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DNA barcoding reveals invasion of two cryptic *Sinanodonta* mussel species (Bivalvia: Unionidae) into the largest Siberian river

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ABSTRACT

This study provides the first record of the two distinct mitochondrial lineages of *Sinanodonta* mussels in the Yenisei River that forms the largest Siberian river basin. It is the first discovery of alien populations of these mussels in Russia. The two mussel lineages are living in sympatry in a river site heated by warm water discharge of the Krasnoyarsk thermal power plant. These lineages represent two cryptic species: *Sinanodonta* aff. *woodiana* lineage E and *S. ovata* Bogatov and Starobogatov, 1996. The population of the first species from the Yenisei shares the invasive haplotype that is widely spread across Europe. The molecular evidence suggests that this lineage originated from the Yangtze River, China. The native distribution of the second species ranges across South Korea, Japan and small basins in the south of Primorsky Krai, Russian Far East. The possible vector of this invasion is the introduction of fish hosts or adult mussels by aquarists. Our results highlight that the populations of *Sinanodonta* species outside their native ranges may represent an overlooked but important threat for freshwater ecosystems in Russia that should be considered an unexpected nation-level ecological problem. Additionally, our discovery reveals the possibility of a successful joint invasion of different *Sinanodonta* species into a single river that may increase negative impacts of invaders on indigenous communities. Some implications of our findings for systematics of the unionid mussels are also discussed.

1. Introduction

The native range of bivalve mollusks of the genus *Sinanodonta* Modell, 1945 covers China, Japan, the south of the Russian Far East (Amur River basin and Primorsky Krai), Korea, and northern Indo-China (Graf and Cummings, 2007; Popa et al., 2007). Among the members of the genus the species *S. woodiana* (Lea, 1834) is the most prominent invader of freshwater ecosystems (Colomba et al., 2013; Zhang et al., 2016) and this species spread rapidly to different parts of the world, including Southeast Asia, Europe, North America, and the Caribbean area (Popa et al., 2007; Guarneri et al., 2014; Donrovich et al., 2017). According to a recent molecular study, there are at least two invasive mtDNA lineages within the genus *Sinanodonta*, i.e., 'temperate' and 'tropical' lineages (Bolotov et al., 2016a).

Invasive bivalves may cause great ecological, evolutionary, and economic impacts on freshwater ecosystems (Sousa et al., 2014). The non-indigenous individuals of *S. woodiana* may compete with native unionoidmussels for food and fish-host and they serve as vectors for the introduction of new parasites and diseases (Sousa et al., 2014; Lopes-Lima et al., 2017a,b; Donrovich et al., 2017). Fairly frequently non-native mollusk species can be found in waterbodies with artificially transformed thermal regimes, which apparently provide them with favorable habitats (Karatayev et al., 2007; Kraszewski, 2007; Crespo et al., 2015; Vinarski et al., 2015). The first reports of *S. woodiana* occurrences in Europe were restricted to thermally polluted water systems (Kraszewski, 2007; Soroka, 2008; Cichy et al., 2016).

In the summer of 2016, the mass media of the city of Krasnoyarsk (Russia) disseminated information on the discovery of some giant bivalves in the Yenisei River at the city beach site, which is heated by the warm water discharge of the Krasnoyarsk Thermal Power Plant (TPP) no. 2. Earlier, in 2010, the finding of "giant pearl mussels" in the vicinities of Krasnoyarsk was reported and discussed in the Russian malacological Internet-forum (http://malacolog.com/forum/viewtopic.php?p=4470). Based on this information, we organized a field trip to the area in July 2016 and managed to collect a sample of these large mussels. Based on the molecular and morphological investigation of

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this sample, we report the first record of the two non-native lineages of *Sinanodonta* spp. in the Yenisei River Basin. The aim of this study was to examine possible phylogenetic relationships and the taxonomic status of *Sinanodonta* spp. from the Yenisei based on DNA sequences and morphological data, and to determine a possible vector for the invasion of *Sinanodonta* spp. lineages into the basin.

2. Materials and methods

2.1. Study area and taxon sampling

Samples of *Sinanodonta* spp. were collected from the Abakanskaya branch of the Yenisei River, Russia (55°59′20.94″N, 92°52′11.80″ E) by snorkeling (Bolotov, Kondakov and Gofarov leg.). The sampling site is located close to the outlet of the warm water of Krasnoyarsk TPP. We have not found other unionoid species at the site, although the duck mussel, *Anodonta anatina* (Linnaeus, 1758), a native species, has been recorded from other parts of the Yenisei River basin (Zieritz et al., 2017; our unpublished data). With a length of 3487 km, the Yenisei River is the largest Siberian watercourse, one of the greatest rivers of Russia and worldwide. The start of ice formation on the Yenisei River has been recorded in November − December with break-up in December. The beginning of the spring ice drift on the Yenisei River has been observed since March (Ivanova, 2015). The water temperature in the Yenisei River during the summer period does not exceed 12°C (Ivanova, 2015). The average air temperature in July varies from +12 to +21 °C, and in January from −14 to −28 °C (Ivanova, 2015). The absolute minimum temperature is −51 °C, and the maximum is +37 °C (Ivanova, 2015).

For molecular analyses, the tissue samples were collected from eight live bivalves. Samples were immediately preserved in 96% ethanol. Additionally, dead shells ($N = 6$) were collected for morphological investigation. A total of 14 specimenswere studied. The length of the live specimens and dead shells were measured to the nearest 0.1 mm with dial calipers. The materials are stored (under the reg. no. Biv 191) in the malacological collection of the Russian Museum of Biodiversity Hotspots of the Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia.

2.2. DNA extraction, amplification and sequencing

The present study includes new molecular data for 13 *Sinanodonta* specimens from Yenisei River (Krasnoyarsk Krai), Gladkaya River (Primorsky Krai) and Vietnam (Table 1). Total genomic DNA was extracted from the alcohol-preserved foot mussel tissue using a NucleoSpin® Tissue Kit (MachereyeNagel GmbH & Co. KG, Germany) and following the manufacturer's protocol. Primers used for amplification of the COI partial sequences were LCO1490 and HCO 2198 (Folmer et al., 1994). The PCR mix contained approximately 200 ng of total cellular DNA, 10 pmol of each primer, 200 mmol of each dNTP, 2.5 ml of PCR buffer (with 10 X 2 mmol $MgCl_2$), 0.8 units of Taq DNA polymerase (SibEnzyme Ltd., Russia), and H_2O, which was added up to a final volume of 25 ml. Thermocycling included one cycle at 95 °C (4 min), followed by 34 cycles of 95 °C (45 s), 50 °C (40 s), and 72 °C (50 s) with a final extension at 72 °C (5 min). Forward and reverse sequence reactions were performed directly on purified PCR products using the ABIPRISM® BigDye™ Terminator v. 3.1 reagents kit and run on an ABI PRISM® 3730 DNA (Thermo Fisher Scientific Inc., Waltham, MA, USA). The resulting sequences were checked using a sequence alignment editor (BioEdit version 7.2.5, Hall, 1999). In addition, 66 COI sequences were obtained from NCBI's GenBank, including two COI sequences of *Margaritifera laosensis* and *Margaritifera dahurica* as outgroup for the phylogenetic analyses (Table 1).

2.3. Phylogenetic analyses

The alignment of the COI sequences was performed directly using the ClustalW algorithm (Thompson et al., 1994). For the phylogenetic analyses, each COI sequence of aligned datasets was trimmed, leaving a 659-bp fragment. Then, identical COI sequences were removed from the dataset using an online FASTA sequence toolbox (FaBox1.41: Villesen, 2007), leaving a total of 33 haplotype sequences (including the two outgroup taxa). For phylogenetic analyses, we used the COI dataset with unique haplotypes. The best models of sequence evolution for each partition were based on corrected Akaike Information Criterion (AICc) of MEGA6 (Tamura et al., 2013) and were as follows: (1) 1 st codon of the COI: HKY; (2) 2nd codon of the COI: TN93 + G ($G = 0.21$); (3) 3rd codon of the COI: HKY. Phylogenetic relationships were reconstructed based on Bayesian inference using MrBayes v. 3.2.6 (Ronquist et al., 2012) at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al., 2010). Four Markov chains, one cold and three heated (temperature = 0.1), were run simultaneously for 25,000,000 generations. The resulting phylogenies were constructed using a tree figure drawing tool (Archaeopteryx v. 0.9901 beta, Han and Zmasek, 2009).

To estimate the number of putative species within the *Sinanodonta* haplotypes, we applied a species delimitation approach by using the Bayesian Poisson Tree Process (bPTP) model (Zhang et al., 2013). Using the Bayesian tree with multiple haplotypes per species, two runs of 100,000 generations were executed with the first 10% discarded as an appropriate burn-in (pre-convergence part) based on the convergence of log-likelihood values.

3. Results

During our field study, we discovered well-established populations of *Sinanodonta* spp. inhabiting the Abakanskaya branch of the Yenisei River near the Krasnoyarsk TPP (Fig. 1). The settlement of mussels was confined to sandy-gravel and sandy-clay substrate. The mean depths at the site with mussel beds ranged between 0.5 and 1.5 m. Living specimens of *Sinanodonta* spp. above and below the outlet of the warm water of the TPP were not found.

Eight individuals of *Sinanodonta* spp. were collected (Fig. 2). Surprisingly, the DNA barcoding revealed that they belong to two distant COI clades, which appear to be different cryptic species, because the mean *p*-distance between these clades is 4.7%. The first clade is *Sinanodonta* aff. *woodiana* lineage E sensu Bolotov et al. (2016a), the 'temperate' invasive lineage, which is widely spread across European countries (Table 1 and Figs. 2 and 3). The sequenced specimens from Yenisei shared the same haplotype (hapE3), which has previously been recorded in non-native European populations of this species in Ukraine, Poland, Hungary and Italy (Table 1).

Based on the molecular sequence data, specimens of the second clade, *Sinanodonta* aff. *woodiana* lineage C sensu Bolotov et al. (2016a), are distributed in South Korea, Japan, and, most importantly, in the Gladkaya River (Primorsky Krai, Russian Far East), the type locality of the species *Sinanodonta ovata* Bogatov and Starobogatov (1996) (Table 1 and Fig. 3). Here, we identify the previously defined lineage C with this nominal taxon that was described on the basis of shell characters only (Bogatov and Starobogatov, 1996). The haplotype, which was found in the Yenisei, is most closely related to those specimens from South Korea (Fig. 3).

The average shell length of collected specimens of *Sinanodonta* aff. *woodiana* lineage E was 128.8 ± 21.2 mm (min–max 109.6-151.7 mm) ($N = 3$) and *S. ovata* was 124.8 ± 9.1 mm (min–max 110.4-131.7 mm) ($N = 5$). We were unable to find any significant morphological differences between these two cryptic species based on the traditional conchological features, i.e., the shape of adductor scars and the shell (Fig. 2).

4. Discussion

This study provides the first record of the presence of two distinct