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The Atlantic-Mediterranean genus *Berghia* Trinchese, 1877
(Nudibranchia: Aeolidiidae): taxonomic review and phylogenetic
analysis

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ABSTRACT

The aeolid genus *Berghia* was described by Trinchese in 1877, with *Berghia coeruleascens* (Laurillard, 1832) as type species. The validity of *Berghia* has been questioned by some authors, who have considered it a junior synonym of *Spurilla* Bergh, 1864. The lack of consensus has caused confusion, blurring the differences between these two genera. A recent molecular phylogeny of Aeolidiidae supported the monophyly of *Berghia* and helped to resolve the controversy about its relationship with *Spurilla*. Here, specimens of *Berghia* from the eastern and western Atlantic were examined from molecular and morphological points of view. *Berghia* was found to be monophyletic, although its relationship with other aeolidiids was not resolved. The morphological studies corroborate our molecular findings and differences in external coloration can distinguish closely related species. So far, *Berghia* comprises 10 species: *B. coeruleascens*, *B. verrucicornis*, *B. norvegica*, *B. benteva*, *B. creutzbergi*, *B. columbina*, *B. rissodominguezi*, *B. stephanieae*, *B. marcusii* and a new species from Senegal, *B. marinae*.

INTRODUCTION

The aeolid genera *Spurilla* Bergh, 1864 and *Berghia* Trinchese, 1877 have been considered to be closely related or, because of their morphological similarities, to be synonymous. Rudman (1982) stated that differences in the ornamentation of the rhinophores, which are perfoliate in *Spurilla* and papillate in *Berghia*, were not significant enough to support these taxa as separate genera, and therefore considered the latter to be a junior synonym of *Spurilla*. However, three years later, Gosliner (1985) considered both genera as valid. Since *Berghia* was erected, up to 14 valid species from around the world have been ascribed or transferred to it: *B. coeruleascens* (Laurillard, 1832), *B. verrucicornis* (A. Costa, 1867), *B. major* (Eliot, 1903), *B. japonica* (Baba, 1933), *B. norvegica* Odhner, 1939, *B. benteva* Er. Marcus, 1958, *B. dela* Er. Marcus & Ev. Marcus, 1960, *B. creutzbergi* Er. Marcus & Ev. Marcus, 1970, *B. australis* (Rudman, 1982), *B. salaamica* (Rudman, 1982), *B. chaka* Gosliner, 1985, *B. columbina* (García-Gómez & Thompson, 1990),

B. rissodominguezi Muniain & Ortea, 1999 and *B. marcusii* Domínguez, Troncoso & García, 2008 (Carmona *et al.*, 2013; Supplementary Material, Table S3). Depending on the author, several of these species have been ascribed to the genus *Spurilla*, but also to the genera *Baeolidia*, *Aeolidiella* and *Anteaeolidiella* (e.g. Er. Marcus, 1958; Miller, 2001; García-García, Domínguez & Troncoso, 2008, for *B. benteva*). Despite this lack of consensus, the validity of *Berghia* and/or its differences from *Spurilla* have never been studied. Only Tardy (1962) conducted a minor revision of this genus, focusing on taxa occurring along the French coast.

Recently, the first phylogenetic analysis of Aeolidiidae (Carmona *et al.*, 2013) supported *Berghia* as monophyletic and concluded that the genus is restricted to the Atlantic Ocean and Mediterranean Sea. The inclusion of *B. stephanieae* (with cerata in rows) showed that the ceratal arrangement is not by itself a good character to define the genus (Carmona *et al.*, 2013), which highlights the need for more detail analyses to find new morphological characters unique to *Berghia*.

The objective of this paper is to provide a phylogenetic hypothesis for the genus *Berghia* based on morphological and molecular data from all described species. A new species from Senegal is described.

MATERIAL AND METHODS

Literature review, source of specimens and morphological examination

A comprehensive review of the literature was conducted to determine the valid names and synonyms for the species recognized in the molecular and morphological analyses.

Samples were obtained using standard scuba diving sampling techniques for opisthobranchs and through the study of museum collections. When possible, two or more specimens of each species were examined anatomically. Specimens were dissected by dorsal incision. Their internal features were examined and drawn using a dissecting microscope with the aid of a camera lucida. Special attention was paid to the morphology of the reproductive system and 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 at the California Academy of Sciences (CASIZ; San Francisco, USA), Museo Nacional de Ciencias Naturales (MNCN; Madrid, Spain), Museo de Zoología de la Universidad de Costa Rica (MZUCR; San José, Costa Rica), Museu de Zoologia da Universidade São Paulo (MZSP; São Paulo, Brazil), Museu Nacional/Universidade Federal do Rio de Janeiro (MNRJ; Rio de Janeiro, Brazil), University Museum of Bergen (ZMBN; Bergen, Norway) and Zoologische Staatssammlung München (ZSM; Munich, Germany).

Number of examined specimens, length and localities for each species are given in Supplementary Material, Appendix 1.

DNA extraction, PCR amplification and sequencing

A total of 50 specimens were successfully sequenced for the cytochrome *c* oxidase subunit I (COI), 58 for the 16S rRNA (16S) and 59 for the Histone-3 (H3) genes. Fifteen sequences were obtained from GenBank (see Supplementary Material Table S1 for full list of samples, localities and voucher references).

Tritonia challengeriana Bergh, 1884 (= *T. antarctica* Pfeffer in Martens & Pfeffer, 1886) was chosen as outgroup, due to its basal taxonomical position within Cladobranchia (Pola & Gosliner, 2010).

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

Partial sequences of COI, 16S and H3 were amplified by polymerase chain reaction (PCR) using the primers: LCO1490 (5'-GGTCAACAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.*, 1994) for COI; 16S ar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16S br-H (5'-CCGGTCTGAACTCAGATCAGT-3') (Palumbi *et al.*, 1991) for 16S rRNA; and H3AD5'3' (5'-ATGGCTCGTACCAAGCAGACVGC-3') and H3BD5'3' (5'-ATATCCTTRGGCATRATRGTGAC-3') (Colgan *et al.*, 1998) for H3. These three gene regions are commonly used in systematic studies of gastropods (e.g. Meyer, 2003; Williams & Reid, 2004; Dinapoli *et al.*, 2006; Frey & Vermeij, 2008; Malaquias & Reid, 2009; Pola & Gosliner, 2010; Pola, Camacho-García & Gosliner, 2012; Carmona *et al.*, 2013; Claremont *et al.*, 2013; Eilertsen & Malaquias, 2013; Ohnheiser & Malaquias, 2013). However, some internal primers for COI were designed for those

specimens that did not amplify with the universal ones (see Carmona *et al.*, 2013: table 1).

PCRs were conducted in 50- μ l volume reactions containing 2 μ l of both forward and reverse primers (10 μ M), 5 μ l of dNTP (2 mM), a gene-dependent amount of magnesium chloride (25 mM), 0.5 μ l of Qiagen DNA polymerase (5 units/ μ l), 10 μ l of 'Q-solution' (5 \times), 5 μ l of Qiagen buffer (10 \times) (Qiagen Taq PCR Core Kit cat. no. 201225) and 1 μ l of genomic DNA. Amounts of magnesium chloride were 7 μ l for COI and 16S, and 4 μ l for H3. 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 mixing 5 μ l of PCR product with 2 μ l 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.

DNA sequence analysis

DNA sequences were assembled and edited using Geneious Pro v. 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. Geneious and MAFFT (Katoh, Asiminos & Toh, 2009) were employed to align the sequences. The alignments were checked by eye using MacClade v. 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 COI, using the cut-off values of Carmona *et al.* (2013) as reference thresholds. Uncorrected *p*-distances between all taxa, and the level of saturation for the first, second and third codon positions (*p*-distances against transitions plus transversions) were calculated in MEGA v. 5.0* (Tamura *et al.*, 2011). No evidence of saturation was found for any codon positions.

The most variable regions of the 16S rRNA alignment were removed using the default settings of Gblocks (Castresana, 2000). The topology and node support in the 16S rRNA gene tree were similar regardless of inclusion or exclusion of 'indel-rich' regions and therefore final analyses were performed with all bases included. After primer removal, sequences of COI, 16S and H3 were trimmed to 658, 463 and 327 base pairs, respectively.

Individual gene analyses and a concatenated analysis were performed. To test for conflicting phylogenetic signal between genes, the incongruence length difference test (ILD) (Farris *et al.*, 1994) was conducted (implemented as the partition homogeneity test in PAUP* v. 4.0b10; (Swofford, 2002). Test settings consisted of 10 random stepwise additions (100 replicates) with TBR branch swapping.

The best-fit models of evolution for each gene were determined using the Akaike information criterion (Akaike, 1974) implemented in MrModeltest v. 2.3 (Nylander, 2004). The GTR + I + G model was selected for the three genes.

Maximum likelihood (ML) analyses were performed using the software RAXML v. 7.0.4 (Stamatakis, Hoover & Rougemont, 2008) and nodal support was assessed with nonparametric bootstrapping (BS) with 5,000 replicates, random starting trees and parameters estimated from each dataset under the model selected for the original dataset. Bayesian inference analyses (BI) were conducted using MrBayes v. 3.1.2b (Ronquist & Huelsenbeck, 2003)

for 5 million generations with two independent runs and sampling frequency of 1,000. The models implemented were those estimated with MrModeltest v. 2.3. The combined dataset was partitioned among genes and the ‘unlink’ command was used to allow all parameters to vary independently within each partition.

Convergence was diagnosed graphically by plotting for each run the likelihood against the number of generations using the software Tracer v. 1.4.1 (Drummond & Rambaut, 2007). For each analysis the first 1,250 trees were discarded (‘burn-in’ period) and nodal support was assessed with posterior probabilities (PP). Only nodes supported by $BS \geq 75$ and $PP \geq 0.90$ are discussed.

Nomenclatural acts

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN (1999). The ZooBank LSIDs (Life Science Identifiers) 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:7DFF9B1C-6426-4124-BEF8-DC03DDB60382.

SYSTEMATIC DESCRIPTIONS

Family Aeolidiidae Gray, 1827

Genus *Berghia* Trinchese, 1877

Type species: *Eolidia coerulescens* Laurillard, 1832, by monotypy

Original diagnosis: “Rhinophores elliptical, perfoliate. Foot corners tentaculiform. Anus latero-dorsal, located right behind the first row of the second group of papillae. Radular teeth pectinate and bilobed. Jaws with a minutely denticulate masticatory process, similar to those of *Spurilla*. Cerata with a circular cross-section.” (Trinchese, 1877: 151; translation from Italian).

Berghia coerulescens (Laurillard, 1832)

(Figs 1A, 2, 3A–B, 4A)

Eolidia coerulescens Laurillard, 1832 (in Guerin-Meneville, 1829–1844): pl. 9, fig. 1.

Berghia coerulescens—Trinchese, 1877: 151.

Spurilla coerulescens—García-Gómez & Thompson, 1990: 324, fig. 1B, table 1.

Eolidia souleyeti Vérany, 1853: 384.

Berghia modesta Trinchese, 1882: 188.

External morphology (Figs 1A, 2): Body elongate, slender, tapering gradually towards tail. Foot corners tentaculiform. Ground colour translucent white. Dull orange patch over pericardial bulge. Each side of head, before rhinophores, with short opaque orange or reddish patches tapering gradually towards base of oral tentacles. Rhinophores dull to bright red/orange, whiter on anterior side. Rhinophores densely papillate on posterior side, white apex. Papillae more or less rounded on posterior side, elongate, perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, larger than rhinophores. Oral tentacles and foot corners same ground colour; former with opaque white tips. Digestive gland epithelium with zooxanthellae. Cerata moderately long, cylindrical, with round apex and uniform diameter throughout most of length. Cerata translucent white, greyish digestive gland. White colour overlaid with iridescent blue pigment, varies in intensity. Apex translucent white with subapical yellow band. Cerata in up to eight arches, extending from rear of rhinophores almost to end of foot, continuing along both sides of pericardial area. Each arch with 4–29 cerata,

decreasing in size towards foot. First three clusters with conspicuous red line on ceratal insertion. Anus cleioproctic, below second right arch. Gonopore among cerata of anterior-most group on right side.

Anatomy: Jaws moderately strong. Masticatory edge with minute denticles (Fig. 3A). Radular formulae: $28 \times 0.1.0$ (ZSM Mol 20070294) and $38 \times 0.1.0$ (MNCN 15.05/63439). Radular teeth progressively smaller towards posterior region of radula, wide and bi-arched with 24–46 elongate, fine and acutely pointed denticles on either side of central cusp. Central cusp triangular and small (Fig. 3B). Oral glands occur dorso-laterally to buccal bulb. Oral glands long, thin, convoluted, with uniform diameter throughout most of length. Salivary glands absent. Reproductive system diaulic (Fig. 4A). Preampullary duct widening into narrow elongate ampulla. Postampullary duct bifurcates into oviduct and vas deferens. Vas deferens elongate, entering wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis kidney-shaped, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Originally described from the French Mediterranean coast (Guerin-Meneville, 1829–1844), this species is also known from different localities along the central and western Mediterranean (including the Adriatic Sea). Atlantic Ocean, from the Brittany coasts to the Canary Islands (Bouchet & Tardy, 1976; Cervera *et al.*, 2004; Debelius & Kuitert, 2007; García-Gómez, Cervera & García-García, 2011; Calado & Silva, 2012). The geographic distribution of this species has been controversial. Some specimens of *B. marcusii* and *B. stephanieae* from the western Atlantic, as well as *B. verrucicornis* and *B. columbina* from the eastern Atlantic were grouped under the colour variability of *B. coerulescens* (Pruvot-Fol, 1953; Marcus, 1957; Ev. Marcus, 1976). Our study clearly shows that the distribution of *B. coerulescens* is restricted to the eastern Atlantic and the Mediterranean Sea.

Remarks: The date of the original description of *B. coerulescens* has often been given as 1830 (e.g. Gofas, 2012) or 1838 (e.g. Sherborn, 1902). According to the provisions of ICZN (1999) Art. 21.7 and research by Cowan (1971), we consider that this species was first described in 1832.

Intraspecific variation was observed in the masticatory border of this species since some of the specimens studied had smooth masticatory edges (ZSM Mol 20070294) (not shown). This kind of variability has been reported in other Aeolidiidae species such as *Spurilla neapolitana* (García-Gómez & Cervera, 1985). Excluding the intraspecific variation of the masticatory edge, our specimens of *B. coerulescens* match those of the original (Tardy, 1962) and subsequent descriptions (Schmekel & Portmann, 1982; García-Gómez, 2002). Since we consider this species as well known and easily distinguishable from other species of this genus, no further comparison with others *Berghia* species is conducted here. The most important morphological characters are listed in Table S2 (Supplementary Material). The oral glands of *B. coerulescens* are described here for the first time.

Berghia verrucicornis (A. Costa, 1867)

(Figs 1B, 2, 3C, D, 4B)

Flabellina verrucicornis A. Costa, 1867: 35, pl. II, fig. 4.

Spurilla verrucicornis—García-Gómez & Thompson, 1990: 323.

Eolis peregrina Delle Chiaje, 1841: 73, fig. 17, 18, 19.

Eolis grossularia Fischer, 1869: 6.

Spurilla margaritae Labbé, 1923: 268.

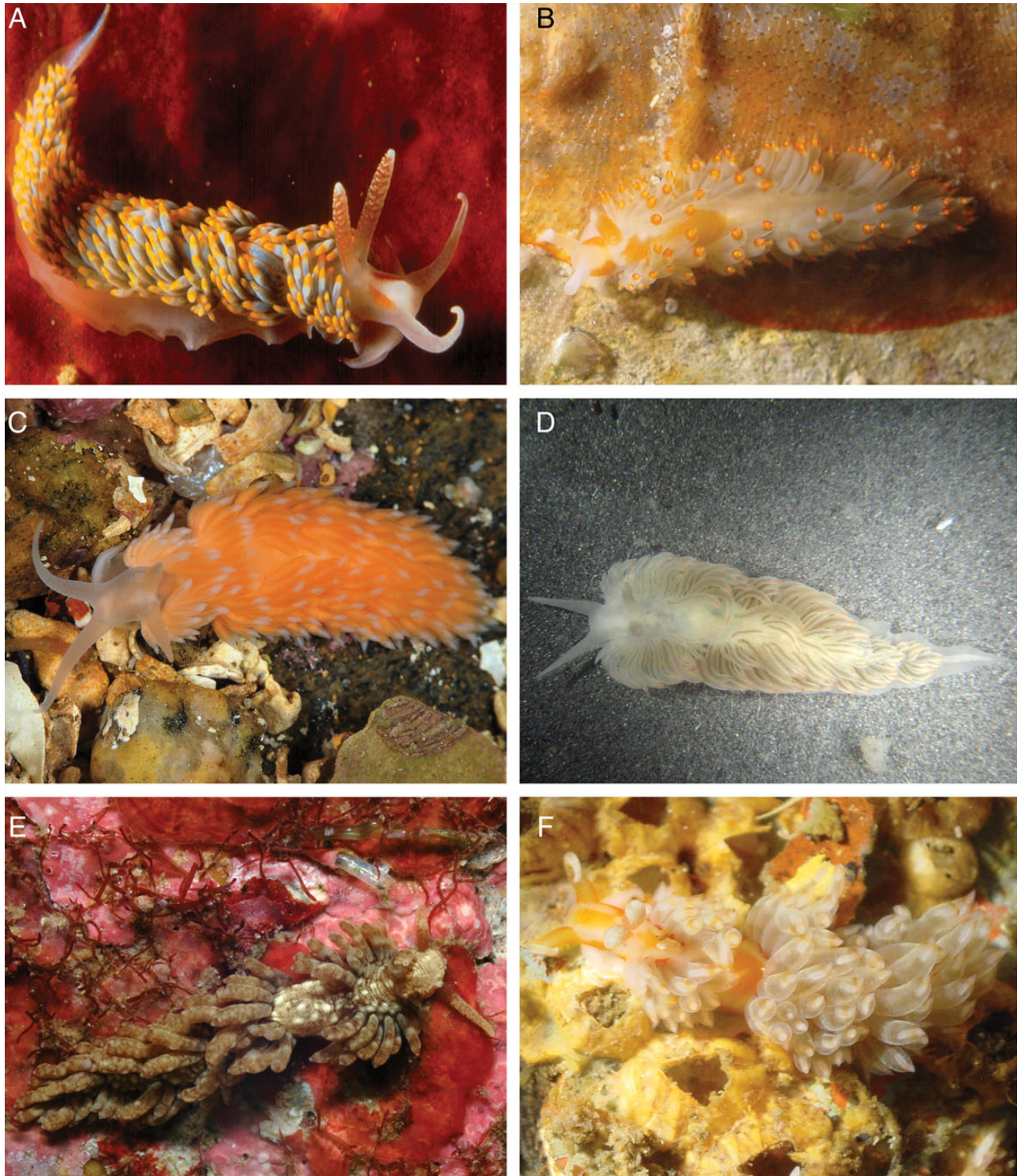


Figure 1. Photographs of living animals of *Berghia* species. **A.** *B. coerulescens*, Italy (photo: G. Villani). **B.** *B. verrucicornis*, Senegal (photo: L. Carmona). **C.** *B. norvegica*, Norway (photo: C. Skauge). **D.** *B. benteva*, Santos, Brazil (photo: C. Magenta). **E.** *B. creutzbergi*, Punta Mona, Costa Rica (photo: Y. Camacho-García). **F.** *B. columbina*, Senegal (photo: L. Carmona).

External morphology (Figs 1B, 2): Body slender, elongate, narrowing towards tail. Foot corners tentaculiform. Ground colour translucent, hyaline white. Each side of head, before rhinophores, with short opaque orange patches tapering gradually towards base of oral tentacles. Rhinophores dull to bright orange, apex white, densely papillate on posterior side. Papillae more or less rounded on posterior side, elongate, perpendicular

to rhinophores on lateral sides (Fig. 2). Oral tentacles elongate, longer than rhinophores. Oral tentacles and foot corners translucent; former with white tips. Cerata translucent, cylindrical, tapering distally with round apex. Digestive gland orange. Subapical band bright, iridescent orange, more intense on edges. Apex white. Cerata in up to six arches, extending from rear of rhinophores, continuing along both sides of pericardial



Figure 2. Lateral view of the papillate rhinophores of *Berghia* (from García-Gómez, 2002).

area and ending in posterior margin of foot. Each arch with 6–15 cerata, decreasing in size towards foot. Anus cleioproctic, below second right arch. Genital opening among cerata of anterior-most group on right side.

Anatomy: Jaws with finely denticulate masticatory edge (Fig. 3C). Radular formulae: $19 \times 0.1.0$ (MNCN 15.05/63441) and $13 \times 0.1.0$ (MNCN 15.05/63443). Radular teeth progressively smaller towards posterior region of radula, wide, bi-arched, with 34–39 elongate, fine and acutely pointed denticles on either side of mid-sized central cusp (Fig. 3D). Oral glands moderately long, dorso-laterally to buccal bulb, widest at proximal end. Salivary glands absent. Reproductive system diaulic (Fig. 4B). Preampullary duct widening into short, thick S-shaped ampulla. Postampullary duct dividing into oviduct and vas deferens. Vas deferens elongate, swollen, entering into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis pear-shaped, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Originally described from Naples (Mediterranean Sea) (Costa, 1867), this species is also known from other Mediterranean localities (including the Adriatic Sea) (Debelius & Kuitert, 2007) as well as the Atlantic coast of France (up to the Brittany coast) (Bouchet & Tardy, 1976) and the Iberian Peninsula (Cervera *et al.*, 2004; García-Gómez *et al.*, 2011), including the Canary Islands (Cervera *et al.*, 2004; García-Gómez *et al.*, 2011), Morocco (Pruvot-Fol, 1953) and Senegal (present study). *Berghia verrucicornis* has been considered an ampho-Atlantic species (Ev. Marcus, 1972; Thompson, 1980; Carroll & Kempf, 1990, among others). In fact, '*B. verrucicornis*' specimens from the western Atlantic were recently described as a new species, *Aelidiella stephanieae*, by Valdés (2005) and transferred to *Berghia*, despite its ceratal arrangement, by Carmona *et al.* (2013) (see Remarks on *B. stephanieae*). The latter contribution as well as our study clearly restricts *B. verrucicornis* to the eastern Atlantic.

Remarks: Pruvot-Fol (1953) identified specimens with two completely different patterns of coloration as *B. coeruleus*. García-Gómez & Thompson (1990) determined that the specimen illustrated in Pruvot-Fol's (1953) plate I, figure 56 corresponded to *B. verrucicornis*,

whereas the second specimen (Plate III, fig. 11) was *B. columbina*. After re-examining Pruvot-Fol's (1953) illustrations we agree with these conclusions.

The masticatory border of the jaws was reported to be either smooth (Schmekel & Portmann, 1982; García-Gómez, 2002) or denticulate (Tardy, 1962). Our specimens have a denticulate masticatory border (Fig. 3C). Hence, intraspecific variation in this character is also found in *B. verrucicornis*.

Our specimens of *B. verrucicornis* agree with the available descriptions of this species (A. Costa, 1867; Tardy, 1962; García-Gómez, 2002). Since this species is well known, only the most important distinctive characters are highlighted in Table S2 (Supplementary Material). The oral glands of *B. verrucicornis* are described here for the first time.

***Berghia norvegica* Odhner, 1939**

(Figs 1C, 3E, F, 4C)

Berghia norvegica Odhner, 1939: 85–89, figs 52–59.

External morphology (Fig. 1C): Body broad, low, head wide. Foot corners tentaculiform. Ground colour translucent white, salmon speckles scattered all over notum. Tuberculate rhinophores translucent with salmon pigment. Tubercles arranged in rows and more dense on posterior side. Oral tentacles translucent, elongate and slightly longer than rhinophores. Foot corners translucent white. The specimen examined here was not well preserved as all cerata were autotomised. Therefore, the information given below is according to Figure 1C and the original description of *B. norvegica* (Odhner, 1939). Cerata moderately long, translucent, slender, with round apex and uniform diameter throughout most of length. Excluding anterior-most group of cerata, digestive gland bright orange with white cnidosacs. Digestive gland of anterior-most group of cerata dull orange, appearing whiter than others. Cerata in up to eight arches followed by nine rows, extending from posterior end of rhinophores almost to posterior end of foot. Anus cleioproctic placed below second right arch. Genital aperture among cerata of anterior-most group on right side.

Anatomy: Buccal mass (jaws and radula) thin, delicate. Masticatory border smooth (Fig. 3E). Radular formula: $18 \times 0.1.0$ (ZMBN 62033). Radular teeth progressively smaller towards posterior region of radula, wide, bi-arched with 15–24 elongate, moderately broad, acutely pointed denticles on either side of medium central cusp (Fig. 3F). A pair of small, translucent oral glands dorsolateral to buccal bulb, widest at proximal end. Salivary glands absent. Reproductive system diaulic (Fig. 4C). Preampullary duct widening into convoluted ampulla. Postampullary duct bifurcating into oviduct and vas deferens. Vas deferens relatively long, entering wider proximal portion of exceptionally large penial sac with unarmed penial papilla. Receptaculum seminis lobed, inserting in anterior oviduct, before oviduct forms female glands. Vagina ventral to penis.

Geographical distribution: To date, only known from Norway (Odhner, 1939; Skauge, 2012; Evertsen & Bakken, 2013).

Remarks: Since Odhner described *B. norvegica* in 1939 this species has been found only twice. The first instance was by Hennig Lemche in 1958, who collected the specimen studied here. This specimen was preserved in formalin and so molecular study was not possible. Although the original description of *B. norvegica* is comprehensive, Odhner (1939) described this species from only two preserved specimens and no information about the coloration of the living animal was provided. Recently, several

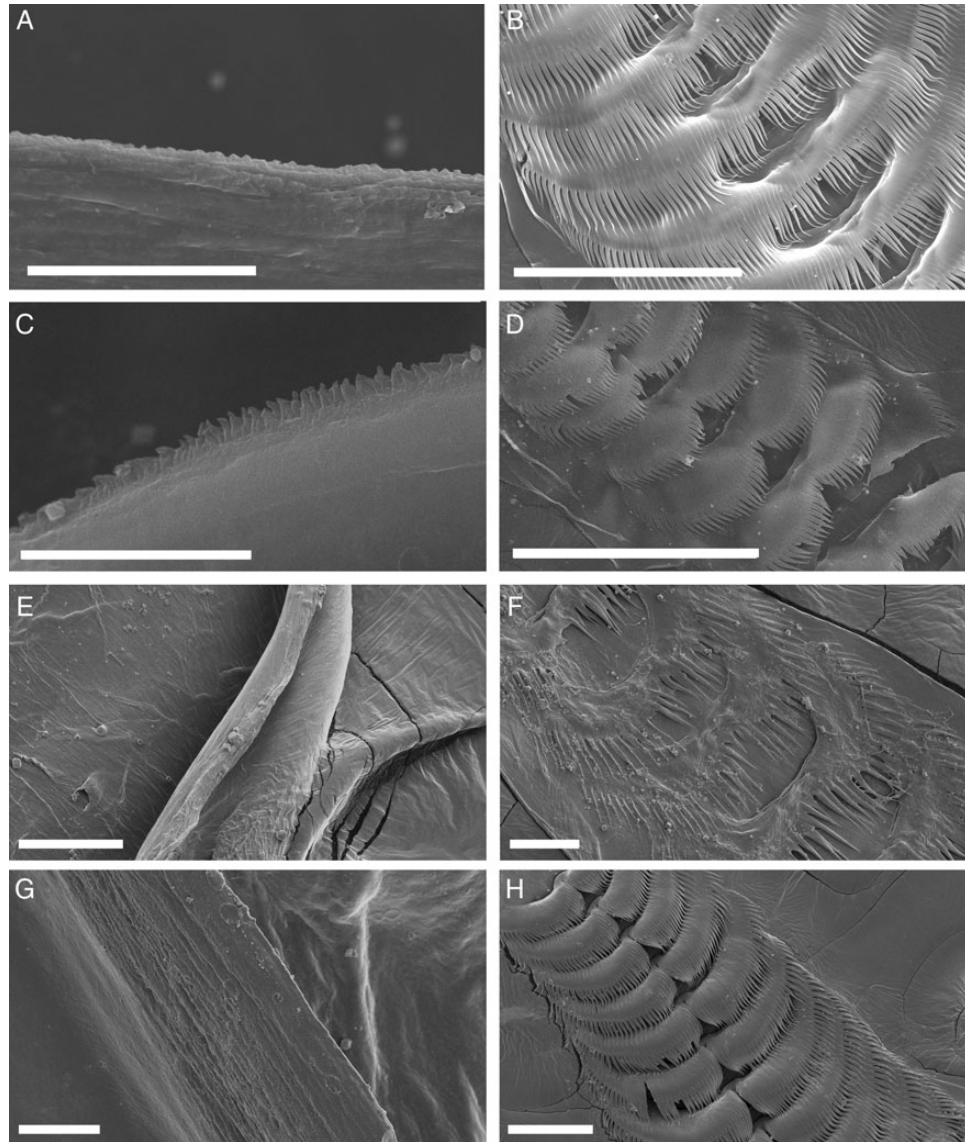


Figure 3. Scanning electron micrographs of *Berghia* species. **A, B.** *B. coeruleus* (ZSM Mol 20070294), Languedoc, France. **A.** Detail of masticatory border. **B.** Radular teeth. **C, D.** *B. verrucicornis* (MNCN 15.05/63441, 6 mm), Grand Piquey, Cap Ferret, France. **C.** Detail of masticatory border. **D.** Radular teeth. **E, F.** *B. norvegica* (ZMBN 62033), Norway. **E.** Detail of masticatory border. **F.** Radular teeth. **G, H.** *B. benteva* (MZSP 104253), Santos, São Paulo, Brazil. **G.** Detail of masticatory border. **H.** Radular teeth. Scale bars: **A** = 50 μm ; **B** = 200 μm ; **C** = 30 μm ; **D** = 150 μm ; **E, F** = 50 μm ; **G** = 20 μm ; **H** = 100 μm .

specimens have been collected during the ‘Nudibranch Safari 2011’ in Norway (Skauge, 2012; Evertsen & Bakken, 2013). These new records allow us to describe the coloration of *B. norvegica*. Moreover, its external appearance makes this species easily recognizable (Table S2 of Supplementary Material).

Considering the original description, the difference in the number of teeth between the paratype (40) and our specimen (18) is notable, although this is likely due to animal size (110 mm in the paratype; 6 mm in studied specimen, ZMBN 62033). The oral glands of *B. norvegica* are reported here for the first time.

***Berghia benteva* (Er. Marcus, 1958)**
(Figs 1D, 2, 3G, H, 4D)

Baeolidia benteva Er. Marcus, 1958: 65, figs 105–111.
Berghia benteva—Ev. Marcus, 1976: 9.

Anteaeolidiella benteva—Miller, 2001: 633.

Aeolidiella benteva—García-García *et al.*, 2008: 189.

Berghia dakariensis—Caballer & Ortea, 2013: 440, figs 1, 2.

External morphology (Figs 1D, 2): Body low, broad, head wide. Tail nearly translucent. Foot may be wider than visceral part of body, foot corners tentaculiform. Body translucent white with opaque white spots scattered all over pericardium. White rhomboidal patch between eyes. Rhinophores, oral tentacles and foot corners translucent white. Rhinophores densely papillate on posterior side. Papillae more or less rounded on posterior side, elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, white tips, larger than rhinophores. Anus cleioproctic, below second right arch. Genital pore placed among cerata of anterior-most group on right side. Cerata long, translucent, slender, with round apex and uniform diameter throughout most of length. Branches of digestive gland brownish with pinkish cnidosac. In anterior-most group of cerata, branches

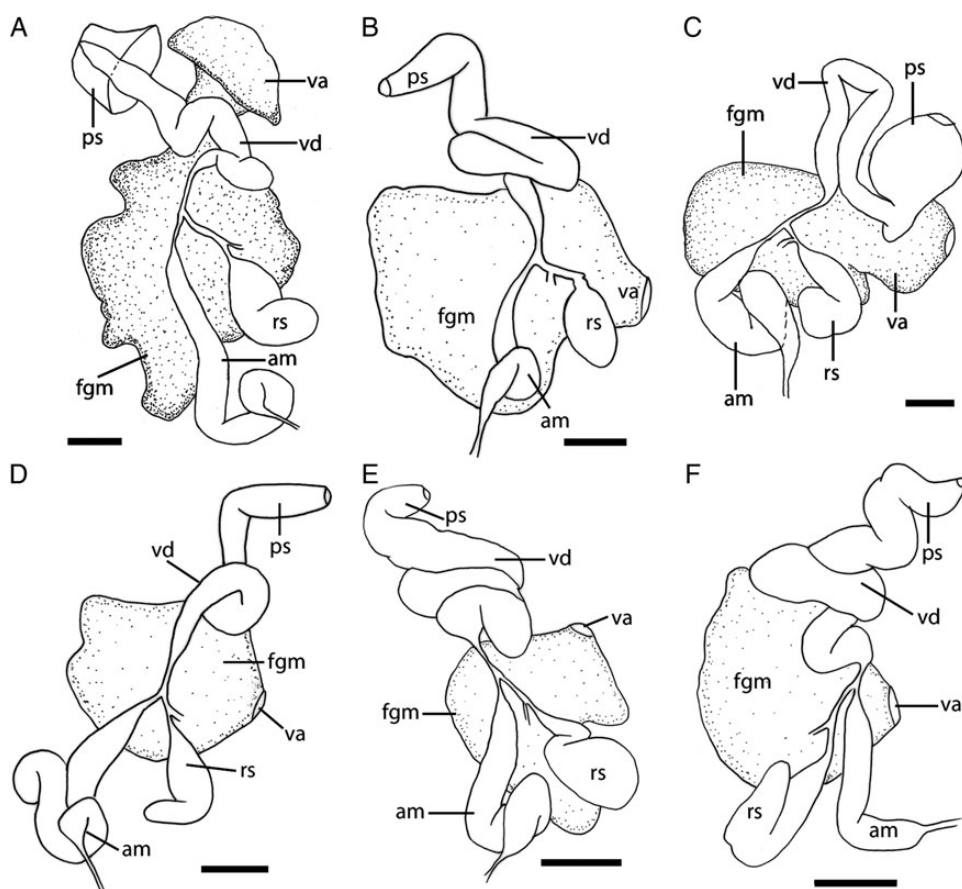


Figure 4. Reproductive system of *Berghia* species. **A.** *B. coeruleascens*, Langedoc, France. **B.** *B. verrucicornis*, Cap Ferret, France. **C.** *B. norvegica*, Norway. **D.** *B. benteva*, Santos, Brazil. **E.** *B. creutzbergi*, Punta Mona, Costa Rica. **F.** *B. columbina*, Huelva, Spain. Scale bars = 0.5 mm. Abbreviations: am, ampulla; fgm, female gland mass; ps, penial sac; rs, receptaculum seminis; va, vagina; vd, vas deferens.

of digestive gland may be thinner and shorter, appearing whiter than others. Cerata in up to seven arches, extending from rear of rhinophores almost to end of foot. Each arch with 3–44 cerata, decreasing in size towards posterior end of foot. First arch triple, arches II–IV double, remaining arches simples.

Anatomy: Masticatory border of paired jaws smooth (Fig. 3G). Radular formula: $22 \times 0.1.0$ (MZSP 104253). Radular teeth progressively smaller towards posterior region of radula, wide, of two arches with 29–38 elongate, fine, acutely pointed denticles on each side of notch (Fig. 3H). Pair of moderately short oral glands dorso-lateral to each side of buccal bulb, of uniform diameter throughout most of length. Salivary glands absent. Reproductive system diaulic (Fig. 4D). Preampullary duct widening into long, thin ampulla. Postampullary duct dividing into oviduct and vas deferens. Vas deferens elongate, penetrating into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis bean-shaped, entering into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Known from Brazil (Er. Marcus, 1958; Ev. Marcus, 1976; Domínguez et al., 2008; García-García et al., 2008) and Senegal (Caballer & Ortea, 2013).

Remarks: When Er. Marcus (1958) described *Baeolidia benteva*, he did not provide any reason for the generic placement. Later, Ev. Marcus (1976) transferred this species to *Berghia* based on its cylindrical cerata, while Miller (2001) considered it as a candidate for *Anteaeolidiella* because of its fork-like ceratal arrangement.

Domínguez, Troncoso & García (2008) retained this species within *Berghia*, but García-García et al. (2008) transferred it to *Aeolidiella*. Morphological characters such as cylindrical cerata in arches and papillate rhinophores, which are shared by most *Berghia* species (see Discussion), support its placement in *Berghia*. Our molecular analyses also support its assignment to this genus.

Recently, Caballer & Ortea (2013) found one specimen of *B. benteva* in Gorée Bay, Senegal. Based on radular information, they identified their specimen as a valid member of the genus *Berghia* according to the classification proposed by Miller (2001). Moreover, these authors considered that *Spurilla dakariensis* Pruvot-Fol, 1953 is conspecific with *B. benteva* and therefore they transferred *S. dakariensis* to *Berghia*, rendering *B. benteva* a junior synonym of *B. dakariensis*. Pruvot-Fol (1953) described *S. dakariensis* from Senegal, but did not provide any information about the external coloration of the animal, the ornamentation of the rhinophores or the arrangement of the cerata, only pointing out the large size of the species and the shape of the jaw and radular teeth. Consequently, Carmona et al. (2013: 8) considered *S. dakariensis* to be a *nomen dubium* since “some forms of *Spurilla neapolitana*, *Spurilla* sp. A and *Spurilla braziliana* could be attributed to *Spurilla dakariensis*”. Additionally, it has been shown that the radular morphology of aeolidiids does not allow unequivocal identification of species (Carmona, 2013).

Our specimens of *B. benteva* agree with the original and subsequent descriptions (Domínguez et al., 2008; García-García et al., 2008; Caballer & Ortea, 2013). Only Domínguez et al. (2008) referred to a larger number of groups of cerata (12–15), but the

specimens studied by the latter authors were twice the size of ours. *Berghia benteva* can be distinguished from the remaining *Berghia* species not only by its coloration, but also by its low and broad body.

***Berghia creutzbergi* Er. Marcus & Ev. Marcus, 1970**

(Figs 1E, 2, 4E, 5A–B)

Berghia creutzbergi Er. Marcus & Ev. Marcus, 1970: 87, figs 145–147.

Spurilla creutzbergi—Redfern, 2001: 181, pl. 120, fig. 745.

Milleroidia ritmica Ortea, Caballer & Espinosa, 2004: 133, figs 2, 4B, pl. 1B.

External morphology (Fig. 1E, 2): Body elongate, slender, tapering gradually towards short tail. Foot corners tentaculiform. Brownish ground colour, opaque white spots over dorsum. In some specimens, white spots can be dense, forming continuous white patch over dorsum. Bases of brown rhinophores very close. Rhinophores papillate, with white-cream apex. Papillae more or less rounded on posterior side, elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, larger than rhinophores. Distal two-thirds cream with brownish base. Cerata moderately short, cylindrical, wider distally. All cerata move from side to side when animal crawls. Cerata brownish with white spots, translucent white apex. Cerata in up to six arches, completely separated from each other, extending from rear of rhinophores almost to end of foot. Each arch with 4–15 cerata, decreasing in size towards posterior end of foot. Anus cleioproctic, below second ceratal right arch. Genital aperture, among cerata of anterior-most group on right side.

Anatomy: Jaws with finely denticulate masticatory border (Fig. 5A). Radular formula: $22 \times 0.1.0$ (MZUCR 8340). Radular teeth progressively smaller towards posterior region of radula, wide, of two arches with 22–32 elongate, fine, acutely pointed denticles on either side of very small central cusp (Fig. 5B). Oral glands small, dorsolateral to buccal bulb, of uniform diameter throughout most of length. Salivary glands absent. Reproductive system diallic (Fig. 4E). Preampullary duct widening into convoluted ampulla. Postampullary duct bifurcating into oviduct and vas deferens. Vas deferens elongate, swollen, entering into wider proximal portion of penial sac with an unarmed penial papilla. Receptaculum seminis pear-shaped, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Tropical western Atlantic; Brazil (Padula & Santos, 2006), Curaçao (Valdés *et al.*, 2006), Venezuela (Valdés *et al.*, 2006), Barbados (Valdés *et al.*, 2006), Bahamas (Redfern, 2001), Cayman Islands (Valdés *et al.*, 2006), Costa Rica (Camacho-García *et al.*, 2014; present study), Cuba (present study) and Florida (Valdés *et al.*, 2006).

Remarks: *Milleroidia ritmica*, which shares many morphological characters with *B. creutzbergi* (Dominguez *et al.*, 2008), was described by Ortea, Caballer & Espinosa (2004) from the Caribbean coast of Costa Rica. These authors considered the long tail, ceratal arrangement, denticulate masticatory border and movement of the animal to be characters that distinguished it from all known Aeolidiidae, although they did not compare it with any *Berghia* species. Some of these characters show intraspecific variation within Aeolidiidae, such as tail length (see the photographs published by Brown, 2006) and the edge of the masticatory border (García-Gómez & Cervera, 1985; present study). Additionally, Ortea *et al.* (2004) depicted a radular tooth with alternate larger and smaller denticles, which has

been considered to be characteristic of *B. creutzbergi* (see below). The authors also included an illustration (their pl. 1B) of the living animal that is similar to *B. creutzbergi*. The side-to-side movement of the cerata is also characteristic of this species (Padula & Santos, 2006; Valdés *et al.*, 2006). Moreover, our specimen of *B. creutzbergi* from Punta Mona, Costa Rica (the type locality of *Milleroidia ritmica*) clusters with the remaining specimens of *B. creutzbergi*. We therefore conclude that the two are conspecific and consider *M. ritmica* a junior synonym of *B. creutzbergi*.

Concerning the radula of *B. creutzbergi*, Er. Marcus & Ev. Marcus (1970) described the presence of larger and smaller denticles, but they examined only a single specimen. This feature was also observed by Padula & Santos (2006). However, our specimen did not show this feature, which seems to be part of the intraspecific variability of the species (see Discussion).

***Berghia columbina* (García-Gómez & Thompson, 1990)**

(Figs 1F, 2, 4F, 5C, D)

Spurilla columbina García-Gómez & Thompson, 1990: 323, figs 1–5.

Berghia columbina—Muniain & Ortea, 1999: 148.

External morphology (Figs 1F, 2): Body elongate, slender, narrowing to posterior end of foot. Foot corners tentaculiform. Ground colour pale grey or white with bright orange surface pigment. Vivid orange mark over pericardial area. Each side of head with a boomerang-shaped bright orange patch, tapering gradually towards base of oral tentacles. Between these patches a diamond bright orange mark, which can merge with pericardial patch to continue uninterrupted to posterior end of foot. White-tipped rhinophores with papillae from dull to bright red/orange. Papillae more or less rounded on posterior side, but elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, larger than rhinophores. Apical two-thirds of oral tentacles red-orange, tips white. Foot corners same as ground colour. Cerata translucent, slender, with round apex and uniform diameter throughout most of length. Digestive gland brown, visible. Ceratal apex translucent white, may have pale orange subapical ring. Cerata in up to eight arches, extending from rear of rhinophores almost to end of foot, continuing along both sides of pericardial area. Each arch with 3–13 cerata, decreasing in size towards foot edge. Red lines on borders of insertion of cerata. Anus cleioproctic, below second right arch. Gonopore among cerata of anterior-most group on right side.

Anatomy: Jaws moderately strong with smooth masticatory edge (Fig. 5C). Radular formula: $14 \times 0.1.0$ (MNCN 15.05/63444). Radular teeth progressively smaller to posterior region of radula, wide, forming two arches with up to 32 elongate, fine, acutely pointed denticles on either side of central cusp. Central cusp triangular, mid-sized (Fig. 5D). Oral glands moderately long, widest at proximal end, dorsolateral to the buccal bulb. Salivary glands absent. Reproductive system diallic (Fig. 4F). Preampullary duct widening into short ampulla. Postampullary duct bifurcates into oviduct and vas deferens. Vas deferens long, convoluted, swollen, inserting into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis large, folded, entering into anterior oviduct before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Originally described from the southwestern coast of the Iberian Peninsula (Atlantic Ocean) (García-Gómez & Thompson, 1990), this species is also known from the Mediterranean coast of Andalusia (southern Spain) (García-Gómez *et al.*, 2011), southern Portugal (Cervera *et al.*, 2004;

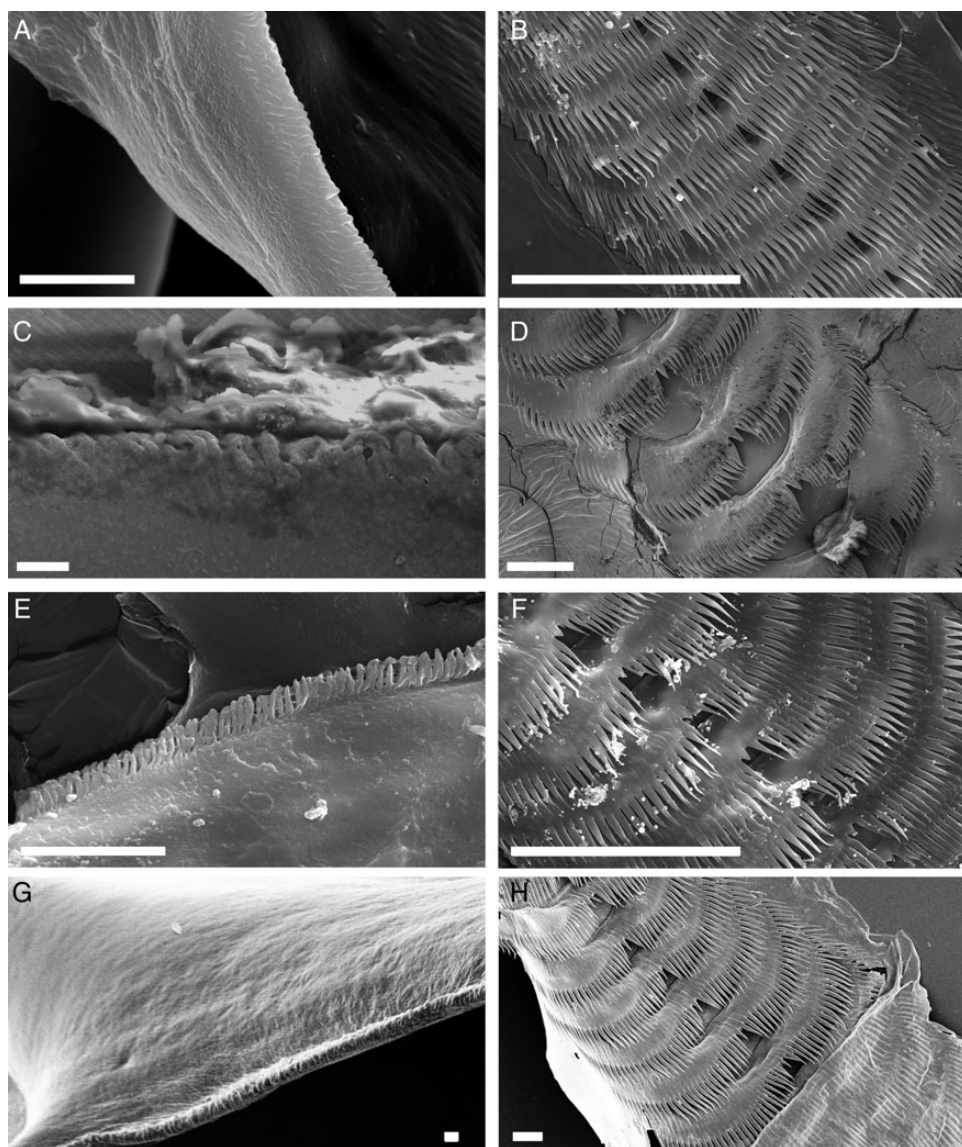


Figure 5. Scanning electron micrographs of *Berghia* species. **A, B.** *B. creutzbergi* (MZUCR 8340), Gandoca-Manzanillo, Provincia de Limón, Punta Mona, Costa Rica. **A.** Detail of masticatory border. **B.** Radular teeth. **C, D.** *B. columbina* (MNCN 15.05/63444), Huelva, SW Spain. **C.** Detail of masticatory border. **D.** Radular teeth. **E, F.** *B. rissodominguezi* (MNCN 15.05/63444), María la Gorda, Cuba. **E.** Detail of masticatory border. **F.** Radular teeth. **G, H.** *B. stephanieae* (CASIZ 185770), Florida. **G.** Detail of masticatory border. **H.** Radular teeth. Scale bars: **A, C, H** = 20 μm ; **B, F** = 300 μm ; **D, E** = 50 μm ; **G** = 2 μm

García-Gómez *et al.*, 2011; Calado & Silva, 2012), the Canary Islands (García-Gómez *et al.*, 2011; Senegal (present study)) and the Atlantic coast of Morocco (Pruvot-Fol, 1953; present study).

Remarks: The specimen examined here perfectly matches with the original description given by García-Gómez & Thompson (1990). *Berghia columbina* could be misidentified as *B. verrucicornis* since both species share part of their geographical distribution and have some morphological similarities such as body colour and the orange patches over the pericardium. The red lines on the borders of the insertion of the cerata found only in *B. columbina* distinguish the two species.

***Berghia rissodominguezi* Muniain & Ortea, 1999**

(Figs 2, 5E–F, 6A, 7A)

Berghia rissodominguezi Muniain & Ortea, 1999: 144, figs 1, 2, pl. 1, fig. A.

External morphology (Figs 2, 6A): Body slender, elongate, tapering gradually to short posterior end of foot. Foot corners tentaculiform. Ground colour translucent white overlaid with opaque orange pigment that may cover the whole notum or part of it (mainly head and pericardial area). Rhinophores bright orange with yellow or cream pigmentation on apical portion. Densely papillate on posterior side. Papillae more or less rounded on posterior side, elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, larger than rhinophores. Oral tentacles and foot corners same ground colour. Oral tentacles with opaque white or yellow tips. The specimen examined was not well preserved. Therefore, the description below is based on Figure 6A (included in Muniain & Ortea, 1999) and on a photograph of one specimen from Colombia (not shown). Cerata moderately elongate, cylindrical, with round apex and uniform diameter throughout most of length; translucent with central reddish-brown, bluish or almost black diverticulum and orange or yellow subapical band; apex translucent white. Cerata in up to eight arches, from rear of rhinophores almost to end of foot,

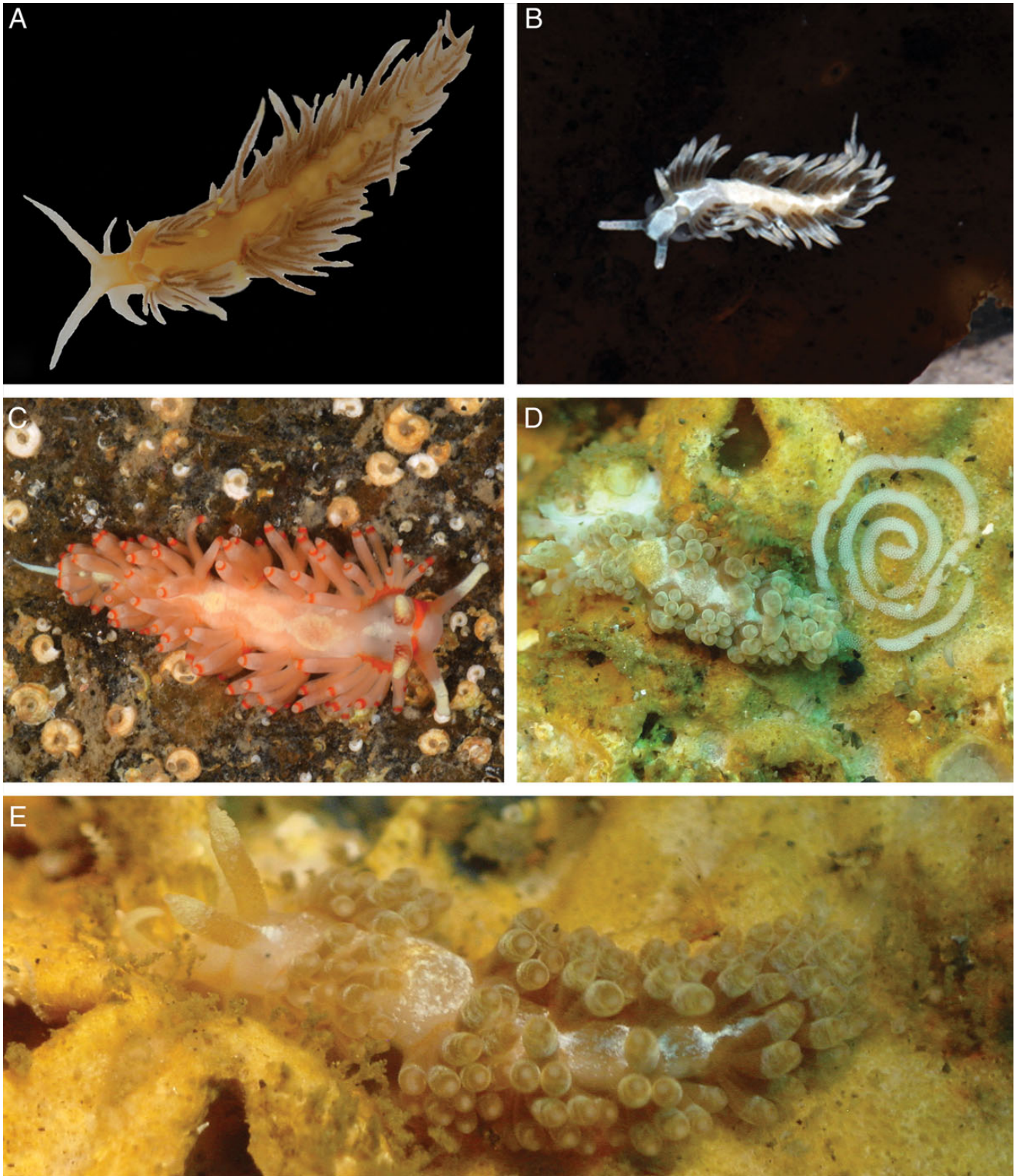


Figure 6. Photographs of living animals of *Berghia* species. **A.** *B. rissodominguezi*, Maria la Gorda, Cuba (photo: M. Pola). **B.** *B. stephanieae*, Florida (photo: T. M. Gosliner). **C.** *B. marcusii*, Santos, Brazil (photo: C. Magenta). **D, E.** *B. marinae* n. sp. (holotype: MNCN 15.05/60094), Senegal (photo: L. Carmona).

continuing along both sides of pericardial area. Each arch with 3–24 cerata, decreasing in size towards foot. Anus cleioproctic located below second right arch, genital aperture among cerata of anterior-most group on right side.

Anatomy: Jaws with denticulate masticatory border (Fig. 5E). Radular formula: $22 \times 0.1.0$ (MNCN 15.05/63445). Radular teeth wide and bi-arched with 28–38 elongate, fine and acutely pointed denticles either side of small central denticle (Fig. 5F).

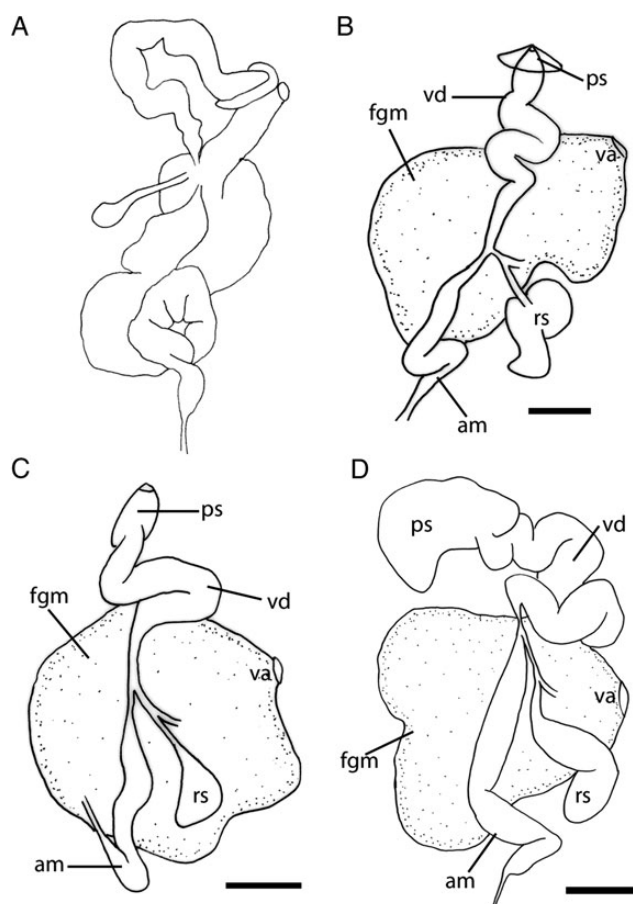


Figure 7. Reproductive system of *Berghia* species. **A.** *B. rissodominguezi* (from Muniain & Ortea, 1999). **B.** *B. stephanieae*, Florida. **C.** *B. marcusi*, Santos, Brazil. **D.** *B. marinae* n. sp., Senegal. Scale bars = 0.5 mm. Abbreviations: am, ampulla; fgm, female gland mass; ps, penial sac; rs, receptaculum seminis; va, vagina; vd, vas deferens.

Oral glands dorsolateral to buccal bulb, shorter than buccal mass. Salivary glands absent. The reproductive system was not sufficiently well preserved for study, but was described in detail by Muniain & Ortea (1999; Fig. 7A).

Geographical distribution: Originally described from Argentina, this species has also been recorded from the Brazilian (Padula *et al.*, 2012) and Caribbean coasts (Valdés *et al.*, 2006).

Remarks: Muniain & Ortea (1999) listed references in which they considered that *B. rissodominguezi* was previously misidentified as other *Berghia* species. Of these, only that to *B. coerulea* by Thompson (1980) is confirmed by us as *B. rissodominguezi*. The remaining reports of *B. coerulea* and *B. verrucicornis* from the western Atlantic mentioned by Muniain & Ortea (1999) are identified here as *B. stephanieae* or *B. marcusi*, or cannot confidently be ascribed to any species.

The holotype described by Muniain & Ortea (1999) had a translucent white body colour, an orange triangle over the head and between the rhinophores, and a red ring below the orange band in the cerata. None of these features are found in our Cuban specimen (Fig. 6A). The specimen from Colombia included in the molecular analyses (Table S1 of Supplementary Material) has a coloration intermediate between that of Muniain & Ortea's specimen and ours from Cuba (not shown). This variability, especially the pigmentation of the notum, has

been observed in other *Berghia* species (e.g. *B. coerulea* and *B. creutzbergi*). Our specimen from Cuba and the holotype share the same coloration of cerata, rhinophores and oral tentacles as well as the morphological features of the radular teeth.

***Berghia stephanieae* (Valdés, 2005)**

(Figs 5G–H, 6B, 7B)

Aeolidiella stephanieae Valdés, 2005: 218, figs 1–3.

External morphology (Fig. 6B): Body elongate, slender, tapering gradually towards posterior end of foot, which may reach 2/3 of body length. Foot corners tentaculiform. Ground colour translucent grey. Entire dorsum with opaque white pigment, forming a triangular patch from anterior-most margin of head to behind eyes. Each side of head may have a triangular ochre patch, tapering gradually to base of oral tentacles. Opaque white pigment on distal half of rhinophores. Rhinophores with small, sparse papillae. Oral tentacles elongate, larger than rhinophores. Oral tentacles and foot corners translucent grey. Former with an opaque white pigment on distal half. Digestive gland epithelium with zooxanthellae. Cerata translucent white, moderately flattened with round apex. Depending on prey, proximal two-third of digestive gland branches from beige to greenish, or brownish ochre; distal one-third diffuse white to iridescent grey, with slight subapical yellow band; apex translucent white. Cerata in up to three groups of rows, extending from rear of rhinophores almost to end of foot, even continuing along both sides of pericardial area. Each row with 2–4 cerata, decreasing in size towards foot. Beige or ochre lines on borders of insertion of cerata. Anus cleioproctic, beneath third group of cerata. Gonopore ventral to second and third rows.

Internal anatomy: Jaws with denticulate masticatory edge (Fig. 5G). Radular formula: 25 × 0.1.0 (CASIZ 185770). Radular teeth progressively smaller to posterior region of radula, wide, bi-arched with up to 31 elongate, fine, acutely pointed denticles either side of triangular, mid-sized central cusp (Fig. 5H). Oral glands dorsolateral to buccal bulb. Oral glands moderately long, with uniform diameter throughout most of length. Salivary glands absent. Reproductive system dialic (Fig. 7B). Preampullary duct widening into short, narrow ampulla. Postampullary duct dividing into oviduct and vas deferens. Vas deferens elongate, entering into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis lobed, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Only known from Florida (Valdés, 2005).

Remarks: *Berghia stephanieae* has been often misidentified as *B. verrucicornis* (e.g. Ev. Marcus, 1972; Carroll & Kempf, 1990; Kempf, 1991; Kempf & Brittsan, 1996). *Berghia stephanieae* is commonly sold commercially as a predator of the aquarium 'weedy' anemone (*Aiptasia*) under the name *B. verrucicornis* (Kempf & Brittsan, 1996; Kempf, 2002; Valdés, 2005; Valdés *et al.*, 2006). Morphological and molecular data clearly show that they are not conspecific. In addition, both species are also distinguished by their respective distributions—eastern Atlantic for *B. verrucicornis* and western Atlantic for *B. stephanieae*.

Valdés (2005) reported the presence of a bursa copulatrix in the reproductive system of *B. stephanieae*. This condition has not previously been described in any other member of the Aeolidiidae. After examining the reproductive system of our specimen, the presence of a bursa copulatrix is not confirmed (Fig. 7B). Furthermore, figure 1B of Valdés (2005) shows the rhinophores

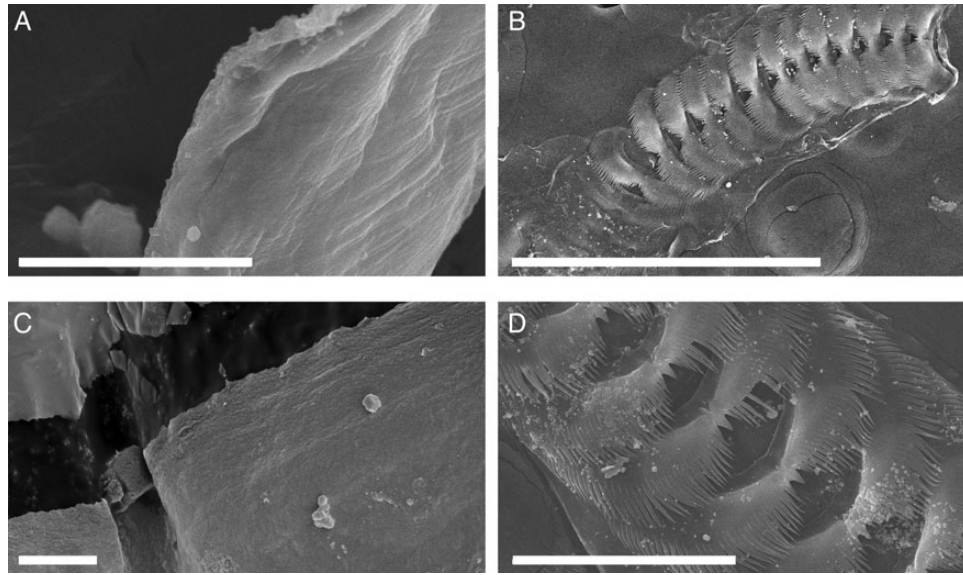


Figure 8. Scanning electron micrographs of *Berghia* species. **A, B.** *B. marcusii* (MZUSP 103225), São Sebastião, São Paulo, Brazil **A.** Detail of masticatory border. **B.** Radular teeth. **C, D.** *B. marinae* n. sp. (MNCN 15.05/60094), Senegal. **C.** Detail of masticatory border. **D.** Radular teeth. Scale bars **A** = 25 μm ; **B** = 500 μm ; **C** = 5 μm ; **D** = 150 μm .

of *B. stephanieae* as inconspicuous lamellae even though the specimen seems to have small and sparse papillae. The inconspicuous lamellae could be a preservational artefact. Excluding these features, our *B. stephanieae* specimen agrees with the original description of this species. The oral glands of *B. stephanieae* are described and reported here for the first time.

***Berghia marcusii* Domínguez, Troncoso & García 2008**

(Figs 2, 6C, 7C, 8A–B)

Berghia marcusii Domínguez, Troncoso & García, 2008: 355, fig. 6, fig. 7a, b.

Berghia sp. B Edmunds, 1968: 213, figs 8b, 9d–g.

External morphology (Fig. 6C): Body slender, elongate, tapering gradually towards posterior end of foot. Anterior margin of foot elongate. Foot corners tentaculiform. Body translucent white. From base of oral tentacles to end of posterior end of foot with opaque white band, which may be broken or may continue uninterrupted. Each side of head with boomerang-shaped bright orange patch tapering gradually to base of oral tentacles. Rhinophores with reddish bases, cream on apical part. Densely papillate on posterior side. Papillae more or less rounded on posterior side, but elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Translucent foot corners. Elongate oral tentacles, larger than rhinophores. Oral tentacles with translucent bases, some red pigment, apical 2/3 cream. Cerata translucent, slender, with round apex and uniform diameter throughout most of length. Cerata with orange subapical band, whitish area below it. Apex translucent white. Digestive gland orange. Cerata in five arches, extending from rear of rhinophores almost to end of posterior end of foot. Each arch with 4–12 cerata, decreasing in size towards foot. Bright orange lines on borders of insertion of cerata. Cleioproctical anus below second right arch. Genital opening among cerata of anterior-most group on right side.

Anatomy: Smooth masticatory edge (Fig. 8A). Radular formula: $18 \times 0.1.0$ (MZUSP 103225). Radular teeth wide, bi-arched with 26 elongate, fine, acutely pointed denticles either side of triangular, large central cusp (Fig. 8B). Oral glands moderately

long, dorsolateral to buccal bulb. Oral glands with uniform diameter throughout most of length. Salivary glands absent. Reproductive system dialytic (Fig. 7C). Preampullary duct widening into moderately long ampulla. Postampullary duct dividing into oviduct and vas deferens. Vas deferens swollen, moderately short, entering into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis bean-shaped, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Known only from Jamaica (Edmunds, 1968, as *Berghia* sp. B) and Brazil (Domínguez *et al.*, 2008).

Remarks: Er. Marcus (1957) identified one specimen from Ubatuba, Brazil as *B. coerulea*. Muniain & Ortea (1999) considered this specimen to be *B. rissodominguezi*, but Domínguez *et al.* (2008: 362) recognized it as *B. marcusii* based on “a yellow and red skin pattern” and “red marks are two lines between tentacles and rhinophores”.

Our specimen clearly matches with the original description of *B. marcusii*. It only differs in the ornamentation of the masticatory border since our specimen lacks denticles (Fig. 8B). We attribute this difference to intraspecific variability, which is found in several *Berghia* species. Additionally, the oral glands of *B. marcusii* are described for the first time in this study.

The coloration of this species makes easy to distinguish it from the remaining *Berghia* species (Table S2 of Supplementary Material).

***Berghia marinae* new species**

(Figs 2, 6D–E, 7D, 8C–D)

LSID urn:lsid:zoobank.org:act:4DE5978E-C1C3-4528-B198-EE8E3BBD4A0C

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

Type material: Holotype: MNCN 15.05/60094, one specimen, dissected, 6.5 mm in length preserved; Tacoma Wreck, Senegal, 08 June 2005, coll. L. Carmona.

Etymology: This species is named after Marina Poddubestkaia, a friend and colleague who kindly provided us several Aeolidiidae species from France.

Diagnosis: This species can be distinguished by its brown cerata with greyish white pigmentation on the distal half (Fig. 6D, E).

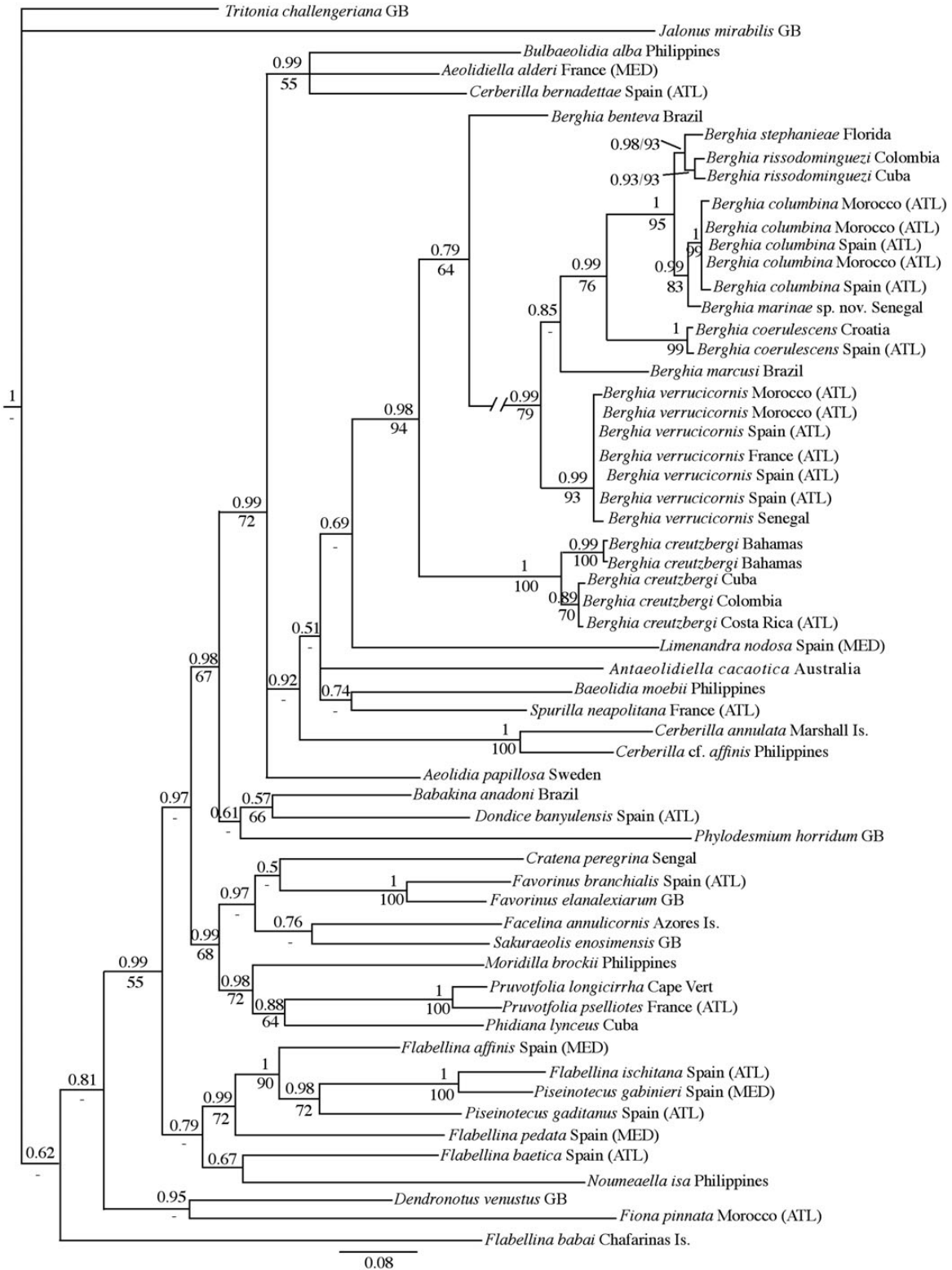


Figure 9. Phylogenetic hypothesis for *Berghia* species based on the combined dataset (H3 + COI + 16S), inferred by Bayesian analysis (BI). Numbers above branches represent posterior probabilities from BI. Number below branches represent bootstrap values from maximum likelihood analysis. Abbreviations: ATL, Atlantic Ocean; MED, Mediterranean Sea; GB, GenBank.

External morphology (Figs 2, 6D–E): Body elongate, slender, tapering gradually to posterior end of foot. Foot corners tentaculiform. Body colour translucent white with bright opaque white speckles on notum. Orange mark from dull to bright orange, covering entire or small part of pericardium. Each side of head with boomerang-shaped dull orange patch, tapering gradually to base of oral tentacles. Rhinophores and oral tentacles brownish with iridescent pale yellow apical portion. Rhinophores densely papillate on posterior side. Papillae more or less rounded on posterior side, but elongate and perpendicular to rhinophores on lateral faces (Fig. 2). Oral tentacles elongate, larger than rhinophores. Foot corners translucent. Cerata slender, with round apex, uniform diameter throughout most of length. Cerata brownish with greyish white pigmentation on distal half. Brown band with thin subapical dull yellow ring. Apex translucent white. Cerata in up to seven arches, extending from rear of rhinophores almost to posterior end of foot. Each arch with 3–20 cerata, decreasing in size towards foot and posteriorly. Dull orange lines on borders of ceratal insertions. Anus cleioproctic below second right arch. Gonopore among cerata of anterior-most group on right side.

Anatomy: Jaws moderately strong. Masticatory border without denticles (Fig. 8C). Radular formula: 22 × 0.1.0 (MNCN 15.05/60094). Radular teeth wide, bi-arched, with 35–39 very elongate, fine, acutely pointed denticles on either side of large central cusp (Fig. 8D). Large oral glands dorsolateral to buccal bulb, widest at proximal end. Without salivary glands. Reproductive system dialucic (Fig. 7D). Preampullary duct widening into elongate, thin ampulla. Postampullary duct bifurcates into oviduct and vas deferens. Vas deferens long and swollen, entering into wider proximal portion of penial sac with unarmed penial papilla. Receptaculum seminis bean-shaped, inserting into anterior oviduct, before latter forms female glands. Vagina ventral to penis.

Geographical distribution: Known only from Senegal (present study).

Spawn: Egg mass (Fig. 6D) white, with up to four complete anti-clockwise whorls.

Remarks: *Berghia marinae* n. sp. is clearly distinguishable from other members of the genus by its coloration (Table S2 of Supplementary Material). Only *B. stephanieae* may have similar coloration of the cerata, but it lacks the orange lines on the ceratal insertion found in *B. marinae* and also has a completely different ceratal arrangement and geographical distribution. Moreover, the molecular phylogenetic hypothesis (Fig. 9) supports the validity of *B. marinae*.

PHYLOGENETIC ANALYSIS

The combined dataset yielded a sequence alignment of 1,448 positions. The ILD test showed incongruence between COI and 16S, H3 and COI, H3 and 16S sequences ($P = 0.01$). Sullivan (1996) and Cunningham (1997) suggested that a significance threshold of 0.05 might be too conservative for the ILD test. Moreover, the shortcomings of this test have been reported by several authors (Dolphin *et al.*, 2000; Barker & Lutzoni, 2002; Darlu & Leconte, 2002; Downton & Austin, 2002) and even when the ILD test shows incongruence between data sets the combined analysis can provide better-resolved trees (Collin, 2003; Vonnemann *et al.*, 2005; Williams & Ozawa, 2006; Malaquias *et al.*, 2009). Hence, concatenated analyses were performed. No saturation was observed across genes and codon positions (not shown). The combined tree provided better

resolution than H3, COI or 16S separately (not shown). Although bootstrap values were lower than the posterior probabilities in deeper nodes, the topologies of the ML trees were congruent with the results yielded by the Bayesian analyses and thus ML trees are not shown. Figure 9 shows the phylogenetic hypothesis based on the combined dataset analysed by BI.

Berghia was monophyletic (PP = 0.98, BS = 94) and *B. creutzbergi* specimens (PP = 1, BS = 100) appeared as the sister taxon to all other *Berghia* species (PP = 0.79, BS = 64). *Berghia benteva* formed the basal branch, whereas *B. stephanieae*, *B. rissodominguezi*, *B. columbina*, *B. marinae* n. sp., *B. coeruleascens*, *B. marcus* and *B. verrucicornis* all clustered together (PP = 0.99, BS = 79).

DISCUSSION

Our analyses confirm the monophyly of *Berghia*, in agreement with Carmona *et al.* (2013). This contribution extends the research by Carmona *et al.* (2013) and suggests the existence of 10 species in this genus. Only eight out of the 14 species initially attributed to and/or transferred to this genus (see Introduction) really belong to *Berghia*. The genus is entirely restricted to the Atlantic Ocean and Mediterranean Sea. Although *B. coeruleascens* and *B. verrucicornis* have been considered ampho-Atlantic, this is a consequence of misidentifications with *B. marcus*, *B. stephanieae* or/and *B. rissodominguezi* (e.g. Er. Marcus, 1957; Ev. Marcus, 1972; Carroll & Kempf, 1990; Kempf, 1991; Kempf & Brittsan, 1996; Kempf, 2002) and none of these species have an ampho-Atlantic distribution. Only *B. benteva* is apparently ampho-Atlantic (Caballer & Ortea, 2013 under the name of *B. dakariensis*), although the occurrence in Senegal of this species, originally described from Brazil, needs to be confirmed by further comparative studies.

The morphological characters that have traditionally been used to separate *Berghia* from other Aeolidiidae were found to be of little use to define the genus. Carmona *et al.* (2013) pointed out the need to find new morphological characters for some aeolidiid genera such as *Berghia* and *Baeolidia*. Different morphological characters such as oral and salivary glands, body shape, morphology of the rhinophoral papillae, as well as the tail have been examined here but they also are phylogenetically uninformative concerning *Berghia*. The great intrageneric variability of the radular teeth (e.g. *B. benteva* vs *B. coeruleascens*), ceratal arrangement (e.g. *B. stephanieae* vs *B. coeruleascens*) and rhinophore ornamentation (e.g. *B. norvegica* vs *B. coeruleascens*), together with the homogeneity of the reproductive system, make it impossible, or at least very difficult, to discover new anatomical synapomorphies. New robust relationships based on molecular analyses without any obvious morphological synapomorphies are emerging in other groups too (Philippe, Lartillot & Brinkmann, 2005; Paps, Bagnà & Riutort, 2009). Among gastropods phylogenetic analyses of various taxa such as *Palustorina* (Reid, Dyal & Williams, 2010), *Drupella* (Claremont, Reid & Williams, 2011), *Roboastra* and *Tambja* (Pola, Cervera & Gosliner, 2007, 2008) have produced similar outcomes.

In terms of morphological characters we conclude that coloration seems to be the most important character for the separation of *Berghia* species (see Table S2 of Supplementary Material). Also, the species of *Berghia* when compared with other aeolidiids are usually slender, with tapering bodies and long tails. The cerata are long, slender, with a round apex. Additionally, except for *B. norvegica* and *B. stephanieae*, the rhinophoral papillae can be useful for identifying this genus. This is supported by our molecular phylogeny as well as by the results obtained by Carmona *et al.* (2013); both phylogenetic hypotheses group all aeolidiid species with these morphological features in *Berghia*.

Revised diagnosis of *Berghia*

Body elongate and slender, with pericardial swelling, in most species, except *B. benteva* and *B. norvegica*. Foot corners tentaculiform. Rhinophores densely papillate, except *B. stephanieae*. Papillae more or less rounded on the posterior side, elongate and perpendicular to rhinophores on their lateral faces/sides. Oral tentacles longer than rhinophores. Except in *B. stephanieae* and *B. norvegica*, the cerata are arranged in arches. Cerata cylindrical, usually long, slender, with a round apex and uniform diameter throughout most of their length. Reproductive aperture within the anterior-most group on the right side. Reproductive system diaulic. Oral glands composite, moderately large and tubular. Anus cleioproctic. Radular teeth bilobed and deeply indented, normally with a medium-sized central cusp. Presence of denticles on the masticatory edge varies intraspecifically.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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