

## Abstract

Histatins are a family of salivary proteins with anti-fungal and anti-bacterial properties. We studied the complex and recent evolution of *HTN1* and *HTN3*, two similar genes that code for members of the histatin family. Using BLAST, which searches genomes for similar sequences, we found *HTN1* and *HTN3* in humans, nonhuman Great Apes (e.g., chimpanzees), and Old World monkeys. Our comparative analyses indicated that *HTN3* evolved in an early placental mammal ancestor possibly through a duplication of neighboring *STATH* gene. *HTN1* evolved later, through duplication of *HTN3* in the ancestor of Great Apes and Old World monkeys. We were able to find *HTN3*, but not *HTN1* in New World monkey genomes, an out-group for the Great Apes and Old World monkeys. We also documented the differences that directly changed the protein sequence between *HTN1* and *HTN3*, through which we will extend our analyses to functional impact of these genes in primate evolution.

## Introduction

- Salivary genes, *HTN1* and *HTN3*, code for histatin proteins which have anti-microorganism properties.
- They have evolved from a series of duplication events in the secretory calcium-binding gene cluster, which include genes that code for milk, enamel, and salivary proteins.
- HTN diverged from *STATH*, an earlier evolved salivary gene, between 40-50 million years ago.
- HTN divergence into *HTN1* and *HTN3* occurred relatively recently, 15-30 million years ago.

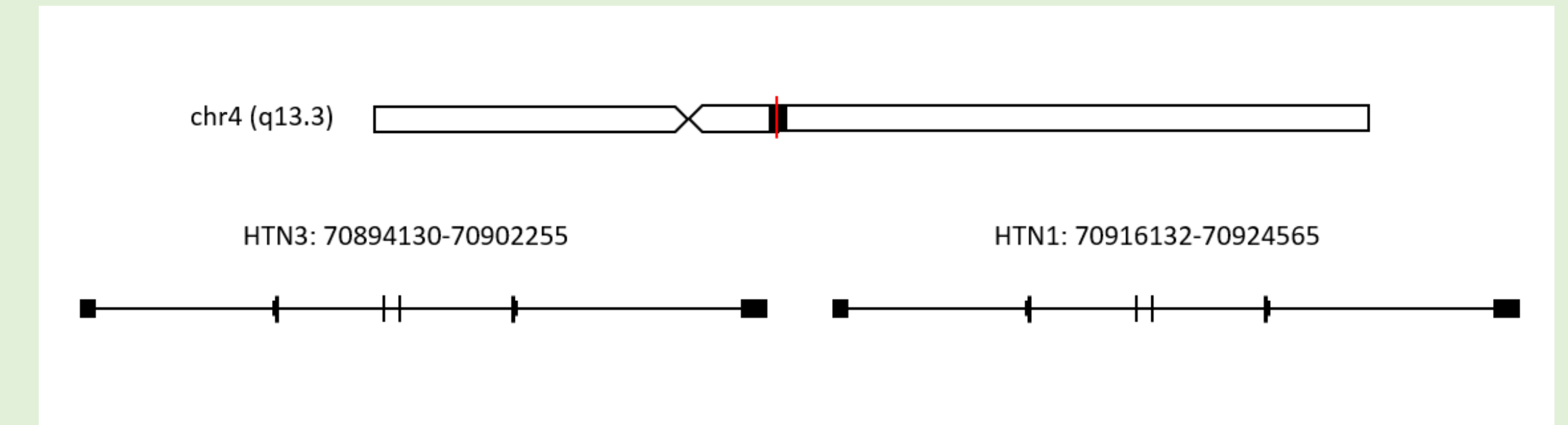


Figure 1: Genomic location of *HTN1* and *HTN3* genes

## How similar are HTN1 and HTN3?

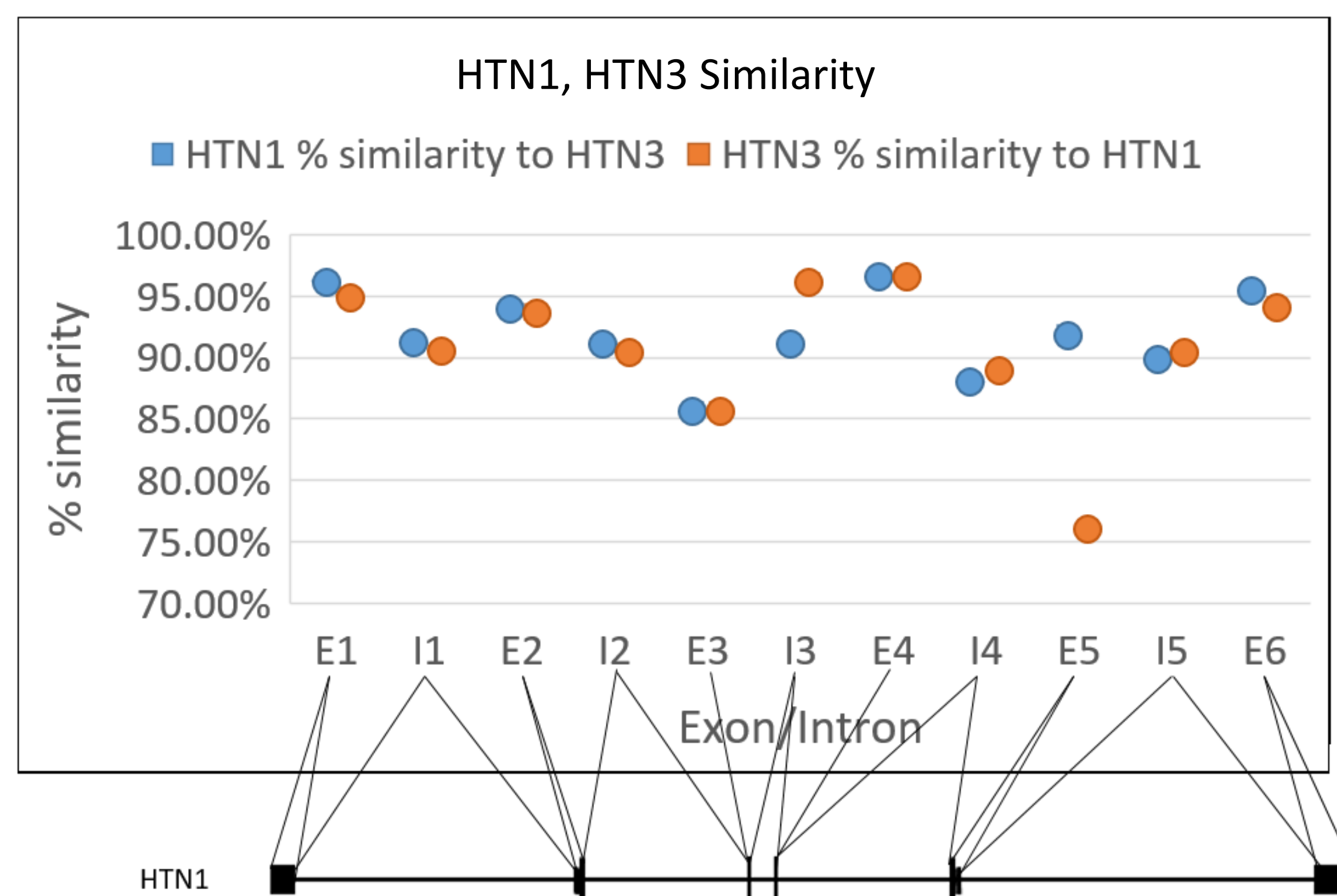
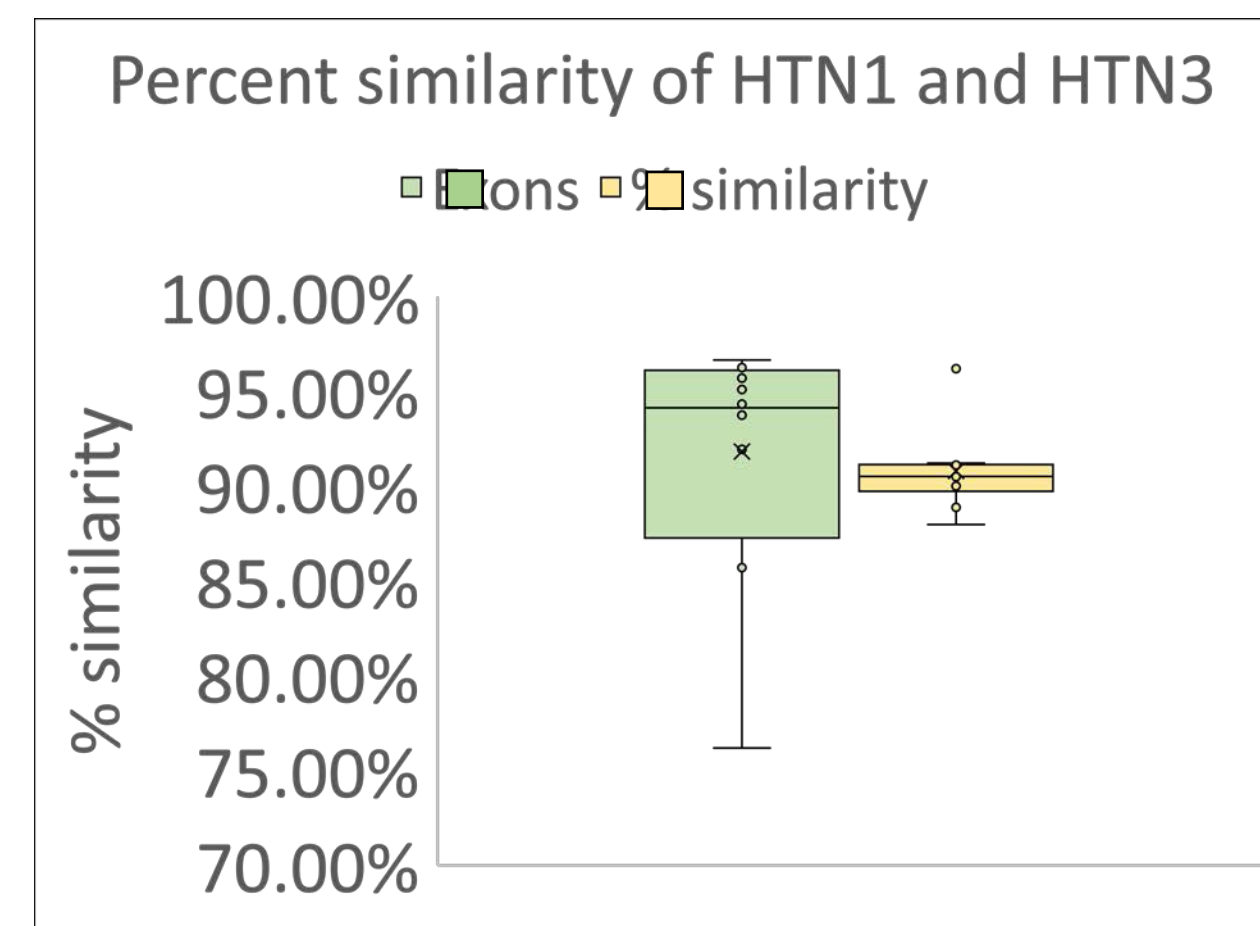


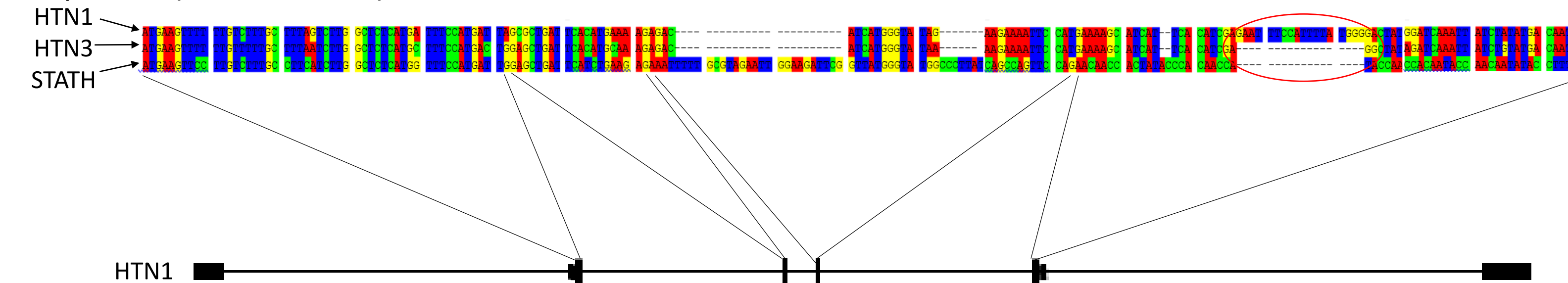
Figure 2 (above): Percent similarity between *HTN1* and *HTN3* for each exon and intron

Figure 3 (right): Box and whisker plot showing variation of exons in comparison to variation of introns  
W=91 p=0.1213



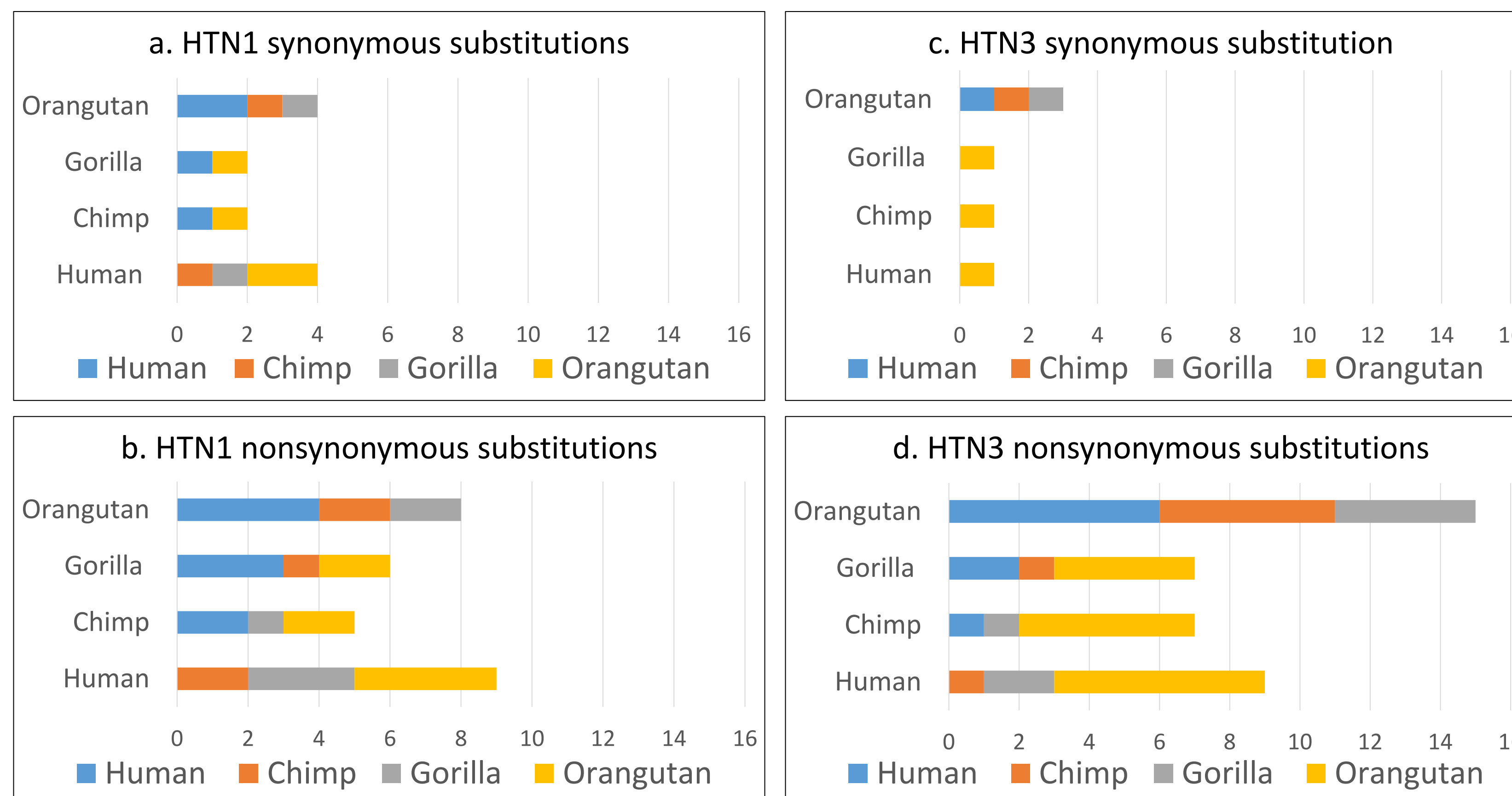
## Which gene, HTN1 or HTN3, is ancestral?

Figure 5: Coding exon DNA sequences for *HTN1*, *HTN3*, and *STATH*. *HTN3* and *STATH* share a missing sequence (circled in red).



## How many synonymous and nonsynonymous substitutions are there in HTN1 and HTN3 between species?

Figure 6a-d: The graphs below display the number of amino acid substitutions in the coding exonic regions of both *HTN1* (a and b) and *HTN3* (c and d).



## When did the ancestral gene evolve?

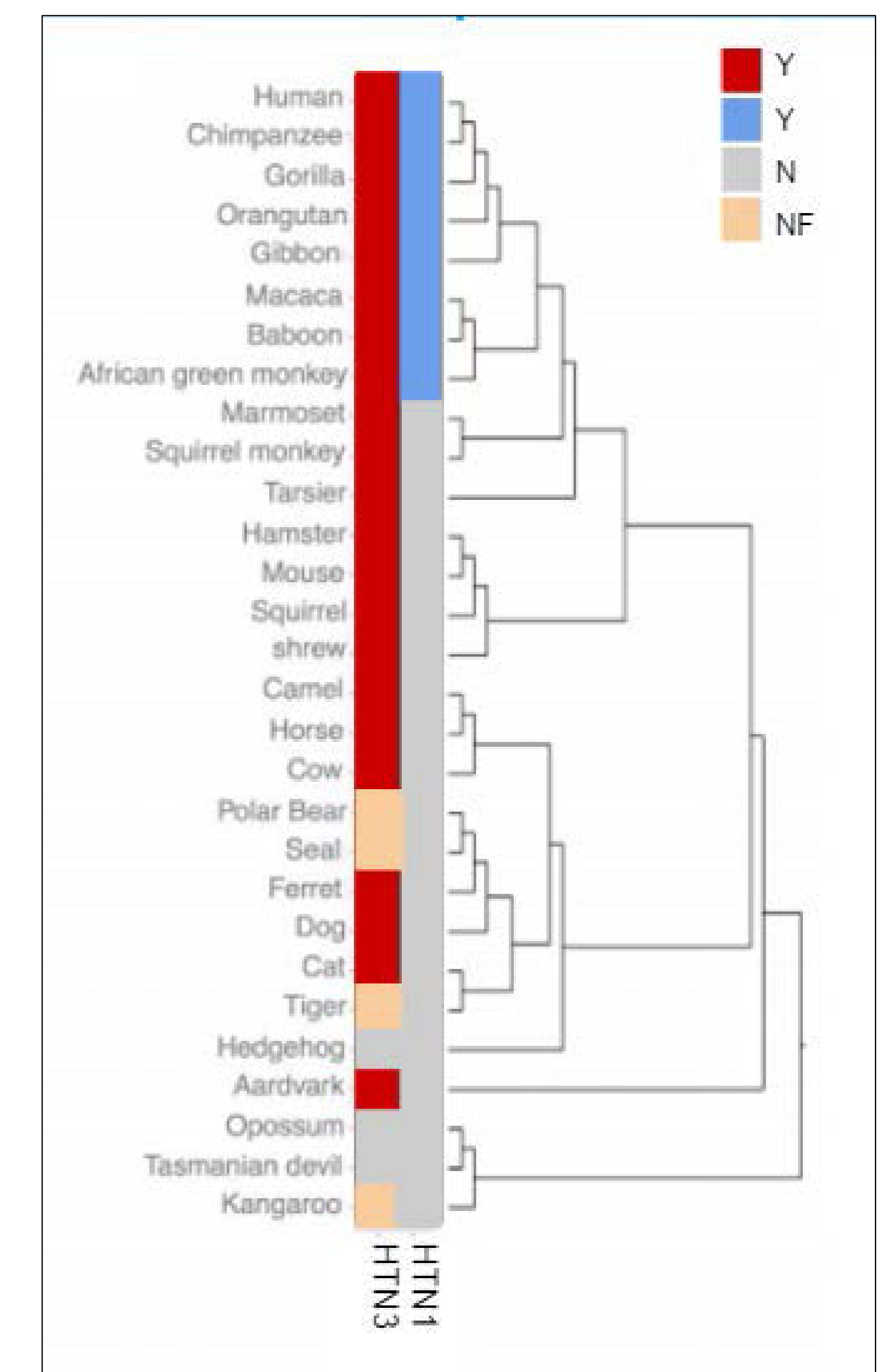


Figure 7: Placental mammals with evidence of *HTN1/HTN3* presence. Lines at the right represent the species tree.

## When did duplication of HTN happen?

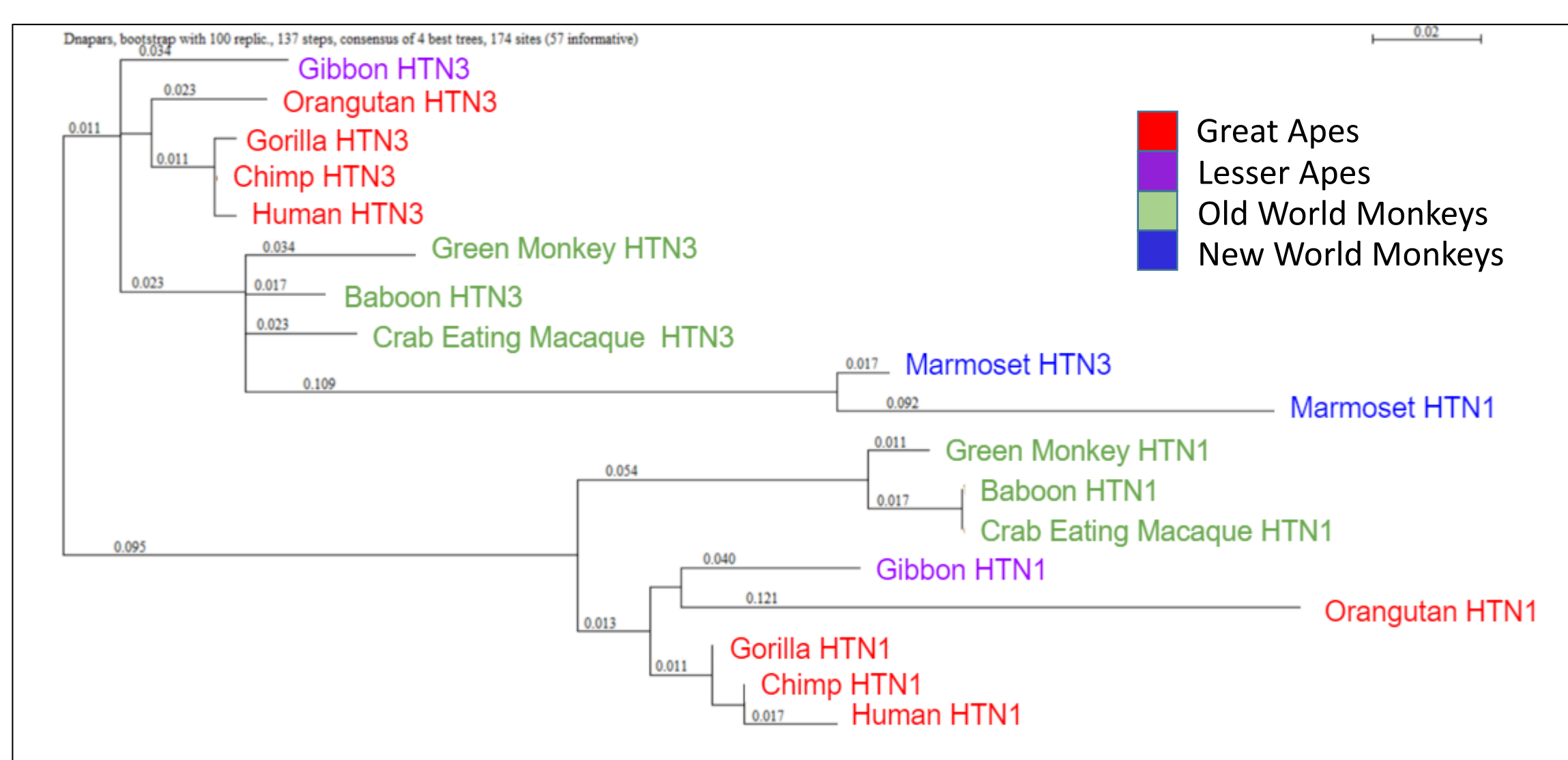


Figure 4: Phylogenetic tree of Humans, Chimp, Gorilla, Orangutan, Gibbon, Old World and New World monkeys *HTN1* and *HTN3* gene.

## Conclusions and Future Directions

- The variation between *HTN1* and *HTN3* is slightly greater in the exons
- We obtained evidence of both *HTN1* and *HTN3* occurrence in Humans, Great Apes, and Old World monkeys, so it is probable that *HTN3* duplication occurred in their most common ancestor of these species.
- New World monkeys only have one HTN gene, *HTN3*.
- HTN3* is the ancestral HTN form. This is the most parsimonious explanation for our observation that *HTN3* is more closely related to *STATH* than *HTN1*.
- Evidence of some form of *HTN3* is found in early placental mammals.
- Documentation of the nonsynonymous and synonymous substitutions in *HTN1* and *HTN3* between primate species indicates positive adaptive human selection and will extend future research of the evolutionary function of both histatin genes.

## References

Kawasaki, K., Weiss, K. 2002. Mineralized tissue and vertebrate evolution: The secretory calcium-binding phosphoprotein gene cluster. *PNAS*. **100(7)**: 4060-4065.