DISTRIBUTION PATTERN, REPRODUCTIVE BIOLOGY, CYTOTAXONOMIC STUDY AND CONSERVATION OF *RAFFLESIA MANILLANA* IN MT. MAKILING, LAGUNA, PHILIPPINES

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YAHYA AF, HYUN JO, LEE JH, CHOI TB, SUN BY & LAPITAN PG. 2010. Distribution pattern, reproductive biology, cytotaxonomic study and conservation of *Rafflesia manillana* in Mt. Makiling, Laguna, Philippines. We investigated the population of *Rafflesia manillana* in Mt. Makiling, Laguna, Philippines. Results of field surveys in 2006, 2007 and 2008 and the cytotaxonomic study in 2003 were used to determine the reproductive biology, ecological characteristics and distribution pattern of *R. manillana*. Problems and effective conservation approaches for the species were also identified. Three subpopulations were located at 436 to 834 m elevation. *Rafflesia manillana* produced flowers and fruits from March till July, with more male flowers formed compared with female. The cytotaxonomic data confirmed the observed phenology of *R. manillana*. Mitotic chromosome number was 2n = 22. In Mt. Makiling, compared with *Rafflesia* in other locations, the threat to *R. manillana* was caused more by its sex ratio imbalance, unsuccessful pollination and seed dispersal, and habitat disturbances caused by tropical typhoons rather than direct human exploitation. The successful conservation of this species requires information on reproduction and dispersal of *R. manillana* and the biology of its host plant (*Tetrastigma harmandii*), which this study attempted to elucidate. Efforts of conservation should focus on the protection and restoration of the habitat of *R. manillana* which have been damaged by typhoons. An aggressive public awareness programme on the ecological state of Mt. Makiling and *R. manillana* should be enhanced.

Keywords: Ecological characteristics, habitat disturbance, human exploitation, host plant, *Tetrastigma harmandii*, tropical typhoon

YAHYA AF, HYUN JO, LEE JH, CHOI TB, SUN BY & LAPITAN PG. 2010. Corak taburan, biologi pembiakan, kajian sitotaksonomi dan pemuliharaan *Rafflesia manillana* di Gunung Makiling, Laguna, Filipina. Kami mengkaji populasi *Rafflesia manillana* di Gunung Makiling, Laguna, Filipina. Keputusan tinjauan lapangan pada tahun 2006, 2007 dan 2008 serta kajian sitotaksonomi pada tahun 2003 diguna untuk menentukan biologi pembiakan, ciri ekologi dan corak taburan *R. manillana*. Masalah dan pendekatan pemuliharaan yang berkesan untuk spesies ini turut dikenal pasti. Tiga subpopulasi ditemui pada ketinggian 436 m hingga 834 m. *Rafflesia manillana* menghasilkan bunga dan buah dari bulan Mac hingga Julai dan bunga jantan lebih banyak daripada bunga betina. Data sitotaksonomi mengesahkan fenologi yang dicerap bagi *R. manillana*. Bilangan kromosom mitosis ialah 2n = 22. Jika dibandingkan dengan *Rafflesia* di lokasi lain, ancaman terhadap *R. manillana* di Gunung Makiling lebih diakibatkan oleh ketidakseimbangan nisbah jantina, pendebungaan serta penyebaran biji benih yang tidak berhasil dan juga gangguan habitat yang lebih banyak diakibatkan oleh taufan tropika berbanding eksploitasi manusia secara langsung. Kejayaan pemuliharaan *R. manillana* memerlukan maklumat tentang pembiakan serta penyebaran spesies ini dan juga biologi tumbuhan perumahnya (*Tetrastigma harmandii*). Inilah yang ingin dijelaskan oleh kajian ini. Usaha pemuliharaan seharusnya bertumpu kepada perlindungan serta pemulihan habitat *R. manillana* yang rosak akibat taufan. Program kesedaran umum yang agresif tentang keadaan ekologi Gunung Makiling dan *R. manillana* hendaklah ditingkatkan.

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INTRODUCTION

Mt. Makiling in Laguna, Philippines, is a natural habitat for *Rafflesia manillana* and is one of the earliest forest reserves established in the country. With an area of 42.4 km^2 surrounded by 16.5 km^2 of buffer zone, Mt. Makiling is situated approximately 65 km south-east of Metro Manila. The unprecedented supertyphoons in the recent past have brought unusually heavy rainfall that threatened the ecological state of this mountain and its biodiversity. Mt. Makiling is also surrounded by rapidly expanding human settlements, agricultural croplands and urban and industrial development that impinge on the natural functioning of the mountain.

Rafflesia manillana is categorised as an endangered species in the 1997 IUCN Red List of Threatened Plants (Walter & Gillet 1998). The small population of this species in Mt. Makiling (one of two sites in Luzon Island) was thought to be extinct after the site was bulldozed for geothermal exploration in 1982 (Fernando *et al.* 2001). However, during field expeditions to the mountain between 2002 and 2004, *R. manillana* was found to be present and thriving with its host (Abraham *et al.* 2004).

As a holoparasitic species, *R. manillana* is more host dependent and more prone to extinction. The availability and quality of the host plant *Tetrastigma harmandii* and the habitat that supports the existence of *R. manillana* and its host limit the survival of this plant. A good understanding of the bio-interaction between *R. manillana*, its host plant and habitat is needed in formulating holistic conservation approaches for *R. manillana* in Mt. Makiling. So far, two major studies of biodiversity decision-support system for the Mt. Makiling Forest Reserve have been initiated, namely, (1) the biodiversity assessment and conservation of Mt. Makiling Forest Reserve and (2) the biodiversity information system, particularly on *R. manillana* (AKECU 2004, Cruz *et al.* 2007).

The reproductive biology, ecological characteristics and distribution pattern of *R. manillana* and its host plant *T. harmandii* were investigated in Mt. Makiling Forest Reserve. This article combines results of field observations in the years 2006, 2007 and 2008 with available information related to *R. manillana* and the cytotaxonomic study conducted in 2003 in order to identify current problems and to plan effective conservation approaches for this species.

MATERIALS AND METHODS

Field surveys were conducted in Mt. Makiling since 2002 to determine the distribution pattern, ecological characteristics and the reproductive biology of *R. manillana* in the area. Recent surveys were conducted from 2006 till 2008. The surveys were conducted during the months of May and July in 2006 and March in 2007 involving three areas, namely, Dampalit subwatershed, Greater Sipit subwatershed and Malaboo and in April 2008, in Sipit only. The number of patches and flowers per patch found in the areas were counted, photographed and recorded. The stage of the life cycle of the plant during the survey and the prevailing condition of the habitat and the host plant *T. harmandii* were also noted and recorded.

Cytotaxonomic investigation was conducted in 2003 as part of a biodiversity conservation project for *R. manillana*. Samples of young male bud of *R. manillana* were collected from Mt. Makiling on 18 May 2002 (voucher specimen: Fernando 1620, 1621) and on 6 March 2003 (diameter of bud ca. 5.8 cm). The materials collected were immediately fixed in modified Carnoy's solution in the field, left for 24 hours at room temperature and, later in the laboratory, transferred to 70% ethyl alcohol and stored under refrigeration before use. Slide preparations were made from squashed young anther sac from bud stained with two or three drops of 2% acetocarmine. Chromosome count was obtained from squashed sporogenous cells and developing sterile cells in the anther sac.

RESULTS AND DISCUSSION

Pattern and extent of endangerment

There were three subpopulations of *R. manillana* located in Mt. Makiling; one subpopulation was found in the Dampalit subwatershed, another in the Greater Sipit subwatershed and the third in Malaboo (Figure 1). We observed a total of 17 patches in the surveys. In the 2007 survey, 15 patches were located—8 in Dampalit, 2 in Sipit and 5 in Malaboo. In the survey in Sipit in July 2006, two patches of *R. manillana* were found which included one of the two reported in the 2007 survey. Another patch was located during the survey in April 2008. A total of four patches have so far been found in the Sipit area.

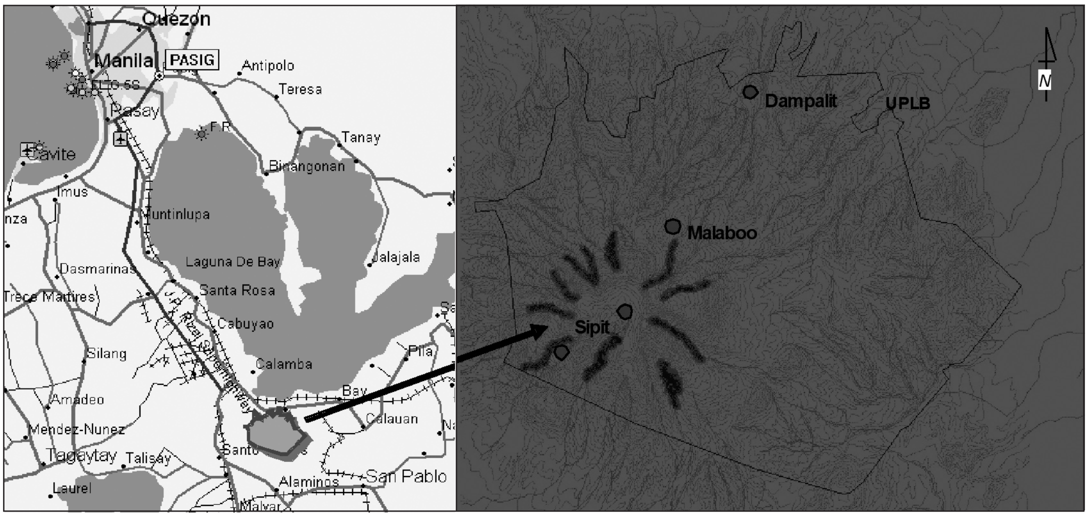

Figure 1 Relative location of the patches (●) of *R. manillana* in Dampalit, Malaboo and Sipit, Mt. Makiling with respect to the landslides (/)

Table 1 shows the number of flowers per patch of *R. manillana* subpopulations in Mt. Makiling. In the 2006 survey, four wilted female flowers (fruits) were found, three in Dampalit and one in Sipit. Five individual female flowers were found during the 2007 survey, one fresh and four wilted. Male flowers were dominantly found in all patches. Figure 2 shows the different stages of development of *R. manillana* subpopulations observed during the field surveys.

Dampalit had the most number of patches (i.e. eight) compared with the other two subpopulations. The number of flowers, on the other hand, was highest in Malaboo (32). There were 34 individuals of *T. harmandii* sampled during the 2007 survey. Of these, 15 were infected by *R. manillana*. Although *T. harmandii* is distributed from 480 m above sea level (asl) to the summit of Mt. Makiling, which is more than 1000 m asl, infected *T. harmandii* were found only in a specific area of each subpopulation, within approximately a 50 m-diameter area. Interestingly, surveys in Sipit showed that the patches were near trails in low-lying portions of the area. We are not certain if infection can be facilitated by human traffic and the downward movement of air or water that may carry *R. manillana* to low-lying areas.

The subpopulation in Dampalit was within a thickly vegetated area dominated by trees at an elevation of 436 to 516 m asl. On the other hand, the patches of the species found in Sipit spread from an elevation of 569 to 834 m asl but was also thickly vegetated. As explained above, the patches were near creeks and in relatively low-lying portions of the area. The Malaboo subpopulation was found in an area from 643 to 661 m asl elevation.

Problems related to the conservation of *R. manillana* in Mt. Makiling

Survival probability

A number of factors that contribute to the low survival of *R. manillana* are insect infestation and factors that determine pollination success, which include pollinator availability, distance between patches if flowers emerged in separate patches or subpopulations, synchrony of flower opening and susceptibility for pollination.

Tetrastigma harmandii produces a phellogen-like layer around the embryonic shoots of *R. manillana* (Brown 1912). This layer is believed to act as a selector agent in the early stage of *R. manillana* life cycle by cutting off the embryonic shoot from its food supply and killing them before they are large enough to cause any appreciable distortion in the host plant tissue. However, in this study, several host plants produced many buds or flowers in one flowering season (Figures 2b and 3a). Although sporadic growing buds was observed in a patch, the number of buds that finally developed into flowers was small. A detailed study of bud development and

Table 1 Number of flowers per patch of *R. manillana* subpopulations in Mt. Makiling

Subpopulation	Number of patches surveyed	2006	2007	2008[1]
Dampalit	8			
FB		3	45	–
DB		1	7	–
FF		1 (male)	7 (6 male, 1 female)	–
WF		17 (3 fruits)	7 (1 fruit)	–
Sipit	4			
FB		> 14	0	> 18
DB		0	0	2
FF		0	0	0
WF		8 (1 fruit)	3 (fruit)	5 (1 fruit)
Malaboo	5			
FB		0	6	–
DB		1	0	–
FF		0	8 (male)	–
WF		21	3	–

[1]Observation was only conducted in Sipit; DB = dead buds; FB = fresh buds (ready to bloom); FF = fresh flower; WF = wilted flower

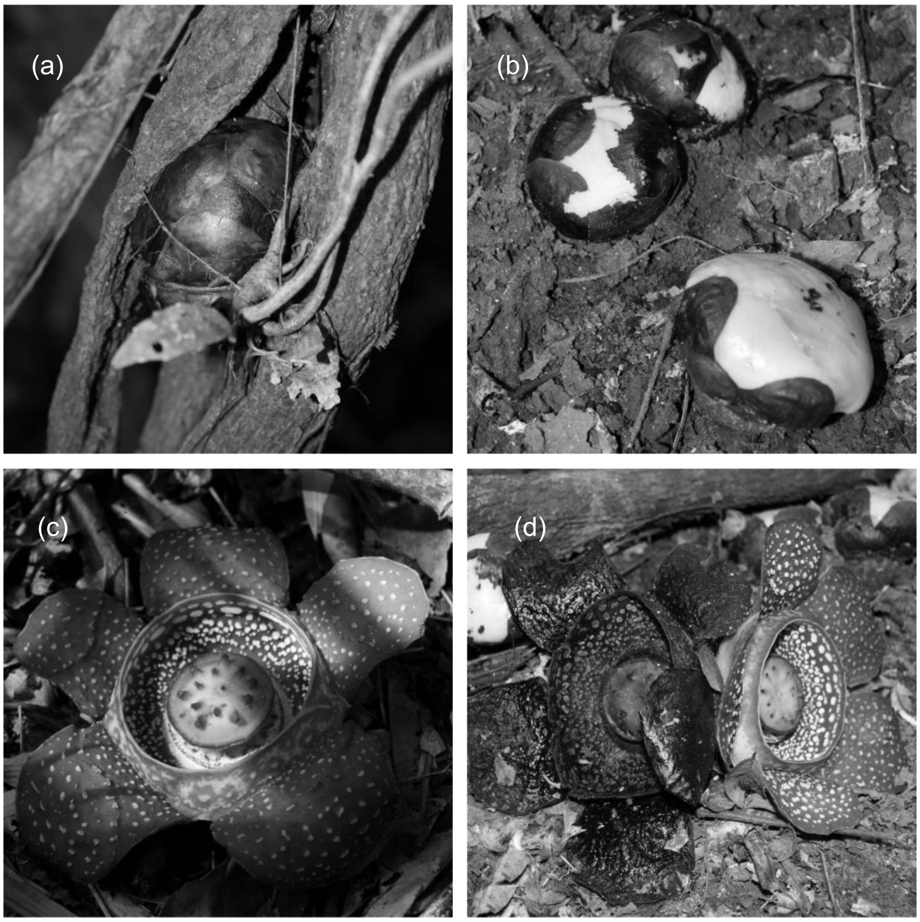

Figure 2 (a) Aerial bud of *R. manillana* observed at Malaboo area, (b) buds, (c) blooming male flower and (d) wilted and blooming flowers of *R. manillana* observed at Dampalit area during field survey in March 2007

flowering showed that more than 60% of the buds eventually failed (Figure 3b), generally during the rainy season from June till September (Abraham *et al.* 2004).

Similar to Brown (1912) and other researchers, we also observed insects and larvae infesting buds in the subpopulations of *R. manillana* in Mt. Makiling. Figure 3(c) shows unopened fleshy petals of *R. manillana* with larvae inside. Until now, there has been no report on organisms other than insects infesting the buds of *R. manillana* .

The problem of low coincident blooming of male and female flowers, though both flowers are produced in the same host plant (Figures 2d and 3d), also contributed to the low survival of *R. manillana*, resulting in low success of pollination and fertilisation. Among the 15 fresh flowers observed in the 2007 survey, only one flower was female (Table 1). A large sex ratio imbalance is another common problem in *Rafflesia* population (Nais 2001). Thus, the rarity of *R. manillana* may be due to the fact that its flowers are frequently not pollinated (Brown 1912). Also, besides seeds being damaged by insects and larvae as mentioned before, there was no evident means of seed dispersal and the number of infected host plants was thus low.

The absence of chlorophyll in this plant makes it more dependent on the host plant *T. harmandii*. The host as a climber, on the other hand, depends on another host, a tree, for support to reach the light in the forest canopy (Marvier & Smith 1997, Nais 2001). Any disturbance or exploitation of the host tree and/or host plant will, therefore, have an effect on *R. manillana*.

Habitat destruction

Numerous cases showed that habitat destruction has been implicated in the decline of a number

Figure 3 (a) Sporadic growing buds in one patch of *R. manillana*, (b) failing bud (c) part of infected unopened fleshy petals with larvae inside and (d) the only female flower found blooming among buds and male flowers in all patches during the field survey in Dampalit in March 2007

of parasitic plant populations (Skinner & Pavlik 1994). However, there is no literature detailing the correlation of *R. manillana* population, its early distribution in Mt. Makiling and its habitat characteristics. Our current data and that from Abraham *et al* (2004) show that the distribution of *R. manillana* in the forested slopes of Mt. Makiling is shrinking. In this study, the population of *R. manillana* was spread in areas between 436 and 900 m asl when before, the first collection of this species was obtained from 400 m asl (Brown 1912). Most of the subpopulations observed in this survey are considered as conservation hotspots because they are located in areas threatened by wildlife poaching, illegal collection of non-timber products as well as poaching of saplings and poles (AKECU 2004). The presence of most patches along trails used as access into the forest by people predisposes the population to further damage.

Natural events such as typhoons have also caused destruction of *R. manillana* habitat in Mt. Makiling. The super typhoon Xangsane that hit the country on 28 September 2006 was the worst in 11 years, thrashing the northern islands with heavy rains and strong winds, and causing widespread flooding and landslides. An area of about 4.5 km^2 in Mt. Makiling Forest Reserve was also affected (Lapitan 2007), including the area where some patches of *R. manillana* were located before. This changed the physical environment of the habitat especially that of the two patches each in Sipit and Dampalit.

The typhoon also changed the physical environment of the areas. The light intensity that penetrated through the defoliated canopies had increased significantly, which caused greater variability in the environment (Brokaw & Grear 1991, Fernandez & Fetcher 1991). Air and soil temperatures in the area subsequently increased while relative humidity decreased compared with a closed-canopy forest (Fetcher *et al.* 1985, Denslow 1987). The change in air temperature brought about by direct exposure to sunlight when the forest canopy opened also affected the species. Being endothermic in nature, *Rafflesia* maintains the temperature of the internal part of its flowers a few degrees (1–6 K) above the air temperature of the environment to attract pollinator flies (Patiño *et al.* 2002). If the increase in temperature is within the range that *R. manillana* tolerates, the condition should still be favourable to the species.

The change in the canopy also reduced canopy interception of rain and increased the surface run-off in the area (Putz *et al.* 2001, Susswein *et al.* 2001). *Rafflesia* buds emerge from the stems of its host in shaded understorey of the forest. The erosive effects of direct rainfall and the increased surface run-off can kill some *R. manillana* buds.

Reproductive biology

Results of the three surveys revealed that flowers and fruits of *R. manillana* in Mt. Makiling are produced from March till July. The flowers and fruits developed from tiny buds that were initiated several months to about a year back. All the three subpopulations were with flowers when surveyed in March 2007.

In the Sipit area, in July 2006, fruits, rotting flowers and buds were found at 834 m asl. In April 2008, another patch of wilted flowers and buds were found within a kilometer away. In Malaboo, fresh flowers were also found in March 2007 while wilted or rotting flowers, in May 2006.

Cytotaxonomic characteristics

From the male buds collected on 18 May 2002, sterile cells undergoing active mitotic division were found. The mitotic chromosome number of *R. manillana* was 2n = 22 (Figure 4). This is the first report for this species. However this value is not the same as the value (2n = 24) reported for *R. arnoldi* (Olah 1960). Therefore, it is concluded that aneuploid series may exist within the genus and more chromosome counts for other species are needed to infer the basic chromosome number of the genus. Meiotic configurations were also observed from the bud collected on 8 March 2003. Bivalent chromosomes were formed during prophase (Figure 5).

These cytotaxonomic data confirmed the observed phenology of *R. manillana*. The division of sex cells was observed in samples collected in March while mitosis was observed in samples collected later in the month of May. March and April are the months when flowering of *R. manillana* occurs and towards the end of May, fruit set and fruit development begin.

Conservation approaches

The conservation of *R. manillana* has to be given priority since it is a highly endangered species.

Figure 4 Mitotic configuration of *R. manillana* showing 22 chromosomes (× 400)

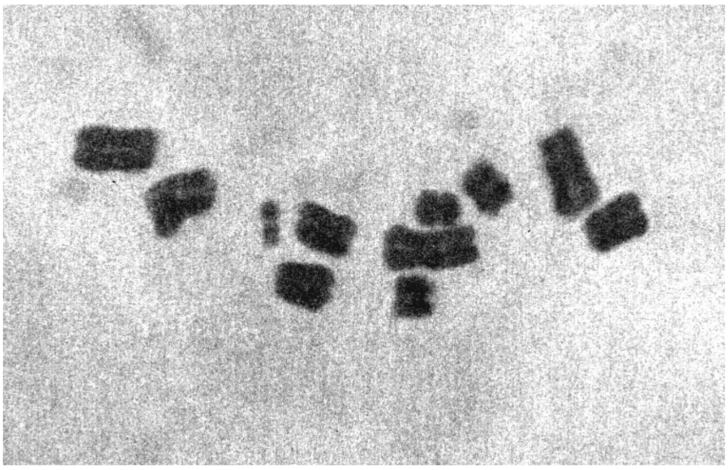

Figure 5 Meiotic configuration of *R. manillana* showing 11 bivalent chromosomes (× 400)

The effectiveness of conservation programmes covering both *ex situ* and *in situ* approaches for *Rafflesia* has been assessed previously. *Ex situ* conservation has not been a viable option for *R. manillana* (Meijer 1997), even though success in the propagation of *R. keithii* has been reported (Nais 2007). A wider knowledge of the species is vital for its *in situ* conservation (Heywood & Dulloo 2005).

Unlike other locations where direct human exploitation is a major problem, *Rafflesia* in Mt. Makiling faces more threats due to its biological characteristics and habitat disturbances. Specific information on reproduction and dispersal of this species is, however, still inadequate. Thus, the first step towards the effective conservation of this species in this mountain would be to fully understand its biology. The conservation genetics of the species should also be fully investigated if conservation approaches for *R. manillana* in Mt. Makiling is to succeed.

The data so far gathered from these studies revealed that the flowering of *R. manillana* in Mt. Makiling occurred during dry months. While this condition is favourable for the flowers as strong typhoons that damage the plants are less likely to occur during this time, no data is available to substantiate the claim that this condition affects the dispersal of seeds from the plant. This should be investigated together with other factors that affect the actual dispersal of the seeds.

The threats to the habitat of *R. manillana* are mainly caused by natural phenomenon and indirectly by illegal harvesting/poaching of other forest products in the area. Unfortunately illegal activities and adverse effects of natural events have been difficult to predict with accuracy and to monitor, given the facilities and resources currently available. The conservation of the species in Mt. Makiling can be approached by protecting and regularly monitoring the patches that have been located particularlly those in areas where illegal activities have occurred. Meanwhile, patches affected by landslide should be prioritised for rehabilitation to minimise the extent of damage, especially on the host plant and the host tree.

The success of *R. keithii* propagation by inoculating the seed into a young *T. diepenhorstii* may be limited only to fruit-producing *Rafflesia* species. The inoculation of *R. manillana* into its host plant *T. harmandii* has not been done for the population in Mt. Makiling. Our results showed that only 15 of 34 plants found in Mt. Makiling were infected with *R. manillana*. Thus, we need to identify the factors and host plant characteristics that would be essential for the establishment of *R. manillana* in *T. harmandii*. The *in situ* propagation of the species can be facilitated if this information is available. An assessment of biological characteristics of the host plant *T. harmandii* should, therefore, be undertaken to understand *R.* manillana–host plant interaction. Vegetative reproduction of the infected host plant can also be considered to obtain new stocks of host plant that would carry *R. manillana*.

The conservation of *R. manillana* could be facilitated by raising the awareness of the local community and visitors to Mt. Makiling on the importance of the species and its growth as an indicator of the ecological state of the mountain. The conservation of the habitat can subsequently be used very effectively to promote the conservation of *R. manillana*. Considering the important socioeconomic and ecological functions of Mt. Makiling, particularly to its different stakeholders, the support for and even the success of the conservation of Mt. Makiling would be easier to advocate.

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