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FURTHER EVIDENCE FOR DIVERSIFICATION WITHIN THE *DIOGENES PUGILATOR* COMPLEX (ANOMURA, DIOGENIDAE) IN THE MEDITERRANEAN AND BLACK SEAS

BY

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ABSTRACT

We document an unrecognised genetic lineage within the European hermit crab *Diogenes pugilator* species complex. Specifically, we propose the revalidation of the previously synonymised species *D. ponticus*, for specimens previously identified as *D. pugilator* from the Adriatic, Ionian, Aegean, and Black Seas. Analyses of the mitochondrial genes COI and 16S rRNA resulted in a clear genetic separation to East Atlantic and Western Mediterranean populations of *Diogenes*. Morphological analyses confirmed differences in the individuals assigned to *D. ponticus* compared to the congeners *D. curvimanus* and *D. pugilator* and thereby a concordance between molecular and morphological characters. Despite the relatively high morphological resemblance to *D. armatus*, the molecular differentiation and the remarkable geographic segregation within the species complex suggest at least one speciation or radiation event in the past, underlining the growing importance of molecular methods in the identification of species and evolutionarily significant units. Major oceanographic processes that are known to shape genetic variability and population genetic structure in the marine environment are pointed out as possible structuring factors for the observed intrageneric divergence. The evidence of long evolutionary independence is therefore considered sufficient to

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re-establish the validity of *D. ponticus* as a valid species in the northern-central and north-eastern Mediterranean Sea and the Black Sea and is redescribed accordingly.

Key words. — Biogeography, comparative sequence analysis, *Diogenes ponticus*, molecular systematics, morphological comparison, taxonomy

ZUSAMMENFASSUNG

In dieser Studie dokumentieren wir eine bisher unerkannte genetische Einheit innerhalb des Artenkomplexes des europäischen Einsiedlerkrebses *Diogenes pugilator*. Insbesondere schlagen wir die Wiedereinführung des zuvor synonymisierten Artnamens *D. ponticus* für Exemplare aus der Adria, dem Ionischen Meer, der Ägäis und dem Schwarzen Meer vor, die zuvor als *D. pugilator* identifiziert wurden. Analysen der mitochondrialen Gene COI und 16S rRNA ergaben eine klare genetische Abgrenzung zu *Diogenes* Populationen des Ost-Atlantik und des westlichen Mittelmeers. Morphologische Betrachtungen bestätigten Unterschiede zwischen den Individuen, die *D. ponticus* zugeordnet wurden, verglichen mit *D. curvimanus* und *D. pugilator* und damit eine Konkordanz zwischen molekularen und morphologischen Merkmalen. Trotz der vergleichsweise großen morphologischen Ähnlichkeit zu *D. armatus* deuten die molekulare Differenzierung, als auch die bemerkenswerte geographische Aufspaltung innerhalb des Artenkomplexes auf mindestens ein Speziations- oder Radiationsereignis in der Vergangenheit hin. Sie unterstreichen somit die wachsende Bedeutung molekularer Methoden in der Bestimmung von Arten und evolutionär bedeutsamen Einheiten. Es wird auf wichtige ozeanografische Prozesse, von denen bekannt ist, daß sie die genetische Variabilität und genetische Struktur von Populationen im marinen Lebensraum beeinflussen, als mögliche Strukturierungsfaktoren für die beobachtete intragenerische Divergenz hingewiesen. Die nachgewiesene langjährige evolutionäre Unabhängigkeit wird daher als ausreichend angesehen, um die Gültigkeit von *D. ponticus* als gültige Art im nordzentralen und nordöstlichen Mittelmeer, sowie im Schwarzen Meer wiederherzustellen und ist entsprechend neu beschrieben.

Schlüsselwörter. — Biogeographie, *Diogenes ponticus*, molekulare Systematik, morphologischer Vergleich, Taxonomie, vergleichende Sequenzanalyse

INTRODUCTION

Taxonomic inventories of all life forms and the environments they inhabit are considered a powerful tool in handling global environmental challenges, such as the undeniable global loss of biodiversity that has been recognised for decades now (Cracraft, 2002; Cochrane et al., 2016). However, while a growing interest in the study and conservation of biodiversity can be perceived worldwide, an estimated percentage of approximately 90% of all species remain undescribed or unrecognised (Blaxter, 2016). Given the inherent limitations of morphology-based diversity estimates (Hebert et al., 2003), modern taxonomy now seeks to integrate as many sources of information as possible. Molecular approaches such as DNA barcoding have shown to be effective tools for species discovery, contributing to current biodiversity assessments and classifications (Hebert et al., 2003; Dos Santos & Tixier, 2017; Rodrigues et al., 2017). The mitochondrial first coding subunit of the cytochrome oxidase subunit I (COI) and the 16S large subunit rRNA mitochondrial (16S rRNA) gene have proven their suitability as molecular markers for the phylogenetic analysis in a variety of organisms, including decapod crustaceans (Schubart et al., 2000; Hebert et al., 2003; Mantelatto et al., 2006; Matzen da Silva et al., 2011a, b; Thiercelin & Schubart, 2014; Lin et al., 2015; Almón et al., 2022a, b). Moreover, both markers have been revealed to be useful for discerning highly divergent (Deli et al., 2018a, b) and cryptic genetic lineages within species complexes of decapod crustaceans (Reuschel et al., 2010; Weiss et al., 2018). The combination of molecular and morphological analyses, along with other potential inputs, provides an essential criterion of evolutionary independence for species recognition, including hermit crabs (Heethoff et al., 2011; Keikhosravi & Schubart, 2014; Negri et al., 2014; Thiercelin & Schubart, 2014; Purroy et al., 2016; Landschoff & Gouws, 2018; Li et al., 2020; Almón et al., 2022a, b).

The genus *Diogenes* Dana, 1851 is one of the most diverse genera of the hermit crab family Diogenidae, exhibiting high morphological diversity and a widespread geographical distribution (Almón et al., 2022a, b). Until recently, the species *D. pugilator* (Roux, 1829) was considered to be distributed throughout the Mediterranean Sea, but also widespread in other regions such as the Eastern Atlantic and the Black Sea (Almón et al., 2022a, b). Through a revision of many specimens of *Diogenes pugilator* s. l. from the Atlantic and the Mediterranean, Almón et al. (2022a, b) found a pronounced genetic separation between five distinct morphotypes, revealing the existence of a species complex, that is considered to include different species previously assigned to *D. pugilator*. One of the morphotypes identified was recognised as *D. pugilator* s. str., which led to the redescription of the species, while a second morphotype was assigned to the previously synonymised and now resurrected species *D. curvimanus* (Clément, 1874). The three other morphotypes identified in their samples were described as new species: *D. arguinensis* Almón, Cuesta & García-Raso, 2022, *D. armatus* Almón, Cuesta, Schubart & García Raso, 2021, and *D. erythromanus* Almón, Cuesta & García-Raso, 2022 (see Almón et al., 2022a, b), leaving open the possibility of the existence of additional morphotypes within the species complex in other areas yet to be identified. The species described so far, although with some degree of overlap, show well-defined geographic distribution patterns (fig. 1).

In this study, the previously described genetic separation of the European species *D. armatus*, *D. curvimanus*, and *D. pugilator* s. str. is confirmed, also revealing the existence of another distinct lineage within the *D. pugilator* species complex based on specimens collected in the northern-central and north-eastern Mediterranean Sea as well as the Black Sea, which have not been analysed in previous studies. The congruence between molecular and morphological characters was examined following the work of Almón et al. (2022a), where the relevance of characters such as the shape and spination of chelipeds and pereopods for species identification within the species complex was highlighted.

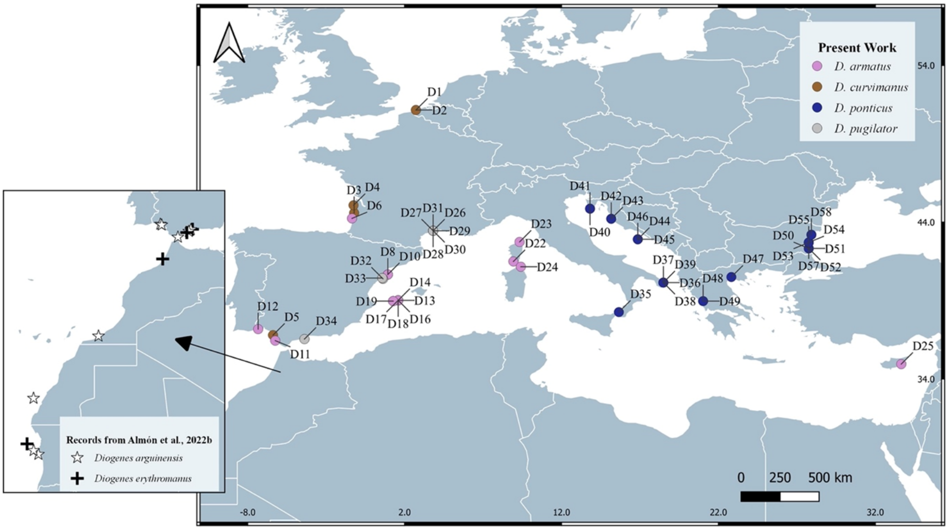

Fig. 1. Map of *Diogenes* sampling locations, including records of Almón et al. (2022b). Colours and shapes indicate species of specimen sampled, numbers identify specimen and correspond to those in table AI in the Appendix.

MATERIAL AND METHODS

The specimens of *Diogenes* used in this analysis were collected and donated from locations across Europe including France, mainland Spain, Ibiza, Corsica, Sicily, Italy, Croatia, Greece, Cyprus, and Bulgaria. Collection sites of all specimens included in the present study are listed in table AI in the Appendix. Some of the sequences used for molecular comparisons were provided by Almón et al. (2022a, b), with corresponding specimens listed in table AII in the Appendix. All specimens were preserved in 75% ethanol. Genomic DNA was extracted and purified through application of the Puregene method (Gentra Systems: Minneapolis, MN, U.S.A.). Preferably, anterior pleopods were used for extraction, due to their irrelevance for morphological identification at the species level. In the case of ovigerous females, a clutch of eggs was used for extraction. Type material is part of the Christoph D. Schubart collection that is now being housed in the zoological collection of the Museum of Nature (Leibniz Institute for the Analysis of Biodiversity Change, LIB) in Hamburg (Mercado-Salas et al., 2024).

To determine the genetic diversity and reconstruct the phylogenetic history within European representatives of *Diogenes*, amplifications of the cytochrome oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) mitochondrial genes were carried out with PCRs consisting of 40 cycles with 45 s of denaturation at 95°C, 60 s of primer annealing at 48°C and an elongation phase of 60 s at 72°C (4 min of initial denaturation at 94°C and 5 min final extension at 72°C). The PCR reaction mix contained 8.6 μl ddH_2O, 10 μl of GoTaq® Ready Mix, 0.2 μl of forward (L) and reverse (R) primer, and 1 μl of the specific template DNA adding up to a final volume of 20 μl. Primers used for the amplification of both molecular markers can be found in table I. PCR products were loaded and visualised on 1.5% agarose gel and sent to MACROGEN Europe (Amsterdam, The Netherlands) for sequencing. Chromatograms received by MACROGEN were proofread in Chromas© 2.6.6 (Technelysium, South Brisbane, QLD, Australia). COI and 16S rRNA nucleotide sequences were aligned in BioEdit 7.2.5 (Hall, 1999) using the ClustalW algorithm (Thompson et al., 1994). Additionally, comparative searches in BLAST® (Altschul et al., 1990) were performed to confirm or refute species identity. GenBank accession codes of the newly generated sequences are listed in table AI in the Appendix. After final sequence processing, the alignments of both mitochondrial gene regions were combined in BioEdit to create one concatenated sequence alignment (1845 bp). The software PartitionFinder (v. 1.1.1; Lanfear et al., 2012) generated the best-fit partitioning schemes and models to the corresponding data set using the Akaike Information Criterium (AIC) (Akaike, 1973). For the COI gene, data partitioning by codon was applied to account for different rates of evolution of each codon position (CO1: GTR + G (1st and 2nd partition), HKY (3rd partition); 16S: HKY + I + G).

To obtain a combined phylogenetic tree of Bayesian Inference analysis, the software MrBayes (v. 3.2.7; Huelsenbeck & Ronquist, 2001) with the Markov Chain Monte Carlo method (MCMC; Gamerman & Lopes, 2006) was used with 2.5 million generations. Twenty-five percent of the samples from the MCMC run were discarded as burn-in. Posterior analyses of the generated MCMC trace files were analysed with the software package Tracer (v. 1.7.2; Rambaut et al., 2018). Two haplotype networks were developed using the TCS method (Templeton et al.,1992) (Clement et al., 2000) in the software PopArt (v. 1.7; Leigh & Bryant, 2015). For that, the FASTA format (Goldstein et al., 2014, 2015) of the COI and the 16S rRNA alignments were uploaded separately to the online fasta sequence toolbox FaBox (v. 1.6; Villesen, 2007). The integrated fasta2mrbayes converter creates a file in NEXUS format (Maddison et al., 1997) with data and analysis settings included. To assign each sequence to the geographical sampling coordinates of the associated specimen, a GEOTAGs block was manually added to the received NEXUS format according to the PopART manual. The final NEXUS file was imported into PopART. Final graphic editing was performed in the software Inkscape (v. 1.0).

The morphology of specimens was examined under a stereo zoom microscope (Leica S APO Stereo Zoom; Leica Camera EZ4D) and focused on morphological characteristics used for species identification in *D. pugilator* s. l. Primary characteristics include the shape, proportions, and spination of the left cheliped, shape,

TABLE I
List and sequences of the COI and 16S rRNA primers (L, forward; R, reverse) used for amplification of the mitochondrial genome, with references

Marker	Primer	Sequence (5′ → 3′)	Reference
COI	COL1B	CCWGCTGGDGGWGGDGAYCC	Schubart (2009)
	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart (2009)
	COL6a	TCWACAAATCATAAAGAYATTGG-	Schubart (2009)
	COL6E	ATGCAACGATGATTCTTTTCTAC	Schubart et al. (2023)
	COL8	GAYCAAATACCTTTATTTGT	Schubart (2009)
	COL19	TGTATARGCRTCTGGRTARTC	Present study
	COH1B	TADACTTCDGGRTGDCCAAARAAYCA	Schubart (2009)
	COH6	TGWARAGAAAAAATTCCTA	Schubart & Huber (2006)
	COH900	GTDGGRAYWGCAATAATTAT	Schubart et al. (2023)
	COH7	TGWGAAATTATWCCRAARGC	Schubart (2009)
	COH9G	GTTCTCAGTCCTTTTTTT	Present study
	COH11	GAAGCYCCWGCRTGAGCRATAG	Present study
	COH19	GAAGCYCCWGCRTGAGCRATAG	Present study
16S	16L29	YGCCTGTTTATCAAAAACAT	Schubart et al. (2001) (as 16L2)
	16L12	TGACCGTGCAAAGGTAGCATAA	Schubart et al. (1998)
	16H37	CCGGTYTGAACTCAAATCATGT	Klaus et al. (2006)
	16H2	AGATAGAAACCAACCTGG	Crandall & Fitzpatrick (1996) (as 1472)
	16H10	AATCCTTTCGTACTAAA	Schubart (2009)

and spination of pereopods, the shape of the rostrum, the features of ocular scales, the length of antennae and antennules, length and abundance of setae, as well as the shape and size of tubercles on the whole exoskeleton (Almón et al., 2022a, b). Shield length (sl) measurement of the type specimen is given, measured from the tip of the rostrum to the midpoint of the posterior margin of the shield, using the IC Measure tool (v. 2.0.0.286; The Imaging Source). Moreover, colour patterns on the fresh specimens were examined and photographed right after sampling (fig. A1 in the Appendix).

RESULTS

Molecular comparison

The phylogenetic tree is based on 59 sequences consisting of a combination of partial COI and 16S rRNA sequences, with a maximum length of up to 1845 bp (fig. 2). Almón et al. (2022b) used non-European outgroups and the newly described *Diogenes arguinensis* and *D. erythromanus* to demonstrate the monophyly of the so far recognised European *Diogenes* species. Therefore, only the latter are included in the present analysis to depict the relationship among them.

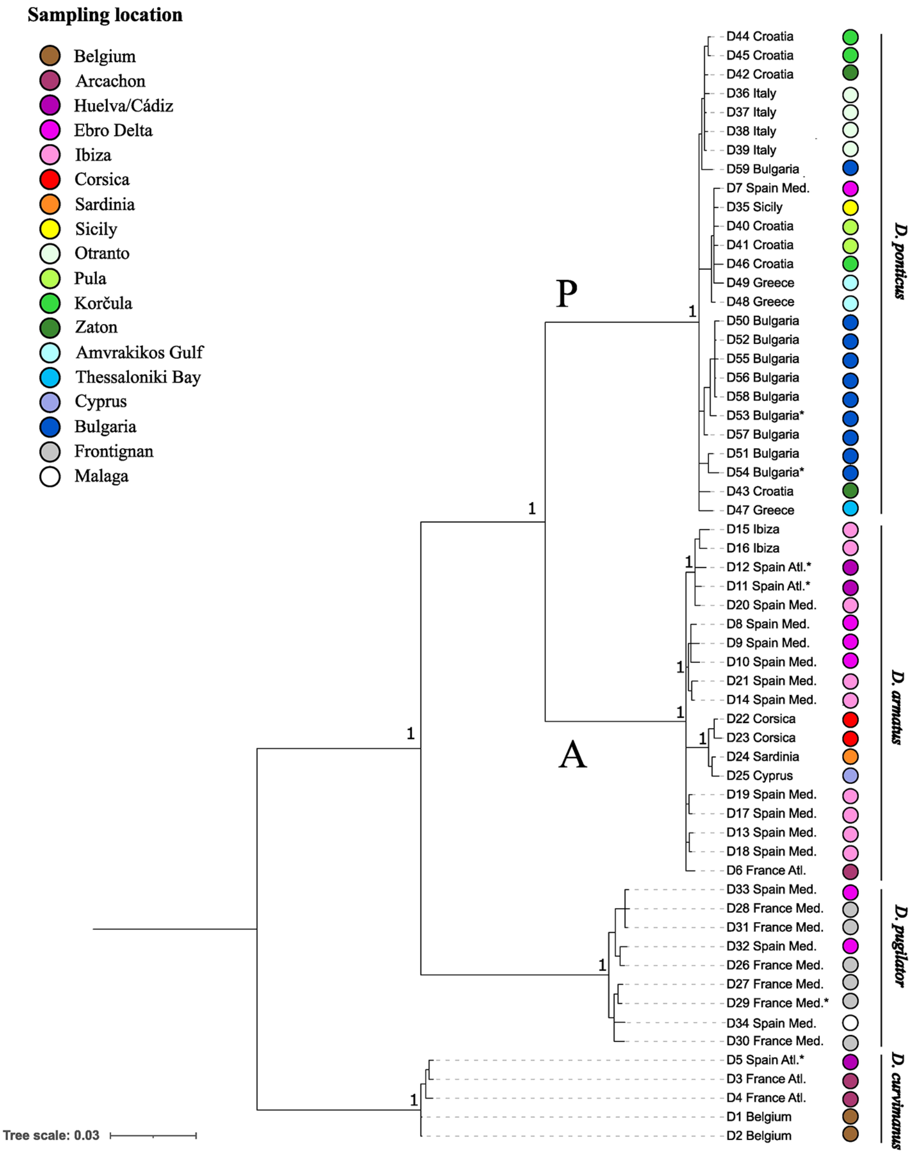

Fig. 2. Bayesian phylogenetic tree of *Diogenes* in Europe based on a concatenated dataset of 1845 base pairs of the mitochondrial genome; all posterior probability values of clusters are higher than 0.5, values equaling 1 are included in bold. Asterisks indicate type specimens and colours identify sampling locations in accordance with those defined for haplotype network analysis. Letters A and P mark the two clades that are now identified as *D. armatus* Almón, Cuesta, Schubart & García Raso, 2021 and *D. ponticus* (Kessler, 1860), respectively.

The phylogeny shows four highly supported main clades with *D. curvimanus* acting as an outgroup. It splits off first with a posterior probability (pp) of 1, represented by individuals from Belgium (D1/2), the French Atlantic (D3/4), and southern Spain (D5) (fig. 2). Among the remaining European specimens from the Mediterranean Sea, the specimens from the Gulf of Lion, French Mediterranean (D26/27/28/29/30/31) and the specimens from the Ebro Delta provided by the ICM-CSIC collections in Barcelona (D32/33), stand together in the next basal group (pp = 1) assigned to *D. pugilator* s. str. (fig. 2).

The remaining Mediterranean specimens form a highly supported monophyletic group (pp = 1), which is subdivided into clades A and P. Clade A includes the assigned holotype of the recently described *D. armatus* (D11) as well as almost all specimens collected from the Western Mediterranean. It also includes one specimen from the French Atlantic (D6), a specimen from southern Spain (D12), and the specimen from Cyprus (D25). Altogether, clade A forms another monophyletic group with high support (pp = 1), and all members standing together in this group can be assigned to *D. armatus* (fig. 2). Within *D. armatus*, the individuals that were assigned as type specimens by Almón et al. (2022a) (D11/12) are the only *D. armatus* specimens that were collected in the Atlantic. They form a highly supported subgroup (pp = 1) with three specimens from Ibiza (D15/16/20). Moreover, the specimens from Corsica (D22, D23) and Sardinia (D24) split off forming a visible subgroup (pp = 1) with the specimen collected in Cyprus (D25) and are therefore the easternmost collected individuals identified as *D. armatus*.

Clade P forms the fourth main cluster (pp = 1) and stands in a sister relationship with *D. armatus* (fig. 2). It is new in the phylogenetic tree, compared to Almón et al. (2022a, b), and comprises specimens from the eastern Mediterranean and the Black Sea including the Italian Peninsula Salento, Croatia, Greece, and the Bulgarian coast. Furthermore, the only sample available from the eastern Sicilian coast and thus the generally accepted border between the Western and Eastern Mediterranean basins, also clusters with the specimens from the Eastern Mediterranean and the Black Sea, as well as one specimen from the Ebro Delta (D7). The specimens whose sequences can be assigned to clade P will be referred to as *Diogenes ponticus* (Kessler, 1860) in the following (see taxonomic remarks), since this name served for the description of individuals from the Black Sea, before their synonymisation in *D. pugilator* (DecaNet eds., 2023).

Since the monophyly of the four groups within the European *Diogenes* has already been confirmed in the phylogenetic tree, the constructed haplotype networks serve to assess the relationship among haplotypes of *D. armatus* and *D. ponticus* (fig. 3). The network depicted in fig. 3A comprises the COI sequences 549 bp of 42 individuals, including 16 individuals assigned to *D. armatus* and 26 individuals assigned to *D. ponticus*, resulting in a total of 35 haplotypes. The

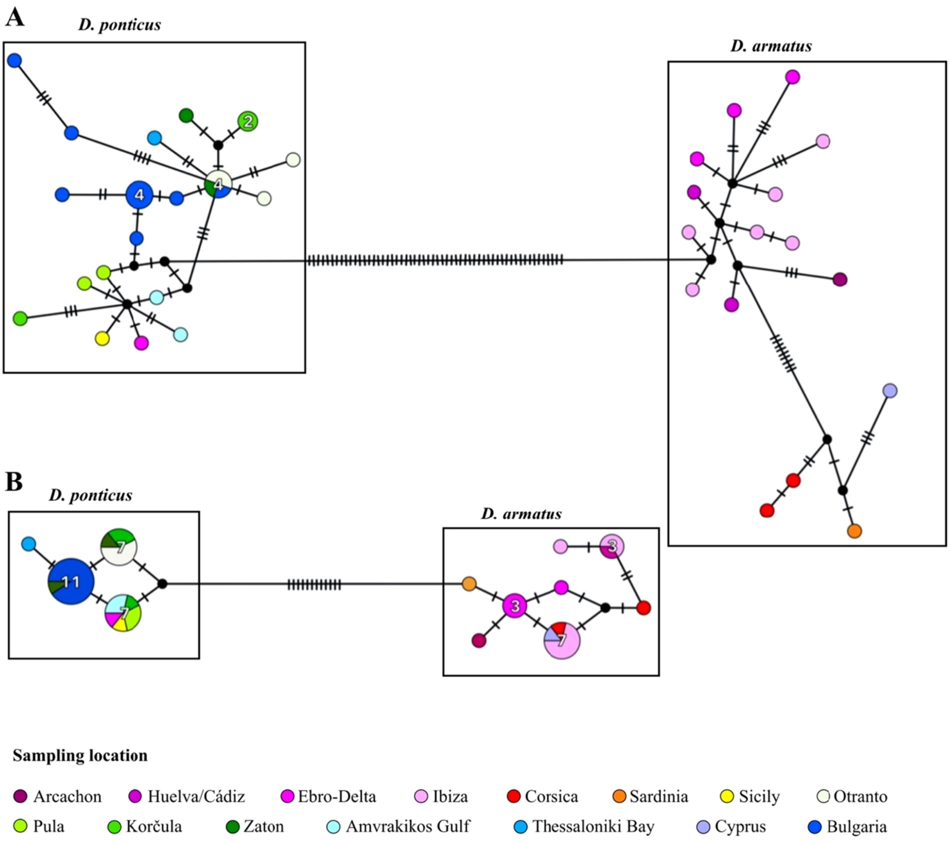

Fig. 3. Haplotype networks of *Diogenes armatus* Almón, Cuesta, Schubart & García Raso, 2021 and *D. ponticus* (Kessler, 1860). Colours identify sampling location; circle sizes depict frequencies of haplotypes, numbers give exact number of individuals sharing a haplotype, no number depicted corresponds to one sample; missing haplotypes are represented by black dots; numbers of mutations between haplotypes visualised with hatch marks; black frames highlight species affiliation. A, 549 base pairs (bp) of the CO1 gene ($N = 42$); B, 442 bp of the 16S gene ($N = 44$).

most important genetic relationships within the network can be summarised as follows: the specimens from the Western Mediterranean Basin and the individual from Cyprus form the first cluster, each of them having an exclusive haplotype separated by a minimum of two mutations. Within this cluster, the specimens from the French Atlantic coast, the Iberian Peninsula, and Ibiza are separated from the individuals from Corsica, Sardinia, and Cyprus by a minimum of 12 mutations. All individuals in this first cluster can be assigned to *D. armatus* and are at least 59 mutation steps away from a second large cluster, which comprises the individuals from the Eastern Mediterranean Basin and the Black Sea, including Sicily, and one individual from the Iberian Peninsula (D7). All the individuals in this second cluster can be assigned to *D. ponticus*: four individuals from Bulgaria share the same haplotype, just as two individuals from Otranto, Italy, one from Zaton, Croatia, and another one from Bulgaria that share another haplotype. Two more individuals from Korčula, Croatia, show the same COI sequence. Other than that, most individuals have exclusive haplotypes. Both main haplogroups, each of them comprising the individuals corresponding to *D. armatus* on the one hand and to *D. ponticus* on the other hand, are very reticulated (i.e., genetically diverse), without any central haplotypes. Apart from the separation within *D. armatus* (French Atlantic, Iberian Peninsula, Ibiza and Corsica, Sardinia, Cyprus), no distinct pattern within the groups can be observed.

The second network depicted in fig. 3B comprises the 16S sequences (442 bp) of 44 individuals, including 18 individuals assigned to *D. armatus* and 26 individuals assigned to *D. ponticus*. Just as in the network based on the COI gene, two main clusters are visible, both consisting of conspicuously fewer haplotypes. The assignment of individuals to each haplogroup corresponds to the one in fig. 3A: The first group includes only individuals from the Western Mediterranean Basin and Cyprus, all of them assigned to *D. armatus*. The second group comprises all individuals from the Eastern Mediterranean Basin and the Black Sea, including Sicily, and the single individual from the Ebro Delta, all of them assigned to *D. ponticus*. The individuals of both groups are separated by at least 13 mutational steps. In the group of *D. armatus*, four individuals from Ibiza, one from Corsica, and one from Cyprus share the most common haplotype in the first group. Other than in the first network based on CO1, there is no visible separation between the Spanish individuals and the individuals from Corsica, Sardinia, and Cyprus. In the second group, all specimens from Bulgaria form the most common haplotype, sharing it with one individual from Zaton, Croatia. The specimen from Thermaikos Gulf, Greece, is the only one standing separately in the second group, however, only one mutation away from the most common haplotype. The remaining individuals included in the second group are equally divided between two other haplotypes, both one mutational step away from the individuals from the Black Sea. One haplotype comprises three individuals from Croatia, two from Amvrakikos Gulf, Greece, the specimen from Sicily, and the individual from the Ebro Delta. The other haplotype includes all individuals from Otranto, Italy, and three more specimens from Croatia.

Morphology

Family DIOGENIDAE Ortmann, 1892

Genus *Diogenes* Dana, 1851

Diogenes ponticus (Kessler, 1860)

(fig. 4, fig. A1)

Synonymy.— *Pagurus ponticus* Kessler, 1860 (by original description); *Diogenes pugilator* Roux, 1829 (in part, including the records in the known range of distribution of *D. ponticus*).

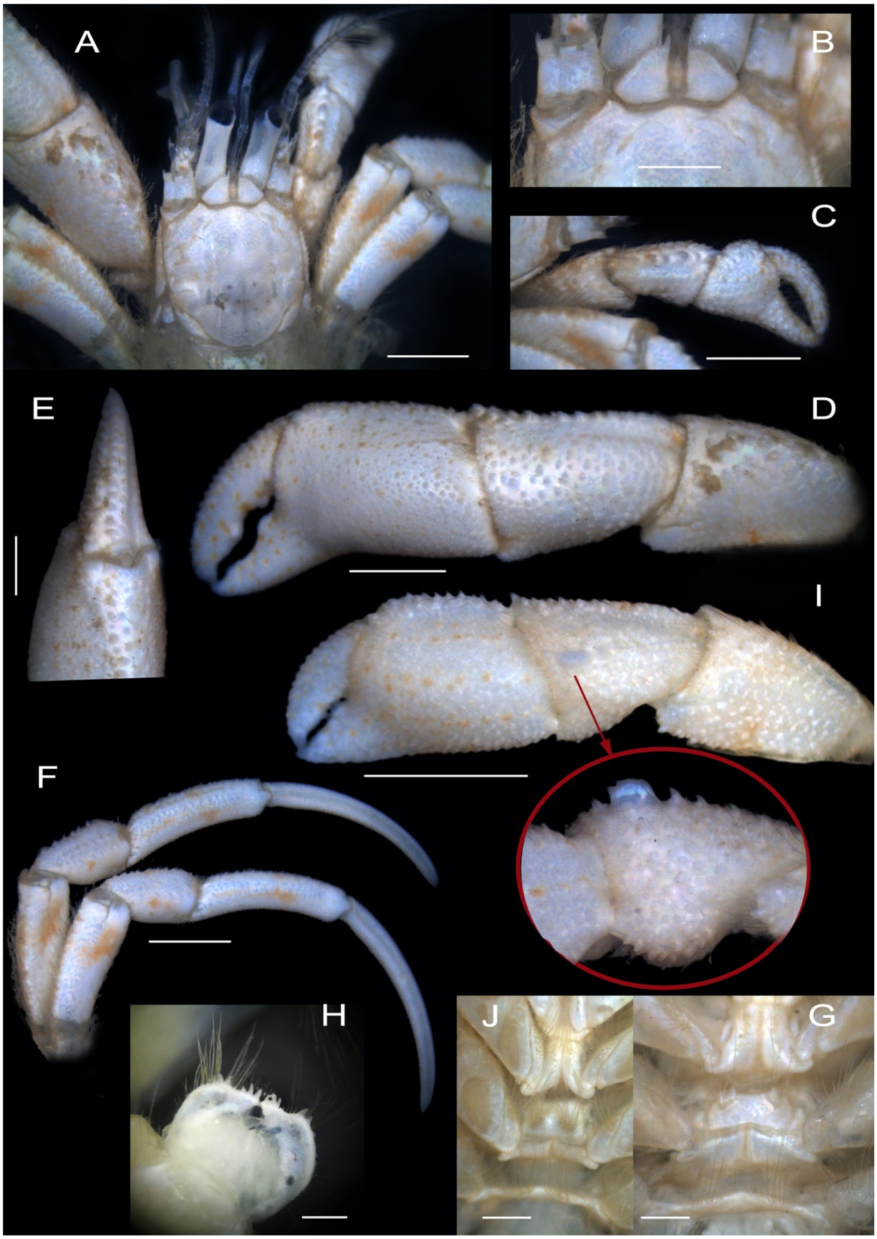

Fig. 4. *Diogenes ponticus* (Kessler, 1860), A-H, J, neotype (ZMH K-64932); I, female topotype (ZMH K-64933); G, holotype of *D. armatus* Almón, Cuesta, Schubart & García Raso, 2021. A, Shield and cephalic appendages, dorsal view; B, rostral lobe and ocular acicles, dorsal view; C, right cheliped, outer view; D, left cheliped dorsal view; E, palm and dactylus of the left cheliped, dorsal view; F, second and third pereopods, outer view; G, sternal plates; H, telson, dorsal view; I, left cheliped, outer view; J, sternal plates. Scales: A, C, D, F, I = 2 mm, B, E = 1 mm, H, G, J = 0.5 mm.

Type material.— Neotype: 1 male, sl 3.6 mm (ZMH K-64932; D53), Bulgaria: Sveti Vlas, 42°42′26.3″N 27°45′13″E, 0.5 m depth, 22 June 2021. Topotype: 1 female (ZMH K-64933; D54), same geographical data as neotype, 22 June 2021.

Material examined.— Spain: Ebro Delta, 1 individual (D6), 40°38′7″N 0°44′47″E, 14 November 2020; Italy: Sicily: Messina: Torre Faro: Punta del Faro, 1 individual (D35), unknown coordinates, 01 July 2010; Italy: Salento: Otranto, 4 individuals (D36/37/38/39), 40°9′15.5″N 18°29′27″E, 01 November 2021; Croatia: Pula: Stoja Bay, 1 individual (D40), 44°51′36″N 13°48′47″E, 10 June 2010; 1 individual (D41), 44°51′36″N 13°48′47″E, 24 October 2018; Croatia: Korčula: Brna: Plaža Istruga, 3 individuals (D44/45/46), 42°54′35″N 16°51′28.1″E, 09 March 2020; Croatia: Nin: Zaton, 2 individuals (D42/43), 44°13′0.7″N 15°9′47.4″E, 01 March 2020; Greece: Amvrakikos Gulf, 2 individuals (D48/49), 38°58′0.9″N 21°1′20.4″E, 11 September 2003; Greece: Thermaikos Gulf: Thessaloniki Bay: Agelohori, 1 individual (D47), 40°30′11.6″N 22°49′13.7″E, 07 November 2021; Bulgaria: Ropotamo Estuary, 3 individuals (D50/51/52), 42°19′40″N 27°45′20″E, 10 June 2010; Bulgaria: Burgas: Nesebar: Sveti Vlas, 5 individuals (D53/54/55/56/57), 42°42′26.3″N 27°45′13″E, 22 June 2021; Bulgaria: Varna: Varna Bay, 2 individuals (D58/59), 43°12′2.3″N 27°55′24″E, 13 September 2021.

Redescription.— Shield (fig. 4A) subquadrate, slightly longer than broad, slightly vaulted anteriorly; margin between rostrum and lateral projections slightly concave; rostral lobe short and rounded, reaching well-behind lateral projections, which are triangular and acutely pointed, with single terminal spine; anterolateral margin sloping, slightly concave, mostly smooth; anterolateral angles rounded, with one marginal spine; posterior margin roundly truncate. Branchiostegites with row of 12-13 acute spines on dorsal margin. Ocular peduncles (fig. 4A) short (about 0.7 times as long as shield, including corneas), moderately stout and slightly compressed medially; rows of thin, short setae on dorsal surface and dense tufts of longer setae mesially. Ocular acicles (fig. 4A, B) subtriangular, with anterior margin slightly convex bearing 13-14 spines (3-4 innermost largest). Intercalary rostriform process significantly shorter than ocular acicles (including spines), smooth. Antennular peduncles overreaching distal corneal margin by about half length of ultimate segment; 3rd segment not broadened distally, subequal in length to penultimate segment, with two longitudinal rows of short setae dorsally, and a tuft of 3-4 longer setae distally; 2nd segment unarmed; basal segment with ventrodistal spine. Antennal peduncles subequal in length to antennular peduncles; 3rd to 5th segments unarmed; 4th about half length of 5th; 2nd segment distolateral outer process stout and acute, without subdistal spines; smaller but strong spine on distomesial angle; 1st segment with spinulose distal margin. Antennal acicle short, subtriangular, reaching slightly below distal margin of 4th antennal segment, bearing a strong terminal spine plus 3 to 4 additional spines on mesial margin. Third maxilliped ischium with crista dentata bearing three prominent distal and two to three smaller proximal spines; exopod peduncle slightly overreaching proximal carpal margin of endopod.

Male left cheliped (fig. 4D, E) much larger than right (fig. 4A, C). Dactylus about as long as palm (measured along upper margin), gently arched, crossing tip of fixed finger; dorsal surface defined by two longitudinal rows of tubercles (those on outer row largest) running over entire dorsal margin; space between rows only slightly concave; upper margin of outer surface with 2 additional rows of small tubercles, first incomplete; scattered tufts of short stiff setae associated with tubercles; upper margin of inner surface with 1 row of small rounded tubercles running parallel to dorsal rows, separated by a conspicuous depression; tufts of short stiff setae associated with tubercles; rest of outer surface slightly convex, with some minute tubercles and tufts of setae associated with some tubercles; cutting edge sinuous with single row of calcareous teeth of different size, ending in calcareous claw. Fixed finger clearly separated from beginning of inflated palm surface by shallow concavity; outer surface with minute blunt tubercles interspersed with rows of sunken tubercles, most with associated tufts of short, stiff setae; inner surface flattened to slightly concave, with 3 rows of sunken tubercles associated with tufts of stiff, dense setae; cutting edge sinuous with single row of calcareous teeth of different size. Palm about 1.3-1.4 times as long as high, robust, and strong; upper margin shorter than carpus; dorsal surface defined by rows of small but strong spines, decreasing in size distally; outer surface clearly inflated throughout, covered with small acute tubercles, largest forming longitudinal rows; moderately long stiff setae irregularly covering the whole outer surface (often associated with largest tubercles); lower margin sinuous, with distal half concave and defined by rows of small acute tubercles over the whole length; inner surface slightly convex, covered with flat, rounded tubercles denser on distal half; largest tubercles forming 2 to 3 longitudinal rows (especially conspicuous when some colour remains); most of larger tubercles with tufts associated of short stiff setae. Carpus clearly longer than high; dorsal margin with 2 to 3 rows of strong spines increasing in size distally; outermost row strongest; outer surface convex, covered with closely spaced acute tubercles increasing in size distally, with scattered stiff setae; disto-outer margin spinulose; lower margin gently protruding distally, forming a faint sinus proximally; inner surface covered by closely spaced, thick rounded tubercles and long stiff setae. Merus longer than high; dorso-distal margin spinulose, with rows of short setae; dorsal margin convex; dorsal surface with rows of acute tubercles decreasing in size distally, with associated tufts of long setae; spinose distal furrow poorly defined; lateral surface covered with small acute tubercles; ventrolateral margin slightly concave proximally, defined by row of obliquely acute tubercles of similar size and associated rows of short setae; mesial surface with weakly calcified U-shaped patch proximally, with faint distal furrow; ventro-mesial margin defined by rows of spines increasing in size distally, connecting with spinose mesial distal margin.

Female left cheliped (fig. 4I): Palm slightly longer than high, thereby differs from male by being more oval, globose and stout; spines and tubercles are generally sharper, with associated long thin setae; lower margin of palm straight to convex throughout on 1st half, slightly concave on distal half. Carpus with medial row of large spines on outer surface, with 2-4 of them fused into a fin-like structure (fig. 4I-inset) that is very conspicuous when present (some specimens showed this structure less developed).

Right cheliped (fig. 4A, C): much shorter than left; dactylus and fixed finger with prominent hiatus, ending in small calcareous claw. Dactylus up to 3× as long as palm dorsal margin, roundly arched; dorsal margin defined by rows of small spines; upper inner and outer margins with row of small spines separated from dorsal rows by concave surface; tufts of long stiff setae associated with each tubercle; outer surface with rows of tubercles associated with tufts of long stiff setae; additional row of tufts of moderately long, stiff setae running parallel and close to cutting edge. Fixed finger outer surface slightly convex, with irregular rows of small acute tubercles with tufts of long stiff setae; cutting edge with single row of small subacute calcareous teeth; lower margin defined by row of acute tubercles extending on to palm; inner surface slightly convex, with three longitudinal rows of small blunt tubercles, each with tufts of short stiff setae. Palm slightly higher than long; dorsal margin defined by rows of low acute tubercles; outer surface with irregular rows of small acute tubercles; lower margin defined by row of small acute tubercles; inner surface slightly convex on distal half, smooth and glabrous. Carpus dorsal margin defined by row of strong spines increasing in size distally; with upper outer and inner margins with additional row of small acute tubercles, separated from dorsal row by concave surfaces; outer surface covered with round flat tubercles and some thin long setae; lower margin gently protruding, forming a prominent sinus proximally; inner surface smooth and glabrous with distal half slightly inflated. Merus dorsal margin with row of obtuse small spines and tufts of long setae; lateral surfaces smooth and glabrous; lower margin defined by small acute tubercles and some long plumose setae.

Second and third pereopods (fig. 4F): subequal in length. Dactylus 1.4 times (P2) or 1.5 (P3) as long as propodus, weakly curved, ending in small corneous claw; dorsal surface unarmed, with rows of moderately long thin setae; ventral surface with rows of longer setae; lateral surface with longitudinal shallow sulcus medially, with associated row of short setae. Propodus slightly (P2) to clearly longer (P3) than merus; each with dorsal margin defined by rows of small spines (P2) or faintly dentate (P3), with rows of setae of different sizes; lateral surface faintly granulated, with rows of setae near dorsal and ventral margins. Carpus dorsal margin defined by 2 rows of small spines increasing in size distally, with tufts of moderately long, thin setae on dorsal, latero-distal and ventral margins; inner and outer lateral surfaces almost smooth and glabrous, with some stout setae on distal half of carpus. Merus dorsal margin defined by row of low, spinose tubercles, with tufts of moderately long setae; ventral margin finely dentate, with distal margin bearing 1 small spine (P2) or unarmed (P3); inner and outer lateral surfaces smooth and glabrous. Ischium smooth except for the faintly serrated ventral margin, with tufts of long setae. Fourth pereopod: Dactylus with row of 14-15 subacute submarginal spiniform setae on ventral margin. Propodus suboval, unarmed. Carpus with spinose protuberance dorsodistally. All segments with tufts of long simple and plumose setae.

Sternal plate subrectangular, bearing 1-2 strong spines on each side (fig. 4G). Male unpaired left pleopods 2-5 uniramous. Female gonopores paired; pleopods 2-4 biramous; 5th without exopod, as in males.

Telson (fig. 4H) barely asymmetrical, with very narrow longitudinal median cleft; left posterior lobe subequal in length but slightly wider than right, with 1-2 strong terminal spines and row of smaller spines on lateral margin, disappearing anteriorly; terminal margins of both lobes almost straight, with 3 spines of similar size near the median cleft; right posterior lobe with 2-3 terminal spines and lateral margin with row of small spines not reaching anterior half of lateral margin.

Colouration.— See fig. A1 in the Appendix. Shield with two brownish-red stains at longitudinal sulci level (in some specimens restricted to two spots on posterior area). Ocular peduncles whitish with proximal 2/3 covered by a diffuse pinkish stain, on top of which a bluish, triangular patch develops, starting proximally and tapering distally; eyeballs velvet black with small honey-yellow stippling. Ocular acicles whitish, light brown apically. Antennular background whitish; third segment with diffuse dark-red longitudinal line and distal complete ring of the same colour; second segment with dark red dorsal and mesial stripes. Antennae slightly translucent, with conspicuous dark-red spot on antennal acicle proximally, and two smaller spots of the same colour, on lateral margins of fourth peduncular segment.

Merus and carpus of left cheliped brownish green in background, with some diffuse reddish-brown areas; dark brown spots on dorsal and outer medial surface of merus (usually forming a complete ring), and dorso-inner and outer, as well as dorsal lower margins of carpus (usually not forming rings). Palm whitish, with dorsal half slightly coloured; purplish path in dorsal surface of dactylus fading distally and extending onto outer and inner surfaces; base of largest tubercles and spines with dark reddish-orange taints; small, dark purple taints at medial dorsal half, base of the junction with dactylus, and dorsodistal area of palm outer surface.

Right cheliped whitish; reddish-brown spots on mid-dorsal and lateral areas of the merus, forming an incomplete ring; two spots of the same colour near proximal and central dorsal areas of carpus; proximal and distal central areas of palm with brownish spots, sometimes faintly extending onto lower distal area and fixed finger; dactylus with dorsal margin tinged in orange, with purplish stain proximally. Second and 3rd pereopods with general whitish colour ventrally, greenish dorsally, except for the propodus, which is also white dorsally; bright reddish-brown thick stripes on distal half of lateral surfaces of merus, narrowing proximally; narrower stripes of the same colour on lower lateral surface of carpus and propodus; additional dark brown spots in dorsal and lateral medial areas of merus, proximal and medial dorsal margin of carpus and dorsal and lateral medial areas of propodus; dactylus with distal half greenish, and proximal area tinged with purple. Both males and females share similar colour patterns.

Habitat.— Sandy beaches and shallow subtidal areas including estuaries, so far collected in up to 22 m of depth.

Distribution.— So far known to inhabit the coasts of the Adriatic (Italy and Croatia), Ionian (Italy, Sicily, and Greece), Aegean (Greece), and the Black Sea (Bulgaria).

Taxonomic remarks

The external morphology of the specimens assigned to *D. ponticus* often resembles that described in *D. armatus* (see Almón et al., 2022a), while it is notably different from the other known European species of the genus. The presence of a disto-outer spine on the fourth segment of the antennal peduncle is exclusive of *D. arguinensis* and *D. erythromanus*, being unarmed in *D. armatus*, *D. curvimanus*, *D. ponticus* and *D. pugilator*. Also, ocular peduncles are smaller in *D. arguinensis* and *D. erythromanus*, with antennules overreaching distal margin of corneas by 0.8-0.9 length of ultimate segment, while among the other four species, only *D. pugilator* shows similar proportions. The inflated palm of *D. ponticus* resembles that of *D. pugilator*, but both species can be separated based on, among other characters, the shape of the palm, which is as long as high in *D. pugilator*, while in *D. ponticus* has a more elongated shape; the ocular scales are formed of 3-4 large spines in both species but the rest of the anterior surface is almost smooth in *D. pugilator* (with tiny tubercles), while in *D. ponticus* it has small but strong spines. The shape of the male left cheliped in *D. curvimanus*, especially the palm, which is much longer than high and with outer surface almost smooth, is diagnostic for the species, being in the other species more subquadrate and with more or less developed spinose crests on the outer surface. The third antennular segment is equally useful for the identification of *D. curvimanus*, since it is the only one with clearly broadened third antennular segment.

Finally, the closest morphologically resembling species to *D. ponticus* is *D. armatus*. Although they share similar general aspects especially when preserved, they show some distinguishing features that allow to separate them. The rostral lobe is rounded and low, slightly produced in *D. ponticus*, while in *D. armatus* is acutely rounded and well-developed. The palm of the left cheliped of *D. ponticus* males is inflated throughout, while in *D. armatus* only the proximal medial half is inflated, showing also a conspicuous proximal spinose crest that is not present in *D. ponticus*. Also, in *D. ponticus* the spination of the chelipeds is less developed and the telson lobes are barely asymmetrical. Finally, the spination of the sternal plate in *D. ponticus* is unique within the European species: In males of the other species this structure is composed of two tufts of long setae (fig. 4J), while *D. ponticus* reveals a group of 1-2 strong spines on each side (fig. 4G).

Relevant morphological differences between the listed specimens of *D. ponticus* and the other European species of *Diogenes* are summarised in table AII in the Appendix. A certain level of morphological variability within the group of specimens listed as *D. ponticus* was observed, as has been described in other species of the genus (Almón et al., 2022a, b). Therefore, a combination of characters is always recommended for species identification. The evidence of genetic and morphological differentiation is presented as a solid foundation to assign the specific name *D. ponticus*, resurrected from the Russian taxonomist Kessler (1860), who described this species from the Black Sea. The species was later synonymised with Roux's *Diogenes pugilator*, under the general opinion of morphological variability and wide distribution of the species. Efforts to locate the type material, or at the very least, to obtain indications from the literature about their possible location, have been unsuccessful, letting us assume that they are most likely lost or were never assigned. Therefore, we designate a neotype (ZMH K-64932) to replace the missing type and thereby restore the validity of this species within the genus *Diogenes*.

DISCUSSION

The results of our molecular analyses indicate a longstanding evolutionary independence of *Diogenes armatus*, *D. curvimanus*, *D. ponticus* and *D. pugilator* s. str., which is reflected in both morphology and colouring patterns, confirming the results of Almón et al. (2022a). Within the European representatives of the genus *Diogenes*, the species *D. curvimanus* holds an ancestral position and exhibits distinct morphological features that are pointed out in the description by Almón et al. (2022a), such as the characteristic shape of the left chela. Findings of *D. curvimanus* are limited to Atlantic waters in our samples (fig. 1). In comparison with other studies that show similar biogeographic patterns in other Mediterranean taxa (Ragionieri et al., 2024), the Almeria-Oran Front (AOF) has been described as the main cause for pronounced genetic differentiation along this area on interspecific and intraspecific levels (Sá-Pinto et al., 2012; Pascual et al., 2017).

The AOF is one of several oceanographic discontinuities along the Spanish coast that emerge due to the entry of lower saline Atlantic waters into the Mediterranean Sea at the Gibraltar Strait (GS), which forms the direct linkage between the Atlantic Ocean and the Mediterranean Sea (Zarokanellos et al., 2022). However, the true distribution range of *D. curvimanus* is unknown and there have been previous records of the species in the Mediterranean Sea (Almón et al., 2022a). Moreover, *D. armatus* is not only restricted to one side of the GS (fig. 1). It spreads from the Mediterranean Sea along the Spanish and French Atlantic coasts and is currently known to reach as far north as Arcachon (Almón et al., 2022a). Our results suggest that the differentiation between the two species, both from a genetic (fig. 2) and morphological point of view (Almón et al., 2022a), is well consolidated and therefore maintained despite living in sympatry.

Similar as the AOF, the Ibiza Channel (IC) and the Balearic Front (BF) are well-known examples of contemporary oceanographic barriers that influence differentiation processes in the Western Mediterranean area (Pascual et al., 2017). Considering the currently known geographical distribution of *D. armatus* and *D. pugilator* s. str. (fig. 1), it is quite conceivable that the IC and the BF may be responsible for preventing gene flow between *D. pugilator* s. str. and populations of *D. armatus* from Ibiza. A comparable current-driven barrier does not exist between the populations from the Ebro Delta in Spain and the French Mediterranean, which is probably why some *D. pugilator* s. str. may occur along the coast of Barcelona and southern Spain (Almón et al., 2022a). A corresponding dispersal of individuals from the French Mediterranean to the southern Spanish coast is presumably favoured by the southwestern direction of the ocean's surface current (Pascual et al., 2017). The genetic separation between *D. pugilator* s. str. and *D. armatus* is well established, and no significant genetic exchange has occurred in recent times, even without being geographically isolated. This is underlined by several morphological characteristics that separate both species and were described by Almón et al. (2022a).

The phylogeny indicates that genetic divergence between *D. armatus* and *D. ponticus* is not as well established as their separation from *D. curvimanus* or *D. pugilator* s. str. (fig. 2). However, the high number of mutations between mtDNA haplotypes of *D. armatus* and *D. ponticus* as well as the presence of distinguishing morphological characters and colouring patterns, clearly confirm a pronounced differentiation between both groups. Moreover, *D. ponticus* is mostly restricted to the northern-central and Eastern Mediterranean and the Black Sea, while almost all specimens of *D. armatus* were collected west of the Siculo-Tunisian Strait (STS). The STS forms the main oceanographic barrier between the Western and the Eastern Mediterranean and has been shown to act as a strong barrier to genetic exchange in numerous population genetic studies on marine invertebrates including decapods (Zitari-Chatti et al., 2008; Mejri, et al., 2009; Gharbi et al., 2011; Ragionieri & Schubart, 2013; Deli et al., 2015, 2020; Ragionieri et al., 2024), The genetic break recorded at the STS supports the impact of particular oceanographic circulation on maintaining genetic differentiation within the species complex, but the finding of *D. armatus* in Cyprus underlines the necessity of long-term studies and higher spatial resolution of sampling to help understand the current geographic distribution patterns in those species included in the *D. pugilator* species complex.

Diogenes ponticus is widespread in the northern-central and East Mediterranean and the Black Sea. The Turkish Straits System, comprised of the Dardanelles, the Bosphorus, and the Marmara Sea, represents a transitional zone connecting the Mediterranean and the Black Sea and plays a significant role in the biology of both basins (Öztürk & Öztürk, 1996). The importance of the narrow Bosphorus Strait to dispersal and gene flow has already been stressed by other authors studying genetic divergence within other European marine decapods such as *Carcinus aestuarii* Nardo, 1847 (Deli et al., 2018a) and *Pachygrapsus marmoratus* (Fabricius, 1787) (Fratini et al., 2016; Çetin et al., 2022), which would suggest a clear biogeographical break between the Black Sea and the Mediterranean in *Diogenes*. Hence, gene flow, e.g. through long-distance dispersal of individual larvae mostly outward through the Bosphorus, cannot be ruled out. Like many marine organisms (D'Aloia et al., 2015), species of the genus *Diogenes* exhibit a bipartite life cycle with long-lived planktotrophic larvae (Pike & Williamson, 1960). Other species of hermit crabs that rely on planktonic larval stages, such as *Pagurus excavatus* (Herbst, 1791) and *Pagurus alatus* (Fabricius, 1775), exhibit high genetic homogeneity and little to no geographic structure (García-Merchán et al., 2012). Moreover, the Bosphorus Strait is known to act as an international maritime transportation artery of high importance linking the Black Sea to the Mediterranean Sea (Akten, 2003; Güler, 2005). Hence, it may promote further passive dispersal and gene flow through transport in the residual ballast water of ships (Zaitsev, 1992; Kideys, 2002; Rata et al., 2018).

CONCLUSIONS

Overall, our results confirm the existence of four genetically differentiated groups within the European genus *Diogenes*. They therefore confirm the validity of *D. armatus* and *D. curvimanus* (see Almón et al., 2022a) and provide new insights into the genetic structure of the genus. Our molecular analyses, paired with the morphological examination, provide a robust basis for the resurrection of a synonymised species of the genus *Diogenes*. Therefore, we propose the resurrection of the name *Diogenes ponticus*, which was described based on individuals from the Black Sea before their synonymisation within *Diogenes pugilator*. The equilibrium between different structuring factors such as geographical barriers, oceanographic processes, and the species' dispersal potential, seems to be responsible for disparate patterns of genetic structure within *Diogenes*. This is further supported by similar findings in other marine Mediterranean decapod species (Ragionieri & Schubart, 2013; Fratini et al., 2016; Deli et al., 2018a, b; Ragionieri et al., 2024). Our conclusions gain even more importance, considering the high human impact on the Mediterranean marine ecoregions and associated habitat loss, degradation, and pollution (Coll et al., 2010). Species with a narrow distribution range are especially threatened (Pimm et al., 2014), which is why the structural aspect of taxonomic biodiversity in a particular and restricted area is central for environmental impact assessments and thereby the protection of its endemic species (Cochrane et al., 2016). Therefore, a detailed understanding and accurate interpretation of processes driving genetic divergence are fundamental to successful spatial conservation management and could additionally provide insights into how populations will respond to the rapidly changing environment in the present-day oceans.

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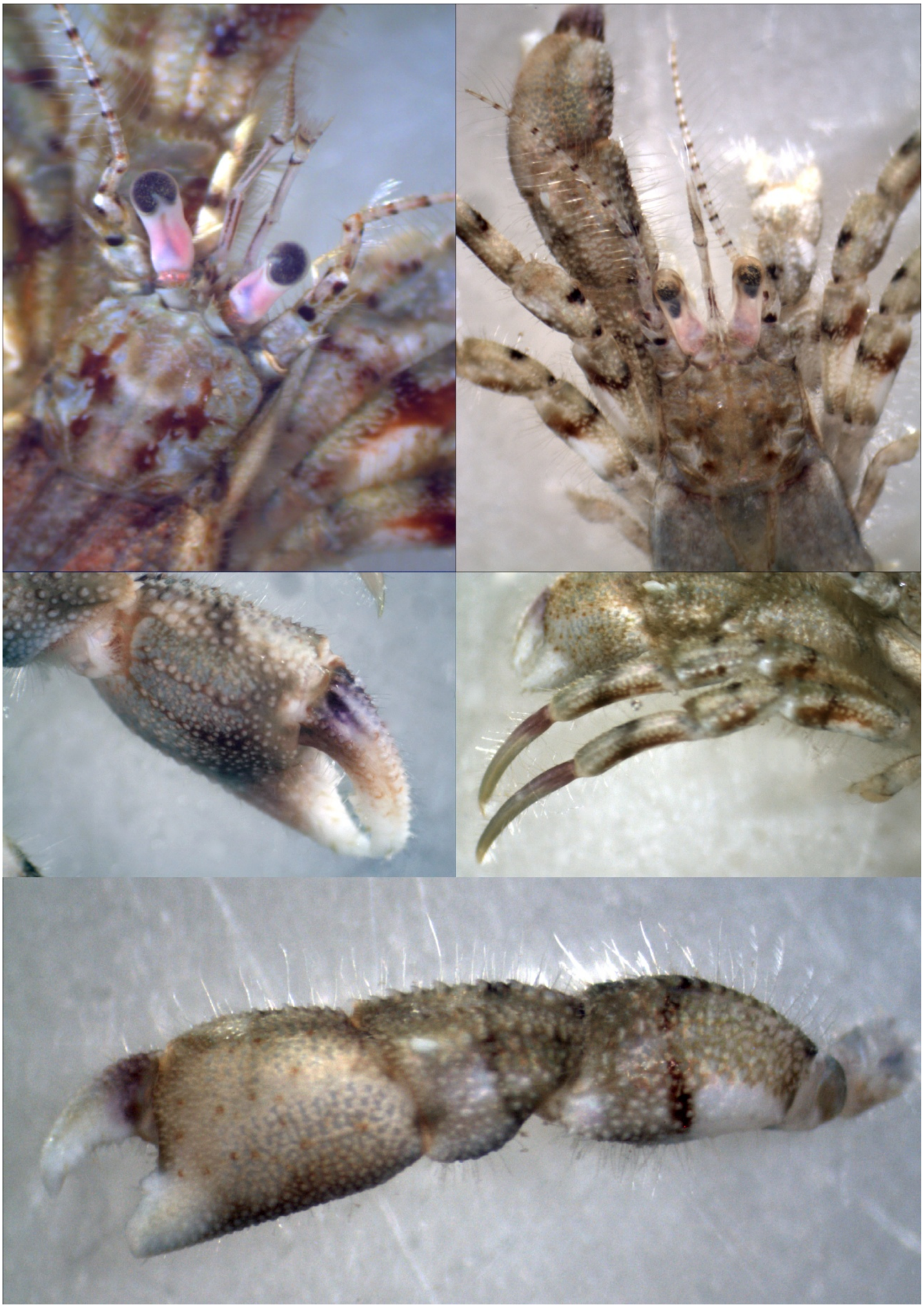

Fig. A1. Live colour images of exoskeleton in different specimens of *Diogenes ponticus*.

TABLE AI

List of *Diogenes* specimens included in the present study, with collection sites and GenBank accession codes for COI and 16S partial sequences

ID	Species	Collection site	16S	COI
D1	*D. curvimanus*	Belgium: Nieuwpoort	*PP866938*	MW776659
D2	*D. curvimanus*	Belgium: Nieuwpoort	*PP866939*	MW776658
D3	*D. curvimanus*	France: Gironde: Bay d'Arcachon	*PP866940*	*PP866891*
D4	*D. curvimanus*	France: Gironde: Bay d'Arcachon	*PP866941*	*PP866892*
D5	*D. curvimanus*	Spain: Guadalquivir: Huelva*	MW791779	MW776663
D6	*D.armatus*	France: Gironde: Bay d'Arcachon	*PP866942*	*PP866893*
D7	*D. ponticus*	Spain: Tarragona: Ebro-Delta	*PP866965*	*PP866910*
D8	*D.armatus*	Spain: Tarragona: Ebro-Delta	*PP866943*	*PP866894*
D9	*D.armatus*	Spain: Tarragona: Ebro-Delta	*PP866944*	*PP866895*
D10	*D.armatus*	Spain: Tarragona: Ebro-Delta	*PP866945*	*PP866896*
D11	*D. armatus*	Spain: Torregorda: Cádiz*	MW791815	MW776704
D12	*D. armatus*	Spain: Isla Canela: Huelva*	MW791814	MW776705
D13	*D.armatus*	Ibiza: Cala Llenya	*PP866950*	*PP866901*
D14	*D.armatus*	Ibiza: Cala Llenya	*PP866951*	*PP866902*
D15	*D.armatus*	Ibiza: Cala Llenya	*PP866949*	*PP866900*
D16	*D.armatus*	Ibiza: Cala Llenya	*PP866952*	*PP866903*
D17	*D.armatus*	Ibiza: Cala Llenya	*PP866953*	*PP866904*
D18	*D.armatus*	Ibiza: Cala Llenya	*PP866954*	*PP866905*
D19	*D.armatus*	Ibiza: Torrent	*PP866946*	*PP866897*
D20	*D.armatus*	Ibiza: Torrent	*PP866947*	*PP866898*
D21	*D.armatus*	Ibiza: Torrent	*PP866948*	*PP866899*
D22	*D.armatus*	Corsica: Roccapina	*PP866955*	*PP866906*
D23	*D.armatus*	Corsica: Golfe St. Florent	*PP866956*	*PP866907*
D24	*D.armatus*	Sardinia: Golfo di Saline	*PP866957*	*PP866908*
D25	*D.armatus*	Cyprus: Larnaca: Oroklini	*PP866958*	*PP866909*
D26	*D. pugilator*	France: Frontignan	MW791804	MW776677
D27	*D. pugilator*	France: Frontignan	*PP866960*	MW776684
D28	*D. pugilator*	France: Frontignan	*PP866961*	MW776681
D29	*D. pugilator*	France: Frontignan*	*PP866962*	MW776683
D30	*D. pugilator*	France: Frontignan	*PP866963*	MW776682
D31	*D. pugilator*	France: Frontignan	*PP866964*	MW776680
D32	*D. pugilator*	Spain: Tarragona: Ebro-Delta	*PP866959*	MW776687
D33	*D. pugilator*	Spain: Tarragona: Ebro-Delta	MW791805	MW776686
D34	*D. pugilator*	Spain: Malága: Torremolino: La Carihuela	MW791796	MW776692
D35	*D. ponticus*	Sicily: Messina: Torre Faro: Punta del Faro	*PP866966*	*PP866911*
D36	*D. ponticus*	Italy: Lecce: Salento: Otranto	*PP866974*	*PP866919*
D37	*D. ponticus*	Italy: Lecce: Salento: Otranto	*PP866975*	*PP866920*
D38	*D. ponticus*	Italy: Lecce: Salento: Otranto	*PP866976*	*PP866921*
D39	*D. ponticus*	Italy: Lecce: Salento: Otranto	*PP866977*	*PP866922*
D40	*D. ponticus*	Croatia: Pula: Stoja Bay	*PP866967*	*PP866912*
D41	*D. ponticus*	Croatia: Pula: Stoja Bay	*PP866968*	*PP866913*

TABLE AI
(Continued)

ID	Species	Collection site	16S	COI
D42	*D. ponticus*	Croatia: Nin: Zaton	*PP866969*	*PP866914*
D43	*D. ponticus*	Croatia: Nin: Zaton	*PP866970*	*PP866915*
D44	*D. ponticus*	Croatia: Korčula: Brna: Plaža Istruga	*PP866971*	*PP866916*
D45	*D. ponticus*	Croatia: Korčula: Brna: Plaža Istruga	*PP866972*	*PP866917*
D46	*D. ponticus*	Croatia: Korčula: Brna: Plaža Istruga	*PP866973*	*PP866918*
D47	*D. ponticus*	Greece: Thermaikos Gulf: Thessaloniki Bay	*PP866980*	*PP866925*
D48	*D. ponticus*	Greece: Amvrakikos Gulf	*PP866978*	*PP866923*
D49	*D. ponticus*	Greece: Amvrakikos Gulf	*PP866979*	*PP866924*
D50	*D. ponticus*	Bulgaria: Ropotamo Estuary	*PP866981*	*PP866926*
D51	*D. ponticus*	Bulgaria: Ropotamo Estuary	*PP866982*	*PP866927*
D52	*D. ponticus*	Bulgaria: Ropotamo Estuary	*PP866983*	*PP866928*
D53	*D. ponticus*	Bulgaria: Burgas: Nesebar: Sveti Vlas*	*PP866984*	*PP866929*
D54	*D. ponticus*	Bulgaria: Burgas: Nesebar: Sveti Vlas*	*PP866985*	*PP866930*
D55	*D. ponticus*	Bulgaria: Burgas: Nesebar: Sveti Vlas	*PP866986*	*PP866931*
D56	*D. ponticus*	Bulgaria: Burgas: Nesebar: Sveti Vlas	*PP866987*	*PP866932*
D57	*D. ponticus*	Bulgaria: Burgas: Nesebar: Sveti Vlas	*PP866988*	*PP866933*
D58	*D. ponticus*	Bulgaria: Varna: Varna Bay	*PP866989*	*PP866934*
D59	*D. ponticus*	Bulgaria: Varna: Varna Bay	*PP866990*	*PP866935*

Asterisks indicate type specimens and sequences generated in this study are shown in italics.

TABLE AII

Summary of some relevant morphological features in the six species of the genus *Diogenes* known to date in European waters

	D. pugilator Roux, 1829	*D. armatus* Almón et al., 2022a	*D. curvimanus* Clément, 1874	*D. arguinensis* Almón, Cuesta & García-Raso, 2022	*D. erythromanus* Almón, Cuesta & García-Raso, 2022	*D. ponticus* Kessler, 1860
Cephalic features						
Shield	nearly as long as broad	slightly longer than broad	slightly broader than long	longer than broad	slightly longer than broad	slightly longer than broad
Rostral lobe	broadly rounded, not well-developed	acutely rounded, well developed	broadly rounded, not well-developed	narrowly rounded, not well-developed	acutely rounded, well-developed	broadly rounded, not well-developed
Ocular acicles	3-5 large spines + 11-12 tiny tubercles	11-12 spines	14-16 small spines	5-6 large + 4-5 small spines	4-5 large + 2-3 small spines	3-4 large + 9-10 small spines
Antennules						
Segment 3	not distally broadened	not distally broadened	distally broadened	slightly broadened distally	slightly broadened distally	not distally broadened
Overreaching distal corneas by...	0.8-0.9 length of seg 3	0.4 length of seg 3	0.4-0.6 length of seg 3	0.8 length of seg 3	0.8-0.9 length of seg 3	0.5 length of seg 3
Antennae						
Peduncular segment 4	unarmed	unnarmed	unarmed	with disto-outer spine	with disto-outer spine	unarmed
Coloured spot	vermillion red spot at base of acicle	brown spot on acicle	greenish-blue spot on segment 4	unknown	diffuse coloration, without defined marks	dark-red spot on acicle

TABLE AII
(Continued)

	D. pugilator Roux, 1829	*D. armatus* Almón et al., 2022a	*D. curvimanus* Clément, 1874	*D. arguinensis* Almón, Cuesta & García-Raso, 2022	*D. erythromanus* Almón, Cuesta & García-Raso, 2022	*D. ponticus* Kessler, 1860
Antennal acicle						
Spines	5	3-4	6-7	5-6	5-6	3-4
Length	slightly below distal margin of antennal segment 4	well below distal margin of antennal segment 4	slightly overreaching the proximal half of antennal segment 4	reaching distal margin of antennal segment 4	almost reaching distal margin of antennal segment 4	slightly below distal margin of antennal segment 4
Palm of left cheliped						
Proportions	as long as high	longer than high	slender, much longer than high	longer than high	higher than long	longer than high
Spination	regularly covereded by small spinose tubercles	spinose, largest spines forming longitudinal rows	almost smooth	small tubercles	small sharp spines	small spinose tubercles, largest forming longitudinal rows
Outer surface	regularly inflated medially without median crest	proximal half elevated, with proximal spinose crest	slightly inflated, without median crest	inflated proximo-medially, with faint proximal crest	medially inflated, with proximal spinose crest	markedly inflated throughout, without proximal crest

TABLE AII
(Continued)

	D. pugilator Roux, 1829	*D. armatus* Almón et al., 2022a	*D. curvimanus* Clément, 1874	*D. arguinensis* Almón, Cuesta & García-Raso, 2022	*D. erythromanus* Almón, Cuesta & García-Raso, 2022	*D. ponticus* Kessler, 1860
Carpus of left cheliped						
Outer surface	with medial row of spines	with medial row of spines	disto-medial row of large tubercles	spinose distally	spinose distally	irregularly tuberculated
Sternal plate	with tuft of long setae on each side	with tuft of long setae on each side	with tuft of long setae on each side	unknown	with tuft of long setae on each side	with 2-3 strong spines on each side
Telson	markedly asymmetrical	asymmetrical	slightly asymmetrical	slightly asymmetrical	markedly asymmetrical	barely asymmetrical

Based on morphological descriptions by Almón et al. (2022a, b) and the current study.

REFERENCES

AKAIKE, H., 1973. Information theory and an extension of the Maximum Likelihood Principle. In: B. N. PETROV & F. CSAKI (eds.), Proc. 2nd Int. Symp. on Information Theory: 267-281. (Akadémiai Kiadó, Budapest).

AKTEN, N., 2003. The Strait of Istanbul (Bosphorus): The seaway separating the continents with its dense shipping traffic. Turk. J. Marit. Mar. Sci., **9**(3): 241-265.

ALMÓN, B., J. A. CUESTA & J. E. GARCÍA-RASO, 2022b. Two new hermit crab species of *Diogenes* (Crustacea: Decapoda: Diogenidae) from Atlanto-Mediterranean coasts of Iberian Peninsula: Poleward migrants or merely overlooked indigenous species? Ecol. Evol., **12**(5): e8844. DOI:10.1002/ece3.8844.

ALMÓN, B., J. A. CUESTA, C. D. SCHUBART, L. ARMENIA & J. E. GARCÍA-RASO, 2022a. Redescription of the hermit crab *Diogenes pugilator* (Decapoda: Anomura) reveals the existence of a species complex in the Atlanto-Mediterranean transition zone, resulting in the resurrection of *D. curvimanus* and the description of a new species. Zool. J. Linn. Soc., **195**(4): 1116-1146. DOI:10.1093/zoolinnean/zlab093.

ALTSCHUL, S. F., W. GISH, W. MILLER, E. W. MYERS & D. J. LIPMAN, 1990. Basic local alignment search tool. J. Mol. Biol., **215**(3): 403-410. DOI:10.1016/S0022-2836(05)80360-2.

BLAXTER, M., 2016. Imagining Sisyphus happy: DNA barcoding and the unnamed majority. Phil. Trans. R. Soc. B: Biol. Sci., **371**: 20150329. DOI:10.1098/rstb.2015.0329.

ÇETIN, C., A. FURMAN, E. KALKAN & R. BILGIN, 2022. Mitonuclear genetic patterns of divergence in the marbled crab, *Pachygrapsus marmoratus* (Fabricius, 1787) along the Turkish seas. PLoS ONE, **17**(4): e0266506. DOI:10.1371/journal.pone.0266506.

CLEMENT, M., D. POSADA & K. A. CRANDALL, 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol., **9**(10): 1657-1659. DOI:10.1046/j.1365-294x.2000.01020.x.

COCHRANE, S. K. J., J. H. ANDERSEN, T. BERG, H. BLANCHET, A. BORJA, J. CARSTENSEN, M. ELLIOTT, H. HUMMEL, N. NIQUIL & P. E. RENAUD, 2016. What is marine biodiversity? Towards common concepts and their implications for assessing biodiversity status. Front. Mar. Sci., **3**(248): 1-24. DOI:10.3389/fmars.2016.00248.

COLL, M., C. PIRODDI, J. STEENBEEK, K. KASCHNER, F. B. R. LASRAM, J. AGUZZI, E. BALLESTEROS, C. N. BIANCHI, J. CORBERA, T. DAILIANIS, R. DANOVARO, M. ESTRADA, C. FROGLIA, B. S. GALIL, J. M. GASOL, R. GERTWAGE, J. GIL, F. GUILHAUMON, K. KESNER-REYES, M.-S. KITSOS, A. KOUKOURAS, N. LAMPADARIOU, E. LAXAMANA, C. M. LÓPEZ-FÉ DE LA CUADRA, H. K. LOTZE, D. MARTIN, D. MOUILLOT, D. ORO, S. RAICEVICH, J. RIUS-BARILE, J. I. SAIZ-SALINAS, C. SAN VINCENTE, S. SOMOT, J. TEMPLADO, X. TURON, D. VAFIDIS, R. VILLANUEVA & E. VOULTSIADOU, 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS ONE, **5**(8): e11842. DOI:10.1371/journal.pone.0011842.

CRACRAFT, J., 2002. The seven great questions of systematic biology: An essential foundation for conservation and the sustainable use of biodiversity. Ann. Mo. Bot. Gard., **89**(2): 127-144. DOI:10.2307/3298558.

CRANDALL, K. A. & J. F. FITZPATRICK, JR., 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst. Biol., **45**: 1-26. DOI:10.1093/sysbio/45.1.1.

D'ALOIA, C. C., S. M. BOGDANOWICZ, R. K. FRANCIS, J. E. MAJORIS, R. G. HARRISON & P. M. BUSTON, 2015. Patterns, causes, and consequences of marine larval dispersal. PNAS, **112**(45): 13940-13945. DOI:10.1073/pnas.1513754112.

DECANET, 2023. DecaNet. *Diogenes pugilator* (Roux, 1829). Available online at https://www.marinespecies.org/aphia.php?p=taxdetails&id=107199 (14 July 2023).

DELI, T., S. GUIZENI, L. BEN ABDALLAH, K. SAID & N. CHATTI, 2020. Chaotic genetic patchiness in the pelagic teleost fish *Sardina pilchardus* across the Siculo-Tunisian Strait. Mar. Biol. Res., **16**(4): 280-298. DOI:10.1080/17451000.2020.1752921.

DELI, T., E. KALKAN, S. Ü. KARHAN, S. UZUNOVA, A. KEIKHOSRAVI, R. BILGIN & C. D. SCHUBART, 2018a. Parapatric genetic divergence among deep evolutionary lineages in the Mediterranean green crab, *Carcinus aestuarii* (Brachyura, Portunoidea, Carcinidae), accounts for a sharp phylogeographic break in the Eastern Mediterranean. BMC Evol. Biol., **18**(1): 1-21. DOI:10.1186/s12862-018-1167-4.

DELI, T., M. PFALLER & C. D. SCHUBART, 2018b. Phylogeography of the littoral prawn species *Palaemon elegans* (Crustacea: Caridea: Palaemonidae) across the Mediterranean Sea unveils disparate patterns of population genetic structure and demographic history in the two sympatric genetic types II and III. Mar. Biodiv., **48**(4): 1979-2001. DOI:10.1007/s12526-017-0711-6.

DELI, T., K. SAID & N. CHATTI, 2015. Genetic differentiation among populations of the green crab *Carcinus aestuarii* (Brachyura, Carcinidae) from the eastern and western Mediterranean coasts of Tunisia. Acta. Zool. Bulg., **67**(3): 327-335.

DOS SANTOS, V. V. & M.-S. TIXIER, 2017. Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). Cladistics, **33**(3): 251-267. DOI:10.1111/cla.12166.

FRATINI, S., L. RAGIONIERI, T. DELI, A. HARRER, I. A. M. MARINO, S. CANNICCI, L. ZANE & C. D. SCHUBART, 2016. Unraveling population genetic structure with mitochondrial DNA in a notional panmictic coastal crab species: Sample size makes the difference. BMC Evol. Biol., **16**(1): 1-15. DOI:10.1186/s12862-016-0720-2.

GAMERMAN, D. & H. F. LOPES, 2006. Markov chain Monte Carlo: Stochastic simulation for Bayesian inference (2nd ed.): 1-342. (Chapman & Hall/CRC, London). DOI:10.1201/9781482296426.

GARCÍA-MERCHÁN, V. H., A. ROBAINAS-BARCIA, P. ABELLÓ, E. MACPHERSON, F. PALERO, M. GARCÍA-RODRÍGUEZ, L. G. DE SOLA & M. PASCUAL, 2012. Phylogeographic patterns of decapod crustaceans at the Atlantic-Mediterranean transition. Mol. Phylogenet. Evol., **62**(2): 664-672. DOI:10.1016/j.ympev.2011.11.009.

GHARBI, A., R. ZITARI-CHATTI, A. VAN WORMHOUDT, M. DHRAIEF, F. DENIS, K. SAID & N. CHATTI, 2011. Allozyme variation and population genetic structure in the carpet shell clam *Ruditapes decussatus* across the Siculo-Tunisian Strait. Biochem. Genet., **49**: 788-805. DOI:10.1007/s10528-011-9450-8.

GOLDSTEIN, T., C. STUDER & R. BARANIUK, 2014. A field guide to forward-backward splitting with a FASTA implementation. arXiv:1411.3406v1 [cs.NA.]: 1-16.

GOLDSTEIN, T., C. STUDER & R. BARANIUK, 2015. FASTA: A generalized implementation of forward-backward splitting. arXiv:1501.04979v3 [cs.MS]: 1-6.

GÜLER, N., 2005. Black Sea maritime transport corridors in relation with EU projects. In: R. MARCET I BARBÉ & C. A. BREBBIA (eds.), Maritime heritage and modern ports: 293-300. (WIT Press, Southhampton).

HALL, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser, **41**: 95-98.

HEBERT, P. D. N., A. CYWINSKA, S. L. BALL & J. R. DEWAARD, 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B: Biol. Sci., **270**(1512): 313-321. DOI:10.1098/rspb.2002.2218.

HEETHOFF, M., M. LAUMANN, G. WEIGMANN & G. RASPOTNIG, 2011. Integrative taxonomy: Combining morphological, molecular and chemical data for species delineation in the parthenogenetic *Trhypochthonius tectorum* complex (Acari, Oribatida, Trhypochthoniidae). Front. Zool., **8**(2): 1-10. DOI:10.1186/1742-9994-8-2.

HUELSENBECK, J. P. & F. RONQUIST, 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, **17**(8): 754-755. DOI:10.1093/bioinformatics/17.8.754.

KEIKHOSRAVI, A. & C. D. SCHUBART, 2014. Revalidation and redescription of *Potamon elbursi* Pretzmann, 1976 (Brachyura, Potamidae) from Iran, based on morphology and genetics. Cent. Eur. J. Biol., **9**(1): 114-123. DOI:10.2478/s11535-013-0203-z.

KESSLER, K. F., 1860. A journey with a zoological goal to the northern shore of the Black Sea and Crimea in 1858: 1-246. [in Russian.] (Kievskiy Universitet, Kiev).

KIDEYS, A. E., 2002. Assessing extent and impact of ship-transported alien species in the Black Sea. CIESM Workshop Monogr., **2**: 79-82.

KLAUS, S., C. D. SCHUBART & D. BRANDIS, 2006. Phylogeny, biogeography, and a new taxonomy for the Gecarcinucoidea Rathbun, 1904 (Decapoda: Brachyura). Org. Divers. Evol., **6**: 199-217. DOI:10.1016/j.ode.2005.09.006.

LANDSCHOFF, J. & G. GOUWS, 2018. DNA Barcoding as a tool to facilitate the taxonomy of hermit crabs (Decapoda: Anomura: Paguroidea). J. Crust. Biol., **38**(6): 780-793. DOI:10.1093/jcbiol/ruy084.

LANFEAR, R., B. CALCOTT, S. Y. W. HO & S. GUINDON, 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol., **29**(6): 1695-1701. DOI:10.1093/molbev/mss020.

LEIGH, J. W. & D. BRYANT, 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol., **6**(9): 1110-1116. DOI:10.1111/2041-210X.12410.

LI, W., P. ŁĄCZYŃSKI, H. ESCALONA, J. EBERLE, L. HUO, X. CHEN, W. HUANG, B. CHEN, D. AHRENS, A. ŚLIPIŃSKI, W. TOMASZEWSKA & X. WANG, 2020. Combined molecular and morphological data provide insights into the evolution and classification of Chilocorini ladybirds (Coleoptera: Coccinellidae). Syst. Entomol., **45**(2): 447-463. DOI:10.1111/syen.12409.

LIN, X., E. STUR & T. EKREM, 2015. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. PLoS ONE, **10**(9): e0138993. DOI:10.1371/journal.pone.0138993.

MADDISON, D. R., D. L. SWOFFORD & W. P. MADDISON, 1997. NEXUS: An extensible file format for systematic information. Syst. Biol., **46**(4): 590-621. DOI:10.1093/sysbio/46.4.590.

MANTELATTO, F. L. M., R. ROBLES, R. BIAGI & D. L. FELDER, 2006. Molecular analysis of the taxonomic and distributional status for the hermit crab genera *Loxopagurus* Forest, 1964 and *Isocheles* Stimpson, 1858 (Decapoda, Anomura, Diogenidae). Zoosystema, **28**(2): 495-506. DOI:10.5281/zenodo.5400460.

MATZEN DA SILVA, J. M., S. CREER, A. DOS SANTOS, A. C. COSTA, M. R. CUNHA, F. O. COSTA & G. R. CARVALHO, 2011b. Systematic and evolutionary insights derived from mtDNA COI Barcode diversity in the Decapoda (Crustacea: Malacostraca). PLoS ONE, **6**(5): e19449. DOI:10.1371/journal.pone.0019449.

MATZEN DA SILVA, J. M., A. DOS SANTOS, M. R. CUNHA, F. O. COSTA, S. CREER & G. R. CARVALHO, 2011a. Multigene molecular systematics confirm species status of morphologically convergent *Pagurus* hermit crabs. PLoS ONE, **6**(12): e28233. DOI:10.1371/journal.pone.0028233.

MEJRI, R., S. LO BRUTTO, O. K. BEN HASSINE & M. ARCUELO, 2009. A study on *Pomatoschistus tortonesei* Miller 1968 (Perciformes, Gobiidae) reveals the Siculo-Tunisian Strait (STS) as a breakpoint to gene flow in the Mediterranean basin. Mol. Phylogenet. Evol., **53**(2): 596-601. DOI:10.1016/j.ympev.2009.04.018.

MERCADO-SALAS, N. F., J. OETTLER, I. A. TOLEDANO CARRASCO & J. J. ASTRIN, 2024. The Christoph Schubart decapod collections. Crustaceana, **97**(5-9): 361-382.

NEGRI, M. P., R. LEMAITRE & F. L. MANTELATTO, 2014. Molecular and morphological resurrection of *Clibanarius symmetricus* (Randall, 1840), a cryptic species hiding under the name for the "thinstripe" hermit crab *C. vittatus* (Bosc, 1802) (Decapoda: Anomura: Diogenidae). J. Crust. Biol., **34**(6): 848-861. DOI:10.1163/1937240X-00002277.

ÖZTÜRK, B. & A. A. ÖZTÜRK, 1996. On the biology of the Turkish straits system. Bull. Inst. Océanogr (Monaco), CIESM Science Ser., **2**: 205-221.

PASCUAL, M., B. RIVES, C. SCHUNTER & E. MACPHERSON, 2017. Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. PLoS ONE, **12**(5): e0176419. DOI:10.1371/journal.pone.0176419.

PIKE, R. B. & D. I. WILLIAMSON, 1960. Larvae of decapod Crustacea of the families Diogenidae and Paguridae from the Bay of Naples. Pubbl. Staz. Zool. Napoli, **21**: 493-552.

PIMM, S. L., C. N. JENKINS, R. ABELL, T. M. BROOKS, J. L. GITTLEMAN, L. N. JOPPA, P. H. RAVEN, C. M. ROBERTS & J. O. SEXTON, 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science, **344**(6187): 1246752. DOI:10.1126/science.1246752.

PURROY, A., T. ŠEGVIĆ-BUBIĆ, A. HOLMES, I. BUŠELIĆ, J. THÉBAULT, A. FEATHERSTONE & M. PEHARDA, 2016. Combined use of morphological and molecular tools to resolve species mis-identifications in the Bivalvia: The case of *Glycymeris glycymeris* and *G. pilosa*. PLoS ONE, **11**(9): e0162059. DOI:10.1371/journal.pone.0162059.

RAGIONIERI, L., S. CANNICCI & S. FRATINI, 2024. Distinct phylogeographic and population genetic patterns of decapod species across Mediterranean biogeographic barriers. Crustaceana, **97**(5-9): 581-597.

RAGIONIERI, L. & C. D. SCHUBART, 2013. Population genetics, gene flow, and biogeographical boundaries of *Carcinus aestuarii* (Crustacea: Brachyura: Carcinidae) along the European Mediterranean coast. Biol. J. Linn. Soc., **109**(4): 771-790. DOI:10.1111/bij.12099.

RAMBAUT, A., A. J. DRUMMOND, D. XIE, G. BAELE & M. A. SUCHARD, 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol., **67**(5): 901-904. DOI:10.1093/sysbio/syy032.

RATA, V., C. GASPAROTTI & L. RUSU, 2018. Ballast water management in the Black Sea's ports. J. Mar. Sci. Eng, **6**(2): 69. DOI:10.3390/jmse6020069.

REUSCHEL, S., J. A. CUESTA & C. D. SCHUBART, 2010. Marine biogeographic boundaries and human introduction along the European coast revealed by phylogeography of the prawn *Palaemon elegans*. Mol. Phylogenet. Evol, **55**(3): 765-775. DOI:10.1016/j.ympev.2010.03.021.

RODRIGUES, M. S., K. A. MORELLI & A. M. JANSEN, 2017. Cytochrome c oxidase subunit 1 gene as a DNA barcode for discriminating *Trypanosoma cruzi* DTUs and closely related species. Parasit. Vectors, **10**(488): 1-18. DOI:10.1186/s13071-017-2457-1.

SÁ-PINTO, A., M. S. BRANCO, P. B. ALEXANDRINO, M. C. FONTAINE & S. J. E. BAIRD, 2012. Barriers to gene flow in the marine environment: Insights from two common intertidal limpet species of the Atlantic and Mediterranean. PLoS ONE, **7**(12): e50330. DOI:10.1371/journal.pone.0050330.

SCHUBART, C. D., 2009. Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: J. W. MARTIN, K. A. CRANDALL & D. L. FELDER (eds.), Decapod crustacean phylogenetics. Crustac. Issues, **18**: 47-65. (CRC Press, Boca Raton).

SCHUBART, C. D., J. A. CUESTA, R. DIESEL & D. L. FELDER, 2000. Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American Grapsoid crabs (Crustacea: Brachyura). Mol. Phylogenet. Evol., **15**(2): 179-190. DOI:10.1006/mpev.1999.0754.

SCHUBART, C. D., J. A. CUESTA & A. RODRÍGUEZ, 2001. Molecular phylogeny of the crab genus *Brachynotus* (Brachiyura: Varunidae) based on the 16S rRNA gene. Hydrobiologa, **449**(1): 41-46. DOI:10.1023/A:1017564229866.

SCHUBART, C. D., T. DELI, G. MANCINELLI, L. CILENTI, A. GIL FERNÁNDEZ, S. FALCO & S. BERGER, 2023. Phylogeography of the Atlantic Blue Crab *Callinectes sapidus* (Brachyura: Portunidae) in the Americas versus the Mediterranean Sea: Determining origins and genetic connectivity of a large-scale invasion. Biology, **12**(1): 35. DOI:10.3390/biology12010035.

SCHUBART, C. D., R. DIESEL & S. B. HEDGES, 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature, **393**: 363-365. DOI:10.1038/30724.

SCHUBART, C. D. & M. G. J. HUBER, 2006. Genetic comparisons of German populations of the stone crayfish, *Austropotamobius torrentium* (Crustacea: Astacidae). Bull. Fr. Peche Piscic., **380-381**: 1019-1028. DOI:10.1051/kmae:2006008.

TEMPLETON, A. R., K. A. CRANDALL & C. F. SING, 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics, **132**(2): 619-633. DOI:10.1093/genetics/132.2.619.

THIERCELIN, N. & C. D. SCHUBART, 2014. Transisthmian differentiation in the tree-climbing mangrove crab *Aratus* H. Milne Edwards, 1853 (Crustacea, Brachyura, Sesarmidae), with description of a new species from the tropical eastern Pacific. Zootaxa, **3793**(5): 545-560. DOI:10.11646/zootaxa.3793.5.3.

THOMPSON, J. D., D. G. HIGGINS & T. J. GIBSON, 1994. Clustal W: Improving the sensitivity of progressive multiples sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., **22**(22): 4673-4680. DOI:10.1093/nar/22.22.4673.

VILLESEN, P., 2007. FaBox: an online toolbox for fasta sequences. Mol. Ecol. Resour., **7**(6): 965-968. DOI:10.1111/j.1471-8286.2007.01821.x.

WEISS, R., Z. TORRECILLA, E. GONZÁLEZ-ORTEGÓN, A. M. GONZÁLEZ-TIZÓN, A. MARTÍNEZ-LAGE & C. D. SCHUBART, 2018. Genetic differentiation between Mediterranean and Atlantic populations of the common prawn *Palaemon serratus* (Crustacea: Palaemonidae) reveals uncommon phylogeographic break. J. Mar. Biol. Assoc. U. K., **98**(6): 1425-1434.

ZAITSEV, Y. P., 1992. Recent changes in the trophic structure of the Black Sea. Fish. Oceanogr., **1**(2): 180-189. DOI:10.1111/j.1365-2419.1992.tb00036.x.

ZAROKANELLOS, N. D., D. L. RUDNICK, M. GARCIA-JOVE, B. MOURRE, S. RUIZ, A. PASCUAL & J. TINTORÉ, 2022. Frontal dynamics in the Alboran Sea: 1. Coherent 3D pathways at the Almeria-Oran front using underwater glider observations. J. Geophys. Res. Oceans, **127**: e2021JC017405. DOI:10.1029/2021JC017405.

ZITARI-CHATTI, R., N. CHATTI, A. ELOUAER & K. SAID, 2008. Genetic variation and population structure of the caramote prawn *Penaeus kerathurus* (Forskäl) from the Eastern and Western Mediterranean coasts in Tunisia. Aquac. Res., **39**: 70-76. DOI:10.1111/j.1365-2109.2007.01874.x.

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