

# REPORT FROM A BOU-FUNDED PROJECT

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## **Pneumatisation and internal architecture of the Southern Cassowary *Casuarius casuarius* casque: a microCT study**

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### **SUMMARY**

Cranial ornaments such as keratinous horns and bony casques are commonplace amongst birds and take a variety of diverse forms. Possible functions include display, thermoregulation, vocalisation and intraspecific combat, yet few hypotheses have been directly tested. Here we investigate the anatomy and mechanics of the casque of the Southern Cassowary (*Casuarius casuarius*), and test functional hypotheses using CT-based virtual dissection. In particular, we determine the nature of pneumatisation within the cassowary casque and explore the extent to which potential void spaces are connected to the airway and sinus system. We find the internal cavity of the casque to be poorly connected to the major sinuses, raising doubts over the degree to which sound could travel through the cranial structure. In light of these new insights into cassowary cranial anatomy, we consider a 'resonance-chamber' function for the casque unlikely, and suggest future research should focus on improving our understanding of cassowary behaviour and associated vocalisations.

### **INTRODUCTION**

Modern birds display a variety of bizarre cranial structures and their role is a source of frequent speculation. Hypothesised functions for the casque of the Southern cassowary (*Casuarius casuarius*) include sexual display, intraspecific fighting, vocalisation, and thermoregulation (Naish & Perron, 2014; and references therein). Yet functional hypotheses are often based on anecdotal evidence and sporadic observations, and are seldom tested in a rigorous manner. Despite a considerable body of research dedicated to interpreting these structures, there remains a paucity of data at the most fundamental level. Confusion and contradiction exists regarding the basic composition of the casques, their internal structure, vascularisation and interconnectivity with the rest of the skull. What limited anatomical data that does exist has been gathered using inherently destructive techniques that rule out the possibility of interpreting the complex 3D architecture of the casque as a whole functional unit.

During the breeding season, cassowaries perform ritualised dances with the opposite sex, during which, 'guttural' low-frequency (20-30 Hz) vocalisations are made (Jones et al., 2003). During this behaviour, individuals have been observed to lower their head in such a way as to point the casque directly towards their partner. This has led to suggestions that the casque may function both as a resonance chamber and as a means of directing sound, potentially advantageous within a dense tropical rainforest habitat (Naish & Perron, 2014). Yet the mechanics of sound production and resonance within such a structure have not been explored.

Avian sound production may be broken down into two functional components: a vibrator, and a resonator. Vibration of membranes in a bird's vocal source, the syrinx, are initially responsible for sound production. However, the resonance properties of the vocal tract, including the trachea, oval cavity and beak also influence the acoustic properties of bird song (Westneat et al., 1993). In order for a structure such as the cassowary casque to contribute to sound resonance, it follows that sound must travel through the structure,

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presumably via interconnectivity with the vocal tract. The degree to which this is case remains untested however.

Elsewhere, a microCT scanned cassowary embryo (<http://www.oucom.ohiou.edu/dbms-witmer/3D-Visualization.htm>) has been found to possess distinct thickening of the frontal bones, potentially indicating a pathway to casque formation via hypertrophy of these skull bones (Naish & Perron, 2014). In light of this embryological data, we hypothesise that pneumatisation of the casque, *if present*, will occur via the frontal sinuses of the skull.

## METHODS

An adult male specimen of the Southern Cassowary (*C. casuarius*) was sourced from the National Museum of Scotland. The individual was a captive zoo animal in the UK and had died of natural causes. As part of a separate dissection of the cadaver, the head and first five cervical vertebrae were removed from the specimen and frozen prior to the study. The specimen was subsequently fixed in neutral buffered formalin, and then stained in a solution of 10% solution of iodine potassium iodide (I<sub>2</sub>KI) for two weeks in order to improve soft tissue contrast. The sample was rinsed in a 10% solution of sodium thiosulphate in order to minimise potential overstaining of the outer dermal surface, and mounted in florist foam prior to scanning.

MicroCT scanning was conducted at the Manchester X-ray Imaging Facility, University of Manchester, utilising the 320/225kV Nikon Custom Bay. The cranium was scanned at a resolution of 119µm, using a voltage of 95kV and current of 245uA. An aluminium filter of 0.5mm thickness was used throughout. The scans were reconstructed in XTek CTPro software (Nikon, Tring U.K) and exported to Avizo (FEI Visualisation Group, Oregon U.S.A.) for further processing. Highly mineralised components of the skull were automatically segmented in Avizo using a global greyscale threshold. Less mineralised components, including aspects of casque, sinuses and braincase were manually segmented using a combination of the threshold paint tool and top-hat tool. Resulting label fields were visualised using the surface render tool of Avizo.

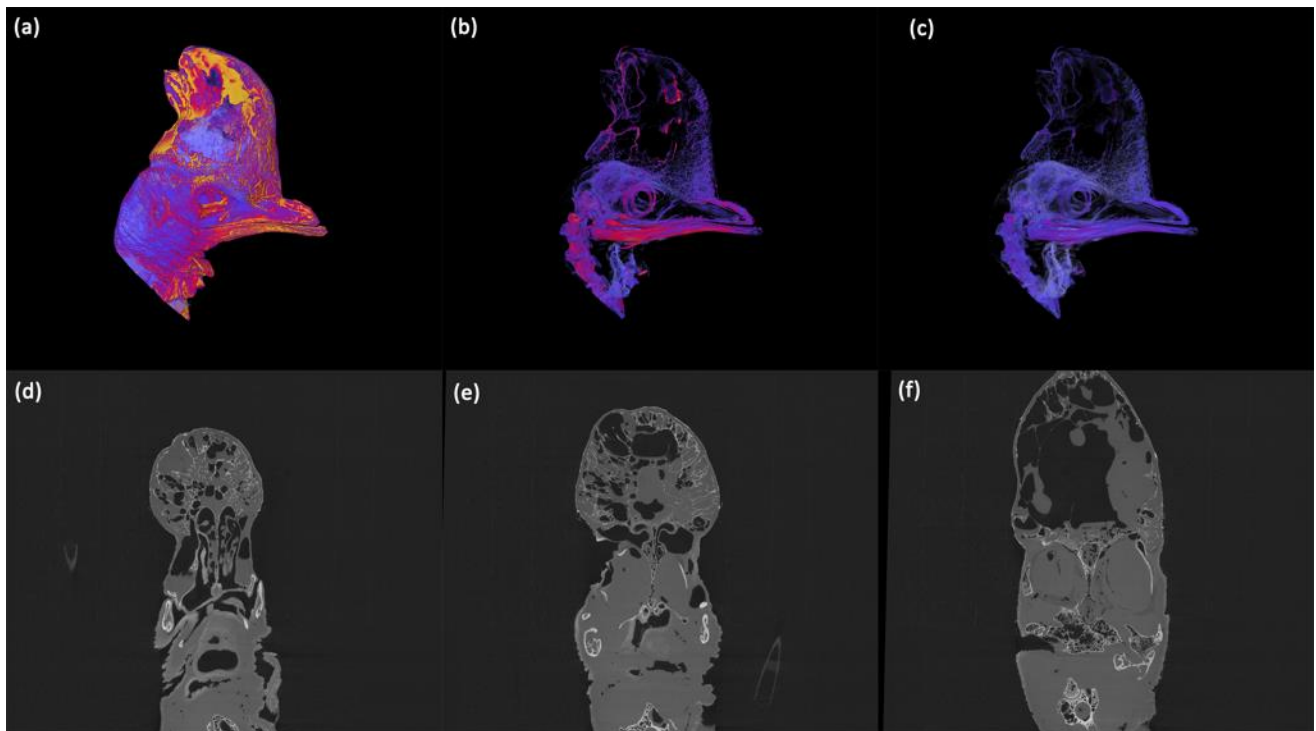
## RESULTS

The extent of the casque is illustrated in Figure 1a-c. Anteriorly, the casque is found to comprise a dense network of mineralised trabeculae (Figure 1d). Posteriorly, the casque becomes enlarged and trabeculae become less frequent and isolated to the dorsal-most interior surface (Figure 1e-f). The casque is largest in its dorsoventral extent directly above the orbit (Figure 1f), where it also contains the greatest volume of void space. The nasal passage, frontal and antorbital sinuses were successfully segmented from the microCT scans (Figure 2a-c, Figure 3b-h). The geometry of the nasal passage and sinuses are very similar to those reconstructed elsewhere in the ostrich (Witmer & Ridgley, 2008). The frontal and antorbital sinuses are located anterior and dorsal to the orbit (Figure 3b-d), but do not extend into the casque space. Void spaces present inside the casque are not directly linked to any nasal or sinus structure. Details of the cranial endocast and endosseous labyrinth were also extracted (Figure 2d-f). Future work will seek to correlate the structure of the cassowary semi-circular canals with the frequency of recorded vocalisations.

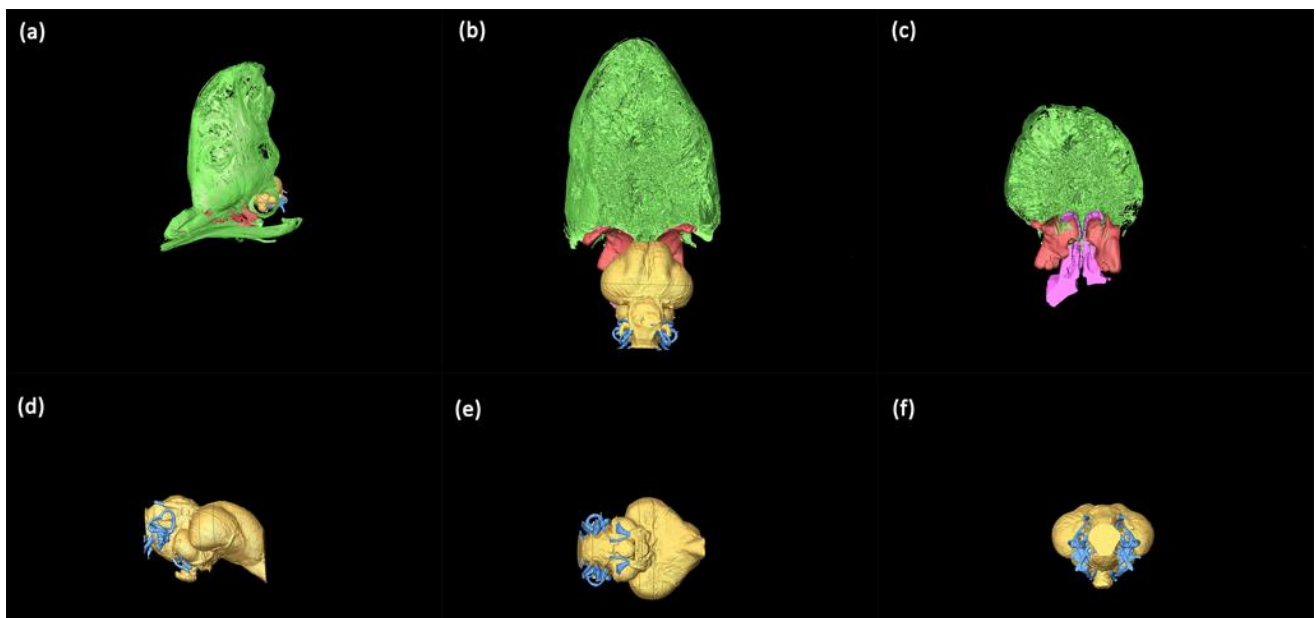
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**Figure 1.** **a-c:** surface renderings of the skull of *C. casuarius*. Colours correspond to relative density values. **d-e:** sequential orthoslices through the skull and casque region in the coronal plane, travelling anteroposteriorly

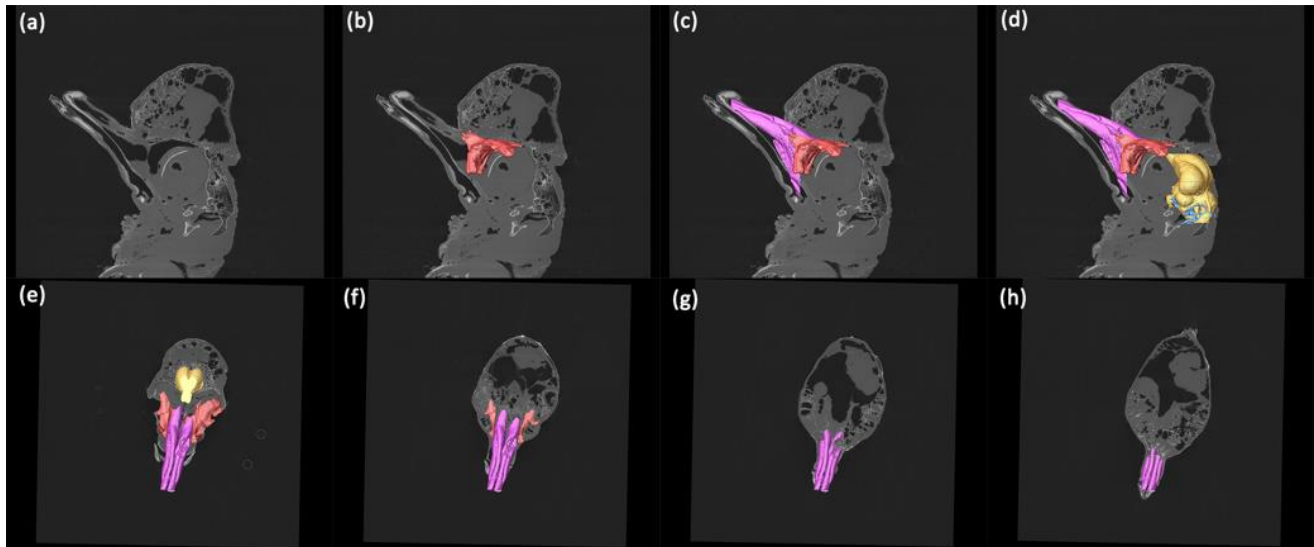


**Figure 2.** **a-f:** segmented labels fields extracted from the microCT data. Green, mineralised skeleton; gold, cranial endocast; blue, semi-circular canals and major endocranial vascular structures; pink, airway; red, frontal and antorbital sinuses. **a:** surface rendering of the segmented skull; **b-c:** coronal plane through casque and associated airways and sinuses; **e-f:** surface renderings of the skull and endosseous labyrinth in sagittal, transverse and coronal view.

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**Figure 3. a-h:** segmented label fields superimposed over original orthoslices. Colours as in Figure 2. **a-d:** sagittal orthoslice through skull and casque; **e-h:** coronal slices through the skull and casque.

## CONCLUSIONS

In summary, we find no obvious evidence for interconnectivity between void spaces within the cassowary casque and the nasal/vocal system. Elsewhere, emu have been documented to make low-frequency calls in the absence of any cranial casque, instead relying upon inflation of their cervical airsacs and tracheal pouch (Eastman, 1969). Given that the tracheal systems of cassowaries and emu share many similarities (Forbes, 1881), we posit that low-frequency calls of cassowary are likewise *generated and resonated* in the trachea rather than the casque. Given the (limited) evidence for female-bias sexual size dimorphism in the casque, future research should focus on quantifying the relationship between casque size *and* shape, and variation in vocalisations in captive individuals. Furthermore, high-speed video cameras may be deployed to visualise potential vibration of the tracheal and/or casque region, in order to further our understanding of the acoustic dynamics of sound production in cassowaries.

## ACKNOWLEDGEMENTS

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