

SHORT COMMUNICATION

Characterization of The Complete Mitochondrial Genomes of Two *Pulex* sp. Samples (Siphonaptera: Pulicidae) from the Qinghai-Xizang Plateau

Meixue Ni^{1,2}, Cidan Zhuoga², Pan Chang³, Mengfang Tian⁴, Xiaoya Li⁵, Wenqiang Tang^{2*} and Qingxia Wu^{1*}

¹Key Laboratory for Prevention and Control of Hydatid Disease in Xizang (Co-constructed by Ministry and Province), Ministry of Agriculture and Rural Affairs, College of Animal Science, Xizang Agricultural and Animal Husbandry University, Linzhi, Xizang 860000; ²Key Laboratory of Animal Parasitology of Xizang, Institute of Animal Husbandry and Veterinary Medicine, Xizang Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850009, China; ³Animal Disease Prevention and Control Center of Jingmen City, Jingmen, 448000; ⁴Bayi District Animal Husbandry and Veterinary Station (Animal Disease Prevention and Control Center), Nyingchi, Xizang Autonomous Region, China 860000; ⁵Nyingchi Agricultural and Rural Affairs Bureau, Linzhi 860000, China

*Corresponding author: iwatetang@126.com (WT); 441594343@qq.com (QW)

ARTICLE HISTORY (26-988)

Received: August 08, 2026
Revised: September 18, 2026
Accepted: September 21, 2026
Published online: September 27, 2026

Key words:

Mitochondrial genome
New species
Phylogeny
Pulicidae

ABSTRACT

The unique ecological environment of the Qinghai-Xizang Plateau has a significant impact on the genetic differentiation of parasitic organisms. However, the information on the mitochondrial genome of ectoparasites on goats in this region is extremely limited. Flea samples were collected from the body surface of grazing goats and the genome was annotated using MITOS2. A phylogenetic tree was constructed using the maximum likelihood method, and a comparative analysis was conducted with known species of the flea family. A total of two complete mitochondrial genome sequences (G2 and G3) were obtained, with lengths of 16,238 bp and 16,274 bp, respectively. They contain 13 protein-coding genes, 20 tRNA genes and 2 rRNA gene fragments. The base composition shows a significant A+T preference (G2: 79.37%; G3: 79.41%). The 13 protein-coding genes use 5 types of start codons (ATG, ATA, ATT, ATC, GTG) and 2 types of stop codons (TAA, TAG). The phylogenetic analysis shows that G2 and G3 are supported by 100% bootstrap support to form an independent branch, which is located within the flea family but is significantly separated from known flea species. The G2 and G3 samples are on independent branches on the phylogenetic tree and have significant genetic distances from other species. The genomic length and base composition also differ from those of reported species. Based on molecular evidence, these two samples may represent a new undescribed species of the flea family, which awaits further morphological verification. This study reports the first mitochondrial genome of ectoparasites on the body surface of goats in the Qinghai-Xizang Plateau region, providing new data for the phylogeny and species diversity of the flea family.

To Cite This Article: Ni M, Cidan Z, Chang P, Tian M, Li X, Tang W and Wu Q, 2026. Characterization of the complete mitochondrial genomes of two *Pulex* sp. samples (Siphonaptera: Pulicidae) from the Qinghai-Xizang Plateau. Pak Vet J, 46(9): 2319-2324. <http://dx.doi.org/10.29261/pakvetj/2026.215>

INTRODUCTION

Fleas (*Siphonaptera*) belong to the class Insecta within the phylum Arthropoda, insects that undergo complete metamorphosis. Fleas are important ectoparasites of domestic and livestock animals and may cause irritation, hypersensitivity, anemia, and other health problems; therefore, accurate identification and characterization of flea species are important for veterinary parasitology and livestock surveillance (Kuntuz

et al., 2025). Fleas are vectors of infectious diseases such as plague and endemic typhus and can also serve as intermediate hosts for certain tapeworms (Driscoll *et al.*, 2020; Harimalala, 2025). In pastoral areas, large populations can disrupt cattle and sheep feeding and resting, reducing fur quality, and cause economic losses to the livestock industry (Li *et al.*, 2025).

Flea mitochondrial genomes usually contain 78-84% A+T, a common feature across different families. These genomes are highly conserved in gene order, reflecting

ancestral arthropod arrangements, with no observed deletions, insertions, or rearrangements. In this study, we sequenced and characterized two complete mitogenomes of fleas collected from domestic goats in Dang Xiong County, Tibet, and examined their phylogenetic position within the family Pulicidae.

MATERIALS AND METHODS

Sample collection: Flea samples were collected on November 23, 2023, from the body surface of grazing goats (n=20), from which a total of flea specimens (n=6) were collected in Ge Da Township, Dang Xiong County, Lhasa City, Qinghai-Xizang Plateau Autonomous Region, at an altitude of approximately 4,560 m. The collected flea specimens were preserved and subsequently used for molecular analysis. Among them, two representative specimens (G2 and G3) were selected for mitochondrial genome sequencing and subsequent phylogenetic analysis.

Mitochondrial sequencing: Total DNA was extracted using a commercial animal tissue kit. Paired-end libraries (PE150) were sequenced on an Illumina platform. Raw reads were quality-filtered, and mitochondrial reads were identified by mapping to the NCBI RefSeq mitochondrial database using minimap2, followed by de novo assembly with SPAdes. Annotation and phylogenetic analysis (Zerek *et al.*, 2024; Haq *et al.*, 2025; Shi *et al.*, 2025). Mitogenomes were annotated using the MITOS2 web server, with manual correction of gene boundaries. Thirteen PCGs were extracted and aligned with homologous sequences from representative Pulicidae species retrieved from GenBank (*Pulex irritans* (NC_063709.1), *Ctenocephalides felis* (NC_049858.1), *Ctenocephalides canis* (NC_063710.1), *Leptopsylla segnis* (NC_072691.1), *Amphalius spirataenius* (OR855715.1), *Thaumapsylla breviceps orientalis* (PP973737.1), *Macrostylophora euteles* (PQ843221.1) and *Bittacus pilicornis* (HQ696578.1). A maximum likelihood (ML) tree was constructed using IQ-TREE under the GTR+G+I model, with 1,000 bootstrap replicates.

RESULTS AND DISCUSSION

The mitochondrial genome obtained through assembly was annotated. The genetic compositions and sequences of the two samples (G2 and G3) were highly consistent (Fig. 1A). The full length of the mitochondrial genome of sample G2 was 16,238 bp and that of sample G3 was 16,274 bp. The average coverage depth of sample G2 was 2,655.26× and that of sample G3 was 2,391.72×. The base compositions of the two samples are shown in Table 1. The A+T content of sample G2 was 79.37% and that of sample G3 was 79.41%, both showing a significant A/T preference. The GC content of sample G2 was 20.62% and that of sample G3 was 20.59% (Fig 1B). Nucleotide skew can reflect the bias of mutations during replication and transcription processes. The AT-skew of G2 is -0.0198 and the GC-skew is -0.2076; the AT-skew of G3 is -0.0183 and the GC-skew is -0.2142. The AT-skew and GC-skew of both samples are negative values, indicating that the content of T is slightly higher than that of A and the content of C is slightly higher than that of G.

This skew pattern is relatively common in insect mitochondrial genomes (Table 2).

A total of 13 protein-coding genes (PCGs) were annotated, including nad1, nad2, nad3, nad4, nad4l, nad5, nad6, cox1, cox2, cox3, atp6, atp8 and cob. The lengths, start codons and stop codons of each gene are shown in Table 3. The total length of the 13 PCGs is 11,121 bp (G2 and G3 are the same), accounting for 68.5% (G2) and 68.3% (G3) of the entire genome. Among them, cox1 is the longest (1,540 bp) and atp8 is the shortest (159 bp). There are five types of initiation codons: ATC (cox1), GTG (cox2), ATT (nad2 and atp8), ATA (nad3, nad5, nad4, nad6) and ATG (the remaining 5). Among the stop codons, 9 are TAA and 4 are TAG (nad3, nad4l, cob, nad1). No incomplete stop codons have been found (Table 3). In addition, the annotations of this sequence identified 20 tRNAs, with a total length of 1,421bp. The names, anticodons and lengths of each tRNA are shown in Table 3. The lengths of the identified tRNAs range from 52 to 71bp, with an average length of 64.6 bp. Among them, trnG(tcc) is the shortest (52 bp) and trnK(ctt) is the longest (71bp). Among the anticodons, there are 11 that start with T, 7 that start with G and 2 that start with C (trnM and trnK).

Table 1: Base composition of the mitochondrial genomes of G2 and G3

Sample	Length (bp)	A (%)	T (%)	G (%)	C (%)	A+T (%)	GC (%)
G2	16,238	38.90	40.47	8.17	12.45	79.37	20.62
G3	16,274	38.98	40.43	8.09	12.50	79.41	20.59

Table 2: Protein-coding genes in the mitochondrial genomes of *Pulex* sp. samples G2 and G3

Gene	Length (bp)	Initiation codon	Stop codon
nad2	1026	ATT	TAA
cox1	1540	ATC	TAA
cox2	681	GTG	TAA
atp8	159	ATT	TAA
atp6	675	ATG	TAA
cox3	789	ATG	TAA
nad3	351	ATA	TAG
nad5	1716	ATA	TAA
nad4	1341	ATA	TAA
nad4l	273	ATG	TAG
nad6	468	ATA	TAA
cob	1164	ATG	TAG
nad1	951	ATG	TAA

Table 3: Transfer RNA genes in the mitochondrial genomes of flea samples G2 and G3

Gene	Anticodon	Length (bp)
trnI	GAT	61
trnQ	TTG	65
trnM	CAT	62
trnW	TCA	58
trnC	GCA	55
trnY	GTA	61
trnL2	TAA	62
trnK	CTT	70
trnD	GTC	60
trnG	TCC	52
trnN	GTT	62
trnS1	TCT	65
trnE	TTC	60
trnF	GAA	60
trnH	GTG	55
trnT	TGT	61
trnP	TGG	59
trnS2	TGA	62
trnA	TGC	59
trnR	TCG	59

The mitochondrial gene map shows the locations of the genes encoding proteins (including subunits of complex I, II, IV and ATP synthase) as well as tRNA genes (such as trnH, trnQ, trnM, trnN). The G2 sample has 24 genes located on the negative strand (-) and 14 genes on the positive strand (+) (Fig. 2). For the G3 sample, except for the cob gene, which is divided into two segments, the rest are the same as in the G2 sample (Fig. 3). This should be an error during the splicing process. Thirteen protein-coding genes and 22 tRNA genes (of which 20 have been sequenced) are densely distributed on the circular genome. There are a few intervening regions between the genes, and no large non-coding regions were found. The GC content fluctuates little throughout the entire length and is consistent with the low GC content of the entire genome (Table 3).

On the mitochondrial phylogenetic tree, all branches have received extremely high support, with 100%. The two samples from this study, G2 and G3, were grouped into a single lineage with 100% support, indicating that their mitochondrial genome sequences are highly similar and that they share a close genetic relationship. The branch consisting of *Pulex irritans*, *Ctenocephalides felis* and *Ctenocephalides canis* is clustered together. Additionally, *Leptopsylla segnis* and *Bittacus pilicornis* are grouped into a smaller branch. G2 and G3, together with the above species, constitute a single lineage group of *Pulex* sp. (Fig. 4).

This study obtained the complete mitochondrial genome sequences of two flea samples collected from the surface of goats in Ge Da Township, Dang Xiong County, Qinghai-Xizang Plateau. The mitochondrial genomes of G2 and G3 are 16,238 bp and 16,274 bp, respectively, differing by 36 bp. This difference is entirely located in the non-coding region. All protein-coding genes and tRNAs have the same length in both samples, and the nucleotide sequences are highly consistent (with only a few SNPs). The length difference is likely due to insertions and deletions in the control region (AT-rich region). This phenomenon is common in mitochondrial genomes. The control region is non-coding and has a high

mutation rate. Length polymorphisms are often observed between individuals and even among geographical populations of the same species.

The A+T content of the two samples was 79.37 and 79.41%, respectively, which was lower than the 81.0% reported for *Pulex irritans*, the 84% for *Xenopsylla cheopis* and the 78.5-81.5% range for *Ctenocephalides felis*.

Both AT-skew and GC-skew were negative values, consistent with most flea species. Negative AT-skew indicates that T is slightly more than A, and negative GC-skew indicates that C is slightly more than G (Xie *et al.*, 2026). This skew pattern is very common in insect mitochondrial genomes and is usually related to mutations in the light and heavy chains during replication (Fan *et al.*, 2011). The absolute value of GC-skew was larger, indicating a more significant deviation of C, which might be related to the distribution of the coding strand in the mitochondrial genome.

In this study, five types of start codons were used among the 13 protein-coding genes: ATG (5 genes), ATA (4 genes), ATT (2 genes), ATC (1 gene) and GTG (1 gene). Such diversity has been reported in the family Pulicidae. For example, in previously reported studies, *cox1* uses GTG (Fu *et al.*, 2024), while in *Ctenocephalides felis*, *cox2* uses TTT (Zhang *et al.*, 2021). Atypical start codons are common in mitochondrial genomes; they usually initiate translation through non-canonical pairing with the mitochondrial initiator tRNA. In our samples, the *cox1* gene was annotated with ATC as the putative start codon. However, because RNA-seq or transcript-level validation was not performed in the present study, the actual translation initiation site could not be experimentally confirmed. Therefore, ATC should be regarded as a predicted/annotated start codon rather than a definitively validated initiation codon. Further transcript-level studies are required to confirm the actual translation initiation site of *cox1* in these flea mitogenomes. *cox2* uses GTG, also a known alternative start codon encoding valine.

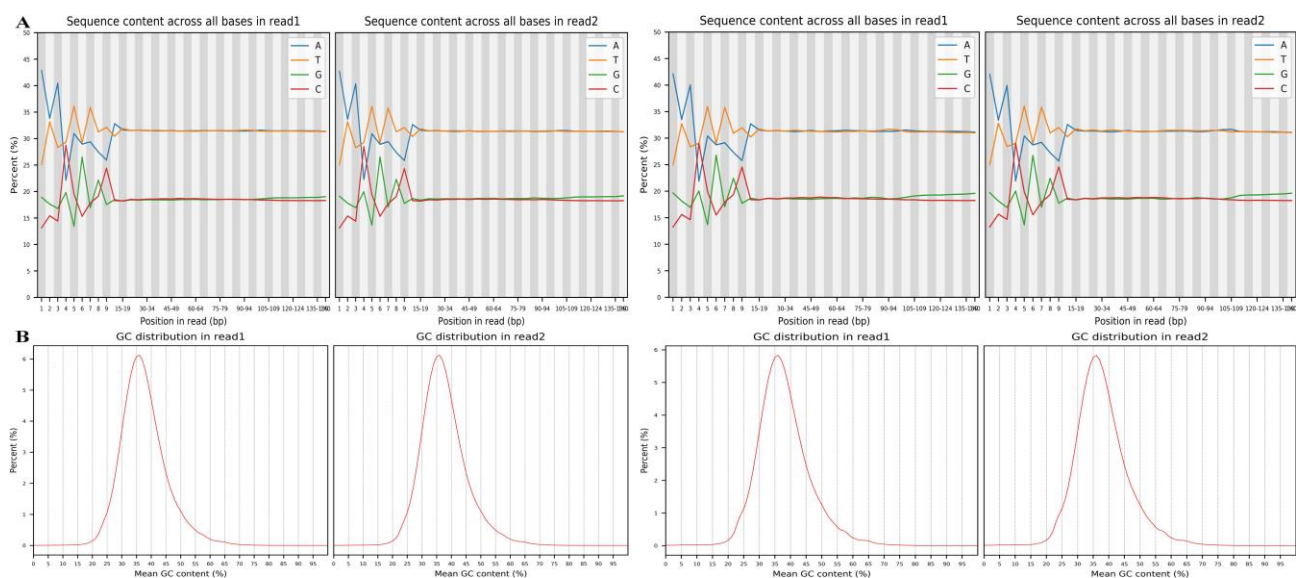
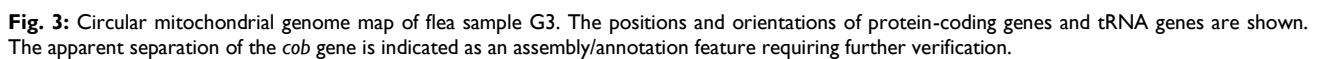
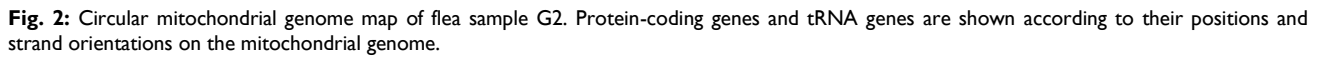


Fig. 1: Sample base composition. A: Base content distribution along the reads for sample G2 (left) and G3 (right). The X-axis represents the position within the reads (bp) and the Y-axis represents the percentage of each base. The proportions of A (blue), T (orange), G (green) and C (red) remained balanced after the initial several base pairs, indicating good sequencing quality. B: Mean GC content distribution of the clean reads for sample G2 (left) and G3 (right). The X-axis represents the GC content (%) and the Y-axis represents the proportion of reads at each GC content level. The distribution approximates a normal curve, indicating a homogeneous library without contamination.



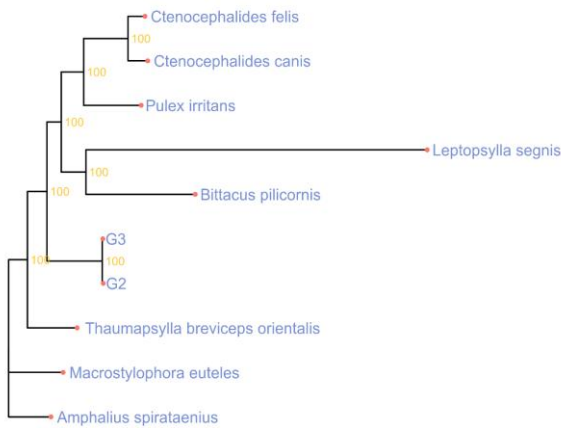


Fig. 4: Maximum-likelihood (ML) phylogenetic tree based on the mitochondrial genome sequences of flea samples G2 and G3 and representative species of the family Pulicidae. The tree was constructed using the GTR+G+I substitution model with 1,000 bootstrap replicates. Bootstrap support values are shown at the corresponding nodes. The G2 and G3 sequences clustered together with strong bootstrap support, indicating their close evolutionary relationship

It is worth noting that in previously reported *Pulex irritans* mitogenomes, *cox1* was found to use ATA as the start codon (Zhang *et al.*, 2022) whereas in our samples it is ATC. Our samples do not seem to match the currently known characteristics of human fleas. We did not perform RNA-seq validation; we cannot determine the actual translation initiation site. Our current practice is to take the first three bases of the sequence as the start codon, which is the common approach in most mitogenome studies (Zang *et al.*, 2026). Regarding stop codons of the protein-coding genes, we observed that nine genes use TAA and four use TAG; no incomplete stop codons (e.g., just T or TA) were found. In some insect mitochondrial genomes, certain genes have stop codons consisting only of T or TA, which are then post-transcriptionally polyadenylated to form a complete TAA.

Molecular approaches based on mitochondrial markers and phylogenetic analysis have also been successfully applied for characterization of veterinary ectoparasites, providing complementary evidence to conventional morphological identification (Ahmad *et al.*, 2025; Kuntuz *et al.*, 2025). The systematic classification of ectoparasitic taxa is supported by phylogenetic analyses based on molecular sequence data, which offer valuable evidence for assessing evolutionary relationships (Koyee and Abdullah, 2019). Based on the comprehensive analysis of mitochondrial genome characteristics and phylogenetic studies, this research determined that the fleas collected from the surface of goats in Ge Da Township, Dang Xiong County, Qinghai-Xizang Plateau Province belong to *Pulex* sp. Genes G2 and G3 clustered together with 100% bootstrap support, forming an independent branch together with human fleas, *Ctenocephalides felis* (cat flea), and *Ctenocephalides canis* (dog flea), constituting a highly supported monophyletic group, indicating that our samples do indeed belong to *Pulex* sp. (Chen *et al.*, 2026).

The mitochondrial genome and phylogenetic analyses indicate that samples G2 and G3 form a distinct and well-supported lineage within the genus *Pulex*.

However, these mitochondrial data alone are insufficient to determine whether the specimens represent an undescribed species. Therefore, we consider the samples as potentially representing a distinct or previously unrecognized lineage of *Pulex* rather than formally assigning them to a new species. Comprehensive morphological examination, together with additional nuclear molecular markers and broader sampling, will be necessary to confirm their taxonomic status and determine whether they represent a distinct species.

Conclusions: The complete mitochondrial genome sequences of two flea samples (G2 and G3) were obtained from the body surface of goats in Ge Da Township, Dang Xiong County, Qinghai-Xizang Plateau Autonomous Region (at an altitude of approximately 4,560m). Phylogenetic analysis placed them as a distinct clade within *Pulex* sp., sister to *Pulex irritans*, suggesting they may represent an undescribed species pending morphological confirmation. This is the first report of flea mitogenomes from the Qinghai-Xizang Plateau, providing new molecular resources for taxonomy and population genetics of ectoparasites in this region.

Data Availability: All the raw data sequences from animals were deposited in NCBI Sequence Read Archive database under the accession number: *Pulex irritans* (NC_063709.1), *Ctenocephalides felis* (NC_049858.1), *Ctenocephalides canis* (NC_063710.1), *Leptopsylla segnis* (NC_072691.1), *Amphalius spirataenius* (OR855715.1), *Thaumapsylla breviceps orientalis* (PP973737.1), *Macrostylophora euteles* (PQ843221.1), and *Bittacus pilicornis* (HQ696578.1).

Ethics statement: All the study operations were conducted under the instructions and approval of the Laboratory Animals Research Centre of Xizang Academy of Agricultural and Animal Husbandry Sciences.

Author contributions: WT, QW: Research idea and methodology; MN, CZ, PC, MT, XL: Reagents, materials and analysis tools; MN, WT, QW: Writing – original draft and preparation; WT, QW: Writing – review and editing; WT, QW: Visualization and supervision; All authors known and approved the final manuscript.

Funding: This study was supported by the "Key Research and Development Program in Xizang Autonomous Region" Project of the Science and Technology Program of Xizang, China (XZ202401ZY0071).

Acknowledge: None.

REFERENCES

- Ahmad M, Sindhu ZD, Zafar MA *et al.*, 2025. Prevalence, risk factors, and molecular characterization of hard ticks in two diverse agro-ecological zones of Punjab, Pakistan. *Pakistan Veterinary Journal* 45: 336-343.
- Chen XD, Hu W, Ma X, *et al.*, 2026. Mitogenome characteristics and phylogenetic analysis of six *Apistogramma* species. *Animals (Basel)* 16(8):1178
- Driscoll TP, Verhoeve VI, Gillespie JJ, *et al.*, 2020. A chromosome-level assembly of the cat flea genome uncovers rampant gene duplication and genome size plasticity. *BMC Biology* 18(1):70.

- Fan S, Hu C, Wen J, et al., 2011. Characterization of mitochondrial genome of sea cucumber *Stichopus horrens*: a novel gene arrangement in Holothuroidea. *Science China Life Sciences* 54:434-441.
- Fu YT, Xun Y, Peng YY, et al., 2024. The complete mitochondrial genome of the rodent flea *Nosopsyllus laeviceps*: genome description, comparative analysis, and phylogenetic implications. *Parasites & Vectors* 17:253.
- Haq SU, Javaid T, Wakid MH, et al., 2025. Global Scale Insights into Genetic Variation in Mitochondrial *cox1* and *nad1* Genes of *Clonorchis sinensis*. *Pakistan Veterinary Journal* 45(2): 640-650.
- Harimalala M, 2025. Current knowledge on fleas (Siphonaptera) associated with human plague transmission in Madagascar. *Journal of Medical Entomology* 62:749-759.
- Koyee QMK and Abdullah SMA, 2019. Phylogenetic and secondary RNA structure analysis of monogenean gill ectoparasites (*Dactylogyrus* spp.) parasitizing certain freshwater fishes. *Polish Journal of Veterinary Sciences* 22:667-675.
- Kuntuz T, Gunes Y, Diren Sigirci B, Anlas C, Sari AB, Bakirel T, Ustuner O. 2025. Investigation of knockdown resistance mutations in *Ctenocephalides felis* samples from the cat populations of Istanbul Province. *Polish Journal of Veterinary Sciences* 28:195-202.
- Li X, Qin Z, Wang H, et al., 2025. Identification of the pathogen *Dorcadia ioffi* Smit and evaluation of the effect of different drugs. *Veterinary Sciences* 12(7):641.
- Shi B, Zhao XL, Tang WQ, et al., 2025. Morphological and Mitochondrial Genome Characterization of *Hypoderma Sinense*: Insights into Evolutionary Relationships Within Oestridae. *Pakistan Veterinary Journal* 45(2): 767-774.
- Xie Y, Bai H, Liu X, et al., 2026. Mitochondrial genome characterization and new insights on phylogenetic relationship of *Chara* (Charophyceae, Charophyta). *BMC Genomics* 27(1):443.
- Zang E, Zhu Y, Ma T, et al., 2026. RhoMitoAnnotator and polypods, bioinformatics tools for the *Rhodiola* mitochondrial gene assembly, annotation and phylogenetic analysis. *International Journal of Molecular Sciences* 27(10):4440.
- Zerek A, Ceylan O, Erdem I, et al., 2024. Molecular detection of two new *Haemoproteus* mitochondrial cytb lineages in ciconiform and charadriiform migratory birds in Turkey. *Pakistan Veterinary Journal* 44(2): 298-305.
- Zhang Y, Fu YT, Yao C, et al., 2022. Mitochondrial phylogenomics provides insights into the taxonomy and phylogeny of fleas. *Parasites & Vectors* 15:223.
- Zhang Y, Nie Y, Deng Y-P, et al., 2021. The complete mitochondrial genome sequences of the cat flea *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) support the hypothesis that *C. felis* isolates from China and USA were the same *C. f. felis* subspecies. *Acta Tropica* 217:105880.