

Identification of Maleo (*Macrocephalon Maleo*) and Gosong Kaki Merah (*Megapodius Reinwardt*) DNA Similarity Level Using Needleman-Wunsch Algorithm

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ARTICLE INFO	ABSTRACT
Article History Received : 17-09-2023 Revised : 07-10-2023 Accepted : 13-10-2023 Keyword Alignment; Needleman-Wunsch; Website; Maleo; Gosong Kaki Merah.	Sequence Alignment is used to determine the similarity of two DNA sequences. <i>Needleman-Wunsch</i> algorithm is one of the global alignment algorithms that uses the entire length of the DNA sequence. In this study, the algorithm is applied to a <i>web-based</i> application system to facilitate in determining the level of similarity of maleo DNA sequences (<i>Macrocephalon maleo</i>) which is endemic to Sulawesi, with a comparison of Gosong Kaki Merah (<i>Megapodius reinwardt</i>) which has a global distribution. The results of the alignment of maleo (<i>Macrocephalon maleo</i>) and Gosong Kaki Merah (<i>Megapodius reinwardt</i>) DNA sequences on a <i>web-based</i> application system have an average similarity level of 83.39% and an average gap value of 8.42%.
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1. INTRODUCTION

Deoxyribonucleic Acid (DNA) is a macromolecule that plays a very important role in the life of all types of living organisms. DNA is a double-stranded polynucleotide that has the characteristics of constituent components including deoxyribose sugar, phosphate groups and nitrogenous bases (adenine, guanine, thymine, and cytosine) (Nur'aini et al., 2019). DNA plays a role as a carrier of genetic information, an intermediary for self-duplication and inheritance of traits, expression of genetic information, forensic fields and detecting diseases. Every organism has different DNA and its own uniqueness. The preservation and uniqueness of DNA is influenced by external factors and internal factors. Most organisms in the inheritance of DNA traits cannot take place perfectly, due to environmental factors such as degeneration and mutation in the DNA sequence (Mustami & Muthiadin, 2021).

DNA sequences can be analyzed using the bioinformatics approach that studies the application of mathematical methods, statistics, and informatics to manage, store and analyze biological information. Sequence alignment is applied to predict or forecast protein shape, DNA secondary structure and gene expression analysis on sequences. The basic method of sequence alignment is used to find the degree of similarity between aligned sequences (Akil, 2020).

Sequence alignment is also known as alignment which is the process of arranging or organizing two or more sequences so that the similarities of the sequences are apparent (Mardika, Setyorini, & Amatullah, 2021). This method is used to study the evolution of sequences from a common ancestor (Sogandi, 2018). So that it can find out the DNA sequences of two or more living things that look different but have the possibility of coming from the same ancestor. In general, sequence alignment is divided into two types: local sequence alignment and global sequence alignment. In local sequence alignment, the sequence used focuses only on pieces of the overall sequence that are considered to have significant similarities. While global alignment uses the entire length of the sequence to be compared (Kusuma, 2019).

In 2018, Alfiana Ririsati Dewi conducted research on the application of the Needleman-Wunsch algorithm to identify mutations in corona virus DNA sequences. From this research,

it can be concluded that the identification of DNA mutations can be used to determine other viruses and corona virus mutations, and can be used in the health sector as a reference for making drugs for diseases resulting from corona virus mutations. In 2021, Jamhuri, Irawan and Mukhlas (2021) conducted research on how to calculate the percentage of similarity of two trajectories using the Needleman Wunsch algorithm and manual calculations based on slices of these trajectories. In the calculation using the Needleman Wunsch algorithm, the stages carried out are interpolating the trajectory, calculating the distance between the coordinate points of the two trajectories, identifying the equivalent distance, converting the trajectory into a sequence of alphabet letters, aligning the sequences, and determining the amount of similarity based on the alignment results. The similarity obtained from the alignment method compared directly with the length of the path shared by the two trajectories reaches more than 90%. In manual work, the Needleman-Wunsch algorithm takes quite a long time. However, along with the rapid development of technology, computers can be created to think intelligently like humans. From here, a system can be made that can work and think like an expert. Therefore, it is necessary to create a website-based system application by applying the Needleman-Wunsch algorithm.

Based on this description, the researcher will conduct sequence alignment to determine the level of similarity of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reinwardt*) DNA sequences using the Needleman-Wunsch algorithm with a website-based. Maleo (*Macrocephalon maleo*) is an endemic animal of Sulawesi (Sugianto, 2010). While gosong kaki merah (*Megapodius reinwardt*) was chosen as a comparison because it is found on a different island. However, it belongs to the same family, Megapodidae, which is a species of bird that buries its eggs, in small hills made of plant litter, in volcanic soil or sand exposed to sunlight to hatch its eggs. This is the basis for the alignment of two different DNAs to determine the level of similarity of the DNA structure in the two bird species (Megapodidae) and become data on the characteristics of Maleo (*Macrocephalon Maleo*) DNA in the fields of biology, health, and bioinformatics. One of them is as a reference for the government to manage maleo (*Macrocephalon maleo*) conservation.

2. METHODS

This study used the Needleman-Wunsch algorithm to determine the DNA similarity level of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reindwardt*). This algorithm was created by Saul Needleman and Christian Wunsch in 1970 (Needleman and Wunsch, 1970). The steps of the Needleman-Wunsch algorithm are:

1. Matrix Initialization

Suppose sequence $A = a_1, a_2, \dots, a_n$ and $B = b_1, b_2, \dots, b_m$, then create a score matrix of size $(n + 1) \times (m + 1)$. Where n is the number of rows representing the length of sequence A, and m is the number of columns representing the length of sequence B. The following is the substitution matrix of sequences A and B with $s = (i, j)$.

		a_1	a_2	$\dots$	a_n
	$s(0,0)$	$s(1,0)$	$s(2,0)$	$\dots$	$s(n, 0)$
b_1	$s(0,1)$	$s(1,1)$	$s(2,1)$	$\dots$	$s(n, 1)$
b_1	$s(0,2)$	$s(1,2)$	$s(2,2)$	$\dots$	$s(n, 2)$
$\dots$	$\dots$	$\dots$	$\dots$	$\dots$	$\dots$
b_m	$s(0,m)$	$s(1,m)$	$s(2,0)$	$\dots$	$s(n, m)$

2. Matrix Filling

Suppose matrix S is a score matrix, then the formula for the elements of matrix S is

$$S(i, j) = \max \begin{cases} S(i-1, j-1) + s(a_i, b_j) \\ S(i-1, j) - d \\ S(i, j-1) - d \end{cases} \quad (1)$$

The following illustrates the calculation of $s(i, j)$ as follows:

$S(i-1, j-1)$	$S(i-1, j)$
$s(a_i, b_j)$	$S(i, j)$
$S(i, j-1)$	$-d$
$-d$	$-d$

Where:

$S(i-1, j-1)$: matrix element S in the upper left diagonal

$S(i, j-1)$: matrix element S on the left $S(i, j)$

$S(i-1, j)$: matrix element S above $S(i, j)$

$S(a_i, b_j)$: substitution matrix element at the residue i in the sequence a and residue j in the sequence b

d : gap penalty/penalty score on virtual symbol

The calculation of filling the penalty gap can be formulated as follows:

$$g_{(i)} = i g_{op}$$

Where $g_{(i)}$ is the penalty gap to $-i$, and i is the index of a sequence. Whereas g_{op} or gap open is the beginning of a negative gap, with the determination of the value is free. Meanwhile, the match and mismatch scoring are

$$S(a_i, b_j) = \begin{cases} \text{match score, if } a_i = b_j \\ \text{mismatch score, if } a_i \neq b_j \end{cases}$$

3. Traceback Step

After the score matrix of size $(n+1) \times (m+1)$ has been completely filled, the maximum alignment score (the sum of all substitution values plus the sum of all gap penalties) of two sequences is the value of the bottom rightmost element of the score matrix, namely $S(n+1, m+1) = s(n, m) = s(i, j)$. $S(i, j)$ is the starting point for traceback until the end point $s(0, 0)$. If $S(i, j) = S(i-1, j-1) + s(a_i, b_j)$ then the trajectory is: $(i, j) \rightarrow (i-1, j-1)$.

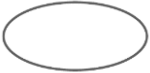
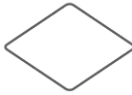
4. Determining the alignment result

- Denote the pair of DNAs as a_i, b_j if the reverse flow starts from a_i, b_j to the upper left corner.
- Inserts a virtual symbol in the vertical sequence and denotes it as $(a_i, -)$ if the backward flow is horizontal.
- Inserts a virtual symbol in the horizontal sequence and denotes it as $(-, b_j)$ if the backward flow is vertical.

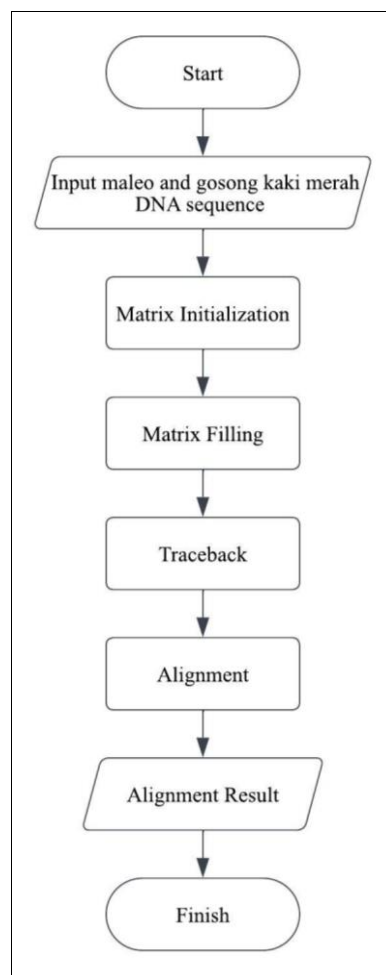
This research also uses flowchart to symbolically describe a procedure to solve a problem. Flowchart functions to provide an overview of the course of a program with the steps of a program. So that other people (not programmers) easily understand the course of a program (Chauduri, 2020).

The flowchart symbols used in this research are as follows:

Table 1. Explanation of symbols in *Flowchart*

Symbol	Explanation
	Flow A symbol that connects one symbol to another or states the flow in a process.
	Process A symbol that shows the processing done by the computer.
	Input/Output Symbols that express <i>input</i> or <i>output</i> processes independent of equipment
	Terminator Symbol that serves as the beginning (<i>start</i>) end (<i>stop</i>) of a program
	Decision Symbol that serves to select a process based on existing conditions.

The *Flowchart* of the *Needleman-Wunsch* algorithm can be seen in the following figure:

**Figure 1.** *Flowchart* of DNA Similarity Level Identification System

At this stage, the design stage of maleo (*Macrocephalon Maleo*) DNA similarity level identification system with gosong kaki merah (*Megapodius Reindwardt*) comparison using the

Needleman-Wunsch algorithm. Starting with inputting DNA sequence data. After the data is inputted, matrix initialization and matrix filling using equation 1 are carried out, then traceback and alignment to get the alignment results of DNA similarity level identification system of maleo (*Macrocephalon Maleo*) and gosong kaki merah (*Megapodius Reindwardt*).

3. RESULT AND DISCUSSION

3.1. Maleo (*Macrocephalon maleo*) DNA Sequence Data

Maleo (*Macrocephalon maleo*) DNA sequence data was obtained from NCBI (National Center for Biotechnology Information) in fasta format. The example of maleo (*Macrocephalon maleo*) DNA sequence data is as follows:

Macrocephalon maleo voucher AMNH DOT 9998 TGFB2 gene, complete sequence with fasta:

```
GTGCCCTCATTCACAGACCTGCGGTGTCCTTGCCCAATGGGTATCTGCCCAGTGCC
CTCACTCCTATGGAGACAGTGCCTGAAGAGGAAGGTTAGGACCTCAGAATGCAG
AAACTGAGTTCCATCTGGTGATTCCCAAGCCTGTCAGCCTCATAATTACAGCCAA
ATGACCACCTCCGCTGTGCTCGTCTCGTCTAATCAGAGAGGCAACTCCAGTAGGA
CCTGGAGAAAAGTGCTTCGTGATCCTCCATTAGCTTTATGAGCAAGCCTGGTGTC
AAAGCATCTCATTTGATTTACAGTATAAGCTGC.
```

3.2. DNA Sequence Data of Gosong Kaki Merah (*Megapodius reindwardt*)

DNA sequence data of Gosong Kaki Merah (*Megapodius reindwardt*) was obtained from NCBI (National Center for Biotechnology Information) in fasta format. The example data of the Gosong Kaki Merah (*Megapodius reindwardt*) DNA sequence is as follows:

Megapodius reinwardt voucher CAS ORN 97012 TGFB2 gene, complete sequence with fasta:

```
GTGCCCTCCACTCCCATGGAGACAGTGCCTGAAGAGGAAGGTTAGGACCTCAGA
ATGCAGAAACTGAGTTCCATGTGGGGTGATTCCCAAGCCTGTCAGCCTCATAATT
ACAGCCAAATGACCACCTGCTGTGCTTGTCTAATCAGAGAGGCAACTCCAGTAGG
ACCTGGAGAAAAGTGCTTCAGTGATCCTCCATTAGCTTTGTATGGGAAGTCCTGC
CGTGTGGAAGCATCTCATTTCAATTCAGTATAAGCTACAATGGATGTGATTTGGT
TGAATTTCTGACTTGCCTGTAAATCATCATTACAGGCAAGCAACTGGATTATGTT
```

3.3. System design

In this process, three diagrams are used, namely data flow diagram (DFD), use case diagram and context diagram. Data flow diagram is a graphical technique used to explain the flow of information and data transformation that moves from data entry (input) to data release (output). Data flow diagram is a tool in describing or explaining the work process of a system (Kristanto, 2018). Use case diagram is a diagram to describe the typical interaction between a user (admin) and a system through a system that is built (Kurniawan dan Syarifuddin, 2020). In use case diagram, there is an actor who interacts with other actors with the information system to be built. These actors can be humans, hardware, other systems, or those that interact with the system (Simatupang dan Sianturi, 2019). Activity diagram is a diagram that describes various activity flows in the system being designed, starting from the beginning of the flow to the end of the decision, and can describe parallel processes that may occur in several executions (Kurniawan dan Syarifuddin, 2020). Activity diagrams explain the sequence of activities in a process on the system as a whole (Booch, Rumbaugh, & Jacobson, 2020). The diagram is to describe the course of the system and is the basic design of the identification system of the DNA similarity level of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reindwardt*) using the web-based Needleman-Wunsch algorithm.

3.4. System Implementation

In this process, the system design will be applied to a website-based application system. The following are the results of the web-based application.

3.4.1. Input implementation

a) Home Page or Main Menu



Figure 2. Main Menu Display

The start page or main menu is the initial display when the user runs the application. On the home page there is one button to start the application. After pressing the start button, the data input page will appear

b) Data Input

Figure 3. DNA Data Input Form

This form is used to input the DNA sequence of maleo (*Macrocephalon Maleo*) and the DNA sequences of gosong kaki merah (*Megapodius reinwardt*), and there is a submit button used by the user after inputting the DNA sequence. In this form, there are also several menus available, namely maleo data menu, gosong kaki merah data menu, and process menu.

3.4.2. Output implementation

a) Result form

The result form is a page that appears after the user presses the submit button. This page serves to display the match, gap, and alignment results of the two DNA sequences.

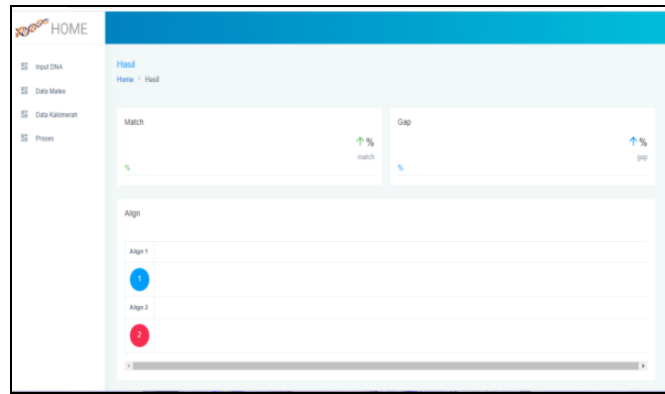


Figure 4. Result Form

3.5. System testing

After making the application has been completed, testing of the application is carried out. System function testing is done with the Blackbox Method to find out whether the functions contained in the system are running properly or not. The Blackbox Method is a method of testing software in terms of functional specifications without testing the design and code on the system to find out whether the functions, inputs and outputs match the required specifications (Cholifah et al., 2018). The results of system testing can be explained as follows:

Table 2. System testing Result

Tested Function	Expectation of Results	Testing Result
Save Maleo Data (Admin Form)	Can Save Maleo Data	Good
Save Gosong Kaki Merah Data (Admin Form)	Can Save Gosong Kaki Merah Data	Good
Maleo and Gosong Kaki Merah Data Input (Data Input Form)	Can Import Maleo and Gosong Kaki Merah Data	Good
Maleo Data Menu (Maleo Data Form)	Can Display Maleo Data	Good
Gosong Kaki Merah Data Menu (Gosong Kaki Merah Data Form)	Can Display Gosong Kaki Merah Data	Good
Process Menu (Proses Form)	Can Display Process Result	Good
Result Page (Result Form)	Can Display Alignment Results	Good

Based on the results of blackbox testing with the test cases above, it can be concluded that in the design of the maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reinwardt*) DNA similarity level identification system application using the Needleman-Wunsch algorithm, there are no process errors and functionally produce results in accordance with the expected.

3.6. Test Results of DNA Sequence Similarity Level of Maleo (*Macrocephalon maleo*) and Gosong Kaki Merah (*Megapodius reinwardt*) Using Needleman-Wunsch Algorithm.

The results of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reinwardt*) DNA similarity level test using Needleman-Wunsch on the system using 10 maleo (*Macrocephalon maleo*) DNA sequences and 10 gosong kaki merah (*Megapodius reinwardt*) DNA sequences by calculating the results of the percentage of matches and the percentage of gaps according to the code on each sequence on maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reinwardt*) body parts. DNA is said to match if the two DNAs are the same, while if there is an exclusion or insertion in one of the DNAs it is said to be a gap. The

results of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reinwardt*) DNA similarity level test are as follows:

Table 3. Test Results of DNA Sequence Similarity Level of Maleo (*Macrocephalon maleo*) and Gosong Kaki Merah (*Megapodius reinwardt*) Using Needleman-Wunsch Algorithm.

Code	DNA Sequence Name		Match (%)	Gap (%)
	Maleo	Gosong Kaki-Merah		
TGFB2	<i>Macrocephalon maleo</i> voucher AMNH DOT 9998 TGFB2 gene, complete sequence	<i>Megapodius reinwardt</i> voucher CAS ORN 97012 TGFB2 gene, complete sequence	81,3	31,69
RDP1	<i>Macrocephalon maleo</i> isolate cons.mal. rhodopsin (RDP1) gene, exons 1 and 2 and partial cds	<i>Megapodius reinwardt</i> isolate cons.mgr. rhodopsin (RDP1) gene, exons 1 and 2 and partial cds	94,64	5,48
TXNDC 12	<i>Macrocephalon maleo</i> voucher AMNH DOT 110103 thioredoxin domain-containing 12 (TXNDC12) gene, intron 6	<i>Megapodius reinwardt</i> voucher CAS ORN 97013 thioredoxin domain-containing 12 (TXNDC12) gene, intron 6	94,78	3,37
NGF	<i>Macrocephalon maleo</i> voucher AMNH DOT 110103 nerve growth factor beta polypeptide (NGF) gene, partial cds	<i>Megapodius reinwardt</i> voucher WAM 22922 nerve growth factor beta polypeptide (NGF) gene, partial cds	96,96	0,13
IRF2	<i>Macrocephalon maleo</i> voucher AMNH DOT 110103 interferon regulatory factor 2 (IRF2) gene, intron 2	<i>Megapodius reinwardt</i> voucher CAS ORN 97013 interferon regulatory factor 2 (IRF2) gene, intron 2	96,68	1,26
TGFB21	<i>Macrocephalon maleo</i> voucher AMNH DOT 9998 TGFB2 gene, complete sequence	<i>Megapodius reinwardt</i> voucher CAS ORN 97012 TGFB2 gene, complete sequence	81,3	31,69
TGFB22	<i>Macrocephalon maleo</i> voucher AMNH DOT 110103 TGFB2 gene, complete sequence	<i>Megapodius reinwardt</i> voucher CAS ORN 97011 TGFB2 gene, complete sequence	97,12	1,61
PAXIP1	<i>Macrocephalon maleo</i> voucher AMNH DOT 9998 PAX interacting protein 1 (PAXIP1) gene, intron 20	<i>Megapodius reinwardt</i> voucher CAS ORN 97011 PAX interacting protein 1 (PAXIP1) gene, intron 20	95,75	4,4
PARK7	<i>Macrocephalon maleo</i> voucher AMNH DOT 9998 Parkinson disease 7 (PARK7) gene, exon 2, intron 2, exon 3 and partial cds	<i>Megapodius reinwardt</i> voucher CAS ORN 96335 Parkinson disease 7 (PARK7) gene, exon 2, intron 2, exon 3 and partial cds	95,31	4,6
FGB	<i>Macrocephalon maleo</i> voucher AMNH DOT 9998 fibrinogen beta chain (FGB) gene, intron 5, exon 6 and partial cds	<i>Megapodius reinwardt</i> voucher CAS ORN 97013 fibrinogen beta chain (FGB) gene, intron 5, exon 6 and partial cds	96,76	0,0
Average Number			83,39	8,42

The results of the DNA similarity level test using 10 sequence data of maleo (*Macrocephalon maleo*) and 10 sequence data of gosong kaki-merah (*Megapodius reinwardt*), can be seen in table 2 which uses a match score value of 1, mismatch of -1, and gap of -2, by calculating the percentage match value and the percentage gap (Muhamad, Ahmad, Asi, & Murad, 2018). Where the percentage match is used to determine the level of similarity of maleo (*Macrocephalon maleo*) and gosong kaki-merah (*Megapodius reinwardt*), while the percentage gap is used to determine the insertion or exclusion in maleo (*Macrocephalon maleo*) and gosong kaki-merah (*Megapodius reinwardt*) DNA. Based on the test results, the similarity level of maleo (*Macrocephalon maleo*) dan gosong kaki-merah (*Megapodius reinwardt*) DNA averaged 83,39% with an average gap value of 8,42%.

4. CONCLUSION

Based on the results and discussion, the following conclusions can be drawn:

- Needleman-Wunsch algorithm can be applied in completing DNA sequence alignment on a web-based application system to determine the level of similarity of DNA maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reindwardt*).
- The DNA sequence similarity level of maleo (*Macrocephalon maleo*) and gosong kaki merah (*Megapodius reindwardt*) in the web-based application system averaged 83.39% and the average gap value was 8.42%.

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