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First Magnetic Resonance Imaging (MRI) of a complete specimen of Bruijn's brush-turkey *Aepypodius bruijnii* (Aves, Megapodiidae)

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The internal anatomy and morphology of Bruijns brush-turkey *Aepypodius bruijnii* (Oustalet, 1880) is unknown to science. When the first complete specimen-in-the-flesh of this enigmatic bird species was available to science in 2002, we took the opportunity to study the visceral anatomy with modern radiological methods (Magnetic Resonance Imaging). It was possible to perform a 'necropsy' by MR imaging with all untouched viscera in situ, before the specimen was skinned and preserved as a dry study skin. The well-developed muscles of the legs were clearly visible. So was the small amount of pectoral muscle. Based on these findings we could confirm the general belief that *Aepypodius bruijnii* goes through life as a terrestrial bird, flying only small distances. Gonads could however not be visualised by MR imaging.

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INTRODUCTION

The internal anatomy and morphology of Bruijns brush-turkey *Aepypodius bruijnii* (OUSTALET, 1880) is unknown to science. Of this enigmatic bird species, no complete specimen or internal organs were preserved till 2002, when a complete freshly dead specimen was secured for science. With this specimen in the hand we took the opportunity to study the visceral anatomy of *Aepypodius bruijnii* with modern radiological methods (Magnetic Resonance Imaging). Here we report our findings.

Magnetic resonance imaging (MRI) is an imaging technique in which the object of interest is placed in a homogeneous static magnetic field. The strength of this magnetic field varies between different machines and ranges from 0.2 (low field) till 9.0T (high field). Recently, 3.0T machines have been introduced for imaging of the human body. Field strengths above 3.0T are in use for experimental imaging. The commonly used 1.5T machine has a magnetic field strength, which is about 20.000 times stronger than the magnetic field of the earth.

With MR detailed images of the anatomy can be acquired. The image information is based on protons, which are abundantly present in any living organism, especially in tissues that contain water and lipids. The protons rotate at a high speed, called precession, and spin just like a top. This processional frequency is proportional to the strength of the applied magnetic field. Therefore at specific field strength the protons resonate at a specific frequency. Due to a different chemical environment different protons will resonate at different frequencies. This difference forms the basis in acquiring different signal intensities of different tissues. Since soft tissues in an organism contain variable amounts of fat and water these tissues produce different signal intensities. This characterization of tissues makes MRI such a potent instrument for imaging.

MR is a non-invasive technique suitable to image soft tissues like muscle, brain and other organs. With this technique being available for humans, it should therefore also be suitable to image other organisms, like *Aepypodius bruijnii*. The high spatial resolution of MR imaging in the study of morphology favours its use in the post-mortem examination of very rare and unique dead animals, allowing preservation of the viscera *in situ*.

In this study we addressed questions with regard to the sex of the specimen and its anatomy in relation to its presumed terrestrial habits. MR images were also related to the findings as found by minimal dissection of the specimen.

MATERIAL AND METHODS

The specimen

Bruijn's brush-turkey (*Aepypodius bruijni*), adult female, collection Natuurhistorisch Museum Rotterdam, catalogue number NMR 9989-01606. This complete specimen was trapped with a snare in July 2002 near Mumes on the island of Waigeo, Papua province, Indonesia. It was presented to Kris Tindige (Papua Bird Club) in Sorong, who kept the bird alive till it died of unknown causes in August 2002. The dead bird was immediately preserved in a freezer. In Sorong, on October 5th, 2002 it was handed over to C.J. Heij and C.W. Moeliker who defrosted the specimen (Fig. 1), took notes on the external morphology, preserved it in alcohol 70% and subsequently transported it to Rotterdam, The Netherlands (Moeliker *et al*. 2003).

Figure 1 The complete specimen of Bruijn's brush-turkey *Aepypodius bruijnii* was defrosted in a hotel room in Sorong, Indonesia and subsequently injected with / preserved in alcohol 70%. [photo C.W. Moeliker]

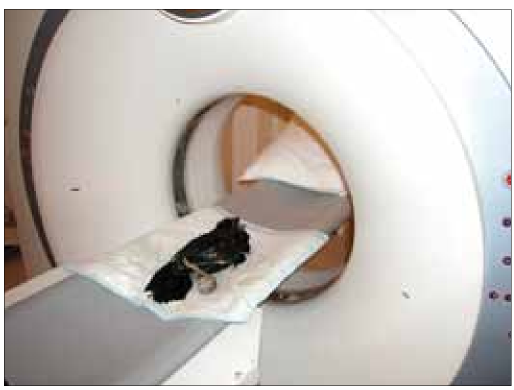

Figure 2 Bruijn's brush-turkey *Aepypodius bruijnii* NMR 9989-01606 subjected to MR imaging. [Photo C.W. Moeliker]

In November 2002 the complete specimen, taken from its alcohol container and dried only superficially, underwent total body MRI and Computer Tomography (Fig. 2). The images obtained during these radiological examinations serve as the first-ever available information of the skeletal system and internal morphology of *Aepypodius bruijnii*.

Eventually, after accurate and comprehensive examination, the bird was skinned and preserved a dry study skin with all intact internal parts kept in alcohol 70%.

The hardware

Specimen NMR 9989-01606 was scanned at the Erasmus MC University Medical Center using a 1.5T Gyroscan MRI (Philips, Best, The Netherlands) to make T2-weighted images (HASTE) in three orthogonal planes. The images of the body were acquired with a head coil, with a field of view (FOV) of 200mm, a slice thickness of 3mm, a matrix of 256 x 256 and two signal averages. This resulted in images with an in-plane resolution of 0.8 mm.

RESULTS

Cross sectional images of *Aepypodius bruijnii* NMR 9989-01606 were made in three orthogonal planes. Selections of the most representative images are shown in Figures 3-10. The images show the abdominal and thoracic organs in situ and allowed study of muscle mass. We refer to the figure captions for descriptions of anatomical details.

DISCUSSION

We show that, in very rare and unique zoological specimens, modern radiological techniques can be used for examination of internal organs and the skeletal-muscular system without dissection. We performed a radiological 'autopsy' without destroying the unique specimen, which subsequently could be preserved for future examination.

The high spatial resolution of MR imaging in the study of human morphology has shown its use in the post-mortem evaluation of unique zoological and human specimens with the possibility of preservation of the intact specimen, for example in the anatomical examination of human conjoined twins (Manzano *et al*. 2001; Sergi *et al.* 1998). In recent years, postmortem MRI is also used in addition to autopsy in humans (Ros *et al.* 1990; Patriquin *et al.* 2001; Ezxawa *et al.* 2003). Our specific aim of subjecting the complete specimen to radiologi-

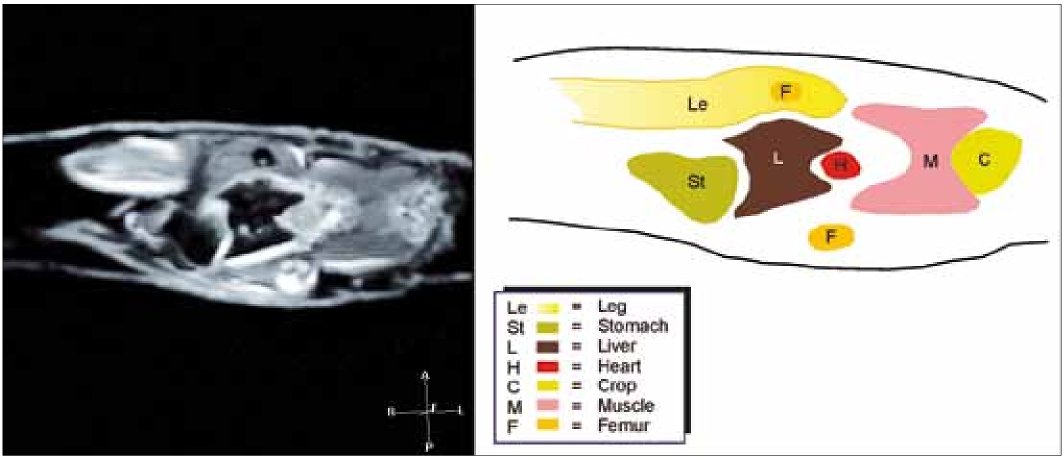

Figure 3 Slice of 8 mm in coronal direction. Right is cranially, left is caudally. In this image several internal organs are visible. Centrally in this image is the butterfly-shaped liver. On the right side of the liver the heart is visible as a circular structure in the indentation. As result of the section plane of the image, the pectoral muscles are also presented in a butterfly form, with cranially situated the crop. This has an inhomogeneous aspect due to the pieces of coconut, partially represented as black dots. On the left side of the image, caudally of the liver, the stomach is situated.

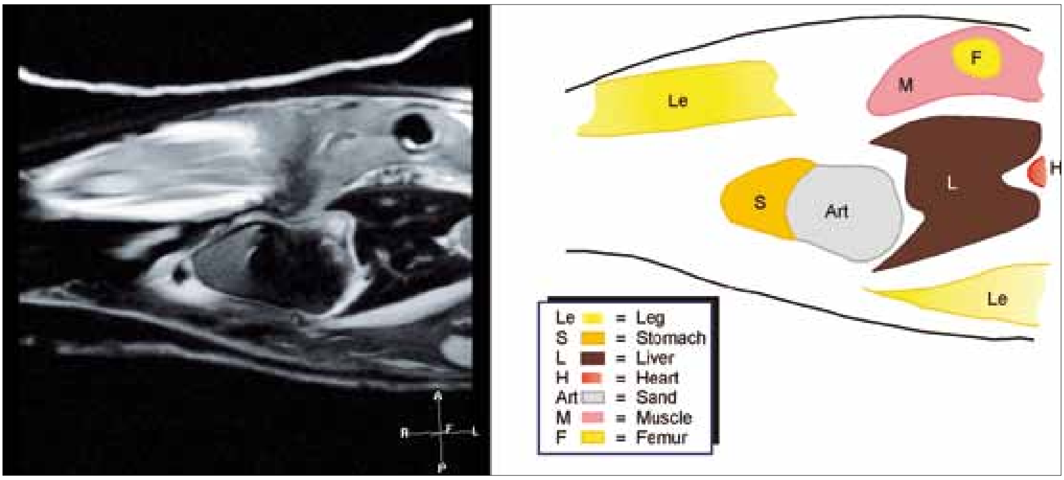

Figure 4 This figure represents a more detailed view of the liver and stomach. The stomach is visible in its entire length. The thick muscular stomach wall is shown on the most caudal part. The rest of the stomach is represented as a large black hole, as result of a MRI artefact. This artefact originates from the large amount of sand in the lumen. On the right side in this image is the butterfly-shaped liver, which partly surrounds the stomach. In the liver white streaks are visible, representing branches of the portal and liver veins.

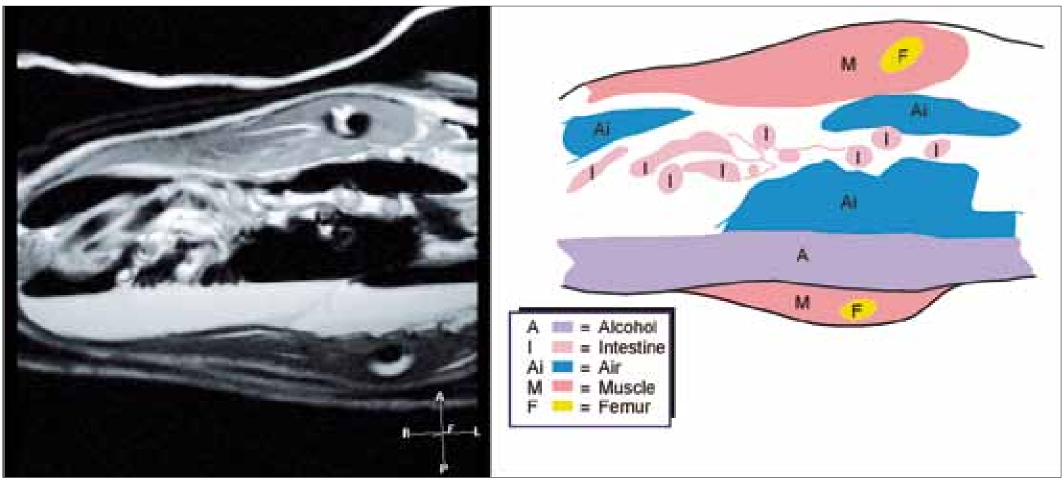

Figure 5 Coronal cross section of the abdominal cavity and lower extremities. An air-fluid level is visible with the air depicted as black and the fluid as a large white area. The fluid (alcohol) was injected for preservation purposes. Centrally in the abdominal cavity the intestine is visible, sometimes as a round cross section, and sometimes as a longitudinal cross section. The muscle mass of the right leg, which is shown in the upper part of the image is larger compared to the muscle mass of the left leg, which is shown in the lower part of the image. The reduction of muscle mass in the left leg is the result of atrophy due to amputation through the tibio-tarsus.

cal examination was to determine the sex of the bird, to visualize the muscle mass and (the position of) internal organs without dissecting the specimen. Since the age of the specimen was not known, a distinction between an immature male, an immature female or an adult female could not be made based on external features. We hoped to determine the sex (and age) of the bird with MRI findings, but were not able to visualize either the testicles or ovaries by radiological examination. However, with more optimized MRI technique smaller details, like the ovaries, might become visible. Eventually, we still had to dissect the preserved specimen for definitive determination of sex and age by direct inspection of the gonads.

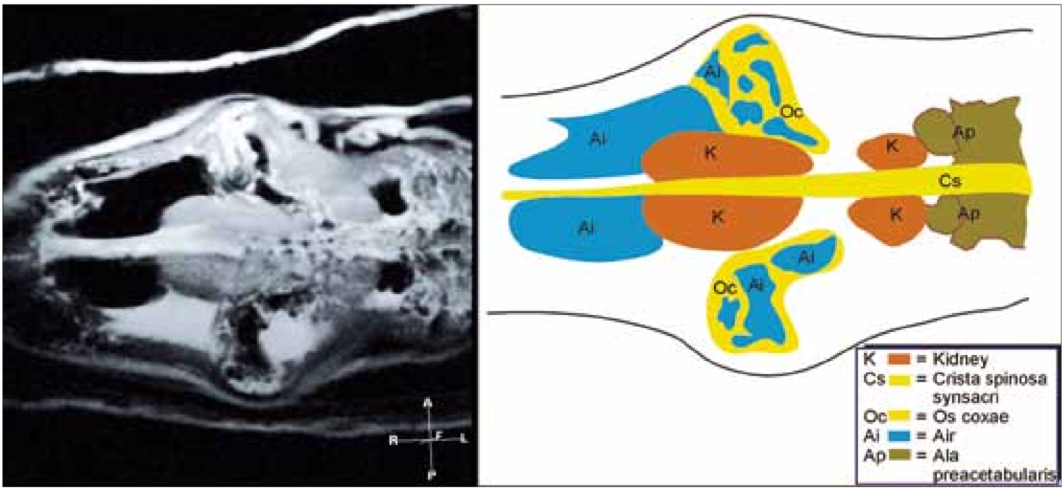

Figure 6 Coronal cross section on the level of the vertebral column and pelvis and kidneys. The *crista spinosa synsacri* is partly visible and located centrally in the image as a straight white column. The crista is continuous with the alae preacetabulares on the right (i.e. cranial) side. Parts of the -(*Alae postacetabulares illi*) are visible in this image as air filled bone structures. The kidneys are situated paravertebrally, shown as lobulated oval structures. Air and fluid in the abdominal cavity surround the kidneys.

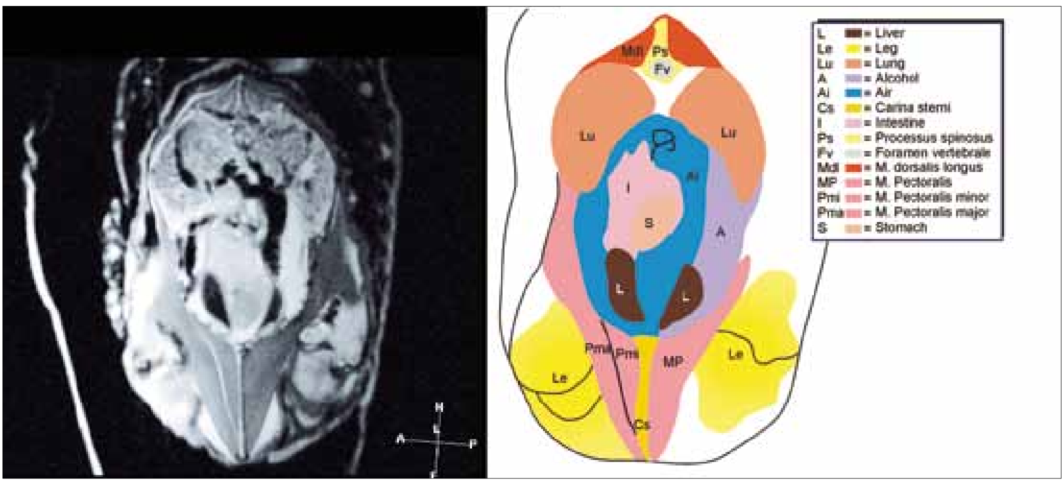

Figure 7 Transverse cross section of the thorax from the *processus spinosus* till the *carina sterni* The *musculus dorsalis longus* is attached to the *processus spinosus*. The minor and major pectoral muscles are attached to the carina sterni. On the left side of the image a line of fat separates these two muscles, which is not visible on the right side of the image. Above the sternum two parts of liver lobes are visible. The vertebral body and ribs with adjacent dorsal musculature border both lungs.

A possibility to sex a bird without dissection could be the use of endoscopic techniques.

The pectoral muscles are clearly visualized. The line between the muscles probably represents a fat plane between the minor and major pectoral muscle (Fig. 7). Compared to species that mainly move around on the wing, the pectoral muscles of *Aepypodius bruijnii* are not well developed. The relative large amount of muscle mass around its leg bones (Fig. 5) support the idea of *Aepypodius bruijnii* (being a megapode, see Jones *et al.* 1995) is a terrestrial bird that only flies occasionally over short distances. This conclusion was also supported by the findings at CT scanning of the skeleton which showed a small sternum, relatively large

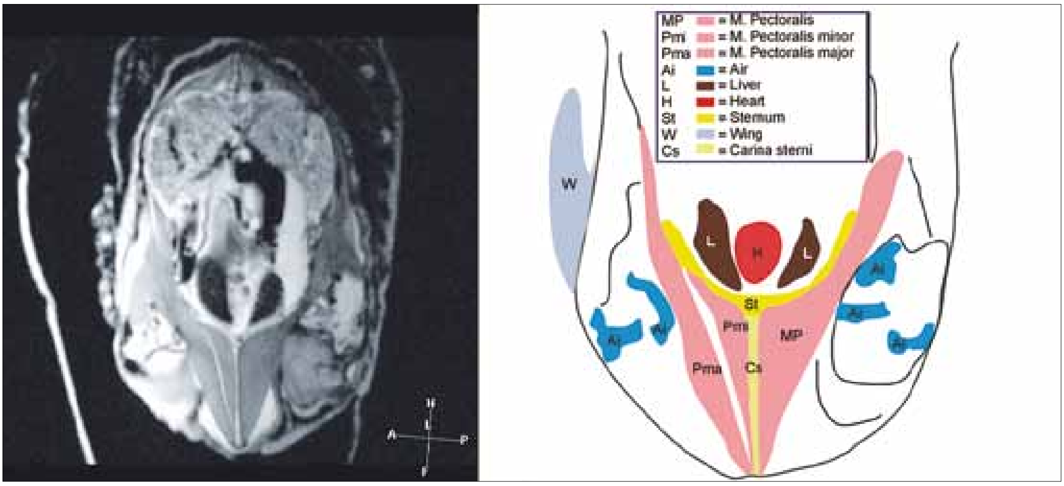

Figure 8 Transverse cross section of the thorax at a more cranially position than in figure 7. The heart is centrally visible above the sternum and in this image bordered by parts of the liver.

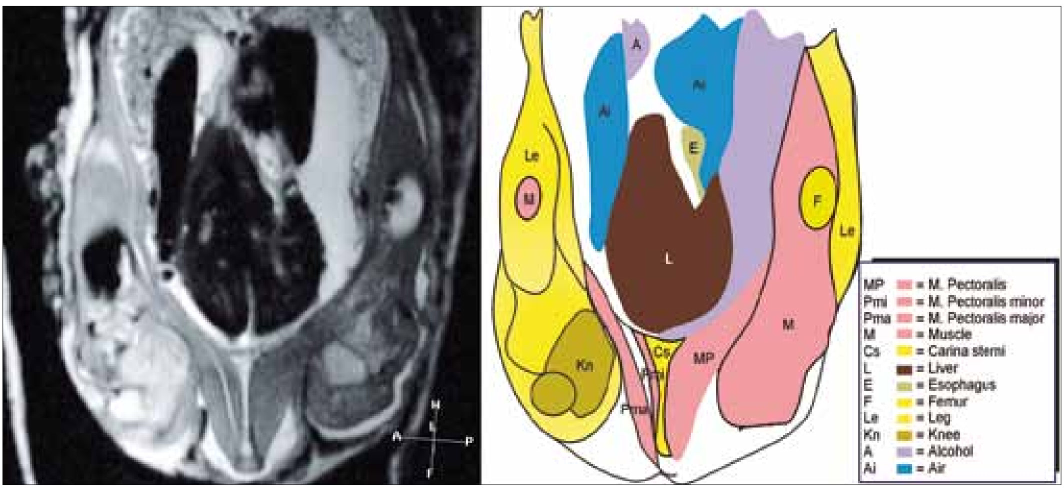

Figure 9 Transverse cross section of the lower thorax/upper abdomen. The minor and major pectoral muscles are smaller in this area than more cranially as shown in figures 7 and 8.

bones of the lower extremity and ossifications along the tibiae representing calcification of the tendon sheaths (Kompanje *et al*. in prep.)

The amount of muscle around the left leg was considerably less than its healthy right leg. The left leg of the brush-turkey was trapped in a snare, which resulted in amputation just below the tibiotarsal joint. This subsequently led to atrophy of the muscles (Fig. 5).

MRI has been used before to obtain high quality images of the entire brain of small passerine birds (Van der Linden *et al.* 1998), to estimate the mass of the pectoral muscles of chickens (Scollan *et al.* 1998), for the assessment of pectoral muscle, abdominal fat and total fat volume in chickens (Kover *et al.* 1998), for imaging intracranial tissue accumulations in domestic ducks (Bartels *et al.* 2001) and for diagnosing hydrocephalus in a grey parrot (Fleming *et al.* 2003). We were, however, not able to find a report of using MR imaging of rare vertebrate museum specimens to prevent damage by traditional dissection.

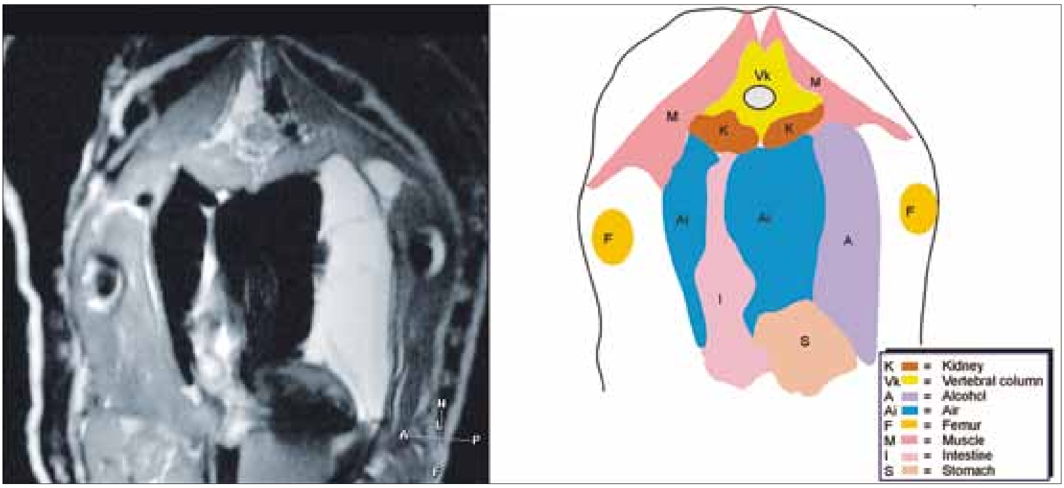

Figure 10 Transverse cross section of the abdominal cavity. Cross-section through a thoracic vertebra, with a remarkable distribution of air (black) on the right side and of bone marrow (white) on the left side in the vertebral body. The spinal cord is centrally visible in the vertebral foramen as an area with a little higher signal intensity than the surrounding cerebrospinal fluid. Right below the transverse processes cross sections of the kidneys are visible.

CONCLUSION

The high spatial resolution of MR imaging in the study of morphology favours its use in the post-mortem examination of this very rare and unique bird, allowing preservation of the viscera in situ. It was possible to perform a ‘necropsy’ by MR imaging with all untouched viscera in situ, before the specimen was skinned and preserved as a dry study skin. The well-developed muscles of the legs were clearly visible. So was the small amount of pectoral muscle. Based on these findings we could confirm the general belief (Jones *et al*. 1995) that *Aepypodius bruijnii* goes through life as a terrestrial bird, flying only small distances. Gonads could however not be visualised by MR imaging.

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