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ORIGINAL ARTICLE

First Insight into the Phylogenetic Diversity of *Bovicola caprae* Infesting Goats of Different Agro-climatic Locations in India

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Abstract

Bovicola caprae is an important obligate ectoparasite of goats worldwide including India. The present study aimed at the molecular confirmation, phylogenetics and population structure analyses of *B. caprae* infesting goats of three different agro-climatic locations in India, by targeting the mitochondrial cytochrome C oxidase subunit 1 (*cox1*) genetic marker. The phylogenetic tree exhibited the presence of two different lineages of *B. caprae*. The sequences generated herein clustered in lineage 2 along with the GenBank™ archived sequences from China and Iran. The sequences generated herein also showed the circulation of sub-lineages of *B. caprae* in India based on the analysis of pairwise genetic distances between sequences and median-joining haplotype network. The population structure analyses revealed low nucleotide (0.00353 ± 0.00291 and 0.02694 ± 0.00363) and high haplotype (0.667 ± 0.314 and 0.618 ± 0.104) diversities for the present study isolates as well as for the complete dataset, respectively, which evinced a recent demographic expansion. High genetic differentiation (F_{ST} value $= 0.97826$) and low gene flow ($N_m = 0.00556$) were also recorded in the different lineages/populations. In conclusion, the present study addressed the research gap and provided the first insight into the phylogenetics of the goat louse *B. caprae* and highlighted the circulation of sub-lineages of the ectoparasite in India.

Keywords *Bovicola caprae* · *cox1* · Goat louse · Molecular confirmation · Phylogenetics · Population structure

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Introduction

In India, goat farming is an integral part of the livestock industry as it is associated with the socio-cultural, economic, agricultural and even religious beliefs of humans (Ajith et al. 2017a, b). The basic purpose behind goat farming is low input and investment and good output in the form of milk, wool and meat (Moudgil et al. 2017). The parasitic (ecto- and endoparasitic) diseases are considered to be one of the major constraints in maintaining the good health of the traditional transhumance and organized goat flocks (Pilarczyk et al. 2021).

Lice infestation in goats, commonly called caprine pediculosis is considered to be a serious problem in goat flocks especially reared in extensive grazing systems (Ajith et al. 2019). Primarily based on feeding habits, goat lice can be classified into two major subgroups; chewing or biting lice including the members of the order Mallophaga and sucking lice consisting of the members of the order Anoplura (Ajith et al. 2019). Goats in India are primarily parasitized by a biting louse, *Bovicola caprae* and a sucking louse, *Linognathus africanus* (Ajith et al. 2017b). Biting or chewing louse of goats, *B. caprae* is an important obligate ectoparasite of goats worldwide including India (Ajith et al. 2017a). However, no serious efforts have been put in for cladistics as well as population structure analysis to ascertain the origin and dispersal of this economically important ectoparasite.

Caprine chewing lice infestation primarily induces irritation, self-excoriation, alopecia, severe pruritus and papulocrustous dermatitis (Taylor et al. 2016; Ajith et al. 2017b). The initial clinical manifestation in the form of severe irritation is attributed to hypersensitivity reactions caused by the antigens present in lice saliva (Ajith et al. 2017b). In line with the pathogenesis associated with other ectoparasites, lice are also incriminated to transmit various *Rickettsia* species in animals and humans (Fournier et al. 2002; Hornok et al. 2010).

In the recent past, extensive studies had been carried out on human head and body lice populations with the help of multiple genetic markers (Ghavami et al. 2020). The researchers targeted both phylogeography as well as demographic dynamics of *Pediculus humanus capitis* (Amanzougaghene et al. 2019; Yingklang et al. 2021). Also, mitochondrial genetic markers played a pivotal role in establishing the different lineages of human head louse based on the dispersal or prevalence in different geographical regions of the world (Ghavami et al. 2020; Yin et al. 2023). However, any such study had not been performed on chewing lice populations of domesticated animals, despite their utmost economic importance. Additionally, understanding the population changes (expansion, a sudden contraction or a bottleneck) based on neutrality tests is also of significant importance to ascertain the evolutionary patterns, spatiotemporal dynamics and genetic exchange (gene flow, migration, genetic drift and genetic selection pressure) in different populations (Yin et al. 2023). The information thus retrieved could be employed for the implementation of the most effective control strategies.

It is also established that agro-climatic regions, breeds, immune status, hygiene and rearing systems significantly impact the distribution and prevalence of lice in goats (Ajith et al. 2017a). Hence, the present study is the first attempt to shed

light on the molecular confirmation, genetic diversity and population structure of *B. caprae* infesting goats of three different agro-climatic locations in India based on mitochondrial cytochrome C oxidase subunit 1 (*cox1*) genetic marker.

Materials and Methods

Study Area and Sample Collection

The present study targeted three geographically distant locations from three different states; Himachal Pradesh, Haryana and Rajasthan (Fig. 1). The study involved Palampur, Himachal Pradesh (32.1109° N, 76.5363° E), located in the north-western Himalayas at an elevation of 1472–2350 m above mean sea level, with an annual average rainfall of 1578 mm. The study involved Fatehabad, Haryana (29.5132° N, 75.4510° E), which is an arid zone, located at an elevation of 207–222 m above mean sea level, with an annual average rainfall of 272 mm. The study also involved the alluvial plain zone of Rajasthan state, i.e. Pali (25.7781° N, 73.3311° E), located at an elevation of 149–1099 m above mean sea level with an annual average rainfall of 525 mm. The lice were collected from local breeds of goats of Himachal Pradesh (Gaddi breed), Haryana and Rajasthan, non-invasively with the help of a pooter and forceps. The collected lice ($n = 10$ from each location) were preserved in 70% ethanol for further analysis.

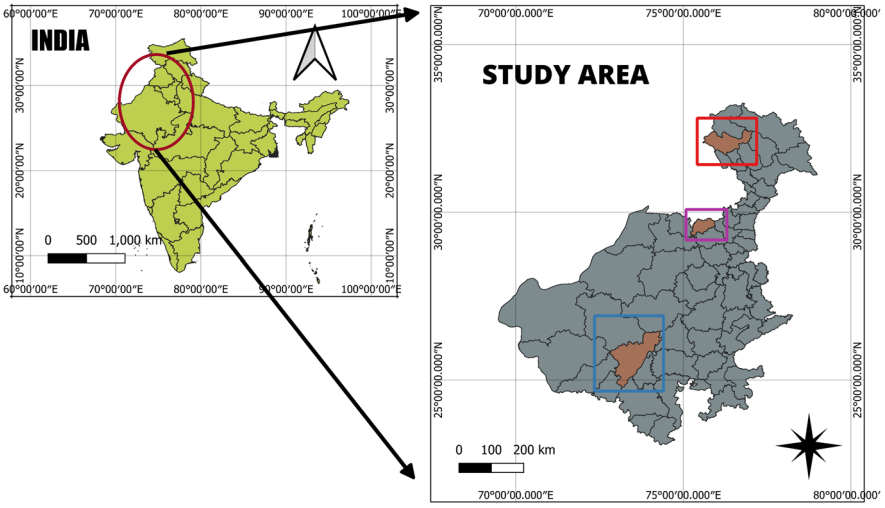

Fig. 1 Map showing the sampling sites of the study area; Palampur (red rectangle) of Himachal Pradesh, Fatehabad (violet rectangle) of Haryana and Pali (blue rectangle) of Rajasthan states (Color figure online)

Genomic DNA Extraction, PCR Amplification and Sequencing

The collected lice specimen ($n = 10$) from the goats of each location were subjected to genomic DNA extraction after cleaning lice twice with normal saline. The genomic DNA was extracted by using Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The retrieved genomic DNA was stored at − 20 °C till further molecular analysis. The amplification of DNA sequences was performed by targeting mitochondrial cytochrome C oxidase subunit 1 (*cox1*) genetic marker through PCR by using the primers; forward: 5'-GGAGGATTTGGAAATTGATTAGTTCC- 3' and reverse: 5'- CCAGGAAGA ATAAGAATATAAACTTC- 3' (Yong et al. 2003). The 25 µL PCR reaction mixture comprised 12.5 µL of master mix (GoTaq Green Master Mix, Promega, Madison, WI, USA), 2.0 µL of each primer (forward and reverse) (10 pmol), 3 µL of genomic DNA template and 5.5 µL of nuclease-free water. The amplification of genomic DNA was carried out in a thermal cycler (Bio-Rad T100™ Thermal cycler, USA) by using the following conditions: initial denaturation (94 °C for 3 min), 40 cycles each of denaturation (94 °C for 30 s), annealing (47 °C for 30 s) and extension (68 °C for 1 min), and then final extension (68 °C for 3 min). The amplified products were electrophoresed through 1.25% agarose gel along with a 100 bp marker (DNAmark™ 100 bp, G-Biosciences, USA) and then visualized under a gel documentation system. To check the specificity of the primers, the genomic DNA of ixodid tick *Rhipicephalus microplus* was used as negative template control in all the amplification reactions. The amplicons of ~ 520 bp size corresponding to partial amplification of the *cox1* gene sequence were subjected to custom sequencing (Biokart India Pvt. Ltd., Bengaluru, Karnataka). Sanger sequencing based on chain termination PCR was performed on the amplified products by using the aforementioned forward and reverse primers. The custom- sequenced products were analyzed for misread sequences, which were eventually aligned using BioEdit version 7.0.5.3 (Hall 1999). The NCBI BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to ascertain the identity of each nucleotide sequence. The consensus sequences generated herein were deposited in the GenBank™ under the accession numbers LC768866-68.

Phylogenetic Analysis

The phylogenetic analysis of the sequences generated in the present study was performed with the GenBank™ archived sequences of *Bovicola* sp. (Table 1), with Molecular Evolutionary Genetic Analysis (MEGA) 11.0.10 software by constructing a phylogenetic tree using Maximum-likelihood method (Tamura et al. 2021). To infer the evolutionary history of *B. caprae* and for the construction of the phylogenetic tree, the best substitution model was found to be Kimura 2-parameter model (Kimura 1980) by using MEGA 11.0.10. Bootstrap analysis was performed using 1000 replicates and the distance scale was estimated at 0.20. The GenBank™ sequence corresponding to the ixodid tick *R. microplus* (LC715165) was used as an

Table 1 Summary of the mitochondrial cytochrome C oxidase subunit 1 (*cox 1*) gene sequences of *Bovicola* sp. derived from the GenBank as well as generated in the present study

S.N.	Accession number	Species	Host	Year	Origin
1	MF927687	*B. caprae*	Goat	2017	China
2	MZ817000	*B. caprae*	Goat	2021	Iran
3	OK135715	*B. caprae*	Goat	2021	Iran
4	OK135716	*B. caprae*	Goat	2021	Iran
5	OK135721	*B. caprae*	Goat	2021	Iran
6	OK135722	*B. caprae*	Goat	2021	Iran
7	OK135723	*B. caprae*	Goat	2021	Iran
8	OK135724	*B. caprae*	Goat	2021	Iran
9	KP256539	*B. caprae*	Goat	2014	Turkey
10	KP256540	*B. caprae*	Goat	2014	Turkey
11	LC768866	*B. caprae*	Goat	2023	India (present study)
12	LC768867	*B. caprae*	Goat	2023	India (present study)
13	LC768868	*B. caprae*	Goat	2023	India (present study)
14	KM260757	*B. tibialis*	California mule deer	2014	USA
15	GU569309	*B. ovis*	Sheep	2010	Japan
16	AF545680	*B. bovis*	Cattle	2002	USA
17	AY594667	*Bovicola* sp.	Galapagos hawk	2004	USA

outgroup to root the tree. A maximum composite likelihood model was employed to estimate the evolutionary divergence based on pairwise genetic distances between the sequences generated herein with the GenBank™ archived *B. caprae* sequences (Tamura et al. 2004) by using MEGA 11.0.10. The comparative sequence analysis between the present study sequences and the GenBank™ archived *B. caprae* sequences from Iran (MZ817000) and China (MF927687) was performed to analyze the variations in the sequences (Hall 1999) by using MEGA 11.0.10.

Median-Joining Network

The relationships between *B. caprae* haplotypes were estimated through median-joining haplotype network analysis by using PopART software (http://popart.otago.ac.nz) (Leigh and Bryant 2015). In total, eleven GenBank™ archived *B. caprae* sequences including the present study sequences were involved in the haplotype network analysis. The misidentified *B. caprae* sequences available in the GenBank™ were not included in the median-joining haplotype network analysis.

Population Dynamics and Genetic Differentiation Indices

Demographic dynamics or population structure analysis involving population diversity indices [number of isolates, number of mutations, average number of pairwise nucleotide differences (K), nucleotide diversity (π), number of haplotypes (H) and haplotype

diversity (Hd)] and neutrality indices (Fu's *Fs*, Fu and Li's *F*, Fu and Li's D and Tajima's *D*), were assessed using DnaSPv6 software (Rozas et al. 2017). DnaSPv6 software was also used for estimating Wright's *F* statistics estimating pairwise genetic difference (F_{ST}), the average number of pairwise nucleotide differences (K_{xy}) and nucleotide substitution per site (D_{xy}). The gene flow (N_m) between the populations was assessed using the Arlequin 3.5.2 software (Nehra et al. 2022). The values of genetic differentiation were defined as high, moderate, low and negligible based on F_{ST} values greater than 0.25, between 0.25 and 0.15, between 0.15 and 0.05, and less than 0.05, respectively (Low et al. 2015). Whereas the level of gene flow was considered as high, intermediate and low, if the values of N_m were recorded greater than 1, between 0.25 and 0.99, and less than 0.25, respectively (Low et al. 2015).

Results

Phylogenetic Analysis

To address the research gap, the present study aimed at genetic diversity and population structure of *B. caprae* infesting goats of three different agro-climatic locations in India based on the mitochondrial *cox1* genetic marker. The BLAST analysis revealed 99.61–100 and 96.13–99.39% nucleotide homology within the sequences generated herein and with the GenBank™ sequences from China and Iran, respectively. The phylogenetic tree demonstrated that the present study sequences along with the GenBank™ archived sequences from China (MF927687) and Iran (MZ817000) clustered in lineage 2, however, arranged in the sister groups at high bootstrap values (Fig. 2). Whereas, all other sequences from Iran formed a sister lineage 1 (Fig. 2). Both the lineages were supported by high bootstrap values (Fig. 2). Additionally, *B. caprae* sequences from Turkey (KP256539-40) sorted with *Bovicola* sp. retrieved from Galapagos hawk in the USA.

The evolutionary divergence studies based on genetic distances revealed no differences between the sequences from Fatehabad (LC768867) and Pali (LC768868), India (Table 2). However, the sequence generated from Palampur, India (LC768866) exhibited slight variation from the aforementioned present study sequences. In addition, the sequences from Iran, belonging to different lineages depicted comparatively high values ranging from 0.552 to 1.5439 (Table 2). The sequences generated herein showed nucleotide polymorphisms at different positions with respect to the sequences from Iran and China (G180A, A187T, C253T, G577T, G669A, G672T, C678T, C690T) (Fig. 3). The sequence from Palampur, India also exhibited substitutions (C253T, G577T) as to other two sequences generated herein from Fatehabad and Pali, India (Fig. 3).

Median-Joining Network Analysis

In totality, 11 sequences of *B. caprae* yielded three different haplotypes (Table 3). Haplotype 3 (Hap_3) containing 6 sequences was the predominant haplotype, which belonged to lineage 1 (Fig. 4). However, there was no central haplotype recorded

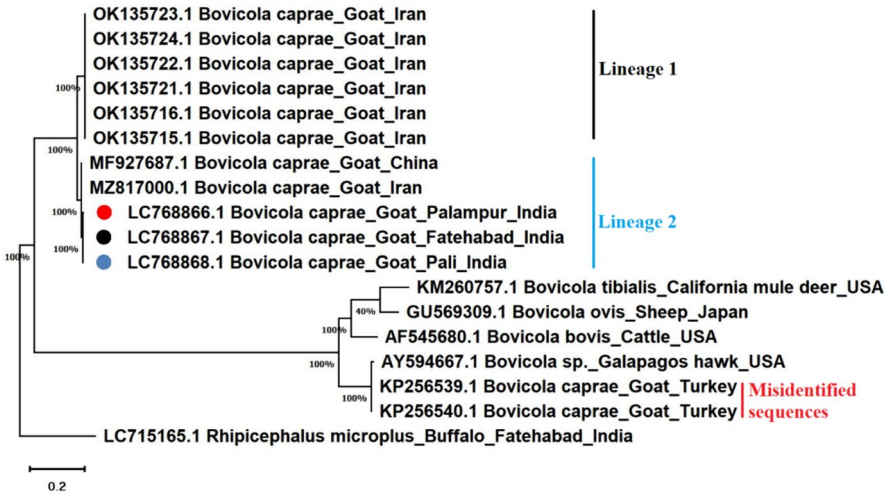

Fig. 2 Maximum-likelihood evolutionary tree inferred from partial mitochondrial *cox1* gene sequence. The percentage of trees in which the associated taxa clustered together/bootstrap values are indicated on each node. The bar represents 0.20 substitutions per site. *Rhipicephalus microplus* (LC715165) is used as an out-group species to root the tree

in the dataset. All the sequences of lineage 1 belonged to Iran. Whereas, lineage 2 comprised haplotypes 1 and 2 with sequences from India, Iran and China. The sequence generated herein from Palampur, Himachal Pradesh formed Hap_1, which separated from other sequences generated in the present study (Hap_2) with two mutational steps. The sequences of lineage 1 were separated from the sequences of lineage 2 by 18 mutational steps (Fig. 4).

Population Structure Analysis

In total, 11 valid/true sequences of *B. caprae* were included for population structure analysis. The overall dataset exhibited a low nucleotide (0.02694 ± 0.00363) and a high haplotype diversity (0.618 ± 0.104). Similarly, the sequences generated in the present study also evinced a very low nucleotide (0.00353 ± 0.00291) and a high haplotype diversity (0.667 ± 0.314). The results of the neutrality tests revealed significantly positive values for Tajima's D (2.22502; $p < 0.05$) and Fu and Li's F (1.60455; $p < 0.05$) and a non-significant positive value for Fu and Li's D (1.13877; $p > 0.10$) and Fu's Fs statistic (8.819).

Gene Flow and Genetic Differentiation Indices

The details of gene flow and genetic differentiation indices are given in Table 4. The low values of inter-population nucleotide differences ($D_a = 0.04762$) and an average number of nucleotide substitutions per site between the populations ($D_{xy} = 0.04868$) depicted high genetic structuring. The pairwise F_{ST} value between the lineages was

Table 2 Estimates of evolutionary divergence between the sequences based on the maximum likelihood model

	LC768866	LC768867	LC768868	KP256539	KP256540	OK135715	OK135716	OK135721	OK135722	MF927687	OK135723	OK135724	MZ817000
LC768866_ Palampur/ India[a]													
LC768867_ Fatehabad/ India[a]	0.0053												
LC768868_ Pali /India[a]	0.0053	0.0000											
KP256539_ Turkey	1.5493	1.5389	1.5389										
KP256540_ Turkey	1.5493	1.5389	1.5389	0.0000									
OK135715_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536								
OK135716_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536	0.0000							
OK135721_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536	0.0000	0.0000						
OK135722_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536	0.0000	0.0000	0.0000					
MF927687_ China	0.0053	0.0000	0.0000	1.5389	1.5389	0.0494	0.0494	0.0494	0.0494				
OK135723_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536	0.0000	0.0000	0.0000	0.0000	0.0494			
OK135724_ Iran	0.0552	0.0494	0.0494	1.5536	1.5536	0.0000	0.0000	0.0000	0.0000	0.0494	0.0000		
MZ817000_ Iran	0.0053	0.0000	0.0000	1.5389	1.5389	0.0494	0.0494	0.0494	0.0494	0.0000	0.0494	0.0494	

"[a]"Represents present study isolate/sequence

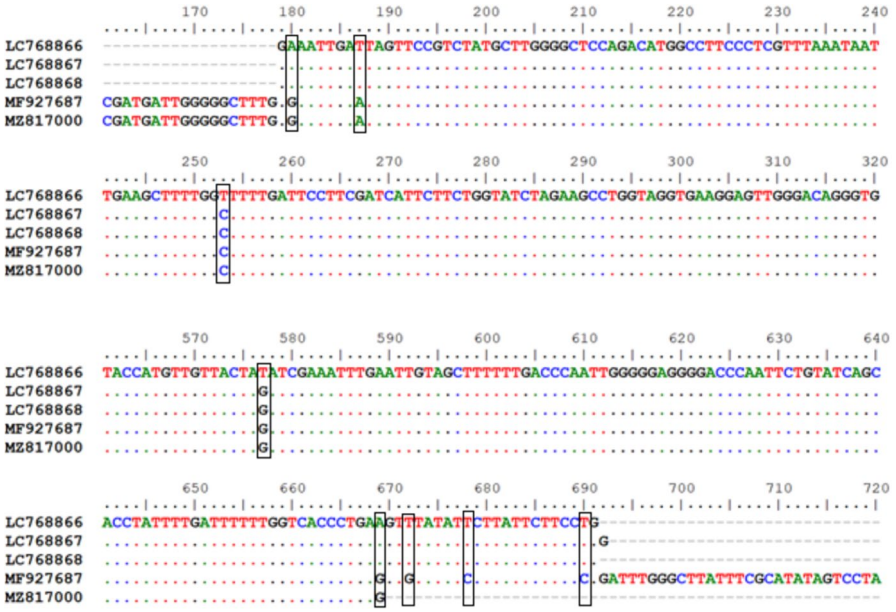

Fig. 3 Comparative multiple sequence alignment of the consensus sequences of the mitochondrial *cox1* gene of *B. caprae* revealed nucleotide polymorphisms in present study isolates (LC768866-68) at different positions (G180A, A187T, C253T, G577T, G669A, G672T, C678T, C690T) with respect to the sequences from Iran (MZ817000) and China (MF927687). The sequence from Palampur, India (LC768866) also exhibited substitutions (C253T, G577T) with respect to other two sequences generated in the present study from Fatehabad (LC768867) and Pali (LC768868), India

Table 3 Various haplotypes of *B. caprae* identified in the present study based on *cox1* gene

Haplotype	Number of sequences	Accession numbers (country)
Hap_1	1	LC768866 (India)
Hap_2	4	LC768867 (India), LC768868 (India), MF927687 (China), MZ817000 (Iran)
Hap_3	6	OK135715 (Iran), OK135716 (Iran), OK135721 (Iran), OK135722 (Iran), OK135723 (Iran), OK135724 (Iran)

observed to be 0.97826, indicating a very high genetic differentiation. The N_m value recorded between the lineages was 0.00556, which was indicative of very low gene flow between the populations.

Discussion

In this study, firstly, the molecular confirmation and cladistics of goat louse *B. caprae* were performed using the *cox1* mitochondrial genetic marker. Secondly, we assessed phylogeography, genetic diversity and demography dynamics of *B. caprae*

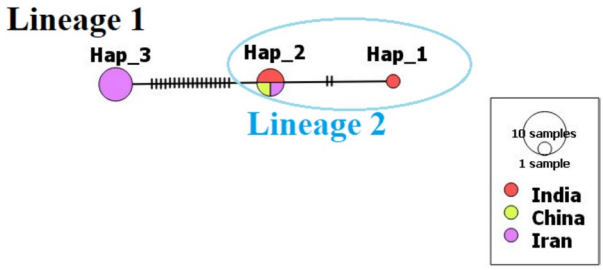

Fig. 4 The median-joining haplotype network of *B. caprae* from different countries based on the partial mitochondrial *cox*1 gene sequence. Each circle depicts a unique haplotype and the circle size is relative to haplotype frequency. Nucleotide differences/haplotype substitutions/mutations are denoted by the hatch marks/bars across the lines connecting the haplotypes with each bar representing a single nucleotide variation. A color code to the country of origin is given (Color figure online)

Table 4 Gene flow and genetic differentiation indices between *B. caprae* lineages based on *cox1* gene

Population 1	Population 2	K_{xy}	G_{st}	N_m	F_{ST}	D_{xy}	D_a
Lineage_1	Lineage_2	18.40000	0.68579	0.00556	0.97826	0.04868	0.04762

K_{xy} Average proportion of nucleotide differences between populations, *Gst* genetic differentiation index based on the frequency of haplotypes, *Nm* gene flow and population migration among population, F_{ST} wright's F statistics, pairwise genetic distance, D_{xy} the average number of nucleotide substitutions per site between populations, *Da* the number of net nucleotide substitutions per site between populations

in India in relation to the isolates reported from different parts of the world. In the past, various studies involving classical light microscopy and scanning electron microscopy had been performed to identify *B. caprae* morphologically (Sebei et al. 2004). To the best of our knowledge, this is the first study to address the research gap associated with phylogenetic characterization and demographic dynamics of *B. caprae* infesting goats in India.

The detailed molecular studies of human louse based on mitochondrial markers resulted in six deeply divergent lineages distributed throughout the globe (Amanzougaghene et al. 2019). However, the perusal of the literature revealed that no such study had been conducted on goat louse *B. caprae*. In the present study, we also targeted the mitochondrial gene to assess the phylogeography of *B. caprae* because the mitochondrial genome is maternally inherited and non-recombining (Ascunce et al. 2013). Whereas, inbreeding in lice of different lineages/clades or haplogroups in sympatric environments may result in the interchange in nuclear genes (Ascunce et al. 2013). Thus, mitochondrial markers had been proved critical in determining evolutionary patterns and similarities between lice species distributed in different geographic regions/locations. The phylogenetic analysis in the present study also revealed the presence of two lineages or haplogroups of *B. caprae* based on the mitochondrial *cox1* gene sequence. However, *B. caprae* sequences involved in the present study did not exhibit geographic isolation. The finding could be attributed to the availability of a limited number of sequences of *B. caprae* in the GenBank™ and hence, the inclusion of only available sequences from Asian countries in the

present study. It is also evident from the phylogenetic findings that the co-occurrence of both the lineages (1 and 2) in Iran may allow interbreeding and interchange in nuclear genes (Ascunce et al. 2013). Furthermore, *B. caprae* sequences reported from Turkey acted like an outgroup and clustered with *Bovicola* sp. recorded from a hawk, indicating the misidentification of the lice species involved.

The pairwise genetic distances among sequences revealed high values further insinuating diverged lineages. The finding supported the observation of the evolutionary tree evincing the diverged lineages of the sequences recorded from Iran to the sequences generated herein. On the contrary, the sequences of the present study exhibited extremely low values within themselves, hence alluding absence of diverged lineages. However, the sequence recorded from Palampur, India also exhibited slight variation, which was indicative of the presence of a sub-lineage of *B. caprae*. The observation was further supported by the nucleotide polymorphism recorded during the comparative sequence analysis. The finding could be attributed to the fact that the endemicity of the parasites might result in nucleotide substitutions or polymorphisms or mutations due to anthropogenic pressure such as transhumance or trading of animals (Solano et al. 2016).

The phylogenetic or evolutionary tree analysis is inadequately explanatory for the variations identified in the sequences included in the study. Owing to the capability of median-joining haplotype network analysis to adjudge even a single nucleotide polymorphism, it is considered to be a better approach for studying the genetic diversity and relationship among the sequences (Dumaidi et al. 2020). Hence, cladogram analysis was performed in the present study to identify the misidentified/misannotated *B. caprae* sequences in the GenBank™. In the present study, the haplogroup corresponding to lineage 2 consisted of sequences from India, China and Iran with the presence of two haplotypes; Hap_1 and Hap_2. Hap_1 represented the sequence from Palampur, India; whereas, Hap_2 comprised the sequences from Fatehabad and Pali, India generated herein and from Iran and China. However, no such differentiation was observed during phylogenetic analysis. In haplogroup/lineage 2, the haplotypes differed with 1–2 mutational steps or nucleotide variations, which indicated the common origin of the sequences due to recent population expansion or genetic bottleneck or selective sweep (Dumaidi et al. 2020). In the median-joining haplotype network analysis, no central haplotype was recorded. The finding further indicated the uncertainty of the existence of a widespread haplotype of *B. caprae* infesting goat populations (Ohiolei et al. 2019). Since, only a few sequences of *B. caprae* corresponding to the mitochondrial *cox1* gene had been submitted to the GenBank™, which eventually restricted the identification of a central haplotype. Detailed geographical studies involving an extensive DNA analysis are a prerequisite for resolving the issue of the identification of a widespread/central or ancestral haplotype (Ohiolei et al. 2021).

The population structure analysis revealed a low nucleotide and a high haplotype diversity for the complete data set as well as for the sequences generated herein, which indicated a recent demographic expansion and low gene flow followed by genetic drifts or bottlenecks (Moudgil et al. 2022). The recent demographic expansion might have resulted in coalescence, which eventually lead to low nucleotide diversity. Also, the amassed mutations might have increased

haplotype diversities (Moudgil et al. 2023). The positive neutrality test values recorded in the present study were suggestive of high polymorphism experienced by the populations. The finding could be incriminated to any factor which had endangered the survival of the lice population such as the use of insecticides/pediculicides (Phadungsaksawasdi et al. 2021).

Lice are not capable of self-dispersion. Anthropogenic short/long-range host mobility especially under transhumance systems could be considered the sole reason behind lice dispersion, which would eventually influence their population structure. The findings of the present study associated with a high value of F_{ST} and a low value of N_m also supported the aforementioned assertion. High genetic differentiation in lice populations could be associated with the biological factors (including the host specificity) and behavioral traits (one-host feeding) of lice.

The present study provided the first molecular insight into the phylogenetic relationship and population structuring of *B. caprae* infesting goats of different agro-climatic locations in India with respect to the goat louse species prevalent in the world. The negative values of the neutrality indices for the complete dataset indicated population expansion of the ectoparasite. Furthermore, the sequences generated herein also showed the circulation of sub-lineages of *B. caprae* in India based on the analysis of pairwise genetic distance between sequences and median-joining haplotype network. The present pilot study is of utmost significance considering the importance of caprine pediculosis and would prove beneficial in designing effective control strategies. Additionally, the results of the present study would also lay the basis for future comprehensive studies involving both biting and sucking lice infesting goats of a wider study area.

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Author Contributions A. D. M. was involved in sample collection, investigation, methodology, phylogenetics, and drafting the manuscript. A. K. N. was involved in sample collection, investigation, and laboratory work. A. S. helped in collection of samples and reviewed the manuscript. S.P. helped in collection of samples and reviewed the manuscript. S. V. was involved in supervision and editing. All the authors contributed to the review.

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Code Availability Not applicable.

Declarations

Conflict of interest The authors report there are no competing interests to declare.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Ethical Approval The research work was carried out with the permission (VCC/IAEC/2022/1679-1705) of Institutional Animal Ethics Committee of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.

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