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Genetic diversity of Muscovy ducks revealed by mtDNA d-loop

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Abstract: *Based on geographical location and previous studies, the Muscovy ducks were grouped into six different populations such as India-Manipuri (IM), Wuyi-China (WC), Yuyao-China (YC), Fujian-China (FC) and Unkown-China (UC) and France. In total, 12 haplotypes were observed from six Muscovy population. The India-Manipuri (IM) population contributes 8 haplotypes. The AMOVA test shows high (75.12%) genetic variation within population. The NJ phylogenetic tree shows the intermingled China, India and France Muscovy populations. In order to find the depth of haplotype differences, the median joining network was constructed whereby H1 haplotype were shared by India and China population. Moreover, except H1 haplotype, other seven haplotypes from IM population were not observed to be shared with China population. Most importantly 33 InDel sites were observed, with regard to that another MJ network was constructed based on the InDel (Insertion-Deletion) sites or including alignment gaps. This InDel MJ network shows that India and China haplotypes separation based on H5 and H1 haplotype, and star-like structure also observed in both the networks. The non-metric multidimensional scaling (NMDS) plot and cluster analysis shows that France population was distinct from other populations and the IM shows closeness with Unknown-China (UC) population. Other three China populations FC, WC and YC clustered together and differ from France and IM-UC groups. The finding of this study would help to understand the maternal genetic evolution of Muscovy duck.*
Key words: *Muscovy, AMOVA, NJ phylogenetic tree, MJ network, NMDS.*

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I. Introduction

Muscovy duck **(***Cairina moschata domestica*) comes under the order of Anseriformes, Anatidae family [1]. The Muscovy duck is a tropical bird well adapted to cooler climates. This is a native duck of South America and the archaeological evidences (bones) shows that wide range of distribution of wild and domestic Muscovy in neotropics stretching areas, which covers the Mexico through the Antilles and Central America, to South America as far South-Western Ecuador and Peruvian coast, and La Plata estuary of Argentina [2, 3, 4, 5, 6]. The Muscovy domesticated from wild Muscovy (*Cairina moschata*) by Colombian and Peruvian Indians. It was then introduced to Europe by Spaniards and Portuguese in 16th century. Finally the Muscovy was introduced by European colonists to India and China in 15th century. In 2016, 48 breeds of Muscovy recorded in worldwide, out of which 23 breeds were reported from pacific Asia region [7]. The most popular feather colour of Muscovy breed in this world is white winged black colour and some other famous breeds are white winged blue, pure black, pure white, black & white, blue and white colours. In India, three types of Muscovy ducks have been identified on the basis of feather colour which include black and white, pure white, and sepia colour feather [8]. Ironically, it's well known by the names of 'Moti' in Odisha and 'Chinae haras' or 'Cina hanhs' or "Bor China", "Bhatt China" in Northeast India, ([8, 9, 10, 11]. In India, the Muscovy is mostly reared in small group of peoples for meat production and they are very poor egg layers i.e. 20 to 40 eggs per year [8]. Vigova and Muscovy are an excellent meat producing ducks in India but they are having poor scavenging ability and high mortality [12, 13]. The Muscovy ducks are crossed with domestic ducks (*Anas platyrhynchos*), in captivity to produce hybrids, known as mulard duck ("mule duck"), this mulard duck possesses big size fatty liver along with unique taste, due to this taste it is very famous among peoples in Spain [14]. The genetic structure and molecular diversity of Muscovy duck is poorly studied in this world. In India, the genetic characterization of 'Moti' (Muscovy) was analysed using microsatellite markers (PIC) from Odisha [10]. The low genetic diversity was observed from 4 different domestic Muscovy population from India using mtDNA cytochrome *b* gene and nuclear DNA CYP2U1 gene [15]. In this study, the genetic diversity was evaluated using mitochondrial DNA d-loop region of three countries Muscovy's such as India, China and France.

II. Materials And Methods

1.1 Data retrieval

A total of 19 samples of d-loop sequences were retrieved from Genbank, out of 19, 13 sequences from India-Manipuri (IM) (accession numbers were GQ922085 to GQ922097), 5 d-loop sequences from China (accession numbers were EU431185 to EU431187, EU755254 and L16769), according to Chen *et al.,* report (2009), this five muscovy dloop sequences were represents 69 samples of four population from China such as Wuyi-China (WC), Yuyao-China (YC), Fujian-China (FC) and Unkown-China (UC), based on this report we have multiplied/duplicated (copy and paste) those five sequences (accessed from GenBank) into 69 sequences and respectively separated to above mentioned four population (WC, YC, FC and UC) also been used for this study [16], and also 1 French domestic Muscovy mtDNA dloop sequence (Accession number: AY112952) was accessed from Genbank and which represents 2 samples according to Donne-Goussé *et al.,* (2002) report also used for further genetic diversity analysis for this study [17]. In total, 84 samples (13 from India, 69 from China and 2 from France were simply duplicated like copy and paste based on previous studies [16, 17] from 19 mtDNA d-loop sequences (retrieved from GenBank) were used for this study (Table 1).

2.2 Molecular diversity analysis

The Muscovy mtDNA d-loop sequences were aligned using Clustal W and edited (trimmed both ends) manually using MEGA 6 programme [18] and the Transition/Transversion bias (*R*) also estimated [19]. The molecular diversity such as number of haplotypes (H), polymorphic sites (S), average number of nucleotide differences (K) and mismatch distribution graph were estimated using Dnasp 5.0 programme [20]. The AMOVA, population pairwise difference (FST) and Number of migration (Nm) of muscovies populations were calculated using ARLEQUIN version 3.5 software [21, 22]. Graphical representations of non-metric Multidimensional Scaling (NMDS) plot, cluster analysis dendrogram and matrix plot were analysed using PAST 3.12 programme to check genetic relationships among Muscovy population based on FST [23].

2.3 Phylogenetic and median joining network analysis

The muscovy mtDNA dloop sequences (84 sequences of 491 bp) were used for phylogenetic analysis using the Neighbor-Joining method by MEGA 6 and out-grouped with mandarin duck (*Aix galericulata*, Accession no: AY988482) [18, 24]. The percentage of replicate trees with which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches [25]. The evolutionary distances were computed using Maximum Composite Likelihood method which was in the units of the number of base substitutions per site [26]. Non-redundant gaps and missing data were eliminated and there were a total of 455 positions in the final dataset used for tree construction. Median-joining (MJ) network was constructed using the NETWORK 4.2 (http://www.fluxusengineering.com/sharenet.htm) [27] in order to obtain further insight into the haplotype relationships between India, France and China Muscovy populations by MJ network analysis.

III. Results

3.1 Molecular diversity analysis

In this study, the retrieved 19 d-loop sequences from GenBank database were multiplied (simply copy and paste) into 84 individuals of Muscovies mtDNA d-loop sequences based on previous studies [16, 17]. These 84 sequences were grouped as six different population based on geographical location (three countries) such as China, India and France, 4 populations from China [Wuyi-China (WC), Yuyao-China (YC), Fujian-China (FC) and Unkown-China (UC)], 1 population from India [IndiaManipuri (IM)] and 1 sequence of 2 samples from France. All these 84 Muscovy d-loop sequences of 6 populations were aligned and trimmed (both ends) up to 491 bp size along with gaps in all population. The missing or alignment gap or InDel (Insertion-Deletion) sites were 33 and the invariable sites were 446, variable sites were 12, so totally 491 sites were used for this study. In total, 12 unique haplotypes were obtained 6 Muscovy population based on 12 variable sites, which 9 sites were singleton variable sites (56, 68, 196, 230, 278, 279, 409, 445, and 174) and 3 sites were parsimony informative sites (91, 242, and 392). The estimated transition/transversion bias (*R*) was 2.25 and the estimated substitution pattern and rates under the Kimura 2- parameter model of the nucleotide frequencies were A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00% [19]. The maximum number of haplotypes 8 was observed from India-Manipuri population (Table.1). The analysis of molecular variance (AMOVA) result indicates 75.12% variation in within population and 24.88% variation in between population, the fixation index FST was 0.24879 statistically highly significant (Table.2). The pairwise genetic difference FST was calculated between 6 populations of Muscovy and the highest pairwise difference (0.72954) was observed between France and WC population was statistically highly significant (Table.3). The second most highest FST was observed between France and YC (0.71604) was also statistically significant. The statistically significant lowest FST was observed between India-Manipuri and Fujian-China (0.24689). Alike, very low non significant negative FST (-0.022) was

obtained between WC and FC. Interestingly, there was no difference (FST) between France and UC population, this result supports the haplotype data both populations (France and UC) shares H4 haplotype. The FST differentiation was graphically represented as a matrix plot respectively in Fig 1a. The pairwise FST data were further utilised to study the relationships among six Muscovy population by Nm (Fig 1b) cluster analysis (Fig 1c) and non-metric multidimentional scaling (NMDS) plot (Fig.1d). The cluster analysis of Unweighted pair-group average (UPGMA) dendrogram was predicted by FST. The NMDS and UPGMA dendrogram analysis results showed that France Muscovy was distinct from rest of other population and the IM shows closeness with UC, whereas the other three China populations FC, WC and YC clustered together. The mismatch distribution analysis of pairwise difference also checked based on the assumption of selective neutrality result to evaluate the possible historical events of population growth and decline. In this study, the mismatch distributions graph was unimodal distribution graphs (Fig 2), which is fully consistent with population expansion [28, 29].

3.2 Phylogenetic tree and network analysis

The Neighbour Joining phylogenetic (NJP) tree was constructed using mtDNA d-loop region sequences (491bp) to find the Muscovy population genetic structure. Numbers on the branches represents the percentage of boots trap values from1000 replications and more than 50% were shown (Fig 3). The Muscovy duck populations were out-grouped with mandarin duck (*Aix galericulata*) [17]. Importantly, the Indian (IM) population were intermingled with China Population. The individuals in populations were distinguished by use of colour codes yellow colour refers to India, indigo blue colour refers to WC, aqua blue belongs to YC, magenta colour refers to FC, brown colour belongs to UC and harlequin green colour belongs to France. This phylogenetic study unveiled one main clade with 3 sub clades, in that sub clade 1 consisted with 15 sequences, of which 14 sequences belongs to H2 haplotype and the 15th sequence/last sequence ChiH5 belongs to H18 haplotype, this branch showed 63% bootstrap value. Sub clade 2 has 5 sequences (YCH3, YCH3a, YCH3b, YCH3c and YCH3d) and showed 63% bootstrap value, representing H3 haplotype. The sub clade 3 showed 50% bootstrap between IMH3 and IMH8 sequences. The results indicate that there were only small differences among individuals of Muscovy from six populations.

The mtDNA d-loop region (491bp) sequences of 84 individuals were used to analyse median joining (MJ) network (Fig.4). The MJ network shows the relationship between haplotypes of India, France and China Muscovy populations based on mutational steps. These haplotypes were connected by mutational steps as shown in Fig 4. The area of each circle was proportional to the frequency of the corresponding haplotypes and the numbers between the haplotype nodes refer to the positions of nucleotide mutations. Different classes of respective populations haplotypes were distinguished by use of colour codes, yellow colour refers to India, indigo blue colour refers to WC, aquablue refers to YC, magenta colour refers to FC, light brown refers to UC and harlequin green colour refers to France. The active 12 haplotypes were distinguished by mutational variation and showed clusters surrounded by star like structure, consistent with recent population expansion. The H1 haplotype was predominant haplotype has 54 frequencies from four populations such as WC (20), YC (21), FC (7) and IM (6) and situated in centre of all haplotype. Likewise, H2 haplotype posses 14 individuals from three population. Most of the haplotypes were singleton mutated haplotypes and extended from H1 haplotype. H2 haplotype extended from H1 haplotype by single mutation step (site: 91) and the H2 possess only China populations such as WC, YC and FC. Similarly, the H12 haplotype extended from H2 haplotype by single mutational step (site: 56) and possess one UC Muscovy sample. H3 haplotype possess five individual from YC population and this also connected with H1 haplotype by single mutational step (site: 392). Likewise, the H4 haplotype connected with H1 with single mutational step (site: 242) and possess 3 individuals from two populations such as France (2 samples) and UC (1 sample). IM population H9 and H10 haplotypes were extended from H1 by two mutational steps, and rest of other haplotypes (H5, H6, H7, H8 and H11) were belongs to IM population extended from H1 by single mutational steps. One median vector (mv1) was located between H10 and H11 haplotype, this median vector (mv) were built by Network software and refers to the ancestor or missing haplotypes.

Similarly, the Fig 5 shows the InDel or alignment gaps or missing sites median joining network structure of six Muscovy populations. Mainly the India and China groups were separated based on H5 and H1 haplotype. The China haplotype H1connected to H5 haplotype with two step mutation sites 267 and 275, the mv5 (median vector) played important role between H1 and H5. The H1 showing maximum haplotype frequencies of 48 individuals from all China population except UC population in this analysis and situated in centre among China based haplotypes (H2, H3 and H4) with the variation of single mutation. The H2 haplotype extended to H18 haplotype by single mutation step (site at 56). France 2 samples were shared the H4 haplotype with UC samples and also connected with H1 haplotype by single mutational step (242). The H5 situated centre of all IM haplotypes and showed star like structure. H5 connected by single mutation step to four haplotypes such as H6, H7, H8 and H9, and further this H5 directly connected to 3 haplotypes with more than one mutation step such as H14, H15 and H17. H5 connected by the way of mv 1, 2, 3 and 6 to 4 haplotypes respectively, they

were H10, H11, H13 and H16. The H13 and mv3 further connected to H12 by the single step mutation intermediate of mv4.

IV. Discussions

In this present study, the 84 individuals of 491bp of Muscovies mtDNA dloop sequences were grouped into six population based on geographical location and previous studies [14, 16] from 3 countries (China - four populations, India - one population and France - one population). A total of 12 haplotypes were observed which eight from IM population and five from China. According to Chen *et al.,* (2009) report China Muscovy population has low genetic diversity and the similar low genetic diversity also was observed in this study. These China population with low genetic diversity results were coincided with cytochrome *b* and microsatellite result of previous studies in China and India on Muscovy [30, 31, 32, 15]. Likewise, the Muscovy genetic diversity was comparatively lower than Chinese domestic mallard duck (*Anas platyrhynchos*) genetic diversity [33, 34]. The IM shows slightly high genetic diversity, the afford said variations of Indian Muscovy samples were already previously reported in moti muscovy RAPD and microsatellite studies [10, 35]. Stai and Highes (2003) reported the comparative analysis of wild and domestic Muscovy based on microsatellite loci, whereby high genetic differences were observed in wild type than domestic Muscovies [36].

4.1 Phylogenetic and Network analysis

The NJ phylogenetic tree [36] shows small differences among individuals with intermingled China, India and France populations whereby sc1 and sc2 represent haplotypes of H2, H12 and H3. In order to find the depth of population differences, median joining network was constructed. India and China haplotypes were intermingled in H1 haplotypes and all other haplotypes were connected with H1 shows star like structure (Fig 4). But the same time (Fig 5) InDel sites MJ network shows separation of India and China population clearly and it was separated based on H5 and H1 haplotype and star like structure also observed around H5. The China haplotype H1 connected from H5 haplotype of India with two mutational steps with mv5. Most importantly the H4 haplotype was shared by UC of CH1 and France Muscovies, which concludes Muscovy in China, has close relationship to France Muscovies than India IM population. This result may infer that India and China Muscovy populations possess slight genetic variations.

V. Figures And Tables

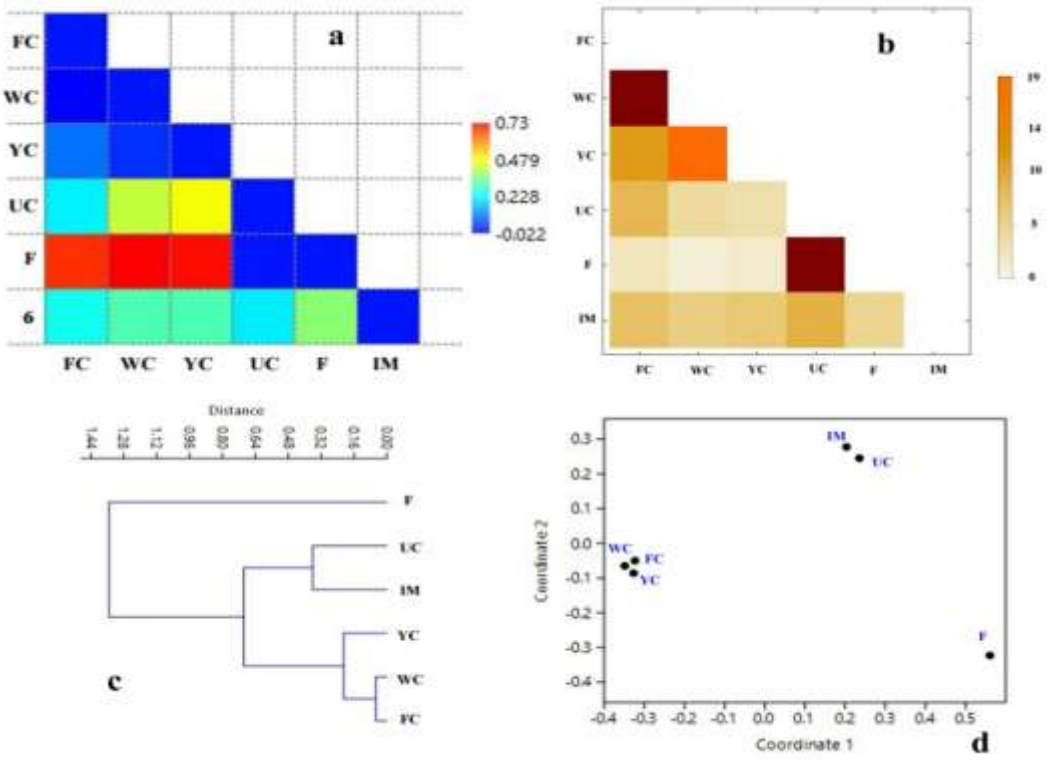

Fig 1 Genetic relationships among Muscovy population. a. Matrix of Fst, b. Matrix of Nm, c. Cluster analysis of Unweighted pair-group average (UPGMA) dendrogram.

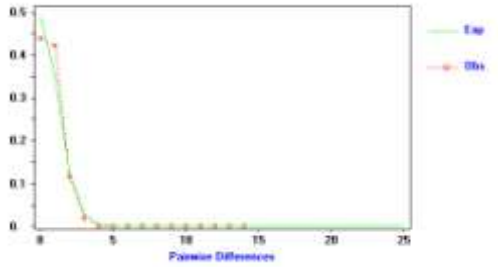

Fig 2 Mismatch distribution graphs for Muscovy populations. The x axis shows the number of pairwise differences, the y axis shows the frequency of the pairwise.

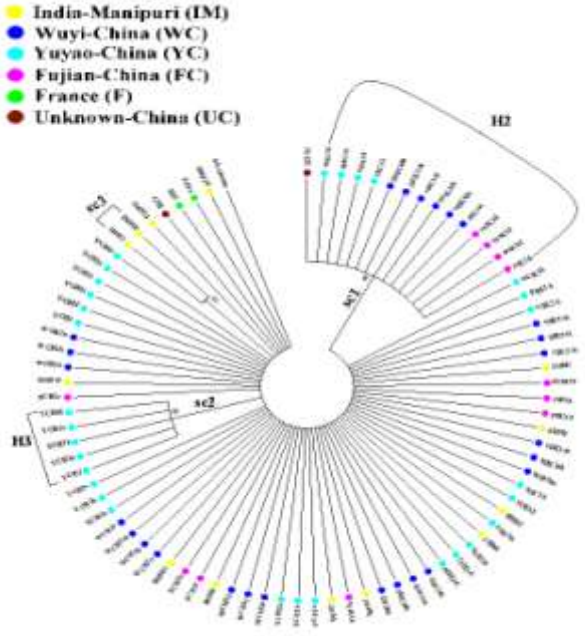

Fig 3 Neighbor-joining phylogenetic tree of Muscovy populations based on the mitochondrial D-loop sequences.

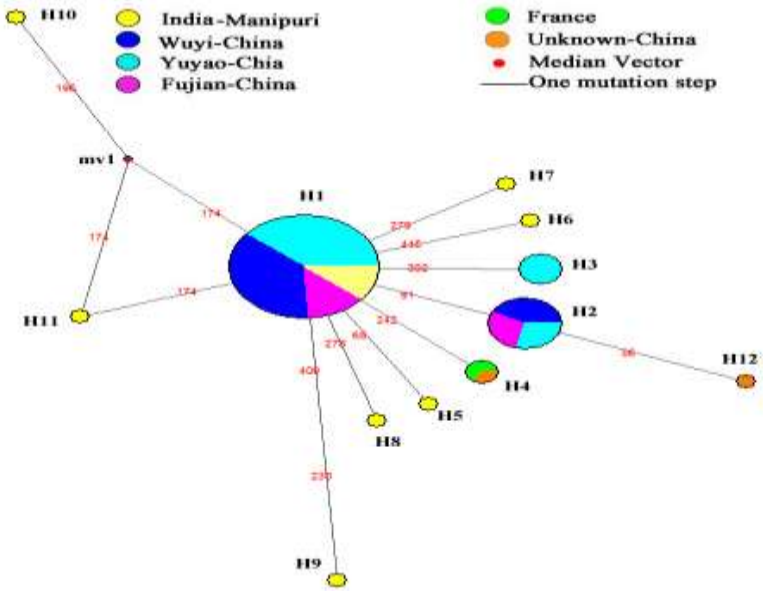

Fig 4 Median-Joining haplotype network of Muscovy populations from India, China and France.

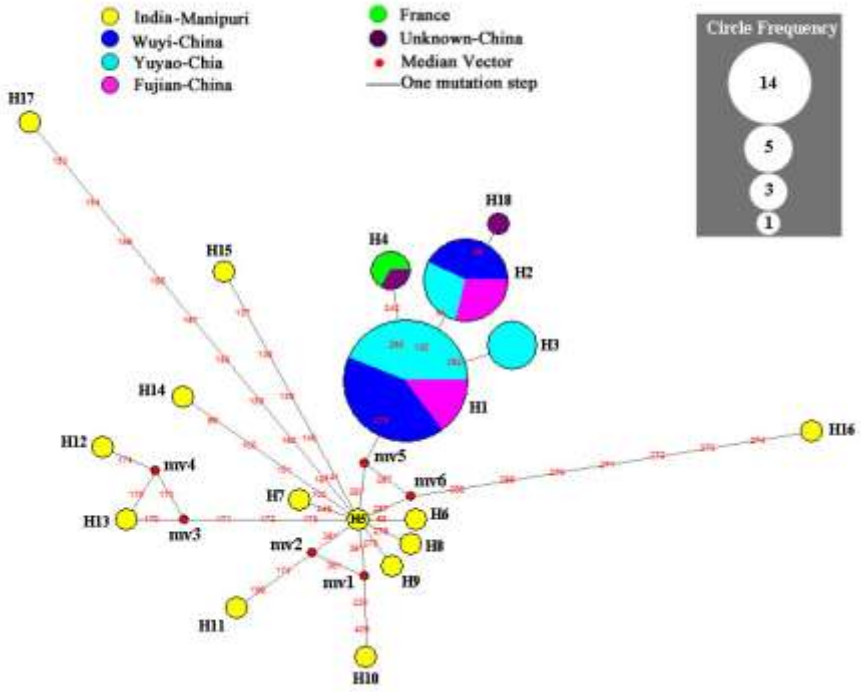

Fig 5 Median-Joining haplotype network of Muscovy populations from India, China and France constructed based on segregation sites includes alignment gap or missing sites

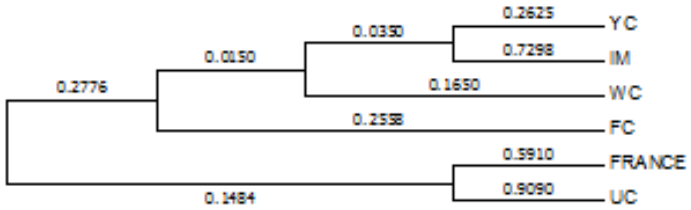

Fig 6 NJ genetic distance tree of Muscovy population based on nucleotide differences (Kxy).

Table 1 Sample size, number of haplotypes and number of polymorphic sites of Muscovy populations

Population	N	Geographic location	H	S	K
Wuyi-China 2 1	26	Wuyi, Zhejiang	2	1	0.36923
Yuyao-China	30	Yuyao, Zhejiang	3	2	0.52644
Fujian-China	11	Fuzhou, Fujian	2	1	0.50909
France	2	France	1	0	0
Unknown-China	2	Unknown	2	3	3.00000
India-Manipuri	13	India	8	8	1.37179
Total/Average	84	China &France and India	12	12	0.71773

N-sample size; H- number of haplotypes; S-number of polymorphic site; K-Average number of nucleotide differences THIS TABLE ADAPTED FROM REFERENCE [16] AND [17]

Table 2 Analysis of molecular variance (AMOVA) of muscovy population based on mtDNA dloop sequence

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Index F_{ST}	P-Value
Among populations	5	15.674	0.20413 Va	24.88	F_{ST}: 0.24879*	0 ($p<0.05$)
Within populations	78	48.076	0.61635 Vb	75.12		
Total	83	63.750	0.82048	100		

*Statistically significant $P<0.05$

Table.3 Population pairwise F_{ST} values between Muscovy populations.

F_{ST}	Fujian-China	Wuyi-China	Yayao-China	Unknown-China	France	India-Manipuri
Fujian-China	0.00000					
Wuyi-China	-0.02202	0.00000				
Yayao-China	0.09016	0.02554	0.00000			
Unknown-China	0.21563	0.41947*	0.47027*	0.00000		
France	0.68264*	0.72954*	0.71604*	0.00000	0.00000	
India-Manipuri	0.24689*	0.30448*	0.30204*	0.20976	0.36550*	0.00000

***Statistically significant P<0.05**

VI. Conclusion

The NJ phylogenetic tree and MJ network results were supports the anomaly between India and other Muscovy populations of China and France. The unimodal mismatch distribution also supports the populations have experienced recent growth. The findings of this study will help to understand the Muscovy duck genetic diversity in this world. If we are increasing the samples size in India and France we could conclude the clear geographically differentiation and maternal genetic evolutionary history of Muscovy duck.

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Authors' contribution

Kameshpandian Paramasivam conceived and designed the study, analysed and prepared the draft of manuscript, Satyanarayana Swamy Vyshnava and Dileep Kumar Kanderi helped for manuscript draft preparation and analysis, Cino Pertoldi contributed critical revision of paper, analysis, draft preparation and moral scientific supports.

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