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Research Progress on *Manglietia ventii*, A Wild Plant Species with Extremely Small Populations

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Abstract *Manglietia ventii* is a wild plant species with extremely small populations endemic to Yunnan, mainly distributed in southeast Yunnan. Due to the continuous deterioration of natural habitats, excessive felling and utilization of human beings, and the decline of breeding ability, the number of individuals in the population has decreased significantly. Through field investigation and literature review, the research status of *M. ventii* in systematics, conservation ecology, reproductive biology, genetic diversity, endangered mechanism and resource protection at home and abroad are systematically reviewed. And the future research direction is prospected. It is necessary to strengthen the research on the basic characteristics of *M. ventii*, explore the transmission route of *M. ventii* and deepen the development and utilization of resources, in order to provide a theoretical support for the protection and sustainable utilization of germplasm resources of *M. ventii*, and provide a reference for the protection of other wild plant species with extremely small populations.

Key words *Manglietia ventii*, Plant species with extremely small populations (PSESP), Research progress

1 Introduction

Manglietia ventii N. V. Tiep, belonging to *Manglietia*, Magnoliaceae, is an evergreen arbor with a height of 36 m and a diameter at breast height of 80 cm, and it is a rare and endangered wild plant with a very narrow range of natural distribution[1-2]. As a relatively primitive *Magnolia*-type plant with a long evolutionary history, it is of great scientific significance to explore the phylogenetic process of Magnoliaceae plants[2]. In addition, the wood of *M. ventii* is a diffuse-porous wood with fine structure and strong resistance to corrosion and pests, being an excellent wood species for industrial buildings and costly furniture[3]. It is characterized by fast growth rate, tall tree form, pure white color and fragrance, and can be used as an excellent garden ornamental tree[4]. Chemical composition studies have shown that monoterpenes and sesquiterpenoids are the main secondary metabolites produced by *M. ventii*, which have high anticancer activity. The plant can also be used medically[5-6]. Thus, *M. ventii* has high value and significance in scientific research, economic utilization, landscape ecology and many other aspects. However, due to long-term and repeated interference, further destruction and loss of suitable habitat and degradation of its own breding ability, *etc.*, the population of *M. ventii* decreases sharply[1-2]. As early as 1999, it was listed as a national class II key protected wild plant. In 2012, it was listed as one of the first batch of wild plant species with extremely small populations priority to be saved and protected in Yunnan Province, and was identified as "endangered (EN)" in the *Red List of Magnoliaceae* published in 2016[7-9]. In recent years, with the development of extremely small population conservation and the in-depth study of *M. ventii*, the research on systematics, conservation ecology, reproductive biology, genetic diversity, endangered mechanism and resource conservation of *M. ventii* have developed rapidly. By summarizing the progress of related work, and looking forward to the future research work, the paper aims to provide a theoretical support for the conservation and sustainable utilization of germplasm resources of *M. ventii*, and provide a reference for the protection research of other wild plant species with extremely small populations.

2 Systematics

In 1980, Tiep[10] collected the specimens of *M. ventii* in Pingbian County, Yunnan Province for the first time. Based on the morphological characteristics of style, carpel and pollen, *M. ventii* was defined as a new species and classified into *Manglietia*, Magnoliaceae, which was rarely reported after publication. According to the type specimen collected in Jinping County, Yunnan Province, academician Wu Zhengyi published a new species in the *Flora of China* and named *M. hebecarpa*[11]. Due to the similarities and differences in the living environment, *M. ventii* and *M. hebecarpa* were once mistaken as two independent species[12]. Later, they were officially classified as the same species by academician Wu Zhengyi in the *Flora of Yunnan* published in

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2006. Therefore, the Latin scientific name of *M. ventii* has synonyms[13]. In addition, due to a large number of overlapping phenomena in the external morphology and internal structure of *Magnolia* and *Manglietia*, there are different views on the definition of genus classification[14-18]. This leads to the following taxonomic treatments of the name of *M. ventii*: *Manglietia hebecarpa*[11], *Magnolia hebecarpa*[12], *Manglietia ventii*[2] and *Magnolia ventii*[9]. The existing research data suggest there are still divergence differences in the classification limits of the family Magnoliaceae. A large number of theoretical bases for the systematic classification position of *Manglietia* have been proposed at home and abroad, but these classification systems have not been widely recognized and supported[19-22]. At present, the classification system of Magnoliaceae proposed by Liu Yuhu[19] is generally adopted in China to separate the genus *Manglietia*. The Latin scientific name *Magnolia ventii*, which is used by foreign scholars to publish relevant literatures, relatively recognizes the system of Nooteboom[20]. Morphology and anatomy is usually the main basis for the establishment of taxonomy system. With the continuous breakthrough and development of biotechnology, different scholars have carried out systematics study on *M. ventii* from the perspectives of palynology, cytology, molecular biology and other disciplines. Sima Yongkang *et al.*[23] suggested that the folding characteristics of young leaves is of great systematical significance. The young leaves of *M. ventii* are folded in half and upright in the bud, which is a typical feature of the genus *Manglietia*. Xue Xiaoming *et al.*[24] observed the pollen morphology of *M. ventii* through scanning electron microscopy, and found that the pollen grain patterns were composed of folds and small perforations, with similar shapes to *M. moto*, *M. patungensis* and *M. decdua* of the same genus. Its pollen grains are bilaterally symmetrical, and the pollen gullies are located at the distal face, reaching the two poles, which are basically similar to the pollen characteristics of other published plants of the genus *Manglietia*[25]. Ye Shanshan *et al.*[26] analyzed the prolamin characteristic bands of *M. ventii* through PAGE technique, and found that *M. ventii* had similar genetic basis with more than 10 species of Magnoliaceae seeds. In order to reveal the evolutionary position of *M. ventii* in Magnoliaceae, Wang Yuchang *et al.*[27] compared the chloroplast genome of *M. ventii* with that of 45 reported Magnoliaceae plants, and the results indicated that *M. ventii* had the closest relationship with *M. aromatic* and *M. megaphylla*. Wang Meng *et al.*[28] studied and analyzed the genetic diversity of various Magnoliaceae plants via ISSR technology, and found that *M. ventii* was relatively close to some species of *Magnolia*, *Manglietia* and *Michelia*, which had obvious similarities and differences with the taxonomy system proposed by Liu Yuhu[19].

To sum up, the main differences in the taxonomy of *M. ventii* lie in the definition of genus within Magnoliaceae. Although morphology, anatomy and cytology provide a lot of favorable evidences for the independence of *Manglietia*, the analysis results from the perspective of molecular biology indicate that there are a lot of overlaps and crossovers between *Manglietia* and *Magnolia*, which should be merged into *Magnolia*. At present, there is no consensus on the definition of genera of Magnoliaceae. We hold that different characters are distributed in a network in various groups of Magnoliaceae. The definition of genera should not be limited to a single character, but should be considered in combination with a variety of characters. Therefore, it is very important to determine the taxonomic status of *M. ventii* by establishing an international unified and objective standard of phylogenetic systematics based on a variety of characters.

3 Conservation ecology

3.1 Physical geographic distribution *M. ventii* is a rare and endangered wild plant with a narrow distribution range, and now is scattered in Jinping Miao, Yao and Dai Autonomous County, Pingbian Miao Autonomous County, Hekou Yao Autonomous County and Yuanyang County in Honghe Prefecture[29-30]. The investigation by related research teams of Kunming Institute of Botany, Chinese Academy of Sciences and Jinping Fenshuiling National Nature Reserve via quadrat sampling method shows that there are about 2 452 plants of *M. ventii*, including 2 024 plants in Jinping County, 366 plants in Pingbian County, 60 plants in Hekou County and 2 plants in Yuanyang County, and most of the plants are outside the reserve[30-31]. Wild plants are mainly distributed in mountain monsoon evergreen broad-leaved forests and near farmlands and villages, 103°31′33″ – 103°51′42″ E, 22°38′29″ – 22°53′56″ N, with an altitude of 800 – 1 500 m[31].

3.2 Habitat characteristics The distribution area of *M. ventii* has distinct dry and wet seasons, which is not hot in summer and warm in winter, with much rainfall and high humidity. The average annual temperature is 16.4 – 22.6 ℃, the annual rainfall is 1 650 – 2 960 mm, and the fog phase is longer[2]. The soil texture for plant growth is mainly acidic mountain yellow soil or yellow brown soil, and the contents of various indexes in different distribution points do not show a similar trend, but the soil fertility changes greatly[32]. *M. ventii* forms mixed forest with *Canarium album*, *Garcinia cowa*, *Gymnosphaera podophylla*, *Castanopsis hystrix*, *Manglietia duclouxii*, *Lindera metcafiana* var. *dictyophylla*, *Parakmeria yunnanensis*, *Sapium discolor*, *Altingia excelsa*, *Macaranga henryi*, *Cinnamomum iners*, *Machilus bombycina*, *Schima wallichii*, *Actinodaphne obovata* and *Artocapus tonkinensis*[31]. It is commonly found in artificial forests, grass and fruit fields and natural secondary forests, as well as along village farmlands and country roads. It exists in the form of single plant, scarce forest or open forest with few saplings around, and its habitat is relatively fragile and easily damaged by human interference[6].

3.3 Genecology The population number of *M. ventii* is small and its distribution points are relatively isolated, so it is not easy to be noticed in the mixed forest with other trees. There are relatively few seedlings and saplings in different distribution areas in

the field. Xu Qing *et al.* [33] speculated that due to the continuous deterioration of natural habitat of *M. ventii*, the light environment required for the growth of its seedlings is affected, thus seedlings are unable to complete natural regeneration. *M. ventii* is mainly distributed near areas with frequent human activities such as roads and villages, so there is more human interference and destruction, which also makes it difficult for its seedlings to survive[31]. Wang Bin[32] conducted dynamic quantitative analysis of *M. ventii* in Jinping County, Pingbian County and Hekou County from distribution pattern, diameter class structure, age structure and population structure; the results showed that the population was an inverted trapezoid, and there were relatively few seedlings and saplings in the population, which was not conducive to the renewal and continuation of the population in the long run.

4 Reproductive biology

Plant reproduction is the core of plant evolution, the most basic behavioral process of plant continuity, and also the basis of biological community change and ecosystem succession. However, reproduction is also a relatively fragile stage in the life cycle of plants. All links in the process of plant reproduction interact with each other. Once an obstacle occurs in one link, it may lead to the failure of the reproduction process, thus resulting in the crisis of population survival[34-37]. Therefore, it is of great significance to explore the endangered mechanism and protect *M. ventii* effectively by understanding its reproductive biological characteristics.

4.1 Reproductive physiology *M. ventii* usually bloom from late April to middle May. Early May is the peak period of blooming, which coincides with the local rainy season. It belongs to protogyny and night flowering plant. The flowers are bell-shaped when they face the ground, which may be to avoid rain erosion during flowering period that will affect the formation of pollen. Meantime, it can provide shelter from the rain when flower-visiting insects arrive. The flowering period of a single flower lasts for about 6 d, and the flowering process of a single flower follows an "open-close-reopen" rhythm, with 5 distinct stages observed during the flowering period of this species (Table 1)[38].

Table 1 Flowering process of *Manglietia ventii*

Stage	Time	Characteristics
Pre-pistillate	12:00 – 18:00	Outer perianth segments of flower buds are relaxed, showing signs of flowering
Pistillate	18:00 – 23:30	Perianth segments open rapidly within 20 min, receptible stigma produces nectar-like secretions, and perianth segments begin to close at about 23:30
Pre-staminate	23:30 – 16:00 of the next day	Stamens have not been dehiscent yet, and stigma receptivity is gradually lost
Staminate	16:00 – 23:00	Single flower opens again, and stamen pollen sac dehisces and scatters powder
Post-staminate	Start at 23:00 and last for 4 – 6 d	Stigma turns dark brown, and perianth segments are pendulous, gradually wilting and slowly shedding

M. ventii is heavily pollinator-dependent and mainly guides insects to pollinate through floral scent, but only weevils (*Sitophilus* sp.) and beetles (*Anomala* sp.) have been found to be effective pollinators[38]. However, due to the existence of small and fragmented habitats, the frequency of insect visits may be low, thus limiting the development of the population[39]. In contrast, artificial cross-pollination can significantly improve seed yield. This indicates that pollen restriction exists in the reproductive and breeding process of *M. ventii*, and it is speculated that the low seed setting rate in the natural population is a result of insufficient pollen deposition[32]. In addition, there are different degrees of abortion in most endangered species of *Manglietia*[40]. Xu Tao *et al.* [41] found that there was also abortive phenomenon in the pollen development of *M. ventii*, and the abortive rate of pollen reached 29%. However, since the pollen quantity of *M. ventii* is large, pollen abortion has limited effect on its low seed setting rate.

4.2 Seed dissemination Seed transmission is a major means for its migration to other places, and is a key stage of natural regeneration of plants[42]. The follicles of *M. ventii* begin to defoliate after the fruit ripens in September, and the seeds are propagated by gravity or wind[6]. However, most of the developed seeds rely on animal propagation to achieve natural renewal, and there is an obvious reciprocity network between plants and animals[43]. Seed transmission will produce different effects due to different animal feeding methods, digestive systems and movement distances[44]. Through field investigation, it is found that some rodents will feed on the seeds of *M. ventii*, so some seeds are damaged and can not germinate. When observing the natural fruit-dropping state, gathering the naturally landing fruit and stripping the seeds, it is found that more seeds are stored in the shell of follicles and can not fall into the soil in time, and such seeds may decay and deactivate due to the lack of suitable germination conditions. This may also result in a lack of seeds under the tree to produce a soil seed bank. In addition, the fleshy red aril of *M. ventii* is visually attractive to birds and animals, which can promote long-distance diffusion of its seeds. However, there has been no report on this aspect of seed transmission of *M. ventii*, thus further research is needed.

4.3 Seed storage and germination Seeds and seedling stage of trees are considered to be relatively vulnerable stages in the life cycle of plants, which is related to the survival and development of the population[45]. Control at harvest time has an important influence on seed quality, and seed germination is closely related to seed quality. Timely harvesting of fruits can effectively improve seed germination rate[46]. The fruits of *M. ventii* are matured from August to September. When follicles change from yellow-green to reddish brown and are slightly dehiscent, with exposure of bright red seeds, the seeds reach morphological maturity[47]. The seeds of *M. ventii* contain volatile aromatic oils which are easy to dry

and shrink and deteriorate, so they are not suitable for dry storage. The seeds could not be stored for a long time, and the germination vigor will be weakened obviously due to storage. Generally, seeds are collected in the current year and sown in January of the next year or sown along with the harvest[48]. Wang Aihua *et al.* [49] also put forward a similar view in their study of *M. crassipes*, in which the germination vigor of all stored seeds was significantly weakened. The regeneration ability of population is affected by the natural germination rate of seeds, and seed characteristics and environmental factors play a decisive role in the germination rate of seeds[50]. Under natural conditions, the germination rate of fresh mature seeds is low, and physiological dormancy exists in seeds, which seriously affected their emergence rate. Wang Bin[32] measured the influence of temperature change on the germination of *M. ventii* seeds; the germination rate of *M. ventii* seeds was the highest under the variable temperature of 25/15 ℃, followed by constant temperature (25 ℃). The germination tests of *M. ventii* seeds treated with different concentrations of gibberellin (50, 100 and 200 mg/L) were compared, and the results showed that the germination rates of *M. ventii* seeds treated with different concentrations of gibberellin were higher than that of the control group, but there was no significant difference between different experimental groups. Based on the effects of gibberellin on seeds of other Magnoliaceae plants[51-52], it is speculated that it might be attributed to short soaking time of gibberellin. The seeds of *M. ventii* treated with different concentrations of cytokinin failed to germinate, showing inhibitory effect compared with the control[32].

4.4 Growth characteristics Chu Yongxing *et al.* [48] proposed that seed germination should be in a relatively moist and shaded habitat, while growth and development of seedlings should be in sufficient light environment according to the characteristics of *M. ventii* seeds. When the light energy absorbed by plants exceeds a certain limit, excessive light energy will lead to photoinhibition of photosynthesis in plants[33,53]. Xu Qing *et al.* [33] proved through experiments that either too strong or too weak light was not conducive to the growth and development of *M. ventii* seedlings, and the optimal light environment was 30% light intensity. Zhang Guangfei *et al.* [53] obtained similar results and proposed that the seedlings of *M. ventii* had certain adaptability to light intensity under the condition of adequate water. Through seed rearing research of *M. ventii*, Zhang Tian *et al.* [54] put forward that July to September was the peak season for seedling growth, and compared with other plants of the same family that were growing at the same time, such as *Magnolia delavayi*, *Manglietia aromatica*, *Pachylarnax sinica* and *Parakmeria yunnanensis*, the growth of *M. ventii* was relatively faster at seedling stage. He Aiman *et al.* [55] studied the growth rule of one-year-old seed seedlings of *M. ventii* and found that the rapid growth period of the plant was from June to August, and that of the leaf was from June to September, while leaf growth began to decline after October.

5 Genetic diversity

Both the population quantity and the effective population quantity of *M. ventii* population are relatively small, which will affect the evolutionary processes of life such as gene flow and genetic drift, resulting in a greater risk of reduced survival rate of plants. The genetic diversity level of population also restricts the adaptive ability of the species to cope with continuous environmental changes to a certain extent[56]. Therefore, it is critical to developing conservation strategies by studying the genetic diversity and genetic structure of *M. ventii*. Wang Bin[32], Li Jiaojie[57] and Wang Meng *et al.* [28] studied the genetic diversity and genetic structure of *M. ventii* using SSR and ISSR molecular marker techniques, respectively, and the results showed that compared with other plants in Magnoliaceae, *M. ventii* maintained a relatively high level of genetic diversity and genetic differentiation. Most of the genetic variation of *M. ventii* existed within populations, while only 27.65% existed between populations[32]. Yang Lin *et al.* [9] analyzed codon preference of chloroplast genome of *M. ventii*, and found that the chloroplast genomes of *M. ventii* preferred codons ending in A/T and codon bias was closely related to GC content.

6 Endangered mechanism

6.1 External factors As an extremely small population endemic to Yunnan Province, *M. ventii* is distributed sporadically on hillsides of evergreen broad-leaved forests in valleys, along roads and near farmlands and villages. Most of the populations show patchy discontinuous distribution, whereas no continuous large area distribution population has been found. The habitat is sensitive and fragile, and the populations are isolated from each other[31]. As the local superior timber species, pilferage and felling of *M. ventii* occur from time to time. The destruction of the living environment of *M. ventii* caused by human activities such as excessive deforestation and underforest planting, as well as local residents' weak awareness of protection, are important reasons for the endangerment of *M. ventii*[6]. In addition, after the seeds of *M. ventii* have matured and fallen off, forest animals will eat them due to the colorful seed coat and delicious germplasm, which also aggravates the endangerment of *M. ventii* population[32].

6.2 Species self factor Although *M. ventii* has a large number of flowers, the flowering period overlapping with the local rainy season hinders the spread of floral scent, and the high dependence on pollinator activities leads to a low natural seed setting rate[38]. There is a phenomenon of irregular bearing, and the seeds have dormancy characteristics, belonging to recalcitrant seeds. Affected by its own characteristics of seedling growth, the population has inbreeding depression, which leads to the critically endangered survival status of *M. ventii*[31-33, 53].

7 Resource protection

7.1 Status quo of resource protection In recent decades, many government agencies and scientific research scholars have made great contributions to the protection of *M. ventii*. With the proposal of wild plant species with extremely small populations, the conservation concepts and methods with population as the basic unit advocate dynamic management and regulation of population quantity and structure of extremely small populations to achieve the goal of stable population development, which provides a scien-

tific guidance for the protection of *M. ventii*[58]. The influence of human activities on *M. ventii* can be reduced by building conservation plots, *ex situ* conservation, near *situ* conservation, germplasm resource conservation and field reintroduction, and other related conservation measures (Table 2), so as to promote the healthy development of *M. ventii* population[30].

Table 2 Protection status of *Manglietia ventii*

Protection type	Distribution of protected areas	Longitude and latitude	Number of individuals/plant
In situ conservation	Yunnan Daweishan National Nature Reserve	103°20′ – 104°03′ E, 22°35′ – 23°07′ N	386
	Jinping *Manglietia ventii* protection area	103°26′ – 103°34′ E, 22°42′ – 22°50′ N	
Ex situ conservation	Pingbian Innovation Company	103°42′ E, 22°57′ N	28
	Kunming Botanical Garden	102°44′ E, 25°08′ N	
	South China Botanical Garden	113°37′ E, 23°18′ N	
	Guangdong Zhanjiang Botanical Garden	110°08′ E, 20°43′ N	
Near *situ* conservation	Pingbian *Manglietia ventii* near *situ* conservation base	103°25′ E, 22°51′ N	2 413
Reintroduction population reconstruction	Jinping Ma'andi *Manglietia ventii* reintroduction test base	103°30′ E, 22°40′ N	700

7.2 Protection strategy

7.2.1 Carrying out scientific research comprehensively and systematically. For the protection of wild plant species with extremely small populations, only relying on a full understanding of their basic characteristics such as ecology and biology, can corresponding effective protection measures be taken scientifically[59]. Therefore, it is necessary to strengthen the related research on the effective protection of *M. ventii*. In addition, cutting propagation and tissue culture experiments of *M. ventii* should be carried out to rapidly expand the population and ensure the protection and sustainable utilization of its germplasm resources[60].

7.2.2 Strengthening the implementation of various protection measures. Climate change could lead to significant evolutionary changes by creating new genetic variations in species. Therefore, in the process of *ex situ* protection, relevant impacts of climate in the migration site should be considered[61-62]. For *in situ* protection, effective management of established protected areas, dynamic monitoring of population and habitat, and scientific collection of basic data should be strengthened. The number of protection districts should be increased as soon as possible, and *M. ventii* scattered in villages and fields should be protected through listing and filing[30].

7.2.3 Improving laws and regulations and strengthening publicity. The related protection policies of wild plant species with extremely small populations should be improved, the feasible program steps should be fully implemented, and the responsibility system of relevant departments should be clarified[63]. The effective conservation of *M. ventii* resource can be eventually achieved by publicizing and educating the significance of resource protection of *M. ventii* in various ways, prohibiting artificial interference such as excessive seed collection and wood felling, and guiding and improving people's protection awareness in the distribution area of *M. ventii*[64].

8 Research expectations

Since *M. ventii* is listed as a priority for the conservation of wild plant species with extremely small populations, people have got a certain understanding of the endangered mechanism of *M. ventii*. Through the joint efforts of a large number of scientific research institutions and researchers, a lot of achievements have been made in the ecological and biological characteristics of *M. ventii*. With the implementation of various protection measures, such as establishment of nature reserves, *ex situ* conservation and listing and filing, the protection of *M. ventii* has also achieved initial results, and its population has been significantly increased. However, there are also some limitations in the study of *M. ventii*. There is little research on basic characteristics and asexual propagation of seeds. Is the low natural germination rate of seeds related to the internal substances? Does asexual propagation expand its population more easily than seed germination? The solution to these problems will have a positive impact on the protection of *M. ventii*. Therefore, the determination of internal components of seeds and the dynamic changes of endogenous hormones, nutrients and enzymes during germination should be strengthened in the future, and cutting propagation and tissue culture experiment of *M. ventii* must be carried out. The researches in the fields of seed transmission, plant photosynthetic characteristics, allelopathic effects, and relationship between roots and microbial communities are inadequate, which belong to the basic exploration stage. The reason may be due to the relatively small number and unconcentrated distribution of wild population, which leads to the difficulty of related data collection. More in-depth researches on these aspects are needed in the future. In addition, the exploitation and utilization of *M. ventii* resources should be deepened. It is also an effective protection measure to strengthen the study on adaptability of introduced species, so that *M. ventii* can be widely used in landscape construction. The analysis of chemical constituents will provide a scientific theoretical guidance for the medicinal development of *M. ventii*. In conclusion, it is necessary to carry out in-depth research on *M. ventii* from various aspects and perspectives and understand its related characteristics comprehensively and deepen resource exploitation, so as to provide a guarantee for the protection and utilization of its germplasm resources.

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