Molecular Identification of the Chinese Pond Mussel *Sinanodonta woodiana* (Lea, 1834) from Mindoro and Leyte Islands, Philippines

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ABSTRACT

The Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) is a large freshwater bivalve species of the family Unionidae and a known invasive alien species. Proper verification of its identity as well as its source population is crucial for the control of its spread. However, its high plastic shell morphology that resembles other non-invasive species of unionids can be an obstacle. The distribution and ecological impact of this invasive unionid is not fully understood and should be further investigated to prevent further spread in the Philippines. In this study, we used the cytochrome oxidase I (*cox1*) gene to verify the identity of putative *S.woodiana* samples collected from Bato Creek in Oriental Mindoro and Lake Danao in Leyte, Philippines and elucidate their source populations. Eighteen cytochrome oxidase subunit 1 (*cox1*) barcodes were generated from samples collected from Lake Danao, Leyte (n=13) and Bato Creek, Oriental Mindoro (n=5). These barcodes were subjected to Basic Local Alignment Search Tool (BLAST) analysis, which showed that the *cox1* sequences from the Philippine samples matched with those of *S. woodiana* (>94%) found in GenBank. The sequences were then aligned with *cox1* sequences of *S. woodiana* and other unionid representatives from GenBank.

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Phylogenetic and haplotype network analyses also showed three haplotypes (Hap 1, 2, and 4) of *S. woodiana* samples from Lake Danao and Bato Creek. Hap 1 and 2 are distinct haplotypes observed in Lake Danao samples while Hap 4 is shared between Lake Danao and Bato Creek samples and have clustered with conspecific specimens from Malaysia and Indonesia, suggesting their potential Island Southeast Asian origin.

Keywords: Unionidae, DNA barcoding, Invasive Alien Species, *Sinanodonta woodiana*

INTRODUCTION

The study of invasive alien species (IAS) cannot be overemphasized, and the negative impact of these organisms cannot be underestimated as they often affect the agricultural sector and cause significant public health problems (Andersen et al. 2004). IAS have been introduced either accidentally or purposely, and many are now uncontrolled in newly colonized ecosystems where they thrive and continually cause significant economic and ecological damage (Anderson 1993). A common example is the introduction of the golden apple snail *Pomacea canaliculata* in the Philippines, which was originally imported as an alternative protein source for farmers but are now considered an agricultural pest due to their uncontrolled proliferation in agricultural lands (Anderson 1993; Halwart 1994). Another freshwater mollusk, the invasive unionid Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834), is now considered a major problem in many countries in Europe, North America and other parts of Southeast Asia (Kraszewski and Zdanowski 2007; Popa and Murariu 2009; Lajtner and Crnčan 2011; Colomba et al. 2013; Kamburska et al. 2013; Soroka et al. 2014). This bivalve species, which closely resembles its non-invasive relatives in the genus *Anodonta*, negatively affects local anodontine populations including *A. anatina* and other unionids in Europe (Guarneri et al. 2014). *S. woodiana*'s invasive capacity is primarily attributed to its high filtering capacity, and the cross-resistance induction of its parasitic glochidium larva to its fish host (Donrovich et al. 2017; Douda and Čadková 2017). These characteristics show that these invasive mussels can become "masters of invasion" due to their high tolerance of various environmental factors such as changes in temperature, moisture, humidity, and utilization of hosts, thus making them able to survive and reproduce in many types of habitats (Mwatawala et al. 2006; Davidson et al. 2011).

Sinanodonta woodiana is a large freshwater dioecious bivalve species of the family Unionidae. This family is a large group of freshwater mussels with several member species endemic in freshwaters of North America, Europe and East Asia. *S. woodiana*'s native distribution include the freshwater basins of Amur River, Hanka Lake, China, Hong Kong, Taiwan, Cambodia, Thailand, and Japan but were also reported in other non-endemic territories in Europe where they are now widespread and have established stable populations (Kraszewski and Zdanowski 2007; Popa and Murariu 2009; Latjner and Crnčan 2011; Colomba et al. 2013; Kamburska et al. 2013; Soroka et al. 2014). The invasion of *S. woodiana* in European freshwaters was due to the introduction of fish stocks infested with the bivalve's parasitic larval glochidium which primarily attaches to the gills of its fish host (Kraszewski 2006; Kraszewski and Zdanowski 2007; Guarneri et al. 2014).

S. woodiana produces large biomass both in natural and colonized areas, and has the ability to tolerate a wide range of physical and chemical factors which contribute to its capacity to invade new aquatic habitats. This bivalve is also a broad host generalist, often able to develop in both co-invasive and native fish hosts (Douda et al. 2012). Moreover, its invasiveness is also associated with its ability to grow fast and produce high numbers of offspring, and to its long life span ranging from 10-15 years (Dudgeon and Morton 1983; Afanasjev et al. 2001; Kraszewski and Zdanowski 2007; Guarneri et al. 2014). Aside from food and fish host competition, *S. woodiana* can quickly dominate both natural and pre-colonized habitat by establishing a strong benthic-pelagic coupling that may result in changes in the biocenoses of organisms affecting the physical characteristics of freshwater systems (Kraszewski and Zdanowsk 2001; Kraszewski and Zdanowski 2007; Guarneri et al. 2014). Proper identification and detection of *S. woodiana* have been a challenge since its shell morphology resembles that of the native species of anodontines such as *Anodonta anatina* (Guarneri et al. 2014). In fact, the taxonomy of *S. woodiana* was originally assigned to the genus *Anodonta* but was later grouped to the more appropriate genus *Sinanodonta* to standardize differences in nomenclature and classification especially for inland water mollusks where anodontines and *S. woodiana* coexist (Kraszewski and Zdanowski 2007; Guarneri et al. 2014).

Morphology-based analysis and molecular tools for species identification have been used for *S. woodiana* in the past. Linear and geometric morphometrics were used for *S. woodiana* samples from Europe and other parts of Asia such as the Philippines (Demayo et al. 2012; Guarneri et al. 2014). Results showed that *S. woodiana*'s phenotype is highly plastic, and the variations in shell dimensions measured were due to the effects of various environmental factors. In the study

of Guarneri et al. (2014), populations of *S. woodiana* in two of Italy's largest lakes, Po and Maggiore, indicated that the species exhibits variation even between populations and may potentially lead to species misidentification. Similar results were reported on geometric morphometrics of *S. woodiana* specimens collected from two separate freshwater bodies (Lake Lanao and Lawis Stream) in Mindanao, Philippines. Variations observed in its shell were attributed to allometry within populations and varying environmental factors were hypothesized to play a major role. Molecular analysis for species identification was also carried out in European *S. woodiana* samples using the general barcode gene cytochrome *c* oxidase subunit 1 (*cox1*) (Guarneri et al. 2014), confirming 99.84-100% similarity of the samples compared using *S. woodiana* accessions in the Barcode of Life Database (BOLD). A more comprehensive analysis using various *S. woodiana cox1* gene accessions in GenBank (https://www.ncbi.nlm.nih.gov/) was generated using Bayesian Inference, which indicated two major lineages detected, namely temperate and tropical, in which samples from the Philippines clustered together with other *S. woodiana* from Indonesia and Malaysia to form the tropical lineage (Vikhrev et al. 2017).

The status of *S. woodiana* distribution in the Philippines is limited and is only known in few locations based on previous reports such as in Mindanao (Demayo et al. 2012). Another introduced unionid species with shell form and shape similarity with *S. woodiana* is *Cristaria plicata*, which is also present in the country but is used for pearl farming (Guerrero et al. 2002). The possibility of introducing the wrong unionid species for pearl farming is highly likely due to the striking resemblance and plastic shell morphology of the two species. Just like *S. woodiana*, *C. plicata* is considered an invasive exotic species and may pose occupational hazard such as injuring workers due to its sharp shell, but is also a source of food and income to people who culture it (Cagauan et al. 2007). *C. plicata* was detected in Taal Lake (Mutia et al. 2017), Lake Oro in Agusan del Sur (Sularte and Jumawan, 2016), and has been reportedly cultured in Nueva Ecija and Laguna for pearl farming (Guerrero 2002).

In this study, we conducted molecular identification through DNA barcoding by using the *cox1* gene from putative *S. woodiana* samples collected from Bato Creek and Lake Danao in the absence of a useful morphological key to confirm species identity. These aforementioned sites have no prior reports on the occurrence of *S. woodiana*. This study also utilized neighbor-joining and maximum likelihood (ML) algorithms and median-joining haplotype network analysis to further reveal the relationships of the two subpopulations in the Philippines with other specimens of *S. woodiana* from other countries accessed from GenBank.

MATERIALS AND METHODS

Sample Collection

S. woodiana samples were bought from local vendors along Lake Danao in the municipality of Ormoc, Leyte and handpicked from the waters along Bato Creek in the municipality of Victoria, Oriental Mindoro (Figure 1). The difference in sampling methodology arose due to a serendipitous discovery of *S. woodiana* in Oriental Mindoro during fieldwork for another unrelated study. Samples were stored in re-sealable plastic bags, placed in a styrofoam chest filled with ice cubes and then transported to the DNA Barcoding Laboratory at the Institute of Biology of the University of the Philippines Diliman in Quezon City. Photographs of the samples were likewise taken. Morphometrics of the Bato Creek, Oriental Mindoro specimens were also taken (Table 1).

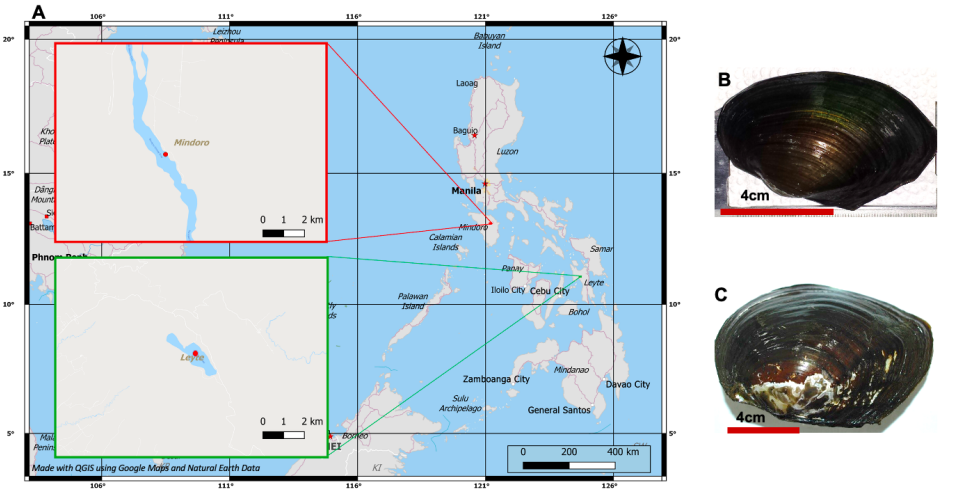

Figure 1. Location of the (A) study sites in Bato Creek, Mindoro and Lake Danao, Leyte. *Sinanodonta woodiana* sample collected from (B) Bato Creek and (C) Lake Danao. Map adapted from ArcGIS base map downloaded from http://www.geoportal.gov.ph/viewer.

Table 1. Measurements of the dorsal shell length of 18 *S. woodiana* specimens presented in this study as well as their corresponding GenBank Accession Numbers.

Specimen	GenBank Accession Number	Dorsal Length (cm)
mindorobv1	MN322559	7.51
mindorobv2	MN322558	4.20
mindorobv3	MN322555	4.33
mindorobv4	MN322557	6.28
mindorobv5	MN322554	4.90
UN1	KX424967	11.5
UN2	KX424968	10.3
UN4	KX424969	11.5
UN5	KX424970	10.0
UN6	KX424976	11.0
UN8	KX424977	11.0
UN10	KX424978	10.0
UN12	KX424979	12.0
SW3	KX424971	*
SW4A	KX424972	*
SW5B	KX424973	*
SW7A	KX424974	*
SW8B	KX424975	*

*No Data

DNA Extraction and Polymerase Chain Reaction

Foot muscle tissue from 13 individuals of *S. woodiana* samples from Lake Danao and 5 individuals from Bato Creek were used. DNA extraction was performed using a commercial DNA extraction kit (Purelink® Genomic DNA Extraction Kit, Invitrogen Life Technologies or PureLink™ Genomic DNA Mini Kit, ThermoFisher Scientific) following the manufacturer's protocol with a final elution of 150 microliters (uL). For DNA quantitation using Nanodrop 2000c (Thermo Scientific), 1 uL was used for each sample. Two primer sets were used for *cox1* amplification, namely HCO-LCO (HCO: 5' TAAACTTCAGGGTGACCAAAAAATCA -3', LCO: 5'- GGTCAACAAATCATAAAGATATTGG -3') (Folmer et al. 1994) and StyHCO-StyLCOii (StyHCO: 5'- GAATTAAAAATATATACTTCTGGGTG -3', StyLCOii: 5'- ACGAATCATAAGGATATTGGTAC -3') (Fontanilla et al. 2017). Either primer pair, which targets the same region of *cox1*, was used for amplification.

For Lake Danao samples, polymerase chain reaction (PCR) was done by preparing a master mixture with the following components: 5 uL 10X PCR Buffer (-$MgCl_2$), 0.5 uL 0.1875 mM dNTP, 1.25 uL for each primer (StyHCO, StyLCOii, respectively), 5 uL Q buffer, 1 uL 25 mM $MgCl_2$, 0.125 uL Taq Polymerase (5U/uL), 8.875 uL nuclease-free dH_2O, and 2 uL DNA for a total of 25 uL per sample using a PCR condition described by Ma et al. (2012) and run in a 96-well thermocycler (MultiGene Optimax).

For the Bato Creek samples, the PCR master mix consisted of 5 uL 10X MyTaq™ PCR Buffer (with 5 mM dNTPs and 15 mM $MgCl_2$), 1 uL of forward and reverse primers (LCO, HCO, respectively), 1 uL of 50 mM $MgCl_2$, 0.125 uL of MyTaq™ DNA Polymerase (5U/uL) (Bioline, United Kingdom), 14.875 uL nuclease dH_2O, and 2 uL of DNA template for a total of 25 uL. PCR conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 36 cycles of denaturation at 92°C for 30 seconds, annealing at 51°C for 30 seconds, extension at 68°C for 2 minutes, and a final extension step at 68°C for 5 minutes run in a 96-well thermocycler (SimpliAmp, Applied Biosystems). The difference in reagents is not expected to affect results since specificity for the target area of *cox1* is dependent on the primers.

Agarose Gel Electrophoresis, Purification and Sequencing

Each PCR product was loaded in a 1% agarose gel stained with 1% ethidium bromide (EtBr), submerged in 0.5X TBE (1 L 5x TBE = 54 g Tris, 27.5 g Boric Acid, 20 mL of 0.5 M EDTA pH 8.0) and exposed to 100 volts of electricity for 30 minutes to separate amplicons (~650-700 bp) using a horizontal gel electrophoresis apparatus (Gel XL ultra v-2 Labnet). The gel was visualized in an ultraviolet transilluminator. Visible bands were cut carefully using sterile scalpel blades and were stored in 2 uL microcentrifuge tubes until purification. The purification process was done using a commercial gel extraction kit (QIAquick® Gel Extraction Kit, QIAGEN and Thermo Scientific™ GeneJET Gel Extraction Kit) following the manufacturer's protocol. A final elution volume of 50 uL was prepared per sample. Samples were sent to 1st Base Malaysia and Macrogen South Korea together with the primers used for single pass sequencing for both forward and reverse sequences.

DNA Sequences *In silico* Analyses

Forward and reverse sequences were assembled using Staden Package version 4.0 (Staden et al. 2000), and each consensus sequence was subjected to nucleotide BLAST® (Altschul and Koonin 1998) for determining related sequences. One hundred ninety *cox1* sequences of unionids were downloaded from GenBank (http://blast.

ncbi.nlm.nih.gov/) and included in the analyses. *S. woodiana cox1* sequences and other unionid *cox1* sequences were downloaded and aligned in BioEdit Sequence Alignment Editor 7.0.9.0 (Hall 1999) using the feature ClustalW (Gibson et al. 1996). Uniform gaps were deleted and the shortest sequence was used as a reference for cutting the edges of the alignment. The sequence alignment file was converted to nexus (.nex) format using DAMBE v. 6.4.81 (Xia 2013, 2017), a format readable by PAUP* version 4.01b10 (Swofford 2002) for tree construction and genetic distance calculation.

The substitution model was determined using jModelTest v.2.1.10 (Darriba et al. 2012), and the model with the best log-likelihood score was chosen using the Akaike Information Criterion (AIC) (Akaike 1973, 1974; Hurvich and Tsai 1993). Neighbor-joining (Saitou and Nei 1987) and maximum likelihood (ML) (Felsenstein 1981) tree construction methods were applied. Bootstrap replication was executed to determine branch support and reliability (bootstrap nreps=1000) (Felsenstein 1985). The generated ML tree was visualized and rooted using Tree Explorer (Tamura 1999), with the bootstrap supports for both tree construction methods incorporated. The tree was rooted on two species under Margaritiferidae, a sister family of Unionidae (Combosch et al. 2017), *Margaritifera margaritifera* (JN243891, DQ060171) and *Margaritifera auricularia* (KC703969, JX046574).

Unique haplotypes were then subjected to median-joining haplotype network analysis using NETWORK v5.0.1.1 (Bandelt et al. 1999) to determine the possible origin of Philippine *S. woodiana*. Haplotype network analysis aimed to determine the relationship between observed haplotypes in a dataset depending on a number of single nucleotide polymorphisms (SNPs) observed in the *cox1* gene.

RESULTS

A total of 18 *cox1* sequences obtained from *S. woodiana* individuals collected from Bato Creek, Mindoro and Lake Danao, Leyte (Figure 1, Table 1) were generated in this study with 21 identified haplotypes from the generated *cox 1* alignment (Figure 3, Table 2). BLAST results (Table 3) showed that the *cox1* sequences matched those of *S. woodiana* found in the GenBank (>94%). ML tree based on TRN+I model of DNA substitution as determined by jModelTest showed the distinct clustering of the Philippine specimens together with other *S. woodiana* specimens from Malaysia and Indonesia with 91% ML bootstraps (Figure 2).

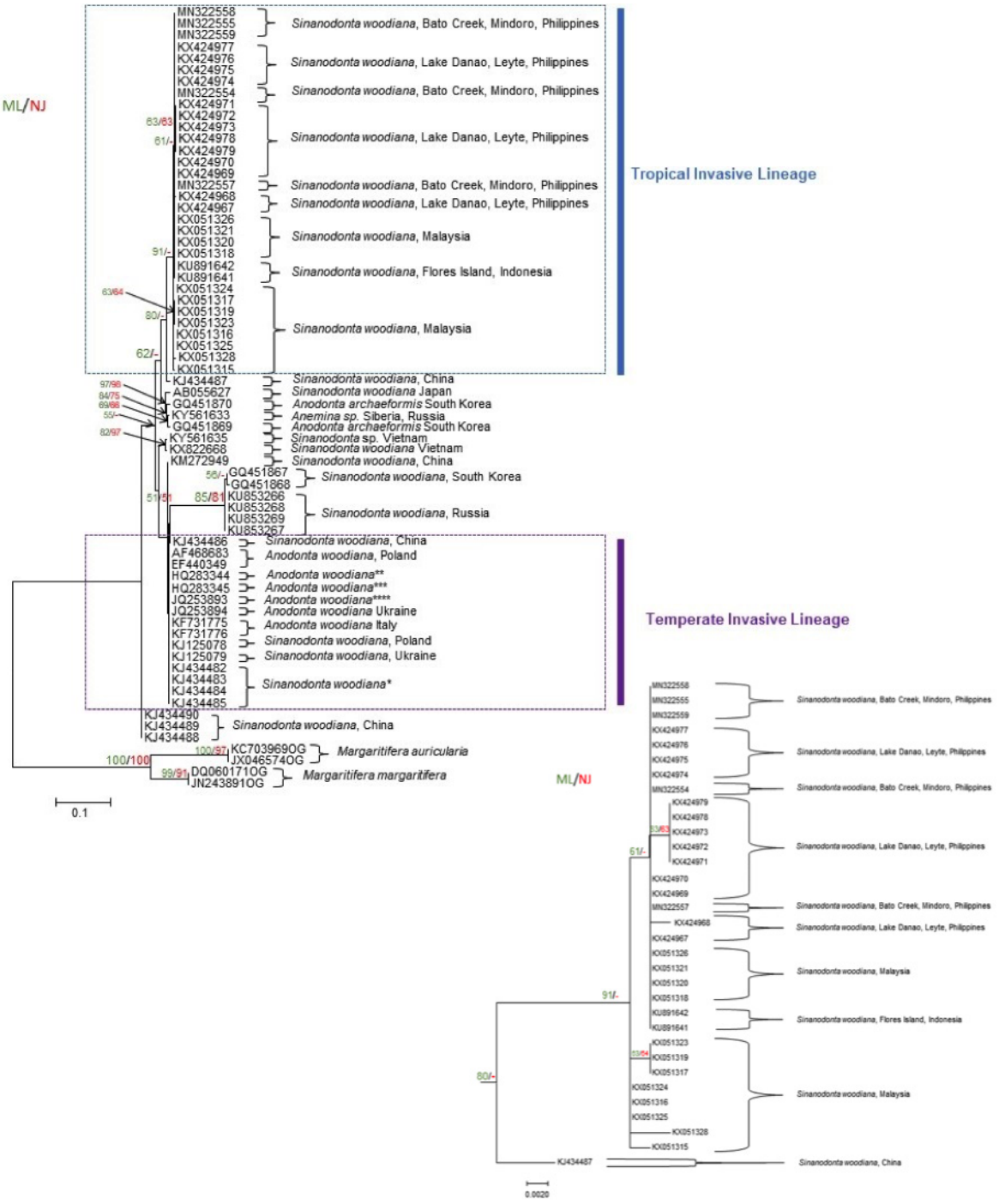

Figure 2. Maximum likelihood tree of *S. woodiana* geographical isolates using the mitochondrial cytochrome oxidase subunit 1 gene (*cox1*) tested in PAUP*. Bootstrap replication was done to test branch reliability (bootstrap nreps = 1000) to nodes with

bootstrap support ≤ 50 were only shown. The tree was rooted on four *cox1* sequences of two species under the Margaritiferidae family [*Margaritifera margaritifera* (GenBank Accession No. JN243891, DQ060171)] [*Margaritifera auricularia* (GenBank Accession No. KC703969, JX046574)]. ML tree on the right is an inset that shows the branches and bootstrap supports of the Tropical Invasive Lineage.

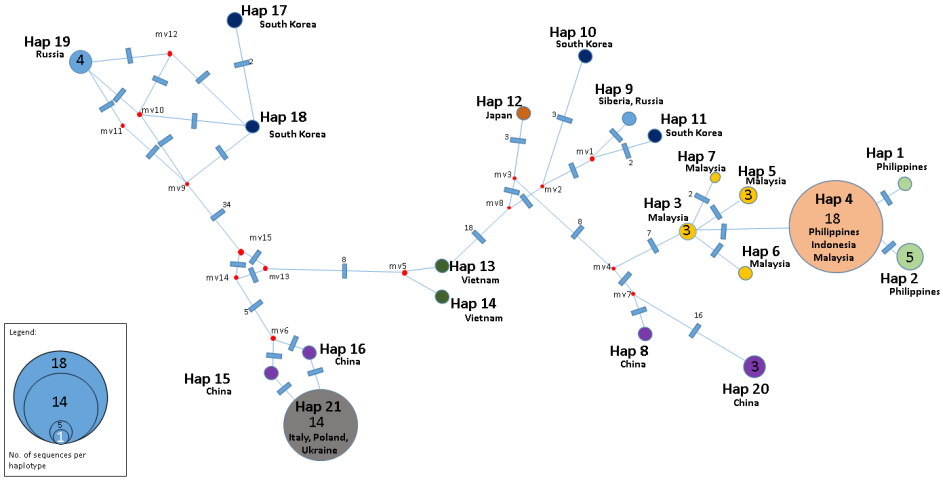

Figure 3. Median-joining network of *S. woodiana* haplotypes of the cytochrome *c* oxidase subunit I gene (*cox1*) from GenBank and sequences generated in this study. A total of 21 haplotypes were observed from the generated 520 bases long *cox1* DNA alignment. Red dots are putative haplotypes. Sequences from Lake Danao split into three haplotypes (Hap 1, Hap 2, Hap 4), while those from Bato Creek, Mindoro Oriental grouped with one of the Lake Danao haplotypes and other *S. woodiana* samples from Malaysia and Indonesia (Hap 4).

Table 2. Composition of the 21 distinct haplotypes used in the median-joining haplotype network analysis. Hap 1, 2, and 4 are haplotypes associated with *S. woodiana* samples collected from Bato Creek and Lake Danao in the islands of Mindoro and Leyte, respectively. Samples with missing locations have no sampling details indicated in GenBank.

Haplotype No.	GenBank Accession Number of Sequences	Species ID	Location
1	KX424968	*Sinanodonta woodiana*	Lake Danao, Leyte, Philippines
2	KX424979, KX424978, KX424973, KX424972, KX424971	*Sinanodonta woodiana*	Lake Danao, Leyte, Philippines
3	KX051325, KX051324, KX051316	*Sinanodonta woodiana*	Malaysia

Table 2. Composition of the 21 distinct haplotypes used in the median-joining haplotype network analysis. Hap 1, 2, and 4 are haplotypes associated with *S. woodiana* samples collected from Bato Creek and Lake Danao in the islands of Mindoro and Leyte, respectively. Samples with missing locations have no sampling details indicated in GenBank. (Cont'n.)

4	KX424977, KX424976, KX424975, KX424974, KX424970, KX424969, KX424967	*Sinanodonta woodiana*	Lake Danao, Leyte, Philippines
	MN322554 MN322559, MN322558, MN322555, MN322557,	*Sinanodonta woodiana*	Bato Creek, Mindoro, Philippines
	KX051326, KX051320, KX051318, KX051321	*Sinanodonta woodiana*	Malaysia
	KU891642, KU891641	*Sinanodonta woodiana*	Indonesia
5	KX051323, KX051319 KX051317	*Sinanodonta woodiana*	Malaysia
6	KX051315	*Sinanodonta woodiana*	Malaysia
7	KX051328	*Sinanodonta woodiana*	Malaysia
8	KJ434487	*Sinanodonta woodiana*	China
9	KY561633	*Anemina* sp.	Russia
10	GQ451870	*Anodonta archaeformis*	South Korea
11	GQ451869	*Anodonta archaeformis*	South Korea
12	AB055627	*Sinanodonta woodiana*	Japan
13	KY561635	*Sinanodonta* sp.	Vietnam
14	KX822668	*Sinanodonta woodiana*	Vietnam
15	KM272949	*Sinanodonta woodiana*	China
16	KJ434486	*Sinanodonta woodiana*	China
17	GQ451868	*Sinanodonta woodiana*	South Korea
18	GQ451867	*Sinanodonta woodiana*	South Korea
19	KU853269, KU853268	*Sinanodonta woodiana*	Russia
	KU853267, KU853266		
20	KJ434489, KJ434488, KJ434490	*Sinanodonta woodiana*	China
21	KJ125079	*Sinanodonta woodiana*	Ukraine
	JQ253894	*Anodonta woodiana*	Ukraine
	KJ125078	*Sinanodonta woodiana*	Poland
	EF440349, AF468683	*Anodonta woodiana*	Poland
	KF731775, KF731776	*Anodonta woodiana*	Italy
	KJ434483, KJ434482,	Sinanodonta woodiana	
	KJ434485, KJ434484		
	JQ253893, HQ283345, HQ283344	Anodonta woodiana	

Table 3. BLASTn results of 18 *S. woodiana* sequences generated from this study.

Accession Number	BLASTn Result	Query Cover	Percent Identity
KX424977	MH319868	100%	100%
KX424976	*Sinanodonta woodiana* UGSB 19578 isolate 24480 cytochrome c oxidase subunit I (COI)		
KX424975			
KX424970			
KX424974			
KX424969			
KX424967			
MN322559			
MN322558			
MN322555			
MN322557			
MN322554			
KX424968	MH319868	99%	100%
	Sinanodonta woodiana UGSB 19578 isolate 24480 cytochrome c oxidase subunit I (COI)		
KX424972	MG742232	100%	100%
KX424971	*Sinanodonta woodiana* cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial		
KX424979			
KX424978			
KX424973			

A total of three haplotypes were observed in all *S. woodiana cox1* sequences generated from this study, two were from Lake Danao (Hap 1 and Hap 2) and one was shared between Lake Danao and Bato Creek (Hap 4). These unique *cox1* sequences were used for haplotype network analysis (Figure 3). Results showed that the Lake Danao and Bato Creek samples of *S. woodiana* probably came from a population of *S. woodiana* from Malaysia introduced to the lake, though the mode of introduction is uncertain and still a subject for investigation. Among all haplotypes, *S. woodiana* from Malaysia and Indonesia are closest to the Lake Danao and Bato Creek specimens based on the level of support for branch reliability on the node where the Philippine specimens diverged from the Chinese *S. woodiana* and on the minimal SNPs observed among Philippine, Chinese, and other Island Southeast Asian (Malaysia and Indonesia) specimens as compared to European specimens (Figure 2).

DISCUSSION

The use of molecular data is crucial for species delineation if traditional taxonomy, which is based on morphological traits, is insufficient. In this study, the presence of the *S. woodiana* is confirmed using DNA barcoding using *cox1* (Hebert et al. 2003).

Phylogenetic and median-joining haplotype network analyses are useful tools for visualizing the relationships of samples with different geographical distributions. The ML tree of *cox1* of unionid taxa showed that the Lake Danao and Bato Creek specimens are conspecific with *S. woodiana*. Median-joining haplotype network analysis visualizes the possible origin of the Philippine *S. woodiana* populations using all distinct haplotypes of *S. woodiana* and creates a network based on mutation events that occurred among haplotypes. The Philippine specimens probably originated from Malaysia. The low haplotype diversity observed from the Lake Danao and Bato Creek samples might be due to founder effect; the same observation has been reported by Soroka et al. (2014) on their analysis of *S. woodiana* samples from Hungary and Poland.

DNA barcoding for species identification is proven useful for *S. woodiana* as the high plasticity of the morphology of *S. woodiana* hinders accurate identification of the species (Kraszewski 2006; Guarneri et al. 2014). In addition, other molecular markers and even the use of microsatellites have been suggested, which may provide useful information on its source and path of invasion and better resolution on the phylogenetic relationship of *S. woodiana* to other unionids (Bogan and Roe 2008; Popa et al. 2011).

The potential host fish species of *S. woodiana* within Lake Danao and Bato Creek system is also a promising area of research. Its possible interaction with local fish stocks such as *Oreochromis niloticus* (Nile tilapia), *Chanos chanos* (milkfish), as well as its known host *Cyprinus carpio* may be of particular interest due to the mussel being a generalist towards its host (Douda et al. 2012). Identification of the host fish species responsible for its invasion success by bearing its glochidium larva could reveal how the species was introduced in the Philippines. Such was the case for Europe where the bivalve utilized *Aristichthus nobilis* (bighead carp), *Ctenopharyngodon idella* (grass carp) and *Hypophthalamichthys molitrix* (silver carp) for its dispersal and propagation (Colomba et al. 2013). The introduction of many species for aquaculture could be the most likely route of introduction of the mussel in the Philippines. Therefore, a survey of freshwater habitats, particularly those utilized for aquaculture using introduced species, is necessary to assess the distribution and spread of this invasive species.

Moreover, further surveys on other endemic unionids with detailed descriptions on morphology, molecular identity, and life history is encouraged so that accurate comparative assessments could be made such as on how invasive exotic unionids like *S. woodiana* impact their distribution and dispersal. This is the case of *Rectidens sumatrensis*, a native unionid in Borneo, for which *S. woodiana* is a major competitor and a significant threat that has dominated freshwater habitats in the island and is likely associated with intentional introductions made for food source and ornamental purposes (Zieritz et al. 2018).

CONCLUSIONS

Invasive alien species are an emerging threat to global biodiversity. Due to the increase in mobility and access to agricultural goods and aquaculture products, opportunistic transport of organisms highly associated with these commodities may likewise occur. In this study, *S. woodiana* was detected from two sites in the Philippines. Phylogenetic and haplotype network analyses revealed that the Philippine populations are most closely related to other Island Southeast Asian haplotypes, such as those from Indonesia and Malaysia, and may even suggest their likely origin from these areas. Introduced fish species bearing the glochidium larva may be the most likely route of introduction in the Philippines. The mussel's resemblance to other native unionid species could also facilitate its further spread. Other potential freshwater areas in the Philippines can be surveyed for the presence of *S. woodiana* to determine its distribution and potential effect to local unionid species.

Moreover, *S. woodiana*'s resemblance to its relatives may facilitate its further spread as it could be mistaken for another unionid, such as the bivalve *Cristaria plicata*, which also belongs to family Unionidae and resembles *S. woodiana* morphologically. Like *S. woodiana*, it is native to East Asia and has been introduced in the Philippines.

Recommendations

Increasing sample size and adding more locations can be performed to provide more comprehensive data on the distribution of *S. woodiana* in the Philippines. Population genetic analysis can also shed light on the potential origin and route of spread in the Philippines. Detection and survey of the presence of the glochidium larva in fish hosts could aid in mitigating the spread of the IAS in the Philippines through its host. Areas with reported *C. plicata* should be prioritized to confirm species identity through barcoding and to survey other native unionid species inhabiting the sites.

Additionally, further investigations should be made on how *S. woodiana* was dispersed in these non-native territories such as in Lake Danao and Bato Creek. Around Lake Danao, for example, *S. woodiana* is being sold and consumed as a major shellfish commodity. Short informal interviews conducted by the authors with the vendors reported that *S. woodiana* was purposely introduced in the lake primarily as a source of food and income for the locals, though these reports should be carefully interpreted and further confirmed by implementing more scientific and systematic methods of extracting information from the locals such as interviews using questionnaires, focus group discussions, and key informant interviews. This can be explored in the future and information from these surveys will supplement biological data in tracing the origin and path of invasion of *S. woodiana*. Furthermore, examining the distribution network of both *S. woodiana* and its potential host fish species in the locality will provide significant information on the path and spread of invasion most especially if there are restocking practices being made using *S. woodiana* or of fish hosts infected with the mussel's glochidium to other freshwater bodies, thus contributing to the further spread.

In the study, *S. woodiana* samples collected from Lake Danao were bought from vendors in the same clump, making it difficult to trace the specific spot in the lake where these mussels were collected. A geographic information systems approach will help us understand its dispersion by mapping sites with stable *S. woodiana* populations in freshwater habitats and in sites where they are recently reported. In this manner, authorities will be able to manage *S. woodiana*'s invasion, protecting more freshwater habitats from colonization.

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Consent for Publication

All authors gave their consent for the publication of this work.

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Conflict of Interest

The authors declare that they have no competing interests.

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