Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 1021 – 1032 (2025)
www.ejabf.journals.ekb.eg

Genetic Evaluation of Local Strain BRAJA 001 Tilapia Resulting from Crossbreeding (Kekar × Jatimbulan Strains)

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ARTICLE INFO

Article History:
Received: June 7, 2025
Accepted: Aug. 30, 2025
Online: Sep. 22, 2025

Keywords:
BRAJA 001,
Tilapia,
Oreochromis niloticus,
Genetic evaluation

ABSTRACT

Improving aquaculture productivity in Indonesia requires the development of locally adapted, high-performance tilapia strains, particularly for saline water environments. This study aimed to genetically evaluate a hybrid tilapia strain, namely BRAJA 001, derived from crossbreeding between the Kekar and Jatimbulan tilapia strains, using the mitochondrial *cytochrome oxidase subunit I* (*co1)* gene as a molecular marker. A total of ten individuals were sampled, and *co1* sequences were amplified, sequenced, and analyzed using BLAST, nucleotide composition profiling, phylogenetic tree reconstruction, and genetic distance estimation. BLAST results confirmed the identity of BRAJA 001 as *Oreochromis niloticus* with 100% similarity. Nucleotide composition showed a consistent base pattern (T: 28.9%, C: 29.8%, A: 24.4%, G: 16.9%) across BRAJA 001 and its parental strains, indicating mitochondrial stability. Phylogenetic analysis grouped BRAJA 001 with its parental lines and other *O. niloticus* populations from different regions, revealing high genetic similarity. Genetic distance values between BRAJA 001 and other *Oreochromis* species remained low (0.051–0.073), supporting its close relationship within the genus. These findings demonstrate that BRAJA 001 maintains genetic integrity while combining desirable traits from both parental strains, establishing a strong foundation for its further development as a superior, saline-tolerant local tilapia strain for sustainable aquaculture.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species worldwide due to its rapid growth, high tolerance to diverse environmental conditions,

and strong consumer demand **(El-Sayed, 2021)**. In Indonesia, developing high-performing local strains is a strategic priority to enhance fish quality and production efficiency across various aquaculture systems, including saline environments. Crossbreeding genetically distinct but locally adapted strains offers a promising approach to combine desirable traits such as fast growth, salinity tolerance, and disease resistance. The Kekar strain is recognized for its robust physiological performance in challenging environments, morphometric traits and broodstock size in Kekar tilapia are closely associated with egg production, emphasizing the importance of selective breeding for strain improvement **(Nasuki *et al.*, 2025)**. The Jatimbulan strain exhibits favorable growth and adaptability. Crossbreeding these two local strains is expected to produce a superior strain, provisionally named BRAJA 001, which integrates the genetic advantages of both parental lines. However, a critical step in this breeding program is the genetic evaluation of the hybrid offspring to ensure genetic stability, purity, and potential for strain fixation.Therefore, a comprehensive molecular evaluation is essential to validate the genetic identity of BRAJA 001 and to support its future application in selective breeding programs.

The genetic evaluation of locally developed strains such as BRAJA 001 is crucial to ensure the sustainability of tilapia breeding programs in Indonesia, since crossbreeding between strains has been shown to enhance genetic diversity and generate heterosis effects that improve growth performance and environmental resilience **(Albar *et al.* 2025)**.

Molecular techniques, particularly mitochondrial DNA markers such as the *cytochrome oxidase subunit* I (*co1*) gene, have become indispensable tools in fish genetics, providing reliable insights into species identification, assessments of genetic diversity, and the reconstruction of phylogenetic relationships **(Hebert *et al.*, 2003; Ward *et al.*, 2005)**. The *co1* gene, commonly used in DNA barcoding, provides a reliable and standardized method for genetic assessment in tilapia. By applying polymerase chain reaction (PCR), sequencing, and bioinformatic tools such as BLAST analysis, nucleotide composition profiling, and phylogenetic tree reconstruction, comprehensive insights into the genetic structure of hybrid populations can be achieved. Similarly, **Syaifudin *et al*. (2025)** confirmed the utility of *co1* for discriminating tilapia populations from Lake Toba, Lake Ranau, and Sukamandi hatchery. At the global level, **Wu and Yang (2012)** highlighted that although the mitochondrial control region (CR) shows higher variability, *co1* remains a robust standard for cross-validation in species identification. More recently, **Raghuwanshi *et al*. (2024)** developed a DNA barcode database for *O. niloticus* using *co1,* further supporting its reliability in aquaculture genetics. Collectively, these findings confirm that *co1* is a powerful molecular marker for characterizing the genetic identity, diversity, and evolutionary relationships of tilapia strains and hybrids.

This study aimed to evaluate the genetic profile of saline tilapia resulting from the cross between the Kekar and Jatimbulan strains using the *co1* gene as a molecular marker. The results are expected to serve as a scientific foundation for the development and stabilization of the BRAJA 001 strain, contributing to the advancement of sustainable local aquaculture in Indonesia.

MATERIALS AND METHODS

This study was conducted from October 2024 to February 2025 using ten local saline tilapia (*Oreochromis niloticus*), consisting of five males and five females, which were the resulting of crossbreeding between the local Jatimbulan and Kekar strains. All specimens were humanely euthanized prior to tissue sampling in accordance with established animal welfare guidelines. Fin tissues were carefully dissected, homogenized, and subjected to genomic DNA extraction using the Geneaid Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan), following the manufacturer's protocol

Amplification of the partial mitochondrial *co1* gene was carried out using the polymerase chain reaction (PCR) technique. Universal fish primers FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) **(Anitasari *et al.*, 2025)**. Each PCR reaction was performed in a 25µL reaction volume, consisting of DNA template, primers, dNTPs, buffer, and Taq polymerase. Thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 15 seconds, with a final extension at 72°C for 6 minutes. PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV transillumination to verify amplification success. Successfully amplified fragments were purified and submitted for bidirectional sequencing to FIRST BASE DNA Sequencing Service.

Data analysis

DNA sequences in FASTA format were complemented with additional reference sequences obtained from GenBank. Sequence alignment was conducted using Mesquite version 3.70, while phylogenetic reconstruction was carried out using the Maximum Likelihood method implemented in MEGA version 11. To assess genetic divergence within and between groups, p-distance values were calculated. Further analyses of genetic variation were performed using DnaSP version 6.

RESULTS

1. Results of the BLAST

The DNA sequences obtained from the samples were analyzed using the BLAST tool available on the NCBI platform to compare them with reference sequences in public

databases. The analysis generated up to 100 sequences with the highest similarity to the query, allowing accurate species identification and genetic comparison. In addition, BLAST provided key parameters such as query coverage and percentage identity, which indicate the extent of alignment and the level of similarity between the sample sequences and the reference sequences. These metrics are essential for validating species identity and assessing the genetic stability of the studied strain, with the detailed results presented in Table (1).

Table 1. Results of NCBI BLAST analysis

No	Description	Query cover	Expectation value	% Identity
1	*Oreochromis niloticus*	100%	0	100.00%
2	*Oreochromis niloticus*	100%	0	100.00%
3	*Oreochromis niloticus*	100%	0	100.00%

2. Nucleotide composition

Nucleotide base composition analysis was conducted using both primary data (saline tilapia) and secondary data (ingroup and outgroup species). This analysis aimed to determine the proportion of nucleotide bases comprising the *co1* gene in each sequence used. The results of the nucleotide composition analysis from both primary and secondary data are presented in Table (2).

Table 2. Nucleotide composition of the *co1* gene in Braja 001 tilapia and comparative strains

No.	Strain/Species	T(U)	C	A	G
1	BRAJA 001 Tilapia	28.9	29.8	24.4	16.9
2	Jatimbulan Tilapia	28.9	29.8	24.4	16.9
3	Kekar Tilapia	28.9	29.8	24.4	16.9
4	*O. niloticus* (IND)	28.9	29.8	24.4	16.9
5	*O. niloticus* (USA)	28.9	29.8	24.4	16.9
6	*O. niloticus* (PLH)	28.9	29.8	24.4	16.9
7	*O. niloticus* (IDN) HM	29.1	30.4	24.1	16.5
8	*O. niloticus* (IDN) KU	28.3	30.1	24.5	17.1
9	*O. aureus*	28.4	30.4	23.5	17.7
10	*O. mossambicus*	28.9	29.3	24.1	17.7
11	*O. jipe*	28.9	29.4	24.4	17.3
12	*O. andersoni*	29.1	29.3	24.3	17.3
	Average	28.8	29.7	24.3	17.2

Overall, the nucleotide composition revealed that thymine (T) accounted for 28.8%, cytosine (C) for 29.7%, adenine (A) for 24.3%, and guanine (G) for 17.2%. The average

A+T content was 53.1%, while the G+C content was 46.9%, indicating a lower proportion of G+C bases compared to A+T bases. One characteristic feature of the *co1* coding gene is that guanine (G) content is typically lower than cytosine (C). In Braja 001 tilapia, the nucleotide composition consisted of 28.9% thymine (T), 29.8%

3. Phylogenetic reconstruction

The phylogenetic analysis based on mitochondrial *co1* sequences revealed the presence of three distinct clades. The first clade includes the BRAJA 001 strain, Jatimbulan strain, Kekar strain, and *Oreochromis niloticus* populations from the Philippines, India, USA, Malaysia, and Indonesia. These groups share a common ancestor and exhibit no significant sequence divergence, indicating strong genetic similarity and conservation across geographical regions. The second clade comprises *O. mossambicus, O. jipe,* and *O. andersoni,* which also share a common ancestor, but show notable sequence divergence at the interspecific level. The third clade consists of *O. aureus,* which displays a high degree of nucleotide sequence divergence from *O. niloticus* and other tilapia species, reflecting its more distant evolutionary relationship. These results are presented in Fig. (1).

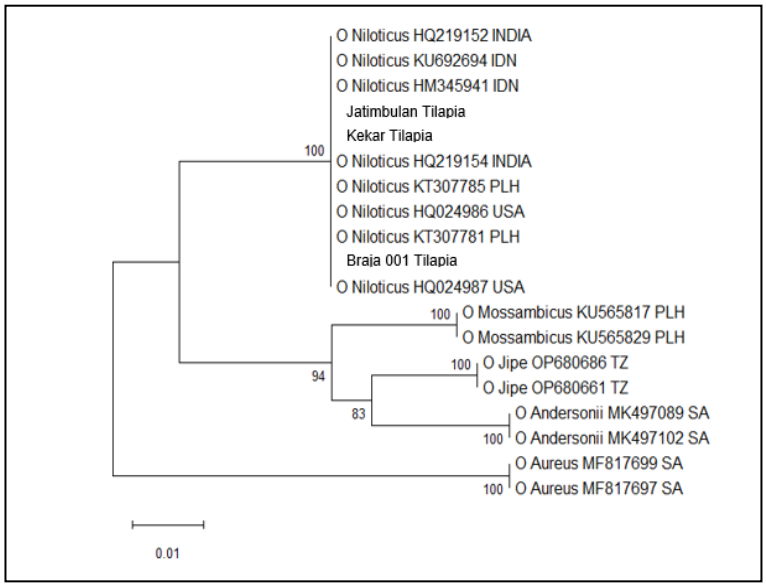

Fig. 1. Phylogenetic tree showing genetic relationships among local strains (Jatimbulan, Kekar, Braja 001)

The BRAJA 001 strain was used as the main research subject to assess whether genetic differences exist between the hybrid (BRAJA 001) and its parental strains, Kekar and Jatimbulan. The BRAJA 001 sequence data were obtained through direct genetic testing, while the sequences of the Kekar and Jatimbulan strains were derived from unpublished data. Comparative sequence data of *O. niloticus* from various countries were retrieved from the NCBI database to examine potential global genetic variation. Other

tilapia species were included to evaluate the genetic distance across different species within the genus.

4. Genetic distance analysis

In this study, the genetic distance among *Oreochromis niloticus* samples was analyzed using population sequence data retrieved from GenBank. The analysis was performed using the MEGA 11 software, employing the between-group mean distance method. The results of the inter-population genetic distance analysis for *O. niloticus* are presented in Table (3).

Table 3. Inter-population genetic distances

O. niloticus	*O. aureus*	*O. mossambicus*	*O. jipe*	*O. andersonii*
O. niloticus				
O. aureus	0,0725			
O. mossambicus	0,0518	0,0679		
O. jipe	0,0514	0,0785	0,0393	
O. andersonii	0,0581	0,0781	0,0369	0,0317

DISCUSSION

The comprehensive molecular analysis of tilapia species using *co1* gene sequences has provided valuable insights into the genetic identity, nucleotide composition, evolutionary relationships, and genetic distances among various tilapia strains and species. The high accuracy of species identification through BLAST analysis is evident from the 100% query coverage, 0.0 E-value, and 100% identity with *O. niloticus*. These parameters confirm that all analyzed samples, including the BRAJA 001 strain, belong to the *Oreochromis niloticus* species. According to **Sajjad *et al.* (2023)**, such BLAST metrics are strong indicators of species-level similarity, where near-complete alignment and minimal E-value signify highly conserved sequences.

The results presented in Table (1) further reinforce this finding, where three representative BLAST hits consistently matched *O. niloticus* with identical values (Query cover = 100%, E-value = 0, Percent identity = 100%). This high degree of similarity strongly suggests the absence of introgression from other *Oreochromis* species, indicating genetic stability and purity of the BRAJA 001 strain. The confirmation of genetic identity through BLAST is critical, since it provides a molecular baseline for subsequent analyses, including phylogenetic reconstruction and genetic distance estimation. Moreover, these findings demonstrate the effectiveness of *co1* barcoding as a universal tool in fish genetics, offering a standardized and reproducible framework for species verification in selective breeding programs.

The uniformity observed in the nucleotide composition among BRAJA 001, Jatimbulan, Kekar, and other *O. niloticus* strains across different countries provides strong evidence of their close genetic relatedness, thereby confirming the genetic stability of the hybrid strain. This pattern reflects that BRAJA 001, developed through the crossbreeding of the Kekar and Jatimbulan strains, inherits a consistent molecular signature that is highly conserved within *O. niloticus*. The predominance of thymine (T) and cytosine (C), accompanied by relatively lower guanine (G) content, is a well-documented feature of the mitochondrial *co1* gene **(Hebert *et al.*, 2003)**.

The overall base composition, with an A+T content of 53.1% and G+C content of 46.9%, aligns with the general characteristics of vertebrate mitochondrial DNA, thereby reinforcing the reliability of the *co1* gene as a molecular marker for DNA barcoding and phylogenetic inference **(Ward *et al.*, 2005)**. Importantly, the near-identical nucleotide composition among BRAJA 001 and its parental strains demonstrates the absence of unexpected mutations or genetic introgression, ensuring that the hybridization process has maintained genetic purity and stability.

As shown in Table (2), BRAJA 001, Jatimbulan, Kekar, and multiple *O. niloticus* populations from Indonesia, the USA, Malaysia, India, and the Philippines all exhibit comparable nucleotide profiles, with only minor variations observed in *O. aureus, O. mossambicus, O. jipe,* and *O. andersoni*. This distinction highlights the clear taxonomic boundaries between *O. niloticus* and other congeneric species, further validating the use of *co1* as a diagnostic marker for species differentiation. For the BRAJA 001 strain, the nucleotide composition consisted of 28.9% thymine (T), 29.8% cytosine (C), 24.4% adenine (A), and 16.9% guanine (G), which closely matches its parental strains and other *O. niloticus* references. Such molecular uniformity underscores the effectiveness of crossbreeding in combining desirable traits while retaining genetic fidelity. From a selective breeding perspective, this stability is critical, as it ensures that BRAJA 001 can be traced, evaluated, and improved without compromising its genetic integrity. Consequently, the nucleotide composition analysis not only validates the identity of BRAJA 001 as *O. niloticus* but also provides a molecular foundation for its future utilization in aquaculture breeding programs aimed at enhancing growth performance, salinity tolerance, and disease resistance.

The phylogenetic reconstruction shown in Fig. (1) indicates a tree scale of 0.01, representing branch length corresponding to a genetic difference of 0.01 substitutions per site. The grouping of species in the phylogenetic tree is based on genetic similarity, which reflects their evolutionary relatedness. Bootstrap values are displayed at each branch and represent the confidence level of the branching. A percentage closer to 100% indicates high confidence typically between 80–100% while values ranging from 50–70% are considered low. The phylogenetic tree also displays taxa in the form of named *Oreochromis* species.

The analysis revealed three distinct clades. The first clade includes the BRAJA 001 strain, Jatimbulan strain, Kekar strain, and *Oreochromis niloticus* from the Philippines, India, USA, Malaysia, and Indonesia. These groups share a common ancestor and show no significant genetic sequence differences. The second clade comprises *O. mossambicus, O. jipe*, and *O. andersoni*, which also share a common ancestor but exhibit genetic sequence differences between species. The third clade includes *O. aureus*, which shows a high degree of nucleotide sequence divergence from *O. niloticus* and other tilapia species.

Phylogenetic analysis using the Neighbor-Joining method revealed three major clades. The first clade grouped BRAJA 001, its parental strains (Jatimbulan and Kekar), and other *O. niloticus* populations from different geographical regions (e.g., India, USA, Philippines, Malaysia, Indonesia), indicating a shared evolutionary lineage and minimal genetic divergence. This clustering pattern is consistent with previous findings that strains of *O. niloticus* across regions often share high genetic similarity due to their common origin and wide translocation history for aquaculture purposes **(Wasso *et al.*, 2025)**.

The second clade comprised *O. mossambicus, O. jipe* and *O. andersoni*, which also share a common ancestor but exhibit interspecific sequence divergence. The third clade, which includes *O. aureus*, showed the highest divergence from the other *Oreochromis* species, particularly *O. niloticus*, supporting earlier reports that *O. aureus* is genetically distinct within the genus **(Macaranas *et al.*, 1986)**. These results align with the genetic distance analysis, where *O. niloticus* was the closest to *O. jipe* (0.0514), *O. mossambicus* (0.0518), and *O. andersoni* (0.0581), and most distant from *O. aureus* (0.0725). All of these distances fall into the low genetic distance category (0.01–0.099), which indicates that, even with species-level divergence, the genetic similarity among these taxa remains high, a pattern consistent with recent cladogenic events within the genus **(Romana-Eguia *et al.*. 2004)**.

The low inter-population genetic distances and high bootstrap support across most nodes suggest that tilapia species retain conserved mitochondrial *co1* regions, making the *co1* gene a suitable marker for identifying genetic structure at both intraspecific and interspecific levels **(Wasso *et al.*, 2025)**. Additionally, the genetic uniformity of BRAJA 001 with its parental strains (Jatimbulan and Kekar tilapia) suggests successful hybridization without significant mitochondrial sequence divergence, further validating the integrity of the hybrid line.

The BRAJA 001 strain was specifically developed through the hybridization of two selected strains: tilapiaJatimbulan and tilapiaKekar. These parental strains were chosen for their superior growth performance, environmental adaptability, and physiological robustness. The genetic analysis confirms that BRAJA 001 retains mitochondrial genetic stability while combining the advantageous traits of both parents. This validates the success of the crossbreeding strategy and provides a strong scientific basis for the development of a new, high-performing tilapia strain.

The BRAJA 001 strain was used as the main research subject to assess whether genetic differences exist between the hybrid (BRAJA 001) and its parental strains, Kekar and Jatimbulan. The BRAJA 001 sequence data were obtained through direct genetic testing, while the sequences of the Kekar and Jatimbulan strains were derived from unpublished data. Comparative sequence data of *O. niloticus* from various countries were retrieved from the NCBI database to examine potential global genetic variation. Other tilapia species were included to evaluate the genetic distance across different species within the genus.

A genetic distance value close to 0 indicates a high level of similarity and close relatedness between populations. Conversely, values approaching 1 suggest greater divergence. A value of 0 denotes that the individuals are from the same population **(Hubert *et al*., 2021)**. Genetic distance is considered low when it ranges from 0.010–0.099, moderate between 0.1–0.99, and high when ranging from 1.00–2.00. Genetic distance is a measure that indicates the degree of genetic divergence between different species or among populations within a species. A low genetic distance value signifies a close genetic relationship, while a high value indicates a more distant genetic relationship. Genetic distance analysis can be categorized into two types namely intra-population and inter-population analyses **(Nei & Kumar, 2000)**.

Inter-population genetic distance refers to the degree of genetic differentiation between two or more distinct populations of fish. This divergence can be observed through variations in allele frequency or differences in DNA sequences among the populations. The greater the genetic distance, the more genetically distant the populations are **(Zhao *et al*., 2021)**. Based on Table (3), the inter-population genetic distance analysis indicates that *Oreochromis niloticus* has the smallest genetic distance with black tilapia (*O. jipe*), at a value of 0.0514. The genetic distance between *O. niloticus* and Mozambique tilapia (*O. mossambicus*) is 0.0518, with three-spotted tilapia (*O. andersoni*) at 0.0581, and with blue tilapia (*O. aureus*) at 0.0725. These genetic distance values are considered low, which suggests a close genetic relationship. This is expected, as all the tested species belong to the same genus, *Oreochromis*. The low inter-population genetic distances indicate that, despite species-level distinctions, these tilapia share a relatively recent common ancestor and retain conserved genetic sequences.

As such, BRAJA 001 represents a promising candidate for future aquaculture development programs in Indonesia. Its confirmed genetic identity as *O. niloticus*, high similarity to international reference strains, and stable mitochondrial profile make it suitable for selective breeding, mass production, and potential commercialization. The development of BRAJA 001 is not only a step forward in genetic resource utilization but also contributes to national goals in strengthening food security through improved aquaculture genetics.

CONCLUSION

This study confirmed the genetic identity of the BRAJA 001 hybrid tilapia using mitochondrial *co1* analysis. Results showed 100% similarity with *Oreochromis niloticus*, identical nucleotide composition to its parental strains, and phylogenetic grouping in a single clade with strong support. Low genetic distance (0.0514–0.0725) indicates close evolutionary relationships, confirming BRAJA 001 as a genetically stable strain with strong potential for selective breeding and aquaculture development.

Acknowledgements

The present study was funded by the Directorate of Innovation and Science & Technology Area (Direktorat Inovasi dan Kawasan Sains & Teknologi – DIKST) under the Strategic Innovation Research Funding Contract, Fiscal Year 2024, with contract number 00320.07/UN10.A0507/B/KS/2024

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