



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

GIULIA FURFARO^{1,2*} & PAOLO MARIOTTINI¹

¹Department of Science, University of “Roma Tre”, Viale G. Marconi 446, I-00146 Rome, Italy.

²Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, Via Prov.le Lecce - Monteroni, 73100 Lecce, Italy.

*Corresponding author: giulia.furfaro@unisalento.it phone: +39.0657336359, orcid.org/0000-0001-8184-2266

Abstract

The diversity of Mediterranean nudibranchs has yet to be thoroughly studied: new species are constantly described, and molecular approaches have revealed some cryptic species. A new facelinid species has been discovered based on specimens collected from the Tyrrhenian Sea (Mediterranean Sea). Integrative results of molecular analyses and of anatomical investigations support the description of *Dondice trainitoi* **sp. nov.** The characteristic chromatic body pattern and the black epithelium covering the masticatory jaws allow an unambiguous identification of the new taxon. Preliminary phylogenetic analyses based on multi-locus molecular markers (nuclear H3 gene and mitochondrial markers 16S rDNA and COI) surprisingly revealed paraphyly of the genus *Dondice* and the need of further studies including more taxa assigned to the currently accepted family Facelinidae. Furthermore, following an integrative taxonomy approach, considerations on the ecological behaviour characterizing most of the species involved in this study provide useful insights for understanding the evolutionary history of this facelinid group.

Key words: Integrative Taxonomy, nudibranch, Facelinidae, Heterobranchia, Tyrrhenian Sea

Introduction

Nudibranch diversity has fascinated amateurs and scientists for decades and is still far from being fully understood. New elusive and rare species have been revealed by recent studies (Evertsen & Bakken 2013; Colucci *et al.* 2015; Trainito *et al.* 2017) and cryptic species have been discovered, challenging traditional morphological species determination (Churchill *et al.* 2014; Furfaro *et al.* 2016a, 2018b; Korshunova *et al.* 2017). Several studies investigating nudibranchs with molecular approaches (DNA barcoding, species delimitation analyses, phylogenetic inference) helped in detecting such hidden diversity, all over the world.

In 2014, during a scientific SCUBA dive on a wreck in the central Tyrrhenian Sea (Mediterranean Sea), two specimens were found which could not be assigned to any known Mediterranean heterobranch species. Their morphology recalled the facelinid *Dondice banyulensis* Portmann & Sandmeier, 1960, with some important differences. Within Nudibranchia, the Facelinidae Bergh, 1889 is one of the most speciose families, showing a great diversity with 34 currently accepted genera (Facelinidae, MolluscaBase 2018a). Focusing on different eolid families, recent molecular works questioned the monophyly of Facelinidae (Gosliner *et al.* 2007; Carmona *et al.* 2015), but phylogenetic relationships among the members of the family are still unresolved.

The genus *Dondice* Marcus, 1958 belongs to the Facelinidae family and was originally described as “*a facalinae with simple jaws, unarmed penis, and produced foot corners*” (Marcus Er. 1958) on the basis of Brazilian specimens of *Caloria occidentalis* (Engels, 1925), which Marcus established as type species of the new genus. Currently, the genus *Dondice* includes four species (Gofas 2004): *D. banyulensis*, *D. galaxiana* Millen & Hermosillo, 2012, *D. occidentalis* Engel, 1925 and *D. parguerensis* Brandon & Cutress, 1985. *Dondice banyulensis* is the only species of *Dondice* occurring in the Mediterranean Sea, while the other taxa show an Eastern Pacific and a Western

Atlantic Ocean distribution. Millen & Hermosillo provisionally placed *D. galaxiana* in the genus *Dondice*, because of some peculiarities shared with *D. occidentalis* (i.e. cerata in arches with multiple rows, a separate and unstalked penial gland and the unarmed penis). Neither further systematic assessment, nor molecular data were produced for this species whose taxonomical position must still be considered uncertain. *Dondice occidentalis*, the type species of the genus, was described by Engel (1925) from specimens living in Montego Bay, Jamaica. To date, its geographical distribution includes Brazil (Sao Paulo), North Carolina, USA, many spots of the Caribbean Sea (seaslug forum: <http://www.seaslugforum.net/find/dondocci>) and Canary islands (OPK: <https://opistobranquis.info/en/guia/nudibranchia/cladobranchia/aeolidioidea/dondice-occidentalis/>). *Dondice parguerensis* is another questioned species: morphologically similar to *D. occidentalis*, it differs from it by coloration, size, number of ceratal groups, larval development and primarily by different preys [*Cassiopea* Péron & Lesueur, 1810 (Cnidaria, Scyphozoa) for *D. parguerensis* and hydroids (Cnidaria) for *D. occidentalis*] (Brandon & Cutress 1985; Gonzalez *et al.* 2013). Anyway, these two *Dondice* species were molecularly analysed by Gonzalez *et al.* (2013) revealing a very low genetic divergence at the 16S mitochondrial molecular marker and no differences at all at the nuclear H3 marker. This unexpected result raised doubts about the validity of the specimens included in the aforementioned work and/or about the validity of the morphological features used as diagnostic for the species and this controversy has yet to be resolved.

In this study we tested the identification of the hypothetical Mediterranean new species, based on external morphology, with a molecular DNA barcoding approach. Furthermore, to investigate the monophyly of the genus *Dondice*, we include members belonging to other genera historically considered close to *Dondice* genus or, accordingly to previous studies, molecularly related to it. In particular, we included in the definitive dataset members of the genera *Babakina* Roller, 1973, *Cratena* Bergh, 1864, *Dicata* Schmekel, 1967, *Facelina* Alder & Hancock, 1855, *Favorinus* Gray, 1850, *Godiva* Macnae, 1954 and *Phyllodesmium* Ehrenberg, 1831. We explored the systematics of the genus *Dondice* and all the possibly evolutionary related genera, following an Integrative Taxonomic method, by comparing results obtained by different molecular and morphological analyses. In particular, by using anatomical data and a multilocus molecular approach with the nuclear H3 gene and the two mitochondrial markers 16S rRNA and COI, we here: i) describe the new taxon *Dondice trainitoi* **sp. nov.**, from the Central Mediterranean Sea; ii) unravel the diversity of the Mediterranean species; iii) explore the phylogenetic relationships among the genus *Dondice* and other related genera.

Materials and Methods

Individuals, egg spawns and the hydroids on which they were feeding were documented *in situ* with high definition photographs. Each collected specimen was photographed, measured, preserved in 95% ethanol (EtOH) and deposited as a voucher at the Department of Science of the Roma Tre University. The holotype MNHN IM-2000-33722 and the paratypes MNHN IM-2000-33723 and MNHN IM-2000-33724 were deposited at the Muséum national d'histoire naturelle (MNHN, Paris, France); other paratypes (RM3_1101, RM3_1102 and RM3_621) and additional specimens (RM3_1034-RM3_1036, RM3_1153, RM3_1536, RM3_1537, RM3_1540 and RM3_1541) are stored at Department of Science, University of Roma Tre, Italy.

Molecular analyses

DNA was extracted from ten individuals belonging to the family Facelinidae (Table 1) from a small piece of tissue, by using the 'salting out' procedure as described in Aljanabi & Martinez (1997). The analysed dataset consisted of a total of 134 sequences, of which 22 newly produced and the remaining retrieved from GenBank, from 47 individuals belonging to 32 different species, including the out group (Table 1). Amplifications were performed by PCR using universal primers: 16Sar-L and 16Sbr-H (Palumbi *et al.* 2001) for the 16S rRNA fragment, LCO1490 and HCO2198 (Folmer *et al.* 1994) for the barcode fragment of the Cytochrome Oxidase subunit I (COI), and H3AD-F and H3BD-R (Colgan *et al.* 1998) for the nuclear Histone 3 (H3) marker. PCR conditions were the same for the three molecular markers and included: 5 min of initial DNA denaturation step at 94°C; 35 cycles of 94°C/30 s (DNA denaturation step), 46–50°C/60 s (annealing step), 72°C/60 s (elongation step); and 7 min of final extension at 72°C (Furfaro *et al.* 2016b). All amplicons were sequenced at the European Division of Macrogen Inc. (Amsterdam, The Netherlands). Sequences from each DNA strain were assembled and edited with Staden Package 2.0.0b9 (Staden *et al.*

TABLE 1. Species name, collection localities, Voucher ID, sequence accession numbers of the specimens analysed and references.

SPECIES	LOCALITY	VOUCHER	H3	16S	COI	REFERENCES
<i>Aeolidiella alderi</i> (Cocks, 1852)	Italy	ZSM Mol20012341	HQ616795	HQ616766	HQ616729	Carmona <i>et al.</i> 2011
<i>Aeolidiella sanguinea</i> (Norman, 1877)	France (Atlantic Ocean)	MNCN/ADN51932	JX087600	JX087538	JX087466	Carmona <i>et al.</i> 2013
<i>Babakina anadoni</i> (Ortea, 1979)	Brazil	MNRJ10893	HQ616775	HQ616709	HQ616746	Carmona <i>et al.</i> 2011
<i>Babakina anadoni</i> (Ortea, 1979)	Galicia, Spain (Atlantic Ocean)	MNCN15.05/46704	HQ616796	HQ616730	HQ616767	Carmona <i>et al.</i> 2011
<i>Babakina indopacifica</i> Gosliner, Gonzalez-Duarte & Cervera, 2007	Luzon, Batangas, Philippines	CASIZ177458	HM162587	HM162678	HM162754	Pola & Gosliner 2010
<i>Cratena peregrina</i> (Gmelin, 1791)	Sabaudia, Latium, Italy	RM3_319	LS483293	LS483282	LS483272	Present study
<i>Dicata odhneri</i> Schmekel, 1967	Ballanera, Algeiras, Spain	BAU2674	LT596569	LT596549	LT596560	Furfaro <i>et al.</i> 2016a
<i>Dicata odhneri</i> Schmekel, 1967	Andalusia, Spain (Mediterranean Sea)	MNCN15.05/53692	-----	HQ616739	HQ616773	Carmona <i>et al.</i> 2011
<i>Dondice banyulensis</i> Portmann & Sandmeier, 1960	Djerba, Tunisia	RM3_129	LS483284	LS483274	LS483267	Present study
<i>Dondice banyulensis</i> Portmann & Sandmeier, 1960	Argentario, Tuscany, Italy	RM3_356	LS483285	LS483275	LS483268	Present study
<i>Dondice banyulensis</i> Portmann & Sandmeier, 1960	Sant'Agostino, Latium, Italy	RM3_290	LS483286	LS483276	LS483269	Present study
<i>Dondice banyulensis</i> Portmann & Sandmeier, 1960	-----	Db_60	-----	GQ403751	GQ403773	Wagele <i>et al.</i> 2010
<i>Dondice occidentalis</i> (Engel, 1925)	Exuma, Bahamas	LACM177715	KC526529	KC526510	-----	Gonzalez <i>et al.</i> 2013
<i>Dondice occidentalis</i> (Engel, 1925)	-----	LACM2003-41.5	JQ699394	JQ699482	JQ699570	Churchill C.K.C. <i>et al.</i> 2013
<i>Dondice occidentalis</i> (Engel, 1925)	Exuma, Bahamas	D252	KC526527	KC526518	-----	Gonzalez <i>et al.</i> 2013
<i>Dondice occidentalis</i> (Engel, 1925)	Jamaica	JG61	KC526534	KC526512	-----	Gonzalez <i>et al.</i> 2013

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TABLE 1. (Continued)

SPECIES	LOCALITY	VOUCHER	H3	16S	COI	REFERENCES
<i>Dondice parguerensis</i> Brandon & Cutress, 1985	La Parguera, Puerto Rico	LACM177705	KC526535	KC526520	-----	Gonzalez <i>et al.</i> 2013
<i>Dondice trainitoi</i> sp.nov.	Civitavecchia, Latium, Italy	RM3_425	LS483287	LS483277	LS483270	Present study
<i>Dondice trainitoi</i> sp.nov.	Civitavecchia, Latium, Italy	RM3_596	LS483288	LS483278	LS483271	Present study
<i>Facelina annulicornis</i> (Chamisso & Eysenhardt, 1821)	Azores Is., Portugal	CASIZ186793	JQ996986	JQ996881	JQ997076	Carmona <i>et al.</i> 2013
<i>Facelina bostoniensis</i> (Couthouy, 1838)	New Hampshire	CAS184184	KY128632	KY128837	KY129046	Cella <i>et al.</i> 2016
<i>Facelina rubrovittata</i> (Costa A., 1866)	Scoglio del Corallo, Tuscany, Italy	RM3_422	LS483294	LS483283	LS483273	Present study
<i>Favorinus branchialis</i> (Rathke, 1806)	Sabaudia, Latium, Italy	BAU2676	LS483292	LT596551	LT596562	Present study; Furfaro <i>et al.</i> 2016a
<i>Favorinus branchialis</i> (Rathke, 1806)	Spain (Atlantic Ocean)	MNCN15.05/53695	HQ616790	HQ616724	HQ616761	Carmona <i>et al.</i> 2011
<i>Flabellina affinis</i> (Gmelin, 1791)	Giannutri Is., Tuscany, Italy	BAU2804	LT718579	LT718527	LT718553	Furfaro <i>et al.</i> 2018b
<i>Flabellina affinis</i> (Gmelin, 1791)	Giannutri Is., Tuscany, Italy	BAU2805	LT718580	LT718528	LT718554	Furfaro <i>et al.</i> 2018b
<i>Godiva quadricolor</i> (Barnard, 1927)	Sabaudia, Latium, Italy	RM3_117	LS483289	LS483279	MG546001	Present study; Furfaro <i>et al.</i> 2018a
<i>Godiva quadricolor</i> (Barnard, 1927)	Sabaudia, Latium, Italy	RM3_153	LS483290	LS483280	MG546002	Present study; Furfaro <i>et al.</i> 2018a
<i>Godiva quadricolor</i> (Barnard, 1927)	Sabaudia, Latium, Italy	RM3_154	LS483291	LS483281	MG546003	Present study; Furfaro <i>et al.</i> 2018a
<i>Godiva quadricolor</i> (Barnard, 1927)	Knysna Lagoon, South Africa	CASIZ176385	HM162589	HM162680	HM162756	Pola & Gosliner 2010
<i>Phyllodesmium briareum</i> (Bergh, 1896)	Batangas, Philippines	CASIZ 177239	HQ010460	HQ010528	HQ010492	Moore & Gosliner 2011
<i>Phyllodesmium colemani</i> Rudman, 1991	Batangas, Philippines	CASIZ 177647	HQ010466	HQ010534	HQ010498	Moore & Gosliner 2011
<i>Phyllodesmium crypticum</i> Rudman, 1981	Batangas, Philippines	CASIZ 180381	HQ010477	HQ010543	HQ010507	Moore & Gosliner 2011
<i>Phyllodesmium horridum</i> (Macnae, 1954)	Cape Region, South Africa	CASIZ176127	HM162590	HM162681	HM162757	Pola & Gosliner 2010

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TABLE 1. (Continued)

SPECIES	LOCALITY	VOUCHER	H3	16S	COI	REFERENCES
<i>Phyllodesmium hyalinum</i> Ehrenberg, 1831		Phy.orig.	-----	GQ403756	GQ403778	Wagele <i>et al.</i> 2010
<i>Phyllodesmium jakobsenae</i> Burghardt & Wägele, 2004	Batangas, Philippines	CASIZ 177576	HQ010456	HQ010524	HQ010489	Moore & Gosliner 2011
<i>Phyllodesmium karenae</i> Moore & Gosliner, 2009	Batangas, Philippines	CASIZ 180384	HQ010478	HQ010544	HQ010508	Moore & Gosliner 2011
<i>Phyllodesmium koehleri</i> Burghardt, Schrödl & Wägele, 2008	Batangas, Philippines	CASIZ 177693	HQ010462	HQ010530	HQ010494	Moore & Gosliner 2011
<i>Phyllodesmium lizardensis</i> Burghardt, Schrödl & Wägele, 2008	Batangas, Philippines	CASIZ 180382	HQ010474	HQ010540	HQ010505	Moore & Gosliner 2011
<i>Phyllodesmium macphersonae</i> (Burn, 1962)	Batangas, Philippines	CASIZ 177493	HQ010453	HQ010522	HQ010487	Moore & Gosliner 2011
<i>Phyllodesmium opalescens</i> Rudman, 1991	Batangas, Philippines	CASIZ 177541	HQ010450	HQ010519	HQ010485	Moore & Gosliner 2011
<i>Phyllodesmium parangatum</i> Ortiz & Gosliner, 2003	Batangas, Philippines	CASIZ 180383B	HQ010476	HQ010542	HQ010506	Moore & Gosliner 2011
<i>Phyllodesmium poindimiei</i> (Risbec, 1928)	Batangas, Philippines	CASIZ 177783	HQ010463	HQ010531	HQ010495	Moore & Gosliner 2011
<i>Phyllodesmium rudmani</i> Burghardt & Gosliner, 2006	Batangas, Philippines	CASIZ 177622	HQ010461	HQ010529	HQ010493	Moore & Gosliner 2011
<i>Phyllodesmium tuberculatum</i> Moore & Gosliner, 2009	Batangas, Philippines	CASIZ 177663	HQ010465	HQ010533	HQ010497	Moore & Gosliner 2011
<i>Tritonia striata</i> Haefelfinger, 1963	Giannutri Is., Tuscany, Italy	BAU2695	LT615407	LT596542	LT596540	Furfaro <i>et al.</i> 2016a
<i>Tritonia striata</i> Haefelfinger, 1963	Le Formiche Is., Grosseto, Italy	BAU2696	LT615408	LT596543	LT596541	Furfaro <i>et al.</i> 2016a

2000). BLASTN (Altschul *et al.* 1990) search was conducted in the GenBank database to confirm the identity of the sequenced fragment and to exclude contamination. Consensus sequences of each individual were aligned together with GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>) sequences using the Muscle algorithm implemented in MEGA 6.0 (Tamura *et al.* 2013). The number of COI base differences per site from averaging over all sequence pairs between groups were calculated. Mean *p*-distances between species were calculated on the COI dataset (including the outgroup) with the use of the program MEGA 6.0. The Automatic Barcode Gap Discovery (ABGD, available at <http://wwwabi.snv.jussieu.fr/public/abgd/>) was carried out to detect the so-called “barcode gap” in the distribution of pairwise distances calculated in a sequence alignment (Puillandre *et al.* 2012a, 2012b). The ABGD analysis was performed on the COI ingroup dataset using the Kimura two-parameter (K2p) model and the following settings: a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 30 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 0.1. The analysis involved 43 COI sequences (588 bp, base pairs of nucleotides), 47 16S sequences (413 bp) and 44 sequences (294 bp) of the nuclear

H3, in the final dataset. We used Gblocks 0.91b (Castresana 2000; Talavera & Castresana 2007) to eliminate poorly aligned positions or hyper-divergent regions of the multiple sequence alignment of the 16S rDNA (resulting 16S dataset of 363bp). We used less stringent options such as ‘allow gap positions (with half) within the final blocks’. For each gene alignment, the best evolutionary model was selected in JModel Test 0.1 (Posada 2008) according to the Bayesian Information Criterion (BIC). Single genes and concatenated datasets (H3, 16S and COI markers) were analysed using Bayesian Inference (BI) and Maximum likelihood (ML) methods implementing the models selected by JModel Test for each gene. BI analyses were carried out with MrBayes 3.2.6 (Ronquist *et al.* 2012) with four Markov-chains of five million generations each, sampled every 1000 generations. Consensus trees were calculated on trees sampled after a burnin of 25%. ML searches were performed using GARLI 0.96 (Zwickl 2006) with a starting tree topology generated by the ML stepwise-addition algorithm. Nodal support was assessed by means of 1000 bootstrap replicates. Nodes in the resulting phylogenetic trees with Bayesian posterior probabilities (PP) $\geq 0.96\%$ and bootstrap values (BS) $\geq 90\%$ were considered ‘highly’ supported, nodes with PP of 0.90-0.95% and BS of 80-89% were considered ‘moderately’ supported (lower support values were considered not significant). We carried out molecular analyses described above, on different datasets (not shown) in order to optimize the definitive dataset by including only taxa with a significant phylogenetic signal within this heterogeneous family group. The definitive in-group consisted of Facelinidae species belonging to the genera *Babakina*, *Cratena*, *Dicata*, *Facelina*, *Favorinus*, *Godiva*, *Phyllodesmium* and members of the families Aeolidiidae Gray, 1827 and Flabellinidae Bergh, 1889. *Tritonia striata* Haefelfinger, 1963 was used as the out-group for the molecular analyses, because of the basal placement within Cladobranchia showed by the genus *Tritonia* Cuvier, 1798 as proposed by Pola & Gosliner (2010).

Morphological analyses

Anatomical observations were conducted on the buccal apparatus and on the reproductive system to assess the status of these commonly used diagnostic morphological characters. The buccal apparatus of *D. trainitoi* **sp. nov.** specimens (n=3) was analyzed by using both optical and Scanning Electronic Microscopies (SEM). Buccal masses were removed and dissolved in a 10% NaOH solution for radulae and jaws extraction. Radulae and jaws were rinsed in water, dried, and mounted for examination by optical microscopy as reported in Furfaro *et al.* (2016b). To obtain high resolution SEM images, dissected radulae were dehydrated in 100% EtOH through a graded series (50-70-90-95-100% EtOH), critical point-dried in a Balzer Union CPD 030 unit, gold coated in an Emitech K550 unit, and finally examined by using the field emission SEM column of the Dualbeam (FIB/SEM) Helios Nanolab (FEI Company, Eindhoven, The Netherlands) at the LIME (Electron Microscopy Interdepartmental Laboratory, University Roma Tre), with secondary electrons and an operating voltage of 5 kV. The reproductive system (from two individuals each, three in the case of the new species) of *D. trainitoi* **sp. nov.**, *D. banyulensis* and *Godiva quadricolor* (Barnard, 1927) respectively were observed and studied under a dissecting microscope.

Results

The first records of the new taxon *D. trainitoi* **sp. nov.** occurred on the June 11th 2014, during a scuba dive at 30-40 m depth on the “Asia” wreck (1 mile off Riva di Traiano Harbour, Civitavecchia, Central Tyrrhenian Sea, Mediterranean, 42°03’15”N, 11°47’45”E). Two years later, two new findings took place in the same locality and seasonal period (June 4th and August 28th 2016). During the years 2017 and 2018, several individuals were observed grazing on hydroids attached to the same wreck. New records occurred at “Liburna” another wreck located about 100 m far from the “Asia” (42°01’47”N, 11°48’31”E). This last finding occurred on hydroids at the same bathymetric range. Picture of one individual belonging to this new species was taken from Portofino promontory in Punta del Faro (44°17’53”N, 9°13’47”E) (Liguria, Italy) on the May 30th 2018. On the August 13th of the same year, two individuals (RM3_1540 and RM3_1541) have been found in Procida Island near Naples (40°45’02.6”N, 14°01’31.6”E) (Campania, Italy). Molecular and morphological analyses were performed on the new species (Fig. 1) and on specimens from other related taxa. The final dataset, comprising all the specimens involved in this study, is reported in Table 1.

Molecular analyses

The ABGD analysis highlighted a barcoding gap between 1% and 5% of genetic distances at the COI mitochondrial marker in the in-group (Fig. 2). Genetic *p*-distances (of the COI dataset) among species analysed, excluding the out-group, were also calculated and results obtained are shown in Table 2. The resulting concatenated alignment

TABLE 2. Mean COI *p*-distances between species included in molecular analyses.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31						
1 - <i>D. banyutensis</i>	-																																				
2 - <i>D. traintoi</i>	18%	-																																			
sp. nov.																																					
3 - <i>D. occidentalis</i>	19%	15%	-																																		
4 - <i>G. quadricolor</i>	18%	20%	18%	-																																	
5 - <i>P. karenae</i>	19%	19%	19%	20%	-																																
6 - <i>P. colemani</i>	21%	19%	20%	18%	20%	-																															
7 - <i>P. jakobsenae</i>	19%	19%	20%	18%	20%	21%	-																														
8 - <i>P. opatzensis</i>	19%	20%	21%	18%	20%	22%	20%	-																													
9 - <i>P. hyalinum</i>	20%	20%	19%	17%	19%	21%	8%	21%	-																												
10 - <i>P. horridum</i>	17%	18%	16%	18%	18%	18%	19%	17%	20%	-																											
11 - <i>P. bitareum</i>	17%	18%	19%	18%	21%	17%	18%	21%	18%	18%	-																										
12 - <i>P. crypticum</i>	18%	19%	21%	19%	19%	18%	17%	18%	15%	19%	19%	-																									
13 - <i>P. tuberculatum</i>	18%	19%	19%	16%	19%	18%	14%	19%	14%	17%	19%	14%	-																								
14 - <i>P. parangatum</i>	21%	22%	20%	19%	19%	20%	19%	19%	21%	20%	22%	17%	18%	-																							
15 - <i>P. koehleri</i>	19%	21%	18%	18%	20%	18%	15%	20%	15%	18%	19%	16%	14%	20%	-																						
16 - <i>P. lizardensis</i>	20%	20%	19%	19%	21%	19%	14%	21%	16%	20%	20%	17%	13%	18%	12%	-																					
17 - <i>P. pointimiei</i>	18%	17%	16%	18%	17%	19%	20%	17%	20%	16%	20%	22%	19%	20%	20%	22%	-																				
18 - <i>P. maiphersonae</i>	21%	20%	17%	17%	19%	18%	19%	18%	20%	17%	20%	22%	18%	21%	21%	21%	14%	-																			
19 - <i>P. rudmani</i>	20%	19%	20%	19%	18%	17%	13%	18%	15%	17%	18%	15%	13%	19%	13%	14%	19%	21%	-																		
20 - <i>D. odhneri</i>	19%	17%	19%	16%	21%	20%	19%	20%	18%	17%	20%	18%	17%	21%	20%	18%	20%	20%	18%	-																	
21 - <i>F. branchialis</i>	20%	19%	19%	20%	22%	20%	21%	23%	22%	20%	22%	22%	20%	21%	20%	20%	19%	21%	20%	-																	
22 - <i>F. bostoniensis</i>	19%	21%	19%	20%	21%	21%	21%	21%	19%	16%	21%	22%	20%	23%	17%	18%	19%	20%	19%	19%	-																
23 - <i>F. annulicornis</i>	21%	21%	21%	20%	21%	22%	18%	21%	20%	19%	20%	21%	20%	22%	21%	23%	22%	20%	21%	22%	20%	-															
24 - <i>F. rubrovittata</i>	17%	17%	19%	19%	18%	20%	18%	21%	17%	17%	16%	18%	18%	23%	18%	20%	18%	18%	18%	19%	19%	15%	17%	-													
25 - <i>C. peregrina</i>	18%	20%	20%	22%	20%	22%	24%	21%	25%	20%	21%	22%	22%	25%	22%	23%	20%	22%	23%	22%	18%	20%	21%	16%	-												
26 - <i>B. anadoni</i>	20%	21%	19%	19%	20%	20%	18%	19%	20%	19%	20%	21%	20%	22%	20%	20%	20%	20%	20%	18%	20%	20%	22%	19%	20%	-											
27 - <i>B. indopacifica</i>	21%	22%	20%	19%	21%	18%	21%	21%	20%	17%	21%	20%	20%	20%	21%	20%	20%	20%	19%	21%	19%	21%	20%	20%	20%	16%	-										
28 - <i>A. sanguinea</i>	20%	18%	18%	19%	18%	19%	19%	20%	20%	18%	19%	20%	19%	19%	21%	20%	19%	20%	19%	18%	20%	20%	19%	19%	17%	18%	18%	-									
29 - <i>A. alderi</i>	20%	20%	19%	19%	20%	20%	22%	20%	20%	17%	20%	21%	19%	20%	19%	21%	20%	19%	20%	17%	20%	20%	19%	20%	19%	19%	17%	16%	14%	-							
30 - <i>F. affinis</i>	19%	20%	20%	20%	21%	19%	21%	22%	19%	19%	20%	22%	21%	23%	22%	23%	21%	19%	21%	19%	20%	20%	21%	20%	20%	19%	17%	16%	14%	-							
31 - <i>T. striata</i>	21%	22%	20%	22%	20%	23%	20%	24%	19%	21%	22%	20%	22%	21%	23%	22%	23%	22%	20%	20%	21%	22%	21%	21%	20%	24%	22%	19%	22%	23%	22%	23%	24%	-			

consisted of 1245 different positions with TIM2ef+G, TPM3uf+I+G and TrN+I+G as the best model of evolution for H3, 16S and COI respectively. The concatenated and partitioned Bayesian inference and Maximum Likelihood analyses were congruent to each other and showed higher statistical support values than single genes analyses. The resulting topology (Fig. 2) highlighted a well-supported monophyletic clade (BI=1; ML=99) grouping all species belonging to *Phyllodesmium* Ehrenberg, 1831. This monophyletic group is clustered (BI=0.98; ML=69) with a grade composed by *G. quadricolor*, the type species of *Godiva*, as sister (BI=0.79; ML=ns) to a clade with three *Dondice* species: *D. occidentalis*, the type species of the genus *Dondice*, showing no genetic differences to *D. parguerensis* (BI=1; ML=98), and *D. trainitoi* **sp. nov.** which is a well-supported clade (BI=1; ML=100). *Dondice banyulensis* is sister, with high statistical support (BI=1; ML=100), to the clade including *Phyllodesmium*, *Godiva* and other *Dondice* species, revealing the genus *Dondice* as non-monophyletic. The latter highly supported monophyletic big group clustered with another clade (BI=0.93; ML=52) composed by *Babakina anadoni* (Ortea, 1979) and *B. indopacifica* Gosliner, Gonzalez-Duarte & Cervera, 2007 (BI=0.99; ML=70) sister to *Dicata odhneri* Schmekel, 1967 (BI=0.59; ML=<50), and altogether sister to two *Aeolidiella* species (BI=0.94; ML=<50). *Flabellina affinis* (Gmelin, 1791) is sister to all the species mentioned above (BI=0.97; ML=60). The big clade which includes members of Aeoliidae, Facelinidae and Flabellinidae families is grouped (BI=0.97; ML=<50) with a clade (BI=1; ML=90) composed by four other Facelinidae species. More in detail this clade is composed by *Facelina bostoniensis* (Couthouy, 1838) and *F. rubrovittata* (Costa A., 1866) (BI=1; ML=95), sister to *Cratena peregrina* (Gmelin, 1791) (BI=0.67; ML=<50) and with *F. annulicornis* (Chamisso & Eysenhardt, 1821) as the sister to all of them. *Favorinus branchialis* (Rathke, 1806) showed a basal placement (BI=1; ML=100) resulting as the sister to all other eolids reported above.

Morphological analyses

Results from optical and SEM microscopical examinations of the masticatory system of *D. trainitoi* **sp. nov.** are shown in figures 3 and 4. Pictures and drawings of the reproductive systems are depicted in figure 5.

TAXONOMY

Family FACELINIDAE Bergh, 1890

Genus *Dondice* Marcus, Er. 1958

***Dondice trainitoi* sp. nov. (Figures 1 A - F)**

Zoobank: urn:lsid:zoobank.org:act:5072B304-A2F2-4AB0-A0EF-6DF26EC0242B

Holotype: MNHN IM-2000-33722, 10 mm in length, Riva di Traiano, Civitavecchia, Latium, Italy, Mediterranean Sea, August 03 2017, 28 m depth, 42°03'15"N, 11°47'45"E.

Paratypes: paratype MNHN IM-2000-33723, 5 mm in length, Riva di Traiano, Civitavecchia, Latium, Italy, Mediterranean Sea, June 11 2016, 30 m depth; paratype MNHN IM-2000-33724, 5 mm long, Riva di Traiano, Civitavecchia, Latium, Italy, Mediterranean, June 04 2016, 32 m depth; paratype RM3_1101, 8 mm in length; paratype RM3_1102, 11 mm in length, Riva di Traiano, Civitavecchia, Latium, Italy, Mediterranean Sea, August 03 2017, 30 m depth; paratype RM3_621, 16 mm in length, Riva di Traiano, Civitavecchia, Latium, Italy, Mediterranean Sea, August 25 2016, 30 m depth. All type material sampled at the "Asia" wreck on hydroids.

Etymology: The species name is after Egidio Trainito, expert in nudibranch biology and Mediterranean marine biodiversity, underwater photographer and good friend of the authors.

Holotype morphological description: The body is slender, with a narrow foot. The anterior part of the foot is bilabiate and extended into well-defined propodial tentacles. The elongate body is translucent cream, with bright white spots along the border of the foot, oral foot corners and tail. A characteristic iridescent light blue band is present on the tail, along the dorsum and the head where it bifurcates into two lines ending at the half part of the oral tentacles. Two other lateral lines of the same iridescent color are present dorsally, starting from the lateral part of the head below the rhinophores, becoming narrower and lighter through the body and terminating in the tail joining the dorsal band. A typical black pigment covering the mandibles is clearly visible through the epithelium. The cerata

are translucent cream with a digestive gland that is yellowish in the basal portion but becoming gradually orange in the sub terminal part. The upper part of the cerata has a brightly yellowish ring larger in the anterior part than in the back where it is tapered. At the base of the rhinophores, the head is diaphanous, allowing the dark eyes to be seen (Figs 1A, C-E).

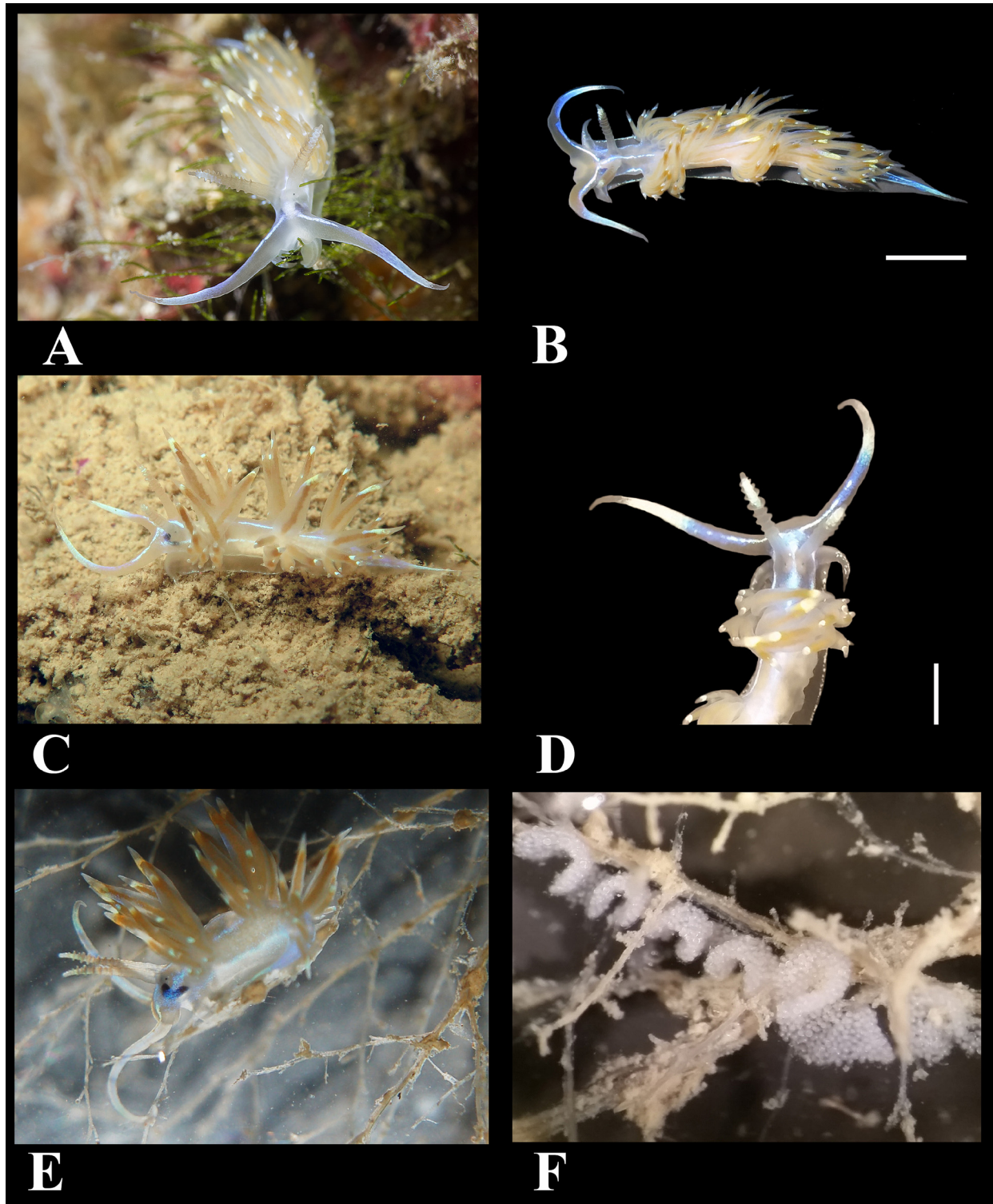


FIGURE 1 *Dondice trainitoi* sp. nov. (A) Specimen from Portofino promontory (44°17'53"N, 9°13'47"E) photographed *in situ* by Michele Solca and not collected. (B) In laboratory photograph of the holotype (Voucher MNHN IM-2000-33722) from 'Asia' wreck (42°03'15"N, 11°47'45"E). (C) Specimen from 'Asia' wreck (42°03'15"N, 11°47'45"E) photographed *in situ* by Jonathan Vulcano and not collected. (D) Cephalic portion of the paratype MNHN IM-2000-33724. (E) Lateral view of the paratype MNHN IM-2000-3372. (F) Egg mass of the paratype MNHN IM-2000-33723. Scale bar = 2 mm (B), = 1 mm (D)

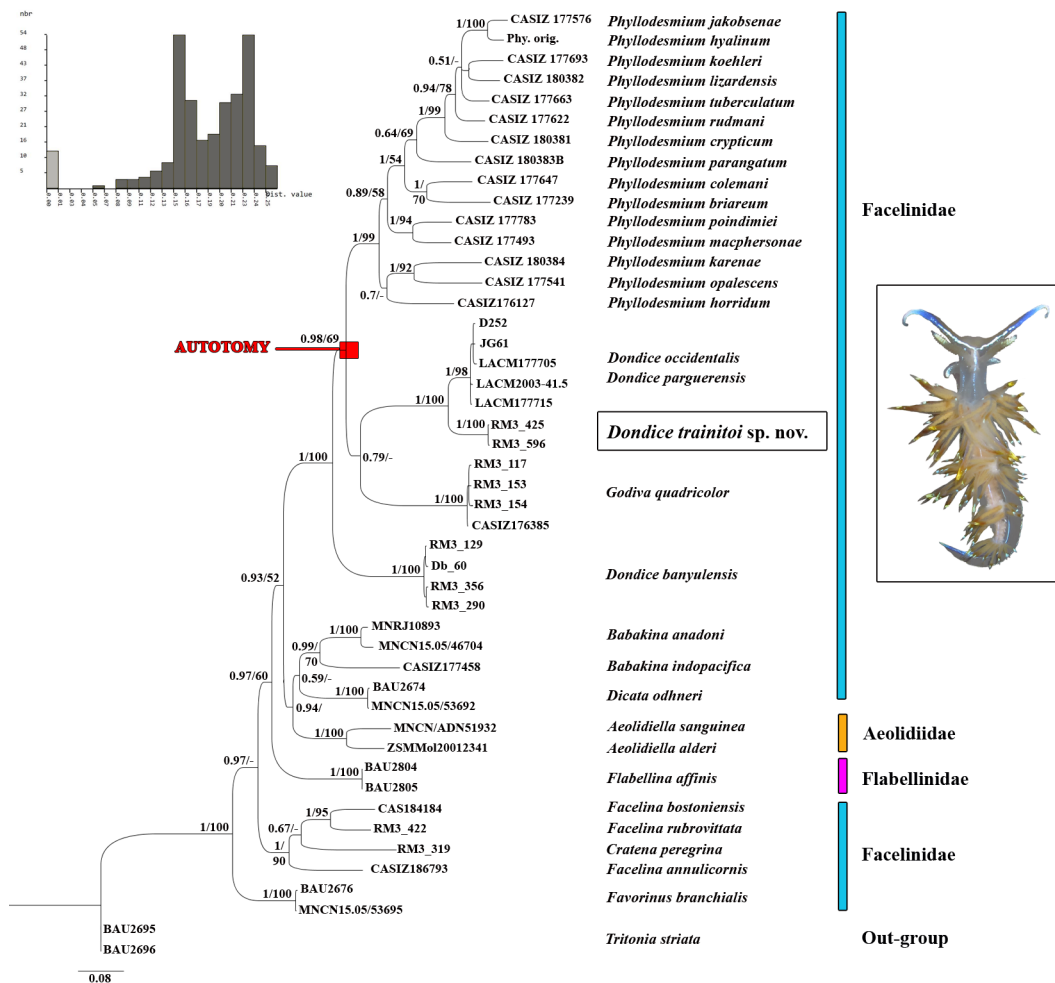


FIGURE 2 The Bayesian tree portrays the phylogenetic relationships based on the H3+16S+COI combined dataset. Numbers at nodes are Bayesian posterior probability (BI, left) and ML bootstrap support (BS, right), respectively. The histogram in the upper left part results from the ABGD analysis at the COI barcoding region showing the distribution of the pairwise estimated genetic distances (K2p) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons.

The rhinophores are lamellate with ten dish-shaped annulations and end with a cylindrical tip (Fig. 1D). The basal portion and the narrow posterior furrow of the rhinophores are smooth. In their upper portion, there is a brightly white/yellowish stripe that is wider in the anterior than in the posterior part. The oral tentacles are longer than the rhinophores, cylindrical and slender with a bright band in the same color of the apical portion of the rhinophores. The notum showed four clusters of cerata composed by two rows, with the exception of the last group that has only one row of small cerata. There is one precardiac cluster with 22 cerata, two median groups with 16 and 14 cerata respectively, and the posterior one with a single row of 5 cerata. The cerata are slender and cylindrical, light orange, and with a yellow ring which is expanded in the front part and jointed in the rear (Fig. 1). The head is slender. The anus is cleioproct, located within the first post-hepatic arch.

Internal anatomy: Three specimens, including the paratype MNHN IM-2000-33724 (Fig. 1D), were examined. The masticatory jaws are covered with a typical black epithelium (Fig. 1E). The underlying chitinous jaws are visible from the muscular lips (Fig. 3A). These structures are pale yellowish and characterized by 16 well-developed, triangular denticles per each side (Figs 3B-D). The radular formula is 8-12 x (0.1.0) (Figs 4A-D). The rachidian tooth has a median cusp, bearing one denticle for each side, and it has 4-5 triangular denticles on each side of the cusp (Figs 4E, F). The reproductive system (Fig. 5) is diaulic. The genital openings are on the right side of the body, between the first and the second group of cerata (Fig. 5B). The long tubular ampulla runs the length of the female gland mass and the hermaphroditic duct then bifurcates into the oviduct and the vas deferens (Figs 5D-F). The male gland terminates into the globular and unarmed penis (Fig. 5F). The penial bulb is smooth and muscular externally.

The vagina is connected to an ovoidal receptaculum seminis and to the convoluted duct of the female gland mass. The female gland mass is white and complex, with a central rounded and a lateral tortuous mass (Fig. 5F).

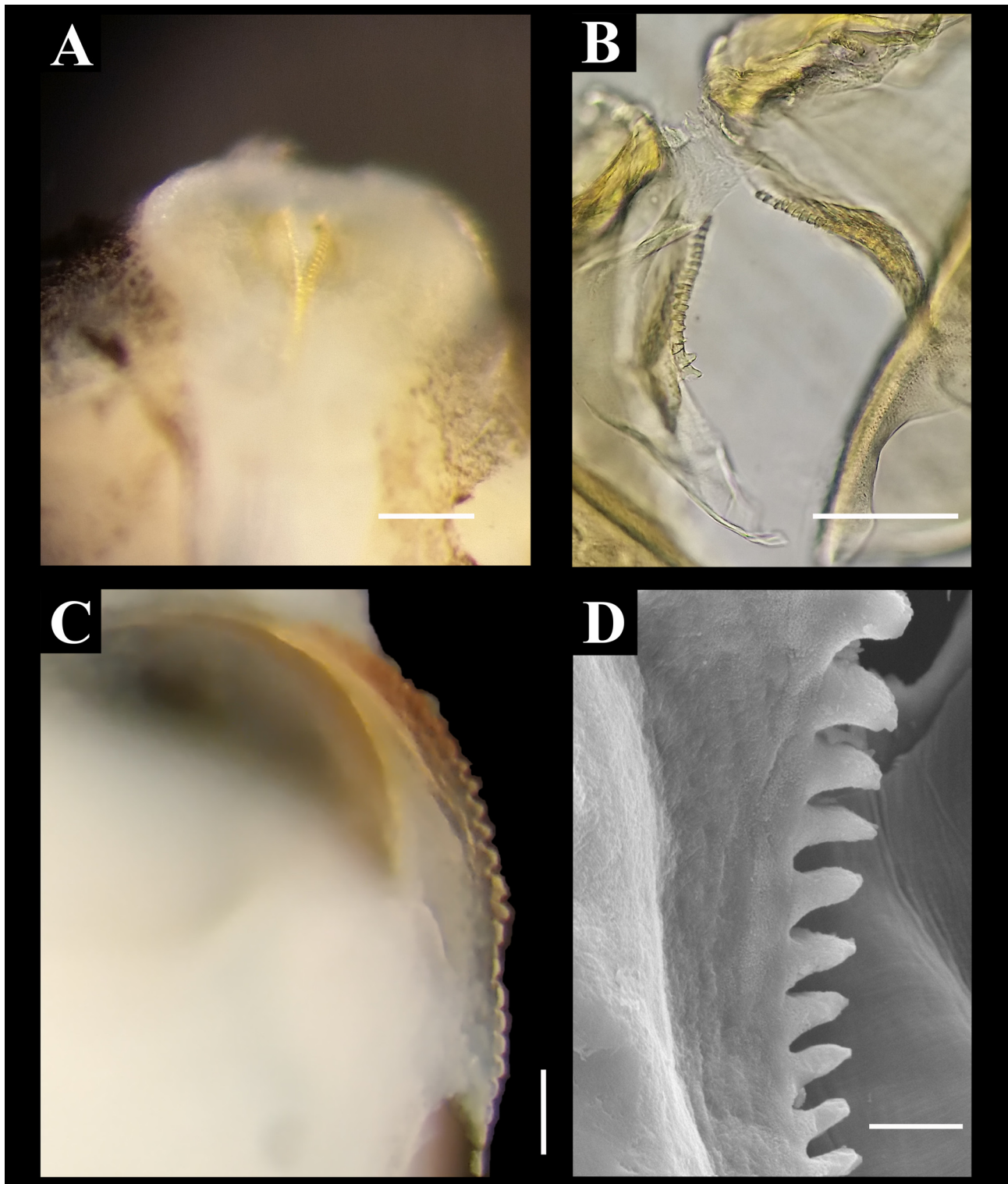


FIGURE 3 Optical (A, B, C) and SEM (D) images of the masticatory jaws of the buccal apparatus of *Dondice trainitoi* sp. nov. at different magnification levels. Scale bar = 100 μ m (A, B), = 40 μ m (C), = 10 μ m (D)

Distribution: Currently, this species is known from the Latium coast (Civitavecchia), type locality, and from Portofino Marine Protected Area (MPA) (Gulf of Genoa, Liguria) and Procida island (Naples, Campania) which are to date respectively the northernmost and the southernmost distribution sites. In particular, most of the findings took place on the ‘Asia’ and the near ‘Liburna’ wrecks, located near the harbour of Civitavecchia where this sea

slug is really abundant (with a total of 15-20 individuals observed per each dive). Four years after the first finding in Civitavecchia, about seven individuals were observed from Portofino MPA, while only two specimens were photographed and collected from Procida Island. The specimens were found feeding on colonies of hydroids covering the artificial substrate, made of torn nets from fishing trawlers, wrapping both wrecks, or on colonies of hydroids in a Coralligenous rocky bottom, from June to early September. The geographic distributions of the species is reported in Figure 6.

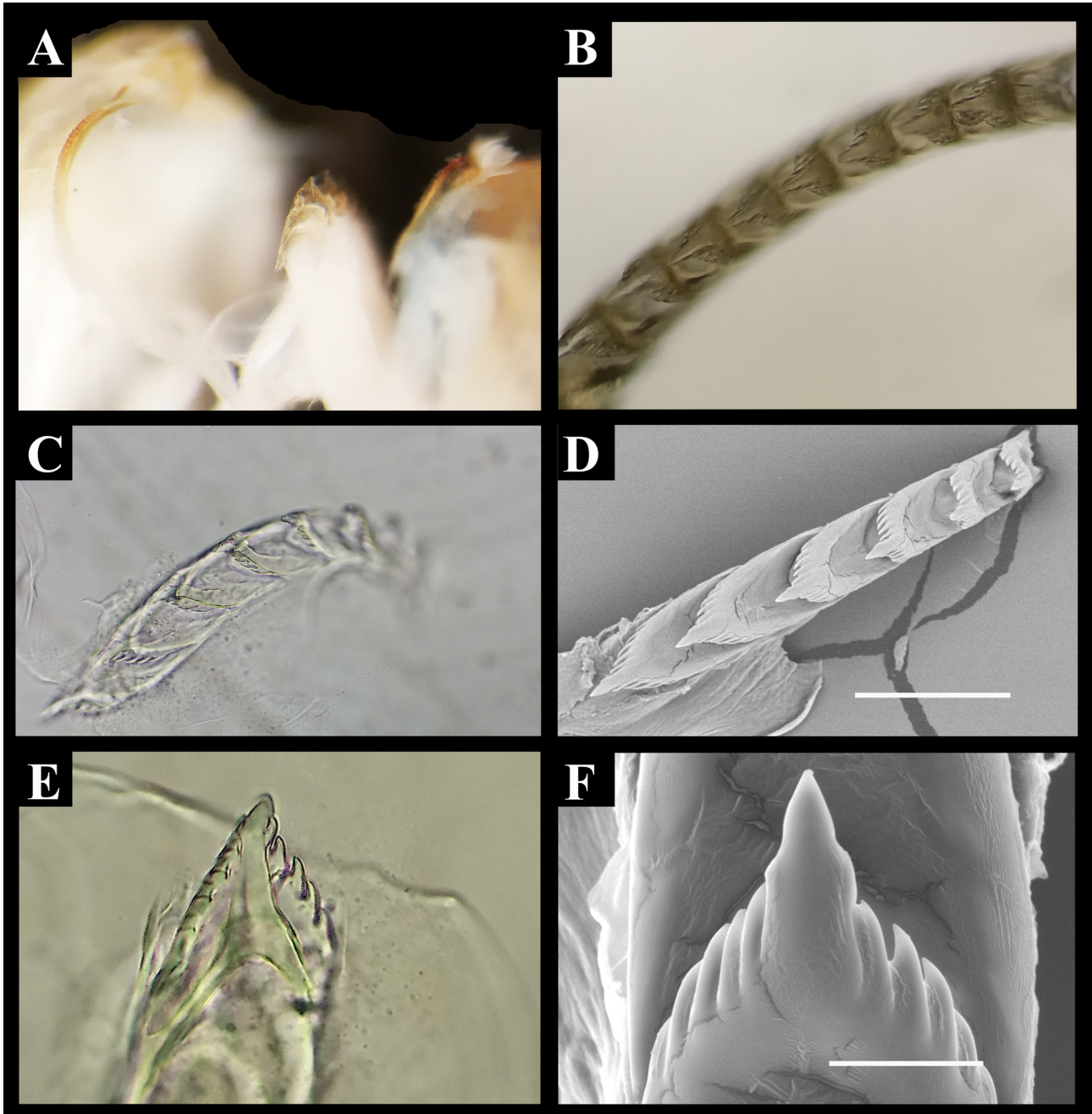


FIGURE 4 Optical (A-C, E) and SEM (D, F) images of the radula of *Dondice trainitoi* **sp. nov.** at different magnification levels. (A-D) Radula. (E, F) Rachidian tooth. Scale bar = 100 μ m (D), = 20 μ m (F).

Remarks: The morphological variability shown by living animals (Fig. 1) consisted in the body length of the specimens (ranging from 8 to 15 mm), rhinophores with usually 10 or 12 (range 6-17) dish-shaped annulations and the notum with 4 or 5 clusters of cerata. The precardiac cluster of cerata could have up to 24 cerata, the two median clusters up to 18 cerata each one and the posterior one with a single row of maximum 6 cerata. The new species shows 15% mean *p*-distance at the COI with *D. occidentalis* and 18% with the Mediterranean *D. banyulensis*. It also differs morphologically from the three formerly known congeners (Table 3). *Dondice trainitoi* **sp. nov.** can be

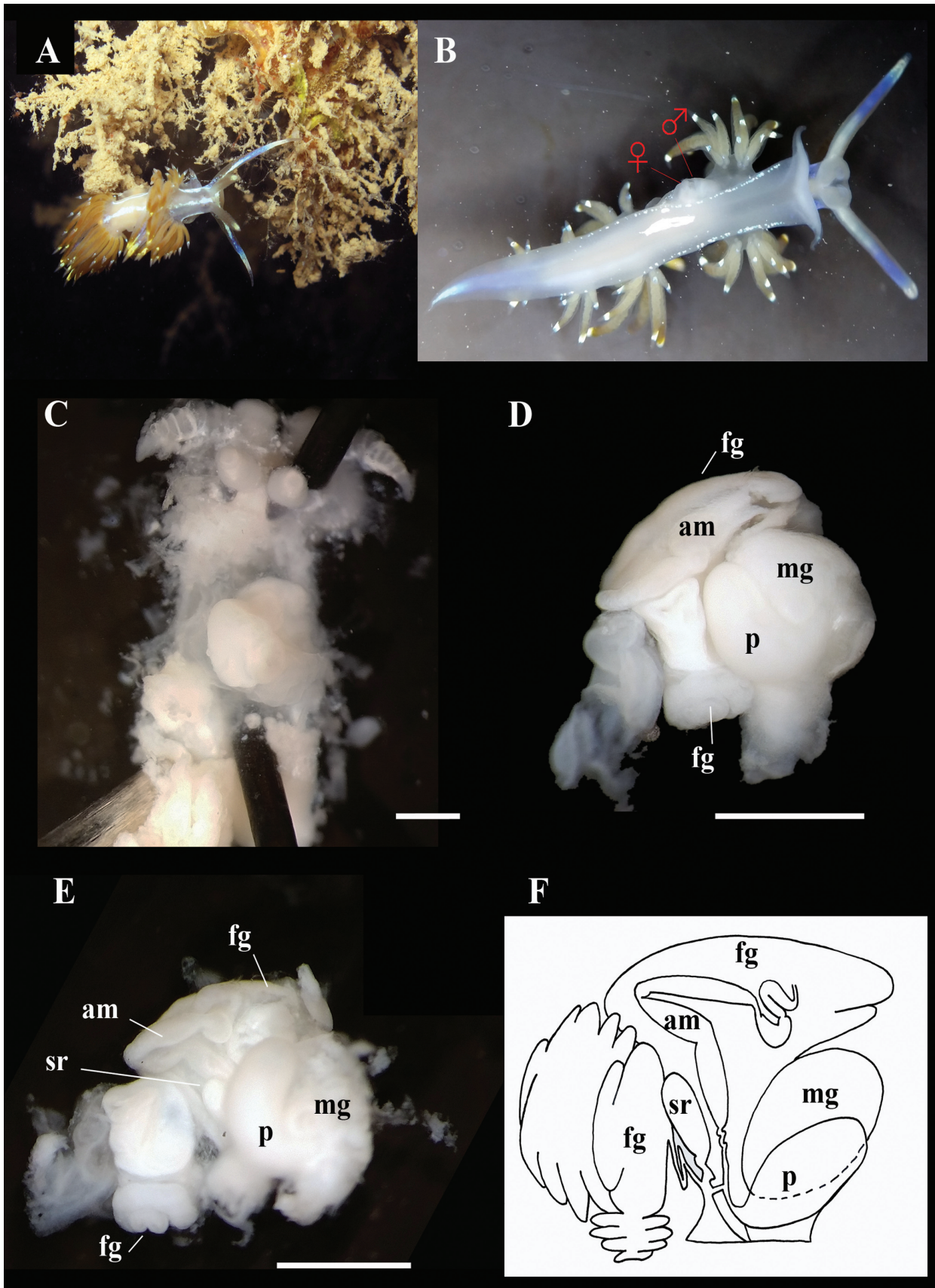


FIGURE 5 Reproductive system of *D. trainitoi* sp. nov. (A) Picture in situ (B) female and male genital openings of the living specimen (voucher RM3_1536) (C-E) pictures from specimens dissected at different steps (F) schematic drawing of the reproductive system. Scale bar: 1 mm. Abbreviations: am=ampulla, fg=female gland, mg=male gland, p=penis, sr=seminal receptacle.



FIGURE 6 Geographical distribution of the Mediterranean *D. trainitoi* **sp. nov.** with the red stars indicating the collection localities. 1= Punta del Faro, Portofino promontory, Liguria (44°17'53"N, 9°13'47"E), 2= 'Asia' wreck, Civitavecchia, Latium (42°03'15"N, 11°47'45"E), 3= Procida Island, Naples (40°45'02.6"N, 14°01'31.6"E).

distinguished from *D. banyulensis* by the presence of the black pigment covering the jaws and forming two black patches clearly visible at the cephalic portion. The only other species which shares this very typical character is the *D. occidentalis* 'sensu Marcus Er.' (1958), who described a specimen from Cananèia (San Paulo, Brazil) which '...differs from the Jamaican specimens in some details. The black pigmented epithelium covering the jaws of our species is very striking, even in living slugs, but apparently is wanting in the Jamaican material as Engel does not mention it.' (Marcus Er. 1958). *Dondice occidentalis* 'sensu Marcus' however, has '...a median red stripe along the head and a stripe along each side' (see the Brazilian specimen photographed by Lindner A. 2014, page 48) that clearly differs from the white ones characterizing *D. trainitoi* **sp. nov.** *Dondice galaxiana* is morphologically entirely different from *D. trainitoi* **sp. nov.**, in fact it has rhinophores with large dish-shaped annulations, the cerata are clustered on raised cushions and its body has rhomboid-shaped patches on the dorsum between the cerata. These features are completely lacking in the new species. *Dondice parguerensis* is morphologically similar to *D. occidentalis* but differing from it for its particular diet which consists of *Cassiopea* sp. and by the epithelium covering the jaws that is brown amber (Brandon & Cutress 1985) and not dark or black as in *D. occidentalis*.

Specimens of the new species were observed and photographed *in situ* feeding on a reddish/orange hydroid belonging to the family Campanulariidae Johnston, 1836. The egg mass is a white lace coiled around the hydroid (Fig. 1F). Interestingly, as known for other facelinid species, *D. trainitoi* **sp. nov.**, when disturbed, autotomized its cerata to distract potential predators.

Discussion

The Facelinidae family is characterized by members showing heterogeneous morphotypes leading to several controversies and to a confused taxonomy. In the last decade, with the advent of molecular techniques, the monophyly of this eolid family was also questioned since many phylogenetic works (Gosliner *et al.* 2007; Carmona *et al.* 2015) have revealed contrasting relationships occurring between members historically assigned to the Facelinidae family highlighting the need to deepen the study this group of molluscs. The new species was here analysed and described by both morphological and molecular approaches and revealed to be strongly related to *D. occidentalis*,

the type species of the genus *Dondice*. Furthermore the new species seems to be morphologically more similar to the Brazilian specimens of *D. occidentalis* described by Marcus Er. (1958) than to the ones originally described by Engel (1925) as *Caloria occidentalis* from Jamaica. In fact, the Mediterranean *D. trainitoi* sp. nov. shares with the Brazilian morphotype of *D. occidentalis*, the black pigment covering the masticatory jaws, anyhow a recent picture of a Brazilian specimen (Lindner 2014) showed some striking differences between the two species supporting their validity, as reported in Table 3.

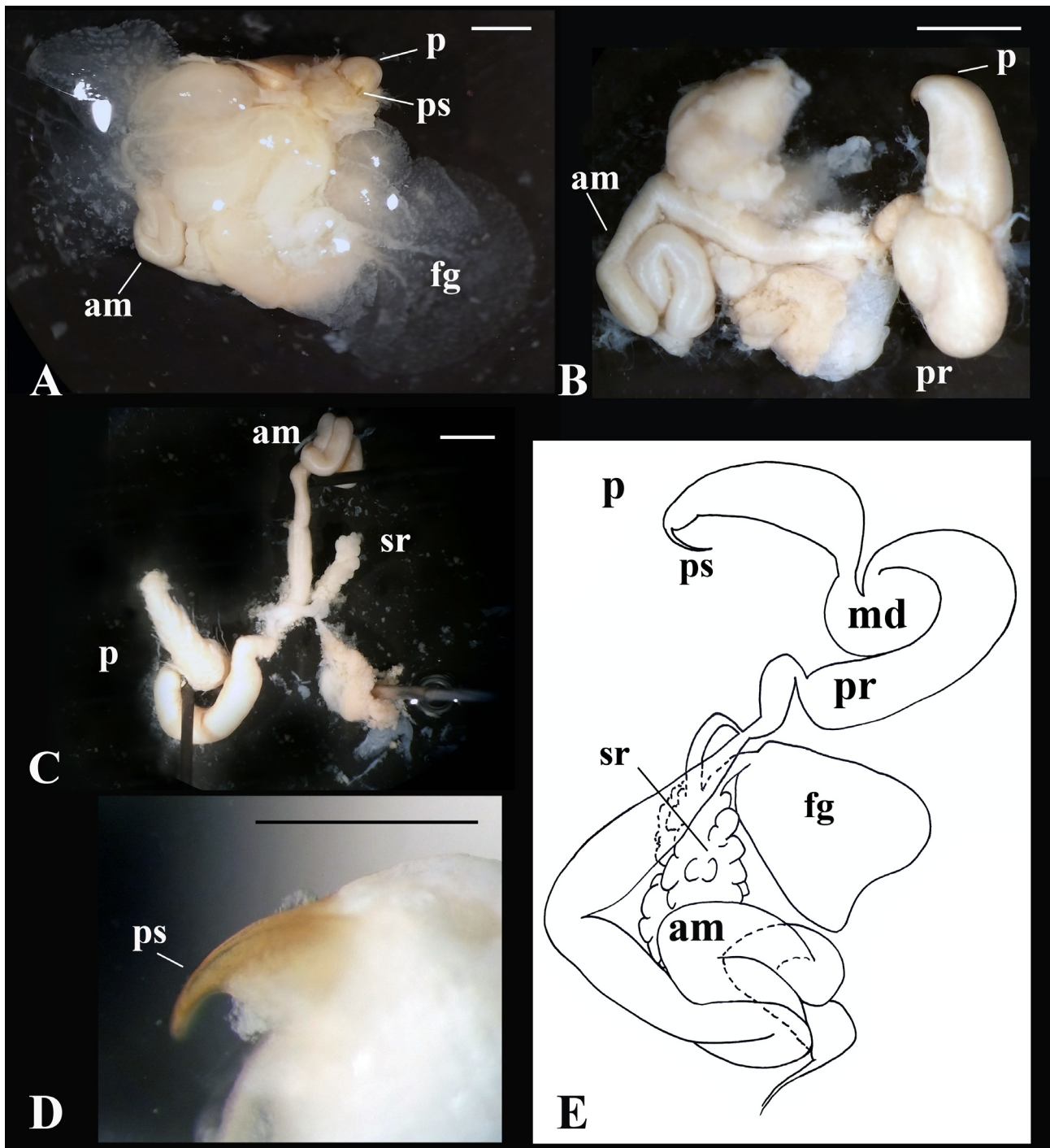


FIGURE 7 Reproductive system of *G. quadricolor*, voucher RM3_792. (A-C) pictures from specimens dissected at different steps (D) schematic drawing of the reproductive system. Scale bar: 1 mm. Abbreviations: am=ampulla, fg=female gland, md= male duct, p=penis, pr=prostate, ps= penis spine, sr=seminal receptacle.

To investigate interactions between species also by molecular methods, phylogenetic relationships of *Dondice* species and other related taxa were explored. Molecular approaches have confirmed the Facelinidae family as non-monophyletic. In particular, *Dondice*, *Godiva* and *Phyllodesmium* genera are grouped in a monophyletic clade with

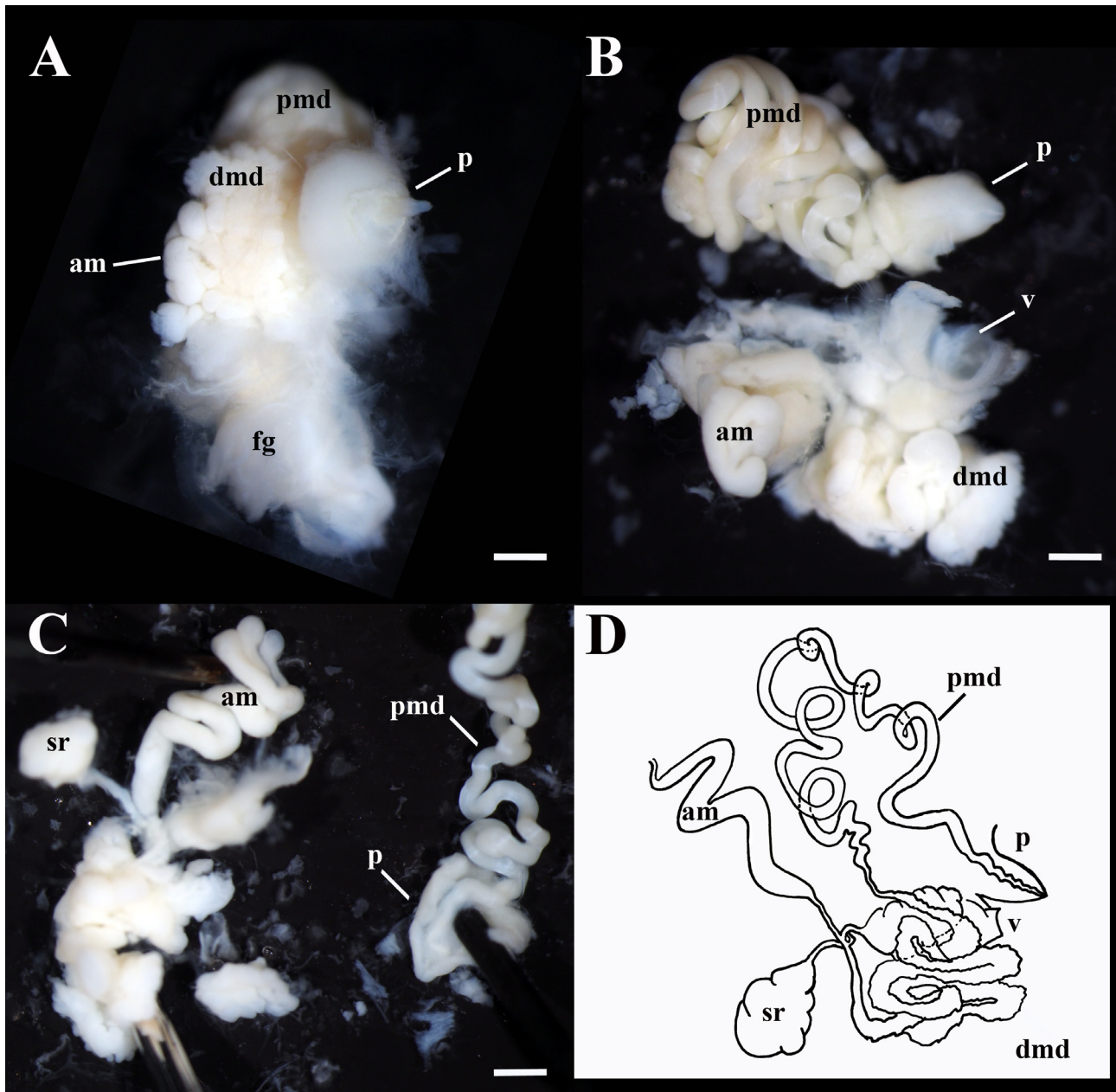


FIGURE 8 Reproductive system of *D. banyulensis*, voucher RM3_197. (A-C) pictures from specimens dissected at different steps (D) schematic drawing of the reproductive system. Scale bar: 1 mm. Abbreviations: am=ampulla, dmd=distal male duct, p=penis, pmd=proximal male duct, sr=seminal receptacle, v=vagina

a strong statistical support but with the genus *Dondice* as paraphyletic. In fact, *D. trainitoi* **sp. nov.** shows a close relationship with the clade composed by the type-taxon *D. occidentalis* and *D. parguerensis* (whose validity as a species remains not clear) but *D. banyulensis* appears, with high statistical support (BI=1; ML=100), as the sister species of a clade including the abovementioned *Dondice* species, *G. quadricolor* and all the *Phylloidesmium* taxa (Fig. 2) supporting the paraphyly of the genus. To investigate these unexpected molecular evidences, we have analysed members of *Godiva* and *Dondice* genera by morphological and anatomical comparison, together with indications obtained by ecological knowledge, in an integrated taxonomic view. Consequently, in order to examine the diagnostic characters used in the past to assign species (Figs 7, 8), we have performed anatomical analyses on *G. quadricolor* and *D. banyulensis* which have a critical role in the molecular topology obtained, (specimens collected during this study and stored at the collection of the Roma Tre University) (see figure captions for the vouchers). Since Marcus Er. (1958) stated that the main morphological feature distinguishing the two genera was the male copulatory organ, unarmed in *Dondice* but armed and with a terminal hook in *Godiva*, we confirmed the presence of the armed penis in *G. quadricolor* (Fig. 7D) and the unarmed penis in *D. banyulensis* (Fig. 8). Interestingly, Ed

TABLE 3. Morphological comparison among species belonging to *Dondice* genus.

	Body colour	Cerata colour	Cerata distribution	Rhinophores	Foot anterior border	Radular formula	Jaws colour	Gonopore	Anus	Penial glands
<i>Dondice banyulensis</i> Portmann & Sandmeier, 1960	Rather clear transparent orange. Deepthick opaque orange covers upper half of rhinophores, head in front of rhinophores, anterior border of the basis of oral tentacles, foot tentacles, forms a sub-apical ring beneath the top of cerata. Upper half of oral tentacles opaque white. Median opaque white line from the anterior border of oral tentacles to end of tail. On either sides a white opaque line from the oral tentacles to the tail connecting the basal region of cerata groups. An incomplete white line on the notum between the median and the lateral ones. Edge of foot lined with bluish iridescent opaque white	A narrow brown vein becomes dark red beneath the very small cnidosac	5 groups horseshoe shaped	L a m e l l a t e along the entire length	Foot corners tentaculiform	18 x 0.1.0	Dark red	On the right beneath the first group of cerata	On the right inside the second group of cerata	Absent
<i>Dondice galaxiana</i> Millen & Hermosillo, 2012	Translucent cream with rhomboid shaped-patches on the dorsum between the cerata. The center of each rhombus is light opaque cream surrounded by a light brown band and contains four equidistant bright turquoise spots forming a square. Near them a larger dark olive-black spot. On the rest of the body random bright white spots. Sides with reddish brown patches and light cream patches with dark brown spots	Light pinkish brown with sub-terminal brown band and dark reddish brown cores. Surface covered with random white spots	1 pericardic group, 2-3 posterior and a row of 2-3 cerata	Short with cylindrical tip, dish shaped annulations	2 tentacles 1 mm length	15-16 x 0.1.0	C o v e r e d with black melanophores in the epithelium	On the right side anterior of the precardic group of cerata	Within the arch of the first posterior group of cerata	Present

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TABLE 3. (Continued)

	Body colour	Cerata colour	Cerata distribution	Rhinophores	Foot anterior border	Radular formula	Jaws colour	Gonopore	Anus	Penial glands
<i>Dondice occidentalis</i> Engel, 1925	Completely lost the colours, and looked very transparent, yellowish-white; everywhere the internal organs shone through clearly	No colour description	7 groups	5-7 rings	2 long up to 5 mm	0.1.0	Dark	On the right side between the two first arches in the interspace behind the limbs of the first of these two	On the right side in the interspace between the limbs of the first of these two pairs of transverse pairs	Present
<i>Dondice occidentalis sensu Marcus</i> Er. 1958	White, with red markings. A median red stripe along the head and a stripe along each side that extends from the tentacle base dorsally to the genital pores, ventrally to the cerata, and caudally to the tail tip. Liver brown or reddish. In the skin white refractive elements	Under the cnidosacs, brilliantly white or pink, the cerata bear an orange-red ring	6 groups	15-18 rings	2 tentacles mm length	21 x 0.1.0	Covered by black epithelium	On the right side under the first arch of cerata	In the center of the second arch of cerata	Present
<i>Dondice parquerensis</i> Brandon & Cutress, 1985	Translucid white; tips of rhinophores, foot tentacles and buccal tentacles refractive white. A white snow line longitudinally and not continuously along the back from the head, between the eyes, up to the tail. Similar lines along the sides circle each group of cerata and join on the tail	Brown terminations of digestive glands inside the cerata. Cnidosac white translucid	2 precardiac groups, 4 postcardiac isolated	5 annulations	2 shorter than buccal tentacles	15 x 0.1.0	Amber	On the right side between first and second group of cerata	Under first group of pericardic cerata	Present

.....Continued next page

TABLE 3. (Continued)

	Body colour	Cerata colour	Cerata distribution	Rhinophores	Foot anterior border	Radular formula	Jaws colour	Gonopore	Anus	Penial glands
<i>Dondice traintittoi</i> sp.nov.	Translucent cream, with brightly white spots along the border of the foot, the oral foot corners and along the tail. A characteristic iridescent light blue band goes from the tail along the dorsum and the head where it bifurcates into two lines ending at the half part of the oral tentacles. Two other lateral lines of the same iridescent colour go dorsally through the body terminating in the lateral part of the head	Light orange, with a yellowish band, expanded in the front part, joins barely in the rear	4 (5) groups, 1 pericardic, and 1 posterior row	Lamellate with usually 10 or 12 (6-17) dish-shaped annulations ending with a cylindrical tip	Well defined propodial tentacles	8-12 x 0.1.0	Black pigment covering the mandibles visible through the epithelium	On the right side between the first and the second group of cerata	On the right side in the middle of the second group of cerata	Present
<i>Godiva quadricolor</i> (Barnard, 1927)	Head pale orange above, with two white streaks from the tentacles to the bases of the rhinophores; tentacles and rhinophores of the same colour as head, with the tips sulphur yellow. Rest of body translucent white, the tail with a narrow median stripe of orange bordered by opaque (faintly blue) white, stomach and intestines showing through the skin a dull blue	Papillae dark brown with a purplish tinge, towards the end a band of bright orange and one of ultramarine blue separated by a narrow band of dark brown, apex sulphur yellow, the ground colour is also sprinkled lightly with sulphur yellow dots just below the orange band	Papillae fusiform in 5 groups, about 30 to 40 in the first, 30 in the second, 25 in the third, 20 in the fourth, and 12 to 15 in the fifth, the outer ones short, the innermost longest (5 to 6 mm. in the preserved state)	Rhinophores slender, simple in life, but wrinkled and seemingly annulate in the preserved specimen	Antero-lateral angles of foot produced, tentaculiform			On the right side immediately below the first group of cerata	On the right side in the middle of the second group of cerata	Absent

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munds (1964) in its work on eolid Mollusca from Jamaica carried out an anatomical comparison of the diagnostic reproductive features of *D. banyulensis* with the ones observed in *D. occidentalis* and *G. quadricolor* and concluding that *D. banyulensis* was more likely to belong to *Godiva* rather than to *Dondice* genus, in line with the systematic hypothesis suggested by our molecular results. In our opinion, the absence or presence of the penial hook could not be considered as diagnostic at the genus level as indeed suggested by other authors (Macnae 1954; Rudman 1980). Furthermore, considering the fact that *Phyllodesmium* and *Dondice* species are characterized by an unarmed penis (Moore & Gosliner 2014) as well as all the species showing a basal placement in the phylogenetic tree (with the exclusion of the *Facelina* ones) we can infer that this particular anatomical feature has evolved independently in *G. quadricolor*.

Our results could suggest unifying the three genera *Phyllodesmium*, *Dondice* and *Godiva* under the single genus *Phyllodesmium*, due to the morphological similarities and the molecular affinities. An important ethological feature could support this hypothesis: the capability to autotomize groups of cerata under stress conditions. This particular skill is shared by all of the *Phyllodesmium* species, by *D. occidentalis*, *D. trainitoi* **sp. nov.** and interestingly by *G. quadricolor*. It is missing instead in *D. banyulensis*, confirming its basal placement within the evolutionary history of the group. Anyhow, considering that *G. quadricolor* did not show a stable position in all the trees recovered from analyses performed on alternative datasets (not shown), we maintain a conservative approach not altering the classification of these three genera (*Dondice*, *Godiva* and *Phyllodesmium*).

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