (Fig. 10G). Radula formula:  $32 \times 7-8.1.7-8$  (W203);  $27 \times 6-7.1.6-7$  (W201),  $29 \times 8-9.1.8-9$  (W196) (Fig. 10A–D). Rachidian tooth strong, triangular, length same as width, bears 12–18 sharp denticles with thin furrows on both sides of the reduced cusp (Fig. 10E). Lateral teeth slightly curved toward midline, bear 4–8 well-defined denticles (Fig. 10F). Outermost lateral teeth form varies from flattened shape to narrow with pointed apex. Innermost lateral teeth thin and highly denticulated. Reproductive system triaulic (Fig. 11C, D). Ototestis large and white, composed from number of rounded lobules, leads to hermaphrodite duct. Ampulla wide and sinuous, merging with oviduct and connecting into prostate. Prostate concentric ring-shaped, consists of 12–14 oval alveolar glands. Distal part of vas deference winding expand into wide, muscular portion. Penis slightly curved, lacking armature. Oviduct connects through insemination duct into female gland complex. Vagina long, convoluted, narrows into rounded seminal receptaculum and then connects into oviduct and female gland mass. Small bursa copulatrix near proximal part of vagina. Female genital aperture opens posterior to penis on right side of body, between first and second pairs of dorsolateral processes.

Ecology: Found subtidally at 2–16 m in depth. Feeds on tecaphoran hydrozoan colonies of the family Campanulariidae (*Obelia* cf. *longissima*). These colonies occupied artificial substrates (ropes) only; we never found them with *Dendronotus* spp. on natural substrates. Reproduction period in June–July. Egg mass is narrow white cord, coiled on hydrozoan colonies.

Distribution: the Sea of Japan: from Rudnaya Bay to Amursky Bay, Sado Isl. Probably has wider distribution.

#### Remarks:

The differences between *D. dudkai* sp.n. and *D. primorjensis* were discussed above. The individuals of *D. dudkai* sp.n. have very similar

internal and external morphology with eastern Pacific species *D. venustus* and amphiboreal *D. frondosus*. Nevertheless, an array of diagnostic differences can be designated in these three species. In contrast with *D. dudkai* sp.n., *D. venustus* possesses unbranched papillae of rhinophoral sheath, very large outgrowth of cardiac prominence, the ampulla is crescent-shaped. *D. frondosus* is distinguished from *D. dudkai* sp.n. by absence of denticles on the masticatory process of jaws, elongated shape of rachidian teeth, flattened shape of outer lateral teeth with no cusp; the ampulla *D. frondosus* is rounded, prostate contains more alveoli (16–40). According to morphological cladistic analysis by Stout *et al.* (2010), morphological differences shown above are sufficient for species delimitation.

Similar colour pattern and morphology is specific also to *D. kamchaticus*, *D. kalikal* as well as some colour variations of *D. lacteus*. Nevertheless these species differ from *D. dudkai* sp.n. in radular morphology. The rachidian tooth in *D. kamchaticus* is smooth, denticles and furrows are absent. *D. kalikal* possesses elongated rachidian tooth bearing tiny denticles along external edge, although the number of denticles is lower while furrows are very fine and nearly invisible. Rachidian tooth in *D. lacteus* possesses large number of small denticles, their furrows are fine, lateral teeth are triangle shaped with small proximal denticles; some individuals are lacking denticles.

The rachidian tooth of *D. dudkai* sp.n. radula possesses well-developed denticles that lengthen with deep furrows; conical cusp is reduced. This pattern of features distinguishes this species from *D. dalli*, *D. kamchaticus*, *D. niveus* (possessing smooth rachidian tooth), *D. robustus*, *D. patricki*, *D. iris* (developed conical cusp), *D. rufus*, *D. lacteus*, *D. kalikal*, *D. albus*, *D. gracilis* (denticle furrows more or less reduced till complete disappearance), *D. subramosus* (sporadic denticles and furrows), *D. albopunctatus* (furrows approach central part of the teeth). Teeth shape in *D. dudkai* sp.n. is well distinguished form *D. noachi* and *D. regius* — spacing between margins in these two species increases toward the cusp. Rachidian tooth in *D.*

*dudkai* sp.n. is similar to *D. venustus*, *D. frondosus*, *D. comteti*, *D. orientalis*. As said above, both *D. frondosus* and *D. venustus* differ from *D. dudkai* sp.n. with the features of internal and external morphology. *D. comteti* possesses elongated rachidian tooth and lower number of lateral ones (4–5). Besides that, this species possesses lower number of dorsolateral appendages (2–4 pairs), with no secondary branching. The number of lateral teeth in *D. orientalis* (2) is a good diagnostic trait to be distinguished from *D. dudkai* sp.n.

## Discussion

As this had been considered earlier, the most common species *D. frondosus* possesses a wide amphiboreal distribution (Robilliard, 1970; Roginskaya, 1987; Baba, 1993). Confirmed southern borders of its distribution range were Cope Cod on the eastern US coast (Lemche, 1948), Californian shore, Bay of Biscay in France, and Sado Island in the Sea of Japan (Baba, 1993). However, in 2010, cladistics and molecular phylogenetic analysis confirmed the validity of *D. venustus* (Stout *et al.*, 2010), a sister species of *D. frondosus* previously considered as its minor synonym. Further in 2015, two new species *D. kalikal* and *D. kamchaticus* were described from the Pacific shore of Kamchatka (Avacha Bay) (Ekimova *et al.*, 2015). Both species possess similar to *D. frondosus* colouration but fundamentally different morphologies of the radula and the reproductive system. Considering these data, an exclusively amphiatlantic distribution is characteristic for ‘amphiboreal’ *D. frondosus* is presumed.

Due to the recent studies on the genus *Dendronotus* taxonomy, populations of the Sea of Japan represent key forms that may shed light on the global distribution range of *D. frondosus*. In this study, it has been shown that at least three *Dendronotus* species possessing similar external morphology to *D. frondosus* occur in north-western part of this sea. These species are characterized with significant differences in radula, jaws, and reproductive system morphology. Molecular phylogenetic analysis of three mark-

ers had also clearly discriminated these species by the means of ABGD analysis and GMYC method. These results are congruent with morphological data, single-gene and concatenated phylogenetic trees.

Discovery of *D. kamchaticus* in Rudnaya Bay of the Sea of Japan extends our knowledge on this species distribution in western Pacific. Two specimens from the Sea of Japan are identical with *D. kamchaticus* types from Kamchatka in either their internal morphology or according to molecular phylogenetic analysis of three gene markers. Nevertheless, the dorsolateral processes branching pattern, one of key species-specific characteristics, differs in both populations. Firstly, this is because different ontogenetic stages were used in the description and this study, as well as cerata morphology may be altered in preserved specimens. New data on *D. kamchaticus* populations may confirm a wider distribution of this species ranged to the north-western Pacific including the Sea of Okhotsk, Commander, Kurile, Sakhalin Islands, northern Japan and Korea.

*D. dudkai* sp.n. had been recorded in three locations of the Sea of Japan: Rudnaya Bay, Vostok Bay and Amursky Bay. All specimens from these locations coincide in their internal and external morphology possessing good discriminative characters from the other species of the genus, as discussed above. Molecular phylogenetic analysis also confirmed discriminative morphological traits. Thus, our results confirm *D. dudkai* sp.n. validity.

Two *D. frondosus* specimens were found in Amursky Bay. Their morphology and molecular markers are identical or quite similar to Atlantic populations of *D. frondosus*. This finding impugns our recent conclusion about exclusively amphiatlantic distribution of this species (Ekimova *et al.*, 2015). Baba (1993) recorded *D. frondosus* from Sado Island. He pointed out the denticles on the masticatory process of the jaw, while this trait is characteristic of *D. dudkai* sp.n., not *D. frondosus* as noted above. Therefore, the presumable distribution area of *D. dudkai* sp.n. includes the entire Sea of Japan, while our finding of *D. frondosus* in Amursky



Bay is the only confirmed record of this species in northwestern Pacific. *D. dudkai* sp.n. may also be distributed outside the waters of the Sea of Japan in south Kuriles and Hokkaido islands. Opisthobranch fauna of Rudnaya Bay, where this species was recorded, has many common species with Kunashir Island and Shiretoko Peninsula due to local currents pattern (Chichvarkhin *et al.*, 2015, 2016).

According to morphological and molecular phylogenetic analyses, *D. frondosus* and *D. dudkai* sp.n. are closely related sister species, although they are clearly distinguished with genetic characters. However, due to our data, it can be supposed that *D. dudkai* sp.n. and *D. frondosus* are sympatric sister species that feed on hydroid cnidarians of the family Campanulariidae. Possessing very consimilar pattern of morphological traits, though being clearly delimited genetically, these species might possess distinct ecological traits. Indeed, immature individuals of *D. frondosus* were found in May when climatic conditions on Amursky Bay were similar to summer temperatures in the boreal and Arctic seas of the Atlantic (e.g. in the White and the Barents seas) while mature individuals of *D. dudkai* sp.n. appear in the end of June when water approaches a higher temperatures of ca. 20 °C. Probably, *D. frondosus* is an amphiboreal species with disjunctive distribution area in Arctic seas while *D. dudkai* sp.n. is a west-Pacific sub-boreal species, and northwestern part of the Sea of Japan is an area of secondary sympatry of both species. This hypothesis may be supported with the fact of recent *D. frondosus* finding being found on the Alaska coast (according to molecular data provided by Ángel Valdés & Tabitha Lindsay, California State Polytechnic University, Pomona, pers. comm.), although the other sister species *D. venustus* is known from the northeastern Pacific coasts that lie to the south of Alaska. The second hypothesis is *D. frondosus* being nevertheless an amphiatlantic species, while it occurs in the Sea of Japan due to anthropogenic invasion being delivered there with ballast waters of ships. This may be explained by single finding of this species in Amursky Bay located in Vladi-

vostok, one of the major international ports in the area.

Due to similarities in external morphological features, the identification of *Dendronotus* species in the North-West part of the Sea of Japan may be a problematic point. The best way to distinguish specimens with a variegated colouration pattern from the Sea of Japan is to use a molecular analysis. However, it is rather expensive, complicated, taking considerable time and therefore not very useful for practical purposes. Therefore, the possibility of the species designation using morphological features becomes more sensible. On the one side, the colouration could be a good diagnostic character for identification of *D. dudkai* sp.n.: all studied specimens possess stripes of white opaque pigment on the dorsal side between the rows of appendages. Therefore, we propose to designate every specimen from the Sea of Japan with similar colouration as *D. dudkai* sp.n. The other good taxonomic characters are the radula morphology and masticatory process denticulation. *D. kamchaticus* could be easily identified by smooth rachidian tooth of radula, while in *D. frondosus* and *D. dudkai* sp.n. the tooth possesses a lot of denticles with deep furrows, which are clearly visible even under a simple microscope. Finally, features of masticatory border can distinguish *D. frondosus* and *D. dudkai* sp.n.: in *D. frondosus* it is smooth, while in *D. dudkai* sp.n. it possess a single row of denticles.

#### ACKNOWLEDGMENTS

We are very grateful to all individuals who helped collect specimens, especially to K. Dudka, T. Antokhina, A. Goloseyev, G.N. Davidovich, A.G. Bogdanov, the staff of the scanning electron microscopic laboratory of the Moscow State University and the staff of Cooperative Far Eastern Center of Electron Microscopy are thanked for providing SEM facilities. I.A. Eki-mova especially thanks A.V. Martynov for useful advices, comments, help and also for giving a deep knowledge on the nudibranch mollusc taxonomy at the beginning of the work. We are grateful to Ángel Valdés and Tabitha Lindsay for advices and providing a new data on *Den-*

*dronotus* composition in the NE Pacific. We specially thank Diego Fontaneto for help with molecular species delimitation analyses. Material collection and SEM studies were supported by the Russian Science Foundation (grant 14-50-00034). Molecular analysis was supported by the Russian Foundation for Basic Research (grant #16-34-00955 mol\_a).

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*Responsible editor E.N. Temereva*