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SHORT NOTE

Palaeoecological reconstructions depend on accurate species identification: examples from South Island, New Zealand, *Pachyornis* (Aves: Dinornithiformes)

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Accurate identification of fossil remains is fundamental to analysis of the composition of New Zealand extinct bird assemblages and their habitats (e.g. wet forest, dry forest, shrubland, seral vegetation, and low and high altitudes) through space and time. Until the advent of ancient genetic analyses in the early 1990s, identification of fossil bird remains was based perforce solely on morphology (Archey 1941; Oliver 1949; Worthy 1988) and morphometrics (Cracraft 1976a, b, c; Worthy 1987, 1989, 1992, 1994). Most research has been focused on moa (Aves: Dinornithiformes), a group of large (20–200 kg) flightless palaeognathous birds (Worthy & Holdaway 2002) presently assigned to nine species in three families (Megalapterygidae; Dinornithidae; Emeidae) (Bunce *et al.* 2009). From the late 1990s (e.g. Cooper *et al.* 2001), the development of moa ancient DNA (aDNA) was rapid and greater reliance is now placed on use of aDNA analyses over morphology and morphometrics (Huynen *et al.* 2003; Bunce *et al.* 2005; Huynen *et al.* 2008; Allentoft *et al.* 2009; Bunce *et al.* 2009; Seabrook-Davison *et al.* 2009; Oskam *et al.* 2010; Allentoft *et al.* 2012; Rawlence *et al.* 2012; Holdaway *et al.* 2014; Huynen *et al.* 2014).

In the moa genus *Pachyornis* (Aves: Dinornithiformes), the validity of the species *P. australis* Oliver, 1949 was accepted on morphological characters in 1989 (Worthy 1989) and then by ancient genetic analysis 20 years later (Bunce *et al.* 2009). It is known mainly from subalpine sites, with outliers in coastal dunes in Southland during the Holocene. Lowland records from Honeycomb Hill in northwest Nelson and the Punakaiki area on the West Coast of the South Island (Worthy & Holdaway 1993) date from the most recent (Weichselian-Otiran) glaciation. The species is important in palaeoecological and palaeoclimatic analyses because it is the only moa thought to be associated solely with high altitudes and cooler temperatures (Worthy & Holdaway 2002; Rawlence *et al.* 2012). As with other species apparently associated with

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a single habitat, such as *Anomalopteryx didiformis* in lowland rain forest (Worthy & Holdaway 2002), the presence of *P. australis* is evidence for its contemporary vegetation.

The post-cranial skeleton of *P. australis* (44–90 kg) has in the past been confused not only with that of its South Island sister species *P. elephantopus*, but also with *Euryapteryx curtus*, a similar-sized (80–90 kg) moa (Worthy 1989); see Worthy & Holdaway (2002) and Brassey *et al.* (2013) for body mass estimates for moa. Before the advent of aDNA, re-identification of individuals on morphological grounds of *E. curtus* from the late Pleistocene at Honeycomb Hill as *P. australis* altered understanding of the regional fauna and relative ecological requirements of both *Pachyornis australis* and *Euryapteryx curtus* (Worthy & Holdaway 1993, 1994, 1995, 1996, 2002).

A survey of the relationships and historical biogeography of South Island *Pachyornis* moa (Rawlence *et al.* 2012) relied almost exclusively on aDNA. They found several instances where individuals had apparently been misidentified on morphology (Table 1). Incorrect identification based on morphology has been reported for other moa taxa at Bell Hill Vineyard, North Canterbury when re-assessed using aDNA (Allentoft *et al.* 2014; Holdaway *et al.* 2014). Although examples of misidentification by genetics are reported rarely, laboratory error and contamination are always possible. Full and accurate documentation of data and methodologies are fundamental to traceability of error. Independent methodologies provide additional means of verification and validation leading to better practice.

The early years of aDNA were plagued by many issues of contamination (Cooper & Poinar 2000; Willerslev & Cooper 2004; Malmström *et al.* 2005; Pruvost *et al.* 2005; Leonard *et al.* 2007). Gilbert *et al.* (2005) cautioned against a "checklist" system of verification of aDNA results [i.e. running through a set of criteria that could be checked off on a list] that was becoming accepted: "these criteria are not foolproof, and we believe that they have, in practice, replaced the use of thought and prudence when designing and executing ancient DNA studies... researchers must take a more cognitive and self-critical approach…[and] must explain, in sufficient enough detail to dispel doubt, how the data were obtained [i.e. the aDNA methodologies and laboratory conditions], and why they should be believed to be authentic [i.e. that the genetic data were actually from the samples, without contamination]" (Gilbert *et al*. 2005: 541).

Research on fossil birds now includes stable isotopic analysis of bone proteins, as well as the morphology, morphometrics of skeletal elements, and ancient mitochondrial and nuclear genetics (Worthy & Holdaway 2002; Holdaway *et al.* 2011; Rawlence *et al.* 2012; Williams *et al.* 2012; Holdaway *et al.* 2013). The stable isotopes of carbon, nitrogen, oxygen, hydrogen, and sulphur are now used routinely in ecological studies of both living and extinct species and ecosystems, providing information on food webs, habitat, and trophic levels that is otherwise difficult – or for extinct systems, impossible – to obtain (Peterson & Fry 1987; Hobson 1999). Stable isotope ratios of bone proteins are more stable than, for example, aDNA and provide information relating to most of the organism's life span and comparable to that available from living organisms. The concept of isotopic niche (Bearhop *et al.* 2004) allows quantitative analysis of ecology for living and extinct species (Williams *et al.* 2012; Holdaway *et al.* 2013), although integration with ecological niches per se is an ongoing process of interpretation (Flaherty & Ben-David 2010).

Stable isotopic analysis can, as well as identifying aspects of the bird's biology and environment, be useful in testing identification of individual moa now that considerable archives of comparative data are available (e.g. Bunce *et al.* 2009; Rawlence *et al.* 2012; Allentoft *et al.* 2014; Holdaway *et al.* 2014). A survey of isotopic data for moa taxa in northwest Nelson revealed nitrogen stable isotopic ratios of three *Pachyornis* individuals that were anomalous, in relation to both their species identification by Rawlence *et al.* (2012), and to isotopic values for both taxa from the rest of the South Island.

Radiocarbon dated individuals of *P. australis* and *P. elephantopus* from deposits in northwest Nelson, the South Island West Coast, western Southland, and Stewart Island identified by morphology or genetically are listed in Table 1. The three *Pachyornis* individuals, from sites on Takaka Hill (Table 1) had been identified as *P. elephantopus* on both morphology (Worthy & Holdaway 1994) and their aDNA (Rawlence *et al.* 2012). The carbon and nitrogen stable isotopic ratios and altitudes of deposition of the three individuals identified by Rawlence *et al.* (2012) as *P. elephantopus* were compared with those of all other dated individuals of both taxa (of all geologic ages) for which both isotopic ratios were known, using R version 3.5.3 (R-Core-Team 2017) (script in Appendix 1) and PAST® Version 3.26 (Hammer *et al.* 2001) statistical software.

In 3-dimensional multivariate kernel distributions, the three individuals lay between two (altitudinal) clusters of *P. australis*, separate by their $\delta^{15}N$ values from the series of *P. elephantopus* (Fig. 1A upper, arrowed circle). When the three individuals were reclassified as *P. australis* they were accommodated in a continuous 95.4% confidence envelope for that species (Fig. 1A, lower).

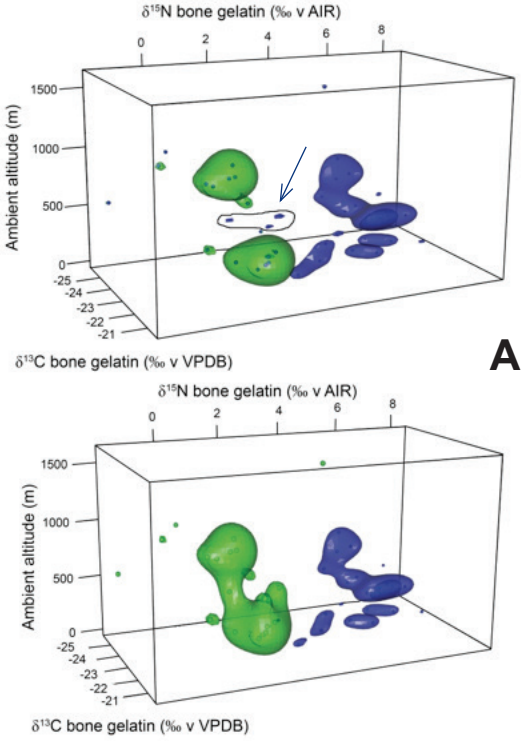
Table 1. Radiocarbon dated specimens of *Pachyornis australis* and *P. elephantopus* used in analysis of species representation in northwest Nelson and Westland: ? shaded, identified genetically as *P. elephantopus* (north), identification questioned here; *, identified by morphology as *P. elephantopus* but as *P. australis* genetically; **, identified by morphology as *P. elephantopus* but potentially *P. australis*; ***, identified genetically as *P. elephantopus* (south) but potentially *P. australis*. Individual from Te Ana Titi (bold) was originally identified as *P. australis* by morphology (Worthy & Holdaway 1993) and not as *P. elephantopus* as listed by Rawlence *et al.* (2012). The Tarakohe Cave individual for which Rawlence *et al.* (2012) list no repository or registration number is one of six individuals from that site in Museum of New Zealand Te Papa Tongarewa (NMNZ), catalogued collectively as S401-410, S454 (Worthy & Holdaway 1994). Other collections are: WCM, Waitomo Museum of Caves; CM, Canterbury Museum; SMAG, Southland Museum and Gallery; OM, Otago Museum.

Original taxon	Sample	Site	Museum/ register	^{14}C lab no.	^{14}C age	δ^{13}C	δ^{15}N
		Takaka Hill					
Pachyornis elephantopus?	A3749	Predator Cave	MNZ S32425	OxA20336	32,230 ± 380	-20.53	1.42
Pachyornis elephantopus?	A3755	Takaka Hill	MNZ DM417E	OxA20293	20,330 ± 90	-21.86	0.9
Pachyornis elephantopus?	A3756	Takaka Hill	MNZ DM417E	OxA20292	14,145 ± 60	-21.67	2.3
*Pachyornis elephantopus***	-	Hawkes Cave	MNZ S28424	NZA3240	13,470 ± 94	-22.2	-
*Pachyornis elephantopus***	-	Kairuru Cave	MNZ S27797	NZA1568	18,950 ± 230	-20.6	-
*Pachyornis elephantopus***	-	Tarakohe Cave	[MNZ S401-410,454]	NZA3047	19,520 ± 130	-22.6	-
*Pachyornis australis**	A3713	Takaka Hill	WCM WO90.47	OxA20291	10,120 ± 45	-24.69	-1.5
Pachyornis australis	A2597	Takaka Hill	NMNZ Unreg.	OxA20290	18,235 ± 80	-23.09	2.00
Pachyornis australis	-	Hawkes Cave	NMNZ S28422	NZA3237	29,011 ± 312	-22.1	-
Pachyornis australis	GU139065	Bone Cave	CM Av21331	OxA12430	10,165 ± 50	-23.17	1.00
		Takaka Valley					
Pachyornis australis	A3739	Irvine's Tomo	MNZ S27881	NZA3049	28,520 ± 20	-23.5	-
Pachyornis australis	A3740	Commentary C	MNZ S35298.1	OxA20294	28,050 ± 300	-25.19	3.40
		Other areas					
Pachyornis australis	GU139066	Charleston	CM Av29445	OxA12431	14,045 ± 65	-22.42	1.99
Pachyornis australis	-	Te Ana Titi	MNZ S28192	NZA2320	25,070 ± 260	-23	-
Pachyornis australis	GU139064	Honeycomb Hill	In situ	OxA12435	18,925 ± 80	-21.47	1.86
*Pachyornis australis**	A3781	Honeycomb Hill	NRS348 in situ	OxA20284	19,575 ± 80	-20.92	1.50
*Pachyornis australis**	A3783	Honeycomb Hill	NRS350 in situ	OxA20285	20,760 ± 90	-21.44	0.88
*Pachyornis australis**	A3726	Honeycomb Hill	MNZ S25863.2	OxA20366	17,645 ± 60	-21.03	1.65
*Pachyornis australis**	A3727	Honeycomb Hill	MNZ S25867	OxA20367	19,335 ± 70	-21.73	2.20
*Pachyornis australis**	A3742	Honeycomb Hill	MNZ S25655	OxA20286	16,860 ± 75	-22.66	0.60
*Pachyornis australis**	A2556	Honeycomb Hill	MNZ 25868	ANU-1611	14,730 ± 170	-21.8	-
*Pachyornis australis**	A2557	Honeycomb Hill	MNZ S25867	ANU-1612	14,950 ± 150	21.2	-
*Pachyornis australis**	A2555	Honeycomb Hill	MNZ S25864	NZA7646	15,000 ± 200	-	-
*Pachyornis australis**	A2594	Magnesite Q	NP 5305.1-2	OxA20289	1,021 ± 26	-24.69	-1.5
*Pachyornis australis**	GU139067	Moa Trap Cave	MNZ S33754	OxA12669	10,450 ± 45	-22.5	-0.8
*Pachyornis australis**	A3766	Moa Arch	NRS324 in situ	OxA20297	10,235 ± 45	-22.32	1.28
*Pachyornis australis**	A3761	Moa Arch	NRS324 in situ	OxA20296	10,265 ± 45	-21.97	0.30
*Pachyornis australis**	A3757	Moa Arch	NRS324 in situ	OxA20595	10,280 ± 45	-25.75	0.60
*Pachyornis australis**	AC923	Cheops Cave	MNZ S41344	OxA20288	1,928 ± 27	-22.24	1.07
*Pachyornis elephantopus***	AC3736	Bulmer Cave	MNZ S23569	OxA20287	564 ± 26	-21.73	3.62
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ6586	14,029 ± 138	-21.47	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ6453	15,677 ± 163	-21.67	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ7323	18,600 ± 230	-	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ7292	20,600 ± 450	-21.44	0.88

Table 1. continued

*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ7642	13,850 ± 140	-	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ6480	14,194 ± 140	-23.35	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZA574	18,300 ± 170	-22.77	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ7647	18,650 ± 250	-	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ6589	14,062 ± 138	-21.54	-
*Pachyornis elephantopus***	-	Honeycomb Hill	-	NZ7675	12,950 ± 450	-21.51	-
*Pachyornis elephantopus***	-	Madonna Cave	MNZ S28064	NZA2505	14,740 ± 110	-22.6	-
*Pachyornis elephantopus***	-	Madonna Cave	-	NZA2446	20,680 ± 160	-22.4	-
*Pachyornis elephantopus****	A2757	Avondale	SMAG 88.95	OxA20326	2,885 ± 28	-22.42	2.28
*Pachyornis elephantopus****	A2759	Riverton	SMAG E80.13	OxA20333	1,336 ± 24	-24.54	3.49
*Pachyornis elephantopus****	GU139071	Stewart Island	OM Av4661	NZA9069	654 ± 56	-22.3	3.18

The $\delta^{15}N$ values of the three individuals were significantly different from those of a sample of 11 other *P. elephantopus* from northwest Nelson (Single factor ANOVA: mean 1.54 v 5.973, $df = 1,12$, $F = 51.521$, $P = 1.12 \times 10^{-5}$, $F_{crit} = 4.747$) but not from those of 13 *P. australis* from the same area (Single factor ANOVA: mean 1.54 v 1.354, $df = 1,14$, $F = 0.0531$, $P = 0.8211$, $F_{crit} = 4.600$). A bi-isotopic plot (Fig. 1B) placed the three individuals clearly within the range for *P. australis*. On this basis, the three birds are most likely to be *P. australis* rather than *P. elephantopus*.



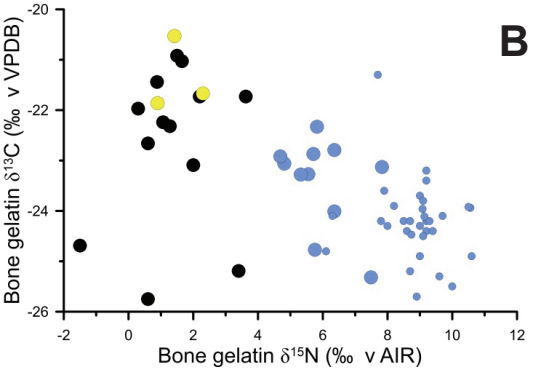


Figure 1. Carbon and nitrogen stable isotopic evidence for mis-identification of three *Pachyornis* moa from sites on Takaka Hill. **A.** 3-dimensional (95.4% confidence interval envelopes) kernel distributions of carbon and nitrogen stable isotopic values of *P. australis* (green) and *P. elephantopus* (blue) in relation to altitude above ambient sea level at time of deposition: upper, three Takaka Hill individuals identified as *P. elephantopus* in Rawlence *et al.* (2012) circled, arrowed; lower, three individuals re-classified as *P. australis*. **B.** bi-isotopic plot for *P. australis* (black) and *P. elephantopus* (blue) with three Takaka Hill individuals identified as *P. elephantopus* in Rawlence *et al.* (2012) high-lighted (yellow). Large symbols for *P. elephantopus* are for data included in Rawlence *et al.* (2012); small symbols are data for *P. elephantopus* in North Canterbury from Allentoft *et al.* (2014) and Holdaway *et al.* (2014).

We do not suggest that stable isotopic ratios are a stand-alone method for taxon identification, but they are certainly an additional, independent criterion that can highlight potential issues in morphological and genetic taxonomy. No method can be taken as inherently error-free, but at present it seems that aDNA might be seen in that light. Hence, it is not a case of stable isotopes versus aDNA and morphology. Rawlence *et al.* (2012)

highlight a significant number of individuals whose identifications by one or other of these methods are in conflict. Stable isotope data can provide measures of the ecology of the different taxa, which, as competitors rarely co-exist, can provide independent evidence that suggests revisiting identifications by one or both standard methods.

Potential issues with stable isotopic ratios in bone protein could include abnormal reliance on a different diet forced on the birds by local circumstances. However, bone proteins are long-lived in an individual and integrate dietary and ecological conditions over many years (Holdaway *et al.* 2011). The ratios are largely independent of plant taxon, especially given that there are few C4 plants in New Zealand and none in the habitats generally used by moa. Similarly, aberrant stable isotopic values are extremely unlikely to result from introgression between the two *Pachyornis* taxa. Hybridisation has never been mooted / recorded for moa, either on the basis of morphology or genetics.

Finally, the distribution of the taxa in space and time supports the new species identification better than the present attributions. In addition, the authors of most of the genetic identifications for *Pachyornis* moa in the deposits at Honeycomb Hill (Rawlence *et al.* 2012) themselves suggest significant levels of mis-identification in the sample. The statistically significant differences – see above – between the $\delta^{15}N$ values of unquestioned *P. elephantopus* and *P. australis* show that the individuals with "anomalous" values can be clearly allocated to the other taxon.

We believe that by using all the available data we have made a strong case for revisiting the genetic and morphological identifications of the individuals concerned, and in general for use of stable isotopic data in future studies. The relationships within *Pachyornis* require further work as the phylogenetic tree provided by Rawlence *et al.* (2012) nests two species within the *P. elephantopus* clade, between separate branches still attributed to *P. elephantopus*. Clearly, if independent data such as from stable isotopes can focus attention on particular issues of identification, this will benefit all moa research. As Gilbert *et al.* (2005), point out there is no basis for an assumption of error-free analysis in aDNA, any more than in any other field.

The potential re-identification of the three *Pachyornis* individuals as *P. australis* on stable isotopic data arising from the present analysis suggested that a wider inspection of the identification was warranted. A starting point was the observation that *P. australis* individuals of all geologic ages exhibited $\delta^{15}N$ values <4‰ (Fig. 2). Morphologically and genetically identified *P. elephantopus* individuals other than the three from Takaka Hill, in contrast, all had $\delta^{15}N$ values >4‰ (Fig. 2). A classification tree analysis (Breiman *et al.* 1984) implemented in the *rpart* package in R, yielded a partition value of $\delta^{15}N = 2.24$‰ (Fig. 2), with three (of 19) *P. australis* and two (of 64) *P. elephantopus* mis-categorised. Two of the three *P. elephantopus* from Takaka Hill fell beneath the 2.24‰ separator and the third, at 2.3‰, within measurement error (Table 1; Fig. 2). Two accepted aDNA-identified *P. australis* and two of the southern individuals here suggested to be *P. australis* fell in the "grey area" between the observational interspecies boundary and the third was, again, within measurement error of the partition value (Table 1; Fig. 2).

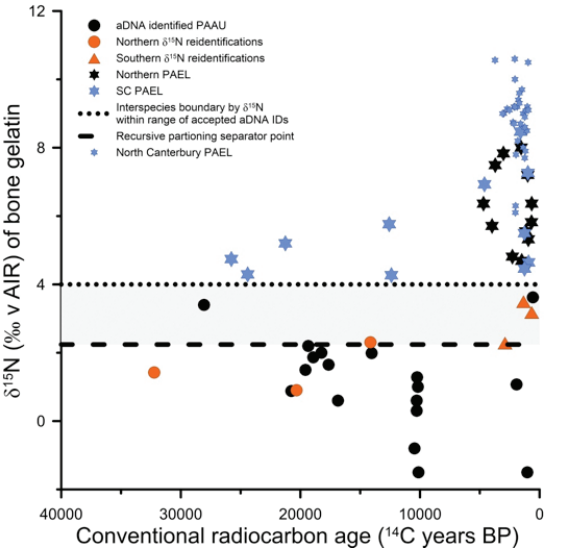


Figure 2. Bone protein nitrogen stable isotopic ratios for *Pachyornis australis* (black circles) and *P. elephantopus* (northern South Island, black stars; southern clade, blue stars) and contested identifications of *P. elephantopus* (Takaka area, orange circles; Southland and Stewart Island, orange triangles) through time. $\delta^{15}N$ values and conventional radiocarbon ages for *P. australis* and contested identifications from Table 1; "northern" and "southern clade" data for *P. elephantopus* from Rawlence *et al.* (2012) and their sources. Abbreviations: PAAU, *P. australis*; PAEL, *P. elephantopus*; SC, southern clade. Interspecies boundary (dotted line) set at the $\delta^{15}N$ value separating the values for presently accepted genetically identified *P. australis* and *P. elephantopus* individuals. All three north-western South Island individuals here assigned to *P. australis* have $\delta^{15}N$ values within the range of accepted identifications of that taxon, as do those for the three southern South Island individuals.

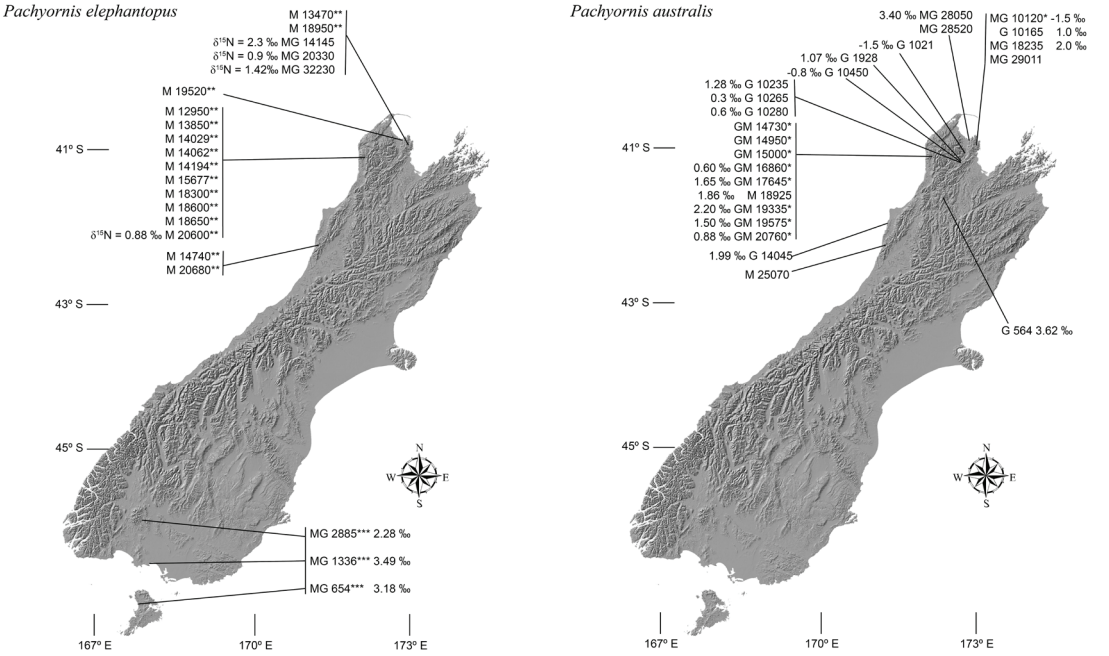


Figure 3. Geographic distribution of *Pachyornis* individuals with the bases for identification, conventional radiocarbon ages (without SD), and bone protein $\delta^{15}N$ values (where available). **A**, for individuals identified as *Pachyornis elephantopus* from fossil deposits west of the South Island Main Divide, in western Southland, and on Stewart Island. M, identified on morphology; G, identified genetically; **, identification on morphology questioned by Rawlence *et al.* (2012); ***, identified both on morphology and by genetic analysis, questioned here on species differences in $\delta^{15}N$ values. **B**, for individuals identified as *Pachyornis australis* from fossil deposits west of the South Island Main Divide, in western Southland, and on Stewart Island. M, identified on morphology; G, identified genetically; *, identification on morphology by Worthy (1989), changed from genetic analysis by Rawlence *et al.* (2012) M25070 (MNZ S28192, Table 1) was identified originally (Worthy & Holdaway 1993) as *P. australis*, not as *P. elephantopus* as listed by Rawlence *et al.* (2012). Digital Elevation Model courtesy School of Earth and Environmental Sciences, University of Canterbury.

There was no relationship between $\delta^{15}N$ values and time for the 18 individuals of unchallenged identification as *P. australis* (Table 1): a generalised linear model of $\delta^{15}N$ against conventional radiocarbon age yielded a P value for zero slope of 0.092; reduced major axis regression, $r^2 = 0.1505$, $t = 1.6834$, permutation $P = 0.1097$; robust regression, r^2 and t as for reduced major axis, permutation $P = 0.117$.

Of the ten *P. elephantopus* from Honeycomb Hill caves, a $\delta^{15}N$ measurement is presently available for only the individual dated by NZ7292. The $\delta^{15}N$ value of 0.88‰ (Table 1) supports the contention that it is *P. australis* (Rawlence *et al.* 2012). Three other individuals identified as *P. elephantopus* had $\delta^{15}N$ values <4‰ (Fig. 2), two from western Southland and a third from Mason Bay, Stewart Island (Table 1; Fig. 3). If the $\delta^{15}N$ value is indeed species-specific, as present data suggest, then SMAG 88.95 (Avondale) and SMAG E 80.13 (Riverton) as well as OM Av 4661 (Mason Bay, Stewart Island) should be identified as *P. australis*. As noted above, Worthy (1989) included Southland dunes in the range of *P. australis*, and Worthy (1998c) recorded one *P. australis* from dunes there. Worthy (1998a) makes a case for the Mason Bay individual having been brought to the island by early Polynesians, there being no other evidence that moa taxa other than *Dinornis robustus* were present there (Worthy 1998b; Holdaway *et al.* 2001). The Mason Bay individual would be the first *P. australis* to be found in an archaeological context (Worthy 1999).

It appears from these analyses that all the *Pachyornis* individuals west of the Divide presently identified as *P. elephantopus* are, instead, *P. australis*. If the remainder are also *P. australis*, too, it would mean that the glacial and interglacial moa faunas would need major re-assessment. The distributions of *P. elephantopus* and *P. australis* (Fig. 3) will need to be combined under *P. australis*. It would also

mean that the systematics of the genus *Pachyornis* will need to be revisited. That in turn would raise the possibility that the canonical association of *P. elephantopus* with *Euryapteryx curtus* as part of a fauna associated with forest-shrubland mosaics (Worthy 1997; Holdaway & Worthy 1997; Worthy & Holdaway 1993, 1994, 1995, 1996; Worthy 1998d; Worthy & Holdaway 2002) during the Holocene in the eastern South Island, occupying similar habitats west of the Divide during the Weichselian-Otiran glaciation, will have to be abandoned. *P. elephantopus* may have been confined always to the eastern South Island, as was *Emeus crassus* (Worthy & Holdaway 2002).

If these changes in identification are confirmed, it will have significant implications for the *Pachyornis* phylogenetic tree presented by Rawlence *et al.* (2012). That tree has issues such as the nesting of *P. geranoides* (North Island) and *P. australis* within a 'fragmented' *P. elephantopus*. Removing the western and southwestern *P. elephantopus* individuals to *P. australis*, as already suggested in part by Rawlence *et al.* (2012), may assist in resolving relationships within the genus. That, in turn, may involve the recognition of other taxa for, for example, the isolated western Southland population.

The re-identifications supported by stable isotopic evidence also caution against automatic acceptance of genetic identifications. State of preservation of bone and its biochemical contents, particularly in older material, and laboratory errors such as mislabelling or inter-sample contamination can and do introduce errors, just as misidentified voucher material can affect identification on morphology (Holdaway & Worthy 1993).

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Appendix 1. Basic R script for generation of 3-dimensional kernel distributions.

```
require (ks)
require (misc3d)
require (rgl)
d<-read.table(file.choose())
Altitude<-d$V1
d13C<-d$V2
d15N<-d$V3
plot3d(x=Altitude,y=d13C,z=d15N,type="s",size=0.9,col="green")
d.dens3d<-kde(x=d,gridsize=c(64,64,64),compute.cont=TRUE)
x.latt<-d.dens3d$eval.points[[1]]
y.latt<-d.dens3d$eval.points[[2]]
z.latt<-d.dens3d$eval.points[[3]]
contour3d(x=x.latt,y=y.latt,z=z.latt,f=d.dens3d$estimate,color="green",level=d.dens3d$cont[95.4],add=TRUE,alpha=0.5)
decorate3d(box = TRUE,axes = FALSE, main = NULL, sub = NULL,top = TRUE, aspect = FALSE, expand = 1.03)
d<-read.table(file.choose())
Altitude1<-d$V1
d13C1<-d$V2
d15N1<-d$V3
plot3d(x=Altitude1,y=d13C1,z=d15N1,type = "s", col="blue",size=1.0,add=TRUE)
d.dens3d<-kde(x=d,gridsize=c(64,64,64),compute.cont=TRUE)
x.latt<-d.dens3d$eval.points[[1]]
y.latt<-d.dens3d$eval.points[[2]]
z.latt<-d.dens3d$eval.points[[3]]
contour3d(x=x.latt,y=y.latt,z=z.latt,f=d.dens3d$estimate,color="blue",level=d.dens3d$cont[95.4],add=TRUE,alpha=0.5)
decorate3d(box = FALSE,axes = FALSE, main = NULL, sub = NULL,top = TRUE, aspect = FALSE, expand = 1.03)
```