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Lichen compounds of common epiphytic Parmeliaceae species deter gastropods both in laboratory and in Central European temperate forests

Ivana ČERNAJOVÁ*, David SVOBODA

Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 12801 Praha 2, Czech Republic

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ABSTRACT

Epiphytic lichens are commonly found with various grazing marks. These are however unequally distributed among species. The extent of the damage caused might be related to lichen secondary metabolites. In the present study we demonstrate that the secondary metabolites of six common epiphytic Parmeliaceae species deter grazing of tree-climbing gastropods in a series of laboratory experiments. We also show that the acetone-rinsing method itself does not affect the palatability of lichens. Subsequently, in a transplantation choice experiment, we show that the genus *Melanohalea*, which does not contain any lichen substances, is strongly preferred. No significant preferences between *Parmelia sulcata* and *Melanelixia glabratula*, species containing different secondary compounds, were found in the experiment. Furthermore, the grazing pressure was shown to be intensive in beech forests of the Central Europe for the first time. Thus, ecological and evolutionary consequences of lichenivory should be considered.

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Introduction

Plant–animal interactions are a popular topic and a wealth of valuable information has been gathered about both herbivory and mycophagy. Much less attention has been paid to lichenivory though it is a common phenomenon. Many vertebrate generalist herbivores (e.g. some Cervidae, the bank vole *Myodes glareolus*, the hoary marmot *Marmota caligata* or the black snub-nosed monkey *Rhinopithecus bieti*) more or less frequently consume lichens (Kirkpatrick et al., 2001; Nybakken et al., 2010; Richardson and Young, 1977).

However, in most ecosystems gastropods and various groups of arthropods such as Lepidoptera, Acari, Collembola, Diplura and Psocoptera, are the most important lichen grazers (Gerson and Seaward, 1977; Lücking and Berner-Lücking, 2000). Recently, a few studies dealing with lichenivory demonstrated that this field offers interesting and challenging study opportunities (e.g. Asplund et al., 2010; Boch et al., 2011; Gauslaa, 2008; Vatne et al., 2010).

Most lichen species produce so-called lichen compounds – carbon-based secondary metabolites almost unique to lichenized fungi (Elix and Stocker-Wörgötter, 2008). When studied

* Corresponding author. Tel.: +420 774 829 113; fax: +420 221 951 645.

E-mail address: ivkacerka@gmail.com (I. Černajová).

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in relation to the lichen grazers, some of them were shown to be toxic to insect larvae while others allowed their normal growth (Emmerich et al., 1993; Giez et al., 1994; Pöykkö and Hyvärinen, 2003; Pöykkö et al., 2005). Even though there is no evidence of toxicity of the lichen substances to gastropods, experimental data strongly support their deterring effects (e.g. Asplund, 2011; Asplund and Wardle, 2013; Gauslaa, 2005).

Variability in the deterring effects was shown to explain preferences among lichen species by grazing gastropods (Benesperi and Tretiach, 2004; Lawrey, 1983). On the other hand, lichens supporting the fastest gastropod growth may be preferred regardless of their lichen compounds (Baur et al., 1994). Similarly, the preferences of natural grazers between four *Lobaria* species could not be reliably explained by the secondary metabolite content (Asplund et al., 2010). Reutimann and Scheidegger (1987) even reported attraction of a mite by the lichen compounds of *Cladonia symphyocarpia*.

The importance of understanding the lichen – lichen compounds – lichen grazers interactions emerges when it comes to the ecological consequences of lichenivory. The grazers' preferences may exert a strong influence on lichen community composition as shown, for example, by Fröberg et al. (2011). It was suggested and experimentally demonstrated that establishment success of *Lobaria pulmonaria* may significantly decrease due to grazing (Asplund and Gauslaa, 2008; Scheidegger, 1995). The grazing pressure also results in various trade-offs, thus constraining the lichen's realized ecological niche and spacial distribution, as shown for *L. pulmonaria* (Asplund and Gauslaa, 2008; Vatne et al., 2010), *Pseudocyphellaria crocata* (Gauslaa, 2008), *Lobaria* spp. (Asplund et al., 2010) or *Nephroma arcticum* (Asplund and Gauslaa, 2010).

In the present study we aimed to further elucidate the interactions of gastropods, lichens and lichen secondary metabolites by means of a series of laboratory feeding experiments using the acetone-rinsing technique (Solhaug and Gauslaa, 1996, 2001), and a field transplantation experiment. Acetone-rinsing allows complete extraction or significant reduction of lichen compounds from dry thalli without affecting their viability (Solhaug and Gauslaa, 1996, 2001). This simple and elegant method is widely used in experiments dealing with lichen secondary metabolites (e.g.

Gauslaa, 2005; Nybakken and Julkunen-Tiitto, 2006; Solhaug et al., 2010).

We focused on eight species of the Parmeliaceae family, which forms an important component of epiphytic lichen communities in Central Europe. A few Parmeliaceae species have previously been used in laboratory feeding studies (Asplund and Wardle, 2013; Gauslaa, 2005) but, to our knowledge, none in field experiments aimed at grazing. To simulate actual interactions we selected two gastropod species that commonly climb tree trunks and feed on epiphytes.

Our aims were: (1) to test the deterrent role of acetone-extractable secondary metabolites in common epiphytic lichen species of the Parmeliaceae against their natural gastropod grazers; and (2) to determine (a) the intensity of grazing activity and (b) the preferences of lichen feeders in the natural conditions of Central European beech forests. We hypothesized that acetone-rinsing would increase palatability of lichen species containing secondary metabolites and that the unprotected species would be preferred by lichen grazers in the subsequent transplantation experiment.

Materials and methods

Lichens

Six species containing various secondary metabolites (*Parmelia sulcata*, *P. saxatilis*, *Parmelina tiliacea*, *Melanelixia glabratula*, *M. subaurifera* and *M. glabra*) and two that are devoid of lichen compounds (*Melanohalea exasperata* and *M. exasperatula*) were selected for the study (Table 1). The presence of the main substances of the studied lichens and the absence of substances in the *Melanohalea* species was confirmed by thin layer chromatography. All the lichens were collected near the SE border of the Muránska Planina National Park in central Slovakia (Table 2, Fig 1) where they are more or less common. Their average size is given in Table 3. If used within 1 week after the collection the thalli were stored air-dry at room temperature, otherwise they were maintained in a freezer which does not affect the viability of air-dried lichens (Honegger, 2003).

Table 1 – Secondary metabolites of the experimental lichens as known from the literature

Lichen	Secondary substances	
	Cortex	Medulla
<i>Parmelia sulcata</i>	Atranorin ^{a,b}	Salazinic ^{a,b} , consalazinic ^b acid
<i>Parmelia saxatilis</i>	Atranorin ^{a,b,c} , chloratranorin ^b	Salazinic ^{a,b,c} , consalazinic ^b , lobaric ^{a,b,c} , norstictic ^{a,c} , lichestic ^c , protocetraric acid ^b
<i>Parmelina tiliacea</i>	Atranorin ^{a,b}	Lecanoric acid ^{a,b}
<i>Melanelixia glabratula</i>	— ^{a,b}	Lecanoric ^{a,b} , 5-methoxylecanoric ^a , skyrin ^a , unknowns ^a
<i>Melanelixia subaurifera</i>	— ^{a,b}	Lecanoric ^{a,b} , subauriferin ^{a,b}
<i>Melanelixia glabra</i>	— ^{b,c}	Lecanoric ^{b,c} a.
<i>Melanohalea exasperata</i>	— ^{a,b}	— ^{a,b}
<i>Melanohalea exasperatula</i>	— ^{a,b}	— ^{a,b}

a Smith et al., 2009.

b Thell and Moberg, 2011.

c Wirth, 1995.

Table 2 – Collection sites of the lichens for the experiments with *Cochlodina cerata*, with *Lehmannia marginata* and the transplantation experiment

Experiment	<i>Cochlodina cerata</i>	<i>Lehmannia marginata</i>	Transplantation
Lichen species	Localities		
<i>Parmelia sulcata</i>	Muránska Zdychava ^a	Muránska Zdychava ^a	Poludnica ^c , Šiance ^b
<i>Parmelia saxatilis</i>	Šiance ^b	Šiance ^b , Paveleková ^e	—
<i>Parmelina tiliacea</i>	Šiance ^b	Šiance ^b , M. Zdychava ^a , Hrdzavá ^d	—
<i>Melanelixia glabrata</i>	Muránska Zdychava ^a	Šiance ^b	Poludnica ^c , Šiance ^b
<i>Melanelixia subaurifera</i>	Muránska Zdychava ^a	Paveleková ^e	—
<i>Melanelixia glabra</i>	Muránska Zdychava ^a	M. Zdychava ^a , Hrdzavá ^d	—
<i>Melanohalea exasperata</i>	Muránska Zdychava ^a	Muránska Zdychava ^a	Muránska Zdychava ^a
<i>Melanohalea exasperatula</i>	Muránska Zdychava ^a	Muránska Zdychava ^a	Muránska Zdychava ^a

a Muránska Zdychava (N48.74558° E20.12539°, alt. 720 m a. s. l.) – bark of various fruit trees (*Prunus avium*, *Prunus domestica*, *Malus domestica* and *Pyrus communis*).

b Šiance (N48.77739° E20.10095°, alt. 900 m a. s. l.) – bark of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) trees.

c Poludnica (N48.76461° E20.04052°, alt. 950 m a. s. l.) – bark of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) trees.

d Hrdzavá (N48.74944° E20.01821°, alt. 650 m a. s. l.) – bark of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) trees.

e Paveleková (N48.75861° E20.00156°, 1050 m a. s. l.) – bark of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) trees.

Snails

We chose two forest gastropod species that climb tree trunks during humid weather: the Clausiliidae snail *Cochlodina cerata* and the Limacidae slug *Lehmannia marginata*, which even climbs up to the tree canopy crowns on rainy days. Both are very common in the area.

Seventy-one adult individuals of *C. cerata* were collected from tree trunks and forest litter at Muránska Zdychava (Fig 1). Sixty-four individuals of *L. marginata* varying in size were collected as they climbed trees on a rainy day at Šiance (Fig 1). On average *C. cerata* weighed 0.126 ± 0.008 g and *L. marginata* 0.490 ± 0.256 g. Prior to the experiments the snails were kept in a humid glass container at room temperature.

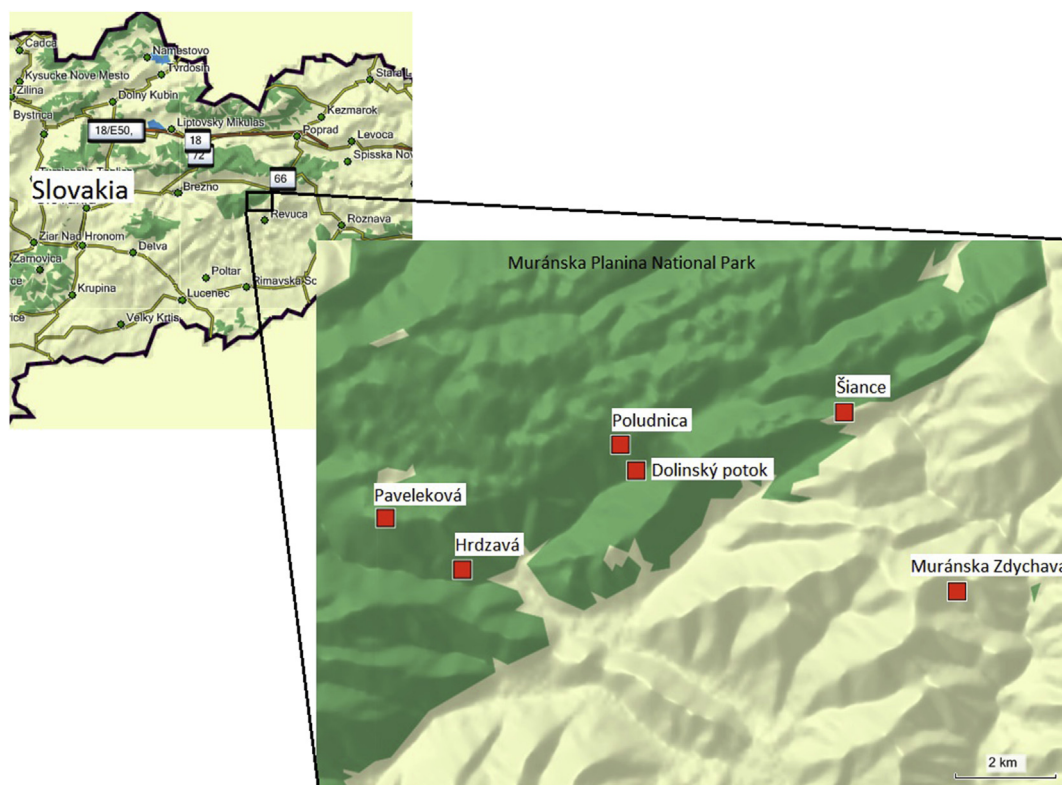


Fig. 1 – Location of the experimental sites (Poludnica, Dolinský potok, Šiance) and the collection sites (Paveleková, Hrdzavá, Poludnica, Šiance, Muránska Zdychava). The map was created using MapSource 6.15.7 (Garmin Ltd. or its subsidiaries 1999–2009).

Table 3 – Comparison of grazing of *Lehmammia marginata* and *Cochlodina cerata* of acetone-extracted and control parts of thalli as calculated on the basis of lichen area or weight. Means \pm standard error and statistical testing results are given

		Original thallus area in mm ²				Area grazed in mm ²				Original thallus weight in mg				Weight grazed in mg				V	t	df	P
		Extracted	Control	Extracted	Control	Extracted	Control	Extracted	Control	Extracted	Control	Extracted	Control								
L. marginata	P. sulcata	753 ± 67	777 ± 56	370 ± 35	281 ± 32	47	0.024	166 ± 21.9	190.4 ± 29.2	45.0 ± 5.3	31.4 ± 5.3	35	0.077								
	P. saxatilis	682 ± 66	666 ± 55	421 ± 47	272 ± 50	47	0.027	132.1 ± 21	139.4 ± 21.6	49.8 ± 6.2	26.9 ± 6.6	47	0.024								
	P. tiliaea	823 ± 60	942 ± 54	448 ± 47	198 ± 37	37	5.776	163.7 ± 15.9	203.6 ± 14.4	48.8 ± 5.7	21.0 ± 5.4	54	0.002								
	M. glabratula	426 ± 36	418 ± 43	342 ± 30	157 ± 21	54	0.002	53.1 ± 5	61 ± 6.4	33.7 ± 2.9	17.1 ± 4.3	3.344	0.004								
	M. subaurifera	342 ± 29	340 ± 35	370 ± 35	215 ± 32	39	3.570	41.8 ± 3.3	47.4 ± 6	33.4 ± 2.1	22.3 ± 4.1	51	0.007								
	M. glabra	444 ± 39	382 ± 21	314 ± 32	257 ± 38	39	0.138	92.6 ± 10	75.5 ± 8	44.9 ± 8.4	33.3 ± 6.5	30	0.204								
C. cerata	M. exasperata	546 ± 34	437 ± 41	390 ± 29	384 ± 40	54	0.184	70.8 ± 8.1	75.7 ± 9.1	56.0 ± 5.6	60.0 ± 5.8	27	0.621								
	M. exasperatula	349 ± 41	370 ± 31	304 ± 31	320 ± 25	54	0.638	33.9 ± 4.9	32.8 ± 4.1	28.2 ± 2.9	25.5 ± 2.2	27	0.635								
	P. sulcata	820 ± 61	828 ± 91	199 ± 30	136 ± 40	54	0.002	155.8 ± 19.2	159.6 ± 28.3	24.1 ± 2.6	11.5 ± 2.5	42	0.012								
	P. saxatilis	991 ± 44	1 150 ± 60	289 ± 25	115 ± 21	54	0.002	177.2 ± 18.1	206.6 ± 17.6	35.9 ± 2.0	11.2 ± 2.2	55	0.001								
	P. tiliaea	484 ± 42	491 ± 28	386 ± 30	25 ± 8	55	0.001	70.8 ± 7.9	81.5 ± 8.5	21.6 ± 1.1	7.2 ± 3.2	53	0.005								
	M. glabratula	415 ± 33	414 ± 37	382 ± 32	37 ± 15	55	0.001	41 ± 5	46 ± 4.1	20.0 ± 1.1	5.0 ± 1.4	55	0.001								
	M. subaurifera	219 ± 33	235 ± 29	211 ± 31	70 ± 21	54	0.002	17.9 ± 3.5	19.9 ± 3.2	15.4 ± 2.4	6.4 ± 1.2	45	0.005								
	M. glabra	439 ± 47	444 ± 64	278 ± 27	76 ± 18	55	0.001	119.7 ± 25	148.6 ± 46.3	25.8 ± 3.6	9.5 ± 1.4	54	0.002								
	M. exasperata	554 ± 54	623 ± 52	239 ± 30	208 ± 45	31	0.770	85.5 ± 10.6	88.9 ± 11.7	20.1 ± 1.5	18.3 ± 2.1	9	0.550								
	M. exasperatula	370 ± 39	383 ± 35	161 ± 29	176 ± 23	31	0.406	41.2 ± 8.2	39.9 ± 6.7	11.7 ± 1.4	12.9 ± 1.7	28	1.000								
	If a paired t-test was performed t-statistic (t) and degrees of freedom (df) are shown, in the case of paired Wilcoxon tests V-statistic (V) is given; P-values (P) are given for both. Significant results (P < 0.05) are highlighted in bold.																				

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Feeding experiments

In the feeding experiments two individuals of a snail species were given a choice between an acetone-rinsed and a control part of a single lichen thallus. With small adjustments we followed the experimental design of Gauslaa (2005). Before the experiment, 10 thalli of each lichen species were carefully separated from the bark, cleaned and air-dried at room temperature for 24 hr. Each lichen thallus was parted into two pieces of approximately the same size. One of them had the secondary metabolites extracted by submerging it in acetone for four 20 min intervals. Afterwards, we let the acetone evaporate for 24 hr at room temperature. All the dried thalli were weighed. Both parts of a thallus were rehydrated, placed into an experimental box (a Petri dish covered with a glass container of 0.72 l), flattened with a piece of glass and photographed.

To reduce the feeding variability between the trials the snails were fed on the same diet (*M. exasperatula* that originated from Muránska Zdychava as well) and then starved for the last 24 hr before the experiment (Hanley et al., 2003; Speiser, 2001). Two random individuals of *C. cerata* were put into each experimental box. Due to great variability in size the individuals of *L. marginata* were divided into two groups and one from each was randomly selected for each trial. During the experimental run the boxes were kept at room temperature and with natural photoperiod but protected from direct sunlight. To maintain the humidity necessary for the gastropod activity we sprayed the boxes with water twice a day. The experiment with *C. cerata* lasted for 72 hr, with *L. marginata* for 48 hr. These time periods were selected as a result of preliminary experiments which indicated this was the time required by the snails to consume the most palatable species completely. After removing the snails from the boxes the lichens were cleaned and photographed. Subsequently, they were left to air-dry for 24 hr and were weighed again. The experiments with *C. cerata* were conducted in May/Jun., 2011, and with *L. marginata* in May/Jun., 2012.

We calculated the biomass consumed as the difference in both thallus weight and area (calculated from the photographs using image processing tool ImageJ <http://imagej.nih.gov/ij/>) before and after grazing. On one hand, weighing was expected to give more accurate results as the lobes may overlap when taking the pictures. On the other hand, the preliminary experiments showed that grazing behavior varied between the lichen species. While in some cases the whole thallus was consumed, in others the lower thallus layers were left intact. Determining consumption on the basis of the area allowed us to consider, for example, thallus parts where only the lower cortex was left, to be grazed. This type of grazing would not result in a marked difference in weight as the lower cortex is the densest and thus the heaviest layer.

Transplantation experiment

Before the experiment the lichens were cleaned and photographed air-dried. Two thalli of *P. sulcata*, two thalli of *M. glabratula*, one thallus of *M. exasperata* and one of *M. exasperatula* were randomly selected and, without separation from the piece of bark they were collected with, in random

positions fixed with epoxy adhesive onto each of the 30 nylon meshes of approximately 15×14 cm.

The species for the transplantation experiment were chosen following the results of the laboratory feeding experiments. The *Melanohalea* species lacking secondary metabolites were heavily grazed in the feeding experiments. The secondary metabolites of *P. sulcata* and *M. glabratula* provide them with protection against the gastropods (see Table 1). However, generally the latter species seemed to be grazed more heavily, regardless of the acetone-rinsing effect. In the transplantation experiment *M. exasperata* and *M. exasperatula* differed neither in the relative area consumed ($V = 32$, $P = 0.684$) nor in the selectivity index ($V = 24$, $P = 0.760$). Consequently, they were treated as one category and in further analyses joined to form the *Melanohalea* group, thus we studied three potential grazing targets in the experiment. We predicted that the *Melanohalea* species would be strongly preferred while we did not make predictions of grazing extent on *P. sulcata* versus *M. glabratula*.

The meshes were transplanted to three localities within the Muránska Planina National Park in the central Slovakia, north-east of the village of Muráň. All three sites are beech forests on calcareous substrata and are less than 5 km from each other (Fig 1). The site Šiance (N48.77756° E20.10112°, alt. 900 m a. s. l.) is the easternmost part of the ridge of the Šiance Natural Reserve bordered by steep scree slopes from both sides. This forest is little affected by human activity. It is dominated by beech (*Fagus sylvatica*) with maple trees (*Acer* spp.), lime trees (*Tilia* spp.) and fir (*Abies alba*); and with hawthorn (*Crataegus* spp.) and dogwood (*Cornus mas*) on the steepest parts of the south-facing slope. The north-facing slope is vegetated by a closed-canopy managed spruce forest. The Dolinský Potok site (N48.76704° E20.05589°, alt. 600 m a. s. l.) is situated at the end of the deep valley of the Dolinský Potok stream which forms the lower border of the Poludnica Natural Reserve. There the beech canopy is mixed with maple trees and firs. The trees used for the experiment were located on both sides, <10 m away from the stream. The Poludnica site (N48.77165° E20.05245°, alt. 950 m a. s. l.) is a more or less flat area above the steep slopes and cliffs of the Poludnica Natural Reserve, and is vegetated by a partially logged, even-aged beech stand.

Ten trees were randomly selected at each site. The meshes were transplanted on 12–14 May, 2012 onto the north-west facing side of beech trunks at the height of 110 cm above the ground. For each tree we recorded the diameter of the trunk at breast height, the perimeter of its base, and estimated the canopy closure using a convex spherical densitometer (R. E. Lemmon, Forest Densimeters, Rapid City, USA). To estimate canopy closure Lemmon's method (1956) modified by Stickler (1959) was used. However, instead of 1.5 % we assigned 1.47 % to each point which results in 99.96 % altogether instead of 102 % and therefore gives a slightly more accurate result. All the meshes were photographed immediately after transplantation and then one more time during the experiment.

The meshes were harvested on 3–4 Sep., 2012 and thus the experiment lasted for 112–114 d. Before taking the meshes down pictures were taken, and again in the laboratory after cleaning.

Area loss was calculated using the image processing tool ImageJ comparing photographs from various stages of the

experiment carefully. The majority of biomass loss was assigned to gastropod activity as the authors had prior experience recognizing the characteristic grazing marks from the laboratory experiments. We also believe that the number of photographs taken of each mesh (altogether five times) allowed us to check for biomass loss due to breakdown. Unlike the laboratory experiments, we used only the area calculations to evaluate the transplantation experiment. This allowed better handling of the thalli as there was no need to separate them from the substratum.

To calculate the preference among the species within a mesh we adopted the selectivity index (Krebs, 1999) which is useful because it takes into account the fact that not all the food types are equally available (the thalli were of different sizes). Basically, it is calculated as the proportion of the percentage of the area consumed of a species to the percentage of the total area consumed of all species in a mesh. It varies from 0 to 1 with the values above 1/3 (=1/number of food types) indicating preference.

Statistical analyses

The difference in grazing between the two thallus parts in the laboratory grazing experiments was tested using a paired t-test or paired Wilcoxon test (if the data were not normally distributed) with the alternative hypothesis of no preference in the case of the *Melanohalea* species and of preference for the acetone-washed thallus part in the case of the species containing secondary metabolites. To test the difference in preference for the species in the transplantation experiment we used the Hotelling's T^2 test as in other studies with a similar experimental design (e.g. Sanchez et al., 2004; Schmidt and Schaefer, 2004 Catalán et al., 2008; Asplund et al., 2010). Sum of the consumption of the two thalli of each species/genus was used. The influence of the locality on total area grazed was tested by analysis of variance using the measured environment parameters (tree diameter, tree base perimeter, canopy closure) as covariates. The calculations were performed in the statistical program R 2.15.2 (R Development Core Team 2011, Vienna, Austria; available at <http://www.R-project.org/>).

Results

Feeding experiments

Grazing was observed in all of the 160 experimental trials (eight lichen species \times two gastropod species \times 10 trials per combination) varying from 5 % to 97 % of lichens' original weight. Acetone-rinsing significantly increased the grazing by *C. cerata* in all six species containing secondary metabolites (Table 3). The grazing of *L. marginata* was significantly increased only in the case of *P. saxatilis*, *P. tiliacea*, *M. glabratula* and *M. subaurifera* (Table 3). The secondary metabolite extraction in *P. sulcata* increased the grazing only if the consumption was calculated by the means of area (Table 3). Thus, a certain degree of chemical defense was confirmed for all the species containing secondary metabolites. For both gastropod species, there was no difference between the consumption of

acetone-rinsed and control parts of thalli in the two species lacking lichen compounds (*M. exasperata* and *M. exasperatula*) (Table 3), indicating random grazing. For both snails the ratio of consumption of the acetone-rinsed thallus part to the total consumption was highest in *P. tiliacea* and *M. glabratula* (Fig 2).

Transplantation experiment

Grazing was observed on lichens in all 30 transplanted meshes. It varied from 1.7 % to 69 % of the original lichen area on a mesh. In 19 meshes the *Melanohalea* species were grazed totally while in four meshes the *P. sulcata* thalli and also in four meshes the *M. glabratula* thalli were left intact.

Natural grazers showed a considerable preference for the *Melanohalea* group (on average 66.1 % of the total lichen area per mesh grazed) and did not distinguish among *P. sulcata* (15.6 %) and *M. glabratula* (18.1 %) (Hotelling's $T^2 = 134.037$, $df = 1$ and 89, $P < 0.001$, Fig 3).

Total grazing (mean area consumed: 50 % at Dolinský potok, 26.8 % at Poludnica, 29.1 % at Šiance, Fig 4) as well as the canopy closure estimate (Dolinský potok 82.3 %, Poludnica 62.5 %, Šiance 77.9 %) significantly differed between the studied localities. The observed grazing increased with increasing canopy closure. Locality and canopy closure together explained 49.3 % of the observed variability in total lichen area consumed in a mesh ($F = 10.07$, $df = 3$ and 25, $P < 0.001$, Table 4). The other two factors measured, tree diameter and tree base perimeter, did not seem to influence total grazing.

Discussion

The deterring role of the acetone-extractable secondary metabolites was demonstrated for all six lichen species. Four of them were protected against both gastropod species, while *M. glabra* deterred *C. cerata* but not *L. marginata*, probably due to low concentration of lecanoric acid, and in *P. sulcata* the results with *L. marginata* were ambiguous. The acetone treatment also significantly increased the palatability of *P. sulcata*, *P. saxatilis* and *P. tiliacea* against the generalist herbivore snail *Cepaea hortensis* in the experiments of Gauslaa (2005) and Asplund and Wardle (2013). Likewise, Asplund (2010) briefly mentions that *L. marginata* is deterred by lichen compounds.

The untreated thallus part was never preferred, thus it can be deduced that none of the studied species contains acetone-extractable substances that attract the studied gastropods. The preference for the acetone-rinsed part seemed to be most pronounced in *M. glabratula* and *P. tiliacea* for both snails (Fig 2). One might assume that of all the lichens tested these two species produce the most effective chemical defense. On the other hand, it has recently been shown that lecanoric acid, the main medullary compound of the two species, may be extracted more readily from the thalli compared to other substances (Asplund and Wardle, 2013). The extraction efficiency varies among lichen species (Solhaug and Gauslaa, 2001; McEvoy et al., 2006) and it may also be different for different substances in various lichen species (Asplund and Wardle, 2013). Considering the species used in our experiments, Asplund and Wardle (2013) found the efficiency to be

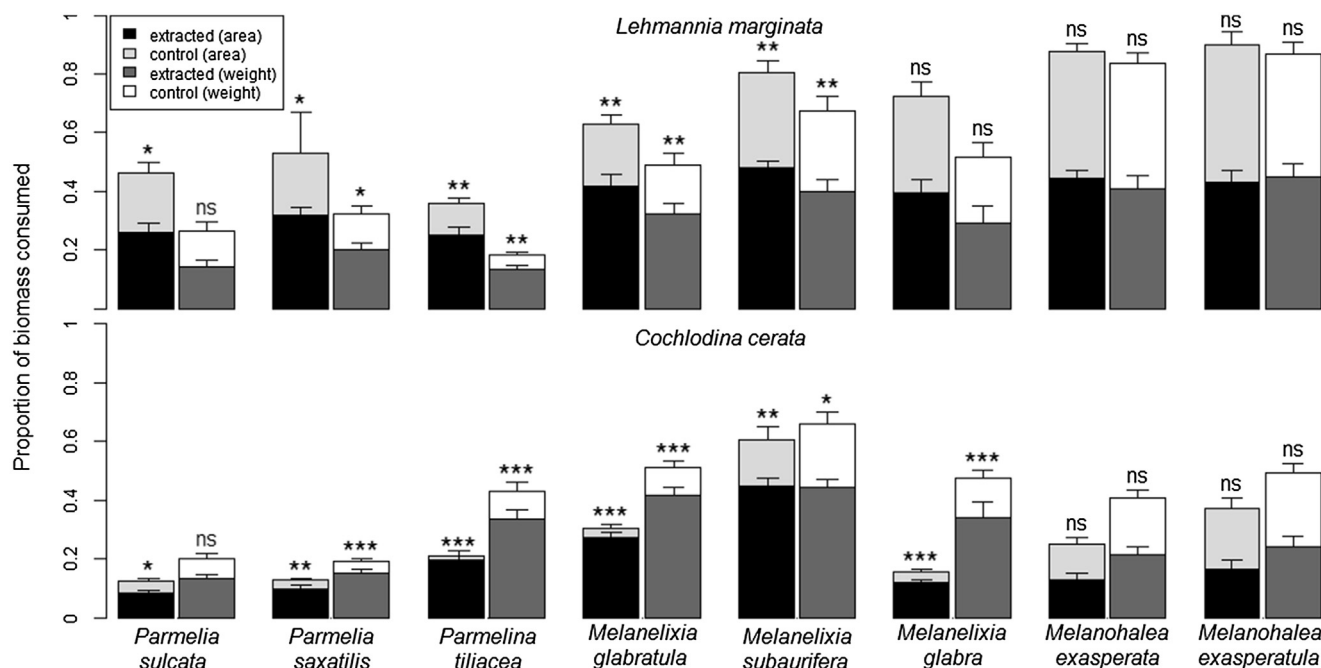


Fig. 2 – Comparison of the proportion of biomass consumed from the acetone-rinsed (lower bar parts) and the control (upper bar parts) thallus part out of total biomass consumed as calculated based on area (left-hand side bars) and weight (right-hand side bars) for both *L. marginata* (upper part) and *C. cerata* (lower part): mean + SE. *, **, *** and ns denote the significance levels of $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$ respectively, for the difference between the acetone-rinsed and control thallus part (paired Wilcoxon's sum rank test).

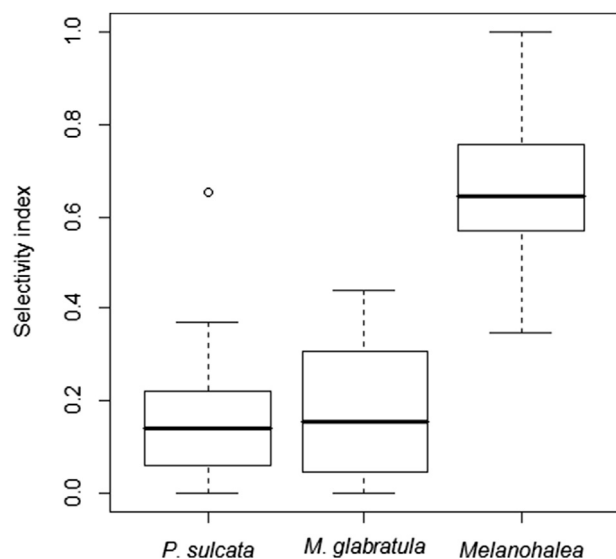


Fig. 3 – The preferences for lichens used in the transplantation experiment calculated as the selectivity index of Krebs (1999) – the proportion of the percentage of the area consumed of a species to the percentage of the total area consumed in a mesh. Values above 0.333 indicate preference. Hotelling's $T^2 = 134.037$, $df = 1$ and 89 , $P < 0.001$, $n = 30$.

more than 60 % for the *P. sulcata* and *P. saxatilis* and more than 90 % for *P. tiliacea*. It remains unknown for the *Melanelixia* species. Nevertheless, in the case of the present study, the results suggest that the efficacy of acetone-rinsing is sufficient to demonstrate the deterring role of the substances. Actual comparison of the strength of preference for the acetone-extracted thallus part between the lichen species would, however, be unreliable.

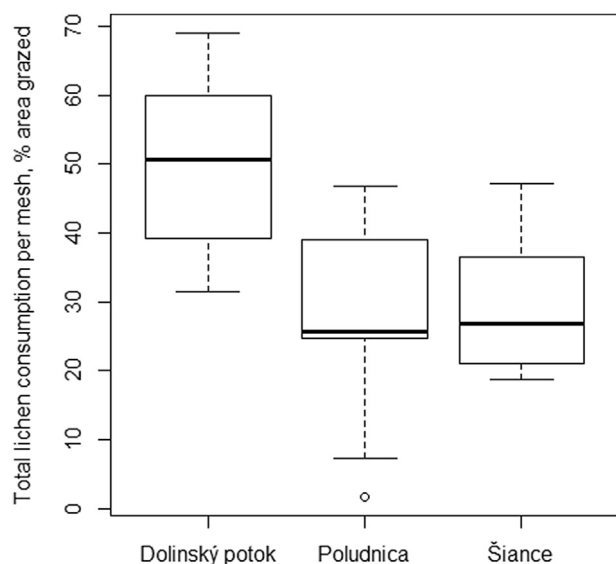


Fig. 4 – Total lichen consumption per mesh expressed as percentage grazed of the original lichen area of the mesh at the three experimental sites, $n = 30$.

Table 4 – One-way ANOVA for total lichen consumption across the three transplantation sites using canopy closure as a covariate

	df	Sum sq	Mean sq	F	P
Locality	2	1 887.8	943.89	7.219	<0.001
Canopy closure	1	2 062.7	2 062.7	15.776	<0.001
Residuals	25	3 268.7	130.75		

$F = 10.07$, $df = 3$ and 25 , $P < 0.001$, adj. $R^2 = 0.493$.

In *M. exasperata* and *M. exasperatula*, the species naturally lacking secondary compounds, no preference for either of the thallus parts was detected, confirming that acetone-rinsing itself does not decrease or in any way affect the palatability of lichens, which is the basic assumption of using the technique for this type of experiment (for example Gauslaa, 2005; Hyvärinen et al., 2000; Reutimann and Scheidegger, 1987).

Which of the compounds are responsible for the observed deterrence can only be speculated. The only substance that appeared individually in the tested lichens is lecanoric acid (in *M. glabra* and some thalli of *M. subaurifera*). Lecanoric acid is a very common lichen substance (Smith et al., 2009) but not much is known about its functions. Nimis and Skert (2006) suggested a deterring role against the coleopteran *Lasioderma serricorne*. It also exhibited antibacterial (Neeraj et al., 2011) and antioxidant (Luo et al., 2009) properties in laboratory conditions. Our results also suggest that certain concentrations of lecanoric acid deter gastropods.

It should be emphasized that the gastropods used in the experiments actually do feed on epiphytic lichens in nature. During the night and on rainy days they climb tree trunks, *L. marginata* even climbs up to the canopy crowns. In other words, these are among the species that impose grazing pressure upon the studied lichens. Therefore, it may be concluded that the secondary metabolites can deter even these grazers adapted to a lichen diet. However, they do not render them absolutely unpalatable as numerous grazing marks can be observed in the field on the studied species that produce secondary metabolites.

As far as we know our transplantation experiment is the first of its kind performed in the continental central-east European environment. The experiment of Asplund et al. (2010) was conducted in *Fraxinus*-dominated stands in SE Norway. Even though grazing marks are commonly observed by lichenologists during their field work in Central Europe as well, the actual grazing level under natural conditions is not much studied. Our experiment documented substantial grazing on lichens at the Muránska Planina National Park. In the relatively short period of 112–114 d lichen grazing was recorded at all 30 meshes, with 35.3 % of the total original lichen thallus area grazed per mesh on average. The study area is quite rich in epiphytes, thus we assume that the experimental meshes did not provide the invertebrates climbing the tree trunks with an unusually attractive food source. Given the grazing intensity recorded we expect a strong influence of grazing on the abundance, distribution and reproductive success of the studied lichens.

Total consumption on meshes was most strongly influenced by locality; it was the highest at the Dolinský Potok site (Fig 4). As this was a deep stream valley it is presumably the site with the highest humidity. Humidity is important for

gastropod activity (Baker, 2001). Canopy closure, the other factor significantly influencing the total consumption in the experiment, might be considered an indirect indicator of humidity also. The less the canopy closure, the more rapid the evapotranspiration. Hence a shorter period of gastropod activity occurs than under a more closed canopy. It has been shown that mollusc grazing on *L. pulmonaria* is more pronounced under denser canopies (Asplund and Gauslaa, 2008). However, Asplund et al. (2010) found no relationship between direct solar radiation and grazing pressure.

Some lichen species were more preferred than others in the transplantation experiment. We expected that the genus *Melanohalea* lacking secondary metabolites would be preferred and indeed a strong preference was observed. In Central Europe these species most commonly occur at exposed habitats such as trunks of solitary, orchard or roadside trees or tree twigs. Their absence in the shade of beech crowns might have two possible interpretations. First, microclimatic requirements of the species – the necessity of higher light intensities might be the cause. Second, grazing pressure consequences should be considered. Their occurrence in more exposed habitats may result from grazing excluding these species from shaded sites, implying an important niche shift caused by gastropod grazing. The idea is consistent with the shaping role of tree-climbing gastropods in epiphytic lichen communities (Asplund et al., 2010).

No preference was observed between *P. sulcata* and *M. glabratula*. However, whether this means that their protection in the form of lichen substances is equally powerful cannot really be decided based on our study. Congruently, previous experiments also suggest that gastropods' preferences cannot be simply explained by the secondary metabolite content (Asplund et al., 2010; Baur et al., 1994). Besides, there are other factors that influence the palatability of lichens, such as thallus structure (Reutimann and Scheidegger, 1987) or nutrient content, and the difference in the snail species' ability to exploit them (Baur et al., 1994; Lawrey, 1983). The thallus of *P. sulcata* is much tougher than that of *M. glabratula* and, therefore, might be expected to be less attractive for lichen-grazing gastropods. Data on the nutrient content of the species used in our study are not available.

In conclusion, the results of the present study show that the secondary metabolites of common epiphytic lichens deter their natural gastropod predators even though they do not exclude grazing. The suitability of the acetone-rinsing technique was also confirmed. The absence of chemical defence explains preferences for certain species. In addition, we have shown that the grazing pressure on epiphytic lichens is intensive and might affect the abundance and distribution of lichen species. Such information about members of the widespread Parmeliaceae from temperate deciduous forests have so far been missing.

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