Diversity of 12S mitochondrial DNA sequences in Iberian and North-west African water frogs across predicted geographic barriers

Taxonomy of Palearctic water frogs has been historically controversial. North African frogs have been considered as a distinct species, *Rana saharica* BOULENGER, 1913 which inhabits the entire Maghreb (e.g., GÜNTHER 1991), as synonymous with Iberian *R. perezi* SEOANE, 1885 (STEINWARZ & SCHNEIDER 1991), or synonymous with *R. perezi* only in the part of Morocco north of the Sahara (BENHACHEM 1988). Most authors now accept *R. perezi* to occur in the Iberian Peninsula, *R. saharica* in north-western Africa (e.g., ARANO et al. 1998), and the hypothesis that the separation of these taxa was due to the opening of the Strait of Gibraltar (BUSACK 1986). BONS & GENIEZ (1996) suggest that *R. saharica* might be a species complex, with different forms in northern and southern Morocco, but BUCKLEY et al. (1996) found little genetic differentiation among populations from Morocco. Based on allozyme and morphometric data ARANO et al. (1998) distinguished two subspecies of *R. saharica*, *R. s. saharica* from Algeria and *R. s. riodeoroi* from Morocco. They also suggest that the north-east Moroccan Moulouya river basin was the probable cause of discontinuity. Thus the major barriers to gene flow would be the Strait of Gibraltar and the Moulouya river basin. Both of these have been considered barriers for many other species of amphibians and reptiles (e.g., BUSACK 1986; ALVAREZ et al. 2000). However recent molecular studies suggest that the opening of the Strait of Gibraltar was not directly related to genetic divergences in the wall lizards of the *Podarcis hispanica* species complex (HARRIS et al. 2002), nor was the Moulouya river basin in *Testudo graeca* (LINNAEUS, 1758) (HARRIS et al. in press).

The aim of this study was to examine genetic diversity within part of the 12S rRNA mtDNA gene of water frogs across both predicted geographic barriers, and also from northern and southern populations in Morocco. This should give insight into if these are real barriers to gene flow, and if *R. saharica* has genetically distinct units that could imply it is a species complex.

Table 1: Sampling localities and codes as used in figure 1.

Species	Code	Locality
Rana perezi	RP1	Laroya, SE Spain
	RP2	Vilar Pouca de Aguiar, NW Portugal
	RP3	Vilar Pouca de Aguiar, NW Portugal
Rana saharica	RS1	Kenitra, Morocco
	RS2	Debdou, Morocco
	RS3	Taza, Morocco
	RS4	Ouarzazate, Morocco
	RS5	Ketama, Morocco
	RS6	Jebel Sirwah, Morocco
	RS7	Bou Ghanem, Tunisia
	RS8	Kesra, Tunisia

Genomic DNA was extracted following standard high-salt protocols. The 12S rRNA fragment was amplified by PCR using the primers published in KOCHER et al. (1989) and conditions described in HARRIS (2001). Sampling localities are given in table 1. The amplified products were sequenced on an automated sequencer (ABI 310 by Amersham Biosciences). New sequences were deposited on Genbank, accession numbers AY332762 - AY332766. Sequences were aligned including those previously published – two *R. saharica* from Tunis and El Fahs, Tunisia (PLÖTNER 1998), and three *R. perezi* (location not mentioned - MARMAYOU et al. 2000; Sierra de la Peña, Spain - PLÖTNER 1998). Three samples of *R. bedriagae* CAMERANO, 1882 (Ansari Mountains, Syria; Ceyhan, Turkey; Amman, Jordan - PLÖTNER et al. 2001) were included as outgroups. Alignment was facile, as only two single base pair insertions were needed. In total, twenty sequences of 346 base pairs were included.

Phylogenetic analysis was performed using PAUP* ver. 4.0b10 software package (SWOFFORD 2003). Using maximum parsimony of the 346 characters, 20 were informative. A 10 replicate heuristic search was performed, and node support estimated by bootstrapping (FELSENSTEIN 1985) with 1000 replicates (fig. 1). An uncorrected neighbour joining analysis gave the same estimate of relationships.

Our results support the genetic distinction between *R. perezi* and *R. saharica*

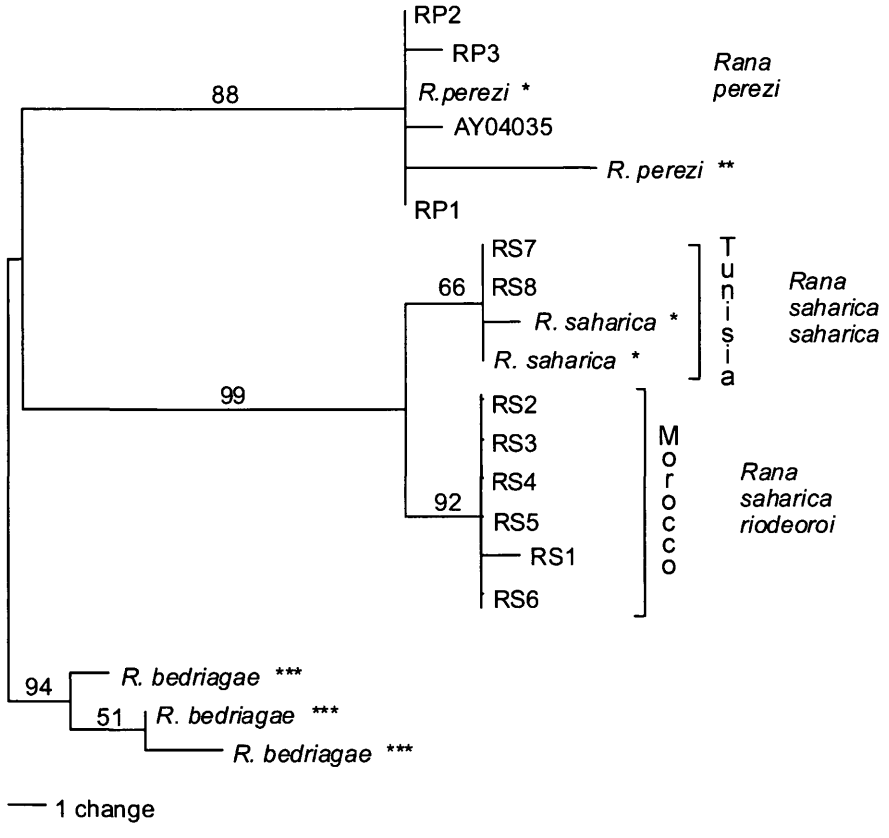

Fig. 1: Maximum parsimony 50% Bootstrap consensus tree of relationships between sampled taxa. Bootstrap values (1000 replicates) are given above nodes. Sequences from: * - PLÖTNER (1998), ** - MARMAYOU et al. (2000), *** - PLÖTNER et al. (2001), AY04035 - unpublished sequence from Genbank. For the localities associated with the sample codes see table 1.

as predicted by allozyme data (ARANO et al. 1998). They further show that, to a lesser extent, that there is a genetic differentiation between Moroccan and Tunisian samples of *R. saharica* that does not conflict with the subspecific differentiation proposed by ARANO et al. (1998). Within Morocco, however, there is almost no genetic differentiation between populations. This therefore does not support the hypothesis that *R. saharica* might be a species complex, and is thus similar to the data derived from protein electrophoretic analysis (BUCKLEY et al. 1996). The sample from Debdou, on the east side of the Moulouya river basin (RS2 in fig. 1) is identical to specimens from the west side using this piece of the mtDNA. Thus while there is a difference between Tunisian and Moroccan specimens, the present barrier between the two forms is not the Moulouya river basin. Extensive sampling across Algeria will be necessary to determine where the barrier does occur.

Within *R. perezi*, the sample from MARMAYOU et al. (2000) is quite distinct from the others. However, examination of the sequence shows that is has four unique mutations within the first nine base pairs of the sequence. This could be an indication that these are sequencing errors rather than true differences; similar errors have been reported in other amphibian sequences on Genbank (HARRIS 2001). Otherwise there

is little genetic differentiation within *R. perezi* across the Iberian Peninsula.

To conclude, our results support the views of ARANO et al. (1998) and PLÖTNER (1998) in separating *R. perezi* from *R. saharica*. Within *R. saharica* there are two distinct clades as suggested by ARANO et al. (1998), but the geographic limit of the two taxa is not the Moulouya river as they predicted. Our results give no indication that *R. saharica* is a species complex within Morocco.

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New records and natural history notes for *Pristidactylus nigroiugulus* CEI, SCOLARO & VIDELA, 2001 from Río Negro and Chubut provinces, Argentina

Lizards of the genus *Pristidactylus* are endemic to Argentina and Chile with ten species now recognized (CEI et al. 2001). Several of these species are poorly known, rare, and possibly endangered. Two such species, *P. casuhatiensis* (GALLARDO, 1968) and *P. volcanensis* LAMBOROT & DÍAZ, 1987, are restricted to small geographic areas of Argentina and Chile respectively, while others, such as *P. fasciatus* (D'ORBIGNY & BIBRON, 1837) and *P. torquatus* (PHILIPPI, 1861) have a large geographic range (LAMBOROT & DIAZ 1987; CEI 1986, 1993; AVILA et al. 2000). In recent years, geographically significant records were made for several species of *Pristidactylus* (AVILA 1994; ETHERIDGE & ESPINOZA 1997; CRUZ et al., 1999; AVILA et al. 2001) showing that the