BioOne COMPLETE

DNA Taxonomy Confirms the Identity of the Widely-Disjunct Mediterranean and Atlantic Populations of the Tufted Ghost Crab Ocypode cursor (Crustacea: Decapoda: Ocypodidae)

Authors: Vecchioni, Luca, Marrone, Federico, Deidun, Alan, Adepo-Gourene, Béatrice, Froglia, Carlo, et al.

Source: Zoological Science, 36(4) : 322-329

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs180191

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

ZOOLOGICAL SCIENCE **36**: 322–329 (2019)
© 2019 Zoological Society of Japan

DNA Taxonomy Confirms the Identity of the Widely-Disjunct Mediterranean and Atlantic Populations of the Tufted Ghost Crab *Ocypode cursor* (Crustacea: Decapoda: Ocypodidae)

Luca Vecchioni[1]*, Federico Marrone[1], Alan Deidun[2], Béatrice Adepo-Gourene[3], Carlo Froglia[4], Arnold Sciberras[5], Michel Bariche[6], Ali Çiçek Burak[7], Maria Foka-Corsini[8], and Marco Arculeo[1]

[1]*University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), via Archirafi 18, 90123 Palermo, Italy*
[2]*University of Malta, Physical Oceanography Research Group, Department of Geosciences, Msida, Malta MSD 2080*
[3]*University of Nangui Abrogoua, Laboratory of Genetic and Bioresources Improvement, Research and Training Unity-, 02 BP 801 Abidjan 02, Côte d'Ivoire*
[4]*CNR – IRBIM u.o.s. Ancona, Largo Fiera della Pesca, I-60125 Ancona, Italy*
[5]*'Arnest`, Arcade Str, Paola, Malta*
[6]*American University of Beirut, Beirut 1107 2020, Lebanon*
[7]*Eastern Mediterranean University (EMUURIC), Underwater Research and Imaging Center, northern Cyprus, Turkey*
[8]*Hellenic Centre for Marine Research, Institute of Oceanography, Hydrobiological Station of Rhodes, Cos Street, 85100 Rhodes, Greece*

The distribution area of the tufted ghost crab *Ocypode cursor* includes two widely separate sub-areas, i.e. the tropical and subtropical Atlantic coasts of Africa and Macaronesia, and the central-eastern Mediterranean basin. The current disjunct distribution of the species is possibly the remnant of a previous wider and continuous distribution area that was fragmented during the Pleistocene, with the disappearance of the species from the temperate Atlantic Ocean and the western Mediterranean basin, and its survival in the warmer areas of the eastern Atlantic and the Mediterranean Sea. Such disjunction is thus compatible with an ancient isolation between the Mediterranean and Atlantic populations of the species, which could in fact constitute two well-characterized independent evolutionary lineages, or even two cryptic species. Unexpectedly, the sequencing of a fragment of the mtDNA COI gene from Mediterranean and Atlantic *Ocypode cursor* allopatric populations showed the cohesion of the species throughout its distribution range, and the nesting of Mediterranean populations within the single Atlantic population studied. This pattern is hereby tentatively ascribed to an incomplete lineage sorting due to the large population sizes of both the Atlantic and Mediterranean subpopulations of the species. The current westward expansion of the species in the Mediterranean Sea originating from the Levantine basin, due to ongoing regional sea warming, follows a typical phalanx dispersal mode.

Key words: disjunct distribution, allopatric populations, phalanx dispersal mode, sea warming, tufted ghost crab

INTRODUCTION

The family Ocypodidae Rafinesque, 1815, includes a group of semi-terrestrial burrowing crabs that inhabits sandy beaches of tropical and temperate seas around the world (Dahl, 1953; Barass, 1963; Strachan et al., 1999), mostly with an allopatric distribution (Lucrezi and Schlacher, 2014). Sakai and Türkay (2013), in a morphological study of the family, divided it into the "ghost crab" subfamily Ocypodinae Rafinesque, 1815, and the "fiddler crab" subfamily Ucinae Dana, 1851, and within the Ocypodinae recognized 22 species. Shih et al. (2016), based on molecular evidences, rearranged this brachyuran family in three subfamilies: Ocy-

* Corresponding author. E-mail: luca.vecchioni@unipa.it
doi:10.2108/zs180191

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

podinae (including the genera *Afruca* Crane, 1975; *Ocypode* Weber, 1795; and *Uca* s.s. Leach, 1814), Gelasiminae Miers, 1886, and Ucidinae Števčić, 2005, with 25 species included in the genus *Ocypode*.

In the Mediterranean area, only the tufted ghost crab, *Ocypode cursor* (Linnaeus, 1758), is known to occur. The distribution of this species is disjunct in two widely separate sub-areas, i.e. an eastern Atlantic subarea encompassing Cape Verde archipelago and the African coasts from northern Namibia to Mauritania, where the species occurs sympatrically with *O. africana* De Man, 1881, and a Mediterranean subarea mostly limited to the eastern Mediterranean basin (Manning and Holthuis, 1981; Lucrezi and Schlacher, 2014).

The current Atlantic–Mediterranean disjunct distribution of the tufted ghost crab is possibly the remnant of a past wider and continuous Atlantic–Mediterranean distribution that experienced a fragmentation in the Pleistocene. The lowering of sea temperature during the Würm glaciation (Thiede, 1978) may have relegated this thermophilous species to the warmer (sub)tropical African Atlantic coast and the Levantine Mediterranean shores, while eradicating it from the colder waters of the western Mediterranean basin. The species has never been observed in the western Mediterranean basin, nor along the Atlantic coasts of Morocco, but according to Bianchi (2007), sea-warming might allow a secondary contact between the Mediterranean and Atlantic sub-populations of the species in the future. In fact, *O. cursor* was not included in the checklist of Sicilian marine decapods by Pipitone and Arculeo (2003), but see Froglia (1995) for its presence at Lampedusa. It is currently expanding westwards in the central Mediterranean basin, where it has been recently observed in southern Sicily and Malta (Froglia, 2010; Zafarana and Nardo, 2016; Deidun et al., 2017) and confirmed for Lampedusa (Prazzi et al., 2017). *Ocypode cursor* is currently listed among the protected marine crustaceans in the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention, 1995) and in the Convention on the Conservation of European Wildlife and Natural Habitats (Berne Convention, 1996-98) (Deidun et al., 2017).

Although no molecular data is available for this species to date, the sharply disjunct distribution of *Ocypode cursor* suggests an absence of gene flow between its Mediterranean and Atlantic populations, and the possible presence of independent allopatric evolutionary lineages, or even cryptic species, currently lumped under this binomen.

In order to test this hypothesis and to investigate the pattern of molecular diversity of *Ocypode cursor* throughout its distribution area, samples of the species from both subareas were collected and genetically analysed sequencing a fragment of the mitochondrial DNA marker cytochrome oxidase subunit I (mtDNA COI). Moreover, specimens of the closely related specie *O. africana* were sampled and included in the analyses. The COI dataset obtained was subsequently used to carry out the molecular identification of collected samples, and for investigating the mtDNA diversity pattern of *O. cursor* throughout its distribution range.

MATERIAL AND METHODS

Sampling activities

Ghost crabs belonging to the species *Ocypode cursor* and *O. africana* were sampled from 2016 to 2018 by active searching of specimens at sunset on sandy beaches in Italy, Malta, Greece, Cyprus and Lebanon (Mediterranean Sea), and Ivory Coast (Atlantic Ocean) (Table 1). The map of the sampling sites (Fig. 1) was realised using the QGIS software v. 2.18.2 (http://www.qgis.org). Collected ghost crabs were identified based on Sakai and Türkay (2013) and one leg was excised from each caught individual before releasing it back alive in the collection site; the loss of one leg does not constitute a harm to the sampled individuals, since they are able to regenerate any lost appendages at the next molt (Das, 2015, and references therein). Sampled legs were fixed *in situ* in 96% ethanol and then stored in freezer at –20°C. Tissue samples are currently stored in the crustacean tissue collection of the University of Palermo, Italy.

DNA extraction and amplification

Prior to DNA extraction, tissue samples were extracted from the excised legs with a scalpel. Tissue samples were then soaked in double-distilled water for 15 minutes and processed for DNA extraction using the BIORON GmbH "Ron's Tissue DNA Mini Kit" following the manufacturer's instructions. The selective amplification of a fragment from the gene encoding for the cytochrome oxidase subunit I (COI) was carried out by the polymerase chain reaction (PCR) using the primers COL6b (5′-ACA AAT CAT AAA GAT ATY GG-3′) and COH6 (5′-TAD ACT TCD GGR TGD CCA AAR AAY CA-3′), described by Schubart and Huber (2006). The PCR mix consisted of 18.05 µl double-distilled water, 2.5 µl Buffer 10X including 15 mM MgCl2 solution, 0.25 µl dNTPs (10 mM of each), 0.9 µl of each primer (10 µM), 0.4 µl Taq Polymerase 5u/µl and 2 µl of DNA template, for a total volume of 25 µl. The thermal cycle consisted of 35 cycles of denaturing (95°C 50 s), annealing (48°C 50 s) and extension (72°C 50 s), followed by seven minutes at 72°C for the final extension step. After PCR, 5 µl of each PCR product were separated by electrophoresis on a 2% agarose gel at 90 V for 20 minutes and visualized with a UV Transilluminator. When PCR products showed a clear and single band of the expected length, they were purified using the Exo-SAP-IT® kit (Affymetrix USB) and sequenced by Macrogen Inc. (Seoul, South Korea) with an ABI 3130xL (Applied Biosystems) sequencer. The same primers used for the PCR were subsequently used for direct sequencing of the PCR product, and the quality of the obtained chromatograms was checked through the measurement of their Phred scores (Richterich, 1998). Only sequences with continuous reads of high-quality bases (QV > 20) were used. Chromatograms were analysed and manually proofread using the software Chromas v. 2.6.2 (Technelysium, Pty. Ltd. 1998, Queensland, Australia) and aligned with ClustalX v. 2.1 (Larkin et al., 2007). MEGA v. 7.0 (Kumar et al., 2016) was used to translate the COI sequences to amino acids in order to check for the possible presence of frameshifts or stop codons, which would indicate the presence of sequencing errors or pseudogenes, a widespread phenomenon among crustaceans (Buhay, 2009).

Molecular identification and phylogeny

The molecular identification of the studied specimens and the phylogenetic analysis of the taxa was performed through Bayesian Inference (BI) and Maximum Likelihood (ML) analyses as implemented in MrBayes v. 3.2.6 (Ronquist et al., 2012) and PhyML v. 3 (Guindon and Gascuel, 2003). In addition to our novel sequences, 15 *Ocypode* spp. sequences and one sequence of *Uca major* Herbst, 1782 were downloaded from GenBank and included in the analyses (see Fig. 2 for the accession numbers of the downloaded sequences) to be used as outgroups. As a measure of branch support, BI posterior probability and ML bootstrap values (Felsenstein,

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

Table 1. List of the sampled sites. n: number of sampled individuals in each site.

Taxon	Country	Code	Location	n	Coordinates (WGS 84)	Sampling date	Origin
Ocypode cursor (Linnaeus, 1758)							
	Cyprus	FMB	Famagusta Bay	7	35.2244 N, 33.9034 E	May 2017	Present work
	Italy	GEL	Gela	3	37.0734 N, 14.2154 E	August 2018	Present work
	Italy	LAM	Lampedusa	1	35.5132 N, 12.5572 E	July 1978	Present work, Specimen reported in Froglia (1995)
	Italy	MEN	Menfi	2	37.5703 N, 12.9491 E	June 2017	Present work
	Italy	SAM	Sampieri	2	36.7207 N, 14.7415 E	August 2017	Present work
	Ivory Coast	ABI	Abidjan	7	5.1916 N, –3.7238 E	May 2017	Present work
	Lebanon	EMA	El Mansouri	4	33.1885 N, 35.1949 E	May 2017	Present work
	Malta	RLH	Ramla L-Hamra	2	36.0615 N, 14.2840 E	July 2016	Present work
	Malta	SBL	San Blas	2	36.0569 N, 14.3006 E	June 2016	Present work
	Rhodes	AGI	Aghios Giorgos	3	35.9144 N, 27.8261 E	September 2016	Present work
Ocypode africana De Man, 1881							
	Ivory Coast	ABI	Abidjan	2	5.1916 N, –3.7238 E	May 2017	Present work
	Liberia	–	Monrovia	1	–	–	Shih et al. (2016)
Ocypode ryderi Kingsley, 1880							
	Mozambique	–	Inharrime	1	–	–	Shih et al. (2016)
Ocypode brevicornis H. Milne-Edwards, 1837							
	India	–	–	1	–	–	Manikantan et al. (2016)
Ocypode cordimanus Latreille, 1818							
	Taiwan	–	Taitung	1	–	–	Wong et al. (2012)
Ocypode sinensis Dai, Song and Yang, 1985							
	Taiwan	–	Kending	1	–	–	Wong et al. (2012)
Ocypode kuhlii De Haan, 1835							
	Australia	–	Christmas Island	1	–	–	Shih et al. (2016)
Ocypode rotundata Miers, 1882							
	Iran	–	Persian Gulf	1	–	–	Shih et al. (2016)
Ocypode mortoni George, 1982							
	China	–	Hong Kong	1	–	–	Wong et al. (2012)
Ocypode stimpsoni Ortmann, 1897							
	Taiwan	–	Hsinchu	1	–	–	Wong et al. (2012)
Ocypode ceratophtalmus (Pallas, 1772)							
	China	–	Hong Kong	1	–	–	Wong et al. (2012)
Ocypode platytarsis H. Milne-Edwards, 1852							
	India	–	–	1	–	–	Manikantan et al. (2016)
Ocypode macrocera H. Milne-Edwards, 1852							
	India	–	–	1	–	–	Manikantan et al. (2016)
Ocypode occidentalis Stimpson, 1860							
	Panama	–	Playa Venado	1	–	–	Shih et al. (2016)
Ocypode gaudichaudii H. Milne-Edwards and Lucas, 1843							
	Panama	–	Culebra	1	–	–	Shih et al. (2016)
Ocypode quadrata (Fabricius, 1787)							
	USA	–	Virginia, Kiptopeke State Park	1	–	–	Aguilar et al. (Unpublished)
Uca major Herbst, 1782							
	Bahamas	–	Pigeon Creek	1	–	–	Shih (2015)

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

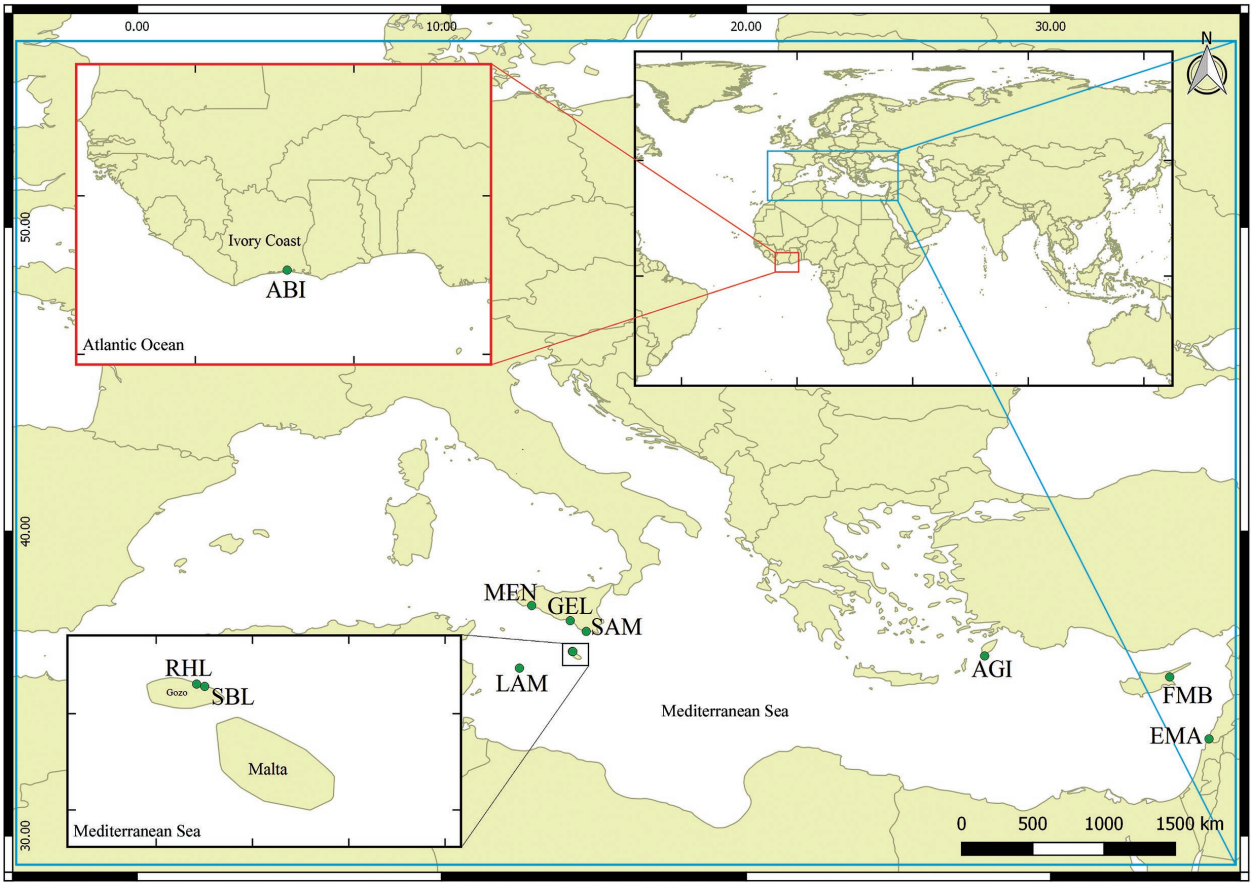

Fig. 1. Map of the sampling sites of *Ocypode cursor* and *O. africana*.

1985) were calculated. For the BI, two independent Markov Chain Monte Carlo analyses were run with 1 million generations (temp.: 0.2; default priors). Trees and parameter values were sampled every 100 generations, resulting in 10,000 saved trees per analysis; in the analysis, convergence was reached (Effective Sample Size (ESS) above 279.09); 2500 trees were conservatively discarded as "burn-in". The choice of the best evolutionary model was made using PartitionFinder v. 1.0.1 (Lanfear et al., 2012) according to the Akaike Information Criterion (AIC; Akaike, 1974). The BI and ML analyses were performed using a General Time-Reversible model of sequence evolution with a proportion of invariant sites (GTR+I; nst=6).

Molecular diversity pattern in *Ocypode cursor*

Sampling sites where *O. cursor* samples were collected were *a priori* partitioned into three geographical groups: (i) Eastern Mediterranean, i.e. Aegean and Levant seas (ii) Central Mediterranean, i.e. Strait of Sicily, and (iii) Eastern Atlantic Ocean. These geographical groups are hereafter referred to as 'E_MED', 'C_MED', and 'ATL' (see Table S1). A haplotype network including all the available *O. cursor* COI sequences was constructed through the software Popart (v. 1.7, (http://popart.otago.ac.nz), using the Minimum spanning method (Kruskal, 1956) (Fig. 3). In addition, haplotype diversity and nucleotide diversity were computed with the software DnaSP v. 5 (Librado and Rozas, 2009). In order to estimate the significance of the pair-wise differences in haplotype diversity between the three geographical groups, we used a series of functions, developed by Thomas et al., (2002) and available at http://www.ucl.ac.uk/mace-lab/resources/software ("TEST_h_DIFF"), through the statistical software "R" (v. 3.2.1, https://www.R-project.org).

RESULTS

In total, 35 *Ocypode* samples were collected from 10 coastal sites located in the Mediterranean Sea and the Atlantic Ocean (Table 1 see also Fig. 2 for their GenBank Accession Numbers). Only *Ocypode cursor* was observed in the Mediterranean Sea, whereas both *O. cursor* and *O. africana* were found sympatrically in Ivory Coast.

The obtained novel COI sequences aligned unambiguously and translated without stop codons, thus suggesting that no pseudogenes were erroneously amplified. After having trimmed the tails not present in all the specimens, a 555-bp long COI fragment was obtained. A total of 12 COI haplotypes were observed. Alignment is available from the first author on request.

BI and ML trees showed concordant topologies which support the morphological identification of the analysed specimens as *Ocypode cursor* and *O. africana* (Fig. 2), which proved to be sister species constituting a well-supported clade.

The *O. cursor* haplotype network showed that there are two major haplotypes shared by two groups, plus some rarer haplotypes shared by a single or by few individuals which are restricted to single areas (Fig. 3 and Supplementary Table S1).

Values of the Haplotypes diversity (Hd), nucleotide diversity (Pi) and the associated p-values related to the three geographical groups ("E_MED", "C_MED", "ATL") are

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

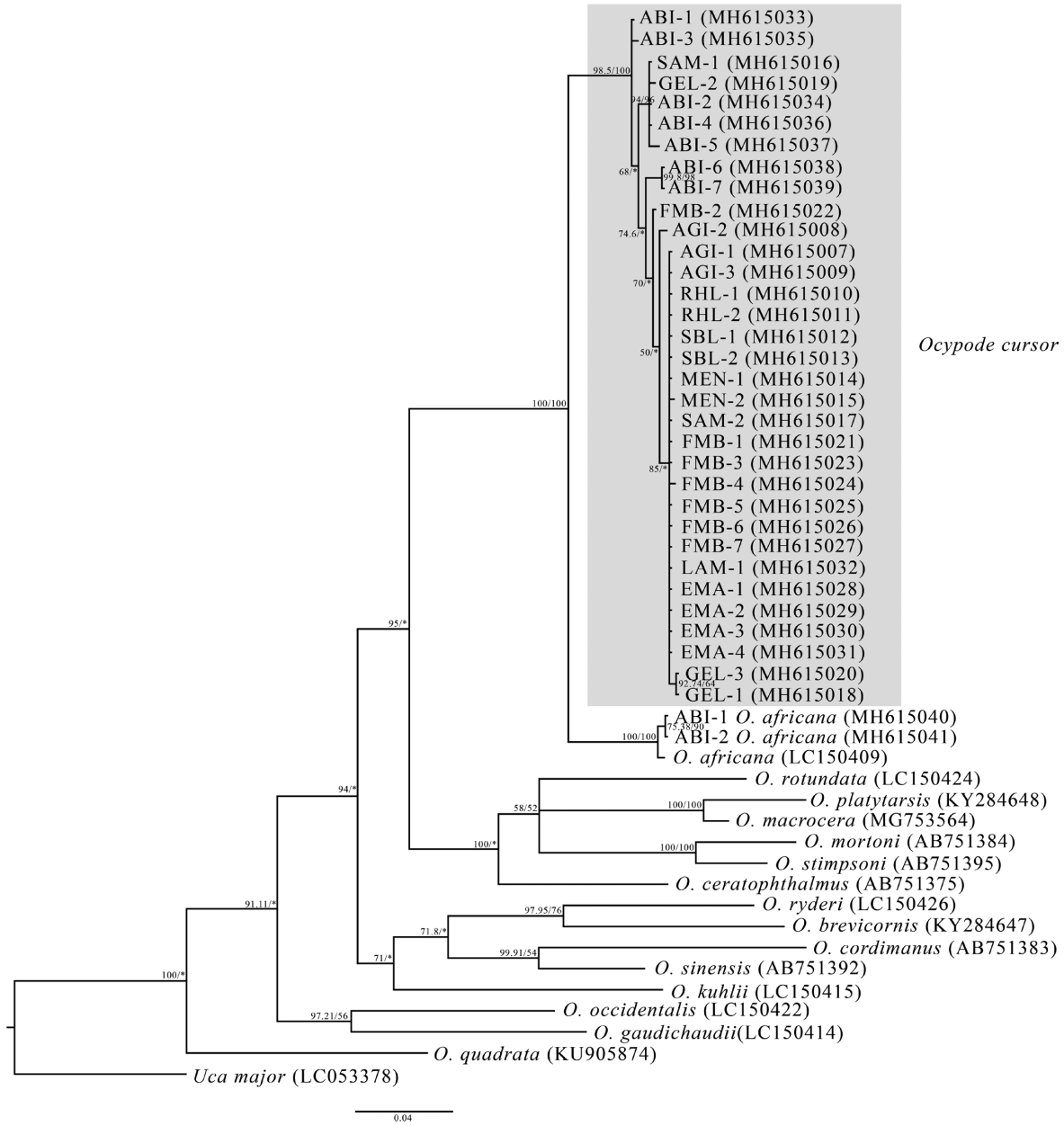

Fig. 2. Bayesian phylogram (95% majority rule consensus tree) of *Ocypode* spp. based on a fragment of 555 bp of the mitochondrial cytochrome oxidase 1 (COI). *Uca major* was used as outgroup. Node statistical support is reported as nodal posterior probabilities (BI)/bootstrap values (ML). Asterisks indicate a bootstrap support value lower than 50. The acronyms refer to the codes shown in Table 1. GenBank Accession Numbers are shown in brackets.

listed in Table 2.

DISCUSSION

The presence of *Ocypode cursor* on the shores of the Levant Sea was already known to Aristotle, (4th century BC) who, in part IV of his "History of Animals" commented "In the neighbourhood of Phoenice there are found on the beach certain crabs that are nicknamed the 'horsemen', from their running with such speed that it is difficult to overtake them" (translation in Cresswell, 1878). Until the past century, the known Mediterranean distribution of *O. cursor* was restricted to the warmer sector of the eastern basin, from Egypt to Israel, southern Turkey and the South-East Aegean Islands (Holthuis and Gottlieb, 1958; Glaubrecht, 1992), with a single old record from Greek mainland, near Megara (Guerin, 1832). In recent years, a westward expansion of the species has been observed in the Mediterranean, with records from Southern Sicily, Malta and Pelagie Islands (see Deidun et al., 2017 for a complete list). Along the West African coasts, the species is recorded from Namibia to Mauritania, including the Cape Verde archipelago (Manning and Holthuis, 1981), with the northernmost record being from Cap Blanc (Ras Nouadhibou) in Mauritania (Monod, 1956), whereas the species is absent from the shores of Morocco and of the

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

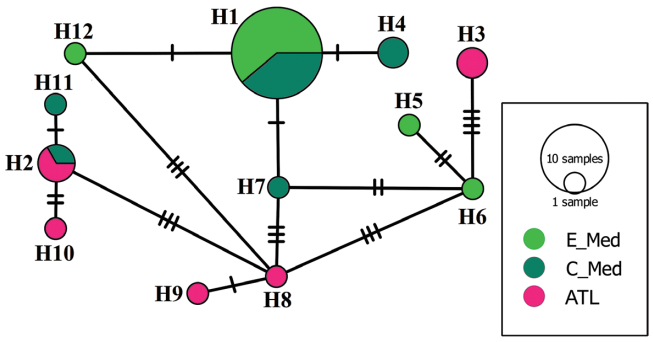

Fig. 3. Minimum Spanning Haplotype Network based on a fragment of 555 bp of mtDNA COI. Dashes indicate substitution steps. See Supplementary Table S1 for the haplotype codes.

Table 2. Summary of the Haplotype diversity (Hd), nucleotide diversity (π) and p-values obtained from the three geographical groups considered: East Mediterranean area, 'E_MED', Central Mediterranean area, 'C_MED', and Eastern Atlantic Ocean, 'ATL'.

Group	Hd	π	p-value
"E_MED"	0.396	0.001	–
"C_MED"	0.666	0.003	–
"ATL"	0.904	0.007	–
"ATL + E_MED + C_MED"	–	–	0.332
"E_MED + C_MED"	–	–	0.314
"ATL + E_MED"	–	–	< 0.0001
"ATL + C_MED"	–	–	< 0.0001

whole West Mediterranean area.

This study represents the first molecular approach towards the DNA taxonomy and phylogeography of the ghost crabs of Mediterranean Sea and Eastern Atlantic Ocean. Samples originating from both current distribution subareas of *Ocypode cursor* proved to be strictly conspecific, constituting the sister taxon of *O. africana*, with which it coexists throughout its eastern Atlantic distribution subarea. Present molecular data thus fully confirm the morphology-based identification of the tufted ghost crab populations, and the disjunct nature of its distribution area (Fig. 2).

No clear geographical pattern was observed in the distribution of the *O. cursor* haplotypes, nor any distinct Mediterranean vs. Atlantic haplogroups were observed; conversely, one haplotype proved to be shared by Mediterranean and Atlantic specimens (Fig. 3). Moreover, in spite of the smaller size of the studied Atlantic sample, Mediterranean *O. cursor* sequences nested within the Atlantic ones (Fig. 2). The absence of obvious molecular structuring of the species in spite of the presence of widely-disjunct allopatric populations is typical of those species whose populations have not been sundered by long-term biogeographical barriers and that are tightly connected in history (Avise, 2000). This seems to be the case of the tufted ghost crab, which probably had a continuous East Atlantic–Mediterranean distribution and an extensive gene-flow, until the last Pleistocene glaciation, as indicated by the abundant fossil remains of *O. cursor* found in a Tyrrhenian layer in Mallorca Island (West Mediterranean) (Via Boada, 1966). During the last glaciation (Würm), the lowering of the sea surface temperature, which was particularly pronounced in the central and western Mediterranean (Thiede, 1978), brought about the extirpation of the species in these sectors of the basin, while it survived in the warmer East Mediterranean refugia and along the tropical West African shores. The isolation of the two subpopulations of the species, however, has not been long enough to allow a complete lineage sorting of ancestral haplotypes for the studied mitochondrial DNA marker, thus resulting in the shallow, sympatric clades reported in Fig. 2.

At the Mediterranean Sea scale, no significant genetic differences were observed between *Ocypode cursor* samples from the Levantine basin and those from the recently-colonized central Mediterranean area, most likely colonized through larval drift, (see Fig. 2 and Table 2) This is in good agreement with the phalanx dispersal model (Hewitt, 1996), which describes the effects of slow, massive post-glacial expansions from refugia, keeping a significant connectivity between the source population(s) and the recently-colonized areas. The ongoing increase in sea surface temperatures (Shaltout and Omstedt, 2014; Pastor et al., 2017) may favour further spreading of the tufted ghost crab along the Mediterranean shores of North Africa and Italy, following a general pattern observed for several other coastal and marine taxa (e.g., Bianchi and Morri, 1993; Grubelic et al., 2004; Lejeusne et al., 2010; Caracappa et al., 2017; Sacco et al., 2017). However, the upwelling of cold waters along the Atlantic coast of North-West Africa (Pelegrí and Benazzouz, 2015) might act as an effective barrier to the northward expansion of the Atlantic subpopulation of the species and to the drift of its larvae into the western Mediterranean through the Gibraltar strait. Therefore, the reestablishment of a continuity between the Mediterranean and East Atlantic populations of *O. cursor* seems unlikely, at least in the near future.

ACKNOWLEDGMENTS

The research was partially supported by a financial grant from the Research Committee of the University of Malta and by the fund "PJ_RIC_FFABR_2017_010077" of the Italian Ministry of Education, Universities and Research (MIUR). The authors are indebted to Mr. Jeffrey Sciberras for his assistance during field sampling trips conducted in the Maltese Islands.

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

AD and MA planned and headed the project. LV, FM, AS, CF, ACB, BAG, MFC, MB, carried out the field work. LV and FM carried out the laboratory work and the analyses. LV and FM wrote a first draft of the manuscript, which was discussed and improved by all of the authors.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available online. (URL: https://bioone.org/journals/supplementalcontent/10.2108/zs180191/10.2108.zsj.36.322.s1.pdf)

Supplementary Table S1. Summary of the Haplotype and Geographical groups.

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

REFERENCES

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19: 716–723

Avise JC (2000) Phylogeography: the History and Formation of Species. Harvard University Press, Cambridge, MA

Barass R (1963) The burrows of *Ocypode ceratophthalmus* (Pallus) (Crustacea, Ocypodidae) on a tidal wave beach at Inhaca Island, Mozambique. J Anim Ecol 32: 73–85

Barcelona Convention (1995) Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean. Barcelona, Spain. Available at: http://ec.europa.eu/environment/marine/international-cooperation/regional-sea-conventions/barcelona-convention/index_en.htm

Bianchi CN (2007) Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia 580: 7–21

Bianchi CN, Morri C (1993) Range extensions of warm-water species in the northern Mediterranean: evidence for climatic fluctuations? Porcupine Newsletter 5: 156–159

Buhay JE (2009) "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. J Crustacean Biol 29: 96–110

Caracappa S, Persichetti MF, Piazza A, Gentile A, Marineo S, Currò V, et al. (2017) A new record and molecular characterization of the Green Turtle *Chelonia mydas* (Testudines, Cheloniidae) in Sicilian coastal waters. Rev Biol Mar Oceanogr 52: 163–167

Council of Europe (1979) Convention on the Conservation of European Wildlife and Natural Heritage. Bern, Switzerland. Available at: http://conventions.coe.int/Treaty/EN/Treaties/Html/104.htm

Cresswell R (1878) Aristotle's History of animals. In ten books. George Bell and Son, London: ix + 1–326

Dahl E (1953) Some aspects of the ecology and zonation of the fauna of sandy beaches. Oikos 4: 1–27

Das S (2015) Morphological, molecular, and hormonal basis of limb regeneration across Pancrustacea. Integr Comp Biol 55: 869–877

Deidun A, Crocetta F, Sciberras A, Sciberras J, Insacco G, Zava B (2017) The protected taxon *Ocypode cursor* (Linnaeus, 1758) (Crustacea: Decapoda: Ocypodidae) documenting its well-established presence in the central Mediterranean. Eur Zool J 84: 96–103

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791

Froglia C (1995) Crustacea Malacostraca III. (Decapoda). In "Checklist Delle Specie Della Fauna Italiana" Ed by A Minelli, S Ruffo, S La Posta, Calderini, Bologna. 31: 1–17

Froglia C (2010) Crustacea, Malacostraca, Decapoda. Biol Mar Medit 17: 519–534

Glaubrecht M (1992) On the chronology of the horseman crab *Ocypode cursor* (Linnaeus, 1758) in Eastern Mediterranean and the first evidence in SW-Anatolia. Zool Jahrb Abt Syst 119: 563–567

Grubelic I, Antolić B, Despalatović M, Grbec B, Paklar GB (2004) Effect of climatic fluctuations on the distribution of warm-water coral *Astroides calycularis* in the Adriatic Sea: news records and review. J Mar Biol Assoc UK 84: 599–602

Guérin FE (1832) Crustacés. In: Brullé A. - Des Animaux articules. Expedition Scientifique de Morée 3: 30–50

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696–704

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linnean Soc 58: 247–276

Holthuis LB, Gottlieb E (1958) An annotated list of the Decapod crustacea of the Mediterranean coast of Israel, with an appendix listing the Decapoda of the Eastern Mediterranean. B Res Counc Israel 7B: 1–126

Kruskal JB (1956) On the shortest spanning subtree of the graph and the travelling salesman problem. Proc Amer Math Soc 7: 48–57

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33: 1870–1874

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29: 1695–1701

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948

Lejeusne C, Chevaldonné P, Pergent-Martini C, Boudouresque CF, Thierry P (2010) Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends Ecol Evol 25: 250–260

Librado P, Rozas J (2009) DnaSP version 5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452

Lucrezi S, Schlacher TA (2014) The ecology of ghost crabs. Oceanogr. Mar Biol 52: 201–256

Manning RB, Holthuis LB (1981) West African Brachyuran Crabs (Crustacea: Decapoda). Smithson Contr Zool 306: xii + 1–379

Monod TH (1956) Hippidea et Brachyura ouest-africains. MIFAN 45: 1–674

Pastor F, Valiente JA, Palau JL (2017) Sea Surface Temperature in the Mediterranean: Trends and Spatial Patterns (1982–2016). Pure Appl Geophys 175: 4017–4029

Pelegrí JL, Benazzouz A (2015) Coastal upwelling off North-West Africa. In "Oceanographic and Biological Features in the Canary Current Large Marine Ecosystem" Ed by L Valdés, I Déniz-González, IOC-UNESCO, Technical Series 115: 93–103

Pipitone C, Arculeo M (2003) The marine Crustacea Decapoda of Sicily (central Mediterranean Sea): a checklist with remarks on their distribution. Ital J Zool 70: 69–78

Prazzi E, Billeci V, Maraventano G, Sanguedolce F, Sorrentino G (2017) Presenza del granchio fantasma *Ocypode cursor* (Linnaeus, 1758) (Malacostraca Decapoda Ocypodidae) nell'Isola di Lampedusa (Isole Pelagie, Canale di Sicilia). Naturalista Sicil 41(1): 95–99

QGIS Development Team (2016) QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: http://www.qgis.org/

R Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org

Richterich P (1998) Estimation of errors in "raw" DNA sequences: A validation study. Genome Res 8: 251–259

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, et al. (2012) MrBayes v. 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61: 539–542

Sacco F, Marrone F, Lo Brutto S, Besbes A, Nfati A, Gatt M, et al. (2017) The Mediterranean Sea hosts endemic haplotypes and a distinct population of the dolphinfish *Coryphaena hippurus* Linnaeus, 1758 (Perciformes, Coryphaenidae). Fish Res 186: 151–158

Sakai K, Türkay M (2013) Revision of the genus *Ocypode* with the description of a new genus, *Hoplocypode* (Crustacea: Decapoda: Brachyura). Mem Queensl Mus 56: 665–793

Schubart CD, Huber MGJ (2006) Genetic comparisons of German populations of the stone crayfish, *Austropotamobius torrentium* (Crustacea: Astacidae). Bull fr pêche piscic 380–381: 1019–1028

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut

Shaltout M, Omstedt A (2014) Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia 56: 411–443

Shih HT, Ng PKL, Davie PJF, Schubart CD, Türkay M, Naderloo R, et al. (2016) Systematics of the family Ocypodidae Rafinesque, 1815 (Crustacea: Brachyura), based on phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of *Uca* Leach, 1814, sensu lato and its subgenera. Raffles Bull Zool 64: 139–175

Strachan PH, Smith RC, Hamilton DAB, Taylor AC, Atkinson RJA (1999) Studies on the ecology and behaviour of the ghost crab, *Ocypode cursor* (L.) in Northern Cyprus. Sci Mar 63: 51–60

Thiede J (1978) A glacial Mediterranean. Nature 276: 680–683

Thomas MG, Weale ME, Jones AL, Richards M, Smith A, Redhead N, et al. (2002) Founding mothers of Jewish communities: geographically separated Jewish groups were independently founded by very few female ancestors. Am J Hum Genet 70: 1411–1420

Vía Boada L (1966) Abundantes restos de *Ocypoda cursor* (crustáceo decápodo) en el Cuaternario de Mallorca. Acta Geol Hisp 1: 22–24

Zafarana MA, Nardo A (2016) Sulla presenza di granchio fantasma *Ocypode cursor* (L.) (Malacostra Decapoda Ocypodidae) nel litorale sabbioso del golfo di Gela. Naturalista Sicil 40: 329–333

(Received November 29, 2018 / Accepted March 1, 2019)

Downloaded From: https://bioone.org/journals/Zoological-Science on 29 Apr 2024
Terms of Use: https://bioone.org/terms-of-use Access provided by American University of Beirut