

Phylogeographic link between Sicilian and Corso-Sardinian *Testudo h. hermanni* confirmed

GABRIELE GIACALONE^{1,2}, MARIO LO VALVO¹, UWE FRITZ³

¹ *Laboratorio di Zoologia applicata, Dipartimento di Biologia Animale, Università degli Studi di Palermo, Via Archirafi 18, I-90123 Palermo, Italy.*

² *Present address: Dipartimento di Botanica, Università degli Studi di Catania, Via Antonino Longo 19, I-95125 Catania, Italy.*

³ *Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, A.B. Meyer Building, Königs-brücker Landstr. 159, D-01109 Dresden, Germany. Corresponding author. E-mail: uwe.fritz@senckenberg.de.*

Submitted on: 2008, 12th October; revised on 2009, 20th August; accepted on 2009, 31st August.

Abstract. Our study confirms that Sicilian and Corso-Sardinian *Testudo h. hermanni* share a certain mtDNA haplotype, while tortoises from peninsular Italy harbour slightly different haplotypes. When the fossil record is considered, this striking pattern agrees well with the idea of local extinction in Corsica and Sardinia and later replacement by tortoises originating elsewhere, either by natural oversea dispersal or translocation by man.

Keywords. *Testudo hermanni*, phylogeography, Corsica, Sardinia, Sicily.

Traditionally, the fauna of the islands Corsica, Sardinia and Sicily is considered to constitute the Tyrrhenian faunistic province (La Greca, 1995). However, while Corsica and Sardinia are known to share many endemic or closely related taxa, Sicily represents a distinct entity (Lanza and Vanni, 1987; Lanza, 1988; Cheylan, 1992). Most of Sicily's zoogeographic affinities point at the Italian peninsula (Cheylan, 1992), although some endemic taxa are known (Stöck et al., 2008), one of which (*Bufo siculus*) suggests a link to North Africa. Also other taxa support this relationship between Sicily and North Africa (La Greca, 1990, 1995; Cheylan, 1992).

Fritz et al. (2006) demonstrated that two Sicilian *Testudo hermanni hermanni* harboured a mitochondrial haplotype also occurring on Corsica and Sardinia, an unexpected finding when it is considered that tortoises from the Italian peninsula possess distinct haplotypes. In order to find out whether the two individuals studied by Fritz et al. (2006) might represent introductions from Corsica or Sardinia, we obtained blood samples of *T. h. hermanni* from five other Sicilian populations (Fig. 1) and sequenced most of the mitochondrial cytochrome *b* gene (cyt *b*), the marker used by Fritz et al. (2006). In addition, we sequenced part of the faster evolving mitochondrial control region (CR; Table 1) from two Sicilian, three Corsican, and two Sardinian tortoises.

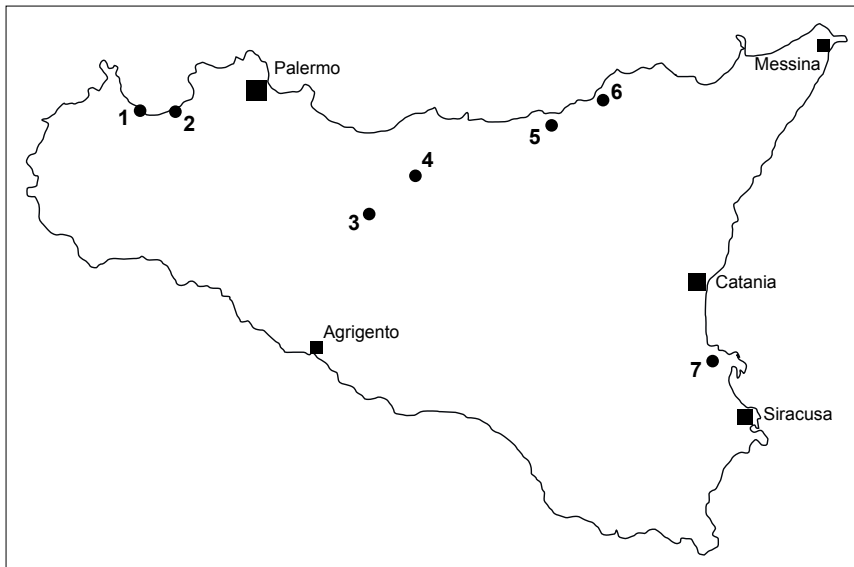


Fig. 1. Collection sites of Sicilian *Testudo hermanni hermanni* samples used in this study and in Fritz et al. (2006). Site numbers refer to Table 1.

Table 1. Geographic origin of *Testudo hermanni* samples used in this study, Sicilian samples from Fritz et al. (2006) and the studied mtDNA fragments. Sample numbers refer to the Tissue Collection of the Museum of Zoology Dresden (MTD T); site numbers, Fig. 1.

Sample	Site	Cyt <i>b</i>	CR	Reference
2140	1 – Castellammare del Golfo (Palermo), Sicily	+	-	Fritz et al. (2006)
2141	2 – Balestrate (Palermo), Sicily	+	-	Fritz et al. (2006)
4078	3 – M. M. Belsito (Madonie), Sicily	+	-	This study
4075	4 – Collesano-Campofelice (Madonie), Sicily	+	-	This study
4077	4 – Collesano-Campofelice (Madonie), Sicily	+	-	This study
4082	4 – Collesano-Campofelice (Madonie), Sicily	+	-	This study
4074	5 – Caronia (Nebrodi), Sicily	+	-	This study
4079	6 – S. Agata di Militello (Nebrodi), Sicily	+	+	This study
4083	7 – Augusta (Siracusa), Sicily	+	-	This study
4084	7 – Augusta (Siracusa), Sicily	+	+	This study
4085	7 – Augusta (Siracusa), Sicily	+	-	This study
4086	7 – Augusta (Siracusa), Sicily	+	-	This study
1921	Porto Vecchio, Corsica	-	+	This study
1927	Casabianda, Corsica	-	+	This study
1936	Fontane du Salario (Ajaccio), Corsica	-	+	This study
1114	Porto Palmas (Sassari), Sardinia	+	+	Cyt <i>b</i> : Fritz et al. (2006), CR: this study
1115	Bay of Porto Ferro (Sassari), Sardinia	+	+	Cyt <i>b</i> : Fritz et al. (2006), CR: this study

Laboratory procedures followed Fritz et al. (2006). For amplification and sequencing of an approximately 1000 bp long fragment of *cyt b* the primers CytbG (Spinks et al., 2004), mt-f-na, mt-c-For2, and mt-E-Rev (Fritz et al., 2006) were used; for an approximately 850 bp long CR fragment, the primers Thr-L15569 and Phe-H26 (Palkovacs et al., 2003). An ABI 3130 Genetic Analyzer was used for automatic sequencing in both directions. Obtained sequences were checked by eye and aligned. *Cyt b* sequences were compared with published data (Fritz et al., 2006). Using tcs 1.21 (Clement et al., 2000), a parsimony network was constructed from a 1008 bp long alignment including all 31 previously published Western Mediterranean *T. h. hermanni* sequences (Fritz et al., 2006) plus our 10 new sequences from Sicily (Fig. 2). The alignment of the CR sequences comprised 855 bp.

All of our 10 Sicilian *T. h. hermanni* harboured the same *cyt b* haplotype (H5) as the two Sicilian tortoises studied by Fritz et al. (2006). With respect to the CR, all Corsican and Sardinian tortoises shared the same haplotype; this haplotype was also found in one Sicilian tortoise. The second Sicilian individual (MTD T 4079) possessed a similar haplotype differing in only one mutational step (position 815 of our alignment: T instead of A). The two CR haplotypes are deposited under accession numbers FN298446-FN298447 in GenBank.

Compared to the eastern subspecies *T. h. boettgeri*, the phylogeographic structure of Western Mediterranean *T. h. hermanni* is strikingly shallow (Fritz et al., 2006). This is unexpected because the fossil record provides evidence for its long presence and wide distribution in the Western Mediterranean (Delfino, 2002; Morales Pérez and Sanchis Serra, 2009). Amongst others, fossils are known from the Middle Pleistocene of Corsica (Hervet, 2000,

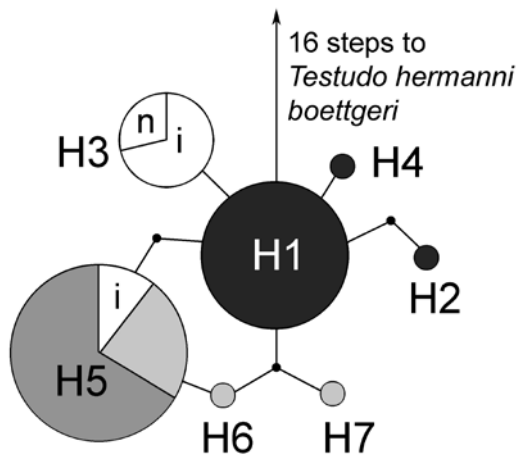


Fig. 2. Parsimony network of mtDNA haplotypes (*cyt b* fragment, 1008 bp) of Western Mediterranean *Testudo hermanni hermanni*, using the dataset of Fritz et al. (2006) and 10 new Sicilian sequences. Haplotype nomenclature follows Fritz et al. (2006). Symbol size corresponds to haplotype frequency; missing haplotypes, dots. Each line between symbols represents one mutation step. The arrow indicates the connection to Eastern Mediterranean *T. h. boettgeri* haplotypes. Black symbols, peninsular Italy and southern France (Var); dark grey, Sicily; light grey, Corsica and Sardinia; white, Spain (i: introduced populations or populations known to comprise also allochthonous individuals, n: native population). H1: n = 12, H2: n = 1, H3: 7, H4: n = 1, H5: n = 18, H6: n = 1, H7: n = 1. When only Western Mediterranean haplotypes are considered, H1 is under coalescent theory ancestral to H2-H7 (outgroup probability of H1: 0.3175).

2001; Hervet and Salotti, 2000) and from the Plio-Pleistocene boundary of Sardinia (Abbazzi et al., 2004). On the Italian peninsula, the oldest findings date back to the Pliocene; the oldest fossils from Sicily are from the Middle Pleistocene (Delfino, 2002), suggesting that Sicily was colonized later than Corsica and Sardinia. Consequently, some extent of genetic differentiation of the island tortoises and a closer relationship of the Sicilian and the peninsular Italian populations should be expected, but not between Sicily and Corso-Sardinia.

The weak genetic differentiation of Western Mediterranean tortoises might be related to a major climatically caused extinction event some 38000-39500 years ago, wiping out many populations (Morales Pérez and Sanchis Serra, 2009). However, while our study confirms that Sicilian and Corso-Sardinian *T. h. hermanni* are not clearly differentiated in the studied genetic markers, they are slightly distinct from peninsular Italian tortoises (and from native Spanish tortoises; Fig. 2). This pattern supports the survival of several local populations in the Western Mediterranean, in agreement with fossil evidence (Delfino, 2002; Morales Pérez and Sanchis Serra, 2009) for instance on the Italian peninsula and Sicily. Furthermore, the genetic identity of Corsican, Sardinian, and Sicilian tortoises agrees also well with the idea of local extinction in Corsica and Sardinia and later replacement by tortoises originating elsewhere, either by natural oversea dispersal or translocation by man.

ACKNOWLEDGEMENTS

Thanks for introducing Gabriele Giacalone to lab work go to Anna Hundsdörfer and Anke Müller. Massimo Delfino helped with information about the fossil record. The samples of Corsican and Sardinian tortoises were provided by Marc Cheylan and Wolfgang Wegehaupt. Dr. F.P. Faraone assisted during field work.

REFERENCES

- Abbazzi, L., Angelone, C., Arca, M., Barisone, G., Bedetti, C., Delfino, M., Kotsakis, T., Marcolini, F., Palombo, M.R., Pavia, M., Piras, P., Rook, L., Torre, D., Tuveri, C., Valli, A.M.F., Wilkens, B. (2004): Plio-Pleistocene fossil vertebrates of Monte Tuttavista (Orosei, eastern Sardinia, Italy), an overview. *Riv. Ital. Paleontol. Stratigraf.* **110**: 681-706.
- Cheylan, M. (1992): Synthèse biogéographique. In: *Batraciens et reptiles de Corse*, p. 105-119. Delaugerre, M., Cheylan, M., Eds, Ministère de l'Environnement et Région Corse, Paris.
- Clement, M., Posada, D., Crandall, K.A. (2000): TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657-1660.
- Delfino, M. (2002): *Erpetofauna italiane del Neogene e del Quaternario. Tesi per il conseguimento del titolo di Dottore di Ricerca in Paleontologia.* Università degli Studi di Modena e Reggio Emilia.
- Fritz, U., Auer, M., Bertolero, A., Cheylan, M., Fattizzo, T., Hundsdörfer, A.K., Martín Sampayo, M., Pretus, J.L., Široký, P., Wink, M. (2006): A rangewide phylogeography

- of Hermann's tortoise, *Testudo hermanni* (Reptilia: Testudines: Testudinidae): implications for taxonomy. *Zool. Scr.* **35**: 531-543.
- Hervet, S. (2000): Tortues du Quaternaire de France: critères de détermination, répartition chronologique et géographique. *Mésogée* **58**: 3-47.
- Hervet, S. (2001): Étude du peuplement chélonien de la Corse à partir de la répartition des tortues fossiles de la Méditerranée occidentale. *Bull. Soc. Sci. histor. natur. Corse* **696/697**: 147-163.
- Hervet, S., Salotti, M. (2000): Les tortues pléistocènes de Castiglione (Oletta, Haute-Corse) et la preuve de leur indigénat en Corse. *C.R. Acad. Sci., Sér. II, Sci. Terre Planètes* **330**: 645-651.
- La Greca, M. (1990): The insect biogeography of West Mediterranean islands. *Atti Conv. Lincei* **85**: 469-491.
- La Greca, M. (1995): Il concetto di fauna e le caratteristiche della fauna italiana. *Atti Conv. Lincei* **118**: 13-28.
- Lanza, B. (1988): Hypothèses sur les origines de la faune herpétologique corse. *Bull. Ecol.* **19**: 163-170.
- Lanza, B., Vanni, S. (1987): Hypotheses on the origins of the Mediterranean island batrachofauna. *Bull. Soc. Zool. France* **112**: 179-196.
- Morales Pérez, J.V., Sanchis Serra, A. (2009): The Quaternary fossil record of the genus *Testudo* in the Iberian Peninsula. Archaeological implications and diachronic distribution in the western Mediterranean. *J. Archaeol. Sci.* **36**: 1152-1162.
- Palkovacs, E.P., Marschner, M., Ciofi, C., Gerlach, J., Caccone, A. (2003): Are the native giant tortoises from the Seychelles really extinct? A genetic perspective based on mtDNA and microsatellite data. *Mol. Ecol.* **12**: 1403-1413.
- Spinks, P.Q., Shaffer, H.B., Iverson, J.B., McCord, W.P. (2004): Phylogenetic hypotheses for the turtle family Geoemydidae. *Mol. Phylogenet. Evol.* **32**: 164-182.
- Stöck, M., Sicilia, A., Belfiore, N.M., Buckley, D., Lo Brutto, S., Lo Valvo, M., Arculeo, M. (2008): Post-Messinian evolutionary relationships across the Sicilian channel: Mitochondrial and nuclear markers link a new green toad from Sicily to African relatives. *BMC Evol. Biol.* **8**: 56.