https://creationismeweersproken.blogspot.com/2023/01/de-rode-panda-encserhati-12.html

THE RED PANDA AND CSERHATI (12): PHYLOGENETIC TREES BASE ON MTDNA

Cserhati presents three 'hierarchical trees' based on mitochondrial DNA. He uses the complete mtDNA as reported in GenBank for 52 species: 15 species and subspecies of the bear family, the two subspecies of the red panda, three species of skunks, 30 species of the mustelid family, and now also two species of the raccoon family, the raccoon itself and the coati.

Cserhati uses three different methods to obtain his 'hierarchical trees'. Cserhati does not call his results a phylogenetic tree or phylogeny, see his captions to his figures.

So hierarchical trees, with three phylogenetic methods: UPGMA, NJ and ML. Phylogenetic trees, whatever Cserhati avoids to call them.

UPGMA and NJ use the differences = distances, between sequences.

UPGMA is the simplest and oldest method to construct a phylogenetic tree from sequence data. The major drawback of UPGMA is that this method assumes that the rate of change of the sequences is the same over time and across all lineages, ie over the entire phylogenetic tree. UPGMA produces an implicitly rooted tree.

In his Figure 4, Cserhati provides a UPGMA phylogenetic tree



Musteloidea

Figure 1: Figure 4 from Cserhati BMC Genomics. UPGMA phylogenetic tree.



Figure 2: Figure 4 from Cserhati BMC Genomics sequence of splits within Musteloidea indicated.

The legend to his Figure 4 as given by Cserhati is:

UPGMA-based hierarchical tree for the 52 species analyzed in the mtDNA study, based on sequence identity metrics. Mustelids and ursids form two large clades, and mephitids, procyonids forming two small groups. <u>Ailurus fulgens</u> and A<u>ilurus fulgens and Ailurus fulgens styani</u> appear either to form their own clade, or loosely associate with mustelids.

" or loosely associate with mustelids."

Very loosely associated.

This Figure 4 clearly shows that the family Ailuridae, with the two red panda subspecies *Ailurus fulgens* and *Ailurus fulgens styani*, appears as the sister group of the family Mustelidae. So their own clade, here as a sister group to the mustelids. Remarkably, the Ailuridae appear as a sister group to the mustelids in the presence of the Procyonidae, the raccoon family; this has no precedent in the literature.

Neighbor-Joining takes the distances between sequences, pairs the two sequences with the smallest distance, and continues with the pair as if it were a taxonomic unit. NJ always searches where the smallest distance can be found. The NJ method gives unrooted trees, without any assumption about rate of change over time.

An NJ program might plot an unrooted tree as a cladogram: the program then inserts a fictitious root to be able to establish a lay-out. It is up to the investigator using NJ to ensure that the tree is rooted, by providing an outgroup. Cserhati writes that he uses the default settings, and that means, no outgroup. An unrooted tree was plotted as a cladogram: the lengths of the horizontal lines are not a representation of the number of differences between the species. The layout gives on its own no conclusion about the phylogeny. Cserhati indicates how well substantiated the splits are, but that does not establish the phylogeny.



Figure 3: Figure 5 Cserhati BMC Genomics. Neighbour Joining phylogenetic tree plotted as cladogram. The horizontal distance between successive splits is the same; only the topology of the tree is of importance.

Cserhati's description of his result shows that he does not grasp the difference between a layout and a phylogenetic tree:

Mustelidae forms a well-defined clade, with almost all branch points supported with a bootstrap value of 100. <u>Nasua nasua</u> and <u>Procyon lotor</u> form a smaller clade right next to Mustelidae. The three mephitids, <u>Conepatus chinga</u>, <u>Mephitis</u> <u>mephitis</u> and <u>Spilogale putorius</u> also form a small clade, well separated from the other clades. The NJ method places Ailurus next to Ursidae, suggesting that they possibly form a monophyletic group. However, the node connecting Ailurus with Ursidae only has a bootstrap value of 45.

A phylogenetic tree is a mobile, and we can start flipping the Mephitidae, the skunks, upwards. We also have an unrooted tree, so we can put a root

between the bears Ursidae as outgroup and all the other species, all of which belong to the Musteloidea. The low bootstrap value of the node between bears and *Ailurus* also argues in favor of placing the root between *Ailurus* and bears.

We take the bears as an outgroup to the Musteloidea, and walk along the lines to find the successive splits in the Musteloidea. Then one of the well-known phylogenetic trees of the Musteloidea emerges, namely the tree in Flynn et al (2000).



Figure 4: Figure 5 Cserhati BMC Genomics rooted between bear family and superfamily Musteloidea; successive divisions in the Musteloidea numbered.

In blog post 2, a list of articles is presented that found this classification of the Musteloidea. The outcome of the NJ phylogenetic tree of Cserhati is part of this. Cserhati is misled by the layout that comes out of his default program.





Maximum Likelihood seeks a phylogenetic model that best fits the data. An initial tree is needed

Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances

This implies that Cserhati started by making an unrooted tree - and never got a root to root the phylogenetic tree. There is no outgroup in this ML phylogenetic tree.



Figure 6. Figure 6 from Cserhait BMC Genomics. Maximum Likelihood phylogenetic tree. The horizontal distance between successive splits now represents genetic distance

Cserhati's description of his result now indicates that the red panda may form a clade of its own:

Here Mustelidae, Procyonidae, and Mephitidae all form their own clades with a likelihood value of at least 94%. As opposed to the NJ tree, here Ailurus is separated from Ursidae suggesting that it might form its own clade as well.

The two red panda species form their own clade, also with a likelihood values of 94% (Figure 6).

The topology of the bears and the red panda is the same in Cserhati's NJ tree as in Cserhati's ML tree: the difference is that now the genetic distance is visible. Cserhati's "opposed" and "separated" is based on insufficient understanding of layout versus topology of the phylogenetic trees.

Of course we can consider the bears again as an outgroup to the Musteloidea, and place the root of the phylogenetic tree between bears and Musteloidea.



Figure 7. Figure 6 Cserhait BMC Genomics. Maximum Likelihood phylogenetic tree rooted between bear family and superfamily Musteloidea; successive divisions in the Musteloidea numbered.

Following the lines of the ML phylogenetic tree to find the successive splits in the Musteloidea yields the same classification as the NJ phylogenetic tree, namely the one in Flynn et al (2000).

Cserhati provides three phylogenetic trees on mtDNA of 15 bears and 37 musteloids. In all three trees the red panda forms its own group, a clade, the family Ailuridae. The red panda as a separate family was proposed in 1996 by Ledje and Arnason; since then, the red panda's status as a family Ailuridae has not been debated (except by Cserhati).

UPGMA gives a different phylogenetic tree, with the red panda Ailuridae and not the raccoon family Procyonidae as sister group of Mustelidae. The NJ phylogenetic tree and the ML phylogenetic tree give the Procyonidae as a sister group to the Mustelidae, as commonly found. The NJ and ML trees also provide a classification within the Mustelidae, in the placement of otters, weasels, badgers and martens, that is consistent with results elsewhere in the literature.

Cserhati concludes:

The mtDNA results as well as the maximum likelihood tree appear to place Ailurus fulgens into a monophyletic group.

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Perhaps: The clustering of the 52 species on their mtDNA and all three phylogenetic analyses of those 52 species on their mtDNA give the family Ailuridae, the red panda, as its own monophyletic group.

Cserhati, M., 2021, A tail of two pandas – whole genome k-mer signature analysis of the red panda (Ailurus fulgens) and the Giant panda (Ailuropoda melanoleuca), BMC Genomics 22: 228

Ledje C, & Arnason U. (1996) Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene. Journal of Molecular Evolution 43:641–649.