Intersection of historical museum collections and modern systematics: a relict population of the Arctic nudibranch *Dendronotus velifer* G.O. Sars, 1878 in a Swedish fjord

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Abstract

Based on morphological, bathymetric and molecular data comparing recently collected Arctic and North Atlantic specimens with morphological and bathymetric data on historical museum specimens, a unique relict population of the deep-water mollusc *Dendronotus velifer* G.O. Sars, 1878 (Gastropoda: Nudibranchia) is shown to have existed in the deepest section of the Swedish Gullmar Fjord (the only true silled fjord in Sweden) at least until the middle of the 20th century. This population is more than 1500 km away from the nearest point in the species' distributional range in the Arctic Ocean today. Using an integrative approach incorporating the data mentioned above, including genetic distances, from recently collected specimens taken from the Arctic Ocean, *D. velifer* is validated and its species status is restored, for the first time in more than a century after being regarded as a junior synonym of *D. robustus*. The bathymetric data for historical and recently collected specimens of *D. velifer* demonstrate significant differences compared to the shallow-water species *D. robustus*. The findings support the necessity of a stronger protection for the unique marine habitats of the Gullmar Fjord.

Contents

Introduction	303
Materials and methods	304
<i>Historical museum specimens</i>	304
<i>Specimens collected on recent expeditions</i>	306
<i>Morphological analysis</i>	306
<i>Molecular and statistical analyses</i>	306
Results	306
<i>Molecular analysis of Arctic material of Dendronotus robustus and D. velifer</i>	306
<i>Statistical analysis of the bathymetric distribution</i>	309
Discussion	310
<i>Integration of molecular and morphological data</i>	311

<i>Statistical analysis of the bathymetric distribution</i>	313
<i>Taxonomic evaluation</i>	313
<i>Species differentiation, bathymetric distribution and biogeographic range</i>	314
<i>Implications for nature conservation</i>	315
<i>Importance of natural history collections</i>	315
Acknowledgements	316
References	316
Appendix	318
<i>Morphological analysis and redescription of D. velifer</i>	318

Introduction

An accurate taxonomy is indispensable for evaluating the conservation status and biogeographic pattern of species (Bickford et al., 2006; Martynov et al., 2017). Modern molecular analyses are able to reveal considerable inconsistencies within morphology-based systematics and thus may have a direct influence on the conservation status of a species and on the perception of its distributional range. A potentially threatened species cannot be properly understood, or even recognised, without a thorough taxonomic background study. While for large well-known vertebrate animals a taxonomic placement is usually well established today, this is not the same for numerous invertebrates, especially marine ones. An incorrect taxonomy may affect the estimation of the actual number of species in a protected area, and thus may cause an underestimation of the conservation needs of that area.

Here we present a case from an outstanding group of marine invertebrates, the nudibranch molluscs (Gastropoda: Opisthobranchia), and discuss the importance of a correct taxonomic basis for the conservation

perspectives and evaluation of biogeographic patterns within Scandinavian fjords.

Nudibranch molluscs are important model subjects for various disciplines, including phylogenetics, neurophysiology and also the emerging field of marine natural products (Nuzzo et al., 2012; Goodheart et al., 2015; Katz, 2016). They are now routinely included in well-illustrated field guides (Picton and Morrow, 1994; Malmberg and Lundin, 2015; Hayward and Ryland, 2017) and owing to their attractiveness they have recreational importance in national parks and other conservation areas (e.g., Bertsch, 2014; Garcia-Mendéz and Camacho-Garcia, 2016; Mehrotra and Scott, 2016; Nimbs et al., 2016). The species of the nudibranch genus *Dendronotus* occur in the Northern European and Arctic seas, generally on rocky and stony habitats in shallow waters less than 50 m deep (Thompson and Brown, 1984; Thollessen, 1998; Korshunova et al., 2016). Most have relatively slender bodies and they feed on hydroids. One of the most notable exceptions is a species that inhabits soft bottoms at depths greater than 50 m, which has a relatively broad body and an omnivorous diet (Roginskaya, 1987). This species has generally been called *Dendronotus robustus* Verrill, 1870, and has been assessed as having a very broad circumpolar distribution (Ekimova et al., 2015). In the northeast Atlantic Ocean, it occurs from the Vega archipelago in Hordaland on the northwestern Norwegian coast (Evertsen and Bakken, 2005), northwards to the Barents Sea in the Arctic Ocean, and across to the Kara Sea and the White Sea in Russia (Martynov and Korshunova, 2011). On the northwestern side of the Atlantic Ocean it occurs from Cape Cod to Greenland, and to the Bering Strait (Verrill, 1870; G.O. Sars, 1878; Odhner, 1907, 1939; Ekimova et al., 2015). On Arctic latitudes, as in the Barents Sea, it occurs in relatively shallow depths, but in the southernmost parts of its range it occurs at greater depths.

A species that is similar to *D. robustus* apparently had an isolated population in the Gullmar Fjord on the Swedish west coast at least until the early 1940s. This southernmost occurrence has not been appropriately described in the literature. There are no other reports or findings from any other areas in Swedish waters or the British Isles, where the nudibranch fauna is now well known (e.g., Thompson and Brown, 1984; Picton and Morrow, 1994; Malmberg and Lundin, 2015). The nearest present occurrence is on the northwestern Norwegian coast, some 1500 km from of the Gullmar Fjord, except for two historical findings - one specimen from the innermost part of the Oslo Fjord in 1912, and one specimen from the Hardanger Fjord, south of Ber-

gen, collected sometime between 1860 and 1920. Both of these specimens are deposited at the Oslo Museum of Natural History. *Dendronotus robustus* was listed for Denmark (Jensen and Knudsen, 1995), but without a description that could confirm the identification. The isolated population in the Gullmar Fjord was apparently rather abundant if one considers how many specimens collected in the late 1800s were deposited at the Gothenburg Natural History Museum (GNM) and the Swedish Natural History Museum (NRM).

To re-evaluate the taxonomic status of the population in the Gullmar Fjord, we use an integrated analysis applying morphological and molecular methods for both very shallow water populations and the deep-water specimens currently known as “*D. robustus*” from across almost its entire geographic range. We compare the bathymetric distribution of the historical specimens from Gullmar Fjord with both shallow-water and deep-water populations using a statistical test. The reliability of an integrated molecular and morphological approach has recently been proved by the discovery in Norway and Great Britain of a large new nudibranch species within one of the world’s best studied European marine faunas (Korshunova et al., 2017). The resultant conclusions are highly relevant from both biogeographic and conservation perspectives, and important for the ongoing discussion regarding the conservation of the Gullmar Fjord.

In the present study we show that there are two distinct species previously mixed up under the name “*D. robustus*” (e.g. Ekimova et al., 2015). These species are *D. robustus* Verrill, 1870 and *D. velifer* G.O. Sars, 1878. According to our present analysis the Gullmar Fjord accommodates the species *D. velifer* G.O. Sars, 1878, and not the true *D. robustus*. The synonymy of the latter species, plus the designation of a lectotype, and a revised morphological description, are given in the Appendix to this paper.

Materials and methods

Historical museum specimens

A total of 117 specimens of the *Dendronotus robustus* species complex (Table 1) from the Gothenburg Natural History Museum (GNM), the Swedish Museum of Natural History, and the Oslo Natural History Museum (NHM-UIO) were investigated, including a study of both external and internal features. The majority of the specimens had been examined and determined as *Den-*

Figure 1. *Dendronotus velifer*, two historical specimens from the Gullmar Fjord. a–c: habit of specimen 26 mm in length (GNM 3026); f–h: habit of specimen 13.5 mm in length, dorsal, ventral and lateral views respectively (GNM 3027); d–e: radula of specimen GNM 3026; i–k: radula of specimen GNM 3027, central and lateral teeth respectively; l, jaw; n, o: lectotype, Barents Sea, Vadsø, NHM-UIO nr. 16174. m: genital openings and copulative apparatus. Scale bars: d, i: 100 μ m; e, j, k: 30 μ m; n, o: 5 mm. Photographs by Tatiana Korshunova (a–h, m) and Karsten Sund (n, o).

dronotus robustus by the nudibranch specialist Nils Odhner between the 1910s to the 1930s. Five of the specimens from the Gothenburg Natural History Museum were sent as loan to the Zoological Museum in Moscow for morphological investigation. Two specimens (GNM 3026 and 3027) were dissected and SEM-studies of their radula were performed (Figure 1, Table 1). Additionally, AM checked all previously identified as *D. robustus* specimens while he visited GNM in August 2017.

Specimens collected on recent expeditions

A total of 15 specimens of the *Dendronotus robustus* species complex were collected alive during marine biological expeditions in the Barents, Kara, and Laptev seas (Table 2; Figs. 2, 3) in this study and earlier. These specimens were all deposited in the Zoological Museum of Moscow State University (ZMMU, Op-296, Op-343, Op-344, Op-348, Op-390, Op-391, Op-392, Op-393, Op-546, Op-547).

Morphological analysis

The external and internal morphology was studied under a stereomicroscope and using a full-frame digital camera (Nikon D-810). The buccal mass of each specimen was extracted and processed in 10% sodium hypochlorite solution to extract the radula and the jaws. The jaws of each species were analysed under a stereomicroscope and then photographed. The radulae were coated and examined and photographed using a scanning electron microscope (CamScan). The reproductive systems were also examined using the stereomicroscope.

Molecular and statistical analyses

All 15 specimens of the *Dendronotus robustus* species complex from the Barents, Kara, and Laptev seas were sequenced in this study and earlier for the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S, and sequences of one specimen were obtained from GenBank (see Table 2 for list of samples, localities, and voucher references). No historical museum specimens were able to be sequenced because their extracted DNA was in a degraded state.

Small pieces of foot tissue were used for DNA extraction with Diatom™ DNA Prep 100 kit by Isogene Lab, according to the manufacturer's protocols. Extracted DNA was used as a template for the amplification of partial sequences of the COI and 16S. The primers that were used for amplification were LCO1490

(GGTCAACAAATCATAAAGATATTGG (Folmer et al., 1994)); HCO2198 (TAAACTTCAGGGTGAC-CAAAAAATCA (Folmer et al., 1994)); 16SarL (CGC-CTGTTTAACAAAAACAT (Palumbi et al., 2002)); 16SR (CCGRTYTGAAGCTCAGCTCAG (Puslednik and Serb 2008). Polymerase chain reaction (PCR) amplifications were carried out in a 20 µL reaction volume, which included 4 µL of 5x Screen Mix (Eurogen Lab), 0.5 µL of each primer (10 µM stock), 1 µL of genomic DNA, and 14 µL of sterile water. The amplification of COI was performed with an initial denaturation for 1 min at 95°C, followed by 35 cycles of 15 sec at 95°C (denaturation), 15 sec at 45°C (annealing temperature), and 30 sec 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 sec at 95°C (denaturation), 15 sec at 52°C (annealing temperature), and 30 sec at 72°C, with a final extension of 7 min at 72°C. Sequencing for both strands proceeded with the ABI PRISM® BigDye™ Terminator v. 3.1. Sequencing reactions were analysed using an Applied Biosystems 3730 DNA Analyzer. Protein-coding sequences were translated into amino acids for confirmation of the alignment. All sequences were deposited in GenBank (Table 2).

Original data and publicly available sequences were aligned with the MUSCLE algorithm (Edgar 2004). Separate analyses were conducted for the data sets; the resulting alignments being 658 bp for COI, 443 bp for 16S. The program Mega7 (Kumar et al., 2016) was used to calculate the uncorrected p-distances between all the sequences and distances within and between groups.

To evaluate the genetic distribution of the different haplotypes the haplotype network for both the COI and for 16S genes were reconstructed using the Population Analysis with Reticulate Trees (PopART, <http://popart.otago.ac.nz>) with the TCS network method. Usefulness of the haplotype network analysis for the delineation of the nudibranch species have been demonstrated recently (e.g. Padula et al., 2014; Furfaro et al., 2016) Bathymetric data were evaluated statistically using nonparametric Mann-Whitney rank sum tests.

Results

Molecular analysis of Arctic material of Dendronotus robustus and Dendronotus velifer

The present molecular analysis shows that there are two distinct species under the name *Dendronotus robustus*,

Table 1. Historical specimens of *Dendronotus velifer* G.O. Sars, 1878 from Swedish and Norwegian museums.

Museum	Museum registration number	Number of specimens	Locality	Date	Depth, m	Collector	Previous identification and name of identifier
Gothenburg Natural History Museum	Gastropoda 3024	11	Skår, Gullmar Fjord	1887-07-01	100–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3025	8	Skår, Gullmar Fjord	1887-07-22	100–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3026, dissected	1	Alsback, Gullmar Fjord	1887-07-17	100–118	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3027, dissected	1	Alsback, Gullmar Fjord	1887-07-11	100–118	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3028	2	Finsbo udde, Gullmar Fjord	1889-07-18	–	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3029	6	Finsbo udde, Essevik, Gullmar Fjord	1887-06-22	–	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3030	7	Skåreskär, Gullmar Fjord	1887-06-23	100–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3031	3	Skåreskär, Gullmar Fjord	1887-06-28	90–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3032	4	Skåreskär, Gullmar Fjord	1887-06-29	90–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3033	1	Skåreskär, Gullmar Fjord	1887-06-30	90–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3034	1	Gullmar Fjord	1887-07-28	–	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3035	8	Gullmars-viken, Gullmar Fjord	1889-08-01	35–90	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3036	2	Darns-huvud, Gullmar Fjord	1889-07-11	60–85	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3037	1	Hälleback, Gullmar Fjord	1889-07-08	60–110	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3652	2	Skår, Gullmar Fjord	1887-07-01	100–116	A. Stuxberg	<i>D. robustus</i> by N. Odhner
	Gastropoda 3038	6	Kristine-berg, Gullmar Fjord	September – October 1905	–	L.A. Jägerskiöld	<i>D. robustus</i> by N. Odhner
Swedish Museum of Natural History	Gastropoda 3652	3	Skår, Gullmar Fjord	1917-07-01	90–116	R. Wahrberg	<i>D. velifer</i> by K. Lundin April 2017
	198	4	Saltkälle-fjorden, innermost section of the Gullmar Fjord	August 1894	–	–	<i>D. robustus</i> by N. Odhner

Museum	Museum registration number	Number of specimens	Locality	Date	Depth, m	Collector	Previous identification and name of identifier
	199	1	Gullmar Fjord	Not stated, late 1800s	–	–	<i>D. robustus</i> by N. Odhner
	200	4	Gullmar Fjord	1893, 1896	–	–	<i>D. robustus</i> by N. Odhner
	201	1	Flatholmen, Gullmar Fjord	August 1891	–	O. Carlgren	<i>D. robustus</i> by N. Odhner
	202	9	Skår, Gullmar Fjord	1895		N. Odhner	<i>D. robustus</i> by N. Odhner
	8072 (1116)	1	Gullmar Fjord	1908-08-17	–	N. Odhner	<i>D. robustus</i> by N. Odhner
	8682 (1435)	1	Kristine-berg marine biological station, Gullmar Fjord	July 1920	–	N. Odhner	<i>D. robustus</i> by N. Odhner
	8968 (1455)	1	Kristine-berg Marine Biological Station, Gullmar Fjord	July 1913	–	N. Odhner	<i>D. robustus</i> by N. Odhner
	10595 (1813)	1	Saltkälle-fjorden innermost part of the Gullmar Fjord	1941	53	S. Bock	<i>D. robustus</i> by N. Odhner
	10599 (1817)	1	Smör-kullen, Gullmar Fjord	1941	40–71	S. Bock	<i>D. robustus</i> by N. Odhner
	10600 (1818)	1	Smör-kullen, Gullmar Fjord	1941	40–71	S. Bock	<i>D. robustus</i> by N. Odhner
	10676 (1854)	1	Smör-kullen, Gullmar Fjord	1941	40–71	S. Bock	<i>D. robustus</i> by N. Odhner
Oslo Natural History Museum	16174	10	Vadsø	–	70-180	G.O. Sars	G.O. Sars original identification <i>D. robustus</i> by N. Odhner
	16175	2	Tromsø	–	–	M. Sars	M. Sars original identification <i>D. robustus</i> by N. Odhner
	16176	1	Sunde, Hardanger Fjord	–	–	G.O. Sars	G.O. Sars original identification <i>D. robustus</i> by N. Odhner
	16177	1	Oslo Fjord, Bunde-fjord	winter-spring 1912	–	G.O. Sars	G.O. Sars original identification <i>D. robustus</i> by N. Odhner

corresponding to *D. robustus* Verrill, 1870 and *D. velifer* G.O. Sars, 1878. Of the sampled specimens, 11 belonged to the former species and five to the latter. Minimal uncorrected p-distances for the COI gene are significantly different between both species, with a

range of $8.74\% \pm 1.24\%$. Distances within *D. robustus* group are within 0.71%. Distances within *D. velifer* group are up to 0.54%, but distances between *D. robustus* and *D. velifer* groups reach 9.34%. Results obtained by PopART both for the COI and for 16S genes showed

Figure 2. Recently collected *Dendronotus velifer* specimens from the Arctic, which were utilised for the molecular study. a–b: specimen 49 mm in length (preserved), Laptev Sea (ZMMU Op-546), dorsal and lateral views; h–j: specimen 23 mm in length preserved, Kara Sea (ZMMU Op-348), dorsal, lateral and ventral views respectively. c–g: internal characters of the specimen in a–b: radula, central and lateral teeth, and lateral teeth only respectively; n: jaw 9.6 mm in length; k–m: internal characters of the specimen in h–j: radula, central and lateral teeth and lateral teeth only, respectively. Scale bars: c, d, e, g, k: 100 μ m; f: 50 μ m; l: 20 μ m; m: 10 μ m. Photographs of specimens (a, b, h–j) by O. Zimina.

a network of haplotypes that clearly clustered into two groups coincident with the species *D. robustus* and *D. velifer* (Figure 4a, b).

Statistical analysis of the bathymetric distribution

The comparison of the bathymetric distribution of specimens of *Dendronotus robustus* and *D. velifer* which were involved in the molecular taxonomic anal-

ysis (see above) reveal significant statistical differences ($p=0.002$, Figure 4c). The bathymetric data for historical specimens of *D. velifer* and for recently collected *D. robustus* demonstrate statistically significant differences ($p < 0.001$, Figure 4c; right lilac and left blue bars). At the same time, the bathymetric data between historical specimens of *D. velifer* and the recently collected specimens of *D. velifer* do not show any significant differences (Figure 4c).

Figure 3. *Dendronotus robustus* specimens from the Arctic, which were utilised for the molecular study. a, b: habit of living *D. robustus* 35 mm in length, Barents Sea, dorsal and lateral views respectively (ZMMU Op-343); d, e: habit of living *D. robustus* 19 mm in length (ZMMU Op-344), Barents Sea, dorsal and ventral views, respectively; c: internal characters of Arctic *D. robustus* specimen ZMMU Op-343: radula of specimen ZMMU Op-344, central and lateral teeth; f–g: radula of specimen ZMMU Op-344, central and lateral teeth respectively; h: jaw of specimen ZMMU Op-343. Scale bars: c: 100 μm ; f, g: 30 μm . Photographs by T. Korshunova.

Discussion

Dendronotus robustus is often considered a circumpolar and deep-water species (Robilliard, 1970; Ekimova et al., 2015). However, based on the combined distribution of all specimens it has supposedly an extremely broad range encompassing the whole Arctic fringe of

Eurasia and North America. This distributional hypothesis has never been tested using modern molecular approaches. Remarkably, the type locality of *Dendronotus robustus* is at an intertidal location at Grand Manan Island in the Canadian Eastern Atlantic (Verrill, 1870), whereas most specimens of supposed *D. robustus* come from considerably deeper waters, usually greater than

Table 2. List of recently collected specimens of *D. velifer* (Figure 2a–e) and of *D. robustus* (Figure 2j–m) which were utilised for the molecular study.

Species	Registration	Locality	Depth, m	GenBank accession no.	
				COI	16S
<i>D. robustus</i>	ZMMU Op-343	Russia: Barents Sea	5–10	KM397002	KM397084
<i>D. robustus</i>	ZMMU Op-344	Russia: Barents Sea	5–10	KM397003	KM397085
<i>D. robustus</i>	ZMMU Op-390-1	Russia: Barents Sea	13	KM396963	KM397045
<i>D. robustus</i>	ZMMU Op-390-2	Russia: Barents Sea	13	KM396964	KM397046
<i>D. robustus</i>	ZMMU Op-390-3	Russia: Barents Sea	13	KM396965	KM397047
<i>D. robustus</i>	ZMMU Op-390-4	Russia: Barents Sea	13	KM396966	KM397048
<i>D. robustus</i>	ZMMU Op-390-5	Russia: Barents Sea	13	KM396968	KM397051
<i>D. robustus</i>	ZMMU Op-391	Russia: Barents Sea	18	KM396970	KM397053
<i>D. robustus</i>	ZMMU Op-392-1	Russia: Barents Sea	19	KM396967	KM397049
<i>D. robustus</i>	ZMMU Op-392-2	Russia: Barents Sea	19	KY996408*	KY996404*
<i>D. robustus</i>	ZMMU Op-393	Russia: Barents Sea	17	KM396969	KM397052
<i>D. velifer</i>	ZMMU Op-546	Russia: Laptev Sea	43	KY996409*	KY996405*
<i>D. velifer</i>	ZMMU Op-547	Russia: Barents Sea	308–321	KY996410*	KY996406*
<i>D. velifer</i>	ZMMU Op-348	Russia: Kara Sea	21	MF685027*	KY996407*
<i>D. velifer</i>	ZMMU Op-296	Russia: Barents Sea	135–137	KC660038	-
<i>D. velifer</i>	NF11	Canada: Newfoundland	231–212	KU695599	-

* - sequenced for this study.

100 m. In spite of such obvious ecological contradictions, to assess a correct taxonomic name for the Swedish Gullmar Fjord population, we investigated both very shallow water populations previously identified as *D. robustus* from the Barents Sea and compared them with the deep-water specimens from almost the whole geographic range from North American Eastern Atlantic waters to the Arctic Laptev Sea.

Integration of molecular and morphological data

We discovered significant molecular differences between shallow-water and deep-water specimens of putative *D. robustus*. These molecular differences agree with the disparity in the colour features in the first description of true shallow-water *D. robustus* from the intertidal (Verrill, 1870) and the deep-water specimens. Verrill (1870: 406) described the colour of *D. robustus* as “pale greyish, thickly sprinkled with small yellow spots”, whereas the colour of the deep-

water Norwegian specimens, previously described by G.O. Sars (1878: 315) from 109–183 m under the name of *Dendronotus velifer* G.O. Sars, 1878, is a “bright reddish with varied white spots” (“*Color late rufescens, punctis albidis variegates*”).

As shown in the present study, such molecular and morphological differences between shallow-water and deep-water specimens persist over a very large biogeographic range, from the American Eastern Atlantic to the Arctic Laptev Sea. Significantly, a molecular and morphological data set has recently become available for a deep-water specimen from off Newfoundland (Valdés et al., 2017). This specimen matches, both its external features (i.e. rather bright and uniform reddish colour) and its molecular data, with our deep-water specimens from about 100 m and deeper from the Barents, Kara and Laptev seas. It does not match our shallow water Barents Sea specimens, which represent a different species with more greyish to brownish-yellowish colour. Although these differences are

Figure 4. The haplotype networks (a–b) based on COI (a) and 16S (b) molecular data showing genetic mutations occurring within *Dendronotus* species. (c) Statistical test of the reliability of the bathymetric distribution patterns of *D. robustus* (blue bar) and *D. velifer* (red and lilac bars): comparison of the specimens involved in a molecular study (blue and red bars) with historical specimens (lilac bar) of *D. velifer* from Swedish and Norwegian museums. ** $p < 0.01$, *** $p < 0.001$. See text for details.

somewhat subtle, the greyish colour mentioned in the first description of true *D. robustus* from the Canadian Atlantic fits with the shallow-water species from the Barents Sea (Figure 3a, b, d, e), and not with the more uniformly red colour of the deep sea *D. velifer* from the various Arctic seas, including the Laptev and Kara seas (Figure 2a, b, h–j).

Therefore, both the morphological and the molecular data clearly suggest that two species are actually confused under the name *D. robustus* in the North Atlantic Ocean – one lives in shallow water from the intertidal to approximately 20 m depth and the other lives deeper, at depths of 50–300 m, most commonly at 80–150 m. The integrative, molecular and morphological data unambiguously suggest that the shallow-water and the deep-water specimens belong to different species, and therefore we hereby restore the name *Dendronotus velifer* G.O. Sars, 1878 for the deep-water species and retain *D. robustus* for the shallow-water species. Both species share a similar body shape and a soft-bottom habitat, usually with an almost omnivorous diet (including cnidarians, which is typical of other dendronotoids, but also polychaetes and even sunken terrestrial beetles; see Roginskaya, 1990).

Statistical analysis of the bathymetric distribution

Based on robust molecular data to distinguish between the shallow and deep species from other parts of the North Atlantic Ocean (Figure 4a, b), the historical specimens from Swedish Gullmar Fjord most probably belong to the deep-water species *D. velifer* and not to the shallow water *D. robustus*. This is because most of the specimens from the Gullmar Fjord were found in depths of 100–118 m, and never shallower than 53 m. This agrees with the general upper bathymetric limit for *D. velifer* at 40–60 m. At some localities in the Arctic seas where the layer of cold water can occur close to the surface, e.g. in the Kara Sea and the White Sea, *D. velifer* can be found in shallow depths, around 15–20 m, thus overlapping with the potential range of *D. robustus*. However, it is important to note that the true *D. robustus* was never found in Kara and White seas, except for the subarctic Barents Sea, which is influenced by the Gulfstream. Furthermore, in the Barents Sea *D. robustus* apparently never goes down to the shallowest depth (43 m) reported here for *D. velifer*. Thus, despite the possibility that the lower bathymetric limit of *D. robustus* might hypothetically overlap with the upper bathymetric limit of *D. velifer* at a general scale, these two species are unlikely to occur syntopically. Also,

for the correct evaluation of the taxonomic placement of the Gullmar Fjord *Dendronotus* specimens, it is crucial that despite specimens of *D. velifer* (having general preference for deeper waters) in some localities are able to appear much shallower (for example following by low temperatures in shallow waters in many Arctic regions, e.g. in the Kara and White seas), *D. robustus* has never been recorded at depths greater than 20 m. Thus, along with morphological arguments, the results of the statistical analysis of the bathymetric distribution (Figure 4c) strongly support the hypothesis that it was *D. velifer* that occurred in the Gullmar Fjord and the southern Norwegian localities.

Taxonomic evaluation

Further support for the hypothesis that the Swedish deep-water specimens belong to *D. velifer* is their reddish colour (Odhner, 1907: 19). However, in the same publication Odhner (1907) listed *D. velifer* as a synonym of *D. robustus*, without any specific discussion on the issue, probably due to the similarity of the broad body in both species. But Odhner evidently omitted noting the differences in colour (reddish versus greyish-yellowish colour), and importantly also the considerable differences in bathymetric distribution (100–300 m versus intertidal), and hence, in ecological patterns. Previously, Bergh (1900) had also uncritically synonymised *D. robustus* and *D. velifer* under the former name. Furthermore, Odhner subsequently reported *D. velifer*, mostly from some deep Norwegian fjords (e.g. Sandnessjøen, Nordland fylke, June 1938, coll. O. Björlykke, 200 m, see Odhner 1939, also Odhner, 1922, 1926), under the name *D. robustus*. Thus, it was accepted by most of the subsequent researchers and incorporated in regional lists and identification keys (e.g. Roginskaya, 1987; Martynov and Korshunova, 2011; Ekimova et al., 2015). Apart from the differences in coloration (which compared to some other *Dendronotus* species actually appear to be more stable in this species pair, but still may represent some difficulties for identification in the field) and considerable differences in the molecular data (Figure 4a, b), *D. robustus* and *D. velifer* can also be distinguished by radular features (Appendix; Figs. 1–3). In particular, specimens of *D. robustus* prove to have higher central (rachidian) teeth (Figure 3c, f) and considerably more denticulated lateral teeth, especially in smaller specimens (Figure 2c, f, k). *Dendronotus velifer* in contrast possesses lower central teeth and less denticulated lateral teeth. The lateral teeth in larger specimens of *D.*

velifer are almost smooth with only traces of reduced denticles on some of them (Figure 1e), whereas smaller specimens of *D. velifer* may possess more denticulated teeth, which are considerably less denticulated (Figure 1j, k) than in *D. robustus* of comparable size (Figure 3g). These data on the Swedish Gullmar specimens are remarkably consistent with the original description of *D. velifer* by G.O. Sars (1878: 315), wherein he mentions of the lateral teeth: “*uncini utrinque 15, laeves, vel vestigium modo indistinctum denticulorum hic et illic exhibentes*” (= 15 lateral teeth, smooth with indistinct rudimentary denticles on the lateral teeth).

Dendronotus velifer also appears to have fewer dorsolateral appendages than *D. robustus*: even in quite a large specimen from the Laptev Sea (49 mm) there are only five pairs of dorsolateral appendages (Figure 2a, b), and likewise in specimens from the Gullmar Fjord, including a small specimen of 13.5 mm length (Figure 1a–h). In contrast, even in a subadult (19 mm) specimen of *D. robustus* (Figure 3d, e) from the Barents Sea, there are six dorsolateral processes. In the larger (35 mm) *D. robustus* from the Barents Sea, the number of dorsolateral appendages is seven. These data partly agree with the original descriptions of *D. robustus* and *D. velifer*: Verrill (1870) indicated six pairs of dorsolateral appendages in a 50 mm long specimen of *D. robustus*, whereas G.O. Sars mentioned five to six dorsolateral appendages for several specimens with a maximum length of 90 mm. According to these and our data, it is likely that *D. velifer* usually has no more than five dorsolateral appendages compared to the usual six appendages of *D. robustus*, even found in small specimens. As an exception, large specimens of *D. velifer* may have an additional sixth appendage, whereas *D. robustus* with half the size of the largest *D. velifer* possess up to at least seven pairs. Furthermore, the frontal digitate appendages (processes) on the oral veil are relatively much longer in *D. robustus* (Figure 3a–d) than in *D. velifer* (Figure 2a, b, h–j). Such relative differences, after additional testing, may prove to be reliable morphological features to distinguish *D. velifer* and *D. robustus*.

Dendronotus velifer differs from the three other congeneric species that can occur in the same geographic area (i.e. *D. frondosus* (Ascanius, 1774), *D. lacteus* (W. Thompson, 1840), and *D. niveus* Ekimova et al., 2015) by its wedge-shaped body, which is considerably expanded in comparison to the more slender body that is only slightly expanded anteriorly in the other three species. Furthermore, *D. velifer* has a considerably broader oral veil and it lacks lateral papillae

on the rhinophoral sheaths, compared to the three congeners. Internally these three species also differ from *D. velifer* by their radular characters. *Dendronotus velifer* differs from a tropical Pacific Ocean species with a similarly wide anterior body, *D. patricki* Stout et al., 2011, by its reddish colour with scattered white dots and stripes (*D. patricki* is uniformly pinkish to reddish brown, without any white spots on the dorsum, except for the tips of the appendages) and *D. velifer* also differs from *D. patricki* by the less protracted cusp of the central tooth, more numerous lateral teeth per row, and by the presence of the reduced denticles on the lateral teeth (Figure 1e, j, k). Along the Swedish western coast *D. lacteus* and *D. frondosus* are common in the subtidal zone from the surface down to 30 m depth. None of these species have been confidently recorded from the deep section of the Gullmar Fjord, although the known bathymetric preferences of *D. lacteus* and *D. niveus* potentially allow these species to inhabit the deeper parts of the Gullmar Fjord. Should these species be found sympatrically with *D. velifer* in the Gullmar Fjord, it will be easy to distinguish them by body shape, colour and the radula. So far, true *D. robustus* has never been positively reported from the shallow areas of Norway or Sweden. Material from the Norwegian Trondheim Fjord, previously identified as *D. robustus* by Friele and Grieg (1901), was examined by Odhner and revised as *D. frondosus*.

Species differentiation, bathymetric distribution and biogeographic range

As shown above, the molecular analysis confirms that *D. robustus* and *D. velifer* are separate species, and that they are clearly differentiated by morphology. The bathymetric analysis also shows a clear separation in depth preference. The historical specimens from Gullmar Fjord have a morphology that is consistent with the recently collected Arctic *D. velifer* specimens and they show a clear preference to the greater depths around 100 m. Thus, these specimens can be confidently identified as *D. velifer*. A further finding relevant for this case, is the recent report of a single specimen identified as *D. “robustus”* collected from around 200 m depth off the North Atlantic coast of North America (Valdés et al., 2017) that fully agrees with *D. velifer* according to our molecular analysis. Therefore, it is possible that *D. velifer* might occur in more southward locations like the Gullmar Fjord if temperature and other ecological conditions permit. The isolated population of *D. velifer* in the Gullmar

Fjord could therefore possibly have been a relict population from an earlier glacial period with colder water. At that time the species could have had a more southerly distribution.

The maximum depth of the Gullmar Fjord is about 118 m and many of the *D. velifer* specimens were taken from the deepest section of the fjord. The deeper parts of the fjord (90–118 m), inside the sill, have fully saline water and naturally stable conditions (Svansson, 1975; Filipsson et al., 2005). In the present study it is statistically proven (Figure 4c) that *D. velifer* differs significantly from *D. robustus* by its preference for greater depths and hence for lower seawater temperatures. Therefore, it can be supposed that small, statistically insignificant differences in the depth preferences between the Arctic specimens of *D. velifer* (Figure 4c) and the specimens from the Gullmar Fjord (Figure 4c) are due only to the depth limit of the Fjord. If the Gullmar Fjord were deeper, *D. velifer* would extend to depths of at least 200 m, and beyond. Two historical findings of single specimens in the fjords of Norway – the Oslo Fjord and the Hardanger Fjord – provide a link from the Gullmar Fjord in Sweden to the northwestern Norwegian coast, and further to the main range of *D. velifer* through almost all of the Arctic from the Barents to the Laptev Sea and further, to the Bering Strait.

Implications for nature conservation

The Gullmar Fjord has been impacted by bottom trawling and other disturbances since the 1900s. At about the midpoint of the fjord, close to the bay of Skår, one of Sweden's busiest ferry services has been running since the late 1960s, operating the largest car-driven car ferries in the country. During the day these ferries ply back and forth across the fjord every 20 min. This causes constant turbulence of the bottom sediments on the slopes and, consequently has at least locally, a considerable impact on the fjord's ecosystem. This impact, together with bottom trawling for shrimps in the habitat for *D. velifer* might have caused the decline and local extinction of the species. Furthermore, at some periods during later part of the 1970s and early 1980s there were some periods of low oxygen levels in the deep trench of the fjord, which must have added stress to the organisms living there. The collections of *D. velifer* from the 1940s were in the innermost and relatively shallow sections of the fjord, in which the bottom environment could have been comparatively less affected, at least for some time, and be more stable. It is unknown if such a stability still exists in places, which

may imply that a population of *D. velifer* could still be present. This would then be in parallel with that of the long-absent aeolid nudibranch *Flabellina borealis* (Odhner, 1922), which was recently rediscovered in the fjord (Lundin and Malmberg, 2016).

The two historical records of single specimens of *D. velifer* in the Oslo Fjord in southeastern Norway and in the Hardanger Fjord on the southwest may represent a link from the Gullmar Fjord in Sweden to the northwestern Norwegian coast. The Bunnfiorden area has a threshold at 60 m, and an inner deep trench that goes down to 150 m. It is adjacent to parts of the city of Oslo and its southeastern suburbs. The water in the deep trench is of poor quality, hence we can hardly expect any *D. velifer* to have survived here since the early 1900s. The locality at Sunde, in the Hardanger Fjord on the southwestern coast of Norway is close to the mouth of the fjord, and thus it has better environmental conditions.

Although we cannot report living specimens of *Dendronotus velifer* from the Gullmar Fjord now, this study is the first that summarises all findings of this species and the first that uses morphological, molecular and ecological evidence to assess the taxonomic position of the predominantly Arctic *D. velifer*. Such an integrative assessment is important for conservation since there is an ongoing discussion on establishing a national marine park within the Gullmar Fjord. The relatively recent record of *D. velifer* in the Gullmar Fjord would certainly strengthen the arguments in favour of a marine park to protect the whole area.

Importance of natural history collections

The present study highlights the necessity of close linkages between historical museum specimens and actual biological patterns and processes including refinement of the taxonomic placement and biogeographic ranges. Therefore, it further contributes to a general recognition of taxonomy as a central biological discipline with high integrative potential for multi-purpose applications. This is not the first case in which a study of historical specimens in museum collections can be applied to retrieve past species records and to reconstruct historical base lines regarding the distribution ranges of species, which can produce relevant information for the conservation of these species and their habitats (e.g. Shaffer et al., 1998; Pyke and Ehrlich, 2010; Rainbow, 2009; Drew, 2011; Ellis et al., 2011; Hoeksema et al., 2011; Lips, 2011; Hoeksema, 2015). The historical records of the present study should stimulate researchers to survey Gullmar Fjord

and other localities for the present occurrence of *D. velifer* and for additional rarely encountered species that may be locally threatened.

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References

- Bergh R. 1900. Nudibranchiate Gasteropoda. *The Danish In-golf-Expedition, Copenhagen* 2: 1–49.
- Bertsch H. 2014. Biodiversity in La Reserva de la Biósfera Bahía de los Ángeles y Canales de Ballenas y Salsipuedes: Naming of a new genus, range extensions and new records, and species list of Heterobranchia (Mollusca: Gastropoda), with comments on biodiversity conservation within Marine Reserves. *The Festivus* 46: 158–177.
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL., Meier R, Winker K, Ingram KK, Das I. 2006. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22: 148–155.
- Drew J. 2011. The role of natural history institutions and bioinformatics in conservation biology. *Conservation Biology* 25: 1250–1252.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nuclear Acid Research* 32: 1792–1797.
- 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.
- Ellis SL, Incze LS, Lawton P, Ojaveer H, MacKenzie BR, et al. 2011. Four regional marine biodiversity studies: approaches and contributions to ecosystem-based management. *PLOS ONE* 6: e18997.
- Evertsen J, Bakken T. 2005. Nudibranch diversity (Heterobranchia, Gastropoda) along the coast of Norway. *Fauna Norvegica* 25: 1–52.
- Filipsson HL, Björk G, Harlandb R, McQuoidc MR, Nordberg K. 2005. A major change in the phytoplankton of a Swedish sill fjord – a consequence of engineering work? *Estuarine, Coastal and Shelf Science* 63: 551–560.
- Friele H, Grieg JA. 1901. Mollusca III. *Den Norske Nordhavsexpedition 1876-1878* 28: 1–131.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology Biotechnology* 3: 294–299.
- Furfaro G, Picton B, Martynov A, Mariottini P. 2016. *Diaphorodoris alba* Portmann & Sandmeier, 1960 is a valid species: molecular and morphological comparison with *D. luteocincta* (M. Sars, 1870) (Gastropoda: Nudibranchia). *Zootaxa* 4193: 304–316.
- García-Mendéz K, Camacho-García YE. 2016. New records of heterobranch sea slugs (Mollusca: Gastropoda) from Isla del Coco National Park, Costa Rica. *Revista de Biología Tropical* 64 (Suppl. 1): S205–S219.
- Goodheart JA, Bazinet AL, Collins AG, Cummings MP. 2015. Relationships within Cladobranchia (Gastropoda: Nudibranchia) based on RNA-Seq data: an initial investigation. *Royal Society Open Science* 2: 150–196.
- Hayward PJ, Ryland JS. 2017. *Handbook of the marine fauna of north-west Europe. Second edition*. Oxford University Press, Oxford.
- Hoeksema BW. 2015. Latitudinal species diversity gradient of mushroom corals off eastern Australia: a baseline from the 1970s. *Estuarine, Coastal and Shelf Science* 165: 190–198.
- Hoeksema BW, van der Land J, van der Meij SET, van Ofwegen LP, Reijnen BT, van Soest RWM, de Voogd NJ. 2011. Unforeseen importance of historical collections as baselines to determine biotic change of coral reefs: the Saba Bank case. *Marine Ecology* 32: 135–141.
- Jensen K R., Knudsen J. 1995. *Annotated checklist of recent marine molluscs of Danish waters*. Jorgen Knudsen, Copenhagen.
- Katz PS. 2016. Evolution of central pattern generators and rhythmic behaviours. *Philosophical Transactions of the Royal Society B* 371: 20150057.
- Korshunova T, Sanamyan N, Zimina O, Fletcher K, Martynov A. 2016. 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., Martynov A., Bakken T., Picton B. 2017. External diversity is restrained by internal conservatism: New nudibranch mollusc contributes to the cryptic species problem. *Zoologica Scripta*, doi 10.1111/zsc.12253.
- 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.
- Lips KR. 2011. Museum collections: mining the past to manage the future. *Proceedings of the National Academy of Science of the United States of America* 108: 9323–9324.
- Lundin K, Malmberg K. 2016. Nya fynd av nakensnäckor vid svenska västkusten. *Fauna och Flora* 111: 11–13.
- Malmberg K, Lundin K. 2015. *Svenska nakensnäckor*. Waterglobe Productions, Halmstad.
- Martynov A, Korshunova T. 2011. *Opisthobranch molluscs of the seas of Russia. A colour guide to their taxonomy and biology*. Fiton, Moscow.

- Martynov A, Hasegawa K, Korshunova T. 2017. The Japanese Red Data book marine mollusk *Japonacteon nipponensis* and a *Japonacteon* population from Russia belong to the same species: Molecular evidence and recommendations for conservation. *Global Ecology and Conservation* 9: 82–89.
- Mehrotra R, Scott CM. 2016. Species inventory of sea slugs (Gastropoda: Heterobranchia) for Koh Tao, Thailand, with 25 first records for Thai waters. *Marine Biodiversity* 46: 761–771.
- Nimbs MJ, Larkin M, Davis TR, Harasti D, Willan RC, Smith SDA. 2016. Southern range extensions for twelve heterobranch sea slugs (Gastropoda: Heterobranchia) on the eastern coast of Australia. *Marine Biodiversity Records* 9: 27.
- Nuzzo G, Ciavatta ML, Kiss R., Mathieu V., Leclercqz H, Manzo E, Villani G, Mollo E, Lefranc F, D'Souza L, Gavagnin M, Cimino G. 2012. Chemistry of the nudibranch *Aldisa andersoni*: Structure and biological activity of phorbazole metabolites. *Marine Drugs* 10: 1799–1811.
- Odhner N. 1907. Northern and Arctic invertebrates in the collection of the Swedish state museum. *Kungliga Svenska Vetenskapsakademiens Handlingar* 41: 1–114.
- Odhner N. 1922. Norwegian Opisthobranchiate Mollusca in the collections of the zoological museum of Kristiania. *Meddelelser fra Det Zoologiske Museum Kristiania* 1: 1–47.
- Odhner N. 1926. Nudibranchs and lamellariids from the Trondhjem fjord. *Det Kongelige Norske Videnskabers-Selskabs Skrifter* 2: 1–36.
- Odhner N. 1939. Opisthobranchiate Mollusca from the western and northern coast of Norway. *Det Kongelige Norske Videnskabers-Selskabs Skrifter* 1: 1–93.
- Padula V, Araújo AK, Matthews-Cascon H, Schrödl M. 2014. Is the Mediterranean nudibranch *Cratena peregrina* (Gmelin, 1791) present in the Brazilian coast? Integrative species delimitation and description of *Cratena minor* n. sp. *Journal of Molluscan Studies* 80: 575–584.
- Palumbi SR, Martin AP, Romano S, McMillan WO, Stice L, Grabowski, G. 2002. *The simple fool's guide to PCR*. University of Hawaii, Honolulu.
- Picton BE, Morrow C. 1994. *A field guide to the nudibranchs of the British Isles*. Immel Publishing, London.
- Puslednik L, Serb JM. 2008. Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. *Molecular Phylogenetics and Evolution* 48: 1178–1188.
- Pyke GH, Ehrlich PR. 2010. Biological collections and ecological/environmental research: a review, some observations and a look to the future. *Biological Reviews* 85: 247–266.
- Rainbow PS. 2009. Marine biological collections in the 21st century. *Zoologica Scripta* 38 (Suppl. 1): 33–40.
- Robilliard GA. 1970. The systematics and some aspects of the ecology of the genus *Dendronotus*. *The Veliger* 12: 433–479.
- Roginskaya IS. 1987. *Order Nudibranchia Blainville, 1814*. In: Molluscs of the White Sea. Keys to the Fauna of USSR 151: 155–201.
- Roginskaya IS. 1990. Additional data on the food and feeding of *Dendronotus robustus* Verrill, 1879 (Nudibranchia: Dendronotidae) from Dvinsky Bay of the White Sea. In: A. P. Kuznetsov (eds) *Feeding and bioenergetics of marine bottom invertebrates*. Academy of Sciences of the U.S.S.R., P. P. Shirshov Institute of Oceanology, Moscow, pp. 93–110, 158.
- Sars GO. 1878. *Bidrag til kundskaben om Norges Arktiske fauna. I. Mollusca Regionis Arcticae Norvegiae. Oversigt Over de I Norges Arktiske Region Forekommende Bløddyr*. Brøgger, Christiania.
- Shaffer HB, Fisher RN, Davidson C. 1998. The role of natural history collections in documenting species declines. *Trends in Ecology and Evolution* 13: 27–30.
- Svanesson A. 1975. *Physical and chemical oceanography of the Skagerrak and the Kattegat. I Open sea conditions*. Fishery Board Sweden, Institute of Marine Research.
- Thollessen M. 1998. Discrimination of two *Dendronotus* species by allozyme electrophoresis and the reinstatement of *Dendronotus lacteus* (Thompson, 1840) (Nudibranchia, Dendronotoidea). *Zoologica Scripta* 27: 189–195.
- Thompson TE, Brown GH. 1984. *Biology of opisthobranch molluscs*. Vol. 2. The Ray Society Publications, London.
- Valdés A, Murillo FJ, McCarthy JB, Yedinak N (2017) New deep-water records and species of North Atlantic nudibranchs (Mollusca, Gastropoda: Heterobranchia) with the description of a new species. *Journal of Marine Biological Association of the United Kingdom* 97: 303–319.
- Verrill AE. 1870. Contributions to zoology from the museum of Yale College. N° 8. – Descriptions of some New England Nudibranchiata. *American Journal of Science and Arts* 50: 405–408.

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Appendix 1

Morphological analysis and redescription of Dendronotus velifer G.O. Sars, 1878

A syntypic specimen (Figure 1n-o) of *D. velifer* from the type locality in the Barents Sea (Vadsø, Norway) in the collection at the Oslo Natural History Museum (Accession number 16174) is hereby designated as lectotype, to fix and stabilize the name. This is the best preserved specimen of the original series collected by G.O. Sars on which the original description of *D. velifer* was based. A short redescription based on both this lectotype and additional specimens, is given below.

Dendronotus velifer G.O. Sars, 1878

Dendronotus velifer G.O. Sars, 1878: 238–239, pl. 28, Figure 2, pl. 15, Figure 4.

Dendronotus robustus sensu Bergh 1900 – Odhner 1907 (non Verrill 1870).

Dendronotus robustus auct. Partim = mixture of *D. robustus* and *D. velifer*.

Body broad, preserved specimens ranged from 13.5–49 mm (up to 90 mm in first description) in length (Figures 1, 2). 6–12 main branched appendages on oral veil, 4–5 appendages on rhinophoral stalks, 12–15 rhinophoral lamellae, small papilla lateral to rhinophore either present or absent, 4–5 pairs of dorsolateral appendages present (90-mm specimen has sixth rudimentary pair according to first description), 30–35 distinct lip papillae. Dorsolateral appendages with relatively short primary stalk, moderately branched secondary branches and elongate tertiary branches (Figure 1a, f). Length of dorsal processes of jaws approximately one-third total length of jaw (Figures 1l; 2). Masticatory border of jaw with fine denticles. Radular formula: 29–36 × 3–13.1.13–3 (up to 15 lateral teeth in first description). Central (rachidian) tooth strongly denticulated and bearing up to 26 distinct denticles (Figures 1d, i; 2c, f, k). Denticles with deep furrows. Lateral teeth short, slightly curved, smooth or sometimes bearing 1–7 rudimentary denticles (Figures 1e, j, k; 2g, l, m). Ampulla 2-looped. Prostate discoidal, consisting of alveolar glands. Distal part of vas deferens moderate in length, transiting to long, thin, copulatory organ. Bursa copulatrix large, rounded, elongated, seminal receptaculum placed distally.