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THE RED PANDA AND CSERHATI (16): CONCLUSIONS AND DISCUSSION IN THE CRSQ ARTIKEL

In his CRSQ article "*Classification of the Enigmatic Red Panda (Ailurus fulgens) Based on Molecular Baraminology-Based Analysis*" Cserhati aims to place the red panda in a baramin on the basis of moleculair methods:

Thus, molecular baraminology is the study of the created kinds from a molecular biology perspective.

An important point is that 'kinds', baramin, were created separately. For creationists it follows from 'created separately' that differences between the 'kinds' must still be visible today.

Hence, there is continuity between species within a kind, and discontinuity between two separate kinds

not ex hypothesi, but per axioma.

A 'kind' is identified by finding a 'holobaramin', a hypothesis for a baramin.

Cserhati translates the idea of 'continuous' into statistics, into groups formed by statistical clusters.

All (three) of these groups show statistically significant continuity within themselves and discontinuity with all other species in this study.

Cserhati's question is whether the red panda clusters consistently with other species. If so, the red panda can be assigned to a holobaramin with these species.

Conclusion WGKS analyse

The WGKS analysis produced three clusters, the cat family Felidae, the bear family Ursidae and the superfamily Musteloidea. Cserhati considers these clusters to be hypotheses for holobaramin:

Based on this result, three putative baramins can be defined: felids covering the family Felidae (eleven species), ursids covering the family Ursidae (five species),

and musteloids, a superfamily including Mephitidae and Mustelidae (twelve species).

The red panda is *a*pparently moved into the family Mustelidae, without further explanation. Not the family Mustelidae, but the superfamily Musteloidea is here considered a holobaramin.

Conclusion mtDNA analysis

Cserhati arrives at five clusters based on mtDNA; the five clusters correspond to the five families of the standard taxonomy: bears, skunks, raccoons, red panda and mustelids. That should have led to five holobaramin, with the red panda as its own holobaramin, but Cserhati doesn't draw this conclusion. He searches for literature that seems to support the placement of the red panda among the mustelids, and gives two references.

1 However, some authors have found that <u>A. fulgens</u> is related to mustelid species. For example, Peng et al. (2017) placed <u>A. fulgens</u> next to <u>Martes americana</u>, the American marten in an analysis of 13 concatenated mtDNA proteins.

Peng et al (2017) use the superfamily Musteloidea as an outgroup for their study of bears, with one species from each of the four families of the Musteloidea: the American marten for the Mustelidae, the striped skunk for the Mephitidae, the raccoon for the Procyonidae and the red panda for the Ailuridae. In one of their two analyses, Peng et al (2017) list the red panda *Ailurus fulgens* as related to the American marten, but as a sister group of the American marten, not as a mustelid. In the other analysis, Peng et al (2017) find the red panda as a sister group of the striped skunk. Peng et al (2017) is an evolutionary study, and then relatedness is not limited to the family, but relatedness also exists between families.

2 Based on a study of the 12S rRNA, the 16S rRNA, and cytochrome-b, Flynn et al. (2000) also classified <u>A. fulgens</u> as a mustelid.

Flynn et al (2000) classified the red panda not as a mustelid, but as a musteloid. Flynn et al (2000) wrote:

Rather, evidence from nucleotide sequences strongly support placement of the red panda within a broad Musteloidea (sensu lato) clade, including three major lineages (the red panda, the skunks [mephitids], and a clearly monophyletic clade of procyonids plus mustelids [*sensu stricto*, excluding skunks])

Cserhati is inaccurate in reading and makes errors in citing.

Cserhati searches in vain for support for his idea that the red panda belongs to the mustelids; such support cannot found in these two articles.

Conclusion amino acid sequence in the protein cytochrome b.

Cserhati arrives at three clusters: cats, bears and mustelids + red panda; and two separate species, the striped skunk and the coati.

All three larger clusters show statistically significant continuity among themselves and discontinuity with all other clusters.

No silhouette plots are provided to substantiate three clusters of cats, bears and mustelids + pandas and two non-clustered species. As a result, it is also unclear whether mustelids + red panda can be considered a holobaramin.

Three different conclusions from three analyses

Cserhati is faced with three different outcomes in his three analyses. He tries to reconcile them in line with his third finding, that of cytochrome b, a clustering of mustelids with the red panda.

1 comparing results for WGKS and cytochrome b

For the WGKS data, Cserhati now mentions the possibility that there are four clusters, the three clusters (bears, cats and mustelids + red panda) and the Western spotted skunk *Spilogale gracilis* from the family Mephitidae separately. This was the possibility he gave in the BMC Genomics article, but did not mention in this CRSQ article. For cytochrome b Cserhati gives the same three clusters, and now the coati *Nasua nasua* of the family Procyonidae and the striped skunk *Mephitis mephitis* of the family Mephitidae separately.

2 comparing results for mtDNA and cytochrome b

The gene for cytochrome b is located on the mitochondrion. Cserhati wants to compare changes in part of the mitochondrion with changes in the mitochondrion as a whole; and comparing differences in an amino acid sequence with differences in a DNA sequence. Of course, there is much less difference between species in the amino acid sequence than in the DNA of cytochrome b. In fact, there is so little difference between the species that the amino acid sequence of cytochrome b hardly provides enough differentiation between the species to work with. This can be clearly seen in the low resolution and fuzzy patterns in figure 4 of the CRSQ article (figures 15 and 16

blog post 15). A difference between the mtDNA and the cytochrome b analysis does not occur because mtDNA has too many mutations, but because the "structurally conservative" (page 80) cytochrome b protein gives too few changes for a clear analysis. Table 1 shows that there are relatively few differences between species in cytochrome b.

TABEL 1	minimum correlation	mean correlation
WGKS	0.666	0.838
mtDNA	0.751	0.816
cytochrome b	0.852	0.921

3 comparing results for mtDNA and WGKS

Cserhati mentions the possibility that mtDNA does not provide good material for an analysis because it mutates too quickly. To demonstrate rapid mtDNA mutation, he compares the mitochondrial DNA of cytochrome b with an exon of the same length of a gene RAG1 (Recombination Activating Gene 1) that is located in the nuclear genome. It is not mentioned why RAG1 was chosen. More mutations have occurred in the DNA for cytochrome b than in the DNA for RAG1.

This is another indication that mtDNA mutates faster than nuclear DNA.

This is not formulated correctly. Cserhati's comparison is of two protein-coding DNA sequences, one on the mitochondrion and one in the nuclear DNA. Thus, the comparison of mutation rate is not for all DNA in mitochondria and nucleus, but only for protein-coding DNA.

This difference in mutation rate is a well-known phenomenon. The textbook 'Fundamentals of Molecular Evolution' (1990) states on page 86 that the mutation rate in mammalian mtDNA genes is about 10x higher than in nuclear genes (exact quote at the end here). This has since been found dozens of times. A reference to a textbook would have sufficed.

However, then Cserhati says:

This can explain why the WGS, mtDNA, and cytochrome-b results are divergent.

If there were more changes in the mtDNA than in WGKS, the mean crossspecies correlation and the minimum cross-species correlation should be greater for WGKS than for mtDNA. Table 1 shows the mean and minimum of the correlations between all species pairs of bears and Musteloidea in the three analyses. WGKS and mtDNA have about the same range in interspecies correlations.

Although WGKS is a kind of measure over the entire genome, it only depends to a small extent on base pair mutations in protein coding genes. The mutation rate in the nuclear protein coding genome is not decisive for the interspecies differences reflected in the WGKS.

The difference between the detailed heatmap of the mtDNA analysis and the coarse scale heatmap of the WGKS analysis is not because there are more mtDNA mutations. The WGKS results have less resolution than the mtDNA analysis, because there are only few species in the WGKS analysis. Therefore, it is difficult to find clusters within the superfamily Musteloidea.

family	WGKS	mtDNA
Ursidae	5	15
Mustelidae	10	30
Procyonidae	0	2
Mephitidae	1	3
Ailuridae	1	2
	17	52

Alternative conclusion

Another possibility would have been to regard the superfamily Musteloidea as a holobaramin. Cserhati chooses not to do that.

The superfamily Musteloidea as holobaramin is the first outcome of the WGKS analysis. It is also the result of the mtDNA analysis, if we keep the two clusters bears and Musteloidea as indicated by the silhouette plots (see blog post 15). For the amino acid sequence of cytochrome b, it is impossible to say how good the possibility of a cluster of Musteloidea is, in the absence of silhouette plots. The heatmap leaves open the possibility of a Musteloidea cluster.

The discussion of the CRSQ article

Cserhati prefers his interpretation of the WGKS analysis.

The WGKS analysis seems to bring the strongest results, since it is a global analysis of the entire genome. According to this analysis, <u>A. fulgens</u> belongs to the

mustelid holobaramin. Also, <u>M. mephitis</u> could either belong to the mustelid holobaramin, or it could either belong to another holobaramin, due to the minimal differences in average silhouette width values.

As part of the results of the WGKS analysis, Cserhati gave a Musteloidea baramin (pages 78, 79) based on clustering of 10 species from the family Mustelidae, the red panda *Ailurus fulgens* and the western spotted skunk *Spilogale gracilis*. (*Mephitis mephitis* represents the family Mephitidae in the other two analyses; Cserhati is confused about his own data). If *Spilogale gracilis* is removed from that cluster, the red panda will continue to cluster with the 10 species of mustelids.

Cserhati is allowed to call any cluster a holobaramin – as holobaramin is a fantasy term -, but the problem is 'mustelid'. Calling the cluster 'mustelid' suggests that the red panda is part of the Mustelidae family, and Cserhati fails to demonstrate that. A clustering does not give a classification.

Cserhati gives a reference to a study by Nie et al (2002); Cserhati presumably intended to indicate this citation to argue a close relationship between red panda and the family Mustelidae.

Genomically, <u>A. fulgens</u> shares several apomorphic chromosome fusions with mustelids, namely F2+C1p and A1p+C1q (Nie, 2002). However, <u>A. fulgens</u> differs in several other chromosomal rearrangements indicating that it diverged early from the mustelids.

Nie et al (2002) examined the chromosomes of the domestic cat, the red panda and five species of the marten family. Nie et al (2002) considered the domestic cat, the red panda and the five mustelids to belong to three different families. In a study based on these five species the mustelids and the red panda are more similar to each other than any of these are to the domestic cat; as is obvous given the phylogenetic tree of the order Carnivora. The domestic cat comes from the main division Feliformia of the order Carnivora, the mustelids and the red panda from the alternative main division, the Caniformia. Procyonidae and Mephitidae are not present in the study by Nie et al (2002) - and therefore nothing whatsoever can be said about how related the red panda and mustelids are. The study by Nie et al (2002) cannot be used to place the red panda closer to the mustelids than to the other families of the superfamily Musteloidea. Cserhati's phrase "indicating that it (red panda) diverged early from the mustelids" is from Cserhati, not from Nie et al (2002).

There is another possible conclusion: that mtDNA gives the best results.

Alternatively, <u>A. fulgens</u> could be the only known member of its own holobaramin, as mentioned in the Introduction and supported by the mtDNA results.

Cserhati interpretation of five clusters in mtDNA correspond to the scientific classification: species from five families were present in the mtDNA analysis. In fact, the mtDNA heatmap shows the neatest results.

Summary and Conclusion

Based on all of these considerations, it is likely that <u>A. fulgens</u> belongs to the mustelid holobaramin, and not the ursid holobaramin.

The problem here is the use of scientific terms such as 'mustelid' and 'ursid'. A 'mustelid' holobaramin contains a cluster of the family Mustelidae and the family Ailuridae. Everyone is free to provide clusters with names of their own choice: everyone is free to call a cluster of choice a holobaramin. After all, clustering is not biology, but statistics, and clustering is different from classification. Only, the suggestion is here that the scientific family Mustelidae is defined differently, namely including the red panda *Ailurus fulgens.* That would pose a scientific problem.

Cserhati, M., 2021, Classification of the Enigmatic Red Panda (Ailurus fulgens) Based on Molecular Baraminology-Based Analysis, Creation Research Society Quarterly 58 (2): 76-84

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Li, W-H, & D.Graur (1990) Fundamentals of Molecular Evolution. Sinauer Ass, Inc. pg 86: "The synonymous rate of substitution in mammalian mitochondrial genes has been estimated to be 5.7×10 -8 substitutions per synonymous site per year (Brown et al 1982). This is about 10 x the value for synonymous substitutions in nuclear protein-coding genes. "

Peng, R., B. Zeng, X. Meng, B. Yue, Z. Zhang, and F. Zou. 2017. The complete mitochondrial genome and phylogenetic analysis of the giant panda (*Ailuropoda melanoleuca*). Gene 397(1–2):76–83.

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