Not only mosses – lemming winter diets as described by DNA metabarcoding

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Abstract

The temporal dynamics of most tundra food webs are shaped by the cyclic population dynamics of lemmings. While processes during winter may be behind the recent disruptions of lemming cycles, lemming winter ecology is poorly known. We present here the first DNA metabarcoding data on the winter diet of Norwegian lemmings (*Lemmus lemmus*), based on feces collected after a winter of population increase. Prostrate willows, mosses, and graminoids dominated the species winter diet, indicating that the conventional idea of lemmings as moss-specialists should be revised. The behavior of lemming-plant models in theoretical studies is conditional on the assumptions of mosses being their main winter food item. As shrubs have been excluded from the framework of these models, incorporating them in future modeling studies should nuance our understanding on how plants affect lemmings. We also sampled diet of a few individuals found dead on top of the snow. These individuals had relatively empty stomachs and had, prior to death, relied heavily on mosses. This apparent lack of abundant good quality indicates spatial heterogeneity in local food availability during the population increase phase.

Key words: Arctic, bryophyte, *Lemmus lemmus,* prostrate *Salix,* snowbed, winter

Introduction

The temporal dynamics of most tundra food webs are shaped by the cyclic population dynamics of lemmings, considered as key species in the Arctic (Ims and Fuglei 2005). Wintertime processes are crucial for lemming population dynamics (Gilg et al. 2009; Bilodeau et al. 2013a) and changes in snow properties may be behind the recent disruptions of lemming population cycles in Fennoscandia (Kausrud et al. 2008; Ims et al. 2011). Yet, winter ecology of lemmings is poorly known as Arctic winters up to nine months long and snow packs up to several meters thick, combined with often difficult access to remote field sites, make data collection challenging.

Lemming grazing during the periodic peaks can have a profound effect on vegetation (Virtanen 2000; Olofsson et al. 2012) and interactions with food plants have been suggested to be behind the cyclic dynamics (Turchin et al. 2000; Oksanen et al. 2008). Interactions between lemmings and their food resources can be expected to be most pronounced during winter. No new plant growth occurs during this period, snow conditions may limit access to some food items, and individuals tend to concentrate at locations with favorable snow conditions such as snowbeds (Duchesne et al. 2011). However, descriptions of lemming winter diet are scarce (but see Soininen et al. 2015b).

We present here the first DNA metabarcoding (Taberlet et al. 2012) analysis of the winter diet of Norwegian lemmings (*Lemmus lemmus*). The species feeds on a range of mosses, graminoids, forbs and shrubs during summer (Tast 1991; Saetnan et al. 2009; Soininen et al. 2013) but is thought to rely heavily on mosses during the winter (Kalela et al. 1961; Koshkina 1961; Calandra et al. 2015). Previous descriptions of the species winter diet are based on a

58 cafeteria experiment (Kalela et al. 1961), a combination of microhistological analyses of
59 stomach content and grazing signs on vegetation (Koshkina 1961) and stable isotopes
60 analyses of tooth tissue (Calandra et al. 2015). Compared to these methods, DNA
61 metabarcoding enables taxonomically detailed analyses of a large number of samples and
62 allows for more precise and spatially extensive assessments of variability of herbivore diets
63 (Soininen et al. 2009).
64
65 To describe Norwegian lemming winter diet in low Arctic landscapes, we analyzed feces
66 collected in their winter habitat during a year of population peak in Finnmark, northeastern
67 Norway. We complement these data with samples collected from individuals found dead on
68 top of the snowpack during the same winter. To achieve taxonomically detailed information
69 on both vascular plants and bryophytes, we used two different primer sets to identify the
70 ingested plants (Taberlet et al. 2007) and compared the recovered plant DNA in feces to
71 reference libraries of Arctic and boreal vascular plants (Sønstebø et al. 2010; Willerslev et al.
72 2014b) and bryophytes (Soininen et al. 2015b).
73
74 **Material and methods**
75 **Study area and samples**
76 All samples were collected in northeastern Norway (70-71° N, 28-31° E), from snowbed
77 habitats where monitoring of Norwegian lemmings has been conducted since 2009 using
78 feces removal plots. The snowbeds are distributed among three different watershed areas;
79 Komagdalen (KO), Vestre Jakobselv (VJ) and Ifjordfjellet (IF). Within the watersheds, the
80 sampled snowbeds are spread across an area of 32km^2, 18km^2, and 16 km^2 at KO, VJ and IF,
81 respectively. They cover an altitudinal gradient of approximately 150 to 200m, from valley

bottoms with willow thicket to barren highlands. Snowbeds occur in small-scale topographic depressions, where the snowpack can be more than 4m thick in winter and persist until late July. Characteristic plants are mosses (*Dicranum sp.* and *Polytrichum sp.*), a prostrate willow (*Salix herbaceae*), graminoids (*Carex bigelowii*, *Avenella flexuosa*), and low statured forbs (e.g. *Bistorta vivipara*).

Populations of Norwegian lemmings peaked in the area autumn 2011, followed by a population crash during the winter 2011-2012 (Ims et al. 2013). To assess the species winter diet during an increase phase of the population cycle (i.e. winter 2010-2011), we sampled feces soon after snowmelt in 2011. In each snowbed (n= 18, 18, and 16 snowbeds for KO, VJ and IF, respectively) we collected a sample of 5-20 pellets, aiming at five pellets from each feces removal plot within a snowbed (n=4 plots per snowbed). However, this was sometimes impossible as some snowbeds had few pellets. Thus, three of the samples had only one pellet. The feces removal plots were cleaned the previous time in July 2010. We assume that the feces collected in July 2011 represent winter 2011 instead of summer/autumn 2010, because i) snowbeds are typically winter habitats of the Norwegian lemming, and ii) we excluded feces that had clear signs of decomposition, i.e. feces potentially originating from summer 2010. Further, we assume that the feces did not originate from after snowmelt in 2011 as the sampling was conducted relatively soon after snowmelt (on average 17 days, as the average snowmelt date of the sampled snowbeds was June 23rd and the average sampling date July 10th). In addition, during snowmelt the snowbed habitats are very wet, colder than the ambient air, provide little fresh plant foods, and lemmings seem to move away from these habitats before the snow melts (Bilodeau et al. 2013b).

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107 We also collected dead individuals opportunistically in March 2011 (n=6 individuals found on
108 top of the snow, all from VJ). We initially aimed to sample stomach content of these
109 individuals, but as the stomachs were mainly empty we sampled pellets from the intestines
110 (n=1 stomach content and n=5 samples of pellets). The number of samples is summarized in
111 Online Resource 1, Supplementary Table S1.

112

113 **Diet analysis**

114 We analyzed 58 samples for this study as a part of a larger batch of samples (n=192), using
115 DNA metabarcoding. Other parts of the dataset have previously been described in Soininen
116 et al. (2013); Soininen et al. (2014); Soininen et al. (2015a). The method is based on
117 amplifying and high-throughput DNA sequencing a targeted plastid DNA region (P6-loop of
118 the chloroplast *trn*L (UAA) intron) with universal primers for plants. (Taberlet et al. 2007;
119 Soininen et al. 2009). We used two complementary primer pairs, *g-h* which targets seed
120 plants and *c-h* which is universal to plants, to get data on both vascular plants and
121 bryophytes (Taberlet et al. 2007). See details in Online Resource 1, Supplementary Text S1.

122

123 Sequence reads were analyzed using the OBITools software package (Boyer et al. 2016). As
124 taxonomic reference libraries for the primer pair *g-h*, we first used a combined library of 815
125 Arctic (Sønstebø et al. 2010) and 835 boreal vascular plant species (Willerslev et al. 2014b).
126 For the *c-h* primer pair, we used the same taxonomic reference libraries of Arctic and boreal
127 vascular plant species, supplemented with a library of 455 Arctic and boreal bryophyte
128 species (Soininen et al. 2015b). Sequences that matched poorly against these references
129 were further compared with references retrieved from the EMBL Nucleotide Sequence

Database (version 111, available at http://www.ebi.ac.uk/embl/). We then carefully checked these taxonomic assignments using both the known regional flora and the reference libraries coverage of all relevant taxa. See details in Online Resource 1, Supplementary Text S1.

The resulting datasets consisted of a sequence count per taxon and sample, from which we calculated the proportion of different taxa in each sample. Even though DNA metabarcoding data for plants probably reflects herbivore diets well (Soininen et al. 2009; Willerslev et al. 2014a), the amount of DNA sequences per sample may be biased for some taxa (Soininen et al. 2009; Pompanon et al. 2012). Hence, we also report the number of samples in which a given taxon was found. We used the *c-h* dataset to compare the proportions of seed plants, ferns and fern allies (i.e. vascular non-seed plants) and bryophytes (i.e. mosses and liverworts) in the diet and to assess the proportions of different bryophyte taxa. We used data from primer pair *g-h* to assess the proportions of seed plant taxa. Preliminary multivariate analyses (PCA on centered log-ratio transformed proportions of families with >1% mean proportion of the diet) revealed little differences in Norwegian lemming diets between the three watershed areas. Furthermore, the difference in sample size between snowbeds (n=50) and dead individuals (n=6) was large. For these reasons, we here focus on descriptive analyses.

Results

Taxonomic precision of diet data

A total of 12 190 sequences were obtained with the *g-h* primer pair (210 sequences/sample on average) and 19 199 sequences with the *c-h* primer pair (343 sequences/sample on average). We removed two samples from the dataset because we were unable to amplify

any DNA with the *c-h* primer from them. Overall, 98.2% of the sequences were identified at the family level, 60.1% at the genus level and 17.1% at the species level. The large amount of sequences assigned to the family level were mainly assigned to Salicaceae, a common plant family in the study area and for which the *g-h* region is almost identical between members of this group (Sønstebø et al. 2010). Excluding this family, 77.0% of sequences were identified to the genus level. However, as only the genus *Salix* is present in the study area, we considered all sequences assigned to Salicaceae to belong to this genus.

Composition of Norwegian lemming winter diet

For the samples collected during the population cycle increase phase (i.e. snowbed samples), we retrieved 17 species, 29 genera and 25 families of vascular plants, and 9 species, 18 genera and 13 families of bryophytes (Table 1; Online Resource 1, Table S2). Proportion of vascular plants was on average 0.54 (range from 0.03 to 0.99) (Figure 1a). The most common family was Salicaceae. Other common vascular plant families were Poaceae and Polygonaceae. The vascular plant component of Norwegian lemming diets thus encompassed deciduous shrubs, grasses and forbs (Figure 1a). The three most common moss families were Polytrichaceae, Dicranaceae and Rhabdoweisiaceae. In the study area, all of these families are mainly represented by acrocarpous species, with Polytrichaceae growing as scattered stems, while the two other families usually form carpets. We obtained very similar results by using the frequency of occurrence instead of relative abundance (Table 1; Online Resource 1, Table S2). Plant family composition differed little between the three watershed areas (Online Resource 1, Figure S1). In the samples collected from dead individuals , bryophytes of the family Dicranaceae largely dominated the diet while the mean proportion of vascular plants was 0.30 (range from zero to 0.97) (Figure 1b).

178

179 **Discussion**

180 We found that mosses, grasses, and willows dominated the winter diet of the Norwegian
181 lemming in snowbed habitats during the increase phase of the population cycle. This
182 indicates that vascular plants have a more prominent role in the species winter diet than
183 previously assumed. Use of food plants varied little between the sampled watershed areas.
184 In contrast, dead individuals sampled on top of the snow pack had relied heavily on mosses.
185 This suggests that Norwegian lemming winter diets may differ substantially between
186 individuals remaining in their normal subnivean habitat and individuals dispersing on the
187 snow surface.

188

189 Based on DNA metabarcoding, we were able to identify food items that have previously
190 been considered unimportant. Furthermore, we were able to describe Norwegian lemming
191 winter diet at an unprecedented level of taxonomic detail, showing a previously undescribed
192 diversity of food items. DNA metabarcoding has previously been used to successfully
193 describe diets in a semi-quantitative way in various herbivores (Kowalczyk et al. 2011;
194 Newmaster et al. 2013; Willerslev et al. 2014a), including lemmings (Soininen et al. 2013;
195 Soininen et al. 2015a). Still, DNA metabarcoding of faeces has several potential biases, in
196 particular differential PCR amplification between taxa and differential digestion between
197 plant taxa (Pompanon et al. 2012). The abundance of *Salix* in our results is unlikely to be an
198 artifact due to preferential amplification of short fragments. The DNA fragment amplified by
199 the primer pair *g-h* for *Salix* is of similar length (56bp) as that of the two most abundant
200 grass genera we identified (*Avenella* and *Festuca*, 52bp in the species occurring in the study
201 area; *A. flexuosa*, *F. rubra,* and *F. ovina*). Furthermore, differential digestion is unlikely a

major problem in small rodents, as there is a good correspondence of DNA metabarcoding data between samples collected from stomach and rectum of the same individuals (Soininen 2012). In ruminants, DNA metabarcoding has been compared with known diets, recorded by animal-born video footage Newmaster et al. (2013) or by controlling the diet of a captive individual (Willerslev et al. 2014a; Nakahara et al. 2015). While population-level average diets were found to have good correspondence (Newmaster et al. 2013), the correspondence of individual-level diets appears to be variable (Willerslev et al. 2014a; Nakahara et al. 2015). For small rodents, the method has been evaluated in terms of its correspondence with microhistology, the two methods yielding a taxonomically similar picture of small rodent diets (Soininen et al. 2009). We thus believe that our results reflect actual diet proportions of Norwegian lemmings rather well, although assessing the quantitative correspondence between food intake and DNA metabarcoding would be required to confirm this.

The most common food item of the Norwegian lemmings' winter diet was the vascular plant family Salicaceae. Although we could not identify the species with DNA metabarcoding, we do know that the predominant species within the family Salicaceae in the snowbed habitats in northern Norway is the prostrate *Salix herbaceae*. Our findings thus contrast most previous studies on Norwegian lemming diets, which have highlighted the importance of mosses and grasses during winter (Kalela et al. 1961; Koshkina 1961; Calandra et al. 2015) and summer (Stoddart 1967; Hansson 1969; Tast 1991; Saetnan et al. 2009). Yet, the biomass of prostrate willows in snowbeds is affected by Norwegian lemmings (Moen et al. 1993; Virtanen 2000), supporting our interpretation of these plants as important winter food for the species. Accordingly, a recent DNA metabarcoding study of winter diets of two other

lemming species from Arctic Canada showed that *Salix* was an important winter food item for both the collared lemming, *Dicrostonyx groenlandicus*, and brown lemming, *Lemmus trimucronatus* (Soininen et al. 2015a). The conventional wisdom that lemmings are " moss-eaters, in particular so during the critical winter period" (cf. Turchin et al. 2000) has had a profound implication for how their dynamics have been modelled in theoretical studies (Turchin et al. 2000; Turchin and Batzli 2001). In these studies, the destabilizing effect of plants on lemmings is conditional on the plants re-growth corresponding to logistic growth. This has been argued to apply for mosses but not graminoids, whereas woody plants were excluded from this modeling framework (Turchin and Batzli 2001). Hence, further development of rodent-plant interaction models would benefit from considering how the functional diversity of vascular plants in lemming diets would best be incorporated.

We found three moss families to be common in the Norwegian lemmings' winter diet, namely Rhabdoweisiaceae, Polytrichaceae and Dicranaceae. This contrasts with the summer diet, where Dicranaceae has been found to be the dominant moss in the same study area (Soininen et al. 2013). In addition, the species appears also to use more mosses during winter than summer, as indicated by a higher mean proportion of bryophytes (50% in this study vs 32% in Soininen et al. 2013). The use of mosses seems thus to be more important and diversified during winter. The winter diet differs from the summer diet in terms of the diversity and importance of vascular plants: winter diet contains i) larger proportion of *Salix*, ii) a lower vascular plant diversity, and iii) lower proportion of the grass *A. flexuosa*. Norwegian lemmings thus appear to compensate for the low availability of herbaceous plants in winter by feeding more on woody plants and mosses. Such seasonality contrasts the findings by Calandra et al. (2015) who found little differences between summer and

250 winter diets based on stable isotope analyses of Norwegian lemming teeth. However, the
251 isotopic signatures of for instance mosses, forbs and shrubs overlap largely (Calandra et al.
252 2015). Thus, seasonal differences in diet taxonomic composition do not necessarily result as
253 a change in the isotopic diet. Even though we found no clear indication of regional
254 differences in diets, it is possible that some of the variation (e.g. the proportion of vascular
255 plants that ranged almost between zero and one [0.03 and 0.99]) could be caused by local
256 differences in available vegetation. Yet, a proper assessment of active selection or
257 alternatively, avoidance, of plants and potential seasonal patterns in it, would require
258 comparisons of available biomass and ingested biomass.
259
260 Interestingly, the samples collected from dead individuals that were found on top of the
261 snow pack show a contrasting diet composition. These relied heavily on *Dicranum* mosses,
262 while other food items were scarce in their diets. The samples collected from the snowbeds
263 represent an average diet of several individuals, across a longer time window and larger
264 spatial scale, in the normal subnivean winter habitat of Norwegian lemmings. In contrast,
265 the diet description of the dead individuals represents the last meal of these individuals that
266 were likely searching for better grazing grounds as we found them on top of the snow. In
267 particular, the mostly empty stomachs and the difference in diet composition compared to
268 the feces samples from snowbeds suggest a lack of abundant good quality food prior to
269 death. Indeed, limited access to food due to poor snow conditions (Kausrud et al. 2008) and
270 overgrazing of food resources (Turchin et al. 2000) have been assumed to cause population
271 crashes in lemmings, and similar causes could explain the movement of individuals on top of
272 the snow during the increase phase. Although some of the differences in the diet between
273 the two sets of samples could be due to lower sample size, they indicate spatial

heterogeneity in local food availability during the population increase phase. Consequently, lemming-plant interactions may show substantial spatial heterogeneity during a given population cycle phase.

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Conflict of interest

We would like to mention that Ludovic Gielly is one of the co-inventors of a patent concerning *g-h* primers and the subsequent use of the P6 loop of the chloroplast *trn*L (UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers.

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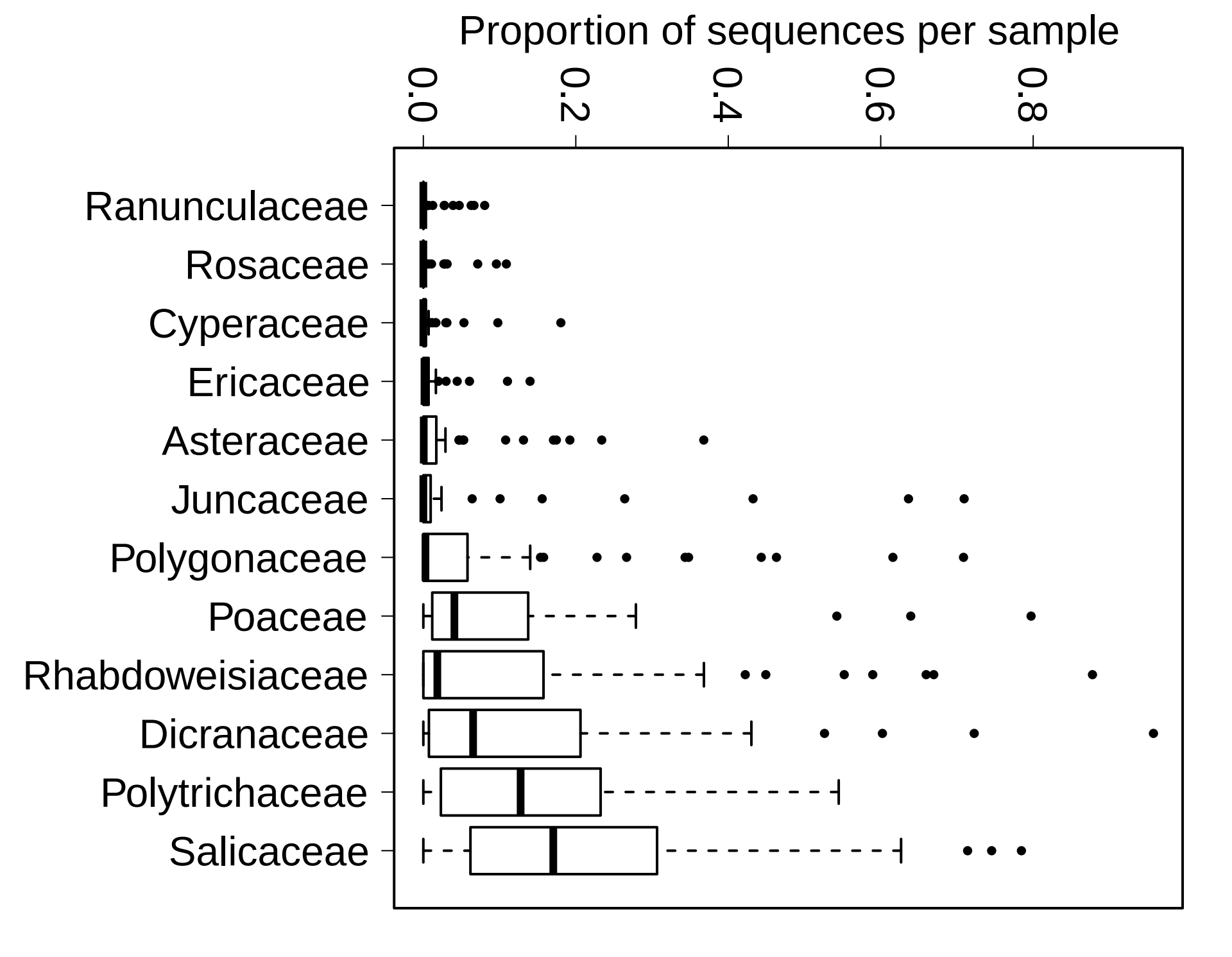
Table 1. Composition of winter diets of Norwegian lemmings (*Lemmus lemmus*) during a population cycle increase phase (mean proportion of DNA sequences of fecal pellets analyzed primer pairs *g-h* and *c-h*) in northern Norway (n=50 snowbeds). At each taxonomic level, also the proportions from lower levels are included. Only taxa with mean proportion >0.01 are included. Column frequency refers to the number of samples in which the taxa were found. When this differed between family and genus resolution data, both values are given.

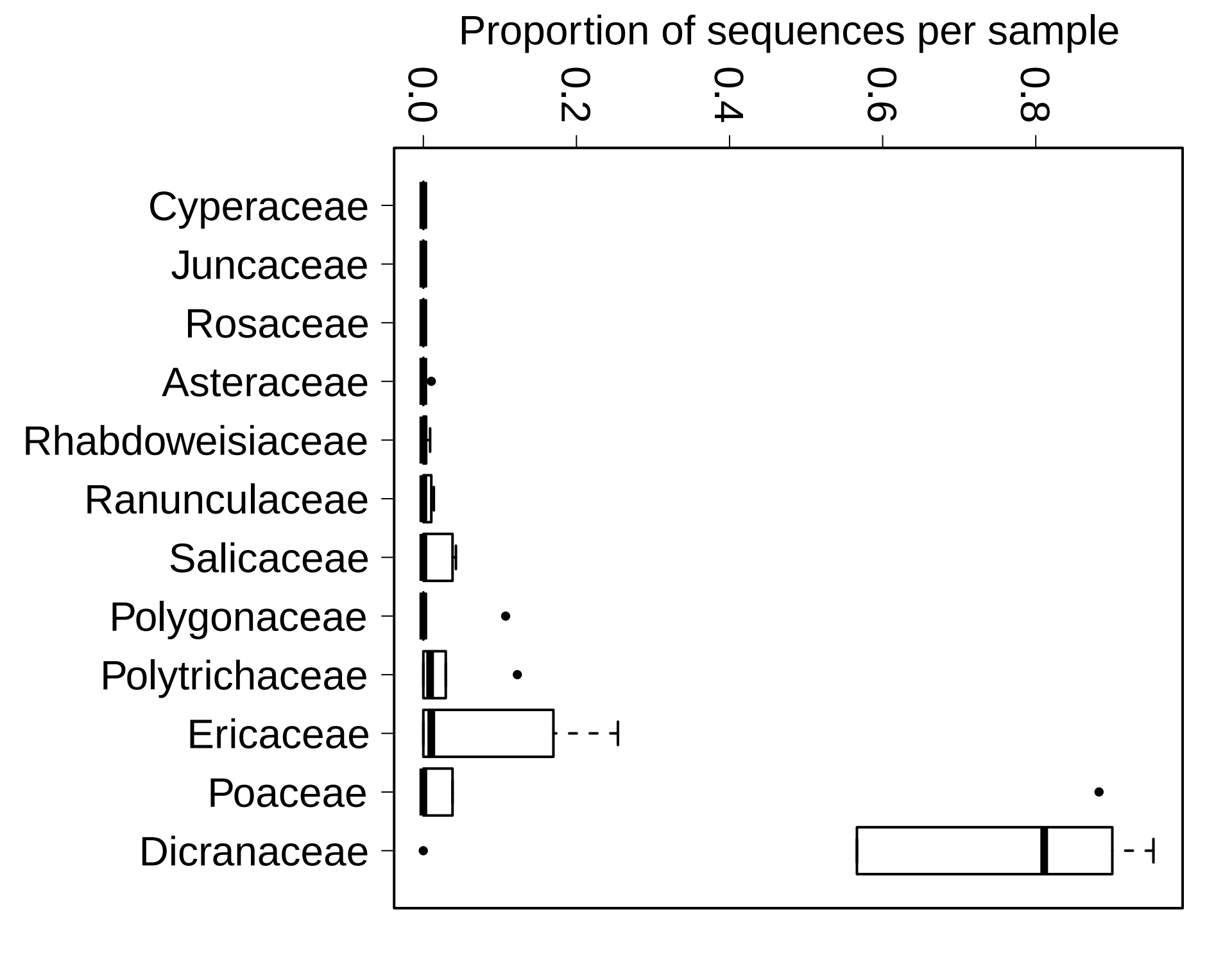
Family	Genus	Species	Mean (±SE)	Frequency
Vascular plants				
Salicaceae	*Salix*		0.21 (±0.03)	45
Poaceae			0.10 (±0.02)	46
	Avenella	*Avenella flexuosa*	0.04 (±0.01)	40
	Festuca		0.01 (±0.00)	15
Polygonaceae			0.09 (±0.02)	27
	Rumex		0.02 (±0.01)	18
	Bistorta	*Bistorta vivipara*	0.07 (±0.02)	20
Juncaceae			0.05 (±0.02)	23
	Juncus		0.04 (±0.02)	16
	Luzula		0.01 (±0.01)	11
Asteraceae			0.03 (±0.01)	25
Ericaceae	*Empetrum*	*Empetrum nigrum*	0.01 (±0.00)	26/20
Cyperaceae	*Carex*		0.01 (±0.00)	22/21
Rosaceae			0.01 (±0.00)	12
Ranunculaceae	*Ranunculus*		0.01 (±0.00)	12
Bryophytes				
Polytrichaceae			0.16 (±0.02)	47
	Polytrichum		0.08 (±0.01)	44
	Psilopilum		0.03 (±0.01)	12
Dicranaceae			0.15 (±0.03)	42
	Dicranum		0.14 (±0.03)	40
Rhabdoweisiaceae			0.14 (±0.03)	37
	Kiaeria		0.10 (±0.02)	36
	Kiaeria	*Kiaeria glacialis*	0.01(±0.01)	9
Hylocomiaceae	*Pleurozium*	*Pleurozium schreberi*	0.01 (±0.00)	5/4

Figure captions

Figure 1. Proportion of plant families in winter diets of Norwegian lemmings (*Lemmus lemmus*). Families are arranged with increasing mean proportion towards the right. Families with mean proportion < 0.01 are omitted from the figure. Families to the left of the vertical line are vascular plants, to the right mosses.

- **a.** Feces samples from snowbeds (n= 50 snow beds).
- **b.** Samples from intestines (n=5) and stomachs (n=1) of dead lemmings.


Proportion of sequences per sample
0.0
0.2
0.4
0.6
0.8
Ranunculaceae
Rosaceae
Cyperaceae
Ericaceae
Asteraceae
Juncaceae
Polygonaceae
Poaceae
Rhabdoweisiaceae
Dicranaceae
Polytrichaceae
Salicaceae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Rhabdoweisiaceae
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Salicaceae
Polygonaceae
Polytrichaceae
Ericaceae
Poaceae
Dicranaceae