Amphibia-Reptilia 30 (2009): 483-492

Relationships of *Podarcis* wall lizards from Algeria based on mtDNA data

Alexandra Lima[1,2]*, Catarina Pinho[1], Said Larbes[1,3], Miguel A. Carretero[1], José Carlos Brito[1], D. James Harris[1,2]

Abstract. Recent molecular studies indicate that *Podarcis* wall lizards occurring in the southern region of the Iberian Peninsula and in North Africa, from south Morocco to eastern Tunisia, constitute a monophyletic group composed of several highly differentiated forms that appear to be incipient species. However, Algerian populations, which are geographically intermediate, have not been investigated so far. In this study we determine the levels of genetic variability between Algerian populations and other North African populations, using a more extensive sampling scheme covering most of the distribution range in this area. Our results show that North African *Podarcis* present high genetic diversity, comprising at least five highly divergent lineages. Two of these lineages were only detected in Algeria, which harbours most of the genetic diversity found within *Podarcis* from North Africa.

Keywords: 12S RNA, Algeria, NADH4, North Africa, phylogeography, *Podarcis*.

Introduction

Podarcis wall lizards, the most common lacertid genus in Europe, have attracted the attention of researchers due to the high level of morphologic variability and cryptic genetic diversity, which are not always in agreement. Much of this variability was found within European peninsulas and Mediterranean islands where the main clades of this genus occur (Harris and Arnold, 1999; Oliverio, Bologna and Mariottini, 2000). Although most studies have been focused in Europe, an important part of the range of this genus, the North African region, has remained virtually unstudied until recently (Harris et al., 2002; Pinho, Harris and Ferrand, 2003, 2007b; Busack, Lawson and Arjo, 2005; Pinho, Ferrand and Harris, 2006). Due to its palaeogeographic complexity, this region is now attracting considerable interest for biogeographic studies on herpetofauna occurring on both sides of the Strait of Gibraltar, like *Podarcis* lizards. Such studies explore the possible vicariant role of this strait. *Podarcis* are found across Northwestern Africa, from south Morocco to Algeria and Tunisia, where its presence is highly dependent on humidity, Mediterranean vegetation and low temperatures (Kaliontzopoulou et al., 2008). However, the phylogeny and phylogeography of African *Podarcis* have not been studied in the same detail as their European counterparts and the scarce studies available are biased to Moroccan populations.

Podarcis vaucheri (Boulenger, 1905) (type locality: Tanger, North Morocco) a former member of the *P. hispanica* species complex, is now considered to occur in both Morocco and Southern Spain (i.e., Montori et al., 2005). The specific status of this form was first suggested based on mtDNA data (Oliverio, Bologna and Mariottini, 2000) and gained additional support from protein electrophoresis (Pinho, Harris and Ferrand, 2003, 2007a; Busack, Lawson and Arjo, 2005) and other mitochondrial markers (Pinho, Ferrand and Harris, 2006). The populations inhabiting Southern Spain are considered to be the result of an ancient transma-

---

1 - CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
2 - Departamento de Zoologia-Antropologia, Faculdade de Ciências da Universidade do Porto, 4099-002 Porto, Portugal
3 - Département de Biologie, Faculté des Sciences Biologiques et Agronomiques, Université M. Mammeri, Tizi-Ouzou, Algérie
*Corresponding author; e-mail: allima@gmail.com

rine colonization from Africa dated to 2.8 mya (Pinho, Ferrand and Harris, 2006). Within Morocco, *P. vaucheri* displays considerable levels of nucleotide and haplotype diversity, higher than other Iberian *Podarcis* studied, such as *P. bocagei* or *P. carbonelli* (Pinho, Harris and Ferrand, 2007b). Up to eight geographically consistent haplotype groups are associated with the main Moroccan mountain massifs and are hypothesized to have diverged as the result of the humid-dry cycles that occurred during the glaciations (Pinho, Harris and Ferrand, 2007b). Most of the biogeographic studies of North African reptiles focused on Morocco and have also found genetic sub-structuring in this region related to the Atlas and Rif mountain chains that separate coastal from inland clades. This is the case in the Ocellated lizard *Lacerta tangitana* (Paulo et al., 2008), the Spiny-footed lizards *Acanthodactylus erythrurus* (Harris, Batista and Carretero, 2004) and *Acanthodactylus pardalis* group (Fonseca et al., 2008) or the Spiny-tailed lizards *Uromastyx acanthinura* (Harris, Vasconcelos and Brito, 2007). In the Moorish gecko *Tarentola mauritanica* (Harris et al., 2004a, 2004b) and in the terrapin *Mauremys leprosa* (Fritz et al., 2005) there are distinct northern and southern clades, while north-west and south-east clades exist in the Agamid lizard *Agama impalearis* (Brown, Suárez and Pestano, 2002).

Initially, *P. vaucheri* was considered to be the only representative of the *Podarcis* genus occurring in North Africa (Oliverio, Bologna and Mariottini, 2000). However, given the cryptic genetic variability detected in other regions of the distribution of *Podarcis* (i.e., Poulakakis et al., 2003; Podnar, Mayer and Tvrtkovic, 2005), it would be reasonable to expect that in such a wide range as North Africa, with a rich palaeogeographic and palaeoclimatic history, these lacertids should also present higher variability. Indeed, two other forms were found: one in an isolated mountain in Southern Morocco, Jebel Sirwah, and another in Tunisia. Surprisingly, according to mtDNA, these two lineages are even more closely related to each other than to other populations of *P. vaucheri* occurring in Morocco (Harris et al., 2002; Pinho, Ferrand and Harris, 2006). Guillaume (1987) already reported morphologic differences between *Podarcis* populations inhabiting Tunisia and Morocco, distinguishing a “Tunisian form”. Protein electrophoretic data analysis confirmed that *P. vaucheri* from Morocco is highly differentiated from the form inhabiting Tunisia (Pinho, Ferrand and Harris, 2004; Pinho, Harris and Ferrand, 2007a). Moreover, the clustering of Jebel Sirwah with Tunisia is the only one, among Ibero-Maghrebian populations, supported by mtDNA, allozymes and nuclear genealogies (Pinho, Harris and Ferrand, 2008).

More recently, following the need to clarify the uniqueness of the lineages detected and the extent of their ranges, studies have increased the sampling schemes used to encompass a more complete view of the genetic diversity of North African reptile species. They have found important genetic diversity harboured by Eastern populations of some species, with an emergent pattern: the differentiation between Eastern and Western Maghreb, with the frequent clustering of Eastern Algerian with Tunisian samples. This is the case of *Natrix maura* in which the populations of Morocco are associated with those from central and western Algeria, while eastern Algerian populations cluster with Tunisian ones (Barata, Harris and Castilho, 2008). Exceptions also exist, for example, *Macroprotodon mauritanicus* occurring in Algeria separates from other samples from Tunisia and Spain (Carranza et al., 2004). In fact, the region comprising Algeria and Tunisia is considered a distinct “area of endemism” (de Jong, 1998).

Despite this eastern versus western Maghrebian phylogeographic pattern being widely recovered, it also might correspond to a partial view as populations from Algeria are often poorly sampled or completely lacking due to logistic difficulties of sampling there. When samples have been available, highly divergent

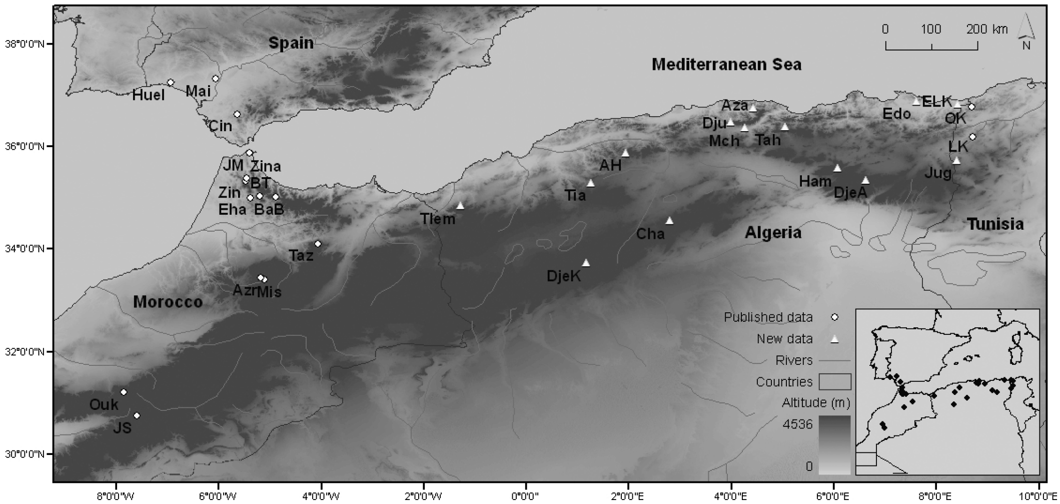

Figure 1. Map showing the sampled localities of *Podarcis* analysed in this study. Codes attributed correspond to those in table 1 and fig. 2.

lineages were often found to occur in Algeria, as in the case of *Tarentola mauritanica* (Harris et al., 2004a, 2004b) and *Psammophis schokari* (Rato et al., 2007). Taking this into account and the high cryptic variability found in *Podarcis*, a systematic sampling covering most the range of Maghrebian *Podarcis* is needed to detect the major genetic variability, establish the uniqueness of divergent lineages and their ranges. Presently, the extremes of North African *Podarcis* distribution that have been sampled, in south Morocco and Tunisia, have been shown to be genetically differentiated from *P. vaucheri*. However, the geographically intermediate Algerian forms have not been investigated so far. Sampling in Algeria is important to assess the western limits of the eastern lineages, and vice-versa, as for example, it is unknown if the populations inhabiting Algeria are representative of *P. vaucheri* or of different lineages (Pinho, Harris and Ferrand, 2007b).

In this study we determine the genetic variability between Algerian populations and compare it to other North African *Podarcis* populations. To accomplish our objective we used mtDNA markers, partial 12S rRNA and NADH dehydrogenase subunit 4 gene sequences, combining published and new data.

Methods

We performed an extensive sampling including 13 new localities from Algeria, some of which were only discovered after being predicted by recent Geographic Information Systems (GIS) models (Kaliontzopoulou et al., 2008). These models provided potential distribution maps with high degree of resolution and field work addressed to these localities confirmed the previously unsuspected presence of these lizards and allowed the collection of samples. The geographic locations and number of individuals sampled for this study are given in fig. 1 and table 1. Total genomic DNA was extracted from muscle tissue of a small piece of the tail of the individuals, according to standard protocols (Sambrook, Fritsch and Maniatis, 1989). We amplified and sequenced portions of two mitochondrial genes: 12S rRNA using primers 12Sa and 12Sb (Kocher et al., 1989), and NADH subunit 4 (NADH4) gene using the primer pair ND4 and Leu (Arévalo, Davis and Sites, 1994). The amplification conditions for 12S were carried in 25 μl volumes containing 2.5 μl of 10× reaction buffer, 3.0 mM of $MgCl_2$, 0.4 mM of each dNTP, 0.4 μM of each primer, 1 U of Ecotaq DNA polymerase and approximately 100 ng of genomic DNA. For NADH4, the conditions were similar except that we used: 3.2 mM of $MgCl_2$, 0.2 μM of each primer and approximately 50 ng of genomic DNA. The PCR reaction for 12S rRNA consisted of a pre-denaturing step of 3 min at 94°C followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 30 s, and a final extension step of 3 min at 72°C. In the case of NADH4 the conditions were similar but the annealing temperature was 54°C, the extension of 40 s and the final extension step was also longer (4 min). The amplified fragments were enzymatically purified and sequenced using the same primers. All previously published NADH4 and 12S sequences of *Podarcis* from North Africa and of *P. vaucheri* from the Iberian Peninsula that were available for

Table 1. List of sampled localities analysed in this study with corresponding sample and population (pop) codes and accession numbers of each individual sequences (new data signed with asterisks). Sequence data for most samples analysed from Morocco, Tunisia and Spain were previously published (Harris et al., 2002; Pinho, Ferrand and Harris, 2006, 2007b).

Pop code	Sample code	Country	Locality	Coordinates	GenBank acession numbers	
					12S	NADH4
Tah*	Tah1	Algeria	Tahament, 20 km NW Sétif	N36°22′ E05°03′	GQ856109	GQ856084
	Tah4				GQ856110	GQ856085
AH*	AH1	Algeria	Ain Harhar, 3 km SW Theniet el-Had	N35°52′ E01°56′	GQ856111	GQ856086
	AH3				GQ856112	GQ856087
Mch*	Mch1	Algeria	M'Chedallarh, 39 km E Bouira	N36°22′ E04°16′	GQ856113	GQ856088
	Mch2				GQ856114	GQ856089
Dju*	Dju939	Algeria	Djurjura, 30 km S Tizi-Ouzou	N36°28′ E03°59′	GQ856115	GQ856090
	Dju938				GQ856116	GQ856091
Tia*	Tia1	Algeria	10 km S Tiaret	N35°17′ E01°15′	GQ856117	GQ856092
	Tia2				GQ856118	GQ856093
Tlem*	Tlem15	Algeria	5 km S Tlemcen	N34°50′ W01°17′	GQ856119	GQ856094
	Tlem16				GQ856120	GQ856095
Cha*	Cha22	Algeria	Charef, 50 km W Djelfa	N34°33′ E02°47′	GQ856121	GQ856096
	Cha23				GQ856122	GQ856097
DjeK*	Djek17	Algeria	Djebel Ksel, 20 km NE El-Biadh	N33°43′ E01°10′	GQ856123	GQ856098
	Djek18				GQ856124	GQ856099
DjeA*	DjeA31	Algeria	Djebel Aurés, 46 km SW Khenchela	N35°21′ E06°37′	GQ856124	GQ856100
	DjeA34				GQ856126	GQ856101
Ham*	Ham1	Algeria	Hamla, 5 km NW Batna	N35°34′ E06°04′	GQ856127	GQ856102
	Ham2				GQ856128	GQ856103
Edo*	Edo33	Algeria	Edough, 25 km W Annaba	N36°52′ E07°37′	GQ856129	GQ856104
ELK*	Elk32	Algeria	El Kala, 87 km E Annaba	N36°50′ E08°24′	GQ856130	GQ856105
Aza*	Aza879	Algeria	Azazga, 37 km E Tizi-Ouzou	N36°45′ E04°25′	GQ856131	GQ856106
	Aza881				GQ856132	GQ856107
Jug*	Jug2	Tunisia	Yughurta Table	N35°43′ E08°24′	GQ856133	GQ856108
LK	LK6	Tunisia	Le Kef	N36°11′ E09°42′	DQ081071	DQ081177
OK	OK1	Tunisia	Oued Kebir	N36°46′ E08°41′	DQ081072	DQ081178
Huel	Elv1	Spain	Huelva	N37°15′ W06°57′	AY134397	DQ081176
Mai	E16084	Spain	Mairena del Aljarafe	N37°19′ W06°04′	AY134407	EF081076
Cin	Cin1	Spain	Guadalcacín	N36°38′ W05°39′	AY134402	EF081075
Taz	E29058	Morocco	Taza	N34°13′ W04°01′	AY134415	EF081082
Ouk	Ouk7	Morocco	Oukaïmeden	N31°12′ W07°51′	AY134401	DQ081172
Mis	MisD	Morocco	Mischleiffen	N33°31′ W05°05′	AY134710	EF081099
Azr	E31052	Morocco	Azrou	N33°25′ W05°13′	AY134737	EF081094
BaB	E29056	Morocco	Bab-Berred	N34°58′ W04°55′	AY134414	EF081110
	E29055				AY134413	EF081111
BT	BT6	Morocco	Bab Taza	N35°03′ W05°12′	AY134404	DQ081174
Zin	E29054	Morocco	15 km SW Zinat	N35°19′ W05°29′	AY134412	EF081112
	E29053				AY134411	EF081113
Zina	E29052	Morocco	8 km SW Zinat	N35°23′ W05°28′	AY134410	EF081115
JS	JS1	Morocco	Jebel Sirwah	N30°44′ W07°36′	AY134395	DQ081180
JM	E31051	Morocco	Jebel Musa	N35°52′ W05°24′	AY134424	EF081115
Eha	E290510	Morocco	El Had	N35°00′ W05°23′	AY134417	EF081114
Mot	Mot1	Spain	Motilla del Palancar	N39°34′ W01°53′	AY134400	DQ081168
And	And8	Spain	Puebla de D. Fadrique	N37°54′ W02°24′	DQ081069	DQ081169
Pod	Pod12	Spain	Granada	N37°11′ W03°36′	AF469427	DQ081170
MTA	MTA1	Spain	Tanes	N43°12′ W05°24′	DQ081074	DQ081182
Gua	Gua1	Spain	Guadarrama	N40°40′ W04°48′	DQ081073	DQ081181

the same individuals were included in the analysis. These included samples from Southern Spain, Morocco and Tunisia. We included five individuals as outgroups: two of *Podarcis muralis* and three of *Podarcis hispanica sensu stricto* following Pinho, Ferrand and Harris (2006). The sequences were aligned by eye using the program BioEdit version 5.0.9 (Hall, 1999). Sequence alignment was unambiguous as only the alignment of the 12S sequences required the inclusion of one insertion. The concatenated datasets were imported to PAUP* 4.0b10 (Swofford, 2002). All identical haplotypes were removed. The software ModelTest v.3.06 (Posada and Crandall, 1998) was used to choose the model of nucleotide substitution more appropriate for our dataset according to the Akaike Information Criterion (AIC). We used it to estimate a tree by Maximum Likelihood (ML) method (Felsenstein, 1981), heuristic searches performed with 10 replicates of random sequence addition and TBR branch swapping. Support values for each node were estimated by bootstrapping (Felsenstein, 1985) with 100 pseudoreplicates. The concatenated dataset was also analyzed by Bayesian methods using the software MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) running on the free webserver BioHPC (http://cbsuapps.tc.cornell.edu/mrbayes.aspx). These analyses were conducted using the same model of substitution previously selected. The analysis was run for 10^7 generations using four Markov chains, starting with random trees and sampled every 1000 generations. This generated an output of 10^4 trees. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenbeck and Bollback, 2001). The performance of the Markov chain Monte Carlo (MCMC) inference was evaluated by likelihood trace plots using Tracer software (Rambaut and Drummond, 2007) to ensure stationarity of the Markov chains and also by a more comprehensive convergence diagnostic analysis using the AWTY software (Wilgenbusch et al., 2004) which allows a graphical exploration of the MCMC convergence over replicated runs (Nylander et al., 2008). The stationarity was reached after approximately 5×10^5 generations, so the first 10^3 trees were discarded ("burn-in" of 10%). This data collected at stationarity, 9001 post-burnin trees of each run, were used to estimate posterior nodal probabilities and a summary phylogeny.

Results

The complete data set included mtDNA sequences from 48 individuals representing 35 sampled populations, including the outgroups. The concatenated dataset was 1010 bp long, 349 bp corresponding to 12S rRNA and 661 bp to NADH4. A total of 257 sites were variable, of which 221 were parsimony-informative. The new sequences analysed were deposited in GenBank with accession numbers GQ856084 to GQ856133. Most populations had unique haplotypes except three cases in which we found shared haplotypes between localities M'Chedallarh (Algeria) and Djurjura (Algeria), Jebel Musa (Morocco) and population Zina (8 km SW Zinat, Morocco), and between localities Huelva (Spain) and Mairena del Aljarafe (Spain).

According to the Akaike Information Criterion (AIC), the most appropriate model of evolution for our data was the general-time-reversible model with gamma-distributed rate of variation across sites ($\alpha = 0.8186$) and a proportion of invariable sites (0.5561; GTR + I + G model). The Maximum Likelihood analysis run under this model found two equally likely trees that had almost identical topologies. One of these trees ($\ln L = -3942.64$) was identical to the consensus topology recovered by the Bayesian analysis (fig. 2).

As previously found, the Iberian populations of *P. vaucheri* differ from those in Morocco, although they are grouped together with these, forming a cluster distinct from the remaining lineages. This major clade includes all the Moroccan populations, except the one from Jebel Sirwah, and also North-western Algerian populations. Among this later clade there were included not only the populations from the West Tellian Atlas that are well connected to Morocco but also those of the West Saharan Atlas that comprise of Mediterranean isolates surrounded by arid vegetation. A second clade is composed by divergent lineages. Namely, as expected, the Jebel Sirwah population constitutes a distinct lineage, apparently confined to this locality. Also in North-central Algeria, a new highly divergent lineage was detected in a single location, Azazga. The sampled populations which were geographically closest to this locality belong to the *P. vaucheri* clade. Another new lineage was detected in Eastern-central Algeria, in two different locations, apparently restricted to the Batna region, another Mediterranean isolate. This new distinct lineage forms a sub-clade that groups with the Eastern Algerian

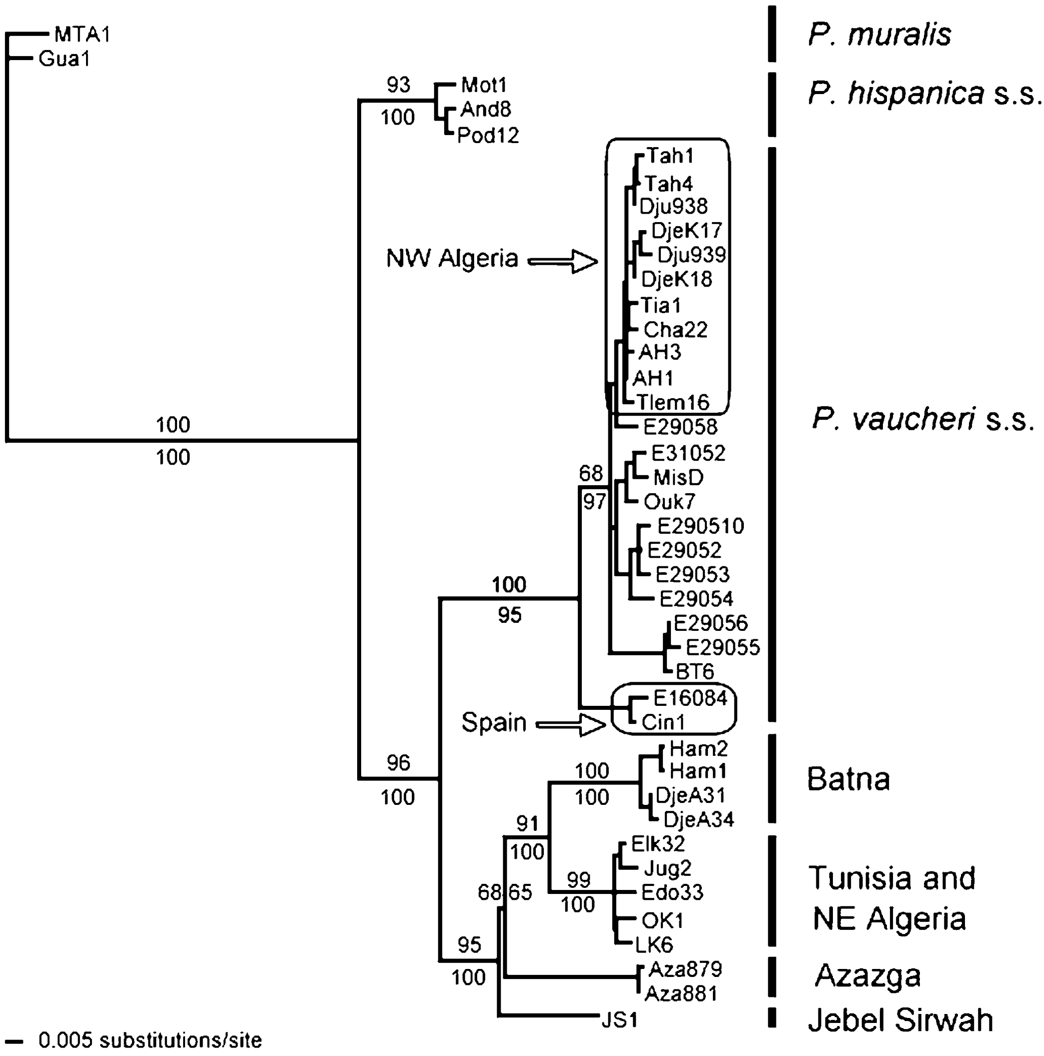

Figure 2. Maximum-likelihood phylogenetic tree of the concatenated dataset of partial 12S and NADH4 genes. The regions corresponding to each clade and current taxonomic divisions are indicated. Numbers above branches are bootstrap values and below are Bayesian posterior probabilities values.

and Tunisian populations, previously refered to as "Tunisian form". All of these lineages are highly genetically divergent from each other, as indicated by the uncorrected *p* distance values between these and *P. vaucheri sensu stricto* clade, which range from 0.073 of Jebel Sirwah to 0.089 of Azazga. These values are comparable to that of *P. hispanica sensu stricto* and *P. vaucheri* (0.085). The populations of *P. vaucheri* in Southern Spain are much closer related to their relatives occurring in Morocco, as the p distance is only 0.037. All these lineages are also well supported both by bootstraps and Bayesian posterior probabilities units (fig. 2).

Discussion

Previous studies had identified three distinct *Podarcis* lineages in North Africa but due to an important sampling gap and the high genetic diversity of the genus, it was expected to find more. Indeed, our extended sampling in Algeria has enabled us to identify two new lineages within

North African *Podarcis*, in addition to the three clades detected in previous studies (Harris et al., 2002; Busack, Lawson and Arjo, 2005; Pinho, Ferrand and Harris, 2006; Pinho, Harris and Ferrand, 2007a, 2008). One occurs in Azazga, in the North-Western region, being currently limited to this locality as the most geographically close sampled populations belong to the *P. vaucheri* clade, although no obvious geographical or ecological barriers seem to exist between them. The second new lineage is represented by two sampled populations from the Hamla and Aurés Massifs in the Batna region, forming a differentiated subclade related to the "Tunisian form" previously found. This "Tunisian form" is also present in coastal Eastern Algeria, while the form that seems to be most common in North Africa, *P. vaucheri sensu stricto*, is only found in Western Algeria.

Despite the discovery of these two new genetically distinct lineages, our results show that the relationships between the lineages previously found are unaffected by the increased sampling. *P. vaucheri* "type I" and "type II" *sensu* Busack, Lawson and Arjo (2005) are still recovered. The western Algerian populations form a sub-clade within *P. vaucheri sensu stricto* although not particularly divergent (uncorrected *p* distance value of 0.021) even for the two isolates from the Western Saharan Atlas.

The pattern found in this study, of divergent lineages between Eastern and Western Maghrebian populations, is a common trend in many herpetologic species groups, such as *Timon* (Paulo et al., 2008), *Acanthodactylus erythrurus* (Harris, Batista and Carretero, 2004), *Acanthodactylus pardalis* (Fonseca et al., 2008), *Trogonophis wiegmanni* (Mendonça and Harris, 2007) and *Malpolon* (Carranza, Arnold and Pleguezuelos, 2006).

However, until now few studies have found high complexity within herpetofauna inhabiting Algeria. An exception is the case of the genus *Pleurodeles*, as mtDNA and morphometric analyses found more diversity than previously considered (Carranza and Arnold, 2004). Two different clades belonging to the recently revalidated *P. nebulosus* are present in North-West and in Central North Algeria, this one ranging into Tunisia where a different clade also occurs. Moreover, a very divergent lineage restricted to the Edough Peninsula, currently identified as *P. poireti*, was found in North-East Algeria. These lineages form the sister clade to Moroccan and Iberian populations, known as *P. waltl*. It was suggested that *P. poireti* may have differentiated in the fossil island of Djebel Edough during the Messinian crisis, while permitted to diverge in the continent by Pleistocene climatic fluctuations (Carranza and Arnold, 2004; Carranza and Wade, 2004). Although the new *Podarcis* lineages were found in the neighbouring mountainous region of Kabylie and not in the Edough Massif, these results highlight the importance of extending sampling into Algeria. Nevertheless, none of the analysed species until now has shown geographic substructuring similar to North African *Podarcis*.

Divergence between lineages has often been associated to the climatic oscillations that occurred during the Pleistocene which alternately isolated and joined Mediterranean regions in North Africa (Prentice et al., 2000). This may well explain the divergence within the North African *Podarcis* West clade (Pinho, Harris and Ferrand, 2007b) although the divergence within the Eastern clade seems to be higher. Thus, populations in Western Saharan Atlas (as Djebel Ksel) seem to have become isolated very recently as a result of the last aridification episode. However, if one accepts the calibration point of 2.8 mya for the divergence between Morocco and the Iberian Peninsula (Pinho, Ferrand and Harris, 2006), then the split between the two main clades in North Africa and the separation of Jebel Sirwah, Batna and Tunisian forms is much older, that is, occurred during the Miocene. In the absence of plate separations in the region during this period, one possible event promoting vicariance in *Podarcis* may have been the progressive aridification during the Miocene (van Dam, 2006) as is suspected to have occurred in the Iberian Peninsula (Pinho, Ferrand and Harris, 2006).

The divergence between these new Algerian lineages is expected to be reflected to some degree in the patterns of morphologic variability displayed. In fact, a recent detailed morphologic study comparing two Algerian populations, one belonging to the *P. vaucheri sensu stricto* clade and another comprising one population of the Batna lineage here defined, found significant differences in biometric and pholidotic characters (Larbes, unpublished data). These populations inhabit different habitats, separated by 350 km, opposing a typical Mediterranean mountain massif, Djurdjura, to a continental mountain range with semi-arid to cold climate as is the case of Belezma, near Djebel Aurés. Although the significant morphologic differences found could be in part attributed to the influence of this climatic discrepancy acting as a promoting agent of intraspecific variability (Vanhooydonck and Van Damme, 1999), strong pholidotic differences frequently agree with phylogenetic divergence having taxonomic importance (Bruschi et al., 2006; Lymberakis et al., 2008) and in this case they also co-occur with phylogenetic divergence. This is an interesting result and it is expected that similar distinctions can be made between the other new lineages here reported. These questions are currently being studied by an ongoing wide biogeographic project on Algerian herpetofauna (Larbes, pers. comm.).

To conclude, at least five distinct *Podarcis* lineages exist in North Africa, two of which are reported here for the first time. All of them are present in Algeria, with the exception of the Moroccan "Jebel Sirwah form". The continuing estimation of related populations in Southern Morocco and Tunisia remains an interesting biogeographic phenomenon. A similar connection was also observed in the case of *Mauremys leprosa* populations from southern Morocco that were found to be closely related to Algerian and Tunisian ones (Fritz et al., 2005). But exceptions to this pattern have already been detected, such as the case of *Natrix maura* in which Jebel Sirwah populations do not diverge from other Moroccan populations (Barata, Harris and Castilho, 2008).

Even though the forms found are very divergent to the unique currently recognized *Podarcis* species in North Africa, *P. vaucheri*, more data should be analysed, especially nuclear markers and morphology, before any taxonomic recommendations can be made. However it seems likely that, as in the Iberian Peninsula, various incipient species exist within the *P. vaucheri* complex in North Africa.

Acknowledgements. This project received partial funding from projects PTDC/BIA-BDE/74349/2006 and POCTI/BIA/BDE/55865/2004 attributed to D.J. Harris and M.A. Carretero, respectively, by Fundação para a Ciência e Tecnologia (FCT), Portugal. J.C. Brito and D.J. Harris have contracts (Programme Ciência 2007) from FCT. M.A. Carretero and C. Pinho held post-doctoral contracts (SFRH/BPD/27025/2006 and SFRH/BPD/28869/2006) from FCT. During part of this work A. Lima received a grant from project POCTI/BIA/BDE/55865/2004 funded by FCT. Fieldwork was also supported by a grant to J.C. Brito from the National Geographic Society, number 7629-04. The authors recognize that this work could not be possible without the annually field trips to North Africa and so wish to thank to all the researchers and volunteers that helped during the field work. We are also very grateful to Rachid Rouag (Institut d'Agronomie at Centre Universitaire d'El Tarf) for sending us samples from two locations in Algeria.

References

Arévalo, E., Davis, S.K., Sites, J.W. (1994): Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst. Biol. **43**: 387-418.

Barata, M., Harris, D.J., Castilho, R. (2008): Comparative phylogeography of northwest African *Natrix maura* (Serpentes: Colubridae) inferred from mtDNA sequences. Afr. Zool. **43**: 1-7.

Brown, R.P., Suárez, N.M., Pestano, J. (2002): The Atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. Mol. Phylogenet. Evol. **24**: 324-332.

Bruschi, S., Corti, C., Carretero, M.A., Harris, D.J., Lanza, B., Leviton, A. (2006): Comments on the Status of the Sardinian-Corsican Lacertid Lizard *Podarcis tiliguerta*. Proceedings Calif. Acad. Sci. **57**: 215-236.

Busack, S.D., Lawson, R., Arjo, W.M. (2005): Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex. Amphibia-Reptilia **26**: 239-256.

Carranza, S., Arnold, E.N. (2004): History of West Mediterranean newts, *Pleurodeles* (Amphibia: Salamandridae), inferred from old and recent DNA sequences. System. Biodivers. **1**: 327-337.

Carranza, S., Wade, E. (2004): Taxonomic revision of Algero-Tunisian *Pleurodeles* (Caudata: Salamandridae) using molecular and morphological data. Revalidation of the taxon *Pleurodeles nebulosus* (Guichenot, 1850). Zootaxa **488**: 1-24.

Carranza, S., Arnold, E.N., Pleguezuelos, J.M. (2006): Phylogeny, biogeography, and evolution of two Mediterranean snakes, *Malpolon monspessulanus* and *Hemorrhois hippocrepis* (Squamata, Colubridae), using mtDNA sequences. Mol. Phylogenet. Evol. **40**: 532-546.

Carranza, S., Arnold, E.N., Wade, E., Fahd, S. (2004): Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from Northwest Africa. Mol. Phylogenet. Evol. **33**: 523-532.

de Jong, H. (1998): In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. Biol. J. Linn. Soc. Lond. **65**: 99-164.

Felsenstein, J. (1981): Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17**: 368-376.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

Fonseca, M.M., Brito, J.C., Rebelo, H., Kalboussi, M., Larbes, S., Carretero, M.A., Harris, D.J. (2008): Genetic variation among spiny-footed lizards in the *Acanthodactylus pardalis* group from North Africa. Afr. Zool. **43**: 8-15.

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

Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95-98.

Harris, D.J., Arnold, E.N. (1999): Relationships and evolution of wall lizards, *Podarcis* (Reptilia, Lacertidae) based on partial mitochondrial DNA sequences. Copeia **3**: 740-754.

Harris, D.J., Batista, V., Carretero, M.A. (2004): Assessment of genetic diversity within *Acanthodactylus erythrurus* (Reptilia: Lacertidae) in Morocco and the Iberian Peninsula using mitochondrial DNA sequence data. Amphibia-Reptilia **25**: 227-232.

Harris, D.J., Vasconcelos, R., Brito, J.C. (2007): Genetic variation within African spiny-tailed lizards (Agamidae: *Uromastyx*) estimated using mitochondrial DNA sequences. Amphibia-Reptilia **28**: 1-6.

Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. J. Biogeogr. **29**: 1257-1262.

Harris, D.J., Batista, V., Carretero, M.A., Ferrand, N. (2004a): Genetic variation in *Tarentola mauritanica* (Reptilia: Gekkonidae) across the Strait of Gibraltar derived from mitochondrial and nuclear DNA sequences. Amphibia-Reptilia **25**: 451-459.

Harris, D.J., Batista, V., Lymberakis, P., Carretero, M.A. (2004b): Complex estimates of evolutionary relationships in *Tarentola mauritanica* (Reptilia: Gekkonidae) derived from mitochondrial DNA sequences. Mol. Phylogenet. Evol. **30**: 855-859.

Huelsenbeck, J.P., Bollback, J.P. (2001): Empirical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. **50**: 351-366.

Huelsenbeck, J.P., Ronquist, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17**: 754-755.

Kaliontzopoulou, A., Brito, J.C., Carretero, M.A., Larbes, S., Harris, D.J. (2008): Modelling the partially unknown distribution of wall lizards *Podarcis* in North Africa: ecological affinities, potential areas of occurrence and methodological constraints. Can. J. Zool. **86**: 992-1001.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Vilablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. **86**: 6196-6200.

Lymberakis, P., Poulakakis, N., Kaliontzopoulou, A., Valakos, E., Mylonas, M. (2008): Two new species of *Podarcis* (Squamata; Lacertidae) from Greece. Syst. Biodivers. **6**: 307-318.

Mendonça, B., Harris, D.J. (2007): Genetic variation within *Trogonophis wiegmanni* Kaup 1830. Belg. J. Zool. **137**: 239-242.

Montori, A., Llorente, G.A., Alonso-Zarazaga, M.A., Arribas, O., Ayllón, E., Bosch, J., Carranza, S., Carretero, M.A., Galán, P., García-París, M., Harris, D.J., Lluch, J., Márquez, R., Mateo, J.A., Navarro, P., Ortiz, M., Pérez-Mellado, V., Pleguezuelos, J.M., Roca, V., Santos, X., Tejedo, M. (2005): Lista patrón actualizada de la herpetofauna española. Conclusiones de nomenclatura y taxonomía para las especies de anfibios y reptiles de España. Montori, A., Llorente, G.A., Eds, Barcelona, Asociación Herpetológica Española.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford. D.L. (2008): AWTY (are we there yet?): a system for graphical exploration of MCMC convergente in Bayesian phylogenetics. Bioinformatics **24**: 581-583.

Oliverio, M., Bologna, M.A., Mariottini, P. (2000): Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). J. Biogeogr. **27**: 1403-1420.

Paulo, O.S., Pinheiro, J., Miraldo, A., Bruford, M.W., Jordan, W.C., Nichols, R.A. (2008): The role of vicariance vs. dispersal in shaping genetic patterns in ocellated lizard species in the western Mediterranean. Mol. Ecol. **17**: 1535-1551.

Pinho, C., Harris, D.J., Ferrand, N. (2003): Genetic polymorphism of 11 allozyme loci in populations of wall lizards (*Podarcis* sp.) from the Iberian Peninsula and North Africa. Biochem. Genet. **41**: 343-359.

Pinho, C., Ferrand, N., Harris, D.J. (2004): Genetic variation within the *Podarcis hispanica* species complex – new evidence from protein electrophoretic data. In: The Biology of Lacertid Lizards. Evolutionary and Ecological Perspectives, Collecció Recerca, Vol. 8, p. 269-277. Pérez-Mellado, V., Riera, N., Perera, A., Eds, Menorca, Spain, Institut Menorquí d'Estudis.

Pinho, C., Ferrand, N., Harris, D.J. (2006): Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. Mol. Phylogenet. Evol. **38**: 266-273.

Pinho, C., Harris, D.J., Ferrand, N. (2007a): Comparing patterns of nuclear and mitochondrial divergence in a cryptic species complex: the case of Iberian and North African wall lizards (*Podarcis*, Lacertidae). Biol. J. Linn. Soc. Lond. **91**: 121-133.

Pinho, C., Harris, D.J., Ferrand, N. (2007b): Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation. Mol. Ecol. **16**: 1191-1205.

Pinho, C., Harris, D.J., Ferrand, N. (2008): Non-equilibrium estimates of gene flow inferred from nuclear genealogies suggest that Iberian and North African wall lizards (*Podarcis* spp.) are an assemblage of incipient species. BMC Evol. Biol. **8** (63).

Podnar, M., Mayer, W., Tvrtkovic, N. (2005): Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. Mol. Ecol. **14**: 575-588.

Posada, D., Crandall, K.A. (1998): Modeltest: testing the model of DNA substitution. Bioinformatics **14**: 817-818.

Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E.D. (2003): Molecular phylogeny and biogeography of the wall-lizard *Podarcis ehrardii* (Squamata, Lacertidae). Mol. Phylogenet. Evol. **28**: 38-46.

Prentice, I.C., Jolly, D., BIOME 6000 Participants (2000): Mid-Holocene and glacial-maximum vegetation geography of the northern continents and Africa. J. Biogeogr. **27**: 507-519.

Rato, C., Brito, J.C., Carretero, M.A., Larbes, S., Shacham, B., Harris, D.J. (2007): Phylogeography and genetic diversity of *Psammophis schokari* (Serpentes) in North Africa based on mitochondrial DNA sequences. Afr. Zool. **42**: 112-117.

Rambaut, A., Drummond, A.J. (2007): Tracer v.1.4. http://beast.bio.ed.ac.uk/Tracer.

Ronquist, F., Huelsenbeck, J.P. (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572-1574.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning: A Laboratory Manual. New York, Cold Spring Harbour Press.

Swofford, D.L. (2002): PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0.b10. Sunderland, MA, Sinauer Associates.

van Dam, J.A. (2006): Geographic and temporal patterns in the late Neogene (12-3 Ma) aridification of Europe: the use of small mammals as palaeoprecipitation proxies. Palaeogeogr. Palaeoclimatol. Palaeoecol. **238**: 190-218.

Vanhooydonck, B., Van Damme, R. (1999): Evolutionary relationships between body shape and habitat use in lacertid lizards. Evol. Ecol. Res. **1**: 785-805.

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L. (2004): AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available at http://ceb.csit.fsu.edu/awty.

Received: October 15, 2008. Accepted: April 4, 2009.