Note: this is a preprint version of the exchange published in *Folia primatologica*, 72 (6): 342 – 344.

To the Editor:

In a recent contribution to your journal, Montagnon and colleagues (Montagnon et al., 2001) have reported DNA sequences from the mitochondrial cytochrome *b* gene that were putatively sequenced from a specimen of the extinct Malagasy primate *Megaladapis*. The authors conclude that *Megaladapis* groups with the five species of *Lepilemur* sampled by their study with 100% bootstrap support. In their report, the authors comment upon results published by myself and colleagues (Yoder et al., 1999) in which we also claim to have obtained sequences from the same extinct taxon for the same gene. I wish to both clarify their interpretation of our report as well as to comment on their results and lack of agreement with those from our study.

In the first place, Montagnon et al. misinterpret our conclusions. They state that we propose grouping *Megaladapis* with *Paleopropithecus*, when in fact, we state clearly in the abstract (p.1) that "Our analyses suggest that *Palaeopropithecus* is sister to the living indrids as predicted by morphological studies. Contrary to morphological data, however, *Megaladapis* appears to belong to an independent lemuriform lineage rather than form a clade with *Lepilemur*." Indeed, it is this last sentence that is most interesting with respect to the Montagnon et al. results. In an analysis of a 550 bp alignment of cytochrome *b* sequences, our study found that several potential topologies could be supported, depending on the taxon sample and how the data were analyzed (e.g., transversion weighting, equal weighting, etc.). Although we did publish one tree (out of six) that shows a clade formed by *Paleopropithecus* and *Megaladapis*, we carefully explained why we believe this result to be false.

This misinterpretation of our conclusions is actually rather trivial, however. The more significant issue is why do the results of these two studies, for a roughly-equivalent amount of data, the same genetic marker, and a similar selection of taxa, differ so markedly? It is probable that the answer lies more in the realm of lab technique than in the realm of species sampling. By examining the methods of the Montagnon et al. study, one is forced to recognize the possibility that *Megaladapis* is grouping with *Leplilemur* due to contamination. In our study, we employed most of the extraordinary contamination control methods mandated by the rigors of ancient DNA analysis (Cooper and Poinar, 2000), including a physically-isolated ancient DNA laboratory, verification by independent personnel in a geographically-separated laboratory (T. Parsons, Armed Forces DNA Identification Laboratory), as well as by the acquisition of DNA sequences from multiple individuals of *Megaladapis*. With regard to the latter precaution, we have obtained homologous cytochrome *b* sequences for three individual *Megaladapis* specimens (Université d'Antananarivo, Département de Paléontologie et d´Anthropologie Biologique catalog numbers 4543, 4821, and 4822), finding that the sequences match expectations for different individuals from the same species (i.e., they are extremely similar, though not identical). None of these controls were employed by the Montagnon et al. (2001) study. Rather, the putative *Megaladapis* sequence comes from a single individual that was presumably processed in the same laboratory wherein biochemical analysis of modern lemurs, including *Lepilemur*, is conducted. Although the point raised by Montagnon et al. is laudable, that species sampling can have a large effect on phylogenetic analysis, it nonetheless seems that the most reliable ancient DNA sequence data come from the Yoder et al. (1999) study. Those data indicate that *Lepilemur* and *Megaladapis* do not share an especially close phylogenetic relationship, and therefore, do not warrant shared recognition as a separate family Megaladapidae.

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Yoder, A. D., B. Rakotosamimanana, and T. J. Parsons. 1999. Ancient DNA in subfossil lemurs: methodological challenges and their solutions*in* New Directions in Lemur Studies. Plenum Press (B. Rakotosamimanana, H. Rasaminanana, J. Ganzhorn, and S. Goodman, eds.)., New York.

To The Editor :

In a letter to the Editor, A.Yoder reported that we misinterpreted the conclusions of the paper concerning the respective phylogenetic position of *Megaladapis* and *Paleopropithecus*. Although *Megaladapis* and *Palaeopropithecus* appeared to be grouped in one of the trees obtained by Yoder et al., in their final conclusion these authors indicate that "*Palaeopropithecus* joins the *Indri* clade and that *Megaladapis* is another independent long branch..... and not sister to *Lepilemur*...". In our paper the term grouping was effectively miss-used.

I agree with Yoder that the risk of the contamination is one of the major problems in ancient DNA studies; in this view our extractions and amplifications on *Megaladapis* were carefully performed and the final amplified sequences tested. The *Megaladapis* sequence submitted to EMBL was checked against all Mammals sequences on EMBL (including more than 200 *Lepilemur* sequences determined in our Laboratory) using the FASTA33-t software. The results clearly indicate that the *Megaladapis* sequence differs from all the haplotypes determined in our lab and that the maximum identity scores which was found ,with two *lepilemur* sequences, differed by 14 and 15 nucleotides. This result argues against a contamination by living *Lepilemur* sequences.

The remaining open question is now how we can explain the discrepancies observed between the data of the two teams and I agree also with Yoder that the answer lies probably in the realm of lab technique. One explanation could be found in the differences of DNA extraction techniques used in the two labs The method used in Strasbourg , especially developed by Ludes for ancient DNA analyses, and fruitfully used for human bones from ancient necropoles;(Fily M.L. et al 1998); allows to obtain a maximum of DNA fragments of which some could be amplifiied with the same primers as those used for living species. Thus it was us possible to align complete sequences of the same length without undetermined nucleotides and we consider that ,at the moment, the most reliable ancient DNA sequence data of *Megaladapis* come from the Montagnon et al (2001)study.

A future collaborative exchange of information and samples between the two labs should probably help to resolve this problem.

Montagnon et al.

References cited:

Montagnon, D., B. Ravaoarimanana, B. Rakotosamimanana, and Y. Rumpler. 2001. Ancient DNA from Megaladapis edwardsi (Malagasy Subfossil): Preliminary Results Using Partial Cytochrome b Sequence. Folia Primatol 72:30-2.

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