Electronic Supplementary Material – Text S1

**Nocturnal giants: evolution of the sensory ecology in elephant birds and other palaeognaths inferred from digital brain reconstructions**

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**1. Methods**

**(a) Sampling, scanning and rendering**

We reconstructed digital endocasts for at least one representative of each major crown clade palaeognath lineage. Specimen numbers and scanning parameters are provided in electronic supplementary material, table S1. Elephant bird endocasts were reconstructed from two specimens, MNHN F 1910-12 and an uncatalogued specimen from MNHN. Each specimen is a partial skull, preserving mostly the braincase. However, the endocranial space is well-preserved in both specimens, allowing taxonomic assignment based on comparison with the elephant bird cerebrotypes characterized by Wiman and Edinger [1]. We reconstructed the endocast of the Heavy-footed Moa from MNHN 1875-602, an undescribed partial skull. We reconstructed the endocasts the tanager *Tiaris bicolor* (Passeriformes) and shorebird *Charadrius vociferous* (Charadriiformes, from Smith and Clarke [2]) as neognath outgroups for reconstructions of ancestral relative optic lobe size.

All extant palaeognaths, the uncatalogued elephant bird specimen, the tanager and the shorebird were scanned at the University of Texas High-Resolution X-ray Computed Tomography Facility (UTCT), Austin, Texas, USA. MNHN F 1910-12 (elephant bird) and MNHN 1875-602 (Heavy-footed Moa) were scanned at Viscom France, Saint-Ouen-L’Aumône, France. Raw scan data are available at doi.org/10.5061/dryad.7519042 and scanning parameters are provided in Table S1. Data segmentation and visualization were completed in Avizo 9 (FEI) following the best practices suggested by Balanoff et al. [3]. Our ostrich specimen was preserved with soft tissue and was scanned twice, the first attempt incompletely capturing the pituitary fossa and the second incompletely capturing the wulst (electronic supplementary material, table S1). All anatomical measurements were taken from the more recent scan. We reconstructed the endocast from both these datasets and combined them to visualize the full neuroanatomy (electronic supplementary material, figure S2).

**(b) Phylogenetic hypothesis**

Our analyses used the phylogenetic supertree of Burleigh et al. [4], who used a supermatrix comprising 29 nuclear and mitochondrial loci to investigate the relationships among 36 palaeognaths and 6678 neognaths. However, because that study excluded elephant birds, we grafted the elephant bird-kiwi-cassowary-emu clade of Yonezawa et al. [5] onto the Burleigh et al. [4] tree in R version 3.3.3 [6] using the bind.tree() function in the ape package [7]. Yonezawa et al. [5] investigated the relationships of 22 extant and recently extinct palaeognaths, including elephant birds, as well as 19 neognath taxa using whole mitochondrial genomic and partial nuclear genomic data. Relative branch lengths within this clade were maintained from the donor Yonezawa et al. [5] tree but the total clade height was scaled to match the total height of the same clade from the receiving Burleigh et al. [4] tree. To reconcile the taxa from our data with the taxa in the tree, we substituted the tinamou *Tinamus solitarius* for the congeneric *Tinamus guttatus*, and we substituted *Aepyornis maximus* for the composite taxon *Aepyornis*. The original, unmodified tree files from Burleigh et al. [4] and Yonezawa et al. [5] are provided in electronic supplementary material, tree files 1 and 2.

**(c) Olfactory bulb size**

The standard metric for the relative size of the olfactory bulb is the olfactory ratio (OR), the ratio of the greatest linear lengths of the olfactory bulb and cerebral hemisphere, regardless of measurement orientation [8]. To investigate the relationship of olfactory bulb size to cerebral hemisphere size among palaeognaths, we measured olfactory bulb and cerebral hemisphere length for our samples directly from the 3D data using Avizo 9. Bang and Cobb [8] provided olfactory measurements for 108 neognaths. 88 of these are also included in the tree and so were included for comparison. Lithornithidae, an extinct clade of volant stem palaeognaths from the Paleogene [9], were not included in the tree and so were excluded from our phylogenetic comparative analyses but were included in non-phylogenetic analyses. Zelenitsky et al. [10] reported the OR for the lithornithid *Lithornis plebius* but did not provide either olfactory bulb length or cerebral hemisphere length. However, we measured the length of each from their figure 1 [10], which yielded an OR of 0.36, as reported in the original study. Our final dataset comprised 13 palaeognaths and 90 neognaths (electronic supplementary material, table S2). The tree modified tree used in analyses of olfactory bulb size data is provided in electronic supplementary material, tree file 3.

Generalized least squares (GLS) regressions using log-transformed data were calculated for relative olfactory bulb size in R version 3.3.3 [6] using the gls() function in the nlme package [11], optimizing random effects maximum likelihood. To account for the statistical non-independence of species data, phylogenetic GLS regression was used to compare the dataset to the grafted Burleigh et al. [4] tree, pruned to include only those taxa represented in our dataset using the drop.tip() function in the ape package [7]. The tree file used in this analysis is provided in electronic supplementary material, dataset S1. To test alternative evolutionary models, phylogenetic GLS regressions used Brown motion (BM) and Ornstein-Uhlenbeck (OU) processes. We performed phylogenetic GLS assuming BM process using the corBrownian() function in the APE package [7]. Phylogenetic GLS assuming an OU process used a Martins Correlation Structure created using the corMartins() function in the APE package; initial alpha set to 0.5 (value = 0.5) and was allowed to vary (fixed = FALSE). Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to compare among GLS methods for palaeognaths alone, neognaths alone and palaeognaths+neognaths (electronic supplementary material, table S3). In all phylogenetic GLS analyses, elephant birds were represented by *A. maximus*. To include all palaeognath taxa, we ran an additional non-phylogenetic GLS analysis on palaeognaths alone, including both elephant bird taxa as well as *L. plebius*.

To investigate patterns of relative olfactory bulb size evolution across palaeognaths, we reconstructed olfactory ratio ancestral states in R using the contmap() function and 95% confidence intervals were estimated using the fastAnc() function, both in the phytools package v. 0.6 [12]. Because the two taxa of elephant bird studied here exhibited different olfactory ratios, we ran two alternative analyses, with elephant birds represented by either *A. maximus* or *A. hildebrandti*; otherwise, the reconstructions used the same tree and data as the phylogenetic GLS analyses.

To investigate the relationship between olfactory ratio and habitat type among palaeognaths, we used both phylogenetic and non-phylogenetic one-way analysis of variance (ANOVA) to test for significant variation in relative olfactory bulb size between open- and forest-dwelling taxa. Phylogenetic ANOVA was run in R using the aov.phylo() function in the GEIGER package [13] with the same tree as the phylogenetic GLS analyses. Non-phylogenetic ANOVA was run in R using the aov() function in the R stats package [6] both including and excluding *L. plebius* and *A. hildebrandti*. To account for the possibility that *A. hildebrandti* occupied open grasslands rather than forests, alternative ANOVAs were run with *A. hildebrandti* coded as either occupying open or forested habitat.

**(d) Optic lobe size**

To investigate the relationship of optic lobe size to brain size, we measured the surface area of a single optic lobe and of the entire endocast (excluding innervation and vasculature) directly from the 3D data using Avizo 9 for our sample. To the best of our knowledge, comparable data for neognaths are not available in the literature, and so the tanager *T. bicolor* and shorebird *C. vociferus* comprised our neognath outgroup. Our final dataset comprised 11 palaeognaths and two neognaths (electronic supplementary material, table S2). To investigate patterns of relative optic lobe size evolution across palaeognaths, we reconstructed ancestral states for the ratio of optic lobe surface area to brain surface area following the methods described for olfactory ratio. The modified tree used in analyses of optic lobe size data is provided in electronic supplementary material, tree file 4.

**2. Results**

**(a) Endocast Description and Comparison.**

**(i) Telencephalon**

The morphology and relative size of the olfactory bulb vary widely across palaeognaths. In both elephant bird endocasts reconstructed here, the bulb is large and pedunculated, projecting from the anterior pole of the cerebrum and lacking clear bifurcation into separate bulbs (figure 1), as in the Southern Cassowary, Emu and Greater Rhea (electronic supplementary material, figures S1,2). All physical elephant bird endocasts reconstructed by Wiman and Edinger [1] exhibited bifurcated olfactory bulbs. However, as the authors noted, the destructive nature of their methods likely impacted the fidelity of the olfactory bulb reconstructions. Thus, our reconstructions likely better represent the true olfactory bulb shape. The olfactory bulb in MNHN F 1910-12 is longer relative to cerebral hemisphere length than MNHN uncatalogued and projects anteriorly from the anterior pole of the telencephalon (figure 1), a condition like the cerebrotypes described by Wiman and Edinger [1] for the largest elephant bird species, *A. maximus* and *A. medius*. By contrast, the bulbs of MNHN uncatalogued are smaller and project from the anterior pole of the telencephalon at a slight ventrally deflected angle (figure 1), as in the smaller species, *A. hildebrandti* and *Mullerornis agilis*. Among other palaeognaths, the shape of the bulb in the kiwi is unique among birds, forming a broad “cap” on the anterior pole of the telencephalon (electronic supplementary material, figure S1). The bulbs are bifurcate and adjacent to each other at the midline in tinamous (electronic supplementary material, figure S3) and the Common Ostrich (electronic supplementary material, figure S2). The bulbs are also bifurcate in the Heavy-footed Moa but are set apart from each other (electronic supplementary material, figure S2).

In elephant birds and most other palaeognaths, the anteroposterior length of the cerebellum is more than half that of the cerebrum (figure 1 and electronic supplementary material, figures S1-3). Consequently, the telencephalon is proportionally small in these birds compared with most neognaths. The exceptions to this pattern among palaeognaths include the highly-derived kiwi (electronic supplementary material, figure S1) and the volant tinamous (electronic supplementary material, figure S3). The palaeognath-like condition is often observed in flightless extant taxa, including the recently extinct alcid *Pinguinus impennis* (Great Auk) and the Miocene-Pleistocene pan-alcid *Mancalla* in Charadriiformes [13], as well as the Miocene stem penguin *Paraptenodytes antarcticus* in Sphenisciformes [14]. The kiwi differs from all other palaeognaths by exhibiting a greatly developed telencephalon comprising nearly the entire long axis of the brain in lateral view (electronic supplementary material, figure S1). As with the olfactory bulbs, the two elephant bird specimens show variation in the relative anteroposterior length of the telencephalon. The telencephalon of MNHN F 1910-12 (figure 1) is approximately the same length as the cerebellum, as in Wiman and Edinger’s [1] *A. maximus*, whereas that of MNHN uncatalogued (figure 1) is longer than the cerebellum, as in *A. hildebrandti* and *M. agilis*.

The shape of the anterolateral margins of the cerebral hemispheres in dorsal view is where the most marked differences occur between MNHN F 1910-12 and MNHN (figure 1). The cerebral hemispheres of MNHN F 1910-12 are rounded, imparting a semicircular shape with a blunt anterior pole (figure 1), as in *A. maximus* and *A. medius* [1]. The Solitary, Chilean and Red-wing Tinamous, as well as the Common Ostrich, are similar (electronic supplementary material, figures S2,3). By contrast, the anterolateral margins of MNHN uncatalogued are extremely curved, exaggerating the semicircular shape into a square-like shape (figure 1), as in *A. hildebrandti* (figure 1) [1] and the Heavy-footed Moa (electronic supplementary material, figure S2). In the kiwi*,* this condition is modified into a semi-oval due to elongation of the telencephalon (electronic supplementary material, figure S1). By contrast, in the Southern Cassowary, Emu and Greater Rhea (electronic supplementary material, figures S1,2) the anterolateral margins of the cerebral hemispheres are straight and the anterior pole is more pointed in dorsal view, imparting a heart-like shape to the telencephalon. In the Brown Tinamou, the anterolateral margins are markedly notched (electronic supplementary material, figure S3), a condition unique among our sample. In all palaeognaths except the kiwi, the interhemispherical fissure widens posteriorly, forming a wide V-shape in dorsal view that opens into the cerebellum (figure 1 and electronic supplementary material, figures S1-3). In the kiwi, interhemispherical fissure is shallow and the contact between the cerebellar hemispheres and the cerebrum is rounded in dorsal view (electronic supplementary material, figure S1).

The wulst (sagittal eminence) is well developed across Palaeognathae with well-demarcated lateral valleculae (grooves), except the kiwiand Greater Rhea (figure 1 and electronic supplementary material, figures S1-3). Although the dorsal part of MNHN uncatalogued (figure 1) is missing, the posteroventral-most part is preserved and shows trivial difference from MNHN F 1910-12 (figure 1); the wulst is well-developed and anteriorly positioned in both endocasts. This agrees with Wiman and Edinger [1], who reported little variation in wulst morphology or position among elephant birds. Among other palaeognaths, the wulst is especially well developed in the Heavy-footed Moa (electronic supplementary material, figure S2), where it extends anteroposteriorly across the entire dorsal surface of the cerebral hemispheres, unlike any other palaeognath. The wulst in the Greater Rhea is less developed dorsally with shallower lateral valleculae than the other palaeognaths (electronic supplementary material, figure S2). The wulst in the kiwi is lesser developed still with no appreciable valleculae (electronic supplementary material, figure S1). The wulst of the kiwi is also positioned at the posterior pole of the telencephalon, a unique condition among palaeognaths. The interhemispherical fissure is U-shaped in MNHN F 1910-12, MNHN uncatalogued, Southern Cassowary, Emu, Heavy-footed Moa and Greater Rhea (figure 1 and electronic supplementary material, figures S1-3) [15]. By contrast, the fissure is V-shaped in tinamous and the Common Ostrich (electronic supplementary material, figures S2,3).

**(ii) Diencephalon**

The external surface of the endocranial diencephalon is limited to the foramen containing cranial nerve II (optic nerve) and the pituitary. Osteological correlates for the optic nerve in the endocranium are variable across palaeognaths, confounding comparisons. However, the optic nerve canals in both elephant birds are well developed and do not appear to contact the pituitary fossa (figure 1), a condition shared among all elephant birds described by Wiman and Edinger [1] as well as by the Emu, Heavy-footed Moa, tinamous and Common Ostrich (electronic supplementary material, figures S1-3). In the kiwi and Southern Cassowary, this canal and the pituitary fossa are closely associated (electronic supplementary material, figure S1). In the kiwi, the canal for the optic nerve is greatly reduced (electronic supplementary material, figure S1). In the Greater Rhea, skeletal correlates for the optic nerve were absent. The pituitary fossa is proportionally very large in palaeognaths except for the kiwi and Greater Rhea (figure 1 and electronic supplementary material, figures S1-3)*,* which exhibit small fossae as in most neognaths. The pituitary fossa of MNHN F 1910-12 is relatively larger and more bulbous than that of MNHN uncatalogued (figure 1), a condition most like Wiman and Edinger’s [1] *A. maximus*. The pituitary fossa of MNHN uncatalogued is most like Wiman and Edinger’s [1] *A. hildebrandti*.

**(iii) Mesencephalon**

The optic lobes in both elephant bird specimens are highly reduced (Fig. 1). This reduction is exaggerated in MNHN F 1910-12 to a degree otherwise known only in kiwis(electronic supplementary material, figure S1) among extant birds [16]. By contrast, the optic lobes of MNHN uncatalogued are present as faint convexities on the ventral surface of the mesencephalon, smaller relative to the telencephalon than any other known bird save for MNHN F 1910-12 and kiwis. The proportionally larger optic lobes in the smaller elephant bird specimen are consistent with the relationship observed by Wiman and Edinger [1] between optic lobe size and body size among elephant bird species. Optic lobe size is variable across the other palaeognaths; in the Heavy-footed Moa, they are also greatly reduced and are smaller than the pituitary fossa. The lobes are proportionally larger in the Southern Cassowary, Emu and Common Ostrich (electronic supplementary material, figures S2,3). In the Greater Rhea (electronic supplementary material, figure S3) and especially the tinamous(electronic supplementary material, figure S3), the optic lobes are larger still, approaching the condition observed in most neognaths.

**(iv) Metencephalon**

As mentioned above, the cerebellum is proportionally large in palaeognaths except tinamous and especially the kiwi (figure 1 and electronic supplementary material, figures S1-3). The cerebellum is proportionally longer in MNHN F 1910-12 than in MNHN uncatalogued (figure 1) and its size and shape are comparable to Wiman and Edinger’s [1] *A. medius* and especially *A. maximus*. By contrast, the cerebellum of MNHN uncatalogued (figure 1) is more like Wiman and Edinger’s [1] *A. hildebrandti*. The dorsal projection of the cerebellum is high in all palaeognaths, projecting to the dorsoventral midpoint of the cerebral hemispheres in the kiwi and above the midpoint in the other palaeognaths (figure 1 and electronic supplementary material, figures S1-3). Although folds are present in the cerebella of all extant palaeognaths, impressions for these folds are absent in both elephant birds reported here (figure 1), as well as the Southern Cassowary, Emu, Heavy-footed Moa,Greater Rhea and Common Ostrich (electronic supplementary material, figures S1-3). Folds are not present in our specimen of kiwi (electronic supplementary material, figure S1) but have been reported by others [15], indicating they are variable within kiwis. Cerebellar folds are present in tinamous(electronic supplementary material, figure S3). The occipital sinus not demarcated on the endocast of any palaeognath except the Greater Rhea (figure 1 and electronic supplementary material, figures S1-3).

The size and shape of the floccular fossa varies across palaeognaths. MNHN F 1910-12, MNHN uncatalogued, Southern Cassowary, Emu and Common Ostrich all exhibit large, elongate fossae with blunt distal margins (figure 1 and electronic supplementary material, figures S1-3). Among these, elephant birds stand apart with straight, rectangular fossae, though they are proportionally slenderer in the smaller MNHN uncatalogued (figure 1). In the other palaeognaths, by contrast, the fossae exhibit a hooked shape with more rounded ends (electronic supplementary material, figures S1-3). The condition in Greater Rhea is also elongate and hooked but is markedly pointed (electronic supplementary material, figure S2). The floccular fossae are reduced in the kiwi and tinamous; they are elongate in the former and short in the latter (electronic supplementary material, figures S1,3). The Heavy-footed Moa lacks appreciable floccular fossae (electronic supplementary material, figure S2) [15]. The cerebella of the elephant birds exhibit shallow, circular depressions surrounding the floccular fossae, corresponding to the anterior semicircular canal of the inner ear. Among the elephant bird cerebrotypes described by Wiman and Edinger [1], MNHN F 1910-12 (figure 1) exhibits a depression more like *A. maximus* than *A. medius*; the depression in *A. medius* is deeper than the condition observed here.

**(b) Olfactory bulb size and habitat**

For all GLS regressions of olfactory bulb length vs. cerebral hemisphere length of palaeognaths alone, neognaths alone and all birds, phylogenetic GLS assuming an OU process was preferred based on BIC and AIC over both phylogenetic GLS assuming a BM process and non-phylogenetic GLS (electronic supplementary material, table S7). However, for these analyses, phylogenetic GLS regressions assuming an OU process and non-phylogenetic GLS regressions had identical slopes and intercepts (electronic supplementary material, table S5). Thus, we used non-phylogenetic GLS regressions for all of our main analyses, allowing us to include taxa not in our tree (i.e. multiple elephant birds and *Lithornis plebius*).

**3. Supplementary figures**

**Figure S1.** Comparison of endocasts of elephant bird *Aepyornis* *maximus* (MNHN F 1910-12), elephant bird *Aepyornis hildebrandti* (MNHN, uncatalogued specimen), kiwi (*Apteryx* sp.) and Southern Cassowary (*Casuarius casuarius*) in (*a*) left lateral, (*b*) anterior, (*c*) ventral and (*d*) dorsal views. Colors: blue, brain; green, inner ear; red, vasculature; yellow, cranial nerves. Scale bars = 1 cm.

**Figure S2.** Comparison of endocasts of Emu (*Dromaius novaehollandiae*), Heavy-footed Moa (*Pachyornis elephantopus*), Greater Rhea (*Rhea americana*) and Common Ostrich (*Struthio camelus*) in (*a*) left lateral, (*b*) anterior, (*c*) ventral and (*d*) dorsal views. Dashed line in Common Ostrich differentiates between two reconstructions of the same specimen. Colors: blue, brain; green, inner ear; red, vasculature; yellow, cranial nerves. Scale bars = 1 cm.

**Figure S3.** Comparison of endocasts of Brown Tinamou (*Crypturellus obsoletus*), Chilean Tinamou (*Nothoprocta perdicaria*), Red-winged Tinamou (*Rhynchotus rufescens*) and Solitary Tinamou (*Tinamus solitarius*) in (*a*) left lateral, (*b*) anterior, (*c*) ventral and (*d*) dorsal views. Colors: blue, brain; green, inner ear; red, vasculature; yellow, cranial nerves. Scale bars = 1 cm.

**Figure S4.** Plots of olfactory ratio (olfactory bulb size vs. cerebrum size) for (*a*) palaeognaths alone, analyzed with *Aepyornis hildebrandti* and *Lithornis plebius*; (*b*) palaeognaths alone, analyzed without *A. hildebrandti* and *L. plebius*; (*c*) neognaths alone and (*d*) palaeognaths and neognaths together, analyzed without *A. hildebrandti* and *L. plebius*. Regression lines: black, non-phylogenetically corrected GLS; red, phylogenetic GLS with Brownian motion; green, phylogenetic GLS with Ornsein-Uhlenbeck process (overlapped by black in *b*-*d*). Colors: brown, ostrich; dark blue, lithornithid; green, rhea; light blue, tinamous; orange, elephant birds; pink, emu; purple, moa; red, kiwi; yellow, cassowary. Shapes: circles, included in regression; diamonds, excluded from regression.

**Figure S5.** Ancestral state reconstruction of olfactory ratio (olfactory bulb size vs. cerebrum size) for 11 palaeognaths and 88 neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [5] grafted onto the tree of Burleigh et al. [4]. Elephant birds are represented by *A. maximus*.

**Figure S6.** Ancestral state reconstruction of olfactory ratio (olfactory bulb size vs. cerebrum size) for 11 palaeognaths and 88 neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [4] grafted onto the tree of Burleigh et al. [3]. Elephant birds are represented by *A. hildebrandti*.

**Figure S7.** Ancestral state reconstruction of optic lobe surface area vs. total brain surface area for 11 palaeognaths and two neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [5] grafted onto the tree of Burleigh et al. [4].

**4. Supplementary tables**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| species | common | specimen number | scanner make/model | scan date | scan location | X-ray power (kv/mA) | pixel dimensions | # slices | voxel size (um) |
| Aepyornis maximus | elephant bird | NMNH F 1910-12 | X8060 NDT (Viscom) | 6/25/2010 | VF | 100/0.17 | 841x904 | 630 | 138 |
| Aepyornis hildebrandti | elephant bird | uncatalogued | NSI | 10/30/2015 | UTCT | 200/0.13 | 1700x154 | 1525 | 48.1 |
| Apteryx sp. | kiwi sp. | AMNH 18456 | BIR | 12/21/2001 | UTCT | 150/0.19 | 512x512 | 983 | 164.4 |
| Casuarius casuarius | Southern Cassowary | TMM M-12033 | BIR | 5/23/2013 | UTCT | 250/2.8 | 1024x1024 | 379 | 230 |
| Dromaius novaehollandiae | Emu | TMM M-12678 | BIR | 10/28/2013 | UTCT | 150/0.115 | 994x1131 | 1936 | 91.7 |
| Pachyornis elephantopus | Heavy-footed Moa | MNHN 1875-602 | X8060 NDT (Viscom) | 6/25/2010 | VF | 70/0.8 | 645x815 | 968 | 125 |
| Nothoprocta perdicaria | Chilean Tinamou | UMNH 23838 | Xradia (Zeiss) | 5/12/2014 | UTCT | 120/10.0 | 1012x1012 | 1518 | 42.39 |
| Tinamus solitarius | Solitary Tinamou | TMM M-10445 | NSI | 3/1/2017 | UTCT | 150/0.17 | 778x1345 | 1896 | 42.1 |
| Crypturellus obsoletus | Brown Tinamou | TMM M-10444 | NSI | 3/1/2017 | UTCT | 150/0.17 | 877x861 | 1968 | 28.5 |
| Rhynchotus rufescens | Red-wing Tinamou | TMM M-10443 | NSI | 3/1/2017 | UTCT | 150/0.17 | 778x1345 | 1896 | 42.1 |
| Rhea americana | Greater Rhea | TMM M-6721 | BIR | 2/1/2006 | UTCT | 120/0.2 | 512x512 | 456 | 181 |
| Struthio camelus | Common Ostrich | TMM M-14237 | NSI | 8/8/2016 | UTCT | 150/0.17 | 1083x1012 | 1947 | 94.7 |
| Struthio camelus | Common Ostrich | TMM M-14237 | BIR | 4/24/2012 | UTCT | 250/3.5 | 1024x1024 | 486 | 250 |
| Tiaris bicolor | Black-faced Grassquit | USNM 33768 | NSI | 5/2/2016 | UTCT | 150/0.1 | 1118x1224 | 1833 | 12.3 |
| Charadrius vociferus1 | Killdeer | NCSM 18305 | BIR | 6/4/2010 | UTCT | 200/0.16 | 1024x1024 | 1183 | 52.93 |

**Table S1.** Specimen information and CT scanning parameters for scanned samples used in this study. 1From Smith and Clarke Abbreviations: AMNH, American Museum of Natural History, New York City, New York; BIR, Bio-Imaging Research, Inc, Lincolnshire, Illinois; NCSM, North Carolina State Museum of Natural Sciences, Raleigh, North Carolina; NMNH, National Museum of Natural History, Paris, France; NSI, North Star Imaging, Irvine, California; TMM, Texas Memorial Museum, Austin, Texas; UMNH, Utah Museum of Natural History, Salt Lake City, Utah; UTCT, University of Texas High-Resolution X-ray Computed Tomography Facility, University of Texas at Austin, Austin, Texas; VF, Viscom France, Saint-Ouen-L’Aumône, France.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| taxon | OLA | TBA | OptR | OBL | CHL | OR |
| Casuarius\_casuarius | 2.99 | 81.12 | 0.04 | 11.27 | 42.12 | 0.27 |
| Dromaius\_novaehollandiae | 2.34 | 52.52 | 0.04 | 8.37 | 31.51 | 0.27 |
| Apteryx\_sp | 0.24 | 27.01 | 0.01 | 12.00 | 35.00 | 0.34 |
| Aepyornis\_maximus | 1.42 | 119.38 | 0.01 | 11.74 | 44.40 | 0.26 |
| Aepyornis\_hildebrandti |  |  |  | 9.97 | 41.07 | 0.24 |
| Pachyornis\_elephantopus | 1.29 | 65.03 | 0.02 | 7.80 | 36.55 | 0.21 |
| Struthio\_camelus | 2.58 | 77.09 | 0.03 | 6.88 | 39.10 | 0.18 |
| Rhea\_americana | 2.08 | 45.06 | 0.05 | 7.37 | 33.40 | 0.22 |
| Rhynchotus\_rufescens | 1.01 | 12.91 | 0.08 | 2.36 | 17.32 | 0.14 |
| Nothoprocta\_perdicaria | 0.75 | 8.82 | 0.09 | 2.51 | 13.82 | 0.18 |
| Tinamus\_solitarius | 1.06 | 13.56 | 0.08 | 5.10 | 16.24 | 0.31 |
| Crypturellus\_obsoletus | 0.85 | 9.97 | 0.08 | 4.66 | 14.59 | 0.32 |
| Lithornis\_plebius |  |  |  | 5.61 | 15.77 | 0.36 |
| Tiaris\_bicolor | 0.26 | 4.26 | 0.06 |  |  |  |
| Charadrius\_vociferus | 0.49 | 7.11 | 0.07 |  |  |  |
| Corvus\_brachyrhynchos |  |  |  | 1.30 | 26.00 | 0.05 |
| Colaptes\_auratus |  |  |  | 1.50 | 18.00 | 0.08 |
| Phalacrocorax\_urile |  |  |  | 2.00 | 21.00 | 0.10 |
| Morus\_bassanus |  |  |  | 2.50 | 26.00 | 0.10 |
| Pelecanus\_occidentalis |  |  |  | 3.00 | 31.00 | 0.10 |
| Urocolius\_macrourus |  |  |  | 1.00 | 10.30 | 0.10 |
| Picoides\_pubescens |  |  |  | 1.50 | 15.00 | 0.10 |
| Amandava\_amandava |  |  |  | 1.00 | 10.00 | 0.10 |
| Phalacrocorax\_auritus |  |  |  | 3.00 | 29.00 | 0.10 |
| Cinclus\_cinclus |  |  |  | 1.50 | 14.00 | 0.11 |
| Coracina\_melanoptera |  |  |  | 1.50 | 12.50 | 0.12 |
| Haliastur\_indus |  |  |  | 2.50 | 20.00 | 0.13 |
| Turnix\_suscitator |  |  |  | 1.00 | 8.00 | 0.13 |
| Oriolus\_xanthornus |  |  |  | 2.00 | 15.10 | 0.13 |
| Meleagris\_gallopavo |  |  |  | 2.50 | 18.50 | 0.14 |
| Motacilla\_flava |  |  |  | 1.50 | 11.00 | 0.14 |
| Fratercula\_arctica |  |  |  | 2.50 | 18.00 | 0.14 |
| Bonasa\_umbellus |  |  |  | 2.00 | 14.00 | 0.14 |
| Celeus\_brachyurus |  |  |  | 2.00 | 14.00 | 0.14 |
| Pandion\_haliaetus |  |  |  | 3.00 | 21.00 | 0.14 |
| Phalacrocorax\_carbo |  |  |  | 2.90 | 20.00 | 0.15 |
| Upupa\_epops |  |  |  | 2.00 | 13.60 | 0.15 |
| Charadrius\_semipalmatus |  |  |  | 1.50 | 10.00 | 0.15 |
| Fregata\_magnificens |  |  |  | 3.00 | 20.00 | 0.15 |
| Hirundo\_rustica |  |  |  | 1.50 | 10.00 | 0.15 |
| Mergus\_serrator |  |  |  | 3.00 | 20.00 | 0.15 |
| Milvus\_migrans |  |  |  | 3.00 | 20.00 | 0.15 |
| Megascops\_asio |  |  |  | 2.70 | 18.00 | 0.15 |
| Uria\_lomvia |  |  |  | 2.70 | 18.00 | 0.15 |
| Gallus\_gallus |  |  |  | 2.00 | 13.00 | 0.15 |
| Limnodromus\_griseus |  |  |  | 2.00 | 13.00 | 0.15 |
| Megalaima\_asiatica |  |  |  | 2.00 | 13.00 | 0.15 |
| Larus\_argentatus |  |  |  | 3.00 | 19.00 | 0.16 |
| Phalacrocorax\_sulcirostris |  |  |  | 3.00 | 19.00 | 0.16 |
| Dicrurus\_adsimilis |  |  |  | 2.13 | 13.20 | 0.16 |
| Coragyps\_atratus |  |  |  | 4.00 | 24.00 | 0.17 |
| Lanius\_schach |  |  |  | 2.50 | 15.00 | 0.17 |
| Motacilla\_madaraspatensis |  |  |  | 2.10 | 12.60 | 0.17 |
| Scolopax\_minor |  |  |  | 2.50 | 15.00 | 0.17 |
| Pygoscelis\_adeliae |  |  |  | 5.00 | 30.00 | 0.17 |
| Pelecanoides\_georgicus |  |  |  | 2.00 | 11.30 | 0.18 |
| Tephrodornis\_pondicerianus |  |  |  | 2.20 | 12.30 | 0.18 |
| Bubo\_virginianus |  |  |  | 4.50 | 25.00 | 0.18 |
| Pitta\_brachyura |  |  |  | 2.20 | 12.10 | 0.18 |
| Centropus\_sinensis |  |  |  | 3.00 | 16.40 | 0.18 |
| Apus\_affinis |  |  |  | 1.40 | 7.50 | 0.19 |
| Cypsiurus\_parvus |  |  |  | 1.40 | 7.50 | 0.19 |
| Chaetura\_pelagica |  |  |  | 1.50 | 8.00 | 0.19 |
| Merops\_orientalis |  |  |  | 1.50 | 8.00 | 0.19 |
| Rallus\_longirostris |  |  |  | 3.00 | 16.00 | 0.19 |
| Anas\_platyrhynchos |  |  |  | 4.00 | 21.00 | 0.19 |
| Asio\_flammeus |  |  |  | 3.50 | 18.00 | 0.19 |
| Anas\_carolinensis |  |  |  | 3.00 | 15.00 | 0.20 |
| Falco\_peregrinus |  |  |  | 3.40 | 17.00 | 0.20 |
| Gallinula\_chloropus |  |  |  | 3.00 | 15.00 | 0.20 |
| Gavia\_immer |  |  |  | 5.00 | 25.00 | 0.20 |
| Hydrophasianus\_chirurgus |  |  |  | 2.60 | 13.00 | 0.20 |
| Phaethon\_aethereus |  |  |  | 3.00 | 15.00 | 0.20 |
| Puffinus\_gravis |  |  |  | 6.00 | 30.00 | 0.20 |
| Rallus\_elegans |  |  |  | 3.20 | 16.00 | 0.20 |
| Nycticorax\_nycticorax |  |  |  | 4.30 | 21.30 | 0.20 |
| Porphyrio\_porphyrio |  |  |  | 4.00 | 19.00 | 0.21 |
| Coccyzus\_americanus |  |  |  | 1.90 | 9.00 | 0.21 |
| Phoenicopterus\_minor |  |  |  | 5.00 | 23.00 | 0.22 |
| Vanellus\_indicus |  |  |  | 3.10 | 14.10 | 0.22 |
| Tachybaptus\_ruficollis |  |  |  | 3.00 | 13.50 | 0.22 |
| Amaurornis\_phoenicurus |  |  |  | 3.50 | 15.50 | 0.23 |
| Columba\_livia |  |  |  | 2.90 | 12.70 | 0.23 |
| Fulica\_americana |  |  |  | 4.00 | 17.00 | 0.24 |
| Polysticta\_stelleri |  |  |  | 4.50 | 19.00 | 0.24 |
| Gallirallus\_australis |  |  |  | 4.60 | 19.00 | 0.24 |
| Opisthocomus\_hoazin |  |  |  | 4.00 | 16.50 | 0.24 |
| Steatornis\_caripensis |  |  |  | 3.70 | 15.00 | 0.25 |
| Fulica\_atra |  |  |  | 4.30 | 17.30 | 0.25 |
| Caprimulgus\_vociferus |  |  |  | 2.50 | 10.00 | 0.25 |
| Cathartes\_aura |  |  |  | 6.00 | 24.00 | 0.25 |
| Aix\_sponsa |  |  |  | 5.50 | 21.50 | 0.26 |
| Rallus\_limicola |  |  |  | 3.20 | 12.50 | 0.26 |
| Podiceps\_auritus |  |  |  | 4.00 | 15.00 | 0.27 |
| Fulmarus\_glacialis |  |  |  | 5.70 | 21.00 | 0.27 |
| Daption\_capense |  |  |  | 5.50 | 20.00 | 0.28 |
| Phoebastria\_nigripes |  |  |  | 8.00 | 28.00 | 0.29 |
| Pachyptila\_desolata |  |  |  | 4.10 | 14.00 | 0.29 |
| Puffinus\_opisthomelas |  |  |  | 5.00 | 17.00 | 0.29 |
| Puffinus\_pacificus |  |  |  | 5.50 | 17.80 | 0.31 |
| Oceanodroma\_leucorhoa |  |  |  | 3.30 | 10.00 | 0.33 |
| Oceanites\_oceanicus |  |  |  | 3.60 | 10.80 | 0.33 |
| Pagodroma\_nivea |  |  |  | 6.70 | 18.00 | 0.37 |

**Table S2.** Olfactory bulb size and optic lobe size data used in this study. OLA, optic lobe surface area (cm2); TBA, total brain area (cm2); OptR, ratio of optic lobe surface area to total brain surface area; OBL, olfactory bulb length (longest axis regardless of orientation, cm); CHL, cerebral hemisphere length (longest axis regardless of orientation, cm); OR, olfactory ratio. 1Data provided in this study. 2Data from Zelenitsky et al. [9]. All other data from Bang and Cobb [7].

**Table S3**. Ancestral olfactory ratios and 95% confidence intervals using the Burleigh et al. [4] tree for 11 palaeognaths and 88 neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [5] grafted onto the tree of Burleigh et al. [4] (Fig. S5). Elephant birds are represented by *A. maximus*. Node numbers correspond to tree in Tree file S3.

|  |  |  |
| --- | --- | --- |
| node | ancestral olfactory ratio | 90% CI |
| 100 | 0.20 | 0.07 |
| 101 | 0.20 | 0.06 |
| 102 | 0.19 | 0.04 |
| 103 | 0.19 | 0.03 |
| 104 | 0.19 | 0.04 |
| 105 | 0.18 | 0.04 |
| 106 | 0.18 | 0.04 |
| 107 | 0.16 | 0.05 |
| 108 | 0.14 | 0.04 |
| 109 | 0.13 | 0.03 |
| 110 | 0.13 | 0.03 |
| 111 | 0.13 | 0.03 |
| 112 | 0.15 | 0.02 |
| 113 | 0.14 | 0.03 |
| 114 | 0.14 | 0.03 |
| 115 | 0.14 | 0.03 |
| 116 | 0.13 | 0.03 |
| 117 | 0.13 | 0.03 |
| 118 | 0.18 | 0.04 |
| 119 | 0.18 | 0.04 |
| 120 | 0.17 | 0.05 |
| 121 | 0.16 | 0.06 |
| 122 | 0.14 | 0.05 |
| 123 | 0.12 | 0.04 |
| 124 | 0.12 | 0.03 |
| 125 | 0.16 | 0.06 |
| 126 | 0.17 | 0.04 |
| 127 | 0.17 | 0.04 |
| 128 | 0.18 | 0.04 |
| 129 | 0.15 | 0.06 |
| 130 | 0.14 | 0.03 |
| 131 | 0.20 | 0.05 |
| 132 | 0.17 | 0.05 |
| 133 | 0.17 | 0.05 |
| 134 | 0.17 | 0.05 |
| 135 | 0.16 | 0.04 |
| 136 | 0.16 | 0.05 |
| 137 | 0.15 | 0.04 |
| 138 | 0.15 | 0.04 |
| 139 | 0.18 | 0.05 |
| 140 | 0.20 | 0.04 |
| 141 | 0.20 | 0.04 |
| 142 | 0.20 | 0.04 |
| 143 | 0.21 | 0.05 |
| 144 | 0.24 | 0.05 |
| 145 | 0.20 | 0.05 |
| 146 | 0.20 | 0.05 |
| 147 | 0.19 | 0.04 |
| 148 | 0.19 | 0.04 |
| 149 | 0.20 | 0.04 |
| 150 | 0.19 | 0.04 |
| 151 | 0.19 | 0.04 |
| 152 | 0.19 | 0.04 |
| 153 | 0.19 | 0.04 |
| 154 | 0.18 | 0.05 |
| 155 | 0.15 | 0.05 |
| 156 | 0.14 | 0.05 |
| 157 | 0.12 | 0.04 |
| 158 | 0.13 | 0.03 |
| 159 | 0.14 | 0.03 |
| 160 | 0.20 | 0.04 |
| 161 | 0.27 | 0.04 |
| 162 | 0.28 | 0.04 |
| 163 | 0.28 | 0.04 |
| 164 | 0.28 | 0.03 |
| 165 | 0.27 | 0.03 |
| 166 | 0.27 | 0.03 |
| 167 | 0.27 | 0.03 |
| 168 | 0.26 | 0.03 |
| 169 | 0.30 | 0.03 |
| 170 | 0.29 | 0.03 |
| 171 | 0.20 | 0.05 |
| 172 | 0.20 | 0.06 |
| 173 | 0.22 | 0.04 |
| 174 | 0.22 | 0.03 |
| 175 | 0.22 | 0.03 |
| 176 | 0.24 | 0.02 |
| 177 | 0.22 | 0.03 |
| 178 | 0.23 | 0.03 |
| 179 | 0.23 | 0.03 |
| 180 | 0.19 | 0.01 |
| 181 | 0.19 | 0.07 |
| 182 | 0.21 | 0.03 |
| 183 | 0.20 | 0.03 |
| 184 | 0.21 | 0.03 |
| 185 | 0.21 | 0.03 |
| 186 | 0.16 | 0.06 |
| 187 | 0.14 | 0.04 |
| 188 | 0.21 | 0.07 |
| 189 | 0.23 | 0.06 |
| 190 | 0.24 | 0.06 |
| 191 | 0.27 | 0.05 |
| 192 | 0.27 | 0.05 |
| 193 | 0.27 | 0.05 |
| 194 | 0.24 | 0.06 |
| 195 | 0.24 | 0.05 |
| 196 | 0.19 | 0.05 |
| 197 | 0.27 | 0.05 |

**Table S4**. Ancestral olfactory ratios and 95% confidence intervals using the Burleigh et al. [4] tree for 11 palaeognaths and 88 neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [5] grafted onto the tree of Burleigh et al. [4] (Fig. S6). Elephant birds are represented by *A. hildebrandti*. Node numbers correspond to tree in Tree file S4.

|  |  |  |  |
| --- | --- | --- | --- |
|  | F value | P value | phylogenetic P value |
| without *A. hildebrandti* | 17.73 | < 0.01 | < 0.001 |
| with *A. hildebrandti* as open-dwelling | 21.14 | < 0.001 | - |
| with *A. hildebrandti* as forest-dwelling | 17.62 | < 0.01 | - |

**Table S5.** F and P values from one-w­­­ay ANOVAs of olfactory ratio vs. habitat type among 11 palaeognaths. Analyses including *A. hildebrandti* also included *L. plebius*. All P values indicate significant differences in olfactory ratio between open- and forest-dwelling taxa.

|  |  |  |
| --- | --- | --- |
| node | ancestral olfactory ratio | 95% CI |
| ­­­­14 | 0.050 | 0.020 |
| 15 | 0.057 | 0.030 |
| 16 | 0.046 | 0.021 |
| 17 | 0.045 | 0.025 |
| 18 | 0.045 | 0.026 |
| 19 | 0.031 | 0.014 |
| 20 | 0.028 | 0.011 |
| 21 | 0.038 | 0.022 |
| 22 | 0.051 | 0.032 |
| 23 | 0.068 | 0.051 |
| 24 | 0.076 | 0.061 |
| 25 | 0.072 | 0.056 |

**Table S6.** Ancestral ratios of optic lobe surface area vs. total brain surface area and 95% confidence intervals for 11 palaeognaths and two neognaths using the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [5] grafted onto the tree of Burleigh et al. [4]. Node numbers correspond to Tree file S4.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | AIC | BIC | slope | intercept |
| all birds - UC | -76.09 | -68.36 | 1.05 | -0.81 |
| all birds - BM | -106.87 | -99.15 | 0.68 | -0.28 |
| all birds - OU | **-74.09** | **-63.79** | 1.05 | -0.81 |
| palaeognaths - UC | -3.64 | -3.05 | 1.08 | -0.74 |
| palaeognaths - BM | -4.98 | -4.39 | 0.91 | -0.52 |
| palaeognaths - OU | **-1.64** | **-0.85** | 1.08 | -0.74 |
| neognaths - UC | -69.36 | -62.00 | 0.91 | -0.64 |
| neognaths - BM | -96.69 | -89.33 | 0.64 | -0.31 |
| neognaths - OU | **-67.36** | **-57.54** | 0.91 | -0.64 |
| palaeognaths - UC1 | - | - | 0.98 | -0.59 |

**Table S7.** Bayesian (BIC) and Akaike (AIC) information criteria, slopes and y-intercepts for standard and phylogenetic generalized least squares (GLS) of olfactory bulb size vs. cerebral hemisphere size among palaeognaths alone, neognaths alone and all birds combined. 1Analyzed all palaeognaths, including both elephant birds and *L. plebius*. NC, non-phylogenetically correct GLS; BM, phylogenetic GLS assuming a Brownian motion process; OU, phylogenetic GLS assuming an Ornstein-Uhlenbeck process. Phylogenetic GLS used the tree from Burleigh et al. [4]. Best scores for each subset in bold.

**5. Tree files**

**Tree file S1.** Unmodified tree file from Burleigh et al. [3].

**Tree file S2.** Unmodified tree file from Yonezawa et al. [4].

**Tree file S3.** Tree file from Burleigh et al. [3] modified to include the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [4] and pruned to include on taxa in our olfactory bulb dataset.

**Tree file S4.** Tree file from Burleigh et al. [3] modified to include the elephant bird-kiwi-cassowary-emu clade (including branch lengths) from Yonezawa et al. [4] and pruned to include on taxa in our optic lobe dataset.

**6. References**

1. Wiman C, Edinger T. 1942 Sur les cranes et les encephales d’Aepyornis et de Mullerornis. *Bull. Acadamie Malgache* **42**, 1–50.

2. Smith NA, Clarke JA. 2012 Endocranial Anatomy of the Charadriiformes: Sensory System Variation and the Evolution of Wing-Propelled Diving. *PLoS ONE* **7**, e49584. (doi:10.1371/journal.pone.0049584)

3. Balanoff AM *et al.* 2016 Best practices for digitally constructing endocranial casts: examples from birds and their dinosaurian relatives. *J. Anat.* **229**, 173–190. (doi:10.1111/joa.12378)

4. Burleigh JG, Kimball RT, Braun EL. 2015 Building the avian tree of life using a large-scale, sparse supermatrix. *Mol. Phylogenet. Evol.* **84**, 53–63. (doi:10.1016/j.ympev.2014.12.003)

5. Yonezawa T *et al.* 2017 Phylogenomics and morphology of extinct paleognaths reveal the origin and evolution of the ratites. *Curr. Biol.* **27**, 68–77. (doi:10.1016/j.cub.2016.10.029)

6. R Core Team. 2018 *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

7. Paradis E, Claude J, Strimmer K. 2004 APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)

8. Bang B, Cobb S. 1968 The size of the olfactory bulb in 108 species of birds. *The Auk* **85**, 55–61. (doi:10.2307/4083624)

9. Houde P. 1988 *Paleognathous Birds from the Early Tertiary of the Northern Hemisphere*. Cambridge, Massachusetts: Nuttall Ornithological Club.

10. Zelenitsky DK, Therrien F, Ridgely RC, McGee AR, Witmer LM. 2011 Evolution of olfaction in non-avian theropod dinosaurs and birds. *Proc. R. Soc. B Biol. Sci.* **278**, 3625–3634. (doi:10.1098/rspb.2011.0238)

11. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017 *nlme: linear and nonlinear mixed effects models*.

12. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things): phytools: R package. *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)

13. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008 GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131. (doi:10.1093/bioinformatics/btm538)

14. Ksepka DT, Balanoff AM, Walsh S, Revan A, Ho A. 2012 Evolution of the brain and sensory organs in Sphenisciformes: new data from the stem penguin Paraptenodytes antarcticus. *Zool. J. Linn. Soc.* , 202–219. (doi:10.1111/j.1096-3642.2012.00835.x)

15. Early C, Ridgely R, Porter W, Cerio D, Witmer L. 2016 The skull and endocranial anatomy of the extinct giant moa Dinornis robustus (Aves: Palaeognathae) and implications for the behavioral role of vision in moa. *Anat. Rec.* **299**, **Special Feature**, 223–224.

16. Martin GR, Wilson K-J, Wild JM, Parsons S, Kubke MF, Corfield J. 2007 Kiwi Forego Vision in the Guidance of Their Nocturnal Activities. *PLoS ONE* **2**, e198. (doi:10.1371/journal.pone.0000198)