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Live samples of the mussels *Sinanodonta* aff. *woodiana* (Lea, 1834) settled in the river, Qala-I-Zal, Afghanistan, in 2020.

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DNA barcoding revealed the presence of the invasive freshwater mussel *Sinanodonta* aff. *woodiana* (Lea, 1834) in Afghanistan

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ABSTRACT

Chinese pond mussels are known as a successful invasive species, spreading rapidly almost around the world. Here, we report the first record of *Sinanodonta* aff. *woodiana* (Lea, 1834) in Afghanistan. The species was observed in the Amu Darya River in Qala-I-Zal, Kunduz Province. DNA barcoding revealed that the Afghan population belongs to the temperate invasive lineage that is widespread in Europe. Consistent with our phylogenetic data, the haplotype network showed that the alien population in Afghanistan shared the same COI haplotype as non-indigenous populations from Kazakhstan, Siberia, European Russia, Myanmar and Europe, suggesting a common origin from a single source population in China. *Sinanodonta* aff. *woodiana* seems to have been introduced into Afghanistan via waterways from the Middle Asia and/or fish hosts. The presence of individuals between one to eight years old indicates that *S.* aff. *woodiana* can survive and breed in the environmental conditions of the Amu Darya River in Afghanistan. Further spread and colonisation of *S.* aff. *woodiana* in the Amu Darya is not unexpected due to of some human-mediated dispersal events and waterways in the region. Some identified invasive populations of *Sinanodonta* sp. based on the available databases are under discussion, as there are at least eight lineages within *Sinanodonta* complex, each could indicate a separate cryptic species. Further work should be addressed to evaluate the taxonomy of this morphologically variable group.

KEY WORDS
Sinanodonta aff. *woodiana*, Afghanistan, Amu Darya, COI haplotype, invasive species, new record.

RÉSUMÉ
La présence de la moule d'eau douce envahissante Sinanodonta *aff.* woodiana (*Lea, 1834) en Afghanistan révélée par le barcoding.*
Les anodontes chinoises sont connues comme des espèces envahissantes qui se propagent rapidement presque partout dans le monde. Ici, nous rapportons la première signalisation de *Sinanodonta* aff. *woodiana* (Lea, 1834) en Afghanistan. L'espèce a été observée dans la rivière Amu Darya à Qala-I-Zal, province de Kunduz. Selon les données de barcoding, la population afghane appartient à la lignée invasive tempérée qui est largement répandue en Europe. Conformément à nos données phylogénétiques, le réseau d'haplotypes montre que la population exotique en Afghanistan partage le même haplotype COI que celui des populations non indigènes du Kazakhstan, de Sibérie, de Russie européenne, du Myanmar et d'Europe, suggérant une origine commune à partir d'une population source unique en Chine. *Sinanodonta* aff. *woodiana* semble avoir été introduite en Afghanistan par des voies navigables depuis l'Asie centrale et/ou des poissons hôtes. La présence d'individus âgés de un à huit ans indique que *S.* aff. *woodiana* peut survivre et se reproduire dans les conditions environnementales du fleuve Amu Darya en Afghanistan. On peut s'attendre à ce que *S.* aff. *woodiana* continue à se propager et à coloniser d'autres territoires par des dispersions d'origine humaine et le long des voies navigables de la région. Certaines populations de *Sinanodonta* sp. reconnues envahissantes à partir des bases de données disponibles sont en cours de discussion, car il existe au moins huit lignées au sein du complexe *Sinanodonta*, chacune pouvant correspondre à une espèce cryptique distincte. D'autres travaux devraient être entrepris pour évaluer la taxonomie de ce groupe morphologiquement variable.

MOTS CLÉS
Sinanodonta aff. *woodiana*, Afghanistan, Amu Darya, haplotype COI, espèces envahissantes, signalisation nouvelle.

INTRODUCTION

Freshwater bivalves are considered as one of the most threatened groups in animals worldwide (Lydeard *et al.* 2004), but also comprise many detrimental exotic species (Karatayevy *et al.* 2007), so that some of them are known to be the main invaders of freshwater habitats (Sousa *et al.* 2008; Lajtner & Crnčan 2011; Karatayev *et al.* 2015). The freshwater mussel *Sinanodonta woodiana* (Lea, 1834), known as the Chinese pond mussel, is regarded as one of those invasive species which is widely introduced almost around the world (Bogan *et al.* 2011a). It is a large-sized benthic filter-feeding species of the family Unionidae Rafinesque, 1820, which is resistant to pollution (Li *et al.* 2015; Douda & Cadkova 2017). *Sinanodonta woodiana* is a habitat generalist, and extremely modified and artificial ecosystems with high siltation are known to be appropriate for its populations (Paunovic *et al.* 2006). The species prefers silty and clay sediments, turbid and relatively warm (30-33°C) waters and is usually found in slow and standing-water ecosystems (Soroka 2005; Zettler & Jueg 2006). As a hyper-successful invader (Sousa *et al.* 2014), *S. woodiana* may have adverse effect on indigenous mussels via competition for space, host and food. Its populations can alter physical and biological properties of freshwater habitats (Douda *et al.* 2012; Guarneri *et al.* 2014). The species glochidia may also adversely impact the growth and physiological conditions of infected fishes (Douda *et al.* 2017).

Molecular studies have indicated that *S. woodiana* is rather a species complex that includes several divergent mitochondrial DNA lineages (Bolotov *et al.* 2016; Kondakov *et al.* 2018). Its native range is believed to be the river basins of Amur and Yangtze in China and Easten Russia (Watters 1997; Graf 2007; Kondakov *et al.* 2018; Zieritz *et al.* 2018), Korea (Graf 2007), Kampuchea (doubtful; Bogan *et al.* 2011b), Thailand (refuted by Brandt (1974)), Taiwan, Japan and Hong Kong (Popa *et al.* 2007). However, two species-level lineages of the complex (tropical and temperate) are considered as successful invaders, sharing thoroughly allopatric non-native areas (Bolotov *et al.* 2016).

To date, *S. woodiana* has been reported in many parts of Europe including Hungary (Petro 1984; Sárkány-Kiss 1986), Romania (Sárkány-Kiss 1986; Popa *et al.* 2007), France (Girardi & Ledoux 1989; Mouthon 2008), Slovakia (Kosel 1995), Czech Republic (Beran 1997; Kubín 2013), Austria (Reischutz 1998), Poland (Bohme 1998; Spyra *et al.* 2016), Italy (Manganelli *et al.* 1998; Cappelletti *et al.* 2009; Kamburska *et al.* 2013; Cilenti *et al.* 2019), Ukraine (Yurishinets & Korniushin 2001), Serbia (Paunovic *et al.* 2005), Germany (Gloer & Zeittler 2005), Bulgaria (Hubenov 2006), Sweden (Von Proschwitz 2008), Moldova (Munjiu & Shubernetski 2008), Belgium (Packet *et al.* 2009), Spain (Pou-Rovira *et al.* 2009), Croatia (Lajtner & Crnčan 2011; Beran 2020), Montenegro (Tomović *et al.* 2013), European Russia (Kondakov *et al.* 2020a) and Greece (Karaouzas *et al.* 2022). The species has also been observed in North and Central America and in the West Indies, including the Dominican Republic, Costa Rica (Watters 1997), probably Panama (Watters 1999), the United States (Bogan *et al.* 2011b), Guatemala (Watters & Coltro 2014), as well as some areas in Asia outside the native range, including Kazakhstan (Uvaliyeva 2001; Kondakov *et al.* 2020b), probably Turkey (Ercan *et al.* 2012), Myanmar (Vikhrev *et al.* 2017), the Philippines, Singapore (Watters 1998, 1999), Indonesia (Djajasasmita 1982), Uzbekistan (Kondakov *et al.* 2018), Malaysia (Bogan & Schilthuizen

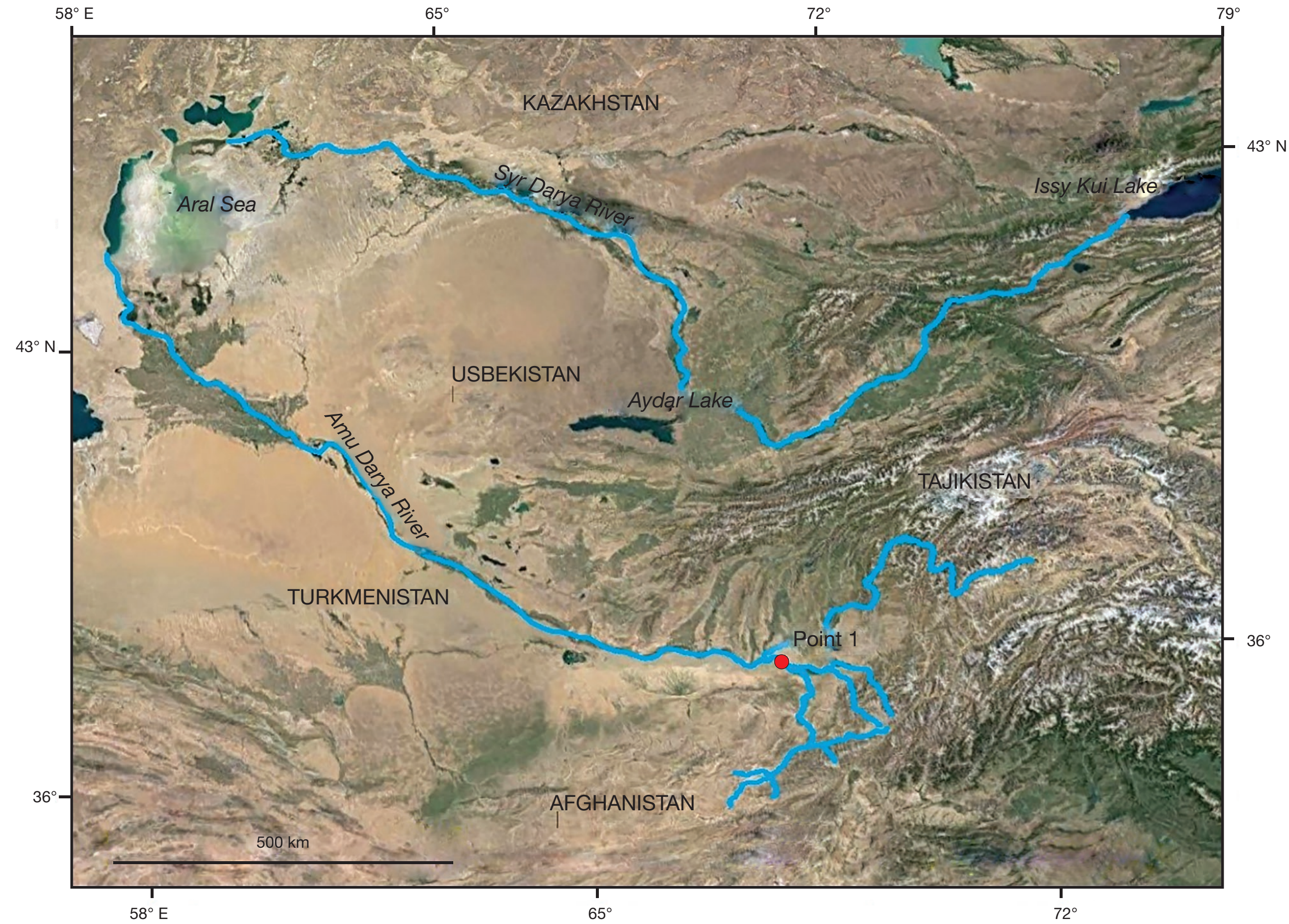

Fig. 1. — Map illustrating Amu Darya River position; Point 1 (**red circle**) indicates sampling site of *Sinanodonta* aff. *woodiana* (Lea, 1834) in Amu Darya River in Kunduz Province, Afghanistan.

2004; Zieritz *et al.* 2016), Iraq (Bogan *et al.* 2021) and also Eastern and Western Siberia (Bespalaya *et al.* 2018; Kondakov *et al.* 2020a). The invasive populations in Europe, Siberia, Uzbekistan and Kazakhstan are considered to belong to the temperate lineage (Bespalaya *et al.* 2018; Kondakov *et al.* 2018, 2020a, b) while the populations invaded to the Southeast Asia and also Costa Rica belong to the tropical one (Bolotov *et al.* 2016; Zieritz *et al.* 2018; Bauer *et al.* 2021), possibly introduced through host fish from Taiwan or southern areas of continental China (Watters 1997; Djajasasmita 1982). A non-indigenous population of the temperate lineage has been also discovered in Myanmar, Irrawaddy River basin (Vikhrev *et al.* 2017). However, there are only limited molecular sequences available on online databases and the phylogenetic affinity of many other indigenous and non-indigenous populations of the *S. woodiana* complex is still unknown (Bolotov *et al.* 2016; Kondakov *et al.* 2018).

In the early summer 2020, during our project on Unionidae mussels in the Middle East and Afghanistan, we received a report from an Afghan colleague in which local people of the village Safikot in Kunduz province declared that there were some large mussels in the river. Based on the photos received, our team arranged a field trip with the help of Afghanistan colleagues to this region in August 2020 and finally collected samples from Amu Darya River. Here we report the first record of the alien freshwater mussel *Sinanodonta* aff. *woodiana* in Afghanistan and provide the first molecular and morphological data on the Afghan population of this invasive species.

MATERIAL AND METHODS

Study area and sampling

The study location is Amu Darya River, near Safikot, Qala-I-zal, Kunduz Province, Afghanistan (36°57'34.97"N, 68°33'3.47"E) (Fig. 1). With a length of 2400 km, the Amu Darya is a major river in middle Asia and Afghanistan. This river forms part of Afghanistan northern border with Uzbekistan, Tajikistan and Turkmenistan. The fieldwork was carried out on 10 August 2020. The mussel specimens were collected by hand from the river. A small piece of foot was dissected from each mussel for molecular studies. The pieces of tissue were placed in ethanol (96%). Mussel shells were also collected to study morphological traits. The specimens were deposited at Aquatic laboratory of the Fisheries and Environment Faculty, Gorgan University of Agricultural Sciences and Natural Resources.

Morphological analysis

Biometric variables of mussel shells (N = 28), including length (L), height (H) and width (W), were measured to the nearest 0.1 mm using an AACO caliper. Morphometric indices, i.e., shell elongation (EI = H/L ratio × 100) and convexity (CI = W/L ratio × 100) were also measured. The age of each specimen was determined by counting the annual growth rings that were obviously visible on the shells. We also classified our samples into four classes according to length (Afanasjev *et al.* 2001; Spyra *et al.* 2012) as follows: L less than 5 cm (very small), 5-10 cm (small), 10-15 cm (medium) and more than 15 cm (large).

DNA extraction, PCR and sequencing

DNA was extracted from each alcohol-preserved tissue (N = 6) using a standard high-salt method (Sambrook *et al.* 1989) slightly modified. DNA quality and concentration were assessed by agarose gel electrophoresis (1%) and a Biophotometer Spectrophotometer (Eppendorf, Germany).

The rimers used to amplify the cytochrome c oxidase subunit I (COI) partial sequences were LCO22me2 (5'-GGTCAACAAAYCATAARGATATTGG-3') and HCO700dy2 (5'-TCAGGGTGACCAAAAAAYCA-3') (Walker *et al.* 2006, 2007). DNA fragments were amplified in a 25 µL reaction mixture containing 1 µL DNA (20-160 ng/µL), 15 µL Taq 2× Master Mix Red-$MgCl_2$: 1.5 mM (Amplicon), 1 µL of each primers and 7 µL ddH_2O. The PCR program included 4 min at 94°C, 40 cycles at 94°C (30 s), 50°C (40 s) and 72°C (60 s), followed by 10 min at 72°C. Products were checked by agarose gel (1.5%) electrophoresis in TBE buffer (1X). Finally, the amplicons were sequenced using an ABI 3730XL automatic sequencer (Applied Biosystems, 3730/3730xl DNA Analyzers Sequencing, Bioneer, Korea) with the same primers.

Molecular analysis

The sequences obtained were studied using a sequence alignment editor in BioEdit 7.2.5 (Hall 1999). We extracted 185 COI sequences of *Sinanodonta* sp. and related taxa from the NCBI GenBank (Table 1). Multiple sequence alignment using ClustalW was implemented in BioEdit. Sequences were trimmed and a 616-bp fragment was left. Identical sequences were deleted using the online tool FaBox 1.41 (Villesen 2007). Phylogenetic tree was finally constructed using 37 unique sequences. Two taxa of *Margaritifera dahurica* (Middendorff, 1850) (KJ161516) and *M. laosensis* (I. Lea, 1863) (JX497731) were also applied as outgroups.

We reconstructed the phylogenetic relationships among the taxa studied on the basis of Bayesian inference by MrBayes v3.2.2 (Huelsenbeck & Ronquist 2001). The best-fitting nucleotide substitution models based on Akaike information criterion (Akaike 1973) were assessed by MrModelTest v3.7 (Posada & Crandall 1998) in PAUP v4.0 (Swofford 2003). Two parallel runs were conducted independently. Each included one cold and three heated Metropolis coupled MCMC chains. The program was run for 10 million generations and sampled once every 10 000 generations with a 20% burn-in fraction. The phylogenetic tree was finally visualized using FigTree v1.4.2 (Rambaut 2008). We reconstructed the phylogenetic tree using the TIM + I + G model (Fig. 3)

Genetic divergence on the basis of *P*-distance was determined through MEGA 6.0 (Tamura *et al.* 2013). A median joining network was also constructed through PopArt v1.7 (Leigh & Bryant 2015) to study relationships among haplotypes.

RESULTS

Superfamily Unionoidea Rafinesque, 1820
Family Unionidae Rafinesque, 1820
Genus *Sinanodonta* Modell, 1945

Sinanodonta woodiana (Lea, 1834)
(Fig. 2)

Symphynota woodiana Lea, 1834: 42, pl. 5, fig. 12.

Sinanodonta woodiana – Graf & Cummings 2007: 305.

Common name. — Chinese pond mussel.

First record of the invasive chinese pond mussel in Afghanistan

A well-established population of *Sinanodonta* was detected in Amu Darya River (ADR) in Afghanistan. No other Unionidae species were found in the sampling site. The *Sinanodonta* settlement was confined to muddy-sandy substrates. The depth at the sampling area was about 60 cm. At the site with bivalve bed, the water flow was so low and there was a dense covering of macrophytes. The water temperature was 27°C on the sampling day.

Morphological traits

The valves (SING) pattern (pl) of the specimens were slightly elongated with brown/olive greenish periostracum (Fig. 2). The youngest and oldest specimens were one and eight years old respectively, with a mean age of 4.7 years. Morphometric traits of the specimens collected from the Amu Darya River in Afghanistan are given in Table 2. All size classes were observed in the collected samples except the large one (Fig. 2b). Most of the individuals were placed in small size class (N = 21) while the remaining ones (N = 7) were of medium and very small size (Table 2). The length, width and height of the shell of collected samples varied respectively from 33.82 to 127.40 mm, 8.91 to 43.01 mm, and 23.42 to 71.63 mm. The convexity and elongation indices of the specimens also ranged from 26.34 to 39.11 and from 55.79 to 69.24 mm, respectively.

Molecular data

DNA barcoding confirmed the invasion of the freshwater bivalve *S. woodiana* into Afghanistan. Six 671-bp long fragments of Cytochrome oxidase subunit I were obtained from the *S. woodiana* individuals and deposited in the NCBI GenBank (Table 1). BI analysis of COI confirmed the presence of the alien species *S. woodiana* in Afghanistan. Our specimens

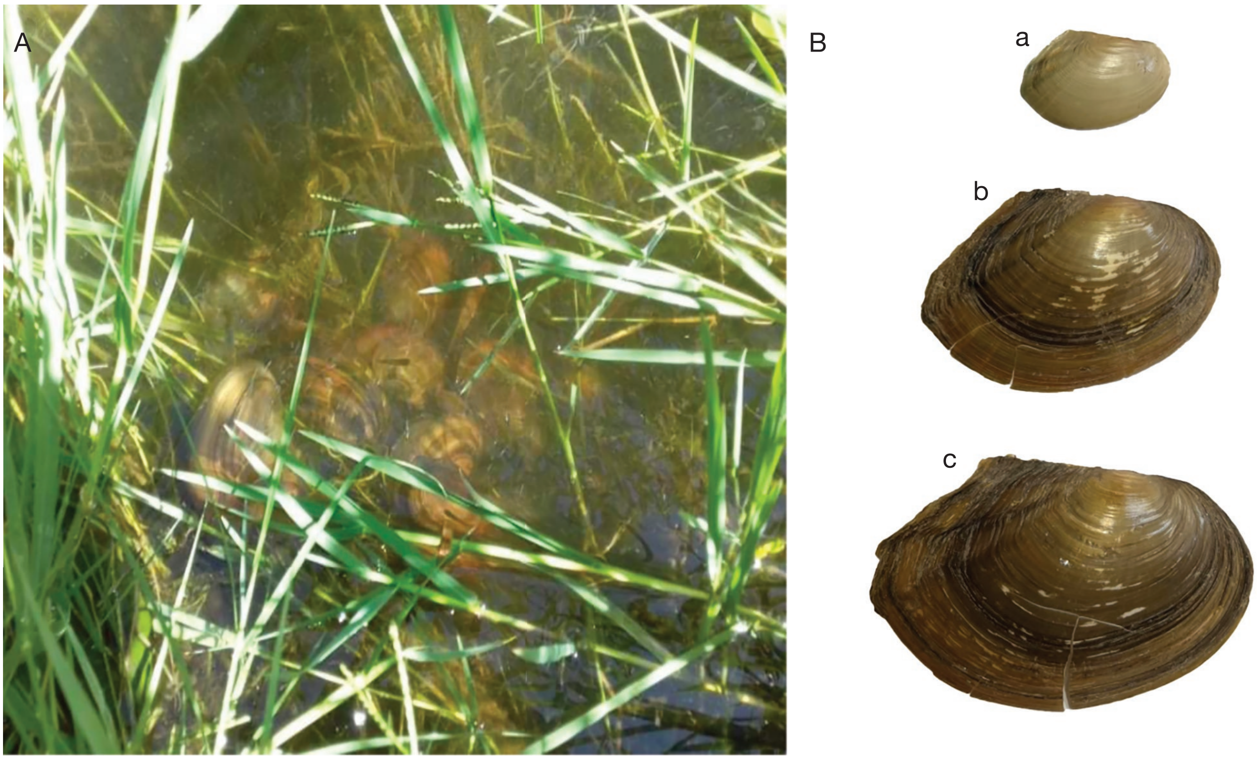

FIG. 2. — Non-indigenous *Sinanodonta* aff. *woodiana* (Lea, 1834) in Afghanistan: **A**, live samples of the mussels settled in the river, Qala-I-Zal (10 August 2020); **B**, shell exterior view of the mussels: **a**, **b** and **c** represent very small, small and medium size classes, respectively.

TABLE 1. — COI sequences used for molecular studies in the present study

Taxon	Region	Population	Lineage/Hap. Code	Acc. no.	Reference
S. aff. *woodiana* (Lea, 1834)	Afghanistan	Non-indigenous	E/E1	OP279027	This study
S. aff. *woodiana*	Afghanistan	Non-indigenous	E/E1	OP279028	This study
S. aff. *woodiana*	Afghanistan	Non-indigenous	E/E1	OP279029	This study
S. aff. *woodiana*	Afghanistan	Non-indigenous	E/E1	OP279030	This study
S. aff. *woodiana*	Afghanistan	Non-indigenous	E/E1	OP279031	This study
S. aff. *woodiana*	Afghanistan	Non-indigenous	E/E1	OP279032	This study
S. aff. *woodiana*	Germany	Non-indigenous	E/E1	OU070149	GenBank
S. cf. *woodiana*	Iraq	Non-indigenous	A/A6	LC656037	Nuah and Mukhlif (GenBank)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424967	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A1	KX424968	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424969	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424970	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424976	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424977	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A2	KX424978	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A2	KX424979	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A2	KX424971	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A2	KX424972	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A2	KX424973	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424974	Fornillos *et al.* (2020)
S. cf. *woodiana*	Philippines	Non-indigenous	A/A3	KX424975	Fornillos *et al.* (2020)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013177	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013178	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013179	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013180	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013181	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013183	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013182	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013185	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013186	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013190	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013191	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013192	Kondakov *et al.* (2020a)

Table 1. — Continuation.

Taxon	Region	Population	Lineage/Hap. Code	Acc. no.	Reference
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013193	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013196	Kondakov *et al.* (2020a)
S. aff. *woodiana*	European Russia	Non-indigenous	E/E1	MT013197	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013156	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013162	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013163	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013167	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013168	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013172	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013173	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Western Siberia	Non-indigenous	E/E1	MT013175	Kondakov *et al.* (2020a)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809929	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809930	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809931	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809932	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809933	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809934	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809935	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809936	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809937	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Kazakhstan	Non-indigenous	E/E1	MN809938	Kondakov *et al.* (2020b)
S. aff. *woodiana*	Eastern Siberia	Non-indigenous	E/E1	KY978735	Bespalaya *et al.* (2018)
S. aff. *woodiana*	Eastern Siberia	Non-indigenous	E/E1	KY978736	Bespalaya *et al.* (2018)
S. aff. *woodiana*	Eastern Siberia	Non-indigenous	E/E1	KY978738	Bespalaya *et al.* (2018)
S. aff. *woodiana*	Uzbekistan	Non-indigenous	E/E5	MG581711	Kondakov *et al.* (2018)
S. aff. *woodiana*	Uzbekistan	Non-indigenous	E/E5	MG581712	Kondakov *et al.* (2018)
S. cf. *woodiana*	Germany	Non-indigenous	A/A3	MH319868	Stelbrink *et al.* (2019)
S. aff. *woodiana*	Myanmar	Non-indigenous	E/E1	MF497807	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Myanmar	Non-indigenous	E/E1	MF497808	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Myanmar	Non-indigenous	E/E1	MF497809	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	KF731775	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	KF731776	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	KF731777	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414328	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414329	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414330	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414331	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414332	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414333	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414334	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414335	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414336	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414337	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E2	MF414338	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414339	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414340	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414341	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414342	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414343	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414344	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414345	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414346	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414347	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414348	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414349	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414350	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414351	Froufe *et al.* (2017)
S. aff. *woodiana*	Italy	Non-indigenous	E/E1	MF414352	Froufe *et al.* (2017)
S. aff. *woodiana*	Ukraine	Non-indigenous	E/E1	JQ253893	GenBank
S. aff. *woodiana*	Ukraine	Non-indigenous	E/E1	JQ253894	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	AF468683	Soroka (2005)
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	EF440349	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	HQ283344	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	HQ283345	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	HQ283346	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	HQ283347	GenBank
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	HQ283348	GenBank
S. aff. *woodiana*	Hungary	Non-indigenous	E/E1	KJ125079	Soroka *et al.* (2014)
S. aff. *woodiana*	Poland	Non-indigenous	E/E1	KJ125078	Soroka *et al.* (2014)
S. aff. *woodiana*	China	Indigenous	E/E1	KJ434482	GenBank

Table 1. — Continuation.

Taxon	Region	Population	Lineage/Hap. Code	Acc. no.	Reference
S. aff. *woodiana*	China	Indigenous	E/E7	KJ434483	GenBank
S. aff. *woodiana*	China	Indigenous	E/E1	KJ434484	GenBank
S. aff. *woodiana*	China	Indigenous	E/E1	KJ434485	GenBank
S. aff. *woodiana*	China	Indigenous	E/E9	KJ434486	GenBank
S. aff. *woodiana*	China	Indigenous	B/B1	KJ434487	GenBank
S. aff. *woodiana*	China	Indigenous	D/D1	KJ434489	GenBank
S. aff. *woodiana*	China	Indigenous	D/D1	KJ434490	GenBank
S. aff. *woodiana*	China	Indigenous	D/D1	KJ434488	GenBank
S. aff. *woodiana*	China	Indigenous	E/E1	MG463060	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E6	MG463068	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E7	MG463069	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E1	MG463070	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E4	MG463073	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E9	MG463075	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E8	MG463076	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E1	MG463078	Huang *et al.* (2019)
S. aff. *woodiana*	China	Indigenous	E/E3	MG463080	Huang *et al.* (2019)
S. aff. *woodiana*	Vietnam	Indigenous	H/H1	KY561635	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Vietnam	Indigenous	H/H3	KY978744	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Vietnam	Indigenous	H/H2	KY978745	Vikhrev *et al.* (2017)
S. aff. *woodiana*	Vietnam	Indigenous	H/H3	KX822668	Lopes-Lima *et al.* (2017)
S. cf. *woodiana*	Indonesia	Indigenous	A/A3	KU891641	Bolotov *et al.* (2016)
S. cf. *woodiana*	Indonesia	Indigenous	A/A3	KU891642	Bolotov *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A4	KX051328	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A3	KX051326	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A5	KX051325	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A8	KX051324	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A7	KX051323	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A7	KX051322	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A3	KX051321	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A3	KX051320	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A7	KX051319	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A3	KX051318	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A7	KX051317	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A8	KX051316	Zieritz *et al.* (2016)
S. cf. *woodiana*	Malaysia	Non-indigenous	A/A9	KX051315	Zieritz *et al.* (2016)
S. lauta (E. von Martens, 1877)	Japan	Indigenous	C/C5	AB055627	GenBank
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809939	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809940	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809941	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809942	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809943	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809944	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809945	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809946	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809947	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809948	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809949	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809950	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809951	Kondakov *et al.* (2020b)
S. lauta	Kazakhstan	Non-indigenous	C/C2	MN809952	Kondakov *et al.* (2020b)
S. lauta	European Russia	Non-indigenous	C/C2	MT013184	Kondakov *et al.* (2020a)
S. lauta	European Russia	Non-indigenous	C/C2	MT013187	Kondakov *et al.* (2020a)
S. lauta	European Russia	Non-indigenous	C/C2	MT013188	Kondakov *et al.* (2020a)
S. lauta	European Russia	Non-indigenous	C/C2	MT013189	Kondakov *et al.* (2020a)
S. lauta	European Russia	Non-indigenous	C/C2	MT013194	Kondakov *et al.* (2020a)
S. lauta	European Russia	Non-indigenous	C/C2	MT013195	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013157	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013158	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013160	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013161	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013164	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013165	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013176	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013166	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013169	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013170	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013171	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013174	Kondakov *et al.* (2020a)
S. lauta	Western Siberia	Non-indigenous	C/C2	MT013159	Kondakov *et al.* (2020a)

Table 1. — Continuation.

Taxon	Region	Population	Lineage/Hap. Code	Acc. no.	Reference
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY561633	Bespalaya *et al.* (2018)
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY978739	Bespalaya *et al.* (2018)
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY978740	Bespalaya *et al.* (2018)
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY978741	Bespalaya *et al.* (2018)
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY978737	Bespalaya *et al.* (2018)
S. lauta	Eastern Siberia	Non-indigenous	C/C2	KY978741	Bespalaya *et al.* (2018)
S. lauta	Russian Far East	Indigenous	C/C4	KY978743	Bespalaya *et al.* (2018)
S. lauta	Russian Far East	Indigenous	C/C4	KY978742	Bespalaya *et al.* (2018)
S. lauta	South Korea	Indigenous	C/C1	GQ451869	GenBank
S. lauta	South Korea	Indigenous	C/C3	GQ451870	GenBank
S. schrenkii (I. Lea, 1870)	Russia	Indigenous	G/G1	KU853266	Sayenko *et al.* (2017)
S. schrenkii	Russia	Indigenous	G/G2	KU853267	Sayenko *et al.* (2017)
S. schrenkii	Russia	Indigenous	G/G2	KU853268	Sayenko *et al.* (2017)
S. schrenkii	Russia	Indigenous	G/G1	KU853269	Sayenko *et al.* (2017)
S. lucida (Heude, 1878)	China	Indigenous	F/F1	KX822667	Lopes-Lima *et al.* (GenBank)
Anemina arcaeformis (Heude, 1877)	China	Indigenous	-/I3	KJ434481	Uyang *et al.* (GenBank)
A. arcaeformis	China	Indigenous	-/I1	KJ434479	Uyang *et al.* (GenBank)
A. arcaeformis	China	Indigenous	-/I2	KJ434480	Uyang *et al.* (GenBank)

Table 2. — Morphometric features of *Sinanodonta* aff. *woodiana* (Lea, 1834) from Afghanistan. Abbreviation: **SD**, standard deviation.

Size class	No. of individuals		Length	Height	width	Elongation index	Convexity index
Very small	3	Min-Max	33.82-45.43	23.42-26.18	8.91-12.44	57.62-69.24	26.34-27.38
		Mean ± SD	40.39 ± 14.9	24.96 ± 11.6	10.95 ± 8.2	62.38 ± 14.5	26.77 ± 12.6
Small	21	Min-Max	69.44-96.14	41.06-62.28	20.66-37.43	58.02-67.31	28.64-39.11
		Mean ± SD	83.60 ± 17.4	53.75 ± 12.8	28.32 ± 11.9	61.22 ± 15.6	33.72 ± 13.8
Medium	4	Min-Max	106.11-127.40	69.12-71.63	38.44-43.01	55.79-65.13	33.36-36.22
		Mean ± SD	118.22 ± 21.8	70.46 ± 15.2	41.21 ± 14.7	59.81 ± 14.8	34.91 ± 13.2

Table 3. — Genetic divergences (mean uncorrected *P*-distance %) among the *Sinanodonta* sp. lineages. Symbol: *****, the lineage that includes Afghan samples.

	Lineage A	Lineage B	Lineage C	Lineage D	Lineage E*	Lineage F	Lineage G
Lineage B	2.1	–	–	–	–	–	–
Lineage C	3.7	2.8	–	–	–	–	–
Lineage D	5.4	3.7	4.9	–	–	–	–
Lineage E	5.7	5.3	4.9	4.9	–	–	–
Lineage F	13.9	12.9	13.5	12.9	12.5	–	–
Lineage G	9.8	10.2	10.6	9.5	8.2	12.7	–
Lineage H	6	4.9	4.8	4.0	4.2	12.9	8.5

exhibited the same haplotype as previously recorded for non-indigenous individuals in Kazakhstan, Eastern and Western Siberia, European Russia, Myanmar, Hungary, Italy, Poland, Germany and Ukraine (Hap E1). This haplotype was placed in the same clade along with eight other haplotypes from Uzbekistan, Italy and China with strong bootstrap support, applying to be the temperate invasive lineage (Lineage E; *Sinanodonta* aff. *woodiana*) (Fig. 3).

Based on our molecular data, there are at least eight mitochondrial lineages within the *Sinanodonta* complex (Fig. 3). The mean COI *P*-distances among the mitochondrial lineages in the *Sinanodonta* complex are given in Table 3. The highest mean *P*-distance was observed among the lineages F and A (13.9%), while the lowest value was observed among the lineages A and B (2.1%). The distance between the lineage E comprising our specimens and other lineages ranged between 4.2 and 12.5%. This value was also 5.7% between the temperate and tropical invasive lineages. The mean distance within the temperate lineage comprising nine unique haplotypes was also 0.3.%.

The median joining network was constructed based on 99 COI sequences of *S.* aff. *woodiana* within the temperate invasive lineage (Lineage E) (Fig. 4). Consistent with our phylogenetic data, in the haplotype network, our specimens and those from China (KJ434482, KJ434484 and KJ434485), and non-indigenous individuals from Kazakhstan, Eastern and Western Siberia, European Russia, Myanmar, Hungary, Italy, Poland, Germany (OU070149) and Ukraine were lumped together into a same haplotype. This haplotype also weakly separated from non-indigenous individuals of Uzbekistan and Italy (MF414338) by only one substitution.

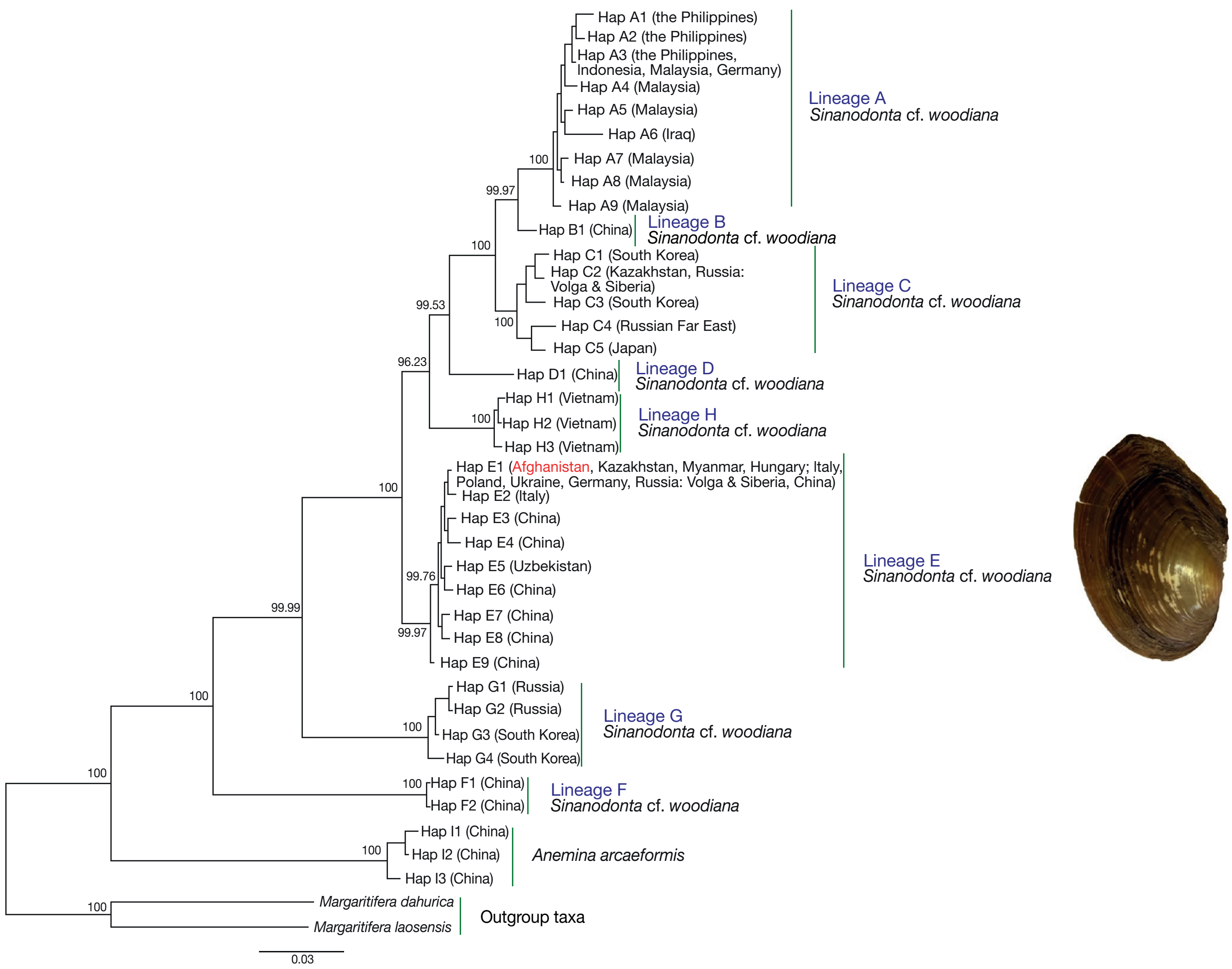

FIG. 3. — The Bayesian phylogenetic tree on the basis of 37 unique COI sequences of *Sinanodonta* sp. and related taxa, including one new sequence from Afghanistan (**red**) and 36 sequences from GenBank. *Margaritifera laosensis* (I. Lea, 1863) and *M. dahurica* (Middendorff, 1850) are the outgroups. The numbers above branches show the bootstrap support value. The scale bar represent the branch lengths.

DISCUSSION

Our study confirms the invasion of the freshwater bivalve *Sinanodonta woodiana* to South Asia, Afghanistan. No species of the genus *Sinanodonta* has yet been reported from any other region of Southern of Asia. However, *S. woodiana* has previously been reported from Middle Asia including Uzbekistan (Kondakov *et al.* 2018) and Kazakhstan (Kondakov *et al.* 2020b) as well as the Middle East region including Iraq (Bogan *et al.* 2021) and probably Turkey (Ercan *et al.* 2012).

According to our phylogenetic analysis, the invasive population found in Afghanistan belongs to the temperate invasive lineage (Lineage E, *Sinanodonta* aff. *woodiana*). The primary source of the temperate lineage which is now widespread in Europe, is debatable. However, it has been suggested that this lineage probably originated from a founder event and the introduction of host fish from the Yangtze Basin (Watters 1997; Bolotov *et al.* 2016; Bespalaya *et al.* 2018; Kondakov *et al.* 2018). Consistent with our phylogenetic data, the haplotype network also revealed that the alien population found in Afghanistan shared similar COI haplotype as that previously reported for non-indigenous populations from Kazakhstan, Siberia, European Russia, Myanmar and Europe suggesting the common origin from a single source population in China (Kondakov *et al.* 2018, 2020b; Huang *et al.* 2019).

Fish host carrying the glochidia is regarded as the primary vector for *Sinanodonta* sp. introduction into non-native regions (Watters 1997; Spyra *et al.* 2016; Bespalaya *et al.* 2018; Kondakov *et al.* 2020b). In Afghanistan, Asian carps including *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Ctenopharyngodon idella* (Valenciennes, 1844), *Hypophthalmichthys nobilis* (Richardson, 1845) and *Cyprinus carpio* Linnaeus, 1758 have been repeatedly introduced to some parts including Qala-I-Zal for aquaculture activities. Asian carps have been recognized to serve as hosts for glochidia (Von Proschwitz 2008; Tomović *et al.* 2013). These fish species introduction to the river may become possible via migrating from hatchery and fish farms. Currently, we have no information about the fish fauna present

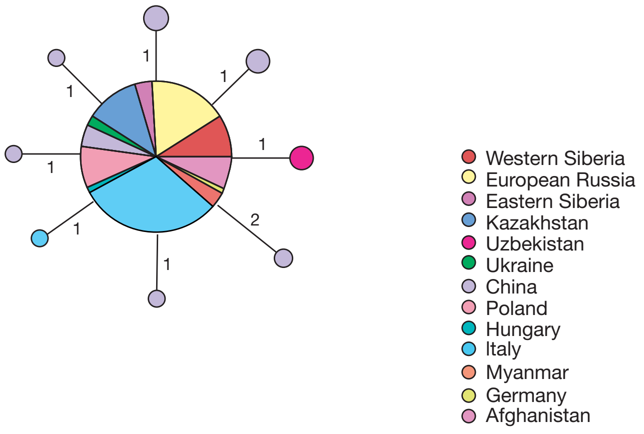

FIG. 4. — Median joining network for COI sequences of *Sinanodonta* aff. *woodiana* (N = 100; Lineage E (Table 1)). The numbers near branches show the number of mutation sites.

in Amu Darya River in Afghanistan but the introduction of *S.* aff. *woodiana* to Afghanistan may be closely related to the introduction of Asian carps as glochidia hosts. Besides, viable population of *S. woodiana* has been previously reported from Uzbekistan (Syr Darya, Zarafshan and Amu Darya Rivers). This species was observed in 1960-1965 in Uzbekistan with *H. molitrix* and *C. idella* imported from China (Kondakov *et al.* 2018). Unfortunately, there are no molecular data on *S. woodiana* population in Syr Darya but taking into account the waterways between Uzbekistan and Afghanistan (Fig. 1), we can also propose a scenario in which the invasive population in Afghanistan may originate from Middle Asia, where *S. woodiana* sharing a unique COI haplotype was found in Amu Darya in Uzbekistan (Kondakov *et al.* 2018). In this regard, during Autumn, flood sometimes occur in Amu Darya from Uzbekistan to Afghanistan which may cause the movement of mussels in the river. In addition, we recently received photos attesting that the mussel is also present in two other parts of Amu Darya in Kunduz Province. As this river also forms part of Afghanistan northern border with Tajikistan and Turkmenistan, the mussel may be present in these countries. However, no information is yet available on the presence of this invasive mussel in Tajikistan and Turkmenistan. The other possible means of introducing invertebrates including *Sinanodonta* sp. into non-native regions is considered to be the aquarium trade (Von Proschwitz 2008; Duggan 2010), but as this trade is not common in Afghanistan, this explanation for *S. woodiana* invasion is not considered here.

The individuals we collected at Amu Daryan were between one and eight years old. The presence of eight-year-old individuals means that the invasive settlement lives for at least seven to eight years, suggesting a rather recent introduction in 2012. However, due to the lack of information available in Afghanistan and the fact that area of initial invasion is unknown, it is difficult to determine the exact date and source of the introduction of *S. woodiana* into this country. We, therefore, can only propose two scenarios in which *S. woodiana* was introduced to Afghanistan at the glochidia stage with Asian carps imported into the country and/or was introduced from neighbouring countries via the Amu Darya River. However, it is possible that *S. woodiana* has arrived via some intermediate stations and if so, more freshwater areas may be invaded by this species.

Water temperature is suggested to be a main parameter playing a pivotal role in *Sinanodonta* sp. growth and survival (Kraszewski & Zdanowski 2001; Kraszewski 2007; Liu *et al.* 2014). It is stated that temperature may affect gametogenesis, incubation and glochidia development of *S. woodiana* (Araujo & Ramos 2000; Galbraith & Vaughn 2009). The thermophilic character of this species was previously suggested by various lines of evidence (Kraszewski 2007; Kraszewski & Zdanowski 2007; Demayo *et al.* 2012; Spyra *et al.* 2016) but *S. woodiana* has now colonised freshwater ecosystems with low temperature (Kamburska *et al.* 2013). In fact, the species is currently known to be resistant to unfavourable situations even when temperature of water decreases below 0°C (Domagala *et al.* 2007; Lajtner & Crnčan 2011; Labęcka & Domagala 2016, this study). In this regard, Kraszewski & Zdanowski (2007) reported the highest length of 241 mm for *S. woodiana* in the warmest parts of the heated Konin lake system in Poland, while it was 125 mm in moderate temperature areas. Here, in the case of Amu Darya in Qala-I-Zal where the weather temperature sometimes exceeds 45°C in summer and drops below 0°C in winter, the maximum shell lengths of the collected specimens was 127.40 mm and no individuals were observed in the large size class (Table 2). Here, the absence of large size mussels cannot be related to the temperature: more studies on indigenous and non-indigenous populations are needed to exactly determine the role of ecological parameters including temperature on the length growth and ecological preference of this invasive species. Besides temperature, the presence of fish host and proper interaction with it is also essential for stimulating large larval release of Unionidae (Haag & Warren-Junior 2003) but as mentioned above, we currently do not have enough information on the fish fauna of the Amu Darya River in Afghanistan. However, the morphological data of our specimens (Table 2) reveals the presence of very small class individuals with the age of one and two years, suggesting successful reproduction and glochidia development of *S.* aff. *woodiana* in the sampling region. Velocity of water, substrate as well as the presence of macrophytes are also considered to affect the density and biomass of *S. woodiana* (Kraszewski & Zdanowski 2007). It was previously stated that this species prefers sandy beds with moderate water velocity (Kraszewski & Zdanowski 2007), but the studies by Paunovic *et al.* (2006) and Lajtner & Crnčan (2011) indicated that *S. woodiana* prefers the habitats with low water flow or even without it as wells as the beds dominated by muddy and salt-silt. The region where we collected the mussels had a very low water flow with dense covering of macrophytes and the bed was dominated by silt substrate. It seems that the details of the environmental preference of *S. woodiana* and the effect of habitat features on this invasive species are not yet clear and require further study in the future.

Although the density and biomass of *S. woodiana* in an invaded area like the Amu Darya River may be significantly different from its indigenous habitats, our record of one to eight-year-old individuals indicates that *S. woodiana* can be able to survive and breed in the natural environment of the Amu Darya in Afghanistan. This is in conflict with the theory that alien species are unable to spread beyond regions with variable water temperatures and therefore constitute a threat for indigenous fauna (Najberek *et al.* 2013). The resistance of *S. woodiana* to low levels of temperature and its capability to adapt to fluctuation could enhance its opportunities to colonise new freshwater ecosystems, as previously suggested by Douda *et al.* (2012). As mentioned above, we have recently found that *S. woodiana* inhabits two other areas of the Amu Darya in Afghanistan. Considering this discovery and several introductions of Asian carps into Afghanistan together with the establishment of viable populations of the species in the Amu Darya in Uzbekistan, rapid spread and colonisation of this invasive bivalve in Amu Darya as a major river in the Middle Asia and Afghanistan is not unexpected in next future. It is also possible that the non-indigenous population of *S.* aff. *woodiana* we found in Amu Darya may serve as a source for future human-mediated spread events. The possible spread of invasive *S. woodiana* may produce some adverse impacts on invaded areas. As an ecosystem engineer, *S. woodiana* can produce changes in the whole system such as aquatic composition and structure, as previously reported for *Dreissena polymorpha* (Pallas, 1771). As a wide host generalist (Douda *et al.* 2012), *S. woodiana* glochidia may adversely effect on the growth and physiological traits of host fishes (Douda *et al.* 2017). This species may also be a vector for introducing parasites and diseases (Sousa *et al.* 2014; Lopes-Lima *et al.* 2017). Moreover, the higher resistance to metal pollution and thermal stress (Bielen *et al.* 2016) and the more successful development the glochidia of *S. woodiana* lead to a competitive superiority of this species over indigenous mussels (Sousa *et al.* 2014; Huber & Geist 2019). Because of high temperatures during most of the year (exceeding 20°C), it could also be assumed that this river can support the establishment and further expansion of the invasive *S. woodiana* along this river system in Afghanistan. Therefore, sampling at different seasons and locations seems necessary to better estimate the actual distribution structure and habitat preference of *S. woodiana* in the Amu Darya River.

In the future, we will study the mussel and fish fauna of the Amu Darya river in Afghanistan and possible spread of invasive *S. woodiana* in different locations of this river. However, the most significant work at the global level is the exact determination of the taxonomic status of *Sinanodonta* sp. as the current status is yet under discussion due to insufficient molecular data for native and non-native populations as well as the high morphological resemblance among the cryptic taxa (Bolotov *et al.* 2016; Sayenko *et al.* 2017; Bespalaya *et al.* 2018; Kondakov *et al.* 2018, 2020b). In fact, large morphological variations may complicate *Sinanodonta* sp. relationships causing species misidentification (Sano *et al.* 2017). According to the COI distances obtained in the present study (Table 3), each clade in our phylogenetic tree could correspond to a separate cryptic species, indicating at least eight species-level lineages within the *Sinanodonta* complex, but it is possible that more species-level lineages could be detected based on a larger molecular data set. In the case of lineage C, the recorded sequences from South Korea and Japan in GenBank were misidentified as *Anodonta arcaeformis* (Heude, 1877) and *S. woodiana.* Previously reported sequences from South Korea (lineage G) were also misidentified as *S. woodiana* in GenBank, whereas they are two haplotypes of *S. schrenkii* (I. Lea, 1870) (Hap. G3 and G4, Fig. 3). However, many nominal taxa are regarded as junior subjective synonyms of the Asian pond mussel (Graf & Cummings 2015) and revising their taxonomy with respect to recently obtained molecular information will be a complicated work. In the previous study by Bolotov *et al.* (2016), the mean *P*-distance within the tropical lineage was 0.3% and no intraspecific variation was observed within the temperate invasive lineage. In our study, mean COI *P*-distances observed within the tropical and temperate lineages were 0.7 and 0.3% respectively, each comprising nine unique haplotypes. High intraspecific variation has been recorded however (Källersjö *et al*. 2005; Soroka 2010; Prié & Puillandre 2014). Overall, the morphospecies of the family Unionidae require revision on the basis of integrative taxonomy together with studying topotypes and type series as well as inter- and intraspecific diversity of genetic and morphological traits. Valuable steps in the right direction have been taken by Bolotov *et al.* (2015), Klishko *et al.* (2017) and Sayenko *et al.* (2017). Nevertheless, further work should be conducted to assess the taxonomy of this morphologically variable group.

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