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Disordered Translocation is Hastening Local Extinction of the Chinese Giant Salamander

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Abstract Biodiversity is declining globally by an unprecedented extinction rate. This is especially true for amphibians, accounting for 24.3% of all threatened vertebrates. As the largest extant amphibian species in the world, wild populations of the Chinese giant salamander (Genus *Andrias*) (CGS) have decreased dramatically because of overexploitation and habitat degradation. Translocation has become an important strategy for restoring threatened wild populations worldwide. However, disordered translocation usually has negative effects on the native populations. We provide an overview of CGS translocation and show that disordered translocation can increase local population extinction. Nearly four times the estimated number of wild individuals have been released across China. There are three types of translocation used for CGS, namely, reinforcement, reintroduction and ecological replacement, the last of which accounts for over one-third of translocations. Our genetic screening revealed that most released individuals were not from local populations, with one to four lineages detected in every release site (n = 6). This disordered translocation can potentially reduce the genetic integrity of original populations. Hence, we suggest suspending current CGS translocation activities immediately, until more robust measures can be developed and implemented to improve the current translocation program, especially with respect to lineage identification and the identification of appropriate release sites.

Keywords Chinese giant salamander, genetic test, translocation, conservation, wild population

1. Introduction

Global biodiversity is in decline and we are currently experiencing unprecedented rates of extinction, for example, current rates of extinction are about 1000 times the likely background rate of extinction and future rates are likely to be 10 000 times higher (Pimm *et al*., 2014). Amphibians are disproportionately the most threatened vertebrate group, with 41% of species considered threatened (Hoffmann *et al*., 2010). In the face of rapid biodiversity loss, effective strategies for the conservation of threatened species are urgently required (Bainbridge *et al*., 2014; Taylor *et al*., 2017).

Translocation has become an important tool to manage wildlife over the past few decades, which involves the human-mediated, intentional movements of living organisms from one area to another (Armstrong and Seddon, 2007; IUCN/SSC, 2013; Batson *et al*., 2015; Swan *et al*., 2019). If the primary objective is a conservation benefit, the translocation is called a conservation translocation. A conservation translocation is usually undertaken with the goal of improving the conservation status of the target species locally or globally, and/or restoring

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natural ecosystem functions or processes (IUCN/SSC, 2013). Whilst translocations are being used with increased frequency, there is an obvious bias towards mammalian and avian taxa (Seddon *et al*., 2005; Bajomi *et al*., 2010).

The Chinese giant salamander (CGS) have attracted widespread attention because they represent an evolutionary ancient and distinct amphibian lineage and have few close relatives. Also, they are the largest extant amphibian species in the world, with a maximum length and weight of 2 m and 50 kg, respectively (Zhao, 1998). Historically, this species was widely distributed in the middle and lower tributaries of the Yangtze River, Yellow River, and Pearl River, including 17 provinces (or municipalities) in central, southern and southwestern China (Wang *et al*., 2004). However, the CGS populations have declined dramatically since the 1950s due to habitat loss and overharvesting for food (Wang *et al*., 2004). The CGS is listed as a National Special Protected Animal (category II) and as Critically Endangered on the IUCN Red List of Threatened Species (Liang *et al*., 2004), Biodiversity Red List of China and in included in CITES Appendix I (Jiang *et al*., 2016; CITES, 2020). Therefore, the Chinese government has heavily invested to protect and conserve the CGS. So far, there are more than fifty nature reserves associated with protection of CGS in China (Liang *et al*., 2013). Hunting wild CGS is prohibited and breeding the species in captivity requires permission. Recent field surveys have shown that the CGS has been critically depleted or extirpated in most of their original habitats (Xu *et al*., 2018; Turvey *et al*., 2018).

With the development of CGS farming industry, the CGS farms can supply sufficient source populations for translocation programs of CGS, which provides hope and opportunity to save this threatened species. By the end of 2014, there were 2622 CGS farms in China and up to 1 249 000 individuals across the country (China Aquatic Wildlife Conservation, 2015). As a result, government-promoted translocations for the protection of the CGS have been conducted widely in China. In 2014, the Ministry of Agriculture and Rural Affairs of the People's Republic of China (MARAPRC) enacted the technical specification for the stock enhancement of Chinese giant salamander (SC/T 9414-2014). A lack of detailed knowledge on CGS population genetics meant that previous translocations did not consider CGS from different river basins represented as different lineages and the species was treated as a single population. Current studies revealed that the CGS is comprised of at least seven lineages (Yan *et al*., 2018; Liang *et al*., 2019). Turvey *et al*. (2019) argued that the wild populations from Pearl River/Nanling regions should be restored as a valid species *Andrias sligoi* (Boulenger, 1924). Mix-source reintroductions can lead to outbreeding depression in hybrid offspring or reduce the genetic integrity of the original populations (Huff *et al*., 2011). Therefore, a detailed understanding of CGS translocations is important as it will guide future conservation management decisions.

In this study, we aimed to (a) understand the current CGS translocations including the number of individuals involved and the locations where CGS were released, and the translocation type, (b) to conduct genetic screening on released individuals and assigned them to CGS lineages or species in order to assess the effectiveness and risk of current translocations, and (c) to make recommendations for future CGS translocations.

2. Materials and Methods

2.1. Release data collection The CGS often grabs the attention of the general public when released because it is regarded as a flagship species in China. Therefore, all CGS translocation news are generally released to the public. Data on CGS translocation were obtained from the internet (mainly media outlets) using the search terms Chinese giant salamander, giant salamander, and baby fish, along with the keywords reintroduction, translocation, release, and stock enhancement. The time frame of the search was from the last century to the end of October 2019. The language of the search was Chinese (Mandarin). We also obtained supplementary data from two fishery bureaus, including the Fishery Bureau of Tongjiang County, Sichuan and the Fishery Bureau of Cili County, Hunan. Following the definitions of IUCN translocations, we classified CGS translocations into 3 different types. Reinforcement, as the intentional movement and release of CGS into these existing populations of conspecifics; reintroduction, as the intentional movement and release of CGS inside their indigenous ranges from which they have disappeared; ecological replacement, as the intentional movement and release of CGS outside their indigenous ranges to perform a specific ecological function.

2.2. Survey area and methodology We conducted sampling surveys at six release sites: Kaihua (4532 released individuals), Zhejiang; Yuexi (1890 released individuals), Anhui; Liannan (400 released individuals), Guangdong; Heyuan (1480 released individuals), Guangdong; Tongjiang (4600 released individuals), Sichuan, and Wanyuan (110 released individuals), Sichuan. These sites represent three distinct distribution regions of Chinese giant salamander in Turvey *et al.* (2019): the southwestern (Pearl/Nanling), northern (Yangtze/Sichuan) and southeastern (Huangshan and Zhejiang). Released individuals were collected in natural habitat through visual encounter methods and wire-mesh baited traps in September 2018 and 2019. The visual encounter methods consisted of wading, nocturnal spotlighting, and netting. Wire-mesh baited traps are reasonably effective for surveying *Andrias japonicus* and *Cryptobranchus alleganiensis*

alleganiensis (Foster *et al.*, 2008; Brigger *et al.*, 2013; Turvey *et al.*, 2018). We selected survey areas according to habitat conditions during the day and searched for CGSs in the evening (from 18:30 to 23:00). The traps consisted of 110 cm × 60 cm × 30 cm rectangular boxes made of 1.5 cm plastic-coated hardware cloth and a funnel trap. Fresh fish and chicken were used as bait for trapping (Browne *et al.*, 2011). The traps were set in the upper, middle, and lower reaches of the surveyed river and checked them the next day (between 5:00 and 7:00 AM).

2.3. Habitat suitability model To infer the potential distribution range of the CGS under current climatic conditions, we produced a habitat suitability model according to CGS locality records and environmental data (Chen *et al.*, 2018). We combined GPS information on the current distribution of the species and environmental variable modeling to project its historical distribution.

For distribution information, we obtained sampling records of wild individuals from the literatures (Murphy *et al.*, 2000; Fei *et al.*, 2006; Zheng, 2006; Li *et al.*, 2009; Luo *et al.*, 2009; Guo *et al.*, 2011; Yang *et al.*, 2011; Chen *et al.*, 2017; Wu *et al.*, 2017; Wang *et al.*, 2018; Liang *et al.*, 2019) and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) and excluded duplicate GPS sites within each pixel (30 arc-seconds, 1 km × 1 km). Climatic predictor variables were downloaded from WorldClim (www.worldclim.org/current). We downloaded maps of Chinese administrative areas, elevation, and land use from DIVA-GIS (available at http://www.diva-gis.org/Data). Vegetation cover was extracted from the land use data. Human footprint data were downloaded from http://sedac.ciesin.columbia.edu/wildareas/. To ensure consistency in climate layer resolution, we increased the resolution of last glacial maximum (LGM) layers from 2.5 min to 30 arc-seconds by the 'resample' method in ArcGIS v.10.3. According to the sampling records, all of China was selected as the range of interest.

Before constructing the habitat suitability model, bioclimatic raster layers were clipped into the study area and converted to ASC II format for use in MaxEnt. To prevent overfitting, we used the band collection statistic of the spatial analysis tool to calculate the correlation coefficients between bioclimatic layers in ArcGIS v.10.3 and removed the variables with strong correlations (correlation coefficient ≥ 0.8) and low contributions to the habitat suitability model with 19 bioclimatic factors used for preliminary analysis. Twelve bioclimatic predictor variables were ultimately included in our habitat suitability model: elevation, vegetation cover, human footprint data, annual mean temperature, mean diurnal range, isothermality, temperature seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation seasonality, and precipitation of the coldest quarter.

The habitat suitability model was constructed by MaxEnt v.3.4.1 (Phillips *et al.*, 2018) with 8 uncorrelated bioclimatic data layers using the maximum entropy method based on the optimal model, with 25% of occurrence records as the test set and 75% as the training set. Other conditions included 10 000 background points, a logistic output format and 10 cross-validation replicates for each run, with the average of those replicate models representing the final prediction results. The area under the receiver operating characteristic curve (AUC) was used to evaluate the overall fit of the model, and AUC values > 0.9 indicated that the model fit well (Araujo and Guisan, 2006); the higher the AUC score is, the stronger the predictive power of the model. Jackknife analysis was employed to identify which bioclimatic factors were most important and contributed the most to the model.

2.4. Molecular sampling and sequencing We swabbed oral mucosa cells or collected exfoliated skin cuticles from caught and trapped individuals. All animals were released after tissue collection at the original site of capture. The collected tissues were immediately stored in 95% ethanol at −20°C for DNA extraction.

DNA was extracted using a TIANamp Genomic DNA Kit (Qiagen). Three mitochondrial DNA (mtDNA) fragments from the complete mitochondrial cytochrome b (*Cytb*) gene, the partial control region (D-loop), and the partial cytochrome C oxidase 1 (*COI*) gene were amplified by polymerase chain reaction (PCR). The primers and cycling parameters were as described by literatures (Tao *et al.*, 2005, 2006; Che *et al.*, 2012). PCR products were purified using a Qiagen PCR purification kit and sequenced on an ABI 3100 automated sequencer. We also downloaded nucleotide sequences available in recent studies (Yan *et al.*, 2018; Liang *et al.*, 2019) and other references from GenBank (Table S1) and aligned them with ClustalW built into BioEdit version 7.1.9 (Thompson *et al.*, 1997) using the default parameters. Alignments were also visually checked for ambiguous alignment in MEGA version 7.0.26 (Tamura *et al.*, 2013). All new sequences were deposited in GenBank with the accession numbers listed in Table S1.

2.5. Phylogenetic inference Bayesian inference (BI) was used to reconstruct phylogenetic relationships. The optimal partitioning scheme and best-fitting nucleotide substitution model for each partition of the mtDNA genomes were estimated using PartitionFinder v.2.1.1 (Lanfear *et al.*, 2016). Bayesian analyses were conducted in MrBayes v.3.2.2 (Ronquist *et al.*, 2012) executed in the CIPRES Science Gateway (http://www.phylo.org/index.php). All searches were performed with two independent runs, each initiated with a random tree. Each run consisted of four Markov chain Monte Carlo (MCMC) chains (three heated chains and one cold chain) estimated for 5 million generations and sampled every 1000 generations. Stationarity was assessed in Tracer 1.6, and the first 25% of samples were discarded as burn-in (Rambaut *et al*., 2014). The remaining trees were used to create a 50% majority-rule consensus tree and estimate Bayesian posterior probabilities (BPPs).

3. Results

We collected a total of 268 records of CGS translocations from official reports (internet searches, 233 records), and fishery stations (35 records), and found that the first releases were undertaken in the Zhangjiajie National Nature Reserve, in Hunan Province, in 2002. By the end of October 2019, a total of 287 840 farmed individuals were released into the wild across 98 counties and cities (Figure 1A, 1C) in central and southern China. All records of CGS being released were distrbuted in 16 provinces or municipalities (Figure 1B). In fact, the numbers of released animals may likely be an underestimate as not all releases were reported. Even so, this number is almost four times the estimated wild population size of *Andrias* species in China (75 000) (Liang *et al*., 2013). The number of released animals increased gradually between 2008 and 2012. This was followed by a sharp increase in the number of released animals between 2013 and 2015. The number of released animals peaked in 2016 and has subsequently declined (Figure 1A). The greatest number of individuals were released in Shaanxi Province since 2002, reaching up to 94 464 in total; by contrast, the minimum number of released individuals occurred in Beijing, where 200 individuals were released (Figure 1B).

There are three types of CGS translocation according to definitions of IUCN: reinforcement (40.8%), reintroduction (24.5%) and ecological replacement (34.7%) (Figure 1C). This meant more than one-third of releases took place at sites outside the indigenous range of CGS. Our habitat suitability model showed that the suitable habitat of CGS was concentrated in central and southern China and was scattered in the east. However, more than 10% of the release sites were not inside predicted suitable habitat (Figure 1C).

In total, 48 CGS individuals were recaptured from five of the six sites we sampled for released animals. The details of sites and number of individuals captured at each site were as followed: Yuexi, Anhui (5 adults, 2 larvae); Liannan, Guangdong (3 adults, 2 larvae); Kaihua, Zhejiang (28 adults, 5 larvae); Heyuan, Guangdong (1 adult, 1 larva) and Wanyuan, Sichuan (1 adult).

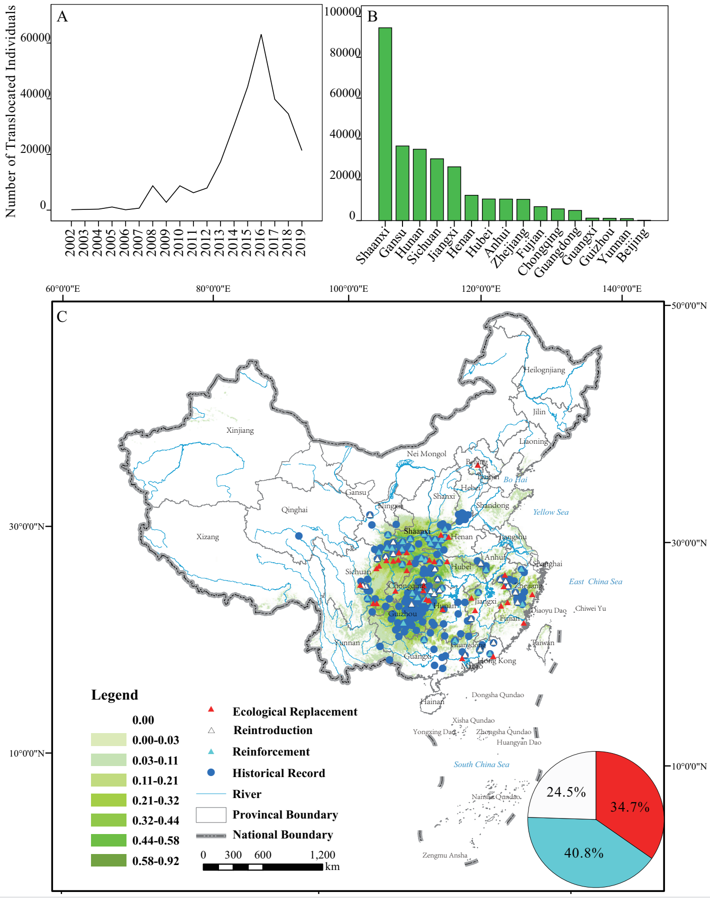

Figure 1 Translocation overview of the Chinese giant salamander. (A) Trends in the translocations of Chinese giant salamanders from 2002–2019. (B) The number of translocated giant salamanders across different Provinces in China. (C) Translocation sites (triangles) and types of the Chinese giant salamanders. The green areas present the predicted suitable habitat for Chinese giant salamander. The pie chart shows the proportion of three translocations.

Our molecular data showed that all the animals we captured could be assigned to non-native lineages defined by recent studies (Yan *et al*., 2018; Liang *et al*., 2019). Released individuals from Kaihua belonged to four non-native clades, namely, B, C, D and U1, described in Yan *et al*. (2018) (Figure 2). A non-indigenous lineage from Shaanxi was released in Liannan and Heyuan (B clade; Figure 2). Also, Wanyuan's animal clustered into a clade with northern Guangxi (U1 clade; Figure 2), and Yuexi's individuals represent three different non-local clades (U1, U2 and B; Figure 2).

4. Discussion

4.1. Factors that may influence CGS translocation The success of amphibian translocations are related to multiple factors, such as habitat condition, age, food limitation, disease, the number of animals released, etc. (Griffiths and Pavajeau, 2008; Germano and Bishop, 2009). Generally, translocations are most successful when more than 1000 individuals were released (Germano and Bishop, 2009). On the contrary, most reports indicated that causes of amphibian translocation failure were homing and migration of introduced individuals out of release sites and poor habitat (Germano and Bishop, 2009). Current studies show that some CGS translocations have not succeeded in restoring wild CGS populations (Luo *et al*., 2009; Lin *et al*., 2017; Lu *et al*., 2020). Turvey *et al*. (2018) reported that dead individuals of CGS were found at one translocation site. Researchers suggested that most translocations of CGS failed to restore the wild populations due to the limitation of factors such as water quality, food supply, human disturbance, release season, individual age, poaching, and subsequent management measures (Luo *et al*., 2009; Lin *et al*., 2017; Zhang *et al*., 2017; Turvey *et al*., 2018). Thus, a variety of factors influence the success rate of translocation of CGS. So far, the number of released individuals of CGS (287 840) has far exceeded the number of wild individuals (75 000) (Liang *et al*., 2013), but release sites are outside the indigenous range (ecological replacement) and outside the predicted suitable habitat which accounted for more than 33% and 10% of sites, respectively. In general, our habitat suitability model is similar to the previously published model (Chen *et al*., 2018), both models predicted that suitable CGS habitat was primarily located in central and southern China. However, there were some differences between our model and the Chen *et al*. (2018) model. Our suitable habitats were mainly distributed in Shaanxi, Guizhou, Hunan, Sichuan, Chongqing, Hubei, Henan, and Zhejiang, while the suitable habitats from Chen *et al*. (2018) were concentrated in Guizhou, Hunan, Zhejiang, Hubei, Anhui, and Sichuan. This difference could be the result of the additional predictive variables (i.e. human footprint) and the inclusion of more historical records in our model. In fact, the conservation biologists' original intention was to restore wild CGS population by reinforcement or reintroduction and not to conduct ecological replacement. Currently, there are no good

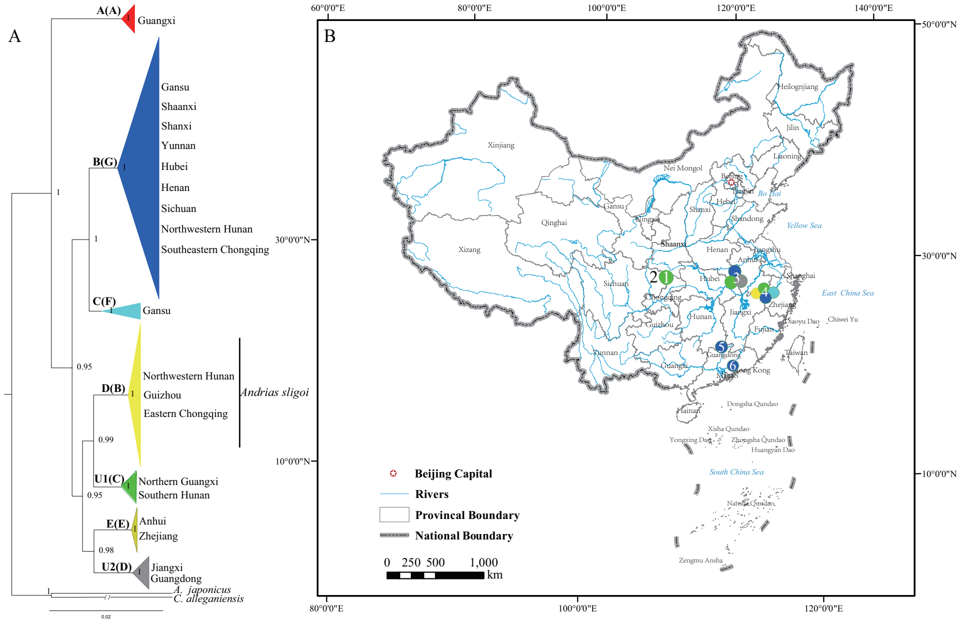

Figure 2 Bayesian tree based on mtDNA and six sampling release sites of Chinese giant salamander. (A) A simplified Bayesian inference tree based on concatenated mtDNA (Cytb, D-loop and COI). Numbers near branches indicate Bayesian posterior probabilities. The capital letters outside and inside the brackets show corresponding clades for Chinese giant salamander in Yan *et al*. (2018) and Liang *et al*. (2019), respectively. Clade D corresponds to *A. sligoi* in Turvey *et al*. (2019). The distribution areas for different clades are marked after subulate branches. (B) The sampling release sites of Chinese giant salamander. The numbers represent sampling sites, including Wanyuan, Tongjiang, Yuexi, Kaihua, Liannan, and Heyuan in numerical order. The colored circles correspond to the different clades in the left.

management measures (e.g. monitoring and habitat protection) after releasing CGS back into the wild. Therefore, whether these translocations will succeed or not is completely uncertain and requires mark-recapture studies.

In recent years, the scale of CGS translocation has declined since its peak in 2016. This decline might be associated with fluctuations of CGS market value. From 2008 to 2010, the CGS price gradually increased and reached peak at the end of 2010, when the highest price was 3000 RMB/kg (Cheng, 2015). During this period the CGS industry expanded and was able to provide a greater number of both larvae and adult animals for translocation. In 2011, driven by huge profits, many farmers started to raise CGS (Cheng, 2015), and since 2012, the price of CGS drastically declined (Li *et al*., 2018). However, because the sexual maturity cycle of captive takes about 4 years (Luo *et al*., 2005). The supply of salamander exceeded the demand in 2016, which was when the CGS translocations reached the largest scale. Meanwhile, the price of CGS has almost bottomed out in 2017 (140 RMB/kg), therefore the CGS farming industry has gone into decline (Li *et al*., 2018). Subsequently, the number of CGS translocations also declined.

4.2. The risk of current CGS translocation All the populations of CGS were treated as a single species during the previous translocations, but recent studies revealed that there are at least seven distinct lineages (Yan *et al*., 2018; Liang *et al*., 2019). Furthermore, a new study revealed that the wild populations from Pearl River/Nanling regions were a valid species *Andrias sligoi* (Turvey *et al*., 2019). In this study, even though we only recaptured 48 individuals from six release sites (Kaihua, Yuexi, Liannan, Heyuan, Tongjiang, and Wanyuan), all individuals belonged to non-native genetic lineages. For example, the native wild populations from Zhejiang belong to clade E according to the recent results (Yan *et al*., 2018; Liang *et al*., 2019), however, all sampled released individuals were divided into four non-native genetic lineages (B, C, D and U1) in Kaihua, Zhejiang, where the clade D is the representative of *Andrias sligoi*. This is quite contrary the genetic purity of karst cave populations (Wang *et al*., 2017). We don't know if our captures were all the original released individuals or their offspring that were born in the wild. But our results suggest that some released individuals have survived at least four years (e.g. 952 individuals were released in Kaihua, Zhejiang in October 2015.) and could compromise the genetic integrity of the native population. Meanwhile, some surveys suggest that the released individuals have established new populations in the wild, such as in Guangdong, but no evidence suggested that interbreeding has occurred between the released and wild populations (Su, 2018). However, interbreeding has already occurred in *Andrias japonicus* in Japan owing to the introduction of *Andrias davidianus* (Wang *et al*., 2015), which resulted in severe genetic contamination in the wild. With so many non-native genetic lineages identified at our sampling sites, there is a strong indication that the extensive commercial farming has led to the movement of animals between farms throughout the range of CGS in China (Yan *et al*., 2018), and their progeny are being released into the wild without genetic testing. Due to their overwhelming advantage of population size, there is a great possibility that non-native strains or species may eventually eradicate the unique evolutionary characteristics of original populations. Although some hybrids are more fit than their parents and the pursuit of hybrid vigor or "heterosis" has been important in agriculture and animal breeding (Edmands, 1999), empirical work indicates that multi-generational outbreeding depression can be sufficiently severe in some cases (e.g., Houde *et al*., 2011; Huff *et al*., 2011). In addition, mixing genetically, divergent sources are often proposed to attenuate genetic diversity in reintroduced populations that may result from small effective population sizes (Huff *et al*., 2011). However, outbreeding depression is a possible negative tradeoff in hybrid offspring for mixing sources (Huff *et al*., 2011). Outbreeding becomes unhelpful with regard to the restoration of the wild population of CGS due to adaptability decline in hybrid offspring. Therefore, the current mixed-source translocation could accelerate the extinction of wild local CGS.

Besides, the release of non-native lineages may lead to the risk of ecological invasion. In our investigation, more than one-third of release sites were not within the historical distribution of CGS according to the historical records. So, these released individuals should be treated as an alien invasive species at these sites. There may be competitive or predator-prey relationships between released individuals and local aquatic animals and this could destroy the local ecological balance.

4.3. Translocation suggestions Based on our findings, we suggest that the administrative department suspend CGS translocation and collect genetic information for all released populations across the country in order to take immediate remedial measures. Meanwhile, for standardizing the procedures of CGS translocation, we recommend that the following points should be considered:

Establish dedicated conservation breeding facilities. Although CGS farms can be used for translocation programs, commercial breeding activities have resulted in extensive movements of CGS between different farms (Cunningham *et al*., 2016). Some evidence suggests that the introgression has occurred in some sampled individuals from different localities (Yan *et al*., 2018). This study showed that different non-native lineages were present at the same translocation site, where released animals were all from CGS farms. Furthermore, the stocks held on the private farms may be unsuitable for translocation due to the potential risk of pathogens (e.g. *Andrias* *davidianus ranavirus*, *Aeromonas hydrophila*) and the unknown provenance of the salamanders held on the farms. Therefore, establishing dedicated conservation breeding facilities for CGS of known provenance should be conducted urgently (Turvey *et al.*, 2018, 2019). Current release programs must improve to identify the origin of captive animals and prevent extramural introductions of non-native lineages or different species (Turvey *et al.*, 2019).

Pre-preparation for releasing. Best practices for CGS translocation needs to involve selection of appropriate release sites (Luo *et al.*, 2009; Lin *et al.*, 2017), assessment of habitat (Lin *et al.*, 2017), selection of animals to be released (Lin *et al.*, 2017), genetic screening of animals to be released (Lin *et al.*, 2017), pre-release health screening (SC/T 9414-2014), determination of release scale and time (Luo *et al.*, 2009), adaptability training for releasing individuals (Gao *et al.*, 2017; Kenison & Williams, 2018), measurement of body parameters, marking with passive integrated transponder (PIT) tags (Dodd, 2009; Liu *et al.*, 2020) and preparation of appropriate food (e.g. small fish, shrimp) (Luo *et al.*, 2009). Since the 1950s, and especially in the 1980s or human activities have caused damage, loss and reduction of *Andrias davidianus* habitat (Zhang *et al.*, 2002). Our results indicate that some historical sites where CGS were present are no longer suitable for CGS due to the expansion of the human footprint and pollution. Release sites should be chosen according to the current distribution ranges and habitat suitability model, rather than historical distribution records of CGS. After determining the appropriate translocation sites, habitat assessement, including water quality, food supply, human disturbance and predators are required, because these environmental factors can affect the survival, growth and development (Luo *et al.*, 2009; Zhang *et al.*, 2017). Released individuals need be to strictly selected, as older individuals have higher survival (Luo *et al.*, 2009; Zhang *et al.*, 2016). As mentioned above, all animals destined for release should be subject to genetic screening to avoid genetic contamination. To increase survival, we suggest that the suitable release time in autumn and spring, because the food is plentiful and the water temperature is suitable during this period (Luo *et al.*, 2009; Lin *et al.*, 2017). Sex ratios of the released individuals should be between 1:1 and 1:1.5, which is consistent with the sex ratio of natural reproduction of giant salamanders (Luo *et al.*, 2009). The number of translocated individuals should be determined by the carrying capacity of release site for CGS, because the translocation may fail if the scale of release is too large. The healthy and experienced individuals will likely adapt to new environments quickly. Quarantine and adaptability training are also critical for increasing the survival (Gao *et al.*, 2017). Adaptability training mainly includes acclimatization to foods commonly found in their proposed release sites, the acclimatization to an array of environmental parameters that individuals will be exposed to at release sites (e.g. running water, temperature and photoperiod, etc. Gao *et al.*, 2017), and behavioral adaptation (Crane and Mathis, 2011; Gao *et al.*, 2017). Body condition should be assessed for translocated animals, therefore the development and validation of a body scoring system is an important area of future research. Lastly, released individuals could be traced if they are marked with passive integrated transponders (PIT).

Releasing and post release. Releasing cohorts should comprise of individuals of the same size or age class. Continuously track and monitor the released animals. We suggest that the animals should be monitored twice a week after releasing, and the monitoring frequency can be reduced after a month (based on our preliminary translocation project of CGS). The follow-up monitoring should continue for 5 years, since CGSs take at least three or four years to mature (Luo *et al.*, 2005). Meanwhile, protecting the release sites from habitat destruction and poaching is essential. Any CGS deaths at release sites should be investigated and if possible, mitigated. Finally, all release programs should be systematically evaluated in order to continuously improve the translocations of CGS. Currently there is little evidence that CGS translocation results in the establishment of viable populations of native CGS; we suggest that translocations can only be deemed successful once there is evidence of breeding for several generations and the establishment of self-sustaining populations that have originated from translocated stock.

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Appendix

Table S1 Detailed information of samples and referenced sequences for genetic analysis in this study. Letters in front of brackets are corresponding clades in Yan *et al.* (2018). The capital letters in brackets present corresponding clades in Liang *et al.* (2019).

Species	Locality	Specimen voucher	MtDNA lineage	GenBank Accession No.			Remarks
				Cytb	D-loop	COI	
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS01	B(G)	MT268836	MT268788	MT240265	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS02	B(G)	MT268837	MT268789	MT240266	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS03	B(G)	MT268838	MT268790	MT240267	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS04	B(G)	MT268839	MT268791	MT240268	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS05	U1(C)	MT268840	MT268792	MT240269	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS06	B(G)	MT268841	MT268793	MT240270	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS07	B(G)	MT268842	MT268794	MT240271	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS08	U1(C)	MT268843	MT268795	MT240272	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS09	B(G)	MT268844	MT268796	MT240273	This Study
A. sligoi	Gutianshan, Kaihua, Zhejiang	GTS10	D(B)	MT268845	MT268797	MT240274	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS11	B(G)	MT268846	MT268798	MT240275	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS12	B(G)	MT268847	MT268799	MT240276	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS13	B(G)	MT268848	MT268800	MT240277	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS14	B(G)	MT268849	MT268801	MT240278	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS15	B(G)	MT268850	MT268802	MT240279	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS16	C(F)	MT268851	MT268803	MT240280	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS17	B(G)	MT268852	MT268804	MT240281	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS18	B(G)	MT268853	MT268805	MT240282	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS19	B(G)	MT268854	MT268806	MT240283	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS20	B(G)	MT268855	MT268807	MT240284	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS21	B(G)	MT268856	MT268808	MT240285	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS22	B(G)	MT268857	MT268809	MT240286	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS23	B(G)	MT268858	MT268810	MT240287	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS24	B(G)	MT268859	MT268811	MT240288	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS25	B(G)	MT268860	MT268826	MT240289	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS26	B(G)	MT268861	MT268827	MT240290	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS27	B(G)	MT268862	MT268828	MT240291	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS28	B(G)	MT268863	MT268829	MT240292	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS29	B(G)	-	MT268830	MT240293	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS30	B(G)	MT268864	MT268831	MT240294	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS31	B(G)	MT268865	MT268832	-	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS32	U1(C)	MT268866	MT268833	MT240295	This Study
A. davidianus	Gutianshan, Kaihua, Zhejiang	GTS33	B(G)	MT268867	MT268834	MT240296	This Study
A. davidianus	Heyuan, Guangdong	GDHY1	B(G)	MT268868	MT268812	MT240297	This Study
A. davidianus	Heyuan, Guangdong	GDHY2	B(G)	MT268869	MT268813	MT240298	This Study
A. davidianus	Liannan, Guangdong	GDLN1	B(G)	MT268870	MT268814	MT240299	This Study
A. davidianus	Liannan, Guangdong	GDLN2	B(G)	MT268871	MT268815	MT240300	This Study
A. davidianus	Liannan, Guangdong	GDLN3	B(G)	MT268872	MT268816	MT240301	This Study
A. davidianus	Liannan, Guangdong	GDLN4	B(G)	MT268873	MT268817	MT240302	This Study
A. davidianus	Liannan, Guangdong	GDLN5	B(G)	MT268874	MT268818	MT240303	This Study
A. davidianus	Yuexi, Anhui	AHYX1	U1(C)	MT268875	MT268819	MT240304	This Study
A. davidianus	Yuexi, Anhui	AHYX2	B(G)	MT268876	MT268820	MT240305	This Study
A. davidianus	Yuexi, Anhui	AHYX3	U2(D)	MT268877	MT268821	MT240306	This Study
A. davidianus	Yuexi, Anhui	AHYX4	U2(D)	MT268878	MT268822	MT240307	This Study
A. davidianus	Yuexi, Anhui	AHYX5	U2(D)	MT268879	MT268823	MT240308	This Study
A. davidianus	Yuexi, Anhui	AHYX6	B(G)	-	MT268824	MT240309	This Study
A. davidianus	Yuexi, Anhui	AHYX7	U2(D)	MT268880	MT268825	MT240310	This Study
A. davidianus	Wanyuan, Sichuan	SCWY01	U1(C)	MT268881	MT268835	MT240311	This Study
A. davidianus	Fengxian, Shaanxi	KIZ020235	B(G)	MH051410	MH051482	MH051336	Yan *et al.* (2018)
A. davidianus	Changan, Shaanxi	11041	B(G)	-	MH051483	MH051337	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14528	B(G)	MH051411	MH051484	MH051338	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14529	B(G)	MH051412	MH051485	MH051339	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14530	B(G)	MH051413	MH051486	MH051340	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14531	B(G)	MH051414	MH051487	MH051341	Yan *et al.* (2018)

(Continued Table S1)

Species	Locality	Specimen voucher	MtDNA lineage	GenBank Accession No.			Remarks
				Cytb	D-loop	COI	
A. davidianus	Baoji, Shaanxi	KIZYPX14532	B(G)	MH051415	MH051488	MH051342	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14533	B(G)	MH051416	MH051489	MH051343	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14534	B(G)	MH051417	MH051490	MH051344	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14535	B(G)	MH051418	MH051491	MH051345	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14537	B(G)	MH051419	MH051492	MH051346	Yan *et al.* (2018)
A. davidianus	Baoji, Shaanxi	KIZYPX14538	B(G)	MH051420	MH051493	MH051347	Yan *et al.* (2018)
A. davidianus	Yuanqu, Shanxi	11051	B(G)	MH051421	MH051494	MH051348	Yan *et al.* (2018)
A. davidianus	Yuanqu, Shanxi	11052	B(G)	MH051422	MH051495	MH051349	Yan *et al.* (2018)
A. davidianus	Yuanqu, Shanxi	11053	B(G)	MH051423	MH051496	MH051350	Yan *et al.* (2018)
A. davidianus	Lushi, Henan	KIZYPX44113	B(G)	MH051424	MH051497	MH051351	Yan *et al.* (2018)
A. davidianus	Qingchuan, Guangyuan, Sichuan	KIZ020236	B(G)	MH051425	MH051498	MH051352	Yan *et al.* (2018)
A. davidianus	Qingchuan, Guangyuan, Sichuan	KIZYPX25999	B(G)	MH051426	MH051499	MH051353	Yan *et al.* (2018)
A. davidianus	Qingchuan, Guangyuan, Sichuan	KIZYPX25990	C(F)	MH051427	MH051500	MH051354	Yan *et al.* (2018)
A. davidianus	Qingchuan, Guangyuan, Sichuan	KIZYPX25991	C(F)	MH051428	MH051501	MH051355	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2505	D(B)	MH051430	MH051503	MH051357	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2506	D(B)	MH051431	MH051504	MH051358	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2507	D(B)	MH051432	MH051505	MH051359	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2508	D(B)	MH051433	MH051506	MH051360	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2509	D(B)	MH051434	MH051507	MH051361	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2513	D(B)	MH051435	MH051508	MH051362	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2514	D(B)	MH051436	MH051509	MH051363	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2515	D(B)	MH051437	MH051510	MH051364	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2516	D(B)	MH051438	MH051511	MH051365	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2517	D(B)	MH051439	MH051512	MH051366	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2518	D(B)	MH051440	MH051513	MH051367	Yan *et al.* (2018)
A. sligoi	Xinglong, Chongqing	KIZYPX2519	D(B)	MH051441	MH051514	MH051368	Yan *et al.* (2018)
A. sligoi	Zheng‘an, Zunyi, Guizhou	KIZZA2	D(B)	MH051442	MH051515	MH051369	Yan *et al.* (2018)
A. sligoi	Zheng’an, Zunyi, Guizhou	KIZZA9	D(B)	MH051443	MH051516	MH051370	Yan *et al.* (2018)
A. sligoi	Leishan, Guizhou	KIZYPX10535	D(B)	MH051445	MH051518	MH051372	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10518	D(B)	MH051446	MH051519	MH051373	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10519	D(B)	MH051447	MH051520	MH051374	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10521	D(B)	MH051448	MH051521	MH051375	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10522	D(B)	MH051449	MH051522	MH051376	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10523	D(B)	MH051450	MH051523	MH051377	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10524	D(B)	MH051451	MH051524	MH051378	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10525	D(B)	MH051451	MH051525	MH051379	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10526	D(B)	MH051453	MH051526	MH051380	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10527	D(B)	MH051454	MH051527	MH051381	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10528	D(B)	MH051455	MH051528	MH051382	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10529	D(B)	MH051456	MH051529	MH051383	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10530	D(B)	MH051457	MH051530	MH051384	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10531	D(B)	MH051458	MH051531	MH051385	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10532	D(B)	MH051459	MH051532	MH051386	Yan *et al.* (2018)
A. sligoi	Guiding, Guizhou	KIZYPX10533	D(B)	MH051460	MH051533	MH051387	Yan *et al.* (2018)
A. davidianus	Mao‘ershan, Guilin, Guangxi	KIZYPX10536	A(A)	MH051461	MH051534	MH051388	Yan *et al.* (2018)
A. davidianus	Mao’ershan, Guilin, Guangxi	KIZGXDN3	A(A)	MH051462	MH051535	MH051389	Yan *et al.* (2018)
A. davidianus	Mao‘ershan, Guilin, Guangxi	KIZGXDN4	A(A)	MH051463	MH051536	MH051390	Yan *et al.* (2018)
A. davidianus	Mao’ershan, Guilin, Guangxi	KIZGXDN5	A(A)	MH051464	MH051537	MH051391	Yan *et al.* (2018)
A. davidianus	Mao‘ershan, Guilin, Guangxi	KIZ020273	A(A)	MH051465	MH051538	MH051392	Yan *et al.* (2018)
A. davidianus	Mao’ershan, Guilin, Guangxi	KIZ022435	A(A)	MH051466	MH051539	MH051393	Yan *et al.* (2018)
A. davidianus	Mao‘ershan, Guilin, Guangxi	KIZ020272	A(A)	MH051467	MH051540	MH051394	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	11036	E(E)	MH051468	MH051541	MH051395	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	11037	E(E)	MH051469	MH051542	MH051396	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	11038	E(E)	MH051470	MH051543	MH051397	Yan *et al.* (2018)

(Continued Table S1)

Species	Locality	Specimen voucher	MtDNA lineage	GenBank Accession No.			Remarks
				Cytb	D-loop	COI	
A. davidianus	Huangshan, Anhui	11039	E(E)	MH051471	MH051544	MH051398	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	11040	E(E)	MH051472	MH051545	MH051399	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	KIZYPX6151	E(E)	MH051473	MH051546	MH051400	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	KIZYPX6152	E(E)	MH051474	MH051547	MH051401	Yan *et al.* (2018)
A. davidianus	Huangshan, Anhui	KIZYPX6153	E(E)	MH051475	MH051548	MH051402	Yan *et al.* (2018)
A. davidianus	Pearl River	11046	C(F)	-	MH051550	MH051404	Yan *et al.* (2018)
A. davidianus	Farm-bred (Guangxi)	CGS1009	U1(C)	MH051478	MH051552	MH051406	Yan *et al.* (2018)
A. davidianus	Farm-bred (Guangxi)	CGS947	U2(D)	MH051479	MH051553	MH051407	Yan *et al.* (2018)
A. davidianus	Farm-bred (Guizhou)	CGS725	U1(C)	MH051480	MH051554	MH051408	Yan *et al.* (2018)
A. davidianus	Farm-bred (Jiangxi)	CGS291	U2(D)	MH051481	MH051555	MH051409	Yan *et al.* (2018)
A. davidianus	Qinzhou, Tianshui, Gansu	GSTS240	C(F)	KU131042	KU131042	KU131042	Yan *et al.* (2018)
A. davidianus	Yinge, Taibai, Baoji, Shaanxi	SXTBYG677	B(G)	KU131058	KU131058	KU131058	Liang *et al.* (2019)
A. davidianus	Taibaihe, Taibai, Baoji, Shaanxi	SXTB202	B(G)	KU131040	KU131040	KU131040	Liang *et al.* (2019)
A. davidianus	Zhashui, Shangluo, Shaanxi	SXSLZS672	B(G)	KU131057	KU131057	KU131057	Liang *et al.* (2019)
A. davidianus	Lishan, Yuanqu, Shanxi	SXYQLS371	B(G)	KU131047	KU131047	KU131047	Liang *et al.* (2019)
A. davidianus	Wangwoshan, Jiyuan, Henan	HNJY390	B(G)	KU131048	KU131048	KU131048	Liang *et al.* (2019)
A. davidianus	Mabian, Leshan, Sichuan	SCMB244	B(G)	KU131043	KU131043	KU131043	Liang *et al.* (2019)
A. davidianus	Niujie, Yiliang, Zhaotong, Yunnan	YNYL551	B(G)	KU131053	KU131053	KU131053	Liang *et al.* (2019)
A. sligoi	Huolu, Wulong, Chongqing	CQWL481	D(B)	KU131051	KU131051	KU131051	Liang *et al.* (2019)
A. sligoi	Longshan, Xiangxi, Hunan	HNLS55	D(B)	KU131052	KU131052	KU131052	Liang *et al.* (2019)
A. sligoi	Yongding, Zhangjiajie, Hunan	HNWMY48	D(B)	KU131050	KU131050	KU131050	Liang *et al.* (2019)
A. davidianus	Sangzhi, Zhangjiajie, Hunan	HNSZSDJ82	B(G)	KU131061	KU131061	KU131061	Liang *et al.* (2019)
A. davidianus	Jin'gan, Yichuan, Jiangxi	JXJA336	U2(D)	KU131044	KU131044	KU131044	Liang *et al.* (2019)
A. davidianus	Xiuning, Huangshan, Anhui	AHHS695	E(E)	KU131060	KU131060	KU131060	Liang *et al.* (2019)
A. davidianus	Qingyuan, Lishui, Zhejiang	ZJLSQY680	E(E)	KU131059	KU131059	KU131059	Liang *et al.* (2019)
A. sligoi	Guiding, Qiannan, Guizhou	GZGDYX583	D(B)	KU131054	KU131054	KU131054	Liang *et al.* (2019)
A. davidianus	Ziyuan, Guilin, Guangxi	GXZY587	U1(C)	KU131055	KU131055	KU131055	Liang *et al.* (2019)
A. davidianus	Xing'an, Guilin, Guangxi	GXXA609	A(A)	KU131056	KU131056	KU131056	Liang *et al.* (2019)
A. davidianus	Jinggangshan, Ji'an, Jiangxi	JXJGS352	U2(D)	KU131045	KU131045	KU131045	Liang *et al.* (2019)
A. davidianus	Lianzhou, Qingyuan, Guangdong	GDLZ365	U2(D)	KU131046	KU131046	KU131046	Liang *et al.* (2019)
A. sligoi	Farm-bred (Longsheng, Guangxi)	-	D(B)	AJ492192	AJ492192	AJ492192	Zhang *et al.* (2003)
A. davidianus	Shaoxing, Zhejiang	-	B(G)	KT119359	KT119359	KT119359	Feng *et al.* (2016)
A. davidianus	Qimen, Anhui	HS16091	E(E)	KX268733	KX268733	KX268733	Xu *et al.* (2016)
A. davidianus	Xiuning, Huangshan, Anhui	HS16092	E(E)	KX298240	KX298240	KX298240	Xu *et al.* (2018)
A. davidianus	Zhangjiajie, Hunan	HS16093	B(G)	KX298241	KX298241	KX298241	Xu *et al.* (2018)
A. davidianus	Jingzhou, Hubei	HS16094	B(G)	KX298239	KX298239	KX298239	Xu *et al.* (2018)
A. davidianus	Jinzhai, Lu'an, Anhui	HS16095	U1(C)	KX298242	KX298242	KX298242	Xu *et al.* (2018)
A. japonicus				AB208679	AB208679	AB208679	Okamoto *et al.* (2005)
C. alleganiensis				AB445785	AB445803	KU985766	Matsui *et al.* (2008)