

# 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.

Dear authors, I post here some comments (I made "comment(s)" to some part(s)/figures/etc. of the "Text") that I expect you find useful. I accessed the manuscript on October 25<sup>th</sup>.

## Introduction

Text: "...the lack of DNA or difficulties in extracting DNA for old preserved specimens presents further obstacles to accurately identifying the type species..."

Comment: I am not a native English speaker, but I think it would be "to accurately identify". Also, from this sentence, it seems to be understood that the only way to identify a species is through DNA. I believe that both internal and external anatomy should be recognized as a valid way to recognize species (e.g., external measures, skin, measures on bones, detailed descriptions on anatomy). I saw that some lines below you stated that the DNA is a "crucial source of information"; that's better for me!

Text: "...Furthermore, older preserved material often presents greater difficulty in DNA extraction from remaining tissues and may feature outdated, incomplete, or non-standardized species descriptions..."

Comment: What does "remaining tissues" mean? Did you want to say "preserved tissues"? And how does "...older preserved material..." "...may feature outdated, incomplete, or non-standardized species descriptions..."?

## Figure 1.

Comment: It would be nice to see an image of the entire specimen.

Text: "...In addition to the holotype, specimens from six other *Ctenomys* species were included in this study (Appendix I) ..."

Comment: Could you indicate these species here, in the main text?

Text: "...alongside related complete mitochondrial genomes of six species of ctenomids analyzed for the first time in this study..."

Comment: Replace ctenomids by ctenomyids (here, and in other places along the text).

Text: "...cover all nine species groups within the genus..."

Comment: Parada et al. described 8 groups (Fig. 3)

Figure 3 legend: "...the tree was rooted with representatives of Octodontids..."

"...Terminal labels indicate species names and their corresponding identification (including GenBank accession numbers) ..."

Comment: It should be "octodontids". The corresponding identification is the GenBank accession number, isn't it? (Legend of Fig. 4 has the format I am thinking of). Why did you include a "sp" specimen (xingu)?

Figure 4.

Comment: It is read "C. scaglia", please add the ending "i"

Text: "...The voucher CML431..."

Comment: Which is this voucher?

Figure 5 legend. "...nine species group (as defined by Parada et al., 2011..."

Comment: In some places, you state that Parada and colleagues defined 8 or 9 groups.

Figure 6.

Comment: Why did you use a satellite image instead of the map shown in Fig. 2?

### 3.2. Quantitative morphological comparison

Comment: There is no image showing shape variation among species (i.e., deformation grids)

Comment: Why did you not use more than one specimen (of *C. brasiliensis*) for MG analyses? Can you give a list of "referred specimens" in the taxonomy section? And which localities are those referred to in the Distribution section?

### Discussion

Text: "...Th. Bischofp..."

Comment: "Th. Bischoff"?

Text: "...According to Nehring[44], the three specimens of ctenomyids from Brazil, initially identified by Th. Bischofp as *Ctenomys brasiliensis* Blainville[20], were subsequently examined by himself[44]."

Comment: This sentence is not clear (to me). Nehring examined three specimens that were initially identified by Bischofp(f)? Which are these three specimens? Are those of the first paragraph? Maybe you can unify both paragraphs.

Text: "...Surprisingly, he did not include *C. brasiliensis* in this comparative analysis..."

Comment: That's because Nehring considered the first(?) three specimens described as *C. brasiliensis* (including the holotype?) as *C. minutus*?

Text: "...Therefore, taxonomic decisions regarding this issue cannot rely solely on morphometry, whether through linear measurements or geometric morphometric analyses..."

Comment: It would be nice to see some figure(s) showing cranial morphology of the holotype of *C. brasiliensis*, *C. minutus*, and some other species. I agree that measures and/or GM data are not completely useful for taxonomic purposes that usually rely on detailed anatomical descriptions.

Appendix I.

Comment: What are the letters and numbers in parenthesis (e.g. "(CML 431)")? I think they are the voucher identification (i.e. collection ID)? Could you clarify this?

Table SD1.

Comment: Why are Genbank accession numbers and the voucher for the holotype not indicated?

Supplementary Data SD3, Data Table

Comment: In the name list, the species name should be separated from "C." with a space.

Text: "...7-8- anteriormost point of the root of the zygomatic arch..."

Comment: I do not think that the anteriormost point of the root of the zygomatic arch can be seen in dorsal view (at least, not always). Why not define these points as the intersection between the lateral margin of the rostrum and the anterior margin of the zygomatic arch (or something similar)?

Text: "...9- suture between nasals and frontals..."

Comment: I think it would be better to define this point as the posterior end of the nasals' suture.

Text: "6-7- anteriormost point of the root of the zygomatic arch; 8-9- anteriormost point of the orbit in the inferior zygomatic root..."

Comment: Are there different roots of the zygomatic arch? And I am not sure that 8-9 correspond to the orbit but rather to the space for muscular accommodation. It can be defined as the posterior extension (together with landmark 2) of the inferior root of the zygomatic arch.

Text: "...13- posterior extremity of the III molar alveolus..."

Comment: It is unusual to see the third molar named as "III"

Text: "...14- posterior extremity of the suture between the palatines..."

Comment: Can it always be observed? You can define it as "posterior (midsagittal) tip of the palate" or "Middle point of the palatine torus"

Comment about selected points: Why did you not use the posterior part of the cranium? Is it because the holotype MNHN-ZM-MO-1988-271 is a partial skull? Can you show some pictures of it (in the views analyzed at least)?

Results.

Comment: Why did you show only the first two PCs?