Haplocladium microphyllum (Hedw.) Broth. capsules as food for *Agrotis* sp. (Lepidoptera) larvae

Yuan Fang, Rui-Liang Zhu

School of Life Science, East China Normal University, Shanghai, China

Bryophytes usually have anti-feeding properties to defend against microbial and herbivore attack; however, the consumption of *Haplocladium microphyllum* (Hedw.) Broth. capsules by *Agrotis* sp. larvae is rather common in Shanghai in the spring. To test whether *H. microphyllum* is the only moss eaten, and why the gametophytes of *H. microphyllum* are not eaten, a series of quantitative experiments were carried out to understand the feeding habits of *Agrotis* larvae on the given moss materials at three growth stages of larval life. The results show that the larvae can feed on the capsules of six moss species to different degrees: *Funaria hygrometrica* Hedw., *H. microphyllum, Physcomitrium sphaericum* (C.F.Ludw. ex Schkuhr) Brid., *Trematodon longicollis* Michx., *Ditrichum pallidum* (Hedw.) Hampe, and *Pogonatum inflexum* (Lindb.) Sande Lac. The capsules of the first four species were grazed heavily by the larvae, compared with limited consumption of the latter two (*D. pallidum* and *P. inflexum*), which even induced a high mortality rate among the larvae. With the growth of the larval instar, the daily demand for moss capsules by the larvae increases gradually. The lipid content of the capsules may play an important role when the larva selects its feeding target.

Keywords: *Funaria hygrometrica*, Moss, Phenolic compounds, *Physcomitrium sphaericum*, *Trematodon longicollis*

Introduction

Bryophytes were traditionally considered to suffer rarely from herbivore and insect attack, possibly owing to having few nutrients, being tough and rich in phenolic compounds (Liao, 1993; Glime, 2006). The consumption of bryophytes by slugs (Richardson, 1981; Davidson *et al.*, 1990; Frahm & Kirchhoff, 2002; Glime, 2006), cranefly (Richardson, 1981), aphids (Gerson, 1969), pillbugs or woodlice (Glime, 2006), ants (Loria & Herrnstadt, 1980), bison (Richardson, 1981), butterflies (Mallet & Singer, 1986; DeVries, 1988), reindeer (Richardson, 1981) and lemmings (Collins & Oechel, 1974) is known mainly from cold environments, especially in arctic tundras or deserts (Richardson, 1981; Gerson, 1982; Glime, 2006). There are only a few reports of direct consumption of bryophytes in subtropical and tropical regions, e.g. thrips (Johansen *et al.*, 1983), mites (Flechtmann, 1985), pheasants (Wang *et al.*, 2011), lepidopteran larvae and micro-snails (Maciel-Silva & dos Santos, 2011).

In recent years, RLZ found that numerous capsules of *Haplocladium microphyllum* (Hedw.) Broth. on university campuses and in public parks in Shanghai, China, were missing, and only setae could be found in spring (Figure 1A and B). The disappearance of a large number of *H. microphyllum* capsules in the subtropical city aroused our great interest. The present paper aims to answer the following questions: (1) what ate the moss capsules? and (2) why were these capsules targeted as a food material?

Correspondence to: Rui-Liang Zhu, Department of Biology, School of Life Science, East China Normal University, 3663 Zhong Shan North Road, Shanghai 200062, China. Email: lejeunea@163.com

Materials and Methods

Investigated area and experimental design

In order to determine what ate the capsules, a 5 m × 5 m lawn area with rich *H. microphyllum* populations whose capsules were partially missing, was selected as an area for investigation (31°13′768″N, 121°24′209″E, alt. 12 m) on our campus. Invertebrates, seed plants, and bryophytes around *H. microphyllum* in the selected area, were investigated, collected, and brought to the laboratory for further experiments, including feeding tests, and chemical analyses. The observation that the seta apices seemed to be gnawed suggested that the most probable suspect was an invertebrate. Thus, invertebrates in the lawn around the destroyed mosses were collected. After the capsule consumer was confirmed, moss capsules of other species were offered to determine whether *H. microphyllum* was the sole

DOI 10.1179/1743282012Y.0000000008

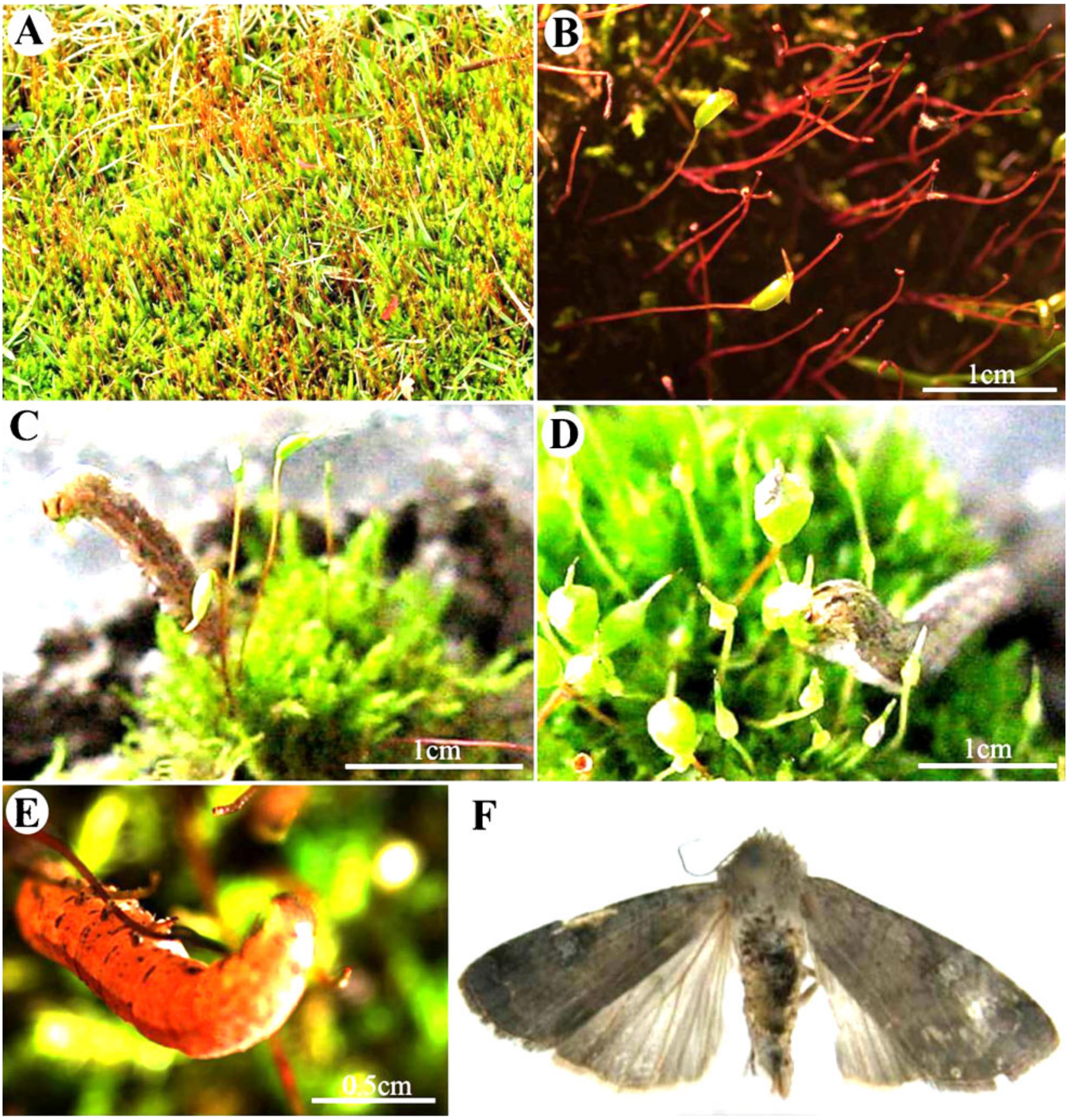

Figure 1 **(A) The sampled area with rich *Haplocladium microphyllum* populations on the campus of East China Normal University, Shanghai, China. (B) A large number of *H. microphyllum* capsules consumed by *Agrotis* sp. larvae in the sampled area. (C) *Agrotis* sp. larva consuming capsules of *H. microphyllum.* (D) *Agrotis* sp. larva consuming capsules of *Physcomitrium sphaericum.* (E) *Agrotis* sp. larva consuming capsules of *H. microphyllum.* (F) Adult insect of *Agrotis* sp., ventral view.**

moss that the invertebrate could feed on. Second, the main chemical components, soluble sugar, proteins, phenols and lipids of capsules and other parts, including setae and gametophytes were compared to reveal the main factor which may have induced consumption of the moss capsules. Besides mosses, newly fallen leaves in the investigated area were also collected, as the larvae were always hidden in them.

Plant material

Moss materials used in this research include six species: *Haplocladium microphyllum* from the selected area, *Funaria hygrometrica* Hedw. and *Physcomitrium sphaericum* (C.F.Ludw.) Fürnr. from outside the area under investigation on our campus, *Trematodon longicollis* Michx. from lower altitudes (low banks) in Zhejiang Province, *Pogonatum inflexum* (Lindb.) Sande Lac. and *Ditrichum pallidum* (Hedw.) Hampe from higher altitudes (Tianmushan Nature Reserve) in Zhejiang. All voucher specimens are listed in the appendix.

Seed plant materials, including fallen leaves of *Platanus acerifolia* (Ait.) Willd and *Ginkgo biloba* L., and fresh leaves of *Ophiopogon bodinieri* H.Lev. were collected from the selected area on our campus.

Animal material

Experimental animals of *Agrotis* sp. (Lepidoptera: Noctuidae) larvae, ant (*Camponotus tokioensis*), pill-bug (Armadillidiidae), snail (Endodontidae), slug (Philomycidae), earthworm (Lumbricidae), grubworm (Scarabaeidae), and nematode (Mononchidae) were collected from the selected area where the moss capsules were partially consumed in March and April 2009. However, the results of early feeding tests, involving all of the taxa mentioned, confirmed that the only predators on moss capsules were the larvae of *Agrotis* sp., and the other taxa were eliminated from further testing. The larvae of *Agrotis* sp. were separated into three groups, early-instar, middle-instar and late-instar, according to their head capsule width. When mortality occurred within the experimental period, additional individuals were collected from the same selected area to meet the experimental need. The voucher specimens were kept in the herbarium of the Institute of Zoology, Chinese Academy of Sciences.

Feeding tests

In order to determine whether larvae of *Agrotis* sp. are capable of feeding on other moss capsules, five other moss species with fresh and mature or almost mature capsules were offered to larvae. Thirty capsules of each moss species, including its gametophytes, were placed on the sterilized artificial soil (1 cm thick) surface of each pot. Eighteen pots were tested in total. The number of capsules consumed was counted after 24 h. The capsules of *H. microphyllum*, *F. hygrometrica*, and *P. sphaericum* were offered to larvae of different instars in order to estimate the degree to which they fed on them. The larvae were kept without food in darkness for 5 h before the experiment. Agar (1 cm thick) was placed at the bottom of each tank (15.2 cm in diameter, 6 cm in height). Each capsule was cut at the base of the seta, and then inserted into the agar with a piece of wet filter paper in each tank to avoid desiccation of the mosses and maintain the tank humidity. Forty-five tanks were tested in total. The number of consumed capsules was counted after 24 h. The whole process of consumption in the daytime was videoed and observed in the laboratory with the aid of a digital camera (Canon G9).

Chemical analysis

Comparative analyses were conducted on capsules, which were cut just below the apophysis, and the remaining setae and gametophytes. The material was washed and dried at 80°C for two days. All the material was crushed to powder with a mortar and pestle in 5 ml liquid nitrogen immediately before these analyses.

Soluble sugar in each sample was determined by anthrone-ethyl acetate colorimetry (Loewus, 1952) with sucrose as the standard. Lipids of all the samples were extracted in a Soxhlet extractor with petroleum ether for 10 hours, and the filter paper bags were weighed before and after the extraction to calculate the net lipid weight of each sample. Protein in each moss was determined by the Bradford (1976) method with bovine serum albumin (BSA) as the standard. Samples (50 mg) were treated as described by Glime (2006). The absorbance at 595 nm was measured. The total phenol determinations were made from aqueous solutions with Folin-Ciocalteus reagent, following the method of Li *et al.* (2008) with gallic acid used as the standard.

Results

What ate the capsules of H. microphyllum?

After an overnight feeding, over half of *H. microphyllum* capsules in the tank containing the *Agrotis* sp. larva were lost, whilst in the tanks with the other invertebrates *H. microphyllum* was not eaten, even when the trials were extended up to seven days to promote hunger. The larvae raised themselves among the setae, using their mandibles to check the capsules, climbed up the seta with their thoracic legs, then held the seta at the base of the capsule, and began to gnaw with their mouthparts (Figure 1C–E).

The larvae also fed on the capsules of five other moss species, *F. hygrometrica*, *P. sphaericum*, *T. longicollis*, *P. inflexum*, and *D. pallidum* (Table 1). According to the degree of larval appetite for them, the mosses could be divided into two groups. One is comprised of four moss species, *H. microphyllum*, *F. hygrometrica*, *P. sphaericum*, and *T. longicollis*, whose capsules were mostly consumed by the larvae. The other group contained *D. pallidum* and *P. inflexum*. The data on *Agrotis* sp. larval consumption of *D. pallidum* show intra-group differences, and only a few *P. inflexum* capsules were eaten (Table 1). This moss group induced high mortality of larvae (81.8%), compared with the former group where no deaths occurred.

The results of quantitative research on the degree of destruction of the moss capsules by *Agrotis* sp. larvae (Figure 2) indicate that the daily consumption

Table 1 Comparison of consumption of six moss species eaten after 24 hours by three *Agrotis* sp. larvae belonging to different growth stages

	Number of capsules		
Moss species	Total	Eaten	Remaining
Haplocladium microphyllum	30+30+30	30+30+30	0+0+0
Funaria hygrometrica	30+30+30	30+30+30	0+0+0
Physcomitrium sphaericum	30+30+30	30+30+30	0+0+0
Trematodon longicollis	30+30+30	30+30+28	0+0+2
Ditrichum pallidum	30+30+30	30+2+3	0+28+27
Pogonatum inflexum	30+30+30	5+4+7	25+26+23

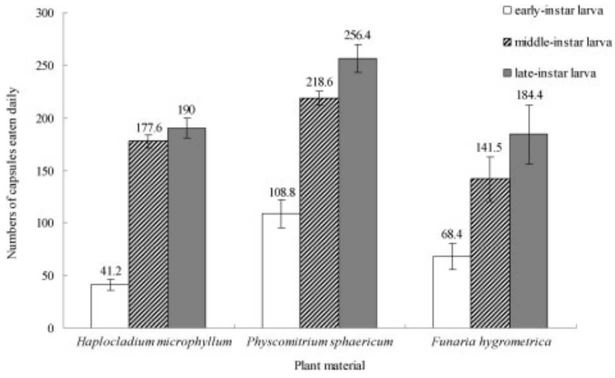

Figure 2 Mean numbers of capsules consumed after 24 hours by *Agrotis* sp. larva at three growth stages.

of moss capsules by larvae is large, even by the early-instar larvae, and increases gradually with instar growth. Each late-instar larva, kept without food in darkness for 5 h before the experiment, consumed about 190 capsules of *H. microphyllum* daily, when only *H. microphyllum* was given in the laboratory (Figure 2).

Why were the capsules of mosses treated as a food material?

The contents of lipids, soluble sugar, phenol, and protein in different parts of the moss samples are shown in Figure 3A. The lipid content of *H. microphyllum* capsules is the highest among the materials analyzed (Figure 3A). The protein content in all parts of the mosses investigated is very low, and the highest content (0.05%) is in the seta and gametophyte extract of *P. sphaericum* (Figure 3B2). The chemical analysis, however, revealed that the capsules of three moss species (*H. microphyllum, F. hygrometrica*, and *P. sphaericum*) have a significantly higher ($P<0.001$) content of phenolic compounds on a dry weight basis than the setae and gametophytes (Figure 3B1), which the larvae avoided eating.

Discussion

Although many lepidopteran larvae eat mosses at all latitudes (Prins, 1982; Richardson 1981; Maciel-Silva & dos Santos, 2011), the heavy consumption of *H. microphyllum* capsules by *Agrotis* larvae in subtropical cities in spring has never been reported. The known accounts of the feeding habits of lepidopteran larvae suggested that only the following four families are known to consume bryophyte capsules: Micropterygidae (Gerson, 1969), Mnesarchaeidae (Grehan, 1984), Lithosiidae (Liu, 1989), and Geometridae (Maciel-Silva & dos Santos, 2011). Thus, this is the first record of Noctuidae larvae that feed on mosses.

The larvae ate the moss capsules of the five other species to varying degrees. In view of the daily consumption of moss capsule number by larvae (Figure 2), *H. microphyllum* and *P. sphaericum* seem more palatable to *Agrotis* sp. larvae. Only the capsules of *H. microphyllum,* however, suffered from heavy consumption in the field in Shanghai at almost all the sites we observed. Two other species (*P. sphaericum* and *F. hygrometrica*) in Shanghai were seldom damaged. A possible reason may be that *P. sphaericum* and *F. hygrometrica* usually only grow on introduced soil in Shanghai. They may not have been encountered by the larvae in the field. *H. microphyllum* is the commonest species in Shanghai and occurs extensively in lawns. The larvae consumed a huge number of *H.*

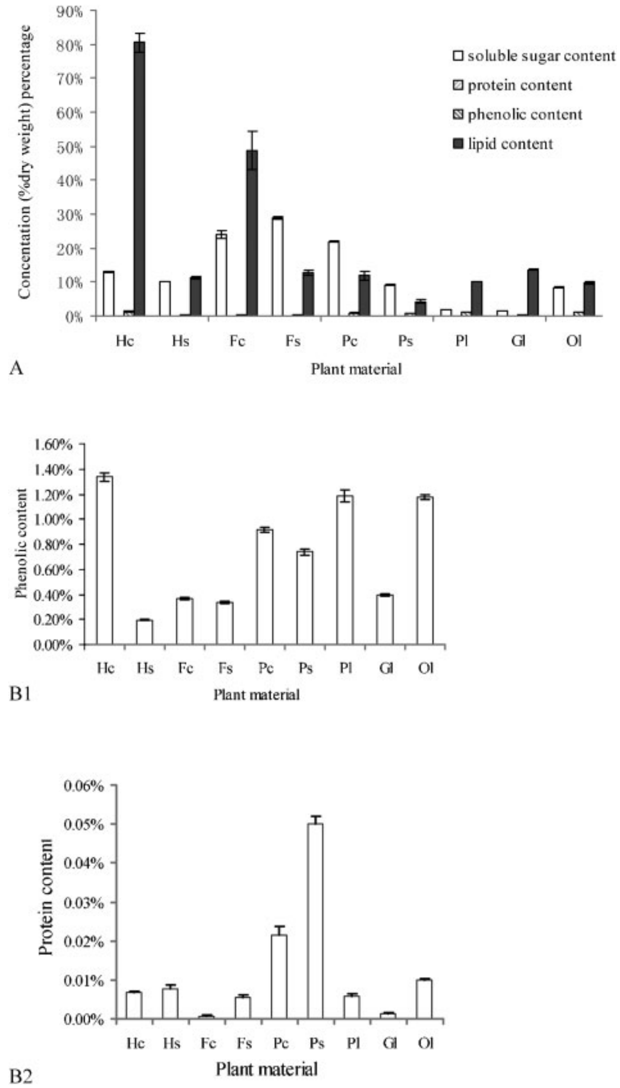

Figure 3 Comparison of two tissue parts of three mosses (*Haplocladium microphyllum*, *Funaria hygrometrica*, *Physcomitrium sphaericum*), the fallen leaves of *Platanus acerifolia* and *Ginkgo biloba*, and the fresh leaves of *Ophiopogon bodinieri* on chemical component percentages. (A) Four main chemical component contents; (B1) phenolic content; (B2) protein content. Hc=*H. microphyllum* capsule extract, Hs=*H. microphyllum* seta and shoot extract, Fc=*F. hygrometrica* capsule extract, Fs=*F. hygrometrica* seta and shoot extract, Pc=*P. sphaericum* capsule extract, Ps=*P. sphaericum* seta and shoot extract, Pl=*Platanus hispanica* fallen leaf extract, Gl=*Ginkgo biloba* fallen leaf extract, Ol=*Ophiopogon japonicus* fresh leaf extract.

microphyllum capsules daily; however, the gametophytes were very rarely grazed. Some Brazilian Lepidoptera larvae (Geometridae sp.), however, prefer leaves of a moss (*Hypopterygium tamarisci*), and do not eat its capsules (Maciel-Silva & dos Santos, 2011).

Phenolics have always been considered as effective deterrents to molluscan feeding in bryophytes (Liao, 1993; Glime, 2006). Liao (1993) proposed that a high concentration of phenolics results in increased deterrence of herbivory based on the 'apparency' theory (Feeny, 1976; Rhoades & Cates, 1976). In the field, capsules are more frequently damaged than shoots (Davidson *et al*., 1990). That may be because they have higher lipid content than other parts, providing a rich energy resource, possibly affording the larvae a better protection against the cold of early spring (Prins, 1982). In our experiments, to look for possible reasons why capsules were attractive, chemical analysis of soluble sugar, protein, lipid and phenolic contents was applied. In the three species tested (*H. microphyllum, F. hygrometrica*, and *P. sphaericum*), although the phenolic content (Figure 3B1) is higher than that of the other parts, the lipid content of capsules is also much higher than that of the other parts (Figure 3A). On a dry weight basis the lipid content of capsules is higher than that of phenolic compounds: 80.8% lipids against 1.4% of phenolics. As concluded by earlier authors (Glime, 2006; Cornelissen *et al.*, 2007), we suggest that the high lipid content, which is likely to provide more energy, may play an important role when the larvae select *H. microphyllum* capsules as their feeding target in early spring.

Acknowledgements

The authors are grateful to Professsor Janice M. Glime of Michigan Technological University for her sincere help in improving the manuscript and correcting the English, and Professor L.-Z. Li of Shanghai Normal University for determining *Agrotis* sp. This research was sponsored by the National Natural Science Foundation of China (grant nos. 30825004 and 31170190), the Program of Shanghai Subject Chief Scientist (no. 08XD14016), and 211 Project for the East China Normal University.

Taxonomic Additions and Changes: Nil.

References

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248−54.

Collins, N.J. & Oechel, W.C. 1974. The pattern of growth and translocation of photosynthate in the tundra moss *Polytrichum alpinum*. *Canadian Journal of Botany*, 52: 355−63.

Cornelissen, J.H.C., Lang, S.I., Soudzilovskaia, N.A. & During, H.J. 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*, 99: 987−1001.

Davidson, A.J., Harborne, J.B. & Longton, R.E. 1990. The acceptability of mosses as food for generalist herbivores, slugs in the Arionidae. *Botanical Journal of the Linnean Society*, 104: 99−113.

DeVries, P. 1988. The use of epiphylls as larval hostplants by the tropical rionid butterfly, *Sarota gyas*. *Journal of Natural History*, 22: 1447−50.

Feeny, P. 1976. Plant apparency and chemical defence. *Recent Advances in Phytochemistry*, 10: 1−40.

Flechtmann, C.H.W. 1985. *Eustigmaeus bryonemus* sp. n., a moss feeding mite from Brazil (Acari, Prostigmata: Stigmaeidae). *Revista Brasileira de Zoologia*, 2: 387−91.

Frahm, J.-P. & Kirchhoff, K. 2002. Antifeeding effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against the slug *Arion lusitanicus*. *Cryptogamie Bryologie*, 23: 271−5.

Gerson, U. 1969. Moss-Arthropod associations. *Bryologist*, 72: 495−500.

Gerson, U. 1982. Bryophytes and invertebrates. In: A.J.E. Smith, ed. *Bryophyte Ecology*. London: Chapman & Hall, pp. 45−57.

Glime, J.M. 2006. Bryophytes and herbivory. *Cryptogamie Bryologie,* 27: 191−203.

Grehan, J. R. 1984. The host range of *Aenetus virescens* (Lepidoptera: Hepialidae) and its evolution. *New Zealand Entomologist*, 8: 52−61.

Johansen, R.M., Retana-Salazar, A.P. & Mojica-Guzman, A. 1983. A review of the New World bryophyte-feeding genus *Wegenerithrips* Johansen 1983 (Insecta, Thysanoptera, Thripidae). *Senckenbergiana Biologica*, 85: 61−83.

Li, J., Nie, J.-Y., Li, H.-F., Xu, G.-F., Wang, X.-D., Wu, Y.-L. & Wang, Z.-X. 2008. On determination conditions for total polyphenols in fruits and its derived products by Folin-phenol methods. *Journal of Fruit Science*, 25: 126−31. (In Chinese)

Liao, C.-L. 1993. Chemical defence in bryophytes with high apparency. *Bryological Times*, 75: 1−4.

Liu, Y.-F. 1989. Insect fauna at Jianfengling in Hainan island-Lithosiidae. *Scientia Silvae Sinicae*, 25: 175−9. (In Chinese)

Loewus, F.A. 1952. Improvement in anthrone method for determination of carbohydrates. *Analytical Chemistry*, 24: 219.

Loria, M. & Herrnstadt, I. 1980. Moss capsules as food of the harvest ant *Messor*. *Bryologist*, 83: 524−5.

Maciel-Silva, A.S. & dos Santos, N.D. 2011. Detecting herbivory in two mosses from an Atlantic Forest, Brazil. *Journal of Bryology*, 33: 140−7.

Mallet, J. & Singer, M.C. 1986. Moss-feeding by a satyrine butterfly. *Journal of Research on the Lepidoptera*, 24: 392.

Prins, H.H. 1982. Why are mosses eaten in cold environments only? *Okios*, 38: 374−80.

Rhoades, D.F. & Cates, R.G. 1976. A general theory of plant herbivore chemistry. *Recent Advances in Phytochemistry*, 10: 168−213.

Richardson, D.H.S. 1981. *The Biology of Mosses*. Oxford: Blackwell.

Wang, N., Tan, B.C. & Davison G.W.H. 2011. Bryophytes as a major dietary component for the Blood Pheasant, *Ithaginis cruentus. Journal of Bryology*, 33: 257–60.

Appendix

Voucher specimens used in this study.

A species name is followed by the locality and collection number. All specimens are housed in HSNU.

Haplocladium microphyllum, CHINA, Shanghai, East China Normal University, old campus, on soil, *R.-L. Zhu et al. 20090320-1*.

Ditrichum pallidum, CHINA, Zhejiang, Linan, Tianmushan Nature Reserve, on soil, 1390 m, *R.-L. Zhu et al. 20090421-100*.

Funaria hygrometrica, CHINA, Shanghai, East China Normal University (old campus), on soil, *R.-L. Zhu et al. 20100307-1*.

Physcomitrium sphaericum, CHINA, Shanghai, East China Normal University (old campus), on soil, *Y. Fang 2009315-1*.

Pogonatum inflexum, CHINA, Zhejiang, Linan, Tianmushan Nature Reserve, on soil, 1390 m, *R.-L. Zhu et al. 20090421-3.*

Trematodon longicollis, CHINA, Zhejiang, Shaoxing, Qianqing Town, on soil, *Y. Fang 20100329-1*.