



# First record of *Arion vulgaris* Moquin-Tandon, 1855 (Arionidae) from Armenia

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## Abstract

## Background

*Arion vulgaris* Moquin-Tandon, 1855 is amongst the fastest-spreading terrestrial slugs Europe-wide. In recent years, it has been recorded in Canada, Mexico and continues to expand eastwards into Eurasia. Renowned for its high invasiveness, combatting its swift spread creates significant challenges in organising effective preventative measures.

## New information

This study presents the first record of *Arion vulgaris* from Armenia, which is the second record of this species' invasion of the Caucasus. In 2022, a substantial population of *A. vulgaris* was observed close to the City of Stepanavan, which is also the first record in Armenia of the family Arionidae. How the species was introduced to Armenia remains

unknown. Identification of *Arion vulgaris* was conducted, based on external and genital morphology and mitochondrial CO1 (cytochrome c oxidase subunit 1) gene sequencing, revealing notable similarities with Central European clades. Our results confirm the introduction and distribution of *A. vulgaris* to Armenia. Invasion of such species into Armenia will require additional monitoring and would be aided by further research on Armenia's mollusc fauna in the future.

## Keywords

Gastropoda, biological invasions, Caucasus, terrestrial molluscs

## Introduction

*Arion vulgaris* Moquin–Tandon, 1855 stands prominently as one of Europe's most notorious pests, included in a list of the 100 most dangerous invasive species of Europe (Rabitsch 2009). The native range of *A. vulgaris* is considered somewhere from southwest France to southwest Germany (Pfenninger et al. 2014, Zemanova et al. 2016, Zajaç et al. 2019).

*Arion vulgaris* feeds on vegetation in gardens and orchards and causes serious agricultural damage (Grimm et al. 2000). This pest is characterised by a large body size, high reproductive capacity and survival in adverse conditions (Kozłowski 2007). In addition, *A. vulgaris* might not have natural enemies in newly-invaded territories, but a parasitic nematode species (*Phasmarhabditis hermaphrodita*) can infect slugs, which might be useful for biological control (Iglesias et al. 2001, Rae et al. 2007, Kozłowski et al. 2014). Some predatory beetles may also be effective against them (Hatteland 2010, Hatteland et al. 2010, Hatteland et al. 2011). Individuals live for approximately one year, but some may live for two years. Individuals become mature in spring or summer. The mating period usually starts in June and may last until December, depending on climatic conditions (Kozłowski 2007). Egg-laying reaches its peak around August or September. Most adults die in autumn after reproduction. Young individuals hatch in late autumn and then enter the soil and overwinter (Davies 1987, Kozłowski 2007, Hatteland et al. 2013).

The species is currently invasive in several countries and has also been found in the Caucasus Region (Schikov and Komarov 2021). The first record of this species in the Caucasus was in north Ossetia–Alania in 2019 (Schikov and Komarov 2021). However, the first record from Russia was in 2009 in gardens in Moscow (Schikov 2016). Additionally, it was reported from Türkiye in 2017 in the City of Isparta (Yıldırım and Gürlek 2017), but this observation needs to be verified because only the external appearance was examined (Reise et al. 2018).

This study documents the species' occurrence in Armenia for the first time.

## Materials and methods

### Sampling

Species sampling was performed from the beginning of September in 2022, in Gyulagarak Village, Lori Province, northern Armenia. Sampling was repeated during the summer season of 2023 and over 30 specimens of *Arion vulgaris* were sampled from the same area. Individuals were in high abundance in vegetable gardens, where potatoes, tomatoes, cucumbers and other vegetables were usually planted by the local people. The species distribution is considered to be the areas surrounding Gyulagarak Village and Stepanavan City. An individual of a species resembling *A. vulgaris* was found in a forested area in the City of Dilijan by Prof. S. Pipoyan. However, due to the mixed forest habitat and the fact that there was neither genetic nor thorough anatomical proof that this was an *A. vulgaris* individual, we do not include Dilijan as a distribution point for the species at this time.

Sampling in Gyulagarak was performed by hand and individuals were kept alive until they were transported to the Yerevan State University, Laboratory of Invertebrate Zoology. Specimens for sequencing were immersed in 70% ethanol and later transferred to 96% ethanol for better preservation. The ethanol was changed until the point at which no more discoloration occurred. Specimens for dissection were preserved by submerging them in a 70% ethanol solution, ensuring the fixation of the specimens for subsequent analysis.

A total of thirteen specimens were used for this study: nine vouchers were used for sequencing and three were used for dissection. Specimen CaBOL-1013790 was used to illustrate the external morphology of the species. The three dissected ethanol-preserved voucher specimens are deposited in the Hungarian Natural History Museum, Budapest (inventory numbers: HNHM 105451a, HNHM 105451b and HNHM 105451c). Specimens CaBOL-1013789, CaBOL-1013790, CaBOL-1013791, CaBOL-1013792, CaBOL-1013793, CaBOL-1013794, CaBOL-1013795, CaBOL-1013796 and CaBOL-1013797 were used for the sequencing. The specimens that were used for sequencing are part of the Malacological collection of the Department of Zoology at Yerevan State University, stored under the institute museum ID: YSU\_MOL\_MA.

### Morphology, dissection and photography

Examination of external and genital morphological traits was based on the works of Wiktor (1983), Rowson et al. (2014) and Reise et al. (2020).

Dissection was performed under a Zeiss Stemi 305 stereomicroscope. The genital organs of the slugs were photographed using a Canon EOS 2000d camera with Tamron SP AF 90 mm F/2.8 Di MACRO 1:1 macro objective lens. One camera-mounted flash with two studio flash units (BlitzBirne Mikrosat) were used on the left and right side of the subject, respectively, using white umbrellas for reflection.

## DNA extraction, PCR and sequencing

The total genomic DNA was extracted from each sample using a HiGene™ Genomic DNA Prep Kit (For Whole Blood, Bacterium, Plant, Animal Tissue, Fungus), following the manufacturer's protocol (catalogue number: GD141-100, BIOFACT Co., Ltd., Daejeon, Korea).

Partial sequences of the cytochrome oxidase subunit 1 (CO1) were amplified via polymerase chain reaction (PCR), employing the primer pairs LCO1490-JJ 5'-GGTCAACAAATCATAAAGATATTGG and HCO2198-JJ 5'-TAAACTTCAGGGTGACCA AAAAATCA (Folmer et al. 1994). The thermal conditions comprised initial denaturation at 95°C for 1 minute, followed by the first cycle set (15 cycles) of 94°C for 30 seconds, annealing at 55°C for 1 minute (decreasing by 1°C per cycle) and extension at 72°C for 1 minute and 30 seconds. The second cycle set (25 cycles) involved steps of 94°C for 35 seconds, 45°C for 1 minute, and 72°C for 1 minute and 30 seconds, followed by a single cycle at 72°C for 3 minutes and a final extension step at 72°C for 5 minutes, followed by indefinite hold at 4°C. PCR amplicons were observed on 1.5% agarose gels with 2 µl of PCR product. Sequencing of the unpurified PCR products in both directions was carried out at the Beijing Genomics Institute (Hong Kong, CN) using the amplification primers.

## Sequence processing

Analysis of nine sequences of specimens from garden sites, (sequence length 655 bp), as well as the quality check of the sequences, was conducted using Geneious Prime 2023.2.1 (<http://www.geneious.com>). The haplotype data file was generated using DnaSP 6 (Rozas et al. 2017) and resulted in three unique haplotypes. A sequence similarity search was conducted using the BLAST tool of NCBI directly in Geneous Prime 2023.2.1. The most closely related lineages of *A. vulgaris* were extracted and aligned with our haplotypes. A Neighbour-joining tree (genetic distance model: Tamura–Nei) was constructed to observe the relationship of both our and previously found haplotypes (Nei and Kumar 2000, Tamura et al. 2004).

The GenBank IDs of the nine voucher specimens used in this research are as follows: CaBOL-1013797:PP465469, CaBOL-1013796:PP465470, CaBOL-1013795:PP465471, CaBOL-1013794:PP465472, CaBOL-1013793:PP465473, CaBOL-1013792:PP465474, CaBOL-1013791:PP465475, CaBOL-1013790:PP465476, CaBOL-1013789:PP465477.

## Taxon treatment

### *Arion vulgaris* Moquin-Tandon, 1855

#### Materials

- a. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda;

order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; lifeStage: subadult; catalogNumber: HNHM 105451a; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Dissection; language: Armenian; collectionID: HNHM 105451a; datasetID: HNHM; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 8DC67D2A-7463-5B91-AD92-866FA1C4F7EE

- b. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; lifeStage: subadult; catalogNumber: HNHM 105451b; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Dissection; language: Armenian; collectionID: HNHM 105451b; datasetID: HNHM; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 9DF99457-A965-56DC-9DC9-11FC16B6E43F
- c. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; lifeStage: juvenile; catalogNumber: HNHM 105451c; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Dissection; language: Armenian; collectionID: HNHM 105451c; datasetID: HNHM; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: AEE31151-C361-5C48-A8BD-7062A2E3F3A8
- d. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-

- Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013789; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 09-12-22; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013789; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 2F28EE20-0497-58C4-A62D-7BB43361BEA9
- e. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013790; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 07-01-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013790; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 8C474C5B-2524-5391-83FE-BDFC3FFC931A
- f. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013791; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 07-01-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013791; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 41A510F9-AE0E-58AA-BBCB-7EF286C982A0
- g. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex:

- hermaphrodite; catalogNumber: CaBOL-1013792; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 07-01-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013792; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: AA9DA143-4568-5355-AA23-A89C3E62CF8C
- h. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013793; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013793; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 3F851C25-634C-518D-906B-BD0EF6E01698
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- j. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013795; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013795; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: A99D5774-E829-59EE-9051-C66146807805

- k. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013796; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013796; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 99DC1334-2758-5445-93BB-5B44804D06C0
- l. scientificName: *Arion vulgaris*; originalNameUsage: *Arion vulgaris*; nameAccordingTo: sensu; acceptedNameUsage: Spanish slug; taxonomicStatus: accepted; higherClassification: Eukaryota; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Stylommatophora; family: Arionidae; taxonRank: species; vernacularName: Spanish slug; genus: *Arion*; specificEpithet: *vulgaris*; scientificNameAuthorship: Moquin-Tandon, 1855; locationID: Stepanavan; higherGeography: Lesser Caucasus; continent: Eurasia; country: Armenia; countryCode: ARM; stateProvince: Lori; locality: Gyulagarak village, Lori province, ARM; decimalLatitude: 40.9686; decimalLongitude: 44.4675; georeferenceProtocol: GPS; samplingProtocol: hand sampling; habitat: garden; fieldNumber: Arion\_001; fieldNotes: First record of invasion; individualCount: 1; sex: hermaphrodite; catalogNumber: CaBOL-1013797; recordedBy: Meri Arzumanyan; identifiedBy: Meri Arzumanyan; dateIdentified: 08-08-23; identificationQualifier: Sequence; language: Armenian; collectionID: CaBOL-1013797; datasetID: CaBOL; institutionCode: YSU\_MOL\_MA; collectionCode: Molluscs; basisOfRecord: PreservedSpecimen; occurrenceID: 0584E305-B066-58AE-90AF-05A73BE2DB4B

## Description

Adult living specimens were 70–110 mm long when fully extended. Collected individuals were unicolour brown with an orange hue without bands, the tubercles were coarse, the foot fringe was orange or brown (same shade as body colour) with thin vertical black lines, the head had an orange tint and the tentacles were black (Fig. 1).

Samples for dissection were collected during the summer of 2023. Although all three dissected specimens were subadults or juveniles, the main genital characters were visible. The main distinctive character is the dilated anterior (i.e. closer to the genital pore) part of the oviduct, which contains a longitudinal ligula that has two elongated flanks. The posterior part of the oviduct is short and not dilated. The bursa is somewhat elongated and almost as wide as their ducts. The vas deferens is short and the epiphallus is slightly wider than the vas deferens in all cases (Fig. 2).



Figure 1. [doi](#)

*Arion vulgaris* specimen from Gyulagarak (CaBOL-1013790). The photograph was taken by M. Arzumanyan.

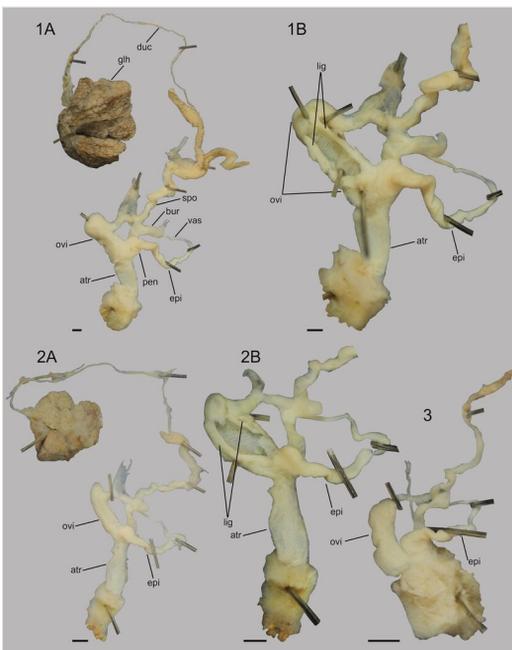


Figure 2. [doi](#)

Genital tract of three specimens deposited in the Natural History Museum of Budapest: 1: HNHM105451a, 2: HNHM105451b, 3: HNHM105451c. 1B and 2B: same specimens as in A with oviduct opened 1A: Abbreviations: atr = atrium, bur = bursa copulatrix, duc = ductus hermaphroditicus, epi = epiphallus, ghl = glandula hermaphroditica, pen = penis, lig = ligula, ovi = oviductus, spo = spermoviductus. All scale bars = 1 mm.

## Distribution

The map of Armenia presented in Fig. 3 highlights the specific sampling locations where *Arion vulgaris* was recorded. In 2022, the species was documented for the first time in Armenia in Gyulagarak.

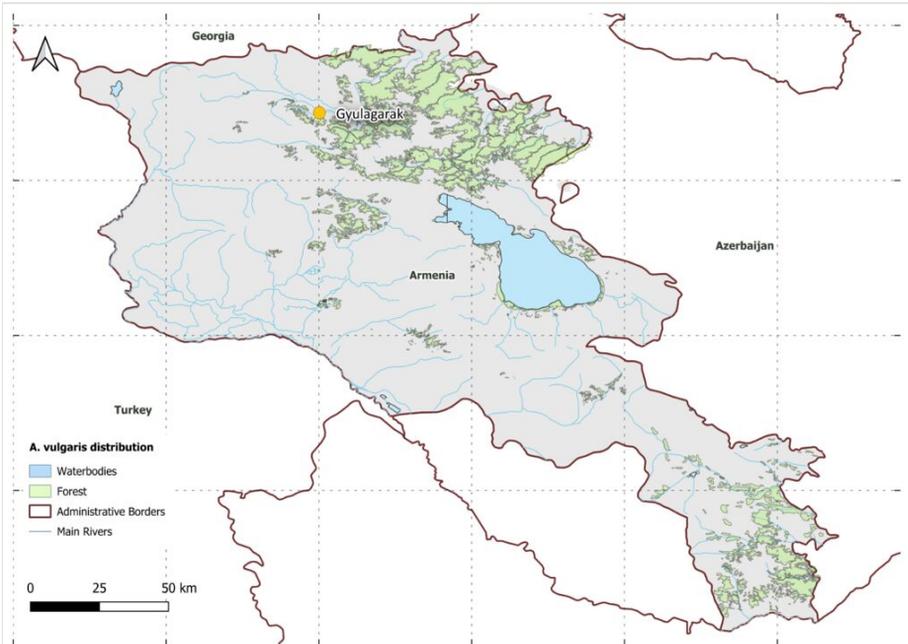


Figure 3. [doi](#)  
Sampling locations of *Arion vulgaris* in Armenia.

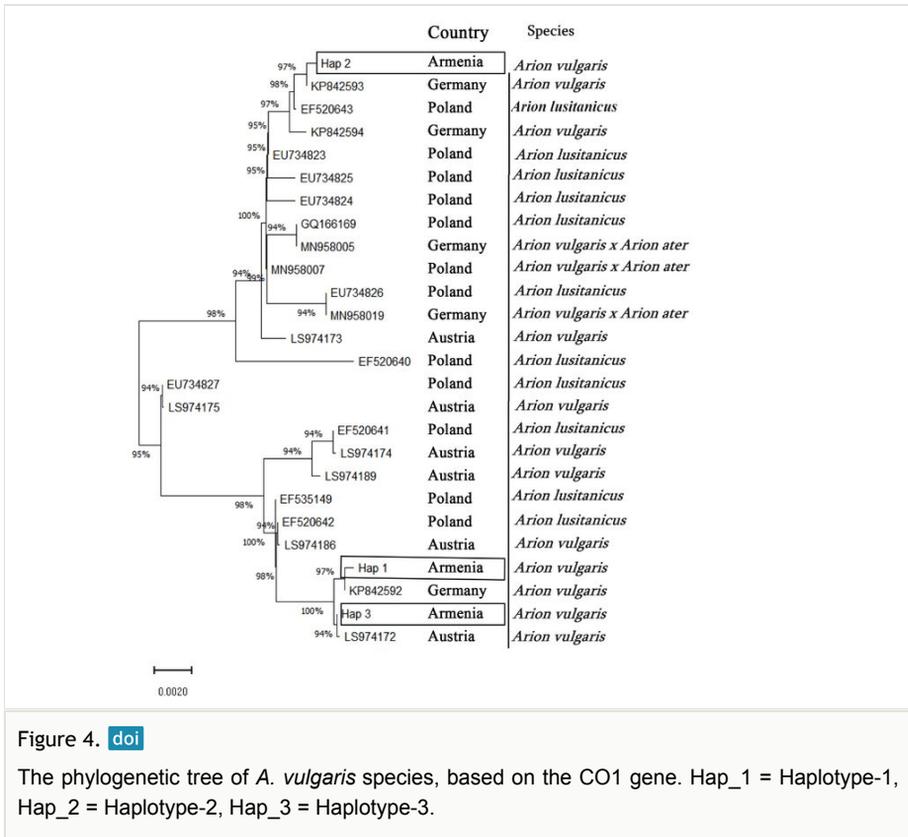
## Taxon discussion

First record of *Arion vulgaris* in Armenia.

## Molecular analyses

Phylogenetic analysis has shown that there are three distant haplotypes of *A. vulgaris* occurring in Gyulagarak Village in Armenia. Haplotype-1 consists of barcoding results of six specimens: CaBOL-1013789, CaBOL-1013790, CaBOL-1013791, CaBOL-1013793, CaBOL-1013795, CaBOL-1013797; Haplotype-2 two specimens: CaBOL-1013792, CaBOL-1013796; and Haplotype-3 only one: CaBOL-1013794. Haplotype-1 and Haplotype-3 are phylogenetically close to each other and to specimens from Germany and Austria. Haplotype-2 is close to specimens from Germany and is phylogenetically further from Haplotype-1 and Haplotype-3 (Fig. 4). This indicates that the species has been introduced from Central Europe. Most of the GenBank records used for the phylogeny in Fig. 4 were for *A. vulgaris* specimens, especially those records from Austria and Germany. For most of the Polish data, taken

from Soroka et al. (2009), where the species' anatomical description is similar to *A. vulgaris*, *A. vulgaris* might be misidentified as *A. lusitanicus*. There are three *A. vulgaris* and *A. ater* hybrids – two from Germany and one from Poland – shown in Fig. 4.



## Discussion

Our results confirm the first record of *A. vulgaris* for Armenia. External morphological characters and genital anatomy of the species complied with external and genital traits of *A. vulgaris* (Wiktor 1996, Rowson et al. 2014). However, the bursa copulatrix is usually egg-shaped with a short duct in adults (Wiktor 1996); in our specimens, the bursa was elongated. This difference was probably due to the fact that the collected specimens were not fully mature.

From neighbouring Türkiye, *Arion ater* s.l. was first recorded in 2017 (Reise et al. 2018). The taxonomy of some large *Arion* species is extremely problematic due to external morphological similarities and, potentially, hybridisation (Reise et al. 2020). Dissection is required to identify species. The ligula are shorter and can be found in the upper atrium in *Arion ater* s.l., but the ligula are elongated and situated in the dilated oviduct in the case of *A. vulgaris*. The specimens from Armenia fit the species description of *A. vulgaris* well, and

based on molecular data, Armenian samples are also similar to European *A. vulgaris*. However, the individual photographed near Dilijan, which was not from a garden, but was seen in mixed forest habitat, might suggest *A. ater* occurs in Armenia as well. This is currently unconfirmed though and anatomical and genetic analyses are required before we can say for certain.

*Arion vulgaris* has been confused with *Arion lusitanicus* Mabille, 1868, which is a species endemic to Portugal. After this confusion was disentangled, based on genital morphological differences (Castillejo 1997) and although a molecular study suggests they are separate species (Quinteiro et al. 2005), the name *A. lusitanicus* auct. non Mabille, 1868 was used for a while for the invasive species with a Europe-wide distribution. However, the name *A. vulgaris* had been used more and more frequently for the invasive species, finally becoming its valid name (Balashov 2018, Kadolsky et al. 2018, Reise et al. 2020).

The first specimen of *A. vulgaris* from Armenia was detected in Gyulagarak Village. It was found in mixed gardens where different types of vegetables were planted. Based on our observations, individuals are inactive during the day, but during the night, they actively emerge from their hiding places and start to disperse and feed. An individual representing a possible second population of the species was photographed in 2023 in Dilijan by Prof. S. Pipoyan, but this is an unconfirmed sighting of *A. vulgaris*. The current record of the species from Gyulagarak is the second from the Caucasus (Schikov and Komarov 2021) and the first Arionidae for Armenia.

*Arion vulgaris* is a dangerous invasive species that can cause damage to domestic gardens (Proschwitz 1997, Hulme 2009a, Zemanova et al. 2017) and is one of the fastest spreading terrestrial molluscs. In Table 1 and Fig. 5, the distribution of *A. vulgaris* in Eurasia is presented across several decades. However, it should be noted that, for France and Germany, the species is considered to be native (Zemanova et al. 2016).

Table 1.

Distribution and first records of *Arion vulgaris* by year around the world. \*Note that the Spanish record probably belongs to *A. lusitanicus*, a Portuguese endemic species and not to the invader *A. vulgaris*.

Country	Year of first observation	Reference
France	Native	Moquin-Tandon (1855)
Germany	Native	Schmid (1970)
Spain*	Unconfirmed data	Mabille (1868)
Portugal*	Unconfirmed data	Mabille (1868)
Türkiye	Unconfirmed data	Yıldırım and Gürelek (2017)
UK	1954	Kerney (1999)
Switzerland	1956	Turner et al. (1998)
Italy	1965	van Regteren Altena (1971)

Country	Year of first observation	Reference
Bulgaria	1966	van Regteren Altena (1971)
Slovenia	1970	Wiktor (1996)
Austria	1971	Reischütz and Stojapal (1972)
Norway	1972	Reischütz and Stojapal (1972)
Belgium	1973	Risch and Backeljau (1989)
Netherlands	1973	de Winter (1989)
Sweden	1975	Proschwitz (1989)
Croatia	1983	Wiktor (1996)
Ireland	1984	Anderson (2010)
Hungary	1985	Varga (1986)
Poland	1987	Kozłowski and Kornobis (1994)
Finland	1990	Valovirta (2001)
Czech Republic	1991	Dvořák and Horsák (2003)
Denmark	1991	Proschwitz and Winge (1994)
Slovakia	1992	Reischütz (1994)
Faroe Islands	1996	Bloch (2003)
Montenegro	2002	Vuksa et al. (2003)
Serbia	2002	Vuksa et al. (2003)
Iceland	2003	Ingimarsdóttir and Ólafsson (2005)
Macedonia	2003	Stankovic et al. (2006)
Ukraine	2007	Sverlova and Gural (2008)
Estonia	2009	Palginõmm (2009)
Canada	2009	L'Heureux et al. (2023)
Mexico	2009	Araiza-Gómez et al. (2021)
Russia	2009	Schikov (2016)
Latvia	2010	Rudzīte et al. (2010)
Romania	2012	Papureanu et al. (2014)
Lithuania	2013	Skujienė (2013)
Russia, North Ossetia-Alania	2019	Schikov and Komarov (2021)
Belarus	2021	Ostrovsky (2022)
Armenia	2022	Current Study

Based on the map (Fig. 5), it is clear that the species has been spreading in all directions from its native range. It is likely only a matter of time before it will be recorded in Georgia, Azerbaijan, Iran and the eastern part of Türkiye. Considering that species introduction mechanisms have not been recorded, there are several possibilities as to how it could have entered Armenia. If we assume the natural range expansion of the species, it may have entered Armenia from Georgia in the north or from the eastern region of Türkiye to

the west. Invasive species are usually understudied, but due to the lack of confirmed records from Georgia and eastern Türkiye, this hypothesis remains unconfirmed. Obtaining haplotype samples from a wider range of locations and a more comprehensive phylogenetic analysis of these could help to determine where the possible routes into Armenia are.

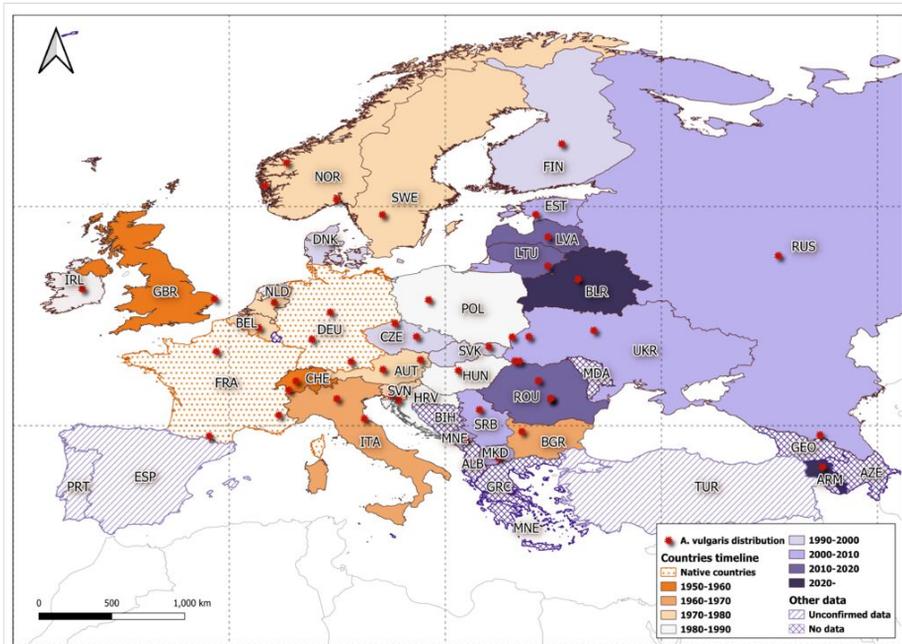


Figure 5. [doi](#)

Distribution and dispersal of *A. vulgaris* across Eurasia by decade.

However, a more likely scenario than natural distribution is the human-assisted spread of the species (Meyerson and Mooney 2007). Due to increased international transportation, agricultural or horticultural products provide a fast and convenient way for invasive species to spread (Cowie et al. 2008, Hulme 2009b). Slugs can easily hide in plant pots and a sufficient amount of moisture makes them capable of surviving long-distance travel. This is not just the case for adults; juveniles and unhatched eggs in the soil of plant pots enhance the distribution of invasive molluscs further. As a consequence, botanical gardens and garden centres are potential hotspots for new introductions (Turóci et al. 2023).

Monitoring new invasions of *A. vulgaris* is crucial, as species invasions into new territories can have several negative consequences: for example, the impact on agriculture via reduced yields (Grimm et al. 2000) or on ecosystems as a result of habitat modification and loss of native species (Proschwitz 1997, Knop and Reusser 2012, Zemanova et al. 2017, Reise et al. 2020, Hutchinson et al. 2021). *Arion vulgaris* is also an intermediate host for several cardiopulmonary metastrongyloid nematodes that cause respiratory or systemic disease in canids and felids (Ferdushy et al. 2010, Gismervik et al. 2015, Lange et al. 2018

, Penagos-Tabares et al. 2019, Antzée-Hyllseth et al. 2020). Additionally, *A. vulgaris* may also be a potential vector of plant pathogens, such as genus *Phytophthora* de Bary, 1876 (Telfer et al. 2015) and pathogenic bacteria of humans, such as Enterobacteriaceae (Stalder et al. 2014, Gismervik et al. 2015).

However, monitoring inhabited areas and areas into which *A. vulgaris* has possibly been newly introduced can help as a means to survey the population and this enables us to assess whether occupied ecosystems are suitable for this species. We emphasise that prevention of new introductions and eradication of populations of invasive species are very important and provide great challenges for the future.

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## Author contributions

Meri Arzumanyan designed the project and wrote the manuscript. Gohar Zhamakochyan conducted DNA extraction and PCR. Hasmik Torosyan prepared maps for the article. Arevik Ghrmajyan worked on raw sequence data and prepared them for further analysis. Marine Arakelyan and Siranush Nanagulyan coordinated the project fieldwork, data sampling and contributed to paper writing. Lusine Margaryan contributed to the data collection for the species' worldwide distribution. Sargis Aghayan prepared the phylogenetic tree, wrote the methodological part of the manuscript related the sequences and DNA extraction, Robert B Davis provided methodological support for phylogenetic trees, coordinated and edited the manuscript and Ágnes Turóci conducted specimen dissections and edited the manuscript.

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