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Phylogenetic placement of the Mount Bamboutos endemic skink, *Trachylepis mekuana* (Chirio and Ineich, 2001)

MICHELE M. KAMENI N.[1], WALTER P. TAPONDJOU N.[2], WERNER CONRADIE[3,4,*]

[1] *Laboratory of Zoology, University of Yaoundé I, Cameroon*

[2] *Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA.*

[3] *Port Elizabeth Museum (Bayworld), 23 Beach road, Humewood 6013, Gqeberha, South Africa*

[4] *Department of Nature Conservation Management, George Campus, Nelson Mandela University, George, South Africa*

[*]*Corresponding author.* Email werner@bayworld.co.za

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Abstract. African skinks of the genus *Trachylepis* is one of the most diverse genera of lizards in Africa. Although, many species have not been validated phylogenetically in recent years. In this study we evaluate the phylogenetic status of the Cameroon Volcanic Line endemic montane skink, *Trachylepis mekuana.* We recover this species as part of the larger *Trachylepis varia* group, with sister relationship to the morphological similar skinks from Eastern Africa *Trachylepis megalura* and central Africa *Trachylepis raymondlaurenti*. Low sequence divergence (<1.6% *16S*) have been observed among the three species. Based on some

morphological and colouration differences, as well as mostly allopatric distribution we regard them as good species.

Keywords. Endemic, Volcanic, Scincidae, montane, allopatric

Trachylepis is one of the most diverse Scincidae genera in Africa, with a taxonomically convoluted history and many unresolved species complexes. A recently published review of this genus in Angola has raised the number of species in the genus to ~94 species (Ceríaco et al., 2024).

In 2015, an unusual plainly coloured *Trachylepis* skink with an exceptionally long tail was found in Cangandala National Park, Angola (Ceríaco et al., 2016, 2018), which was subsequently described as a new species, *T. raymondlaurenti* (Marques et al., 2019). The new species was reported to be differentiated from its closest congener *T. megalura* by 4% *16S* sequence divergence, the fact that the supranasals are always separated, and a uniform greyish dorsal colouration versus fine longitudinal black or white dorsolateral stripes dorsally. However, in a later study on the *Trachylepis* of Angola (Ceríaco et al., 2024), the authors reported a lower (2.12%) *16S* uncorrected p-distance between these two species, but a large *RAG1* uncorrected p-distance of 3.42%.

In a broad-scale phylogenetic study of the *Trachylepis* genus, Weinell et al. (2019) recovered the two above-mentioned species (*T. raymondlaurenti* ~labelled as *T. megulara* and *T. megalura*) as part of the larger *T. varia* group. Within this group *T. varia* has undergone a substantial revision, in which previous works recovered a widespread species complex (*T. varia* complex) comprising seven well differentiated lineages (Weinell and Bauer, 2018). Consequently, some of these have subsequently be allocated to older names (*T. laevigata*, *T. damarana*, *T. albopunctata*), while others remain undescribed due to lacking material.

Nevertheless, authors of the above studies have not made further mention to the close sister relationship between *T. megalura* and the rest of the *T. varia* group.

Of special interest is that when *T. raymondlaurenti* was described, the authors did not make any comparison to *T. mekuana* from Mount Bamboutos in Cameroon, which was documented to be morphologically closely related to *T. megalura* (Chiro and Ineich, 2003). *Trachylepis mekuana* shares morphological similarities to *T. raymondlaurenti* in that the supranasals are mostly separated, yet it differs in having a bolder dorsal colouration versus uniform greyish in the latter. In an independent study looking at the phylogenetic status of the Lygosominae, *16S* sequences of *T. mekuana* (as *Mubuya* sp.) were included in their analyses (Honda et al., 2003). Another study looking at the Central and West Africa *Trachylepis* published *ND2* sequences of *T. mekuana* (Allen et al. 2019). None of these sequences were incorporated into the broad scale study of Weinell et al. (2019) or in the recent Angolan *Trachylepis* revision (Ceríaco et al., 2024). Consequently, the close morphological similarities of *T. mekuana* with *T. megalura* and *T. raymondlaurenti*, combined with the above-mentioned discrepancies in genetic results, have motivated a deeper re-analysis of the group using all the available genetic data of these three species to shed light on the taxonomic and phylogenetic position of this group.

To this aim, previously published sequences of *16S*, *ND2*, *RAG1* and *KIF24* genes (Table S1; Honda et al. 2003; Marques et al. 2019; Allen et al. 2019; Weinell et al. 2019) were obtained from GenBank, including four newly generated *16S* sequences of *T. mekuana*, and aligned in MEGA v.7.0.27 (Tamura et al., 2013), using the ClustalW v.1.6 alignment method (Thompson et al., 1994) with default parameters. The final dataset comprised 51 samples (Table S1), including *T. laevis* as the outgroup taxon. Separate alignments were created for each gene, and a concatenated dataset, was created using SequenceMatrix v.1.8.2 (Vaidya et al., 2011). The best-fitting models and partition schemes were determined using ModelFinder

implemented in IQ-TREE (Chernomor et al., 2016; Minh et al., 2021). The following settings were used: *-p* partition file (each partition with its own evolution rate), a greedy strategy and the FreeRate heterogeneity model excluded (only invariable site and Gamma rate heterogeneity considered) (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017). The following models and partition schemes were used: GTR+F+I (*16S*), GTR+F+I+G4 (*ND2*), and HKY+F+I (*RAG1 + KIF24I*). A maximum likelihood (ML) phylogeny was generated in IQ-TREE, using a random starting tree and the best-fitting model schemes selected for each dataset as selected above. The ultrafast bootstrap approximation (UFBoot) method (Hoang et al., 2017) was implemented using 1000 replicates and minimum correlation coefficient of 0.99. For accuracy, the analysis was run twice to ensure that independent ML searches recovered the same topologies. The ML phylogeny was rooted with *Trachylepis laevis* and visualised using FigTree v.1.4.4 (Rambaut, 2018). Nodes with bootstrap support (BS) ≥ 95% were regarded as well supported. Finally, an uncorrected pairwise distance (p-distance) analysis was conducted in MEGA X (Kumar et al., 2018) for the *16S* and *ND2* gene. The hyper-variable region of the *16S* gene was retained. Sequences were grouped according to species, and pairwise distance analyses were conducted using uniform rates, pairwise deletion and 500 bootstrap replicates.

The phylogenetic reconstructions recovered a similar topology within the *T. varia* group compared to previous studies (Weinell et al., 2019; Ceríaco et al., 2024), showing a well-supported sister relationship between the *T. megalura* group and the rest of the *T. varia* complex (Figs. 1 and S1). The only difference is the inclusion of *T. mekuana* in this study. *Trachylepis mekuana* was recovered as sister to *T. raymondlaurenti*, although not well-supported. In turn, these two species were recovered as a well-supported sister clade to *T. megalura*. The uncorrected *16S* p-distance obtained between these three species varies between 0.9 – 1.6% *16S*. This is well below the interspecific threshold observed between the other species of the *T. varia* complex (4.2 – 9.6 %, average 6.1 ± 1.1% *16S* average uncorrected p-

distance; Table 1). Similarly, the uncorrected *ND2* p-distance sequence divergence between *T. mekuana* and *T. raymondlaurenti* was 9.4% (Table 2), also well below the interspecific threshold observed between the other species of the *T. varia* complex (13.5 – 20.4%, average 16.5 ± 1.6% *ND2* average uncorrected p-distance).

Morphologically, these three species are reported to differ only in their dorsal colouration (striped dorsal pattern, with a number of fine longitudinal black or white stripes and a distinct white dorsolateral stripe in *T. megalura*; *T. mekuana* is similar in colouration to *T. megalura* but the dorsal stripes are more defined; uniform greyish-brown with no stripes in *T. raymondlaurenti*), and head scalation (supranasals always in contact in *T. megalura versus* never in contact in *T. raymondlaurenti* and *T. mekuana* [except paratype female in narrow contact]). In addition, the three species occur mostly allopatrically, except at the Upemba National Park area in southern Democratic Republic of the Congo, where both *T. raymondlaurenti* and *T. megalura* occur sympatrically (Marques et al., 2019). In the Port Elizabeth Museum, a *T. megalura* specimen from north of Shabeli, Ethiopia (PEM R08590) shares morphological characters with *T. mekuana* (supranasals separated and similar dorsal colour pattern). This agrees with the conclusions of Chirio and Ineich (2003), who refer to specimens from Koffole, Ethiopia that share morphological similarities with *T. mekuana*. Other specimens of interest here include the undescribed *Trachylepis* sp. observed by Kameni et al. (2022) on Mt. Bamboutos, occurring at lower elevation than *T. mekuana*. The two individuals observed present the habitus and uniform colouration of *T. raymondlaurenti* and occur sympatrically with *T. mekuana*. These population warrants further investigation.

Although the sequence divergence between the species in the *T. megalura* group are on the low side, the minor morphological and colouration differences in conjunction with the mostly allopatric distribution provides multiple lines of evidence to retain all three species as valid. Wider sampling, especially of the Ethiopian population, may help shedding further light

on this group and potentially discover additional cryptic species in this group. Of further interest is the close relationship between reptile species from the high lying areas of Cameroon and East African. Other species showing similar patterning as *T. mekuana* and *T. megalura* is: *Lepthosiaphos koutoui versus L. kilimensis* and *Trachylepis nganghae versus* undescribed *Trachylepsis* species from Uganda (Ineich and Chirio, 2004). These species pairs are separated by a large geographical gab with no records in-between due to a lack of suitable montane habitat and potential sampling effort.

In conclusion, this study phylogenetic placed *T. mekuana* in the *T. megalura* subgroup and eluded to the low sequence divergence among species in this group.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 16188

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TABLES

Table 1. Mean sequence divergences (uncorrected p-distances) within the *Trachylepis varia* complex (group names based on Weinell and Bauer 2018) for *16S* gene, given as percentages. The numbers in the diagonal grey boxes represent the mean intraspecific sequence divergences, numbers below the diagonal grey boxes represent the mean interspecific sequence divergences, while numbers above the diagonal grey boxes represent standard errors of the interspecific sequence divergences. Important values have been framed. n/c – was not possible to estimate sequence divergences.

	16S	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*T. varia* C	0.7	*0.9*	*0.9*	*1.0*	*0.9*	*1.0*	*1.0*	*1.2*	*1.4*	*1.4*	*1.3*
2	*T. varia* D	4.1	0.0	*1.0*	*1.1*	*1.0*	*1.3*	*1.2*	*1.3*	*1.3*	*1.4*	*1.3*
3	*T. varia* sensu stricto	4.9	5.6	0.0	*1.0*	*0.8*	*1.0*	*1.0*	*1.2*	*1.3*	*1.4*	*1.3*
4	*T. albopunctata*	5.6	6.2	4.9	0.6	*0.9*	*1.1*	*0.9*	*1.3*	*1.3*	*1.4*	*1.2*
5	*T. varia* E	4.7	5.1	4.4	4.8	1.5	*1.0*	*0.9*	*1.2*	*1.3*	*1.3*	*1.2*
6	*T. varia* G	5.2	7.7	4.7	5.5	5.5	n/c	*1.1*	*1.3*	*1.4*	*1.5*	*1.4*
7	*T. damarana*	5.9	7.0	5.5	4.6	4.7	6.0	1.0	*1.2*	*1.4*	*1.4*	*1.3*
8	*T. laevigata*	8.4	8.3	8.0	8.5	7.5	8.6	8.3	0.2	*1.3*	*1.3*	*1.3*
9	*T. raymondlaurenti*	9.9	9.5	9.2	9.1	8.7	10.1	10.0	8.2	n/c	*0.4*	*0.6*
10	*T. mekuana*	10.4	10.0	9.7	9.6	9.2	10.6	10.4	8.3	0.9	n/c	*0.6*
11	*T. megalura*	9.4	9.1	8.9	8.8	8.0	9.8	9.6	7.7	1.5	1.6	0.0

Table 2. Mean sequence divergences (uncorrected p-distances) within the *Trachylepis varia* complex (group names based on Weinell and Bauer 2018) for *ND2* gene, given as percentages. The numbers in the diagonal grey boxes represent the mean intraspecific sequence divergences, numbers below the diagonal grey boxes represent the mean interspecific sequence divergences, while numbers above the diagonal grey boxes represent standard errors of the interspecific sequence divergences. Important values have been framed. n/c – was not possible to estimate sequence divergences.

	ND2	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	*T. varia* D	0.2	*1.4*	*1.2*	*2.0*	*1.4*	*1.4*	*1.6*	*1.6*
2	*T. varia* sensu stricto	15.0	2.6	*1.4*	*1.8*	*1.4*	*1.5*	*1.7*	*1.7*
3	*T. albopunctata*	13.5	16.1	5.6	*1.8*	*1.2*	*1.4*	*1.5*	*1.5*
4	*T. varia* G	14.9	16.4	15.5	n/c	*2.0*	*2.0*	*2.2*	*2.3*
5	*T. damarana*	16.3	18.2	14.8	16.2	1.6	*1.4*	*1.6*	*1.6*
6	*T. laevigata*	17.9	20.4	18.6	17.9	16.3	0.6	*1.6*	*1.5*
7	*T. raymondlaurenti*	22.0	21.4	23.3	19.9	22.3	22.0	n/c	1.1
8	*T. mekuana*	22.4	22.2	22.5	22.9	22.3	21.2	9.4	0.0

CAPTIONS TO FIGURES

Fig. 1. Maximum Likelihood (ML) concatenated phylogeny (*16S*, *ND2*, *RAG1*, *KIF24*), showing relationships between the *Trachylepis varia* group and the placement of *Trachylepis mekuana*.