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THE TAXONOMIC IDENTITY OF CENTRAL AND NORTHERN EUROPEAN *SIMULIUM* (*NEVERMANNIA*) (DIPTERA, SIMULIIDAE): MOLECULAR, MORPHOLOGICAL, AND ECOLOGICAL DATA

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The taxonomic identity of Central and Northern European *Simulium* (*Nevermannia*) (Diptera, Simuliidae): molecular, morphological, and ecological data. Zinchenko, M. O., Sukhomlin, K. B., Zinchenko, O. P. & Tepliuk, V. S. — DNA barcoding based on the cytochrome c oxidase subunit I (COI) gene is increasingly employed in blackfly taxonomy to resolve identification challenges within species complexes. This study evaluates two competing hypotheses: whether the populations of the subgenus *Nevermannia* in Central Continental Europe represent three distinct species (*Simulium angustitarse*, *S. lundstromi*, and *S. volhynicum*) as traditionally recognised, or a single, highly plastic species complex. To test these models, samples were collected from the Ukrainian Polissia (Volyn Region) and compared with datasets from Finland, Germany, Bulgaria, and the United Kingdom. While populations in the Carpathians and the Balkans may exhibit greater genetic divergence, our results demonstrate that the populations from Volyn are conspecific with those from Finland — the type locality of *S. angustitarse*. Despite notable morphological and ecological variation, the lack of significant molecular differentiation across this Continental European range supports the hypothesis of a single, highly plastic species complex. Consequently, *Simulium angustitarse* Lundström, 1911 is established as a senior synonym of *Simulium volhynicum* (Usova & Sukhomlin, 1990), **syn. n.**

Key words: black flies, Ukraine, mitochondrial DNA, cytochrome c oxidase subunit I (COI), taxonomy, genetic distance, phenotypic plasticity.

Introduction

The family Simuliidae is a family of the nematoceran Diptera belonging to the infraorder Culicomorpha (Wiegmann et al., 2011). It comprises over 2,430 living species globally, arranged within 31 genera (Adler, 2025). Females of many species are hematophagous, posing significant health and economic challenges for both humans and domestic animals. Effective control measures and accurate knowledge of species distribution, biology, and behaviour depend heavily on precise identification methods. While traditionally based on morphology, modern studies on the European black fly fauna increasingly adopt an integrative taxonomic approach (see Ruiz-Arrondo et al. (2018) and Adler (2025) for references).

The taxonomy of Simuliidae is notoriously challenging due to the paradoxical combination of interspecific morphological homogeneity and high intraspecific variability (Crosskey, 1981; Jedlička, 1982). As noted by Kúdelá & Jedlička (2002), this leads to two frequent taxonomic errors: the erroneous merging of distinct species into a single 'species' or, conversely, the artificial splitting of highly variable species into several separate taxa. Within the *Nevermannia* subgenus, the pupal respiratory organs (spiracular gills) remain among the most critical diagnostic characters, with their branching patterns and basal trunk shapes serving as primary identification features. However, the inherent plasticity of these organs often creates 'unsolved taxonomical problems,' necessitating a reassessment of traditional morphological boundaries through molecular tools to distinguish true reproductive isolation from eco-phenotypic variation.

The *Simulium* (*Nevermannia*) *ruficorne* species group includes 66 species primarily distributed in the Afrotropical Region, with a limited number of representatives in the Palaearctic, Oriental, and Australasian Regions. In Europe, the group is represented by five nominal species, of which *Simulium ruficorne* Macquart, 1838 and *S. pinhaoi* Santos Grácio, 1985 are recorded only from Portugal; *Simulium angustitarse* (Lundström, 1911) is reported from Finland (type locality) to Turkey and from Ireland and Morocco to Siberia; *S. lundstromi* (Enderlein, 1921) described from England and reported from Finland to Turkey and from Ireland and Morocco to European Russia (Adler, 2025); and *S. volhynicum* (Usova & Sukhomlin, 1990) described from north-western Ukraine and then recorded also from Belarus (Kaplich et al., 2015). Traditionally, the latter three taxa have been regarded as distinct species based on morphological differences observed across all developmental stages, including the structure of adult genitalia, leg colouration, larval mandibular teeth, and the branching patterns of pupal gills (Usova & Sukhomlin, 1990).

The 658-bp barcoding region of the mitochondrial cytochrome c oxidase subunit I (COI) gene has emerged as the globally standardised molecular marker for DNA barcoding across the Class Insecta, owing to its high diagnostic resolution. In the study of Simuliidae, the application of COI sequences has become a fundamental tool for resolving morphological ambiguities and detecting cryptic lineages. While extensive barcoding has been conducted in North America (Rivera & Currie, 2009; Conflitti et al., 2013) and Thailand (Pramual et al., 2011, 2014, 2021; Low et al., 2020), European datasets were initially limited to regional assessments, such as those identifying 22 species in Spain (Ruiz-Arrondo et al., 2018). However, molecular

taxonomy has been particularly instrumental in addressing the complexities of the *Simulium reptans* species group, where research in the United Kingdom (Day et al., 2008), Lithuania (Bernotienė & Stunžėnas, 2009), and Central Europe (Kúdela et al., 2014) has revealed both cases of conspecificity and cryptic diversity. More recently, comprehensive studies in Slovakia (Dušinský et al., 2006; Jedlička et al., 2012; Kúdela et al., 2018; Kúdelová et al., 2023), Armenia (Werner & Kampen, 2012; Andrianov et al., 2015), and Ukraine (Zinchenko et al., 2021; Sukhomlin et al., 2022) have further expanded the molecular dataset, revealing complicated taxonomic relationships among geographically distant populations.

Within the subgenus *Nevermannia*, *S. angustitarse*, *S. lundstromi*, and *S. volhynicum* constitute a species complex isolated from the rest of the *ruficorne* species group. Although traditionally treated as distinct, their morphological characters have yet to be tested against molecular data to evaluate whether they represent true species or environmental plasticity.

Our preliminary attempts to obtain COI sequences for the three nominal species via Sanger sequencing provided initial indications of a lack of correlation between molecular data and morphological traits. However, as these sequences were fragmentary and inconsistent in length, it was impossible to provide definitive proof of this observation at that stage. To resolve these discrepancies and conclusively evaluate the true extent of phenotypic variability, the present study employed a more robust analysis to obtain complete, standardised 658-bp COI barcodes from specimens strictly conforming to the morphological diagnoses of each morphospecies.

This research evaluates two competing hypotheses: whether the European species of the *ruficorne* group — from the Ukrainian Polissia to the type locality of *S. angustitarse* in Finland — (1) represent three distinct species based on their diverse morphological and biological traits, or (2) a single, highly plastic species complex as indicated by preliminary molecular evidence. While acknowledging that populations in the Carpathians and the Balkans may exhibit greater genetic divergence, this study focuses on the taxonomic identity of Continental populations using an integrative approach.

Material and Methods

Sampling and field observations were conducted at six locations across the Volyn and Ivano-Frankivsk regions of Ukraine during the spring and summer periods (April–November) of 2011, 2017, and 2024. The study sites included the Konopelka River (Muravyshche: 50.9305° N, 25.5925° E), the Putylivka River (Palche: 50.7682° N, 25.7599° E; Stavok: 50.4641° N, 25.4814° E), the Pistynka River (Sheshory: 48.3340° N, 24.9890° E), and water bodies in Polapy (51.1955° N, 23.5939° E) and Sokyrychi (50.5149° N, 25.2953° E) (Fig. 1). Immature stages (larvae and pupae) were collected from submerged vegetation in running waters with varying flow velocities to encompass the full spectrum of morphological variation within the *S. angustitarse* complex.

Taxon sampling and identification. A total of 19 specimens belonging to the *Simulium angustitarse* complex were collected from six localities in Western

Ukraine between 2011 and 2024. The sampling covered various life stages, including last instar larvae (n = 15) and pupae (n = 4). Initial morphological identifications were performed using standard taxonomic keys; specifically, specimens were assigned to *S. angustitarse*, *S. volhynicum*, or *S. lundstromi* following the detailed descriptions in Kaplich et al. (2015) (see Supplementary Material. Appendix S3). However, these taxa were subsequently reconsidered to be conspecific based on the integrated molecular and morphological evidence presented herein. For molecular analysis, specimens were fixed in 96% ethanol immediately upon collection and stored at 4 °C to prevent DNA degradation. In the laboratory, specimens were identified and cleaned using a stereomicroscope.

Initial DNA Extraction and Sanger Sequencing. Initial DNA processing was conducted by the first author at the University of Oslo (Norway), following the protocols recommended in the EPPO PM7/129 Standard (EPPO, 2016). Total DNA was extracted from individual larvae and pupae using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions for animal tissue. PCR amplification of the 658-bp fragment of the mitochondrial COI gene was performed using the primer combination LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994).

The PCR master mix, including 20 mg/mL BSA, was prepared according to the EPPO Standard. The thermal profile consisted of initial denaturation at 95°C for 2 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 1 min; with a final extension at 72 °C for 10 min and a hold step at 8 °C. Success was verified via electrophoresis on a 1% agarose gel stained with GelRed (Biotium, Inc., Fremont, CA, USA). Amplified fragments were sequenced both directions using the ExoStar kit. Sequence assembly was performed by aligning complementary strands; however, these preliminary sequences were fragmentary and short (approximately 200 bp), with a portion of them deposited in GenBank for initial reference (accession numbers PX775817–PX775819). The limitations of these fragmentary data necessitated the collection of additional material and the implementation of a more robust analytical approach to obtain complete barcodes.

High-throughput MinION Sequencing (2024 Phase). For the current study, a second set of 16 samples (PX775801– PX775816) representing all identified morphotypes was processed at the Museum für Naturkunde, Berlin (Germany). For each specimen, either the whole body (larva or pupa) or its parts were incubated in lysis buffer. Following extraction, the remaining cuticular parts and sclerotised structures were recovered and preserved in 70–96% ethanol for subsequent morphological verification.

Genomic DNA was extracted using the non-destructive methods described by Korlević et al. (2021). The COI gene was amplified using the LCO1490 primer and a modified jgHCO2198 primer (5'-TAN ACY TCN GGR TGN CCR AAR AAY CA-3') (Geller et al., 2013). Both primers were tagged with unique 9-bp sequences to enable multiplexing (Srivathsan et al., 2024).

Each 15 μL PCR reaction contained 7 μL of 2X CWBio master mix (Beijing, China), 1 μL of 1 mg/mL BSA, 1 μL of 10 μM LCO1490, 2 μL of 10 μM modified jgHCO2198, and 4 μL of DNA template. The PCR conditions involved initial dena-

turation at 95°C for 5 min; 35 cycles of 94°C for 1 min, 45 °C for 2 min, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. Amplification success was monitored on a 1% agarose gel. Pooled PCR products (6 μL each) were cleaned using CleanNGS beads (CleanNA, Waddinxveen, The Netherlands) at a 0.7 ratio. DNA concentration was quantified using the Qubit™ dsDNA BR Assay Kit (Invitrogen, California, USA).

Libraries were prepared using SQK-LSK114 ligation sequencing kits (Oxford Nanopore Technologies, Oxford, UK) with 50 ng of pooled amplicons. Sequencing was performed on GridION and PromethION platforms using an R10.4 Flowcell for approximately 15 hours. Basecalling of the resulting Fast5 files was conducted via Dorado v0.9.6 on the Super Accuracy (SUP) setting. Demultiplexing and generation of the final FASTA files were performed using ONTBarcoder 2.2 (Srivathsan et al., 2024).

Vouchering and data availability. All processed specimens are deposited as physical vouchers, stored in ethanol, in the entomological collection of the Museum für Naturkunde, Berlin (ZMB) and are associated with unique catalogue numbers in the series ZMB:VK250711-17 through ZMB:VK250711-32.

The COI sequences obtained in this study have been deposited in GenBank under the accession numbers PX775801–PX775819. Detailed metadata, including geographic coordinates (formatted as decimal degrees), collection dates, and life stages, are provided in the supplementary materials and the GenBank source modifiers.

Sequence Retrieval and Reference Data. To supplement the newly generated dataset, reference COI sequences for the *ruficorne* species group were retrieved from GenBank and the Barcode of Life Data System (BOLD). These included sequences formally identified as both *Simulium angustitarse* and *S. lundstromi* from various European localities. These reference data were essential for evaluating the genetic consistency of the species across their continental range. A comprehensive list of the accession numbers and BOLD Process IDs for all retrieved and newly generated sequences is provided in the Supplementary Material (Appendix S1).

Data Analysis and Phylogenetics. In total, 16 full-length COI sequences were successfully generated from nine samples (probes) each containing 1–3 larvae and pupae. These sequences represent the three morphospecies identified in the initial morphological assessment: *S. angustitarse*, *S. lundstromi*, and *S. volhynicum*, collected from the Volyn and Ivano-Frankivsk regions. The newly generated sequences, along with the reference data retrieved from GenBank and BOLD, were aligned using the MUSCLE algorithm in MEGA 11 (Tamura et al., 2021).

The best-fit nucleotide substitution model for the dataset was identified as the Tamura 3-parameter model (Tamura, 1992) with a Gamma distribution and a proportion of invariable sites (T92+G+I), based on the Bayesian Information Criterion (BIC). To evaluate the phylogenetic relationships and taxonomic consistency of the continental populations, a Neighbor-Joining (NJ) tree was constructed with 1,000 bootstrap replicates to assess nodal support. Additionally, intraspecific and interspecific genetic distances were calculated using p-distances to determine the level of divergence between the local populations from Ukraine and the reference sequences from Northern and Central Europe.

Results

Molecular Characterisation and Phylogeny. The molecular analysis involved 39 mitochondrial COI sequences (3 of them used as an outgroup), resulting in a final dataset of 658 nucleotide positions (Supplementary Files: Appendix 2). The sampling integrated full-length barcodes obtained via MinION sequencing (2024) with archival fragments (211 bp) from 2017, ensuring temporal and geographic consistency. To assess the taxonomic boundaries of the *S. angustitarse* species complex, additional sequences of closely related taxa were retrieved from GenBank and BLAST and included as comparative lineages (Fig. 2).

The Neighbor-Joining (NJ) analysis revealed a highly supported and cohesive 'Core Continental Clade' encompassing all investigated sequences obtained from black flies collected in Ukraine and the reference material from Northern Europe. It should be noted that the seemingly scattered positioning of the short (211 bp) fragments (SimMZ17–19) within this clade is a clear computational artifact; these sequences are entirely identical across their shared positions with the full-length barcodes, and thus their placement reflects the limits of the NJ algorithm within a genetically monomorphic complex.

Genetic Distance Analysis. Evolutionary divergence estimates (Table 1) demonstrate a clear hierarchy of genetic relationships, effectively separating the core continental lineages from the outgroup and isolated populations.

Baseline Divergence and the Identity of *S. angustitarse*. The evolutionary divergence between the British populations (England_angustitarse) and the Carpathian lineag (Carpath_angustitarse), geographically distant populations definitively identified as *S. angustitarse,* was found to be 2.71% (Table 1). This value represents the maximum internal variation within a single widely distributed species across the European continent and serves as a crucial reference point for the entire complex.

Genetic Affinity of Continental Morphotypes. Our molecular analysis reveals a striking lack of genetic differentiation between the three morphotypes (*angustitarse, lundstromi,* and *volhynicum*) collected in Ukraine. Despite their distinct morphological features, all specimens from the Volyn core clustered into a single, genetically monomorphic clade (average intra-group distance of 0.33%).

This "Core Continental Clade" also includes reference sequences from Finland (Finland_lundstromi), which represents the type locality of *S. angustitarse* (Lundström, 1911). Although the Finnish sample is identified as "*S. lundstromi*" in public databases, its origin from the terra typica of *S. angustitarse* and its negligible genetic distance (0.09%) from our Ukrainian material provide a critical taxonomic anchor. This identity demonstrates that the "continental" populations traditionally assigned to "*S. lundstromi*" or "*S. volhynicum*" are, in fact, morphological variants of *S. angustitarse*. The Finnish population is genetically very close to the Ukrainian Volyn samples, with a distance of only 0.30% to the clade representing the "volhynicum" morphotype and 0.55% to the "lundstromi" morphotype clade.

In contrast, the sequences from the United Kingdom (the type locality of *S. lundstromi*) form a separate, well-supported cluster with a genetic distance of approximately 1.8–2.1% from the continental core. This confirms that the true *S. lund-*

stromi is an insular taxon, whereas the continental diversity previously attributed to three species represents the phenotypic plasticity of a single widespread species, *S. angustitarse*.

Conclusion on Conspecificity. Historically, most continental specimens, including those from Ukraine, were morphologically identified as "*S. lundstromi*". The molecular evidence, however, necessitates a revision of this practice. Given that the Finnish lineage (assumed to be "the true *S. angustitarse*") clusters so tightly with the Volyn and Carpathian samples, it is evident that all these populations are conspecific. Therefore, according to the principle of priority, they all are assigned the name of the senior synonym, *Simulium* (*Nevermannia*) *angustitarse* (Lundström, 1911).

The southern periphery: Bulgaria and Turkey. Our analysis included several fragmentary sequences from Bulgaria and Turkey, initially identified as *S. lundstromi* (BOLD: GMBUD093-14) and *S. ibleum* (GenBank: OK073994.1). These sequences represent populations at (or beyond) the southern margin of the species' known range. Despite their fragmentary nature, these samples exhibit a genetic distance of approximately 1.57–1.67% from the Northern / Eastern European core (Finland–Ukraine). This level of divergence is significantly lower than the 2.71% distance observed between English and Carpathian populations, suggesting that these south-eastern lineages are also conspecific with *S. angustitarse*. *Simulium ibleum* (Rivosecci, 1966) is a Mediterranean member of the *ruficorne* species group, originally described from Sicily and subsequently recorded from Turkey. The COI sequence for this taxon used in our analysis is incomplete (560 bp), which may partly account for its high genetic affinity to the rest of the *S. angustitarse* samples.

The stable genetic divergence (0.89%) observed between two sympatric British lineages identified as *S. angustitarse* and *S. lundstromi* warrants further investigation. Given the widespread occurrence of cryptic speciation within the family Simuliidae, it is plausible to hypothesise that these lineages may represent distinct cytoforms. In many black fly species complexes, such consistent molecular gaps often correlate with specific chromosomal rearrangements (polytene chromosome inversions). However, determining whether these British populations constitute true sibling species or reflect deep mitochondrial phylogeographic structure would require a detailed cytogenetic analysis, which remains beyond the scope of the present study.

Future research. These findings highlight the necessity of more extensive DNA barcoding studies across the entire Palaearctic range of *S. angustitarse*. Systematic sampling outside Central Europe is required to fully map the genetic landscape of this species and to determine the precise limits of its evolutionary integrity. Other genetic markers or cytotaxonomic data can be also investigated to better understand the taxonomy of black flies.

Outgroup and Monophyly. The analysis is robustly supported by the clear separation of the outgroup (*Simulium vernum*), which shows genetic distances of 12.86–16.13% from the *S. angustitarse* complex. This level of divergence confirms the monophyly of the species complex and validates the use of COI as a reliable marker for resolving synonymy within this complex. The sequences PX775817–PX775819 appear at the base of the 'Core Continental Clade', basal to the common lineage of the English samples and the rest of the clade. However, these sequences are identical to

Table 1. **Estimates of evolutionary divergence (*p*-distances in %) between the investigated groups**

Group	1	2	3	4	5	6	7
1. Carpath angustitarse	–						
2. Volyn volhynicum	1.06	–					
3. Volyn lundstromi	1.21	0.43	–				
4. Finland lundstromi	1.40	0.30	0.55	–			
5. England lundstromi	2.80	2.10	2.22	2.14	–		
6. England angustitarse	2.71	1.80	2.08	2.00	0.89	–	
7. Bulgaria & Turkey	1.74	1.61	1.57	1.67	3.14	2.76	–

Table 2. **Average intra-group genetic variability, %**

Group	Intra-group Divergence, %
Volyn volhynicum	0.26
Volyn lundstromi	0.72
England angustitarse	0.18
Carpath angustitarse	1.22

each other and to most other local sequences (except for PX775801, 02, 03, 10, and 14, which differ by a single nucleotide); thus, their topological deviation is merely a Neighbour-Joining artefact resulting from their limited length (211 bp).

Discussion of Molecular Results. The molecular data provide two critical insights into the taxonomy of the *S. angustitarse* complex: 1. Continental Unity: The genetic distance between the diverse Ukrainian morphotypes and the Finnish reference (near the type locality of *S. angustitarse*) is a mere 0.30–0.43%. This provides definitive evidence that *S. volhynicum* and the continental forms traditionally assigned to *S. lundstromi* are conspecific with *S. angustitarse*. 2. Insular Isolation: British populations consistently diverge from the continental core by 1.8–2.1%, reinforcing the status of the "true" *S. lundstromi* as an insular endemic restricted to the British Isles.

Morphological vs. Genetic Divergence. The NJ tree shows a discrepancy between traditional morphological characters and mitochondrial COI sequences, suggesting potential phenotypic plasticity within the complex.

Intermingled Morphotypes. Specimens identified as the 'volhynicum' morphotype (e. g., PX775808, 10, 15, 16) are found on the same terminal branches as those identified as 'angustitarse' (PX775803) or 'lundstromi' (PX775812, 13). It is noteworthy that minor intra-populational variation was observed even within single samples. For instance, the sequence PX775814 exhibits six nucleotide substitutions compared to PX775812 and PX775813 from the same probe, representing a divergence of approximately 0.91%. This level of individual polymorphism is typical for mitochondrial DNA in Simuliidae and further illustrates that the genetic diversity within the 'Core Continental Clade' remains well within the expected limits for a single, cohesive species, not exceeding the threshold for species delimitation.

Implication: This distribution strongly supports the hypothesis that the morphological characters previously used to distinguish these taxa represent manifestations of phenotypic plasticity — responses to specific ecological niches (flow velocity, oxygen levels, substrate) rather than markers of reproductive isolation.

Taxonomic Status and Nomenclature Revision

Simulium* (*Nevermannia*) *angustitarse (Lundström, 1911)

Simulium angustitarse Lundström, 1911: 12 (type-locality: Helsinge, Finland).

For other synonymy, see: Adler (2025)

Chelocnetha volhynica Usova & Sukhomlin, 1990: 146 (type-locality: Vyzhivka River, Ukraine), **syn. n.**

Nevermannia volhynica: Yankovsky, 2002: 360; Kaplich et al., 2012: 116; 2015: 139.

Simulium volhynicum: Vasiliev & Sukhomlin, 2022: 545, 546.

Simulium (*Nevermannia*) *volhynicum*: Adler & Crosskey, 2008: 58; Adler, 2025: 76.

Chelocnetha volhnica: Usova & Sukhomlin, 1990: 148: incorrect original spelling (figure 2 legend).

Chelocnetha volinica: incorrect subsequent spelling.

Simulium lundstromi auctt. (non Enderlein, 1921): records from Continental Europe (Finland, Ukraine, apparently Belarus, Bulgaria and other countries).

Summary of Molecular Evidence. The integrative analysis of the mitochondrial COI gene fragments provided a definitive resolution to the taxonomic uncertainty surrounding the *Simulium* (*Nevermannia*) *angustitarse* complex in Eastern Europe. Our data demonstrate that the populations of Polissia regions, historically identified as *S. volhynicum* (Usova & Sukhomlin, 1990) and *S. lundstromi* (Enderlein, 1921), are genetically nearly identical to the Finnish population of *S. angustitarse* (Lundström, 1911), with a negligible divergence of only 0.30–0.55%.

Conspecificity and Eco-phenotypes. This high genetic affinity, being significantly lower than the 2.71% baseline divergence observed between distant Carpathian and British lineages, confirms that all continental specimens investigated represent a single, cohesive species. Consequently, the various morphological forms previously treated as distinct species are herein interpreted below as eco-phenotypes or morphotypes (I, II, and III). These variations are interpreted here as ecophenotypic modifications — likely representing plastic responses to varying ecological factors.

Taxonomic status. *Simulium volhynicum* was originally described based on a holotype larva and two paratype larvae (SIZK), but the diagnosis was actually

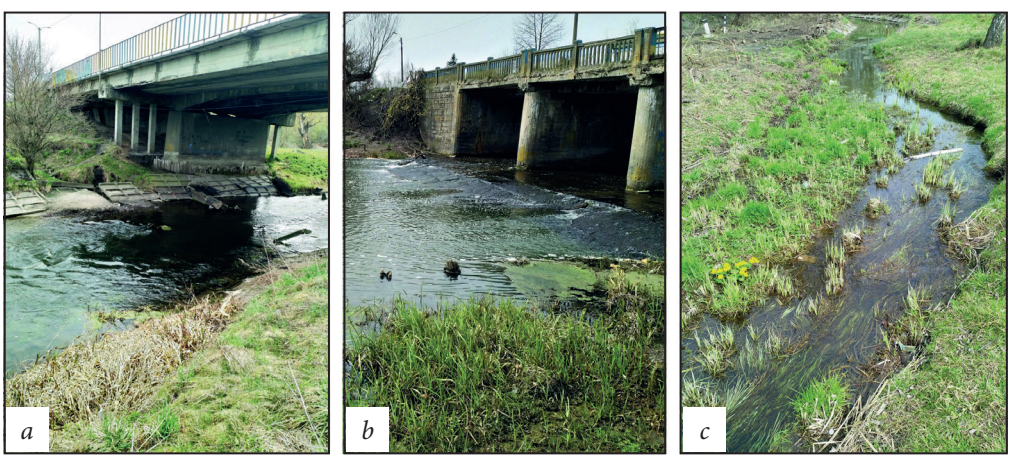


Fig. 1. Location of material collection: *a* — Konopelka River; *b* — Putylivka River; *c* — reclamation canals

based on comparison of characters of some non-type adult specimens with *S. latigonium* (Rubzov, 1956) from St. Petersburg (Russia, close to Finnish border), which Adler (2025) considered to be a junior synonym of *S. lundstromi.*

Original diagnosis (translated from Usova & Sukhomlin, 1990). "Morphologically, our forms resemble *Ch. latigonium* Rubz., 1956. However, significant differences in the structure of the male and female terminalia, as well as in larval morphology, justify the recognition of our forms as a new species. Our forms differ from the description provided by Rubzov (1956) as follows:

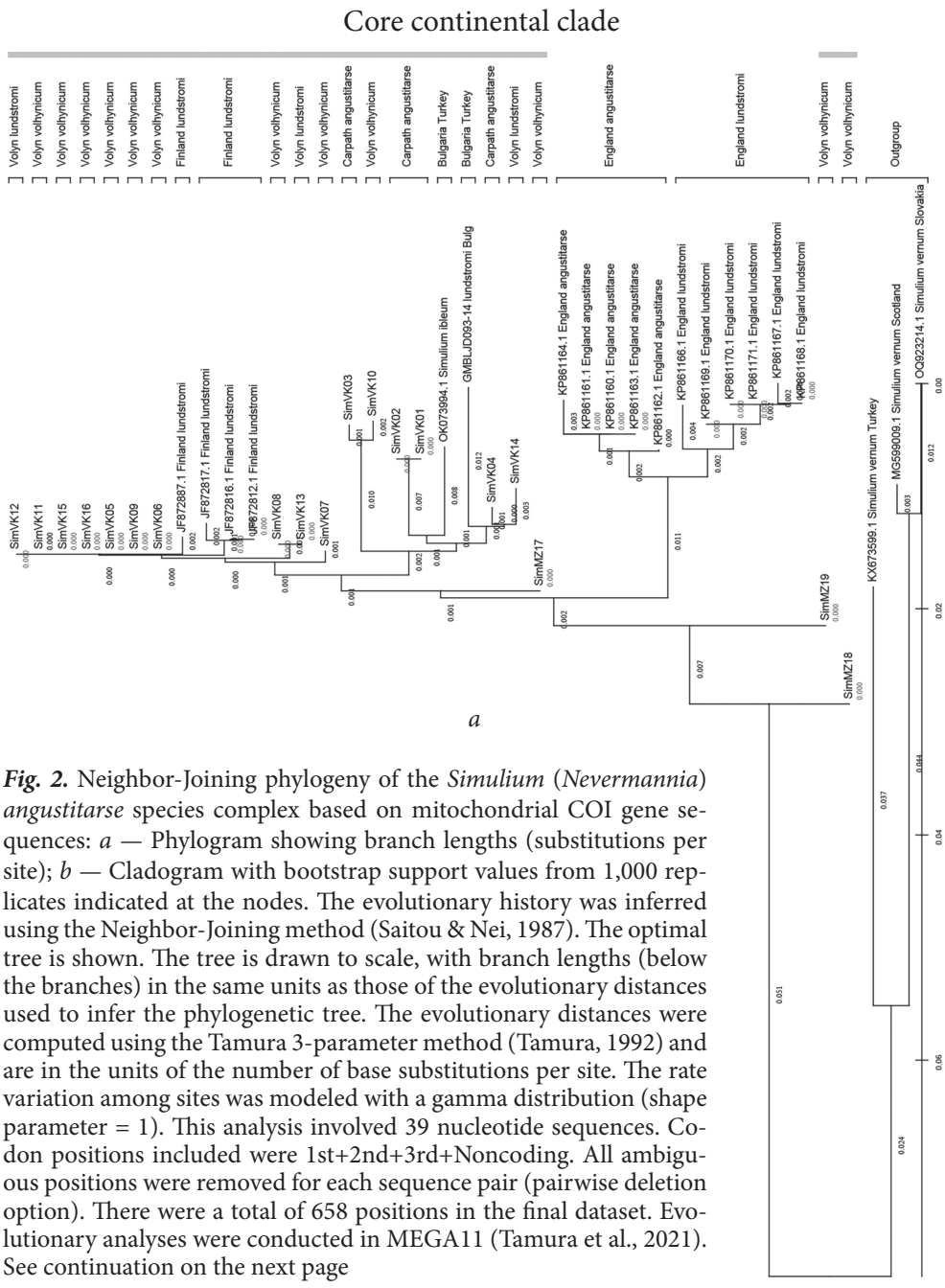
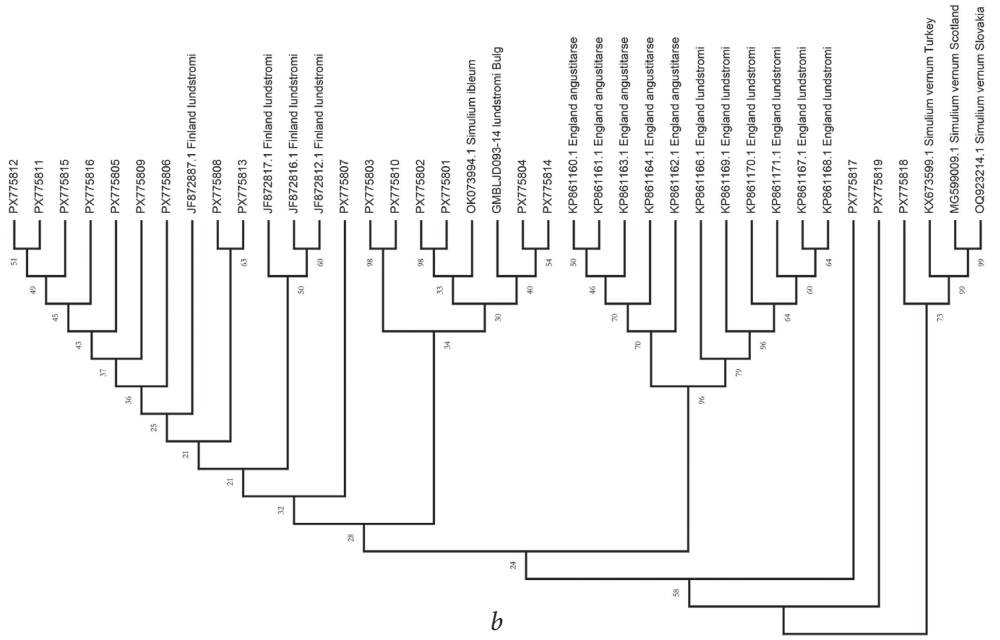


Fig. 2. Neighbor-Joining phylogeny of the *Simulium* (*Nevermannia*) *angustitarse* species complex based on mitochondrial COI gene sequences: *a* — Phylogram showing branch lengths (substitutions per site); *b* — Cladogram with bootstrap support values from 1,000 replicates indicated at the nodes. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree is shown. The tree is drawn to scale, with branch lengths (below the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura, 1992) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). This analysis involved 39 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 658 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).
See continuation on the next page



b

Terminalia (♀): anal plates oval; genital fork with a shorter and thicker stem, expanded at the apex; branches of the genital fork long, not twisted, diverging at an angle of 50–60°.

Terminalia (♂): gonosternum sharply narrowing toward the posterior margin, the keel expanding toward the middle and the posterior margin; gonocoxites trapezoidal, narrowed at the base; gonostyli with almost parallel margins.

Larva: postgenal cleft small and square, characteristically surrounded by strong dark pigmentation extending almost to the submentum; antenna long and slender, with all segments of equal length; primary fan with 44–46 rays (compared to 64 rays in the description [of *S. latigonium*] by Rubzov, 1956)."

The phylogenetic position of the Ukrainian samples relative to the Finnish population (divergence of 0.30%) serves as a decisive argument for revising the taxonomy of the complex. Given that Finland is the type locality for *S. angustitarse*, the near-identity of our morphotypes with this "taxonomic anchor" proves that Ukrainian morphotype identified as *Simulium volhynicum* (Usova & Sukhomlin, 1990) is conspecific. Traditional morphological diagnostics, which treated these forms as distinct species for decades, proved vulnerable to convergent variability. Our data confirm that for the subgenus *Nevermannia*, molecular markers are more reliable for resolving synonymy than traditional larval and pupal traits, which are heavily influenced by environmental factors.

Nomenclature. The names *Simulium angustitarse* Lundström, 1911 and *Chelocnetha volhynica* Usova & Sukhomlin, 1990 are considered to be **synonyms**. The name *S. lundstromi* must be restricted to the genetically isolated insular populations of the United Kingdom, while the continental records previously assigned to this name are referred to as *S. angustitarse*. The historical reliance on a comparison with *Simulium latigonium* Rubzov, 1956 as the primary diagnostic reference, while omitting direct comparisons with the Finnish populations of *S. angustitarse* (Lund-

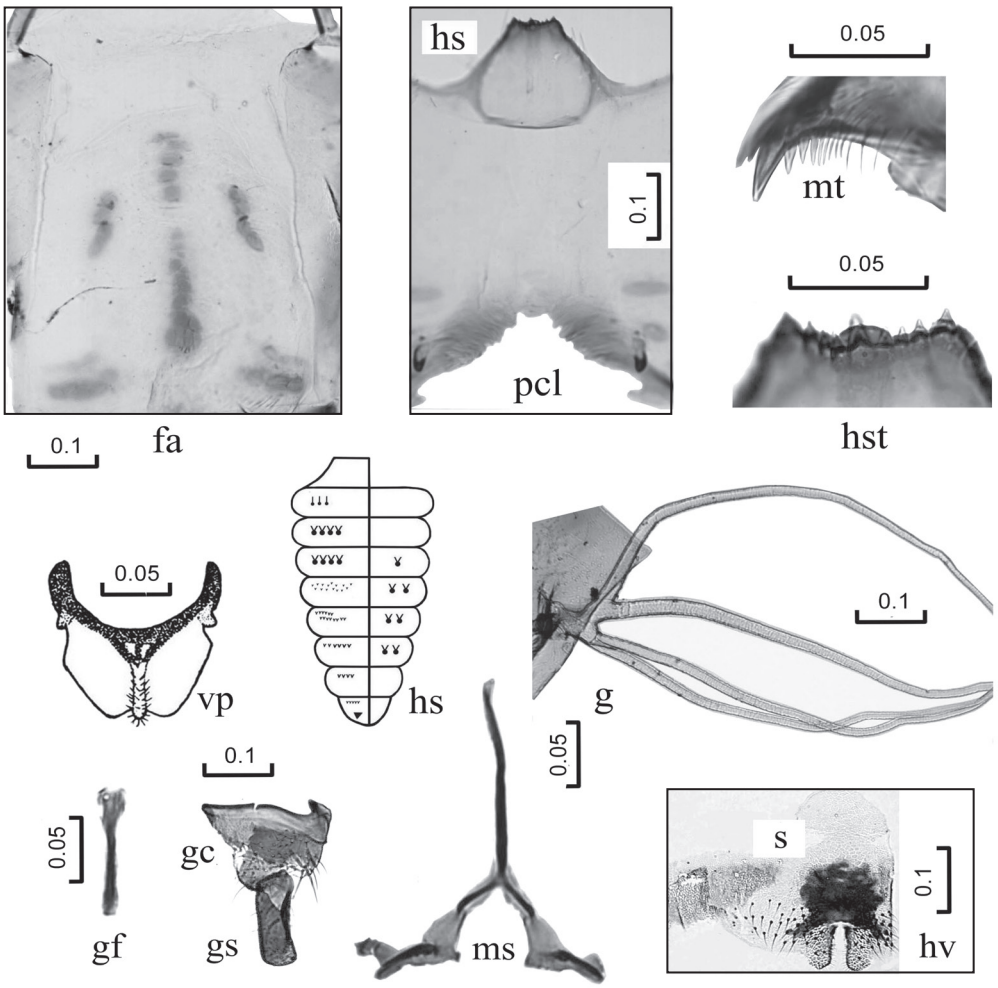


Fig. 3. Morphological structures of the immature stages in the *Simulium* (*Nevermannia*) *angustitarse* complex. Morphotype I ("*angustitarse*-type"): fa — frontoclypeal apotome, g — gill, gf — genital fork, gc — gonocoxite, gs — gonostylus, hs — hypostoma, hsc — hooks and spine comb of the pupal abdomen, hst — hypostomal teeth, hv — hypogynial valves, ms — median sclerite, mt — mandibular teeth, pcl — postgenal cleft, s — sternite VIII, vp — ventral plate

ström, 1911) and the British lineages of *S. lundstromi* (Enderlein, 1921), has significantly clouded the taxonomic landscape. This methodological gap necessitates a comprehensive revision also of the species status of *S. latigonium*. Within the context of our current integrative study, this lack of comparative data from type localities or reference insular populations is addressed through the synthesis of both traditional morphology and modern molecular evidence.

Based on this revised framework, the following section provides a comprehensive morphological description of *S. angustitarse*, encompassing the full range of its observed phenotypic variation.

Diagnoses of Morphotypes. Despite the genetic uniformity revealed by the COI analysis, the populations of *Simulium angustitarse* in the Volyn Region exhibit pronounced phenotypic plasticity. We distinguish three main morphotypes that correlate with specific ecological conditions and were previously treated as distinct "species".

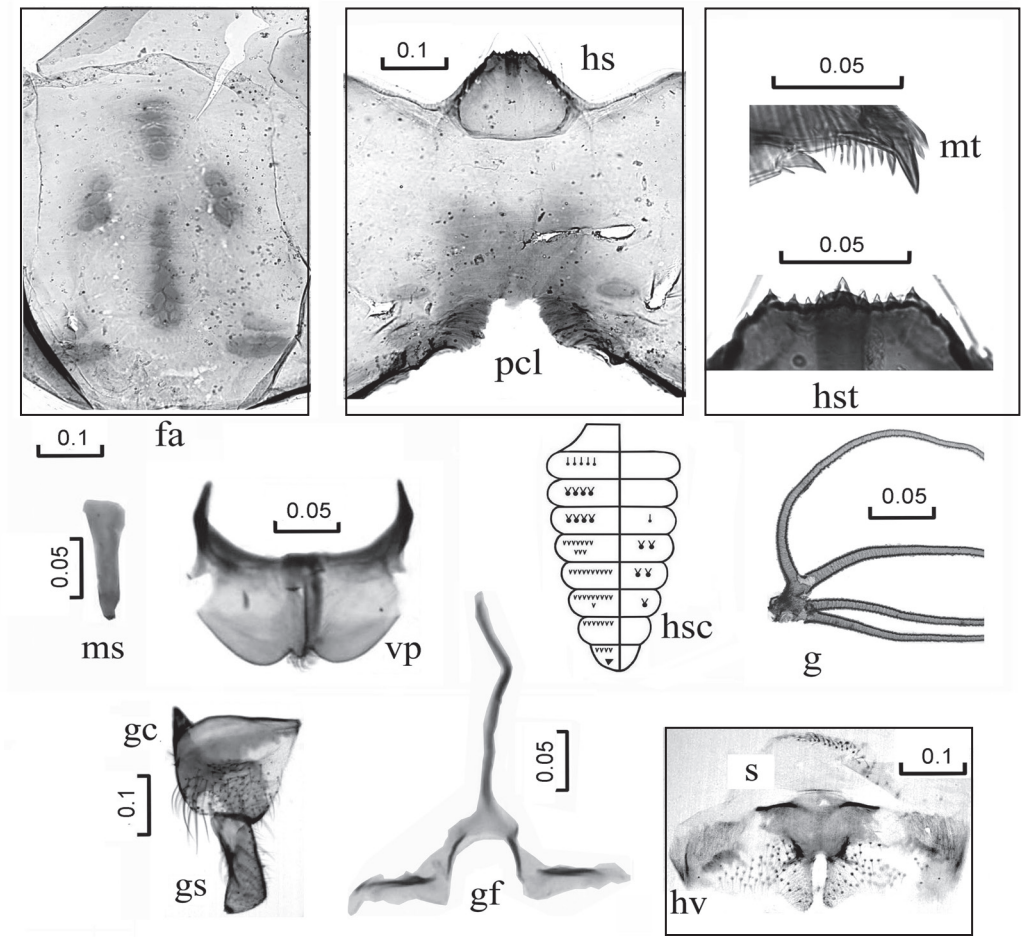


Fig. 4. Morphological structures of the immature stages in the *Simulium* (*Nevermannia*) *angustitarse* complex. Morphotype II ("*volhynicum*-type"). For legend, see Fig. 3

Morphotype I ("angustitarse"-type) (Fig. 3). Characteristic of small to medium streams with moderate flow and submerged vegetation. Head. Face dark grey with sparse silver pubescence. Antennae 11-segmented, entirely dark brown to black. Maxillary palps with the sensory vesicle in the third segment occupying approximately one-third of the segment's length. Thorax. Scutum uniformly black, dull, covered with dense, short, golden-yellow recumbent hairs. Scutellum with long, erect black bristles. Halter pale yellow to creamy white. Legs. Fore coxae yellow; all femora yellowish-brown with darkened distal apices. Fore basitarsus elongated and slender, not expanded. Wing. Radial sector simple; basal cell absent. Costa with a mix of fine hairs and short black spines. Terminalia. Gonocoxite slightly longer than wide, rectangular in ventral view. Gonostyle subtriangular, tapering significantly towards the apex, bearing a single apical tooth. Ventral plate broad, with a moderately developed median keel and short, divergent basal arms.

Morphotype II ("volhynicum"-type) (Fig. 4). Typically found in slow- flowing rivers and drainage channels with dense aquatic macrophytes. Head. Face light grey, heavily pollinose. Antenna dark, but the first two segments (scape and pedicel) often notably paler, brownish-yellow. Thorax. Scutum black

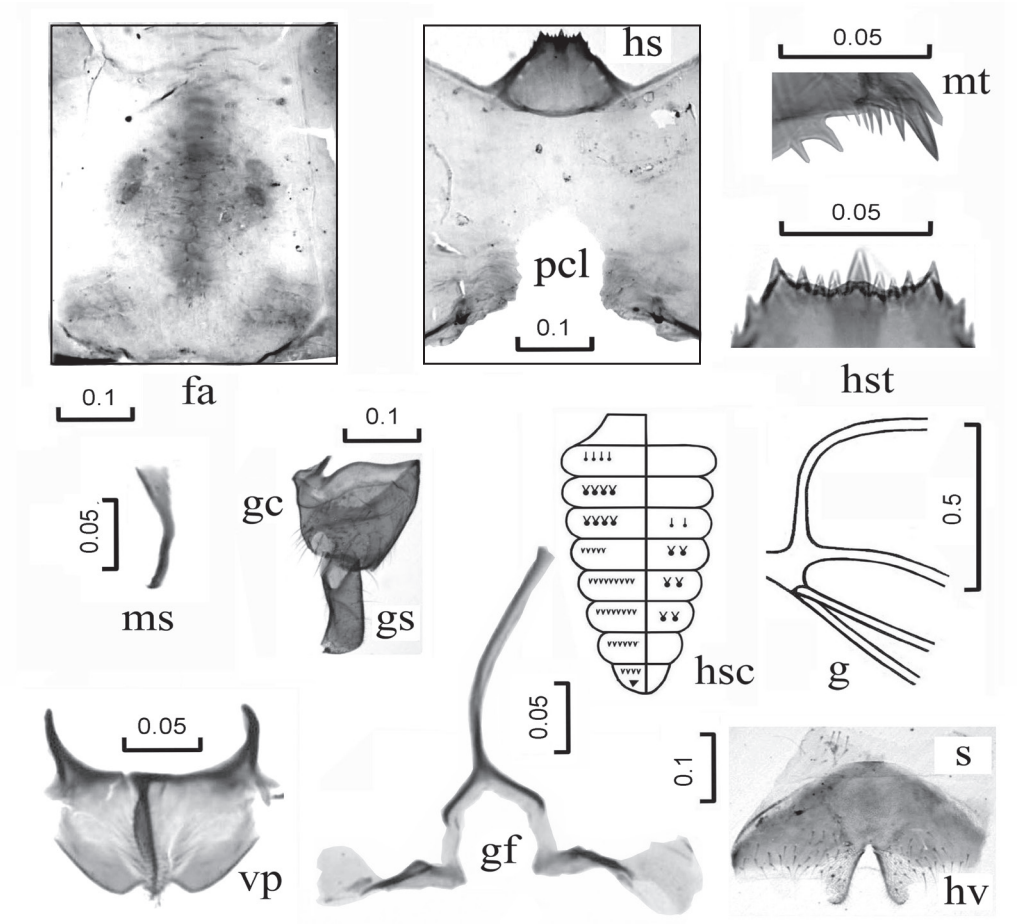


Fig. 5. Morphological structures of the immature stages in the *Simulium* (*Nevermannia*) *angustitarse* complex. Morphotype III ("*lundstromi*-type"). For legend, see Fig. 3

with a more pronounced silver-grey pruinosity under certain lighting. Pubescence consisting of pale golden hairs. Halter bright white. Legs. Similar to Morphotype I, but the yellow colouration on the mid and hind femora often more extensive, occupying the basal two-thirds. Wing. Veins pale yellow; hairs on the stem vein predominantly dark. Terminalia. Ventral plate slightly narrower than in Morphotype I, with the median keel being more prominent and sharply defined. Gonostyle slightly broader at the base.

Morphotype III ("lundstromi-type", continental form) (Fig. 5). Occurring in larger river systems; larvae characterised by distinct head capsule patterns. Head. Face dark, antenna entirely dark. Sensory vesicle of the maxillary palp slightly larger and more rounded than in the previous types. Thorax. Scutum black with dense golden pubescence. Halter yellow. Legs. Legs generally darker; femora and tibiae with extensive dark brown infuscation, yellow areas restricted to the extreme bases. Wing. Costa and radial veins robust, brownish-black. Terminalia. Gonostyle robust, with a slightly curved outer margin. Ventral plate with a broad, flattened body and a short, stout keel.

Discussion

Taxonomic implications. The species complex under study involves three nominal taxa with contrasting distributional patterns: *Simulium (Nevermannia) angustitarse* and *S. lundstromi* are characterised by vast, broadly overlapping Palaearctic ranges, while *S. volhynicum* has traditionally been treated as a narrow regional endemic. Crucially, the taxonomic identity of these taxa is anchored in their respective type localities: Finland for *S. angustitarse* and England for *S. lundstromi*.

The most significant outcome of this integrative study is the profound discordance between the high level of morphological plasticity and the remarkable genetic homogeneity observed in the populations of *Simulium angustitarse*. Despite the clear differentiation of three morphotypes based on classical characters — such as the branching patterns of pupal filaments and the depth of the larval postgenal cleft — molecular analysis of the COI gene confirms that they belong to a single, unified genetic pool. Specifically, the genetic distance between the "volhynicum" and "lundstromi" morphotypes in the Volyn region (0.43%) is comparable to intra-group variability and remains significantly lower than the divergence between geographically distant lineages from the UK and the Carpathians (2.71%).

Based on these findings, we propose that the described Morphotypes I, II, and III represent "eco-phenotypes" — adaptive forms arising in response to specific environmental conditions rather than markers of reproductive isolation. Morphotype I is associated with moderate-flow streams, Morphotype II (volhynicum-type) exhibits modifications characteristic of slow-flowing waters with abundant macrophytes, and Morphotype III is primarily linked to larger river systems. Such plasticity likely allows *S. angustitarse* to effectively exploit a wide range of ecological niches across its vast Palaearctic range without undergoing speciation.

Conclusions

Integrative analysis of the mitochondrial COI gene demonstrates that the morphological diversity within the *Simulium (Nevermannia) angustitarse* complex across Central and Eastern Europe is intraspecific. The genetic distances observed between the investigated morphotypes (0.30–0.55%) are significantly lower than established interspecific thresholds for Simuliidae, confirming that they belong to a single, unified genetic pool. According to the principle of priority and genetic identity with populations from the type locality in Finland, *Simulium (Nevermannia) volhynicum* (Usova & Sukhomlin, 1990) is formally established as a junior synonym of *Simulium (Nevermannia) angustitarse* (Lundström, 1911).

Furthermore, continental European populations previously identified as *S. lundstromi* based on morphological characters are shown to be conspecific with *S. angustitarse*. The "true" *S. lundstromi* (Enderlein, 1921) is identified as a genetically isolated insular taxon restricted to the British Isles, diverging from the continental core by approximately 1.8–2.2%. The three identified morphotypes (I, II, and III) are interpreted as eco-phenotypes that represent adaptive plastic responses to specific environmental conditions, as no evidence of reproductive isolation was observed at the mitochondrial level. These findings highlight the limitations of purely morphological approaches in the study of the

subgenus *Nevermannia* and underscore the reliability of molecular markers for resolving complex taxonomic uncertainties and synonymy. While preliminary data from Bulgaria and Turkey suggest maintained genetic integrity across a vast geographic range, further extensive barcoding across the Palaearctic is required to fully map the species' phylogeographic structure.

Data Availability Statement. Data are publicly available in GenBank Databases.

Conflicts of Interest. The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the authors used Gemini (Google LLC) in order to improve the language and readability of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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