INTRODUCTION

The individuals of a species within a population are not typically identical, and variation in DNA sequences and the resulting differences are regarded as genetic diversity, which are also known as polymorphisms (Ellegren & Galtier, 2016). This genetic diversity is important for the evolution of species as it allows for adaption to a particular environment (Väinölä & Johannesson, 2017). Mechanisms causing increased genetic diversity among species remain largely unknown (Leffler et al., 2012). Species in the wild with effectively large population sizes typically have high genetic diversity, as predicted by the neutral mutation hypothesis (Casillas & Barbadilla, 2017). By
contrast, self-fertilizing, inbred, and/or domesticated species have comparatively less genetic diversity (Cutter, Jovelin, & Dey, 2013). Recently, a correlation between life-history traits and level of genetic diversity within species across phylogeny has been elucidated (Romiguier et al., 2014).

Ascidians, also known as sea squirts, are the most abundant class of the subphylum Tunicata and are distributed along shorelines worldwide (Shenk & Swalla, 2011). They are sessile marine invertebrates belonging to the subphylum prochordata and are widely used as a model organism for developmental and evolutionary studies (Lemaire, 2011). Ascidians exhibit multiple morphological characteristics, from small colonial to colorful and large solitary forms. They are divided into three major well-accepted orders, namely, Phlebobranchia, Aplousobranchia, and Stolidobranchia, based on the branchial sac morphology of the adults (Stolfi & Brown, 2015). However, within the phylogenetic tree, Phlebobranchia and Aplousobranchia show a closer relationship with Thaliacea, which is also a Tunicata class different from Asciidae, whereas Stolidobranchia remains a distinct and monophyletic group (Delsuc et al., 2018). Over the course of several decades, the Ascidiae have been shown to be an important class for ecological species because of their invasive potential (Zhan, Briski, Bock, Ghobooli, & Maclsaac, 2015) along with their ability to adapt to new environments (Hawes et al., 2019). Transportation of ascidians attached to ship hulls as fouling material and within the ballast water of ships has enabled them to invade new territories (Lambert, 2007). This phenomenon has major impacts on local marine biodiversity as well as aquaculture industries. Therefore, the Asciidae were recently considered as important model species for the study of nonindigenous species (NIS) worldwide (Zhan et al., 2015).

The mitochondrial genome is highly conserved across bilaterian phyla and typically contains 37 genes (Boore, 1999), with only 13 protein-coding genes. Among them, cox1 is used as a DNA barcode for species identification (Hebert, Ratnasingham, & deWaard, 2003), genetic diversity patterns, and population genetic analysis (Moritz, Dowling, & Brown, 1987). In the recent decades, several Tunicata mitochondrial genomes have been sequenced (Gissi, Iannelli, & Pesole, 2004; Griggio et al., 2014; Singh et al., 2009; Yokobori, Oshima, & Wada, 2005; Yokobori, Watanabe, & Oshima, 2003). The sequencing data revealed that Tunicata species have high mutation rates compared with other animals (Griggio et al., 2014). Gene arrangements in the mitogenome of Tunicata are not conserved, suggesting high plasticity (Gissi et al., 2010). A mitogenomic approach has been used for cryptic species identification within Ciona (Iannelli, Pesole, Sordino, & Gissi, 2007), as well as for population genetic study for several ascidian species [Goldstien et al., 2011; Lopez-Legentil, Turon, & Planes, 2006; Villalobos, Lambert, Shenkar, & Lopez-Legentil, 2017; Zhan et al., 2012].

Here, we identified three ascidian species Ciona robusta (Hoshino & Tokioka, 1967), Ciona savignyi (Herdman, 1882), and Styela clava (Herdman, 1882) from northeast of China and examined their genetic diversity patterns. A comparative analysis of these species isolated from China with the samples elsewhere in the world using cox1 as a genetic marker was also performed to distinguish native from invasive ascidian populations.

## 2 MATERIALS AND METHODS

### 2.1 Animal collection

Adult animals were collected from the Rongcheng Bay area (37°55′N, 122°12′E China) and were maintained in the laboratory in seawater tanks with aeration and constant illumination. Ascidian species were identified morphologically. C. robusta, C. savignyi, and S. clava adults were dissected, and internal tissues were collected for DNA extraction and sequencing.

### 2.2 DNA extraction, cox1 amplification, and sequencing

Tissue sections were deposited into Eppendorf tubes containing digestion buffer and were incubated overnight at 50°C with shaking. In the following day, samples were treated with RNase for 15 min at 37°C to remove total RNA, and the phenol-chloroform extraction method was used for genomic DNA extraction. Around 500 μl of phenol-chloroform was mixed and incubated at room temperature for 5 min with rocking. The mixture was then centrifuged for 10 min at room temperature. The upper aqueous layer was transferred to a fresh tube, and 1 ml ethanol was added for DNA precipitation. Tubes were centrifuged again for 10 min at room temperature, and the supernatant was discarded. DNA pellets were air-dried and then resuspended in water. DNA quality was checked on a 1% agarose gel, and concentration was measured using a Nanodrop spectrophotometer. The primers for the amplification of cox1 from C. robusta, C. savignyi, and S. clava were designed according to published mitogenomes (Gissi et al., 2004; Griggio et al., 2014; Yokobori et al., 2003) as follows: (5′-CATATAGTTGAAACTATAAGATTC-3′, 5′-AGCCTTAAATACTGGTGAAG-3′), (5′-ATGTATAATTGATTAAATC-3′, 5′-TCTTTTCATAACTGGAGATAC-3′), and (5′-ATGAGTTGTTGG-3′, 5′-ATGTATAATTGATTAATC-3′, 5′-ATGAGTGTTGG-3′, 5′-ATGTATAATTGATTAATC-3′, 5′-ATGAGTGTTGG-3′, 5′-ATGTATAATTGATTAATC-3′, 5′-ATGAGTGTTGG-3′, 5′-ATGTATAATTGATTAATC-3′, 5′-ATGAGTGTTGG-3′). The sequencing data revealed that Tunicata species have high mutation rates compared with other animals (Griggio et al., 2014). Gene arrangements in the mitogenome of Tunicata are not conserved, suggesting high plasticity (Gissi et al., 2010). A mitogenomic approach has been used for cryptic species identification within Ciona (Iannelli, Pesole, Sordino, & Gissi, 2007), as well as for population genetic study for several ascidian species [Goldstien et al., 2011; Lopez-Legentil, Turon, & Planes, 2006; Villalobos, Lambert, Shenkar, & Lopez-Legentil, 2017; Zhan et al., 2012].

Here, we identified three ascidian species Ciona robusta (Hoshino & Tokioka, 1967), Ciona savignyi (Herdman, 1882), and Styela clava (Herdman, 1882) from northeast of China and examined their genetic diversity patterns. A comparative analysis of these species isolated from China with the samples elsewhere in the world using cox1 as a genetic marker was also performed to distinguish native from invasive ascidian populations.

### 2.3 Phylogenetic analysis

The cox1 sequences from three ascidian populations at different regions of the world (Table S1) were retrieved from the NCBI database to build multiple sequence alignments using ClustalW (Thompson, Higgins, & Gibson, 1994) hosted by MEGA7.0 (Kumar, Stecher, & Tamura, 2016). Only the unique haplotype datasets were used for the multiple sequence alignments (Table S1). Neighbor-Joining (NJ) and maximum parsimony (MP) methods were employed to construct a phylogenetic tree with 1,000 bootstrap estimations in the default
setting using MEGA7.0 (Kumar et al., 2016). The barcode region of the cox1 sequence (accession no. HM151268.1) of Halocynthia roretzi was used as an out-group.

### 2.4 Genetic diversity, neutrality test, and population structure

Multiple sequence alignments of cox1 from three ascidians were performed separately in ClustalW (Thompson et al., 1994) hosted by MEGA7.0 (Kumar et al., 2016) using default settings. Genetic diversity parameters, including haplotype number (H), haplotype diversity (Hd), nucleotide difference (K), mutation number per sequence (θ), number of segregating sites (S), and nucleotide diversity (Pi), were estimated using DnaSP software (Rozas, Sanchez-DeBarrio, Messeguer, & Rozas, 2003). For the neutrality test, Tajima’s D and Fu and Li’s D* methods were used, with an additional evaluation using DnaSP software (Rozas et al., 2003). Relationships among the three ascidians cox1 haplotypes found globally, including those from China (Table S1), were determined using a median-joining method in the network software (Bandelt, Forster, & Rohl, 1999). To infer the population structure and understand the connectivity between native and invasive ascidian populations, we performed molecular variance (AMOVA) analysis using cox1 haplotypes from samples available in the database (Table S1) as well as those in this study using the ARLEQUIN 3.11 software (Excoffier & Lischer, 2010) with 1,000 permutations.

### 3 RESULTS

#### 3.1 Identification of three ascidians by morphological characteristics

All three ascidian samples were collected from the Rongcheng Bay area, which is a part of the Yellow Sea in Northeast China (black arrow, Figure 1a). The overall adult morphology between Ciona spp. and S. clava consisted of a vase-like shape. The tunic in Ciona spp. was soft and semitransparent, whereas in S. clava was hard and dark brown (Figure 1b–d). Consequently, the gonoducts were clearly visible by naked eye in Ciona spp. (arrows in Figure 1b,c), but were not visible in S. clava adults (Figure 1d). All three species were solitary, sessile, and attached on a submerged surface. In addition, there were other distinguishing features between Ciona spp. as the outer tunic of C. robusta was comparatively thicker and rougher than that of C. savignyi. Red coloration was present at the tip of the sperm duct in C. robusta (arrowhead, in Figure 1b), which was not observed in C. savignyi. We also examined the egg morphology to identify and distinguish the three species. The eggs of Ciona spp. had follicle cells on the outer covering, and the length of the follicle cells in C. robusta was longer (arrow, Figure 1e) than that in C. savignyi (arrow, Figure 1f). By contrast, no outer follicle cells were present on the eggs of S. clava (Figure 1g).

#### 3.2 Molecular identification of three ascidians by the cox1 gene

We cloned the full length of the cox1 gene from genomic DNA of 50 individuals of the three ascidian species. Each resulting sequence was subjected to BLASTN (Altschul, Gish, Miller, Myers, & Lipman, 1990), with the results indicating that these sequences belonged to the three respective ascidian species. The open reading frames of the cox1 sequence from three species were variable. We identified a deletion polymorphism of cox1 in C. savignyi, but not in C. robusta and S. clava. For instance, only a single 1,560 and 1,543 bp-length of cox1 sequence was identified in C. robusta and S. clava, respectively, whereas two different lengths of cox1 (1,545 and 1,548 bp) were identified in C. savignyi. All these sequences were deposited in the NCBI database, and the accession numbers are listed in Table S1.

We also retrieved the cox1 barcode sequences from the NCBI database (Table S1). Only the cox1 barcode regions of unique haplotypes were used for multiple sequence alignments and phylogenetic tree construction. The resulting phylogenetic trees allowed us to delineate different haplotypes among all of the samples. In the C. robusta tree, we found that the haplotypes (H_1 to H_9) from China did not form a single clade in either NJ (Figure 2a) or MP (Figure 2b) trees, but rather clustered with some haplotypes from individuals originating from Korea and the USA. Similarly, the haplotypes (H_1 to H_16) of C. savignyi from China did not cluster in a single clade in either NJ (Figure 3a) or MP (Figure 3b) trees. Instead, they grouped with other haplotypes from Korea and the USA. In addition, NJ (Figure 4a) and MP (Figure 4b) trees did not resolve the haplotypes (H_1 to H_14) of S. clava from China into a single clade either. Conversely, they formed a cluster with some haplotypes from New Zealand and the USA, which were invasive populations.

#### 3.3 Haplotype identification and diversity of the cox1 gene marker

We used the cox1 gene for molecular diversity analysis. Nine haplotypes were identified among 14 C. robusta samples, 14 haplotypes among 19 S. clava samples, and 16 haplotypes among 17 C. savignyi samples (Table 1). The results of the comparative analysis using different genetic diversity parameters also revealed that C. savignyi was diverse compared with C. robusta and S. clava. The haplotype diversity (Hd) was comparatively higher in C. savignyi (0.993 + 0.038) than that in C. robusta (0.912 + 0.059), and S. clava (0.947 + 0.038). Similarly, the detected average number of nucleotide difference (K) in C. savignyi (20.618) was higher than that in C. robusta (8.143) and S. clava (11.550). Nucleotide diversity (Pi) and average number of mutations (θ) were also relatively higher in C. savignyi (0.02630, 0.05061) compared with C. robusta (0.01094, 0.01811) and S. clava (0.01919, 0.03097), respectively (Table 1).

Next, we carried out the neutrality test. The Tajima’s D values were negative for all three species (Table 2), but these values were significant.
only in \textit{C. savignyi} population \((p < .05)\), indicating that there was a excess of low-frequency polymorphisms, and the \textit{C. savignyi} population was expanding. However, in the \textit{C. robusta}/\textit{S. clava} populations, the values were not statistically significant, indicating that these two species populations did not deviate from the neutral expectations. Similarly, for \textit{Fu} and \textit{Li}'s \(D^*\) statistic, negative values were observed in all three species (Table 2). The values from \textit{C. robusta} and \textit{C. savignyi} were statistically significant, whereas those from \textit{S. clava} were not \((p > .10)\). The results from these two analytical approaches indicate that the population of \textit{C. savignyi} is undergoing positive selection and expansion.
3.4 | Connectivity between native and non-native ascidians

We divided the three ascidian species populations into native and invasive groups, with populations located within eastern Asian countries like China, Japan, and Korea being considered as native groups. Since these species are believed to have originated from this region (Lambert & Lambert, 1998), while the rest of the populations from other regions were grouped as invasive populations. Network analysis revealed that there were three haplogroups (1, 2, and 3) in *C. robusta* (Figure 5a) and *C. savignyi* (Figure 5b), respectively. No haplogroups were found for *S. clava* (Figure 5c). In the *C. robusta* network, we found native populations in haplogroup 1, and haplogroup 3 consisted of invasive populations. On the other hand, haplogroup 2 was comprised mainly of native populations, including those from China, but few haplotypes were shared from invasive populations as well. Haplogroups 1 and 2 were connected with haplogroup 3 (Figure 5a). Similarly, in the *C. savignyi* network (Figure 5b), we found native populations in haplogroup 1, but haplogroup 2 was entirely composed of only native populations, and haplogroup 3 consisted only of invasive populations. By contrast, there were no haplogroups present in the *S. clava* network, and all haplotypes from both native and invasive populations, including those from China, were connected to each other (Figure 5c).

We also performed hierarchical AMOVA using cox1 haplotypes from both native and invasive populations of three ascidian species. There was no clear structure between native and invasive populations in *C. robusta* (11.94%, CT = 0.11941) and *C. savignyi* (6.58%, CT = 0.06582), but these values were not statistically significant (Table 3). In addition, we recorded a negative value for *S. clava* (−8.01%, CT = −0.08014), indicating that there was no population differentiation (Table 3). By contrast, among populations of *C. robusta* (66.99%, SC = 0.78928), *C. savignyi* (70.64%, SC = 0.75622), and *S. clava* (30.65%, SC = 0.28373), there were significant variations, with the highest level of variation appearing in *C. savignyi* (Table 3). Surprisingly, within these variations, the highest value was recorded for *S. clava* (77.37%, ST = 0.22632), followed by *C. savignyi* (22.77%, ST = 0.77226) and *C. robusta* (21.07%, ST = 0.76671; Table 3).

4 | DISCUSSION

In the present study, we identified three ascidian species from the Northeast China using both morphological characteristics and genetic marker analysis. The tunic of *Ciona* spp. is soft and semitransparent, whereas that of *S. clava* is relatively rough and opaque. Since the tunic is mainly composed of a cellulose-like material resembling that of plants (Nakashima, Yamada, Satou, Azuma, & Satoh, 2004), we assume that tunic composition varies among different species. In addition, *Ciona* spp. absorb more water, as demonstrated by dry tunic weight (Tarallo, Yagi, Oikawa, Agnisola, & D’Onofrio, 2016), and potentially as a result, this organ became semitransparent in nature. Furthermore, *C. robusta* is comparatively larger in size than *C. savignyi*. Recently, it was also revealed that the morpho-physiological properties play an essential role in the control of size between these two ascidians (Tarallo et al.,...
Hence, we can use these characters to distinguish between them. It is also interesting to note that there is a red coloration at the tip of the sperm duct in *C. robusta*, which is absent in *C. savignyi* (Lee & Shin, 2014). The evolutionary and functional property of this pigmentation is not yet known. Strikingly, egg morphology also varies among these three species. For instance, long follicle cells are present on the outer covering of *C. robusta* eggs, comparatively shorter follicular cells overlay *C. savignyi* eggs, and no outer follicle cells are present on *S. clava* eggs. Generally, the ascidian egg consists of two layers of follicle cells, with a vitelline coat next to the egg membrane and several test cells between them (Satoh, 2014). These outer follicle cells are vacuolated and elongated and are speculated to provide buoyancy to eggs in seawater (Kessel, 1967). This may help ascidian eggs disperse by the water current and thereby be transported to distant places. Follicle cells are also the first contact of sperm entry, and it is widely known that they function to prevent self-fertilization via a chemical reaction (Pinto, Desantis, Marino, & Usui, 1995). Long follicle cells might have enabled a higher dispersal rate of *C. robusta*. This characteristic might also inhibit more self-fertilization in comparison to *C. savignyi* and *S. clava*.

The genetic marker *cox1* has been widely used for identification and characterization of genetic diversity (Hebert & Gregory, 2005). On the basis of barcode region of the *cox1* gene from these three ascidian species as well as other available sequences in the databases, we constructed the phylogenetic trees to infer their identification, which showed that the Ciona spp. from China was closely related to native populations, mostly from Korea to Japan. This result indicates
FIGURE 4  Phylogenetic trees of S. clava population constructed using cox1 bar code fragment. The haplotypes (H_1 to H_14) are from this study. (a) Population phylogeny of 38 haplotypes constructed by neighbor-joining (NJ) method. (b) Maximum parsimony (MP) method was applied to construct population phylogeny from 38 haplotypes. Bootstrap values of more than 50% values are presented at the nodes.

TABLE 1  Summary statistics of genetic diversity from three ascidian species

| Species         | Sample size | No of haplotype (H) | Haplotype diversity (Hd) + SD | Nucleotide difference (K) | No of mutation per site (θ) | No of segregating sites (S) | Nucleotide diversity (Pi) |
|-----------------|-------------|---------------------|-------------------------------|---------------------------|-----------------------------|-----------------------------|--------------------------|
| Ciona robusta   | 14          | 9                   | 0.912 ± 0.059                | 8.143                     | 0.01811                     | 41                          | 0.01094                  |
| Ciona savignyi  | 17          | 16                  | 0.993 ± 0.023                | 20.618                    | 0.05061                     | 122                         | 0.02630                  |
| Styela clava    | 19          | 14                  | 0.947 ± 0.038                | 11.550                    | 0.03097                     | 60                          | 0.01919                  |

TABLE 2  Test for neutrality from three ascidian species

| Species         | Tajima's D test | Significance (p) | Fu and Li's D* statistic | Significance (p) |
|-----------------|-----------------|------------------|-------------------------|------------------|
| Ciona robusta   | -1.67103        | >.05             | -2.28224                | <.50*            |
| Ciona savignyi  | -1.92716        | <.05*            | -2.40874                | <.05*            |
| Styela clava    | -1.47586        | >.10             | -1.98094                | >.10             |
Native ascidian
Invasive ascidian
that the Ciona spp. samples collected here from China are indeed native ascidians, and these were not introduced from other geographical areas. However, S. clava formed a clade with invasive populations. We also found that some haplotypes from invasive populations formed a cluster with native populations. This result indicates that there was incursion of native and invasive ascidian populations to different parts of the world. A similar phylogenetic method was used for ascidian identification in other geographical regions as well (Iyappan, Ananthan, & Sathishkumar, 2016; Jaffarali, Akram, & Arshan, 2018; Lee & Shin, 2014; Smith, Cahill, & Fidler, 2010).

The ascidians are marine organisms with a relatively high level of genetic diversity (Leffler et al., 2012), and there exist differences in levels of genetic diversity among the ascidians themselves (Tsagkogeorga, Cahais, & Galtier, 2012). How these differing levels of genetic diversity are maintained remains unknown. Our current analyses confirmed that these ascidians have a high level of genetic diversity, with C. savignyi exhibiting a comparatively high level of genetic diversity at the molecular level. One possible explanation might be that C. savignyi has a large effective population size, with differing life-history traits compared to C. robusta and S. clava. Of note, a previous genome-wide study also revealed that C. savignyi exhibited the highest level of genetic diversity (Small, Brudno, Hill, & Sidow, 2007). Other comparative studies on ascidians also confirmed that they have different evolutionary rates (Berna & Alvarez-Valin, 2014). This could be another reason causing the different levels of genetic diversity among these three species. In addition, the neutrality tests showed that C. robusta and S. clava are undergoing neutral evolution, and C. savignyi is experiencing population expansion and positive selection. This also explains why C. savignyi exhibits a higher level of genetic diversity compared with C. robusta and S. clava. Given the widespread distribution of ascidians, it is possible to exhibit high genetic diversity across populations. This kind of observation is also seen in a wide range of other organisms (Ellegren & Galtier, 2016).

Another important characteristic feature of ascidians is their invasive potential. Some ascidian species are dispersed to different geographical or ecological niches because of both anthropogenic and natural causes and are hereby considered as invasive species (Lambert & Lambert, 1998). We compared the global cox1 haplotypes of these three ascidians to understand their connectivity and population genetic structure. Global haplotypes were divided into native and invasive populations. The network analysis indicated that Ciona spp. formed haplogroups with separate native and invasive populations, although some haplotypes were shared. However, in the network of S. clava, there was no such haplogroup formation as all of its haplotypes were interconnected, suggesting extensive incursion for this species in different geographical areas. A previous global study of S. clava also suggested its extensive incursion, in which it was categorized as invasive species (Goldstien et al., 2011). In addition, a regional study of this species indicated the multiple sources of incursions (Goldstien, Schiel, & Gemmell, 2010). The results of the hierarchical AMOVA analysis of the three species here were also consistent with the network analysis. We found a weak population genetic structure in Ciona spp. and less genetic

### TABLE 3 AMOVA of three ascidian populations by mitochondrial cox1 sequences

| Species          | Source of variance          | df | Sum of squares | Variance components | Percentage of variation | Fixation indices | p Values          |
|------------------|----------------------------|----|----------------|---------------------|------------------------|------------------|------------------|
| **Ciona robusta** | *Between native and invasive ascidians* |    |                |                     |                        |                  |                  |
| (n = 66)         | Among group                 | 1  | 1664.677       | 15.24744            | 11.94                  | CT = 0.11941       | .30205 ± .01599 |
|                  | Among population within group| 4  | 3,479.895      | 85.53321            | 66.99                  | SC = 0.78928       | .00000 ± .00000 |
|                  | Within populations           | 60 | 1614.337       | 26.90561            | 21.07                  | ST = 0.76071       | .00000 ± .00000 |
| **Ciona savignyi**| *Between native and invasive ascidians* |    |                |                     |                        |                  |                  |
| (n = 59)         | Among group                 | 1  | 1551.825       | 10.18461            | 6.58                   | CT = 0.06582       | .28446 ± .01465 |
|                  | Among population within group| 3  | 3,745.571      | 109.31788           | 70.64                  | SC = 0.75622       | .00000 ± .00000 |
|                  | Within populations           | 54 | 1903.003       | 35.24079            | 22.77                  | ST = 0.77226       | .00000 ± .00000 |
| **Styela clava** | *Between native and invasive ascidians* |    |                |                     |                        |                  |                  |
| (n = 45)         | Among group                 | 1  | 8.899          | −0.37431            | −8.01                  | CT = −0.08014      | .00684 ± .00231 |
|                  | Among population within group| 5  | 52.702         | 1.43135             | 30.65                  | SC = 0.28373       | .01173 ± .00437 |
|                  | Within populations           | 38 | 137.310        | 3.61343             | 77.37                  | ST = 0.22632       | .00684 ± .00231 |
differentiation in *S. clava* populations. An occasional gene flow between native and invasive populations of ascidians might have occurred previously, most likely via ship transport. We clearly show that the *C. robusta* and *S. clava* invasive potential is attributed to the neutral genetic diversity, whereas the invasive potential of *C. savignyi* might not be due to neutral evolution, but rather by population expansion and positive selection. Previous work indicated that a neutral force plays a role in the biological invasion and subsequent structuring of a population (Daleo, Alberti, & Iribarne, 2009), but equally natural selection within biological invasion was also well characterized (Lee, 2002). It is worth noting that our analysis was based on the small sample size, because of the fewer collection sites. Increase of collection sites and sample sizes could be more accurate for the population genetic evaluation, but would not change the conclusion. Our current study reveals a global relationship between native and invasive populations and has implications in understanding the invasive potential of these three species. Thus, our work provides approaches useful for risk evaluation and management of invasive species.

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**CONFLICT OF INTEREST**

The authors report no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Punit Bhattachan: Conceptualization (equal); data curation (equal); formal analysis (equal); visualization (equal); writing-original draft (equal); writing-review and editing (equal). Runyu Qiao: Data curation (equal); formal analysis (equal); validation (equal). Bo Dong: Conceptualization (equal); formal analysis (equal); funding acquisition (lead); project administration (lead); resources (lead); supervision (lead); writing-review and editing (lead).

**DATA AVAILABILITY STATEMENT**

All DNA sequences were deposited in NCBI: Genbank accessions MK012337–MK012341 and MN890028–MN890047.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section.

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