

Burnaia Miller, 2001 (Gastropoda, Heterobranchia, Nudibranchia): a facelinid genus with an Aeolidiidae's outward appearance

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Abstract In recent years, several morphological and molecular analyses have been undertaken to study the phylogenetic systematics of Aeolidiidae members. The monospecific genus *Burnaia* could not be included in the previous analysis, due to the lack of material. This study includes two specimens of *Burnaia helicochorda* from Australia and places them in their systematic position using two mitochondrial and one nuclear genes (COI and 16S, and H3, respectively). A description of its anatomy is also included with colour pictures of the animal and scanning electron micrographs of radula and jaws. Based on our results, *B. helicochorda* does not belong to Aeolidiidae since it appears nested among some facelinids.

Keywords Molluscan diversity · Molecular phylogeny · Taxonomy · Cladobranchia · Morphological convergence

Introduction

Burnaia helicochorda (Miller 1987), originally ascribed to *Aeolidia* Cuvier, 1798, was considered a member of Aeolidiidae although the author did not provide any reason for that systematic position. According to Miller (1987, 2001), this species was of easy identification by its external colouration, large body, lamellate rhinophores, and cerata in arches. Actually, because of all these morphological aspects, Miller (2001) decided to create a new genus, *Burnaia*, for this species. Since it was first described, *B. helicochorda* has not been reported many times (Rudman 2001; Grove 2015), and for this reason, Carmona et al. (2013), who conducted a molecular phylogeny of the family Aeolidiidae, could not include material of this not very common aeolid.

Recently collected material from Victoria, Australia, permitted us to study the systematic position of this species. This fresh material allowed us to use a molecular approach as well as to re-examine the morphology and anatomy of *B. helicochorda*, providing for the first time scanning electron photographs of the radula and jaws.

Materials and methods

Molecular work

DNA extraction, amplification, and sequencing

Two specimens of *B. helicochorda* were studied. One hundred twenty-four additional sequences were obtained from GenBank (see Table 1 for full list of samples, localities, and vouchers). *Tritonia challengeriana* Bergh, 1884, was chosen as out-group because of its basal

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Table 1 List of specimens used for phylogenetic analyses

Family	Species	Locality	Collection dates	Voucher	GenBank accession Nos.		
					COI	16S	H3
Tritoniidae Lamarck, 1809	<i>Tritonia challengeriana</i> Bergh, 1884	Bouvetoya (EA, GB)	30 Jun 04		HM162718	HM162643	HM162550
Dendronotidae Allman, 1845	<i>Dendronotus venustus</i> MacFarland, 1966	Santa Monica (California, GB)	Dec 07		HM162709	HM162630	HM162536
Proctonotidae Gray, 1853	<i>Janolus mirabilis</i> Baba and Abe, 1970	Philippines (GB)	19 May 09		HM162750	HM162674	HM162583
Aeolidiidae Gray, 1827	<i>Aeolidia papillosa</i> (Linnaeus, 1761)	Sweden	14 Sep 11	MNCN/ ADN: 51929	JX087534	JX087462	JX087596
	<i>Aeolidiella alderi</i> (Cocks, 1852)	France (MED)	26 Jul 02	ZSM Mol 20020982	HQ616765	HQ616728	HQ616794
	<i>Anteaeolidiella cacaotica</i> (Stimpson, 1855)	Eastern Australia	14 Feb 10	MNCN/ ADN: 51922	JX087528	JX087455	JX087590
	<i>Baeolidia moebii</i> Bergh, 1888	Philippines	16 Apr 08	CASIZ 177602	HQ616770	HQ616733	HQ616799
	<i>Berghia coerulescens</i> (Laurillard, 1830)	Croatia	03 Dec 04	ZSM Mol 20041584	JQ997049	JQ996845	JQ996946
	<i>Bulbaeolidia alba</i> (Risbec, 1928)	Philippines	21 May 09	CASIZ 180387	JQ997012	JQ996805	JQ996904
	“ <i>Cerberilla</i> ” <i>annulata</i> (Quoy and Gaimard, 1832)	Marshall Is.	24 Jul 00	CASIZ 182227	–	JQ996866	JQ996967
	“ <i>Cerberilla</i> ” <i>bernadettae</i> Tardy, 1965	Spain (EA)	06 Apr 108	MNCN/ ADN: 51957	JX087555	JX087489	JX087625
	“ <i>Cerberilla</i> ” cf. <i>affinis</i> (Quoy and Gaimard, 1832)	Philippines	16 May 09	CASIZ 180421	–	JQ996863	JQ996964
	<i>Limenandra nodosa</i> Haefelfinger and Stamm, 1958	Balearic Is. (Spain, MED)	Sep 2007	MNCN/ ADN 24.923	HQ616768	HQ616731	HQ616797
<i>Spurilla neapolitana</i> (delle Chiaje, 1841)	Italy	17 Mar 09	MNCN/ ADN: 51976	JX087584	JX087521	JX087659	
Babakinidae Roller, 1973	<i>Babakina anadoni</i> (Ortea, 1979)	Brazil	Feb 06	MNRJ 10893	HQ616746	HQ616709	HQ616775
	<i>Babakina indopacifica</i> Gosliner, González-Duarte and Cervera, 2007	Philippines (GB)	20 Mar 08	–	HM162754	HM162678	HM162587
Facelinidae Bergh, 1889	<i>Burnaia helicochorda</i> Miller, 1987	Victoria, Australia	26 Oct 07	NMV F152957	–	–	KT200150
		Victoria, Australia	13 Feb 08	NMV F155816	KT200148	KT200147	KT200149
	<i>Cratena peregrina</i> Gmelin, 1791	Senegal	30 May 05	MNCN 15.05/ 53691	HQ616752	HQ616715	HQ616781
	<i>Dondice banyulensis</i> Portmann and Sandmeier, 1960	Spain (EA)	26 May 09	MNCN 15.05/ 53693	–	HQ616740	HQ616804
		Spain (MED, GB)	–	–	AF249782	–	–
<i>Facelina annulicornis</i> (Chamisso and Eysenhardt, 1821)	Azores Is. (Portugal)	11 Jun 02	CASIZ 186793	JQ997076	JQ996881	JQ996986	

Table 1 continued

Family	Species	Locality	Collection dates	Voucher	GenBank accession Nos.		
					COI	16S	H3
	<i>Favorinus branchialis</i> (Rathke, 1806)	Spain (EA)	26 Jun 07	MNCN 15.05/ 53695	HQ616761	HQ616724	HQ616790
	<i>Favorinus elenalexiarum</i> García and Troncoso, 2001	Costa Rica (EP, GB)	17 Apr 07	–	HM162755	HM162679	HM162588
	<i>Godiva quadricolor</i> (Barnard, 1927)	South Africa (EA, GB)	09 Jan 08	–	HM162692	HM162602	HM162508
	<i>Hermosita hakunamatata</i> (Ortea, Caballer and Espinosa, 2003)	Mexico	17 Feb 06	CASIZ 174088	KP143674	KP143670	KP143678
	<i>Moridilla brockii</i> Bergh, 1888	Philippines	29 Apr 11	CASIZ 186245	JQ997083	JQ996888	JQ996994
	<i>Noumeaella isa</i> Marcus and Marcus, 1970	Philippines	01 May 11	CASIZ 186249	JQ997084	JQ996889	JQ996995
	<i>Phidiana lynceus</i> Bergh, 1867	Cuba	21 Jul 08	MNCN/ ADN: 51995	JX087562	JX087497	JX087633
	<i>Phyllodesmium horridum</i> (Macnae, 1954)	South Africa (EA, GB)	03 Jan 08	–	HM162757	HM162681	HM162590
	<i>Pleurolidia juliae</i> Burn, 1966	Philippines	05 May 05	CASIZ 186217	JQ997094	JQ996899	JQ997007
	<i>Protaeolidiella atra</i> Baba, 1955	Japan	17 Feb 04	NSMT-Mo 78853	KP143676	KP143672	KP143680
		Japan	18 Feb 04	NSMT-Mo 78852	KP143675	KP143671	KP143679
	<i>Pruvotfolia longicirra</i> (Eliot, 1906)	Cape Verde	Mar 10	MNCN 15.05/ 53703	HQ616760	HQ616723	HQ616789
	<i>Pruvotfolia pselliotes</i> (Labbé, 1923)	France (EA)	05 Sep 04	MNCN 15.05/ 53705	HQ616762	HQ616725	HQ616791
	<i>Sakuraeolis enosimensis</i> (Baba, 1930)	California (GB)	13 Dec 07	–	HM162758	HM162682	HM162591
Fionidae Alder and Hancock, 1855	<i>Fiona pinnata</i> (Eschscholtz, 1831)	Morocco (EA)	22 Dec 10	MNCN/ ADN: 51997	JX087558	JX087492	JX087628
Flabellinidae Bergh, 1881	<i>Flabellina affinis</i> (Gmelin, 1791)	Balearic Is. (Spain, MED)	14 Jul 07	MNCN 15.05/ 53696	HQ616753	HQ616716	HQ616782
	<i>Flabellina babai</i> Schmekel, 1972	Chafarinas Is. (MED)	25 Feb 07	MNCN 15.05/ 53698	HQ616754	HQ616717	HQ616783
	<i>Flabellina baetica</i> García-Gómez, 1984	Spain (EA)	14 Jan 05	MNCN 15.05/ 53699	HQ616755	HQ616718	HQ616784
	<i>Flabellina ischitana</i> Hirano and Thompson, 1990	Morocco (EA)	07 Mar 08	MNCN 15.05/ 53700	HQ616756	HQ616719	HQ616785
	<i>Flabellina pedata</i> (Montagu, 1815)	Spain (MED)	13 Oct 07	MNCN 15.05/ 53702	HQ616758	HQ616721	HQ616787

Table 1 continued

Family	Species	Locality	Collection dates	Voucher	GenBank accession Nos.		
					COI	16S	H3
Piseinotecidae Edmunds, 1970	<i>Piseinotecus gabinieri</i> (Vicente, 1975)	Spain (MED)	13 Oct 07	MNCN/ ADN: 52000	JX087561	JX087495	JX087631
	<i>Piseinotecus gaditanus</i> Cervera, García-Gómez and García, 1987	Spain (EA)	20 Jun 07	MNCN 15.05/ 53704	HQ616759	HQ616722	HQ616788
	<i>Piseinotecus</i> sp.	Philippines (GB)	22 Apr 08	–	HM162694	HM162604	HM162510

ATL Atlantic Ocean, EA eastern Atlantic Ocean, EP eastern Pacific, GB GenBank, MED Mediterranean

phylogenetic position within Cladobranchia (Pola and Gosliner 2010). Tissue samples were taken from the foot. Extraction, amplification, purification, and sequencing of portions of the COI, 16S rRNA, and H3 genes followed the methods described in Carmona et al. (2013, 2014a, b). Sequence reactions were run on a 3730XL DNA sequencer (Applied Biosystems). Sequences were verified by forward and reverse comparisons and have been deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analyses

Sequences were assembled and edited with Geneious Pro version 4.7.6 (Drummond et al. 2009), aligned in MAFFT (Kato et al. 2009), and further checked using MacClade version 4.06 (Maddison and Maddison 2005). The most variable regions from the 16S rRNA alignment were removed using the default settings in Gblocks (Talavera and Castresana 2007). Excluding “indel-rich” regions, the tree was in general very similar when including the variable regions. Therefore, final analyses were performed including all bases. Sequences of COI, 16S, and H3 were trimmed to 657, 445, and 327 base pairs, respectively.

The best-fit evolutionary model (GTR + I + G for the three genes) was determined in MrModeltest version 2.3 (Nylander 2004), using the Akaike information criterion (Akaike 1974). MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) was used for Bayesian inference analysis and to estimate posterior probabilities (PP) for node support with two runs of 10,000,000 generations each. Convergence was checked in TRACER version 1.5 (Drummond and Rambaut 2007) with a burn-in of 25 %. Maximum likelihood (ML) analyses were performed using the software RAXML version 7.0.4 (Stamatakis 2006), and node support was assessed with nonparametric bootstrapping (BS) with 5000 replicates, random starting trees, and parameters estimated from each data set under the model

selected for the original data set. Only nodes supported by $BS \geq 70$ (Hillis and Bull 1993) and $PP \geq 0.95$ (Alfaro et al. 2003) are discussed.

Source of specimens and morphology

The specimens studied were borrowed from the Museum Victoria (Melbourne, Australia). Specimens were dissected by dorsal incision. Their internal features were examined and drawn under a stereoscopic microscope with the aid of a camera lucida. The buccal mass was removed and soaked in a 10 % sodium hydroxide solution to dissolve the connective and muscle tissue, leaving only the radula and jaws. Both, radula and jaws, were then rinsed in water, dried, and mounted for examination under a scanning electron microscopy (SEM).

Results

Phylogenetic analysis

The combined data set yielded a sequence alignment of 1429 positions. No saturation was observed across genes and codon positions, not even in the third position (not shown). The resulting combined tree provided better resolution than H3, COI, or 16S separately (not shown). Figure 1 shows the phylogenetic hypothesis based on the combined data set constructed by Bayesian inference. The topology of the ML tree was identical (not shown), although the bootstrap values were lower than the PP in deeper nodes.

Burnaia helicochorda and the remaining species of Aeolidiidae included in this study clustered in two distinct and well-separated clades ($PP = 1$, $BS = 100$, and $PP = 0.99$, $BS = 72$, respectively). *B. helicochorda* were nested among different facelinid species ($PP = 0.99$,

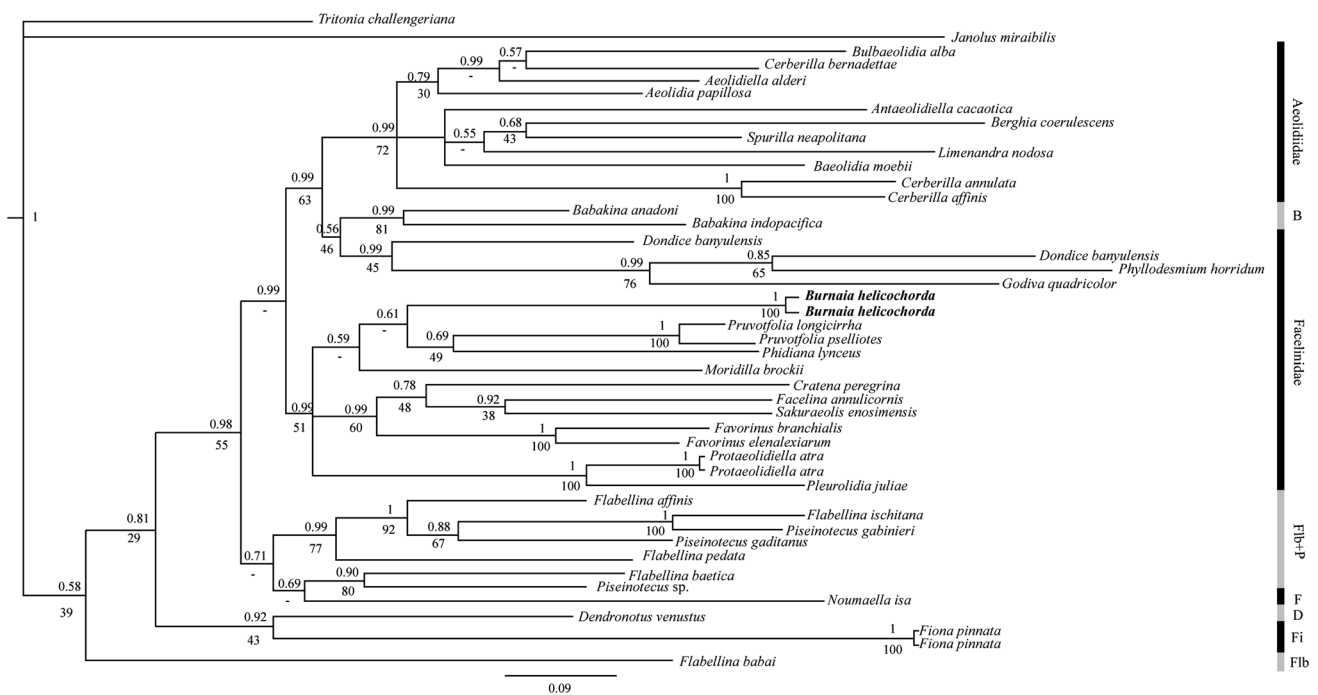


Fig. 1 Phylogenetic hypothesis for *B. helicochorda* based on the combined data set (H3 + COI + 16S) inferred by Bayesian analysis (BI). Numbers above branches represent posterior probabilities from

BI. Numbers below branches represent bootstrap values from ML. *B. helicochorda* specimens in bold. *B* Babakiniidae, *Fbl* Flabellinidae, *P* Piseinotecidae, *F* Facelinidae, *D* Dendronotidae, *Fi* Fionidae

BS = 51): *Pruvotfolia longicirra* (Eliot, 1906), *P. pselliotes* (Labbé, 1923), *Phidiana lynceus* Bergh, 1867, *Moridilla brockii* Bergh, 1888, *Cratena peregrina* (Gmelin, 1791), *Facelina annulicornis* (Chamisso and Eysenhardt, 1821), *Sakuraeolis enosimensis* (Baba, 1930), *Favorinus branchialis* (Rathke, 1806), *Favorinus elenalexiarum* Garcia and Troncoso, 2001, *Protaeolidiella atra* Baba, 1955 and *Pleurolidia juliae* Burn, 1966. However, the relationships between them were not resolved, and Facelinidae was not recovered as monophyletic.

Diagnosis of *Burnaia* following Miller (2001):
 Body long, narrow, oral tentacles moderately long, tapered, rhinophores longish, lamellate almost to base; cerata cylindrical, long, mobile (diverticula loosely spiralled), arranged in horseshoe-shaped arches, in single rows; cleioproctic, anus in centre of second arch, renal pore abanal, immediately in front of arch, reproductive apertures at tip of rear limb of first arch; foot narrow, rounded at front end extending as short, blunt lobes; oral glands small, club-shaped; radular tooth a very low, wide arch, blade narrowish, denticles almost uniform; jaw masticatory process complexly denticulate.

- Burnaia helicochorda* (Miller 1987) (Figure 2)
- Aeolidia helicochorda* Miller 1987: 391, figs. 1–5.

Type locality: Goat Island Bay, New Zealand.
Type material: The holotype was deposited at the Museum of New Zealand (M.87451) (Miller (1987)).
Material examined (NMV F155816), one specimen, adult, mature, partially dissected, 11 mm in length preserved, Victoria, Australia, collected by Leon Altoff, 13.ii.08; (NMV F152957), one specimen, adult, mature, 5 mm in length preserved, Victoria, Australia, collected by Leon Altoff, 26.x.07.

Geographical distribution Originally described from North of New Zealand (Miller 1987), this species has been also reported in eastern Australia (Rudman 2001; present study) and Tasmania (Grove 2015).

External morphology (Fig. 2a, b) The body is elongate and narrow when fully extended, tapering gradually to the fairly short tail. The foot corners are rounded. The body colour is translucent white, but the dorsal surface may have orange pigmentation, which is denser from the area immediately posterior to the head to the posterior end of the body. The rhinophores, oral tentacles, and foot corners are also covered with orange. From the anterior base of the rhinophores to the edge of the head, there is longitudinal orange band that joins a U-shaped mark, which surrounds the edge of the head and runs forward to the base of the oral tentacles. The rhinophores, which are a bit shorter than the oral tentacles, are

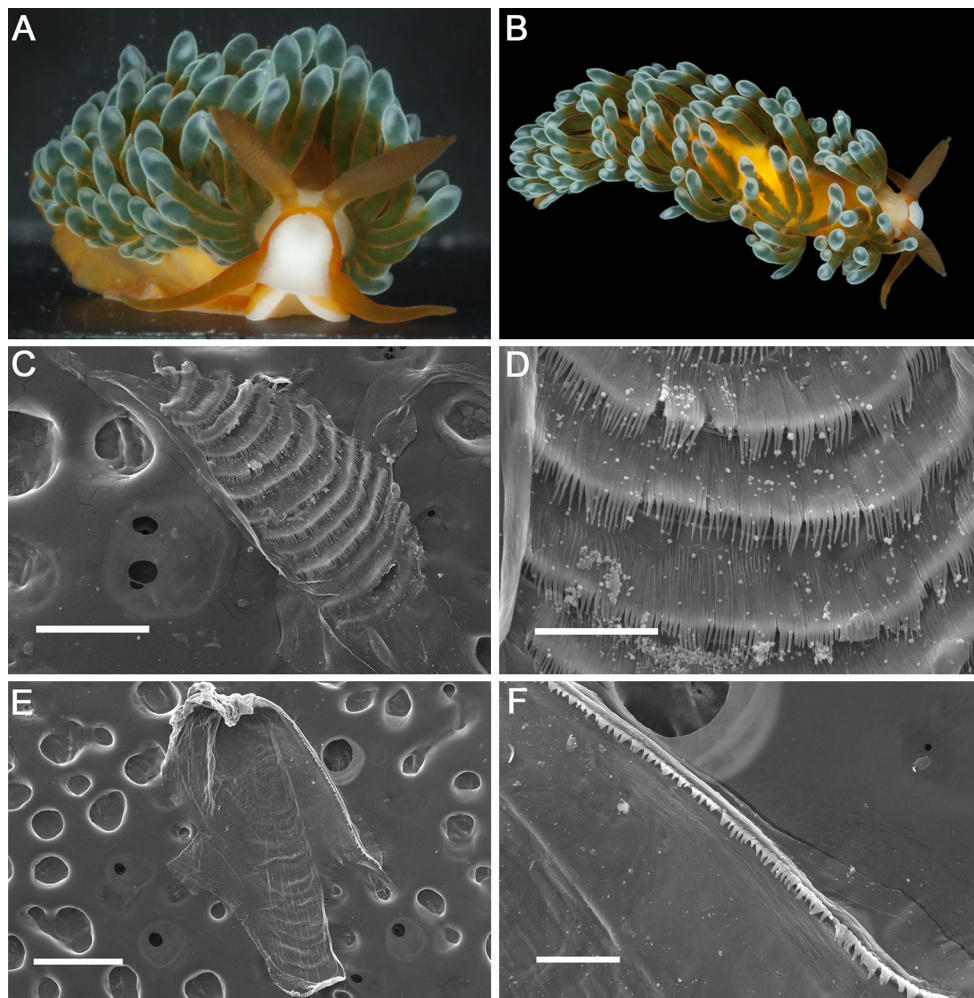


Fig. 2 *Burnaia helicochorda* (Miller 1987). **a, b** Frontal and dorsal view of the living animal, photograph by L. Altoff. **c, d** Scanning electron photographs (NMV F155816). **c** General view of the radula,

scale bar 125 μm . **d** Radular teeth, *scale bar* 75 μm . **e** Complete view of the jaw, *scale bar* 0.5 mm. **f** Detailed view of the masticatory border, *scale bar* 125 μm

perfoliate, bearing up to 18 lamellae. A pair of black eyes is visible at the posterior side of the rhinophores base.

The cerata are arranged in arches, with gaps between the arches that decrease posteriorly. Each arch contains between two and 11 cerata. They extend from behind the rhinophores to the posterior end of the body. The ceratal length is variable, but all are slender, cylindrical, with a small swelling at the apical part. The cerata are translucent, but covered by orange pigmentation. The olive green ramifications of the digestive gland are visible through the ceratal wall and have several constrictions over their length. The distal 1/3 of the cerata is opaque greyish green. On the right side of the body, the gonopore is situated among the cerata of the anteriormost group. The anus is on the right side of the body, in a cleioproctic position, located within the second ceratal arch.

Anatomy (Fig. 2c–f) The radula is uniseriate ($11 \times 0.1.0$, NMV F155816). The teeth decrease in size

towards the posterior region of the radula (Fig. 2c). The radular teeth are pectinate with 58–124, fine, elongate, and acutely pointed denticles (Fig. 2d). The jaws are translucent and oval (Fig. 2e). The masticatory border of the jaws is finely denticulate (Fig. 2f). The oral glands occur dorso-laterally to the buccal bulb. They are moderately short and increase in size towards the posterior end. Salivary glands were not observed.

The reproductive system was not examined so as not to damage the specimen, but was described in detail by Miller (1987, Fig. 5).

Discussion

The inclusion of *B. helicochorda* within the paraphyletic Facelinidae represents an intriguing and unexpected outcome. As Miller (1987, 2001) pointed out, in body shape *B.*

helicochorda resembles *Limenandra nodosa* and *L. fusiformis*. In addition, the pectinate radula could be considered as the typical radula of the Aeolidiidae members (Carmona et al. 2014a, b, c). However, our molecular study separates *B. helicochorda* from Aeolidiidae and places it in a clade composed by facelinids. Carmona et al. (2013) and more recently Carmona et al. (2015) found out a similar situation with the species *P. atra* and *P. juliae*, formerly considered as primitive members of Aeolidiidae and now clearly situated within Facelinidae. This last outcome was supported by the feeding habits of these two species, since they prey on hydroids instead of sea anemones and other anthozoans that represent the characteristic prey of aeolidiids (Gosliner et al. 2008; Carmona et al. 2013). Nevertheless, we were unable to find information about the diet of *B. helicochorda*. Finally, in terms of morphology and internal anatomy, our study suggests re-evaluation of the apparent morphological synapomorphies that have traditionally united the aeolidid genera and species is needed. A more general and comprehensive revision of the Facelinidae and Aeolidida is also necessary given the fact that Facelinidae is not monophyletic (Carmona et al. 2013, 2015; present study).

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