

Research Article

The Mediterranean Sea as a gateway for invasion of the Red Sea: the case of the Indo-West Pacific head-shield slug *Chelidonura fulvipunctata* Baba, 1938

Manuel António E. Malaquias^{1,*}, Andrea Zamora-Silva¹, Dyana Vitale², Andrea Spinelli², Sergio De Matteo², Salvatore Giacobbe², Deneb Ortigosa³ and Juan L. Cervera³

¹Phylogenetic Systematics and Evolution Research Group, Section of Taxonomy and Evolution, Department of Natural History, University Museum of Bergen, University of Bergen, PB 7800, 5020-Bergen, Norway

²Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres, 31, 98166 Messina, Italy

³Department of Biology, Faculty of Marine and Environmental Sciences, University of Cádiz, International Campus of Excellence on the Sea (CEIMAR), Polígono del Río San Pedro s/n, Apdo. 40, 11510 Puerto Real (Cádiz), Spain

*Corresponding author

E-mail: Manuel.Malaquias@uib.no

Received: 1 December 2015 / Accepted: 9 March 2016 / Published online: 7 April 2016

Handling editor: Vadim Panov

Abstract

The number of tropical species established in the Mediterranean Sea has risen at an unprecedented rate in the last 40–50 years, often entering the basin from the Red Sea and to a lesser extent by shipping or mariculture. The taxonomic identity of these alien species can be difficult to determine and DNA barcoding can help to clarify the status of questionable species. One of these cases is the W-shaped mark Aglajidae slug usually identified as being the Indo-West Pacific species *Chelidonura fulvipunctata* Baba, 1938 but was first described in the Mediterranean Sea as a native species under the name *C. mediterranea* Swennen, 1961. A Bayesian phylogeny using the barcode marker cytochrome *c* oxidase subunit I and an ABGD species delimitation analysis unequivocally showed that specimens from the eastern and central Mediterranean Sea are conspecific with specimens from the Indian and Pacific Oceans. In this work, we hypothesize that *C. fulvipunctata* (presently occurring in the entire Mediterranean Sea and Indo-West Pacific; only once recorded in the Red Sea) has entered the Red Sea through the Mediterranean Sea. Thus the Red Sea can also be a receiver of tropical species that have arrived in the Mediterranean by other routes than Lessepsian immigration, with the Suez Canal acting as a “revolving door” allowing both species of Indo-Pacific origin to enter the Mediterranean and species established in the Mediterranean resilient to tropical/subtropical environmental conditions to move into the Red Sea.

Key words: alien species, biodiversity, Cephalaspidea, DNA barcode, Mollusca, species range shifts

Introduction

The influx of tropical and subtropical species into the Mediterranean Sea has reached an unprecedented magnitude over the last 40–50 years. There are about 212 non-native molluscan species reported in the basin, 21 being opisthobranchs (reviewed by Gofas and Zenetos 2003; Gosliner et al. 2008a; Zenetos et al. 2010). Three main routes are often proposed to explain the arrival of these exotic elements; the most common is the influx from the Red Sea through the Suez Canal, followed by introduction via shipping on ships’ hulls or in ballast water, and to a less extent mariculture (Gofas and Zenetos 2003). Most of these exotic species have been recognized based

on morphological similarities with their tropical counterparts.

Barcoding methodologies have seldom been applied but could help to clarify the status of questionable species (*sensu* Gofas and Zenetos 2003). For example the taxonomic identity of the Mediterranean W-shaped mark slug (Aglajidae) is uncertain. Originally described as a native species *Chelidonura mediterranea* Swennen, 1961; it was later identified as the Indo-West Pacific species *Chelidonura fulvipunctata* Baba, 1938. No similar head-shield slug occurs in the Atlantic Ocean, and there is a single record from the Red Sea in the Gulf of Aqaba (reported online; Koretz 2005). The Red Sea is a well-studied basin surveyed by a wealth of

expeditions from as early as the second half of the eighteenth century, which has resulted in a large number of studies on the opisthobranchs of the region (see reviews by Yonow 2008; 2012). It is therefore significant that none have reported the occurrence of *C. fulvipunctata*.

Swennen (1961) described the species *C. mediterranea* from a single specimen collected in the Bay of Antalya, Turkey, in the eastern Mediterranean Sea. The specimen was 18 mm in length with a whitish foot and greyish-brown background colour with orange spots scattered over the dorsum, a yellowish-white W-shaped mark on the anterior part of the cephalic shield, and a median dorsal stripe of similar colour. The presence of a W-shaped mark led Swennen (1961) to compare this species with the Indo-West Pacific *C. fulvipunctata* (type-locality: Seto, Kii Peninsula, Japan; Baba 1938) which, despite being known for its striking chromatic variability (Gosliner 1987; Wells and Bryce 1993; Nakano 2004; Gosliner et al. 2015), was thought to never have a median dorsal stripe. The dorsal stripe is, however, present on specimens from Hawaii (Gosliner 1980) and *C. mediterranea* has since been considered to be a junior synonym of *C. fulvipunctata*. In all subsequent reports, the W-shaped mark head-shield slug in the Mediterranean Sea has been identified as the Indo-West Pacific species.

It took more than 20 years for additional specimens of *Chelidonura* slug with a W-shape mark be found in the Mediterranean Sea: Mienis and Gat (1987) reported an occurrence for the shores of Israel and Perrone and Sammut (1997) and Sammut and Perrone (1998) found three additional specimens in the Maltese islands. All these specimens lacked the median dorsal line and resembled Indo-West Pacific morphs that occur, for example, in Japan and South Africa and typically have a black background and many orange dots scattered over the body (Gosliner 1987; Perrone and Sammut 1997; Nakano 2004). More recently, Tsiakkios and Zenetos (2011) cited the presence of this slug in Cyprus and illustrate two specimens with different chromatic patterns; one lighter with a striking cephalic W-mark and a darker one with an almost imperceptible cephalic W-mark. Horst (2015) reported the first occurrence in the western Mediterranean Sea from the coast of France and Karachle et al. (2016) report its occurrence in the Balearic Islands off the coast of Spain.

In this paper, we review the historical records and provide new records of *C. fulvipunctata* in the Mediterranean Sea, and we use DNA barcoding to test the conspecificity of Mediterranean and Indo-West Pacific specimens. We discuss the possible means of entrance of this species in the Mediterranean

Sea and the role of this basin as a possible gateway for other species of tropical origin to enter the Red Sea as opposed to the traditional colonization of the Mediterranean Sea from the Red Sea.

Methods

Sampling, DNA extraction, amplification, and sequencing

Samples from Mozambique (ZMBN 94192.1, ZMBN 94192.2) and Italy (ZMBN 106843, ZMBN 106844, ZMBN 106845) were collected by the authors, whereas those from Marshall Islands (ZMBN 106813, ZMBN 106814) and Cyprus (MNCN/ADN: 86334) have been donated by colleagues (see Acknowledgements). The DNA sequence of the specimen from Lizard Island, Australia, was obtained from GenBank.

The specimen from Cyprus was collected by divers using SCUBA on 21 October 2015 on a rocky wall in water 2 m deep, and it was crawling on algae. Specimens from Italy were collected by snorkeling in water 0.9 to 1.5 m deep at Capo Peloro Lagoon, NE Sicily, Mediterranean Sea, between March–June 2015. This shallow Lagoon is a transitional environment formed by two interconnected basins, Lago di Faro and Lago di Ganzirri, each of them communicating with the Strait of Messina and is characterized by bottoms of coarse sand and shell debris with coralline algae and artificial rocky banks. Three specimens were collected and preserved in 96% ethanol and an additional 12 specimens were observed. All specimens were found crawling actively, but mating and egg-laying were not witnessed.

DNA was extracted from tissue obtained from parapodial lobes using the Qiagen DNeasy Blood and Tissue Kit following the protocol recommended by the manufacturer. Partial sequences of the mitochondrial gene cytochrome *c* oxidase subunit I (COI; ca. 660 bp) were amplified using the universal primers: LCO1490 (F) GGTCACAAATCATAA-AGATATTGG and HCO2198 (R) TAAACTT-CAGGGTGACCAAAAATCA (Folmer et al. 1994), following the protocol described by Malaquias et al. (2009). The quality and quantity of PCR products were assessed by gel-electrophoresis following standards methods (see Eilertsen and Malaquias 2013). Successful PCR products were purified according to the EXO-SAP method described by Eilertsen and Malaquias (2013). Sequence reactions were run on an ABI 3730XL DNA Analyser (Applied Biosystems).

Acronyms: ZMBN, Department of Natural History, University Museum of Bergen, University of Bergen, Norway. MNCN/ADN: DNA collection, Museo Nacional de Ciencias Naturales, Madrid, Spain.

Phylogenetic analyses and molecular species delimitation

The programme Geneious (v. 6.1.4 Biomatters Ltd.) was used to inspect, edit, and assemble the chromatograms of the forward and reverse DNA strands. All sequences were blasted in GenBank to check for contamination. Single gene sequences were aligned with Muscle (Edgar 2004a, b) implemented in Geneious. Alignments were trimmed to a position at which at least 50% of the sequences had nucleotides and missing positions at the ends were coded as missing data (N). Saturation was tested for the first, second, and third codon positions in MEGA5 (Tamura et al. 2011) by plotting uncorrected pairwise distances against total substitutions (transitions + transversions). The jModeltest software (Darriba et al. 2012) was used to find the best-fit model of evolution under the Akaike information criterion (Akaike 1974).

The Bayesian analyses were performed in MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using two parallel runs of 5 million generations with sampling every 100 generations and a burn-in set to 20%. Convergence of runs was inspected in Tracer v1.5 (Rambaut and Drummond 2007). The consensus phylogram was annotated and converted to graphics in FigTree v1.3.1 (Rambaut and Drummond 2009).

An Automatic Barcode Gap Discovery (ABGD) species delimitation analysis (Puillandre et al. 2012) was performed via the web interphase at <http://www.abi.snv.jussieu.fr/public/abgd/abgdweb.html> (version of 16 February 2016). ABDG was run by selecting Kimura 2-parameter distance (K2P) with transition/transversion ratio (TS/TV) equal to 2 and with a fasta file input of the alignment, with default values for Pmin, Pmax, and relative gap width.

Results

The specimens of *C. fulvipunctata* used in this study exhibited a wide range of colour patterns (Figure 1). The background colour ranged from ivory white to nearly black; however, all specimens showed the W-shaped mark that characterizes the species.

No evidence of saturation was observed and therefore all three codon positions were included in the genetics analysis. The best-fit model of evolution selected was the TVM+I+G with I=0.55 and G=0.64. Both the specimens of *C. fulvipunctata* from the Mediterranean Sea and those from the Indo-West Pacific clustered together in the phylogenetic tree (PP = 1; Figure 2), with maximum uncorrected *p*-

distances of 3.4% between a specimen from Italy and Lizard Island and 3.1% between two specimens from Italy. Minimum differences ranged between 0–0.2% and were found between the specimen from Cyprus and the two specimens from Mozambique (Table 1).

The ABGD species delimitation method retrieved seven “initial partitions” and four “recursive partitions” with eleven species each rendering all samples of *C. fulvipunctata* monospecific. Only when values of intraspecific divergence (*P*) were lower than 0.0028 was a higher number of groups suggested (13) and only by the recursive analysis resulting in the split of *C. fulvipunctata* in three different lineages; two represented by single specimens from Italy and a third with all other Mediterranean and IWP specimens. According to Pullandre et al. (2007) higher splitting is expected at lower *P* values (*P* < 0.01); in which case, the method is prone to suggest less realistic species hypotheses scenarios (Figure 3, Table 2).

Literature data and our results reveal the species to now be present in the entire Mediterranean basin. After its first appearance in the eastern Mediterranean in Turkey in 1959, the species was observed in Israel in 1986 and in Cyprus in 2006. The first observations in the central Mediterranean Sea date from 1993 and 1998 in Malta and we now report it from Sicily, Italy. It was only in 2014 and 2015 that the species was found in the western Mediterranean, namely off the coast of France and in the Spanish Balearic Islands (Figure 4; Table 3).

The observation of a relatively large number of living adult specimens (15 specimens) between March and June 2015 at Lago di Faro, Sicily, Italy, suggests that this species is now established in the Mediterranean Sea.

Discussion

Our results show unequivocally that specimens of the W-shaped mark head-shield slug occurring in the Mediterranean Sea and those occurring in the Indo-West Pacific belong to the same species. Since the oldest name available for this species is *C. fulvipunctata*, the name *C. mediterranea* is confirmed as a junior synonym. Synonymy was previously suggested by Gosliner (1980) based on similarity of colour patterns between the morphotype described by Swennen (1961) from Turkey and a specimen from Hawaii. The question then becomes: how could *C. fulvipunctata* have entered the Mediterranean Sea? A priori, the most logical explanation is a route through the Suez Canal, since this pathway is responsible for most of the recent changes in faunal composition of the Mediterranean Sea (Gofas and

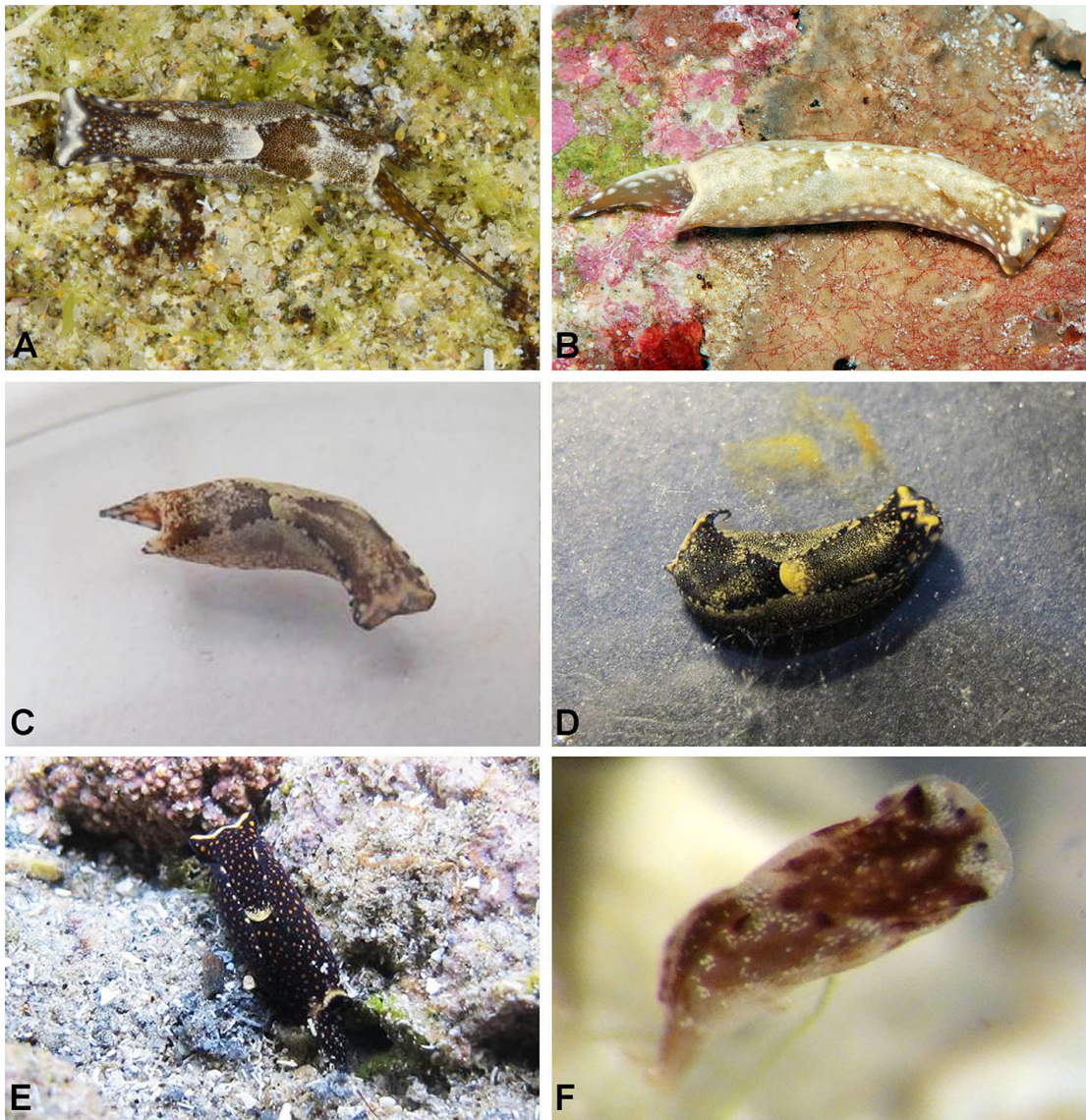


Figure 1. Diversity of coloration of phenotypes of *Chelidonura fulvipunctata* used in the present study. **A**, Mozambique (ZMBN94192; GenBank: KU183994, KU183995; photo credit: M.A.E. Malaquias). **B**, Marshall Is. (ZMBN106814; GenBank: KU183999; photo credit: S. Johnson and J. Johnson). **C**, Italy (ZMBN106845; GenBank KU184001; photo credit: A. Spinelli). **D**, Italy (ZMBN106844; GenBank: KU183997; photo credit: S.D. Matteo). **E**, Italy (ZMBN106843; GenBank: KU184000; photo credit: S.D. Matteo). **F**, Cyprus (MNCN/ADN:86334; GenBank: KU183998; photo credit: M. Poddubetskaia).

Zenetos 2003; Zenetos et al. 2010); a pathway first referred to as Lessepsian migration by Por (1978). However, the single observation (by Koretz 2005) of *C. fulvipunctata* in the Red Sea, a well-studied marine realm (Yonow 2008; 2012), conflicts with this hypothesis.

The first reports of *C. fulvipunctata* in the Mediterranean Sea were from the eastern basin (Turkey and Israel) and recent observations confirm that the species is slowly expanding westwards (Figure 4,

Table 3), which is consistent with a Lessepsian introduction. However, Gofas and Zenetos (2003) have drawn attention to the fact that several Indo-Pacific molluscan immigrants not known in the Red Sea were reported first in the Mediterranean from the southern coast of Turkey and from Cyprus, and only later were observed and eventually become established in the Levantine coast and Egypt. Therefore, their pattern of spreading is unlikely to be a progression from the Suez Canal.

Table 1. Uncorrected *p*-distances for the COI gene between all samples of *Chelidonura fulvipunctata* and *Chelidonura* sp. from Hawaii.

Species / uncorrected <i>p</i> -distance	<i>Chelidonura</i> sp. Hawaii KU183993	<i>C. fulvipunctata</i> Lizard I. AM421896	<i>C. fulvipunctata</i> Mozambique KU183995	<i>C. fulvipunctata</i> Mozambique KU183994	<i>C. fulvipunctata</i> Marshal Is KU183996	<i>C. fulvipunctata</i> Italy KU183997	<i>C. fulvipunctata</i> Cyprus KU183998	<i>C. fulvipunctata</i> Marshal Is KU183999	<i>C. fulvipunctata</i> Italy KU184000
<i>Chelidonura</i> sp. Hawaii KU183993									
<i>C. fulvipunctata</i> Lizard I. AM421896	0.096								
<i>C. fulvipunctata</i> Mozambique KU183995	0.100	0.008							
<i>C. fulvipunctata</i> Mozambique KU183994	0.098	0.010	0.002						
<i>C. fulvipunctata</i> Marshal Is KU183996	0.100	0.011	0.004	0.006					
<i>C. fulvipunctata</i> Italy KU183997	0.102	0.011	0.004	0.006	0.008				
<i>C. fulvipunctata</i> Cyprus KU183998	0.100	0.008	0.000	0.002	0.004	0.004			
<i>C. fulvipunctata</i> Marshal Is KU183999	0.098	0.010	0.002	0.004	0.006	0.006	0.002		
<i>C. fulvipunctata</i> Italy KU184000	0.096	0.034	0.027	0.029	0.027	0.031	0.027	0.025	
<i>C. fulvipunctata</i> Italy KU184001	0.100	0.031	0.023	0.025	0.027	0.027	0.023	0.021	0.019

Table 2. Automatic Barcode Gap Discovery (ABGD). Specific composition of the eleven and thirteen groups hypotheses.

Eleven groups hypothesis		Thirteen groups hypothesis	
Group	Species composition	Group	Species composition
1	<i>C. alisonae</i>	1	<i>C. alisonae</i>
2	<i>C. hirundinina</i>	2	<i>C. hirundinina</i>
3	<i>Aglaja tricolorata</i>	3	<i>Aglaja tricolorata</i>
4	<i>C. flavolobata</i> + <i>C. electra</i> + <i>C. amoena</i>	4	<i>C. flavolobata</i> + <i>C. electra</i> + <i>C. amoena</i>
5	<i>C. inornata</i>	5	<i>C. inornata</i>
6	<i>C. tsurugensis</i> + <i>C. sandrana</i>	6	<i>C. tsurugensis</i> + <i>C. sandrana</i>
7	<i>C. berolina</i>	7	<i>C. berolina</i>
8	<i>C. africana</i>	8	<i>C. africana</i>
9	<i>C. varians</i>	9	<i>C. varians</i>
10	<i>Chelidonura</i> sp.	10	<i>Chelidonura</i> sp.
11	All specimens of <i>C. fulvipunctata</i>	11	All specimens of <i>C. fulvipunctata</i> but two
-	-	12	<i>C. fulvipunctata</i> , Italy, KU184000
-	-	13	<i>C. fulvipunctata</i> , Italy, KU184001

There are a number of examples of this pattern of Indo-Pacific species bypassing the Red Sea when colonizing the Mediterranean Sea. One example is the Persian conch *Conomurex persicus* (Swainson, 1821), a species native to the Persian Gulf and southern Arabia and absent from the Red Sea, that was first reported in the Mediterranean in 1978 from

Turkey and is today present in Israel, Greece, Cyprus, and Lebanon, and it has become invasive in most of these places (Oliverio 1995; Gofas and Zenetos 2003; Zenetos et al. 2003). Other examples of this pattern are the shelled gastropod *Oscilla jocosa* Melvill, 1904; the nudibranch *Melibe viridis* (Kelaart, 1858), and the bivalve *Saccostrea cucullata* (Born, 1778) all

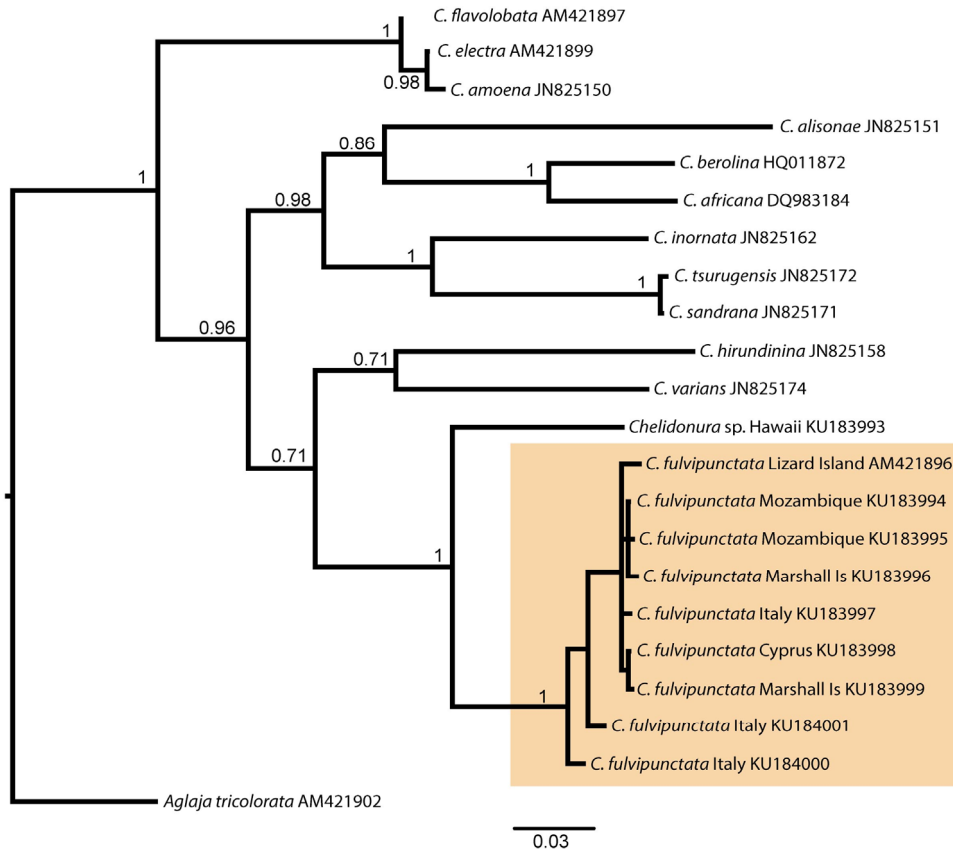


Figure 2. Bayesian phylogenetic tree based on the COI mitochondrial gene. Figures above branches are posterior probabilities. Coloured square highlights the cluster of specimens of *Chelidonura fulvipunctata*. Species names are followed by geographic origin of samples and GenBank Accession numbers.

present in areas of the Indo-Pacific and Mediterranean Sea, but absent from the Red Sea basin (Gofas and Zenetos 2003; Zenetos et al. 2003; Yonow 2008). All of these examples represent benthic species with limited mobility making colonization of the Mediterranean Sea while bypassing the Red sea by natural means unlikely.

It has been proposed that shipping can explain the presence of species with disjunct geographic distributions, *i.e.* present in the Mediterranean and Indo-Pacific, but absent in the Red Sea. Gofas and Zenetos (2003) suggested that an occurrence first reported in a harbour could be regarded as evidence consistent with this pathway. Several localities in Turkey are important commercial and recreational harbours, which is the case of Selimiye (type locality of *C. mediterranea*) a former traditional boat-builders' village popular with yacht owners.

Another interesting case is the invasive Indian Ocean and Red Sea bivalve species *Brachidontes pharaonis* (P. Fischer, 1870) where the use of molecular

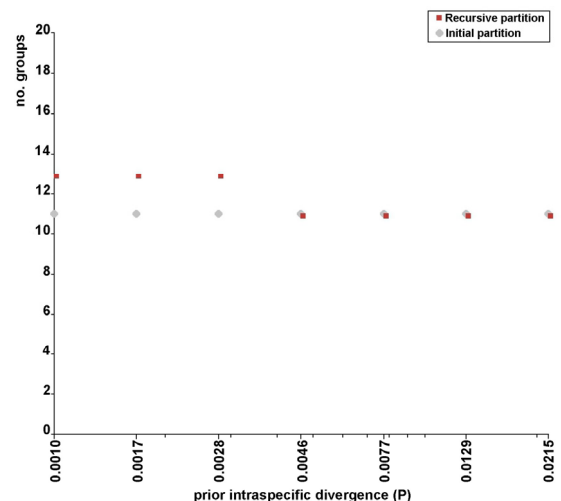


Figure 3. Results of the Automatic Barcode Gap Discovery (ABGD) analysis showing the 14 partitions recovered (initial and recursive). Nearly all partitions suggested the existence of 11 species (= No of groups) with all representatives of *C. fulvipunctata* rendered monospecific. Only recursive partitions rendered a higher number of species (13) when P values were ≤ 0.0028 .

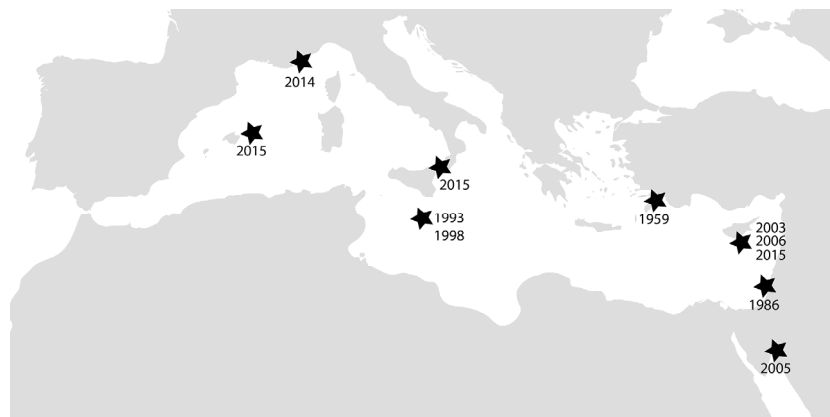


Figure 4. Distribution of *Chelidonura fulvipunctata* in the Mediterranean Sea and Red Sea. Dates refer to the years when specimens were observed and/or collected.

Table 3. Records of *Chelidonura fulvipunctata* in the Mediterranean Sea (we consider the occurrence of this species in Lebanon not confirmed based on Crocetta et al. 2013). *Geographical coordinates estimated based on sampling locations mentioned in original articles.

Locality	Ecology	No specimens	Length (mm)	First occurrence	Citation	Coordinates
Turkey: Selimiye, Antalya	0.5 m; under a stone	1	18	4 May 1959	Swennen (1961)	* 36°45'52.85"N–31°23'26.19"E
Israel: Ashqelon	12 m; on a rock	1	10	2 August 1986	Mienis and Gat (1987)	* 31°40'53.60"N–34°33'5.39"E
Malta: Ghar Lapsi	3 m; under a sea urchin	1	28	19 August 1993	Perrone and Sammut (1997)	* 35°49'38.16"N–14°25'29.11"E
Malta: north-west side (S27) and south-west side of Malta I. (S31)	?	2	33 & ?	No date specified	Sammut and Perrone (1998)	* S27: 35°59'5.02"N–14°19'32.68"E * S31: 35°49'3.22"N–14°27'31.80"E
Cyprus: Cyclops Cave (Protaras) and Agios Georgios Alamanou (Limassol)	3 m (crawling on sand) & 9 m (on rocks with algae)	2	?	June 2003 & December 2006	Tsiakkuros and Zenetos (2011)	Protaras: 34°59'10.34"N–34°04'35.8"E Limassol: 34°42'22.9"N–33°13'11.7"E
France: Agay, Saint-Raphael	3 m; under rocks	1	10–12	1 November 2014	Horst (2015)	43°25'34.81"N–06°52'34.55"E
Balearic Is: S'Algar, Menorca I., Spain	5 m; crawling on sandy-gravel	1	10	21 August 2015	Karachle et al. (2016)	39°49'772"N–04°18'183"E
Cyprus: Limassol, off Mediterranean Beach Hotel	2 m; crawling on algae	1	2	21 October 2015	Present study	34°42'27.3528"N–33°07'53.2380"E
Italy: Lago di Faro Messina, Sicily	0.9–1.5 m; crawling on soft bottom	15	26–30	March–June 2015	Present study	38°15'39.16"N–15°37'38.41"E

techniques revealed that Red Sea haplotypes are present in the Mediterranean Sea, but non-Red Sea haplotypes are also rather common (Shefer et al. 2004). This suggests that the presence of this species in the Mediterranean Sea is the combination of ship transport from distant populations and natural Lessepsian immigration (presumably of pelagic larvae) from the Red Sea.

Still, the species *C. fulvipunctata* is a difficult case because it is established in the Mediterranean Sea (Sciberras and Schembri 2007; Zenetos et al. 2010; Karachle et al. 2016; present work) and widely distributed in the Indo-West Pacific (Gosliner et al. 2008b), yet there is but a single report for the Red Sea (Koretz 2005) despite the large number of studies on opisthobranchs in this realm (Yonow

2008). It is also noteworthy that the Red Sea observation comes from Eilat in the Gulf of Aqaba in the northern part of the basin and was made nearly half a century after the species was first reported in the Mediterranean Sea, and after it was recorded in eastern Mediterranean localities such as Cyprus, Israel, and Turkey and in the central Mediterranean island of Malta. Therefore, we propose a reinterpretation of the observations available for this species. We consider, in light of present data and geographical distribution of *C. fulvipunctata*, the presence of this species in the Red Sea to be more parsimoniously explained by an expansion from the Mediterranean into the Red Sea rather than dispersal from the Indo-West Pacific to the Red Sea. While the Red Sea is typically regarded as a donor of species, with the Suez Canal acting as the main gateway for tropical/subtropical marine species to move into the Mediterranean (Zenetos et al. 2010), we postulate that this man-made seaway can act as a “revolving door”. This revolving door would allow both species of Indo-Pacific/Red Sea affinity to enter the Mediterranean and species established in the Mediterranean resilient to tropical/subtropical environmental conditions that may have arrived by shipping or mariculture to enter the Red Sea. The Red Sea is certainly the main donor of alien species present in the Mediterranean (Zenetos et al. 2010), but we hypothesize that the Red Sea can also be a receiver of species, in this case of Indo-Pacific origin, but not ruling out colonization by species of tropical Atlantic origin. The presence of fish species from warm Atlantic waters in the Mediterranean is well documented (Golani et al. 2002), but it is much less significant for all other taxonomic groups (Zenetos et al. 2010). For example, there are less than 10 species of molluscs of tropical/sub-tropical Atlantic origin established in the Mediterranean; all of them believed to have entered the basin by shipping through the Strait of Gibraltar (Gofas and Zenetos 2003; Zenetos et al. 2010). To date, none of these have been detected in the Red Sea.

A possible way to verify our hypothesis could be by means of a haplotype network analysis comparing populations from the Mediterranean with those from the Red Sea and localities in the Indo-West Pacific in a similar fashion to the study by Shefer et al. (2004) for the bivalve *Brachidontes pharaonis*. Shared presence of unique haplotypes between Red Sea and Mediterranean animals could be interpreted as evidence for Lessepsian immigration, and therefore, would reject our hypothesis. Otherwise, because of the relative isolation of the Red Sea (DiBattista et al. 2015; Hui et al. 2016), similarity between haplotypes from all three geographical sources could be inter-

preted as invasion of the Mediterranean from non-Lessepsian routes with later expansion into the Red Sea. At present the difficulty to test this hypothesis is obvious, mainly because the species seem to be rare, or even non-existent, in the Red Sea and a minimum of 10–30 specimens per site would be required to yield significant results. Yet, this approach could be useful to ascertain the Indo-West Pacific area of origin of our Mediterranean specimens and help to understand which shipping routes may have been responsible for the introduction of this species in the Mediterranean basin.

Acknowledgements

We are grateful to S. Johnson and J. Johnson for samples and images of *C. fulvipunctata* from the Marshall Islands, to M Poddubetskaia for the image and specimen of *C. fulvipunctata* from Cyprus, to M. Martin-Hervás (U. Cadiz) for help with image editing, and to A. Zenetos (Hellenic Centre for Marine Research) for valuable discussions on the invasive routes of alien species in the Mediterranean Sea. At the University of Bergen, Norway we thank L. Lindblom and K. Meland (Marine Biodiversity Laboratory) for help with molecular work. This work received financial support from the National Council of Science and Technology, Mexico (scholarship CONACYT/BAZS/188890 granted to the second author and scholarship CONACYT/JDOG/210391 granted to the penultimate author), University of Bergen, and University of Cadiz. This is CEI-MAR journal publication 126.

References

- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723, <http://dx.doi.org/10.1109/TAC.1974.1100705>
- Baba B (1938) Opisthobranchia of Kii, middle Japan. *Journal of the Department of Agriculture, Kyusyu Imperial University* 6: 1–19
- Crocetta F, Zibrowius H, Bitar G, Templado J, Oliverio M (2013) Biogeographical homogeneity in the eastern Mediterranean Sea – I: the opisthobranchs (Mollusca: Gastropoda) from Lebanon. *Mediterranean Marine Science* 14(2): 403–408, <http://dx.doi.org/10.12681/mms.404>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772, <http://dx.doi.org/10.1038/nmeth.2109>
- DiBattista JD, Choat JH, Gaither MR, Hobbs J-PA, Lozano-Cortés DF, Myers RF, Paulay G, Rocha LA, Toonen RJ, Westneat MW, Berumen ML (2015) On the origin of endemic species in the Red Sea. *Journal of Biogeography* 43: 13–30, <http://dx.doi.org/10.1111/jbi.12631>
- Edgar RC (2004a) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797, <http://dx.doi.org/10.1093/nar/gkh340>
- Edgar RC (2004b) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 113, <http://dx.doi.org/10.1186/1471-2105-5-113>
- Eilertsen MH, Malaquias MAE (2013) Systematic revision of the genus *Scaphander* (Gastropoda, Cephalaspidea) in the Atlantic Ocean, with a molecular phylogenetic hypothesis. *Zoological Journal of the Linnean Society* 167: 389–429, <http://dx.doi.org/10.1111/zoj.12013>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299

- Gofas S, Zenetos A (2003) Exotic molluscs in the Mediterranean basin: current status and perspectives. *Oceanography and Marine Biology: an Annual Review* 41: 237–277
- Golani D, Orsi-Relini L, Massuti E, Quignard JP (2002) CIESM Atlas of exotic species in the Mediterranean. Volume 1. Fishes, F. Briand (ed.). Monaco: CIESM
- Gosliner TM (1980) Systematics and phylogeny of the Aglajidae (Opisthobranchia: Mollusca). *Zoological Journal of the Linnean Society* 68:325–360, <http://dx.doi.org/10.1111/j.1096-3642.1980.tb01925.x>
- Gosliner T (1987) Nudibranchs of Southern Africa. A guide to opisthobranch molluscs of southern Africa. Sea Challengers & Jeff Hamann Pub., 136 pp
- Gosliner TM, Cervera JL, Ghiselin MT (2008a) Scientific exploration in the Mediterranean region biodiversity of the Mediterranean opisthobranch gastropod fauna: historical and phylogenetic perspectives. *Proceedings of the California Academy of Sciences* 59 (supplement 1): 117–137
- Gosliner TM, Behrens DW, Valdés Á (2008b) Indo-Pacific nudibranchs and sea slugs: a field guide to the world's most diverse fauna. Sea Challengers Natural History Books, California, 426 pp
- Gosliner TM, Valdés Á, Behrens DW (2015) Nudibranchs & Sea Slugs Identification. Indo-Pacific. New World Publications, Inc., Jacksonville, Florida, USA, 408 pp
- Horst B (2015) Premier signalement d'un spécimen vivant de *Chelidonura fulvipunctata* (Gastropoda: Cephalaspeida: Aglajidae) en Méditerranée occidentale, sur les côtes françaises. *Xenophora* 150: 18–20
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755, <http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Hui M, Kraemer WE, Seidel C, Nuryanto A, Joshi A, Kochzius M (2016) Comparative genetic population structure of three endangered giant clams (Cardiidae: Tridacna species) throughout the Indo-West Pacific: implications for divergence, connectivity and conservation. *Journal of Molluscan Studies*, <http://dx.doi.org/10.1093/mollus/eyw001>
- Karachle PK, Angelidis A, Apostolopoulos G, Ayas D, Ballesteros M, Bonnici C, Brodersen MM, Castriota L, Chalari N, Cottalorda JM, Crocetta F, Deidun A, Đodo Ž, Dogrammatzi A, Dulčić J, Fiorentino F, Gönülal O, Harmelin JG, Insacco G, Izquierdo-Gómez D, Izquierdo-Muñoz A, Joksimović A, Kavadas S, Malaquias MAE, Madrenas E, Massi D, Micarelli P, Minchin D, Ōnal U, Ovalis P, Poursanidis D, Siapatis A, Sperone E, Spinelli A, Stamouli C, Tiralongo F, Tunçer S, Yaglioglu D, Zava B, Zenetos A (2016) New Mediterranean Biodiversity Records (March 2016). *Mediterranean Marine Science* 17: 230–252
- Koretz B (2005) *Chelidonura fulvipunctata* from the Red Sea. [Message in Sea Slug Forum (Apr 23)]. Australian Museum, Sydney. Available from <http://www.seaslugforum.net/find/13596>
- Malaquias MAE, Mackenzie-Dodds J, Bouchet P, Gosliner T, Reid DG (2009) A molecular phylogeny of the Cephalaspeida *sensu lato* (Gastropoda: Euthyneura): Architectibranchia redefined and Runcinacea reinstated. *Zoologica Scripta* 38: 23–41, <http://dx.doi.org/10.1111/j.1463-6409.2008.00354.x>
- Mienis HK, Gat G (1987) A record of the Indo-Pacific species *Chelidonura fulvipunctata* from the Mediterranean coast of Israel (Opisthobranchia, Aglajidae). *Levantina* 67: 709–711
- Nakano R (2004) Opisthobranchs of Japan Islands. Rutles, Inc., Tokyo, Japan, 304 pp
- Oliverio M (1995) The status of the living Mediterranean Strombus or: What is a lessepsian migrant? *Notiziario del CIESMA* 16: 35–40
- Perrone AS, Sammut C (1997) Opisthobranchia of the genus *Chelidonura* Adams, 1850 (Cephalaspeida) from the Isle of Malta. *Basteria* 61: 61–70
- Por FD (1978) Lessepsian migration: the influx of Red Sea biota into the Mediterranean by way of the Suez Canal. *Ecological Studies* 23: 1–228, http://dx.doi.org/10.1007/978-3-642-66728-2_1
- Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* 21: 1864–1877, <http://dx.doi.org/10.1111/j.1365-294X.2011.05239.x>
- Rambaut A, Drummond A (2007) Tracer v.1.5. <http://beast.bio.ed.ac.uk/Tracer>
- Rambaut A, Drummond A, (2009) FigTree v1. 3.1. Computer Program and Documentation Distributed by the Author. <http://tree.bio.ed.ac.uk/software>
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574, <http://dx.doi.org/10.1093/bioinformatics/btg180>
- Sammut C, Perrone AS (1998) A preliminary check-list of Opisthobranchia (Mollusca, Gastropoda) from the Maltese islands. *Basteria* 62: 221–240
- Sciberras M, Schembri PJ (2007) A critical review of records of alien marine species from the Maltese Islands and surrounding waters (Central Mediterranean). *Mediterranean Marine Science* 8: 41–66, <http://dx.doi.org/10.12681/mms.162>
- Shefer S, Abelson A, Mokady O, Geffen ELI (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? *Molecular Ecology* 13(8): 2333–2343, <http://dx.doi.org/10.1111/j.1365-294X.2004.02227.x>
- Swennen C (1961) On a collection of Opisthobranchia from Turkey. *Zoologische Mededelingen* 38: 41–75
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739, <http://dx.doi.org/10.1093/molbev/msr121>
- Tsiakkios L, Zenetos A (2011) Further additions to the alien mollusc fauna along the Cypriot coast: new Opisthobranchia species. *Acta Adriatica* 52: 115–124
- Wells FE, Bryce CW (1993) Sea slugs of Western Australia. Western Australia Museum, Perth, 184 pp
- Yonow N (2008) Sea Slugs of the Red Sea. Pensoft Publishers, Sofia, Bulgaria, 304 pp
- Yonow N (2012) Opisthobranchs from the western Indian Ocean, with descriptions of two new species and ten new records (Mollusca, Gastropoda). *Zookeys* 197: 1–129, <http://dx.doi.org/10.3897/zookeys.197.1728>
- Zenetos A, Gofas S, Russo G, Templado J (2003) CIESM Atlas of Exotic Species in the Mediterranean. Vol. 3 Molluscs. F. Briand Ed., CIESM Publishers, Monaco, 376 pp
- Zenetos A, Gofas S, Verlaque M, Cinar ME, Garcia JE, Bianchi CN, Morri C, Azzurro E, Bilecenoglu M, Froglija C, Siokou I, Violanti D, Sfriso A, San Martin G, Giangrande A, Katagan T, Ballesteros E, Ramos-Esplá A, Mastrotoataro F, Oca-a O, Zingone A, Gambi MC, Streftaris N (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterranean Marine Science* 11: 381–493, <http://dx.doi.org/10.12681/mms.87>