

Phylogenetic Analysis of *Manihot* sp. Based on *matK* and *rbcL* Markers

Waki'atil Rosida¹, Fuad Bahrul Ulum¹, Mukhamad Su'udi^{1*}

¹Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Jember 68212, Indonesia.

*Correspondence Author: msuudi.fmipa@unej.ac.id

Abstract

Cassava (*Manihot* sp.) is a staple crop of major economic and ethnomedicinal importance. Understanding the phylogenetic relationships within the genus *Manihot* is essential for clarifying its taxonomy and supporting genetic improvement programs. This study aimed to reconstruct the phylogeny of several *Manihot* species using two chloroplast DNA markers, *matK* and *rbcL*. Sequence data were retrieved from the GenBank database, and *Ricinus communis* was designated as the outgroup. Phylogenetic analyses were performed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods, each with 1,000 bootstrap replications to assess branch support. The resulting trees consistently clustered *Manihot esculenta* within a well-supported monophyletic group, indicating a close genetic relationship with other congeners. Distinct clades corresponding to genetic similarity were also observed among the analyzed species. These findings enhance current knowledge of *Manihot* systematics, providing molecular evidence that supports its taxonomic classification. Furthermore, the results contribute valuable insights for cassava breeding and conservation efforts through improved understanding of its evolutionary relationships.

Keywords: Cassava, GenBank, *Manihot esculenta*, Neighbor Joining, Phylogenetic

Introduction

Cassava (*Manihot* sp.) is a vital food crop that serves as a staple source of carbohydrates for millions of people, particularly in tropical and subtropical regions. It thrives in a wide range of environments, including arid and nutrient-poor soils, making it one of the most resilient and adaptable crops worldwide (Nurdjanah et al., 2020). Beyond its role as a dietary staple and rice substitute, cassava is also a significant agricultural commodity contributing to global trade. Major cassava-producing countries include Indonesia, Nigeria, the Democratic Republic of the Congo, Brazil, and Thailand, where it plays an important socioeconomic role in both rural livelihoods and food security (Reskhi Firdaus et al., 2016).

In addition to its nutritional value, cassava has long been recognized for its medicinal properties. In several traditional medicine systems, such as those practiced in Nigeria, the leaves and roots are used to treat abscesses, tumors, conjunctivitis, and wounds. Phytochemical studies have shown that cassava leaves contain various bioactive compounds, including alkaloids, tannins, saponins, flavonoids, glycosides, and phenolic compounds (Akmalia et al., 2020). Cassava's ease of cultivation, pest resistance, and tolerance to drought make it superior to many other staple crops such as rice. Because it is an indigenous and underutilized food resource, comprehensive characterization of cassava's genetic diversity is essential for both

conservation and crop improvement (Sikteubun et al., 2022).

Molecular phylogenetic analysis provides a reliable framework for understanding evolutionary relationships within plant taxa. Among the most widely used genetic markers for plant systematics are the chloroplast genes *matK* and *rbcL*. These markers, recommended by the Consortium for the Barcode of Life (CBOL) Plant Working Group (2009), have demonstrated high universality and discriminatory power for plant DNA barcoding. The *matK* gene, located within the chloroplast genome, has high substitution rates and is useful for resolving relationships across diverse taxonomic levels (Su'udi et al., 2023). Similarly, the *rbcL* gene, which encodes ribulose-1,5-bisphosphate carboxylase/oxygenase, is a conserved locus with high amplification success and phylogenetic informativeness (Afifah et al., 2025).

Using publicly available sequence data from NCBI, this study aims to elucidate the phylogenetic relationships among species within the genus *Manihot* based on *matK* and *rbcL* markers. The findings are expected to enhance understanding of *Manihot* taxonomy and evolutionary history while providing a molecular foundation to support cassava breeding programs aimed at improving productivity, resilience, and genetic conservation.

Materials and Methods

Nucleotide Data Collection

This study utilized publicly available nucleotide sequence data retrieved from the GenBank database of the National Center for Biotechnology Information (NCBI). Searches were conducted using the keywords “Manihot” combined with either “*matK*” or “*rbcL*.” The dataset included accessions representing various Manihot species distributed globally, with particular focus on Manihot esculenta. All retrieved sequences were downloaded in FASTA format.

To provide an evolutionary reference, *Ricinus communis* (Euphorbiaceae) was selected as the outgroup taxon, as it belongs to the same family as Manihot. The compiled sequence files were subsequently processed for multiple alignment and phylogenetic analysis using MEGA version 11 (Tamura et al., 2021).

Phylogenetic Trees Construction

Sequence alignments were performed using the ClustalW algorithm (Thompson et al., 1994) implemented in MEGA 11 with default parameters. Phylogenetic trees were reconstructed using two

complementary approaches: the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods. The robustness of each node was evaluated by bootstrap analysis with 1,000 replicates.

Pairwise genetic distances were calculated to determine evolutionary divergence among taxa, with smaller distances indicating closer relationships. The NJ method was employed for its computational efficiency and reliability in handling large datasets (Kumar et al., 2018), while ML analysis provided higher statistical accuracy in model-based inference of phylogenetic relationships. The resulting trees were visualized and compared to assess topological consistency between methods.

Results and Discussion

A total of 19 nucleotide sequences for the chloroplast genes *matK* (700–800 bp) and *rbcL* (500–1,500 bp), along with one outgroup species (*Ricinus communis*), were retrieved from the NCBI GenBank database (Tables 1 and 2). BLAST searches confirmed that all selected accessions belonged to the genus Manihot, representing various cultivars and geographic origins, including Asia, Africa, North America, and Europe.

Table 1. List of *Manihot* spp. *matK* gene sequences obtained from NCBI GenBank

No	Name	Accession Number	Source
1	<i>Manihot esculenta</i>	MK510204.1	Thailand
2	<i>Manihot esculenta</i> cultivar Pirun1	MK834340.1	Thailand
3	<i>Manihot</i> sp.	MT832458.1	United Kingdom
4	<i>Manihot esculenta</i> cultivar Huaybong60	MK834338.1	Thailand
5	<i>Manihot esculenta</i> cultivar Kasetsart50	MK834337.1	Thailand
6	<i>Manihot esculenta</i> cultivar Rayong90	MK834336.1	Thailand
7	<i>Manihot esculenta</i> cultivar Rayong72	MK834335.1	Thailand
8	<i>Manihot aesculifolia</i>	JQ587462.1	Canada
9	<i>Manihot aesculifolia</i>	JQ587463.1	Canada
10	<i>Manihot dichotoma</i>	MT370644.1	United Kingdom
11	<i>Manihot esculenta</i> cultivar Rayong9	MK834331.1	Thailand
12	<i>Manihot esculenta</i> cultivar Rayong7	MK834330.1	Thailand
13	<i>Manihot esculenta</i> cultivar Rayong5	MK834329.1	Thailand
14	<i>Manihot caerulea</i>	MT370643.1	United Kingdom
15	<i>Manihot aesculifolia</i>	JQ587460.1	Canada
16	<i>Manihot esculenta</i> cultivar Rayong1	MK834326.1	Thailand
17	<i>Manihot esculenta</i> cultivar Hanatee	MK834342.1	Thailand
18	<i>Manihot esculenta</i> cultivar Pirun2	MK834341.1	Thailand
19	<i>Manihot esculenta</i> cultivar Huaybong80	MK834339.1	Thailand
20	<i>Ricinus communis</i>	MW843833.1	China

Among the *matK* accessions, the majority of sequences were from Thailand, particularly cultivars such as Rayong, Kasetsart, Huaybong, Pirun, and Hanatee. The Rayong cultivar is recognized as one of the earliest released varieties from Kasetsart University, characterized by narrow leaves and yellowish stems. Kasetsart, a hybrid derived from Rayong 1 × Rayong 90, is known for

its light green foliage, light brown stems, and high starch yield (Ha et al., 2016). The Huaybong cultivar displays large tuberous roots and upright growth, whereas Pirun exhibits moderate height, grayish-brown stems, and strong drought tolerance (Malik et al., 2020).

relationships and geographic variation within the genus.

No	Name	Accession Number	Source
1	<i>Manihot carthagenensis</i> isolate Mozambique	MK430314.1	Brazil
2	<i>Manihot esculenta</i> isolate Mozambique	MK430313.1	Brazil
3	<i>Manihot esculenta</i> isolate Kenya	MK430304.1	Brazil
4	<i>Manihot esculenta</i> isolate Tanzania	MK430292.1	Brazil
5	<i>Manihot esculenta</i> isolate MS2	MW960605.1	China
6	<i>Manihot esculenta</i> isolate DNAS	MK475659.1	USA
7	<i>Manihot grahamii</i> isolate PECB125	MG718146.1	Brazil
8	<i>Manihot aesculifolia</i>	JQ591493.1	Canada
9	<i>Manihot esculenta</i> isolate Kenya	MK430302.1	Brazil
10	<i>Manihot esculenta</i>	LT576833.1	United Kingdom
11	<i>Manihot esculenta</i>	MK510193.1	Thailand
12	<i>Manihot esculenta</i>	MW164974.1	Nigeria
13	<i>Manihot aesculifolia</i>	JQ591492.1	Canada
14	<i>Manihot esculenta</i>	MW164975.1	Nigeria
15	<i>Manihot grahamii</i>	AY794875.1	USA
16	<i>Manihot esculenta</i> isolate Mozambique	MK430306.1	Brazil
17	<i>Manihot esculenta</i> isolate Tanzania	MK430289.1	Brazil
18	<i>Manihot aesculifolia</i>	JQ591492.1	Canada
19	<i>Manihot esculenta</i>	JQ591497.1	Canada
20	<i>Ricinus communis</i>	OL537090.1	USA

Huaybong, Kasetsart, Rayong, and Hanatee formed a well-supported monophyletic clade with a bootstrap value of 100%, indicating strong evolutionary relatedness. Moreover, *Manihot* sp. grouped closely with *M. aesculifolia*, supported by a moderate bootstrap value of 74%, suggesting a probable shared ancestor. These findings support the concept of monophyly, where species exhibiting similar genetic and biochemical characteristics descend from a common lineage (Hidayat et al., 2008).

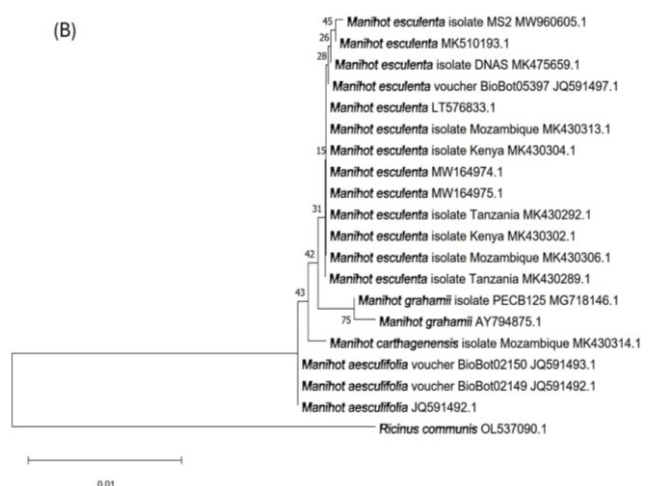
(A)

Phylogenetic tree (A) showing relationships between *Manihot* and *Ricinus* species. The tree is rooted on the left and branches to the right. Bootstrap values are indicated at the nodes. The scale bar at the bottom left represents 5.00 substitutions per site.

Species and bootstrap values (from top to bottom):

- Manihot esculenta* cultivar Pirun2 MK834341.1
- Manihot esculenta* cultivar Huaybong80 MK834339.1
- Manihot esculenta* cultivar Rayong11 MK834332.1
- Manihot esculenta* cultivar Rayong1 MK834326.1
- Manihot esculenta* cultivar Rayong5 MK834329.1
- Manihot esculenta* cultivar Rayong7 MK834330.1
- Manihot esculenta* cultivar Rayong9 MK834331.1
- Manihot esculenta* cultivar Rayong72 MK834335.1
- Manihot esculenta* cultivar Rayong90 MK834336.1
- Manihot esculenta* cultivar Kasetsart50 MK834337.1
- Manihot esculenta* cultivar Huaybong60 MK834338.1
- Manihot esculenta* cultivar Hanatee MK834342.1
- Manihot esculenta* cultivar Pirun1 MK834340.1
- Manihot dichotoma* MT370644.1 (30)
- Manihot caerulescens* MT370643.1
- Manihot aesculifolia* JQ587462.1 (100)
- Manihot aesculifolia* JQ587460.1 (70)
- Manihot* sp. MT832458.1 (38)
- Manihot aesculifolia* JQ587463.1 (74)
- Ricinus communis* MW843833.1

Scale bar: 5.00



29

In contrast, the NJ phylogeny constructed from *rbcL* sequences (Figure 1B) showed that *M. grahamii* clustered with its conspecific accessions, supported by a bootstrap value of 75%, whereas *M. esculenta* sequences were grouped together but with relatively lower bootstrap values (15–42%), indicating weaker resolution at the species level. These results demonstrate that the *matK* marker provides higher discriminatory power than *rbcL* for resolving intra-generic relationships in *Manihot*. The superior resolution of *matK* may be attributed to its higher substitution rate and greater

phylogenetic informativeness in angiosperms (Su'udi et al., 2023).

Comparative Phylogenetic Inference Using Maximum Likelihood

Phylogenetic reconstruction using the Maximum Likelihood (ML) method (Figure 2) revealed overall topological patterns consistent with the NJ analysis, though with variations in bootstrap support. The ML approach, known for its higher statistical accuracy and model-based inference, provided improved confidence estimates for most clades (Tamura et al., 2021).

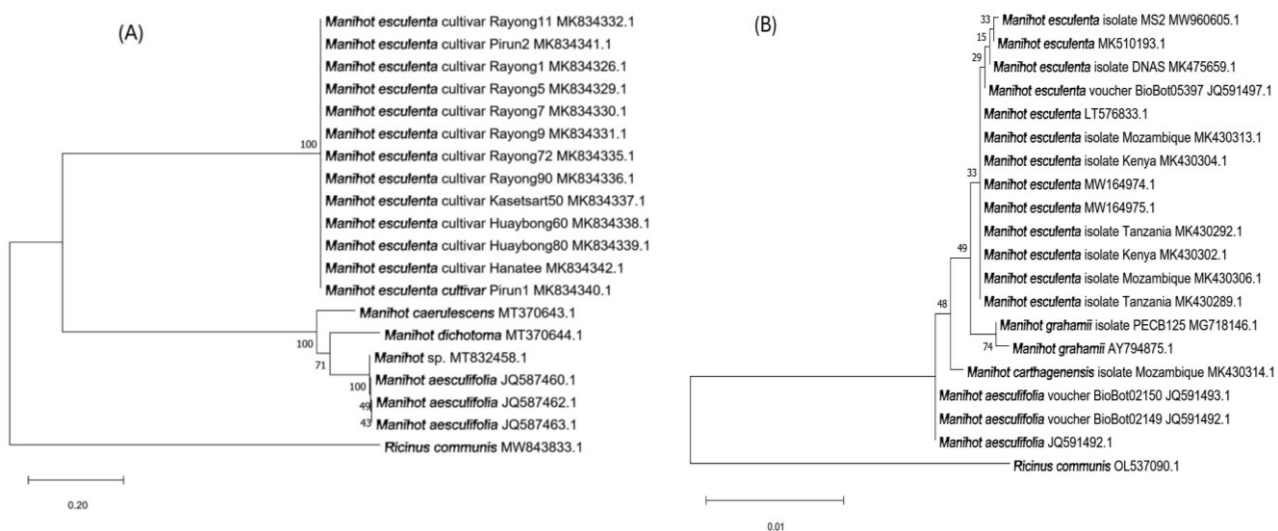


Figure 2. Phylogenetic trees of *Manihot* spp. constructed using the Maximum Likelihood (ML) method based on (A) *matK* and (B) *rbcL* gene sequences.

The evolutionary distances among species were visualized through branch length variation: longer branches indicated greater genetic divergence, while shorter branches reflected closer evolutionary proximity (Anafarida & Badruzsaufari, 2020). The ML tree revealed that *M. esculenta* accessions from Thailand clustered tightly with a bootstrap value of 100%, confirming their close genetic affinity. Similarly, *M. grahamii* accessions from Brazil and the United States formed a moderately supported clade (bootstrap = 74%). Bootstrap values between 70–100% suggest stable clades, whereas values below 70% indicate potential topological uncertainty.

The ML-based topology successfully separated *Manihot* species into distinct clades corresponding to their genetic and geographic relationships. The high confidence observed for *matK*-based groupings compared to *rbcL* supports its superior utility for species-level resolution in cassava phylogenetics. Previous research has shown that

matK often outperforms *rbcL* due to its higher substitution rate and alignment variability, enabling clearer delineation of closely related taxa (Simon et al., 2022).

In summary, both markers successfully reconstructed the phylogeny of *Manihot* species, but *matK* demonstrated greater discriminatory power, while the ML method provided higher accuracy and better clade support compared to NJ. These findings confirm that the integration of *matK* and *rbcL* markers, analyzed under robust phylogenetic frameworks, provides valuable insights into the evolutionary relationships and genetic diversity within the genus *Manihot*.

Acknowledgments

The authors acknowledge the Biotechnology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, for providing research facilities and scientific support.

References

- Affiah, N., Susilowati, A., & Purwanto, E. (2025). Morphological and genetic variation of cassava (*Manihot esculenta*) based on DNA markers barcoding *rbcL* and ITS. *Asian Journal of Agriculture*, 9(1), 255–263. <https://doi.org/10.13057/asianjagric/g090127>
- Akmalia, H., Zulfitri, & Ridwan, A. (2020). Daya Hambat Ekstrak Etanol Daun Ubi Kayu (*Manihot esculenta* Crantz) Varietas Mentega Terhadap Methicilin-Resistant *Staphylococcus aureus* (MRSA) Secara in Vitro. *Kedokteran Nanggroe Medika*, 3(3), 11–22.
- Anafarida, O., & Badruzsaufari, B. (2020). Analisis Filogenetik Mangga (*Mangifera* Spp.) Berdasarkan Gen 5,8S Rrna. *Ziraa'Ah Majalah Ilmiah Pertanian*, 45(2), 120. <https://doi.org/10.31602/zmip.v45i2.3001>
- CBOL plant working group. (2009). A DNA barcode for land plants. *The Proceedings of the National Academy of Sciences (PNAS)*, 106(31), 12794–12797. <https://doi.org/10.1111/1755-0998.12194>
- Dharmayanti, I. (2011). Filogenetika Molekuler: Metode Taksonomi Organisme Berdasarkan Sejarah Evolusi. *Wartazoa*, 21(1), 1–10. <https://doi.org/10.2307/2799276>
- Gaffar, S., Sumarlin, S., Haryono, M. G., & Pidar, H. (2021). Penentuan Jenis dan Status Konservasi Pari Layang-Layang yang Didaratkan Di TPI Gunung Lingkas Kota Tarakan Dengan Pendekatan Molekuler. *Biotropika: Journal of Tropical Biology*, 9(1), 80–87. <https://doi.org/10.21776/UB.BIOTROPIKA.2021.009.01.09>
- Ha, C. D., Ngoc Quynh, L. T., Hien, N. T., Ly Thu, P. T., Ham, L. H., & Dung, L. T. (2016). Morphological characterization and classification of cassava (*Manihot esculenta* Crantz) in Vietnam. *Tap Chi Sinh Hoc*, 38(3). <https://doi.org/10.15625/0866-7160/v38n3.8570>
- Hidayat, T., Kusumawaty, D., Kusdianti, Yati, D. D., Muchtar, A. A., & Mariana, D. (2008). Analisis Filogenetik Molekuler pada *Phyllanthus niruri* L. (*Euphorbiaceae*) Menggunakan Urutan Basa DNA Daerah Internal Transcribed Spacer (ITS). *Jurnal Matematika Dan Sains*, 13(1), 16–21. https://www.researchgate.net/publication/280566991_Analisis_Filogenetik_Molekuler_pada_Phyllanthus_niruri_L_Euphorbiaceae_Menggunakan_Urutan_Basa_DNA_Daerah_Internal_Transcribed_Spacer_ITS
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/MOLBEV/MSY096>
- Lestari, D. A., Azrianingsih, R., & Hendrian, H. (2018). Filogenetik Jenis-jenis *Annonaceae* dari Jawa Timur Koleksi Kebun Raya Purwodadi Berdasarkan Coding dan Non-coding sekuen DNA. *Journal of Tropical Biodiversity and Biotechnology*, 3(1), 1. <https://doi.org/https://doi.org/10.22146/jtbb.28308>
- Malik, A. I., Kongsil, P., Nguyễn, V. A., Ou, W., Sholihin, Srean, P., Sheela, M. N., López-Lavalle, L. A. B., Utsumi, Y., Cheng, C., Kittipadakul, P., Nguyễn, H. H., Ceballos, H., Nguyễn, T. H., Gomez, M. S., Aiemnaka, P., Labarta, R., Chen, S., Amawan, S., ... Ishitani, M. (2020). Cassava breeding and agronomy in Asia: 50 years of history and future directions. *Breeding Science*, 70(2), 145–166. <https://doi.org/10.1270/jsbbs.18180>
- Nurdjanah, S., Susilawati, S., Hasanudin, U., & Anitasari, A. (2020). Karakteristik Morfologi Dan Kimiawi Beberapa Varietas Ubi Kayu Manis Asal Kecamatan Palas, Kabupaten Lampung Selatan Berdasarkan Umur Panen Yang Berbeda. *Jurnal Agroteknologi*, 14(02), 126. <https://doi.org/10.19184/j-agt.v14i02.17383>
- Reskhi Firdaus, N., Dewi Hayati, P., Peminatan Pemuliaan Tanaman, B., Studi Agroekoteknologi, P., Pertanian, F., Tangah, K., Anai, B., Pariaman, P., & Selatan, P. (2016). KARAKTERISASI FENOTIPIK UBI KAYU (*Manihot esculenta* Crantz) LOKAL SUMATERA BARAT Phenotypic Characterization of Cassava (*Manihot esculenta* Crantz) Landrace in West Sumatera. *Jurnal Agroteknologi*, 10(01), 104–116.
- Rusinko, J., & McPartlon, M. (2017). Species tree estimation using Neighbor Joining. *Journal of Theoretical Biology*, 414(November 2016), 5–7. <https://doi.org/10.1016/j.jtbi.2016.11.005>
- Sikteubun, F. S., Patty, J. R., & Polnaya, F. (2022). Identifikasi Karakter Morfologi Varietas Lokal Ubi Kayu (*Manihot esculenta* Crantz) di Kecamatan Leihitu, Kabupaten Maluku Tengah Identification of Morphological Characters of Cassava (*Manihot esculenta* Crantz) Local Varieties in Leihitu District, Ce. 18(2), 116–122. <https://doi.org/10.30598/jbdp.2022.18.2.116>
- Simon, M. F., Mendoza Flores, J. M., Liu, H. L., Martins, M. L. L., Drovetski, S. V., Przelomska, N. A. S., Loiselle, H., Cavalcanti, T. B., Inglis, P. W., Mueller, N. G., Allaby, R. G., Freitas, F. de O., & Kistler, L. (2022). Phylogenomic analysis points to a South American origin of *Manihot* and illuminates the primary gene pool of cassava. *New Phytologist*, 233(1), 534–545. <https://doi.org/10.1111/nph.17743>
- Su'udi, M., Puspito, A. N., Arimurti, S., Hasanah, M.L. & Arum, A. (2022). Karakterisasi Molekuler Gen HAP3 pada Tanaman Ubi Kayu (*Manihot esculenta* Crantz). *Indonesian Journal of Biotechnology and Biodiversity*, 6(2), 68–76. DOI: <https://doi.org/10.47007/ijobb.v6i2.136>

- Su'udi, M., Setyati, D., Khoirunnisa, G. A., Afidah, Y., & Ulum, F. B. (2023). The *matK* Mini-barcode as a Potential Molecular Identification Tool for Medicinal Orchids. *Life Science and Biotechnology*, 1(1), 21. <https://doi.org/10.19184/lb.v1i1.39851>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>