

First coprolite evidence for the diet of *Anomalopteryx didiformis*, an extinct forest ratite from New Zealand

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Abstract: Evidence of diet has been reported for all genera of extinct New Zealand moa (Aves: Dinornithiformes), using preserved gizzard content and coprolites, except the forest-dwelling *Anomalopteryx*. Skeletal features of the little bush moa (*Anomalopteryx didiformis*) have led to competing suggestions that it may have either browsed trees and shrubs or grubbed for fern rhizomes. Here, we analyse pollen assemblages from two coprolites, identified by ancient DNA analysis as having been deposited by *Anomalopteryx didiformis*. The pollen results, together with identified fragments of leaf cuticles from the coprolites, support the hypothesis that *Anomalopteryx didiformis* browsed trees and shrubs in the forest understorey.

Keywords: Ancient DNA; Dinornithiformes; moa; pollen

Introduction

The diets of New Zealand's extinct moa (Aves: Dinornithiformes) have long been a topic for speculation (Haast 1872; Buick 1931). Following early descriptive reports of plant material from putative moa gizzard contents (Forbes 1892; Hamilton 1892), empirical studies of gizzard content (Gregg 1972; Burrows et al. 1981; Wood et al. 2007) and coprolites (Horrocks et al. 2004; Wood et al. 2008) have revealed aspects of the diets of South Island giant moa (*Dinornis robustus*), eastern moa (*Emeus crassus*), coastal moa (*Euryapteryx curtus*), heavy-footed moa (*Pachyornis elephantopus*) and upland moa (*Megalapteryx didinus*); sensu the nomenclature of Bunce et al. (2009) and Gill et al. (2010). The studies have revealed that these moa species were relatively generalist herbivores, consuming a wide range of plant types from trees and shrubs to ground-cover herbs. However, the little bush moa (*Anomalopteryx didiformis*) remains a significant gap in the current understanding of moa feeding ecology, as it is the sole moa genus for which no empirical evidence of diet has been reported.

The little bush moa was a relatively short and gracile moa species (adults 50–90 cm tall at the back, 26–64 kg). The distribution of its fossil bones suggests *Anomalopteryx didiformis* was restricted to lowland closed-canopy forest (Fig. 1) (Worthy 1990; Bunce et al. 2009). In contrast, all other moa species except crested moa *Pachyornis australis* (which was restricted to subalpine herbfields; Tennyson & Martinson 2006) occupied a broader range of habitats, with most ranging from forest to shrubland, grassland and herbfields (Worthy & Holdaway 2002). Anatomically derived hypotheses for the feeding ecology of *Anomalopteryx didiformis* (based on the relatively robust jaw with a secateur-like overlap of the mandible and premaxilla, large temporal fossae (Fig. 1), and large gizzard volume) have mostly agreed that the species probably browsed forest understorey trees and had a fibre-rich

diet (Atkinson & Greenwood 1989; Worthy & Holdaway 2002; Lee et al. 2010; Thorsen et al. 2011). Early in the history of moa research, Richard Owen recognised adaptations in the bone morphology of moa, including *Dinornis parvus* (now *Anomalopteryx didiformis*), that he related to digging and scratching, specifically suggesting that their foot structure was sufficient for and probably used for the digging up of nutritious fern rhizomes (Owen 1883a,b). This view was also held later by Kooyman (1991), who also suggested possible similarities with the feeding habits of kiwi (*Apteryx* spp.). Here we report the discovery of the first coprolites identified as being from *Anomalopteryx didiformis*, and present analyses of their pollen content to help to resolve the diet of this enigmatic species.

Materials and methods

The coprolites were recovered from a rock shelter on Mt Nicholas Station, near the western shore of Lake Wakatipu, South Island, New Zealand (45°06'16.7" S, 168°26'43.4" E; c. 400 m above sea level). The discovery of moa feathers, bones, and the 'excrement of a large bird' in a cave at Mt Nicholas was reported by White (1876). In February 2009, we re-located this cave following the description and measurements of the site given by White (1876) (Fig. 2). More than 100 years of burrowing by rabbits (*Oryctolagus cuniculus*) had significantly disrupted the stratigraphy at the site. Further exploration of the area revealed several more caves of similar size at the same elevation (c. 60 m above lake level). At the east end of the conical hill in which the cave described by White (1876) is located (Fig. 2), is an area of dry sandy ground sheltered by a rock wall. We excavated a pit measuring 100 × 50 cm, orientated perpendicular to, and extending out from, the wall, and uncovered approximately 40 coprolites (Figs 2 and 3).

Identification of the depositor species was based on ancient

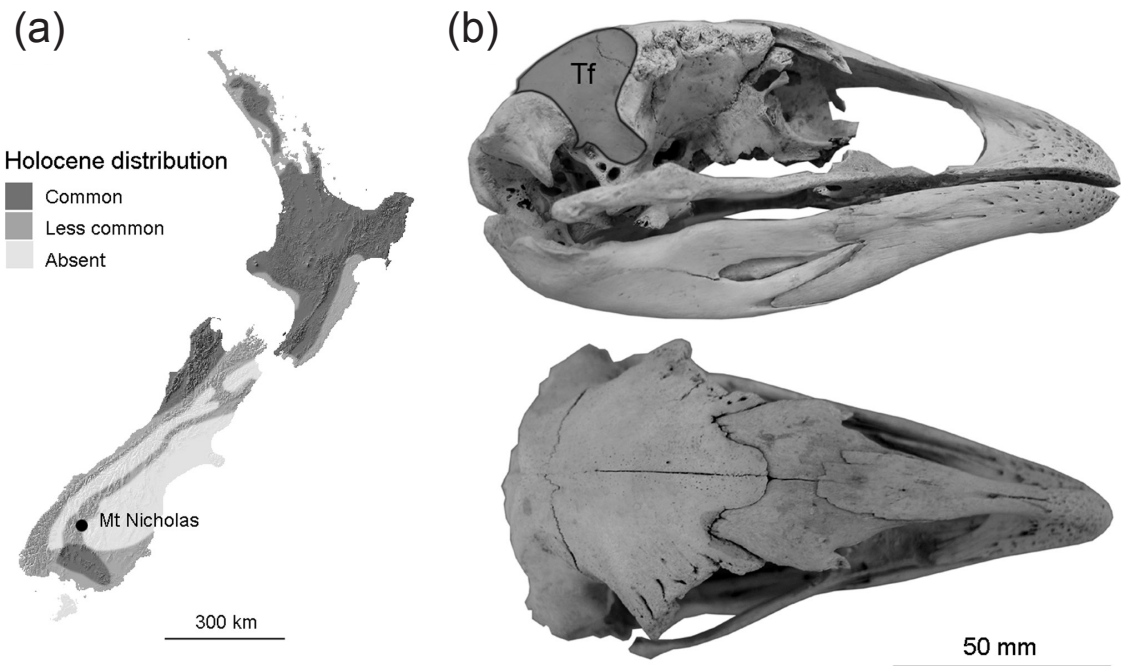


Figure 1. (a) Holocene distribution of little bush moa (*Anomalopteryx didiformis*) based on fossil bone records (adapted from Worthy & Holdaway (2002)); (b) lateral and dorsal views of *A. didiformis* skull, showing large temporal fossa (Tf).

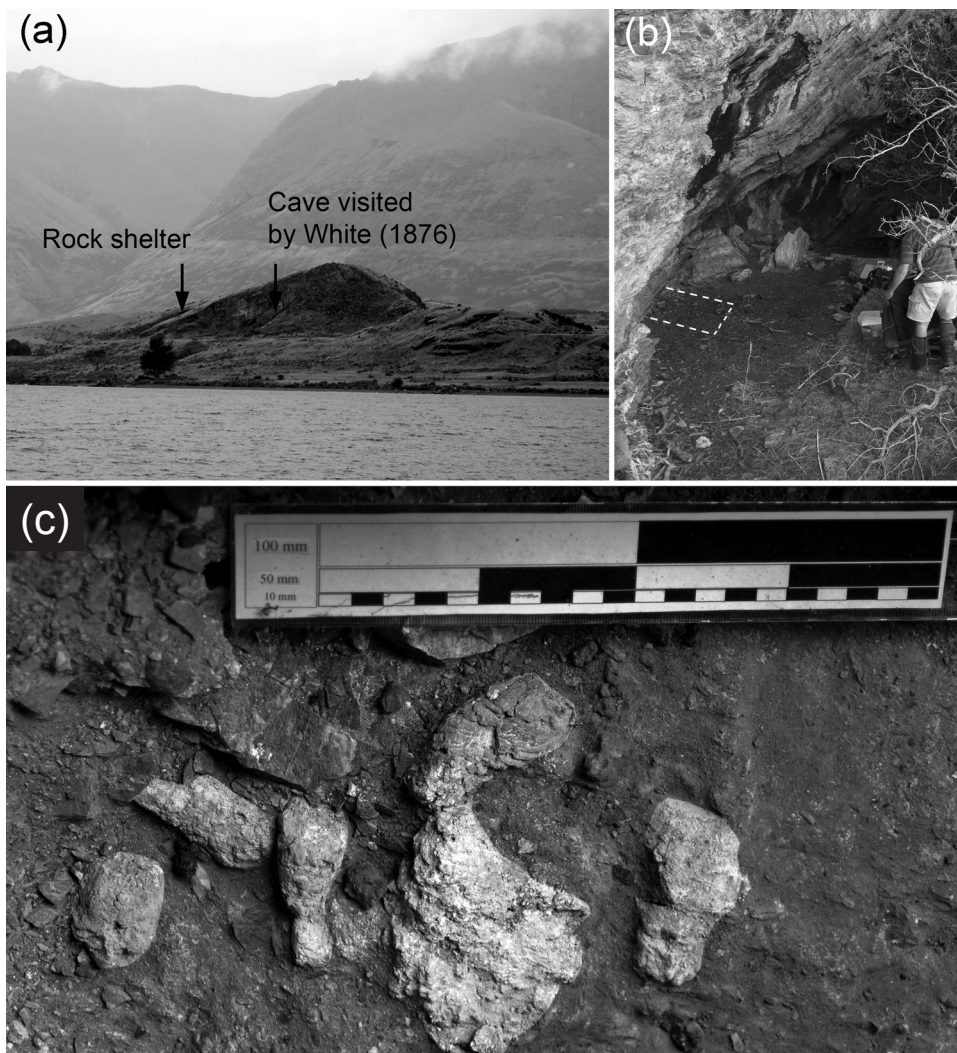


Figure 2. (a) Location of rock shelter at Mt Nicholas Station on the western shore of Lake Wakatipu, South Island, New Zealand, in relation to the cave described by White (1876); (b) Eastern end of rock shelter showing position of excavation pit; (c) Concentration of several moa coprolites within excavation pit (background sediment darkened to highlight coprolites).

DNA analysis, performed at the Australian Centre for Ancient DNA at the University of Adelaide. DNA was extracted from each coprolite once following methods described by Wood et al. (2008). We used three primer pairs to amplify diagnostic sections of the moa control region (Fig. 4). First, we used Moa 262fw (5' GCGAAGACTGACTAGAAGC 3') and Moa 294rv (5' CGTACTGTTCAAATCTCGC 3') to amplify 31 bp that can distinguish each moa species except *Euryapteryx curtus* and *Anomalopteryx didiformis*. Second, to distinguish these two species we designed the primers Andi/Eugr fw (5' GCCATACGTTTCATTAGTTATACACC 3') and Andi/Eugr rv (5' AGATATTAAGRCCCTCGGCGA 3') to amplify a diagnostic 22-bp fragment. Third, if the 31-bp fragment could not be amplified using the primers Moa 262fw and Moa 294rv, we used Moa 204fw (5' AGATTTATARCTCGGACA 3') and Moa 294rv to amplify 11 bp that also allow discrimination of each moa species except *Euryapteryx curtus* and *Anomalopteryx*

didiformis. All these primer pairs are highly specific to moa, and the primers 204fw, 262fw, and 294rv have previously been used in ancient DNA studies on the group (e.g. Cooper et al. 2001; Bunce et al. 2003; Wood et al. 2008; Rawlence et al. 2009). Coprolite sequence identification was performed using BLAST (Basic Local Alignment Search Tool; Altschul et al. 1990) (100% identity), and by alignment with sequences from moa specimens of known identity.

Subsamples of coprolites were processed for pollen analysis by soaking in hot KOH for 10 min, treatment with HCl, acetolysis, separation of inorganics by lithium polytungstate flotation (at specific density 2.2), staining, and mounting on a microscope slide. Spikes of exotic *Lycopodium* spore tablets were used to allow quantification of pollen concentrations. Counts of at least 280 pollen grains were made from each coprolite. Fragments of leaf cuticle on the pollen slides were identified using the cuticle photographic collection at the

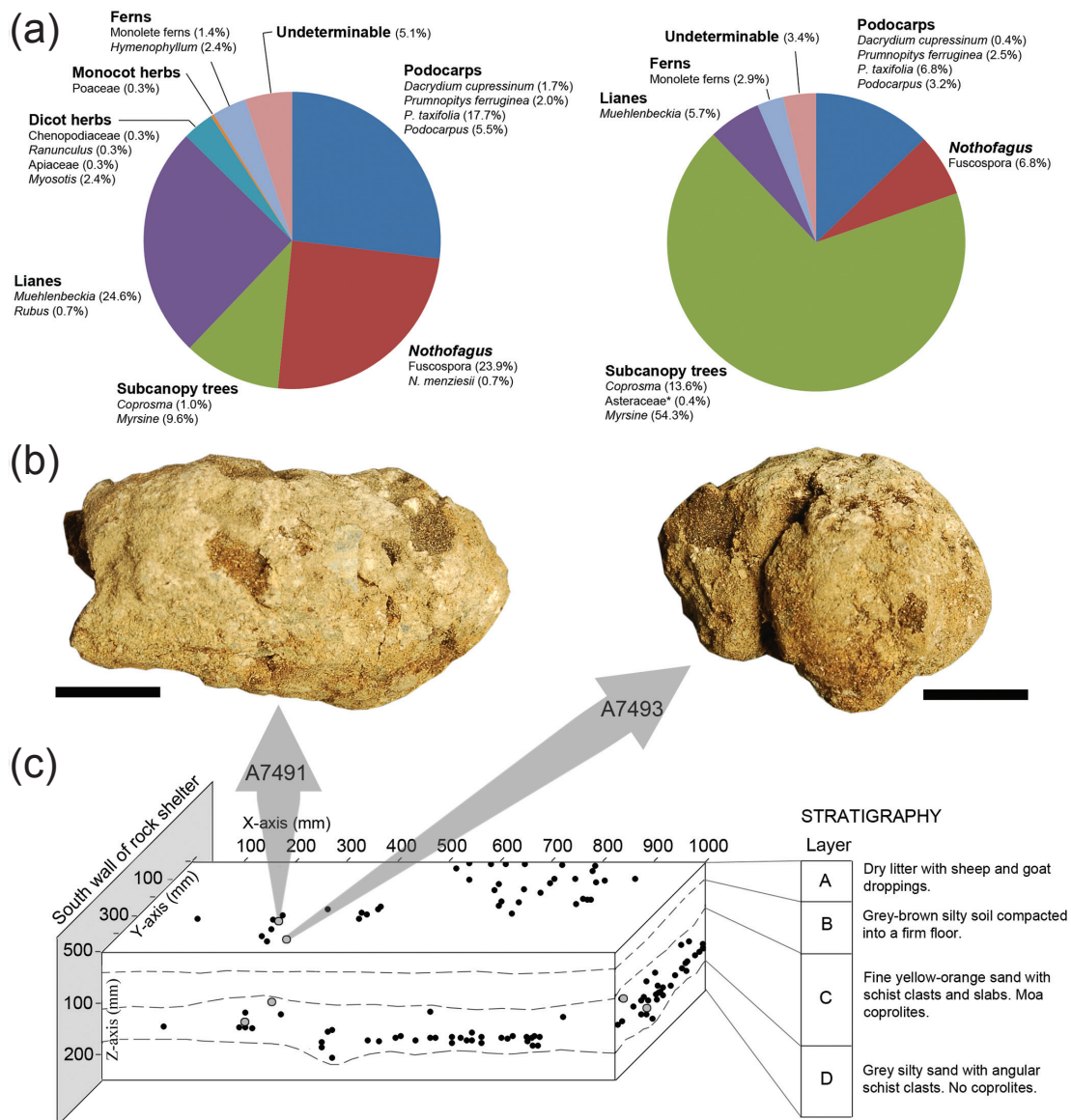


Figure 3. (a) Pollen assemblages from little bush moa (*Anomalopteryx didiformis*) coprolites from rock shelter at Mt Nicholas Station, South Island, New Zealand. Note *Asteraceae is tentatively assigned to *Olearia*, within subcanopy trees, but may also represent dicot herbs; (b) *Anomalopteryx didiformis* coprolites (scale is 10 mm); (c) Pit excavated in the floor of the rock shelter at Mt Nicholas Station, showing stratigraphy and location of moa coprolites projected onto X–Y, X–Z, and Y–Z planes.

Allan Herbarium, Landcare Research (Lincoln, New Zealand).

Results and discussion

Moa DNA was successfully amplified from four coprolites. Two of the coprolites were identified as being from *Dinornis robustus* and will not be discussed here. Coprolite A7493 was positively identified as being from *Anomalopteryx didiformis* (Fig. 4). Coprolite A7491 was identified as being from either *Euryapteryx curtus* or *Anomalopteryx didiformis* (Fig. 4). Despite this uncertainty, we infer that it is likely to be *Anomalopteryx didiformis* based on the close proximity to A7493 in the excavation pit (Fig. 3), and the fact the site was formerly forested (as discussed below) and therefore had the preferred habitat of this moa species. In contrast, *Euryapteryx curtus* preferred drier eastern regions (Tennyson & Martinson 2006). Few other moa remains have been found in the Mt Nicholas area, and the identities of most of those that have are unverified (Worthy 1998). However, the former presence of *Megalapteryx didinus* is indicated by green eggshell attributable to this species (Gill 2006) collected by White (1876) and most skeletal specimens from the Lake Wakatipu area are *M. didinus* (e.g. Lydekker 1891; Oliver 1949). Fossil bone assemblages described by Worthy (1998) from West Wanaka Station, 60 km to the northwest of Mt Nicholas but with a similar climatic and palaeovegetation history, were dominated by *Megalapteryx didinus*, *Dinornis robustus*, and *Anomalopteryx didiformis*.

Plant macrofossils recovered from the sediment between coprolites provide evidence of a forested environment while the moa occupied the area, and include leaf fragments of silver beech (*Nothofagus menziesii*) and bush lawyer (*Rubus schmidelioides*), and seeds of mataī (*Prumnopitys taxifolia*), miro (*P. ferruginea*) and *Coprosma* spp. Avian bones and feathers collected from the Mt Nicholas area by White (1876) are also typical of species from a forest fauna, and include parakeet (*Cyanoramphus* sp.), owl-nightjar (*Aegotheles novaezealandiae*), robin (*Petroica australis*), and saddleback (*Philesturnus carunculatus*) (Worthy 1998).

Radiocarbon dating of six coprolites (two *Anomalopteryx didiformis*, one *Dinornis robustus*, three unidentified) show the assemblage represents a short period of deposition in the site c. 800–500 years prior to human settlement (95% CI calibrated age range of 1521–1185 BP) (Table 1). The 95% CI calibrated age ranges for the two *Anomalopteryx didiformis* coprolites overlap (Table 1), suggesting they could have been

deposited by the same individual bird; however, the coprolite pollen assemblages differ (Fig. 3) indicating they were not deposited during the same defecation event. The pollen concentrations in the coprolites were high (800 000 grains g⁻¹ in A7493 and 950 000 grains g⁻¹ in A7491), possibly indicative of deposition during spring or summer months. Both pollen assemblages contain significant components from trees and shrubs (87.9% in A7493 and 62.1% in A7491), including podocarps, beech (*Nothofagus*), *Myrsine*, and *Coprosma*. Herbs and ferns comprise relatively minor components of the pollen assemblages but include plant taxa typical of those that may grow around large rock outcrops within a forest (e.g. *Ranunculus*, *Myosotis*, and Poaceae). The liane *Muehlenbeckia* was an important component of the pollen assemblages from the coprolites, and is known to have been consumed by other moa species (Trotter 1970; Burrows et al. 1981; Wood et al. 2008). Fragments of leaf cuticle on the pollen slides were predominantly *Myrsine* and podocarp (Fig. 5).

Attempts to amplify plant DNA from the coprolites using the generic angiosperm rbcL primers h1aF and h2aR (Poinar et al. 1998) were unsuccessful. This is possibly due to the larger size of this amplicon (95 bp excluding primers) compared with the successfully amplified moa DNA fragments (≤ 31 bp). An exponential decrease in DNA abundance relative to fragment size is often observed in ancient samples (Adler et al. 2011). However, in the absence of plant DNA, the microfossils provide dietary information. While coprolite pollen assemblages may include wind-blown pollen that do not necessarily reflect actual diet (but have merely settled on the leaves of other plant taxa), at least 2 of the important taxa from the coprolites (*Myrsine*, *Muehlenbeckia*) are locally dispersed and insect-pollinated (Moar et al. 2011), indicating that these plants were likely to have been directly ingested. The case for *Anomalopteryx* having browsed on *Myrsine* is strengthened by the abundance of *Myrsine* leaf cuticle present in the samples. These are likely to be either *Myrsine divaricata* (a small-leaved divaricating shrub) or *M. australis*. We found no evidence for a subterranean or fern rhizome-rich diet as suggested by Owen (1883a, b) and Kooyman (1991). No fern tissues or rhizomatous fibres were observed in the coprolites. Both these were noted in winter dung samples from South Island takahē (*Porphyrio hochstetteri*), a species known to feed on fern rhizomes during winter (Wilmshurst 2003). Fern spores were a minor component of the coprolite pollen/spore assemblages (2.9% and 3.8% respectively) and would have been expected to be in much higher concentrations had the birds been foraging in forest soil beneath ferns (e.g. James &

Table 1. Radiocarbon dates and 95% Confidence Interval (CI) calibrated age for moa coprolites from rock shelter at Mt Nicholas Station, South Island, New Zealand.

Lab no.	Identity	Radiocarbon age	Error	Δ ¹³ C	Calibrated age BP (95% CI)
Wk28323	Probably <i>Anomalopteryx didiformis</i> (A7491)	1440	30	-28.9	1352–1265
Wk28324	<i>Anomalopteryx didiformis</i> (A7493)	1527	30	-29.0	1410–1302
Wk28325	Unidentified moa	1415	30	-31.8	1338–1185
Wk28326	Unidentified moa	1538	30	-29.9	1484–1301
Wk28327	Unidentified moa	1478	30	-30.3	1377–1289
Wk28328	<i>Dinornis robustus</i>	1582	34	-27.6	1521–1343

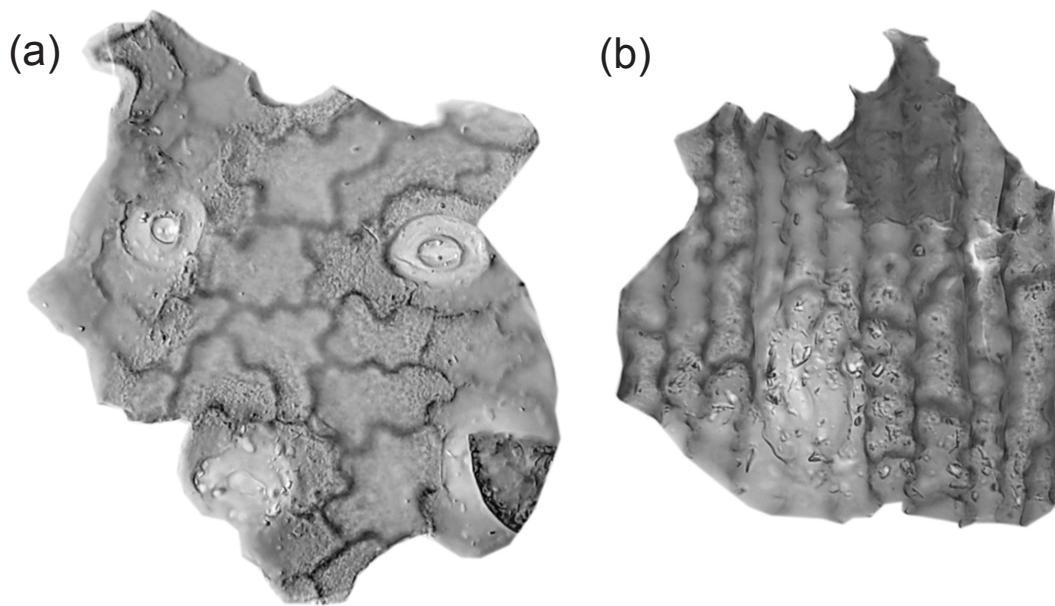


Figure 5. Predominant leaf cuticle types from *Anomalopteryx didiformis* coprolites: (a) *Myrsine*; (b) podocarp.

Burney 1997; Wilmshurst 2003).

Overall, our results are consistent with the currently accepted inference that the diet of *Anomalopteryx didiformis* consisted largely of fibrous material taken from trees and shrubs in the forest understorey (Atkinson & Greenwood 1989; Worthy & Holdaway 2002; Tennyson & Martinson 2006). Interestingly, this feeding ecology appears to have been partly shared with at least two other species of moa: *Megalapteryx didinus* as reported by Horrocks et al. (2004), which has a considerably more gracile skull and a far smaller volume of gizzard stones (THW unpubl. data) even though they were similar sized birds to *Anomalopteryx*; and *Dinornis robustus* (Burrows et al. 1981). However, these species also grazed herbs in non-forest vegetation communities (Horrocks et al. 2004; Wood et al. 2008). The discovery and analysis of further *Anomalopteryx* coprolites from other sites across New Zealand will be necessary to understand the full dietary range of this bird, the factors partitioning its niche from those of other moa species, and its likely ecological role within New Zealand's prehuman forest communities.

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References

- Adler CJ, Haak W, Donlon D, Cooper A, The Genographic Consortium 2011. Survival and recovery of DNA from ancient teeth and bones. *Journal of Archaeological Science* 38: 956–964.
- Altschul S, Gish W, Miller W, Myers E, Lipman D 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Atkinson IAE, Greenwood RM 1989. Relationships between moas and plants. *New Zealand Journal of Ecology* 12 (suppl.): 67–96.
- Buick TL 1931. The mystery of the moa: New Zealand's avian giant. New Plymouth, Thomas Avery under the auspices of the Board of Maori Ethnological Research. 357 p.
- Bunce M, Worthy TH, Ford T, Hoppitt W, Willerslev E, Drummond A, Cooper A 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. *Nature* 425: 172–175.
- Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev W, Haile J, Shapiro B, Scofield RP, Drummond A, Kamp PJJ, Cooper A 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. *Proceedings of the National Academy of Sciences (USA)* 106: 20646–20651.
- Burrows CJ, McCulloch B, Trotter MM 1981. The diet of moas based on gizzard contents samples from Pyramid Valley, North Canterbury, and Scaifes Lagoon, Lake Wanaka, Otago. *Records of the Canterbury Museum* 9(6): 309–336.
- Cooper A, Fox-Lalueza C, Anderson S, Rambaut A, Austin J, Ward R 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* 409: 704–707.
- Forbes HO 1892. On a recent discovery of the remains of extinct birds in New Zealand. *Nature* 45: 416–418.
- Gill BJ 2006. A catalogue of moa eggs (Aves: Dinornithiformes). *Records of the Auckland Museum* 43: 55–80.
- Gill BJ, Bell BD, Chambers GK, Medway DG, Palma RL, Scofield RP, Tennyson AJD, Worthy TH 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica. 4th edn. Wellington, Te Papa Press in association with the Ornithological Society of New Zealand and. 464 p.
- Gregg DR 1972. Holocene stratigraphy and moas at Pyramid Valley, North Canterbury, New Zealand. *Records of the Canterbury Museum* 9: 151–158.
- Haast J 1872. Moas and moa-hunters. Address to the Philosophical Institute of Canterbury. *Transactions and Proceedings of the New Zealand Institute* 4: 66–107.
- Hamilton A 1892. Notes on moa gizzard stones. *Transactions and Proceedings of The New Zealand Institute* 24: 172–175.

- Horrocks M, D'Costa D, Wallace R, Gardner R, Kondo R 2004. Plant remains in coprolites: diet of a subalpine moa (*Dinornithiformes*) from southern New Zealand. *Emu* 104: 149–156.
- James HF, Burney DA 1997. The diet and ecology of Hawaii's extinct flightless waterfowl: evidence from coprolites. *Biological Journal of the Linnean Society* 62: 279–297.
- Kooyman B 1991. Implications of bone morphology for moa taxonomy and behavior. *Journal of Morphology* 209: 53–81.
- Lee WG, Wood JR, Rogers GM 2010. Legacy of avian-dominated plant–herbivore systems in New Zealand. *New Zealand Journal of Ecology* 34: 28–47.
- Lydekker R 1891. Catalogue of the fossil birds in the British Museum (Natural History). London, British Museum (Natural History). 368 p.
- Moar NT, Wilmshurst JM, McGlone MS 2011. Standardizing names applied to pollen and spores in New Zealand Quaternary palynology. *New Zealand Journal of Botany* 49: 201–229.
- Oliver WRB 1949. The moas of New Zealand and Australia. *Dominion Museum Bulletin* 15. 205 p.
- Owen R 1883a. On *Dinornis* (Part XXIII): containing a description of the skeleton of *Dinornis parvus*, Owen. *Transactions of the Zoological Society of London* 11: 233–256.
- Owen R 1883b. On *Dinornis* (Part XXIV): containing a description of the head and feet, with their dried integuments, of an individual of the species *Dinornis didinus*, Owen. *Transactions of the Zoological Society of London* 11: 257–261.
- Poinar HN, Hofreiter M, Spaulding WG, Martin PS, Stankiewicz BA, Bland H, Evershed RP, Possnert G, Pääbo S 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* 281: 402–406.
- Rawlence NJ, Wood JR, Armstrong KN, Cooper A 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa. *Proceedings of the Royal Society B* 276: 3395–3402.
- Tennyson AJD, Martinson P 2006. Extinct birds of New Zealand. Wellington, Te Papa Press. 180 p.
- Thorsen MJ, Seddon PJ, Dickinson KJM 2011. Faunal influences on New Zealand seed dispersal characteristics. *Evolutionary Ecology* 25: 1397–1426.
- Trotter MM 1970. Archaeological investigations in the Aviemore area, South Island. *Records of the Canterbury Museum* 8: 439–453.
- White T 1876. Notes on Moa Caves, etc., in the Wakatipu District. *Transactions and Proceedings of the New Zealand Institute* 8: 97–102.
- Wilmshurst JM 2003. Establishing long-term changes in takahe winter feeding grounds in Fiordland using pollen analysis. *Science for Conservation* 228. Wellington, Department of Conservation. 25 p.
- Wood JR 2007. Moa gizzard content analyses: Further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves, *Dinornithiformes*). *Records of the Canterbury Museum* 21: 27–39.
- Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: *Dinornithiformes*). *Quaternary Science Reviews* 27: 2593–2602.
- Worthy TH 1990. An analysis of the distribution and relative abundance of moa species (Aves: *Dinornithiformes*). *New Zealand Journal of Zoology* 17: 213–241.
- Worthy TH 1998. Quaternary fossil faunas of Otago, South Island, New Zealand. *Journal of the Royal Society of New Zealand* 28: 421–521.
- Worthy TH, Holdaway RN 2002. The lost world of the moa: prehistoric life of New Zealand. Christchurch, Canterbury University Press. 718 p.

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