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TAXONOMIC POSITION OF TETROPS PETERKAI SKOŘEPA, 2020 (COLEOPTERA: CERAMBYCIDAE) AND ITS CRYPTIC DISTRIBUTION

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Abstract. In this study, we sequenced the cytochrome c oxidase subunit I (COI) gene from *Tetrops peterkai* specimens collected in northern Ukraine, obtaining the first barcodes for this species from the region. These barcodes revealed major genetic differences, supporting the distinction between *T. peterkai* and its sibling *Tetrops praeustus* and refuting the early suggestion to synonymize them. These findings underscore the need to formally restore the name *Tetrops peterkai* Scořepa, 2020, **nom. res.** Our analysis reveals wide distribution of *Tetrops peterkai* across Northern and Eastern Europe and Northern Balkans, including the first barcode-based records in Bulgaria, Estonia, Finland, France, Norway, and Sweden. In contrast, *Tetrops praeustus* is more common in southern and western Europe. The ranges of both species broadly overlapping in Central and Eastern Europe. Additionally, the *Tetrops* species introduced to North America has been identified as *Tetrops peterkai*, likely originating from France.

Keywords: the longhorn beetles, molecular phylogeny, cryptic biodiversity, cladistics, integrative taxonomy, sequencing, barcoding.

1. INTRODUCTION

Tetrops Kirby, 1826, is a small Palearctic genus comprising at least ten valid species. Taxonomically, the genus is divided into two subgenera. The first, the nominative subgenus *Tetrops* Kirby, 1826, includes at least six species, primarily distributed across the Western Palearctic region, extending as far as Mongolia. The second, *Mimosophronica* Breuning, 1943, contains at least four species found mainly in the Eastern Palearctic and Western Asia (Danilevsky, 2020; Tavakilian. & Chevillotte, 2024).

In Europe, *Tetrops* is represented by four species: *Tetrops praeustus* (Linnaeus, 1758), *Tetrops gilvipes* (Faldermann, 1837), *Tetrops starkii* Chevrolat, 1859, and *Tetrops peterkai* Skořepa, 2020. Due to the high morphological similarity among these species, most published data on their distribution, biology, and ecology are reported under the generalized name of the classic Linnaean species, *Tetrops praeustus* (Sláma, 2020; Hass & Pütz, 2023). However, this categorization is generally recognized as artificial, a view shared by most researchers. Attempts to address this issue have led to the subdivision of most of these species into subspecies (Tavakilian. & Chevillotte, 2024). For example, *T. praeustus* is currently divided into at least five subspecies; *T. gilvipes* encompasses six subspecies; and *T. starkii* is represented by two subspecies (Danilevsky, 2020; Tavakilian. & Chevillotte, 2024). This classification is based on observed geographic variation within each species, though it remains unconfirmed by molecular phylogenetic and phylogeographic data. Such studies are yet to be conducted.

Tetrops peterkai is a recently described species from Central Europe (Skořepa, 2020), previously mistaken for *T. praeustus*. However, the two species are distinguishable by the structure of male terminalia and several external morphological features. According to the original description, *T. peterkai* is distributed in the Czech Republic, Slovakia, Austria, and Germany. It was later recorded in Ukraine, with a strong likelihood of occurrence in Poland (Zamoroka, 2023). Nevertheless, its overall distribution and taxonomic status remain uncertain.

In the present study, we sequenced the gene of cytochrome c oxidase subunit I (COI) from several specimens of *T. peterkai* collected in northern Ukraine during expeditions in 2022. From these samples, we obtained two reliable and high-quality COI sequences. As a result, we confirmed that *T. peterkai* and *T. praeustus* are indeed two distinct species, showing clear genetic differentiation.

2. MATERIALS AND METHODS

Sample collections. Samples for sequencing were collected in May–June 2022 in the Volyn Region of Ukraine (northwestern part of the country). For the analysis, whole *Tetrops* imago specimens were preserved in 96% ethanol in field conditions, labeled, and transported to the laboratory. Prior to DNA extraction, samples were stored in ethanol at -20°C.

DNA extraction. Since *Tetrops* imagos are very small (3 mm), we used the thoracic muscles for DNA extraction by separating the pronotum from the rest of the body and placing it in a separate well of 96-well PCR plate. Then we added 25 μL of lysis buffer C, 200 mM Tris pH 8.0, 25 mM EDTA pH 8.0, 0.05% Tween-20 and 0.4 mg/ml Proteinase K (Korlević et al., 2021), to the tube. The mixture was homogenized to ensure complete lysis buffer distribution with following incubation of the samples at 56°C for 2 hours to promote thorough cell lysis and DNA release. After incubation, we diluted aliquot of lysate 10 times and did all consecutive PCR steps with unpurified DNA solution to save time and consumables (Korlević et al., 2021; Makunin et al., 2022).

PCR. We applied indexed universal indexed COI primers HCO and LCO for PCR (Srivathsan et al., 2021). Master Mix mixture was prepared as followed: 5 μL of Master Mix (MM), 0.3 μL of the Forward Primer (Pr F), 0.3 μL of the Reverse Primer (Pr R), 3.2 μL of distilled water, 0.7 μL of the DNA template, and 0.5 μL of Dimethyl sulfoxide (DMSO). This combination brings the total reaction volume to 10 μL per sample. The mixture was gently mixed to avoid introducing bubbles in a thin-walled PCR tube. The PCR was conducted with an initial denaturation at 95°C for 2 minutes, followed by 25-35 cycles of denaturation at 95°C for 30 seconds, annealing with 40°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension at 72°C for 5 minutes was applied to complete the amplification. The 2 μL of each amplified samples were proceeded immediately with gel electrophoresis for DNA detections and the rest volumes were stored at 4°C for the following sequencing.

Library preparation and Sequencing.

We followed protocol of Oxford Nanopore Technology DNA barcoding workflow developed by Srivathsan and coauthors (2021). In brief, individual PCR products amplified with unique combination of indexed primers were mixed, purified and prepared according to the ONT protocols and read on flow cell. After base calling all the further bioinformatic steps (demultiplexing, consensus sequence construction) were done using ONTBarcoder 2.0.

Phylogenetic analysis. The analysis was conducted using Seaview 5.0, a cross-platform software designed for multiple sequence alignment, molecular phylogenetic analysis, and tree reconciliation (Gouy et al., 2021). Sequence alignments were generated using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm integrated into Seaview 5.0. Alignments were subsequently reviewed and manually edited to address regions with missing data and to exclude unalignable positions. Phylogenetic trees were inferred using the maximum-likelihood approach implemented in the PhyML algorithm (Guindon et al., 2010). The analysis employed the general time-reversible (GTR) model of sequence evolution, with branch support assessed using the approximate likelihood-ratio test (aLRT) based on the log-likelihood ratio between the current tree and the most likely alternative (Anisimova & Gascuel, 2006; Guindon et al., 2010). Tree optimization was performed using a combination of nearest-neighbor interchange (NNI) and subtree pruning and regrafting (SPR) algorithms. Additionally, the neighbor-joining (BioNJ) algorithm was utilized to optimize tree topology for branch length estimation (Gascuel, 1997).

Table 1. The additional barcodes used in the study

Species identified in the current study	Barcode name in genomic databases	GenBank voucher number	BOLD systems voucher number	Origin
Tetrops peterkai	T. gilvipes adlbaueri	–	COANT1538-22	Czech Republic
Tetrops peterkai	T.praeustus	–	TDAOE2279-23	Austria
Tetrops peterkai	T.praeustus	–	GMBSO1158-18	Bulgaria
Tetrops peterkai	T.praeustus	–	OPPEE6021-17	Canada
Tetrops peterkai	T.praeustus	–	OPPEC5002-17	Canada
Tetrops peterkai	T.praeustus	–	OPPEE7091-17	Canada
Tetrops peterkai	T.praeustus	–	OPPEE7099-17	Canada
Tetrops peterkai	T. praeustus	–	OPPOC183-17	Canada
Tetrops peterkai	T. praeustus	–	OPPOC296-17	Canada
Tetrops peterkai	T. praeustus	–	BARSK251-16	Canada
Tetrops peterkai	T. praeustus	–	CNRGK672-15	Canada
Tetrops peterkai	T. praeustus	–	PHMAL230-10	Canada
Tetrops peterkai	T. praeustus	–	OPPEC4250-17	Canada
Tetrops peterkai	T. praeustus	–	OPPEC4977-17	Canada
Tetrops peterkai	T. praeustus	–	OPPEE7078-17	Canada
Tetrops peterkai	T. praeustus	–	OPPOC2982-17	Canada
Tetrops peterkai	T. praeustus	–	RRSSA1043-15	Canada
Tetrops peterkai	T. praeustus	–	OPPOC2568-17	Canada
Tetrops peterkai	T. praeustus	–	RRSSA2371-15	Canada
Tetrops peterkai	T. praeustus	–	PPEC4978-17	Canada
Tetrops peterkai	T. praeustus	–	OPPEC4993-17	Canada
Tetrops peterkai	T. praeustus	–	ROUGE1924-17	Canada
Tetrops peterkai	T. praeustus	–	SSROC4042-15	Canada
Tetrops peterkai	T. praeustus	–	CERLF576-08	Canada
Tetrops peterkai	T. praeustus	KM285770.1	–	France
Tetrops peterkai	T. praeustus	KR486593.1	–	Canada
Tetrops peterkai	T. praeustus	KT615264.1	–	Canada
Tetrops peterkai	T. praeustus	MF639495.1	–	Canada

Tetrops peterkai	T. praeustus	MF640407.1	–	Canada
Tetrops peterkai	T. praeustus	MG054964.1	–	Canada
Tetrops peterkai	T. praeustus	MG058445.1	–	Canada
Tetrops peterkai	T. praeustus	MG059846.1	–	Canada
Tetrops peterkai	T. praeustus	MG061362.1	–	Canada
Tetrops peterkai	T. praeustus	–	COLFH227-14	Estonia
Tetrops peterkai	T. praeustus	–	COLFH1523-16	Finland
Tetrops peterkai	T. praeustus	–	COLFH1525-16	Finland
Tetrops peterkai	T. praeustus	–	COLFH1579-16	Finland
Tetrops peterkai	T. praeustus	–	COLFH1524-16	Finland
Tetrops peterkai	T. praeustus	–	LEFIJ8599-19	Finland
Tetrops peterkai	T. praeustus	–	LEFIJ8598-19	Finland
Tetrops peterkai	T. praeustus	–	COLFA620-12	Finland
Tetrops peterkai	T. praeustus	KJ967255.1	–	Finland
Tetrops peterkai	T. praeustus	MZ632603.1	–	Finland
Tetrops peterkai	T. praeustus	MZ633609.1	–	Finland
Tetrops peterkai	T. praeustus	MZ656721.1	–	Finland
Tetrops peterkai	T. praeustus	MZ658860.1	–	Finland
Tetrops peterkai	T. praeustus	–	PSFOR447-13	France
Tetrops peterkai	T. praeustus	–	GCOL285-16	Germany
Tetrops peterkai	T. praeustus	–	GCOL441-16	Germany
Tetrops peterkai	T. praeustus	–	GCOL286-16	Germany
Tetrops peterkai	T. praeustus	KU908989.1	–	Germany
Tetrops peterkai	T. praeustus	KU911352.1	–	Germany
Tetrops peterkai	T. praeustus	KU916245.1	–	Germany
Tetrops peterkai	T. praeustus	–	NOCLP2079-20	Norway
Tetrops peterkai	T. praeustus	KJ967464.1	–	Sweden
Tetrops peterkai	T. starkii	–	GBCOC360-12	Germany
Tetrops peterkai	T. starkii	–	FBCOG1398-12	Germany
Tetrops peterkai	T. starkii	–	GBCOU561-13	Germany
Tetrops peterkai	T. starkii	KM446254.1	–	Germany
Tetrops peterkai	T. starkii	KM447580.1	–	Germany
Tetrops peterkai	T. starkii	KM450609.1	–	Germany
Tetrops peterkai	T. starkii	KU911828.1	–	Germany
Tetrops praeustus	T. praeustus	–	TDAOE2312-23	Austria
Tetrops praeustus	T. praeustus	–	GMBUA356-14	Bulgaria
Tetrops praeustus	T. praeustus	–	GMBUA345-14	Bulgaria
Tetrops praeustus	T. praeustus	–	GMBSO1078-18	Bulgaria
Tetrops praeustus	T. praeustus	KM286337.1	–	France
Tetrops praeustus	T. praeustus	–	PSFOR988-14	France
Tetrops praeustus	T. praeustus	–	GCOL1475-16	Germany
Tetrops praeustus	T. praeustus	–	GCOL10331-16	Germany
Tetrops praeustus	T. praeustus	–	GCOL10337-16	Germany
Tetrops praeustus	T. praeustus	–	GCOL12722-16	Germany
Tetrops praeustus	T. praeustus	–	GCOL328-16	Germany
Tetrops praeustus	T. praeustus	–	GCOL3776-16	Germany
Tetrops praeustus	T. praeustus	–	GBCOC567-12	Germany
Tetrops praeustus	T. praeustus	–	FBCOG999-12	Germany

Tetrops praeustus	T. praeustus	–	GBCOD703-13	Germany
Tetrops praeustus	T. praeustus	–	GCOL307-16	Germany
Tetrops praeustus	T. praeustus	HQ953425.1	–	Germany
Tetrops praeustus	T. praeustus	KM440548.1	–	Germany
Tetrops praeustus	T. praeustus	KM448032.1	–	Germany
Tetrops praeustus	T. praeustus	KM451230.1	–	Germany
Tetrops praeustus	T. praeustus	KU906235.1	–	Germany
Tetrops praeustus	T. praeustus	KU907158.1	–	Germany
Tetrops praeustus	T. praeustus	KU907334.1	–	Germany
Tetrops praeustus	T. praeustus	KU909290.1	–	Germany
Tetrops praeustus	T. praeustus	KU914532.1	–	Germany
Tetrops praeustus	T. praeustus	KU917980.1	–	Germany
Tetrops praeustus	T. praeustus	–	GBCOU4403-14	Hungary
Tetrops praeustus	T. praeustus	–	GBCOU4464-14	Hungary
Tetrops praeustus	T. praeustus	–	UZINS213-23	Slovakia
Tetrops praeustus	T. praeustus	KJ967190.1	–	Sweden
Tetrops praeustus	T. praeustus	–	COLFF265-13	Sweden
Tetrops praeustus	T. praeustus	–	WFEN924-23	United Kingdom
Tetrops starkii	T. starkii	–	COLFE1537-13	Estonia
Tetrops starkii	T. starkii	KJ965662.1	–	Estonia
Tetrops starkii	T. starkii	–	GCOL11393-16	Germany
Tetrops starkii	T. starkii	–	GCOL13394-16	Germany
Tetrops starkii	T. starkii	–	GCOL318-16	Germany
Tetrops starkii	T. starkii	–	GCOL3413-16	Germany
Tetrops starkii	T. starkii	–	GCOL3414-16	Germany
Tetrops starkii	T. starkii	–	GCOL3415-16	Germany
Tetrops starkii	T. starkii	–	GCOL3416-16	Germany
Tetrops starkii	T. starkii	–	GCOL368-16	Germany
Tetrops starkii	T. starkii	–	GCOL6145-16	Germany
Tetrops starkii	T. starkii	KU906859.1	–	Germany
Tetrops starkii	T. starkii	KU908741.1	–	Germany
Tetrops starkii	T. starkii	KU909409.1	–	Germany
Tetrops starkii	T. starkii	KU910669.1	–	Germany
Tetrops starkii	T. starkii	KU912491.1	–	Germany
Tetrops starkii	T. starkii	KU913095.1	–	Germany
Tetrops starkii	T. starkii	KU913505.1	–	Germany
Tetrops starkii	T. starkii	KU915437.1	–	Germany
Tetrops starkii	T. starkii	KU918394.1	–	Germany
Tetrops starkii	T. starkii	KJ964167.1	–	Sweden
Tetrops starkii	T. starkii	–	COLFE1500-13	Sweden

3. RESULTS

As a result of this study, we obtained the first barcodes for *T. peterkai* from Ukraine. Moreover, we were able to link these barcodes to *T. peterkai* specimens that were unequivocally identified based on external morphology and terminalia structure (Fig. 1). The barcodes obtained differ by 0.3%,

corresponding to two nucleotide substitutions: T178C, where thymine is replaced by cytosine at position 178, and T424A, where thymine is replaced by adenine at position 424.

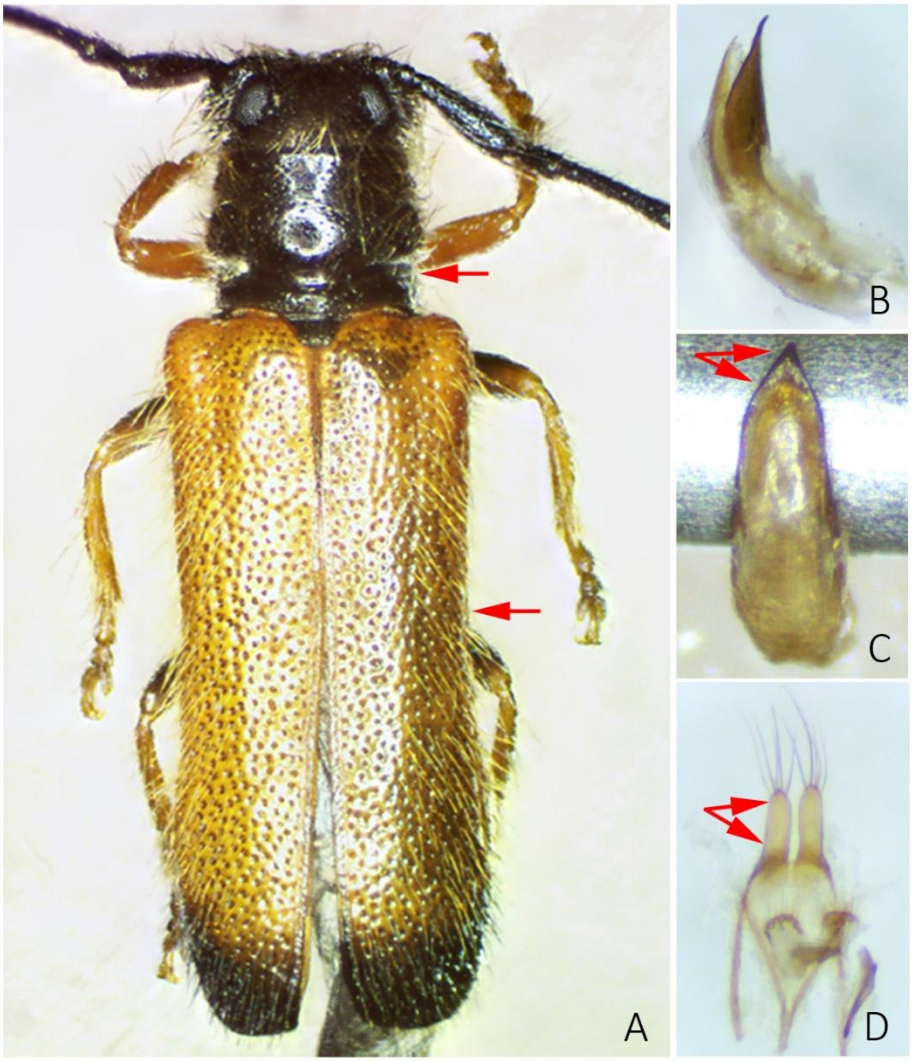

Fig. 1. *Tetrops peterkai* male external habitus and genitalia from Ukraine (after Zamoroka, 2023); Key distinguishing features are indicated by arrows (for details see Skořepa, 2020)

Upon comparing our COI sequence with existing barcodes in genomic databases, we identified at least 62 barcodes that are highly similar or identical to the *T. peterkai* barcodes obtained in our research (Fig. 2). It should be noted that the mentioned samples with barcodes stored in genomic databases are known as three different species, although in fact they belong to the same species. These include 54 samples identified as *T. praeustus*, six samples identified as *T. starkii*, one sample identified as *T. gilvipes adlbaueri*, and one sample assigned only to the genus level as *Tetrops sp.* Since most of these barcodes were generated before *T. peterkai* was established as a distinct species in 2020, it is understandable that many samples were morphologically classified as *T. praeustus*. However, the presence of *T. starkii* and *T. gilvipes adlbaueri*, which are readily distinguishable by male terminalia morphology, indicates potential misidentifications likely based solely on external morphology, possibly involving females.

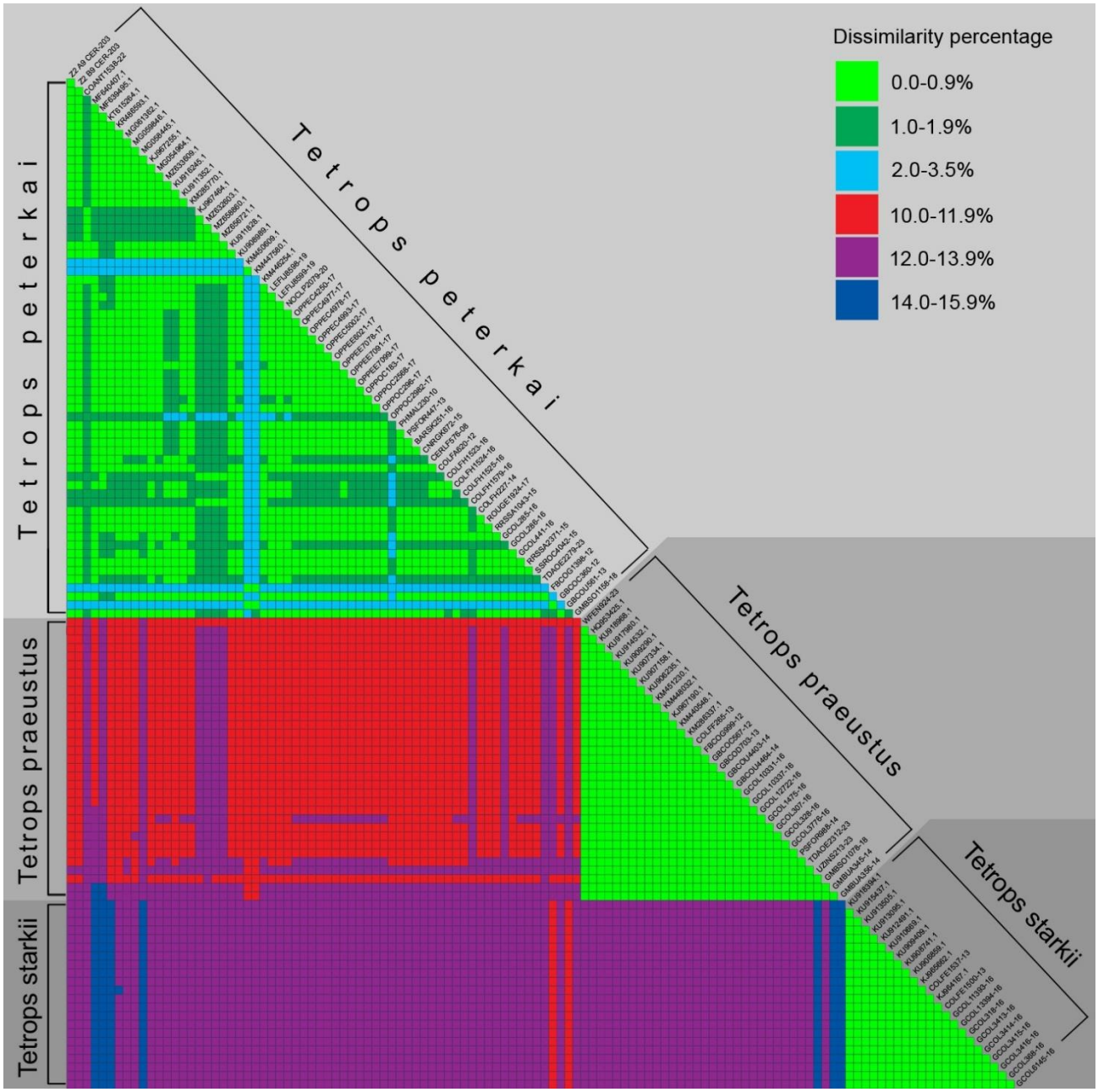

Fig. 2. Pairwise percentage variation in COI sequences within the studied species

It is also worth noting the genetic variability observed among *T. peterkai* barcodes, with some samples showing differences of 2.0-3.5%. This is in stark contrast to the genetic homogeneity of *T. praeustus* and *T. starkii*. Such genetic variability in *T. peterkai* may suggest a complex evolutionary history of the species and its migration from different glacial refugia during Holocene as we described for other Cerambycid species (Zamoroka et al., 2019, 2024). Currently, there is insufficient data to provide a definitive answer to this question.

The phylogenetic tree we constructed (Fig. 3) shows strong support (SH≥0.9) for the distribution of 119 individual *Tetrops* sequences into three major clades. The most basal clade is *T. starkii* with 22 sequences. The crown part of the tree consists of two clades: 1) *T. praeustus* with 35 sequences, and 2) *T. peterkai* with 62 sequences. The average genetic divergence between species pairs is 13.6% for *T. starkii* and *T. praeustus*, 11.9% between *T. praeustus* and *T. peterkai*, and 13.2% between *T. peterkai* and *T. starkii*.

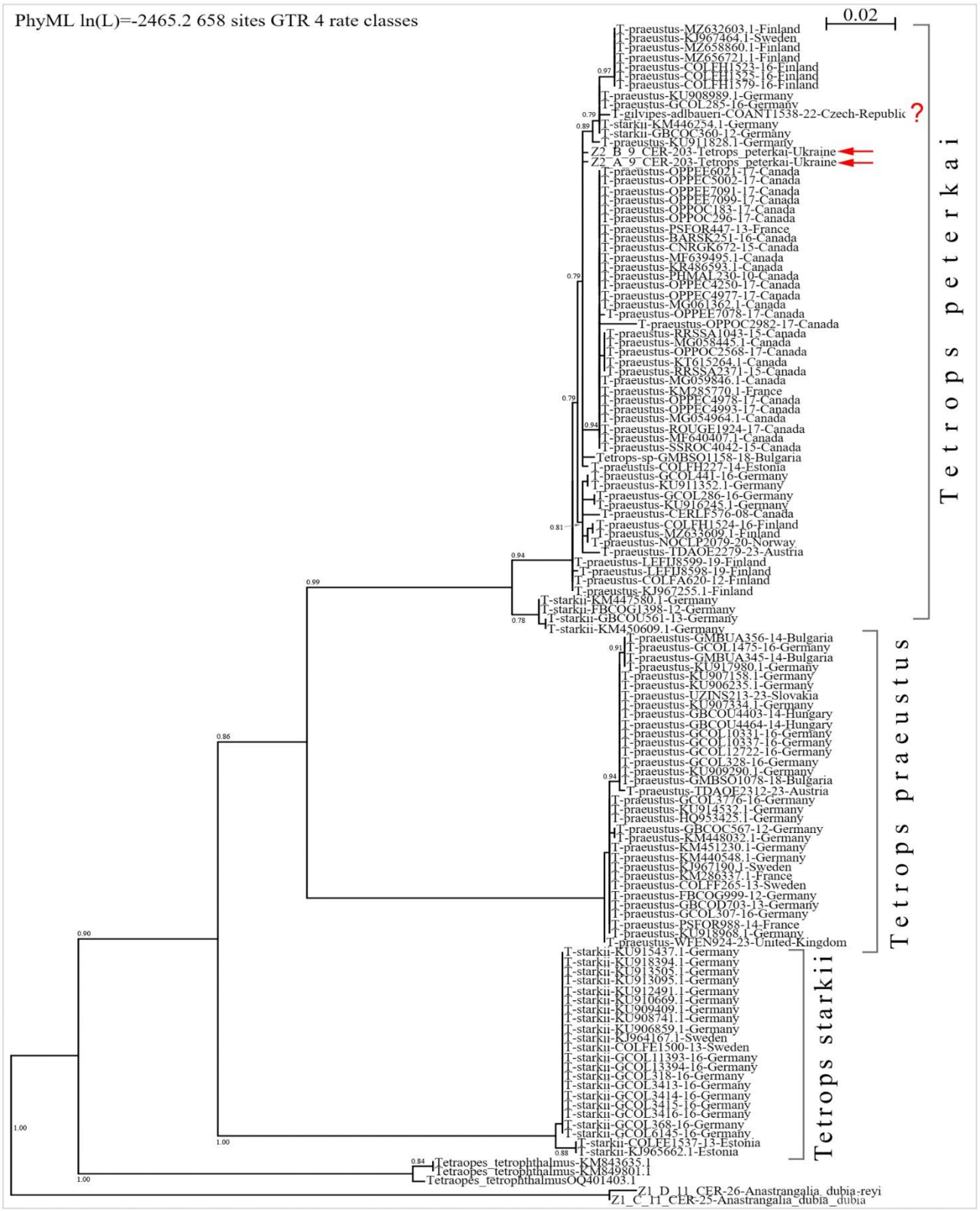

Fig. 3. COI tree illustrating the phylogenetic hypothesis of relationships within genus *Tetrops*; the branch support SH-like values are shown with the threshold rule SH>0.70. Original labels for sequences stored in GenBank and BOLDSYSTEMS are shown. The first reliable sequences of *T. peterkai* are labeled by arrows, and doubtful identification of *T. gilvipes adlbaueri* is labeled by the question mark.

4. DISCUSSION

The results of our study clearly indicate that *T. praeustus* is not a monophyletic species, but rather consists of at least two sibling species that are morphologically very similar to each other. One of these belongs to the classical Linnaean *T. praeustus*, while the other is the recently described *T. peterkai*. Our findings have, for the first time, enabled a robust linkage between morphologically identified *T. peterkai* specimens and their corresponding barcodes, integrating them with numerous pre-existing COI nucleotide sequences. This has finally enabled a revision and resolved the confusion surrounding barcodes available in the genomic databases GenBank and BOLDSYSTEMS. This confusion emerged prior to the discovery and description of *T. peterkai* in 2020. Prior to this, all barcode samples were labeled as *T. praeustus*. As a result, 87% of all barcodes from the *T. peterkai* clade were classified as *T. praeustus*. However, among the analyzed sequences, there are also those identified as *T. starkii* (4 sequences) and *T. gilvipes adlbaueri* (1 sequence). This can only be explained by errors in identification based on external morphology. We exclude the possibility of hybridization between *T. peterkai* and *T. starkii*, as well as between *T. peterkai* and *T. gilvipes adlbaueri*, in contrast to previous cases of hybridization observed in other Cerambycidae taxa (Zamoroka et al., 2019), due to the significant genetic differentiation between *Tetrops* species. It should be noted, however, that the actual phylogenetic relationships of *T. gilvipes* with other *Tetrops* species remain unclear due to the lack of the serial sequences for this species.

Another unresolved issue is the uncertainty regarding which species, *T. praeustus* or *T. peterkai*, C. Linnaeus originally described as *T. praeustus*. This ambiguity arises because both *T. praeustus* and *T. peterkai* are found in southern Sweden (see below). It is highly likely that both species were present in Linnaeus's collection; however, he evidently did not distinguish between them and likely considered them a single species. This question requires further investigation. Overall, the situation regarding the taxonomic status of *T. peterkai* and *T. praeustus* is analogous to that of *Leiopus nebulosus* (Linnaeus, 1758) and *Leiopus linnei* Wallin, Nylander & Kvamme, 2009, which are also morphologically very similar but genetically distinct (Wallin et al., 2009). Similarly, it was unclear which of the species had been described by C. Linnaeus. To address this, Wallin and colleagues (2009) designated one of the existing species as the classical Linnaean species and proposed a new name for the other. They also determined the COI nucleotide sequences for each of the identified morphospecies, *L. nebulosus* and *L. linnei*, enabling molecular identification of these species. Their research serves as an excellent example of the synergy between classical morphology and modern molecular biology. In fact, *L. linnei* was the first species of the longhorn beetles described using integrative taxonomy methods, including both morphological and molecular analyses (Wallin et al., 2009).

The results we obtained clearly demonstrate the distinctiveness of *T. peterkai* as a species, which is supported both morphologically and molecularly. Our findings stand in stark contrast to the unfounded suggestion to synonymize *T. peterkai* and *T. praeustus* (Lazarev, 2024b). For the sake of completeness, we provide the full quote from Lazarev (2024b): "*Tetrops praeustus praeustus (Linnaeus, 1758) = T. peterkai Scořepa, 2020, syn. nov. = T. praetermitus Sláma, 2020, syn. nov. The morphological differences described by the authors of each name are completely insignificant*." Lazarev (2024b) made a nomenclatural act without proper justification or evidence, relying solely on subjective reasoning. This leads us to believe that the author either lacked *T. peterkai* specimens or, if they did, they were incorrectly identified. We previously published information on the recording of *T. peterkai* in Ukraine (Zamoroka, 2023), including illustrations of the habitus and genital morphology, which

clearly align with those presented in the original description (Skořepa, 2020). For three of the 17 specimens collected in Ukraine, we extracted and sequenced DNA, obtaining two high-quality sequences with a large number of reads, as described in the current study. The synthesis of our results with materials available in genomic databases showed that *T. peterkai* and *T. praeustus* are two distinct species, fully corroborating the morphological data. Therefore, we deem it appropriate to undertake a nomenclatural act to restore the name *T. peterkai* Scořepa, 2020, **nom. res.** Furthermore, we must note that Lazarev (2024a) has repeatedly made nomenclatural acts without proper justification or evidence, relying solely on subjective reasoning, particularly in cases of molecular phylogeny. Such actions are detrimental to the establishment of evolutionary relationships between taxa and the implementation of an integrated natural classification for *Cerambycidae*.

Our barcodes analysis allowed us to reveal geographic patterns (Fig. 4) in the distribution of *T. peterkai* and *T. praeustus*. In the recent publications it is reported the distribution of *T. peterkai* in Central Europe, including the Czech Republic, Slovakia, Germany, and Austria (Skořepa, 2020; Sláma, 2020; Hass & Pütz, 2022). Later, we discovered *T. peterkai* in the northwestern part of Ukraine (Zamoroka, 2023). Our current results have shown that *T. peterkai* is more widely distributed across Europe, including Bulgaria, Estonia, Finland, France, Norway, and Sweden, where it is reported for the first time. In comparison to *T. praeustus*, the distribution of *T. peterkai* is significantly shifted toward the northern and eastern parts of the European continent. In contrast, *T. praeustus* occupies the southern and western parts of Europe. The ranges of both species broadly overlap in Central and Eastern Europe. It remains unclear whether *T. praeustus* is present in Northeastern Europe, or if all literature data should be attributed to *T. peterkai*. This requires further investigation with large-scale revisions of existing collection materials and the collection of new specimens, including those for molecular identification. It should also be noted that the *Tetrops* species introduced to North America belongs to *T. peterkai* and most likely originated from France. This is supported by the grouping of the specimen KM285770.1 (GenBank) from France with a series of sequence samples from Canada (Fig. 3). The difference between the sequences of these samples ranges from 0.0% to 0.2%, which suggests that they are identical.

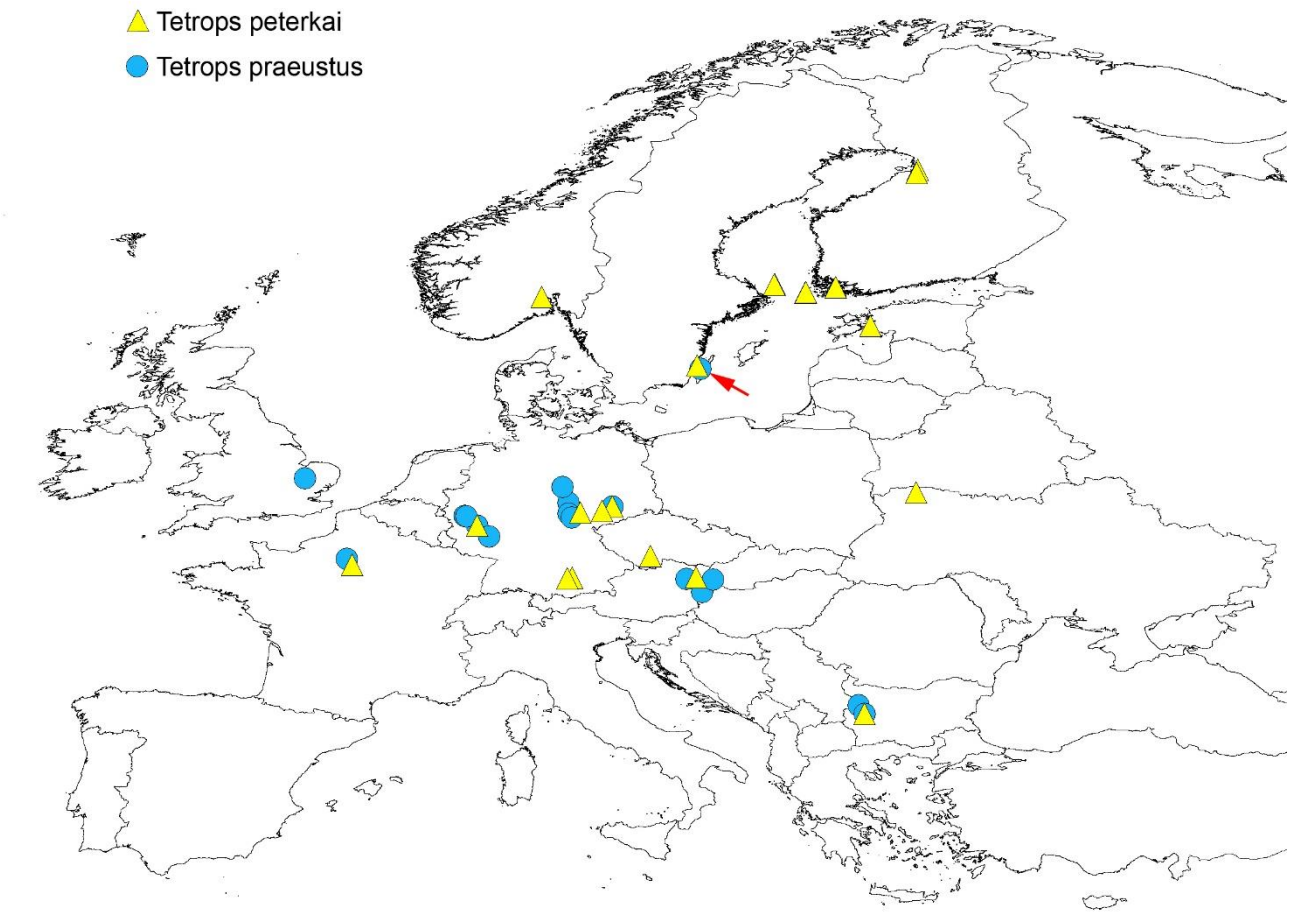

Fig. 4. Geographical patterns of *T. peterkai* and *T. preustus* currently known from barcodes in Europe. The arrow indicates presence of both species in the southern Sweden

5. CONCLUSION

In summary, we demonstrate that *T. praeustus* is not monophyletic but comprises at least two morphologically similar sibling species: *T. praeustus* and *T. peterkai*. We also linked our *T. peterkai* specimens with pre-existing COI sequence data in GenBank and BOLDSYSTEMS, clarifying their relationships and uncovering the taxonomic position of the species. This enables us to reveal distinct geographic distribution patterns for *T. peterkai* and *T. praeustus*. *Tetrops peterkai* exhibits a broader and more northerly distribution across Europe, including several first records in northern and eastern regions, whereas *T. praeustus* is largely confined to southern and western Europe, with overlapping ranges in Central and Eastern Europe.

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Андрій Заморока, Олександр Зіненко. Таксономічне становисько Tetrops peterkai Skořepa, 2020 (Coleoptera: Cerambycidae) і його криптичне розповсюдження. *Журнал Прикарпатського національного університету імені Василя Стефаника. Біологія*, 11 (2024), С6–С19.

Невеликий палеарктичний рід *Tetrops* Kirby, 1826 налічує щонайменше десять валідних видів, розподілених між двома підродами: західнопалеарктичним *Tetrops* і східнопалеарктичним *Mimosophronica* Breuning, 1943. На Європейському континенті рід представлений щонайменше чотирма видами: *Tetrops praeustus* (Linnaeus, 1758), *Tetrops gilvipes* (Faldermann, 1837), *Tetrops starkii* Chevrolat, 1859, and *Tetrops peterkai* Skořepa, 2020, яким притаманна надзвичайна морфологічна схожість. Це зумовило значну плутанину і помилкові визначення у матеріалах, а також низку описів нових видів, які періодично синонімізуються, що спричинює тривалі наукові дискусії. *Tetrops peterkai* – нещодавно описаний вид із Центральної Європи, якого раніше помилково вважали за *Tetrops praeustus*. Обидва види відрізняються низкою ознак у зовнішні морфології, однак більш надійно їх можна розрізнити за будовою терміналій самців. З моменту опису, довкола видового статусу *Tetrops peterkai* триває дискусія і навіть була спроба його синонімізувати із *Tetrops praeustus*. У чинному дослідженні ми просеквенували ген цитохром с оксидази I (COI) декількох особин *Tetrops peterkai*, зібраних у Волинській області України впродовж низки експедицій 2022-го року. В результаті ми отримали дві якісні нуклеотидові послідовності (баркоди) з великою кількістю прочитань, що дало нам можливість їх порівняти із

матеріалами доступними у геномних базах даних. Подальший аналіз отриманих баркодів продемонстрував, що вид, який вважався *Tetrops praeustus* насправді представляє собою два різні види, один з яких відповідає власне *Tetrops praeustus*, а інший – *Tetrops peterkai*. Таким чином, наше дослідження довело самостійність видового статусу *Tetrops peterkai*, що спонукало нас здійснити номенклатурний акт із формального відновлення назви: *Tetrops peterkai* Scoťera, 2020, **nom. res.** відповідно до норм Міжнародного Зоологічного Кодексу. Окрім того, наш аналіз баркодів дав змогу виявити закономірності географічного розповсюдження обох видів у Європі. Зокрема ареал *Tetrops peterkai* зміщений до північної частини Європи, а *Tetrops praeustus* – до південної. Обидва види широко перекриваються у Центральній Європі. Додатковим результатом нашого дослідження стало те, що усі відомі баркоди інвазійного *Tetrops* із Північної Америки належать саме *Tetrops peterkai* і є ідентичними до його популяцій, розповсюджених на півночі Франції, звідки вид ймовірно був ввезений у Новий Світ.

Ключові слова: скрипунові жуки, молекулярна філогенія, криптичне біорозмаїття, інтегративна таксономія, секвенування, баркодинг