The Linnean Society of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2016, **177**, 481–506. With 13 figures

Aeolidia papillosa (Linnaeus, 1761) (Mollusca: Heterobranchia: Nudibranchia), single species or a cryptic species complex? A morphological and molecular study

KAREN KIENBERGER[1], LEILA CARMONA[1,*], MARTA POLA[2], VINICIUS PADULA[3,4], TERRENCE M. GOSLINER[5] and JUAN LUCAS CERVERA[1]

[1]*Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus de Excelencia Internacional del Mar (CEI·MAR), Universidad de Cádiz, Polígono Río San Pedro, s/n, Ap. 40, 11510, Puerto Real, Cádiz, Spain*
[2]*Departamento de Biología, Edificio de Biología, Campus de Excelencia Internacional UAM+CSIC, Universidad Autónoma de Madrid, C/Darwin, 2, 28049 Madrid, Spain*
[3]*SNSB, Zoologische Staatssammlung München, Münchhausenstraße 21, 82147 Munich, Germany*
[4]*Biocenter/Ludwig Maximilian, University of Munich, Großhadernerstraße 2, Planegg-Martinsried, 82152 Munich, Germany*
[5]*Department of Invertebrate Zoology, California Academy of Sciences, 55 Music Concourse Drive, Golden Gate Park, San Francisco, CA, 94118, USA*

Received 20 May 2015; revised 10 November 2015; accepted for publication 11 November 2015

Abstract

Aeolidia papillosa (Linnaeus, 1761) is a well-known aeolidiid species that has been reported to have a worldwide distribution in cold–temperate waters, mainly from the northern hemisphere. Molecular tools have recently shown that most cosmopolitan species usually belong to a taxonomic species complex. Here we used integrative taxonomy to test the range of distribution of *A. papillosa*, and to assess the existence of a putative species complex that has been traditionally included as a single species under the name *A. papillosa*. Maximum-likelihood and Bayesian analyses of partial DNA sequences of the mitochondrial cytochrome *c* oxidase subunit I and *16S* rRNA genes, and the nuclear gene *histone 3*, were used to infer phylogenetic trees. Automatic Barcode Gap Discovery (ABGD) species delimitation analyses and morphological study complemented the phylogenetic approach. Our results show that *A. papillosa* is a cosmopolitan and an amphi-Atlantic species, being distributed in the eastern and western Atlantic as well as in the eastern Pacific; however, some specimens from the UK and the Netherlands, together with specimens from Portugal, Galicia, and France, as well as the Californian and Oregon populations, emerge as two pseudocryptic species described herein: ***Aeolidia filomenae* sp. nov.** and ***Aeolidia loui* sp. nov.**, respectively. Finally, the specimens from Chilean coasts, previously attributed to *A. papillosa*, belong to a different species, *Aeolidia campbellii* (Cunningham, 1871), that is a senior synonym of *Aeolidia serotina* Bergh, 1873.

doi: 10.1111/zoj.12379

ADDITIONAL KEYWORDS: *Aeolidia campbellii* – *Aeolidia papillosa* – Gastropoda – Heterobranchia – Nudibranchia – phylogeny – sibling species.

INTRODUCTION

Many of the original descriptions of aeolidid nudibranchs written by classic authors are incomplete, making it difficult, if not impossible, to recognize

*Corresponding author. Present address: Department of Marine Sciences, University of Gothenburg, Box 460, 40530, Gothenburg, Sweden. E-mail: leila.carmona.barnosi@marine.gu.se

these species, even if they were encountered again. A major problem in dealing with the taxonomy of the Aeolidiidae is the availability of ancient names that provide confusing statements on type localities or species names. With an incomplete description and a suggested cosmopolitan distribution, *Aeolidia papillosa* (Linnaeus, 1761) is likely to represent an exemplary case of these issues.

Aeolidia Cuvier, 1798 is the type genus of Aeolidiidae, with only two or three species currently recognized as valid (e.g. Schrödl, 2003; Gofas, 2014). *Aeolidia papillosa*, the type species, and originally described from the Norwegian Sea, can be regarded as well known because it has been intensively studied over a long period of time (e.g. Hancock & Embleton, 1845; Edmunds *et al.*, 1974, 1976; Edmunds, 1983; Thompson & Brown, 1984; McFarland & Muller-Parker, 1993). Over the years, species from different localities of temperate–cold waters have been synonymized as *A. papillosa*: *Doris bodoensis* Gunnerus, 1770; Norwegian Sea; *Doris papillosa* Müller, 1776; North Sea; *Doris vermigera* Turton, 1807; British Isles; *Eolis rosea* Alder & Hancock, 1842; *Eolis obtusalis* Alder & Hancock, 1842; British Isles; *Aeolis murrayana* MacGillivray, 1843; Scotland; *Eolis papillosa* Hancock & Embleton, 1845; British Isles; *Eolis plumata* Dalyell, 1853; Scotland; *Eolis farinacea* Gould in Stimpson, 1853; New England (USA), and *Aeolidia papillosa* var. *pacifica* Bergh, 1879; Alaska Peninsula (USA). As a consequence of all these synonyms, different morphotypes and colour patterns have been explained as great intraspecific colour variability within this species. In addition, the continuing controversy about the validity of *Aeolidia collaris* Odhner, 1921; *Aeolidia serotina* Bergh, 1873; and *Aeolidia herculea* Bergh, 1894 (Er. Marcus, 1959; Schrödl, 1996, 1997, 2003; Martynov & Korshunova, 2011) has blurred the morphological characteristics of *A. papillosa* and therefore the limits among these species.

One of the most puzzling aspects of *A. papillosa* is its disjunct geographical range. Presently, it is accepted that its geographical distribution ranges from Norway (type locality), Iceland (Platts, 1985), and Greenland (Rosenberg, 2009) to California (Er. Marcus, 1961b; McDonald, 1983), passing through the Atlantic coast of the USA (Bleakney, 1996), Alaska (Dall, 1884), the Barents Sea (Russia) (Martynov & Korshunova, 2011), and northern Japan (Baba, 1935; Nakano, 2004; Debelius & Kuiter, 2007). Furthermore, there are some records from the west and east coasts of South America (Er. Marcus, 1959; Schrödl, 1996, 1997, 2003; Fig. 1). Developments in molecular biology, population genetics, and phylogeographic methods have boosted research on diversity and evolution in the marine environment. Molecular methods have highlighted the presence of several cryptic species in Heterobranchia (Carmona *et al.*, 2014a,b; Cooke *et al.*, 2014; Espinoza, DuPont & Valdés, 2014; Padula *et al.*, 2014; Pola, Roldán & Padilla, 2014a; Shipman & Gosliner, 2015; Wilson & Burghardt, 2015). Therefore, the wide and discontinuous geographic range of *A. papillosa*, as well as its variability in coloration, suggests that we may be dealing with more than a single species.

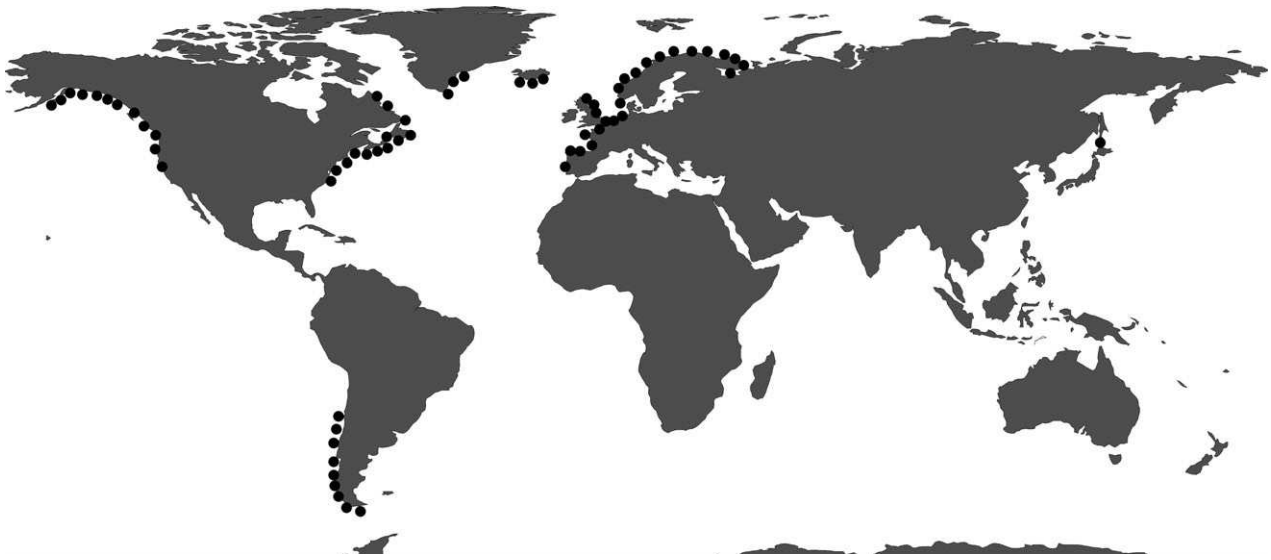

Figure 1. Currently accepted geographical distribution of '*Aeolidia papillosa*' (Platts, 1985; Schrödl, 1996, 1997, 2003; Debelius & Kuiter, 2007; Rosenberg, 2009).

In this paper, we attempt to clarify the taxonomic status of different populations of *A. papillosa* based on an integrative taxonomic approach. Specimens collected from almost the complete range of distribution of the target species have been used, including both hemispheres. Molecular data from two mitochondrial genes, cytochrome *c* oxidase I (*COI*) and *16S* ribosomal RNA (*16S*), and from one nuclear gene, *histone 3* (*H3*), were used in order to infer the phylogenetic hypothesis. Additionally, Automatic Barcode Gap Discovery (ABGD) species delimitation analyses, and the study of morphological traits, such as external morphology, radula, jaws, and the reproductive system, have been examined to supplement and compare against the molecular results.

MATERIAL AND METHODS

Literature review

A comprehensive review of the literature was conducted to determine the valid names for the species recognized in the molecular and morphological analyses. After the description of the type species, all available names for *Aeolidia* species are organized and discussed in this paper according to the year of publication. In the synonymy lists, references to the original description of the valid name and all synonyms as well as the first proposed change of binomen are included, but subsequent references are not.

Nomenclatural acts

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the International Code of Zoological Nomenclature (ICZN). The ZooBank Life Science Identifiers (LSIDs) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix (http://zoobank.org/). The LSID for this publication is: LSID urn:lsid:zoobank.org:pub:BC5635FF-D804-4782-9B07-AB81F8FBCB07.

Morphology

Specimens were dissected by dorsal incision. The internal features were examined and drawn under a dissecting microscope with a camera lucida. Special attention was paid to the morphology of the reproductive system, and the oral and salivary glands. The buccal mass was removed and dissolved in 10% sodium hydroxide until the radula was isolated from the surrounding tissue. The radula was then rinsed in water, dried, and mounted for examination by scanning electron microscopy (SEM).

Voucher specimens are held either at the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), California Academy of Sciences, San Francisco, USA (CASIZ), Zoologische Staatssammlung München, Munich, Germany (ZSM), and Zoological Museum of Moscow State University, Moscow, Russia (ZMMU), being named in agreement with the most recent classification of Aeolidiidae (Carmona *et al.*, 2013).

Molecular work

Taxon sampling

Samples were obtained by collection from the intertidal zone and by scuba-diving, and through the study of museum collections. Fifty-seven specimens of the '*Aeolidia papillosa*' species complex (including seven specimens of *Aeolidia filomenae* sp. nov., six specimens of *Aeolidia loui* sp. nov, and 27 specimens of the true *A. papillosa*) and 104 sequences from 36 different species obtained from GenBank (for the full list of samples, localities, and voucher references, see Table 1) that were used for phylogenetic inference. *Tritonia challengeriana* Bergh, 1884 was chosen as a distant out-group, whereas the remaining closely related species were chosen based on the work of Carmona *et al.* (2013).

DNA extraction, amplification, and sequencing

DNA was extracted from foot tissue of specimens preserved in 70–100% ethanol, except in the cases of small animals where the whole specimen was used. The DNeasy Blood & Tissue Kit (09/2001; Qiagen, Valencia, CA, USA) was used for DNA extraction.

Partial sequences of *COI*, *16S*, and *H3* were amplified by polymerase chain reaction (PCR) using the primers: LCO1490 (5′–GGTCAACAAATCATAAAGAT ATTGG–3′) and HCO2198 (5′–TAAACTTCAGGGTG ACCAAAAAATCA–3′) (Folmer *et al.*, 1994), for *COI*; 16S ar–L (5′–CGCCTGTTTATCAAAAACAT–3′) and 16S br–H (5′–CCGGTCTGAACTCAGATCACGT–3′) (Palumbi *et al.*, 1991), for *16S* rRNA; and H3AD5′3′ (5′–ATGGCTCGTACCAAGCAGACVGC–3′) and H3 BD5′3′ (5′–ATATCCTTR GGCATRATRGTGAC–3′) (Colgan *et al.*, 1998), for *H3*. These three gene regions are commonly used in systematic studies of heterobranchs (e.g. Eilertsen & Malaquias, 2013; Ohnheiser & Malaquias, 2013; Ortigosa *et al.*, 2014; Pola *et al.*, 2014a,b; Hallas & Gosliner, 2015; Shipman & Gosliner, 2015); however, several internal primers for *COI* and *H3* were designed for specimens that did not amplify with the universal primers (see Table 1 of Carmona *et al.*, 2013).

Polymerase chain reactions (PCRs) were conducted in 25μl reactions containing 1 μl of both forward and

Table 1. List of specimens used for phylogenetic analyses

Family	Species	Locality	Collection dates	Voucher	GenBank accession nos		
					COI	*16S*	*H3*
Tritoniidae (Lamarck, 1809)	*Tritonia challengeriana* (Bergh, 1884)	Bouvetoya (EA, GB)	30 June 2004		HM162718	HM162643	HM162550
Dendronotidae (Allman, 1845)	*Dendronotus venustus* (MacFarland, 1966)	Santa Monica (California, GB)	December 2007		HM162709	HM162630	HM162536
Proctonotidae (Gray, 1853)	*Janolus mirabilis* (Baba & Abe, 1970)	Philippines (GB)	19 May 2009		HM162750	HM162674	HM162583
Aeolidiidae (Gray, 1827)	*Aeolidia campbellii* (Cunningham, 1871)	Chile	11 May 2002	ZSM 20020700	KF317849	KF317837	KF317859
		Chile	16 March 2007	ZSM HF4(1280)	–	–	KF317860
	***Aeolidia filomenae* sp. nov.**	France (EA)	1 June 2009	MNCN 15.05/74470	JX087531	JX087459	JX087593
		France (EA)	18 June 2009	MNCN 15.05/74472	JX087532	JX087460	JX087594
		France (EA)	19 October 2012	MNCN 15.05/74478	KU160589	KU160563	–
		France (EA)	10 November 2012	MNCN 15.05/74477	KU160588	KU160562	KU160606
		the Netherlands	14 April 2012	MNCN 15.05/74474	KU160596	KU160570	KU160608
		Portugal	16 May 2014	MNCN 15.05/74480	KU160595	KU160569	–
		Scotland	7 March 2013	MNCN 15.05/74481	–	–	KU160605
		Scotland	18 April 2014	MNCN 15.05/74479	KU160586	KU160560	–
		Spain (EA)	17 March 2011	CASIZ 187742	JQ997037	JQ996832	JQ996933
		Spain (EA)	17 March 2011	CASIZ 187742	–	–	KU160599
		Spain (EA)	1 June 2011	MNCN/ADN: 51928	JX087533	JX087461	JX087595
	***Aeolidia loui* sp. nov.**	California (North)	26 May 2001	CASIZ 184504	JQ997035	JQ996830	JQ996931
		California (North)	28 Jan 2010	CASIZ 182214	JQ997036	JQ996831	JQ996932
		California (Central)	12 December 2012	MNCN 15.05/74482	KU160571	–	KU160598
		California (South)	April 2014	MNCN 15.05/74486	KU160590	KU160564	–
		Oregon	June 2006	MNCN 15.05/74483	KU160591	KU160565	KU160607
		Oregon	31 January 2014	MNCN 15.05/74484	KU160592	KU160566	–
		Oregon	31 January 2014	MNCN 15.05/74485	KU160593	KU160567	–
		Oregon	16 May 2014	MNCN 15.05/74487	KU160594	KU160568	–

Table 1. *Continued*

Family	Species	Locality	Collection dates	Voucher	GenBank accession nos		
					COI	*16S*	*H3*
	Aeolidia papillosa (Linnaeus, 1761)	Alaska	6 May 2012	MNCN 15.05/65212	KF317850	KF317838	KF317861
		Alaska	6 May 2012	MNCN 15.05/65213	KF317851	KF317839	KF317862
		Alaska	6 May 2012	MNCN 15.05/65210	–	KF317840	KF317863
		Alaska	6 May 2012	MNCN 15.05/65209	–	KF317841	KF317864
		Denmark	21 January 2014	MNCN 15.05/71562	KU160574	KU160545	–
		Denmark	21 January 2014	MNCN 15.05/71563	–	KU160546	–
		Denmark	21 January 2014	MNCN 15.05/71564	–	KU160547	–
		Denmark	21 January 2014	MNCN 15.05/71565	KU160575	KU160548	–
		Denmark	21 January 2014	MNCN 15.05/71566	KU160576	KU160549	–
		Denmark	21 January 2014	MNCN 15.05/71567	KU160577	KU160550	–
		Denmark	21 January 2014	MNCN 15.05/71569	KU160578	KU160551	–
		Denmark	21 January 2014	MNCN 15.05/71570	KU160579	KU160552	–
		Denmark	21 January 2014	MNCN 15.05/71571	KU160580	KU160553	–
		Denmark	21 January 2014	MNCN 15.05/71572	KU160581	KU160554	–
		Maine	9 August 2009	CASIZ 182329	JQ997038	JQ996833	JQ996934
		Massachusetts	November 2010	CASIZ 187760	JQ997039	JQ996834	JQ996935
		Massachusetts	November 2010	CASIZ 187760	JQ997040	JQ996835	JQ996936
		Massachusetts	26 October 2011	CASIZ 187841	JQ997041	JQ996836	–
		Massachusetts	26 October 2011	CASIZ 187841	JQ997042	–	JQ996937
		the Netherlands	14 April 2012	MNCN 15.05/65211	KF317852	KF317842	–
		the Netherlands	14 April 2012	MNCN 15.05/65214	KF317853	KF317843	KF317865
		the Netherlands	14 April 2012	MNCN 15.05/65215	KF317854	KF317844	KF317866
		the Netherlands	14 April 2012	MNCN 15.05/65216	KF317855	KF317845	KF317867
		Scotland	24 November 2012	MNCN 15.05/71559	KU160582	KU160556	KU160602
		Scotland	24 November 2012	MNCN 15.05/71560	KU160583	KU160557	KU160603
		Scotland	18 April 2014	MNCN 15.05/71574	KU160587	KU160561	–
		Scotland	24 November 2012	MNCN 15.05/71558	–	KU160555	KU160601
		Scotland	24 November 2012	MNCN 15.05/71561	KU160584	KU160558	KU160604
		Shetland Islands	–	MNCN 15.05/71573	KU160585	KU160559	–
		Sweden	14 September 2011	MNCN/ADN: 51929	JX087534	JX087462	JX087596
		Sweden	14 September 2011	MNCN/ADN: 51930	JX087535	JX087463	JX087597
		Sweden	14 September 2011	MNCN 15.05/65217	KF317856	KF317846	KF317868
		Washington	1 December 2010	MNCN 15.05/65208	KF317857	KF317847	KF317869

Table 1. *Continued*

Family	Species	Locality	Collection dates	Voucher	GenBank accession nos		
					COI	*16S*	*H3*
		Washington	1 December 2010	MNCN 15.05/65207	KF317858	KF317848	KF317870
		Washington	1 December 2010	MNCN/ADN: 51931	JX087536	JX087464	JX087598
		Barents Sea, Russia	August 2012	ZMMU Op-441	KU160597	–	–
	Aeolidiella alderi (Cocks, 1852)	France (MED)	26 July 2002	ZSM Mol 20020982	HQ616765	HQ616728	HQ616794
	Anteaeolidiella cacaotica (Stimpson, 1855)	Eastern Australia	14 February 2010	MNCN/ADN: 51922	JX087528	JX087455	JX087590
	Baeolidia moebii (Bergh, 1888)	Philippines	16 April 2008	CASIZ 177602	HQ616770	HQ616733	HQ616799
	Berghia benteva (Marcus, Er. 1958)	Brazil	31 July 2010	MZSP 96473	–	–	KF273245
	Berghia coerulescens (Laurillard, 1830)	Croatia	3 December 2004	ZSM Mol 20041584	JQ997049	JQ996845	JQ996946
	'*Cerberilla*' *annulata* (Quoy & Gaimard, 1832)	Marshall Islands	24 July 2000	CASIZ 182227	–	JQ996866	JQ996967
	'*Cerberilla*' *bernadettae* (Tardy, 1965)	Spain (EA)	6 April 20 l08	MNCN/ADN: 51957	JX087555	JX087489	JX087625
	'*Cerberilla*' cf. *affinis* (Quoy & Gaimard, 1832)	Philippines	16 May 2009	CASIZ 180421	–	JQ996863	JQ996964
	Limenandra nodosa (Haefelfinger & Stamm, 1958)	Balearic Islands (Spain, MED)	September 2007	MNCN/ADN 24.923	HQ616768	HQ616731	HQ616797
	Spurilla neapolitana (Delle Chiaje, 1841)	France (EA)	16 August 2006	MNCN/ADN: 51969	JX087574	JX087514	JX087650
Babakinidae (Roller, 1973)	*Babakina anadoni* (Ortea, 1979)	Brazil	February 2006	MNRJ 10893	HQ616746	HQ616709	HQ616775
Facelinidae (Bergh, 1889)	*Cratena peregrina* (Gmelin, 1791)	Senegal	30 May 2005	MNCN 15.05/53691	HQ616752	HQ616715	HQ616781

Table 1. *Continued*

Family	Species	Locality	Collection dates	Voucher	GenBank accession nos		
					COI	*16S*	*H3*
	Dondice banyulensis (Portmann & Sandmeier, 1960)	Spain (EA)	26 May 2009	MNCN 15.05/53693	–	HQ616740	HQ616804
		Spain (MED, GB)	–	–	AF249782	–	–
	Facelina annulicornis (Chamisso & Eysenhardt, 1821)	Azores (Portugal)	11 June 2002	CASIZ 186793	JQ997076	JQ996881	JQ996986
	Favorinus branchialis (Rathke, 1806)	Spain (EA)	26 June 2007	MNCN 15.05/53695	HQ616761	HQ616724	HQ616790
	Favorinus elenalexiarum (García & Troncoso, 2001)	Costa Rica (EP, GB)	17 April 2007	–	HM162755	HM162679	HM162588
	Godiva quadricolor (Barnard, 1927)	South Africa (EA, GB)	9 January 2008	–	HM162692	HM162602	HM162508
	Moridilla brockii (Bergh, 1888)	Philippines	29 April 2011	CASIZ 186245	JQ997083	JQ996888	JQ996994
	Noumeaella isa (Marcus & Marcus, 1970)	Philippines	1 May 2011	CASIZ 186249	JQ997084	JQ996889	JQ996995
	Phidiana lynceus (Bergh, 1867)	Cuba	21 July 2008	MNCN/ADN: 51995	JX087562	JX087497	JX087633
	Phyllodesmium horridum (Macnae, 1954)	South Africa (EA, GB)	3 January 2008	–	HM162757	HM162681	HM162590
	Pruvotfolia longicirrha (Eliot, 1906)	Cape Verde	March 2010	MNCN 15.05/53703	HQ616760	HQ616723	HQ616789
	Pruvotfolia pselliotes (Labbé, 1923)	France (EA)	5 September 2004	MNCN 15.05/53705	HQ616762	HQ616725	HQ616791
	Sakuraeolis enosimensis (Baba, 1930)	California (GB)	13 December 2007	–	HM162758	HM162682	HM162591

Table 1. *Continued*

Family	Species	Locality	Collection dates	Voucher	GenBank accession nos		
					COI	*16S*	*H3*
Fionidae (Alder & Hancock, 1855)	*Fiona pinnata* (Eschscholtz, 1831)	Morocco (EA)	22 December 2010	MNCN/ADN: 51997	JX087558	JX087492	JX087628
Flabellinidae (Bergh, 1881)	*Flabellina affinis* (Gmelin, 1791)	Balearic Islands (Spain, MED)	14 July 2007	MNCN 15.05/53696	HQ616753	HQ616716	HQ616782
	Flabellina babai (Schmekel, 1972)	Chafarinas Islands (MED)	25 February 2007	MNCN 15.05/53698	HQ616754	HQ616717	HQ616783
	Flabellina baetica (García-Gómez, 1984)	Spain (EA)	14 January 2005	MNCN 15.05/53699	HQ616755	HQ616718	HQ616784
	Flabellina ischitana (Hirano & Thompson, 1990)	Spain (EA)	26 March 2009	MNCN 15.05/53697	HQ616757	HQ616720	HQ616786
	Flabellina pedata (Montagu, 1815)	Spain (MED)	13 October 2007	MNCN 15.05/53702	HQ616758	HQ616721	HQ616787
Piseinotecidae (Edmunds, 1970)	*Piseinotecus gabinieri* (Vicente, 1975)	Spain (MED)	13 October 2007	MNCN/ADN: 52000	JX087561	JX087495	JX087631
	Piseinotecus gaditanus (Cervera, García-Gómez & García, 1987)	Spain (EA)	20 June 2007	MNCN 15.05/53704	HQ616759	HQ616722	HQ616788
	Piseinotecus sp.	Philippines (GB)	22 April 2008	–	HM162694	HM162604	HM162510

ATL, Atlantic Ocean; EA, eastern Atlantic Ocean; MED, Mediterranean; PAC, Pacific.

reverse primers (10 μM), 2.5 μl of dNTP (2 mM), a gene-dependent quantity of magnesium chloride (25 mM), 0.25 μl of Qiagen DNA polymerase (5 units μl^{-1}), 5 μl of 'Q–solution' (5×), 2.5 μl of Qiagen buffer (10×) (Qiagen Taq PCR Core Kit cat. no. 201225), and 2 μl of genomic DNA. Magnesium chloride volumes were: 3.5 μl for *COI* and *16S*; and 2 μl for *H3*. The amplification of *COI* was performed with an initial denaturation for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at 44°C (annealing temperature), and 1 min at 72°C, with a final extension of 7 min at 72°C. The *16S* amplification began with an initial denaturation for 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 44°C (annealing temperature), 1 min at 72°C, with a final extension of 7 min at 72°C. *H3* amplification was performed with an initial denaturation for 3 min at 95°C, followed by 40 cycles of 45 s at 94°C, 45 s at 50°C (annealing temperature), 2 min at 72°C, with a final extension of 10 min at 72°C.

Successful PCRs were purified by mixing 5 μl of PCR product with 2 μl of ExoSAP-IT (usb.affymetrix.com). Samples were incubated at 37°C for 15 min, followed by an inactivation step at 80°C for 15 min. Sequence reactions were run on a 3730XL DNA sequencer (Applied Biosystems). All new sequences have been deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analyses

DNA sequences were assembled and edited using GENEIOUS PRO 4.7.6 (Drummond *et al.*, 2009). All the sequences were checked for contamination with BLAST (Altschul *et al.*, 1990), implemented in the GenBank database. MAFFT (Katoh, Asimenos & Toh, 2009) was employed to align the sequences. The alignments were further checked by eye using MacClade 4.06 (Maddison & Maddison, 2005). Protein-coding sequences were translated into amino acids for confirmation of alignment. Pairwise uncorrected p–distance values between each taxon were calculated for the *COI* gene, using the cut-off values from Carmona *et al.* (2013) as reference thresholds. Saturation was visually inspected in MEGA 5.0 (Tamura *et al.*, 2011) by plotting for all specimens and the out-group the total number of pairwise differences (transitions and transversions) against uncorrected p–distances. For the *COI* and *H3* genes, saturation was further examined separately for the first, second, and third codon positions.

The most variable regions from the *16S* rRNA alignment were removed using the default settings in Gblocks (Talavera & Castresana, 2007). Excluding 'indel-rich' regions, the tree was generally poorly resolved with lower node support. Therefore, final analyses were performed including all bases.

Sequences of *COI*, *16S*, and *H3* were trimmed to 658, 446, and 327 base pairs, respectively.

Individual gene analyses and a concatenated analysis were performed. The best-fitting models of evolution for each gene and codon position were determined using the Akaike information criterion (Akaike, 1974), implemented in MrModeltest 2.3 (Nylander, 2004). The general time-reversible model GTR + I + G was selected for the three genes.

Maximum-likelihood (ML) analyses were performed using RAxML 7.0.4 (Stamatakis, Hoover & Rougemont, 2008), and node support was assessed with non-parametric bootstrapping (BS) with 5000 replicates, random starting trees, and parameters estimated from each data set under the model selected for the original data set. Bayesian inference analyses (BI) were conducted using MrBayes 3.1.2b (Ronquist & Huelsenbeck, 2003) for 10 million generations with two independent runs and a sampling frequency of 1000. The models implemented were those estimated with MrModeltest 2.3. The combined data set was partitioned among genes and positions, and the "unlink" command was used to allow all parameters to vary independently within each partition.

Convergence was diagnosed graphically by plotting the likelihood against the number of generations for each run using TRACER 1.4.1 (Drummond & Rambaut, 2007). For each analysis the first 2500 trees were discarded ('burn-in' period) and node support was assessed with posterior probabilities (PPs). Only nodes supported by bootstrap values ≥ 70 (Hillis & Bull, 1993) and posterior probabilities ≥ 0.95 were considered statistically significant (Alfaro, Zoller & Lutzoni, 2003).

Species delimitation analyses

To define species, we used an integrative approach including tree topologies, pairwise uncorrected p–distance, and ABGD, as well as the morphological and anatomical data. Pairwise uncorrected p–distance values between each taxon were calculated for the *COI* gene using PAUP* 4.0b 10.0 (Swofford, 2002). The ABGD method (Puillandre *et al.*, 2012) was run for *COI* and for all of the *Aeolidia* specimens included in this study. The ABGD settings were as follows: P_{min} = 0.001, P_{max} = 0.1, steps = 10, X = 1.0, Nb bins = 20, and Jukes Cantor (JC69) and Kimura (K80). In addition, the NeighborNet network (Bryant & Moulton, 2004) was constructed using SplitsTree 4 (Huson & Bryant, 2006), and an unrooted statistical parsimony network was generated for *COI* using TCS 1.21 (Clement, Posada & Crandall, 2000) with a 95% connection limit. The resulting tree was converted to graphics in FigTree 1.4.0, and final adjustments were performed in Adobe Illustrator CS5.

RESULTS

Molecular results

The combined data set of three genes yielded a sequence alignment of 1431 positions. We obtained almost 100 new sequences: 22 for *H3*, 35 for *COI*, and 38 for *16S*. No saturation was observed across genes and codon positions (not shown). The combined tree provided better resolution than *H3*, *COI*, or *16S* separately (see the complete trees in Figure S1). The *COI* gene better resolved the relationships at the species and genus level, followed by *16S*, whereas *H3* did not provide any resolution, including S*purilla neapolitana* (Delle Chiaje, 1841) and *Facelina annulicornis* (Chamisso & Eysenhardt, 1821) among the *Aeolidia* specimens. These results arise from unlinked genes, as they can present different evolutionary histories (Huelsenbeck, Bull & Cunningham, 1996; Maddison, 1997; Rokas *et al.*, 2003). Many studies that focused on Heterobranchia have shown that combined analysis can provide better-resolved trees (e.g. Malaquias & Reid, 2008; Carmona *et al.*, 2013; Camacho-García *et al.*, 2014; Oskars, Bouchet & Malaquias, 2015), which is clearly the case in the current study.

Although bootstrap values were lower than posterior probabilities in larger clades, the topologies of the ML trees were congruent with the results yielded by Bayesian analysis, and thus the ML trees are not shown. Figure 2 shows the *Aeolidia* phylogenetic hypothesis based on the combined data set represented by BI (for the complete tree, see Figure S2). Figure 2 also illustrates the results obtained from different delimitation approaches used in this study.

The *Aeolidia* species included in this study clustered together with maximum support (PP = 1, BS = 100). This clade splits into four subclades, but the relationship among them remained unresolved. The identity of the real *A. papillosa* was based on the placement of the specimen closer to the type locality, Norway. All the specimens of the real *A. papillosa* clustered together (PP = 0.94, BS = 91), regardless of whether they were from the Atlantic or the Pacific oceans. Specimens from California and Oregon (*A. loui* sp. nov.) constituted a clade with high support (PP = 1, BS = 97), as well as the two specimens of *A. campbellii* included in this study (PP = 1, BS = 99). The last clade included the specimens of *A. filomenae* sp. nov. from Portugal, north of Spain, the Atlantic coast of France, Scotland, and one specimen from the Netherlands (PP = 1, BS = 94). The minimum genetic distance (uncorrected p–distance for *COI*) among the specimens of these four clades ranged from 8.4 to 18.2%, supporting the existence of four different species.

The ABGD analyses revealed the existence of cryptic diversity in the '*Aeolidia papillosa*' species complex. These analyses recovered the same four groups as in the phylogenetic analyses, with *p* values ranging from 0.005 to 0.06. The ABGD grouping results were independent of the chosen model (Jukes Cantor or Kimura). The NeighbourNet graph created by SplitsTree (Fig. 3) depicts a low level of conflict among *Aeolidia* species because of the lack of many parallel edges of equal lengths. In addition, the SplitsTree analysis supports the results obtained by the tree reconstructions and the ABGD analyses. Regarding true *A. papillosa*, 15 haplotypes were identified among the 31 specimens sequenced for *COI*. The haplotype network (Fig. 4) separated the *A. papillosa* specimens into three groups: North Atlantic plus Baltic Sea; Barents Sea; and Northeast Pacific.

SYSTEMATICS

Nudibranchia Blainville, 1814
Cladobranchia Willan & Morton, 1984
Family Aeolidiidae Gray, 1827
Genus *Aeolidia* Cuvier, 1798

Type species. Limax papillosa Linnaeus, 1761.

Diagnosis of the genus *Aeolidia*, according to Cuvier (1798):

Qui ont le corps comme les tritonies; mais leurs organs de la respiration sont des espèces de feuilles ou d'écailles membraneuses, rangées comme des tuiles des deux côtés du dos.

The morphology of the *Aeolidia* body resembles that of tritonids; however, the respiratory system is similar to leaves or membranous scales, arranged like tiles on both sides of the back.

Diagnosis of the genus *Aeolidia* according to Thompson & Brown (1984):

Body depressed, broad; anterior foot angles acute; cerata little flattened, arise in regular, numerous, close and transversal rows; long and agile oral tentacles; rhinophores smooth; each radular tooth forms a smoothly curved arch bearing regularly graded denticles; smooth-edged jaws; anus cleioprotic; penis unarmed.

Aeolidia papillosa (Linnaeus, 1761) (Figs 5A–F, 6A–H, 7A)

Limax papillosa Linnaeus, 1761: 508.
Doris bodoensis Gunnerus, 1770: 170, figs 11–16.
Doris papillosa Müller, 1776: 229.
Doris vermigera Turton, 1807: 133.
Eolidia papillosa Johnston, 1835: 376.
Eolis rosea Alder & Hancock, 1842: 34.

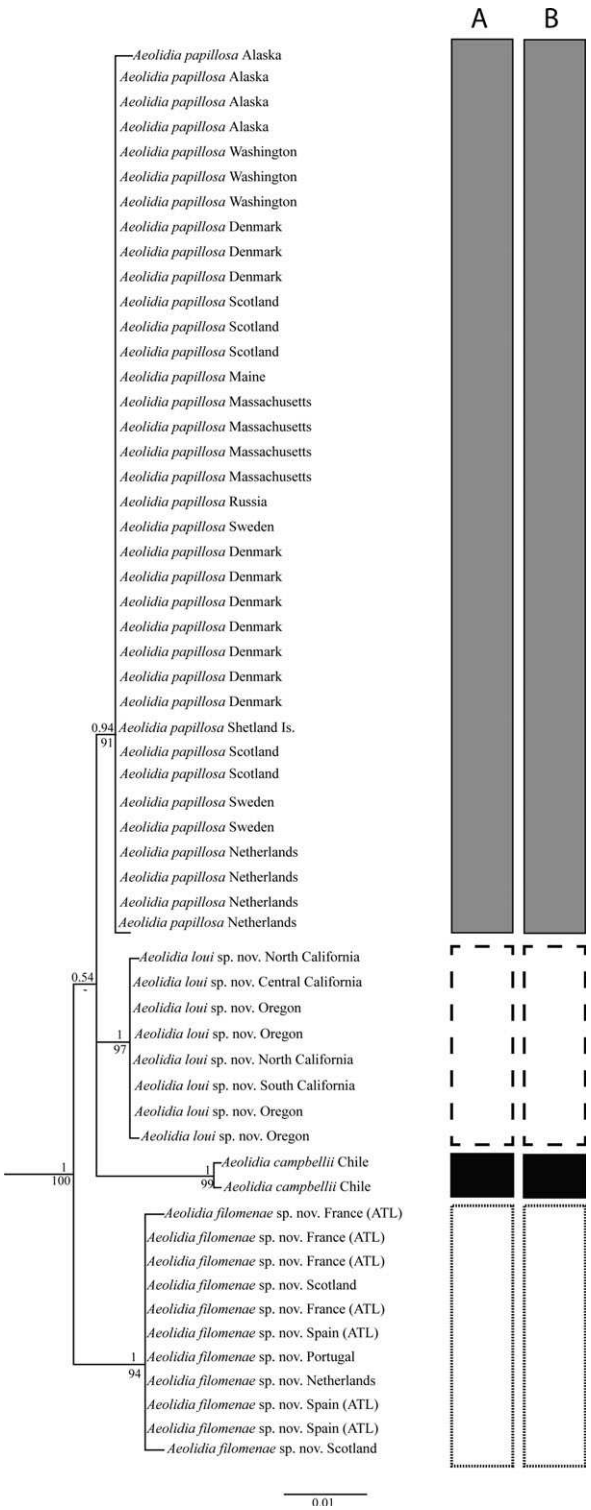

Figure 2. Phylogenetic hypothesis for the genus *Aeolidia* based on the combined data set (*H3* + *COI* + *16S*) inferred by Bayesian analysis (BI). Numbers above branches represent posterior probabilities from BI. Numbers below branches represent bootstrap values from ML. The results from different species delimitation methods are also plotted. Abbreviations: A, ABGD, based on the *COI* data set, with both models (Jukes Cantor and Kimura); B, number of species based on SplitsTree results; EA, eastern Atlantic Ocean; grey rectangle, *Aeolidia papillosa*; dashed rectangle, ***Aeolidia loui* sp. nov.**; black rectangle, *Aeolidia campbellii*; pointed rectangle, ***Aeolidia filomenae* sp. nov.**

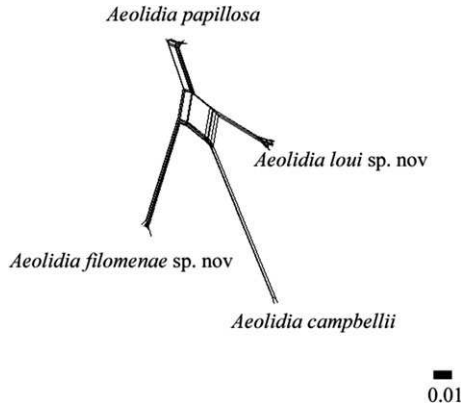

Figure 3. NeighbourNet graph of the *COI* sequences.

Eolis obtusalis Alder & Hancock, 1842: 34.
Aeolis murrayana MacGillivray, 1843: 70.
Eolis papillosa MacGillivray, 1843: 70.
Eolis plumata Dalyell, 1853: 300, plate XLIV, figs 1–2.
Eolis farinacea Gould in Stimpson, 1853: 25.
Aeolidia papillosa var. *pacifica* Bergh, 1879: 75, plate 1, figs 1–6.

Material examined. MNCN 15.05/65218, one specimen, dissected, 10 mm in length preserved, Sweden, Tjärno, ix.10, collected by Juan Lucas Cervera Currado; MNCN 15.05/65219, one specimen, dissected, 8 mm in length preserved, Sweden, Tjärno, ix.10, collected by Juan Lucas Cervera Currado; MNCN 15.05/65211, one specimen, dissected, 40 mm in length preserved, Netherlands, Eastern Scheld, iv.12, collected by Peter H. van Bragt; MNCN 15.05/65210, one specimen, dissected, 40 mm in length preserved, USA, Alaska, Cook Inlet, Katchemak Bay, v.12, collected by Katrin Iken; MNCN 15.05/65209, one specimen, dissected, 23 mm in length preserved, USA, Alaska, Cook Inlet, Katchemak Bay, v.12, collected by Katrin Iken; MNCN 15.05/65208, one specimen, dissected, up to 35 mm in length preserved, USA, Washington State, Gig Harbor, xii.10, collected by David Behrens; MNCN 15.05/65207, one specimen, dissected, up to 30 mm in length preserved, USA, Washington State, Gig Harbor, xii.10, collected by David Behrens; CASIZ 182329, one specimen, dissected, USA, Maine, viii.09, collected by Larry Harris; CASIZ 187841, one specimen, dissected, 10 mm in length preserved, USA, Massachusetts, x.11 collected by Larry Harris; CASIZ 187760, one specimen, dissected, 15 mm in length preserved, USA, Massachusetts, xi.10, collected by Larry Harris.

Geographical distribution. Amphiboreal species, common along the Atlantic coasts of Europe and North America. *Aeolidia papillosa* ranges from the Norwegian shores to the Netherlands, and is also known from all around the coasts of the British Isles (Alder & Hancock, 1852; Thompson & Brown, 1984; Picton & Morrow, 1994) and on the southern coastlines of the Barents Sea (southern margin of the Arctic Ocean) (Platts, 1985; Martynov & Korshunova, 2011). This species is extremely common on the West Atlantic coast of New England, USA (Bleakney, 1996; Shine, 2012), and as far south as North Carolina (Er. Marcus, 1961a). It also occurs along the coast of the eastern Pacific, from Washington (USA), through British Columbia, Canada, up to Alaska (O'Donoghue, 1921; MacFarland, 1966; McDonald, 1983; Behrens & Hermosillo, 2005). Although it could not be verified in this study, it is also reported from the Asiatic coast of Sakhalina and the east cost of Hokkaido, Japan (Baba, 1935; Nakano, 2004).

Type locality. Norwegian Sea, Eastern Atlantic.

Type material. To our knowledge no type material remains in any of the primary Swedish museums or institutions. Therefore, we designate the specimen MNCN 15.05/65217 from Sweden, Tjärno, as the neotype.

External morphology (Fig. 5A–F). The specimens may reach up to 120 mm in length. The body is broad and relatively low, narrowing to the posterior end of the foot. The foot corners are tentaculiform.

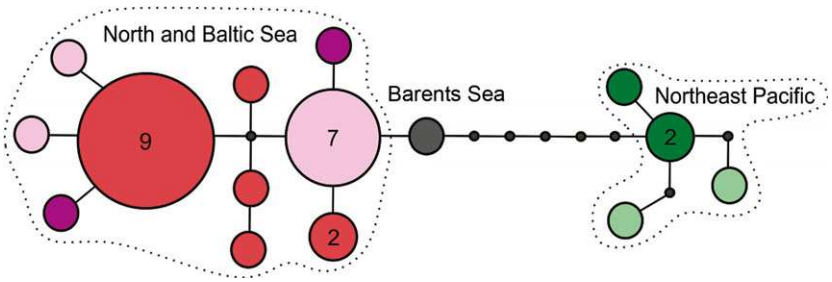

Figure 4. *COI* haplotype network for *Aeolidia papillosa*: red, Denmark; pink, Scotland; purple, Sweden; dark grey, Russia; green, Washington; bright green, Alaska. Numbers indicate the haplotype frequency, when higher than one.

Figure 5. Different morphotypes of *Aeolidia papillosa*: A, specimen from Sweden, Tjärno, photo by Marta Pola, MNCN 15.05/65206; B, specimen from Russia, Barents Sea, Kandalakshsky Bay, photo by Alexander Martynov; C, specimen from the Netherlands, Eastern Scheld, photo by Peter H. van Bragt, MNCN 15.05/65211; D, specimen from USA, Massachusetts, photo by Larry Harris, CASIZ 187760; E, specimen from USA, Washington State, Gig Harbor, photo by David Behrens, MNCN 15.05/65207; F, specimen from USA, Alaska, Cook Inlet, Katchemak Bay, photo by Katrin Iken, MNCN 15.05/65212.

The background colour is extremely variable: from light white–beige, through mustard brownish, to reddish brown or dark brown (Fig. 5A–F). Flecks are scattered all over the surface of the notum. These flecks are slightly darker than the general colour, varying in their intensity and density within a single specimen. A white Y–shaped or triangular mark extending from the oral tentacles to the pericardial area between the rhinophores may be present (Fig. 5B). The rhinophores are conical, blunt, and smooth. They are a little bit darker than the general body colour, lighter at the apices, with light flecks all over their surface. The oral tentacles are translucent, longer than the rhinophores, and occasionally exhibit white tips.

The cerata are elongate and thin, never flattened, with a uniform diameter for most of their length. Those in the anterior region and near the posterior end of the foot are smaller than those in the middle. A bare zone from behind the rhinophores to the pericardium is found. The cerata have a darker coloration than the rest of the body. The apex is white–beige. The digestive gland is visible throughout the body wall. The cerata are arranged in numerous and densely packed rows (up to 25), making the indistinct rows hardly recognizable. Each row contains between eight and 12 cerata, decreasing in size towards the posterior end of the foot. The cleioproctic anus is located between the ninth and tenth row of the right side. The gonopore is situated between the

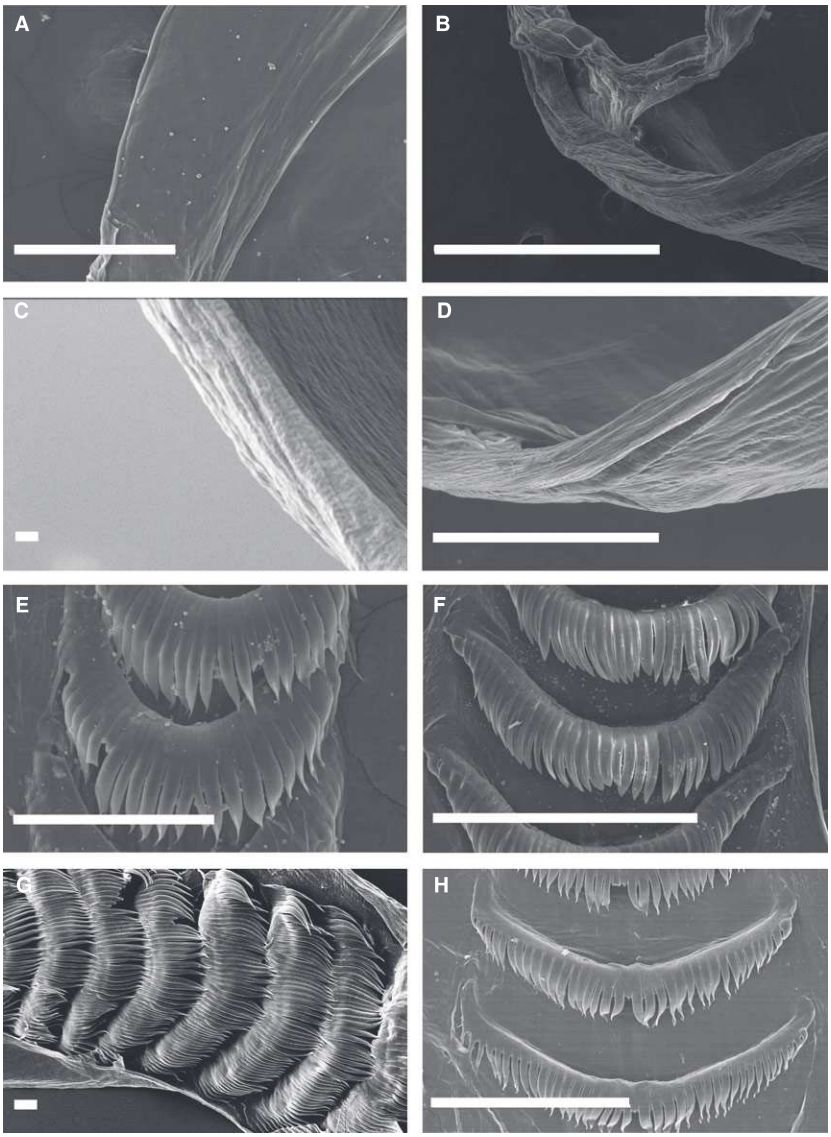

Figure 6. Scanning electron micrographs of the radula and masticatory edge of four *Aeolidia papillosa* specimens: A, detailed view of the masticatory border of the specimen from Sweden (MNCN 15.05/65218), scale bar 250 μm; B, detailed view of the masticatory border of the specimen from the Netherlands (MNCN 15.05/65211), scale bar 500 μm; C, detailed view of the masticatory border of the specimen from Maine (CASIZ 182329), scale bar 3 μm; D, detailed view of the masticatory border of the specimen from Alaska (MNCN 15.05/65210), scale bar 1000 μm; E, radular teeth of the specimen from Sweden (MNCN 15.05/65218), scale bar 150 μm; F, radular teeth of the specimen from the Netherlands (MNCN 15.05/65211), scale bar 500 μm; G, radular teeth of the specimen from Maine (CASIZ 182329), scale bar 10 μm; H, radular teeth of the specimen from Alaska (MNCN 15.05/65210), scale bar 500 μm.

sixth and eighth anterior rows of cerata on the right side.

Internal anatomy (Figs 6A–H, 7A). The jaws are strong and rounded, with a smooth masticatory edge (Fig. 6A–D). The radular formulae are 18 × 0.1.0 (MNCN 15.05/65218, 10 mm; CASIZ 182329), 24 × 0.1.0 (MNCN 15.05/65210, 40 mm), and 31 × 0.1.0 (MNCN 15.05/65211, 40 mm). The teeth are progressively smaller towards the posterior region of the radula and pectinate with 25–58 denticles (Fig 6E–H), which are relatively broad. One of the specimens from Alaska has a minute central cusp (Fig. 6H). Salivary glands were not found. Oral glands are absent.

The reproductive system is diaulic (Fig. 7A). The preampullary duct widens into the narrow and elon-

gate ampulla. The ampulla bifurcates into the oviduct and the vas deferens. The vas deferens is moderately long and enters the wider proximal portion of the penial sac. The penial papilla is devoid of any armature. The pear-shaped receptaculum seminis joins the oviduct and enters the female gland mass. The vagina opens ventrally adjacent to the penis.

Remarks. Aeolidia papillosa was first described as *Limax papillosus* (Linnaeus, 1761). Under the variability of its colour pattern, a large number of specific names, mostly from north-western Europe, were erected. Many of these names are currently considered as junior synonyms. In fact for specimens of *A. papillosa* from the British coasts, Alder & Hancock (1852) noted 'that its variability, in form and colour, has led to raise some fake species, especially when these descriptions were based on juveniles with a lower number of papillae'.

This species has been recorded from the north-western Atlantic as well as from the north-eastern and north-western Pacific (Fig. 1). Our study confirms that specimens from the north-western Atlantic and the north-eastern Pacific coasts attributed to *A. papillosa* belong to the same species as those from Sweden (close to the type locality). Therefore, we can conclude that *Eolis farinacea* (first described in the Gulf of Maine, north-west Atlantic) and *A. papillosa* var. *pacifica* from Alaska (north-east Pacific) are two junior synonyms of *A. papillosa*. Regarding the presence of this species in Japan (Baba, 1935; Nakano, 2004), further studies are needed in order to confirm that the Japanese population is conspecific with *A. papillosa*.

Finally, our specimens of *A. papillosa* agree with the available descriptions of this species (e.g. Alder & Hancock, 1852; Shine, 2012). The only difference was the presence of salivary glands, as noted by Bergh (1879); however, this disagreement could arise from subjective misinterpretation, as these glands are very faint and small, and therefore they are very easy to miss during the dissecting process.

Aeolidia campbellii (Cunningham, 1871) (Figs 7B, 8, 9A, B)

Eolis campbellii Cunningham, 1871: 484, pl. 58, fig. 5.

Aeolidia serotina Bergh, 1873: 618. plate IX, figs 14–17; plate X, figs 4–12.

Aeolidia campbellii (Cunningham, 1871): Carcelles & Williamson, 1951: 318.

Aeolidia papillosa var. *serotina* Bergh, not *Aeolidia serotina* Bergh, 1873; Er. Marcus, 1959: 81, figs 191–196.

Material examined. ZSM 20020700, one specimen, dissected, 25 mm in length preserved, Chile, Bahía de Coliumo, Dichato, v.02, collected by Michael Schrödl; ZSM 20041026, two specimens, dissected, 20 and 22 mm in length preserved, Chile, Región de

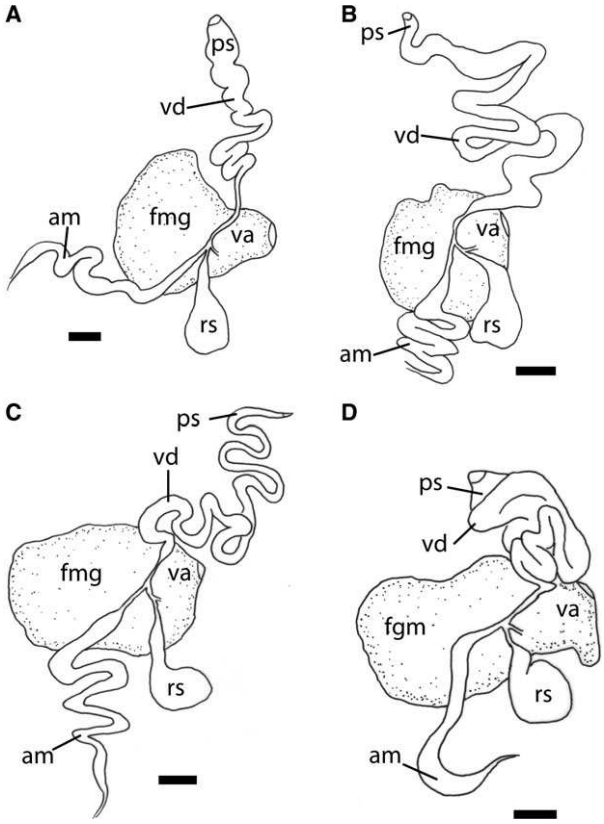

Figure 7. Reproductive system: A, *Aeolidia papillosa*, the Netherlands (MNCN 15.05/65211); B, *Aeolidia campbellii* (ZSM 20041026, 20 mm), Chile; C, ***Aeolidia filomenae* sp. nov.**, France; D, ***Aeolidia loui* sp. nov.** (CASIZ 102425), California, USA. Scale bars: 2.0 mm. Abbreviations: am, ampulla; fgm, female gland mass; ps, penial sac; rs, receptaculum seminis; va, vagina; vd, vas deferens.

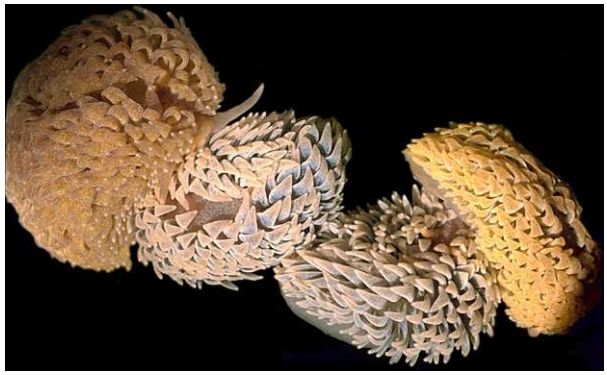

Figure 8. Photograph of live specimen of *Aeolidia campbellii*, taken by Michael Schrödl.

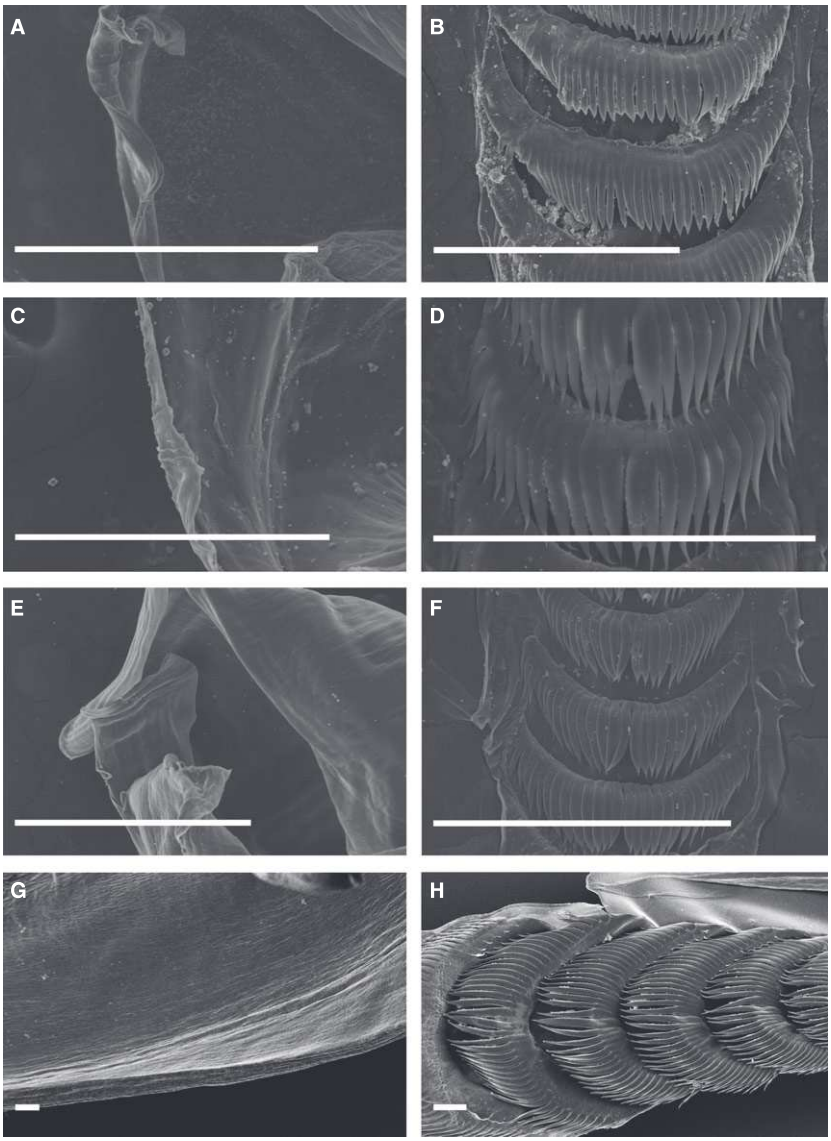

Figure 9. Scanning electron micrographs. A, B, *Aeolidia campbellii* (ZSM 20041026, 20 mm); A, detailed view of the masticatory border, scale bar 1000 μm; B, radular teeth, scale bar 500 μm. C, D, ***Aeolidia filomenae* sp. nov.** (MNCN 15.05/74470); C, detailed view of the masticatory border, scale bar 300 μm; D, radular teeth, scale bar 200 μm; E, F, ***Aeolidia filomenae* sp. nov.** (MNCN 15.05/74475); E, detailed view of the masticatory border, scale bar 500 μm; F, radular teeth, scale bar 400 μm; G, D, ***Aeolidia loui* sp. nov.** (CASIZ 182214); G, detailed view of the masticatory border, scale bar 10 μm; H, radular teeth, scale bar 30 μm.

Magallanes y de la Antártida Chilena (Off Islas Barnevelt), xi.94, collected by Michael Schrödl.

Type locality and habitat. Swallow Bay, north-east of Tierra del Fuego (Chile) (Cunningham, 1871). Found in hard substrates with algae and anemones (Schrödl, 2009). This species feeds on *Antholoba achates* (Drayton in Dana, 1846) (Cnidaria, Actinaria) (Schrödl, 2009).

Type material. According to Cunningham (1871) it was deposited in the British Museum, London. Our research revealed that the type material probably never arrived at the British Museum. The type material of *A. serotina* Bergh, 1873 exists in the Zoological Museum in Copenhagen (ZMUC Gas-2045 and ZMUC Gas-2046). As the older name (*A. campbellii*), which takes precedence over *A. serotina* (see remarks), lacks type material, we designate the specimen ZSM 20020700 (Chile) as the neotype, for its proximity to the type locality, according to the ICZN (1999, article 75.3).

Geographical distribution. So far, this species is only present in South America, in the Falklands Islands, and from the Argentinian and Chilean Patagonia to Valparaiso (Schrödl, 2003; as *A. papillosa*).

External morphology (Fig. 8). The body is broad and relatively low, narrowing to the posterior end of the foot. The background colour is highly variable; it ranges from white or greyish to pale pink or pale purple, transitioning to ochre or orange–brown. White flecks are scattered all over the surface of the notum. The rhinophores are conical, blunt, and smooth. They are a darker shade of the body colour, lighter on the apices, and exhibit light flecks all over their surface. The oral tentacles are translucent, longer than the rhinophores, and with white tips.

The cerata are flattened, broader at their base. Those at the anterior region and near the posterior end of the foot are smaller than those in the middle. A bare zone from behind the rhinophores to the pericardium is present. The cerata have the same colour as the background. They are also covered by white speckles and have white tips. The cerata are arranged in numerous, densely packed and oblique rows (up to 20). Each row contains up to 25 cerata each, decreasing in size towards the posterior end of the foot. The anus is cleioproctic and is situated between the tenth and 11th rows of the right side. The genital aperture is located between the seventh and eighth row of cerata on the right side.

Internal anatomy (Figs 7B, 9A, B). The jaws have a smooth masticatory edge (Fig. 9A). The radular formula is 25 × 0.1.0 (ZSM 20041026, size 20 mm). The radular teeth are progressively smaller towards the posterior region of the radula and have 33–38 denticles (Fig. 9B). Salivary glands were not found. Oral glands are absent.

The reproductive system is diaulic (Fig. 7B). The preampullary duct widens into the narrow, elongate ampulla. The ampulla divides into the oviduct and the vas deferens. The vas deferens is quite long and enters the wider proximal portion of the penial sac. The penial papilla is devoid of any armature. The pear-shaped receptaculum seminis joins the oviduct and enters the female gland. The vagina opens ventrally to the penis.

Remarks. Eolis campbellii was described by Cunningham in 1871, 2 years before *Aeolidia serotina* (Bergh, 1873). Cunningham (1871) erected this new species based on a single specimen from Swallow Bay, north-east of Tierra del Fuego (Chile). Although the original description is quite brief, the author described the coloration of the living animal (as pale pink with chocolate brown spots) and also provided a drawing of the specimen (Fig. 10). In 1873, when Bergh described *A. serotina* from

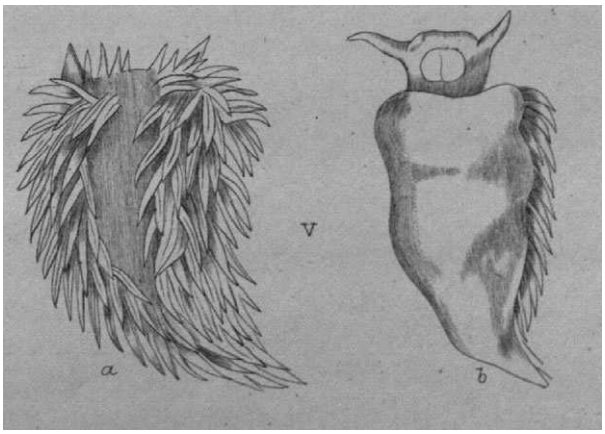

Figure 10. Original drawing of *Aeolidia campbellii*, from Cunningham (1871).

Valparaiso (Chile), he did not compare that new species with *E. campbellii*. In fact, the only reference Bergh made to *E. campbellii* is in the catalogue of the Aeolididae species (1884), where *E. campbellii* (included as *A. campbelli*) appears with a question mark. Since then, the latter species has passed almost unnoticed. According to Er. Marcus (1959), Cunningham's specimen could not even be identified to generic level, but plate VIII, figure 5, of Cunningham's publication is clearly similar to *Aeolidia*. Schrödl (2003) regarded *A. campbellii* as a junior synonym of *A. papillosa*, rendering the latter species, together with *A. collaris* the only valid Aeolidia species of South America. However, figure 52 (p. 107) of that contribution depicts a pale pink '*Aeolidia papillosa*', which matches with the original description of *A. campbellii*.

Since Bergh (1873), the validity of *A. serotina* has been questioned. In fact, Bergh (1894) stated that *A. serotina* was a junior synonym of *A. papillosa* and soon thereafter resurrected it (Bergh, 1898). Additionally, Eliot (1907) attributed three specimens of *Aeolidia* from the Falklands Islands to *A. serotina*, whereas Er. Marcus (1959) considered *A. serotina* as a variety of *A. papillosa*. Recently, Forcelli (2000) identified four specimens from Patagonia and Southern Chile as *A. serotina*. Based on the great variability in body coloration and ceratal shape, Schrödl (2003) rejected the validity of *A. serotina* and attributed his Chilean material to *A. papillosa*. In addition, the author pointed out that two of the four specimens illustrated by Forcelli (2000: in particular, the specimens of the second and third photograph on p. 130) may belong to a different species because the cerata are cylindrical and translucent. Six years later, Schrödl (2009) pointed out the necessity of a molecular approach in order to clarify the identity of the variable '*Aeolidia papillosa*' from Chile. Some of

Schrödl's material (2003) could be studied here from a molecular and morphological approach. The morphology and anatomical features of the specimens dissected in this study agree with those described by Bergh (1873, 1894, 1898), Eliot (1907), Er. Marcus (1959), and at the same time with the original description of *A. campbellii*. Only specimens illustrated by Forcelli (2000) in the second and third photograph look different to ours in terms of external coloration and ceratal shape, and therefore their identity needs to be determined in further studies. Additionally, Bergh (1873) described the presence of salivary glands in *A. campbellii* (called *A. serotina* by the author). Nevertheless, this mismatch could have resulted from an accidental removal during dissection. In conclusion, we can determine that *A. serotina* is a junior synonym of *A. campbellii* as, according to the ICZN (1999, article 23.9.2), *A. serotina* cannot take precedence over *A. campbellii*.

On the other hand, morphological and anatomical differences between *A. papillosa* and *A. campbellii* are difficult to find because of the great level of variability found in both species. Most of the specimens of *A. campbellii* present flattened and leaf-like cerata, whereas in *A. papillosa* the cerata are usually cylindrical and are not flattened in cross section. In accordance with Schrödl (2003), however, Chilean and Magellanic specimens show some variation in terms of ceratal shape, and therefore this difference between *A. campbellii* and *A. papillosa* should be regarded with caution. Besides genetic distance, some taxa are separated based on geographical differences (e.g. Johnson & Gosliner, 2012; Carmona *et al.*, 2014c), which so far seem to be the only consistent distinction between these two species.

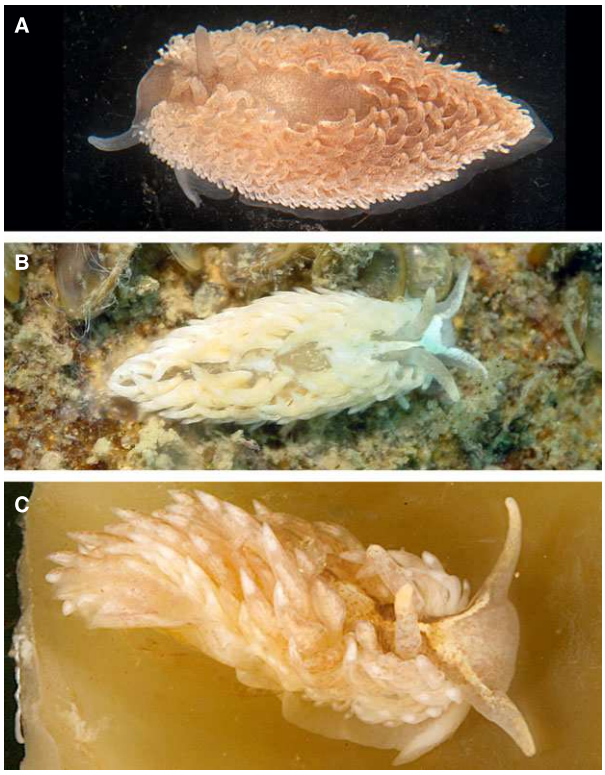

Figure 11. Photographs of live ***Aeolidia filomenae* sp. nov.** specimens: A, specimen from the Netherlands, Eastern Scheld, photo by Peter H. van Bragt (MNCN 15.05/74474); B, specimen from France, Arcachon Bay, Cap Ferret, photo by Marina Poddubetskaia (MNCN 15.05/74471, 12 mm); C, specimen from Spain, Galicia, Ribeira, photo by Jacinto Pérez Dieste (MNCN 15.05/74475, 12 mm).

AEOLIDIA FILOMENAE SP. NOV.

LSID URN:LSID:ZOOBANK.ORG:ACT:F99139A8-04B3-4F70-AA72-9F037C8FF05A

(FIGS 7C, 9C–F, 11A–C)

Aeolidia sp. A Carmona *et al.*, 2013: 6.

Material examined. Holotype: MNCN (15.05/74475), one specimen, dissected, 25 mm in length alive, dissected, Spain, Galicia, A Coruña, Galicia Ribeira, iii.11, collected by Jacinto Pérez Dieste. Paratype: MNCN (15.05/74474), one specimen, dissected, 45 mm in length alive, the Netherlands, Eastern Scheld, iv.12, collected by Peter H. van Bragt. Other material: MNCN (15.05/74472), one specimen, dissected, 25 mm in length alive, France, Cap Ferret, v.09, collected by Marina Poddubetskaia; MNCN (15.05/74470), one specimen, dissected, 6 mm in length alive, France, Cap Ferret, v.09, collected by Marina Poddubetskaia; MNCN (15.05/74471), one specimen, dissected, 12 mm in length alive, France, Cap Ferret, v.09, collected by Marina Poddubetskaia; CASIZ 187742, one specimen, dissected, 25 mm in length alive, Spain, Galicia, A. Coruña, Galicia Ribeira, iii.11, collected by Jacinto Pérez Dieste; MNCN (15.05/74473), one specimen, dissected, 17 mm preserved, Portugal, Cosata da Arrábida, Cabo Alfonso, v.12, collected by Goncalo Calado.

Type locality and habitat. Spain, Galicia, A. Coruña. Found under *Laminaria* spp. in 6 m of water.

Geographical distribution. From Scotland (Picton & Morrow, 1994; as *A. papillosa*; present study) to southern Lisbon (Portugal; present study), including the Netherlands (present study).

Etymology. This species is dedicated to Matilde Filomena López González, born in Galicia (Spain) and grandmother of the third author of this paper.

External morphology (Fig. 11A–C). The body is broad and relatively low, with a pointed posterior end of the

foot. The foot corners are tentaculiform. The background colour is variable, ranging from white, light beige, salmon colour to greenish. White or light-brown flecks are scattered all over the body. A white Y–shaped mark that runs from the oral tentacles to the pericardial area, passing between the rhinophores, may be present. This Y–shaped mark can be very evident and intense opaque white, or less noticeably beige or light brown. White or beige flecks may partly cover the Y–shaped pattern. The rhinophores are conical, blunt, and smooth. They are translucent with white or light-brown spots. The eyes are visible at the base of the rhinophores in lighter specimens (Fig. 11B). The oral tentacles are elongate, translucent, with opaque white or beige–brown flecks, depending on the general colour. Some specimens have white or beige tips, whereas the remaining species have translucent tips.

The cerata are flattened, broader at their base, and curved inwards. Those at the anterior region and near the posterior end of the foot are smaller than those in the middle. A bare zone from behind the rhinophores to the pericardium is present. The cerata have a lighter coloration than the rest of the body. Their apexes are white. The white–beige digestive gland is visible throughout the body wall. The cerata are arranged in up to 16 oblique rows, with each row including up to eight cerata. The anus is cleioproctic, situated between the ninth and tenth row of the right side. The gonopore is located between the fourth and fifth anterior rows of cerata.

Internal anatomy (Figs 7C, 9C–F). The jaws are more delicate than in *A. papillosa*, with a smooth masticatory edge (Fig. 9C, E). The radular formulae are 15 × 0.1.0 (MNCN 15.05/74470, 6 mm) and 16 × 0.1.0 (MNCN 15.05/74475, 25 mm). The teeth are progressively smaller towards the posterior region of the radula and pectinate with 25–31 denticles (Fig. 9D, F), which are relatively broad. Salivary glands were not found. Oral glands are absent.

The reproductive system is diaulic (Fig. 7C). The preampullary duct widens into the narrow and elongate ampulla. The ampulla bifurcates into the oviduct and the vas deferens. The vas deferens is extremely long and enters the wider proximal portion of the penial sac. The penial papilla is devoid of any armature. The pear-shaped receptaculum seminis joins the oviduct and enters the female gland. The vagina opens ventrally to the penis.

Remarks. Our study reveals that the specimens from several localities from the Atlantic coast of Europe, previously attributed to *A. papillosa* (Carmona *et al.*, 2013), belong to a different species. The accepted variability in the colour pattern of the former species masked the existence of a second and pseudocryptic European species of this genus. Anatomically, both species *A. papillosa* and *A. filomenae* sp. nov. are very similar, but there are several consistent differences. The jaws of *A. filomenae* sp. nov. are more delicate than in *A. papillosa*. In addition, the number of radular teeth is significantly higher in *A. papillosa*: whereas *A. papillosa* can have 31 teeth, *A. filomenae* sp. nov. has a maxiumum of 16 teeth. Regarding the reproductive system, the vas deferens in *A. filomenae* sp. nov. is much longer than the vas deferens of *A. papillosa*. Also, some external differences have been observed. The cerata of *A. filomenae* sp. nov. are more flattened, slightly hook shaped, and usually show a paler coloration than the rest of the body, whereas the cerata in *A. papillosa* are usually darker and more slender.

After a careful examination of all the available literature concerning *A. papillosa*, it was not possible to identify our species amongst any of the specific European names considered as synonyms of *A. papillosa*; however, considering the geographical range of *A. filomenae sp. nov.*, there are several names that could match our species although their descriptions are very poor, vague, or almost inexistent. These names are:

?*Doris spinis mollibus hirsute* Baster, 1762: 81, plate X.

?*Eolis cuvierii* Lamarck, 1819: 302.

Eolidia zetlantica Forbes & Goodsir, 1839: 647.

Aeolis lesliana MacGillivray, 1843: 70.

Aeolidia papillosa L., not *A. papillosa* (L., 1761): Walton, 1908: 227.

Eolis papillosa var. *albina* Dautzenberg & Durouchoux, 1913: 8.

Aeolidia papillosa L., not *A. papillosa* (L., 1761): Ortea, 1980: 73.

Aeolidia papillosa L., not *A. papillosa* (L., 1761): Picton & Morrow, 1994: 130.

Aeolidia filomenae sp. nov. is sympatric to *A. papillosa* in the Netherlands and the British Isles.

AEOLIDIA LOUI SP. NOV.

LSID URN:LSID:ZOOBANK.ORG:ACT: D79111E0-0229-4C75-82DA-33C86AF49E5F

(FIGS 7D, 9G, H, 12A–E)

Aeolidia herculea, not *A. herculea* Bergh, 1894; Smith & Gordon, 1948: 181.

Aeolidia papillosa, not *A. papillosa* (L., 1761); Er. Marcus, 1961b: 54, plate 10, figs 193–195.

Aeolidia papillosa herculea, not *A. herculea* Bergh, 1894; MacFarland, 1966: 370, plate 72, figs 1–8.

Aeolidia sp. B: Carmona *et al.* (2013: 6).

Material examined. Holotype: CASIZ 182214, one specimen, dissected, 30 mm in length preserved,

Figure 12. Photographs of live ***Aeolidia loui* sp. nov.** specimens: A, holotype, specimen from USA, California, photo by Terrence M. Gosliner (CASIZ 182214); B, specimen from USA, California, photo by Patricia Álvarez-Campos and Greg Rouse; C, specimen from USA, Oregon, photo by Nancy Treneman; C, specimen from USA, California, photo by Gary McDonald; E, detail of the rhinophores of the specimen from USA, California, photo by Dave Behrens.

USA, California, Marin Country, Duxbury Reef, i.10, collected by Terrence M. Gosliner. Paratype: CASIZ 102425, one specimen, dissected, 28 mm in length preserved, USA, California, vii.76, collected by T. Pennington and D. Thoney. Other material: CASIZ 104504, one specimen, dissected, 20 mm in length preserved, USA, California, v.05, collected by Rebecca Johnson and Christine Piotrowski; CASIZ 168044, one specimen, dissected, 20 mm in length preserved, USA, California, iv.03, collected by R. Ayres, C. Brown, M. Walton, and S. Lattanzio.

Type locality and habitat. USA, California, Marin Country, Duxbury Reef. Found in intertidal area, within tide pools and under the rocks.

Geographical distribution. To date, this species is distributed from Cape Arago, Oregon, to San Diego, California, USA.

Etymology. This species is dedicated to Lou Timothée Ménélik von Graffenried Kienberger, first nephew of the first author of this paper.

External morphology (Fig. 12A–E). The body is broad and relatively low, narrowing to the posterior end of the foot. The foot corners are tentaculiform. The coloration is variable, ranging from translucent white (Fig. 12A–C) to bright orange or brown (Fig. 12D). Over the dorsum there are opaque white marks that may be covered by light-ochre and brown flecks, and/or spots. The white marks may form a somewhat uniform patch that runs, like the Y–shaped mark, from the head to the posterior end of the foot. The rhinophores and oral tentacles present the same colour as the body and light tips. The rhinophores are conical, blunt, and covered by irregular warts (Fig. 12E). The eyes are visible at

the base of the rhinophores. The oral tentacles are elongate and translucent, with opaque white pigments.

The cerata are somewhat bristly, flattened, and broader at their base, with pointed tips. Those at the anterior region and near the posterior end of the foot are smaller than those in the middle. There is a bare zone from behind the rhinophores to the pericardium. The cerata are translucent and have the same coloration as the background colour of the body. White pigment or spots cover both edges of the cerata. This pigmentation may reach the proximal two-thirds of the cerata. The ochre, greenish, or brownish digestive gland is visible throughout the body wall. The cerata are arranged in up to 24 rows, and are extremely densely packed. Each row has between four and 30 cerata, decreasing in size towards the posterior end of the foot. Behind the pericardium the cerata rows join across the back, forming an arch that goes from one side of the body to the other. The cleioproctic anus is situated between the ninth and tenth rows on the right side, whereas the genital aperture is located between the right fourth and fifth rows.

Internal anatomy (Fig. 7D, 9G–H). The jaws have a smooth masticatory edge (Fig. 9G). The radular formula is 16 × 0.1.0 (CASIZ 182214, 30 mm). The teeth are progressively smaller towards the posterior region of the radula and present 41–45 denticles (Fig. 9H). Salivary glands were not found. Oral glands are absent.

The reproductive system is diaulic (Fig. 7D). The preampullary duct widens into the narrow, elongate, and moderately short ampulla. The ampulla bifurcates into the oviduct and the vas deferens. The vas deferens is quite long and convoluted, entering the wider proximal portion of the penial sac. The penial papilla is devoid of any armature. The somewhat rounded receptaculum seminis joins the oviduct and enters the female gland. The vagina opens ventrally to the penis.

Remarks. In the past, specimens of *Aeolidia* collected from Californian shores were named *A. papillosa*, *A. herculea* Bergh, 1894; or *A. papillosa herculea* MacFarland, 1966; by several authors (Smith & Gordon, 1948; Er. Marcus, 1961b; MacFarland, 1966). Ernest Marcus (1961b) attributed the Californian specimens of *Aeolidia* to *A. papillosa*, describing them as pink with occasionally red cerata. Additionally, their cerata were flattened, broader at their base, leaving a free space in the anterior region just over the heart. Marcus (1961b) also illustrated the radular teeth of these specimens. Some years later, specimens from Monterey Bay and Waddel Creek Reef were attributed to *A. papillosa herculea* by MacFarland (1966). He provided detailed information about the coloration of the living animal (dull rose or mauve) and its radular teeth.

As the coloration, the ceratal shape, and the morphology of the radular teeth of *A. loui* sp. nov. match with the specimens found by Er. Marcus (1961b) and MacFarland (1966), we concluded that all are conspecific and belong to an undescribed species.

Regarding *A. herculea*, this species is a deep-water aeolid (Behrens, 2004). Therefore, all of the specimens studied by the above authors, together with

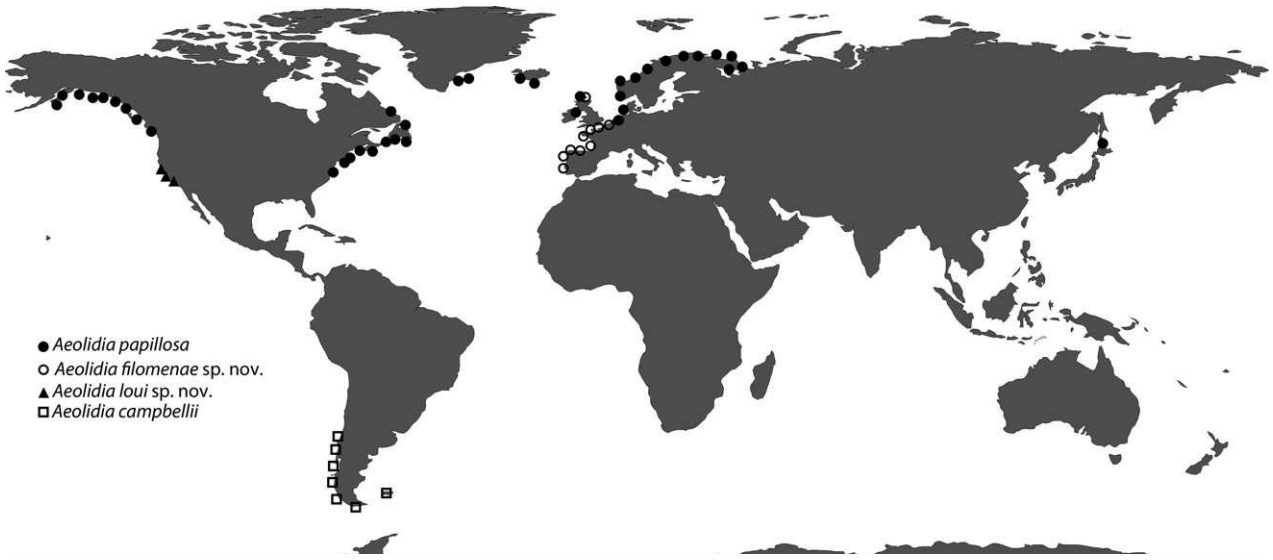

Figure 13. Distribution of *Aeolidia papillosa*, *Aeolidia campbellii*, ***Aeolidia filomenae* sp. nov.**, and ***Aeolidia loui* sp. nov.**

those of Smith & Gordon (1948) and McDonald (1983), cannot be attributed to *A. herculea* because they were collected from shallow waters. Gosliner & Behrens (1996) described *A. farallonensis* from material collected at 1405–1491 m depth. This species is currently considered as the junior synonym of *A. herculea* (Martynov & Korshunova, 2011). We consider that *A. herculea* and *A. loui* sp. nov. are not the same species, as our specimens reside in shallow waters. Also, there are significant morphological differences between these two species, especially in the position of the anus: whereas *A. herculea* is pleuroproctic, *A. loui* sp. nov. has a cleioproctic anus situated between the ninth and tenth rows on the right side.

The bristly cerata and the warty rhinophores of *A. loui* sp. nov. easily distinguish it from the remaining *Aeolidia* species. This is the first time that an *Aeolidia* species is described with 'papillate' rhinophores. Moreover, it should be mentioned that this ornamentation of the rhinophores is lost when the specimens are preserved. This would explain why Er. Marcus (1961b) and MacFarland (1966) depicted *A. loui* sp. nov. rhinophores as smooth.

DISCUSSION

What has been traditionally known as a single species, *A. papillosa*, has been shown to be part of a species complex of four sibling species. *Aeolidia papillosa* still has a wide geographical distribution range. Besides being amphi-Atlantic, this species is also present in the Pacific Ocean, along the coasts of Alaska and Washington State. The validity of *A. campbellii* is here confirmed by both morphological and molecular data. Thus far, this species, which has been considered a junior synonym of *A. papillosa* (Schrödl, 2003), is restricted to the Southern Hemisphere from the coast of Chile and Argentina (Fig. 13). Moreover, an additional pseudocryptic species, *A. filomenae* sp. nov., overlaps in part of its distribution with *A. papillosa*, where they are sympatric in the Netherlands and in Scotland. On the other hand, so far *A. papillosa* does not appear to overlap with the other north Pacific pseudocryptic species *A. loui* sp. nov.; however, more specimens of both species from the Pacific coast of Canada and the states of Washington and Oregon are needed in order to completely determine whether any overlap exists between these two species.

Morphologically, these species are all rather similar, but the warty rather than smooth rhinophores of *A. loui* sp. nov. are consistently distinctive for that species. Upon revising the pertinent literature it seems evident that several authors (e.g. Er. Marcus, 1961b; MacFarland, 1966) previously found this species, but that it was always identified as *A. papillosa*. Although these warts are quite conspicuous in living specimens (Fig. 12E), they are completely lost after preservation (L. Carmona, pers. observ.). This could be the reason why rhinophorial ornamentation has not been observed over the years.

In order to complete and clarify the relationship among all *Aeolidia* species, further studies, including material of *A. herculea* and *A. collaris*, and more specimens of *A. campbellii*, are needed.

ACKNOWLEDGEMENTS

We are deeply grateful to all of the individuals that helped to collect and provided specimens, sequences, and images for this study, such as A. Martynov, M. Schrödl, P. Álvarez-Campos, G. Rouse, D.W. Behrens, J. Goddard, N. Treneman, J. Anderson, R. Shucksmith, K. Iken, L. Harris, P.H. van Bragt, J. Pérez Dieste, M. Poddubetskaia, and G. Calado. This work was supported by several research grants [CGL2006-05182/BOS and CGL2010-17187, Spanish Ministry of Economy and Competitiveness (including the early Ministry of Sciences and Innovation) to J.L. Cervera; California Academy of Sciences, National Science Foundation (DEB 0329054 PEET) to T.M. Gosliner; and a Research Grant of the Malacological Society of London to L. Carmona. J.L. Cervera and M. Pola are indebted to P. Sundberg and the Swedish Taxonomy Initiative for the invitation to the Lovén Centre for Marine Science (Tjärno, Sweden). Vinicius Padula was funded by a PhD grant from the CNPq-Brazil and DAAD-Germany. Sequencing of ZSM specimens was supported by DFG SCHR667/13-1. This is CEI·MAR journal publication 111.

REFERENCES

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19:** 716–723.

Alder J, Hancock A. 1842. Descriptions of several new species of nudibranchs Mollusca found on the coast of Northumberland. *Annals & Magazine of Natural History* **9:** 31–36.

Alder J, Hancock A, 1854 for 1852. *A monograph of the British nudibranchiate Mollusca: with figures of all the species*. London: Ray Society.

Alfaro ME, Zoller S, Lutzoni F. 2003. Bayes or bootstraps? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* **20:** 255–266.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215:** 403–410.

Baba K. 1935. The fauna of Akkeshi Bay. I. Opistobranchia. *Journal of the Faculty of Science, Hokkaido Imperial University* **3:** 115–125.

Baster J. 1762. Opuscula subseciva, observationes miscellaneas de animalculis et plantis quibusdammarinis, eorumque ovariis et seminibus continentia, Tom. II. Liber I.: 53–97.

Behrens DW. 2004. Pacific coast nudibranchs, supplement II new species to the Pacific coast and new information on the oldies. *Proceedings of the California Academy of Sciences* **55:** 11–54.

Behrens DW, Hermosillo A. 2005. *Eastern Pacific nudibranchs. A guide to the Opisthobranchs from Alaska to Central America*. Monterey, California: Sea Challengers.

Bergh R. 1873. *Beiträge zur Kenntniss der Aeolidiaden I*. Wien: Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien, 597–628.

Bergh R. 1879. On the Nudibranchiate Gasteropod Mollusca of the North Pacific Ocean, with Special Reference to Those of Alaska (Part 1). *Proceedings of the Academy of Natural Sciences of Philadelphia*. Philadelphia: Academy of Natural Sciences, 71–77.

Bergh R. 1884. Malacologische Untersuchungen. In: Reisen im Archipel der Philippinen von Dr. Carl Gottfried Semper. Zweiter Theil. Wissenschaftliche Resultate. Band 2, Theil 3, Heft 15, 647–754, pls. 69–76.

Bergh R. 1894. Die Opisthobranchien. Reports on the dredging operations off the west coast of Central America to the Galapagos, to the west coast of Mexico, and in the Gulf of California, in charge of Alexander Agassiz, carried on by the U.S. Fish Commission steamer "Albatross," during 1891, Lieut. Commander Z. L. Tanner, U.S.N., commanding. *Bulletin of the Museum of Comparative Zoology, Harvard* **25:** 125–129.

Bergh R. 1898. Die Opisthobranchier der Sammlung Plate. *Zoologische Jahrbücher Supplement* **4:** 539–544.

Bleakney S. 1996. *Sea slugs of Atlantic Canada and the Gulf of Maine*. Halifax: Nimbus.

Bryant D, Moulton V. 2004. Neighbor-net. An agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution* **21:** 255–265.

Camacho-García YE, Ornelas-Gatdula E, Gosliner TM, Valdés Á. 2014. Phylogeny of the family Aglajidae (Pilsbry, 1895) (Heterobranchia: Cephalaspidea) inferred from mtDNA and nDNA. *Molecular Phylogenetics and Evolution* **71:** 113–126.

Carcelles AR, Williamson SI. 1951. Catalogo de los moluscos marinos de la Provincia Magallanica. *Revista del Instituto Nacional de Investigacion de las Ciencias Naturales (Anexo al Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"), Ciencias Zoologicas, Buenos Aires* **2:** 225–383.

Carmona L, Pola M, Gosliner TM, Cervera L. 2013. A tale that morphology fails to tell: a molecular phylogeny of Aeolidiidae (Aeolidida, Nudibranchia, Gastropoda). *PLoS ONE* **8:** 13. doi:10.1371/journal.pone.0063000.

Carmona L, Bhave V, Salunkhe R, Pola M, Gosliner TM, Cervera JL. 2014a. Systematic review of *Anteaeolidiella* (Mollusca, Nudibranchia, Aeolidiidae) based on morphological and molecular data, with a description of three new species. *Zoological Journal of the Linnean Society* **171:** 108–132.

Carmona L, Lei BR, Pola M, Gosliner TM, Valdés Á, Cervera JL. 2014b. Untangling the *Spurilla neapolitana* (Delle Chiaje, 1841) species complex: a review of the genus *Spurilla* Bergh, 1864 (Mollusca: Nudibranchia: Aeolidiidae). *Zoological Journal of the Linnean Society* **170:** 132–154.

Carmona L, Pola M, Gosliner TM, Cervera JL. 2014c. The end of a long controversy: systematics of the genus *Limenandra* (Mollusca: Nudibranchia: Aeolidiidae). *Helgoland Marine Research* **68:** 37–48.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1660.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston S, Macaranas J, Edgecombe GD, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46:** 419–437.

Cooke S, Hanson D, Hirano Y, Ornelas-Gatdula E, Gosliner TM, Chernyshev AV, Valdés Á. 2014. Cryptic diversity of *Melanochlamys* sea slugs (Gastropoda, Aglajidae) in the North Pacific. *Zoologica Scripta* **43:** 351–369.

Cunningham RO. 1871. Notes on the reptiles, Amphibia, fishes, Mollusca, and Crustacea obtained during the voyage of H.M.S. 'Nassau' in the years 1866-69. London.

Cuvier G. 1798. *Tableau Élémentaire de l'Histoire Naturelle des Animaux*. Paris: Baudouin.

Dall WH. 1884. Contributions to the history of the Commander Islands. No. 3. Report on the Mollusca of the Commander Islands, Bering Sea, collected by Leonhard Stejneger in 1882 and 1883. *Proceedings of the United States National Museum* **7:** 340–349.

Dalyell JG. 1853. The powers of the creator displayed in the creation: or, observations on life amidst the various forms of the humbler tribes of animated nature: with practical comments and illustrations. London.

Dautzenberg P, Durouchoux P. 1913. Les mollusques de la Baie de Saint-Malo. *La Feuille des Jeunes Naturalistes* **5:** 1–24.

Debelius H, Kuiter RH. 2007. *Nudibranchs of the world*. Frankfurt, Germany: IKAN-Unterwasserarchiv.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009. GENEIOUS v4.6. Available at: http://www.geneious.com/

Edmunds M. 1983. Advantages of food specificity in *Aeolidia papillosa*. *Journal of Molluscan Studies* **49:** 80–81.

Edmunds M, Potts GW, Swinfen RC, Waters VL. 1974. The feeding preferences of *Aeolidia papillosa* (L.) (Mollusca, Nudibranchia). *Journal of the Marine Biological Association of the United Kingdom* **54:** 939–947.

Edmunds M, Potts GW, Swinfen RC, Waters VL. 1976. Defensive behaviour of sea anemones in response to predation by the opisthobranch mollusc *Aeolidia papillosa* (L.).

Journal of the Marine Biological Association of the United Kingdom **56:** 65–83.

Eilertsen MH, Malaquias MAE. 2013. Systematic revision of the genus *Scaphander* (Gastropoda, Cephalaspidea) in the Atlantic Ocean, with a molecular phylogenetic hypothesis. *Zoological Journal of the Linnean Society* **167:** 389–429.

Eliot CNE. 1907. Nudibranchs from New Zealand and the Falkland Islands. *Proceedings of the Malacological Society of London* **7:** 327–361.

Espinoza E, DuPont A, Valdés Á. 2014. Molecular data reveal an undescribed cryptic species of *Costasiella* Pruvot-Fol, 1951 (Euthyneura: Sacoglossa: Limapontidae) in the Bahamas. *American Malacological Bulletin* **32:** 173–182.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Forbes E, Goodsir J. 1839. Notice of zoological researches in Orkney and Shetland during the month of June, 1839. *Athenaeum* **618:** 647.

Forcelli DO. 2000. *Moluscos Magallánicos: Guía de Moluscos de Pataognia y Sur de Chile*. Buenos Aires, Argentina: Vásquez Mazzini Editores.

Gofas S. 2014. *Aeolidia*, Cuvier, 1798. World Register of Marine Species. Available at: http://www.marinespecies.org/aphia.php?p=taxdeposteriorendofthefoots&id=137630 (accessed on 17 July 2014)

Gosliner TM, Behrens DW. 1996. Two new species of nudibranch mollusks from the Gulf of the Farallones and Cordell Bank National Marine Sanctuaries, Central California. *The Veliger* **39:** 348–353.

Gunnerus JE. 1770. Nogle smaa rare og meestendeelen nye norske søedyr. *Skrifter som udi det Kiøbenhavnske Selskab af Lærdoms og Videnskabers Elskere ere Fremlagte og Oplæste i Aarene 1765, 1766, 1767, 1768, og 1769* **10:** 166–176.

Hallas JM, Gosliner TM. 2015. Molecular phylogenetics and evolution family matters: the first molecular phylogeny of the Onchidorididae. *Molecular Phylogenetics and Evolution* **88:** 16–27.

Hancock A, Embleton D. 1845. On the Anatomy of *Eolis*, a genus of Mollusks of the order Nudibranchiata. *The Annals and Magazine of Natural History, Including Zoology, Botany and Geology* **15:** 1–10, 77–88.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42:** 182–192.

Huelsenbeck JP, Bull JJ, Cunningham CW. 1996. Combining data in phylogenetic analysis. *Trends in Ecology & Evolution* **11:** 152–158.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23:** 254–267.

ICZN. 1999. *International Code of Zoological Nomenclature*, 4th edn. London: International Trust for Zoological Nomenclature.

Johnson RF, Gosliner TM. 2012. Traditional taxonomic groupings mask evolutionary history: a molecular phylogeny and new classification of the chromodorid nudibranchs. *PlosOne* **7:** 1–15.

Johnston G. 1835. Illustrations in British zoology. *London, Magazine Natural History & Journal of Zoology, Botany, Mineralogy, Geology & Meteorology* **8:** 376–380.

Katoh K, Asimenos G, Toh H. 2009. Multiple alignment of DNA sequences with MAFFT. *Methods in Molecular Biology* **537:** 39–64.

Lamarck JBPA. 1819. Histoire naturelle des animaux sans vertèbres, presentant les caracteres generaux et particuliers de ces animaux, leur distribution, leur classes, leurs familles, leurs genres, et al citation des principales especes qui s'y rapportent; precedee d'une Introduction offrant la determination des caracteres essentiels de l'Animal, sa distinction du vegetal et des autres corps naturels, enfin, l'Exposition des Principes fondamentaux de la Zoologie. *Paris, Chez L'Auteur, au Jardin du Roi* **6:** 1–343.

Linnaeus CV. 1761. *Fauna svecica: sistens animalia sveciae regni: mammalia, aves, amphibia, pisces, insecta, vermes, distributa per classes & ordines, genera & species, cum differentiis specierum, synonymis auctorum, nominibus incolar Caroli Linnaei*. Stockholmiae: Sumtu & Literis Direct. Laurentii Salvii.

MacFarland FM. 1966. Studies of opisthobranchiate mollusks of the Pacific coast of North America. *Memoirs of the California Academy of Sciences*. San Francisco: The Academy, 370–373.

MacGillivray W. 1843. *A history of the molluscous animals of the counties of Aberdeen, Kincardine, and Banff; to which is appended an account of the animals of the same district*. London: Cunningham & Mortimer.

Maddison WP. 1997. Gene trees in species trees. *Systematic Biology* **46:** 523–536.

Maddison DR, Maddison WP. 2005. *MacClade 4., v. 4.08 for OSX*. Sunderland, MA: Sinauer Associates.

Malaquias MAE, Reid DG. 2008. Functional morphology of the gonoduct of the gastropod *Bulla striata* Bruguière, 1792 (Opisthobranchia: Cephalaspidea): evidence for a monaulic system. *Acta Zoologica* **89:** 205–210.

Marcus Er. 1959. Reports from the Lund University Chile Expedition 1948-49, No. 36. *Lamellariacea und Opisthobranchia. Lunds Universitets Arsskrift (Ny Följd)* **55:** 81–84.

Marcus Er. 1961a. Opisthobranchia from North Carolina. *Journal of the Elisha Mitchell Sciences Society* **77:** 141–151.

Marcus Er. 1961b. Opisthobranch mollusks from California. *The Veliger* **3:** 54.

Martynov A, Korshunova T. 2011. Opisthobranch molluscs of the seas of Russia, a colour guide to their taxonomy and biology. Fiton.

McDonald GR. 1983. A review of the nudibranchs of the California coast. *International Journal of Malacology* **24:**114–276.

McFarland FK, Muller-Parker G. 1993. Photosyntesis and retention of zooxanthellae and zoochlorellae within the aeo-

lid nudibranch *Aeolidia papillosa*. *The Biological Bulletin* **184:** 223–229.

Müller OF. 1776. Zoologiae Danicae prodromus: seu Animalium Daniae et Norvegiae indigenarum; characteres, nomina, et synonyma imprimis popularium. Havniae.

Nakano R. 2004. *Opisthobranchs of Japan Islands*. Tokyo: Rutles Inc.

Nylander JAA. 2004. *MrModeltest v2.3. Program distributed by the author*. Uppsala: Evolutionary Biology Centre, Uppsala University.

Odhner NH. 1921. Mollusca from Juan Fernandez and Easter Island. In: Skottsberg C, ed. *Natural History of Juan Fernandez and Easter Island*. Uppsala: Almqvist and Wiksells Boktryckeri, Vol. **3:** 219–254.

O'Donoghue CH. 1921. Nudibranchiate Mollusca from the Vancouver Island Region. *Transactions of the Canadian Institute* **13:** 199–200.

Ohnheiser LT, Malaquias MAE. 2013. Systematic revision of the gastropod family Philinidae (Mollusca: Cephalaspidea) in the north-east Atlantic Ocean with emphasis on the Scandinavian Peninsula. *Zoological Journal of the Linnean Society* **167:** 273–326.

Ortea JA. 1980. Sobre la biología de *Aeolidia papillosa* (L.) (Mollusca, Nudibranchia) en Asturias. *Boletín de Ciencias Naturales, Instituto de Estudios Asturianos* **25:** 73–76.

Ortigosa D, Pola M, Carmona L, Padula V, Schrödl M, Cervera JL. 2014. Redescription of *Felimida elegantula* (Philippi, 1844) and a preliminary phylogeny of the European species of *Felimida* (chromodorididae). *Journal of Molluscan Studies* **80:** 541–550.

Oskars TR, Bouchet P, Malaquias MAE. 2015. A new phylogeny of the Cephalaspidea (Gastropoda: Heterobranchia) based on expanded taxon sampling and gene markers. *Molecular Phylogenetics and Evolution* **89:** 130–150.

Padula V, Araújo AK, Matthews-Cascon H, Schrödl M. 2014. Is the Mediterranean nudibranch *Cratena peregrina* (Gmelin, 1791) present on the Brazilian coast? Integrative species delimitation and description of *Cratena minor* n. sp. *Journal of Molluscan Studies* **80:** 575–584.

Palumbi SR, Martin A, Romano S, Owen Macmillan W, Stice L, Grabowski G. 1991. *The simple fool's guide to PCR*. Honolulu: Department of Zoology, University of Hawaii.

Picton BE, Morrow CC. 1994. *A Field Guide to the nudibranchs of the British Isles*. London: Immel Publishing Limited.

Platts E. 1985. An annotated list of the North Atlantic Opisthobranchia (excluding Thecosomata and Gymnosomata), In: Just H, Edmunds M, eds. *North Atlantic nudibranchs (Mollusca) seen by Henning Lemche: with additional species from the Mediterranean and the north east Pacific. Ophelia*, Supplementum 2, 150–170.

Pola M, Roldán P, Padilla S. 2014a. *Okenia* (Nudibranchia: Goniodorididae) reveals a new cryptic species from New South Wales (Australia). *Journal of the Marine Biology Association of the United Kingdom* **94:** 587–598.

Pola M, Padula V, Gosliner TM, Cervera JL. 2014b. Going further on an intricate and challenging group of nudibranchs: description of five novel species and a more complete molecular phylogeny of the subfamily Nembrothinae (Polyceridae). *Cladistics* **30:** 607–634.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21:** 1864–1877.

Rokas A, Williams BL, King N, Carroll SB. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* **425:** 798–804.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Rosenberg G. 2009. Malacolog 4.1.1: A Database of Western Atlantic Marine Mollusca. [WWW database (version 4.1.1)]. Available at: http://www.malacolog.org/.

Schrödl M. 1996. Nudibranchia y Sacoglossa de Chile: Morfología exterior y distribución. *Gayana Zoología* **60:** 17–62.

Schrödl M. 1997. Range extensions of Magellanic nudibranchs (Opisthobranchia) into the Peruvian faunal province. *The Veliger* **40:** 38–42.

Schrödl M. 2003. *Sea Slugs of Southern South America*. Hackenheim: ConchBooks.

Schrödl M. 2009. Opisthobranchia – sea slugs. In: Häussermann V, Försterra G, eds. *Marine benthic fauna of Chilean Patagonia*. Puerto Montt: Nature in Focus, 506–542.

Shine J. 2012. *Nudibranchs of the Northeast*. Somerville, Massachussets: Blue Sphere Pubs.

Shipman C, Gosliner T. 2015. Molecular and morphological systematics of *Doto* Oken, 1851 (Gastropoda: Heterobranchia), with descriptions of five new species and a new genus. *Zootaxa* **3973:** 57–101.

Smith AG, Gordon M Jr. 1948. The marine mollusks and brachiopods of Monterey Bay, California, and vicinity. *Proceedings of the California Academy of Sciences*, Series 4, **16:**181.

Stamatakis A, Hoover P, Rougemont J. 2008. A fast bootstrapping algorithm for the RAxML web-servers. *Systematic Biology* **57:** 758–771.

Stimpson W. 1853. Synopsis of the marine Invertebrata of Grand Manan: or the region about the mouth of the Bay of Fundy, New Brunswick. *Smithsonian Contributions to Knowledge* **6:** 1–66.

Swofford DL. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland, MA: Sinauer. 142 pp.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56:** 564–577.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28:** 2731–2739.

Thompson TE, Brown GH. 1984. *Biology of opisthobranch molluscs*. London: Ray Society.

Turton W. 1807. British fauna, containing a compendium of the zoology of the British Islands: arranged according to the Linnean system. *Swansea* **1:** 133.

Walton CL. 1908. Nudibranchiate collected in the North Sea by the s.s. "Huxley" during July and August, 1907. *Journal of the Marine Biological Association of the United Kingdom* **8:** 227–240.

Wilson NG, Burghardt I. 2015. Here be dragons – phylogeography of *Pteraeolidia ianthina* (Angas, 1864) reveals multiple species of photosynthetic nudibranchs (Aeolidina: Nudibranchia). *Zoological Journal of the Linnean Society* **175:** 119–133.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Molecular phylogeny inferred from partial sequences of the individual nuclear gene *H3* (A), and the mitochondrial genes *COI* (B) and *16S* (C) by Bayesian analysis.

Figure S2. Phylogenetic hypothesis based on the combined data set (*H3* + *COI* + *16S*), inferred by Bayesian analysis (BI).