Protection and Utilization of Chinese Giant Salamander Germplasm Resources

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Abstract Chinese giant salamander (*Andrias davidianus*) is salamander germplasm resource that only distributed in China. It is the material basis of the evolution principle research, genetic research and artificial breeding. Since the 1990s, overharvesting, water pollution and habitat changes have seriously destroyed the germplasm resources of *A. davidianus*, their population density has decreased since then. Strengthening the protection and rational utilization of *A. davidianus* germplasm resources are key factors in impacting the healthy, stable and sustainable development of *A. davidianus* industry. Under the above background, we discussed the current problems with the protection for *A. davidianus* germplasm resources, put forward suggestions for the conservation of *A. davidianus* germplasm resources. It is expected to provide a reference to effective protection for *A. davidianus* germplasm resources, and provide a scientific foundation for further studies, so as to promote the high-quality development of related industry.

Key words Chinese giant salamander (*Andrias davidianus*), Germplasm resources, Protection, Utilization

1 Introduction

Chinese giant salamander (*Andrias davidianus*) is the largest extant amphibian in the world. It is widely distributed in China historically, while has been classified as endangered due to habitat destruction and hunting by human[1]. The economic value, protection status and special evolutionary position from aquatic to terrestrial animal of *A. davidianus* have been widely reported[2-5]. It is regarded as a kind of rare and precious tonic in many countries and regions due to its good meat quality and high nutritional value[6]. The miraculous effects of *A. davidianus* such as strengthening stomach and helping digestion[7] created great interest and provided a strong market drive, therefore, *A. davidianus* is commercially cultured[8] and allowed to reach the market, which provides an effective way to develop the rural economy and augment farmers' income.

The distribution of aquatic germplasm resources in China is extremely extensive[9]. However, due to the comprehensive impact of social and economic development, ecological environment destruction, overharvesting and many other factors, the aquatic germplasm resources in natural water have decreased sharply[10]. The disordered exchange of seedlings has polluted the species gene pool, the genetic background as well as genetic structure of many economic species is confused[11], *A. davidianus* was no exception since the late 1990s, and has reached a very serious level[12-15]. Up to the year 2015, there are only 36 national aquatic original breeding institutions in China, and no national preservation agency has been built for *A. davidianus*. Germplasm resources are lack of scientific protection, financial, technical strength and scientific research team are relatively scattered. *A. davidianus* breeding enterprises have no strength to carry out scientific and technological research on original species protection.

2 Status of *A. davidianus* germplasm resources

2.1 Reduction of *A. davidianus* wild germplasm resources We have identified three reasons to explain the decline in *A. davidianus* resources. Overharvesting is a main reason[16], poaching is accompanied and this eventually led to a plunge in the wild germplasm resources of *A. davidianus*. In the 1980s, there were about 300 000 wild *A. davidianus* individuals distributed in Lushi County, Henan Province. In the 1990s, high profits in the illegal market led to large numbers of wild *A. davidianus* being poached, which caused huge damage to the wild populations. Secondly, the deterioration of water ecological environment destroyed the living environment of *A. davidianus*[17]. Large areas of forest vegetation were destroyed by mining since the 1990s (*e.g.* Luanchuan County and Lushi County in Henan Province), mineral processing has caused water pollution, which threaten the survival of *A. davidianus* directly. Thirdly, river damming, road building and some other human activities destroyed the river ecosystem, local adaptation is driven by environment components and results in locally adapted phenotypes[18]. In fact, some rivers have even been landfill, habitat loss causing great damage to the survival of *A. davidianus*.

2.2 Weak basic work of researches on *A. davidianus* germplasm resources and insufficient research depth In the past 20 years, conservation efforts such as breeding in captivity, release back into nature and national reserves has been carried out, captive breeding of *A. davidianus* has been widely studied, which drove the maturity of breeding technology, while the collection,

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protection and research of *A. davidianus* germplasm resources are still in the initial stage. The germplasm conservation work is scattered and not systematic. Most of them do not have a standardized management system and long-term funding support. Basically, these strategies did not play the role of germplasm bank in serving the development of seed industry. Although a lot of efforts have been done on the basic research of germplasm resources, the research on population genetic structure, molecular markers and biodiversity is still scarce, and the genetic background of *A. davidianus* breeding in many places is still not clear[19]. The existing germplasm identification methods still mainly stay on morphological classification, and even rely on visual judgment. The existence of multiple species complicates the situation, the lack of molecular level identification methods and population specific genetic markers has caused technical difficulties in further protecting and studying the genetic resources of *A. davidianus*. Due to the disordered exchange of breeding parent and juveniles, as well as poor breeding management, the germplasm is seriously mixed and the gene bank of *A. davidianus* is polluted, which brings difficulties to the study on genetic background and genetic structure of *A. davidianus* germplasm. The above factors have caused the degradation of breeding *A. davidianus* germplasm, serious decline in traits can be observed, such as reduction of growth rate, deterioration of quality, decreased disease resistance, which greatly affected production.

2.3 Weak protection of *A. davidianus* germplasm resources Only 17 nature reserves have been established in China, they are located in Hunan Province (2), Hubei Province (2), Guangdong Province (1), Sichuan Province (2), Jiangxi Province (1), Henan Province (5), Shaanxi Province (2), Gansu Province (1) and Chongqing City (1). It should be pointed out that Zhangjiajie Chinese Giant Salamander Reserve in Hunan Province is the only national *A. davidianus* nature reserve[20]. These indicate that the protection of *A. davidianus* germplasm resources is weak and insufficient.

Given that few institutions were engaged in the conservation of *A. davidianus* germplasm resources, a provincial *A. davidianus* fishery germplasm resources protection area was established (2013) in Kaihua County, Quzhou City of Zhejiang Province, intend to protect the wild resources of *A. davidianus*, enrich the biodiversity and improve the ecological environment. The *Document of Agricultural Office of Fisheries* ([2014] No. 47) announced the seventh batch of national aquatic germplasm resources protection zones on July 22, 2014, among them, Gansu Hongchang National Aquatic Germplasm Resources Protection Zone involved species include *A. davidianus* and the northern mountain stream salamander (*Batrachuperus pinchonii*). Yunnan Province approved the establishment of the Guangnan Chinese Giant Salamander Provincial Aquatic Germplasm Resources Protection Zone in 2016, aiming to carry out breeding experiment and protect wild *A. davidianus* resources.

Although these reserves and original breeding farms have played a certain role in the protection of *A. davidianus* germplasm resource, in view of the huge amount of *A. davidianus* germplasm resources in China[21], the number of protection sites that have been built and continuously supported is still limited. The coverage is insufficient, and related system is no completed. In addition, equipment of these institutions is backward, they do not have sufficient scientific research conditions and technical strength. It is not possible for them to conduct in-depth research on germplasm conservation. Their work is mainly to maintain the survival and reproduction of this group, and lacks systematic resource utilization plans and conservation planning. The above conditions lead to hybridization, promiscuity and backcrossing occurring frequently, causing serious degradation of the excellent properties of artificially preserved germplasm resources.

3 Hypothesis of research

In view of the main problems in *A. davidianus* germplasm resources, and in combination with the actual situation of the large coverage of *A. davidianus* resources in China, we proposed the following work ideas, aiming to make full use of the limited wild *A. davidianus* germplasm resources:

(i) It is recommended to strengthen the investigation, collection and preservation of *A. davidianus* germplasm resources, conduct further field investigation to collect wild germplasm resources, explore more about its biological and ecological characteristics, reproductive physiology, endocrine changes, and complete the research on seedling breeding technology. At the same time, studies on the ecological environment of germplasm resources and the origin preservation technology of *A. davidianus* should be carried out. We also should establish different types of main breeding resource reserves or original breeding farm, carry out research on conservation techniques of *A. davidianus* resources. For the protection of endangered resources, effective protection measures should be taken, to strengthen the construction of their nature reserves and germplasm resources reserves[22].

(ii) The identification and evaluation of *A. davidianus* germplasm resources as well as germplasm innovation should be carried out on a large scale. It is the very time to build *A. davidianus* germplasm resources identification platform for important economic traits of breeding and production (including high yield, high quality, stress resistance, disease resistance and nutrient efficient utilization), study the precise evaluation technology of traits urgently needed in breeding, improve the identification standards of biological and economic traits of preserved resources. In an attempt to ensure the accuracy of identification and evaluation results as well as the effectiveness of resource utilization, researches should be carried out at three levels: genetic diversity, species diversity and ecosystem diversity[23]. Further research should be carried out on the conservation of original superior species, the construction of excellent families, the screening of molecular markers, and the mining of controlling genes for traits. More attention should be paid to the basic work of breeding. For example, the collection, cultivation, screening, pedigree establishment, germplasm identification, trait analysis and seed conservation of basic groups. It is necessary to develop the purification and rejuvenation of *A. davidianus* with the construction of excellent provenance retention capacity simultaneously, carry out large-scale innovative research on germplasm resources, and strive to realize the rapid transformation from resource advantage to variety advantage, so as to provide a steady stream of new materials for the breeding of *A. davidianus*

and the development of *A. davidianus* proliferation industry.

(iii) It is recommended to pay attention to the safe preservation and protection system construction of germplasm resources, and ensure the safe preservation and early warning monitoring of germplasm resources is the most important task. Regular genetic monitoring of germplasm resources stored in genetic breeding centers and breeding farms should be conducted to determine the "inflection point" of genetic diversity declines, so that germplasm with low or declining genetic diversity can be timely renewed and rejuvenated. Establish the monitoring and early warning system for the original habitat protection site (area) of *A. davidianus* as soon as possible, so that the agricultural administrative departments at all levels can grasp the protection information in time. It is necessary to accelerate the establishment and improvement of *A. davidianus* germplasm resources conservation system, so as to realize the important economic value and endangered species resources of origin protection.

(iv) The management, maintenance and operation of conservation facilities such as the resources germplasm banks, genetic breeding centers, original breeding farms and seedling breeding agencies of *A. davidianus* germplasm are lack of stable financial support, which leads to the weak protection of *A. davidianus* germplasm resources, and there is a risk that some of the protected germplasm resources will be lost again. We should strengthen the construction of germplasm resources facilities and R&D investment, establish a sound management mechanism to achieve the balance of conservation and utilization, so that the *A. davidianus* germplasm resources can become a real sustainable public green health resource.

4 Building public resource service platform for *A. davidianus*

4.1 Establishing a public welfare platform for *A. davidianus* germplasm resources It is necessary to build germplasm resource specimen bank, cell bank, pathogen bank and gene bank facilities with different geographical distribution based on the distribution characteristics of *A. davidianus*, and focus on expanding the base for breeding and preserving live resources. To strengthen the construction of *A. davidianus* original (good) breeding farms, it is recommended to build six national *A. davidianus* original breeding farms in Zhangjiajie (Hunan Province), Shennongjia (Hubei Province), Hanzhong (Shaanxi Province), Zunyi (Guizhou Province), Jing'an (Jiangxi Province) and Lushi (Henan Province). On this basis, 15 to 20 excellent *A. davidianus* breeding farms above the provincial level should be established to avoid the pollution of *A. davidianus* germplasm resources from the source, and to ensure the quality of this industry development.

4.2 Establishing an *A. davidianus* germplasm resource information sharing service platform We need to strengthen the integration of resources and improve the quality of resources. Based on the existing aquatic germplasm database of the National Aquatic Germplasm Resources Platform, formulate and improve the technical procedures for the collection, sorting and preservation of *A. davidianus* germplasm resources, and the description standards of *A. davidianus* germplasm resources. It is necessary to realize the digital expression and standardized integration of living, cell, sperm, DNA and other resources, improve various data quality control standards and germplasm resource parameter standards, design various databases and data structures, improve the remote data entry and maintenance systems, database management systems, and *A. davidianus* germplasm resource portal systems. It is recommended to improve the carrier efficiency of the platform, strengthen public services, improve the national germplasm information sharing service platform and website system, improve the sharing and utilization efficiency of *A. davidianus* germplasm resources, promote the protection and utilization of China's aquatic wildlife resources, and lay a solid foundation for promoting the socialization of *A. davidianus* germplasm resource. The Henan Aquatic Germplasm Resource Bank has been established in Henan Province, which has preserved 2 000 living *A. davidianus* individuals. All the germplasm resource information has been included in the National Fishery Germplasm Resource Platform Sharing System. Meanwhile, Henan Province is building an agricultural germplasm resource sharing information platform, and the information of Henan aquatic germplasm resource bank has also been included in the platform information sharing system.

4.3 Establishing the testing and evaluation system of *A. davidianus* germplasm resources It is recommended to establish the microsatellite DNA fingerprints of *A. davidianus* in various original breeding farms, improved breeding farms and *A. davidianus* nature reserves. There may be only one kind of *A. davidianus* in the history, while due to crustal changes, the original living environment has been moved and the population has been isolated, forming different water systems and living plates[24]. After a long period of differentiation, *A. davidianus* distributed in different water systems and different plates will be different, that is to say, the identified microsatellite sites of *A. davidianus* in different water systems or plates have their own specific sites[25], which can produce unique spectral bands of the strain. The spectral bands of each site are expressed by numerical codes (*i. e.* digital fingerprint), by which we can identify *A. davidianus* from different water systems or plates one by one.

In order to facilitate the recovery of wild *A. davidianus* resources, release farm-bred individuals back into nature environment should continue. Artificial release of *A. davidianus* must use pure-native species. Before releasing, health assessments should be completed. Moreover, some seedlings should be marked with molecular markers[26], at least with coded metal markers, so as to master the germplasm status and releasing effect evaluation of *A. davidianus*, the largest extant amphibians[8].

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