







Article

Similar Ones Are Not Related and Vice Versa—New *Dendronotus* Taxa (Nudibranchia: Dendronotidae) from the North Atlantic Ocean Provide a Platform for Discussion of Global Marine Biodiversity Patterns [†]

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[†] <http://urn:lsid:zoobank.org:act:A2FF984D-B84C-4AD2-A686-56485F5F1FB8>.

Abstract: One new species of the genus *Dendronotus* (Nudibranchia: Dendronotidae) is described from Norway and Northern Ireland, as well as from the adjacent North Sea, and one new subspecies of *Dendronotus arcticus* is described from Norway by applying a combination of fine-scale morphological and molecular phylogenetic data. The present case demonstrates multilevel morphological and molecular similarities and differences considering on the one hand a grouping of three similar looking sympatric taxa (*D. yrjargul*, *D. arcticus gartensis* n. subsp. and *D. keatleyae* n. sp.), and on the other hand two different looking apparently allopatric subspecies (*D. arcticus arcticus* and *D. arcticus gartensis* n. subsp.). The type species of the genus, *D. frondosus*, which is the commonest dendronotid in Norway and the United Kingdom, consistently demonstrates substantial molecular and fine-scale morphological differences from *D. keatleyae* n. sp. The present study, apart from providing purely taxonomic information, also provides new data for a broad discussion of global biodiversity patterns.

Keywords: multilevel organismal diversity; molecular phylogeny; phylogeography; species delimitation; taxonomy



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1. Introduction

Biodiversity studies, including a fundamental understanding of taxonomic richness, are among the main aims of biology [1–3], especially in our time of pollution of marine habitats and global climate change [4–7]. The nudibranch fauna of the northern Atlantic Ocean, spanning from Norway to the United Kingdom, has been historically assessed as rich and diverse [8–12]. Moreover, in recent years further and previously undetected diversity has been documented from Norway, the United Kingdom and neighbouring northern European localities [13–18]. A particular focus in recent years in connection with these biodiversity studies has been the family Dendronotidae and genus *Dendronotus* Alder and Hancock, 1845, which includes common shallow water species and for which a series of major revisions including the description of several new species has been conducted [19–24]. A new species of *Dendronotus* that spans both insular Europe (the United Kingdom) and European continent (Norway) was recently described as *D. europaeus* [25].

Increasingly, there has been a practice of recognising and celebrating the considerable contribution made by citizen scientists to the discovery of hidden worldwide diversity [26].

All this makes a solid base for the continuation of research on nudibranch biodiversity in northern Europe, with collaboration between scientists and citizen scientists. As an example of this partnership, recent sampling in the Ørland region and in Northern Ireland have resulted in the discovery of previously unrecognised ‘new’ diversity within the genus *Dendronotus* [23]. The specimens investigated here are compared with *D. yrjargul* Korshunova et al., 2020 that occurs sympatrically in the Ørland region of Norway. Both have subdued colouration, but they form a distinct grouping based on molecular phylogenetic data. In Northern Ireland, these species co-occur with the three common European species *D. frondosus* (Ascanius, 1774), *D. lacteus* (Thompson, 1840) and *D. europaeus* Korshunova et al. 2017, but all are readily distinguished by molecular markers, and also by fine-scale morphological differences. Moreover, in the Ørland region, additional *Dendronotus* specimens were also discovered, which are strongly similar externally to the brightly coloured *D. yrjargul*, but they are related to the geographically distant *Dendronotus arcticus* Korshunova et al., 2016 according to molecular data. This intricate combination of the degree of morphological disparity and molecular divergence is providing a solid platform for better understanding the diversity within *Dendronotus* and other organisms [19,23,27,28], and this contribution continues this theme.

2. Materials and Methods

2.1. Data Collection

Specimens of *Dendronotus* were collected by V. V. Grøtan, K.B. Johnson, H. Jensen and A.V. Martynov in the vicinity of Trondheim, Ørland, Norway (63°41' N, 9°39' E), and in Portrush in Northern Ireland (55°12' N, 6°39' E) by B. Picton and L. Keatley. Others were collected in the North Sea during research cruises conducted by the Thünen Institute of Sea Fisheries (Hamburg, Germany) on board the research vessels ‘Walther Herwig III’ and ‘Solea’ within the framework of the VECTORS European Union project, the International Bottom Trawl Survey (IBTS), the German Small-scale Bottom Trawl Survey (GSBTS), and R.V. ‘Senckenberg’ [29,30]. The specimens that were studied have been stored in the mollusc collections at the Norwegian University of Science and Technology (NTNU), NTNU University Museum (NTNU-VM) [31], Senckenberg Natural History Museum, Frankfurt, Germany (SMF), and Zoological Museum, Moscow State University (ZMMU). ZooBank registration: urn:lsid:zoobank.org:pub: A2FF984D-B84C-4AD2-A686-56485F5F1FB8.

2.2. Morphological Analysis

External and internal morphology was studied under a stereomicroscope with either a Nikon D-810 digital camera or Nikon Z6 digital camera attached. The radula and jaws of each specimen was extracted and processed in a 10% sodium hypochlorite. The jaws and reproductive systems were analysed under a stereomicroscope. The jaws and radulae were coated, then examined and photographed using a scanning electron microscope (either CamScan, Camscan Electron Optics Ltd., Cambridge, UK; or a JSM, JEOL Ltd., Tokyo, Japan, or a Quattro S, Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.3. Molecular Analysis

Nine specimens were sequenced for the mitochondrial genes cytochrome c oxidase sub-unit I (COI) and 16S rRNA. The DNA extraction procedure, PCR amplification options, and sequencing have been previously described in detail in Korshunova et al. [21–23,25]. All the procedures were carried out without protocol modifications. Extracted DNA was used as a template for the amplification of partial sequences of the COI and 16S, using the primers: LCO1490 (GGTCAACAAATCATAAAGATATTGG, [32]); HCO2198 (TAAACTTCAGGGT-GACCAAAAAATCA, [32]); 16SarL (CGCCTGTTTAACAAAAACAT, [33]); 16SR (CCGR-TYTGAAGCTCAGCTCAG, [34]). COI sequences were translated into amino acids to verify coding regions and avoid improper base-calling. All new sequences were deposited in GenBank (Table 1, highlighted in bold). Four specimens collected in Northern Ireland were

sequenced for COI via a commercial laboratory (Canadian Centre for DNA Barcoding, BOLD systems) also using standard Folmer primers [32].

Table 1. List of specimens used for molecular analyses.

Species Name	Voucher	Locality	COI	16S
<i>Dendronotus arcticus arcticus</i>	ZMMU: Op-561	Russia: Laptev Sea	KX788140	KX788129
<i>D. arcticus arcticus</i>	ZMMU: Op-562	Russia: Laptev Sea	KX788141	KX788130
<i>D. arcticus arcticus</i>	ZMMU: Op-563	Russia: Laptev Sea	KX788142	KX788131
<i>D. arcticus arcticus</i>	ZMMU: Op-564	Russia: Laptev Sea	OQ588822	OQ594740
<i>D. arcticus arcticus</i>	ZMMU: Op-356	Russia: Kara Sea	KP984995	KP984989
	(the correct voucher number)			
<i>D. arcticus gartensis</i> n. subsp.	ZMMU: Op-804	Norway	OQ588823	OQ594741
<i>D. arcticus gartensis</i> n. subsp.	ZMMU: Op-802	Norway	OQ588824	OQ594742
<i>D. frondosus</i>	ZMMU: Op-380	Norway: Gulen	KM396976	KM397056
<i>D. frondosus</i>	ZMMU: Op-589	Norway: Gulen	KY391833	KY391853
<i>D. frondosus</i>	ZMMU: Op-591	Norway: Gulen	KY391835	KY391855
<i>D. frondosus</i>	ZMMU: Op-592	Norway: Gulen	KY391836	KY391856
<i>D. frondosus</i>	CCMMO069-21	UK: N. Ireland	OQ588829	-
<i>D. frondosus</i>	CCMMO071-21	UK: N. Ireland	OQ588830	-
<i>D. kalikal</i>	ZMMU: Op-284.1	Russia: Kamchatka	KC660026	KC611285
<i>D. kalikal</i>	ZMMU: Op-284.2	Russia: Kamchatka	KC660025	KC611286
<i>D. kalikal</i>	ZMMU: Op-284.3	Russia: Kamchatka	KM396988	KM397070
<i>D. kalikal</i>	ZMMU: Op-283	Russia: Kamchatka	KC660024	KC611284
<i>D. kalikal</i>	ZMMU: Op-285	Russia: Bering Strait	KC660027	KC611287
<i>D. kalikal</i>	ZMMU: Op-349.1	Russia: Kamchatka	KM396986	KM397068
<i>D. kalikal</i>	ZMMU: Op-349.2	Russia: Kamchatka	KM396987	KM397069
<i>D. kalikal</i>	ZMMU: Op-657	Russia: Kuril Islands	MK302458	MK302453
<i>D. kalikal</i>	WS9131	Russia: Kuril Islands	MN138314	MN138079
<i>D. kalikal</i>	WS9132	Russia: Kuril Islands	MN138315	MN138080
<i>D. kalikal</i>	ZMMU: Op-808	Russia: Kuril Islands	OQ588825	OQ594743
<i>D. yrjargul</i>	ZMMU: Op-718	Norway: Norwegian Sea	MT654641	MT655313
<i>D. yrjargul</i>	NTNU-VM-76302	Norway: Norwegian Sea	MT654642	MT655314
<i>D. yrjargul</i>	NTNU-VM-76306	Norway: Norwegian Sea	MT654643	MT655315
<i>D. yrjargul</i>	NTNU-VM-76308	Norway: Norwegian Sea	MT654644	MT655316
<i>D. yrjargul</i>	NTNU-VM-76305	Norway: Norwegian Sea	MT654645	MT655317
<i>D. yrjargul</i>	WS9116	Russia: Kara Sea	MN138317	MN138082
<i>D. yrjargul</i>	WS9113	Russia: Kara Sea	MN138316	MN138081
<i>D. yrjargul</i>	WS9117	Russia: Kara Sea	MN138318	MN138083
<i>D. yrjargul</i>	WS9103	Russia: White Sea	MN138311	MN138076
<i>D. yrjargul</i>	WS9126	Russia: White Sea	MN138312	MN138077
<i>D. yrjargul</i>	WS9127	Russia: White Sea	MN138313	MN138078
<i>D. keatleyae</i> n. sp.	NTNU-VM-82154	Norway	OQ588826	OQ594744
<i>D. keatleyae</i> n. sp.	NTNU-VM-82155	Norway	OQ588827	OQ594745
<i>D. keatleyae</i> n. sp.	CCMMO067-21	UK: N. Ireland	OQ588828	-
<i>D. keatleyae</i> n. sp.	MT09656	North Sea	KR084744	-
<i>D. keatleyae</i> n. sp.	MT09657	North Sea	KR084934	-
<i>Duvaucelia plebeia</i>	ZMMU: Op-572	Norway	KX788134	KX788122
<i>Duvaucelia odhneri</i>	CASIZ: 176219	South Africa	HM162716	HM162641

Before analyses, all sequences were validated via BLAST comparisons. A preliminary analysis was undertaken with a full dataset used in a recently published synoptic review of the Dendronotidae, with the addition of new molecular data obtained in this study (in total 226 sequences, Supplementary Material S1), using the same methodology [23]. Separate analyses were conducted for COI (658 bp), 16S (465 bp), and the concatenated dataset (1123 bp). To conduct a detailed and comparative analysis, data newly obtained in this study together with data for closely related taxa, demonstrating similar external morphology, were selected, based on the preliminary analysis. In total, 79 sequences were selected of 42 specimens (including data for two outgroup specimens). All sequences were aligned with MAFFT [35]. Separate analyses were conducted for COI (658 bp), 16S (464 bp), and the concatenated dataset (1122 bp). Evolutionary models for each dataset were selected using MEGA11 [36]. For full dataset analyses, the HKY+I+G was chosen for the COI dataset, the GTR+I+G for the 16S dataset, and the GTR+I+G model for the concatenated COI+16S dataset. For selected dataset analyses, the HKY+G model was chosen for the COI dataset, GTR+I+G for the 16S dataset, and HKY+G model for the concatenated COI+16S dataset. Two different phylogenetic methods, Bayesian inference (BI) and maximum likelihood (ML), were used to infer evolutionary relationships. Bayesian estimation of posterior probability was performed in MrBayes 3.2 [37]. Four Markov chains were sampled at intervals of 500 generations. Analysis was started with random starting trees and 5×10^7 generations. Maximum likelihood-based phylogeny inference was performed in RAxML 7.2.8 [38] with bootstrap in 1000 pseudo-replications. Final phylogenetic tree images were rendered in FigTree 1.4.2 (<http://tree.bio.ed.ac.uk>, accessed on 25 November 2018). Species delimitation was performed using different methods: ABGD (automatic barcode gap detection [39], <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>, accessed on 1 June 2022), ASAP (assemble species by automatic partitioning [40], <https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>, accessed on 1 June 2022), single-threshold GMYC (general mixed Yule coalescent [41], <https://species.h-its.org/gmyc/>, accessed on 1 June 2022), PTP (Poisson tree processes [42]), and the refined multi-rate PTP (mPTP) version (multiple Poisson tree process [43], <https://mptp.h-its.org/#/tree>, accessed on 1 June 2022). The ABGD analysis was performed with the following settings: a priori for the maximum value of intraspecific divergence between 0.001 and 0.1, 10 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 1. The alignments were analysed using both ABGD and ASAP, separately, using both Jukes-Cantor (JC69) and Kimura (K80) proposed models. GMYC, PTP and mPTP were performed on the Bayesian trees from the BEAST package (<http://beast2.org/>, accessed on 1 June 2022). All these analyses were conducted for the COI and 16S datasets of *Dendronotus* specimens, separately. The program MEGA11 [36] also was used to determine the uncorrected p-distances.

3. Results

3.1. Systematics

Order Nudibranchia Cuvier, 1817

Family Dendronotidae Allman, 1845

Genus *Dendronotus* Alder & Hancock, 1845

Type species *Amphitrite frondosa* Ascanius, 1774.

Diagnosis

Cladobranch nudibranchs with distinct dorsolateral appendages, but appendages can be secondarily partially reduced; dorsolateral appendages can bear distinct (but again sometimes partially reduced) branches. Oral veil with branched appendages. Jaws usually with set of tightly packed, ridge-like, semi-discoid structures, with denticles. Radula commonly with more than eight lateral teeth in adult specimens. Central tooth with cusp integrated within lateral denticles or within body of tooth if denticles are absent; denticle-bearing section of central teeth separated from sides by usually distinct shoulder, thus central teeth with triangular top and squarish base. Prostate with conspicuous alveolar glands, commonly represented by a discoid structure varying from flattened to compressed spherical structure, rarely non-discoidal and linear. Copulatory organ essentially conical, with various ratios of length and width and degrees of tapering [based on 23].

Dendronotus keatleyae n. sp.

The intended authors of this new taxon are Korshunova, Grøtan, Johnson, Bakken, Picton and Martynov. As a patronym, the name is intended as a noun in apposition.

Figure 1

ZooBank registration:

<http://urn:lsid:zoobank.org:act:EA2995B0-BFE3-4A1C-AB52-F625767243C9>.

Etymology

Named after Seasearch diver Libby Keatley, who attracted the attention of one of the authors (BP) to a specimen of this new species during an ascidian training course at Portrush, Northern Ireland.

Material examined

Type material. *Holotype*. NTNU-VM-82155, Norwegian Sea, mouth of Trondheimsfjord, Ørland, Breivika, 63°41'8.66" N 9°39'58.79" E, 18 June 2020, 28 m depth, on hydroids growing on rocks, collector Viktor V. Grøtan.

Paratypes. 1 individual, NTNU-VM-82154, Norwegian Sea, mouth of Trondheimsfjord, Ørland, Uthaug, Rogna, 28 January 2019, 25 m depth, on hydroids growing on rocks, collector Viktor V. Grøtan. 1 individual, CCMMO067-21, Northern Ireland, Portrush, 6 June 2021, 10–30 m depth, collectors Bernard Picton and Libby Keatley. 2 individuals, MT09656, North Sea, 24 March 2012, depth 47.4 m depth, collector not specified.

Diagnosis

Oral veil with 5–6 branched appendages; lateral papilla present on rhinophoral sheath; 7 pairs of dorsolateral appendages with elongate primary stalk, moderately branched elongate secondary branches, short tertiary branches; colour (in life) semi-translucent white to pale greyish-brown, commonly also brownish spots and broken line-like patterns, including small white to yellowish spots and pointed tubercles, some specimens almost without any additional spots and lines; tips of appendages covered with white to yellowish contrasting pigment, no intense golden-yellow pigment; central tooth of radula with deep to partially reduced furrows, moderately denticulated, 11 distinct (to reduced) denticles on each side; ampulla massive, large; prostate discoidal, with 10–20 alveolar glands; copulatory organ moderately long, partially curved; vas deferens moderate in length; bursa copulatrix large, rounded, seminal receptaculum placed distally.

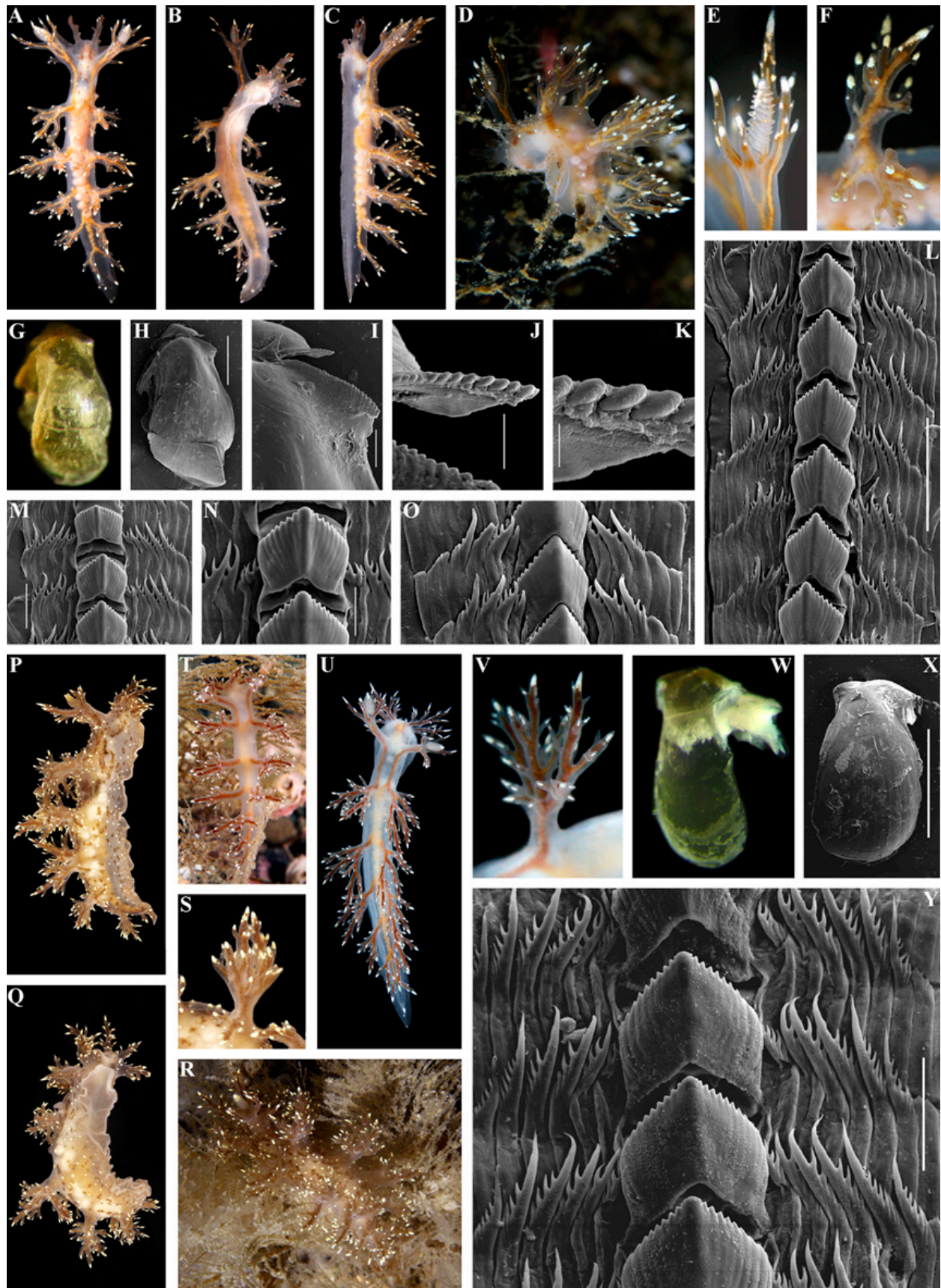


Figure 1. *Dendronotus keatleyae* n. sp. (A–L) Holotype, NTNU-VM-82155, Norway: (A) dorsal view;

(B) ventral view; (C) lateral view; (D) in situ; (E) rhinophore; (F) dorsolateral appendages, details; (G) jaw, light microscopy; (H) jaw, SEM, scale bar 500 μm ; (I) denticles on the masticatory edge, SEM, scale bar 30 μm ; (J) discoid structures on the masticatory edge, SEM, scale bar 30 μm ; (K) details of discoid structures on the masticatory edge, SEM, scale bar 10 μm ; (L) radula, posterior part, SEM, scale bar 100 μm ; (M) radula, posterior part, details, SEM, scale bar 50 μm ; (N) radula, posterior part, details, SEM, scale bar 30 μm ; (O) radula, anterior part, SEM, scale bar 40 μm ; (P) Paratype, CCMMO067-21, the United Kingdom, lateral view of a living specimen; (Q) the same, latero-ventral view; (R) the same, living specimen in situ. (S) the same, dorsolateral appendages, details; (T–Y) Paratype, NTNU-VM-82154, Norway: (T) living specimen in situ; (U) living specimen, dorsal view; (V) the same, dorsolateral appendages, details; (W) jaw, light microscopy; (X) jaw, SEM, scale bar 1 mm; (Y) radula, posterior part, details, SEM, 50 scale bar μm . Photographs of living animals by Viktor V. Grøtan and Bernard Picton. SEM micrographs and light microscopy images by Alexander Martynov.

Description

Body elongate, living specimens (Figure 1) up to 35 mm in length when live. Holotype 25 mm when live. Oral veil with 5–6 main appendages, all branched. Up to 6 appendages on rhinophoral stalks. Rhinophores with 11–14 lamellae; rhinophoral sheath with a lateral papilla. Adult with 7 pairs of dorsolateral appendages (including one rudimentary appendage posteriorly); each appendage with elongate primary stalk, moderately branched elongate secondary branches and short tertiary branches (Figure 1A–D,P). Basal colour semi-translucent white to pale greyish-brown; some specimens almost without any additional spots or lines (only very rare small white spots) or with more distinct brownish spots and broken line-like patterns, including small white and yellowish spots and pointed tubercles; upper part of dorsolateral appendages not covered with bright golden-yellow pigment; tips of appendages covered with white to yellowish bright contrasting pigment. Lip papillae up to 10. Jaws strong, oval, dorsal processes of jaws inclined posteriorly (Figure 1G–K,S,T). Masticatory border of jaw with ridge-like discoidal denticles (Figure 1I–K). Radular formula $33\text{--}34 \times 0\text{--}9.1.9\text{--}0$ (the zero represents the state at the anteriormost section of the radula where the laterals can be completely absent). Central (rachidian) tooth moderately denticulated, bearing up to 11 distinct denticles on each side (Figure 1L–O,Y); denticles sometimes reduced. Denticles with deep (to partially reduced) furrows. Slightly curved, bearing up to 8 denticles, elongated lateral teeth (Figure 1L–O,Y). Ampulla of reproductive system massive, large (Figure 2A). Bursa copulatrix large, rounded, with seminal receptaculum placed distally. Prostate discoidal, consisting of about 10–20 alveolar glands. Distal part of vas deferens moderate in length, transitioning to moderately long, partially curved copulatory organ.

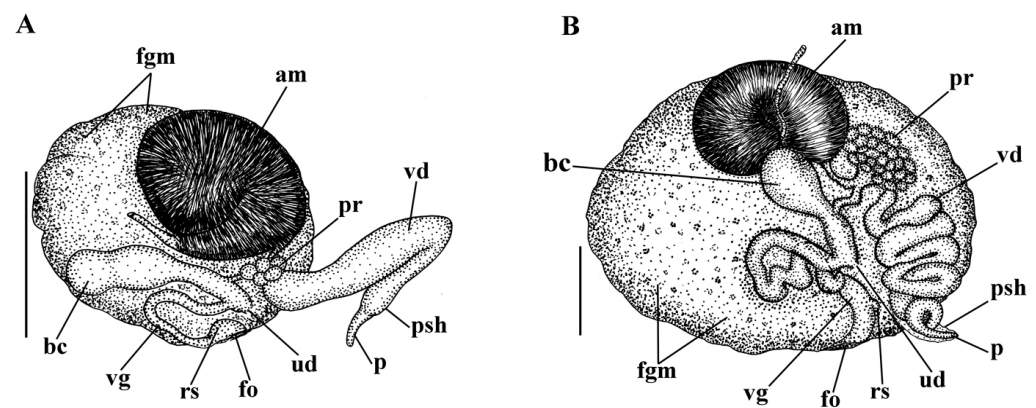


Figure 2. Reproductive systems of *Dendronotus keatleyae* n. sp. (A) and *Dendronotus arcticus gartensis* n. subsp. (B); Abbreviations: am, ampulla; bc, bursa copulatrix; fgm, female gland mass; fo, female opening; p, penis (=copulatory organ); pr, prostate; psh, penial sheath; rs, receptaculum seminis; ud, uterine duct; vd, vas deferens; vg, vaginal duct. Scale bars: 1 mm.

Distribution and ecological observations

Dendronotus keatleyae n. sp. is presently known from the Norwegian Sea to the North Sea and the United Kingdom (only Northern Ireland to date). In Norway, the distribution is at least from Ørland to Oslofjord. This species occurs at upper subtidal depths and is associated with hydroids; our specimens were observed associated with the following four species of leptothecate hydroids—*Abietinaria abietina* (Linnaeus, 1758), *Sertularia argentea* Linnaeus, 1758, *Thuiaria thuja* (Linnaeus, 1758) and *Hydrallmania falcata* (Linnaeus, 1758).

Comparative remarks

According to the molecular phylogenetic analysis (see the Molecular phylogeny section), *Dendronotus keatleyae* n. sp. is placed in a distinct clade, but it shows few external morphological differences from *D. frondosus* (Ascanius, 1774) with which it co-occurs—hence, the title of this paper. However, the presence of small white or yellowish spots and markings, as well as the bright, white or yellowish tips to the lateral appendages—even in the case of almost uniform morphs—distinguishes *D. keatleyae* n. sp. from *D. frondosus* (Figure 1, Table 2). Additionally, specimens of *D. keatleyae* n. sp. bearing brownish irregular lines on the body (Figure 1A,P) are consistently distinguished from *D. frondosus*. The other sympatric taxa, *Dendronotus yrjargul* Korshunova et al., 2020 and *D. arcticus gartensis* n. subsp. newly described below, are readily distinguished from *D. keatleyae* n. sp. by the presence of striking golden-yellow colouration on the upper part of dorsolateral appendages and by their shape (Figures 1, 3 and 4). *Dendronotus europaeus* Korshunova et al., 2017 and *D. lacteus* (W. Thompson, 1840), which also commonly occur in northern Europe, differ from *D. keatleyae* n. sp. by the shape of their dorsolateral appendages and the colour pattern (see review in [23,25]).

The radula of *D. keatleyae* possesses relatively deep furrows and demonstrates fine-scale differences from *D. frondosus*, *D. yrjargul* (Figure 3, Table 2) and *D. arcticus gartensis* n. subsp. (Figure 4, Table 2). The radula of adult *D. keatleyae* n. sp. differs from *D. yrjargul* and *D. arcticus gartensis* n. subsp. in the presence of more distinct furrows (and corresponding ribs) compared to *D. keatleyae* n. sp. However, we must emphasise that the radula of juveniles, and especially that of subadult individuals (which can be retained at the anterior end of the adult radula), are fundamentally similar across the majority of *Dendronotus* species. This is due to the ontogenetic phenomenon of phylotypic periods [44]. The radulae of the sympatric *D. lacteus* and *D. europaeus* strongly differ from that of *D. keatleyae* n. sp., either by the presence of foliated furrows on the central teeth (*D. lacteus*) or by the considerably reduced furrows (*D. europaeus*) [23,25]. *Dendronotus keatleyae* n. sp. is differentiated from all the other *Dendronotus* species by a combination of external colouration and radular features [23].

Table 2. Key morphological characters of *Dendronotus* taxa. For a comparison with other species of the genus *Dendronotus*, see the detailed synopsis of Korshunova et al. (2020, [23]).

	Colouration	Body Length	Dorsolateral Appendages (Pairs)	Appendages of Oral Veil	Appendages of Rhinophoral Stalks	Rhinophoral Lamellae	Radula	Ampulla	Bursa Copulatrix	Prostate	Copulatory Organ (Penis)	Distribution	References
<i>Dendronotus frondosus</i>	Brownish to reddish-brown, often with small white and yellow specks, but usually without opaque white stripes between dorsolateral processes, to completely white translucent specimens	Up to 50 mm (live)	Up to 6, with relatively long primary stalk; moderately branched secondary branches bear elongate tertiary branches	Up to 7	Up to 5	Up to 12	Up to 42 rows of teeth. Central tooth with deep furrows and with up to 14 (common range 8–12) distinct denticles. Up to 10 (usually up to 8) lateral teeth, bearing up to seven denticles	Voluminous, folded	Large, oval to rounded	Up to 30 alveolar glands	Relatively long, curved	Boreal waters of North Atlantic; do not penetrate to the true Arctic further than easternmost Barents Sea	Korshunova et al., 2020 [23]
<i>Dendronotus arcticus arcticus</i>	Brownish, highly consistent in all observed specimens, with scattered distinct opaque white dots on dorsal side and appendages; upper part of the dorsolateral appendages not covered with golden yellow pigment	Up to 19 mm (preserved)	Up to 6, with a moderate primary stalk; moderately branched secondary branches, and short tertiary branches	Up to 8	Up to 6	Up to 18	Up to 39 rows of teeth. Central tooth with up to 14 small denticles and reduced furrows. Up to nine lateral teeth, bearing up to 9 denticles	Folded	Large and rounded	Up to 30 alveolar glands	Long and twisted	Restricted to the true Arctic waters: Laptev Sea, Kara Sea, easternmost part of Barents Sea adjacent to Kara Sea	Korshunova et al., 2016 [22]; present study

Table 2. Cont.

	Colouration	Body Length	Dorsolateral Appendages (Pairs)	Appendages of Oral Veil	Appendages of Rhinophoral Stalks	Rhinophoral Lamellae	Radula	Ampulla	Bursa Copulatrix	Prostate	Copulatory Organ (Penis)	Distribution	References
<i>Dendronotus arcticus gartensis</i> n. subsp.	Semi-translucent, highly consistent in all observed specimens, with bright yellow spots scattered dorsally and laterally, supplemented with dark brownish to deep violet markings, and similarly coloured cores of the dorsolateral appendages; upper part of the dorsolateral appendages covered with bright golden yellow to yellowish pigment	Up to 40 mm (live)	Up to 6, with elongate and pointed primary stalk, secondary branches reduced towards the tip of primary stalk and short tertiary branches, general spinose appearance	Up to 8	Up to 7	Up to 13	Up to 46 rows of teeth. Central tooth moderately denticulated and bearing up to 20 distinct denticles from each side. Denticles with relatively deep furrows. Up to eight lateral teeth, bearing up to eight denticles	Large, folded	Large, rounded	Up to 40 and more alveolar glands	Long, strongly convoluted	Restricted to the boreal waters of middle Norway	Present study

Table 2. Cont.

	Colouration	Body Length	Dorsolateral Appendages (Pairs)	Appendages of Oral Veil	Appendages of Rhinophoral Stalks	Rhinophoral Lamellae	Radula	Ampulla	Bursa Copulatrix	Prostate	Copulatory Organ (Penis)	Distribution	References
<i>Dendronotus keatleyae</i> n. sp.	Semi-translucent white to pale greyish-brown, in some specimens almost without any additional spots and lines (only very rare small white spots) or with more distinct brownish spots and broken line-like patterns, including small yellowish spots and pointed tubercles; upper part of dorsolateral appendages not covered with bright golden-yellow pigment; tips of appendages covered with white to yellowish bright contrasting pigment	Up to 35 mm (live)	Up to 7, with elongate primary stalk, moderately branched elongate secondary branches and short tertiary branches	Up to 6	Up to 6	Up to 14	Up to 34 rows of teeth. Central tooth moderately denticulated and bearing up to 11 distinct to reduced denticles from each side. Denticles with relatively deep to reduced furrows. Up to nine lateral teeth, bearing up to eight denticles	Massiv, large	Large, rounded	About 10–20 alveolar glands	Moderately long, partially curved	Middle to southern Norway, North Sea, the UK	Present study

Table 2. Cont.

	Colouration	Body Length	Dorsolateral Appendages (Pairs)	Appendages of Oral Veil	Appendages of Rhinophoral Stalks	Rhinophoral Lamellae	Radula	Ampulla	Bursa Copulatrix	Prostate	Copulatory Organ (Penis)	Distribution	References
<i>D. yrjargul</i>	Semi-translucent white to greyish, with characteristic two thin wavy subparallel brownish-orange to brownish lines running from the head nearly to the tail. There are no specimens with brownish basal colour; upper part of the dorsolateral appendages covered with bright golden-yellow to yellowish pigment. Foot bordered with thin brownish orange line	Up to 95 mm (live)	Up to 8, with relatively short primary stalk, strongly branched secondary branches and elongate tertiary branches	Up to 6	Up to 5	Up to 30	Up to 50 rows of teeth. Central (rachidian) tooth moderately denticulated and bearing up to 16 distinct denticles. Denticles with deep furrows. Up to 9 lateral teeth, bearing up to 7 denticles	Kidney-shaped, thickened	Large, rounded	Up to 50 alveolar glands	Long, thick, curved	Middle Norway to Kara Sea	Korshunova et al., 2020 [23]; present study

Table 2. Cont.

	Colouration	Body Length	Dorsolateral Appendages (Pairs)	Appendages of Oral Veil	Appendages of Rhinophoral Stalks	Rhinophoral Lamellae	Radula	Ampulla	Bursa Copulatrix	Prostate	Copulatory Organ (Penis)	Distribution	References
<i>Dendronotus kalikal</i>	Brownish, with creamy to whitish areas, dorsum, dorsolateral appendages, and upper sides of foot bear brownish orange lines and dots, partly arranged in blurred subparallel pattern, no golden-yellow pigment on the upper part of dorso-lateral appendages	Up to 15 mm (preserved)	Up to 5 (plus 2–3 smaller), with relatively long primary stalk, moderately branched thin secondary branches	Up to 5	Up to 5	Up to 14 mm	Up to 31 rows of teeth. Central tooth with deep furrows and with up to 18 distinct denticles. Up to eight lateral teeth, bearing up to 8 denticles	Moderately voluminous, folded	Large, considerably elongate, narrowly-oval	Up to circa 20 alveolar glands	Relatively long, slightly curved	Kurile Islands to Bering Strait	Korshunova et al., 2019 [27], 2020 [23]

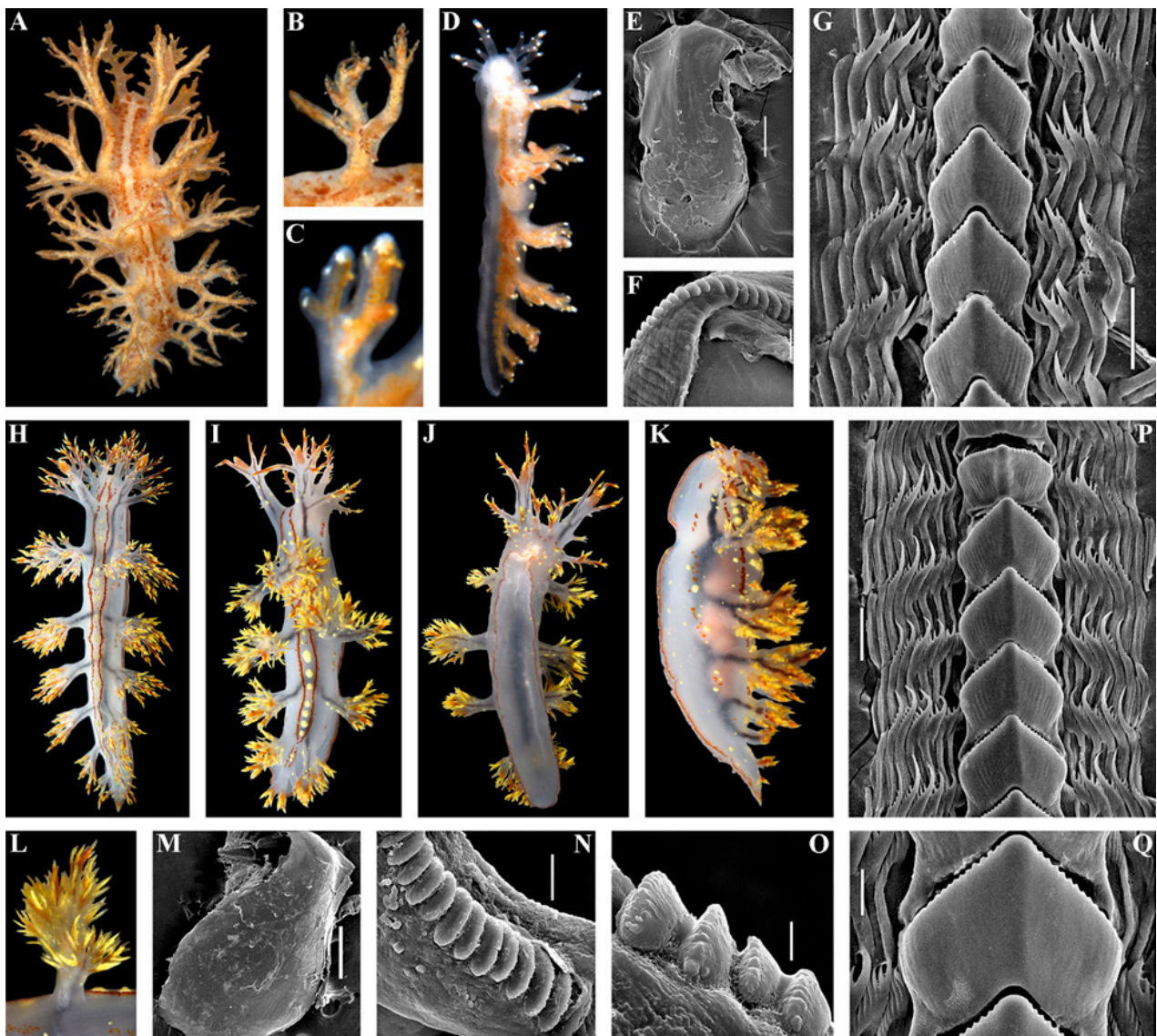


Figure 3. *Dendronotus kalikal* (A–G): (A) holotype of *D. kalikal* in life (ZMMU Op-283), Kamchatka, dorsal view; (B) dorsolateral appendages, holotype; (C) specimen (ZMMU Op-808) from Urup Island, the Kuril Islands, dorsolateral appendages; (D) *D. kalikal* in life, specimen from Urup Island; (E) jaw of *D. kalikal*, specimen (ZMMU Op-657) from Matua Island, the Kuril Islands, SEM, scale bar, 200 µm; (F) discoid structures on the masticatory edge of the same specimen, SEM, scale bar 20 µm; (G) anterior part of radula of the same specimen, SEM, scale bar 50 µm. *Dendronotus yrjargul* (H–Q): (H) holotype of *D. yrjargul* in life (NTNU-VM-76306), Norway, dorsal view; (I) *D. yrjargul* in life (ZMMU Op-809), Norway, dorsal view; (J) living specimen (ZMMU Op-809), Norway, ventral view; (K) living specimen (ZMMU Op-809), Norway, lateral view; (L) *D. yrjargul* in life (ZMMU Op-809), Norway, dorsolateral appendages; (M) jaws of paratype (NTNU-VM-76302), SEM, scale bar 500 µm; (N) discoid structures on the masticatory edge of the same paratype, SEM, scale bar 20 µm; (O) details of discoid structures on the masticatory edge of the same paratype, SEM, scale bar, 5 µm; (P) anterior part of radula of holotype, SEM, scale bar, 50 µm; (Q) central teeth of radula of paratype (NTNU-VM-76302), SEM scale bar, 20 µm. Photographs of living animals by Viktor V. Grøtan, Tatiana Korshunova and Alexander Martynov. SEM micrographs and light microscopy images by Alexander Martynov.

Dendronotus arcticus arcticus Korshunova et al., 2016.

Figure 4A–F.

Dendronotus arcticus Korshunova et al. 2016 [22] (pp. 26–28), Figures 1 and 3; Korshunova et al. 2020 [23] (p. 21), Figure 7.

Material examined

Type material. *Holotype*. ZMMU Op-561, Laptev Sea, R/V “Dalnie Zelentsy”, station O-48, 74°34.9′ N–74°35.1′ N, 115°43.4′ E–115°42.2′ E, 4 October 2014, 15 m depth, benthic trawl, collector O.L. Zimina. *Paratypes*. 3 individuals, ZMMU Op-562–Op-564, same data as holotype.

Diagnosis

Oral veil with 6–8 branched appendages; lateral papilla present; 5–6 pairs of dorsolateral appendages; dorsolateral appendages non-spinose, with a moderate primary stalk, moderately branched secondary branches, and short tertiary branches; colour in life brownish with scattered white dots, upper part of the dorsolateral appendages not covered with golden yellow pigment; central tooth weakly denticulated, bearing up to 14 small denticles with reduced furrows; lateral teeth short and slightly curved; ampulla folded twice; prostate with 25–30 alveolar glands; vas deferens moderate in length; bursa copulatrix large and rounded; small seminal receptaculum placed distally; penis long and twisted.

The complete description of *D. arcticus* is included below because we have separated it into two subspecies, this description being assignable to the subspecies named *D. a. arcticus*.

Description

Holotype 19 mm in life. Body elongate, up to 19 mm in length when live (Figure 4A–C). Oral veil bearing 6–8 branched appendages. Rhinophoral stalk bearing 5–6 appendages; 15–18 rhinophoral lamellae, branched rhinophoral lateral papilla present; 15–25 lip papillae. Dorsolateral appendages in 5-pairs, each with a moderate primary stalk, moderately branched secondary branches, short tertiary branches (Figure 4A–C). Basal colour highly consistent in all observed specimens, brownish with scattered distinct opaque white dots on notum, as well as tips of lateral appendages, oral appendages, lip papillae and rhinophores; upper part of the dorsolateral appendages not covered with golden yellow pigment (Figure 4A–C). Dorsal processes of jaws inclined posteriorly at approximately longitudinal axis to body of jaw (Figure 4D,E). Masticatory processes apparently bear indistinct denticles. Radula formula $39 \times 3-9.1.9-3$. Central tooth weakly denticulated with up to 14 small denticles (Figure 4F) with reduced furrows. Lateral teeth short, slightly curved, with up to 9 elongate denticles (Figure 4F). Ampulla folded twice; prostate consisting of 25–30 alveolar glands; vas deferens moderate in length expanding to voluminous penial sheath; penis long and twisted; vagina long and twisted; bursa copulatrix large, rounded, elongate; small seminal receptaculum placed distally.

Distribution and ecological observations

Central and eastern coastal waters of the Arctic Ocean. Inhabits soft substrates (sands, muds) with gravel and small stones.

Comparative remarks

See below under *Dendronotus arcticus gartensis* n. subsp.

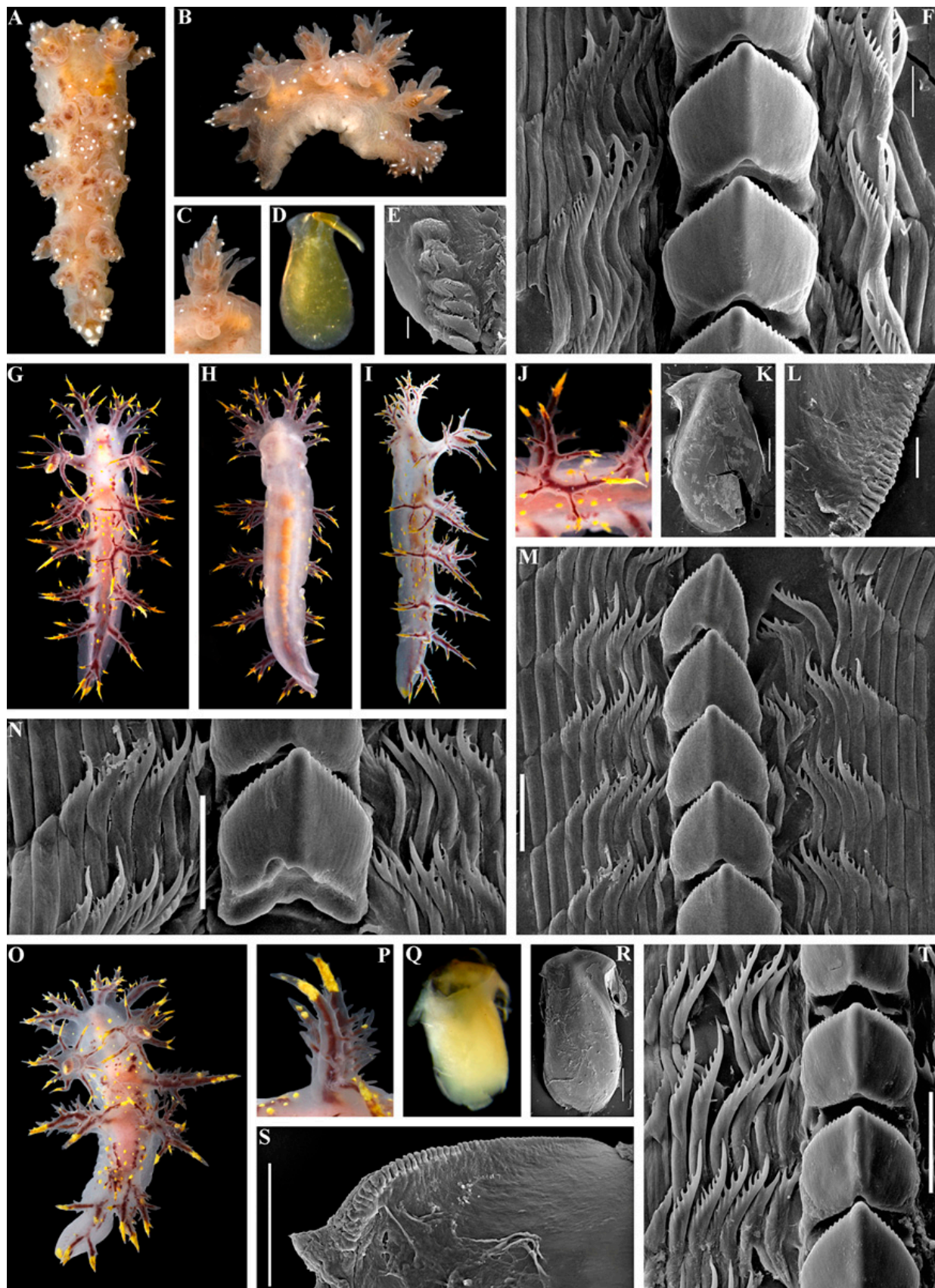


Figure 4. *Dendronotus arcticus arcticus* (A–F): (A) holotype *D. arcticus arcticus* in life (ZMMU Op-561)

from the Laptev Sea, dorsal view; (B) paratype *D. arcticus arcticus* in life (ZMMU Op-562) from the Laptev Sea, lateral view; (C) dorsolateral appendages of the same paratype; (D) holotype jaws, light microscopy; (E) the same, discoid structures on the masticatory edge, SEM, scale bar 10 µm; (F) holotype, posterior part of radula, SEM, scale bar 30 µm. *Dendronotus arcticus gartensis* n. subsp. g–t: (G) holotype in life (ZMMU Op-802) from Norway, dorsal view; (H) holotype in life, ventral view; (I) the same, lateral view; (J) the same, dorsolateral appendages; (K) holotype, jaws, SEM, scale bar, 500 µm; (L) the same, discoid structures on the masticatory edge SEM, scale bar 50 µm; (M) holotype, radula, posterior part, SEM, scale bar, 50 µm; (N) the same, posterior part, details, SEM, scale bar 50 µm; (O) paratype ZMMU Op-807 from Norway, dorsal view; (P) the same, dorsolateral appendages; (Q) paratype ZMMU Op-807, jaws, light microscopy; (R) the same, jaws, SEM, scale bar, 500 µm; (S) the same, discoid structures on the masticatory edge, SEM, scale bar 200 µm; (T) radula, posterior part, SEM, scale bar 50 µm. Photographs of living animals by Viktor V. Grøtan, Alexander Martynov and Olga Zimina. SEM micrographs and light microscopy images by Alexander Martynov.

Dendronotus arcticus gartensis n. subsp.

The intended authors of this new taxon are Korshunova, Grøtan, Johnson, Bakken, Picton and Martynov. The name is adjectival.

Figures 2B and 4G–T.

ZooBank registration:

<http://urn:lsid:zoobank.org:act:3399D8E6-D563-49FE-A24B-371EF38E4723>

Etymology

Dendronotus arcticus gartensis n. subsp. is named after the island Garten in Ørland municipality, where the type locality of Breivika is located. The subspecific name is intended to be adjectival.

Material examined

Type material. *Holotype*. (ZMMU Op-802), Norwegian Sea, mouth of Trondheimsfjord, Ørland, Breivika, 1 January 2020, 26 m depth, on hydroids growing on rocks, collector Viktor V. Grøtan.

Paratypes (all same locality and substrate as holotype). 1 individual, NTNU-VM-82156, 16 February 2020, 28 m depth, collector Viktor V. Grøtan. NTNU-VM-82157, 14 March 2021, 28 m depth, collector Viktor V. Grøtan. 1 individual, NTNU-VM-82158, 15 April 2020, 28 m depth, collector Viktor V. Grøtan. 1 individual, ZMMU Op-803, 28 January 2019, 26 m depth, collector Viktor V. Grøtan. 1 individual, ZMMU Op-804, 1 January 2020, 26 m depth, collector Viktor V. Grøtan. 1 individual, ZMMU Op-805, 10 February 2022, 26 m depth, collector Viktor V. Grøtan. 1 individual, ZMMU Op-806, 10 February 2022, 25 m depth, collector Viktor V. Grøtan. 1 individual, ZMMU Op-807, 3 April 2022, 25 m depth, collectors Alexander Martynov, Viktor V. Grøtan & Heine Jensen.

Diagnosis

Oral veil with 5–8 branched appendages; lateral papilla present on rhinophoral sheath; up to 6 pairs of spinose dorsolateral appendages with elongate and pointed primary stalk, secondary branches reduced towards the tip of primary stalk and short tertiary branches; colour semi-translucent, with bright yellow spots and dark brownish to deep violet markings, cores of dorsolateral appendages covered with bright golden-yellow pigment; central tooth of radula with relatively deep furrows, moderately denticulated, bearing up to 20 distinct denticles on each side; ampulla large, folded; prostate large, discoidal, with more than 40 alveolar glands; vas deferens long; bursa copulatrix large, rounded; seminal receptaculum placed distally; copulatory organ long, strongly convoluted.

Description

Holotype 40 mm in life. Body moderately broad, living specimens up to 40 mm in length (Figure 4G–I,O). Oral veil with 5–8 main branched appendages. Lip papillae up to 20. Up to 7 appendages on rhinophoral stalks. Rhinophores with 10–13 lamellae;

branched lateral papilla present on rhinophoral sheath. Dorsolateral appendages up to 6 pairs (including one rudimentary) in adult specimens; spinose in appearance, with elongate and pointed primary stalk, secondary branches reduced towards the tip of the primary stalk and short tertiary branches (Figure 4 G–J,O,P). Basal colour semi-translucent, highly consistent in all observed specimens, with bright yellow spots scattered dorsally and laterally, supplemented with dark brownish to deep violet markings, and similarly coloured cores of the dorsolateral appendages; upper part of dorsolateral appendages covered with bright golden-yellow pigment. Jaws strong, oval; dorsal processes of jaws inclined posteriorly to the longitudinal axis of jaw itself (Figure 4K,Q,R); masticatory border of jaw with ridge-like denticles (Figure 4L,S). Radular formula: 33–46 × 0–8.1.8–0 (however, the laterals can be completely absent in juveniles and this is reflected in the “0” in the formula). Central (rachidian) tooth moderately denticulated, bearing up to 20 distinct denticles on each side (Figure 4N,M,T); denticles with relatively deep furrows. Lateral teeth elongate, slightly curved, bearing up to 8 denticles (Figure 4N,M,T). Ampulla large, folded (Figure 2B). Prostate large discoidal, consisting of more than 40 alveolar glands. Distal part of vas deferens long, transitioning to long, strongly convoluted penis. Bursa copulatrix large, rounded; seminal receptaculum placed distally.

Distribution and ecological observations

This new subspecies occurs in the Norwegian Sea, where it is only known so far from the Trondheimsfjord region in middle Norway. It inhabits rocky and stony substrates. The new subspecies is associated with hydroids, including possibly *Abietinaria abietina*. Its spawn is a convoluted and moderately narrow, slightly pinkish cord.

Comparative remarks

Dedronotus arcticus gartensis n. subsp. shows a remarkable similarity to *D. yrjargul* in the colouration of the body and dorsolateral appendages (Figures 3 and 4, Table 1); however, it is significantly different from *D. yrjargul* according to our molecular phylogenetic data (Figure 5). There are fine morphological differences between these taxa in the numbers of dorsolateral and oral veil appendages, as well as details of the central radular teeth (Figure 4). *Dendronotus arcticus arcticus* ranges from the core of the Arctic region [22], including the Laptev and Kara Seas, to the easternmost part of the Barents Sea adjacent to the Kara Sea. By contrast, *D. arcticus gartensis* n. subsp. occurs only in the boreal waters of mid-Norway.

Significantly, *Dendronotus arcticus arcticus* and *D. arcticus gartensis* n. subsp. share much molecular phylogenetic data but are readily differentiated by external morphology and colouration (Figure 4, Table 2). In particular, all the specimens of *D. arcticus arcticus* we studied have brownish basal colouration. Their dorsolateral appendages are not considerably spinose in general appearance and they lack golden-yellow upper parts and white spots on the dorsal side (Figure 4A,B and Figure 6), whereas *D. arcticus gartensis* n. subsp. has translucent basal colouration, spinose dorsolateral appendages with golden-yellow upper parts and bright yellow spots on the dorsal side (Figure 4G,I,O,P). There are also potential fine-scale morphological differences between these subspecies internally in the relatively greater number of denticles on the rachidian teeth, copulatory organ and shape of the ampulla, and the number of prostatic alveoles (Figure 2B [22]).

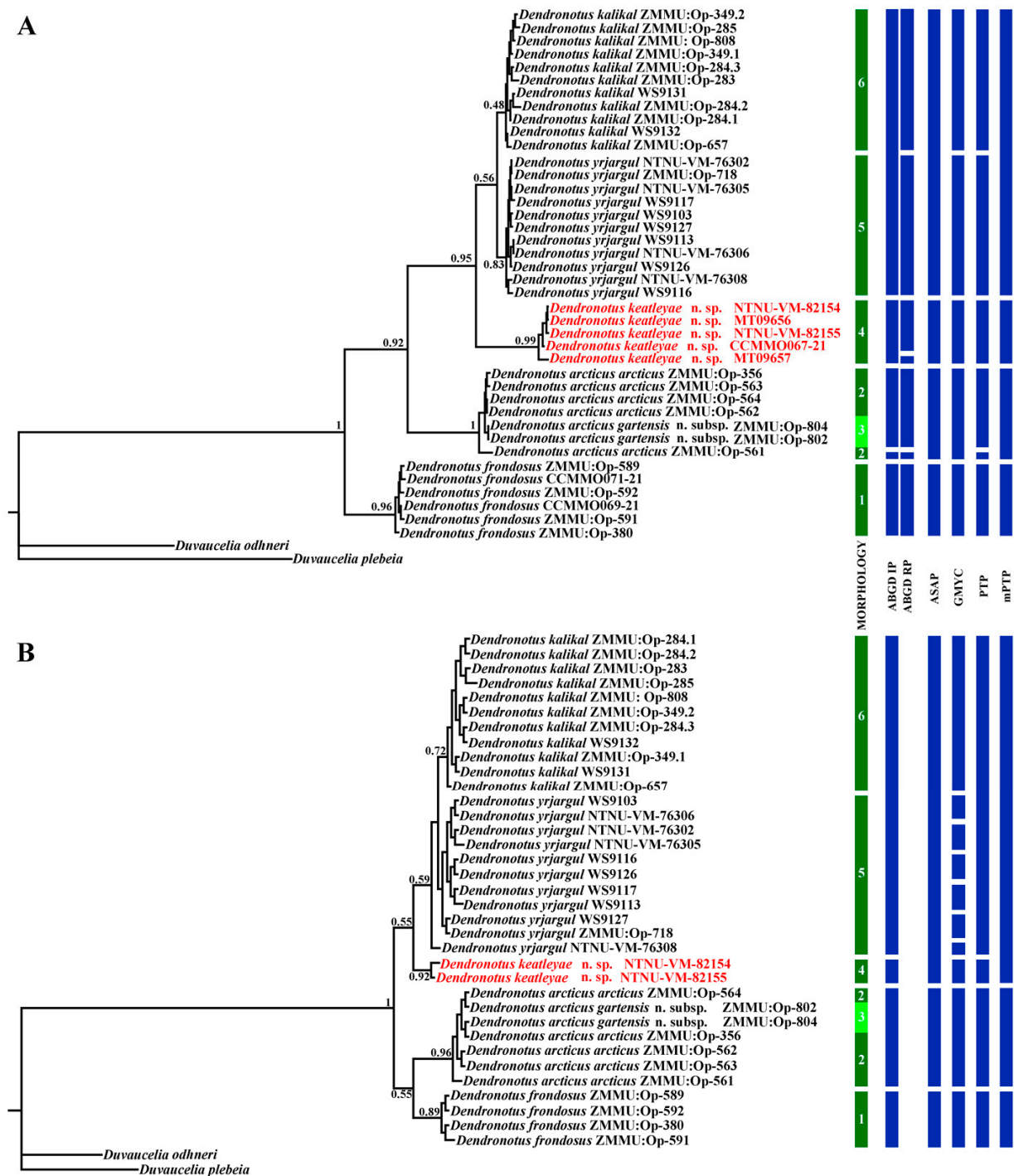
The separation of such morphologically distinct forms as subspecies meets the requirements of both the International Code of Zoological Nomenclature [45] and general taxonomic practice considering that, regardless of molecular differences, subspecies status commonly implies a geographic separation. The hydrological regimes where these Arctic and boreal subspecies occur are considerably different and they might contribute to the formation of these differences: *D. arcticus arcticus* lives commonly in the freezing waters of the high Arctic, whereas *D. arcticus gartensis* n. subsp. inhabits boreal Norwegian waters that are warmed by the North Atlantic Current (which is part of the Gulf Stream), which never freezes. Further explanations of the (assumedly) convergent *D. yrjargul* and *D. arcticus gartensis* n. subsp. could include some other factors, such as common food stuffs,

or hydrological peculiarities, or composition of elements in the seawater. Although we have accumulated a significant amount of data on the nudibranch molluscs of the subarctic Barents Sea and northern Norway, we have never detected either *D. arcticus gartensis* n. subsp. or *D. arcticus arcticus* there [31,46,47]. Another significant piece of evidence for the separate status is that truly Arctic *D. arcticus arcticus* apparently displays no colour variation, being consistently brownish without golden-yellow upper parts of the dorsolateral appendages (Figure 4). By contrast, the common European *D. frondosus* displays considerable colour variation, but without any geographic structure [23]. Additionally, the sympatric *D. keatleyae* n. sp. differs considerably from *D. arcticus gartensis* n. subsp. in external and internal morphological characters, as well as molecular data (Figures 1 and 5). No other described *Dendronotus* species (see synopsis in [23]) displays a close similarity to *D. arcticus gartensis* n. subsp.

3.2. Molecular Phylogeny

The phylogenetic analyses, taken in combination with the dataset used in the recently published synoptic review of the family Dendronotidae and newly obtained data (for *Dendronotus frondosus*, *D. kalikal*, and a potential new *Dendronotus* species), confirmed the species composition of the genus *Dendronotus* with an additional clade for a potential new species (Supplementary Material S1). Five *D. keatleyae* n. sp. specimens from Norway, the United Kingdom and the North Sea clustered in a highly supported clade (BS = 100, PP = 1). Newly sequenced specimens of *D. frondosus* from the United Kingdom (co-occurring with *D. keatleyae* n. sp. and displaying similar coloration) nested within the highly supported *D. frondosus* clade (BS = 100, PP = 1). A newly sequenced specimen *D. kalikal* from Russia (Kuril Islands) nested within the moderately supported *D. kalikal* clade (BS = 79, PP = 0.97). Two specimens of *D. arcticus gartensis* n. subsp. that displayed very similar colouration to *D. yrjargul*, nested within highly supported *D. arcticus* clade (BS = 100, PP = 1). The species delimitation analysis performed with the ABGD tool from recursive partitions showed more potential species in the *D. arcticus* and *D. keatleyae* n. sp. clades compared with results from initial partitions.

As mentioned in the methods section above, detailed analyses based on a consolidation of external and internal morphological, molecular and geographical data were performed for the *D. arcticus* species complex, *D. frondosus*, and *D. keatleyae* n. sp. together with the closely related *D. yrjargul* and *D. kalikal*. A phylogenetic analysis was performed using 40 specimens of *Dendronotus*, plus two outgroup species. Separate analyses were conducted for COI, and 16S gene markers (Figure 5), as well as the concatenated dataset. Bayesian inference (BI) and maximum likelihood (ML) analyses based on the combined dataset yielded similar results (Figure 6). Five *Dendronotus keatleyae* n. sp. specimens from Norway, the United Kingdom and the North Sea clustered in a well-supported clade (PP = 0.98, BS = 92). The clade comprising specimens of *D. keatleyae* n. sp. has the closest position to the clades formed from specimens identified as *D. yrjargul* and *D. kalikal*. Based on the COI marker, the intragroup distance within *D. keatleyae* n. sp. ranged from 0–1.52%. The intergroup distances between *D. keatleyae* n. sp. and *D. kalikal* ranged from 5.81–7.37%, and that between *D. keatleyae* n. sp. and *D. yrjargul* ranged from 5.71–6.67%. The intergroup distances between *D. keatleyae* n. sp. and *D. arcticus arcticus*, *D. arcticus gartensis* n. subsp. and *D. frondosus* ranged from 9.98–11.0%, 10.6–11.1%, 11.0–11.9%, respectively. Two specimens of *D. arcticus gartensis* n. subsp. from Norway nested within a well-supported clade (PP = 1, BS = 96) together with five *D. arcticus arcticus* from the Laptev and Kara Seas. Based on the COI marker, the intragroup distance within *D. arcticus arcticus* ranged from 0–1.82%, and two specimens *D. arcticus gartensis* n. subsp. showed 100% similarity between themselves. The intergroup distances between *D. arcticus gartensis* n. subsp. and *D. arcticus arcticus* ranged from 0.3–0.62%. Thus, regarding the COI marker, no significant differences were found between *D. arcticus gartensis* n. subsp. and *D. arcticus arcticus*.



Two distance-based methods of species delimitation (ABGD, ASAP) and three tree-based methods (GMYC, PTP, and mPTP) were applied. None of these five methods of species delimitation produced congruent results or resolved the same number of partitions (Figure 5). Regarding the COI marker dataset and COI phylogenetic tree, the species number estimated by ABGD tool from recursive partitions, GMYC and PTP were closer to the number of taxa morphologically identified (Table 2) but the ABGD tool from the initial partitions, ASAP (asap-score 1.0) and mPTP, revealed a lower number of lineages than those identified by morphology. ABGD and PTP produced more delimitations, separating the *D. arcticus arcticus* ZMMU:Op-561. The species number estimated by GMYC was congruent with the number of species morphologically identified. Also, because in nudibranch molluscs the resolution of COI at the intraspecific level is greater than that for the 16S gene marker, different numbers of species were revealed in the COI and 16S gene marker datasets, using different methods. Regarding the 16S marker dataset and 16S phylogenetic tree, the species number estimated by ABGD, ASAP (asap-score 1.0), PTP and mPTP was underestimated compared to the number of species identified by morphology (Table 2), whereas 16S marker analysis with the GMYC tool had a higher estimate.

Dendronotus keatleyae n. sp., *D. arcticus* and *D. frondosus* were recognised by all five of these methods. In both the preliminary and detailed phylogenetic analyses, *D. arcticus arcticus* and *D. arcticus gartensis* n. subsp. nested together in a highly supported separate clade. *Dendronotus arcticus gartensis* n. subsp. was not recognised as separate from *D. arcticus* in the analyses using single gene markers, but nevertheless it has distinctive morphology and a restricted distribution. *Dendronotus kalikal* and *D. yrjargul* clustered in separate clades in all analyses. Additionally, *D. kalikal* and *D. yrjargul* were recognised as separate species by the ABGD tool from recursive partitions, GMYC and PTP. Externally, *D. yrjargul* differs readily from *D. kalikal* by a combination of off-white semitransparent body with two, thin, dorsal, subparallel, brownish lines and the fact that its dorso-lateral appendages are golden-yellow to yellowish. Considerable differences between these two species exist in the reproductive system and ontogenetic pattern. Individuals of *D. yrjargul* reach a much larger size (95 mm) when mature, and in specimens of 20–30 mm the reproductive system is still very weakly developed. By contrast, *D. kalikal* achieves sexual maturity at a considerably smaller size (individuals with lengths of 7–10 mm showed a well-developed reproductive system [23]).

Finally, *D. yrjargul* ranges from the Norwegian Sea to the Kara Sea, whereas *D. kalikal* ranges in the northwestern Pacific Ocean from Kamchatka to the northern Kurile Island and the Bering Strait (Figure 6). Thus, *D. kalikal* and *D. yrjargul* are identifiable by molecular data (although possibly recently diverged, as indicated by the slight molecular divergence), and are also consistently separated by morphological features as well as geographical distribution.

Based on the combination of morphological, molecular and geographical data, *D. frondosus*, *D. arcticus*, *D. yrjargul* and *D. kalikal* were confirmed as distinct species. Additionally, *D. keatleyae* n. sp. and *D. arcticus gartensis* n. subsp. were confirmed as a distinct species and distinct subspecies, respectively.

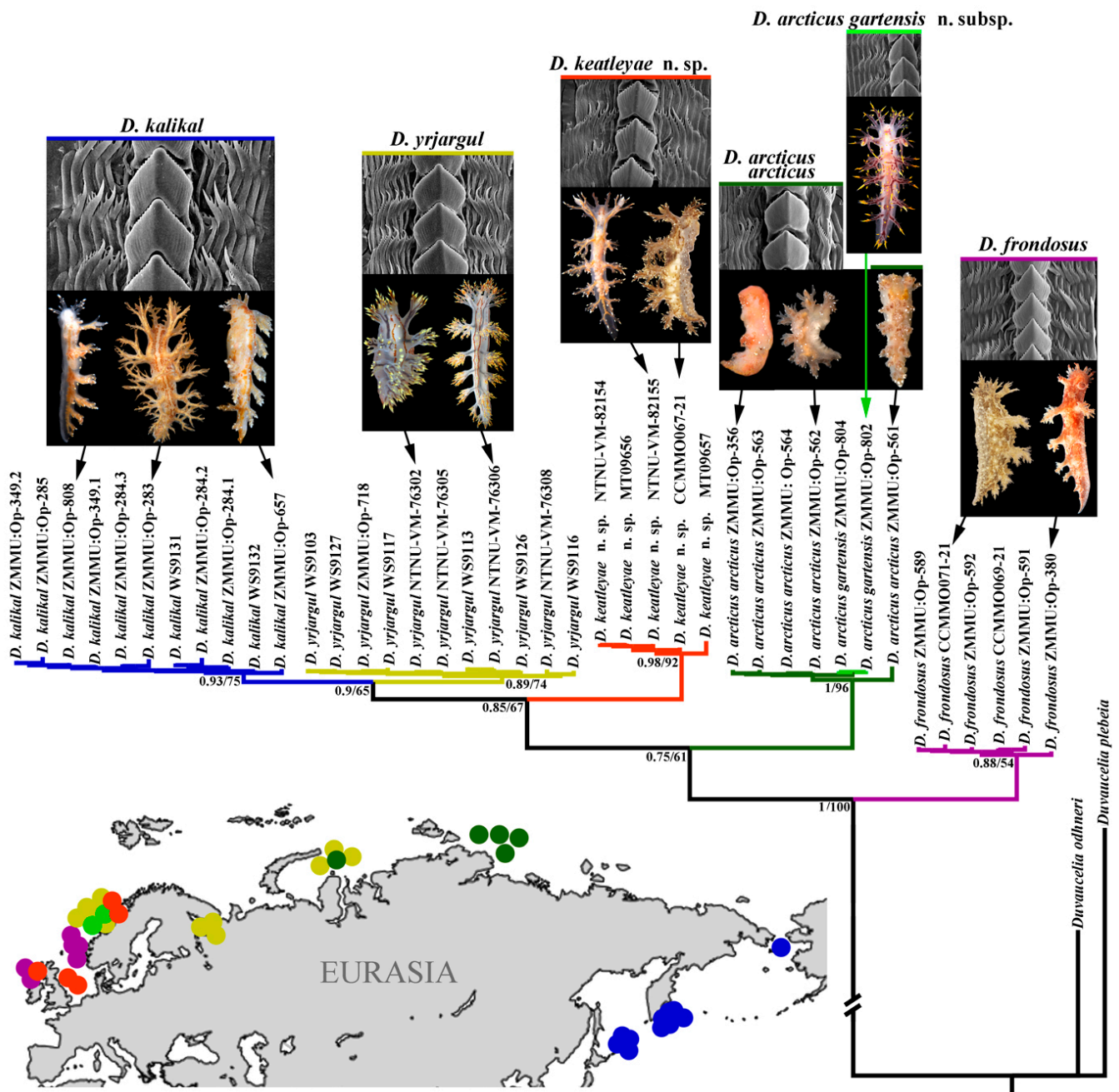


Figure 6. Phylogenetic tree based on concatenated molecular data (cytochrome c oxidase subunit I + 16S) represented by Bayesian inference (BI). Numbers represent posterior probabilities from BI and bootstrap values for maximum likelihood. For each species, colour pattern examples and single low variable radular characteristics are presented. Map showing the distribution of *Dendronotus* species, used for molecular phylogenetic analysis.

4. Discussion

The broader issue of integrating morphological and molecular data to reach conclusions about species delimitation has a considerable history under two different names—“total evidence” [48,49] or “integrative taxonomy” [50–53]. The multilevel diversity approach (MOD, see most recent review in [44]) fundamentally differs from “integrative taxonomy” and explicitly emphasises multiple biological levels instead of the contrasting distinction only between morphological and molecular levels, because in spite of the original desire “to integrate” various disciplines [50], in real taxonomic practice an “integration”

implies a primacy of molecular data over morphological data [54]. However, an immense amount of recent data clearly highlights that both genetic and epigenetic processes influence the formation of any organism [55–57], and therefore genetic data cannot be used as the sole basis of modern taxonomy to “delimit units of life’s diversity”. Thus, any proposals for putative absence of “morphological differences” [58–60] in reality implies insufficient fine-scale comparison [16,61–66]. Therefore, the naming of ‘morphodiversity’, contrary to the guidelines of the “integrative taxonomy” [50], is among the most crucial of aims of taxonomy, from which we will approach a true understanding of “units of life’s diversity”.

Furthermore, recently doubts have been cast on the standard “barcoding gene” CO1 [67,68]. These doubts have far more serious consequences for the entire phylogenetic field than these practical studies implied. Because either “bin” barcoding distance [69], commonly considered as slightly higher than two percent is indicative as a “species level boundary” regardless of other organism genes and various multilevel, “phenotypic” traits, or we should if not completely abandon the importance of molecular phylogenetic data, at least significantly downgrade their importance for taxonomy.

A highly intricate combination of hidden diversity, introgression and gene flow in various organismal groups [70–73] also prevent any straightforward demarcation of biodiversity into ‘cryptic’ and ‘non cryptic’ taxa, a topic which is also tightly connected with the putatively strict divisions of any organismal traits into strictly ‘genetic’ and ‘phenotypic’ features. While a detailed discussion on this topic would take several separate papers, it is very important to highlight here that these recent controversies obviously show that instead of common modern practice to use a molecular phylogenetic tree as a backbone for taxonomic decisions and ignore any other evidence as “phenotypic variations” (and continue to call such a genetic-centred approach “integrative ones”), we should gradually change our comprehension of the world’s biodiversity towards its multilevel understanding, in which both genetic and phenotypic differences and similarities will be employed in the resulting taxonomic classification. By this, it is clear that multilevel organismal diversity is a truly whole-encompassing, complex discipline, whereas so-called “integrative taxonomy” is not.

The present example of *Dendronotus* demonstrates a genuinely multilevel complex of both morphological and molecular similarities and differences (Figures 1–6) in two groupings within this genus. On the one hand, there is a set of closely related species that co-occur in the Ørland region of Norway (comprising *D. yrjargul*, *D. arcticus gartensis* n. subsp. and *D. keatleyae* n. sp.) (Figures 1–6). On the other hand, there are two subspecies that are apparently allopatric (*D. arcticus arcticus* and *D. arcticus gartensis* n. subsp.) (Figures 4 and 6). Our observations of these taxa immediately undermine any straightforward assertion of a ‘cryptic’ species complex, because there is a mosaic of similarities and fine-scale differences among the species studied here. For example, whilst *D. yrjargul* and *D. arcticus gartensis* n. subsp. can be superficially described as ‘cryptic’ (compare Figures 3H–K and 4G–I,O), detailed morphological analysis clearly reveals consistent fine-scale differences, including the absence of subparallel brown lines and bright yellow spots on the dorsal side of *D. arcticus gartensis* n. subsp., as well as differences in relative number of dorso-lateral and oral veil processes (Figures 3 and 4). *Dendronotus keatleyae* n. sp. (Figure 1), in turn, can be differentiated morphologically from the closely related *D. frondosus*, *D. lacteus* and *D. europaeus* by both external and internal features (see details in [23,25], and present study). Developing such fine-scale differences helps us to better understand biodiversity. Three of the authors of this paper (TB, VVG, KBJ) have taken photographs of specimens for more than two decades from other parts of the Trondheimsfjord now identified as *D. arcticus gartensis* n. subsp. and *D. yrjargul*. Previously, they were identified as *D. frondosus* as that species was considered to possess great intraspecific morphological variation [16]. Additionally, *D. yrjargul* was photographed recently in the NW Atlantic (Maine, ME, USA; Newfield A., Feick J., personal communication).

The congruence between morphological data and molecular phylogenetic results has long been discussed [74,75]. Nowadays, incongruent delimitation scenarios indicated by different species delimitation methods are actively discussed [43,76,77]. In this study, several commonly used molecular species delimitation methods were applied. Compared with

morphological results, different analyses were conducted using two different barcoding fragments potentially over- or underestimating the number of putative species [78], yet here the results estimated by GMYC for the COI tree were congruent with the number of species identified morphologically. One new species was identified and four previously described species (supported by morphological diagnosis) were confirmed based on different species delimitation methods.

Specimens of *D. keatleyae* n. sp., *D. yrjargul* and *D. arcticus gartensis* n. subsp., co-occurring in the Ørland region of Norway, were confirmed as separate taxa by different delimitation methods, as well as specimens of *D. keatleyae* n. sp. and *D. frondosus* co-occurring in Northern Ireland (Figure 6). The recently described *D. yrjargul* demonstrates considerable external and internal morphological differences from *D. kalikal*, including an up to 10 times larger adult body size, and hence different maturation patterns, very consistent colouration, a larger number of radular teeth and denticles on the central teeth, very different shape of bursa copulatrix, larger number of prostatic alveoles, and it always forms a separate molecular phylogenetic clade despite relatively low genetic differences [23]; present study, Table 2, Figures 3, 5 and 6.

Our study, therefore, reveals the complex combinations of morphological and molecular similarities and differences (Figures 1–6), which contradict the simplistic division into ‘cryptic’ or ‘non-cryptic’ species groups, which only superficially appear indistinguishable until detailed integration of niche, molecular data, or other lines of evidence reveal clearly different and distinguishable species lineages [79]. This contrast led to a misleading distinction between ‘cryptic’, ‘pseudocryptic’ and just ‘ordinary’ species [80–87]; see detailed discussion in [16,18,63,88,89]. This consideration is of crucial importance for practical taxonomy. The issue of ‘cryptic’ species would not arise if the MOD approach was used because the ontogenetic properties of any organism imply it is impossible to have an identical, “indistinguishable” morphology [90,91], which is commonly claimed as a basis for the cryptic species concept [92]. “Integrative taxonomy” [50] has not been able to overcome these significant inconsistencies, whereas the multilevel organismal diversity approach [44] is specifically targeted to encompass any potential organismal properties in taxonomic framework and fundamentally avoids the pitfalls of ‘cryptic’ versus ‘non-cryptic’ species. Thus, the present study, apart from providing purely taxonomic information, also provides new data for a broad discussion of global biodiversity patterns.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15040504/s1>.

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