

## Article

# Mediterranean Matters: Revision of the Family Onchidorididae (Mollusca, Nudibranchia) with the Description of a New Genus and a New Species <sup>†</sup>

Giulia Furfaro <sup>1,\*</sup>, Egidio Trainito <sup>2</sup>, Marco Fantin <sup>3</sup>, Marcella D'Elia <sup>4</sup>, Enric Madrenas <sup>5</sup> and Paolo Mariottini <sup>6</sup>

<sup>1</sup> Department of Biological and Environmental Sciences and Technologies DiSTeBA, University of Salento, Via Prov. le Lecce-Monteroni, 73100 Lecce, Italy

<sup>2</sup> Genoa Marine Centre-Stazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia e Biotecnologie Marine, Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy

<sup>3</sup> Shoreline Soc. Coop. Area di ricerca Grignano, 34149 Trieste, Italy

<sup>4</sup> Department of Mathematics and Physics "Ennio de Giorgi", University of Salento, Via Prov. le Lecce-Monteroni, 73100 Lecce, Italy

<sup>5</sup> VIMAR (Vida Marina), C/Rocafort 246, 08029 Barcelona, Spain

<sup>6</sup> Department of Science, University of "Roma Tre", Viale G. Marconi 446, 00146 Rome, Italy

\* Correspondence: giulia.furfaro@unisalento.it; Tel.: +39-08322-98660

<sup>†</sup> The LSID (Life Science Identifier) for each new species and new genus in this publication:  
urn:lsid:zoobank.org:act:67C7064C-9DB5-45FE-910E-7D51153B33F4;  
urn:lsid:zoobank.org:act:2639236D-A114-4043-9C11-F7EC77825AF1.

**Abstract:** The Mediterranean Sea hosts a great Nudibranchia diversity and has proved to be particularly intriguing in the case of the family Onchidorididae, a group of dorid nudibranchs that lately increased its diversity with the addition of one recently described Mediterranean species. The Onchidorididae family has a troubled systematic history to date, characterized by uncertainties and genera that are considered valid or not, according to the different authors. This confused taxonomy reflects the lack of a broad and comprehensive view on the phylogenetic relationships occurring between Onchidorididae members, an incorrect interpretation of the diagnostic morphological characters, and a poor knowledge of important biological aspects characterizing the different genera included in the family. To shed some light on the systematics of Onchidorididae, an integrative taxonomic revision was carried out involving morphological, ecological, and molecular analyses on an updated dataset. Mediterranean specimens and species were added to the dataset of the already known Onchidorididae and a new species from the Adriatic Sea (Central Mediterranean Sea) is described here. Furthermore, historical controversies are clarified due to the discovery of new important synapomorphies useful to define genera belonging to the Onchidorididae family and to describe a new genus. Finally, the taxonomic status of all the known Onchidorididae species is investigated and discussed, filling the gap of knowledge on neglected species.

**Keywords:** Heterobranchia; Nudibranchia; phylogeny; evolution; egg masses; integrative taxonomy



**Citation:** Furfaro, G.; Trainito, E.; Fantin, M.; D'Elia, M.; Madrenas, E.; Mariottini, P. Mediterranean Matters: Revision of the Family Onchidorididae (Mollusca, Nudibranchia) with the Description of a New Genus and a New Species. *Diversity* **2023**, *15*, 38. <https://doi.org/10.3390/d15010038>

Academic Editor: Maxim V. VinarSKI

Received: 27 November 2022

Revised: 24 December 2022

Accepted: 26 December 2022

Published: 29 December 2022



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The Mediterranean Sea is considered a hot spot of marine biodiversity with high levels of endemism and cryptic species, and new species continually revealed [1–3]. New methods, combining techniques from different biological fields such as morphology, ecology, chemistry, and genetics, helped to reveal the hidden Mediterranean diversity, but the gap in knowledge is still far from being filled. In this context, mollusks are the second largest phylum after arthropods, with around 100,000 described species, and constitute a potential reservoir of hidden biodiversity. In fact, it could be presumed that still another 100,000 species remain to be described [4], with nearly 75% of them belonging to the class

Gastropoda [5]. Mollusks belonging to this class are characterized by several morphological synapomorphies, of which the single spiralized shell is the most distinct. However, not all Gastropoda have a shell in their adult stage: in the Nudibranchia clade, this important solid, defensive structure is lost after metamorphosis. This lack of external protection is the main driving force that allowed for the evolution of remarkable ethological, chemical, and physiological defensive strategies. The soft body opened the possibility of a great adaptive radiation which made possible the colonization of a large number of marine habitats, from rocky tide pools and shallower waters to the mesophotic zone, the deep sea, and dark caves [6–10].

The family Onchidorididae Gray, 1827 includes 5 genera: *Acanthodoris* Gray 1827; *Adalaria* Bergh, 1878; *Atalodoris* Iredale & Donoghue, 1923, *Onchidoris* Blainville, 1816; and *Onchimira* Martynov, Korshunova, N. Samayan & K. Samayan, 2009; and includes a total of 56 currently accepted species [11]. Except for the genus *Acanthodoris*, which includes 15 accepted species and three dubious ones (one *nomen dubium* and two *taxa inquirenda*) [12,13], and the recently described monospecific *Onchimira* genus, represented by *Onchimira cavifera* Martynov, Korshunova, N. Samayan & K. Samayan, 2009, the rest of the genera show a troubled systematic history. In fact, *Adalaria*, *Atalodoris*, and *Onchidoris* (including respectively, 7, 17, and 16 currently accepted species) have undergone numerous revisions over the last few years, producing dissimilar systematic assessments, according to the different opinions of various authors [14,15]. The validity of the genus *Adalaria* is nowadays disputed; Hallas & Gosliner [14] treated it as a synonym of *Onchidoris* whereas Martynov et al. [15] considered it as a valid genus. Furthermore, the former authors established the genus *Knoutsodonta* to include all Onchidorididae without a rachidian tooth, but this diagnostic character was previously stated as the synapomorphy of the genus *Atalodoris*, originally described by Iredale and Donoghue [16], and for this reason, was recently re-established [17] and is currently considered as valid. This taxonomical instability contributes to the gap of knowledge still existing on the useful characters to define genera and to be considered as species-diagnostic. Additionally, this factor makes it difficult to shed light on the geographical distribution of the species and on the evolutionary history occurring among the family. In this framework of uncertainty, the fact that the external morphology of many species has important traits of similarity is not secondary, as is the fact that some species have been found very few times after their description or never observed with certainty. Finally, for many of these species, the description is so succinct that it prevents their recognition without wide margins of doubt. In this scenario, the Mediterranean Sea is particularly important as it is the area where many species were originally described, where many endemic species are hosted, and where many taxonomists worked in the past, generating, in some cases, several synonyms that could become central in the case of unknown taxa [18–20]. On the other hand, the Mediterranean proves to be a key area for the comprehension of the taxonomic relationships within the Onchidorididae, both for the endemic component and the recent description of new species of Atlantic–Mediterranean distribution [17,21,22]. The findings from Mediterranean samples of individuals not assignable to any previously described species among known species provided the opportunity to unravel deep evolutionary relationships between Onchidorididae, solving the controversial systematics of this family. Therefore, through an integrative taxonomic approach, the aims of the present study are to (i) investigate the possible occurrence of Mediterranean species not known to science yet; (ii) clarify the troubled systematics of the Onchidorididae family, adding Mediterranean individuals and species to the already known molecular dataset; (iii) investigate and define characters that are diagnostic at the genus taxonomic level; (iv) propose an updated revision of the family Onchidorididae based on a more comprehensive dataset in terms of specimens, species, and molecular markers used; and (v) describe the biogeographic distribution of the Onchidorididae species.

## 2. Materials and Methods

Onchidorididae species were investigated, observed, photographed, and hand-collected in different Mediterranean regions by scuba diving at different depths from the west to the east coasts of the Mediterranean Sea (Table 1). Ten individuals were collected belonging to five different species. Sequences from other extra Mediterranean specimens were obtained from GenBank. Collected samples were photographed in situ and in laboratory, preserved in 95% ethanol (EtOH) for molecular analyses, and deposited in the Department of Science of the Roma Tre University collection (Vouchers RM3\_ID number).

**Table 1.** List of the species names, vouchers, collection localities, and of the COI, 16S, and H3 GenBank accession numbers of the Onchidorididae species and the outgroup included in the present study. Specimens included in the concatenated molecular analysis are highlighted with an asterisk (\*). In bold, the sequences obtained in the present study. In parentheses, the names of the species as they appear in GenBank.

Species	Voucher	Locality	COI	16S	H3
<i>Adalaria proxima</i> (as <i>Onchidoris proxima</i> )	CCDB22758-H04	Canada, British Columbia, Howe Sound	MG422023	————	————
	USNM:IZ:1523836	USA, Dutch Harbor, City Spit Dock	MZ580694	————	————
	CCDB22758-H05	Canada, British Columbia, Howe Sound	MG422901	————	————
	USNM:IZ:1523835	USA, Dutch Harbor, City Spit Dock	MZ580693	————	————
	ZMMU:Op-596	Russia	KY996413	————	————
	CASIZ 183931A *	Passamaquody Bay Eastport: Washington Co., Maine	KM219677	KJ653674	KM225827
(as <i>Onchidoris proxima</i> )	CASIZ 183921A *	Passamaquody Bay Eastport: Washington Co., Maine	KM219676	KJ653673	KM225826
(as <i>Onchidoris loveni</i> )	NTNU 65511B *	Norway, Mausund	KP340395	KP340300	————
(as <i>Onchidoris loveni</i> )	NTNU 65511A *	Norway, Mausund	KP340394	KP340299	————
(as <i>Onchidoris loveni</i> )	NTNU 66880A *	Norway, Gløssvika	KP340393	KP340297	————
<i>Adalaria rossica</i>	ZMMU:Op-548	Russia	KY996416	————	————
	ZMMU:Op-550	Russia	KY996415	————	————
<i>Adalaria slavi</i>	ZMMU:Lc-37459 *	Russia	MN224050	MN224074	————
	MIMB34211	Russia, Avacha Bay, Kamchatka	MF958446	————	————
<i>Atalodoris camassae</i> sp. nov.	MNHN-IM-2000-38594 *	Slovenia, Fiesa	<b>OQ001347</b>	<b>OP965547</b>	<b>OQ096455</b>
	RM3_1993 *	Slovenia, Fiesa	<b>OQ001348</b>	<b>OP965548</b>	<b>OQ096456</b>
	RM3_1991 *	Slovenia, Fiesa	<b>OQ001349</b>	<b>OP965549</b>	<b>OQ096457</b>
<i>Atalodoris oblonga</i> (as <i>Knoutsodonta oblonga</i> )	MN 3010A *	Mewstone, Skomer	KP340410	————	KP340430
<i>Atalodoris pictoni</i> (as <i>Knoutsodonta pictoni</i> )	BAU02982 *	Ireland, South of Inishgalloon, Purteen, Keel, Achill Island	LT840347	<b>OP965550</b>	<b>OQ096458</b>
	BAU02983 *	Ireland, South of Inishgalloon, Purteen, Keel, Achill Island	LT840348	<b>OP965551</b>	<b>OQ096459</b>
	BAU02985 *	Italy, Trieste, Sistiana	LT840346	<b>OP965552</b>	<b>OQ096460</b>
	BAU02986 *	Italy, Sardinia, Porto San Paolo	LT840344	<b>OP965553</b>	————
	BAU02987 *	Italy, Sardinia, Porto San Paolo	LT840345	<b>OP965554</b>	————
	CASIZ 208194 *	Scotland	KP340411	KP340316	KP340432
	<i>Atalodoris pusilla</i> (as <i>Knoutsodonta pusilla</i> ) (as <i>Onchidoris bilamellata</i> )	RM3_2098 *	Spain, Costa Brava, Girona, Punta del Ferro	<b>OQ001350</b>	<b>OP965555</b>
Gastr 8972V	Sweden, Skagerrak	MG934901	————	————	
MT09252	North Sea	KR084801	————	————	
<i>Idaliadoris brasiliensis</i> (as <i>Onchidoris brasiliensis</i> )	BNHS-Opistho-336 *	————	KC255226	KC255225	————
<i>Idaliadoris bouvieri</i>	RM3_1156 *	Croatia, Rijeka	<b>OQ001351</b>	————	<b>OQ096462</b>
<i>Idaliadoris cervinoi</i> (as <i>Knoutsodonta depressa</i> )	CASIZ 186769A *	Spain, Huelva	KP340409	KP340315	KP340428
<i>Idaliadoris depressa</i> (as <i>Knoutsodonta depressa</i> )	Gastr 8969V	Sweden, Skagerrak	MG934879	————	————

Table 1. Cont.

Species	Voucher	Locality	COI	16S	H3
<i>Idaliadoris neapolitana</i>	RM3_1116 *	Italy, Sardinia, Gulf of Olbia, Lu Carragioni	OQ001353	OP965557	OQ096464
	RM3_1115 *	Italy, Sardinia, Gulf of Olbia, Lu Carragioni	OQ001354	————	OQ096465
	RM3_750 *	Italy, Trieste, Sistiana	OQ001355	OP965558	————
	RM3_779 *	Italy, Sardinia, Porto San Paolo	OQ001356	OP965559	OQ096466
<i>Idaliadoris perlucea</i>	RM3_231 *	Italy, Latium, M.P.A. Secche di Tor Paterno	OQ001352	OP965556	OQ096463
<i>Onchidoris bilamellata</i>  (as <i>Onchidoris muricata</i> )	USNM:IZ:1503309	USA, Dutch Harbor; City Spit Dock	MZ580513	————	————
	BMBM-0089	USA, Washington, San Juan County, San Juan Island, Snug Harbor	MH242876	————	————
	USNM:IZ:1529241	USA, Dutch Harbor	MZ580910	————	————
	USNM:IZ:1523834	USA, Captains Bay; North Pacific Fuel Dock	MZ580692	————	————
	USNM:IZ:1529283	USA, Dutch Harbor	MZ580915	————	————
	USNM:IZ:1523838	USA, Captains Bay; Little South America	MZ580696	————	————
	USNM:IZ:1523837	USA, Dutch Harbor; City Spit Dock	MZ580695	————	————
	USNM:IZ:1529244	USA, Dutch Harbor	MZ580913	————	————
	CASIZ_101555 *	California, Peninsula Point, Marin Co.	KP340408	KP340312	————
	CASIZ 184190 *	New Hampshire, Rockingham Co.	————	KP340313	KP340426
	10NBMOL-10020	Canada, New Brunswick, St. Andrews, Passamaquoddy Bay	KF644026	————	————
	10NBMOL-10019	Canada, New Brunswick, St. Andrews, Passamaquoddy Bay	KF643873	————	————
	10BCMOL-00203	Canada, British Columbia, Bamfield, Wizard Islet	KF643475	————	————
10NBMOL-10018	Canada, New Brunswick, St. Andrews, Passamaquoddy Bay	KF643245	————	————	
CASIZ 188593 *	Washington, Kitsap Co., Puget Sound	————	KP340314	KP340427	
<i>Onchidoris evincta</i>	CASIZ 187758B*	Washington, Kitsap Co., Puget Sound	KP340391	KP340294	————
	CASIZ 186817 *	Washington, Kitsap Co., Puget Sound	KP340389	KP340292	————
	CASIZ 187758A*	USA, Washington, Kitsap County, Puget Sound	KP340390	KP340293	————
<i>Onchidoris expectata</i>  (as <i>Onchidoris macropompa</i> )	ZMMU:Op-595	Russia	KY996411	————	————
	ZMMU:Op-593	Russia	KY996412	————	————
	MIMB34210 *	Russia, Avacha Bay, Kamchatka	MF958423	MF958292	————
<i>Onchidoris jannae</i> (as <i>Knoutsodonta jannae</i> ) (as <i>Knoutsodonta jannae</i> )	CASIZ 175578 *	California, San Mateo Co., Pillar Point	KP340392	KP340296	KP340415
	CASIZ 142450 *	Alaska, Prince William Sound	————	KP340295	KP340414
<i>Onchidoris muricata</i>	BMBM-0550	USA, Washington, San Juan County, San Juan Island, FHL Dock	MH242877	————	————
	AC19-30	Russia, Nevelsk, Sakhalin Is., Sea of Japan	KX951697	————	————
	AC19-32	Russia, Kievka Bay, Sea of Japan	KX951696	————	————
	AC19-31	Russia, Kievka Bay, Sea of Japan	KX951695	————	————
	AC17-40	Russia, Rudnaya Bay, Sea of Japan	KX951694	————	————
	AC17-24	Russia, Rudnaya Bay, Sea of Japan	KX951693	————	————
	AC16-19	Russia, Rudnaya Bay, Sea of Japan	KX951692	————	————
	AC16-18	Russia, Rudnaya Bay, Sea of Japan	KX951691	————	————
	BIOUG < CAN >: WS130	Russia	HM386493	————	————
	ZMMU:Op-597	Russia	KY996414	————	————
	BIOUG < CAN >: WS125	Russia	HM386492	————	————
	Gastr 8524V	Sweden, Kattegatt	MG935180	————	————
	————	Scotland, Clachan Seil	AY345033	————	————
	MT07703	North Sea	KR084489	————	————
	CASIZ 184185A *	New Hampshire, Rockingham Co., New Castle Portsmouth Bay	KM219681	KJ653677	KM225831
CASIZ 181312 *	California, Monterey Co., Asilomar	KM219680	KJ653676	KM225830	
10BCMOL-00318	Canada, British Columbia, Haida Gwaii, Juskalta Narrows	KF643468	————	————	
———— *	Sweden, Bohuslan, Kristineberg	AJ223271	AJ225196	————	
<i>Ancula gibbosa</i>	CASIZ 182028 *	USA, Cumberland County, Maine	KP340388	KP340291	KP340413
<i>Trapania lineata</i>	RM3_698 *	Italy, Sardinia, Secca La Mandria	OQ001357	OP965560	OQ096467

### 2.1. Morphological Analyses

The photographic documentation in situ and in the laboratory was obtained with high resolution digital cameras (mainly 16–24 megapixels) and post-produced with Camera Raw and Photoshop CS6. External morphological observations and anatomical dissections were carried out under the stereomicroscope Nikon SMZ800N equipped with the Nikon Digital Sight 1000 camera. Reproductive systems were observed and photographed to produce a final schematic and descriptive drawing. Anatomical observations of the buccal apparatus were carried out by removing the buccal masses and using proteinase K solution (20 mg/mL) for the digestion of the biological tissues and the extraction of the chitinous structures. Radulae were rinsed in water, dried, and mounted for examination by optical and scanning electron microscopy. To obtain high-resolution scanning electron microscope (SEM) images, dissected radulae were gold-coated in an Emitech K550X Sputter Coater system (Emitech Ltd., Ashford, UK) and, finally, examined by using a JSM-6480LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) at the Laboratorio di Microscopia Elettronica (Department of Mathematics and Physics, University of Salento, Italy), with secondary electrons and an operating voltage of 20 kV.

### 2.2. Molecular Analyses

DNA was extracted from the body tissues using the “salting out” procedure [23]. First, a small piece of tissue was cut from the tail and placed in a tube where it was heated for 1 h at 40 °C. In the following step, 430 µL of cell lysis buffer and 20 µL of proteinase K were added to the dried tissue. The samples were then left in a thermoblock overnight at 56 °C. Next, samples were vortexed and centrifuged at 13,200 × g rpm for 10 min. After this first centrifugation, the liquid supernatant was carefully pipetted into new tubes. Afterwards, 160 µL of NaCl 5 M was added to the samples; these were gently vortexed and centrifuged for 10 min at 13,200 × g rpm. The supernatant was carefully collected and placed into the final tubes and 500 µL of cold isopropanol was added. Next, samples were gently vortexed and centrifuged under the same conditions used in the previous steps and, finally, the supernatant was discarded, letting the DNA pellet adhere to the wall of the tubes. An amount of 1 mL of 80% EtOH was added, and the tubes were centrifuged for the last time for 10 min at 13,200 × g rpm. The supernatant was carefully discarded again, and the samples were left to dry for 1–2 h at room temperature. Finally, dried samples were diluted with 60–100 µL of purified H<sub>2</sub>O. In total, 2 different mitochondrial gene regions, COI and 16S, and the nuclear H3 were amplified. The universal primers, LCO1490 and HCO2198 [24], and 16Sar-L and 16Sbr-H [25] were used for the COI and 16S mitochondrial markers, respectively, while H3AD-F and H3BD-R universal primers [26] were used for nuclear H3. The temperature profile for the PCR reactions was the same for the 3 molecular markers starting with the initial denaturation step at 94 °C, which lasted 5 min. This step was followed by 35 cycles consisting of 30 s at 94 °C for the denaturation step, 60 s at an annealing temperature of 46–50 °C, and 60 s at an elongation temperature of 72 °C. After this cycle, temperature was kept for another 7 min. at 72 °C. Once all these steps were completed, the whole reaction was cooled down to a temperature of 10 °C. The PCR reaction mix had a final volume of 20 µL and consisted of 14.6 µL of dH<sub>2</sub>O, 4.0 µL of 5 × FIREPol Mastermix (5 × reaction buffer (0.4M Tris-HCl, 0.1M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% w/v Tween-20), 12.5 mM MgCl<sub>2</sub>, 1 mM dNTP), 0.2 µL each of forward and reverse primers (20 µM), and 1.0 µL DNA. The quality of all the PCR products obtained was controlled on 1.2% agarose gel. Samples were sequenced by MacroGen Europe.

Before the sequences were used for the alignment, they were controlled with the basic local alignment search tool (BLAST) to exclude possible contamination. Sequences were aligned with GenBank sequences using the muscle algorithm implemented in MEGA 6.0 [27]. Four different alignments were generated: three single-gene datasets (COI, 16S, and H3) and one with the three genes concatenated and partitioned (ConcDNA). Primer regions were always removed from the final alignments. The best-fitting evolutionary model for each of the four datasets (three single-gene, and one concatenated and partitioned) was

determined using JModelTest version 2.1.10 under the BIC model [28]. To generate the concatenated and partitioned dataset, the program DnaSP 6.12.03 [29] was used. The mean  $p$ -distances between groups were calculated using MEGA 6.0 [27]. Different kinds of species delimitation analyses were carried out. We used ASAP [30,31] (available at <http://wwwabi.snv.jussieu.fr/public/abgd/> (accessed on 11 November 2022)) to detect the barcode gap in the distribution of pairwise distances calculated on COI sequence alignment. The ASAP analysis was performed on the ingroup dataset using the Kimura two-parameter (K2P) genetic distance and the default settings parameters. The Species Identifier program [32] was used to calculate maximum intraspecific and minimum interspecific distances ( $p$ -distance) and for clustering sequences based on pairwise distances. To assess the number of putative species in our COI dataset, we used the Poisson tree processes model as implemented in the PTP web server [33] applied on the Bayesian tree. This species delimitation method outperforms other methods based on single-locus molecular phylogenies [33].

Bayesian inference and maximum likelihood phylogenetic analyses were carried out to investigate the phylogenetic evolutionary relationships. Bayesian inference analysis (BI) was performed using the program MrBayes (v. 3.2.6) [34], applying a Bayesian posterior likelihood methodology. Each of the 4 runs were conducted with 4 MCMC (Markov chain Monte Carlo) for 5 million generations, a sample frequency of 1 tree per 1000 generations and a burn-in of 25%. Maximum likelihood analysis was performed using raxmlGUI 1.5b2 [35], a graphical front-end for RAxML 8.2.1 [36], with 100 independent ML searches and 1000 bootstrap replicates. The species *Trapania lineata* Haefelfinger, 1960 was selected as the outgroup species for both analyses.

### 3. Results

#### Systematics

Order Nudibranchia Cuvier, 1817

Family Onchidorididae Gray, 1827

Genus *Atalodoris* Iredale & O'Donoghue, 1923

*Atalodoris camassae* sp. nov. Furfaro & Trainito

Figure 1A–H.

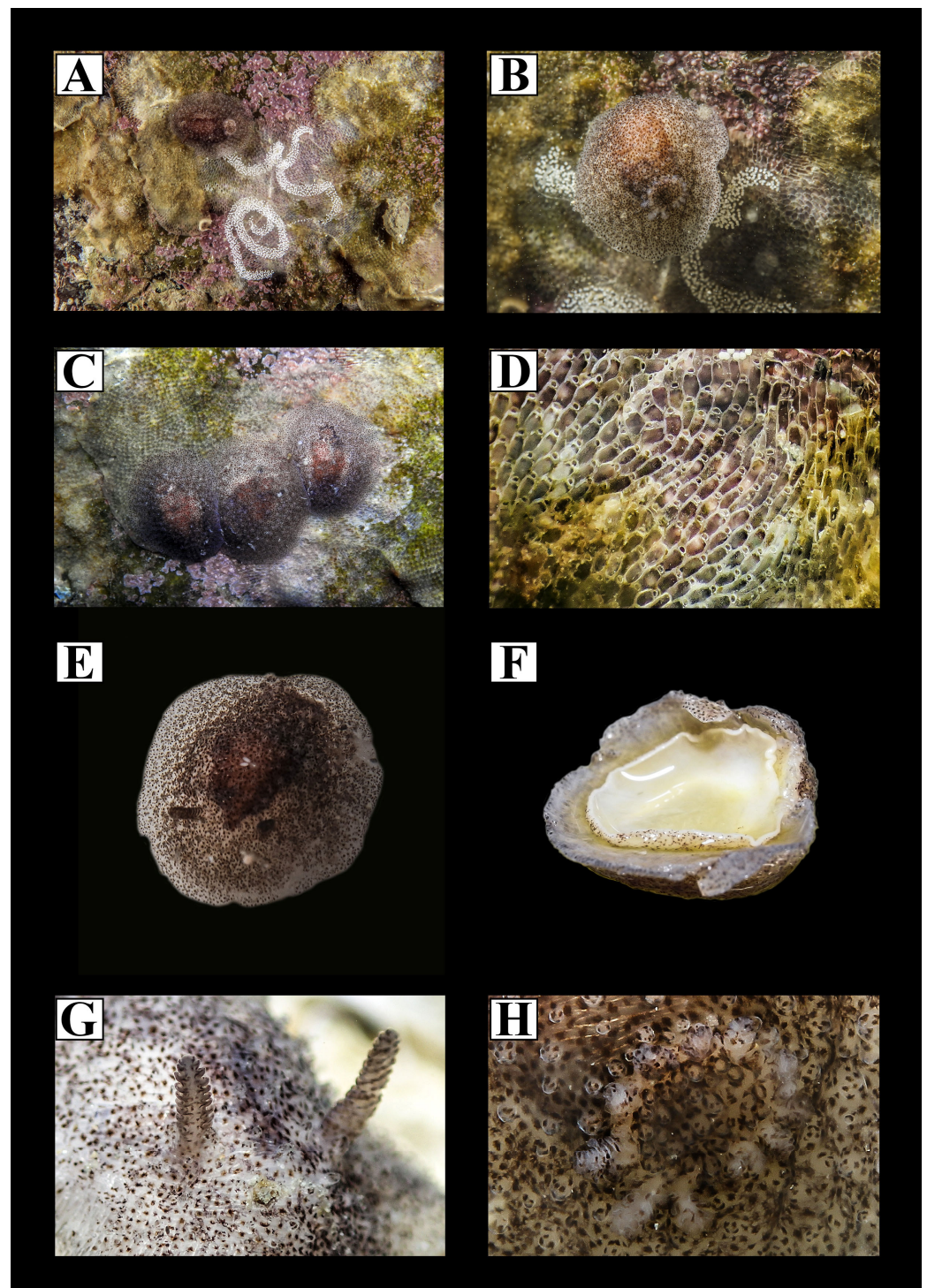
Zoobank: urn:lsid:zoobank.org:act:67C7064C-9DB5-45FE-910E-7D51153B33F4.

*Etymology*: The name is in honor of the deceased Barbara Camassa, a great underwater photographer, nudibranchs enthusiast who helped unveil several North Adriatic species, and a good friend of the coauthors MF, GF, and ET.

*Holotype*: sample MNHN-IM-2000-38594, 10 mm in length, preserved in ethanol 99%, and deposited in the collection of the Natural History Museum of Paris (France; Voucher MNHN-IM-2000-38594).

*Paratypes*: paratype RM3\_1991, 11 mm in length, preserved in ethanol 99%, dissected, and deposited in the zoological collection of the Department of Science of the University of Roma Tre (Italy); paratype RM3\_1993, 13 mm in length, preserved in ethanol 99%, and deposited in the zoological collection of the Department of Science of the University of Roma Tre (Italy); paratype RM3\_1994, 13 mm in length, preserved in ethanol 99%, dissected, and deposited in the zoological collection of the Department of Science of the University of Roma Tre (Italy).

*Type locality*: Northern Adriatic Sea (Mediterranean Sea), Fiesa, Slovenia, 6 m depth, 45°31' N 13°35' E, 7 March 2020.



**Figure 1.** *Atalodoris camassae* sp. nov. (A,B) In situ pictures of the holotype specimen (voucher MNHN-IM-2000-38594) from Fiesa, Slovenia in 11 March 2020 and its typical egg masses. (C) In situ pictures of the three topotypical specimens (voucher RM3\_1991 and two uncatalogued specimens) collected from Fiesa, Slovenia in 7 March 2020. (D) The bryozoan *Calpensia nobilis* eaten by *A. camassae*, Fiesa, Slovenia 11 March 2020. (E) Dorsal view of the paratype specimen (voucher RM3\_1991) collected from Fiesa, Slovenia. (F) Ventral view of the paratype specimen (voucher RM3\_1994) collected from Fiesa, Slovenia. (G). Detail of the rhinophores of *A. camassae*. Specimen collected from Fiesa, Slovenia 5 April 2021. (H) Detail of the gills of *A. camassae* sp. nov. Specimen collected from Fiesa, Slovenia 5 April 2021.

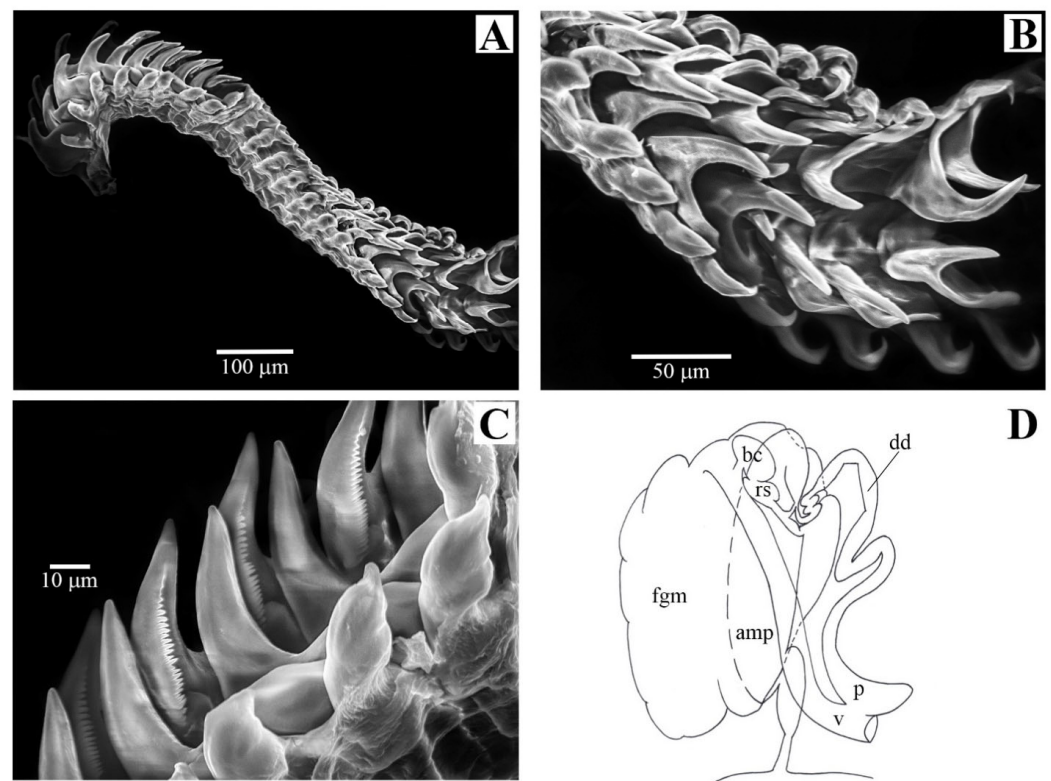
*External morphology:* Body: oval and depressed. Mantle: broad, typically oval, and completely surmounting the foot, which is never visible, except by turning the individual upside down. Mantle entirely covered by rounded, globular tubercles, sometimes with a narrower base than the larger diameter; the tubercles tend to become smaller towards the edges. Background color: hyaline off-white with a thick covering of dark irregular brown spots, with a lighter brown halo; there are also on the tubercles, rhinophores, and gills. In transparency, crossed spicules are visible, appearing as bright streaks. Reverse of the mantle: off-white hyaline and free of spots. In the central area of the back, the halos of the spots are more widespread and, due to transparency, a dark area is formed in correspondence with the mass of the internal organs; the dark area has an elliptical shape and forms a kind of tip that reaches the space between the two rhinophores, where it stops. The foot is covered laterally with small dark spots without a halo, while the sole is completely devoid of them and is hyaline off-white in color. The retractable gills are placed posteriorly in correspondence with the posterior portion of the darker portion of the back; they form an almost complete circle. They are 9–12 in number, bipinnate, with alternating lamellar pinnules; the anterior gills are longer than the posterior ones, with a progressive decrease in size. The gills are hyaline in the internal part and free of spots, with a candid base, while in the external portion they are darker and spotted. When contracted, they appear dark and practically indistinguishable from the tubercular mantle. They surround the perianal area, which is papillated and spotted, but lighter than the mantle due to the absence of halos around the spots. Rhinophores have 7–12 dark lamellae on the hyaline spine and are retractable, appearing dark when contracted.

*Internal anatomy:* The rachidian tooth is absent. The radular formula is  $25-28 \times 1.1.0.1.1$ . The radular teeth are almost translucent and colorless (Figure 2a–c). The first lateral teeth are strong and possess a wide base and a triangular cusp—almost straight—with 24–30 small denticles along the internal surface (Figure 2c). The second lateral teeth are smaller than the others and shaped like rectangular plates, with a downward-directed cusp on the lower outside corner (Figure 2a–c). The oval and elongated ampulla shows two curves, one near the ovotestis and the second in the most distant part. The swollen tube of the ampulla is connected through a short duct to the bursa copulatrix and the seminal male duct. The bursa copulatrix leads to distal part of the female duct, in connection with the seminal receptaculum, ending in the vagina and female opening. The prostate is a narrow, smooth, and convoluted tube that widens slightly near the vas deferens, which makes a series of curves and ends with the unarmed penis (Figure 2d). A large, globular female gland mass connects to the vaginal duct near the seminal receptaculum and the bursa copulatrix. The vagina is wide and long (Figure 2d).

*Distribution:* North Adriatic Sea; Gulf of Trieste; Fiesa, Slovenia. Currently, only specimens from the type locality are known.

*Remarks.* The species was first observed in March 2020, when the samples mentioned here were collected in the type locality. The specimens, together with others not collected, were found exclusively on bryozoans of the species *Calpensia nobilis* (Esper, 1796) (Cheilostomatida; Flustrina) encrusting on various specimens of *Pinna nobilis* (Linnaeus, 1758), settled on a bed about 6 m depth, made up of silty mud. In the same locality and in the same conditions, other specimens were observed in April 2020 and March 2021. Specimens were observed feeding, mating, and spawning characteristic spiral strings, almost flat on the substrate, with egg capsules apparently out of order. Frequently, egg cords were laid on top of each other, even from different specimens. The count of the capsules of various spawns reported an average quantity of about 1400 capsules per string. In March 2022, no specimens were observed in the same locality, probably coinciding with the fact that all the *Pinna nobilis* were found dead, as a consequence of the action of pathogens starting in the Mediterranean in 2016 and still ongoing [37].





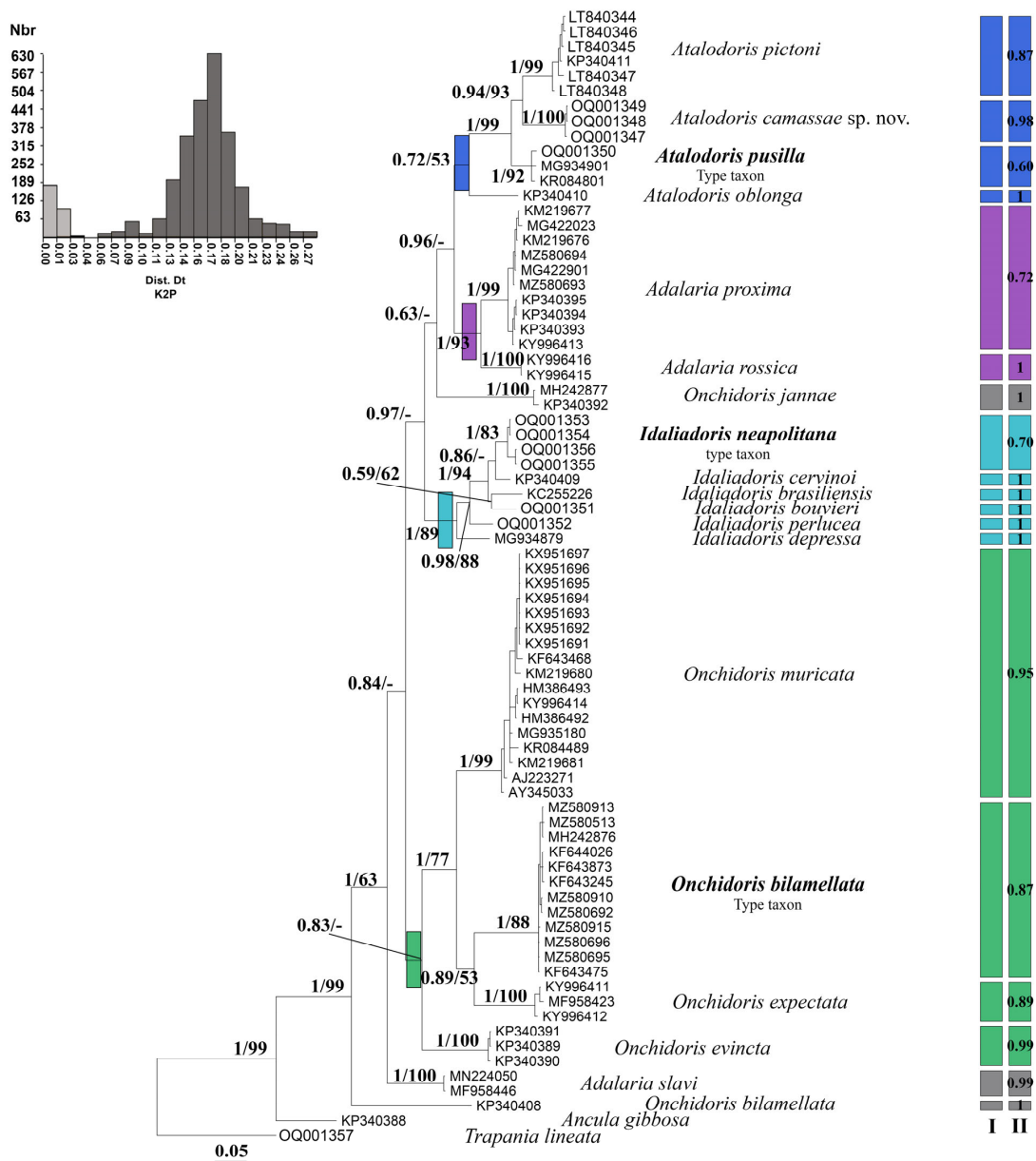
**Figure 2.** Radula and reproductive system of *Atalodoris cammassae* sp. nov. (A) SEM picture of the entire radula. (B) SEM image at a higher magnification level showing the lateral teeth. (C) Detail of the denticulated first lateral teeth. (D) Schematic drawing of the reproductive system; am = ampulla, bc = bursa copulatrix, dd = deferent duct, fgm = female gland mass, p = penis, rs = receptaculum seminis, v = vagina.

#### Molecular Analyses

Molecular analyses involved 77 Onchidorididae and 2 outgroup specimens for a total of 136 sequences (130 ingroup and 6 outgroup) (Table 1). The COI single dataset included 75 sequences—10 obtained in the present study. The 16S single dataset contained 36 sequences, 14 of which were from the present study. The histone 3 single-gene dataset comprised 25 sequences, almost half of them (13) obtained in the present study.

Four different alignments were generated: three single-gene datasets (COI, 16S, and H3 datasets) and one concatenated (COI + 16S + H3) and partitioned dataset (ConcDNA). The COI, 16S, and H3 single-gene datasets were 630 bp, 406 bp, and 305 bp, respectively, and the ConcDNA dataset was 1342 bp. The best evolutionary models resulting from the COI, 16S, and H3 single-gene datasets were TPM1uf + I + G, TPM2uf + I + G, and TPM3uf + I, respectively. All the resulting trees provided congruent results, but with a different ability to resolve phylogenetic relationships at different taxonomic levels. Bayesian supports lower than 0.50, and bootstrap values lower than 50 in the maximum likelihood analyses were considered as not supported. The single-gene analyses carried out using the 16S and the H3 molecular marker were congruent with COI and the concatenated and partitioned analysis but, as expected [20,38,39], with low statistical support; for this reason, these analyses are not shown here. The COI single-gene dataset was used to investigate at the species taxonomic level and to identify species using both genetic distance methods and monophyly (Figure 3). In fact, Bayesian inference (BI) and maximum likelihood (ML) analyses were congruent and revealed 19 monophyletic clades; this was also confirmed by the species delimitation analyses (Figure 3). The barcoding analysis revealed errors in the sequence submission involving the COI sequence with accession number MF958423, deposited in GenBank as *O. macropompa* but perfectly matching sequences of *O. expectata* obtained from the typical materials, and another GenBank sequence reported as *Onchidoris*

*bilamellata* (COI accession number: KP340408), which shows 99.85% homology with *Doris montereyensis* J. G. Cooper, 1863. In fact, this *O. bilamellata* sequence was found to be sister to all the other Onchidorididae that were grouped in a strongly supported monophyletic clade (BI = 1, ML = 99). Sister to the latter clade was a monophyletic clade including all the other Onchidorididae species, and with *Adalaria slavi* as the basal taxon (Figure 3). All the remaining Onchidorididae were divided in two large monophyletic clades, one (BI = 0.89, ML = 53) grouping the species belonging to the *Onchidoris* genus and the other (BI = 0.97, ML ≤ 50) assembling all the remaining genera.

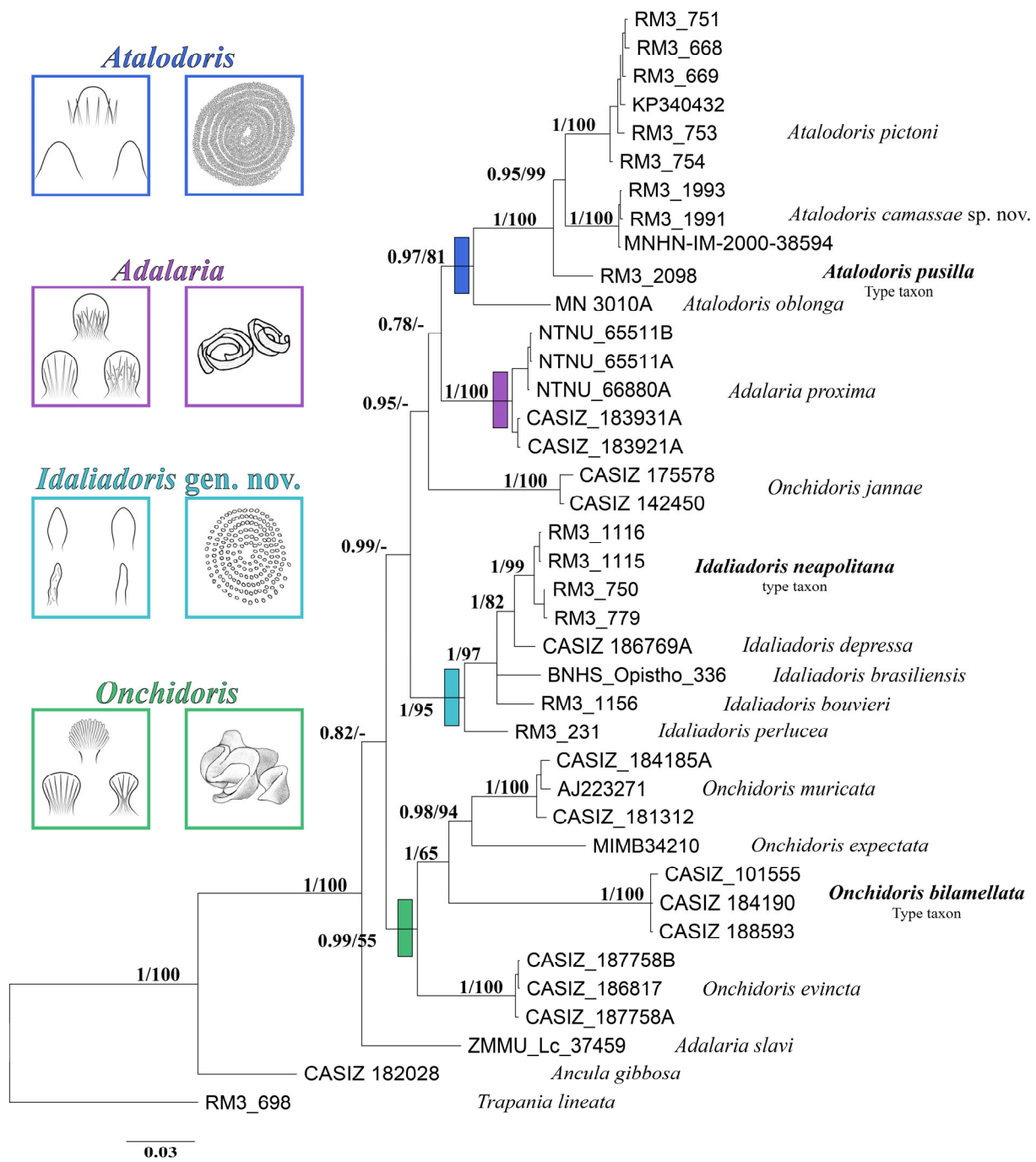


**Figure 3.** Bayesian inference tree based on the COI sequence dataset. Numbers at nodes indicate Bayesian posterior probability (BPP; left) and bootstrap support from maximum likelihood analysis (BP; right). BPP < 0.50 and BP < 50% are not reported. The histogram shows the distribution of the pairwise genetic distances (K2P) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons. The “-” symbol indicates unsupported values. On the right side, results are reported (with colored vertical rectangles) from ASAP and Species Identifier programs “I”, and those from bPTP analysis “II”.

The *Onchidoris* monophyletic clade is divided in two strongly supported sister clades, one with *O. evincta* (BI = 1, ML = 100) and the other (BI = 1, ML = 77) which, in turn, is split in two well-supported monophyletic clades, the first one (BI = 0.89, ML = 53) containing the type species of the genus, *O. bilamellata* (BI = 1, ML = 88) and *O. expectata* (BI = 1, ML = 100), and the second one with *O. muricata* (BI = 1, ML = 99) (Figure 3). The *Onchidoris* clade is sister to a monophyletic clade (BI = 0.97, ML  $\leq$  50) which includes all the other genera investigated here. This large clade is, in turn, divided into two monophyletic clades, one statistically supported (BI = 1, ML = 89), that is proposed here as a new genus named *Idaliadoris* gen. nov. (see below description), formed by *I. depressa* (Alder & Hancock, 1842) comb. nov. as the sister of a clade which, in turn, has *I. perlucea* Ortea & Moro, 2014 comb. nov. as basal (BI = 0.98, ML = 88) to another monophyletic clade (BI = 1, ML = 94). This latter clade groups two clades, the first one composed by the sister species *I. bouvieri* (Vayssiere, 1919) comb. nov. and *I. brasiliensis* (Alvim, Padula & Pimenta, 2011) comb. nov. (BI = 0.59, ML = 62), and the second one showing a GenBank sequence identified as *O. depressa* as the sister species with low support (BI = 0.86, ML  $\leq$  50) to *I. neapolitana* (Delle Chiaie, 1841) comb. nov. (BI = 1, ML = 83), the type species of the *Idaliadoris* gen. nov. The last large clade (BI = 0.63, ML  $\leq$  50) has *O. jannae* (BI = 1, ML = 100) as the sister to a monophyletic clade (BI = 0.96, ML  $\leq$  50) grouping *Adalaria* and *Atalodoris* genera. The *Adalaria* monophyletic clade (BI = 1, ML = 93) is composed of *A. rossica* (BI = 1, ML = 100) and includes the type taxon *A. proxima* (BI = 1, ML = 99) as sister species. The latter monophyletic clade includes GenBank sequences erroneously identified as *A. loveni*. The monophyletic clade that groups all *Atalodoris* species shows *A. oblonga* as the sister to a clade (BI = 1, ML = 99) that, in turn, has the type species *A. pusilla* (BI = 1, ML = 92) as sister to a monophyletic clade (BI = 0.94, ML = 93) composed by *A. pictoni* (Furfaro & Trainito, 2017) (BI = 1, ML = 99) and the new species *A. camassae* sp. nov. (BI = 1, ML = 100).

Bayesian and maximum likelihood analyses carried out on the concatenated and partitioned (ConcDNA) dataset were congruent with single-gene analysis but showed improved ability to investigate at deeper phylogenetic relationships (Figure 4).

In fact, the genera included in the present study were all found to be statistically supported, with BI = 1 ML = 100, BI = 0.97 ML = 81, BI = 1 ML = 95, and BI = 99 ML = 55 for *Adalaria*, *Atalodoris*, *Idaliadoris* gen. nov., and *Onchidoris*, respectively. *Adalaria slavi* is basal and sister (BI = 1, ML = 100) to all the other Onchidorididae included in the analyses. The genus *Onchidoris* is sister to a monophyletic group (BI = 0.99, ML  $\leq$  50) that includes both *Idaliadoris* gen. nov. and another monophyletic clade (BI = 0.95, ML  $\leq$  50) formed by *O. jannae*, sister to the monophyletic clade grouping *Adalaria* and *Atalodoris*. Finally, the phylogenetic analyses revealed the consistency of the shape of the egg masses and the dorsal papillae as important diagnostic characters at a genus taxonomic level, with typical shapes of both eggs and papillae each consisting in a synapomorphy of the relative genus (Figure 4).



**Figure 4.** Bayesian phylogenetic tree based on the ConcDNA dataset (COI, 16S, H3). Bayesian posterior probability (BPP; left) and bootstrap support from maximum likelihood analysis (BP; right) are indicated at each node. The “-” symbol indicates unsupported values. The colored squares on the left show schematic drawings of the egg masses and the dorsal papillae diagnostic for each genera.

#### 4. Discussion

Investigations based on an integrative taxonomy approach were carried out on an extended Onchidorididae dataset which included additional Mediterranean specimens and species, and the molecular markers commonly used were increased. This investigation laid the foundations for proposing a new scheme for the complex systematics of the Onchidorididae family. In fact, recent and conflicting systematic revisions have not clarified the evolutionary relationships between the different genera and have introduced confusion and instability. Furthermore, the Mediterranean contribution was not explored even if the

Mediterranean Sea was revealed to be particularly important for this group of dorid sea slugs, due to the recent discovery of new Onchidorididae species inhabiting this semi-closed basin and the adjacent Northeastern Atlantic [17,22].

Molecular investigations carried out in this study using the molecular markers mostly used in nudibranchs (i.e., COI, 16S, and H3) revealed a phylogenetic history that did not reflect the proposed systematics based on traditional diagnostic morphological characters, such as the radula. In fact, phylogenetic reconstruction carried out by concatenating and partitioning the three molecular markers revealed the presence of well-defined monophyletic clades (Figure 4) that were not considered before. The lack of a broad view of the whole family contributed to the general confusion regarding the genera that should be considered as valid or not. For this reason, phylogenetic analyses were followed by an in-depth study that considered in parallel morphological, ecological, ontogenetic, and molecular characters from all the known Onchidorididae species. This widespread revision allowed us to finally find important key characters able to define useful synapomorphies of each genus that perfectly match with the monophyletic clades obtained by phylogenetic analyses. These key characters are: (i) the shape of the egg masses (Figure 4), which show four different states in the four different genera analyzed here; (ii) the type of development, which involves planktotrophic or lecithotrophic larvae characteristic for the four genera; and (iii) the type of dorsal papillae (Figure 4). Even if the shape of the egg masses and the type of dorsal papillae were revealed to be very useful to identify different genera, data on the development of several species are still lacking and further observations are required to confirm this character as effective in delimiting Onchidorididae genera. In this regard, the taxonomic status of two species analyzed here remains unclear. They are *Onchidoris jannae* and *Adalaria slavi*, both conservatively maintained unaltered due to the low statistical support of the molecular analysis and the lack of knowledge of important characters. While for *O. jannae* it was possible to retrieve data on the type of larva and the number of eggs capsulae per spawn, for *A. slavi* these useful data are still lacking. For this reason, it could be supposed that *O. jannae* should move to the *Adalaria* genus or may represent a possible separate genus, hypotheses that are worthy of further in-depth investigations. Regarding *A. slavi*, molecular results suggest that it does not belong to the *Adalaria* genus and should be moved to a new genus; however, considering the lack of knowledge of the biology of the species, more data are also needed to resolve its systematic status.

*Adalaria* Bergh, 1878.

The genus *Adalaria* was originally described by Bergh [40] and, according to the present study, includes seven species, all showing a boreal distribution (Table 2). *Adalaria loveni* is currently considered as the type species. However, the validity of *A. loveni* is questioned here. In fact, this species was described eight years after *A. proxima* by the same authors (Alder and Hancock, see Table 2) based on a single specimen that was reported as weakly different from the sympatric *A. proxima*. As mentioned above, sequences deposited in GenBank as *A. loveni* are genetically indistinguishable from *A. proxima*, supporting the hypothesis of a possible case of synonymy. However, we reconstructed the taxonomic history of *A. loveni* and we found that from the very first steps the recognition of the specimens reported in the extensive bibliography has mainly had as a reference the comparison with *O. muricata* and not with *A. proxima*. The small number of samples for which molecular data are currently available is insufficient to definitively state that *A. loveni* is a synonym of *A. proxima*. Therefore, until a consistent collection of samples, coming also from the type locality, will make available more supported data, *A. loveni* is here conservatively considered as valid.

**Table 2.** Comparison of external morphology (body, rhinophores, and gills), radular formula, geographical distribution, and development between all the species included in the genus *Adalaria*. ‘P’ stands for planktotrophic larvae.

Species Attributed to the Genus <i>Adalaria</i> Bergh 1878						
Species	Body	Rhinophores	Gills	Radular Formula	Distribution	Ontogenesis N Capsules
<i>Adalaria loveni</i> (Alder & Hancock, 1862)	White, sometimes pale yellow	White	White	2–46 × 12–13.1.1.1.1.13–12	Great Britain, W. Scotland, to Norway	?
<i>Adalaria proxima</i> (Alder & Hancock, 1854)	White to yellow orange	Same as body	Same as body	39–50 × 13–9. 1.1.1.9–13	Amphiboreal: North Atlantic, North Pacific	P 2470
<i>Adalaria olgae</i> Martynov, Korshunova, Sanamyan & Sanamyan, 2009	Intense lemon yellow, invariable	Semitransparent white	Semitransparent white	30–31 × 3–4.1.1.1.4–3	Northwest Pacific	?
<i>Adalaria rossica</i> Martynov & Korshunova, 2017	Yellowish white, inconspicuous white dots	Yellowish	Yellowish	46 × 1–10.1.1.1.1.10–1	Arctic Ocean	?
<i>Adalaria tschuktschica</i> Krause, 1885	Yellowish white when preserved	Yellowish white when preserved	Yellowish white when preserved	30–32 × 6–8.1.1.1.8–6	Arctic Ocean to Northern Bering Sea, NW Pacific	?
<i>Adalaria ultima</i> Martynov & Korshunova, 2017	Yellowish white, inconspicuous white dots	Yellowish	Yellowish	37 × 1–10.1.1.1.1.10–1	Arctic Ocean, Okhotsk Sea	?

#### *Atalodoris* Iredale & O’Donoghue, 1923.

This genus includes eight valid species (Table 3), all with Mediterranean and/or Atlantic distribution. The type species is *Atalodoris pusilla*. In the Mediterranean Sea, we consider it confused with *Atalodoris albonigra* (Pruvot-Fol, 1951). This latter taxon was thus summarily described based on a single 2.5 mm long specimen: “Cette très petite espèce se distingue nettement de toutes les autres par son coloris remarquable. Longueur 2.5, largo 1.5 mm. Le voile est étiré sur les côtés, un peu anguleux. La forme n’est pas très plate. Radula: 1-1-0-1-1; la dominante légèrement denticulée au sommet. La couleur est blanche, translucide, mais presque complètement recouverte par un dessin noir en relief, qui laisse seulement apparaître un peu, au milieu du dos et à travers le pied, la couleur rousse du foie. Méditerranée: Banyuls; un seul échantillon” [41]. This description is totally insufficient to differentiate it from *A. pusilla*. Subsequently, the species was redescribed based on five specimens of 4–5 mm by Schmekel [18], using the same general approach used in the first summary description: some inconsistencies in the description of the various specimens support the idea of a possible synonymy with *A. pusilla*. After Schmekel’s findings in the Gulf of Naples, the species was reported in numerous checklists, which refer to the reports of Pruvot-Fol [41] and Schmekel [18]. However, it was found again only by Perrone [42] and, subsequently, in Catalonia [43]. Perrone’s description does not correspond to *A. albonigra*, but to *A. pictoni*. The same identification error concerns the only report of *A. pusilla* (as *Onchidoris pusilla*) in the Ligurian Sea [44], which must be ascribed to *A. pictoni*. Finally, the specimens reported for Catalonia [45] correspond to *A. pusilla* in the molecular analysis, but also in the external morphology so succinctly described by Alder and Hancock: “Fulvous, thickly covered with dark brown spots, ovate, depressed; cloak with numerous conical tubercles; tentacles slender, conical, white; branchial plumes 9, pure white, obtuse, set in a rather open circle. Length 3/10 inch (= about 7.5 mm) and represented in Plate 13” [46].

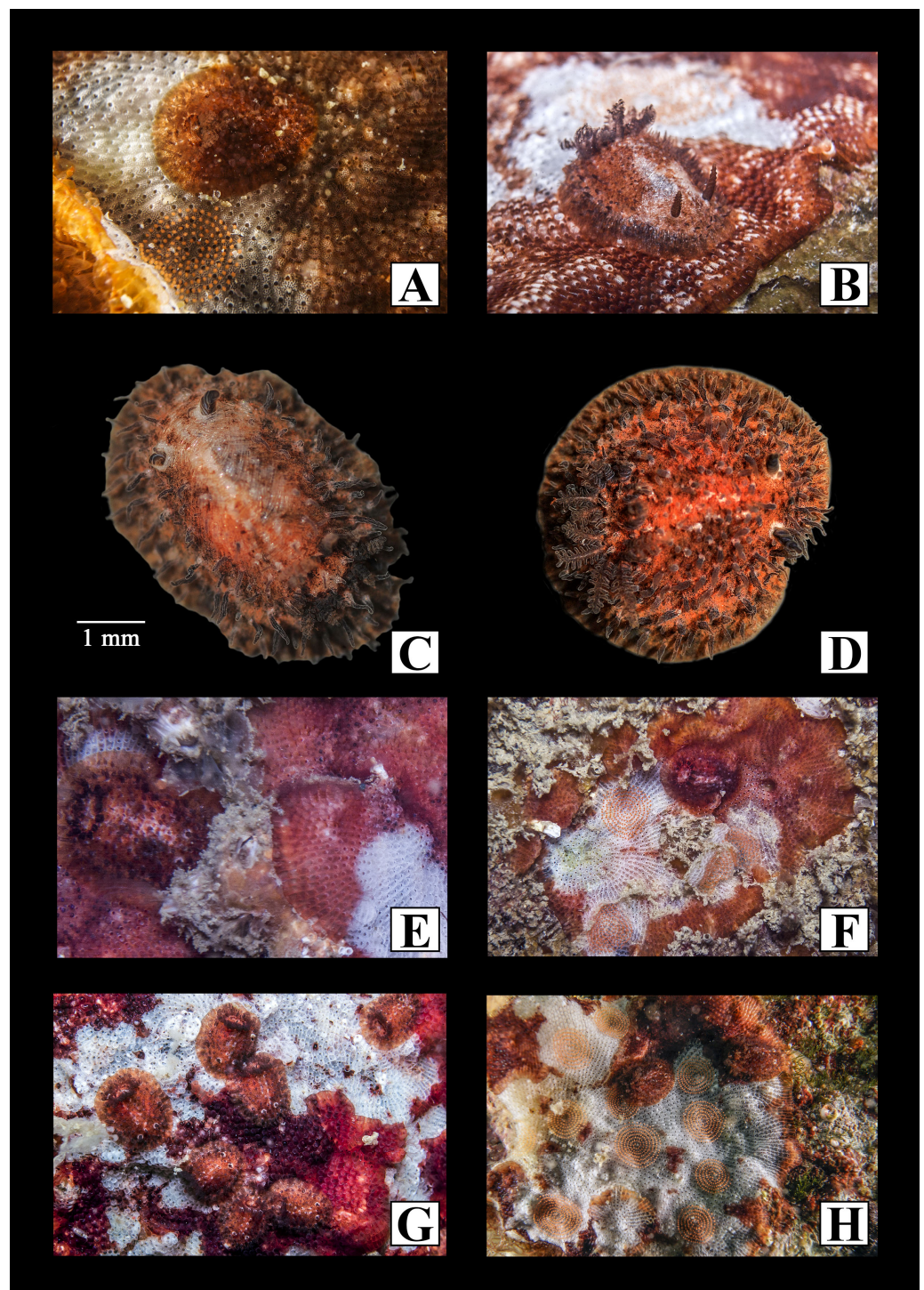
**Table 3.** Comparison of external morphology (body, rhinophores, and gills), radular formula, geographical distribution, and development between all the species included in the genus *Atalodoris*. ‘P’ stands for planktotrophic larvae.

Species Attributed to the Genus <i>Atalodoris</i>						
Species	Body	Rhinophores	Gills	Radular Formula	Distribution	Ontogenesis N° Capsules
<i>Atalodoris camassae</i> sp. nov.	Hyaline off-white with a thick covering of dark irregular brown spots (salt and pepper) with a lighter brown halo. Central area darker in correspondence with internal organs, ending around the gills and with a pointed end between the rhinophores	Hyaline spine with dark lamellae	Hyaline in the internal part, darker and spotted in the external portion	? × 1.1.0.1.1	Mediterranean endemic?	P 1400
<i>Atalodoris butanito</i> Ortea, 2021	Orange with reddish brown speckles that are less sparse in the central region; the entire edge of the mantle is blotched brown, with a continuous series of spicules running perpendicular to the edge	Yellowish with dark leaflets	Yellowish	48 × 1.1.0.1.1	Northeast Atlantic	P 3000 eggs × cm
<i>Atalodoris inconspicua</i> (Alder & Hancock, 1851)	White or pale brown, often tinged with a purple hue, covered by small specks of brown pigment	Pale yellow	White	29 × 1.1.0.1.1	Northeast Atlantic	P
<i>Atalodoris oblonga</i> (Alder & Hancock, 1845)	Grey with a few darker blotches on the back	Yellowish	Yellowish white	28 × 1.1.0.1.1	Northeast Atlantic	P
<i>Atalodoris pictoni</i> (Furfaro & Trainito, 2017)	Uniform dark brown, almost black, with small bluish white speckles scattered along the mantle	White	Dark	25–28 × 1.1.0.1.1	Northeast Atlantic, Mediterranean	<i>p</i> > 9000
<i>Atalodoris pusilla</i> (Alder & Hancock, 1845)	Dense dark brown pigment spots responsible for dark appearance	Transparent	Transparent	21–29 × 1.1.0.1.1	Northeast Atlantic, Mediterranean	P
<i>Atalodoris reticulata</i> (Ortea, 1979)	Whitish, intensely pigmented in green at center, fading at borders; whitish longitudinal bands forming a net with bands running from tubercles surrounding brown and green patches	Large brown stains	Pigmented dirt cream	51–54 × 1.1.0.1.1	Northeast Atlantic	P
<i>Atalodoris sparsa</i> (Alder & Hancock, 1846)	Pale brown mantle with regularly spaced, darker blotches forming patches	Blotched with olive-brown	Colorless	32–36 × 1.1.0.1.1	Northeast Atlantic, Mediterranean	P

It should be considered that in specimens of such small dimensions (from 2.5 to 7.5 mm) the pigmentation of the mantle may appear with a prevalence of brown/black in the smallest specimens, but with dark spots on a creamy white background in those three times larger. Furthermore, the coloration and structure of gills and rhinophores correspond between the two taxa. Based on these considerations and on the fact that the samples described by Pruvot-Fol [41], Schmekel [18], and Perrone [42] are not available, we consider *A. albonigra* as the junior synonym of *A. pusilla*, according to the International Commission on Zoological Nomenclature, ICZN. Furthermore, a new species is here described belonging to this genus, *A. camassae* sp. nov., which shares the same synapomorphies of the congeneric species (see species description above).

*Idaliadoris* gen. nov. Furfaro & Trainito.

Type species: *Idaliadoris neapolitana* (Delle Chiaje, 1841) (Figure 5).



**Figure 5.** *Idaliadoris neapolitana* comb. nov. (A) Voucher RM3\_779; Porto San Paolo, Sardinia, 26 December 2016. (B) Voucher RM3\_750; Sistiana, Friuli-Venezia Giulia, 12 July 2016. (C) Voucher RM3\_1115; Lu Carragioni, Gulf of Olbia, Sardinia, 24 March 2017. (D) Voucher RM3\_1115; Lu Carragioni, Gulf of Olbia, Sardinia, 25 March 2017. (E) Gulf of Naples, Campania. (F) Gulf of La Spezia, Liguria. (G) Tegnuè di Chioggia, Veneto. (H) Lu Carragioni, Gulf of Olbia, Sardinia, 1 April 2017.

Zoobank: LSID urn:lsid:zoobank.org:act:2639236D-A114-4043-9C11-F7EC77825AF1.

The body is oval, and the mantle completely covers the foot. Notum spiculate with visible spicules in transparency are covered by loose, soft, long, and more or less swollen tubercles. It possesses linear, lamellar, long, and retractable rhinophores in low sheaths. The gills are pinnate, forming an almost complete semicircle around the anus. Its sexual opening



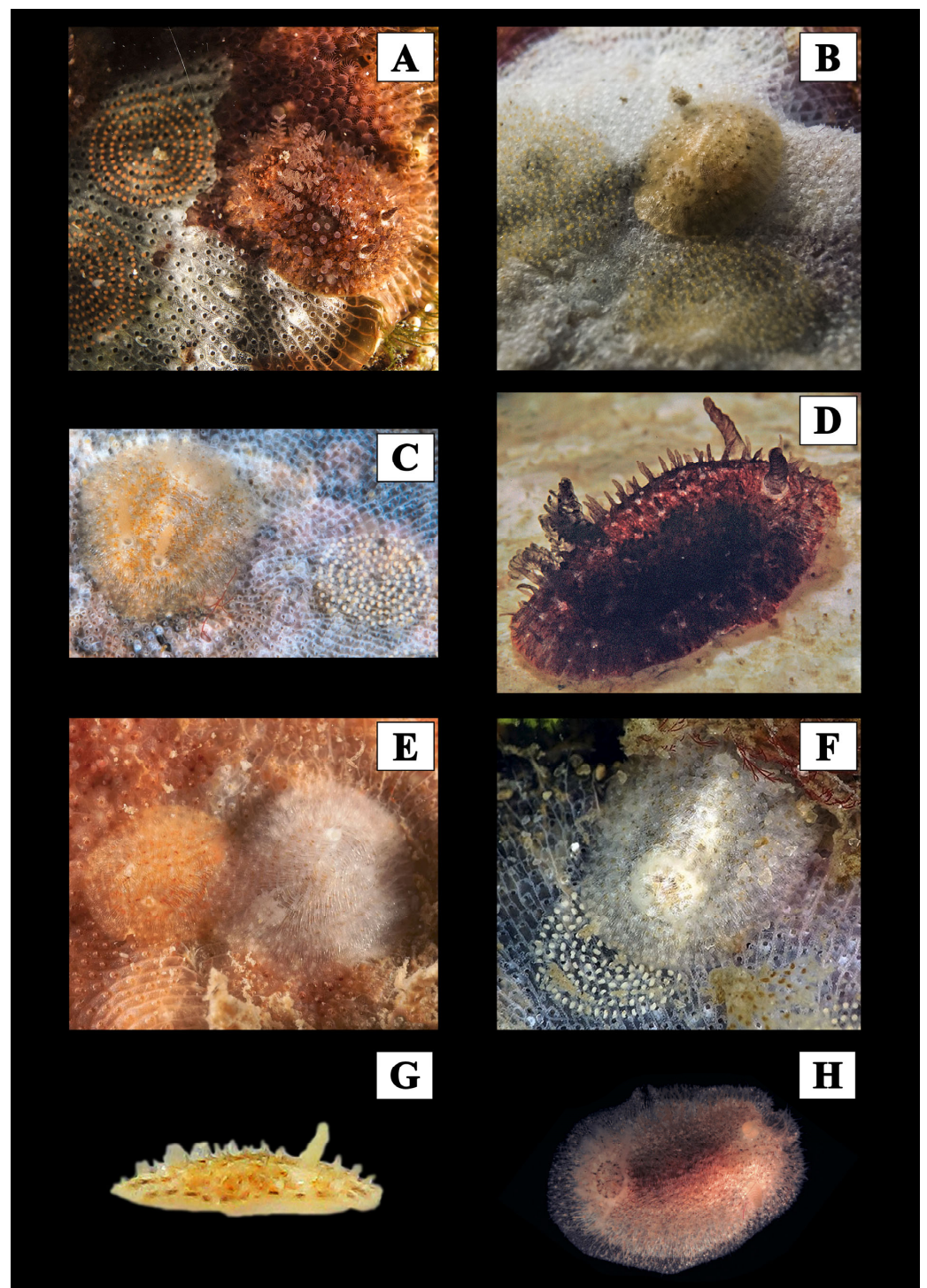
is on the right side. Its radular formula is  $X \times 1.1.0.1.1$ . It is lecithotrophic with characteristic spawns of no more than 400 large ovarian capsules, similar in color to the notum.

*Origin of the name:* The type species *Idaliadoris neapolitana* was described with the original combination *Idalia neapolitana*, but the generic epithet was not usable, as it was previously assigned to another genus *Idalia* (Leuckart, 1828). The union between the genus used in the original description with “doris” maintains a connection with the taxonomic history and the belonging to the suborder Doridina.

Eight species are included in this genus (Figure 6), all of them showing the same shape of the egg masses and the lecithotrophic larva (Table 4). Furthermore, a morphological trait is common to the species of the genus: it is the shape of the dorsal papillae, which in all species has a more or less swollen, finger-like appearance. *Idaliadoris neapolitana* comb. nov. is designated as the type species of the genus. Furthermore, for the first time, *I. bouvieri* comb. nov. is analyzed and sequences are deposited. This species could have been underestimated due to its similarity with *I. neapolitana*; for this reason, further in-depth study is necessary to better understand its real spread and distribution. *Idaliadoris perlucea* comb. nov. is included in this genus based on a sample (voucher RM3\_231) (Figure 6G) not collected from the type locality which, however, shares important diagnostic characters, such as the structure of the dorsal papillae and the egg masses.

**Table 4.** Comparison of external morphology (body, rhinophores, and gills), radular formula, geographical distribution, and development between all the species included in *Idaliadoris* gen. nov. ‘L’ stands for lecithotrophic larvae.

Species Attributed to the Genus <i>Idaliadoris</i> gen. nov.						
Species	Body	Rhinophores	Gills	Radular Formula	Distribution	Ontogenesis N° Capsules
<i>Idaliadoris bouvieri</i> comb. nov. (Vayssière, 1919)	Pale translucent pink with scattered red brown patches distributed in three longitudinal bands	Yellowish pink	Yellowish pink	$40 \times 1.1.0.1.1$	Med. endemic?	L 240
<i>Idaliadoris brasiliensis</i> comb. nov. (Alvim, Padula & Pimenta, 2011)	Greyish white or yellowish orange, both translucent, with a pattern of scattered dark brown (sometimes orange) tiny spots, tending towards the mid-line	Translucent white	Translucent white	$16-17 \times 1.1.0.1.1$	Southeast Atlantic	L 116
<i>Idaliadoris cervinoi</i> comb. nov. (Ortea & Urgorri, 1979)	Orange, centrally intense, fading at the borders; some specimens’ borders almost whitish	Orange with dark brown lamellae	Dark brown circled by a light area	$? \times 1.0.1$	Northeast Atlantic	L
<i>Idaliadoris depressa</i> comb. nov. (Alder & Hancock, 1842)	Pale or translucent brown with scattered orange or purple-brown speckles	Translucent	Translucent	$33-34 \times 1.1.0.1.1$	Northeast Atlantic, Mediterranean	L 354
<i>Idaliadoris maugensis</i> comb. nov. (Burn, 1958)	Pale pattern with shades or colors of yellow and/or orange	Yellowish	Yellowish	22 rows	Southwest Pacific	L 129
<i>Idaliadoris neapolitana</i> comb. nov. (Delle Chiaie, 1841)	Pale reddish or pale brown covered with dense and intense red or purple/brown; at the notal edge, pigment becomes linear	Dark	Dark	$24 \times 1.1.0.1.1$	Mediterranean endemic	L 212
<i>Idaliadoris perlucea</i> comb. nov. (Ortea & Moro, 2014)	Transparent with brown speckles	Translucent white	Translucent	?	Northeast Atlantic, Mediterranean	L 354
<i>Idaliadoris tridactila</i> comb. nov. (Ortea & Ballesteros, 1982)	Whitish with orange or dark reddish spots; in between rhinophores and gills, up to six irregular lines formed by this pigment	Yellow or light pink	Yellow	$12 \times 1-1-0-1-1$	Northeast Atlantic	L 354



**Figure 6.** Species belonging to *Idaliadoris* gen. nov. (A) *I. neapolitana* comb. nov., Porto San Paolo, Sardinia. (B) *I. bouvieri* comb. nov. Rjeka, Croatia. (C) *I. brasiliensis* (Adapted with permission from Ref. [47]. 2011, *Journal of the Marine Biological Association of the United Kingdom*). (D) *I. cervinoides* (Adapted with permission from Ref. [17] 2021, *Revista de la Academia Canaria de Ciencias*). (E) *I. depressa* comb. nov., L'Escala, Catalonia, Spain. (F) *I. maugensis* comb. nov., Sunshine Coast, Australia (photo courtesy of G. Cobb). (G) *I. perlucea* comb. nov. (voucher RM3\_231), M.P.A. 'Secche di Tor Paterno', Latium, Italy. (H) *I. tridactyla* (Adapted with permission from Ref. [17] 2021, *Revista de la Academia Canaria de Ciencias*).

*Onchidoris* Blainville, 1816.

Of the 17 species currently assigned to the genus *Onchidoris*, we consider 2 as valid, and 4 others as *taxa inquirenda* for possible synonymy (Table 5); we also consider the remaining 11 species to be *nomina dubia* (see discussion below and Table 6). Table 5 shows *O. bilamellata* and *O. muricata*, whose validity is confirmed, and the questioned four species that require further investigations based on morphological and/or molecular analysis of type material or of new samples from the type localities. The first pair of doubted species includes *O. evincta* as the possible junior synonym of *O. hystricina*. In fact, the original description of *O. evincta* (as *Adalaria evincta* Millen, 2006) does not contrast with that provided by Bergh [40] for *Lamellidoris hystricina*, now nested in the genus *Onchidoris*. The only detectable difference is in the radular formula, but Bergh himself noted that his examination of the radula was imperfect, since he was unable to examine it in detail. Unfortunately, Millen did not compare *O. evincta* with *O. hystricina*, but the tubercles on the notum, which are covered by dense filiform processes, described independently both in *O. evincta* and in *O. hystricina*, are very characteristic and diagnostic at the species level, since they are absent in any other species of the group. For this reason, we believe that *O. evincta* could be considered a junior synonym of *O. hystricina*, but due to the lack of comparison with type material, we provisionally consider the two species as valid. Finally, as occurred for *A. proxima* and *A. loveni* (see comments above), there is the case of *O. macropompa* and *O. expectata*. In fact, even if the sequence deposited in GenBank as *O. macropompa* perfectly matches those of *O. expectata*, further investigation on type material or on specimens from the type locality is needed to confirm the validity of the former species. For this reason, we provisionally continue to consider both species as valid.

**Table 5.** Comparison of external morphology (body, rhinophores, and gills), radular formula, geographical distribution, and development between all the species included in *Onchidoris*. ‘P’ stands for planktotrophic larvae.

Species Attributed to the Genus <i>Onchidoris</i>						
Species	Body	Rhinophores	Gills	Radular Formula	Distribution	Ontogenesis N° Capsules
<i>Onchidoris bilamellata</i> (L., 1767)	Light brown or yellowish grounds with numerous dark brown spots	Dark at the base lighter on top	Dark at the base lighter on top	23–30 × 1.1.1.1.1	Amphiboreal	P 60.000
<i>Onchidoris evincta</i> Millen, 2006	White, rarely yellowish	White or yellowish	White or yellowish	4–39 × 3–6.1.1.1.6–3	Northeast Pacific, British Columbia	?
<i>Onchidoris expectata</i> Martynov & Korshunova, 2017	Opaque white	White	White	35–38 × 1.1.1.1.1	Northwest Pacific	?
<i>Onchidoris hystricina</i> (Bergh, 1878)	Uniformly whitish shining spicules	Whitish	Whitish	40 ×	East Pacific (Alaska + California)	?
<i>Onchidoris macropompa</i> Martynov, Korshunova, Sanamyan & Sanamyan, 2009	Off-white transparent	White	White	35–38 × 1.1.1.1.1	Northern Pacific	?
<i>Onchidoris muricata</i> (Müller, 1776)	White to yellow	White	White	20–36 × 1.1.1.1.1	Amphiboreal	P 2500

**Table 6.** List of the species that should be considered as *nomina dubia*.

List of <i>Nomina Dubia</i>					
Species	Original Genus	Status	Synonymy	Distribution	References
<i>Onchidoris aureopuncta</i> (Verrill, 1901)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	Possible synonym of <i>Cadlina laevis</i>	Central West Atlantic	/
<i>Onchidoris lactea</i> (Verrill, 1900)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	ND	Central West Atlantic	[48]
<i>Onchidoris miniata</i> (Verrill, 1901)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	ND	Central West Atlantic	/
<i>Onchidoris olivacea</i> (Verrill, 1900)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	ND	Central West Atlantic	[48]
<i>Onchidoris quadrimaculata</i> (Verrill, 1900)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	ND	Central West Atlantic (Bermuda)	/
<i>Onchidoris derjugini</i> (Volodchemko, 1941)	<i>Adalaria</i>	<i>Nomen dubium</i>	Possible senior synonym of <i>Adalaria jannae</i>	Sea of Japan	[49]
<i>Onchidoris diademata</i> (Agassiz, 1850)	<i>Lamellidoris, Doris</i>	<i>Nomen dubium</i>	<i>Archidoris tuberculata</i>	Northwest Atlantic	[50–52]
<i>Onchidoris grisea</i> (Stimpson, MS, Gould, 1870)	<i>Lamellidoris</i>	<i>Nomen dubium</i>	ND	Northwest Atlantic	[53]
<i>Onchidoris tenella</i> (Agassiz, 1850) (Gould, 1870)	<i>Lamellidoris, Doris</i>	<i>Nomen dubium</i>	Doridacea, according to Trott [54]	Northwest Atlantic	[53]
<i>Onchidoris beringi</i> (Volodchemko, 1941)	<i>Lamellidoris, Adalaria</i>	<i>Nomen dubium</i>	ND	North Pacific	[49]
<i>Onchidoris spiculoides</i> (Volodchemko, 1941)	<i>Lamellidoris, Adalaria</i>	<i>Nomen dubium</i>	Possible synonym of <i>Adalaria tschuktschica</i>	North Pacific	[49]

Finally, in addition to the species treated so far, 11 species described by 4 different authors are currently contemplated within the family Onchidorididae and in the genus *Onchidoris*. Of these, not only have no more probative findings occurred, but their descriptions are either insufficient or exclude their belonging to the genus and even to the family. Table 6 summarizes these species, indicating possible synonyms, the geographical area of description, and the reference bibliography, when it exists. At the current state of knowledge, we propose them as *nomina dubia* (Table 6) until new discoveries from typical localities can be traced back to the succinct descriptions.

Even if the diversity of the Mediterranean nudibranchs is still far from being comprehensively unveiled, several steps forward have been made thanks to integrative methods [20,38,39]. In fact, it is now increasingly clear how morphological and anatomical identification of nudibranch species is based on characteristics that are quite variable, difficult to compare, and not always present [20,39,55]. Thus, the integration with data obtained by other approaches is necessary. In the Onchidorididae systematics, the shape of the egg masses was revealed to be a useful diagnostic character allowing one to distinguish between genera. This result is particularly interesting and opens new perspectives that should be considered in future systematic revisions focused on other Nudibranchia. In fact, even if the radula, the hard structure part of the buccal apparatus that nudibranchs

typically use for feeding, is nowadays considered one of the main diagnostic characters, it is undeniable that in some cases [39] it could be less informative or even completely lacking (as in particular genera like *Calma* Alder & Hancock, 1855; *Doriopsilla* Bergh, 1880; *Melibe* Rang, 1829, etc.), in which cases, additional valid diagnostic characters are desirable.

For all these reasons, the study of marine biodiversity, even if facilitated by the advent of new advanced technologies, cannot ignore the in vivo study and in situ observation of the organisms in their own habitats.

## 5. Conclusions

The systematic revision here described, based on biological, morphological, and molecular (COI, 16S, and H3 molecular markers) data, allowed the proposal of a valid solution to some historical controversies within the Onchidorididae family. In particular, the Onchidorididae genera were investigated and clarified, with the introduction of a new genus, *Idaliadoris* gen. nov., described here. New synapomorphies, shape of the egg masses, and type of larva were proposed at the genus taxonomic level and new combinations for the species were accordingly provided. Finally, a new *Atalodoris* species from the Adriatic Sea, *A. camassae* sp. nov., was described based on external morphology, internal anatomy, and molecular identification. To date, this is the most updated revision of the Onchidorididae family considering all the known Onchidorididae species and shedding light on the evolutionary history of this group of dorid nudibranchs. Furthermore, it provides insights on the need to include in vivo studies and in situ observations of the organisms analyzed in order not to overlook important aspects of their biology that may be useful for reconstructing the systematics of the various groups.

**Author Contributions:** Conceptualization, G.F., E.T. and P.M.; methodology, G.F.; formal analysis, G.F., M.D. and E.T.; investigation, E.T. and M.F.; resources, E.T., M.F. and E.M.; data curation, G.F. and E.T.; writing—original draft preparation, G.F.; writing—review and editing, G.F., E.T. and P.M.; supervision, P.M.; funding acquisition, G.F. and P.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Publicly available datasets were analyzed in this study. This data can be found here: [<https://www.ncbi.nlm.nih.gov/>].

**Acknowledgments:** This paper is dedicated to the late Barbara Camassa (Sistiana, Trieste), who collected samples from the Gulf of Trieste (Adriatic Sea) and whose contribution to the growing body of knowledge about Mediterranean nudibranchs has yet to be fully acknowledged. The authors are grateful to Robert Laurence Westmore (Bielefeld, Nordrhein-Westfalen, Germany), a native English speaker who proofread the English language of the manuscript. The authors wish to thank Saul Ciriaco (Trieste, Italy) and Mauro Doneddu (Sassari, Italy) for their support and wise advice, Irena Frkovic (Koper, Slovenia) for providing useful underwater pictures of the new species, Gary Cobb (Queensland, Australia) for the permission to use the photograph in Figure 5F, Michele Solca (Milano, Italy) for post-producing the SEM images, Stefano Trainito (Padova, Italy) for the drawings of the dorsal tubercles, Lisa Faresi (Arpa Friuli) for her contribution in finding and collecting the new species, and the Miramare Marine Protected Area for logistic support. The collection of samples in the Tavolara-Punta Coda Cavallo Marine Protected Area (Olbia, Italy) was authorized by the managing body, which is here thanked. GF wishes to thank the Scubalandia Team for technical underwater support. Finally, the authors thank the three anonymous reviewers that helped improve the quality of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## References

1. Coll, M.; Piroddi, C.; Steenbeek, J.; Kaschner, K.; Ben Rais Lasram, F.; Aguzzi, J.; Ballesteros, E.; Bianchi, C.N.; Corbera, J.; Voultsiadou, E.; et al. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLoS ONE* **2010**, *5*, e11842. [CrossRef] [PubMed]
2. Moullec, F.; Barrier, N.; Drira, S.; Guilhaumon, F.; Marsaleix, P.; Somot, S.; Ulses, C.; Velez, L.; Shin, Y.J. An end-to-end model reveals losers and winners in a warming Mediterranean Sea. *Front. Mar. Sci.* **2019**, *345*, 1–19. [CrossRef]
3. Renda, W.; Amati, B.; Bogi, C.; Bonomolo, G.; Capua, D.; Dell'Angelo, B.; Giulia, F.; Riccardo, G.S.; Rafael, L.P.; Oliverio, M.; et al. The new Checklist of the Italian Fauna: Marine Mollusca. *Biogeogr. J. Integr. Biogeogr.* **2022**, *37*, 1–14. [CrossRef]
4. Strong, E.E.; Gargominy, O.; Ponder, W.F.; Bouchet, P. Global Diversity of Gastropods (Gastropoda; Mollusca) in Freshwater. *Hydrobiologia* **2008**, *595*, 149–166. [CrossRef]
5. Brown, K.M. 10—Mollusca: Gastropoda. In *Ecology and Classification of North American Freshwater Invertebrates*, 2nd ed.; Thorp, J.H., Covich, A.P., Eds.; Academic Press: San Diego, CA, USA, 2001; pp. 297–329. ISBN 978-0-12-690647-9.
6. Furfaro, G.; Vitale, F.; Licchelli, C.; Mariottini, P. Two Seas for One Great Diversity: Checklist of the Marine Heterobranchia (Mollusca; Gastropoda) from the Salento Peninsula (South-East Italy). *Diversity* **2020**, *12*, 171. [CrossRef]
7. Chimienti, G.; Angeletti, L.; Furfaro, G.; Canese, S.; Taviani, M. Habitat, morphology and trophism of *Tritonia callogorgiae* sp. nov., a large nudibranch inhabiting *Callogorgia verticillata* forests in the Mediterranean Sea. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2020**, *165*, 103364. [CrossRef]
8. Canessa, M.; Bavestrello, G.; Cattaneo-Vietti, R.; Furfaro, G.; Doneddu, M.; Navone, A.; Trainito, E. Rocky substrate affects benthic heterobranch assemblages and prey/predator relationships. *Estuar. Coast. Shelf Sci.* **2021**, *261*, 31. [CrossRef]
9. Trainito, E.; Fantin, M.; Manganelli, E.; Furfaro, G. What are you doing here? Investigating on an unexpected association in shallow Mediterranean dark caves sheds light on the diet of *Marionia blainvillea* (Mollusca, Gastropoda, Nudibranchia). *Turk. J. Zool.* **2021**, *45*, 550–556. [CrossRef]
10. Riccardi, A.; Colletti, A.; Virgili, R.; Cerrano, C. Diversity and behavior of sea slugs (Heterobranchia) in the rocky tide pools of Conero Riviera (western Adriatic Sea). *Eur. Zool. J.* **2022**, *89*, 856–869. [CrossRef]
11. MolluscaBase eds. 2022. MolluscaBase. Onchidorididae Gray, 1827. World Register of Marine Species. Available online: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=175> (accessed on 24 November 2022).
12. MolluscaBase eds. 2022. MolluscaBase. *Acanthodoris* Gray, 1850. World Register of Marine Species. Available online: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=138284> (accessed on 24 November 2022).
13. Hallas, J.M.; Simison, W.B.; Gosliner, T.M. Dating and biogeographical patterns in the sea slug genus *Acanthodoris* Gray, 1850 (Mollusca, Gastropoda, Nudibranchia). *Mol. Phylogenetics Evol.* **2016**, *97*, 19–31. [CrossRef]
14. Hallas, J.M.; Gosliner, T.M. Family matters: The first molecular phylogeny of the Onchidorididae Gray, 1827 (Mollusca, Gastropoda, Nudibranchia). *Mol. Phylogenetics Evol.* **2015**, *88*, 16–27. [CrossRef] [PubMed]
15. Martynov, A.; Sanamyan, N.; Korshunova, T. Review of the opisthobranch mollusc fauna of Russian Far Eastern seas: Pleurobranchomorpha, Doridida and Nudibranchia. *Bull. Kamchatka State Tech. Univ.* **2015**, *34*, 62–87. (In Russian)
16. Iredale, T.; O'Donoghue, C.H. List of British nudibranchiate Mollusca. *Proc. Malacol. Soc. Lond.* **1923**, *15*, 195–233.
17. Ortea, J. Nueva especie del género *Atalodoris* Iredale & O'Donoghue, 1923 (Mollusca: Nudibranchia) colectada en la Concha de Artedo, Asturias, dedicada al periodista deportivo José María García. *Rev. De La Acad. Canar. De Cienc.* **2021**, *33*, 97–106.
18. Schmekel, L.; Portmann, A. *Opisthobranchia des Mittelmeeres*; Springer Science & Business Media: Berlin, Germany, 1982.
19. Furfaro, G.; Mariottini, P. A New *Dondice* Marcus Er. 1958 (Gastropoda: Nudibranchia) from the Mediterranean Sea Reveals Interesting Insights into the Phylogenetic History of a Group of Facelinidae Taxa. *Zootaxa* **2020**, *4731*, 1–22. [CrossRef]
20. Furfaro, G.; Schreier, C.; Trainito, E.; Pontes, M.; Madrenas, E.; Girard, P.; Mariottini, P. The Sea Slug *Doriopsilla areolata* Bergh, 1880 (Mollusca, Gastropoda) in the Mediterranean Sea: Another Case of Cryptic Diversity. *Diversity* **2022**, *14*, 297. [CrossRef]
21. Martynov, A.; Korshunova, T. World's northernmost and rarely observed nudibranchs: Three new onchidoridid species (Gastropoda: Doridida) from Russian seas. *Zootaxa* **2017**, *4299*, 391–404. [CrossRef]
22. Furfaro, G.; Trainito, E. A new species from the Mediterranean Sea and North-Eastern Atlantic Ocean: *Knoutsodonta pictoni* n. sp. (Gastropoda Heterobranchia Nudibranchia). *Biodivers. J.* **2017**, *8*, 725–738.
23. Aljanabi, S.M.; Martinez, I. Universal and Rapid Salt-Extraction of High Quality Genomic DNA for PCR-Based Techniques. *Nucleic Acids Res.* **1997**, *25*, 4692–4693. [CrossRef]
24. 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.
25. Palumbi, S.R.; Martin, A.; Romano, S.; McMillan, W.O.; Stice, L.; Grabowski, G. *The Simple Fool's Guide to PCR*; Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii: Honolulu, HI, USA, 2002.
26. Colgan, D.J.; McLauchlan, A.; Wilson, G.D.F.; Livingston, S.P.; Edgecombe, G.D.; Macaranas, J.; Cassis, G.; Gray, M.R. Histone H3 and U2 SnRNA DNA Sequences and Arthropod Molecular Evolution. *Aust. J. Zool.* **1999**, *46*, 419–437. [CrossRef]
27. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef] [PubMed]
28. Posada, D. JModelTest: Phylogenetic Model Averaging. *Mol. Biol. Evol.* **2008**, *25*, 1253–1256. [CrossRef]
29. Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sánchez-Gracia, A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. [CrossRef] [PubMed]

30. Puillandre, N.; Lambert, A.; Brouillet, S.; Achaz, G. ABGD, Automatic Barcode Gap Discovery for Primary Species Delimitation. *Mol. Ecol.* **2012**, *21*, 1864–1877. [[CrossRef](#)]
31. Puillandre, N.; Modica, M.V.; Zhang, Y.; Sirovich, L.; BOISSELIER, M.-C.; Cruaud, C.; Holford, M.; Samadi, S. Large-Scale Species Delimitation Method for Hyperdiverse Groups. *Mol. Ecol.* **2012**, *21*, 2671–2691. [[CrossRef](#)]
32. Meier, R.; Shiyang, K.; Vaidya, G.; Ng, P.K.L. DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success. *Syst. Biol.* **2006**, *55*, 715–728. [[CrossRef](#)]
33. Zhang, J.; Kapli, P.; Pavlidis, P.; Stamatakis, A. A General Species Delimitation Method with Applications to Phylogenetic Placements. *Bioinformatics* **2013**, *29*, 2869–2876. [[CrossRef](#)]
34. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)]
35. Silvestro, D.; Michalak, I. RaxmlGUI: A Graphical Front-End for RAXML. *Org. Divers. Evol.* **2012**, *12*, 335–337. [[CrossRef](#)]
36. Stamatakis, A. RAXML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [[CrossRef](#)] [[PubMed](#)]
37. Šarić, T.; Župan, I.; Aceto, S.; Villari, G.; Palić, D.; De Vico, G.; Carella, F. Epidemiology of noble pen shell (*Pinna nobilis* l. 1758) mass mortality events in Adriatic Sea is characterised with rapid spreading and acute disease progression. *Pathogens* **2020**, *9*, 776. [[CrossRef](#)] [[PubMed](#)]
38. Furfaro, G.; Salvi, D.; Mancini, E.; Mariottini, P. A Multilocus View on Mediterranean Aeolid Nudibranchs (Mollusca): Systematics and Cryptic Diversity of Flabellinidae and Piseinotecidae. *Mol. Phylogenet. Evol.* **2018**, *118*, 13–22. [[CrossRef](#)] [[PubMed](#)]
39. Furfaro, G.; Salvi, D.; Trainito, E.; Vitale, F.; Mariottini, P. When Morphology Does Not Match Phylogeny: The Puzzling Case of Two Sibling Nudibranchs (Gastropoda). *Zool. Scr.* **2021**, *50*, 439–454. [[CrossRef](#)]
40. Bergh, L.S.R. Malacologische Untersuchungen. In *Reisen im Archipel der Philippinen*; Semper, C. (1868). II Theil. Wissenschaftliche Resultate, 1. Available online: <https://curiosity.lib.harvard.edu/expeditions-and-discoveries/catalog/38-990041866000203941> (accessed on 25 December 2022).
41. Pruvot-Fol, A. Mollusques Opisthobranches. *Faune De Fr. Paris* **1954**, *58*, 1–460.
42. Perrone, A.S. Opistobranchi (Aplysiomorpha, Pleurobrancoomorpha, Sacoglossa Nudibranchia) del litorale Salentino (Mare Jonio) (Elenco-contrib. secondo). *Thalass. Salentina* **1986**, *16*, 19–42.
43. Ballesteros, M.; Madrenas, E.; Pontes, M. “Knoutsodonta albonigra” a OPK-Opistobranquis. 2022. Available online: <https://opistobranquis.info/ca/1HCSO> (accessed on 25 November 2022).
44. Betti, F.; Bava, S.; Cattaneo-Vietti, R. Composition and seasonality of a heterobranch assemblage in a sublittoral, unconsolidated, wave-disturbed community in the Mediterranean Sea. *J. Molluscan. Stud.* **2017**, *83*, 325–332. [[CrossRef](#)]
45. Ballesteros, M.; Madrenas, E.; Pontes, M. “Knoutsodonta pusilla” in OPK-Opistobranquis, 2022. Available online: <https://opistobranquis.info/ca/JuGvP> (accessed on 25 November 2022).
46. Alder, J.; Hancock, A. Notice of a new genus and several new species of nudibranchiate Mollusca. *Ann. Mag. Nat. Hist.* **1845**, *16*, 311–316. [[CrossRef](#)]
47. Alvim, J.; Padula, V.; Pimenta, A.D. First record of the genus *Onchidoris* (Gastropoda: Nudibranchia: Onchidorididae) from the South Atlantic Ocean, with the description of a new species from Brazil. *J. Mar. Biol. Assoc. UK* **2011**, *91*, 505–511. [[CrossRef](#)]
48. Verrill, A.E. Additions to the fauna of the Bermudas from the Yale Expedition of 1901, with notes on other species. *Trans. Conn. Acad. Arts Sci.* **1901**, *11*, 15–62.
49. Martynov, A.V. *Marine and Brackish Water Gastropoda of Russia and Adjacent Countries: An Illustrated Catalogue*; Kantor, Y.I., Sysoev, A.V., Eds.; KMK Scientific Press Ltd.: Moscow, Russia, 2006; 372p.
50. Abraham, P.S. Revision of the anthobranchiate nudibranchiate Mollusca, with descriptions or notices of forty-one hitherto undescribed species. *Proc. Zool. Soc. Lond. Year* **1877**, *196*, 27–30.
51. Gould, A.A. *Report on the Invertebrata of Massachusetts*, 2nd ed.; Published Agreeably to an Order of the Legislature; Wright and Potter: Cambridge, UK, 1870; pp. 225–253, pls. XVI–XXII.
52. McDonald, G. Bibliographia Nudibranchia. Third online edition, 2021. Available online: [eScholarship.org](https://eScholarship.org) (accessed on 25 December 2022).
53. Balch, F.N. Rectification of the nomenclature of the Nudibranchiata in Lermond’s “Shells of Maine”. *Nautica* **1910**, *23*, 100–102.
54. Trott, T.J. Cobscook Bay inventory: A historical checklist of marine invertebrates spanning 162 years. *Northeast. Nat.* **2004**, *11*, 261–324. [[CrossRef](#)]
55. Prkić, J.; Furfaro, G.; Mariottini, P.; Carmona, L.; Cervera, J.L.; Modica, M.V.; Oliverio, M. First record of *Calma gobioophaga* Calado and Urgorri, 2002 (Gastropoda: Nudibranchia) in the Mediterranean Sea. *Mediterr. Mar. Sci.* **2014**, *15*, 423–428. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.