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RESEARCH

Ecology and taxonomic identification of āwheto | vegetable caterpillar in Hawke's Bay, Aotearoa | New Zealand

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Abstract: Āwheto, also known as the vegetable caterpillar, is a taonga (treasure) of Aotearoa | New Zealand, produced when endemic *Ophiocordyceps* fungi infect ghost moth (Hepialidae) larvae and produce fruiting bodies extending from the infected larvae to above the forest floor. Despite its cultural importance, āwheto ecology is not well documented or understood. Partnering with kaitiaki (guardians), we paired mātauranga (Māori knowledge)-informed soil DNA assays with systematic field surveys to resolve āwheto ecology in a montane native forest. ITS2/18S gene metabarcoding detected *Ophiocordyceps* sequences in 92% of random soil samples, however āwheto were found in higher density clustered at a subset of three of these sites (and at 25% of randomly sampled sites). Āwheto occurred exclusively in tall red-beech (*Fuscospora fusca*) forest with partial canopy (c. 75%), deep litter, high bryophyte cover, and minimal bare ground (< 10%). Indicator analysis highlighted pōkākā (*Elaeocarpus hookerianus*) and Hall's tōtara (*Podocarpus laetus*) as positive associates; occurrence was also more likely in west facing higher altitude sites. Sanger sequencing of stromata and moth larvae cadavers was used to identify *Ophiocordyceps robertsii* and its sole host, *Dumbletonius unimaculatus*, with low intraspecific genetic variation. Stroma persisted for > 10 months; motion-detection camera monitoring showed frequent deer visits caused negligible disturbance. These findings demonstrate that, although *O. robertsii* is widespread in soil, successful āwheto formation depends on specific micro-habitats and host distribution. Integrating molecular detection with mātauranga Māori offers a reliable, non-invasive tool for locating āwheto and provides a baseline for kaitiakitanga-led conservation.

Keywords: āwheto, entomopathogen, ghost moth, Hepialidae, *Ophiocordyceps robertsii*, vegetable caterpillar

Introduction

Āwheto, also known as the vegetable caterpillar, represents an interaction between endemic fungi of the genus *Ophiocordyceps* and native ghost moth caterpillars (Hepialidae) in Aotearoa | New Zealand. The fungus infects late instar larvae, mummifies them within their burrows, and produces a slender stroma that protrudes above the soil to release spores. Māori have long regarded āwheto as a taonga, using it for tā moko (tattoo) pigment, food, and rongoā medicine (Riley, 1994; Fuller et al. 2005).

Ecological information on āwheto is scarce. Existing knowledge is largely anecdotal or from a study by Casonato and Hill (2022), that hints at associations with native trees such as hīnau (*Elaeocarpus dentatus*) and tawa (*Beilschmiedia tawa*) (Casonato & Hill 2022). Often, the species identity of the fungus and the host are not known. Two fungal species, *Ophiocordyceps robertsii* and *Ophiocordyceps hauturu* are known to form āwheto, with *O. robertsii* thought to be widespread in forest soils (Dingley 1953). Seven genera of ghost moths, six of which are endemic, are found in Aotearoa | New Zealand. The larvae of one species, the Pūriri moth (*Aenetus virescens*), reside in trees (Dugdale 1994). Larvae of other species, such as *Aoraia enysii* (Butler 1877), *Dumbletonius characterifer* (Walker 1865), and *Dumbletonius unimaculatus* (Salmon 1948), inhabit the forest floor and are potential hosts for āwheto (Dugdale 1994, 1996).

Reliable data are still lacking on āwheto habitat requirements and distribution patterns, and the utility of molecular tools for detecting both fungus and host. In collaboration with a Māori entity (name withheld to maintain anonymity of land block; hereafter referred to as kaitiaki

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[trustees] at their request), we first combined molecular methods with mātauranga (Māori knowledge) to guide our surveys and we then used ecological assessments to describe the āwheto habitat. The study addresses three key research aims: (1) To evaluate whether systematic molecular and field survey methods can reliably locate āwheto populations in native forests, (2) To determine if *O. robertsii* is the dominant fungal species responsible for āwheto formation, and (3) To identify environmental factors, including vegetation composition, soil characteristics, and host moth distribution, that most influence the presence of āwheto. The research improves ecological understanding of āwheto and supports practical conservation and management strategies.

Methods

Āwheto search strategy

Kaitiaki invited Manaaki Whenua – Landcare Research scientists to undertake research as manuhiri (visitors) within a specific forestry block in the eastern North Island. To avoid potential exploitation of this taonga, the location details are not disclosed in this paper. The forestry block (c. 15 000 ha) comprised extensive stands of mixed indigenous forest primarily at higher elevations, surrounded by a commercial plantation of *Pinus radiata* at lower elevations. The indigenous forest area is regularly subjected to an aerial pest control programme. We made visits to the area in February, April, and December 2021.

To determine whether *Ophiocordyceps* spp. were present, soil samples were collected by kaitiaki at 25 random sites during late 2019 and early 2020, scattered across both indigenous forest areas and the *P. radiata* plantation. Sites where *Ophiocordyceps* spp. sequences were detected in the soil samples were considered potential āwheto sites. The final selection of search sites was made in collaboration with kaitiaki by incorporating mātauranga Māori with historical information as well as forest and abiotic site characteristics.

In February 2021, we searched for āwheto aboveground structures. Teams explored 100-metre radial transects along random bearings from the selected sites. If āwheto were found, the search was extended into adjacent areas. Structured āwheto searches were also undertaken on stratified random 20 × 20 m vegetation plots (see below) by surveying the forest 0.5 m either side of the internal centre lines of the 20 × 20 m plot area. Travel to and from vegetation plots provided additional opportunities for unstructured searches.

Characterising āwheto habitat

To characterise the forest habitat of āwheto, permanent 20 × 20 m vegetation plots centred on the densest āwheto populations were established (three subjectively located plots). A standard Recce vegetation description (Hurst et al. 2022) was undertaken on each plot. A Recce vegetation description documents all plant species within a bounded area; each species is recorded into a series of height tiers: tier 1 (> 25 m), tier 2 (12–25 m), tier 3 (5–12 m), tier 4 (2–5 m), tier 5 (0.3–2 m) and tier 6 (< 0.3 m); if present in a height tier a species is given a cover class score based on its abundance over a horizontal plane within that tier.

Stratified random plots (n = 20) were established and surveyed during the final visit to the area in December 2021 to better understand the distribution of āwheto in the research area and to look for further relationships between āwheto and their habitat. A master sample was used to randomly sample the section of the research area dominated by indigenous forest (Van Dam-Bates et al. 2018). A map was clipped of forested areas by LCDB v5.0, categories Broadleaved Indigenous Hardwoods and Indigenous Forests, within 300 m of roads (1:500k; LINZ 2022a) or tracks (1:50k; LINZ 2022b). The plot locations were generated by overlaying a grid and grid intersection. At each random plot a Recce vegetation description on a 20 × 20 m plot area was undertaken (Hurst et al. 2022).

Āwheto longevity and animal monitoring

Whenever clusters or isolated individuals of āwheto were discovered, they were georeferenced, and at our three primary sites they were marked using both pink flagging tape and permolat on an aluminium peg. This method enabled the tracking of individual āwheto throughout the research period. To investigate the risk of āwheto predation or disturbance by animals, 15 individuals were caged to prevent predation and disturbance across the three primary sites. Two motion detection cameras were set up at two sites, targeting groups of both caged and uncaged āwheto individuals. The cameras operated in two periods: from February to April 2021 (one site) and from April to October 2021 (both sites). Additionally, 15 chew cards were distributed across the main sites to assess the presence or abundance of non-native mammals between February and October 2021.

Sample collection and processing

Soil samples were collected by scooping approximately 50 ml of the top 10 cm of soil into falcon tubes. After transport to the laboratory, they were stored at −20 °C for up to one month until analysis.

Four representative āwheto structures were carefully excavated from the forest ground and stored at 4 °C upon arrival at the laboratory. A 10 mg subsample was sliced from the caterpillar cadaver with a sterile scalpel blade for subsequent DNA extraction and host identification. The stroma tips were removed with a sterile scalpel blade. In addition, stroma tips were also removed on site from āwheto that were not excavated and stored at 4 °C upon arrival at the laboratory. Stroma samples were used for fungal cultivation and subsequent DNA extraction and fungal identification. The āwheto structures were dried and deposited in the New Zealand Fungarium - Te Kohinga Hekaheka o Aotearoa (PDD).

Fungal isolation and cultivation

To cultivate and identify *Ophiocordyceps* spp. from the stroma samples, methods outlined by Casonato and Hill (2022) were followed with modifications. The culture medium comprised 1.95% potato dextrose broth, 1.5% agar, and 0.1% yeast extract. Incubation occurred at 20 °C and 80% humidity, following a 12:12 h light-dark cycle.

Three successful methods were employed to generate *Ophiocordyceps* spp. cultures: (1) Approximately 1 cm of perithecia-containing stroma was sliced longitudinally and placed inside the lid of a Petri dish containing the culture media. The closed Petri dish was then incubated upside down. Once the discharged spores sporulated on the culture media (typically within 24 to 48 hours), the lid containing the stroma was replaced with a clean lid. The Petri dish was flipped and incubated for an additional week. (2) The stroma was cut longitudinally using a sterile scalpel blade to extract the perithecia. These perithecia were then plated onto the

culture media for incubation. (3) A fine, slightly hooked needle was used to penetrate the ostiole, allowing the removal and transfer of spores from the perithecia on to the culture media for incubation.

The *Ophiocordyceps* spp. cultures were incubated for up to six months before a single hyphal tip was transferred onto fresh culture media to establish a pure culture. To create culture stocks, agar plugs were frozen in 50% glycerol at −80 °C. The pure cultures were maintained on the culture media and deposited at the International Collection of Microorganisms from Plants (ICMP). A reference culture of *O. robertsii* was obtained from the ICMP (ICMP16396) and preserved in the same manner until DNA extraction.

Host collection and identification

Given the likely host caterpillar inhabits the forest floor, several pitfall traps were set on the forest floor in the vicinity of known āwheto sites to capture ghost moth caterpillars. Given males of *Dumbletonius unimaculatus*, a likely host, fly in the earlier part of the night, and are easily attracted to lights, light traps were also deployed from dusk onward and through the night in an attempt to collect adult moths. Several 1 × 1 m holes were also dug in a vicinity of located āwheto to find ghost moth caterpillars in their burrows.

DNA extraction, PCR, and sequencing

From the soil, DNA was extracted using MN Soil DNA manual extraction kit (Macherey-Nagel, Düren, Germany). Samples were lysed using a combination of SL1 and SX lysing buffers and incubated at 65 °C for 20 min with shaking. Samples were then homogenised using a GenoGrinder (SPEX SamplePrep, Metuchen, NJ, USA) with 1750 clamp strokes $\cdot min^{-1}$ for 20 min in 5 min intervals. The rest of the extraction protocol followed the manufacturer's instructions. DNA was eluted in 2 × 50 µl of PCR grade H_2O.

From the fungus, DNA was extracted using two-month-old pure cultures, carefully scraping mycelium off the surface to avoid agar, using the Fungi/Plant Isolation kit (Norgen Biotech, Thorold, Canada), per the manufacturer's instructions.

From āwheto caterpillar tissue, DNA was extracted using MN XS Tissue kit (Macherey-Nagel) according to the manufacturer's protocol and eluted in 2 × 15 µl. As reference, a leg of *Dumbletonius unimaculatus* (Hepialidae) was obtained from the New Zealand Arthropod Collection (NZAC, accession number: OQ699135) and processed in the same manner.

For fungal identification in DNA from fungal cultures, PCRs were performed to amplify a 450 bp fungi-specific ITS2 gene region with primers fITS7 (5′-GTGARTCATCGAATCTTTG) (Ihrmark et al. 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC) (White 1990), a 576 bp ITS1 region with primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) (White 1990) and ITS4, and a 324 bp 18S rRNA gene region with primers #3 (5′-GYGGTGCATGGCCGTTSKTRGTT) and #5RC (5′-GTGTGYACAAAGGBCAGGGAC) (Drummond et al. 2015). To identify the insect host in āwheto and insect samples, PCRs were performed to amplify a 710 bp COI region with primers LCO1290 (5′-GGTCAACAAATCATAAAGATATTGG) and HCO12198 (5′- TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994). The 20 µl reactions contained 1 µg rabbit serum albumin, 10 µl 2 × KAPA Plant 3G Buffer (Kapa Biosystems, Wilmington, MA, USA), 0.2 µl KAPA Plant 3G enzyme, 500 nM of each target primer, and 2 µl of the DNA extract as a template. The cycling conditions were 95 °C for 3 min and 30 cycles of 95 °C for 30 sec, 53 °C for 30 sec, and 72 °C for 30 sec; followed by 72 °C for 2 min. PCR products were analysed by Sanger sequencing.

DNA from soil samples were used to amplify the 450 bp ITS2 and 324 bp 18S regions as described above. The PCR products were used as templates in a second PCR reaction to prepare the samples for Illumina sequencing. The 25 µl reactions contained 2 µg rabbit serum albumin, 12.5 µl 2 × KAPA Hifi ReadyMix Buffer (Kapa Biosystems), 500 nM of Illumina-tagged sequencing adaptors, and 1 µl of the first PCR reaction as a template. The cycling conditions were 95 °C for 3 min and five cycles of 98 °C for 15 s, 65 °C for 15 s, and 72 °C for 15 s; followed by 72 °C for 1 min. The amplicons were treated with SPRIselect magnetic beads (Beckman Coulter, Brea, CA, USA) to remove primer dimers and high molecular weight DNA, according to the manufacturer's instructions. The amplicons underwent quantification using the Qubit assay (ThermoFisher Scientific, Waltham, MA, USA) and were subsequently pooled in equimolar amounts. The combined library was then sequenced using a 2 × 300 bp MiSeq run conducted by Otago Genomics at the University of Otago, Aotearoa | New Zealand.

Bioinformatics

Sequences obtained from Sanger sequencing were processed and aligned in GeneiousPro®10.2.6 (Biomatters Ltd., Auckland, NZ). The sequences were identified by blastn against the NCBI nucleotide database (October 2020) or by comparison to collection reference sequences.

For bioinformatic analysis of Illumina sequences, a pipeline described by Dopheide et al. (2023) was followed. In brief, demultiplexed sequences were merged, adaptors and primers were trimmed, sequences were quality filtered and denoised into Amplicon Sequence Variants (ASVs). Amplicon Sequence Variants were further filtered for chimeras using VSEARCH (v2.14) (Rognes et al. 2016) and cutadapt (v3.5) (Martin 2011). Taxonomic classification of fungal 18S and ITS2 ASVs was conducted using Blast (Zhang et al. 2000) and RDP naïve Bayesian classifier (Wang et al. 2007), respectively. This classification employed Blast nt and UNITE fungal ITS database (sh_general_release_10.05.2021) (Nilsson et al. 2018). Additionally, the ITS2 and 18S sequences from *O. robertsii* (ICMP16396) were utilised as references for the Illumina dataset.

Statistical analysis

To assess vegetation and environmental correlations with āwheto presence, we used (1) indicator species analysis, to assess whether any plant species showed strong fidelity to āwheto presence/absence, and (2) model selection to determine the environmental variables that best predicted āwheto presence/absence. We assessed āwheto presence/absence only, even though count data were available, as we considered this was appropriate for the number of replicates available. Following the indicator species and environmental variables assessment, we present an ordination to show overall patterns in vegetation and āwheto presence/absence.

Indicator species analysis

Indicator species analyses were originally proposed by Dufrene and Legendre (1997), and calculate the indicator value, based

on fidelity and relative abundance of species in clusters. We used the "labdsv" package (Roberts 2016) and function *indval* to undertake the indicator species analysis. The significance of each species' indicator value was measured by a site randomisation procedure. In our case, the clusters were two: one cluster was plots where āwheto was present, and the other cluster, plots where āwheto was absent. The response variable was continuous cover percentages for each plant species. We selected the plant species where indicator value for the clusters was significant ($p < 0.05$). We present average abundance and frequency (i.e. presence/absence for each plant species across plots) for these indicator species by āwheto presence/absence in the results, including boxplots.

Environmental variables assessment

To assess the variables that were most associated with āwheto presence and absence, we used model selection comparing a global model of all relevant variables (mean top height, canopy percentage, altitude, drainage, plot slope, sin(aspect), cosine(aspect), physiography, litter cover, and bare ground cover). We used the *dredge* function from package "MuMIn" (Bartoń 2023), specifying that candidate models could only have a maximum of one variable. We imposed this constraint due to the number of observations we were working with. We created a model selection table from the best models, using the criteria that the change in Akaike's information criterion (delta AICc) was < 2 (Burnham & Anderson 2002). There were three models that were favoured over the null (intercept-alone) model. We present boxplots of the variables from these top three models.

Overall composition

We undertook a non-metric multidimensional scaling (NMDS) ordination of the vegetation using the binary version of Jaccard distance and then tested the fit of environmental variables to the ordination; we used the functions *metaMDS* and *envfit* from the R package "vegan" to do so (Oksanen et al. 2022). The stressplot for the ordination is shown in Appendix S1 in Supplementary Material. The environmental fit assumes a linear relationship between continuous variables and the ordination; we visually inspected the results of non-linear analysis using *ordisurf* from package "vegan" to assess whether any non-linear relationships of relevance to āwheto presence/absence were detected. No such effects were detected, and therefore we present (1) the ordination with an overlay of presence/absence of āwheto and associated ellipses (using "vegan" function *ordiellipse*); (2) the ordination with species positions overlain; and (3) the ordination with the environmental fit overlain.

Results

Āwheto molecular signals in soils

After sequence trimming and filtering, the total number of reads were 19.3 million in the 18S and 5.6 million in the ITS2 datasets. Sequencing failed for two 18S samples, and the remaining 25 samples had 384 419 reads on average. Sequencing failed for eight ITS2 samples, and the remaining 19 samples had 147 458 reads on average. The total number of operational taxonomic units (OTUs) were 10 842 for 18S and 5281 for ITS2.

In the 18S dataset, two 18S OTUs were identified as *O. robertsii*, both matching the same GenBank accession (*O. robertsii* strain YHORZT007 from China, accession KC561978). OTU1924 was 88.8% identical (331 bp) and OTU4418 was 98.4% identical (327 bp) to KC561978. OTU4418 was found in 23 out of 25 samples (Fig. 1a). The average number of 18S reads in the 23 *O. robertsii* positive samples was 149.9 (min = 2, max = 1191). The 18S sequence of OTU4418 was 98.1% identical to the ICMP16396 *O. robertsii* reference (320 bp).

In the ITS2 dataset, no OTUs were identified as *O. robertsii*. There were matches to other *Ophiocordyceps* species (*Ophiocordyceps rubiginosiperitheciata*, *Ophiocordyceps formicarum*, *Ophiocordyceps prolifica*, and unidentified), but they were short or had low identities (e.g. 34 bp with 100% identity or 160 bp with 89% identity). Screening the data with the ITS2 sequence from the ICMP16396 *O. robertsii* reference identified OTU3429 with 86.6% identity over 256 bp. The main difference between the sequences was a missing 24 bp GC region in OTU3429. Blastn of OTU3429 on GenBank found the highest identity with *Hirsutella rhossiliensis* (an asexual species in the same family Ophiocordycipitaceae, 99.6% ID, 265 bp, AY745253.) Screening the ITS2 OTU taxonomic assignments for 100% matches to Ophiocordycipitaceae identified three more OTUs in our dataset: OTU1742 (*Purpureocillium lavendulum*, LS422085), OTU233 (*Elaphocordyceps*

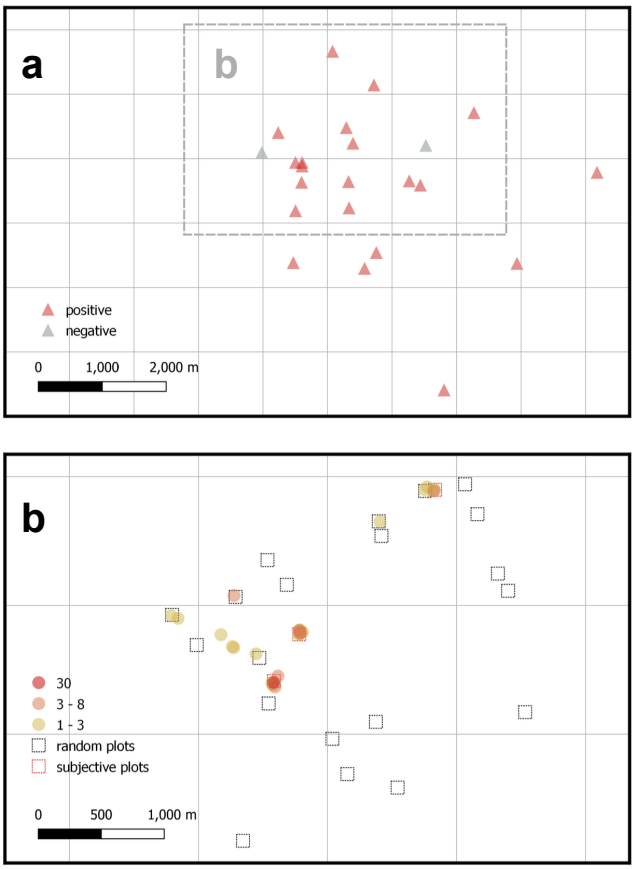

Figure 1. Soil sampling and āwheto distribution. (a) triangles indicate soil sampling sites and *Ophiocordyceps* spp. detection. Red indicates a positive DNA signal and grey a negative DNA signal. The dashed rectangle denotes the region that is magnified in panel b. (b) dashed squares indicate plot locations. Red are subjective locations and black are random locations. Circles indicate individuals and clusters of āwheto. Yellow = 1–3 āwheto, pink = 4–8 āwheto, crimson = 30 āwheto. Symbols are not to scale.

subsessilis, LS422399), and OTU1054 (*Elaphocordyceps* sp., LS422157). The four Ophiocordycipitaceae ITS2 OTUs were found in 12 of the 19 ITS2 samples, and these samples were also positive for *O. robertsii* according to the 18S data, see above (Fig.1a). The average number of ITS2 reads in the 12 Ophiocordycipitaceae positive samples was 94.8 (min = 2, max = 474).

These results were discussed in wānanga (workshop) with kaitiaki who also contributed knowledge of the land, and natural and cultural history. Wānanga resulted in identification of nine field sites used in our first field survey.

Āwheto distribution, abundance, and longevity

Three site visits in February, April, and December 2021 yielded a total of 104 observations of individual āwheto. These findings comprised mainly clustered āwheto, with the largest cluster being 30 individuals, but isolated individuals were also found (Fig. 1b). Most of the āwheto (79%) were found at three search sites identified by the soil DNA survey and consultation with kaitiaki. At six search sites (sites with positive soil signal), and in fifteen out of twenty random vegetation plots, no āwheto were found, noting that after the initial discovery (at the first search site) the initial search focus was narrowed to sites with similar vegetation and topography to the first site. Five out of twenty random vegetation plots had āwheto, in total ten āwheto in an area of 8000 m² (Fig. 1b). Based on this, we make the conservative (given the limited search effort in each plot) estimate of 12–13 āwheto per hectare across the indigenous forest-dominated research area.

In terms of longevity, all but three of the āwheto discovered in February and April were successfully rediscovered in December. The missing three āwheto were uncaged. Among the remaining 101 āwheto, only two appeared to have undergone physical decay during the 10-month study period.

Fungal and moth identification

The fungal stroma visible on the forest floor (Fig. 2a) typically measured up to 20 cm in length and was covered by dark brown, spore-containing perithecia on the upper part (Fig. 2c). Perithecia were generally oval, measuring 0.4–0.5 × 0.6–0.75 mm,

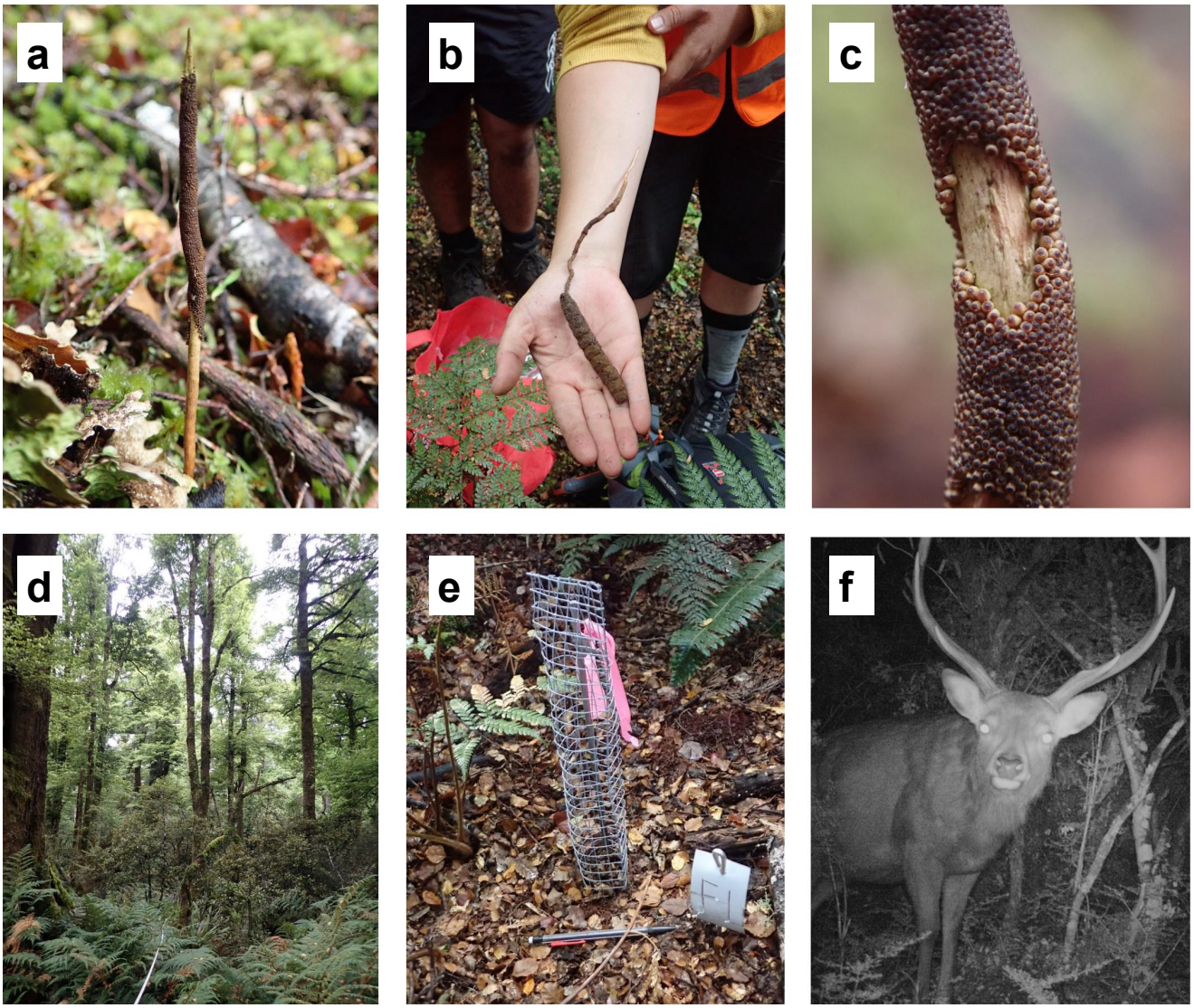

Figure 2. (a) *O. robertsii* stroma on the forest floor; (b) an āwheto structure with the mummified *Dumbletonius* spp. caterpillar and protruding fungal stroma; (c) āwheto perithecia on a stroma; (d) typical tawhairaunui forest where āwheto was found; (e) a cage around āwheto stroma; (f) an 8 point red deer stag visiting an āwheto site.

discrete yet densely packed. In total, five pure fungal cultures were derived from the āwheto structures. Live cultures were submitted to the ICMP collection (ICMP 24365, 24366, 24367, 24368, 24369). The ITS1, ITS2, and 18S sequences were identical between the isolates, except for a single base pair deletion in the ITS1 of strain ICMP24365. The highest sequence identities of ITS1 in GenBank ranged from 99.6% to 100% (510 bp alignment) compared to five *O. robertsii* strains from New Zealand (accession numbers: KC167174, KC167175, MW836814, MW836788, MW832244). After collecting samples from the caterpillar cadaver and the fungal stroma of four excavated complete āwheto structures, they were deposited in the PDD collection (PDD 119184, 119185, 119186, 119187). Attempts to capture live larvae and adult moths were unsuccessful. Host identification could be made, however, by molecular analysis and comparing the sequences to a known *D. unimaculatus* (Hepialidae) from the NZAC (accession number: OQ699135). DNA was successfully extracted from two cadavers and their COI sequences exhibited the closest sequence identity (99.1%, 641 bp alignment) to the reference *D. unimaculatus*.

Āwheto habitat indicator species analysis

From the 23 Recce vegetation descriptions completed, 27 vascular plant species were assessed for the indicator species analysis. Two were found to be positively associated with āwheto presence (at $p < 0.05$). These species were pōkākā (*Elaeocarpus hookerianus*; indicator value = 0.6601, p = 0.011) and Hall's tōtara (*Podocarpus laetus*; indicator value = 0.6065, p = 0.030). Pōkākā median presence was 0 (average 0.25) where āwheto were absent, and median presence was 1 (average 0.71) where āwheto were present (Fig. 3a). Tōtara median presence was 0 (average 0.38) where āwheto were absent, and median presence was 1 (average 0.86) where āwheto were present (Fig. 3a). In terms of plant species cover, pōkākā median cover was 0% (average < 1%) where āwheto were absent, and median cover was 2% (average = 4.6%) where āwheto were present (Fig. 3b). For tōtara, median cover was 0% (average 2.1%) where āwheto were absent and median cover was 5% (average 5.1%) where āwheto were present (Fig. 3b).

Environmental variables assessment

In our model selection exercise, we found three univariable models (see Methods) that outperformed the null model (Appendix S2). In order of selection, these were bare ground coverage (negatively correlated with āwheto presence), altitude (positively correlated with āwheto presence), and aspect as it represents easterly-westerly directions; āwheto presence was correlated with plots facing a more westerly aspect (Fig. 4).

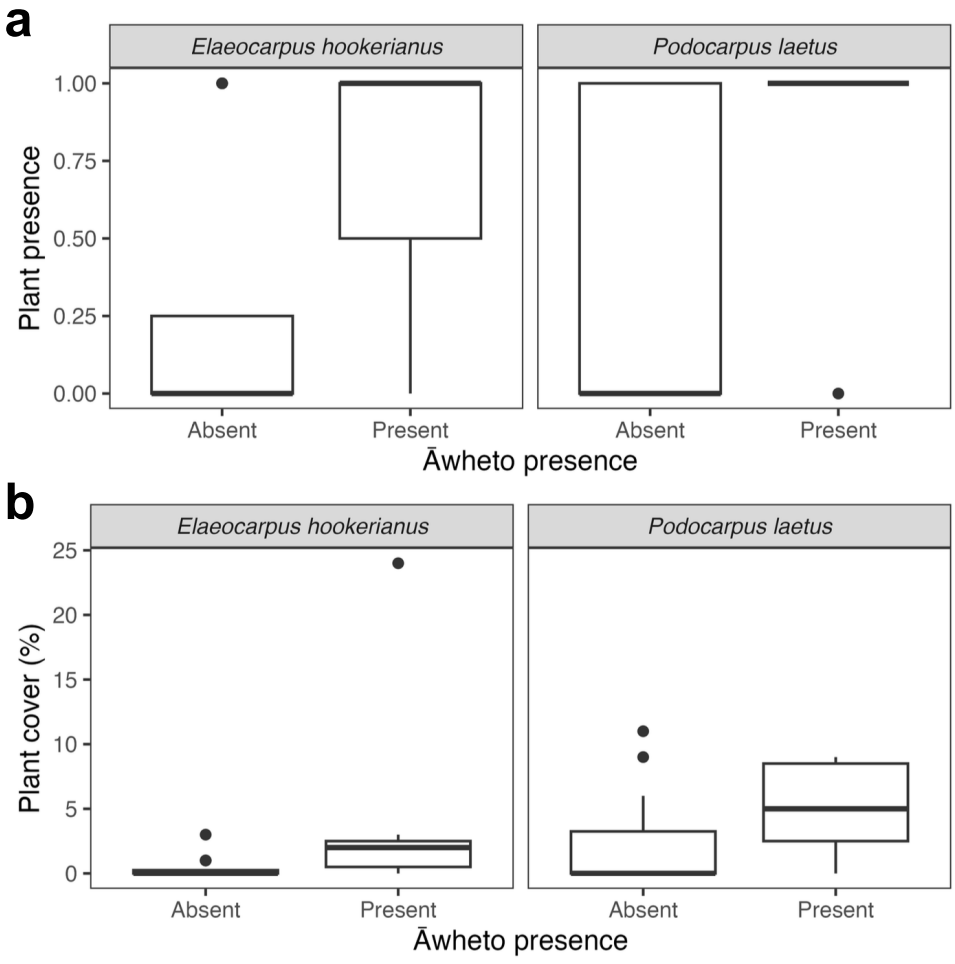

Figure 3. (a) Boxplot of plant species presence, by *āwheto* presence, for indicator species. (b) Boxplot of plant species abundance, by *āwheto* presence, for indicator species.

Overall forest vegetation composition

The ordination of vegetation (using binary presence/absence of vegetation) using two dimensions had a stress value of 0.155. Vegetation plots in which āwheto were present appeared to be more tightly constrained than those from which āwheto were absent, as judged from the relative ellipse sizes (Fig. 5). The species on the left side of Figure 5 (a and b) are primarily tree ferns, and the species on the right side include species more common to mid-successional stages or forest assembly. Consistent with the environmental variable analysis (above), the variable with the highest explanatory power (highest r2 value; see Table 1) was bare ground coverage, followed by sin(aspect), representing the east-west components of aspect. Increasing values of sin(aspect) were associated with the area of the ordination from which āwheto were present (Fig. 5).

Animal presence

The 15 chew cards deployed between February and December did not detect any presence of possums or rodents. Motion detection cameras were set up at two sites encompassing groups of āwheto, including both caged and uncaged individuals. The recorded detection events and species are detailed in Table 2. Among the observed species, deer were the most frequently detected (40 visits), followed by pigs (11 visits) and blackbirds (9 visits). Deer were noted to investigate both the caged and uncaged āwheto, often lingering at these sites, and showing curiosity towards the cameras (Fig. 2f). No signs of pig rooting or disturbance were observed in the vicinity of āwheto at any time.

Motion detection cameras were triggered four times by large moths, potentially *D. unimaculatus*. Alongside the animals recorded on the motion detection cameras, animal browsing was observed at most sites, primarily consisting of deer browse on species such as *Carex uncinata*, *Coprosma foetidissima*, *Coprosma macrocarpa*, *Dicksonia lanata*, *Microlaena avenacea*, *Raukaua simplex*, and *Rytidosperma gracile.*

Discussion

Āwheto host and entomopathogen

This study confirmed *Dumbletonius unimaculatus* as the definitive host of āwheto, based on molecular analysis of caterpillar cadavers. Despite efforts to capture adult ghost moths and caterpillars, none were collected during this study. This

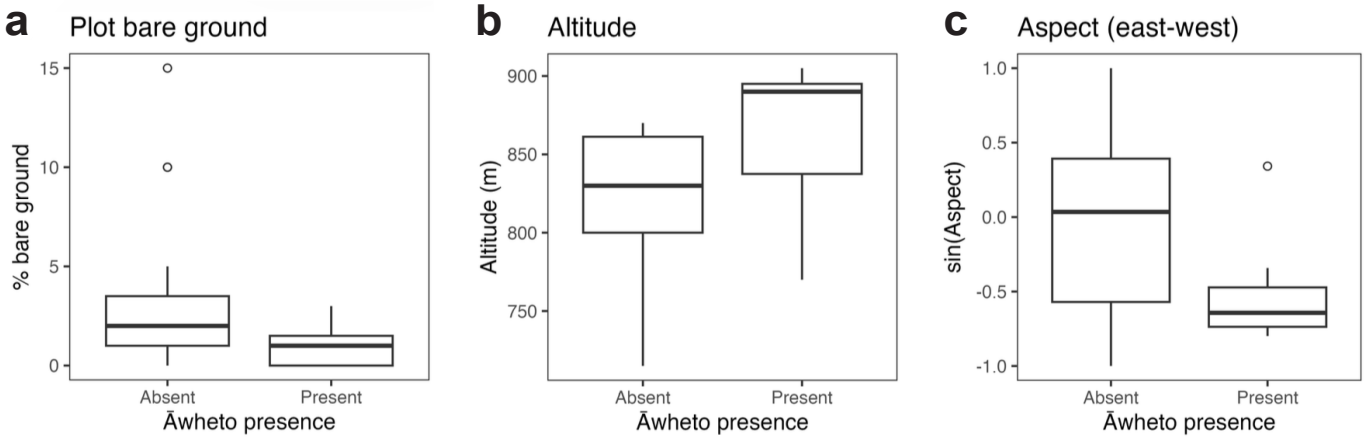

Figure 4. (a) Boxplot of percentage of bare ground in each plot (%) by *āwheto* presence/absence. (b) Boxplot of altitude of each plot by *āwheto* presence. (c) Boxplot of aspect (radians) sin-transformed, to represent the east-west dimensions of aspect, by *āwheto* presence. For sin (aspect), values range between −1 and +1, with +1 directly east and −1 directly west.

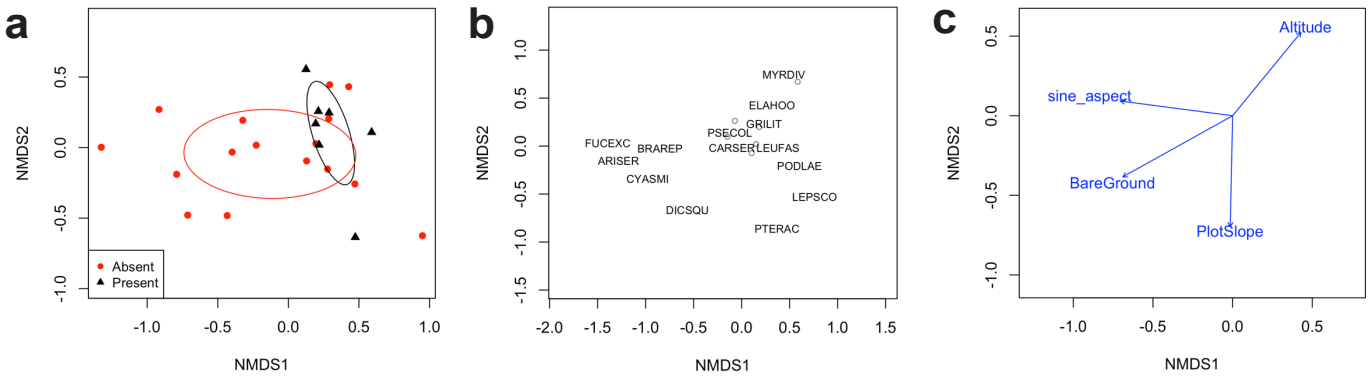

Figure 5. (a) NMDS ordination of 23 vegetation plots, with *āwheto* presence (red) and absence (black) indicated by point colours. Ellipse shows standard deviation of the points. (b) Species positions within the ordination. Only species with > 3 occurrences were considered for inclusion; grey points indicate a species name to avoid overwriting other species names. (c) Environmental covariates overlain on the ordination; only significant variables ($p < 0.05$) are shown.

Table 1. Results of assessment of fit between ordination and selected environmental variables.

Variable	NMDS1	NMDS2	r2	Pr(> r)
MeanTopHeight	0.224	0.975	0.085	0.382
CanopyPercentage	−0.601	−0.799	0.047	0.602
BareGround	−0.874	−0.487	0.470	0.005
Litter	0.061	0.998	0.200	0.111
Altitude	0.634	0.773	0.346	0.015
PlotSlope	−0.021	−1.000	0.367	0.008
sine_aspect	−0.992	0.130	0.383	0.009
cosine_aspect	−0.292	−0.956	0.164	0.175

Table 2. Summary of animals captured by motion detection cameras at āwheto sites.

Animal common	Animal scientific	Total number of visits
Red or sika deer	*Cervus elaphus* or *Cervus nippon*	40
Pig	*Sus scrofa*	11
Blackbird	*Turdus merula*	9
Hedgehog	*Erinaceus europaeus*	6
Moth	Hepialidae family	4
Possum	*Trichosurus vulpecula*	4
Thrush	*Turdus philomelos*	2
Mustelid	*Mustela* species	1
Rat	*Rattus* species	1
North Island robin	*Petroica longipes*	1
Ruru	*Ninox novaeseelandiae*	1

was likely due to the seasonal timing of adult emergence and limited extent of our search for moths and caterpillars. There is only limited knowledge of *D. unimaculatus* ecology. The moth is commonly found in warm temperate forests throughout the North Island and on offshore islands, but is unknown from the South Island (Dugdale 1994). Males fly in the earlier part of the night, mainly from late December to April, and are easily attracted to lights, arriving almost immediately at dusk. Larvae live in vertical shafts in the soil and only emerge to the surface during the night to feed on leaf litter (Dugdale 1994).

The fungal cultures isolated from āwheto were identified as *Ophiocordyceps robertsii*. The three sequenced taxonomic markers were identical for all isolates, apart from one single base pair deletion. Our molecular soil data indicated that *O. robertsii* could be detected throughout the forest block in all except two soil samples. However, āwheto itself was not as widely spread. This result indicates the ability for *O. robertsii* to disperse across large distances. Not much is known about the natural reservoir of *O. robertsii*, however, similar results were obtained in China, where researchers reported 36 out of 40 soil sampling sites contained molecular signals of *O. sinensis* (Yang et al. 2021). In another study, researchers investigated the presence of *O. sinensis* biomass on plant roots, stems, and leaves, as well as collected caterpillars' body parts (Lei et al. 2015). They focussed on the plants that are a known food source and habitat of *Thitarodes* spp. ghost moth caterpillars. Their results found fungal biomass present in all plant and caterpillar tissues; however there were significant differences in biomass abundance between plant species, indicating that certain food sources are more likely to contribute to *O. sinensis* infection (Lei et al. 2015). *Dumbletonius unimaculatus* larval diet has only been observed to consist of dead leaves, and no evidence of the root-feeding habit suggested by Hudson (1928) was found on Kapiti Island by Grehan (Grehan et al. 1983). This supports the current hypothesis of *O. robertsii* spreading through spores on the forest floor rather than by being consumed as an endophyte of food plants. However, research on *O. robertsii* presence in or on New Zealand plants and transmission experiments would be needed to confirm this hypothesis. If spore dispersal on the forest floor is the primary mechanism of dispersal by *O. robertsii*, then there are many additional questions concerning dispersal distances, vectors, and viability to better understand how it achieves such broad coverage. Through cultivation methods it was observed that the spores are very sticky, and it is possible that the fungus could use other ground dwelling insects as vectors, but without causing infection. Additionally, many ascomycetes forcibly eject their ascospores from pressurised asci (Trail 2007). Once in the air column, these spores could become entrained and carried by wind currents, potentially facilitating long-distance aerial dispersal, as has been suggested for other *Ophiocordyceps* species (de Bekker et al. 2021).

Āwheto habitat and forest ecology

In the current study, āwheto were only found in tall forest (mean height of 18 m) dominated by tawhairaunui | red beech (*Fuscospora fusca*) and with partially open canopies (mean cover of 75%). Considering the widespread presence of *O. robertsii* in soil, *āwheto* distribution appears strongly influenced by the habitat preferences of *D. unimaculatus*. Partially open canopies likely provide optimal conditions for moth flight and egg deposition, making moth habitat suitability

a significant factor influencing *āwheto* distribution (Dugdale 1994). However, further ecological research is required to confirm this relationship.

In terms of forest plant composition, of all the species analysed, two were considered to be potential indicator species. These species were pōkākā and Hall's tōtara, both of which were positively associated with āwheto presence. As discussed above, Casonato and Hill (2022) inferred associations between *āwheto* and hinau, the sister species of pōkākā within the genus *Elaeocarpus*. Given the abundance of *O. robertsii* in soil, the link between āwheto and *Elaeocarpus* spp. could either be a causative link between *D. unimaculatus* and pōkākā (or native *Elaeocarpus* species in general) potentially as a larval food source—adults have no functional mouthparts (Dugdale 1994)—or it may simply reflect shared environmental preferences. There are no documented records linking *D. unimaculatus* behaviour or life history with either pōkākā or Hall's tōtara. Greater replication of Recce vegetation descriptions (plots) within the site is required to further examine relationships between forest plant species and *D. unimaculatus*.

Across all plots litter coverage was high (mean 76%). The consistent presence of bryophytes (mean 25%) also indicates greater humidity within the substrate given known positive correlations between humidity and bryophyte cover and diversity (Frego 2007). The presence of leaf litter and bryophytes provide optimal conditions for Lepidoptera larval establishment and development. Most arthropod groups have been shown to inhabit the litterfall and fermentation horizons of the forest floor (McColl 1974), and most Lepidoptera larvae will feed in the litterfall horizon, particularly the lower part thereof (Dugdale 1994). Future research should include measurements of litter depth, thickness of horizons, and analysis of litter nutrient metrics to understand the qualities of the leaf litter strata that support *D. unimaculatus* and by association *āwheto*. Vegetative cover of shrubs and herbaceous plants, as opposed to bare ground, might also have some impact on protecting *āwheto* from non-native mammal disturbance. Red deer or their faecal pellets were frequent in *āwheto* areas. The animals were often captured on cameras lingering at sites with *āwheto* and investigating them, but we did not observe any physical damage to *āwheto* from deer. Deer may have a significant impact on forest ecosystems via selective browse of palatable species in the forest understorey and trampling of litter macrofauna, far exceeding that of extinct taxa like moa (Forsyth et al. 2010; Wood et al. 2020). The impact of deer on the *āwheto* habitat may be neutral or positive, by providing an open understory for the adult moth, or negative, by trampling caterpillars on the ground.

Plots at higher altitudes were positively associated with āwheto presence at the study sites. This result may be a proxy for other topographic, abiotic, or biotic variables. Within the research area, higher altitudes are more likely to sample flatter hill/mountain summits, which in themselves are likely to be drier habitats with a slightly different suite of forest plant species. Similarly, there is a strong tendency for āwheto to be associated with west-facing sites. Rainfall is extremely variable in spring and summer in the region of this study when westerly winds prevail (Chappell 2013). The ordination indicated that vegetation in which āwheto plots were present appeared to be a subset of the full vegetation community measure. This is consistent with the environmental variable analysis that east-west element of aspect seems to be associated with vegetation community overall, as well as āwheto presence/absence. The forest plant species on the right side of ordination include species more common in locally drier environments or that would typically occupy flat, drier sites, including the likes of pōkākā, Hall's tōtara, tall mingimingi (*Leucopogon fasciculatus*), putaputawētā (*Carpodetus serratus*), horopito (*Pseudowintera colorata*), and weeping māpou (*Myrsine divaricata*). But, as for the indicator species analysis, it is unclear whether there are specific biotic elements within this forest type that support *D. unimaculatus* or there might simply be shared environmental preferences. With only 23 forest plots these results are preliminary, however this remains a valuable step forward in beginning to understand āwheto and *D. unimaculatus* ecology.

Āwheto life history

We observed āwheto individuals in the study environment over a period of ten months (Fig. 2). During each of the three visits, in late summer, mid-autumn, and early summer, āwheto structures at different maturity stages co-occurred: short stromata with immature perithecia (spore-containing structures), fully developed stromata with mature perithecia, and structures with decaying/disintegrating stromata. This suggests a non-seasonal fruiting body emergence and sporulation, and it is in contrast with the strongly seasonal lifecycle of *Ophiocordyceps sinensis,* which infects caterpillars in winter with stroma emerging in late spring or summer (Wang & Yao 2011). Given the prolonged larval development period of *D. unimaculatus* (Dugdale 1994), infection by *O. robertsii* could potentially occur at various stages of larval growth. Previous research on *O. sinensis* has indicated that infection can persist internally for months before the emergence of visible fruiting bodies (Zhong et al. 2014; Zhong et al. 2016; Guo et al. 2017).

We observed little change to individual āwheto stromata over ten months and therefore speculate that they could survive and sporulate for multiple years. A few individuals appeared very underdeveloped, without visible perithecia on the stroma, indicating that this most likely represents an early stage of āwheto development. In an ant-associated *Cordyceps* spp. it was described that these young stages of fruiting body are more susceptible to hyperparasitism, with much shorter life spans than observed in mature specimens (Andersen et al. 2012). Long-term observations will be necessary to better describe the development and longevity of *O. robertsii* fruiting bodies.

Conservation management and threats

Non-native mammal predation and climate change appeared to be principal threats to āwheto populations. Although no direct predation on āwheto was observed in this study, it is well documented that moth larvae, including *D. unimaculatus*, are vulnerable to predation by rodents (Dugdale 1996; Fitzgerald et al. 1996), hedgehogs (Jones et al. 2005) and possums (Cowan & Moeed 1987). Climate projections indicate significant warming, reduced rainfall, and increased frequency of extreme events in Hawke's Bay, potentially degrading āwheto habitats through altered canopy structure and reduced litter moisture. Elevated climatic risks at higher elevations, where āwheto occurrences are more frequent at the study sites, highlight the urgency of targeted conservation and habitat management strategies (Vittoz et al. 2001; Hashiba et al. 2014; Woolley et al. 2020).

Conclusions

This study demonstrates how the bringing together of mātauranga Māori, field and quantitative ecology, and molecular science can improve the understanding of ecology and

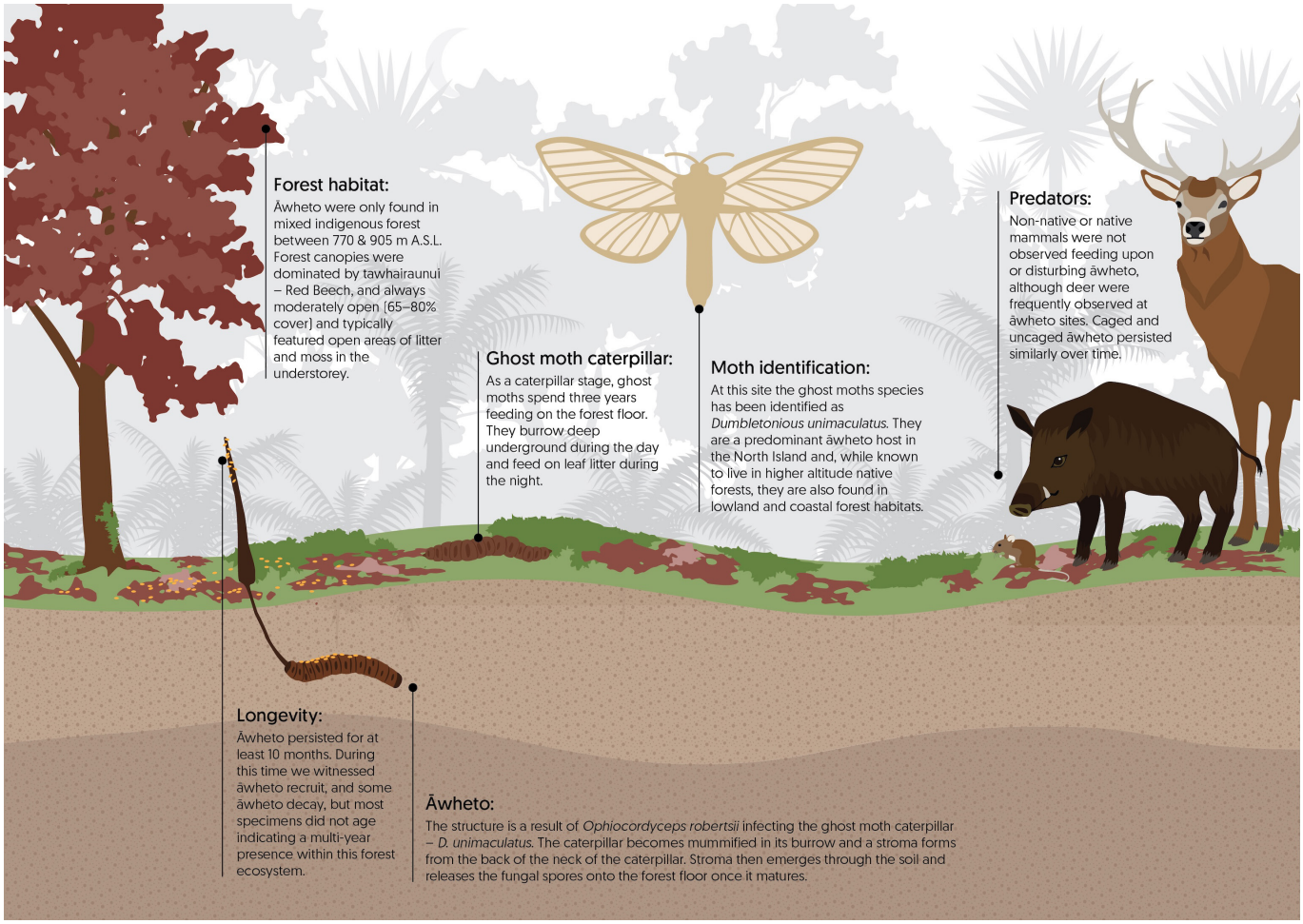

Figure 6. Conceptual summary of āwheto (*O. robertsii* infecting the ghost moth *D. unimaculatus*) ecology and key findings from this study, showing the host life cycle, forest habitat, persistence of stromata, and the persistence of āwheto in the presence of introduced mammals.

potential management of a taonga (Fig. 6). Within this study we rediscovered āwheto at site, used molecular techniques to identify the partners in the parasitism, *D. unimaculatus* and *O. robertsii*, and began to examine the ecology and life histories of both species. We know from this system that *O. robertsii* is widespread and the distribution of the host moth *D. unimaculatus* is the main driver of āwheto formation. Further research should target moth ecology with increased surveys of āwheto, *D. unimaculatus* populations, and the forest habitat they occupy, along with expanding the methods described above to other sites.

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Additional information and declarations

Author contributions: EB, CL, AJF, NH, MW, MN and SO developed the concept for the paper. AJF, CL, EB, FW and MN collected the field samples. EB, CL, AJF, RH and OB wrote the manuscript with substantial help and revisions from PB, PJ, MW and NH. AB, CD, FW and CL analysed the samples in the laboratory and generated data. EB, CL, AJF and OB analysed the data and produced final figures. Photos: AJF.

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Data and code availability: GPS coordinates and molecular data obtained in this study are not publicly available, on request of kaitiaki due to cultural and privacy reasons. Should the reader need access to data, they can contact the corresponding author who will pass their request to kaitiaki for consideration.

Ethics: No ethics permission was required to undertake this study.

Conflicts of interest: The authors declare that there are no conflicts of interest.

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Supplementary material

Additional supporting information may be found in the online version of this article.

Appendix S1. Stressplot for the ordination shown in main text. Number of dimensions is two. The ordination stress is shown within the plot.

Appendix S2. Model selection table of models where delta AICc < 2. Altitude (M.A.S.L.), Bare G (% bare ground), Canopy (% canopy cover above 1.35 m), sin(aspect) (the east-west dimension of aspect, calculated by sin transforming aspect in radians).

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