Conservation of Wild Plants with Extremely Small Populations under the Delineation of Ecological Protection Red Lines

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Abstract The implementation of the ecological protection red line serves as a crucial protective barrier for the conservation of wild plants with extremely small populations by delineating specific geographical areas designated for their conservation. The implementation of a comprehensive set of conservation measures aimed at establishing a biodiversity conservation network for wild plants with extremely small populations has laid the foundation for the health and stability of ecosystems, as well as the harmonious coexistence of humans and the natural environment. Research on the conservation of wild plants with extremely small populations serves as a critical foundation for informing and enhancing the effective conservation of such populations. This paper provides a systematic examination of the theoretical and practical conservation status of certain wild plants with extremely small populations and discusses the conservation trends of these populations within the framework of ecological protection red line delineation. In the future, strategies for the conservation of wild plants with extremely small populations should be integrated with an analysis of the causes of endangerment. This approach will provide a robust foundation for applied research focused on the conservation of these vulnerable plant populations.

Key words Wild plants with extremely small populations, Conservation research, Cause of endangerment, Conservation countermeasure

0 Introduction

The ecological protection red line is a critical zone within the ecological framework that possesses significant ecological functions and requires stringent protection. It serves as both a fundamental boundary and a lifeline for ensuring and sustaining national ecological security[1]. Since 2005, China has implemented a series of policies and regulations aimed at enhancing environmental governance and ecological protection in response to ecological challenges, including ecosystem degradation and the diminished carrying capacity of natural resources and the environment, which have been exacerbated by rapid industrialization. In 2011, the State Council released the *Opinions on Strengthening Key Environmental Protection Work*, which for the first time established the requirement to delineate a national-level ecological protection red line. This initiative was progressively elevated to a strategic decision aimed at vigorously advancing the construction of ecological civilization. In accordance with the requirements for the implementation of ecological protection red line delineation, China issued the *Guidelines for the Delineation of Ecological Protection Red Line* in 2017. This document serves as a foundational framework for the delineation process and further elucidates the principles of delineation, the procedural steps involved, the technical processes, and the criteria for the resulting outcomes. Eventually, following the assessment and optimization of all departments across various levels, it has been determined that the ecological protection red line encompasses approximately 18% of the national land area designated for various types of nature reserves, 90% of terrestrial ecosystems, and 85% of key populations of wild flora and fauna[2]. This finding underscores that the delineation of the ecological protection red line offers a significant opportunity for the conservation of wild plants with extremely small populations.

The introduction of the concept of wild plants with extremely small populations, along with the implementation of rescuing conservation efforts, exemplifies the delineation of ecological red lines, the protection of biodiversity, and the maintenance of ecosystem balance. Most wild plants with extremely small populations are subject to national and local protection due to their significant ecological, economic, and cultural values[3]. These plants may be utilized for various purposes, including crop improvement, medicinal applications, ornamental use, or they may possess unique eco-evolutionary significance[4]. Rescuing conservation plays a vital role in preserving unique genetic and biological values, which is of considerable importance for biodiversity conservation both in China and the world[5]. Furthermore, understanding the processes that lead to the formation of wild plants with extremely small populations, as well as the associated conservation techniques, is essential for studying their potential adaptability to global climate change[6].

To date, the conservation of wild plants with extremely small populations has been actively pursued. Yunnan Province has established 30 conservation plots for wild plants with extremely small populations following years of dedicated efforts. These initiatives have successfully relocated and conserved a total of 61 species of wild plants, comprising over 100 000 individual plants[7-8]. Addi-

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tionally, the province has reintroduced 16 species, with populations exceeding 30 000 individuals. Based on a survey of field habitat and spatial distribution of resources, the Qinling National Botanical Garden established three distinct living environments for *Ulmus elongata* L. K. Fu & C. S. Ding and *Elaeagnus mollis* Diels. Additionally, several thousand seedlings of both *U. elongata* and *E. mollis* were cultivated through seed propagation and branch cuttings to enhance the population size. This paper reviews theoretical and practical studies concerning the conservation of wild plants with extremely small populations, contextualized within the framework of ecological red line delineation. It analyzes and discusses the relationship between ecological red line delineation and the conservation of these vulnerable plant species. The aim is to provide references for future conservation efforts, facilitating targeted research on mechanisms of endangerment and the implementation of application-oriented conservation practices.

1 Conservation of wild plants with extremely small populations

1.1 Theoretical research (Table 1) Research focusing on the age structure and dynamic renewal of populations and communities, the adaptive analysis of genetic and physiological characteristics in conjunction with environmental factors, and a comprehensive understanding of plant evolution from a reproductive biology perspective are critical for elucidating the ecological requirements, viability, and developmental trends of wild plants with extremely small populations[9-10]. Furthermore, they are significant strategies for addressing the challenges associated with the endangered extinction of such wild plants. Several studies have examined the populations and communities of *Acer amplum* subsp. *catalpifolium* (Rehder) Y. S. Chen, *Sinojackia huangmeiensis* J. W. Ge & X. H. Yao, *Firmiana major* (W. W. Sm.) Hand.-Mazz., *Acer miaotaiense* Tsoong, *etc.* Zhang Yuyang *et al.* [11] discovered that in *A. amplum*, the presence of dominant species in various growth layers competes for substantial growth resources. This competition adversely affects the distribution of seedling and young tree grades, leading to a reduced seed germination rate and challenges in population renewal. Furthermore, the overall fusiform shape of the population structure, along with a higher emergence rate, constitutes significant factors influencing species composition and community structure, which have yet to attain a stabilization stage[12]. *F. major* was previously believed to be extinct in its natural habitat. However, it has been rediscovered in the vicinity of the Niru River Valley in Shangri-La. The wild population of *F. major* spans an area of 0.7 km^2 and comprises over 21 000 individual plants. This finding enhances confidence in the field surveys and conservation efforts directed towards other extremely small populations[13].

The *Sixth Assessment Report on Climate Change* published by the Intergovernmental Panel on Climate Change (IPCC) indicates that the likelihood of a global temperature increase exceeding 1.5 ℃ by the year 2040 is nearing 50%. This projection suggests that global warming will have a direct impact on Earth's flora and the diverse forms of life that depend on these ecosystems. Furthermore, the Arecaceae family serves as a significant bio-indicator of global warming[14]. Research on biome theory indicates that global warming is projected to place 25% of existing species at risk of extinction by the year 2100. As a crucial component of natural ecosystems, the extinction of a wild plant with extremely small population may trigger a chain reaction resulting in the loss of 10 to 30 additional species[15-16]. Zhu *et al.* [17] conducted an evaluation of 443 cases of biodiversity conservation that are aimed at adapting to climate change on a global scale. Their findings indicated that species-specific management strategies, which achieved a population recovery rate of 73%, were significantly more effective than generalized soil and water conservation management approaches, which resulted in a population recovery rate of only 22%. Wang *et al.* [18] synthesized 1 268 cases of species conservation from 2005 to 2023. They proposed that the establishment of germplasm banks for endangered species, along with advanced genome and microclimate modeling and scenario analysis, can effectively predict and mitigate the impacts of future climate warming on species with extremely small populations. In addition, models examining the interactions among plants, soil, microorganisms, and pathogens may be advantageous for the recovery of endangered species[19]. Furthermore, light intensity, water stress, soil physicochemical properties, and stand type are all critical factors influencing the capacity of plants to adapt to changes in their habitat[20-21].

Currently, extremely small populations are encountering significant challenges related to the loss of genetic diversity and inbreeding. These issues, to varying extents, diminish both the continuity of the population and the evolutionary potential of the species[22]. Habitat fragmentation is a significant factor influencing species dispersal. The restricted population size is susceptible to inbreeding, which leads to a decrease in gene flow and ultimately impedes the maintenance of genetic diversity within populations[23]. Several studies have indicated that in populations characterized by relatively low genetic diversity, such as *Rhododendron meddianum* Forrest and *Lirianthe fistulosa* (Finet & Gagne P.) N. H. Xia & C. Y. Wu, the collection of samples from diverse populations, coupled with the minimization of human interference and the enhancement of *ex situ* conservation efforts, can contribute to the restoration of population size and the stabilization of genetic diversity[24]. Furthermore, the relationship between genetic diversity and the risk of species extinction is not characterized by a straightforward negative correlation. This relationship is influenced by various factors, including the biological characteristics of species, soil seed banks, and mutation rates, *etc.* [25]. These factors also contribute to the reappearance of previously declared extinct plant species. Sun Yarui *et al.* [26] conducted an analysis of the biological characteristics of *Petrocosmea grandiflora* Hemsl. utilizing genomic karyotype analysis. Their findings indicated that the stigma of *P. grandiflora* exhibited optimal fertilization potential during the fully open stage of the corolla, at which time the fertilization rate and seed setting rate following pollination approached 70%. The preservation of genetic diversity is essential for species to effectively respond to global changes and human activities, thereby enhancing their resilience. This is evidenced by the fact that certain individuals possess superior stress resistance or harbor specific genes. In the face of alterations in the external environment, particular genotypes are likely to be favored, thereby facilitating active resistance against the invasion and adaptation of specific pathogens or parasites[27]. Conversely, increased genetic diversity can enhance the genetic stability of species. In the face of significant environmental changes, genetic diversity offers a broader range of options, thereby mitigating the risk of mass extinction[28]. In response to the international call for protecting the genetic diversity of at least 90% of species, Ruegg *et al.*[29] integrated the principles of biodiversity and population genetics to stimulate the interplay between genetic diversity and habitat loss. This framework is presently being utilized in the investigation of small flowering plants, including *Oenanthe javanica* (Blume) DC., as well as various animal and plant species such as *Empidonax traillii*.

Reproduction is a critical phase in the life cycle of plants and serves as a vital link for population renewal and maintenance. The germination of seeds and spores constitutes a fundamental process in species reproduction and *in situ* conservation. Wade *et al.*[30] gathered germination data from 28 wild plants with extremely small populations and demonstrated that spore storage is a cost-effective strategy for the *ex situ* conservation of ferns. Currently, over 60% of species necessitate seed cryopreservation to ensure the long-term preservation of genetic information. Furthermore, optimizing seed soaking duration, temperature, and the appropriate concentration of gibberellin can address the challenges that hinder seed production and germination[31]. The bulbils of *Cystopteris chinensis* (Ching) X. C. Zhang & R. Wei are attached to the veins at the apex of the pinnules. New plants can be propagated by cultivating these bulbils in soil culture for approximately 10 d under optimal conditions[32]. The utilization of both sexual and asexual reproduction methods mitigates the risk of species extinction in natural habitats. Research on the mechanisms underlying the endangerment of wild plants with extremely small populations is typically categorized into intrinsic factors (such as low genetic diversity and reproductive disorders) and extrinsic factors (including habitat fragmentation, climate change, and human interference). The primary causes of endangerment and extinction among 28 wild plants with extremely small populations designated as first-level national protection are identified as low reproductive capacity and diminished population competitiveness. Furthermore, the impact of extrinsic factors is predominantly attributed to human interference[33]. The combined impacts of climate change and anthropogenic factors have resulted in a diminished capacity for adaptability in *Ostrya rehderiana* Chun, reaching a critical threshold. Likewise, the low seed germination rates and inadequate stress resistance observed in the population of *Horsfieldia hainanensis* Merr. have hindered its development into juvenile trees. Consequently, the extremely low rate of natural regeneration has contributed to a gradual decline in population size over the past decade.

Table 1 Theoretical research on some wild plants with extremely small populations

Theoretical research	Latin name	Family	Genus	Distribution area	Protection level	Threat	Protection countermeasure	Reference
Population, community structure and dynamic characteristics	*Firmiana major*	Malvaceae	*Firmiana*	Niru River Valley, Shangri-La, Yunnan Province	Extinct in the wild (EW), national level II	Less flowering and fruiting	Shifting from the single species protection to the protection of species habitats and ecosystems	[13]
	Rhododendron griersonianum	Ericaceae	*Rhododendron*	Western Yunnan	Critically endangered (CR), national level II	Limitation of population renewal by human activities	Strengthening the publicity of protection	[12]
	Sinojackia microcarpa	Styracaceae	*Sinojackia*	Wushitan Forest Area, Zhejiang Province	Critically endangered (CR), national level II	Serious human logging activities in history	Strengthening the enclosure and protection of the population and building a core germplasm resource nursery	[50]
	Acer miaotaiense	Sapindaceae	*Acer*	Shennongjia Forestry Area, Hubei Province	Vulnerable (VU), national level II	Small population size, narrow distribution range, weak anti-interference ability	Carrying out management and care at the sapling stage and long-term monitoring of populations and communities	[51]
	Acer amplum subsp. *catalpifolium*	Sapindaceae	*Acer*	Mount Emei and Dujiangyan, Sichuan Province	Least concern (LC), national level II	Strengthening the research on biological characteristics and human defense	Carrying out research on biological and reproductive characteristics and strengthening the protection of individuals and habitats	[11]
Environmental adaptability	*Taxus contorta*	Taxaceae	*Taxus*	Jilong Prefecture, Tibet	Endangered (EN), national level I	Short sunshine hours	Strengthening research on *ex situ* conservation	[52]

(To be continued)

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Theoretical research	Latin name	Family	Genus	Distribution area	Protection level	Threat	Protection countermeasure	Reference
	Abies ziyuanensis	Pinaceae	*Abies*	The middle and upper parts of a few high mountains in Guangxi, Hunan and Jiangxi	Endangered (EN), national level I	Changes in soil volume under intraspecific competition conditions	Enhancing the nitrogen content in the soil and strengthening the monitoring of seedling growth	[53]
	Taxus cuspidata	Taxaceae	*Taxus*	Muling Nature Reserve, Heilongjiang Province	Least concern (LC), national level I	Sharp decline in wild populations due to excessive logging of natural forests	Restoring population habitats	[54]
	Ilex kaushue	Aquifoliaceae	*Ilex*	Hubei, Hunan, and southeastern Yunnan	National level II	Climate warming in the future	Increasing human intervention measures and promoting the natural renewal and spread of the populations	[55]
Genetic diversity	*Cypripedium palangshanense*	Orchidaceae	*Cypripedium*	Wolong Nature Reserve	Endangered (EN), national level II	Relatively weak gene flow among communities	Establishing core protected area	[56]
	Carya sinensis	Juglandaceae	*Carya*	Sandu County, Guizhou Province	Endangered (EN), national level II	Relative low level of genetic variation	Promoting sexual reproduction among different genotypes and establishing outcrossing protocols	[57]
	Petrocosmea grandiflora	Gesneriaceae	*Petrocosmea*	Mengzi City, Yunnan Province	Endangered (EN)	Pollen restriction	Conducting pollination when the stigma is most receptive during the fully open stage of the corolla	[26]
	Cycas dolichophylla	Cycadaceae	*Cycas*	Tianyang County, Baise City, Guangxi Province	Near threatened (NT), national level I	Low degree of genetic differentiation among populations	Adopting artificial pollination	[58]
	Cephalostachyum mannii	Poaceae	*Cephalostachyum*	Gongshan County, Yunnan Province	Key species in Yunnan Province	Significant inbreeding	Adopting reasonable population management and breeding plans	[59]
Reproductive biology	*Cystopteris chinensis*	Cystopteridaceae	*Cystopteris*	Erlang Mountain, Tianquan County, Sichuan Province	Extinct in the wild (EW), national level I	High interspecific competition, low photosynthetic capacity, and special habitat	Enhancing growth monitoring and research on bulbil propagation	[32]
	Quercus sichourensis	Fagaceae	*Quercus*	Funing and Xichou, Yunnan Province	Critically endangered (CR), national level I	Low temperature for seedling raising	Controlling temperature for seed germination not lower than 20 ℃	[60]
	Urophysa rockii	Ranunculaceae	*Urophysa*	Jiangyou City, Sichuan Province	Critically endangered (CR), national level I	The flowering phenology and the reproductive system have reproductive disorders in adapting to special environments	Combined with the evidence of pollination ecology	[61]
	Dendrobium flexicaule	Orchidaceae	*Dendrobium*	Chengkou County, Chongqing City	Critically endangered (CR), national level I	Reproductive disorder	Strengthening the research on artificial propagation and cultivation techniques	[62]
	Lirianthe odoratissima	Magnoliaceae	*Lirianthe*	Wenshan Prefecture, Yunnan Province	Critically endangered (CR), national level II	Inhibitors in seeds that prevent seed germination	250 mg/L GA_3 can break seed dormancy	[31]
Endangered mechanism	*Ostrya rehderiana*	Betulaceae	*Ostrya*	Tianmu Mountain Reserve, Lin'an, Zhejiang Province	Critically endangered (CR), national level I	Human interference and climate change	Scientifically designing artificial hybridization strategies and enhancing the reproductive capacity of wild individuals	[27]
	Horsfieldia hainanensis	Myristicaceae	*Horsfieldia*	The area around Ningming and Fangcheng in Guangxi	National level II	Human interference and climate change	Improving the light transmittance of the forest	[28]

Table 2 Practical research on some wild plants with extremely small populations

Theoretical research	Latin name	Family	Genus	Distribution area	Protection level	Threat	Protection countermeasure	Reference
In situ protection	*Diospyros sutchuensis*	Ebenaceae	*Diospyros*	Simian Mountain, Chongqing City	Critically endangered (CR), national level I	Extreme deficiency of seedlings in its original habitat	Enhancing the environment of *in situ* protection and increasing the number of young seedlings	[40]
	Bhesa robusta	Centroplacaceae	*Bhesa*	Beihai and Fangchenggang, Guangxi Province	Critically endangered (CR), national level I	Insufficient lighting and poor natural renewal ability of the community	Removing the shading trees on the upper layer of the community to ensure the lighting rate	[41]
	Pinus wangii	Pinaceae	*Pinus*	Malipo County, Yunnan Province	Critically endangered (CR), national level II	Insufficient supply of seedlings leads to difficulty in revitalizing wild populations	Increasing soil moisture content in habitats	[63]
	Rhododendron protistum var. *giganteum*	Ericaceae	*Rhododendron*	Baoshan Administration Bureau of Gaoligong Mountain, Yunnan Province	Critically endangered (CR)	Strong limitations in population distribution	*In situ* protection of remaining populations	[64]
	Cypripedium macranthos	Orchidaceae	*Cypripedium*	Wuling Mountain National Nature Reserve, Hebei Province	Endangered (EN), national level II	Tourists trampling, indiscriminate mining and digging	Setting up video surveillance and promoting science popularization	[42]
	Geodorum densiflorum	Orchidaceae	*Geodorum*	Guizhou, Guangxi, and Yunnan	Endangered (EN)	Significant overlap with the ecological niches of most species	Establishing protective zone for *on-situ* protection	[65]
Ex situ conservation and preservation of germplasm resources	*Betula halophila*	Betulaceae	*Betula*	Altay Prefecture, Xinjiang	Extinct in the wild (EW), national level II	Human interference and climate change	Relocating to Shandong for conservation and setting up wind barriers during the sensitive period of the seedlings	[45]
	Vatica guangxiensis	Dipterocarpaceae	*Vatica*	Mengla County, Yunnan Province	Critically endangered (CR), national level I	Low seed germination rate	Carrying out seed germination in the *ex-situ* areas	[66]
	Lirianthe odoratissima	Magnoliaceae	*Lirianthe*	Wenshan Prefecture, Yunnan Province	Critically endangered (CR), national level II	Excessive human interference	Carrying out the reconstruction of *ex situ* protected areas for wild populations	[67]
	Nyssa yunnanensis	Nyssaceae	*Nyssa*	Puwen Experimental Forest Farm, Yunnan Province	Endangered (EN), national level I	Severe destruction of the original habitat	Establishing protective zone	[21]
	Paeonia rockii subsp. *atava*	Paeoniaceae	*Paeonia*	Taibai Mountain, Shaanxi Province	Vulnerable (VU), national level II	Defects in its own natural reproductive capacity and changes in the natural environment	Conducting scientific research and breeding	[68]
	Acer amplum subsp. *catalpifolium*	Sapindaceae	*Acer*	Sichuan Province	Least concern (LC), national level II	Large variation of seed thickness	The populations in Pingwu and Leibo areas are the preferred places for high-quality germplasm resources	[11]
Reintroduction into natural habitat	*Petrocosmea qinlingensis*	Gesneriaceae	*Petrocosmea*	Lueyang County, Hanzhong City, Shaanxi Province	Critically endangered (CR), national level II	Relatively short overlapping period of pollen and stigma vitality	Reintroduction into natural habitats and artificial breeding	[69]
	Taxus wallichiana var. *chinensis*	Taxaceae	*Taxus*	Southern Gansu, southern Shaanxi, and Sichuan	Vulnerable (VU), national level I	Slow growth and poor regenerative ability	Simulating the growth environment outdoors in the nursery	[68]
	Ulmus elongata	Ulmaceae	*Ulmus*	Guanshan National Nature Reserve, Jiangxi Province	Vulnerable (VU), national level II	Extremely underdeveloped samara	Increasing the population size through reintroduction to natural habitat	[70]

(To be continued)

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Theoretical research	Latin name	Family	Genus	Distribution area	Protection level	Threat	Protection countermeasure	Reference
	Davidia involucrata	Nyssaceae	*Davidia*	Shaanxi, Sichuan, and Chongqing	National level I	Severe phenomenon of seed abortion	The application of rapid propagation techniques enables the mass production of seedlings for reintroduction into natural habitat	[68]
Artificial breeding	*Gleditsia japonica* var. *velutina*	Fabaceae	*Gleditsia*	Guangji Temple on Mount Heng in Nanyue	Critically endangered (CR), national level II	Weak reproductive ability	Establishing rapid propagation technology system for tissue culture	[71]
	Yulania sinostellata	Magnoliaceae	*Yulania*	Songyang County, Zhejiang Province	Critically endangered (CR), national level II	Less fruiting number and difficulty in asexual reproduction	Carrying out layerage propagation experiments and establishing their layerage propagation methods	[72]
	Firmiana danxiaensis	Malvaceae	*Firmiana*	Danxia Mountain Nature Reserve	Critically endangered (CR), national level II	Lack of artificial breeding technology	Using *Firmiana simplex* as an anvil to enhance cold resistance	[68]
	Petrocosmea grandiflora	Gesneriaceae	*Petrocosmea*	Mengzi City, Yunnan Province	Endangered (EN)	Wild populations are threatened to varying degrees	Conducting research on asexual reproduction techniques	[73]

1.2 Practical research (Table 2) China integrates ecosystems that possess significant and representative ecological functions into its legal protection framework by delineating ecological protection red lines. *In situ* conservation maintains the reproduction and evolution of organisms in a concrete manner and serves as a crucial method for safeguarding wild plants and their habitats[34]. Currently, there are no established protection concepts or objectives specifically targeting wild plants with extremely small populations in China. Consequently, the protection strategies developed in this context have increasingly served as a model advocated by other countries[35]. The alterations in soil chemical properties and the loss of habitat on slopes are significant limiting factors that impact the growth of plant species on cliff walls. A high correlation, quantified at 0.94, exists between the population abundance of *Pachyphytum caesium* Kimnach & R. C. Moran and the degree of landscape fragmentation. Consequently, the conservation of plant habitats on cliff walls has been incorporated into the *Guidelines for the Conservation of Native Wildlife Species in Mexico—List of Endangered Species*[36]. The reconstruction of the *in situ* conservation area for the extremely small population of the fern *Ophioglossum vulgatum* L. in Sardinia, Italy, along with the establishment of long-term monitoring efforts, has resulted in a nearly 50% recovery of populations of other small ferns, including *O. vulgatum*, in recent years[37]. Over the past five decades, *Glyptostrobus pensilis* (Staunton ex D. Don) K. Koch, an aquatic conifer, has been extensively distributed throughout the Pearl River Delta. Recent investigations conducted at 42 wild distribution sites of *G. pensilis* indicate that only approximately 350 individuals of this species survive in China, alongside around 120 individuals in Vietnam and approximately 300 individuals in Laos[38]. Furthermore, in the regions bordering the western part of North America and the northern region of Vietnam, there exist remnants of extremely small populations, including *Craigia yunnanensis* W. W. Sm. & W. E. Evans, *Dipteronia sinensis* Oliv., and *Cathaya argyrophylla* Chun & Kuang. These populations require immediate and comprehensive conservation efforts, which should encompass the investigation of the genetic structure of the remaining populations as well as the establishment of *ex situ* conservation populations[39].

The regions in China characterized by a high density of wild plants with extremely small populations include southeastern Yunnan, southwestern Guangxi, and southwestern Hainan Island. Zhang Zejin *et al.*[5] sorted out high-precision distribution maps for 120 species of wild plants with extremely small populations. Their findings indicate that the establishment of national and provincial nature reserves has successfully encompassed over 30% of these species. Notably, Yunnan Province and Guangxi Province have effectively included the majority of their native wild plants with extremely small populations through the establishment of various nature reserves. The habitats of wild plant populations, including *Diospyros sutchuensis* Yang[40], *Bhesa robusta* (Roxb.) Ding Hou[41], *Cypripedium macranthos* Sw.[42], and *Pseudotsuga sinensis* Dode[43], are increasingly threatened by human activities. The establishment of conservation zones and micro-protected areas has significantly enhanced the number of plant communities and habitat populations. *Ex situ* conservation and the preservation of germplasm resources are effective conservation strategies that require relatively low resource investment. These methods serve as crucial backups for *in situ* conservation efforts and the reintroduction of species into their natural habitats. Furthermore, it is essential for botanical gardens to prioritize activities related to the introduction, cultivation, and conservation of plant species. Yunnan Province has established *ex situ* conservation areas for 61 plant species with extremely small populations, including *Nyssa yunnanensis* W. Q. Yin ex H. N. Qin & Phengklai and *Betula halophila* Ching ex P. C. Li[44-45]. East China Normal University has collected a total of 1 500 germplasm resources, which encompass 9 species with extremely small populations, such as *G. pensilis* and *O. rehderiana*. The Xishuangbanna Tropical Botanical Garden, affiliated with the Chinese Academy of Sciences, conducted *ex situ* conservation experiments on 38 species of nationally key protected plants. The findings indicated that 92% of the plants exhibited strong adaptability, with a flowering and fruiting rate reaching 84%. The majority of the plants successfully completed their generational transition under *ex situ* conservation conditions[46]. Furthermore, the comparison of life history and genetic diversity between *ex situ* and wild plant populations can provide a critical foundation for assessing the rationality of *ex situ* conservation.

Reintroduction into natural habitats functions as a critical bridge between *ex situ* and *in situ* conservation for wild plant species. This process is the primary objective of *ex situ* conservation and is considered the most effective method for the restoration of wild populations. Currently, China has successfully completed more than 300 plant reintroduction projects involving 206 species. The Qinling International Botanical Garden has cultivated seedlings of *Davidia involucrata* Baill. and *Taxus wallichiana* var. *chinensis* (Pilg.) Florin through the method of branch cuttings. As a result, 1 000 plants have been successfully reintroduced into their natural habitat under controlled artificial care conditions. For rare and endangered plant species characterized by limited survival capabilities and adaptability, the likelihood of successful reintroduction into their natural habitats has been enhanced through the improvement of environmental conditions, including light, temperature, and water, as well as through the implementation of robust human management and protection strategies. From the standpoint of assessing the long-term outcomes of such reintroductions, the establishment of a scientific monitoring system is essential for evaluating the self-repair mechanisms of the population and the stability of ecological communities. Liu *et al.* [47] conducted a scientific assessment of nearly 200 domestic reintroductions into their natural habitat and discovered that over half of the individual species lacked survival records. This finding suggests that the establishment of continuous field monitoring in the later stages is an effective method for verifying the ultimate success of reintroduction into natural habitats. Large-scale reproduction serves as a critical component in the conservation of plant species, and addressing the challenges associated with reproductive bottlenecks is of paramount importance in efforts to alleviate the plight of endangered populations of wild plants with extremely small populations. The propagation and preservation of rare and endangered plant species are typically achieved through various methods, including seed propagation, seedling cultivation, cuttings, and tissue culture, all of which aim to maintain genetic diversity. The project titled on Conservation and Restoration Techniques for Wild Plants with Characteristically Extremely Small Populations successfully cultivated nearly 300 000 seedlings across approximately 30 species through the combined methods of sexual and asexual reproduction[48]. However, approximately 35% of the seeds from certain endangered species, including *Paphiopedilum emersonii* Koop. & Cribb, *Gleditsia japonica* var. *velutina* L. Chu Li, and *Firmiana danxiaensis* H. H. Hsue & H. S. Kiu, are unable to survive under standard survival conditions during sexual reproduction. Consequently, investigating the optimal conditions of light, temperature, and humidity during seed storage and germination is an essential prerequisite for the successful advancement of artificial plant breeding[49].

2 Delineation of ecological protection red lines to assist in the protection of wild plants with extremely small populations

2.1 Building a solid barrier for the protection of wild plants with extremely small populations

2.1.1 Compulsory protection at the legal level. The ecological protection red line is a mandatory protective boundary established for areas that are ecologically significant and ecologically vulnerable, aimed at ensuring national ecological security and the conservation of biodiversity. This concept has now been integrated into both the *Environmental Protection Law* and the *National Security Law*. The delineation of ecological protection red lines includes the integration of habitats for wild plants with extremely small populations into a legally mandated protection framework at the national level. The comprehensive implementation guidelines entail specific management strategies for the habitats of these wild plants. This approach not only enhances the protection of species with extremely small populations but also offers robust legal assurances for their survival and recovery.

2.1.2 Overall maintenance of ecosystem. The delineation of ecological protection red lines is conducted through a top-down technical approach, thereby ensuring the systematic and comprehensive governance and maintenance of ecosystems. The document titled *Several Opinions on Delineating and Strictly Maintaining Ecological Protection Red Lines* and *the Guidelines for Delineating Ecological Protection Red Lines* (HBST [2017] 48) explicitly articulate that ecological protection red lines serve not only to safeguard specific species but also to prioritize the overall health of entire ecosystems. This approach fosters a stable and diverse ecological environment, which is essential for the conservation of wild plants with extremely small populations, thereby facilitating the natural recovery of these populations and promoting genetic exchange.

2.1.3 Long-term planning and dynamic management. The implementation of the ecological protection red line promotes long-term planning initiatives. China has made substantial contributions to the achievement of biodiversity conservation goals through the establishment of national botanical gardens, which facilitate *ex situ* conservation, and national parks, which support *in situ* conservation efforts. With ongoing comprehensive research on wild plants with extremely small populations, the strategy for ecological protection red lines has been promptly adjusted to enhance the effectiveness of conservation efforts for these vulnerable species. Furthermore, activities within the designated red line areas are subject to strict and dynamic regulation. Regular monitoring and assessment of the flora within the protected zones facilitate the effective implementation of protection measures, while also enabling the timely identification and resolution of potential threats.

2.1.4 Social participation and policy support. The publicity and implementation of the ecological protection red line have significantly increased public awareness regarding the conservation of wild plants with extremely small populations. This initiative has fostered public engagement in conservation activities and has created a synergistic effect in conservation efforts, which is essential for the long-term preservation of these vulnerable wild plant species.

Furthermore, with the implementation of the ecological protection red line policy, the government may enhance financial investment in conservation projects aimed at wild plants with extremely small populations. This initiative would provide essential resources for research, monitoring, and the construction and management of protective facilities for these vulnerable populations.

2.2 Providing an innovative model for the protection of wild plants with extremely small populations

2.2.1 Scientific assessment and precise positioning. The ecological protection red line, which is grounded in technical methods for evaluating the significance of ecological conservation, quantitatively assesses and spatially delineates ecosystem service functions, biodiversity (including critical habitats, ecological corridors, and distribution areas of extremely small populations), as well as ecological vulnerabilities. This initiative is a significant achievement and a major institutional innovation in the construction of ecological civilization, and its scientific validity has been widely acknowledged. By conducting a scientific assessment of the richness and uniqueness of biodiversity, accurately identifying the distribution areas and ecological requirements of extremely small populations, and implementing targeted conservation measures for the habitats of wild plants with extremely small populations, it is ensured that these areas, which possess special ecological functions and significant biodiversity value, are effectively incorporated within the ecological protection red line.

2.2.2 Enhancement of habitat connectivity. Ecological corridors play a vital role in linking isolated wild plants with extremely small populations. The *Technical Guidelines for the Delineation of Ecological Protection Red Lines* consider the systematicness of natural ecology, ensuring that all necessary areas are appropriately delineated to prevent habitat fragmentation. Furthermore, these guidelines underscore the importance of preserving the connectivity of ecological corridors. This approach facilitates the connectivity of fragmented habitats for wild plants with extremely small populations, promotes their migration and genetic exchange, and enhances the stability and resilience of these populations. Furthermore, the establishment and protection of ecological corridors are of paramount importance for maintaining genetic diversity and preventing population decline due to inbreeding.

2.2.3 Scientific research and monitoring. The ecological protection red line area, which will serve as a focal point for future ecological conservation efforts, encompasses various studies, including population dynamics, genetic diversity, and mechanisms of endangerment. This area offers both theoretical support and an optimal setting for the formulation and implementation of scientific conservation strategies aimed at wild plants with extremely small populations. The *Technical Specifications for Ecological Protection Red Line Supervision—Ecological Condition Monitoring*, currently under pilot implementation, have explicitly delineated the monitoring methods, technical processes, and content relevant to ecologically fragile areas. This framework provides a solid basis for the scientific and continuous monitoring of extremely small populations, thereby enhancing the understanding of their ecological needs and contributing to the formulation of more effective conservation strategies.

2.2.4 International cooperation and demonstration effect. China's practice in delineating ecological protection red lines has generated innovative thoughts for global biodiversity conservation, thereby fostering advancements in this field. The successful initiatives undertaken to protect wild plants with extremely small populations have emerged as exemplary models for international communication. Notable examples include the conservation and propagation of *Acer yangbiense* (Y. S. Chen & Q. E. Yang), *Pachylarnax sinica* (Y. W. Law) N. H. Xia & C. Y. Wu), and *C. yunnanensis*. Meanwhile, the ecological protection red line system is designed to preserve ecological functions, maintain the nature of natural environments, and prevent the reduction of protected areas. The innovative aspects of this system have established a benchmark for the conservation of wild plants with extremely small populations on a global scale.

3 Factors contributing to the endangerment of wild plants with extremely small populations and corresponding conservation strategies

3.1 Factors contributing to the endangerment of wild plants with extremely small populations The rapid expansion of China's economy has resulted in a heightened demand for wild plant resources, consequently exacerbating the destruction of plant habitats. Currently, there are 3 879 higher plant species classified as threatened in China, which represents 10.84% of the total number of species[74]. This article synthesizes findings from theoretical research in the reproductive and genetic biology of plants with extremely small populations, alongside practical studies involving both *in situ* and *ex situ* conservation methods, and delineates the primary factors contributing to the endangerment of these extremely small populations. (i) The factors contributing to the endangerment of wild plants with extremely small populations are multifaceted and varied. Habitat destruction is the primary driver of species endangerment and even potential extinction[75]. In China, the area of natural forests has experienced a significant reduction, leading to the loss or fragmentation of habitats for certain wild plant species. This has resulted in a marked decline in species numbers, thereby complicating their chances of survival. (ii) The excessive utilization of resources constitutes a significant contributing factor to the decline of various plant species. The population of plants possessing unique medicinal, ornamental, or timber values has experienced a marked decrease due to overexploitation[76]. Specifically, all parts of *Camptotheca acuminata* Decne. contain camptothecin, which exhibits notable anti-cancer properties. However, the species has suffered considerable damage as a result of resource overutilization, leading to a wild population of fewer than 100 individuals[77]. Besides, *Pterocarpus indicus* Willd., *Thuja koraiensis* Nakai, *Hopea chinensis* (Merr.) Hand.-Mazz., *Euryodendron excelsum* Hung T. Chang, and various members of the Orchidaceae family have experienced overexploitation due to local economic interests, leading to a gradual decline in their populations[78]. (iii) Certain plant species are progressively being eliminated due to their vulnerable survival capabilities. Their unique growth cycles and reproductive strategies render them highly sensitive to environmental fluctuations and hinder their ability to adapt to anthropogenic influences. Consequently, the population sizes of these species are either gradually declining or approaching extinction, as exemplified by *Abies beshanzuensis* M. H. Wu and members of the Magnoliaceae family, such as *P. sinica*[79]. (iv) The existing protection measures lack sufficient specificity. While certain wild plants with extremely small populations have been incorporated into the protection list, there is a notable deficiency in comprehensive research pertaining to population genetics, reproductive biology, and community ecology. This gap in knowledge has contributed to the precarious survival status of the majority of these extremely small populations. Furthermore, wild plants with extremely small populations encounter challenges including limited geographic distribution, significant human interference, and the accumulation of harmful mutations. The synergistic impact of these factors has intensified the endangered status of these species.

3.2 Countermeasures for the protection of wild plants with extremely small populations In light of the preceding analysis and summary regarding the factors contributing to the endangerment of wild plants with extremely small populations, the following protective measures are proposed with the objective of restoring these vulnerable plant species.

3.2.1 Habitat restoration and reconstruction. The restoration of ecosystem functions in habitats that have experienced varying degrees of destruction is achieved through a combination of vegetation restoration, soil remediation, and the recovery of microbial communities. In cases of mild to moderate habitat degradation, artificial interventions such as enclosure, tending, reseeding, and replanting are implemented to facilitate the natural recovery of vegetation. Additionally, techniques such as tree planting, irrigation, and grass planting are employed to enhance the self-recovery of soil health[80]. In the case of severely degraded habitats, the restoration of the original vegetations is achieved through methods such as artificial seedling planting and direct seeding, which are informed by the original vegetation types. Engineering interventions, including soil replacement, the creation of fish scale pits, and the enhancement of surface cover, are employed to improve and restore soil conditions. Additionally, microbial resources associated with the conservation objectives are introduced into the degraded habitats to facilitate the recovery of relevant microbial communities and the broader ecosystem in which they reside[81]. Furthermore, wild plants with extremely small populations are at a heightened risk of extinction. The restoration of habitats is of paramount importance, and effective management of water resources serves as a critical component in this process. Miyun Reservoir, a critical surface drinking water source and strategic water resource reserve in Beijing, has implemented habitat restoration, water ecological restoration, and monitoring initiatives through the collaborative efforts of various departments. *Fritillaria maximowiczii* Freyn, a second-class protected plant in China, has experienced a remarkable increase in its population, expanding from approximately 20 individuals to over 2 000[82].

3.2.2 Implementation of precise management. For wild plants with extremely small populations, precise management entails the implementation of management and protection strategies tailored to local conditions. This approach is informed by research into the mechanisms of endangerment, utilizing scientific methods such as population assessments, community and habitat investigations, genetic adaptability studies, evaluations of genetic diversity, and analyses of reproductive biology. Additionally, technical measures, including *in situ* and *ex situ* conservation, reintroduction into the natural habitat, and long-term monitoring, are employed to ensure that these populations are sustained and that their habitats are effectively enhanced[83-85]. The investigation into the distribution, abundance, and ecological and biological characteristics of wild plants with extremely small populations provides insights into their life history, reproductive behaviors, ecological requirements, and the threats they encounter. This understanding is essential for the development of scientifically informed and effective conservation strategies. The conservation strategies encompass the establishment of a comprehensive monitoring system for plants with extremely small populations, as well as the implementation of dynamic monitoring and systematic assessment of the number, distribution, and habitat conditions of target plant species, aiming to enhance the understanding of their survival status and trends in population changes. Simultaneously, it is essential to implement dynamic update management for the list of extremely small populations. Populations that have undergone re-investigation and have been re-classified as endangered should be promptly incorporated into the list, and protection strategies should be adjusted accordingly. Priority should be given to safeguarding wild plants with extremely small populations that are at imminent risk of extinction.

3.2.3 Improvement of sustainable management. (i) Strict collection restrictions and bans. During critical periods or in specific areas, it is imperative to implement stringent collection restrictions or even an outright ban on the harvesting of wild plants with extremely small populations. Such measures are essential to facilitate the recovery and growth of these populations. Furthermore, it is necessary to enhance the supervision of collection activities to deter illegal harvesting practices and safeguard wild plant resources. (ii) Ecological compensation mechanisms and community participation. For communities that rely on natural resources, it is essential to establish ecological compensation mechanisms. These mechanisms should incentivize local populations to engage in conservation initiatives and diminish their reliance on wild plants with extremely small populations by promoting alternative livelihoods. (iii) Artificial breeding and development of alternatives. The development of cultivated alternatives for economically valuable species, particularly through horticultural propagation, serves to meet market demands while alleviating pressure on wild populations. Promoting the use of these cultivated alternatives is essential for decreasing reliance on and demand for wild plants, ultimately contributing to the conservation of wild populations. (iv) Implementation of training and education activities. The public's awareness regarding the conservation of wild plants with extremely small populations can be enhanced through educational initiatives and information dissemination campaigns. Additionally, it is essential to mitigate non-essential consumption behaviors, particularly concerning these vulnerable wild plants with extremely small populations, and to actively advocate for the utilization of sustainably sourced products[86].

3.2.4 Enhancement of long-term survival ability. It is essential to develop scientifically robust conservation strategies and establish long-term survival mechanisms that are grounded in a comprehensive understanding of the distribution patterns of genetic diversity in wild plants with extremely small populations. *P. sinica* is a wild plant with extremely small population, which is exclusively distributed in southeastern Yunnan. Lei *et al.*[87] conducted a comprehensive analysis of the whole genome of *P. sinica* and identified genetic bottlenecks and deleterious mutations as the primary factors contributing to the formation of its extremely small population. In this context, it is recommended that additional artificial heterosis strategies involving diverse populations and individuals should be developed for *P. sinica* in order to mitigate the loss of genetic diversity associated with inbreeding. Regarding *in situ* and *ex situ* conservation, which is particularly relevant for extremely small populations, it may be advantageous to prioritize the conservation of populations exhibiting a high frequency of endemic haplotypes[88]. As the degree of habitat fragmentation intensifies, it becomes imperative to implement measures such as artificial assisted pollination, the alleviation of seed dormancy, and the timely replenishment of seed banks. These actions are essential for maintaining genetic connectivity among populations and ensuring population stability[86]. Populations characterized by extremely low numbers and heritability, which are in critical need of immediate conservation efforts, should be preserved *in situ*. This can be achieved by collecting germplasm resources, such as seeds, pollen, tissues, organs, and other propagating materials, from populations exhibiting high genetic diversity[89]. In the later stages, this approach can be integrated with the development of ecological corridors to improve connectivity among species, facilitate gene flow between populations, and strengthen the adaptability and resilience of these populations.

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