Cold adaptation in mammals: mechanisms and genomic insights from bats

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Abstract

Cold environments impose strong energetic constraints on mammals, driving adaptations including thermogenesis, insulation and torpor. Bats are the only mammals capable of powered flight and inhabit different climates, yet the evolutionary basis of their cold tolerance remains poorly understood. In this study, we screened 571 records and retained 137 studies on mammalian cold adaptation. While physiological research focused mainly on non-shivering thermogenesis and metabolic regulation, studies were concentrated in the Palearctic and Nearctic regions, with Rodentia predominating. In contrast, bats were comparatively underrepresented. Among 478 reported genes, most were reported only once. Using thermogenesis-related genes derived from the review, we tested 33 nuclear genes across published bat genomes for positive selection. Site-level evidence consistent with positive selection was limited to six genes. Meanwhile, none of the branch-site tests across predefined cold-associated lineages remained statistically significant. Notably, uncoupling protein 1 (UCP1), the most frequently reported gene in the cold adaptation literature, showed no significant evidence of positive selection in any model. Overall, thermogenesis-related genes frequently emphasized in the literature did not show a common pattern of coding-sequence positive selection across cold-associated bat lineages. Future studies should examine whether regulatory, expression-level or other genomic mechanisms contribute to cold adaptation in bats.

Keywords: cold adaptation, bats, thermogenesis, positive selection, mammals

1. Introduction

Accelerating climate change is altering environmental conditions at an extraordinary pace, affecting species globally, leading to range shifts, changes in adaptive responses, habitat loss, and in some cases, extinctions [1]. Species inhabiting cold environments are particularly vulnerable to climate change because they have lower thermal tolerance, limiting their ability to cope with rising temperatures and often forcing them to migrate to higher latitudes or

altitudes [2,3]. Moreover, cold-adapted species can be competitively displaced as rising temperatures drive the range expansion of warm-adapted species [4–7]. These processes can reshape species interactions by altering food webs and food availability [5,7–9]. In addition, rising temperatures can disrupt key physiological adaptations of cold-adapted species. For example, warmer winters can shorten the duration of torpor in hibernating mammals, leading to ecological mismatches in the phenology of prey-predator interactions. Earlier arousal from hibernation results in increased metabolic rate, and limited food sources or prey causes faster depletion of fat reserves [10,11].

Cold adaptation is a diverse set of traits involving morphological, physiological, and genetic mechanisms that allow organisms to survive in cold environments [12,13]. In mammals, these mechanisms include metabolic rate adjustments, energy generation through thermogenesis, and morphological adaptations such as insulation, which help maintain energy balance [14]. Morphologically, mammals living in colder regions have denser fur or thick blubber layers for thermal insulation [15,16]. Furthermore, many cold-adapted mammals tend to have a larger body size, increasing the volume-to-surface ratio, a pattern known as Bergmann's Rule [17,18]. A similar biogeographical pattern described as Allen's rule is observed in cold-adapted animals. These animals have shorter limbs and smaller ears to minimize cold-exposed surface area, reducing heat loss [19]. Although these patterns have been widely observed in terrestrial mammals, numerous studies have documented exceptions across multiple taxa such as bats, marine mammals, and rodents [20–22].

While morphological adaptations reduce heat loss, physiological mechanisms enable mammals to actively maintain heat balance at low temperatures [14,18]. As endotherms, mammals can maintain a stable body temperature through metabolic heat production [14,23]. For example, during cold exposure, skeletal muscles produce heat through shivering thermogenesis. Shivering often occurs alongside non-shivering thermogenesis (NST), primarily mediated by brown adipose tissue (BAT) and produces heat independent of muscle contractions [24,25]. This mechanism is primarily observed in placental mammals and appears to be absent in marsupials and birds [26]. In addition, cold-adapted mammals often have a higher basal metabolic rate, contributing to increased heat production [14,27–29]. However, maintaining metabolic heat production is energetically costly. To mitigate such costs, animals use the vasoconstriction mechanism, which reduces the need for continuous heat production by minimizing blood flow towards the skin [15]. This mechanism is critical for the conservation of body heat, which can also prevent hypothermia [30]. Some mammals reduce their metabolic

activity in the short term to conserve energy under cold stress. For example, Djungarian hamsters use daily torpor, temporarily suppressing their metabolism by up to 65% during winter [31]. During prolonged energetic stress, some mammals enter a seasonal hypometabolic state known as hibernation [32]. Beyond temporary metabolic states, mammals can undergo acclimatization, enabling them to exhibit reversible long-term morphological and physiological adjustments to endure cold environments [33]. For instance, higher altitude populations of deer mice have increased vascularization in BAT, increasing oxygen availability [34]. Moreover, mammals exhibit behavioural adaptations such as migration to avoid unfavourable environments, hence increasing their survival rate [12,35].

Morphological and physiological adaptations are regulated by complex molecular mechanisms such as thermogenesis, lipid metabolism, and cellular responses [12,36]. As an example, cold exposure triggers lipid metabolic reprogramming that supports thermogenesis and cellular membrane fluidity under low temperatures [37]. These responses involve coordinated regulation of a wide range of genes and signalling pathways associated with energy metabolism and thermal homeostasis. Furthermore, several genes have been recurrently reported in such pathways and are proposed as candidate genes associated with cold adaptation [38]. Among the identified genes are those involved in thermogenesis and mitochondrial regulation, including uncoupling protein 1 (UCP1) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A), which play important roles in fatty acid β-oxidation, mitochondrial biogenesis, and heat production during cold exposure [39]. Another putative candidate gene is the cold-sensing ion channel protein gene, transient receptor potential cation channel subfamily M member 8 (TRPM8), involved in calcium signalling and thermoregulatory responses [40]. Recent studies have used transcriptomic, comparative genomic and evolutionary approaches, including positive selection analysis, to investigate genes associated with cold adaptation [38,41–44]. Together, these studies have identified changes in genes and regulatory pathways involved in energy metabolism, thermogenesis and cold-stress responses. However, the specific genes implicated in cold adaptation appear to vary among taxa and across different adaptive strategies.

Despite growing interest in cold adaptation, significant gaps remain in our understanding, particularly regarding wild mammal species. Bats represent a substantial portion, over 20% of all mammalian species, while cold adaptation remains poorly understood [45,46]. Among mammals, bats are the only taxonomic group capable of sustained flight and represent a particularly interesting group in the context of cold adaptation. Bats face unique

thermoregulatory challenges due to their high surface-area-to-volume ratio and high energy demands due to flight. Similar to other mammals, bats have evolved a range of mechanisms to cope with cold stress, such as migration, torpor and hibernation. [47–49]. Species living in cold environments are predominantly insectivorous, and insect availability is strongly temperature-dependent. Bat activity therefore fluctuates seasonally and nightly with ambient temperature [49,50]. During cold conditions, the energy demand increases while food availability falls. This dual challenge makes bats a particularly informative organism for studying cold adaptation. Although cold adaptation has been extensively studied in fish, birds, and mammals, the molecular pathways enabling bats to cope with low temperatures are less well characterized [15,16,38]. Furthermore, cold-adapted species may be disproportionately affected by climate change due to their limited heat tolerance and dispersal capacity, making this an urgent area of study [3].

In this review, we aim to synthesize current knowledge on the physiological and genetic bases of cold adaptation, with particular emphasis on key genes associated with thermoregulatory traits. These include processes such as thermogenesis, regulation of metabolic rate, and acclimatization across diverse mammalian taxa. In addition, we seek to identify putative candidate genes linked to cold adaptation and evaluate their evolutionary significance by testing for signals of positive selection in bats. To achieve this, we will analyse 30 published reference genomes from a range of bat species, encompassing both cold- and warm-adapted taxa. This comparative approach allows us to investigate how natural selection has shaped genetic adaptations to different thermal environments in bats.

2. Material & Methods

(a) Systematic literature search

We conducted a systematic literature search in the Web of Science Core Collection to identify studies investigating cold adaptation in mammals [51]. The search was performed on 13 June 2025 at 17.00 using the following query: ALL = ("cold adaptation" OR "cold tolerance" OR "thermal adaptation" OR "acclimatization" OR "cold stress" OR “thermoregulatory adaptation” OR "cold-adaptation" OR "cold-adapted" OR "cold adapted") AND ALL = ("mammal" OR "mammalian" OR "mammals") AND ALL = ("physiology" OR "physiological" OR "molecular" OR "genetic" OR "genomic" OR "proteomic" OR "transcriptomic" OR "metabolomic" OR "metabolic" OR "microbiome" OR "brown adipose tissue" OR "BAT" OR "thermogenesis" OR "hibernation" OR "torpor" OR "vasoconstriction" OR "insulation" OR

"fur" OR "blubber" OR "arctic" OR "antarctic") NOT ALL=("diabetes" OR "obesity" OR "hypothermia"). The literature search followed PRISMA guidelines to ensure transparency in the study selection (figure s1) [52].

Initially, we screened the titles and abstracts to remove: review articles, book chapters, studies not focusing on cold adaptation, and behavioural-only studies without physiological or molecular analyses. After filtering, the remaining studies were retained for metadata extraction and downstream analyses. For each remaining publication, we manually extracted a set of metadata variables describing the study. These included: publication year, country, continent, biogeographical region, taxonomic classification (species, genus, family, order, class), study type (genetic, physiological, or both), study system (wild / non-model organism, model organism, mixed), physiological traits investigated (e.g., thermogenesis, hibernation, metabolic rate), and genes studied. We later grouped physiological traits into broader categories according to shared physiological functions associated with cold adaptation. These categories included thermoregulation, metabolic regulation, heat production, torpor/hibernation, and acclimation. To identify research trends in our remaining dataset, we generated summary statistics and visualizations to describe taxonomic representation across mammalian orders, the most frequently studied physiological traits, and the most frequently studied genes. Analyses and figures were produced in R v4.4.1 using packages primarily from the tidyverse ecosystem and ggplot2, with maps used for geographic visualizations[53–55]. The R scripts are provided in the electronic supplementary material.

(b) Candidate gene selection

The studies from our curated dataset reported 478 unique genes through different study designs using transcriptomic, candidate gene, and genome-scan approaches. The majority of these reflect genes correlated with cold response instead of components of cold adaptation pathways such as thermogenesis. Most of the reported genes spanned pathways from thermogenesis to immune response. Instead of assessing all genes individually, we performed a functional enrichment analysis using ShinyGO v0.85.1 on 30.01.2026 to identify significantly enriched pathways in the candidate gene dataset [56]. We selected the human annotation considering its extensive functional annotation. Since our gene list consists of many different species, mapping to the human annotation maximizes the proportion of genes that can be assigned to the pathways. Enrichment returned 228 significantly enriched KEGG pathways (table S2). Thermogenesis (hsa04714) was the second most significantly enriched pathway overall (FDR

163 = 1.7×10^{-21}), ranking below Pathways in cancer (hsa05200). We then decided to restrict our
164 analysis to genes assigned to a specific pathway, the thermogenesis pathway (KEGG
165 hsa04714), which has a well-defined role in cold adaptation and all 37 genes assigned to it
166 formed the candidate panel [57–59]. Retained genes were screened for encoding compartment,
167 and four mitochondrially encoded (ATP6, ND3, ND4, COX2) genes were excluded from
168 further analysis as the mitochondrial genome acts as a single non-recombining linkage unit
169 with its own genealogy [60]. Remaining 33 nuclear genes span the principal components of the
170 thermogenic pathway: oxidative phosphorylation (ATP5F1A, ATP5F1B, ATP5MC1,
171 NDUFA1, NDUFB8, NDUFS2, NDUFV2, COX4I1, COX7A1, UQCRB, SDHA, SDHB),
172 fatty-acid transport and oxidation (ACSL1, CPT1A, CPT1B, CPT2, SLC25A20), lipid storage
173 (PLIN1), uncoupling (UCP1), transcriptional regulation (PPARG, PPARGC1A, PRDM16,
174 CREB5, CREB3L1, CREB3L4), cAMP signalling (ADCY3, ADCY7, ADCY8), growth-
175 factor signalling (FGFR1, SOS1, SOS2) and metabolic sensing (PRKAA1, SIRT6) (full gene
176 names and functional categories can be found in table S3, KEGG; hsa04714) [57,58].

177 **(c) Ortholog identification and recovery**

178 To construct the candidate gene dataset, we first assembled a reference query protein set for
179 the 37 candidate genes identified above from the greater mouse-eared bat (*Myotis myotis)*
180 reference genome (NCBI RefSeq assembly: GCF_014108235.1) [61]. The reference set is used
181 to find orthologs in publicly available bat genomes. We used this dataset as queries in BLASTP
182 searches using BLAST v2.17.0 [62]. RefSeq mammalian proteins were downloaded from
183 NCBI in June 2026 and were used to create a local BLAST database. This database was
184 searched using a BLASTP approach, restricting our search to bats (Chiroptera, taxid: 9397)
185 with an E-value threshold of $1x10^{-10}$. To avoid paralog selection, we constructed gene-specific
186 annotation pattern files. We filtered hits if the sequence title matched the pattern, percent
187 identity and query coverage were ≥ 70%, and if the subject/query length ratio was between
188 0.70 and 1.30. We discarded sequences annotated as “PARTIAL” or “LOW QUALITY
189 PROTEIN”. After initial filtering, we selected a single protein sequence per species and gene
190 through a custom Python script (supplementary file). Rather than taking the longest isoforms
191 of each gene, we selected the isoform whose length was closest to the query sequence because
192 selecting the longest isoform in many cases resulted either in a paralogue or an isoform
193 containing a non-homologous N-terminus. We manually retained two accessions failing a
194 single filter, both for *Cnephaeus nilssonii*, because no alternative candidate was available for
195 this species in both genes. In cAMP responsive element binding protein 3 like protein 1 gene

(CREB3L1), the sequence label carried “LOW QUALITY PROTEIN” but showed 97.5% identity and complete coverage. Relatedly, in cytochrome c oxidase subunit 7A1 (COX7A1), the sequence fell outside the length ratio range but showed 73% identity and 97.5% coverage. Protein and protein-coding sequences (CDS) of each selected protein accession were downloaded from NCBI using EDirect v13.4 [63]. Retrieved sequences were aligned using MAFFT v7.505 using “--auto” parameter [64,65]. We visually inspected the protein alignments in AliView v1.31 [66]. We changed four accessions having non-homologous regions caused by alternative isoform models with compatible alternatives. In three gene-taxon combinations, the absence of a usable orthologue could not be solved by replacement. Hence, we excluded these taxa from the cAMP responsive element binding protein 3 like 4 (CREB3L4) and COX7A1 from the corresponding gene alignments only: CREB3L4: *Pteropus vampyrus*, *Rousettus aegyptiacus*; COX7A1: *Cnephaeus nilssonii* (table S6). We further validated orthologs by reciprocal best-hit searches against the complete *Myotis myotis* proteome (61,156 sequences). In each case (951/951), the best hit corresponded to the target gene, confirming our ortholog retrieval method (supplementary tables 4 and 5). We then re-aligned our corrected accession set. To preserve the codon reading frame for further analyses, codon-based alignments were generated using PAL2NAL v14, converting protein alignments into their corresponding nucleotide sequences into codon alignments suitable for evolutionary analyses [67]. For alignment trimming, we followed [68], using Gblocks v0.91b for codon-based trimming, with -t=c -b1=(n+1)/2+2 -b2=(n+1)/2+4 -b3=8 -b4=15 -b5=a parameters, where n is the number of sequences in the alignment. Because block-based trimming operates on alignment columns and cannot detect errors confined to individual taxa, trimmed codon alignments were additionally filtered for sequence-specific erroneous segments using TAPER v1.02 [69], applied to all genes and all taxa. Alignment filtering and sequence-specific masking statistics are shown in table S7.

Gene trees were inferred from the trimmed codon alignments with IQ-TREE2 v2.3.6 automated model selection with 1000 ultrafast bootstrap and 1000 SH-aLRT replicates [70–72]. Individual gene trees were first generated for each locus and subsequently combined using ASTRAL V5.7.8 with default settings to infer a species tree [73,74]. For each gene, we pruned the species tree to the taxa present in that gene’s alignment. As codon models estimate branch lengths internally, we removed branch lengths and support values manually [75]. ASTRAL species tree was visualized using the ape and ggtree packages in R [76,77]. Trees are shown

rooted at the split between Yangochiroptera and Yinpterochiroptera for representation purposes only; all codon-model analyses were treated as unrooted.

Since cold adaptation in bats is observed in more than one vespertilionid lineage, we identified and tested seven foreground branches rather than a single a priori set (table 1). These comprised: the *Cnephaeus nilssonii* terminal branch alone (H1); the *Cnephaeus* / *Eptesicus* stem branch (H2); the *Cnephaeus* and *Eptesicus* terminal branches (H3); the stem branch leading to the *Myotis* clade (H4); the terminal branches of all seven *Myotis* species (H5); the entire Myotis clade comprising its stem and all internal branches (H6); and the Vespertilionidae stem branch (H7) (table 1). Stem and whole-clade labels were applied only when the requested foreground taxa formed a monophyletic group in the pruned gene tree. Gene–hypothesis combinations failing these conditions were not tested, so the number of tests per family is 198 rather than the maximum 231.

Sites containing gaps or ambiguous nucleotides were removed automatically during downstream analyses using the cleandata = 1 option in PAML. Tests for positive selection were conducted using codeml from the PAML package v4.9 [78]. All analyses were performed using codon-based substitution models (seqtype = 1) with the F3×4 codon frequency model (CodonFreq = 2). Unless otherwise specified, default parameters were used. Key parameter settings comprised: runmode = 0, fix_blength = 0, seqtype = 1, CodonFreq = 2, fix_kappa = 0, kappa = 2, fix_alpha = 1, alpha = 0, cleandata = 1. To identify codon sites evolving under positive selection across the phylogeny, several site models implemented in PAML were applied. Site models were fitted to each gene alignment using the unlabelled pruned species tree. Positive selection at the site level was assessed with the following likelihood ratio tests: M1a (Nearly Neutral) vs M2a (Positive Selection), M7 (Beta) vs M8 (Beta + ω), and M8a vs M8 [79,80]. For each foreground definition, branch-site model A was fitted in its null (ω_2 fixed at 1) and alternative (ω_2 estimated) forms and compared by likelihood ratio test [81]. In addition, two-ratio branch models (model = 2, NSsites = 0) were fitted for each foreground and compared with M0. Likelihood ratio tests were used to evaluate statistical significance, calculated with $2\Delta\ln L = 2(\ln L_{alt} - \ln L_{null})$ formula through a custom Python script. Statistical significance was assessed using likelihood ratio tests. Site model comparisons M1a vs M2a and M7 vs M8 were assessed with two degrees of freedom, and the branch models against χ^2 with one degree of freedom. For M8a vs M8 and for branch-site model A tests, in which ω is tested at the boundary of its range, the null distribution was taken as a 50:50 mixture of a point mass at zero and χ^2 [81]. P-values were corrected for multiple testing using the Benjamini-

Hochberg False Discovery Rate (FDR) procedure applied separately within each test family [82], implemented in statsmodels v0.14.5 through a Python script [83]. Site model comparisons were corrected independently of each other (M1a vs M2a, M7 vs M8, M8a vs M8; n = 33 genes each), and branch-site and branch tests were corrected within their own families (n = 198 each).

Both nominal p-values and BH-adjusted q-values are reported; significance of the results was assessed at $q < 0.05$. Sites under positive selection were identified from Bayes empirical Bayes (BEB) posterior probabilities under M8 and branch-site model A, at thresholds of 0.95 and 0.99 [84]. BEB sites are interpreted only for comparisons that were significant after FDR correction in which $\omega > 1$, since a site estimated at $\omega < 1$ provides no evidence for positive selection[75,78]. A complete workflow summary can be found in figure s2. All constructed figures were further edited using Inkscape v.1.4.4[85].

3. Results

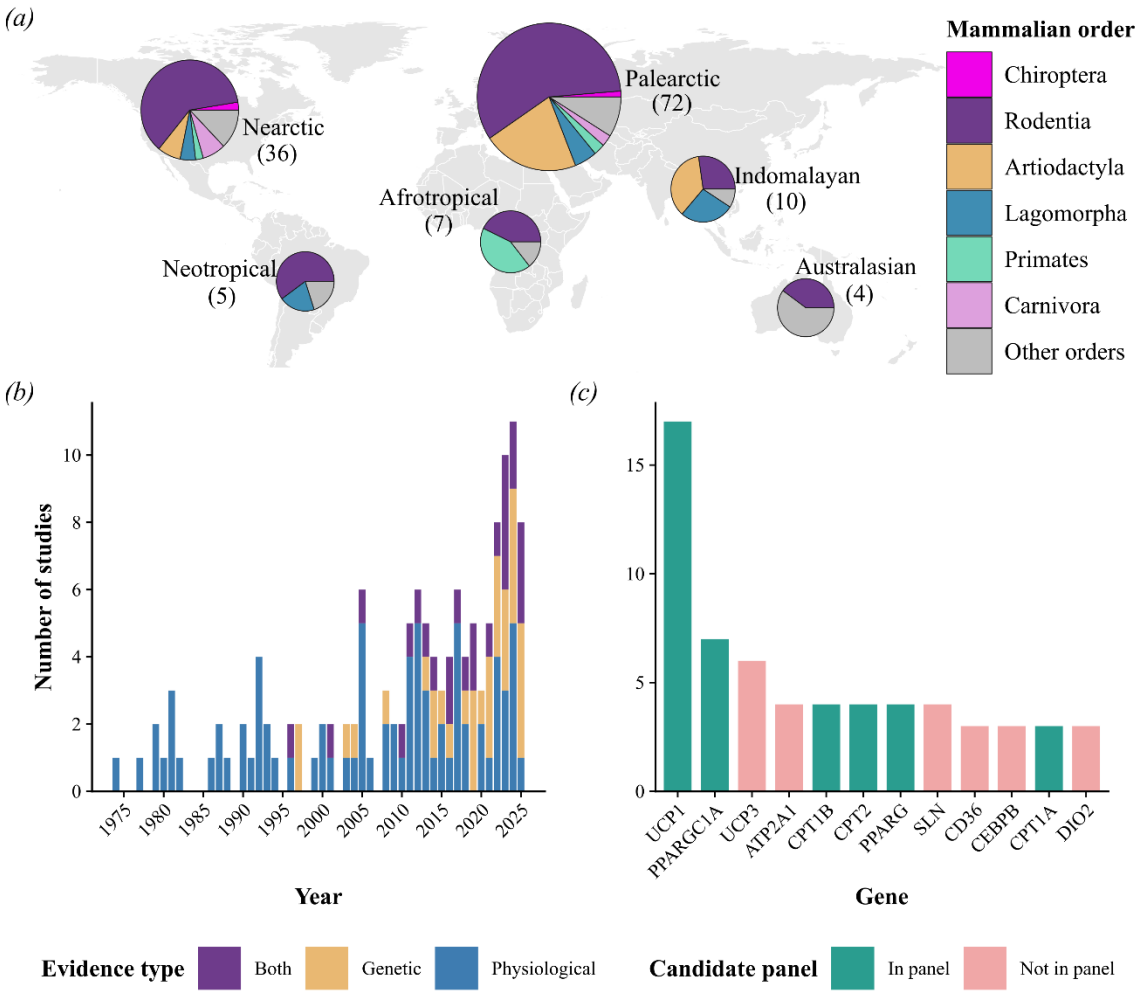

Figure 1. Publication trends within the mammalian cold adaptation literature. (a) Geographic and taxonomic distribution of mammalian cold adaptation studies across major biogeographical regions. Pie charts represent the proportional contribution of mammalian orders within each region. Numbers in parentheses indicate total studies per region. (b) Number of studies shown per year between 1974 and 2025. Colours indicate study type categories including genetic, physiological and combined studies. (c) Occurrence of the 12 most frequently reported genes in the reviewed literature. Genes included in the final curated candidate gene dataset are shown in green.

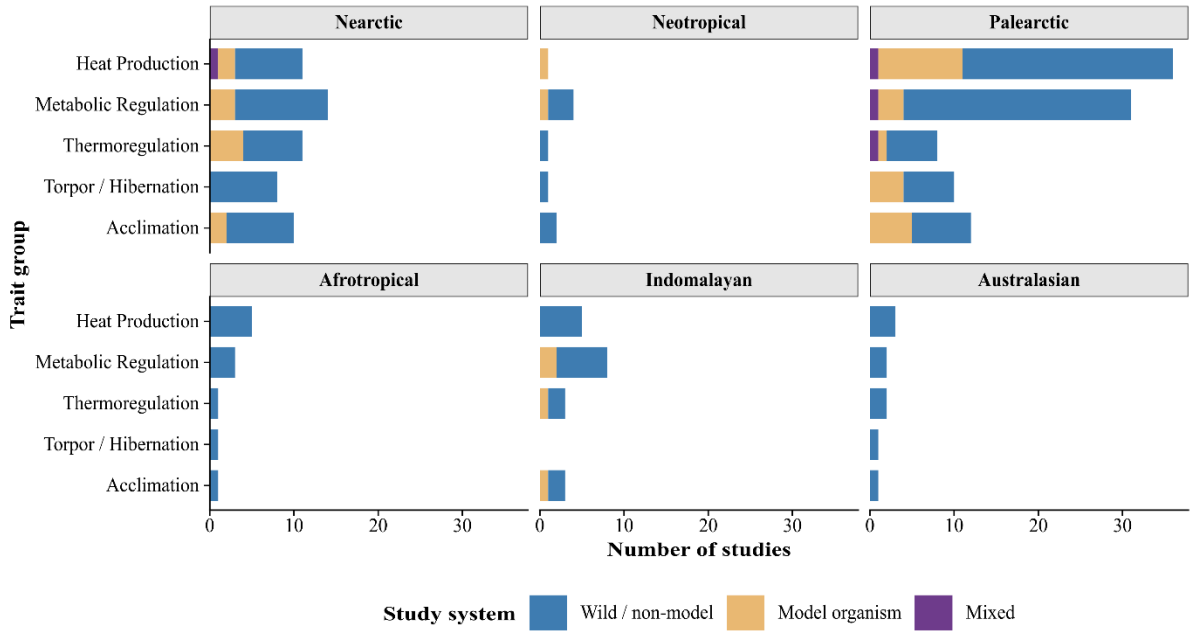

Figure 2. Distribution of grouped physiological traits across biogeographical regions, separated by study systems within the literature. Colours indicate categorized study systems.

(a) Systematic review

The initial Web of Science search resulted in a total of 571 publications. After screening and excluding irrelevant studies, the final curated paper dataset comprised 137 studies investigating physiological and genetic mechanisms associated with mammalian cold adaptation. The full table can be found in supplementary table 1. These studies were published between 1974 and 2025 and represented 30 countries across six major biogeographical regions. The number of studies investigating mammalian cold adaptation increased substantially over time (figure 1b). More specifically, relatively few studies were published before the 1990s, whereas publication

frequency increased consistently during the early 2000s and accelerated further during the last decade. Throughout this period, physiological studies dominated the literature; however, the number of studies investigating genetic mechanisms increased after 2010, corresponding with the broader usage of genomic and transcriptomic approaches together in ecological and evolutionary research. In detail, among the included studies, 80 focused primarily on physiological mechanisms, 32 investigated genetic mechanisms, and 25 combined both approaches (figure 1b). Most studies were conducted on wild or non-model mammals (72.3%), whereas studies using model organisms represented 24.8% of the dataset (table S1). Geographically, research effort was unevenly distributed, as shown in figure 1a. The majority of studies were conducted in the Palearctic (n = 72) and Nearctic (n = 36) regions, whereas comparatively few studies originated from the Afrotropical, Indomalayan, Australasian, and Neotropical regions. Taxonomically, the literature encompassed 19 mammalian orders, 62 families, 129 genera, and 289 species entries. Across all regions, Rodentia represented the most frequently studied mammalian order, accounting for the largest proportion of the dataset. Other commonly represented orders included Artiodactyla, Carnivora, and Lagomorpha. In contrast, several mammalian lineages were represented by only a small number of studies. Although Chiroptera was represented in the dataset, studies focusing on bats were limited compared to frequently investigated groups such as Rodentia and Artiodactyla (figure 1a).

The physiological mechanisms investigated across the dataset were dominated by traits associated with metabolic regulation and heat production (figure 2). Particularly, grouped physiological categories related to thermogenesis, metabolic regulation, and NST were the most frequently represented across all biogeographical regions. Studies investigating BAT, metabolic rate, acclimation, torpor, and hibernation were also common. In contrast, traits associated with insulation and heat conservation, including fur characteristics and heat loss reduction, were less frequently investigated. Across geographical regions, physiological studies generally outnumbered genetic studies, although mixed physiological-genetic approaches became more common in recent years. Turning to genetic mechanisms, 52 studies reported specific genes associated with cold adaptation, representing 478 unique genes in total (table S1). Exceptionally, 426 (89.1%) genes were reported in a single study, indicating that only a small portion of the genes were investigated repeatedly (figure 1c). The UCP1 gene was by far the most frequently studied, appearing in 17 studies, followed by PPARGC1A (7), UCP3 (6), ATP2A1 (4), CPT1B (4), CPT2 (4), PPARG (4), and SLN (4). These studies also had uneven taxonomic distribution.

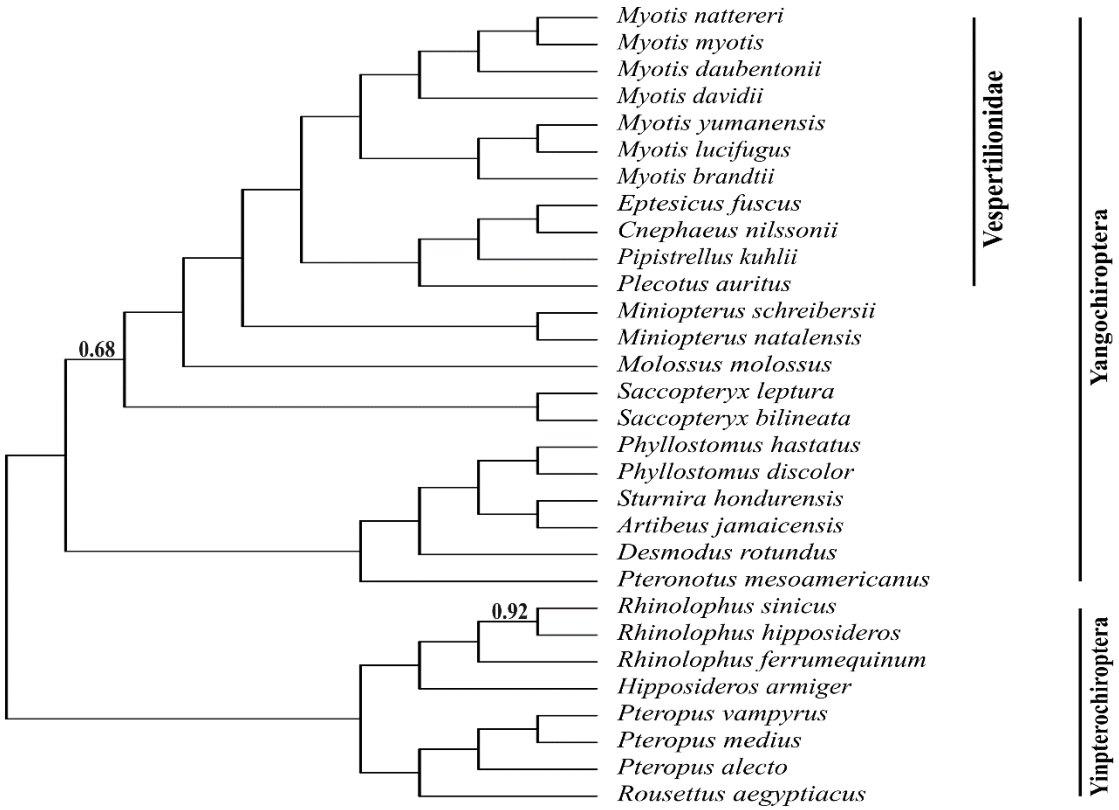

Figure 3. ASTRAL species tree inferred from the 33 nuclear candidate genes. ASTRAL local posterior probabilities are shown only for nodes with support values below 0.95. The family Vespertilionidae and the suborders Yangochiroptera and Yinpterochiroptera are shown on the right.

Table 1. Foreground branches labelled for branch-site tests.

Symbol	Foreground	Type	Branches labelled
H1	*Cnephaeus nilssonii*	terminal	1
H2	*Cnephaeus* + *Eptesicus MRCA*	stem	1
H3	*Cnephaeus, Eptesicus*	terminal	2
H4	*Myotis MRCA*	stem	1
H5	*seven Myotis species*	terminal	7
H6	*Myotis clade whole clade*	whole clade	stem + internal
H7	*Vespertilionidae MRCA*	stem	1

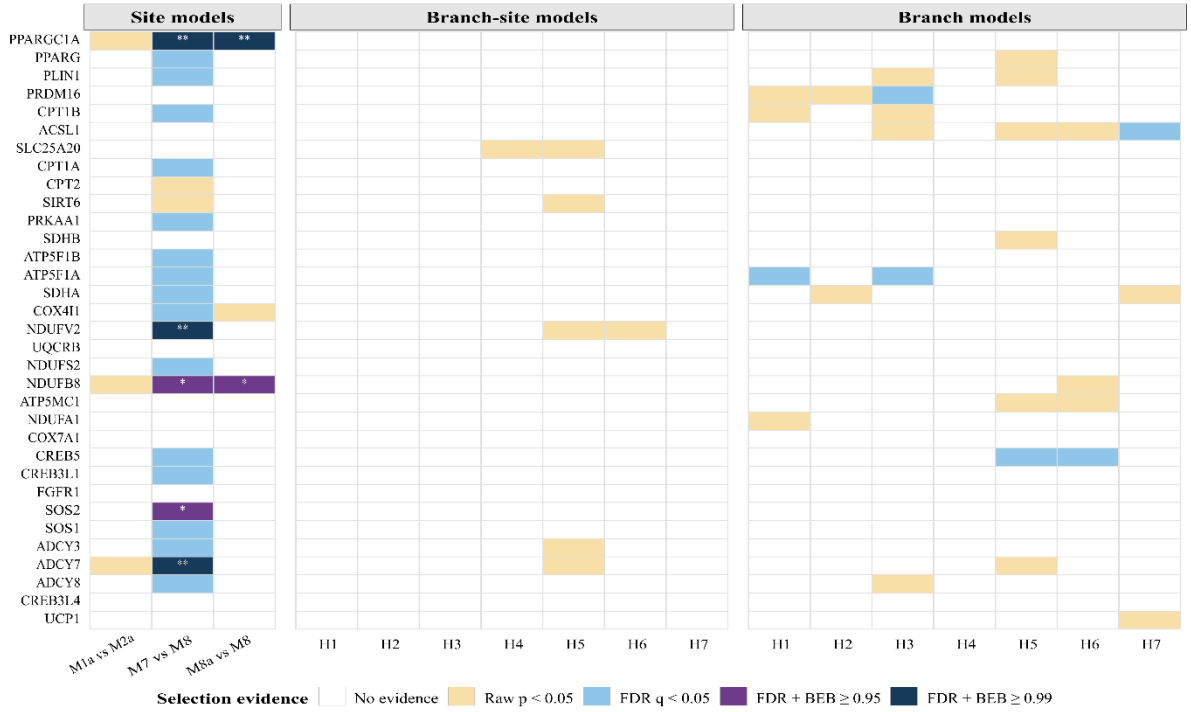

Figure 4. Heatmap summarizing codon-model tests across candidate genes. Cell colours indicate significance of each test: FDR-significant tests ($q < 0.05$) with at least one BEB-supported site are shown for genes where $\omega > 1$. * BEB posterior probability ≥ 0.95; ** ≥ 0.99.

Table 2. Significant site-model results for the six genes in the M7 vs M8 test after FDR correction and an estimated $\omega > 1$. q denotes the FDR-adjusted p-value, and the proportion of sites refers to the estimated proportion assigned to the $\omega > 1$ site class under M8. BEB-supported positively selected sites are reported for posterior probabilities ≥ 0.95 (* ≥ 0.95; ** ≥ 0.99).

Gene	2ΔlnL	q	ω	Proportion of sites	Positively selected sites
NDUFB8	10.01	0.014	3.280	0.019	138V*
PPARGC1A	30.60	1.25×10^{-6}	2.002	0.040	388V*, 436P**, 438A*
COX4I1	13.29	0.004	1.816	0.042	–
SOS2	15.05	0.002	1.291	0.014	1167Y*
ADCY7	32.82	4.92×10^{-7}	1.189	0.008	6R**
NDUFV2	12.44	0.005	1.135	0.135	2F**

(b) Orthologue recovery and phylogenetic reconstruction of candidate genes

Orthologues were recovered for all 33 nuclear candidate genes across 24–30 bat species per gene, yielding 951 sequences in total (table S5). Taxon representation varied among genes. UCP1 had the lowest representation with 24 species. The reference genome assemblies used for the analyses can be found in table S4.

The ASTRAL species tree, used as the fixed phylogenetic framework, recovered the tree topology consistent with previously published bat phylogenies (figure 3)[86–88].

(c) Site and lineage-specific codon-model analyses

Three site-model comparisons varied in the number of genes that remained statistically significant after FDR correction (figure 4). The M1a vs M2a tests resulted in no significant genes, whereas the M8a vs M8 test resulted in two statistically significant genes. By contrast, the M7 vs M8 test resulted in 20 statistically significant genes. Among 20 genes significant under the M7 vs M8 tests six had estimated $\omega > 1$ values: NDUFB8, PPARGC1A, COX4I1, SOS2, ADCY7 and NDUFV2 (figure 4 and table 2). Five had ≥ 0.95 BEB support on the site-level tests except the COX4I1 gene (figure 4). Moreover, two genes, PPARGC1A and NDUFB8, were statistically significant in M8a vs M8 tests, providing the strongest site-model support for positive selection. Site model parameter estimates for all 33 genes are provided in table S9.

Several genes had estimated $\omega > 1$ values under M8 despite non-significant M7 vs M8 comparisons (table S8). Notably, UCP1, which was often reported in the mammalian cold adaptation literature, had an estimated $\omega = 1.5$ for the additional site class under the M8 model, but the M7 vs M8 test was not significant ($p = 0.962$, $q = 1$). Furthermore, M1a versus M2a was also non-significant ($p = 1.0$), providing no site-model support for positive selection in UCP1 (table S8 and S9).

At the lineage-specific site level, none of the 198 branch site tests remained significant after FDR correction across the seven foreground hypotheses (figure 4, table S8). Unlike the branch-site tests, six of 198 tests were statistically significant after FDR correction. These tests involved four genes: CREB5 under H5 and H6, ATP5F1A under H1 and H3, ACSL1 under H7, and PRDM16 under H3 (figure 4 and table 1). However, foreground ω estimates ranged from 0.04 to 0.46 and remained below 1 in all significant tests, indicating differences in the strength of purifying selection rather than evidence of positive selection (table S8).

4. Discussion

(a) Cold-adaptation literature is biased

Our synthesis shows that mammalian cold adaptation research is strongly shaped by taxonomic, geographic, and mechanistic biases (figures 1 and 2). Although our curated dataset covered a wide taxonomic range of mammals, research was concentrated predominantly in the order Rodentia, and studies were mostly conducted within the Palearctic and Nearctic regions (figure 1a). Our approach using only English-language search terms might have limited the number of publications found in other languages and contributed to the apparent Nearctic and Palaearctic bias. This pattern points to the historical dominance of rodents in laboratory experiments on thermoregulation and metabolism, as well as to the concentration of research efforts and funding in temperate and northern ecosystems, where severe winter conditions are predictable [89,90]. As a result, our current understanding of mammalian cold adaptation may be biased toward mechanisms characteristic of a relatively small subset of taxa rather than the complete diversity of mammalian responses to cold environments. This concentration does not, however, reflect reliance on laboratory model organisms: 72.3% of studies used wild or non-model mammals (figure 2), suggesting that the taxonomic bias arises primarily from which wild taxa are studied.

In the literature, mammalian cold adaptation is extensively studied in environments that have seasonal winters and prolonged cold exposure, potentially emphasizing mechanisms such as torpor, NST, and BAT activity [24,37,49]. In contrast, comparatively fewer studies have examined cold tolerance in tropical, high-altitude, or climatically variable systems, where selective pressures and energetic constraints may differ substantially [27,91–93]. Thus, geographic bias toward northern temperate regions may also affect how cold adaptation itself is interpreted within the literature, leaving important alternative physiological strategies comparatively underrepresented in current research.

The predominance of thermogenesis-related and metabolism-related traits across the literature further reflects the strong emphasis on energetic regulation in mammalian cold adaptation literature (figure 2). In particular, the high frequency of studies investigating BAT, NST, and metabolic rate may partly reflect the experimental tractability of these systems and their strong association with measurable physiological responses to cold exposure. Rodent model organisms have been especially influential in establishing thermogenic pathways as a primary framework for understanding cold adaptation. However, this concentration on canonical

thermogenic mechanisms may unintentionally narrow the scope of the field. Traits associated with insulation, circulatory regulation, and other mechanisms of heat conservation were comparatively less represented despite their potential ecological importance in many mammalian systems. This imbalance suggests that current literature may favour mechanisms that are experimentally accessible rather than those that are most important across all taxa.

A similar pattern emerged in the genetic literature. Although 478 unique genes associated with cold adaptation were reported, research attention was strongly concentrated on a limited number of repeatedly studied thermogenesis-related genes, particularly UCP1 and PPARGC1A (figure 1c). This focus is unsurprising given the established importance of these genes in mitochondrial metabolism and heat production [39,94]. Moreover, repeated research on a relatively small set of candidate genes may also show broader limitations of traditional candidate-gene approaches. Most studies focused on previously characterized thermogenic genes and pathways, and potentially may leave important genes outside these pathways understudied. Nevertheless, an important proportion of the genes can be categorized into similar metabolic and thermogenic pathways. This may indicate that key pathway systems were studied repeatedly rather than specific genes. The dominance of UCP1 in this literature makes its behaviour in our own analysis particularly notable.

Although a wide range of taxa are represented within the literature, studies on bats are relatively limited. This is particularly notable given their ecological diversity and unusual energetic demands, as bats are the only mammals capable of powered flight [45,46]. These characteristics make bats informative systems for studying how metabolism, thermoregulation, and environmental adaptation are connected. This motivated our analysis of coding-sequence evolution in thermogenesis-related genes across bat lineages. Nevertheless, bats remain understudied compared to rodents and other mammal groups, likely due to challenges associated with field sampling, limited genomic resources for non-model species, and historical dominance of model-organism studies. As genomic resources for bats continue to expand, comparative analyses across cold-adapted and non-cold-adapted lineages will provide important opportunities to explore alternative mechanisms of mammalian cold adaptation.

(b) Limited evidence for coding-sequence adaptation in bats

Our codon-model analyses did not reveal a widespread pattern of positive selection across thermogenesis-related genes in cold-adapted bat lineages. Instead, evidence for adaptive

evolution across the 33 candidate genes was limited. Across the predefined cold-associated foreground lineages, no significant evidence of episodic positive selection was detected.

Parallel to the site-level support, two-ratio branch models did detect shifts in ω on foreground branches in six tests; however, these differences reflected changes in the strength of selective constraint rather than positive selection, as all foreground estimations remained below 1. The lack of significant branch-site results suggests that the predefined cold-associated lineages did not exhibit strong evidence of episodic positive selection in the candidate gene dataset. However, this does not prohibit the possibility of other types of molecular adaptations which could not be detected by these models [78]. In contrast, these differences did not indicate positive selection. A similar pattern has previously been reported in hibernating bats. Han et al. [95] found no evidence for positive selection of branch-site in the PPARα gene among hibernating bat lineages but detected a stronger selective constraint in one of the lineages. Additionally, the same study also showed that during torpor, PPARα expression increases, suggesting that, at the molecular level, hibernation may include changes in selective constraint and may not necessarily result in positive selection in the coding sequence.

The functional distribution of genes under positive selection was involved in different components of the thermogenesis pathway. These included genes involved in mitochondrial regulation (PPARGC1A), oxidative phosphorylation (NDUFB8, NDUFV2 and COX4I1) and signalling pathways (ADCY7 and SOS2) (table S3). However, since site models do not test specific cold-adapted lineages, these signals cannot be directly related to cold adaptation.

Apart from coding-sequence evolution, molecular changes associated with cold adaptation may also occur at the level of gene expression and regulation. For example, Villanueva-Canas et al. [96] identified shared metabolic pathways among hibernating mammals but found no enrichment of positive selection signal within the coding sequence of hibernation-related genes. Correspondingly, Faherty et al. [97] found significant changes in gene expression during torpor and active state in dwarf lemurs even though they had no statistical support for positive selection of hibernation-related genes. Together, these findings suggest that changes in gene regulation may contribute to cold-adaptation-related phenotypes even when positive selection within the coding sequence is limited.

Importantly, UCP1 provides an example of this distinction pattern. Although it has a prominent role in the mammalian cold adaptation literature, UCP1 showed no significant positive selection evidence in our site, branch-site, and branch model analyses. The lack of selection

signal does not imply that UCP1 has a limited role in cold adaptation. Previous analyses of the UCP gene family showed that these genes are largely conserved under strong purifying selection, with positive selection restricted to a single site [98]. Hence, the absence of positive selection in UCP1 in our analyses may be because of the conserved nature of the protein.

(c) Limitations

Our systematic literature review was based on a single bibliographic database searched using English-language search terms. Studies focusing on hibernation, torpor, or seasonal physiology without explicitly using cold adaptation terminology may have been underrepresented. Consequently, this may have contributed to the taxonomic and geographical biases observed within the literature. Secondly, for the molecular analyses, our approach was based on a candidate gene dataset rather than a genome-wide dataset. We selected only a portion of the curated genes, and the selected genes reflected the existing research focus on well-studied pathways. Thus, our candidate-gene dataset cannot provide a complete picture of the genetic basis of cold adaptation. Moreover, our codon model approach cannot detect regulatory or expression-level changes. The predefined foreground lineages may not correspond to all cold adaptation events.

5. Conclusion

With this study, we combined a systematic review of mammalian cold adaptation with analysis of candidate thermogenesis-related genes in bats. The review revealed strong mechanistic, taxonomic and geographic biases in the literature, particularly towards rodents, northern temperate regions and thermogenic mechanisms such as NST and BAT. Furthermore, bats remained comparatively underrepresented. Across the candidate genes identified within this study, our molecular analyses provided limited evidence for positive selection. Taken together, these findings suggest that the molecular basis of mammalian cold adaptation is likely to be more diverse than the current literature emphasis on a small number of canonical thermogenic genes implies, involving lineage-specific coding changes as well as regulatory, expression-level and polygenic responses. Future studies integrating whole-genome and transcriptomic data could test whether regulatory and expression-level changes contribute to cold adaptation in bats.

Author contributions (CRediT taxonomy)

Gamze Özbay: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing.

Thomas M. Lilley: Conceptualization, Supervision, Writing – review & editing.

Veronika N. Laine: Conceptualization, Supervision, Writing – review & editing.

Chris Heilakka: Data curation, Writing – review & editing.

Mariia Matlova: Data curation, Writing – review & editing.

Ella Sippola: Data curation, Writing – review & editing.

Alexandra Streicher: Data curation, Writing – review & editing.

Tanya Troitsky: Data curation, Writing – review & editing.

Victoria G. Twort: Data curation, Writing – review & editing.

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Competing interests

The authors declare no competing interests.

AI use declaration

AI-assisted tools were used to improve the readability and language of the manuscript and to assist with debugging and optimizing custom Python and R scripts. ChatGPT (OpenAI) and Grammarly were used for these purposes. All code suggestions were reviewed, tested, and validated by the authors. AI tools were not used to generate scientific interpretations or conclusions. The authors take full responsibility for the content of the manuscript.

Data availability

Data supporting the findings of this study are provided within the article and its electronic supplementary material.

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Figure S1. PRISMA diagram illustrating the screening process for publications identified in the Web of Science database. *Created in BioRender. Özbay, G. (2026) https://BioRender.com/6qot9j0*

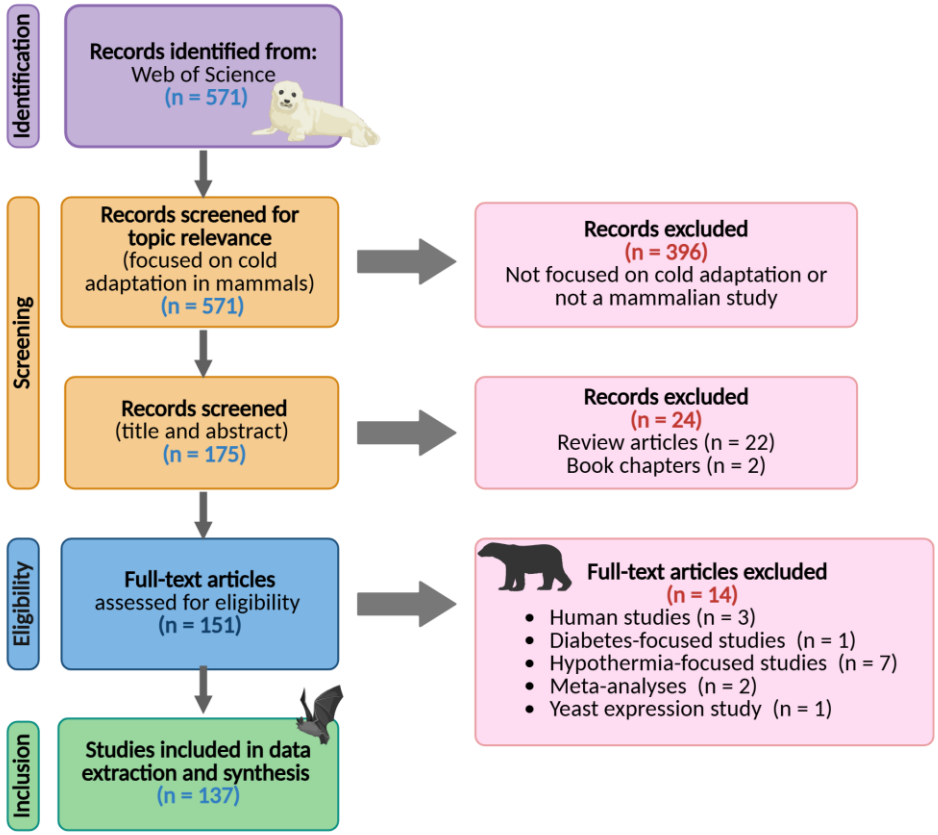

Figure S2: Overview of the workflow for candidate-gene selection, sequence recovery and codon model analyses. *Created in BioRender. Özbay, G. (2026) https://BioRender.com/m3annco*