Dendrodorididae (Heterobranchia, Nudibranchia) from Persian Gulf
with a description of a new species of Doriopsilla
and remarks on the family

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Abstract. The family Dendrodorididae has a global distribution, with prevalence in tropical and subtropical intertidal zones. Three species of Dendrodorididae were collected from the intertidal zone of the northern coast of the Persian Gulf in Iran. Based on anatomical, histological, and molecular investigations they can be assigned to Dendrodoris fumata, Dendrodoris nigra, and a new species of Doriopsilla, D. aroni sp. nov. Molecular analyses of CO1 and 16S, including all genera of Dendrodorididae, members of the sister taxon Phyllidiidae, and other dorid outgroups resulted in a polyphyletic genus Dendrodoris, which is in contrast to the nuclear gene studies. Our molecular results confirm the differentiation between Dendrodoris rubra and D. fumata. Dendrodoris nigra, D. fumata, and D. krusensternii each consist of several clades, indicating cryptic species complexes requiring further investigation. We describe the presence of bacteria for the first time in the vestibular gland of D. fumata. Validation of the specimens of Doriopsilla from the Persian Gulf as a new species is supported by haplotype networking, genetic distance, and ABGD analyses of mitochondrial genes. Our CO1 analysis confirms a previous hypothesis that Cariopsilla is a junior synonym of Doriopsilla.

Keywords. Dendrodoris, Iran, histology, species delimitation, haplotype networking.

Introduction

Nudibranchia Cuvier, 1817, known from all marine geographic areas, are usually found from the intertidal zone to the deep waters in different types of habitats (Klussmann-Kolb et al. 2008; Yonow 2015). Its highest diversity with approximately 2000 species is reported from tropical areas of the Indo-Pacific Ocean, but the diversity is assumed to be much higher (Gosliner et al. 2018). Some geographic areas experienced much more attention in the last years, mainly the species-rich Coral Triangle (e.g.,
Eisenbarth et al. 2018; Gosliner et al. 2018; Papu et al. 2022), while other areas are much less studied, e.g., the Iranian coastline. Most recently, Amini-Yekta & Dekker (2021) published a checklist on Gastropoda Cuvier, 1795 recorded from the Persian Gulf and the Gulf of Oman. Within this checklist, 44 nudibranch species are mentioned of 850 gastropods in total. Thus, the species number is considerably smaller than in other tropical areas, e.g., the Red Sea, Mozambique, Maldivian Archipelago, or the Coral Triangle (Yonow 2008; Tibiriçá et al. 2017; Papu et al. 2020; Cunha et al. 2023). One reason for this could be the lower number of collection campaigns in the Persian Gulf, another the harsher conditions with higher average temperature and salinity compared to the Indian Ocean (Rezai et al. 2016; Maniei et al. 2020). Only few nudibranch groups can be found regularly in the intertidal flats worldwide, and one of these is the family Dendrodorididae O’Donoghue, 1924 (1864). This family comprises three genera, *Dendrodoris* Ehrenberg, 1831, *Doriopsilla* Bergh, 1880, and *Cariopsilla* Ortea & Espinosa, 2006 (MolluscaBase 2023a). Of the 44 species of *Dendrodoris* listed in the World Register of Marine Species (WoRMS Editorial Board 2023) (https://www.marinespecies.org), only two are recorded from the Persian Gulf and the Gulf of Oman: *Dendrodoris fumata* (Rüppell & Leuckart, 1830) and *Dendrodoris nigra* (W. Stimpson, 1855) (Mousavipoor 2013; Fatemi & Attaran 2015; Amini-Yekta et al. 2016, 2019; Rezai et al. 2016; Abdollahi et al. 2020; Fatemi et al. 2021). *Dendrodoris krusensternii* (Gray, 1850) is listed in The Global Biodiversity Information Facility (GBIF Secretariat 2023a) with one record from the Gulf of Oman. The genus *Doriopsilla* has 26 species registered in WoRMS with two species, *Doriopsilla nigrocera* Yonow, 2012 and *Doriopsilla cf. miniata* from the Persian Gulf only, and an unidentified species of *Doriopsilla* from the Iranian coastline (Nithyanandan 2012; Yonow 2012; Rezai et al. 2016).

Members of the Dendrodorididae do not possess a radula or jaws, a feature which the taxon shares with the five genera of the family Phyllidiidae Rafinesque, 1814 and the monotypic Mandeliidae Á. Valdés & Gosliner, 1999 (Valdés & Gosliner 1999; Brodie 2001; Papu et al. 2022). These three families are currently united under the superfamily Phyllidioidea Rafinesque, 1814 since a close relationship was confirmed by morphological (Valdés & Gosliner 1999) and molecular studies (Valdés 2003; Thollesson 2020; Furfaro et al. 2022; Papu et al. 2022).

In recent years, species diversity and numbers of species have increased especially by molecular studies, in which cryptic speciation and/or variation becomes much more obvious than in morphological studies (Hirose et al. 2014; Nimbs & Smith 2021; Furfaro et al. 2022). This also holds true for the Phyllidioidea. Stoffels et al. (2016) and especially Papu et al. (2022) demonstrated the underestimation of species numbers within the Phyllidiidae; Furfaro et al. (2022) revealed cryptic variation in *Doriopsilla areolata* Bergh, 1880. Interestingly, some of these studies were able to describe small morphological variations for the molecularly well-defined species. This is of interest especially in taxa like *Dendrodoris*, which can exhibit considerable variations in external colours and patterns but have anatomical similarities, often causing taxonomic confusion, e.g., between *D. fumata* and *D. nigra* (Hirose et al. 2014). DNA sequencing enables species identification despite these variations in colour patterns or other morphological features, and species delimitation when morphological variability is lacking (Hirose et al. 2014). In the present study, we examined dendrodorid specimens collected on the Iranian intertidal coastline of the Persian Gulf, using anatomical and histological methods. Their assignment to *Dendrodoris nigra*, *D. fumata*, and a new species of *Doriopsilla* was confirmed by analysing their partial CO1 and 16S rRNA gene sequences. The inclusion of all published sequences of the Dendrodorididae genera, various sequences covering all genera of the sister taxon Phyllidiidae, and several other dorids in our comprehensive study allowed us to address putative cryptic variations within the involved species of *Dendrodoris*, as well as to delimit the new Iranian species of *Doriopsilla* from similarly coloured species. Additional network analyses were performed to provide further evidence for the delimitation of the species of *Doriopsilla* that hardly differ in their anatomical features.
Material and methods

In total, 12 specimens of *D. nigra*, 18 specimens of *D. fumata*, and 6 specimens of *Doriopsilla aroni* sp. nov. were collected from the southern coast of Iran (details summarised in Table 1). Sampling was undertaken in the intertidal zone during low tide at Bandar Lengeh (26°33′29″ N, 54°52′50″ E), and Lavan Island (26°48′20.99″ N, 53°16′4.80″ E) in February to April 2015 and March 2016 (Fig. 1A–C). Some animals were photographed alive using a digital camera (Canon SX160IS). The material is deposited in the Leibniz Institute for the Analysis of Biodiversity Change – Zoological Museum Hamburg (ZMH).

Institutional abbreviations

LIB = Leibniz Institute for the Analysis of Biodiversity Change, Bonn and Hamburg, Germany
ZMH = LIB, Zoological Museum Hamburg, Germany

Anatomical observation

For histological analyses, one specimen of *Dendrodoris nigra* (FM48, Table 1), the genital system of one *D. fumata* (FM49, Table 1), and one specimen of *Doriopsilla aroni* sp. nov. (FM50, Table 1) were embedded in hydroxyethyl methacrylate (Heraeus Kulzer GmbH) for serial sectioning. Sections (2.5 µm) were stained with toluidine blue and photographed subsequently under a ZEISS Microscope (Imager.Z2m). One specimen of *D. aroni* (FM47, Table 1) was dissected under a stereo microscope (Wild M8 ZOOM).

DNA extraction, PCR, and DNA sequencing

In total, we sequenced 11 *Dendrodoris nigra*, 17 *D. fumata*, and 5 *Doriopsilla aroni* sp. nov. specimens. To retrieve the CO1 and 16S sequences from our newly collected material, we used the primer designed by Palumbi *et al.* (1991) and Astrin & Stüben (2008), and the settings of the amplification, trimming and quality control as described in Papu *et al.* (2022). Sequences are deposited in GenBank with the accession numbers listed in Table 1.

Phylogenetic reconstruction

All available CO1 and 16S sequences of the family Dendrodorididae (248 sequences) were downloaded from GenBank (for more details see Supp. file 1: Table S1) and added to our alignment. Five species of the family Phyllidiidae and other dorids were included to better understand the relationship of the genera within Phyllidiidea. Two members of the Bathydidoridae Bergh, 1891 were used to root the trees (Supp. file 1: Table S1). Additionally, all available nuclear sequences of the 18S and H3 genes of species of *Doriopsilla*, *Dendrodoris*, and phyllidiid, as well as several dorid specimens, with two sequences

Fig. 1. Information on collection sites of specimens of Dendrodorididae O'Donoghue, 1924 (1864). A. Location of collection sites along the Iranian coast line. B. Bandar Lengeh, collection site exposed at low tide. C. Lavan Island, collection site exposed at low tide.
Table 1 (continued on next three pages). Information on the specimens and type material used for histology and/or DNA barcoding in this study.

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<th>Species</th>
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of Bathydorididae as outgroup were downloaded from GenBank (Supp. file 1: Table S1) and analysed separately from the two mitochondrial gene data sets (Table 2).

Sequences were edited using BioEdit ver. 7.2.6.1 (Hall 1999) and aligned using MAFFT (Katoh et al. 2002) in Geneious ver. 7.1.9 (Kearse et al. 2012). After trimming, the alignments of the mitochondrial genes comprised 501 bp for 16S, 640 bp for CO1 and 1141 bp for the concatenated dataset. The alignments of the nuclear genes comprised 2082 bp for 18S and 345 bp for H3. In-depth genetic analyses were partially performed on reduced datasets by confining the complete CO1 and 16S datasets to only Dendrodoris (with two additional 16S sequences of D. areolata: ON229526 and ON229532) or Doriopsilla sequences; see Table 2 for details of these alignments.

Maximum likelihood (ML) analyses were run in IQ-TREE (Nguyen et al. 2014; Trifinopoulos et al. 2016) using the online ver. 1.6.3 on a webserver (http://iqtree.cibiv.univie.ac.at/), with the GTR model for all genes and gene data sets (see Table 2). Support values were calculated based on 1000 ultrafast bootstrap replicates and the approximate likelihood ratio test (SH-aLRT) (2000 replicates). Dendroscope ver. 3.5.8 (Huson & Scornavacca 2017) and Inkscape ver. 0.92 (https://inkscape.org/en/) were used to edit the phylograms.

**Species delimitation and genetic distances**

For species delimitation, we analysed CO1, 16S, 18S, and H3 datasets and the Doriopsilla subset applying the Automatic Barcode Gap Discovery (ABGD) methodology (Puillandre et al. 2011) (Table 2). This program was also used recently for investigation of Mediterranean and Atlantic species of Dendrodoris (Galià-Camps et al. 2022). We used the default settings under the Kimura K80 model. The relative gap width, denoted as X, varies across different datasets; details are provided in Table 2. The minimum and

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**Table 2.** Summary of molecular datasets used in this study and performed analyses with indication of respective figures in text and supplement. Relative gap width (X) used in the ABGD species delimitation test is provided in brackets.

<table>
<thead>
<tr>
<th>Number of sequences (Dendrodorididae/other dorids/outgroups)</th>
<th>Phylogeny</th>
<th>ABGD Test</th>
<th>Genetic distance analysis</th>
<th>Haplotype analysis</th>
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</thead>
<tbody>
<tr>
<td>Concatenated 16S and CO1 complete dataset</td>
<td>247/19/2  Bathydorids</td>
<td>Fig. 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CO1 complete dataset with all Dendrodorididae</td>
<td>202/19/2  Bathydorids</td>
<td>Fig. 12</td>
<td>Fig. 12</td>
<td>No</td>
</tr>
<tr>
<td>16S complete dataset with all Dendrodorididae</td>
<td>198/19/2  Bathydorids</td>
<td>Fig. 11</td>
<td>Fig. 11</td>
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</tr>
<tr>
<td>18S Dendrodorididae</td>
<td>17/10/2   Bathydorids</td>
<td>Supp. file 2: Fig. S1</td>
<td>Supp. file 2: Fig. S1</td>
<td>No</td>
</tr>
<tr>
<td>H3 Dendrodorididae</td>
<td>115/10/2  Bathydorids</td>
<td>Supp. file 2: Fig. S2</td>
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</tr>
<tr>
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<td>71/0/—    Bathydorids</td>
<td>No</td>
<td>Yes</td>
<td>S2</td>
</tr>
<tr>
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<td>132/0/—   Bathydorids</td>
<td>No</td>
<td>Yes</td>
<td>S3</td>
</tr>
<tr>
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<td>98/0/3    Dendrodoris</td>
<td>Supp. file 2: Fig. S3</td>
<td>Supp. file 2: Fig. S3</td>
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<tr>
<td>CO1 subset only Doriopsilla</td>
<td>76/0/4    Dendrodoris</td>
<td>Supp. file 2: Fig. S4</td>
<td>Supp. file 2: Fig. S4</td>
<td>4</td>
</tr>
</tbody>
</table>

---
maximal pairwise uncorrected p-distances of the reduced 16S and CO1 subsets between and within species or main clades were also calculated with this program (Table 2).

**Haplotype networks of *Doriopsilla***

A statistical parsimony analysis (Templeton *et al.* 1992) was performed on the reduced *Doriopsilla* subset using the program TCS 1.21 (Clement *et al.* 2000) in PopART (Leigh & Bryant 2015) (Table 2). Settings used were a 95% connection limit and 5000 iterations. In the haplotype analysis, we also included and visualised geographical information available for each specimen.

**Results**

**Taxonomic descriptions**

Class Gastropoda Cuvier, 1795  
Subclass Heterobranchia Burmeister, 1837  
Order Nudibranchia Cuvier, 1817  
Family Dendrodorididae O’Donoghue, 1924 (1864)

**Genus *Dendrodoris*** Ehrenberg, 1831

**Type species**

*Dendrodoris limbata* Cuvier, 1804 (type by subsequent designation).

*Dendrodoris nigra* (W. Stimpson, 1855)  
Figs 2A–B, 3–4, 5A

*Dendrodoris nigra* – Yonow 2012: 53, fig. 57 (Oman) and references therein.

**Diagnosis**

Colouration usually black or dark grey. Body soft, without spicules; mantle margin delicate; more than 5 gill branches; oral tentacles absent. Ptyalin glands and salivary glands present; oesophagus characterised by a highly glandular epithelium. Penis with spines; vestibular gland present (Valdés *et al.* 1996; Brodie *et al.* 1997; Valdés & Gosliner 1999; Wägele *et al.* 1999; Yonow 2012).

**Material examined**

IRAN • 12 specs; Bandar Lengeh; 26°33′29″ N, 54°52′50″ E; intertidal; Mar.–Apr. 2015; Fatemeh Maniei leg.; ZMH 141488.

**Description**

**General appearance.** Body length of the 12 preserved animals: 10–24 mm. Live animals elongate with a soft body, the almost smooth mantle margin forming a wavy edge. Two colour variants observed: black mantle, with many white to yellow spots and with a pale red band around the mantle (Fig. 2A), or black mantle with white spots and a thick distinct red submarginal band around the notum margin and around foot margin, bordered by an outer yellow band (Fig. 2B). Rhinophores always black with a white apex and ca 12 lamellae on each clavus. Six pinnate gills present, arranged in a circle around the anus and curved inwards in life.

**Histology.** One black specimen with a distinct red submarginal band (length of preserved animal 19 mm) was examined histologically. The slide series is deposited in the histology collection of the LIB, Museum Koenig Bonn, without a number.
INTEGUMENT. Epidermis of the mantle composed of cuboidal cells with mucus cells interspersed; mucus cells with homogeneously stained purple or with dark purple grana (acid mucopolysaccharides) (Fig. 3A). Epithelium of hypnotum with cuboidal or flat cells and fewer glandular cells. Melanin grana lying subepidermal, distributed in patches.

Fig. 2. External appearance of live animals. A. Dorsal view of *Dendrodoris nigra* (W. Stimpson, 1855) with black mantle and yellow spots. B. *Dendrodoris nigra* with black mantle, white spots, and a thick distinct red submarginal band; inset shows ventral view. C. *Dendrodoris fumata* (Rüppell & Leuckart, 1830) black form. D. *Dendrodoris fumata* red form. E. *Dendrodoris fumata* pale brown form. F. Dorsal view of *Doriopsilla aroni* sp. nov.; inset shows ventral view.
**Digestive system.** Mouth area surrounded by a thick violet stained glandular layer containing high columnar cells. Oral tube with cuboidal to columnar cells, interspersed with reddish stained glandular cells (Fig. 3B). A paired ptyalin gland present with separate ducts leading into one central muscular duct finally opening dorsally into the oral tube, close to the transition into the pharynx; cells of ptyalin gland with pyknotic nuclei and no visible contents, thus creating a spongy appearance. Ducts inside the gland composed of glandular cells with pale bluish stained grana. Labial disc with reddish staining.

**Fig. 3.** Histological cross sections of epidermis, digestive system, and part of the genital system of *Dendrodoris nigra* (W. Stimpson, 1855). A. Mantle. B. Head area with parts of the digestive system; the oral tube is marked with a star. C. Section behind the head region with parts of the genital system. D. Stomach with folded wall (arrow). E. Intestine. F. Ampulla. Abbreviations: Am = Ampulla; E = Empty unicellular gland; Ep = Epithelium; G = glandular vesicle with mucopolysaccharides (stained violet); LD = Labial disc; Oe = Oesophagus; Ph = Pharynx; Sgl = Salivary gland; Vgl = Vestibular gland; Rs = Receptaculum seminis.
subepithelial glandular cells, and without cuticle (Fig. 3B). The pharynx with a triangular-shaped lumen. Epithelium consisting of cuboidal to elongate cells, covered at least in the proximal part of pharynx by a very thin cuticle. Glandular cells absent. Two muscular layers surrounding the pharyngeal epithelium; the inner one with fibres oriented transversely, which enlarge the lumen when contracted; the outer layer with fibres arranged circularly, which serve to reduce the lumen of the pharynx, and lengthen it (Fig. 3B). Salivary glands small, with small spherical cells filled with pale bluish stained grana and large nuclei (Fig. 3C). Oesophagus thicker in the cross section than the pharynx. Epithelium of the oesophagus highly folded, consisting of high columnar glandular cells staining light to dark violet (Fig. 3C). In

**Fig. 4.** Histological cross sections of sensory organs and genital, circulatory, and excretory systems of *Dendrodoris nigra* (W. Stimpson, 1855). **A.** Prostate gland. **B.** Vestibular gland with high microvilli edge (arrows). **C.** Statocyst with several otoconia (arrow) and eye. **D.** Blood gland. **E.** Kidney, digestive gland, and gonad. **F.** Syrinx; note the highly folded interior. Abbreviations: Dgl = Digestive gland; Ey = Eye; Go = Gonad; Ki = Kidney;
some areas these cells with granular, in others with homogeneously stained contents. Ciliated cells interspersed. Stomach sac-like and fully embedded within digestive gland, with folded epithelium; cells elongate and ciliate (Fig. 3D). Digestive gland lobulated or folded on dorsal side, with gonad and kidney lying in these folds. Intestine arising from the embedded stomach; its folded epithelium surrounded by an inner circular and outer longitudinal muscle layer. Epithelial cells of the intestine elongate, ciliated, and non-glandular (Fig. 3E). No caecum and typhlosole observed.

**Reproductive system.** A general outline of the reproductive system is given in Fig. 5A. Gonad forming thick layer on dorsal side above the digestive gland, composed of separate areas of oocyte and spermatocyte production. Sausage-shaped ampulla filled with autosperm. Epithelium of ampulla formed by cuboidal cells (Fig. 3F). Prostatic part of vas deferens with subepithelial glandular layer, gland cells with pale bluish stained grana (Fig. 4A). Penis very long, without cuticle, but with spines. Receptaculum seminis surrounded by muscle layer; sperm heads oriented or attached to the rather flat epithelial cells (Fig. 3C). Bursa copulatrix filled with degrading sperm, prostatic material and few eggs; epithelium partly composed of apocrine secreting cells. Bursa connecting to short distal vaginal duct, the latter opening in a common vestibulum with vas deferens. Nidamental glands already large despite the small size of the animal, consisting of three areas, staining in different shades from red to bluish and dark violet. Vestibular gland large, partly lying in the body cavity, partly in the lateral body tissue (Fig. 3C); connecting to distal oviduct. Its heavily folded epithelium consisting of small, dark blue stained cells with a thick layer of microvilli in which symbiotic bacteria appear to reside (Fig. 4B).

**Nervous system.** Cerebropleural complex at the transition of pharynx into the oesophagus. Statocyst with many otoconia (Fig. 4C).

**Circulatory system.** Ventricle very muscular. Blood gland close to cerebropleural complex, containing small, dark blue, stained cells (Fig. 4D).

**Excretory system.** Kidney lying intermingled between gonad and digestive gland; with large cuboidal cells with no stained contents (Fig. 4E). Syrinx very large, forming bulb-like structure with highly folded epithelium and long cilia (Fig. 4F).

**Additional features**

Body cavity surrounded by a very thick muscle layer, especially in the hind part of the body. A large retractor muscle originating close to the gills, dividing into two main muscles leading ventrally towards the anterior part, finally fusing with a strong ventral muscle layer in the anterior body. Small gill glands with pale bluish cytoplasm and large nuclei present at the base of the gills, opening into the gill pocket.

*Dendrodoris fumata* (Rüppell & Leuckart, 1830)

Figs 2C–E, 5B, 6

*Dendrodoris fumata* – Yonow 2012: 52, fig. 56 (Saudi Arabia) and references therein.

**Diagnosis**

Body colour pale brown to translucent red/orange to almost black; specimens with paler colours often with irregular dark patches on notum. Body soft, without spicules; more oval in shape when crawling and with a more wavy mantle margin than in *D. nigra*. Five or six large gills. Ptyalin glands and salivary glands present. Penis with spines. In the present study we confirm the presence of a vestibular gland.
Material examined

IRAN • 12 specs; Bandar Lengeh; 26°33′29″ N, 54°52′50″ E; intertidal; Mar.–Apr. 2015; Fatemeh Maniei leg.; ZMH 141489 • 6 specs; Lavan Island; 26°48′20.99″ N, 53°16′4.80″ E; Mar. 2016; Fatemeh Maniei leg.; ZMH 141489.

Description

General appearance. Body length of preserved animals: 15–32 mm. Body of investigated live animals elongate, with soft and smooth notum. Margin of the notum wavy, thin, and wider than in *D. nigra*. Body colour variable: completely black with traces of a submarginal red-orange band around the anterior end of the mantle (Fig. 2C), red (Fig. 2D) or pale brown (Fig. 2E). The red and the pale brown animals with a large number of dark brown patches on the dorsal surface, except at the margin. Rhinophores of the same colour as the dorsal surface, but always with a white apex. Clavus with 10–16 lamellae in larger animals. Five large, branched gills, usually somewhat darker than the dorsal notum.

Histology. Reproductive system of one specimen (red form, 24 mm in length, collected in Bandar Lengeh 2015) was extracted and investigated separately by histological means. The slide series is deposited in the histology collection of the LIB, Museum Koenig Bonn (without number).

Reproductive system. A general outline of the reproductive system is given in Fig. 5B. Gonad intermingled with the digestive gland and kidney. Ampulla filled with autosperm; epithelium thin with cuboidal cells (Fig. 6A). Non-prostatic part of vas deferens with elongate and ciliated epithelial cells. The prostate gland partly accompanying vas deferens, composed of epithelium with large glandular cells, staining pale blue; thick layer of subepithelial glandular cells present with small, dark blue, stained cells (Fig. 6A). Penis equipped with spines and thick cuticular layer (Fig. 6B). Vagina and vas deferens opening into vestibulum. Vagina with thick folded walls (Fig. 6C). Receptaculum seminis with epithelium composed of small cuboidal cells and surrounded by thick muscle layer (Fig. 6D); with sperm

Fig. 5. Schematic outline of genital system of species of *Dendrodoris* Ehrenberg, 1831. A. *D. nigra* (W. Stimpson 1855). B. *D. fumata* (Rüppell & Leuckart, 1830). Abbreviations: Am = Ampulla; Bc = Bursa copulatrix; Gn = Gonad; P = Penis; Pr = Prostate gland; Rs = Receptaculum seminis; Va = Vaginal duct; Vgl = Vestibular gland.
Fig. 6. Histological cross sections of genital system of *Dendrodoris fumata* (Rüppell & Leuckart, 1830). A. Gonad, ampulla, and prostate. B. Penis with spines (arrows) inside the muscular sheath. C. Vaginal duct and vas deferens. D. Receptaculum seminis. E. Gonad, prostate, bursa copulatrix, receptaculum seminis, proximal oviduct and a part of nidamental gland. F. Strongly folded vestibular gland (*) starting from less folded oviduct. G. Vestibular gland. H. Vestibular gland. Note the thick microvilli fringe. Abbreviations: Am = Ampulla; Bc = Bursa copulatrix; Gn = Gonad; Ngl = Nidamental gland; Ov = Oviduct; P = Penis; Pr = Prostate gland; Rs = Receptaculum seminis; Va = Vaginal duct; Vd = Vas deferens.
partly attached to the wall and partly lying in the lumen. Bursa copulatrix filled with sperm remnants; its thin epithelium composed of cuboidal cells, without underlying muscle layer (Fig. 6E). Female gland with three distinguishable parts: capsule, membrane, and mucous gland staining from red to dark violet (Fig. 6E). Large vestibular gland attached to distal oviduct; partly lying in lateral body tissue and partly in body cavity; internally highly folded, whereas oviduct less folded in this area (Fig. 6F). Epithelium of vestibular gland with small elongate cells, but thick microvillous border, in which bacteria are assumed to reside (Fig. 6G–H). Distal oviduct tube-like, with small mucus cells, and opening outwards next to vestibulum. A nematode and another unidentified parasite were observed in the vicinity of the genital opening (Fig. 6F).

**Genus Doriopsilla Bergh, 1880**

**Type species** *Doriopsilla areolata* Bergh, 1880 (type species by monotype).

*Doriopsilla aroni* sp. nov.  
urn:lsid:zoobank.org:act:9EE37DDA-5EBD-4D95-A124-44495753DB69  
Figs 2F, 7–9

**Diagnosis**  
Size up to 40 mm or slightly longer. Coloration cream yellow to deep orange or vermilion; a tiny white line along the rim of the gill pocket present. Dorsal notum covered by tubercles, in which many spicules intrude. Spicules also forming a thick layer around visceral cavity. Oral tentacles fused. Five gill plumes present; small gill glands present at their base. Anus eccentric. Pyloric gland present. Prostate gland forming a large mass. Penial hooks arranged in longitudinal lines in the proximal part of eversible penis.

**Etymology**  
*Doriopsilla aroni* sp. nov. is named after the first author’s young son, Aron.

**Material examined**  
**Holotype**  
IRAN • 38 mm; Bandar Lengeh; 26°33′29″ N, 54°52′50″ E; intertidal; 14 Feb. 2015; Fatemeh Maniei leg.; ZMH 141485.

**Paratypes**  
IRAN • 3 specs; same data as for holotype; ZMH 141486 • 1 spec.; same data as for holotype; ZMH 141487 histological slide series.

**Description**  
**General appearance.** Body length of preserved animals: holotype 38 mm, paratypes 20–40 mm. Colour of living animals varying from cream yellow to deep orange or vermilion. White pigmentation in the form of dots or lines on notum or rhinophores absent (Fig. 2F), except for a thin white line around the rim of the gill pocket. Body oval. Notum covered with rounded spiculate tubercles of approximately the same size, becoming smaller only along mantle margin; their colour sometimes a little paler than the notum. Hyponotum nearly translucent or cream in colour, stiffened by a network of spicules (Fig. 2F). Cylindrical rhinophores with 11–13 lamellae, same colour as tubercles but with a brighter apex. Five gill plumes (two pointing forward and three backward) located in a gill pocket. Anus eccentric to the left of the gill. Oral tentacles small and fused. Fleshy muscular foot rounded anteriorly and more acute posteriorly.
HISTOLOGY. The preserved specimen ZMH 141487 used for histological examination had a length of 40 mm. The slide series is deposited in the collection of the LIB, Zoological Museum Hamburg.

INTEGUMENT. Dorsal notum epithelium with many single subepithelial glandular cells staining red to violet and glandular follicles composed of several large bluish gland cells. These glandular follicles

Fig. 7. Histological sections of *Doriopsilla aroni* sp. nov. A. Cross section of tubercles containing spicules. B. Cross section of the mantle with spicules; two parasites can be seen in the notum tissue. C. Cross section of oesophagus and part of cerebropleural/pedal ganglia with statocyst. D. Cross section of oesophagus and opening of intestine into stomach with pyloric gland. E. Cross section of muscular oesophagus. F. Cross section of highly ciliated and muscular intestine. Abbreviations: Cplg = Cerebropleural ganglion; G = Glandular vesicle containing mucopolysaccharides (stained in violet); GF = Glandular follicles surrounded by muscle layers; In = Intestine; Oe = Oesophagus; P = Parasite; Peg = Pedal ganglion; Pgl = Pyloric gland; Sp = Spicules; St = Statocyst with otoconia.
surrounded by muscle layers (Fig. 7A). Spicules mainly present in the tubercles, around the visceral cavity (Fig. 7A–B), and in the foot.

Fig. 8. Schematic outline of digestive and genital system of *Doriopsilla aroni* sp. nov. A. Digestive system. B. Genital system and separate schematic outline of inverted penis. C. Penial spines of different shapes. Abbreviations: Bc = Bursa copulatrix; Cplg = Cerebropleural ganglion; In = Intestine; MO = Mouth opening; MP = Muscular portion of oesophagus; Ngl = Nidamental gland; Oe = Oesophagus; Ov = Oviduct; P = Penis; Peg = Pedal ganglion; Ph = Pharynx; Pgl = Pyloric gland; Pr = Prostate gland; PS = Penial sheath; Rem = Retractor muscle; Rs = Receptaculum seminis; Sp = Spine; Va = Vagina; Vd = Vas deferens; Ves = Vestibulum.
Digestive system. A schematic outline of the digestive tract is given in Fig. 8A. Mouth opening non glandular. Proximal oral tube highly folded with few epithelial mucus glands staining reddish. Subsequent part widening, with increasing number of epithelial acid mucus cells. Distal part of oral tube widening considerably, surrounded by muscle fibres and dorsally with subepithelial glands staining violet. Gradual transition into pharynx. Pharynx oval, surrounded by muscle fibres and with a slightly folded, glandular epithelium. Subepithelial glands reaching into muscle layer. No cuticle observed. Transition of pharynx into oesophagus marked by the location of the ganglionic nervous system (Fig. 7C). At this transition,

Fig. 9. Genital system of Doriopsilla aroni sp. nov. A. Highly lobulated prostate gland. B. Transition of penis into penial sheath. C. Detail of penis showing the area, where spines start; note the various shapes. D. Fertilisation chamber containing a few eggs. E. Bursa copulatrix. F. Empty receptaculum seminis in vicinity of bursa copulatrix. Abbreviations: Bc = Bursa copulatrix; Pr = Prostate gland; Rs = Receptaculum seminis; Va = Vaginal duct; Vd = Vas deferens.
two lateral retractor muscles attached. Salivary glands absent. Tubular oesophagus with Y-shaped interior lumen and no glandular epithelium (Fig. 7D), subsequently entering a highly muscular portion without Y-shaped lumen (Fig. 7E) and with few subepithelial glands, staining pale blue. Two small retractor muscles arising from this muscular part. After the muscular part oesophagus transiting into a small tube before entering the stomach. Stomach completely embedded in digestive gland; characterised by folded and ciliated epithelium. Transition into intestine with folds, but without a distinct typhlosole (Fig. 7D–E). Pyloric gland opening immediately after transition of stomach into intestine; internally folded but without glandular cells and filled with stomach contents (Fig. 7D). Interior lining of the intestine highly folded and ciliated, especially in the anal papilla. Intestine surrounded by a muscular layer throughout its course (Fig. 7F). Besides the oral gland layer, no ptyaline or salivary glands present. No caecum found.

Reproductive system. A schematic outline of the genital system is provided in Fig. 8B. Gonad in mature phase with sperm and eggs formed in the same follicles. A distinct ampulla not found. Vas deferens leading into strongly lobulated, bulky prostate gland (Fig. 9A), showing pristine glandular areas, with small cells and large nuclei, other areas with elongate cells and vesicles with blue stained droplets, as well as some areas where the prostatic material was already exuded. Distal vas deferens (ejaculatory duct) coiled, lying in muscular sheath (Fig. 9B); penis long, inverted, with hooked spines only in the proximal part, which lies in the wider part of the penial sheath. Spines arranged in longitudinal lines along the proximal penis. Size of spines ca 200 µm, some of them smaller and thinner in the region where spines start. Distal part without spines (Figs 8B–C, 9C). Bursa copulatrix stalked and filled with decaying sperm and prostatic material; epithelium thin and characterised by apocrine secreting cells (Fig. 9E). Muscular receptaculum seminis empty, indicating a recent fertilisation process (Fig. 9F). Fertilisation chamber with a few eggs (Fig. 9D). Female glands well developed with typical three glandular parts staining violet or reddish; opening into a joint non glandular vestibulum with vas deferens and the vaginal duct.

Nervous system. Statocyst between cerebropleural and pedal ganglia, with many otoconia (Fig. 7C).

Circulatory and excretory system. Blood gland large, surrounding the central nervous system and also the anterior part of the oesophagus. Syrinx highly folded and rather small (compared to species of Dendrodoris). Kidney thin, covering gonad and digestive gland.

Additional features
Body cavity surrounded by a thick muscle layer, especially in the hind part of the body. One thick gill retractor starting close to gills and running ventrally towards anterior part of body. Small gill glands with pale bluish cells present at the base of gills, opening into gill pocket. A few unidentified parasites lying in the notal tissue, as well as close to the oral tube (Fig. 7B).

Phylogenetic analyses and species delimitation

Dendrodoris

Phylogenetic analyses of the mitochondrial gene datasets (concatenated 16S and CO1 Fig. 10, 16S Fig. 11, CO1 Fig. 12) including all three recognised genera of the family Dendrodorididae and several other dorid genera result in the polyphyly of the genus Dendrodoris, as members of the Phyllidiidae and other dorids cluster together with several species of Dendrodoris. This is in contrast to the analyses of the nuclear gene datasets 18S and H3 where Dendrodoris is monophyletic (Supp. file 2: Figs S1–S2).

Eleven species of Dendrodoris cluster in the mitochondrial analyses with a bootstrap value of 100. This subclade, containing D. nigra and D. fumata, is characterised by a long branch that separates it from all remaining members of the Dendrodorididae (Figs 10–12). The genetic distance of this long branch
Fig. 10. Phylogenetic reconstruction of Dendrodorididae O’Donoghue, 1924 based on a concatenated data set (CO1 and 16S), with Bathydoris aioca Er. Marcus & Ev. Marcus, 1962 and Prodoris clavigera (Thiele, 1912) as outgroup. Terminal taxa at species level partly collapsed (triangles coloured for clarity) and specimen numbers written in brackets. Species in red are newly sequenced in this study. Numbers indicate bootstrap values for maximum likelihood (ML) test. Localities are provided for species closely related to the Iranian specimens.
Fig. 11. Phylogenetic reconstruction of Dendrodorididae O’Donoghue, 1924 based on 16S data set, with Bathyodoris aioca Er. Marcus & Ev. Marcus, 1962 and Prodoris clavigera (Thiele, 1912) as outgroup. Terminal taxa at species level partly collapsed (triangles coloured for clarity) and specimen numbers written in brackets. Species in red are newly sequenced in this study. Species more related to the Iranian sequences are mentioned with their localities. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and bootstrap values for maximum likelihood (ML) respectively. Values less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test.
Fig. 12. Phylogenetic reconstruction of Dendrodorididae O’Donoghue, 1924 based on complete CO1 data set, with *Bathydoris aicca* Er. Marcus & Ev. Marcus, 1962 and *Prodoris clavigera* (Thiele, 1912) as outgroup. Terminal taxa on species level partly collapsed (triangles coloured for better clarity) and specimen numbers written in brackets. Species written in red are new in this study. Species more related to the Iranian sequences are mentioned with their locality. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and bootstrap values for maximum likelihood (ML) respectively. Values less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test.
to other dendrodorids ranges from approximately 45% (CO1 *D. limbata* (Cuvier, 1804) to *D. citrina* (Cheeseman, 1881)) to 70% (group B to *D. krusensternii* (Gray, 1850) clade g (Supp. file 1: Table S3)). These other dendrodorids always show a closer relationship to *Doriopsilla*, members of the Phyllidiidae, and other dorid species than with members of the long branch (Figs 10–12).

The ABGD test identifies 11 groups within *Dendrodoris* 16S sequences. The groups partly represent recognised species, but *Dendrodoris temarana* Pruvot-Fol, 1953, *Dendrodoris grandiflora* (Rapp, 1827), *Dendrodoris senegalensis* Bouchet, 1975, and *Dendrodoris herytra* Á. Valdés & Ortea, 1996 are united into one clade (Fig. 11) (group A, Supp. file 1: Table S2). *Dendrodoris fumata* (only our sequences available) as well as *D. nigra* are distinct from all other species of *Dendrodoris* (Fig. 11). Intraspecific variability in 16S sequences is less than 6% and interspecific variability ranges from 5% (between *D. krebsii* (Mörch, 1863) and group A) to more than 50% (between long- and short-branched clades) (Supp. file 1: Table S2). Both *D. nigra* (Persian Gulf, Philippines, and Australia) and *D. fumata* (only sequences of specimens from the Persian Gulf are available) have their shortest genetic distance to group A (20–24% and 10–13%, respectively) (Supp. file 1: Table S2).

The ABGD test based on the *Dendrodoris* CO1 sequences identifies 22 groups with more pronounced distances than in the 16S analysis (compare Supp. file 1: Tables S2 and S3). The single and apparent barcode gap for intraspecific variability in the CO1 dataset is less than 3% and interspecific variability is more than 11%. Acknowledged species are usually recognised as such, e.g., the recently resurrected *D. temarana* (see Galià-Camps et al. 2022), or even split into clades. This is the case for *D. nigra* (clades a–c), with specimens from the Persian Gulf (including our specimens) forming a separate species (clade c), the specimens from Japan, Hawaii, and Australia united in clade a, and specimens from Singapore (unidentified species, accession number MN690568) and the Philippine Islands united in clade b. These clades show an interspecific genetic distance of 11–13. *Dendrodoris fumata* also splits into subclades: all specimens from the Persian Gulf (including our specimens), Gulf of Oman, and those from the Red Sea are united into one clade (clade e), whereas the single specimen from Australia is considered a separate species (clade f). The genetic distance of 4–6% (Supp. file 1: Table S3) is rather small between these two clades. Another clade (clade d) unites sequences of *D. fumata* from Hawaii and probably Singapore, and sequences from Japanese specimens, the latter assigned to *D. rubra* (Kelaart, 1858) by Hirose et al. (2014). Here, the genetic differences between clades e and f lies between 8–11% and 9–10%, respectively. *Dendrodoris krusensternii* is divided into five clades (g–k) (Fig. 12) with a genetic difference of 6–13% between these clades.

**Doriopsilla**

The genus *Doriopsilla* is monophyletic in the analyses using separate mitochondrial and nuclear gene data sets, but not in the concatenated dataset (16S and CO1, Fig. 10) with the long branch of *Dendrodoris* nested within the genus. *Cariopsilla pharpa* (Er. Marcus, 1961) (only one CO1 sequence available) groups with species of *Doriopsilla* (Figs 10, 12).

Specimens of *Doriopsilla aroni* sp. nov. from the Persian Gulf always form a monophyletic group (bootstrap support in concatenated, 16S, and CO1 analyses = 100, 98, and 100, respectively). In the concatenated and 16S analyses (Figs 10–11), the new species is the sister group of *Doriopsilla miniata* (Alder & Hancock, 1864) (bootstrap values 94 and 86, respectively). In the CO1 analysis, our specimens form a sister taxon to an undescribed *Doriopsilla* sp. from Spain, but with minor support (57), and these two are sister to *D. miniata* (Fig. 12).

*Doriopsilla aroni* sp. nov. is not considered a separate species in the ABGD tests based on the 16S datasets (Figs 11, S3), but forms a clade (group 1) together with sequences from various species distributed or collected in the Mediterranean and the African West coast: *D. areolata*, *D. miniata*, *D. pelseneeri*

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th><em>D. spaldingi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0–9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>12–16</td>
<td>0–8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>11–15</td>
<td>17–19</td>
<td>0–4</td>
<td></td>
</tr>
<tr>
<td><em>D. spaldingi</em></td>
<td>12–16</td>
<td>16–18</td>
<td>13–14</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Intra- and interspecific pairwise uncorrected p-distances of *Doriopsilla* Bergh, 1880 based on the CO1 data subset including only *Doriopsilla*. Ranges between minimum and maximum distances are given as percentages.

<table>
<thead>
<tr>
<th></th>
<th><em>D. sp.</em> (Spain)</th>
<th><em>D. rarispinosa</em></th>
<th><em>D. areolata</em></th>
<th><em>D. pelseneeri</em></th>
<th><em>D. miniata</em></th>
<th><em>Doriopsilla aroni</em> sp. nov.</th>
<th><em>D. gemela</em></th>
<th><em>D. bertschi</em></th>
<th><em>D. albopunctata</em></th>
<th><em>D. davebehrensi</em></th>
<th><em>D. fulva</em></th>
<th><em>D. spaldingi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. sp.</em> (Spain)</td>
<td>0</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. rarispinosa</em></td>
<td>10</td>
<td>0–2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. areolata</em></td>
<td>9–10</td>
<td>5–7</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. pelseneeri</em></td>
<td>10</td>
<td>5–7</td>
<td>2–4</td>
<td>0–4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. miniata</em></td>
<td>11</td>
<td>6–7</td>
<td>7–8</td>
<td>6–8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Doriopsilla aroni</em> sp. nov.</td>
<td>13–14</td>
<td>10–11</td>
<td>11–12</td>
<td>10–12</td>
<td>11</td>
<td>0–1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. gemela</em></td>
<td>18–20</td>
<td>18–20</td>
<td>18–19</td>
<td>18–21</td>
<td>17–18</td>
<td>21–22</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. bertschi</em></td>
<td>20</td>
<td>16–18</td>
<td>18</td>
<td>18–19</td>
<td>18</td>
<td>22</td>
<td>11–12</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. davebehrensi</em></td>
<td>24</td>
<td>19–21</td>
<td>21–23</td>
<td>21</td>
<td>22</td>
<td>22–23</td>
<td>16–17</td>
<td>17–18</td>
<td>8–10</td>
<td>0–1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. fulva</em></td>
<td>24–25</td>
<td>19–21</td>
<td>19–21</td>
<td>18–20</td>
<td>19–21</td>
<td>21–22</td>
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<td>8–10</td>
<td>8–10</td>
<td>0–1</td>
<td></td>
</tr>
<tr>
<td><em>D. spaldingi</em></td>
<td>22</td>
<td>20</td>
<td>22</td>
<td>21–22</td>
<td>19</td>
<td>23–24</td>
<td>16–18</td>
<td>17</td>
<td>21–22</td>
<td>18–19</td>
<td>20–21</td>
<td>0</td>
</tr>
</tbody>
</table>
d’Oliveira, 1895, *D. rarispinosa* Pruvot-Fol, 1951, and an unidentified specimen of *Doriopsilla* from Spain (Fig. 11, black bar). The maximum genetic distance between the sequences within this group (group 1, Table 3) is 9%. In this analysis the other species of *Doriopsilla* are also not recognised as distinct species and instead form larger clades with a genetic divergence between a minimum of 17%

**Fig. 13.** Haplotype network of *Doriopsilla* Bergh, 1880 sequences from the CO1 data subset (for the corresponding tree see Supp. file 2: Fig. S4). Coloured nodes on the haplotype network correspond to specific geographic localities and the size of the coloured nodes corresponds to the number of available sequences. Hash marks on the haplotype network denote mutational steps.
to a maximum of 19% divergence (Fig S3, 3, groups 2 and 3). Only the single _Doriopsilla spaldingi_ Á. Valdés & Behrens, 1998 sequence is not united with other species.

ABGD tests using the CO1 data sets (all dendrodoridids, as well as the _Doriopsilla_ subset) recognise our specimens from Iran as a separate species. Using the reduced _Doriopsilla_ subset, 12 groups are identified with intraspecific variability less than 4% and interspecific variability between 5–25% (4, Supp. file 2: Fig. S4). All species were recognised, including all the re-instated/re-investigated Mediterranean/Atlantic species by Furfaro _et al._ (2022). The gap between _Doriopsilla aroni_ sp. nov. and the closely related species _D. rarispinosa_, _D. areolata_, _D. pelseneeri_, and _D. miniata_ is 10–12%.

**Haplotype networking**

The haplotype network analysis based on the _Doriopsilla_ CO1 subset (Fig. 13) shows considerable distances between _Doriopsilla aroni_ sp. nov. and all other clades of _Doriopsilla_, reflecting geographic distance (Supp. file 2: Fig. S5). None of the Iranian haplotypes are found in any other group, indicating that there seems to be no connectivity to other populations of _Doriopsilla_ and vice versa. The closest relationship is to _D. rarispinosa_, _D. pelseneeri_, and _D. areolata_ from the Mediterranean Sea, and to two _D. miniata_ from Japan. However, all these species are separated by many mutational steps from the Iranian specimens (Fig. 13). According to the rather linear network, gene flow between the different localities in the Mediterranean and Atlantic appears to be limited, resulting in these separate species. All species with a distribution along the Eastern Pacific coastline (_D. gemela_ Gosliner, Schaefer & Millen, 1999, _D. spaldingi_, _D. fulva_ (MacFarland, 1905), _D. davebehrensi_ Hoover, Lindsay, Goddard & Á. Valdés, 2015, _D. albopunctata_ (J.G. Cooper, 1863), and _D. bertschi_ Hoover, Lindsay, Goddard & Á. Valdés, 2015; Fig. 13) are separated by many mutations from the Mediterranean/Indo Pacific species.

The haplotype network analysis based on the 16S dataset partly confirms the results of the CO1 analysis but shows a more network-like structure than a linear one. Only few mutational steps separate the new species from Mediterranean species, as well as specimens of _D. miniata_ from South Africa (Figs 14, Supp. file 2: Fig. S6). One specimen from Spain identified as _D. areolata_ (see Valdés 2002) is interesting, because it groups with the Mexican _D. bertschi_ (Fig. 14). This could be a case of mistaken identity, but this does not explain why a Mediterranean specimen clusters with Pacific Ocean specimens.

**Discussion**

Within the Dendrodorididae, the mitochondrial gene 16S seems quite conservative compared to the CO1 (minimum interspecific variability 5% and 11%, respectively) and does not recognise several acknowledged species. This led first to synonymisations within the genus _Doriopsilla_ (see Goodheart & Valdés 2013), which were subsequently resurrected (Furfaro _et al._ 2022). We therefore focus on the CO1 analysis, as in the most recent studies on dendrodorid species (Furfaro _et al._ 2022; Galià-Camps _et al._ 2022) or other nudibranchs (e.g., Maroni & Wilson 2022 on _Doris kerguelenensis_ (Bergh, 1884) species complex).

**Dendrodoris**

Hallas _et al._ (2017) and Korshunova _et al._ (2020) recognised the divergence of certain species of _Dendrodoris_ (_D. nigra_, _D. fumata_, _D. arborescens_ (Collingwood, 1881), and _D. guttata_ (Odhner, 1917)) from other species of _Dendrodoris_ when applying mitochondrial genes. We here identified six more species which cluster in this long branch taxon. Like the analysis of nuclear genes in dorids by Hallas _et al._ (2017), our investigation of all published dendrodoridid 18S and H3 sequences covering more of the taxa under consideration confirm a monophyletic _Dendrodoris_ clade.
Three species of *Dendrodoris* have been frequently recorded from the Persian Gulf in the past, namely *D. nigra*, *D. fumata* (Yonow 2012; Mousavipoor 2013; Fatemi & Attaran 2015; Amini-Yekta et al. 2016, 2019; Rezai et al. 2016; Abdollahi et al. 2020; Fatemi et al. 2021), and a specimen that was putatively assigned to *Dendrodoris coronata* Kay & Young, 1969 by Yonow (2012).

*Dendrodoris nigra* has sometimes been considered a junior synonym of *D. fumata* (Gohar & Soliman 1967). Our molecular analyses on both mitochondrial genes including many specimens provide further evidence that *D. nigra* and *D. fumata* are two separate species (e.g., Brodie et al. 1997; Valdés & Gosliner 1999; Yonow 2012; Tibiriçá et al. 2017; Gosliner et al. 2018). Results from Galià-Camps et al. (2022), Ballesteros (2023), and our CO1 analyses indicate cryptic variation in the widespread species *D. nigra*. We can separate at least three different clades (clades a–c in Supp. file 1: Table S3) with a

![Haplotype network of Doripsilla Bergh, 1880 sequences from the 16S data subset](image)

**Fig. 14.** Haplotype network of *Doripsilla* Bergh, 1880 sequences from the 16S data subset (for the corresponding tree see Supp. file 2: Fig. S3). Coloured nodes on the haplotype network correspond to specific geographic localities and the size of the coloured nodes corresponds to the number of analysed sequences. Hash marks on the haplotype network denote mutational steps.
genetic difference of ~10%, which is very close to the genetic difference between recognised species (e.g., 12% between *D. krebsii* and *D. grandiflora*). Since the type locality of *D. nigra* lies in Japan, clade a (including specimens from Hawaii, Japan, and Australia) would therefore best match the distribution of the type description of *D. nigra*. Thorough morphological descriptions are mainly based on material from Australia or Hawaii (Valdés et al. 1996; Valdés & Gosliner 1999; Wägele et al. 1999; Brodie 2001).

The only histological descriptions are based on material from Australia (Wägele et al. 1999), which also formed part of the same collection as the *D. nigra* sequences from Australia included in this study (Fig. 11, clade a in Fig. 12). Since the molecular analyses revealed cryptic variations in the species and considered the specimens from the Persian Gulf as a separate clade (clade c in Fig. 12), the analysis of the morphology/histology for comparison is of interest. The oral tube of the Iranian *D. nigra* specimen was surrounded by reddish stained glandular cells, a character not previously mentioned in *D. nigra* descriptions. In our specimen, a sac-like stomach was observed, while Valdés (2002: 622) mentioned an undifferentiated stomach for radula-less dorids. Our specimens have spines on the penis, whereas the three specimens investigated by Wägele et al. (1999, Australia) lack these spines. Presence or absence of a cuticle was not mentioned in that study, but our specimen does not have a cuticle on the penis, in which the spines are embedded. Brodie et al. (1997) discussed the variation of penial spines in species of *Dendrodoris* and did not consider these spines a useful trait, as their presence/absence can be influenced by ontogeny; it cannot be excluded that the spines in the animals investigated by Wägele et al. (1999; largest animal with 53 mm in length) were dissolved in formalin preservative. The well-developed and large nidamental glands, as well as the large vestibular gland, indicate that our histologically investigated specimen was fully mature, despite a small size of 19 mm. The smallest Australian animal examined by Wägele et al. (1999) measured 23 mm and did not show any differentiation of the nidamental gland into capsule, membrane, and mucous gland. Furthermore the vestibular gland was not yet developed either. These morphological characters support the molecular data that the clade c, comprising only specimens from the Persian Gulf, is a separate lineage from clade a, with specimens mainly from the Pacific Ocean. However, descriptions of specimens represented in clade b from the Philippines and Singapore, as well as more sequences from adjacent geographic areas, are necessary before assigning new names to these clades.

CO1 analyses confirm *D. fumata* and *D. rubra* as two distinct species in a sister-group relationship (Hirose et al. 2014; Fatemi et al. 2021). The type locality of *D. rubra* is in Trincomalee, on the north east coast of Sri Lanka (Kelaart 1858). Taking into account the localities of the available *D. rubra* sequences, this species is more frequently collected in the western Pacific, whereas *D. fumata*, with its type locality in the Red Sea (Rüppell & Leuckart 1830) is more common in the Indian Ocean (Tibiriçá et al. 2017; Gosliner et al. 2018) and Red Sea (Yonow 2008). *Dendrodoris fumata* is also mentioned in studies from Iran (Fatemi & Attaran 2015; Rezai et al. 2016; Fatemi et al. 2021). Fatemi et al. (2021) included a short external description (in Persian language) with an illustration of one specimen of *D. fumata*, which matches our material. In addition to the sequences from Iranian specimens, one sequence is available from Australia, which is considered a separate species in our ABGD test (CO1 dataset, genetic divergence of 4–6% to the other *D. fumata* sequences). Brodie et al. (1997), Valdés & Gosliner (1999), and Brodie (2001) provided anatomical details of *D. fumata* and information about its colour morphs. The presence or absence of penial hooks was considered to vary in the different colour forms whereas the black colour form seems to lack spines (Brodie et al. 1997). These hooks were also described as present in the grey and red colour forms in the older literature (Eliot 1904; Gohar & Soliman 1967; Ev. & Er. Marcus 1970; Edmunds 1971; Brodie et al. 1997) and this confirms our observations on the reddish specimen that we investigated by histological means. Additionally, our specimen with a length of 23 mm had a thick cuticle on the penis, a feature not previously mentioned. Only Brodie (2001) provided some histological results on this species and detected the vestibular gland for the first time in this species, which is difficult to see; we also confirm the presence of a vestibular gland in *D. fumata.*
Klussmann-Kolb & Brodie (1999) described symbiotic bacteria within the epithelium of the vestibular gland in *D. nigra*. We also observed the typical fringe of microvilli in our specimen, as well as in the specimen of *D. fumata* and therefore conclude that *D. fumata* also houses bacteria (of unknown function) in the vestibular gland epithelium. Future studies incorporating molecular data on specimens from other geographic areas, together with information on colour, size of specimens, and presence/absence of penial spines and cuticle may reveal ontogenetic differences or the presence of cryptic variations not evident from these studies.

Based on our extensive molecular analyses we confirm the results of Galià-Camps *et al.* (2022) and Ballesteros (2023), that *D. grandiflora* and *D. temarana* are separate species. Two specimens assigned to *D. grandiflora* by Almada *et al.* (2016) not included in previous analyses appear to be a misidentification and belong to *D. temarana*.

We identified five clades in *Dendrodoris krusensternii*, with a partly disjunct distribution. The two sequences from Japan (type locality, see Valdés & Fahey 2006) are considered a separate species (clade j) from the three Australian clades (clades g–i) and the single specimen from New Zealand (clade k). Therefore, a re-investigation of *Dendrodoris denisoni* and *D. gunnamatta* that were synonymised with *D. krusensternii* (Valdés & Fahey 2006; Nimbs & Smith 2021) is needed. Further species considered as synonyms of *D. krusensternii*, i.e., *D. clavulata* (Alder & Hancock, 1864) and *D. gemmacea* (Alder & Hancock, 1864), should also be included in future investigations.

**Doriopsilla**

*Doriopsilla pharpa* Er. Marcus, 1961 is listed under the genus name *Cariopsilla* in the World Register of Marine Species (WoRMS Editorial Board 2023), MolluscaBase (MolluscaBase 2023b), or GBIF (GBIF Secretariat 2023b), as well as in biodiversity studies (e.g., Ortea & Buske 2018). We can confirm the conclusion of Valdés & Hamann (2008) that *Cariopsilla* is a junior synonym of *Doriopsilla*.

Only a few studies record species of *Doriopsilla* from the Persian Gulf (Nithyanandan 2012; Yonow 2012; Rezai *et al.* 2016). Yonow (2012) described a new species, *D. nigrocera*, from Jubail, Dahwat ad Daffi (eastern Persian Gulf, Saudi Arabia); however, no sequences are available. The species description is based on the external description of one specimen and mentions the black rhinophores as a unique and distinguishing feature (shared only by an undescribed species of *Doriopsilla* from Papua New Guinea illustrated in Gosliner *et al.* 2018). The colour of *D. nigrocera* is similar to our specimens but the tubercles are covered in opaque white lines, which distinguish *D. nigrocera* from our specimens in addition to the black rhinophores. A single specimen of an unidentified species of *Doriopsilla* from the northern region of the Persian Gulf was listed by Rezai *et al.* (2016), but no description is available, therefore species assignment is not possible. Probably the three specimens from the south coast of the Persian Gulf (Kuwait) described and illustrated by Nithyanandan (2012) as *D. aroni* sp. nov. belong to our new species, as these specimens also lack the reticulated white lines on the mantle that are so characteristic of *D. miniata*.

Distinctness of our new species is reflected in the sequence divergence of 10–14% (CO1) to the closest related species. The values are even larger than between valid species: the lowest value is 5–7% between *D. areolata*, *D. pelseneeri*, and *D. rarispinosa* from the Mediterranean/Atlantic region, and 6–7% between *D. miniata* specimens from Japan and *D. rarispinosa* (Mediterranean region). Similar to *D. miniata*, *D. areolata* and *D. rarispinosa* show a typical white network on the notum, which was never observed in our five specimens of *D. aroni* sp. nov. The only other species that lacks this white pigmentation in form of a network or lines and dots on the notum is *D. pelseneeri*, which, however, has a distribution restricted to the Atlantic shoreline of Spain and Portugal (Furfaro *et al.* 2022) and has a genetic divergence of 10–12% from *D. aroni* (this study). According to our molecular data, *D. miniata*,
whose original description was based on specimens from the east coast of India, is closely related to *D. aroni*. The length of our preserved specimens (up to 40 mm) is in a similar range as the *D. miniata* described by Alder & Hancock 1864 (35 mm) and the living specimens described by Gosliner *et al.* (2008) and Ávila *et al.* (1992) (30 and 40 mm). These authors as well as Marcus (1961) and Hirose *et al.* (2014) described *D. miniata* with different colours from pale orange to deep orange and always with a characteristic pattern of opaque white lines. However, the original description by Alder & Hancock (1864), which was based on preserved specimens, did not mention this white pattern. Since living specimens of *D. miniata* are always described or illustrated with white lines (several specimens from India illustrated in GBIF have white lines and patterns), and taking into account the molecular results, we consider our specimens sufficiently distinguishable by the absence of the white lines on the notum except the tiny line around the gill pocket.

If we compare the geographical distribution of our specimens with the distribution of the 26 recognised species, and also consider our molecular data, we can exclude all species that only occur in the western Atlantic and on the eastern Pacific coast: *Doriopsilla albopunctata*, *D. bertschi*, *D. davebehrensi*, *D. elitae* Valdés & Hamann, 2008, *D. fulva*, *D. gemela*, *D. rowena* Er. Marcus & Ev. Marcus, 1967, *D. spaldingi*, *D. espinosai* Valdés & Ortea, 1998, *D. evanae* Ballesteros & Ortea, 1980, *D. ciminoi* C. Ávila, Ballesteros & Ortea, 1992, *D. nigrolineata* Meyer, 1977, and *D. tishae* Valdés & Hamann, 2008. Many species can also be excluded because of the presence of distinct white spots, white tubercles, white lines, or a white reticulate network on their back: the Mediterranean species *D. areolata* and *D. rarispinosa* (see Furfaro *et al.* 2022), the Atlantic species *D. pelseneeri*, *D. carneola* (see Burn 1962, 1966), *D. capensis* Bergh, 1907 (Bergh 1907; Furfaro *et al.* 2022; Perrone 2001; Valdés & Gosliner 1999), and the Australian species *D. aurea* (Quoy & Gaimard, 1832), *D. carneola* (Angas, 1864), and *D. peculiaris* (Abraham, 1877).

Other species show a completely different coloration compared to our new species. *Doriopsilla debruini* Perrone, 2001 from the west coast of South Africa is externally characterised by a series of large dark brown spots on a pale brown mantle (Perrone 2001), in contrast to the yellow to red colour of *D. aroni* sp. nov. With regards to the anatomy, *D. debruini* is described with probably few penis hooks (“hardly observed”, Perrone 2001: 62), which is also incongruent with the well-developed spines on the penis of *D. aroni*.

*Doriopsilla pallida* Bergh, 1902 was described from the Gulf of Thailand based on one preserved individual (6 mm in length; Bergh 1902). The colour of the living specimen is not known. However, for the only specimen available to him, Bergh (1902) described the presence of ptyalin glands below the pharynx, a feature that is characteristic of the genus *Dendrodoris* and not of *Doriopsilla*.

Further undescribed species of *Doriopsilla* illustrated in various biodiversity studies (e.g., Tibiriçá *et al.* 2017; Gosliner *et al.* 2018) usually have white lines or dots on the dorsal notum, and/or are recorded from localities rather far away from our type locality of *D. aroni* sp. nov.

Anatomical features are less suited to delimit these species (see also Valdés & Ortea 1997) as well as our new species. One feature that may differ is the armature of the penis. The penis of *D. areolata* is covered with numerous long and curved spines, which have a narrow base (Valdés & Ortea 1997): the size of the spines described in that paper is ~400 µm, and thus double the size of the spines in *D. aroni* sp. nov., which are, however, similar in shape. In his original description of *D. areolata* Bergh (1880) described the arrangement of the spines as being in ~15 longitudinal lines, which is similar to our new species. The spines of *D. pelseneeri* have a long base and straight cusp (Valdés & Ortea 1997) and clearly differ in their unusual shape. Pruvot-Fol (1951) described and illustrated hooked spines in an irregular
arrangement in *D. rarispinosa*, which also differs from *D. aroni*. Future analyses should include detailed information of penial spines to better understand their intra- and interspecific variability.

**Conclusions**

Our study confirms previous results on the presence of dendrodorid species in the Persian Gulf, but also broadens species diversity. With *Doriopsilla aroni* sp. nov., four dendrodorid species are now documented from the Gulf (*Dendrodoris nigra*, *D. fumata*, *Doriopsilla nigrocera*, *Doriopsilla aroni*), and a fifth (*Dendrodoris coronata*) needs confirmation. Whether our specimens of *D. nigra* form a subclade or can even be assigned to a new species still has to be decided. Our thorough histological investigations of the anatomy indicate morphological differences that may help to describe the molecularly well-defined clades in the future.

The few earlier studies and our investigation on Iranian marine heterobranch taxa have shown that studies in the Persian Gulf reveal species not previously recorded from this area, but also new and probably endemic species (Nithyanandan 2012; Yonow 2012; Mousavipoor 2013; Fatemi & Attaran 2015; Rezai et al. 2016; Maniei et al. 2020; Amini-Yekta & Dekker 2021; Fatemi et al. 2021). The number of Nudibranchia is still much lower than in other (adjacent) geographic regions (see, e.g., Yonow 2012, 2015; Tibiriçá et al. 2017; Cunha et al. 2023). However, future collecting efforts in the subtidal of the Iranian coastline might reveal many more taxa, including new species. “The only real conclusion is that much remains unknown and additional research is badly needed” (Yonow 2012: 79).

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**Supplementary files**

**Supp. file 1.** Supplementary tables. [https://doi.org/10.5852/ejt.2024.943.2595.11837](https://doi.org/10.5852/ejt.2024.943.2595.11837)

Table S1. Data of Dendrodorididae O'Donoghue, 1924 sequences retrieved from NCBI GenBank with additional information on names when changed subsequently in literature (column 2), with locality information, references and accession numbers.


Table S3. Intra- and interspecific pairwise uncorrected p-distances of *Dendrodoris* Ehrenberg 1831 based on CO1 data set. Ranges between minimum and maximum distances are given as percentages. Group B: *Dendrodoris temarana* Pruvot-Fol, 1953 and *Dendrodoris grandiflora* (Rapp, 1827) (only 2 sequences).

**Supp. file 2.** Supplementary figures. [https://doi.org/10.5852/ejt.2024.943.2595.11839](https://doi.org/10.5852/ejt.2024.943.2595.11839)

Fig. S1. Phylogenetic reconstruction of Dendrodorididae O’Donoghue, 1924 (1864) based on the 18S data set, with *Prodoris clavigera* (Thiele, 1912) as outgroup. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and bootstrap values for maximum likelihood (ML) respectively. Numbers less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test.

Fig. S2. Phylogenetic reconstruction of Dendrodorididae O’Donoghue, 1924 (1864) based on the H3 data set, with *Bathydoris aioca* Er. Marcus & Ev. Marcus, 1962 and *Prodoris clavigera* (Thiele, 1912) as outgroup. Terminal taxa on species level partly collapsed (triangles coloured for better clarity) and specimen numbers written in brackets. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and bootstrap values for maximum likelihood (ML) respectively. Numbers less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test. Note that the genera *Dendrodoris* Ehrenberg, 1831 and *Doriopsilla* Bergh, 1880 are monophyletic.

Fig. S3. Phylogenetic reconstruction of the genus *Doriopsilla* Bergh, 1880 based on the 16S data subset, with *Dendrodoris* Ehrenberg, 1831 species (from short branch) as outgroups. Terminal taxa on species level partly collapsed (triangles coloured for better clarity) and specimen numbers written in brackets. Numbers before and after slash indicate approximate likelihood ratio test (SH-
aLRT) and bootstrap values for maximum likelihood (ML) respectively. Numbers less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test.

Fig. S4. Phylogenetic reconstruction of the genus *Doriopsilla* Bergh, 1880 based on the COI data set, with *Dendrodoris* Bergh, 1880 species (from short branch) as outgroup. Terminal taxa on species level partly collapsed (triangles coloured for better clarity) and specimen numbers written in brackets. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and bootstrap values for maximum likelihood (ML) respectively. Numbers less than 50 are removed. Coloured bars indicate species delimitation resulting from ABGD test.

Fig. S5. Distribution map of *Doriopsilla* Bergh, 1880 species (COI sequences) included in our study. Species and their respective localities highlighted in the same colour as in Fig. 13. Outline world map downloaded from https://www.outline-world-map.com/blank-thick-white-world-map-b3c

Fig. S6. Distribution map of *Doriopsilla* Bergh, 1880 species (16S sequences) included in our study. Species and their respective localities highlighted in the same colour as in Fig. 14. Outline world map downloaded from https://www.outline-world-map.com/blank-thick-white-world-map-b3c