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SHORT NOTES

Genetic variation within *Trogonophis wiegmanni* Kaup 1830

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The fossorial Amphisbaenid *Trogonophis wiegmanni* is the only extant representative of the family Trogonophidae in North Africa, occurring in the North of the Maghreb, from southwest Morocco to northeast Tunisia. Its elongate limbless body, reduced vision, compact skull and enhanced hearing and olfactory capabilities are morphological adaptations for burrowing (1). It is not commonly observed due to its fossorial habits and thus the distribution remains poorly known. Most authors accept the existence of two subspecies: *T. w. wiegmanni* and *T. w. elegans* (2;3). Morphologically, the two subspecies are very similar except for their colouration patterns. *Trogonophis w. wiegmanni* presents a ground yellow colour and *T. w. elegans* a whitish, grey or pink one (3). Nevertheless, the yellow pigmentation of *T. w. wiegmanni* disappears after a very short period of time in alcohol, so the two forms in preserved specimens cannot be separated morphologically (3). *Trogonophis w. wiegmanni* is found in central and eastern Morocco and into western Tunisia (3). The western range limit is thought to be the oriental mountains of the Rif and Medium Atlas (2). It is rarely found above 900m (3). This subspecies occupies relatively dry regions, with its western range limit apparently coinciding with the 600mm isohyet (3). *Trogonophis w. elegans* is endemic to Morocco, found in the north and west of the Atlas mountain chains up to an altitude of 1600m (2;3). This form lives in relatively moist regions influenced by the temperate Atlantic climate with an eastern limit apparently coinciding with the 700mm isohyet (3). These apparently very different ecological demands of both forms suggest a considerable step towards speciation (3). In fact, in a recent checklist, GANS (4) considers them as two different species: *T. wiegmanni* and *T. elegans*. There are two principal mountain chains in Morocco, the Rif mountains in the north of the country and further south in the country the Atlas, Medium Atlas, High Atlas and Anti-Atlas mountains that extend in a northeast/southwest direction. The two forms are considered to be separated by the Atlas mountains and there is no evidence of hybridization (2). The Atlas mountain chain was formed towards the end of the Miocene and at this time the Rif formed an island, separated from continental Africa (5). The Miocene Atlas uplift may provide a general explanation of differentiation and speciation in many northwest African species (6). Such a hypothesis of vicariance has been suggested to explain the observed differentiation in various species such as *Acanthodactylus erythurus* (7) and *Agama impalearis* (6). Many reptile species in this area, that have been studied phylogeographically, revealed unexpected high levels of mitochondrial DNA (mtDNA) sequence variation. In *Lacerta perspicillata* 12s rRNA uncorrected distances detected were between 5.2 and 6.6% (8) and in *Tarentola mauritanica* the 16s rRNA uncorrected genetic distances between subspecies reached 8%, and 5% between the north and south Morocco populations (9;10). In all studies the authors suggest that some of the genetic lineages identified were probably distinct species. In *Acanthodactylus erythrurus,* genetic distances between all populations range up to 3.1% within 12s rRNA, which was considered as substantial intraspecific variation (11). In *Agama impalearis*, the Agamid lizard, the 16s rRNA maximum uncorrected intraspecific divergence was 2.6% (6) which the authors considered that, combined with other factors, supported the recognition of two species. Thus there is a real need to assess other species in this region for cryptic genetic variation.

Phylogeographic studies of amphisbaenians are almost non-existent, however, in a recent study in another amphisbaenian, *Rhineura floridana,* very high genetic distances were observed between the two populations studied (9.27%) and also within one of the populations (7.34%) suggesting that the taxonomy should be reviewed (12).

The aim of this study was to assess the levels of genetic variation within *Trogonophis wiegmanni* using mtDNA sequence data, and determine if the proposed subspecies are monophyletic. We expect also to contribute towards the clarification of the recent alternative taxonomical hypotheses. By comparing this species with other recent phylogenetic studies of reptiles in North Africa, a biogeographic pattern of genetic variation across various species could be overlaid against predicted geological barriers to gene flow.

The number and geographic locations of the specimens used in this study are given in Table 1 and Fig. 1. Specimens collected in the field were identified to subspecies following BONS & GENIEZ (2). Digital photographs were taken, and then individuals were released after tail tips were collected. Total genomic DNA was extracted from tissue samples following the SAMBROOK et al. (13) protocol. Two mitochondrial gene regions were amplified, sequenced and analyzed: fragments of the 12s rRNA gene (387bp) and 16s rRNA gene (486bp). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb and 16SL and 16SH from KOCHER et al. (14). Amplification conditions were the same as described by HARRIS et al. (15). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus. Mitochondrial DNA sequences

were aligned by eye. Aligned sequences of the combined partial gene regions were 873 base pairs long. The data were imported into PAUP* 4.0b10 (16) for phylogenetic analysis. For the analysis of the combined data maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference were used. The approach outlined by HUELSENBECK & CRANDALL (17) was used to test 56 alternative models of evolution, employing PAUP* 4.0b10 and Modeltest (18). Once a model of evolution was chosen under the Akaike Information Criterion, following BUCKLEY & POSADA (19), it was used to estimate a tree using ML (20) with random sequence addition (100 replicates, TBR branch-swapping) and support for nodes estimated by bootstrapping with 100 replicates (21). 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 100 replicates (21). The Bayesian analysis was implemented using MrBayes version 2.01 (22) which calculates Bayesian posterior probabilities using a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. Bayesian analyses were conducted with random starting trees, run 0.5×10^6 generations, and sampled every 100 generations using the general-time reversible model of evolution with a model of among site rate variation. Two independent replicates were conducted and inspected for consistency to check for local optima (23).

Following BONS & GENIEZ (2) *T. w. wiegmanni* has a discontinuous distribution in Morocco; in the Debdou plateau north to the Mediterranean, and in the Middle Atlas mountains. Our new record of two specimens from the Moulouya river basin (Tr62, Tr72) largely fills the gap between these areas, indicating the distribution is probably continuous.

TABLE 1

Sample code and locality of specimens used for this study

Species	Locality	Code
Trogonophis wiegmanni elegans	Morocco- Oulad Brahim	Tr 2
Trogonophis wiegmanni elegans	Morocco - Oulad Brahim	Tr 3
Trogonophis wiegmanni elegans	Morocco - Oulad Brahim	Tr 4
Trogonophis wiegmanni elegans	Morocco- Asilah	Tr 5
Trogonophis wiegmanni elegans	Morocco- Moulay Idriss	Tr 6
Trogonophis wiegmanni wiegmanni	Morocco- Ain Beni Mathar	Tr 62
Trogonophis wiegmanni wiegmanni	Morocco- Tirnest	Tr 72
Trogonophis wiegmanni elegans	Morocco- Al jadida	Tr 1R
Trogonophis wiegmanni wiegmanni	Tunisia- El Kef	Tr 2R
Trogonophis wiegmanni wiegmanni	Morocco- Moulouya river mouth	Tr 139R
Trogonophis wiegmanni wiegmanni	Morocco- Berkane Oujda	Tr 761
Trogonophis wiegmanni wiegmanni	Morocco- Berkane Oujda	Tr768

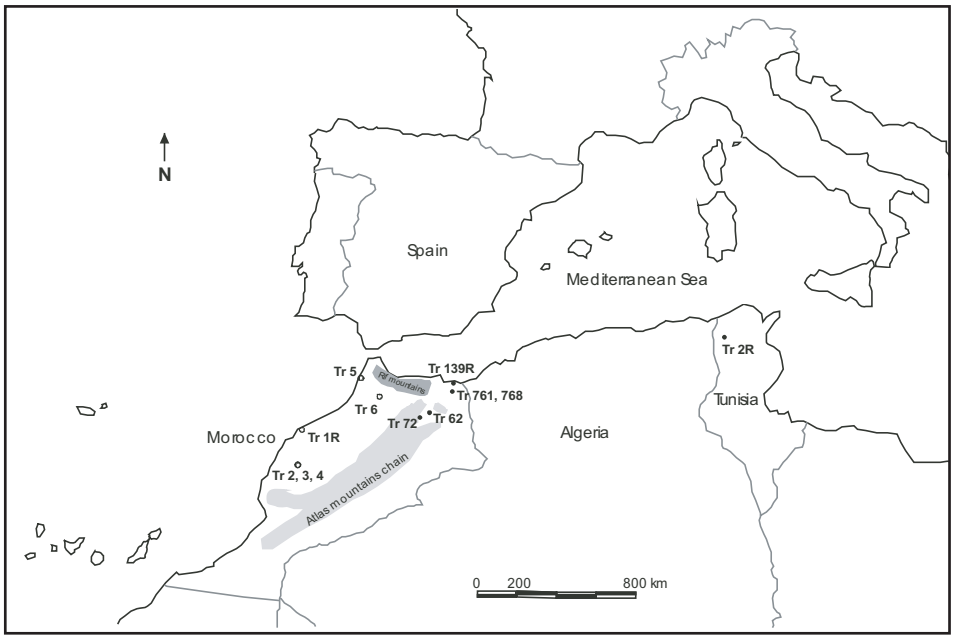

Fig. 1. – Map showing the sampling locations of *T. wiegmanni* sequenced for this study. *T. w. wiegmanni* is represented in full circles and *T. w. elegans* is represented in open circles. Codes are given in Table 1.

In total, 13 taxa were included for a total of 873 base pairs. Alignment was facile for both datasets, only 7 single base, 2 double and one 4 base pair insertions were needed. Sequences have been submitted to GenBank with accession numbers EF545712 to EF545735. *Trogonophis* is a monotypic genus; the closely related *Diplometopon zarudnyi* (24) was used to root the trees. We concluded that the General Time Reversible with a proportion of invariable sites and a discrete approximation of the γ distribution was the most appropriate model. A heuristic search incorporating this model inferred one tree of –ln 2146. Maximum parsimony estimated 3 trees of 198 steps the strict consensus of which was identical to the ML analysis, but less well resolved (Fig. 2). One hundred and three characters were parsimony informative. The estimate of phylogeny obtained using Bayesian analyses was identical to the ML tree. Our results provide evidence of two deep lineages corresponding to *T. w. elegans* and *T. w. wiegmanni* from Morocco (Bayesian probabilities and ML bootstraps for each: 0.97/86 and 0.94/64, respectively). The mean genetic distance between haplotypes in these two clades was high (3.8%). Evidence for the monophyly of these two clades was found in all the analyses.

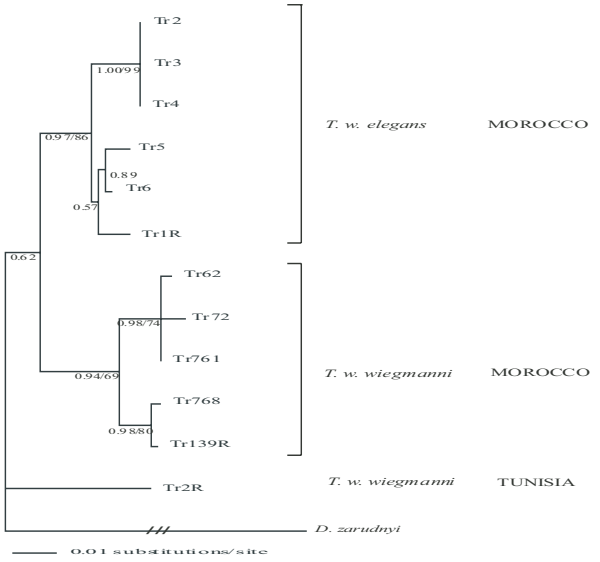

Fig. 2. – Tree derived from the ML analysis using the GTR+G+I model. All analyses produced identical relationships to the one shown. Below the branches, Bayesian posterior probabilities and bootstrap values for ML are indicated (posterior probabilities/ML bootstraps). For both analyses, only bootstrap values above 50% are represented.

The sample from Tunisia, thought to belong to *T. w. wiegmanni,* appears as a distinct lineage in all estimates of relationships. Concerning the genetic divergence, levels are high between the two Moroccan clades and the Tunisian individual (4.6% between the Tunisian individual and all the Morocco samples). The Tunisian individual is more divergent from *T. w. wiegmanni* (4.8%), the subspecies it is considered to belong to, than from *T. w. elegans* (4.4%).

Based on our analysis of 12s and 16s rRNA we confirm the existence of two monophyletic groups approximately separated by the Atlas mountains in Morocco. Compared to other species previously studied, the divergence between the two clades (3.8% for the combined data, 3.8% for 16s rRNA and 2.6% for 12s rRNA uncorrected distances) and between the Tunisian sample and the Moroccan groups (4.6% for the combined data, 4.8% for 16s rRNA and 4.3% for 12s rRNA uncorrected distances) can be considered high. Although not as high as the distances observed for *Tarentola mauritanica* (5% and 8% for 16srRNA uncorrected distances) and *Lacerta perspicillata* (12s rRNA uncorrected distances between 5.2 and 6.6%) that were considered cryptic species, divergences were higher than the ones reported for *Acanthodactylus erythrurus* (genetic distances between all populations range from 0.08 to 3.1% for 12S rRNA) and for *Agama impalearis* (16s rRNA maximum uncorrected intraspecific divergence of 2.6%). In the *Agama impalearis* phylogeographic study the sequence variation detected was considered surprisingly large and combined with other factors used to suggest the existence of distinct species (6).

In Morocco, this high genetic diversity combined with the well supported monophyly of the two mtDNA lineages and the apparently different ecological demands of both forms again raises the question of whether the two main clades could be considered distinct species. Furthermore, the morphological variation and the lack of morphological intermediate forms between subspecies (2) although weak, corroborates the two genetic lineages. Further sampling near the contact zone would be useful to confirm this. Field observations are also needed to better assess the ranges of the two forms, as our new records clearly indicate.

Concerning the Tunisian sample, our results do not conform to its inclusion in either of the Moroccan clades. The magnitude of mtDNA variation between the Tunisian sample and the other groups suggest that it belongs, at least, to a different subspecies possibly even to a different species. Unfortunately, the *T. w. wiegmanni* type locality has been described as restricted to Algeria (25). Since Algerian samples have not been included in this analysis, the correct nomenclature remains uncertain. Obviously, more sampling from Algeria and Tunisia will be crucial in evaluating the structure of the species *T. wiegmanni*, especially within *T. w. wiegmanni*. A detailed assessment of morphological variation is also needed, in particular between the Tunisian form and Moroccan *T. w. wiegmanni*. Only after this should the taxonomy be redefined.

These results are another example of high genetic variation within North African reptiles and indicate again the need for assessment of other Moroccan species in order to keep evaluating the diversity of this biogeographical complex region (9) and also to further address comparative biogeographic questions.

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