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Interhaplotypic distance within the *Monkeypox* virus group and the phylogenetic relationship with the *orthopoxvirus* genus

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Abstract: In this work are used 59 haplotypes of the gene of interferon-alpha-beta receptor *of Monkeypox* virus, *Buffalopox* virus, *Camelpox* virus, *Cowpox* virus, *Ectromelia virus*, *Rabbitpox* virus, *Vaccinia* virus *and Variola* virus, recuperated the GenBank. All sequences were publicly available on the National Biotechnology Information Center (NCBI) platform. The results indicate that the methods of genetic attribution and maximum likelihood presented responses that had not been observed with the use of other methodologies. These results suggest that evolutionary actors such as the retention of ancestral polymorphism, are the responsible by the diversity among the groups studied. The *Buffalopox, Ectromelia and Rabbitpox* groups did not present significant structuring level and *the Cowpox group* presented the highest degree of haplotypic diversity. The Monkeypox *group* presented a poorly polymorphic pattern that, by not increasing the genetic variability of the group, also does not contribute to large variations in its protein products.

Keywords: Phylogeny, Bioinformatics, *Orthopoxvirus*, *Monkeypox*

1. Introduction

Over evolutionary time, *Orthopoxviruses* adapted to limit the immune response in the human host. Viral strategies to avoid immunological interference may influence even the adverse effects of smallpox vaccination. This occurred through mechanisms that weakened the action of effectors that had the function, inhibiting antiviral effects, which conferred immunoregulatory activity. Among these, we mention interferons and their cellular receptors. (Stanford *et al*, 2007; Randall and Goodbourn, 2008).

Interferons (IFNs) are natural cell signaling glycoproteins that belong to the cytokine class and that participate in cell control and replication, besides being modifiers of the immune response, with antiviral, antiproliferative and immunomodulatory effects (Priyanka *et al*, 2014). IFNs can be divided into three distinct groups. Type I includes IFN-α (alpha) and β (beta), produced by epithelial cells and fibroblasts, contribute to the first line of antiviral defense (Ank *et al*, 2006) specific receptors of its cell surface, called IFN-α / β receptor (IFNAR), is present in all type I IFNs, and consists of the IFNAR1 and IFNAR2 chains. IFN-ε (epsilon), IFN-κ (kappa) and IFN-ω (omega) also fall under type I and are present in humans (Ank *et al*, 2006).

Poxviruses encode proteins that serve as invasion strategies in the immunological functions of interferon (IFN),

and one of them is the expression of the IFN/BP protein that prevents the interaction of IFN with cell receptors. Detailed interaction studies were performed with VARV and MPXV, and the expression of IFN/BP to identify the adaptation to the human IFN system, where it was possible to observe that the Monkeypox strain efficiently inhibited the antiviral activity of IFN-i types tested, as well as the smallpox strain also specifically inhibited the IFN-i activity. (Alcami *et al*, 2000).

Progress in molecular biology and genomics has improved understanding of viral infection and monkeypox replication, which has been shown to be a virus with a relatively large genome, with about 196,858 base pairs and which form a good part of the genetic material needed for viral replication in the cellular cytoplasm. Viral types and strains differ in the entry of the virus that occurs immediately after an interaction between several viral ligands and receptors on the cell surface, for example, condroitine sulfate and heparine sulfate (Chung *et al*, 1998; Lin *et al*, 2000). However, few studies have employed tools to test the host response to infections with poxvirus, in general, specifically in the case of Monkeypox **(**Abdulnaser *et al*, 2010).

Trying to understand evolutionary aspects of the Interferon gene receptor and its probable behavior in Monkeypox infection, we from the team of the Laboratory of Population Genetics and Computational Evolutionary Biology (LaBECom-UNIVISA)performed a phylogeny work in a PopSet with 59 sequences of the interferon-alpha-beta receptor gene, available in the database of the National Biotechnology Information Center (NCBI).

2. Methodology

Databank: 59 haplotypes of the interferon-alpha-beta gene of *Monkeypox virus, Buffalopox virus, Camelpox virus, Cowpox virus, Ectromelia virus, Rabbitpox virus, Vaccinia virus and Variola virus,* were recovered from GENBANK (https://www.ncbi.nlm.nih.gov/popset/60547302?report=fasta) on August 6, 2022. These haplotypes had 1429 base pairs and aligned using the MEGA X program (Kumar *et al*., 2018), had ambiguous sites, lost data and excluded gaps generating content with 1188bp analyzable.

Phylogenetic analyses: Nucleotide sequences previously described were used for phylogenetic analyses. The sequences were aligned using the MEGA X program (Kumar *et al*., 2018) and gaps were extracted for the construction of phylogenetic trees.

Minimum Coverage Network (MSN) among haplotypes: In LaBECom, this tree is generated using the operational taxonomic units (OTUs). This tree is calculated from the paired distance matrix using a modification of the algorithm described in Rohlf (1973).

Haplotypic inferences: We use these inferences for haplotypic or genotypic data with unknown gamtic phase. Following our protocol, inferences are estimated by observing the relationship between haplotype i and xi times its number of copies, generating an estimated frequency (^pi). With genotype data with unknown ethical gam phase, haplotype frequencies are estimated by the maximum probability method, and can also be estimated using the expected Maximization (DM) algorithm.

3. Results and Discussion

General properties of interferon-alpha-beta receptor gene sequences in the *orthopoxviruses* studied

59 haplotypes of the interferon-alpha-beta receptor gene of Monkeypox virus, Buffalopox virus, Camelpox virus, Cowpox virus, Ectromelia virus, Rabbitpox virus, Vaccinia virus and Variola virus, were recovered from GENBANK (https://www.ncbi.nlm.nih.gov/popset/60547302) on August 6, 2022. These haplotypes had 1429 base pairs and that after being aligned using the MEGA X program (Kumar *et al*., 2018), all their ambiguous sites, lost data, gaps and were excluded generating a segment with only 1188 conserved and polymorphic sites. Of these, only 195 sites were informative parsimony and their graphic representation could be seen in a logo built with the PROGRAM WEBLOGO 3. (CROOKS *et al*., 2004), where the size of each nucleotide. proportional to their frequency for certain sites. (Figure 1).

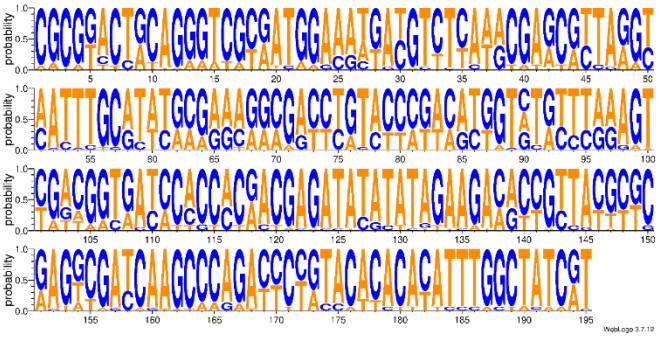

Figure 1: Graphic representation of 195 parsimonious-informative sites of interferon-alpha-beta receptor gene sequences.

Using the UPGMA method for the 195 parsimony-informative sites, it was possible to understand that the 59 haplotypes comprised eight distinct groups, with greater sharing of haplotypes between *Cowpox and Vaccinia groups.* The maximum likelihood maps found the presence of phylogenetic signal for all data sets in the six groups studied, presenting more than 70% of the quartets resolved (data not shown). The GTR+I+G evolutionary model was the one that best represented the differences between groups, with a *significant bootstrap value* (65%) supporting clade separation (Figure 3).

Inter-haplotypic distance of the genus Orthopoxvírus

All groups studied revealed some degree of Inter-haplotípic sharing (Figure 2) but the Cowpox group presented the highest number of polymorphic sites (Figure 4).

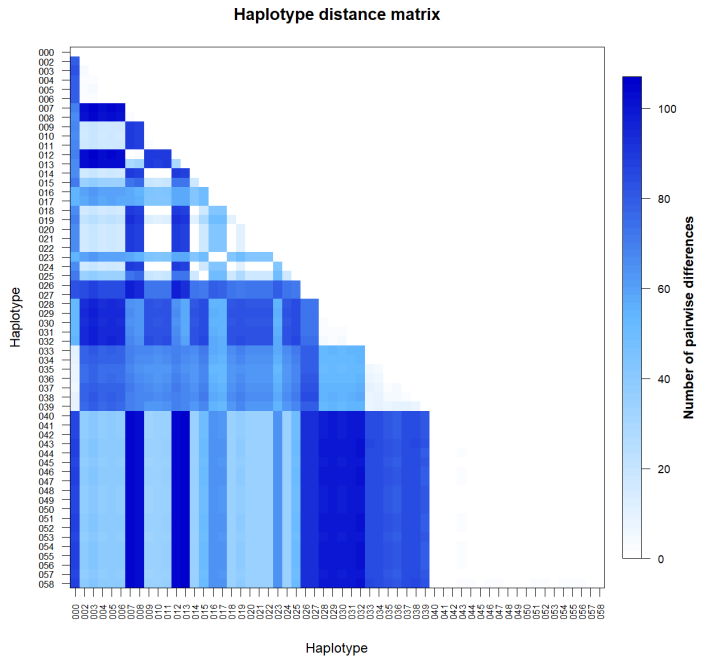

Figure 2. Interhaplotipic distance matrix and number of polymorphic sites of interferon-alpha-beta receptor gene sequences. Featured haplotypes with greater distance. *Generated by the statistical package in R language using the output data of the Software Arlequin version 3.5.1.2.

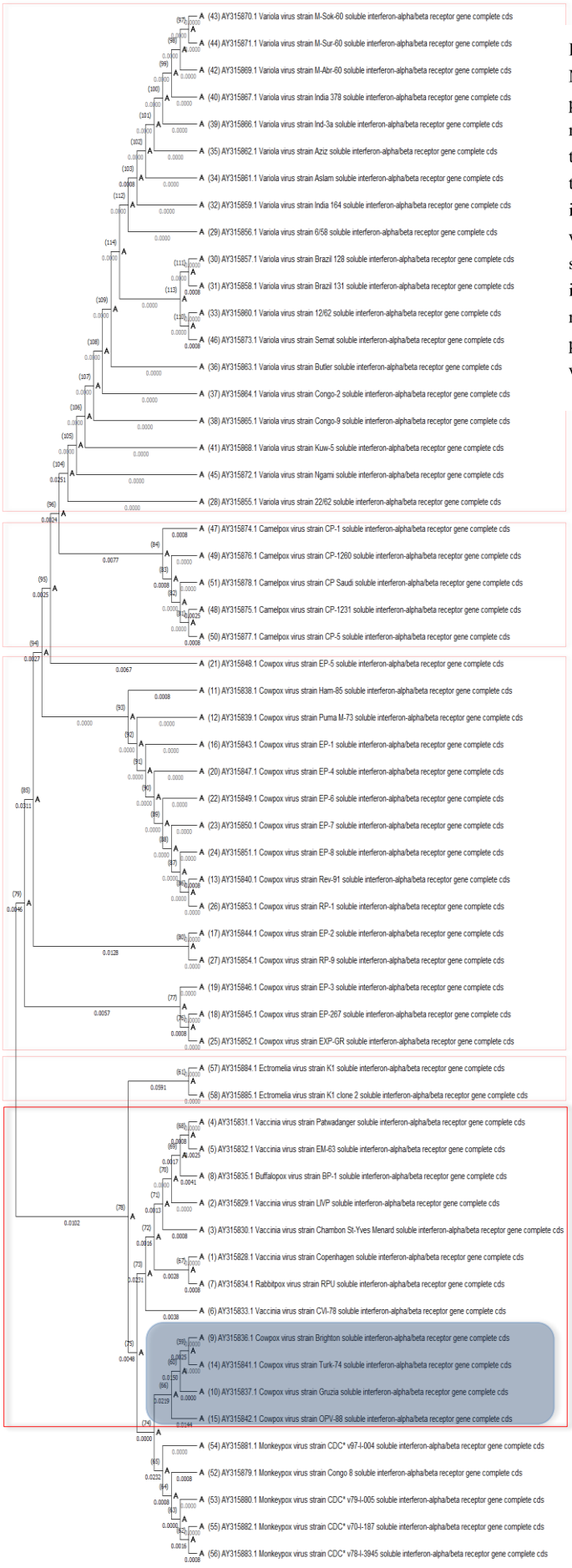

Figure 3. Ancestral states were inferred using the Maximum Likelihood method [1] and Tamura 3-parameter model [2]. The tree shows a set of possible nucleotides (states) at each ancestral node based on their inferred likelihood at site 1. For each node only the most probable state is shown. The initial tree was inferred using the method. The rates among sites were treated as a Gamma distribution with invariant sites using 5 Gamma Categories (Gamma with invariant sites option). This analysis involved 59 nucleotide sequences. There were a total of 1188 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [3]

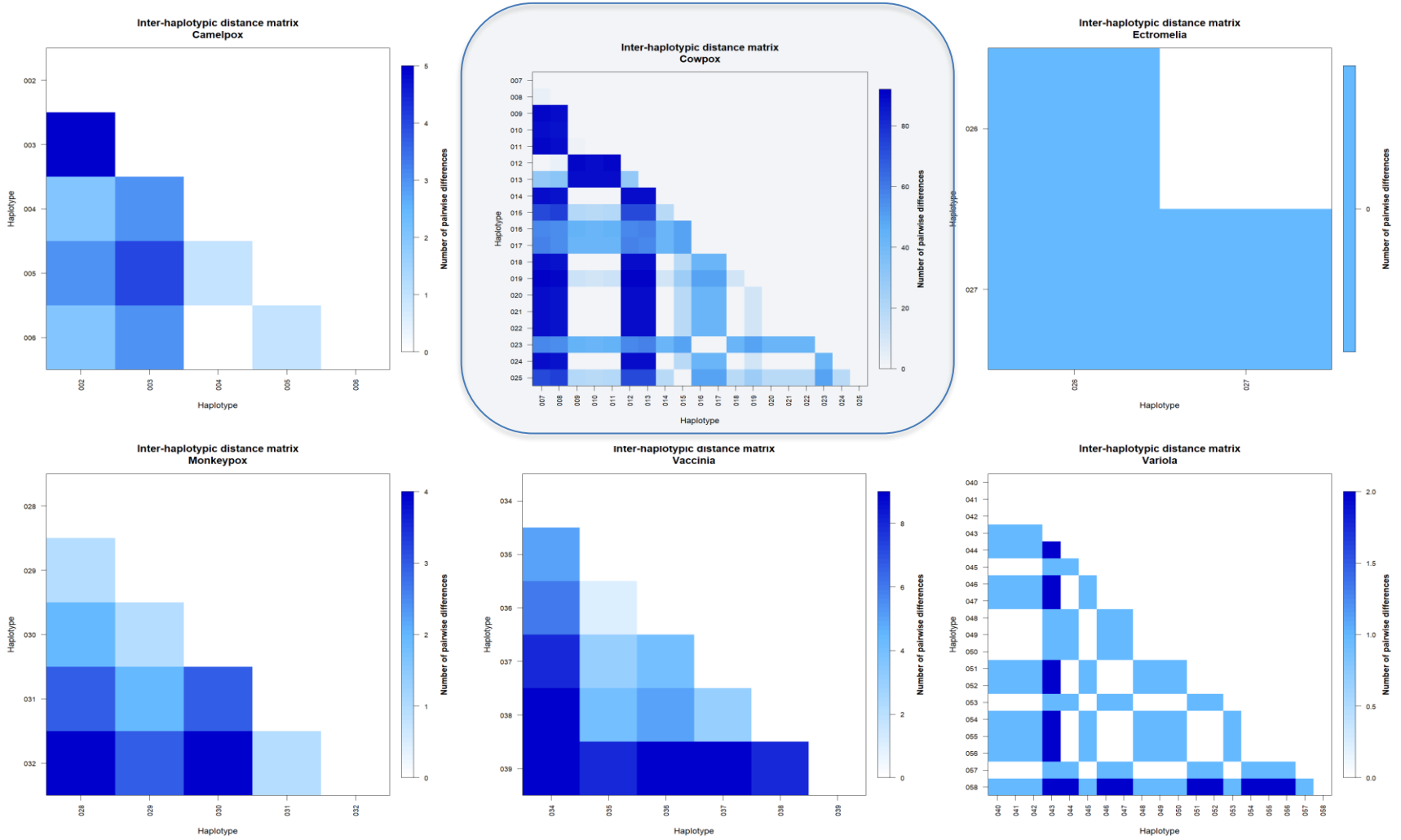

Figure 4. Interhaplotypic distance matrix and number of polymorphic sites found in interferon-alpha-beta receptor gene sequences (d). *Generated by the statistical package in R language using the output data of the Software Arlequin version 3.5.1.

4. Conclusions

The *Buffalopox, Ectromelia and Rabbitpox* groups did not present significant structuring level perhaps because they did not have considerable interhaplotypic variation, these being the main components in moderate and high structuring for *the Cowpox and Vaccinia groups*. These data suggest that the high degree of structuring present in Cowpox may be related to a loss of intermediate haplotypes throughout generations, associated with the absence of gene flow between the groups.

These structuring levels were also supported by simple phylogenetic pairing methodologies such as UPGMA and more complex as the haplotype network, which in this case, with a discontinuous pattern of genetic divergence between the groups (supporting the occurrence of geographical isolation stemming from past fragmentation events), was observed a large number of branches with many mutational steps. These mutations were possibly fixed by drift due to the founding effect, which accompanies the behavior of dispersion and/or loss of intermediate haplotypes throughout the generations.

The *Monkeypox group* had a somewhat polymorphic pattern with subtle differences in its internal haplotypic sharing. The inter-haplotypic variations were all hierarchized in their covariance components: by their intra- and inter-individual differences or by their intra- and intergroup differences, generating a pattern that supports the idea that the significant differences found for this group were shared more in their number than in their form, since the result of the estimates of the mean evolutionary divergence found within and between them were not significant. In the *Monkeypox group* specifically, existing polymorphisms do not contribute to large variations in their protein products, since they show slow and almost always non-silent mutations that do not significantly increase the genetic variability of this group specifically.

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