

Scientific note

## The mitochondrial genome of the booid snake *Sanzinia volontany* and its first record from northeastern Madagascar

(Squamata, Serpentes, Sanziniidae)

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Madagascar boas comprise four endemic species, which were traditionally included in the mostly Neotropical family Boidae (Vences et al. 2001, Raxworthy & Glaw 2022) but are now classified in their own family Sanziniidae (Pyron et al. 2014). *Sanzinia volontany* was originally described as a western subspecies of *S. madagascariensis* by Vences & Glaw (2004) but subsequently elevated to species level due to the comparatively large genetic distance to its sister species (Orozco-Terwengel et al. 2008, Reynolds et al. 2014). *Sanzinia volontany* is widespread in western and far northern Madagascar but has never been genetically confirmed from the Northeast of the island (Glaw & Vences 2007, Scherz et al. in press).

On 7 December 2012, we discovered an adult snake of the genus *Sanzinia* along a trail in the Fanambana forest (-13.6200, 50.0012; ca. 130 m a.s.l.), ca. 30 km south of the town of Vohémar in northeastern Madagascar. The individual was captured and released after taking photographs and a tissue sample for DNA sequencing. The snake had a brown ground colouration typical for the western species *Sanzinia volontany* (Fig. 1).

Sequencing data was generated by the sequencing service of the company AIM (Advanced Identification Methods, Leipzig, Germany) from a tissue sample of this individual (SOH 219). We obtained 3.5 million raw reads from library preparation with the Illumina DNA prep kit and sequencing on an Illumina MiSeq platform with 2 × 300 bp and 600 cycles. Through annotation in Geneious 2023.2.1, we obtained a circularized mitogenome, representing the first mitochondrial genome of a



Fig. 1. *Sanzinia volontany* from the Fanambana forest in northeastern Madagascar.

Malagasy booid snake (Fig. 2; GenBank accession number PQ492719). We annotated protein-coding and ribosomal tRNAs. The mitogenome has a length

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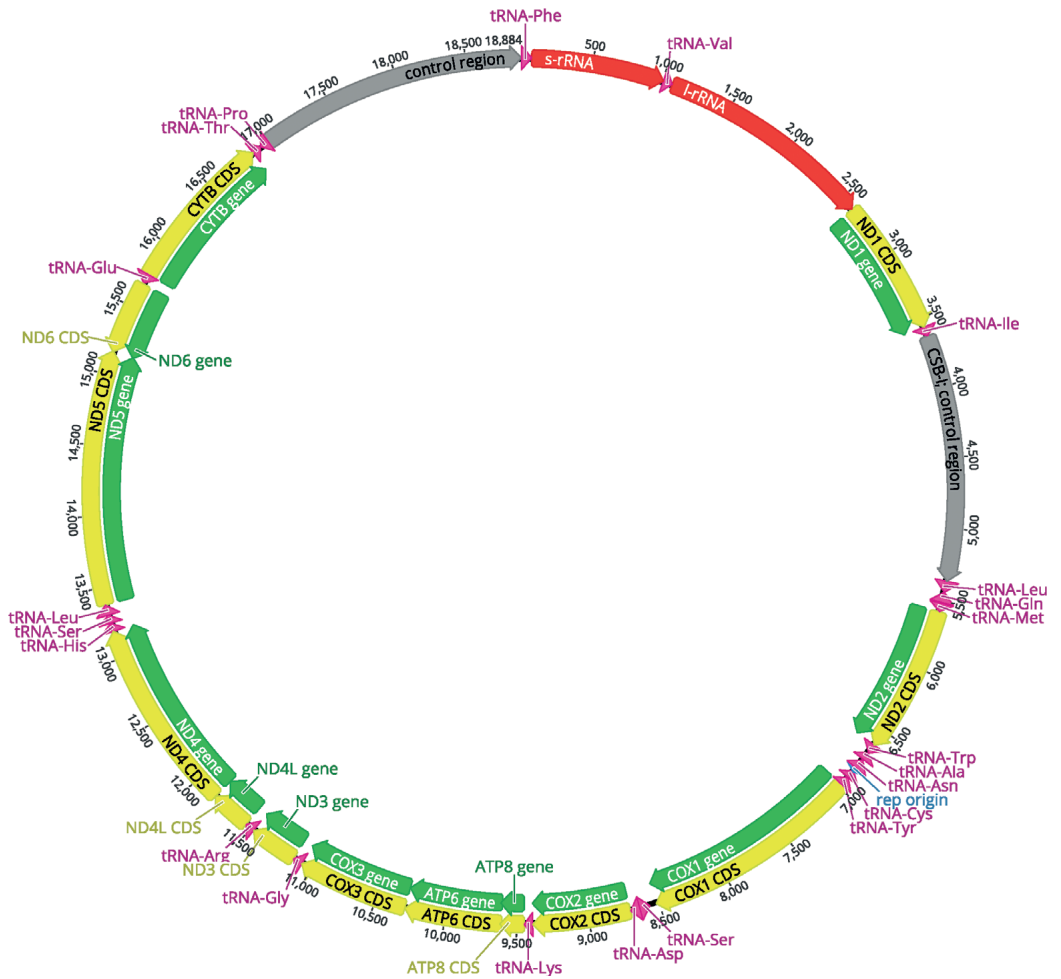
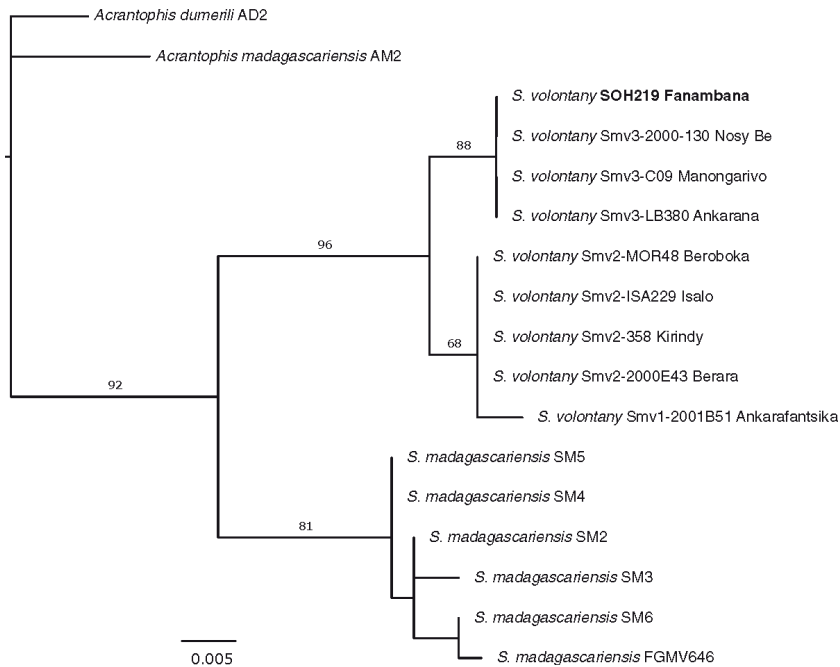


Fig. 2. The circularized mitogenome of *Sanzinia volontology* from the Fanambana forest.

of 18884 base pairs and the same gene order as the booid snake *Eryx tataricus* (MK780743) and *Boa constrictor* (NC007398). A comparison with partial 16S sequences of other *Sanzinia* individuals from GenBank in a phylogenetic tree reconstructed in IQtree 1.6.12 with 1000 ultrafast bootstrap repeats after alignment in MAFFT v.7 (Katoh et al. 2009, Nguyen et al. 2015, Minh et al. 2020) revealed its nesting in *S. volontology* (Fig. 3), and its haplotype was identical with individuals from the northern clade (Nosy Be, Manongarivo and Ankarana). The combined evidence of the typical *volontology*-like colouration and its mitochondrial DNA leaves no doubts that this individual belongs to *S. volontology*, resulting in a substantial range extension for this species into the northeastern coastal area. This larger distribution range is further supported by

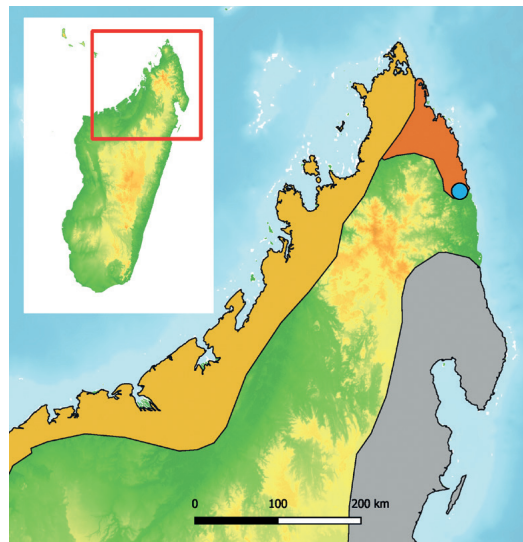
additional *Sanzinia* records from the region north of Fanambana, which have been tentatively assigned to *S. volontology* by Raselimanana et al. (2018). These records refer to the protected areas Loky-Manambato, Analamerana, and Andrafiamera-Andavakoera, but so far no genetic data or photographs were available to confirm these tentative species determinations.

The Fanambana forest is located 95 km northeast of the nearest genetically identified population of *Sanzinia madagascariensis* in Marojeje National Park and 120 km southeast of the nearest genetically identified population of *S. volontology* in the Ankarana special reserve. The potential contact or hybrid zone of both species is therefore expected to be somewhere between Fanambana and Marojeje (Fig. 4).



**Fig. 3.** A phylogenetic tree calculated from DNA sequences of the mitochondrial 16S rRNA gene (alignment length 489 bp) for 14 samples from GenBank and one sample from Fanambana (bold) of the booidean snake genus *Sanzinia*. DNA sequences of *Acrantophis dumerilli* and *A. madagascariensis* were used as outgroup. Bootstrap support values are given above branches.

The Sorata massif (now included in the new protected area COMATSA Nord) and its adjacent mountain chains running to the south are connected with the Tsaratanana massif and form a strong biogeographic barrier, which might prevent most faunal exchanges between the west (Sambirano region) and east coast in northern Madagascar. It is therefore likely that most of this exchange has occurred north of these mountains, for example through the corridor between the Sorata massif and the east coast. The Fanambana forest, which is located in this corridor area, might therefore harbour a unique faunal assemblage of species from the east coast, the Sambirano region and from the north, and is of special interest for biogeographical research. However, the degraded rainforest of Fanambana is not listed in the protected area book of Goodman et al. (2018) and is apparently still unprotected, although most of the low-altitude rainforest in this area has already been lost. During our short visit to this forest, we observed ongoing logging activity, with men extracting large logs from the forest. Though brief, our survey revealed that the Fanambana forest might harbour a rich herpetofauna beside *S. voluntary* and is certainly worth protecting as a nature reserve to ensure its long-term conservation.



**Fig. 4.** Map of the ranges of *Sanzinia voluntary* and *S. madagascariensis* in North Madagascar. Ochre: previously known range of *S. voluntary*, blue circle: Fanambana forest, brown: suggested range extension of *S. voluntary* based on the new record, grey: previously known range of *S. madagascariensis*.

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