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Double trouble. A cryptic first record of *Berghia marinae* Carmona, Pola, Gosliner, & Cervera 2014 in the Mediterranean Sea

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Abstract

In 2014, *Berghia marinae* Carmona, Pola, Gosliner & Cervera, 2014 from Senegal was described along with the revision of the genus *Berghia* Trinchese, 1877. In this study, we establish a second record for the senegalese species *B. marinae* in the Mediterranean Sea, 4,000 Km away from its type location. The morphological mismatch from the original description hampered its identification, and thus, a molecular approach was needed. Multilocus phylogenetic trees were inferred from Maximum-likelihood and Bayesian analyses based on partial DNA sequences of the mitochondrial cytochrome c oxidase subunit I and 16S rRNA genes, and the nuclear gene histone-3. Species delimitation analyses were performed to support the phylogenetic results and a new morphological description is provided complementing earlier information on this barely known species.

Keywords: Cryptic species; Marine Biodiversity; Species distribution; Mollusca.

Introduction

The first records of Heterobranch sea slugs (formerly known as opisthobranchs) in the Mediterranean Sea date back to the 18th century. Since then, these organisms have been heavily researched, with descriptions and/or reports of the species providing a deeper knowledge of this group of molluscs from the Mediterranean Sea. Moreover, since the beginning of the 20th century, the presence of exotic species of opisthobranchs in the Mediterranean has become more evident (Zenetos *et al.,* 2004). The observation of new species increased in the 1960s, 1970s and 1980s, mainly due to the migration of species from the Red Sea through the Suez Canal (Lessepsian migrants) as well as the advances in scuba diving and more careful observations of the aquatic medium (Zenetos *et al.*, 2004; Borg *et al.*, 2009; Yokes *et al.*, 2012).

Several opistobranch checklists, including alien species, have been published and updated for specific areas (Ortea *et al.*, 2001; Ballesteros *et al.,* 2016; Zenetos *et al.* 2016), countries (Cervera *et al.*, 2004; Calado & Silva, 2012; Crocetta *et al.* 2013;) or for the whole Mediterranean Basin (Schmekel & Portmann, 1982; Cattaneo-Vietti *et al.*, 1990; Trainito & Doneddu, 2014). On most of these checklists, species have been distinguished using external morphology and, in some cases, are based on the internal anatomy. However, molecular methodologies have facilitated the detection of species complexes, in parallel with the description of new species and even genera among the marine Heterobranchia (Bond *et al.,* 2001; Xavier *et al.,* 2010; Carmona *et al.*, 2011; Carmona *et al.*, 2014a; Churchill *et al.*, 2014; Padula *et al.*, 2014; Kienberger *et al.*, 2016; Furfaro *et al.*, 2017; Korshunova *et al.*, 2017; Zamora-Silva & Malaquias, 2017).

The genus *Berghia* Trinchese, 1877 was recently revised (Carmona *et al.*, 2014b). The authors concluded that this genus does not have any clear morphological synapomorphy and proposed the external colouration as the main feature to differentiate and delimit species. Moreover, a new species from Senegal was described: *Berghia marinae* Carmona, Pola, Gosliner, & Cervera, 2014, increasing the number of species for the genus to 14. This species is closely related to *B. columbina* García-Gómez & Thompson, 1990, with the two presenting few external differences, being colouration the only significant differentiating trait. *B. columbina* has orange highlights in the cerata, while *B. marinae* presents a brownish opaque colour on these structures. In the Mediterranean Sea, only three *Berghia* species have been reported thus far: *B. coerulescens* Laurillard 1832, *B. columbina* and

B. verrucicornis A. Costa, 1867 (Cervera *et al.*, 2004). While *B. coerulescens* and *B. verrucicornis* are found in the western Mediterranean Sea and eastern Atlantic Ocean, *B. columbina* is currently found in the Atlantic Ocean and rarely found in the Alboran area (García-Gómez *et al.*, 2011).

A rare specimen of this genus was found on the Catalan coast of Mataró (NE Spain). This specimen showed ambiguous traits characteristic of *B. columbina* and *B. marinae*, and thus it was impossible to make a conclusive identification, leading to the use of molecular techniques. Two mitochondrial markers (COI and 16S) and one nuclear marker (H3), which are widely used in heterobranch studies (Cella *et al.*, 2016; Furfaro *et al.*, 2017; Zamora-Silva & Malaquias, 2017), were sequenced for this specimen and included in a public dataset. In addition, three different species delimitation analyses and a *p*-distance calculation using the mitochondrial gene COI, were carried out to determine the identity of the specimen.

Material and Methods

Examined material

18-IV-2017. A single specimen was obtained among *Posidonia oceanica* (Linnaeus) Delile, 1813 at 15m depth while SCUBA diving in La Trencada, Mataró (41.52733º N, 2.466667º E) (NE Spain). The live specimen measured 8mm in length when it was completely extended. The specimen was photographed alive and then preserved in absolute ethanol.

Extraction, amplification and DNA sequencing

The whole organism was used for extraction. The DNeasy Blood & Tissue kit (09/2001; Qiagen, Valencia, CA, USA) was used for DNA extraction. Cytochrome Oxidase Subunit I (COI), ARN ribosomal 16S and Histone 3 fragments were amplified using the polymerase chain reaction (PCR) with the primers L1490 and Bergh_COI_intR for COI (Folmer *et al.*, 1994; Carmona *et al.*, 2013 respectively), AR-L and BR-H for 16S (Palumbi *et al.*, 1991), and H3AD5'3 'I H3BD5'3' for the H3 marker (Colgan *et al.*, 1998).

PCRs were performed in 10 µL reactions, containing 5 µL of Amplitaq Gold 360º (Thermo Fisher Scientific Inc.), 0.5 µL of forward primer and 0.5 µL of reverse primer, both at 10 µM, 1 µL of genomic DNA at variable concentrations (approx. 30 µM), and 3 µL of milliQ water. Amplification of the three genes was performed with a denaturation phase of 5 minutes at 95 °C followed by 40 cycles of 30 seconds at 95 °C, 30 seconds at 42 °C (annealing phase) and an elongation phase of 45 seconds at 72 °C, with a final elongation phase of 5 minutes at 72 ºC. An S1000 thermal cycler (Bio-Rad Laboratories, Inc.) was used to perform the PCRs. Successful PCRs were purified by mixing 2 µL of ExoSAP-IT (Thermo Fisher Scientific Inc) at a 1:10 dilution of the total PCR product. The samples were incubated at 37 ºC for 30 minutes followed by an inactivation phase of 15 minutes at 80 ºC. Once cleaned, samples were sent for sequencing by the Sanger method to the University of Barcelona scientific-technical services (CCiTUB) equipped with an ABI Prism 3730XL DNA sequencer (Applied Biosystems).

Phylogenetic analyses

DNA sequences were assembled with SeqMan (Swindell & Plasterer, 1997) and edited with MEGA7 (Kumar *et al.*, 2016). All sequences were confirmed to be free of contamination by BLAST (Altschul *et al.*, 1997), implemented in the GenBank database. The coding sequences were translated into amino acids to confirm alignment. These sequences were deposited in GenBank under the accession numbers: MK468733 for COI, MK468734 for 16S, MK468735 for H3. In addition, 74 additional sequences from 31 specimens from GenBank previously used in Carmona *et al.* (2014) and Borges *et al.* (2016) were obtained and reanalysed to compare the sequences belonging to the collected organism (Table 1). MUSCLE (Edgar, 2004), implemented in MEGA7, was used to align the sequences.

Analyses of the partial gene sequences were carried out separately and concatenated. The nucleotide substitution models that best suited the alignments of the three markers were determined using the Akaike Information Criterion (AIC) (Akaike, 1974), implemented in the JmodelTest2 (Darriba *et al.,* 2015). The GTR+I+G model was chosen for the three genes. Maximum-likelihood analyses were carried out with RAxML7.0.3 software (Stamatakis, 2006), where the support of the nodes was advised by non-parametric bootstrapping (BT) with 5000 replicates, random initial trees, and the estimated parameters for each data set according to the chosen evolutionary model. Bayesian inference analyses were carried out by MrBayes 3.1.2b software (Ronquist & Huelsenbeck, 2003) for 10 million generations with two independent runs with sampling every 1000 generations. In both Maximum-likelihood and Bayesian Inference analyses, the combined data set was divided into the different genes, allowing all parameters to vary independently from each partition. The resulting trees were manipulated with Figtree 1.4.2 (Drummond & Rambaut, 2007) and Figtree 1.3.1 (Rambaut, 2009).

Species Delimitation

Multiple methodologies were used to confirm the identification of the studied specimen. Genetic p-distances were calculated for the COI marker with MEGA7 (Kumar *et al.*, 2016) assuming the Kimura-2 parameters nucleotide substitution-rate model. In addition, ABGD analyses were performed with the ABGD web version (http://wwwabi.snv.jussieu.fr/public/abgd/) (Puillandre *et al.*, 2012). Pmin and Pmax values were established as 0.001 and 0.5 respectively, with a total of 1000 steps.

Table 1. Information of organisms and molecular sequences obtained from GenBank. For each organism, original papers, locality and accession numbers are shown. Numbers following the species names belong to these in Figures 2 and 3.

Organism identifcation	Original Paper	Locality	COI	16S	H3
Baeolidia salaamica 1	Carmona,L 2014	Philippines	JQ997047	JQ996843	JQ996944
Baeolidia salaamica 2	Carmona,L 2014	Philippines	JQ997062	JQ996859	JQ996960
Baeolidia salaamica 3	Carmona,L 2014	Philippines		JQ996860	
Baeolidia salaamica 4	Carmona,L 2014	Japan		JQ996862	JQ996962
Baeolidia salaamica 5	Carmona,L 2014	Hawaii	JQ997048	JQ996844	JQ996945
Berghia benteva 1	Carmona,L 2014	Brazil			KF273245
Berghia coerulescens 1	Carmona,L 2014	Croatia	JQ997049	JQ996845	JQ996946
Berghia coerulescens 2	Carmona,L 2014	Spain: Andalucia		JX087470	JX087604
Berghia columbina 1	Carmona,L 2014	Morocco	JX087542	JX087472	JX087606
Berghia columbina 2	Carmona,L 2014	Morocco	JX087543	JX087473	JX087607
Berghia columbina 3	Carmona,L 2014	Spain: Andalucia	JX087544		JX087608
Berghia columbina 4	Carmona,L 2014	Spain: Andalucia	JX087545	JX087474	JX087609
Berghia columbina 5	Carmona,L 2014	Morocco		JX087471	JX087605
Berghia creutzbergi 1	Carmona,L 2014	Colombia	JX087546	JX087477	JX087614
Berghia creutzbergi 2	Carmona,L 2014	Cuba	JX087547	JX087478	JX087615
Berghia creutzbergi 3	Carmona,L 2014	Bahamas		JX087475	JX087612
Berghia creutzbergi 4	Carmona,L 2014	Bahamas		JX087476	JX087613
Berghia marcusi 1	Carmona,L 2014	Brazil	KF273244	KF273243	KF273246
Berghia marinae 1	Carmona,L 2014	Senegal	JX087549	JX087480	JX087617
Berghia rissodominguezi 1	Carmona,L 2014	Cuba	JX087552	JX087484	JX087621
Berghia rissodominguezi 2	Carmona,L 2014	Colombia		JX087483	JX087620
Berghia sp.		Spain: Catalonia	MK468733	MK468734	MK468735
Berghia stephaniae 1	Carmona,L 2014	USA: Florida	JQ997044	JQ996839	JQ996940
Berghia verrucicornis 1	Carmona,L 2014	Morocco: Agadir	HQ616749	HQ616712	HQ616778
Berghia verrucicornis 2	Carmona,L 2014	Spain: Andalucia	HQ616750	HQ616713	HQ616779
Berghia verrucicornis 3	Carmona,L 2014	Senegal		JX087488	JX087610
Berghia verrucicornis 4	Carmona,L 2014	Morocco		JX087485	JX087622
Berghia verrucicornis 5	Borges, L 2016	Portugal: Algarve	KU496627		
Berghia verrucicornis 6	Borges, L 2016	Portugal: Algarve	KU496628		
Berghia verrucicornis 7	Borges, L 2016	Portugal: Algarve	KU496629		
Berghia verrucicornis 8	Carmona,L 2014	Spain: Andalucia	JX087553	JX087486	JX087623
Berghia verrucicornis 9	Carmona,L 2014	Spain: Andalucia	JX087554	JX087487	JX087624

Simple distance, Jukes & Cantor and Kimura-2 parameters were used as nucleotide substitution models and compared to each other to confirm the best barcoding detection.

The rooted COI consensus tree obtained by MrBayes was used for a bPTP analysis, performed via web version (http://species.h-its.org/ Zhang J. 2013-2015). The run was executed with a total of 100.000 generations, with an initial burn-in of 0,1% of the iterations performed. The value of thinning was established by default as 100.

To perform the GMYC analysis, two ultrameric trees for the COI gene were obtained by using BEAST 2.0 (Drummond & Rambaut, 2007). Priors and models established for the trees were determined based on previ-

ous knowledge, using the associated software BEAUti. The nucleotide substitution model selected was HKY. The chosen clock model was the uncorrelated relaxed with lognormal distribution. Two different priors were determined for the trees: Yule process and Birth-Death process. No specific tree was selected as a starting tree. A total of 150M of generations were run with a sampling every 1.000 generations. The BEAUti output was executed with BEAST, with a 10% burn-in. Normality, low standard deviation and good values of ESS for each statistic of the resulting ultrameric tree were checked with Tracer v 1.6 (Rambaut *et al.*, 2014). Once these values were considered to be correct, both files for each model were joined using LogCombiner v.2 (Rambaut & Drummond, 2014). GMYC analyses were then carried out with R (R Development Core Team, 2011) and Rstudio (Rstudio Team, 2015), using the packages "rncl" (Michonneau *et al.*, 2016), "ape" (Paradis *et al.*, 2004), "MASS" (Ripley, 2011), "paran" (Dinno, 2012), and "splits" (Ezard *et al.*, 2009).

Results

Taxonomy

Subclass Heterobranchia Burmeister, 1837
Order Nudibranchia Cuvier, 1817
Family Aeolidiidae Gray, 1827
Genus *Berghia* Trinchese, 1877
Berghia marinae Carmona, Pola, Gosliner & Cervera, 2014

Morphology

The specimen collected in Mataró (NE Spain) (Fig. 1) has a long, narrow, whitish and translucent body except for the post-cardiac dorsal area, which is light brown due to the digestive gland. Bright orange patches are observed on both sides of the head. The oral tentacles are translucent and have white or slightly yellow granulations in their distal half. Rhinophores are orange in their lower half and moving up this colour becomes yellowish. The apex of the rhinophores is white. The rhinophores are covered with small papillae from almost the base to near the apex, which is white. The eyes are visible behind the base of the rhinophores. There are seven groups of cerata on each side of the back. Each insertion of the cerata has bright orange pigments. The cerata are elongated and sharp at the tip. Inside them, the digestive gland is light brown and occupies almost the entire interior of the cerata. A subapical yellowish band and fine whitish dots on the surface of the cerata is also visible. The cnidosacs are white. The foot is broad and almost translucent, and in its anterior part it has well-developed propodial palps. The tail is long and sharp.

Sequence analyses

After the primer deletion, the COI, 16S and H3 sequences were trimmed respectively to the GenBank sequence length obtaining a total of 658 bp for COI, 445 bp for 16S (including variant sites), and 328 bp for H3. Sequence alignment and gene concatenation yielded a combined dataset of 1431 (including variable sites) base pairs in length. The combined tree provided better resolution than H3, COI, or 16S independently. The COI gene best resolved the relationships at the species and generic levels, followed by 16S, while H3 provided little or no resolution. Bayesian inference and maximum likelihood analyses yielded the same topology, and thus only the BI trees are presented.

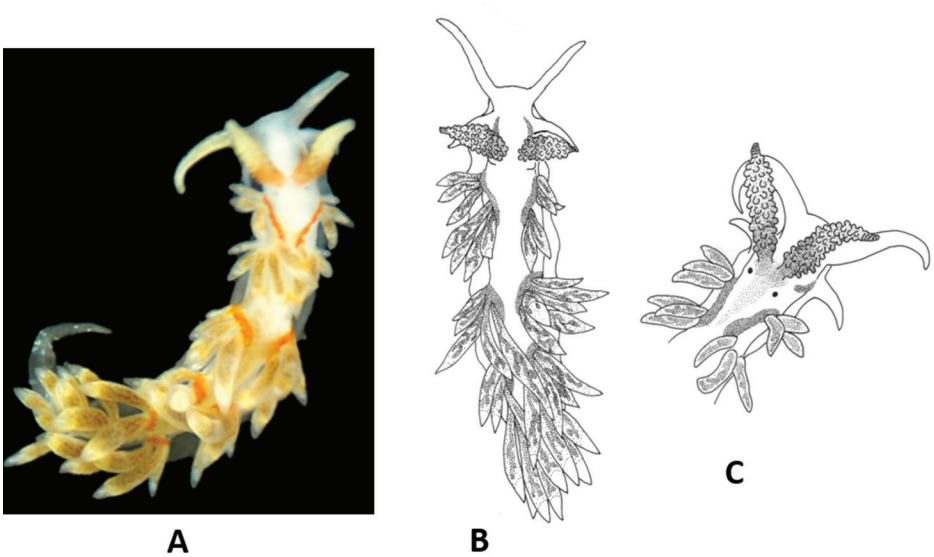

Fig. 1: *Berghia marinae* Carmona, Pola, Gosliner & Cervera, 2014 representations. Photograph of living animal (A), drawing of the entire animal (B) and details of the rhinophoric structure (C).

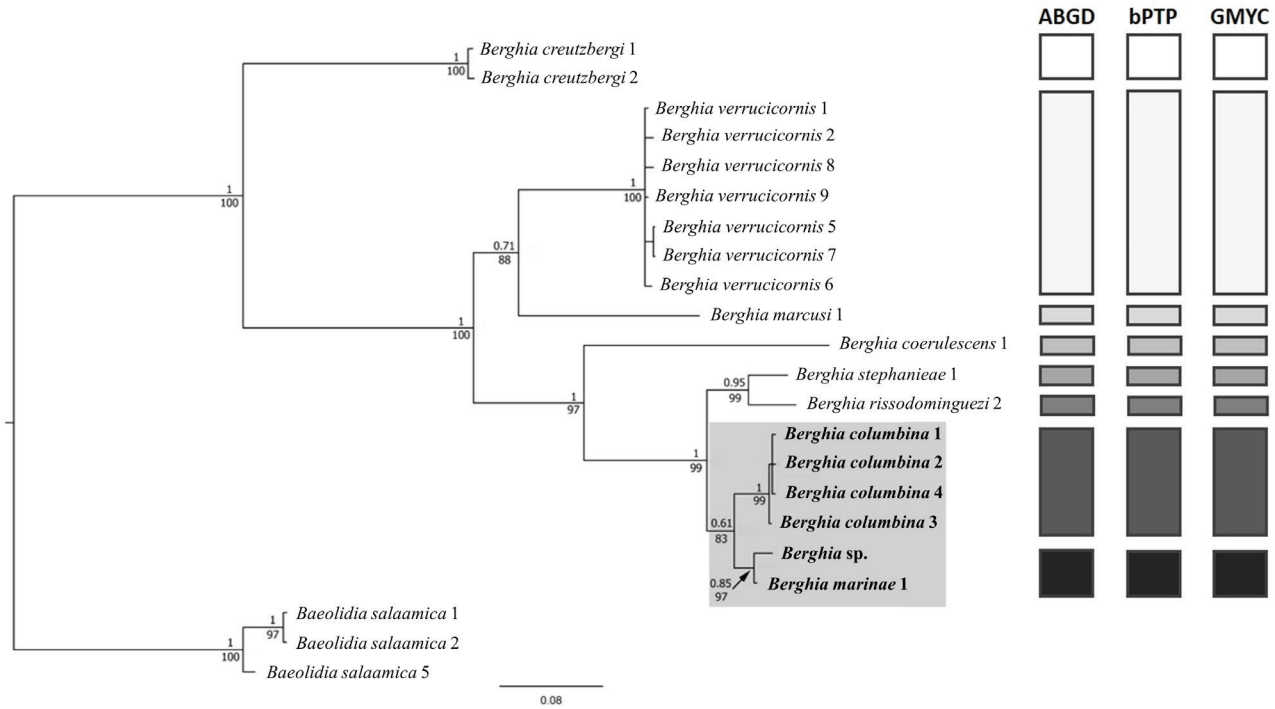

Fig. 2*:* Bayesian topology of the phylogenetic tree obtained with the COI marker. Represented on the upper side of the nodes is the PP and on the bottom side the BT values. On the right, the results of the different species delimitation analyses are shown.

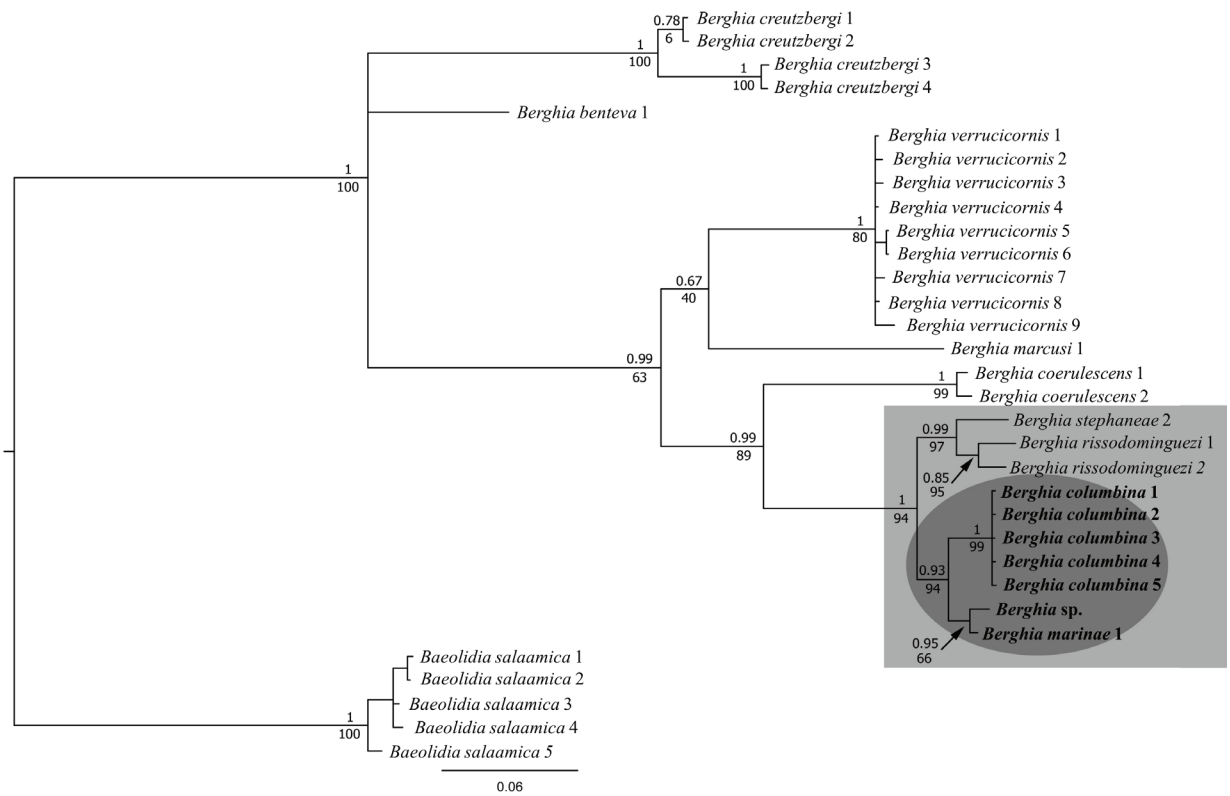

Fig. 3: Bayesian topology of the phylogenetic tree obtained with COI+16S+H3 markers. Represented on the upper side of the nodes is the PP values and on the lower side the BT values.

Phylogenetic analyses

The phylogenies obtained from each molecular marker place the studied specimen together with *Berghia marinae* from Senegal (Fig. 2), except in the case of the H3 marker, where it is located with the species *B. marinae* and *B. columbina*. However, the obtained nodes are not supported for any of the independent analyses (COI: PP=0.61, BT=83; 16S: PP=0.71, BT=91; H3: PP=0.87, BT=53). The clade made up of *B. marinae* and *B. columbina* is sibling to *B. rissodominguezi* Muniain & Ortea, 1999 and *B. stephanieae* (Valdés, 2005), being supported by COI and 16S genes (COI: PP=1, BT=99, 16S: PP=1, BT=98) but not by H3.

The concatenated phylogeny (Fig. 3) shows that the specimen collected from Mataró, *Berghia* sp. joins *B. marinae* from Senegal with consistent Bayesian node support values (PP=0.95, BT=66). This species is sister to *B. columbina*. This sibling species clade clusters together with *B. stephanieae* and *B. rissodominguezi* with high support values (PP=1, BT=99). *Berghia coerulescens* appears as a sibling species to the clade that includes *B. marinae*, *B. columbina, B. stephaniae* and *B. rissodominguezi* (PP=0.99, BT=89). All of the species named previously are sister to a clade that comprises *B. verrucicornis* and *B. marcusi* Domínguez, Troncoso & García, 2008 (PP=0.93, BT=66). Finally, the─basal node splits in a politomy made up of *B. creutzbergi* Er. Marcus & Ev. Marcus, 1970, *B. benteva* (Er. Marcus, 1958) and the clade including all the other *Berghia* species.

Species delimitation

The minimum *p*-distance value (Table 2) between the specimen collected on the Catalan coasts and *B. marinae* is 0.013. This value is much higher when comparing the Catalan *Berghia* sp. to *B. columbina* (0.046-0.048), *B. rissodominguezi* or *B. stephaniae* (0.09 both). The minimum genetic distance (uncorrected p–distance for COI) between *Berghia* sp. and the remaining species of the genus ranges from 0.189 to 0.221.

The ABGD output (Fig. 2) indicates eight species as the most suitable scenario for our data, independently from the chosen model (Jukes and Cantor, Tamura 2-parameters and simple distance). This model displays the *Berghia* sp. found in Mataró together with *B. marinae*.

On the other hand, the bPTP analysis shows that *Berghia* sp. from Mataró joins *B. marinae* with a PP value of 0.8, and is sister to *B. columbina* (PP=0.92).

Finally, both Yule and Birth-Death models obtained by BEAST presented high BSS values, normality in the residue, and a low standard deviation. The Yule model, nevertheless, when was used for GMYC analyses, showed a low p-value. Data given with this model was considered unsatisfactory due to the low statistical support. The Birth-Death model presented satisfactory p-values and the results were taken into consideration. From this analysis, eight putative species were distinguished, combining *Berghia* sp. with *B. marinae*, and separating these two specimens from all other species.

Discussion

Phylogenetic analyses

Phylogenetic analyses based on the mitochondrial genes COI and 16S cluster the specimen found in Mataró with *B. marinae* from Senegal. However, when the nuclear gene H3 was used, our specimen was sibling to the *B. columbina* and *B. marinae* clade. While these results may be ambiguous, analyses representing unlinked genes may provide different genetic evolutionary stories. Thus, combined analyses provide better-resolved trees (Huelsenbeck *et al.,* 1996; Maddison, 1997). Here, concatenated data is supported in the majority of the nodes for both Maximum likelihood and Bayesian Inference. In this case, the specimen found in Mataró belongs to *B. marinae,* and is a sibling species to *B. columbina,* as previously suggested by Carmona *et al.* (2014b).

Double crypsis

The specimen found on the Spanish coast of Mataró shows a different colouration pattern compared to ─the original description of *B. marinae*, and externally resembles *B. columbina*. This presents a new case study for cryptic species living sympatrically, not only due to the resemblance between the two different species, but because of different chromatic patterns observed within the same species. Multiple hypotheses have been proposed about why cryptic species live together in the

Table 2. Genetic distances among the species most related to *Berghia* sp.

	***Berghia* sp.**	***Berghia marinae***	***Berghia columbina***	***Berghia stephanieae***	***Berghia rissodominguezi***
***Berghia* sp.**					
B. marinae	0.013				
B. columbina	0.046-0.048	0.036			
B. stephanieae	0.09	0.075	0.073-0.075		
B. rissodominguezi	0.09	0.084	0.077-0.079	0.062	

same area, such as niche overlapping and coexistence in nearby areas (Fiser *et al.*, 2018). B*erghia columbina* and *B. marinae*, which are sibling species with low genetic divergence, are found in overlapping areas. Evolution is not only driven by phenotypic characters, but also by anatomical ones, as well as chemical compounds involved in reproduction, ecology and/or behaviour. Hence, sister clades can show identical phenotypes because their evolutionary characters are hidden (Faulkner & Ghiselin, 1983; Harvell, 1990). Nonetheless, our specimen is clearly different from the specimen found in Senegal. Mimetism and aposematism play a crucial role in aeolidids nudibranchs. For instance, *Spurilla neapolitana* (Delle Chiaje, 1841) is completely mimetic to the anemone where it feeds. Moreover, *S. neapolitana* is able to retain its nematocysts and zooxanthellae.This way it is able to camouflage in front of their potential predators and defend itself with the anemone stinging cells. (Marín & Ros, 1991). In *Cratena peregrina* (Gmelin, 1791), the opposite strategy is found, and vivid colours represent a warning signal to potential predators (Aguado & Marín, 2007). Something similar may be occurring in *B. marinae*, in which the strategy used by the Senegal population is to be unnoticed, while the Mediterranean population, with its vivid colours, warns its predators in the same way as *B. columbina*.

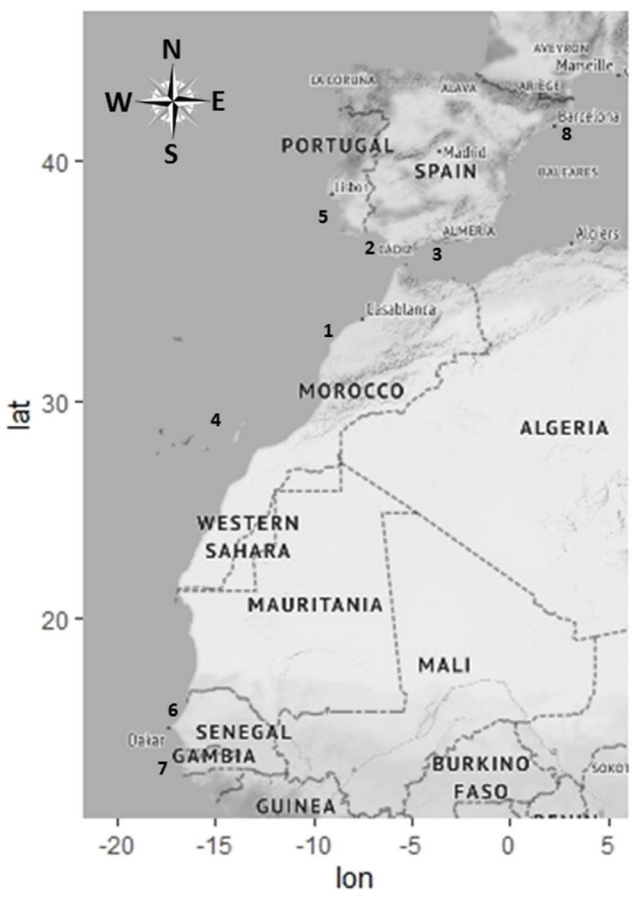

Fig. 4: Locations where *B. columbina* and *B. marinae* species have been found. The locations are numbered in chronological order: *B. columbina*. 1: Atlantic coast of Morocco (Pruvot-Fol, 1953, as *Berghia coerulescens*); 2: southwest coast of Spain (García-Gómez & Thompson, 1990); 3: southeast coast of Spain (Moreno & Templado, 1998); 4: Canary Islands (Ortea et al, 2001); 5: southern Portugal (Calado & Silva, 2012); 6: Senegal (Carmona et al, 2014b). *B. marinae*. 7: Senegal (Carmona *et al.*, 2014b); 8: northeast Spain (present study).

Geographical Distribution

Until now, *B. marinae* had only been reported in Senegal, and thus its presence in the Mediterranean presents new questions. Was this species present in the Mediterranean Sea, hidden by crypsis and presumed to be *B. columbina,* or is this species allochthonous, settling only recently in the Mediterranean Sea? Figure 4 shows the localities where *B. columbina* and *B. marinae* have been found thus far. There are other examples of nudibranch species whose range occupies the Mediterranean Sea and part of the Atlantic Ocean (*Polycera quadrilineata* (O. F. Müller, 1776), *Polycerella emertoni* A. E. Verrill, 1880**,** *Flabellina affinis* (Gmelin, 1791), *Doriopsilla areolata* Bergh, 1880) (Eyster, 1980; Templado *et al.*, 1990; García & Bertsch, 2009; Camps & Prado, 2018). On the other hand, several species of Heterobranchia considered widely distributed, were ultimately found to have hidden species complexes and their geographic location had been limited to a given area (Alexander & Valdés, 2013; Churchill *et al.,* 2014; Hoover *et al.,* 2015*;* McCarthy *et al.*, 2017). Due to this problem, records of *B. columbina* for the Mediterranean Sea can be the result of misidentifications caused by resemblance with *B. marinae*. Further research and a broader taxon sampling should be carried out for this species to corroborate its ecological state.

Conclusions

This specimen of *B. marinae* from the Catalan coast is the first record of the species on the Mediterranean coasts and the second one globally. The great geographical distance between this sample and its species holotype, as well as the difference in habitat, opens the debate about whether this species is alochthonous or if its distribution range is wider than was originally described, overlapping with the range of *B. columbina* and *B. verrucicornis*. However, since the genetic divergence between *B. marinae* and *B. columbina* is based on just two organisms and due to the external morphological similarity between the organism found in Mataró and the one found in Senegal (holotype), further investigation will be required to totally clarify their relationships.

In the study of marine heterobranchs certain groups of species should be studied with some caution, especially when identifying closely-related species. Some species with chromatic variability have actually been shown to include different cryptic species (see for example Furfaro *et al*., 2016). Similarly, morphologically similar specimens may also belong to different species (see for example Korshunova *et al*., 2019). In all of these cases, molecular analyses are necessary to provide a better understanding of the species taxonomy.

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