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Research Article

The origin and dispersal pathway of the spotted sea hare *Aplysia dactylomela* (Mollusca: Opisthobranchia) in the Mediterranean Sea

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Abstract

The spotted sea hare *Aplysia dactylomela* Rang, 1828 is a large and conspicuous opisthobranch sea slug that since 2002 has rapidly colonized the eastern Mediterranean, establishing populations in numerous localities. The source of the Mediterranean populations has been the subject of debate, with two main hypotheses considered (Atlantic and Red Sea origin). A recent study on the taxonomy of *A. dactylomela* has shown that the spotted sea hare is a complex of at least two genetically distinct species (*A. dactylomela* in the Atlantic and *A. argus* in the Indo-Pacific), facilitating the correct identification of Mediterranean specimens by molecular means. We used sequence data from the mitochondrial cytochrome oxidase I gene to identify the Mediterranean individuals for the first time and to infer their origin. Our results confirmed that all the specimens collected in the Mediterranean belong to *A. dactylomela* and therefore have an Atlantic origin. The limited sample size does not allow identification of the dispersal pathway of *A. dactylomela* into the Mediterranean, but the colonization sequence is consistent with a "natural" dispersal event. This hypothesis is evaluated in light of local surface circulation patterns. Possible causes for the recent and rapid invasion of the eastern Mediterranean by *A. dactylomela* are discussed.

Key words: Aplysiidae; Atlantic Ocean; population genetics; haplotype network

Introduction

Aplysia dactylomela Rang, 1828 is a large species of sea hare (Opisthobranchia: Aplysiidae) reaching up to 200 mm in length with a distinctive pattern of dark rings on a yellowish-cream background color. This species was considered to have a world-wide native range in tropical and subtropical regions, including: the tropical Indo-Pacific from the Red Sea and South Africa to the Hawaiian Islands and Panama (Gosliner et al. 2008); and both the eastern and western Atlantic Ocean (Ortea and Martínez 1990; Cervera et al. 2004; Valdés et al. 2006). However, in a recent paper Alexander and Valdés (2013) revealed that the pan-tropical *A. dactylomela* is comprised of at least two genetically distinct species, *A. argus* (Rüppell and Leuckart, 1828) distributed in the tropical Indo-Pacific Ocean, and *A. dactylomela* found in the Atlantic Ocean. The two species are similar externally, thus genetic information is the most reliable tool to distinguish them (Alexander and Valdés 2013).

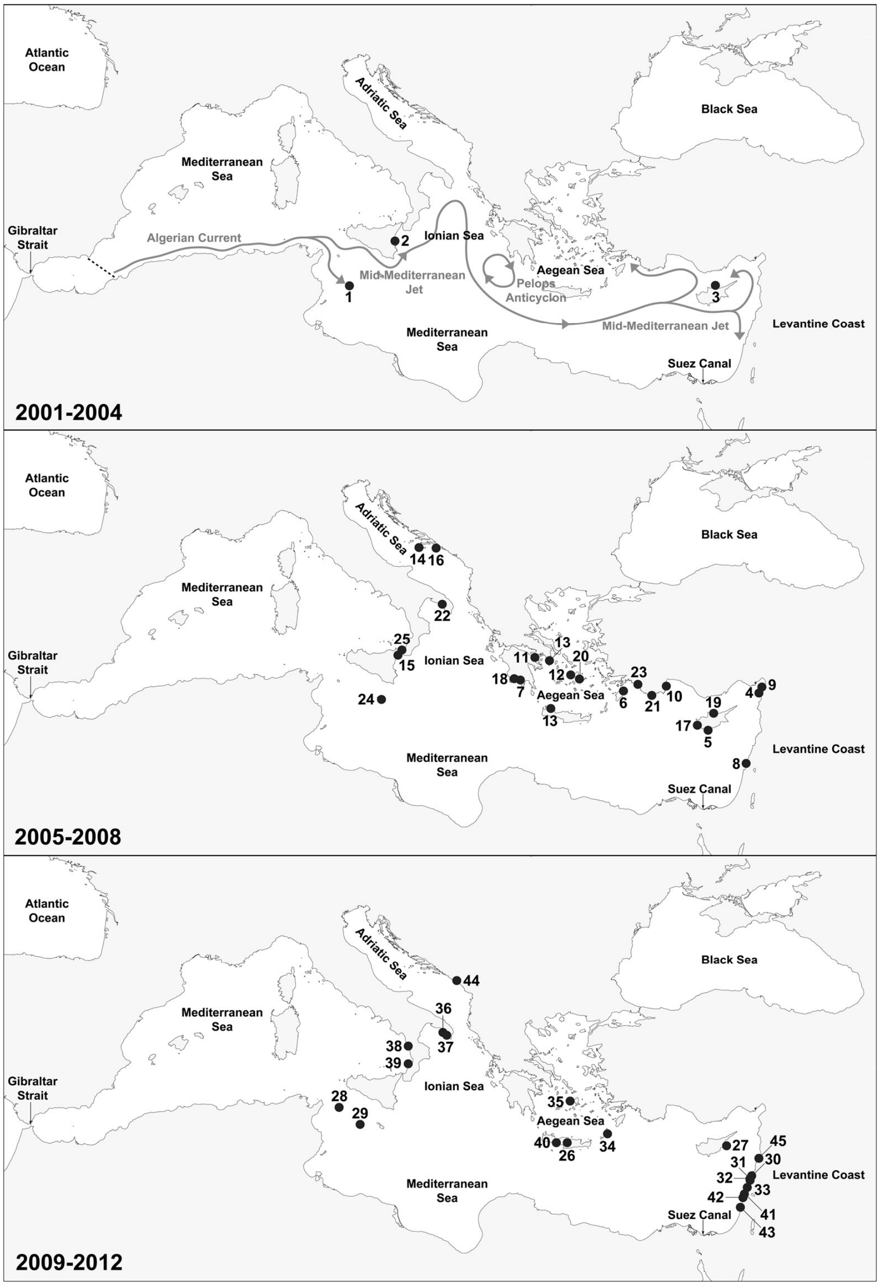

Figure 1. First records of *Aplysia dactylomela* in the Mediterranean during three time periods: 2001-2004, 2005-2008, and 2009-2012 based on Crocetta and Galil (2012), Crocetta et al. (2013) and references therein. Top map includes a simplified scheme of the main Mediterranean ocean current systems mentioned in the discussion (in grey), based on Garofalo et al. (2010) and Abudaya (2013). Locality codes with date of first record in chronological order – 1: Lampedusa (2002), 2: Catania, Sicily (2003), 3: Girne, Cyprus (2004), 4: Meydan Köyü, Turkey (2005), 5: Akrotiri, Cyprus (2005), 6: Rhodes, Greece (2005), 7: Stoupa (2005), 8: between Akhziv and Rohs Hanikra, Israel (2005), 9: İskenderun Bay, Turkey (2005), 10: Üç Adalar, Turkey (2006), 11: Chania, Crete, Greece (2006), 12: Paros, Greece (2006), 13: Saronikos, Greece (2006), 14: Sušac, Croatia (2006), 15: Taormina, Sicily (2006), 16: Mljet, Croatia (2006), 17: Kissonerga, Cyprus (2006-2008), 18: Kalamaki, Greece (2007), 19: Kayalar, Cyprus (2007), 20: Koufonissi, Greece (2007-2008), 21: Kaş, Turkey (2007), 22: Gulf of Taranto (2008), 23: Fethiye, Turkey (2008), 24: Cirkewwa, Malta (2008), 25: Strait of Messina (2008), 26: Crete, Greece (2009), 27: Makronisos, Cyprus (2009), 28: Pantelleria Island (2009), 29: Linosa Island (2009), 30: Saadiyat, Lebanon (2009), 31: Khaizaran, Lebanon (2010), 32: Tyr, Lebanon (2010), 33: Akhziv, Israel (2010), 34: Karpathos, Greece (2010), 35: Antiparos, Greece (2010), 36: Gallipoli, Italy (2011), 37: Santa Maria al Bagno, Italy (2011), 38: Diamante, Cosenza, Italy (2011), 39: Baia di Riaci, Italy (2011), 40: Hora Sfakion, Greece (2011), 41: Rosh Hanikra, Israel (2011), 42: Nahariya, Israel (2011), 43: Michmoret, Israel (2011), 44: Boka Kotorska, Montenegro (2011), 45: Ramkine Island, Lebanon (2011). Locality data deposited in the EASIN database (http://easin.jrc.ec.europa.eu/)."

Specimens assigned to *Aplysia dactylomela* were only reported from the Mediterranean Sea recently, starting with a 2002 record from the island of Lampedusa in the Sicily Channel (Trainito 2003), followed by a 2003 record from eastern Sicily (Scuderi and Russo 2005), and a rapid expansion into the Adriatic and the central and eastern Mediterranean (Crocetta and Galil 2012) where is now well established (Zenetos et al. 2012) (Figure 1). All Mediterranean animals have a characteristic pattern of black rings, which makes them easily recognizable in the field. No other Mediterranean species of sea hare has a similar color pattern, thus it is unlikely that this species was present and undetected in the Mediterranean much earlier than the initial 2002 record. There is a general consensus that specimens identified as *A. dactylomela* are non-native in the Mediterranean (Pasternak and Galil 2010; Çinar et al. 2011; Crocetta 2012; Pećarević et al. 2013) but because their true identity (whether they belong to *A. dactylomela* or *A. argus* or both) is unknown, the origin of the Mediterranean populations remains open to debate.

One of the most intriguing aspects of the colonization of the Mediterranean by spotted sea hares is the unusual sequence of events, starting in a relatively isolated island in the central Mediterranean (Lampedusa) followed by a very rapid dispersal (between 2005 and 2008) into the eastern Mediterranean and the Adriatic and a slower spread in the center and periphery of the range between 2009–2012 (Figure 1). Before there was available genetic evidence showing that *Aplysia dactylomela* was a species complex, a number of papers attempted to elucidate the origin of the Mediterranean populations. For example, some authors discussed the possibility that the Mediterranean spotted sea hares were an Erythraean alien (from the Red Sea) that entered the Mediterranean via the Suez Canal (e.g., Yokeş 2006; Crocetta and Colamonaco 2010; Kout 2012; Crocetta and Galil 2012). The Red Sea migration hypothesis is supported by the fact that the species is not known from the western Mediterranean (Figure 1). But, if this hypothesis were correct, spotted sea hares should have been detected earlier in the Levantine coast as most other Erythraean aliens (Gofas and Zenetos 2003; Galil and Zenetos 2002; Crocetta and Galil 2012). Other authors have suggested a possible “natural” dispersal from Atlantic populations (di Silvestro et al. 2010; Turk and Furlan 2011) with veliger larvae carried by the Algerian Current into the central Mediterranean (Sicily Channel). This hypothesis is supported by some color similarities between the Mediterranean and the Atlantic specimens (Rudman 2005; Turk and Furlan 2011), the early record from Lampedusa, and its presence in areas removed from shipping traffic (Crocetta and Galil 2012; Zenetos et al. 2013). A third hypothesis is that *A. dactylomela* was introduced into the Mediterranean from either the Atlantic Ocean or the Indo-Pacific region by human mediated vectors such as ballast water, sea chests, aquaculture, or aquarium trade (Schembri et al. 2010; Katsanevakis et al. 2013).

To understand how spotted sea hares colonized the Mediterranean Sea, we first must determine their correct taxonomic identity. In this paper we attempted to do so using sequence data from the mitochondrial cytochrome oxidase I gene. We studied several Mediterranean individuals collected from localities covering most of the non-native range and compared them with both Atlantic and Indo-Pacific specimens collected throughout the native range of *A. dactylomela* and *A. argus*.

Methods

Specimen collection

A total of 23 specimens were sequenced for the cytochrome c oxidase I (COI) mitochondrial gene. Specimens were collected from 3 populations in the Mediterranean: Crete, Greece (3 specimens), Turkey (4 specimens), and Italy (6 specimens), 5 populations in the Atlantic Ocean: the Bahamas (2 specimens), Jamaica (1 specimen), US Virgin Islands (1 specimen), Brazil (1 specimen), and the Canary Islands, Spain (1 specimen), and 2 populations in the Indo-Pacific: the Hawaiian Islands, USA (2 specimens) and Reunion, France (2 specimens). An additional sequence from China and two from Florida, USA, were obtained from GenBank (HQ834119, AF343427 and DQ991927 respectively). Most specimens from the Atlantic Ocean and the Indo-Pacific region were confidently assigned to *A. dactylomela* and *A. argus*, respectively, by Alexander and Valdés (2013) based on molecular and morphological evidence. Twenty-four full-length sequences were included in the analyses (Table 1).

Table 1. List of specimens studied in this paper including locality, collecting date, GenBank accession numbers and voucher numbers. Dashes represent missing data. One asterisk (*) represents partial sequences not included in some analyses, two asterisks (**) denote specimens identified morphologically by Alexander and Valdés (2013). Abbreviations: CPIC, California State Polytechnic University Invertebrate Collection; HU, Haliç Üniversitesi; MZSP, Museu de Zoologia Universidade de São Paulo, LSGB, Laboratory of Shellfish Genetics and Breeding, Ocean University of China, Qingdao; NHMC, Natural History Museum of Crete.

Locality	Date	Voucher #	Accession #	Haplotype #
Antalya, Turkey	July 27, 2007	HU-421	KF041209	H2
Antalya, Turkey	Aug 24, 2011	HU-1500	KF041210	H2
İskenderun Bay, Turkey	Nov 2, 2005	HU-66	KF041208	H2
İskenderun Bay, Turkey	Sep 23, 2012	HU-1510	KF041211	H2
Ricadi, Vibo Valentia, Italy	Aug 13, 2011	CPIC-00871	KF028651	H2
Palinuro, Salerno, Italy	Aug 29, 2012	CPIC-00872	KF028652	H4
Palinuro, Salerno, Italy	Aug 29, 2012	CPIC-00873	KF028653	H12
Messina, Italy	Sep 10, 2012	-	KF028654	H2
Messina, Italy	Sep 10, 2012	-	KF028655	H5
Torre Ovo, Taranto, Italy	Oct 20, 2012	-	KF028656	H10
Kouremenos, Lasithi, Crete, Greece	Aug 18, 2012	NHMC-52.96	KF028657	H3
Legrena, Attiki, Greece	Dec 2012	-	KF028658	H6
Legrena, Attiki, Greece	Dec 2012	-	KF028659	H6
Itapuã, Salvador, Bahia, Brazil	Oct 25, 2012	MZSP-108772	KF569904*	-
Hull Bay, St. Thomas, US Virgin Is**	Apr 12, 2002	CPIC-00120	JX560146*	-
Stocking Island, Exuma, Bahamas**	Dec 26, 2009	CPIC-00234	JX560145	H9
Stocking Island, Exuma, Bahamas**	Dec 31, 2008	CPIC-00093	JX560144	H1
Montego Bay, Jamaica**	Apr 14, 2011	CPIC-00650	JX560143	H6
Florida, USA	-	-	DQ991927	H7
Florida, USA	-	-	AF343427	H8
El Socorro, Tenerife, Canary Islands	Nov 22, 2011	CPIC-00875	KF028660	H11
Hekili Point, Maui, Hawaiian Islands**	Jun 13, 2011	CPIC-00297	JX560142	H13
Napili Bay, Maui, Hawaiian Islands**	Jun 13, 2011	CPIC-00302	JX560150	H16
Beihai, Guangxi, China	-	LSGB-25902	HQ834119	H17
Trois Bassins Reef, Reunion	Feb 15, 2013	CPIC-00876	KF028649	H15
Trois Bassins Reef, Reunion	Feb 15, 2013	CPIC-00877	KF028650	H16

DNA extraction, PCR and sequencing

Specimens were preserved in 70% or 99% ethanol. DNA was extracted from a small piece of foot tissue using a hot Chelex ® protocol, then amplified by polymerase chain reaction (PCR) using the COI HCO2198/LCO1490 universal primers (Folmer et al. 1994). PCRs were performed in a 50 µL reaction volume containing 0.25 µL 5u/µL taq polymerase, 5.00 µL 10x buffer, 5.00 µL 25 mM $MgCl_2$, 1.00 µL 40 mM dNTPs, 1.00 µL each 10 mM primer, 34.75 µL H_2O, and 2.00 µL extracted DNA. Reaction conditions involved an initial denaturation of 95°C for 3 min, 35 cycles of 94°C for 45 s, 45°C for 45 s, and 72°C for 2 min, followed by a final elongation step of 72°C for 10 min. PCR products were run on gel electrophoresis to confirm the presence of DNA fragments of appropriate size (700 bp), and positive products were cleaned using Montage PCR Cleanup Kit (Millipore). The DNA concentration of purified samples was then determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific). Sequencing was outsourced to the Eton Biosciences (San Diego, CA). Sequences were assembled and edited using the software GENEIOUS PRO 4.7.4 (Biomatters Ltd.). Geneious also was used to extract the consensus sequences and to align COI using the default parameters.

Population genetics

Sequences were trimmed to match the length of the sequence from China available in GenBank (648 bp) of the original 658 bp. Partial sequences from Brazilian and US Virgin Island specimens were not included in the following analyses. A haplotype network was constructed using TCS 1.21 (Clement et al. 2000) with a 95% connection limit. ARLEQUIN 3.5 (Excoffier and Lischer 2010) was used to calculate F_{st} values as a measure of pairwise differences between the three populations

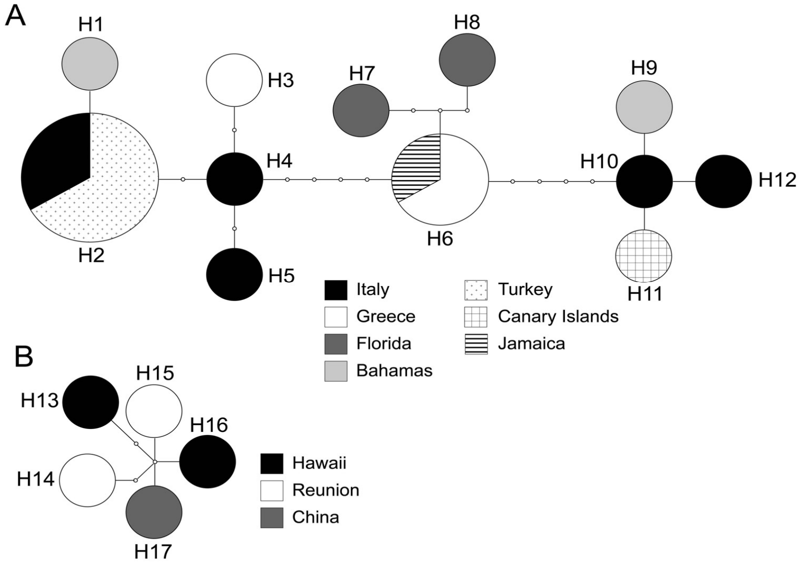

Figure 2. COI haplotype networks produce by TCS with the geographic location in which each haplotype was found. The relative size of the charts is proportional to the number of sequences. A. Mediterranean and Atlantic haplotypes (*A. dactylomela*). B. Indo-Pacific haplotypes (*A. argus*). Haplotype numbers as in Table 1.

(Mediterranean, Atlantic and Indo-Pacific) as well as the nucleotide diversity (π) and haplotype diversity (h) of each population. The significance of the pairwise F_{ST} value was estimated by performing 16,000 permutations.

Phylogenetic analysis

A Bayesian phylogenetic analysis was conducted to assess the posterior probability support for the placement of Mediterranean specimens in *A. dactylomela* or *A. argus*, using the same data set as in the haplotype network. The Akaike information criterion (Akaike 1974) was executed in MrModeltest (Nylander 2004) to determine the best-fit model of evolution (GTR+G). The Bayesian analyses were executed in MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001), with no outgroup (unrooted). The Markov chain Monte Carlo analysis was run with two runs of six chains for fifty million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus tree/s. Convergence of runs was diagnosed using the program Tracer 1.3 (Rambaut and Drummond 2003).

Results

The haplotype network analysis revealed the presence of two distinct networks with no connection between them (Figure 2), one including Indo-Pacific samples (*A. argus*) and the other consisting of all Mediterranean and Atlantic samples (*A. dactylomela*). There was no geographic structure within the networks. The *A. dactylomela* network consisted of 3 haplotype groups separated by 4 substitutions each. Each of these groups contained haplotypes recovered from western Atlantic and Mediterranean specimens. One of the haplotype groups included specimens collected in the Bahamas and the Canary Islands together. The *A. argus* network consisted of three haplotype groups separated by only one substitution, one included a specimen from the Hawaiian Islands, another included a Hawaiian as well as an Indian Ocean and a Chinese specimen, and the third included a specimen from the Indian Ocean.

The F_{ST} analysis results (Table 2) substantiated the differentiation between both Atlantic and Mediterranean populations of *A. dactylomela* from the Indo-Pacific population (*A. argus*), but confirmed that Atlantic and Mediterranean population were not significantly distinct. The levels of nucleotide diversity (Table 3) varied between the three populations with *A. argus* having the lowest and the Atlantic population of *A. dactylomela* the highest diversity.

The Bayesian phylogenetic analysis confirmed the population genetics results and revealed the existence of two well supported clades (Figure 3), one containing Indo-Pacific sequences (*A. argus*) and the other Atlantic and Mediterranean sequences

Table 2. Results of the F_{ST} analysis with 16,000 permutations (lower triangular) and *P* values (upper triangular). Significant *P* value after Bonferroni correction $P < 0.008$. In bold are non-significant values.

	Atlantic	Mediterranean	Indo-Pacific
Atlantic	-	0.05218±0.0017	0.00256±0.0004
Mediterranean	**0.16970**	-	0.00012±0.0001
Indo-Pacific	0.84765	0.87473	-

Table 3. Nucleotide (π) and haplotype (*h*) diversity and number of haplotypes for all three populations examined.

Population	Nucleotide Diversity (π)	Haplotype Diversity (*h*)	Number of haplotypes
Atlantic	0.0121 (±0.0074)	1.0000 (±0.0962)	6
Mediterranean	0.0085 (±0.0048)	0.7949 (±0.1091)	7
Indo-Pacific	0.0043 (±0.0031)	1.0000 (±0.1265)	5

(*A. dactylomela*). The *A. dactylomela* clade contained two well-supported subclades each including a combination of Atlantic and Mediterranean sequences.

Discussion

The origin of the Mediterranean populations

This study demonstrates unequivocally that the Mediterranean specimens belong to *A. dactylomela* and therefore the Mediterranean populations originated in the Atlantic Ocean. The molecular data showed that all haplotypes recovered from the Mediterranean samples (covering the entire known longitudinal range of the species in this sea) were either identical or very similar to haplotypes found in Atlantic populations and clustered together in the phylogenetic analysis. Additionally, the F_{ST} analysis confirmed that there was no significant genetic differentiation between Mediterranean and Atlantic populations of *A. dactylomela*. However, this study cannot identify the source region of the population. Part of the problem is the apparent lack of geographic structure in the Atlantic populations, which suggests this species has a broad dispersal potential as is found in many other species of Aplysiidae (Switzer-Dunlap and Hadfield 1977). Conversely, *A. argus,* the Indo-Pacific sister species to *A. dactylomela*, is genetically and morphologically distinct (Alexander and Valdés 2013), and no Indo-Pacific haplotypes were found in Mediterranean samples. Although the introduction or dispersal of *A. argus* into the Mediterranean is possible, we have found no evidence that it had occurred.

The timing and possible causes of the invasion

With the available data, the hypothesis of a human-mediated introduction of *A. dactylomela* in the Mediterranean from Atlantic populations cannot be discarded. However, the presence of broad genetic representation of Atlantic haplotype groups in the Mediterranean and the relatively high nucleotide diversity in Mediterranean populations is consistent with a “natural” dispersal of a large number of individuals through the Gibraltar Strait. Similar episodic and discontinuous expansions of marine species such as the green crab *Carcinus maenas* (Linnaeus, 1758) in the Pacific coast of North America have been attributed to larval dispersal (Darling 2011).

If this case is the result of a “natural” dispersal, several important questions need to be answered: 1) Why did *A. dactylomela* begin to spread into the Mediterranean so recently? 2) Why did it first appear in Lampedusa and spread into the eastern Mediterranean, completely bypassing the western Mediterranean and the European Atlantic coast? 3) Why did it spread so rapidly in the eastern Mediterranean? Although a definitive answer to these questions may not be possible at this point, several hypotheses discussed below imply the possibility that recent physical and biological changes may have facilitated the invasion process.

The Atlantic and Mediterranean biotas have been physically connected through the Gibraltar Strait for approximately 5.33 million years, since the opening of the Atlantic-Mediterranean connection at the end of the Messinian Salinity Crisis

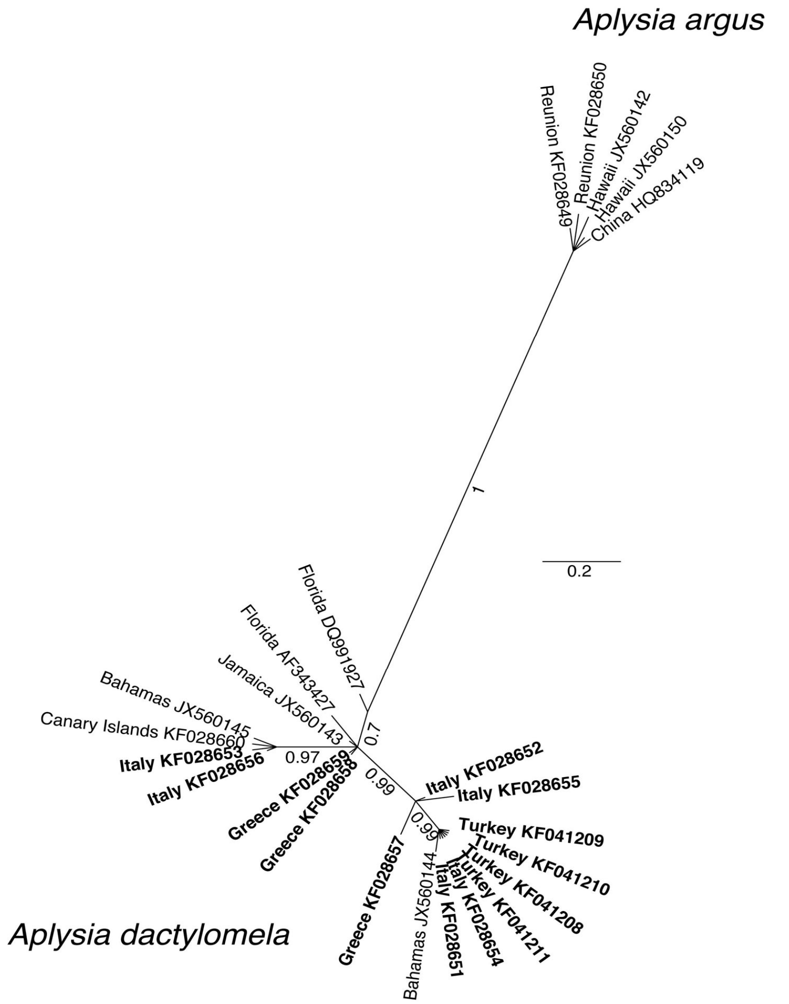

Figure 3. Unrooted Bayesian consensus tree produced by MrBayes with posterior probabilities for each branch. Mediterranean samples highlighted in bold. Values > 0.95 are considered significant.

(Patarnello et al 2007), and most Mediterranean marine animals and plants have an Atlantic origin (Pallary 1907; Harzhauser et al. 2007). Despite the physical connection between the two basins, evidence from several species suggests the existence of barriers to dispersal preventing the spread of some Atlantic species into the Mediterranean. For example, according to Vermeij (2012), the cold Canary Current and Saharan upwelling have historically prevented the dispersal of tropical West African species into the Mediterranean. Vermeij (2012) suggested that warming resulting from current global climate change should reduce the strength of this barrier, as occurred during the productive and warm Early Pliocene, facilitating the spread of the West African biota into the Mediterranean. Another powerful barrier, albeit not impermeable, is the Almería-Oran Front (Patarnello et al 2007), which allows dispersal of some species from west to east (Schunter et al. 2011). The Almería-Oran Front is a quasi-permanent oceanic front and an associated sea current that separate the water flowing in from the Atlantic Ocean from the more saline Mediterranean Sea water (Tintoré et al 1988). Circulation patterns in this complex oceanographic region are altered by weather conditions, with mild winters weakening the strength of the system (Tintoré et al. 1994). The increased frequency of mild winters as a consequence of the current period of global climate change might have lessened the effectiveness of the Almería-Oran barrier, allowing the dispersal of *A. dactylomela* larvae into the Mediterranean. Should *A. dactylomela* larvae have arrived into the Mediterranean by a weakening of the Canary

Current or the Almería-Oran front, or both, the powerful Algerian Current would be able carry them rapidly into the central Mediterranean bypassing most of the western Mediterranean, as suggested by di Silvestro et al. (2010). The long-lived sub-basin scale gyre eddies associated with the Algerian Current trap and transfer pelagic larval assemblages into the Sicily Channel (Siokou-Frangou et al. 2010; Elmaidi et al. 2010) where a Mid-Mediterranean Jet rapidly splits in two main branches affecting the North African coast and southern Sicily (Figure 1). The first branch reaches Lampedusa, and the second follows the Sicily continental slope (Poulain et al. 2012), producing persistent vortexes on the Adventure Bank and Ionian Shelf that favor larval recruitment (Garofalo et al. 2010). Further east, the northern Mid-Mediterranean Jet branch reaches the northern Ionian Sea originating an anti-cyclonic gyre that flows along the Greek continental slope, and forms a small vortex south of Pelops before reaching the eastern/Levantine basin (Figure 1). There, the Mid-Mediterranean Jet turns eastwards, affecting the Turkish and Greek coasts and originating a complex system of cyclonic-anticyclonic eddies and fronts. This ocean circulation system is consistent with the sequence of records documenting the dispersal and colonization of *A. dactylomela* and seems to explain the apparent absence of the species from the western Mediterranean, providing additional support for the "natural" dispersal hypothesis. Other records, from the southern and central Adriatic Sea are consistent with the northwards cyclonic flux, which affects the Balkan coasts throughout the Otranto Channel. Similarly, recent records in the south Tyrrhenian Sea could be due to spreading throughout the Strait of Messina, which is known as an area of plankton accumulation (Guglielmo et al. 1995).

In addition to oceanographic changes, other factors may have facilitated the dispersal and establishment of *A. dactylomela* in the central and eastern Mediterranean. In the last few decades, increases in water temperatures have favored the entry and dispersal of tropical species in the Mediterranean Sea (Occhipinti-Ambrogi 2007; Por 2009; Raitsos et al. 2010). This warming was proposed as the main cause of the recent spread of *Percnon gibbesi* (H. Milne-Edwards, 1853) (see Katsanevakis et al. 2011), a warm-water Atlantic crab that has rapidly colonized both the western and eastern Mediterranean following a similar dispersal pattern to that of *A. dactylomela*. Other significant ecological changes, such as the introduction of non-native species and eutrophication, have disproportionally affected the eastern Mediterranean versus other parts of the Mediterranean basin and might have facilitated the invasion of *A. dactylomela*. The presence of numerous alien species has substantially altered eastern Mediterranean biotas reducing functional biological diversity (Galil 2000; Galil and Zenetos 2002; Galil 2007), which can further accelerate the invasion process by facilitating the arrival of new invaders (Fridley et al. 2007). Another factor to consider is the possibility that recently introduced species of seaweeds might have facilitated the establishment of herbivorous species including sea hares (Mollo et al. 2008). Although *Aplysia dactylomela* held in aquarium in Barbados were observed to feed preferentially on *Ulva* (=*Enteromorpha*) and *Cladophora* when given a choice of several seaweeds (Carefoot 1987), in the Mediterranean it seems to feed on *Laurencia* sp. (Yokeş 2006). Some Mediterranean species of *Laurencia* have been found to be non-native (Furnari et al. 2001). *Aplysia dactylomela* has also been found on dense algal film with the presence of the invasive species *Caulerpa racemosa* (Crocetta and Colamonaco 2010). The eclectic taste of *A. dactylomela* may have allowed it to take advantage of multiple food sources including possibly native and non-native seaweeds. Finally, nutrient enrichment from land and river runoff has primarily affected the generally nutrient-poor regions of the eastern Mediterranean and Adriatic Sea dramatically increasing productivity and altering ecological conditions (Caddy et al. 1995). Eutrophication is known to promote algal growth (Ryther and Dunstan 1971), which in turn can favor the spread and establishment of herbivorous invasive species. In summary, several factors independently or in synergy might have transformed biotic and abiotic conditions sufficiently to allow the dispersal and rapid establishment of *A. dactylomela* in the eastern Mediterranean. As Por (2009) noted, "while attention is concentrated almost entirely on the Indo-Pacific Lessepsian migrants, there is also an increasing settlement by tropical Atlantic newcomers entering the Mediterranean through the Straits of Gibraltar." Whether we refer to this process as "natural" or not is up for debate.

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