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Prey Selection by Pitcher Plant, *Nepenthes khasiana* in Meghalaya, India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SGM and MP did field visit and collected the data. Author GA identified the species. Author PS did field visit, collected the data and compiled it. Author AB framed the research work, performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Nepenthes khasiana, an endangered pitcher plant endemic to Meghalaya and southern Assam, India seems to develop a pitcher for trapping small animals as their prey to supplement the nutrient deficiency which occurs in the soil. A study on the enigmatic meat-eating choice of *N. khasiana* is scarce. Hence, this study was conducted to find out their prey species covering all three hills of Meghalaya, India. A total of 90 pitcher samples (30 from each hill) were collected randomly and preserved in 70% alcohol which was further identified at the lower taxa level. A total of 71 species were identified up to the species level while another five were identified up to their genus level. Among this, 90.8% of species were insects (69 species) while the remaining 9.2% (7 species) were

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non-insect species. All these seven non-insect species belonged to single order Arachnida. The majority of them (71.1%, 54 species) were capable of flying while the remaining 28.9% (22 species) were incompetent to fly. Those species which were incompetent to fly belonged to orders Araneae, Dermaptera, Diptera, Hemiptera, Lepidoptera, and Odonata. The prey constituted the highest 23 species belonging to the order Hymenoptera followed by 11 species under Coleoptera, 9 species under Diptera, 7 species under Araneae, 6 species each under Hemiptera and Blattodea, 5 species each under Orthoptera and Lepidoptera, 2 species under Odontata and 1 species each under Mantodea and Dermaptera. The orders Hymenoptera and Diptera together contributed 83.3% of the total feeding spectrum. This further indicates the sustainable management of biodiversity securing the abundance of animal prey species for the conservation of pitcher plants in this landscape.

Keywords: Pitcher plant; Nepenthes khasiana; carnivorous plant; prey species; feeding spectrum; conservation.

1. INTRODUCTION

The pitcher plant is a type of carnivorous plant that has evolved to live in nutrient-deficient soil. Therefore, some plants developed the carnivorous habit of trapping prey in the pitchers, which is a modification of the leaf tip through the process of evolution [1]. This special trapping mechanism was developed for trapping animals especially insects to compensate for nutrient deficiency with protein from animals. Therefore, the plants obtain some of their essential nutrients from consuming insects that fall into the trap.

The family Nepenthaceae comprises approx. 134 species of carnivorous pitcher plants [2-4] whose leave tip portion develops highly specialized pitcher-shaped leaves that trap small tiny animals' especially insect prey [1,5-11]. This insect trapping mechanism is a pitfall mechanism where no moving plant parts are involved in the trapping process [11]. However, the plant may certainly attract and kill its prey through the active production of attractive colors, sugary nectar, and even sweet scents [12].

Nepenthes khasiana (Fig. 1), an endangered plant [13] is categorized as an Appendix-I species by CITES [14] which is enlisted under the Negative List of Export by the Govt. of India [15]. A recent study reported that the colonization of *Nepenthes* species occurred in South East Asia from an ancient Indian stock (*Nepenthes khasiana*) during 8.16–15 Mya [16]. This pitcher plant is endemic to India and is reported from Meghalaya state [17-24]. Very recently, its distribution was also reported in the Dima Hasao area of Assam state of India [25]. However, the species is in verse of extinction due to habitat destruction [26], trade due to medicinal properties [27] and other anthropogenic causes [28,29].

The trap of a pitcher plant is a cylindrical rod-shaped structure that is formed by the modification of the leaf tip that consists of a deep cavity filled with liquid which has a slippery coating from the insides. Once the insect falls into the pitcher, the insects lose their footing due to anti-adhesive surfaces of the peristome [30] and the inner pitcher wall [31-34]. Several Nepenthes species have epicuticular wax crystals on the upper part of the inner pitcher wall that helps in the retention of captured prey inside the pitcher. However, this anti-adhesive surface on the inner pitcher wall is more effective in the retention of the captured prey than the physical trapping [30]. The pitchers contain liquids that in some species are highly viscoelastic, and therefore play an important role in retaining the prey [32,35]. This liquid comprises not only enzymatic products, acids, and alcohols but also products that render the interior of the pitcher walls slippery to prevent trapped insects from escaping [36,37].

The liquid of the pitcher contains digestive fluid at the bottom part which is not only responsible for drowning the insect but also decomposes gradually [36-37]. Once an insect is drowned in the pitcher, the process of digestion begins. The digestive glands present on the lower part of the inner pitcher wall secrete some oily substances which lures the prey inside the pitcher. In some species of Nepenthes, these enzymes are so powerful that they can be digested by small

Fig. 1. Pitcher plant, *Nepenthes khasiana* – an endangered species endemic to Meghalaya and Dima Hasao area of Assam, India

insects in a few hours [8]. However, those species which do not produce their digestive enzymes may rely upon symbiotic bacteria or other organisms to digest their prey [8] and absorb nutrients from the plant [12]. After the entire digestive process is completed, the prey is converted into the solution of amino acid, peptides, phosphate, and ammonium, and finally, the pitcher plants get all the nutrition from this and the absorption of these nutrients are taken place through multicellular glands on the inner pitcher wall [38].

Nepenthes pitcher plants employ several different mechanisms to ensure prey attraction. They also attract and trap invertebrate prey using nectar-secreting pitchers [39]. Very recently, Kurup et al. [40] found that though most insectivorous plants use nectar, color, and olfactory cues to attract their prey traps, the fluorescence emission from fresh *N. khasiana* pitcher serves as a critical factor in attracting arthropods and other species and later trapped. Pant and Bhatnagar [41] stated that the digestive glands in *N. khasiana* occur in the inner surface of the lower portion of the pitcher. Venugopal [42] conducted an extensive study on the different types of glands while Devi et al. [43] conducted a study on the development and ultrastructure of digestive glands located in the inner part of the pitcher concerning their mode of secretion of enzymes in *N. khasiana*.

Several studies are there on the prey and inquilines of different taxa present in different species of pitcher plants. Among these, presence of prokaryotic [44], fungi [44-46], algae [45], protozoa [45], vermiform [47-49], Crustacea [50-54], Arachnida [11,46-48,55-57], insect (*Hymenoptera* [45,55,58,59]; *Lepidorptera* [55]; *Odontata* [60]; *Diptera* [55,61-63], amphibian [46,52,62-65]. All these studies were conducted on various species of Nepenthes. However, the study on this aspect of *N. khasiana* species is scarce, and the only study on this species was conducted in two locations in Garo Hills of Meghalaya [66]. Therefore, this present study aimed to find out the prey composition of *N. khasiana* and inquiline species in some areas covering all three hills in Meghalaya.

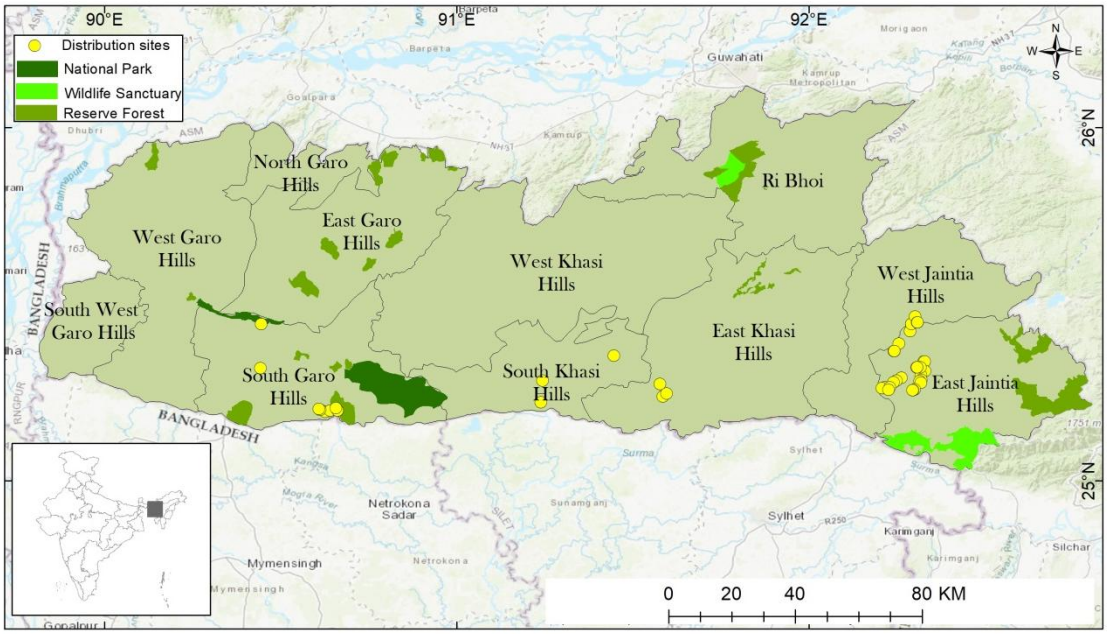

Fig. 2. Map of Meghalaya, India showing the different hills where this study was conducted

2. STUDY AREA

The present study was conducted in Meghalaya state (between 24º 58'N and 26º 07'N latitude and 89º 48'E and 92º 51'E longitude) located in the North-eastern part of India (Fig. 2) covering all three hills (Garo, Khasi, and Jaintia). The state is rimmed on the North by Goalpara, Kamrup, and Nagaon districts of Assam, on the East by Karbi Anglong and North Cachar Hills District of Assam, and on the South and West Bangladesh. The state has 22,429 sq. km of which 8510 sq. km. is covered with forest. The elevation of the plateau stretches between 150m and 1961m. The Khasi Hills encompass the central part of the plateau and the Jaintia Hills in the eastern section of the plateau whereas the western section embodies the Garo Hills. Shillong Peak is the paramount point in the State with an altitude of 1961 meters.

The presence of various types of forests like tropical moist and dry deciduous forests, tropical evergreen forests, tropical semi-evergreen forests, grass and Savannas, temperate forests, and sacred groves make the habitat suitable for a large variety of mammals, birds, and plants.

The climate of Meghalaya is humid but changes with the monsoon. The western part of the state receives an average yearly rainfall of around 2600 mm while annual rainfall is between 2500 to 3000 mm in northern Meghalaya. Southeastern Meghalaya gains annual showers of above 4000 mm. Cherrapunji experiences the highest rainfall of around 12000 mm annually and thus, Meghalaya is the wettest state in India.

The climate is neither too hot during the summer nor too cold during the winter. The climate of the state is dry between November and April, while between May and October, it rains heavily. The winter season drops down in December and lasts till February. Summer then slinks in and lingers till May and then the rainy season takes over the Meghalaya climate.

The Khasis, Jaintia, and Garos are the prime inhabitants of this state. The Khasi people occupy the majority of the population of the eastern part of Meghalaya whereas the Garo people are the second-largest tribe in Meghalaya.

3. METHODOLOGY

Sampling sites were identified based on the present distribution of pitcher plants found during distributional surveys conducted in all three hills of Meghalaya. A total of 30 numbers of pitcher samples were collected randomly from each hill during June to October, 2022 totaling 90 samples (Plate-1). Sample sites were randomly selected

and fluid from one pitcher per plant was collected to avoid pseudo replication [67]. At least a 100m gap was maintained between two samples if more than one sample was collected from the same site. During the collection of pitcher samples, an equal number of terrestrial and aerial pitchers was selected to maintain uniformity among the samples.

Pitcher samples were collected from a full-growing open pitcher and the collection was made by simply inverting the pitcher into a glass bottle. Then the samples were fixed in ethanol (70% final concentration) and transported to the laboratory. For identification of the prey and inquiline species, first of all, the pitcher sample was placed into a petri dish and then observed under a dissection microscope and determined up to the lowest taxonomic level. As digestion results in the degradation of the prey body parts, only the heads were counted to avoid duplicate counts. For this, the heads of the prey and inquilines were removed one by one by using a thin paintbrush to ensure correct counting.

4. RESULTS

4.1 Prey Composition

A total of 71 species were identified up to the species level while another five were identified up to their genus level. There are possibilities of having more species as the dipteran larvae (both Chrysops and Cyprus mosquito) could not be identified due to their larval stage. Among this, 90.8% of species were insects (n=69 species) while the remaining 9.2% (n=7 species) were non-insect species. All these seven non-insect species belonged to single order Arachnida, but three different families namely, *Salticidae Theridiidae* and *Thomisidae*. On the other hand, those species which belong to class Insecta comprised 37 families (*Acrididae, Alydidae, Anthocoridae, Apidae, Arctiidae, Blaberidae, Blattidae, Calliphoridae, Calopterygidae, Chironomidae, Chlorocyphidae, Chrysomelidae, Coccinellidae, Crabronidae, Crysomelidae, Culicidae, Curculionidae, Drosophilidae, Ectobiidae, Elateridae, Forficulidae, Formicidae, Gryllidae, Hymenopodidae, Meloidae, Muscidae, Nymphalidae, Pentatomidae, Phoridae, Pompilidae, Pyralidae, Pyrrhocoridae, Scarabaeidae, Sphecidae, Stratiomyidae, Termitidae, Tettigoniidae, and Vespidae) under 10 orders (Blattodea, Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Odonata and Orthoptera*).

Among the prey and inquiline found inside the pitcher, the majority (71.1%, n=54 species) of them were capable of flying while the remaining 28.9% (n=22 species) were incompetent to fly. The number of species that were capable and incapable to fly varies in different orders (Fig. 3). Those species that were incompetent to fly were belonging to 6 orders (*Araneae, Dermaptera, Diptera, Hemiptera, Lepidoptera, and Odonata) and 7 families namely, Calopterygidae, Chironomidae, Culicidae, Formicidae, Nymphalidae, Salticidae, Theridiidae and Thomisidae. However, all the species that were capable to fly belonged to 9 orders (Blattodea, Coleoptera, Dermaptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Odonata, and Orthoptera) 32 families (Acrididae, Alydidae, Anthocoridae, Apidae, Arctiidae, Blaberidae, Blattidae, Calliphoridae, Chlorocyphidae, Chrysomelidae, Coccinellidae, Crabronidae, Culicidae, Curculionidae, Drosophilidae, Ectobiidae, Elateridae, Gryllidae, Hymenopodidae, Meloidae, Muscidae, Pentatomidae, Phoridae, Pompilidae, Pyralidae, Pyrrhocoridae, Scarabaeidae, Sphecidae, Stratiomyidae, Termitidae, Tettigoniidae, and Vespidae*).

4.2 Prey Assemblages

The study revealed that a maximum number of 23 species belonging to the order Hymenoptera fall victim as prey species (found inside the pitcher) followed by 11 species under Coleoptera, 9 species under Diptera, 7 species under Araneae, 6 species each under Hemiptera and Blattodea, 5 species each under Orthoptera and Lepidoptera, 2 species under Odontata and 1 species each under Mantodea and Dermaptera (Fig. 4). This indicates that the Order Hymentoptera itself contributed 30.2% of the total carnivory prey selection of *N. khasiana* in the present studied area.

On the other hand, a family-wise selection showed that a highest of 9 species belonged to the family Forficulidae were selected as prey followed by 5 species in Sphecidae, 4 species each belonged to Vespidae and Culicidae, 3 species each belonged to Pyralidae, Pentatomidae, Apidae, Acrididae and Chrysomelidae while 1 species each belonged to 22 number of different families (Fig. 5). This

indicates that the species belonged to 9 families namely; Forficulidae, Sphecidae, Vespidae, Culicidae, Pyralidae, Pentatomidae, Apidae, Acrididae, and Chrysomelidae comprised about 47.3% of the total prey species for *N. khasiana* in the present study.

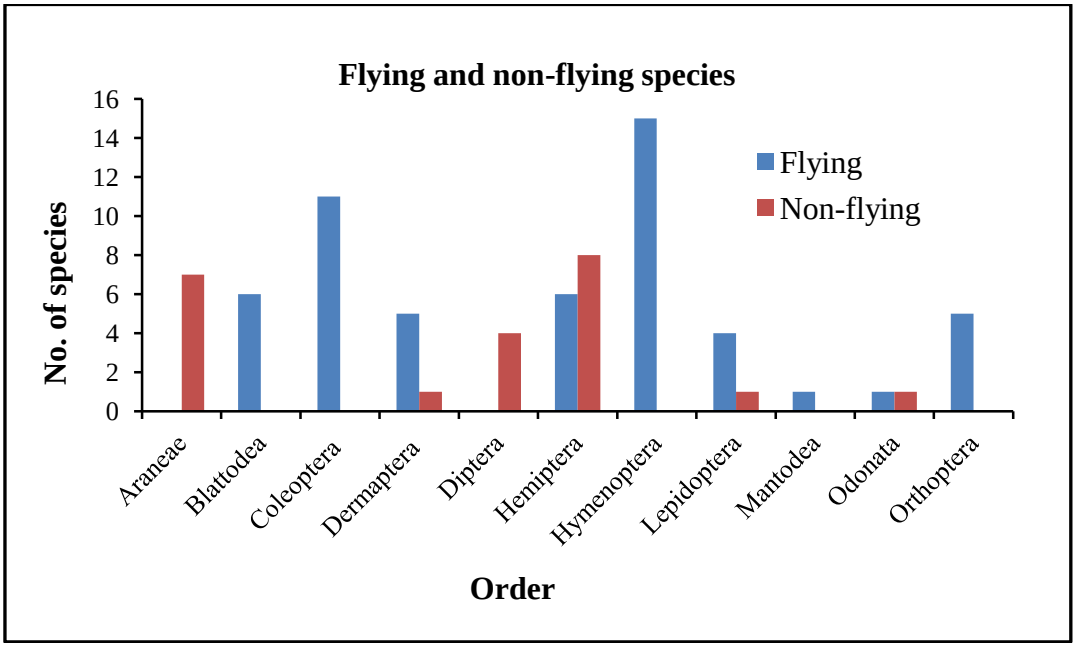

Fig. 3. Comparative account between flying and non-flying species found inside the pitcher

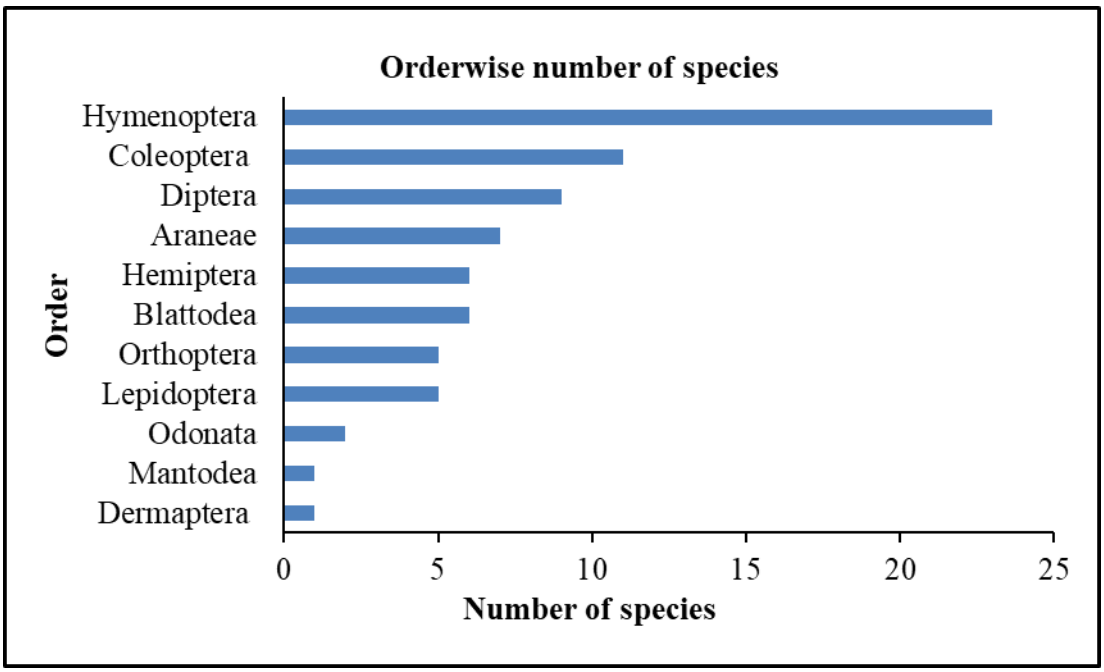

Fig. 4. Selection of different prey species by *N. khasiana* concerning the order

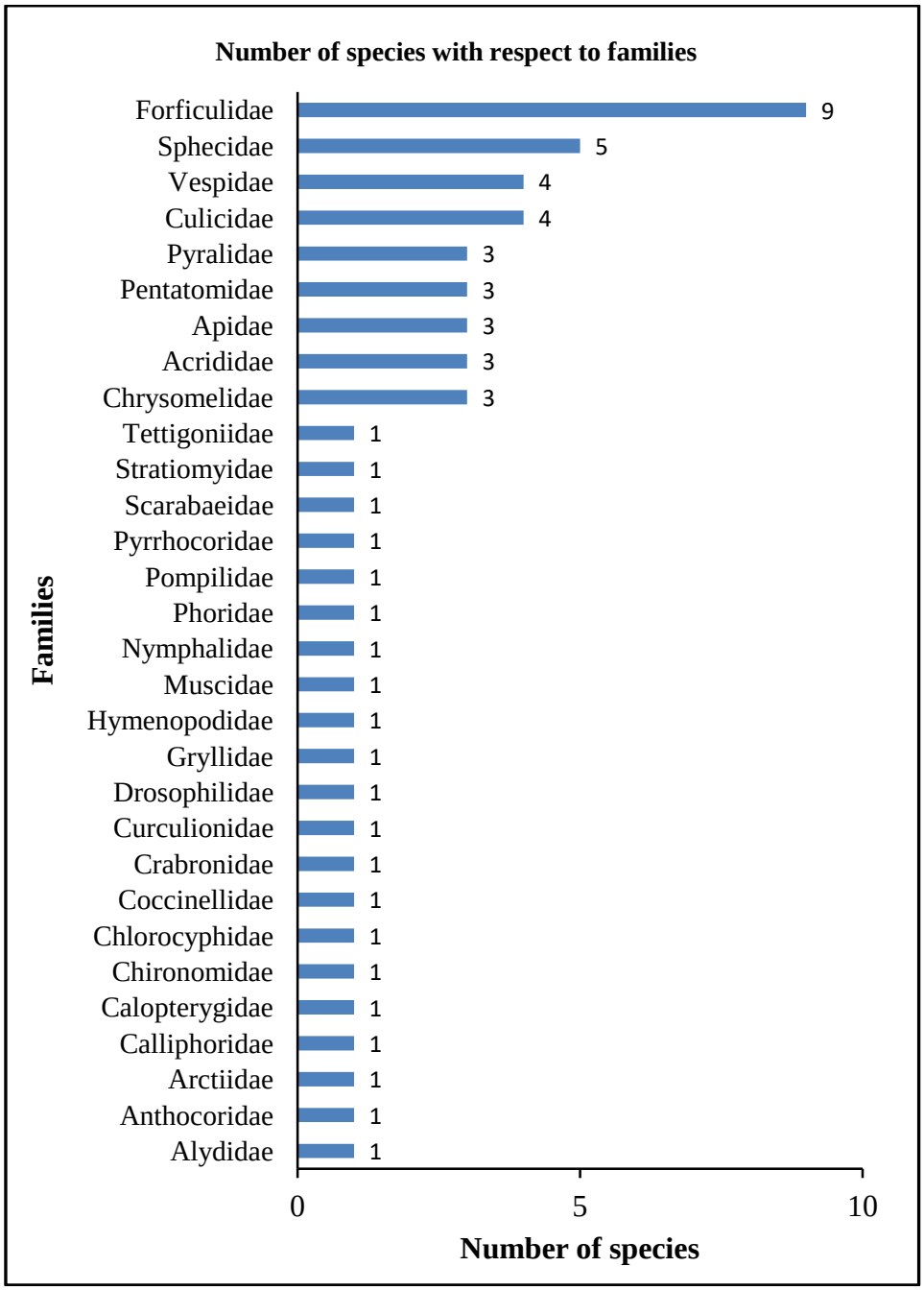

Fig. 5. Selection of different prey species by *N. khasiana* concerning families

4.3 Feeding Spectrum

In terms of dominance (number of preys with that of species), the Order Diptera contributed 45.5% of the carnivore feeding spectrum. However, the Diptera together with Hymenoptera contributed 83.3% of the total feeding spectrum. However, the remaining 16.7% of the feeding spectrum was contributed by the prey belonging to 9 different orders (Coleoptera, Orthoptera, Blattodea, Araneae, Hemiptera, Lepidoptera, Mantodea, Odonata and Dermaptera) (Fig. 6). This indicated that the species belonging to Diptera and Hymenoptera played a major role in the carnivore feeding spectrum of *N. khasiana*.

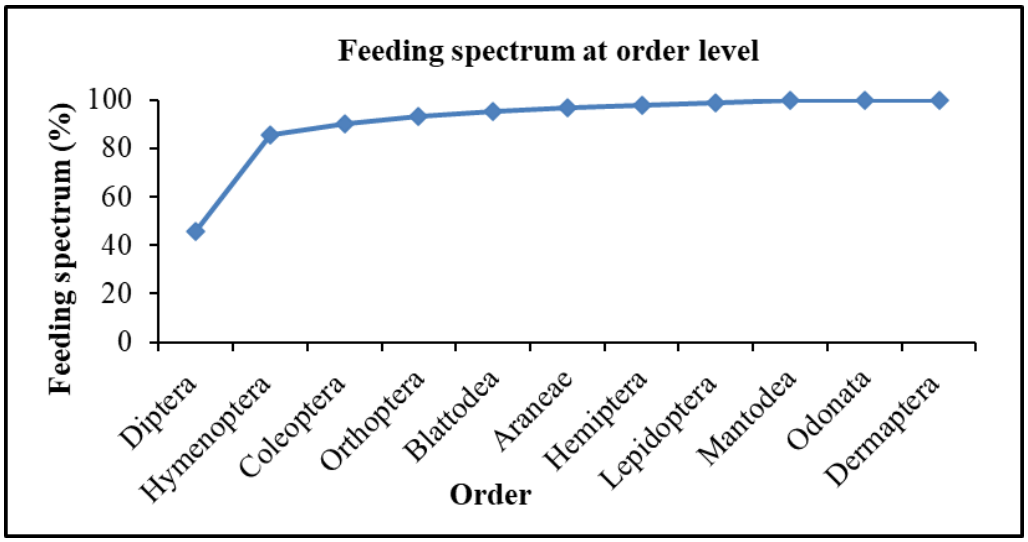

Fig. 6. Feeding spectrum of *N. khasiana* at the order level

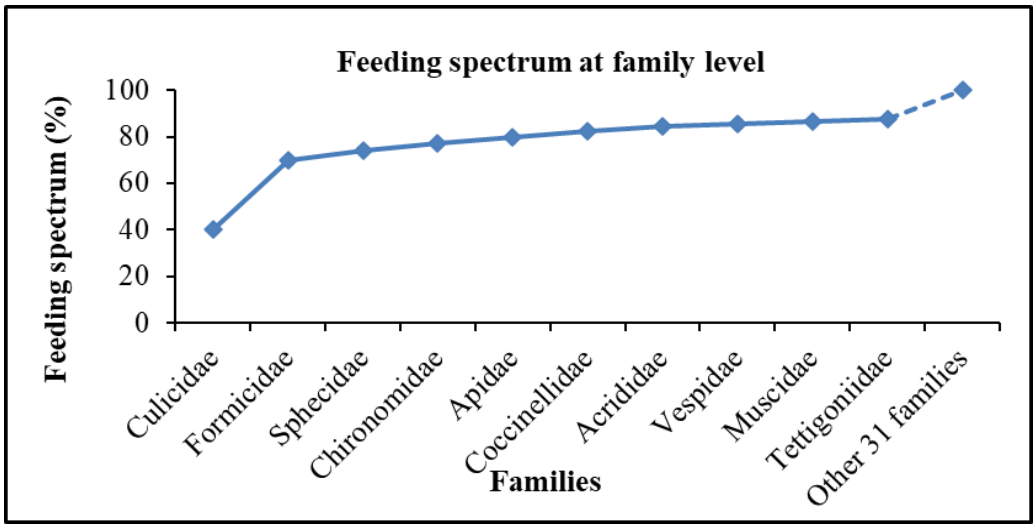

Fig. 7. Feeding spectrum of *N. khasiana* at the family level

Similarly, the prey belonging to the family Culicidae contributed 40.3% of the total carnivore-feeding spectrum of *N. khasiana*. However, the family Culicidae along with Formicidae contributed a total 70.0% feeding spectrum. The remaining 30.0% of the feeding spectrum was contributed by 39 families (Fig. 7). This further indicates that the families Culicidae and Formicidae played a major role in the feeding spectrum of the studied species.

5. DISCUSSION

It is now well established that some plant develops carnivory behavior and capture prey species to supplement inorganic nutrients, especially nitrate (N) and phosphate (P) when the soil is deficient with such inorganic compound [68-70] and the net benefit of this carnivory behavior appears to be marginal that vary from species to species [71]. Therefore, different species of carnivores develop a great variety of pitcher morphologies to cope with the local environmental parameters [72]. Therefore, different species of *Nepenthes* developed different nitrogen acquisition strategies as part of the adaptation under extreme environmental conditions [73-76] which are occasionally detritivores [77,78] or partially coprophagous [79,80] than purely carnivorous strategies. In *N. albomarginata,* the rim of white trichomes below the peristome attracts some termites and therefore, the plant obtains more than 50% of its total nitrogen from them [81,82]. Similarly, some

pitcher plants present visual and olfactory signals that attract small creatures. There is a report about the radiation of UV reflection by *N. rafflesiana* [39] which exude a strong sweet scent [7,83,84]. Therefore, a large number of small animals are being trapped by the pitcher plant which differs among different *Nepenthes* species [1,6,85]. Few studies reported the composition of the prey in detail [1,7,39,86,87]. Previous studies conducted in Borneo found that *N. pudica* trapped 40 different taxa [88]. However, the study conducted on *N. khasiana* reported 55 numbers of morphospecies [66]. Finding 76 numbers of species in all three hills of Meghalaya, India further added the prey assemblages. However, the majority of the studies conducted on Nepenthes species reported that the prey consisted of insects [5,9-11]. Similar results were also reported in *N. madagascariensis where* the prey constituted 96.9% of insects. A previous study conducted on *N. khasiana* reported 53 numbers of insects (96.3%) out of a total of 55 prey species [66]. The present study found a comparatively higher number of insect species (69 species) as compared to some previous studies on Nepenthes species and reported more non-insect species. This may be because of the sampling period which was done during the flowering season that yielded a much higher number of prey species.

Studies are also available on the ratio of flying and non-flying trapped species. On average, 75-85% of all the trapped species were flying insects. The study conducted on *N. raffelesiana* also found a higher number of winged prey bases [7]. In the present study, we also confirmed a higher number of flying species (71.1%, 54 out of 76) being trapped in *N. khasiana*. All the non-flying trapped species belonged to ants under Formicidae and spiders under Salticidae, Theridiidae, and Thomisidae families (order: Araneae) which is consistent with the previous studies in other species of Nepenthes [7]. Almost similar findings were reported in a previous study conducted on *N. khasiana* where flying insects constituted 85% of the total trapped animals [66]. The presence of a higher number of flying insects further indicates that the pitcher successfully mimics flowers.

There was a wide variation in prey in different species of carnivores. Studies conducted on *N. rafflesiana* plants reported prey species belonging to 63 families of arthropods. At the order level, Hymenoptera (Formicidae species) constituted up to 50.3% of the prey [83]. In *N. madagascariensis*, 80.2% of the insect prey belonged to the family Formicidae and 94.3% of its prey animals belonged to three taxa: Formicidae (80.2%), Diptera (9.7%) and Coleoptera (4.4%) [89]. A previous study conducted on *N. khasiana* found that the order Hymenoptera was the most dominant in prey species (17 species) followed by Diptera (11 species) and Coleoptera (10 species) [66]. A similar trend is also found in the present study which further supports the finding of major carnivore species of the world. This is because ants are one of the most important prey species of carnivorous plants [90]. In *S. purpurea*, ants account for 75% of the prey [91]. Similar findings were also reported in *S. rubra* and *S. minor* [92], Darlingtonia [93], Heliamphora [90,94] and Cephalotus [5,95]. Studies conducted on various species of Nepenthes also found a similar trend. In *N. bicalcarata,* ants along with termites were the most abundant prey, and an average of 35 animals were caught per trap [63]. In *N. rafflesiana*, twenty-three species of ants were identified as trapped species and constituted the most important prey of the lower (89.3% of prey) and upper pitchers (40.8%) [7,83]. However, in *N. albomarginata* termites were mostly trapped followed by ants [81-82].

However, in terms of trapped abundance, the family Diptera was the most important group in the present study of *N. khasiana*. The Dipteran contributed 45.5% of the total feeding spectrum followed by Hymenoptera 39.8% accounting total of 85.3%. A previous study conducted on *N. rafflesiana* also found a similar phenomenon [83]. This finding further indicates that though there is a wide prey spectrum, only a few groups of species play a major role in supplementing the nutrient deficiency of the pitcher plant. On the other hand, a very low rate of prey capture by *N. khasiana* as compared to the diversity may be because of two reasons, the passive trapping mechanism which preyed only the accidental pitfall arthropods, and the second reason may be due to non-closure of pitcher cap due to which the arthropods often come out of the trap after getting into accidental fall. The pitcher could trap only those arthropods which fail to come out of the pitcher due to pitcher fluid.

Table 1. Species found in the pitcher fluid of *N. khasiana* in various locations of Garo, Khasi and Jaintia Hills of Meghalaya, India

Order	Family	Common name	Scientific name
Araneae	Salticidae	Banded phintella	*Phintella vittata*
	Theridiidae	Common house spider	*Parasteatoda tepidariorum*
	Salticidae	Fighting spider	*Thiania bhamoensis*
	Thomisidae	Goldenrod crab spider	*Misumena vatia*
	Thomisidae	Red ant spider	*Amyciaea forticeps*
	Salticidae	Spider spp. 1	*Myrmachne orientalis*
	Salticidae	Spider spp. 2	*Epius indicus*
Blattodea	Blattidae	American cockroach	*Periplaneta americana*
	Blaberidae	Cockroach	*Stictolampra plicata*
	Ectobiidae	German cockroach	*Blattella germanica*
	Termitidae	Termite spp. 1	*Odontotermes singsiti*
	Termitidae	Termite spp. 2	*Macrotermes khajuriai*
	Termitidae	Termite spp. 3	*Pseudocapritermes tikadari*
Coleoptera	Meloidea	Blister beetle	*Epicauta spp*
	Chrysomelidae	Bruchid beetle	*Callosobruchus chinensis*
	Elateridae	Click beetle	*Alaus spp*
	Chrysomelidae	Cucumber beetle	*Diabrotica undecimpunctata*
	Scarabaeidae	Dung beetle	*Onthophagus dama*
	Scarabaeidae	Green beetle	*Mimela Splendens*
	Coccinellidae	Lady bird beetle	*Coccinella septempunctata*
	Chrysomelidae	Leaf beetle	*Trirhabda bacharidis*
	Chrysomelidae	Mint beetle	*Chrysolina coerulans*
	Chrysomelidae	Pumkin beetle	*Aulacophora foveicollis*
	Curculionidae	Weevil	*Sitophilus granarius*
Dermaptera	Forficulidae	Earwing	*Forficula auricularia*
Diptera	Culicidae	Mosquito larva (Anopheles)	*Anopheles quadrimaculatus*
	Culicidae	Mosquito larva (Chrysops)	*Chrysops sp.*
	Culicidae	Mosquito larva (Culex)	*Culex sp.*
	Culicidae	Mosquito-Yellow fever mosquito	*Aedes aegypti*
	Chironomidae	Chironomous larva	*Chironomous plumosus*
	Drosophilidae	Fruitfly	*Drosophila melanogaster*
	Muscidae	Housefly	*Musca domestica*
	Calliphoridae	Oriental latrine fly	*Chrysomya megacephala*
	Phoridae	Phorid fly	*Megaselia scalaris*
Hemiptera	Anthocoridae	Common flower bug	*Anthocoris nemorum*
	Pentatomidae	Green bug	*Chinavia hilaris*
	Pentatomidae	Green stink bug	*Nezara viridula*
	Pyrrhocoridae	Indian Cotton stainer bug	*Dysdercus cingulatus*
	Pentatomidae	Rice Black Bug	*Scotinopara coartata*
	Alydidae	Rice Gandhi Bug	*Leptocorisa acuta*
Hymenoptera	Formicidae	Ant spp.	*Camponotus parius*
	Formicidae	Black carpenter ant	*Camponotus pennsylvanicus*
	Formicidae	Black garden ant	*Lasius niger*
	Stratiomyidae	Black soldier fly	*Hermetiinae illucens*
	Formicidae	Crazy ant	*Paratrechina longicornis*
	Formicidae	Little black ant	*Monomorium minimum*
	Formicidae	Silky shield ant	*Meranoplus bicolor*
	Formicidae	Tropical fire ant	*Atta geminata*
	Formicidae	White-footed ant	*Technomyrmex albipes*
	Vespidae	Asian giant hornet	*Vespa mandarinia*
	Sphecidae	Black wasp	*Sphex pensylvanicus*
	Apidae	Bumblebee	*Bombus lapidarius*
	Apidae	Common Blue-headed Bee	*Amegilla cingulata*
	Sphecidae	Cricket hunter wasp	*Chlorion aerarium*
	Crabronidae	Eastern Cicada killer Wasp	*Sphecius speciosus*
	Sphecidae	Great Black wasp	*Sphex ichneumoneus*
	Apidae	Indian Honey Bee	*Apis indica*
	Vespidae	Paper wasp	*Polistes humilis*
	Sphecidae	Solitary wasp	*Isodontia mexicana*

Order	Family	Common name	Scientific name
	Pompilidae	Spider wasp	*Pompilidae spp*
	Sphecidae	Thread waisted wasp	*Eremnophila aureonotata*
	Vespidae	Windowed carpenter wasp	*Vespa simillima*
	Vespidae	Yellow wasp	*Polistes fuscatus*
Lepidoptera	Nymphalidae	Butterfly larva	*Melanitis leda*
	Pyralidae	Clover hay moth	*Hypsopygia costalis*
	Arctiidae	Hand maiden moth	*Syntomoides amata*
	Pyralidae	The great wax moth	*Galleria mellonella*
	Pyralidae	Rice moth	*Corcyra cephalonica*
Mantodea	Hymenopodidae	Indian Flower Mantis (Grasshopper)	*Creobroter pictipennis*
Odonata	Calopterygidae	Damselfly larva spp.1	*Caliphaea confusa*
	Chlorocyphidae	Damselfly spp.2	*Libellago lineata*
Orthoptera	Acrididae	Common green grasshopper	*Omocestus viridulus*
	Gryllidae	Cricket	*Acheta domesticus*
	Acrididae	Grasshopper spp. 1	*Oxya hyla*
	Tettigoniidae	Great Green Bush-Cricket (Grasshopper)	*Tettigonia viridissima*
	Acrididae	Japanese grasshopper	*Oxya japonica*

Prey species sampled from pitchers of different locations in Garo Hills

Prey species sampled from pitchers of different locations in Jaintia Hills

Prey species sampled from pitchers of different locations in Khasi Hills

Plate 1. Prey samples with fluid collected from the Garo, Khasi, and Jaintia hills of Meghalaya

There are reports which depict that some species use the pitcher as a safe habitat at their developing stages which are called inquiline species. Studies conducted on different species of Nepenthes reported different numbers of inquiline species in the pitcher. A recent study conducted on *N. khasiana* reported three numbers of inquiline species all of which belong to the order Diptera [66]. The present study further confirms the same finding but reported comparatively more numbers of inquiline species. Studies conducted on other species of carnivorous pitcher plants also found Dipteran larvae as inquilines [96]. In general, mosquitoes in Nepenthes usually lay their eggs inside the pitchers. The eggs hatch and the larvae undergo several development stages before they emerge as adults. The adults leave the pitchers, returning to lay eggs. These dipteran species use the pitcher as the safe ground for breeding [8]. The study also reported no presence of metazoan inquilines in all three hills further supporting the finding of a previous study conducted in *N. khasiana* [66]. However, the studies conducted on different species of carnivores found several inquiline species other than the Dipteran species. The study conducted by Steiner [36] reported that small crabs to larvae of moths and tadpoles choose a symbiotic relationship inside the pitcher. Similar findings were also reported during the Malaysian Heritage Endau-Rompin Expedition where the small red crabs (*Geosesarma malayanum*) were found living in the pitcher of *Nepenthes ampullaria* [97]. The small red crab spider, *Misumenops nepenthicola*, lives inside the lowland Nepenthes pitcher in Malaysia and Indonesia. It waits beneath the peristome and ambushes the insect that crawled out from the inner surface of the pitcher and also preys on larvae that live in the pitcher fluid [8]. Even small vertebrates are sometimes mentioned as being captured by Asian *Nepenthes* species [8,97-99]. In the case of frogs, many species, which normally deposit their eggs in tree holes, also use Nepenthes pitchers for this reason [8]. One recent study which was conducted at Kubah National Park, Sarawak proved that this frog has used *N. ampullaria* to lay eggs. The species is an obligate of the pitcher plant which breeds in senescent or mature pitchers [100].

6. CONCLUSION

The study illustrates that the carnivory plants including *N. khasiana* not only require well-developed mechanisms of insect attraction, capture, retention, and digestion but also require a wide composition of prey species to sustain their lives under extreme nutrient deficiency condition. This further indicates the sustainable management of biodiversity securing the abundance of animal prey species for the conservation of pitcher plants in this landscape.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adam JH. Prey spectra of Bornean Nepenthes species (*Nepenthaceae*) in relation to their habitat. Pertanika J Trop Agric Sci. 1997;20:121-34.
2. Jebb MHP, Cheek M. A skeletal revision of *Nepenthes* (*Nepenthaceae*). Blumea. 1997;42:1-106.
3. Cheek M, Jebb M. Nepenthaceae. Flora malesiana, Series I – Seed plants. 2001;15:1-164.
4. McPherson S. Pitcher plants of the Old World. Poole: Redfern Natural History Productions. 2009;2.
5. Lloyd FE 1942. The carnivorous plants. Chronica Botanica Company, Waltham. 352 pp.
6. Kato M, Hotta M, Tamin R, Itino T. Inter- and intraspecific variation in prey assemblages and inhabitant communities in Nepenthes pitchers in Sumatra. Trop Zool. 1993;6:11-25.
7. Moran JA. Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. J Ecol. 1996;84(4):515-25.
8. Clarke CM. The effects of pitcher dimorphism on the metazoan community of the carnivorous plant *Nepenthes bicalcarata* Hook. f. Malay Nat J. 1997;50:149-57.
9. Barthlott W, Porembski S, Fischer E, Gemmel B. First protozoa-trapping plant Found. Nature. 1998;392:447-8.
10. Barthlott W, Porembski S, Seine R, Theisen I. The curious world of carnivorous

plants – A comprehensive guide to their biology and cultivation. Portland, OR: Timber Press. 2007;224.
11. Juniper BE, Robins RJ, Joel DM. The carnivorous plants. San Diego, London: Academic Press; 1989.
12. Mithöfer A. Carnivorous pitcher plants: insights in an old topic. *Phytochemistry*. *Plant-Insect Interactions*. Phytochemistry. 2011;72(13):1678-82.
13. Ved D, Saha D, Haridsan K, Ravikumar K. *Nepenthes khasiana*. The IUCN Red List Threat Species. 2015;2015,e. T48992883A49009685.
14. CITES. UNEP; 2022. The CITES Appendices [cited Jun 12 2023]. Available: https://cites.org/eng/app/index.php
15. Ziemer B. Exciting conservation news: the rare *Nepenthes* collection project! Carnivorous Plant Newsl. 2010;39(3):67.
16. Biswal DK, Debnath M, Konhar R, Yanthan S, Tandon P. Phylogeny and biogeography of carnivorous plant family Nepenthaceae with reference to the Indian pitcher plant *Nepenthes khasiana* reveals an Indian subcontinent origin of Nepenthes colonization in South East Asia during the Miocene epoch. Front Ecol Evol. 2018;6:108.
17. Hooker JD. The flora of British India. Kent; 1886;5.
18. Kanjilal UN, Kanjilal PC, Das A, De RN, Bor NL. Flora of Assam. Shillong: Government Press; 1936.
19. Jain SK, Sastri ARK. Threatened plants of India: A state of art report, Botanical Survey of India; 1980.
20. Joseph J, Mani G. One new species and one new record from India in the genus Utricularia. Bull Bot Surv India. 1982;24:108-11.
21. Haridasan K, Rao RR. Forest flora of Meghalaya. 2nd Vols. Dehra Dun, India: Bishen Singh Mahendrapal Singh; 1987.
22. Choudhury A. Range extension of *Nepenthes khasiana* in the Jaintia Hills, Meghalaya. J Bombay Nat Hist Soc. 2000;97(1):166-7.
23. Mao AA, Kharbuli P. Distribution and status of *Nepenthes khasiana* Hook. f. a rare endemic pitcher plant of Meghalaya, India. Phytotaxonomy. 2002;2:77-83.
24. Devi R, Venugopal N. The status of insectivorous plants in North-East India: their uses and conservation. J Swamy Bot Club. 2006;23(I-4):75-80.
25. Betlu ALS. *Nepenthes khasiana* Hook. f. (Nepenthaceae) from Assam. Curr Sci. 2016;111(8):1311.
26. Devi SP, Kumaria S, Sharma PR, Khoyumthem P, Tandon P. *Nepenthes khasiana* Hook. f. an endangered tropical pitcher plant of India. Indian J Trad Knowl. 2019;18(1):68-75.
27. Minh NP. Antioxidant capacity of pitcher (Nepenthes) extract on oxidative rancidity of sun-dried squid. Food Res. 2021;5(5):167-73.
28. Damayanti F, Mansur M, Roostika I. Diversity of *Nepenthes* spp. in West Kalimantan. Int J Biodivers Conserv. 2011;3(13):705-8.
29. Cross AT, Krueger TA, Gonella PM, Robinson AS, Fleischmann AS. Conservation of carnivorous plants in the age of extinction. Glob Ecol Conserv. 2020;24:e01272.
30. Bohn HF, Federle W. Insect aquaplaning: Nepenthes pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. Proc Natl Acad Sci U S A (PNAS). 2004;1 01(39):14138-43.
31. Juniper BE, Burras JK. How pitcher plants trap insects. New Sci. 1962;13:75-7.
32. Gaume L, Gorb S, Rowe N. Function of epidermal surfaces in the trapping efficiency of *Nepenthes alata* pitchers. New Phytol. 2002;156(3):479-89.
33. Riedel M, Eichner A, Jetter R. Slippery surfaces of carnivorous plants: composition of epicuticular wax crystals in *Nepenthes alata* Blanco pitchers. Planta. 2003;218(1):87-97.
34. Gaume L, Perret P, Gorb E, Gorb S, Labat JJ, Rowe N. How do plant waxes cause flies to slide? Experimental tests of wax-based trapping mechanisms in three pitfall carnivorous plants. Arthropod Struct Dev. 2004;33(1):103-11.
35. Gaume L, Forterre YA. A Viscoelastic deadly fluid in carnivorous pitcher plants. Plos One. 2007;2(11):e1185.
36. Steiner H. Borneo: its mountains and lowlands with their pitcher plants. Trekking from 1992 to 2002. Kota Kinabalu, Sabah: Toihaan Publishing. 2002;136.
37. Dkhar J, Bhaskar YK, Lynn A, Pareek A. Pitchers of *Nepenthes khasiana* express several digestive-enzyme encoding genes, harbor mostly fungi and probably evolved through changes in the expression of leaf

polarity genes. BMC Plant Biol. 2020;20(1):524.
38. Owen TP, Lennon KA, Santo MJ, Anderson AN. Pathways for nutrient transport in the pitchers of the carnivorous plant *Nepenthes alata*. Ann Bot. 1999;84(4):459-66.
39. Moran JA, Booth WE, Charles JK. Aspects of pitcher morphology and spectral characteristics of six Bornean Nepenthes pitcher plant species: Implications for prey capture. Ann Bot. 1999;83(5): 521-8.
40. Kurup R, Johnson AJ, Sankar S, Hussain AA, Sathish Kumar C, Sabulal B. Fluorescent prey traps in carnivorous plants. Plant Biol (Stuttg). 2013;15(3):611-5.
41. Pant DD, Bhatnagar S. Morphological studies in Nepenthes (*Nepenthaceae*). Phytomorphology. 1977;27:13-34.
42. Venugopal N. An interesting observation on the epidermal digestive glands in *Nepenthes khasiana* Hk. f. Curr Sci. 1999;76(1):30-2.
43. Devi KR, Venugopal N, Singha LB. A critical analysis on the digestive glands of endemic and endangered pitcher plant (*Nepenthes khasiana* Hook. f.) of Northeast India. Ne BIO. 2016;7(4):115-21.
44. Okahara K 1933. Physiological studies on Drosera IV: on the function of micro-organisms in the digestion of insect bodies by insectivorouse plants. Scientific Reports of Tohoku Imperial University, 151-68.
45. van Oye P. Zur Biologie der Kanne von Nepenthes melamphora Reinw. Biol Zentralbl. 1921;41:529-34.
46. [46] Ratsirarson J. & Silander J.A. Structure and dynamics in *Nepenthes madagascariensis* pitcher plant micro-communities. Biotropica. 1996;28:218-27.
47. Choo JPS, Koh TL, Ng PKL. Nematodes and arthropodes. In: Tan HTW, editor. *A guide to the carnivorous plants of* Singapore. Singapore: Singapore Science Centre; 1997. p. 141-54.
48. Sota T, Mogi M, Kato K. Local and regional-scale food web structure in *Nepenthes alata* pitchers. Biotropica. 1998;30(1):82-91.
49. Bert W, Tandingan de Ley I, Van Driessche R, Segers H, De Lay P. *Baujardia mirabilis* gen. n., sp. n. from pitcher plants and its phyolgenetic position within Panagroleimide Nematoda: Rhabditida. Nematology. 2003;5:405-20.
50. Rumphius GE. Het Amboinsche kruitboek. Amsterdam: Joannes Burmannus; 1750.
51. Carrow T, Hirschel K, Winkelmann H, Radke R. Fleischfressende Pflanzen: to¨dliche Fallen [scientific film]. ZDF; 1997.
52. Kitching RL. Food webs and container habitats. The natural historyand ecology of phytotelmata. Cambridge: Cambridge University Press; 2000.
53. Reid JW. A human challenge: discovering and understanding continental copepod habitats. Hydrobiologia. 2001;453/454:201-26.
54. Beekmann EM. A note on the priority of Rumphius' observation of decapode crustacea living in Nepenthes. Crustaceana. 2004;77:1019-21.
55. Beaver RA. The communities living in Nepenthes pitcher plants: Fauna and food webs. In: Frank JH, Lounibos LP, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Medford: Plexus Publishing. 1983;129-59.
56. Fashing NJ. *Nepenthecarus*, a new genus of Histiotomatidae Acari: Astigmata inhabiting the pitchers of *Nepenthes mirabilis* Lour. Druce in far north Queensland, Australia. Aust J Entomol. 2002;41:7-17.
57. Hua YJ. Nepenthes and its food web. In: ICP Society, editor. 5th I.C. PS Congress. Lyon. International Carnivorous Plant Society; 2004.
58. Clarke CM, Kitching RL. Swimming ants and pitcher plants: a unique ant–plant interaction from Borneo. J Trop Ecol. 1995;11(4):589-602.
59. Barthlott W, Porembski S, Seine R, Theisen I. Karnivoren. Biologie und Kultur fleischfressender Pflanzen. Stuttgart: Eugen Ulmer; 2004.
60. Corbet PS. Odonata in phytotelmata. In: Frank JH, Lounibos LP, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect. Communities. Medford: Plexus Publishing; 1983. p. 29-54.
61. Mogi M, Yong HS. Aquatic arthropod communities in Nepenthes pitchers: the role of niche differentiation, aggregation, predation and competition in community organization. Oecologia. 1992;90(2):172-84.
62. Cresswell JE. Morphological correlates of necromass accumulation in the traps of an Eastern tropical pitcher plant, *Nepenthes ampullaria* Jack, and observations on the pitcher infauna and its reconstitution

following experimental removal. Oecologia. 1998;113(3):383-90.
63. Cresswell JE. Resource input and the community structure of larval in faunas of an eastern tropical pitcher plant *Nepenthes bicalcarata*. Ecol Entomol. 2000;25(3): 362-6.
64. Lim KKP, Ng PKL. Nepentophilous larvae and breeding habitats of the sticky frog, *Kalophrynus pleurostigma* Tschudi (*Amphibia: Microhylidae*). Raffles Bull Zool. 1991;39:209-14.
65. Ming LT. Chordate. In: Tan HTW, editor. A guide to the carnivorous plants of Singapore. Singapore: Singapore Science Centre.1997;155-7.
66. [66] Karippadath A. Ecology of *Nepenthes khasiana* in South Garo Hills: A study of ecology, associated organisms and trap morphology. M.Sc. [dissertation], eUniversity of Pune. India; 2012.
67. Hurlbert SH. Pseudoreplication and the design of ecological field experiments. Ecol Monogr. 1984;54(2):187-211.
68. Adamec L. Mineral nutrition of carnivorous plants – a review. Bot Rev. 1997;63(3):273-99.
69. Schulze W, Schulze ED, Pate JS, Gillison AN. The nitrogen supply from soils and insects during growth of the pitcher plants Nepenthes mirabilis, Cephalotus follicularis and Darlingtonia californica. Oecologia. 1997; 112(4):464-71.
70. Schulze W, Schulze ED, Schulze I, Oren R. Quantification of insect nitrogen utilization by the Venus fly trap *Dionaea muscipula* catching prey with highly variable isotope signatures. J Exp Bot. 2001;52(358):1041-9.
71. Karagatzides JD, Ellison AM. Construction costs, payback times, and the leaf economics of carnivorous plants. Am J Bot. 2009;96(9):1612-9.
72. Dkhar J, Pareek A. Asymmetric leaves1 and revoluta. Sci Rep. 2019;9(1):6318.
73. Gaume L, Di Giusto B. Adaptive significance and ontogenetic variability of the waxy zone in *Nepenthes rafflesiana*. Ann Bot. 2009;104(7):1281-91.
74. Chin L, Moran JA, Clarke C. Trap geometry in three giant montane pitcher plant species from Borneo is a function of tree shrew body size. New Phytol. 2010;186(2):461-70.
75. Clarke CM, Moran JA, Chin L. Mutualism between tree shrews and pitcher plants. Perspectives and avenues for future research. Plant Signal Behav. 2010;5(10): 1187-9.
76. Moran JA, Hawkins BJ, Gowen BE, Robbins SL. Ion fluxes across the pitcher walls of three Bornean Nepenthes pitcher plant species: flux rates and gland distribution patterns reflect nitrogen sequestration strategies. J Exp Bot. 2010 ;61(5):1365-74.
77. Moran JA, Clarke CM, Hawkins BJ. From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant *Nepenthes ampullaria*. Int J Plant Sci. 2003;164(4):635-9.
78. Pavlovič A. Photosynthetic characterization of Australian pitcher plant *Cephalotus follicularis*. Photosynthetica. 2011;49(2): 253-8.
79. Clarke CM, Bauer U, Lee CC, Tuen AA, Rembold K, Moran JA. Tree shrew lavatories: A novel nitrogen sequestration in a tropical pitcher plant. Biol Lett. 2009;5(5):632-5.
80. Grafe TU, Schöner CR, Kerth G, Junaidi A, Schöner MG. A novel resource-service mutualism between bats and pitcher plants. Biol Lett. 2011;7(3):436-9.
81. Moran JA, Merbach MA, Livingston NJ, Clarke CM, Booth WE. Termite prey spezialisation in the pitcher plant *Nepenthes albomarginata* – evidence from stable isotope analyis. Ann Bot. 2001;88:307-11.
82. Merbach MA, Merbach DJ, Maschwitz U, Booth WE, Fiala B, Zizka G. Mass march of termites into the deadly trap. Nature. 2002;415(6867):36-7.
83. Di Giusto BD, Grosbois V, Fargeas E, Marshall DJ, Gaume L. Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a Nepenthes carnivorous plant from Borneo. J Biosci. 2008;33 (1):121-36.
84. Bauer U, Willmes C, Federle W. Effect of pitcher age on trapping efficiency and natural prey capture in carnivorous *Nepenthes rafflesiana* plants. Ann Bot. 2009;103(8):1219-26.
85. Chin L, Chung AY, Clarke C. Interspecific variation in prey capture behavior by co-occurring Nepenthes pitcher plants: Evidence for resource partitioning or samplingscheme artifacts? Plant Signal Behav. 2014;9(1):e27930.

86. Erber D. Untersuchungen zur Bioz¨onose und Nekroz¨onose in Kannenpflanzen auf Sumatra. Archiv F¨ur Hydrobiologie. 1979; 87:37-48.
87. Jebb M. Some observations on *Nepenthes* in Papua New Guinea (1987). In: Juniper BE, Robins RJ, Joel DM, editors. The carnivorous plants. London: Academic Press. 1989;314-6.
88. Dančák M, Majeský Ľ, Čermák V, Golos MR, Płachno BJ, Tjiasmanto W. First record of functional underground traps in a pitcher plant: *Nepenthes pudica* (Nepenthaceae), a new species from north Kalimantan, Borneo. PhytoKeys. 2022; 201:77-97.
89. Rembold K, Fischer E, Wetzel MA, Barthlott W. Prey composition of the pitcher plant *Nepenthes madagascariensis*. J Trop Ecol. 2010;26(4):365-72.
90. Gonzalez JM, Jaffe K, Michelangeli F. Competition for prey between the carnivorous Bromeliaceae Brocchinia reducta and Sarraceniaceae Heliamphora nutans. Biotropica. 1991;23:602-4.
91. Newell SJ, Nastase AJ. Efficiency of insect capture by *Sarracenia purpurea* (Sarraceniaceae), the northern pitcher plant. Am J Bot. 1998;85(1):88.
92. Givnish TJ. Ecology and evolution of carnivorous plants. In: Abrahamson WG, editor. Plant–animal interactions. New York: McGraw-Hill; 1989; 243-90.
93. Ellison AM. Turning the tables: Plants bite back. 2005;Fall:25-30.
94. Jaffe K, Michelangeli F, Gonzalez JM, Miras B, Christine Ruiz MC. Carnivory in pitcher plants of the genus Heliamphora (*Sarraceniaceae*). New Phytol. 1992; 122(4):733-44.
95. Dakin WJ. The West Australian pitcher plant (*Cephalotus follicularis*) and its physiology. J R Soc West Aust. 1917;4:37-53.
96. Adlassnig W, Mayer E, Peroutka M, Pois W, Lichtscheidl IK. The North American carnivorous plant Sarracenia in Europe. Phyton. 2010;49:279-92.
97. Phillipps A, Lamb A. Pitcher-plants of Borneo. Borneo: National History Publications, Kota Kinabalu. 1996;171.
98. Slack A. Carnivorous plants. Cambridge: MIT Press. 2000;240.
99. Hua YJ, Li K. The special relationship between Nepenthes and tree frogs. Carnivorous Plant Newsl. 2004;33:23-4.
100. Das I, Haas A. New species of *Microhyla* from Sarawak: Old World's smallest frogs crawl out of miniature pitcher plants on Borneo (*Amphibia: Anura: Microhylidae*). Zootaxa. 2010; 2571(1):37-52.