

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

Mauro N. Tammone¹

¹ Independent researcher

Potential competing interests: No potential competing interests to declare.

I have carefully reviewed the manuscript submitted by Renan Maestri and co-authors to the Qeios Open Science Journal. The article addresses an important issue by clarifying longstanding controversies surrounding the holotype of the type species of the genus *Ctenomys*. The study presents valuable data, is well-constructed, and well-written, although I recommend revising the English writing. Its relevance lies in the authors' ability to revisit the holotype specimen and gain significant insights into the location of the original collection site. More importantly, they succeeded in obtaining DNA sequences of the specimen, and this leads to clarifying the taxonomic and phylogenetic position of this material.

Suggestion for Improvement:

Throughout the article, the authors refer to 'ancient DNA.' This term is typically reserved for much older specimens, often associated with prehistoric or archaeological contexts (see Hofreiter et al. 2001; Rizzi et al. 2012; Raxworthy and Smith 2021; Jensen et al. 2022). It serves as a conceptualization of the temporal dimension in which the individual from which the molecular DNA sequences were obtained existed, reflecting its antiquity. In this context, I suggest using "historical DNA," as this term is more appropriately applied to specimens housed in museum collections, which generally date back to the last few centuries.

Hofreiter M, Serre D, Poinar HD, Kuch M, Paabo S. 2001. Ancient DNA. *Nature Reviews Genetics* 2(5):353-359.

Jensen EL, Diez-Del-Molino D, Gilbert MTP, Bertola LD, Borges F, Cubric-Curik V, de Navascues M, Frandsen P, Heuertz M, Hvilsom C et al. 2022. Ancient and historical DNA in conservation policy. *Trends in Ecology & Evolution* 37(5):420-429. 10.1016/j.tree.2021.12.010

Raxworthy CJ, Smith BT. 2021. Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution* 36(11):1049-1060. 10.1016/j.tree.2021.07.009

Rizzi E, Lari M, Gigli E, de Bellis G, Caramelli D. 2012. Ancient DNA studies: new perspectives on old samples. *Genetics Selection Evolution* 44(DOI: 10.1186/1297-9686-1144-1121).

Other minor comments that I suggest to be addressed:

1. Introduction: when saying "Nonetheless, DNA analyses of museum specimens have proven to be a valuable resource for discovering new species and genera[2]" I recommend including also the process of extinctions, as for example a

study presented by me and other co-authors in 2016 on *Ctenomys sociabilis* (Tammone et al. 2016). Tammone MN, Lavin, BR, Pardiñas, UFJ Lacey, EA (2016) Post-extinction discovery of a population of the highly endemic colonial tuco-tuco (*Ctenomys sociabilis*). J Mammal 97:1753–1763.

It is notable that the authors omit this reference, which is one of the first in the genus to sequence museum specimens.

1. It is striking that throughout the manuscript there is no photograph of the skull and skin of the holotype. Including these images would be very useful to illustrate the specimen's phenotype and provide additional documentation. The authors should consider including them.
2. In Figure 2A, the distribution for the genus should be redefined, as in Patagonia these animals have no distribution gaps, at least at the scale of this figure. The figure incorrectly indicates the absence of *Ctenomys* in portions of the provinces of Buenos Aires, Río Negro, Chubut, and Santa Cruz.
3. I agree with the other reviewers that information on the additional (six) samples analyzed should be included in the main text, including the collection site and whether vouchers exist.
4. The sequence of specimen *minutus_BEK NOSW 8* is not included in the cyt b tree but is included in the mitogenome tree. It should be added to the cyt b tree to strengthen the analysis, along with other sequences of *minutus*.
5. There is a possibility that *lami* could also be a synonym of *brasiliensis*. The authors indicate that further analyses are needed, but the supplementary figure SD2 suggests that the *lami* sample was either misidentified or that *lami* is indeed a synonym of *brasiliensis*. I recommend further discussion of this idea.
6. Notably, the discussion is missing a paragraph addressing the importance of considering the inclusion and revision of type material and museum specimens, as seen in studies such as the article on the extinct population of *C. sociabilis* in Patagonia or similar examples from other parts of the world (e.g., Tammone et al. 2016; Abramson et al. 2022).
Abramson N, Petrova T, Dokuchaev N. 2022. Analysis of “historical” DNA of museum samples resolves taxonomic, nomenclature, and biogeography issues: case study of true lemmings. Biological Communications 67(4).
10.21638/spbu03.2022.408