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Molecular and karyological homogeneity in *Trachylepis striata* (Peters 1844) and *T. wahlbergii* (Peters 1869) (Scincidae Reptilia)

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This study provides a genetic characterisation of two skink species, *Trachylepis striata* (Peters 1844) and *Trachylepis wahlbergii* (Peters 1869), by means of karyology and analysis of the 422-bp sequences of the 16S rRNA mitochondrial gene. The two species have an identical karyotype, 2n = 32, NF = 64; no interspecific differences were found in the relative length of the chromosomes and their centromeric index. However, the karyotype of *T. striata* is very different from the one previously described for the same species. For the genetic analysis, we used seven specimens collected in seven different localities. The results indicate that the two species cannot be distinguished by the 16S rRNA mitochondrial gene. In fact, the genetic distance within species is very close to that found among species (2.5%). Moreover, the phylograms obtained with NJ and MP fail to support any monophyletic assemblage suggesting that *T. striata* and *T. wahlbergii* have diverged very recently. Therefore, the specific rank of these two taxa should be re-evaluated.

KEY WORDS: 16S rRNA gene, chromosomal evolution, Lygosominae, *Mabuya*, mtDNA.

INTRODUCTION

The circumtropical genus *Mabuya* Fitzinger 1826, has recently been subjected to revision. Molecular analysis (MAUSFELD et al. 2002) suggested that *Mabuya* consists of several long-separated evolutionary lineages, representing distinct and well

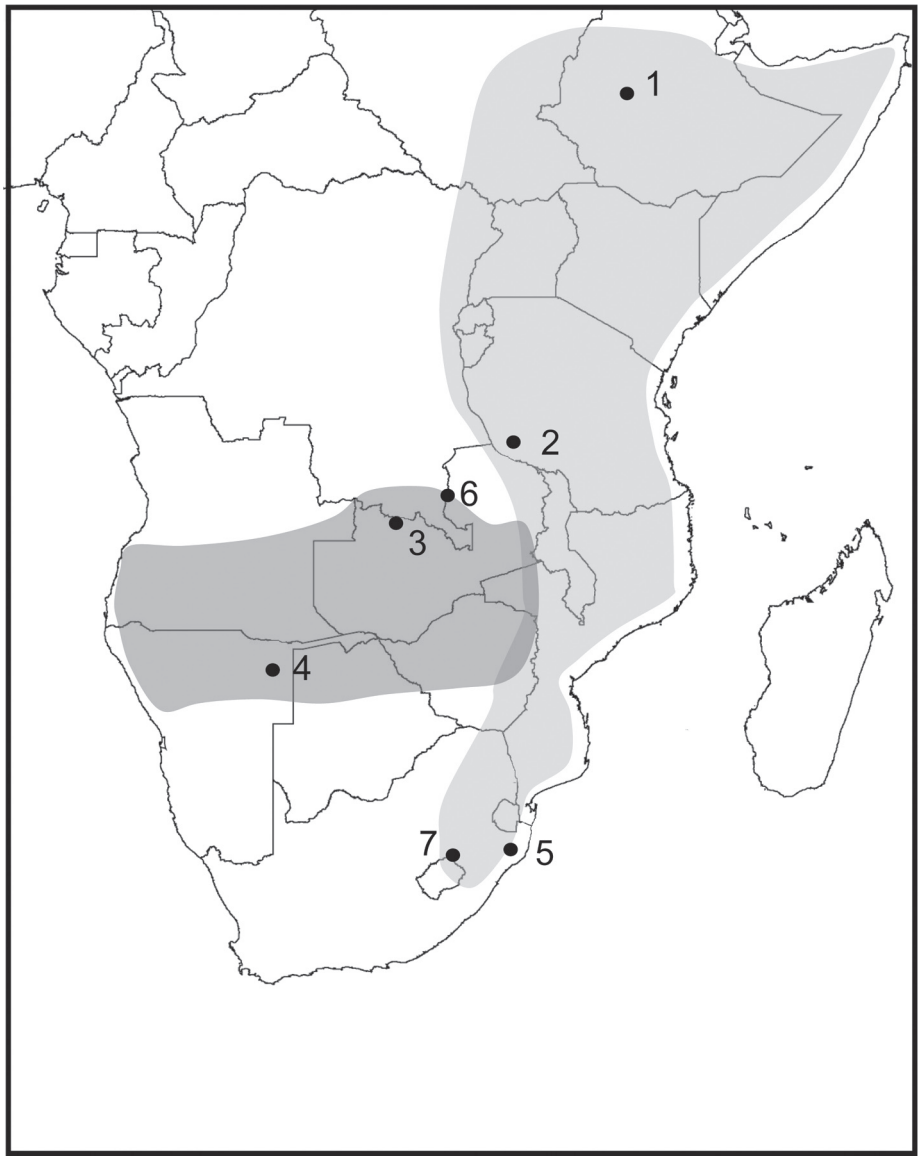

Fig. 1. — Approximate ranges of *T. striata* (light grey) and *T. wahlbergii* (dark grey) on the basis of the last review of the genus by BROADLEY (2000); for number of localities, see Table 1.

supported monophyletic radiations. To reflect their independent origins, the genus *Mabuya* was partitioned into four genera. The Afro-Malagasy species group was assigned to the genus *Euprepis* Wagler 1830, by MAUSFELD et al. (2002). However, reconsideration of the types of this taxon provided another interpretation, giving the oldest available name for this clade as *Trachylepis* Fitzinger 1843 (BAUER 2003).

Two of the most common species in the African savannahs are *T. striata* (Peters 1844) and *T. wahlbergii* (Peters 1869). According to BROADLEY (2000), *T. striata* has a very wide distribution from Sudan southwards to KwaZulu-Natal and westwards to eastern Congo. *T. wahlbergii* has a more restricted range, from southern Angola and northern Namibia to western Zimbabwe and western Mozambique (Fig. 1).

These two taxa were considered subspecies until BROADLEY (2000) suggested that they should be regarded as species because no evidence of a morphological cline occurs at their contact zone in Zimbabwe. In fact, they are morphologically very similar and can only be distinguished by a differing pattern of dorsal coloration and their cephalic lepidosis (BROADLEY 2000). *T. striata* has a red-brown dorsum with a pair of well-defined yellow dorsolateral stripes; subocular scales usually excluded from the lip. *T. wahlbergii* has a pale grey-brown dorsum with pale dorsolateral stripes, a black lateral band which goes from the eye to the shoulder; subocular scales usually reaching the lip.

No genetic data are available to confirm their species rank, with the exception of a sequenced fragment of the 16S rRNA mitochondrial gene for two specimens from distant localities showing a low level of differentiation (MAUSFELD et al. 2000, Table 1).

Studies of the karyotypes of several species of *Trachylepis* have shown that the diploid number is rather conservative (DE SMET 1981, ADEGOKE 1984, ADEGOKE & EJERE 1991, OTA et al. 1996). It typically consists of 32 chromosomes with 4 pairs of large biarmed chromosomes, 6 medium-sized chromosomes and 6 pairs of microchromosomes. In spite of the constant diploid number, the group of medium-sized chromosomes shows a high level of variation when the location of centromeres is considered; consequently, the morphology of these chromosomes may differ among species (ADEGOKE & EJERE 1991). The only known exception to the 2n = 32 karyo-

Table 1.

Specimens of *T. wahlbergii* and *T. striata*. Localities, diploid numbers, GeneBank accession numbers of the 16S rRNA gene sequences and references are indicated.

Species	Locality	Site on map	2n	rRNA gene	Reference
T. wahlbergii	Solwezi (NW Zambia)	3	32	DQ234810	Present study
T. striata	Addis Ababa (Ethiopia)	1	32	DQ234808	Present study
T. striata	Chunya (S Tanzania)	2	—	DQ234809	Present study
T. striata	Munzini, KwaZulu-Natal (South Africa)	5	—	AF153581	MAUSFELD et al. 2000
T. wahlbergii	Grootfontein (Namibia)	4	—	AF153582	MAUSFELD et al. 2000
T. wahlbergii	Kasenga (Zaire)	6	—	AB028791	HONDA et al. 2000
T. striata	Qwaqwa (South Africa)	7	—	AY028889	DANIELS et al. 2002
T. varia	Solwezi (NW Zambia)	—	32	DQ234811	Present study
T. spilogaster	Aranos (Namibia)	—	—	AF153580	MAUSFELD et al. 2000

type among *Trachylepis* is the *T. striata* karyotype described by DALLAI & VEGNI TALLURI (1969), with 28 metacentric macrochromosomes.

In this paper, we provide new data regarding the genetic characterisation of these two species. We describe the karyotype of *T. wahlbergii* for the first time. We report a karyotype for *T. striata* that is very different from the one previously described by DALLAI & VEGNI TALLURI (1969). Moreover, we analyse a fragment of the mitochondrial 16S rRNA gene from other localities of both species. We use the resulting data, together with sequences from the literature, to assess the genetic variation of the two species.

MATERIALS AND METHODS

Chromosomes were obtained from one specimen of *T. striata* (male) and one of *T. wahlbergii* (female). They were collected at the campus of the University of Addis Ababa (Ethiopia) and at Solwezi (northwestern Zambia), respectively (Fig. 1 and Table 1). Chromosomal metaphases were prepared by air drying, following CASTIGLIA (2004), and stained with Giemsa 5%. Twenty metaphase plates were analysed for each individual to assess the diploid number. Five particularly well-spread mitoses were selected for each individual to calculate the relative length of each chromosome (R.L., percentage ratio between the length of each chromosome and the total length of all the chromosomes) and the centromeric index (C.I., ratio between the short arm and total length of a chromosome).

16S rRNA gene sequences were obtained from the same two specimens used for karyotype analysis plus an additional specimen of *T. striata* from southern Tanzania (Table 1). DNA was extracted using the QIAmp tissue extraction kit (Qiagen). The primers 16SA-L (light chain; 59-CGC CTG TTT ATC AAA AAC AT-39) and 16SB-H (heavy chain; 59-CCG GTC TGA ACT CAG ATC ACG T-39) were used to amplify a section of the mitochondrial 16S ribosomal RNA gene (PALUMBI et al. 1991).

The PCR cycling procedure was performed as follows: 34 cycles of denaturation for 90 sec at 95 °C, primer annealing for 60 sec at 50 °C, and extension for 90 sec at 72 °C.

All published sequences of 16S rRNAs of the two species from different African countries were also included in the analysis. All the sequence accession numbers included in the dataset are reported in Table 1 and the localities in Fig. 1. The phylogenetic analysis indicated that the sequence WHITING et al. (2003) described as *T. striata* should be assigned to *Trachylepis spilogaster* (Peters 1882). Therefore, we did not include it in the following analysis. Two specimens were assigned to *T. striata* and *T. wahlbergii* on the basis of their geographic location (localities 6 and 7 in Fig. 1). While locality 7 is clearly within the range of *T. striata*, locality number 6 (Kasenga, Zaire) is near the overlapping zone of the range of the two species; however, HAAGNER et al. (2000) confirmed that only *T. wahlbergii* occurs in the area.

Sequences (480 bp) were deposited at GeneBank (Accession Numbers DQ234808-11). For comparison of the sequences with previously published ones, a shorter fragment of 422 was used for the alignment. Sequences were aligned using ClustalX 1.81 (THOMPSON et al. 1997). The genetic divergence was computed with different distance methods: the Kimura two-parameter model (K2P; KIMURA 1980), the Jukes-Cantor distance (JC; JUKES & CANTOR 1969) and the uncorrected p-distance. These methods provided nearly identical values of differentiation among haplotypes. Therefore, we only used the p-distances for the following analyses.

Maximum Parsimony (MP) and Neighbour-Joining (NJ) methods were used to construct phylogenetic relationships. MP trees were obtained with Paup 4.0b10 using a heuristic search, tree-bisection-reconnection (TBR) swapping and random addition of sequences (10 replicates). MP trees were constructed by weighting transitions and transversions at a ratio of 1:1 and 1:3. NJ trees were obtained using the p-distance with the MEGA program (version 2.1; KUMAR et al. 1993).

The robustness of the nodes was assessed by bootstrap with 10000 replicates for both MP and NJ. Two outgroups were used for NJ: *T. spilogaster* and an unpublished sequence from *Trachylepis varia* (Peters 1867). Only *T. spilogaster* was used in the MP analysis as this species is the sister group of the two species analysed here (MAUSFELD et al. 2000); thus, it represents an optimal outgroup.

RESULTS

Chromosomal analysis

The karyotypes of the two specimens are represented by 32 chromosomes and their Fundamental Number (NF) is 64 (Fig. 2). The chromosomes can be divided into 10 pairs of macrochromosomes and 6 pairs of microchromosomes. However, the distinction between these two groups is not so evident. The macrochromosomes can be further divided into three groups according to ADEGOKE & EJERE (1991). The first group includes 4 pairs of very large metacentrics representing almost 60% of the overall length. The second group is formed by a single pair of medium-sized metacentrics (number 5). Group three is composed of three submetacentrics, very similar in size and arm ratio, and by two pairs of metacentrics. Microchromosomes are almost all biarmed, as can be seen in the prometaphase plates (Fig. 2).

Table 2 reports the RLs and the CIs for each chromosomal pair, separately for each species; the size and morphology of each chromosome are the same in the two species. This excludes the occurrence of pericentric inversions and/or centromeric shifts between the two species.

Table 2.

Relative length and centromeric indices for the chromosomes of *T. striata* and *T. wahlbergii*.

Chr.	*T. wahlbergii*		*T. striata*	
	Length	C.I.	Length	C.I.
1	0.180 ± 0.008	0.432 ± 0.009	0.184 ± 0.005	0.438 ± 0.020
2	0.152 ± 0.005	0.457 ± 0.009	0.158 ± 0.004	0.436 ± 0.023
3	0.122 ± 0.005	0.477 ± 0.009	0.130 ± 0.000	0.480 ± 0.010
4	0.112 ± 0.009	0.465 ± 0.013	0.116 ± 0.005	0.470 ± 0.016
5	0.070 ± 0.000	0.455 ± 0.017	0.068 ± 0.004	0.454 ± 0.030
6	0.050 ± 0.000	0.325 ± 0.061	0.050 ± 0.000	0.324 ± 0.023
7	0.050 ± 0.000	0.315 ± 0.044	0.048 ± 0.004	0.346 ± 0.015
8	0.047 ± 0.005	0.395 ± 0.064	0.044 ± 0.005	0.326 ± 0.044
9	0.035 ± 0.006	0.420 ± 0.062	0.036 ± 0.005	0.472 ± 0.041
10	0.027 ± 0.005	0.475 ± 0.050	0.028 ± 0.004	0.454 ± 0.043
11	0.025 ± 0.006	0.490 ± 0.020	0.024 ± 0.005	0.500 ± 0.000
12	0.027 ± 0.005	0.495 ± 0.010	0.024 ± 0.005	0.500 ± 0.000
13	0.025 ± 0.006	0.487 ± 0.025	0.024 ± 0.005	0.500 ± 0.000
14	0.022 ± 0.005	0.487 ± 0.025	0.022 ± 0.004	0.500 ± 0.000
15	0.022 ± 0.005	0.490 ± 0.020	0.022 ± 0.004	0.500 ± 0.000
16	0.020 ± 0.000	0.490 ± 0.020	0.022 ± 0.004	0.500 ± 0.000

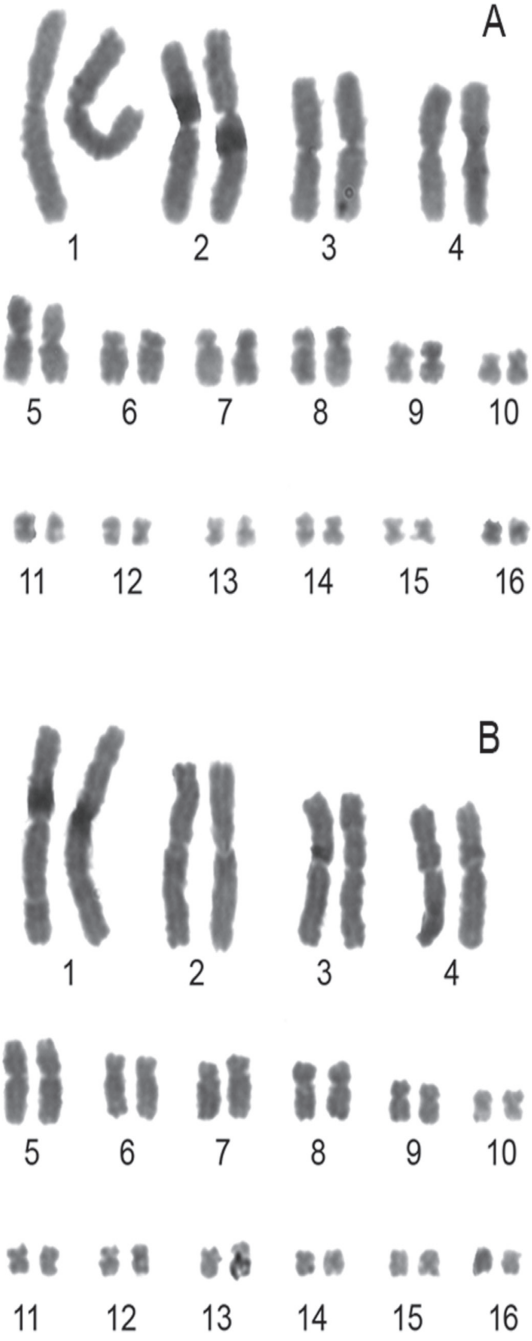

Fig. 2. — A: karyotype of *T. striata,* Ethiopia; B: karyotype of *T. wahlbergii,* NW Zambia.

Table 3.

The p-distance between haplotypes. Numbers in brackets indicate localities in Fig. 1.

Species	1	2	3	4	5	6	7	8
1 — *T. striata*, S Africa (7)	—							
2 — *T. striata*, S Africa (5)	0.005	—						
3 — *T. striata*, Ethiopia (1)	0.029	0.031	—					
4 —- *T. striata*, S Tanzania (2)	0.026	0.029	0.026	—				
5 — *T. wahlbergii*, Zaire (6)	0.019	0.019	0.029	0.026	—			
6 — *T. wahlbergii*, Namibia (4)	0.022	0.024	0.031	0.029	0.024	—		
7 — *T. wahlbergii*, NW Zambia (3)	0.024	0.026	0.029	0.022	0.024	0.026	—	
8 — *T. spilogaster*	0.036	0.034	0.043	0.046	0.046	0.046	0.043	—
9 — *T. varia*	0.091	0.094	0.091	0.089	0.089	0.084	0.082	0.086

16S rRNA gene analysis

All the sequences (422 bp) showed a bias against guanine (A = 34.2-34.9%, C = 23.3-23.5%, G = 19.0-19.5%, T = 22.6-22.8%); such a bias reflects the true mitochondrial genome bias and excludes their integration in the nuclear genome as pseudogenes (Zhang & Hewitt 1996).

Total alignment highlights 40 variable characters (29 non-informative and 11 phylogenetically informative). Thirty-three variable positions were found in the ingroup with one single insertion.

The phylograms obtained with NJ and MP show no significant node supporting any ingroup taxa (Fig. 3). The only exception is represented by two haplotypes from two very close localities in South Africa (Nos 5 and 7) supported by high bootstrap values with NJ (91%) and MP (80%).

Genetic distances among haplotypes vary from 0.5 to 3.1% (average 2.5%) (Table 3). Within *T. striata*, the genetic distance varies from 0.5 to 2.9% (average 2.4%); within *T. wahlbergii*, the genetic distance varies from 2.4 to 2.6% (average 2.5%). Genetic differentiation between *T. striata* and *T. wahlbergii* varies from 1.9 to 3.1% (average 2.5%); thus, inter- and intraspecific distances show comparable values.

DISCUSSION

The comparison of the karyotypes and the phylogenetic analysis of the 16S mtDNA gene demonstrated that the two species are genetically similar. No differences were found in the relative length and centromeric index of the two karyotypes. Thus, these chromosomes are useful characters for the cytotaxonomy of the genus (Adegoke & Ejere 1991, Ota et al. 1996). However, other banding techniques might be useful to detect "cryptic" chromosomal differences, e.g. C-banding or the localisation of nucleolar organizer regions (NORs). For example, in lizards, identical standard-stained karyotypes may differ when the amount and localisation of C-positive heterochromatin or NORs is considered (see In den Bosch et al. 2003).

The prometaphase karyotypes examined made it possible to establish that all microchromosomes were metacentrics. ADEGOKE & EJERE (1991) were not able to reveal the microchromosome morphology in *Trachylepis affinis* (Gray 1838), *Trachylepis maculilabris maculilabris* (Gray 1845) or *Trachylepis quinquetaeniata* (Lichtenstein 1823), probably because it can only be determined in particularly elongated chromosomes. Therefore, we provide evidence of additional chromosomal markers which could be suitable for future investigations.

Apart from these small microchromosomes, *T. striata* and *T. wahlbergii* have the same karyotype as *T. quinquetaeniata* (ADEGOKE & EJERE 1991). This similarity cannot be a synapomorphy because the species are not genetically related (MAUSFELD et al. 2000). Moreover, the same karyotype was also found in *T. varia* (R. CASTIGLIA unpublished data), another species genetically distant from *T. striata*. This widespread karyotype with 2n = 32 and NF = 64 may represent an ancestral condition for all the African *Trachylepis*.

In this context, the finding of a very different karyotype (2n = 28; NF = 56) in a *T. striata* specimen from Kenya (DALLAI & VEGNI TALLURI 1969) merits discussion. The two karyotypes cannot be easily compared in terms of simple chromosomal rearrangements (Rb fusions, pericentric inversions) because they are both formed by metacentric chromosomes. Since this specimen is not available for morphological and genetic analyses (R. DALLAI pers. comm.), it is not possible to establish whether it belongs to a chromosomal variant or was misidentified.

The most striking result of the mitochondrial analysis is that the genetic distances do not support a distinct separation of *T. striata* and *T. wahlbergii*. In fact, the genetic distance within species is very close to that found among species (2.5%). This value is similar to the intraspecific divergence values found in other *Trachylepis* species (MAUSFELD et al. 2004).

Moreover, the resulting phylogeny fails to support any monophyletic group (Fig 3). This could be due to the short sequence used for analysis or to the slow evolutionary rate of the mtDNA 16S RNA gene in comparison with recently diverged populations.

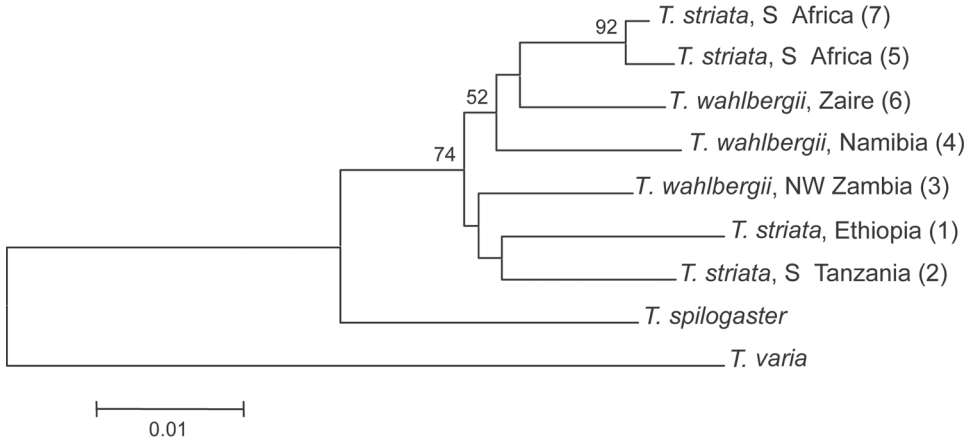

Fig. 3. — NJ tree following the Jukes-Cantor distances using 422 bp of the mitochondrial 16S rRNA gene. Numbers indicate localities as in Table 1.

A similar non-correspondence between morphology and mtDNA haplotypes was recently found by DANIELS et al. (2005). They showed that subspecies in the limbless skink genus *Acontias* traditionally described on the basis of scale pattern and colour pattern probably represent invalid taxonomic units.

In summary, our study indicates that *T. striata* and *T. wahlbergii* share the same karyotype and do not present any differentiation in their 16S mtDNA gene. This emphasizes the need of a correct taxonomic definition. BROADLEY (2000) argued that even minimal morphological differences that are maintained in syntopy, such as in Zimbabwe, are sufficient to merit a species rank. However, this is not a common pattern because an intergradation zone occurs in eastern Zambia and neighbouring Malawi. Therefore, two alternative hypotheses can be proposed. Firstly, ecological constraints rather than past history may have shaped the morphology of these two taxa. The differing morphological characters are probably subject to natural selection or genetic drift and do not reflect their evolutionary history. It is also important to consider that differences in colour and morphology may arise over a short time span within reptile populations subject to natural selection (LOSOS et al. 2000). Secondly, the two taxa diverged very recently and there has not been sufficient time to reach reciprocal monophyly.

Only a genetic analysis at the contact zone, using more variable nuclear loci (microsatellites) or a longer or more variable segment of the mitochondrial DNA, will allow us to choose between these two hypotheses and eventually confirm the specific status of the two taxa.

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REFERENCES

ADEGOKE J.A. 1984. The karyotype of the skink, *Mabuya blandingi*. *Canadian Journal of Genetics and Cytology* 26: 100-103.

ADEGOKE J.A. & EJERE V.C. 1991. Description of the chromosomes of three lizard species belonging to the genus *Mabuya* (Scincidae, Reptilia). *Caryologia* 44: 333-342.

BAUER A.M. 2003. On the identity of *Lacerta punctata* Linnaeus, 1758, the type species of the genus *Euprepis* Wagler, 1830, and the generic assignment of Afro-Malagasy skinks. *African Journal of Herpetology* 52: 1-7.

BROADLEY D.G. 2000. A review of the genus *Mabuya* in southeastern Africa (Sauria, Scincidae). *African Journal of Herpetology* 49: 87-110.

CASTIGLIA R. 2004. First chromosomal analysis for the genus *Lygodactylus* (Gray, 1864): the karyotype of *L. picturatus* (Squamata, Gekkonidae, Gekkoninae). *African Journal of Herpetology* 53: 95-97.

DALLAI R. & VEGNI TALLURI M. 1969. A karyological study of three species of Scincidae (Reptilia). *Chromosoma* 27: 86-94.

Daniels S.R., Heideman N.J., Hendricks M.G., Mokone M.E. & Crandall K.A. 2005. Unraveling evolutionary lineages in the limbless fossorial skink genus *Acontias* (Sauria: Scincidae): are subspecies equivalent systematic units? *Molecular Phylogenetics and Evolution* 34: 645-654.

Daniels S.R., Heideman N.J.L., Hendricks M.G.J. & Willson B.A. 2002. A molecular phylogeny for the South African limbless lizard taxa of the subfamily Acontinae (Sauria: Scincidae) with special emphasis on relationships within *Acontias*. *Molecular Phylogenetics and Evolution* 24: 315-323.

De Smet W. 1981. Description of the orcein stained karyotypes of 36 lizards species (Lacertilia, Reptilia) belonging to the families Teiidae, Scincidae, Lacertidae, Cordylidae and Varanidae (Autarchoglossa). *Acta Zoologica et Pathologica Antverpiensia* 76: 73-118.

Haagner G.V., Branch W.R. & Haagner A.J.F. 2000. Notes on a collection of reptiles from Zambia and adjacent areas of the Democratic Republic of the Congo. *Annals of the Eastern Cape Museums* 1: 1-25.

Honda M., Ota H., Kobayashi M., Nabhitabhata J., Yong H.S. & Hikida T. 2000. Phylogenetic relationships, character evolution, and biogeography of the subfamily Lygosominae (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 15: 452-461.

In den Bosch H.A., Odierna G., Aprea G., Barucca M., Canapa A., Capriglione T. & Olmo E. 2003. Karyological and genetic variation in Middle Eastern lacertid lizards, *Lacerta laevis* and the *Lacerta kulzeri* complex: a case of chromosomal allopatric speciation. *Chromosome Research* 11: 165-178.

Jukes T.H. & Cantor C.R. 1969. Evolution of protein molecules, pp. 21-132. In: Munro H.N., Edit. Mammalian protein metabolism. *New York: Academic Press.*

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.

Kumar S., Tamura K. & Nei M. 1993. MEGA: Molecular Evolutionary Genetics Analysis. *The Pennsylvania State University, University Park, PA* 16802.

Losos J.B., Creer D.A., Glossip D., Goellner R., Hampton A., Roberts G., Haskell N., Taylor P. & Ettling J. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *International Journal of Organic Evolution* 54: 301-305.

Mausfeld P., Schmitz A., Böhme W., Misof B., Vrcibradic D. & Rocha C.F.D. 2002. On the phylogenetic affinity of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean archipelago of Fernando de Noronha (Brazil): necessity of partition the genus *Mabuya* (Scincidae: Lygosominae). *Zoologischer Anzeiger* 241: 281-349.

Mausfeld, P., Schmitz A., Ineich I. & Chirio L. 2004. Genetic variation in two African *Euprepis* species (Reptilia, Scincidae), based on maximum-likelihood and Bayesian analyses: taxonomic and biogeographic conclusions. *Bonner Zoologische Beiträge* 52: 159-177.

Mausfeld P., Vences M., Schmitz A. & Veith M. 2000. First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. *Molecular Phylogenetics and Evolution* 17: 11-14.

Ota H., Hikida T., Matsui M., Hasegawa M., Labang D. & Nabhitabhata J. 1996. Chromosomal variation in the scincid genus *Mabuya* and its arboreal relatives. *Genetica* 98: 87-94.

Palumbi S.R., Martin S.A., Romano S., McMillan W.O., Stice L. & Grabowski G. 1991. The Simple Fool's Guide to PCR. *University of Hawaii, Honolulu*.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876-4882.

Whiting A.S., Bauer A.M. & Sites J.W. Jr 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). *Molecular Phylogenetics and Evolution* 29: 582-598.

Zhang D.-X. & Hewitt G.M. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution* 11: 247-251.