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# Molecular Characterization of the *Forficula tomis* based on the *Cytochrome C Oxidase I* Gene

**Victory Vladimirovna Stolbova, Vladislava Ruslanovna Garbaly, Kseniya Sergeevna Krestonoshina, and Zimfira Yakubovna Zinatullina***

*All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology, Branch of Federal State Institution Federal Research Centre Tyumen Scientific Centre of Siberian Branch of the Russian Academy of Sciences (ASRIVEA – Branch of Tyumen Scientific Centre SB RAS) Institutskaya st. 2, Tyumen, 625041, Russian Federation, Russia*

*Corresponding author's Email: zimfirazi@mail.ru

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**ABSTRACT**

The ovine Earwigs of the genus *Forficula* play a significant role in agriculture and beekeeping, acting as pests and vectors of honey bee pathogens. Despite the ecological and agricultural significance, nucleotide sequences of the species *Forficula tomis* (*F. tomis*) are absent in genetic databases, which complicates its molecular identification. The present study aimed to perform the molecular characterization of the earwig *F. tomis* using the *cytochrome c oxidase I* (*COI*) gene fragment as a molecular marker. A total of 18 earwig specimens were hand-collected from *Apis mellifera* hives in the Tyumen region, Russia. During the cultivation, 9 adults were obtained, which were used in the present study. Morphological analysis confirmed that *F. tomis* is the predominant species of bee hives in the Tyumen region. Sequencing of the *COI* gene fragment made it possible to obtain a reference sequence of *F. tomis*, which, in phylogenetic analysis, formed a separate clade and demonstrated a significant genetic distance (up to 22.9%) with other representatives of the genus *Forficula*. The optimal species differentiation threshold for the group was 11.7%, which significantly exceeds the standard barcode gap (3%) and may indicate long-term evolutionary isolation or the presence of cryptic diversity. The obtained results expand the present understanding of the genetic structure of the genus *Forficula* and have practical significance for monitoring apiary pests, developing methods for molecular identification of bee pests, and understanding their role in the spread of pathogens.

**Keywords:** Beekeeping, C*ytochrome c oxidase I* gene, DNA barcoding, Earwigs, *Forficula tomis*

## INTRODUCTION

The global expansion of invasive insect species, facilitated by human activity, poses significant threats to agriculture, native ecosystems, and managed pollinators. Understanding the biology and spread of these synanthropic pests is critical for developing effective monitoring and control strategies, particularly for cryptic species complexes that are often overlooked (Garnas et al., 2016). Earwigs of the genus *Forficula* are a prime example of such widespread species that have significantly expanded their range. As synanthropic insects, they thrive in human-modified environments, including agricultural fields, gardens, and urban areas, where they are known as pests of agricultural garden (potatoes, beets, soybeans, etc.) and fruit crops (Quarrell et al., 2021; Riedle-Bauer et al., 2024), grain crops (Binns et al., 2021), melons, tobacco, and floriculture (Bey-Bienko, 1936). Beyond their impact on agriculture, earwigs have also become a persistent problem in apiculture. The earwigs are a common component of the fauna of honey bee hives, and during periods of high activity, they can cause significant damage to bee colonies (Domatsky and Domatskaya, 2020). In the hives, they feed on the food reserves of honeybees and bee bread, wax, and bee bread crumbs, small arthropods, and bee corpses (Atakishiyev, 1969; Banaszak, 1980). When present in large numbers, earwig infestations lead to colony weakening and reduced honey production (Domatsky and Domatskaya, 2020). Earwigs are carriers of infectious diseases of honey bees, such as American and European foulbrood (Sidorov, 1968) and honeybee viruses (Atakishiyev, 1969; Domatsky and Domatskaya, 2020). Thus, 5 types of viruses affecting *Apis mellifera* have been isolated from *Forficula auricularia*: deformed wing virus (DWV), Kashmir bee virus (KBV) (Dobelmann et al., 2020), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV), and sacbrood virus (SBV) (Levitt et al., 2013).

The genus *Forficula* belongs to the family Forficulidae and is the most numerous in the order Dermaptera. The genus includes at least 68 species (Steinmann, 1993). The most widespread and most important species of earwigs from this genus is *F. auricularia*, the common or European earwig. The species has a cosmopolitan range, which includes Europe, North Africa, Asia, North America, South America, Australia, and New Zealand (Bey-Bienko, 1936; Kirkland et



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al., 2020; Markova and Maslov, 2024). Numerous studies have been devoted to the study of this species (Bhattarai et al., 2022; Freda et al., 2025; Pasquier et al., 2025). Recent molecular studies have shown that *F. auricularia* is a cryptic species complex (González-Miguéns et al., 2020; Freda et al., 2025). The finding raises a critical question regarding whether similar cryptic diversity occurs in the other species within the genus, particularly in *F. tomis*. It is very important not only from a theoretical but also from a practical point of view, since a recent study found *F. auricularia* DNA in metagenomic analysis of honey from Estonia (Paluoja et al., 2025). Given the high importance of earwigs in bee hives and the emerging opportunity to identify bee pathogens using molecular methods, it is important to know the full composition of the hive's synanthropic fauna, including the presence of cryptic species that carry the main pathogens.

*Forficula tomis* the garden earwig, is a species close to the common earwig, but has been studied to a much lesser extent, even though its role in agriculture and, in particular, in bee hives is no less important (Atakishiyev, 1969). *Forficula tomis* has a much more limited range, distributed in the Southern and central parts of Europe to the West as far as Moldova (Elisovetcaia et al., 2014), in the middle and Southern strip of the European part of Russia (Aleksanov et al., 2023), in Armenia and Turkey (Anlaş, 2012), in the mountains of Central Asia (Bey-Bienko, 1936), in Siberia (Dorzhieva, 2011), Korea, and Japan (Steinmann, 1993). The species was probably brought to Siberia at the beginning of the 20th century by settlers (Bey-Bienko, 1936). Following its introduction, *F. tomis* became the most common earwig species in Siberia and successfully spread to Eastern Siberia, where it was recorded in Transbaikalia in 2006 (Dorzhieva, 2011). In Russia and the former Soviet Union, *F. tomis* is regularly found in bee hives, and has been recorded in hive samples from the Eastern European part of Russia (Volga-Kama region), the Republic of Bashkortostan, and Azerbaijan (Sidorov, 1968; Atakishiyev, 1969; Bakalova, 2011). In Siberia, this species is the only earwig reported to inhabit honeybee hives to date (Stolbova, 2023). Previous studies indicate that *F. tomis* is a recognized pest in apiculture across both Eastern Europe (Atakishiyev, 1969; Banaszak, 1980) and Siberia (Domatsky and Domatskaya, 2020). At the same time, unlike the common earwig *F. auricularia*, this species has been much less studied; in particular, there are no DNA sequences of this species in published scientific articles or the Genbank and Barcode of Life (BOLD) databases (Ratnasingham, 2007). Species identification presents particular challenges when dealing with nymphal-stage earwigs collected from hives, as morphological characteristics are often insufficient for reliable determination. Consequently, comprehensive DNA reference data for all bee-associated pest species are needed to enable accurate molecular identification.

As a molecular marker, the *cytochrome c oxidase I* (*COI*) gene was selected, which is widely used and considered the most convenient molecular marker for identifying insect species (Wang et al., 2017; Kaur and Singh, 2020). The *COI* gene does not contain introns and has a variety of regions, both highly variable, which provide a high level of interspecies polymorphism and low intraspecies polymorphism, and conserved regions that are often used to develop universal PCR primers, which simplifies its use and amplification (Boehme et al., 2012). In addition, the advantage of the *COI* gene is its presence in a large number of copies in the mitochondrial genome, as well as a high rate of evolution, approximately 2-9 times higher than the rate of nuclear genes (Hebert et al., 2003). Taken together, these characteristics allow the analysis of both close and distant phylogenetic relationships and make the *COI* gene an ideal candidate for DNA barcoding, providing rapid, reliable, and cost-effective species identification, even in the case of cryptic and very closely related species, which is difficult with morphological analysis (Kaur and Singh, 2020). The present study aimed to perform the molecular characterization of the earwig *F. tomis* using the *COI* gene fragment as a molecular marker.

## MATERIALS AND METHODS

### Ethical approval

The present study was conducted ethically in accordance with the guidelines of the All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology and national regulations.

### Earwig cultivation

Earwigs were collected from *Apis mellifera* hives in the spring of 2024 from apiaries in the Tyumen region, Russia. A total of 18 specimens at the nymphal stage of development were collected. To obtain adults, earwigs were kept in plastic containers (14×21 cm) on a moistened coconut substrate at a temperature of 20°C and relative humidity of 60%. Dry oatmeal, fresh carrots, and high-protein food for aquarium goldfish of the Goldfish brand (38% protein, 7% fat, 3% plant fibre) were used as a food source (Tomkins, 1999; Markova and Maslov, 2024). During the cultivation, some earwigs (9 specimens) reached the imago stage, and 2 clutches of eggs were obtained. The remaining earwigs died before reaching adulthood due to unspecified causes. Species identification of earwig adults was carried out using a guideline of Bey-Bienko (1936).

**Preparation of the target gene**

A total of 9 adult *F. tomis* specimens (3 males, 6 females) were collected for molecular genetic analysis. Specimens were homogenized whole using a Bioprep-24 device (Hangzhou Allsheng Instruments Co., LTD, China) at +4°C using a diaGene kit (diaGene, Russia) for DNA extraction from animal tissues on spin columns according to the manufacturer's standard instructions provided in the protocol. DNA quantity and quality were determined spectrophotometrically using a Nano-300 (Hangzhou Allsheng Instruments Co., LTD, China). Sample purity was assessed spectrophotometrically by measuring the absorbance ratio at 260 and 280 nm, with ratios between 1.9 and 2.1 indicating high-quality DNA preparations. Amplification was performed on a GeneExplorer GE-96G device (Bioer, China). Amplification conditions for the gene fragment primer: preincubation at 95°C for 5 minutes, then 35 cycles of denaturation for 20 seconds at 95°C, annealing for 20 seconds at 51°C, and elongation for 20 seconds at 72°C. Composition of the reaction mixture (20 µl), including 14.4 µl of deionized water, 4 µl of 5X ScreenMix-HS mix (Evrogen, Russia), and 0.3 µl of forward and reverse primer. In the present study, universal *COI* primers originally designed by Hebert et al. (2003) were employed, as they have become standard markers in DNA barcoding studies (Kim et al., 2012; Mifkova et al., 2023). The primer sequence was taken from the study by Archana et al. (2015), including COIuF - 5'-GGTCAACAAATCATAAAGATATTGG-3' and COIuR 3'-TAAACTTCAGGGTGACCAAAAAATCA-5'. Electrophoresis in a 1.5% agarose gel was used to visualize the amplification results.

**Analysis of sequencing results**

Sequencing of the obtained nucleotide sequences was performed by Evrogen LLC (Russia) using the Sanger method. Electropherogram quality control and base calling were performed by the service provider. The forward and reverse reads were assembled into consensus sequences using the *de novo* assembly algorithm (CAP3) implemented in Unipro Ugene v.52.1. Bioinformatics analysis of the sequences included checking raw data in the Finch TV 1.4.0 program for forward and reverse readings in ABI format. Signal quality and artifacts were visually assessed. During the assembly process, low-quality base calls at the sequencing termini were automatically trimmed. The resulting consensus sequence was manually inspected; no ambiguous bases (N) were present in the final sequences, confirming high read quality. To construct a phylogenetic tree in the MEGA program, 38 nucleotide sequences of various lengths were selected from the NCBI database, representing different *Forficula* species and coinciding with the *COI* gene fragments sequenced by us. The sequences underwent multiple alignment (ClustalW) and trimming to 587 bp. The uniform length of 587 bp was determined by the region flanked by the universal *COI* primers and represents the high-quality, unambiguous consensus sequence obtained after trimming low-quality base calls from the sequencing termini. The approach ensured a consistent and comparable alignment across all taxa, which was a fundamental requirement for robust phylogenetic reconstruction. The selected fragment encompassed a sufficiently variable region of the *COI* gene suitable for resolving interspecific relationships within the genus *Forficula*.

**Phylogenetic tree construction**

The outgroup was selected to root the phylogenetic tree and provide a reference point for inferring evolutionary relationships within the ingroup (*Forficula* spp.). Anisolabis maritima (family *Anisolabididae*) was chosen as it represents a well-established external lineage to the family *Forficulidae*, to which the genus *Forficula* belongs. The taxonomic distance ensures that the outgroup is unequivocally outside the clade of interest, a fundamental criterion for accurate rooting, while remaining sufficiently phylogenetically close to allow for reliable sequence alignment and inference of homology. The species *Anisolabis maritima*, Bonelli, 1832, was consequently used as an outgroup. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method under the p-distance model, with node support assessed by 1000 bootstrap replicates.

To quantitatively assess the genetic divergence between *Forficula* species, pairwise genetic distances were calculated based on multiple nucleotide sequence alignments. The resulting matrix of genetic distances was analyzed to determine the optimal threshold for species differentiation. The optimal threshold was identified using the threshOpt algorithm, which implements the silhouette coefficient maximization method (Rousseeuw, 1987) to iteratively select the threshold value that maximizes the average silhouette coefficient. The method is recommended for threshold-based insect species delimitation using *CO I* barcodes, as it effectively minimizes identification errors compared to arbitrary fixed thresholds (Zhang and Bu, 2022). Furthermore, local minima in the distribution of genetic distances were identified using the localMinima function, which inverts the input data to treat local minima as peaks and applies the sciPy.signal.find_peaks algorithm (Virtanen et al., 2020). The prominence parameter was set to 12.0 to define the minimum height a valley must have relative to its surrounding peaks to be considered significant, effectively filtering out noise-related artifacts.

## RESULTS AND DISCUSSION

Based on morphological criteria, all earwigs collected from apiaries belonged to the species *F. tomis*. Males of *F. tomis* are morphologically quite well distinguished from other species of the genus *Forficula* by the structure of the paired appendages of the last abdominal segment - cerci. In males of *F. tomis*, the cerci (forcipes) do not have an isolated tooth on the inner side, are expanded, and touch in the basal half. The outer sides of the cerci are almost parallel to each other along their entire length; the apical parts of the cerci are moderately thin, slightly bent at the very end (Bey-Bienko, 1936; Figure 1). However, in females of most species of the genus *Forficula*, the cerci are uniform (simple, thin, close together) and therefore have no diagnostic value (Bey-Bienko, 1936; Freda et al., 2025). Morphological species identification of nymphs and females of the genus *Forficula* can be carried out only presumably by additional criteria (color of the head, pronotum, elytra, and ratio of sizes of different body parts), which have wide variability depending on age, ecological, and biological conditions of the habitat. For this reason, to date, there are no identifiers of the genus *Forficula* for females and preimaginal stages of development. Such specimens can be identified only using molecular diagnostic methods.

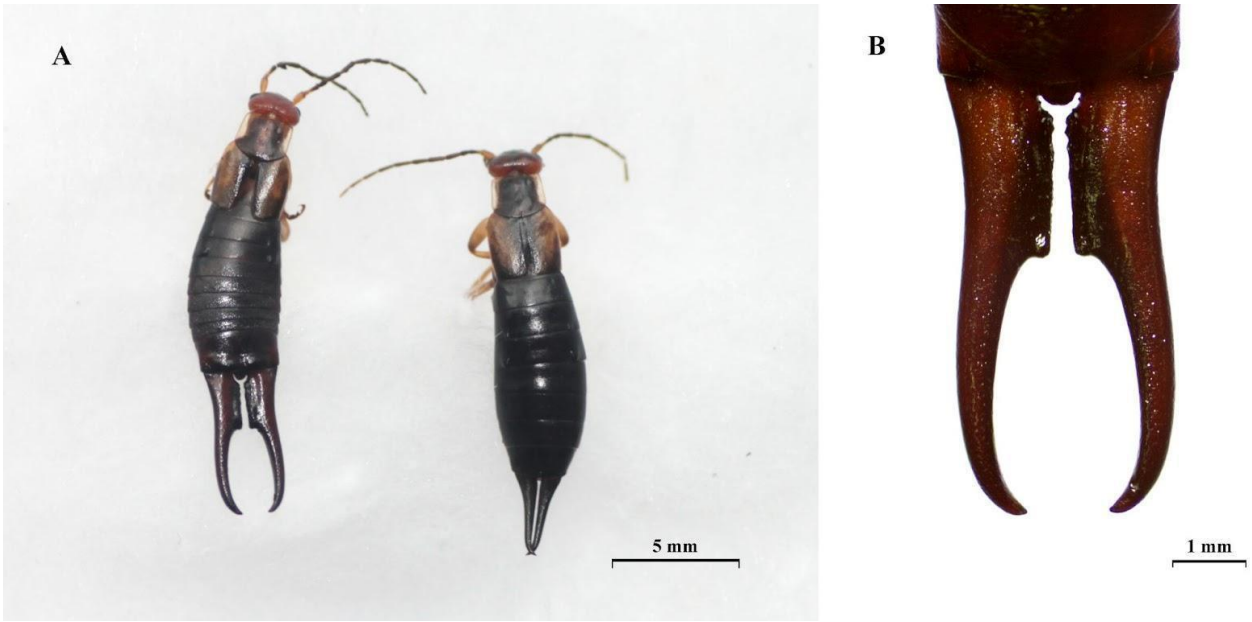



**Figure 1.** *Forficula tomis*, collected from an apiary in the Tyumen region, Russia. **A**: Male (left) and female (right); **B**: Cerci of the male.

Since earwig species are highly morphologically similar and often represent cryptic species complexes, their classification has been revised several times. Initial study by Wirth et al. (1998) revealed that *F. auricularia* comprises a complex of at least two cryptic species. Subsequent study (González-Miguéns et al., 2020) has expanded this number, and the complex is now considered to include at least four species (*F. auricularia*, *F. mediterranea*, *F. aeolica*, *F. dentata*). Given this precedent, it is plausible that the morphologically defined species *F. tomis* may likewise constitute a cryptic species complex. However, to date, there is insufficient information in the databases to verify this assumption, since out of 68 species listed in the identification guides, sequenced DNA sequences are available for only 16 species.

In the present study, as a result of sequencing, the sequences of the *F. tomis COI* gene fragment were obtained for the first time. The combined forward and reverse reads of the samples were subjected to multiple alignment, after which a consensus sequence of 589 bp in length was formed:

5`-
GTCCGGGATGGTTGGGACATCGCTAAGTCTGTTGATCCGAGCCGAGTTGGGGCAACCTGGGGCATTAATTG
GGGATGACCAAATTTACAACGTAATTGTAACCGCACATGCGTTTGTAATGATTTTTTTTATGGTAATGCCA
ATCATGATTGGAGGGTTTGGGAATTGACTGGTTCCCCTGATGCTTAGCGCCCCCGATTGGCATTCCCTCGTA
TAAACAACATAAGCTTTTGATTGCTTCCCCCTTCTTTGATGTTACTACTTTCAGGTAGAATGGTGGATAGCG
GGGCAGGGACAGGGTGAACGGTTTACCCCCCTCTGTCCGGGGCCATTGCTCACGCAGGGGCTTCGGTGGA
TTTGAGAATCTTTTCCCTGCATTTGGCAGGAATTTCCTCTATTTTAGGTGCTGTTAACTTTATCACAACCGTA
ATCAACATGCGCCCAGTAGGATTAAGCCTAGAACGGATGCCTTTGTTTGTTTGGTCAGTAGCTATCACTGC
TTTGTTGTTATTGTCTTTGCCCGTATTGGCGGGGGCCATTACTATGCTTTTAACCGATCGAAACCTAAATAC
CTCCTTTTTTGACCCTG-3`

Analyses with ORFfinder confirmed the absence of stop codons and indels, supporting the conclusion that the obtained sequence represents a functional mitochondrial *COI* gene and not a nuclear pseudogene (numt). In BLAST alignment, the highest similarity was with *F. dentata* (80.44%). The surprisingly low sequence identity for congeneric species could be attributed to several non-exclusive factors. These include the potential presence of extensive cryptic

diversity within the genus *Forficula* that remains underrepresented in genetic databases, a long evolutionary history of divergence between *F. tomis* and other sequenced congeners, or the possibility that current taxonomic boundaries within the genus do not fully reflect the deep genetic divergences. The notably high genetic distance observed supports the hypothesis that *F. tomis* represents a highly divergent lineage. The consensus amino acid sequence for the *COI* gene fragment:

>ORF 585 nt / 194 aa

MVGTSLSLLIRAELGQPGALIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLSAPDMAFPRMN
NMSFWLLPPSLMLLLSGSMVDSGAGTGWTVYPPLSGAIAHAGASVDLSIFSLHLAGISSILGAVNFITTVINMRP
VGLSLERMPLFVWSVAITALLLLLSLPVLAGAITMLLTDRNLNTSFFDP

The consensus sequence of *F. tomis* obtained in the present study formed a distinct clade on the phylogenetic tree, which appeared as a sister group to the clade comprising *F. ruficollis* (Portugal) and *F. mikado* (Japan). Bootstrap support for this node was 77% (Figure 2), which is generally considered moderately high support in phylogenetic analyses. The formed clade was positioned distantly from both *F. auricularia* and *F. dentata*. The tree was rooted using *Anisolabis maritima* (*Anisolabididae*), which, as expected, formed a long external branch (branch length = 0.1506), confidently separating it from all representatives of the family *Forficulidae*. The substantial genetic divergence confirms the correct choice of an outgroup and provides a reliable reference point for interpreting the internal topology of the genus *Forficula*.

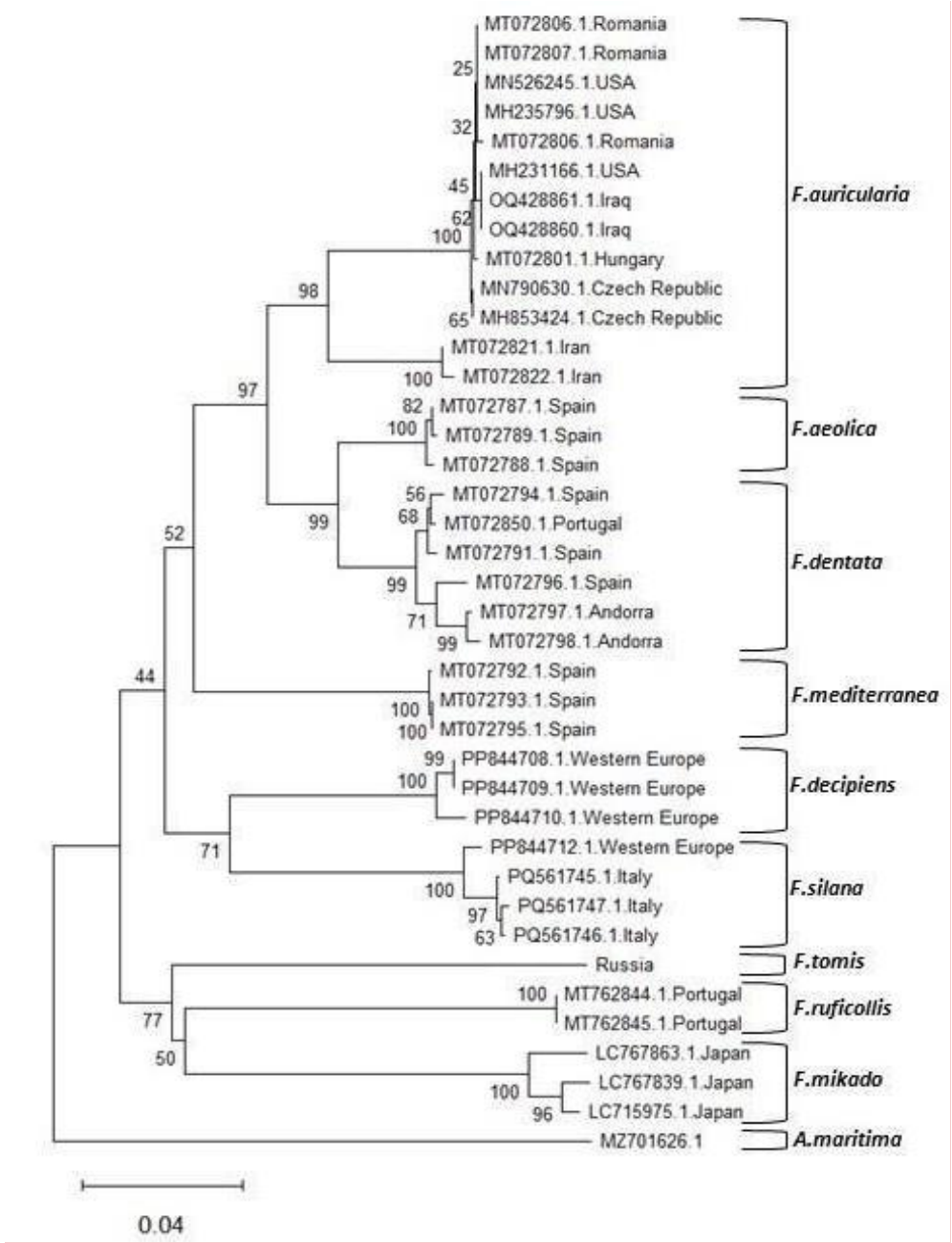
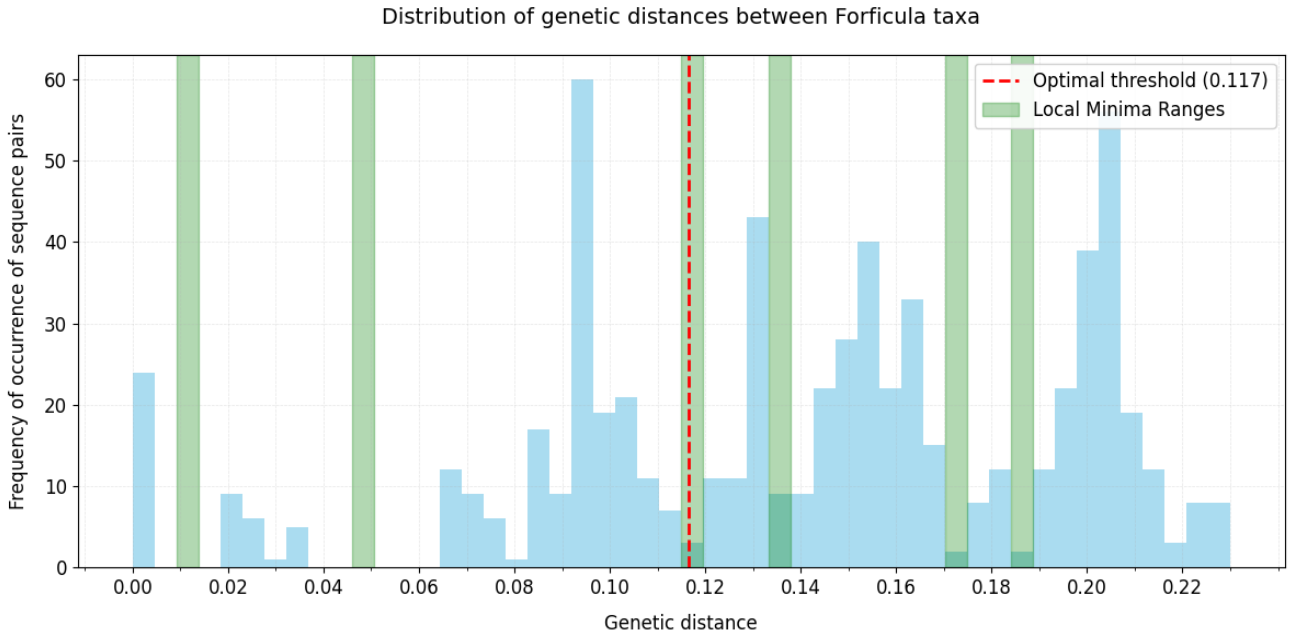



**Figure 2.** Phylogenetic tree of the genus *Forficula*, with the species *A. maritima* used for the outgroup. Construction method: Neighbor-Joining, evolutionary distance model: P-distance, node stability assessment: 1000 bootstrap replicates.

The substantial genetic gap, evidenced by both the BLAST results and the phylogenetic reconstruction, is likely caused by the low representation of suitable nucleotide sequences from relevant geographic regions in the NCBI database and the generally low level of study of earwigs in Russia and adjacent areas. It is probable that with expanded geographic sampling and a more comprehensive database, the phylogenetic relationships within the genus will be resolved with greater accuracy.

The analysis of genetic distances showed that the maximum distance (0.2290) was observed between the sequence *F. tomis* (Russia) and the sequence *F. silana* (Western Europe) with NCBI accession number PP844712.1. The median of distances was 0.1482, and the standard deviation was 0.0550. The threshOpt algorithm determined the optimal threshold at 0.117, which corresponded to the silhouette index of 0.606 (> 0.5 stable values). The "localMinima" algorithm identified 6 local minima, which can serve as potential thresholds for separating species (Graph 1). The six local minima identified in the genetic distance distribution likely correspond to genetic boundaries between major lineages within the genus *Forficula*. Given the limited taxonomic sampling (9 species) in the current analysis, these minima cannot be unambiguously assigned to known species complexes without additional data. However, their positions are consistent with the deep genetic divergences observed in the phylogenetic tree (Figure 2) and may represent both described species and potential cryptic diversity. For instance, the minima at the lower end of the range (~ 0.01-0.08) may reflect intraspecific variation or recent speciation events within complexes such as *F. auricularia*, while the minima at higher values (~ 0.11-0.19) likely correspond to divergence between well-established species (e.g., *F. auricularia* and *F. dentata*). The pattern underscores the complex phylogenetic structure of the genus and highlights the need for a more comprehensive taxon sampling to validate these preliminary boundaries.




**Graph 1.** The distribution of pairwise genetic distances among *Forficula taxa*. Blue bars show the frequency of sequence pairs in ranges of genetic distances, and green bars indicate ranges of local minima. The red dotted line at 0.117 indicates the optimal threshold.

The success of DNA barcoding is often related to the presence of a barcoding gap. If intraspecific divergence does not overlap with interspecific divergence, DNA barcodes can effectively identify specimens. DNA barcoding loses its effectiveness when the gap in the barcode becomes small or absent (Meyer and Paulay, 2005). Thresholds of sequence divergence, such as 2% or 3%, have been proposed to group specimens into preliminary species (Hebert et al., 2004; Smith et al., 2005). The obtained threshold of 11.7% (Graph 1) was significantly higher than the standard threshold (barcode gap) of 3%, but it is not always reliable in delimiting species boundaries, especially in groups with cryptic species such as *Forficula* and other insects. The presence of cryptic species and high levels of divergence mean that the gap is often greater than 3%, making the use of a universal threshold problematic. For example, the interspecific genetic distance in Molytinae (Coleoptera: Curculionidae) was exactly 11% in (Ren and Zhang, 2024), and the average interspecific divergence in scale insects (Hemiptera: Diaspididae) was 10.07% in (Niu et al., 2024). It should be noted that about a quarter of insect species have high intraspecific genetic variability (> 3%). Due to the high intraspecific genetic variability in insects, false positive results can easily occur when determining species boundaries based on the *COI* gene thresholds (Zhang and Bu, 2022). To obtain reliable results for phylogeny reconstruction, it is necessary to supplement studies with genetic markers such as *COII*, *16s rRNA*, *ITS II*, *28Sr RNA*, and others, increase the sample size,

and expand studies to other regions (Huemer and Wieser, 2023). Therefore, the high threshold observed in the present study is not anomalous but rather underscores the limitations of applying a universal distance threshold for species delimitation in genetically structured groups (Ren and Zhang, 2024). It highlights the necessity of integrative taxonomic approaches for *Forficula*, combining multiple molecular markers (e.g., nuclear genes, transcriptomes), morphology, ecology, and other data sources to achieve robust species boundaries (Janzen et al., 2017).

## CONCLUSION

Morphological analysis confirmed that *F. tomis* is the sole earwig species inhabiting hives in Tyumen apiaries. Tyumen *F. tomis* specimens form a distinct clade, with BLAST analysis revealing significant genetic distance between *F. tomis* and other representatives of the *Forficula* genus. The identified optimal species differentiation threshold (11.7%) significantly exceeds the generally accepted barcode gap value of 3%. While *COI* variability suffices for genus-level identification, species-level phylogenetic resolution requires supplemental markers. Adaptive methods ("threshOpt" and "localMinima") uncovered complex genetic differentiation patterns, underscoring the necessity of integrative approaches for understudied insect groups, especially in such poorly studied regions as Siberia. Future studies should prioritize comparative morphology of *F. tomis*, nuclear marker analysis, and expand sampling across Northern Eurasia.

## DECLARATIONS

### Authors' contributions

All authors participated in writing the article, searching for information, and editing the text. Victory Vladimirovna Stolbova collected the earwigs and performed morphological identification of species, Vladislava Ruslanovna Garbaly performed bioinformatics analysis, translation, and editing of the publication, Kseniya Sergeevna Krestonoshina conducted molecular genetic studies, was responsible for strategic planning, Zimfira Yakubovna Zinatullina was engaged in project administration and its conceptualization, and collected materials. All authors checked and approved the final version of the manuscript.

### Competing interests

The authors have not declared any conflicts of interest.

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### Ethical considerations

All authors declare that this paper is original, conducted solely by the authors, and it has not been submitted and published elsewhere.

### Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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