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Authors: Vikhrev, Ilya V., Konopleva, Ekaterina S., Gofarov, Mikhail Y., Kondakov, Alexander V., Chapurina, Yulia E., et al.

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A Tropical Biodiversity Hotspot Under the New Threat: Discovery and DNA Barcoding of the Invasive Chinese Pond Mussel *Sinanodonta woodiana* in Myanmar

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Ilya V. Vikhrev^{1,2}, Ekaterina S. Konopleva^{1,2}, Mikhail Y. Gofarov^{1,2}, Alexander V. Kondakov^{1,2}, Yulia E. Chapurina^{1,2}, and Ivan N. Bolotov^{1,2}

Abstract

A well-established invasive *Sinanodonta woodiana* population was discovered in a floodplain lake of the upstream section of the Irrawaddy River basin, Kachin State, Myanmar. The DNA barcoding reveals that the population belongs to the temperate invasive mtDNA lineage and represents the same cytochrome c oxidase subunit I haplotype, which has been recorded in the invasive European populations. It is the most southern location of a population pertaining to this highly invasive haplotype known to date. The actual distribution of this alien species in Myanmar is still unknown, although it appears to be rather not widespread. Its possible dispersal through the country may affect native benthos communities, which include many unique endemic taxa. However, further expansion of the temperate lineage across South East Asia is supposed to be limited due to a specific environment of tropical floodplain freshwater systems that appears too warm for this cryptic taxon.

Keywords

Unionidae, Irrawaddy River, the Indo-Burma biodiversity hotspot, alien species, biological invasions, mitochondrial DNA

Introduction

The Chinese pond mussel, *Sinanodonta woodiana* (Lea, 1834) is a well-known invasive species of the Unionidae, which is widely spread almost around the world together with its alien fish hosts (Douda, Vrtílek, Slavík, & Reichard, 2012; Lajtner & Crnčan, 2011; Lopes-Lima et al., 2017; Watters, 1997). Recent molecular studies reveal that *S. woodiana* is rather a complex of several closely related species because it comprises at least seven deeply divergent mtDNA lineages (Bolotov et al., 2016). Among them, the two lineages could only be considered as invasive. The first one is the tropical invasive lineage, which is currently widespread across the Malay Peninsula, the Philippines, and through Indonesia to the Lesser Sunda Islands (Bolotov et al., 2016; Zieritz et al., 2016). The origin of this successful invader is uncertain, but several authors placed it within Taiwan or the southern regions of continental China based on the analysis of primary sources for introduced fishes (Bolotov et al., 2016; Djajasasmita, 1982;

Watters, 1997). The second one is the temperate invasive lineage, which has broad nonnative range in Europe, but most likely originated from the Yangtze drainage basin (Bolotov et al., 2016; Watters, 1997).

S. woodiana may affect native mussel populations, including the negative impact via cross-resistance of host fishes (Donrovich et al., 2017; Sousa, Novais, Costa, & Strayer, 2014). Adult *Sinanodonta* mussels can compete effectively with indigenous taxa for space, food, and host fishes. Their ability to modify natural

¹Northern Arctic Federal University, Arkhangelsk, Russian Federation

²Federal Center for Integrated Arctic Research, Russian Academy of Sciences, Arkhangelsk, Russian Federation

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Corresponding Author:

Ekaterina S. Konopleva, Federal Center for Integrated Arctic Research, Russian Academy of Sciences, Severnaya Dvina Emb. 23, Arkhangelsk 163000, Russian Federation.
Email: es.konopleva@gmail.com



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Table 1. List of the Mitochondrial COI Sequences of *Sinanodontia* spp. Examined in the Present Study.

Species	mtDNA lineage of Bolotov et al. (2016)	Haplotype code	Specimen locality	COI GenBank acc. no.	Voucher no.	Data source
<i>Sinanodontia</i> aff. <i>woodiana</i>	E	hapE3	A floodplain lake near Bhamo, Irrawaddy River basin, Myanmar	MF497809	RMBH biv269	This study
<i>S. aff. woodiana</i>	E	hapE3	A floodplain lake near Bhamo, Irrawaddy River basin, Myanmar	MF497807	RMBH biv269/15	This study
<i>S. aff. woodiana</i>	E	hapE3	A floodplain lake near Bhamo, Irrawaddy River basin, Myanmar	MF497808	RMBH biv269/16	This study
<i>S. aff. woodiana</i>	E	hapE3	Ukraine	JQ253894	isolate PB23	GenBank
<i>S. aff. woodiana</i>	E	hapE3	Ukraine	JQ253893	isolate PB22	GenBank
<i>S. aff. woodiana</i>	E	hapE1	China	KJ434486	isolate I79ANWE5	GenBank
<i>S. aff. woodiana</i>	E	hapE3	China	KJ434485	isolate I77ANWE4	GenBank
<i>S. aff. woodiana</i>	E	hapE3	China	KJ434484	isolate I21ANWE3	GenBank
<i>S. aff. woodiana</i>	E	hapE2	China	KJ434483	isolate I19ANWE2	GenBank
<i>S. aff. woodiana</i>	E	hapE3	China	KJ434482	isolate I17ANWE1	GenBank
<i>S. aff. woodiana</i>	E	hapE3	Poland	AF468683	N/A	Soroka (2005)
<i>S. aff. woodiana</i>	E	hapE4	China	KM272949	2014-phc-si-wo-001	Zhang et al. (2016)
<i>S. aff. woodiana</i>	E	hapE3	Poland	HQ283344	isolate AW19	Soroka (2010a)
<i>S. aff. woodiana</i>	E	hapE3	Poland	HQ283345	isolate AW22	Soroka (2010a)
<i>S. aff. woodiana</i>	E	hapE3	Poland	HQ283346	isolate AW124	Soroka (2010a)
<i>S. aff. woodiana</i>	E	hapE3	Poland	HQ283347	isolate AW123	Soroka (2010a)
<i>S. aff. woodiana</i>	E	hapE3	Poland	HQ283348	isolate AW125	Soroka (2010a)
<i>S. aff. woodiana</i>	E	hapE3	Poland	EF440349	2	Soroka & Burzyński (2010b)
<i>S. aff. woodiana</i>	E	hapE3	Poland	KJ125078	354	Soroka, Urbańska & Andrzejewski, (2014)
<i>S. aff. woodiana</i>	E	hapE3	Hungary	KJ125079	375	Soroka et al. (2014)
<i>S. aff. woodiana</i>	E	hapE3	Italy: Lake Maggiore	KF731775	M21	GenBank
<i>S. aff. woodiana</i>	E	hapE3	Italy: River Po	KF731776	P15	GenBank
<i>S. aff. woodiana</i>	E	hapE3	Italy: River Po	KF731777	P32	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Indonesia: West Flores, Wae Racang River	KU891641	wood1	Bolotov et al. (2016)
<i>S. cf. woodiana</i>	A	hapA8	Indonesia: West Flores, Wae Racang River	KU891642	wood2	Bolotov et al. (2016)
<i>S. cf. woodiana</i>	A	hapA1	Malaysia	KX051328	S003	Zieritz et al. (2016)

(continued)

Table 1. Continued

Species	mtDNA lineage of Bolotov et al. (2016)	Haplotype code	Specimen locality	COI GenBank acc. no.	Voucher no.	Data source
<i>S. cf. woodiana</i>	A	hapA8	Malaysia: Perlis, Tasik Melati	KX051326	X128	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA2	Malaysia: Selangor; unnamed sungai	KX051325	X134	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA3	Malaysia: Selangor; Tasik Semenyih	KX051324	X227	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA4	Malaysia: Perlis, unnamed sungai	KX051323	X117	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA4	Malaysia: Kedah, Sg. Pendang	KX051322	X130	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA8	Malaysia: Johor, Chohong, Kesang, Taman Bekoh Jaya	KX051321	BIV1793	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA8	Malaysia: Pahang, Lipis, Jelai, Kampung Jeram Besu	KX051320	BIV1691	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA4	Malaysia: Perak, Kinta	KX051319	BIV1605	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA8	Malaysia: Malacca, Chohong, Kesang, Kampung Chohong	KX051318	BIV1778	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA4	Malaysia: Negeri Sembilan, fish pond	KX051317	X224	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA3	Malaysia: Kelantan, Sg. Semerak	KX051316	X205	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA5	Malaysia: Selangor; abandoned mining pool	KX051315	X149	Zieritz et al. (2016)
<i>S. cf. woodiana</i>	A	hapA6	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424979	HL_UN12	GenBank
<i>S. cf. woodiana</i>	A	hapA6	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424978	HL_UN10	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424977	HL_UN8	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424976	HL_UN6	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424975	HL_SW8B	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424974	HL_SW7A	GenBank
<i>S. cf. woodiana</i>	A	hapA6	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424973	HL_SW5B	GenBank
<i>S. cf. woodiana</i>	A	hapA6	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424972	HL_SW4A	GenBank

(continued)

Table 1. Continued

Species	mtDNA lineage of Bolotov et al. (2016)	Haplotype code	Specimen locality	COI GenBank acc. no.	Voucher no.	Data source
<i>S. cf. woodiana</i>	A	hapA6	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424971	HL_SW3	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424970	SH_UN5	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424969	SH_UN4	GenBank
<i>S. cf. woodiana</i>	A	hapA7	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424968	SH_UN2	GenBank
<i>S. cf. woodiana</i>	A	hapA8	Philippines: Lake Danao Natural Park Ormoc Leyte	KX424967	HL_UN1	GenBank
<i>S. aff. woodiana</i>	B	hapB1	China	KJ434487	107ANWPI	GenBank
<i>Sinanodonta ovata</i> (Bogatov & Starobogatov, 1996)	C	hapC5	Russia: Primorsky Krai, Gladkaya River ^a	KY978743	RMBH biv 225_2	This study
<i>S. ovata</i>	C	hapC5	Russia: Primorsky Krai, Gladkaya River ^a	KY978742	RMBH biv 225_1	This study
<i>S. ovata</i>	C	hapC3	Russia: Krasnoyarsk, Yenissei River	KY561633	RMBH biv 191_1	This study
<i>S. ovata</i>	C	hapC1	South Korea	GQ451870	isolate C82	GenBank
<i>S. ovata</i>	C	hapC2	South Korea	GQ451869	isolate C8	GenBank
<i>S. ovata</i>	C	hapC4	Japan	AB055627	N/A	GenBank
<i>S. aff. woodiana</i>	D	hapD1	China	KJ434490	176ANWPA	GenBank
<i>S. aff. woodiana</i>	D	hapD1	China	KJ434489	164ANWP3	GenBank
<i>S. aff. woodiana</i>	D	hapD2	China	KJ434488	110ANWP2	GenBank
<i>Sinanodonta amurensis</i> (Moskvicheva, 1973)	G	hapG1	South Korea	GQ451868	C72	GenBank
<i>S. amurensis</i>	G	hapG2	South Korea	GQ451867	C7	GenBank
<i>S. amurensis</i>	G	hapG3	Russia: Razdolnaya River	KU853266	348	Sayenko, Soroka & Kholin (2017)
<i>S. amurensis</i>	G	hapG4	Russia: Amur River	KU853267	349	Sayenko et al. (2017)
<i>S. amurensis</i>	G	hapG4	Russia: Khanka Lake	KU853268	384	Sayenko et al. (2017)
<i>S. amurensis</i>	G	hapG3	Russia: Khanka Lake	KU853269	395	Sayenko et al. (2017)
<i>S. aff. woodiana</i>	H	hapH2	Vietnam	KX822668	NCSM84916	Lopes-Lima et al. (2017)
<i>S. aff. woodiana</i>	H	hapH1	Vietnam: Hanoi	KY561635	RMBH 91_1	This study
<i>S. aff. woodiana</i>	H	hapH2	Vietnam: Hanoi	KY978744	RMBH 91_2	This study
<i>S. aff. woodiana</i>	H	hapH3	Vietnam: Hanoi	KY978745	RMBH 91_3	This study
<i>S. lucida</i> (Heude, 1877)	F	hapF1	China	KX822667	N/A ^a	Lopes-Lima et al. (2017)

(continued)

Table 1. Continued

Species	mtDNA lineage of Bolotov et al. (2016)	Haplotype code	Specimen locality	COI GenBank acc. no.	Voucher no.	Data source
<i>S. lucida</i>	F	hapF2	China	KF667529	01-ALF-2013	Song et al. (2016)
<i>Anemina arcaeformis</i> (Heude, 1877)	I	hapI1	China	KJ434481	175ANA3	GenBank
<i>A. arcaeformis</i>	I	hapI2	China	KJ434480	49ANA2	GenBank
<i>A. arcaeformis</i>	I	hapI3	China	KJ434479	47ANA1	GenBank
<i>A. arcaeformis</i>	I	hapI4	China	KF667530	01-AAF-2013	GenBank
<i>Margaritifera laosensis</i> ^b (Lea, 1863)	–	–	Laos: Phongsali Province, Nam Pe River, Nam Ou River Basin,	JX497731	d0002/1	Bolotov et al. (2016)
<i>M. dahurica</i> ^b (Middendorff, 1850)	–	–	Russia: Primorsky kray, Ilistaya River (Amur drainage)	KJ161516	d0088/6	Bolotov et al. (2016)

Note. COI = cytochrome c oxidase subunit I.

^aThe type locality of this species. N/A = not available.

^bThese species were used as an out-group for Bayesian analysis.

ecosystems influences biological, physical, and chemical parameters of water environment (Bolotov et al., 2016; Douda et al., 2012; Guarneri et al., 2014; Sousa, Gutiérrez & Aldridge, 2009, 2014; Watters, 1997). An infection of fishes with the glochidia of *Sinanodonta* may negatively affect their physiological conditions, size, and weight (Douda et al., 2017).

In the present correspondence, we report the first occurrence of *S. woodiana* from Myanmar. In addition, we discuss the possibility of the further spread of this lineage of *S. woodiana* species complex across river systems of Southeast Asia and its possible effects on the native freshwater communities.

Methods

Data Collection

Available cytochrome c oxidase subunit I (*COI*) sequences of *S. woodiana* species complex and its sister taxa were downloaded from the BOLD IDS and NCBI's GenBank, resulting in 68 sequences from Europe, China, Russia, South Korea, Japan, Malaysia, the Philippines, and Indonesia (Table 1). The *COI* sequence, which was provided for the *S. aff. woodiana* specimen from Romania (NCBI's GenBank acc. no. JQ435822), was not included (see Bolotov et al., 2016). We have analyzed three available specimens of *S. woodiana*, which were collected from a floodplain lake of the upstream section of the Irrawaddy River basin, Kachin State, Myanmar in November 2016 during the join fieldworks with specialists of Flora & Fauna International and Department of Fisheries of Myanmar (Table 1). In addition, six new samples of *S. woodiana* complex from Vietnam and Russia were also sequenced. Sequences of *Margaritifera dahurica* and *M. laosensis* were used as out-groups (GenBank acc. nos. KJ161530 and KR006699, respectively).

Laboratory Protocols and Phylogenetic Analysis

A total genomic DNA was extracted from the alcohol-preserved mussel foot tissue using the NucleoSpin® Tissue Kit (Macherey Nagel GmbH & Co. KG, Germany), following the manufacturer's protocol. Primers used for amplification of the *COI* partial sequences were LCO1490 and HCO 2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). The polymerase chain reaction (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 × 2 mmol MgCl₂), 0.8 units of Taq DNA polymerase (SibEnzyme Ltd., Russia), and H₂O, which was added up to a final volume of 25 ml. Thermocycling included one cycle at 95°C (4 min), followed by 34 cycles at 95°C (45 s), 50°C (40 s), and 72°C (50 s) with a final extension at 72°C

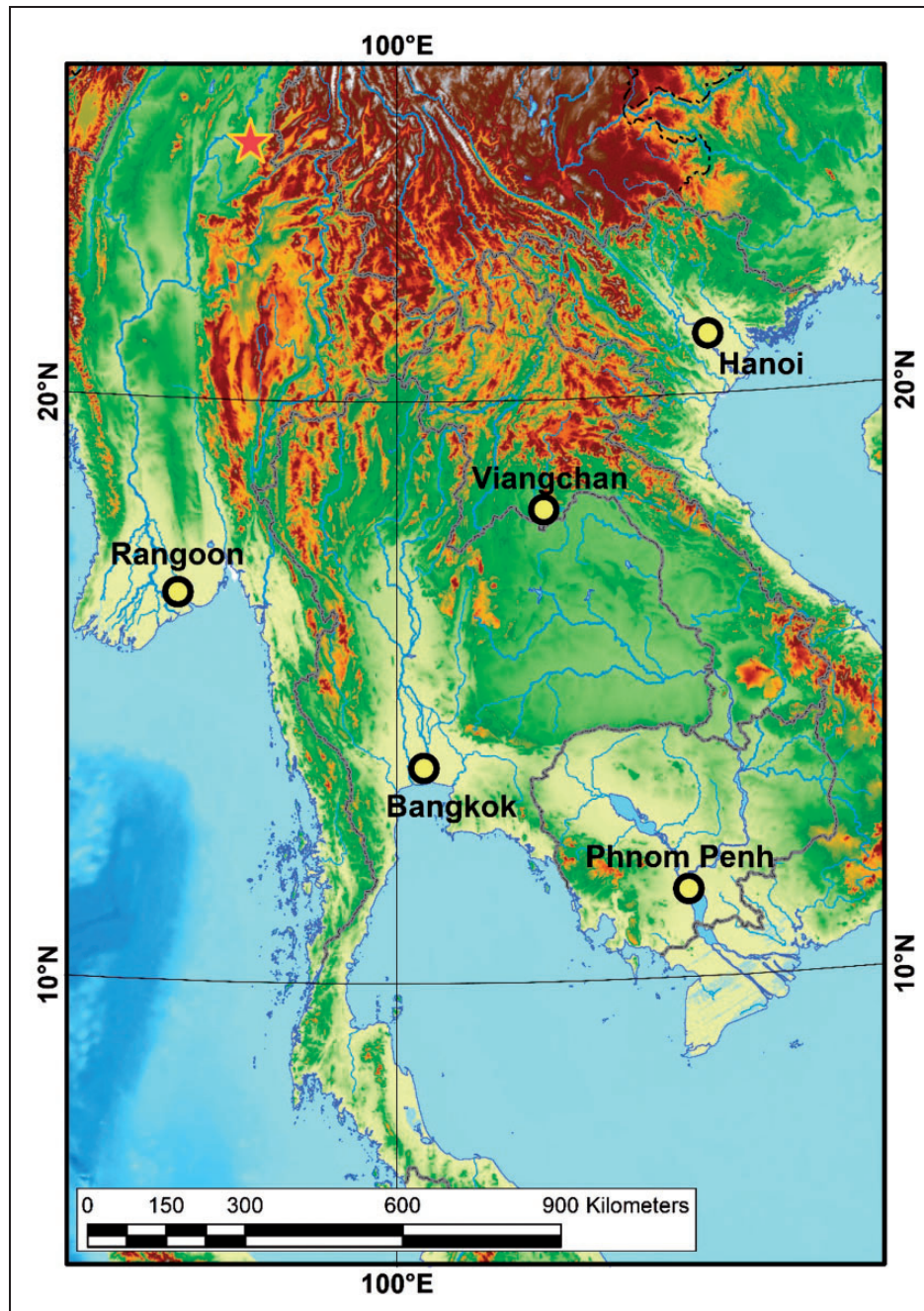


Figure 1. Map of our new record (red asterisk) of the temperate invasive lineage of *Sinanodonta* aff. *woodiana* from Myanmar.

(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 analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The resulting sequences were checked using a sequence alignment editor (BioEdit v. 7.2.5; Hall, 1999).

The alignment of the *COI* sequences was performed using the ClustalW algorithm (Thompson, Higgins, &

Gibson, 1994). For the phylogenetic analyses, each *COI* sequence of aligned data set was trimmed, leaving a 659-bp fragment. Then, identical *COI* sequences were removed from the data set using an online FASTA sequence toolbox (FaBox1.41; Villesen, 2007), leaving 35 haplotype sequences (including the two out-group taxa). For phylogenetic analyses, we used the *COI* data set with unique haplotypes. The best evolutionary models for each partition calculated on the base of corrected



Figure 2. Habitat of *Sinanodonta* aff. *woodiana* in Myanmar: A floodplain lake near Bhamo, the Irrawaddy River drainage basin, Kachin State, Myanmar.

Akaike Information Criterion through MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) were as follows: (a) first codon of the *COI*: HKY; (b) second codon of the *COI*: TN93 + G ($G = 0.21$); (c) third 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, Pfeiffer, & Schwartz, 2010). Four Markov chains, one cold and three heated (temperature = 0.1), were run simultaneously for 25,000,000 generations. The resulting phylogeny was constructed using a tree figure drawing tool (Archaeopteryx v. 0.9901 beta; Han & Zmasek, 2009).

Results

A well-established population of *S. aff. woodiana* was discovered in a floodplain lake of the upstream section of the Irrawaddy River basin, Kachin State, Myanmar (Figures 1 to 3). The mean shell length is 110.3 mm (90.2–128.5 mm, $N = 20$).

The three sequenced specimens of *S. aff. woodiana* belong to a single *COI* haplotype, which is identical to that recorded from invasive populations in Europe and from native populations in China (Figure 4). This haplotype belongs to the temperate invasive lineage of Bolotov et al. (2016), which also comprises three additional haplotypes from China.

The mean *COI*p-distance within the temperate invasive lineage varies from 0.15% to 0.66%.

Populations from Malaysia, Indonesia, and Philippines belong to the tropical invasive lineage of Bolotov et al. (2016; see Figure 4). The sequenced specimens from Singapore are also representatives of this lineage, although they were obtained from ornamental trade (Ng et al., 2016). In contrast, the populations from Vietnam belong to a separate lineage that appears to be a native species, which inhabits the Red River basin (see Figure 4).

Discussion

Among countries of Southeast Asia, representatives of *S. woodiana* species complex were recorded from Malaysia, Indonesia, Singapore, Philippines, Cambodia, and Vietnam but were unknown from Myanmar, Thailand, and Laos (Bolotov et al., 2016; Ng et al., 2016; Zieritz et al., 2016, 2017).

Our sequenced specimens of *S. woodiana* from Irrawaddy basin surprisingly reveal the same *COI* haplotype that was recorded from invasive European populations and that belongs to the temperate lineage of Bolotov et al. (2016).

The taxonomy of *S. woodiana* species complex is unclear because it includes several divergent mtDNA lineages, each of which may represent a separate cryptic

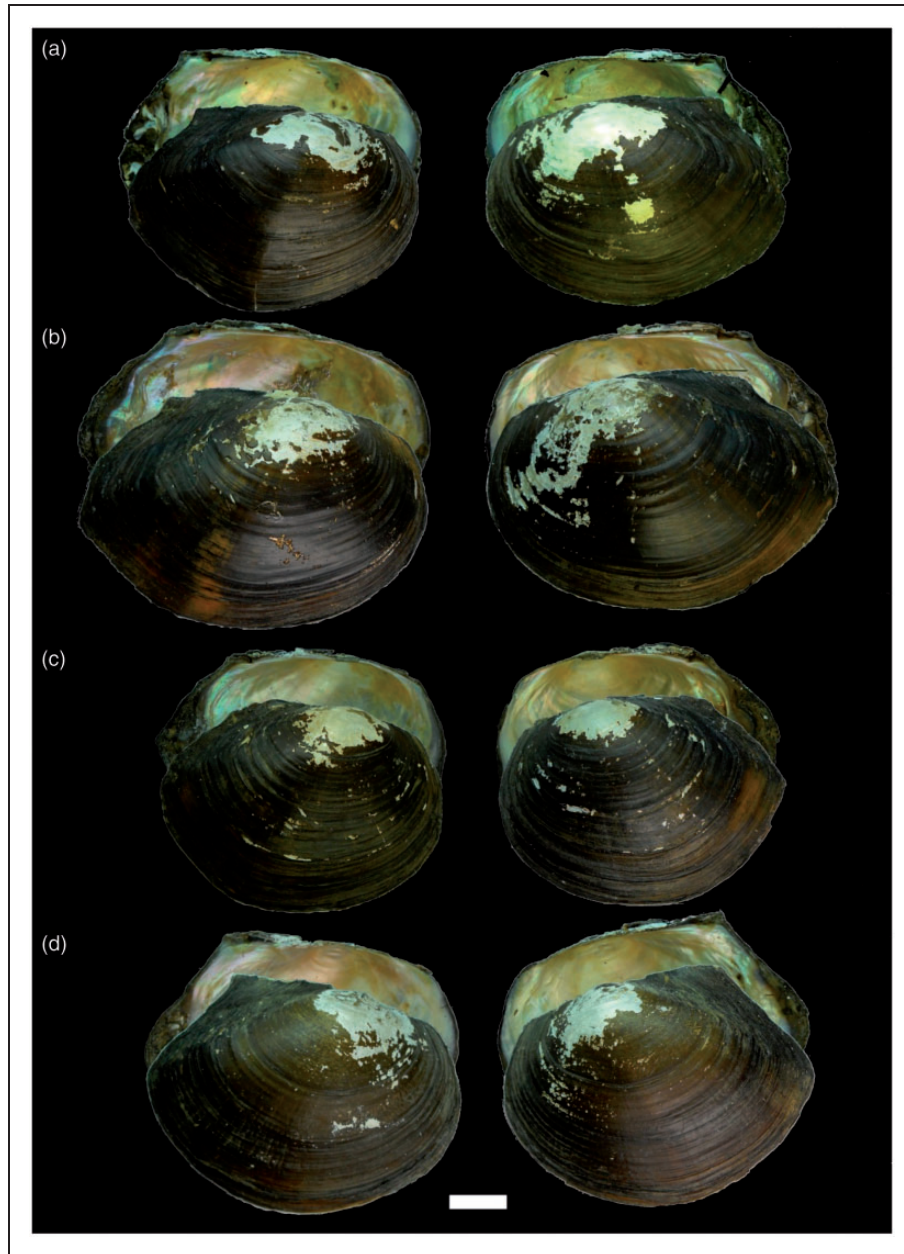


Figure 3. Shells of *Sinanodonta* aff. *woodiana* from a floodplain lake near Bhamo, the Irrawaddy River drainage basin, Kachin State, Myanmar: (a) Specimen no. RMBH biv269/15*, (b) Specimen no. RMBH biv269/16*, (c) Specimen no. RMBH biv269/9, and (d) Specimen no. RMBH biv269/6. An asterisk indicates sequenced specimens (Table 1). Scale bar = 2 cm.

species (Bolotov et al., 2016; Froufe et al., 2017). Moreover, there is a plethora of nominal taxa that were synonymized with *S. woodiana*. With respect to an integrative approach (Konopleva, Bolotov, Vikhrev, Gofarov, & Kondakov, 2017), a taxonomic revision of such a species complex should be based on molecular sequence data obtained from the topotypes of each nominal taxon because old museum lots with dry shells are

not appropriate for the extraction of high-quality DNA. The holotype of *S. woodiana* was collected from the Pearl River near Guangzhou (USNM: voucher no. 86380, the type locality: “Canton, China”), but any *Sinanodonta* sequences from this region, which may serve as reference sequences for the species, are not available.

Finally, the genus *Sinanodonta* should be a focus of the extensive taxonomic revision, along with efforts to

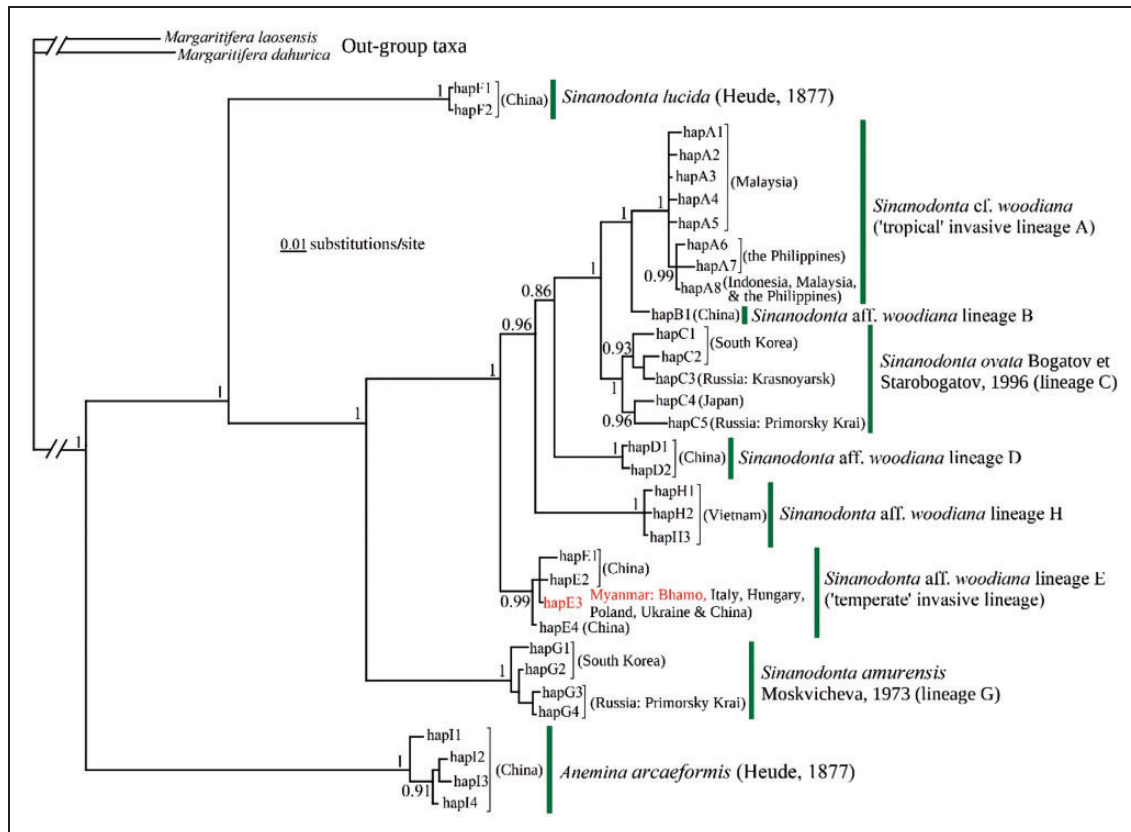


Figure 4. The majority-rule consensus phylogenetic tree of *Sinanodonta* spp. and sister taxa recovered from Bayesian inference analysis of an alignment comprising 33 unique COI haplotypes. In addition, haplotypes of *Margaritifera dahurica* and *M. laosensis* were used as out-groups (GenBank acc. nos. KJ161530 and KR006699, respectively). Numbers near nodes are Bayesian posterior probabilities. Haplotype codes and lineage codes correspond to Table 1. The scale bar indicates the branch length.

estimate the distribution of invasive lineages and to detect the environmental threats and risks from alien mussel impacts.

Implications for Conservation

The actual distribution of *S. woodiana* in Myanmar is still unknown, although it seems to be rather local, because it was not recorded anywhere during our extensive field-works across the country (Bolotov et al., 2017a, 2017b). However, its possible spread through the country may affect native benthos communities, which include many unique endemic taxa of the Unionidae (Bolotov et al., 2017b). Our survey in the Bhamo area has shown that small lakes, which harbor the majority of mussel species in this region, may represent a suitable habitat for *S. woodiana*. The Chinese pond mussel could successfully inhabit these waterbodies and could compete with native mussels for resources and host fishes (Bolotov et al., 2016; Zieritz et al., 2016). Negative effects of the invasive *S. woodiana* on freshwater ecosystem functioning have

been reported from Europe and Malaysia (Donrovich et al., 2017; Sousa et al., 2014; Zieritz et al., 2016). In Malaysia, this alien species replaced the native unionid assemblages in many river systems (Zieritz et al., 2016). However, we assume that further expansion of the temperate lineage across Southeast Asia may be limited due to the specific environment of tropical floodplain freshwater systems. These environmental conditions appear too warm for this cryptic taxon, which most likely originated from temperate China (see Figure 4). In contrast, a possible invasion of the tropical lineage into large freshwater basins of Myanmar (e.g., the Irrawaddy, Salween, and Sittaung drainage basins) seems to be much more dangerous because this lineage is adapted well to the environment of tropical monsoon freshwater systems, and it may therefore spread throughout the country.

Appearance of the Chinese pond mussel in Myanmar requires regular monitoring and other environmental measures from the government and conservation organizations. Dramatic declining of native species in Malaysia as a result of *S. woodiana* invasion is an example of

lacking of special measures for the invasion management (Zieritz et al., 2016). We assume that current local range of *S. woodiana* in Myanmar provides benefits for invasion control and eradication efforts.

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