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The Maghreb as a Hotspot of Diversity for the Freshwater Crab Genus *Potamon* (Decapoda, Potamidae)

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Abstract

The Maghreb region of North Africa, located at the intersection of the Palaearctic and Afrotropical zones, is a biodiversity hotspot for terrestrial and freshwater taxa, including the freshwater crab of genus *Potamon* Savigny, 1816. Recent molecular studies have suggested the presence of two distinct *Potamon* species in the region: *Potamon algeriense* Bott, 1967, and an as-yet undescribed taxon, *Potamon* sp. However, comprehensive data on their distribution, genetic structure, and conservation status are still lacking. In the present study, we integrate new field collections from Algeria and Morocco (2021–2023) with molecular analyses of mitochondrial (COI, ND1) and nuclear (28S rDNA) markers to assess species boundaries and genetic diversity within *Potamon* across the Maghreb. Phylogenetic reconstructions based on Maximum Likelihood and Bayesian Inference consistently support the presence of two well-differentiated *Potamon* lineages in the region, corresponding to *P. algeriense* in western and central Maghreb, and *Potamon* sp. in eastern Algeria and Tunisia. While *Potamon* sp. exhibits low intra-specific genetic variation, *P. algeriense* displays a deeply structured mitochondrial lineage composition, forming four geographically coherent subclades, each corresponding to distinct hydrological regions. In light of this, it would be advisable to revise the IUCN assessment to include both species and updated information on their distribution.

Keywords: *Euthelphusa*; northeast Algerian phylogeographic break; *Potamon algeriense*; IUCN status; North Africa

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1. Introduction

The Maghreb is a well-defined geographical region surrounded by effective natural barriers. It is located in western Africa between the Sahara, the largest hot desert in the world, the Mediterranean Sea, and the Atlantic Ocean. Although it is part of the African continent, its biota is similar to the Euroasiatic one, and the region is thus ascribed to the Palaearctic biogeographical region [1]. In fact, since the Tertiary period, the area has had several directly facilitated connections with the European land masses: at the end of the Miocene, the Messinian salinity crisis put the Maghreb in direct contact with southern Europe [2,3], and, during Pleistocene, marine regressions facilitated exchanges to and from the Iberian Peninsula [4,5]. Moreover, its location along the southern shores of the Mediterranean Sea protected the area from the most intense effects of Quaternary glaciations, and the whole area acted as a refuge, where west Mediterranean terrestrial and freshwater biota could survive through Pleistocene climatic upheavals [6]. Conversely, its contacts with the Afrotropical region and its biota were much weaker, and nearly negligible, especially since the formation of the Sahara Desert (e.g., [7]).

Its complex palaeogeographical and climatic history, and its current complex topography, made the Maghreb a hotspot for terrestrial and freshwater biological diversity [8]. Local freshwater diversity was further enhanced by the relative climatic stability of the area during the Quaternary period, which allowed the long-term persistence in situ of existing lineages and populations, and the intrinsically fragmented nature of inland waterbodies, especially in mountainous and semi-arid areas, which favours processes of allopatric differentiation [9]. However, the inland water fauna of the Maghreb is currently severely threatened by anthropogenic pressures, including overexploitation of water resources, wetland reclamation for agricultural and urban development, biological invasions, and the accelerating effects of climate change [10–12]. These impacts are especially severe for those species dependent on permanent freshwater habitats, where the balance between human activities and ecosystem stability is most precarious [13,14].

The crabs belonging to the genus *Potamon* Savigny, 1816, are among the most iconic Maghrebian freshwater taxa. They inhabit running and lentic water bodies throughout the area, where they are an important part of the trophic web [15]. Recent molecular studies proved that at least two different species occur in the area, *Potamon algeriense* Both, 1967 and *Potamon* sp., the latter still pending a formal description due to the lack of adult specimens for its morphological characterisation [16]. *Potamon algeriense* occurs in Morocco and most of Algeria, whereas *Potamon* sp. is to date known to occur only in northern Tunisia and the Annaba province in eastern Algeria [16,17]. However, no accurate data about the distribution of the two *Potamon* species occurring in Maghreb are to date available. Based on the available data, freshwater crabs' populations are reported discontinuously for the whole Maghreb (e.g., [15,17–19]), but a recent synopsis is missing and the limit between the distribution areas of the two species has been described only tentatively [16]. In the light of the wide distribution range of the species, *Potamon algeriense* (at that time including also the *Potamon* sp. populations from Tunisia and eastern Algeria) was considered of "least concern" (LC) in the last available IUCN evaluation [19].

No assessment about the status and trend of Tunisian and Algerian *Potamon* spp. populations is currently available, whereas existing data about some Moroccan populations suggest an ongoing decline of the species in this country (e.g., [20–25]).

The current distribution of the two *Potamon* species inhabiting the Maghreb region remains insufficiently characterised, and there is a notable lack of data regarding the conservation status of their extant populations. This study aims to enhance the understanding of the distribution and genetic diversity of *Potamon* species across North Africa by presenting newly validated occurrence records for both species. Furthermore, given the significant molecular structuring previously observed within *Potamon algeriense sensu stricto*, we investigated the potential presence of additional hitherto unrecognised *Potamon* species in the region, or alternatively, the existence of highly divergent evolutionary lineages within *P. algeriense*.

2. Materials and Methods

Novel *Potamon* populations were sampled in Algeria and Morocco from 2021 to 2023. No samplings were carried out in Tunisia for this research. Samples were collected by means of baited funnel traps and direct collection of individuals found under rocks and other shelters. Each site was characterised through its toponym and its geographical WGS84 coordinates. A GIS-based map showing the distribution of the sampled site was produced using the QGIS software v. 3.30.2 (https://www.qgis.org/, last accessed on 8 July 2025 [26]). Following Beddek et al. [27] and Marrone et al. [16], the mid-distance between the nearest populations belonging to the two different *Potamon* species occurring in Maghreb was considered the geographical limit between species distribution areas.

Collected individuals were identified in situ according to their habitus following [15,28], fixed in 90% ethanol, and stored in the authors' collections; in some cases, upon field identification, one walking appendage was excised from caught individuals before releasing them back alive in the collection sites (see [29]).

One walking appendage from each specimen was removed and used for DNA extraction. Tissue vouchers are deposited at the University of Palermo (Italy) under the responsibility of (FM).

Prior to DNA extraction, a small fragment of muscle tissue was carefully dissected from the excised walking appendages and subsequently soaked in double-distilled water for 5 min to remove potential ethanol residuals. Genomic DNA was then extracted using the BIORON GmbH "Ron's Tissue DNA Mini Kit"), following the manufacturer's standardised protocol.

Partial sequences of mitochondrial genes encoding cytochrome oxidase subunit 1 (COI) and NADH dehydrogenase subunit 1 (ND1) were amplified via polymerase chain reaction (PCR). The amplification employed the primer pairs COL6B and COH6 [30] for COI, and 16L11 and NDH5 [31] for ND1, as described in Vecchioni et al. [32]. Moreover, a fragment of the nuclear 28S ribosomal DNA was amplified through PCR in selected specimens using the primers and the protocols outlined in Keikhosravi & Schubart [33].

Following PCRs, 5 µL of each PCR product was subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide. Electrophoresis was conducted at 90 V for 30 min, and DNA bands were visualised using a UV transilluminator. PCR products displaying a clear, single band of the expected size were subsequently purified using the Exo-SAP-IT® kit (Affymetrix USB, USA) and sent for sequencing at Macrogen Europe (Milan, Italy), employing an ABI 3130xl genetic analyser (Applied Biosystems). The same primer pairs employed in the PCR amplification were also used for direct sequencing of the produced amplicons. Sequence quality was assessed by evaluating their Phred scores [34], and only those sequences displaying continuous stretches of high-quality bases (QV > 20) were retained for further analyses. The resulting chromatograms were analysed and manually proofread using MEGA11 software [35].

Obtained sequences were deposited in GenBank under the accession numbers reported in Table 1.

Table 1. Taxonomy, origin and GenBank accession numbers (A.N.) of the analysed specimens from Maghreb. N: number of collected specimens; *: 28S sequence which was excluded from the analyses for its insufficient length; §: COI and 28S sequences which were excluded from the analysis since they were likely erroneous or mislabelled (see text for details).

Taxon	Subclade	Country	Site	Hydrographical Basin	Latitude	Longitude	Date	N	COI	ND1	28S	Source
Potamon algeriense	1	Algeria	Wadi Dib—Beni Haroun reservoir	Wadi El Kebir	36.56619	6.29691	26/10/2023	4	PX057708-PX057711	PX099053-PX099056	PX057999/PX058000/-/-	Present work
	1	Algeria	Wadi Agrioun	Wadi Agrioun	36.48946	5.27753	(2009-2019)	1	-	MZ507394	-	[17]
	1	Algeria	Mezouara	Wadi Soummam	36.64608	4.61209	(2009-2019)	1	-	MZ507386	-	[17]
	1	Algeria	Wadi Chiffa	Wadi Mazafran	36.47430	2.75030	2013	2	MN622982/MN622985	MN639343/MN639346	-	[16]
	2	Algeria	Ghrib reservoir	Wadi Chelif	36.16140	2.56060	2015	2	MN622983/MN622984	MN639344/MN639345	MN621358/-	[16]
	2	Algeria	Ghrib reservoir	Wadi Chelif	36.16189	2.56028	22/10/2023; 25/11/2023	5	PX057698-PX057702	PX099043-PX099047	PX057996/-/-/-/-	Present work
	2	Algeria	Chelif reservoir	Wadi Chelif	35.98367	0.41444	15/10/2023	5	PX057703-PX057707	PX099048-PX099052	PX057997/PX057998/-/-/-	Present work
	3	Algeria	Beni Bahdel reservoir	Wadi Tafna	34.71183	−1.50411	14/10/2023; 23/11/2023	5	PX057693-PX057697	PX099038-PX099042	PX057994/PX057995/-/-/-	Present work
	4	Morocco	Gorges du Zegzel	Wadi Moulouya	N.A.	N.A.	2005	1	EU908247§	EU908268	HM244838*/HQ223109	[36–38]
	4	Morocco	Wadi Zegzel	Wadi Moulouya	34.84490	−2.34270	31/04/19	1	MN622991	MN639352	-	[16]
	4	Morocco	Wadi Zegzel	Wadi Moulouya	34.84490	−2.35350	31/04/19	1	MN622990	MN639351	MN621360	[16]
	4	Morocco	Wadi Safsaf	Wadi Moulouya	34.90760	−2.63570	30/04/2019	1	MN622989	MN639350	-	[16]
	4	Morocco	Wadi Sebra	Wadi Moulouya	34.88630	−2.66250	30/04/2019	1	MN622988	MN639349	-	[16]
	4	Morocco	Al Hoceima	Wadi Boujbar	35.23288	−3.94347	(2009-2019)	2	-/MZ506928	MZ507390-MZ507391	MZ507137§-MZ507138§	[17]
	4	Morocco	Allal El Fassi reservoir	Wadi Sebou	33.92011	−4.67242	24/10/2021	1	PX057690	PX099035	PX057991	Present work
	4	Morocco	Sidi Bouali	Wadi Sebou	33.77342	−4.70639	25/05/2022	1	PX057691	PX099036	PX057992	Present work
	4	Morocco	Wadi Aggai	Wadi Sebou	33.82767	−4.85142	07/03/2021	1	PX057686	PX099031	PX057987	Present work
	4	Morocco	Ain Smen	Wadi Sebou	33.96569	−5.02181	13/02/2021	1	PX057688	PX099033	PX057989	Present work
	4	Morocco	Wadi Fes	Wadi Sebou	34.04000	−5.06200	23/10/2021	1	PX057689	PX099034	PX057990	Present work

Table 1. *Cont.*

Taxon	Subclade	Country	Site	Hydrographical Basin	Latitude	Longitude	Date	N	COI	ND1	28S	Source
Potamon algeriense	4	Morocco	Bourgaiz	Wadi Sebou	33.92050	−5.08833	04/04/2021	1	PX057687	PX099032	PX057988	Present work
	4	Morocco	Wadi Laou	Wadi Laou	35.42216	−5.11442	(2009-2019)	4	-	MZ507382-MZ507385	MZ507131§-MZ507134§	[17]
	4	Morocco	Ain Ablouz	Wadi Sebou	33.91158	−5.13942	04/11/2023	1	PX057692	PX099037	PX057993	Present work
	4	Morocco	Ouazzane	Wadi Loukos	34.82121	−5.62973	(2009-2019)	1	-	MZ507392	MZ507139§	[17]
	4	Morocco	Wadi Oum Er-Rbia	Wadi Oum Er-Rbia	32.43280	−6.51620	10/05/2019	1	MN622987	MN639348	MN621359	[16]
	4	Morocco	Wadi Tissakht	Wadi Oum Er-Rbia	31.99694	−6.71472	(2009-2019)	3	MZ506936/-/MZ506943	MZ507407/MZ507408/-	MZ507150§/MZ50715§/MZ507164§	[17]
	4	Morocco	Wadi Oum Er-Rbia	Wadi Oum Er-Rbia	32.29610	−7.02590	05/05/2019	1	MN622986	MN639347	-	[16]
	4	Morocco	Bzou	Wadi Oum Er-Rbia	32.12131	−7.03885	(2009-2019)	3	-	MZ507387-MZ507389	MZ507135§/MZ507136§/-	[17]
Potamon sp.	-	Algeria	Wadi Seybouse	Wadi Seybouse	36.82750	7.75830	2013	2	MN622977/MN622972	MN639338/MN639333	-	[16]
	-	Tunisia	Lebna reservoir	Wadi Lebna	36.74050	10.92290	2011	2	MN622981/MN622980	MN639342/MN639341	MN621357/-	[16]
	-	Tunisia	Wadi Joumine	Lake Ichkeul	37.05187	9.67969	(2009-2019)	1	-	MZ507398	-	[17]
	-	Tunisia	Cap Serrat	Ziatine reservoir	37.20610	9.23220	2011	1	MN622974	MN639335	-	[16]
	-	Tunisia	Wadi Béja	Wadi Medjerda	36.74415	9.20691	(2009-2019)	1	-	MZ507395	MZ507141§	[17]
	-	Tunisia	Wadi Maaden	Wadi El Zouara	36.91180	9.10940	2011	1	MN622973	MN639334	MN621356	[16]
	-	Tunisia	Wadi Belif	Wadi El Zouara	37.03170	9.09960	2011	3	MN622975/MN622976/MN622978	MN639336/MN639337/MN639339	-	[16]
	-	Tunisia	Firnanah	Wadi Medjerda	36.65869	8.70733	(2009-2019)	2	-	MZ507393/MZ507397	MZ507140§/-	[17]
	-	Tunisia	Wadi Ghrib	Wadi Medjerda	36.61630	8.68590	2011	1	MN622979	MN639340	-	[16]
	-	Tunisia	Hammam Bourguiba	Wadi Bou Namoussa	36.77354	8.56668	(2009-2019)	1	-	MZ507396	-	[17]

All existing ND1, COI, and 28S *Potamon* sequences from Maghreb available on GenBank were downloaded to be included in the analyses. These were adopted from the papers by Jesse et al. [36–38], Marrone et al. [16], and Ghanavi et al. [17] and are reported in Table 1. Moreover, selected ND1, COI, and 28S sequences of the other *Potamon* species belonging to the *Potamon* subgenus *Euthelphusa*, i.e., *P.* (*E.*) *fluviatile* Herbst, 1785 and *P.* (*E.*) *pelops* Schubart & Klaus, 2010, were downloaded and included in the analyses as comparative material and are listed in Table S1. For the 28S marker, sequences significantly shorter than 500 bp were pruned from the dataset. *Potamon* (*Potamon*) *potamios* (Olivier, 1804) was chosen as an outgroup in the phylogenetic analyses, and its COI, ND1 and 28S sequences were downloaded accordingly.

Based on available sequences, three datasets were built. The first one included ND1 sequences (“ND1 dataset”). This allowed us to include 108 *Euthelphusa* sequences and 1 *Potamon potamios* sequence to be used as the outgroup, i.e., 40 published Maghrebian sequences, 26 novel sequences from Maghrebian individuals investigated in the frame of present work, and 42 sequences of the European species *P. fluviatile* and *P. pelops*.

A second dataset included all the individuals for which both ND1 and COI sequences were available (“mtDNA dataset”). In the mtDNA dataset, we could include all the sequences produced by Marrone et al. [16], and the novel ones produced in the frame of present work; conversely, only two of the individuals studied by Ghanavi et al. [17] could be included in the dataset, since individuals C045 and C068 are the only ones for which both COI and ND1 sequences were available (see [17]). The COI sequence produced by Jesse et al. [38] for a Moroccan *P. algeriense* individual from “Gorges du Zegzel” was discarded since it was highly divergent from those of other *P. algeriense* individuals collected in the same site and likely erroneous (see comments in [16]); accordingly, the Moroccan individual studied by Jesse et al. [36–38] could not be included in the “mtDNA dataset”. The mtDNA dataset thus included sequences from 89 *Euthelphusa* individuals, i.e., 22 published Maghrebian individuals, 26 novel Maghrebian individuals investigated in the frame of present work, and 41 individuals belonging to the European species *P. fluviatile* and *P. pelops*. ND1 and COI *Potamon potamios* sequences were included in the analyses to be used as the outgroup.

Finally, we created a third dataset including the available *Euthelphusa* 28S sequences of sufficient length (“28S dataset”). This originally led to the inclusion of 14 novel sequences from the Maghreb, 20 published sequences from the Maghreb, and 10 published sequences belonging to the European species *P. fluviatile* and *P. pelops*. However, after pruning some sequences of uncertain identity (see below), the final “28S dataset” included 14 novel sequences from Maghreb, 6 published sequences from Maghreb, and 4 sequences belonging to the European species *P. fluviatile* and *P. pelops*. A 28S *Potamon potamios* sequence was included in the analysis to be used as the outgroup.

For each dataset, all analysed sequences were aligned with MEGA11 software through the ClustalW method. Novel COI sequences were translated into amino acids to check for any possible presence of frameshifts or stop codons, eventually highlighting the presence of sequencing errors or pseudogenes.

For all datasets, the software packages MrBayes v. 3.2.7 [39] and PhyML v. 3.0 [40] were used for inferring phylogenetic relationships through Bayesian Inference of phylogeny (BI) and Maximum Likelihood (ML) analysis. As support measures for the nodes, bootstrap values (BS) were calculated with 1000 replicates in the ML trees, whereas in the BI tree, the posterior probability values (PP) were reported. For each marker, the optimal evolutionary model was selected from those analysed by MrBayes, using the Bayesian model choice criteria (nst = mixed, rates = invgamma) and then selected also in the ML analysis. The best-fit models for the analysed datasets proved to be a General Time-Reversible model of sequence evolution with a proportion of invariable sites for the COI dataset (GTR + I), and a Hasegawa, Kishino, and Yano model with a proportion of invariant sites (HKY + I) for both the ND1 and the 28S. The "mtDNA dataset" was partitioned according to the best-fit model of evolution for each marker.

In the BI analyses, two independent Markov chain Monte Carlo analyses were carried out for 1,000,000 generations (temp.: 0.2; default priors) with sampling every 1000 generations; the first 2500 trees were discarded as a burn-in process, and a consensus tree was constructed (Effective Sample Size (ESS) greater than 200 was reached in all the analyses performed). The potential scale reduction factor (PSFR) was comprised between 1.000 and 1.004 for all parameters, thus assessing the convergence of the runs.

Haplotype networks of the "28S dataset" were built using the software PopART v.1.7 by the University of Otago, New Zealand, implementing the median-joining network algorithm as suggested by Bandelt et al. ([41]).

Following Marrone et al. [16], DNA polymorphism, including the haplotype diversity (h) and nucleotide diversity (π), was calculated based on the "mtDNA dataset" for the two *Potamon* species occurring in the Maghreb through the use of DnaSP v.6.12.03 [42]. Novel sequences are reported in bold.

3. Results

In total, 7 new *Potamon* individuals were analysed from seven distinct locations in Morocco, and 19 further *Potamon* individuals were sampled from four sites in Algeria (Table 1). All samples were collected from previously unstudied sites, with the only exception of Ghrib reservoir (Algeria), where some individuals had been previously collected and investigated by Marrone et al. [16]. In comparison with the sampling localities reported by Jesse et al. [38], Marrone et al. [16], and Ghanavi et al. [17], the newly surveyed sites encompass regions that were previously unsampled (Table 1), thereby providing an almost continuous geographic coverage across the Maghreb, from the Béni Mellal-Khénifra region in Morocco to the Cap Bon Peninsula in Tunisia (Figure 1).

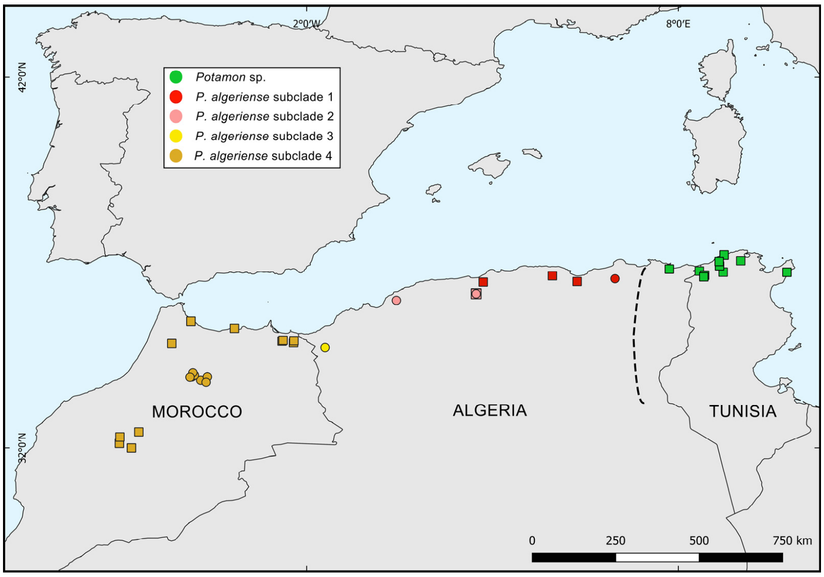

Figure 1. Geographic location of the studied sites. See Table 1 for the coordinates of the sites and for information on the *Potamon* Savigny, 1816 species collected in each site. Squares and circles indicate published and novel sample sites, respectively. Different colours refer to the different species and subclades found in the study area based on molecular data (see text for details). Black dashed line corresponds to the updated suture zone between *Potamon algeriense* and *Potamon* sp.

Overall, 26 COI and ND1 and 14 28S novel *Potamon* sequences were produced in the frame of this work. No stop codons were detected through the translation of the novel and published COI sequences into amino acids showing the existence of a conserved amino acid sequence shared by all the Maghrebian specimens, with the only exception of the likely erroneous COI sequence produced by Jesse et al. [38] (see comment above, and [16]).

Among the *Potamon* sequences downloaded from GenBank, one Maghrebian and some *P. fluviatile* and *P. pelops* 28S sequences (produced by [36,38]) were discarded because their length was significantly shorter than that of the sequences produced in the frame of present work. Sequences available on GenBank but not included in the analyses due to their insufficient length are marked with an asterisk in Tables 1 and S1.

After aligning novel and GenBank sequences, and the trimming of the sequence tails which were not present in all the specimens, we obtained a 522 bp aligned fragment for COI and a 535 bp aligned fragment for ND1. These were concatenated and re-aligned, resulting in a combined mtDNA dataset 1057 bp long, which was used as an input for BI and ML analyses, and for assessing haplotype and nucleotide diversity of the studied taxa. For the 28S gene, an aligned fragment of 551 bp in length was obtained.

Bayesian and ML analyses results for the ND1 and mtDNA datasets (Figures 2 and 3) consistently show the occurrence of four taxa of species level within the subgenus *Euthelphusa*. These are divided into two sister groups, one occurring in Europe and the other occurring in Maghreb, including two species each. The two European species are *Potamon fluviatile* and *P. pelops*, and the two species occurring in Maghreb are *P. algeriense* and *Potamon* sp. Based on the combined mitochondrial DNA dataset, pairwise genetic distances among *Euthelphusa* species range from 6% to 12%, as estimated under the selected evolutionary models (Table 2).

Table 2. Estimates of evolutionary divergence among Maghrebian *Potamon* species. Values reported above the diagonals were calculated using the Kimura 2-parameter model (gamma distributed). Values reported below the diagonals were calculated using the uncorrected p-distance.

	***Potamon* sp.**	***P. algeriense***	***P. pelops***	***P. fluviatile***
***Potamon* sp.**		0.08	0.12	0.10
P. algeriense	0.06		0.13	0.12
P. pelops	0.10	0.09		0.07
P. fluviatile	0.11	0.10	0.07	

Within the taxa belonging to the Maghrebian species group, a sharp difference can be observed. *Potamon* sp. is characterised by a striking molecular homogeneity, which sharply contrasts with the molecular structuring observed within *Potamon algeriense* (Figures 2 and 3, Table 3). Within *Potamon algeriense*, four major subclades can be observed (Figures 2 and 3, Table 1); these are divided following a longitudinal fashion (Figure 1) and show a different degree of internal molecular structuring, ranging from very low (subclade 3) to significantly high (subclade 4).

Table 3. Sample size (n), number of haplotypes (H_n), haplotype (H_d) and nucleotide (π) diversity reported for the Maghrebian *Potamon* species, based on the "mtDNA dataset".

Taxon	n	H_n	H_d	π
Potamon sp. and *P. algeriense*	48	26	0.962	0.03343
Potamon sp.	10	5	0.822	0.00230
Potamon algeriense	38	21	0.950	0.02251

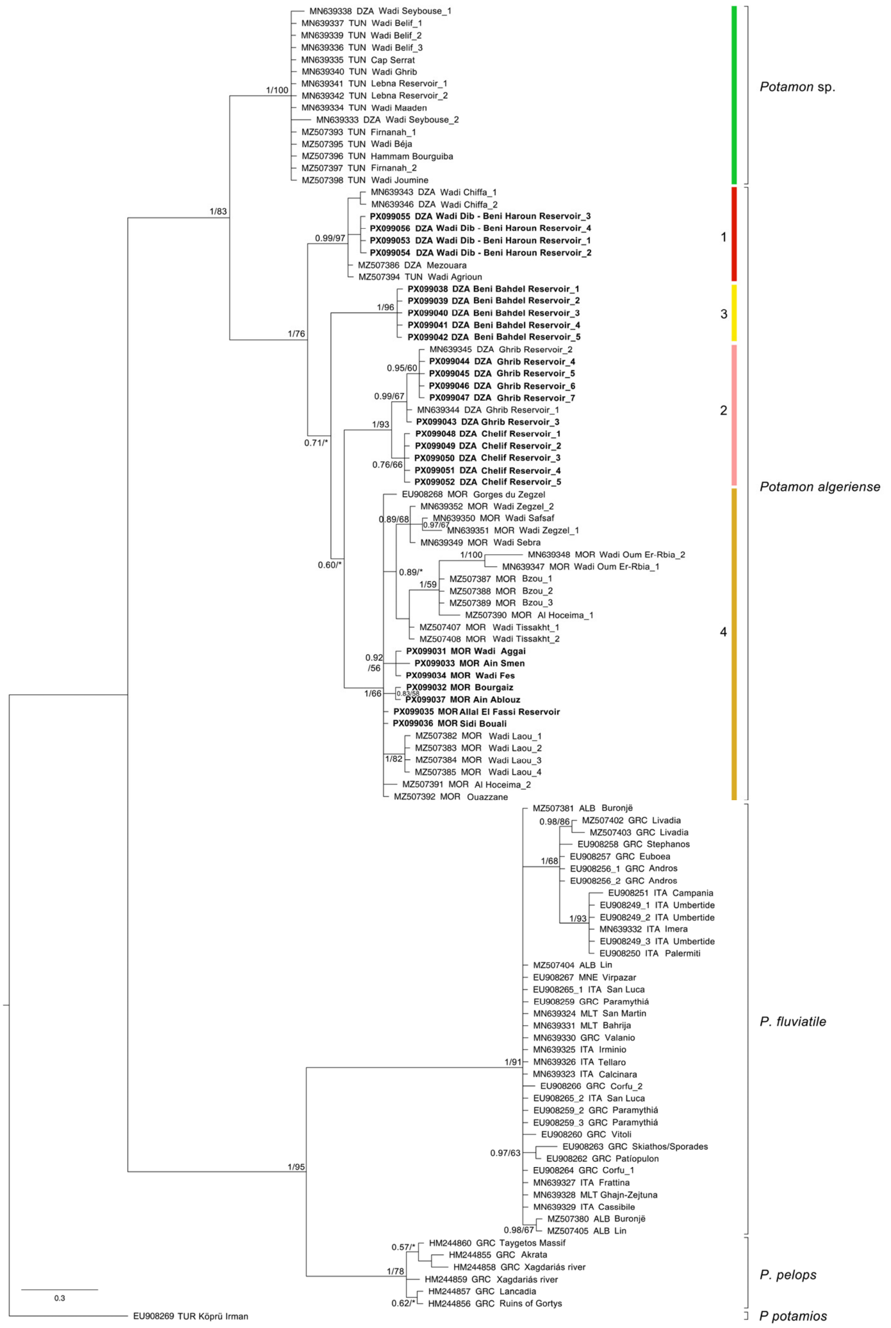

Figure 2. Bayesian phylogram (95% majority rule consensus tree) of *Potamon* spp. based on the "ND1 dataset". *Potamon potamios* (Olivier, 1804) was used as an outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). Asterisks indicate a bootstrap support value lower than 50. Square brackets group the samples according to the current taxonomy of the genus. Rectangles refer to *Potamon* sp. clade and to *Potamon algeriense* subclades (see text for details). The analysed specimens are reported using the GenBank accession numbers listed in Table 1 and the ISO 3166-1 alpha-3 code for the country. Novel sequences are reported in bold.

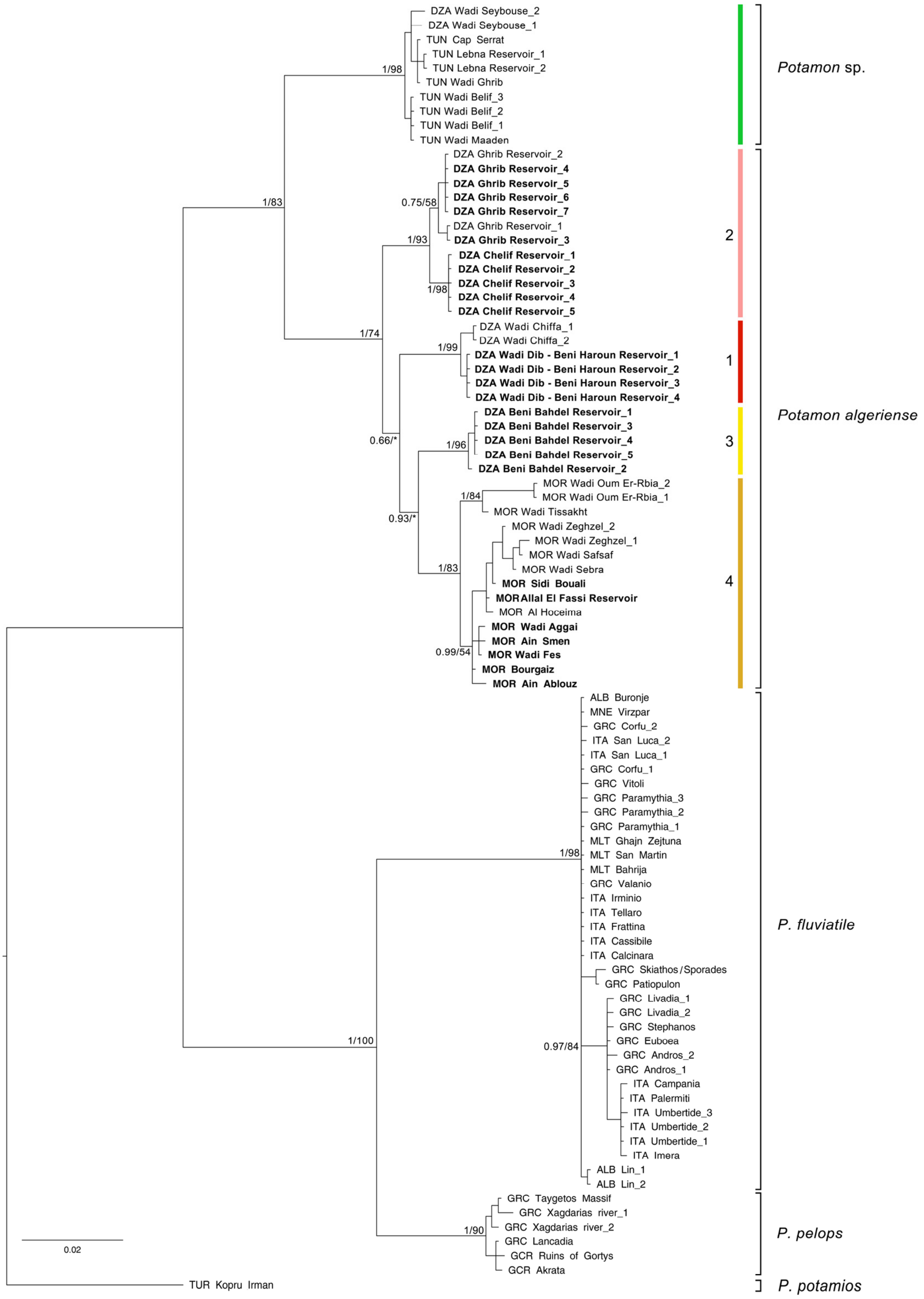

Figure 3. Bayesian phylogram (95% majority rule consensus tree) of *Potamon* spp. based on the concatenated "mtDNA dataset". *Potamon potamios* (Olivier, 1804) was used as an outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). Asterisks indicate a bootstrap support value lower than 50. Square brackets group the samples according to the current taxonomy of the genus. Rectangles refer to *Potamon* sp. clade and to *Potamon algeriense* subclades (see text for details). The analysed specimens are reported using the sites' names listed in Table 1 and the ISO 3166-1 alpha-3 country codes. Novel sequences are reported in bold.

Unexpectedly, some of the 28S sequences labelled as *Potamon algeriense* and *Potamon* aff. *algeriense* by Ghanavi et al. [17] resulted identical to the private *Potamon fluviatile* 28S haplotype, whereas others proved to be identical with the commonest *Potamon algeriense* s.l. haplotype reported by other authors and the present work (Figure S1). We ascribe this unexpected result to the mislabelling of at least some of the 28S sequences uploaded on GenBank by Ghanavi et al. [17], and thus opted to discard all 28S sequences provided by these authors and re-run the analyses with a pruned dataset. The BI/ML analyses and haplotype network built on the pruned database (Figure 4) showed the occurrence of three 28S haplotypes in the whole Maghreb, with the commonest haplotype shared by the two species *Potamon algeriense* s.s. and *Potamon* sp.; two rarer 28S haplotype were observed in two populations of *Potamon algeriense*, i.e., in one individual from Oum Er-Rbia (Morocco) and in one individual from Beni Bahdel reservoir (Algeria).

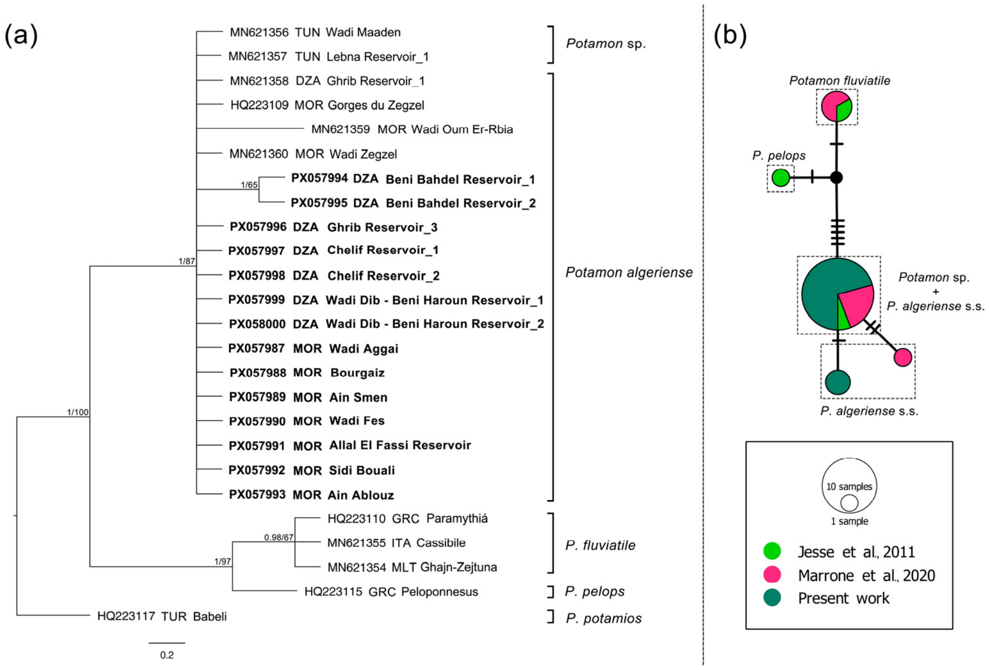

Figure 4. (**a**) Bayesian phylogram (95% majority rule consensus tree) of *Potamon* spp. based on the "28S dataset". *Potamon potamios* (Olivier, 1804) was used as an outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). Rectangles group the samples according to the current taxonomy of the genus. The analysed specimens are reported using the GenBank accession numbers listed in Table 1 and the ISO 3166-1 alpha-3 country codes. Novel sequences are reported in bold. (**b**) Median-joining haplotype network based on a 551 bp long fragment of the nuclear 28S ribosomal DNA. Dashes indicate substitution steps. Each circle represents a haplotype, and its size is proportional to its frequency. Dashed rectangles indicate the current taxonomy of the genus. Colours refer to the origin of the data.

4. Discussion

Based on the analysis of mitochondrial DNA, the existence of two deeply divergent *Potamon* species in Maghreb is fully supported. The existence of two native freshwater crab species in Maghreb is thus confirmed by the extended sampling coverage included in the frame of the present study. Conversely, no additional species or major evolutionary lineages were identified within the study area in the current work.

Our novel Algerian sample from "Wadi Dib—Beni Haroun reservoir" (Mila Province, Algeria) moves 100 km eastward from the validated distribution limit of *Potamon algeriense*, thus bringing the species only 200 km apart from the westernmost validated occurrence locality of *Potamon* sp., i.e., "Wadi Seybouse" (Annaba Province, Algeria) (Figure 1).

No clear geographical pattern of molecular diversity was observed based on 28S sequences; however, this result is likely due to the fact that 28S is an extremely conservative marker, which is poorly informative for investigating phylogenetic relationships at specific or intra-specific level. Conversely, novel and previously published mitochondrial DNA data strongly corroborate the pronounced interspecific divergence previously reported by other authors for Maghrebian freshwater crabs based on geographically distant samples [16,17]. These findings suggest that the observed genetic distances between clades are unlikely to be artefacts of insufficient sampling, thereby excluding the presence of a clinal gradient of genetic diversity within a single taxon. Instead, the data robustly support the existence of two distinct and divergent taxa in the Maghreb.

The occurrence of different, but closely related, sister species in eastern and western Maghreb is in line with a widespread biogeographical pattern observed in the region, according to which a longitudinal diversification of the taxa, with the occurrence of distinct species or lineages at the two extremes of the region, can be often observed [27]. A pattern nearly identical to the one described here for the genus *Potamon* had already been reported for other freshwater taxa (e.g., [43–45]) and, lacking evident geographical or palaeogeographical discontinuities in the study area, it is ascribed to the aridification of the region since the Miocene and the occurrence of Pleistocene dry cycles [46] or, alternatively, Miocene marine transgression [47], which promoted the fragmentation of originally continuous distributions and the survival of relict populations in disjunct western and eastern refugia, where allopatric diversification phenomena took place.

Present data thus show that Maghrebian freshwater crabs conform to the biogeographic patterns previously proposed for North African fauna (e.g., [27]). Originating from a common ancestor, likely derived from the eastern Mediterranean region [17], *Euthelphusa* populations first divided into a Maghrebian and a European major lineage. These were later both subject to further diversification phenomena leading to the existence of the sister species *Potamon fluviatile* and *Potamon pelops* in Europe [36], and *Potamon algeriense* and *Potamon* sp. in the Maghreb [16].

A major phylogeographic break can be observed in the eastern Maghreb and led to the speciation event resulting in *Potamon algeriense sensu stricto* and the as-yet undescribed species, *Potamon* sp., distributed in Tunisia and Numidia. In addition, some further, minor phylogeographic discontinuities within the western part of the range contributed to intra-specific diversification within *P. algeriense* s.s.

In fact, a very high genetic diversity can be observed within *Potamon algeriense* s.s., with an intra-specific nucleotide and haplotype diversity significantly higher than those observed in the European species of the *Potamon* subgenus *Euthelphusa* [16,17], which is here ascribed to the long-term in situ persistence of Maghrebian populations, and the fragmented nature of permanent water bodies network and their biota in the Maghreb (Table 3). Four major subclades, each of which contains a noteworthy haplotype and nucleotide diversity, can be singled out within the species (Figures 2 and 3, Table 3). These major *P. algeriense* subclades can be found in the inferred trees based both on the "ND1 dataset" and "mtDNA dataset", although their mutual relationships are not fully resolved. Subclade 1 includes investigated populations from Mila to Blida provinces (Algeria), subclade 2 includes two sampling sites along the Wadi Chelif (located in Mostaganem and Ain Defla provinces, Algeria), subclade 3 includes several samples collected in Beni Bahdel reservoir (Tlemcen province, Algeria), and subclade 4 includes all the studied Moroccan populations (Figure 1, Table 1). The apparent geographical proximity of the Algerian sites "Wadi Chiffa" (subclade 1) and "Ghrib reservoir" (subclade 2) is misleading when actual hydrological connectivity is taken into account. Although these sites are separated by only 38 km in a straight line, they belong to distinct hydrographic basins, i.e., Wadi Mazafran and Wadi Chelif, respectively.

In contrast, the three sites belonging to subclade 2, despite being separated by distances of up to 200 km, are all situated within the same river basin.

In sharp contrast with *Potamon algeriense*, the *Potamon* species occurring in the easternmost part of Maghreb shows a shallow genetic structure, which is reminiscent of that observed in the European species of the subgenus (Figures 2 and 3, see also [17,36]). The marked difference in genetic diversity observed between *Potamon algeriense* and *Potamon* sp. is likely attributable to the more restricted geographic distribution of this last species and to the greater ecological and landscape homogeneity of the area it inhabits. This contrasts with the broader extent, higher ecological heterogeneity, and topographic complexity characterising the distribution range of *P. algeriense*.

5. Conclusions

Based on the diversity pattern of freshwater crabs in the Maghreb, it is necessary to update the IUCN red list to include both species and to redraw the distribution map of *Potamon algeriense*, which currently also includes the area where *Potamon* sp. occurs [19]. In this context, the morphological characterisation and formal taxonomic description of *Potamon* sp. from eastern Maghreb are also needed. Moreover, the pronounced molecular structuring observed within *Potamon algeriense* supports the recognition of its four major subclades as independent Evolutionarily Significant Units (ESUs). Preserving these units is crucial to safeguard the species' genetic diversity, which is an outcome of its long evolutionary history and a key resource for future adaptation and evolution, particularly in the face of anthropogenic pressures on freshwater ecosystems and global environmental change.

Given the paucity of ecological and demographic data on Maghrebian *Potamon* populations, coordinated efforts involving standardised field surveys, habitat assessments, and long-term monitoring programmes are imperative. Such initiatives will be crucial for developing informed conservation strategies and for safeguarding the unique and vulnerable freshwater biodiversity of the Maghreb.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17080562/s1, Figure S1: (a) Bayesian phylogram (95% majority rule consensus tree) of *Potamon* spp. based on the "28S dataset". *Potamon potamios* (Olivier, 1804) was used as an outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). Rectangles group the samples according to the current taxonomy of the genus. The analysed specimens are reported using the GenBank accession numbers listed in Table 1 and the ISO 3166-1 alpha-3 country codes. Novel sequences are reported in bold. (b) Median-joining haplotype network based on a 551 bp long fragment of the nuclear 28S ribosomal DNA. Dashes indicate substitution steps. Each circle represents a haplotype, and its size is proportional to its frequency. Dashed rectangles indicate the current taxonomy of the genus. Colours refer to the origin of the data. Sequences produced by Ghanavi et al. [17] are divided among those ascribed to *P. fluviatile* (in orange, "Pflu") and those ascribed to *P. algeriense* s.l. (in blue, "Palg"). See text for details; Table S1: Taxonomy, origin and GenBank accession numbers (A.N.) of the analysed *Potamon* specimens from Europe. *: Sequences which were excluded from the analyses for their insufficient length; §: 28S sequences which were excluded from the analysis since likely erroneous or mislabelled (see text for details).

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References

1. Wallace, A.R. *The Geographical Distribution of Animals*; Harper: New York, NY, USA, 1876.
2. Marabuto, E.; Pina-Martins, F.; Rebelo, M.T.; Paulo, O.S. Ancient Divergence, a Crisis of Salt and Another of Ice Shaped the Evolution of the West Mediterranean Butterfly *Euchloe tagis*. *Biol. J. Linn. Soc.* **2020**, *131*, 487–504. [CrossRef]
3. Trájer, A.J.; Sebestyén, V.; Padisák, J. The Impacts of the Messinian Salinity Crisis on the Biogeography of Three Mediterranean Sandfly (Diptera: Psychodidae) Species. *Geobios* **2021**, *65*, 51–66. [CrossRef]
4. Carranza, S.; Arnold, E.N.; Pleguezuelos, J.M. Phylogeny, Biogeography, and Evolution of Two Mediterranean Snakes, *Malpolon monspessulanus* and *Hemorrhois hippocrepis* (Squamata, Colubridae), Using MtDNA Sequences. *Mol. Phylogenet. Evol.* **2006**, *40*, 532–546. [CrossRef] [PubMed]
5. Gaubert, P.; Machordom, A.; Morales, A.; López-Bao, J.V.; Veron, G.; Amin, M.; Barros, T.; Basuony, M.; Djagoun, C.A.M.S.; San, E.D.L.; et al. Comparative Phylogeography of Two African Carnivorans Presumably Introduced into Europe: Disentangling Natural versus Human-Mediated Dispersal across the Strait of Gibraltar. *J. Biogeogr.* **2011**, *38*, 341–358. [CrossRef]
6. Dinis, M.; Merabet, K.; Martínez-Freiría, F.; Steinfartz, S.; Vences, M.; Burgon, J.D.; Elmer, K.R.; Donaire, D.; Hinckley, A.; Fahd, S.; et al. Allopatric Diversification and Evolutionary Melting Pot in a North African Palearctic Relict: The Biogeographic History of *Salamandra algira*. *Mol. Phylogenet. Evol.* **2019**, *130*, 81–91. [CrossRef] [PubMed]
7. Husemann, M.; Schmitt, T.; Zachos, F.E.; Ulrich, W.; Habel, J.C. Palaearctic Biogeography Revisited: Evidence for the Existence of a North African Refugium for Western Palaearctic Biota. *J. Biogeogr.* **2014**, *41*, 81–94. [CrossRef]
8. Jaskuła, R. The Maghreb—One More Important Biodiversity Hot Spot for Tiger Beetle Fauna (Coleoptera, Carabidae, Cicindelinae) in the Mediterranean Region. *Zookeys* **2015**, *482*, 35–53. [CrossRef]
9. Bespalaya, Y.V.; Vinarski, M.V.; Aksenova, O.V.; Babushkin, E.S.; Gofarov, M.Y.; Kondakov, A.V.; Konopleva, E.S.; Kropotin, A.V.; Mabrouki, Y.; Ovchankova, N.B.; et al. Phylogeny, Taxonomy, and Biogeography of the Sphaeriinae (Bivalvia: Sphaeriidae). *Zool. J. Linn. Soc.* **2024**, *201*, 305–338. [CrossRef]
10. Mabrouki, Y.; Taybi, A.F.; El Alami, M.; Berrahou, A. Biotypology of Stream Macroinvertebrates from North African and Semi Arid Catchment: Oued Za (Morocco). *Knowl. Manag. Aquat. Ecosyst.* **2019**, *420*, 17. [CrossRef]
11. Mabrouki, Y.; Taybi, A.F.; Skalli, A.; Sánchez-Vialas, A. Amphibians of the Oriental Region and the Moulouya River Basin of Morocco: Distribution and Conservation Notes. *Basic Appl. Herpetol.* **2019**, *33*, 19–32. [CrossRef]
12. Taybi, A.F.; Mabrouki, Y.; Piscart, C. Distribution of Freshwater Alien Animal Species in Morocco: Current Knowledge and Management Issues. *Diversity* **2023**, *15*, 169. [CrossRef]
13. Taybi, A.F.; Mabrouki, Y.; Berrahou, A.; Legssyer, B. Spatio-Temporal Typology of the Physico-Chemical Parameters of a Large North African River: The Moulouya and Its Main Tributaries (Morocco). *Afr. J. Aquat. Sci.* **2020**, *45*, 431–441. [CrossRef]
14. Taybi, A.F.; Mabrouki, Y.; Dakki, M.; Berrahou, A.; Millán, A. Longitudinal Distribution of Macroinvertebrate in a Very Wet North African Basin: Oued Melloulou (Morocco). *Ann. Limnol.* **2020**, *56*, 17. [CrossRef]
15. Cumberlidge, N. Freshwater Crabs of Africa: Biodiversity, Distribution, and Conservation. In *The Diversity of Life in African Freshwaters: Under Water, Under Threat. An Analysis of the Status and Distribution of Freshwater Species Throughout Mainland Africa*; Darwall, W.R.T., Smith, K.G., Allen, D.J., Holland, R.A., Harrison, I.J., Brooks, E.G.E., Eds.; IUCN: Cambridge, UK; Gland, Switzerland, 2011; pp. 178–199.
16. Marrone, F.; Vecchioni, L.; Deidun, A.; Mabrouki, Y.; Arab, A.; Arculeo, M. DNA Taxonomy of the Potamid Freshwater Crabs from Northern Africa (Decapoda, Potamidae). *Zool. Scr.* **2020**, *49*, 473–487. [CrossRef]
17. Ghanavi, H.R.; Rahimi, P.; Tavana, M.; Rezaei Tavabe, K.; Jouladeh-Roudbar, A.; Doadrio, I. The Evolutionary Journey of Freshwater Crabs of the Genus *Potamon* (Decapoda: Brachyura: Potamidae). *Mol. Phylogenet. Evol.* **2023**, *180*, 107690. [CrossRef]
18. Gauthier, H. *Recherches Sur la Faune des Eaux Continentales de L'algerie et de la Tunisie*; Minerva: Alger, Algeria, 1928.
19. Cumberlidge, N. *Potamon algeriense*. Available online: https://www.iucnredlist.org/species/134365/3945207 (accessed on 8 July 2025).
20. Taybi, A.F.; Mabrouki, Y.; Berrahou, A.; El Abd, A.A. Bio–Ecology of *Potamon algeriense* (Herbst, 1785) (Crustacea, Decapoda) in Eastern Morocco. *Anim. Biodivers Conserv.* **2018**, *41*, 267–274. [CrossRef]
21. Fadlaoui, S.; Melhaoui, M. Population Structure of the Freshwater Crab *Potamon algeriense* (Bott, 1967) Inhabiting Oued Zegzel, (Northeast of Morocco). *Int. J. Ecol.* **2019**, *2019*, 7281725. [CrossRef]

22. Fadlaoui, S.; Melhaoui, M. Diet Composition of the North African Freshwater Crab, *Potamon algeriense* (Bott, 1967) in Oued Zegzel (Northeast of Morocco). *Mar. Freshw. Behav. Physiol.* **2022**, *55*, 73–86. [CrossRef]
23. Fadlaoui, S.; El Asri, O.; Bouterfas, M.; Melhaoui, M. Effects of Physicochemical Variables of Superficial Waters on the Abundance of the North African Freshwater Crab *Potamon algeriense* (Bott, 1967). *J. Toxicol.* **2021**, *2021*, 6669919. [CrossRef] [PubMed]
24. Mabrouki, Y.; Gourari, K.; Taybi, A.F. Atlantic Blue Crab *Callinectes sapidus* Rathbun, 1896 (Portunidae, Malacostraca) Could Lead to Local Extinction of Few Endemic Freshwater Species in the Eastern Morocco. *Povolz. Ekol. Zh* **2025**, *2025*, 91–99. [CrossRef]
25. Fadlaoui, S.; Mahjoub, M.; El Asri, O.; Melhaoui, M. Allometric Growth of the Freshwater Crab *Potamon algeriense* (Bott, 1967) (Decapoda, Brachyura, Potamidae) in Oued Zegzel, a Mountain Stream, in the Northeast of Morocco. *Int. J. Zool.* **2019**, *2019*, 5168639. [CrossRef]
26. QGIS Development Team QGIS Geographic Information System. Open-Source Geospatial Foundation Project. Available online: https://www.qgis.org/ (accessed on 2 July 2025).
27. Beddek, M.; Zenboudji-Beddek, S.; Geniez, P.; Fathalla, R.; Sourouille, P.; Arnal, V.; Dellaoui, B.; Koudache, F.; Telailia, S.; Peyre, O.; et al. Comparative Phylogeography of Amphibians and Reptiles in Algeria Suggests Common Causes for the East-West Phylogeographic Breaks in the Maghreb. *PLoS ONE* **2018**, *13*, e0201218. [CrossRef]
28. Brandis, D.; Storch, V.; Turkay, M. Türkay Michael Taxonomy and Zoogeography of the Freshwater Crabs of Europe, North Africa, and the Middle East (Crustacea, Decapoda, Potamidae). *Senckenb. Biol.* **2000**, *80*, 5–56.
29. Vecchioni, L.; Marrone, F.; Deidun, A.; Adepo-Gourene, B.; Froglia, C.; Sciberras, A.; Bariche, M.; Burak, A.Ç.; Foka-Corsini, M.; Arculeo, M. DNA Taxonomy Confirms the Identity of the Widely-Disjunct Mediterranean and Atlantic Populations of the Tufted Ghost Crab *Ocypode cursor* (Crustacea: Decapoda: Ocypodidae). *Zoolog. Sci.* **2019**, *36*, 322–329. [CrossRef] [PubMed]
30. Schubart, C.D.; Huber, M.G.J. Genetic Comparisons of German Populations of the Stone Crayfish, *Austropotamobius torrentium* (Crustacea: Astacidae). *BFPP—Bull. Fr. Peche Piscic.* **2006**, *380–381*, 1018–1028. [CrossRef]
31. Schubart, C.D. Mitochondrial DNA and Decapod Phylogenies; The Importance of Pseudogenes and Primer Optimization. In *Decapod Crustacean Phylogenetics*; Martin, J.W., Crandall, K.A., Felder, D.L., Eds.; Taylor & Francis/CRC Press: Boca Raton, FL, USA, 2009; pp. 47–65.
32. Vecchioni, L.; Deidun, A.; Sciberras, J.; Sciberras, A.; Marrone, F.; Arculeo, M. The Late Pleistocene Origin of the Italian and Maltese Populations of *Potamon fluviatile* (Malacostraca: Decapoda): Insights from an Expanded Sampling of Molecular Data. *Eur. Zool. J.* **2017**, *84*, 575–582. [CrossRef]
33. Keikhosravi, A.; Schubart, C.D. Description of a New Freshwater Crab Species of the Genus *Potamon* (Decapoda, Brachyura, Potamidae) from Iran, Based on Morphological and Genetic Characters. *Crustac. Monogr.* **2014**, *19*, 115–133. [CrossRef]
34. Richterich, P. Estimation of Errors in "'Raw'" DNA Sequences: A Validation Study. *Genome. Res.* **1998**, *8*, 251–259. [CrossRef]
35. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
36. Jesse, R.; Schubart, C.D.; Klaus, S. Identification of a Cryptic Lineage within *Potamon fluviatile* (Herbst) (Crustacea:Brachyura:Potamidae). *Invertebr. Syst.* **2010**, *24*, 348–356. [CrossRef]
37. Jesse, R.; Grudinski, M.; Klaus, S.; Streit, B.; Pfenninger, M. Evolution of Freshwater Crab Diversity in the Aegean Region (Crustacea: Brachyura: Potamidae). *Mol. Phylogenet. Evol.* **2011**, *59*, 23–33. [CrossRef]
38. Jesse, R.; Pfenninger, M.; Fratini, S.; Scalici, M.; Streit, B.; Schubart, C.D. Disjunct Distribution of the Mediterranean Freshwater Crab *Potamon fluviatile*-Natural Expansion or Human Introduction? *Biol. Invasions* **2009**, *11*, 2209–2221. [CrossRef]
39. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. Mrbayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef] [PubMed]
40. Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* **2010**, *59*, 307–321. [CrossRef] [PubMed]
41. Bandelt, H.-J.; Forster, P.; Röhl, A. Median-Joining Networks for Inferring Intraspecific Phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. [CrossRef]
42. Rozas, J.; Ferrer-Mata, A.; Sanchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sanchez-Gracia, A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. [CrossRef]
43. Veith, M.; Mayer, C.; Samraoui, B.; Barroso, D.D.; Bogaerts, S. From Europe to Africa and Vice Versa: Evidence for Multiple Intercontinental Dispersal in Ribbed Salamanders (Genus *Pleurodeles*). *J. Biogeogr.* **2004**, *31*, 159–171. [CrossRef]
44. Stuckas, H.; Velo-Antón, G.; Fahd, S.; Kalboussi, M.; Rouag, R.; Arculeo, M.; Marrone, F.; Sacco, F.; Vamberger, M.; Fritz, U. Where Are You from, Stranger? The Enigmatic Biogeography of North African Pond Turtles (*Emys orbicularis*). *Org. Divers. Evol.* **2014**, *14*, 295–306. [CrossRef]
45. Dufresnes, C.; Beddek, M.; Skorinov, D.V.; Fumagalli, L.; Perrin, N.; Crochet, P.A.; Litvinchuk, S.N. Diversification and Speciation in Tree Frogs from the Maghreb (*Hyla meridionalis* Sensu Lato), with Description of a New African Endemic. *Mol. Phylogenet. Evol.* **2019**, *134*, 291–299. [CrossRef] [PubMed]

46. deMenocal, P.B. African Climate Change and Faunal Evolution during the Pliocene-Pleistocene. *Earth Planet. Sci. Lett.* **2004**, *220*, 3–24. [CrossRef]
47. Joleaud, L. Étude Géologique de La Région de Bône et de La Calle. *Bull. Serv. Cart. Géolog. L'algér.* **1936**, *II*, 199.