TUHINGA

Records of the Museum of New Zealand Te Papa Tongarewa

MORPHOLOGY, MYOLOGY, COLLAGEN AND DNA OF A MUMMIFIED UPLAND MOA, *Megalapteryx didinus* (AVES: DINORNITHIFORMES) FROM NEW ZEALAND

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MUSEUM
OF NEW ZEALAND

Tuhinga: *Records of the Museum of New Zealand Te Papa Tongarewa* is a peer-reviewed publication, ISSN 1173-4337. It is published at irregular intervals by the Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, New Zealand. It supersedes the following publications: (in 1993) *Museum of New Zealand Records* (1171-6908); (in 1992) *National Museum of New Zealand Records* (0110-943X); *Dominion Museum Records; Dominion Museum Records in Ethnology*.

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No. 4, pp. 1-26, 9 figs (1995)

MORPHOLOGY, MYOLOGY, COLLAGEN AND DNA OF A MUMMIFIED UPLAND MOA, *Megalapteryx didinus* (AVES: DINORNITHIFORMES) FROM NEW ZEALAND

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ABSTRACT: Study of a naturally mummified 600-yr-old *Megalapteryx didinus* (Owen, 1883) permits description of cranial myology, stapedial morphology, DNA and collagen content of the skin. After rehydration of the head and neck, the structure of nine cranial muscles could be determined, including most of the jaw muscles, the **M. orbicularis palpebrarum** and the **M. dermotemporalis**. The **postorbital** and **external jugomandibular ligaments** are also described. The stapes is unusual among ratites in that it has a kink in the middle of its shaft, a small basal fossa and no struts. The shaft joins the footplate over a large area, differing distinctly from *Apteryx*, but most closely approaching *Dromaius*. Collagen is preserved in the skin and, although severely degraded, appears to have kept its helical configuration and much of its original immunological reactivity. DNA preserved appears to be that of *Megalapteryx* and not of bacterial origin. It reacted strongly with *Apteryx* genomic DNA.

INTRODUCTION

The *Nelson Evening Mail* of 2 March 1943 records the discovery of "moa bones in cave" in an account of an expedition led by Dr W.R.B. Oliver, Director of the Dominion Museum in Wellington (now the Museum of New Zealand Te Papa Tongarewa). Oliver, with the help of local enthusiasts, had unearthed parts of skeletons of several moas in a cave at Tarakohe near Takaka, in New Zealand's South Island.

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[1] A full list of authors' affiliations is on page 26.

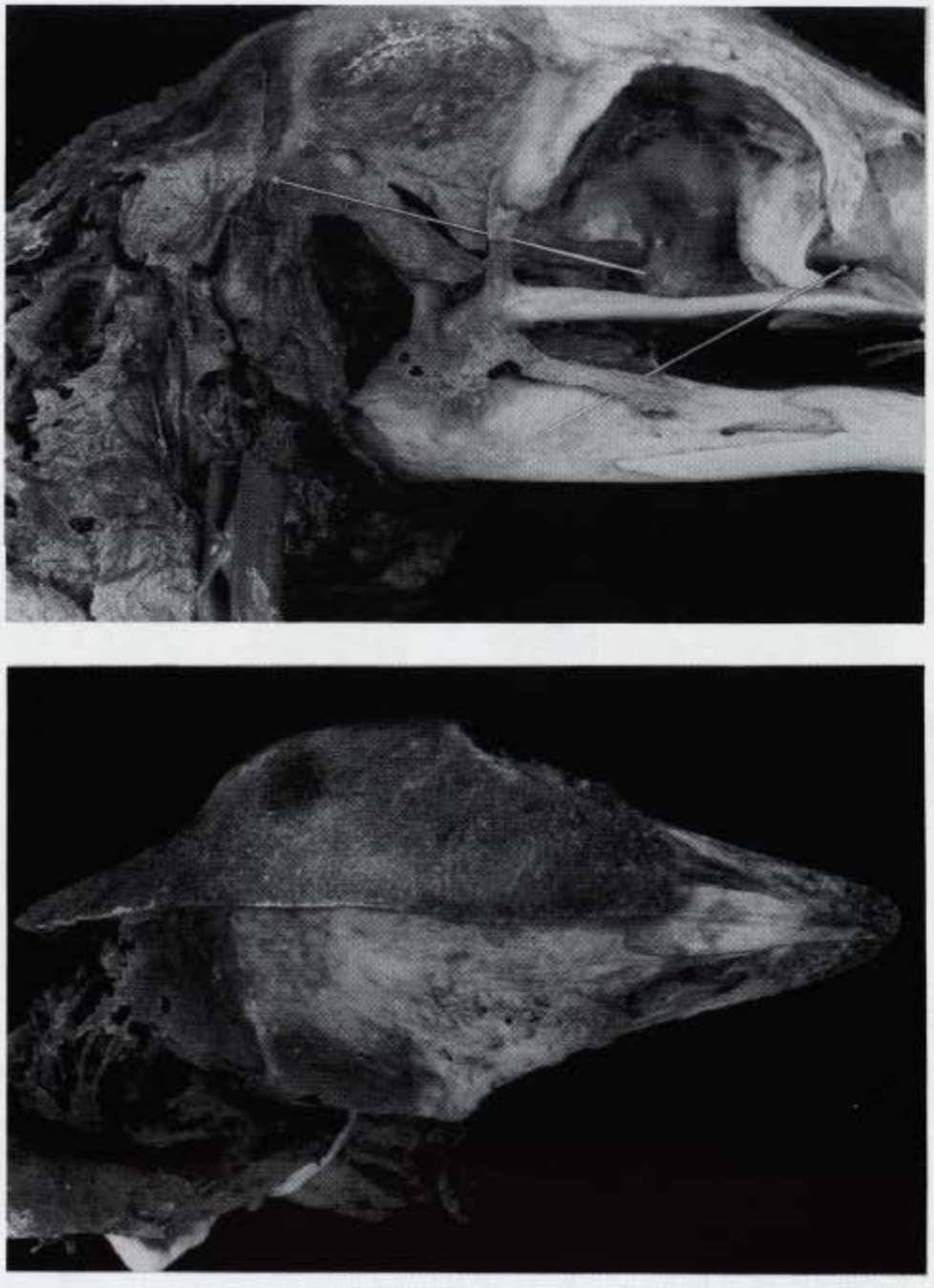
The most important part of the expedition, however, was not this excavation (described in Oliver, 1949: 18, fig.13) but the acquisition of a skeleton of a small species of moa, *Megalapteryx didinus* (Owen), which "had been collected at Cromwell, Otago, many years ago, and was in the possession of Mr D.W. Simpson, of Takaka ... Evidently it [the skeleton] had lain in a dry cave since the bird died as the head and upper part of the neck still is covered with flesh and skin" – so read Oliver's manuscript notes on the 1943 expedition in the Museum of New Zealand. The specimen (MNZ S400, formerly DM 400) was crated and sent to Wellington, finally arriving there by 15 March 1943. A letter from Oliver to Mr Simpson, on this date, records payment of £5 for it.

According to a manuscript note written by Dr R.A. Falla (Director of the Dominion Museum after Oliver) dated 23 September 1957, which accompanies the specimen, it "was obtained at Cromwell by Mrs [R.H.] Simpson's grandfather Mr James Campbell (of Nelson) who arrived in New Zealand in 1863. It is not recorded when or how he got the moa. The skeleton was given to Dr Oliver by Mr and Mrs [R.H.] Simpson's son Mr D.W. Simpson." Unfortunately, the specimen had been collected long before Oliver's visit to Takaka, and the exact locality in the Cromwell area from which it was obtained was never pinpointed.

The Cromwell *Megalapteryx* mummy (referred to during the present study as "Oliver" in reference to both Oliver Cromwell 1599-1658, Lord Protector of England, and Walter Reginald Brook Oliver 1883-1957, Director of the Dominion Museum) is indeed a rare object. Only three other mummified specimens of this genus of moa are known – the Queenstown head and legs in the Natural History Museum, London (figured in Owen, 1883: pls 59-61; Oliver, 1949: fig.8; Stevens *et al.*, 1988: 114; Anderson, 1989: figs 5.12, 5.13; Holdaway and Worthy, 1991: 55; McCulloch and Cox, 1992: 40), the articulated leg from Waikaia, Old Man Range, in the Otago Museum, Dunedin (figured in Oliver, 1949: fig. 9; Anderson, 1989: fig. 5.9; Holdaway and Worthy, 1991: 59) and the Mt Owen, northwest Nelson, feet and associated remains in the Museum of New Zealand (Worthy, 1989a: figs 1,2; Anderson, 1989: fig. 4.8).

Very few mummified remains of other moa genera exist, and these are listed and reviewed in Worthy (1989a) and Anderson (1989). The most significant are: for the genus *Dinornis*, the Tiger Hill partially mummified skeleton and the Galloway, Knobby Range, leg; for *Emeus*, the Earnscleugh Cave neck and leg; for *Pachyornis*, the Nevis leg, and for *Anomalopteryx*, the recently found, partially mummified skeleton from Echo Valley. All this mummified material, except the Mt Owen remains, is from Otago and Southland in the southern South Island. The photograph (Forrest, 1987: fig. 2) of the partially articulated skeleton of *Anomalopteryx* lying *in situ* in the Echo Valley dry rock shelter in Southland is the only partially articulated moa mummy photographed *in situ*. DNA has been extracted and amplified from most of these mummified specimens (Cooper *et al.*, 1992).

Fig. 1– Mummified head and neck of Upland Moa *Megalapteryx didinus* MNZ S400 before rehydration and dissection. (Distance from crown of skull to base of neck, about 180 mm.) *M. didinus* feathers, MNZ S 27950, are presumed to have been collected in 1949 by R.A. Falla from a cave in Takahe Valley, Fiordland. (Main shaft of upper left double-shafted feather, about 75 mm.) Both illustrations by P. Trusler.



Rehydrated cranial mummy of *Megalapteryx didinus* (MNZ S400) during dissection. Fig. 2 (above), right lateral view with upper pin under postorbital ligament and lower pin under anterior slip of external jugomandibular ligament. Fig. 3 (below), top view with skin on right side of head removed.

With the support and encouragement of the Museum of New Zealand Te Papa Tongarewa, a detailed study of the Cromwell *Megalapteryx* mummy was undertaken by several researchers in Victoria (Australia), New York, and Wellington between 1985 and 1988. Samples of the dried muscle and skin were taken for biochemical analysis at both Monash University and Victoria University of Wellington, the mummified head and neck was extensively photographed in black and white, and in colour, stereo pairs at the Museum of New Zealand, and photographed and drawn (Fig. 1) at Monash University, and only then was it rehydrated in preparation for dissection. Dissection was carried out on one side of the head only, in order to preserve the other side undisturbed. The skin and feather bases were examined for feather lice without success (R. Palma, Museum of New Zealand, pers.com.), and after a six-month rehydration programme at Monash University, dissection was carried out at Columbia University over a period of two years. All parts of the head and neck of S400 were then returned to the Museum of New Zealand. During 1991-93 further aspects of the morphology of the head and neck of S400 were studied at the Museum of New Zealand, and the associated post-cranial skeleton, with some attached skin and ligaments, described.

This report presents the combined results of these various team studies of the mummified Upland Moa *Megalapteryx didinus* MNZ S400 between 1985 and 1993.

DISTRIBUTION AND HABITS OF *Megalapteryx*

Megalapteryx didinus was a reasonably small moa species, estimated at about 25 kg (Anderson, 1989) and was restricted to the South Island of New Zealand (Worthy, 1990). Its remains (usually bones) have been found frequently and it was the commonest moa in alpine elevations, and its unusual feathering from bill to the base of the ankle may be an adaptation for coping with the cooler temperatures and humid conditions of its habitat (Worthy, 1990). Therefore, although *Megalapteryx* has often been called the "bush moa", the preferred name is now the Upland Moa. Although several species, generally two, have been proposed for the genus, Worthy (1988a) demonstrated that the genus was monotypic. Preference for remote upland South Island habitats may have resulted in *Megalapteryx* having been one of the last genera of moa to exist in New Zealand, and it is interesting that the only moa skin recorded in an archaeological context was a narrow strip of *Megalapteryx* skin sewn in a cloak along with weka skins and found in an Otago burial cave about 1890 (Anderson, 1989). *M. didinus* feathers from a Fiordland cave are shown in Figure 1.

IDENTIFICATION OF MNZ S400

The characters of both the cranial and poscranial skeletal material of MNZ S400 confirm the identification of this partially mummified specimen as an Upland Moa *Megalapteryx didinus* (Owen, 1883).

Six genera of moa, in two families (Emeidae [= Anomalopterygidae of Oliver and some authors] and Dinornithidae)

including 11 species, are currently recognised (Turbott, 1990). Using even the most superficial suite of characters S400 is readily referable to the monotypic genus *Megalapteryx*.

The following selection of familial traits place it in the Emeidae: the cranium is about as wide as high (in dinornithids about twice as wide as high), there are 27 presacral vertebrae (29 or 30 in dinornithids), the tibiotarsus is not elongate with respect to the femur, and the tarsometatarsus is shorter than the femur, as shown by the legbone length ratio - femur : tibiotarsus : tarsometatarsus of 1 : 1.5 : 0.7 (1 : 2.0 : 1.1 in dinornithids), and on the caudal femur surface the *Linea intermuscularis caudalis* ends distally in a single complex tuberosity with the *interna* and *externa* branches continuous with each other (not two distinct tuberosities with no connection of the *externa* and *interna* branches).

Within the Emeidae, the narrow, pointed premaxilla immediately excludes the genera *Emeus* (one species) and *Euryapteryx* (two species). The shape, size and relative proportions of the legbones also exclude these genera, and *Pachyornis* (three species) as well. *Megalapteryx* (with its single species *M. didinus*) and the monotypic genus *Anomalopteryx* remain the only taxa to which S400 might be assigned, but a comparison of these two taxa allows specific assignment of the mummified specimen to the former (Oliver, 1949; Worthy, 1988a, 1988b).

The following comparison between *Megalapteryx didinus* and the Little Bush Moa *Anomalopteryx didiformis* (Owen, 1844) allows conclusive identification of S400 to *M. didinus*.

Skull: premaxilla narrower and more sharply pointed in *Megalapteryx* than in *Anomalopteryx*; mandibular rami less robust and more obviously downcurved towards the tip; postorbital width greater than rather than subequal to that across zygomatic processes; temporal fossae narrower and not excavated ventrally on the anterior margin; temporal ridge separated from the lambdoidal by a narrow gap rather than convergent upon the lambdoidal; orbital margins of nasals convergent towards premaxilla rather than parallel; maxillary antrum nearly collapsed, rather than expanded and robust; preorbital plate angled, diverging from the rostrum at a wide angle.

Sternum: precostal processes triangular and more sharply pointed; costal region relatively more elongate; posterior notches (between xiphoid and lateral processes) less deep; coracoidal depressions shallower and less distinct.

Pelvis: plane of escutcheon steeper; preacetabular region narrower; bases of ischia and pubes (forming internal, ventral borders of acetabulae) both meet with synsacrum at nearly a right angle rather than forming a U-shaped junction.

Femur: shaft more slender; proximal and distal ends relatively narrower; rotular groove narrower; fossa above fibular condyle anterior to popliteal fossa, unlike that which is level with popliteal fossa in *Anomalopteryx*.

Tibiotarsus: shaft more slender; proximal and distal ends relatively narrower; procnemial ridge straight, rather than curved.

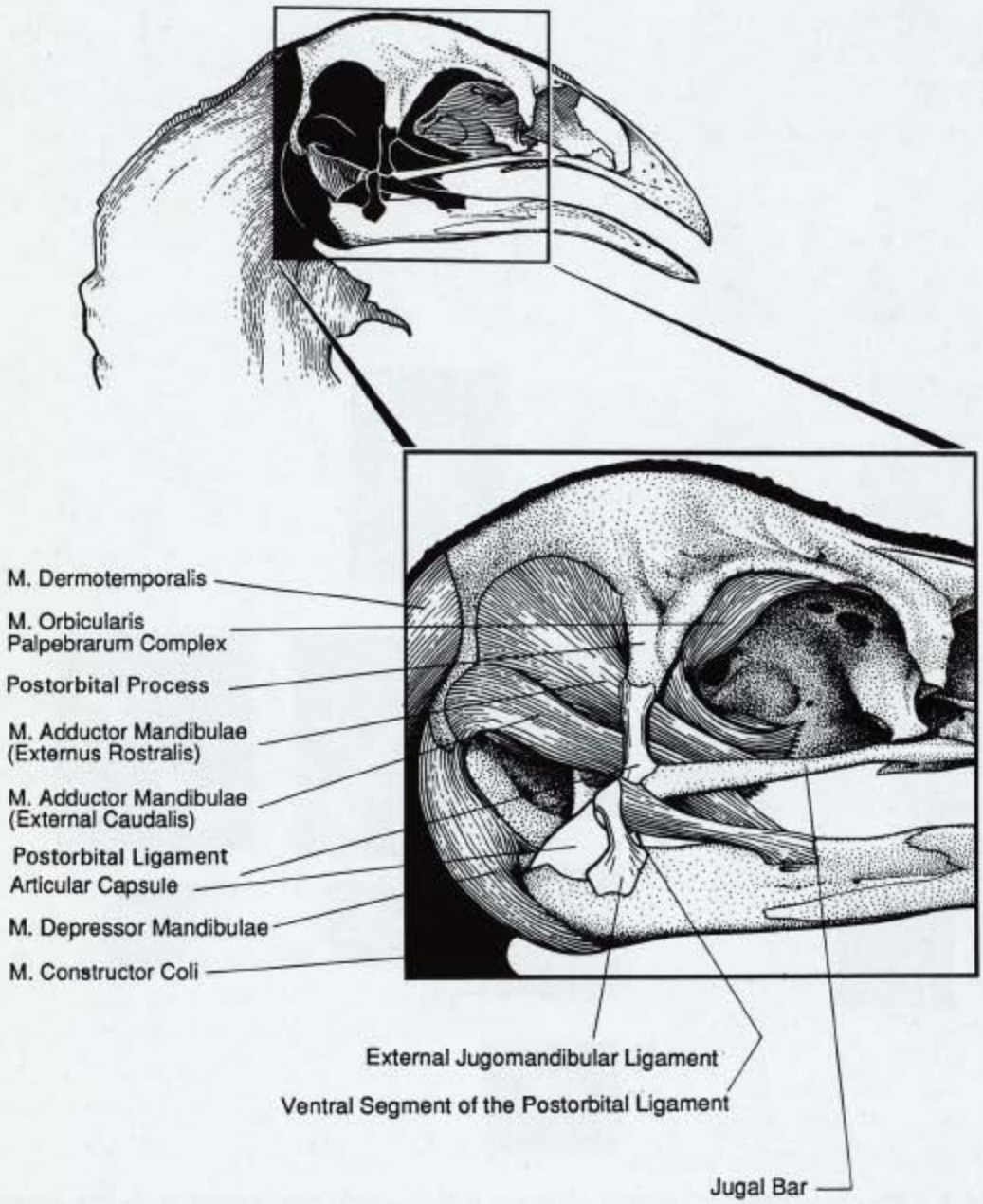


Fig. 4 – Partially dissected head of *Megalapteryx didinus* (MNZ S400), lateral view showing cranial myology. Illustration by P. Trusler.

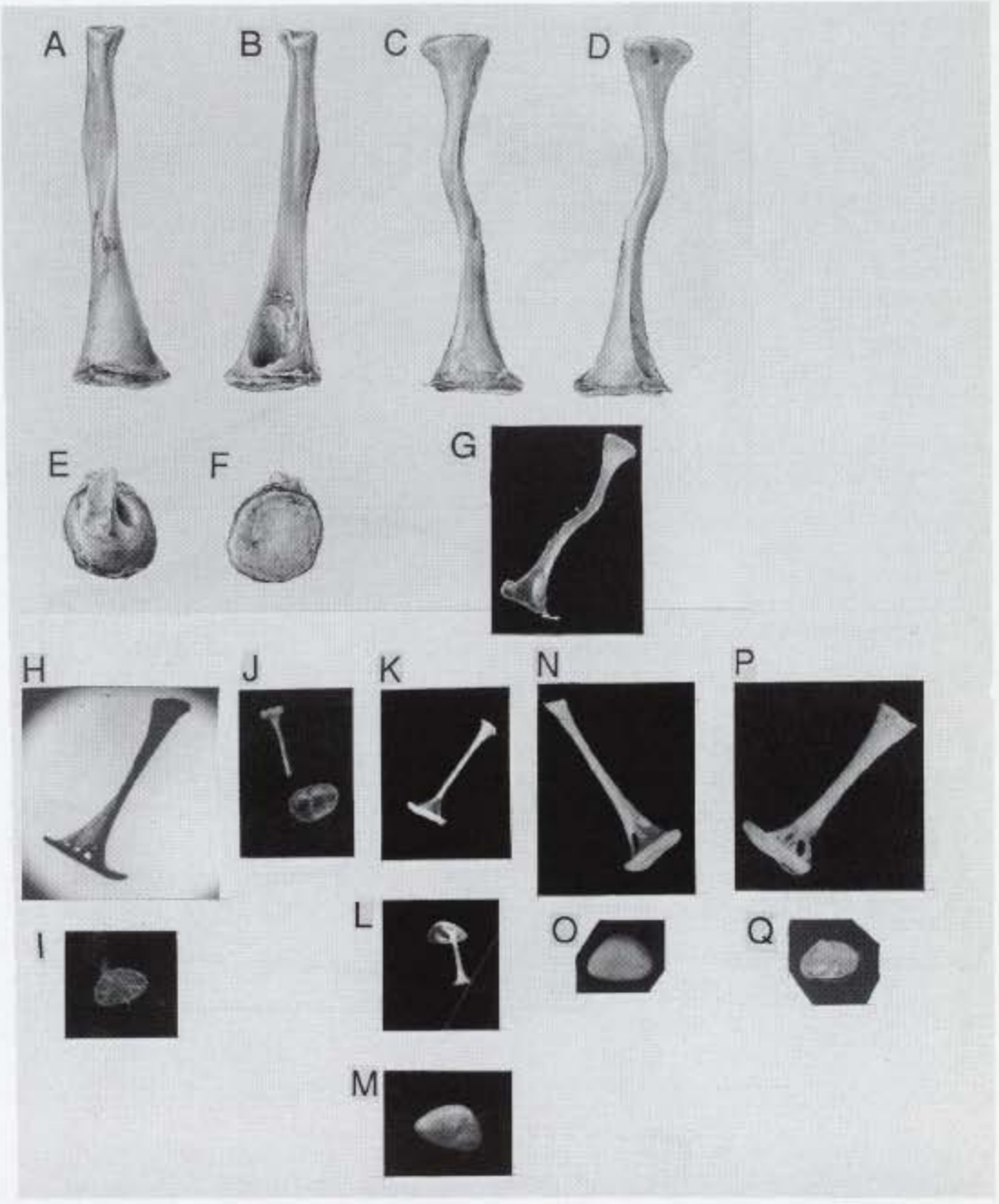


Fig. 5 – Stapes of *Megalapteryx didinus* (MNZ S400) compared with the stapes of other birds thought to be related to moas. A-G, *M. didinus* (MNZ S400, right stapes, 6 mm in length); H-I, *Rhynchotus rufescens* (AMNH 6605, left, 2.08 mm in length) a tinamou; J, *Apteryx* (AMNH 4437, right, 2.96 mm in length) a kiwi; K-M, *Struthio camelus* (AMNH 1173, left, 7.76 mm in length) Ostrich; N-O, *Pterocnemia* (AMNH 12262, left, 4.8 mm in length) a rhea; P-Q, *Dromaius novaehollandiae* (Monash University collection, left, 5.9 mm in length) Emu.

Tarsometatarsus: shaft relatively narrower; medullarterial foramen on sloping medial surface of hypotarsal ridge in a distinct, deep, sharply-angled depression (rather than opening directly to the sloping surface without a deep fossa).

DESCRIPTION OF *Megalapteryx didinus* MNZ S400

The mummified head and neck of *Megalapteryx didinus* from Cromwell were first illustrated by Oliver (1949: fig. 6) in a black and white photograph of the right side (republished in Oliver, 1955: 568), with this view shown in different black and white photographs in several later publications (e.g. Falla, 1974: 70; Anderson, 1989: fig. 5.5), and in colour photographs by Brewster (1987: 33) and McCulloch (1993: 4). A ventral view photograph showing the cricoid bone *in situ* was published in Scarlett (1975: 249). Figure 1 in the present work is a drawing of the head and neck from the right side before rehydration and dissection. Note the insect holes in the neck skin and tissue described in the **Cranial Myology** section below.

The photographic and graphic record of the S400 head shows that the maxillary process of the nasal was lost on the right side at some time between the late 1940s, when the photograph for Oliver (1949) was taken at the Dominion Museum, and late 1972, when Michael Trotter of the Canterbury Museum took the photograph used by Falla (1974).

The Brewster (1987) and Anderson (1989) photographs are from the Museum of New Zealand's 1985 stereo coverage and confirm the absence of the right maxillary process. Oliver's sketch (1949: fig. 121) of the right preorbital plate of S400 shows clearly the form of the missing process; S400's left maxillary process is present and in place, and will be illustrated and described in a further study by two of the present authors (T.H. Worthy and J.C. Yaldwyn).

The head and neck, and the other parts of *Megalapteryx didinus* S400 are described in outline by Oliver (1949: 146, 151, 153-155), who also published photographs in the same work of the pelvis (figs 123-125), the left femur and tibiotarsus joined by ligaments (fig. 126 right), and the left tarsometatarsus with one phalanx of digit II joined by a ligament (fig. 126 lower left). Oliver (1949) also provides some measurements of the skull, sternum, pelvis and leg bones of S400 (tables 24-26). Two photographs of the S400 pelvis were republished by Oliver (1955: 584).

The head and neck after dissection is held at the Museum of New Zealand in a mixture of ethanol and glycerine, and now consists of the cranium, upper and lower mandibles, and attached first nine cervical vertebrae with skin and tissue on left side, and skin reflected back from the head and dissected tissue on right side (Figs 3-5). The neck is still partly enclosed in damaged skin and tissue.

The first nine cervical vertebrae are linked in position of articulation by more or less complete ligaments (among them **ligamentum elasticum transversum, lig. elasticum interlaminare, lig. elasticum obliquum, lig. collaterale, lig. interspinosum, membrana interlaminaris** – see Baumel, 1979:172). Post-mortem drying, shrinkage and rupture of

certain of these ligaments has resulted in the neck, in this region, becoming twisted. The amount of twist reaches 90 degrees at the 9th vertebra, with the ventral surface of this element now facing left.

Also held detached in ethanol are the bony sclerotic ring of the right eye, the right stapes, a long slender hyoid bone (ceratobranchial) with attached terminal ligament, parts of the larynx (cricoid and arytenoids), and a connected section of tracheal rings from the throat.

Held dry is the other slender ceratobranchial, a piece of dried throat skin showing feather pits and with an attached section of tracheal rings and some dry tissue, and another piece of dried skin with an obviously straight cut edge. This latter piece of skin has numerous feather pits some bearing feather shafts up to about 16 mm in length.

The cranial myology and the stapes are described in separate sections below, while the sclerotic ring, the turbinals, the ceratobranchials, cricoid and arytenoids, and the tracheal rings will be described with the maxillary process of the nasal in a further study by Worthy and Yaldwyn.

The remainder of *Megalapteryx didinus* S400 consists of dry skeletal elements (vertebrae, pelvis, right femur, most of left leg, sternum, several ribs) with some attached dried skin and ligaments. Terminology used generally follows Baumel (1979) supplemented by Cracraft (1971).

Including the nine vertebrae in liquid preservative, there is a total of 27 presacral vertebrae and this is the number typical of all emeid moas (see Oliver, 1949: 35; Worthy 1989b: 170). Numbers 1-9 are conjoined (as described above) with the first (atlas) articulating with the skull; numbers 10 and 11 are free but have remnants of connective tissue attached; number 12 is attached by thin fibres to number 13; numbers 13 and 14 are firmly joined by several ligaments and have a sheath of parchment-like skin covering their right lateral and dorsal surfaces; number 15 is free; numbers 16 and 17 are conjoined by ligaments and some skin; the remainder (18-27) are loose (some have a veneer of cartilage and/or connective tissue on various of their articular surfaces, for example remnants of menisci coating surfaces of the synovial joints between the vertebrae).

The pelvis is complete and bears fixed ribs on fused pelvic vertebra 29. Connective tissue of the **capsula articularis** (the joint capsule of Cracraft, 1971: 181) lines all but the posterior surface of the left acetabulum. The proximal portion of the **teres ligament (lig. capitis femoris)** remains inserted on the anteroventral rim of the foramen acetabuli, but no trace of the **posterior acetabular ligament** has been preserved. No caudal vertebrae are present.

The right femur has both the trochanter and anterior face of the internal condyle eroded. Part of the right tibiotarsus of *Megalapteryx didinus* S400 was originally present but was used for bone collagen radiocarbon dating at the New Zealand Institute of Nuclear Sciences in 1988. There are no other bones of the right leg of S400 present in the collection.

The left femur, tibiotarsus, fibula, tarsometatarsus and some phalanges of

S400 are present. The femur is attached to the fibula by a ligament, and the fibula is firmly and closely attached to the tibiotarsus by ligaments. The tarsometatarsus is not attached to the tibiotarsus but has the 1st phalanx of digit II attached by ligaments to the inner trochlea.

On the femur a veneer of connective tissue remains on parts of the head (caput femoris) and lateral trochanteric surface. The distal portion of **Musculus iliotrochantericus posterior (gluteus profundus)**, comprising a dense mass of fibres up to 30 mm long, inserts on a curved ridge on the lateral surface of the femur just distal to the trochanter. A thin veneer of connective tissue covers most of the articular and lateral surfaces of the femoral condyles.

Portions of several ligaments and muscles of the knee joint are preserved:

(a) Part of the strong, strap-like (60 x 10 x 3 mm) **lateral ligament (lig. collaterale laterale)** is complete between its insertions on the lateral side of the fibular condyle of the femur and the lateral side of the proximal end of the fibula. A parallel portion of similar size remains inserted on the femur but its connection to the fibula has been severed.

(b) The **medial ligament (lig. collat. mediale)** is represented by only a few fibres at its point of insertion on the medial condyle of the femur.

(c) The **anterior cruciate ligament (lig. cruciatum craniale)**, inserted on the distolateral part of the popliteal area of the femur is represented by a mass of fibres up to 25 mm in length. Remnants of various other interjoint ligaments are also present.

(d) The **posterior cruciate ligament (lig. cruc. caudale)**, attached to the lateral side of the rotular groove of the femur, consists of fibres up to 20 mm long.

(e) The proximal portion (tendinal attachment) of **M. tibialis cranialis** remains inserted in the fovea tendineus on the lateral condyle of the femur.

The fibula is held firmly in its position against the fibular crest of the tibiotarsus by **lig. interosseum tibiofibulare**. Portions of **lig. meniscofibulare caudale** remain attached to the posteromedial surface of the head of the fibula.

On the tarsometatarsus substantial fibrous remnants (presumably analogous to the ossified tendon which continues from the achilles tendon in the turkey – see Harvey *et al.*, 1968: 220) remain fused to the proximal and lateral parts of the lateral hypotarsal ridge, and a few fibres remain on the distomedial surface of the medial hypotarsal ridge. Collateral ligaments remain on the proximal end of phalanx 1 of digit II which is still attached to the tarsometatarsus by a medial ligament firmly inserted in the medial fovea lig. collateralis of the trochlea for digit II (trochlea metatarsi secundus). A thin veneer of connective tissue adheres to the articular surfaces of the trochleae for digits II and III.

The distal extremity of attached digit II phalanx 1 shows marks of probable rat gnawing. There are 5 loose phalanges from the left foot: I/2 also with probable rat gnawing, II/3 unguis with tip abraded, III/3 with medial side abraded, IV/2 and IV/3.

The sternum is present but the left xiphial area and the left posterior lateral process are damaged. There is also a very dark, almost black, stain or burn mark internally on the right upper quarter of the body of the sternum. The MNZ S400 sternum conforms in general size and shape with the range of *Megalapteryx didinus* sterna in the Museum of New Zealand collections.

Detailed comparison with sternum MNZ S443 (from a cave in Takahe Valley, Fiordland, collected by R.A. Falla *et al.*, August 1949) selected more or less at random as a representative *M. didinus* sternum, showed that there are some reasonably obvious minor differences. The craniolateral processes are somewhat less angular, and the posterior lateral processes are less robust than those of S443. Beneath (ventral to) its anterior margin S400 has a rounded protuberance (preserved on the right side only) of which there is no trace in S443. In S400 the coracoidal sulci are simple, shallow, rounded depressions, while in S443 the sulci take the form of moderately deep transverse grooves bordered above and below by discernible, if rather rounded, dorsal and ventral lips. These differences are considered to be individual and intra-specific.

The following ribs are present: left 2nd complete with fused uncinate process, left 4th with its uncinate process free, left 5th lacking distal end, left 6th complete, right 3rd proximal end only, right 4th complete, right sternal 4th complete, and right 6th lacking distal end.

CRANIAL MYOLOGY

The Cromwell *Megalapteryx didinus* cranial mummy (MNZ S400) was rehydrated for six months in a solution of 50% glycerine, 25% ethanol, 23% distilled water and 2% phenoxyethanol, which expanded and softened the muscles, and softened the collagenous and skin tissues (Figs 2-4). The mummy was kept refrigerated during this period to retard any form of decay. It should be emphasized that following rehydration of the moa mummy, dissection of its cranial muscles and ligaments was almost identical to working on these structures in a preserved specimen of a Recent bird. Our ability to dissect and describe the cranial muscles and ligaments of this specimen was limited by the damage caused by insect larvae and by earlier dissections on the still dehydrated specimen as described below.

The primary dissection used as a basis for the myological descriptions was carried out by W. Bock and P. Vickers-Rich at Columbia University. P. Trusler dissected the specimen further as he prepared the illustrations. Histological sections, which helped establish the ligamental nature of the jugomandibular ligament, were prepared by K.L. Bock.

Not all cranial muscles were still preserved in S400 at the time of our dissection. The superficial surfaces of most or all cranial muscles appeared to have been preserved in the mummy, but these muscles have a number of small holes which were almost certainly produced by insect larvae. The interiors of all muscles were destroyed, presumably by the action of insect larvae, which had consumed the muscular tissue

before the specimen became completely dried.

We suspect that the surface of the moa dried rapidly after death, so that the superficial layer of the cranial and cervical muscles could not be eaten by these insect larvae and hence were preserved in the mummy. Considerable damage was done to the floor of the mouth, the tongue apparatus and the palatal region, including the associated muscles of this specimen during an earlier, unrecorded dissection conducted on the dry mummy while it was held at the Dominion Museum.

It must be emphasized that it is imperative for mummified subfossil material, such as S400, to be rehydrated before dissection. Rehydration has long been used on dehydrated Egyptian mummies, to ensure maximum information with minimum damage to the specimen. Because of the extensive insect damage to the interiors of the extant cranial muscles, we confined our dissections to the superficial aspects of the muscles, and made no attempt to separate the parts of complex muscles, such as the **M. adductor mandibulae externus**, or to determine internal fibre arrangement of those muscles. Almost certainly nothing remains of the internal structure of these muscles.

Preservation and/or earlier dissection of *M. didinus* S400 made it difficult to determine the limits of and/or exact demarcation between some muscles. Thus, it was impossible to determine the exact area of origin of the **M. pseudotemporalis superficialis**. It was also difficult to ascertain precise muscle architecture in all cases. We were, however, able to identify two major ligaments (**external jugomandibular** and the **postorbital ligament**) and ten muscles, which are described here for the first time for this order of birds.

Ligaments

The **postorbital ligament** consists of distinct dorsal and ventral segments as found in other palaeognathous birds; these segments are slightly offset from one another with the ventral portion being slightly posterior to the dorsal portion. The dorsal **postorbital ligament** courses directly ventral from the ventral tip of the postorbital process and attaches on to the dorsolateral surface of the jugal bar. The ventral segment originates from the lateral surface close to the posterior end of the jugal bar and extends straight ventrally to attach to the lateral surface of the mandible. It is a very thin band, narrow in its midsection and flaring broadly ventrally.

The **external jugomandibular ligament** arises from the lateral surface of the jugal bar between the attachments of the dorsal and ventral segments of the postorbital ligament, and extends forward to attach on to the dorsolateral surface of the mandibular ramus just lateral to the insertion of the adductor mandibular muscles. It is a thick band, appearing fleshy and resembling a muscle; indeed, we first mistook this ligament for a muscle in our dissections. Histological sections of this structure showed no indications of muscular tissue, but resembled collagenous fibre. A similar thick **external jugomandibular ligament** is present in other palaeognathous birds (e.g. *Rhea, Stru-*

thio) as shown by subsequent dissections of these taxa.

The **internal jugomandibular ligament** could not be found, and presumably it is lacking, as it is in other palaeognathous birds.

Muscles

The **M. depressor mandibulae** originates from the posterior surface of the occipital bone, dorsal and posterior to the external auditory meatus and ventral to the origin of the **M. adductor mandibulae externus**. It inserts on the posteroventral surface of the mandible. It is mostly parallel-fibred.

Two parts of the **M. adductor mandibula externus** can be distinguished in this specimen. The **M.a.m.e. caudalis** originates from the ventral part of the temporal fossa. Its posterior end extends as far as the posterior end of the **M.a.m.e. rostralis**. The **caudalis** inserts on a small coronoid process on the dorsal edge of the mandibular ramus. Its fibre arrangement could not be determined because of insect damage. The **rostralis** originates from the dorsal three-quarters of the temporal fossa, which is separated from the lower quarter by a distinct ridge. Its insertion has also been destroyed by insect action. This muscle is pinnate.

The origin of the **M. pseudotemporalis superficialis** could not be observed, but it appears to arise from the posterolateral surface of the bony orbit and from the anterodorsal area of the temporal fossa. It inserts on the pseudotemporal process, a small mamelon or tubercle on the internal surface of the mandible just anterior to the quadrate articulation. All that is preserved of this muscle is the large tendon of insertion with a small number of muscle fibres attached to it. The **superficialis** appears to be partly parallel-fibred where preserved, but this appearance is only illusionary. As this muscle inserts by a thick tendon it must be pinnate as in almost all other birds.

The **M. pseudotemporalis profundus** originates along the lateral surface of the quadrate over a broad area, possibly by an aponeurosis. It inserts anterior to the **M. pseudotemporalis superficialis** on the medial surface of the mandible. The **profundus** is parallel-fibred. Many of the structural details of the **M. adductor mandibulae externus** and the **M. pseudotemporalis** complexes cannot be determined. These muscles and their component parts could not be separated from one another in certain areas because of extensive insect and human damage. Where these muscles can be separated as distinct units, they have been so indicated on the illustrations.

The origin of the large and complex **M. pterygoideus** is not preserved because of human damage. All that remains of its insertion are a few fibres on the medial surface of the mandibular ramus and a few on the internal process of the mandible as well as a tendinal attachment to the distal tip of the internal process of the mandible. The architecture of this muscle cannot be determined.

The origin of the **M. protractor pterygoidei et quadrati** is either not preserved or it could not be observed on the specimen because of its deep position behind the quadrate. We did not attempt to dissect the deep lying portions of this muscle for fear of causing unnecessary damage to the specimen without gaining

significant useful information. The **M. protractor** inserts along the dorsal and medial surface of the orbital process of the quadrate. Its architecture could not be discerned.

The **M. orbicularis palpebrarum inferioris et superioris** originate from a thin aponeurosis from the posterior rim of the orbit. They are attached to a tendon which extends posteriorly beneath the postorbital process to tendinous material between the **M. adductor mandibulae externus caudalis** and **rostralis**.

The **M. dermotemporalis** is a thin muscle, riddled with insect damage in this specimen. It originates from the lateral edge of the occipital plate just posterior to the origin of the **M. adductor mandibulae externus**; its architecture cannot be determined, but it is presumably parallel-fibred as in other birds. The origin of this muscle from the lateral edge of the occipital plate is typical for palaeognathous birds.

STAPES

This description is based on the right stapes (Fig. 5A-G) removed from the *Megalapteryx didinus* MNZ S400 mummy dissected by W. Bock and P. Vickers-Rich. Terminology follows that outlined by Feduccia (1975).

The stapes is about 6 mm long. Its most distinctive feature is the curvature of the shaft. The distal half of the shaft is convex posteriorly, while the medial half is concave posteriorly. The distal (or lateral) end is anteroposteriorly compressed, but not as much so as in the living Emu *Dromaius novaehollandiae* (Latham) – Fig. 5P-Q. No distal processes are developed, the distal end being rounded. This is quite distinct from the stapes of kiwi *Apteryx* (Fig. 5J), in which the ends of the distal moiety are extended, forming a distinct distal process and a distinct distal condyle. The distal end in *Apteryx* is also not so compressed and is concave laterally, rather than being flattened.

The distal fossa of the *Megalapteryx* stapes is a shallow and broad excavation, not deep. Located centrally in the distal fossa is a single, well-developed foramen. When viewed anteriorly, the distal (or lateral) half of the shaft is quite flattened, while the more medial part is more rounded in cross-section.

In posterior view, the shaft is flattened over much of its midsection, gradually becoming concave posteriorly towards the medial end. The more distal or lateral moiety is convex posteriorly. The shaft gradually widens from the midpoint towards the footplate; there is a deep, well developed basal fossa.

In *Apteryx* the shaft is much the same width throughout its length, lacks any curvature, and joins the footplate without any noticeable widening. The basal fossa is quite small, and two more minor fossae occur in the area where the shaft joins the footplate (these are completely lacking in *Megalapteryx*).

In *Megalapteryx*, the shaft of the stapes runs directly into the footplate, connecting with it around the edges of the footplate except where excavated by the basal fossa. No struts are present, because the shaft merges as a solid sheet of bone with the edges of the footplate.

In contrast, in *Apteryx* the shaft joins the footplate in a very restricted area near the centre of the footplate. In *Apteryx*, also,

the footplate is very thin and excavated laterally, with a marginal lip developed around its periphery, very unlike the more bulbous footplate in *Megalapteryx*. The footplate disc of *Megalapteryx* is nearly circular in outline, and is pierced internally by a prominent foramen that opens into the tympanic membrane area. The foramen is in a shallow depression. A similar depression is present in *Apteryx*.

In *Dromaius*, reminiscent of the condition in *Megalapteryx*, the stapedial shaft joins the footplate over a broad area, mainly around the periphery. The bone is, however, much thinner than in *Megalapteryx*, and it has been reduced, leaving windows in certain areas; the basal fossa is large; the distal end is not nearly as expanded as is the basal end, thus differing from *Megalapteryx* where the two ends have more nearly the same expansion. In *Dromaius*, the stapes is much more delicate, even though very similar in size, especially the basal end; the basal disc is more triangular in shape, not as rounded as in *Megalapteryx*. *Dromaius* has a straight stapedial shaft, thus differing considerably from the curved condition in *Megalapteryx*.

In the ostrich *Struthio* (Fig. 5K-M), the shaft merges with the margin on the footplate, not joining it centrally. The bone near this merger is thin and pierced by several basal fossae, whereas in *Megalapteryx* only a single, enlarged fossa is present. There is some individual variation in the number of basal fossae; for example AMNH (American Museum of Natural History) 2775 possesses several fossae, whereas AMNH 1507 possesses only a single fossa, as in *Megalapteryx*. Perhaps this is correlated with age, with the several fossae being present in the juveniles. Further study is needed here to determine what is controlling the variation within this single species. The footplate is quite robust, not delicate, and somewhat elliptical in outline. The distal end is not greatly expanded and lacks any emphasized distal process. A well developed distal fossa is present, and this continues for some distance along the shaft medially as a distinct groove.

In the rhea *Pterocnemia* (Fig. 5N-O) the shaft joins the footplate in a number of broad-based struts. Some of these struts join near the margin, but others join in the middle of the footplate. This is a very different arrangement from that in *Megalapteryx* and, for that matter, in *Struthio*. The shaft is straight, and the distal end is considerably narrower than the footplate (only one-third the width). The shaft is distinctly flattened, not as rounded in cross-section near its midpoint as in *Struthio*, and it lacks a distinct distal fossa. The footplate is quite robust and possesses a distinct depression on the medial side of the disc. The footplate is elliptical to nearly rectangular in outline.

In tinamous, such as *Rhynchotus* (Fig. 5H-I), *Eudromia*, and *Nothoprocta*, the shaft joins the footplate either as a single rod or several struts that are restricted to near the middle of the footplate. The shaft is straight and decidedly narrower distally than medially. The footplate is quite variable in shape, being circular or triangular or even elliptical in outline. Basal fossae are present, but small, the footplate is delicate, not robust, and often

possesses a distinct depression on the medial surface.

In summary the stapes of *Megalapteryx* is distinct from that of all other ratites, particularly in that the shaft is kinked, not straight. There is some similarity between *Megalapteryx*, *Dromaius* and *Struthio*, in that the shaft joins the footplate over a wide area around the periphery, quite unlike the condition in *Apteryx*, rheas and tinamous where the shaft joins at the centre of the footplate. This, and the many other noted differences between the stapes of *Megalapteryx* and *Apteryx*, support the DNA evidence for no close relationship between moas and kiwis within the ratites (Cooper *et al.*, 1992 and this paper).

BIOCHEMICAL AND GENETIC INFORMATION

Collagen, detectable in the dried skin of *Megalapteryx didinus* S400 by immunofluorescence (Fig. 6) on acetone-fixed sections, was extracted by M.J. Rowley at Monash University, Victoria, using standard methods (Chung & Miller, 1974; Miller, 1971) and further characterised by polyacrylamide gel electrophoresis (PAGE), immunoblotting and in a solid phase radioimmunoassay. Separation by SDS-PAGE using a 10% gel showed a smear of protein throughout the gel, with bands of molecular weights 39, 41 and 44, but no evidence of the high molecular weight bands characteristic of collagen (Fig. 7). These bands, and the background smear of protein were removed by pretreatment of the sample with collagenase, at 1 mg/ml overnight at 37°C in a Tris buffer, pH 7.5, containing 0.2M NaCl and 0.002M $CaCl_2$ (data not shown).

After Western blotting, the high molecular weight region of the protein smear reacted weakly with antibody, but the three protein bands did not stain specifically. Samples were tested also by Western blotting after electrophoresis on a 3% PAGE gel and electrotransfer to nitrocellulose at pH3. Under these conditions, designed to retain native collagen (Ramshaw & Werkmeister, 1988), a single spot of protein was observed, of slightly greater mobility than that of a native collagen control, of molecular weight 300 kDa. This protein spot reacted strongly with the antibody to collagen on the Western blot (Fig. 7).

Collagen was detectable also in a solid phase radioimmunoassay (Rowley *et al.*, 1986). Using a rabbit antibody which reacted strongly with native collagen, but weakly with denatured collagen, this reactivity was removed by heat treatment at 45°C, which would denature the collagen (Table 1 and Appendix 1). These studies indicated that the dried skin contained significant quantities of residual collagen. Although obviously severely degraded, as evidenced from the SDS-PAGE protein profile, the collagen appeared to retain its helical configuration, and retained much of its original immunological reactivity. This reactivity was illustrated using an assay in which the reaction of moa collagen with a sheep antiserum to mixed avian collagens was inhibited by varying concentrations of collagens from other avian and mammalian species. The reactivity was inhibited by most collagens

tested, but most strongly by moa collagen itself (Fig. 8 and Appendix 1).

The data indicate that the surviving moa collagen can be used for studies of immunological cross-reactivity, and work is in progress to analyse cross-reactivities between moa collagen and other avian collagens in order to reexamine phylogenetic relationships between moas and other birds.

DNA has been detected in known *Megalapteryx* mummies (Cooper *et al.*, 1992), and the data presented here represent results from MNZ S400.

DNA from the *M. didinus* S400 mummy was prepared by A. Cooper and G.K. Chambers at Victoria University of Wellington from ~250 mg of dry moa skin and muscle by standard treatment with proteinase K and detergent under reducing conditions and purified by phenol extraction and ethanol precipitation (Gill *et al.*, 1985; Graham, 1978). The amount of DNA extracted, as assessed by absorbance readings at 260 nm, and comparative ethidium bromide fluorescence quantitation, was about 5% of that expected from fresh avian erythrocytes.

To determine whether the DNA extracted was of moa origin, or resulted from postmortem bacterial contamination, a series of hybridization experiments was performed (Fig. 9). The extracted DNA was digested with the restriction endonuclease Hae III (BRL), separated by electrophoresis and transferred to a nylon membrane. The membrane was allowed to react with an appropriate radioactively-labelled DNA, and the amount of radioactive probe bound was determined by autoradiography. The radioactive probe was

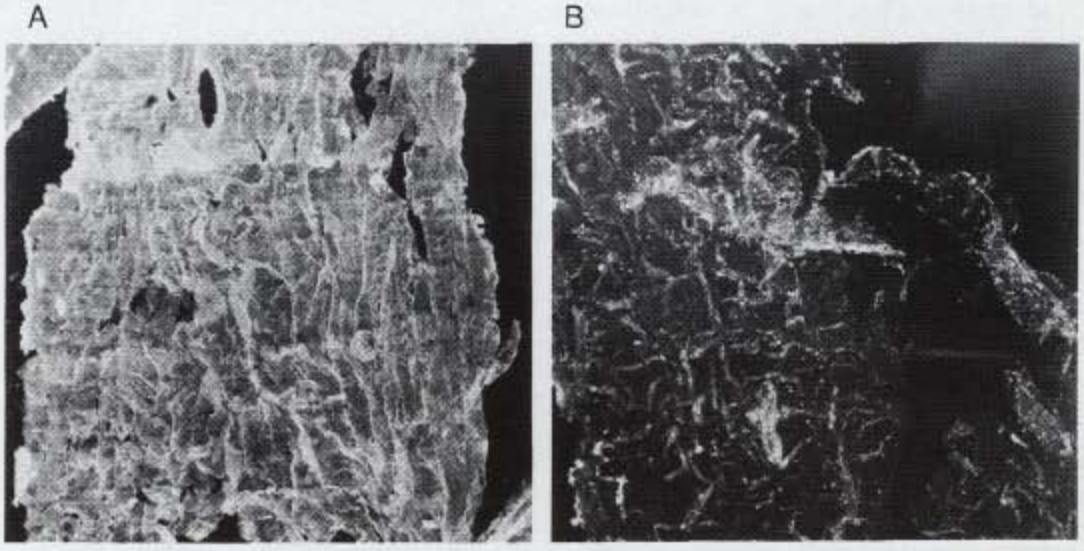


Fig. 6 – Immunofluorescence staining for collagen in frozen sections of *Megalapteryx didinus* skin (MNZ S400). A, using an antibody to avian collagen and goat anti-rabbit μgG conjugated with fluorescein isothiocyanate. B, using normal rabbit serum and fluorescein isothyocyanate-conjugated goat anti-rabbit μgG.

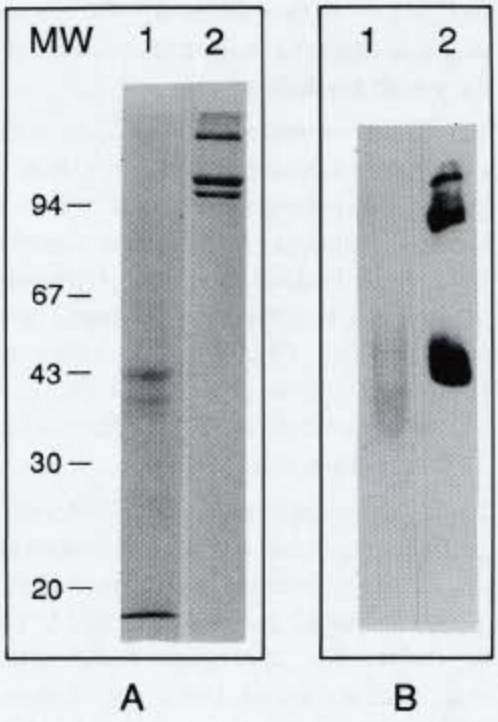


Fig. 7 – Polyacrylamide gel electrophoresis and Western blotting of *Megalapteryx didinus* (MNZ S400) collagen extract. A, electrophoresis on 10% SDS-PAGE, stained with Coomassie blue: 1, *Megalapteryx* pepsin extract. 2, Emu *(Dromaius novaehollandiae)* collagen control, 10 μg. B, Western blot of proteins separated on a 3% gel for native collagen. After autoradiography for 24 hours, there was strong reaction with both moa collagen (1) and the collagen control (2), and the background radioactivity was very low.

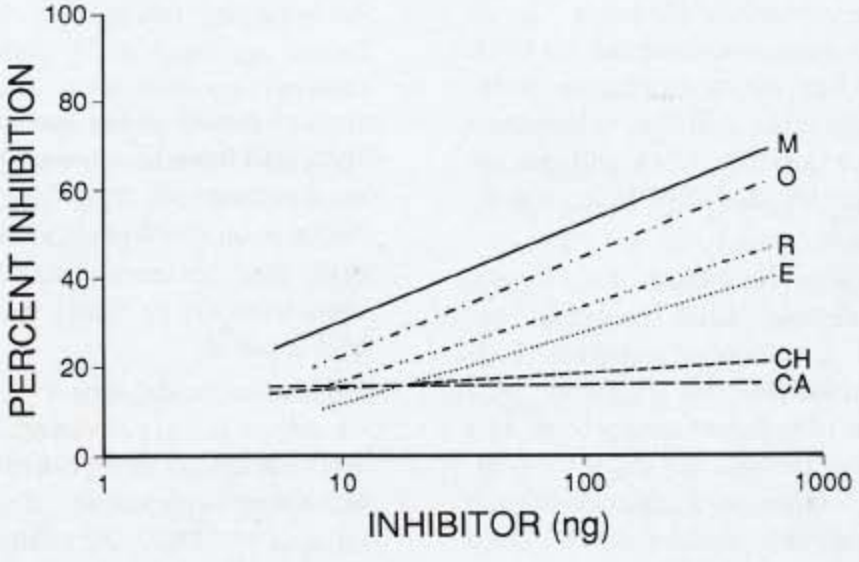


Fig. 8 – Inhibition of the reaction between *Megalapteryx didinus* (MNZ S400) collagen and sheep antiserum to avian collagen. Inhibitors shown are collagens from moa (M), ostrich (O), rhea (R), emu (E), cassowary (CA), and chicken (CH). For methods used in the preparation of this figure see Appendix 1.

stripped from the membrane using 0.1 x SSC, 0.5% SDS washes at 95°C, and a single membrane was probed sequentially with multiple probes.

The DNA extracted was probed with radioactively-labelled North Island Brown Kiwi *Apteryx australis mantelli* Bartlett genomic DNA, moa sample DNA, New Zealand Blue Duck *Hymenolaimus malacorhynchos* (Gmelin) genomic DNA, *Pseudomonas syringae* (bacterial) whole genomic DNA, and human hypervariable minisatellite probe, 33.15 (Jeffreys *et al.*, 1985).

During the experiment other DNA samples were included on the membranes to illustrate the specificity of the probe used. These included both prokaryote DNA *(Pseudomonas syringae, Escherichia coli)* and eukaryote DNA *e.g.* human, kiwi, blue duck, New Zealand Kaka *Nestor meridionalis* (Gmelin). In all cases, the results indicated that the DNA extracted from the moa tissue was closer genetically to the kiwi than to human or blue duck genomic DNA and did not contain prokaryotic DNA to any significant extent.

These experiments indicate that *Megalapteryx didinus* S400 contained extractable quantities of relatively high molecular weight DNA fragments. This DNA was of sufficient quality to act as a satisfactory template for the DNA polymerase I (Klenow fragment) enzyme used to radioactively label the DNA as a probe.

Moreover, hybridization experiments indicated that the likely origin of the DNA was the moa itself, and the DNA was unlikely to have resulted from bacterial contamination.

In subsequent experiments reported elsewhere (Cooper *et al.*, 1992), we have shown that segments of *Megalapteryx didinus* mitochondrial genes can be amplified from DNA extracted from this specimen. Sequence analysis of amplified mitochondrial 12S ribosomal RNA genes reveals that *M. didinus* is part of a monophyletic group with other ratite birds but within this group *M. didinus*, and moas as a whole are, surprisingly, only distantly related to kiwis.

DATING THE CROMWELL *Megalapteryx* SPECIMENS

The extraordinary preservation of some specimens of the Upland Moa *Megalapteryx didinus*, particularly of the type specimen in the Natural History Museum, London, and MNZ S400, has led some workers (Oliver, 1949; Falla, 1962, 1974) to consider this to have been the last surviving moa species. In fact, the known age range of *M. didinus* is the same as many other moas. The youngest known remains of this species are 300-400-year-old midden bones from Papatowai in Southland. The oldest are about 20,000 years BP (Worthy, 1988a). However, sites containing terrestrial fossil bones known to be older are very rare in New Zealand.

Mummification depends on preservation conditions and is not directly related to specimen age. Worthy (1989a) dated the Mt Owen mummified *Megalapteryx* remains at 3350 ± 70 yrBP. *Megalapteryx didinus* MNZ S400 has been dated by ^{14}C technique using bone collagen to provide conventional radiocarbon age (Libby half-life = 5568 yr) estimates of 646 ± 95 yrBP (NZA 408) and 690 ± 120 yrBP (NZA 409).

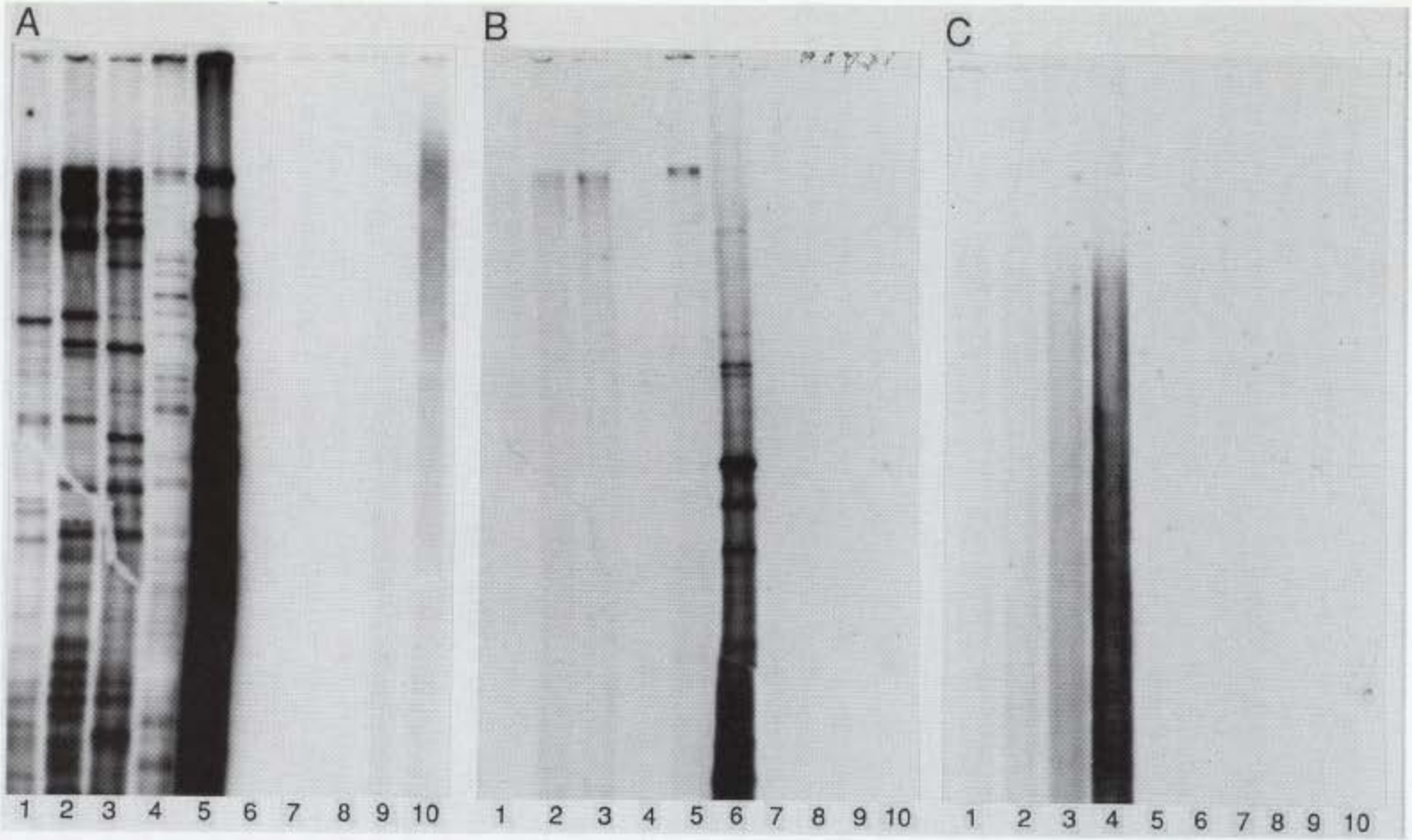


Fig. 9 – Autoradiographs of A, DNA from *Megalapteryx didinus* (MNZ S400) probed with Human minisatellite 33.15 DNA. This binds strongly to kiwi DNA (Lanes 1-3), Blue Duck DNA (Lane 4), Human DNA (Lane 5) and also *Megalapteryx* DNA (Lanes 7-10), but not to *Pseudomonas syringae* DNA (Lane 6). B, the same filter probed with *Pseudomonas syringae* DNA. This binds only to *P. syringae* (Lane 6), not to kiwi (Lanes 1-3), or *Megalapteryx* (Lanes 7-10). C, a different filter (K/M) with different DNA samples probed with *Megalapteryx* DNA. This binds to kiwi DNA (Lanes 1-4), but not to *P. syringae* (Lane 6), Human (Lane 7), ox 174 (Lane 8) and weakly to *Megalapteryx* DNA (Lanes 9-10). Longer exposure showed that the probe binds well to the *Megalapteryx* DNA. The weak signal is thought to relate to the small amount of *Megalapteryx* DNA present initially on the gel. THUS, THE DNA EXTRACTED FROM THE MOA TISSUE IS OF MOA ORIGIN, AND DOES NOT RESULT FROM BACTERIAL CONTAMINATION.

Despite S400 being among the youngest dated moa bones from any natural deposit (cf. 620 ± 60 yrBP for *Pachyornis* bone from Kawarau Valley, Central Otago, and 663 ± 51 yrBP for moa eggshell from the Redcliffe moa nesting site, near the Rakaia Gorge, Canterbury, see McCulloch and Trotter, 1979: 278) the age of these *Megalapteryx* remains demonstrate that such mummies cannot be taken as evidence of a post-European survival of this moa, nor of *M. didinus* having been the last surviving moa species.

SUMMARY AND CONCLUSION

The mummified specimen of *Megalapteryx didinus* from Cromwell, Central Otago, New Zealand (MNZ S400) was radiocarbon dated as between 600 and 700 years old (two dates) and so lived 300 to 400 years before moas became extinct throughout New Zealand. Its extraordinary preservation of soft-tissue structures enabled the studies of cranial musculature and stapedial morphology, and preliminary biochemical studies reported here. The soft tissues of this *M. didinus* cranium, although severely insect damaged, allowed the description of two major ligaments and ten muscles for the first time in the Dinornithiformes. Their morphology is similar to that of other ratites in, for example, the presence of an **external jugomandibular ligament** and absence of an **internal jugomandibular ligament**, and in the origin of the **M. dermotemporalis** on the lateral edge of the occipital plate.

The stapes of *M. didinus* is distinct from those of all other ratites. There is some similarity with that of *Dromaius* and *Struthio*, but little with *Apteryx*, *Pterocnemia* and tinamous. The dried skin of MNZ S400 was shown to contain significant quantities of collagen and DNA, sufficiently well preserved for ongoing studies of phylogenetical relationships which have been reported elsewhere.

In conclusion, the cranial musculature and stapedial morphology, and collagen and DNA studies on this specimen, show that *Megalapteryx didinus* is a member of the monophyletic group of ratites, but is very distinct from all extant members, and different especially from the kiwis with which it was sympatric.

New material of the Upland Moa has been found, and older material restudied, in the last 10 years so that we now know something more about *Megalapteryx didinus* than just the appearance of its bones and skeleton. Biochemical and morphological data gathered during this time reinforce the conclusion that moas, though clearly part of the palaeognathous radiation (Bock, 1963; Bock and Bühler, 1990), are very distinct from other birds, and only distantly related to kiwis (Cooper *et al.*, 1992).

Until further, broader-based, comparative studies are available, the full meaning of this new data, as to the position of the moas within the ratites and the palaeognathous radiation, remains unclear.

AUTHORS' RESPONSIBILITIES

The series of studies that form the basis of this report was initiated by Vickers-Rich and Yaldwyn in 1985; general work on preparation, illustration and the format of the project was done by Vickers-Rich and Trusler in Victoria, Australia; the dissection, cranial myology and stapedial morphology were done in New York by

Bock and Vickers-Rich; the collagen study was carried out by Rowley in Victoria, Australia, and the DNA study carried out by Cooper and Chambers in Wellington; work on cranial and postcranial osteology was done in Wellington by Millener, Worthy and Yaldwyn; the initial manuscript report was prepared by Vickers-Rich, and the final manuscript was put together by Millener, Worthy and Yaldwyn, though all authors have contributed to it.

Worthy and Yaldwyn take responsibility for making all necessary alterations and additions arising out of the reports of the referees for improvements in the text of the manuscript.

ACKNOWLEDGEMENTS

We thank the Trustees of the National Museum of New Zealand, now the Museum of New Zealand Te Papa Tongarewa (MNZ), for allowing us to begin the study of this important mummified specimen of *Megalapteryx didinus* in 1985; W.N. Wilson (MNZ), C. Tarka (American Museum of Natural History, New York), I. Stewart and S. Morton (Monash University, Victoria) for their generous help with photography; D. Gelt for assistance in graphics; C. Williams for measurements; J.A. Bartle and other staff at MNZ for their assistance in acquiring comparative material, historical correspondence and field notes; B.J. Gill and G.S. Park (Auckland Institute and Museum) and L. Christidis (Museum of Victoria, Melbourne) for the loan of comparative material; K.L. Bock for histological assistance; M.M. Trotter (Canterbury Museum, Christchurch) for information on *Megalapteryx* and moa dating; J.T. Darby (Otago Museum, Dunedin) for providing additional *Megalapteryx* comparative material; P. Komarower for assistance in specimen and manuscript preparation, and Lee Ratapu for typing the manuscript.

Financial assistance allowing completion of this project was provided by the Museum of New Zealand, the Chapman Fund of the American Museum of Natural History, Andrew Isles Natural History Books, and Monash University. M.J. Rowley was supported by an Australian National Research Fellowship.

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Table 1:

Substrate	Concentration	Radioactivity bound (cpm)	
		Native	Denatured
Moa, pepsin extract	50 μg/ml	9441	2438
Control pepsin solution	50 μg/ml	169	145
Emu collagen	1 μg/ml	20,000	513

APPENDIX 1: Notes on methods used in testing collagen samples from *Megalapteryx didinus* MNZ S400 presented in Table 1 and Figure 8.

Table 1: Samples were tested in a solid phase RIA in which plates were coated with the substrate at 4°C. (native), or at 50°C. to denature the collagen. Collagen was detected using a rabbit antiserum to chicken collagen which reacted strongly with native collagen, but weakly with denatured collagen. Antibody binding was detected using ^{125}I-labelled Protein A. Heat treatment significantly reduced the binding of radioactivity with the moa extract, suggesting that the collagen fragments retain considerable helical structure.

Figure 8: A solid phase radioimmunoassay was carried out in which plates coated with *Megalapteryx* collagen, 20 μg/ml overnight at 4°C were reacted with a sheep antiserum to avian collagen at a standard dilution of 1 : 50,000, and with varying amounts of collagen as inhibitor, in doubling dilutions from 600 μg/well. The amount of antibody bound was measured using ^{125}I-labelled donkey anti-sheep IgG (Silenus, Hawthorn, Australia). Percent inhibition was obtained by comparison of the amount of radioactivity bound in the presence of inhibitor and the amount bound in the absence of any inhibitor. For each inhibitor, the linear regression line was obtained using the programme Sigmaplot (Jandel Scientific, Sausalito, CA).

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Keywords: Birds, New Zealand, upland moa, DNA analysis

ISSN 1173-4337

P.O. Box 467, Wellington, New Zealand
Published: 30 June 1995
Editor/Typesetter: M. M. Cresswell