

1024 **Supplemental Information**

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1026 Supp. 1. Brief description of diceCT methods employed on the male kiwi skeleton prior to histological  
1027 processing and sampling.

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1029 Diffusible iodine-based contrast-enhanced CT (diceCT) leverages the radiopacity of Lugol's iodine  
1030 (iodine-potassium iodide, I<sub>2</sub>KI) along with the relatively low cost and high spatial resolution of micro-  
1031 computed tomography ( $\mu$ CT) imaging to visualize soft tissues. During osmosis lipids and sugars present  
1032 within soft structures hold aqueous polymers of triiodide (I<sub>3</sub><sup>-</sup>). Various soft tissues contain varying  
1033 amounts of fats and carbohydrates, causing triiodide to binds in tissue-specific ways. Although bone and  
1034 connective tissues do not stain well, muscle tissue, epithelia, glands, and neurons neurons readily take in  
1035 iodine, and, thus, become radiodense. This allows for soft-tissue anatomy to be visualized using X-ray  
1036 technologies like computed tomography imaging. DiceCT has been repeatedly used to image avian soft  
1037 tissues.

1038 Here a captive adult male (14.3 years old) *Apteryx mantelli* was imaged to capture skeletal and soft-tissue  
1039 anatomy in 3D. The specimen was frozen after necropsy, which included thoracoabdominal organ  
1040 removal. Upon thawing it was immediately fixed in 10% neutral buffered formalin for 28 days. Following  
1041 fixation, the head was removed from the body at the third cervical vertebra. Head and body imaging and  
1042 preparation were treated separately.

1043 The specimen was first  $\mu$ CT scanned to image skeletal anatomy at DENTSPLY R&D in Tulsa, OK on a  
1044 2013 Nikon 225 XT H microcomputed tomography system (Nikon Corp., Shinagawa, Tokyo, JPN). For  
1045 all scans the samples were double-wrapped in heat-sealed polyethylene bags to prevent dehydration  
1046 during scanning and physically stabilized using polyethylene foam within a plastic mounting unit. The  
1047 skull was scanned at 80.06 microns resolution (isometric voxels), using 150 kilovolts (kV), 61 micro-  
1048 amperages ( $\mu$ A), 708 millisecond (ms) exposure timing, no multi-frame averaging, without a filter, and on  
1049 a tungsten reflection target for 37 minutes. The post-cranial skeleton was scanned at 107.9 microns  
1050 resolution (isometric voxels), using 150 kilovolts (kV), 61 micro-amperages ( $\mu$ A), 708 millisecond (ms)

1051 exposure timing, no multi-frame averaging, without a filter, and on a tungsten reflection target for 74  
1052 minutes in two sections. The two resulting TIFF image stacks were fused, using the “3D Stitching”  
1053 feature of ImageJ (National Institutes of Health, Bethesda, Maryland, USA).

1054 Subsequently, both head and body were fully submerged in I<sub>2</sub>KI for staining. The head was stained in a  
1055 5% weight-by-volume (w/v) solution of I<sub>2</sub>KI for 21 days (refreshed once), followed by a 3% w/v solution  
1056 for 28 days (not refreshed). Fast scans were undertaken during the staining period to evaluate stain  
1057 uptake. Two final CT dataset (following staining completion) were captured at the MicroCT Imaging  
1058 Consortium for Research and Outreach (MICRO, Fayetteville, Arkansas, USA) on a 2018 Nikon 225 XT  
1059 H microcomputed tomography system. The first included the entire head at 59.99 microns resolution  
1060 (isometric voxels), using 201 kV, 310  $\mu$ A, 267 ms exposure timing, four-times multi-frame averaging,  
1061 with a 0.125 mm copper filter, and on a tungsten rotating target for 113 minutes in two sections. The pair  
1062 of TIFF stacks were fused using ImageJ. The second diceCT cranial dataset focused on the brain and  
1063 roots of the cranial nerves. It was captured at 32.32 microns resolution (isometric voxels), using 200 kV,  
1064 400  $\mu$ A, 354 ms exposure timing, eight-times multi-frame averaging, with a 0.125 mm copper filter, and  
1065 on a tungsten rotating target for 150 minutes.

1066 The body was stained in a 5% (w/v) solution of I<sub>2</sub>KI for 42 days (one refresh). Fast scans were undertaken  
1067 during the staining period to evaluate stain uptake. The final dataset (following staining completion) was  
1068 captured at MICRO at 121.14 microns resolution (isometric voxels), using 165 kV, 300  $\mu$ A, 354 ms  
1069 exposure timing, four-times multi-frame averaging, with a 0.125 mm copper filter, and on tungsten  
1070 reflection target for 221 minutes in two sections. The pair of TIFF stacks were fused using ImageJ.

1071 Following successful diceCT imaging, the head and body were destained using alternating baths of 1%  
1072 w/v solutions of sodium thiosulfate and de-ionized water until tissues returned to normal coloration  
1073 (approximately 12 weeks).

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