Consolidated data on the phylogeny and evolution of the family Tritoniidae (Gastropoda: Nudibranchia) contribute to genera reassessment and clarify the taxonomic status of the neuroscience models *Tritonia* and *Tochuina*

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**Abstract**

Nudibranch molluscs of the family Tritoniidae are widely used neuroscience model systems for understand the behavioural and genetic bases of learning and memory. However species identity and genus-level taxonomic assignment of the tritoniids remain contested. Herein we present a taxonomic review of the family Tritoniidae using integration of molecular phylogenetic analysis, morphological and biogeographical data. For the first time the identity of the model species *Tritonia tetraquetra* (Pallas, 1788) and *Tritonia exsulans* Bergh, 1894 is confirmed. *T. tetraquetra* distributes across the large geographic and bathymetric distances in the North-Eastern (NE) and North-Western (NW) Pacific. In turn, at NE Pacific coasts the separate species *T. exsulans* is commonly occurred. Thus, it reveals a misidentification of *T. tetraquetra* and *T. exsulans* species in neuroscience applications. Presence of more hidden lineages within NW Pacific *T. tetraquetra* is suggested. The long lasting confusion over identity of the species from the genera *Tritonia* and *Tochuina* is resolved using molecular and morphological data. We also disprove a common indication about “edible *T. tetraquetra*” at the Kuril Islands. It is shown that *Tochuina* possesses specialized tritoniid features and also some characters of “arminacean nudibranchs”, such as *Doridoxa* and *Heterodoris*. Diagnoses for the families Doridoxidae and Heterodorididae are provided. Taxonomy of the genus *Doridoxa* is clarified and molecular data for the genus *Heterodoris* presented for the first time. A taxonomic synopsis for the family Tritoniidae is provided. A new genus among tritoniid taxa is proposed. Importance of the ontogeny-based taxonomy is highlighted. The cases when apomorphic characters considerably modified in a crown group due to the paedomorphosis are revealed. Tracing of the character evolution is presented for secondary gills—a key external feature of the family Tritoniidae and traditional dendronotacean nudibranchs.
Introduction

A remarkable nudibranch family Tritoniidae has an intricate phylogenetic position within Nudibranchia [1–5]. For several decades species of the genera Tritonia and Tochuina have been no less useful model systems for studies neural basis of behaviour than Aplysia [6–12]. The brain of tritoniids contains giant neurons which can be reliably identified by their behavioural functions. Studies of how the brain controls behaviour are conducting, as well through identify homologous neurons in different mollusc taxa [10–13]. It can shed light on evolution the neural basis of behaviour but requires precise taxa identification.

The most commonly used for neuroscience purposes was a North Pacific species previously known under the name Tritonia diomedea Bergh, 1894 [14–16], which recently was showed to be a junior synonym of Tritonia tetraquetra (Pallas, 1788) [17, 18]. However, though the latter name now is accepted [19–21] many questions have remained. Particularly, the large geographic and bathymetric ranges of T. tetraquetra in the northern Pacific were not tested using both morphological and molecular data. Tritonia tetraquetra is also frequently mentioned among few examples of apparent involvement of a nudibranch species in a culture of indigenous ethnic groups. This species was originally described from the Kuril Islands and the genus name Tochuina was proposed to be derived from Ainu language [22, 23]. Thus, members of the family Tritoniidae are important for several fields, however their taxonomic and phylogenetic placement needs in a clarification. Here we for the first time summarize available morphological, molecular and biogeographic data across the North-Easter (NE) and the North-Western (NW) Pacific “Tritonia tetraquetra” group of species and the genus Tochuina to clarify their taxonomic status and phylogenetic relationships (Figs 1–7).

Recently we presented phylogenetic data and ancestral character state reconstruction for aeolidacean nudibranchs using the family Tritoniidae as an outgroup [24]. In order to place the model species of the genera Tritonia and Tochuina in a broad phylogenetic framework, in the present study we investigated the internal and external phylogenetic relations of the family Tritoniidae. This is particularly relevant because the tritoniiid genus Tochuina shows an intriguing morphological similarity to the presumably distantly related non-tritoniid taxa Doridoxa and Heterodoris [25]. This similarity was never explored with application of the modern molecular data. Here we therefore present phylogenetic analysis with a broad taxon sampling and ancestral character state reconstruction for the superfamilies Dendronotoidea and Tritonioida to trace the formation of key features of one of the most basal nudibranchs.

Materials and methods

Sample data

Material for this study was obtained by scuba diving in NW Pacific (Kamchatka, Russia, 52° 50’ N 158° 42’ E), NE Pacific (British Columbia, Canada, 50° 53’ N 125° 37’ W, 50° 36’ N, 126° 49’ W; Washington, USA, 47° 35’ N 122° 33’ W), NE Atlantic (Norway, 60° 57’ N 05° 07’ E), by dredging in NW Atlantic and the Barents Sea, and preserved in 99% ethanol for morphological and molecular investigations. For morphological study several previously collected formalin-fixed samples during dredging operations in the Sea of Japan (Peter the Great Bay) were used. Specimens are stored in the Zoological Museum of Moscow State University (ZMMU). Type specimens were examined from the Natural History Museum of Denmark and Swedish Museum of Natural History. No permission was necessary to obtain samples in the field and to access the museum collections.

Morphological analysis

External and internal morphology was studied under a stereomicroscope, using a Nikon D-810 digital camera and scanning electron microscopes. The buccal masses were extracted and
Fig 1. A. Phylogenetic relationships of Tritoniidae, Doridoxidae, Heterodorididae, Doridomorphidae, Arminidae and Dendronotoidea based on COI + 16S + H3 concatenated dataset inferred by Bayesian inference (BI). Numbers above branches represent posterior probabilities from BI; numbers below branches indicate bootstrap values for Maximum Likelihood. B. Scheme of the potential morphological transformations of the rhinophores and oral veil that supported by the present phylogenetic data (see discussion in the text). Oral veil and derivates highlighted in yellow colour.

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Fig 2. *Tritonia tetraquetra* (Pallas, 1788). A. Figure of high body (laterodorsal view) with bilobed oral veil and without distinct notal edge of "Limax" *tetraquetra* from the original description by Pallas (1788 [38]). B. Figure of elongate-oval jaws of "Limax" *tetraquetra* from the original description by Pallas (1788 [38]). C–E. Dorsal, ventral and lateral views respectively of living neotype (ZMMU Op-719) of *Tritonia tetraquetra* from Kamchatka (120 mm in length). F. Details of bilobed oral veil of living neotype of *T. tetraquetra*. G. Elongate-oval jaw of specimen *T. tetraquetra* from Kamchatka (length 170 mm, live), collected on the same date with neotype (SEM). H. Same specimen, overview of masticatory process of jaw. I, J. Same specimen, details of elongate bristle-like elements of masticatory process. K. Same, details of polygonal structures on the masticatory process. L. Same specimen, general overview of radula (SEM). M. Same, details of outer lateral teeth. N. Same, details of middle lateral teeth. O, P. Same, details of central part of the radula with central teeth and inner laterals. Q, R. Dorsal and and ventral views respectively of the lectotype *T. diomedea* Bergh, 1894 (junior synonym of *T. tetraquetra*) (ZMUK, GAS-2034, 30 mm in length). S. Elongate-oval jaws of lectotype *T. diomedea*. T. Label of the type of *T. diomedea* (ZMUK, GAS-2034). U. Jaw from first description of *T. diomedea* (from Bergh, 1894, [50]). V. Details of details of elongate bristle-like elements of masticatory process from the first description of *T. diomedea*. Scale bars: G, L–2 mm, H–500 μm, I–K–100 μm, M–P–200 μm. All bibliographic excerpts are not in copyright.

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Molecular analysis

For molecular analysis a small pieces were used for DNA extraction with Syntol S-Sorb kit by Syntol Company, according to the producer’s protocols. In total, 8 specimens were successfully sequenced for the mitochondrial genes cytochrome c oxidase subunit I (COI), 16S rRNA, and the nuclear genes Histone 3 (H3). Partial sequences were amplified by PCR using the primers: LCO 1490 (GGTCAACAATCTAAGATATTTG, [26]); HCO 2198 (TAAACTTCAGGGTGACC AAAAAATCA, [26]); 16S arL (GGCCTTTATCAAAAAACAT, [27]); 16 S R (CCGRTYTGACT CAGCTCACG, [28]); H3 AF (ATGGCTCGTACCAAGCAGCAGG, [29]) and H3 AR (ATAT CCTTGGGCATATGGTGAC, [29]). Extracted DNA was used as a template for the amplification of partial sequences. 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 secs at 95°C (denaturation), 15 secs at 45°C (annealing temperature), and 30 secs at 72°C, with a final extension of 7 mins at 72°C. The 16S amplification began with an initial denaturation for 1 min at 94°C, followed by 40 cycles of 15 secs at 94°C (denaturation), 15 secs at 52°C (annealing temperature), and 30 secs at 72°C, with a final extension of 7 mins at 72°C. The amplification of H3 began with an initial denaturation for 1 min at 95°C, followed by 40 cycles of 15 secs at 95°C (denaturation), 15 secs at 50°C (annealing temperature) and 30 secs at 72°C, with a final extension of 7 mins 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. Additional sequences were obtained from GenBank (see Table 1 for a full list of samples, localities, and voucher references). Original data and publicly available sequences were aligned with the MAFFT algorithm [30]. COI and H3 sequences were translated into amino acids for confirmation of the alignment. Separate analyses were conducted for COI (657 bp), 16S (425 bp), H3 (327 bp), and concatenated data (1409 bp). Gblocks
0.91b [31] was applied to discard poorly aligned regions for the 16S data set using less stringent options (allow smaller final blocks, gap positions within the final blocks, and less strict flanking positions), in total, 13% of the positions were eliminated. Evolutionary models for each data set were selected using MrModelTest 2.3 [32]. 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 [33]. Four Markov

Fig 4. Tritonia exsulans Bergh, 1894. A. Dorsal view of living neotype of T. exsulans (NE Pacific) (ZMMU Op-720). B, C. Ventral and lateral views of preserved neotype of T. exsulans (13 mm in length). D, E. Living specimen of T. exsulans from NE Pacific before involvement to the neurobiological experiments. F. Jaw of neotype (SEM). G. Masticatory process of the same jaw. H. Details of massive conical elements of masticatory process of the same jaw. I. General overview of radula of neotype (SEM). J. Details of central part of the radula with central teeth and inner laterals. K. Details of central teeth. L. Details of outer laterals. M. Details of massive conical elements of masticatory process in the first description of T. exsulans (from Bergh, 1894 [50]). N. Central teeth in the first description of T. exsulans (from Bergh, 1894 [50]). O. Lateral teeth in the first description of T. exsulans (from Bergh, 1894 [50]). Scale bars: F, I–500 μm, G, J, L–100 μm, H–10 μm, K–50 μm. All bibliographic excerpts are not in copyright.

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Fig 5. *Tritonia psoloides* Aurivillius, 1887 and *Tritonia primorjensis* Minichev, 1971. A–C. Dorsal, lateral and ventral views of the preserved type specimen of *T. psoloides* respectively (SMNH 6875, length ca. 45 mm). D. Jaw of type of *T. psoloides* (light microscopy). E. Label of the type of *T. psoloides* (SMNH 6875). F. Central and lateral teeth of *T. psoloides* from the original description in Aurivillius, 1887 [51]. G–R. External views and internal characters of the preserved...
chains were sampled at intervals of 1000 generations. Analysis was started with random starting trees and $5 \times 10^6$ generations. Maximum likelihood-based phylogeny inference was performed in RAxML 7.2.8 [34] with bootstrap in 1000 pseudo-replications. Final phylogenetic tree images were rendered in FigTree1.4.2. Nodes in the phylogenetic trees with Bayesian posterior values $\geq 0.96$ and bootstrap values $\geq 90$ were considered 'highly' supported. Nodes with $0.90–0.95$ and $80–89$ accordingly were considered 'moderately' supported (lower support values were considered not significant). The program MEGA7 [35] was used to calculate the uncorrected p-distances between all the sequences. Automatic Barcode Gap Discovery (ABGD) [36] (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) was used to estimate Tritonia sensu lato, Tochuina and Doridoxa species divergence. Alignment from the COI marker was submitted and processed in ABGD using the Jukes-Cantor (JC69) and Kimura (K80) models with the following settings: a prior 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. Ancestral character state reconstruction for the secondary gill traits were run using Parsimony ancestral states in Mesquite v3.10 [37], based on the topology of the best tree from the Bayesian analysis of a concatenated dataset.

Nomenclatural acts
The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub:0D2A82C2- AA25-4B93-96A8-7158D56F6477. The electronic edition of this work was published in a journal with an ISSN and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results and discussion
Molecular analysis
The phylogenetic analysis was performed using sixty-one specimens of the family Tritoniidae, and forty-five related taxa. The dataset consisted of two hundred and fifty eight nucleotide sequences. The HKY + I + G model was chosen for the 16S; the GTR + I + G model was chosen for COI, H3, and for the combined dataset. The resulting combined tree provided better resolution than COI, 16S, or H3 separately (Fig 1A, S1 Fig). Bayesian Inference (BI) and Maximum Likelihood (ML) analyses based on the combined dataset for the mitochondrial COI and 16S, and the nuclear H3 genes yielded similar results (Fig 1A).
The molecular phylogenetic analyses support the existence of the several main clades within the family Tritoniidae. "Tritonia" manicata, "T." lineata, "T." striata, "T." plebeia, and "T." nilsodhneri show close evolutionary relationships and clustered together with the maximal support (PP = 1, BS = 100) in a separate clade that attributes here as Duvaucelia (restricted) clade.

"Tritonia" bayeri, "T." myrakeenae, "T." pickensi, and "T." hamnerorum form separate clade (PP = 1, BS = 76) that attributes here as Tritonicula gen. nov. clade. All another Tritonia species clustered together in a clade that is sister to Duvaucelia (restricted) clade. All five Tritonia exsulans, five T. festiva, three T. tetraqueta and T. cf. psoloides clustered together in separate clade with high support (PP = 1, BS = 99), that branched to three clades. The first clade (PP = 1, BS = 99) includes T. tetraqueta and T. cf. psoloides is sister to the clade (PP = 1, BS = 92) divided into two subclades: T. exsulans and T. festiva. The ABGD analysis of the Tritonia sensu lato COI data set run with two different models revealed fifteen potential species, include T. tetraqueta, T. exsulans, T. festiva, T. cf. psoloides, and some others (see Fig 1A).
Fig 7. *Doridoxa walteri* (Krause, 1892) and *Heterodoris robusta* Verrill et Emerton 1882 –non-tritoniid taxa that showing external (presence of partly opened rhinophoral sheath connected to a non-bilobed oral veil) and internal characters (unicuspids central teeth, numerous hamate laterals) similar to the tritoniid genus *Tochuina*. A. *Doridoxa walteri*, morphological data from original description in Krause, 1892 [109], including external view, radular teeth and jaws. B. *Doridoxa ingolfiana* Bergh, 1899 (junior synonym of *D. walteri*, see text for details), morphological data from original description in Bergh, 1899 [105], including external view, radular teeth and jaws. C. “*Doridoxa ingolfiana var.*” from original description in Bergh, 1899 [105] (junior synonym of *D. walteri*, see text for details), showing denticulated central teeth, D–J. External views and internal characters of *Doridoxa walteri*, preserved specimen (ZMMU Op-721, length 16 mm) from the Barents Sea (eastern Spitzbergen region), type locality of *D. walteri*. D. Ventrolateral view. E. Details of oral veil connected to the partly opened rhinophoral sheath, essentially similar to the pattern of oral veil in *Tochuina*. F. Jaws (light microscopy). G. General overview of radula (SEM). H. Central part of the radula with central and lateral teeth showing presence of both denticulated and non denticulated central teeth. I. Details of the larger central teeth with distinct denticulation. J. Details of the smaller central teeth without denticulation. K, L. *D. walteri*, dorsal and ventral views respectively, preserved specimen from the Barents Sea (eastern Spitzbergen region, ZMMU Op-722, length 17 mm). M. *D. walteri*, lateroventral view, preserved specimen (length 14 mm) from the Barents Sea showing details of oral veil connected to the partly opened rhinophoral sheaths. N. Same specimen of *D. walteri*, central part of the radula with larger central teeth without distinct denticulation on lateral teeth. O. Same specimen of *D. walteri*, details of the larger central teeth with and without denticulation on the same teeth. P–X. External views and internal characters of *Heterodoris robusta*, specimen off Greenland and Canadian waters (ZMMU Op-723, length 39 mm) showing details of non bilobed oral veil connected to the partly opened rhinophoral sheaths. P–R. Dorsal, ventral and lateral views respectively (live). S. Details of oral veil (preserved). T. Jaws (light microscopy). U. General overview of radula with unicuspids central teeth and numerous hamate laterals, similar to the tritoniid *Tochuina* (SEM). V. Details of the outer lateral teeth. W. Details of the central part of the radula with central and inner lateral teeth. X. Central part of the radula with central and lateral teeth showing presence of both smooth and weakly denticulated central teeth. Scale bars: G, U– 300 μm, J– 50 μm, N– 80 μm, O– 40 μm. All bibliographic excerpts are not in copyright.

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uncorrected p-distances of the COI marker which separate *T. tetraquetra* from *T. exsulans*, *T. festiva*, and *T. cf. psoloides* are 10.96%, 11.4%, and 7.5% respectively. Minimum uncorrected p-distances of the COI marker which separate *T. exsulans* from *T. festiva*, and *T. cf. psoloides* are 9.09% and 10.88% respectively. Minimum uncorrected p-distances of the COI marker between *T. festiva* and *T. cf. psoloides* are 11.63% (Table 2). Results the ABGD analysis from recursive partitions show two potential species in group “*T.* nilsodhneri” from two different regions, Europe and South Africa, whereas results from initial partitions show only one. The specimens previously identified as “*T. nilsodhneri*” from South Africa therefore likely belong to an undescribed species, pending further revision. Publicly available COI sequences *T. challengeri ana* and *T. antarctica* were revealed by ABGD analysis as two different potential species. *Tritonia antarctica* therefore needs a separate study, including molecular, morphological, and geographical data.

Highly supported clade *Tritoniopsis* (PP = 1, BS = 99) is sister to *Marianina rosea* (PP = 1, BS = 76). All *Marionia* clustered together (PP = 1, BS = 100) in a separate clade. Clade *Tochuina* is sister (PP = 1, BS = 77) to *Tritoniella belli* clade. It is important to note that “*Tritonia* nigritigris” has the closest position to the clade *Tochuina gigantea* and clustered together with the maximal support (PP = 1, BS = 100). The ABGD analysis of the COI data set revealed two potential species: “*Tritonia* nigritigris and *Tochuina gigantea*”. Minimum uncorrected p-distances of the COI marker between *Tochuina gigantea* and “*Tritonia* nigritigris” are 15.83% (Table 2).

Heterodoris robusta and *Doridomorpha gardineri* clustered in two distinct and separated sister clades (PP = 1, BS = 97) that formed the sister group (PP = 0.99, BS = 70) to the *Doridoxa* clade. Two *Doridoxa walteri* and *D. ingolfiana* clustered together in clade with maximal support (PP = 1, BS = 100). ABGD analysis of the COI data set revealed one potential species, combined *D. walteri* and *D. ingolfiana*. Uncorrected p-distances of the COI marker within the *Doridoxa walteri* and *D. ingolfiana* range from 0.15% to 0.91%, indicating that they are the same species (Table 2).

### Analysis of taxonomic history of the North Pacific model species tritoniids

The systematics of the North Pacific species of the family Tritoniidae has a long history.
Table 1. GenBank accession numbers for all sequences used in this study.

| Species name                      | Voucher | Locality    | COI           | 16S          | H3          |
|-----------------------------------|---------|-------------|---------------|--------------|-------------|
| *Armina scotti* Mehrotra, Caballer & Chavanich, 2017 | -       | Thailand    | KX538792      | KX538793     | -           |
| *Armina scotti* Mehrotra, Caballer & Chavanich, 2017 | CASIZ177535 | Philippines | HQ010504      | HQ010539     | HQ010473    |
| *Armina scotti* Mehrotra, Caballer & Chavanich, 2017 | CASIZ177534 | Philippines | HM162696      | HM162606     | HM162512    |
| *Bornella johnsonorum* Pola, Rudman & Gosliner, 2009 | CASIZ175407 | Marshall Islands | JN869445 | JN869401 | JN869419 |
| *Bornella stellifera* (A. Adams & Reeve [in A. Adams], 1848) | CASIZ169989 | Hawaii | HM162703 | HM162623 | HM162529 |
| *Bornella valdae* Pola, Rudman & Gosliner, 2009 | CASIZ176832 | South Africa | HM162706 | HM162626 | HM162532 |
| *Crosslandia viridis* Eliot, 1902 | CASIZ192342 | Saudi Arabia | KF871637 | KF871685 | KF871661 |
| *Doto amyra* Er. Marcus, 1961 | CASIZ181213 | USA: California | KJ486703 | KJ486768 | KJ486674 |
| *Dendronotus dalli* Bergh, 1879 | ZMMU:Op-295 | Kamchatka | KM397001 | KM397083 | KM397094 |
| *Dendronotus irisi J. G. Cooper, 1863* | LACM 174194 | USA: California | KX058082 | GU339188 | KX058110 |
| *Dendronotus frondosus* (Ascanius, 1774) | ZMMU:Op-380 | Norway | KM396976 | KM397056 | KM397111 |
| *Bornella johnsonorum* Pola, Rudman & Gosliner, 2009 | CASIZ179493 | Philippines | JN869451 | JN869407 | JN869430 |
| *Bornella stellifera* (A. Adams & Reeve [in A. Adams], 1848) | CASIZ179375 | Philippines | HM162698 | HM162616 | HM162522 |
| *Doridomorpha gardineri* Eliot, 1903 | CASIZ178233 | Malaysia | HM162695 | HM162605 | HM162511 |
| *Doridoxa walteri* (Krause, 1892) | ZMMU:Op-721 | Russia: Barents Sea | MW139263 | MW144285 | MW158320 |
| *Doridoxa walteri* (Krause, 1892) | ZMMU:Op-722 | Russia: Barents Sea | MW139262 | MW144284 | - |
| *Doridoxa “ingolfiana”* Bergh, 1899 | COPC01052 | Canada: Newfoundland | KX871640 | KX871688 | - |
| *Doto coronata* (Gmelin, 1791) | LACM 174934 | Mexico | JN869406 | JN869433 | - |
| *Doto millbayana* Lemche, 1976 | BELUM Mn33135 | UK | KJ486723 | KJ486762 | KJ486653 |
| *Doto dunnii* Lemche, 1976 | DUN1 | Mediterranean | KX274292 | KX274318 | KX274300 |
| *Doto koenneckeri* Lemche, 1976 | CASIZ178247 | Portugal | HM162673 | HM162658 | HM162567 |
| *Duvaucelia lineata* (Alder & Hancock, 1848) | GNM8890 | Sweden | MG934992 | - | - |
| *Duvaucelia manicata* (Deshayes, 1853) | - | - | KY629602 | KY629592 | KY629606 |
| *Duvaucelia nilsodhneri* Marcus Ev., 1983 | Med1 | Mediterranean | KY629600 | KY629585 | KY629611 |
| *Duvaucelia cf. nilsodhneri* Marcus Ev., 1983 | Atl2 | Atlantic | KY629597 | KY629591 | KY629605 |
| *Duvaucelia plebeia* (G. Johnston, 1828) | CASIZ176218A | South Africa | KP153294 | KP153261 | KP153327 |
| *Duvaucelia plebeia* (G. Johnston, 1828) | CASIZ176218B | South Africa | KP153295 | KP153262 | KP153328 |
| *Duvaucelia striata* (Haefelfinger, 1963) | BAU2696 | Italy | LT596541 | LT596543 | LT615408 |
| *Duvaucelia striata* (Haefelfinger, 1963) | BAU2695 | Italy | LT596540 | LT596542 | LT615407 |
| *Hancockia californica* MacFarland, 1923 | CASIZ175722 | Costa Rica | HM162702 | HM162702 | HM162527 |
| *Hancockia californica* MacFarland, 1923 | LACM 174934 | Mexico | JN869452 | JN869408 | JN869433 |
| *Hancockia uncinata* Hesse, 1872 | SRR372669 | United Kingdom | KX889735 | MK100971 | - |
| *Heterodoris robusta* Verrill & Emerton, 1882 | ZMMU:Op-723 | Canada and Greenland | MW139261 | MW144283 | MW158318 |
| *Lomanotus sp.1* | CASIZ 177751 | Philippines | JN869453 | JN869409 | JN869434 |
| *Lomanotus sp.2* | LACM174962 | Mexico | HM162715 | HM162640 | - |
| *Lomanotus vermiumformis* Eliot, 1908 | SRR3726706 | Panama | KX889740 | MK100978 | - |

(Continued)
| Species name                  | Voucher | Locality          | COI        | 16S        | H3         |
|------------------------------|---------|-------------------|------------|------------|------------|
| Lomanotus vermiformis Eliot, 1908 | CASIZ175963 | Mexico           | -          | -          | JN869435   |
| *Luisella babai* (Schmekel, 1972) | MNCN15.05/53698 | Spain           | HQ616783   | HQ616754   | HQ616717   |
| Marianinia rosea (Pruvot-Fol, 1930) | CASIZ175746 | Philippines      | HM162733   | HM162656   | HM162565   |
| Marionia abrahamorum F. V. Silva, Herrero-Barrencu, Pola & Cervera, 2019 | MB28005053 | Gulf of Guinea   | MH892390   | MH892386   | MH892392   |
| Marionia arborescens Bergh, 1890 | CASIZ177578 | Philippines      | HM162722   | HM162646   | HM162554   |
| Marionia blainvillea (Risso, 1818) | CASIZ176812 | Portugal         | HM162721   | HM162645   | HM162553   |
| Marionia blainvillea (Risso, 1818) isolate 2 | - | - | KY629604 | KY629593 | KY629613 |
| Marianita rosea (Pruvot-Fol, 1930) | CASIZ177540 | Philippines      | HM162725   | HM162648   | HM162557   |
| Marionia elongoviridis V. G. Smith & Gosliner, 2007 | CASIZ177308 | Philippines      | HM162724   | -          | HM162556   |
| 'Marionia gemmii' Almón, Pérez & Caballer, 2018 | - | Spain: Galicia   | K584069    | K584068    | -          |
| Marionia levis Eliot, 1904 | CASIZ192357A | Saudi Arabia     | KP153284   | KP153251   | KP153317   |
| Marionia sp.1 | MB28005057 | Spain: Gulf of Cadiz | MH892391 | MH892389 | MH892395 |
| Melibe leonina (Gould, 1852) | SRR1950947 | USA: CA          | KX889741   | KX889741   | -          |
| Melibe leonina (Gould, 1852) | - | USA: CA, Monterey | KP764764   | KP764764   | -          |
| Notobryon wardi Odhner, 1936 | CASIZ177540 | Philippines      | JN869454   | JN869411   | JN869437   |
| Notobryon sp. | CASIZ176363 | South Africa     | HM162713   | HM162636   | HM162543   |
| Notobryon thompsoni Pola, Camacho-Garcia & Gosliner, 2012 | CASIZ176362 | South Africa     | JN869456   | JN869413   | JN869439   |
| Pleurobranchaea meckeli (Blainville, 1825) | - | Mediterranean Sea, Spain | FJ917499 | FJ917439 | EF133470 |
| Pleurobranchaea meckeli (Blainville, 1825) | - | Spain: Gerona    | AY345026   | AY345026   | -          |
| Pleurobranchus variaus Pease, 1860 | CPC00351 | USA: Hawaii      | KMS521700  | KMS521597  | KMS521625  |
| Samia takashigei Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl & Picton, 2017 | ZMMU:Op-530 | Japan | MFS523384 | MFS523463 | MFS523009 |
| Scyllaea pelagica Linnaeus, 1758 | CASIZ184317 | Philippines      | JN869459   | JN869416   | JN869443   |
| Scyllaea fulva Quoy & Gaimard, 1824 | SRR3726701 | French Polynesia | KX889746   | MK100991   | -          |
| Tethys fimbria Linnaeus, 1767 | - | Spain: Tarragona | AY345035 | AY345035 | EF133468 |
| Tochuina gigantea (Bergh, 1904) | ZMMU:Op-726 | Canada: British Columbia | MW139260 | MW144282 | MW158321 |
| Tochuina gigantea (Bergh, 1904) | - | USA: Washington  | MH243006   | -          | -          |
| Tochuina nigrigris (Valdés, Lundsten & N. G. Wilson, 2018) | LACM 3553 | USA: CA          | MH756138   | MH756133   | -          |
| Tritonia cf. antarctica Pfeffer in Martens & Pfeffer, 1886 | - | Antarctica, Ross Sea | GQ292052 | -          | -          |
| Tritonia challengeriana Bergh, 1884 | CASIZ171177 | Atlantic Ocean: Bouvet Island | HM162718 | HM162643 | HM162550 |
| Tritonia challengeriana Bergh, 1884 | CASIZ189419 | Atlantic Ocean: Falkland Islands | KP153310 | KP153277 | KP153343 |
| Tritonia exsulans Bergh, 1894 | ZMU: Op-720 | USA: Washington  | MW139259   | MW144281   | -          |
| Tritonia exsulans Bergh, 1894 | - | Canada: Vancouver | KP764765 | KP764765 | -          |
| Tritonia exsulans Bergh, 1894 | LACM:2004–16.3 | USA: California | - | GU339203 | -          |
| Tritonia exsulans Bergh, 1894 | LACM: DISCO:4046 | North Pacific Ocean | BOLD: AAW7932 | - | - |
| Tritonia exsulans Bergh, 1894 | - | USA: Washington  | GQ292050   | -          | -          |
| Tritonia festiva (Stearns, 1873) | CASIZ186478 | USA: CA          | KP153291   | KP153258   | -          |
| Tritonia festiva (Stearns, 1873) | SRR1950941 | USA: CA          | KX889748   | MK100994   | -          |
| Tritonia festiva (Stearns, 1873) | - | USA: Washington  | GQ292051   | -          | -          |
| Tritonia festiva (Stearns, 1873) | CASIZ174491 | USA: Oregon      | HM162719   | -          | HM162551   |
| Tritonia festiva (Stearns, 1873) | CASIZ173748 | USA: CA          | -          | KP153270   | KP153336   |
| Tritonia hombergii Cavier, 1803 | ZMMU: Op-724 | Norway           | MW139258   | MW144280   | MW158319   |

(Continued)
Table 1. (Continued)

| Species name | Voucher | Locality       | COI      | 16S      | H3     |
|--------------|---------|----------------|----------|----------|--------|
| Tritonia hombergii Cuvier, 1803 | MT09685 | North Sea | KR084797 | -        | -      |
| Tritonia hombergii Cuvier, 1803 | GNM: Gastr8763V | Sweden | MG934917 | -        | -      |
| Tritonia hombergii Cuvier, 1803 | GNM: Gastr 8761V | Sweden | MG935087 | -        | -      |
| Tritonia tetractera (Pallas, 1788) | SIO-BIC M12395 | USA | MH756139 MH756134 MH756145 |
| Tritonia tetractera (Pallas, 1788) | ZMMU:Op-719 | Russia: Kamchatka | MW139257 MW144279 |
| Tritonia tetractera (Pallas, 1788) | ZMMU:Op-725 | Canada: British Columbia | MW139256 MW144278 |
| Tritonia cf. poloides Aurivillius, 1887 | CASIZ181055 | Costa Rica | -        | HM162642 HM162549 |
| Tritoniella belli Eliot, 1907 | N31D | Antarctica | GU227111 GU227002 |
| Tritoniopsis elegans (Audouin, 1826) | CASIZ99298 | Japan | KPI53314 KPI53281 KPI53347 |
| Tritoniopsis frydis Er. Marcus & Ev. Marcus, 1970 | CASIZ181156 | Bermuda | KPI53311 KPI53278 KPI53344 |
| Tritoniopsis frydis Er. Marcus & Ev. Marcus, 1970 | SRR1950954 | USA: Florida | KX889749 MK08234 |
| Tritoniopsis sp. | CASIZ191453A | Papua New Guinea | KPI53312 KPI53279 KPI53345 |
| Tritoniopsis sp. | CASIZ191453B | Papua New Guinea | KPI53313 KPI53280 KPI53346 |

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Table 2. Intraspecific (highlighted in bold) and interspecific uncorrected p-distances (range, %) for COI marker in species of genera Tritonia, Tochuina, and Doridoxa.

| Tritonia exsulans | Tritonia festiva | Tritonia tetractera | Tritonia cf. poloides | Tritonia hombergii | Tritonia challengerianna | Tritonia cf. antarctica | Tochuina gigantea | Tochuina nigritigris | Doridoxa walteri | Doridoxa "ingolfiana" |
|------------------|------------------|---------------------|---------------------|-------------------|------------------------|------------------------|-----------------|--------------------|----------------|----------------------|
| 0–1.01 | 9.09–10.46 | 10.96–11.80 | 10.88–11.09 | 16.64–17.35 | 15.83–16.72 | 16.28–16.49 | 18.11–18.72 | 20.24–20.57 | 21.16–21.75 | 20.55–21.08 |
| Tritonia festiva | 9.09–10.46 | 0.61–2.81 | 11.4–12.94 | 11.63–12.2 | 15.0–16.89 | 15.37–16.88 | 15.22–16.91 | 19.17–19.79 | 18.35–19.94 | 18.84–20.55 | 18.18–19.94 |
| Tritonia tetractera | 10.96–11.80 | 11.4–12.94 | 0–0.46 | 7.5–8.07 | 15.04–16.13 | 15.37–15.76 | 15.86–16.28 | 18.57–19.18 | 19.48–19.94 | 19.63–19.79 | 19.33–19.48 |
| Tritonia cf. poloides | 10.88–11.09 | 11.63–12.2 | 7.5–8.07 | - | 15.2–15.76 | 15.2–15.38 | 15.51 | 17.64–17.82 | 17.82 | 18.57 | 18.2 |
| Tritonia hombergii | 16.64–17.35 | 15.0–16.89 | 15.04–16.13 | 15.2–15.76 | 0–0.64 | 18.72–20.38 | 17.76–18.39 | 17.44–18.42 | 18.82–19.79 | 20.8–21.18 | 20.16–20.55 |
| Tritonia challengerianna | 15.83–16.72 | 15.37–16.88 | 15.37–15.76 | 15.2–15.38 | 18.72–20.38 | 2.71 | 13.32–13.74 | 17.83–18.42 | 17.99–19.03 | 20.55–21.82 | 20.7–21.82 |
| Tritonia cf. antarctica | 16.28–16.49 | 15.22–16.91 | 15.86–16.28 | 15.51 | 17.76–18.39 | 13.32–13.74 | - | 18.39–18.6 | 18.6 | 20.08–20.3 | 19.66 |
| Tochuina gigantea | 18.11–18.72 | 19.17–19.79 | 18.57–19.18 | 17.64–17.82 | 17.44–18.42 | 17.83–18.42 | 18.39–18.6 | 0.15 | 15.83–15.98 | 20.7–21.0 | 20.24–20.4 |
| Tochuina nigritigris | 20.24–20.57 | 18.35–19.94 | 19.48–19.94 | 17.82 | 18.22–19.79 | 17.99–19.03 | 18.6 | 15.83–15.98 | - | 21.61–21.77 | 20.85 |
| Doridoxa walteri | 21.16–21.75 | 18.84–20.55 | 19.63–19.79 | 18.57 | 20.8–21.18 | 20.55–21.82 | 20.08–20.3 | 20.7–21.0 | 21.61–21.77 | 0.15 | 0.76–0.91 |
| Doridoxa "ingolfiana" | 20.55–21.08 | 18.18–19.94 | 19.33–19.48 | 18.2 | 20.16–20.55 | 20.7–21.82 | 19.66 | 20.24–20.4 | 20.85 | 0.76–0.91 | - |

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Peter Simon Pallas [38] described the first northern Pacific tritonii under the name *Limax tetraquetra* (*now Tritonia tetraquetra*) from the North Kuril Islands (Fig 2A and 2B). Pallas did not collect himself the materials that he published in 1788. The collection and descriptions were performed by Georg Wilhelm Steller. He was a naturalist who participated in the Vitus Bering’s Second Kamchatka Expedition [39]. Steller had a short trip to three northernmost Kuril Islands in May–June 1743 [40]. Detailed descriptions of invertebrates (including *Tritonia tetraquetra*), fishes and the sea-otter were made by Steller during Kuril and South Kamchatka voyages. After Steller’s premature death some of his data were published by Pallas [39, 41].

Only after a century, *Limax tetraquetra* was attributed to the genus *Tritonia* by Bergh in 1879 [42] and this species name was applied to a specimen from Unalaska Island. On the first line of the description of *T. tetraquetra* Bergh ([42]: p. 98) noted that “this species was detected by Pallas...”. However, an incorrect comparison with the Pallas’s description is given in a footnote: “the form of the mandibulae is rather similar to the figure in Pallas ([38]: Fig 22)” ([42]: p. 102). The jaws (mandibulae) of *T. tetraquetra* as described by Bergh [42: p. 102] considerably different from the jaws shape in the original description of *L. tetraquetra* by Pallas ([38]: Fig 22) (Fig 2B). No any other evidence for the similarity of Unalashka’s and Kuril’s specimens was given. Later on, Bergh (1904, [43]) described a new species *Tritonia gigantea* based on a collection from Unalaska Island too. *Tritonia gigantea* description is a nearly identical to the *T. tetraquetra* description from Bergh (1879, [42]).

According to the original description of *Limax tetraquetra* by Pallas ([38]: 237–239) this species possesses bilobed oral veil with distinct processes, a high body without traces of lateral edges of notum (Fig 2A) and elongate-oval jaws (Fig 2B). To contrary, *T. tetraquetra* and *T. gigantea* described by Bergh in 1879 and 1904 [42, 43] have non-bilobed oral veil without distinct appendages (only with tubercles), low body with strongly projected lateral sides of notum (Fig 3A and 3B) and nearly square short jaws (Fig 3F). Thus Bergh in 1879 [42] incorrectly attributed Pallas’s *Limax tetraquetra* to the genus *Tritonia*. However, all subsequent authors [15, 22, 44–48] followed Bergh’s decision. Odhner [23] in a review of the family Tritoniidae, proposed a new genus *Tochuina Odhner, 1963 for Tritonia gigantea* sensu Bergh, 1879 in a combination “*Tochuina tetraquetra*”. *Tritonia gigantea* was considered as a synonym of “*Tochuina tetraquetra*” [47, 48].

Unlike the genus *Tochuina* (Fig 3), the outlined above characters of the real *Limax tetraquetra* shared much in common with the genus *Tritonia* (Fig 2). The inappropriate application of the characters of the genus *Tochuina to Limax tetraquetra* persisted until recently. Afterwards, *Limax tetraquetra* was re-assigned as *Tritonia tetraquetra*, and type species of the genus *Tochuina* was re-designated to *Tochuina gigantea* [17, 18]. In the present study we for the first time present morphological data on the *Tochuina gigantea* holotype from the Natural History Museum of Denmark (ZMUK GAS-2011, not suitable for molecular research) (Fig 3A–3D) and molecular and morphological data for the recently collected specimen *Tochuina gigantea* from British Columbia (Fig 3J–3R). Results clearly confirm that *T. gigantea* displays morphological characters that were incorrectly assigned to *Limax tetraquetra* by Bergh [42] and Odhner [23]. “*Tritonia* gigantea (original binomen in Bergh, 1904, [43]) thus belongs to the genus *Tochuina* and according to the present analysis shows a separate phylogenetic lineage within the family Tritoniidae, distantly related to the genus *Tritonia* (see Fig 1A and 1B and a taxonomic synopsis below).” *Limax* tetraquetra (original binomen in Pallas, 1788 [38]) instead belongs to a clade closely related the type species of the genus *Tritonia, T. hombergii* Cuvier, 1803 (Fig 1A).

Only a single large-sized species of *Tritonia* was previously recognized from the shallow waters of the NW Pacific and Kuril Islands, the type locality of *Tritonia tetraquetra* [17, 18, 49]. It is *Tritonia diomedea* Bergh, 1894. This species was described by Bergh (1894 [50]) based
on deep-sea specimens from California and the Shumagin Islands. In the same publication Bergh [50] also described a shallow water species from California under the name *Tritonia exsulans* (Fig 4). This species was recognized in a few works [45–47] but then *T. exsulans* was commonly considered as a synonym of *T. diomedea* [15]. This decision was made because of a putative absence of distinguishing characters between *T. diomedea* and *T. exsulans*. Three more species were described from NW Pacific, namely *Tritonia psoloides* Aurivillius, 1887 [51], *T. septemtrionalis* (Baba, 1937) [52], and *T. primorjensis* Minichev, 1971 [53] (Fig 5). One more species, *T. gilberti* (MacFarland, 1966) [47], was described from NE Pacific. All of them were considered as synonym of *T. diomedea* [15] without detailed analysis. This decision has persisted in the literature on the North Pacific fauna until recently, including colour guides, ecological and neurobiological experimental works.

Among the large North Pacific tritoniids, species in *Tritonia* and *Tochuina* genera were most widely used as neuroscience model systems. Majority of the neurobiological research in *Tritonia* were done both on NE and NW coasts of the North Pacific (see list of works in [15]), using the species name “*T. diomedea* Bergh, 1894”. “*Tochuina* tetraquetra (Pallas, 1788)” sensu Bergh 1879, not Pallas 1788, has been also studied for neural basis of behaviour [8, 13].

**Morphological and molecular delimitations of *Tritonia* model species from the northern Pacific**

Species delimitation based on morphological characters between *T. tetraquetra* sensu Pallas not Bergh (previously identified as “*T. diomedea*”) and *T. exsulans* was uncertain [47, 48] or considered as not reliable [15]. In the present study robust molecular differences (Fig 1A), and considerable differences in fine morphology of the masticatory edges of the jaws between real *Tritonia tetraquetra* and *T. exsulans* were revealed (Figs 2I–2K, 4G and 4H). Bergh in 1894 ([50]: plate 4, Fig 2) clearly figured smaller irregular and polygonal elements for *Tritonia diomedea* that transit to larger tablet-like narrow endings of the tightly packed long bristle-like elements on the masticatory edge of the jaws (Fig 2V). These elements can be also described as long, narrow, flattened spines (Fig 2I–2K), but not as massive cones (Fig 4G and 4H). The details of the masticatory edge of *T. diomedea* readily differ from distinct conical elements throughout entire masticatory edge, without table-like endings in *Tritonia exsulans* (Fig 4G, 4H and 4M). In the original description of *T. diomedea* “4–5 quincunx rows of small cones gradually change into a number of fine tablets on the outside” are mentioned (Bergh, 1894 [50]: p. 148) (Fig 2V). This description well matches to our scanning electron microscopic data for *T. tetraquetra*: initially polygonal and irregular conical rows of elements (Fig 2I–2K) ended up with long, narrow plates. This structure after dissection of the masticatory edge turned to be tightly packed clusters of bristle-like, elongated elements (Fig 2H and 2I). The polygonal elements (with missing bristles, Fig 2K) are figured also in [19] for a *Tritonia* sp. from the deep waters (587–610 m) off Oregon. The mentioned *Tritonia* sp. molecularly matches to our confirmed specimens of *T. tetraquetra* from shallow waters of Kamchatka and British Columbia (Fig 1A). These important results are the first confirmation that *Tritonia tetraquetra* has a very broad geographic and bathymetric range, as it was suggested earlier [17, 18]. Instead, *T. exsulans* (Bergh, 1894 [50]: plate 3, Fig 11) (Fig 4M) has the massive distinct conical elements embedded into masticatory edge and it is impossible to reveal any elongate bristle-like elements after dissection of the edge (Fig 4G and 4H). Bergh [50] also clearly figured these elements in *T. exsulans* ([50]: plate 3, Fig 11) and described it (compare to *T. diomedea*) in a different way: “10–12 rows of polygonal flat plates, of which those of the 3–4 innermost rows forming stronger, lower oblique cones” ([50]: 151) (Fig 4M). McDonald in 1983 [15] dismissed taxonomic importance of these differences using previously published light microscopic data.
In the present study these differences were confirmed using scanning electron microscopic data (Figs 2G–2K and 4F–4H). The patterns of the masticatory edges elements are stable and easily identifiable in both first descriptions of *T. diomedea* and *T. exsulans* respectively [50]. Thus, *Tritonia tetraquetra* (and its junior synonym *T. diomedea*) is a different from *T. exsulans* species.

According to the integrative molecular and morphological data *T. tetraquetra* and *T. exsulans* definitely represent two distinct separate species (Figs 1A, 2 and 4). There is only available syntype of *T. diomedea* in the Natural History Museum of Denmark (ZMUK, GAS-2034) that originated from a location in California (Fig 2Q–2T). This type specimen comes from essentially the same depth (676 m vs 587–610 m) with a molecularly confirmed specimen of *T. tetraquetra* from geographically close to the California waters of Oregon (“*Tritonia sp.‘” in [19]: 410; present study, Figs 1A and 6). All reliable records of *T. exsulans* [47, 50, present study] do not exceed the depth about 100 meters. Instead, the confirmed *T. tetraquetra* (Figs 1A and 6) has a very broad bathymetric range, from shallow waters (5–10 m) up to at least 610 m depth.

Another syntype specimen of *T. diomedea* mentioned in Bergh [50] from deep waters of Alaska (the Shumagin Islands) is lost [54]. The saved syntype of *T. diomedea* (ZMUK, GAS-2034) from deep waters of California is designated here as lectotype of *T. diomedea* (Fig 2Q–2S) to avoid a potential confusion of “*T. diomedea*” in original sense of Bergh [50] with some potential hidden molecular lineages within *T. tetraquetra*.

Based on the present analysis we therefore able to reveal a clear presence of two species in the waters of NE Pacific that formerly were known under the name of “*T. diomedea*”; *T. tetraquetra* (= *T. diomedea*) and *T. exsulans* (Figs 1A and 6). *T. exsulans* is more warm water adapted species because distributes to the southern California and never reliably recorded from the colder NW Pacific. Herein the molecular data throughout all the geographic range of *T. exsulans* from California to British Columbia applied (Fig 6). Therefore it is reliably estimated that *T. exsulans* was also used for neurobiological experiments, commonly hold at the Friday Harbor Laboratories [8, 20]. Because in the region of the Friday Harbor *T. tetraquetra* and *T. exsulans* can co-occur (Fig 6), both species could be used as model systems for neurobiological and related research at NE Pacific coast.

Another known nominative species of *Tritonia* from NW Pacific, *T. septemtrionalis* (Baba, 1937) [52] was described from the relatively shallow waters (82 m) of the Okhotsk Sea near Kamchatka region. According to the morphological data, depicted in Baba ([52]: Fig 1F), *T. septemtrionalis* shows a not dissected massive of the long bristles on masticatory edges of the jaws. According to morphological and distributional data *T. septemtrionalis* can be therefore considered as a junior synonym of *T. tetraquetra*. Present molecular data (Fig 1A) confirmed only true *T. tetraquetra* in the shallow waters of the neighbouring Kamchatka region.

Further molecular lineages, closely related to real *T. tetraquetra* can be potentially revealed in NW Pacific. For example, in the present study we revealed the publicly available sequence from the Bering Sea that somewhat diverges from *T. tetraquetra*, but definitely belongs to the same clade (Figs 1A and 6). Because the only single sequence is available on GenBank and we have no possibility conduct morphological analysis, we can not absolutely confidently confirm this species. However, potentially it belongs to *T. psoloides* Aurivillius, 1887 [51], a species of *Tritonia* that was described from the Bering Sea. The type specimen of *T. psoloides* comes from the depth about 140 m, whereas potential *T. psoloides* specimen comes from 402 m depth (Fig 6). Therefore the same species may habitat in the same locality and similar bathymetric range. The type specimen of *T. psoloides* is in the Swedish Museum of Natural History and was available for the present study (Fig 5A–5E). The external appearance (Fig 5A–5C), radular teeth (Fig 5F) and bathymetric distribution of *T. psoloides* ([51]: p. 373, plate 13, Fig 20; present study) are generally consistent with *T. tetraquetra*. Therefore, *T. psoloides* is an available name...
for that potentially hidden lineage (Fig 1A) close to *T. tetraquetra* in the Bering Sea. We therefore do not consider *T. psoloides* as a synonym of *T. tetraquetra* and preliminary assign a specimen from the Bering Sea with available molecular data to *T. cf. psoloides* (Figs 1A and 6) pending a further study.

There is one more species from NW Pacific, *Tritonia primorjensis* Minichev, 1971, described from the Sea of Japan (Fig 5G–5V). Molecular data were not available for this species due to formalin fixation, but morphologically *T. primorjensis* is consistent with *T. tetraquetra* in absence of the white lines and presence of bristle-like elements on the masticatory edges of the jaws ([53]; present study, Fig 5M and 5N). Minichev ([53]: p. 282) thus correctly indicated the presence of the clusters of narrow masticatory elements (Fig 5S and 5T), but incorrectly estimated its differences from *T. tetraquetra* (= *T. diomedea*). We specially studied the morphology of the jaws in potential *T. primorjensis* from the Sea of Japan. The specimens of *T. primorjensis* were collected in the Peter the Great Bay (the Sea of Japan) and used by Soviet neurophysiologists in the 1960s – 1980s for experimental works. We confirm here the basic similarity of *T. primorjensis* to *T. tetraquetra* using our scanning electron microscopic data (Figs 2I, 2J, 5M and 5N) and correctness of the Minichev’s description of the masticatory edge of the jaws with long thin spines (Fig 5T). However, *T. primorjensis* differs from *T. tetraquetra* by longer spines at the masticatory edges (Figs 2I, 2J, 5M and 5N) and more intensive reddish-orange body colouration compare to the orange (with a slight reddish tinge) or yellow *T. tetraquetra* from British Columbia and Kamchatka. *T. primorjensis* potentially represents a further hidden lineage close to *T. tetraquetra*. Therefore *T. primorjensis* not included into synonymy of *T. tetraquetra* (a taxonomic diagnosis see below). The smaller difference in colouration is consistent with the modern agenda of the fine-scale species differentiation, but needs an additional testing in this *Tritonia* species complex. Thus, Russian neurophysiologists worked exclusively on a species that closely related to *T. tetraquetra*, but definitely not on *T. exsulans*, which does not occur in NW Pacific (Figs 1A and 6). Renewed taxonomic diagnoses for *T. tetraquetra* and *T. exsulans* are provided below.

**Taxonomic diagnoses of *Tritonia tetraquetra* and *T. exsulans***

*Tritonia tetraquetra* (Pallas, 1788)

Fig 2

*Limax tetraquetra* Pallas, 1788 [38]: 237–239, pl. 5, Fig 22

*Tritonia tetraquetra* (in original sense of Pallas, 1788)–Martynov, 2006a [17]: 280, pl. 134 F–H; Martynov, 2006b [18]: 69

*Tritonia diomedea* Bergh, 1894 [50]: 146–150, pl. 2, Figs 10, 11, pl. 3, Figs 6–10, pl. 4, Figs 1–5

*Tritonia diomedea*–O’Donoghue, 1921 [55]: 151–152, pl. 7, Figs 1–3; Volodchenko, 1955 [56]: 249, pl. 48, Fig 3; Veprintsev et al., 1964 [6]: 327–336, Figs 1, 2; Borovyagin and Sakharov, 1968 [7]: 3 (a mistake in spelling (“diomedia” vs. correct “diomedea” appeared after O’Donoghue (1921) [55] in numerous further publications, the list in McDonald, 1983 [15])

*Tritonia diomedea* sensu Thompson, 1971 [47] and McDonald, 1983 [15] and auct.–partim. (mixture with *T. exsulans*)

*Duvaucelia (Duvaucelia) septemtrionalis* Baba, 1937 [52]: 391–392, text Figs 1a–1e

Non *Tritonia tetraquetra* sensu Bergh, 1879 [42] and auct. (= *Tochuina tetraquetra* sensu Odhner, 1963 [23])
Diagnosis. Colouration commonly yellow-orange to dark orange with a slight reddish tinge. No white lines along edges of lateral side between dorsolateral appendages and on oral veil. White line on edge of foot absent. Masticatory processes of jaws with clusters of bristle-like thin elongate plates. Radular formula ca. 40–73 x 150–50.1.50–150. Seminal receptacle small, oval, with long thin stalk and large wide, rounded bag-like base. Copulative organ massive, folded. Body length up to 300 mm. Confirmed bathymetric range about 1–700 m (potentially to about 1000 m).

Remarks. Type materials for *T. tetraquetra* are not traceable [17]. Neotype for *T. tetraquetra* is designated here (NW Pacific, Kamchatka Peninsula, ca. 10 m depth, stones, 11.08.2008, coll. Tatiana Korshunova, Alexander Martynov (ZMMU Op-719), live length 120 mm, preserved length 80 mm. Sources of the morphological data used for the diagnosis are [17, 38, 50] and present study (Fig 2C–2S). Details of taxonomic and phylogenetic position of *T. tetraquetra* and differences from related species see above.

**Tritonia exsulans** Bergh, 1894, reinstated

* Tritonia exsulans* Bergh, 1894 [50]: 150–152, pl. 3, Figs 11–12, pl. 4, Fig 6; O’Donoghue, 1921 [55]: 152–154, pl. 7, Figs 4–6; MacFarland 1966 [47]: 226–235, pl. 30, Figs 9, 10, pl. 39, Fig 7, pl. 43, Figs 20–26; pl. 44, Figs 3, 4; pl. 45, Figs 9–13.

?*Duvaucelia gilberti* MacFarland, 1966 [47]: 223, 224, 235–243, pl. 30, Figs 1–2, pl. 43, Figs 27–36, pl. 44, Fig 5, pl. 45, Fig 6.

Non *Tritonia exsulans* sensu Thompson, 1971 [48] (mixture with *Tritonia tetraquetra*)

Non *Tritonia exsulans* sensu McDonald, 1983 [15] and auct. (incorrect synonymy with *T. tetraquetra*)

Non *Tritonia exsulans* sensu Baba, 1937 [57] (uncertain species attribution, possible *T. tetraquetra*)

Diagnosis. Colouration commonly pinkish to reddish salmon. White lines along edges of lateral side between dorsolateral appendages and on oral veil present. White line on edge of foot present. Masticatory processes of jaws with oval to conical strong elements, no thin clusters of bristle-like elements. Radular formula ca. 39–55 x 64–82.1.82–64. Seminal receptacle relatively large with long stalk and apparently with bag-like base. Copulative organ elongate with a circular fold. Body up to 200 mm. Confirmed bathymetric range about 5–100 m.

Remarks. Type materials for *T. exsulans* are not traceable (ZMUK type collection [54]). Neotype is designated here (NE Pacific, Port Orchard, Rich Passage, Washington, USA, 9.1 m depth, stones, 29.04.2017, coll. Karin Fletcher (ZMMU Op-720, live length 20 mm, preserved length 13 mm). Sources of the morphological data used for the diagnosis are [47, 50, 55] and present study (Fig 4A–4L). *Duvaucelia gilberti* (currently accepted as *Tritonia gilberti*) was described from the same geographic area with *T. exsulans* and MacFarland mentioned that in both species “The color is similar” ([47]: p. 242). The apparent main difference between *T. exsulans* and *T. gilberti* is in morphology of the copulative apparatus ([47]: p. 243), but listed differences are uncertain because indicated presence of variously expressed circular folds in both species. MacFarland [47] also found a minute armature in *T. exsulans* copulative organ, but this was not indicated in the original description of *T. exsulans* [42] and was not confirmed by further studies [48]. The presence of a widened base of the receptaculum seminis did not figured by MacFarland for *T. exsulans*, but indicated for *T. gilberti* ([47]: plate 44, 5). Therefore, with some reservation *T. gilberti* is considered here as a junior synonym of *T. exsulans*. 
Uncorrected p-distances values of the COI marker within the *T. exsulans* group range from 0 to 1.01% (Table 2), indicating low heterogeneity within all available *T. exsulans* molecular data on a broad geographic range from California to British Columbia.

### Origin of the generic name Tochuina and the role of Tritonia tetraquetra in human culture: A clarification and refuting

In the beginning of the original description of *Limax tetraquetra* from the northern Kuril Islands, Pallas ([38]: 237) indicated that “...ubi crudum coctumque edunt et Tochui appellant incolae.” (= [this species] was eaten as raw or cooked by local inhabitants). This short indication has been repeated as evidence that Ainu people (major inhabitants of the Kuril Islands at that time) was eating *T. tetraquetra* [22, 42, 58]. Incorrect subsequent spelling ”tochni” for the Ainu word (originally spelled by Pallas as ”tochui”) was also appeared in the listed works. Odhner [23] for the first time after Pallas [38] used original spelling *tochui* to name newly proposed genus *Tochuina* Odhner, 1963. Because of a long term confusion over real identity the Pallas’s original name *Limax tetraquetra* and misidentify it with the different tritoniid *Tochuina gigantea* [43] (see details above) the potential including of a large tritoniid into human diet was incorrectly attributed not to *Tritonia tetraquetra*, but to *Tochuina gigantea* [22, 42, 58]. The clarification of this issue is necessary to resolve persisted taxonomic confusion between *Tritonia* and *Tochuina* and this is therefore important part of the present study. The details are provided below.

Ainu were indigenous people of the Kuril Islands having their highly isolated language and unusual for Northeastern Asian ethnic groups external appearance [59, 60]. Fishing and hunting of marine mammals were important for the Ainu [61]. Besides, they collected a number of marine invertebrates for food. Several particular names for shelled molluscs have been attested for the Ainu language [62]. However, majority of nudibranchs including tritoniids, possess a strong chemical-based defense system [63]. Therefore proposals for an edible *Tritonia* (“Tochuina”) initially appeared as unrealistic. Pallas in the first description of *T. tetraquetra* [38] mentioned the vernacular Ainu name “tochui”. We need to investigate is that name was really used in Ainu language for nudibranch molluscs, or this is a case of a subsequent incorrect usage. For this purposes we used several existed Ainu dictionaries, including a compilative Dobrotvorsky dictionary [62] and a special dictionary with a comprehensive list of the Ainu names for animals [64]. The dictionary compiled by M.M. Dobrotvorsky encompasses words from many sources, including old Japanese and Russian sources and his own lexical data from the Sakhalin region [62]. Notably, in [62] we were able to identify at least two Ainu words which are considerable similar to the mentioned in Pallas [38] word *tochui*. These words are *totsui* and *togoi* [62]. The word *totsui* is indicated as “a molluscous animal”, but without mentioning of a particular species. Another potential word, *togoi* defined as “as a sea inhabitant with two teats; Ainu eat its viscera...”, with an addition “...a soft-bodied animal?[sic]” ([62]: p. 326). Such features can be reliably associated with two siphons of solitary ascidians, but not with molluscs. For instance, *this can be attributed to a common North Pacific ascidian species Halocynthia aurantium* (Pallas, 1788). *H. aurantium* was described (based on materials from Steller), remarkably, in the same publication of Pallas ([38]: pp. 246–247), a few pages after description of *T. tetraquetra*. *Halocynthia aurantium* shares with *T. tetraquetra* similar orange colouration and Steller definitely collected ascidian *H. aurantium* and nudibranch *T. tetraquetra* during the same expedition to the northern Kuril Islands. Thus, Steller could be informed by Ainu people with a vernacular ascidian name (similar to *totsui/togoi*) because nudibranch *T. tetraquetra* has the same colour and after taken out of water was similar to ascidian *H. aurantium*. It is also possible that a subsequent confusion arose after Steller applied the indigenous
name for ascidian or nudibranch in his diaries. Ascidian *H. aurantium* is still used for food in the neighbouring Japanese Islands and available on the modern Japanese fish markets [65], which was confirmed during our recent expeditions to Japan.

Furthermore, a word similar in spelling to *totsui* and *tochui* (トツ°イ in katakana) is recorded in a comprehensive Ainu dictionary of the animal names [64] precisely for ascidians, and in a modern Japanese source [66] also for an ascidian species, *Halocynthia roretzi* (Drasche, 1884). Importantly, in XIX century the chordate ascidians still were incorrectly assigned to "molluscs". That explain the indication of "molluscous or soft-bodied animals" for both *togoi* and *totsui* ([62]: pp. 326, 334). Thus, similarity of both Ainu words mentioned in the Dobrotvorsky’s dictionary as well as *totsui* in the Japanese sources [64, 66] to the Ainu word *tochui* indicated in the Pallas’s original description of *T. tetraquetra* can not be considered as an occasional one. Differences in spelling *tochui/totsui/togoi* can be potentially explained by dialectal differences, previously attested for the Kuril, Sakhalin and Hokkaido Ainu language [67] or by peculiarities of the Latin transliteration by Steller (or subsequently by Pallas) of an original spoken Ainu word. The Ainu dialect of the North Kuril Islands (type locality of *T. tetraquetra* [38]) is extinct, but available data indicate its mixing lexical composition with several Ainu dialects (including Sakhalin), and also a closer relationship with some Hokkaido dialects [e.g., 68]. All these data provide a strong evidence that while Ainu people reported to Steller the name *tochui/totsui/togoi* they mentioned an edible ascidian (most likely, *H. aurantium*), and not inedible nudibranch *T. tetraquetra*. We therefore disprove the common indication [22, 23, 42] about “edible Tritonia tetraquetra”. As a taxonomically important implication, the name *tochui* was incorrectly applied by Odhner ([23]: p. 50) to the species that currently known as *Tochuina gigantea*, and not *Tritonia tetraquetra* in the original sense of Pallas. All above information confirms that tritoniid species found by Steller on the Kuril Islands is *Tritonia*, but not a *Tochuina*.

**Consolidated data on phylogeny and taxonomy of the family Tritoniidae**

Currently the classification of the family Tritoniidae is ambiguous and a modern taxonomic synopsis integrating morphological and molecular data is lacking. Odhner [23] and Marcus [69] recognized several genera and subgenera among tritoniid taxa. Thompson and Brown [1], Gosliner and Ghiselin [70] nevertheless used single genus *Tritonia*. However, some genera, e.g. *Tritoniopsis* Eliot, 1905 is continued to use currently. Instead, other taxa, e.g. *Tochuina* are sometimes omitted [19] in spite of the current valid status [71]. The genus *Marionia Vayssière, 1877* is different from other tritoniid taxa because of presence of stomach plates, but otherwise contains numerous species with disparate morphology, and molecular phylogenetic data show within *Marionia* at least two distinct clades [5, 72]. The taxonomy of the type tritoniid genus *Tritonia* has been especially controversial. Several disparate morphological groups supported by molecular data currently falls within single genus *Tritonia*. Thus, an assessment of the taxonomic placement of the model species of the North Pacific tritoniids is impossible without a broad-scope phylogenetic framework for the family Tritoniidae. Otherwise it is not possible clearly indicate for what reason the model species *Tritonia tetraquetra* was assigned to the genus *Tochuina* for a long time and why *Tochuina* has some intriguing external and internal similarities to the non-tritoniid species *Doridoxa* Bergh, 1899 and *Heterodoris* Verrill et Emerton, 1882 (Figs 1A, 1B, 3 and 7). Furthermore, a morphologically highly aberrant genus *Marianina* (having the bifid cerata-like processes instead of typically branched tritoniid dorsolateral appendages) is a sister to *Tritoniopsis*. The genus *Tritoniopsis* in turn is morphologically more similar to the type species of the genus *Tritonia* but phylogenetically is more close to the genera *Marianina, Tochuina* and *Tritoniella* (Fig 1A and 1B). Thus, unbalanced
and a non-integrative classification regarding morphological and molecular data on various tritoniid lineages will emerge if the all tritoniid diversity will be united into a single genus *Tritonia*. Therefore, herein the taxonomic synopsis of the family Tritoniidae is presented and accommodates modern morphological and molecular discrepancies.

Previously, several working rules for integration of morphological and molecular data to produce a consistent classification were outlined [73]. One of the main principle, it is avoidance of large taxa containing many species. Another important rule, it is separation of a morphologically aberrant taxon, when it phylogenetically nested within taxa with disparate morphology. The present phylogeny of the family Tritoniidae is highly relevant case for application of such proposals. If a broad-taxa approach was applied, an inevitable synonymization of the almost all tritoniid diversity (including taxa *Tochuina* and *Marianina*) into a single genus must be used. The argumentation on the “presence of intermediate forms” [70] is not a relevant one because evolution does not proceed with an “intermediate” forms in its straightforward understanding. Phylogeny shows a complicated mosaic of various characters at many levels of phylogenetic differentiation in an ontogenetic framework. The evolutionary key linking taxa can be found, e.g. *Onchimira* that links cryptobranch and phanerobranch dorid nudibranchs [74], but this is not a reason for synonymization of morphologically highly disparate taxa. A potential lumping decision will mask taxonomic diversity and can be very impractical for potential descriptions on any further new species. The strict apomorphy-based diagnoses are also problematic. Any apparent apomorphy (as in case of tritoniids, a broad body or tricuspid central teeth) can change or disappear in a crown group due to the ontogenetic process of paedomorphosis [24]. For example it can be reliably applied to the sister tritoniid genera *Marianina* and *Tritoniopsis* demonstrating the profoundly different morphology at adults stages (Fig 1A and 1B). The application of narrow-defined genera makes focus on hidden diversity among particular, smaller lineages. Recognition of the monotypic genera is also in line with the consistent morphological and molecular units. This proposal is a practical one, because even supporters of a lumping classification now separate a morphologically disparate monotypic nudibranch genus *Bonisa* from a large paraphyletic assemblage of the genus *Janolus* [75].

Small genera and families are the reliable way to propose maximally coherent taxonomic units to accommodate morphological and molecular data [73, 76]. The long term confusion between two large common North Pacific tritoniid taxa *Tritonia tetraquetra* (previously known as “*Tritonia diomedea*”) and *Tochuina gigantea* (previously known as “*Tochuina tetraquetra*”) well illustrates the need to use this approach. The misidentification between these two distantly related tritoniid clades, *Tochuina* and *Tritonia* has persisted for more than a century. In the present study using combination of morphological data from the type specimen and recent molecular data we finally evidently demonstrate the significant differences between *Tochuina* and *Tritonia* in a broad phylogenetic framework (Figs 1–6). Compare to the genus *Tritonia*, genus *Tochuina* has distinctive features as presence of numerous, small, branched dorsolateral appendages, oral veil without elongate processes, and unicuspid central teeth (see Figs 2–4). Other genera of tritoniids, e.g. *Tritoniopsis* and *Tritoniella* may also possess unicuspid central teeth, but they differ from *Tochuina* by combination of other features. These characters include presence of fewer large branched appendages in *Tritoniopsis* and absence of distinctly branched dorsolateral appendages in *Tritoniella*. Such diagnoses are more complex than traditional ones (based on a searching for strict differences), but much finer encompasses the molecular and morphological data. Also, the genus *Tochuina* shows external similarity to the distantly related non-tritoniids *Doridoa* and *Heterodoris* (compare Figs 3H–3L, 7A, 7D, 7E, 7K–7M and 7P–7S). A well recognized common species of the North Pacific tritoniids, *Tritonia festiva* (Stearns, 1873), though largely was not involved the neurobiological works, but closely related to the important model species, *T. tetraquetra* and *T. exsulans* (Figs 1A and 6).
These similarities and differences need to be addressed. Family Tritoniidae needs a synopsis based on the available integrative data. Following valid genera (in alphabetical order) are proposed to recognize within the family Tritoniidae. The adult characters are listed in the diagnoses below because juvenile features can be different considerably. In addition in this study were specially investigated phylogenetic placement of Doridoxidae and Heterodorididae (Figs 1A, 1B and 8), and respective taxonomic diagnoses of these families are presented after synopsis of the family Tritoniidae.

**Taxonomic synopsis of the family Tritoniidae Lamarck, 1809**

**Genus Duvaucelia** Risso 1826, restricted

= Candiella Gray, 1850

Type species. *Duvaucelia gracilis* Risso, 1826 (= *Tritonia manicata* Deshayes, 1853)

Diagnosis. Body narrow. Dorsolateral appendages distinct, branched, relatively few in number. Notal edge indistinct. Anterior corners of notum absent. Rhinophoral sheaths closed, without lateral opening and appendage. Oral veil not bilobed with few long processes. Anal opening usually placed towards anterior part of lateral side. Jaws oval. Masticatory edge with conical elements. Central radular teeth tricuspid. Moderate number of rows of lateral teeth, about 20–30 per half row. Stomach plates absent. Seminal receptacle relatively large, oval with long stalk and without large bag-like base. Copulative organ conical, without distinct folds. Usually small forms with body length no more than ca. 30 mm. Sources of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. *Duvaucelia lineata* (Alder et Hancock, 1848), *Duvaucelia manicata* (Deshayes, 1853), *Duvaucelia nilsodhneri* (Marcus Ev., 1983) comb. nov., *Duvaucelia plebeia* (G. Johnston, 1828), *Duvaucelia striata* (Haefelfinger, 1963),* Duvaucelia taliartensis* (Ortea et Moro, 2009) comb. nov.

Remarks. This is a maximal supported clade in the present phylogenetic analysis (Fig 1A) that constitutes a narrowly-defined group of a few species of tritoniids. This group also shows very consistent morphological features, such as relatively small size, not bilobed small oral veil and moderate number of lateral teeth. This narrowly-defined genus has support from biogeographical data since it encompasses species that inhabits mostly European temperate and subtropical waters. Therefore, the older genus *Duvaucelia* in the restricted sense is resurrected here (see list of included species above). For a species *Tritonia taliartensis* molecular or internal morphological data are not available from the original description [77], but general external appearance (small size, 7.5 mm and narrow body) similar to the species of the restricted genus *Duvaucelia*.

**Genus Marianina** Pruvot-Fol, 1931

= Aranucus Odhner, 1936

Type species. *Marianina rosea* (Pruvot-Fol, 1930) (= *Aranucus bifidus* Odhner, 1936)

Diagnosis. Body very narrow. Dorsolateral appendages distinct, bifid, cerata-like, few in number. Notal edge indistinct. Anterior corners of notum absent. Rhinophoral sheaths closed, without lateral opening and with distinct fleshy lateral appendage. Oral veil with two long processes pointed forward and two shorter processes directed laterally. Anal opening placed towards anterior part of lateral side. Jaws oval. Masticatory edge with small granule-like denticles. Central radular teeth with strong triangular cusp and distinct lateral denticles. Small number of rows of lateral teeth (about 5–6 per half row). Stomach plates absent. Seminal receptacle large, oval with short stalk and without large bag-like base. Copulative organ elongated, with a rounded tip, without distinct folds. Small forms with body length no more than 15 mm. Sources of the morphological data used in the diagnosis are indicated in the Table 3.
Consolidated data on phylogeny of the family Tritoniidae clarify taxonomic status of the neuroscience models.
Species composition. *Marianina khaleesi* (Silva, de Azevedo et Matthews-Cascon, 2014) comb.nov., *Marianina rosea* (Pruvot-Fol, 1930).

Remarks. The highly aberrant genus *Marianina* with bifid but not branched dorsolateral appendages is phylogenetically sister to the genus *Tritoniopsis* (Figs 1A and 8). The genus *Tritoniopsis* however has a “typical” tritoniid external morphology. This proves necessity of a fine-scale distinguishing of the genera within the family Tritoniidae. The radular patterns of adult *Marianina rosea* highly resemble early juvenile teeth of other complex at adult stage tritoniids [78, 79], and also species from the distantly related family Dendronotidae [80, 81]. According to the molecular phylogenetic analysis (Fig 1A) *Marianina* with simple external morphology (narrow body with non-branched appendages, Figs 1A, 1B and 8) is a sister taxon to the tritoniid *Tritoniopsis* with a complex external morphology (broad body with strongly branched appendages). The juvenile appearance of some set of characters and simultaneous phylogenetic placement inside of a group with generally complex morphology is a robust evidence for the paedomorphosis [24]. The disparate morphology as in *Marianina* and phylogenetic relation to a complex group is characteristic for paedomorphic taxa and commonly resulting in proposals for higher taxonomic groupings. Two genera were proposed two times for the same paedomorphic tritoniid species: *Marianina* Pruvot-Fol, 1930 [82] and *Aranucus* Odhner, 1936 [46]. The separate family Aranucidae was later synonymyzed with Marianiniidae (now considered as a synonym of Tritoniidae). The process of paedomorphosis likely dominated in the evolution of the genus *Marianina*. Intriguingly, there is a species, currently assigned to the genus *Tritonia*, *T. khaleesi* Silva, Azevedo et Matthews-Cascon, 2014 which shows a remarkable combination of small size (up to 12 mm), a gracile external shape with just two long and two short processes of the oral veil, the radula with central teeth bearing strong triangular cusp with lateral denticles and a few lateral teeth (max 5) (83): pp. 580–582). These characters are significantly similar to these in *Marianina rosea* [46, 84, 85]. Reproductive systems are quite similar in both taxa, with distinct prostate, relatively large receptaculum seminis on a short stalk and copulative organ without distinct folds [46, 83]. “*Tritonia*” *khaleesi* demonstrates a very similar to *Marianina* strongly paedomorphic radular morphology. The only difference that *T. khaleesi* shows partly branched dorsolateral appendages, but some appendages are already unbranched (83): pp. 580–581). *Marianina rosea* and *T. khaleesi* also share similar fleshy lobe at the lateral sides of rhinophoral sheaths. A small tritoniid “*Tritonia*pickensi” (Marcus and Marcus, 1967) also shows partly similar to *Marianina rosea* and “*T. khaleesi*” few elongate processes of oral veil and a lateral lobe at rhinophoral sheaths. However radula of “*Tritonia*pickensi” has common for tritoniids tricuspid central teeth, whereas central teeth of “*T. khaleesi*” is very similar to *Marianina*. While there is a possibility that *T. khaleesi* is independently from *Marianina* acquired paedomorphic radular morphology, taking into
Table 3. Diagnostic characters of the genera in the family Tritoniidae.

| Genus                  | Body                      | Oral veil                              | Rhinophoral sheaths | Notal edge                                      | Dorsolateral appendages | Lateral teeth of radula | Central teeth | Stomach plates | Copulative organ | References                  |
|------------------------|---------------------------|----------------------------------------|---------------------|------------------------------------------------|-------------------------|-------------------------|---------------|----------------|-------------------|--------------------------------|
| **Duvaucelia Risso 1826** | Narrow, commonly up to 30 mm in length | Oral veil not bilobed with few long processes | Closed, without appendage; Anterior corners of notum absent | Indistinct | Distinct, branched, relatively few in number | Moderate in number, about 20–30 per half row | Tricuspid | Absent | Conical, without distinct folds (here and below conical structures of various length are implied, including "flagelliform" ones) | [1, 69; 77, 90] |
| **Marianina Pruvo-Fol, 1931** | Very narrow, commonly up to 15 mm in length | Oral veil not bilobed with pair of long oral-tentacle like processes and pair of shorter appendages | Closed, with distinct appendage; Anterior corners of notum in front of rhinophores absent | Indistinct | Distinct, bifid, cerata-like, few in number | Small in number, about 5–6 per half row | With strong triangular cusp and distinct lateral denticles | Absent | Elongated, with rounded tip, without distinct folds | [46, 84, 85] |
| **Mariona Vayssière, 1877 (a heterogeneous assemblage)** | Broad to moderately narrow, up to 200 mm in length, but small forms also present | Oral veil bilobed or not, with numerous long processes | Closed, without appendage; Anterior corners of notum absent or indistinct | Notal edge commonly remains as narrow folds between of dorsolateral appendages | Distinct, branched, from few to numerous | From moderate to extremely large number (from 15 to more than 150 teeth) per half row in various species | Tricuspid | Present | Conical or bulbous, without distinct fold | [3, 72, 87, 88 89, 90] |
| **Paratritonia Baba, 1949** | Narrow, up to 30 mm in length | Oral veil not bilobed with few long processes | Closed, without appendage; Anterior corners of notum absent | Indistinct | Distinct, branched, few in number | Numerous, 100–110 per half row | Unicuspid | Present | Unknown | [91] |
| **Tochuina Odhner, 1963** | Broad, up to 300 mm in length | Oral veil not bilobed without processes | Partly open laterally, lateral appendage absent; Anterior corners of notum present | Distinct | Indistinct, branched, numerous | Numerous, up to 200 teeth per half row | Unicuspid | Absent | Conical, without distinct folds | [17, 19, 23, 47, 48, 69; present study] |
| **Tritonia Cuvier, 1798** | Broad, up to 300 mm in length | Oral veil bilobed with numerous long processes | Closed, without lateral opening and distinct appendage; Anterior corners of notum absent or indistinct | Indistinct | Distinct, branched, moderate to relatively few in number | Numerous, up to 200 teeth per half row | Tricuspid | Absent | Massive, cylindrical or conical, with variously expressed folds | [1, 23, 46, 47, 50, 53, 69, 93–98, 106; present study] |

(Continued)
consideration several significant similarities in different characters, it is more probable that T. khaleesi is more closely related to M. rosea. We therefore transferred here “T.” khaleesi into the genus Marianina (Marianina khaleesi (Silva et al., 2014) comb. nov.) with a reservation. When the molecular data for M. khaleesi will be available, it will be possible to further conclude either it is a sister species to M. rosea, or a separate new genus within some other tritoniid lineage. In any scenario, compare to M. rosea, M. khaleesi demonstrates remnants of the branching pattern of the dorsolateral appendages, which is common to the majority of tritoniids. This is very consistent with the results of the molecular analysis shows that simplified paedomorphic Marianina is sister to the externally complex Tritoniopsis (Fig 1A and 1B), which already acquired the central teeth with a strong pointed cusp.

Genus Marionia Vayssiére, 1877

Type species. Tritonia blainvillea Risso, 1818 (= Marionia berghii Vayssiére, 1879)

Diagnosis. Body broad to moderate. Dorsolateral appendages distinct, branched, from few to numerous in number. Notal edge commonly remains as narrow folds between of dorsolateral appendages. Anterior corners of notum absent or indistinct. Rhinophoral sheaths closed, without lateral opening and appendage. Oral veil not bilobed or few long processes. Anal opening usually placed towards middle or posterior part of lateral side. Jaws oval. Masticatory edge with elements of various degree and numbers. Central radular teeth tricuspid. From 15 to more than 150 lateral teeth per half row in various species. Stomach plates present. Seminal receptacle very large with long stalk and without bag-like large base. Copulative organ conical or bulbous, without distinct folds. Usually large forms with body length

Table 3. (Continued)

| Genus                      | Body            | Oral veil                  | Rhinophoral sheaths | Notal edge | Dorsolateral appendages | Lateral teeth of radula | Central teeth | Stomach plates | Copulative organ | References |
|----------------------------|-----------------|----------------------------|---------------------|------------|-------------------------|------------------------|---------------|-----------------|-----------------|------------|
| Tritonicula gen. nov.      | Narrow, commonly up to 20 mm in length | Oral veil not bilobed with few long processes | Closed, with elongate lateral appendage in some species; Anterior corners of notum absent | Indistinct | Distinct, branched, few in number | Small in number, up to 11 per half row | Tricuspid | Absent | Conical or rounded, without distinct folds | [14, 70, 78, 99] |
| Tritonodoxa Bergh, 1907    | Narrow, up to 30 mm in length | Oral veil not bilobed with few processes | Closed, without lateral appendage; Anterior corners of notum indistinct | Distinct | Absent | Numerous, up to 75 per half row | Tricuspid | Absent | Conical, without distinct folds | [100] |
| Tritoniella Eliot, 1907    | Narrow to moderate, up to 80 mm in length | Oral veil not bilobed or indistinctly bilobed, without processes | Closed, with lateral expansion; Anterior corners of notum indistinct | Distinct | Absent or indistinct, non-branched | Numerous, more than 50 per half row | Unicuspid or tricuspid | Absent | Conical, with distinct circular fold or bulge | [102] |
| Tritoniopsis Eliot, 1905   | Moderate, up to 50 mm in length | Oral veil not bilobed with numerous long processes | Closed, sometimes with lateral appendage; Anterior corners of notum absent | Indistinct | Distinct, commonly very large, branched, relatively few to moderate in number | Moderate to numerous in number, about 30–50 per half row | With prominent triangular cusp and fine lateral denticles, or smooth | Absent | Conical, without distinct folds | [69, 103, 104] |

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more than 30 mm (up to 200 mm), but several species apparently are small-sized. Sources of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. *M. abrahamorum* Silva, Herrero-Barrenueva, Pola et Cervera, 2019, *M. albotuberculata* Eliot, 1904, *M. arborescens* Bergh, 1890, *M. babai* Odhner, 1936, *M. bathy-carolinensis* Smith et Gosliner, 2005, *M. blainvillea* (Risso, 1818), *M. cabindae* White, 1955, *M. chloanthes* Bergh, 1902, *M. cucullata* (Couthouy, 1852), *M. cyanobranchiata* (Ruppell et Leuckart, 1828), *M. dakini* (O’Donoghue, 1924), *M. distincta* (Bergh, 1905), *M. echinomuriceae* Jensen, 1994, *M. elongoviridis* Smith et Gosliner, 2007, *M. elongoreticulata* Smith et Gosliner, 2007, *M. fulvicola* Avila, Kelman, Kashman et Benayahu, 1999, *M. gemmii* Almón, Pérez et Caballer, 2018, *M. ghanensis* Edmunds et Carmona, 2017, *M. hawaiiensis* (Pease, 1860), *M. koi* Angulo-Campillo et Bertsch, 2013, *M. levis* Eliot, 1904, *M. limceana* Silva, de Meirelles et Matthews-Cascon, 2013, *M. olivacea* Baba, 1937, *M. ramosa* Eliot 1904, *M. rubra* (Rüppell et Leuckart, 1828), *M. pellucida* Eliot, 1904, *M. platycenea* (Willan, 1988), *M. pusa* Er. Marcus et Ev. Marcus, 1968, *M. pustulosa* Odhner, 1936, *M. semperi* Jensen, 1994, *Marionia tedi* Ev. Marcus, 1983, *M. vanira* Ev. Marcus et Er. Marcus, 1966, *M. viridescens* Eliot 1904.

Remarks. The genus *Mariona* in spite of the external similarity to the genus *Tritonia* possesses such a very distinctive feature as the solid chitinous stomach plates. However this large genus currently is united only by this single character. Intriguingly, stomach plates which are very similar to *Mariona* also present in a distantly related dendronotoidean family Tethyidae [86] (Figs 1A and 8). This again raises a question that taxonomy should be based not only on the drastically different characters, but on a fine-scale diagnostics. In this respect, an extremely broad range of other characters, including number of dorsolateral appendages (ranged from 7 to 100 pairs) and lateral teeth (ranged from 15 to 150 teeth) were mentioned for the genus *Mariona* [3, 71, 87–90]. This diversity exceeds ranges of character “variations” (at adult stage) within putatively the same “*Mariona*” genus. The available names such as *Marioniopsis* and *Paratrionia*, after clarification to which clades they actually belong can be used for further genus-level delineation among “*Mariona*”. For example, the so far monotypic genus *Paratrionia* possesses small-sized species with few dorsolateral appendages but with a large number of the radular lateral rows ([91]: pp. 84–86). Some other small-sized species of “*Mariona*” can be included into that genus, when molecular data will became available for the type genus *Paratritonia lutea* Baba, 1949. In case they will be inconsistent with *P. lutea* (for this species the unicuspid central teeth was reported [91]), new genera will be needed to separate for such forms.

Importantly, according to all available data *Mariona* invariably forms a separate clade from other tritoniids [5, 19, 72, 92; present study, Fig 1A]. Therefore, potentially *Mariona* with related genera *Marioniopsis* and *Paratrionia* (and more genera which have to be separated) should be placed in a separate family. In this connection, there is another considerable issue with the genus *Mariona* that affects all tritoniids. The majority of the available phylogenetic analyses [5, 72, 92] shows that *Mariona* renders the family Tritoniidae paraphyletic regarding Dendronotoidea. A revision of the large *Mariona* clade is out of scope of the present paper and pending a separate study.

**Genus Paratritonia** Baba, 1949

*Type species. Paratritonia lutea* Baba, 1949.

*Diagnosis. Body narrow. Dorsolateral appendages distinct, branched, few in number. Notal edge indistinct. Anterior corners of notum absent. Rhinophoral sheaths closed, without lateral opening and appendage. Oral veil not distinctly bilobed with few long processes. Anal opening usually placed towards middle or posterior part of lateral side. Jaws oval. Masticatory edge with teeth-like elements of various degree and numbers. Central radular teeth unicuspid with additional denticles. Numerous rows of lateral teeth (100–110 per half row). Stomach plates ...
present. Anterior and posterior portions of digestive gland fused. Reproductive organs unknown. Small forms with body length up to 30 mm. Source of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. Only type species so far was included [91].

Remarks. See under genus Marionia.

Genus Tochuina Odhner, 1963

Type species. Tritonia gigantea Bergh, 1904 (= “Tritonia tetraquetra” sensu Bergh, 1879 non Pallas, 1788)

Diagnosis. Body broad. Dorsolateral appendages indistinct, branched, numerous. Notal edge distinct, well-defined. Anterior corners of notum present, distinct. Rhinophoral sheaths partly open laterally; lateral appendage absent. Oral veil not bilobed without processes or with small tubercles. Anal opening placed towards middle or posterior part of lateral side. Jaws square. Masticatory edge smooth or possibly with indistinct elements. Central radular teeth unicuspid. Additional fine denticles often present on central teeth. Numerous rows of lateral teeth, more than 50 (up to 200) per half row. Stomach plates absent. Seminal receptacle relatively large with long stalk and without bag-like base. Copulative organ conical, without distinct folds. Large forms with body length more than 30 mm and include very large form up to 300 mm. Sources of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. Tochuina gigantea (Bergh, 1904), Tochuina nigritigris (Valdés, Lundsten et Wilson, 2018) comb. nov., Tochuina nigromaculata (Roginskaya, 1984) comb. nov.

Remarks. The incompletely closed rhinophoral sheaths and the well defined anterior notal corners of T. gigantea (Figs 3I and 3J) represent considerable similarity with the non-tritoniid taxa Doridoxa walteri (Krause, 1892) [93] (Figs 7A, 7D, 7E and 7K–7M) and Heterodoris robusta Verrill et Emerton 1882 (Fig 7P–7S), see also Discussion). In the present study we for the first time perform the molecular analysis with inclusion of the type species of the genus Tochuina, T. gigantea (previously incorrectly identify as “Tochuina tetraquetra”). As results, we robustly reveal that a recently described species which was assigned to “Tritonia”, T. nigritigris from NE Pacific [19] forms instead a maximal supported clade with the type species of the genus Tochuina (Fig 1A). The species T. nigritigris is therefore transfered to the genus Tochuina. Valdés et al. [19] while described T. nigritigris did not mention that this species is very similar (including name) to the species that was described considerably earlier by Roginskaya in 1984 [94] as Tritonia nigromaculata also from the deep sea of the North Pacific (Fig 35–3Z). Here we present the first SEM images of the radula of a paratype of “Tritonia” nigromaculata (Fig 3W–3Z). The central teeth are unicuspid with additional denticles, the cusp is massive and long (Fig 3Y). These patterns are very similar to the original descriptions of T. nigromaculata [94] and T. nigritigris [19]. It should be specially noted, that the present detailed SEM study (Fig 3Y1 and 3Y2) did not reveal any peculiar “articulated” appendage of the central cusp (depicted in the original description of T. nigromaculata [94]: fig b on the p. 100). Also, the cusp is smooth (both in the anterior/middle and posterior parts of the radula, Fig 3W, 3X, 3Y1 and 3Y2), whereas originally the cusp was described as a “denticulated spine” [94: p. 101]. In addition, the thinner and delicate apical part of the cusp appears somewhat bent in the posteriormost teeth (Fig 3Y2) after standard processing of the radula in sodium hypochlorite solution. Nonetheless, the posteriormost teeth also do not possess an “articulated” cusp (Fig 3Y2). These differences are clearly due to a misinterpretation of the light microscopic data in the original description of T. nigromaculata [94]. This is significant because the incorrect data on the morphology of the cusp were used to consider the central teeth of T. nigromaculata as a unique structure of a “neotenous” origin [94: p. 104]. In reality, similar unicuspid teeth with a long, smooth cusp (partially denticulated mostly at the base, and without any “articulated spines”) are present in the different non-tritoniid taxa Doridoxa and Heterodoris (Fig
7H–7], 7W and 7X), and the type species of the tritoniid genus Tochuina (Fig 3Q). The similarity of the unicuspid central teeth between “Tritonia” nigromaculata and Tochuina gigantea was previously underestimated [94]. The paedomorphosis is important evolutionary force [24] and attested in this study for several tritoniids. However, the case of T. nigromaculata suggests not only a paedomorphic trait in a particular Tochuina species, but rather a common phylogenetic pattern between Tritoniidae, Doridoxidae and Heterodorididae (see details below).

“Tritonia” nigritigris was indicated [19: p. 407] as “the most similar species...” with a species Tritonia newfoundlandica, which however is highly inconsistent with T. nigritigris because of the substantial differences in the oral veil, dorsolateral appendages and radula. Instead, both T. nigritigris and T. nigromaculata share similar non-bilobed oral veil without long processes, numerous small dorsolateral appendages, and unicuspid central teeth. “Tritonia” nigromaculata and T. nigromaculata also inhabit similar depths around 2000 m in the respective locations of the north-eastern and north-western Pacific, and potentially may represent the same species. In the present study we therefore correct the assessment in [19], and include “Tritonia” nigromaculata into the genus Tochuina. Morphologically, both T. nigritigris and T. nigromaculata share with the type species of the genus Tochuina (Fig 3A–3R) numerous small dorsolateral appendages, absence of frontal veil appendages and unicuspid central tooth (Fig 3S–32). The usual for Tochuina gigantea partially opened rhinophoral sheaths, instead are less pronounced in T. nigromaculata and T. nigritigris. “Tritoniopsis” aurantia Mattox, 1955 was considered as a synonym of T. gigantea [69], that needs to be checked with additional data.

**Genus Tritonia Cuvier, 1798, restricted**

= Sphaerostoma MacGillivray, 1843
= Lateribranchiacea Stearns, 1873
= Candellista Iredale et O’Donoghue, 1923

Type species. *Tritonia hombergii* Cuvier, 1803

**Diagnosis.** Body broad to moderate. Dorsolateral appendages distinct, branched, relatively few to moderate in number. Notal edge commonly indistinct. Anterior corners of notum absent or indistinct. Rhinophoral sheaths closed, without lateral opening and distinct appendage. Oral veil bilobed with numerous long processes. Anal opening usually placed towards middle or posterior part of lateral side. Jaws oval. Masticatory edge with strong conical or elongate bristle-like elements. Central radular teeth tricuspid. Additional fine denticles may present irregularly on central teeth. Usually many rows of lateral teeth, more than 50 (up to 200) per half row. Stomach plates absent. Seminal receptacle small or larger, oval with long stalk and a bag-like large base. Copulative organ massive, cylindrical or conical, with variously expressed folds. Usually large to moderate in size forms with body length more than 30 mm (including very large species up to 300 mm). Sources of the morphological data used in the diagnosis are indicated in the Table 3.

**Species composition.** Tritonia antarctica Pfeffer in Martens et Pfeffer, 1886, T. australis (Bergh, 1898), T. bollandi Smith et Gosliner, 2003, T. challengeriana Bergh, 1884, T. coraliumrubri Doneddu, Sacco et Trainito, 2014, T. dantarti Ballesteros et Avila, 2006, T. episcopalis Bouchet, 1977, T. exsulans Bergh, 1894, T. festiva (Stearns, 1873), T. griegii Odhner, 1922, T. hombergii Cuvier, 1803, T. incerta Bergh, 1904, T. indecora Bergh, 1907, T. ingolfiana (Bergh, 1899), T. newfoundlandica Valdés, Murillo, McCarthy et Yedinak, 2017, T. odhneri Er. Marcus, 1959, T. olivacea Bergh, 1905, T. pallescens Eliot, 1906, T. pallida Stimpson, 1855, T. primorjensis Minicheck, 1971, T. tetraquetra (Pallas, 1788), T. psoloides Aurivillius, 1887, T. vorax (Odhner, 1926).

**Remarks.** Based on the present molecular phylogenetic analysis (Fig 1A) we restrict the genus Tritonia to several predominantly large forms with a numerous processes on the bilobed oral veil and a large number of the lateral teeth. In our present analysis this morphological
group forms a clade that closely related to the type species *T. hombergii* and including these model species *T. tetraqueta* and *T. exsulans* (Figs 1–6). The genus *Tritonia*, even in the restricted form, still represents a partly heterogeneous taxonomic assemblage that pending further revision. In addition, the supports of molecular clades inside of the apparent “genus” *Tritonia* are not always high (Fig 1A). More lineages are deserved separate genus status. For example a species currently assigned to *Tritonia, T. flemingi* (Powell, 1937) is inconsistent with the morphological data on the type and closely related to it *Tritonia* species. Particularly, “*T.* flemingi” is a very small species, with non-bilobed oral veil with only few processes, and indistinct dorsolateral appendages [95, 96]. By these characters, “*T.* flemingi” partly approaches the genera *Duvaucelia* and *Tritonica* gen. nov., but because of significant geographic gap it may represents an independent lineage of small-sized tritoniids. Therefore “*T.* flemingi” does not include here into the genus *Tritonia* pending molecular phylogenetic data to propose a separate genus for this species. For several other species, including for instance a deep-sea *T. griegi* Odhner, 1922, the molecular data are also still unavailable, and inclusion of these taxa into the genus *Tritonia* needs to be confirmed. Also not all included species are consistent morphologically with the type species *T. hombergii* from the northern Atlantic and related North Pacific *T. tetraqueta* and *T. exsulans*. For instance, Valdés et al. [19] compared two recently described species, *T. newfoundlandica* with *T. nigritigris*. However, according to the present analysis the deep-sea species from NE Pacific *T. nigritigris* belongs to the genus *Tochuina*, which is distinctly related to the genus *Tritonia* (Fig 1A). The North Atlantic *T. newfoundlandica* instead shows presence of the bilobed veil with distinct processes and tricuspid central teeth [97], which are common in the genus *Tritonia*, but not in *Tochuina*. We therefore keep *T. newfoundlandica* in the genus *Tritonia*. Because of several genus-level synonyms of the genus *Tritonia* are available (see synonymy above), some of them will be needed to resurrect in a future. In addition, the present *Tritonia* species composition is inconsistent biogeographically. While the type species *T. hombergii* from the northern Atlantic, and the North Pacific species such as *T. tetraqueta, T. exsulans* and *T. festiva* are closely related with a maximal support (Fig 1A), another lineage of the Antarctic and Subantarctic species *Tritonia cf. antarctica* and *T. challengeriana* forms a basal clade with lower support to other *Tritonia* species (Fig 1A). *Tritonia cf. antarctica* and *T. challengeriana* previously were synonymized (a taxonomic review in [98]). However present analysis shows them as two distinct species (Fig 1A). According to the present molecular phylogeny, the Antarctic and Subantarctic tritoniid species likely represent a different genus, that needs to be addressed in a separate study. Myrella Odhner, 1963 (= *Microlophus* Mabille et Rochebrune, 1889, non *Microlophus* Duméril et Bibron, 1837) [23] is an available potential genus name for the clade of the Antarctic and Subantarctic tritoniids.

**Genus Tritonica** gen. nov.

*Urn:lsid:zoobank.org:act:7E6448CD-78DA-4A65-8141-0A9EB40D0D0C.*

Type species. *Tritonia hamnerorum* Gosliner et Ghiselin, 1987

Etymology. From common family stem *Tritoni-* and a diminutive Latin suffix–*cula* in reference to a “little *Tritonia*” because the new genus contains so far only small, gracile species.

Diagnosis. Body usually narrow. Dorsolateral appendages distinct, branched, relatively few in number. Notal edge commonly indistinct. Anterior corners of notum absent. Rhinophoral sheaths closed, without lateral opening, in some species with elongate lateral appendage. Oral veil not bilobed with few long processes. Anal opening usually placed towards middle lateral side, or partly moves to dorsal side. Jaws oval. Masticatory edge with delicate tubercle-like elements or more distinct conical plates. Central radular teeth tricuspid. Additional fine denticles may present irregularly on central teeth. Few rows of lateral teeth, so far reported no more than 11 per half row. Stomach plates absent. Copulative organ conical or rounded, without distinct folds.
Small forms with body length no more than 20 mm. Sources of the morphological data used in
the diagnosis are indicated in the Table 3.

Species composition. **Tritonidoxa bayeri** Ev. (Marcus et Er. Marcus, 1967) comb. nov., **Tritonidoxa hamnerorum** (Gosliner et Ghiselin, 1987) comb. nov., **Tritonidoxa myrakeenae** (Bertsch et Osuna, 1986) comb. nov., **Tritonidoxa pickensi** Ev. (Marcus et Er. Marcus, 1967) comb. nov., **Tritonidoxa wellsi** (Er. Marcus, 1961) comb. nov.

Remarks. In the present analysis we confirm a separate and morphologically distinct clade
(Fig 1A and 1B) from both **Tritonia** s. str. and **Duvaucelia** s. str. This group contains generally
smaller-sized species (with body length no more than 20 mm) such as "T." bayeri Ev. Marcus
et Er. Marcus, 1967, "T." hamnerorum Gosliner et Ghiselin, 1987, "T." myrakeenae Bertsch et
Osuna, 1986 and "T." pickensi Ev. Marcus et Er. Marcus, 1967 [70, 78, 99]. All these species
share small size, small number oral veil processes and a very small number of rows of lateral
teeth (around 10 per half radular row) in adult specimens. This group of species is separated
here as a new genus. This combination distinguishes **Tritonicula** gen. nov. from both generally
large-bodied **Tritonia** and small-sized **Duvaucelia**. The genus **Tritonia** possesses in adult speci-
mens bilobed oral veil with numerous processes and more than 50 lateral teeth per row.

Small-sized **Duvaucelia** commonly has larger number of lateral teeth than **Tritonicula** gen.

ov. (more than 20 lateral teeth per row), though in few species this number can be smaller.
Also species of the genus **Duvaucelia**, compare to **Tritonicula** gen. nov. does not have lateral
extension on the rhinophoral sheaths. In addition, the new genus is consistent biogeographi-
cally. All included species inhabit warm waters of the American Atlantic and the Caribbean, or
neighbour tropical Pacific waters. **Tritonia wellsi** was originally described from North Carolina
and further founded in Brazil and placed in the subgenus **Tritonidoxa** [14]. However, the real
genus **Tritonidoxa** demonstrates completely different characters including absence of dorsolat-
eral appendages [100]. Small size, few oral veil processes and no more than 10 lateral teeth per
row of "T." wellsi are consistent with **Tritonicula** gen. nov. We include this species into new
genus with reservation because of absence of molecular data. A species "T." khaleesi (for which
there are no available molecular data) which is included here to the genus **Marianina** (see
above) can be also potentially related to **Tritonicula** gen. nov. According to our analysis it is
likely that **Duvaucelia** and **Tritonicula** gen. nov. acquired small body size and smaller number
of the lateral teeth independently (Fig 1A). This is an example of importance of fine-scale taxo-
nomic delimitation not only at the species level (as in the case of the delimitation of the model
species **T. tetraquetra** and **T. exsulans**), but also at the genus level, because otherwise such con-
vergent evolutionary events will be considerably masked.

**Genus Tritonidoxa** Bergh, 1907

Type species. **Tritonidoxa capensis** Bergh, 1907

Diagnosis. Body narrow. Dorsolateral appendages absent. Notal edge distinct. Anterior cor-
ners of notum indistinct. Rhinophoral sheaths closed, without opening laterally and lateral
appendage. Oral veil not bilobed with few processes. Anal opening placed towards posterior
part of lateral side. Jaw oval. Masticatory edge with conical elements. Central radular teeth tric-
suspids. Large number of rows of lateral teeth (ca. 75 per half row). Stomach plates absent. Sem-
inal receptacle relatively large. Copulative organ conical, without folds. Body length about 30
mm. Source of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. Only type species, **T. capensis** [100].

Remarks. There are more species that could potentially be included in **Tritonidoxa** [69, 78,
101]. However all they are inconsistent with characters of the type species, first of all absence
of the dorsolateral appendages. Thus, only single type species is included here.

**Genus Tritoniella** Eliot, 1907

Type species. **Tritoniella sinuata** Eliot, 1907
Diagnosis. Body narrow to moderately broad. Dorsolateral appendages absent or indistinct, non-branched. Notal edge distinct. Anterior corners of notum indistinct. Rhinophoral sheaths closed, without lateral opening, but expanded laterally. Oral veil not bilobed or indistinctly bilobed, without processes. Anal opening placed towards middle part of lateral side. Jaws square. Masticatory edge with fine tubercles. Central radular teeth in adults unicuspid or tricuspid. Additional distinct cusps may present on central teeth. Numerous rows of lateral teeth, commonly more than 50 per half row. Stomach plates absent. Seminal receptacle relatively large with long stalk and without large bag-like base. Copulative organ conical, with distinct circular fold or bulge. Large forms up to 80 mm. Source of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. Tritoniella belli Eliot, 1907 (= T. sinuata Eliot, 1907).

Remarks. The Antarctic genus Tritoniella well distinguishes from the majority of tritoniids by absence of distinctly branched dorsolateral appendages. Phylogenetically it is related to the genus Tochuina and important for the present study. Here it is shown that the Antarctic Tritoniella forms a sister clade to the North Pacific genus Tochuina (Fig 1A), but considerably differs from the latter by external morphological features. Therefore, without broader framework it is impossible to assess taxonomic position of particular model species. Furthermore, all so far known species of the genus Tochuina always possess unicuspid central tooth (Fig 3), whereas Tritoniella may possesses both unicuspid and tricuspid central teeth [102]. These data clearly demonstrate that straightforward taxonomic diagnoses like a dilemma “presence/absence” of a character should be substituted with a fine-scale diagnostics. This case also help for taxonomic studies to become really integrative one and consistent with the novel molecular data.

Genus Tritoniopsis Eliot, 1905
= Tritoniopsilla Pruvot-Fol, 1933

Type species. Tritoniopsis brucei Eliot, 1905

Diagnosis. Body moderately broad. Dorsolateral appendages distinct, commonly very large, branched, relatively few to moderate in number. Notal edge indistinct. Anterior corners of notum absent. Rhinophoral sheaths closed, without lateral opening, sometimes with a small lateral appendage. Oral veil not distinctly bilobed with numerous long processes. Anal opening usually placed towards middle part of lateral sides. Jaws square. Masticatory edge with conical elements. Central radular teeth elongate, with prominent elongate cusp, smooth of with a number of small, regularly arranged lateral denticles. Number of rows of lateral teeth moderate to numerous (ca. 30–50 per half row). Stomach plates absent. Seminal receptacle relatively large, oval with long stalk and without large bag-like base. Copulative organ conical, without distinct folds. Small to medium forms with body length from ca. 20 to 50 mm. Sources of the morphological data used in the diagnosis are indicated in the Table 3.

Species composition. T. brucei Eliot, 1905, T. cincta (Pruvot-Fol, 1937), T. elegans (Audouin, 1826), T. frydis Er. Marcus et Ev. Marcus, 1970.

Remarks. Type species of the genus Tritoniopsis, T. brucei Eliot, 1905 comes from temperate southern Atlantic (Gough Island). Other currently assigned to the genus Tritoniopsis species come from tropical and subtropical waters. The molecular data are not available for T. brucei and phylogetic placement of the genus Tritoniopsis is based on other included species (Fig 1A). Tritoniopsis characterizes by the central tooth with a prominent triangular cusp, often with lateral denticles [103, 104], which is distinctive from the common in Tritoniidae tricuspidate teeth. The genus Tritoniopsis is a good example of the necessity of a fine-scale assessment of the genera within the family Tritoniidae. According to the phylogenetic data Tritoniopsis is closely related to the profoundly aberrant, paedomorphic genus Marianina (present study, Fig 1A and 1B), but external morphology of Tritoniopsis is consistent with the majority of other
Tritoniidae [104]. In addition to the taxonomic synopsis of the family Tritoniidae, diagnoses and remarks for the phylogenetically related but morphologically different non-tritoniid families Doridoxidae and Heterodorididae (addressed in the present study, Figs 1, 7 and 8) are provided below.

**Diagnoses of the families Doridoxidae and Heterodorididae**

**Family Doridoxidae** Bergh, 1899

Diagnosis. Body moderately broad. Dorsolateral appendages absent. Notal edge distinct, well-defined. Anterior corners of notum present, distinct. Rhinophoral sheaths partly open laterally; lateral appendage absent. Rhinophores perfoliate. Oral veil not bilobed without processes. Anal opening placed towards posterior part of lateral side. Jaws square. Masticatory edge smooth or possibly with indistinct elements. Central radular teeth with distinct, broad, triangular cusp. Additional fine denticles may present on central teeth. Moderate number of lateral teeth, up to ca. 30 per half row. Stomach plates absent. Digestive gland without distinct external lobes. Seminal receptacle with long stalk and without bag-like base. Copulative organ conical, without distinct fold. Medium to small-sized forms with body length ca. 20–30 mm. The sources of the morphological data are in [93, 97, 105–109] and present study (Fig 7A–7O).

Composition. The genus *Doridoxa* Bergh, 1899 contains confirmed species *D. walteri* (Krause, 1892) (= *Doridoxa ingolfiana* Bergh, 1899, syn. nov., see details below) and a second poorly known South African species *D. benthalis* Barnard, 1963 [110].

Remarks. The genus *Doridoxa* and family Doridoxidae until recently was among the most enigmatic and poorly known nudibranch taxa (see details in the Discussion section). Molecular data obtained in the present study and available in GenBank clearly suggest that *Doridoxa ingolfiana* Bergh, 1899 [105, 106] is a junior synonym of *Doridoxa walteri* (Krause, 1892) [108] (= *D. ingolfiana* Bergh, 1899 syn. nov.). The external and internal morphology of the described from the Barents Sea *D. walteri* [108] (Fig 7A and 7D–7O) is essentially similar to the previously redescribed *D. ingolfiana* from the waters of the northern Atlantic [107, 108, Fig 7B and 7C]. In the original description of *D. ingolfiana* (including "*D. inglofiana* var.") the presence of both smooth and denticulate central teeth is indicated [105, 106] (Fig 7B and 7C). Our conclusions based on *D. walteri* from the type locality region (Barents Sea off the East Spitzbergen) well agreed with the original description of *D. walteri* in Krause [109] (outlined on Fig 7A) and further records [93, 111]. Exactly as reported for *D. ingolfiana*, *D. walteri* has both smooth and denticulate central teeth (Fig 7H–7J, 7N and 7O). Maximal uncorrected COI p-distances between *D. walteri* and "*D. ingolfiana*" are 0.91% (Table 2), and according ABGD analysis they are the same species. The taxonomic placement of *Doridoxa benthalis* Barnard, 1963 [110] needs to be clarified since only single poorly preserved specimen is available [107]. *D. benthalis* does not demonstrate considerable similarity [110] to the type species of the genus *Doridoxa* and may represent a separate taxon of the genus or family levels.

**Family Heterodorididae** Verrill et Emerton, 1882

(= Atthilidae Bergh, 1899)

Diagnosis. Body moderately broad. Dorsolateral appendages non-branched or absent. Notal edge distinct, well-defined. Anterior corners of notum present, distinct. Rhinophoral sheaths partly open laterally; lateral appendage absent. Rhinophores perfoliate. Oral veil not bilobed without processes. Anal opening placed towards posterior part of lateral side. Jaws oval. Masticatory edge smooth or possibly with indistinct elements. Central radular teeth unicusp with a narrow, weak cusp. Additional fine denticles may present on central teeth. Numerous lateral teeth, more than 50 (up to 200 and more) per half row. Stomach plates absent. Digestive gland with several external lobes. Seminal receptacle with long stalk and
without bag-like base. Copulative organ conical or cylindrical, without distinct fold. Medium sized forms with body length up to ca. 35 mm. The sources of the morphological data are in [104, 107, 112–115] and present study (Fig 7P–7X).

Composition. The genus *Heterodoris* Verrill et Emerton, 1882 (= *Atthila* Bergh, 1899, see details below) contains two confirmed species: *H. robusta* Verrill et Emerton 1882 and *H. anthipodes* Willan, 1981.

Remarks. The genus *Heterodoris* and the family Heterodorididae show intriguing similarity to the family Doridoxidae (which was previously widely discussed as a key taxon for nudibranch phylogeny [107], Fig 7) and to the tritoniid genus *Tochuina* (Fig 3). However prior to this study (Fig 1A) the molecular data on Heterodorididae were never presented and incorporated into a molecular phylogeny. Present phylogenetic analysis shows complex pattern of the molecular relations and morphological disparities (Fig 1A and 1B). The family Heterodorididae is revealed as the sister group of the morphologically very different dorid-like Doridomorphidae (Fig 1A). The family Doridoxidae (morphologically similar to Heterodorididae), in its turn, comes as the sister group of Doridomorphidae+Heterodorididae clade (Fig 1A). Together Doridoxidae, Heterodorididae, and Doridomorphidae are sister to the morphologically highly disparate family Arminidae (Fig 1A). The family Arminidae differs from Heterodorididae and Doridoxidae substantially, by presence of joined rhinophores with vertical lamellae and oral veil with a strong transversal fold (Fig 1A and 1B, see also Discussion). Thus, in case of an artificial taxonomic unification of Heterodorididae with Arminidae or Doridoxidae, the synonymyization of Arminidae and drastically different family Doridomorphidae will also be required. This will be a counterproductive decision, because instead of producing of a fine-scale taxonomic system, different taxa will be lumped into a large assemblage without support of apomorphies. Therefore, the present study confirms both *Doridoxa* and *Heterodoris* warrant separate families Doridoxidae Bergh, 1899 and Heterodorididae Verrill et Emerton 1882 (reinstated) respectively. The external similarity between the families Doridoxidae and Heterodorididae, as well as similarity to the tritoniid genus *Tochuina* are likely due to the pleiomorphic similarity of the rhinophoral sheaths and oral veil patterns (see details below).

Internally families Doridoxidae and Heterodorididae are different in presence of an entire (except for microscopical branches [107]) digestive gland in Doridoxidae and subdivided into few lobes [113] in Heterodorididae. However, for the second species *H. anthipodes* the lobed digestive gland was not reported [114] and this character needs in a further investigation.

The genus *Atthila* Bergh, 1899 and the family Atthilidae, introduced by Bergh [106], are synonyms of *Heterodoris* Verrill et Emerton, 1882 and the family Heterodorididae Verrill et Emerton 1882 respectively. It was already proposed [113], but later on it was suggested to restore the genus *Atthila* without a study of additional materials [115]. However, both *Atthila* and *Heterodoris* show the same incompletely closed rhinophoral sheaths integrated to the oral veil [106, 113, present study]. This character was misleadingly described for *Atthila* as a “two-lobed edge of rhinophoral cavity” [106, 115]. *Atthila* and *Heterodoris* also come from the same geographic region of the western part of the North Atlantic and similar depth range (800–1500 meters) [106, 111, present study]. The conspecificity of deeper records of *H. robusta* from more southern localities in the north-eastern Atlantic [116] and exact number of species of the genus *Heterodoris* worldwide needs in a further investigation, including clarification of the species status “*Heterodoris ingolfiana*” [106, 116]. In this study several specimens of *Heterodoris robusta* from the type locality region of both *Heterodoris* and *Atthila* (the northern Atlantic coast of North America) were investigated. It was discovered that both denticulated and non-denticulated central teeth may occur in the same exemplar (Fig 7W and 7X). This is important result because prior to this study the denticulated versus non- denticulated central tooth was proposed as a main distinguishing character between *Atthila* and *Heterodoris* [115].
Also, denticulation on the central teeth of *H. robusta* represents by weak folds (Fig 7W and 7X) and could be omitted in course of a light microscopic study by Odhner [113]. Furthermore, variation of the body length [115] and internal characters (number and patterns of the lateral teeth) do not show any significant differences between *Heterodoris* and *Atthila*. Thus, there are no reliable characters to distinguish *Heterodoris* and *Atthila* and the latter genus is considered as synonym of *Heterodoris*.

**Phylogenetic relations of the family Tritoniidae to other nudibranch groups**

The family Tritoniidae was recently shown as more closely related to the family Arminidae than to other traditional dendronotacean nudibranchs [4, 117]. Arminidae have no branched notal appendages and possess different rhinophores and anterior notum. The placement of the family Tritoniidae together with Arminidae renders traditional Dendronotacea paraphyletic. However, the taxon selection of the arminids and related families was limited and previously included only common shallow water arminid genera *Armina* and *Dermatobranchus*. The tritoniid genus *Marionia* and related taxa were also not included into that previous analysis [117]. In the present study the non-tritoniid genera *Doridoxa* and *Heterodoris* (Figs 1 and 7) from the deep sea of the North Atlantic were included into phylogeny of the family Tritoniidae for the first time. These non-tritoniid taxa are important for a tritoniid phylogeny consolidation. *Doridoxa* and *Heterodoris* have protruding anterior corners of the notum and rhinophoral sheaths with a lateral opening considerably similar to the tritoniid genus *Tochuina* (Figs 3 and 7). Arminidae have joined rhinophores without sheaths and usually with vertical lamellae (instead of horizontal ones in *Doridoxa* and *Heterodoris*) (Figs 1 and 7); these patterns are very different from Tritoniidae. By presence of perfoliated rhinophores both *Doridoxa* and *Heterodoris* are also different from the tritoniid *Tochuina*. However, the unicuspid central teeth of the radula in *Doridoxa* and *Heterodoris* is substantially similar to the tritoniid genera *Tochuina* and *Tritoniopsis* (Figs 3 and 7). This highlights the necessity to include *Doridoxa* and *Heterodoris* into molecular study of the family Tritoniidae. The molecular data on the North Atlantic *Heterodoris robusta* and for *Doridoxa walteri* from the Barents Sea are presented here for the first time (Figs 1 and 8).

According the molecular phylogenetic data, *Doridoxa*, *Heterodoris* and *Doridomorpha* clades are sister to the non-tritoniid family Arminidae (Fig 1). Similar externally *Tochuina* (Fig 3) comes as a basal lineage within the family Tritoniidae (Fig 1). Two phylogenetically distantly related lineages as *Doridoxidae* and *Heterodorididae* from one hand and tritoniid *Tochuina* on other hand have similar widely separated rhinophores with not completely closed rhinophoral sheaths integrated to the oral veil (Figs 1, 3 and 7). Instead, the peculiar joined rhinophores are restricted solely to the family Arminidae (Fig 1A and 1B). The transversal fold of the oral veil (Fig 1B) is also unique feature of the family Arminidae. Furthermore, among majority of the members of the distantly related superfamilies Tritonioidea and Dendronotioidea the separate non-joined rhinophores and oral veil without transversal fold are common character states (Fig 1A and 1B). The widespread presence of non-joined rhinophores supports separate rhinophores as plesiomorphic condition for Tritonioidea and Dendronotioidea in which lateral rhinophoral sheath opening was closed during subsequent phylogenetic diversification (Fig 1B). Available ontogenetic data for the type species of the genus *Tritonia* show earlier appearance of separate rhinophores integrated to the oral veil [79] and also support plesiomorphic state of this character. Taking together, the morphological and molecular phylogenetic data indicate separate rhinophores as a common ancestral character instead of a convergence. At the same time the structure of rhinophores in *Doridoxa* and *Heterodoris*...
(perfoliated, horizontal lamellae) differ from *Tochuina* (vertical, commonly branched lamellae typical of tritoniids). These taxa belong to different families, which acquired specialized characters in course of the phylogeny (Fig 1).

The majority of tritoniids do not have well-defined anterior corners of notum at adult stages (Fig 1A and 1B). However, early juveniles of *Tritonia* possess distinct notal anterior corners and rhinophores integrated to the oral veil [79]. During subsequent development of *Tritonia* towards adult stages, anterior corners of notum considerably reduced, whereas rhinophoral sheaths instead become distinct, but still integrated to the posterior part of the oral veil [79]. All members of Doridoxidae and Heterodorididae at the adult stages have well-defined anterior corners of notum integrated with the rhinophores and posterior part of the oral veil (Figs 1 and 7), thus similar in this respect to the early juveniles of *Tritonia*. This is a reliable ontogenetic evidence for the plesiomorphic state of the distinct anterior corners of notum and ancestral integration of the oral veil and rhinophores. Among tritoniids only few genera, e.g. *Tochuina* and *Tritoniella* possess distinct corners of the anterior notum at the adult stages (Fig 1A and 1B). The well-defined anterior corners of notum in combination with closed rhinophoral sheaths present also in the other families (e.g. Curnonidae from Antarctic and Lemindidae from South Africa), which are distantly related to the family Tritoniidae [115, 118–121]. These ontogenetic and molecular phylogenetic data confirm ancestral pattern of the anterior corners of the notum integrated with the separated rhinophores and the oral veil (Figs 1, 3, 7 and 8) for Tritonioida and Dendronotoidea. This conclusion is further supported by the currently widely accepted close relationship of the nudibranch molluscs with the group Pleurobranchida [2, 25, 122]. All members of Pleurobranchida do not have paired branched dorsolateral appendages, but possess distinct notum (including anterior parts). Therefore, any reductions of the notum including its anterior corners are derived states. Pleurobranchids also have enrolled rhinophores and a lateral gill. The lateral gill is completely reduced in all non-dorid nudibranchs [2, 24]. The genera *Doridoxa* and *Heterodoris* and tritoniid *Tochuina* are lacking the lateral gill, but by patterns of the separate rhinophoral sheaths integrated to the oral veil and presence of the anterior notal corners demonstrate similarity to the enrolled rhinophores of Pleurobranchaeidae integrated into the oral veil [25].

The enigmatic nudibranch *Doridoxa* has always been considered as a crucial taxon to produce a general classification of the nudibranch molluscs [107]. In the present study *Doridoxa* and another poorly known but externally similar taxon *Heterodoris* were included into a broad-scope phylogeny together with the family Tritoniidae. Phylogenetic analysis evidently shows that Tritoniidae evolutionary close to Arminidae, *Doridoxa*, *Heterodoris*, and *Doridomorpha* (Fig 1A). Previous morphological consideration that *Heterodoris* does not belong to the family Arminidae [123] is confirmed in the present study by the molecular data (see also details in the remarks above). This is important because currently *Heterodoris* formally was included into the family Arminidae [124], possibly because it was mentioned in a morphology-based study on the species of *Armina* and *Dermatobranchus* [125].

In a previous analysis [108] *Doridoxa* (without inclusion of *Heterodoris*) was placed with a lower support in a basal position to the majority of the non-dorid nudibranch families. The present analysis places Doridoxidae and Heterodorididae as a sister group to Arminidae (Fig 1A). This is new evidence that the joined rhinophores in Arminidae is a secondary condition, as it was proposed before [25]. The secondary joining of the rhinophores in Arminidae is accompanied by a correlative transversal folding of the oral veil (Fig 1B). This special feature was attested for the majority of arminids [123, 125], but remained morphologically unexplained. The present phylogenetic data support morphological transformation of the ancestral broad oral veil with a smooth (or a finely tuberculated) frontal edge into
various number of the distinct elongate processes. To explain this process clearly, a scheme of the potential morphological transformations of the rhinophores and oral veil that supported by the present phylogenetic data is given on the Fig 1B. The oral veil and its derivates are in yellow colour. In a recent study [24] we showed using phylogenetic analysis and ancestral character state reconstruction that pair of oral tentacles can be evolutionary reversed into oral veil-like structure via paedomorphosis. In this study we show a potential way of the evolutionary formation of the oral tentacles (as one of the key novelties of the nudibranch molluscs) in course of the process of peramorphosis (Fig 1A and 1B) (for a definition of the term see [24]). In support of this implication, within the family Tritoniidae (which usually possesses a broad oral veil), a genus Marianina acquired a pair of long, tentacle-like oral processes, whereas other oral veil appendages were considerably reduced (Fig 1B). These phylogenetic data evidently supported the independent formation of the oral tentacles in several major nudibranch lineages. The partly enrolled anterior corners of the oral veils of Pleurobranchida are present in Tritoniidae and probably were additional precursors for the formation of the oral tentacles. Here we also evidently show that another enigmatic family Doridomorphidae (with a single genus Doridomorpha) is nested together with Doridoxa and Heterodoris clades (Fig 1A). Doridomorphidae thus secondarily acquired dorid-like external shape but via a different ontogenetic mechanism: the oral veil of ancestors of Doridomorpha was completely merged with the frontal part of the notum (Fig 1B, the derivates of oral veil coloured in yellow in every depicted taxon, including Doridomorpha). In true dorids the oral veil is instead enclosed by the frontal notal edge [126]. Doridomorpha does not show any distinct oral veil around the mouth under the putative “anterior notal edge” [127, 128]. This is a direct morphological indication that oral veil in Doridomorphidae was fused with the notum. The frontal notal edge of Doridomorpha is anterior part of the modified and fused oral veil. In dorids the frontal edge instead is the true notum and separate oral veil is placed under the frontal notum [126].

A division of the traditional dendronotacean nudibranch into superfamilies Dendronotoidea and Tritinoidea is used currently [129]. These superfamilies are highly disbalanced by its taxonomic composition. Dendronotoidea includes eight families (Bornellidae Bergh, 1874, Dendronotidae Allman, 1845, Dotidae Gray, 1853, Hancockiidae MacFarland, 1923, Lomontidae Bergh, 1890, Phylliroidae Menke, 1830, Scyllaeidae Alder et Hancock, 1855, Tethydidae Rafinesque, 1815). Tritinoidea contains only single family Tritoniidae, supposedly related to Arminidae [4, 117]. The present study is also contributed to this problem. By inclusion the molecular phylogenetic data in analysis on Doridoxa and Heterodoris was shown that these taxa (without branched dorsolateral appendages) together with Doridomorphidae and Arminidae come as a sister clade to tritoniids (Fig 1A). The tritoniid genus Tochuina is similar to the non-tritoniid Doridoxa and Heterodoris by the rhinophoral and radular patterns but already possesses numerous small dorsolateral branched appendages (secondary gills) at the edge of the notum (Figs 3, 7 and 8). The secondary gills (often represented by dichotomously branched appendages) are a key external character of the family Tritoniidae and a majority of the traditional dendronotacean nudibranchs [2]. According to the present molecular phylogenetic data, the small branched dorsolateral appendages of Tochuina could be evolutionary predecessors for the large distinct dorsolateral appendages in other tritoniids (Figs 1 and 8). Antarctic tritoniid Tritoniella (without evident branched appendages) is sister to the North Pacific genus Tochuina (Fig 1A). It may imply that acquisition of the branched appendages for more efficient respiratory function took place among large-bodied basal tritoniids independently from other traditional dendronotaceans. However it will require special explanation because considerable ontogenetic similarities between radulae of Dendronotidae and Tritoniidae [78, 79] contradict to the paraphyly of traditional dendronotaceans. The unstable
phylogenetic position of the tritoniid *Marionia* (with distinct branched appendages) either basal to all tritoniids [19, present study] or rendering Tritoniidae paraphyletic [5, 72, 92] also does not support this scenario. The tracing of the dorsolateral appendages and secondary gills evolution is therefore important both for the family Tritoniidae phylogeny and for a broad-scope understanding of the evolutionary pathways among nudibranch molluscs. In the present study we reconstruct the character evolution for secondary gills in Tritoniidae and Dendronotoidea applying Mesquite software [37] (Fig 8). We conclude that another scenario for the secondary gills evolution is also plausible. This scenario implies that common ancestor of a monophyletic group that includes all traditional dendronotaceans plus Doridoxidae, Heterodorididae, Doridomorphidae and Arminidae already acquired small respiratory dorsolateral appendages (Figs 1 and 8). These ancestral respiratory structures could be represented by not yet completely differentiated appendages at the edge of the notum. Such appendages could combine both small branched processes (as in the modern *Tochuina*) and short cerata-like structures, sometimes partly branched (as in the modern *Lomanotus*) (Fig 8). Further evolutionary differentiation led to appear distinctly branched appendages in Tritoniidae and Dendronotoidea from one hand and elongate cerata in the aeolidacean lineage on the other hand ([24, 73]; present study, Figs 1 and 8; see details of the coding of the character states in the legend to Fig 8). Both branched and non-branched appendages serve as protective structures and secondary gills since they increase an effective body surface for respiration [1, 86, 130, 131]. The appendages can be reduced in various nudibranch lineages when the respiration remains efficient without special structures. An evident case of the reduction of the branched appendages and a secondary formation of the cerata-like structures is occurred in a paedomorphic tritoniid genus *Marianina* (body length up to 15 mm) (Figs 1 and 8). A very small dorid nudibranch *Vayssierea* (a maximal length 6 mm) is entirely lacking any gills [74]. A majority of Dendronotoidea and Tritoniidae (of various sizes) have no distinct notal edge but possess branched appendages (Fig 8). Therefore, distinct notal edges of the gill-less *Doridoxa* and *Heterodoris* serve as a respiratory structure. The oxygen-rich environment of cold waters [132] can promote gigantism [133, 134]. However, among cold-water species of the family Tritoniidae only an exceptionally large-sized *Tochuina* (up to 300 mm in length) possesses small branched appendages and distinct notal edges at the same time (Fig 3H–3L). The model species *Tritonia tetraquetra* and *Tritonia exsulans* from northern Pacific are also very large (up to 300 mm), but lacking a distinct notal edge and have large branched appendages (Figs 2D–2F and 4A–4E). Instead, Antarctic *Tritoniella* (up 80 mm in length) does not possess distinct branched appendages, and hence can respire using only ample notum and short unbranched/weakly branched notal processes. Therefore if in the oxygen-rich cold water environment a reducing of body size is occurred, special respiratory structures can become unnecessary. *Doridoxa* and *Heterodoris* two times smaller than *Tritoniella* (up to 30–40 mm in length), and either completely devoid of any special respiratory structures or possesses only weak appendages (Fig 7D, 7K–7M and 7P–7S). Therefore, in the Doridoxidae-Heterodorididae lineage (Figs 1, 7 and 8) a reduction of the more complex ancestral respiratory appendages could be occurred. The irregular short processes along the notal edge in *Heterodoris* (Fig 7P–7S) may represent remnants of more complex ancestral appendages. The highly modified tropical Arminidae instead acquired lamellae under the notal edge as secondary respiratory structures [86, 123] in the warm water oxygen-deficient environment [132, 135]. Thus, based on the available morphological and phylogenetic evidences several evolutionary scenarios for traditional dendronotaceans (including the family Tritoniidae) are possible to propose. Therefore, currently accepted higher level system of the nudibranch molluscs is an interim one and require further investigations.
Conclusions

A consolidate phylogeny of the nudibranch family Tritoniidae is presented. Large tritoniid that previously was recorded from the Canadian, Russian and US Pacific coasts and commonly used as model system for neurobiological research comprises at least two species. One species is *Tritonia tetraquetra* (Pallas, 1788) and characterizes externally by commonly orange colour without white lines (Fig 2). Another species is *Tritonia exsulans* Bergh, 1894 and commonly has pink or salmon colour with white lines on the oral veil and between dorsolateral appendages (Fig 4). *Tritonia tetraquetra* inhabits a wider geographic and bathymetric range, at least from Oregon in NE Pacific to Kamchatka and the Kuril Islands in NW Pacific, at the depth about 1 m to 700 m (potentially up to 1000 m). *Tritonia exsulans* instead has a restricted geographic range and so far reliably recorded from California to British Columbia in shallow waters, in a range circa 5–100 m. Described from deeper waters (more than 500 m) off Alaska and California *Tritonia diomedeae* Bergh, 1894 shares same morphological characters with molecularly confirmed *T. tetraquetra* (Figs 1 and 2) and therefore previously [17, 18] was correctly synonymised with *T. tetraquetra*. The complicated history of a long-term confusion between type species of another tritoniid genus, *Tochuina, T. gigantea* (Fig 3) and real *Tritonia tetraquetra* is cleared. The present phylogenetic analyses for the first time shows that recently described "*Tritonia* nigritigris" from NE Pacific belongs to the genus *Tochuina* and not to *Tritonia* (Fig 1A). Importance of a fine-scale taxonomic diagnostics as the reliable way to integrate complex morphological and molecular phylogenetic patterns in an ontogenetic framework is demonstrated using the case of the family Tritoniidae and presenting the respective taxonomic synopsis. Inclusion of the non-tritoniid taxa *Doridoxa* and *Heterodoris* into phylogenetic analysis (Figs 1, 7 and 8) provides explanation for their similarity to the tritoniid genus *Tochuina* and contributes to the general phylogeny of the nudibranch molluscs.

Supporting information

S1 Fig. Phylogenetic relationships of tritoniidae, doridoxidae, heterodorididae, doridomorphidae, arminidae and dendronotoidea in single COI, 16S, and H3 gene trees inferred by Bayesian inference. Some branches are collapsed.

(TIF)

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References

1. Thompson TE, Brown GH. Biology of opisthobranch molluscs, vol. 2. London: Ray Society; 1984.
2. Wägele H, Willan RC. Phylogeny of the Nudibranchia. Zool J Linn Soc. 2000; 130: 83–181. https://doi.org/10.1006/zjls.1999.0214
3. Willan RC. The taxonomy of two host-specific, cryptic dendronotoid nudibranch species (Mollusca: Gastropoda) from Australia including a new species description. Zool J Linn Soc. 1988; 94: 39–63. https://doi.org/10.1111/j.1096-3642.1988.tb00881.x
4. Goodheart JA, Bazinet AL, Valdes A, Collins AG, Cummings MP. Prey preference follows phylogeny: evolutionary dietary patterns within the marine gastropod group Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). BMC Evol Biol. 2017; 17: 221. https://doi.org/10.1186/s12862-017-1066-0 PMID: 29073890
5. Almón B, Pérez JC, Caballer M. Expect the unexpected: a new large species of Marionia (Heterobranchia: Nudibranchia: Tritoniidae) from western Europe. Invert Syst. 2018; 32: 892–908. https://doi.org/10.1071/IS17073
6. Veprintsev BN, Krasts IV, Sakharov DA. Neurous cells of the nudibranch molluscs of Tritonia diomedia Bergh. Biophysicika. 1964; 9: 327–336.
7. Borovyagin VL, Sakharov DA. Ultrastructure of giant nerve cells of Tritonia (an atlas). Moscow: Nauka; 1968.
8. Willows AOD. Behavioral acts elicited by stimulation of single, identifiable brain cells. Science. 1967; 157: 570–574. https://doi.org/10.1126/science.157.3788.570 PMID: 6028923
9. Willows AOD, Hoyle G. Correlation of behavior with the activity of single identifiable neurons in the brain of Tritonia. Neurobiology of Invertebrates. Budapest: Akad. Kiado; 1968. pp. 443–461. https://doi.org/10.1007/978-1-4615-8618-0_31
10. Willows AOD. Giant brain cells in mollusks. Sci Amer. 1971; 224: 68–75. https://doi.org/10.1038/scientificamerican0271-68 PMID: 5545195
11. Beck JC, Cooper MS, Willows AOD. Immunocytochemical localization of pedal peptide in the central nervous system of the gastropod mollusc Tritonia diomedia. J Comp Neur. 2000; 425: 1–9. https://doi.org/10.1002/1096-9861(20000911)425:1<1::aid-cne1>3.0.co;2-y PMID: 10940937
12. Barkan CL, Zornik E. Feedback to the future: motor neuron contributions to central pattern generator function. J Exper Biol. 2019; 222. https://doi.org/10.1242/jeb.193318 PMID: 31420449
13. Newcomb JM, Katz PS. Homologues of serotonergic central pattern generator neurons in related nudibranch molluscs with divergent behaviors. J Comp Physiol A. 2007; 193: 425–443. https://doi.org/10.1007/s00359-006-0196-4 PMID: 17180703
14. Er Marcus. Opisthobranch mollusks from California. Veliger. 1961; 3: 1–85. https://www.biodiversitylibrary.org/item/134668#page/7/mode/1up
15. McDonald G. A review of the nudibranchs of the California coast. Malacologia. 1983; 24: 114–276. https://www.biodiversitylibrary.org/item/47247#page/130/mode/1up
16. Schwarz JS, McCullagh GB. Detection of substrate-associated odour cues versus prey-associated cues by the oral veil in Tritonia diomedia. Mar Fresh Beh Phys. 2016; 49: 93–107. https://doi.org/10.1080/10236244.2015.1123870
17. Martynov A.V. Nudipleura. In: Kantor Yu.I., Sysoev A.V.(Eds.). Marine and brackish water Gastropoda of Russia and adjacent countries: an illustrated catalogue. Moscow, KMK Scientific Press. 2006, pp. 267–294.
18. Martynov AV. Opisthobranch mollusca of Russia: the neglected diversity of cold waters, p. 69. In: 72nd Annual American Malacological Society & 39th Annual Western Malacological Society, 29 July-3 August, 2006, Abstracts and Program, University of Washington, Seattle, WA.
19. Valdés Á, Lundsten L, Wilson NG. Five new deep-sea species of nudibranchs (Gastropoda: Heterobranchia: Cladobranchia) from the Northeast Pacific. Zootaxa. 2018; 4526: 401–433. https://doi.org/10.11646/zootaxa.4526.4.1 PMID: 30651499
20. Murray JA, Jones AP, Links AC, Willows AOD. Daily tracking of the locomotion of the nudibranch *Tritonia tetraquetra* (Pallas 1788 = *Tritonia diomede a* Bergh 1894) in nature and the influence of water flow on orientation, crawling, and drag. Mar Fresh Beh Phys. 2011; 44: 265–288. https://doi.org/10.1080/10236244.2011.629463

21. MolluscaBase. *Tritonia tetraquetra* (Pallas, 1788). Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=851422 on 2020-03-29.

22. Behrens DW, Hermosillo A. Eastern Pacific nudibranchs, a guide to the opisthobranchs from Alaska to Central America. Monterey: Sea Challengers; 2005.

23. Odhner NH. On the taxonomy of the family Tritoniidae (Mollusca: Opisthobranchia). The Veliger 1963; 6: 48–52. https://www.biodiversitylibrary.org/item/134153#page/18/mode/1up

24. Martynov A, Lundin K, Picton B, Fletcher K, Malmberg K, Korshunova T. Multiple paedomorphic lineages of soft-substrate burrowing invertebrates: parallels in the origin of *Xenocratera* and *Xenoturbella*. PLoS ONE; 2020; 15: e0227173. https://doi.org/10.1371/journal.pone.0227173 PMID: 31940379

25. Martynov AV. From ‘tree-thinking’ to ‘cycle-thinking’: ontogenetic systematics of nudibranch molluscs. Thalassas; 2011; 27: 193–224. ISSN 0212-5919.

26. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech. 1994; 3: 294–299. PMID: 7881515

27. Palumbi SR, Martin AP, Romano S, McMillan WO, Stice L, Grabowski G. The simple fool’s guide to PCR. Honolulu: University of Hawaii; 2002.

28. Puslednik L, Serb JM. Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. Mol Phy Evol. 2008; 48: 1178–1188. https://doi.org/10.1016/j.ympev.2008.05.006

29. Colgan D, Macarana J, Cassidy G, Gray MR. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust J Zool. 1998; 4: 419–437. https://doi.org/10.1071/ZO98048

30. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res. 2002; 30: 3059–3066. https://doi.org/10.1093/nar/gkf436 PMID: 12136088

31. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 2007; 56: 564–577. https://doi.org/10.1080/10635150701472164 PMID: 17654362

32. Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. Bayesian phylogenetic analysis of combined data. Syst Biol. 2004; 53: 47–67. https://doi.org/10.1080/10635504909264699 PMID: 14965900

33. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012; 61: 539–542. https://doi.org/10.1093/sysbio/sys029 PMID: 22357727

34. Stamatakis A, Hoover, P, Rougemont JA. Rapid bootstrap algorithm for the RAxML web-servers. Syst Biol. 2008; 75: 758–771. https://doi.org/10.1080/1063550802429642

35. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33: 1870–1874. https://doi.org/10.1093/molev/msw054 PMID: 27004904

36. Puillandre N, Lambert A, Brouillet S, Achaz G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol Ecol. 2012; 21, 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x PMID: 21883587

37. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 3.10. 2016. Available at http://mesquiteproject.org

38. Pallas PS. 1788. Marina varia nova et rariora. Nova Acta Acad. Sci. Imp. Petrop. 1788; 2: 229–250. https://www.biodiversitylibrary.org/page/1009567#page/351/mode/1up

39. Stejneger L. Georg Wilhelm Steller. The pioneer of Alaskan Natural History. Cambridge, Massachusetts: Harvard: University Press; 1936.

40. Pekarskiy P. Archives investigations on the picture of non existing now animals *Rhytina borealis*. Mem Russ Imp Acad Sci. 1869; 15: 1–33.

41. Andreev AI. Sketches on historical sources of Siberia. 2. G.W.Steller. Moscow–Leningrad, Nauka; 1965, 229–286 pp.

42. Bergh R. On the nudibranchiate gastropod mollusca of the North Pacific Ocean, with special reference to those of Alaska, pt. 1. Proc Acad Nat Sci Phil. 1879; 31: 71–132. https://www.biodiversitylibrary.org/item/17663#page/7/mode/1up
43. Bergh R. Malacologische untersuchungen. In: Reisen im Archipel der Philippinen von Carl Semper. Zweiter Theil. 1904; 6: 1–56. https://www.biodiversitylibrary.org/item/50273#page/14/mode/1up

44. O’Donoghue CH. Notes on the nudibranchiate Mollusca from the Vancouver Island region. III. Records of species and distribution. Trans Roy Can Inst. 1922; 14: 145–167.

45. O’Donoghue CH. Notes on the nudibranchiate Mollusca from the Vancouver Island region. IV. Trans Roy Can Inst. 1924; 15: 1–33.

46. Odhner NH. Nudibranchia Dendronotacea—A revision of the system. Mém Mus Roy d’Hist Natur Belg. 2; 1936: 1057–1128.

47. MacFarland FM. Studies on opisthobranchiate mollusks of the Pacific Coast of North America. Mem Cal Acad Sci. 1966; 6: 1–546. https://www.biodiversitylibrary.org/bibliography/3949/#summary

48. Thompson TE. Tritoniidae from the North American Pacific coast. The Veliger. 1971; 13: 333–338. https://www.biodiversitylibrary.org/item/134687#page/7/mode/1up

49. Martynov AV. Opisthobranch molluscs of the coastal waters of Commander Islands with the notes on their fauna in Far-Eastern seas of Russia. In: Bottom fauna and flora of the Commander Islands. Vladivostok, Dalnauka; 1997, 230–241 pp.

50. Bergh R. Die Opisthobranchien. Reports on the dredging operations off the West Coast of Central America to the Galapagos. Bull Mus Comp Zool. 1894; 25: 125–233. https://www.biodiversitylibrary.org/item/24909#page/8/mode/1up

51. Aurivillius C. Öfversigt öfver de af Vega-expeditionen insamlade arktiska hafsmollusker. II. Placophora och Gastropoda. Vega-Expeditionens Vetensk Iakt Bearb Delt Res Forsk. 1887; 4: 311–383. https://www.biodiversitylibrary.org/item/131224#page/7/mode/1up

52. Baba K. Duvacellua septemtrionalis nov. sp., a nudibranchiate mollusc from the Sea of Okhotsk. Zool Mag. 1937; 49: 391–392.

53. Minichev Yu S. Tritonia primorjensis sp. nov. (Gastropoda, Opisthobranchia), an object for neurophysiological and biophysical investigations studies. Zool Zhur. 1971; 50: 282–284.

54. ZMUK. The digitized type collection of the Natural History Museum of Denmark. 2020. Accessed through http://www.daim.snm.ku.dk/The-digitized-type-collection.

55. O’Donoghue CH. Nudibranchiate Mollusca from the Vancouver Island region. Trans Roy Can Inst. 1921; 13: 147–209.

56. Volodchenko Nl. Opisthobranchia. Atlas of invertebrates of the Far-Eastern seas of USSR. Ed. Pavovsky E.N. Moscow: Nauka Publ; 1955.

57. Baba K. Opisthobranchia of Japan (I). J Dep Agr Kyushu Imp Univ. 1937; 5: 195–236.

58. Behrens DW. Pacific coast nudibranchs, a guide to the opisthobranchs of the northeastern Pacific. 1980. California, Sea Challengers.

59. Lee S., Hasegawa T. Evolution of the Ainu language in space and time. PLoS ONE 2013; 8: e62243. https://doi.org/10.1371/journal.pone.0062243 PMID: 23638014

60. Fukazawa M. Ainu language and Ainu speakers. Routledge Handbook of Japanese Sociolinguistic; 2019.

61. Takase K. Diet of the Kuril Ainu as evidenced from charred materials adhering to ceramic surfaces. J Faculty Humanities and Human Sci, Hokkaido Univ. 2020; 15: 37–47. http://hdl.handle.net/2115/77453

62. Dobrotvorsky MM. Ainu-Russian Dictionary. Kazan: University Typography; 1875.

63. Wägele H, Klussmann-Kolb A, Verbeek E, Schrödl M. Flashback and foreshadowing—a review of the taxon Opisthobranchia. Org Div Evol 2014; 14: 133–149. https://doi.org/10.1007/s13127-013-0151-5

64. Chiri M. Classified dictionaries of the Ainu language. Vol. 2, Animals. Tokyo: Japan Citizens Culture Institute; 1962. PMID: 14020887

65. Lambert G, Karney R, Rhee WY, Carman MC. Wild and cultured edible tunicates: a review. Man Biol Inv. 2016; 7: 59–66. https://doi.org/10.3391/mbi.2016.7.1.08

66. Hokkaido Research Organization. 2020. Accessed through: https://www.hro.or.jp/list/fisheries/marine/att/o7u1kr000000enzenz.pdf

67. Vovin AV. A Reconstruction of proto-Ainu. Leiden: Brill; 1993.

68. Ono Y. Reconsideration of “Major division” of Ainu dialects: a statistical reanalysis of Asai (1974). North Lang Stud. 2020; 10: 231–254. http://hdl.handle.net/2115/77595

69. Ev Marcus. The western Atlantic Tritoniidae. 1983. Biol Zool. 6: 177–214.

70. Gosliner TM, Ghiselin MT. A new species of Tritonia (Opisthobranchia: Gastropoda). Bull. Mar. Sci. 1987; 40: 428–436.
Consolidated data on phylogeny of the family Tritoniidae clarify taxonomic status of the neuroscience models

71. MolluscaBase. Tochuina Odhner, 1963. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=248350 on 2020-03-31 MolluscaBase.

72. Silva FV, Herrero-Barreucua A, Pola M, Cervera JL. Description of a new species of Marionia (Gastropoda: Heterobranchia: Tritoniidae) from the Gulf of Guinea (eastern Atlantic Ocean). Bull Mar Sci. 2019; 95: 431–447. https://doi.org/10.5343/bms.2018.0096

73. Korshunova TA, Martynov AV, Bakken T, Evertsen J, Fletcher K, Mudianta IW, et al. Polyphyly of the traditional family Flabellinidae affects a major group of Nudibranchia: aeolidacean taxonomic reassessment with descriptions of several new families, genera, and species (Mollusca, Gastropoda). ZooKeys. 2017; 717: 1–139. https://doi.org/10.3897/zookeys.717.21885

74. Korshunova TA, Fletcher K, Picton B, Lundin K, Kashio S, Sanamyan N, et al. The Emperor's Cadlina, hidden diversity and gill cavity evolution: new insights for the taxonomy and phylogeny of dorid nudibranchs (Mollusca: Gastropoda). Zool J Linn Soc. 2020; 189: 762–827, https://doi.org/10.1093/zoolinnean/zza126/5741605

75. Pola M, Hallas JM, Gosliner TM. Welcome back Janolidae and Antipellia: Improving the understanding of Janolidae and Madrellidae (Cladobranchia, Heterobranchia) with description of four new species. J Zool Syst Evol Res. 2019; 57: 345–368. https://doi.org/10.1111/jzs.12257

76. Korshunova TA, Martynov AV, Picton BE. Ontogeny as an important part of integrative taxonomy in tergipedid aeolidaceans (Gastropoda: Nudibranchia) with a description of a new genus and species from the Barents Sea. Zootaxa. 2017; 4324: 1–22. https://doi.org/10.11646/zootaxa.4324.1.1

77. Ortea J, Moro L, Bacallado JJ, Sanchez JJ, Telle A, Herrero R. Nuevas aportaciones al inventario de las babosas marinas del archipiélago canario (Mollusca: Opisthobranchia y Sacoglossa). Vieraea. 2009; 37: 105–117.

78. Ev Marcus, Er Marcus. American opisthobranch mollusks Part I, Tropical American opisthobranchs, Part II, Opisthobranchs from the Gulf of California. Studies in Tropical Oceanography, Miami. 1967; 6: 1–256.

79. Thompson TE. Studies on the ontogeny of Tritonia hombergi Cuvier (Gastropoda Opisthobranchia). Phil. Trans. Roy. Soc. Lond. B 1962; 245: 171–218. https://doi.org/10.1098/rstb.1962.0009

80. Martynov A, Fujiwara Y, Tsuchida S, Nakano R, Sanamyan N, Sanamyan K, et al. Three new species of the genus Dendronotus from Japan and Russia (Mollusca, Nudibranchia). Zootaxa 2020; 4747: 495–513. https://doi.org/10.11646/zootaxa.4747.3.4 PMID: 32230098

81. Korshunova T, Bakken T, Grettan V, Johnson K, Lundin K, Martynov A. A synoptic review of the family Dendronotidae (Mollusca: Nudibranchia): a multilevel organisinal diversity approach. Contr Zool. 2020; 1–61, https://doi.org/10.1163/18759866-bja10014

82. Pruvot-Fol A. Diagnose provisoires (incomplètes) des espèces nouvelles et liste provisoire des mollusques nudibranches recueillis par Mme. A. Pruvot-Fol en Nouvelle Calédonie (Ile des Pins). Bul Mus Nat d’Hist Natur, Paris. 1930; 2: 229–232.

83. Silva FV, de Azevedo MV., Matthews-Cascon H. A new species of Tritonia (Opisthobranchia: Nudibranchia: Tritoniidae) from the tropical South Atlantic Ocean. J Mar Biol Assoc UK. 2014; 94: 579–585. https://doi.org/10.1017/S0025315413001586

84. Carlson CH, Hoff PJ. External description of a living Aranucus bifidus (Odhner, 1936) (Opisthobranchia: Dendronotacea). The Veliger. 1973; 15: 172–173.

85. Burn RF. The taxonomy and distribution of Marianina rosea (Pruvot-Fol, 1930) and Thecacera pacifica (Bergh, 1883) comb. nov. (Opisthobranchia: Dendronotacea and Doridacea). The Veliger. 1974; 16: 30–36. https://www.biodiversitylibrary.org/item/134247#/page/7/mode/1up

86. Rudman WB. Suborder Dendronotina. In: Beesley PL, Ross GJB, Wells A, eds. Mollusca: the southern synthesis. Fauna of Australia, Vol. 5, part B, Chapter 16. Melbourne: CSIRO Publishing; 1998, 990–1001 pp.

87. Avila C, Kelman D, Kashman Y, Benayahu Y. An association between a dendronotid nudibranch (Mollusca, Opisthobranchia) and a soft coral (Octocorallia, Alcyonaria) from the Red Sea. J Nat Hist. 1999; 33: 1433–1449. https://doi.org/10.1080/002229999299833

88. Silva FV, Oliveira De Meirelles CA, Matthews-Cascon H. A new species of Marionia (Opisthobranchia: Nudibranchia: Tritoniidae) from south Atlantic Ocean. J Mar Biol Assos UK 2013; 93: 1617–1624. https://doi.org/10.1017/S0025315412001671

89. Smith VG, Gosliner TM. A new species of Marionia (Gastropoda: Nudibranchia) from the Caroline Islands. Proc Calif Acad Sci. 2005; 56: 66–75.

90. Schmekel L, Portmann A. Opisthobranchia des Mittelmeeres, Nudibranchia und Sacoglossa. Fauna e fora del Golfo di Napoli. 1982; 40: 1–410.

91. Baba K. Opisthobranchia of Sagami Bay collected by His Majesty The Emperor of Japan. Tokyo: Iwanami Shoten; 1949.
92. Hulett RE, Mahguib J, Gosliner TM, Valdés Á. Molecular evaluation of the phylogenetic position of the enigmatic species *Trivetta papalotla* (Bertsch) (Mollusca: Nudibranchia). Invert Syst. 2015; 29: 215–222. https://doi.org/10.1071/IS15002
93. Martynov AV, Korshunova TA. Opisthobranch molluscs of the seas of Russia. A colour guide to their taxonomy and biology. Moscow: Fiton Press; 2011.
94. Roginskaya IS. A new species of deep-sea Tritoniidae (Opisthobranchia, Nudibranchia, Dendronotoidea) from the slope of Kurile Islands. Trans Shirshov Inst Ocean Acad USSR. 1984; 119: 99–105.
95. Powell A. New species of nudibranchiate mollusca from Auckland waters. Rec Auck Mus Inst. 1937; 2: 119–124. https://www.jstor.org/stable/42905972
96. Willan RC, Morton JE. Marine Molluscs part II—Opisthobranchia. 1984. University of Auckland, Leigh Marine Laboratory.
97. Valdés Á, Murillo FJ, McCarthy JB, Yedinak N. New deep-water records and species of North Atlantic nudibranchs (Mollusca, Gastropoda: Heterobranchia) with the description of a new species. J Mar Biol Assoc UK. 2017; 97: 303–319. https://doi.org/10.1017/S0025315416000394
98. Wägele H. The morphology and taxonomy of the Antarctic species of *Tritonia Cuvier, 1797* (Nudibranchia, Dendronotoidea). Zool J Linn Soc. 1995; 113: 21–46. https://doi.org/10.1016/0162-3893(95)00032-2
99. Bertsch H, Osuna AM. A new species of *Tritonia* (Nudibranchia) from southern California and Baja California. Nautilus 1986; 100: 46–49. https://www.biodiversitylibrary.org/page/8277274#page/60/mode/1up
100. Bergh R. The Opisthobranchiata of South Africa. Trans South Afr Phil Soc. 1907; 17: 1–144. https://doi.org/10.1080/21560382.1907.9526085
101. Odhner NH. Die Opisthobranchien. Zool Res Swed Antarctic Exped 1901–1903. 1926; 2: 1–100.
102. Wägele H. On the anatomy and zoogeography of *Tritoniella bellii* Eliot, 1907 (Opisthobranchia, Nudibranchia) and the synonymy of *T. sinuata* Eliot, 1907. Polar Biol. 1989; 9: 235–244.
103. Eliot C. The Nudibranchiata of the Scottish National Antarctic Expedition. Trans Roy Soc Edinburgh. 1905; 41: 22. https://doi.org/10.1017/S0080456800035493
104. Baba K. Taxonomic study on *Tritoniopsis elegans* (Audouin, 1826) from Seto, Japan (Nudibranchia—Dendronotoidea). Publ Seto Mar Biol Lab. 1969; 16: 395–398. https://repository.kulib.kyoto-u.ac.jp/dspace/handle/2433/175562
105. Bergh R. Nudibranchiate Gasteropoder. Den Danske Ingolf-Exped. 1899; 2: 1–46.
106. Bergh R. Nudibranchiate Gasteropoder. The Danish Ingolf-Exped. 1900; 2: 1–49.
107. Schrödl M, Wägele H, Willan RC. Taxonomic redescription of the Doridoxidae (Gastropoda; Opisthobranchia), an enigmatic family of deep water nudibranchs, with discussion of basal nudibranch phylogeny. Zool Anz. 2001; 240: 83–97. https://doi.org/10.1078/0044-5231-00008
108. Mahguib J, and Valdés A. Molecular investigation of the phylogenetic position of the polar nudibranch *Doridoxa* (Mollusca, Gastropoda, Heterobranchia). Pol Biol. 2015; 38: 1369. https://doi.org/10.1007/s00300-015-1700-5
109. Krause A. Mollusken von Ostspitzbergen. Zool Jahrb Abth Syst Geogr Biol Thier. 1892; 6: 339–376.
110. Barnard KH. Deep sea Mollusca from west of Cape Point, South Africa. Ann South Afr Mus. 1963; 46: 407–452.
111. Roginskiyaya IS. On the systematic status and distribution of *Dermatobranchus walteri* (Krause, 1892) (Nudibranchia Arminidae). Zool Zhur. 1969; 48: 1320–1324.
112. Verrill AE. Catalogue of Marine Mollusca added to the fauna of the New England region, durin the past ten years. Trans Conn Acad Sci Art. 5: 447–599. https://doi.org/10.5962/bhl.title.12964
113. Odhner NH. Nudibranchs and lamellariids from the Trondhjem Fjord. Kong Norsk Vidensk Selsk Skrift, NR. 2. Med Trondh Biolog Stat NR. 1926; 24: 1–36.
114. Willan R.C. A new abyssal arminacean nudibranch from New Zealand. NZ J Zool. 1981; 8: 325–330. https://doi.org/10.1080/03014223.1981.10430612
115. Griffiths RJ. Description of a new South African arminacean and the proposed re-instatement of the genus *Attthila* Bergh (Mollusca, Opisthobranchia). Ann South Afr Mus. 1985; 95: 269–280. https://www.biodiversitylibrary.org/page/40682756#page/335/mode/1up
116. Bouchet P. Opisthobranches de profondeur de l’Océan Atlantique. II: Notaspidea et Nudibranchiata. J Moll Stud. 1977; 43: 28–66. https://doi.org/10.1093/oxfordjournals.mollus.a065359
117. Goodheart JS, Bleidießel S, Schillo D, Strong EE, Ayres DL, Preisfeld A, et al. Comparative morphology and evolution of the cnidosac in Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). Front Zool. 2018; 15: 43. https://doi.org/10.1186/s12983-018-0289-2 PMID: 30473719

118. Wägele H. Studies on the morphology and anatomy of the Antarctic nudibranch genera Pseudotritonia Thiele, 1912 and Telarma Odhner, 1934 with a discussion of the family Charcotidae Odhner, 1926 (Nudibranchia, Opisthobranchia). Zool J Linn Soc. 1991; 101: 359–389. https://doi.org/10.1111/j.1096-3642.1991.tb00657.x

119. Wägele H, Barnes DKA., Bullough LW. Redescription of Charcotia granulosa Vayssiere, 1906 (Nudibranchia, Arminoidea, Charcotiidae) from Signy Island, Antarctica. J Moll Stud. 1995; 61: 197–207. https://doi.org/10.1093/mollus/61.2.197

120. Pola M., Gosliner T. M. The first molecular phylogeny of cladobranchian opisthobranchs (Mollusca, Gastropoda, Nudibranchia). Mol. Phyl. Evol. 2010; 56: 931–941. https://doi.org/10.1016/j.ympev.2010.05.003 PMID: 20460158

121. Bouchet P, Rocroi J-P, Hausdorf B, Kaim A, Kano Y, Nützel A, et al. Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia 2017; 61: 1–526.

122. Pola M., Gosliner T. M. The first molecular phylogeny of cladobranchian opisthobranchs (Mollusca, Gastropoda, Nudibranchia). Mol. Phyl. Evol. 2010; 56: 931–941. https://doi.org/10.1016/j.ympev.2010.05.003 PMID: 20460158

123. Kölb A, Wägele H. On the phylogeny of the Arminidae (Gastropoda, Opisthobranchia, Nudibranchia) with considerations of biogeography. J Zool Syst Evol Res. 1998; 36: 53–64. https://doi.org/10.1111/j.1439-0469.1998.tb00777.x

124. MolluscaBase. Arminidae Iredale & O’Donoghue, 1923 (1841). Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=2087 on 2020-04-08.

125. Gosliner TM, Fahey SJ. Previously undocumented diversity and abundance of cryptic species: a phylogenetic analysis of Indo-Pacific Arminidae Rafinesque, 1814 (Mollusca: Nudibranchia) with descriptions of 20 new species of Dermatobranchus. Zool J Linn Soc. 2012; 161: 245–356. https://doi.org/10.1111/j.1096-3642.2010.00649.x

126. Martynov AV, Korshunova TA. A new deep-sea genus of the family Polyceridae (Nudibranchia) possesses a gill cavity, with implications for the cryptobranch condition and a ‘Periodic Table’ approach to taxonomy. J Moll Stud. 2015; 81: 365–379. https://doi.org/10.1093/mollus/eyv003

127. Eliot CNE, Evans TJ. Doridoeides gardineri: a doridiform cladohapatic nudibranch. Quart. J Micr. Sci 1908; 52: 279–299. https://www.biodiversitylibrary.org/page/15268124#page/295/mode/1up

128. Hoon T.C. New record of the nudibranch Doridomorpha gardineri in Singapore. Singapore Biodiver Rec. 2015: 151–153.

129. MolluscaBase. Nudibranchia. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=1762 on 2020-04-08

130. Graham JB. Ecological and evolutionary aspects of integumentary respiration: body size, diffusion, and the Invertebrata. Amer Zool., 1988; 28: 1031–1045. https://doi.org/10.1093/icb/28.3.1031

131. Neusser TP, Hanke F, Haszprunar Gerhard, Jörgen KM. ‘Dorsal vessels’? 3D-reconstruction and ultrastructure of the renopericardial system of Elysia viridis (Montagu, 1804) (Gastropoda: Sacoglossa), with a discussion of function and homology. J Moll Stud. 2019; 85: 79–91, https://doi.org/10.1093/mollus/eyy049

132. Mahaffey C, Palmer M, Greenwood N, Sharple J. Impacts of climate change on dissolved oxygen concentration relevant to the coastal and marine environment around the UK MCCIP Sci Rev. 2020: 31–53. https://doi.org/10.14465/2020.arc02.ox

133. Chapelle G, Peck LS. Polar gigantism dictated by oxygen availability. Nature. 1999; 399: 114–115. https://doi.org/10.1038/20099

134. Woods AH, Moran AL. Reconsidering the oxygen-temperature hypothesis of polar gigantism: successes, failures, and nuance. Integr Comp Biol. 2020; icaa088, https://doi.org/10.1093/icb/icaa088 PMID: 32573680

135. Oschlies A, Wolfgang K, Landolfi A, Kähler P. Loss of fixed nitrogen causes net oxygen gain in a warmer future ocean. Nat Commun. 2019; 10: 2805. https://doi.org/10.1038/s41467-019-10813-w PMID: 31243270