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Status of *Nepenthes khasiana* Hook. f. (Pitcher plant) in Meghalaya: A review

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Abstract

Carnivorous plants derive some or most of their nutrients from trapping and consuming small insects and spiders. About 600 species of carnivorous plants are reported to grow mainly in tropical or semi-tropical areas throughout the world. The majority of terrestrial carnivorous plants grow in bog and fen soils in which they encounter persistent unfavorable conditions. The soils where the carnivorous plants grow are characterized by very low nutrients such as nitrogen, phosphorus and alkali ions, as well as high acidity. *Nepenthes khasiana* Hook f. is endemic to Meghalaya and is distributed throughout the state from West to East Khasi hills, Jaintia hills, East, West and South Garo hills from 1000 to 1500 m altitude. Habitat destruction, decimation of species and fragmentation of large contiguous populations into isolated small and scattered ones have rendered them increasingly vulnerable to inbreeding depression, high infant mortality and susceptibility to environmental change, ultimately leading to extinction. It has now become imperative to give impetus on the conservation of *N. khasiana* for saving this species from extinction. The present paper explains the current status of *Nepenthes khasiana* and ongoing work done so far in conservation, propagation and diversity estimation of the species.

Keywords: pitcher plant, propagation, Meghalaya, uses, medicine

Introduction

Unique bio-geographical partitioning with high rainfall and plenty of sunlight makes North East India remarkably rich in floral diversity. The North East region is rightly called the 'cradle of flowering plants'. The *Nepenthes*, popularly known as 'pitcher plant' or 'monkey cups', is a genus of carnivorous plants. The popular name of this species 'monkey cups' refers to the fact that monkeys have been observed drinking rainwater from these plants. '*Nepenthes*' literally means 'without grief' and in Greek mythology, it is a drug that quells all sorrows with forgetfulness. *Nepenthes khasiana*, the only representative of the genus *Nepenthes* in India, belongs to monotypic family Nepenthaceae and has a polyploid chromosome number ($2n=80$). Karyomorphological studies were not possible in this species due to the relatively small size of the chromosomes (Devi *et al.*, 2012) [6]. It is an endangered, dioecious member of the carnivorous plants and is endemic to the state of Meghalaya (Mandal and Mukherjee 2011) [21]. It grows in association with *Licuala peltata*, *Calamus erectus*, *Lithocarpus dealbata* and fern species like *Alsophila gigantea*, *Dicranopteris lanigera*, *D. splendens*, *Thelypteris lakhimpurensis* and other species (Singh *et al.*, 2011) [30].

The plant species can trap hundreds of individual insects of different species. The 'insectivorous plant' possess an extraordinary habit of adding to their supplies of nitrogenous salts by capturing and digesting the proteins of trapped insects. To catch the insects, they have developed curious mechanisms like closing of traps, formation of attractive pitchers, using sensitive sticky hairs or grabbing with tentacles, thereby ensnaring the unwary insects (Sharief and Murthy, 2010; Venugopal and Devi, 2003) [28]. The aboriginal people of Meghalaya have a long association with this plant. Khasi people call it 'Tlew-rakot' meaning 'devouring plant'; Jaintia's call it 'Kset-phare' meaning 'lidded fly net' and Garo's call it 'Me.mang Koksi' meaning 'basket of theghost'.

Distribution

The genus is mostly distributed in the Malay Archipelago with the greatest diversity in Borneo, Sumatra and Phillipines with many endemic species. Pitcher plants are also found growing in India, Madagascar, Sri-Lanka, Borneo, Malaysia, New Guinea, Thailand, Vietnam and Queensland in Australia (Ghosh and Ghosh, 2012) [9]. *Nepenthes khasiana* is the only known pitcher plant species which is found in India having a very localised distribution. Isolated subpopulations are known to occur in Jarain, Jowai and Umtra areas of the Jaintia

Hills and Baghmara, Balpakram and Maheshkola area of the South Garo Hills, Lawbah and Mawlynong region of EastKhasi Hills region of Meghalaya (Ghosh and Ghosh, 2012) ^[9]. Nevertheless, *Nepenthes khasiana* exhibits considerable genetic diversity and is endemic to the state of Meghalaya (Mao and Kharbuli 2002, Ghosh and Ghosh, 2012) ^[22, 9]. Fragmented subpopulations are recorded from

Belpara, Balpakram, Baghmara and Nokrek in Garo hills and Sutnga of Jaintia hills in Meghalaya. In the CAMP workshop organised at Guwahati in 2003, it was collectively agreed by experts that 40% of the wild population from the natural habitat in Meghalaya had declined over the past 30 years. The fragmented sub-population of *N.khasiana* distribution is shown in Fig.1.

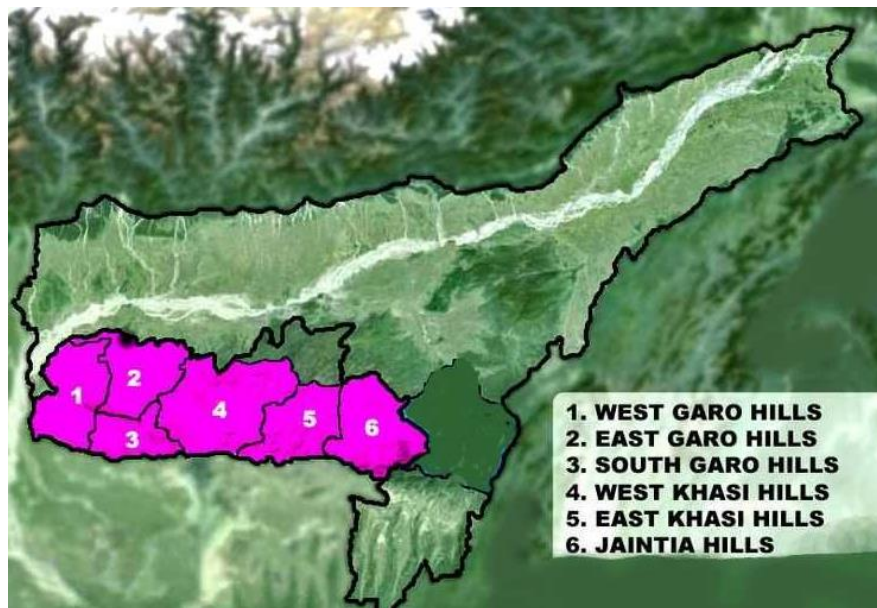


Fig 1: Distribution map of *Nepenthes khasiana* Hook. In Meghalaya state

Status and conservation strategies

The curious and unique pitcher shaped plant is in great demand for its ornamental value and also for medicinal properties and thus, have led to its further exploitation. The population is severely fragmented due to human habitation, construction of roads and urbanization. The area of occupancy (AOO) is estimated as 250 km². Therefore, the species is assessed as Endangered. Owing to its specific growing conditions and its over exploitation, the species is under threat and a sanctuary known as Baghmara Pitcher Plant wildlife Sanctuary in East Garo Hills, Meghalaya has been set up for the conservation of this endemic species. *N. khasiana* is a protected species, classified as 'Endangered' and is included in the Appendix- I of CITES and Negative List of Exports of the Government of India (Sharief and Murthy, 2010; Venugopal and Devi, 2003; Zeimer, 2010) ^[28]. The gene pool in the cultivated plants is exceedingly small and as a result no new material from India has entered horticulture for decades. Therefore, if the gene pool of cultivated plants cannot be increased, effective protection and conservation within the native range of the species is vital (Clarke 2014).

There is a need of effective conservation efforts such as trade regulation and sustainable collection. The pitcher plant sanctuary in Jarain, Jaintia hills and Baghmara in East Garo hills region of Meghalaya needs more focused attention for the conservation of *Nepenthes khasiana*. The natural habitat of the species is disturbed by coal mining, limestone mining, stone quarrying, forest fires (Prasad and Jeeva 2009) ^[26], human population growth coupled with unsustainable patterns of consumption, increasing production of waste and pollutants, deforestation, urban development, developmental projects, road laying, forest fire, jhum cultivation and poor seed germination ability. Habitat destruction, decimation of species and fragmentation of large contiguous subpopulations into isolated small and scattered ones have rendered them

increasingly vulnerable to inbreeding depression, high infant mortality and susceptibility to environmental stochasticity (Mandal and Mukherjee 2011, Verma *et al.* 2014) ^[21, 35]. Conservation strategy needs more attention towards managing disturbance to the natural habitat of the species (Singh *et al.* 2011) ^[30].

Few studies of *N. khasiana* which were carried out in the forests of Meghalaya show the dominance of *N. khasiana*. The anthropogenic disturbance was also reported to affect *N. khasiana* (Rao *et al.*, 1983; Tripathi *et al.*, 2008) ^[27, 32]. In the tropical region, the forests were rated according to the magnitude of anthropogenic disturbances (i.e., undisturbed, mildly disturbed and highly disturbed) and it was observed that highly disturbed forest were the ones where *N. khasiana* was intensively exploited for fodder, fuel and other necessary requirements by the villagers. Singh *et al.* 2011 ^[30] carried out studies to analyze the effect of human disturbance on *Nepenthes khasiana* in Nokrek Biosphere Reserve. They concluded that the inevitable pressure due to commercialization of the *N. khasiana* is leading to severe destruction of the species.

In order to conserve the species, cultivation and propagation of this endangered species is of utmost importance. It has been demonstrated by several workers that propagation of *N. khasiana* is possible through tissue culture, stem cuttings and seeds. Nongrum *et al.* (2012) ^[24] determined the genetic diversity by using Random Amplified Polymorphic DNA (RAPD) markers among three populations of *N. khasiana* Hook f. The values of gene flow ($N_m = 1.284$) and the diversity among populations of 0.280 demonstrates higher genetic variation within the population. AMOVA or analysis of molecular variance revealed a low level of genetic variation (21.96%) among the populations. This study indicates that some variation still exists within and between the existing populations of *N. khasiana*, thus, these highly

diverse populations could provide materials for re-establishing of this important rare and threatened species.

In another study, Bhau *et al.* (2009) [3] studied multi-locus analysis using PCR based Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers for the first time to assess the genetic diversity of *N. khasiana* Hook. f. The result of cluster analysis by using UPGMA method showed that the groups based on pooled RAPD-ISSR genetic similarity were more similar than the groups based on RAPD. Furthermore, genetic similarity reveals variability within the population at Jarain of Jaintia hills, while between populations the Baghmara region differs from the others with at least 40% dissimilarity.



Fig 2: The pitcher plant (*Nepenthes khasiana* Hook.)



Fig 3: Pitcher plant in damp swampy areas



Fig 4: Pitcher plant along the road side

Morphological description

N. khasiana grows mostly in acidic, moist and nutrient deficient soils and sometimes in sandy areas, where the medium is light and airy. The plant grows as a climbing or scrambling vine which ranges from few centimeters to several meters in height (Bordoloi, 1977) [4]. The long foliage leaves radiate out in a rosette fashion from a central climbing stem (Ghosh and Ghosh, 2012) [9]. The lamina is linear, elliptic or narrowly oblong, up to 46 cm long and 10 cm wide. The apex of the leaf is acute or obtuse and the base is attenuate and sub-petiolate to petiolate. The petiole is winged, up to 13 cm long and 2.5 cm wide, and clasps the stem, often becoming strongly decurrent. The stem, midrib and tendril may be green, yellow, orange or red, especially in direct sunlight. The upper surface of the lamina is often dark green, whilst the lower surface is very pale.

The entire pitcher (both lower and upper) is tinged with exciting colours (Di Giusto *et al.*, 2009) [8]. The outer side of the pitcher is yellowish green, sometimes mottled with faint red or orange blotches. The inner side of the pitcher is yellow, orange or pink and the lid often has a red underside. *N. khasiana* flowers between June to October and the fruit is capsular, 20-25 mm long. A mature fruit contains 500 or more seeds, light in weight and have long wings to be carried away by wind.

N. khasiana plants have pitfall traps which are passive traps. Insects are attracted by the brightly coloured pitchers covered with colourful lid projecting over the mouth or by the odour of the nectar. Nectar is secreted from the glands at the entrance of the pitcher as well as lower surface of the lid. Each pitcher trap contains copious amount of liquid and is very slippery. Once inside, the insect cannot get a grip on the walls of the pitcher because a flaky wax on the interior surface peels off as it struggles to climb and eventually the insects fall. The motion caused by the struggle stimulates digestive glands to release a proteolytic enzyme and a digestive acid. These two secretory materials helps in digestion of the prey. The end product of the digestion provides the plant with much needed nitrogen which is absorbed by the walls of the pitcher.

Propagation

Vegetative propagation of *Nepenthes* comes in two main forms: tissue culture (Nongrumet *et al.*, 2008; Devi *et al.*, 2013) and stem cuttings (Anonymous, 2014). *In vitro* multiplication for large scale propagation of *N.khasiana* has been achieved using seeds to conserve this rare and unique pitcher plant of India (Nongrumet *et al.*, 2008). An efficient *in vitro* protocol for large scale multiplication of *N. khasiana* has been developed by Devi *et al.* (2012) [6] from nodal stem segments. Their studies revealed that the highest shoot proliferation of 19.16 ± 0.23 shoots/explant was recorded in half strength MS medium supplemented with 2.5 mg/l kinetin, 2.0 mg/l BAP, 3% sucrose and 0.8% agar. The best rooting was achieved in half strength MS medium supplemented with 2.0 mg/l α -naphthalene acetic acid with an average of 9.04 ± 0.46 root/shoot. While the large-scale *Nepenthes* nurseries use tissue culture to generate huge numbers of plants, it is not recommended due to the difficulty associated with this method. The far more common method of *Nepenthes* propagation is stem cuttings.

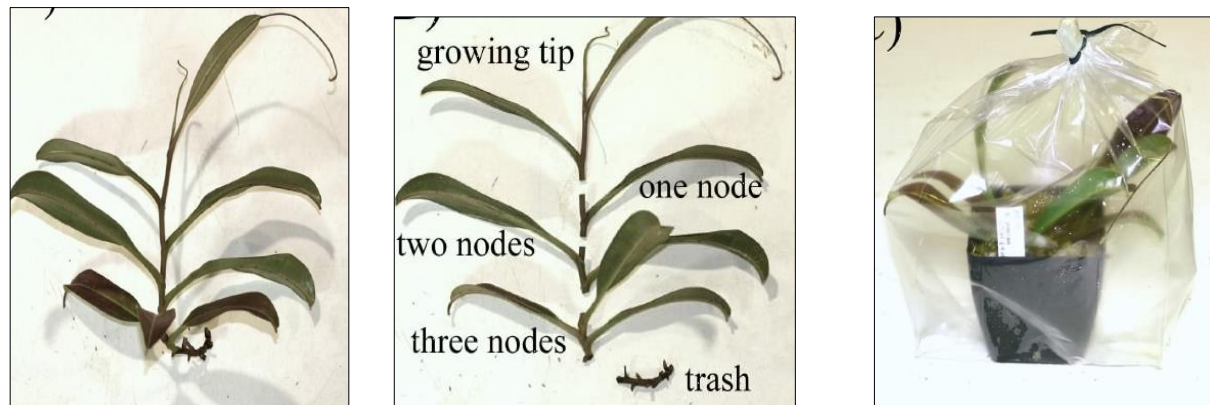


Fig 5: Propagation of *Nepenthes khasiana* through stem cuttings

Nepenthes cuttings can be taken at any time of the year, but the cuttings should be taken from an actively growing parent plant. The ideal *Nepenthes* propagation situation is a plant that has a tall climbing stem with widely-separated leaves and a new basal rosette of leaves. In this case, the climbing stem (either the whole stem or just the tip) can be taken as cutting material leaving the parent plant with an intact basal rosette. The stem on the parent plant will generally re-sprout near the tip assuming there are still green leaves on that stem. It will take the parent plant a few months to re-sprout and look presentable again.

For rooting medium a loose neutral to slightly acidic medium with little or no nutrients that drains well can be used. The medium should be damp, but not sopping wet or sitting in water. The *Nepenthes* cutting will only grow a new stalk from a growth bud that is just above a leaf axis, so all cuttings must include a leaf node or a growing tip. The roots will form at the location of the cut on the bottom of the cutting. The roots do not form (at least initially) at the leaf node. The stem can be cut into 1, 2 or 3 node cuttings; all of which have their advantages. Typically 1-node cuttings are made from the stem segments near the growing tip and 2-node or 3-node cuttings are made from segments that are low on the stem and are not as vigorous. *One-node* cuttings are best reserved for easy-to-propagate species and/or very recently grown material just below the growing tip. *Two-node* cuttings are sections of stems with two green leaves and growth buds. It is preferred over more one-node cutting. *Three-node* cuttings are the same as two-node cuttings except that 2 leaves can remain on the cutting--remove the bottom leaf. Two and three node cuttings are best for the older sections of the stem that are not quite as robust as the recent sections. Depending on the vigor of the parent plant, the species and the exact rooting conditions, it can take a period between a month to a year. Once the plants have rooted and started to grow, then the plants are potted in the growing pot and kept in a terrarium (or partially opened plastic bag) to transition the cuttings to the lower humidity conditions outside.

Khuraijam and Roy 2015 developed and demonstrated successful propagation technique of *N.khasiana* through seeds for easy to propagation along with detailed information on precautions to be taken during the adoption of the techniques. The 100 percent germination was observed in Medium III made of coir or cocopeat only, while Medium I (chopped sphagnum moss and coir or coco peat in a ratio 1:5) and Medium II (chopped sphagnum moss, coir or coco peat and sand in a ratio 1:3:3) had 85-90% germination. Temperature should be strictly maintained between 25-30°C, kept moist in semi shaded place and should not be exposed to direct

sunlight. The techniques does not require any sophisticated equipments, however, it requires certain precautions that need to be carefully followed for the proper plant development.

Uses of *Nepenthes khasiana*

a) Ornamental uses

Carnivorous plants are a fascinating group of plants, and have long been the subject of popular interest. Due to the various shapes and sizes of leaves of carnivorous plants there are wide ranges of choice for landscaping purposes. Furthermore, their leaf colorvariation as well as their beautiful flowers makes these plants ornamental materials with a high commercial potential. However, most carnivorous plants are not recommended for outside landscaping purpose because they require nutrient-poor and acidic soils. Accordingly, carnivorous plants can be used only for special outdoor landscaping such as swamps and bogs. Thus, carnivorous plants are utilized more for interior landscaping purposes. Their use as pot plants is most popular, and growing them in hanging baskets or terrariums is also gaining popularity. This is especially true because a terrarium is an excellent container for carnivorous plants. This species also collected from the wild and kept at home as ornamental plant (Mukerjee *et al.* 1984). The species is cultivated worldwide (Clarke 2014).

b) Medicinal properties

N.khasiana is intensively exploited by the Garo, Khasi and Jaintia tribal inhabitants for their medicinal use and as a source of income for their daily basic needs. Khasi and Garo tribes used the fluid of unopened pitcher as eye drops to cure cataract and night blindness, as well as in treating stomach troubles, diabetes, ear and gynecological problems (Singh and Mudgal, 1999; Ghosh and Ghosh, 2012)^[31, 9]. They also used paste of pitcher and its contents to cure leprosy (Ghosh and Ghosh, 2012)^[9]. The powdered roots and pitcher is applied in skin diseases and the pitcher with the juice and crushed insects is administered to cholera patients. It is also potentially used to treat diabetes (Shil *et al.* 2010). Jaintia's use *N. khasiana* for treating stomach troubles, inflamed skin and gynaecological problems. The local people are the primary collectors who collect plants from the nearby area and sell them to nearby markets for a cheap price (Rs. 50-100/plant). The whole plant is traded under the name 'Tiewrako' and 'Me.mangkoksi'. The uncontrolled habitat destruction, acid mine drainage associated with coal mining and over-collection for medicinal and ornamental uses have threatened this species (Bordoloi, 1977; Prasad and Jeeva, 2009; Tandon *et al.*, 2009; Singh *et al.*, 2011; Ghosh and Ghosh, 2012; Verma *et al.*, 2014)^[4, 26, 30, 9, 35].

Especially important is plumbagin, a kind of naphthoquinone found in genera *Nepenthes*, *Drosera*, and *Dionaea* (Crouch *et al.*, 1990; Shin *et al.*, 2007; Zenk *et al.*, 1969)^[5, 29, 36], which has various effects: insect antifeedant (Kubo *et al.*, 1980; Tokunaga *et al.*, 2004)^[15, 33], cardiotoxic (Itoigawa *et al.*, 1991)^[10], anticancer (Parimara and Sachdanandam, 1993)^[25], antimicrobial (Didry *et al.*, 1994)^[7], antimalaria (Likhitwitayawuid *et al.*, 1998)^[16] and antifungal (Shin *et al.*, 2007)^[29]. Among the various medicinal properties of the plant, the digestive juice of the unopened pitcher plant *N. khasiana* Hook. is used as an eyedrop for cataract and night blindness (Behera *et al.*, 2007)^[2].

Future prospects

Carnivorous plants, due to the unusual ability of ingesting insects and other prey, have attracted much attention and curiosity. However, so far their use has been limited to aesthetic purposes since their ornamental value is high. Because they have desirable leaf shapes and patterns as well as striking flowers, they can make good houseplants if grown under light without fertilization. Watering is the most critical factor when growing vigorous carnivorous plants. Recently however, various functional and physiologically active substances have been identified in many carnivorous plants. In the near future, many carnivorous plants will certainly be utilized for the isolation of medicinal and pharmaceutical substances. For this purpose, a stable and mass supply of the carnivorous plants is of the utmost importance. New techniques in micropropagation have made possible the stable mass supply of carnivorous plants (Jayaram and Prasad, 2005; Joe *et al.*, 2003; Kim *et al.*, 2003, 2005; Lee, 2005; Lee and Kwon, 2005; Lee *et al.*, 2003a, b)^[11, 12, 14, 13, 13, 17, 18, 18, 19].

N. khasiana is one of the most endangered plant species and presently, a few populations of this plant survive in the wild. The main reasons – extensive mining, shifting cultivation, habitat destruction have rendered *N. khasiana* increasingly vulnerable in its native land. However, the main threat to its existence is posed by the human collectors who uproot the whole plant for sale domestically and export clandestinely outside. Before it reaches the extreme point of extinction, export of these plants for commercial gains have been banned by both the Central and the State Governments.

At present, various *in situ* and *ex situ* conservation measures have been implemented from time to time by various organizations and institutions. Pitcher plant is a rare and symbolic plant of Meghalaya. Government is, therefore, is taking steps not only to preserve it but also to make sure that it is well-protected and maintained. *N. khasiana* from Shillong has been grown, maintained and conserved for the past 37 years in the National Orchidarium and Experimental Garden of Botanical Survey of India, Yercaud.

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