GENETIC DIVERSITY AND GENETIC STRUCTURE OF DECALOBANTHUS BOISIANUS IN HAINAN ISLAND, CHINA

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Abstract
Decalobanthus boisianus is a native plant of Hainan Island, China, which has caused considerable damage to tropical forest ecosystems in recent decades. Understanding the genetic diversity and structure of this species can facilitate uncovering the molecular mechanism of its invasive ability. Here, we collected 77 individuals of D. boisianus spanning 8 distribution areas with a gradient of human disturbance intensity (i.e., low, moderate, and high disturbance intensity groups) to assess patterns of genetic diversity and structure using inter simple sequence repeat (ISSR) markers. We found that a total of 220 loci were scored with 13 primers using ISSR methods, and that 198 loci were polymorphic. The genetic diversity of D. boisianus among these eight forests decreased with increasing human disturbance intensity. Over 70% of the total genetic variation was present within populations, while less than 30% of variation was found among populations. There was a high gene flow (1.27) among them due to a lack of effective geographic barriers. The mean Nei’s genetic distance of D. boisianus populations was found to be relatively small (i.e., 0.07), and the average genetic similarity of the eight populations was high (i.e., 0.93). Our findings indicate that the genetic diversity of D. boisianus correlated to human disturbance density, and that D. boisianus populations in Hainan Island have frequent gene exchange. We suggest that reduce deforestation to decrease human disturbance may be a good way to prevent the invasion of D. boisianus.

KEYWORDS
gene flow, genetic diversity and structure, human disturbance intensity, sexual and clonal reproduction

1 | INTRODUCTION

Invasive species are a major problem across the globe and pose a multitude of threats to natural ecosystems (Carboneras et al., 2017). Despite massive efforts to understand the factors influencing invasion potential of introduced species, we still lack a predictive framework for determining which species are most likely to become invasive. This may be due to the fact that most research on this topic has focused on life-history traits and not on genetic diversity. Recently, many studies have demonstrated that genetic diversity of invasive plant species leads to their strong ability to adapt to environmental change and facilitates their capacity to spread to...
novel habitats (Bozkurt, Muth, Parzies, & Haussmann, 2015; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008; Templeton, 1994). Therefore, it is essential to investigate the genetic diversity and structures of invasive plants to better understand how these plants successfully invade and cause ecological damages.

Environmental pressure is seen as an important factor accounting for plant reproductive strategies, which can further influence levels of genetic diversity in the species (Cook, 1985; Eriksson, 1997; Philbrick & Les, 1996; Sinclair et al., 2016). For example, sexual reproduction typically increases genetic diversity in a population. In contrast, clonal reproduction allows species to rapidly colonize available habitats, but often leads to a decline in genetic diversity (Ellstrand & Roose, 1987; Hamrick, Linhart, & Mitton, 1979; Schmid, Bazzaz, & Weiner, 1995). Several studies have shown that environmental pressures often have negative consequences for genetic diversity. Rusterholz, Kissling, and Baur (2009) demonstrated that soil disturbance induced by human trampling reduced the frequency of sexual reproduction for Anemone nemorosa populations, resulting in lower levels of genetic diversity for these populations. Likewise, Avellina et al. (2009) demonstrated that the genetic diversity of Briza media was negatively associated with environmental pressures caused by high population densities of humans.

Decalobanthus boisianus (Gagnep.) A. R. Simões & Staples is a liana that distributed in Hainan Island and Guangzhou City. It also has three varieties including Decalobanthus boisianus var. boisiana, Decalobanthus boisianus var. fulvopiola, and Decalobanthus boisianus var. sumatrania, which distributed in Yunnan and Guangxi Province of China, as well as Laos, Vietnam, Malaysia, Kalimantan Island, and Sumatra Island (Flora of China Editorial board, 1995; Wang et al., 2005; Wang, Peng, Li, & Zhou, 2009; Staples, 2010). This species has recently moved from genus Merremia Hall. f. to Decalobanthus Ooststr since the former now only included polyphyletic species because of the weak support for monophyly and strong support for polyphyly of this genus (Simões & Staples, 2017). Therefore, D. boisianus is more closely related to species such as Decalobanthus peltatus (L.) A. R. Simões & Staples which is also a harmful weeds, Decalobanthus borneensis (Merr.) Simões & Staples, Decalobanthus eberhardtii (Gagnep.) Simões & Staples, and Decalobanthus pacificus (Ooststr.) Simões & Staples, ect. D. boisianus is adapted to a wide range of ecological conditions, due to its ability to undergo both sexual and clonal reproduction (Lian et al., 2007; Liu, 2007; Wang et al., 2009; Wu, Liang, Chen, Li, & Cao, 2007). A number of studies assumed that viable seeds of the species can be transported over streams (Wu et al., 2007; Wang et al., 2009), which helps D. boisianus spread long distance. The species is thus characterized by rapid growth and a strong competitive ability (Huang et al., 2015), which allow it to easily invade plantation areas, shrublands, and secondary forests with low canopy density (Wang et al., 2009; Huang et al., 2015), covering more than 2000 ha areas in Hainan Island. After invasion, it often kills a large number of trees and understory plants with low canopy coverage (Huang et al., 2013). Although researchers suggest that D. boisianus can be taken advantage and used for vegetation recovery in barren mountains, as well as leaves feeding livestocks (Sun, Shen, Wan, & Xie, 2006), it has been listed as a major forest harmful pest in China due to the serious damage to secondary forests that the species causes (State Forestry Administration, China).

In this study, we explored genetic diversity and structures of D. boisianus in eight Nature Reserves in Hainan Island. According to field surveys and interviews with local residents, we found that D. boisianus distributed in areas with relatively high human disturbance intensity. Human disturbance intensity has been proved to affect genetic diversity of plant species (Avellina et al., 2009; Ledo & Schnitzer, 2014; Rusterholz et al., 2009). Increases in human disturbance intensity across the eight distributed areas of D. boisianus likely led to a decrease in its genetic diversity. We thus hypothesized that genetic diversity of D. boisianus was high in areas with the low human disturbance intensity, and vice versa.

2  | MATERIALS AND METHODS

2.1  | Sample collection

Samples of D. boisianus were collected from eight forest sites in Hainan Island. A total of 8–10 individuals were randomly selected in each forest site, with at least 100 m intervals between adjacent individuals to reduce possibility of sampling clones. For each individual, three to four fresh leaves were collected and placed in a zip-lock plastic bag with 20 g of silica gel. In total, 77 individuals from the eight populations were collected.

The eight forests are located in Wuzhishan National Nature Reserve (WZS), Bawangling National Nature Reserve (BWL), Yinggeling National Nature Reserve (YGL), Diaoluoshan National Nature Reserve (DLS), Jianfengling National Nature Reserve (JFL), Limushan National Nature Reserve (LMS), Baishiling Nature Reserve (BSL), and Ganshiling Nature Reserve (GSL) (Figure 1). We took the proportion of natural primary forest area occupying the whole land area in each region as a measure of the degree of human disturbance, and assumed that the greater proportion of natural primary forest means the smaller degree of human disturbance, and vice versa (FAO, 2005; Sabatini et al., 2018) (Table 1). In light of the human disturbance intensity, we categorized D. boisianus in these eight forests into three groups, including low disturbance intensity group (WZS), moderate disturbance intensity group (YGL, BWL, JFL, and DLS), and high disturbance intensity group (LMS, BSL, and GSL).

2.2  | DNA extraction and PCR amplification

DNA was extracted from our 77 samples using the Plant DNA Isolation Kit (Foregene Co., Ltd., Chengdu, China). We followed the manufacturer’s protocol using 25–30 mg of dried leaf material. DNA quality and quantity were determined visually by comparisons with the DNA marker DL2000 on 1.0% (W/V) agarose gel electrophoresis. Samples were then stored at −20°C prior to PCR amplification.

A set of 100 ISSR primers was synthesized by SinoGenoMax Co., Ltd. according to the sequences obtained from the University
of British Columbia (Biotechnology Laboratory, University of British Columbia, primer set #9: http://www.biotech.ubc.ca/services/naps/primers/Primers.pdf). An initial experiment was performed to determine the suitable primer and reaction conditions. One sample randomly selected from each eight *D. boisianus* populations to test the preliminary number of polymorphic loci with 100 primers and to optimize the reaction and amplification procedure for PCR. Finally, we obtained optimal reaction and amplification procedures as well as 13 primers that produced a high number of variable and readable loci (Table 2).

The PCR amplification was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA) in 20 μl reaction volumes containing 2 μl of DNA (50 ng/μl), 2 μl of primer (10 μM), 6 μl of ddH2O, and 10 μl of 1X Taq-Plus PCR Forest Mix (included 0.1 U/μl Taq-Plus DNA Polymerase Forest Reaction Buffer, 1.5 mM MgCl2, 200 μM of each dNTP). Polymerase chain reaction started with an initial activation step at 94°C for 4 min, followed by 45 cycles of denaturation at 94°C for 45 s, annealing at 52–54°C for 50 s (depending on primer, Table 2), extension at 72°C for 60 s, and then a final extension at 72°C for 6 min, followed by cooling at 4°C until recovery of the samples (Table 2).

The amplified products were separated on a 1.5% (W/V) agarose gel in 1× TAE buffer staining by GoldView (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) with a setup of 100V, 200 mA and 50 min and then visualized and photographed under ultraviolet light. A 2000 bp DNA ladder (Real times Biotechnology Co. Ltd., Beijing, China) was used to estimate the molecular weights in the Alphalmager—2200 UV Transilluminator (NatureGene Corp., Beijing, China).

### 2.3 | Data analysis

Only bands that were clear and reproducible were used to construct data matrices. The amplified bands were scored as presence (1) or absence (0) of a particular band for each primer. These data were then used to assemble the data matrix of ISSR phenotypes.

### 2.4 | The genetic diversity of the eight *D. boisianus* populations

We calculated the proportion of polymorphic loci (PPL), Shannon’s diversity index (I), Nei’s gene index (H), the observed number of alleles (Na), and the effective number of alleles (Ne) to assess levels of genetic diversity among our populations using the POPGENE program v. 1.32 (Francis, Rong, & Boyle, 1999). The PPL is an important indicator of genetic diversity and of the adaptability of populations to a particular environment. Shannon’s index is based on the band phenotypic frequency and is used to estimate the genetic diversity.
2.5 | The genetic structure of the eight *Decalobanthus boisianus* populations

Genetic differentiation among population (Gst) represents the portion of the total genetic diversity found among populations (Nei, 1972), and the number of migrants (gene flow, Nm) measures the degree of genetic differentiation among populations (Wright, 1950). Here, Gst, Nm, total genetic diversity (Ht), and gene diversity within populations (Hs) were used to determine the genetic differentiation among the eight populations of *D. boisianus*. A matrix of genetic similarity and Nei’s genetic distances were used to depict genetic relationship among the eight *D. boisianus* populations (Nei, 1972). Populations with high genetic similarity and low Nei’s genetic distances value indicate a close genetic relationship. All parameters were calculated using the POPGENE program v. 1.32 (Francis et al., 1999).

We also explored spatial structures of *D. boisianus* with a Bayesian model-based cluster analysis, using the STRUCTURE program version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) and calculated the appropriate number of clusters of populations (K) (Evanno, Regnaut, & Gouet, 2005). Groups were chosen that best fit the genetic variability observed in the dataset, irrespective of the number of populations sampled within and between populations according to King and Schaal’s method (King & Schaal, 1989). H is an index to reflect the gene diversity based on Hardy–Weinberg hypothesis (Nei, 1972). Other indices such as Na and Ne indicate the observed and effective numbers of alleles that are maintained in the populations (Johnson, 1974). Here, we compared the differences in genetic diversity of the eight *D. boisianus* populations across different human disturbance intensity using ANOVA analysis.

### TABLE 1 Site conditions of the eight *Decalobanthus boisianus* populations

| Group | Code | Population | Sample size | Longitude (°E) | Latitude (°N) | Altitude (m) | Proportion of natural primary forests (%) |
|-------|------|------------|-------------|----------------|---------------|-------------|------------------------------------------|
| A     | WZS  | Wuzhishan National Nature Reserve, Wuzhishan County | 10 | 109°37’–109°41’ | 18°43’–18°54’ | 300–800 | 54.20 |
| B     | JFL  | Jianfengling National Nature Reserve, Ledong County | 10 | 108°47’–108°49’ | 18°40’–18°43’ | 200–650 | 18.50 |
| C     | GSL  | Ganshiling Nature Reserve, Sanya City | 9  | 109°38’–109°40’ | 18°22’–18°23’ | 100–300 | 0 |
| D     | BSL  | Baishiling Nature Reserve, Qionghai County | 10 | 110°22’–110°23’ | 19°09’–19°10’ | 100–300 | 0 |

### TABLE 2 Primers selected from the UBC (University of British Columbia) used for ISSR amplification

| Primers | Sequence | Annealing temperature (°C) | Fragment size (bp) | Loci number | Polymorphic loci number | Proportion of polymorphic loci (%) |
|---------|----------|----------------------------|--------------------|-------------|-------------------------|-----------------------------------|
| UBC825  | (AC)₈ T  | 52                         | 200–1,300          | 15          | 13                      | 87                                |
| UBC826  | (AC)₈ C  | 52                         | 200–1,500          | 19          | 18                      | 95                                |
| UBC827  | (AC)₈ G  | 52                         | 150–1,300          | 19          | 19                      | 100                               |
| UBC836  | (AG)₈ YA | 52                         | 150–1,800          | 17          | 13                      | 76                                |
| UBC841  | (GA)₈ YC | 52                         | 150–1,200          | 16          | 12                      | 75                                |
| UBC842  | (GA)₈ YG | 52                         | 150–1,200          | 17          | 15                      | 88                                |
| UBC849  | (GT)₈ YA | 52                         | 200–1,800          | 12          | 12                      | 100                               |
| UBC851  | (GA)₈ YG | 52                         | 300–1,800          | 17          | 15                      | 88                                |
| UBC856  | (AC)₈ YA | 54                         | 200–1,300          | 18          | 14                      | 78                                |
| UBC880  | (GGAGA)₃  | 54                         | 150–1,300          | 19          | 15                      | 79                                |
| UBC888  | BDB(CA)₂  | 54                         | 150–1,100          | 17          | 15                      | 88                                |
| UBC891  | HVH(TG)₁  | 54                         | 200–1,400          | 19          | 15                      | 79                                |
| UBC899  | CAT(GT)₁ CATTTGTTCCA | 54 | 200–1,100 | 15 | 15 | 100 |
TABLE 3 Genetic diversity of eight populations of Decalobanthus boisianus based on 220 ISSR loci

| Population | Polymorphic loci | PPL (%) | Na    | Ne    | I     | H     |
|------------|------------------|---------|-------|-------|-------|-------|
| YGL        | 126              | 57.27   | 1.57  | 1.24  | 0.24  | 0.15  |
| BWL        | 128              | 58.18   | 1.58  | 1.29  | 0.27  | 0.17  |
| JFL        | 94               | 42.73   | 1.43  | 1.24  | 0.21  | 0.14  |
| GSL        | 76               | 34.55   | 1.35  | 1.20  | 0.17  | 0.11  |
| LMS        | 87               | 39.55   | 1.40  | 1.22  | 0.20  | 0.13  |
| WZS        | 146              | 66.36   | 1.66  | 1.33  | 0.31  | 0.20  |
| DLS        | 110              | 50.00   | 1.50  | 1.24  | 0.22  | 0.14  |
| BSL        | 81               | 36.82   | 1.37  | 1.22  | 0.19  | 0.13  |
| Mean (± SD) | 106              | 48.18   | 1.48 ± 0.11 | 1.25 ± 0.04 | 0.23 ± 0.04 | 0.15 ± 0.03 |
| Species level (± SD) | 198          | 90.00   | 2.00 ± 0.00 | 1.33 ± 0.33 | 0.33 ± 0.22 | 0.21 ± 0.17 |

Note: H: Nei’s gene index; I: Shannon’s diversity index; Na: the observed number of alleles; Ne: the effective number of alleles; PPL: the proportion of polymorphic loci.

(Andreakis, Kooistra, & Procaccini, 2009). After initial pilot runs of variable burn-in and run-length, 20 independent runs were performed at K = 2–8 with 10,000 MCMC repetitions and a burn-in period of 10,000 iterations, using no prior information and assuming correlated allele frequencies and admixture model in this study. The STRUCTURE output was further interpreted by STRUCTURE HARVESTE (Earl & VonHoldt, 2012). The CLUMPP version 1.1 (Jakobsson & Rosenberg, 2007) were used with greedy algorithms, with 1,000 random input orders and 1,000 repeats to calculate the average pairwise similarity of runs. The clustered output was visualized using the software Distruct version 1.1 (Rosenberg, 2004). Finally, we conducted cluster analysis for the eight populations with the MEGA v.10.0.1 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) based on the Nei’s genetic distances and bootstrap analysis with 5,000 replications using the unweighted pair-group method of averages (UPGMA).

Geographic distances (in kilometers) among populations were estimated by using toolbox in ArcGIS 10.3 (Esri: California, CA, USA) according to the coordinate (the longitude and latitude) of each population. A Mantel test was performed to assess isolation-by-distance with 1,000 random permutations, to test the significance of the relationship between ΦST and geographic distances. The distribution of whole genetic diversity was estimated using the molecular variance (AMOVA), taking populations and individuals as different levels. The significance of ΦST genetic differentiation was assessed with 1,000 random permutations. The principal coordinate analysis (PCoA) was carried out for the genetic relationships among populations. These analyses above (Mantel test, AMOVA, and PCoA) were carried out using the GenALEX6.5 program (Peakall & Smouse, 2012).

3 | RESULTS

3.1 | PCR amplification

A total of 220 unique loci ranging in size from 150 to 1800 bp were scored from ISSR marker products. From these, 198 (90.00%) were polymorphic (Table 3). A mean of 18 loci was generated per primer, in which the number of loci varied from 13 to 21. The UBC827, UBC849, and UBC899 primers had the highest proportion of polymorphic loci (100%) per D. boisianus population, whereas UBC841 had the lowest one (75%).

3.2 | Genetic diversity

Among the eight D. boisianus populations collecting from the eight forest sites, the mean values of the proportion of polymorphic loci, the observed number of alleles (Na), the effective number of alleles (Ne), Shannon’s diversity index (I), and Nei’s gene index (H) were 48.18%, 1.48 ± 0.11, 1.25 ± 0.04, 0.23 ± 0.04, and 0.15 ± 0.03, respectively (Table 3). At the species level, PPL, Na, Ne, I, and H were 90.00%, 2.00 ± 0.00, 1.33 ± 0.33, 0.33 ± 0.22, and 0.20 ± 0.17, respectively (Table 3).

We also found the genetic diversity varies significantly with human disturbance intensity, with the high, medium, and low genetic diversity observing in low-intensity group (D. boisianus population in WZS), moderate intensity group (D. boisianus population in YGL, BWL, JFL, DLS), and high-intensity group (D. boisianus population in LMS, BSL and GSL), respectively (Polymorphic loci: F_{(2,8)} = 18.89, p = 0.003; PPL: F_{(2,8)} = 18.89, p = 0.003; Na: F_{(2,8)} = 19.08, p = 0.003; Ne: F_{(2,8)} = 23.05, p = 0.002; I: F_{(2,8)} = 21.34, p = 0.002; and H: F_{(2,8)} = 24.54, p = 0.001).

3.3 | Genetic structure

Among the eight populations, the total gene diversity index (H) and gene diversity within population index (Hs) were 0.21 ± 0.03 and 0.15 ± 0.01, respectively. The genetic differentiation index among the eight populations (Gst) was 0.28. AMOVA analysis showed that a relatively large proportion of genetic variation (75.67%) occurred within the eight D. boisianus populations, whereas only 24.33% of genetic variation was observed among the eight populations (ΦST = 0.24, p < 0.001; Table 4). The historical mean number of migrants (Nm) was 1.27, indicating the effective gene flow among the eight D. boisianus populations.
The mean value of genetic similarity among the eight *D. boisianus* populations was high (0.93), while the genetic distances among the eight *D. boisianus* populations were relatively low (0.07), indicating the close relationships among the eight *D. boisianus* populations (Table S1). The Mantel test’s results ($r = 0.39, p = 0.10$) indicated that there were no significant correlations between Nei’s genetic distances and geographic distances.

STRUCTURE analysis showed that delta K displayed two peaks ($K = 3$ and $K = 5$) but the maximum one was obtained at $K = 3$ (Figure 2a), suggesting the eight *D. boisianus* populations can be

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**TABLE 4** Analysis of molecular variance (AMOVA) for 8 populations of *Decalobanthus boisianus* based on 220 ISSR loci

| Source of variation | df  | SSD   | MSD   | Variance component | Total variance (%) | $p^*$ |
|---------------------|-----|-------|-------|--------------------|--------------------|------|
| Among populations   | 7   | 574.09| 82.01 | 6.45               | 24.33              | <0.001|
| Within populations  | 69  | 1382.71| 20.04 | 20.04              | 75.67              | <0.001|
| Total               | 76  | 1956.81| 102.05| 26.48              | 100                |       |

*Note. df, the degree of freedom; SSD, the sum of squared deviations; MSD, the mean squared deviations. *Indicated significance tests after 1,000 permutations.*
clustered into three groups, including group 1 (populations in BWL, WZS, DLS, BSL, and LMS), group 2 (populations in JFL and GSL) and group 3 (population in YGL). These three groups, moreover, were confirmed using UPGMA dendrogram (Figure 3) and PCoA analyses (Figure 4).

4 | DISCUSSION

4.1 | Genetic diversity of *D. boisianus* varies with human disturbance intensity

Genetic diversity of the eight *D. boisianus* populations significantly increase with the decrease in human disturbance intensity (Polymorphic loci: $F_{(2,8)}^{(2,8)} = 18.89, p = 0.003$; PPL: $F_{(2,8)}^{(2,8)} = 18.89, p = 0.003$; Na: $F_{(2,8)}^{(2,8)} = 19.08, p = 0.003$; Ne: $F_{(2,8)}^{(2,8)} = 23.05, p = 0.002$; I: $F_{(2,8)}^{(2,8)} = 21.34, p = 0.002$; H: $F_{(2,8)}^{(2,8)} = 24.54, p = 0.001$), which proves our hypotheses. Human disturbance may influence the balance between sexual and clonal reproduction of *D. boisianus* populations (Hamrick et al., 1979; Loveless & Hamrick, 1984), to alter genetic diversity of *D. boisianus* populations (Cook, 1985; Eriksson, 1997; Sinclair et al., 2016). For example, *D. boisianus* in LMS, BSL and GSL with high-intensity human disturbances may allocate resources to clonal reproduction, which helps it rapidly expand to novel habitats. But the clonal reproduction produces the same offspring gene and leads to low genetic diversity (Ellstrand & Roose, 1987; Philbrick & Les, 1996; Kudoh, 1999). In contrast, low-intensity human disturbances allow WZS population to allocate more resources to sexual reproduction, thereby maintaining higher level of genetic diversity (Ellstrand & Roose, 1987; Philbrick & Les, 1996; Kudoh, 1999). In contrast, low-intensity human disturbances allow *D. boisianus* populations to allocate more resources to sexual reproduction, thereby maintaining higher level of genetic diversity (Ellstrand & Roose, 1987; Philbrick & Les, 1996; Kudoh, 1999). Our results are consistent with Aveliina et al. (2009), which demonstrated that human activity leads to a decrease in genetic diversity for *Briza media*. Although our study shows the relationships between the genetic diversity of *D. Boisiana* and human disturbance intensity, the cause of the conversion between sexual and clonal reproduction still need large-scale manipulative experiments.

4.2 | Genetic structure of *D. boisianus* in Hainan Island

In our study, both Nei’s genetic differentiation index (Gst = 0.28) and AMOVA values (24.88%) indicated that there was a large proportion of genetic variation (over 70%) present within the eight *D. boisianus* populations, while less than 30% of genetic variation was observed among the eight populations. These results are consistent with the previous reports from other invasive species like *Carduus acanthoides* L. (Bohumil, Petr, Dana, Petr, & Ivana, 2009), *Ambrosia artemisiafolia* L. (Kočiš et al., 2015), and *Spartina densiflora* Brongn. (Castillo et al., 2018) but are less than most clonal invasive species, such as *Eupatorium catarium* Veldkamp (Li, Li, & Liu, 2014), *Praxelis clematidea* R.M.King & H.Rob. (Wang, Huang, Downie, Chen, & Chen, 2015), *Galinsoga quadriradiata* Ruiz & Pav. (Li, Qi, Yao, & Liu, 2015), *Mikania micrantha* H.B.K. (Li, Dong, & Zhong, 2007), and *Alternanthera philoxeroides* Griseb. (Ye, Li, Cao, & Ge, 2003). These results indicate that a very low genetic differentiation of the eight *D. boisianus* populations is observed on Hainan Island. One possible reason is that the effective gene flow (1.27) among the eight populations result from the high dispersal ability of *D. boisianus* to against barriers throughout the expansion of this species (Opedal et al., 2017; Wright, 1950). Indeed, the small mean value of Nei’s genetic distance (0.07) and the high mean genetic similarity index (0.93) both indicate the close relationship among *D. boisianus* populations. Although little is known for the dispersal mode of this species, many studies have assumed that viable seeds of *D. boisianus* may be transported over streams to achieve long-distance transmission (Wu et al., 2007; Wang et al., 2009). Moreover, several species that are closely related to *D. boisianus*, such as *D. peltata*, *Merremia palmeri* Hallier f., and *Merremia ellenbeckii* Pilg, were found that could be transported over streams (Francisco

![Figure 3: UPGMA dendrogram based on Nei’s genetic distances among 8 populations of *Decalobanthus boisianus*. BSL: population sampled from Baishiling Nature Reserve; BWL: population sampled from Bawangling National Nature Reserve; DLS: population sampled from Diaoluoshan National Nature Reserve; GSL: population sampled from Ganshiling Nature Reserve; JFL: population sampled from Jianfengling National Nature Reserve; LMS: population sampled from Limushan Nature Reserve; WZS: population sampled from Wuzhishan National Nature Reserve; YGL: population sampled from Yinggeling National Nature Reserve.](image-url)
& Clara, 1997; Taylor & Kumar, 2016; Thulin, 2010). Value for \( \phi_{st} \) (0.24) of \( D. \) boisianus is very close to those of hydrophilous plants (0.25) (Nybom, 2004), too. Hence, this stream dispersal mode may therefore generate the effective gene flow across large distances for \( D. \) boisianus. On the other side, intense storms on Hainan Island can also increase opportunities for propagules dispersal of \( D. \) boisianus and move them long distances across the landscape, downstream, and across catchments (Diez et al., 2012, Murphy & Metcalfe et al., 2016), and therefore accelerated the movement and gene exchange frequency among \( D. \) boisianus populations. Therefore, we should try to limit the spread of seeds and propagules of this weed. Further research is needed to identify the optimal management to control this species, for example, introduction of host-specific natural enemies and proper disposal of this species.

UPGMA dendrogram, Bayesian analysis and PCoA analysis all show that the eight \( D. \) boisianus populations are categorized to three different groups (Figures 2, 3, and 4), which is not match the three groups categorized by the human disturbance intensity (Table 1), suggest that human disturbance cannot affect genetic structure of \( D. \) boisianus populations. In spite of geographic distances is nonsignificant (Mantel test, \( r = 0.34, p = 0.09 \)) with genetic distances of this species, some nearby populations have clustered together, such as WZS with LMS and BSL, JFL with GSL populations. This implied that the horizontal expansion of clones might also lead to gene flow among nearby populations of that species and made their genetic relationships closer.

5 | CONCLUSION

Generally, we found that genetic diversity of \( D. \) boisianus was correlated to the human disturbance intensity, which has been proved to be a major factor governing plant invasion. High dispersal ability and adaptive potential of \( D. \) boisianus may be important factors that were attributed to its large distances expansion and cause serious damage to the ecosystems. So far, artificial removal and chemical controls have been implemented to kill and prevent \( D. \) boisianus in the study areas, but were seldom effective. We suggest reducing deforestation to decrease human disturbance to forests for one hand. In addition, effective efforts should be taken to remove \( D. \) boisianus, and restore the secondary forests.
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AUTHOR CONTRIBUTIONS

Huan Jiang, Wenxing Long, Hui Zhang, Chengneng Mi, Tao Zhou, and Zongzhu Chen wrote the paper. Huan Jiang, Wenxing Long performed the laboratory work; Hui Zhang and Zongzhu Chen analysed the data; Huan Jiang, Wenxing Long, Hui Zhang, Chengneng Mi, Tao Zhou, and Zongzhu Chen wrote the paper.

DATA ACCESSIBILITY

Inter Simple Sequence Repeat (ISSR) data for Decalobanthus boisiannus is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.7nq1636

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