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Herbivore Damage to Ferns Caused by a Chrysomelid Beetle from Lower Gangetic Plains of West Bengal, India

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Abstract.—This paper records the occurrence of a polyphagous beetle, *Schenklingia bhaumiki* (Coleoptera: Chrysomelidae), feeding on ten fern species growing in the Lower Gangetic Plains of West Bengal, India viz., *Christella dentata*, *Ampelopteris prolifera*, *Cyclosorus sp.*, *Pteris vittata*, *Nephrolepis cordifolia*, *N. exaltata*, *Adiantum philippense*, *Drynaria propinqua*, *Pyrrosia adnascens* and *Phymatosorus scolopendria* for the first time. The adult beetles are leaf surface scraper and skeletonize the lamina. The larvae are leaf miners and produce linear–blotch mines between the epidermal layers with continuous spiral black frass. Young leaves of all ten species of ferns are significantly less damaged than mature ones indicating that both the adults and the larvae attack leaves of all ages. Herbivore damage of the beetle infested ferns ranged from 1.94% to 25.47% and 2.68% to 54.86% for scraping feeding and mining feeding respectively. Among the host ferns, the members of Thelypteridaceae viz., *Christella* (Scraping feeding 25.47%; mining feeding 54.86%), *Ampelopteris* (Scraping feeding 24.10%; mining feeding 53.60%) and *Cyclosorus* (Scraping feeding 16.06%, mining feeding 27.12%) suffered maximum herbivore damage. Interspecific variation of plant size and biogeographic range of the fern species are not related to herbivore damage. Insects may perhaps attack fewer ferns than angiosperms, but there is no evidence that ferns are generally less damaged than angiosperms.

Pteridophytes, especially ferns in a broad sense, are among the most primitive land plants and are generally considered to be difficult plants for herbivores to exploit (Eastop, 1973; Hendrix, 1977, 1980; Cooper-Driver, 1978; Soo Hoo and Fraenkel, 1964; Kaplains *et al.*, 1967). This underutilization of ferns by herbivores has been attributed to host resistance factors such as texture (Soo Hoo and Fraenkel, 1964), toxins (Muenscher, 1939), amino acid deficiency (Smith and Agiza, 1951), poor nutritional composition (Moon and Pal, 1949), and the presence of cyanogens (Lawton, 1976) and thiaminase (Somogyi, 1949). Although it has also been suggested by Auerback and Hendrix (1980), Balick *et al.* (1978) and Gerson (1979) that this assumption may not be well founded and may be due to less documentation of herbivory on ferns. Fossilized ferns showing damage attributed to herbivores are known from the Carboniferous (Smart and Hughes, 1973) to the Upper Triassic (Ash, 1999, 2000). Recent work on fern herbivory, however has revealed that a fairly large number of insects of different groups efficiently utilize fern hosts for their growth and development (Mound, 1967; Room *et al.*, 1981; Ottosson and Anderson, 1983; Lawton and MacGarvin, 1985; Mohan-Daniel and Chandrasekar, 1986; Misra *et al.*, 1986; Kraus *et al.*, 1993; Bera *et al.*, 1994; Bera and Ghorai, 1995a, b, 1997a, b, 1999a, b; Gilman and Cooper-Driver, 1998; Pemberton, 1998; Patra and Bera, 2002; Bera *et al.*, 2003; Mehltreter and Tolome, 2003, Barker *et al.*, 2005). The objective of this study was to contribute new data to the field of insect-fern interactions and to demonstrate that although insects may attack fewer ferns than angiosperms, ferns are not totally free from insect attack. This paper presents a first report of interactions between a chrysomelid beetle and ferns in India.

Materials and Methods

Beetle-fern interactions were recorded from ten different sites in the lower Gangetic Plains of West Bengal (21°25′–26°50′ N, 86°30′–89°85′ E), India during May 1999–December 2000. Field photographs were taken while the adult beetles and their larvae were feeding on the ferns, at which time they were caught alive, kept in small vials, and then sent to the Coleoptera Department, Zoological Survey of India, Calcutta, for identification. Infested host ferns were preserved on herbarium sheets numbered (CU/ IFI 1–11) and deposited in the pteridophyte repository, Department of Botany, Calcutta University, Calcutta.

Feeding habit, occurrence and abundance of the beetle on different fern hosts were studied directly in the field. Abundance and numbers of beetles per leaf were estimated by counting the number of adult beetles present on each leaf of the host ferns once a week during each month of the study period. Mean values of beetles/leaf/month were calculated for each species. Monthly average temperature and rainfall for the study area and mean number of beetle/leaf/month were graphically represented to establish the influence of environmental parameters on the seasonal abundance of the beetle. Herbivore damage in terms of leaf area lost and and total leaf area were estimated by placing individual leaflets on millimeter graph paper, tracing the outline of both the leaflets as well as their damaged areas and then counting the included squares. Finally, mean herbivore damage as a percentage of leaf area was calculated. In each case a minimum of 50 plants were studied. Herbivore damage in young and mature leaves were compared using student's t- test. Correlation coefficients were used to establish the relationship between leaf age and herbivore damage. Measurement of the longest leaf, which we considered as a measure of the plant size, was also taken. Leaves of less than 3 months were considered to be young whereas those of more than 3 months were considered mature. In order to test whether the beetle and its larvae fed only on ferns as opposed to angiosperms, a feeding trial was conducted. Adult beetles and their larvae were exposed to randomly selected terrestrial and aquatic ferns and angiosperms growing in the study area. These feeding trials were conducted on three occasions for a minimum of seven days in nylon gauze cages enclosed in perforated polythene sheets. Control sets with beetles and their recorded host ferns were also maintained.

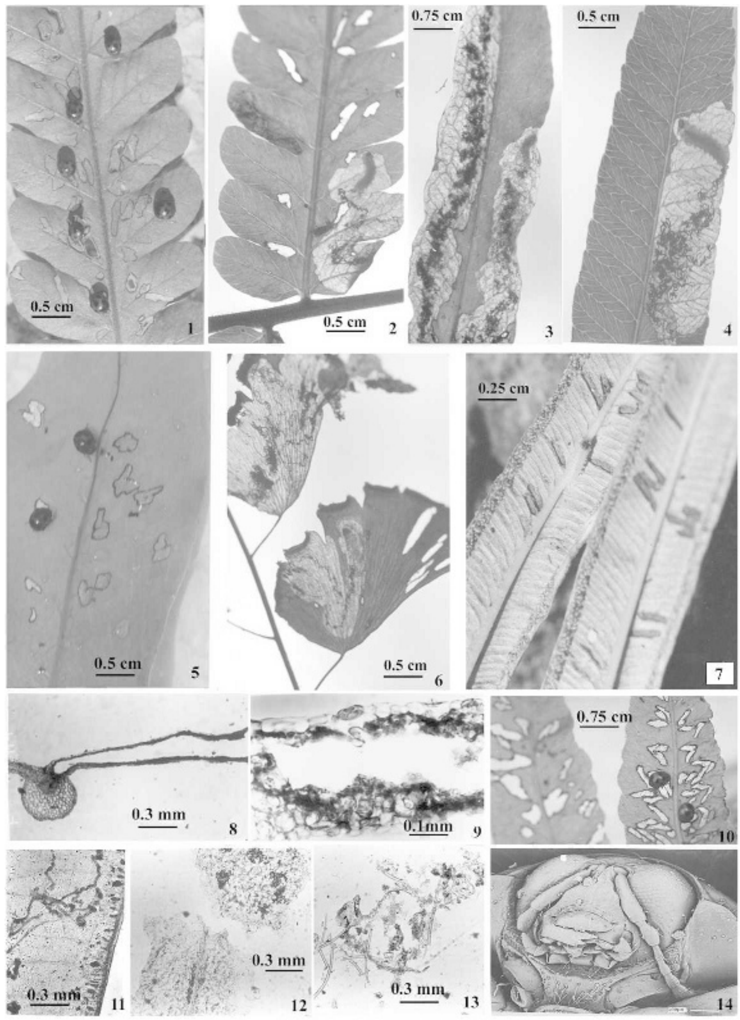
For scanning electron microscopic studies the beetles were dried, coated with gold and scanned using a Leica Leo S-440 electron microscope and photographed with a High Resolution Record Unit (HRRU) using 35 mm automatic camera. Protein, total amino acids, total sugar, and total phenol of young and mature leaves were estimated following the methods employed by Lowry *et al*. (1951), Lee and Takahasi (1966), Somogyi (1945), and Malik and

Singh (1980) respectively. Fecal pellets, frass, of feeding insects, were dissolved in a small amount of water to form a suspension. Drops of this suspension were mounted in glycerin jelly and examined under the microscope for residual undigested plant parts.

Results

The adult beetles. and larvae found feeding on different species of ferns of Lower Gangetic plains of West Bengal were identified as *Schenklingia bhaumiki* Basu and Sengupta.(C. R. Basu collection 18.iv. 1976, ZSI, Calcutta). Csiki and Heikertinger (1940) proposed the name *Schenklingia* for *Eucycla*. The genus has 25 species distributed in India, Sri Lanka, Taiwan, Indonesia, New Guinea, Solomon Island and Micronesia (Basu and Sengupta, 1981). The Indian species of the genus include *Schenklingia bhaumiki*, *S. heteropunctata* and *S. himalayensis* (Basu and Sengupta, 1981). The adult beetles are small (2.25 mm in length, 1.90 mm in width), somewhat rounded, strongly convex, shiny, and reddish brown with 5 pairs of black spots on the elytra (Basu and Sengupta, 1981). Earlier, Basu and Sengupta (1981) collected adults of the *Schenklingia bhaumiki* for their taxonomic studies from ferns in Darjeeling (West Bengal) and Sikkim Himalayas. There are three larval instars but the external morphologies of the larvae are more or less the same across all instars. Full grown larvae are 7×1.5 mm, considerably flattened and show characteristics of leaf miners. They have a dark head, and strong, well developed mandibles, each with two sharp chitinous blades. The body is yellow with transverse furrows and ridges. The larval period is 42 days and the third instar larva is pupated on leaflets and lasts up to 14 days.

The adult beetles are host surface scrapers and the damage they cause is very distinctive. They are confined primarily to the central portions of the fronds and tissues between the veins are selectively eaten, partially skeletonizing the lamina, leaving it riddled with holes (Figs. 1, 2, 5 & 10). The upper and lower leaflet surfaces of the host ferns are indiscriminately scraped by the adult beetles (Fig. 14) leaving the veins intact. Subsequent to the removal of some of the laminar tissue, much of the remaining tissue becomes necrotic and brown, giving the infested region a distinctive pattern (Figs. 1, 2, 5, 7 & 10). The beetle larvae are leaf miners, and eat tissue between the upper and lower epidermis. The damage appears as linear-blotch mines with continuous spiral black frass trails (Figs. 2, 3, 4 & 6). Initially the mines are about 1.2 mm–1.5 mm broad, but as the larvae grow, the trails gradually become broader (10 mm–15 mm or more). A mine constructed by one larva is generally confined to one side of the midrib (Figs. 3 & 4) but occasionally may cross it. Inside the mine, the larvae are capable of moving rapidly backward and forward and when removed from the mine, they are capable of re-entering the leaflet. They are leaf defoliators and may consume the whole laminar tissue of the frond leaving only a light-colored, wrinkled upper and lower epidermis behind (Figs. 3, 4, 6, 8 & 9).



FIGS. 1, 5, 10. Beetle infestation on *Christella dentata*, *Drynaria propinqua* and *Ampleopteris prolifera*. Figs. 2, 3, 4, 6. Linear blotch mines showing continuous spiral black frass on the leaflets of *Christella dentata* (fig.2), *Drynaria propinqua*(fig.3), *Ampelopteris prolifera*(fig.4) and *Adiantum philippense*(fig.6) caused by beetle larvae. Fig. 7. Feeding scars on the abaxial leaflet surface of *Pteris vittata*; note larva on the costa. Figs. 8, 9. Sections of mined leaflets from *Pteris vittata* and *Drynaria propinqua* showing loss of mesophyll tissue. Fig. 11. Mined leaflet of. *Drynaria propinqua* with continuous spiral black frass deposited by larvae. Figs. 12, 13. Fecal matter from the mining larvae and adult beetles showing undigested remains of mesophyll tissue and acicular trichomes with fragments of laminar tissue respectively. Fig. 14. SEM *Schenklingia bhaumiki* showing mouthparts of adult beetle.

Young leaves of the ten fern species were significantly less damaged than mature leaves in both scraping and mining feeding ($t > 3.44$, $P < 0.05$; $t > 3.83$, $P < 0.05$; respectively) (Table 1). The occurrence and abundance of the beetle was purely seasonal. They started appearing in June, became very abundant

TABLE 1. Herbivore damage (%) of young and mature leaves of ten Indian fern species of Lower Gangetic Plains of West Bengal fed by the adult beetle *Schenklingia bhaumiki* (scraping feeding) and its larvae (mining feeding), expressed as percent leaf area loss. Data are mean ± standard errors, N = 50.

	Scraping feeding		Mining feeding	
Species	Young	Mature	Young	Mature
1.*Christella dentata*	2.69 ± 0.2	25.47 ± 1.66	2.95 ± 0.2	54.86 ± 0.7
2.*Ampelopteris prolifera*	1.35 ± 0.1	24.10 ± 1.7	2.12 ± 0.3	53.60 ± 0.2
3.*Cyclosorus sp.*	0.36 ± .02	16.06 ± 1.2	0.69 ± 0.03	27.12 ± 0.6
4.*Adiantum philippense*	Not found	13.22 ± 1.2	Not found	23.19 ± 0.1
5.*Pteris vittata*	0.53 ± .05	7.07 ± 0.7	1.21 ± 0.1	19.37 ± 0.9
6.*Nephrolepis cordifolia*	0.36 ± .01	8.25 ± 1	0.53 ± .02	14.72 ± 0.1
7.*Nephrolepis exaltata*	0.35 ± .01	6.15 ± 1.2	0.35 ± 0.04	9.25 ± 0.2
8.*Pyrrosia adnascens*	0.27 ± .01	3.5 ± 1	Not found	Not found
9.*Drynaria propinqua*	0.29 ± .04	2.79 ± 1	0.50 ± .05	22.55 ± 0.1
10.*Phymatosorus scolopendria*	Not found	1.94 ± 0.3	Not found	2.68 ± 0.2

from July to mid August, decreased in numbers from the end of August, and completely disappeared by December. This seasonal behavior of the beetle was found to be correlated with atmospheric temperature and precipitation (Fig. 15). The number of adult beetles per leaf was high in July–August when monthly average temperature (mean maximum) and precipitation were 25°C–31°C and 14.1 mm respectively. With the decrease of atmospheric temperature and precipitation in the subsequent months the number of beetles per leaf

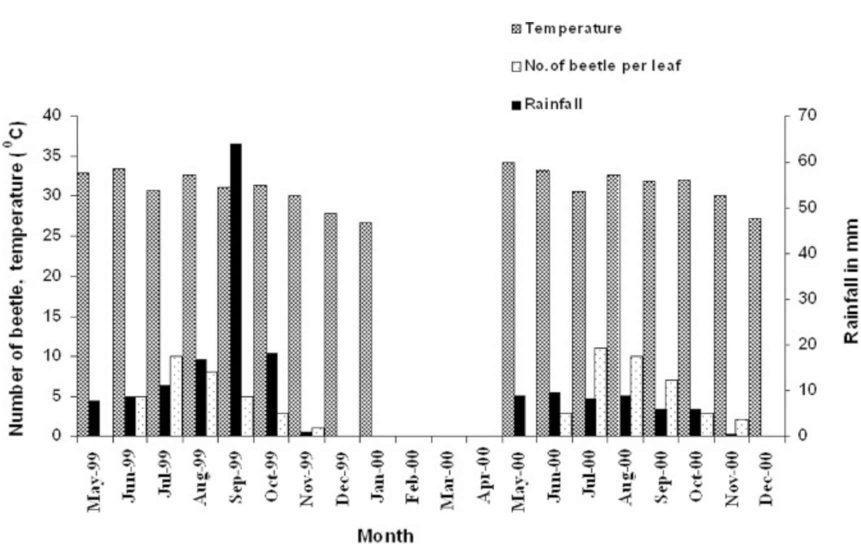


FIG. 15. Correlation between seasonal abundance of adults of *Schenklingia bhaumiki* on the most preferred host fern *Christella dentata* with mean maximum temperature and average monthly rainfall in Kolkata, India (1999–2000).

decreased and completely disappeared in December when atmospheric temperature felt sharply to 22°C and precipitation became almost nil.

The interspecific variation of plant size was not correlated with herbivore damage to the host ferns (r = − 0.07) (Table 3). The beetle was specific to ferns and fed exclusively on selected species of ferns of Lower Gangetic Plains of West Bengal. No feeding occurred on aquatic ferns (*Marsilea sp.*, *Azolla sp.*, *Salvinia sp.*) or angiosperms (*Oryza sp.*, *Colocasia sp.*, *Solanum sp.*, *Lantana sp.*, and *Parthenium* sp.) growing in association with the host ferns, when tested in vitro where the beetles and larvae were provided with only single food sources.

Biochemistry of the fern frond varied quantitatively with its age. The results of the present investigation showed that young leaves of the most preferred (*Christella*, *Ampelopteris*, *Cyclosorus*) and the least preferred (*Phymatosorus*) host fern species have higher amounts of phenol, sugar, and protein than in mature leaves. Total amino acid levels ran in the reverse direction (Table 4). Analysis of fecal pellets of adult beetles showed the presence of undigested remains of cuticle, epidermal hairs, and mesophyll tissue (Fig. 13) whereas analyses of larval frass showed the remains of mesophyll tissue only (Fig. 12).

Discussion

Strong and Levin (1979) hypothesized that ferns are subject to less herbivory than angiosperm herbs, shrubs and trees. If ferns are less frequently attacked by herbivores, it can be hypothesized that they should show less damage than angiosperms. The results of the present study do not support this hypothesis. In the present study, leaf damage in ten fern species ranged from 1.94% to 25.47% and from 2.68% to 54.86% for scraping and mining feeding activities respectively. These data are comparable to reported levels of leaf damage on angiosperms of 10.9% (Coley and Aide,1991) and on ferns of 5.8, 6.1, 11.1% (Mehltreter and Tolome, 2003), and 38% (Balick *et al.*, 1978). Although these variations in reported herbivore damage may be a consequence of differences among species in plant phenology, changes in herbivore pressure at different study sites, or diverse applied methodologies, the general hypothesis that ferns are generally less damaged by herbivores than angiosperms lacks evidence.

The herbivores caused more damage to mature fern fronds than young ones. Ottosson and Anderson (1983) and Lawton (1976) suggested that nutritional quality and levels of plant protection compounds are lower in mature fronds than in young fronds. When phenol is sufficiently synthesized it combines with sugars form tannins. These tannins combine irreversibly with available protein to form indigestible complexes, which result in nutritional deficiency of the host plants. This in turn affects the percentage of infestation and host preference (Raman and Ananthakrishnan, 1986). In the present study, a higher abundance of beetles and a higher herbivore damage were recorded on *Christella dentata* than on *Phymatosorus scolopendria* (Table 1 & 2). High concentrations of phenol and sugar, along with available protein, in *Phymatosorus* appear to act as repellent to beetle infestation. This interspecific

TABLE 2. Feeding behavior of the adult beetle on different species of ferns in West Bengal, India. Data are mean ± standard deviation.

	Host	Feeding surface	Insect abundance/leaf
Thelypteridaceae	*Christella dentata*	Upper	5.6 ± 3.2
	Ampelopteris prolifera	Upper	4.4 ± 2.6
	Cyclosorus sp.	Lower	3.9 ± 2.3
Adiantaceae	*Adiantum philippense*	Upper	2.8 ± 1.5
Pteridaceae	*Pteris vittata*	Lower	2 ± 1
Nephrolepidaceae	*Nephrolepis cordifolia*	Upper	1.7 ± 1.09
	Nephrolepis exaltata	Upper	1.3 ± 1.1
Polypodiaceae	*Pyrrosia adnascens*	Upper	1.5 ± 0.7
	Drynaria propinqua	Lower	1.08 ± 0.64
	Phymatosorus scolopendria	Lower	1 ± 0

variation in biochemistry makes *Christella* and *Phymatosorus* the most and the least preferred hosts, respectively. Similarly, the higher levels of herbivory in mature fern fronds may be attributed to differences in biochemical composition (protein, phenol and sugar) compared to young fronds (Table 4). Whereas, these data help explaine the differences in herbivory between *Christella* and *Phymatosorus*, they fail to explain interspecific variation in herbivory among the thelypterid ferns, *Christella*, *Ampelopteris* and *Cyclosorus*. Thus the involvement of certain morphological features of thelypterid ferns in the regulation of herbivory cannot be ruled out.

Two correlations between plant features and herbivore damage have been reported in literature. First, herbivore damage may increase with plant size (Marquis, 1992), perhaps because larger plants offer more resources and are easier to locate. Second, the biogeographic range of the plant species appears to affect the number of plant-animal interactions. Species with wider ranges should have more herbivores (Strong, 1979; Cooper-Driver, 1985) and consequently may suffer more damage. The present study however does not confirm these hypotheses. There is insufficient data to draw a final conclusion but comparisons of plant size, biogeographic range, and herbivore damages of the ferns in the present study show no such trends (Table 3).

Physical factors such as temperature, light, precipitation and humidity help regulate insect abundance because insects are cold-blooded animals and their growth, development, occurrence and abundance are largely dependent on these physical factors. In the present study, the number of beetles per host was higher during the rainy season, July to August, when the atmospheric conditions were amenable to their growth and development but insect numbers gradually declined at the end of rainy season. This study showed that herbivore damage is higher in thelypterid ferns (Table 1) than other host ferns of this study, perhaps due to lower phenol content, and thus may be considered to be the preferred host. From the present insect- fern interaction, it is clear that certain species of ferns are susceptible to the attack of the beetle *Schenklingia bhaumiki* and that aquatic ferns (*Azolla*, *Salvinia*, *Marsilea*) and certain angiosperms (*Oryza*, *Lantana*, *Colocasia*, *Parthenium*, *Solanum*) were

TABLE 3. Herbivore damage of mature leaflets, biogeographic range and maximum size of ferns from Lower Gangetic Plains of West Bengal, India.

Host	Herbivore damage % ± s.d.	Biogeographic range	Maximum leaf size (cm)
Christella dentata	25.47 ± 1.66	Throughout the tropics and subtropics	80
Ampelopteris prolifera	24.10 ± 1.75	India, Nepal, Bhutan, Burma, Philippines, China, South Africa, Australia, New Caledonia	68
Cyclosorus sp.	16.06 ± 1.2	India, Nepal, Bhutan, Burma, Philippines, China, South Africa, Australia, New Caledonia	90
Adiantum philippense	13.22 ± 1.2	Throughout the tropics and subtropics	40
Pteris vittata	7.07 ± 0.7	Throughout the tropics and subtropics	40
Nephrolepis cordifolia	8.25 ± 1	Throughout the tropics and subtropics	105
Nephrolepis exaltata	6.16 ± 1.2	Throughout the tropics and subtropics	132
Pyrrosia adnascens	3.5 ± 1	India, China, Formosa, Malaysia to Polynesia	13
Drynaria propinqua	2.79 ± 1	India, Burma, China, Malay Peninsula, Malesian Islands	65
Phymatosorus scolopendria	1.94 ± 0.3	India, China, Formosa, Malaysia to Polynesia	134

not susceptible to the beetle infestation. This may be due to nutritional inadequacy or to high levels of deterrent chemicals. The parameters responsible for such selective feeding of the beetle are, however, yet to be determined.

Included among the ferns studied are those with aesthetic (*Ampelopteris prolifera*, *Christella dentata*, *Adiantum philippense*, *Nephrolepis cordifolia*), food (*Ampelopteris prolifera*), and potential medicinal value (*Adiantum philippense*, *Nephrolepis cordifolia*, *Drynaria propinqua*) (Vasudeva, 1999). Young leaves of *Ampelopteris prolifera* and *Nephrolepis cordifolia* are cooked as leafy vegetables by local tribal people. The fronds of *Adiantum philippense* are used to fight fever, dysentery, asthma and bronchitis. Powdered rhizomes are used for dog bites and snakebite by local tribal people. Rhizomes of *Nephrolepis cordifolia* are mixed with water dropped from hair while bathing and administered to women orally once during their menstrual period for permanent sterility (Henry *et al.*, 1996). Thus herbivore damage to these

TABLE 4. Biochemical analysis of young and mature leaves of the most and least preferred host ferns of *Schenklingia bhaumiki*. Bracketed figures correspond to values for young leaves.

Species	Amino acids (mg/g)	Phenol (mg/g)	Sugar (mg/g)	Protein (mg/g)
Christella dentata	17.55 (13.56)	0.123 (1.45)	68.42 (76.11)	3.10 (6.25)
Ampelopteris prolifera	42.25 (32.5)	0.29 (1.55)	74.26 (91.55)	3.94 (11.36)
Cyclosorus sp.	38.11 (31.10)	0.137 (1.73)	113.5 (119.33)	2.50 (3.75)
Phymatosorus scolopendria	12.00 (18.30)	32.5 (56.0)	560 (725)	1.20 (2.50)

economically important ferns is of concern to the beneficiaries in Gangetic West Bengal, India and measures should be taken to insure their survival in the wild.

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