

# Review of: "Ancient DNA Clarifies the Identity and Geographic Origin of the Holotype of the Genus *Ctenomys*"

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**Potential competing interests:** No potential competing interests to declare.

The work by Mestri and colleagues addresses two areas of significant interest within Latin American mammalogy. On one hand, it focuses on the taxonomy and historical distribution of caviomorph rodent species, which have been studied from countless biological aspects and remain relevant due to the vast diversity present. On the other hand, it highlights the application of novel methodologies that are clearly necessary in cases like this, where traditional taxonomy and conventional methodologies have failed to provide reliable answers from an evolutionary perspective. The study utilizes ancient DNA alongside increasingly modern and comprehensive sequencing techniques, contributing valuable information to historical phylogeographic studies.

The work is very interesting, methodologically well-structured, careful, and clearly provides relevant information. I commend the authors and recommend its publication. I would like to make a few minor comments and one more substantial comment regarding the discussion.

**Minor Comments:**

- It is preferable not to reference a map in the introduction if it is merely a comment, as the map is figure 2 and figure 1 follows.
- Similarly, in the morphological quantitative analysis section, it is unnecessary to reference table 2 in Materials and Methods (MyM) because table 2 contains results; it does not show the number of individuals and their provenance, where referencing it would be useful.
- Again, in the discussion, the references to figures are confusing. Figure 2 is mentioned followed by figure 1; however, this last mention lacks justification in that context and should refer to figure 2 (unless it is a typographical error).

**Major Comment in Discussion:**

The paragraph related to the karyology of voucher 431 of *C. minutus* under discussion is interesting because it presents information from various data sources that is crucial for a better characterization: "This particular area coincides with the closest DNA sequence found for *C. minutus* (voucher 431) compared to *C. brasiliensis* in our current study (marked in red in Fig. 2), sharing the Cyt b-haplotype H1. Voucher 431 has a diploid number of  $2n=46a$ . *Ctenomys minutus* exhibits high karyotype polymorphism, with chromosomal variation categorized into eight parental karyotypes ( $2n=50a$ ,  $48c$ ,  $46a$ ,  $48a$ ,  $42$ ,  $46b$ ,  $48b$ , and  $50b$ ). According to Lopes et al. , the  $2n=46a$  mtDNA haplogroup belongs to the Coastal and Barros

Lake regions, including specimens collected at Gaivota Beach, Passo de Torres, Guarita Beach, Barco Beach, and Tramandaí.”

However, this paragraph does not continue to expand or compare information related to the specimen studied and analyzed in this work (obviously because it is not possible to perform karyology on a museum specimen from which neither blood nor bone marrow can be extracted for chromosomal analysis). Therefore, it unfortunately loses relevance and even confuses the reader in the discussion section. To enhance the utility and significance of the cytogenetic information, which is clearly interesting in tuco-tucos, a section on the karyology of the species group could be incorporated. This would provide greater support for the aforementioned paragraph, which I reiterate is interesting as context regarding the variability of *Ctenomys* and the influence of chromosomal polymorphisms on the geographical distributions of the species; however, mentioned in this way, it loses value.