SALAMANDRA **51**(3) | 221–230 | 30 October 2015 | ISSN 0036–3375

Demographic structure and genetic diversity of *Mauremys leprosa* in its northern range reveal new populations and a mixed origin

Carmen Palacios[1,2,6], Cristina Urrutia[1,2], Nikolai Knapp[1,2], Marc Franch Quintana[3], Albert Bertolero[4], Gael Simon[1,2], Louis du Preez[5] & Olivier Verneau[1,2,5]

[1] Univ. Perpignan Via Domitia, Centre de Formation et de Recherche sur les Environnements Méditérranéens (CEFREM), UMR 5110, F-66860, Perpignan, France

[2] CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens (CEFREM), UMR 5110, F-66860, Perpignan, France

[3] Univ. Barcelona, Dept. Biol. Animal, Av. Diagonal 645; 08028 Barcelona, Spain

[4] Ecosistemes Aquàtics IRTA, Ctra. Poble Nou km 5.5; 435840 Sant Carles de la Ràpita, Spain

[5] Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

Corresponding author: Carmen Palacios, e-mail: carmen.palacios@univ-perp.fr

Manuscript received: 20 February 2014
Accepted: 2 February 2015 by Philipp Wagner

Abstract. Freshwater turtle species are still poorly understood, and many species are in decline due to unsustainable trade as well as human alteration and degradation of freshwater ecosystems. *Mauremys leprosa* is a freshwater chelonian endemic to the Mediterranean Basin. Whereas the fossil record demonstrates that this species used to be distributed to well beyond the Spanish border in France, it is today restricted to the border region with Spain, at the Baillaury River in the Pyrenees, with some isolated observations from slightly farther into France. The species consequently holds an "Endangered" status according to the French IUCN Red list. Here we report for the first time the presence and demographic structure in its northern range and demonstrate that its distribution expands beyond the Pyrenees Mountains, throughout French Catalonia. Sequence analyses of the mitochondrial DNA (mtDNA) cytochrome b (cyt b) gene from 216 specimens mainly from France and Spanish Catalonia resulted in a patchwork pattern of haplotypes that supports a mixed origin of the species in France. We encountered two extreme haplotypes, with specimens with the endemic Spanish Catalonian haplotype A18 belonging to *M. leprosa leprosa* and others being clearly referable to *M. leprosa saharica* (cyt b haplotypes from clade B) that is otherwise typical from below the Atlas Mountain Range in Morocco. Short- and long-term directions for research as well as conservation management actions are suggested for this insufficiently studied species.

Key words. *Mauremys leprosa*, freshwater turtles, mtDNA cytochrome b, demography, species conservation, turtle trade.

Introduction

Freshwater turtle species are still poorly understood and in decline due to human alteration and degradation of freshwater ecosystems (Moll & Moll 2004). Since the 1980s, freshwater chelonians have suffered from "the terrible turtle trade" (see, e.g., van Dijk et al. 2000), i.e., they are poached and sold as pets and sometimes released into the environment when they are no longer wanted, with the potential of becoming a threat to native species (Cadi & Joly 2004, Polo-Cavia 2008, 2009, 2010a, 2010b, 2012). The Mediterranean pond turtle, *Mauremys leprosa* (Schweigger, 1812), is a freshwater species that mainly inhabits streams and ponds with riparian vegetation (da Silva 2002). The species is present in large parts of North Africa and on the Iberian Peninsula (Iverson 1992, Segurado et al. 2005) and to a minimal extent in France. It is classified as "Vulnerable" in both the European Red List of Reptiles (Cox & Temple 2009) and Spanish Red List (da Silva 2002) with its decline being due to the loss of suitable freshwater ecosystems and deteriorating water quality in the Mediterranean. Until recently, *M. leprosa* lacked a conservation status in France, but it is now listed as "Endangered" (UICN France & MNHN 2008) due to the fragmentation and scarcity of its populations (criterion B1a) and its continuous decline in its range as a result of habitat degradation and poor demographical structure (criterion B1b (i, ii, iii, iv, v)). Based on the fossil record, *M. leprosa* is believed to have originated in North Africa at least during the Pliocene (more than 2.5 Mya) and subsequently dispersed to the Iberian Peninsula in the early Pleistocene or late Pliocene (de Lapparent de Broin 2001). Its genetic diversity has confirmed this demographic expansion (Fritz et al. 2006). During the course of the species' expansion, only the Atlas Mountains in Morocco may have posed a barrier to its dispersion, impeding genetic exchange, as is shown by its genetic differentiation on both sides of the Atlas (Fritz et al. 2005, 2006). In France, the oldest fossil remains date back to the Holocene, about 4,000 years ago (Cheylan 1982). Remains of the species dating back to the 1st century BC, the 2nd century AD (Cheylan & Poitevin 2003) and the 11th century (Maufras & Mercier 2002) have also been found. These archaeological sites are all located in the Languedoc-Roussillon region, well beyond the Pyrénées-Orientales (PO) province to the northeast of the Pyrenees (Figs 1a and 1b). Given these data and the currently supposed scarcity of the species in France, Cheylan & Vacher (2010) considered *M. leprosa* the most endangered reptile in France.

Several *M. leprosa* subspecies have been proposed (Schleich 1996, Doucotterd & Bour 2002) based on morphological characteristics, but according to genetic differences of the mitochondrial DNA cytochrome b gene (cyt b), only two subspecies are currently recognised (Fig. 2): *Mauremys leprosa leprosa* (Schweigger, 1812) (cyt b haploclade A) on the Iberian Peninsula and from north of Atlas Mountains in Morocco, and *M. l. saharica* Schleich, 1996 (haploclade B) in Algeria, Tunisia and mainly from south of the Atlas Mountains in Morocco (Fritz et al. 2006, Fritz & Havas 2007). The northernmost geographic limit of the species is the Baillaury River (France) in the Pyrenees Mountains on the border with Spain. This population is widely recognised as indigenous to France based on records dating from the previous century until today (Knoepffler 1979b, Geniez & Cheylan 2005). Some individuals have also been observed or even captured in France at several localities beyond the Pyrenees Mountains, along the Mediterranean coast (Courmont & Rodriguez 2004, Geniez & Cheylan 2005, Cheylan & Vacher 2010, Cheylan & Verneau 2012). Nevertheless, no studies regarding the genetics of any French specimens, and/or the demographic structure of any French populations, have been published to date. In this paper, we report on the distribution and demographic structure of the species in France, identify the cyt b haplotype of specimens from France and Spanish Catalonia and compare them to other available *M. leprosa* sequences (Fritz et al. 2006).The aim is to gain insights into the origin and genetic diversity of *M. leprosa* populations in its northern range in order to guide future research and conservation management.

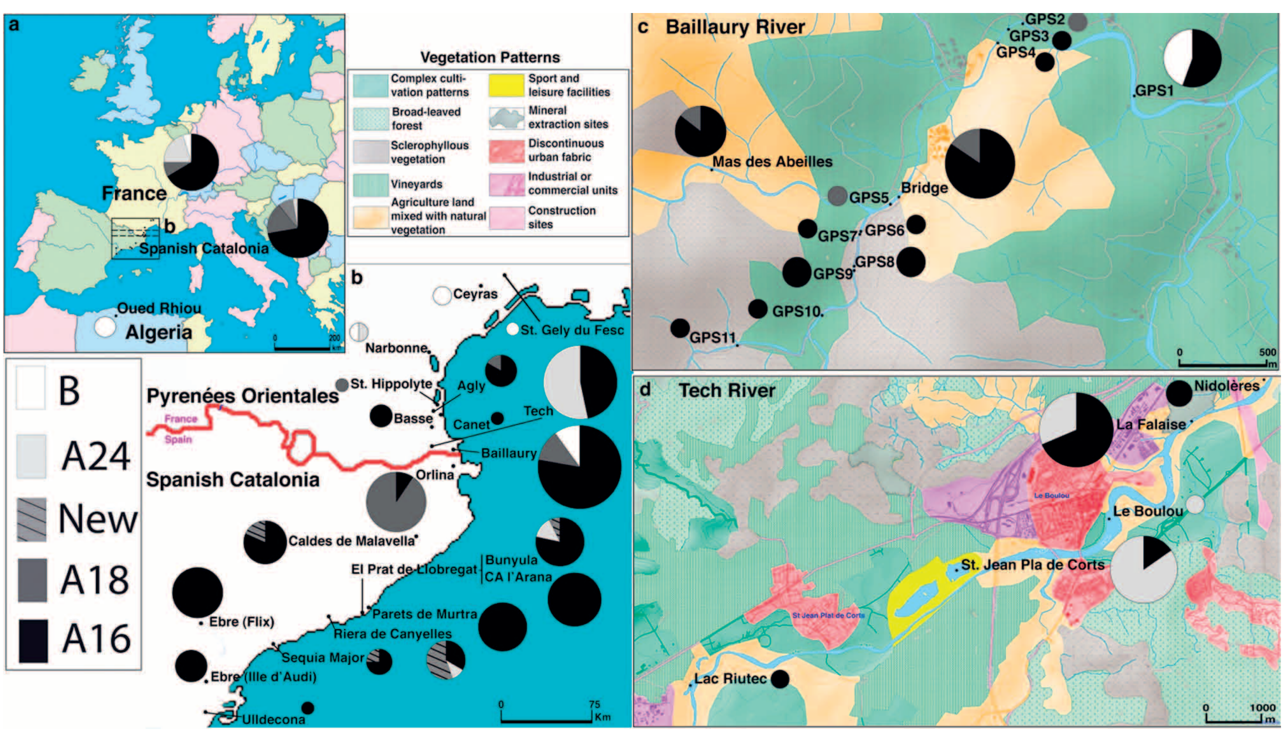

Figure 1. Geographic locations and mtDNA cyt b haplotype abundances of the different populations of *M. leprosa* in the northern parts of its range. a) General overview of sample sites; b) sample sites from this study; c + d) sample sites of the two largest French metapopulations. Haplotype legend denotes "B" for B clade haplotypes and "New" for haplotypes that this study found to be new (see Table 1 for details). Raw data including GPS coordinates and individual specimen numbers are summarised in Table S1. Pie charts are sized proportionally to the number of mtDNA cyt b haplotypes found at each site except for Algeria, in a) magnified for improved visualisation. Maps were obtained from OpenStreetMap and vegetation layers from the Corine Land Cover dataset (European Environmental Agency).

Materials and methods

Study area

In France, fieldwork was conducted in wetlands beyond the Pyrenees Mountains along the Mediterranean coast (Figs 1a and 1b), where *M. leprosa* was suspected to have expanded its range from Spain (Cheylan & Verneau 2012). The Baillaury River, from where the only French population is known, is a Mediterranean river with a typically irregular course that runs along the Albères Mountains in the Eastern Pyrenees, 11 km distant from the closest known Spanish population at the Orlina River (Fig. 1b) (Knoepffler 1979a). Vegetation is composed here mainly of reeds, and a wild riparian forest develops between vineyards (Fig. 1c). Specimens from a turtle farm located in Sorède (PO province) and from Algeria (Figs 1a and b, Table 1) were also sequenced for comparison.

In Spanish Catalonia (Fig. 1a), where the species is widely distributed at altitudes below 200 m (Llorente et al. 1995), nine populations were sampled, covering a range of habitats typically occupied by this species. The Orlina is a river where an expansive riparian forest dominates the landscape (Fig. 1b). This locality has an abundant and well-preserved population of *M. leprosa* free of reintroductions. The Caldes de Malavella population inhabits a well conserved area that is probably free of reintroductions (Franch Quintana 2003). In the Delta de Llobregat plain, Bunyola, Ca l'Arana and Parets de Murtra are localities composed of wetlands, ponds and canals. The Fauna Recovery Centres released specimens of different origins to reinforce these populations during the 1990s (Franch Quintana et al. 2007). The Riera de Canyelles is a typical Mediterranean seasonal stream that forms bodies of water only during autumn and spring. Local farmers have recently released some specimens of *M. leprosa* from the south of Spain into the area. Reeds dominate the Sèquia Major canal locality. According to local people, *M. leprosa* was not known in this area until recently. At the Ebro River, three localities were sampled (Fig. 1b). The population inhabiting the Flix Dam (Reserva Natural de Fauna Salvatge de Sebes) and the small ponds and canals of Ulldecona are most likely unaffected by releases. In contrast, six individuals of unknown origin were sampled for blood before they were introduced to the Illa Audí (Reserva Natural de Fauna Salvatge de les Illes de l'Ebre) (Fig. 1b).

Fieldwork and demography structure

Field captures were performed in France and Spanish Catalonia between 2006 and 2010. Trapping of turtles was performed by using baited crayfish traps that were left overnight. Captured individuals were marked by cutting notches in the peripheral scutes of the carapace (Plummer, 1989) and sexed according to secondary sexual characters (Pérez et al. 1979). Accurate locality data of individuals captured in the field were obtained using a Global Position System (GPS) navigator eTrex Vista HCx (Garmin). Fifty to 200 µl of blood were obtained by means of the occipital sinus vein puncture technique (Martínez-Silvestre et al. 2002) or otherwise by the coccygeal vein puncture technique using an insulin-type syringe. Blood was stored either pure at -80°C or fixed in 95% ethanol and then stored at -18°C until it was oven-dried at 40°C for 24–36 h. Straight carapace length was measured to facilitate the classification of specimens into age classes. Specimens were considered immature if the carapace length was less than 70 mm, because sex-indicative characters could not be discriminated below this size. We compared the size distributions of two populations according to carapace length by taking juveniles, males, and females together and performing a two-sample Kolmogorov-Smirnov test with R software (R Development Core Team 2011). Chi-square (χ^2) tests to compare sex ratios between populations were also performed using R software.

Figure 2. The two *M. leprosa* subspecies. Left: *M. l. saharica* from Oued Massa at Toulou (29°57'15,87" N, 9°39'15,70" W) (photo by Andrej Funk). Right: *M. l. leprosa* from Flix Dam (Reserva Natural de Fauna Salvatge de Sebes) (photo by AB).

Table 1. Abundance matrix of mtDNA cyt b haplotypes of *M. leprosa* specimens in sampled populations. Their relative frequencies are indicated for French and Spanish Catalonia. Haplotypes A26–A29, A31, and A32 are new from this study.

	Haplotypes											
Populations	A16	A18	A24	B5	B6	A26	A27	A28	A29	A31	A32	Total
Turtles farm	10	2	1	0	0	0	0	0	0	0	1	14
Algeria	0	0	0	0	1	0	0	0	0	0	0	1
Ceyras	0	0	0	2	0	0	0	0	0	0	0	2
St. Gely du Fesc	0	0	0	1	0	0	0	0	0	0	0	1
Narbonne	0	0	1	0	1	0	0	0	0	0	0	2
French Catalonia												
St Hippolyte	0	1	0	0	0	0	0	0	0	0	0	1
Agly	5	1	0	0	0	0	0	0	0	0	0	6
Canet	1	0	0	0	0	0	0	0	0	0	0	1
Basse (Thuir)	3	0	0	0	0	0	0	0	0	0	0	3
Tech main watercourse	13	0	6	0	0	0	0	0	0	0	0	19
Tech (St. Jean Pla de Corts)	2	0	11	0	0	0	0	0	0	0	0	13
Baillaury (Banyuls)	32	5	0	4	0	0	0	0	0	0	0	41
SUBTOTAL	56	7	17	4	0	0	0	0	0	0	0	84
Haplotype relative frequency (%)	67	8	20	5	0	0	0	0	0	0	0	
Spanish Catalonia												
Orlina	2	19	0	0	0	0	0	0	0	0	0	21
Caldes de Malavella	9	0	0	0	0	0	0	1	1	0	0	11
Parets de Murtra	14	0	0	0	0	0	0	0	0	0	0	14
Ca L'Arana	17	0	0	0	0	0	0	0	0	0	0	17
La Bunyula	11	0	2	0	0	0	0	0	0	1	0	14
Riera de Canyelles	3	0	1	0	0	5	0	0	0	0	0	9
Sequia Major	3	0	0	0	0	0	1	0	0	0	0	4
Ebre (Flix)	15	0	0	0	0	0	0	0	0	0	0	15
Ebre (Ille Audí)	6	0	0	0	0	0	0	0	0	0	0	6
Ulldecona	1	0	0	0	0	0	0	0	0	0	0	1
SUBTOTAL	81	19	3	0	0	5	1	1	1	1	0	112
Haplotype relative frequency (%)	72	17	3	0	0	4	1	1	1	1	0	
TOTAL	147	28	22	7	2	5	1	1	1	1	1	216

DNA extraction, PCR, and sequencing

We extracted DNA from frozen blood following the Blood & Body Fluid DNA Protocol of the E.Z.N.A.® Tissue DNA or MicroElute Kits (Omega bio-tek), which is optimised for the use of fresh or frozen blood. Ethanol-dissolved blood samples were dried using a Speed Vac® Plus lyophiliser (Savant), and blood pellets were subsequently dissolved in 400 µl of PBS Buffer and stored at 4°C for 48 hours before DNA extraction. The concentration of extracted DNA was measured with a Nanodrop 2000 spectrophotometer (Thermo Scientific).

Before PCR amplification, all DNA samples were adjusted to a concentration of 30 ng/µl. The mtDNA cyt b gene was amplified with forward mt-a-new (5'-CTCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3') (Lenk & Wink 1997) and reverse H-15909 (5'-AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG-3') primers (Fritz et al. 2005). Amplification reactions consisted of a mix with a final volume of 30 µl containing 1 µl of each DNA sample, 200 µM of each dNTP (Promega), 0.4 µM of each primer, 1.5 mM $MgCl_2$, 6 µl Taq-buffer, and 1 unit of GoTaq FlexiDNA Polymerase (Promega). The PCR was conducted in a Mastercycler Eppendorf® with the following settings: denaturation step of 2 min 30 sec at 95°C; 35 cycles of 30 sec each at 95°C, 40 sec at 49°C, 1 min 10 sec at 72°C; and one final extension step of 6 min at 72°C. The resultant PCR products were verified in 1% agarose gel, stained with ethidium bromide. The amplified DNA was then purified with the Wizard SV Gel and PCR Clean-up System (Promega) and sent for sequencing with both PCR primers to GATC (Biotech, France).

Sequence analysis

Reverse and forward sequences were assembled by using the Sequencher™ software (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequence chromatograms were carefully inspected and sequences were corrected manually at ambiguous sites. The edited cyt b gene sequences were aligned with MEGA version 4 (Tamura et al. 2007) to identify variable positions of different haplotypes. New haplotypes were named following the nomenclature outlined by Fritz et al. (2006), which includes the clade plus a correlative number. New sequence haplotypes were submitted to GenBank (accession numbers are KF791559–KF791564). Intra-population diversity indices, such as mitochondrial haplotype (H) and nucleotide (π_n) diversities and mean number of pairwise differences (π), were calculated for each population by using ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010). French and Spanish intra-population diversity indices with and without A24 and/or B haplotypes were compared using a Mann-Whitney-Wilcoxon non-parametric test in R software. Given the slow evolutionary rate of the cyt b gene in this species, a network of haplotypes is sufficient to show their divergence. Haplotypes were connected in the most parsimonious way (minimum distance between haplotypes) by using NETWORK 4.6.0.0 (www.fluxus-engineering.com) and applying the Median Joining algorithm (Bandelt et al. 1999).

Results

Demographic structure of *M. leprosa* in its northernmost distribution range

At the Baillaury River (Fig. 1c), the species was well represented with 108 individuals captured in 2010. The population presented an equilibrated age structure when compared to the structure of the little-impacted Orlina River population from Spanish Catalonia (two-sample Kolmogorov-Smirnov test D = 0.32, p = 0.30). With respect to gender composition, both populations show similar sex ratios biased in favour of males (Baillaury: 1:1.4; Orlina: 1:1.2; $\chi^2 = 0.06$, df = 1, p = 0.81). We also found forty-eight specimens at the Tech River, where only five specimens had been captured previously (Courmont & Rodriguez 2004). This is an irregularly routed but continuously flowing Mediterranean river in the Roussillon Plain adjoined by wetlands with vegetation dominated by dense reeds as well as a well-developed riparian forest (Fig. 1d). Along the Tech River's main bed, two turtles were captured each at two different sites, i.e., Riutec and Le Boulou, four at Nidolères, and sixteen at La Falaise (Fig. 1d). At St. Jean Pla de Corts, which is an artificial pond annexed to the river that is used for recreational activities (Fig. 1d), 29 turtles were captured. Captures from 2009 to 2010 at the Tech River main course yielded only adults, with a sex ratio of 1:1.6 in favour of males (Fig. 3) that was not significantly differ-

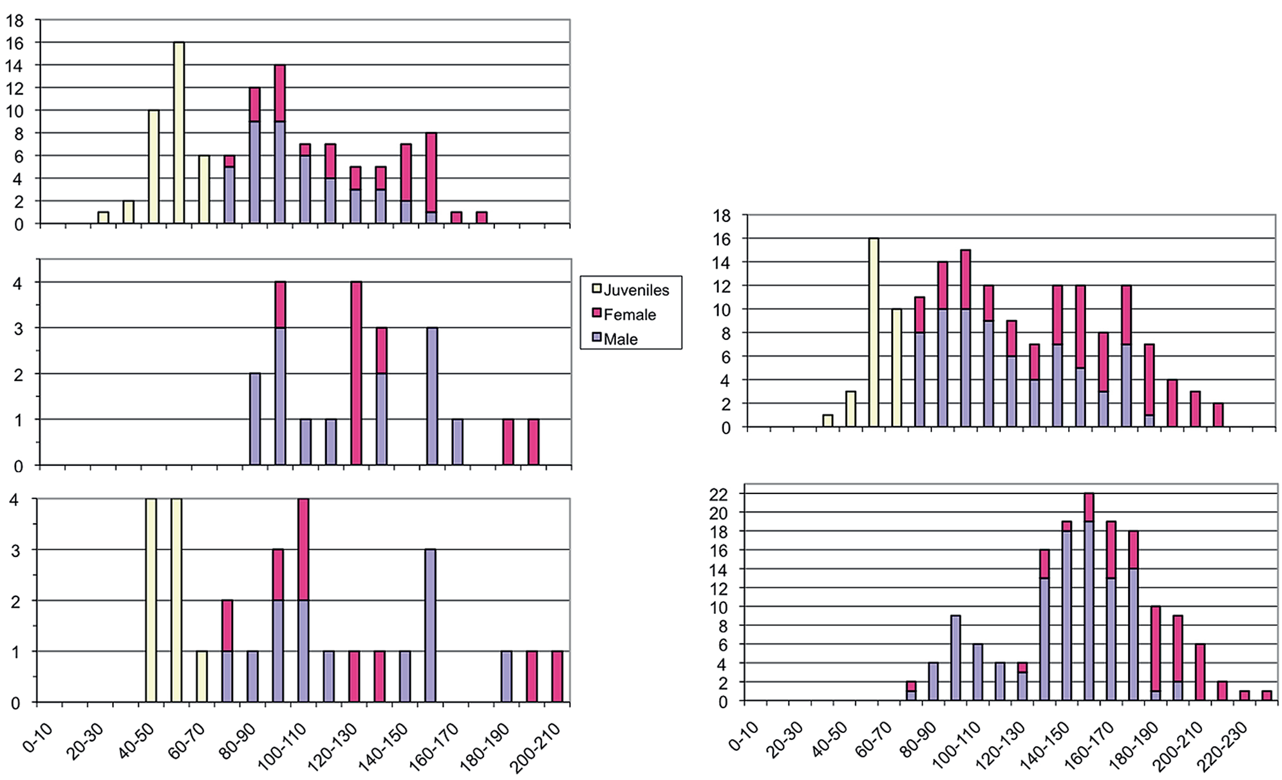

Figure 3. Age class structure based on carapace lengths. Left: Age class structure for the major French *M. leprosa* populations from this study: the Baillaury River in 2010, the Tech River main course in 2009–10, and the artificial pond at St Jean Pla de Corts in 2010. Right: age class structure for two Spanish populations: the Orlina River and Delta de Llobregat (from Franch Quintana et al. 2007).

ent from the Baillaury and Orlina populations (p = 0.92 and p = 0.70, respectively). The population at St. Jean Pla de Corts included juveniles and exhibited a heterogeneous age structure (Fig. 3). However, the sex ratio in favour of males (1:1.5) was not significantly different from the two previous populations (p = 0.84 and p = 0.95). Moreover, a two-sample comparison of carapace length distributions from the Baillaury and Tech Rivers (St. Jean Plat de Corts and main course taken together) populations produced an insignificant difference, too (D = 0.42, p = 0.07). In 2008, the presence of the species was recorded in an artificial channel in a swamp area at St. Hippolyte (Fig. 1b), where ten turtles were captured. In September 2010, no specimens were found at this site. Other singular catches of the species comprise eight individuals found at the Agly River in 2010, where stream water is calm forming anastomoses, six specimens at the Basse River in Thuir in 2008 and 2010, and one individual caught in a small artificial pond in Canet, which is annexed to the Têt River (Fig. 1b).

Genetic analysis of specimens from France and Spanish Catalonia

Among the 216 specimens that were genetically investigated (Table 1), we identified 12 different haplotypes from 933 usable nucleotide sites of the cyt b gene (Tables 1 and S1). We assigned the majority of haplotypes to clade A (*M. l. leprosa,* Fig. 4), which is known to occur throughout the Iberian Peninsula as well as north of the Atlas Mountain range (Fritz et al. 2006). However, some individuals had haplotypes referable to clade B and thus to *M. l. saharica* (Fig. 4, Table 1), occurring south of the Atlas Mountains (Fritz et al. 2006). We found one specimen with haplotype B6 in Algeria (Fig. 1a). In France, haplotypes referable to clade B were found mainly in locations north of French Catalonia in Ceyras, Narbonne, and St. Gély du Fesc (Table 1, Fig. 1b), except for four specimens with haplotype B5 found at a single site on the Baillaury River (Table 1, Fig. 1c). The dominant haplotype from clade A was A16, which was detected in 81 specimens from Spanish Catalonia and 57 from French Catalonia, in addition to the ten from the turtle farm (Table 1, Fig. 1a). Seven new haplotypes from this study (Table 1) differed from A16 by only one mutation each (Fig. 4). Five of them, namely A27, A28, A29, A31, and A32, were encountered only once (Tables 1 and S1). A32 was found at the turtle farm (Table 1), while the remaining ones were detected in terrapins occurring at Spanish sites (Fig. 1b), including five specimens of the new A26-haplotype found at one locality, Canyelles (Table 1). We also found specimens with haplotypes A18 and A24. Haplotype A18, which was dominant in the Spanish border population of the Orlina River (Fig. 1b), was previously thought to be endemic there (Fritz et al. 2006). We found A18-individuals at the sites Baillaury, Agly, and St. Hippolyte as well as at the turtle farm (Fig. 1b). Haplotype A24 had so far been encountered only in one individual in northern Morocco (Fritz et al. 2006). It differs from A16 at two mutational points (Fig. 4) and, together with A25, they are thought to represent an ancient branch within clade A that never crossed the Strait of Gibraltar (see Fig. 4). We found 17 specimens of haplotype A24 on the Tech River in France (Fig. 1d), three at the Spanish sample sites of Bunyola and Canyelles, one at the turtle farm, and another one in Narbonne (Fig. 1b, Table 1). Intra-population diversity indices (H, π_n, π) for all potentially natural populations with more than five individuals are shown in Table 2. The average gene diversity (H), or the probability of finding two different haplotypes in a sample when chosen ran-

Table 2. Diversity indices (H – haplotype diversity; π_n – nucleotide diversity; π – mean number of pairwise differences) calculated for potentially natural populations with more than 5 individuals with and without exotic B5 and potentially exotic A24 haplotypes. Average diversity values are reported for French and Spanish Catalonian populations. Probability-values of the Mann-Witney-Wilcoxon statistical test for differences between both groups of populations are also shown.

	Diversity indexes					
	With exotics			Without exotics		
Populations	H	π_n ($\times10^{-3}$)	π	H	π_n ($\times10^{-3}$)	π
Turtles farm						
Algeria	-	-	-	-	-	-
Ceyras	-	-	-	-	-	-
St. Gely du Fesc	-	-	-	-	-	-
Narbonne	-	-	-	-	-	-
French Catalonia						
St Hippolyte	-	-	-	-	-	-
Agly	0.33	0.4	0.33	0.33	0.4	0.33
Canet	-	-	-	-	-	-
Basse (Thuir)	-	-	-	-	-	-
Tech main watercourse	0.47	1.00	0.94	0	0	0
Tech (St. Jean Pla de Corts)	0.36	0.70	0.73	0	0	0
Baillaury (Banyuls)	0.38	2.00	1.84	0.24	0.26	0.24
Mean values	0.38	1.03	0,96	0,14	0,17	0,14
Spanish Catalonia						
Orlina	0.18	0.19	0.18	0.18	0.19	0.18
Caldes de Malavella	0.35	0.60	0.55	0.35	0.60	0.55
Bunyula	0.39	0.70	0.67	0.17	0.18	0.17
Ca L'Arana	0	0	0	0	0	0
Parets de Murtra	0	0	0	0	0	0
Riera de Canyelles	0.64	1.00	1.00	0.54	0.57	1.00
Sequia Major	-	-	-	-	-	-
Ebre (Flix)	0	0	0	0	0	0
Ebre (Ille Audí)	-	-	-	-	-	-
Ulldecona	-	-	-	-	-	-
Mean values	0.22	0.36	0.34	0.18	0.22	0.27
Mann-Whitney-Wilcoxon Test (p-values)	0.29	0.10	0.11	0.92	0.92	0.92

domly, and the average molecular diversity indices (π_n, π), were much higher in French than in Spanish populations except when haplotypes A24 and B5 (Table 2) or A24 or B5 (not shown) were eliminated from the analysis. The Mann-Whitney-Wilcoxon test confirmed this difference (Table 2). P-values were close to 1 when A24 and B haplotypes were eliminated from the analysis, which indicates a small difference between both groups of populations.

Discussion

Expansion of the Mediterranean pond turtle's known northern geographic distribution

Our results comprise the first survey of *M. leprosa* in France and demonstrate that the northern distribution expands beyond the Pyrenees Mountains all along the French Catalonian coast (Fig. 1b). We demonstrate that there are two well-established populations. Firstly, the already known population on the Baillaury River (Fig. 1c) where more than 200 individuals have been captured since 1990 (Verneau 2007, Verneau 2009, Hardy 2010, Verneau 2010). Secondly, a population that was discovered in the course of this study at the Tech River. The species is spatially structured as metapopulations at both locations (Figs 1c, d), i.e., groups of spatially separated populations that are potentially connected by the main course of the river (Hanski & Simberloff 1997). Both metapopulations showed an equilibrated demographical structure (Fig. 3) and no significant differences in sex ratio when compared to the well-preserved Orlina River population (Fig. 3). The small number of captures in spite of several survey campaigns and the apparent absence of juveniles on the Tech River main course (Fig. 3), lead us to hypothesise that the St. Jean Pla de Corts subpopulation might provide recruitments to the other Tech River subpopulations (Fig. 1d). *Mauremys leprosa* was

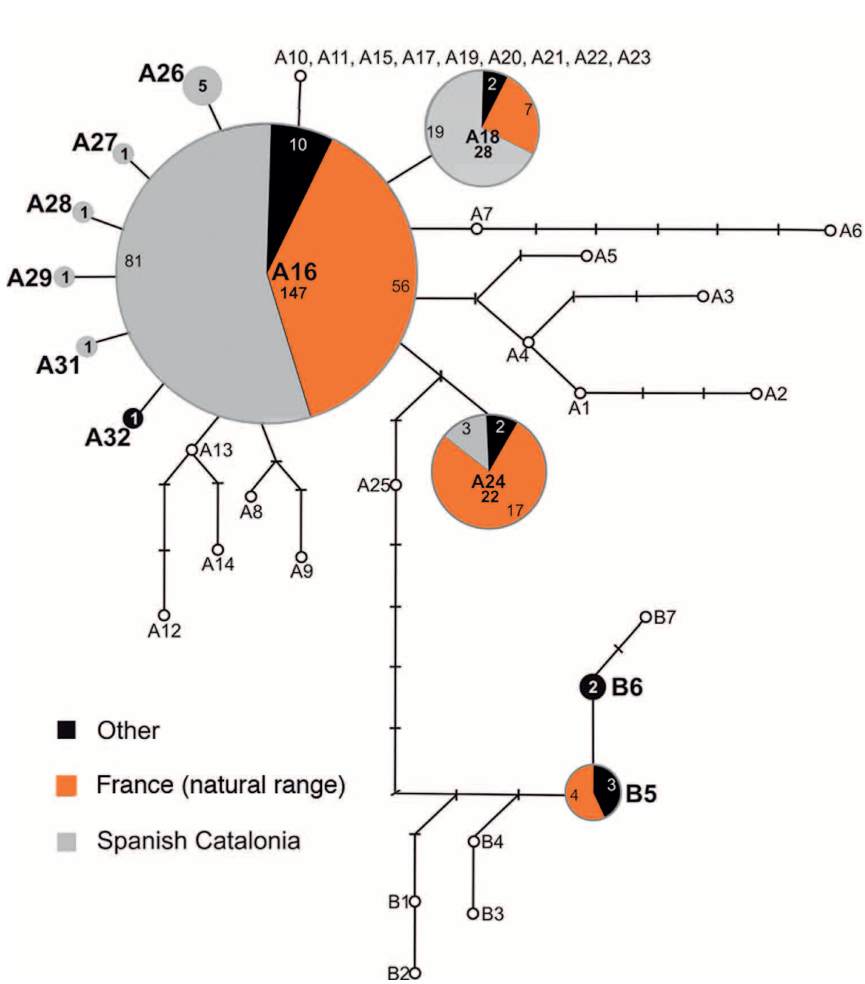

Figure 4. Minimum Spanning Network of *M. leprosa* mtDNA cyt b haplotypes. Coloured pies represent the distribution and frequency of identified haplotypes in Spanish and French Catalonia as well as in "Other" sites from this study (see Table 1). White circles represent known haplotypes of this species not observed in this study. A10, A11, A15, A17, A19, A20, A21, A22, and A23 are all distinct haplotypes that have been grouped for convenience; they all differ from A16 by one single mutation. Dashes represent missing haplotypes, each line between dashes or pies represents one mutational step.

also captured farther north, at Canet on the Têt River, and at Agly and the Basse Rivers, and in a small pond at St. Hippolyte, in areas with habitats similar to the previously mentioned populations, i.e., those with relatively calm waters and dense riparian vegetation (Fig. 1b). Salty water, as indicated by the many marine crabs caught in the crayfish traps, may explain the absence of captures at St. Hippolyte in 2010, as the species does not tolerate salinity above a critical value (Keller 1997, Bertolero & Oro 2009, Maran 2010), indicating that the species might have vacated the site. Captures from this study in predictable habitats call for further demographic surveys to conclude the establishment of the species in those northern wetlands. Beyond the region of French Catalonia, captures are scarce and limited to isolated localities (Fig. 1b), which is most likely the result of random releases of turtles.

A mixed origin in the northern range

Our genetic analyses aimed to shed light on the phylogeography of the populations of *M. leprosa* living at its northern range limits. Its presence on the Baillaury River was already known prior to the previous century's turtle trade, and we have encountered several individuals with A18 haplotypes in this area (Fig. 1c). Given that A18 was previously only found in Spanish Catalonia (Fritz et al. 2006), these results advocate the hypothesis of its presence on the Baillaury River being due to ancient dispersal from Spain, supporting the hypothesis that the Pyrenees Mountains have not always been an insurmountable geographical barrier for the species, as appears to be the case with the Atlas Mountains in Morocco (Fritz et al. 2005, 2006). Furthermore, we found a similar relative abundance of the A16 haplotype between French and Spanish Catalonian populations (Table 1) and a widespread presence of A18 in French Catalonia (Figs 2, 4). These results favour the nativity hypothesis of the French populations, particularly on the Baillaury, Tech, Basse, and Agly Rivers (Fig. 1). We therefore promote two exclusive explanations for the origin of the species in France: i) an ancient natural origin in France expanding from Spain, as illustrated by the fossil record (Cheylan 1982, Cheylan & Poitevin 2003), or ii) extinction and a more recent northern expansion. Nevertheless, a mixed origin in this country cannot be ruled out due to the presence of A24 and particularly B haplotypes in the Tech and Baillaury Rivers populations, respectively. With respect to A24, and given the limited data available from Spain and Morocco, we cannot discard the possibility that this haplotype may be more widespread in Europe than suspected. However, we found a higher relative abundance of the A24 haplotype in France (20%) than in Spain (3%, Table 2), and molecular diversity indices of French populations were exacerbated compared to those of Spanish populations except when A24 and/or B haplotypes were eliminated from the analysis (Table 2). Secondly, the B haploclade denotes another subspecies, *M. l. saharica* (Fig. 2). Translocation to reinforce natural populations or the illegal trade in *M. leprosa* specimens and their unauthorised random release, as is known to happen with other turtle species (van Dijk et al. 2004, Moll & Moll 2004, Velo-Antón et al. 2011), are therefore the most plausible explanations for the presence of these haplotypes in France. Furthermore, only singular individuals with A24 or B haplotypes have been encountered at isolated northern French locations (Fig. 1b), supporting the hypothesis that *M. leprosa* is present naturally only up to St. Hippolyte in the area known as French Catalonia.

Conservation management and research perspectives in France

This is the first comprehensive study of *M. leprosa* in France, where it is classified as 'Endangered' (UICN France & MNHN 2008). Several allochthonous mtDNA cyt b haplotypes were detected. We found several individuals with haplotype B5 indicative of *M. l. saharica* (Figs 1c, 4) concentrated in one location at the Baillaury River (Fig. 1c). A high abundance of the most likely exotic haplotype A24 was also encountered on the Tech River (Table 1, Fig. 1b). This haplotype constitutes nowadays a paradox, as it has previously been found only in one individual in northern Morocco (Fritz et al. 2006). A more thorough study of the genetic diversity of this species, particularly in Spain and Morocco, will likely help to explain this phenomenon. Even supposedly autochthonous haplotypes, such as A16 or A18, could potentially be the result of translocation, as corroborated in this study by the presence of these haplotypes at the turtle farm, where specimens are brought to by people who kept them as pets (Table 1). To identify the exact origin of French specimens, it will be necessary to study the genetic structure of the entire distribution range of the species using appropriate genetic markers. The dynamics of the Tech River metapopulation require further research to identify migration and gene flow patterns between subpopulations and understand the higher number of A24 individuals at St. Jean Pla de Corts. Subsequent modelling of population dynamics will better guide conservation management of the species in this area (Ovaskainen et al. 2002). In order to guide decision-making regarding whether exotic haplotypes should be eliminated, it will be necessary to perform experimental research on the local adaptation of endemic haplotypes. If hybridisation between exotic and endemic haplotypes occurs, experimental research aiming to find signs of outbreeding depression or hybrid vigour will help with decisions concerning allochthonous haplotypes. If evidence of local adaptation or absent hybrid vigour is found, it would be advisable to eliminate exotic haplotypes (Edmands 2007, Huff et al. 2011). Inversely, controlled hybridisation between endemic and exotic haplotypes would be advisable if signs of inbreeding depression are observed (Frankham 1995, Edmands 2007). Nevertheless, due to the historical value of the Baillaury River population (Knoepffler 1979b), it is advisable to extirpate specimens belonging to subspecies *M. l. saha-*

rica from this area. Last but not least, efforts should be focused on informing the public of the risks associated with poaching or releasing turtles, as they might threaten native turtles for the reasons pointed out above.

Demographic structure and genetic diversity insights suggest a more widespread distribution of *M. leprosa* in France than recently reported (Cheylan & Vacher 2010), but one that is much smaller than suggested by the fossil record (Cheylan 1982). We propose to maintain the current 'Endangered' status of *M. leprosa* in France (UICN France & MNHN 2008) until research regarding the present distribution, possible population expansion and growth, and connectivity between populations will be completed.

Acknowledgements

We are grateful for permits to capture turtles issued by the Servei de Protecció i Gestió de la Fauna (Environmental Department of the Catalan government) to AB, by the Port Aventura and Consorci of Llobregat Delta to MF, and by the Ministère de l'Ecologie, de l'Energie, du Développement Durable et de la Mer to OV. We are indebted to the herpetology group of Universitat de Barcelona, Ms. Malirach ("Vallée des Tortues", Sorède), Centre de Reproducció de Tortugues de l'Albera (Spain), The Reserva Natural de Fauna Salvatge de Sebes (especially P.J. Jiménez, M. Viñas), and to N. Kaid (UPVD). This work has been supported by: projects entrusted to OV and LDP (CNRS PICS N°4837 2010–2012; Protea 2011–2012 from the Ministère des Affaires Étrangères et Européennes et de l'Enseignement Supérieur et de la Recherche), to OV (CEN L-R project 2010) and CP (CNRS PEPS N° FG/cb D156 2011); invited professorial grants to LDP (UPVD-L-R 2009–2010); and a project assigned to L. Courmont (Groupe Ornitologique du Roussillon) and OV in 2010 for sampling in the Pyrenees Mountains. We also thank T. Gendre and collaborators (CEN L-R) and V. Rigaud (La Tartuga) for blood samples from Hérault and Aude.

References

Bandelt, H. J., P. Forster & A. Röhl (1999): Median-joining networks for inferring intraspecific phylogenies. – Molecular Biology and Evolution, **16**: 37–48.

Bertolero, A. & D. Oro (2009): Conservation diagnosis of reintroducing Mediterranean pond turtles: what is wrong? – Animal Conservation, **12**: 581–591.

Cadi, A. & P. Joly (2004): Impact of the introduction of the red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). – Biodiversity & Conservation, **13**: 2511–2518.

Cheylan, M. (1982): Présence de la Clemmyde lépreuse dans le chalcolithique de la grotte de la Salpêtrière. – Études Quaternaire Languedociennes, **2**: 29–33.

Cheylan, M. & F. Poitevin (2003): Les tortues du site de Lattara (IVe s. av. n. è. – IIe s. de n. è.). Intérêt archéozoologiques et biologiques. – Lattara, **16**: 137–145.

Cheylan, M. & J. P. Vacher (2010): L'émyde lépreuse. – pp. 261–265 in: Vacher J. P. & M. Geniez (eds): Les reptiles de France, Belgique, Luxembourg et Suisse. – Biotope/MNHN, Paris.

Cheylan, M. & O. Verneau (2012): L'Émyde lépreuse. – pp. 210–216 in: Geniez P. & M. Cheylan (eds): Les amphibiens et les reptiles du Languedoc-Roussillon et régions limitrophes. Atlas biogéographique 2nd edn. – Biotope, Paris.

Courmont, L. & P. Rodriguez (2004): Une nouvelle station d'Emyde lépreuse *Mauremys leprosa* dans les Pyrénées-Orientales. – Meridionalis, **6**: 60–65.

Cox, N. A. & H. J. Temple (2009): European Red List of Reptiles. – Office for Official Publications of the European Communities, Luxemburg, 34 pp.

da Silva, E. (2002): *Mauremys leprosa*. – pp. 143–146 in: Pleguezuelos, J. M., R. Marquez & M. Lizana (eds): Atlas y libro rojo de los anfibios y reptiles de España. – Dirección General de Conservación de la Naturaleza-Asociación Herpetologica Española (2ª impresión), Madrid.

de Lapparent de Broin, F. (2001): The European turtle fauna from the Triassic to the present. – Dumerilia, **4**: 155–217.

Doucotterd, J.-M. & R. Bour (2002): Nouvelles données sur les sous-espèces de *Mauremys leprosa* dans le centre et le sud du Maroc (Reptilia, Chelonii). – Manouria, **5**: 12–21.

Edmands, S. (2007): Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. – Molecular Ecology, **16**: 463–475.

Excoffier, L. & H. E. L. Lischer (2010): Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. – Molecular Ecology Resources, **10**: 564–567.

Franch Quintana, M. (2003): Caracterització de la tortuga de rierol *Mauremys leprosa* (Schweigger, 1812) a l'Alt Empordà: biometria i cicle biològic. – Master thesis, Universitat de Barcelona.

Franch Quintana, M., G. A. Llorente Cabrera & A. Montori Faura (2007): Primeros datos sobre la biología de *Trachemys scripta elegans* en sintopía con *Mauremys leprosa* en el delta del Llobregat (NE Ibérico). – pp. 85–101 in: GEIB Grupo Especialista en Invasiones Biológicas (ed.): Invasiones biológicas: un factor del cambio global. – EEII 2006 actualización de conocimientos. 2.º Congreso Nacional sobre Especies Exóticas Invasoras "EEI 2006". GEIB, Serie Técnica N.º 3, León.

Frankham, R. (1995): Conservation genetics. – Annual review of genetics, **29**: 305–327.

Fritz, U., M. Barata, S. D. Busack, G. Fritzsch & R. Castilho (2006): Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geoemydidae). – Zoologica Scripta, **35**: 97–108.

Fritz, U., G. Fritzsch, E. Lehr, J.-M. Ducotterd & A. Müller (2005): The Atlas Mountains, not the Strait of Gibraltar, as a biogeographic barrier for *Mauremys leprosa* (Reptilia: Testudines). – Salamandra, **41**: 97–106.

Fritz, U. & P. Havas (2007): Checklist of Chelonians of the World. – Vertebrate Zoology, **57**: 148–368.

Geniez, P. & M. Cheylan (2005): Reptiles et batraciens de France. CD ROM – Educagri, Dijon.

Hanski, I. & D. Simberloff (1997): The metapopulation approach: its history, conceptual domain, and application to conservation. – pp. 5–26 in: Hanski, I. & M. Gilpin (eds): Metapopulation Biology: Ecology, Genetics and Evolution. – Academic Press, San Diego.

Hardy, J.P. (2010): *Mauremys leprosa* en France. – La Tortue, **85**: 50–53.

Huff, D. D., L. M. Miller, C. J. Chizinski & B. Vondracek (2011): Mixed-source reintroductions lead to outbreeding depression in second-generation descendents of a native North American fish. – Molecular Ecology, **20**: 4246–4258.

Iverson, B. J. (1992): A revised checklist with distribution maps of the turtles of the world. – Privately Printed, Richmond. 363 pp.

Keller, C. (1997): Ecología de poblaciones de *Mauremys leprosa* y *Emys orbicularis* en el Parque Nacional de Doñana. Thesis, Universidad de Sevilla.

Knoepffler, L. P. (1979a): *Clemmys caspica leprosa* (Schweigger, 1812), (Chelonien, Testudinoidea, Emydés). La Cistude de Mauritanie. Documents pour un atlas zoogéographique du Languedoc-Roussillon n. 13. – Université Paul Valéry, Montpellier.

Knoepffler, L. P. (1979b): La Cistude de Mauritanie (*Clemmys caspica leprosa* Schweigger, 1812) fait-elle partie de la faune de France? – Bulletin de la Société herpétologique de France, **12**: 22–25.

Lenk, P. & M. Wink (1997): A RNA/RNA heteroduplex cleavage analysis to detect rare mutations in populations. – Molecular Ecology, **6**: 687–690.

Llorente, G. A., A. Montori, X. Santos & M. A. Carretero (1995): Atlas dels amfibis i rèptils de Catalunya i Andorra. – Edicions El Brau, Figueres, 192 pp.

Maran, J. (2010): Observations sur la distribution des tortues du Maroc (Chelonii: Emydidae, Geoemydidae et Testudinidae). – Chéloniens, **19**: 16–34.

Martínez-Silvestre, A., D. Perpiñan, I. Marco & S. Lavin (2002): Venipuncture technique of the occipital venous sinus in fresh-water aquatic turtles. – Journal of Herpetological Medicine and Surgery, **12**: 31–32.

Maufras, O. & C. Mercier (2002): Habitat et terroir du IVe au XIIe s. à Saint-Gilles-le-Vieux (Aimargues, Gard). Archéologie du TGV Méditerranée. Fiches de synthèse, tome 3. Antiquité, moyen âge, époque moderne. – Monographies d'Archéologie Méditerranéenne **10**: 945–972.

Moll, D. & E. O. Moll (2004): The ecology, exploitation and conservation of river turtles. – Oxford University Press, New York. 393 pp.

Ovaskainen, O., K. Sato, J. Bascompte & I. Hanski (2002): Metapopulation models for extinction threshold in spatially correlated landscapes. – Journal of Theoretical Biology, **215**: 95–108.

Pérez, M., E. Collado & C. Ramo (1979): Crecimiento de *Mauremys caspica leprosa* (Schweigger, 1812) (Reptilia Testudines) en la Reserva Biológica de Doñana. – Doñana Acta Vertebrata, **6**: 161–178.

Polo-Cavia, N., P. López & J. Martín (2008): Interspecific differences in responses to predation risk may confer competitive advantages to invasive freshwater turtle species. – Ethology, **114**: 115–123.

Polo-Cavia, N., P. López & J. Martín (2009): Interspecific differences in chemosensory responses of freshwater turtles: consequences for competition between native and invasive species. – Biological Invasions, **11**: 431–440.

Polo-Cavia, N., P. López & J. Martín (2010a): Competitive interactions during basking between native and invasive freshwater turtle species. – Biological Invasions, **12**: 2141–2152.

Polo-Cavia, N., P. López & J. Martín (2010b): Aggressive interactions during feeding between native and invasive freshwater turtles. – Biological Invasions, **13**: 1387–1396.

Polo-Cavia, N., P. López & J. Martín (2012): Effects of body temperature on righting performance of native and invasive freshwater turtles: Consequences for competition. – Physiology & Behavior, **108**: 23–33.

R Development Core Team (2011): R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing, Viena, Austria ISBN 3-900051-07-0.

Schleich, H. (1996): Beitrag zur Systematik des Formenkreises von *Mauremys leprosa* (Schweigger) in Marokko. Teil 1 (Reptilia, Chelonii, Emydidae). – Spixiana, Suppl. **22**: 29–59.

Segurado, P., C. A. Fernández & A. C. Rivera (2005): L'émyde lépreuse *Mauremys leprosa* dans la peninsule ibérique. – Manouria, **8**: 26–27.

Tamura, K., J. Dudley, M. Nei & S. Kumar (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. – Molecular Biology and Evolution, **24**: 1596–1599.

UICN France & MNHN (2008): La Liste rouge des espèces menacées en France – Chapitre Reptiles et Amphibiens de France métropolitaine, Paris.

van Dijk, P., B. Stuart & A. Rhodin (2000): Asian turtle trade: proceedings of a workshop on conservation and trade of freshwater turtles and tortoises in Asia. – Chelonian Research Monograph, **2**: 1–164.

van Dijk, P. P., J. A. Mateo Miras, M. Cheylan, U. Joger, P. Sá-Sousa & V. Pérez-Mellado (2004): *Mauremys leprosa*. – IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2.

Velo-Antón, G., M. García-París & A. Cordero Rivera (2008): Patterns of nuclear and mitochondrial DNA variation in Iberian populations of *Emys orbicularis* (Emydidae): conservation implications. – Conservation Genetics, **9**: 1263–1274.

Velo-Antón, G., M. Wink, N. Schneeweiss & U. Fritz (2011): Native or not? Tracing the origin of wild-caught and captive freshwater turtles in a threatened and widely distributed species (*Emys orbicularis*). – Conservation Genetics, **12**: 583–588.

Veríssimo, J., G. Velo-Antón, S. Lopes, P. Pereira, J. Teixeira & U. Fritz (2013): Cross-amplification of microsatellite loci for the Mediterranean stripe-necked terrapin (*Mauremys leprosa*). – Amphibia-Reptilia, **34**: 1–4.

Verneau, O. (2007): Taxonomie et systématique des parasites d'amphibiens et de tortues d'eau douce en Languedoc-Roussillon. – Scientific Report n. 1 for DIREN Languedoc-Roussillon.

Verneau, O. (2009): Taxonomie et systématique des parasites d'amphibiens et de tortues d'eau douce en Languedoc-Roussillon. – Scientific Report n. 2 for DIREN Languedoc-Roussillon.

Verneau, O. (2010): Taxonomie et systématique des parasites d'amphibiens et de tortues d'eau douce en Languedoc-Roussillon. – Scientific Report n. 3 for DIREN Languedoc-Roussillon.

Supplementary material

Additional information is available in the online version of this article at http://www.salamandra-journal.com

Supplementary table S1. Details of turtle specimens captured and sampled for genetic studies.

Online Supplementary data

Palacios, C., C. Urrutia, N. Knapp, M. F. Quintana, A. Bertolero, G. Simon, L. du Preez & O. Verneau: Demographic structure and genetic diversity of *Mauremys leprosa* in its northern range reveal new populations and a mixed origin. – Salamandra, **51**(3): 221–230.

Supplementary table S1. Details of turtle specimens captured and sampled for genetic studies: locality, turtle ID number, GPS coordinates, blood or tissue sampled for DNA extraction, DNA number, and mtDNA cyt b haplotype are indicated for all catches. Haplotypes in blue are new from this study. Note that for Spanish catches, GPS coordinates are indicated for the entire population.

Population	Turtle Number	Site	GPS North	GPS East	Blood / Tissue	DNA-number	Haplo-type
Turtle Farm (Sorède)	1	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-625	A16
Turtle Farm (Sorède)	2	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-457	A24
Turtle Farm (Sorède)	3	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-626	A16
Turtle Farm (Sorède)	6	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-627	A16
Turtle Farm (Sorède)	7	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-628	A16
Turtle Farm (Sorède)	9	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-629	A16
Turtle Farm (Sorède)	10	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-630	A18
Turtle Farm (Sorède)	11	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB75	A16
Turtle Farm (Sorède)	17	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB76	A16
Turtle Farm (Sorède)	25	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB79	A18
Turtle Farm (Sorède)	35	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB81	A16
Turtle Farm (Sorède)	36	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB82	A16
Turtle Farm (Sorède)	37	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	MiAB83	A16
Turtle Farm (Sorède)	119	Vallée heureuse	42°30'57.26"	2°57'28.54"	Fresh Blood	Mi-581	A32
Outside Catalonia							
Algeria	No number	Oued Rhiou	35°58'25.35"	0°55'14.13"	Tissue	Mi-618	B6
Ceyras (France)	No number	a place called Le Pigné	43°38'56.69"	3°27'36.24"	Blood in alcohol	MiAB143	B5
Ceyras (France)	No number	a place called Le Pigné	43°38'56.69"	3°27'36.24"	Fresh Blood	MiAB147	B5
St. Gely du Fesc (France)	1	Mare 1735	43°42'22.80"	3°47'44.75"	Fresh Blood	MiAB145	B5
Narbonne (France)	1	Carrière	43°09'57.95"	2°56'50.93"	Fresh Blood	MiAB101	A24
Narbonne (France)	2	town centre	43°10'58.36"	3°00'03.73"	Fresh Blood	MiAB102	B6
French Catalonia							
St. Hyppolite	10	………..	42°48'17.21"	2°58'12.22"	Tissue	Mi-617	A18
Agly	71	GPS153	42°45'11.72"	2°56'42.32"	Fresh Blood	MiAB105	A16
Agly	72	GPS154	42°45'08.32"	2°57'00.99"	Fresh Blood	MiAB106	A16
Agly	73	GPS153	42°45'11.72"	2°56'42.32"	Fresh Blood	MiAB107	A16
Agly	74	GPS153	42°45'11.72"	2°56'42.32"	Fresh Blood	MiAB108	A16
Agly	75	GPS152	42°45'15.15"	2°56'29.71"	Fresh Blood	MiAB109	A18
Agly	76	GPS154	42°45'08.32"	2°57'00.99"	Fresh Blood	MiAB110	A16
Canet	1	………..	42°42'28.55"	3°01'23.51"	Fresh Blood	Mi-612	A16
Basse (Thuir)	3	………..	42°38'02.71"	2°46'28.43"	Fresh Blood	MiAB127	A16
Basse (Thuir)	4	………..	42°38'02.71"	2°46'28.43"	Fresh Blood	MiAB128	A16
Basse (Thuir)	68	………..	42°38'02.71"	2°46'28.43"	Fresh Blood	MiAB129	A16
Tech (main course)	17	Nidolères	42°32'25.13"	2°51'39.57"	Fresh Blood	Mi-579	A16
Tech (main course)	38	Nidolères	42°32'25.13"	2°51'39.57"	Fresh Blood	MiAB132	A16
Tech (main course)	20	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-620	A24
Tech (main course)	22	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-824	A24
Tech (main course)	23	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-622	A16
Tech (main course)	24	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB134	A16
Tech (main course)	25	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB135	A16

Population	Turtle Number	Site	GPS North	GPS East	Blood / Tissue	DNA-number	Haplo-type
Tech (main course)	26	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB136	A16
Tech (main course)	27	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-624	A16
Tech (main course)	28	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-825	A16
Tech (main course)	29	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-826	A16
Tech (main course)	30	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	Mi-455	A16
Tech (main course)	32	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB138	A24
Tech (main course)	33	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB139	A24
Tech (main course)	34	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB140	A16
Tech (main course)	35	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB141	A16
Tech (main course)	36	La Falaise	42°32'01.49"	2°50'55.24"	Fresh Blood	MiAB142	A24
Tech (main course)	37	Le Boulou	42°31'22.43"	2°50'10.28"	Fresh Blood	MiAB131	A24
Tech (main course)	39	Lac Riutec	42°30'14.44"	2°46'19.58"	Fresh Blood	MiAB111	A16
Tech (St. Jean Pla de Corts)	40	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB112	A24
Tech (St. Jean Pla de Corts)	41	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB113	A24
Tech (St. Jean Pla de Corts)	42	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB114	A16
Tech (St. Jean Pla de Corts)	43	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB115	A24
Tech (St. Jean Pla de Corts)	44	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB116	A24
Tech (St. Jean Pla de Corts)	45	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB117	A24
Tech (St. Jean Pla de Corts)	46	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB118	A24
Tech (St. Jean Pla de Corts)	47	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB119	A24
Tech (St. Jean Pla de Corts)	48	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB120	A16
Tech (St. Jean Pla de Corts)	49	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB121	A24
Tech (St. Jean Pla de Corts)	50	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB122	A24
Tech (St. Jean Pla de Corts)	51	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB123	A24
Tech (St. Jean Pla de Corts)	66	St Jean Pla de Corts	42°31'0.76"	2°48'46.24"	Fresh Blood	MiAB124	A24
Baillaury (Banyuls)	219	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB90	A16
Baillaury (Banyuls)	220	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB96	B5
Baillaury (Banyuls)	221	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB98	B5
Baillaury (Banyuls)	222	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB100	B5
Baillaury (Banyuls)	223	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB95	A16
Baillaury (Banyuls)	224	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB92	A16
Baillaury (Banyuls)	226	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB99	A16
Baillaury (Banyuls)	227	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB87	A16
Baillaury (Banyuls)	230	GPS1	42°27'59.10"	3°06'10.14"	Fresh Blood	MiAB93	B5
Baillaury (Banyuls)	237	GPS2	42°28'8.52"	3°05'49,56"	Fresh Blood	MiAB94	A18
Baillaury (Banyuls)	239	GPS3	42°28'7.72"	3°05'47.33"	Fresh Blood	MiAB84	A16
Baillaury (Banyuls)	335	GPS4	42°28'6.54"	3°05'45.42"	Fresh Blood	MiAB88	A16
Baillaury (Banyuls)	106	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-827	A16
Baillaury (Banyuls)	138	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-596	A18
Baillaury (Banyuls)	155	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-597	A16
Baillaury (Banyuls)	179	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-598	A16
Baillaury (Banyuls)	184	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-582	A16
Baillaury (Banyuls)	186	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-608	A16
Baillaury (Banyuls)	198	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-599	A16
Baillaury (Banyuls)	199	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-600	A16
Baillaury (Banyuls)	203	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-454	A18
Baillaury (Banyuls)	204	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-828	A16
Baillaury (Banyuls)	205	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-601	A16
Baillaury (Banyuls)	206	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-602	A16
Baillaury (Banyuls)	207	Bridge	42°27'45.38"	3°05'26.70"	Fresh Blood	Mi-583	A16

Population	Turtle Number	Site	GPS North	GPS East	Blood / Tissue	DNA-number	Haplo-type
Baillaury (Banyuls)	202	GPS5	42°27’44.58”	3°05,25.20”	Fresh Blood	Mi-609	A18
Baillaury (Banyuls)	208	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-603	A18
Baillaury (Banyuls)	209	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-604	A16
Baillaury (Banyuls)	210	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-605	A16
Baillaury (Banyuls)	211	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-584	A16
Baillaury (Banyuls)	212	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-606	A16
Baillaury (Banyuls)	213	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-829	A16
Baillaury (Banyuls)	214	Mas Abeilles	42°27’45.99”	3°05’08.77”	Fresh Blood	Mi-607	A16
Baillaury (Banyuls)	234	GPS6	42°27’39.72”	3°05’18.78”	Fresh Blood	MiAB97	A16
Baillaury (Banyuls)	215a	GPS7	42°27’39.84”	3°05’18.84”	Fresh Blood	Mi-610	A16
Baillaury (Banyuls)	231	GPS8	42°27’35.28”	3°05’17.64”	Fresh Blood	MiAB86	A16
Baillaury (Banyuls)	235	GPS8	42°27’35.28”	3°05’17.64”	Fresh Blood	MiAB126	A16
Baillaury (Banyuls)	232	GPS9	42°27’34.62”	3°05’17.70”	Fresh Blood	MiAB89	A16
Baillaury (Banyuls)	233	GPS9	42°27’34.62”	3°05’17.70”	Fresh Blood	MiAB85	A16
Baillaury (Banyuls)	216a	GPS10	42°27’29.22”	3°05’11.70”	Fresh Blood	Mi-611	A16
Baillaury (Banyuls)	236	GPS11	42°27’24.90”	3°04’56.22”	Fresh Blood	MiAB91	A16
Spanish Catalonia							
Orlina	2157	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB296	A18
Orlina	2179	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB282	A18
Orlina	2216	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB440	A18
Orlina	2264	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB278	A18
Orlina	2303	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB281	A18
Orlina	2333	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB295	A18
Orlina	2365	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB290	A18
Orlina	2366	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB286	A18
Orlina	2372	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB436	A18
Orlina	2384	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB288	A18
Orlina	2415	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB273	A18
Orlina	2431	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB441	A16
Orlina	2433	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB276	A16
Orlina	2456	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB289	A18
Orlina	2479	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB280	A18
Orlina	2494	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB284	A18
Orlina	2496	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB292	A18
Orlina	2514	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB275	A18
Orlina	2522	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB277	A18
Orlina	2640	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB439	A18
Orlina	2649	Rabós d’Empordà	42º22’38	03º01’50”	Dried blood	MiAB437	A18
Caldes de Malavella	8223	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB194	A16
Caldes de Malavella	8242	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB195	A16
Caldes de Malavella	8243	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB196	A16
Caldes de Malavella	8244	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB197	A28
Caldes de Malavella	8245	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB198	A16
Caldes de Malavella	8246	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB189	A29
Caldes de Malavella	8248	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB187	A16
Caldes de Malavella	8248	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB188	A16
Caldes de Malavella	8267	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB191	A16
Caldes de Malavella	8268	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB192	A16
Caldes de Malavella	9933	Riera de Santa Maria	41º49’30”	02º46’57”	Dried blood	MiAB193	A16
Parets de Murtra	8304	Gavà	41º17’16”	02º00’54”	Dried blood	MiAB240	A16

Population	Turtle Number	Site	GPS North	GPS East	Blood / Tissue	DNA-number	Haplo-type
Parets de Murtra	8307	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB241	A16
Parets de Murtra	8308	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB242	A16
Parets de Murtra	8316	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB233	A16
Parets de Murtra	8317	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB234	A16
Parets de Murtra	8318	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB235	A16
Parets de Murtra	8321	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB247	A16
Parets de Murtra	8331	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB237	A16
Parets de Murtra	8352	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB251	A16
Parets de Murtra	8353	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB252	A16
Parets de Murtra	8357	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB239	A16
Parets de Murtra	8359	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB243	A16
Parets de Murtra	8364	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB245	A16
Parets de Murtra	8365	Gavà	41º17'16"	02º00'54"	Dried blood	MiAB244	A16
El Prat de Llobregat	8113	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB213	A16
El Prat de Llobregat	8132	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB214	A16
El Prat de Llobregat	4710	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB215	A16
El Prat de Llobregat	7123	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB216	A16
El Prat de Llobregat	4719	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB217	A16
El Prat de Llobregat	4733	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB218	A16
El Prat de Llobregat	4740	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB219	A16
El Prat de Llobregat	4811	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB220	A16
El Prat de Llobregat	8116	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB221	A16
El Prat de Llobregat	8112	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB222	A16
El Prat de Llobregat	8118	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB223	A16
El Prat de Llobregat	4552	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB224	A16
El Prat de Llobregat	4901	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB225	A16
El Prat de Llobregat	4716	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB227	A16
El Prat de Llobregat	4829	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB228	A16
El Prat de Llobregat	4720	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB229	A16
El Prat de Llobregat	4552	CA L'Arana	41º18'10"	02º07'44"	Dried blood	MiAB230	A16
El Prat de Llobregat	411	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB267	A16
El Prat de Llobregat	4000	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB262	A16
El Prat de Llobregat	4449	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB254	A16
El Prat de Llobregat	4453	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB266	A16
El Prat de Llobregat	4812	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB258	A16
El Prat de Llobregat	4816	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB255	A16
El Prat de Llobregat	4817	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB264	A16
El Prat de Llobregat	4818	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB256	A24
El Prat de Llobregat	4819	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB261	A31
El Prat de Llobregat	4824	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB263	A16
El Prat de Llobregat	4826	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB257	A16
El Prat de Llobregat	4828	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB260	A24
El Prat de Llobregat	4830	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB259	A16
El Prat de Llobregat	6117	Bunyula	41º18'31"	02º06'42"	Dried blood	MiAB253	A16
Riera de Canyelles	8134	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB205	A26
Riera de Canyelles	8137	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB206	A26
Riera de Canyelles	8146	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB202	A26
Riera de Canyelles	8147	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB203	A26
Riera de Canyelles	8149	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB210	A26
Riera de Canyelles	8155	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB212	A16

Population	Turtle Number	Site	GPS North	GPS East	Blood / Tissue	DNA-number	Haplo-type
Riera de Canyelles	8163	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB201	A16
Riera de Canyelles	9133	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB200	A24
Riera de Canyelles	xx4x	Riera de Canyelles	41º17'19"	01º43'28"	Dried blood	MiAB208	A16
Sèquia Major	1001	La Pineda - Salou	41º04'37"	01º10'34"	Dried blood	MiAB183	A16
Sèquia Major	1002	La Pineda - Salou	41º04'37"	01º10'34"	Dried blood	MiAB184	A27
Sèquia Major	1003	La Pineda - Salou	41º04'37"	01º10'34"	Dried blood	MiAB185	A16
Sèquia Major	1007	La Pineda - Salou	41º04'37"	01º10'34"	Dried blood	MiAB186	A16
Ebre	1358	Flix	41º14'20"	0º31'28"	Dried blood	MiAB452	A16
Ebre	1360	Flix	41º14'20"	0º31'28"	Dried blood	MiAB464	A16
Ebre	1363	Flix	41º14'20"	0º31'28"	Dried blood	MiAB457	A16
Ebre	1364	Flix	41º14'20"	0º31'28"	Dried blood	MiAB450	A16
Ebre	1368	Flix	41º14'20"	0º31'28"	Dried blood	MiAB446	A16
Ebre	1369	Flix	41º14'20"	0º31'28"	Dried blood	MiAB455	A16
Ebre	1374	Flix	41º14'20"	0º31'28"	Dried blood	MiAB451	A16
Ebre	1376	Flix	41º14'20"	0º31'28"	Dried blood	MiAB461	A16
Ebre	1377	Flix	41º14'20"	0º31'28"	Dried blood	MiAB456	A16
Ebre	1379	Flix	41º14'20"	0º31'28"	Dried blood	MiAB466	A16
Ebre	1380	Flix	41º14'20"	0º31'28"	Dried blood	MiAB462	A16
Ebre	1381	Flix	41º14'10"	0º31'26"	Dried blood	MiAB465	A16
Ebre	1382	Flix	41º14'10"	0º31'26"	Dried blood	MiAB463	A16
Ebre	1391	Flix	41º14'10"	0º31'26"	Dried blood	MiAB454	A16
Ebre	1392	Flix	41º14'10"	0º31'26"	Dried blood	MiAB458	A16
Ebre	1213	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB442	A16
Ebre	1306	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB444	A16
Ebre	1396	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB443	A16
Ebre	1397	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB445	A16
Ebre	1398	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB448	A16
Ebre	Unknown	Illa Audí	40º51'11"	0º31'21"	Dried blood	MiAB467	A16
Ulldecona	1395	…………	40º38'38"	0º29'59"	Dried blood	MiAB447	A16