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How many species of *Siphonaria pectinata* (Gastropoda: Heterobranchia) are there?

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ABSTRACT

Siphonaria pectinata (Linnaeus, 1758) has been considered a widespread species with Amphiatlantic distribution or a case of cryptic taxonomy where sibling species exist. We undertook molecular evaluation of 66 specimens from across its putative distribution range. We examined up to three molecular markers (mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA, and nuclear internal transcribed spacer-2) of putative *S. pectinata*, including populations from the Mediterranean Sea, eastern Atlantic (Spain, Canary Islands, Cape Verde Islands, Cameroon and Gabon) and western Atlantic (Florida and Mexico), covering most of the natural range of the species. While little information could be derived from the shell morphology, molecular data clearly distinguished three lineages with no apparent connectivity. These lineages correspond to what we interpret as three species, two suspected from prior work: *S. pectinata*, restricted to the eastern Atlantic and Mediterranean and *S. naufragum* Stearns, 1872 in Florida and the Gulf of Mexico. A third species has been identified for the Cape Verde Archipelago, for which we use the available name *S. placentula* Menke, 1853.

INTRODUCTION

Estimating the total number of living animal species has proved an onerous task for many reasons, first and foremost the high proportion of unknown (or undescribed) diversity—the so-called Linnean shortfall (Brito, 2010). Only recently have accurate estimates of animal species numbers become available (Appeltans *et al*., 2012; Collen *et al*., 2012; Scheffers *et al*., 2012). Appeltans *et al*. (2012), focusing on marine eukaryotes, estimated that between one-third and two-thirds of marine species could be undescribed. Second, counting the actual total number of described species is also difficult as there is not yet a totally functional repository of species names, and in many cases long lists of synonyms (*c*. 170,000 synonyms were reported for marine eukaryotes by Appeltans *et al*., 2012) exist, often untested.

The genus *Siphonaria* serves as an example for many of the problems listed above about the difficulties of determining the exact number of mollusc species (White & Dayrat, 2012; Dayrat, Goulding & White, 2014). *Siphonaria* taxonomy has been based almost exclusively on the adult shell and numerous synonyms exist for several of its species, for example up to 13 synonyms for *S. pectinata* (e.g. WoRMS: http://www.marinespecies.org/aphia.php?p=taxdetails&id=141470, accessed 23 June 2014), although a recent study has sorted out several Indo-West Pacific species groups (Dayrat *et al*., 2014). Molecular genetic approaches have discovered both cases of high phenotypic plasticity, resulting in synonymization of nominal species (Teske, Barker & McQuaid, 2007) and cases of potential cryptic or sibling species (Kawauchi & Giribet, 2011; Dayrat *et al*., 2014), although in a few cases reproductive characters (Hubendick, 1946), or shell morphology coupled with geography (Dayrat *et al*., 2014), are sufficient for distinguishing some species. Little comparative work exists for the radula of *Siphonaria* species (Hubendick, 1946), although in the siphonariid genus *Williamia* the radula does not differ among species (Ruthensteiner, 2006). In addition, *S. pectinata* has been recently shown to have expanded its distributional range considerably into the Mediterranean Sea (Antit, Gofas & Azzouna, 2009). The eastern Atlantic *S. pectinata* is currently thought to be distributed from Cape São Vicente in Portugal to Angola, including the Canary Islands, and into the western Mediterranean Sea in Alborán, Murcia, Alicante and Algeria, with recent introductions into the Gulfs of Tunisia and Saronikos, Greece (Gofas, Moreno & Salas, 2011).

In a previous report on Atlantic *Siphonaria* we studied two mitochondrial markers from individuals in two populations from Cádiz (Atlantic coast of Spain), one from Kribi (Cameroon) and one from the Florida Keys (USA) (Kawauchi & Giribet, 2011). That study showed that the populations on either side of the Atlantic were sufficiently distinct to constitute possibly separate species, as the genetic variation across the Atlantic was much higher than that across a similar linear distance between the populations in Spain and Cameroon. This was corroborated by Espinosa, Morey-Rubio & Nakano (2015), who suggested that the complex includes two species. However, the first study lacked geographic sampling in intermediate areas between the three examined regions, therefore not allowing for testing the effects of isolation-by-distance, and the second was based on a larger sampling but restricted to a single mitochondrial marker. Here we expand our previous sampling into the recently established

Mediterranean populations of Cabo de Palos (Murcia, Spain), Islas Chafarinas and to Gabon and the Macaronesian archipelagos of Canary and Cape Verde Islands, which could potentially include intermediate haplotypes between the eastern and western Atlantic populations. The addition of samples from intermediate localities was crucial to test our previous conclusion of lack of connectivity between the populations on the two continents. We also included additional samples from the Gulf of Mexico and expanded the set of molecular markers to include the ribosomal internal transcribed spacer-2 (ITS2) for a selected number of individuals from each population.

MATERIAL AND METHODS

Repository institutions

All material examined for molecular and anatomical work is located in the Malacology collection of the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA, USA, and is accessible through the MCZ's dedicated data base MCZbase (http://mczbase.mcz.harvard.edu/SpecimenSearch.cfm). Malacology collection numbers are provided with the MCZ prefix, but many of these specimens can also be queried using the older 'DNA codes' by clicking the 'include other identifiers' option in MCZbase (e.g. *Siphonaria pectinata* MCZ 378735 is equivalent to DNA100660). Individuals from the same lot are indicated with a numerical suffix after the accession number (e.g. DNA100660.1). In addition, we consulted the types of most synonyms of *S. pectinata* in the malacology collections of the Natural History Museum, London and the Muséum national d'Histoire naturelle, Paris.

Specimens

A total of 125 fresh specimens of putative *S. pectinata* were obtained and 66 of these used for molecular study, including populations in the Mediterranean Sea (Cabo de Palos and Islas Chafarinas, Spain), eastern Atlantic (Spain, Canary Islands, Cape Verde Islands, Cameroon and Gabon) and western Atlantic (Atlantic and Gulf of Mexico sides of Florida and Los Tuxlas, Mexico), covering most of the natural range of the species (Table 1).

Molecular markers

We studied two mitochondrial molecular markers, cytochrome *c* oxidase subunit I (COI) and 16S rRNA, which were amplified and sequenced as described by Kawauchi & Giribet (2011). In addition, we amplified and sequenced ITS2 using the primer pair OITS2F (5′-GTA CAA CTC TTA GCG GTG GAT CAC T-3′)-OITS2R (5′-CAG CGG GTT GTC TCG TCT GAT CTG AG-3′) (A.N. Nicolas, personal communication), amplifying between 453 and 472 bp. Polymerase chain reactions (PCR) (25 μl) consisted of 1–2 μl of template DNA, 0.25 μl of each primer (100 μM), 2.5 μl of AmpliTaq 10X PCR buffer containing 0.025 M $MgCl_2$ (Applied Biosystems), 0.5 μl of dNTP's (10 μM) and 0.13 μl of Amplitaq DNA polymerase (Applied Biosystems). The PCRs were carried out using an Eppendorf Mastercycler epgradient thermal cycler, and involved an initial denaturation step (5 min at 94 °C) followed by 35 cycles including denaturation at 94 °C for 30 s, annealing ranging from 47 to 51 °C for 30 s and extension at 72 °C for 50 s, with a final extension step at 72 °C for 7 min. The double-stranded PCR product visualization, clean up, sequencing reactions, visualization and editing of the sequences were performed as described by Kawauchi & Giribet (2011). All sequences generated in this study have been deposited in GenBank under accession numbers KJ407324–KJ407394 (Supplementary Material, Table S1).

Analyses

Phylogenetic analysis of the haplotypic data was accomplished using maximum-likelihood (ML) implemented in RAxML v. 7.0.4 (Stamatakis, 2006), on eight CPUs of a cluster at Harvard University, FAS Research Computing (odyssey.fas.harvard.edu), using *S. concinna* G.B. Sowerby I, 1824, *S. serrata* (Fischer von Waldheim, 1807) and *S. alternata* (Say, 1826) as outgroups. As COI and 16S rRNA are part of the same locus, the analyses were conducted examining both genes independently and concatenated. The 16S rRNA and ITS-2 datasets were aligned using MUSCLE v. 3.6 (Edgar, 2004). The COI and 16S rRNA genes were concatenated using Phyutility (Smith & Dunn, 2008); the ITS2 dataset was analysed separately. For ML searches, a unique GTR model of sequence evolution with corrections for a discrete gamma distribution (GTR + Γ) was specified for each data partition and 100 independent searches were conducted. Nodal support was estimated via 1,000 replicates of rapid bootstrapping (Stamatakis, Hoover & Rougemont, 2008).

Population genetic and demography analyses were conducted in Arlequin v. 3.5 (Excoffier & Lischer, 2010), which was used to calculate diversity indices within population as number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), number of polymorphic sites (N_p) and average number of

Table 1. Collecting data, code and number of individuals of *Siphonaria* collected per site.

MCZ accession no.	DNA#	Collector	Site	Country	Code	*n*
378735	DNA100660	G. Giribet	El Puerto de Santa María, Cádiz	Spain	SP1	2
378736	DNA105664	J.L. Cervera	La Caleta, Cádiz	Spain	SP1	10
378743	DNA106743	J. Templado	Cabo de Palos, Murcia	Spain	SP3	1
378744	DNA106744	J. Templado	Isla Chafarinas	Spain	SP3	4
378740	DNA106488	P. Wirtz	Maspalomas, Gran Canaria, Canary Islands	Spain	SP2	7
378734	DNA104633	L. Benavides, G. Giribet	Kribi, Littoral Province	Cameroon	CA	5
378742	DNA106715	E. Rolán	Cap Santa Clara, Libreville	Gabon	GA	8
378745	DNA106745	J. Templado	Cap Santa Clara, Libreville	Gabon	GA	4
378741	DNA106620	O. Wangensteen	Rife do Pardos, Island of Sal	Cape Verde	CV	8
378733	DNA104886	R. Bieler, S. Clark, G. Giribet, P. Mikkelsen, S. Staubach	Justine beach, Fort Pierce, Hutchinson Island, FL	US	US1	6
378739	DNA106477	J.H. Leal	Sanibel Island, Lee Co., FL	US	US2	9
–	–	I. Vea	Barra de Sóntecomapan, Los Tuxtlas, Veracruz	Mexico	ME	2
					Total	66

pairwise differences (k). The average number of pairwise differences between populations and population pairwise F_{ST}, whose significance was assessed with 1,000 permutations, was also calculated using Arlequin v. 3.5, considering four subdivisions (western Atlantic, Europe, Gulf of Guinea and Cape Verde). The analysis of molecular variance (AMOVA) analyses were calculated after pooling the populations in four larger geographical subdivisions: group 1 (western Atlantic: Mexico, US1, US2), group 2 (Europe: SP1, SP2, SP3), group 3 (Gulf of Guinea: Cameroon, Gabon) and group 4 (Cape Verde: CV) (see Table 1 for abbreviations), and was performed using pairwise differences as measure of divergence, with 16,000 permutations. The haplotype networks, excluding outgroups, were inferred using statistical parsimony implemented in the program TCS v. 1.21 (Clement, Posada & Crandall, 2000), under default parameters and assumptions, at 95% confidence interval, with indels treated as a fifth state. To resolve the loops in the statistical parsimony network we followed Pfenninger & Posada (2002).

RESULTS

From the 66 sampled specimens, the length for each analysed gene after trimming was 658 bp (for COI) and 445–437 bp (for 16S rRNA). The 16S rRNA dataset after alignment had a final length of 450 bp. The combined dataset resulted in 1,098 nucleotides positions, 128 of which (11.6%) were variable. A number was used to designate each haplotype sequence for COI and a letter for 16S rRNA. An alphanumeric code thus represents the combination of both datasets in the concatenated analysis, and a colour code was designated for each population (Fig. 1).

Phylogenetic analyses

Phylogenetic analysis for both mitochondrial markers combined (Fig. 1) supports the monophyly of the putative *S. pectinata* (100% bootstrap proportion, BP), which divides into two basal clades, one comprising all the samples from Cape Verde and another including samples from both sides of the Atlantic (although without significant support, 61% BP), which divides into an eastern Atlantic clade (77% BP)—including samples from Gabon to Spain and the Canary Islands—and a western Atlantic clade (90% BP) from Florida and the Gulf of Mexico (Fig. 1). Relationships among these three groups are unresolved for the individual gene analyses (Supplementary Material, Figs S1, S2). The COI tree has in general a similar topology to that from the concatenated tree, only differing in the monophyly of the Gulf of Guinea samples (Supplementary Material, Fig. S1).

Intragenomic variation has been well documented in the ITS-2 of many invertebrates (e.g. Duran, Giribet & Turon, 2004; Vollmer & Palumbi, 2004). Part of the *Siphonaria* ITS-2 sequences exhibited double peaks and truncated sequences, indicating the existence of heterozygosity. Only sequences with a single copy were used here to generate a phylogeny, and thus the number of samples is smaller than that of the other markers. We recovered a topology (Supplementary Material, Fig. S3) compatible with that of the combined mitochondrial data (Fig. 1), although monophyly of the eastern and of the western Atlantic clades was unsupported; this tree could have alternative rooting positions, since no outgroup was used.

Phylogeography and demography of 'Siphonaria pectinata'

Forty-one COI haplotypes were recovered (Supplementary Material, Fig. S1), the most abundant, haplotype *4*, being represented by 11 individuals from southern Spain (Atlantic and Mediterranean) and the Canary Islands. Two other haplotypes, *17* (4 individuals) and *3* (3 individuals) are also found from the Canary Islands to southern Spain, but none is shared with Gulf of Guinea individuals, which are connected by 7 mutational steps with the European group of haplotypes. In the Gulf of Guinea, two haplotypes, *6* and *34*, are shared by three individuals each, while all other individuals have private haplotypes connected by from 1 to 4 mutational steps to haplotypes *6* or *34*. The distribution of haplotypes is slightly different in the western Atlantic, with four private haplotypes, four haplotypes shared by two individuals and one shared by three individuals; three of these shared hapolytpes are found on both sides of the Florida Peninsula (in the Atlantic and in the Gulf of Mexico). The two specimens from Mexico have private haplotypes. The structure of the Cape Verde specimens is radically different, all haplotypes being private and being connected by between 2 to 11 mutational steps for any pair of adjacent haplotypes.

There was a lack of connectivity (at 95% confidence) of the haplotypic networks of the three clades, Cape Verde, eastern Atlantic and western Atlantic.

Twenty-eight different haplotypes were recovered from the 16S rRNA dataset (Supplementary Material, Fig. S2). Haplotype *z*, from the western Atlantic clade, is the most abundant with 13 individuals from both sides of the Florida peninsula. Haplotype *c* from southern Spain (Atlantic and Mediterranean) and the Canary Islands is shared among 11 individuals. Haplotype *d* is represented in six individuals from the same region; two other haplotypes (*m* and *n*) are found in six individuals from across the Gulf of Guinea and one haplotype (*u*) is found in three individuals from Cape Verde. The remaining haplotypes are represented by one or two individuals each.

The 16S rRNA dataset recovers two independent networks, one for the eastern Atlantic (including Cape Verde) and one for the western Atlantic (at 95% confidence) (Supplementary Material, Fig. S2). A Mediterranean haplotype connects to the Gulf of Guinea group of haplotypes and a Gabon haplotype connects to the Cape Verde group of haplotypes—but no haplotype is shared among individuals in these populations.

The combined dataset (Fig. 1) is largely congruent with the COI dataset, showing three disconnected networks, one for Cape Verde, one for the eastern Atlantic and Mediterranean (subdivided into three main groups of haplotypes, one for the Gulf of Guinea; clearly showing an isolation-by-distance [IBD] effect) and a third one for the western Atlantic populations. Haplogroup *4c* is the most abundant, found in eight individuals, including six from the Canary Islands.

Molecular diversity indices for each marker and for the combined dataset for each population are given in Table 2. Except for haplotypic diversity (h) observed for population US1 (Fort Pierce) in the 16S rRNA dataset, all others show moderate to high haplotypic diversity, ranging from 0.41 (US2 for 16S rRNA) to 1, suggesting that neither of these populations has been recently established. The nucleotide diversity (π_n) in general is low, varying from 0.001 (US2 for 16S rRNA) to 0.015 (CV for COI) in the individual partitions. Pairwise F_{ST} values are generally high and significant for the majority of the population comparisons (Table 3). The AMOVA analysis revealed 85% of variation among groups, whereas only 15.43% occurred within populations (Table 4).

DISCUSSION

Siphonaria taxonomy

A recent check-list lists the available names in *Siphonaria* and their type localities (White & Dayrat, 2012). *Siphonaria pectinata* ranges from the Mediterranean Sea (type locality 'M. Mediterraneo'; Linnaeus, 1758) to Gabon, and includes populations in Morocco (unpublished data), the Canary Islands and Cameroon, among the sampled localities (Fig. 1). The species has a continuous distribution along most of the temperate to tropical Eastern Atlantic

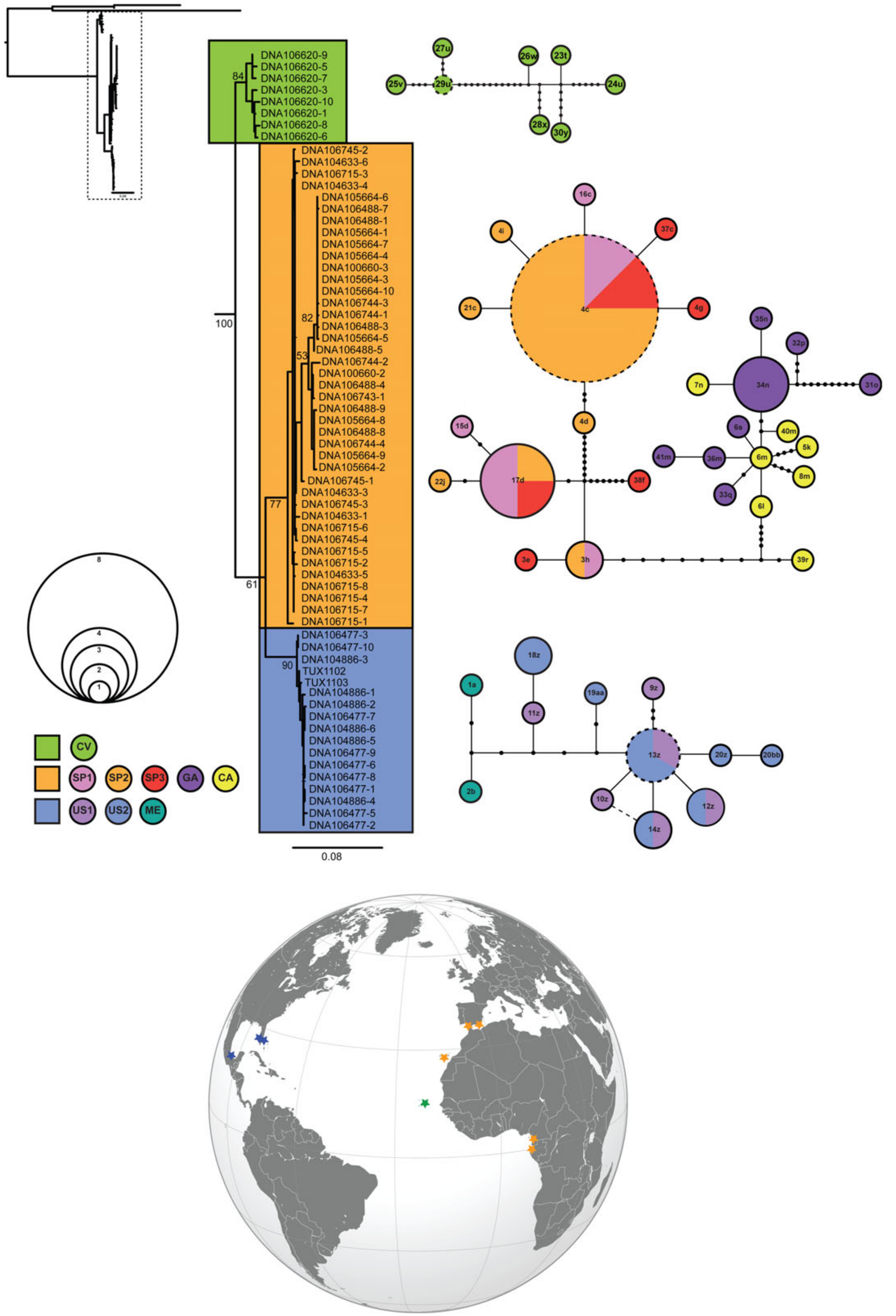

Figure 1. Distribution of the *Siphonaria* samples examined in this study and results from the phylogenetic (ln$L = -4743.853201$) and haplotype diversity analyses. Orange indicates *S. pectinata*, green *S. placentula* and blue *S. naufragum*. Specimen codes follow those of Table 1.

Table 2. Demographic parameters and standard deviations for each *Siphonaria* lineage for COI and 16S genes analysed independently and for the concatenated dataset.

Gene	Site	N	N_h	h	N_p	π_n	k
COI	ME	2	2	1.0000 ± 0.5000	2	0.003040 ± 0.003723	2.000000 ± 1.732051
	US1	6	6	1.0000 ± 0.0962	8	0.004762 ± 0.003307	3.133333 ± 1.884439
	US2	9	6	0.9167 ± 0.0725	9	0.004728 ± 0.003068	3.111111 ± 1.779276
	SP1	12	5	0.6667 ± 0.1409	12	0.007530 ± 0.004437	4.954545 ± 2.592875
	SP2	7	5	0.8571 ± 0.1371	12	0.009263 ± 0.005748	6.095238 ± 3.301981
	SP3	5	5	1.0000 ± 0.1265	12	0.009726 ± 0.006487	6.400000 ± 3.651127
	CA	5	4	0.9000 ± 0.1610	9	0.005471 ± 0.003890	3.600000 ± 2.189554
	GA	12	10	0.9545 ± 0.0569	10	0.003500 ± 0.002318	2.303030 ± 1.354858
	CV	8	8	1.0000 ± 0.0625	24	0.015849 ± 0.009224	10.428571 ± 5.328051
16S	ME	2	2	1.0000 ± 0.5000	1	0.002288 ± 0.003236	1.000000 ± 1.000000
	US1	6	1	0.0000 ± 0.0000	0	0.000000 ± 0.000000	0.000000 ± 0.000000
	US2	9	3	0.4167 ± 0.1907	2	0.001015 ± 0.001123	0.444444 ± 0.433519
	SP1	12	3	0.5303 ± 0.1359	4	0.003718 ± 0.002657	1.621212 ± 1.028975
	SP2	7	5	0.9048 ± 0.1033	6	0.005898 ± 0.004107	2.571429 ± 1.563343
	SP3	5	5	1.0000 ± 0.1265	11	0.011468 ± 0.007834	5.000000 ± 2.921744
	CA	5	4	0.9000 ± 0.1610	4	0.003670 ± 0.003024	1.600000 ± 1.127916
	GA	12	7	0.8636 ± 0.0786	18	0.008841 ± 0.005375	3.863636 ± 2.086316
	CV	8	6	0.8929 ± 0.1113	6	0.004178 ± 0.003050	1.821429 ± 1.167258
Concatenated	ME	2	2	1.0000 ± 0.5000	2	0.001828 ± 0.002239	2.000000 ± 1.732051
	US1	6	6	1.0000 ± 0.0962	8	0.002861 ± 0.001987	3.133333 ± 1.884439
	US2	9	6	0.9167 ± 0.0725	10	0.003044 ± 0.001954	3.333333 ± 1.886042
	SP1	12	5	0.6667 ± 0.1409	16	0.006011 ± 0.003440	6.575758 ± 3.342745
	SP2	7	7	1.0000 ± 0.0764	18	0.007922 ± 0.004776	8.666667 ± 4.561213
	SP3	5	5	1.0000 ± 0.1265	23	0.010420 ± 0.006677	11.400000 ± 6.248044
	CA	5	5	1.0000 ± 0.1265	13	0.004753 ± 0.003234	5.200000 ± 3.026066
	GA	12	10	0.9545 ± 0.0569	27	0.005484 ± 0.003166	6.000000 ± 3.076709
	CV	8	8	1.0000 ± 0.0625	33	0.011197 ± 0.006458	12.250000 ± 6.201925

Abbreviations: N, number of sampled individuals; N_h, number of haplotypes; h, haplotypic diversity; N_p, number of polymorphic sites; π_n, nucleotide diversity; k, mean number of pairwise differences.

Table 3. F_{ST} values for the concatenated dataset.

	ME	US1	US2	SP1	SP2	SP3	CA	GA	CV
ME	0.00000								
US1	0.36779	0.00000							
US2	**0.34958**	−0.05981	0.00000						
SP1	**0.87282**	**0.88787**	**0.89303**	0.00000					
SP2	**0.84309**	**0.87449**	**0.88419**	−0.06713	0.00000				
SP3	**0.80856**	**0.86087**	**0.87534**	0.00577	−0.10751	0.00000			
CA	**0.90457**	**0.91202**	**0.91372**	**0.64046**	**0.58251**	**0.49513**	0.00000		
GA	**0.88218**	**0.88882**	**0.89308**	**0.64352**	**0.60339**	**0.53911**	0.01423	0.00000	
CV	**0.83426**	**0.87025**	**0.88291**	**0.85621**	**0.82962**	**0.80735**	**0.84275**	**0.85921**	0.00000

For site codes see Table 1. Significant values indicated in bold ($P < 0.05$).

Table 4. Hierarchical analysis of molecular variance used to estimate the level of genetic differentiation among and within populations of *Siphonaria*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation (%)
Among groups	3	896.215	18.68496 Va	84.99
Among populations/within groups	5	14.028	−0.09221 Vb	−0.42
Within populations	57	193.408	3.39313 Vc	15.43
Fixation index F_{ST}	0.84567			

and Mediterranean (Espinosa *et al.*, 2015), although some of the Mediterranean range seems to be the result recent man-mediated expansion, as recent invasion as far east as Greece and Tunisia suggests). The species was however long known from Algeria (Monterosato, 1877, as *S. algesirae*; Pallary, 1900, as *S. mouret*; Morrison, 1972), the southern coast of Spain (Hidalgo, 1917) and southern France (Germain, 1913). The genetic structure in specimens from the southern Mediterranean and Chafarinas, although with some majority COI and 16S rRNA haplotypes, does not seem to indicate a recent invasion here either. In addition, the Algerian populations have been the focus of early studies in egg development (Dieuzeide, 1935) and phenotypic plasticity (Cooke, 1911), showing that they have been established there for over a century.

Our Cape Verde population appears distinct in all analyses, and we assign this population to *S. placentula* Menke, 1853. Both this and *S. umbonata* were described by Menke (1853) in his 'Conchylien von St. Vincent mit kritischen Anmerkungen' [Shells from St Vincent with critical comments]. No locality was provided for *S. placentula* while the locality of *S. umbonata* was said to be unknown. White & Dayrat (2012: 67, 69) assigned these species to the Caribbean region, stating "no type locality indicated, but in an article describing shells from St. Vincent, Lesser Antilles", while in fact Menke's 'St Vincent' referred to São Vicente in the Cape Verde archipelago (see for example Groh, 2012). The malacology collection of the MCZ has numerous lots of *Siphonaria* shells from Porto Grande, São Vicente. Given the uncertainty in Menke's type localities, as 'first revisers' we assign the Cape Verdean population to *S. placentula*. Based on its type locality of La Praja (Cap–Vert) [Praia, island of Santiago, Cape Verde] we suggest that *S. milneedwardsi* Locard, 1898 may be a junior synonym.

Only two supposed synomyms of *S. pectinata* have type localities in the western Atlantic. *Siphonaria naufragum* Stearns, 1872 was described from Amelia Island, Florida, on wood from an old wreck (Stearns, 1872). *Siphonaria lineolata* d'Orbigny, 1841 (types in NHMUK) was based on material from Havana, Cuba, and is an available but permanently invalid name preoccupied by *S. lineolata* Sowerby I, 1835 from I. Chiloé (Chile) (White & Dayrat, 2012). Therefore *S. naufragum* appears to be the only available name for the western Atlantic species studied here.

As suggested in earlier work (Kawauchi & Giribet, 2011; Espinosa *et al.*, 2015), and supported by developmental data (Voss, 1959), the western Atlantic populations belong to a different species from the Afro-European *S. pectinata*. It has also been shown that few *Siphonaria* species have a very widespread distribution in the Indo-West Pacific (Dayrat *et al.*, 2014). The range of *S. naufragum* seems to be restricted to the Gulf region of Mexico and Texas to eastern Florida (Voss, 1959; McAlister & Fisher, 1968; Abbott, 1974). Both our COI and 16S rRNA data clearly separate this species from the Eastern Atlantic ones. It is also easily distinguished genetically and morphologically from the sympatric *S. alternata*, described from the southern coast of East Florida, where it has been well studied (Cook, 1971). *Siphonaria alternata* is also found in Bermuda (Sterrer, 1986), from where we obtained our sequence data. Voss (1959) commented on the distribution of these two species along the Florida coast, and considered *S. naufragum* (as *S. pectinata*) the common species on the east coast from Fernandina south to the upper Florida Keys, while in the lower keys S. *alternata* was the common limpet. We corroborate this observation with the specimens we have collected in recent years.

Systematics and biogeography

Our results based on phylogenetic reconstruction, statistical parsimony networks, populations parameters, analysis of gene flow and connectivity identified three groups that are reciprocally monophyletic and that are either disconnected (COI) or connected by a large number of mutational steps (16S rRNA) in the haplotype networks. The Cape Verde group is supported by the analysis of the ITS2 data (which does not contradict the other two groups). One of these groups spans the western Mediterranean, Atlantic Spain and African Atlantic coast until at least the Bight of Biafra, and reaches the oceanic Canary Islands, covering a distance over 6,000 km of more or less contiguous coast. Curiously, the linear distance from Dakar (Senegal) to Cape Verde is barely 650 km, which seems enough to prevent coexistence of haplotypes and results in radically different genetic structure between the two *Siphonaria* species found on each side of this relatively short distance, despite the existence of the Canary and North Equatorial currents in favourable directions.

A recent study of the trochid genus *Phorcus* revealed a distinct but recently originated species in Cape Verde (Donald *et al.*, 2012), subsequently described as *P. mariae* Templado & Rolán, 2012. The Cape Verde archipelago is also well known for an endemic radiation of shallow-water *Conus* species (Cunha *et al.*, 2005, 2008; Duda & Rolán, 2005). It also has an estimated endemicity of about 12% for molluscs (Cosel, 1982) and 10% for coastal reef fishes (Freitas, 2014), but in general, its marine fauna is not well studied in a broader biogeographical context. *Siphonaria* adds one more step towards understanding the level of biological isolation of this oceanic archipelago.

The linear distance between our two nearest localities on each side of the Atlantic and roughly the same latitude, Maspalomas (Canary Islands) and Fort Pierce (Florida), is 6,300 km, a distance comparable to the entire range of the eastern Atlantic species; however, other than some possible stepping stones, such as Madeira, Azores and Bermuda—none of which have *S. pectinata*—there is a large gap of suitable habitat. It is therefore not possible to test for an IBD effect between these populations. Considering the genetic discontinuity despite geographic proximity of the Cape Verde populations and the genetic discontinuity of the Eastern Atlantic and Western Atlantic populations, we conclude that the complex of *S. pectinata* corresponds to three cryptic species, which we treat as valid species.

SUPPLEMENTARY MATERIAL

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

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