

Original Article

Discovery and DNA analysis of the invasive freshwater mussel *Sinanodonta lauta* (Unionidae) in south Iran

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Abstract: Some members of the genus *Sinanodonta* (Modell, 1944) are known as successful invaders of freshwater habitats. Here we report the first record of *Sinanodonta lauta* (Martens, 1877) from Iran and provide morphological and molecular data on this alien population. This species was observed in the Dez River and a fish farm in Khuzestan, south of Iran. The *S. lauta* introduction seems to be closely related to the introduction of Asian carps from East Asia. The individuals collected from the river exhibited two COI haplotypes, probably due to several introduction events of host fish. Based on our haplotype network, the alien individuals found in Iran is closer to the native ones from South Korea (two-three substitutions) compared to other native individuals reported from Japan and Russian Far East. The presence of 10-11 years old specimens in Dez River shows that the species can survive well in the natural environment of southern Iran. Further expansion and colonization of *S. lauta* in south Iran or beyond it are not unexpected due to some human-mediated dispersal events as well as waterways in the region.

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Introduction

Freshwater bivalves of the family Unionidae are key ecological members of aquatic ecosystems due to contributing in purifying water, bioturbating, nutrient cycling and provisioning habitat (Vaughn, 2018). Unionid populations have been considerably declined, with many critically endangered (Lydeard et al., 2004; Böhm et al., 2020). However, some members of the genus *Sinanodonta* are found to be hyper-successful invaders of freshwater habitats (Sousa et al., 2014). *Sinanodonta* comprises several species native to East Asia (Lopes-Lima et al., 2020), but some have been widely introduced across many regions, including Europe, North America, and parts of Asia outside the native range (Lopes-Lima et al., 2016; Bernal et al., 2018; Bauer et al., 2021). Among the species of this genus, *S. woodiana* is regarded as one of the most invasive aquatic macroinvertebrates (Beran, 2020). Recent molecular

studies have shown that *S. woodiana* is a closely related species complex that includes several separate species-level lineages (Kondakov et al., 2018). Two species-level lineages of the complex (temperate and tropical) are known to be successful invaders sharing thoroughly allopatric non-native areas (Bolotov et al., 2016). However, a molecular study on *S. woodiana* complex revealed that *S. lauta* is one more invasive lineage of this complex (Kondakov et al., 2020a).

The native distribution of *S. lauta* ranges across Japan, South Korea, and Russian Far East (Primorye Region) (Lopes-Lima et al., 2020), but its non-native populations have been reported outside the native range, including Eastern Siberia (Yenisei River) (Bernal et al., 2018), European Russia (Volga River), Western Siberia (Ob River) (Kondakov et al., 2020b), Kazakhstan (Ili River) (Kondakov et al., 2020a) and Borneo (Zieritz et al., 2020). The species

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appears to have been indirectly introduced in non-native areas through fish hosts carrying the glochidia or directly by aquarists (Bespalaya et al., 2018; Zieritz et al., 2020). The spread of such filter-feeding nonindigenous mussels represents a significant threat to freshwater ecosystems (Douda et al., 2017) and may cause serious ecological and economic consequences in invaded regions (Sousa et al., 2014). Due to the capability to cope with a broad range of environmental situations (Douda et al., 2012), including hypoxia and pollution (Sárkány-Kiss et al., 2000), fast growth, high reproduction rate (Huber and Geist, 2019), high filtration rate (Douda et al., 2018) and host-generalist glochidia (Douda et al., 2012; Huber and Geist, 2019), invasive *Sinanodonta* populations can outcompete indigenous mussels (Urbańska et al., 2021). These mussels can modify the biological and physical properties of freshwater ecosystems (Douda et al., 2012). The glochidia of *Sinanodonta* can also adversely affect the growth and physiological conditions of fish hosts (Douda et al., 2017).

In the late spring of 2021, we received a report from a fish farm in the south of Iran about the existence of some giant mussels that caused trouble in the aquaculture activity. Based on the information and photos we received, these large mussels seemed to be *Sinanodonta*. Finally, in July 2021, during a field trip to Khuzestan Province in the South of Iran, we found the mussels not only on the fish farm but also in nature as well. Here, we report the first record of invasive *S. lauta* in Iran, describing its morphological traits, taxonomic status and possible affinities of the alien individuals in Iran according to COI gene sequencing data and briefly discuss the possible vectors and expansion in the region.

Materials and methods

Study area and sampling: The study area is the Dez River in Khuzestan Province, south of Iran. The fieldwork was done on 8th and 9th July 2021. *Sinanodonta* samples were collected from Dez River, Dezful (32°13'56.87"N; 48°18'43.96"E) (Fig. 1), by a benthic hand net. No other Unionid mussels

were found in the sampling site. With a length of 400 km, the Dez River is the main tributary of the largest river in Iran, i.e. Karun. The river originates from the mid-Zagros mountains in Lorestan Province, flows to the Khuzestan, and drains into the Karun River. The minimum water temperature of the river is about 14.5°C in winter, and the maximum water temperature also does not exceed 32 in summer. The river bottom substrates range from muddy to stony in different areas (Mohammadi Ruzbahani et al., 2014). The bottom of the sampling site was dominated by silt substrate, with some small-medium-sized stones scattered in some parts. In addition to the river, *Sinanodonta* samples were also collected from a fish farm located in Dezful, Khuzestan Province (32°13'49.85N; 48°19'2.24"E) (Fig. 1a). For molecular studies, a piece of foot tissue was cut from live mussels and immediately preserved in 96% ethanol. Mussel shells of the samples (N = 21 and 11 for Dez and fish farm, respectively) were also collected for morphological studies.

Morphology and age estimation: The biometric variables of each mussel, including shell length (L), height (H) and width (W) were measured to the nearest 0.1 mm by an AACO caliper. The morphological indices of shell convexity (CI = W/L ratio × 100) and elongation (EI = H/L ratio × 100) were also calculated. The age of each *Sinanodonta* individual was determined by counting the growth rings visible on the shell.

DNA extraction, polymerase chain reaction and sequencing: Total genomic DNA was extracted from the foot tissue of each specimen (N = 7 and 5 for Dez and fish farm, respectively) via a high-salt procedure (Sambrook et al., 1989) with a slight modification. DNA quality and quantity were examined via agarose gel (1%) electrophoresis and a Biophotometer Spectrophotometer (Eppendorf, Hamburg, Germany), respectively. We used the primers LCO22me2 (5'-GGT CAA CAA AYC ATA ARG ATA TTGG-3') and HCO700dy2 (5'-TCA GGG TGA CCA AAA AAYCA-3') (Walker et al., 2006, 2007) to amplify the partial sequences of

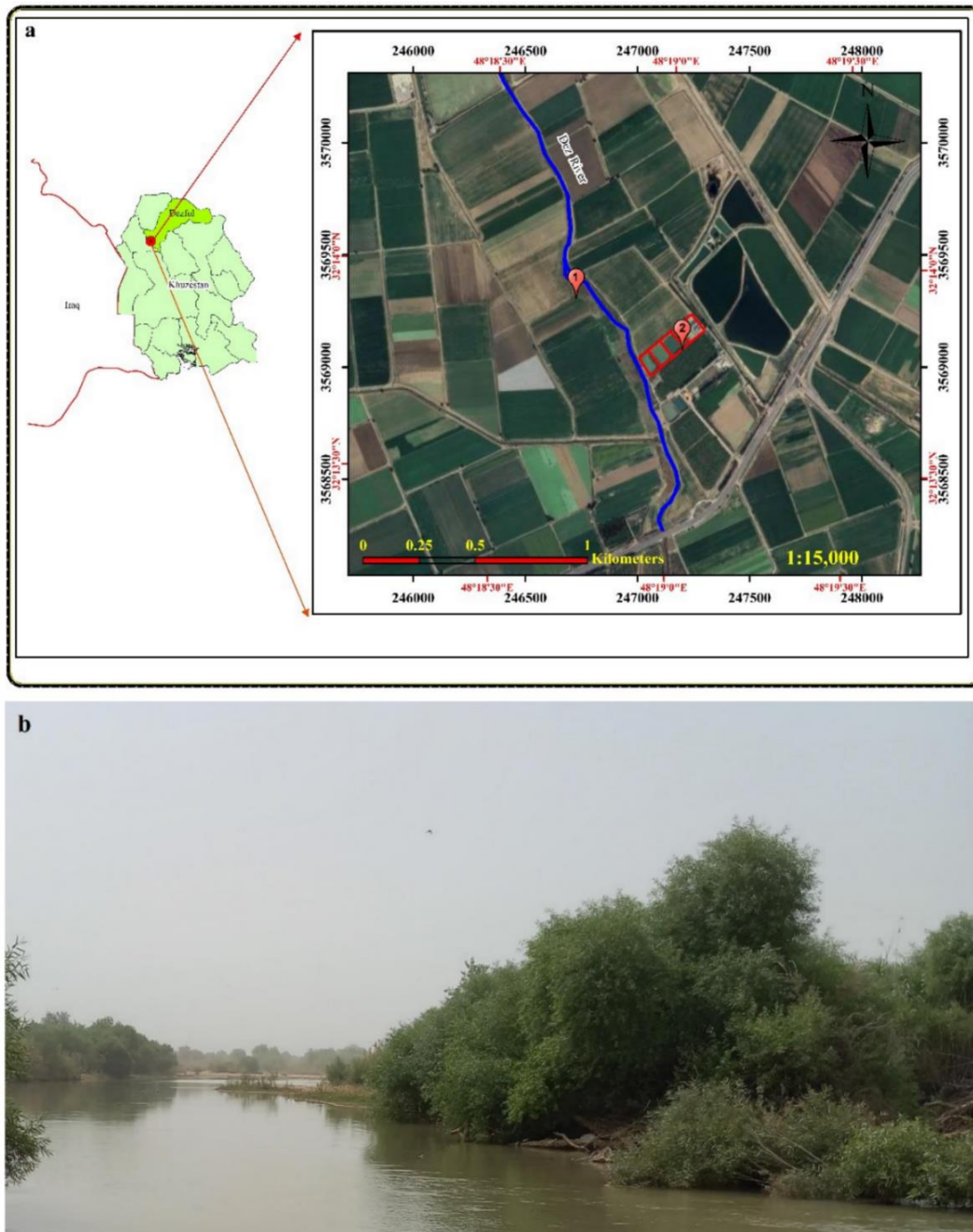


Figure 1. *Sinanodonta lauta*; (a) the study area's location. 1 = sampling site in Dez River, Khuzestan, 2 = fish farm in which the samples were collected. (b) Habitat view of non-indigenous *S. lauta* in a branch of Dez River, Dezfoul, Khuzestan (9th July 2021).

cytochrome c oxidase subunit I (COI) gene. PCR was run on a thermal cycler (Bio-RAD MJ Mini Thermal Cycler, Hercules, CA, USA) in 25 μ l reaction mix containing 1 μ l DNA (20-160 ng/ μ L), 15 μ l Taq 2X master mix red (Amplicon, Denmark), 1 μ l of each primer and 7 μ l PCR grade water. The PCR condition was set as follows: 4 min at 94 $^{\circ}$ C, 40 cycles at 94 $^{\circ}$ C (30 s), 50 $^{\circ}$ C (40 s) and 72 $^{\circ}$ C (60 S), followed by 10 min at 72 $^{\circ}$ C. The products were

checked through agarose gel (1.5%) electrophoresis in TBE buffer (1X). Finally, sequencing was carried out through an ABI 3730XL automatic sequencer (Applied Biosystems, 3730/3730xl DNA Analyzers Sequencing, Bioneer, Korea) using the same primers.

Molecular analysis: The obtained sequences were manually edited in BioEdit 7.0.1 (Hall, 1999). We used 136 COI sequences described in the previous

Table 1. COI sequences used in the present study (for other 136 sequences, see Bolotov et al., 2016 and Kondakov et al., 2020b).

Taxon	Region	population	Lineage	Accession	Reference
<i>Sinanodontia lauta</i>	Iran	Alien	C	OP048111	This study
<i>S. lauta</i>	Iran	Alien	C	OP048112	This study
<i>S. lauta</i>	Iran	Alien	C	OP048113	This study
<i>S. lauta</i>	Iran	Alien	C	OP048114	This study
<i>S. lauta</i>	Iran	Alien	C	OP048115	This study
<i>S. lauta</i>	Iran	Alien	C	OP048116	This study
<i>S. lauta</i>	Iran	Alien	C	OP048117	This study
<i>S. lauta</i>	Iran	Alien	C	OP048118	This study
<i>S. lauta</i>	Iran	Alien	C	OP048119	This study
<i>S. lauta</i>	Iran	Alien	C	OP048120	This study
<i>S. lauta</i>	Iran	Alien	C	OP048121	This study
<i>S. lauta</i>	Iran	Alien	C	OP048122	This study
<i>S. woodiana</i>	Germany	Alien	A	MH319868	Stelbrink et al. (2019)
<i>S. woodiana</i>	Germany	Alien	E	OU070149	GenBank
<i>S. woodiana</i>	Iraq	Alien	A	LC656037	GenBank
<i>S. woodiana</i>	Philippines	Alien	A	KX424967	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424968	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424969	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424970	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424976	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424977	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424978	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424979	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424971	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424972	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424973	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424974	Fornillos et al. (2020)
<i>S. woodiana</i>	Philippines	Alien	A	KX424975	Fornillos et al. (2020)
<i>S. schrenkii</i>	Russia	Indigenous	G	KU853266	Sayenko et al. (2017)
<i>S. schrenkii</i>	Russia	Indigenous	G	KU853267	Sayenko et al. (2017)
<i>S. schrenkii</i>	Russia	Indigenous	G	KU853268	Sayenko et al. (2017)
<i>S. schrenkii</i>	Russia	Indigenous	G	KU853269	Sayenko et al. (2017)
<i>S. lucida</i>	China	Indigenous	F	KX822667	GenBank
<i>Anodontia arcaeformis</i>	China	Indigenous	-	KJ434481	GenBank
<i>A. arcaeformis</i>	China	Indigenous	-	KJ434479	GenBank
<i>A. arcaeformis</i>	China	Indigenous	-	KJ434480	GenBank

studies (Bolotov et al., 2016; Kondakov et al., 2020b). Furthermore, 24 more COI sequences were extracted from NCB I's GenBank (Table 1). Multiple sequence alignment using ClustalW also was implemented in BioEdit. After trimming the sequences, a 616-bp COI fragment was left. Similar sequences were removed through the online tool

FaBox 1.41 (Villesen, 2007). Finally, the phylogenetic tree was constructed based on 36 unique sequences. We also used two taxa, including *Margaritifera dahurica* (KJ161516) and *M. laosensis* (JX497731), as outgroups.

Phylogenetic relationships were reconstructed based on Bayesian inference using MrBayes v3.2.2



Figure 2. *Sinanodonta lauta*; (a) and (b) represent shell interior and exterior view of the samples collected from fish farm and Dez River, respectively.

Table 2. Morphometric features and age of *Sinanodonta lauta* from Iran.

		Length	Height	Width	Convexity index	Elongation index	Age (year)
Fish	Min-Max	125.92-146.58	72.62-91.28	47.70-55.64	37.13-40.4	57.67-64.4	6-10
Farm	Mean±SD	137.23±7.17	85.02±5.36	52.80±3.26	38.46±0.94	61.93±1.68	8.45±1.44
Dez	Min-Max	48.09-152.96	30.16-93.16	13.01-55.92	21.6-38.18	49.01-68.44	2-11
River	Mean±SD	103.03±39.62	62.66±23.48	33.36±18.02	30.90±5.91	61.46±5.2	6.71±2.7

Abbreviations: SD (Standard deviation); Min (Minimum); Max (Maximum)

(Huelsenbeck and Ronquist, 2001). The best-fitting models of nucleotide substitution on the basis of the Akaike information criterion (Akaike, 1973) were estimated through MrModelTest v3.7 (Posada and Crandall, 1998) in PAUP v4.0 (Swofford, 2003). Two parallel runs were independently conducted. 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 20% burn-in fraction. The resulting tree was visualized through FigTree v1.4.2 (Rambaut, 2008).

The genetic divergence based on *P*-distance was assessed in MEGA 6.0 (Tamura et al., 2013). The median-joining network was also constructed through PopArt v1.7 (Leigh and Bryant, 2015) to

study the relationships among haplotypes.

Results

We detected a well-established viable population of the non-indigenous mussel *Sinanodonta* in south of Iran. The shell pattern of our samples was slightly elongated with brown/olive-green periostracum. As some *Sinanodonta* species are morphologically too similar, we used COI sequencing to identify the species. According to the molecular data, *S. lauta* was recorded from Khuzestan, Iran (Fig. 2).

Morphometric data: Morphometric features of the *S. lauta* specimens collected from Dez River (DR) and fish farm (FF) are shown in Table 2. The youngest and oldest individual was 2 and 11 years old, respectively. The largest mussel was 152.96 mm (length) at 11 years old. The convexity index was

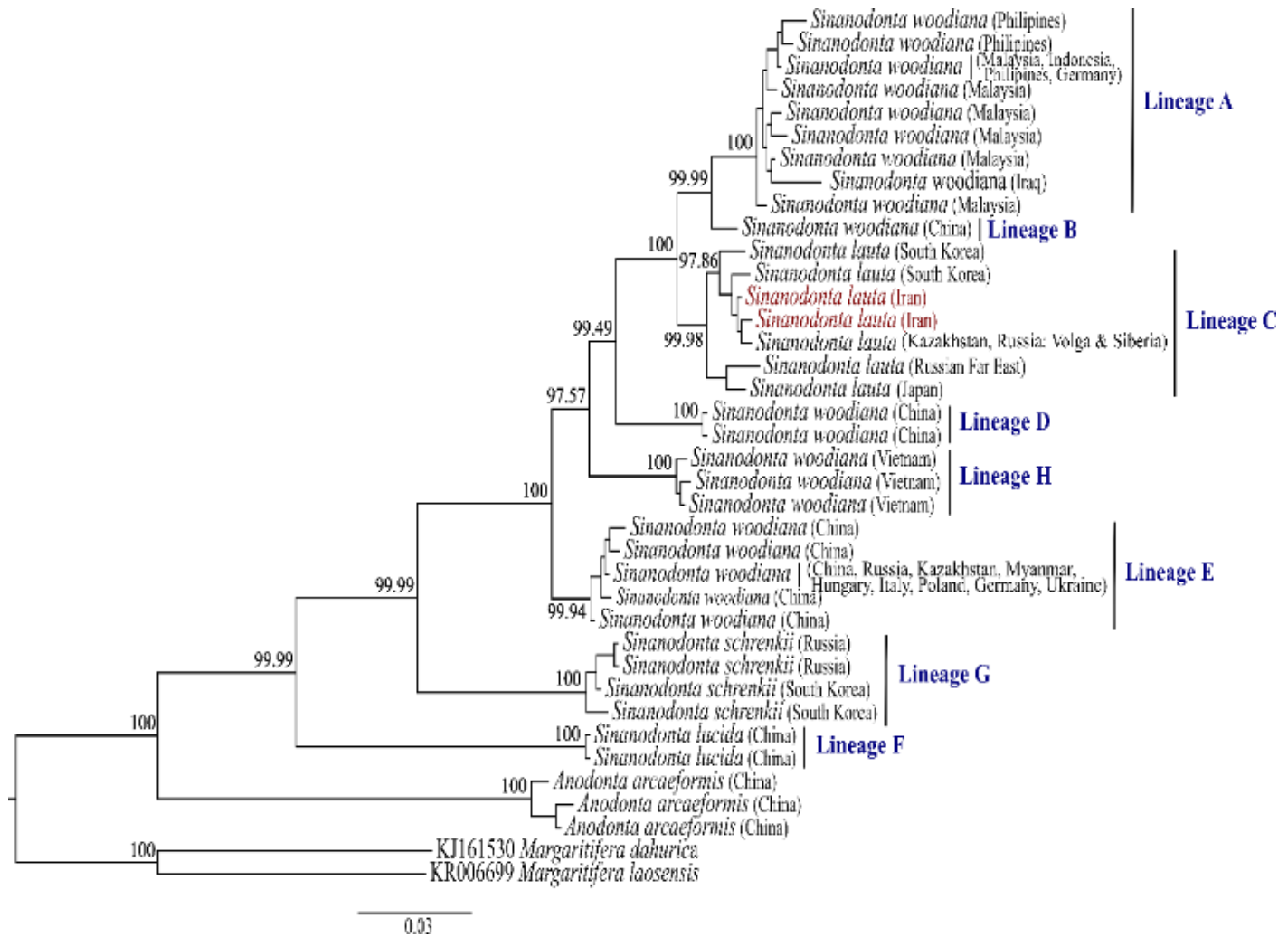


Figure 3. The Bayesian phylogenetic tree on the basis of 36 unique COI sequences of *Sinanodonta* spp. and related taxa; Two sequences of *Margaritifera laosensis* and *M. dahurica* are the outgroups. The numbers above branches represents the bootstrap support values. The scale bar shows the branch lengths.

21.6-38.18 for DR samples and 37.13-40.4 for the samples collected from FF. The elongation index also ranged 49.01-68.44 and 57.67- 64.4 for DR and FF samples, respectively (Table 2).

Molecular data: The COI sequences analysis confirmed the existence of the invasive species of *S. lauta* in Iran. Eight 664-bp and one 621-bp long fragments of the COI gene were acquired from the *S. lauta* specimens and deposited to the NCBI's GenBank (Table 1). We reconstructed the phylogenetic tree under TIM+I+G model (Fig. 3). The specimens from the fish farm belonged to a single haplotype, while the samples collected from Dez River belonged to two haplotypes. Our haplotypes were placed in the same clade with those from Russian Far East, South Korea, Kazakhstan,

Russia (Siberia and Volga), and Japan (Lineage C) with strong bootstrap support (99.98%), and there are seven more mitochondrial lineages (Fig. 3). The mean COI *P*-distances among the *Sinanodonta* spp. lineages are presented in Table 3. This distance ranged from 2 (between lineages A and B) to 13.9 (between Lineages A and F). The distance among lineage C comprising our samples and other lineages ranged from 2.8 to 13.6%. The mean *P*-distance within lineage C was also 1.1%.

We constructed the median-joining network based on the COI sequences of Lineage C (Fig. 4). Consistent with our phylogenetic data, the haplotype network recovered two haplotypes for the Iranian samples. The FF and DR sequences were clustered into a single haplotype separated from the other DR

Table 3. Genetic divergences (mean uncorrected *P*-distance %) among the *Sinanodonta* spp. lineages.

	Lineage A	Lineage B	Lineage C*	Lineage D	Lineage E	Lineage F	Lineage G
Lineage B	2.0						
Lineage C	3.6	2.8					
Lineage D	5.4	3.5	4.8				
Lineage E	5.8	5.5	4.9	4.9			
Lineage F	13.9	13.4	13.6	12.9	12.3		
Lineage G	9.8	10.4	10.6	9.5	8.1	12.7	
Lineage H	6	4.6	4.9	4.0	4.1	12.9	8.5

*The lineage that includes *Sinanodonta lauta*.

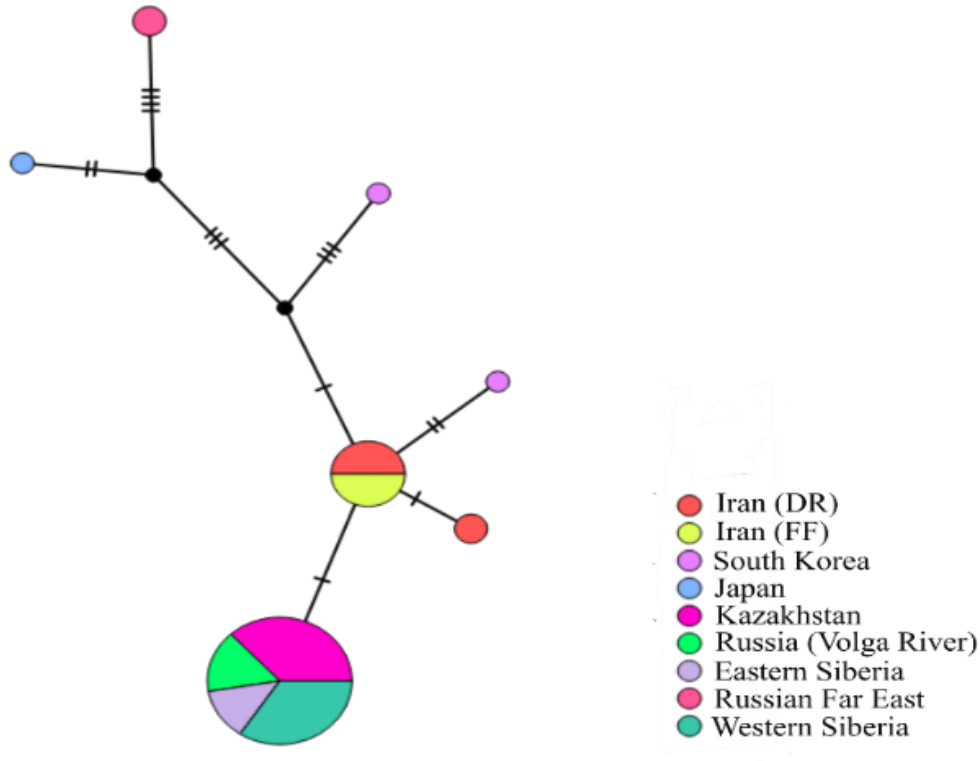


Figure 4. Median joining network for COI sequences of *Sinanodonta lauta* (N=55). Short lines between the haplotypes indicate the number of mutation sites.

haplotype by only one substitution. The Iranian samples weakly differentiated from the respective ancestral haplotype. The samples diverged by only 1 and 2 substitutions from non-native individuals of Kazakhstan and Russia (Volga, Eastern and Western Siberia). The haplotype from Russian Far East was the most divergent, separated by 8-9 mutation sites from Iranian haplotypes (8-9 Substitutions). A haplotype from South Korea was also the closest native haplotype to the alien ones from Iran (2-3 mutation sites).

Discussion

Until now, no species of the genus *Sinanodonta* have

been recorded from Iran. The present study documents the first record of the invasive freshwater mussel *S. lauta* from Iran. There is no record of *S. lauta* in other Middle East countries, but its closely related species *S. woodiana* was previously reported in some regions, including Iraq (Bogan et al., 2021) and Turkey (Ercan et al., 2012). However, in Middle Asia, a well-established population of the alien *S. lauta* was recorded from Kazakhstan (Kondakov et al., 2020a). The species was also recorded from Siberia, European Russia (Bespalaya et al., 2018; Kondakov et al., 2020b), and Borneo (Zieritz et al., 2020). Our finding, together with the previous observations by Bespalaya et al. (2018) and

Kondakov et al. (2020a, b) suggest that this lineage of *Sinanodonta* species complex may have high invasive capability to produce viable populations beyond its native ranges in Japan, South Korea and Russian Far East (Primorye Region) (Lopes-Lima et al., 2020).

The mussels collected from FF had 100% identical COI sequence to some from DR. The farmers of this facility declared that the mussels have been observed since two years ago, after a flood in Dez River. Taking into account the farmers' information, and waterway between this facility and Dez River and the maximum age of the specimens observed in FF (10 years old), it seems that *S. lauta* in this facility has originated from Dez. However, the individuals collected from both the natural environment and fish farm were large, with numerous annual growth rings on the shells and the maximum age of samples was 11 years old, suggesting a rather recent introduction, at least in 2010. Host fish carrying the glochidia is considered as the main vector for introducing *S. lauta* to non-native areas (Bespalaya et al., 2018; Zieritz et al., 2020; Kondakov et al., 2020a). During our survey in Khuzestan, we found that besides the fish farm, we collected the samples, the mussel is also present in at least 2 other aquaculture facilities rearing native (e.g., Common carp) and non-indigenous commercial fish species (e.g., Chinese carps). The farmers in one facility declared that they saw the mussels in 2012 for the first time, one year after setting the facility up. Unfortunately, we could not get sufficient information from the other facility about the exact origin and introduction time of the non-indigenous fish species. However, the farmers of both facilities stated that they imported the fish, including *H. molitrix*, *C. Idella* and *H. nobilis* from East Asia.

Asian carps have been introduced to serve as hosts for glochidia (Von Proschwitz, 2008) and the introduction of *S. lauta* to Iran seems to be closely related to the introduction of carps from East Asia to Iran. The DR samples also exhibited two haplotypes that may be probably due to several introduction

events of fish hosts by different aquaculture facilities in Khuzestan. According to our haplotype network, the alien individuals found in Iran is closer to the native ones from South Korea (two-three substitutions) compared to other native individuals reported from Japan and Russian Far East. However, due to the lack of enough information available, it is not possible to determine the exact origin and time the mussel was introduced to Iran. Currently, the alien *H. molitrix* inhabits the Dez River. The introduction of *H. molitrix* to this River became possible due to the migration from hatcheries and fish farms (Eskandari et al., 2005). Besides that, Cyprinids species, including *C. carp* and alien *H. molitrix* produced in aquaculture facilities in Khuzestan, have been directly released into the Dez River by the Khuzestan Department of Fisheries during the fish stocking program. We, therefore, suggest that this activity by the Fisheries department could facilitate the further introduction of *S. lauta* into nature. Additionally, the species, together with *Anodonta anatina* are newly being sold by aquarists in Khuzestan as water purifiers. The aquarium trade has been considered a possible means of aquatic invertebrates' introduction into non-native areas (Von Proschwitz, 2008; Duggan, 2010). When spoke with the aquarists, they were not aware that they are offering an alien species, and it is being unintentionally sold as *A. anatina*. However, marketing *S. lauta* may accelerate the spread of this alien species in the country.

Water temperature is regarded as a main environmental parameter that may have a main role in the growth and survival rate of *Sinanodonta* spp. (Kraszewski and Zdanowski, 2001; Liu et al., 2014). The thermophilic feature of the closely related species *S. woodiana* was previously suggested by various evidences but less has been studied about *S. lauta*. *Sinanodonta lauta* originates from East Asia with cold winters, and it is suggested that this species may be more cold-resistant than *S. woodiana*. However, it is possible that lower temperatures limit the *S. lauta* to produce viable populations (Kondakov et al., 2020b). Liu et al.

(2014) reported that *S. lauta* from Japan has higher feeding and growth rate in higher temperatures during summer compared to the spring, and temperature is one of the main factors for this species' growth. Our record of 10-11 years old specimens in Dez River shows that the species can well survive in the natural environment of south Iran with the warm weather conditions. Currently, we found a well-established population of *S. lauta* in the Dez River but we suggest that *S. lauta* may be able to produce new populations in other areas of the Tigris River drainage. Considering multiple introductions of Chinese carps from east Asia, stocking Dez River with such species and aquarium trade together with waterways in the region, rapid expansion and colonization of this invasive mussel in Karun or even other parts are not unexpected in the future since some water transport projects from this river to other freshwater basins including Zayandeh River in Isfahan are under construction. The possible expansion of alien *S. lauta* may create important adverse ecological outcomes.

No study has yet been done on the fauna of native freshwater bivalves in this basin. However, Dez and Karun support populations of more than 30 native fish species (Eskandari et al., 2000; Zare et al., 2019; Eagderi et al., 2019, 2022; Abbasi et al., 2020), with some impacted by river dragging, damming, industrial and agricultural pollution and also some invasive species, including *Oreochromis aureus*. We assume that establishing viable populations of *S. lauta* may cause worse outcomes for the region. Theoretically, *Sinanodonta* may be able to produce positive impacts on the aquatic environment, including an increase of water clarification via water filtration and particles deposition or serve as a food resource for vertebrates (Sousa et al., 2014), but these may cause some changes in the whole ecosystem including aquatics composition and structure, as observed in the case of *Dreissena polymorpha*. Douda et al. (2017) also reported that *Sinanodonta* glochidia can adversely impact the growth and physiological properties of the fish host. However, less is known about the host and

ecological preferences of *S. lauta* compared with the widely spread *S. woodiana*. As the spatial niche of *Sinanodonta* may be changed based on the environmental situation, sampling in other seasons and different parts of the river is necessary for a better estimate of the actual distribution structure and habitat preference of *S. lauta* in Dez River.

The taxonomic status of *Sinanodonta* species should be exactly determined since the current status is still under discussion due to the deficiency of molecular information available for indigenous and non-indigenous populations and high morphological similarity among the cryptic taxa (Bolotov et al., 2016; Sayenko et al., 2017; Bepalaya et al., 2018; Kondakov et al., 2018, 2020a). There is high morphological diversity which complicates the relationships among *Sinanodonta* species and may confuse identifying them (Sano et al., 2017). Here, as it is obvious in our phylogenetic tree, the previously reported sequences for *S. woodiana* in South Korea (GQ451867 and GQ451868) are *S. schrenkii* (Fig. 3, Lineage G). Our *S. lauta* haplotypes, together with those from Kazakhstan and Russia were also clustered in the same clade with sequences extracted from GenBank for *A. arcaiformis* (South Korea, GQ451870 and GQ451869) and *S. woodiana* (Japan, AB055627) (Lineage C), representing misidentification of these species. Bepalaya et al. (2018) also declared that with respect to the broad range of the species, *S. lauta* (noted under its junior name *S. ovata*) may indeed represent a junior synonym for some older nominal species reported from South Korea and Japan (Sano et al., 2017). Kondakov et al. (2020a) also stated that the previously recorded species *S. puerorum* and *S. gibba* based on morphological features from Kazakhstan, refer to *S. lauta* and/or *S. woodiana*. Based on the COI distance observed in the present study, each clade in our phylogenetic tree could correspond to a separate cryptic species of the genus *Sinanodonta*. Therefore, there are at least eight species-level lineages in this species complex, but it is possible that more species-level lineages be detected based on an increased molecular data set.

Furthermore, later studies indicate high intraspecific variation within different unionid taxa (maybe more than 3% barcoding threshold) that is usually applied to species delimitation (Källersjö et al., 2005; Soroka, 2010; Prié and Puillandre, 2014). Here, we do not have enough data to discuss the complex systematics, and more information is needed on the basis of both molecular data and morphological characteristics. Altogether, morphospecies of the Unionidae needs complete revising on the basis of the integrative taxonomic method, examining topotypes and type series and also studying the inter- and intraspecific diversity in genetic and morphological features. In this regard, valuable steps have been taken in the right course by Bolotov et al. (2015), Klishko et al. (2017), and Sayenko et al. (2017).

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