Complex patterns of genetic diversity within *Lacerta (Teira) perspicillata*: Preliminary evidence from 12S rRNA sequence data

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Lacerta (Teira) perspicillata (Duméril and Bibron, 1839) is a small montane rock-dwelling lizard, occurring in the western Maghreb (Morocco and Northwest Algeria) and in Menorca, where it has probably been introduced anthropogenically (Mayol, 1997). Intraspecific morphological variation is considerable. Some authors accept the existence of three subspecies, *L. p. perspicillata*, *L. p. pellegrini* and *L. p. chabanaudi* (Bons, 1968) but others consider them only morphotypes (Mayol, 1997). Although its phylogenetic relationships have been highly debated, mitochondrial sequence data suggest it is sister taxon to the Madeiran lizard *Lacerta (Teira) dugesii* (Harris et al., 1998; Oliverio et al., 2000). To investigate genetic diversity within *L. perspicillata* we sequenced part of the 12S rRNA gene from individuals from several populations including all three forms, and compared this to subspecific status.

Specimens collected in the field were identified to subspecies following Bons and Geniez (1996) (table 1). Some voucher specimens were taken (Deposited in the University of Salamanca), but in most cases individuals were released after tail tips were collected. Digital photographs were taken of all individuals, except LpcJ1. Total genomic DNA was extracted from small pieces of tail using standard methods, following Harris et al. (1998). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb from Kocher et al. (1989). Amplification conditions were the same as described by Harris et al. (1998). Amplified fragments were sequenced from both strands on a 310 Applied Biosystem DNA Sequencing Apparatus.

Sequences were aligned using Clustal W (Thompson et al., 1994). A previously published sequence from *Lacerta perspicillata* from Rabat was included (Fu, 2000). Sequences from *Lacerta dugesii*, *Lacerta andreanskyi* and two *Podarcis* were included as outgroups (Brehm et al., 2003; Fu, 2000; Harris and Sá-Sousa, 2002). Aligned sequences were 370 base pairs long. GenBank accession numbers are AY277602 to AY277617.

The data were imported into PAUP* 4.0b10 (Swofford, 2002) for phylogenetic analysis. For the phylogenetic analysis we used maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference. We used the approach outlined by Huelsenbeck and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b3a and Modeltest (Posada and Crandall, 1998) discussed in detail in Posada and Crandall (2001). Once a model of evolution was chosen, it was used to estimate a tree using ML. A MP analysis was carried out (100 replicate heuristic search, TBR branch-swapping) with gaps treated as missing data, and support for nodes estimated by bootstrapping with 1000 replicates (Felsenstein, 1985). The Bayesian analysis was implemented using MrBayes (Huelsenbeck and Ronquist, 2001) which calculates Bayesian posterior probabilities using a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. Bayesian analyses were

Table 1. Specimens included in the analysis with locality and specimen voucher number.

Species	Locality	Code	Reference
Podarcis carbonelli	Portugal		Harris and Sá-Sousa (2002)
Podarcis muralis	Spain		Fu (2000)
Lacerta andreanskyi	Oukaimeden		Fu (2000)
Lacerta dugesii	Porto Santo		Brehm et al. (2003)
	Desertas		Brehm et al. (2003)
	Madeira		Brehm et al. (2003)
	Selvagens		Brehm et al. (2003)
Lacerta perspicillata	Rabat		Fu (2000)
L. p. perspicillata	Debdou	LppD1	This study
	Debdou	LppD2	This study
	Debdou	LppD3	This study
	Menorca	LppM1	This study
	Menorca	LppM2	This study
	Menorca	LppM3	This study
	Menorca	LppM4	This study
	Menorca	LppM5	This study
L. p. pellegrini	Taza	LplT1	This study
	Taza	LplT2	This study
	Taza	LplT3	This study
	Taza	LplT4	This study
L. p. chabanaudi	Mischleiffen	LpcM1	This study
	Taza	LpcT1	This study
	Taza	LpcT2	This study
	Jebel Sirwah	LpcJ1	This study

conducted with random starting trees, run 0.5×10^6 generations, and sampled every 10 generations using a general-time-reversible model of evolution with a gamma model of among site rate variation. In both searches stationarity of the Markov Chain was determined as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value; "burn-in" data sampled from generations preceding this point were discarded. All data collected at stationarity were used to estimate posterior nodal probabilities and a summary phylogeny. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenback and Bollback, 2001).

Including the outgroups 24 sequences were analyzed. We concluded that the TrN model (base frequencies A 0.36, C 0.24, G 0.17, T 0.24, equal transition ratio, transversion ratio A/G 2.93, C/T 6.94), with a gamma distributed rate heterogeneity model (4 rate categories, G = 0.198) was the most appropriate model of evolution for these data (fig. 1). Maximum parsimony analysis found 6 trees of 149 steps, the strict consensus of which was identical to the ML analysis, but less well resolved (fig. 1). Fifty-nine characters were parsimony informative. The estimate of phylogeny obtained using Bayesian analyses was identical to the ML tree (fig. 1).

Our results suggest that *Lacerta dugesii* and *Lacerta perspicillata* are both monophyletic units, and that the two are sister taxa. Within *Lacerta perspicillata* two well supported (100% Bayesian posterior probability) groups were identified in all analyses. The first includes all individuals from Menorca and two from Taza, Morocco. All other sequences of

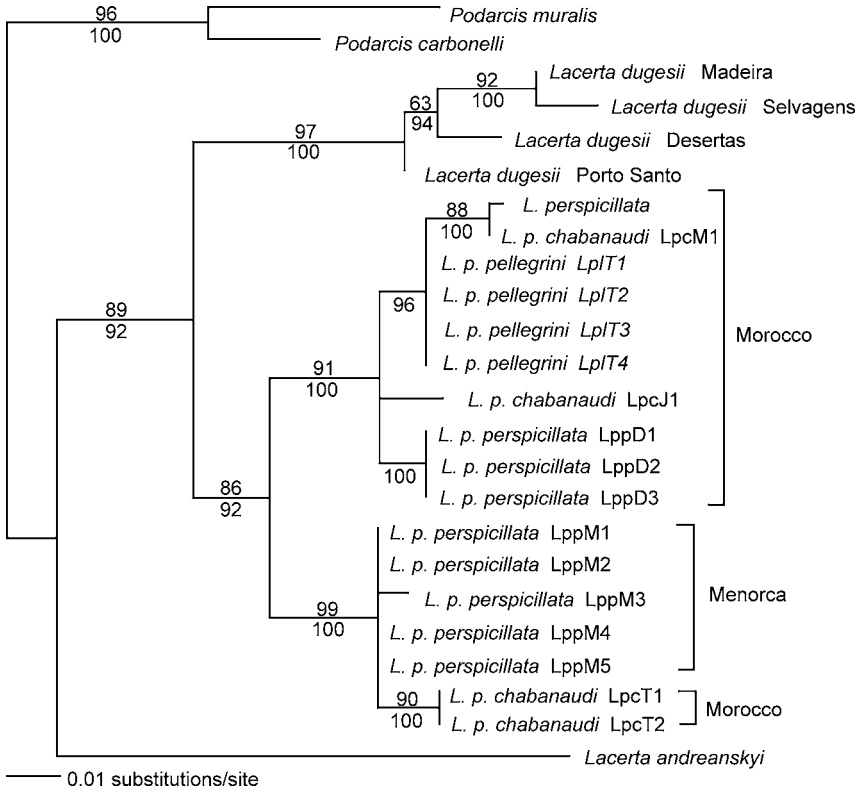

Figure 1. Tree derived from a ML analysis using the model described in the text. Bootstrap values for MP are given above nodes. A Bayesian analysis produced an identical estimate of relationships to that derived from ML. Posterior node probabilities from the Bayesian analysis are indicated below nodes.

Lacerta perspicillata form the second group, including sequences from other individuals from Taza. Uncorrected genetic divergence between these two groups is considerable, between 5.2 and 6.6%. This value compares to 5.2% between *Podarcis muralis* and *Podarcis carbonelli.* Such high genetic diversity, coupled with a lack of morphologically intermediate forms between subspecies (Bons and Geniez, 1996), and the fact that individuals from both groups were collected in strict syntopy (from the same tree) in Taza implies that the two groups probably represent distinct species.

All individuals were identified morphologically in the field to subspecific level, prior to genetic analysis. Some subspecific groupings, however, do not correspond to the clades identified genetically (fig. 1). In all localities other than Taza, all individuals appeared to belong to single subspecies. In Taza the striped subspecies (*L. p. pellegrini*) was found in strict sympatry with the dark, spotted subpecies, *L. p. chabanaudi* (fig. 2). *Lacerta p. chabanaudi* from Taza is genetically closely related to the individuals from Menorca, normally considered *L. p. perspicillata.* Moreover, other individuals identified as *L. p.*

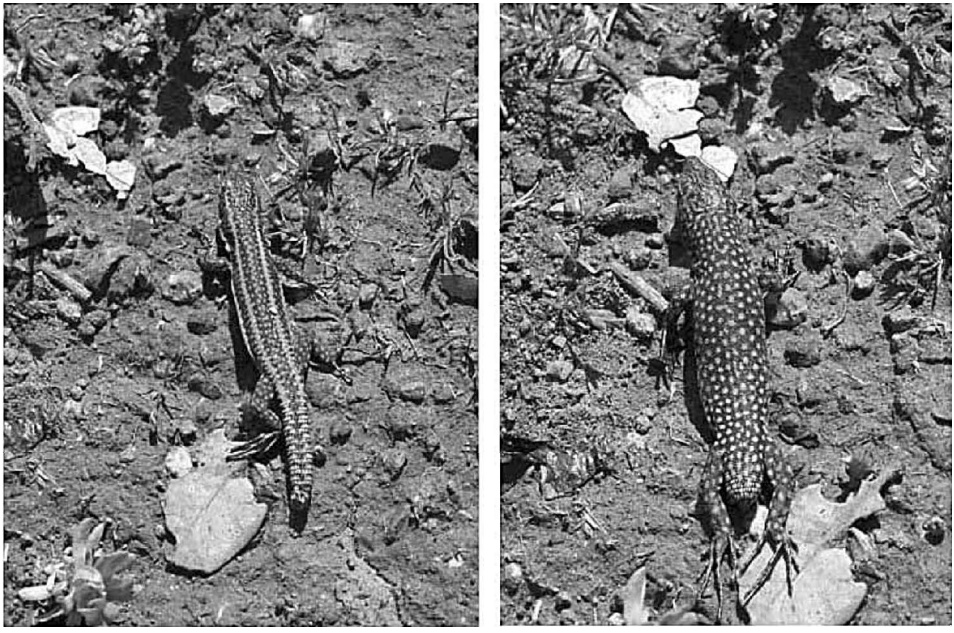

Figure 2. Photographs of two juveniles collected from the same tree in Taza, Morocco, which genetically belong to the two different clades.

chabanaudi are more closely related to *L. p. perspicillata* (individuals from Jebel Sirwah and Debdou), or to *L. p. pelegrini* (individuals from Rabat and Mischleiffen, and Taza).

These data indicate that *Lacerta perspicillata* is probably a species complex. The situation needs to be clarified, since individuals that appear morphologically to be *L. p. chabanaudi* and *L. p. perspicillata* can belong to either of the two genetically distinct groups identified. Extensive sampling, coupled with detailed morphological analyses are needed to clarify the situation further.

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Interspecific communal oviposition and reproduction of four species of lizards (Sauria: Gekkonidae) in the lower Florida Keys

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