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 manuscript titled *"Ancient DNA Clarifies the Identity and Geographic Origin of the Holotype of the Genus Ctenomys"* presents a comprehensive investigation into a long-standing taxonomic issue. By employing a combination of ancient DNA extraction, phylogenetic analysis, and geometric morphometrics, the authors successfully clarified the identity and geographic origin of *Ctenomys brasiliensis*. The work is methodologically sound, well-structured, and provides a strong argument supported by precise results. However, there are several areas where slight improvements in the presentation of methodology, structure, and clarity of the arguments could further strengthen the manuscript.

The methodology used in the manuscript is robust, with a detailed explanation of the DNA extraction, sequencing, and phylogenetic analysis processes. The use of ancient DNA techniques is particularly commendable, given the challenges associated with working on a 200-year-old holotype. The authors successfully navigate these challenges and provide a clear protocol for extracting and analyzing the genetic material. However, the text would benefit from more detailed information regarding the level of DNA degradation and how that influenced the extraction process. Additionally, while the innuPREP Forensic Kit used for DNA extraction is mentioned, it would be helpful to explain why this kit was chosen over other methods, particularly for ancient DNA, to aid reader comprehension. A more in-depth explanation of how the methodology was adapted to suit the nature of the old specimen would further enhance the manuscript's methodological transparency.

The manuscript is structured logically, with a smooth flow from the introduction to the results and discussion. The progression of the study is easy to follow, and the use of subheadings in the methods and results sections enhances clarity. However, the introduction could be expanded to offer a more robust context for why resolving this taxonomic confusion is essential in a broader sense. Connecting the issue of taxonomic clarity with more significant questions, such as biodiversity conservation or species management, would make the study's relevance more apparent to the reader. Furthermore, in the results section, the presentation of data could be refined to make it more accessible. Breaking down complex information into more digestible parts or adding further visual aids could assist the reader in navigating dense material, particularly phylogenetic and morphological data.

The arguments presented in the manuscript are strong and well-supported by the data. The authors provide compelling evidence for synonymizing *Ctenomys minutus* with *Ctenomys brasiliensis*, and the combination of genetic, morphological, and historical geographic data is persuasive. However, the discussion could benefit from an earlier and more explicit statement of the key findings. Presenting the main conclusion—namely, that *Ctenomys minutus* is a junior

synonym of *Ctenomys brasiliensis*—more prominently at the beginning of the discussion would help to ensure that the reader immediately grasps the significance of the findings. Additionally, while the results are robust, it would enhance the discussion to address potential limitations or alternative interpretations of the data briefly. For example, given the historical uncertainties surrounding geographic names, addressing how these factors could influence the interpretation of the data would provide a balanced and critical approach to research.

In conclusion, the manuscript contributes to the field of mammalian taxonomy by resolving long-standing taxonomic ambiguity with a rigorous and multidisciplinary approach. Minor revisions, particularly regarding the expansion of contextual information in the introduction, a more detailed presentation of the methodology, and a more explicit framing of the key findings in the discussion, would elevate the manuscript further. These adjustments would make the work more accessible to a broader audience and enhance the clarity of the manuscript's main arguments. The English used in the manuscript is of a high standard, with no notable issues regarding grammar or phrasing.

The manuscript is well-prepared and provides a robust and well-reasoned argument supported by comprehensive data. After minor revisions, it is highly recommended for publication.