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Stand out from the Crowd: Small-Scale Genetic Structuring in the Endemic Sicilian Pond Turtle

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Abstract: The geographical pattern of genetic diversity was investigated in the endemic Sicilian pond turtle *Emys trinacris* across its entire distribution range, using 16 microsatellite loci. Overall, 245 specimens of *E. trinacris* were studied, showing high polymorphic microsatellite loci, with allele numbers ranging from 7 to 30. STRUCTURE and GENELAND analyses showed a noteworthy, geographically based structuring of the studied populations in five well-characterized clusters, supported by a moderate degree of genetic diversity (F_{ST} values between 0.075 and 0.160). Possible explanations for the genetic fragmentation observed are provided, where both natural and human-mediated habitat fragmentation of the Sicilian wetlands played a major role in this process. Finally, some conservation and management suggestions aimed at preventing the loss of genetic variability of the species are briefly reported, stressing the importance of considering the five detected clusters as independent Management Units.

Keywords: Emydidae; *Emys trinacris*; microsatellites; genetic structuring; Sicily

1. Introduction

The western Palaearctic genus *Emys* Duméril, 1805 contains two species, *Emys orbicularis* (Linnaeus, 1758) and *Emys trinacris* Fritz et al., 2005. *Emys orbicularis* includes a variety of morphologically and genetically defined subspecies [1–8]. Among them, *E. orbicularis hellenica* (corresponding to mitochondrial lineage IV *sensu* Stuckas et al. [6]) and *E. o. galloitalica* (corresponding to mitochondrial lineage V) occur in peninsular Italy and Sardinia [5–7]. *Emys trinacris*, characterized by the mitochondrial lineage III, is endemic to Sicily (Italy) and is the only autochthonous pond turtle occurring there [2,9,10].

The taxonomic status of the Sicilian pond turtle is currently debated. When it was described, species status was inferred by nuclear-genomic fingerprinting [2] and later confirmed using nuclear microsatellite markers [5,7]. However, Speybroeck et al. [11] proposed treating the taxon as a subspecies of *E. orbicularis*, reflecting the weak genetic divergence of *E. trinacris* and *E. orbicularis* compared to other European reptiles. On the other hand, Speybroeck et al. [11] conceded that further distinct species could be currently subsumed under *E. orbicularis*. Among turtles, several taxa with less genetic divergence are currently recognized as full species (e.g., *Graptemys* [12–14]). To avoid unnecessary nomenclatural changes, we here continue to treat *E. trinacris* as a distinct species until new genetic evidence becomes available.

Regardless of the taxonomic status, the Sicilian endemic pond turtle is listed, as well as *Emys orbicularis*, as “EN” (i.e., “Endangered”) in the Italian IUCN Red List of Threatened Species [15].

Moreover, it is included in both Appendix II of the EU Council Directive 92/43/EEC (“Habitats Directive”) and in Appendix II of the “Bern Convention on the Conservation of European Wildlife and Natural Habitats”.

According to several authors, *Emys trinacris* is widely distributed in Sicily, but the distribution is very fragmented due to its limited dispersal capability [7,16,17]. Moreover, populations of *E. trinacris*, as well as of the congeneric *E. orbicularis*, are facing several threats, i.e., habitat loss and fragmentation [2,7,18–21], invasive alien species [22–24], human trading [25], recreational fishing [26], and possibly parasite spill-over [27].

Based on a mitochondrial marker (cytochrome *b* gene) and 15 microsatellite loci, a strong genetic structuring of the Sicilian pond turtle was found throughout the island by Vamberger et al. [7]. In agreement with these results, attention should be paid when individuals are translocated. Such an approach is indicated as pivotal to avoid the loss of genetic variability through a homogenization process. Even though the sampling was adequate in the study by Vamberger et al. [7], some locations were not sampled or represented only by few individuals. Based on 16 nuclear microsatellite loci and a significantly increased sampling, we here reassess the genetic structuring of *E. trinacris* populations in Sicily. We provide a sound population genetic database covering the whole distribution of *E. trinacris* in Sicily for management and conservation actions for this threatened pond turtle.

2. Materials and Methods

2.1. Sampling, DNA Extraction and Selected Loci Amplification

Sicilian pond turtles were collected in permanent water bodies throughout Sicily (Figure S1; Table S1; no precise geographical coordinates are provided since the species is subject to poaching). Turtles were captured using baited hoop traps, immersed in water and equipped with a flotation system, so that turtles and other air-breathing animals that were eventually caught could breathe at the surface [28]. The traps were left fishing for about 48 h per session, and then checked for the possible presence of captured turtles.

Tissue, blood or saliva samples were collected from each turtle. DNA extraction, PCRs and genotyping followed the protocols described in Vamberger et al. [7].

The fragment lengths were determined on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster, CA, USA) using the GeneScan–600 LIZ Size Standard (Applied Biosystems, Foster, CA, USA) and the software PEAK SCANNER 1.0 (Life Technologies, Carlsbad, CA, USA).

All methods and experimental protocols on turtles were conducted in strict accordance with the recommendations of the Italian Ministry of the Environment “Ministero dell’Ambiente e della Tutela del Territorio e del Mare” (U. prot. 000884/PNM 08/05/2014; 0023415/PNM 17/11/2014 and 0006880/PNM 5/04/2016).

2.2. Genetic Cluster Analysis and Diversity Indices

Microsatellite genotypes and sample spatial location data were analysed for all loci and samples using two Bayesian clustering software STRUCTURE 2.3.3 [29,30] and GENELAND 4.0.9 [31]. In STRUCTURE, the admixture model and correlated allele frequencies were used, setting an arbitrary upper bound value of $K = 10$; the most likely number of clusters (K) was determined using the ΔK method [32] with the software STRUCTURE HARVESTER [33] through its online interface (<http://taylor0.biology.ucla.edu/structureHarvester/>). Calculations for each K were repeated 10 times using a MCMC chain of 750,000 generations for each run, including a burn-in of 250,000 generations. Population structuring and individual admixture were visualized using the software DISTRUCT 1.1 [34]. Following Randi [35], individuals with proportions of cluster membership below 80% were treated as having mixed ancestries. Afterwards, data of admixed individuals were removed to avoid introducing noise from alleles of other clusters.

In GENELAND, the geographic information was used to detect spatial delineation of genetic discontinuities. The number of clusters (K) was inferred using 500,000 MCMC iterations with 100 burn-in generations.

In addition, microsatellite data of the inferred clusters were analysed by calculating population genetic diversity indices using CONVERT 1.31 [36] and GENETIX 4.05.2 [37]. Hardy–Weinberg equilibrium (HWE) for all loci and all clusters was tested in GENEPOP ON THE WEB 4.7 [38,39] and the pairwise F_{ST} values were computed through the software ARLEQUIN 3.5.1.2 [40]. Tests for recent bottlenecks were performed with the program BOTTLENECK 1.2.02 [41] using the strict one-step stepwise mutation model (SMM; Ohta and Kimura [42]) as suggested by Piry et al. [41] for microsatellite loci. In order to compare our results, a Discriminant Analysis of Principal Components (DAPCs) was computed using the same dataset, with the package ADEGENET [43,44] for CRAN R 3.6.3.

3. Results

Altogether, 245 samples of the Sicilian pond turtle from 21 different sampling sites were studied (Figure S1; Table S1), including 62 new samples and 183 samples from Vamberger et al. [7], thus covering the whole distribution of the species in Sicily. All studied microsatellite loci were highly polymorphic, with allele numbers ranging from 7 to 30, and a total allele number of 293.

The highest support in both STRUCTURE and GENELAND analyses was found for $K = 5$, suggesting the split of the Sicilian pond turtles into five different clusters: the Nebrodi mountains (cluster A), the Sicilian south-western area (cluster B); south-eastern Sicily (cluster C); the Sicani mountains (province of Palermo; cluster D); north-western Sicily (cluster E) (Figure 1 and Figure S2). DACP analysis (Figure 2) supported the differentiation of the five clusters recognized by STRUCTURE and GENELAND (Figure S2).

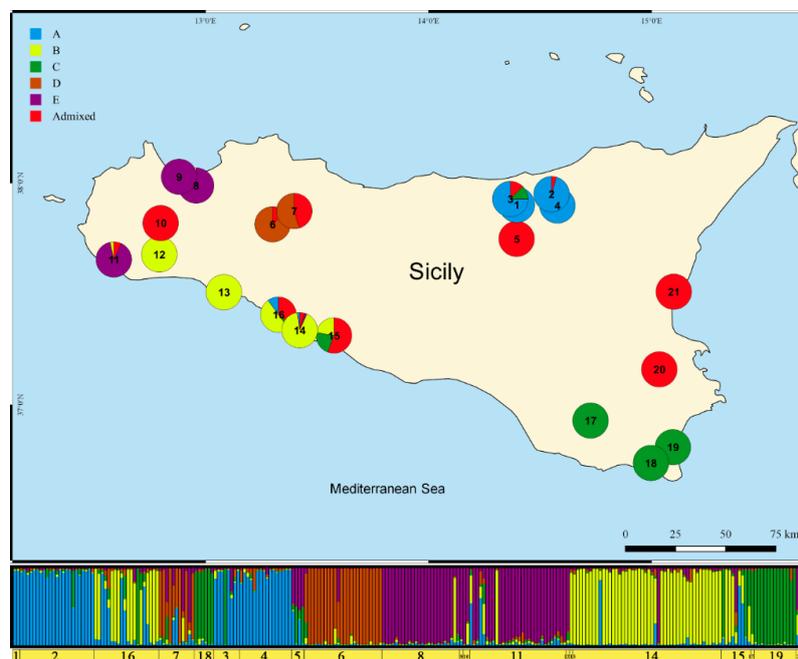


Figure 1. Genotypic structuring of 245 Sicilian pond turtles (*Emys trinacris*) from 21 sampling sites for $K=5$ using 16 microsatellite loci based on the STRUCTURE analysis. Distinct clusters are colour-coded; samples are arranged according to the numbers of the pooled sampling sites (Table S1). Within each cluster, an individual turtle is represented by a vertical segment that reflects its ancestry. Mixed ancestries are indicated by differently coloured sectors corresponding to inferred genetic percentages of the respective cluster. Colours of pooled sampling sites in the map correspond to STRUCTURE clusters; slices represent turtles with mixed ancestries or conflicting cluster assignment (percentages).

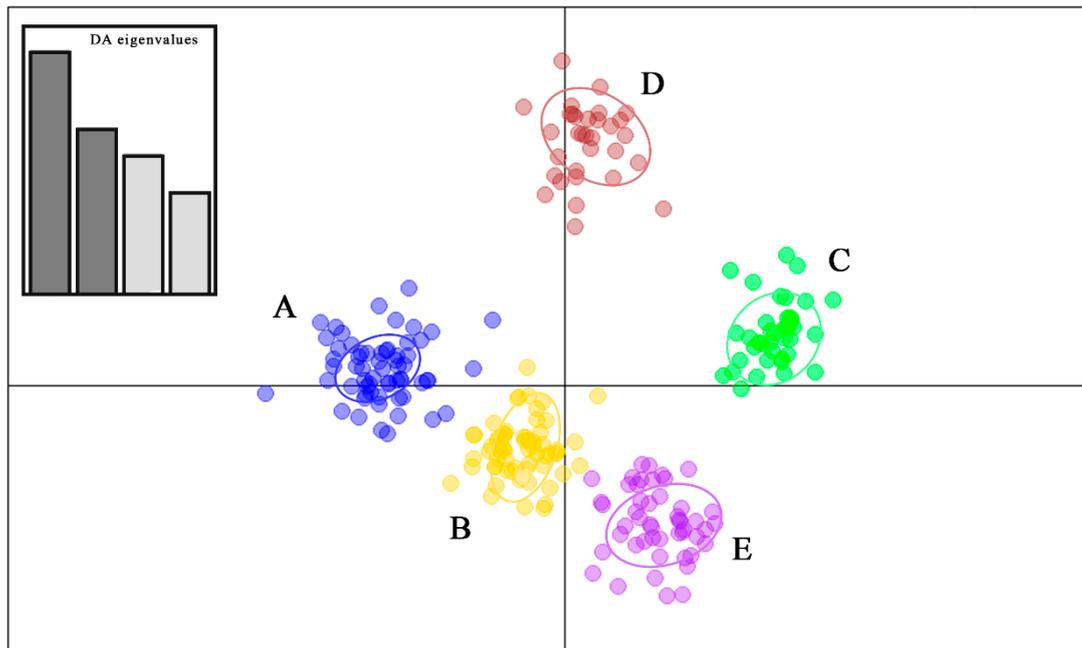


Figure 2. Discriminant Analysis of Principal Components (DAPC) for microsatellite data of the five clusters (A–E) of *Emys trinacris*. The oval outlines correspond to 95% confidence intervals. (DA Eigenvalues: 891, 606.9, 455.1, 326).

Observed heterozygosity (H_o) values (range 0.54–0.66) were lower than the expected ones (H_e , range 0.60–0.76) (Table 1) In addition, all clusters showed evidence of recent bottlenecks (see Table 1).

Table 1. Genetic diversity indices and BOTTLENECK analysis of STRUCTURE clusters of *Emys trinacris*. Colours refer to clusters (see Figure 1). The category admixed individuals comprises turtles with admixed genotypes irrespective of their collection sites.

Cluster	n	n_A	$n_{\bar{A}}$	n_p	H_e	H_o	SMM
K = 5							
A (blue) *	48	140	8.75	13	0.69	0.58	0.02
B (yellow) *	57	136	8.50	10	0.70	0.66	0.02
C (green) *	24	149	9.31	35	0.76	0.60	0.02
D (brown) *	27	84	5.25	6	0.60	0.54	0.03
E (purple) *	53	145	9.66	15	0.68	0.62	0.00
Admixed individuals (red) *	36	186	11.62	11	0.76	0.63	-

n = sample size; n_A = total number of alleles per cluster; $n_{\bar{A}}$ = mean number of alleles per locus; n_p = number of private alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; SMM = stepwise mutation model; * cluster with a significant deviation from Hardy–Weinberg Equilibrium.

Moreover, all identified clusters of *E. trinacris* showed a significant deviation from HWE at all loci and within all clusters defined by STRUCTURE and GENELAND (Table 2). Pairwise F_{ST} values between the five a priori defined cluster, based on the STRUCTURE results, ranged from 0.075 to 0.160 (Table 3); all F_{ST} values were significant with a p-value of 0.05. F_{ST} values indicated moderate genetic differentiation among sampled Sicilian pond turtles, where the highest value was observed between the comparison of the clusters “D” and “C”.

Table 2. Hardy–Weinberg Exact test (Fisher’s method) based on the STRUCTURE clusters of *Emys trinacris*.

Cluster	Hardy-Weinberg Test		
	df	Chi2	Prob
A	32	>205.50	$<3.18 \times 10^{-27}$
B	32	>145.08	$<2.43 \times 10^{-16}$
C	32	>248.29	$<2.70 \times 10^{-35}$
D	32	>131.15	$<5.82 \times 10^{-14}$
E	30	>190.04	$<3.54 \times 10^{-25}$
Admixed individuals	32	>226.94	$<3.07 \times 10^{-31}$

Table 3. Pairwise F_{ST} based on the STRUCTURE clusters of *Emys trinacris*.

Cluster	1	2	3	4	5
1. A	-				
2. B	0.0752	-			
3. C	0.0997	0.1039	-		
4. D	0.1340	0.1320	0.1603	-	
5. E	0.1060	0.0969	0.1357	0.1290	-

4. Discussion

The results obtained in this study suggest the presence of a clear structuring of *Emys trinacris* populations, with a moderate degree of genetic differentiation among the five identified clusters (Table 3). The clusters are supported by moderate genetic diversity indices among them (see Table 1); a similar moderate genetic structuring can be also observed in *E. orbicularis* [45]. Surprisingly however, the genetic structuring only partially agrees with that revealed by Vamberger et al. [7]. While Vamberger et al. [7] found eight well differentiated clusters within *E. trinacris*, our increased sampling covering some new sampling localities concluded that only five clusters exist. Nevertheless, three of them were in full agreement with the ones from Vamberger et al. [7], i.e., clusters A, C, D. Both studies highlighted a moderate genetic structuring of *E. trinacris* in Sicily, with a slightly different grouping of the studied samples, possibly due to an increased sampling scheme and the implementation of a further microsatellite marker, suggesting a long-lasting persistence of turtles in the respective areas of Sicily. In contrast, the subspecies of *E. orbicularis* do not show such a pronounced substructuring (e.g., [7,8]). Stöck et al. [46] found low levels of genetic structuring in the studied Sicilian herpetofauna using two mitochondrial markers, the cytochrome *b* and 16S rDNA genes, and a nuclear marker, the tropomyosin intron. However, it is well known that the current genetic structuring of many other freshwater taxa in Sicily can be ascribed to paleogeographic and paleoclimatic events [47–49]. The pronounced genetic structure in the Sicilian pond turtle could thus be due to the role of Sicily as a “refuge area” during the Plio-Pleistocene climatic fluctuations [50]. The local physiography can maintain genetic diversity (see Vamberger et al. [7]), corresponding to a “refugia within refugia” pattern [51].

In addition, increased habitat fragmentation and the consequent reduction in population size and gene flow levels [52] could also contribute to the current structuring, which shows evidence of recent bottleneck experienced by all the local clusters of the species (Table 1). The paucity of water bodies and habitats suitable for the presence of the species in Sicily prevents the populations from establishing sound metapopulation dynamics, thus precluding significant inter-population gene flow. In some areas, *Emys trinacris* is locally abundant but these regions are separated from other inhabited areas by large gaps where the species is completely absent (e.g., in the Madonie and Peloritani mountain chains).

In recent centuries, the wetlands in Sicily have been significantly modified due to remediation measures to reclaim agricultural land and to fight against malaria (“Provveditorato alle opere pubbliche per la Sicilia” [53]; see also Naselli-Flores and Marrone [23]). During those years, wetlands across Sicily were altered completely by local authorities by re-shaping the surrounding landscape. Furthermore,

according to ISPRA (“Istituto Superiore per la Protezione e la Ricerca Ambientale” [54]), in the last century Sicily has undergone a severe degradation of the wetlands, where 34.5% of all the Sicilian wetlands are in a state of complete degradation and only 5% remain untouched (cf. Table 10 in [54]).

In addition, the presence of invasive species (e.g., *Trachemys scripta* [24,55]) may also contribute to the pronounced genetic fragmentation of *E. trinacris*. To date, *Trachemys scripta* and other non-native emydid taxa are known to occur all over Sicily [23,24,56], and coexist with *E. trinacris* in some of the sampled localities (e.g., Agrigento: Monteallegro, Diga Gorgo; Agrigento: Imera river; Palermo: Bosco Ficuzza; Catania: Simeto river; *pers. obs.*). The invasive pond slider is known to have a negative impact on the local turtle populations, i.e., competing for food, basking and nesting sites [18,57–64], and being vectors for parasites and other pathogens (helminth cross-transmission: [65–67]; *Salmonella*: [68,69]; oxyurid and strongylid nematodes: [70]).

In conclusion, to preserve the genetic variability of *E. trinacris*, protection actions by local management entities are required. According to our improved genetic database, which can now serve in conservation actions, we propose that the five population clusters highlighted by present study should be considered as independent Management Units for Sicily. As already proposed by Vamberger et al. [7], it is necessary to avoid translocating turtles, even when events or activities regarding the restocking or release of treated or seized individuals are carried out. It is thus of outstanding importance to genetically characterize all individuals before any translocation actions can be approved.

Supplementary Materials: The following materials are available online at <http://www.mdpi.com/1424-2818/12/9/343/s1>, Figure S1: Pooled sampling sites of *Emys trinacris*. Consecutive numbers of pooled sites refer to Table S1, Figure S2: Genotypic structuring of 245 Sicilian pond turtles (*Emys trinacris*) from 21 sampling sites for $K = 5$ using 16 microsatellite loci. Samples are arranged according to the numbers of the pooled sampling sites (see Table S1). Colours of pooled sampling sites in the map correspond to GENELAND clusters; slices represent turtles with mixed ancestries or conflicting cluster assignment (percentages). Table S1: Origin of the studied *Emys trinacris* samples. Sample sites values refers to number showed in Figure S1.

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References

1. Lenk, P.; Fritz, U.; Joger, U.; Wink, M. Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Mol. Ecol.* **1999**, *8*, 1911–1922. [[CrossRef](#)] [[PubMed](#)]
2. Fritz, U.; Fattizzo, T.; Guicking, D.; Tripepi, S.; Pennisi, M.G.; Lenk, P.; Joger, U.; Wink, M. A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys* (Reptilia, Testudines, Emydidae). *Zool. Scr.* **2005**, *34*, 351–371. [[CrossRef](#)]
3. Fritz, U.; Guicking, D.; Kami, H.; Arakelyan, M.; Auer, M.; Ayaz, D.; Ayres Fernández, C.; Bakiev, A.; Celani, A.; Havaš, P.; et al. Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*)—An update. *Amphibia-Reptilia* **2007**, *28*, 418–426. [[CrossRef](#)]
4. Fritz, U.; Ayaz, D.; Hundsdörfer, A.K.; Kotenko, T.; Guicking, D.; Wink, M.; Tok, C.V.; Çiçek, K.; Buschbom, J. Mitochondrial diversity of European pond turtles (*Emys orbicularis*) in Anatolia and the Ponto-Caspian Region: Multiple old refuges, hotspot of extant diversification and critically endangered endemics. *Org. Divers. Evol.* **2009**, *9*, 100–114. [[CrossRef](#)]

5. Pedall, I.; Fritz, U.; Stuckas, H.; Valdeón, A.; Wink, M. Gene flow across secondary contact zones of the *Emys orbicularis* complex in the Western Mediterranean and evidence for extinction and re-introduction of pond turtles on Corsica and Sardinia (Testudines: Emydidae). *J. Zool. Syst. Evol. Res.* **2011**, *49*, 44–57. [[CrossRef](#)]
6. 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](#)]
7. Vamberger, M.; Stuckas, H.; Sacco, F.; D'Angelo, S.; Arculeo, M.; Cheylan, M.; Corti, C.; Lo Valvo, M.; Marrone, F.; Wink, M.; et al. Differences in gene flow in a twofold secondary contact zone of pond turtles in southern Italy (Testudines: Emydidae: *Emys orbicularis galloitalica*, *E. o. hellenica*, *E. trinacris*). *Zool. Scr.* **2015**, *44*, 233–249. [[CrossRef](#)]
8. Pöschel, J.; Heltai, B.; Graciá, E.; Franch Quintana, M.; Velo-Antón, G.; Arribas, O.; Valdeón, A.; Wink, M.; Fritz, U.; Vamberger, M. Complex hybridization patterns in European pond turtles (*Emys orbicularis*) in the Pyrenean Region. *Sci. Rep.* **2018**, *8*, 15925. [[CrossRef](#)]
9. Marrone, F.; Sacco, F.; Arizza, V.; Arculeo, M. Amendment of the type locality of the endemic Sicilian pond turtle *Emys trinacris* Fritz et al. 2005, with some notes on the highest altitude reached by the species (Testudines, Emydidae). *Acta Herpetol.* **2016**, *11*, 59–61. [[CrossRef](#)]
10. Scardino, R.; Mazzoleni, S.; Rovatsos, M.; Vecchioni, L.; Dumas, F. Molecular cytogenetics characterization of the Sicilian endemic pond turtle *Emys trinacris* (Testudines, Emydidae). *Genes* **2020**, *11*, 702. [[CrossRef](#)]
11. Speybroeck, J.; Beukema, W.; Dufresnes, C.; Fritz, U.; Jablonski, D.; Lymberakis, P.; Martínez-Solano, I.; Razzetti, E.; Vamberger, M.; Vences, M.; et al. Species list of the European herpetofauna—2020 Update by the Taxonomic Committee of the Societas Europaea Herpetologica. *Amphibia-Reptilia* **2020**, *41*, 139–189. [[CrossRef](#)]
12. Praschag, P.; Ihlow, F.; Flecks, M.; Vamberger, M.; Fritz, U. Diversity of North American map and sawback turtles (Testudines: Emydidae: *Graptemys*). *Zool. Scr.* **2017**, *46*, 675–682. [[CrossRef](#)]
13. TTWG [Turtle Taxonomy Working Group]. *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*, 8th ed.; Chelonian Research Monographs: Lunenburg, MA, USA, 2017; Volume 7, 292p.
14. Thomson, R.C.; Spinks, P.Q.; Shaffer, H.B. Molecular phylogeny and divergence of the map turtles (Emydidae: *Graptemys*). *Mol. Phylogenet. Evol.* **2018**, *121*, 61–70. [[CrossRef](#)]
15. Rondinini, C.; Battistoni, A.; Peronace, V.; Teofili, C. *Lista Rossa IUCN dei Vertebrati Italiani*; Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare: Roma, Italy, 2013.
16. Marrone, F.; Sacco, F.; Kehlmaier, C.; Arizza, V.; Arculeo, M. Some like it cold: The glossiphoniid parasites of the Sicilian endemic pond turtle *Emys trinacris* (Testudines, Emydidae), an example of “parasite inertia”? *J. Zool. Syst. Evol. Res.* **2016**, *54*, 60–66. [[CrossRef](#)]
17. Iannella, M.; Cerasoli, F.; D'Alessandro, P.; Console, G.; Biondi, M. Coupling GIS spatial analysis and Ensemble Niche Modelling to investigate climate change-related threats to the Sicilian pond turtle *Emys trinacris*, an endangered species from the Mediterranean. *PeerJ* **2018**, *6*, e4969. [[CrossRef](#)]
18. Vamberger, M. *European pond turtle (Emys orbicularis) in Slovenia*; Rogner, M., Ed.; Edition Chimaira; European Pond Turtle (*Emys orbicularis*): Frankfurt am Main, Germany, 2009; pp. 191–193.
19. Vamberger, M.; Kos, I. First observations on some aspects on the natural history of European pond turtles *Emys orbicularis* in Slovenia. *Biologia* **2011**, *66*, 170–174. [[CrossRef](#)]
20. Vamberger, M.; Pobjlšaj, K.; Govedič, M.; Debeljak Šabec, N.; Žagar, A. Conservation activities for European pond turtles (*Emys orbicularis*) in Slovenia. *Herpetol. Notes* **2013**, *6*, 123–126.
21. Vamberger, M.; Lipovšek, G.; Šalamun, A.; Cipot, M.; Fritz, U.; Govedič, M. Distribution and population size of the European pond turtle *Emys orbicularis* in Ljubljansko barje, Slovenia. *Vertebr. Zool.* **2017**, *67*, 223–229.
22. Standfuss, B.; Lipovšek, G.; Fritz, U.; Vamberger, M. Threat or fiction: Is the pond slider (*Trachemys scripta*) really invasive in Central Europe? A case study from Slovenia. *Conserv. Genet.* **2016**, *17*, 557–563. [[CrossRef](#)]
23. Naselli-Flores, L.; Marrone, F. Different invasibility of permanent and temporary waterbodies in a semiarid Mediterranean Island. *Inland Waters* **2019**, *9*, 411–421. [[CrossRef](#)]
24. Liuzzo, M.; Termine, R.; Marrone, F. First evidence of an egg-laying attempt of feral *Trachemys scripta* (Schöepff, 1792) in Sicily (Lake Pergusa, Italy). *Herpetol. Notes* **2020**, *13*, 365–368.

25. Mărginean, G.I.; Gherman, E.; Sos, T. The illegal internet-based trade in European pond turtle *Emys orbicularis* (Linnaeus, 1758) in Romania: A threat factor for conservation. *North-Western J. Zool.* **2018**, *14*, 64–70.
26. Vecchioni, L.; Cicerone, A.; Scardino, R.; Arizza, V.; Arculeo, M.; Marrone, F. Sicilians are not easily hooked! First assessment of the impact of recreational fishing on the endemic Sicilian pond turtle *Emys trinacris* (Testudines, Emydidae). *Herpetol. Notes* **2020**, accepted.
27. Arizza, V.; Sacco, F.; Russo, D.; Scardino, R.; Arculeo, M.; Vamberger, M.; Marrone, F. The good, the bad and the ugly: *Emys trinacris*, *Placobdella costata* and *Haemogregarina stepanowi* in Sicily (Testudines, Annelida and Apicomplexa). *Folia Parasit.* **2016**, *63*, 029. [[CrossRef](#)]
28. Ream, C.; Ream, R. The influence of sampling methods on the estimation of population structure in painted turtles. *Am. Midl. Nat.* **1966**, 325–338. [[CrossRef](#)]
29. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959.
30. Hubisz, M.J.; Falush, D.; Stephens, M.; Pritchard, J.K. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Res.* **2009**, *9*, 1322–1332. [[CrossRef](#)]
31. Guillot, G.; Mortier, F.; Estoup, A. GENELAND: A computer package for landscape genetics. *Mol. Ecol. Notes* **2005**, *5*, 712–715. [[CrossRef](#)]
32. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [[CrossRef](#)]
33. Earl, D.A.; von Holdt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Res.* **2012**, *4*, 359–361. [[CrossRef](#)]
34. Rosenberg, N.A. DISTRUCT: A program for the graphical display of population structure. *Mol. Ecol. Notes* **2004**, *4*, 137–138. [[CrossRef](#)]
35. Randi, E. Detecting hybridization between wild species and their domesticated relatives. *Mol. Ecol.* **2008**, *17*, 285–293. [[CrossRef](#)] [[PubMed](#)]
36. Glaubitz, J.C. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol. Ecol. Notes* **2004**, *4*, 309–310. [[CrossRef](#)]
37. Belkhir, K.; Borsa, P.; Chikhi, L.; Raufaste, N.; Bonhomme, F. *GENETIX 4.05, Logiciel Sous Windows TM Pour la Génétique des Populations*; Laboratoire Génome, Populations, Interactions, CNRS UMR 5000; Université de Montpellier II: Montpellier, France, 2004.
38. Raymond, M.; Rousset, F. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* **1995**, *86*, 248–249. [[CrossRef](#)]
39. Rousset, F. GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **2008**, *8*, 103–106. [[CrossRef](#)]
40. Excoffier, L.; Lischer, H.E.L. ARLEQUIN suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res.* **2010**, *10*, 564–567. [[CrossRef](#)]
41. Piry, S.G.; Luikart, G.; Cornuet, J.M. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* **1999**, *90*, 502–503. [[CrossRef](#)]
42. Ohta, T.; Kimura, M. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genet. Res. Camb.* **1973**, *22*, 201–204. [[CrossRef](#)]
43. Jombart, T. ADEGENET: A R package for the multivariate analysis of genetic markers. *Bioinformatics* **2008**, *24*, 1403–1405. [[CrossRef](#)]
44. Jombart, T.; Devillard, S.; Balloux, F. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genet.* **2010**, *11*, 94. [[CrossRef](#)]
45. Velo-Antón, G.; García-París, M.; Cordero Rivera, A. Patterns of nuclear and mitochondrial DNA variation in Iberian populations of *Emys orbicularis* (Emydidae): Conservation implications. *Conserv. Genet.* **2008**, *9*, 1263–1274. [[CrossRef](#)]
46. Stöck, M.; Grifoni, G.; Armor, N.; Scheidt, U.; Sicilia, A.; Novarini, N. On the origin of the recent herpetofauna of Sicily: Comparative phylogeography using homologous mitochondrial and nuclear genes. *Zool. Anz.* **2016**, *261*, 70–81. [[CrossRef](#)]
47. Ferrito, V.; Pappalardo, A.M.; Canapa, A.; Barucca, M.; Doadrio, I.; Olmo, E.; Tigano, C. Mitochondrial phylogeography of the killifish *Aphanius fasciatus* (Teleostei, Cyprinodontidae) reveals highly divergent Mediterranean populations. *Mar. Biol.* **2013**, *160*, 3193–3208. [[CrossRef](#)]

48. 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](#)]
49. Vella, A.; Vella, N. First Population genetic structure analysis of the freshwater crab *Potamon fluviatile* (Brachyura: Potamidae) reveals fragmentation at small geographical scale. *Genet. Aquat. Org.* **2020**, *4*, 49–59. [[CrossRef](#)]
50. Hewitt, G.M. The genetic legacy of the Quaternary ice ages. *Nature* **2000**, *405*, 907–913. [[CrossRef](#)]
51. Gómez, A.; Lunt, D.H. Refugia within Refugia: Patterns of Phylogeographic concordance in the Iberian peninsula. In *Phylogeography of Southern European Refugia*; Weiss, S., Ferrand, N., Eds.; Springer: Dordrecht, The Netherlands, 2007. [[CrossRef](#)]
52. Harrison, S.; Hastings, A. Genetic and evolutionary consequences of metapopulation structure. *Trends Ecol. Evol.* **1996**, *11*, 180–183. [[CrossRef](#)]
53. Consoli, N. *Gl'interventi di Piccola Bonifica Nella Lotta Contro la Malaria in Sicilia*; Provveditorato alle opere pubbliche per la Sicilia. Estratto dalla “*Rivista Sanitaria Siciliana*”; Sanzo, F., Ed.; C. Industria Tipografica Editrice: Moncalieri, Turin, Italy, 1928; p. 18.
54. D’Antoni, S.; Battisti, C.; Cenni, M.; Rossi, G.L. (a cura di) Contributi per la tutela della biodiversità delle zone umide. *Rapp. ISPRA* **2011**, *153*, 461.
55. Sperone, E.; Crescente, A.; Brunelli, E.; Paolillo, G.; Tripepi, S. Sightings and successful reproduction of allochthonous reptiles in Calabria. *Acta Herpetol.* **2010**, *5*, 265–273. [[CrossRef](#)]
56. Marrone, F.; Naselli-Flores, L. A review on the animal xenodiversity in Sicilian inland waters (Italy). *AIOL* **2015**, *6*, 2–12. [[CrossRef](#)]
57. Cadi, A.; Joly, P. Competition for basking places between the endangered European pond turtle (*Emys orbicularis galloitalica*) and the introduced red-eared slider (*Trachemys scripta elegans*). *Can. J. Zool.* **2003**, *81*, 1392–1398. [[CrossRef](#)]
58. Cadi, A.; Delmas, V.; Prévot-Julliard, A.C.; Joly, P.; Pieau, C.; Girondot, M. Successful reproduction of the introduced slider turtle (*Trachemys scripta elegans*) in the south of France. *Aquat. Conserv.* **2004**, *14*, 237–246. [[CrossRef](#)]
59. Pérez-Santigosa, N.; Díaz-Paniagua, C.; Hidalgo-Vila, J. The reproductive ecology of exotic *Trachemys scripta elegans* in an invaded area of southern Europe. *Aquat. Conserv.* **2008**, *18*, 1302–1310. [[CrossRef](#)]
60. Pérez-Santigosa, N.; Florencio, M.; Hidalgo-Vila, J.; Díaz-Paniagua, C. Does the exotic invader turtle, *Trachemys scripta elegans*, compete for food with coexisting native turtles? *Amphibia-Reptilia* **2011**, *32*, 167–175. [[CrossRef](#)]
61. Ficetola, G.F.; Thuiller, W.; Schioppa, E.P. From introduction to the establishment of alien species: Bioclimatic differences between presence and reproduction localities in the slider turtle. *Divers. Distrib.* **2009**, *15*, 108–116. [[CrossRef](#)]
62. Vamberger, M.; Lipovšek, G.; Gregorič, M. First reproduction record of *Trachemys scripta* (Schoepff, 1792), in Slovenia. *Herpetozoa* **2012**, *25*, 76–79.
63. Crescente, A.; Sperone, E.; Paolillo, G.; Bernabo, I.; Brunelli, E.; Tripepi, S. Nesting ecology of the exotic *Trachemys scripta elegans* in an area of Southern Italy (Angitola Lake, Calabria). *Amphibia-Reptilia* **2014**, *35*, 366–370. [[CrossRef](#)]
64. Pearson, S.H.; Arvey, H.W.; Spotila, J.R. Juvenile invasive red eared slider turtles negatively impact the growth of native turtles: Implications for global freshwater turtle populations. *Biol. Conserv.* **2015**, *186*, 115–121. [[CrossRef](#)]
65. Moravec, F.; Vargas-Vázquez, J. Some endohelminths from the freshwater turtle *Trachemys scripta* from Yucatán, México. *J. Nat. Hist.* **1998**, *32*, 455–468. [[CrossRef](#)]
66. Hidalgo-Vila, J.; Ribas, A.; Florencio, M. *Falcaustra donanaensis* sp. nov. (Nematoda: Kathliniidae) a parasite of *Mauremys leprosa* (Testudines, Bataguridae) in Spain. *Parasitol. Res.* **2006**, *99*, 410–413. [[CrossRef](#)]
67. Meyer, L.; Du Preez, L.; Bonneau, E.; Héritier, L.; Quintana, M.F.; Valdeón, A.; Sadaoui, A.; Kechemir-Issad, N.; Palacios, C.; Verneau, O. Parasite host-switching from the invasive American red-eared slider, *Trachemys scripta elegans*, to the native Mediterranean pond turtle, *Mauremys leprosa*, in natural environments. *Aquat. Invasions* **2015**, *10*, 79–91. [[CrossRef](#)]

68. Bringsøe, H. NOBANIS—Invasive Alien Species Fact Sheet, *Trachemys scripta*. Online Database of the North European and Baltic Network on Invasive Alien Species. 2004. Available online: <http://www.nobanis.org> (accessed on 7 July 2020).
69. Shen, L.; Shi, H.; Wang, R.; Liu, D.; Pang, X. An invasive species red-eared slider (*Trachemys scripta elegans*) carrying *Salmonella* pathogens in Hainan island. *Mol. Pathog.* **2011**, *2*, 4. [[CrossRef](#)]
70. Rataj, A.V.; Lindtner-Knific, R.; Vlahović, K.; Mavri, U.; Dovč, A. Parasites in pet reptiles. *Acta Vet. Scand.* **2011**, *53*, 33. [[CrossRef](#)] [[PubMed](#)]



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