

Two invasive species of *Galba* (Gastropoda: Lymnaeidae), new for the Azerbaijan fauna

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ABSTRACT. In this study, we report the discovery of two invasive freshwater mollusc species belonging to the genus *Galba* (Gastropoda: Lymnaeidae) on the territory of Azerbaijan. This is the first record of the species *Galba cubensis* and *G. schirazensis* in this country, based on the analysis of morphological traits and sequencing of the mitochondrial cytochrome c oxidase subunit I gene (COI marker). Also, the phylogeographic analyses of both species were performed based on a median-joining network approach of the COI sequences. The find of *G. cubensis* is the first record of this invasive snail of New World origin in the ex-USSR territory. The taxonomic position of *G. schirazensis* sensu Kruglov et Starobogatov is discussed.

[https://doi.org/10.35885/ruthenica.2025.35\(2\).3](https://doi.org/10.35885/ruthenica.2025.35(2).3)

Два инвазионных вида рода *Galba* (Gastropoda: Lymnaeidae), новых для фауны Азербайджана

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РЕЗЮМЕ. В данном исследовании мы сообщаем об обнаружении двух инвазионных видов пресноводных моллюсков, принадлежащих к роду *Galba* (Gastropoda: Lymnaeidae), на территории Азербайджана. Это первая находка видов *Galba cubensis* и *G. schirazensis* в этой стране, основанная на анализе морфологических признаков и секвенировании фрагмента митохондриального гена субъединицы I цитохром с-оксидазы (маркер COI). Кроме того, проведено изучение филогеографической структуры обоих видов с использованием медианной сети гаплотипов по гену COI. Обнаружение *G. cubensis* является первой находкой этого инвазионного вида родом из Нового Света на территории бывшего СССР. Обсуждается таксономическое положение *G. schirazensis* sensu Kruglov et Starobogatov.

Introduction

The lymnaeid genus *Galba* Schrank, 1803 has almost cosmopolitan distribution and contains more than 30 recent nominal species, many of them have never been subjected to molecular taxonomic scrutiny. Most of these species live in North America, whereas the Old World is relatively depleted with species of *Galba* [Vinarski *et al.*, 2019, 2023; Alda *et al.*, 2021; Aksenova *et al.*, 2023]. Such a taxonomic impediment is even more unfortunate because many species of the genus are of great epidemiologic importance. A classic example is *Galba truncatula* (O.F. Müller, 1774), a vector of the causative agent of fasciolosis, the digenean worm *Fasciola hepatica* (Linnaeus, 1758) [Hurtrez-Boussès *et al.*, 2023]. Some other species of *Galba* are able to transmit this parasite as well [Kruglov, 2005; Correa *et al.*, 2011; Celi-Eraso, 2020; Vázquez *et al.*, 2023]. Another characteristic of *Galba* snails is that many species of this genus are morphologically cryptic, and today it is firmly known that only molecular genetic methods are effective in their identification and detection [Correa *et al.*, 2011; Pointier, 2015; Alda *et al.*, 2018, 2021; Vázquez *et al.*, 2023]. Applications of these methods usually led to improvement in the taxonomy of *Galba*, description of new species and detection of invasive representatives of this genus in new territories [Mahulu *et al.*, 2019; Saito, 2022; Aksenova *et al.*, 2024].

The amount of papers reporting new findings of exotic species of *Galba* in various countries is growing fast [Bargues *et al.*, 2011; Lounnas *et al.*, 2018; Schniebs *et al.*, 2018; Ohari *et al.*, 2020; Alda *et al.*, 2021; Bikashvili *et al.*, 2022; Saito, 2022]. Among lymnaeids, *Galba* spp. appear to be the second most effective invaders, just after *Pseudosuccinea columella* (Say, 1817), a lymnaeid with the broadest known invasive range [Lounnas *et al.*, 2017; Alba *et al.*, 2019; Vinarski *et al.*, 2019, 2023; Alda *et al.*, 2021].

Although the study of freshwater molluscs in Azerbaijan began almost 200 years ago [Ménétriés, 1832], these works were not expanded up to the present time. It is also difficult to give a complete list of aquatic molluscs of the fauna of Azerbaijan, because some Azerbaijani scientists did not present photos of the species along with a list of species in their works. Thus, it is virtually impossible to verify the species identification provided by these authors. At the Institute of Zoology (Baku, Azerbaijan), the museum collection includes only a small number of specimens of land molluscs, there are no samples of freshwater gastropod species. Most old literary sources used outdated taxonomy and nomenclature of snails [e.g., Veisig, 1939; Safarov, 1971; Kasymov, 1972]. For example, A. Alizade in the mid-1940s published a list of freshwater molluscs of Azerbaijan

based on his extensive research, where eight species of Lymnaeidae were included [Alizade, 1945]. Later, other workers repeated his data, placing some of the species into genus *Galba*. Kasymov [1972] listed two species of this genus: *G. truncatula* and *G. palustris* (O.F. Müller, 1774). However, the latter species is now classified within the genus *Stagnicola* Jeffreys, 1830. Based on the information given in the published book “Wildlife of Azerbaijan (protozoans and metazoans)” [2002], 63 species of freshwater molluscs have been included in the malacofauna of Azerbaijan until 2002. It was shown that out of the total number, 25 species and subspecies belonged to the family Lymnaeidae [Kasymov, 1972]. Special research, devoted to the diversity of Lymnaeidae and their epidemiological significance in Azerbaijan [Vakhidov, 1992], revealed five nominal species of the genus *Galba* in the Kura-Araz lowland of Azerbaijan. However, all identifications were made solely on a morphological basis, and the taxonomic identity of some species mentioned by Vakhidov [1992] is unclear. In particular, the true identity of the species listed by the authors as *Lymnaea (Galba) goupili* (Moquin-Tandon, 1856) and *L. (G.) thiesseae* (Clessin, 1879) is unknown.

The aim of this study is to report the recent findings of two species of *Galba* in Azerbaijan. These two species are invasive and new for the Azerbaijan fauna.

Material and methods

The primary material for this study was collected in 2023 in various parts of Republic of Azerbaijan (Fig. 1). A total of 65 snail specimens were collected in five districts: Yevlakh, Lankaran, Sabirabad, Khachmaz, and Shabran.

Molluscs were collected by hand, from stones, submerged objects and other substrates. All individuals were fixed in the field in 96 % ethanol. During the snail collection in Yevlakh District, we estimated the population density by counting the number of individuals per 1 cm².

Shells of *Galba* spp. were morphologically studied in the laboratory following standard protocols of species identification, dissection, and measurements [Kruglov, 2005; Vinarski, Vázquez, 2023]. The collected specimens were represented mostly by juvenile individuals. A total of six of the largest individuals were dissected and measured. Primary identifications were subsequently molecularly verified using genetic markers.

First, young specimens were selected for the study of protoconch morphology. The shells were sequentially cleaned in sodium hypochlorite (NaClO), alcohol, and distilled water, dried, sputtered with gold in Smart Coater with fully automated vacuum and examined in SEM at 15 kV mode. SEM images



FIG. 1. Map of Azerbaijan showing localities for three recorded species of the genus *Galba* in this region: 1. Yevlakh District, artificial ditch in Yevlakh City (645 m a.s.l. Coordinates: 40.61809 N, 47.13274 E. Sampling date: 05.07.2023); 2. Lankaran District, Lankaran River Basin, artificial ditch near Germatuk settlement (15 m b.s.l. Coordinates: 38.71876 N, 48.81939 E. Sampling date: 08.07.2023); 3. Sabirabad District, Kura River Basin, stream near Garaaghaj settlement (20 m b.s.l. Coordinates: 39.927754 N, 48.87490 E. Sampling date: 07.07.2023); 4. Khachmaz District, Khachmaz City vicinity, stream near Agchay River (62 m a.s.l. Coordinates: 41.4289 N, 48.81168 E. Sampling date: 10.07.2023); 5. Shabran District, Zagly village, mountain river (623 m a.s.l. Coordinates: 41.14002 N, 48.81713 E. Sampling date: 01.11.2023). Photos by N.Yu. Snegovaya and O.V. Aksenova.

РИС. 1. Карта Азербайджана с указанием местонахождений трех видов рода *Galba*: 1. Евлахский район, искусственный ручей в г. Евлах (645 м н.у.м. Координаты: 40.61809 N, 47.13274 E. Дата сбора: 05.07.2023); 2. Ленкоранский район, искусственный пруд неподалёку от пос. Герматюк (-15 м н.у.м. Координаты: 38.71876 N, 48.81939 E. Дата сбора: 08.07.2023); 3. Сабирабадский район, ручей рядом с пос. Гараагадж (-20 м н.у.м. Координаты: 39.927754 N, 48.87490 E. Дата сбора: 07.07.2023); 4. Хачмазский район, ручей, впадающий в р. Агчай, в окрестностях г. Хачмаз (62 м н.у.м. Координаты: 41.4289 N, 48.81168 E. Дата сбора: 10.07.2023); 5. Шабранский район, д. Загли, горная река (623 м н.у.м. Координаты: 41.14002 N, 48.81713 E. Дата сбора: 01.11.2023). Фото Н.Ю. Снеговой и О.В. Аксёновой.

of some shells and all protoconchs were obtained using JEOL JCM-6000 (Japan) scanning electron microscope. A total of nine shells were examined using SEM.

Second, total genomic DNA was extracted from 96% ethanol-preserved soft body tissues using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Germany), following the manufacturer's protocol. The performed molecular genetic analysis of freshwater snails included amplification and sequencing of the mitochondrial cytochrome c oxidase subunit I gene (COI marker). The COI sequences were amplified by polymerase chain reaction (PCR)

using the following primers: LCO1490 [Folmer *et al.*, 1994] and C1-N-2329 [Simon *et al.*, 1994]. The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 μ mol of each dNTP, 2.5 μ l of PCR buffer (with 10×2 mmol $MgCl_2$), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Russia), and H_2O was added for a final volume of 25 μ l. Thermocycling was implemented with markers-specific PCR programs as follows: 95 $^{\circ}C$ (4 min), followed by 28 cycles at 94 $^{\circ}C$ (50 sec), 48-50 $^{\circ}C$ (50 sec), 72 $^{\circ}C$ (50 sec) and a final extension at 72 $^{\circ}C$ (5 min). Forward and reverse sequencing were performed on an automatic sequencer (ABI

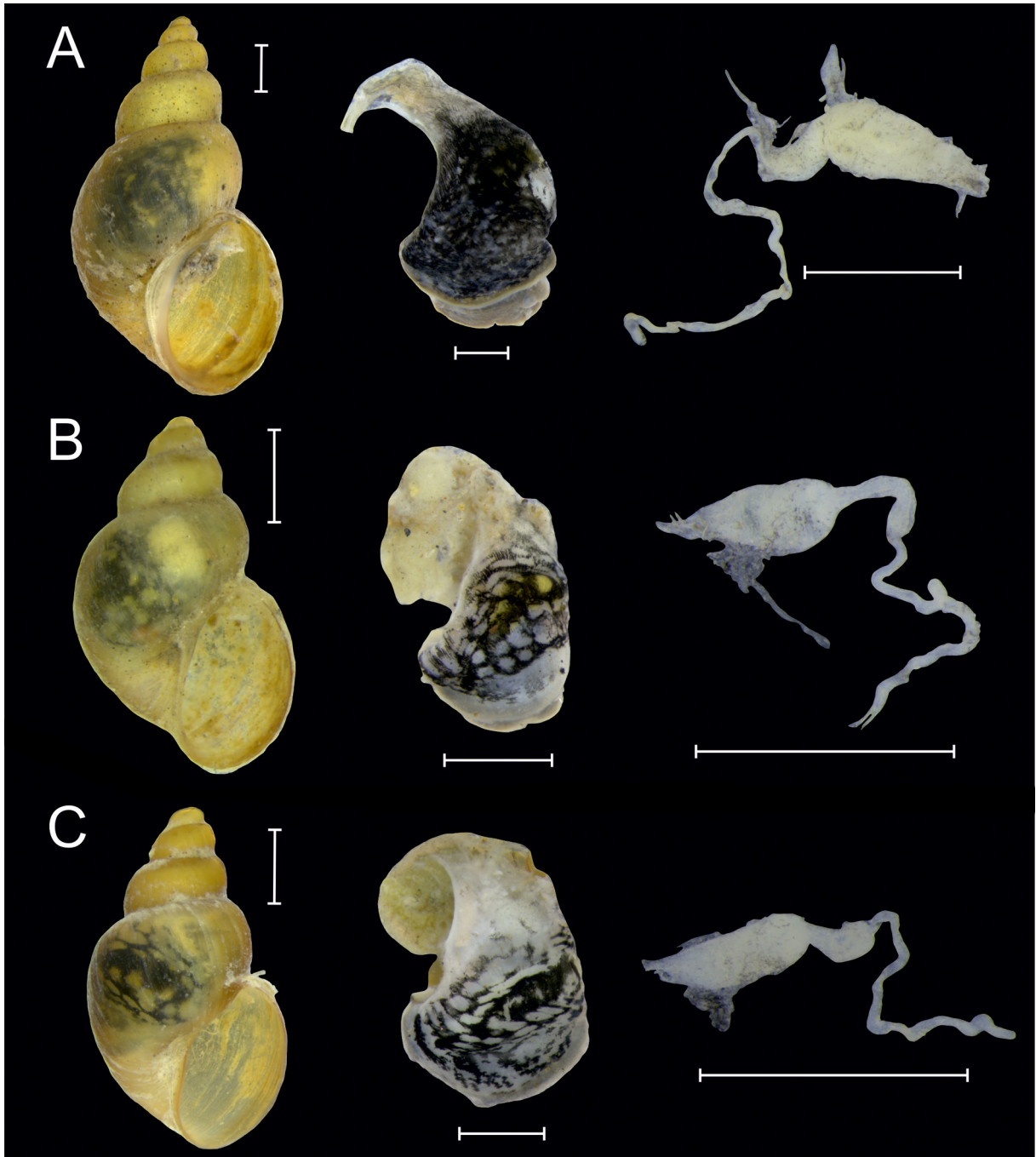


FIG. 2. Shells, soft body and copulatory organs of *Galba* species detected in Azerbaijan. **A.** *Galba cubensis*. **B.** *G. schirazensis*. **C.** *G. truncatula*. Scale bars 1 mm. Photos by O.V. Aksenova.

РИС. 2. Фото раковин, мягкого тела и копулятивных органов трех видов рода *Galba*, найденных в Азербайджане. **A.** *Galba cubensis*. **B.** *G. schirazensis*. **C.** *G. truncatula*. Масштабная линейка 1 мм. Фото О.В. Аксеновой.

PRISM3730, Applied Biosystems) using the ABI PRISM BigDye Terminator v. 3.1 reagent kit. The resulting sequences were checked using a sequence alignment editor BioEdit v. 7.2.5 [Hall, 1999]. A total of nine individuals were sequenced (Table S1).

The sequences were aligned using the MUSCLE algorithm of MEGA X [Kumar *et al.*, 2018]. The phylogeographic analyses of *Galba cubensis* and

G. schirazensis were performed based on a median-joining network approach using Network ver. 4.6.1.3 software with default settings [Bandelt *et al.*, 1999]. In addition to the four generated ones, 79 COI sequences of two *Galba* species were obtained from NCBI GenBank (Table S1). Also, five new sequences were obtained for *G. truncatula*.

The specimens of *Galba* collected during this re-

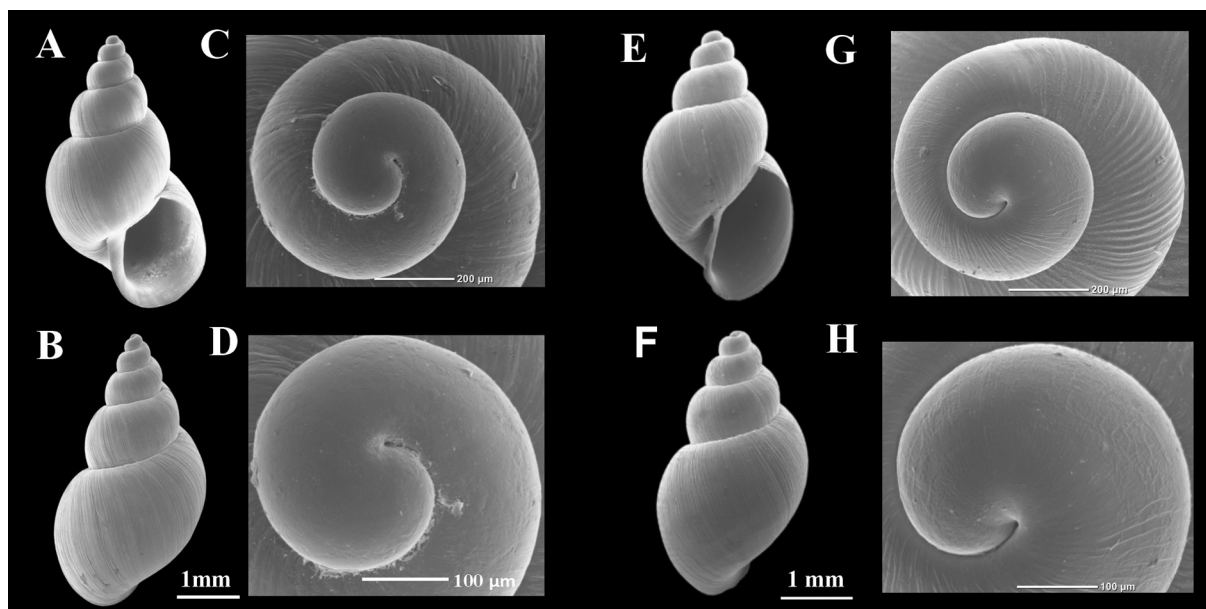


FIG. 3. SEM images of shells of *Galba cubensis* (A-D) and *G. truncatula* (E-H). A-B, E-F. Apertural and abapertural view of the whole shells; C-D, G-H. Apical view of the protoconch with a visible transition between protoconch and teleoconch; Photos by E.N.Tahirova.

РИС. 3. SEM-изображения раковин *Galba cubensis* (A-D) и *G. truncatula* (E-H). A-B, E-F. Вид раковин со стороны устья и с обратной стороны; C-D, G-H. Апикальный вид протоконха с заметным переходом между протоконхом и телеоконхом. Фото Э.Н. Тагировой.

search in Azerbaijan are kept in collections of the Institute of Zoology, Ministry of Science and Education of Republic of Azerbaijan (Baku) (not catalogued), and the Russian Museum of Biodiversity Hotspots, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Russia, Arkhangelsk) (voucher numbers for the specimens of *G. cubensis* – MLym-1292; *G. schirazensis* – MLym-1327; *G. truncatula* – MLym-1290; MLym-1324; MLym-1326).

Results

The study of the collected materials revealed the presence of three distinct species of *Galba* (Fig. 2 and Fig. 3). One of them is identical to *G. truncatula*, which is quite common in many parts of the ex-USSR and adjacent countries [Kruglov, 2005]. The two other species, *G. cubensis* (L. Pfeiffer, 1839) and *G. schirazensis* (Küster, 1863), are exotic and have never been reported from the territory of the Azerbaijan (but see remarks on *G. schirazensis* below). Figure 1 shows the known localities of these three species in Azerbaijan. We cannot add to this figure all the findings of *Galba* known from the literature [i.e. Alizade, 1940; Kasymov, 1972], since *G. truncatula* reported by previous authors could well belong to other, conchologically similar, species.

A single COI haplotype was found in *G. cubensis* from Azerbaijan (Fig. 4). This is the haplotype found also in snails collected in Cuba, Brazil, Spain, and the USA.

G. cubensis shell (Fig. 2 and Fig. 3) is very similar to that of *G. truncatula* and may easily be confused with the latter. However, the surface of the embryonic shell in both species is smooth, whereas the initial part of the teleoconch bears visible axial riblets (Fig. 3 C-D, G-H). The transition from the protoconch to the teleoconch is marked by a notch. In teleoconch whorls, the surface structure becomes more uneven and reticulate. The maximum height of the shell of *G. cubensis* from our samples is 7.91 mm, and the maximum width is 4.54 mm. The maximum height of the aperture is 4.07 mm. The shell has 4.75 whorls separated by a shallow suture. The mantle is black with small light grey dots (Fig. 2 A). The lengths of the praeputium and penis sheath are 1.01 mm and 0.52 mm, respectively. The anterior and posterior width of penis sheath in *G. cubensis* from Yevlakh is 0.23 mm.

During the snail sampling, we observed a rather dense population of the species *G. cubensis* (Fig. 5). About 4-5 individuals of the species were recorded per 1 cm² area.

Specimens of *G. schirazensis* found in Azerbaijan share the same haplotype with conspecific snails collected from a broad array of regions, from France to Iran and Bhutan (see Fig. 4).

The maximum shell height of *G. schirazensis* collected in Azerbaijan is 4.14 mm, and the maximum width is 2.52 mm. The aperture height is 2.12 mm. The shell has 4.25 whorls separated by a shallow suture. The mantle is grey with large blurred black spots (Fig. 2B). The length of the praeputium and

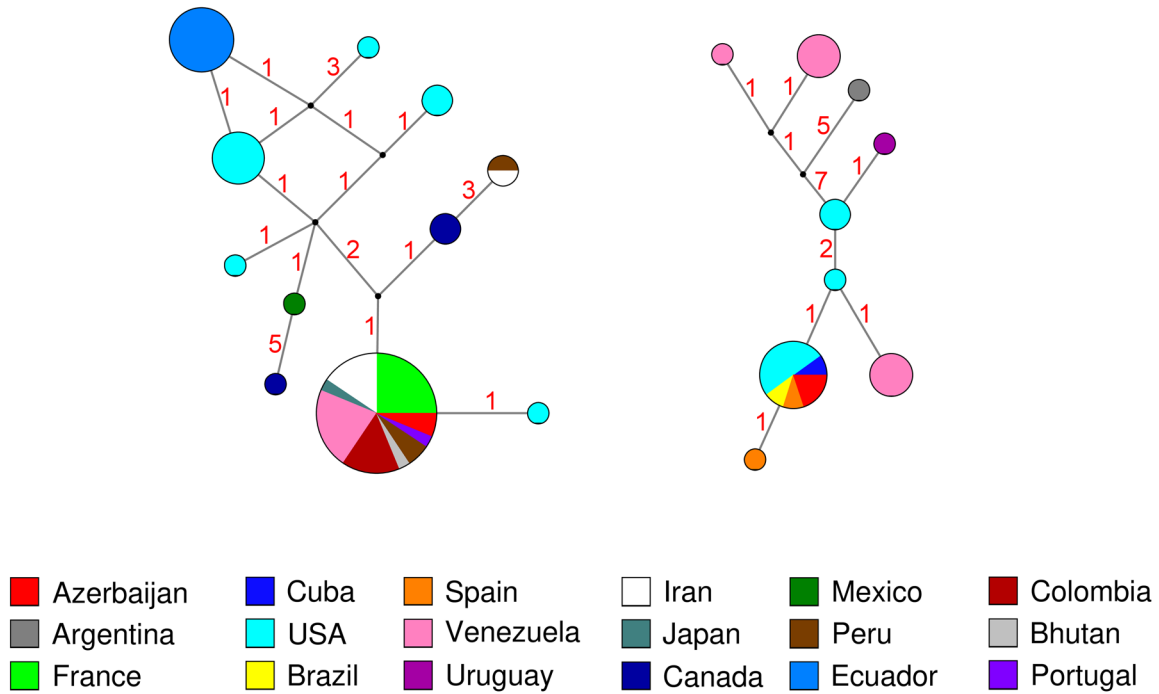
*Galba schirazensis**Galba cubensis*

FIG. 4. Median-joining networks of the *COI* sequences of *Galba schirazensis* ($N = 58$) and *G. cubensis* ($N = 25$). The red numbers near branches indicate the numbers of nucleotide substitutions between haplotypes. Size of circles corresponds to the number of available sequences for each haplotype (smallest circle = 1 sequence). The list of sequences is given in Table S1.

РИС. 4. Медианная сеть гаплотипов, построенная на основе нуклеотидных последовательностей фрагмента гена *COI* для *Galba schirazensis* ($N = 58$) и *G. cubensis* ($N = 25$). Красные цифры рядом с ветвями указывают на количество нуклеотидных замен между гаплотипами. Размер кругов соответствует количеству доступных нуклеотидных последовательностей для каждого гаплотипа (наименьший круг = 1 сиквенс). Список используемых последовательностей приведен в Таблице S1.



FIG. 5. Dense aggregation of *Galba cubensis* beneath the water. Photo by E.N. Tahirova.

РИС. 5. Скопления особей *Galba cubensis*. Фото Э.Н. Тагировой.

phallotheca are 0.84 mm and 0.42 mm, respectively. The anterior and posterior width of phallotheca are 0.8 mm and 0.10 mm, respectively.

Both shell and soft body morphology of *G. trun-*

catula from Azerbaijan studied by us correspond to descriptions of this snail in the literature [Kruglov, 2005; Pointier, 2015; Glöer, 2019]. The maximum shell height of studied specimens is 4.56 mm and the width is 2.65 mm. The aperture height is 2.25 mm. The mantle is light grey with blurred black spots and dots (Fig. 2 C). The lengths of the praeputium and phallotheca are 0.61 mm and 0.27 mm, respectively. The anterior and posterior width of phallotheca are 0.8 mm and 0.13 mm, respectively.

Discussion

This study is the first step in an integrative revision of Azerbaijan's freshwater malacofauna, and even the first results show that the current knowledge on this fauna is unsatisfactory.

Two new exotic species of *Galba* for the fauna of Azerbaijan and one new species for the whole ex-USSR (*G. cubensis*) fauna expand greatly the current knowledge of the diversity of this genus and also can be of interest for helminthologists. Both newly

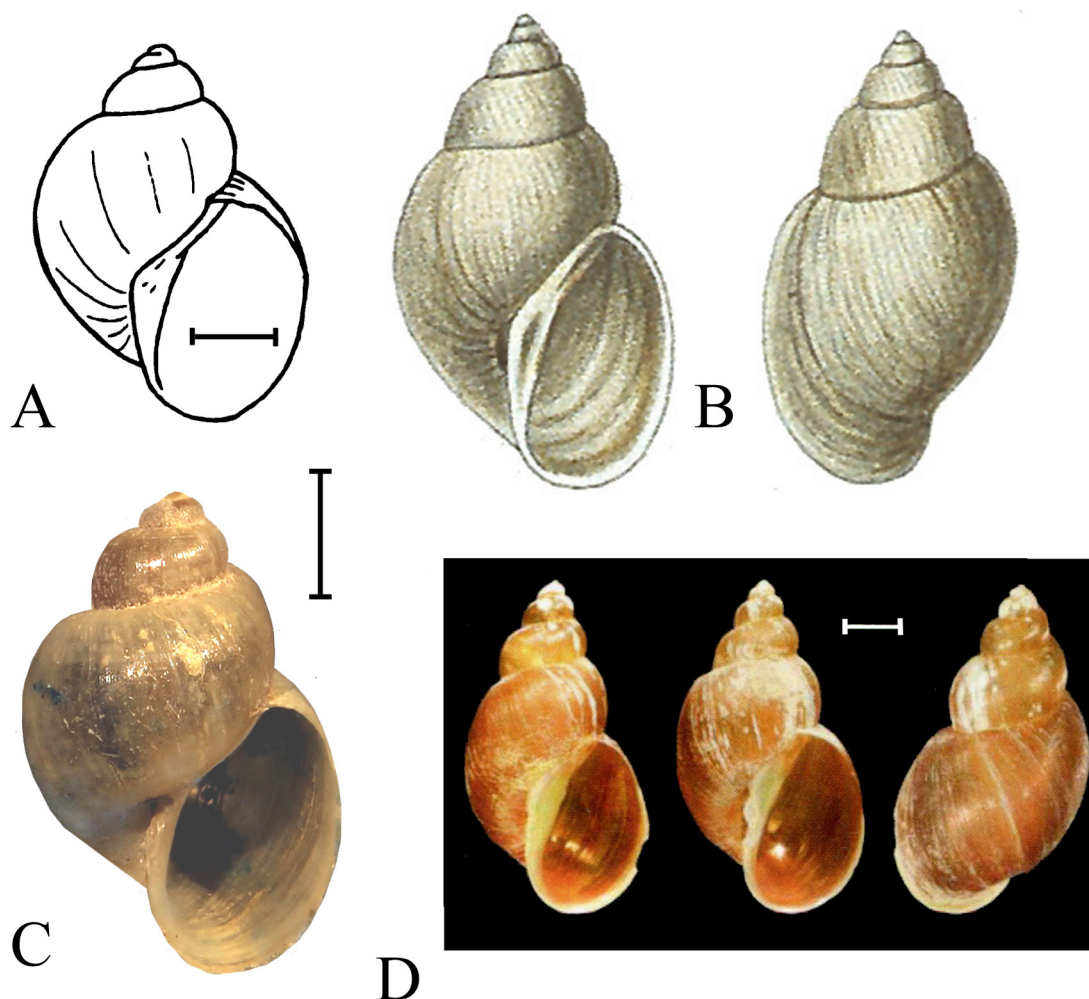


FIG. 6. Shells of *Galba schirazensis* identified by different authors. **A.** After Kruglov, Starobogatov [1993]. **B.** Original image of shell by Küster [1863]. **C.** *G. schirazensis* sensu Kruglov et Starobogatov, Zoological Institute of RAS, St.-Petersburg, not catalogued (locality is unknown). **D.** Molecularly identified specimens from Spain. After Glöer [2019]. Scale bars 1 mm (except for B – scale bar is absent from the original publication). C – photo by M.V. Vinarski.

РИС. 6. Раковины *Galba schirazensis*, определенные разными авторами. **A.** по: Круглов, Старобогатов [1993]. **B.** Оригинальное изображение раковины по: Кюстер [1863]. **C.** *G. schirazensis* sensu Kruglov et Starobogatov, ЗИН РАН (г. Санкт-Петербург), некаталогизирован, (местонахождение неизвестно). **D.** Образцы из Испании, идентифицированные молекулярно-генетическим методом по: Глоер [2019]. Масштабная линейка 1 мм (за исключением **B** – в оригинальной публикации масштабная линейка отсутствует). **C** – фото М.В. Винарский.

registered species have been recorded as vectors of *Fasciola hepatica* in various parts of the world [Vázquez *et al.*, 2023].

Most probably, *G. cubensis* is of New World origin and evolved in the native range of the species. Since the 1920s it has been registered as an invasive snail in such remote parts of Palearctic as Spain and Northern Germany [Boettger, 1929; Wiese, 1991; Schniebs *et al.*, 2018]. Before our study, a record in Spain has remained the only known occurrence of *G. cubensis* in the Old World confirmed by a molecular study. Other findings, made in the 20th century in Germany and Austria, were based on a morphology-only identification and thus need a confirmation. It is worthy to note that all these recordings were made

in greenhouses and other habitats with artificially changed temperature regime [Schniebs *et al.*, 2018]. The concrete mechanisms of *Galba cubensis* dispersal within Europe and to Azerbaijan are unknown, though a hypothesis of unintentional transportation with human vehicles seems the most reliable. The ability of *Galba* snails to use mechanical vehicles for their dissemination is well registered in the literature [Starobogatov, 1970], however other means of this, including ectozoochory with large wild animals, are identified as well [Juhász, Majoros, 2023]. The transfer of *G. cubensis* from the New World to Europe has been ascribed to ‘human-mediated transport with plants or other commercial activities’ [Schniebs *et al.*, 2018. P. 8].

In Spain, this snail species is represented by two haplotypes. One haplotype is invasive and corresponds to the haplotypes known in the Americas, while the other is unique and has so far been found only in Spain (Fig. 4). Although Schniebs *et al.* [2018] wrote that both sequences were identical, in reality they differ from each other by a single nucleotide substitution.

The type locality of *Galba schirazensis* is situated in Iran [Küster, 1863], therefore one may consider it a member of native fauna (i.e. Azerbaijan is adjacent to Iran). Except for Iran, *G. schirazensis* has been recorded from Georgia, Egypt, Japan, Reunion Island, and Spain [Bargues *et al.*, 2011; Lounnas *et al.*, 2018; Alda *et al.*, 2021; Bikashvili *et al.*, 2022; Saito, 2022], which assumes a fairly broad range in the Palearctic. However, the native region of this species remains debatable. Some authors are inclined to think that *G. schirazensis* “has an American origin with recent colonization of the Old World by a genetically uniform strain related to populations from Venezuela and Colombia” [Lounnas *et al.*, 2018, p. 434]. In this case, the species must be considered non-indigenous in Azerbaijan. The distribution of its haplotype, thus, almost coincides with the range of *G. schirazensis* as it is known now.

Another question regards the identity of *Lymnaea* (*Galba*) *schirazensis* described in works of Soviet malacologists [Izzatullaev *et al.*, 1983; Kruglov, Starobogatov, 1985, 1993; Kruglov, 2005]. Indeed, this species was recorded by these authors from different regions of the south USSR (Central Asia, Caucasus), though, according to Vakhidov’s data, no findings from Azerbaijan were known by 1992 [Vakhidov, 1992]. We are not sure that *Lymnaea schirazensis* sensu Kruglov et Starobogatov is identical to *L. schirazensis* sensu Bargues *et al.* [2011]. Izzatullaev *et al.* [1983, p. 395] described “their” *G. schirazensis* as having the lowest shell as compared with all other species of *Galba*, with short and lowered spire. The shape of the shell is almost globose (Fig. 6 A).

This trait distinguishes *G. schirazensis* sensu Kruglov et Starobogatov from *G. truncatula*, which has relatively tall-spined and slender shell. Bargues *et al.* [2011] describe “their” *G. schirazensis* as a snail hardly distinguishable conchologically from *G. truncatula*, as a ‘cryptic species by evolutionary convergence, probably as a consequence of adaptation to a similar amphibious way of life and similar habitats’ [Bargues *et al.*, 2011, p. 20]. Pointier and his coauthors [Pointier, 2015] hold the same opinion. The reproductive systems of *G. truncatula* and *G. schirazensis* sensu Bargues *et al.* [2011] are ‘very similar’ and anatomical traits supposed to distinguish between the two species are ‘thus of poor taxonomical value’ [Pointier, 2015, pp. 116–117]. The original image of *L. schirazensis* shell provided by Küster

[1863] little helps to resolve this question, since conchologically the type shell looks as intermediate between the globose shell of *G. truncatula* sensu Kruglov et Starobogatov and the turriculate shell of *G. truncatula* sensu Bargues *et al.* (see Fig. 6). Irrespectively of the true identity of Küster’s materials (which are probably lost), we may report here the first identification of *G. schirazensis* sensu Bargues *et al.*, 2011 in Azerbaijan. Possibly, this snail can be found in Dagestan and other areas of the South Russia.

Acknowledgement

The study was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation for Russian Museum of Biodiversity Hotspots N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences “Biodiversity, phylogeography and phylogeny of invertebrates in the Arctic and adjacent territories” (project No. FUUW 2025-0005) (to O.V.A., I.S.K., O.V.T., and A.V.Kr.). Molecular genetic analysis of snails was supported by the Russian Science Foundation (project No. 21-74-10155) (to O.V.A. and I.S.K.).

We thank the reviewers and the editor for their constructive comments that significantly improved the original version of the manuscript.

Conflict of interests

The authors have no conflicts of interest to declare.

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