

Elusive but widespread? The potential distribution and genetic variation of *Hyalosaurus koellikeri* (Günther, 1873) in the Maghreb

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Abstract. The genetic variability and the potential distribution under past (Last Glacial Maximum; LGM (MIROC and CCSM simulations)) and present conditions were studied for the anguid *Hyalosaurus koellikeri*, using analyses of two mitochondrial (ND1 and ND2) and one nuclear (PRLR) gene and species distribution modelling (SDM) including 19 geographical coordinates, covering most of its distribution range. Unexpectedly, the genetic results show that *H. koellikeri* presents a very low level of variability both in the mitochondrial and nuclear genes studied. The present predicted distribution of *H. koellikeri* revealed a large potential distribution in both north and eastwards directions, with suitable areas predicted in places where the species has never been reported before, as for instance the Rif Mountains in Morocco, as well as into most parts of northern Algeria and Tunisia. The LGM distribution is even larger compared to the present, with a continuous predicted distribution from Morocco to Tunisia, and even into Libya under the MIROC simulation. The results of the genetic and SDM analyses suggest that the now isolated populations from Debdou and Tlemcen have probably been in contact during the LGM, but its absence from both present and past predicted suitable areas is still a mystery. *Hyalosaurus koellikeri* depends mainly on closed deciduous forests (typically *Cedrus atlantica* and *Quercus* sp.) and open deciduous shrubland with high amounts of annual rainfall. The results of this study and the absence of recent sightings of the species outside the core distribution might indicate a regression of the species. Hence, a reevaluation of the conservation status of the species seems warranted.

Keywords: Anguidae, calibration region, *Hyalosaurus*, Maxent, mtDNA, Pleistocene glaciations, species distribution modelling.

Introduction

The species *Hyalosaurus koellikeri* (Günther, 1873) is the only known anguid living in Africa and was considered to be a Moroccan endemic (Bons and Geniez, 1996) un-

til it was reported for the first time from the Tlemcen region in western Algeria (Mateo, Geniez and Bons, 1998). Bons and Geniez (1996) already hypothesized the possible occurrence of *H. koellikeri* in Algeria based on the discovery of the species in the Debdou area (until then the easternmost locality in Morocco; Mellado and Mateo, 1992). However, old nineteenth century literature sources stated that *Anguis* and *Pseudopus* family Anguidae and *Ophiomorus* family Scincidae were present in Algier, Annaba (= Bône) and Sour el Ghazane (= Aumale) (Gervais, 1836; Strauch, 1861; Olivier, 1894) as hinted in Mateo, Geniez and Bons (1998). In Morocco, *H. koellikeri* is a relatively widespread yet difficult to find species, with a distribution that ranges from the Rabat-Casablanca region to the Middle Atlas region and extends southwards into the High Atlas Mountains (Bons and Geniez, 1996). Isolated populations exist in the Debdou area, Jbel Siroua, Jbel Tazekka, Azemmour

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and around the Souss Valley, although some of these records are old and unconfirmed (Bons and Geniez, 1996). Surprisingly, the species has never been reported from the Rif Mountains despite extensive herpetological exploration in this region (e.g. Real, Pleguezuelos and Fahd, 1997; Donaire and Bogaerts, 2003; Fahd et al., 2005). *Hyalosaurus koellikeri* is associated with semiarid and sub-humid areas with soft, well structured soil and vegetation cover, and is mostly found in *Cedrus atlantica* and *Quercus* sp. forests, where it leads a partially fossorial lifestyle (Bons and Geniez, 1996; Schleich, Kästle and Kabisch, 1996). The species is considered as Least Concern on the current IUCN Red List because of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a higher category (Geniez, 2006). The species was described as *Hyalosaurus koellikeri* by Günther (1873) based on one specimen found when looking, together with Professor Kölliker, at specimens in the notorious Mr. Jamrach's establishment, London, UK. However, the species was subsequently considered by other authors as a member of the genus *Ophisaurus* (Boulenger, 1885, and posterior authors) until Macey et al. (1999) showed that this genus was paraphyletic. As a result, Macey et al. (1999) proposed that all taxa in the Subfamily Anguinae should be considered as part of the genus *Anguis*. However, an alternative solution was also proposed by Macey et al. (1999) according to which the genus *Ophisaurus* should be restricted to the East Asian and North American species, and that the name *Hyalosaurus* would apply to the North African species (*H. koellikeri*), and *Pseudopus* to the European species (*P. apodus*). This taxonomic arrangement was adopted by subsequent authors (de Pous et al., 2010; Gvozdik et al., 2010; Pleguezuelos et al., 2010) and it is also used in the present work. According to Macey et al. (1999), the Subfamilies Gerrhonotinae and Anguinae split 50 mya as a result of the opening of the North Atlantic Ocean in the late Eocene.

Hyalosaurus koellikeri appears to be the sister taxon to the remaining members of the Anguinae, reinforcing the hypothesis of a western origin of this Subfamily with a posterior dispersal to East Asia and finally to North America across the Bering Land Bridge. According to the genetic distances and dating estimates by Macey et al. (1999), *H. koellikeri* diverged from its sister taxa (*Anguis* and *Pseudopus*) more than 10 mya, suggesting a historic presence of the species in North Africa.

Nowadays there is very little knowledge about the species' distribution, ecology and genetics and this paper therefore aims to (1) assess the intraspecific genetic variation, (2) evaluate the 'potential' distribution in the Maghreb under past (Last Glacial Maximum) and present conditions, and (3) identify the environmental niche of the species.

Materials and methods

Taxon sampling and phylogenetic analyses

A total of 10 specimens of *H. koellikeri* covering most of its distribution range were included in the molecular analyses. Sequences from one specimen of *Anguis c. colchica* and one *Pseudopus a. apodus* were downloaded from GenBank and used as outgroups. Specimen data and GenBank accession numbers of the gene fragments sequenced are given in table 1 and a map with all the localities of *H. koellikeri* included in the molecular study is shown in fig. 1. Two mitochondrial and one nuclear fragment were sequenced for most *H. koellikeri* samples. The two mitochondrial genes were ND1 (298 bp) and ND2 (506 bp). Specific primers were designed and used in both amplification and sequencing: ND1-Koel-F1 (5'-AGC ACC CTT TGA CCT AAC AGA G-3') and ND1-Koel-R1 (5'-GAG ATT GGG AAT GAG ATA TGT C-3') for the ND1 fragment and ND2-KO-FV2 (5'-CGA GCC ACA GAA GCC TCA AC-3') and ND2-KO-RV2 (5'-CAT GTT GTT GTT AGG TCT TGG GTA G-3') for the ND2 fragment. PCR conditions for amplification of the two fragments included a first step of 5' at 94°C, followed by 35 cycles of 30'' at 94°C, 45'' at 54°C (ND1) or 48°C (ND2) and 1' at 72°C. A final step of 10' at 72°C was also included. A fragment of 544 bp of the nuclear gene prolactin receptor (*PRLR*) was also sequenced for *H. koellikeri*. This marker was included in the present study because Townsend et al. (2008) suggested that it was the most variable one of their comparative study and, more importantly, it has been used successfully in a recent analysis of the closely related European genus *Anguis* (Gvozdik et al., 2010). Polymerase Chain Reaction (PCR) conditions and primers used for both amplification and

Table 1. Information on the 12 specimens used in the phylogenetic analyses and the 19 localities (indicated with and asterisk) of *Hyalosaurus koellikeri* included in the species distribution modelling (SDM). Localities that were taken from Bons and Geniez (1996) and Mateo, Geniez and Bons (1998) are indicated with a clover (♣).

Species	Specimen code or reference	Locality	Latitude (N)	Longitude (W)	Accession numbers		
					ND1	ND2	PRLR
<i>Hyalosaurus koellikeri</i>	hk1	Morocco	33°24'33	05°14'12	JN400663	JN400667	JN400671
	hk2	Forêt de Cèdres (Azrou)*	34°05'17	04°06'26	JN400663	JN400667	JN400671
	hk3	3.5 km East of Bab Bou Idir (Taza)*	32°34'26	06°02'00	JN400664	JN400668	JN400671
	hk4	El-Ksiba*	33°50'12	04°00'30	JN400663	JN400667	JN400671
	hk5	Tizi Oulmou (Bou Iblane)*	31°14'21	07°48'53	JN400665	JN400669	JN400671
	hk6	Oukaimeden*	33°54'07	03°10'29	JN400663	JN400667	JN400671
	hk7, hk8	Debdou*	33°34'26	05°12'52	JN400663	JN400667	JN400671
	hk9	Ifrane*	34°13'25	06°34'25	JN400666	JN400670	JN400671
	Macey et al., 1999	Fôret Mamora*	34°10'18	06°34'52	AF085621	AF085621	—
		10 km south of Kenitra	30°37'06	07°40'15			
<i>Anguis c. colchica</i> <i>Pseudopus a. apodus</i>		Jbel Siroua*♣	32°56'10	08°02'52			
		Daourat dam*♣	34°50'44	01°20'49			
		Just north of Tahdariat (Algeria)*♣	31°50'55	06°07'49			
		2 km south of Asloun*	31°38'14	06°28'30			
		Taheyda*	32°43'44	05°30'02			
		Just west of Aderass*	34°04'01	05°33'20			
		Fertassa*	34°15'54	06°25'31			
		About 15 km east of Kenitra*	32°01'25	06°43'18			—
		Ouzoud*	33°44'07	04°59'03			
		Imouzzet-Kandar*	33°39'20	07°07'55			
	Macey et al., 1999	Near Ben Slimane*♣	43°40'00	39°38'00	AF085622	AF085622	—
	Macey et al., 1999	Babukal, Krasnodarsky, Russia	43°21'00	46°06'00	AF085623	AF085623	—
		Tersko-Kumskaya Nizmennast,					
		Schelkovskaya, Chechenia					

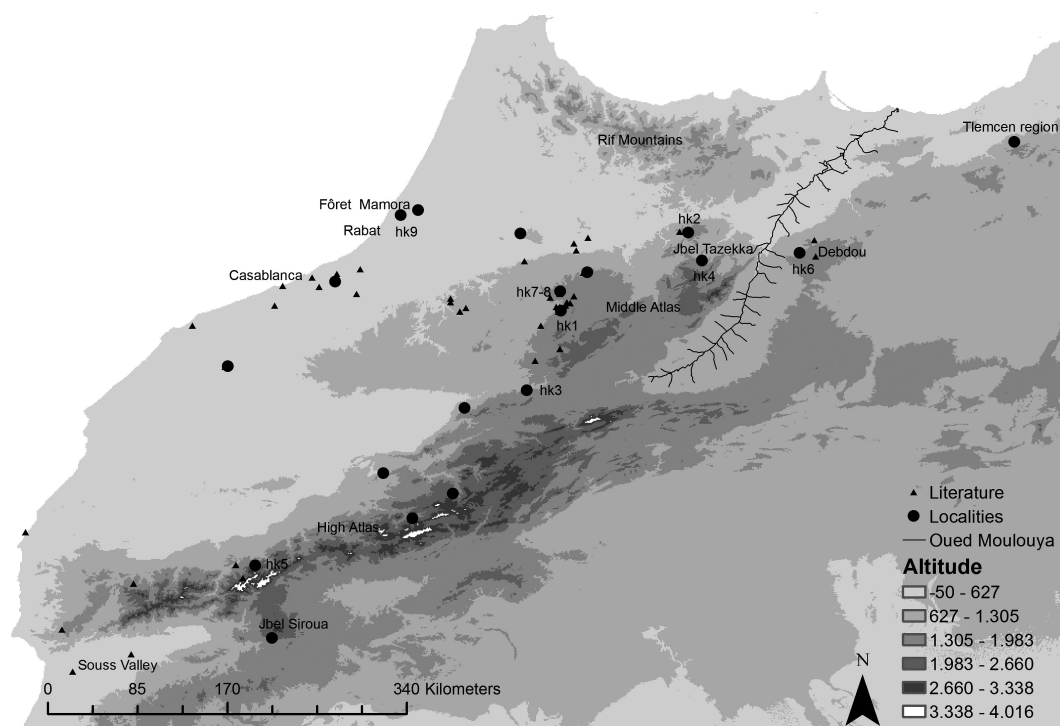


Figure 1. The study area in the Maghreb showing the 19 records of *Hyalosaurus koellikeri* with precise locality data used for the species distribution modelling (SDM). Specimens from some of these localities included in the molecular analyses are also indicated (hk1-9). The main locality names, all available literature records and the Moulouya river basin are indicated.

sequencing of the *PRLR* gene fragment were as in Gvozdik et al. (2010).

The mitochondrial DNA sequences were aligned using ClustalX (Thompson et al., 1997). Translation into amino acids using the vertebrate mitochondrial code yielded no stop codons, suggesting that the sequences were probably all functional. The phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI). JModeltest v. 0.1.1 (Posada, 2008) was used to select the most appropriate model of sequence evolution, under the Akaike information criterion (AIC, Akaike, 1974). The models selected were HKY + I for the ND1 partition, GTR + G for the ND2 partition and GTR + I for the concatenated dataset. Maximum Likelihood analysis of the latter was performed with PhyML v. 3.0 (Guindon and Gascuel, 2003) using the best-fit model GTR + I. Reliability of the ML trees was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications. MrBayes (Huelsenbeck and Ronquist, 2001) was used for BI analysis. The dataset was partitioned and the respective best-fitting models for each gene were applied (see above). Four incrementally heated Markov chains with the default heating values were used. Two independent runs were performed for 2.5×10^6 generations with samplings at intervals of 1000 generations producing 2500 trees. Tracer v. 1.4 (Rambaut and Drummond, 2007) was used to verify that stationarity had been reached and the first 625 trees were discarded before generating the majority rule consensus tree. Basic sequence statis-

tics and genetic distances were calculated with the program MEGA v. 3.0 (Kumar, Tamura and Nei, 2004). Topological incongruence among partitions was tested using a reciprocal 70% bootstrap proportion or a 95% posterior probabilities (pp) threshold (Mason-Gamer and Kellogg, 1996). Topological conflicts were considered significant if two different relationships for the same set of taxa were both supported with bootstrap values $\geq 70\%$ or pp values $\geq 95\%$. A median-joining haplotype network for the ND1 and ND2 concatenated gene fragments was constructed using the Fluxus Phylogenetic Network Analysis software v.4.6.0.0. (Bandelt et al., 1999; <http://www.fluxus-engineering.com>).

Species distribution modelling

A total of 19 geographical coordinates of *H. koellikeri* from Morocco and Algeria were used for species distribution modelling (SDM) (see fig. 1 and table 1). Most species records were collected in the field using GPS devices ($N = 15$) and the additional species records were derived from Bons and Geniez (1996) and Mateo, Geniez and Bons (1998) ($N = 4$). Only literature records and field data with precise locality information from 1980 onwards were used and a minimum distance of ten kilometers was ensured between geographical coordinates to account for spatial autocorrelation of the dataset because this has proven to falsely inflate the accuracy of SDM (Veloz, 2009). Initially, a total of 19 BioClim variables

were downloaded from the WorldClim database version 1.4 (<http://www.worldclim.org/>) to form the present and past (Last Glacial Maximum; LGM) climatic datasets (Hijmans et al., 2005) at a scale of 30 arc seconds (nearly 1×1 km) and 2.5 arc minutes (nearly 5×5 km), respectively. Two general atmospheric circulation models (GCM) were used to generate past climate scenarios for each period: the Community Climate System Model (CCSM, <http://www.cesm.ucar.edu>) and the Model for Interdisciplinary Research on Climate (MIROC, <http://www.ccsr.u-tokyo.ac.jp/~hasumi/MIROC/>). Colinearity among the initial 19 BioClim variables was measured with Pearson's correlation coefficient in SPSS 17 based on 1000 randomly sampled (ensuring a minimal distance) points from Morocco, because colinearity of predictor variables can hinder model interpretation. Nine bioclimatic variables, all of which had a correlation degree lower than 0.80 (Pearson coefficient), were retained. The Jackknife procedure was implemented in Maxent (Maximum entropy modelling) with the remaining nine bioclimatic variables to find the best set of predictor variables following a parsimony approach (Vega et al., 2010) based on the average (AUC) test of ten replicates. The final set of bioclimatic predictor variables for the SDMs consisted of Annual Precipitation (AP) and Mean Temperature of the Driest Quarter (MTDQ). Landcover data (Mayaux et al., 2004) were downloaded from the Global Environment Monitoring database (<http://bioval.jrc.ec.europa.eu>) to assess the potential present distribution of *H. koellikeri* in the Maghreb (18 landcover classes within the study region). Species' geographic distribution was modeled with Maxent v. 3.3.3e (Phillips, Anderson and Shapire, 2006) for the present (using climate variables only (1) and using climate variables with landcover data (2)) and the LGM (climate variables only (3)). It has been shown that Maxent produces high quality predictions that are often more successful when evaluated and compared with other predictive models (e.g. Hernandez et al., 2006; Jiménez-Valverde, Lobo and Hortal, 2008; Giovanelli et al., 2010). Additionally, Maxent has a successful prediction power even when using low sample sizes (Pearson et al., 2007; Wisz et al., 2008). This algorithm uses environmental parameters in combination with geographical coordinates in order to predict the distribution of the species of interest. Maximum entropy is achieved by the constraint that the expected value for each variable under the estimated distribution has to match its empirical average (the mean value of a random set of coordinates within the distribution; Phillips, Anderson and Shapire, 2006). In other words, this means that the model minimizes the relative entropy between two probability densities (one from the presence data and one from the landscape) defined in covariate space (Elith et al., 2011). The model output displays the relative occurrence probability of a species within the grid cells of the study area. Maxent was used with default settings while partitioning the geographical records between training and test samples (75% and 25%, respectively). This technique has been proven to achieve high predictive accuracy (Phillips and Dudík, 2008). Several studies have recently addressed the importance of selecting pseudo-absence or

background locations in SDM (Phillips et al., 2009; VanDerWal et al., 2009; Anderson and Raza, 2010; Giovanelli et al., 2010). Moreover, some of these studies reported that using very large areas for model calibration, especially if the species is absent from these areas, can result in serious ramifications for predictions and performance of SDMs (VanDerWal et al., 2009; Anderson and Raza, 2010; Giovanelli et al., 2010). We therefore followed the suggestion of VanDerWal et al. (2009) and used an exploratory analysis to define the most appropriate calibration region. Final models were calibrated in a study region that encompassed all known localities (fig. 1), as this resulted in more biologically meaningful models (unpublished results, available upon request) and was suggested by previous research (e.g. Phillips, 2008; Warren, Glor and Turelli, 2008; Anderson and Raza, 2010; Godsoe, 2010; Rödder and Lötters, 2010). Subsequently, models were projected onto a larger area.

Ensemble forecasting (e.g. Araújo and New, 2007) of ten models with randomly selected test samples was used to produce predictive distribution maps, which were plotted in logistic format. The "fade-by-clamping" option in Maxent was used to remove heavily clamped pixels from the final models. Furthermore, a comparison of the environmental variables used for projection to those used for training the model were made using visual interpretation of multivariate similarity surface (MESS) pictures and the most dissimilar variable (MoD) (Elith et al., 2010), because hindcasting SDMs into different time periods with possible non-analog climate is not only prone to error (Heikkinen et al., 2006; Williamson, 2006) but it is also ecologically and statistically invalid (Fitzpatrick and Hargrove, 2009; Nogues-Bravo, 2009). The final models were reclassified in ArcGIS 9.3.1 into binary presence-absence maps based on the average lowest presence thresholds (LPT), guaranteeing that all presences are predicted as suitable (Pearson et al., 2007). A visual analysis of all existing *H. koellikeri* records (Bons and Geniez, 1996) with the final predictive model was performed in order to assess the percentile overlay as a measure of model performance. Jackknife testing was used to produce estimates of the average contribution and response of each variable to the model. Thus, each variable was excluded in turn and a model with the remaining variables was created. Additionally, a model using each variable in isolation and a model using all variables were created. All models were tested with receiver operating characteristics (ROC) curve plots, which plot the true-positive rate against the false-positive rate. The average area under the curve (AUC) of the ROC plot of ten models was taken as a measure of the overall fit of each model. Due to the fact that Maxent operates only with presence records, the AUC is calculated using pseudo-absences chosen at random from the study area (Phillips, Anderson and Shapire, 2006). The AUC values range between 0.00 (highly unsuitable) and 1.00 (highly suitable) and display the probability that a randomly chosen presence site will be ranked above a randomly chosen absence site (Phillips, Anderson and Shapire, 2006). Models with AUC values above 0.75 are considered useful (Elith, 2002). Visual analyses of the response curves and graphs of major contributing variables (Austin, 1987) produced by Jackknife analyses were performed in order to assess how the probability of presence of *H. koellikeri* changes

with the range of variation within the predictor variables. Response curves were analyzed from models created using only the corresponding variable.

Results

Phylogenetic analyses

The independent analyses of the two mitochondrial DNA fragments indicated that there were

no topological conflicts (data not shown) and, as a result of that, the two genes (ND1 and ND2) were combined for further analyses. Of the 804 bp of *H. koellikeri* ND1 and ND2 concatenated gene fragments, 27 positions were variable (10 and 17, respectively) and 7 parsimony-informative (2 and 5, respectively). The results of the phylogenetic and network analyses are shown in fig. 2A, B, respectively and indicate

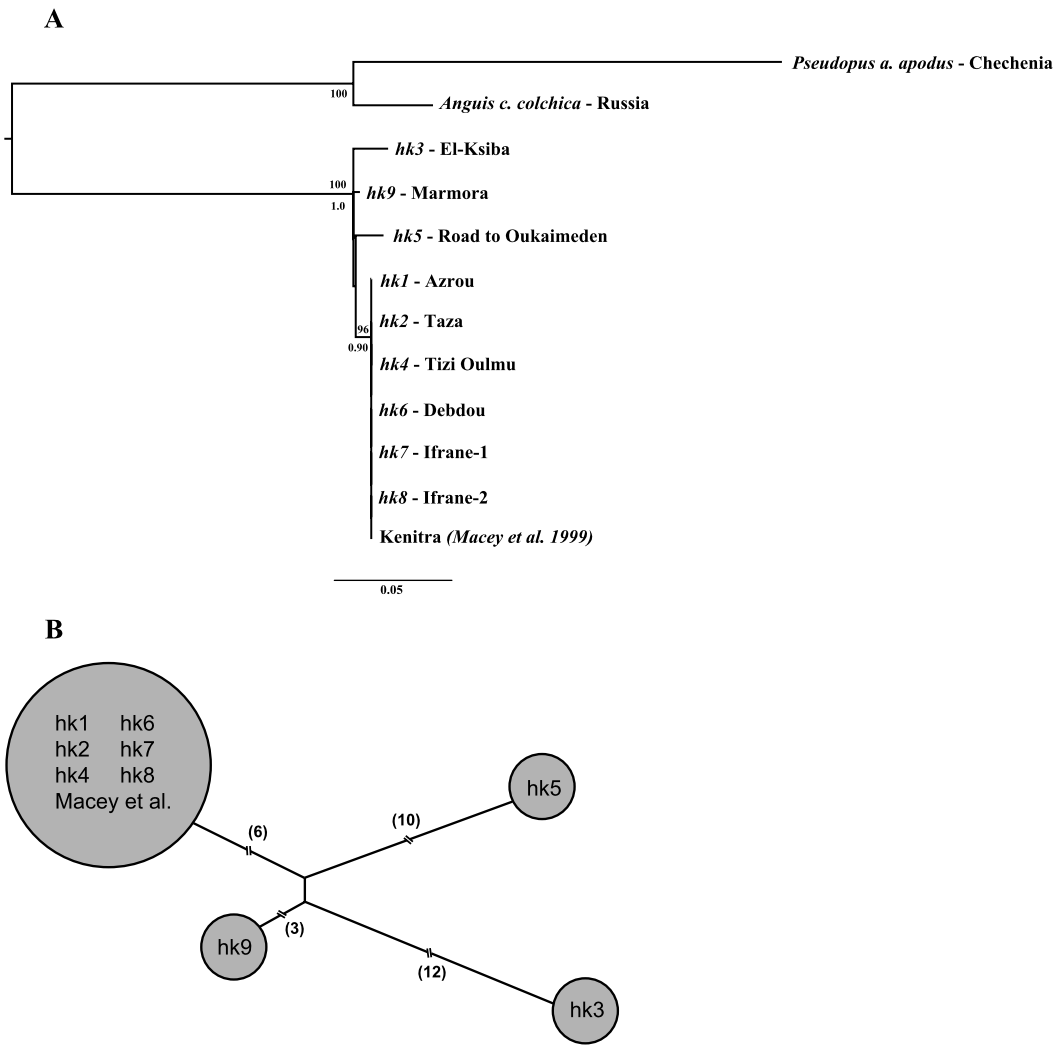


Figure 2. (A) ML tree of *Hyalosaurus koellikeri* inferred using the ND1 and ND2 mtDNA fragments. Numbers above the nodes indicate bootstrap support for the ML analysis and values below the nodes refer to the posterior probability values (pp) under the Bayesian Inference analysis. (B) Median-joining network inferred using a concatenated mtDNA fragment including the ND1 and ND2 gene regions. Grey circles represent different haplotypes and have been drawn proportional to the frequency of each haplotype. Number of mutations between haplotypes greater than one is indicated between brackets. Information on the samples included is shown in fig. 1 and table 1.

Table 2. Uncorrected *p*-distances based on gene fragment ND1 below the diagonal and ND2 above the diagonal.

Uncorrected <i>p</i> -distances (%)	hk1	hk2	hk3	hk4	hk5	hk6	hk7	hk8	hk9	Macey et al.
hk1 – Azrou		0.00	1.78	0.00	1.98	0.00	0.00	0.00	1.19	0.00
hk2 – Taza	0.00		1.78	0.00	1.98	0.00	0.00	0.00	1.19	0.00
hk3 – El-Ksiba	2.68	2.68		1.78	2.17	1.78	1.78	1.78	1.38	1.78
hk4 – Tizi Oulmu	0.00	0.00	2.68		1.98	0.00	0.00	0.00	1.19	0.00
hk5 – Road to Oukaïmeden	1.34	1.34	2.68	1.34		1.98	1.98	1.98	1.98	1.98
hk6 – Debdou	0.00	0.00	2.68	0.00	1.34		0.00	0.00	1.19	0.00
hk7 – Ifrane-1	0.00	0.00	2.68	0.00	1.34	0.00		0.00	1.19	0.00
hk8 – Ifrane-2	0.00	0.00	2.68	0.00	1.34	0.00	0.00		1.19	0.00
hk9 – Marmora	0.67	0.67	2.01	0.67	0.67	0.67	0.67	0.67		1.19
Macey et al., 1999	0.00	0.00	2.68	0.00	1.34	0.00	0.00	0.00	0.67	

that the species consists of four shallow lineages without clear phylogenetic relationships among them. The four haplotypes detected present a very low level of genetic differentiation in both mitochondrial gene fragments, ranging between 0–2.68% in the ND1 and between 0–2.17% in the ND2 (see table 2). In accordance with the low level of genetic variability detected in the mitochondrial genes, all eight sequences of the nuclear gene *PRLR* included in this study were identical and therefore a single haplotype was detected (table 1).

Species distribution modelling

Maximum entropy modelling produced high predictive accuracy models (following Swets, 1988), according to the average testing AUC for the present SDM (climate and landcover) (0.901 ± 0.084) and moderate accuracy for the present SDM (climate variables only) (0.802 ± 0.064) and past SDM (CCSM 0.830 ± 0.082 , MIROC 0.836 ± 0.068). The main predictors of *H. koellikeri* for the present when modelling with climate only are AP (82.7%) and MTDQ (17.3%), while for the SDM with climate and landcover the main predictor variables are AP (48.3%) and landcover (42.8%). Visual interpretation of the response curves (not showed) revealed that the probability of presence of the species increases with increasing rainfall and stabilized from around 600 mm (climate and landcover) and 1200 mm (climate variables only) of rainfall per year. The species has the highest probability of presence

in closed deciduous forests, open deciduous shrubland and croplands, in the specific order given. The SDM models for the present revealed overall similarity, but the SDM made using only climate variables showed less fragmentation in suitable areas and a larger predicted suitable area compared to the SDM that included landcover (fig. 3A, B). The present predicted distribution of *H. koellikeri* revealed, for both models, a large potential distribution in both north and eastward directions, with suitable areas predicted in the Rif Mountains in Morocco, as well as a discontinued distribution into most parts of northern Algeria and Tunisia. Small isolated areas with suitable conditions could also be identified in northern Libya (when including landcover data) and central northern areas in Algeria (fig. 3A, B). The populations from Tlemcen in Algeria and Debdou in Morocco show isolation from the rest of the known localities, which all fall in a continuous distribution of the species in Morocco. Overlay analyses of the final predictive model revealed a full coverage (100%) of all the existing distribution records (Bons and Geniez, 1996).

The LGM distribution shows an increase in comparison to the present predicted distributions with a continuous predicted distribution from Morocco to northern Algeria and Tunisia, and even into Libya under the MIROC simulation (fig. 3C). The two GCMs simulations differed substantially with CCSM predictions being much more restricted in the east-

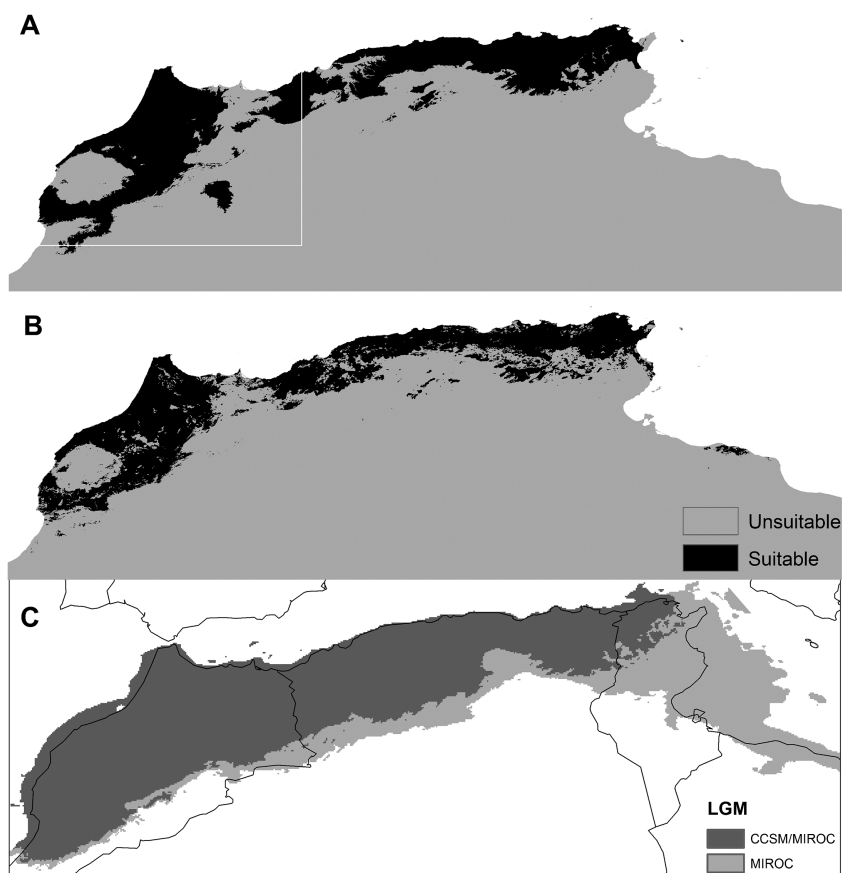


Figure 3. Predictive ‘potential’ distribution models of *Hyalosaurus koellikeri* for the present (using climate variables only) (A), present (using climate variables and landcover) (B) and Last Glacial Maximum (C) based on the LPT threshold. Models were calibrated in the rectangle defined in (A) and projected onto the larger area.

ern part of the Maghreb. Overlap between the two LGM simulations was high in many areas and all predicted areas of the CCSM simulation were also predicted by the MIROC simulation (fig. 3C). No clamping areas could be identified for both the CCSM and MIROC simulations, whereas the MESS and MoD pictures revealed that predictions in the Rif and Atlas Mountains (CCSM/MIROC), as well as large parts of the northern Algerian and Tunisian Mediterranean coast (MIROC) should be treated with caution (unpublished results, available from the authors upon request or from the following link <http://dl.dropbox.com/u/7860230/SuppFilesHyalosaurus.zip>).

Discussion

The calibrated models of *H. koellikeri* for the present potential distribution that included landcover data had a high predictive accuracy (AUC value) compared to the moderately accurate present climate only and past LGM models. The inclusion of landcover data in the present models resulted in the modelling software identifying the species as a ‘vegetation’ specialist compared to a ‘generalist’ with a broad environmental niche that was identified using climatic predictor variables only. Predictions of ecologically specialized species have been shown to be more accurate compared to those of generalist species that occupy a wide variety of habitats (e.g. Jimenez-Valverde et al., 2008;

Beukema et al., 2010; de Pous et al., 2010) and this can explain the substantial differences in model performance. The suitability of AUC values as indicators of model performance is the subject of ongoing debate (e.g. Lobo, Jimenez-Valverde and Real, 2008) but it is still widely used. Additionally, promising model improvement methods have only been developed very recently (e.g. Phillips and Elith, 2010; Warren and Seifert, 2011).

Uncertainties in projections of species distributions have been shown to arise from different sources: initial datasets, statistical methods (SDM) and general circulation models (GCM) (Buisson et al., 2010). VanDerWal et al. (2009) showed that an additional uncertainty in SDM could arise from defining an inappropriate region for model calibration and these findings have been supported by posterior studies (Anderson and Raza, 2010; Giovanelli et al., 2010). Although there is currently no framework or method to define the calibration area (but see Barve et al., 2011), using very large areas for SDM calibration could lead to inflated test statistics, larger predicted distributions, less informative response variables and overfitting of SDMs (see VanDerWal et al., 2009; Anderson and Raza, 2010; Giovanelli et al., 2010). Calibrating our models using a 'small' study region and projecting them onto a larger area resulted in a smaller potential distribution in the Maghreb and lower AUC values compared to calibrating the models using the entire projection region (fig. 3). Furthermore, predictor variable importance between the SDM made using a small and large calibration area differed substantially (unpublished results, available upon request). These findings are in agreement with previous studies (e.g. Giovanelli et al., 2009; VanDerWal et al., 2009) and hence, again, indicate the importance of defining an appropriate calibration region. Projections of species' distributions into other time periods should never be interpreted without assessing the effect of non-analog climate (Anderson and Raza, 2010). Moreover, predictions should not be attempted

at locations with non-analog climate data because no information is known about the species response under these different environments (Fitzpatrick and Hargrove, 2009). Our predictive models therefore excluded the non-analog climate areas using the 'fade-by-clamping' option in Maxent to produce a more conserved prediction of the species' distribution during the LGM, but this had no effect on the final predictive models because no clamped areas were identified. Moreover, the MESS and MoD pictures proved to be a very useful and promising tool in the identification of variables outside their training range. The low level of genetic variation in *H. koellikeri* is striking in contrast to its European sister group *Anguis*. As shown by Gvozdik et al. (2010), there are four clearly differentiated lineages in *Anguis* both at the mitochondrial (ND2) and nuclear (*c-mos* and *PRLR*) levels, which correspond to four different species. In fact, the genetic divergence at the ND2 level between the four species of European *Anguis* ranges between 5.9–9.2%, compared to the maximum of 2.17% in *H. koellikeri*. These results were unexpected, especially as many other Moroccan reptile species with higher vagility also show much higher levels of genetic variation that often represent new (cryptic) species or species complexes (e.g. Harris et al., 2003; Escoriza et al., 2006; Carranza et al., 2008; Fonseca et al., 2008, 2009; Pinho et al., 2008; Rato and Harris, 2008; Perera and Harris, 2010; Rato et al., 2010). The low genetic variation in both mtDNA and nDNA can be explained by a much broader climatic suitability during the LGM that allowed the connection among populations and subsequent homogenization as a consequence of gene flow. The increased potential distribution of *H. koellikeri* during the LGM is a result of wetter and cooler annual climatic conditions in North Africa (Rognon, 1987; Wengler and Vernet, 1992). Additionally, expanded forest cover (Lubell, 2001) and an increase of shrubby vegetation (Fletcher and Sánchez-Gómez, 2008) might have also facilitated the species' dispersal and

subsequent gene flow. Populations in Debdou and Tlemcen, that show isolation under current climate conditions, could migrate to the east and west and were not affected by the present unsuitable Moulouya river valley. Several an-guoid lizards sightings in Algeria have been mentioned in the literature (see introduction) and these sightings extend as far as Annaba in north-eastern Algeria, close to the Tunisian border. The definite presence of the species in Algeria (Mateo, Geniez and Bons, 1998) in the Tlemcen region is therefore probably not exceptional but corroborates a potential wider distribution in Algeria, with suitable conditions for the species also present in northern Tunisia and Libya. Century long herpetological explorations in Morocco have resulted in very few sightings of *H. koellikeri* (Bons and Geniez, 1996 compile 43 locations) and, as an example, the species was found only once by Harris et al. (2008) despite many research trips. The partly fossorial and cryptic lifestyle of the species, together with possible low population densities (de Pous, pers. observ. based on a six month daily basis exploration in Mamora forest and David Donaire pers. observ. based on 12 years of random explorations), and a lack of herpetological explorations in northern Algeria, due to the political instability of the country, might have prevented the species being detected in other suitable areas in the Maghreb. Another plausible scenario is that the species never dispersed further into Algeria because of possible dispersal limitations or competition with other species. This scenario might also explain the absence of the species in the Rif Mountains in Morocco, a region that shows suitable environmental conditions and vegetation but is presumably not occupied by the species. This absence from the Rif Mountains is intriguing and in concordance with several other species that show the same distribution pattern. As an example, *Scelarcis perspicillata* and *Bufo boulengeri* occur in the Middle Atlas and Tellian Atlas but are absent from the Rif Mountains despite suitable condi-

tions for these species (de Pous et al., 2010; but see Donaire et al., 2011).

de Pous et al. (2010) found that only 2.5% of the predicted distribution of *H. koellikeri* is covered by the existing protected area network. The results of this study and the absence of recent sightings of the species outside the core distribution (e.g. Souss Valley) might indicate a regression of the species in dryer habitats. Hence, reevaluating the conservation status of the species seems warranted. *Hyalosaurus koellikeri* depends mainly on closed deciduous forests (typically *Cedrus atlantica* and *Quercus* sp.) and open deciduous shrubland with high amounts of annual rainfall and these findings, therefore, highlight the importance of the conservation value of these habitat types in order to preserve biodiversity over time.

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