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Review of 'Comparing sequences without using alignments: application to HIV/SIV subtyping'

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Master's in Computer Science

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Abstract

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Notations

Notation	Meaning
$[\![\cdot,\,\cdot]\!] \,:\, \mathbb{N}\times\mathbb{N}\to\mathbb{N}$	Integer interval, $[\![a,\,b]\!] = \{a,\,a+1,\,\ldots,\,b\}$

I. Introduction and summary of the article

This article describes the problem in sequence comparison, which arises when attempting to compare a large number of sequences together. Classic sequence alignment methods often struggle to effectively handle certain types of sequence alterations, such as insertions or deletions. Intuitive approaches to address this problem without alignment involve examining the frequency of nucleotides or amino acids. However, this method lacks meaningfulness, as sequences with similar frequencies can still exhibit significant differences. A more sophisticated and effective approach is dealing with what [Did+07] calls N-words (in nowaday's litterature, we call them k-mers). With those, we can compute dissimilarities between sequences [KL94] which can help us show evolutionary relationships between sequences.

I.1 Local decoding method of order N

The first step of this analysis presented by [Did+07] is the local decoding method of order N, or N-local decoding.

Definition I..1 (N-words)

> In a sequence, an N-word is a **contiguous sub-sequence of size** N within the given sequence. The set of N-words for a sequence consists of all its sub-sequences of size N.

Example.

> The set of the 3-words of the sequence $AGTACGT$ is AGT, GTA, TAC, ACG, CGT.

Let $S = S_1S_2\ldots S_i\ldots S_{|S|}$ be a sequence, where i denotes a site (or index) within S. For a given $N\in\mathbb{N}^*$, we consider the set of N-words of S covering the site i.

Definition I..2 (Direct relation)

> Two sites are said **directly related** if they have the same position in two (or more) occurences of the same N-word.

Example.

> (We take the example on Figure 6a in [Did+07]). Let seq1 = CATTG TCCGC **T**GGAC CACAC and seq2 = CACT**T** GGACA CATAC CATGC. We consider the site 11 in seq1 and the site 5 in seq2 (bolded in their definitions), and look at the 5-words covering this site (contained in the sites colored in red).

C	C	G	C	**T**	G	G	A	C
C	C	G	C	T				
	C	G	C	T	G			
		G	C	T	G	G		
			C	T	G	G	A	
				T	**G**	**G**	**A**	**C**

C	A	C	T	**T**	G	G	A	C
C	A	C	T	T				
	A	C	T	T	G			
		C	T	T	G	G		
			T	T	G	G	A	
				T	**G**	**G**	**A**	**C**

> The 5-word TGGAC appears in both these sequences, and the sites 11 in seq1 and 5 in seq2 are both in first position of the 5-word, so these two sites are **directly related**

Definition I..3 (Transitivity and transitive closure)

> Let $\mathcal{R}$ and $\mathcal{R}'$ be binary relations. $\mathcal{R}$ is said to be **transitive** if it respects the following property [Cau21]:
>
> $$a\mathcal{R}b \wedge b\mathcal{R}c \implies a\mathcal{R}c$$
>
> $\mathcal{R}'$ is the **transitive closure** of $\mathcal{R}$ if [Sch77]:
>
> $$\forall a,\,b;\; a\mathcal{R}b \implies a\mathcal{R}'b$$

and

$$\forall a,\, b,\, c;\; a\mathcal{R}b \wedge b\mathcal{R}c \implies a\mathcal{R}'c$$

Definition I..4

We define the (simple) relation between two sites as the transitive closure of the direct relation. Therefore, we say that two sites are related if there is a (finite) chain of direct relations linking those sites.

These related sites can be categorized into distinct equivalence classes, as the relation we described satisfies the properties of an equivalence relation, namely, reflexivity, symmetry, and transitivity. [Did+07] refers to these equivalence classes as N-classes.

Definition I..5 (N-classes)

An N-class can be defined as follows,with a being the identifier of the N-class:

$$C(a) = \{x \in [\![1,\, |S|]\!];\; x \text{ is related with } a\}$$

Consequently, by assigning a unique identifier to each N-class, we can rename every site in the entire sequence by appending the nucleotide (or amino acid) with the identifier of the class to which it belongs. However, there may exist sites that are not related to any other site in any other sequence, resulting in singleton N-classes. To avoid excessive and unnecessary identifiers, which could lead to unreadable representations, we represent these sites solely by their nucleotide or amino acid.

I.2 Dissimilarity matrix and clustering tree

Now that we have our new sequences divided into disjoint N-classes of sites, we aim to compare each sequence. To achieve that, we need to select a measure of (dis-)similarity. [Did+07] chose the one defined in [Did+06].

Let's introduce the following notation. $|s|_x$ is the number of occurences of the identifier x in the sequence s. For each pair of sequence and identifier $(s,\, x)$, we compute the value of $|s|_x$.

Example.

We consider the following rewriten sequences:

seq1	$C\,A\,T_0\,T_1\,G_0\,T_2\,C_0\,C_1\,G\,C_2\,T_3\,G_1\,G_2\,A_0\,C_3\,C_4\,A\,C\,A\,C\,C\,T_0\,T_1\,G_0\,T_2\,C_0\,C_1\,C\,T\,A$
seq2	$C_5\,A_1\,C_6\,T_4\,T_3\,G_1\,G_2\,A_0\,C_3\,A\,C\,A\,T\,A\,C\,C\,A\,T\,G\,C$
seq3	$C_5\,A_1\,C_6\,T_4\,T_3\,C\,T\,T\,T\,C\,C_2\,T_3\,G_1\,G_2\,A_0\,C_3\,C_4\,T\,C\,C$

Table 1: Rewriten sequence (picked from [Did+07])

	seq1	seq2	seq3
A_0	1	1	1
A_1	0	1	1
T_0	2	0	0
T_1	2	0	0
T_2	2	0	0
T_3	1	1	2
T_4	0	1	1
C_0	2	0	0
C_1	2	0	0
C_2	1	0	1
C_3	1	1	1
C_4	1	0	1
C_5	0	1	1
C_6	0	1	1
G_0	2	0	0
G_1	1	1	1
G_2	1	1	1

Table 2: Count of each identified site in each sequence

Then to compute the similarity between each sequences, we apply the following formula:

$$\text{sim}(\text{seq},\, \text{seq}') = \frac{\sum_x \min(|\text{seq}|_x,\, |\text{seq}'|_x)}{\min(|\text{seq}|,\, |\text{seq}'|)}$$

Example.

for i, $j \in \{$seq1, seq2, seq3$\}$:

$$\text{Sim} = (\text{sim}(i,\, j))_{i<j} = \begin{pmatrix} - & \frac{5}{20} & \frac{7}{20} \\ & - & \frac{9}{20} \\ & & - \end{pmatrix} = \begin{pmatrix} - & 0.25 & 0.35 \\ & - & 0.45 \\ & & - \end{pmatrix}$$

The dissimilarity (or distance) can easily be obtained by taking the complement to 1 of the similarity.

I.3 Clustering and trees

Now that we have our mesure of distance between sequences, we can perform an agglomerative hierarchical clustering [And73] that can give us an idea of a potential mutative evolution of HIV and SIV, that can be interpreted as subtypes of these viruses. However, in order to perform this clustering, we need a method to compute the distance between clusters containing multiple sequences. The article does not present one, so we had to design our own, described in Subsection II.2

I.4 Bootstrapping

Finally, in order to have more confidence in the results, the authors use the statistical technique of 'bootstrapping' [Efr79]. In the case of sequence comparison, the method proposed in [Fel80] was considered and adapted to local decoded sequences. The motivation behind using this method is that the results obtained may possess a certain degree of uncertainty due to data limitations or noise.

For each sequence in the original data, a random replicate of the same size is created by randomly selecting sites from the original data with replacement and concatenating them. Once these artificial sets have been generated, the same analysis performed on the original set is carried out on the new set. By comparing the results obtained from the original set with those from the artificial sets, the stability of the method can be assessed, taking into account the inherent uncertainty introduced by the data.

II. Material & Method

II.1 Data Sets

We used two different sequence sets to run our experiments: one given from Gilles Didier, co-author of [Did+07], and another that we retrieved ourselves, both coming from the data base maintened by the 'Los Alamos National Laboratory'[1]. The first set contains 66 sequences writen prior to 2007 [2], and the second one has been created from the query to have 47 sequences sampled in 2016 in Germany (so that we look at mutations in a restrained place and time).

II.2 Cluster merging method

For our cluster merging method, we drew inspiration from centroid linkage methods [DH+73]. When considering a cluster $\mathcal{C}$ containing more than one sequence, we create an artificial sequence with a length equal to that of the shortest sequence within the cluster. This artificial sequence is defined as follows:

$$\widetilde{\text{seq}}_i = \arg\max_{s_i;\, s \in \mathcal{C}} \frac{s_i}{|\mathcal{C}|}, \quad \forall i \in [\![1,\, n]\!],\, n = \min_{s \in \mathcal{C}} |s|$$

In simpler terms, for each site of the artificial sequence, we select the N-class that appears most frequently at the corresponding site across the different sequences in the cluster. If multiple N-classes have the same frequency of occurrence, we choose the first element with the highest frequency. To compute the distance between two clusters, we utilize the same formula as shown in Subsection I.2.

[1] `https://www.hiv.lanl.gov`

[2] The sequences presented in figure 1 of [Did+07], with 4 missing sequences

III. Experiments and Results

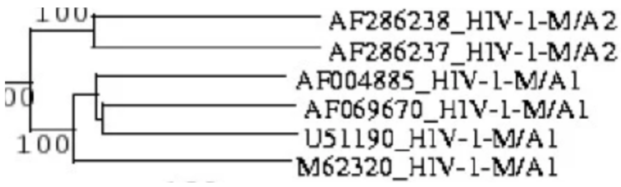
III.1 Original set of sequence

III.1.1 Comparison of the clustering trees

We have followed the procedure outlined in the summary (Section I.). The resulting dissimilarity matrix for the set of sequences can be found in the file named `dissmatrix__66_sequences.txt` within the repository. Since the matrix is too large to be displayed here, please refer to the file for its contents. Additionally, the corresponding cluster tree for this dissimilarity matrix is presented in Figure 3 in the Appendix. It follows the cluster tree from the original article, which can be found in Figure 2.

When looking at both the figures, we can see that they are very alike. Let's take the following sequences in example:

(a) Part of the generated tree



(b) Part of the original tree

We can see on these parts of the trees that the only difference is that, on the generated tree, the sequences `M62320` and `AF004885` are 'siblings' (i.e. formed a cluster alone together at some point of the hierarchical clustering), where in the original tree, they are one 'generation' apart. These kind of changes can be explained by the fact that our clustering method are probably not the same, the one of the article not being presented, and by the fact that we miss 4 sequences from the original set.

The only potentially significant difference between our two trees is that, in ours, the sequence `M31113` is directly linked to the root, with no direct sibling, when it is a lot more deeply inserted in the original tree.

III.1.2 Bootstrapping

We conducted 10 different bootstraps to assess the reliability of our method. The resulting trees can be found at the following path: `Trees/Bootstraps_66_sequences`. All the trees exhibit the same overall structure, with three distinct blocks of sequences that remain separate from each other. Additionally, the sequence `M31113` consistently maintains the same position in all the bootstrap trees. However, some permutations between sequences within the different blocks can still be observed. These permutations tend to occur in the deeper half of the tree for the "large block".

While the bootstrap trees are not identical to each other or to the original generated tree in terms of their structure, our method still accurately distinguishes between the subtypes of HIV and SIV. This indicates the robustness of our approach, despite variations in the specific arrangements of the trees and permutations within the sequence blocks.

III.2 'German' set of sequences

IV. Conclusion

Appendix

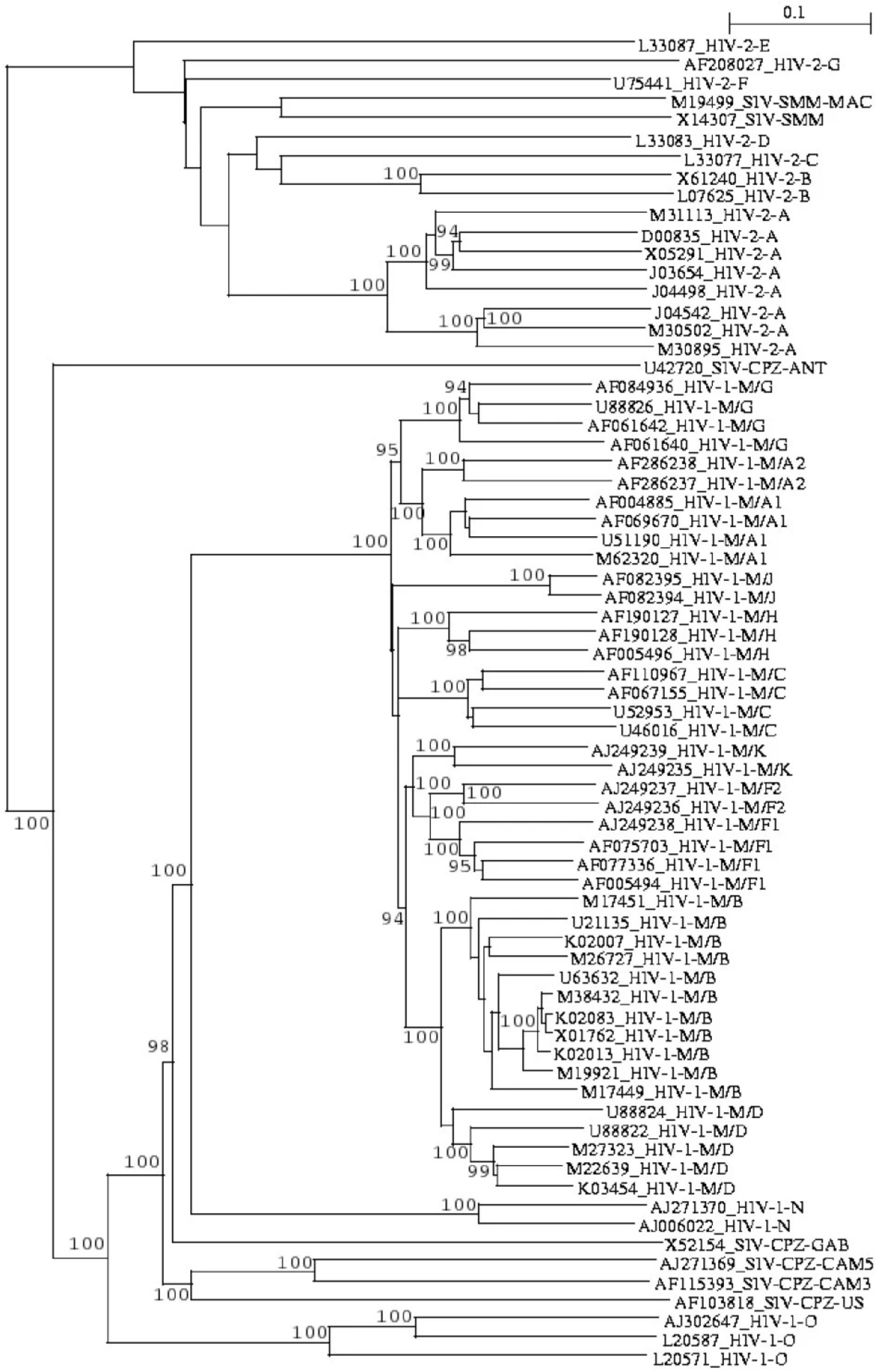


Figure 2: Original tree obtained from the dissimilarity matrix of the sequences of the original article, with $N = 15$

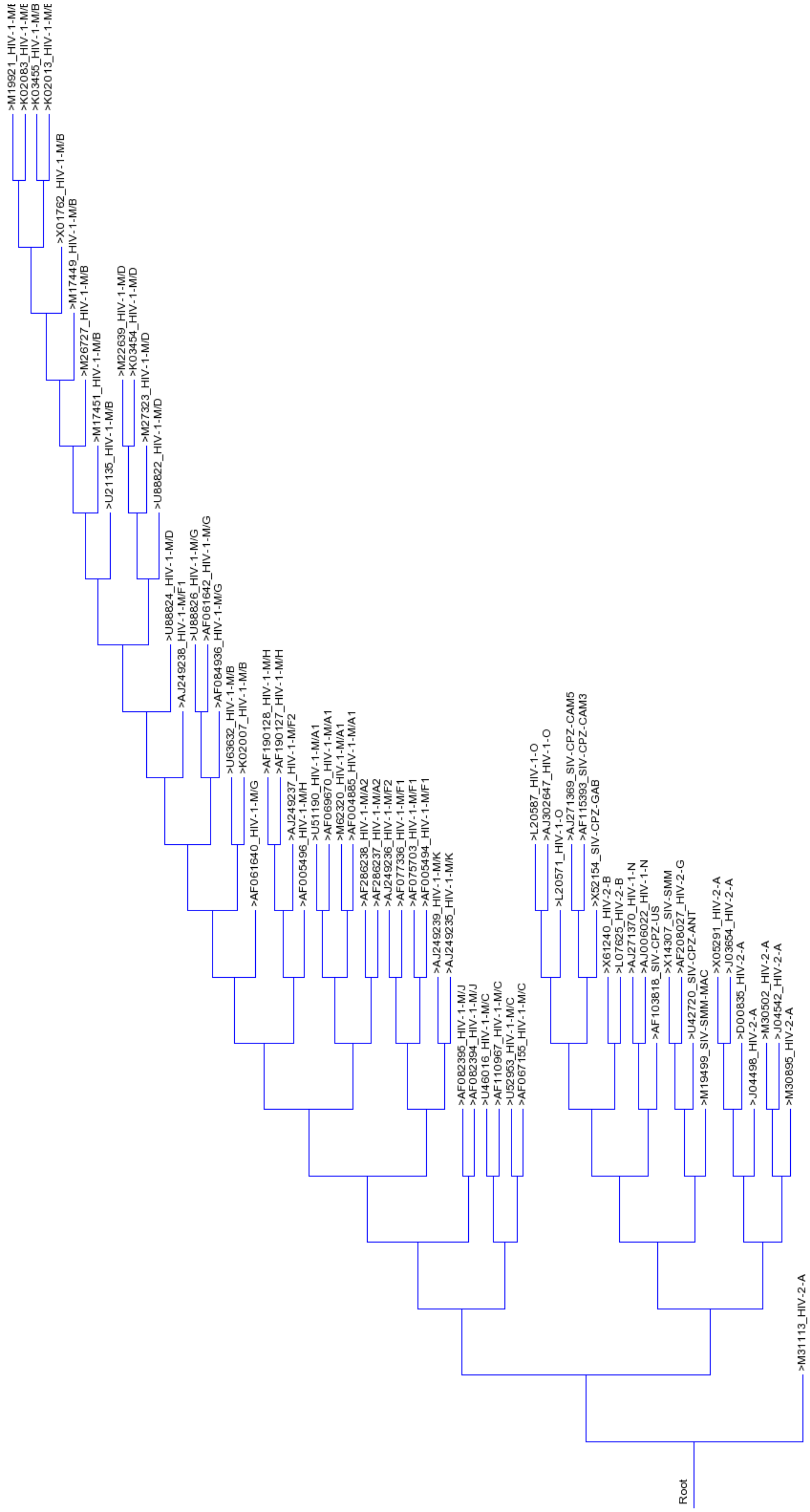


Figure 3: Tree obtained from the dissimilarity matrix of the sequences of the original article, with $N = 15$

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