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Spreading of the Chinese pond mussel, *Sinanodonta woodiana*, across Wallacea: One or more lineages invade tropical islands and Europe

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ABSTRACT

Here we report the first molecular data on an invasive *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) population from Lesser Sundas (Flores Island, Indonesia). The DNA barcoding reveals that this population belongs to a mitochondrial lineage that is widely distributed across Malaysia. The mean *COI* *p*-distance between this 'tropical' lineage and invasive European populations is 5.3 ± 1.0% which clearly corresponds to the interspecific difference. The representatives of such a 'tropical' lineage have likely invaded other Indonesian islands together with the introduction of their host fish species, because our sequenced specimens were taken from a population on the southeastern edge of the current *Sinanodonta* distribution. The molecular identification of invasive populations of the Chinese pond mussels using available online databases such as the BOLD IDS and NCBI's Genbank is doubtful, because there are at least seven distinct mitochondrial lineages, hence each of them could belong to a separate cryptic taxon.

1. Introduction

The Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) is an invasive species, with a native range that expands into East Asia from the Russian Far East (Amur Basin) to Taiwan, Indo-China and Malaysia (Watters, 1997; Popa et al., 2015). Currently, the species has widely spread across many regions, *e.g.*, Europe, the USA, Hispaniola, Costa Rica, Indonesia and Philippines (Djajasasmita, 1982; Watters, 1997; Bogan et al., 2011; Demayo et al., 2012; Soroka et al., 2014; Lopes-Lima et al., 2016). According to some reviews (Djajasasmita, 1982; Watters, 1997), *S. woodiana* is widely ranged across Indonesia and examples have been recorded from Java, Sumatra, Sulawesi, Lombok and Moluccas. All of these invasive populations are considered to belong to a single polymorphic species as based, especially, on the morphological data. Molecular studies of *S. woodiana* are very limited, but a few sequences of the mitochondrial *cytochrome c oxidase* subunit I (*COI*) gene are published in Online databases such as the BOLD Identification System (IDS) and the NCBI's Genbank. The majority of these sequences were not yet discussed, excluding those obtained from certain European populations (Soroka, 2010; Soroka et al., 2014). Despite the broad range of *S. woodiana* in Indonesia, any molecular data regarding these invasive tropical populations are not available.

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The *S. woodiana* is a broad host generalist, which can complete its development on many host fish species (Watters, 1997; Douda et al., 2012). Many introduced and native fish species were reported as suitable hosts for *S. woodiana* glochidia in Indonesia, including the silver carp (*Hypophthalmichthys molitrix*), the Nile tilapia (*Oreochromis niloticus*), the Snakeskin gourami (*Trichopodus pectoralis*), the Philippine catfish (*Clarias batrachus*), etc. (Djajasasmita, 1982; Hamidah, 2012, 2013). The abundant invasive *S. woodiana* populations could impact native mussels and other benthic invertebrates as competitors for food, space and hosts, as a source of parasites, as well as 'ecosystem engineers' that are changing the biological and physical characteristics of freshwater systems (Douda et al., 2012; Sousa et al., 2009, 2014; Lopes-Lima et al., 2016). Generally, invasion by such a large species as *S. woodiana* could affect hydrology, biogeochemical cycling, and biotic interactions through several mechanisms, with impacts ranging from individuals to ecosystems (Sousa et al., 2014). In contradiction to European countries and the USA, the invasive populations of *S. woodiana* in Indonesia are considered as an important protein source to local communities, which has significant economic value (Djajasasmita, 1982; Mujiono, 2011; Hamidah, 2012). The Indonesian populations of this species are actively exploited as a food as well as a feed for cultivated fishes and other animals (Koroh and Lumenta, 2014; Heriyani and Suprapto, 2015).

In the present investigation, we report the recent occurrence of *S. woodiana* from Lesser Sundas (Flores Island, Indonesia) and provide the first molecular data for Indonesian populations of this invasive mussel species. Based on these data, we briefly discuss the phylogenetic affinities of freshwater mussels that invaded the Wallacean Region and its relationships with the European invaders and native Chinese specimens.

2. Material and methods

2.1. Data collection

Available *COI* sequences of *S. woodiana* and closely related taxa were downloaded from the BOLD IDS and NCBI's Genbank (accessed 5.03.16), resulting in 33 sequences of the *COI* gene from Europe, China, South Korea, Japan and Malaysia (Table 1). We did not use the *COI* sequence, which was provided for the *S. woodiana* specimen from Romania (NCBI's Genbank acc. no. JQ435822) because it shows multiple nucleotide substitutions compared with those of other European specimens (Soroka et al., 2014), which might reveal a sequencing error. We have analyzed two available Wallacean specimens of *S. woodiana*, which were collected by a local fisherman from the upstream portion of the Wae Racang River (West Flores, 8°38′25″S, 120°00′44″E, alt. 335 m a.s.l.) in January 2015 during low water level (Supplementary Fig. 1). This population is an important and actively used food source for the local community of the Werang village (M. Albarran Valle, pers. comm.). Sequences of *Margaritifera dahurica* and *Margaritifera laosensis* were used as outgroups (Genbank acc. nos. KJ161530 and KR006699, respectively).

2.2. DNA extraction, PCR and sequencing

A total genomic DNA was extracted from the alcohol-preserved foot mussel 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 et al., 1994). The PCR mix contained approximately 200 ng of total cellular DNA, 10 pmol of each primer, 200 μmol of each dNTP, 2.5 μl of PCR buffer (with 10 × 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 μl. Thermocycling included one cycle at 95 °C (4 min), followed by 34 cycles of 95 °C (45 s), 56 °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 ABI PRISM® 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). The sequence alignment was performed in MEGA6 software (Tamura et al., 2013). Sequences were aligned directly using ClustalW algorithm. Maximum likelihood (ML) analyses were conducted using RAxML v. 8.2.6 HPC Black Box (Stamatakis, 2006) at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al., 2010). Three partitions were used, namely the three codons of the *COI* gene. A unique GTR model was applied for each partition with corrections for a gamma distribution. Nodal support values were estimated using an automatic rapid bootstrapping algorithm (Stamatakis et al., 2008) and the majority-rule consensus tree was constructed from the independent searches. The genetic divergences between separate sequences or these groups were calculated based on a *p*-distance of MEGA6 (Tamura et al., 2013).

2.3. Range mapping

The reliable records of *S. woodiana* from Indonesia, Malaysia and Philippines were obtained from various published sources (Supplement 1). Based on the collected geographical data, the map of occurrences of this bivalve in Southeast Asia was performed using ESRI ArcGIS 10. To clarity of the map, closely located records were merged into single locations.