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Defining the eastern range limit of the semiterrestrial leech *Haemopis elegans* (Hirudinea: Haemopidae) in Ukraine: Morphology, phylogeny, and range modeling

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Abstract. The semiterrestrial leech *Haemopis elegans* Moquin-Tandon, 1846 is widely distributed across Europe, though its eastern range limit remains poorly defined. This study examines the hypothesis that environmental conditions east of the Lviv region in Ukraine are unsuitable for the establishment of *H. elegans*, resulting in its observed range limit. Specimens of *H. elegans* were collected from Pohulianka Park in Lviv, western Ukraine, and their morphological characteristics were examined. In addition, a specimen from Argentré, France, was included in the study. Molecular phylogenetic analysis was performed using mitochondrial DNA markers (cytochrome *c* oxidase subunit I) to confirm species identity, assess genetic diversity, and reveal phylogenetic relationships within the species. To predict the species' potential distribution under current environmental conditions, species distribution modeling was applied using occurrence records and key environmental variables. Morphological analysis confirmed the distinguishing features of *H. elegans*, such as body coloration and reproductive system traits, in comparison to the related and sympatric *H. sanguisuga* (Linnaeus, 1758). Our findings reveal distinct genetic differences among populations within the species' range, including the identification of new haplotypes in Ukraine and France. Both morphological and molecular data support the co-occurrence of these two *Haemopis* species in Ukraine. Species distribution modeling indicates low habitat suitability for *H. elegans* east of its known range, while field surveys and genetic data confirm its absence or discontinuity in those areas.

Keywords. Annelida, Clitellata, morphology, phylogenetics, genetic diversity, species distribution modeling, biogeography, Pohulianka, Argentré, Diohtianets.

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Introduction

Leeches of the genus *Haemopis* Savigny, 1822 are important members of freshwater communities in the western Palearctic and Nearctic regions. Despite their superficial similarity to their blood-feeding relatives in the genera *Hirudo* Linnaeus, 1758 and *Macrobdella* Verrill, 1872, *Haemopis* leeches are macrophagous predators, consuming a variety of invertebrates and smaller vertebrates, such as amphibians (Lukin 1976; Sawyer 1986; Kvist *et al*. 2023).

The semiterrestrial leech *Haemopis elegans* (Hirudinea: Hirudinida: Hirudiniformes: Haemopidae) is widely distributed across Europe, inhabiting both freshwater and moist terrestrial habitats. Originally described by Moquin-Tandon (1846) as *Haemopis sanguisuga* var. *elegans*, the taxon was rediscovered by Grosser (2004) in the floodplain of the Danube in Bavaria, Germany. Since then, its presence has been confirmed in other parts of Germany, Austria, Switzerland, Hungary, and Slovenia (Grosser 2004; Grosser & Pešić 2006; Kutschera *et al.* 2007; Hassl & Kleewein 2011; Jueg & Zettler 2015). Additional records from France (Lecaplain & Noël 2019), Serbia (Grosser & Pešić 2006), and Italy (Bagno 2023) suggest a broader distribution across Europe.

Morphological, ecological, and phylogenetic studies have established *H. elegans* as an independent species, distinct from the related *H. sanguisuga* (Linnaeus, 1758) (Grosser 2004; Kvist *et al*. 2023). According to the International Code of Zoological Nomenclature (ICZN), its correct name is *Haemopis elegans* Moquin-Tandon, 1846. A key ecological distinction of *H. elegans* is its greater tendency to remain on land compared to *H. sanguisuga*. Additionally, *H. elegans* can be differentiated by its much darker black ventral side, in contrast to its lighter dorsum, along with a distinct dark segmentally arranged dorsal pattern (Grosser 2004; Kvist *et al*. 2023).

Historically, research on *H. elegans* has primarily focused on its populations in western and central Europe, while its distribution in eastern Europe, particularly in Ukraine, has yet to be documented. Understanding the genetic diversity and environmental constraints shaping the distribution of *H. elegans* in eastern Europe is crucial for clarifying its range limits and ecological traits.

This study explores the hypothesis that environmental conditions east of the Lviv region in western Ukraine are unsuitable for the establishment of *H. elegans*, resulting in its observed range limit. The hypothesis leads to specific, testable predictions: that species distribution modeling will indicate low habitat suitability for *H. elegans* east of its currently known distribution, and that field surveys and genetic data will confirm its absence or discontinuity in those areas.

We integrated morphological, phylogenetic, and ecological approaches to delineate the geographical distribution of *H. elegans*. Specimens collected from Lviv were examined morphologically and analyzed phylogenetically using a mitochondrial DNA marker, cytochrome *c* oxidase subunit I (COI). Additionally, specimens of *H. elegans* from France and *H. sanguisuga* from areas located east of Lviv were included in the morphological and molecular phylogenetic analyses. Species distribution modeling was also employed to outline the potential distribution of *H. elegans* under current environmental conditions. By combining these methods, this study seeks to clarify the factors that limit the eastern range of *H. elegans*.

Material and methods

Sample collection

Two specimens of *H. elegans* were collected from a wet pedestrian path during an excursion in a planted beech forest in Pohulianka Park (Lviv, Ukraine) on April 30th, 2022. One specimen was pre-relaxed in a 10% ethanol solution before being fixed in 96% ethanol. Both samples were preserved in 96%

ethanol for further morphological and molecular phylogenetic analysis. Additionally, a specimen of *H. elegans* from Argentré, France, collected by Franck Noël in a plant nursery on December 12th, 2018 was examined by molecular methods. For comparison, a specimen of *H. sanguisuga* collected in the Diohtianets River, Vinnytsia, Ukraine, also preserved in 96% ethanol, was included in the study. All specimens are stored in the invertebrate collection of the Department of Zoology and Animal Ecology at V.N. Karazin Kharkiv National University.

Morphological analysis

The redescription of *H. elegans* by Grosser (2004) and the study by Kvist *et al*. (2023) were referenced to identify the species based on morphological characteristics. For measuring key parameters and indices of systematic importance, S. Utevsky's (2014) doctoral dissertation was consulted. The following parameters and indices describe the body shape characteristics that may have systematic value: L represents the total length of the body; D is the maximum width of the body; C1 refers to the horizontal diameter of the posterior sucker; C2 denotes the vertical diameter of the posterior sucker; M is the length of the anterior part of the sucker; R indicates the length of the posterior part of the sucker; D refers to the width at the point where the posterior sucker is attached; L/D is the ratio of the total body length to its maximum width, which characterizes the relative elongation of the body; C1/C2 is the ratio of the horizontal to vertical diameters of the posterior sucker, used to characterize the shape of the sucker; R/M is the ratio of the length of the posterior part of the sucker to the anterior part, which indicates the eccentricity of the sucker's attachment to the urosome. Measurements were taken using Digimizer Version 5.4.1 (MedCalc) and AxioVision rel. 4.8.2.0 (Zeiss). The reproductive system characteristics were examined through dissections.

Distribution analysis

To predict the geographical range of *H. elegans*, data were sourced from scientific publications, the Global Biodiversity Information Facility (GBIF) and the iNaturalist platform. Only records accompanied by photographs clearly showing diagnostic coloration traits of *H. elegans* were included in the analysis (Table S1). The species' range was modeled using MaxEnt software, with spatial mapping performed in QGIS.

It should be noted that citizen science platforms such as iNaturalist have been recognized as valuable sources of biodiversity data, although concerns regarding potential sampling bias are often raised. In the present study, the influence of such bias is considered minimal. All iNaturalist records of *H. elegans* included in our dataset were verified through photo-based identification by taxonomic experts, substantially reducing the likelihood of misidentification. Furthermore, these data were complemented with records from the literature and our own field collections, thereby broadening both the geographic and taxonomic coverage of the dataset. Taken together, these factors suggest that any residual sampling bias in the dataset is unlikely to have significantly influenced the distribution model or the conclusions derived from it.

Environmental variables

Nineteen bioclimatic variables were obtained from WorldClim (version 2.1), derived from monthly temperature and rainfall data interpolated from global land area climate point data spanning 1950 to 2000 (Fick & Hijmans 2017). The data layers were clipped to a geographic extent ranging from 30 to 60 degrees north latitude and from 10 degrees west to 40 degrees east longitude. To reduce collinearity among variables, we selected a subset of less correlated variables with Pearson's correlation coefficients below 0.8, following the recommendations of Kumar & Stohlgren (2009) and Merow *et al*. (2013). The virtualspecies package for R (Leroy *et al*. 2015) facilitated this selection process. The selected bioclimatic variables include: BIO2, Mean Diurnal Range (Mean of monthly (max temp – min temp)); BIO6, Minimum Temperature of the Coldest Month; BIO7, Temperature Annual Range (BIO5–BIO6); BIO8, Mean Temperature of the Wettest Quarter; BIO14, Precipitation of the Driest Month; BIO15, Precipitation Seasonality (Coefficient of Variation); and BIO19, Precipitation of the Coldest Quarter. In addition, the elevation data were used. All layers used in the analysis had a spatial resolution of 2.5 minutes.

The distribution modeling used in this research predicts the species' occurrence based solely on environmental characteristics. Historical factors, such as human-mediated dispersal, cannot be accounted for by this approach. The analysis provides only the likelihood of occurrence under certain environmental conditions. Therefore, inferences regarding historical factors that may have facilitated the species' dispersal must rely on evidence beyond bioclimatic characteristics, such as direct observations of human activity.

Species distribution models

SDMs were fitted using the MaxEnt version 3.4.4 (Phillips *et al*. 2023). MaxEnt is a general purpose algorithm that generates predictions from an incomplete set of information. This approach assumes that the incomplete empirical probability distribution (based on the species occurrences) can be approximated by a probability distribution of maximum entropy subject to certain environmental constraints, and that this distribution approximates the potential geographic distribution of a species (Phillips *et al*. 2006).

To select the best model parameters different models were compared with a combination of the "feature class" and "regularization multiplier" (Morales *et al*. 2017). MaxEnt provides different types of restrictions ("feature class") in the modelling stage such as lineal (L), quadratic (Q), product (P), threshold (T), and hinge (H). Twelve combinations of these features (H, HQ, L, LQ, LQP, LQT, Q, QHP, QHT, QHTP, T and auto-feature and the regularization multiplier β from 1 to 5 by intervals of 1 were used. Combining features classes and regularization multipliers, a total of 60 models were assessed. ENMTools (Warren *et al*. 2010, 2011) was used to calculate the Bayesian (BIC) and sample size corrected Akaike Information Criteria (AICc) for each model. For the final model, we selected the one which (i) shows the lowest AICc, (ii) the largest average the Area under the Curve (AUC), and (iii) considered fewer parameters than observation samples (Gottwald *et al*. 2017). The results suggested that the best combination of parameters was LQP with a regularization multiplier of 1 (Table S2). To generate the final model, the crossvalidate option were used with ten replications.

Relative importance of individual predictors was assessed using jackknife tests that identify the environmental variable with the highest gain (i.e., improvement in penalized average log-likelihood compared to a null model) in the model performance when used in isolation (Elith *et al*. 2011).

Binary maps of suitable habitat were produced. For further analysis, binary maps of suitable habitat based on maximum training sensitivity plus specificity logistic threshold were used as recently recommended (Liu *et al*. 2013, 2016). All output maps were created using the software QGIS 3.22.14 Białowieża (www.qgis.com).

DNA Extraction, Amplification, and Sequencing

Molecular methods were applied to analyze two representatives of *H. elegans* and one *H. sanguisuga*. A small tissue sample was taken from the lateral part of each leech for DNA extraction. The mitochondrial cytochrome *c* oxidase subunit I (COI) gene, a standard fragment for DNA barcoding in animals (Hebert *et al*. 2003), was selected for analysis. DNA was extracted using the GeneElute Mammalian Genomic DNA Miniprep Kit, following the manufacturer's protocol.

Amplification of the COI gene was performed using the following primers (Folmer *et al*. 1994): LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse).

TABLE 1

Haplotype codes, frequencies, GenBank accession numbers, localities, and references used in constructing the network of *Haemopis elegans*.

Haplotype	Haplotype frequency	Accession number	Locality	Reference
1	1	PV210985	Lviv, Ukraine	This research
2	3	OK447798	Ljubljana, Slovenia	Kvist *et al*. (2023)
		OK447799	Ljubljana, Slovenia	
		OK447837	Ribnica, Slovenia	
3	1	PV210986	Argentré, France	This research
4	1	EF125042	Germany	Kutschera *et al.* (2007)

The polymerase chain reaction (PCR) was conducted according to the following protocol: 35 cycles of denaturation at 94°C for 45 sec, annealing at 48°C for 45 sec, and extension at 72°C for 1 min, followed by a final elongation step at 72°C for 3 min. PCR products were purified enzymatically using Exonuclease I and Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and sequenced bidirectionally with amplification primers by Macrogen Europe (Amsterdam, Netherlands). Chromatograms were processed with ChromasPro version 2.1.10 (Technelysium Pty Ltd, Australia), yielding sequences of 658 base pairs.

Phylogenetic analyses

Nucleotide sequence data from Kvist *et al*. (2023) and additional sequences (Kutschera *et al*. 2007; Meng *et al.* 2023) retrieved from GenBank were used to infer phylogenetic relationships within the genus *Haemopis* based on the mitochondrial COI gene. A total of 136 sequences were analyzed. The samples included in the phylogenetic analysis are listed in Table S3.

Sequence alignment was conducted using MAFFT version 7 (Katoh *et al*. 2019) with the “Auto” function, which selects the most appropriate alignment strategy (L-INS-i) based on the dataset. The aligned sequences were translated, and the presence of stop codons as well as amino acid substitutions was verified using MEGA 11 (Tamura *et al*. 2021).

The model selection and tree building were both conducted with the IQ-TREE web server (Nguyen *et al*. 2015; Trifinopoulos *et al*. 2016). ModelFinder (Kalyaanamoorthy *et al*. 2017) was used to select the optimal partitioning scheme (Chernomor *et al*. 2016) and the best-fit models for each codon position based on the Bayesian information criterion (BIC): TN+F+G for the first codon, F81+F+I for the second codon, and TIM3+F+G for the third codon. Phylogenetic relationships were reconstructed based on the Maximum Likelihood method. Statistical support for clades was assessed using ultrafast bootstrapping (Hoang *et al*. 2018) with 1000 replicates, as well as the SH-aLRT (Shimodaira-Hasegawa approximate likelihood ratio test; Guindon *et al*. 2010).

The DNA Sequence Polymorphism program DnaSP (version 6.12.03 x64) was used to identify haplotypes (Rozas *et al*. 2017). Minimum spanning haplotype networks were constructed with PopART (version 1.7) and default settings (Leigh *et al*. 2015). The sequence numbers and their corresponding haplotype codes are provided in Table 1. Between-group and within-groups mean distances were calculated in MEGA 11 with the p-distance method.

Results

Morphology

Body dimensions. In two specimens of *H. elegans* (Figs 1–2), the body length (L) and greatest width (D) exhibit slight variations: the first measures 60.63 mm in length and 10.05 mm in width, while the second measures 68.02 mm and 10.54 mm, respectively. The calculated ratio of body length to width (L/D), reflecting the relative proportions of the body, was 6.03 and 6.45 for the two specimens.

In contrast, a specimen of *H. sanguisuga* (Fig. 3) measured 65.04 mm in length and 8.97 mm in width, resulting in a significantly higher L/D ratio of 7.25 compared to *H. elegans*.

Sucker dimensions. In two specimens of *H. elegans*, the length of the anterior sucker (M), the posterior sucker (R), and the width at the attachment point of the posterior sucker (d) vary slightly. The first specimen has an anterior sucker length of 2.78 mm, a posterior length of 2.20 mm, and a width of 2.64 mm; the second specimen has M = 2.53 mm, R = 2.83 mm, and d = 2.92 mm.

The horizontal (C1) and vertical (C2) diameters of the posterior sucker, along with their ratio, show minor differences. For the first leech, C1 = 2.64 mm, C2 = 6.29 mm, and C1/C2 = 0.42; for the second, C1 = 2.92 mm, C2 = 6.82 mm, and C1/C2 = 0.43.

In *H. sanguisuga*, these parameters differ: C1 = 3.12 mm, C2 = 7.05 mm, and C1/C2 = 0.44.

In addition, the ratio of the dorsal to ventral sucker length was examined. In the first *H. elegans* specimen, R/M = 1.26, while in the second, it is 0.89. For *H. sanguisuga*, this ratio equals 0.88.

Body shape. *Haemopis elegans* are large, elongated leeches with a flattened body. The anterior sucker is weakly developed, while the posterior sucker is more prominent, though small in size (its diameter is smaller than the body's greatest width).

Under rough fixation conditions, the leech contracts, which is especially noticeable in the posterior sucker, leading to significant shape distortion. The second specimen was not pre-relaxed, which likely

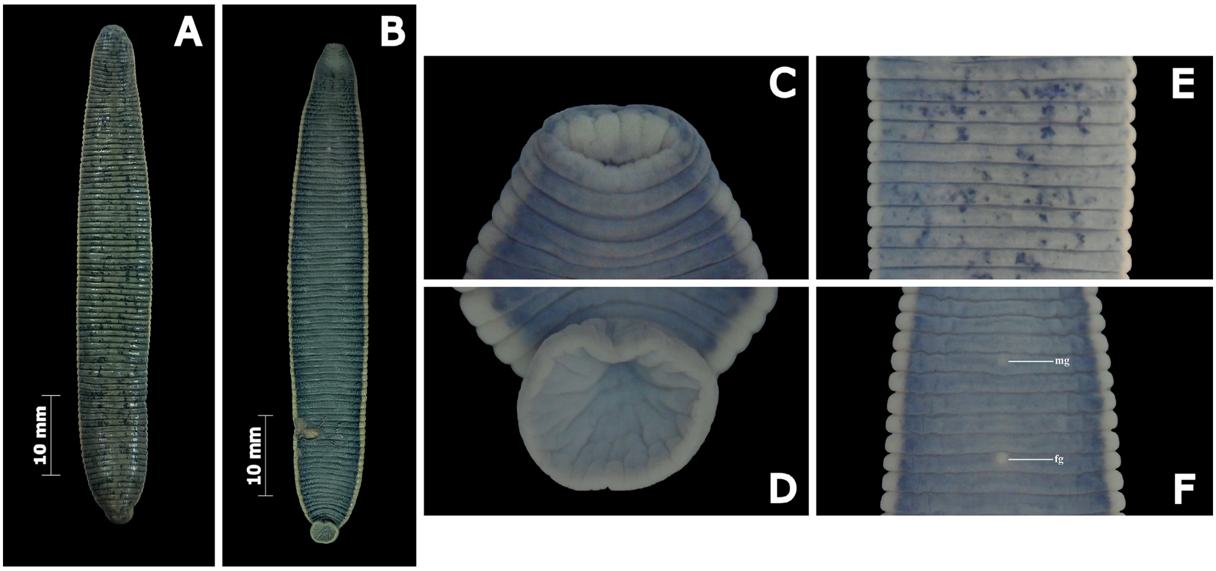

Figure 1 – *Haemopis elegans*, first analyzed specimen. **A**. Dorsal side. **B**. Ventral side. **C**. Anterior sucker. **D**. Posterior sucker. **E**. Dorsal pattern. **F**. Gonopore positions. Abbreviations: fg = female gonopore; mg = male gonopore.

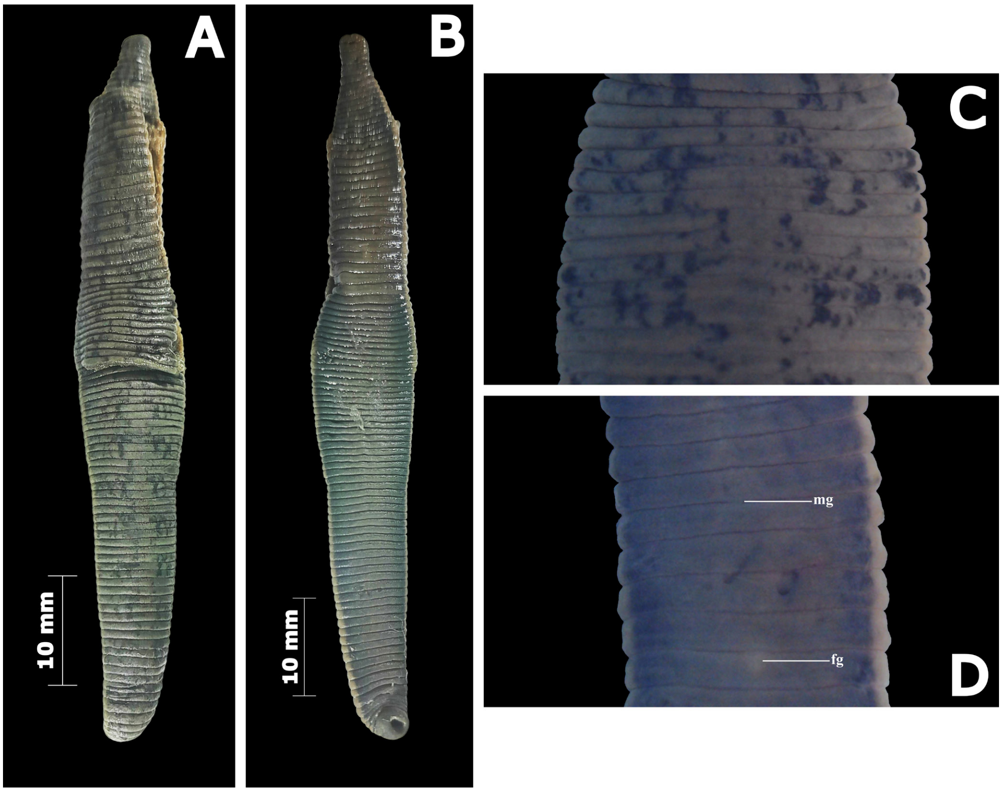

Figure 2 – *Haemopis elegans*, second analyzed specimen, partially dissected for anatomical study. **A**. Dorsal side. **B**. Ventral side. **C**. Dorsal pattern. **D**. Gonopore positions. Abbreviations: fg = female gonopore; mg = male gonopore.

Figure 3 – *Haemopis sanguisuga*. **A**. Dorsal side. **B**. Ventral side. Specimen partially dissected for anatomical study.

explains the differences in its posterior sucker measurements compared to the first, which was fixed by gradually increasing the ethanol concentration.

The body surface is smooth.

Body color. Dorsal surface olive green, with two paramedian clusters of black pigment dots per segment. The intensity of the dorsal pattern varies among individuals of the species. Two yellowish marginal stripes run along body margins, appearing more pronounced on the ventral surface. Ventral surface black in live individuals, lighter in preserved specimens, with paramarginal stripes composed of black spots (Figs 1–2, 4).

Annulation. The annuli are clearly visible. Segment is 5-annulate. The distance between the gonopores is 4 complete rings.

Reproductive system

We compared the reproductive system of the Ukrainian specimen of *H. elegans* with that of a specimen from Slovenia described by Kvist *et al*. (2023) and with *H. sanguisuga* collected by us in Vinnytsia, Ukraine (Fig. 5). The specimens of *H. elegans* share a similar structure of the penis sheath: it is robust, short, folded once. In contrast, *H. sanguisuga* shows significant differences, with a notably longer penis sheath. Regarding the vagina, slight differences were observed within *H. elegans*. In the Slovenian specimen, the vagina is straight, whereas in the Ukrainian specimen, it is approximately the same size but bent to the side. In *H. sanguisuga*, the vagina is more elongated.

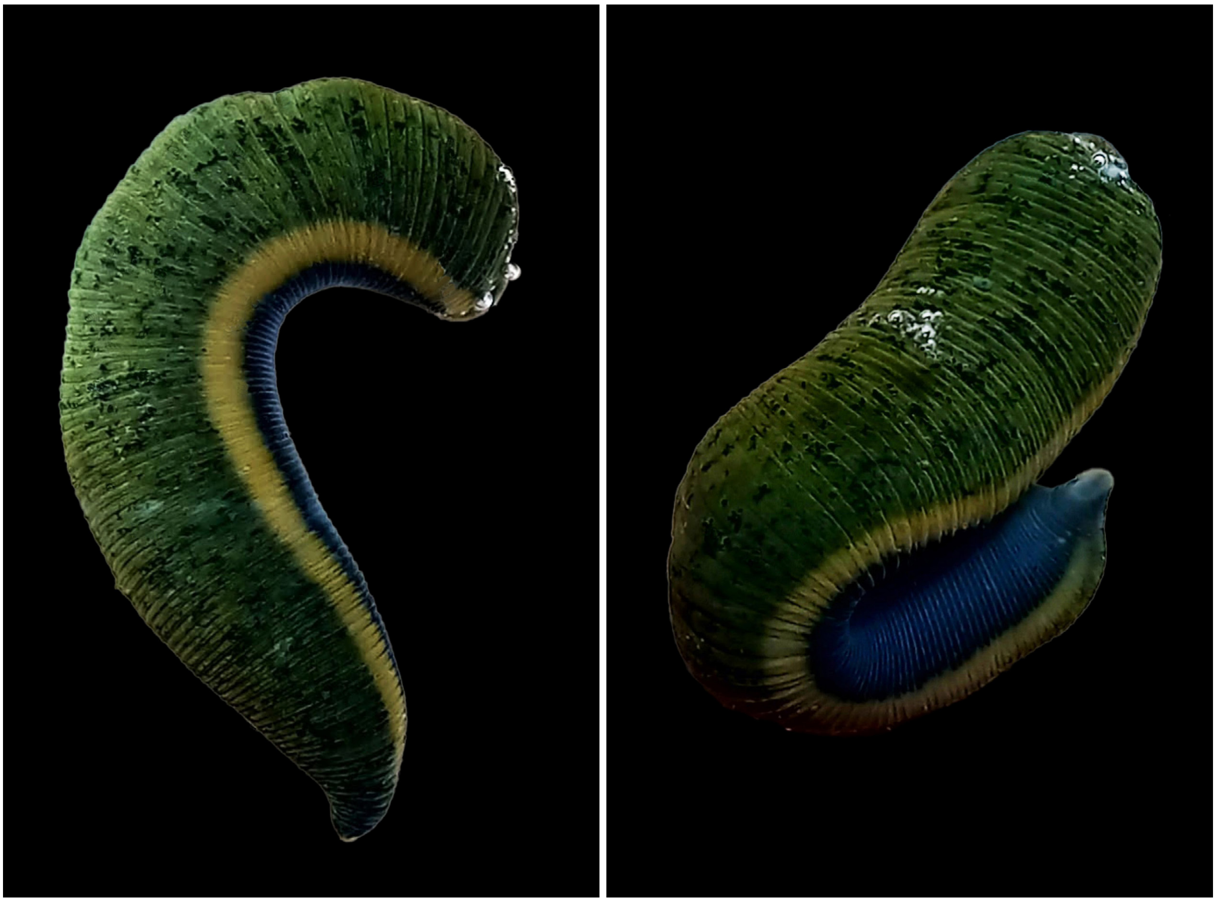

Figure 4 – *Haemopis elegans*, first specimen retaining its natural coloration, shown in varying poses during relaxation.

Phylogenetic relationships

Both the Ukrainian and French samples clustered within the well-supported clade of *H. elegans*. The Ukrainian sample is sister to a clade containing very similar sequences from French and German leeches (Fig. 6). The *H. elegans* clade clearly represents a species-level group. In turn, the Ukrainian *H. sanguisuga* joined the conspecifics within its species' clade.

Network

The COI sequence of the Ukrainian *H. elegans* belongs to a distinct haplotype, closely related to the Slovenian one. The French and German haplotypes are separated by 12 mutational steps from these, possibly indicating distinct evolutionary histories for both lineages (Fig. 7).

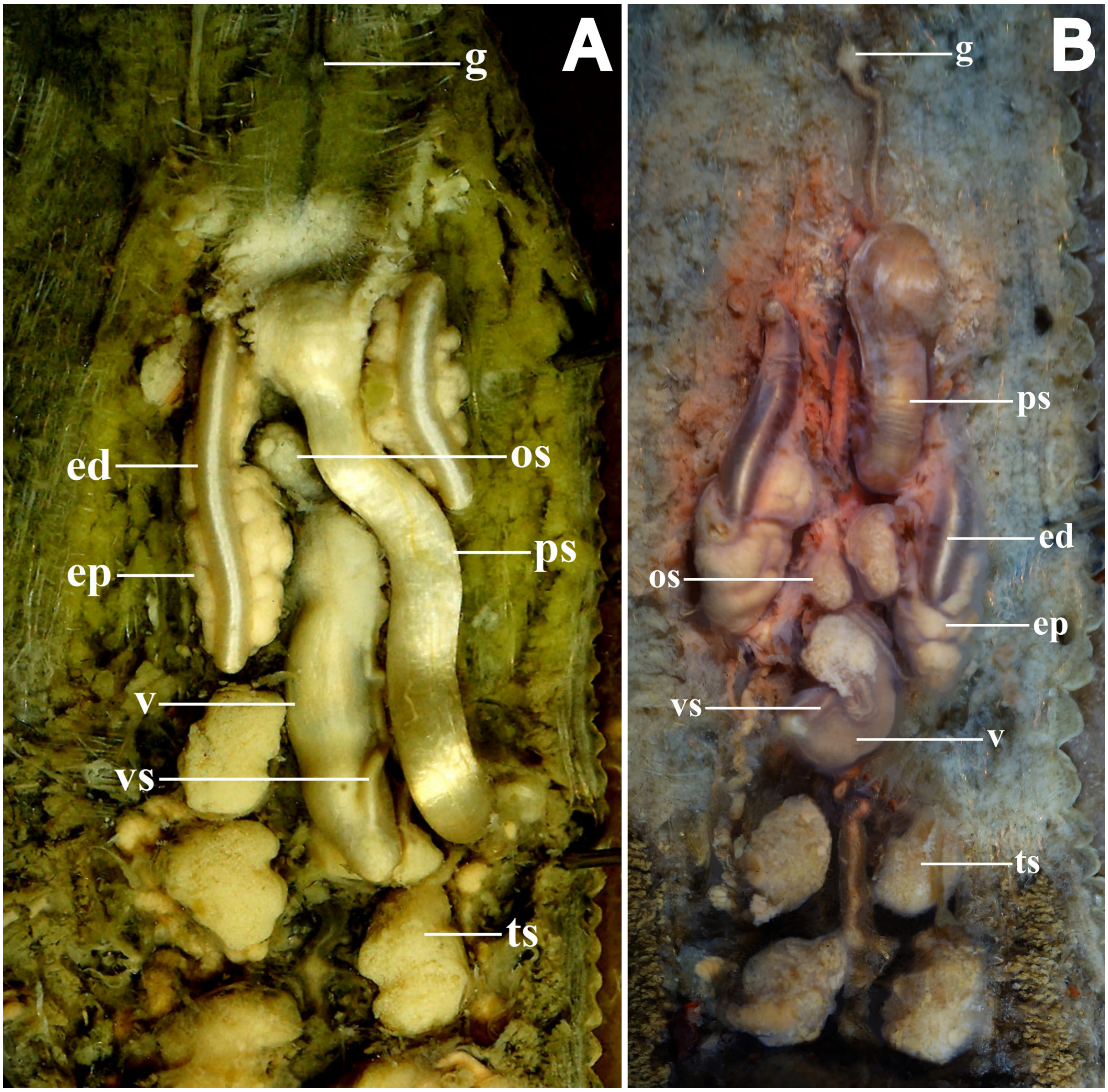

Figure 5 – Male and female reproductive systems. **A**. *Haemopis sanguisuga*. **B**. *Haemopis elegans*. Abbreviations: ed = ejaculatory duct; ep = epididymis; g = ganglion; os = ovisac; ps = penis sheath; ts = testisac; v = vagina; vs = vaginal stalk.

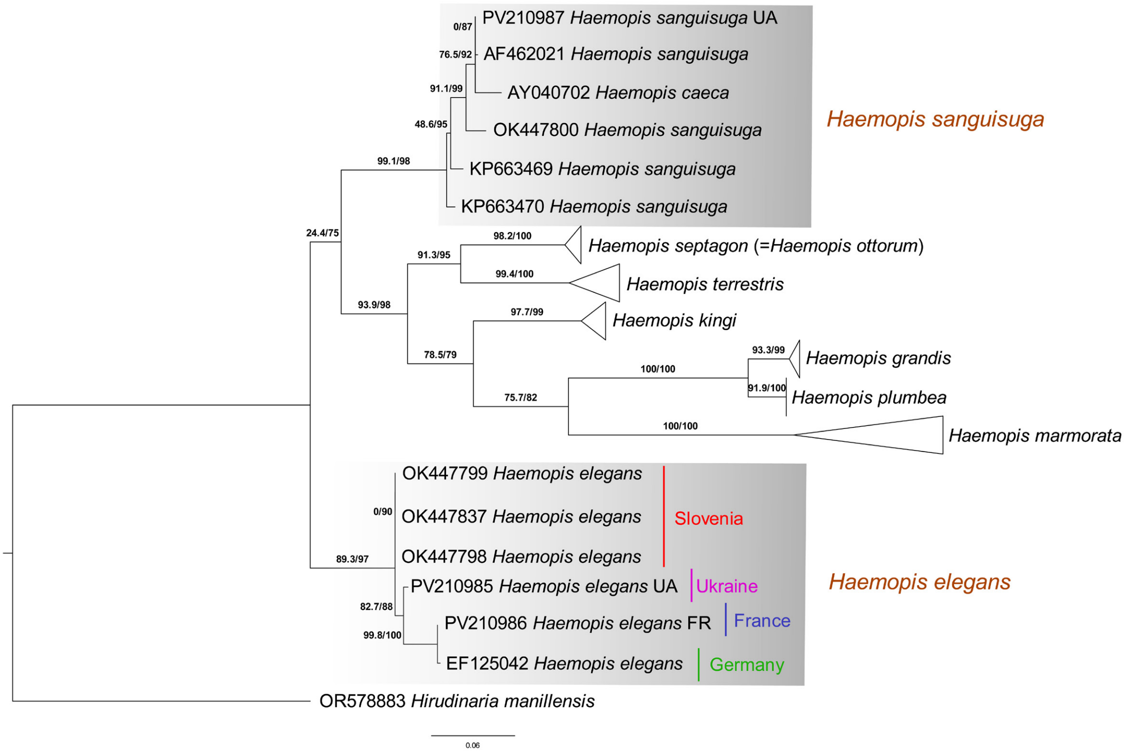

Figure 6 – Maximum Likelihood tree of the genus *Haemopis* based on COI sequences. The tree is rooted with *Hirudinaria manillensis*. Both ultrafast bootstrapping and SH-like approximate likelihood-ratio test (SHaLRT) values are provided for nodes.

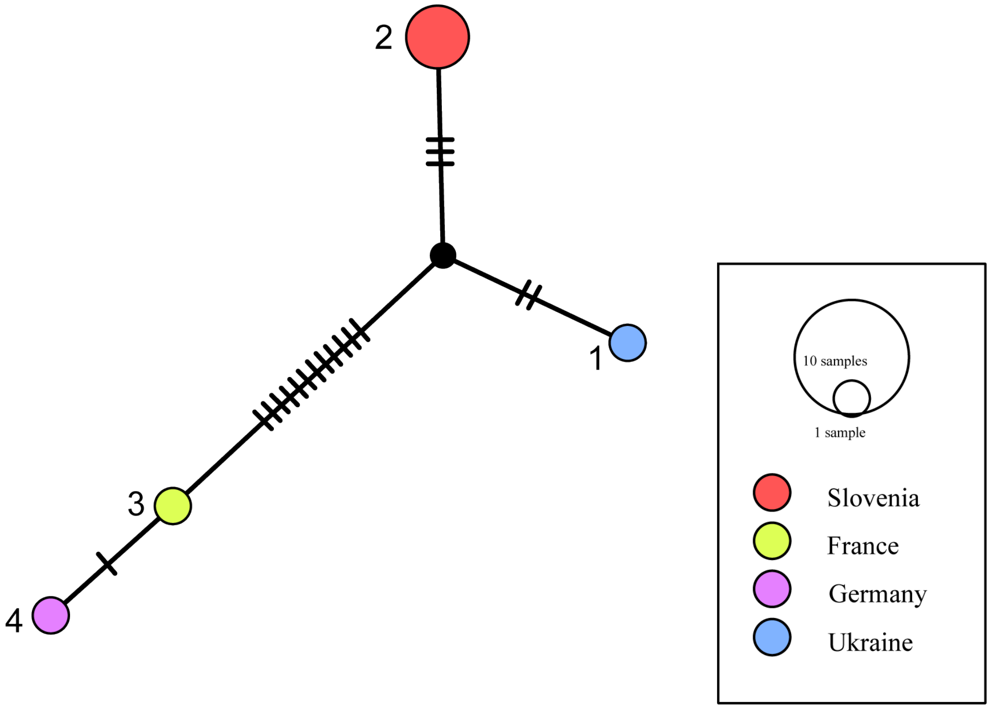

Figure 7 – Minimum spanning network of COI haplotypes of *Haemopis elegans*. Haplotype numbers correspond to the numbers in the Table 1.

Genetic diversity

The between-group mean distance for *H. elegans* and *H. sanguisuga* is 0.097 ± 0.011, whereas their within-group mean distances are 0.015 ± 0.003 and 0.021 ± 0.004, respectively. The p-distance between the Ukrainian and French samples of *H. elegans* is 0.021 ± 0.006, and between the Ukrainian *H. elegans* and *H. sanguisuga*, it is 0.090 ± 0.011.

Distribution analysis

Fifty occurrence points of *H. elegans* were recorded (Fig. 8, Table S1). Average AUC was 0.890 ± 0.0069, indicating good predictive power and discriminatory capacity of the Maxent model (Fig. 9).

The response curves (Figure S1) show how each environmental variable affects the Maxent prediction, i.e., how the predicted probability of presence changes as each environmental variable changes, while all other environmental variables remain at their sample mean. The curves show the average response of 10 Maxent runs. Table S4 gives estimates of the relative contributions of the environmental variables to the Maxent model. The variables that make the greatest contribution to the model are wc2.1_2.5m_eu_bio_14 (Precipitation of Driest Month), and wc2.1_2.5m_eu_bio_8 (Mean Temperature of Wettest Quarter). Figure S2 shows the results of the jackknife test of variable importance. The environmental variable with the highest gain when used in isolation is wc2.1_2.5m_eu_bio_6 (Min Temperature of Coldest Month), which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is the Minimum Temperature of the Coldest Month, which therefore appears to contain most information that is not present in the other variables. Values shown are averages over replicate runs.

Figure 8 – Geographic distribution of *Haemopis elegans* from published sources and new records obtained in this study.

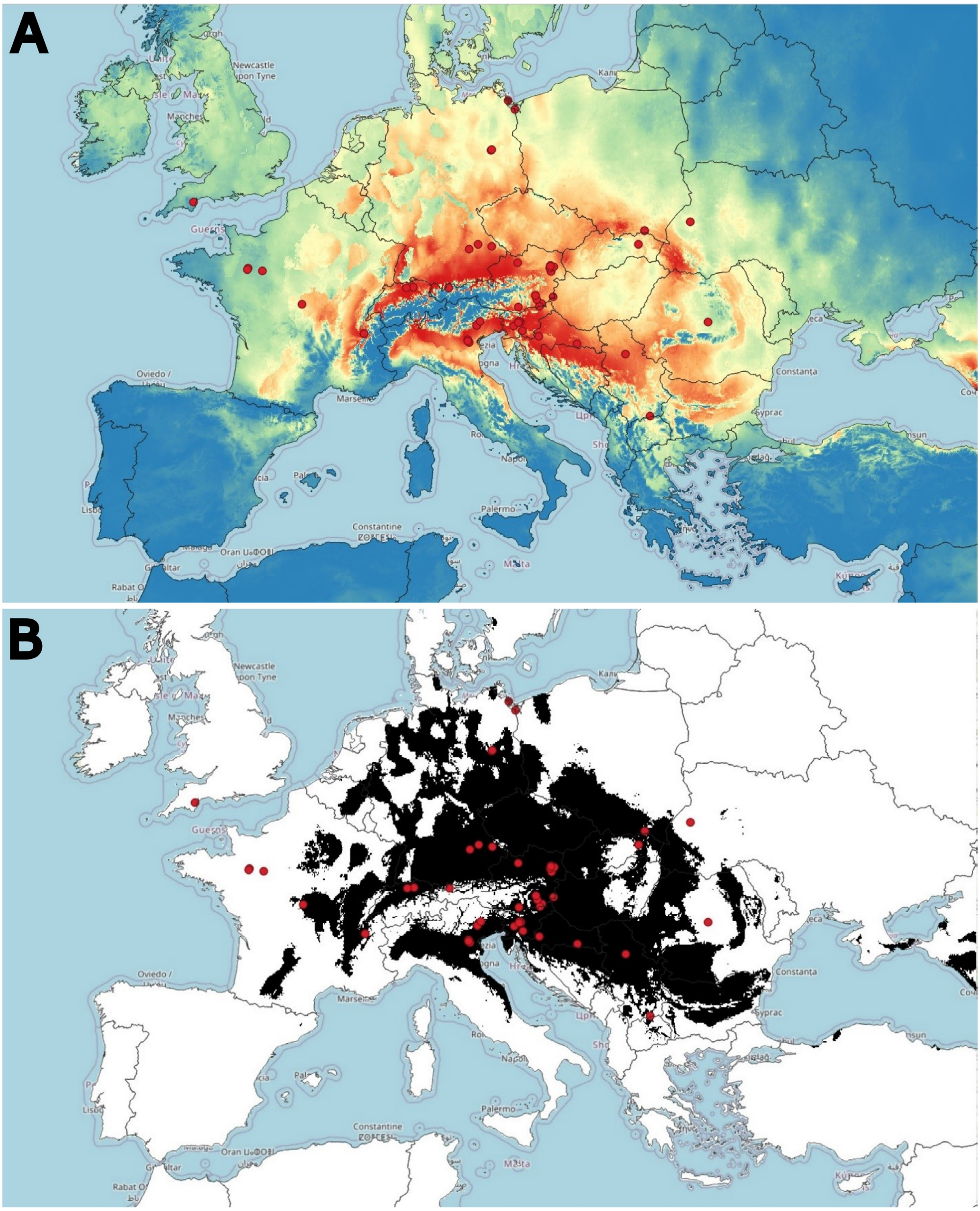

Figure 9 – *Haemopis elegans*. **A**. Predicted probability of the presence of suitable habitats. **B**. Potential geographic distribution.

Discussion

Both morphological characteristics and molecular data indicate that the leeches collected in Lviv should be assigned to *H. elegans*, a species not previously recorded in Ukraine. The specimens display distinctive features of *H. elegans*, including dark ventral coloration and a lighter dorsum with a metameric dark pattern. Moreover, the reproductive system of the Ukrainian *H. elegans* is characterized by a shorter penis sheath and a coiled vagina, in contrast to the longer and straighter reproductive organs found in *H. sanguisuga*. These differences are consistent with those reported by Kvist *et al*. (2023).

Molecular phylogenetic analysis, using the maximum likelihood optimality criterion and COI sequences, placed the Ukrainian and French samples within the species-level clade of *H. elegans*. By contrast, the specimen of *H. sanguisuga* from Vinnytsia, Ukraine, grouped within its respective species clade. The clade of *H. elegans* demonstrates significant phylogenetic structure, with the Ukrainian and French samples showing unique haplotypes. This suggests independent evolutionary histories for the populations and limited gene flow, despite the ability of these leeches to move on land for extended periods, which could have facilitated migration. This pattern of genetic variability differs from the uniform genetic structure observed in blood-feeding leeches, such as species in the genera *Hirudo* and *Limnatis* in the Western Palearctic (Trontelj & Utevsky 2012; Utevsky *et al*. 2022), and *Macrobdella decora* (Say, 1824) in the Nearctic (Kennedy *et al.* 2025). As a predatory macrophagous leech, *H. elegans* is not transported over long distances by vertebrate hosts, unlike blood-feeding hirudinids, which exhibit a more uniform genetic structure due to host-mediated dispersal. It should be emphasized that the inclusion of only two specimens of *H. elegans* (from Ukraine and France) in the molecular analysis limits the assessment of intra-population variation. A broader sampling of individuals across the species' range is essential, as additional molecular data would provide a more robust and comprehensive understanding of the population structure of *H. elegans*.

Both *H. elegans* and *H. sanguisuga* occur in Ukraine and exhibit a sympatric or parapatric distribution pattern, which indicates that the two species may occupy overlapping or adjacent environments in the region. A comparison of their ecological niches shows clear differences in habitat preferences. *Haemopis elegans* remains on land for longer periods than its congener and depends more strongly on stable, moisture-rich terrestrial environments. Its records are concentrated in montane and submontane regions, including the Carpathians, which points to a degree of ecological specialization. In contrast, *H. sanguisuga* follows a more aquatic lifestyle. It occupies a broader range of freshwater habitats, including lowland waters, and shows less dependence on high-moisture or mountain-associated terrestrial environments. These differences likely explain the distinct distribution patterns observed in the two species.

The geographical range of *H. elegans* extends from southwestern Britain to western Ukraine and from Germany to France, northern Italy, and Serbia. More southerly and warmer regions of Europe appear unsuitable for this species. Whereas the records from central and eastern France originate from natural habitats, those from western France are associated with nurseries where plants are frequently transported and exchanged. It is highly probable that the species spreads readily through plant trading. For this reason, *H. elegans* is most likely introduced in western France. Although species distribution modeling identifies environmentally suitable areas, it does not account for historical processes. Therefore, our interpretation of human-mediated dispersal is supported by direct observations of human activity in western France rather than by model outputs.

Our hypothesis – that environmental conditions east of the Lviv region in Ukraine are unsuitable for the establishment of *H. elegans*, resulting in its observed range limit – leads to a number of predictions. Namely, we predicted that species distribution modeling would indicate low habitat suitability for *H. elegans* east of its currently known distribution, and that field surveys and genetic data would confirm its absence or discontinuity in those areas. These predictions were tested and supported by our results:

habitat suitability sharply declines east of Lviv, and *H. elegans* was not detected beyond this region. This distribution pattern is likely driven by the species' preference for moist terrestrial environments, which are increasingly rare in the more continental or arid climates of eastern Ukraine and southern Europe. Our field sampling and molecular analyses support the hypothesis and confirm the expected eastern range limit of the species.

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