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Prey composition of *Utricularia striatula* Sm. (Lentibulariaceae): Lithophytic carnivore Southern Western Ghats, India

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Abstract
The present study analysed physicochemical parameters of water samples, zooplanktons and phytoplanktons correlation coefficient in *Utricularia striatula* L. habitats with number of traps, size and feeding mechanisms of the study species. Physicochemical parameters studies showed positive relationships of micro flora and fauna distribution. The bladder content zooplankton groups of *Rotifera, Cladocera, Copepoda, Annelida, Rhizopoda*, and Insecta as well as phytoplankton members of *Bacillariophyta, Chlorophyta, Cyanophyta,* and *Euglenophyta*. *Utricularia striatula* having large and higher number of bladders, rich in animal and algal morphospecies compared to other *Utricularia* species. The present study concludes feeding ecology studies should be an integral feature of all bladderworts in conservation aspects.

Keywords: Bladderworts, prey, trap, phytoplankton, zooplankton

1. Introduction

Carnivorous plant are typically inhabitants of nutrient-poor environments, and it is well known that organic nitrogen and phosphorus are prominent targets of prey digestion. Presently there are around 600 described species of carnivorous plants from 17 genera described [1], with new species frequently being described (Clarke *et al*. 2003, Clarke and Kruger 2006; Mann 2007; Cheek and Jebb 2009; Fleischmann 2012). Of the 102 carnivorous plant species (of 7 genera) that have been evaluated by the IUCN, seven are listed as critically endangered, 11 are listed as endangered, and 39 are listed as vulnerable (IUCN 2010).

The growing awareness of the importance of carnivorous plants and many habitats for carnivorous plants are threatened by various factors such as agriculture, pesticide, deforestation, drainage, eutrophication, and fire suppression that has come to notice, have given an unprecedented impetus for monitoring and conservation [8, 9, 10]. Until now, most of scientific efforts have been focused mainly on finding the best methods for the efficient protection of a given species recovery plans [11, 12]. Consequently, the conservation of carnivorous plants could benefit humans [13]. Species-targeted conservation is widely implemented all over the world to protect the most interesting carnivorous plants. A variety of recovery approaches and techniques both *in situ* and *ex situ*, have been proposed and implemented for conservation of carnivorous plants.

Bladderworts grow in such a diversity of habitats [14]. They are effective predators of aquatic invertebrates [15, 16, 17] with up to half of the total Nitrogen in *U. vulgaris* being derived from insects [18]. In addition, the aquatic carnivorous plant genus, *Utricularia*, consumes mosquito eggs and larvae [19, 20], and even regularly depredates human *schistosome, miricidia* and *cercariae* [21]. The present study mainly focused on prey composition, the size of the prey, and traps, feeding biology and habitat ecological aspects in conservation activities.

2. Materials and Methods

2.1 Study Area

The Kalakkad-Mundanthurai Tiger Reserve (KMTR) is geographically located at 8° 20´ to 8° 53´ N latitudes and 77° 10´ to 77° 35´ E longitudes in Tirunelveli and Kanniyakumari districts of Tamil Nadu covering an area of 895 km^2 in the Agastiyamalai Biosphere Reserve (2000 km^2) in the Southwestern Ghats of India (Figure 1).

The climate is hot and dry at lower elevations and cool at higher elevations with heavy rain received from northeast monsoon (October to January) and southwest monsoon (June to August). Depending on elevation, the annual rainfall ranges from 1500 to 3400 mm and the temperature varies from 9 to 27°C.

2.2 Study species

Utricularia straitula usually annual small herbs, found in wet rocky slopes and tree trunks, rhizoids up to 2.5 cm long, simple few or altogether absent. Foliar organs simple and obovate shape, veins are dichotomously branched, scape base, scattered on stolons, expanded 5-7 mm. Stolons up to 9 cm, simple, rarely branched and several traps attached serial manner. Traps up to 0.6 - 1.5 mm across, globose to ovoid mouth appendages glandular hairy and divergent lip. Racemes up to 3-6 cm long and erect, up to 10 flowers. Corolla vary in colour, 3 lobed or 5 lobed, often yellow blotched in throat near base, upper lib semi orbicular 0.5-3 mm, lower lib up to 3-5x3-10 mm, spur up to 1-8 mm long, Stamens up to 1-2 mm long, Pistil up to1-3 mm long, Capsules1.5-3.5 mm long and obliquely ovoid, Seeds 0.3 0.4 mm clavate and cylindrical shape attached radially to placentum. Flower and fruiting in July to December (Figure 2).

2.3 Scanning electron microscopic studies

Fresh traps were collected and fixed with formalin–propionic acid–alcohol (FPA) solution finally dehydrated specimen was mounted on carbon stubs, coated with gold ion sputter, specimens were examined and photographed with Hitachi S-2400 model Scanning Electron Microscope (SEM) and studied for Trap and gland terminology [22, 23, 14, 24].

2.4 Physicochemical analysis

The physicochemical analysis for water like pH, total PO_4, Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- were analysed standard procedure [25, 26, 27]. All the statistical analysis was performed using SPSS statistical software 24.0 version of IBM SPSS Statistics.

2.5 Observation and identification of prey and microalgae communities

The micro flora and fauna were sampled immediately adjacent to the sites of plant collection. In June, water samples of from 500 ml to 1000 ml were obtained with a plastic container from within dense colonies of *Utricularia straitula*. The different samples were combined to from 15 ltr. sample that was then concentrated (to about 50 ml) alive by gradually filtered through a 40 µm mesh net. In October, another method was used for collection samples that permitted a high number of organisms to be collected. Specifically, the two types of samples were collected (submerged and floating) each 15 ltr. the study sites and filtered with two types of mesh sizes (< 150 µm and 40 - 150 µm). The collected micro flora and fauna were preserved in a neutral formalin solution. After screening and identification of the organisms, their densities and biomasses were calculated and photographed under Motic Stereo-Zoom Microscope, using an image analysis method [28].

2.6 Trap samples study

For traps, individually dispersed along the stem without any visible structural hierarchy, 10 traps were randomly selected and removed, from 5-10 stolons, in August and October respectively for their morphological analysis and pooled for the study of the prey predation. To do this, each trap from each subsample was opened under a binocular loupe, and all the complete or partially-digested organisms were individually examined under the microscope [29].

3. Results

Physicochemical characteristics of cold water streams revealed that the water temperature range from 18 to 26º C, pH ranged from 7.2 to 8.0, dissolved oxygen from 7.0 to 9.0 mg L^{-1}, alkalinity (carbonate from 0.2-4.0 mg L^{-1}, bicarbonate 0.6 to 2.9 mg L^{-1}, nitrate from 0.18 to 0.20 mg L^{-1}, phosphate 0.22 to 0.2 mg L^{-1}, chloride 0.0 to 6.0 mg L^{-1}, and the maximum range of nutrients such as calcium, magnesium, sodium and potassium has been observed in all sample sites (Table 1).

Scanning electron microscopic studies revealed the traps (0.6-1.2 mm size) have lateral mouth type and expanded upper lip with radiating gland-tipped hairs present in the *U.straitula*. There are three types of external glands: type 1 glands with the domed terminal are on the outer lateral surface, type 2 glands with terminal round, which are around the trap entrance, type 3 glands are on the valve. There are internal glands are present in the two sites, threshold glands are bifids and the chamber glands are quatrifids (Figure 2).

During post monsoon season (October 2016), collected a total of six groups and 20 dominant zooplankton species were identified; 6 of them Rotifera, 4 to Insecta, each three species to Copepod and Rhizopoda, each two species to Cladocera and Ostracoda (Table 1; Figure 3). Similarly, 4 dominant phytoplankton groups were collected, and 18 species identified; viz. 7 of them Chlorophyta, 6 species to Bacillariyophyta, 3 species to Cyanobacteria and 2 species to Euglenophyta (Table 1; Figure 4). The highest number of species was obtained in the samples from (14 species) Kouthalai and (16 species) Upper Kodayar region. We identified six groups of zooplankton (75%) and four groups of phytoplankton (72%) trapped in collected *Utricularia* bladders. Collected traps were dissected out under the microscope and identified the dominate prey compositions are (Rotifera, Insecta and Copepoda) zooplankton communities and indicate the phytoplankton communities were low level in running stream water (Table 2).

Table 1: Morphotype richness of phytoplankton and zooplankton by group in *Utricularia striatula* habitat and traps (August 2016)

Groups	Total Morphotype (habitat)	*Utricularia striatula* (Traps)
Zooplankton		
Rotifera	6	4
Ostracoda	2	2
Copepoda	3	2
Rhizopoda	3	2
Cladocera	2	1
Insecta	4	4

Total	20	15
Phytoplankton		
Cyanobacteria	3	2
Chlorophyta	7	6
Bacillariophyta	6	4
Euglenophyta	2	1
Total	18	13

Table 2: Correlation coefficient between total micro (flora/fauna) and Physic-chemical parameters of *Utricularia striatula* habitats in KMTR (August 2016)

	Temp. °C	**pH**	**C**	**DO**	**PO_4**	**Na^+**	**K^+**	**Mg^{++}**	**Ca^{++}**	**Cl^-**	**TZ sps.**	**TP sps.**
Temp. °C	-											
pH	-0.321	-										
C	-0.156	-0.624	-									
DO	0.818	-0.462	-0.212	-								
PO_4	-0.497	-0.632	0.724	-0.062	-							
Na^+	-0.427	-0.615	0.872	-0.302	0.142	-						
K^+	-0.451	-0.720	0.926	-0.192	-0.236	-0.381	-					
Mg^{++}	-0.524	-0.662	0.748	-0.085	0.548	-0.423	0.482	-				
Ca^{++}	0.512	-0.315	0.228	-0.046	-0.241	0.126	-0.194	0.614	-			
Cl^-	0.502	-0.704	0.782	-0.038	-0.052	-0.058	-0.028	-0.040	0.024	-		
TZ sps.	0.071	0.380	-0.712	0.026	0.184	-0.025	-0.185	0.124	0.142	-0.748	-	
TP sps.	0.713	0.842	-0.985	0.034	0.342	-0.076	0.314	-0.243	0.268	-0.978*	0.484	-

C=carbon; DO=dissolved oxygen; TZ sps.=total zooplankton species; TP sps.= total phytoplankton species.

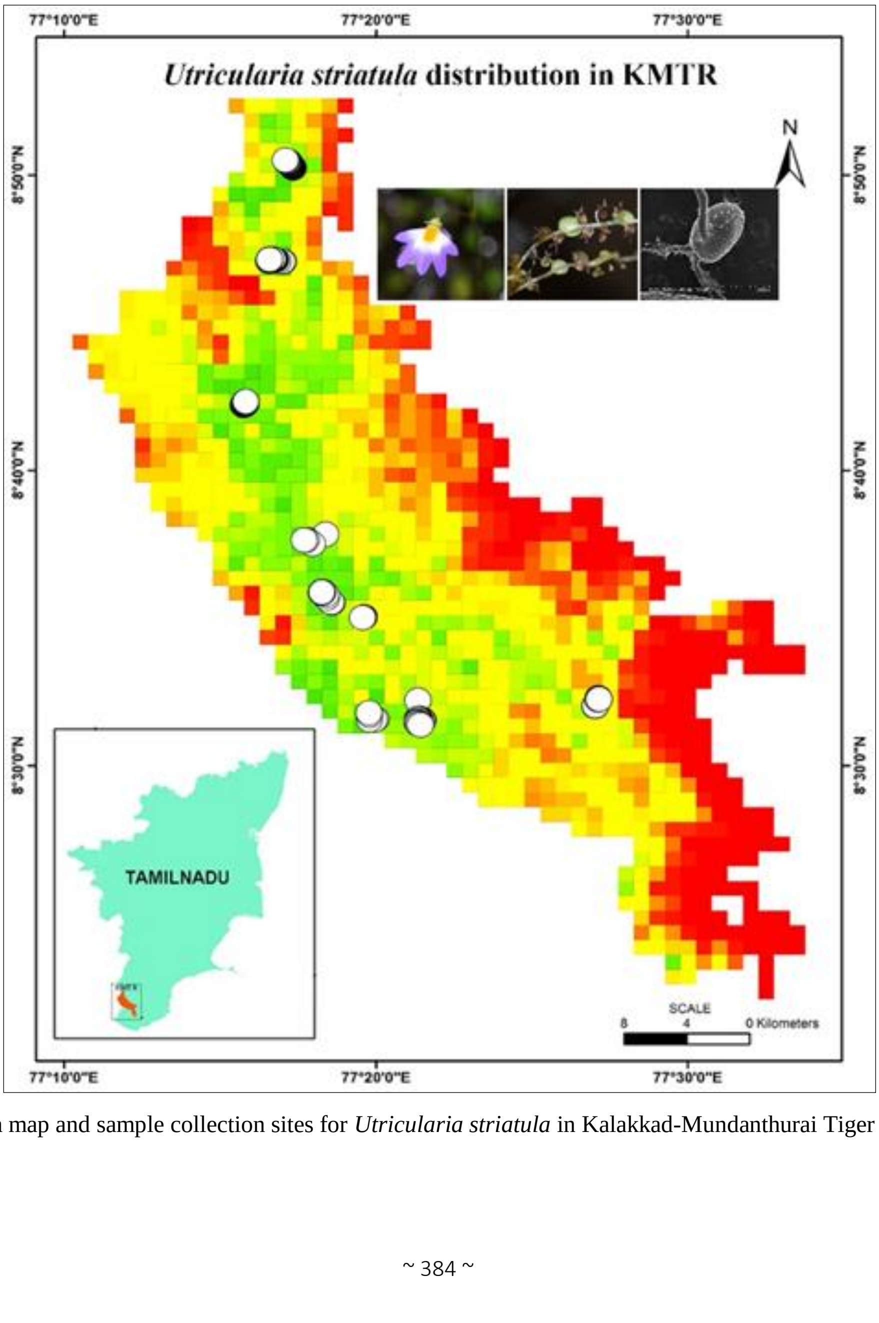

Fig 1: Distribution map and sample collection sites for *Utricularia striatula* in Kalakkad-Mundanthurai Tiger Reserve (KMTR).

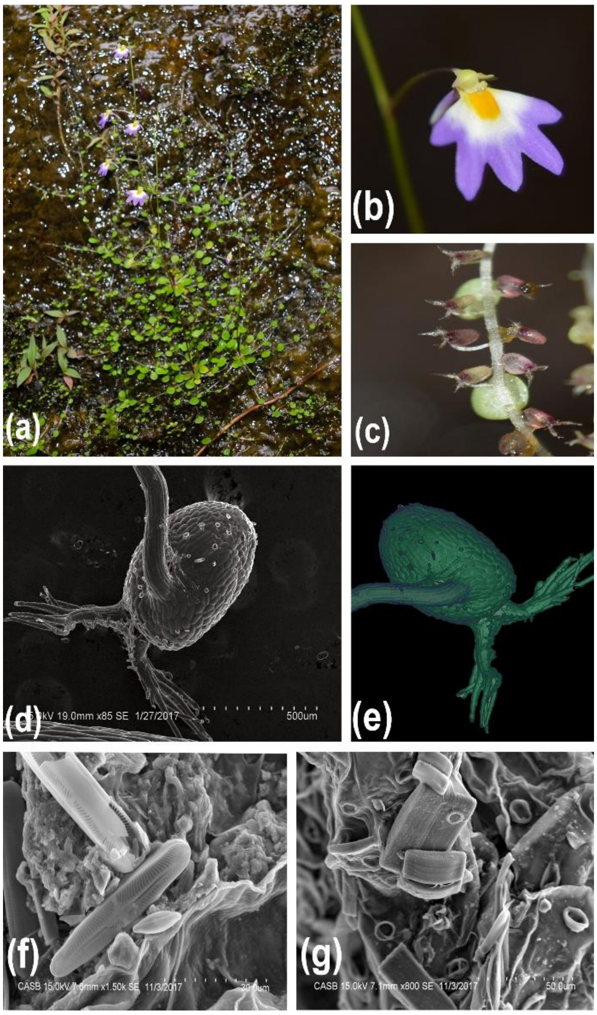

Fig 2: *Utricularia striatula*: (a) habit (b) flower (c) stolon with traps (d) SEM: Trap (original, 500 μm) (e) Trap (pseudo-colour) (f)-(g) Diatom species inside the bladder (original, 30 and 50 μm).

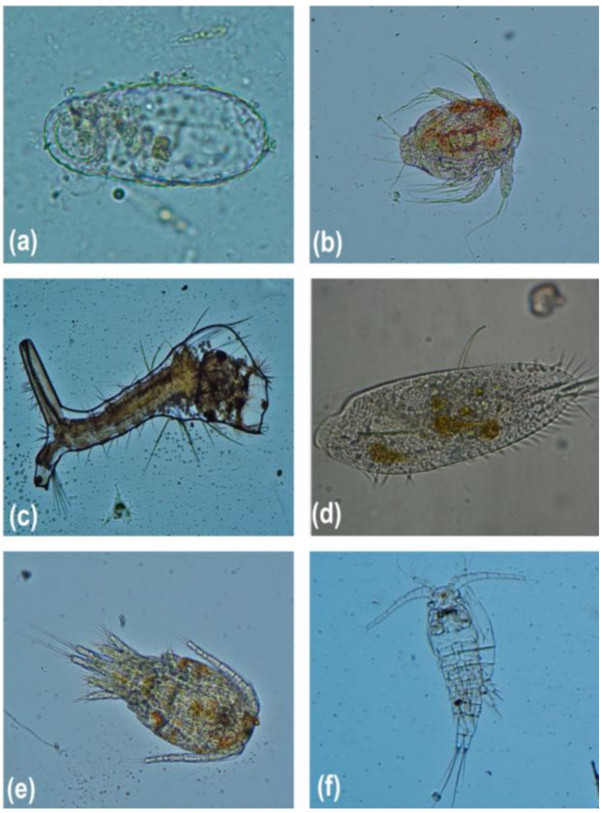

Fig 3: Zooplanktons observed *U. striatula* traps: (a) Mollusca sps. (b) Diaphanosoma sps. (c) Mosquito larvae, (d) Paramecium (e) Halicyclops (f) Mesocyclops.

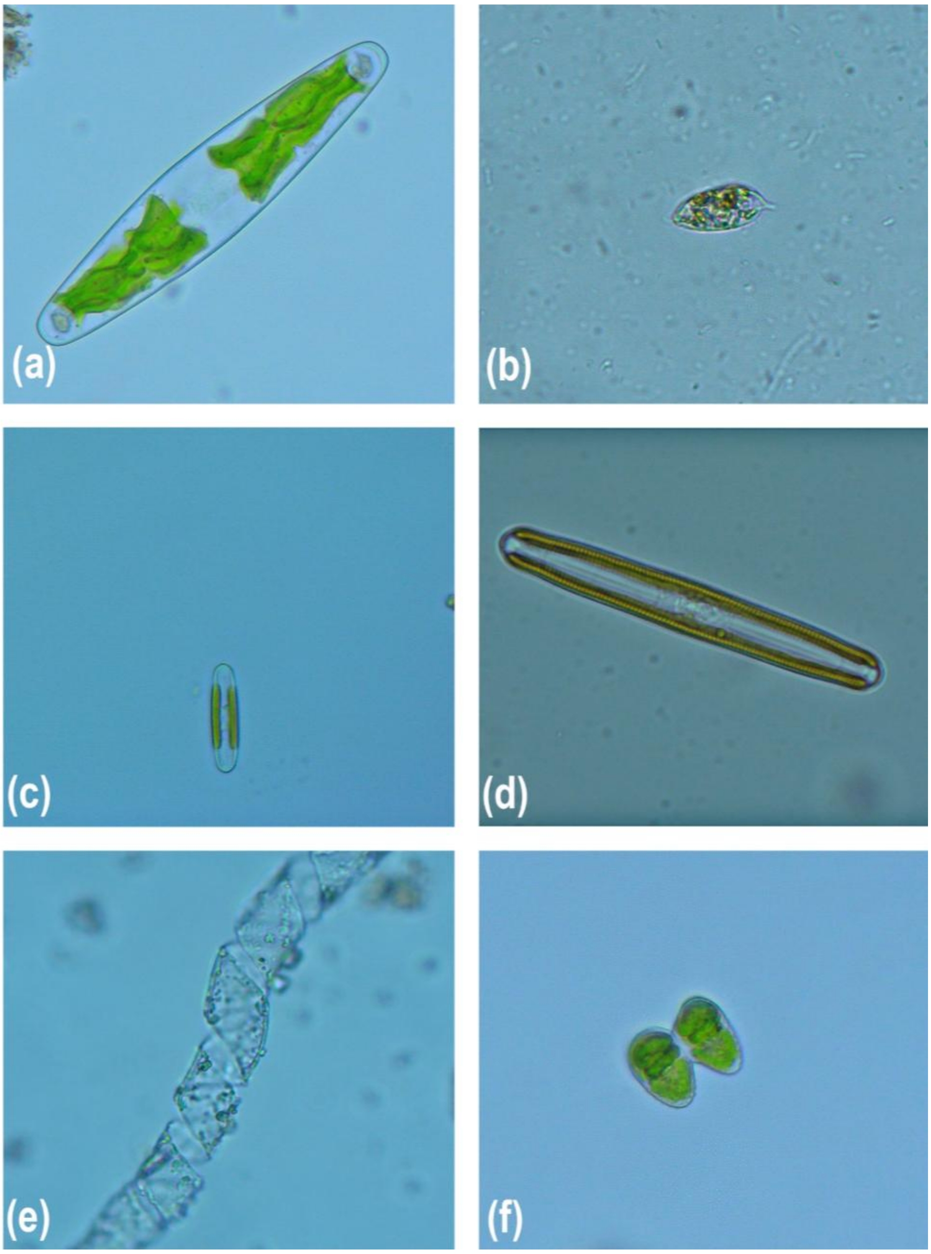

Fig 4: Phytoplanktons observed *U. striatula* traps: (a) Closterium sps. (b) Euglena sps. (c) & (d) Navicula sps. (e) Spirogyra sps. (f) Coelastrum sps.

4. Discussion

The physicochemical parameters showed positive correlation of zooplankton and phytoplankton distribution of the study area in high elevation streams and indicate that *U. striatula* benefits from nutrient-rich waters by uptake of inorganic nutrients from the water, by the production of more traps per unit of shoot length, and by the capture of more prey particles per trap, as nutrient-rich waters harbour more prey organisms.

Prey capture does not compensate completely for an oligotrophic substrate. On the contrary, *Utricularia striatula* benefits both from high nutrient concentrations in the water and from successful prey capture. Thus, the benefit gained from prey is much higher in mesotrophic than in oligotrophic waters. The contribution of zooplankton to the nutrition of the plant is comparable with the benefit gained from phytoplankton. Whereas animals are the main source of Nitrogen, other nutrients or growth factors are derived from prey animals and algae [30, 18].

According to Sharma and Diwan [31] and Arora and Mehra [32] there are 500 species rotifers in Indian water bodies, although only 330 species belonging to 63 genera and 25 families have so far been authenticated. Feeding rate of the *Utricularia* species mainly depend upon zooplankton and phytoplankton maximum levels of habitat structure [33, 34]. In the present study 6 groups of zooplankton (Rotifera, Ostracoda, Copepoda, Rhizopoda, Cladocera and Insecta) and four groups of phytoplankton (Cyanobacteria, Chlorophyta, and Bacillariyophyta and Ugelenophyta) were observed. Similar observation found in the dominant prey species (Oscillatoria and Desmidiaceae) move by gliding, or they float (Anabaena and Zygnemataceae) between the plants without being attached [35].

Epiphytic species (Gloeotrichia, Characiopsis, Tribonema and Oedogonium) were commonly found in the periphyton, but less frequent as prey objects. Zooplankton (Copepods, Mosquito larvae and Cladoceron) and actively swimming species [36] occurred commonly in *Utricularia* traps and also Euglena, which is trapped frequently by *U. uliginosa* [37, 38, 39]. A similar type of observation made by [40, 41] in the phytoplankton and zooplankton diversity in Southern Western

Ghats and Himalayas high altitude mountain fresh water streams.

The research should complement this study by including aspects like a seasonal variation in prey composition and a comparison of prey diversity in different sites. Also, available and total macronutrient concentration should be evaluated because of the huge difference between them in the water and soil [42], bladderworts of the *Utricularia straitula* species may prey upon different organisms in different study habitats [36]. The section *Phyllaria* is represented by eight species in the Himalaya and adjacent western China but one of these *Utricularia striatula*, is successfully widespread species because of their trap size was (0.6 mm - 1.2 mm) and suitable prey size (1mm), compared to the other *Utricularia* species [14, 43].

5. Conclusion

It is concluded that these plants are prime candidates for further research on the complexities of vegetable physiology associated with carnivory, metagenomic surveys of trap microbial communities, novel plant nitrogen/nutrient utilization pathways, the ecology of prey attraction, whole-plant and trap comparative development, and, finally, the evolution of the minimal angiosperm genome.

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