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## THE RED PANDA AND CSERHATI (5): THE INTRODUCTION OF THE BMC GENOMICS ARTICLE

The article by Matyas Cserhati in BMC Genomics has the title: **`A tail of two** pandas— whole genome k-mer signature analysis of the red panda (*Ailurus fulgens*) and the Giant panda (*Ailuropoda melanoleuca*)'

This suggests that the article will be about both the red panda and the giant panda. Cserhatis' abstract in BMC Genomics gives a different impression:

Background: The red panda (Ailurus fulgens) is a riddle of morphology, making it hard to tell whether it is an ursid, a procyonid, a mustelid, or a member of its own family. Previous genetic studies have given quite contradictory results as to its phylogenetic placement.

This tells us two things: the article is about the red panda *Ailurus fulgens*, rather than about the giant panda *Ailuropoda melanoleuca*, and presumably we are getting a genetic study to solve an outstanding morphological problem.



Figure 1 the giant panda Ailuropoda melanoleuca



Figure 2 the red panda Ailurus fulgens

The introduction of an article states more extensively than the abstract what the problem is and also what has been said about that problem before.

1

Cserhati begins by saying that the giant panda and the red panda have been considered related species. Cserhti writes: 'think' not 'thought'.

Some researchers think <u>Ailurus fulgens</u> is a relative of the giant panda (<u>Ailuropoda</u> <u>melanoleuca</u>) based on several physical characteristics. These include an almost exclusive diet of bamboo (both species eat meat on occasion), and have an enlarged radial sesamoid bone, which they use to process bamboo [1, 2].

What do these two articles [1] and [2] say about the relation between the two pandas?

Article [1], Flynn et al (2000), provides a historical overview of taxonomic work on both pandas. The giant panda belongs to the family of bears, Ursidae, as suggested on the basis of morphology by Davis (1964), on the basis of immunology by Sarich (1973), and on the basis of proteins, immunology, chromosomes, and a rough measure of DNA similarity by O'Brien et al (1985). In 1985, the placement of the giant panda with bears was considered a great success for molecular methods. After 1985, the placement of the giant panda is no longer a topic of discussion: the giant panda is a bear.

What about the red panda?. Flynn et al (2000) mention a number of possibilities for the classification of the red panda. Their abstract says:

...whether it (the red panda) should be placed with the bears (ursids), ....,raccoons (procyonids), musteloids or as a monotypic lineage of uncertain phylogenetic affinities

Some of these proposals were already out of circulation in 2000: the red panda as a sister group to the giant panda (1943, 1982) or the red panda as a relative of the bears ((1973, 1989, 1993, 1994). Flynn et al (2000) do not elaborate on this: they only give a historical overview, and in 2000 the relationship of giant panda and red panda was from a molecular point of view not a possibility.

Can a relationship between red panda and giant panda be found in article [2], Hu et al (2017)? The article by Hu et al (2017) is about convergence between the red panda and the giant panda; in other words, about their similarity in their diet of bamboo and their similarity in their false thumb despite being unrelated. Convergence means similarity but never relatedness. Hu et al (2017) write:

The giant panda belongs to the family Ursidae, whereas the red panda belongs to the family Ailuridae within the superfamily Musteloidea.

Hu et al (2017) state in so many words that the red panda and the giant panda are not related; and also where the red panda has been placed since at least 2000, with the Musteloidea.

Cserhati is not very accurate in citing these articles [1] and [2]. Neither gives the red panda as a relative of the giant panda.

## 2

Cserhati proceeds:

According to other opinions, *A. fulgens* has been classified as a member of the family Procyonidae (raccoons).

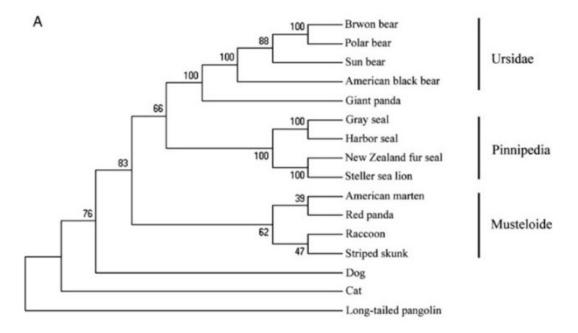
Cserhati does not give a reference here, but in Flynn et al (2000) we come across a number of references, starting with Geoffroy-Saint-Hilaire and Cuvier (1825) with "closely resembling a raccoon (procyonid)". Those references in Flynn et al (2000) show that raccoon and red panda are more often referred to as sister groups than that the red panda would be within the raccoon family Procyonidae. Only Slattery et al (1995) arrive at the placement of the red panda within the raccoon family, in two of their three analyzes of the same material. In later studies, the red panda is no longer found to belong to the raccoon family. Flynn et al (2000) cite earlier work by Flynn & Nedball (1998): they placed the red panda in the superfamily Musteloidea, but not in the raccoon family Procyonidae. Flynn et al (2000) also end up in the superfamily Musteloidea with placement of the red panda in the family Ailurida

3

It would have been logical if Cserhati after citing Flynn et al (2000) would have proceeded with the results of Flynn et al (2000). Cserhati however proceeds in a different direction:

For example, Peng et al. classify A. fulgens either as a mustelid, placing them next to the American marten (<u>Martes americana</u>), or as a mephitid, next to the striped skunk (<u>Mephitis mephitis</u>). This was based on the analysis of 13 concatenated mitochondrial proteins, based on neighbor-joining (NJ) and maximum likelihood (ML) phylogenetic methods, respectively [7].

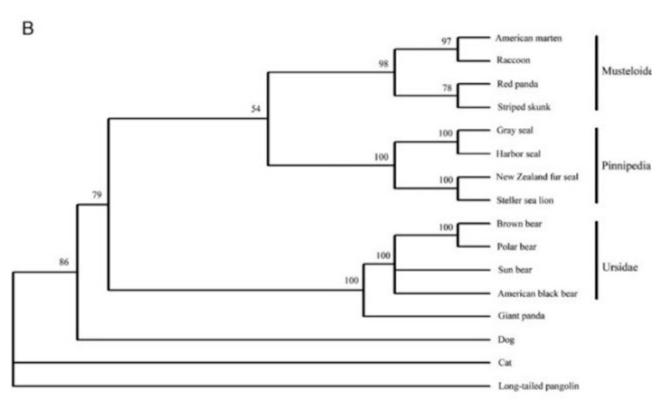
Peng et al (2007) present a phylogenetic analyses of the giant panda, against the background of quite a number of more or less related species. The superfamily Musteloidea is represented by one species per family: the red panda for itself as family Ailuridae, the raccoon for the Procyonidae, the striped skunk for the Mephitides and the American marten for the Mustelidae. Peng gives two analyses.



1 The NJ method:

Figure 3. Figure 2A from Peng et al (2007) : The Ailuridae (represented by the red panda) and the Mustelidae (represented by the American marter) as sistergroups.

The NJ analysis places the red panda as the sistergroup of the American marten; that is, the Ailuridae as the sistergroup of the Mustelidae. The American marten is the only species used from the family Mustelidae; it is therefore impossible to conclude that the red panda belongs to the family Mustelidae: *"classify A. fulgens ... as a mustelid*" is an erroneous interpretation.



2 The ML method:

Figure 4. Figure 2B from Peng et al (2007) The Ailuridae (represented by the red panda) and the Mephitidae (represented by the striped skunk) as sistergroups.

The ML analysis places the red panda as the sistergroup of the striped skunk; that is, the Ailuridae as the sistergroup of the Mephitidae . The striped skunk is the only species used from the family Mephitidae; it is therefore impossible to conclude that the red panda belongs tot het family Mephitidae: *"classify A. fulgens ... as a mephitid*" is an erroneous interpretation. Both interpretations by Cserhati of the results of Peng et al (2007) show faulty reasoning: a basic lack of understanding of phylogenetic trees.

4

Cserhati proceeds with:

Flynn et al. also found that A. fulgens is neither an ursid, nor a procyonid, nor a mephitid, but a mustelid [1].

Cserhati now refers to the conclusions of Flynn et al (2000), the article Cserhati borrowed his historical description from. However, Flynn et al (2000) give their conclusion in their abstract as:

Combined phylogenetic analyses reject the hypotheses that the red panda is most closely related to the bears (ursids) or to the raccoons (procyonids). 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 [Musteloidea *sensu stricto*, excluding skunks])

Flynn et al (2000) place the red panda as family Ailuridae in the superfamily Musteloidea; the red panda is a musteloid. Cserhati pretends that Flynn et al place the red panda in the family Mustelidae; if so, the red panda would be a mustelid.

Cserhati does not know or does not understand that musteloid and mustelid have two very different meanings. A musteloid belongs to the superfamily Musteloidea. A mustelid belongs to the family Mustelidae.

Pretending Flynn et al (2000) called the red panda a mustelid demonstrates great ignorance of taxonomy on the part of Cserhati.

5

Cserhati proceeds with:

Yu and Zhang studied introns 4 and 7 from the nuclear gene ß-fibrinogen (FGB) as well as the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) in 17 species from the order Carnivora. In their results these researchers found that A. fulgens is most closely related to procyonids based on analysis of intron 4 of the FGB gene. But when intron 7 was analyzed, it clustered towards ursids. Classification based on the ND2 gene A. fulgens clustered with mustelids, but these results had poor bootstrapping support. When the two introns were combined with analysis of the genes IRBP and TTR, A. fulgens was closest to mustelids [9].

Cserhati presents the results of Yu & Zhang (2006) per intron.

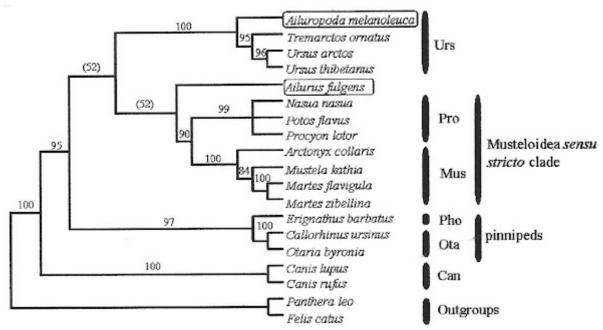


Figure 5 Figure 1 Yu & Zhang (2006): results intron 4

Yu & Zhang's figure 1 shows a phylogenetic tree based on nuclear b-fibrinogen intron 4; the red panda proves the sistergroup of the Procyonidae + Mustelidae (in the figure Musteloidea *sensu stricto*). The layout positions the red panda next to the Procyonidae, but that is only the layout of the tree.

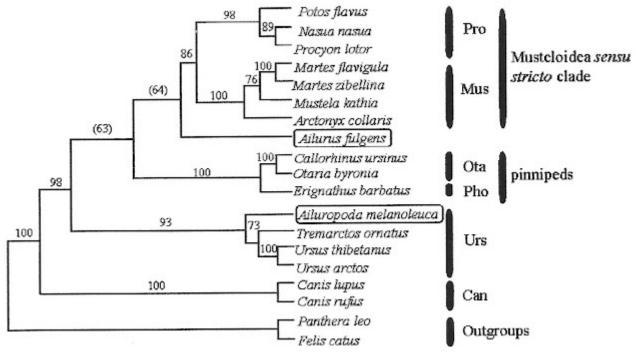


Figure 6. Figure 3 Yu & Zhang (2006): results ND2 gen

In Yu & Zhang's figure 3, het ND2 gen, the red panda is again the sistergroup of the Procyonidae + Mustelidae (Musteloidea *sensu stricto*) in the phylogenetic tree. The layout positions the red panda next to the Mustelidae, but that is only the layout of the tree.

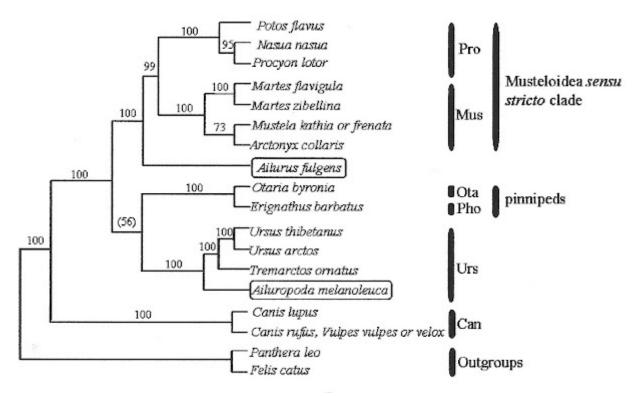


Figure 7. Figure 4 Yu & Zhang (2006): combined analysis

In Yu & Zhangs figure 4, the analysis of the combined IRBP, TTR, b-fibronogen intron 4 and 7 sequence data), the red panda is again the sistergroup of the Procyonidae + Mustelidae (Musteloidea *sensu stricto*) in the phylogenetic tree. The layout positions the red panda next to the Mustelidae, but that is only the layout of the tree.

Cserhati three times confuses the lay-out of the phylogenetic tree with the results of the phylogenetic tree. That is a pretty elementary error.

6

Cserhati proceeds with:

Sato et al. ...., and found that A. fulgens clusters together with procyonids and mustelids, and not with mephitids (skunks and stink badgers) [10].

Sato et al (2009) found that the red panda is the sister group of Musteloidea *sensu stricto*, i.e. of the raccoon family Procyonidae and the marten family Mustelidae together. Sato et al (2009) did not use clustering, but Bayesian analysis.

## 7

Cserhati proceeds with:

Intron analysis is useful, since these sequences are not under selection pressure. An analysis of 22 Kbp of nuclear intron sequences from 16 carnivore species groups A. fulgens with Musteloidea sensu stricto (Mustelidae+ Procyonidae) to the exclusion of mephitids [Yu et al 2011]. These results, however, contradict results coming from mtDNA analyses [DeLisle et al 2005].

Compare the text in Yu et al (2011):

Phylogenetic analyses of the more than 22 kb data set of noncoding intron DNA provided unambiguously strong support for the grouping of Musteloidea sensu stricto and Ailuridae to the exclusion of Mephitidae. ... This result is in contradiction to the mt studies (Ledje and Arnason 1996a, b; Delisle and Strobeck 2005; Arnason et al. 2007), but in agreement with the nuclear studies (Fulton and Strobeck 2006; Sato et al. 2009).

Cserhati almost literally copied Yu et al (2011), but ignores the message.

## Summary:

This introduction was written by someone without any background in taxonomy and phylogeny. As a result, there are gross errors in taxonomy and phylogeny. Cserhati misrepresents the results of Flynn et al (2000), Peng et al (2007), Yu and Zhang (2006). This shows elementary unfamiliarity with taxonomy and with classifying species based on molecular data.

Adopting the abstract and historical part of the introduction by Flynn et al (2000) as a problem statement in 2021 shows no familiarity with the subject of 'red panda' or the subject of 'phylogeny'. Cserhati has a very limited grasp of the literature. The cited articles on phylogeny are at least 10 years old - newer ones are missing. Frequently cited articles such as Flynn et al (2005) with 253 citations, Eizirik et al (2010) with 142 citations or Law et al (2018) with 42 citations are missing from the bibliography.

Flynn et al (2000), Yu and Zhang (2006), Sato et al (2009) and Yu et al (2011) list the families Procyonidae and Mustelidae as sister groups of each other,

together called the Musteloidea *sensu stricto*. In addition, all the articles mentioned give the Musteloidea *sensu lato* as a superfamily with the four families Ailuridae, Mephitidae, Procyonidae and Mustelidae. For Cserhati, too, the question should therefore have been what the order of the Ailuridae, the Mephitidae and the Musteloidea *sensu stricto* is within the Musteloidea *sensu lato*.

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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

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