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Evidence of targeted consumption of mosses by birds in sub-Antarctic South America

NICHOLAS J. RUSSO,*[1] MICHAEL ROBERTSON,[2] ROY MACKENZIE,[3,4] BERNARD GOFFINET[1] AND JAIME E. JIMÉNEZ[2,5,6]

[1]*Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut (Email: nickrusso@g.ucla.edu);* [2]*Department of Biological Sciences, University of North Texas, Denton, Texas, USA;* [3]*Sub-Antarctic Biocultural Conservation Program, University of Magallanes, Puerto Williams;* [4]*Pontifical Catholic University of Chile, Santiago, Chile;* [5]*Advanced Environmental Research Institute, Denton, Texas, USA; and* [6]*Universidad de Magallanes, Punta Arenas, Chile*

Abstract Bryophyte consumption is uncommon among bird species globally and is often presumed incidental. We sought to determine whether herbivorous bird species of the high Andes, including the white-bellied seedsnipe (*Attagis malouinus*) and *Chloephaga* geese (*C. picta* and *C. poliocephala*), consume bryophytes, and if so, how frequently. We collected 26 seedsnipe and 22 goose droppings from alpine and sub-alpine habitats of Navarino Island, Chile and examined their contents for bryophyte diaspores. We detected bryophyte fragments in 84.6% and 90.9% of seedsnipe and *Chloephaga* goose faecal samples, respectively. We also extracted DNA from three bryophyte fragments isolated from goose droppings and sequenced three chloroplast loci for each sample. We inferred through a barcoding analysis that at least one species of *Chloephaga* goose consumes *Polytrichum strictum* and *Notoligotrichum trichodon*. The composition of 11 collected goose droppings was >50% Polytrichaceae bryophyte fragments, suggesting that at least one *Chloephaga* goose species foraged deliberately on moss species of this family. These new observations suggest that bryophytes are part of the diet of some high Andean birds and that birds might disperse bryophytes internally – via endozoochory – in the sub-Antarctic.

Key words: *Attagis*, barcode, bryophyte, *Chloephaga*, diet, endozoochory, sub-Antarctic.

INTRODUCTION

Bryophytes, or seedless nonvascular plants, are crucial to the generation and maintenance of ecological communities by playing pivotal roles in soil formation and nutrient cycling (Vanderpoorten & Goffinet 2009). Due to their low nutritional value and digestibility, however, they are rarely of direct importance to vertebrate herbivores as primary producers – thus, cases of bryophyte consumption by vertebrates are often presumed incidental (Longton 1988; Parsons *et al.* 2007; Glime 2017). Notable exceptions include Arctic herbivores such as birds, lemmings and ungulates, which are known to consume bryophytes regularly or during food-lean times in habitats where herbaceous cover is sparse (Longton 1988; Martin & Hik 1992). The diet of the barnacle goose (*Branta leucopsis*) on Arctic breeding territory, for example, is composed of >90% bryophytes pre-incubation and >40% during incubation and comprises at least 10 bryophyte species (Prop & Vulink 1992; Stech *et al.* 2011).

Evidence of herbivores consuming bryophytes at high latitudes of the Southern Hemisphere is much more limited. Anecdotal foraging observations suggest that sporophytes, or spore-bearing capsules of *Polytrichum juniperinum* Hedw., are an essential diet item of the green eastern rosella parrot (*Platycercus eximius*) in New Zealand, but it is unclear whether bryophytes are important to bird diets further south in Antarctic and sub-Antarctic habitats (Glime 2017).

Avian consumption of bryophytes could contribute to bryophyte dispersal if propagules survive digestion. The structural integrity of the entire plant need not survive digestion because the gametophyte, or vegetative tissue of bryophytes is totipotent, such that viable fragments can regenerate a plant. Indeed, bryophyte gametophyte fragments recovered from the faeces of spectacled flying fox (*Pteropus conspicillatus*) and mallard (*Anas platyrhynchos*) were cultured successfully, thus confirming endozoochory, or dispersal internal to an animal vector, as a potential dispersal mechanism for bryophytes (Parsons *et al.* 2007; Wilkinson *et al.* 2017).

Bryophytes are important to birds as a substrate for feeding and nesting in habitats where they are most abundant (Glime 2017). The Sub-Antarctic Magellanic Ecoregion of southern Chile hosts about 5% of

*Corresponding author.

Present address: Department of Ecology & Evolutionary Biology, University of California, 621 Charles E. Young Drive South, Los Angeles, CA, 90095, USA

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the world's bryophyte species (Rozzi *et al.* 2008), and to our knowledge, Behling *et al.* (2016) were the first to report birds consuming bryophytes in sub-Antarctic South America. Two of Behling *et al.*'s (2016) study species, upland goose (*Chloephaga picta*) and white-bellied seedsnipe (*Attagis malouinus*), are herbivores of herbaceous plants in bryophyte-rich alpine zones of the sub-Antarctic Andes. We continued studying consumption of bryophytes by these two species and the ashy-headed goose (*Chloephaga poliocephala*) and report that seedsnipes and at least one species of *Chloephaga* goose consume bryophytes regularly in alpine areas of Navarino Island, Chile, and that on occasion, bryophyte consumption by at least one species of *Chloephaga* goose is deliberate.

METHODS

Faecal sample evaluation

We collected dry droppings of white-bellied seedsnipe and upland or ashy-headed goose (*C. poliocephala*) opportunistically along the Dientes de Navarino trail of Navarino Island, Magallanes, Chile between 30 November 2017 and 19 January 2018. Seedsnipe droppings are distinctive, cylindrical pellets. Upland goose droppings, however, cannot be distinguished visually from those of the sympatric ashy-headed goose. Although upland goose is typically more abundant at all elevations, we will refer to goose faecal samples as belonging to a *Chloephaga* goose. We sampled from alpine and sub-alpine areas, including lakes and stream banks (410–789 m asl; Fig. 1). Except for two droppings kept frozen before we created a storage protocol, we kept all faecal samples dry at room temperature for two to four weeks prior to examination.

To reduce potential contamination from windborne spores or gametophyte fragments, we removed as much of the hardened outer layer of each faecal sample as possible. We then sliced a 1-mm-thick disc from one end of each goose and seedsnipe faecal sample and diluted it in filtered, deionised water. We examined the contents of each disc under a compound microscope at 40× magnification, collected all plant fragments that possessed bryophyte characteristics and photographed each specimen.

We isolated two sporophyte fragments from one goose dropping and a sporangium fragment from a second goose dropping and extracted DNA from the specimens using a modified cetyltrimethylammonium bromide (CTAB)-spermidine lysis method (Doyle & Doyle 1990). Both droppings visually contained abundant bryophyte sporophyte fragments and were collected from two different sites. We amplified three plastid loci (i.e. the intergenic spacers *psb*A-*trn*H and *atp*B-*rbc*L, and the *trn*L-*trn*F region, comprising the *trn*L intron, the 3′exon and the *trn*L-*trn*F intergenic spacer) for the three bryophyte fragments following a nested PCR approach. We targeted loci using one pair of primers in the first PCR and amplified its products for the second PCR using internal primers (Appendix S1). All PCR amplifications were performed based on the following profile: a hot start denaturation step of 3 min at 94°C, followed by 40 cycles of denaturation (1 min, 94°C), annealing (1 min, 54°C) and extension (1 min, 70°C), ended by a final extension step of 10 min. PCR products were cleaned using the ExoSAP-IT protocol (USB–Affymetrix, Cleveland OH, USA) and sequenced using the internal

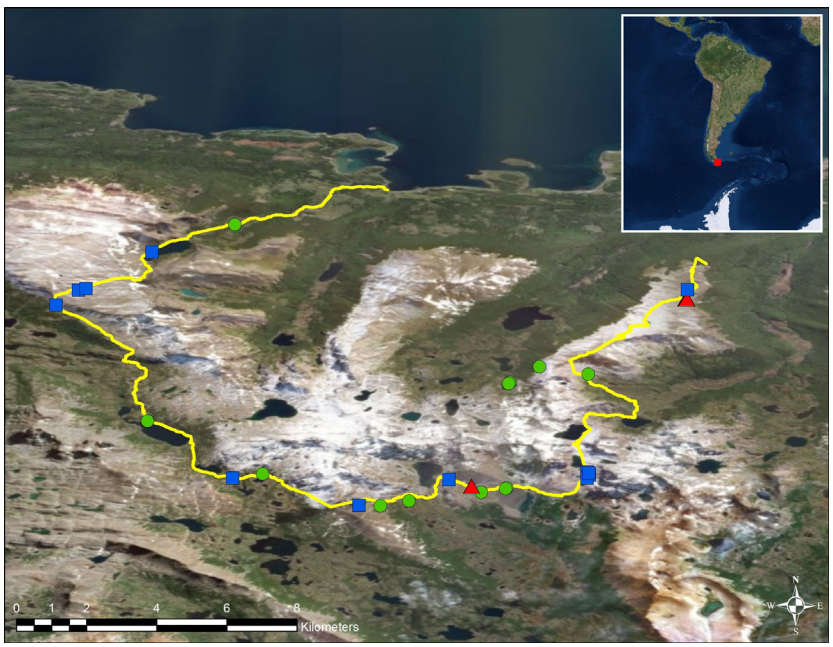

Fig. 1. Collection sites of *Chloephaga* goose and white-bellied seedsnipe faecal samples along the Dientes de Navarino trail (yellow) of Navarino Island, Chile. Green circles correspond to goose dropping collection sites, blue squares correspond to seedsnipe dropping collection sites, and red triangles correspond to sites where droppings of both species were collected.

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primers (Appendix S1) on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City CA, USA). Sequences were edited manually and assembled in contigs using Sequencher v. 4.9 (Gene Codes Corp.; http://www.genecodes.com/). Comparison of the sequences obtained for two sporophyte fragment specimens to those in GenBank using the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed a match to sequences of *Notoligotrichum* G.L.Sm. (Polytrichaceae). To confirm this, we extracted DNA from dried tissue of five sub-Antarctic specimens of *Notoligotrichum* (*N. minimum* (Cardot) G.L. Sm.; *N. tapes* (Müll. Hal.) G.L. Sm.; *N. tapes var. apiculatum* (Cardot) Schiavone; and two *N. trichodon* (Hook. f. & Wilson) G.L. Sm.) and amplified and sequenced the three plastid loci following the same protocol as above. We compared sequences from faecal specimens to those of reference specimens using a sequence similarity matrix in BioEdit v. 7.2.5 (Hall 1999; latest version downloaded from https://bioedit.software.informer.com), after trimming to the shortest sequence (303 bp for *atp*B-*rbc*L, 178 bp for *psb*A-trnH and 263 bp for *trn*L-*trn*F).

RESULTS AND DISCUSSION

We detected bryophyte fragments in 22 of 26 seedsnipe faecal samples (84.6%) and in 20 of 22 (90.9%) goose faecal samples (Fig. 2). We recovered a total of 550 putative bryophyte fragments, including 238 from seedsnipe droppings and 312 from geese. Ten of the bryophyte fragments were vegetative stem fragments with intact leaves (nine from goose faeces and one from seedsnipe faeces). Eleven goose faeces were composed of approximately 50–95% sporophyte fragments by visual estimation of the crushed contents of the entire faecal sample. These findings suggest that *Chloephaga* geese and white-bellied seedsnipe regularly consume bryophytes in sub-Antarctic Chile, and that on occasion, at least one *Chloephaga* goose species feeds primarily on bryophyte sporophytes.

Of the eleven goose faeces with high sporophyte content, three were collected from a wet meadow on the Dientes de Navarino trail (55°01′12.7″S, 67°42′21.6″W) and were composed of approximately 50–80% sporophyte fragments (Fig. 3). The nematodontous peristome, a ring of microscopic teeth lining the mouth of the spore-bearing capsule, was still present on some capsules, and its architecture linked them to the family Polytrichaceae (Goffinet *et al.* 2009). We inferred two bryophyte fragments extracted from one of the droppings as *Notoligotrichum trichodon* after comparing sequences of three plastid loci (*psb*A-*trn*H, *trn*L-*trn*F and *atp*B-*rbc*L) with those of reference *Notoligotrichum* specimens generated for this study. Overlap of sequences of our unknown bryophyte fragments was consistently larger with those of *N. trichodon* (97–100%) than the other sympatric species of the genus. *Notoligotrichum trichodon* occurs on wet or moist soil in meadows above tree line on Navarino Island, Chile (Buck & Goffinet 2010).

The remaining eight goose faeces with high sporophyte content were collected from a wet meadow on the Dientes de Navarino trail (54°56′57.7″S, 67°45′02.2″W) and were composed of approximately 80–95% bryophyte sporophytes. Again, these fragments were visually identifiable as belonging to the family Polytrichaceae. We inferred the representative sporangium fragment from one of the droppings as belonging to *Polytrichum strictum* after a BLAST search of the *trn*L-*trn*F sequence revealed a 100% overlap with five homologous sequences of this species (GenBank

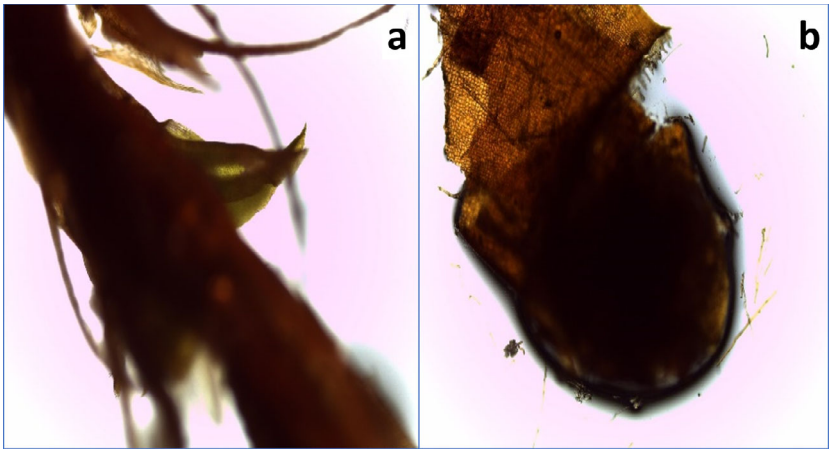

Fig. 2. Bryophyte fragments recovered from faeces of upland or ashy-headed goose on Navarino Island, southern Chile. All specimens were photographed at 40×. (a) Stem fragment and leaves of Polytrichaceae spp. (b) Sporangium of *Polytrichum strictum*.

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Fig. 3. *Chloephaga* goose faeces collected on Navarino Island, Magallanes, Chile, visibly containing reddish setae (sporophyte stalks) of *Notoligotrichum trichodon*.

Accession Numbers: GU569759.1; GU569758.1; AF257782.1; MF180583.1; MF180580.1).

Our observations of abundant moss fragments in 11 *Chloephaga* goose faecal samples from two sites suggest that at least one species of *Chloephaga* goose deliberately consumed bryophytes of the family Polytrichaceae. *Polytrichum strictum* often forms extensive mats with abundant long sporophytes bearing a conspicuous capsule (Goffinet *et al.* 2012), such that ingestion of sporophytes merely as a by-catch when feeding on vascular plants is unlikely. *Notoligotrichum trichodon* is small, but still forming homogenous mats, with yellowish sporophytes contrasting well against the dark mineral soil. Prins (1982) speculated that herbivores consume bryophytes in polar habitats for their arachidonic acid content, which is not available in vascular plants and thought to maintain the integrity of cell membranes in cold temperatures. Birds in the Arctic and sub-Arctic may, however, consume bryophytes when other sources of food are unavailable, such as when migrants reach the breeding territory before vascular plants begin flourishing (Hohman 1985; Prop & Vulink 1992). One study suggested that moss spore capsules are important to the chick diet of willow ptarmigan (*Lagopus lagopus*; Martin & Hik 1992). As we are unable to determine either the age of the geese that deposited faeces containing abundant sporophyte fragments or the time of year the bryophytes were eaten, we suggest further research on the diet of upland and ashy-headed goose across life stages and throughout the annual cycle.

Bryophytes establish new individuals and populations following the dispersal and establishment of spores or vegetative fragments. Wind is likely the predominant vector above tree line (e.g. Robinson & Miller 2013) whereas in forests, passerine birds may play an important role in shaping the spatial distribution of bryophytes by carrying propagules in their plumage (Chmielewski & Eppley 2019). Seasonal movements of seedsnipes and geese could provide the mechanism for dispersal of Polytrichaceae among habitats suitable for establishment. Although white-bellied seedsnipes spend the breeding season in alpine zones, they have been recorded further north and at sea level in winter (J. Jiménez, pers. Obs., 2018). Individual upland geese have been shown to displace northward up to 2789 km during migration, thus potentially interacting with mosses over a broad land area (Pedrana *et al.* 2018). Germination experiments of bryophytes recovered from bird faeces are necessary to explore the potential for birds to disperse bryophytes via endozoochory.

Although most evidence of birds consuming bryophytes is anecdotal, the regularity with which upland or ashy-headed goose and white-bellied seedsnipe consume bryophytes warrants empirical studies on the potential for bird–bryophyte interactions to shape sub-Antarctic ecosystems through herbivory and endozoochoric dispersal. Very little is known about the white-bellied seedsnipe diet (Fjeldså & Kirwan 2019), and this study suggests that they consume bryophytes regularly. Behavioural studies could confirm whether bryophyte consumption by seedsnipes is intentional. Bryophytes are thought to be unimportant to herbivore diets due to their low nutritional value, but our study shows that, on occasion, at least one *Chloephaga* goose species targets sporophytes of Polytrichaceae mosses as a food source.

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AUTHOR CONTRIBUTIONS

Conceptualization-lead, data curation-lead, formal analysis-lead, funding acquisition-equal, investigation-

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lead, methodology-lead, project administration-lead, resources-equal, software-equal, supervision-lead, validation-lead, visualization-lead, writing-original draft-lead, writing-review & editing-lead: N.R. conceptualization-equal, data curation-equal, formal analysis-supporting, investigation-equal, methodology-equal, project administration-equal, resources-equal, software-equal, supervision-equal, validation-equal, visualization-equal, writing-original draft-supporting, writing-review & editing-supporting: M.R. formal analysis-supporting, investigation-supporting, methodology-supporting, project administration-supporting, resources-equal, software-supporting, validation-equal, writing-review & editing-supporting: R.M. conceptualization-equal, formal analysis-supporting, funding acquisition-supporting, investigation-equal, methodology-equal, project administration-equal, resources-equal, software-equal, supervision-supporting, validation-supporting, visualization-equal, writing-review & editing-supporting: B.G. conceptualization-supporting, funding acquisition-lead, investigation-supporting, methodology-supporting, project administration-supporting, resources-equal, writing-review & editing-supporting: J.J.

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SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Primers used in PCR and sequencing reactions in this study.

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Supplemental Data

Table S1. Primers used in PCR and sequencing reactions in this study. Internal primers used for nested PCR are marked with a footnote (†).

Region	Primer	Sequence 5'-3'	Reference
*atp*B-*rbc*L	atpb-0	TCCTCTCATYAARCCATCTG	New
	rbcl-0	GGAGTCATWCGAAATGCTGCT	New
	atpb-1a†	ACRTCTAATACRGGWCCAATAA	modified from Chiang *et al*. (1998)
	rbcl-1a†	AACACCAGCTTTAAATCCAA	modified from Chiang *et al*. (1998)
*psb*A-*trn*H	psbA-F0	GCTGGTGTATTCGGTGGC	New
	trnH-R1	GAACGACGGGAATTGAAC	(Pedersen and Hedenäs 2003)
	psbA-F1†	CTGCTCACGGTTACTTTG	New
	trnH-R0†	ACTGCCTTAATCCACTTG	New
*trn*L-F	trnF-0	AGYGCWGATTTTCAAGAACG	New
	trnC-0	TACAAGTGCGGTGCTCTGAC	New
	trnF-1†	ATTTGAACTGGTGACACAAG	modified from Taberlet *et al*. (1991)
	trnC-1†	CGGAATTGGTAGACGCTACG	modified from Taberlet *et al*. (1991)

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