

**Charles University**

**Faculty of Science**

Study programme: Zoology



**Mgr. Miroslava Loudová**

Population Genetics and Speciation Processes of European Hedgehogs

Populační genetika a speciační procesy evropských ježků

Doctoral thesis

Supervisor: Mgr. Barbora Černá Bolfíková, Ph.D.

Advisor: prof. RNDr. Pavel Hulva, Ph.D.

Praha, 2025

**Prohlášení:**

Prohlašuji, že jsem závěrečnou práci vypracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 16. 2. 2025

**Declaration of originality:**

I declare that this thesis or its substantial part has not been submitted to obtain the same or any other academic degree. I have written it independently based on the material cited in the text and in consultation with my supervisor.

Prague, 16<sup>th</sup> February 2025



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**Abstract:**

Hedgehogs of the genus *Erinaceus* are key models for studying population genetics, phylogeography, and speciation in the Western Palearctic. This thesis integrates genome-wide data to reconstruct phylogenetic relationships within the Erinaceidae family, revealing that the divergence of *E. europaeus*, *E. roumanicus*, *E. concolor*, and *E. amurensis* occurred during the Pleistocene, driven by climatic fluctuations. Phylogeographic analyses highlight the role of glacial refugia in shaping postglacial expansions, with distinct mitochondrial lineages corresponding to the Iberian, Apennine, and Balkan refugia. A newly identified Asian lineage of *E. roumanicus* suggests a Ponto-Caspian refugium. Contact zones between *E. europaeus* and *E. roumanicus* in Central Europe and the Russian-Baltic region demonstrate asymmetric gene flow and limited hybridization, likely constrained by reproductive barriers. In populations in sympatry we identified skull size reduction, suggesting competitive interactions and convergent character displacement. Additionally, landscape genetics reveals fine-scale population structure influenced by geographic barriers and anthropogenic factors. These findings contribute to a deeper understanding of hedgehog evolutionary history, speciation processes, and the impact of environmental and genetic factors on population differentiation.

**Key words:** *Erinaceus*, phylogeography, population genetics, secondary contact zone

## **Abstrakt:**

Ježci rodu *Erinaceus* slouží jako modelový druh pro studium populační genetiky, fylogeografie a speciace ve západním Palearktu. Tato práce obsahuje studii zabývající se rekonstrukcí fylogenetických vztahů v rámci čeledi Erinaceidae, přičemž odhaluje, že divergence *E. europaeus*, *E. roumanicus*, *E. concolor* a *E. amurensis* proběhla během pleistocénu v důsledku klimatických oscilací. Fylogeografické analýzy zdůrazňují roli glaciálních refugií při formování postglaciálních expanzí, přičemž odlišné mitochondriální linie odpovídají iberskému, apeninskému a balkánskému refugiu. Nově identifikovaná asijská linie *E. roumanicus* naznačuje existenci ponto-kaspického refugia. Kontaktní zóny mezi *E. europaeus* a *E. roumanicus* ve střední Evropě a v rusko-baltském regionu vykazují asymetrický tok genů a omezenou hybridizaci, pravděpodobně ovlivněnou reprodukčními bariérami. V sympatrických populacích jsme identifikovali redukci velikosti lebky, což naznačuje konkurenční interakce a konvergentní posun znaků. Kromě toho krajinná genetiky odhaluje jemnou populační strukturu ovlivněnou geografickými bariérami a antropogenními faktory. Tyto poznatky přispívají k hlubšímu porozumění evoluční historii ježků, procesům speciace a vlivu environmentálních a genetických faktorů na diferenciaci populací.

**Klíčová slova:** *Erinaceus*, fylogeografie, populační genetiky, sekundární kontaktní zóna

## List of publications (and author contribution statement):

Černá Bolfíková B, Eliášová K, Loudová M, Kryštufek B, Lymberakis P, Sándor AD, *et al.* (2017). Glacial allopatry vs. postglacial parapatry and peripatry: The case of hedgehogs. *PeerJ* **4**: e3163.

- performed the laboratory work, contributed to samples/reagents/materials, contributed to the final version

Ploi K, Curto M, Černá Bolfíková B, Loudová M, Hulva P, Seiter A, *et al.* (2020). Evaluating the Impact of Wildlife Shelter Management on the Genetic Diversity of *Erinaceus europaeus* and *E. roumanicus* in Their Contact Zone. *Animals* **10**: 1452.

- data curation, investigation, writing—review & editing

Černá Bolfíková B, Evin A, Knitlová MR, Loudová M, Sztencel-Jabłonka A, Bogdanowicz W, *et al.* (2020). 3D geometric morphometrics reveals convergent character displacement in the central european contact zone between two species of hedgehogs (genus *Erinaceus*). *Animals* **10**.

- contributed to genetic characterization of the samples, contributed to the final version

Eliášová K, Lucas Lledó JI, Grau JH, Loudová M, Bannikova AA, Zolotareva KI, *et al.* (2022). Contrasting levels of hybridization across the two contact zones between two hedgehog species revealed by genome-wide SNP data. *Hered* **2022** *1295* **129**: 305–315.

- performed the laboratory work, contributed to samples/reagents/materials, contributed to the final version

Loudová M, Eliášová K, Curto M, Baránková L, Meimberg H, Hulva P, & Černá Bolfíková B. Microsatellite sequencing reveals cryptic landscape patterns in European hedgehogs. *Journal of Vertebrate Biology* (submitted).

- designed the study, performed the laboratory work, analysed the data, contributed to samples/reagents/materials, drafted the manuscript, and contributed to the final version

## Taxonomy and distribution range

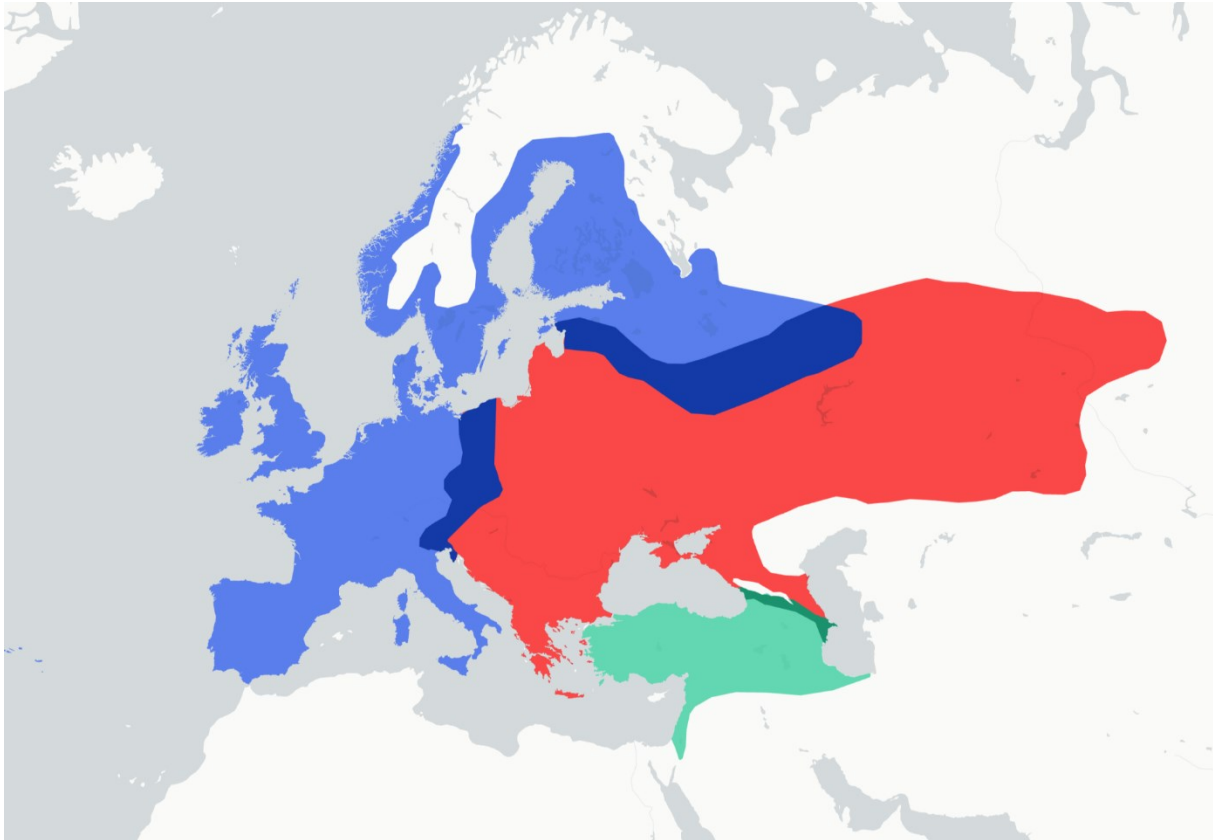
The order Eulipotyphla includes insectivorous mammals classified into four families: Erinaceidae (hedgehogs and gymnures), Talpidae (moles), Soricidae (shrews), and Solenodontidae (solenodons). The Erinaceidae family consists of 27 species divided into two distinct subfamilies: Galericinae (moonrats and gymnures), and Erinaceinae (spiny hedgehogs) (Wilson & Mittermeier 2018, Shi et al. 2023).

Four species fall into the genus *Erinaceus* and all of them naturally occur in the Palearctic region. Less known *E. amurensis* (Schrenk, 1859) inhabits East Asia (Cassola 2016) and other three species live in Western Palearct. These are *E. europaeus* (European Hedgehog; Linnaeus, 1758), *E. roumanicus* (Northern White-breasted Hedgehog; Barrett-Hamilton, 1900) and *E. concolor* (Southern White-breasted Hedgehog; Martin, 1837). *Erinaceus concolor* can be found in Turkey, Transcaucasia and Levant (Amori et al. 2021a). *Erinaceus europaeus* inhabits southwestern and western Europe, including the British Isles, Italy, central Europe to Poland, and extends north through Scandinavia into northwestern Russia. This species was also introduced into many islands, like the Azores or New Zealand (Gazzard & Rasmussen 2024, Pipek et al. 2020). *Erinaceus roumanicus* occurs in central, southeastern, and eastern Europe extending to Kazakhstan (Amori et al. 2021b). The distribution of European species is shown more precisely on the map (Fig. 1).

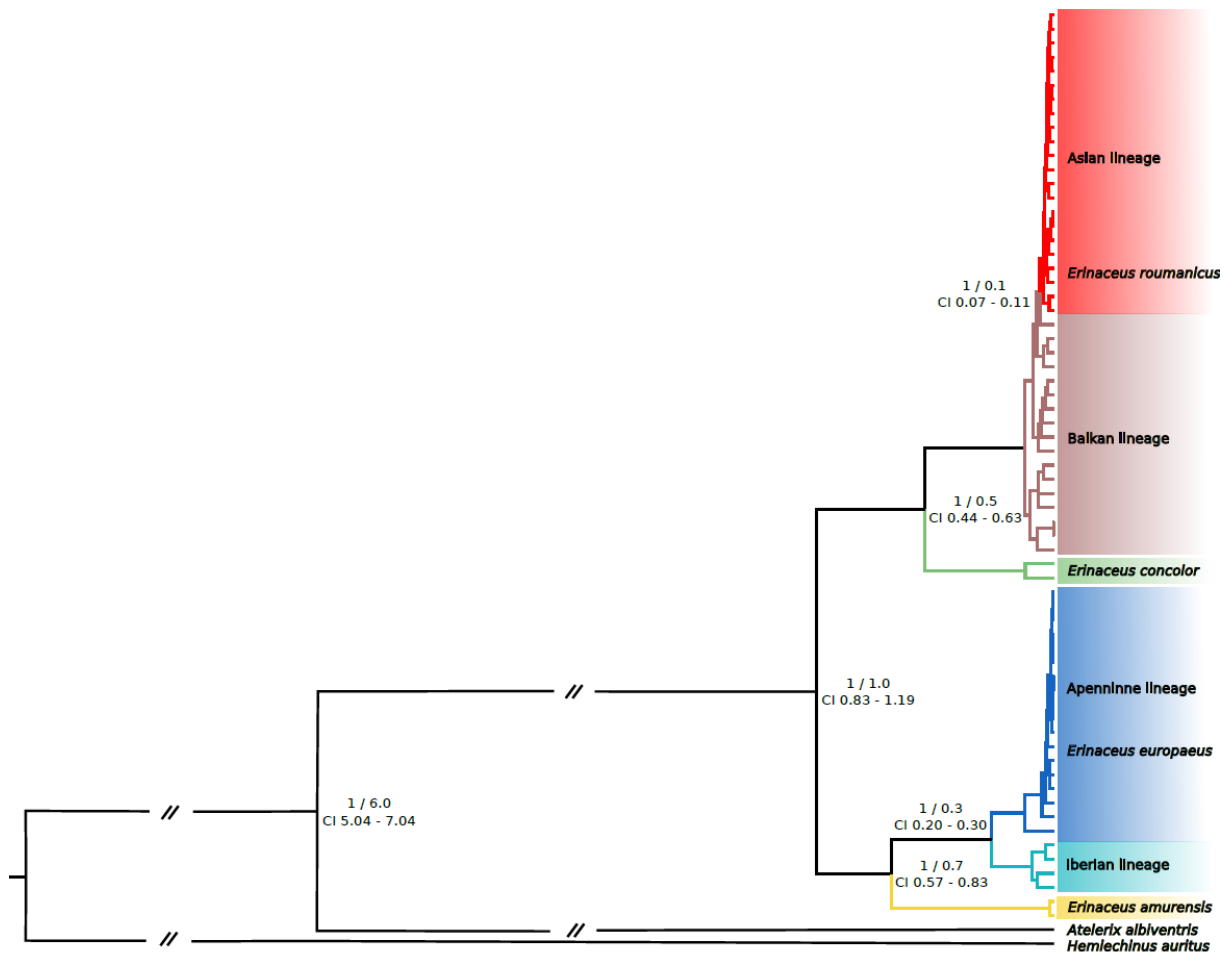
Phylogeographic research can uncover cryptic lineages, often prompting a reevaluation of taxonomic classifications. This was the case for hedgehogs, where *E. roumanicus* was historically considered a subspecies of *E. concolor* (as *Erinaceus concolor roumanicus*). However, molecular analyses (e.g., Berggren et al. 2005; Seddon et al. 2001, 2002) provided strong evidence for distinct genetic lineages, leading to the taxonomic revision and recognition of *E. roumanicus* as a separate species.

In our study, we have reconstructed the phylogenetic relationships within the Erinaceidae family using genome-wide data for the first time (Fig. 2), providing greater resolution and stronger support than previous studies (Eliášová et al. 2022). Traditional phylogeographic studies utilizing a limited number of genetic markers proposed that speciation occurred prior to the Pleistocene (Hewitt 1999, Seddon et al. 2001). However, our genome-wide SNP analysis suggests that the divergence of all four species took place during the Pleistocene, indicating

that climatic fluctuations were likely the primary driver of their speciation. Both the Iberian and Apennine lineages of *E. europaeus*, as well as the Balkan lineage of *E. roumanicus* proposed by Seddon et al. (2001) and Hewitt (1999), were identified. Moreover, a new Asian lineage of *E. roumanicus* was detected (Fig.2), suggesting that the divergence between the Asian and Balkan lineages began during the last glacial period (Eliášová et al. 2022).



**Fig. 1 Map** of hedgehog species in the genus *Erinaceus* occurring in the Western Palearctic. The species are represented by the following colours: blue represents *E. europaeus*, red represents *E. roumanicus*, and green represents *E. concolor*. Contact zones are illustrated with dark shades. Figure is based on an IUCN distribution map of species (Amori et al. 2021a, 2021b; Gazzard & Rasmussen 2024) and it was created via Mapshaper and Kepler.gl.



**Fig. 2 Bayesian phylogeny** analysis of the genus *Erinaceus*, *Atelerix* and *Hemiechinus*. Posterior probabilities from MrBayes indicating support of each node are reported before the forward slash symbol while the age of each node is reported after. CI represents 95% HPD intervals estimated and the estimates are given in million of years.

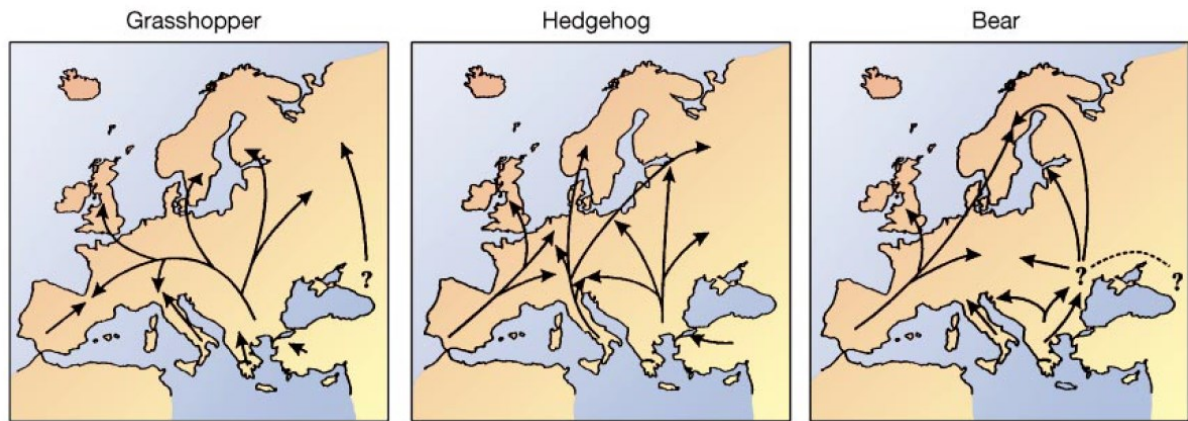
## Phylogeography

Historically, fossils and pollen data have formed the basis for studying the history of species distribution (Taberlet et al. 1998). With the advancement of genetic methods starting in the 1970s, these methods began to be used to describe intraspecific genetic variation across different taxa of organisms. For animals, mitochondrial DNA (mtDNA) has been particularly useful due to its structural and inheritance characteristics. As a haploid and clonally inherited genome, mtDNA has an effective population size ( $N_e$ ) that is four times smaller than that of nuclear DNA, leading to a higher rate of lineage sorting. Moreover, its elevated mutation rate

enhances the resolution of phylogeographic studies, allowing for the reconstruction of past demographic events and population histories with greater detail. These properties have made mtDNA an essential marker in early phylogeographic research, providing insights into species' evolutionary trajectories, migration patterns, and historical refugia (Hewitt 2001, 2004). However, despite its advantages, mtDNA represents only the maternal lineage and can be influenced by selection or introgression, necessitating the integration of nuclear markers for a more comprehensive understanding of genetic structure and evolutionary history. This advancement led to the emergence of phylogeography, a discipline that investigates the historical processes shaping the spatial distribution of genetic lineages over time (Avise et al. 1987, Avise 1998).

The Quaternary period is characterized by climatic oscillations, with ice ages periodically interrupted by relatively short humid warm interglacial periods, such as the one we are experiencing today (Hewitt 1996). Glacial periods seem to have had a significant impact on most organisms in temperate regions, but the extent of their influence on species distributions depended on latitude and topographical features (Hewitt 1996, 2004). In the northern hemisphere, extension of ice sheets influence the species ranges mostly in north-south direction and in Europe, three main refugia have been initially identified on the Mediterranean southern peninsulas: the Iberian, Apennine, and Balkan (Hewitt 1996). Such contractions and expansions of species ranges had an impact on their genetic diversity and population structure (Emerson & Hewitt 2005).

As mid-sized insectivores with low basal metabolic rates, hedgehogs are highly responsive to temperature fluctuations and ecosystem productivity, so they can serve as a classic model for Mediterranean refugial dynamics. Studies on the genus *Erinaceus* have revealed postglacial expansions from all major European refugia: the Iberian and Apennine Peninsulas (including Sicily) for *E. europaeus* and the Balkan Peninsula for *E. roumanicus*, as four corresponding mitochondrial clades were identified (Černá Bolfíková et al. 2020, Hewitt 2001; Santucci et al. 1998; Seddon et al. 2001). It is suggested that *E. concolor* had refugia in northern Turkey or the southern Caucasus (Seddon et al. 2002).



**Fig. 3** Three paradigm postglacial colonizations from southern Europe deduced from DNA differences for the grasshopper, *Chorthippus parallelus*, the hedgehog, *Erinaceus europeus/roumanicus*, and the bear, *Ursos arctos*. The main refugial areas, Iberia, Italy, the Balkans and Caucasus, contributed differently to the repopulation of northern parts (Hewitt 2004).

With advancements in genetic techniques and additional species being investigated, additional extra-Mediterranean refugia have been identified for thermophilic species, particularly in Eastern Europe (Kotlík et al. 2006). Or phenomena known as refugia-within-refugia occurring in Iberian peninsula, where multiple glacial refugia exist within each larger refugial area (Abellán & Svenning 2014). Furthermore, microrefugia have been recognized in Central Europe, including the Carpathian Basin and the Alps (Schmitt & Varga 2012). This is consistent with our findings, as an Asian lineage of *E. roumanicus* with a probable refugium in the Ponto-Caspian region was recognized (Eliášová et al. 2022).

Investigating refugial areas and recolonization routes in detail is crucial for understanding the fine-scale processes shaping genetic diversity and population structure. Although hedgehogs are well-established models of postglacial recolonization, the specific mechanisms influencing their population differentiation have not been fully explored using high-resolution genetic markers and detailed sampling. Our study examined the impact of glacial isolation and postglacial expansion on the population structure of the Northern White-breasted hedgehog (*Erinaceus roumanicus*), with a particular focus on processes occurring at the edges of its distribution. Analysis of mitochondrial and nuclear markers across Central Europe, the Balkan Peninsula, and surrounding islands revealed pronounced population subdivision, especially in insular populations of the Mediterranean and the Central European contact zone with *E.*

*europaeus*. The genetic diversity varied across regions, with low diversity in Crete and the highest genetic distances observed in Romania. The complex genetic structure of post-refugial populations, particularly in the Pannonian Basin and along the Adriatic and Pontic coasts, suggests that range expansion is shaped not only by geographic barriers such as the Carpathians but also by microevolutionary forces acting during both glacial contraction and interglacial expansion (Černá Bolfíková et al. 2017).

## Contact zones and hybridization

Speciation is an evolutionary process that includes splitting of parental lineage into descendent lineages and finally leads to reproductive isolation of the divergent species. Allopatric speciation, characterized by the complete geographic isolation of lineages, was considered the most common type of speciation (Coyne & Orr 2004). However, advancements in genomic methodologies have increasingly demonstrated that speciation with gene flow is more frequent than previously assumed (Smadja & Butlin 2011). Recent advancements have improved methods for detecting hybridization and ancestry inference, yet challenges remain in estimating gene flow magnitude and timing, hybrid fitness, and recombination effects (Payseur & Rieseberg 2016).

The isolation of species in glacial refugia facilitated allopatric speciation, while rapid post-glacial recolonization may have led to parapatry and the establishment of secondary contact zones (Bolfíková & Hulva 2012).

The first contact zone of European hedgehogs (*E. europaeus*) and Northern white-breasted hedgehog (*E. roumanicus*) is located in Central Europe, and the second one is in the Russian-Baltic region. As the contact zones differ in ages, a valuable framework for investigating speciation dynamics and the mechanisms of reproductive isolation is offered.

The Central European contact zone was established approximately 10,000 – 12,000 years ago in response to Neolithic deforestation and extends across Poland, Germany, the Czech Republic, Austria, and Italy (Bolfíková & Hulva 2012, Seddon et al. 2001). This broad sympatric area exhibits a mosaic hybridization pattern, primarily influenced by ecological differences between the two species. Historically, *E. europaeus* dominated the Czech Republic (Kratochvíl

1966, 1975); however, recent genetic analyses suggest a continuous range expansion of *E. roumanicus* into this region (Bolfíková & Hulva 2012). Although both species occasionally co-occur, hybridization remains rare (Bolfíková & Hulva 2012, Černá Bolfíková et al. 2017, Curto et al. 2019, Eliášová et al. 2022).

The Russian-Baltic contact zone is considered to be more recent than the Central European zone and is likely associated with climatic shifts during the Sub-Boreal period (5,000 – 2,000 years ago) (Hewitt 1999; Seddon et al. 2001; Sommer 2007). This zone extends across Latvia, Estonia, and Russia. In the Russian-Baltic region, approximately 20% of the Moscow population exhibits genetic admixture (Zolotareva et al. 2021).

The previously observed variation in hybridization levels in two contact zones was confirmed in our genome-wide analysis. Also asymmetrical gene flow from *E. europaeus* into *E. roumanicus* has been observed in both zones, likely due to pre- and/or post-mating reproductive barriers that limit hybridization and introgression towards *E. europaeus*. No loci that could be potentially influenced by selection causing reproductive isolation in the Russian-Baltic contact zone were identified (Eliášová et al. 2022).

## **Phenotypic variation**

Phenotypic traits are shaped by both abiotic and biotic factors. Under sympatric conditions, although environmental variables remain consistent, ecological, microallopatric, or trophic differentiation may still occur (Coyne & Orr 2004). Furthermore, as speciation advances, the integration of ancestral species into ecological networks evolves, with increasing competition between sister species, acting as a driver of niche differentiation (Reifová et al. 2011).

In our study we used longitudinal transect sampling spatially crossing region in Central Europe to eliminate the effect of clinal variation. In allopatric populations, *E. europaeus* exhibits a larger skull size and distinct cranial and mandibular morphology compared to *E. roumanicus*. However, in sympatric populations, individuals of both species display reduced skull sizes and increased morphological similarity, particularly in mandible shape. Our findings do not support a significant role for hybridization or clinal variation in driving these morphological patterns. Instead, we propose that competitive interactions between the species do not induce divergent selection strong enough to promote character displacement. Rather, in resource-limited environments, selection appears to favor a convergent reduction in skull size. Given

the complex adaptive landscape, this size reduction may also be influenced by predation pressure in ecosystems undergoing mesopredator release, as well as other anthropogenic factors. Since the mandible is primarily associated with feeding mechanics, whereas the cranium has broader functional roles, we interpret the observed mandibular similarity as an adaptation to overlapping dietary niches, indicative of convergent character displacement (Černá Bolfíková et al. 2020).

## **Landscape genetics**

Landscape genetics aims to understand how geographical and environmental factors shape genetic variation. Unlike traditional population genetics, which relies on predefined populations, landscape genetics adopts a spatially explicit approach, allowing genetic variation to be studied at both individual and population levels without the need for predefined units. This approach provides deeper insights into population connectivity, dispersal patterns, and local adaptation (Manel et al., 2003).

Our analysis uncovered fine-scale population structure in hedgehogs that was previously undetected using microsatellites. This improved resolution was attributed to the higher number of loci analysed with SSR-GBAS and the larger sample size. Bayesian clustering and landscape genetics approaches revealed a distinct southern cluster in South Bohemia and additional genetic differentiation along a west-east gradient. In *E. roumanicus*, increased sampling exposed further subpopulation divisions, particularly along the southern Czech border. Geographic barriers such as mountain ranges and rivers, along with human-made structures like roads and fences, likely influence genetic structure by restricting dispersal and reducing gene flow. Anthropogenic factors, including wildlife rescue and translocation, may blur these genetic boundaries, though further research is needed (Loudová et al. submitted).

Although European hedgehogs have been a subject of significant interest, there remains ample opportunity for further research and exploration in this field.

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## List of Annexes:

### Annex I.

Černá Bolfíková B, Eliášová K, Loudová M, Kryštufek B, Lymberakis P, Sándor AD, *et al.* (2017). Glacial allopatry vs. postglacial parapatry and peripatry: The case of hedgehogs. *PeerJ* **4**: e3163.

### Annex II.

Ploi K, Curto M, Černá Bolfíková B, Loudová M, Hulva P, Seiter A, *et al.* (2020). Evaluating the Impact of Wildlife Shelter Management on the Genetic Diversity of *Erinaceus europaeus* and *E. roumanicus* in Their Contact Zone. *Animals* **10**: 1452.

### Annex III.

Černá Bolfíková B, Evin A, Knitlová MR, Loudová M, Sztencel-Jablonka A, Bogdanowicz W, *et al.* (2020). 3D geometric morphometrics reveals convergent character displacement in the central european contact zone between two species of hedgehogs (genus *Erinaceus*). *Animals* **10**.

### Annex IV.

Eliášová K, Lucas Lledó JI, Grau JH, Loudová M, Bannikova AA, Zolotareva KI, *et al.* (2022). Contrasting levels of hybridization across the two contact zones between two hedgehog species revealed by genome-wide SNP data. *Hered* **2022 1295** **129**: 305–315.

### Annex V.

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