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Leptin and leptin receptor genes of domestic animals in the context of phylogeny, polymorphism and molecular genotyping

A. Saienko*, T. Buslyk*,**, D. Dubinin*, O. Tsereniuk*, S. Korinnyi*, V. Teniaiev*, M. Peka*, ***

*Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine, Poltava, Ukraine
**Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine
***V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

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Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine, Shvedska Mohyla St., 1, Poltava, 36009, Ukraine. Tel.: +38-063-395-55-31. E-mail: pigbreeding@ukr.net

Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Vasyl Stus St., 38, Lviv, 79034, Ukraine. Tel.: +380-32-260-07-95. E-mail: inenbiol@mail.lviv.ua

V. N. Karazin Kharkiv National University, Svobody Sq., 4, Kharkiv, 61022, Ukraine. Tel.: +38-057-707-55-00. E-mail: pekapoltava@gmail.com

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The leptin signaling system, involving *LEP* and *LEPR* genes, plays a central role in the regulation of energy balance, metabolism, reproduction, immunity, thermoregulation, and production traits in mammals. Despite their functional importance and general evolutionary conservation, the extent and distribution of genetic variation within these genes across domestic animal species remain insufficiently characterized. In this study, we integrated comparative phylogenetic analysis with variant annotation and development of molecular tools for genotyping *LEP* and *LEPR* polymorphisms. Phylogenetic trees reconstructed from coding sequences of both genes showed consistent clustering of closely related taxa, indicating the presence of a measurable phylogenetic signal in these loci. Analysis of Ensembl Variation data revealed pronounced differences in the number and distribution of variants among domestic animal species and humans. Across all analyzed species, intronic variants were predominant, while coding variants represented a smaller fraction of total variation. Among livestock species, cattle, sheep, and pigs harbored comparatively higher numbers of annotated missense and synonymous substitutions. A detailed structural characterization of porcine *LEP* and *LEPR* genes was performed, including exon-intron organization and transcript annotation, along with mapping of coding polymorphisms across functional gene regions. Based on these data, PCR-based genotyping systems were developed for all coding exons of both genes, including primer sets and restriction enzymes selected for PCR-RFLP analysis of specific allelic variants. The proposed assays enable cost-effective screening of both established and newly annotated polymorphisms and provide a practical framework for molecular genetic studies in pig breeding populations. The developed methodological platform may support future association studies aimed at identifying functionally relevant variants and integrating them into marker-assisted selection programs for economically important traits.

Keywords: *LEP* gene; *LEPR* gene; leptin signaling; genetic variant; polymorphism; phylogenetics; mammals; domestic animals; marker-assisted selection.

Introduction

The functioning of adipose tissue and the regulation of energy homeostasis are of fundamental importance for the evolutionary adaptation and survival of mammals (Kajimura, 2015; Mauvais-Jarvis, 2024). Secreted predominantly by adipocytes, the peptide hormone leptin acts as a multifunctional adipokine that regulates appetite, metabolism, immune response, and reproductive function (La Cava & Matarese, 2004; Deck et al., 2017; Velazquez, 2025). Leptin is encoded by the *LEP* gene (also known as the obesity gene, *Ob*) and exerts its biological effects through interaction with a specific transmembrane leptin receptor encoded by the *LEPR* gene (also known as *Ob-R*). Leptin is also synthesized locally in various organs (including the lungs, blubber, and bone marrow), where it triggers JAK-STAT3 and PI3K signaling cascades (Hammond et al., 2005). Acting similarly to cytokines, it participates in the fine-tuning of innate and adaptive immune mechanisms by enhancing lymphocyte proliferation, stimulating macrophages, and promoting effector chemokine secretion (Huertas & Bhattacharya, 2025). Recent studies highlight the important contribution of leptin to adaptive thermogenesis, justifying its classification as a novel thermolipokine (Kaiyala et al., 2016; Trayhurn & Arch, 2020; Genchi et al., 2021).

Leptin regulatory mechanisms and the primary structure of the *LEP* and *LEPR* genes exhibit a high level of conservation among a wide range of mammals (Prokop et al., 2012). However, despite this general conservation, accumulating data indicate notable interspecific and intraspecific variations in the sequences of these genes (Gaucher et al., 2003, Yang et al., 2008, Yu et al., 2011; Bakshi & Rai, 2022). Contemporary studies show that the genes of the leptin system have undergone differential evolutionary pressure and positive selection across various vertebrate phylogenetic lineages, which is associated with adaptation to specific ecological niches, dietary types, or thermoregulation. A high velocity of sequence evolution in leptin loci has been explicitly demonstrated in primates (Gaucher et al., 2003), cetaceans and pinnipeds (Hammond et al., 2005; Yu et al., 2011), pikas (Yang et al., 2008), the spotted snakehead (Bakshi & Rai, 2022), and the Antarctic toothfish (Wang et al., 2021).

The study of animals across diverse temperature zones using stepwise multiple regression revealed that cold may act as the primary environmental driver stimulating the adaptive evolution of leptin (Yang et al., 2008). Such temperature-driven adaptive evolution of leptin plays an important role in ecological adaptation to extreme environments, as evidenced by the emergence of unique functional motifs and amino acid variations that assist nonhibernating mammals in managing the significant energetic demands of cold habitats.

Certain cytokines and their receptors evolve in a coordinated manner, consistent with the ligand-receptor coevolution hypothesis. Consequently, it is expected that *LEPR* genes in vertebrates also exhibit adaptive evolution similar to *LEP*. In alignment with this expectation, the occurrence of both pervasive and episodic diversifying selection across leptin and extra- and intracellular domains of the leptin receptor substantiates the coevolutionary dynamics of this ligand-receptor pair in teleost lineages (Bakshi & Rai, 2022). However, in contrast to previous findings, the study by Yu et al. (2011) has not revealed positive selection in the *LEPR* gene in cetaceans and pinnipeds. This supports the suggestion that the biological role of leptin

varies in a species-specific manner. Population and phylogenetic studies on the structure of the leptin and leptin receptor genes, alongside the analysis of their associations with phenotypic traits, have also been conducted in various domestic animal species, including cattle (Konfortov et al., 1999; Kuswati et al., 2022, Wang et al., 2022), sheep (Girmay et al., 2022; Macé et al., 2022), buffalo (Silva et al., 2021), pigs (Pérez-Montarelo et al., 2015; Balatsky et al., 2022; Suárez-Mesa et al., 2024), and goats (Senturk et al., 2024). The LEP gene in livestock exhibits considerable polymorphism, with multiple studies confirming its impact on feed intake (Marie et al., 2001), growth performance (Kuswati et al., 2025), carcass traits (Kuswati et al., 2022), and meat quality (Wang et al., 2022, Suárez-Mesa et al., 2024). The identification of candidate and causal mutations within the *LEP* and *LEPR* genes, which coordinate energy reserve management, provides new perspectives for marker-assisted selection in farm animals to enhance their evolutionary and production adaptation to climatic and nutritional challenges.

Thus, the variability and evolutionary dynamics of the *LEP* and *LEPR* genes represent one of important directions targeted by both natural and artificial selection. In view of this, the analysis of genetic polymorphism and phylogenetic relationships based on the nucleotide sequences of these genes allows for a deeper understanding of the mechanisms of mammalian macro- and microevolution, as well as the assessment of the functional potential of various allelic variants.

Therefore, the objective of this study was to conduct a phylogenetic analysis based on the *LEP* and *LEPR* genes in mammals, to investigate polymorphisms in these genes across several domestic animal species, and to develop approaches for molecular genotyping based on these genes.

Materials and methods

To investigate the variability of the *LEP* and *LEPR* genes and to perform phylogenetic analysis, the Ensembl Genome Browser release 115 was used (Hunt et al., 2018; Dyer et al., 2025). Gene variation was analyzed in the following species: human (*LEP*: ENSG 00000174697, *LEPR*: ENSG00000116678), pig (*LEP*: ENSSSCG 00000040464, *LEPR*: ENSSSCG00000025188), cow (*LEP*: ENSBTAG00000014911, *LEPR*: ENSBTAG00000005910), sheep (*LEP*: ENSOARG00020045130, *LEPR*: ENSOARG 00020092532), goat (*LEP*: ENSCHIG00000019616, *LEPR*: ENSCHIG 00000023226), horse (*LEP*: ENSECAG00000030283, *LEPR*: ENSECAG00000013863), dog (*LEP*: ENSCAFG00845025165, *LEPR*: ENSCAFG00845029124), and cat (*LEP*: ENSFCTG 00005007327, *LEPR*: ENSFCTG00005017591). Variants in the *LEP* and *LEPR* genes were classified according to their predicted consequences for transcripts and proteins (McLaren et al., 2016). The following categories were analyzed separately: 5′-UTR, 3′-UTR, missense, synonymous, start-lost, stop-lost, stop-gained, stop-retained, frameshift, in-frame insertion/deletion, and intronic variants.

Phylogenetic analysis was performed using coding sequences of the *LEP* and *LEPR* genes obtained from the Ensembl Genome Browser for orthologous *LEP* genes from 86 mammalian species and for orthologous *LEPR* genes from 91 mammalian species (Kinsella et al., 2011). Sequence alignment was conducted using the MUSCLE algorithm (Edgar, 2004) implemented in MEGA11 software (Tamura et al., 2021). Phylogenetic trees were subsequently constructed using the Maximum Likelihood method with the Tamura-Nei model (Tamura & Nei, 1993).

A detailed analysis of the structural organization of the porcine *LEP* and *LEPR* genes was then performed, and the distribution of missense and synonymous variants across individual exons was determined. Based on the identified polymorphisms, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping systems (Dai & Long, 2015) were developed. A specific primer pair was designed for each exon containing a protein-coding region using the Primer3Plus software (Untergasser et al., 2007), enabling its amplification by PCR (Waters & Shapter, 2014). The theoretical melting temperature (T_m) of each primer was evaluated using three independent approaches: direct calculation in Primer3Plus during primer design, Thermo Fisher Scientific Multiple Primer Analyzer (Breslauer et al., 1986), and NEB T_m Calculator v1.17.0 (Santa-Lucia et al., 1998). Potential primer dimer formation was assessed using Multiple Primer Analyzer. For each missense and synonymous polymorphism, suitable restriction endonucleases capable of discriminating between allelic variants were identified, where possible, using NEBcutter v3.0.23 (Vincze et al., 2003). Expected restriction fragment sizes generated from digestion of the polymerase chain reaction (PCR) amplicons were determined using the Restriction Analyzer tool.

Experimental validation of selected theoretically developed PCR-RFLP genotyping systems was performed using biological samples obtained from Large White pigs kept at the State Enterprise Experimental Farm Stepne of the Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine (Stepne village, Poltava District, Poltava Oblast, Ukraine). Genomic DNA was extracted using Quick-DNA / RNA MagBead reagents designed for high-throughput magnetic bead-based DNA and RNA isolation (Zymo Research, USA). PCR was conducted in a total reaction volume of 25 μL using reagents from New England Biolabs (USA) in 0.5 mL Eppendorf (Germany) microcentrifuge tubes and a Biometra (Germany) thermal cycler. The resulting amplicons were digested with the appropriate restriction endonucleases according to the manufacturer's recommendations (New England Biolabs, USA). The restriction digestion mixture (15.2 μL H_2O, 2.8 μL buffer, 8.0 μL PCR product, and 1.0 μL restriction endonuclease) was incubated for 3 h at 37 °C in a thermostatic incubator. Following enzymatic hydrolysis, specific DNA fragments were generated, enabling discrimination of different pig genotypes. PCR amplicons and restriction fragments were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide (Galindo-Murillo & Cheatham, 2021). Fragment sizes were estimated using a pBR322/MspI DNA ladder (New England Biolabs, USA). Gel images were documented using a Canon digital camera (Japan).

Results

In this study, phylogenetic trees were reconstructed using the coding sequences of the *LEP* (Fig. 1) and *LEPR* (Fig. 2) genes from representative mammalian species. As shown in the resulting topologies, both genes provide sufficient phylogenetic signal to resolve relationships among closely related taxa, generally enabling the correct clustering of species within genera, and, to some extent, families and orders. Considering that the analyses were based exclusively on coding regions, which are typically more conserved than noncoding sequences, the discriminatory power of both *LEP* and *LEPR* is noteworthy. However, the inferred phylogenies do not fully recapitulate the currently accepted relationships among major mammalian lineages. This suggests that, although *LEP* and *LEPR* are informative markers for lower-level phylogenetic analyses, their utility for resolving deep evolutionary relationships among higher mammalian taxa is limited.

The analysis of sequence variation in the *LEP* and *LEPR* genes based on Ensembl Variation release 115 revealed substantial differences among species both in the total number of variants and in their distribution across functional categories according to their annotated consequences for transcripts and proteins (tables 1, 2, Fig. 3). The number of known variants was markedly higher in humans than in domestic animal species, reflecting the much greater extent of human genomic studies and variant cataloging efforts. Among domestic animals, pigs, cattle, and sheep generally exhibited higher numbers of known variants in both genes than goats, horses, dogs, and cats, likely reflecting differences in the availability of genomic data and the intensity of molecular genetic studies in these species.

In the *LEP* gene, intronic variants represented the most abundant class in all species examined (Table 1, Fig. 3c). For example, 642 intronic variants were identified in pigs, 714 in cows, and 5,178 in humans. The second most numerous category comprised variants located in the 3′-UTR. In humans, 1,213 such variants were found, whereas among domestic animals the highest number was observed in cows (257 variants), followed by pigs (65 variants); considerably fewer variants were identified in the remaining species (Table 1, Fig. 3b).

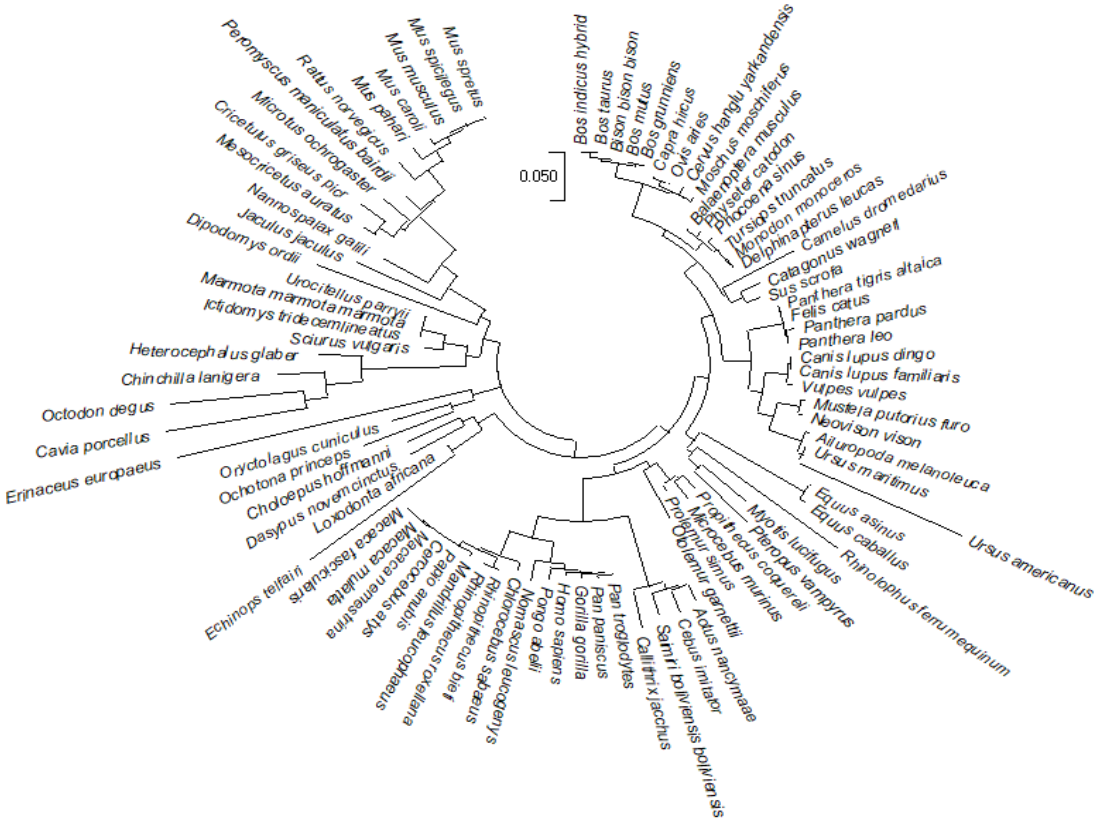

Fig. 1. Phylogenetic tree of 86 mammalian species constructed from coding sequences of the *LEP* gene using the maximum likelihood method under the Tamura-Nei substitution model

Fig. 2. Phylogenetic tree of 91 mammalian species constructed from coding sequences of the *LEPR* gene using the maximum likelihood method under the Tamura-Nei substitution model

Table 1

Number of variants in the *LEP* gene in different domestic animal species and humans according to their annotated consequences for the coding transcript and protein

Variant consequence	Pig	Cow	Sheep	Goat	Horse	Dog	Cat	Human
5'-UTR	–	16	2	–	–	3	–	30
Start-lost	–	1	–	–	2	–	–	–
Missense	4	28	16	3	2	2	–	140
Synonymous	6	15	10	3	5	–	–	92
Inframe deletion	–	–	–	–	–	–	–	1
Inframe insertion	–	–	–	–	–	–	–	1
Frameshift	–	–	–	–	–	–	–	8
Stop-lost	–	–	–	–	–	–	–	1
Stop-gained	–	1	–	–	–	–	–	6
Stop-retained	–	–	–	–	–	–	–	1
3'-UTR	65	257	30	20	19	29	4	1213
Intronic	642	714	456	122	181	171	36	5178

Note: variants with splice-related consequences are not reported separately because most of them are also annotated as intronic or exonic (missense or synonymous) variants; dashes indicate the absence of variants with the corresponding consequence in Ensembl Variation release 115 for the respective species.

Table 2

Number of variants in the *LEPR* gene in different domestic animal species and humans according to their annotated consequences for the coding transcript and protein

Variant consequence	Pig	Cow	Sheep	Goat	Horse	Dog	Cat	Human
5'-UTR	3	4	–	–	15	12	–	108
Start-lost	–	–	–	–	–	–	–	1
Missense	20	86	54	15	15	10	–	1017
Synonymous	23	44	45	21	8	6	1	391
Inframe deletion	–	–	1	–	–	–	–	5
Inframe insertion	–	–	–	–	–	–	–	1
Frameshift	–	2	1	–	–	3	–	49*
Stop-lost	–	–	–	–	–	–	–	1*
Stop-gained	–	9	–	–	–	–	–	35*
3'-UTR	35	–	–	–	–	–	2	1754
Intronic	2314	3669	2386	810	1657	938	27	87244

Note: variants with splice-related consequences are not reported separately because most of them are also annotated as intronic or exonic (missense or synonymous) variants; dashes indicate the absence of variants with the corresponding consequence in Ensembl Variation release 115 for the respective species; of the 49 frameshift variants identified in the human *LEPR* gene, five are additionally annotated as stop-gained variants and one as a stop-lost variant; consequently, these variants are counted twice in the corresponding columns of the table.

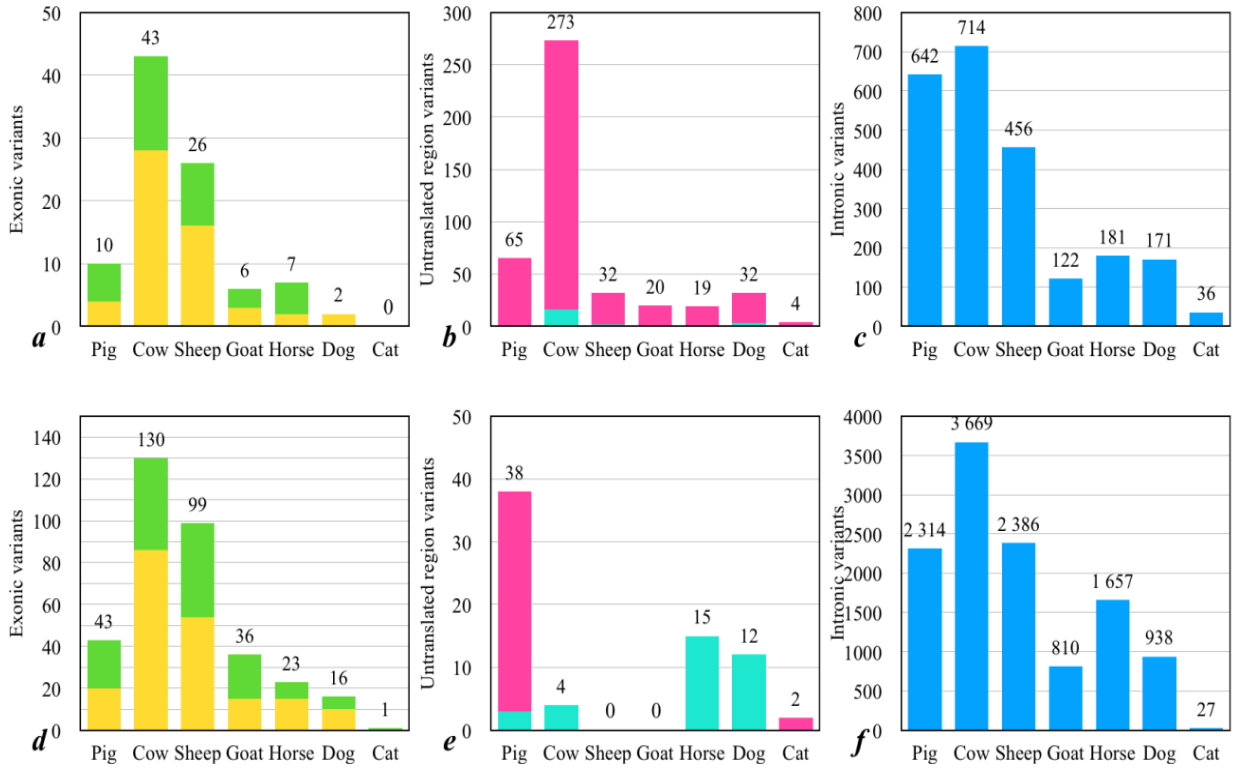

Fig. 3. Distribution of *LEP* and *LEPR* gene variants with different annotated consequences across animal species: *a* – exonic variants (missense and synonymous) in the *LEP* gene; *b* – untranslated region variants (5′-UTR and 3′-UTR) in the *LEP* gene; *c* – intronic variants in the *LEP* gene; *d* – exonic variants (missense and synonymous) in the *LEPR* gene; *e* – untranslated region variants (5′-UTR and 3′-UTR) in the *LEPR* gene; *f* – intronic variants in the *LEPR* gene; missense variants are shown in yellow, synonymous variants in green, 5′-UTR variants in cyan, 3′-UTR variants in magenta, and intronic variants in blue; the total number of variants represented in each column is indicated above the corresponding bar

The number of variants in the 5′-UTR was substantially lower than in the 3′-UTR, which is consistent with the shorter length of the 5′-UTR in the *LEP* gene. Accordingly, 5′-UTR variants were detected only in cows, sheep, dogs, and humans. Exonic variants (missense and synonymous substitutions) were less frequent (Fig. 3a), reflecting both the greater evolutionary conservation of coding regions and their smaller size relative to introns in *LEP* gene. Among domestic animals, cows exhibited the highest number of coding variants, including 28 missense and 15 synonymous variants, followed by sheep with 16 missense and 10 synonymous variants, and pigs with 4 missense and 6 synonymous variants. Variants predicted to have a major effect on protein structure, such as start-lost and stop-gained mutations, were rare and were detected only in cows and humans. Overall, the human *LEP* gene harbored substantially more known variants than that of any domestic animal species, including numerous missense (140), synonymous (92), intronic, and 3′-UTR variants, as well as several frameshift and termination-altering mutations (Table 1).

A similar distribution pattern was observed for the *LEPR* gene, although the total number of variants was considerably higher than for *LEP* (Table 2, Fig. 3d, 3f), reflecting the larger size and more complex genomic structure of the *LEPR* gene. Intronic variants again constituted the predominant category, with 2,314 variants identified in pigs, 2,386 in sheep, and 3,669 in cows, whereas the human *LEPR* gene contained more than 87,000 intronic variants (Table 2, Fig. 3f). Coding variation was also more extensive in *LEPR* than in *LEP*. Cows exhibited the highest number of missense variants among domestic animals (86), followed by sheep (54) and pigs (20), while synonymous variants were most numerous in sheep (45), cows (44), and pigs (23) (Table 2, Fig. 3d). Variants located in untranslated regions were relatively uncommon in domestic animals, although 35 variants in the 3′-UTR were identified in pigs and 15 variants in the 5′-UTR in horses (Table 2, Fig. 3e). As in the *LEP* gene, mutations predicted to have major functional consequences (such as frameshift, stop-gained, and stop-lost variants) were rare and were identified only in cows, sheep, and dogs among the domestic animal species examined. By contrast, the human *LEPR* gene displayed a much broader spectrum of functional variation, including 1,017 missense variants, 391 synonymous variants, 49 frameshift variants, and several other mutations affecting start and stop codons (Table 2).

Overall, both genes exhibited a predominance of noncoding variation, particularly within intronic regions, whereas coding variants accounted for a relatively smaller proportion of the total observed polymorphism in *LEP* and *LEPR* genes.

A detailed analysis of the structure and variation of the *LEP* and *LEPR* genes was subsequently performed using the domestic pig (*Sus scrofa domesticus*), an economically important livestock species. The *LEP* gene (ENSSSCG00000040464) is located on chromosome 18 at coordinates 18:20,106,868-20,123,323 on the reverse strand and encodes a single transcript, ENSSSCT00000055031.3, with a total length of 3,100 bp. This transcript is translated into a protein consisting of 167 amino acids. According to UniProt, a 21-amino-acid signal peptide is cleaved from the N-terminus during posttranslational processing, resulting in a mature leptin protein of 146 amino acids.

The porcine *LEP* gene comprises three exons. The first exon, 26 bp in length, consists entirely of a portion of the 5′-UTR and does not contain any protein-coding sequence. The second exon is 172 bp long, including 28 bp of the 5′-UTR and 144 bp of coding sequence. The third exon is 2,902 bp long, of which 360 bp constitute the coding region, while the remaining sequence forms the 3′-UTR. The exons are separated by two introns measuring 11,006 and 2,350 bp, respectively.

Several polymorphic sites are present within the coding region of the porcine *LEP* gene. Specifically, exon 2 contains three missense and two synonymous variants, whereas exon 3 contains one missense and four synonymous variants. Detailed information on the exonic structure of the porcine *LEP* gene and the distribution of coding variants is presented in Table 3.

Table 3

Distribution of missense and synonymous variants within exons of the porcine *LEP* gene

Exon	Localization (chromosome 18, reverse strand)	Variants	
		missense	synonymous
1	20,123,323-20,123,298	–	–
2	20,112,291-20,112,120	rs1113239558 (c.55G>A, p.Val19Ile), rs695579307 (c.59A>T, p.Glu20Val), rs1110706811 (c.106A>G, p.Lys36Glu)	rs789415220 (c.30G>A, p.Leu10=), rs793436160 (c.63C>T, p.Ala21=)
3	20,109,769-20,106,868	rs701423985 (c.400G>A, p.Val134Ile)	rs45431504 (c.214T>C, p.Leu72=), rs45431505 (c.426G>A, p.Thr142=), rs45431507 (c.462G>T, p.Leu154=), rs793411477 (c.465G>A, p.Gln155=)

Note: for all variants, HGVS nomenclature relative to the reference transcript and protein is provided in parentheses.

The porcine *LEPR* gene (ENSSSCG00000025188) is located on the reverse strand of chromosome 6 and spans the genomic region 6:146,801,954-146,895,999. Three transcripts have been annotated for this gene: ENSSSCT00000028665.4 (5,631 bp), ENSSSCT 00000075980.2 (5,332 bp), and ENSSSCT00000089477.2 (2,767 bp), which encode protein isoforms consisting of 1165, 895, and 814 amino acids, respectively. The first transcript is designated as the canonical transcript. According to UniProt, the encoded protein contains a 21-amino-acid signal peptide at the N-terminus; consequently, the mature leptin receptor consists of 1,144 amino acids.

The canonical porcine LEPR transcript comprises 21 exons. The 5′-UTR is encoded by the first four exons, whereas the 3′-UTR is located within the final exon (exon 21). The coding sequence extends from exon 4 through exon 21. A total of 43 variants are located within the coding region of the canonical transcript. The distribution of missense and synonymous variants among individual exons is presented in Table 4. Next, a system for molecular genotyping of exonic polymorphisms in the porcine *LEP* and *LEPR* genes was developed to enable analysis using PCR-based methods, including PCR-RFLP and DNA sequencing. Primers were designed for each exon containing coding sequence (with the exception of *LEP* exon 1 and LEPR exons 1-3, which contain only 5′-UTR sequences). The primers proposed for amplification of porcine *LEP* gene exons are listed in Table 5, whereas those designed for the *LEPR* gene are presented in Table 6. For each primer, the theoretically calculated Tm obtained using three different calculation methods is provided, together with chromosomal localization, GC content, and the expected amplicon size generated by each primer pair.

To enable genotyping of variants in the porcine *LEP* and *LEPR* genes by the PCR-RFLP method, suitable restriction endonucleases were identified. The selected allele-specific restriction enzymes for *LEP* and *LEPR* gene variants are presented in tables 7 and 8, respectively. These tables also include the expected allele-specific restriction fragments generated after digestion of PCR products amplified from individual exons. For some variants, discrimination between alleles was possible using multiple non-isoschizomeric restriction enzymes. However, for certain polymorphisms, no suitable restriction enzyme capable of distinguishing between alternative alleles was identified. Genotyping of such variants may instead be performed using DNA sequencing, TaqMan assays, or other molecular methods.

To experimentally validate the selected primer systems, fragments corresponding to *LEP* exon 2 and *LEPR* exons 13 and 14 were amplified by PCR. This testing demonstrated that the designed primers generated amplicons of the expected size, as confirmed by agarose gel electrophoresis (Fig. 4). However, the optimal annealing temperature differed from some of the theoretical predictions. For all three primer pairs, the optimal annealing temperature was 60 °C, which was slightly higher than the temperatures predicted by the

Primer3Plus tool but lower than those calculated using the Thermo Fisher Scientific and NEB calculators. Therefore, additional optimization of annealing temperature and other PCR conditions may be required for the remaining primer systems presented in tables 5 and 6 to ensure robust amplification.

Table 4
Distribution of missense and synonymous variants within exons of the porcine *LEPR* gene

Exon	Localization (chromosome 6, reverse strand)	Variants	
		Missense	synonymous
1	146,895,975-146,895,872	–	–
2	146,895,534-146,895,490	–	–
3	146,868,369-146,868,241	–	–
4	146,866,806-146,866,731	–	–
5	146,861,270-146,860,941	rs1112829936 (c.115C>A, p.Pro39Thr), rs45435516 (c.206T>C, p.Met69Thr), rs45435517 (c.217T>A, p.Ser73Thr), rs45435518 (c.218C>T, p.Ser73Leu), rs1110725385 (c.260T>C, p.Phe87Ser)	–
6	146,859,064-146,858,941	–	–
7	146,851,768-146,851,560	rs1108786909 (c.512G>A, p.Gly171Glu), rs691502408 (c.658G>A, p.Ala220Thr), rs1108637163 (c.700G>A, p.Val234Ile)	rs1111253840 (c.507A>G, p.Leu169=), rs705711832 (c.603G>A, p.Ser201=)
8	146,847,268-146,847,123	rs700555031 (c.735G>A, p.Met245Ile)	–
9	146,844,549-146,844,405	rs1108059605 (c.976T>C, p.Phe326Leu)	rs710321903 (c.900C>T, p.Pro300=), rs3476132417 (c.945C>G, p.Gly315=), rs3476529436 (c.948A>C, p.Pro316=)
10	146,838,530-146,838,240	rs693100909 (c.1145A>C, p.Asp382Ala)	rs1109832824 (c.1089A>G, p.Ser363=), rs1107895955 (c.1158C>T, p.Asp386=)
11	146,838,073-146,837,956	–	–
12	146,835,328-146,835,129	–	rs1112189652 (c.1464C>T, p.Cys488=), rs1113846231 (c.1554G>T, p.Pro518=)
13	146,831,701-146,831,553	rs1111622347 (c.1747T>C, p.Trp583Arg)	rs1112698255 (c.1677G>A, p.Glu559=), rs1107758149 (c.1689C>T, p.Phe563=)
14	146,830,438-146,830,279	rs1112637715 (c.1835G>A, p.Arg612His)	rs699829555 (c.1863T>C, p.Tyr621=)
15	146,829,663-146,829,581	rs709596309 (c.1987C>T, p.Leu663Phe)	–
16	146,826,239-146,826,023	rs695414377 (c.2149G>A, p.Val717Ile)	rs1113347176 (c.2143C>T, p.Leu715=), rs702359732 (c.2172C>T, p.Ser724=)
17	146,824,054-146,823,872	rs3472805163 (c.2323A>G, p.Lys775Glu)	rs712243224 (c.2229A>G, p.Ser743=), rs694867280 (c.2370C>G, p.Ser790=)
18	146,822,204-146,822,109	–	–
19	146,820,219-146,820,117	–	rs703986375 (c.2529G>A, p.Val843=)
20	146,818,651-146,818,576	–	rs1109964279 (c.2619T>C, p.Asp873=)
21	146,804,600-146,801,954	rs1113972516 (c.2875C>T, p.Arg959Cys), rs1109261799 (c.3059C>T, p.Pro1020Leu), rs3475047780 (c.3194G>A, p.Gly1065Glu), rs792804682 (c.3235T>G, p.Tyr1079Asp)	rs704399896 (c.2754G>A, p.Ser918=), rs1107415284 (c.2769T>G, p.Val923=), rs694660564 (c.2841T>C, p.Asp947=), rs1108340001 (c.3060G>A, p.Pro1020=), rs1107659194 (c.3330C>T, p.Phe1110=)

Note: for all variants, HGVS nomenclature relative to the reference transcript and protein is provided in parentheses; *variants rs45435517 and rs45435518 are typically in linkage disequilibrium and, when present together, jointly result in the S73I amino acid substitution.

Table 5
Characteristics of primer pairs designed for amplification of coding exons of the porcine *LEP* gene

Exon	Primer	Primer sequence	Localization	T_m (calculated), °C			GC content, %	Product size, bp
				Primer3Plus	Thermo Fisher	NEB		
2	F	5'-AAGCTCCCTTTGATCCGCAT-3'	18:20112379-20112398 (-)	59.7	67.2	67.0	50.0	310
2	R	5'-AGTTCGACCTTGTCTCCCAG-3'	18:20112089-20112108 (+)	59.0	63.7	66.0	55.0	
3	F	5'-GCAGAGCCAACATCTCTCTC-3'	18:20109804-20109823 (-)	58.1	62.3	65.0	55.0	463
3	R	5'-GAAGCTCAGGTTTCTTCCCC-3'	18:20109361-20109380 (+)	58.2	64.3	66.0	55.0	

Note: F – forward primer, R – reverse primer; primer pairs were not designed for exon 1 of the porcine *LEP* gene because this exon corresponds entirely to the 5′-untranslated region (5′-UTR) and does not contain coding sequences.

Table 6
Characteristics of primer pairs designed for amplification of coding exons of the porcine *LEPR* gene

Exon	Primer	Primer sequence	Localization	T_m (calculated), °C			GC content, %	Product size, bp
				Primer3Plus	Thermo Fisher	NEB		
4	R	5'-GTGAAATGCTGATATTGATCCAGAG-3'	6:146866924-146866948 (-)	58.1	64.9	63.0	40.0	319
4	F	5'-TAACCGAAACAGGACATGCG-3'	6:146866630-146866649 (+)	58.6	66.2	65.0	50.0	
5	R	5'- TGGACAATGGAAGAGCTTTGT-3'	6:146861409-146861429 (-)	57.8	63.9	64.0	42.9	588
5	F	5'-TCCATACAGGCATCTATTTCACA-3'	6:146860842-146860864 (+)	57.4	63.5	63.0	39.1	
6	R	5'-ACTACCAGTTAGAGCCTGCA-3'	6:146859159-146859177 (-)	58.1	59.7	65.0	50.0	340
6	F	5'-AGGGATAGAGGAGCTTTCAGA-3'	6:146858838-146858858 (+)	57.3	61.4	64.0	47.6	
7	R	5'-TCCAGTGCTGTCCCATCTAT-3'	6:146851800-146851819 (-)	57.8	62.6	65.0	50.0	314
7	F	5'-ACCTGTTTGTGTGACTGATGT-3'	6:146851506-146851526 (+)	57.7	60.1	64.0	42.9	
8	R	5'-ACCACTTAGGGAGAAGGAAA-3'	6:146847460-146847479 (-)	55.3	59.7	62.0	45.0	517
8	F	5'-AATGAAAGCCAGTGACATGA-3'	6:146846963-146846982 (+)	55.2	61.3	61.0	40.0	
9	R	5'-ATAGATGCCAGCAGTGGTTG-3'	6:146844863-146844882 (-)	58.0	63.2	65.0	50.0	515
9	F	5'-GTCCCCTGTCAATAGAAGCG-3'	6:146844368-146844387 (+)	58.1	63.9	65.0	55.0	
10	R	5'-CGCTTACTAACTGTACACGAAC-3'	6:146838683-146838704 (-)	57.2	58.6	63.0	45.5	504
10	F	5'-TCCTATGGAGCGAAATGAAGA-3'	6:146838201-146838221 (+)	56.5	63.9	63.0	42.9	

Exon	Primer	Primer sequence	Localization	T_m (calculated), ℃			GC content, %	Product size, bp
				Primer3Plus	Thermo Fisher	NEB		
11	R	5'-CACCATCGCTATGCTGAGTT-3'	6:146838251-14683827 (-)	58.1	63.3	65.0	50.0	422
11	F	5'-TCTACCTCCTTCTTGCAGGA-3'	6:146837849-146837868 (+)	57.4	62.0	65.0	50.0	
12	R	5'-GGTCGTGATATAGCTGGGAATT-3'	6:146835470-146835491 (-)	57.7	63.2	64.0	45.5	633
12	F	5'-GACTACCAGATTTCATTTCCGG-3'	6:146834859-146834880 (+)	56.9	63.7	63.0	45.5	
13	R	5'-AGTTGTAGGGGATCAAATGAGA-3'	6:146831841-146831862 (-)	56.5	61.7	63.0	40.9	435
13	F	5'-AGCGACAGAATAGAGGAGGA-3'	6:146831428-146831447 (+)	57.3	61.1	64.0	50.0	
14	R	5'-CCCTTTAGCTGCATGTGTTAG-3'	6:146830470-146830490 (-)	57.0	61.7	63.0	47.6	216
14	F	5'-AGACCTTTTACATCCGTGACA-3'	6:146830275-146830295 (+)	57.0	61.5	63.0	42.9	
15	R	5'-ATGTACAACTTGGGCTCCTTG-3'	6:146829675-146829695 (-)	58.2	63.2	65.0	47.6	207
15	F	5'-GCAGAGTCCAGAAATGACTTCAT-3'	6:146829489-146829511 (+)	58.7	63.8	65.0	43.5	
16	R	5'-CTGCCTACCAAGGGGAAATG-3'	6:146826311-146826330 (-)	58.2	65.8	66.0	55.0	415
16	F	5'-CGACACAGAGAATCAGAGGC-3'	6:146825916-146825935 (+)	58.1	63.1	65.0	55.0	
17	R	5'-AGCGGAGAACACAGTTTTGA-3'	6:146824313-146824332 (-)	57.7	62.9	64.0	45.0	514
17	F	5'-TGCAGCATCACACTAATTCAGA-3'	6:146823819-146823840 (+)	58.1	63.8	64.0	40.9	
18	R	5'-GGTGAATTTGGAGTGCTGAGAT-3'	6:146822388-146822409 (-)	58.6	64.6	65.0	45.5	413
18	F	5'-GGACTCTCCCCTGATTCCAT-3'	6:146821997-146822016 (+)	58.2	64.6	66.0	55.0	
19	R	5'-GCTTCTGAGTTGCGTGAATT-3'	6:146820234-146820253 (-)	57.0	62.4	63.0	45.0	211
19	F	5'-ACTTCAGTGTTTCCGAATTGG-3'	6:146820043-146820063 (+)	56.7	63.2	63.0	42.9	
20	R	5'-GGCACAGACAGTTTAGTGGA-3'	6:146818740-146818759 (-)	57.5	60.9	64.0	50.0	262
20	F	5'-ATGTGAAGCTCTAACAGGCC-3'	6:146818498-146818517 (+)	57.6	61.4	65.0	50.0	
21	R	5'-CCAGTTGATTTGTGACGACTTG-3'	6:146804710-146804731 (-)	58.1	64.8	64.0	45.5	1011
21	F	5'-ACCCTTAATATGACTCATCCCA-3'	6:146803721-146803742 (+)	56.0	61.4	62.0	40.9	

Note: F – forward primer, R – reverse primer; primer pairs were not designed for exons 1-3 of the porcine *LEPR* gene because these exons correspond entirely to the 5′-untranslated region (5′-UTR) and do not contain coding sequences.

Table 7

Restriction enzymes for PCR-RFLP genotyping of porcine *LEP* gene variants and predicted allele-specific restriction fragments

Variant	Exon	Restriction enzyme	Restriction fragments, bp
rs1113239558 (c.55G>A)	2	HpyCH4IV (A/CGT)	G: 188+122 A: 310
rs793436160 (c.63C>T)	2	AluI (AG/CT)	C: 307+3 T: 193+114+3
		TaqI (T/CGA)	A: 304+6 G: 239+65+6
rs1110706811 (c.106A>G)	2	BsmBI (CGTCTC(1/5))	A: 310 G: 235+75
		BsmAI (GTCTC(1/5))	A: 230+59+21 G: 176+59+54+21
rs45431504 (c.214T>C)	3	HinfI (G/ANTC)	T: 463 C: 343+120
		Nt.BstNBI (GAGTC(4/none))	T: 463 C: 335+128
rs701423985 (c.400G>A)	3	BtsCI GGATG(2/0)	G: 355+95+13 A: 200+155+95+13
		BsaHI (GR/CGYC)	G: 308+155 A: 463
rs45431505 (c.426G>A)	3	BtgI (C/CRYGG)	G: 332+131 A: 463
		BsaJI (C/CNNGG)	G: 126+99+70+61+43+32+10+9+6+6+1 A: 126+102+99+61+43+10+9+6+6+1
rs45431507 (c.462G>T)	3	HpyCH4V (TG/CA)	G: 311+91+49+12 T: 311+103+49
		Hpy188III (TC/NNGA)	G: 281+117+65 T: 191+117+90+65
		Nt.BspQI (GCTCTTC(1/none))	G: 463 T: 374+99
rs793411477 (c.465G>A)	3	SfcI* (C/TRYAG)	G: 358+93+12 A: 358+105
		PstI* (CTGCA/G)	G: 362+89+12 A: 362+101

Note: restriction fragments were predicted in silico for PCR amplicons generated using the primer pairs listed in Table 5; for each polymorphism, the restriction enzyme listed produces distinct digestion patterns for alternative alleles; when multiple restriction enzymes are shown for the same variant, each enzyme can be used independently for allele discrimination; restriction fragments are reported in descending order of size; variants for which no suitable allele-discriminating restriction enzyme was identified are not included in the table; *these restriction endonucleases can be used to distinguish alleles for rs793411477 only if the reference G allele for rs45431507 is present.

Table 8

Restriction enzymes for PCR-RFLP genotyping of porcine *LEPR* gene variants and predicted allele-specific restriction fragments

Variant	Exon	Restriction enzyme	Restriction fragments, bp
rs1112829936 (c.115C>A)	5	SphI (GCATG/C)	C: 355+233 A: 588
rs45435517 (c.217T>A)	5	MluCI (/AATT)	T: 132+124+119+67+55+40+37+14 A: 251+124+67+55+40+37+14
rs1108786909 (c.512G>A)	7	Sau3AI (/GATC)	G: 218+68+28 A: 286+28
		HinfI (G/ANTC)	G: 314 A: 246+68
rs691502408 (c.658G>A)	7	HpyCH4III (ACN/GT)	G: 314 A: 217+97
rs1108637163 (c.700G>A)	7	HpyCH4IV (A/CGT)	G: 255+59 A: 314
		AclI (AA/CGTT)	G: 255+59 A: 314
rs700555031 (c.735G>A)	8	NdeI (CA/TATG)	G: 278+239 A: 517
rs710321903 (c.900C>T)	9	XmaI (C/CCGGG)	C: 382+133 T: 515
rs3476132417 (c.945C>G)	9	BsaJI (C/CNNGG)	C: 260+122+86+47 G: 260+133+122
rs3476529436 (c.948A>C)	9	MspI (C/CGG)	A: 383+132 C: 383+84+48
rs1109832824 (c.1089A>G)	10	TaqI (T/CGA)	A: 283+121 G: 267+121+116
rs1107895955 (c.1158C>T)	10	Sau3AI (/GATC)	C: 256+248 T: 256+169+79
		BstEII (G/GTNACC)	C: 333+171 T: 504
rs1112189652 (c.1464C>T)	12	BsrI (ACTGG(1/-1))	C: 409+224 T: 633
rs1107758149 (c.1689C>T)	13	Hpy188III (TC/NNGA)	C: 268+167 T: 248+167+20
rs1111622347 (c.1747T>C)	13	HpyCH4III (ACN/GT)	T: 172+131+69+63 C: 241+131+63
		BtsIMutI (CAGTG(2/0))	T: 187+127+121 C: 248+187
		MspA1I (CMG/CKG)	T: 266+169 C: 169+135+131
		AciI (CCGC(-3/-1))	T: 268+167 C: 167+137+131
rs1112637715 (c.1835G>A)	14	HinP1I (G/CGC)	G: 133+83 A: 216

Variant	Exon	Restriction enzyme	Restriction fragments, bp
		HhaI (GCG/C)	G: 135+81 A: 216
rs699829555 (c.1863T>C)	14	BsrI (ACTGG(1/-1))	T: 87+72+57 C: 87+72+49+8
rs695414377 (c.2149G>A)	16	MscI (TGG/CCA)	G: 438 A: 252+186
rs702359732 (c.2172C>T)	16	AciI (CCGC(-3/-1))	C: 164+161+113 T: 274+164
		HpyCH4V (TG/CA)	C: 311+127 T: 159+152+127
rs712243224 (c.2229A>G)	17	Tsp45I (/GTSAC)	A: 301+291 G: 592
rs694867280 (c.2370C>G)	17	Nt.CviPII (/CCD)	C: 92+43+40+40+38+35+35+24+22+21+20+19+17+16+15+13+13+12+12+11+9+8+8+8+7+5+4+3+2 G: 92+43+40+40+38+35+35+26+24+21+20+19+17+16+15+13+13+12+12+11+9+8+8+8+7+5+3+2
rs703986375 (c.2529G>A)	19	MluCI (/AATT)	G: 109+71+67+65 A: 109+71+65+55+12
rs1109964279 (c.2619T>C)	20	HpyCH4IV (A/CGT)	T: 300 F: 169+131
rs704399896 (c.2754G>A)	21	Nt.CviPII (/CCD)	Multiple fragments
rs1107415284 (c.2769T>G)	21	Nt.CviPII (/CCD)	Multiple fragments
		Hpy188III (TC/NNGA)	T: 287+230+221+213+44+10+6 C: 287+236+221+213+44+10
		MboI (/GATC)	T: 430+299+282 C: 581+430
rs694660564 (c.2841T>C)	21	BstYI (R/GATCY)	T: 712+299 C: 1011
		Sau96I (G/GNCC)	T: 828+183 C: 712+183+116
		AvaII (G/GWCC)	T: 1011 C: 712+299
rs1113972516 (c.2875C>T)	21	HpyCH4V (TG/CA)	C: 1011 T: 674+337
rs1109261799 (c.3059C>T)	21	Nt.CviPII (/CCD)	multiple fragments

Note: restriction fragments were predicted *in silico* for PCR amplicons generated using the primer pairs listed in Table 6. For each polymorphism, the restriction enzyme listed produces distinct digestion patterns for alternative alleles; when multiple restriction enzymes are shown for the same variant, each enzyme can be used independently for allele discrimination; restriction fragments are reported in descending order of size; variants for which no suitable allele-discriminating restriction enzyme was identified are not included in the table.

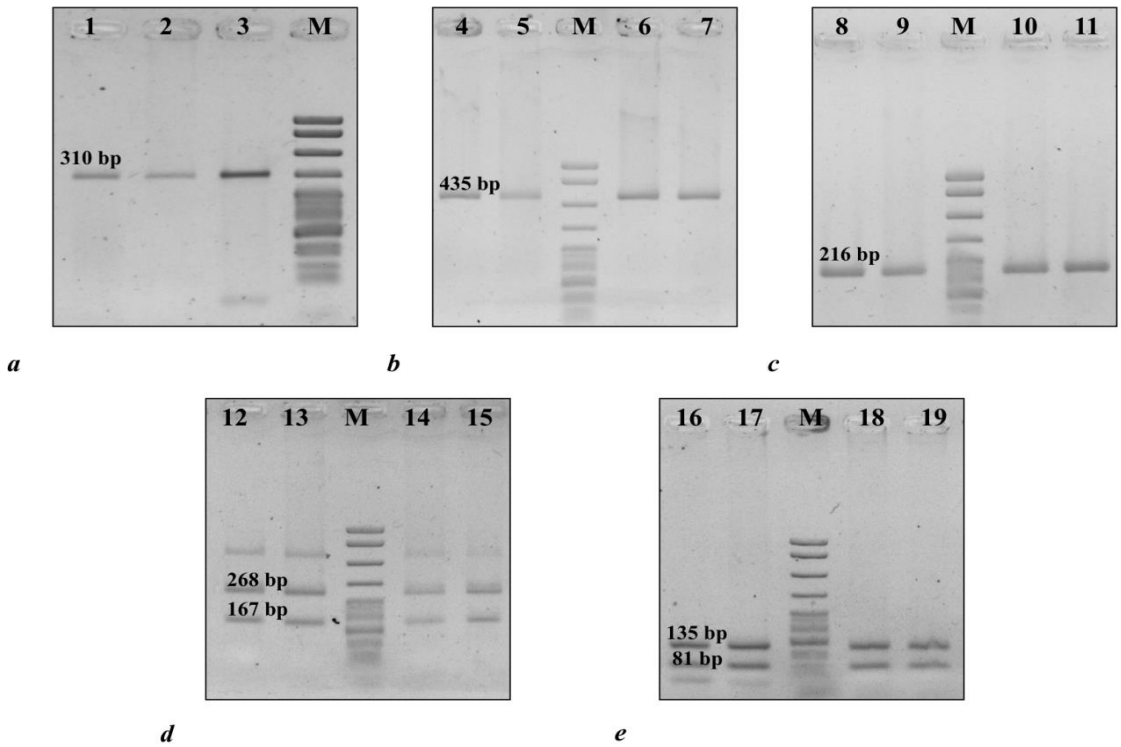

Fig. 4. Electropherograms of PCR amplicons and restriction fragments in agarose gel: *a* – PCR amplicons of exon 2 of the porcine *LEP* gene (lanes 1-3; 310 bp); *b* – PCR amplicons of exon 13 of the porcine *LEPR* gene (lanes 4-7; 435 bp); *c* – PCR amplicons of exon 14 of the porcine *LEPR* gene (lanes 8-11; 216 bp); *d* – restriction fragments of exon 13 amplicons of the porcine *LEPR* gene digested with AciI in the presence of the reference T allele of rs1111622347 (lanes 12-15; fragment sizes 268 + 167 bp); *e* – restriction fragments of exon 14 amplicons of the porcine *LEPR* gene digested with *HhaI* in the presence of the reference G allele of rs1112637715 (lanes 16-19; fragment sizes 135 + 81 bp); M – pBR322/MspI DNA ladder

Restriction digestion was also performed using amplicons of exons 13 and 14 of the porcine *LEPR* gene to demonstrate the activity of the AciI restriction enzyme, which is informative for the rs1111622347 variant in exon 13, and the HhaI restriction enzyme, which is informative for the rs1112637715 variant in exon 14. The resulting restriction fragments following gel electrophoresis are shown in Figure 4. The observed fragment patterns corresponded to the expected digestion profiles of the reference alleles of the rs1111622347 (T allele) and rs1112637715 (G allele) variants.

It should be noted that, for a number of LEP and LEPR polymorphisms, alternative restriction enzymes listed in tables 7 and 8 may be useful in practice. In several cases, restriction enzymes specific for both the major and minor alleles are available. For example, for the rs1111622347 polymorphism in exon 13 of the *LEPR* gene, the restriction enzymes *HpyCH4III* and *BtsIMutI* recognize an additional restriction site in the presence of the T allele, whereas *MspA1I* and *AciI* recognize an additional restriction site in the presence of the C allele.

Discussion

The results obtained in this study provide new insights into both the evolutionary aspects and the genetic variability of the leptin signaling system in domestic animal species. In general, *LEP* and *LEPR* are regarded as highly conserved genes (Prokop et al., 2012) because of their central roles in energy homeostasis, appetite regulation (Liu

et al., 2023), reproduction (Priyadarshini et al., 2015), immunity (Bernotiene et al., 2006; Mukherjee et al., 2023), and thermoregulation (Kajimura et al., 2015; Chen et al., 2022). Nevertheless, our analyses demonstrate that substantial variation exists among mammalian species and that these genes retain sufficient phylogenetic signal to resolve relationships among closely related taxa.

Phylogenetic trees were reconstructed from the coding sequences of *LEP* and *LEPR* generally grouped taxa at the species and genus levels in accordance with currently recognized taxonomic relationships. However, several discrepancies were observed in the clustering of higher mammalian lineages. In particular, the expected sister-group relationship between *Laurasiatheria* and *Euarchontoglires* (Phillips & Shazwani Zakaria, 2021), as well as the relationship between *Euarchonta* and *Glires* within *Euarchontoglires* (López-Torres et al., 2020), was not consistently recovered in the reconstructed phylogenies. On the one hand, the inability of either marker to fully recapitulate the accepted higher-level mammalian phylogeny may reflect the strong functional constraints acting on protein-coding regions, which limit the accumulation of phylogenetically informative substitutions. On the other hand, it may also reflect methodological limitations associated with reconstructing deep evolutionary relationships from relatively short coding sequences. Several previous studies have likewise examined the balance between conservation and variability in *LEP* and *LEPR* across different taxonomic groups (Denver et al., 2011; Prokop et al., 2012; Morini et al., 2015; Londraville et al., 2017), highlighting the value of these genes for investigating evolutionary processes.

At the same time, variation in *LEP* and *LEPR* is important not only from an evolutionary perspective but also because of its influence on phenotypic diversity. Consequently, these genes may serve as targets of both natural and artificial selection. Given their central role in metabolic regulation, polymorphisms within *LEP* and *LEPR* have frequently been investigated as genetic markers of economically important traits in domestic animals. In cattle, *LEP* and *LEPR* polymorphisms have repeatedly been associated with body weight (Kuswati et al., 2022; Kurlyana et al., 2023), energy metabolism, feed intake, and feed efficiency (Lagonigro et al., 2003; Giblin et al., 2010), reproductive performance (Trakovická et al., 2013; Pramono et al., 2026), carcass and meat quality traits (Wang et al., 2022), and milk production characteristics (Matteis et al., 2012; Trakovická et al., 2013; Pramono et al., 2026). In sheep and goats, polymorphisms within *LEP* and *LEPR* have been associated with body weight and growth rate (Rojib et al., 2026), feed intake and body reserves (Jonas et al., 2016; Avondo et al., 2019; Macé et al., 2022), reproductive performance (Wang et al., 2011; Lakhssassi et al., 2020; El-Shorbagy et al., 2022; Yang et al., 2025), and milk production traits (Avondo et al., 2019; El-Shorbagy et al., 2022).

Pigs are among the most extensively studied livestock species with respect to associations between *LEP* and *LEPR* gene variants and economically important traits. Previous studies have demonstrated that both *LEP* and *LEPR* are major candidate genes affecting feed intake (Kennes et al., 2001), reproductive performance and growth efficiency (Kulig et al., 2001; de Oliveira Peixoto et al., 2006; Sun et al., 2009), and pork quality traits (Balatsky et al., 2018; Balatsky et al., 2022; Limón-Morales et al., 2025). One of the most extensively investigated variants is the missense polymorphism rs709596309 (c.1987C>T) in the porcine *LEPR* gene, which results in the L663F substitution and has been associated with intramuscular fat deposition, saturated fatty acid content, and backfat thickness (Galve et al., 2012; Pérez-Montarelo et al., 2012; Balatsky et al., 2016; Suárez-Mesa et al., 2024). In addition, several other *LEPR* polymorphisms have been studied, including the missense variants rs45435516 (c.206T>C), resulting in the T69M substitution; rs45435517 (c.217T>A) and rs45435518 (c.218C>T), which jointly encode the S73I substitution; and rs693100909 (c.1145A>C), resulting in the D382A substitution. Synonymous variants rs710321903 (c.900C>T) and rs694660564 (c.2841T>C) have also been investigated (Ovilo et al., 2005; Balatsky et al., 2018; Balatsky et al., 2022). In the *LEP* gene, the most frequently studied polymorphisms include the intronic variant rs344615147 (c.145-590T>A), the 3′-UTR variant rs337366389 (c.*238T>C), and the synonymous variant rs45431504 (c.214T>C) (Kennes et al., 2001; de Oliveira Peixoto et al., 2006; Balatsky et al., 2022).

An important finding of the present study is the markedly different extent of documented genetic variation among species. Humans exhibited substantially higher numbers of known variants in both *LEP* and *LEPR* than any domestic animal species. However, this observation is likely attributable, at least in part, to the much greater scale of sequencing efforts and variant cataloging in humans. Furthermore, our results demonstrate the predominance of intronic and untranslated-region variants across all the species examined. This pattern is consistent with genome-wide observations in mammals and reflects both the larger size of noncoding regions and the stronger purifying selection acting on protein-coding sequences (Lindblad-Toh et al., 2011; Hubé & Francastel, 2015; Peña-Martínez & Rodríguez-Martínez, 2024). At the same time, coding variants, particularly missense variants, are of special interest because they can directly alter the structure and function of the encoded proteins and therefore represent the most obvious candidates for causal effects on phenotypic traits (Peka & Balatsky, 2024). As discussed above, missense variants are among the most extensively studied polymorphisms in the porcine *LEPR* gene, and several have been associated with economically important production traits (Ovilo et al., 2005; Balatsky et al., 2018; Balatsky et al., 2022). Although missense variants occur less frequently than variants located in noncoding regions, they are considerably more common than frameshift, stop-gained, stop-lost, and start-lost variants in domestic animals. Consequently, the identification and characterization of missense variants within livestock populations may represent a particularly promising approach for future marker-assisted selection programs.

In the present study, a comprehensive characterization of coding variants within the porcine *LEP* and *LEPR* genes was performed, providing a valuable resource for molecular breeding applications. Most previously published association studies have focused on a limited number of known polymorphisms in these genes. By contrast, the present analysis systematically cataloged all currently annotated coding variants and developed PCR-based approaches for their genotyping. The resulting sets of primers and restriction enzymes for PCR-RFLP analysis provide a cost-effective framework for screening both established and newly identified polymorphisms in breeding populations. This is particularly relevant for breeding programs in which access to high-throughput sequencing technologies may be limited. It should be noted that part of the foundation for this work was established in a previous study (Saienko et al., 2023), in which primers targeting exon 2 of the *LEP* gene were proposed for genotyping the rs1110706811 (c.106A>G) variant using the TaqI restriction enzyme. All other primers and restriction enzymes reported here were characterized within the framework of the present study. An additional strength of the developed genotyping system is its flexibility. For several variants, multiple alternative restriction enzymes were identified, allowing laboratories to select the most suitable assay according to enzyme availability, cost, and experimental conditions. More broadly, the methodological resources generated in this study may facilitate future association analyses aimed at identifying functional *LEP* and *LEPR* variants that influence economically important traits in livestock species.

A promising direction for future research is the development of similar genotyping systems for other domestic animal species, including cattle, sheep, goats, and horses. Further studies should focus on identifying the most informative coding polymorphisms within the *LEP* and *LEPR* genes of pigs and other livestock species, determining their frequencies in breeding populations, and evaluating their associations with economically important traits related to production, reproduction, and health.

Conclusions

In this study, a comprehensive analysis of the *LEP* and *LEPR* genes was performed, combining phylogenetic reconstruction based on the coding sequences of these genes in mammals, characterization of

genetic variation in domestic animal species and humans, and the development of practical molecular genotyping tools for domestic pigs. The phylogenetic analysis demonstrated that *LEP* and *LEPR* are sufficiently informative markers for evolutionary studies and can be used to reconstruct phylogenetic relationships among closely related taxa. The analysis of genetic variation revealed substantial interspecific differences in both the number and distribution of variants within *LEP* and *LEPR*. In all the species examined, noncoding variants, particularly intronic polymorphisms, predominated over coding variants. Nevertheless, considerable numbers of missense and synonymous variants were identified in livestock species, especially in cattle, sheep, and pigs.

A detailed structural characterization of the porcine *LEP* and *LEPR* genes was conducted, including analyses of exon-intron organization, transcript structure, and the distribution of coding variants across exons. Based on this information, PCR-based genotyping systems were developed for all coding exons of both genes, and allele-specific restriction endonucleases were identified for PCR-RFLP analysis. The molecular tools developed in this study may facilitate future association analyses aimed at identifying functional candidate *LEP* and *LEPR* variants, evaluating their potential causal effects on economically important traits, and supporting marker-assisted breeding programs designed to improve livestock productivity.

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The authors declare no conflict of interest.

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