Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2011) **77**: 401–412. doi:10.1093/mollus/eyr036
Advance Access publication date: 11 October 2011

AMPHI-ATLANTIC DISTRIBUTIONS AND CRYPTIC SPECIES IN SACOGLOSSAN SEA SLUGS

LEILA CARMONA[1], MANUEL ANTÓNIO E. MALAQUIAS[2], TERRENCE M. GOSLINER[3], MARTA POLA[4] AND JUAN LUCAS CERVERA[1]

[1]*Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro, s/n, Ap.40, 11510 Puerto Real (Cádiz), Spain;*
[2]*Phylogenetic Systematics and Evolution Research Group, Bergen Museum, Natural History Collections, University of Bergen, PB 7800, 5020 Bergen, Norway;*
[3]*Department of Invertebrate Zoology, California Academy of Sciences, 55 Music Concourse Drive, Golden Gate Park, San Francisco, CA 94118, USA; and*
[4]*Laboratorio de Biología Marina, Departamento de Biología, Universidad Autónoma de Madrid, Edificio de Biología, C/ Darwin, 2, 28049 Madrid, Spain*

Correspondence: L. Carmona; e-mail: leila.carmona@uca.es

(Received 16 February 2011; accepted 29 July 2011)

ABSTRACT

Nearly 13% of opisthobranch gastropods in the Atlantic Ocean are regarded as amphi-Atlantic (i.e. species occurring on both eastern and western coastlines of the Atlantic realm). This assumption has been broadly based on morpho-anatomical similarities and has rarely been tested within a molecular phylogenetic framework. Among opisthobranchs, the order Sacoglossa is renowned for its difficult and confusing species taxonomy, particularly in some genera like *Elysia*, where several cases of cryptic speciation have been documented. Moreover, the width of the Atlantic realm poses a serious challenge to the idea of trans-Atlantic dispersal, particularly in nonplanktotrophic species. The species *Bosellia mimetica*, *Elysia timida* and *Thuridilla picta* are three sacoglossan opisthobranchs with amphi-Atlantic status, but doubts have been raised about the conspecificity of eastern and western populations. Here we used methods to test molecular phylogenetic whether the Atlantic Ocean constitutes a barrier for dispersal and hence whether eastern and western populations belong to the same species. We used the criteria of divergence and reciprocal monophyly, supported by concordant genealogically independent genetic markers, to define species. Maximum-likelihood and Bayesian analyses of partial DNA sequences of the mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA genes and nuclear gene Histone-3 (H3) were used to produce phylogenetic trees. We conclude that the eastern Atlantic species *B. mimetica* is not amphi-Atlantic, although the H3 gene did not recover reciprocal monophyly between western and eastern populations, perhaps in a result of incomplete lineage-sorting. *Elysia timida* is the only species showing an amphi-Atlantic distribution, but possibly due to recent introduction in the tropical western Atlantic. *Thuridilla picta* is restricted to the western Atlantic and therefore not amphi-Atlantic. Populations of '*T. picta*' inhabiting the Macaronesian archipelagos of Azores and Madeira are a colour form of the eastern Atlantic *T. hopei*. Our results highlight the difficulty surrounding systematics of Sacoglossa sea slugs and provide further evidence that cryptic speciation is common in the group.

INTRODUCTION

Oceanographic, palaeontological and developmental data are often used to explain trans-Atlantic dispersal and amphi-Atlantic geographical ranges in molluscs (i.e. species inhabiting both eastern and western coastlines of the Atlantic Ocean) (Williams & Reid, 2004; Malaquias & Reid, 2009; Claremont *et al.*, 2011). Scheltema (1971, 1979, 1995) showed that passive dispersal is highly important to the distribution of shallow-water molluscan species. The larvae of marine gastropods are capable of frequent long-distance dispersal; they can therefore be expected to serve as agents for colonizing new regions and as vectors of gene flow between widely separated populations. The fossil record shows that trans-Atlantic expansions seem to have occurred since the late Oligocene (Vermeij, 1995; Maestrati & Lozouet, 1996; Petuch, 1997) and to have increased since the Pliocene after the closure of the Isthmus of Panama, when the Gulf Stream became stronger and Atlantic circulation intensified (Vermeij & Rosenberg, 1993; Briggs, 2003; Teske, Cherry & Matthee, 2004; Williams & Reid, 2004).

Opisthobranchs are a diverse group of gastropods occurring worldwide in marine habitats, with few representatives in fresh-

water environments (Schrödl & Neusser, 2010). Some species have restricted geographical ranges (e.g. *Haminoea fusari* from the Mediterranean Sea, Alvarez, García & Villani, 1993), whereas others are broadly distributed, sometimes over more than one ocean basin (e.g. the circumtropical *Aplysia dactylomela*; Cervera *et al*., 2004; Gosliner, Behrens & Valdés, 2008). This array of geographical patterns has been earlier discussed by Edmunds (1977), who suggested that the distribution of opisthobranch species could depend on food availability, water temperature, ocean currents, duration of planktonic life and anthropogenic interference (e.g. shipping).

Amphi-Atlantic distributions are regarded as moderately common in opisthobranchs. The first account focused on all Atlantic Gastropoda recognized a modest 11 species of amphi-Atlantic opisthobranchs (García-Talavera, 1983), but a recent survey of opisthobranchs alone recognized 134 species occurring on both sides of the Atlantic Ocean, which equals 12.6% of the total opisthobranch diversity in this realm (García & Bertsch, 2009).

Anthropogenic impacts resulting from the aquaculture trade and shipping are becoming important vectors of introduction of exotic marine species (Streftaris & Zenetos, 2006; Zenetos *et al*., 2008, 2011; Cervera *et al*., 2010). Several examples have been documented in opisthobranchs, such as the presence in Europe of the Asian cephalaspid species *Haminoea japonica* resulting from the oyster-spat trade from North America (Gosliner & Behrens, 2006), the introduction by shipping in San Francisco Bay, USA, of the New Zealand species *Philine auriformis*, with negative impacts on the local fauna (Gosliner, 1995), and the occurrence in the Mediterranean Sea of the Indian Ocean species *Melibe fimbriata* which is unknown in the Red Sea (Thompson & Crampton, 1984).

Despite these facts and the lack of obvious barriers to gene flow across the Atlantic, it is evident that the present width of this ocean basin poses a serious challenge to the idea of regular gene flow between populations inhabiting both margins. Between 60 and 300 days are necessary to accomplish drift across the Atlantic (Scheltema, 1971). This is beyond the larval life span of opisthobranchs with planktotrophic larvae (usually 15–42 days, Schaefer, 1996); even the known capacity for some species to delay metamorphosis (Thompson, 1958, 1962; Hadfield & Karlson, 1969) is not an entirely convincing argument due to the temporal gap. Passive rafting of adults is a possibility that cannot be discarded; this has often been raised as a speculative means of long-distance dispersal but direct evidence is usually lacking (Fraser, Nikula & Waters, 2011) and to our knowledge no case has been documented in opisthobranchs.

Research on evolution and diversity patterns in the marine environment has been greatly stimulated in the past two decades by developments in molecular biology, population genetics and phylogeographic methods. Molecular methods are very powerful for detecting sibling species and have frequently shown that cosmopolitan or widely distributed 'species' consist of a taxonomic complex of multiple evolutionary partitions (e.g. Knowlton, 1993, for a general review; Meyer, 2003; Williams & Reid, 2004; Reid *et al*., 2006; Claremont *et al*., 2011). Molecular tools have not yet been widely used in species-level phylogenies of the Opisthobranchia, but recently their application showed the presence of two distinct allopatric species in *Bulla striata* complex, previously regarded as an amphi-Atlantic species (Malaquias & Reid, 2008, 2009) and led to the recognition of cryptic species in the cephalaspidean genus *Melanochlamys* in New Zealand (Krug *et al*., 2008).

The sacoglossans *Bosellia mimetica* Trinchese, 1891, *Elysia timida* (Risso, 1818) and *Thuridilla picta* (Verrill, 1901) are three species of opisthobranchs regarded as amphi-Atlantic (Valdés *et al*., 2006). The first two species have as their type locality the Mediterranean Sea, respectively Capri (Italy) and Nice (France), whereas *T. picta* was described from Bermuda (western Atlantic). These species have a temperate/tropical distribution and occur on shallow environments with the green algae upon which they feed. *Bosellia mimetica* has a planktotrophic larva (based on specimens from eastern Florida; Clark & Jensen, 1981) and *E. timida* has either direct or lecithotrophic development (Rahat, 1976; Bonar, 1978; Marín & Ros, 1989, 1992, 1993; Jensen, 2001); the development of *T. picta* is unknown but its congeneric Atlantic species *T. hopei* has lecithotrophic larvae and field observations here revealed that eggs of the latter species resemble those of *T. picta* (Jensen, 2001).

Although the amphi-Atlantic distribution of these three species are generally accepted, doubts have been raised about the conspecificity of eastern and western populations of *E. timida* and *T. picta*, because of consistent chromatic differences, the significance of which is difficult to judge based on morpho-anatomical comparisons alone (Ortea, Moro & Espinosa, 1997; Malaquias *et al*., 2009).

In this study we apply for the first time a molecular phylogenetic framework to sacoglossan species with contrasting larval development, to test the hypothesis that the width of the Atlantic realm does not constitute an insurmountable barrier for dispersal, and that therefore populations of these snails on both eastern and western coastlines of the Atlantic Ocean belong to the same species.

MATERIAL AND METHODS

Species concept and genetic divergence thresholds

We used the criteria of divergence and reciprocal monophyly supported by concordant genealogically independent genetic markers to define species (Knowlton, 2000; Wheeler & Meier, 2000; Avise, 2004; Reid *et al*., 2006; Malaquias & Reid, 2009). The use of genetic thresholds to distinguish between species is difficult to apply because different groups of organisms, even within the same genus or family, can have different rates of molecular evolution (Hebert, Ratnasingham & Waard, 2003; Williams, Reid & Littlewood, 2003). Yet, several attempts have been made to establish cut-off values for molluscs. Hebert *et al*. (2003) suggested the mean cytochrome *c* oxidase subunit I (COI) distance between sister species in molluscs to be $11.1 \pm 5.1\%$ (mean uncorrected p-distance) and Malaquias & Reid (2009) based on an integrative taxonomic revision of the opisthobranch genus *Bulla* established a cut-off value of 10% between sister species (uncorrected p-distance for COI gene). We use these cut-off values as reference thresholds.

Taxon sampling

Samples were obtained using standard scuba diving sampling techniques for opisthobranchs and through the study of museum collections. Sixty-two specimens from eight species of sacoglossans were used for phylogenetic inference. A total of 29 specimens were successfully sequenced for the COI, 32 for the 16S rRNA (16S) and 34 for the Histone-3 (H3) genes. Forty-three additional sequences were obtained from GenBank (see Table 1 for full list of samples, localities and voucher references). *Oxynoe antillarum* was chosen as outgroup due to its basal taxonomical position within the Sacoglossa (Jensen, 1996; Händeler & Wägele, 2006; Händeler *et al*., 2009). Identifications were performed by the senior authors based on external morphology and coloration.

DNA extraction, amplification and sequencing

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 of Qiagen (Qiagen, Valencia, CA, USA; 09/2001) was used for DNA extraction.

Partial sequences of COI, 16S and H3 were amplified by PCR using the primers: LCO1490 (5′-GGTCAACAAATCA TAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGG GTGACCAAAAAATCA-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′- ATGGCTCGTACC AAGCAGACVGC-3′) and H3BD5′3′ (5′-ATATCCTTR GGC ATRATRGTGAC-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).

PCRs were conducted in a 50 μl volume reactions containing 2 μl of both forward and reverse primers (10 μM), 5 μl of dNTP (2 mM), a gene-dependent amount of magnesium chloride (25 mM), 0.5 μl of Qiagen DNA polymerase (250 U), 10 μl of 'Q-solution' (5×) and 5 μl of Qiagen buffer (10×) (Qiagen Taq PCR Core Kit cat. no. 201225). Magnesium chloride amounts were 7 μl for COI and 16S, and 4 μl for H3. Amplification of COI was performed with an initial denaturation for 1 min at 95°C, followed by 35 cycles of 30 s at 95°C, 45 s at 48°C (annealing temperature) and 1 min at 72°C, with a final extension of 3 min at 72°C. The 16S amplification began with an initial denaturation for 4 min at 95 followed by 9 touchdown-cycles of 45 s at 94°C, 45 s at 56°C and 1.5 min at 72°C, followed by 25 amplification cycles of 45 s at 94°C, 45 s at 48°C (annealing temperature), 1.5 min at 72°C, with a final extension of 4 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.

Sequence alignment and phylogenetic analyses

DNA sequences were assembled and edited using Geneious Pro 4.7.6 (Drummond *et al*., 2009). Clustal_X (Thompson *et al*., 1997) was employed to align the sequences. The alignments were further optimized 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 the COI gene (Table 2). Saturation was visually inspected in MEGA v. 5.0 (Tamura *et al*., 2011) by plotting for all specimens including outgroup 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. No evidence of saturation was found even in third codon positions.

The 16S rRNA alignment showed one indel-rich region of 30 bp (between positions 237–267). 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 657, 466 and 333 bp, respectively.

Individual gene analyses and a concatenated analysis were performed. To test for conflicting phylogenetic signal between genes, the incongruence length difference (ILD) test (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 and maxtrees set to 1,000.

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 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 node support was assessed with non-parametric 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 2 million generations, with three 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 500 trees were discarded ('burn-in' period) and node support was assessed with posterior probabilities (PP). Only nodes supported by BS $\geq$ 75 and PP $\geq$ 0.90 are discussed.

RESULTS

The combined dataset yielded a sequence alignment of 1,419 positions. The ILD test showed no significant conflicting signal between the three genes ($P = 0.55$). Individual gene trees (Fig. 1) were nearly congruent with the concatenated tree (Fig. 2) and, therefore, results are described based on the latter; when relevant, reference is also made to the individual gene trees. Moreover, the topologies of the ML trees were entirely congruent with the results yielded by Bayesian analyses and thus ML trees are not shown. No saturation was observed across genes and codon positions (not shown).

Regarding the genus *Elysia* the phylogenetic analyses revealed contrasting differences with our provisional identification of specimens (Table 1). Specimens of '*E. timida*' branched off in four different clades, all with maximum support (PP = 1, BS = 100%; Fig. 2). One of these clades includes all specimens from the MED, which is the type locality of *E. timida*, plus one individual from the Florida Keys (FK) in the Caribbean Sea (maximum uncorrected p-distance = 3.5% for COI between MED and FK specimens). Each of the remaining four specimens, previously identified as *E. timida*, belong to different clades and likely correspond to three distinct species, hereafter referred as *Elysia papillosa*, *E.* cf. *cornigera* and *Elysia* sp. (Figs 1, 2; Table 1; see Discussion).

Specimens of *Bosellia* clustered in two separated clades with maximum support (PP = 1, BS = 100%; Fig. 2). One clade includes a Mediterranean specimen of *B. mimetica* (the type locality) sister to a specimen from the Bahamas (PP = 1, BS = 100%; uncorrected p-distance = 7.3% for COI), and the other contains a specimen from Cuba previously identified as *B. mimetica* (hereafter referred to as *Bosellia* sp.), and a specimen of *B. marcusi* from the Bahamas. The 16S tree does not

Table 1. List of sacoglossan specimens used for phylogenetic analyses.

Species		Locality	Voucher	GenBank accession nos		
Provisional id.	Revised id.			COI	16S	H3
Bosellia marcusi		Bahamas (GB)		DQ471254	DQ480191	DQ534783
Bosellia mimetica	*Bosellia marcusi*	Cuba	MNCN 15.05/53674	HQ616839	HQ616810	HQ616864
Bosellia mimetica		France (MED, GB)		—	EU140873	—
Bosellia mimetica		Mataró, Spain (MED, GB)		—	EU140872	—
Bosellia mimetica		Mediterranean Sea (GB)		GQ996657	—	—
Bosellia mimetica		Menorca (Spain, MED)	MNCN 15.05/53675	HQ616838	HQ616809	HQ616863
Bosellia mimetica		Tossa, Spain (Med, GB)		—	EU140874	—
Bosellia mimetica	*Bosellia* sp.	Bahamas (GB)		DQ471215	DQ480203	DQ534793
Bosellia mimetica	*Bosellia* sp.	Bahamas (GB)		DQ471214	—	—
Bosellia mimetica	*Bosellia* sp.	Bahamas (GB)		DQ471213	—	—
Bosellia mimetica	*Bosellia* sp.	Bahamas (GB)		DQ471212	—	—
Bosellia mimetica	*Bosellia* sp.	Bahamas (GB)		—	DQ480202	—
Bosellia marcusi	*Bosellia* sp.	Cuba	MNCN 15.05/53673	—	HQ616834	HQ616888
Elysia papillosa	*Elysia* cf. *cornigera*	Bermuda	CASIZ 181086	HQ616841	HQ616812	HQ616866
Elysia timida	*Elysia* cf. *cornigera*	Cuba	MNCN 15.05/53677	HQ616846	HQ616817	HQ616871
Elysia timida	*Elysia* cf. *cornigera*	Cuba	MNCN 17013	—	HQ658122	HQ658128
Elysia timida	*Elysia* cf. *cornigera*	Florida Keys (GB)		DQ471246	DQ480167	DQ534766
Elysia timida	*Elysia* cf. *cornigera*	Florida Keys (GB)		—	DQ480168	—
Elysia timida	*Elysia* cf. *cornigera*	Florida Keys (GB)		—	DQ480166	—
Elysia timida	*Elysia* cf. *cornigera*	Mexico (ATL)	UNAM 2995	—	HQ616835	HQ616889
Elysia crispata		Florida Keys (GB)		DQ471225	DQ480199	DQ534788
Elysia papillosa		Bahamas/Florida Keys (GB)		DQ471230	DQ480190	DQ534782
Elysia papillosa		Bahamas/Florida Keys (GB)		DQ471229	—	—
Elysia papillosa		Bahamas/Florida Keys (GB)		—	DQ480189	—
Elysia papillosa		Bermuda	CASIZ 181165	HQ616840	HQ616811	HQ616865
Elysia papillosa		Bermuda	CASIZ 181144	HQ616843	HQ616814	HQ616868
Elysia papillosa		Bermuda	ZMBN 82998	HQ616842	HQ616813	HQ616867
Elysia papillosa		Colombia	MNCN 15.05/53678	HQ616860	HQ616831	HQ616885
Elysia papillosa		Colombia	MNCN 15.05/54986	—	HQ658121	HQ658127
Elysia timida	*Elysia papillosa*	Cuba	MNCN 24.922	HQ616844	HQ616815	HQ616869
Elysia cf. *papillosa*	*Elysia* sp.	Colombia	MNCN 15.05/54993	HQ616859	HQ616830	HQ616884
Elysia cf. *papillosa*	*Elysia* sp.	Colombia	MNCN 15.05/53676	—	HQ616837	HQ616891
Elysia crispata	*Elysia* sp.	Colombia	MNCN 15.05/54990	—	HQ658120	HQ658126
Elysia timida	*Elysia* sp.	Cuba	MNCN 15.05/53679	HQ616845	HQ616816	HQ616870
Elysia subornata		Bermuda	CASIZ 181085	HQ616848	HQ616819	HQ616873
Elysia subornata		GB		DQ471283	—	—
Elysia timida		Florida Keys (GB)		DQ471248	DQ480169	DQ534767
Elysia timida		France (MED)	CASIZ 184305	HQ616856	HQ616827	HQ616881
Elysia timida		France (MED)	CASIZ 184306	HQ616857	HQ616828	HQ616882
Elysia timida		France (MED, GB)		—	EU140857	—
Elysia timida		Menorca (Spain, MED)	MNCN 15.05/53680	HQ616847	HQ616818	HQ616872
Elysia timida		Menorca (Spain, MED)	MNCN 15.05/54992	—	—	HQ658130
Elysia timida		Menorca (Spain, MED)	MNCN 17014	HQ616858	HQ616829	HQ616883
Elysia timida		Menorca (Spain, MED)	MNCN 15.05/54989	HQ658124	—	HQ658131
Elysia timida		Roses, Spain (MED, GB)		—	EU140858	—
Elysia timida		Tossa, Spain (MED, GB)		—	EU140859	—
Oxynoe antillarum		GB		FJ917483	FJ917425	DQ534807
Thuridilla bayeri		GB		DQ471279	DQ480208	DQ534796
Thuridilla picta	*Thuridilla hopei*	Azores	ZMBN 81680	HQ616850	HQ616821	HQ616875
Thuridilla picta	*Thuridilla hopei*	Azores	ZMBN 81680	HQ658123	—	HQ658129
Thuridilla hopei		Elba, Spain (MED, GB)		—	EU140881	—
Thuridilla hopei		France (MED)	CASIZ 184307	HQ616854	HQ616825	HQ616879
Thuridilla picta	*Thuridilla hopei*	Madeira	MNCN 15.05/53685	HQ616853	HQ616824	HQ616878
Thuridilla hopei		Menorca (Spain, MED)	MNCN 15.05/53682	HQ616849	HQ616820	HQ616874
Thuridilla hopei		Mataró, Spain (MED, GB)		—	EU140882	—

Continued

Table 1. *Continued*

Species		Locality	Voucher	GenBank accession nos		
Provisional id.	Revised id.			COI	16S	H3
Thuridilla hopei		Western Andalucia (Spain, ATL)	MNCN 17015	HQ616855	HQ616826	HQ616880
Thuridilla mazda		Mexico (ATL)	UNAM 3027	—	HQ616836	HQ616890
Thuridilla picta		Bermuda	ZMBN 83023	HQ616851	HQ616822	HQ616876
Thuridilla picta		Bermuda	ZMBN 83023	HQ658125	—	—
Thuridilla picta		Colombia	MNCN 15.05/53683	HQ616861	HQ616832	HQ616886
Thuridilla picta		Colombia	MNCN 15.05/54991	HQ616862	HQ616833	HQ616887
Thuridilla picta		Cuba	MNCN 17016	HQ616852	HQ616823	HQ616877

We include both the species names resulting from our morpho-chromatic identification (provisional id.) and the names after analyses (revised id.; this only when changes have occurred). Abbreviations: ATL, Atlantic Ocean; GB, GenBank; id., identification; MED, Mediterranean Sea.

Table 2. Maximum and minimum COI gene pairwise uncorrected p-distances between sacoglossan species of the same genus (analysis conducted in PAUP* v. 4.0b10).

	B. marcusi	*B. mimetica*	*Bosellia* sp.	*E. cornigera*	*E. papillosa*	*E. timida* (MED)	*E. timida* (WA)	*Elysia* sp.	*T. hopei* (EA)	*T. hopei* (MED)	*T. picta* (WA)
Bosellia marcusi (WA)	0.0	21.3–22.9	19.6–21.2	14.4–16.3	14.4–16.9	16.7–19.3	17.1–17.4	17.2–18.1	14.7–16	15.4–15.5	15.1–16
Bosellia mimetica (MED)	21.3–22.9	0.0	7.3–8.5	19.8–20.7	18.6–20.4	21.3–24	21.2–21.6	18.8–19.3	19.1–20.1	19.1–20.1	19.1–19.9
Bosellia sp. (WA)	19.6–21.2	7.3–8.5	0.0	18.6–17.6	17.6–18.9	19.2–19.8	19.5–19.8	16.7–17.3	17.4–18.9	17.3–18.1	17.6–18.8
Elysia cornigera	14.4–16.3	19.8–20.7	18.6–17.6	0.0	16.8–18.6	9.2–10.6	9.7–10.63	17.4–11.7	15–16.3	15–16.3	15.7–17.3
Elysia papillosa	14.4–16.9	18.6–20.4	17.6–18.9	16.8–18.6	0.0	16–19.3	16.3–17.4	10.1–10.5	16.1–17.5	16.7–16.9	16.6–17.2
Elysia timida (MED)	16.7–19.3	21.3–24	19.2–19.8	9.2–10.6	16–19.3	0.0	0.8–3.5	17.4–20.7	15.1–19.1	15.7–19.1	16–19.3
Elysia timida (WA)	17.1–17.4	21.2–21.6	19.5–19.8	9.7–10.6	16.3–17.4	0.8–3.5	0.0	17.8–18.2	15.2–16.2	15.9–16.2	16.2–16.7
Elysia sp.	17.2–18.1	18.9–19.3	16.7–17.3	17.4–11.7	10.1–10.5	17.4–20.7	17.8–18.2	0.0	16.1–17.5	16.7–16.9	16.7–17.2
Thuridilla hopei (EA)	14.7–16	19.1–20.1	17.4–18.9	15–16.3	16.1–17.5	15.1–19.1	15.2–16.2	16.1–17.5	0.0	0.7–1.3	4.8–6.6
Thuridilla hopei (MED)	15.4–15.5	19.1–20.1	17.3–18.1	15–16.3	16.7–16.9	15.7–19.1	15.9–16.2	16.7–16.9	0.7–1.3	0.0	4.8–5.5
Thuridilla picta (WA)	15.1–16	19.1–19.9	17.6–18.8	15.7–17.3	16.6–17.2	16–19.3	16.2–16.7	16.7–17.2	4.8–6.6	4.8–5.5	0.0

Abbreviations: EA, Eastern Atlantic; MED, Mediterranean Sea; WA, Western Atlantic.

recover reciprocal monophyly between eastern and western specimens of '*B. mimetica*' but does not contradict it (Fig. 1B), whereas in the more slowly evolving nuclear gene H3 these specimens formed a single cluster with maximum support (Fig. 1C).

All specimens of *Thuridilla picta* from the western Atlantic formed a clade that received maximum support (PP = 1, BS = 100%). This clade was reciprocally monophyletic (PP = 1, BS = 100%) with a clade of eastern Atlantic and MS specimens of *T. hopei* plus specimens previously thought to be *T. picta* from the Macaronesian archipelagos of the Azores and Madeira (PP = 0.86, BS = 85%). Genetic distance between the western and eastern Atlantic clades and between Macaronesian specimens and eastern continental specimens are 4.8–6.6% and 0.7–1.3%, respectively (uncorrected p-distances for COI). Reciprocal monophyly between western and eastern specimens was recovered by the combined and COI gene analyses. In the 16S analysis, Macaronesian specimens branched off separately from the eastern Atlantic specimens and in the H3 analysis support for the western Atlantic clade was low (PP = 0.7, BS = 68%). However, reciprocal monophyly was not contradicted by the results of the latter two genes.

DISCUSSION

Bosellia mimetica

Our results showed the existence of a complex of three sibling species within *Bosellia mimetica* and suggest that *B. mimetica* is not amphi-Atlantic, but restricted to the eastern side of the

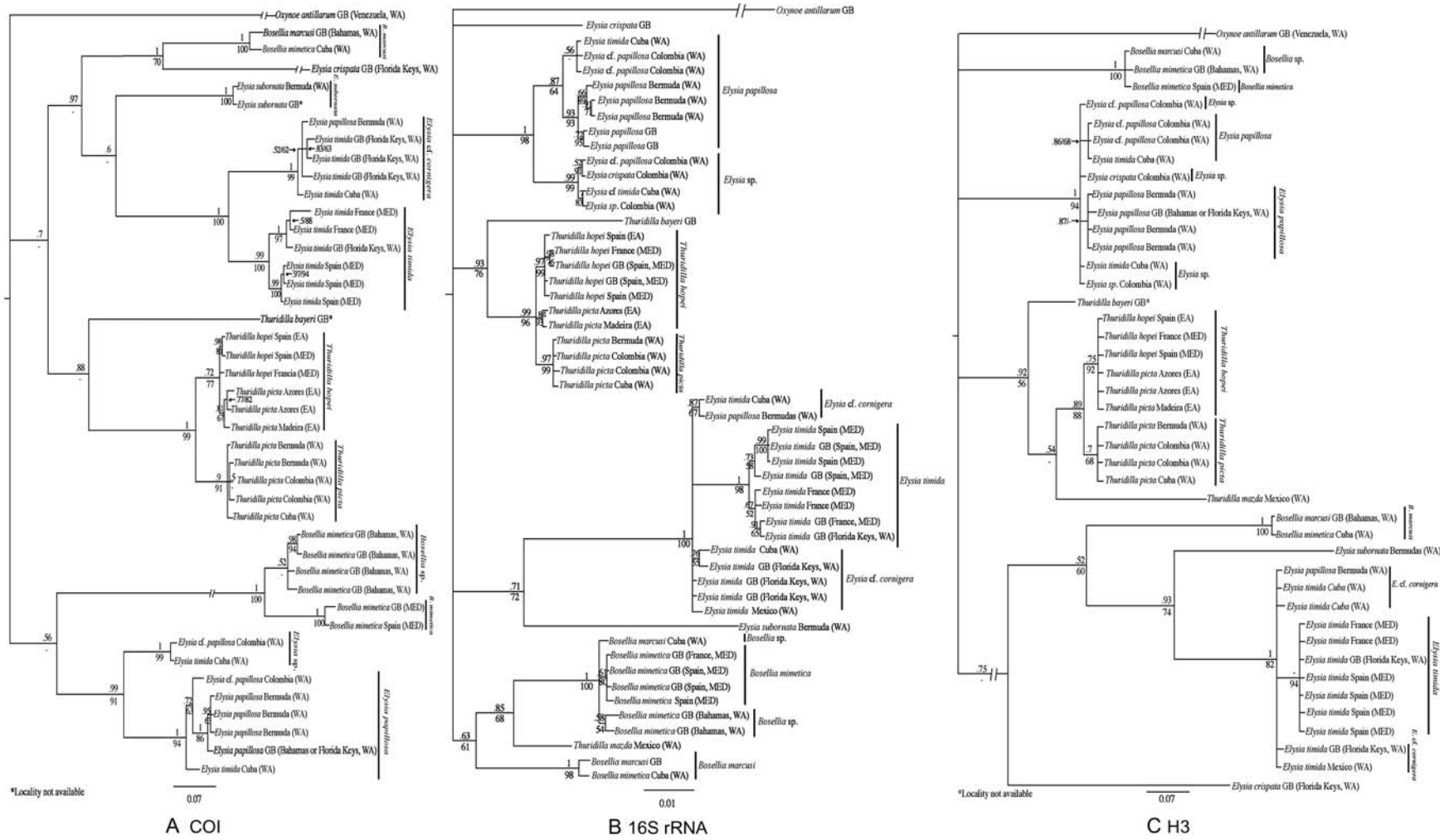

Figure 1. Molecular phylogenies of sacoglossan species inferred from partial sequences of the individual mitochondrial COI (**A**) and 16S rRNA (**B**) and nuclear H3 (**C**) genes by Bayesian analysis (BI). Figures above branches represent PP from BI and figures below branches indicate bootstrap percentage from ML. Abbreviations: EA, eastern Atlantic Ocean; GB, GenBank; MED, Mediterranean; WA, western Atlantic Ocean. Species names on right side of vertical bars refer to revised classification.

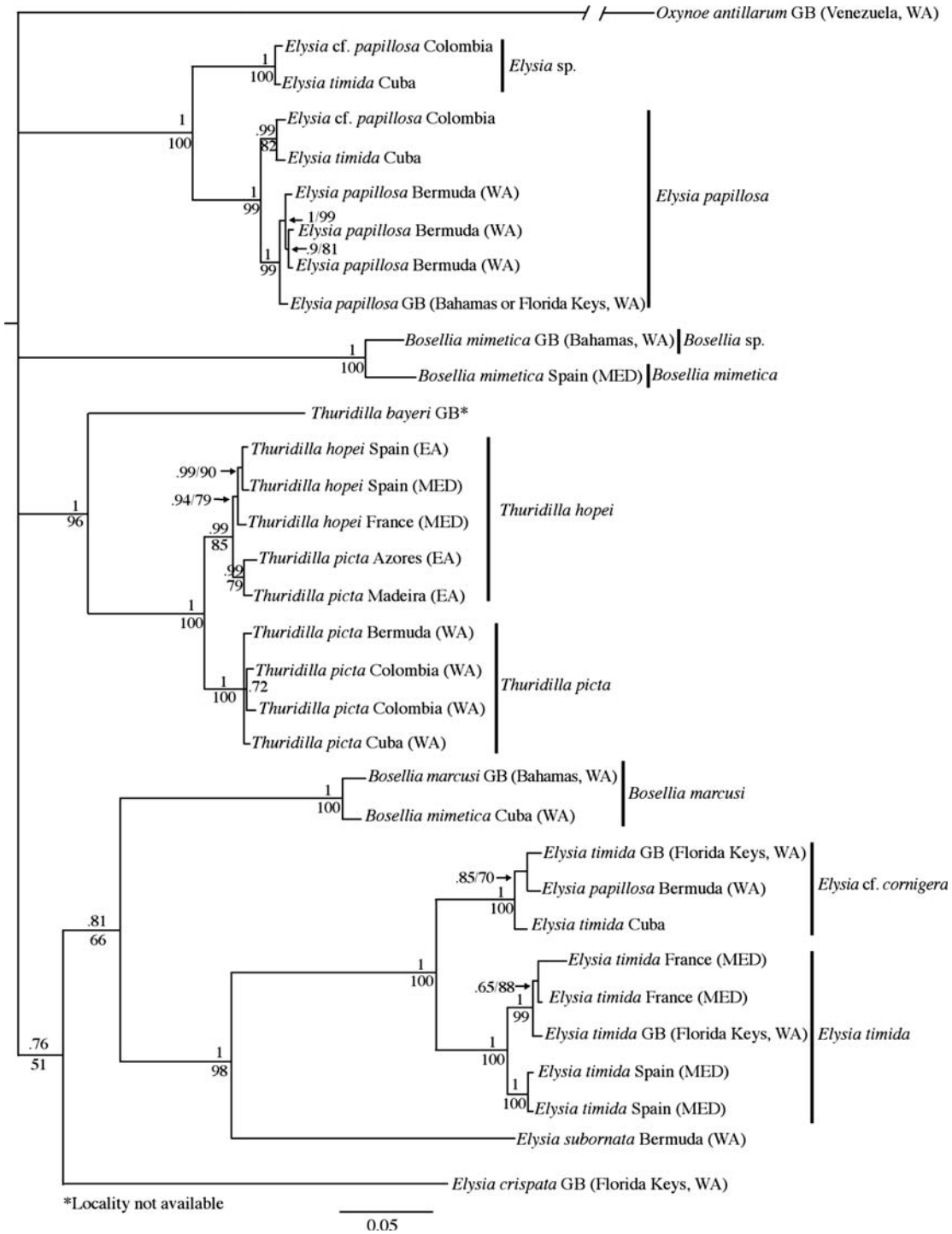

Figure 2. Phylogenetic hypothesis based on the combined dataset (COI + 16S + H3) inferred by Bayesian analysis (BI). Figures above branches represent PP from BI and figures below branches indicate bootstrap values from ML. Abbreviations: EA, eastern Atlantic Ocean; GB, GenBank; MED, Mediterranean; WA, western Atlantic Ocean. Species names on right side of vertical bars refer to revised classification.

Atlantic Ocean. Specimens from the Mediterranean Sea (type locality) were sister to western Atlantic specimens in the individual analyses of the mitochondrial COI (uncorrected p-distance = 7.3% between individuals from both sides of the Atlantic; this figure is within the threshold defined for other species) and combined analysis of the three genes. However, the nuclear H3 gene did not retrieve reciprocal monophyly between eastern and western Atlantic specimens of '*B. mimetica*'; this might result from incomplete lineage-sorting since H3 is known to be a conservative gene in opisthobranchs (Dinapoli *et al.*, 2006).

Dinapoli *et al.* (2006) concluded that H3 has good resolution at genus level, which is confirmed by our results. Furthermore, our phylogenetic hypotheses do not support the monophyly of the genus *Bosellia*, because specimens of '*B.*' *marcusi* branched off separately in the trees. As a result of a broader phylogenetic analysis of the Plakobranchidae, Bass & Karl (2006) have already hypothesized that this genus is not monophyletic.

Figure 3. Images of living animals. **A.** *Thuridilla hopei*, Menorca, Spain ($H = 2$ mm; MNCN 15.05/53682). **B.** *Thuridilla hopei*, Madeira ($H = 9$ mm; MNCN 15.05/53685). **C.** *Thuridilla hopei*, Azores ($H = 23$ mm; ZMBN 81680). **D.** *Thuridilla picta*, Bermuda ($H = 11$ mm; ZMBN 81680). **E.** *Thuridilla picta*, Colombia ($H = 5$ mm; ZMBN 81680). **F.** *Thuridilla picta*, Cuba ($H = 10$ mm; MNCN 15.05/53684).

Recently, Händeler *et al*. (2009) and Wägele *et al*. (2010) suggested that '*B*.' *marcusi* is a derived *Elysia* that this been traditionally ascribed to the genus *Bosellia* due to its morphological similarity, produced by the secondary fusion over the dorsum of the parapodia.

Elysia timida

The amphi-Atlantic status of *E. timida* is confirmed by our results, because specimens from its type locality (the Mediterranean Sea) cluster together with a specimen from the FK (western Atlantic). Additionally, the genetic distance between eastern and western specimens (uncorrected p-distance = 3.5%) is below the threshold here considered for different species. However, it is possible that the presence of *E. timida* in the western Atlantic results from a recent introduction, either by rafting of adults or anthropogenic activities, because the species was only first recognized in the western Atlantic during the late 1990s (Ortea *et al*., 1997). Moreover its direct development, or alternatively lecithotrophic larvae, makes trans-Atlantic dispersal unlikely even during Plio-Pleistocene periods of stronger ocean currents.

A larger-scale study of the haplotype diversity of eastern and western populations may shed light on whether the amphi-Atlantic distribution is ancient or has resulted, as hypothesized here, from a recent invasion. If the latter is correct, we predict the presence of greater diversity and older haplotypes in the original population (eastern Atlantic), whereas the younger population (western Atlantic) would contain primarily a subset of haplotypes found in the original population.

There is an ongoing problem surrounding the complex '*E. timida/cornigera*' in the Caribbean. The species *E. cornigera* was described by Nuttall (1989), who suggested it was closely related to *E. timida* based on morphological and ecological similarities. Later Ortea *et al*. (1997), based on morpho-anatomical and behavioural data, considered *E. cornigera* to be a synonym of the eastern Atlantic *E. timida*. Our results show unequivocally that both species are valid and sister to each other. This highlights the difficulties of discriminating between some species of *Elysia* without a molecular phylogenetic framework.

The genus *Elysia* is an example of the complex and confusing taxonomy of Sacoglossa. Our molecular data showed that western Atlantic specimens previously recognized as *E. timida*, based on morphological and chromatic similarities, belong to four different species. This confirms an entrenched suspicion among opisthobranch researchers that the genus contains many cryptic species, as previously pointed out by Nuttall (1989) for western Atlantic *Elysia* species using allozymes.

Thuridilla picta

It has been a prevalent view that specimens of *Thuridilla* from the central and eastern Atlantic Macaronesian archipelagos of the Azores (Fig. 3C) and Madeira (Fig. 3B; and also Canaries; not tested here) are conspecific with the western Atlantic *T. picta* (Fig. 3D; type locality Bermuda) (e.g. Valdés *et al*., 2006; Malaquias *et al*., 2009). This assumption has been based on chromatic similarities between specimens from these disjunct geographical areas. However, our results do not support the amphi-Atlantic status of *T. picta*. The data revealed that insular specimens belong to the eastern Atlantic and Mediterranean species *T. hopei*. Genetic distance between eastern and western populations is at the lower limit of the threshold for separating species, but combined and COI analyses support reciprocal monophyly, which is not contradicted by the 16S and H3 gene analyses (Figs 1, 2; Table 2). A reassessment of the chromatic variability of *T. hopei* (Fig 3A–C) showed that, unlike its sister western Atlantic sibling *T. picta* (Fig 3D–F), the former shows high levels of intraspecific chromatic variation, ranging from the most common colour pattern illustrated in Figure 3A to patterns similar to those found in western Atlantic specimens of *T. picta* (Poddubetskaia, 2002; Horst, 2008, 2009). This is the case for Macaronesian specimens that have a colour pattern similar to the western Atlantic *T. picta*, but are conspecific with the eastern Atlantic *T. hopei* (Fig. 3).

This result yields support for the biogeographic affinities of the Azorean marine fauna with that of the Lusitanian and Mediterranean provinces. This biogeographic affinity goes against prevailing oceanographic currents of western Atlantic origin, but has been found by several authors for numerous taxonomic groups (e.g. fish, amphipods, demosponges; see Ávila, 2000 for an account). Nevertheless, this biogeographic affinity has rarely been tested in a molecular framework (Muss *et al*., 2001 for the blennid fish *Ophioblenius atlanticus*; Ó Foighil *et al*., 2001 for the bivalve *Lasaea*; present study). The processes that drive this pattern are still poorly understood and remain one of the most exciting biogeographic challenges in North Atlantic biogeography.

CONCLUSIONS

Empirical and field observations indicate that *Elysia timida*, *Thuridilla hopei* and *T. picta* are either direct or lecithotrophic developers. The type of development is unknown for *Bosellia mimetica*, but the western Atlantic *Bosellia* sp. is planktotrophic (previously recognized as *B. mimetica*; Clark & Jensen, 1981; Marín & Ros, 1992; Jensen, 2001). Even for planktotrophic developers, trans-Atlantic dispersal under normal oceanographic conditions should be unlikely (see Introduction), but the idea is even more challenging for direct or lecithotrophic organisms. Dispersal by rafting is a possibility but, as emphasized earlier, evidence is lacking. An alternative hypothesis is that the ancestral lineages had planktotrophic development that favoured stochastic trans-Atlantic dispersal during periods of stronger currents during the Plio-Pleistocene, with subsequent change of development type. This is strengthen by the fact that poecilogony (simultaneous occurrence of alternative larval morphs—such as direct and planktotrophic—produced by the same organism) is prevalent among sacoglossan sea slugs (Bouchet, 1989; Ellingson & Krug, 2006).

The results presented in this work expose further the complexity of sacoglossan systematics, showing that cryptic species are prevalent in the Sacoglossa. An integrative taxonomic approach (including DNA and geographical data coupled with morpho-anatomy) is crucial in order to understand the diversity and evolutionary history of the group.

ACKNOWLEDGEMENTS

We are deeply grateful to all individuals that helped to collect and provided specimens and images to this study: A. Luque, G. Calado, G. Villani, J. Templado, J.A. Fayos, J. Ortigosa, M. Poddubetkaia, N. Ardila, C. Megina and V. Padula. Many thanks are due to W. Sterrer (Bermuda Aquarium and Museum of Zoology, BAMZ), B. Williams (Bermuda Institute of Oceanographic Sciences), T.M. Trott (Acting Senior Marine Resources Officer, Bermuda Government), M. Biscoito and T.M. Freitas (Marine Biological Station of Funchal), M.E. Ibarra and M. Ortiz-Touzet (Centro de Investigaciones Marinas, Universidad de la Habana) and J. Gonçalves, G. Carreira and R. S. Santos (Department of Oceanography and Fisheries, University of Azores) for welcoming, housing and for facilitating permits during the expeditions in Bermuda,

Madeira, Cuba and the Azores. Two anonymous referees provided useful insights that helped to improve the manuscript. This work was supported by several research grants (CGL2006-05182/BOS and CTM2008-05228-E/MAR, Spanish Ministry of Science and Innovation; Institut Menorquí d'Estudis, Balearic Is; Bermuda Zoological Society, California Academy of Sciences, Lindsay Field Research Fund; Norwegian Meltzer's Foundation Proj. No. 803108). This is contribution no. 182 from the Bermuda Biodiversity Project, Bermuda Aquarium, Museum & Zoo.

REFERENCES

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

ALVAREZ, L.A., GARCÍA, F.J. & VILLANI, G. 1993. A new Mediterranean species of *Haminaea* Leach, 1820 (Gastropoda: Opisthobranchia: Cephalaspidea). *Journal of Molluscan Studies*, **59**: 339–345.

ÁVILA, S.P. 2000. Shallow-water marine molluscs of the Azores: biogeographical relationships. *Arquipélago, Life and Marine Sciences*, **Supplement 2**: 99–130.

AVISE, J.C. 2004. *Molecular markers, natural history, and evolution*. Edn 2. Sinauer Associates, Sunderland, MA.

BASS, A.L. & KARL, S.A. 2006. Molecular phylogenetic analysis of genera in the family Plakobranchidae (Mollusca: Opisthobranchia: Sacoglossa). *Records of the Australian Museum*, **69**: 61–68.

BONAR, D.B. 1978. Morphogenesis at metamorphosis in opisthobranch molluscs. In: *Settlement and metamorphosis of marine invertebrate larvae* (F.S. Chia & M.E. Rice, eds), pp. 177–196. Elsevier, New York.

BOUCHET, P. 1989. A review of poecilogony in gastropods. *Journal of Molluscan Studies*, **55**: 67–78.

BRIGGS, J.C. 2003. Marine centres of origin as evolutionary engines. *Journal of Biogeography*, **30**: 1–18.

CERVERA, J.L., CALADO, G., GAVAIA, C., MALAQUIAS, M.A.E., TEMPLADO, J., BALLESTEROS, M., GARCÍA-GÓMEZ, J.C. & MEGINA, C. 2004. An annotated and updated checklist of the opisthobranchs (Mollusca: Gastropoda) from Spain and Portugal (including islands and archipielagos). *Boletín del Instituto Español de Oceanografía*, **20**: 1–122.

CERVERA, J.L., TAMSOURI, N., MOUKRIM, A. & VILLANI, G. 2010. New records of two alien opisthobranch molluscs from the north-eastern Atlantic: *Polycera hedgpethi* and *Godiva quadricolor*. *Marine Biodiversity Records*, **3**: 1–4; doi:10.1017/S1755267210000102.

CLAREMONT, M., WILLIAMS, S.T., BARRACLOUGH, T.G. & REID, D. 2011. The geographic scale of speciation in a marine snail with high dispersal potential. *Journal of Biogeography*, **38**: 1016–1032.

CLARK, K. & JENSEN, K.R. 1981. A comparison of egg size, capsule size, and development patterns in the order Ascoglossa (Sacoglossa) (Mollusca: Opishtobranchia). *International Journal of Invertebrate Reproduction*, **3**: 57–64.

COLGAN, D., McLAUCHLAN, A., WILSON, G.D.F., LIVINGSTON, S.P., EDGECOMBE, G.D., MACARANAS, J., CASSIS, G. & GRAY, M.R. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, **46**: 419–437.

DINAPOLI, A., TAMER, C., FRASSEN, S., NADUVILEZHATH, L. & KLUSSMANN-KOLB, A. 2006. Utility of H3-Genesequences for phylogenetic reconstruction—a case study of heterobranch Gastropoda. *Bonner Zoologische Beiträge*, **55**: 191–202.

DRUMMOND, A.J., ASHTON, B., CHEUNG, M., HELED, J., KEARSE, M., MOIR, R., STONES-HAVAS, S., THIERER, T. & WILSON, A. 2009. *Geneious v4.6*. Available from: http://www.geneious.com/.

DRUMMOND, A.J. & RAMBAUT, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**: 214.

EDMUNDS, M. 1977. Larval development, oceanic currents, and origins of the Opisthobranch fauna of Ghana. *Journal of Molluscan Studies*, **43**: 301–308.

ELLINGSON, R.A. & KRUG, P.J. 2006. Evolution of poecilogony from planktotrophy: criptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. *Evolution*, **60**: 2293–2310.

FARRIS, J.S., KÄLLERSJÖ, M., KLUGE, A.G. & BULT, C. 1994. Testing significance of incongruence. *Cladistics*, **10**: 315–319.

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.

FRASER, C.I., NIKULA, R. & WATERS, J.M. 2011. Oceanic rafting by a coastal community. *Proceedings of the Royal Society of London*, **278**: 649–655.

FREY, M.A. & VERMEIJ, G.J. 2008. Molecular phylogenies and historical biogeography of a circumtropical group of gastropods (Genus: *Nerita*): implications for regional diversity patterns on the marine tropics. *Molecular Phylogenetics and Evolution*, **48**: 1067–1086.

GARCÍA, F.J. & BERTSCH, H. 2009. Diversity and distribution of the Gastropoda Opisthobranchia from the Atlantic Ocean: a global biogeographic approach. *Scientia Marina*, **73**: 153–160.

GARCÍA-TALAVERA, F. 1983. *Los moluscos gasterópodos anfiatlánticos. Estudio paleo y biogeográfico de las especies bentónicas litorales*. Publicaciones de la Universidad de La Laguna. Monografías, no. 10.

GOSLINER, T.M. 1995. Introduction and spread of *Philine auriformis* (Gastropoda: Opisthobranchia) from New Zealand to San Francisco Bay and Bodega Harbor. *Marine Biology*, **122**: 249–255.

GOSLINER, T.M. & BEHRENS, D.W. 2006. Anatomy of an invasion: systematics and distribution of the introduced Opisthobranch snail, *Haminoea japonica* Pilsbry, 1895 (Gastropoda: Opisthobranchia: Haminoeidae). *Proceeding of the California Academy of Sciences*, **57**: 1003–1010.

GOSLINER, T.M., BEHRENS, D.W. & VALDÉS, Á. 2008. *Indo-Pacific nudibranchs and sea slugs: a field guide to the world's most diverse fauna*. California Academy of Sciences/Sea Challengers Natural History Books, San Francisco.

HADFIELD, M.G. & KARLSON, H. 1969. Externally induced metamorphosis in a marine gastropod. *American Zoologist*, **9**: 1122.

HÄNDELER, K., GRZYMBOWSKI, Y.P., KRUG, P.J. & WÄGELE, H. 2009. Functional chloroplasts in metazoan cells—a unique evolutionary strategy in animal life. *Frontiers in Zoology*, **6**: 1–18.

HÄNDELER, K. & WÄGELE, H. 2006. Preliminary study on molecular phylogeny of Sacoglossa and a compilation of their food organisms. *Bonner Zoologische Beiträge*, **55**: 231–254.

HEBERT, P.D.N., RATNASINGHAM, S. & WAARD, J.R.D. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society*, **270**: S96–S99.

HORST, D. 2008. *Thuridilla hopei* from the French Mediterranean. [Message in] Sea Slug Forum. Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/21023.

HORST, D. 2009. Re: *Thuridilla hopei* under predation attack. [Message in] Sea Slug Forum. Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/22520.

JENSEN, K. 1996. Phylogenetic systematics and classification of the Sacoglossa (Mollusca, Gastropoda, Opisthobranchia). *Philosophical Transactions of the Royal Society of London*, **351**: 91–122.

JENSEN, K. 2001. Review of reproduction in the Sacoglossa (Mollusca, Opisthobranchia). *Bollettino Malacologico*, **37**: 81–98.

KNOWLTON, N. 1993. Sibling species on the sea. *Annual Review in Ecology and Systematics*, **24**: 189–216.

KNOWLTON, N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*, **420**: 73–90.

KRUG, P.J., MORLET, M.S., ASIF, J., HELLYAR, L.L. & BLOM, W.M. 2008. Molecular confirmation of species status for the rare cephalaspidean *Melanochlamys lorrainae* (Rudman, 1968), and comparison with its sister species *M. cylindrical* Cheesman, 1881. *Journal of Molluscan Studies*, **74**: 267–276.

MADDISON, D.R. & MADDISON, W.P. 2005. *MacClade 4., v. 4.08 for OSX*. Sinauer Associates, Sunderland, MA.

MAESTRATI, P. & LOZOUET, P. 1996. Les Glycymerididae (Mollusca: Bivalvia) de l'Oligocène superior (Chattien) du Basin de l'Adour, France. *Annalles de Paléontologie (Vert-Invert.)*, **82**: 3–25.

MALAQUIAS, M.A.E., CALADO, G.P., PADULA, V., VILLANI, G. & CERVERA, J.L. 2009. Molluscan diversity in the North Atlantic Ocean: new records of opisthobranch gastropods from the Archipelago of Azores. *Marine Biodiversity Records*, **2**: 1–9.

MALAQUIAS, M.A.E. & REID, D.G. 2008. Systematic revision of the recent species of Bullidae (Mollusca: Gastropoda: Cephalaspidea), with a molecular phylogenetic analysis. *Zoological Journal of the Linnean Society*, **153**: 453–543.

MALAQUIAS, M.A.E. & REID, D.G. 2009. Tethyan vicariance, relictualism and speciation: evidence from a global molecular phylogeny of the opisthobranch genus *Bulla*. *Journal of Biogeography*, **36**: 1760–1777.

MARÍN, A. & ROS, J.D. 1988. Los Sacoglossos (Mollusca: Opisthobranchia) del sudeste ibérico. Catálogo de las especies y presencia de cloroplastos algales en las mismas. *Iberus*, **8**: 25–49.

MARÍN, A. & ROS, J. 1989. The chloroplast-animal association in four Iberian sacoglossan opisthobranchs: *Elysia timida*, *Elysia translucens*, *Thuridilla hopei* and *Bosellia mimetica*. *Scientia Marina*, **53**: 429–440.

MARÍN, A. & ROS, J.D. 1992. Dynamics of a peculiar plant-herbivore relationship: the photosyntetic ascoglossan *Elysia timida* and the chlorophycean *Acetabularia acetabulum*. *Marine Biology*, **112**: 677–682.

MARÍN, A. & ROS, J.D. 1993. Ultrastructural and ecological aspects of the development of chloroplast retention in the sacoglossan gastropod *Elysia timida*. *Journal of Molluscan Studies*, **59**: 95–104.

MEYER, C.P. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, **79**: 401–459.

MUSS, A., ROBERTSON, D.R., STEPIEN, C.A., WIRTZ, P. & BOWEN, B.W. 2001. Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. *Evolution*, **55**: 561–572.

NUTTALL, T.R. 1989. A new *Elysia* (Opisthobranchia: Ascoglossa) from Florida Keys. *Veliger*, **32**: 302–307.

NYLANDER, J.A.A. 2004. *MrModeltest v2.3*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ó FOIGHIL, D., JENNINGS, R., PARK, J.P. & MERRIWETHER, D.A. 2001. Phylogenetic relationships of mid-oceanic ridge and continental lineages of *Lasaea* spp. (Mollusca: Bivalvia) in the northeastern Atlantic. *Marine Ecology Progress Series*, **213**: 165–175.

ORTEA, J., MORO, L. & ESPINOSA, J. 1997. Nuevos datos sobre el género *Elyisia* RISSO, 1818 (Opisthobranchia: Sacoglossa) en el Atlántico. *Revista de la Academia Canaria de Ciencias*, **9**: 141–155.

PALUMBI, S.R., MARTIN, A., ROMANO, S., OWEN MACMILLAN, W., STICE, L. & GRABOWSKI, G. 1991. *The simple fool's guide to PCR*. Department of Zoology, University of Hawaii, Honolulu.

PETUCH, E.J. 1997. *Coastal paleoceanography of eastern North America*. Kendall Hunt Publishing, Dubuque, IA.

PODDUBETSKAIA, M. 2002. (Jul 30) *Thuridilla hopei* from French Mediterranean. [Message in] Sea Slug Forum. Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/7551.

POLA, M. & GOSLINER, T.M. 2010. The first molecular phylogeny of cladobranchian opisthobranchs (Mollusca, Gastropoda, Nudibranchia). *Molecular Phylogenetics and Evolution*, **56**: 931–941.

RAHAT, M. 1976. Direct development and symbiotic chloroplasts in *Elysia timida* (Mollusca: Opisthobranchia). *Israel Journal of Zoology*, **25**: 186–193.

REID, D.G., LAL, K., MACKENZIE-DODDS, J., KALIGIS, F., LITTLEWOOD, D.T.J. & WILLIAMS, S.T. 2006. Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography*, **33**: 990–1006.

RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

SCHAEFER, K. 1996. Review of data on cephalaspid reproduction, with special reference to the genus *Haminaea* (Gastropoda: Opisthobranchia). *Ophelia*, **45**: 17–37.

SCHELTEMA, R.S. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biological Bulletin*, **140**: 284–322.

SCHELTEMA, R.S. 1979. Dispersal of pelagic larvae and the zoogeography of Tertiary marine benthic gastropods. In: *Historical biogeography, plate tectonics, and the changing environment*. (J. Gray & A.J. Boucot, eds), pp. 391–397. Oregon State Univ. Press, Corvallis, Oregon.

SCHELTEMA, R.S. 1995. The relevance of passive dispersal for the biogeography of Caribbean mollusks. *American Malacological Bulletin*, **11**: 99–115.

SCHRÖDL, M. & NEUSSER, T.P. 2010. Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linneana Society*, **158**: 124–154.

STAMATAKIS, A., HOOVER, P. & ROUGEMONT, J. 2008. A fast bootstrapping algorithm for the RAxML web-servers. *Systematic Biology*, **57**: 758–771.

STREFTARIS, N. & ZENETOS, A. 2006. Alien marine species in the Mediterranean—the 100 'worst invasives' and their impact. *Mediterranean Marine Science*, **7**: 87–118.

SWOFFORD, D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods)*. Version 4. Sinauer, Sunderland, MA.

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* (in press). doi:10.1093/molbev/msr121.

TESKE, P.R., CHERRY, M.I. & MATTHEE, C.A. 2004. The evolutionary history of seahorses (Syngnathidae: Hippocampus): molecular data suggest a west Pacific origin and two invasions of the Atlantic Ocean. *Molecular Phylogenetics and Evolution*, **30**: 273–286.

THOMPSON, T.E. 1958. The natural history, embryology, larval biology, and post-larval development of *Adalaria proxima* (Alder and Hancock) (Gastropoda: Opisthobranchia). *Philosophical Transactions of the Royal Society*, **242B**: 1–58.

THOMPSON, T.E. 1962. Studies on the ontogeny of *Tritonia hombergi* Cuvier (Opisthobranchia). *Philosophical Transactions of the Royal Society of London*, **24**: 171–218.

THOMPSON, T.E. & CRAMPTON, D.M. 1984. Biology of *Melibe fimbriata*, a conspicuous opisthobranch mollusc of the Indian Ocean, which has now invaded the Mediterranean Sea. *Journal of Molluscan Studies*, **50**: 113–121.

THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**: 4876–4882.

VALDÉS, Á., HAMANN, J., BEHRENS, D.W. & DUPONT, A. 2006. *Caribbean sea slugs: a field guide to the opisthobranch mollusks from the tropical northwestern Atlantic*. Sea Challengers Natural History Books, Gig Harbor, WA, USA.

VERMEIJ, G.J. 1995. Morphology and possible relationships of Ecphora (Cenozoic Gastropoda: Muricidae). *Nautilus*, **109**: 120–126.

VERMEIJ, G.J. & ROSENBERG, G. 1993. Giving and receiving: the tropical Atlantic as a donor and recipient region for invading species. *American Malacological Bulletin*, **10**: 181–194.

WÄGELE, H., STEMMER, K., BURGHARDT, I. & HÄNDELER, K. 2010. Two new sacoglossan sea slug species (Opisthobranchia, Gastropoda): *Ercolania annelyleorum* sp. nov. (Limapontioidea) and *Elysia asbecki* sp. nov. (Plakobranchoidea), with notes on anatomy, histology and biology. *Zootaxa*, **2676**: 1–28.

WHEELER, Q.D. & MEIER, R. (eds), 2000. *Species concepts and phylogenetic theory: a debate*. Columbia University Press, New York.

WILLIAMS, S.T. & REID, D.G. 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. *Evolution*, **58**: 2227–2251.

WILLIAMS, S.T., REID, D.G. & LITTLEWOOD, D.T.J. 2003. A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism and biogeography of the Southern Ocean. *Molecular Phylogenetics and Evolution*, **28**: 60–86.

ZENETOS, A., KATSANEVAKIS, S., POURSANIDIS, D., CROCETTA, F., DAMALAS, D., APOSTOLOPOULOS, G., GRAVILI, C., VARDALA-THEODOROU, E. & MALAQUIAS, M. 2011. Marine alien species in Greek Seas: additions and amendments by 2010. *Mediterranean Marine Science*, **12**: 95–120.

ZENETOS, A., VASSILOPOULOU, V., SALOMIDI, M. & PURSANIDIS, D. 2008. Additions to the marine alien fauna of Greek waters (2007 update). *Marine Biodiversity Records*, **1**: 1–8; doi:10.1017/S1755267207009281.