Reassessment of distribution and conservation status of freshwater turtles (Testudines) in Tunisia

Rahma Attia El Hili, Olivier Verneau, Jamel Jrijer, Mohamed Sghaier Achouri

SALAMANDRA **56**(4): 362–372
30 October 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Reassessment of distribution and conservation status of freshwater turtles (Testudines) in Tunisia

Rahma Attia El Hili[1,2,3], Olivier Verneau[2,3,4], Jamel Jrijer[1] & Mohamed Sghaier Achouri[1]

[1)] University of Tunis El Manar, Faculty of Sciences of Tunis, Laboratory of Diversity, Management and Conservation of Biological Systems, LR18ES06, Tunis, Tunisia

[2)] Université Perpignan Via Domitia, Centre de Formation et de Recherche sur les Environnements Méditerranéens, Perpignan, France

[3)] CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens, Perpignan, France

[4)] Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

Corresponding author: Rahma Attia El Hili, e-mail: rahma-hili@hotmail.fr

Manuscript received: 18 September 2019
Accepted: 8 May 2020 by Andreas Schmitz

Abstract. Assessing the extant distribution of wildlife species is a crucial step towards promoting their protection and management in the light of global biodiversity decline, and even more so when threatened, vulnerable, rare, or endemic taxa are concerned. In this context, we present an overview of the biogeography of two freshwater turtle species, namely the Mediterranean pond turtle *Mauremys leprosa* and the European pond turtle *Emys orbicularis*, as a basis for Conservation Action Plans. *Mauremys leprosa* is widely distributed in the north and centre of Tunisia and has viable populations in most of the study sites. Based on the evaluation of its biogeography, the size, structure and genetics of four populations, it appears that this species is currently not threatened in Tunisia. In contrast, *E. orbicularis*, which is represented in eastern Algeria and Tunisia by an endemic, undescribed subspecies, was absent from 11 historical sites in Tunisia, and only isolated small populations and scattered individuals were present in the extreme north of the country. Our study thus suggests that this subspecies is declining in Tunisia and its status is probably close to Endangered (EN). Therefore, it needs special attention and urgent conservation measures.

Key words. Tunisia, *Mauremys leprosa*, *Emys orbicularis*, biogeography, genetic variation, size structure.

Introduction

Chelonians (Testudines) have existed on earth for more than 200 million years since the Triassic Era (Ernst & Lovich 2009). Most recent estimates indicate that there are just 360 extant species (Rhodin et al. 2018), while birds, by comparison, comprise several thousand species.

Worldwide, turtles are exposed to intense anthropogenic pressure (Rhodin et al. 2018, Stanford et al. 2018) due to their relatively late sexual maturity, low reproductive output, and high juvenile mortality (Iverson 1991, Congdon et al. 1993). Major threats are habitat modification and loss, biological invasion, and illegal collection and trade (Klemens 2000). Based on the IUCN Red List (2018), 61% of the 251 turtle and tortoise species are currently classified as 'threatened' (Rhodin et al. 2018), which renders Testudines one of the most endangered vertebrate groups of the world (Rhodin et al. 2018, Stanford et al. 2018). Thus, without strategic conservation plans, several species could become extinct in the current century (Buhlmann et al. 2009).

Tunisia is home to six turtle species amongst which are three marine species off the Tunisian coast, viz. the loggerhead sea turtle *Caretta caretta* (Linnaeus, 1758), the leatherback sea turtle *Dermochelys coriacea* (Vandelli, 1761), and the green sea turtle *Chelonia mydas* (Linnaeus, 1758), two freshwater turtle species, viz. the Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) and the European pond turtle *Emys orbicularis* (Linnaeus, 1758), and a single terrestrial species, namely the Moorish tortoise *Testudo graeca* Linnaeus, 1758. Although all these species have been included in the list of rare and endangered Tunisian species since 2006 (JORT n 60 of 28/7/06; art. 209–210 of the Forest Code modified by law 2009-59 of 20/7/09), no specific legislation for turtle protection has as yet been implemented. However, several monitoring studies on the loggerhead sea turtle have been conducted in Tunisia as part of the Action Plan for the conservation of Mediterranean marine turtles (CAR/ASP & PNUE/PAM 2014). This species has attracted special interest because it nests along the Tunisian coasts. Recently, some conservation programs have also been developed for the Moorish tortoise in the shape of vulnerability assessment and wildlife rehabilitation in response to poaching. For their part, the distribution and conservation status of Tunisian freshwater turtles are still unclear because these have remained poorly studied. Only a few attempts to these effects were made back in the 1950–1970s, focusing on the geographical distribution (Loveridge & Williams 1957, Blanc 1978), the biology (Rouault & Blanc 1978), and the parasitic fauna (Mishra & Gonzalez 1978) of these two species.

To the best of our knowledge, the Mediterranean pond turtle is widely distributed in North Africa, from northwestern Libya to the south of Morocco and southern Europe, namely across the Iberian Peninsula (Barth et al. 2004, Rhodin et al. 2017). It is also found in the south of France in the Eastern Pyrenees Department (Palacios et al. 2015, Rhodin et al. 2017). Based on differences in their mitochondrial DNA and morphology, this species can be divided into two distinct subspecies (Fritz et al. 2006, Verissimo et al. 2016, Rhodin et al. 2017). *Mauremys leprosa leprosa* (haploclade A) occurs in the north of the Atlas Mountains in Morocco and in Portugal, Spain and France (Fritz et al. 2006, Palacios et al. 2015, Verissimo et al. 2016); whereas *M. l. saharica* (haploclade B) occurs in the south of the Atlas Mountains in Morocco, and north of the Atlas Mountains in Algeria and Tunisia and southern France, with the latter being the result of recent introductions (Fritz et al. 2006, Palacios et al., 2015, Verissimo et al. 2016). Conversely, the European pond turtle is represented only in isolated habitat fragments in the northern African Maghreb region, whereas it is relatively widely distributed in Europe and western Asia (Fritz 2003). Nine lineages have been identified from cytochrome *b* sequences, with each of them characterizing a distinct subspecies (Lenk et al. 1999, Fritz et al. 2007, 2009, Velo-Antón et al. 2008, Stuckas et al. 2014, Vamberger et al. 2015). North African populations were identified as *E. o. occidentalis* in earlier years (Fritz et al. 2007), but it was subsequently demonstrated that only Moroccan populations belong to this taxon and that eastern Algerian and Tunisian populations represent an undescribed distinct subspecies (Stuckas et al. 2014). With the latter thus possibly representing an endemic taxon it is all the more important to assess their status in both countries to establish a basis for conservation measures.

In an evolving world challenged by frequent and unpredictable global changes, the emergence of biotic and abiotic stresses can contribute to the impoverishment or even extinction of animal populations. In this context, several studies were focused on the impact of environmental changes on freshwater turtles, like the impacts of invasive turtle species (Cadi & Joly 2003, 2004, Polo-Cavia et al. 2008, 2009, 2010, Pearson et al. 2015) and their platyhelminth parasites (Meyer et al. 2015, Héritier et al. 2017a) on freshwater ecosystems, water pollution (Loulida et al. 2018, Héritier et al. 2017b, Venancio et al. 2013, El Hassani et al. 2019, Slimani et al. 2017), and climate change (Chessman 2011). With data on the geographical distribution of freshwater turtles in Tunisia dating back to the 1950s and no further survey results being available, updating knowledge on these turtles in Tunisia is crucial for assessing their conservation status and developing appropriate measures for their protection. Therefore, the present study provides information on the current distributions of *M. leprosa* and *E. orbicularis* in Tunisia and their classification based upon mitochondrial data. It also reports on the size and structure of four *M. leprosa* populations in different Tunisian habitats. It thus aims to provide a starting point for defining monitoring protocols for each species and for developing a sustainable conservation plan for freshwater turtles at national and regional levels in Tunisia.

Materials and methods

Study area

Tunisia is situated in the north of Africa, borders Algeria in the west and Libya in it's the southwest. It has a long seafront along the Mediterranean Sea in the north and east. Despite its relatively small size, it harbours a wide environmental diversity with a contrasting topography. This relief is composed of the eastern extension of the Atlas Mountains from the Algerian border to the Cape Bon peninsula in the east, a plain along the eastern Mediterranean coast, and desert in the southern regions. Tunisian climate varies from humid in the northwestern regions to desert in the south of the country. Climatic variations are mainly due to the Tunisian Atlas Mountains, which act as a barrier between regions influenced by the Mediterranean Sea and those influenced by the Sahara desert.

Fieldwork sampling and data collection

Sampling sites were selected according to the distribution range of each species cited in Loveridge & Williams (1957) and Blanc (1978), but also from various other sources such as turtle sightings by local people in the wild, the presence of freshwater resources, and the importance of habitats in terms of biodiversity conservation (e.g., nature reserves). Our survey was carried out between March 2017 and September 2018, with fieldwork peaking from April through June during the two years. We searched 48 potentially suitable habitats for freshwater turtles across different regions in Tunisia except for arid and Saharan regions where water is very scarce. Turtles were captured using non-harmful traps set for periods extending from one to three days. Traps were baited with sardines and fixed in place in such manner that trapped turtles were still able to breathe. Collected turtles were marked with marginal cuts on the carapace for Capture-Mark-Recapture (CMR). Data recorded included the sexual identities of captured animals, morphometrics (length, width and height of the carapace and length of the plastron), and identity of recaptured animals. Blood samples were collected from the caudal sinus at the base of the tail using a 1-ml syringe, preserved in 98% ethanol, and stored at -20°C until molecular processing. Captured turtles were eventually released at the exact place of their capture.

Population sizes and sex ratios were estimated only for four populations selected as to be representative in terms of geographical location (north/south) and water quality (natural *versus* polluted), for which purpose at least 50 specimens were collected per site. Straight carapace length was the single measure to classify specimens into three categories: juvenile, adult male, and adult female. Sexing was based on secondary sexual characters (e.g., plastron morphology and cloaca position) (Pérez et al., 1979, Servan et al., 1989), thus the term "adult" was used for turtles that could be sexed. Based on the carapace lengths (CL) taken of mature females in different natural habitats (Combescot 1954, Pérez el al. 1979, Da Silva 1995, Alarcos Izquierdo et al. 2009, Naimi et al. 2012), mature females are much larger than adult males in our study.

Genetic analyses

Total genomic DNA extractions were carried out from blood samples with the DNeasy Blood & Tissue (Qiagen) and E.Z.N.A® Tissue DNA Kits (Omega Bio-tek) following manufacturers' protocols. The cytochrome *b* gene was amplified using the forward primer mt-a-new (5'-CTCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3') (Lenk & Wink 1997) and the reverse primer H-15909 (5'-AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG-3') (Lenk et al. 1999). PCRs were carried out in a final volume of 25 µl and performed in a Mastercycler Eppendorf under the following conditions: a denaturation step of 5 min at 95°C; 35 cycles of 30 sec at 94°C, 30 sec at 54°C and 1 min at 72°C; and a final extension of 10 min at 72°C. The success of the PCR was checked by electrophoresis on a 1% agarose gel containing ethidium bromide, and PCR products were sent to the company Genoscreen (Lille, France) for sequencing. Chromatograms were carefully checked with the software Chromas and sequences were manually corrected if necessary. The obtained cytochrome *b* gene sequences were aligned using MEGA X software (Kumar et al. 2018) with sequences extracted from GenBank and reflecting the variability already detected within both turtle species. This was done to assess the variability along sequences and to identify the turtle haplotypes occurring in Tunisia.

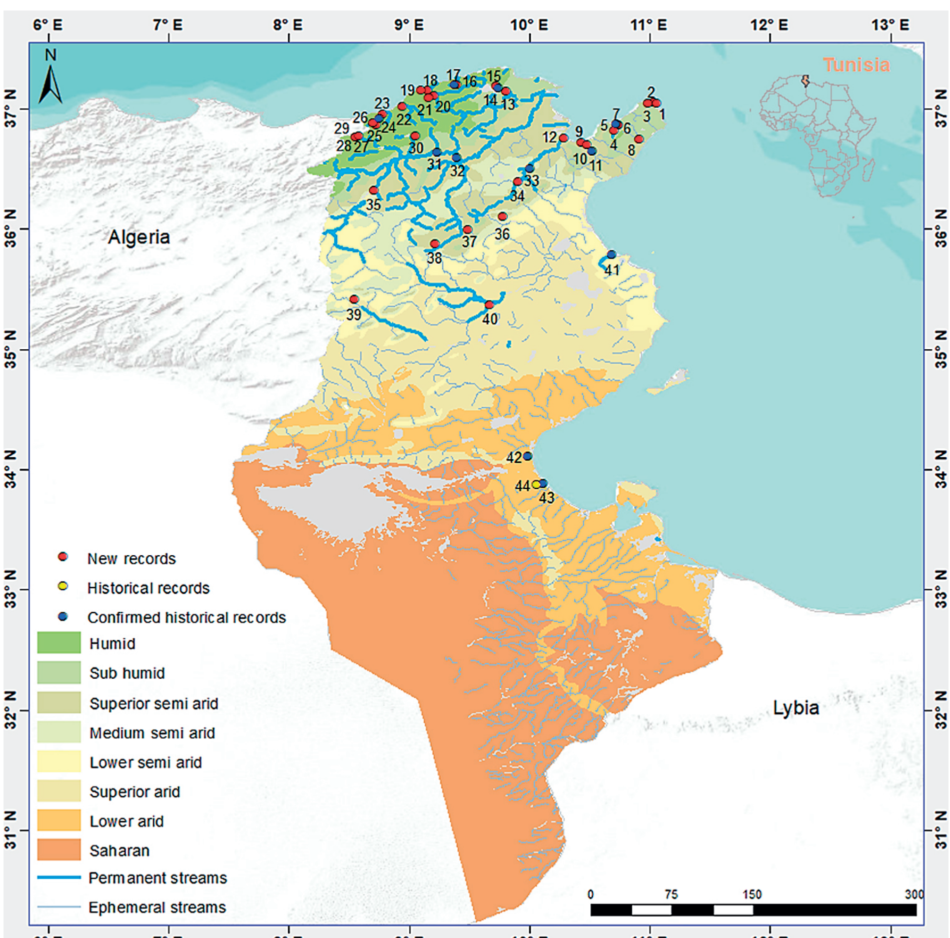

Figure 1. Distribution map of *Mauremys leprosa* in Tunisia based on historical and new records. Numbers refer to the localities specified in Table 1.

Table 1. Sampling effort and GPS records of actual and historical turtle occurrences in Tunisia. See Fig. 1A, B for the mapping of locality records. Sampling effort = Number of traps × number of sampling nights; * Historical record of *M. leprosa* (Posner 1988, Blanc 1978, Loveridge & Williams 1957); ∆ Historical record of *E. orbicularis* (Posner 1988, Blanc 1978, Loveridge & Williams 1957, Nouira 2001); • Traps were moved between two sampling sessions and set again at the same locality; – No data available.

Species	Latitude DD	Longitude DD	Locality	Habitat type	Sampling effort	Number of captures or observations	Number of recaptures
M. leprosa	37.05083	11.02944	1. Oued El Ain	Pond	8 × 3	32	6
M. leprosa	37.04694	11.05722	2. Haouaria dam	Dam	5 × 2•	4	0
M. leprosa	37.04639	10.98667	3. Ain Halloufa	Watercourse	5 × 1	6	–
M. leprosa	36.820278	10.703056	4. Oued Laabid dam	Dam	10 × 1	15	–
M. leprosa	36.8625	10.72583	5. Oued Laabid village	Watercourse	10 × 2	40	4
M. leprosa	36.8575	10.74139	6. Oued Gargoura Argoub	Watercourse	5 × 1	15	–
M. leprosa	36.8675	10.72472	7. Oued El Abid*	Watercourse	10 × 2•	57	0
M. leprosa	36.739444	10.913889	8. Libna dam	Dam	10 × 1	6	0
M. leprosa	36.7125	10.42944	9. Oued Borj Cedria	Watercourse	5 × 1	2	–
M. leprosa	36.69433	10.48007	10. Oued Soliman	Watercourse	5 × 1	4	–
M. leprosa	36.641111	10.518056	11. Oued Grombelia	Watercourse	5 × 1	1	–
M. leprosa	36.755556	10.284722	12. Oued Miliane	Watercourse	5 × 1	2	–
M. leprosa	37.14167	9.80417	13. Oued Manzel Bourguiba	Pond	10 × 2•	22	0
M. leprosa	37.17139	9.74222	14. Ichkeul lake*	Lake	5 × 1	2	–
M. leprosa	37.19222	9.72056	15. Beni Toun pond	Pond	10 × 3•	76	0
M. leprosa	37.19667	9.40194	16. El Amaria	Pond	10 × 3	62	7
M. leprosa	37.195	9.37889	17. Oued Sajnen*	Watercourse	10 × 2•	22	0
M. leprosa	37.15278	9.09833	18. Majen Chitana	Pond	10 × 1	2	–
M. leprosa	37.155556	9.155278	19. Oued Sidi Mechreg	Watercourse	5 × 1	9	–
M. leprosa	37.11059	9.21056	20. El Hania	Pond	10 × 4	44	7
M. leprosa	37.08528	9.15806	21. Oued El Hania	Watercourse	10 × 2•	12	0
M. leprosa	37.01639	8.94694	22. Sidi Barrak dam	Dam	9 × 2•	9	0
M. leprosa	36.94889	8.78389	23. Tabarka golf course	Pond	5 × 1	10	–
M. leprosa	36.91917	8.75333	24. Oued El Kabir*	Watercourse	5 × 1	6	–
M. leprosa	36.865	8.72528	25. Tabarka dam	Dam	5 × 2 •	26	0
M. leprosa	36.88167	8.70278	26. Oued Zarga dam	Dam	5 × 2•	5	0
M. leprosa	36.77139	8.57944	27. Oued Hammam Bourguiba	Watercourse	2 × 1	1	–
M. leprosa	36.7625	8.55167	28. Large Hammam Bourguiba pond	Pond	10 × 2•	23	0
M. leprosa	36.76583	8.56583	29. Small Hammam Bourguiba Pond	Pond	5 × 1	2	–
M. leprosa	36.76861	9.05694	30. Oued Amdoun	Watercourse	2 × 1	1	–
M. leprosa	36.63278	9.23139	31. Oued Mastouta*	Watercourse	Observation only	5	–
M. leprosa	36.58694	9.39667	32. Sidi Salem dam*	Dam	5 × 1	3	–
M. leprosa	36.4975	10.005278	33. Bir Msherga dam*	Dam	5 × 1	1	–
M. leprosa	36.388056	9.906111	34. Oued El Fahs	Watercourse	Observation only	4	–
M. leprosa	36.31309	8.70747	35. Guelta Zargua	Pond	Observation only	1	–
M. leprosa	36.09556	9.78278	36. Nebhana dam	Dam	Observation only	1	–
M. leprosa	35.992222	9.488056	37. Lakhmes dam	Dam	Observation only	1	–
M. leprosa	35.87144	9.21424	38. Oued Saboun	Watercourse	Observation only	15	–
M. leprosa	35.41344	8.54292	39. Oued Fousana	Watercourse	Observation only	2	–
M. leprosa	35.3675	9.672222	40. Sidi Saad dam	Dam	5 × 1	3	–
M. leprosa	35.78056	10.68056	41. Oued Hamdoun*	Watercourse	5 × 2•	10	0
M. leprosa	34.10861	9.98361	42. Oued El Akarit*	Watercourse	5 × 3	97	6
M. leprosa	33.87861	10.11139	43. Oued Gabes*	Watercourse	5 × 1	7	–
M. leprosa	33.87361	10.05833	44. Chenini Oasis*	Watercourse	Observation only	0	–

Species	Latitude DD	Longitude DD	Locality	Habitat type	Sampling effort	Number of captures or observations	Number of recaptures
E. orbicularis	37.11059	9.21056	20. El Hania	Pond	10 × 4	8	4
E. orbicularis	37.01639	8.94694	22. Sidi Barrak dam	Dam	9 × 2•	2	0
E. orbicularis	36.8675	10.72472	7. Oued El Abid$^{\Delta}$	Watercourse	10 × 2•	0	–
E. orbicularis	36.739444	10.913889	8. Libna dam$^{\Delta}$	Dam	10 × 1	0	–
E. orbicularis	36.69433	10.48007	10. Oued Soliman$^{\Delta}$	Watercourse	5 × 1	0	–
E. orbicularis	36.641111	10.518056	11. Oued Grombelia$^{\Delta}$	Watercourse	5 × 1	0	–
E. orbicularis	37.17139	9.74222	14. Ichkeul lake$^{\Delta}$	Lake	5 × 1	0	–
E. orbicularis	36.91917	8.75333	24. Oued El Kabir$^{\Delta}$	Watercourse	5 × 1	0	–
E. orbicularis	35.3675	9.672222	40. Sidi Saad dam$^{\Delta}$	Dam	5 × 1	0	–
E. orbicularis	36.72111	10.6325	45. Oued Berzik$^{\Delta}$	Watercourse	10 × 1	0	–
E. orbicularis	36.945	8.76278	46. Oued El Amor$^{\Delta}$	Watercourse	5 × 1	0	–
E. orbicularis	37.1192	9.7206	47. Oued El Melah$^{\Delta}$	Watercourse	10 × 1	0	–
E. orbicularis	36.7697	8.73361	48. Oued Ezzen $^{\Delta}$	Watercourse	5 × 1	0	0

Results

Distribution range, size, structure and sex ratio of *M. leprosa*

Mauremys leprosa was captured and/or observed throughout Tunisia in all prospected watercourses but one, i.e., Chenini Oasis (Fig. 1 and Table 1). Our sampling effort at each of the prospected sites yielded a capture success that was rather good at most of the sites, suggesting that population sizes could be quite important (Table 1). A total of 622 individuals were captured at 36 sites and 29 more were observed at 7 sites (Table 1). The largest populations were recorded in Oued El Abid in the northeast, in Beni Toun pond, and in El Amaria in the northwest, and in Oued El Akarit in the southeast (Table 1).

CLs ranged from 30 to 90 mm injuveniles, from 93 to 185 mm in adult males, and from 95 to 210 mm in adult females. A total of 57 turtles were captured in Oued El Abid in September 2017 and June 2018. This population was composed for about 80.7% of adults and for 19.3% of juveniles with a sex ratio of 1:0.64, i.e., slightly in favour of females (Table 2 and Fig. 3). A total of 76 turtles were captured at Beni Toun pond in June 2017 and May 2018. This population was composed for about 65.79% of adults and for 34.21% of juveniles with a sex ratio of 1.4:1, i.e., strongly in favour of males (Table 2 and Fig. 4). A total of 55 turtles were captured at El Amaria in July 2017 and 2018. This population was composed for about 85.5% of adults and for 14.5% of juveniles with a sex ratio of 1:0.62, i.e., strongly in favour of females (Table 2 and Fig. 5). A total of 91 turtles were captured at Oued El Akarit in June 2017 and August 2018. Here, the turtles were concentrated in small bodies of water due to the main part of the watercourse having dried up. This population was composed for about 82.4% of adults and for 17.6% of juveniles with a sex ratio of 1:0.53, i.e., strongly in favour of females (Table 2 and Fig. 6).

Distribution range of *E. orbicularis*

Despite our high sampling effort in several parts of Tunisia, *E. orbicularis* was captured in only two of the 48 prospected sites from which it had never before been mentioned (Fig. 2 and Table 1). Captures of *E. orbicularis* were less abundant than those of *M. leprosa*, with only eight adults collected in El Hania and two specimens at Sidi Barrak dam (Table 1). Population size and sex ratios were therefore not assessed for this species.

Genetic diversity of *M. leprosa* and *E. orbicularis*

Only six specimens of *M. leprosa* representative of six distinct populations, sampled at the localities Oued El Ain, Oued El Abid, El Hania, El Amaria, the large Hammam Bourguiba pond, and Oued El Akarit, were genetically analysed. A single haplotype, i.e., B6 (Genbank accession number XXX) was identified, which characterizes *M. l. saharica* from eastern Algeria and Tunisia (Fritz et al. 2006) (Table 3). All ten blood samples collected from *E. orbicularis* at El Hania and Sidi Barrak dam were processed. A single haplotype, i.e., IXa (Genbank accession number YYY), was identified, which, according to Stuckas et al. (2014), characterizes an undescribed subspecies of *E. orbicularis* that occurs in Algeria and Tunisia.

Discussion

Vulnerability assessment of *M. leprosa* in Tunisia

The Mediterranean pond turtle is classified as 'vulnerable' (Vu) in both the European and Mediterranean IUCN red lists of threatened species (Cox et al. 2006, 2009). Though that species has also its own status in France (UICN France et al. 2015) and Spain (Pleguezuelos et al. 2002) where

Table 2. Proportion of adult males to females of *M. leprosa* surveyed at four sites in Tunisia. See Table 1 for GPS coordinates of localities.

Locality	Type of sites	Males (M)	Females (F)	M/F
7. Oued El Abid	Manmade	18	28	1.0:0.64
15. Beni Toun pond	Natural	29	21	1.4:1.00
16. El Amaria	Natural	18	29	1.0:0.62
42. Oued El Akarit	Manmade	26	49	1.0:0.53

it is also classified as a vulnerable species, due to the lack of data in Tunisia no status was attributed to this species. In northwestern Africa, this species is known to be tolerant of rather adverse conditions and to concentrate in places with limited freshwater reserves (Bertolero & Busack 2017, El Hassani et al. 2019). In Morocco, it occurs in very different ecosystems and habitats, including coastal rivers, mountain streams, oases, and ephemeral rivers on the northern fringes of the Sahara desert (Lovich et al. 2010, Bertolero & Busack 2017). Comparatively little information currently exists on its distribution range in Algeria (Rozet 1833, Nasri et al. 2008, Mamou et al. 2014). During our study period, *M. leprosa* was found to be common in several regions of Tunisia where it occurs in almost all water bodies. We also confirmed its presence in 10 historical localities (Loveridge & Williams 1957). It had previously been recorded from the Chenini Oasis in

Table 3. Cytochrome *b* haplotypes characterizing *M. leprosa* and *E. orbicularis* in Tunisia. See Table 1 for GPS coordinates of localities.

Species	Locality	Haplotype
M. leprosa	1. Oued El Ain	B6
M. leprosa	7. Oued El Abid	B6
M. leprosa	16. El Amaria	B6
M. leprosa	20. El Hania	B6
M. leprosa	28. Large Hammam Bourguiba pond	B6
M. leprosa	42. Oued El Akarit	B6
E. orbicularis	20. El Hania	IXa
E. orbicularis	22. Sidi Barrak dam	IXa

the arid region, but was found to be absent there during our study. According to local people, it is no longer present here due to the fact that watercourses tend to seasonally dry up in this region. Its overall distribution extends from the subhumid northern mountainous regions to the arid south (Fig. 1 and Table 1). This species was also found to be not selective in terms of environmental conditions. It was abundant in highly polluted watercourses (Oued Laabid Village, Oued Borj Cedria, Oued Soliman, Oued Grombelia, Oued Manzel Bourguiba, Oued Hamdoun, and Oued Gabes), in those with reduced water levels (El Hania, Oued El Akarit), and in brackish water (Ichkeul lake and Oued El Akarit). The *M. leprosa* populations at Oued El Abid, Oued El Akarit, El Amaria, and Beni Toun exhibited a high

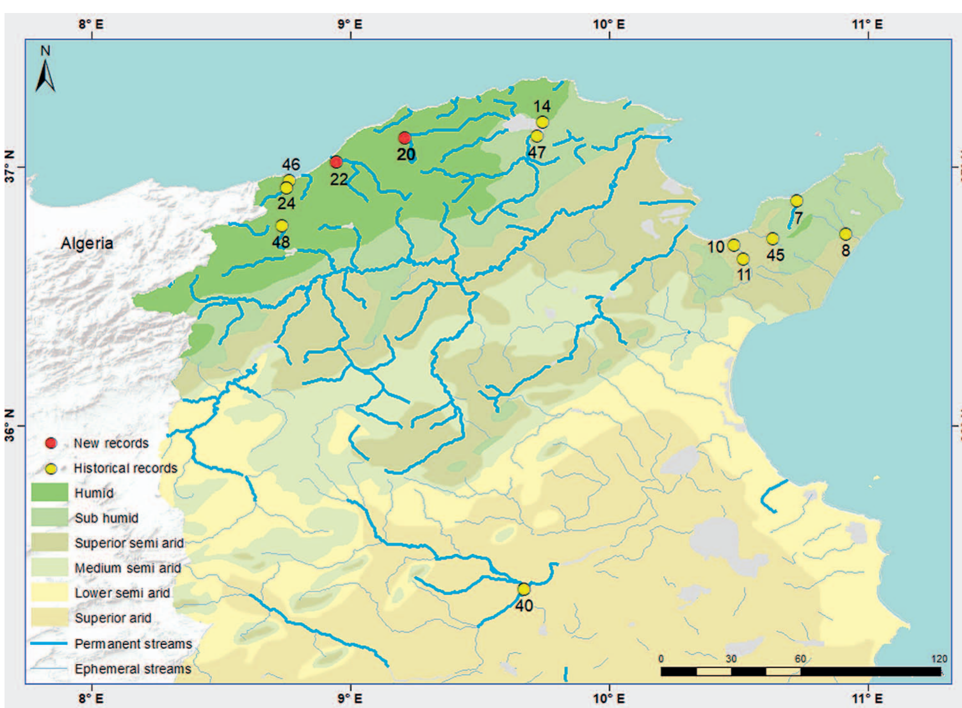

Figure 2. Distribution map of *Emys orbicularis* in Tunisia based on historical and new records. Numbers refer to the localities specified in Table 1.

percentage (more than 70%) of adult specimens. However, differences in our sampling methodology could have impacted on the capture rates of juveniles. Given the fact that adults and juveniles do not necessarily occupy the same aquatic habitats at the same locality, exploiting different resources and preferring different temperature ranges, light intensities, and water depths (Reese 1986), capture results may have been biased by where traps were set (Zuffi 2000, Mosimann & Cadi 2004). Overall, if differential mortality between sexes and/or age groups existed in turtles, the observed variation in sex ratios could be due to sampling biases, as unbalanced sex ratios seem to be common in populations of turtles (Bury 1979, McKnight et al. 2017). This deserves more in-depth analysis by means of CMR (Capture-Mark-Recapture) field studies.

Our genetic study of 6 *M. leprosa* individuals revealed the presence of a single haplotype B6 (Table 3) in the northern regions of Tunisia (Fig. 1 and Table 1), which is characteristic of *M. l. saharica* (clade B). The limited number of examined *M. leprosa* specimens might be the reason why only a single haplotype was found. This haplotype was also identified in *M. leprosa* populations in the east of Algeria and at one site in the northwest of Tunisia in the phylogeographic study of *M. leprosa* in North Africa (Fritz et al. 2006). Although haplotype B7 was found in a single specimen from eastern north Tunisia (Fritz et al. 2006) and haplotype B3 in different northern regions (Verissimo et al. 2016), the presence of a homogenous genetic cluster over a large geographical range suggests an absence of significant landscape barriers that could hamper the dispersal of this species. Our evaluation of our findings regarding biogeography, population size and structure and the genetics of *M. leprosa leprosa* suggest this taxon not to be threatened in Tunisia at present. Nevertheless, it requires careful monitoring due to the drying up of water bodies following global temperature increases, draining of swamps for agricultural uses, and illegal trade, all of which could affect its stability in the long term.

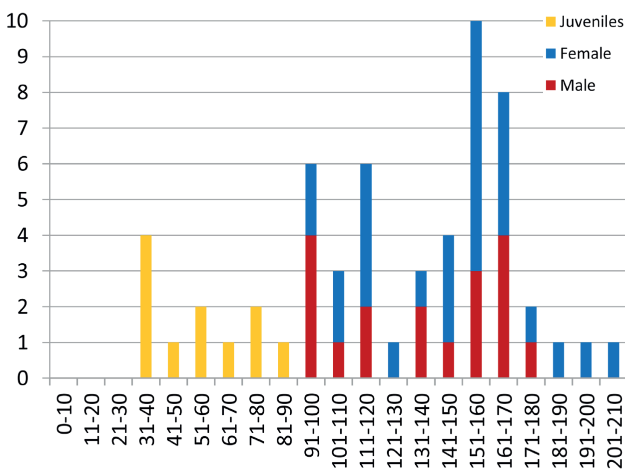

Figure 3. Sizes of *M. leprosa* based on carapace lengths at Oued El Abid.

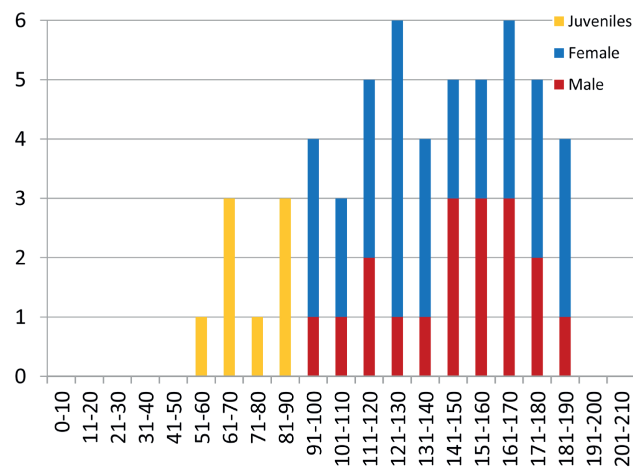

Figure 5. Sizes of *M. leprosa* based on carapace lengths at El Amaria.

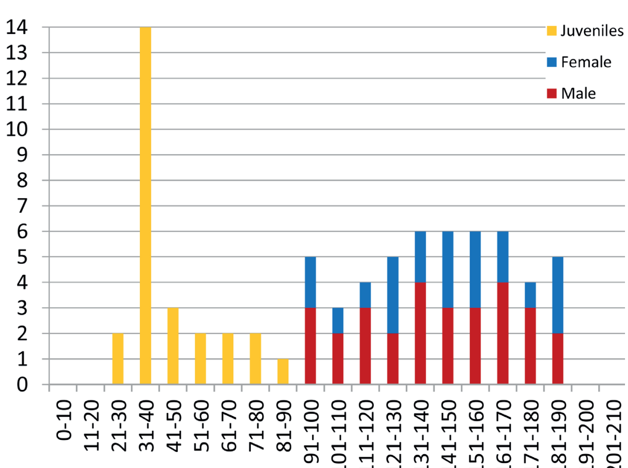

Figure 4. Sizes of *M. leprosa* based on carapace lengths at Beni Toun pond.

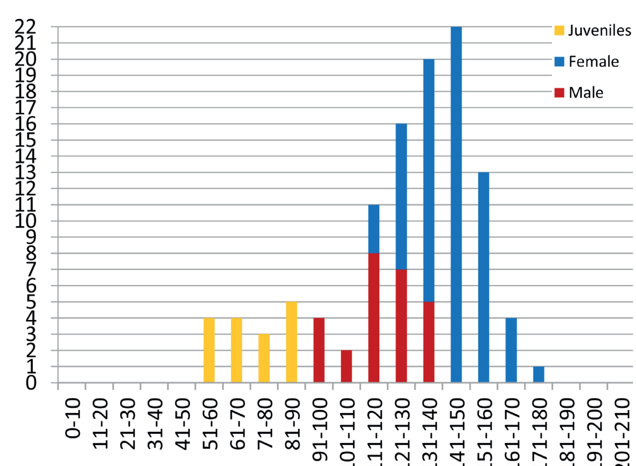

Figure 6. Sizes of *M. leprosa* based on carapace lengths at Oued El Akarit.

Vulnerability assessment of *E. orbicularis* in Tunisia

The European pond turtle is classified as 'near-threatened' (NT) in both the European and Mediterranean IUCN red lists of threatened species (Cox et al. 2006, 2009). However, its situation seems more problematic in Tunisia. Whereas the species was mentioned in the List of Rare and Endangered Wildlife in the Tunisian Forest Code of 2017–2018, no data on its ecological aspects existed and it was not afforded any protection measures in Tunisia. In North Africa, the distribution of *E. orbicularis* is fragmented (Velo-Antón et al. 2015). In Morocco, its distribution is limited to mountainous areas in the western Rif and the Middle Atlas, with isolated individuals having been recorded from the Gharb plain and the eastern Rif (Velo-Antón et al. 2015). In Algeria, populations have been confirmed for the wetland of El Kala in the extreme northeast of the country close to the Tunisian border (Tiar-Saadi et al. 2017; Stuckas et al. 2014). In Tunisia, this species was reported from 11 localities (Fig. 2 and Table 1) (Posner 1988, Blanc 1978, Loveridge & Williams 1957, Nouira 2001). The Oued El Abid population was deemed the most stable and its habitat was considered one of the last intact ones and probably the best for its protection (Nouira 2001). No further reports about the presence or absence of this species at these localities have been published since Nouira (2001), and despite several searching sessions in the field during this study, *E. orbicularis* was neither observed nor captured at any of the 11 historical localities where it was previously encountered. Therefore, it has become either extinct at these localities or is present only in very small numbers of individuals. A few individuals were collected in a small pond in Sajnen near El Hania as well as around the Sidi Barrak dam, though. Our observations suggest that *E. orbicularis* may be more sensitive than *M. leprosa* to environmental changes and its populations appear to be declining in Tunisia.

The genetic study we conducted on *E. orbicularis* revealed a single haplotype, IXa (Table 1), at the two localities where it was found, which confirms it as the undescribed Tunisian subspecies reported by Stuckas et al. (2014). This subspecies comprises three haplotypes, namely IXa, which is a common haplotype in Algeria and Tunisia, IXb, which is limited to northeastern Algeria, and IXc, which marks the turtles collected in northeastern Tunisia (Stuckas et al. 2014). Our unsuccessful search for *E. orbicularis* in the northeast of Tunisia during this study suggests this endemic subspecies, and especially the haplotype Ixc, to be endangered in this region. Protection and conservation of its habitat must therefore be given high priority to prevent its extinction.

While our study suggests that the population of the European pond turtle in Tunisia is declining with a status probably close to Endangered (EN), it does not facilitate the identification of the specific reasons for this situation. However, we noted that habitats from which *E. orbicularis* was previously recorded in the literature (loc. cit.) have since undergone significant changes as a result of the construction of dams, which can have negative impacts on freshwater biodiversity (Vörösmarty et al. 2010, Liermann et al. 2012). Our study is a first step towards the definition of longer-term conservation objectives and priorities and it creates a scientific basis for further ecological study.

Acknowledgements

The fieldwork of the present study was funded by a Rufford Small Grant for Nature Conservation. We thank S. Moslah, B. Ben Jemaa, Notre Grand Bleu Association, Tunisian Wildlife Association for assistance and companionship during fieldwork. We also thank the General Directorate of Forests in Tunisia for authorizing us to temporarily capture freshwater turtles.

References

Alarcos Izquierdo, G., J. Madrigal González, M. E. Ortíz-Santaliestra, M. J. Fernández-Beneitez, M. F. Flechoso del Cueto & M. Lizana Avia (2009): Caracterización de una población de *Mauremys leprosa* en un arroyo temporal en la provincia de Salamanca, al noroeste de la Península Ibérica. – Revista Española de Herpetología**, 23**: 129–140.

Barth, D., D. Bernhard, G. Fritzsch & U. Fritz (2004): The freshwater turtle genus *Mauremys* – a textbook example of an east-west disjunction or a taxonomic misconcept? – Zoologica Scripta, **33**: 213–221.

Bertolero, A. & S. D. Busack (2017): *Mauremys leprosa* (Schoepff in Schweigger 1812) – Mediterranean pond turtle, Spanish terrapin, Mediterranean stripe-necked terrapin. – pp. 1–19 in: Rhodin, A. G. J., J. B. Iverson, P. P. van Dijk, K. A. Buhlmann, P. C. H. Pritchard & R. A. Mittermeier (eds): Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group. Chelonian Research Monographs, **5**: 102.1–19.

Blanc, C. P. (1978): Notes sur les reptiles de Tunisie. III. Distribution et perspectives de protection des tortues terrestre et dulçaquicoles. – Archive Institut Pasteur de Tunis, **55** (1–2): 51–65.

Buhlmann, K. A., T. S. B. Akre, J. B. Iverson, D. Karapatakis, R. A. Mittermeier, A. Georges, A.G.J. Rhodin, P. P. Van dijk & J. W. Gibbons (2009): A global analysis of tortoise and freshwater turtle distributions with identification of priority conservation areas. – Chelonian Conservation and Giology, **8**: 116–149.

Bury, R. B. (1979): Population ecology of freshwater turtles. – pp. 571–602 in: Harless, M. & H. Morlock (eds): Turtles : perspectives and research. – Wiley Interscience.

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 and Conservation, **13**: 2511–2518

Cadi, A. & P. Joly (2003): Competition for basking places between the endangered European pond turtle (*Emys orbicularis galloitalica*) and the introduced red-eared turtle (*Trachemys scripta elegans*). – Canadian Journal of Zoology, **81**: 1392–1398.

Cagle, F. R. (1939): A system for marking turtles for future identification. – Copeia, **3**: 170–173.

CAR/ASP & PNUE/PAM (2014): Suivi de la nidification de la tortue marine *Caretta caretta* sur les îles Kuriat en Tunisie (2014). Par Imed JRIBI et Mohamed Nejmeddine BRADAI. Ed. CAR/ASP – Projet MedMPAnet, Tunis: 24 p.

Chessman, B. C. (2011): Declines of freshwater turtles associated with climatic drying in Australia. – Wildlife Research, **38**: 664.

Combescot, C. H. (1954): Sexualité et cycle génital de la tortue d'eau algérienne, *Emys leprosa* Schw. – Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord, **45**: 366–377.

Congdon, J. D., A. E. Dunham & R. C. Van Loben Sels (1993): Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. – Conservation Biology, **7**: 826–833.

Cox, N., J. Chanson & S. Stuartn (2006): The status and distribution of reptiles and amphibians of the Mediterranean basin. – IUCN, Gland, Switzerland and Cambridge, UK, v + 42 pp.

Cox, N. A. & H. J. Temple (2009): European red list of reptiles. – Office for Official Publications of the European Communities, Luxembourg.

Da Silva, E. (1995): Notes on clutch size and egg size of *Mauremys leprosa* from Spain. – Journal of Herpetology, **29**: 484–485.

EL Hassani, M. S., E. M. El Hassan, T. Slimani & X. Bonnet (2019): Morphological and physiological assessments reveal that freshwater turtle (*Mauremys leprosa*) can flourish under extremely degraded-polluted conditions. – Chemosphere, **220**: 432–441.

Fritz, U. (2003): Die Europäische Sumpfschildkröte.– Laurenti, Bielefeld, 224 pp.

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., 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., D. Guicking, H. Kami, M. Auer, D. Ayaz, A. G. Bakiev, A. Celani, A. C. Rivera, G. Džukic, S. Fahd, P. Havaš, U. Joger, V. F. Khabibullin, L. F. Mazanaeva, P. Široký, A. Tripepi & M. Wink (2007): Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*). – Amphibia-Reptilia, **28**: 418–426.

Fritz, U. & P. Havas (2007): Checklist of chelonians of the world. – Vertebrate Zoology, **57**: 149–368.

Fritz, U., D. Ayaz, A. K. Hundsdörfer, T. Kotenko, D. Guicking, M. Wink, C. V. Tok, K. Cicek & J. Buschbom (2009): 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. – Organisms Diversity and Evolution, **9**: 100–114.

Ernst, C. H. & J. E. Lovich (2009): Turtles of the United States and Canada, 2nd Edition. – Johns Hopkins University Press.

Gibbons, J. W. (1990): Turtle studies at SREL: a research perspective. pp 19–44 in: Gibbons J. W. (ed.): Life history and ecology of the slider turtle. – Smithsonian Institution Press.

Godfrey, M. H. & L. M. Campbell (2000): Turtle conservation. – Smithsonian Institution Press, 334.

Héritier, L., A. Valdeón, A. Sadaoui, T. Gendre, S. Ficheux, S. Bouamer, N. Kechemir-Issad, L. Du Preez, C. Palacios & O. Verneau (2017): Introduction and invasion of the red-eared slider and its parasites in freshwater ecosystems of Southern Europe: risk assessment for the European pond turtle in wild environments. – Biodiversity and Conservation, **26**: 1817–1843.

Héritier, L., A. L. Meistertzheim & O. Verneau (2017): Oxidative stress biomarkers in the Mediterranean pond turtle (*Mauremys leprosa*) reveal contrasted aquatic environments in southern France. – Chemosphere, **183**: 332–338.

Iverson, J. B. (1991): Patterns of survivorship in turtles (order Testudines). – Canadian Journal of Zoology, **69**: 385–391.

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

Klemens, M. W. (2000): Turtle conservation. – Smithsonian Institution press, pp. 1–4.

Kumar, S., G. Stecher, M. Li, C. Knyaz, & K. Tamura (2018): MEGA X: Molecular evolutionary genetics analysis across computing platforms. – Molecular Biology and Evolution, **35**: 1547–1549.

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

Lenk, P., U. Fritz, U. Joger & M. Wink (1999): Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). – Molecular Ecology, **8**: 1911–1922.

Liermann, C. R., C. Nilsson, J. Robertson & R. Y. Ng, (2012): Implications of dam obstruction for global freshwater fish diversity. – BioScience, **62**: 539–548.

Loulida, S., M. Naimi & M. Znari (2018): Stress environnemental et instabilité du développement de l'émyde lépreuse *Mauremys leprosa* (Schweigger, 1812) dans des environnements contrastés en termes de pollution dans le bassin du Tensift, Centre Ouest du Maroc. – Journal International Sciences et Technique de l'Eau et de l'Environnement, 1737–6688.

Loveridge, A. & E. E. Williams (1957): Revision of the african tortoises and turtles of the suborder Cryptodira. – Bulletin of the Museum of Comparative Zoology, **115**: 163–557.

Lovich, J. E., M. Znari, M. A. A. Baamrane, M. Naimi & A. Mostalih (2010): Biphasic geographic variation in sexual size dimorphism of turtle (*Mauremys leprosa*) populations along an environmental gradient in Morocco. – Chelonian Conservation and Biology, **9**: 45–53.

Mamou R., A. Boissinot, M. Bensidhoum, M. Amroun & F. Marniche (2014): Inventaire de l'herpétofaune du sud de la Kabylie (Bouira et Bordj Bou Arreridj). – Revue Ivoirienne Sciences et Technologie, **23**: 259–273.

McKnight, D. T. & D. B. Ligon (2017): Correcting for unequal catchability in sex ratio and population size estimates. – PLOS ONE, **12**: e0184101.

Meyer, L., L. Du Preez, E. Bonneau, L. Héritier, M. F. Quintana, A. Valdeón, A. Sadaoui, N. Kechemir-Issad, C. Palacios & O. Verneau (2015): Parasite host-switching from the invasive American red-eared slider, *Trachemys scripta elegans*, to the native Mediterranean pond turtle, *Mauremys leprosa*, in natural environments. – Aquatic Invasions **10**: 79–91.

Mishra, G. S. & J. P. Gonzalez (1978): Les parasites des tortues d'eau douce en Tunisie. – Archive de l'Institut Pasteur de Tunis, **55**: 303–326.

Mosimann, D. & A. Cadi (2004): On the occurrence and viability of the European pond turtle (*Emys orbicularis*) in the Moulin-de-Vert (Geneva, Switzerland): 50 years after first introduction. – Biologia, **14**: 109–112.

Naimi, M., M. Znari, J. E. Lovich, Y. Feddadi & M. Abdeljalil Ait Baamrane (2012): Clutch and egg allometry of the turtle *Mauremys leprosa* (Chelonia: Geoemydidae) from a polluted peri-urban river in west-central Morocco. – The Herpetological Journal, **22**: 43–49.

Nasri, H., S. El Herry & N. Bouaïcha (2008): First reported case of turtle deaths during a toxic *Microcystis* spp. bloom in lake Oubeira, Algeria. – Ecotoxicology and Environmental Safety, **71**: 535–544.

Nouira, S. (2001): Conservation des zones humides littorales et des écosystèmes côtiers: Cap Bon. Projet de conservation des zones humides littorales et des écosystèmes côtiers. – MedWestCost Publication.

Palacios, C., C. Urrutia, N. Knapp, M. F. Quintana, A. Bertolero, G. Simon, L. du Preez & O. Verneau (2015): Demographic structure and genetic diversity of *Mauremys leprosa* in its northern range reveal new populations and a mixed origin. – Salamandra, **51**: 221–230.

Pedall, I., H. Schäfer, U. Fritz & M. Wink (2009): Isolation of microsatellite markers in the *Emys orbicularis* complex and development of multiplex PCR amplification. – Conservation Genetics, **10**: 725–727.

Pearson, S. H., H. W. Avery & J. R. Spotila (2015): Juvenile invasive red-eared slider turtles negatively impact the growth of native turtles: Implications for global freshwater turtle populations. – Biological Conservation, **186**: 115–121.

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

Pleguezuelos J. M., R. Márquez & M. Lizana (2002): Atlas y libro rojo de los anfibios y reptiles de España. – Dirección General de Conservación de la Naturaleza-Asociación Herpetologica Española (2ª impresión), pp 587.

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

Polo-Cavia, N., P. Lopez & J. Martin (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., T. Engstrom, P. López & J. Martín (2010): Body condition does not predict immunocompetence of western pond turtles in altered versus natural habitats. – Animal Conservation. **13**: 256–264.

Posner, S. D. (1988): Biological diversity and tropical forests in Tunisia. – Agency of the International Development, Washington, DC, USA, p. 241.

Reese, D. A. (1986): Comparative demography and habitat use of western pond turtles in northern California: The effects of damming and related alterations. – PhD thesis, University of California, Berkeley.

Rhodin, A., G, J., J. B. Iverson, R. Bour, U. Fritz, A. Georges, H. B. Shaffer & P. P. van Dijk (2017): Turtles of the world: annotated checklist and Atlas of taxonomy, synonymy, distribution, and conservation status, 8th ed. Monograph 7 of Rhodin, A. G. J., J. B. Iverson, P. P. van Dijk, R. A. Saumure, K. A. Buhlmann, P. C. H. Pritchard & R. A. Mittermeier (eds): Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group. – Chelonian Research Monographs, **7**: 1–292.

Rhodin, A. G., C. B. Stanford, P. P. Van Dijk, C. Eisemberg, L. Luiselli, R. A. Mittermeier et al. (2018): Global conservation status of turtles and tortoises (order Testudines). – Chelonian Conservation and Biology, **17**: 135–161.

Rouault, J. & C. P. Blanc (1978): Notes on the reptiles of Tunisia. V. Biometric characteristics of *Mauremys caspica leprosa* (Schweigger, 1812) (Reptilia: Emydidae). – Archive de l'Institut Pasteur de Tunis, **55**: 337–357.

Rozet, C. A. (1833): Voyage Dans la Régence d'Alger, V1–2. Reprint (2010). – Kessinger Legacy Reprints, Kessenger Publishing, pp. 634.

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

Servan J., P. Zaborski, M. Dorizzi & C. Pieau (1989) : Détermination du sexe ratio adulte de la tortue *E. orbicularis* dans des populations provenant de 7 étangs de Brenne (Indre, France). – Canadian Journal of Zoology, **67**: 1279–1284.

Shaffer, H. B., M. Gidiş, E. McCartney-Melstad, K. M. Neal, H. M. Oyamaguchi, M. Tellez & E. M. Toffelmier (2015): Conservation genetics and genomics of amphibians and rep tiles. – Annual Review Animal Biosciences, **3**: 113–138.

Slimani, T., M. S. El Hassani, E. H. El Mouden, M. Bonnet, P. Bustamante, F. Brischoux, M. Brault-Favrou & X. Bonnet (2017): Large-scale geographic patterns of mercury contamination in Morocco revealed by freshwater turtles. – Environmental Science and Pollution Research, **25**: 2350–2360.

Stanford, C. B., A. G. J. Rhodin, P. P. van Dijk, B. D. Horne, T. Blanck, E. V. R. Goode, Hudson, R. A. Mittermeier, A. Currylow, C. Eisemberg, M. Frankel, A. Georges, P. M. Gibbons, J. O. Juvik, G. Kuchling, L. Luiselli, H. Shi, S. Singh & A. Walde (2018): Turtles in trouble: The world's 25+ most endangered tortoises and freshwater turtles. – Turtle Conservation Coalition, p. 79.

Stuckas, H., G. Velo-Anton, S. Fahd, M. Kalboussi, R. Rouag, M. Arculeo, F. Marrone, F. Sacco, M. Vamberger & U. Fritz (2014): Where are you from, stranger? The enigmatic biogeography of North African pond turtles (*Emys orbicularis*). – Organisms, Diversity & Evolution, **14**: 295–306.

Tiar-Saadi, M., G. Tiar, Z. Bouslama & P. Široký (2017): First data on the population of the European pond turtle *Emys orbicularis* at Lake Tonga, El Kala national park, Algeria. – Biologia, **72**: 819–824.

UICN France, MNHN & SHF (2015): La Liste rouge des espèces menacées en France. – Chapitre Reptiles et Amphibiens de France Métropolitaine. Paris, France.

Vamberger, M., H. Stuckas, F. Sacco, S. D'Angelo, M. Arculeo, M. Cheylan, C. Corti, M. Lo Valvo, F. Marrone, M .Wink & U. Fritz (2015): 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*). – Zoology Scripta, **44**: 233–249.

Van Dijk, P. P., J. A. M. Miras, M. Cheylan, U. Joger, P. Sasousa & V. Perez-Mellado (2004): IUCN (ed.): IUCN red list of threatened species. Version 2013. 2. – International Union for Conservation of Nature (IUCN), Gland, Switzerland.

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 & W. Fritz (2011a): 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.

VeloAntón, G., B. El Marnisi, U. Fritz & S. Fahd (2015): Distribution and conservation status of *Emys orbicularis* in Morocco. – Vertebrate Zoology, **65**: 131–134.

Venancio, L. P. R., M. I. A. Silva, T. L. da Silva, V. A. G. Moschetta, D. A. P. de Campos Zuccari, E. A. Almeida & C. R. Bonini-Domingos (2013): Pollution-induced metabolic responses in hypoxia-tolerant freshwater turtles. – Ecotoxicology and Environmental Safety, **97**: 1–9.

Veríssimo, J., M. Znari, H. Stuckas, U. Fritz, P. Pereira, J. Teixeira, M. Arculeo, F. Marrone, F. Sacco, M. Naimi, C. Kehlmaier & G. Velo-Antón (2016): Pleistocene diversification in Morocco and recent demographic expansion in the Mediterranean pond turtle *Mauremys leprosa*. – Biological Journal of the Linnean Society, **119**: 943–959.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. Reidy Liermann & P. M. Davies (2010): Global threats to human water security and river biodiversity. – Nature, **467**: 555–561.

Zuffi, M. A. L. (2000): Biology of the conservation of the European pond turtle *Emys orbicularis* (L.) of Italy. – Biologiezentrum Linz/Austria, **149**: 219–228.