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Molecular data and updated morphological description of *Flabellina rubrolineata* (Nudibranchia: Flabellinidae) from the Red and Arabian seas

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ABSTRACT. *Flabellina rubrolineata* was believed to have a wide distribution range, being reported from the Mediterranean Sea (non-native), the Red Sea, the Indian Ocean and adjacent seas, and the Indo-West Pacific and from Australia to Hawaii. In the present paper, we provide a redescription of *Flabellina rubrolineata*, based on specimens collected near the type locality of this species in the Red Sea. The morphology of this species was studied using anatomical dissections and scanning electron microscopy. To place this species in the phylogenetic framework and test the identity of other specimens of *F. rubrolineata* from the Indo-West Pacific we sequenced COI, H3, 16S and 28S gene fragments and obtained phylogenetic trees based on Bayesian and Maximum likelihood inferences. Our morphological and molecular results show a clear separation of *F. rubrolineata* from the Red Sea from its relatives in the Indo-West Pacific. We suggest that *F. rubrolineata* is restricted to only the Red Sea, the Arabian Sea and the Mediterranean Sea and to West Indian Ocean, while specimens from other regions belong to a complex of pseudocryptic species.

Молекулярно-генетический анализ и обновленное таксономическое переописание *Flabellina rubrolineata* (Nudibranchia: Flabellinidae) из Красного и Аравийского морей

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РЕЗЮМЕ. *Flabellina rubrolineata* долгое время считался широко распространенным циркумтропическим видом, распространенным в Средиземном море (в результате вселения), Красном море, Индийском океане и связанным с ним морях, в тропических и субтропических водах западной части Тихого океана, прибрежных водах Австралии и Гавайских островов. В данной работе мы приводим обновленное таксономическое описание данного вида по образцам, собранным вблизи типового местонахождения вида в Красном море. Морфология была изучена с применением анатомических вскрытий и сканирующей электронной микроскопии. Для сравнения этих экземпляров с особями *F. rubrolineata* из Индо-Вест Пацифики был проведен молекулярно-филогенетический анализ по четырем маркерам: COI, H3, 16S и 28S с применением байесовского метода и метода максимального правдоподобия. Полученные результаты свидетельствуют, что особи *F. rubrolineata* из Красного моря отличаются от образцов из Индо-Вест Пацифики как по морфологическим, так и по молекулярным данным. Таким образом, мы предполагаем, что *F. rubrolineata* обитает только в Красном, Аравийском, Средиземном морях и близлежащих регионах, в то время как в других областях он представлен комплексом псевдокриптических видов.

Introduction

The implementation of molecular methods in systematics and organisms identification has uncovered a vast assortment of cryptic species and

species complexes, from what were believed to be single vicarious species with plastic morphology [Martin, Bermingham, 2000]. In many cases extremely large distribution ranges may be a good indicator for the discovery of cryptic species complex. For nudibranch molluscs this situation is also extremely common, as many widely distributed species were divided into several cryptic or pseudocryptic species in different taxa: various *Chromodoris* species [Layton *et al*., 2018; Tibiriçá *et al*., 2020], *Diaulula sandiegensis* (Cooper, 1983) [Lindsay *et al*., 2016], *Dendronotus frondosus* (Ascanius, 1774) [Stout *et al*., 2010; Ekimova *et al*., 2015, 2019], *Spurilla neapolitana* (Delle Chiaje, 1841) [Carmona *et al*., 2014], *Fiona pinnata* (Eschscholtz, 1831) [Trickey *et al*., 2016], *Hermissenda crassicornis* (Eschscholtz, 1831) [Lindsay, Valdés, 2016], *Aeolidia papillosa* (Linnaeus, 1761) [Kienberger *et al*., 2016], and many others. Opposite situation is also plausible, *i.e.* fionid species *Cuthona nana* (Alder & Hancock, 1842) was suggested to be a species complex with *Cuthona divae* (Er. Marcus, 1961) inhabiting North East Pacific, *Cuthona hermitophila* Martynov, Sanamyan & Korshunova, 2015 described from North West Pacific and *C. nana* which distribution range was restricted to North Atlantic [Martynov *et al*., 2015]. However, dedicated analyses of the three species suggested that they are conspecific and should be united under the name *C. nana* [Cella *et al*., 2016; Chichvarkhin *et al*., 2016].

Nudibranch *Flabellina rubrolineata* (O'Donoghue, 1929) is one of the widest distributed species within the family Flabellinidae *s.l*. It was initially described from the Suez region in the Red Sea [O'Donoghue, 1929] and further reported from the Mediterranean Sea [Gat, 1993; Yokes, Rudman, 2004], the Red Sea [Yonow, 2000, 2008], Indian Ocean and adjacent seas [Gul, 2019; Sreeraj *et al*., 2012a,b, 2013; Tibiriçá *et al*., 2017], the Indo-West Pacific [Gosliner, Kuzirian, 1990; Gosliner, Willan, 1991; Martynov, Korshunova, 2012; Gosliner *et al*., 2015; Yonow, 2017; Papu *et al*., 2020], subtropical waters of Korea [Jung, Park, 2015], Japan [Baba, 1955], and Australia [Wells, Bryce, 1993; Edgar, 1997; Burn, 2006; Larkin *et al*., 2018]. However, in most recent nudibranch identification guide for the Indo-West Pacific [Gosliner *et al*., 2018] the range of *F. rubrolineata* was restricted to the type locality (Red Sea) and adjacent areas (Mediterranean Sea, Arabian Sea). It was therefore suspected that similar animals found in other localities represent a complex of cryptic species, precipitating a necessity of taxonomic revision. At the same time all publicly available sequences under the name "*Flabellina rubrolineata*" belong to samples collected from the Indo-West Pacific. Detailed morphological descriptions (excluding the initial description by O'Donoghue [1929]) and scanning electron micrographs of radula and jaws were also made based on material from tropical waters [Gosliner, Willan, 1991; Korshunova *et al*., 2017]. Therefore, the main goal of our study is to provide first molecular data for *Flabellina rubrolineata* from its type locality and to update its formal taxonomic description.

Material and methods

Material

Specimens were collected during snorkeling and scuba diving in two localities: 1. Red Sea, Egypt, Hurghada, 09.05.2014, 4 specimens of *F. rubrolineata*; 2. Arabian Sea, Gulf of Aden, Gulf of Tadjoura, Djibouti, 06.12.2019, 1 specimen of *F. rubrolineata* (Table 1). Each animal was relaxed and photographed using a Sony NEX-5N camera and then fixed in 96° ethanol. Voucher specimens and DNA samples are stored in the collection of Invertebrate Zoology Department, Lomonosov Moscow State University.

Morphological analysis

The external morphology of specimens was studied under a stereomicroscope. The buccal mass of each specimen was extracted and soaked in proteinase K solution for 2 hours at 55°C to dissolve connective and muscle tissues, leaving only the radula and the jaws. The coated radulae and jaws were examined and photographed using the scanning electron microscope CamScan-S4 (Cambridge, UK). The reproductive system of both species was examined using the stereomicroscope.

Taxon sampling for molecular analysis

The molecular dataset assembled by Korshunova *et al*. [2017] for the genus *Coryphellina sensu* Korshunova *et al.* [2017] was used (24 sequences available from GenBank). Also, available sequences for species *F. rubrolineata* from GenBank were also implemented in the analysis (Table 1). *Coryphella verrucosa* (M. Sars, 1829), *Edmundsella pedata* (Montagu, 1816), *Flabellina affinis* (Gmelin, 1791) and *Flabellinopsis iodinea* (Cooper, 1863) were chosen as outgroups. *Tritonia pickensi* Ev. Marcus & Er. Marcus, 1967 and *Tritonia plebeia* G. Johnston, 1828 were chosen as distant outgroups.

DNA extraction, amplification and sequencing

DNA was extracted from small pieces of foot tissue using PALL™ AcroPrep 96-well plates by *PALL Corp.* [Ivanova *et al.*, 2006]. Extracted DNA was used as a template for amplification of partial cytochrome *c* oxidase subunit I (COI), 16S rRNA (16S), histone H3 (H3) and 28S rRNA (28S) using standard primers:

Table 1. Specimens used in the present study with information on vouchers and GenBank accession numbers.
Таблица 1. Изученные особи с указанием регистрационных номеров ГенБанка.

Original ID	Revised ID	Voucher	Location	GB accession numbers			
				COI	16S	H3	28S
Flabellina rubrolineata	*Flabellina rubrolineata*	IE-fr1	Arabian Sea, Djibouti	MT420426	MT419862	MT419777	MT419823
Flabellina rubrolineata	*Flabellina rubrolineata*	IE-fr2	Red sea, Egypt	MT420427	MT419863	MT419776	MT419824
Flabellina rubrolineata	*Flabellina rubrolineata*	IE-fr3	Red sea, Egypt	-	MT419864	-	MT419825
Flabellina rubrolineata	*Flabellina rubrolineata*	IE-fr4	Red sea, Egypt	-	MT419865	-	MT419826
Flabellina rubrolineata	*Flabellina rubrolineata*	IE-fr5	Red sea, Egypt	-	-	-	-
Flabellina rubrolineata	*Flabellina* sp. 1	CAS177287	Philippines	KY129061	KY128852	KY128646	-
Flabellina rubrolineata	*Flabellina* sp. 1	ZMMU Op-132	Vietnam	MF523381	MF523437	MF523306	MF523504
Flabellina rubrolineata	*Flabellina* sp. 2	n/a	Queensland, Australia	KJ001316	KJ018915	-	-
Flabellina rubrolineata	*Flabellina* sp. 3	ZFMK262	Lizard Island, Australia	MK091277	MK100963	-	-
Flabellina lotos	*Flabellina lotos*	ZMMU Op-515	Japan	MF523387	MF523462	MF523312	MF523528
Flabellina exoptata	*Flabellina exoptata*	CAS178322	Malaysia	KY129053	KY128844	KY128638	
Flabellina exoptata	*Flabellina exoptata*	ZMMU Op-116	Vietnam	MF523380	MF523438	MF523305	MF523505
Flabellina arveloi	*Flabellina arveloi*	CAS179418	São Tome and Principe	KY129048	KY128839	KY128634	-
Flabellina arveloi	*Flabellina arveloi*	CAS179419	São Tome and Principe	KY129049	KY128840	KY128633	-
Coryphella verrucosa		ZMMU Op-520	White Sea	MF523374	MF523412	MF523299	MF523488
Coryphella verrucosa		ZMMU Op-521	Barents Sea	MF523375	MF523421	MF523300	MF523494
Edmundsella pedata		NTNU VM 65498	Norway	MG452603	MG452648	MG452566	-
Flabellina affinis		MNCN15.05/53 696	Spain	HQ616753	HQ616716	HQ616782	-
Flabelinopsis iodinea		CAS181313a	California	KY129056	KY128847	KY128641	-
Tritonia pickensi		CAS175718	California	HM162717	HM162642	HM162549	
Tritonia plebeia		ZMMU Op-572	Norway	KX788134	KX788122	-	KX788132

HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') [Folmer *et al*., 1994]

LCO1498 (5'-GGTCAACAAATCATAAAGATATTGG-3') [Folmer *et al*., 1994]

16SarL (5'- CGCCTGTTTAACAAAAACAT-3') [Palumbi, 1996]

16SR (5'-CCGRTYTGAACTCAGCTCACG-3') [Puslednik, Serb, 2008]

H3AF (5'-ATGGCTCGTACCAAGCAGACVGC-3') [Colgan *et al*., 1998]

H3AR (5'-ATATCCTTRGGCATRATRGTGAC-3') [Colgan *et al*., 1998]

28S C1 (5'-ACCCGCTGAATTTAAGCAT-3') [Lê *et al*., 1993]

28S C2 (5'- TGAACTCTCTCTTCAAAGTTCTTTTC-3') [Lê *et al*., 1993]

Polymerase chain reactions were carried out in a 25-μL reaction volume, which included 5 μL of 5x Taq Red Buffer by *Eurogen Lab*, 0.5 μL of HS-Taq Polymerase by *Eurogen Lab,* 0.5 μL of dNTP (50 μM stock), 0.3 μL of each primer (10 μM stock), 1 μL of genomic DNA and 17.7 μL of sterile water. The amplification was performed with an initial denaturation for 1 min at 95°C followed by 35 cycles of 15 s at 95°C (denaturation), 30 s at 45°C (annealing) (52°C in case of 16S marker) and 45 s at 72°C (elongation) with a final extension of 7 min at 72°C. Sequencing for both strands proceeded with the *Big Dye Terminator v3.1* sequencing kit by Applied Biosystems, the same primers as for PCR were used. Sequencing reactions were analyzed using ABI 3500 Genetic Analyser (*Applied Biosystems*) at N.K. Koltsov Institute of Developmental Biology (Moscow, Russia). All new sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

Raw reads for each gene were assembled and checked for improper base-calling using Geneious-Pro 4.8.5 (Biomatters, Auckland, New Zealand). Original data and publicly available sequences were aligned with the MUSCLE [Edgar, 2004] algorithm in MEGA7 [Kumar *et al*., 2016]. Protein-coding sequences were translated into amino acids to verify coding sequences. The resulting alignments were of 663 bp for COI, 412 bp for 16S, 327 bp for H3 and 343 bp for 28S. Indel-rich regions of the 16S marker were removed using the default settings in Gblocks [Talavera, Castresana, 2007]. Phylogenetic analysis was conducted for all datasets concatenated. Sequences were concatenated by a simple biopython script [Chaban *et al*., 2019]. The best-fitting nucleotide evolution model was tested in the MEGA7 toolkit based on the Bayesian information criterion (BIC) for each partition. The best-fitting model for COI partition was GTR+G+I, for 16S partition – HKY+G+I, for H3 and 28S partitions the best model was K2+G. Phylogenetic reconstruction was performed applying evolutionary models for partitions separately. The Bayesian estimation of posterior probability was performed in MrBayes 3.2 [Ronquist, Huelsenbeck, 2003]. Markov chains were sampled at intervals of 500 generations. The analysis was started with random starting trees and $2x10^7$ generations. Maximum likelihood-based phylogeny inference was performed in HPC-PTHREADS-AVX version of RaxML [Stamatakis, 2014] with ultrafast bootstrapping (UFBoot approzimination approach) [Minh *et al*., 2013] in 1000 pseudoreplicates under GTRCAT model of nucleotide evolution. Bootstrap values were placed on the best tree found with SumTrees 3.3.1 from DendroPy Phylogenetic Computing Library Version 3.12.0 [Sukumaran, Mark, 2010]. Final phylogenetic tree images were rendered in FigTree 1.4.0 and further modified in Adobe Illustrator CC2015.

Species delimitation

To confirm the status of recovered monophyletic clades and singletons as distinct species we used the Automatic Barcode Gap Discovery (ABGD) method [Puillandre *et al*., 2012]. COI sequences were aligned for ABGD analysis with the MUSCLE [Edgar, 2004] algorithm in MEGA7 [Kumar *et al*., 2016]. The analysis was run on the online version of the program (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with the default settings except X value that was decreased to 0.5. Uncorrected *p*-distances were calculated using MEGA7 [Kumar *et al.*, 2016] software.

Results

Phylogenetic analysis

We obtained 12 new sequences of different Flabellinidae *s.l.* species (Table 1). Sequence alignment of concatenated COI, 16S, H3 and 28S loci included 1734 positions. The topology of resulting concatenated trees from Bayesian Inference (BI) and Maximum likelihood (ML) analyses were mostly congruent, but several relationships were not supported in BI (Fig. 1). Species with papillate rhinophores from tropical waters (representatives of the genus *Coryphellina sensu* Korshunova *et al*. [2017]) clustered together in a monophyletic well-supported group (PP=1; ML=99). On both reconstructions specimens of *F. rubrolineata* from Philippines (CAS177287) and Vietnam (ZMMU Op-132) were recovered sister to *Flabellina lotos* (Korshunova *et al*., 2017) from Japan, however, these relationships did not receive high support. *Flabellina rubrolineata* collected in the Indo-West Pacific (specific locality is unknown) was recovered sister to these two species (PP = 1; ML = 100). *Flabellina rubrolineata* from the Red Sea and the Arabian Sea

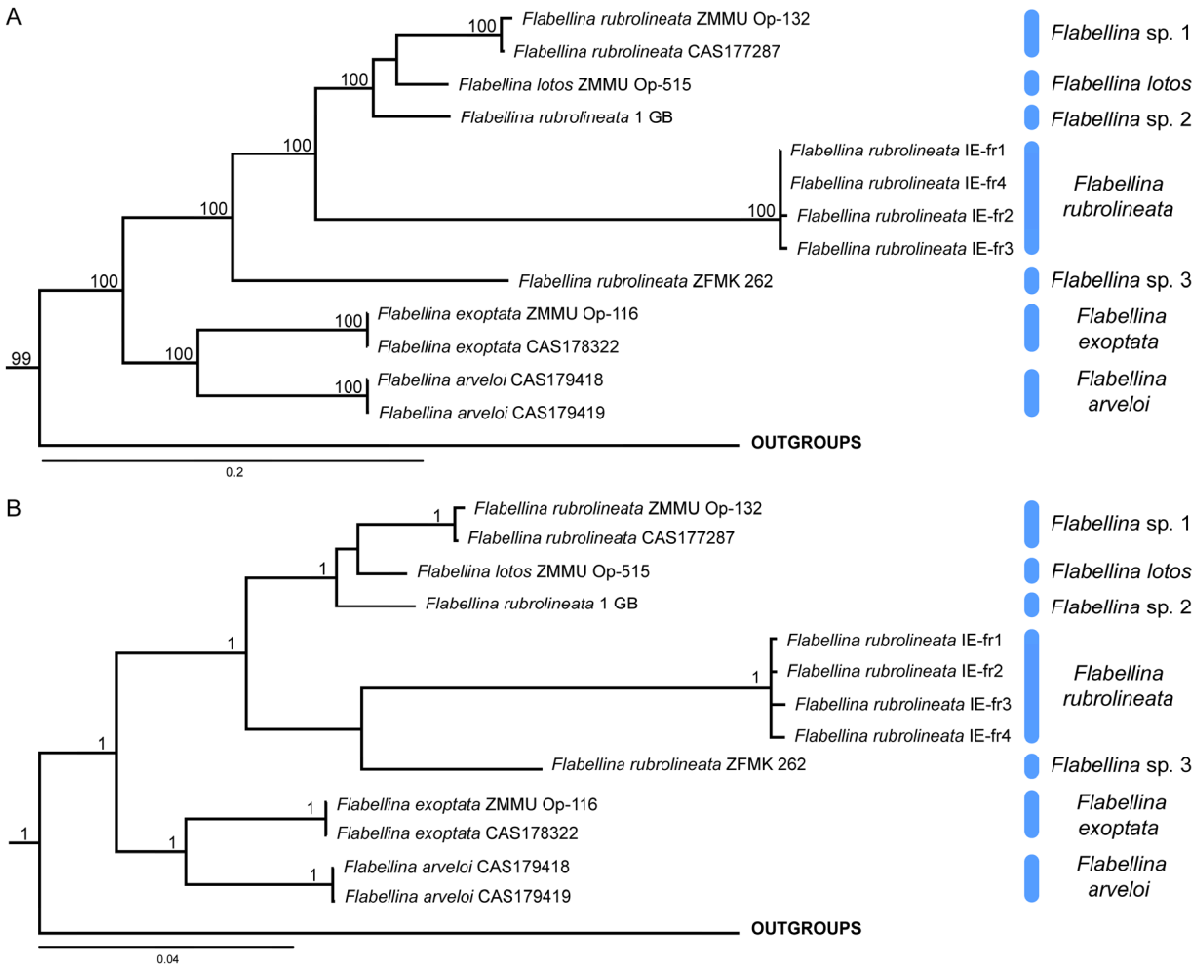

FIG. 1. Part of a molecular phylogenetic tree for the family Flabellinidae based on the concatenated dataset, showing phylogenetic relationships of different specimens initially identified as *Flabellina rubrolineata*. Blue boxes on the right represent the results of the ABGD test. For each species the revised ID is provided. **A**. Maximum Likelihood phylogenetic tree. Numbers above branches represent bootstrap values (>90). **B**. Bayesian phylogenetic tree. Numbers above branches show posterior probabilities (>0.95).

РИС. 1. Фрагмент молекулярно-филогенетического дерева для семейства Flabellinidae, построенного на основании комбинированного датасета, демонстрирующее филогенетические отношения различных особей, предварительно определенных как *Flabellina rubrolineata.* Голубые блоки справа обозначают результат делимитационного ABGD теста. Для каждого вида дано ревизованное название. **A**. Дерево, построенное методом максимального правдоподобия. Значения над ветвями обозначают поддержки бутстрепа (>90). **B**. Дерево, построенное при помощи байесовского алгоритма. Значения над ветвями обозначают апостериорные вероятности (>0.95).

clustered together in a highly supported clade (PP = 1; ML = 100) and showed sister relationships to the clade united Indo-West Pacific *F. rubrolineata* and *F. lotos*. Another *F. rubrolineata* specimen from Australia, was recovered sister to this large group (ML = 100) or showed unsupported sister relationships to *F. rubrolineata* from the type locality in the BI analysis. Overall, these results indicate that *F. rubrolineata* from the Red and the Arabian seas represents a distinct species, which differs from specimens collected in the Indo-West Pacific region and Australia.

Species delimitation

ABGD test of representatives of the genus *Coryphellina sensu* Korshunova *et al*. [2017] recovered 7 initial partitions (P = 0.0010-0.1110). These groups corresponded to clades and derived singletons recovered in the molecular phylogenetic analysis (Fig. 1). Uncorrected interspecific *p*-distance values (Table 2) varied from 8.8% (between *F. lotos* and *Flabellina* sp. 2) to 17.06% (between *F. rubrolineata* and *Flabellina* sp. 3). Intraspecific *p*-distances in case of *F. rubrolineata* were only 1.17% and in other species these values did not exceed 1%.

Table 2. Uncorrected *p*-distances calculated in MEGA7 (%) for COI marker.

Таблица 2. Нескорректированные *p*-дистанции для маркера COI, посчитанные в программе MEGA7 (в %).

No	Species	1	2	3	4	5	6
1	*Flabellina rubrolineata*						
2	*Flabellina lotos*	12.28					
3	*Flabellina* sp. 1	13.55	8.18				
4	*Flabellina* sp. 2	12.38	8.88	9.11			
5	*Flabellina* sp. 3	17.06	14.01	15.65	17.06		
6	*Flabellina arveloi*	15.42	14.02	14.49	12.38	17.76	
7	*Flabellina exoptata*	16.59	12.85	14.72	13.79	16.82	13.08

Taxonomic description

Suborder Cladobranchia
Family Flabellinidae Bergh, 1889
Flabellina McMurtrie, 1831
Flabellina rubrolineata (O'Donoghue, 1929)
(Figs 2-4)

Material examined: IE-fr1, Arabian Sea, Gulf of Aden, Gulf of Tadjoura, Djibouti, 11°41.219'N, 43°11.651'E, 13 m, on hydroids, 06.12.2019 (1 spm). IE-fr2-5, Red sea, Egypt, 27°13.122'N, 33°50.555'E, 1 m, on hydroids, 09.05.2014 (4 spms).

Description based on studied specimens. *External morphology* (Fig. 2). Length (preserved) from 8 to 12 mm. Body slender, foot slender with long anterior corners. Oral tentacles 2-3 times longer than rhinophores. Rhinophores highly papillate, bearing up to 50 papillae on their inner side. Cerata arranged in distinct groups, up to seven groups per row. First group largest, with 8-12 cerata in group. Cerata cylindrical or finger-shaped, pointed distally. Digestive gland diverticula cylindrical, fills about ½-2/3 of ceratal volume. Well-defined discontinued notal edge under ceratal groups. Anus pleuroproctic, reproductive openings lateral, below first group of cerata.

Coloration. Background color translucent-white or milky-white. Digestive gland diverticula in cerata from bright-orange and pinkish-orange (Fig. 2B-D) to intense violet, almost black (Fig. 2A). Cnidosac area covered by bright orange pigment with intense lilac or purple subapical rings. Cerata and interceratal areas usually covered by sparse white opalescent speckling. Prominent thick purple or lilac line beginning between oral tentacles and continuing to tail on dorsal side. Two other pigment lines of same color located laterally under notal edge, continuing from head to tail. All three lines merging on dorsal side of tail. Rhinophores same color as body, with lilac tips and light-orange patches underneath them, oral tentacles covered by sparse white opalescent powder with lilac subapical rings.

Buccal mass (Fig. 3). Jaws typical for flabellinids, composed by two triangle plates with triangle masticatory process. Masticatory process with numerous sharp denticles, arranged in up to 8 rows (Fig. 3 A, B). Radula triseriate, radular formula: 27-34x1.1.1. Rachidian tooth elongated-triangular with short conical central cusp, bearing from 5 to 8 large denticles with deep furrows on both sides. Cusp sharp, small, in one specimen bifurcated tip was found (Fig. 3G). Cusp slightly compressed by adjacent denticles. Lateral teeth triangular with elongated cusp and 8-11 denticles on inner edge, in specimen IE-fr3 lateral teeth lack inner denticles (Fig. 3E, F). Outer surface with slight striation, and in several specimens it possesses 2-3 reduced denticles (Fig. 3 D, F). Base of teeth almost right angled proximally, oblong distally, with attenuated processes varying in length from short (Fig. 3C, D) to long (Fig. 3K).

Reproductive system (Fig. 4). Ampulla large, sausage-shaped. Proximal seminal receptacle bilobed. Vas deference loops and widens distally before entering penial sac, presenting prostatic area. Mucous gland lays distally into vagina, albumen and membrane glands next to proximal seminal receptacle, their connection to vagina is not clear. Proximal seminal receptacle small, opened into vagina distally. Penis small, conical, unarmed.

Distribution. Red Sea, Arabian Sea, West Indian Ocean [Gosliner *et al*., 2018; present study], Mediterranean Sea (not native) [Gat, 1993; Yokes, Rudman, 2004].

Remarks. Red and Arabian seas animals differ clearly from Indo-West Pacific specimens identified as *F. rubrolineata* by molecular data. Morphologically Red and Arabian seas animals possess distinct coloration with translucent white body, pink-orange to purple coloration of the digestive gland in cerata, and bright-orange markings of cnidosac

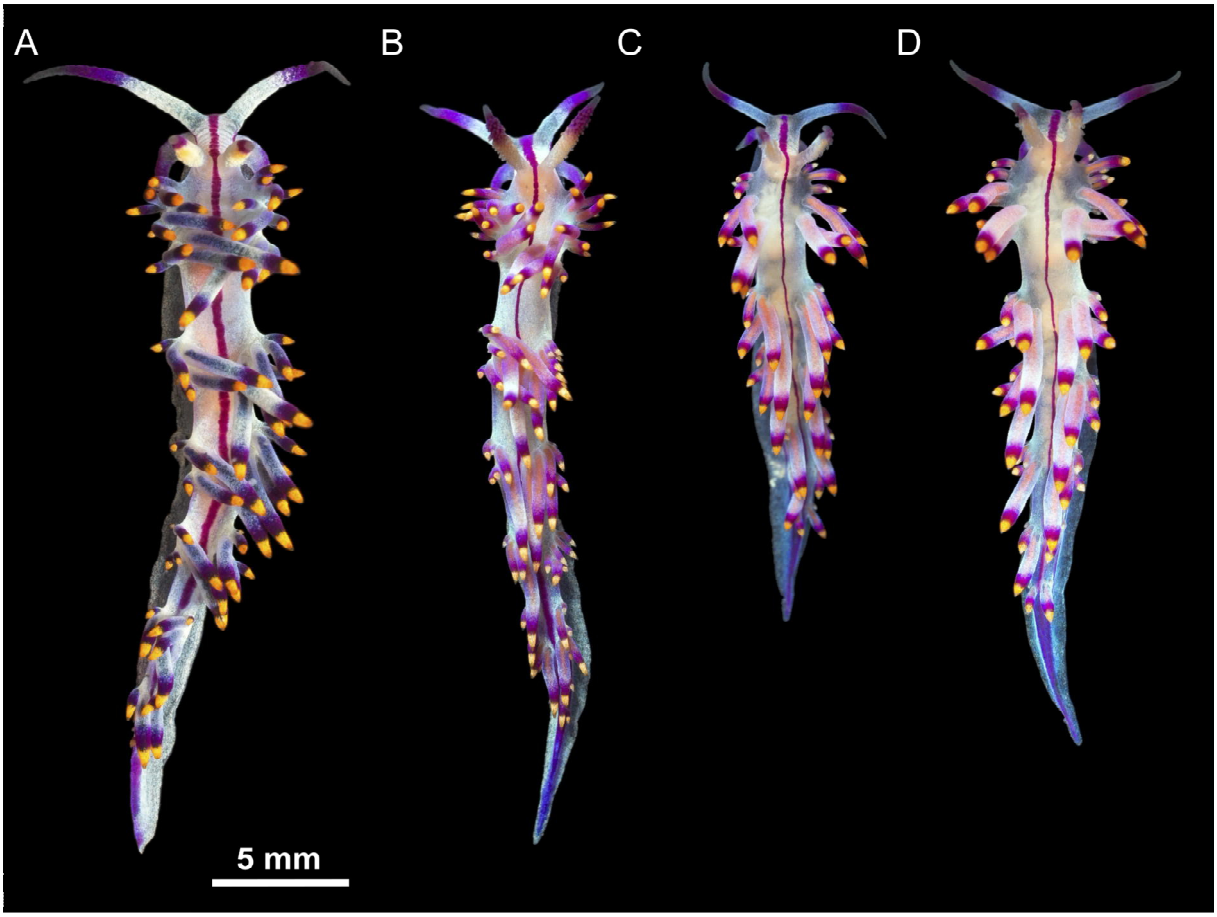

FIG. 2. Living specimens of *Flabellina rubrolineata.* **A**. IE-fr1. **B**. IE-fr2. **C**. IE-fr3. **D**. IE-fr4.

РИС. 2. Прижизненные фотографии *Flabellina rubrolineata.* **A**. IE-fr1. **B**. IE-fr2. **C**. IE-fr3. **D**. IE-fr4.

areas. Dorsal and lateral purple lines continue from head to tail. Indo-West Pacific specimens usually display a different external morphology: dorsal and lateral lines often discontinuous [Coleman, 2001; Gosliner *et al*., 2008], overall coloration may vary from white and light-orange and pink [Korshunova *et al*., 2017; Sreeraj *et al*., 2012a,b; Kaligis *et al.*, 2018] to intensive purple and violet [Coleman, 2001]. Regarding internal morphology, we failed to find any consistent differences between our specimens and Indo-West Pacific and Australian ones, whose internal characters were illustrated in Gosliner and Willan [1991] and Korshunova *et al*. [2017]. Those specimens possess lateral teeth with very long process attenuated basally, however this character was found to be variable in Red Sea specimens (Fig. 3D, F, H, K). Denticulation of the lateral teeth is also a variable character (Fig. 3D, F). Also, no distinct differences in configuration of the reproductive system was found [Gosliner, Willan, 1991; Korshunova *et al*., 2017; present study].

Discussion

As discussed in several recent works the alpha-taxonomy of the family Flabellinidae *s.l.* is controversial and different generic divisions have been proposed [Gosliner, Willan, 1991; Korshunova *et al*., 2017; Furfaro *et al.,* 2018; Gosliner *et al*., 2018]. According to recent revision by Korshunova *et al*. [2017], the genus *Coryphellina* O'Donoghue, 1929 was resurrected for the species *Flabellina rubrolineata* and Indo-West Pacific flabellinids with papillate rhinophores. However, Gosliner *et al*. [2018] suggested retaining most of Flabellinidae *s.l.* diversity, except representatives of the families Samlidae, Unidentidae and Apataidae in the genus *Flabellina* McMurtrie, 1831 until a dedicated revision would be undertaken. For ease of comparison and comprehension in the present study we use a traditional version of Flabellinidae *s.l.* taxonomy as published in Furfaro *et al.* [2018]. Nevertheless, our molecular analysis supports close phylogenetic re-

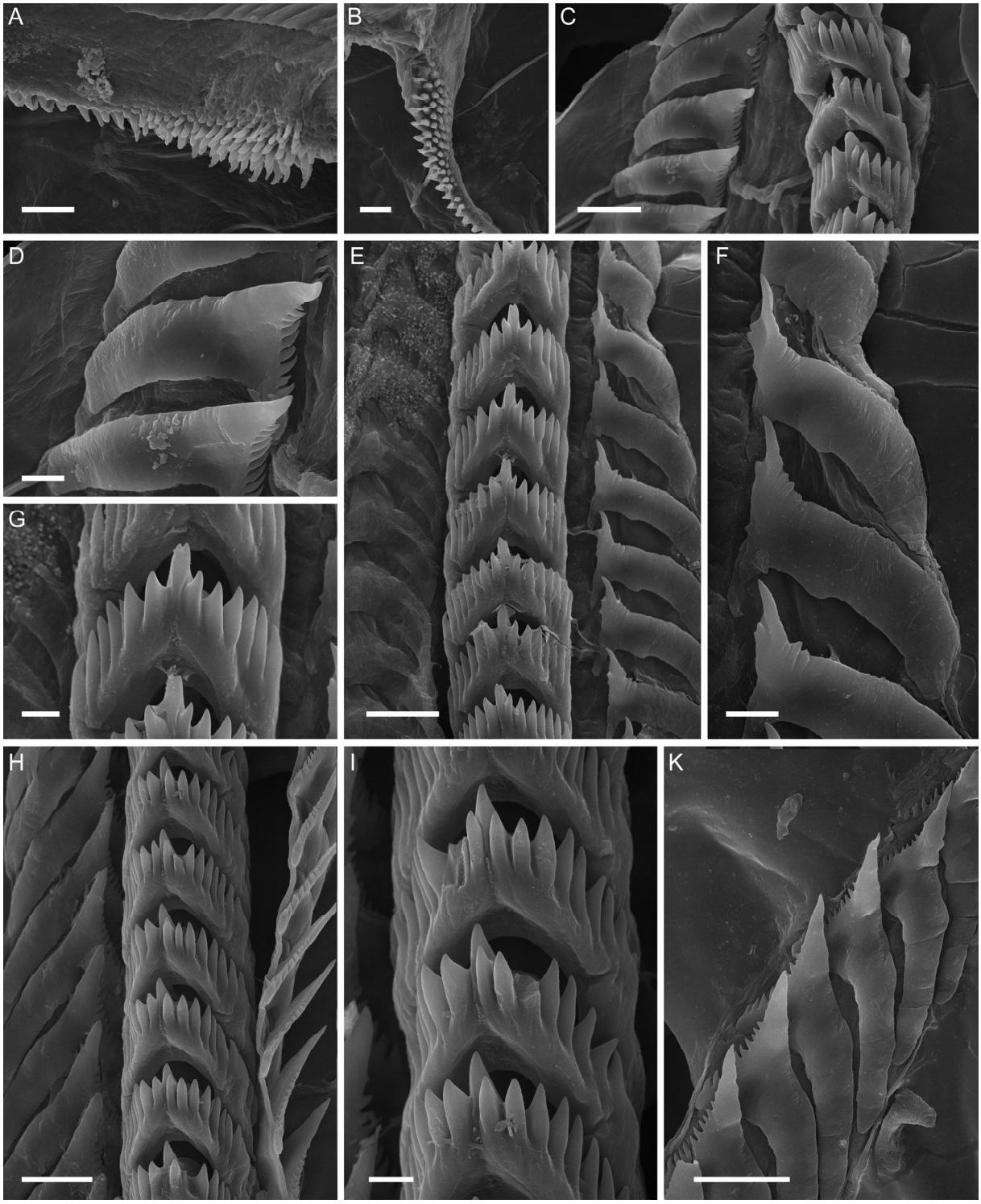

FIG. 3. SEM micrographs of jaws and the radula of *Flabellina rubrolineata.* **A**. IE-fr2, masticatory process of jaws. **B**. IE-fr3, masticatory process of jaws. **C**. IE-fr2, middle radular portion. **D**. IE-fr2, lateral teeth, note short attenuated process. **E**. IE-fr3, middle radular portion. **F**. IE-fr3, lateral teeth, note absence of inner denticles. **G**. IE-fr3, rachidian tooth, note bifurcated central cusp. **H**. IE-fr4, middle radular portion. **I**. IE-fr4, rachidian teeth. **K**. IE-fr4, lateral teeth, note long attenuated process. Scale bars: A-C, E, H, K – 30 μm; D, F, G, I – 10 μm.

РИС. 3. Микрофотографии челюстей и радулы *Flabellina rubrolineata* (СЭМ). **A**. IE-fr2, жевательный отросток челюстей. **B**. IE-fr3, жевательный отросток челюстей. **C**. IE-fr2, средний участок радулы. **D**. IE-fr2, латеральные зубы. **E**. IE-fr3, средний участок радулы. **F**. IE-fr3, латеральные зубы. **G**. IE-fr3, центральный зуб. **H**. IE-fr4, средний участок радулы. **I**. IE-fr4, центральные зубы. **K**. IE-fr4, латеральные зубы. Масштабные линейки A-C, E, H, K – 30 μm; D, F, G, I – 10 μm.

lationships of tropical flabellinids with papillate rhinophores, which is congruent to Korshunova *et al.* [2017] taxonomical scheme, as those species form a well-supported monophyletic group apart of the rest flabellinid diversity (Fig. 1).

Our molecular results show a clear separation of *F. rubrolineata* specimens collected in the Red Sea and the Arabian Sea from other specimens identified as *F. rubrolineata,* collected from the Indo-West Pacific and Australia (Fig. 1). These results support the suggestion by Gosliner *et al*. [2018] that "true" *F. rubrolineata* is restricted to the Red and Arabian seas and also non-natively occurs in the Mediterranean Sea, while in other regions it represents a complex of cryptic or pseudocryptic species. Externally *F. rubrolineata* shows similar coloration with unique combination of continuous dorsal and lateral lines, translucent white body and purple pigment of subapical areas and hepatic diverticula. Internally our specimens show very variable features of the radula (Fig. 3) with reduced denticles on lateral teeth, bifurcated central cusp of the rachidian teeth and different length of the attenuated process. Similar observations were given in the original description of *F. rubrolineata* by O'Donoghue [1929], who mentioned abnormal denticulation of the rachidian teeth and length of attenuated process of the lateral teeth. This variability should be taken in account for further studies of the *F. rubrolineata* species complex in other regions.

We were not able to compare the morphology of *F. rubrolineata* and *Flabellina* sp. 2 and *Flabellina* sp. 3 since only their DNA sequences were published in respective papers [Cheney *et al.,* 2014; Goodheart *et al.,* 2018]. Regarding *Flabellina* sp. 1 from Vietnam and Philippines [Cella *et al*., 2016; Korshunova *et al*., 2017] it differs from *Flabellina rubrolineata* in coloration pattern: *Flabellina* sp. 1 has orange-red patches on dorsolateral sides between ceratal groups and bands underneath cnidosac areas are red [Korshunova *et al*., 2017] in contrast to white body and violet or lilac bands in *F. rubrolineata.* At the same time internal traits like the morphology of radula and the reproductive system are similar in these two species. Specimens from Papua New Guinea and Australia studied by Gosliner and Willan [1991] also had different coloration, with opaque white patches on the dorsum and purple to red dorsal and dorsolateral lines and subapical ceratal rings. In addition, these specimens have less cerata in each group than *F. rubrolineata* from the Red Sea [Gosliner, Willan, 1991]. In any case, all these specimens showed identical internal morphology to *F. rubrolineata,* suggesting external morphology is the most important diagnostic trait for species separation within *F. rubrolineata* species complex.

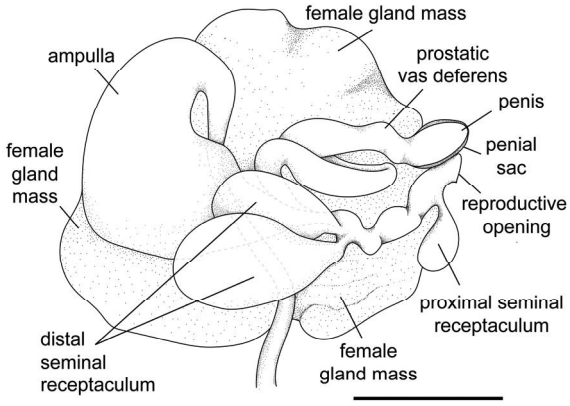

FIG. 4. Configuration of the reproductive system in *Flabellina rubrolineata* (IE-fr2). Scale bar: 0.7 mm.

РИС. 4. Морфология половой системы *Flabellina rubrolineata* (IE-fr2). Масштабная линейка 0,7 мм.

Both *Flabellina* sp. 2 and *Flabellina* sp. 3 were found in Australian waters, and, according to images of "*F. rubrolineata"* from Australia [Coleman, 2001], species diversity in this region may be even higher. *Flabellina* sp. 1 is restricted to the South China Sea, being found in Southern Vietnam and Philippines [Cella *et al*., 2016; Korshunova *et al*., 2017]. In addition, a large variety of "*F. rubrolineata*" color morphs are known from this area and other regions of the Indo-West Pacific [Coleman, 2001; Gosliner *et al*., 2015; Yonow, 2017; Papu *et al*., 2020]. While most of these morphs may represent several undescribed species, some of pink and violet color morphs with discontinuous dorsal and lateral lines may correspond to *Coryphellina lotos,* which was recently described from Japan [Korshunova *et al*., 2017]. Also, Risbec's "variété violaceé" of "*Flabellina" ornata* (Risbec, 1928) may correspond to pink or violet morphs with continuous longitudinal lines, since "variété violaceé" of "*Flabellina" ornata* has distinct pink to violet coloration and three continuous red lines [Risbec, 1928; Gosliner, Willan, 1991].

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