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Original research article

Genome-wide assessment of suspected domestic cat introgression in the critically endangered Tsushima leopard cat

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ABSTRACT

The Tsushima leopard cat, an insular felid endemic to the Tsushima Island, Japan, is listed as Critically Endangered in the Japanese Red List, with an estimated population size of approximately 100 individuals. Recently, a short-tailed individual was detected, raising concerns over its possible hybrid origins because such a phenotype has not been documented in leopard cats but is relatively common in domestic cats. However, whether this phenotype reflects domestic introgression or variation within the wild population remains unknown. We hypothesized that recent introgression would produce a detectable domestic ancestry signal in the focal individual. We therefore analyzed whole-genome data from the focal individual, 15 additional Tsushima leopard cats, five domestic cats from Tsushima Island, and four Amur leopard cats as continental wild references. Principal component analysis placed the focal individual within the Tsushima leopard cat cluster. ADMIXTURE assigned the focal individual almost entirely to the Tsushima leopard cat ancestry component, and neither *f*3 nor *f*4 admixture statistics supported recent domestic cat introgression. Mitochondrial DNA analysis showed that the focal individual shared a haplotype with the Tsushima leopard cats and was distinct from the domestic cat clade. The same genetic pattern was observed across the sampled population, with no evidence of substantial recent hybridization in the focal individual or nearby wildcats. To the best of our knowledge, this is the first report of a short-tailed individual in the genus *Prionailurus*. These findings highlight the value of genome-wide evaluation in resolving suspected hybrid cases in endangered wild felids.

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1. Introduction

Hybridization with domestic animals is a major concern in conservation genetics because it may erode the evolutionary distinctiveness of small and isolated wild populations (Rhymer and Simberloff, 1996). This concern also extends to wild felids, where suspected hybrid individuals may trigger difficult management decisions in rescue, monitoring, and breeding contexts. In practice, such suspicion often arises from unusual phenotypes, although morphology alone cannot reliably distinguish hybrid ancestry from within-population variations or rare developmental traits (Nussberger et al., 2013).

The Tsushima leopard cat (*Prionailurus bengalensis euptilurus*) is an insular population of the leopard cat restricted to the Tsushima Island, Japan. It is listed as Critically Endangered in species on the Japanese Ministry of the Environment Red List 2020 (Ministry of the Environment, Japan, 2020). Molecular phylogenetic analyses have placed the Tsushima leopard cat within *P. b. euptilurus*, the Amur leopard cat subspecies distributed across the Russian Far East, the Korean Peninsula, and neighboring Japanese islands (Masuda and Yoshida, 1995; Tamada et al., 2008). The population is currently recognized as a regional isolate of this northern lineage (Kitchener et al., 2017). Located between Kyushu and the Korean Peninsula, Tsushima has a land area of approximately 700 km^2. However, the Tsushima leopard cat persists as a small and vulnerable population (Izawa et al., 2009); the most recent population survey estimated approximately 90–100 individuals on Kamijima, the northern main island of Tsushima, and confirmed at least 17 individuals on Shimojima, where the species has recently expanded its distribution (Ministry of the Environment, Japan, 2026). Recent whole-genome analyses have also revealed a prolonged demographic decline, markedly reduced heterozygosity, and elevated genomic inbreeding relative to continental leopard cat populations (Ito et al., 2025; Ito and Inoue-Murayama, 2019). Owing to its restricted range, small population size, and continuous exposure to anthropogenic pressures, maintaining the genetic integrity of this population is a central conservation priority.

Domestic cats (*Felis catus*), including free-ranging and feral individuals, occur on the Tsushima Island. They have long been associated with conservation risks, including interspecific pathogen transmission (Makundi et al., 2018; Nishimura et al., 1999) and broader ecological impact on the island's wildlife (Izawa et al., 2009). Potential introgression by domestic cats is a biologically plausible concern on the Island (Izawa and Doi, 1991; Tamada et al., 2005). At least three Tsushima leopard cats with apparent tail abnormalities were identified through field surveys conducted across Island during 2024–2025, of which only one was captured for clinical and genomic examination. (Supplementary Fig. 1). The occurrence of tail abnormalities in multiple individuals makes independent acquired causes, such as traumatic injuries, a less parsimonious explanation for all cases and raises the possibility of a shared heritable or developmental basis. To our knowledge, this phenotype has not been documented in Tsushima leopard cats or other leopard cat populations, although shortened tails are relatively common in domestic cats (Buckingham et al., 2013). The biological capacity for hybridization between *Prionailurus bengalensis* and domestic cats is evidenced by the presence of the Bengal cat breed, in which at least some hybrid lineages are reproductively viable and some individuals retain phenotypic characteristics resembling those of the leopard cat (Menotti-Raymond et al., 1999; Yosida, 1982). Therefore, the coexistence of wild and domestic cats within the same island landscape makes the hybrid origin a reasonable hypothesis that warrants direct genomic evaluation.

In felids, tail abnormalities have been reported in a range of caudal phenotypes, including taillessness, that is, complete absence of the tail, shortened tails with reduced tail length associated with fewer caudal vertebrae, and kinked tails, that is, angular tail deformities resulting from vertebral malformations. In addition to those reported in domestic cats, tail abnormalities have been documented in several wild felids, including cheetahs (*Acinonyx jubatus*) (Schmidt-Küntzel et al., 2018), European wildcats (*Felis silvestris silvestris*) (Lioy et al., 2022), Asiatic Golden Cat (*Catopuma temminckii*) (Wang et al., 2022) and Florida panthers (*Puma concolor coryi)* (Onorato et al., 2024). In Florida panther, kinked tails and other morphological abnormalities were common in the highly inbred population and declined following genetic rescue (Onorato et al., 2024; Roelke et al., 1993). In domestic cats, short- and kinked-tail phenotypes result from mutations at genetically independent loci such as *TBXT* and *HES7* (Buckingham et al., 2013; Lyons et al., 2016; Xu et al., 2016). The repeated occurrence of short-tailed phenotypes through separate mutational events in domestic cats illustrates that the disruption of axial developmental pathways can recurrently produce this morphology (Xu et al., 2016). Whether analogous mutations occur spontaneously in wild felids remains unclear. However, the underlying developmental pathways are conserved across mammals, and naturally occurring caudal anomalies have been reported in multiple vertebrate taxa (Stott et al., 1993). In addition to heritable causes, tail shortening may result from a traumatic injury followed by healing.

As phenotypic abnormalities alone cannot reliably distinguish hybrid ancestry from alternative developmental, genetic, or traumatic causes, population-level genomic evaluation is essential for interpreting unusual individuals. This approach is particularly important in conservation settings, where accurate classification can directly inform management decisions. However, population-level genomic evaluations that place phenotypically unusual individuals in the context of nearby conspecifics remain limited, leaving it unclear whether suspected introgression is restricted to a focal individual or reflects broader gene flow within the local population. In this study, we used nuclear genome-wide and mitochondrial DNA data to evaluate whether a short-tailed Tsushima leopard cat (STI) showed evidence of recent domestic cat introgression and whether genomic signals of hybridization were detectable more broadly in nearby conspecifics sampled from the same local context. We further investigated whether the genomic data could offer insights into tail abnormalities by screening candidate variants associated with abnormal tail morphology.

2. Materials and methods

2.1. Study design and samples

To investigate whether Tsushima leopard cats, including a short-tailed individual, showed evidence of recent domestic cat

introgression, we analyzed genome-wide single nucleotide polymorphism (SNP) data from 25 individuals assigned to 4 groups. This included 15 Tsushima leopard cats that were used as a comparison set, 1 short-tailed Tsushima leopard cat of particular phenotypic interest (Fig. 1A), 5 domestic cats from Tsushima Island (DOMs), and 4 leopard cats sampled in Amur (ALCs).

An STI was captured as part of a survey capture program, with permission from the Ministry of Environment, Japan. Several additional STIs were recognized in the camera-trap images but were not captured and were excluded from further examination. After capture, an external examination and radiography were performed. Although the typical tail length of male Tsushima leopard cats is 245.0 ± 20.14 mm (Ohdachi et al., 2009), the STI showed a shortened tail measuring 104 mm—approximately half the reported typical length. Radiographic examination suggested that the distal caudal vertebrae were not clearly delineated, with a possible lesion or damage around the 9th–10th caudal vertebra—approximately half of that observed in a long-tailed Tsushima leopard cat examined for comparison, in which 19 caudal vertebrae were identified, and lower than the number typically reported for domestic cats (approximately 20–24) (Pollard et al., 2015) (Fig. 1B–D). As the animal was intended for release, the coat was not clipped and the skin was not examined in detail. However, no obvious external signs of trauma were observed. Radiography suggested a possible previous fracture in a part of the caudal vertebrae, and the cause of the shortened tail could not be determined from clinical findings alone. No obvious differences in coat color or other morphological characteristics were identified relative to typical Tsushima leopards.

The other 15 Tsushima leopard cat samples (TLCs) comprised individuals obtained through rescue or conservation management activities, including animals recovered after road traffic accidents. All the cats were phenotypically long-tailed, although radiographic confirmation was available for only one cat examined for clinical purposes (Table S1). DOM samples obtained from local cats on the Tsushima Island were presented to a veterinary hospital for routine neutering. Gonadal tissues from both male and female cats that would otherwise have been discarded after surgery were collected and used in this study. Tail abnormalities were observed in three cats (Table S1). ALC genomes were obtained from publicly available genomic databases (Table S1) and included as an external wild reference representing the broader *P. bengalensis* lineage.

All the tissue and blood samples were obtained opportunistically during clinical, rescue, necropsy, and conservation management procedures. These samples were collected between 2020 and 2025 by the Ministry of the Environment as part of legally authorized rescue and conservation management activities conducted independently of the present study. No animals were handled specifically for this study. The secondary use of archived samples in the present study was reviewed and approved by the Ethics Committee of Anicom Specialty Medical Institute, Inc. (ID: 2022–03) in 2022, before the samples were used for research.

2.2. Genomic dataset preparation

Genomic DNA was extracted from each tissue or blood sample using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Sequencing libraries were prepared using the Illumina DNA PCR-Free Prep (Illumina, San Diego, CA, USA) or NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) and sequenced

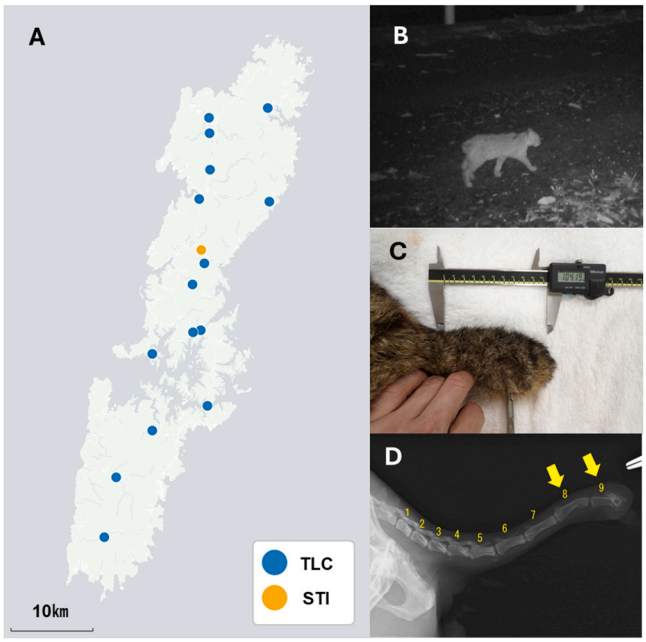

Fig. 1. Sampling locations of the analyzed Tsushima leopard cats and morphological characterization of the focal short-tailed individual (STI). (A) Sampling locations of Tsushima leopard cats used in this study. Blue circles indicate reference Tsushima leopard cats (TLC), and the orange circle indicates STI. (B) Camera-trap image of the focal individual. (C) External appearance of the shortened tail, with tail length measured using calipers. (D) Radiograph of the tail of the focal individual. Yellow arrows indicate the distal caudal vertebrae associated with the shortened-tail phenotype.

on a NovaSeq 6000 or NovaSeq X Plus platform (Illumina). Raw base-call files were converted to FASTQ files using bcl2fastq2 according to the manufacturer's protocol.

All the genomic data, including public ALC datasets, were used for subsequent processing. Low-quality bases, adapter sequences, and short reads were removed using fastp v0.22.0. Reads were then mapped to the Leopard cat reference genome assembly (Bredemeyer et al., 2021) (Fcat_Pben_1.1_paternal_pri; GCF_016509475.1) using DRAGEN v4.0.3 (Illumina). Variant calling was performed in the genomic variant call format (gVCF) mode for each sample. All the gVCF files were joint-genotyped using the DRAGEN joint genotyper. The default DRAGEN hard-filtering settings were applied to small variants. The resulting joint VCF file was used for downstream population genetic analysis.

The merged VCF was processed with bcftools v1.23 through the following sequential filtering steps to produce a high-quality biallelic SNP dataset. Multi-allelic sites were first decomposed and left-aligned against the reference genome using the bcftools norm (-m -any, followed by -f). Non-variant and artifactual records were excluded, including sites with NON_REF or * ALT alleles, and sites annotated using the PloidyConflict or RMxNRepeatRegion flag. Individual genotype calls were masked as missing (. /.) when the read depth (DP) was < 8 or the genotype quality (GQ) was < 20, using the bcftools setGT plugin. To reduce the influence of repetitive or multi-mapping regions, we retained only the sites that overlapped uniquely mappable genomic regions with a mappability score of 1 (GCF_016509475.1_mappability.bedgraph.mp1). The dataset was then restricted to biallelic SNPs using bcftools view (-m2 -M2 -v snps). Sites carrying the DRAGEN hard-filter flag SNPFILT (DP ≤ 5) were removed. We further excluded sites that met any of the following hard-filtering criteria based on GATK recommendations: QD < 2.0, MQ < 40.0, FS > 60.0, SOR > 3.0, MQRankSum < -12.5, or ReadPosRankSum < -8.0. Sites with a missing genotype rate $> 10\%$ across all 25 individuals (F_MISSING > 0.10) were also removed. The final filtered dataset consisted of 3879,588 biallelic SNPs across 25 individuals.

2.3. SNP filtering for population-genetic analyses

Population genetic analyses were conducted using a filtered SNP dataset derived from the final high-quality joint VCF. To reduce marker redundancy before principal component analysis (PCA) and ADMIXTURE (Alexander et al., 2009), linkage disequilibrium pruning was performed in PLINK (v1.90b6.21) (Chang et al., 2015) using a sliding window of 50 SNPs, a step size of 10 SNPs, and an r2 threshold of 0.1. After linkage disequilibrium (LD) pruning, 112,184 SNPs were retained for PCA, kinship, ADMIXTURE analyses, and admixture statistics. The full high-quality SNP dataset was retained for admixture statistics.

2.4. PCA, kinship, and genomic diversity

PCA was conducted using the LD-pruned SNP dataset to visualize the genomic relationships among TLCs, DOMs, and ALCs. PCA was performed to assess whether the STI occupied an intermediate genomic position between DOMs and TLCs using PLINK2 (v2.00a5.10LM).

To further characterize the genomic position of the STI within the sampled TLCs, we examined pairwise relatedness and genomic inbreeding. Pairwise kinship was estimated using the KING robust relationship estimate (Manichaikul et al., 2010), the make-king option was implemented in PLINK2, and was visualized as a kinship matrix.

Genomic inbreeding is summarized as F_{ROH}, calculated as the proportion of the autosomal genome contained within runs of homozygosity (ROH). ROH were identified using PLINK software. These analyses were treated as descriptive complements to PCA and ADMIXTURE rather than direct tests of hybridization inference using ADMIXtools and searching for potential candidate variants.

2.5. Mitochondrial DNA maximum likelihood phylogenetic analysis

To evaluate the phylogenetic placement of the STI within the mitochondrial genealogy, we performed a maximum-likelihood (ML) analysis using 79 biallelic SNPs identified in the mitochondrial genome contig NC_028301.1 (16,701 bp; *Prionailurus bengalensis* reference sequence included in GCF_016509475.1). For each individual, a mitochondrial haplotype sequence consisting of these 79 sites was extracted from the quality-controlled VCF and heterozygous calls, if any, were treated as missing data (N). Twenty-five analyses included all twenty-five individuals. Model selection and ML tree inference were performed using IQ-TREE v3.0.1 with the -m TEST option. Branch support was assessed using 1000 ultrafast bootstrap replicates. The best-fitting substitution model was HKY + F.

2.6. ADMIXTURE analysis

ADMIXTURE v1.3.1 was used to determine whether the STI occupied an intermediate ancestry position between DOMs and TLCs and whether a comparable signal was present in additional TLCs from the same local context. Individual ancestry proportions were estimated using ADMIXTURE with a thinned SNP dataset. The values of K from 1 to 6 were evaluated, and the best-supported partition was identified using cross-validation error. Unsupervised and supervised analyses were also conducted. In the supervised analysis, DOMs and TLCs were specified as reference groups to test whether the STI showed any detectable DOM-associated ancestry components.

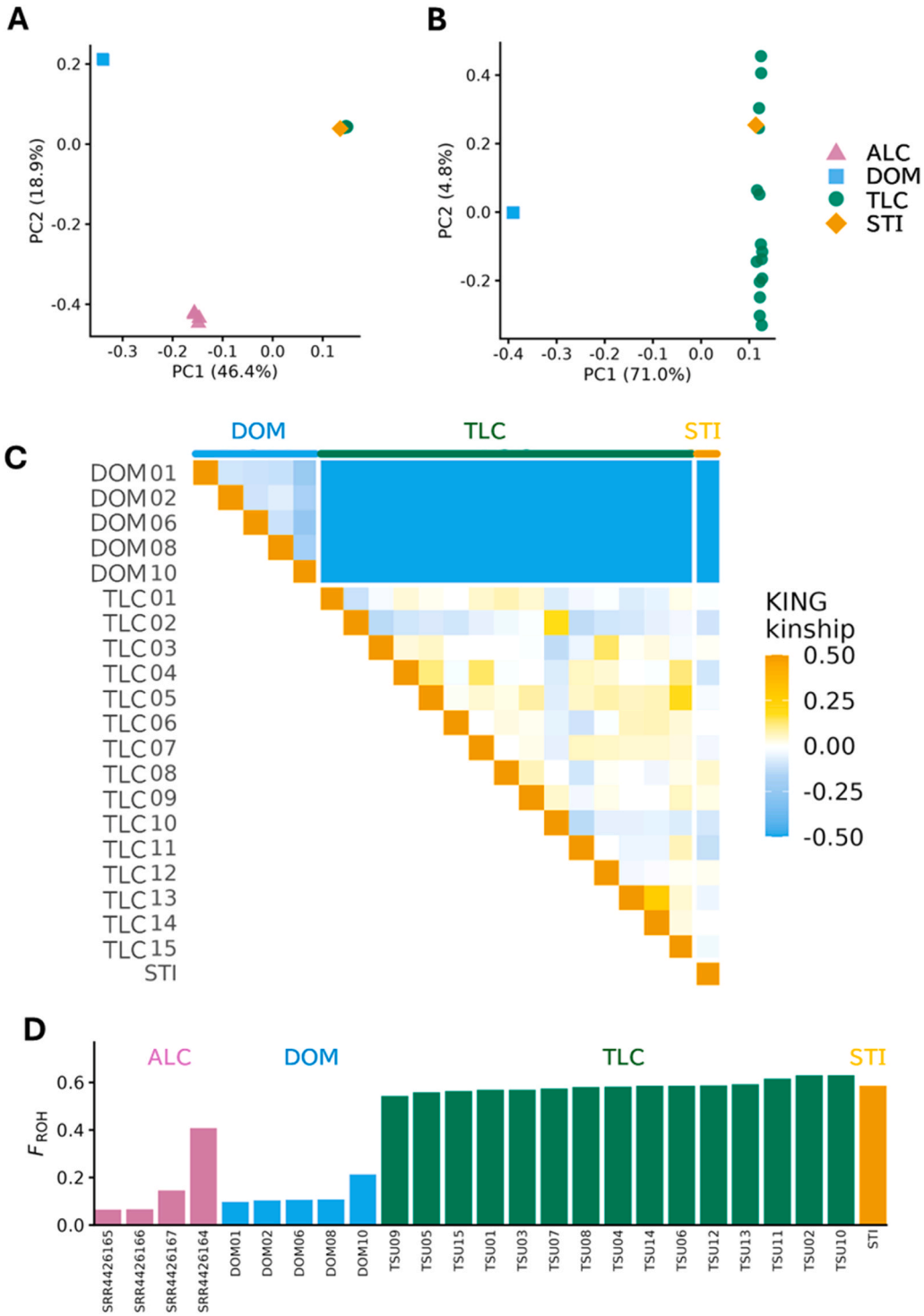

Fig. 2. Principal component analysis, kinship, and genomic inbreeding of Tsushima leopard cats (TLC), domestic cats from the Tsushima Island (DOM), and Amur leopard cats (ALC). (A) Principal component analysis including ALCs, DOMs, a comparison set (TLCs), and the short-tailed individual (STI). (B) Principal component analysis excluding ALC. (C) Pairwise kinship matrix among sampled individuals. (D) Genomic inbreeding estimate (F_{ROH}).

2.7. Admixture statistics

To test for admixture in the STI, we calculated f -based statistics using ADMIXtools v8.0.2. The three-population statistic was formulated as f_3 (DOM; ALC, X), where X represented either the STI or other TLC. This statistic was used to compare genome-wide allele-sharing patterns between ALC and each X individual relative to DOMs, and to assess whether the STI fell outside the range of variation observed among the reference TLCs.

We further calculated the individual-based f_4 statistics, f_4 (DOM, ALC; X, Y), where X represents the reference. We also calculated f_4 statistics using the two reference TLCs with the highest and lowest f_3 (DOM, ALC, and X) estimates as anchors. We calculated f_4 (DOM, ALC; TLC_high, Y) and f_4 (DOM, ALC; TLC_low, Y), where Y represents either the STI or the reference TLC. This analysis was used to assess whether the STI fell within the background variation of DOM–ALC allele-sharing patterns observed among the reference TLCs. Statistical support was evaluated using block-jackknife Z-scores, and results with $|Z| < 3$ were considered insignificant.

2.8. Searching for potential candidate variants of short-tail phenotype

From the set of QC-passed callable variants, we screened for variants in the candidate short-tail genes *HES7* and *TBXT*. Variant annotation was performed with reference to the Asian leopard cat assembly Fcat_Pben_1.1_paternal_pri (GCF_016509475.1). Predicted functional consequences were assessed using SnpEff 5.4c. We prioritized variants annotated as HIGH or MODERATE impact, with a focus on putative loss-of-function variants, such as nonsense and frameshift mutations.

3. Results

3.1. STI clusters with TLCs in PCA and shows comparable genomic inbreeding

We analyzed genome-wide data from 25 individuals, including 15 TLCs, 1 STI, 5 DOMs, and 4 ALCs. Whole-genome sequencing generated mean coverages of 9.36–10.46 × for ALCs, 8.84–15.14 × for DOMs, 12.86–27.64 × for TLCs, and 36.06 × for the STI (Table S1). After variant filtering, the final high-quality dataset contained 3879,588 SNPs.

PCA separated DOMs, TLCs, and ALCs. In PCA, including ALCs (Fig. 2A), DOMs and TLCs were separated along PC1. Meanwhile, ALCs formed a distinct cluster. In PCA, excluding ALCs (Fig. 2B), DOMs and TLCs remained separated. In both analyses, the STI fell within the TLC cluster, rather than in an intermediate position between TLCs and DOMs. Its placement was consistent with the variation observed among other TLCs, indicating no genome-wide shift toward DOM ancestry.

Similarly, the pairwise kinship analysis showed that the STI did not deviate from the TLC comparison set (Fig. 2C). The kinship coefficients between STI and DOMs were low and comparable to those observed in other TLCs and DOMs. No close relatives of the STI were identified among the TLC individuals. Genomic inbreeding, measured as F_{ROH}, was higher in TLCs (mean = 0.585, range = 0.544–0.636) than in DOM (mean = 0.126, range = 0.097–0.213) and ALC (mean = 0.172, range = 0.065–0.409) (Fig. 2D). The STI showed a similar level of genomic inbreeding ($F_{ROH} = 0.587$), which fell well within the range observed in the TLC comparison set.

3.2. Mitochondrial DNA-based phylogeny places the STI in the TLC clade

Maximum likelihood phylogenetic analysis of 79 mitochondrial SNPs revealed 3 major groups (Fig. 3A). DOMs formed a strongly supported monophyletic clade distinct from all the TLCs and ALCs. All TLCs, including the STI, were placed within a single clade and shared the same mitochondrial haplotype. The ALCs formed a sister group to the TLC clade, which is consistent with their closer phylogenetic affinity to *Prionailurus bengalensis* than to DOMs. The placement of STI within the TLC clade rather than within the DOM clade did not support DOM matrilineal ancestry in this individual.

3.3. ADMIXTURE supports $K = 2$ and assigns the STI almost entirely to the TLC component

A dataset of 112,184 LD-pruned SNPs was used for ADMIXTURE analysis. Cross-validation supported $K = 2$ as the best-fit partition of the dataset (Fig. 3B). Under this partition, the DOMs and TLCs formed clearly differentiated ancestry clusters. The STI was assigned almost entirely to the ancestry component of the TLC in both unsupervised and supervised analyses (Fig. 3C). In the supervised analysis, the estimated DOM ancestry in the STI group was less than 0.01% (Fig. 3D). The remaining TLCs showed the same overall pattern, with no detectable DOM-associated ancestry across the sampled local populations.

3.4. Admixture statistics do not support recent DOM introgression in STI

Next, we tested whether STI showed a formal genomic signature of the admixture. The f_3 statistic, formulated as f_3(DOM; ALC, X), yielded a positive value for all tested individuals, including the STI ($f_3 = 0.803$) and all 15 TLCs (range = 0.729–1.026; Fig. 3E); TLC09 and TLC01 showed the lowest and highest f_3 values, respectively. The f_3 statistics were consistently positive, and the STI fell within the range observed in the reference TLC. Although this configuration was not a formal admixture f_3 test with the STI as the target, the results provided no evidence that the STI showed an exceptional genome-wide affinity pattern consistent with recent DOM introgression.

To assess whether STI showed an anomalous affinity to DOMs relative to individual TLCs, we calculated f_4 (DOM, ALC; TLC_ref, X)

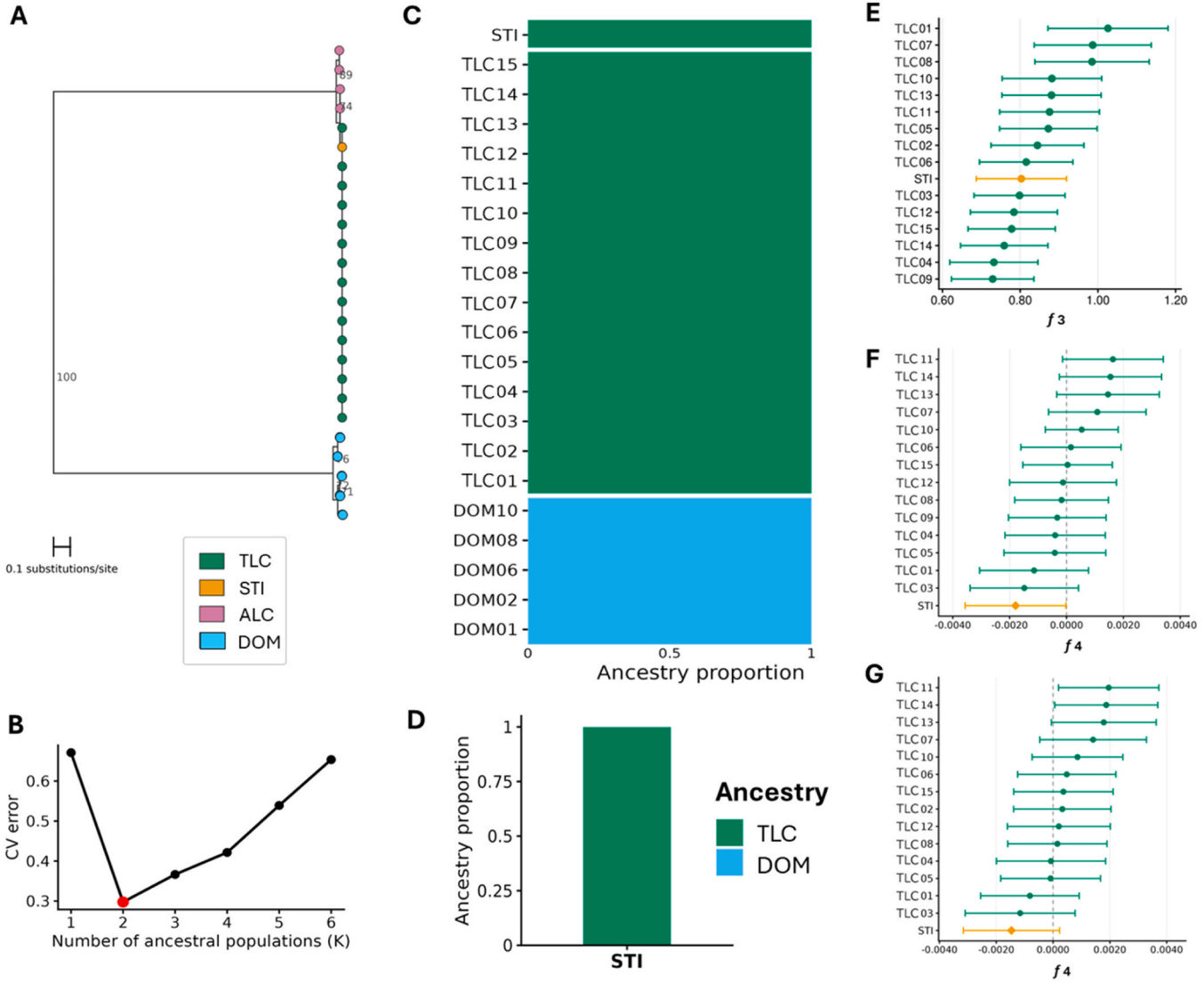

Fig. 3. Mitochondrial and nuclear genomic analyses to identify admixture. (A). Maximum-likelihood phylogeny based on 79 mitochondrial single nucleotide polymorphisms (SNPs). (B) ADMIXTURE cross-validation error across tested K values, showing $K = 2$ as the best-supported partition of the dataset. (C) Unsupervised ADMIXTURE bar plot at $K = 2$ for domestic cats (DOMs), Tsushima leopard cats (TLCs), and the focal short-tailed individual (STI). (D) Supervised ADMIXTURE assignment of STI, using DOMs and TLCs as reference populations. (E) f 3 statistics, f 3(DOM; ALC, X), showing the position of STI relative to TLCs. (F) f 4 statistics, f 4(DOM, ALC; TLC02, TLC_i). (G) f 4 statistics, f 4(DOM, ALC; TLC09, TLC_i). Error bars indicate plus or minus two standard errors. ALC, Amur leopard cats.

using TLC02 and TLC09 as fixed references, which showed the highest and lowest f_3 values, respectively (Fig. 3 F–G). For f_4 (DOM, ALC; TLC02, X), the only comparison reaching $|Z| \geq 2$ was X = STI ($f_4 = -0.00179$, $Z = -2.02$). The negative value indicated that STI showed slightly less DOM-related allele sharing than TLC01 along the DOM–ALC allele-frequency contrast, rather than a greater affinity to DOMs. For f_4 (DOM, ALC; TLC09, X), the STI was not significant ($f_4 = -0.00147$, $Z = -1.73$). These results suggest that STI is not an outlier with respect to DOM–ALC allele-sharing patterns and that comparable or stronger heterogeneity is present among the reference TLCs.

3.5. *No candidate variants with predicted functional impact were detected in TBXT or HES7*

Within the *TBXT* gene body, four variants were identified in the STI. None was predicted to have a HIGH or MODERATE impact; all were classified as MODIFIER variants and annotated as intronic or 3′ UTR variants, and were therefore not expected to alter the protein sequence (Table S2). In *HES7*, no variants were detected in the quality- filtered variant. These results provide no evidence that STI phenotype in STI can be explained by protein-altering variants of *TBXT* or *HES7*, including previously reported high-impact variants associated with STI phenotypes in DOMs.

4. Discussion

Our mitochondrial and nuclear genome-wide analyses consistently failed to support recent DOM introgression in the sampled TLCs, including the STI. Across all the analyses, the focal individual clustered with TLCs rather than with DOMs, showing no detectable DOM-associated ancestry in PCA and ADMIXTURE, and was not supported as admixed by either f -based test. These concordant results indicate that the unusual STI phenotype is not accompanied by a genome-wide signature of recent hybridization with DOMs. Hybridization with DOM in wild felids is a recognized conservation problem in wild felid systems, such as European wildcats, where introgression can be an extensive and complicated management decision (Howard-McCombe et al., 2023). Therefore, concern over a possible hybrid origin for a phenotypically unusual TLC is reasonable. However, in the present case, the STI did not correspond to a detectable DOM ancestry. This distinction is particularly consequential for the TLC, a small and isolated population under active conservation management, in which incorrect phenotype-based inferences can disproportionately affect management decisions.

Tail abnormalities have been reported in several felid, including Florida panthers and European wild cats, for which a heritable basis has been suggested (Lioy et al., 2022). To the best of our knowledge, this is the first report of a short-tailed individual in the genus *Prionailurus* although the genetic basis and heritability of the phenotype remain unknown.

Screening of *TBXT* and *HES7*, the two loci known to underlie short- and kinked-tail phenotypes in domestic cats (Buckingham et al., 2013; Lyons et al., 2016; Xu et al., 2016), identified no candidate variants with a predicted functional impact. These findings indicate that the phenotype observed in the STI is unlikely to be attributable to the previously characterized mutations associated with tail abnormalities in domestic cats at these loci. In Florida panthers, kinked tails have been treated as a phenotypic correlate of inbreeding depression and their frequency declines following genetic rescue (Aguilar-Gómez et al., 2025; Onorato et al., 2024). However, the causative variant or variants remain unidentified. Although the Tsushima leopard cat population is characterized by elevated genomic inbreeding, the STI did not exhibit a higher level of genomic inbreeding than the other individuals examined in this study. Thus, our results do not support unusually high individual inbreeding as a specific explanation for the phenotype of the STI, although they cannot exclude an effect of deleterious variants segregating within the highly inbred population. Developmental abnormalities and traumatic injury also cannot be excluded based on a single short-tailed individual, and the present study provides no direct evidence that the phenotype is heritable. Therefore, the underlying cause of the short-tail phenotype remains unresolved.

This study has some limitations. Although our analyses do not support recent DOM introgression, they cannot exclude older or highly localized introgression at levels below the detection threshold of the present dataset. The f-statistic framework and ADMIXTURE are well suited for detecting introgression from recent generations but have limited power to identify ancient or low-level admixtures, particularly in small datasets. Given the small sample size of the present study ($n = 15$ TLCs), caution is warranted when interpreting the absence of a signal as definitive evidence against all forms of introgression. Although sampling was conducted broadly across the Tsushima Island, several additional individuals with shortened or otherwise abnormal tails were identified in camera-trap photographs, but could not be captured and were therefore unavailable for clinical or genetic examination. The occurrence of multiple individuals with apparent tail abnormalities suggests that such phenotypes may not be restricted to a single case. However, qualitative inspection of the available photographs also suggested possible variation in the degree of tail shortening among individuals (Supplementary Fig. 1). As differences in body posture, viewing angle, image quality, and the absence of a standardized scale precluded reliable morphological comparison, it remains unclear whether these observations represent a single recurring phenotype or multiple forms of tail abnormality arising from different causes. Clarifying whether these phenotypes share a common heritable basis will require detailed phenotypic characterization and genetic sampling of additional short-tailed individuals, which was beyond the scope of the present study.

Nevertheless, the present study demonstrated the value of a genome-wide evaluation of suspected hybrid individuals in endangered wild felids. By integrating a phenotypically unusual individual with neighboring sampled wildcats and relevant comparative groups, this approach provides a stronger basis for conservation decision-making than phenotype-based inference alone. In the present case, genomic evidence did not support substantial recent DOM introgression in STI or the sampled local population. This emphasizes the importance of genetic assessment when unusual phenotypes raise concerns about hybrid ancestry. To the best of our knowledge, this study provides the first clinical and genomic characterization of an short-tailed individual in the genus *Prionailurus*. This adds to the known phenotypic diversity of this group. As tail abnormalities have been associated with inbreeding in other felids, their occurrence

also warrants attention in small, isolated populations. These findings highlight the need to document morphological anomalies in endangered wild felid populations.

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CRediT authorship contribution statement

Yuki Matsumoto: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Takuma Kaito:** Writing – review & editing, Resources, Funding acquisition. **Koki Taniguchi:** Resources, Funding acquisition. **Daijiro Hata:** Resources. **Ryotaro Kaneko:** Resources. **Yui Habe:** Resources. Keisuke Matsushita: Resources. **Noriyoshi Akiyama:** Methodology, Investigation, Data curation. **Yushi Koshida:** Resources. **Takae Shimizu-Matsumoto:** Validation, Methodology. **Miho Inoue-Murayama:** Writing – review & editing, Supervision, Funding acquisition. **Hideyuki Ito:** Writing – review & editing, Supervision, Resources, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT and Claude to assist with reading and summarizing relevant literature and improving the clarity of the manuscript text. After using these tools/services, the authors reviewed and edited the content as needed, and they take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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Data archiving

Raw sequence reads were deposited in the DDBJ Sequence Read Archive under the accession number BioProject PRJDB42204.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2026.e04365.

Data Availability

I have shared the IDs to link the data.

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