EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES ZOOLOGY

B

ISSN
2090-0759

WWW.EAJBS.EG.NET

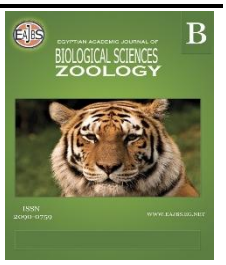
Vol. 17 No. 2 (2025)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 17(2) pp:1-12 (2025)*
DOI: 10.21608/EAJBSZ.2025.446617

Egypt. Acad. J. Biolog. Sci., 17(2):1-12(2025)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
Res. Ethics No.: 03-2025-0020
http://eajbsz.journals.ek b.eg/



Molecular Genetic Variations of Some Rabbit Breeds Using Small Mitochondrial rRNA Sequences

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ARTICLE INFO
Article History
Received:8/7/2025
Accepted:10/8/2025
Available:14/8/2025

Keywords:
12S rRNA, Egyptian rabbit, Genetic distance and Rabbit breeds.

ABSTRACT

With the help of small mitochondrial rRNA (*12S rRNA*), the genetic diversity of five different rabbit breeds in Egypt was estimated in the current study. Slight difference was found between *12S rRNA* sequences of the five different rabbit breeds where, the sequence size was 923 bp in four breeds and 924 bp in New Zealand White breed. The final alignments consisted of 924 bp., of them 913 conserved sites. The accession numbers for these sequences in GenBank/NCBI were (OR210201-OR210205). The nucleotide frequency average was (A) 36%, (T) 23.68%, (C) 22.36%, and (G) 17.96%. All breeds had an average A+T rating of 59.68%. The genetic distance values amongst breeds extended from 0.000 to 0.0033. The (0.0033) percentage was observed amongst the breed New Zealand White and REX breed. As well as high genetic variations were observed between New Zealand White breed and the rest breeds. The information provided by the *12S rRNA* sequences demonstrated that the New Zealand White breed was genetically unique from the other breeds. The investigation's findings validate the suitability and qualification of *12S rRNA* for observing breed-specific genetic variation in rabbits.The generation of genetic maintenance and amelioration strategies for Egyptian rabbit genetic resources may benefit from this data in the future.

INTRODUCTION

Rabbits are considered a widespread micro-livestock species and described as a source of providing protein, fibre, furthermore they are experimental models and companionship (Vietmeyer, 1985 and Omotoso *et al*., 2019).

Genetic variation is known as the differences and variations within and amongst breeds of the same species. Several factors affected genetic variation like genetic drift, migration, mutation, and selection (Talle *et al*., 2005 and Omotoso *et al*., 2019). With a view to adapt the breeds the variable markets, and to fulfill the needs of consumers, assess the genetic variation in rabbits play a significant role in planning appropriate of breeding delineation (Groeneveld *et al*., 2010).

In order to overcome the spread of diseases and to respond to environmental changes, genetic variation plays a significant role in ameliorating the quality and features of the existing breeds (Galal *et al*., 2013).

Rabbit breeds in Egypt include local varieties and imported foreign breeds that have been used since the mid-20th century for breeding and crossbreeding to enhance productive and reproductive efficiency (Emam *et al*., 2020)

For researching origin and diversity, mtDNA is a favorable marker due to three advantages: rapid mutation rates, maternal inheritance, and availability in large numbers per cell, facilitating its isolation (Gupta *et al*., 2015). High mutation rates are crucial for the variation that takes place in the species over time, and maternal inheritance is important for identifying the animal's ancestor or ancestors (Owuor *et al*., 2019). Many studies illustrated the wide employing of *12S rRNA* gene in the phylogenetic relationship studies amongst variable levels taxa such as species, genera, and families (Ledje and Arnason, 1996; Murphy and Collier, 1996, 1997; Gatesy *et al*., 1997 and Halanych and Robinson, 1997). Compared to the whole mitochondrial genome, 12S rRNA gene is preserved and evolving more slowly (Palumbi, 1996 and Di Finizio *et al*., 2007).

In this vein, this study aimed to utilize small mitochondrial rRNA sequences (*12S rRNA)* to study the molecular variations of some domestic rabbit breeds and appreciate the molecular linkages amongst these breeds.

MATERIALS AND METHODS

Study Animals:

To perform this study, we used twenty-five rabbits from five breeds: Baladi Black (BB), Chinchilla, Flander, New Zealand White, and REX. All samples were healthy. The tissues of muscle were collected and conserved at -20ºC to use in DNA isolation.

DNA Isolation:

By following the manufacturer's instructions of Mini kit (Qiagen, Hidden, Germany), the total DNA was isolated from the muscle tissues.

PCR Amplification and Sequencing:

To amplify partial sequence of *12S rRNA* primers described by (Wang *et al*., 2000) were used. The sequences of primers were, 12SR: TTTCATGTTTCCTTGCGGTAC, 12SL: AAAGCACGGCACTGAAGATGC. The final reaction volume for the PCR reactions was 50 µL and it contained 25 µL of PCR master mix along with 1 µL (10 pmol) of each forward and reverse primer, and 1 µL DNA template. A preliminary denaturation at 95 °C for 4 minutes was followed by 35 cycles of denaturation, annealing, and extension at 95 °C, 54 °C, and 72 °C, respectively, for 1 minute, with a final elongation at 72 °C for 5 minutes. The PCR results were examined using an ethidium bromide-containing 1.2% agarose gel. In all breeds, the PCR reaction produced a single band. The sequence process was performed by Macrogen (South Korea). The sequence regions of *12S rRNA* from five different rabbit breeds were deposited in the (GenBank/NCBI) in order to obtain the accession numbers for each breed.

Genetic Distance and Phylogenetic Tree:

The alignment was finalized utilized MUSCLE (Edgar, 2004). The genetic distance was carried out by MEGA version 7 (Kumar *et al*., 2016) with 1000 bootstrap iterations (Felsenstein, 1985). Two methodologies; Minimum-Evolution, and Neighbour-Joining were applied for phylogenetic rebuilding to compare the harmony of the breeds and species. Through Kimura 2-parameter distances (Kimura, 1980) the sequence divergences were calculated.

RESULTS

The PCR reaction resulted in a single band in each breed. The NCBI was supplied with the partial *12S rRNA* sequence from the five breeds in order to obtain the accession numbers (OR210201-OR210205). Slight difference was found between *12S rRNA* sequences

of the five different rabbit breeds where, the sequence size was 923 bp in four breeds and 924 bp in New Zealand White breed. The final alignments consisted of 924 bp. of which ten variable site regions (**Table 1 and Fig. 1).**

Table 1. Basic feature of sequence alignment in five Rabbit breeds using for *12S rRNA* gene.

Aligned sites	Constant sites	Variable sites	Best fit model	Evolutionarily invariable (+I)	Gamma distribution (+G)
924	913	10	GTR+G+I	0.00	0.18

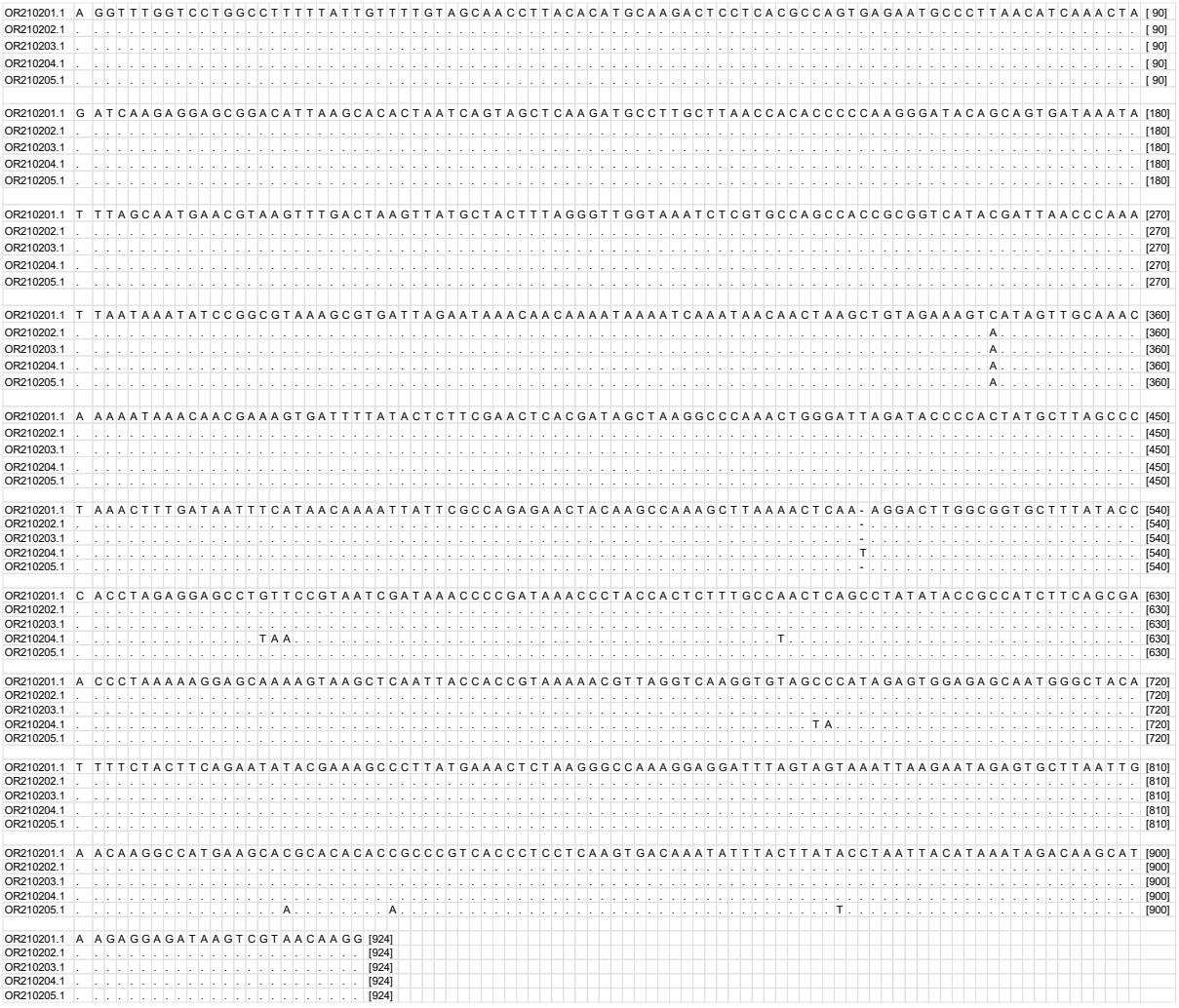

Fig. 1. Alignment of *12S rRNA* sequences amongst five Rabbit breeds.

The nucleotide frequency average was thymine (T) 23.68%, cytosine (C) 22.36%, adenine (A) 36%, and guanine (G) 17.96%. The A+T percentage was bigger than C+G in all breeds. The average value of A+T was 59.68% (Table 2).

Table 2. Nucleotide frequencies and its average of *12S rRNA* sequence in five Rabbit breeds.

No.	Breed	Base pair length	Nucleotide Number %				A+T Content (%)
			T%	C %	A%	G%	
1-	**Baladi Black**	923	23.62	22.54	35.86	17.98	59.48
2-	**Chinchilla**	923	23.62	22.43	35.97	17.98	59.59
3-	**Flander**	923	23.62	22.43	35.97	17.98	59.59
4-	**New Zealand White**	924	23.81	22.19	36.15	17.85	59.96
5-	**REX**	923	23.73	22.21	36.08	17.98	59.81
	Average %	-	23.68	22.36	36	17.96	59.68

The Kimura two-parameter distance model varied from 0.0000 to 0.0033%. The highest P-distance (0.0033) was recorded between New Zealand White and REX breeds. The most related breeds were Chinchilla and Flander (Table 3).

Table 3. Genetic distance amongst five Rabbit breeds.

No.	Breed	1	2	3	4	5
1	**Baladi Black**		0.0011	0.0011	0.0029	0.0022
2	**Chinchilla**	0.0011		0.0000	0.0027	0.0020
3	**Flander**	0.0011	0.0000		0.0027	0.0020
4	**New Zealand White**	0.0077	0.0066	0.0066		0.0033
5	**REX**	0.0044	0.0033	0.0033	0.0099	

Dendrogram (Fig. 2) consisted of two main cluster; (A) contained New Zealand White breed, and (B) contained the rest breeds. Data of *12S rRNA* sequences of five rabbit breeds were utilized to account the genetic similarity (Table 4) and (Fig. 3). The relationship amongst the five rabbit breeds was performed using genetic similarity presented in (Table 4) and Dendrogram as (Fig. 2) and revealed low genetic similarity between New Zealand White breed and the rest breeds. While the highest genetic similarity value 0.99949 was found between Chinchilla and Flander breeds.

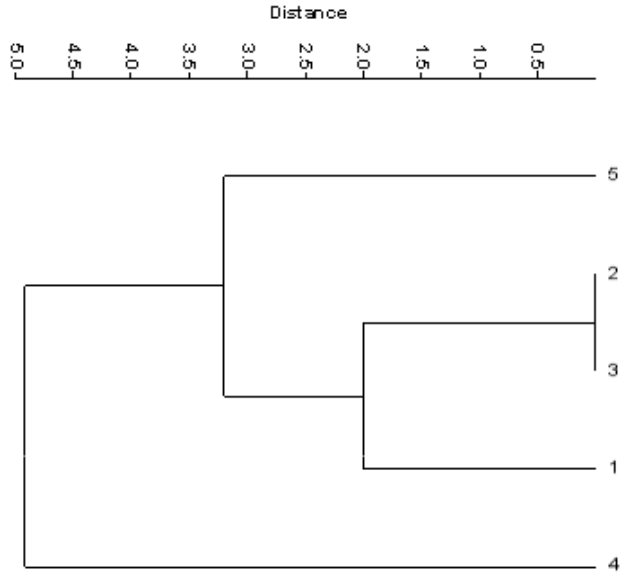

Fig. 2. Dendrogram demonstrating the relationship among five rabbit breeds based on the sequenced regions of *12S rRNA*, where 1- Baladi Black, 2- Chinchilla, 3-Flander, 4- New Zealand White, and 5- REX.

Table 4. The genetic similarity amongst five Rabbit breeds.

No.	Breed	1	2	3	4	5
1	**Baladi Black**	1	0.99782	0.99618	0.98435	0.98928
2	**Chinchilla**	0.99782	1	0.99949	0.98847	0.99388
3	**Flander**	0.99618	0.99949	1	0.98975	0.99557
4	**New Zealand White**	0.98435	0.98847	0.98975	1	0.98621
5	**REX**	0.98928	0.99388	0.99557	0.98621	1

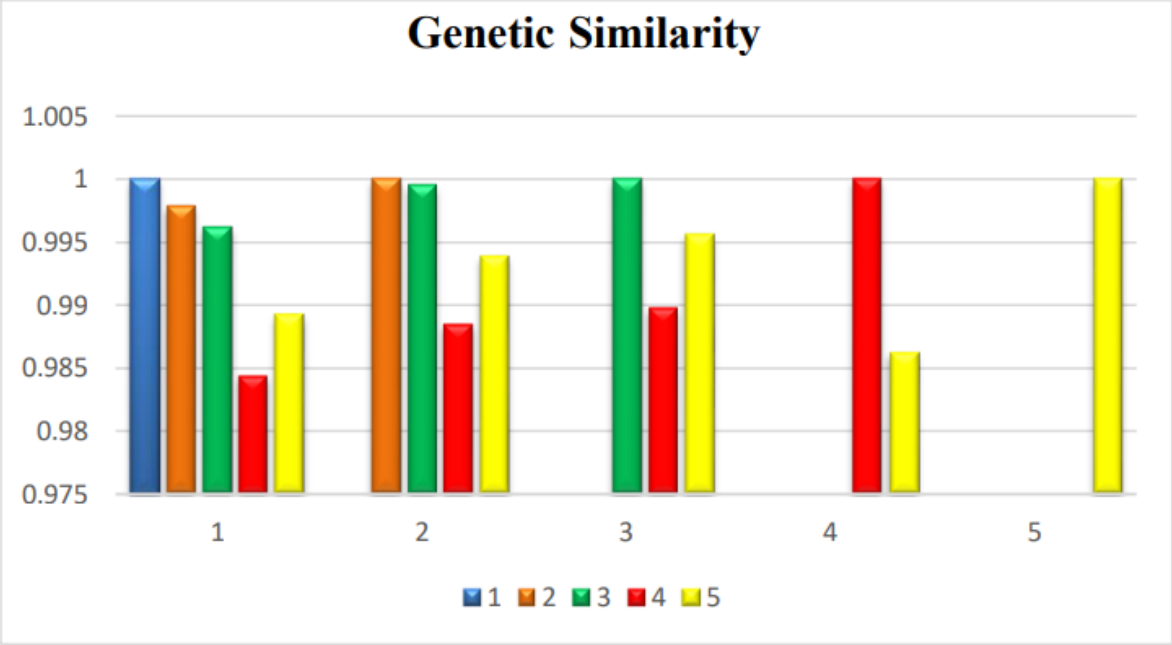

Fig. 3. The genetic similarity amongst five Rabbit breeds. Where 1- Baladi Black, 2- Chinchilla, 3-Flander, 4- New Zealand White, and 5- REX.

Phylogenetic Analysis:

Two methodologies; Minimum-Evolution, and Neighbor-Joining were applied for phylogenetic rebuilding to compare the harmony of the breeds and species (**Table 5**). With some alterations in the support parameters, the two phylogenetic methodologies generated findings that were similar and displayed: (1) species of the outgroup were found in a separate cluster. (2) *Oryctolagus cuniculus* from GenBank (MN518689.1) was related to the included domestic rabbit breeds (Fig. 4 and 5).

Table 5. The five Rabbit breeds and their linked species with the outgroup from the GenBank/ NCBI employing 12S rRNA gene.

No.	Species	Accession number
1	*Oryctolagus cuniculus* breed Baladi Black	OR210201.1
2	*Oryctolagus cuniculus* breed Chinchilla	OR210202.1
3	*Oryctolagus cuniculus* breed Flander	OR210203.1
4	*Oryctolagus cuniculus* breed New Zealand White	OR210204.1
5	*Oryctolagus cuniculus* breed Rex	OR210205.1
6	*Oryctolagus cuniculus*	MN518689.1
7	*Sylvilagus floridanus*	AY012126.1
8	*Lepus californicus*	KJ397614.1
9	*Lepus corsicanus*	KJ397606.1
10	*Lepus arcticus*	KJ397607.1
11	*Lepus timidus*	OQ270737.1
12	*Lepus arcticus*	KY786038.1
13	*Lepus sinensis formosus*	OM334908.1
14	*Sylvilagus dicei*	KU057256.1
15	*Sylvilagus brasiliensis*	MH115197.1
16	*Sylvilagus andinus*	KU057258.1
17	*Sylvilagus obscurus*	KU057247.1
18	*Sylvilagus transitionalis*	KU057250.1
19	*Sylvilagus audubonii*	KU057237.1
20	*Sylvilagus bachmani*	KU057238.1
21	*Sylvilagus aquaticus*	U58927.1
22	*Pentalagus furnessi*	AB058603.1
23	*Sylvilagus nuttallii*	U63886.1
24	*Brachylagus idahoensis*	U58921.1
25	*Ochotona curzoniae*	AF326253.1
26	*Ochotona hyperborea*	AY012127.1
27	*Ochotona princeps*	AF390540.1

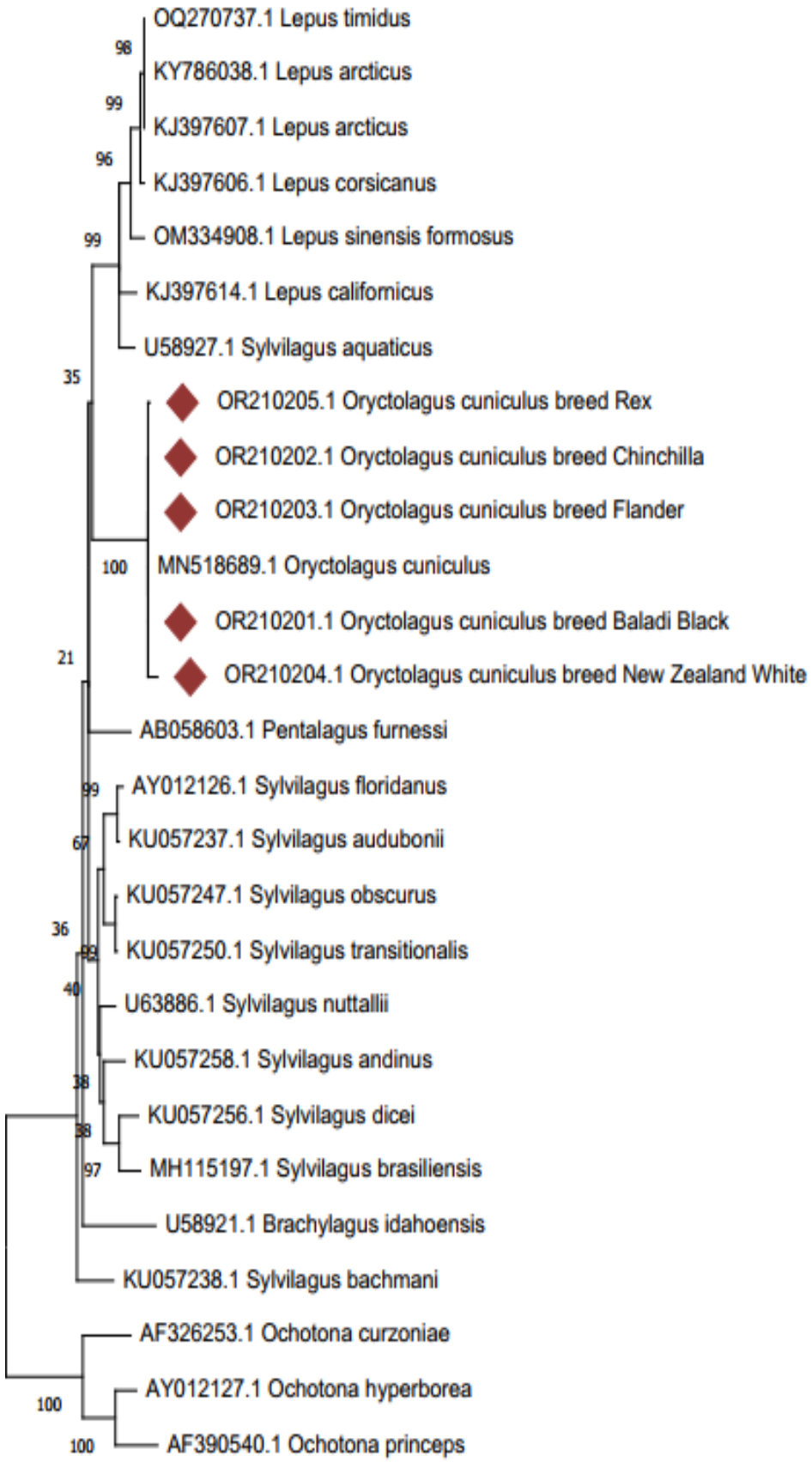

Fig. 4. Minimum-Evolution tree amongst five Rabbit breeds and their linked species with the outgroup.

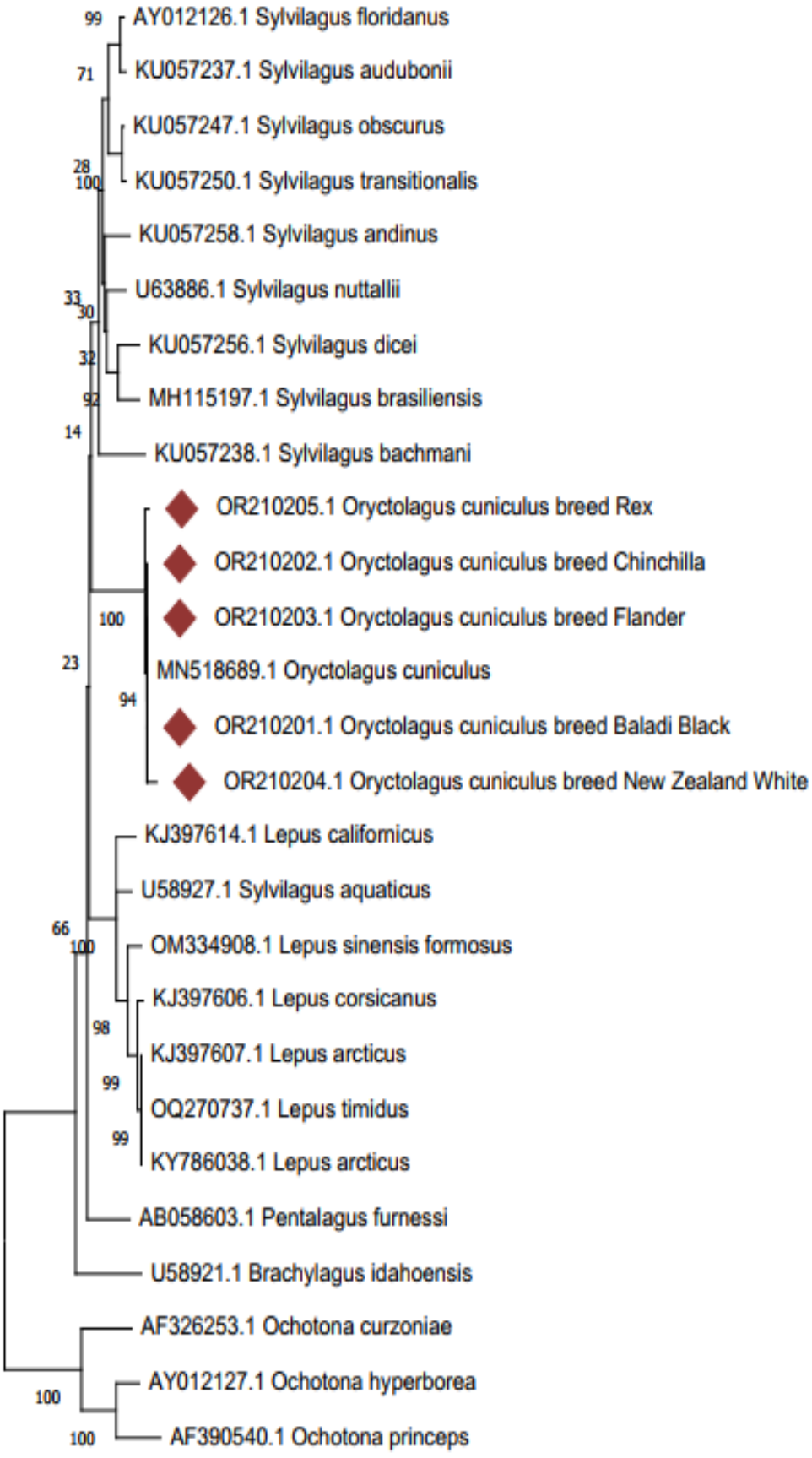

Fig. 5. Neighbor-Joining amongst five Rabbit breeds and their linked species with the outgroup.

DISCUSSION

Illustrate the genetic variations amongst breeds help in proposed conservation and breeding strategies. It supplies us with suitable data to comprehend domestication and evolution history. In addition, it increase researches that interested in making relation among phenotype and genotype (Ben Larbi *et al*. 2012 and Badr *et al*. 2016).

The study of rabbit genetic diversity was widely achieved by many molecular markers like RAPD and SRAP (Mohamed and Abdelfattah, 2018); the mitochondrial DNA (Emam *et al*., 2020); microsatellites (Adeolu et al., 2021); and single-nucleotide polymorphisms (SNPs) (Ballan *et al*., 2022).

Mitochondrial genome (mtDNA), markers are helpful in examining species evolution and genetic diversity. Furthermore, mtDNA is considered as a molecular technique for studying the phylogenetic links and genetic variation amongst various breeds (Slaska *et al*., 2014 and Ahmed *et al*., 2022). Owuor *et al*. (2019) appreciate the genetic variabilities and the origins of the domesticated rabbits in Kenya utilized mtDNA genetic marker. Emam *et al*. (2020) determine the status of genetic variability and phylogeny for some local rabbit breeds in Egypt and Spain based on cytochrome b gene sequences. Allam and Mahrous (2021) utilized *16S rRNA* to assess the molecular linkages of some rabbit breeds and reveal the molecular linkages amongst some rabbit breeds of *Oryctolagus cuniculus* and some species of family Leporidae. Ahmed *et al*. (2022) studies the genetic variation of the mitochondrial DNA D-loop in some rabbit breeds.

The alignment of *12S rRNA* sequence involved 924 bp. of them 913 conserved sites (Figure1). This was agree with Van der Kuyl *et al*. (1995) and Saikia *et al*. (2016) who stated that mitochondrial genes in various animal species were characterized by highly conserved which help in design universal primers used in mitochondrial genes amplification. In this study we used primers described by Wang *et al*. (2000) which were generated successfully one band in each rabbit breed.

According to El-Sabrout and Aggag (2015) the genetic similarity consider as an indicator of the rate of homogeneity and inbreeding within studied genotype. The finding of *12S rRNA* revealed that Chinchilla and Flander breeds were the most related breeds where genetic similarity value was 0.99949 in this study. This was consistent with findings of Allam and Mahrous (2021) who observed strong genetic similarities between Chinchilla and Flander using *16S rRNA* sequences.

The results of *12S rRNA* revealed that the New Zealand White breed genetically far from the others, this agree with El Sayed (2010) who found that New Zealand White and Hyplus were genetically different breeds.

Based on the calculated genetic similarity, Baladi Black breed display high genetic variation from the New Zealand White breed, this was concordant with Grimal *et al*. (2012) who observed structurally separation between four Egyptian breeds and the Spanish New Zealand White line. In the same context Badr *et al*. (2019) applied microsatellite markers in four rabbit breeds and noticed that the New Zealand White breed was more distinctly from Gabali, Baladi Red, and Baladi Black breeds.

Genetic variation in domestic breeders permits evolving new advantages for environmental changes or diseases adaptation Galal *et al*. (2013). El-Sabrout and Aggag (2015) illustrated that the Alexandria breed was more distinctly from California and New Zealand breeds and expected that due to the genetic distance between Alexandria and California breeds, the crossbreeding between them will support the development of new features and improvement reproductive capacity of rabbits furthermore enhance new characteristics that make them more adapted to the changeable environment condition. The data of *12S rRNA* sequences in this investigation illustrated that the New Zealand White breed was more distinctly from the rest breeds. Based on this data, it is probable that the crossbreeding between

the New Zealand White breed and each other breeds will improve the reproductive ability of rabbits and obtain several new characteristics that make them more adapted to the changeable environment condition.

The finding of this investigation affirms the applicability and qualification of *12S rRNA* in estimating the genetic variation of rabbit breeds. This data may be useful in future in the development of genetic amelioration and maintenance programs for Egyptian rabbit genetic resources.

Conclusion

The *12S rRNA* gene sequences demonstrated that the New Zealand White breed was genetically unique from the other breeds. This study also showed the ability and effectiveness of the *12S rRNA* sequences to evaluate the genetic diversity among rabbit breeds. Additionally, this study will assist researchers understand the variation in specific rabbit reproductive traits and promote future research on rabbit genetic resources.

List of Abbreviation

12S rRNA	Small Mitochondrial rRNA
A	Adenine
C	Cytosine
T	Thymine
G	Guanine
NCBI	National Center for Biotechnology Information

Declarations:

Ethical Approval: All experimental protocols were approved by Animal Experiments Committee of South Valley University, Faculty of Science (Permit No.: 006/03/2023).

Competing interests: The authors declare no competing interests.

Author's Contributions: All authors are equal in contribution.

Funding: This research did not receive external funding.

Availability of Data and Materials: The datasets analysed during the current study are available in the [GenBank/NCBI] repository, accession numbers [OR210201-OR210205].

Acknowledgements: We thank Faculty of Science, South Valley University, where all experimental protocols were approved by Animal Experiments Committee.

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