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.

This article illustrates the potential contribution of modern techniques for analysing ancient DNA to recurrent questions concerning the origin of specimens deposited in museums, in this case that of the first described specimen of the polyspecific genus of South American rodents *Ctenomys, the h*olotype of *C. brasiliensis* Blainville 1826. The exercise might seem gratuitous if it did not have repercussions on the correct taxonomic nomenclature according to the rules of anteriority. As the precise location of the type specimen (holotype) deposited at the Muséum National d'Histoire Naturelle (Paris) is particularly imprecise, the very identity of this species and its relationship with the other species of the genus described subsequently has been a recurring question in all the taxonomic literature concerning this genus for almost two centuries. Previous attempts to resolve the problem by geometric morphological analysis alone have proved unsuccessful, as the holotype of *C. brasiliensis* is very close to morphologically very similar species of the*torquatus* group within the genus *Ctenomys*. This article therefore uses ancient DNA techniques to sequence the complete mitogenome of the holotype obtained from a fragment of skin preserved dry for two centuries and to compare it with the complete mitogenomes of 6 other species of the genus obtained using standard techniques (Fig.3). A comparison extended to a larger number of *Ctenomys* species but limited to Cyt-b alone, thanks to the sequences available in Genbank, was also carried out (Fig.4). In addition, a morphological comparison of the holotype skull with those of individuals belonging to closely related species in the *torquatus* group was carried out.

The analysis clearly shows that the mitogenome of the holotype of*C. brasiliensis* is identical to that of *C. minutus*, an endemic species of the states of Rio Grande do Sul and Sta. Catarina. Further analysis of the Cyt-b haplotype network of 11 individuals related to *C. minutus* (Fig.6) shows that the holotype of*C. brasiliensis* has a sequence identical to that of one of the *minutus* individuals from the centre of the range of this species.

The authors therefore logically conclude that the geographical origin of the holotype is very probably within the range of*C. minutus*. They added to their analysis a number of historical considerations to explain the haphazard localisations that had prevailed until now. They also carried out a complementary morphological analysis (Procrustes distances), which confirmed that the holotype of *C. brasiliensis* is very close to the other species of the*torquatus* group. They therefore proposed that *C. minutus* should be placed in synonymy with*C. brasiliensis*, thus resolving a controversy that has existed for over a century.

Conclusion and remarks

The work reported here is technically of very high quality, and in view of the results, the proposed taxonomic revision put forward by the authors can be accepted as valid. It does, however, attract a number of comments that should be addressed in the paper if it is to be truly complete:

1- The authors implicitly make the assumption that the mitogenome is a faithful reflection of phylogeny, yet it is recognised that in certain groups of organisms at least, horizontal capture or transfer of mitochondria is commonplace, so that two different closely related taxa may momentarily share the same mitochondria. If this is not the case in *Ctenomys*, this should be mentioned and explained.

2- The Cyt-b of *C. minutus* shows great molecular diversity (Fig.6). Haplotype H8, for example, has around 30 nucleotide substitutions compared with the *C. brasiliensis* holotype (H1), according to the haplotype network. Compared with the 1140 bp of Cyt-b, this represents a divergence of around 3%. The question raised is therefore that of the uniqueness of *C. minutus* as a single species, and as it is not clearly indicated where the holotype used for the initial description of *C. minutus* comes from, the present proposal could prove invalid in the future if *C. minutus* were itself split into 2 or more species, especially as there are apparently several chromosomal races in *C. minutus*.

3- To give the reader an idea of any close sympatry or parapatry that may have distorted the results, due, for example, to mitogenome transfer, Fig. 2C lacks the ranges of closely related species in the *torquatus* group, particularly those that are genetically or morphologically very close, such as *C. lami* (Fig. 5) and *C. roigi* (Table 2) respectively.

Minor points:

- The name "August Saint-Hillaire," which appears in the text, should be spelled correctly as "Auguste de Saint-Hilaire."

- The MNHN (Paris) should be referred to as the "Muséum National d'Histoire Naturelle" and not as the "National Museum of Natural History," which is an institution based in Washington, DC.

- Apparently, the links to the additional data are missing on the corresponding PDF.

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