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**Article**

# Tracing the origin of "Durdubak": DNA barcoding reveals that the widely used fish bait is a recently described leech species from Türkiye

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**Abstract**
In this study, a DNA barcoding method was successfully applied to identify the taxonomic affiliation and the geographical origin of the so-called “Durdubak” leeches, which are commonly used as fish bait in Serbia and the Balkans and are imported to Western and Central Europe for this purpose. Our results indicate that the "Durdubak" specimens imported from Serbia to Germany in 2006, and previously identified morphologically as *Trocheta danastrica* Stschegolew, 1938, molecularly belong to *T. kesirvenensis* Sağlam, Saunders & Shain, 2025, a species previously known only from the Aegean region of Türkiye. This study demonstrates that traceability of leeches used as fish bait may become increasingly important in the future, as fraudulent labeling or misidentification not only undermines consumer confidence in the bait market but also poses a threat to populations of native leech species.

**Key words** Hirudinea, Serbia, fish bait, DNA barcoding, bioinvasion.

## Introduction

Leeches (Hirudinida) represent a relatively small monophyletic group, with about 830 species described to date (Grosser *et al*. 2024). The majority inhabit freshwater environments, while fewer species are marine, and only a small number are adapted to terrestrial life (Sket & Trontelj 2008). Many leech species are active predators, feeding on insect larvae, snails, and other small invertebrates, whereas others are ectoparasitic and blood-feeding. Leeches are found on all continents except Antarctica, with the greatest diversity occurring in the Holarctic region (Sket & Trontelj 2008).

Some leech species, especially *Hirudo medicinalis*, have long been used in medicine, particularly for bloodletting and wound healing. However, the range of leech applications extends far beyond medicine, including environmental monitoring and use as bait in freshwater fishing. In many parts of the world, leeches are commonly used as bait, particularly in freshwater angling. In the Balkans, they are especially popular for catching large fish such as perch, catfish, large carp, and barbel.

One group of leeches commonly used as bait in this region is referred to by the local folk name "Durdubak" (also known as "durbok" or "turbok"). This name typically applies to large, predatory, non-blood-sucking leeches, likely belonging to the genera *Haemopis* or *Trocheta*. These leeches have been exported from Serbia to various European countries under the name "Durdubak" mainly as bait for *Silurus glanis* (Linnaeus, 1758) (see Grosser & Epshtein 2009).

The taxonomic affiliation of "Durdubak" with *Trocheta danastrica* Stschegolew, 1938 was clarified by Grosser & Epshtein (2009). In 2006, they examined two specimens imported from Serbia to Germany under this name. Although originally suspected by the importer to be *Haemopis sanguisuga*, the specimens were identified as members of the genus *Trocheta*. Grosser & Epshtein (2009) assigned them to *T. danastrica* Stschegolew, 1938, the largest known European leech species, originally described from the Dniester River near Odesa, Ukraine (Stschegolew 1938).

The distribution of *T. danastrica* remains poorly understood. Nesemann & Neubert (1999), who redescribed the species as *Trocheta* sp., reported its presence in the Aegopotamos region, which includes parts of Hungary, Romania, northern Greece, and Anatolia. Khomenko *et al*. (2018) noted the Dnieper Delta as the easternmost confirmed range of *T. danastrica*.

More recently, the taxonomic status of populations attributed to *T. danastrica* has been challenged through molecular analyses. Khomenko *et al*. (2020) demonstrated that the genus *Trocheta* exhibits high cryptic diversity. They showed that specimens from the Crimean Peninsula, long misidentified as *T. danastrica*, actually represent a distinct species, *T. blanchardi* Khomenko, Utevsky, Utevsky & Trontelj, 2020, which shares close phylogenetic relationship with Anatolian species. Subsequently, Sağlam *et al*. (2025) described another new species from the *T. danastrica* complex, *T. kesirvenensis* Sağlam, Saunders & Shain, 2025, based on populations from the Aegean region of Türkiye (Balıkesir and Antalya).

In this study, we used DNA barcoding to clarify the taxonomic affiliation, and consequently the possible geographic origin, of the so-called "Durdubak" leeches, which are commonly used as fish bait in Serbia and have recently been increasingly exported to other countries, posing a potential bioinvasion risk.

## Material and Methods

Leeches were collected by hand netting and immediately preserved in 96% ethanol for the purpose of molecular analyses. The specimens used for molecular study are listed in Table 1. After non-destructive DNA extraction, the specimen vouchers were stored in 96% ethanol and morphologically examined.

**Molecular and DNA barcode analyses**

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding, Guelph, Canada (CCDB; http://ccdb.ca/)). In the later institution, the specimens were sequenced for the barcode region of COI using standard invertebrate DNA extraction, amplification and sequencing protocols (see for details: Ivanova *et al*. 2007; Ivanova & Grainger 2007a, b).

Consensus sequences were made available in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007). In this study DNA was extracted from one specimen from Serbia and four specimens from Türkiye listed in Table 1. For all other species, COI sequence data were downloaded from the respective sequence data archives, i.e., *Trocheta blanchardi* (n = 5; Ukraine; Khomenko *et al*. 2020), *T. cylyndrica* Örley, 1886 (n = 1; Ukraine; Khomenko *et al*. 2020), *T. cylyndrica* (n = 1; Bosnia and Herzegovina), *T. dalmatina* Sket, 1968 (PV938092; Montenegro), *T. danastrica* (n = 4; Khomenko *et al*. 2020), *T. haskonis* Grosser, 2000 (n = 1; Germany; Pfeiffer *et al*. 2005), *T. kesirvenensis* (n = 5; Sağlam *et al*. 2025), *Trocheta* sp. (KY989486.2; Iran; Darabi-Darestabi *et al.* 2021), *Trocheta* sp. (ON098217.1; Azerbaijan; Farzali & Saglam 2021) and *Trocheta* sp. (n = 1; Austria). *Erpobdella octoculata* (Linnaeus, 1758) from Albania was used as the outgroup.

Sequence alignments were performed using MUSCLE (Edgar 2004). Intra- and interspecific genetic distances were calculated based on the Kimura 2-parameter model (K2P; Kimura 1980), using MEGA 11 software (Tamura *et al*. 2021). The latter software was used to calculate Neighbour-Joining (NJ) trees based on K2P distances (standard for barcoding studies) using pairwise deletion for missing data. Branch support was calculated using nonparametric bootstrap (Felsenstein 1985) with 1000 replicates and shown next to the branches.

Additionally, the sequence data were analyzed using the Assemble Species by Automatic Partitioning (ASAP) method (Puillandre *et al*. 2012). We used the online ASAP version (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) with default settings and K2P distance model.

**Table 1**. Details of DNA barcoded specimens, including localities and coordinates of sampling sites, and the barcode index number codes. BOLD data presented here was last accessed on 14th July 2025.

| **Locality** | **Coordinates** | **Sample ID** | **Process ID** | **BIN** |
|---|---|---|---|---|
| Serbia, no locality | unknown | LCH_TRO | LCHME050-25 | |
| Türkiye, Burdur, spring near Çavdir | 37.14478º N, 29.656534º E | CCDB-48501-G07 | HYDCG174-24 | BOLD:ADR5337 |
| Türkiye, Burdur, stream near Söbüce | 37.287872º N, 30.067743º E | CCDB-48501-G10 | HYDCG177-24 | |
| Türkiye, Isparta, stream near Çukurköy | 37.651257º N, 30.81791º E | CCDB-48501-G08<br>CCDB-48501-G09 | HYDCG175-24<br>HYDCG176-24 | BOLD:AGH6595 |

## Results

Since no mitochondrial DNA sequences were previously available for "Durdubak" we extracted DNA from a specimen that had been morphologically studied by Grosser & Epshtein (2009) and successfully obtained a mitochondrial COI gene sequence of 603 bp (Genbank id: PV938091; BOLD id: LCHME050-25). In addition, new COI sequences were generated for four *Trocheta* specimens collected by the first author in the Aegean region of Türkiye (see Table 1). These five newly obtained sequences were supplemented with published COI data for other *Trocheta* species available in the BOLD and GenBank databases.

The neighbor-joining phylogenetic tree (Figure 1) yielded the following results: the sequenced "Durdubak" specimen from Serbia, along with the four specimens from western Türkiye, clustered within the *Trocheta kesirvenensis* clade. Furthermore, the ASAP species delimitation analysis grouped the Serbian "Durdubak" sequence and the *T. kesirvenensis* sequences from Türkiye into the same molecular operational taxonomic unit (MOTU).

These results clearly indicate that the "Durdubak" specimen from Serbia, previously described morphologically by Grosser & Epshtein (2009), molecularly belong to *Trocheta kesirvenensis*, a species that, until now, was only known from the Aegean region of Türkiye (Sağlam *et al*. 2025).

## Discussion

Identification of leeches using traditional methods is challenging, primarily because it is time-consuming, and because closely related species are often difficult to distinguish morphologically. As an alternative, methods such as DNA barcoding have proven successful in identifying leech species, particularly in detecting cryptic species (e.g., Khomenko *et al*. 2020; Pešić & Grosser 2021; Grosser *et al*. 2023; Sağlam *et al.* 2025). However, the lack of local reference DNA barcodes presents a significant

obstacle to the effective use of barcoding for species identification, including efforts to trace the geographic origin of leeches exported to other regions.

In this study, using molecular methods, we confirmed that leeches known locally as "Durdubak" which were exported from Serbia to Germany for use as fish bait, belong to *Trocheta kesirvenensis*, a species previously known only from the Aegean region of Türkiye (Sağlam *et al*. 2025).

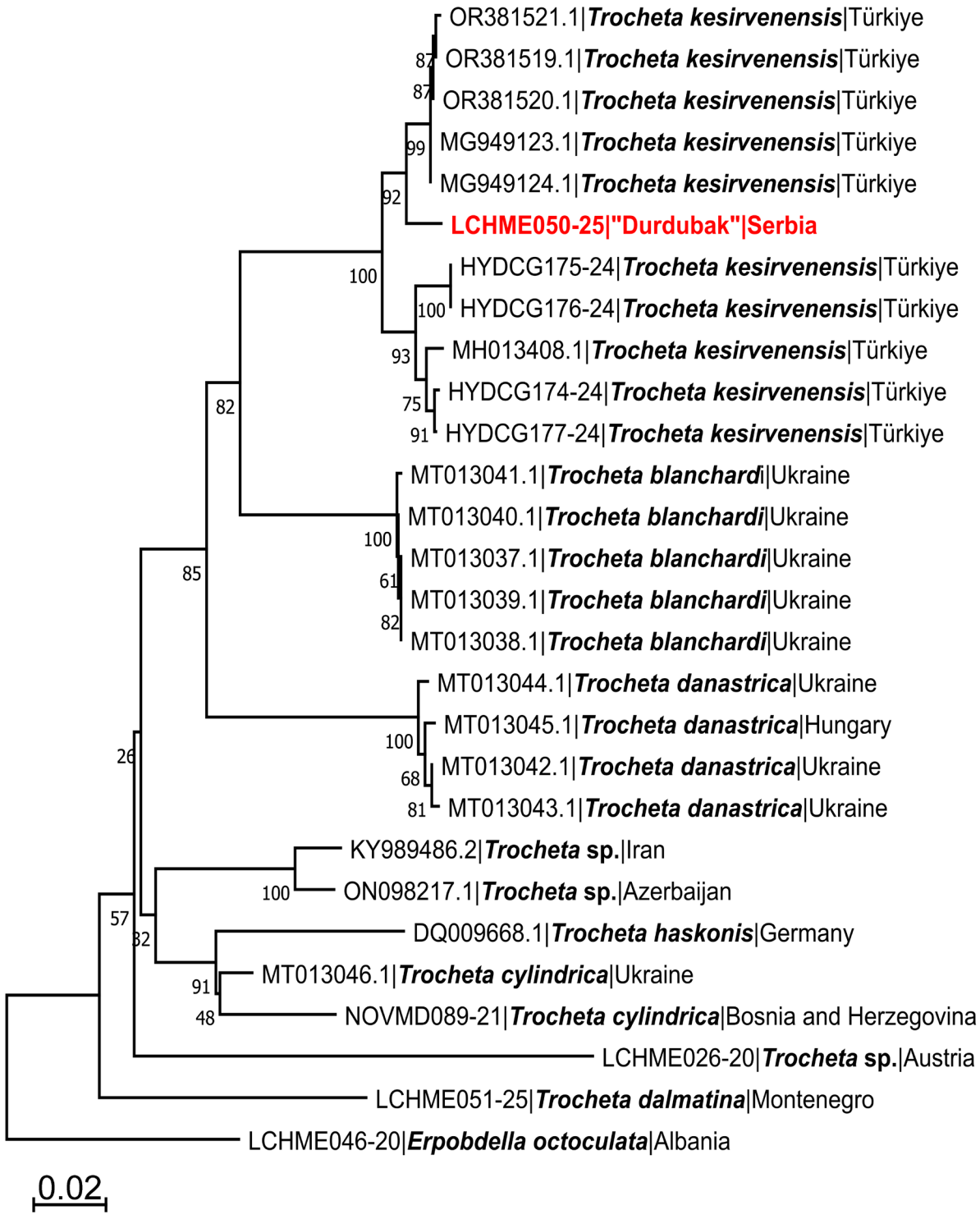


**Figure 1**. Neighbor-Joining tree of the genus *Trocheta* obtained from 27 nucleotide COI sequences; sequence of "Durdubak" specimen from Serbia is marked in red. There were a total of 697 positions in the final dataset. *Erpobdella octoculata* from Albania was used as outgroup.

Grosser & Epshtein (2009) were the first to associate "Durdubak" with *Trocheta danastrica*. Based on the origin of imported specimens from Serbia, they assumed that this species was also widespread in the country, possibly in the Sava River basin (Grosser & Epshtein 2009; Grosser *et al*. 2014). According to the available literature (Grosser & Epshtein 2009; Khomenko *et al*. 2018), *T. danastrica* was formerly common in the Dniester River. Stschegolew (1938) reported that locals used to catch large numbers of *T. danastrica* to use as bait.

For this study, we examined a large number of "Durdubak" leeches purchased from a local bait harvester in Niš, southern Serbia. The collected specimens closely match the description provided by Grosser & Epshtein (2009). According to the harvester, these leeches were collected from moist soil along the banks of the Nišava River, near the city of Niš.

Our study highlights the need for results obtained through traditional identification methods (e.g., morphological and anatomical analyses) to be aligned with findings from genetic research. The clarification of the taxonomic status of "Durdubak" leeches reveals several intriguing aspects. Morphologically, the "Durdubak" specimens examined by Grosser & Epshtein (2009) appear more similar to *Trocheta danastrica* than to the genetically identical *T. kesirvenensis*. For instance, the genital atrium of the "Durdubak" specimens studied by Grosser & Epshtein (2009) closely resembles that of *T. danastrica* from Hungary (described as *Trocheta* sp. in Nesemann & Neubert 1999) and from the Dnieper River (Grosser & Epshtein 2009) but differs from the genital atrium described in the original diagnosis of *T. kesirvenensis* (see Sağlam *et al*. 2025, fig. 6A). A similar discrepancy is noted in the presence of papillation: dorsal papillae are observed in *T. danastrica* from Hungary and in the specimens studied by Grosser & Epshtein (2009), whereas the original description of *T. kesirvenensis* mentions papillae only on the ventral side (Sağlam *et al*. 2025).


**Figure 2. A, C —** "Durdubak" leeches collected from local bait collectors on sale at the local market in Niš (Serbia). **B —** Genital atrium of the specimen from Niš, Serbia. Photos by Dj. Milošević (A, C) and V. Pešić (B).

Although our material was collected in Serbia (near Niš), the exact geographical origin of "Durdubak" leeches, commonly purchased from local bait harvesters in Serbia and exported abroad, remains uncertain. Conversations with local collectors in Niš (southern Serbia) indicate that most leeches are gathered from moist soil along major rivers such as the Nišava and Zapadna Morava, though some of the material is reportedly imported from neighboring North Macedonia (pers. comm. with local

bait harvesters). Further research, with more extensive sampling on a larger scale, is needed to clarify whether the leeches sold under the name "Durdubak" are imported from Türkiye or originate from local Serbian populations.

There are no reliable records indicating how many Durdubak specimens are collected within Serbia or neighboring countries. However, online listings for bait sales and local accounts suggest that live predatory leeches are actively exported from Serbia to other countries, pointing to extensive and largely unregulated leech harvesting (Živić *et al*. 2015). In addition to overharvesting, another concern, first raised by Grosser & Epshtein (2009), is the growing use of leeches as fish bait, which increases the risk of introducing non-native species into new ecosystems. This could pose a threat to native, particularly endemic, leech species already under pressure from habitat degradation, water pollution, and the spread of invasive species that impact aquatic invertebrate communities (Grosser & Epshtein 2009). Fraudulent labeling or misidentification of leeches also undermines consumer confidence in the fish bait market. Although few efforts have been made to trace the geographic origins of bait organisms, this study is the first to apply such a method to leeches. It underscores the growing importance of traceability in the use of leeches as fish bait.

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