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Cover: Green Bee-eater with colour pencils and watercolor wash by Elakshi Mahika Molur.



Assessment of population, habitat, and threats to *Cycas pectinata* Buch.-Ham. (Cycadaceae), a vulnerable cycad in Bhutan

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Abstract: *Cycas pectinata* Buch.-Ham. is an ancient gymnosperm that is now confined to pockets of habitats in the Indian subcontinent but was once widely distributed. Its decline is attributable to habitat loss, and has reached the point where *C. pectinata* is listed as 'Vulnerable' in the IUCN Red List. *C. pectinata* is the only species of *Cycas* found in Bhutan, and in this biodiversity-rich area it is present as a relic of great scientific and conservation value because of its rarity and long evolutionary history. Although it is well known in India, it has not been studied in detail in Bhutan. This study assessed populations and threats to *C. pectinata* in two places in Bhutan. Field visits were made to document the distribution, habitats, and associated threats to the populations. Plants were observed growing in steep rugged terrain in the open Chir Pine forest. Populations are significantly threatened due to human activities such as habitat destruction and over collection as ornamental plant. Possible expansion of populations is naturally threatened by low seed production and by predators.

Keywords: Conservation, dioecious, gymnosperm, habitat loss, IUCN Red List, ornamental, population, regeneration, threats.

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Author contributions: ST and TW conceptualised and designed the study, including data collection and manuscript writing. KW, JD, and TN contributed in developing field method, field data collection, and manuscript drafting.

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INTRODUCTION

Cycas is an evergreen, palm-like, dioecious tree with a robust trunk (Grierson & Long 1983; Lindstrom & Hill 2007; Khuraijam & Singh 2015). The trunk bears an apical crown of pinnately compound leaves with few spines in the petiolar region. Male cycads bear large, cylindrical ovoid yellow or green male cones having numerous microsporophylls with long apical spines (Lindstrom & Hill 2007; Khuraijam & Singh 2015). Female plants bear compact closed cones with numerous broadly subulate pectinate megasporophylls which bear 2–6 ovules.

The Scottish surgeon and botanist Francis Buchanan-Hamilton first described *Cycas pectinata* in 1824 from “Camrupae orientalis” which is probably part of modern-day Assam in northeastern India (Buchanan-Hamilton 1824; Grierson & Long 1983; Lindstrom & Hill 2007; Nguyen 2010; Khuraijam & Singh 2018). The family Cycadaceae Pers. includes the sole genus *Cycas* L. with 118 accepted species (Lindstrom & Hill 2007; Calonje et al. 2023). Cycads are one of the most ancient gymnosperms, originating in the early Permian period 280 million years ago or possibly in the late Carboniferous period (300–325 MYA). This long evolutionary history makes them of great scientific and conservation value (Zheng et al. 2017). The genetic information contained in cycads is important for palaeontology, palaeoclimatology, and palaeogeography (Feng et al. 2016; Zheng et al. 2017). Additionally, cycads are thought to be the earliest gymnosperm lineage, reaching the greatest diversity during the Jurassic-Cretaceous period, approximately 200–65 MYA (Feng et al. 2016). Cycads are bridges in major evolutionary transition in plants, and remain indispensable for understanding the origin and subsequent evolution of seed plants (Feng et al. 2016; Zheng et al. 2017).

Cycads, once more widely distributed, are now primarily distributed in Africa, Asia, Australia, and South & Central America (Feng et al. 2016; Zheng et al. 2017; Khuraijam & Singh 2020). The current distribution of *C. pectinata* extends from Nepal to northeastern India and Bangladesh (Lindstrom & Hill 2007; Khuraijam & Singh 2020), generally recorded within the elevation range of 600–1,300 m (Khuraijam & Singh 2014, 2020), mostly restricted to subtropical and tropical regions. From Bhutan, *C. pectinata* was first documented by Grierson & Long (1982, 1983) from two districts: Mongar and Trashigang (Now Trashigang) growing at elevations of 925 and 1,100 m. Subsequently, four additional localities for the species have become known (Tobgay et al. 2019), though none of these populations has been studied in detail.

C. pectinata in Bhutan is mostly confined to small geographically isolated populations in the rugged terrain of river gorges; this isolation may pose threats to genetic viability in the future (Yang et al. 1996). Although more than half of the Bhutan's total land area is protected for conservation (Wildlife Conservation Division 2016), only one known location of *C. pectinata* is in a protected area, making the species less likely to enjoy full protection by law. Habitat suitability modeling under climate change scenarios also does not indicate the protection of the species from known locations (Pradhan & Chettri 2017). In Bhutan species like *C. pectinata* is threatened by developmental activities like the construction of new and widening of existing roads, hydropower projects, and electric transmission lines. Such activities increase threats not only through habitat destruction but by facilitating exploitation through the collection for ornamental purposes and for food by the increasing human population in the localities. In Bhutan, despite knowledge of the localities of *C. pectinata*, detailed studies on these have not been undertaken. Although 68 globally threatened floral and faunal species are legally protected in Bhutan, *C. pectinata* is not included in this list (Biodiversity Statistics of Bhutan 2017).

Today, cycads are by far the most threatened plants (Donaldson 2003; Zheng et al. 2017). Around 62% of Cycad species are listed in IUCN Red List as threatened (Nagalingum et al. 2011). *C. pectinata*, though considered to be one of the most common and widespread cycads, is suffering a significant current population decrease due to habitat loss. The IUCN estimates there to be around 200,000–250,000 individuals of *C. pectinata* in the world; it is listed as ‘Vulnerable’ in the IUCN Red List (Nguyen 2010). The species is under severe threat with its total population declining due to an estimated 30% of its natural habitat having been destroyed over the past 90 years (Pradhan & Chettri 2017). More of its suitable habitats are very likely to be further reduced under predicted climate change scenarios (Pradhan & Chettri 2007; IPCC 2007).

Important threats to Cycads in general are collection from the wild for various purposes across the globe. *Cycas* has probably been used by people since prehistoric times in some regions and they have been traded for many different purposes (Donaldson 2003; UNEP-WCMC 2009; Williamson et al. 2016). The most common uses have been for food, medicine, and cultural practice (Bhowmik & Datta 2014; Khuraijam & Singh 2015). *Cycas* also has a long history of use as ornamental plants in Asia and they have now become popular garden and specialist collector plants in other parts of the world. By far the greatest

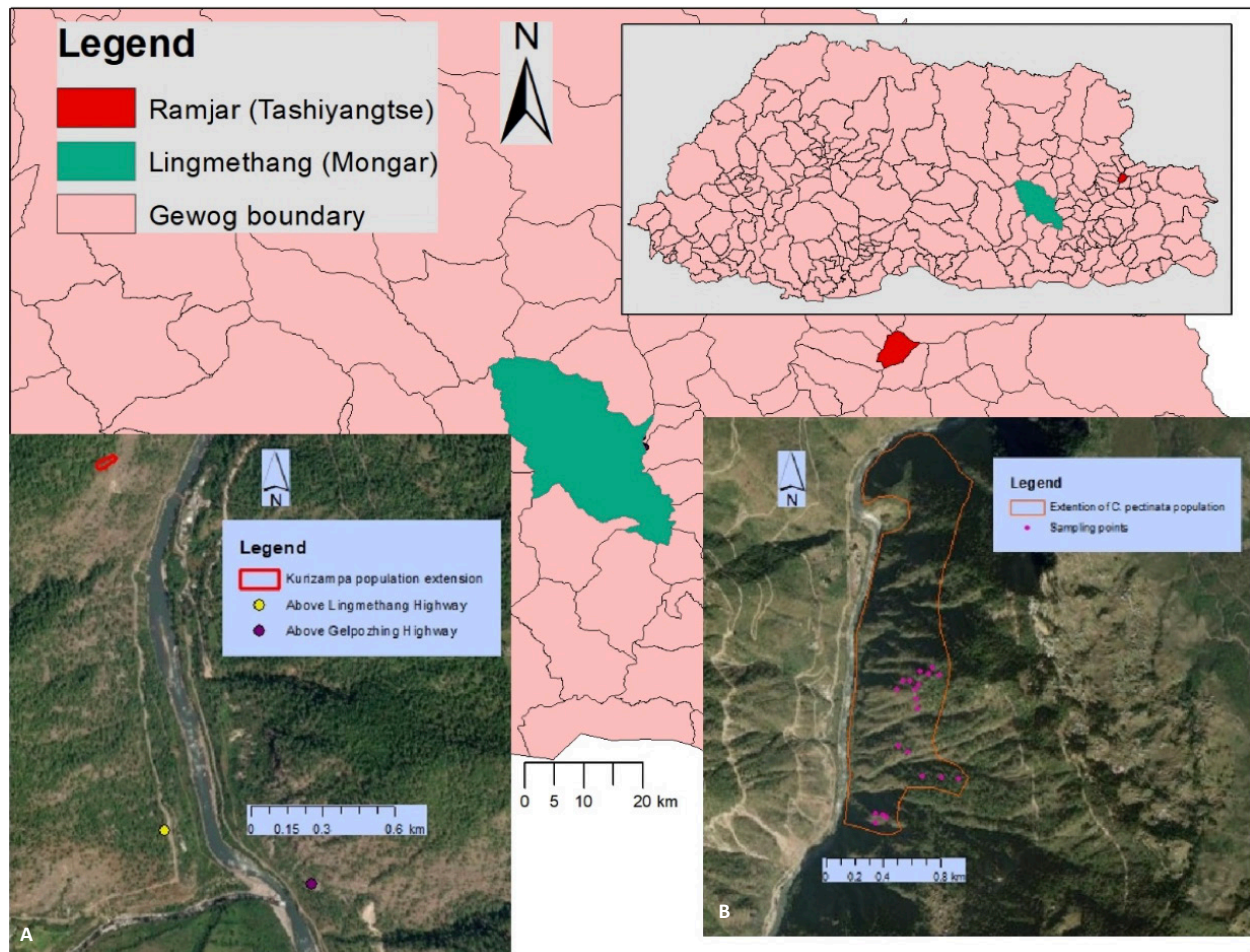


Image 1. Study sites: A—Kurizampa & Lingmethang | B—Ramjar.

global trade of *Cycas* is for ornamental purpose, with more than 30 million plants being traded between 1977 and 2001 (Bhima 2003). This study has assessed natural populations and threats to *C. pectinata* populations from two places in Bhutan.

METHODS AND METHODS

Study sites

Bhutan is part of the eastern Himalayan global biodiversity hotspot known for species richness with more than 11,000 known species (National Biodiversity Centre 2019). The high variation in climatic conditions across the small geographic area has supported diverse biodiversity. The IUCN (2019) has listed 99 species from Bhutan as threatened which include 43 plant species, 13 species are vulnerable to extinct including *C. pectinata*. This study has assessed the population, habitat, immediate threats and necessary conservation measures of *C. pectinata* from known locations in two districts of Bhutan (Trashy Yangtse

and Mongar). In Trashy Yangtse, the population of the study species grows on the eastern bank of the Dangme Chhu River opposite Gomphu Kora at Ramjar within Trashy Yangtse District. The place name is “Bawoongshing-pek” meaning the hill of *C. pectinata*. Another population was assessed from Lingmethang and Kurizampa in the Mongar district. Both the historical and extant population in this site lies on the bank of Kurichuu River (Image 1A). The population here is easily accessible with a national highway passing nearby. Three populations within the locality have been assessed. The vegetation in both the study locations is dominated by large Chir Pine *Pinus roxburghii* Sarg. forming a canopy and *Cymbopogon flexuosus* (Nees ex Steud., Will. Watson) forming dominant ground vegetation (Table 1). Commonly associated shrubs include *Rhus paniculata* Wall. ex G. Don., *Phyllanthus emblica* L., *Ficus bengalensis* L., *Asparagus racemosus* Willd. (Wild asparagus), *Zanthoxylum* L., *Grewia* L., and other commonly associated ground vegetation are *Artemisia vulgaris* L. and *Chromola odorata* (L.) R.M. King & H. Rob. Ramjar study site is steep with hilly terrain

and rocky outcrops while in Kurizampa the study site was on a hilltop.

Data collection

Population assessment in Ramjar locality (787–1,394 m) was carried out by laying random quadrats in between approximately 1.5 km². A total of 20 quadrats measuring 40 m² were laid out. In Kurizampa all the individuals were counted. All the *C. pectinata* plants within the quadrat were counted and morphometric measurements were taken along with the geocode for the quadrat. Male and female plants were identified based on cone morphology. Each plant was measured in height, girth, and base. Plants with stem well-formed above ground measuring >5 cm in height were recorded as individual plant and plants with less than two leaves and stem not seen above ground were categorized as saplings. The total number of leaves on each plant was counted along with the measurement of the length of selected leaves from each tree and leaf samples were collected from each tree and the number of pinna counted along with a length of petiole measurement. Those small plants height less than 5 cm were categorized as seedlings. All the quadrats and site of the population were assessed for any signs of threats.

Population structure determination

Cycads do not produce annual growth rings which in other trees are generally used to estimate age. For this species, the age of the plant can be estimated by its height, as in other *Cycas* species (Jian et al. 2006).

RESULTS

Population structure

A total of 566 individuals were recorded from Ramjar, Trashy Yangtse; 25 were identified as female and 43 as male, with the remainder lacking cones at the time of field sampling (Table 2). Most of the female plants recorded were mature with fully mature ovules, and male plants bore remnants of male cones. The shortest male cone-bearing plant measured 50 cm, and shortest female 55 cm.

The average density of plants measuring more than 5 cm in stem height within a quadrat was 27 (SD ± 13.42). In an estimated 1.5 km² of *C. pectinata* habitat, the total number of individuals were estimated to be around 25,000. The density distribution of plants show a significant correlation with elevation (p -value 0.045 and $R^2 = 0.51$); with a gradient of increasing density towards lower elevation.

In Mongar study area, three different populations were identified. The entire population in Kurizampa comprise 62 recorded individuals with stem height measuring >5 cm, and 74 saplings. Of these were three plants with female cones and seven with male cones. The male cone bearing plant has a minimum height of 80 cm and female plants 56 cm. Another site in this region which includes an area above Lingmethang highway had recorded only one male plant, and another site above Gyelpozhing highway recorded three individuals all without any sign of coning. Age classification of individual plants measured in the field shows *C. pectinata* population has an opposite pyramid age structure in Ramjar site. There are older plants but fewer younger ones. In contrast, the population from Kurizampa has more young individuals than older plants (Figure 2).

Coning and regeneration

In both populations, the number of plants bearing cone is very low compared to the total number of adult and old individuals which all have the potential of bearing one type of cones. Further, the percentage of plants bearing a female cone is lower than that of the male cone bearing plants. Population in Ramjar site have 2% and 11.75% of female and male coning individuals, respectively (Table 1). Regeneration from bulbils is common in *Cycas* species. A total of 36 individuals were recorded with a total of 105 bulbils, mostly associated with fallen trunk remains. One fallen trunk was recorded with three bulbils in the Kurizampa site.

Threats to the populations

Socio-economic developmental activities are common in both study sites. These activities do not show an imminent threat to *Cycas* population in Ramjar. Activities for a hydropower project on the other side of the river at Dangmechu are completely separated by the river. Other activities like road construction took place away from the area where *Cycas* was recorded. Threats to *C. pectinata* population in Kurizampa and nearby places are directly associated with modern socio-economic development activities. This includes human settlement, construction & widening of roads, and hydropower related activities. The site above Lingmethang Highway and Gyelpozhing Highway were represented by only one and three individuals respectively. Small populations above Kurizampa were observed to be frequented by cattle and occasionally by human.

Individuals in both populations had symptoms of pest infection which includes drying of leaves or cutting off pinnules on rachis (Table 3). The young leaf rachis

Table 1. Location and characteristics of *Cycas pectinata* habitat.

Site name	Latitude	Longitude	Elevation range (m)	Habitat	Area (m ²)
Ramjar	27.417	91.56827	791–1,296	Open Chir Pine forest and broad leaf forest with lemongrass	1,514,856
Kurizampa	27.276	91.19106	844–872	Open Chir Pine forest with lemongrass and <i>Chromolaena odorata</i>	2,285
Lingmethang Highway	27.263	91.19059	641	Open Chir Pine forest and lemongrass	
Gyelpozhing Highway	27.26	91.19595	597	Open Chir Pine forest with rocky areas	

bored by larvae (belonging to Lepidoptera) were common in Ramjar site, resulting in death of the crown of young leaves (Image 2A,B).

Additionally, leaf litter accumulating in the crown of leaves has been observed to become inhabited by millipedes resulting in deformed leaf sprouts (Image 2C,D). In Ramjar, Wild Boar is also seen as a threat to *Cycas* populations, leading to seed damage and uprooted young individuals (Image 2G,H), but not observed in the population from Kurizampa and nearby areas. The adult individuals are resistant to fire incidences protected by thick scaly bark. However, frequent disturbances have resulted in growth of invasive species. This was evident in Kurizampa area where plants were overgrown by weed species like *Chromolaena odorata* (L.) R.M.King & H.Rob. (Image 2I,J).

Collection of the plants by humans was not evident in the Ramjar site, except for a few translocated to gardens by local people. Individuals at Kurizampa and nearby areas are threatened by collection. The presence of more *Cycas* plants above Gyelpozhing Highway a couple of years back was confirmed by locals. However, during the present study visit only three individuals were recorded and the other plants had been removed for their ornamental value (Image 2K,L).

DISCUSSION

Population structure

Grouping plants by height approximating their age, the *C. pectinata* population in Ramjar site shows an inverted pyramid age structure where more individuals are in the adult and old categories compared to saplings and seedlings. Similar declining recruitment of seedlings has been observed in populations of other *Cycas* species (James et al. 2018). Low seedling recruitment directly threatens the viability of the population, as fewer individuals remain to replace old plants, which in the long term can reduce the number of plants in the population (Shen et al. 2009; Dian-pei et al. 2012). In

Table 2. Age structure of the two natural population of *Cycas pectinata*.

Site	Ramjar			Kurizampa		
	No. of plants	Cone		No. of plants	Cone	
Tree height class (Stem height)		M	F		M	F
>100 cm	201	32	19	1	1	0
51–100 cm	148	9	7	18	5	3
26–50 cm	102	0	0	15	0	0
6–25 cm	85	0	0	28	0	0
<5 cm	30	0	0	74	0	0

Table 3. Level of threats as determined by the characteristics of the leaves.

Leaf characteristics	Kurizampa	Ramjar
Pest	62 (100%)	158 (62.69%)
Few leaves, not integrated	13	93
Many leaves, some integrated	2	55
Many leaves, integrated	45	64
Nibble	7	22

contrast, the population from Kurizampa showed more sapling recruitments than older plants, a positive sign for sustainability of the population in the area. However, the number of individuals in the different populations must greatly contribute to the viability of the population. Only 62 adult plants constitute the population in Kurizampa.

A population from the Ramjar site was observed with increasing density of plants at a lower elevation with density decreasing with increasing elevation. However, this relation from a Pearson correlation test indicates a weak association $P > 0.05$. ($r = -0.73$). The increasing density at the lower elevation can be associated with the steepness of the habitat and larger seed size, which coupled would facilitate easy roll down by gravity thus concentrating seed germination at lower elevations. Other mode of seed dispersal was also evident in the field, seedling recruitment at elevation ranges higher than the adult trees affirms that seeds dispersal was mediated

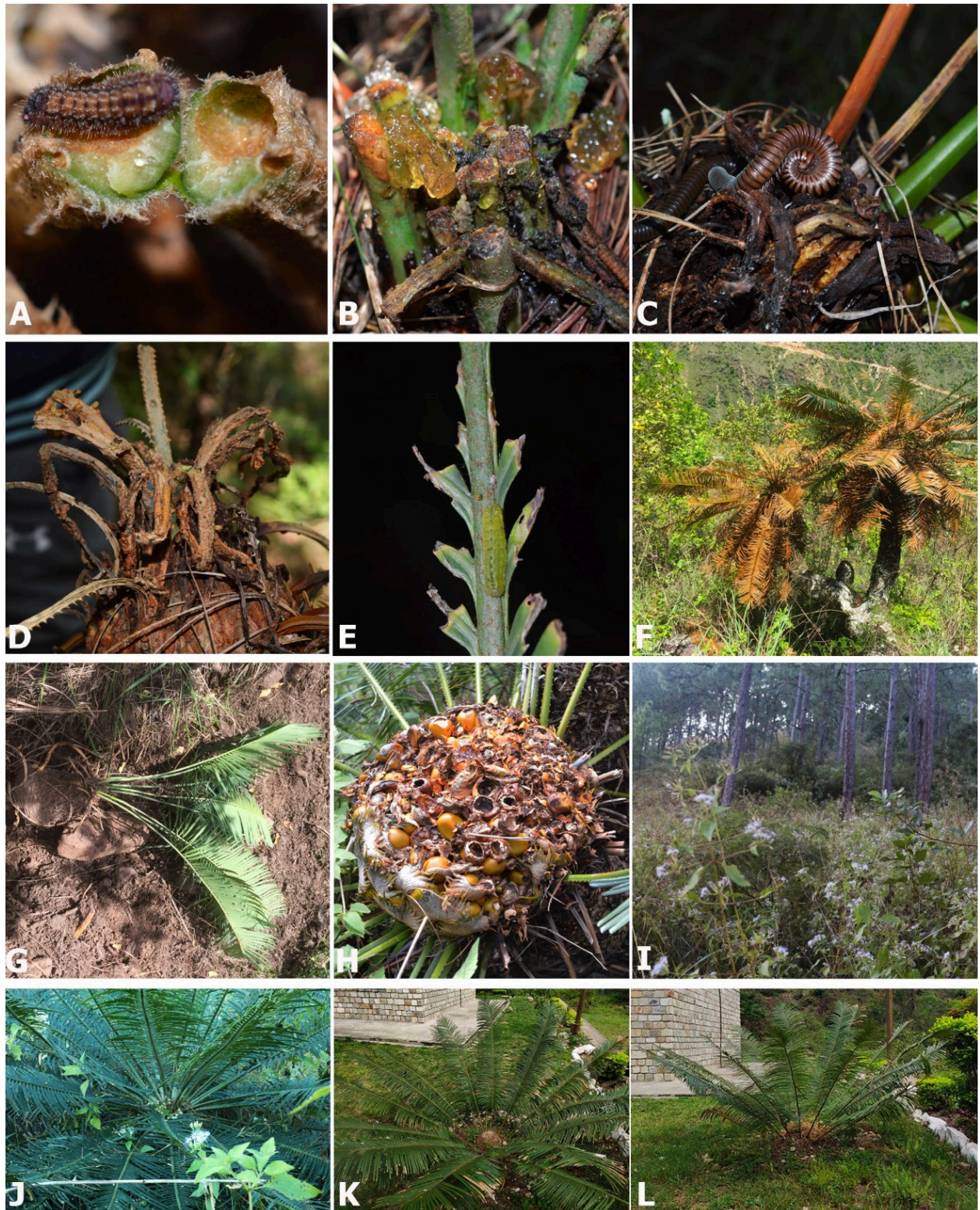


Image 2. *Cycas pectinata*: A & B—Young leaves bored and eaten by larvae (Lepidoptera) | C & D—Millipede inhabiting bases of crown of leaves | E—Remnants of leaf pinnule | F—Plant with leaves showing symptoms of gradual drying of leaves | G—Small plants uprooted by boars | H—Female cone with seed formed being damaged by boar | I & J—Extensive growth of *Chromolaena odorata* in the habitat | K & L—Planted as ornamental plant. © A–E: Tshering Nidup | F–L: Sonam Tobgay.

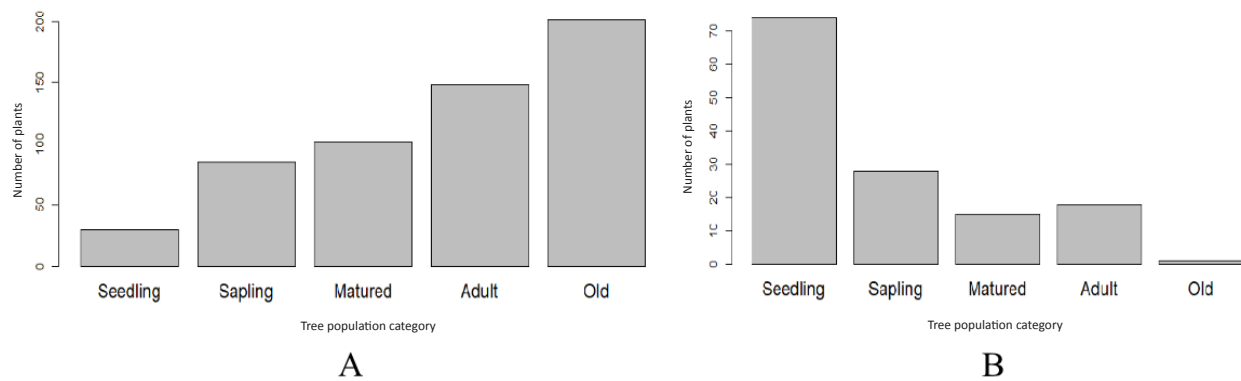


Figure 2. Population structure of *Cycas pectinata* plant form: A—Ramjar in Trashy Yangtseand | B—Kurizmapa in Mongar.

by small mammals such as rodents and fruit-eating bats (Dehgan & Yuen 1983; Yang & Meerow 1996).

Coning and regeneration

Stability of a population will be determined by the number of seed-producing individuals present. In both populations there are fewer coning individuals, particularly female. Only 2% and 16% of adults bore a female cone, compared to 12% and 32% of adult plants bearing a male cone, in respective population from Ramjar and Kurizampa. The trend of declining coning is common in populations of other *Cycas* species (Jian et al. 2006). Determination of sex composition within a population is important, particularly for a dioecious species like *Cycas*, to understand population stability. Many works of literature support a 1:1 ratio as being a theoretical and evolutionarily stable sex ratio for maintaining a dynamic population under natural selection (Yu & Lu 2011; Tarsi & Tuff 2012; Shin et al. 2019). Without comparable numbers of males and females, reproduction opportunities may be limited and population growth stunted (Tarsi & Tuff 2012). Similar observations of a dwindling sex ratio in cycad populations have been made resulting in a reduction of seed production and a reduction of population size (Singh & Singh 2010). Nonetheless, Ueno et al. (2007) claim that in most dioecious plants, either the population sex ratios do not depart from unity, as expected by Fisher's Law (Fisher 1930), or males are more numerous than females, as observed in this study. This difference is attributed to the higher reproductive cost for females producing seeds, resulting in reduced growth rates, higher mortality rates, delayed flowering, lower frequency of future reproduction, and less extensive clonal growth. The ultimate result of reduced seed production means a lower number of recruitments and population showing a decreasing trend.

Threats to the population

Human activities and habitat destruction continue to be the major threats to species diversity including the loss of plants from wild (Zheng et al. 2017; Hossain et al. 2021). Historical evidence confirms *C. pectinata* population in the area above Lingmethang Highway, formally thriving very well, is now found to be represented by only a single male individual. Threats to the *Cycas* population in the area are clearly from anthropogenic disturbances. Population re-settlement driven by developmental activities and associated economic activities have contributed to the loss of population. Activities such as road widening, establishments of gravel grinding machineries, and tunnel construction for hydropower projects have contributed significantly resulting in habitat disturbance.

Loss of *Cycas* populations from one of the study sites near Gyelpozhing Highway is a very recent event. The decrease of *Cycas* population in this location can be evidently attributed to the over-collection of plants for ornamental use. Being easily accessible through roads, the plants were removed by plant collectors. Locals collected the plants as priced specimen for use as ornamental plants in their gardens, a common threat to *Cycas* (Bhima 2003; Chowdhury et al. 2011) beside being culturally associated in using as sources of nutrition (Khuraijam & Singh 2015; Hossain et al. 2021).

Natural threats to the population include those from pest infestation, in particular affecting the young leaves. Such damage to the foliar part of the plants can cause direct deformation of the plant parts and limit the capacity of photosynthesis (Guest 2017).

CONCLUSION

It is clear from this study and others that *C. pectinata* populations are severely threatened and therefore

require priority conservation plans. As anthropogenic activities continue to be a major threat, education and awareness are some of the possible ways to conserve and save species in their natural habitat.

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