

Historical biogeography and mitogenomics of two endemic Mediterranean gorgonians (Holaxonia, Plexauridae)

Angelo Poliseno¹ · Alvaro Altuna² · Carlo Cerrano³ · Gert Wörheide^{1,4,5} · Sergio Vargas¹

Received: 24 June 2016 / Accepted: 6 January 2017 / Published online: 18 January 2017
© Gesellschaft für Biologische Systematik 2017

Abstract Among the Mediterranean plexaurids, four species are endemic and despite their ecological importance, comprehensive studies on the evolution and biogeography of these organisms are lacking. Here, we explore the mitogenomic variability of two endemic, ecologically important Mediterranean *Paramuricea* species. We assess their phylogenetic relationships and provide first insights into their evolution and biogeography. Complete mitogenome sequences of *Paramuricea clavata* and *Paramuricea macrospina* were obtained using long-range PCR, primer-walking and Sanger sequencing. For an enlarged sample of *Paramuricea* species, maximum likelihood and Bayesian phylogenetic trees of the mitochondrial gene *mtMutS* were obtained and used to study the biogeographic history of *Paramuricea* through a statistical Dispersal-Vicariance (S-DIVA) method and a Dispersal Extinction Cladogenesis (DEC) model. Divergence time was estimated under strict and relaxed molecular clock models in

BEAST using published octocoral mutation rates. Our results revealed high nucleotide diversity (2.6%) among the two Mediterranean endemics; the highest mutation rates were found in the *mtMutS*, *Nad4* and *Nad5*. In addition, we found length polymorphisms in several intergenic regions and differences in mitochondrial genome size. The red gorgonian *P. clavata* was closely related to the Eastern Atlantic *Paramuricea grayi* rather than its Mediterranean congener, *P. macrospina*. Our biogeographic results provide evidence for the independent speciation of the Mediterranean species and point to a Miocene origin of the two endemics, highlighting the role played by the Messinian Salinity Crisis in the evolutionary history of Mediterranean organisms.

Keywords Endemic species · Mediterranean · Mitogenomics · *Paramuricea* · Phylogeography · Vicariance · Octocorals

Electronic supplementary material The online version of this article (doi:10.1007/s13127-017-0322-x) contains supplementary material, which is available to authorized users.

✉ Sergio Vargas
sergio.vargas@lmu.de

¹ Department of Earth and Environmental Sciences, Palaeontology and Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner-Straße 10, 80333 Munich, Germany

² INSUB, Zemoria 12, 20013 San Sebastián, Spain

³ Department of Life and Environmental Sciences (DiSVA), Marche Polytechnic University, Via Breccia Bianche, 60131 Ancona, Italy

⁴ GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner-Straße 10, 80333 Munich, Germany

⁵ Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 Munich, Germany

Introduction

The Mediterranean Sea, the largest semi-enclosed sea on Earth, is characterized by unique oceanographic conditions (e.g. limited water exchange, distinctive subsurface-water circulation and deep overturning circulation; see Pinardi et al. 2006; Tanhua et al. 2013) and strong environmental gradients (Danovaro et al. 1999). This wide range of climatic and hydrological conditions within the Mediterranean Sea have been used to define up to 10 biogeographical regions based on the combination of different geological, physical and biological parameters (Bianchi and Morri 2000) and results in the coexistence of temperate and sub-tropical organisms in this basin (Coll et al. 2010).

The Mediterranean is considered a hot spot of marine biodiversity with a high percentage of endemic species (Bianchi

and Morri 2000; Boudouresque 2004). The Mediterranean marine biota is closely related to that of the Atlantic Ocean (Sarà 1985; Bianchi and Morri 2000), and its evolutionary history has been shaped by episodes of isolation and reconnection with the Atlantic. In this regard, evidence for a radiation after the Messinian Salinity Crisis (5.2 Ma)—the isolation and restriction of the Mediterranean due to the closure of marine gateways between the Atlantic ocean and the Mediterranean Sea—has been reported for invertebrates (Sanna et al. 2013) and vertebrates (e.g. Carreras-Carbonell et al. 2005). A second event, the Gelasian Crisis occurred 2.6 Ma (Dov Por 2009) likely affecting the Mediterranean biota and changing its general composition and diversity patterns. Although a wealth of oceanographic and ecological data exist for the Mediterranean Sea (e.g. Sarà 1985; Pinardi et al. 2006; Bas 2009), little is known about the evolutionary history of different Mediterranean taxonomic groups. Among octocorals in particular, the few studies so far published have been mostly focused on the population genetics of economically and/or ecologically relevant species in the region such as the precious coral *Corallium rubrum* (Costantini et al. 2013; Pratlong et al. 2015) and the red gorgonian, *Paramuricea clavata* (see Agell et al. 2009; Mokhtar-Jamaï et al. 2011).

The genus *Paramuricea* Koelliker 1865 (Holaxonia, Plexauridae) has been reported from different latitudes including the Gulf of Mexico (Doughty et al. 2014), North-Western Atlantic (Thoma et al. 2009), Mediterranean (Bo et al. 2012; Angeletti et al. 2014) and South Eastern Australia (Alderslade et al. 2014). The genus currently includes more than 20 species, two of which, *P. clavata* and *P. macrospina*, are endemic to the Mediterranean (Grasshoff 1992; Vafidis 2010). *P. clavata* is one of the most common gorgonians dwelling the coralligenous assemblages of the western Mediterranean. *Paramuricea macrospina* has a more restricted distribution occurring mainly in detritic or sandy deep-sea bottoms up to 200 m deep (Carpine and Grasshoff 1975) and is also common in the oligotrophic Sea of Marmara (Topçu and Öztürk 2015). Despite their ecological importance and their widespread distribution across the Mediterranean basin, the phylogenetic affinities of the Mediterranean *Paramuricea* and their historical biogeography remain largely unstudied. In order to shed light on the molecular (phylo) diversity of the Mediterranean *Paramuricea* and on the evolutionary processes leading to the diversification of these species, here, we (I) sequence the complete mitochondrial genome of the two Mediterranean endemic species, (II) assess their phylogenetic relationships with other members of this widespread genus and (III) infer the biogeographic history of *Paramuricea* to clarify the processes involved in the diversification of the Mediterranean species and shed light on the possible historical events that shaped the evolutionary history of this genus in the Mediterranean.

Materials and methods

Study area, sampling and taxonomic identification

P. macrospina (LT576168, LT576169) and *P. clavata* (LT576167) were collected in the Mediterranean, whereas *Paramuricea grayi* was sampled in Galicia (LT576170) and the Bay of Biscay (LT576171) (North-Eastern Atlantic). All the samples were collected by SCUBA diving at depths ranging from 10 to 90 m and were preserved in absolute ethanol for molecular analyses. Species assignments done in the field were further corroborated by the morphological analysis of the colony and sclerome.

Molecular analyses

Genomic DNA was extracted following three different protocols. The Macherey-Nagel NucleoSpin® Tissue kit (M&N, Düren, Germany), a modified CTAB phenol-chloroform (Doyle and Doyle 1987) and a salting out extraction method (Aljanabi and Martinez 1997) were used to obtain high molecular weight DNA. The quality of the DNA was assessed on a 1.5% agarose gel. Mitochondrial DNA fragments of different sizes (500–4000 bp) were amplified using published octocoral specific primers (i.e. France and Hoover 2002; Sánchez et al. 2003; McFadden and Hutchinson 2004) as well as additional primers designed to bind regions conserved in the octocoral mitochondrial genomes available to date (see Supplementary Table 1). For *P. grayi* (LT576170–LT576170) and *P. macrospina* (LT576169), the 5' end of *mtMutS* was amplified using the primers ND42599F (France and Hoover 2002) and Mut-3458R (Sánchez et al. 2003) following previously published protocols. PCR products were purified using a polyethylene glycol (PEG)-NaCl precipitation and were sequenced with different internal primers (see Supplementary Table 1) using the BigDye® Terminator v3.1 chemistry in an ABI PRISM® 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The mitochondrial genomes were assembled, edited and annotated using Geneious 6.0.5 (Kearse et al. 2012). This program was also used to estimate GC-content and to annotate and create a graphical map of the genomes. Nucleotide diversity (π) between genomes was calculated in DnaSP 5.10.1 using a sliding window of 500 bp (Librado and Rozas 2009). The sequences obtained in this study were deposited in the European Nucleotide Archive (LT576167–LT576171).

Extended phylogenetic analyses

A fragment of about 750 bp of the octocoral mitochondrial gene *mtMutS* was extracted from the mitochondrial genome of the two Mediterranean species and was aligned with the sequences of *P. grayi* here generated and with sequences of other

plexaurids deposited in public sequence repositories (GenBank). A dataset consisting of 166 taxa of which 150 belong to the genus *Paramuricea* was used to assess the phylogeny of the genus *Paramuricea*. This dataset contained many identical genotypes. Thus a second, reduced dataset (see Supplementary Table 2 for the list of species used) was generated including only 26 *Paramuricea* representative species. This dataset was used for phylogenetic inference and biogeographic analyses (i.e. S-DIVA, DEC; see below). All sequences were aligned in MUSCLE (Edgar 2004) with the default options available through Seaview 4.5.3 (Gouy et al. 2010). Alignments used in this study are available at OpenDataLMU (DOI: <http://dx.doi.org/10.5282/ubm/data.89>).

A maximum likelihood tree was inferred in RAxML 7.2.8 (Stamatakis 2006) under the GTR + Γ model (General Time Reversible + Gamma). Branch support was assessed through a rapid bootstrap analysis (Stamatakis et al. 2008) with a random seed number and 1000 pseudo-replicates. For the Bayesian analyses we used BEAST 2.4.4 (Bouckaert et al. 2014) under the best-fit substitution model selected using the AIC (i.e. GTR + Γ + I) in the program jModeltest 2.1.3 (Darriba et al. 2012). The Markov chain Monte Carlo was run for 10,000,000 generations sampling every 1000 generations. Convergence of the Markov chains was assessed in Tracer 1.6 (Rambaut and Drummond 2009). One tenth of the sampled trees (i.e. 1000 trees) were discarded as burn-in and the species *Eunicea fusca*, *Muricea elongata*, *Plexaura homomalla* and *Pseudoplexaura porosa* were used as outgroup in both the ML and Bayesian analyses.

Biogeographic analyses

The geographic distribution of the plexaurid species was based on collection localities gathered from the literature (Sánchez et al. 2003; Wirshing et al. 2005; McFadden et al. 2006, 2011; Thoma et al. 2009; Doughty et al. 2014; Vargas et al. 2014). Seven biogeographical areas were considered: (A) Eastern Pacific (Panama and Galapagos), (B) Caribbean, (C) North-West Atlantic, (D) North-Eastern Atlantic (Galicia, Bay of Biscay), (E) Mediterranean Sea, (F) Central Indo-Pacific (Indonesia, Papua New Guinea, Palau, Western Australia and Philippines) and (G) South-West Pacific (Supplementary Table 2). For simplicity, the Gulf of Mexico and the Caribbean were included in a single geographic area (B). Ancestral distributions were reconstructed using the statistical Dispersal-Vicariance (S-DIVA) method (Yu et al. 2014) and the Dispersal Extinction Cladogenesis (DEC) model (Ree and Smith 2008), both implemented in RASP 3.2. S-DIVA is an extension of classical DIVA (see Ronquist 1997, 2001) that reconstructs ancestral areas using sets of trees (e.g. those samples by the Markov Chain in Bayesian analyses) instead of a fixed topology (Yan et al. 2010). The DEC model

estimates the geographic range of a given species taking into account stochastic processes such as range expansion and contraction (Ree and Smith 2008). Hence, these methods treat tree topology as a nuisance parameter and allow to account for the effect of uncertainty in tree topology on ancestral area optimization. Both methodologies are of special interest in groups, such as octocorals (e.g. Wirshing and Baker 2015; see also Supplementary Fig. 1 and Supplementary Fig. 2), where phylogenetic signal is weak at shallow scales and topological uncertainty is high. In these groups, integrating over a set of possible topologies allows to extract the biogeographic signal that is present in the set of trees independently of whether the summary of the trees (e.g. the consensus) is highly supported or not.

Using either S-DIVA or DEC, the reconstruction of the biogeographic history of *Paramuricea* was performed using a set of 10,000 trees generated for the Bayesian analysis and sampled 1000 random trees from the posterior tree space. Ten percent of the total sampled trees were discarded as burn-in, and the maximum clade credibility (MCC) tree from the BEAST analysis was used as the summary tree to display ancestral area reconstruction. The maximum number of areas allowed per node was four.

Molecular dating

For many soft-bodied organisms such as octocorals, with little or no fossil remains, the estimation of divergence times among lineages rely on the use of geological events or mutation rates to calibrate the molecular clock and provide taxon-ages. We used the Bayesian MCMC analysis implemented in BEAST 2.4.4 (Bouckaert et al. 2014) under a GTR + Γ + I substitution model, and a strict or a relaxed molecular clock model to date the *mtMutS* tree here inferred (see above). Due to the lack of fossils or of information on the substitution rates of Mediterranean gorgonians, we used the only rates so far available for octocorals, calculated for the genus *Leptogorgia*, and inferred dated trees using diverse strategies to explore how different analytical settings affected molecular dating. We assumed a general mutation rate of *mtMutS* ranging between 0.14 and 0.25% Myr⁻¹ (see Lepard 2003 in Thoma et al. 2009 and Pante et al. 2015) and tested a conservative (assuming 0.14% mutation rate) and a speculative (assuming 0.25% mutation rate) molecular dating for *Paramuricea*. In addition, in order to prevent biases derived by the use of a single (fixed) molecular mutation rate, we modelled the clock rate using a log-normal prior with a mean 0.195% \pm 0.055 Myr⁻¹ and a uniform prior ranging between 0.14 and 0.25%. We tested strict and relaxed clock (uncorrelated log-normal) models, and used a Yule model as this is suitable for trees describing species-level processes. For each molecular clock analysis, the Markov chain was run for 10 million generations, sampling every 1000 generations. The effective sample size (ESS) of

each parameter was assessed using Tracer 1.6 (Rambaut and Drummond 2009). Ten percent of the trees were discarded as burn-in, and the remaining set of trees was visualized using DensiTree 2.0 (Bouckaert 2010) (Supplementary Fig. 1 and Supplementary Fig. 2). The MCC tree with mean divergence times and 95% highest posterior density (HPD) interval was summarized in TreeAnnotator. In order to check the influence of the priors on the posterior distribution estimates, each analysis was re-run using ‘empty’ alignments, allowing to sample from the prior (s) only. Each analysis was run for 100 million generations sampling every 1000 generation. A summary of the trace estimates and a comparison between the marginal

prior and posterior densities obtained using different clock models are reported in the Supplementary Fig. 3 and Supplementary Fig. 4.

Results

Mitochondrial genomes

The mitogenomes of *P. clavata* and *P. macrospina* were 18,669 base pairs (bp) and 18,921 bp, respectively (Fig. 1a). The architecture of the two genomes, in terms of genome

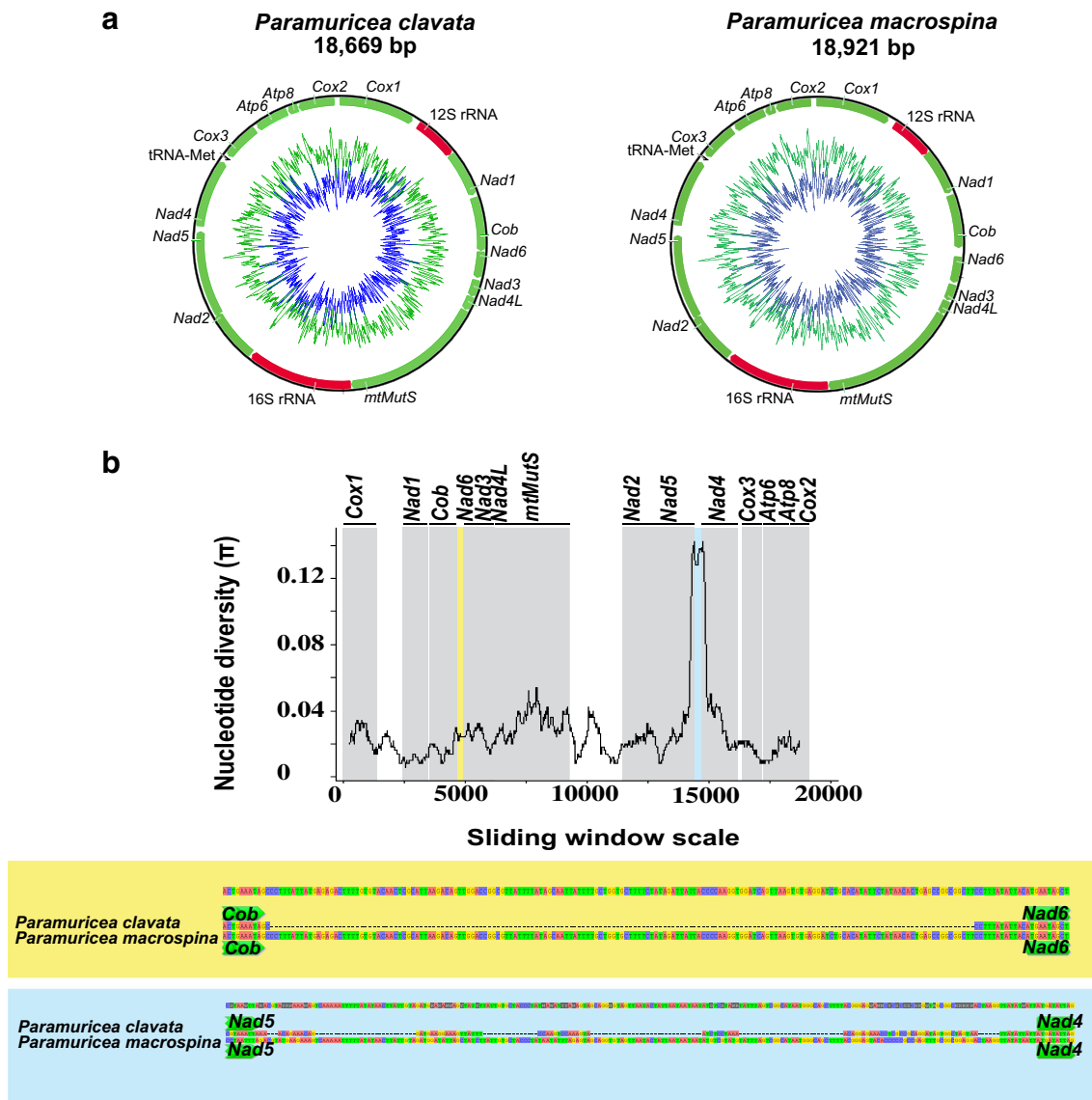


Fig. 1 Mitochondrial genome structure and genes variability. **a** Mitogenomes of *Paramuricea clavata* and *Paramuricea macrospina* with genome size and gene annotation. GC-content and AT-content are shown in blue and green on the inner and outer surface of the ring, respectively. **b** Sliding window analysis of the complete mitochondrial genomes of *P. clavata* and *P. macrospina*. The black line indicates

nucleotide diversity across the mitogenome in a window of 500 bp (10 bp steps). Coding protein genes are in grey; white boundaries represent rDNA genes, tRNA gene and 14 intergenic regions (IGRs). The IGRs between *Cob*-*Nad6* and *Nad5*-*Nad4* are highlighted in yellow and blue, respectively; alignments are showed in the two panels below

content and gene order, was consistent with that of other octocorals with genome arrangement A (see Brockman and McFadden 2012). Both genomes contained all 14 mitochondrial protein-coding genes reported in octocorals, the 12S rDNA (SSU) and 16S rDNA (LSU) and one tRNA gene (tRNA^{Met}). GC-content was 37% in *P. macrospina* and 37.3% in *P. clavata*. Except for *Nad2* and *Nad4*, which overlapped by 13 bp, the remaining genes were separated by intergenic regions (IGRs) of variable length. In both species, the shortest IGRs were 12S rDNA-*Nad1* and 16S rDNA-*Nad2*, which were 4 bp long. The longest IGR was located between *Cox1* and 12S rDNA (150 bp) in *P. clavata* and between *Nad5* and *Nad4* (208 bp) in *P. macrospina*. In total, non-coding regions spanned about 4% of the genome in *P. clavata* and 5.4% in *P. macrospina*. Among the intergenic regions, the highest variability in terms of sequence length was observed between *Cob* and *Nad6* (13 and 178 bp in

P. clavata and *P. macrospina*, respectively). Between *Nad5* and *Nad4*, we found a variable IGR with single base substitutions and insertion/deletion (Fig. 1b). Sequence variability across the two genomes was 2.6%, excluding gaps. The highest level of nucleotide diversity (π) was found within *Nad4* (3.59%), *mtMutS* (3.46%) and *Nad5* (2.75%). The least variable gene was *Atp8* (0.46%) and other conserved regions were identified within *Nad1*, *Cox3* and *Atp6* (Fig. 1b).

Molecular phylogeny

The phylogenetic tree of the partial *mtMutS* gene (~750 nt) showed that the 17 *Paramuricea* species sampled grouped in a clade supported by high bootstrap (BP = 89) and posterior probability (PP = 0.95) values and that the closest related genera were *Echinomuricea*, *Menella* and *Placogorgia* (Fig. 2). Among *Paramuricea*, most of the shallow nodes

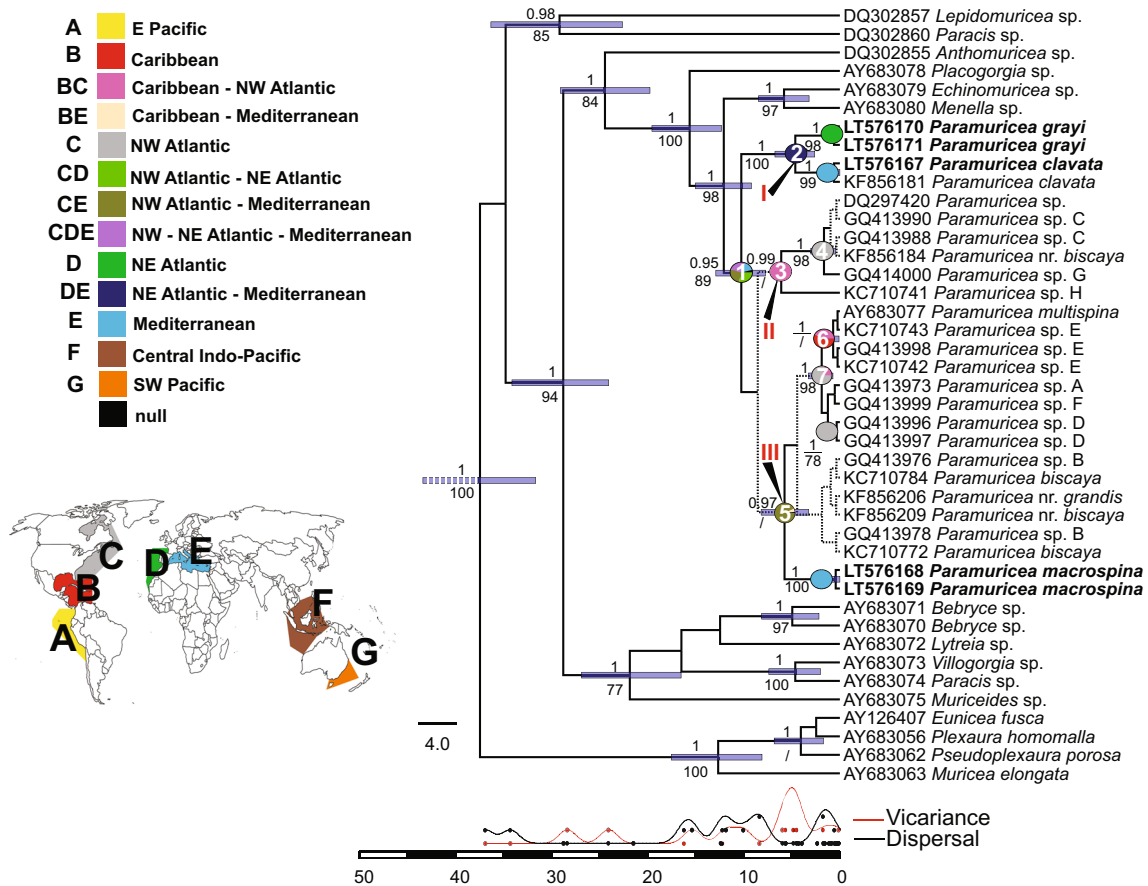


Fig. 2 Phylogenetic relationships, divergence times and ancestral area reconstructions of the genus *Paramuricea*. The tree is a chronogram inferred using a strict molecular clock and conservative mutation rate (0.14% per million year) based on a BEAST Markov chain Monte Carlo (MCMC) analysis. Numbers above and below the nodes represent posterior probability (PP) and bootstrap (BP) values, respectively. Dashed lines indicate branches which lead to nodes with PP <0.95 and BP <70. Roman numbers indicate the three main groups within the genus *Paramuricea*. The Eastern Atlantic and Mediterranean species analysed in this study are shown in bold. The pie charts indicate the ancestral area

reconstruction of a given node based on seven pre-assigned geographic distribution (A-G) for the S-DIVA analysis. The biogeographic regions considered for the analysis are the following: (A) E Pacific (Panama and Galapagos), (B) Caribbean, (C) NW Atlantic, (D) NE Atlantic, (E) Mediterranean Sea, (F) Central Indo-Pacific (Indonesia, Papua New Guinea, Palau, Western Australia and Philippines) and (G) South-West Pacific. Node bars indicate 95% HPD age ranges. The time-event curves for dispersal (black) and vicariance (red) events obtained with RASP 3.2 are shown on the bottom side of the chronogram. The scale bar is in million years

are poorly supported (BP <70; PP <0.95) and the phylogenetic relationships within NW Atlantic specimens were not resolved. Twenty-six *Paramuricea* specimens were divided in three clades: the first (I) includes *P. clavata* and *P. grayi*; the second (II) groups *Paramuricea* sp. (DQ297420), *P. nr. biscaya* (KF856184) and three different *Paramuricea* sp. types (C, G, H) and the third (III) consists of *P. macrospina*, *P. multispina*, *P. biscaya*, *P. nr. biscaya* (KF856209), *P. nr. grandis* and five different *Paramuricea* sp. types (e.g. A, B, D, E, F).

Historical biogeography of Mediterranean *Paramuricea* species

According to our S-DIVA analysis, a total of six dispersal and eight vicariance events occurred during the evolutionary history of the genus *Paramuricea* (Fig. 2). Node 1 included all 26 *Paramuricea* specimens from four main geographic areas (e.g. Caribbean, North-West Atlantic, North-Eastern Atlantic and Mediterranean Sea). S-DIVA assigns this node, the ancestor of *Paramuricea*, to four different ancestral area reconstructions with similar frequency: NW Atlantic + Mediterranean (30%), NW Atlantic + NE Atlantic (25%), NW Atlantic + NE Atlantic + Mediterranean (25%) and Mediterranean (19%). The most probable ancestral range at node 2 (i.e. *P. clavata* + *P. grayi* ancestor) is NE Atlantic + Mediterranean with an occurrence frequency of 100%. This reconstruction suggests that *P. clavata* and *P. grayi* originated through vicariance of this Atlanto-Mediterranean ancestor. S-DIVA reconstructs a Caribbean + NW Atlantic as the ancestral area of node 3 with an occurrence frequency of 100%. This node is further split in two lineages with distributions in the North Atlantic (node 4) and the Caribbean (*Paramuricea* sp. H). For node 5 (i.e. the ancestor of the clade including *P. macrospina*), S-DIVA postulates a NW Atlantic + Mediterranean ancestral range with an occurrence frequency of 93%. This ancestral distribution implies a vicariance led to the separation of *P. macrospina* in the Mediterranean and the ancestor of clades 6 and 7 in the NW Atlantic. These nodes (i.e. 6 and 7) are predicted to have two possible ancestral ranges: Caribbean (61%) and Caribbean + NW Atlantic (39%), and NW Atlantic (72%) and Caribbean + NW Atlantic (28%), respectively. The ancestral distributions obtained using a DEC model were consistent with those obtained by S-DIVA (see Supplementary Fig. 5).

Molecular dating the Mediterranean *Paramuricea* endemics

Using either a conservative or a speculative molecular rate (see Fig. 2 and Supplementary Fig. 6), molecular dating resulted in a divergence time of 4.6 Ma (95% HPD 3–7 Ma) or 2.6 Ma (95% HPD 1–4 Ma), respectively, for *P. clavata*. The

speciation event leading to the diversification of *P. macrospina* from a NW Atlantic + Mediterranean ancestor was dated at 5.8 Ma (95% HPD 3–8 Ma) or 3.2 Ma (95% HPD 2–5 Ma). Similar divergence times were recovered regardless of the clock model and molecular rates used, indicating that the dates obtained are robust to changes in the methods used to infer them. Details on the age estimates for the Mediterranean species are reported in the Supplementary Fig. 7.

Discussion

Mitogenomics, phylogeny and evolution of *Paramuricea*

The genus *Paramuricea* is restricted in the Mediterranean Sea to two endemic species that according to our phylogenetic analyses are not closely related. The level of mitochondrial sequence divergence between these two species was high (~2.6%), well above the 1% threshold proposed for *mtMutS*-based species delimitation in octocorals (McFadden et al. 2011). Intrageneric nucleotide diversity in octocorals changes according to the taxonomic group considered and the molecular marker used. In the Mediterranean *Paramuricea* the *Nad5* and *Nad4* showed the highest mutation rate among mitochondrial protein-coding genes, and the intergenic region between these two genes was remarkably different in terms of size (100–500 bp). For members of *Paramuricea*, this particular mitogenomic region (*Nad5*-*igr*-*Nad4*) may be of interest as a complementary barcode to the *mtMutS* and *Cox1* regions and should be further investigated. Differences in the length of IGRs in non-bilaterian metazoans (i.e. Cnidaria, Ctenophora, Placozoa and Porifera) contribute wide range of size variation observed in the mitochondrial genomes of these animals and is mainly caused by the presence of repetitive elements such as hairpin-forming elements, palindromes and duplicated segmental DNA (Erpenbeck et al. 2009; Lavrov 2010; Park et al. 2011). Octocorals display a wide range of mitochondrial genome sizes—between 18,668 and 20,246 bp among sequenced members of Holaxonia reported in GenBank and the two genomes obtained in this study. Interestingly, neither direct variant repeats (DVRs) nor duplicated segmental DNA, as it has been reported in *Calicogorgia granulosa* (for details, see Park et al. 2011), were found in any of the other published octocoral mitochondrial genomes or those sequenced here leaving open questions about the molecular mechanisms responsible for mitochondrial genome expansion/contraction in octocorals. A broader sampling effort across the subclass may help to better understand mitochondrial genome evolution among octocorals.

Concerning our phylogenetic results, they showed the shallow-water *P. clavata* sister to NE Atlantic *P. grayi* and *P. macrospina* closely related to deep-sea congeners from the Gulf of Mexico and NW Atlantic. Our analyses also

revealed a close genetic relation between the genera *Paramuricea*, *Echinomuricea* and *Placogorgia*, in agreement with previous studies (see Wirshing et al. 2005; McFadden et al. 2011). The genus *Echinomuricea* includes approximately 30 species with a broad geographic distribution, but generally is regarded as limited to the Indo-West Pacific (Williams and Chen 2014). Grasshoff (1977) reassigned the Mediterranean endemic *Echinomuricea klavereni* to *Spinimuricea* and the status of the *Echinomuricea* species from the West Indies and the Gulf of Mexico is not clear and needs revision. Similarly, *Placogorgia* represents another poorly known group in need of taxonomic and systematic treatment. It is interesting to note that these three genera as well as others with thornscale or thornspindle sclerites (i.e. *Muriceides*, *Villogorgia*) were grouped into the same family (Paramuriceidae) by Bayer (1956), now disregarded. The phylogenetic affinity of paramuriceids (sensu Bayer 1956) with similar sclerites highlights the importance of morphological characters in evolutionary studies. For instance, the thornscale sclerites, reported in the family Plexauridae, represent a synapomorphic character that was likely present in the most recent common ancestor of this family.

Historical biogeography, vicariance and speciation

Paramuricea and most members of the family Plexauridae occur in different biogeographic areas such as the Atlantic and Pacific oceans and the Mediterranean Sea. Due to their broad geographic distribution, a Tethyan common ancestor has been proposed for this family (Bayer 1961; Vargas et al. 2010). The Mediterranean is considered to be the evolutionary centre of Tethys fauna remnants (Dov Por 2009) and putative Tethyan relicts have been found in different groups of organisms in this region (Pérès and Picard 1964; Boury-Esnault et al. 1992; Dov Por 2009). These relicts are thought to have survived the Messinian and Gelasian crises (5.2 and 2.6 Ma), during which a dramatic decrease in the number of marine species occurred due to the extreme geological and climatic changes driven by the closing of the strait of Gibraltar and the following desiccation of the Mediterranean Sea (Hsü et al. 1973).

Among octocorals, the existence of early Miocene (~20 Ma) fossils belonging the family Corallidae (Vertino et al. 2014) suggests that a Mediterranean ancestor of this group survived the Messinian crisis 5.2 Ma. In the case of octocorals, where fossils are lacking, it is hard to establish whether Mediterranean species are derived from an Atlantic ancestor (i.e. they speciated after an ancestral range expansion into the Mediterranean Pérès 1985; Sarà 1985) or speciated in situ from a wide spread Atlanto-Mediterranean ancestor. In historical biogeography, however, the distribution of extant species can be used to reconstruct ancestral distributions and clarify the speciation process of taxa when other information

sources are lacking. Our biogeographic analysis suggests that the two Mediterranean endemic species originated from two independent vicariance events and we pose that these events are related to the Messinian and Gelasian crises. Interestingly, our molecular clock estimates are in line with this hypothesis. Based on conservative or speculative mutation rates, the divergence estimates of 4.6 and 2.6 Ma for *P. clavata* and $\sim 5.9 \pm 0.2$ and 3.3 ± 0.1 Ma for *P. macrospina* match the geological age of these crises. Although these estimates are based on mutation rates calculated for the genus *Leptogorgia*, they seem to be robust to variations in the analytical setting used. Hence, the origin of the Mediterranean *Paramuricea* seems to be in agreement with the canonical hypothesis about the role played by the Messinian (and Gelasian) crisis in shaping the evolutionary history of the marine fauna of the Mediterranean. Indeed, after the Messinian crisis the Mediterranean Sea was 'refilled' with Atlantic water (García-Castellanos et al. 2009). According to our results, this event could have provided the conditions for the ancestral vicariance event we detected leading to the diversification of the 'new' Mediterranean biota due to the new environmental conditions offered by this basin. Dispersal has been proposed as the main mechanism responsible for biogeographic patterns in the sea (see, e.g. Lessios and Robertson 2006), yet our results highlight the importance of vicariance as a driving-force for speciation in the ocean. In this respect, recent studies on the evolution and speciation of Mediterranean organisms (e.g. Domingues et al. 2005; Tougaard et al. 2014) reveal that the endemic species are either the result of inter-basin speciation after the Messinian Salinity Crisis or derived from Atlantic ancestor populations which became extinct after the 'colonization' of the Mediterranean.

Despite our phylogenetic trees included some branches with uncertainty, the majority of the deep nodes are well supported and the ancestral reconstruction analysis seems to be robust to topological uncertainty. In this regard, the high occurrence frequency values obtained by S-DIVA indicate that the underlying biogeographic signal is strong enough to overcome the topological uncertainty present in the phylogeny of *Paramuricea* at shallow levels. Finally, we would like to highlight the somewhat unexpected high phylodiversity of the Mediterranean *Paramuricea*, which represent two highly divergent lineages resulting from independent speciation events. Interestingly, this diversity is also reflected in the ecology of the endemic *Paramuricea* species with *P. clavata* occurring along the littoral and *P. macrospina* being restricted to deeper habitats.

We hope our study provides further information on the genus *Paramuricea* in the Mediterranean that can be used to guide conservation planning and decision making in the area by taking into account the evolutionary and biogeographic history of these organisms.

Acknowledgements We thank Andromede Océanologie and Thibault Rauby for help with sampling and to José Aldrey and Marie-Noëlle de Casamajor for sending part of the samples. We would also like to thank an anonymous reviewer for the insightful comments on the paper. This study was possible thanks to the generous support of the LMU München Junior Research Funds to SV through the German Excellence Initiative. SV is indebted to N. Villalobos, M. Vargas, S. Vargas and S. Vargas for their constant support during the realization of this study.

References

- Alderslade, P., Althaus, F., Mcennulty, F., Gowlett-Holmes, K., & Williams, A. (2014). Australia's deep-water octocoral fauna: historical account and checklist, distributions and regional affinities of recent collections. *Zootaxa*, 3796, 435–452.
- Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693.
- Agell, G., Rius, M., & Pascual, M. (2009). Isolation and characterization of eight polymorphic microsatellite loci for the Mediterranean gorgonian *Paramuricea clavata*. *Conservation Genetics*, 10, 2025–2027.
- Angeletti, L., Taviani, M., Canese, S., Fogliani, F., Mastrotoaro, F., Argnani, A., Trincardi, F., Bakran-Petricioli, T., Ceregato, A., Chimenti, G., Macić, V., & Poliseno, A. (2014). New deep-water cnidarian sites in the southern Adriatic Sea. *Mediterranean Marine Science*, 15, 263–273.
- Bas, C. (2009). The Mediterranean: a synoptic overview. *Contributions to Science*, 5, 25–39.
- Bayer, F. M. (1956). Octocorallia. In R. C. Moore (Ed.), *Treatise of invertebrate paleontology, part F Coelenterata* (pp. 167–231). Kansas: Geological Society of America and University of Kansas Press.
- Bayer, F. M. (1961). The shallow-water Octocorallia of the West Indian region. A manual for marine biologists. *Studies of the Fauna of Curaçao and other Caribbean Islands*, 12, 1–373.
- Bianchi, C., & Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin*, 40, 367–376.
- Bo, M., Canese, S., Spaggiari, C., Pusceddu, A., Bertolino, M., Angiolillo, M., Giusti, M., Loreto, M. F., Salvati, E., Greco, S., & Bavestrello, G. (2012). Deep coral oases in the South Tyrrhenian Sea. *PLoS One*, 7(11), e49870. doi:10.1371/journal.pone.0049870.
- Bouckaert, R. (2010). DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics*, 26, 1372–1373.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10(4), e1003537. doi:10.1371/journal.pcbi.1003537.
- Boudouresque, C. F. (2004). Marine biodiversity in the Mediterranean: status of species, populations and communities. *Scientific Report of Port-Cros National Park*, 20, 97–146.
- Boury-Esnault, N., Pasini, M., & Uriz, M. J. (1992). A new *Discorhabdella* (Porifera, Demospongiae), a new Tethyan relict of pre-Messinian biota? *Journal of Natural History*, 26, 1–7.
- Brockman, S. A., & McFadden, C. S. (2012). The mitochondrial genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. *Genome Biology and Evolution*, 4, 994–1006.
- Carpine, C., & Grasshoff, M. (1975). Les Gorgonaires de la Méditerranée. *Bulletin de l'Institut Océanographique de Monaco*, 71, 1–140.
- Carreras-Carbonell, J., Macpherson, E., & Pascual, M. (2005). Rapid radiation and cryptic speciation in Mediterranean triplefin blennies (Pisces: Tripterygiidae) combining multiple genes. *Molecular Phylogenetics and Evolution*, 37, 751–761.
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., Ballesteros, E., Nike Bianchi, C., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Frogliani, C., Galil, B. S., Gasol, J. M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M. S., Koukouras, A., Lampadariou, N., Laxamana, E., López-Fé de la Cuadra, C. M., Lotze, H. K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salina, J. I., San Vicente, S., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., & Voultsiadou, E. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS One*, 5(8), e11842. doi:10.1371/journal.pone.0011842.
- Costantini, F., Carlesi, L., & Abbiati, M. (2013). Quantifying spatial genetic structuring in mesophotic populations of the precious coral *Corallium rubrum*. *PLoS One*, 8(4), e61546. doi:10.1371/journal.pone.0061546.
- Danovaro, R., Dinat, A., Duinevel, G., & Tselepidis, A. (1999). Benthic response to particulate fluxes in different trophic environments: a comparison between the Gulf of Lions–Catalan Sea (western-Mediterranean) and the Cretan Sea (eastern-Mediterranean). *Progress in Oceanography*, 44, 287–312.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
- Doughty, C., Quattrini, A. M., & Cordes, E. E. (2014). Insights into the population dynamics of the deep-sea coral genus *Paramuricea* in the Gulf of Mexico. *Deep Sea Research Part II: Topical Studies in Oceanography*, 99, 71–82.
- Domingues, V. S., Bucciarelli, G., Almada, V. C., & Bernardi, G. (2005). Historical colonization and demography of the Mediterranean damselfish, *Chromis chromis*. *Molecular Ecology*, 14, 4051–4063.
- Dov Por, F. (2009). Tethys returns to the Mediterranean: success and limits of tropical re-colonization. *BioRisk*, 3, 5–19.
- Doyle, J. J., & Doyle, J. L. A. (1987). Rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.
- Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
- Erpenbeck, D., Voigt, O., Wörheide, G., & Lavrov, D. V. (2009). The mitochondrial genomes of sponges provide evidence for multiple invasions by repetitive hairpin-forming elements (RHE). *BMC Genomics*, 10, 591. doi:10.1186/1471-2164-10-591.
- France, S. C., & Hoover, L. L. (2002). DNA sequences of the mitochondrial *COI* gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). *Hydrobiologia*, 471, 149–155.
- García-Castellanos, D., Estrada, F., Jiménez-Munt, I., Gorini, C., Fernández, M., Vergés, J., & De Vicente, R. (2009). Catastrophic flood of the Mediterranean after the Messinian salinity crisis. *Nature*, 460, 778–781.
- Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27, 221–224.
- Grasshoff, M. (1977). Die Gorgonarien des östlichen Nordatlantik und des Mittelmeeres. III Die Familie Paramuriceidae (Cnidaria, Anthozoa). *Meteor Forschungsergebnisse*, 27, 5–76.
- Grasshoff, M. (1992). Die Flachwasser-Gorgonarien von Europa und Westafrika (Cnidaria, Anthozoa). *Courier Forschungsinsitut Senckenberg*, 149, 1–135.
- Hsü, K. J., Ryan, W. B. F., & Cita, M. B. (1973). Late Miocene dessication of the Mediterranean. *Nature*, 242, 240–244.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious basic: an integrated and extendable desktop software

- platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
- Lavrov, D. V. (2010). Rapid proliferation of repetitive palindromic elements in mtDNA of the endemic Baikalian sponge *Lubomirskia baicalensis*. *Molecular Biology and Evolution*, 27, 757–760.
- Lepard, A. (2003). Analysis of variation in the mitochondrial encoded *msh1* in the genus *Leptogorgia* (Cnidaria: Octocorallia) and implications for population and systematic studies. MS thesis, University of Charleston, Charleston, SC.
- Lessios, H. A., & Robertson, D. R. (2006). Crossing the impassable: genetic connections in 20 reef fishes across the Eastern Pacific Barrier. *Proceedings of the Royal Society B*, 273, 2201–2208.
- Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.
- McFadden, C. S., & Hutchinson, M. B. (2004). Molecular evidence for the hybrid origin of species in the soft coral genus *Alcyonium* (Cnidaria: Anthozoa: Octocorallia). *Molecular Ecology*, 13, 1495–1505.
- McFadden, C. S., France, S. C., Sanchez, J. A., & Alderslade, P. (2006). A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein coding sequences. *Molecular Phylogenetics and Evolution*, 41, 513–527.
- McFadden, C. S., Benayahu, Y., Pante, E., Thoma, J. N., Nevarez, P. A., & France, S. C. (2011). Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources*, 11, 19–31.
- Mokhtar-Jamali, K., Pascual, M., Ledoux, J. B., Coma, R., Féral, J. P., Garrabou, J., & Aurelle, D. (2011). From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular ecology*, 20, 3291–3305.
- Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S. C., Boisselier, M. C., & Samadi, S. (2015). Use of RAD sequencing for delimiting species. *Heredity*, 114, 450–459.
- Park, E., Song, J. I., & Won, Y. J. (2011). The complete mitochondrial genome of *Calicogorgia granulosa* (Anthozoa: Octocorallia): potential gene novelty in unidentified ORFs formed by repeat expansion and segmental duplication. *Gene*, 486, 81–87.
- Pérès, J. M. (1985). History of the Mediterranean biota and the colonization of the depth. In R. Margalef (Ed.), *Western Mediterranean* (pp. 200–234). London: Pergamon Press.
- Pérès, J. M., & Picard, J. (1964). Nouveau manuel de bionomie benthique de la mer Méditerranée. *Recueil des Travaux de la Station Marine d'Endoume*, 31, 1–137.
- Pinardi, N., Arneri, E., Crise, A., Ravaoli, M., & Zavatarelli, M. (2006). The physical, sedimentary and ecological structure and variability of shelf areas in the Mediterranean Sea. In A. R. Robinson & K. Brink (Eds.), *The sea* (pp. 1243–1330). Cambridge: Harvard University Press.
- Pratlong, M., Haguenaer, A., Chabrol, O., Klopp, C., Pontarotti, P., & Aurelle, D. (2015). The red coral (*Corallium rubrum*) transcriptome: a new resource for population genetics and local adaptation studies. *Molecular Ecology Resources*, 15, 1205–1215.
- Rambaut, A., Drummond, A. J. (2009). Tracer—MCMC trace analysis tool. Ver. 1. 5. Edinburgh: University of Edinburgh.
- Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57(1), 4–14.
- Ronquist, F. (1997). Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, 46, 195–203.
- Ronquist, F. (2001). DIVA—computer program for MacOS and Win32. Ver 1. 2. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Sánchez, J. A., McFadden, C. S., France, S. C., & Lasker, H. R. (2003). Molecular phylogenetic analyses of shallow-water Caribbean octocorals. *Marine Biology*, 142, 975–987.
- Sanna, D., Cossu, P., Dedola, G. L., Scarpa, F., Maltagliati, F., Castelli, A., Franzoi, P., Lai, T., Cristo, B., Curini-Galletti, M., Francalacci, P., & Casu, M. (2013). Mitochondrial DNA reveals genetic structuring of *Pinna nobilis* across the Mediterranean Sea. *PLoS One*, 8(6), e67372. doi:10.1371/journal.pone.0067372.
- Sarà, M. (1985). Ecological factors and their biogeographic consequences in the Mediterranean ecosystems. In M. M. Apostolopoulou & V. Kiortsis (Eds.), *Mediterranean marine ecosystems NATO conference series* (pp. 1–18). London: Plenum Press.
- Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
- Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, 57, 758–771.
- Tanhua, T., Hainbucher, D., Schroeder, K., Cardin, V., Alvarez, M., & Civitarese, G. (2013). The Mediterranean Sea system: a review and an introduction to the special issue. *Ocean Science*, 9, 789–803.
- Thoma, J. N., Pante, E. G., Brugler, M. R., & France, S. C. (2009). Deep-sea octocorals and antipatharians show no evidence of seascale endemism in the NW Atlantic. *Marine Ecology Progress Series*, 397, 25–35.
- Topçu, E. N., & Öztürk, B. (2015). Composition and abundance of octocorals in the sea of Marmara, where the Mediterranean meets the Black Sea. *Scientia Marina*, 79, 125–135.
- Tougaard, C., Folly, J., & Berrebi, P. (2014). New light on the evolutionary history of the common goby (*Pomatoschistus microps*) with an emphasis on colonization processes in the Mediterranean Sea. *PLoS One*, 9(3), e91576. doi:10.1371/journal.pone.0091576.
- Vafidis, D. (2010) Checklist of class Anthozoa (Phylum Cnidaria). In Coll, M., et al. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS ONE*, 5(8), e11842. doi:10.1371/journal.pone.0011842.
- Vargas, S., Eitel, M., Breedy, O., & Schierwater, B. (2010). Molecules match morphology: mitochondrial DNA supports Bayer's *Lytrea-Bebryce-Heterogorgia* (Alcyonacea: Octocorallia) clade hypothesis. *Invertebrate Systematics*, 24, 23–31.
- Vargas, S., Guzman, H. M., Breedy, O., & Wörheide, G. (2014). Molecular phylogeny and DNA barcoding of tropical eastern Pacific shallow-water gorgonian octocorals. *Marine Biology*, 161, 1027–1038.
- Vertino, A., Stolarski, J., Bosellini, F. R., & Taviani, M. (2014). Mediterranean corals through time: from Miocene to present. In S. Goffredo & Z. Dubinsky (Eds.), *The Mediterranean Sea: its history and present challenges* (pp. 257–274). Dordrecht: Springer.
- Williams, G. C., & Chen, J. (2014). Illustrated key to the shallow-water gorgonians and pennatulaceans of the Verde Island Passage, northern Philippines, including synopses of the taxa and a glossary of terms (Cnidaria: Anthozoa: Octocorallia). In G. C. Williams & T. M. Gosliner (Eds.), *The coral triangle—the Hearst Philippine Biodiversity Expedition 2011* (pp. 67–128). San Francisco: California Academy of Sciences.
- Wirshing, H. H., Messing, C. G., Douady, C. J., Reed, J., Stanhope, M. J., & Shivji, M. S. (2005). Molecular evidence for multiple lineages in the gorgonian family Plexauridae (Anthozoa: Octocorallia). *Marine Biology*, 147, 497–508.
- Wirshing, H. H., & Baker, A. C. (2015). Molecular and morphological species boundaries in the gorgonian octocoral genus *Pterogorgia* (Octocorallia: Gorgoniidae). *PLoS One*, 10(7), e0133517. doi:10.1371/journal.pone.0133517.
- Yan, Y., Harris, A. J., & Xingjin, H. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, 56, 848–850.
- Yu, Y., Harris, A. J., He, X. J. (2014) RASP (Reconstruct Ancestral State in Phylogenies). Ver. 3. 0. Available at <http://mnh.scu.edu.cn/soft/blog/RASP>.