This article was downloaded by: [claudia tambussi]
On: 27 August 2015, At: 08:42
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: 5 Howick Place, London, SW1P 1WG

Click for updates

Journal of Vertebrate Paleontology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ujvp20

Endocranial anatomy of Antarctic Eocene stem penguins: implications for sensory system evolution in Sphenisciformes (Aves)

Claudia P. Tambussi[a], Federico J. Degrange[ab] & Daniel T. Ksepka[cd]

[a] Centro de Investigaciones em Ciencias de la Tierra (Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Córdoba), Avenida Vélez Sársfield 1611, X5016GCA, Córdoba, Argentina,

[b] Centro de Investigaciones Paleobiológicas, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Avenida Vélez Sársfield 299, X5000GCA, Córdoba, Argentina, ;

[c] National Evolutionary Synthesis Center, Durham, North Carolina 27705-4667, U.S.A.

[d] North Carolina State University, Raleigh, North Carolina 27695, U.S.A.

Published online: 26 Aug 2015.

To cite this article: Claudia P. Tambussi, Federico J. Degrange & Daniel T. Ksepka (2015): Endocranial anatomy of Antarctic Eocene stem penguins: implications for sensory system evolution in Sphenisciformes (Aves), Journal of Vertebrate Paleontology

To link to this article: http://dx.doi.org/10.1080/02724634.2015.981635

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Journal of Vertebrate Paleontology e981635 (18 pages)
© by the Society of Vertebrate Paleontology
DOI: 10.1080/02724634.2015.981635

ARTICLE

ENDOCRANIAL ANATOMY OF ANTARCTIC EOCENE STEM PENGUINS: IMPLICATIONS FOR SENSORY SYSTEM EVOLUTION IN SPHENISCIFORMES (AVES)

CLAUDIA P. TAMBUSSI,*,[1] FEDERICO J. DEGRANGE,[1,2] and DANIEL T. KSEPKA[3,4,†]
[1]Centro de Investigaciones em Ciencias de la Tierra (Consejo Nacional de Investigaciones Científicas y Técnicas–Universidad Nacional de Córdoba), Avenida Vélez Sársfield 1611, X5016GCA, Córdoba, Argentina, tambussi.claudia@conicet.gov.ar;
[2]Centro de Investigaciones Paleobiológicas, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Avenida Vélez Sársfield 299, X5000GCA, Córdoba, Argentina, fjdino@gmail.com;
[3]National Evolutionary Synthesis Center, Durham, North Carolina 27705-4667, U.S.A.;
[4]North Carolina State University, Raleigh, North Carolina 27695, U.S.A.

ABSTRACT—Penguins have a more than 60 million year long evolutionary history. Thus, stem lineage fossil taxa are key to understanding their evolution. Here, we present data on three virtual endocasts from stem penguin skulls collected from the Eocene La Meseta Formation of Seymour Island (Antarctica), along with comparative data from extant penguins and outgroups. These fossils appear to belong to three distinct species, and represent both the oldest (34.2 Ma) and the most basal penguin taxa that have yielded endocast data. Data collected from the fossils provide new support for several important shifts in neuroanatomy and cranial skeletal anatomy along the transition from stem to crown penguins, including (1) caudal expansion of the eminentia sagittalis, (2) an increase in the overlap of the telencephalon onto the cerebellum, (3) reduction of the bulbus olfactorius, and (4) loss of the interaural pathway. The large semicircular canal diameters of the Antarctic fossils as well as the more crownward stem penguin *Paraptenodytes antarcticus* together suggest that canal size increased in basal penguins relative to outgroup taxa but later decreased near the crown radiation. As in most other wing-propelled diving birds, the endocasts lack evidence of cerebellar folds and possess a relatively large floccular recess. Several aspects of the endocast morphology, including the exposure of the tectum opticum in dorsal view and the rostral displacement of the eminentia sagittalis away from the border of the cerebellum, are seen neither in crown penguins nor in Procellariiformes (the extant sister clade to Sphenisciformes) and so appear to represent unique characters of these stem taxa.

INTRODUCTION

Sphenisciformes (penguins) provide one of the classic examples of the evolution of a secondarily aquatic ecology in tetrapods. Penguins are one of four known lineages of flightless wing-propelled diving birds, along with the extinct Plotopteridae (Olson and Hasegawa, 1979) and the Mancallinae (Lucas Auks) and Great Auk lineages, which independently transitioned to flightlessness (Smith, 2011). As the only surviving clade to exploit this niche, penguins provide our most direct source of information on how this shift in locomotor behavior is reflected in the anatomy of the brain and sensory organs. Although the neuroanatomical transitions associated with the evolution of flight have been subject to extensive research (e.g., Jerison, 1968; Hopson, 1977; Larsson et al., 2000; Alonso et al., 2004; Northcutt, 2011; Bhullar et al., 2012; Balanoff et al., 2013), the shift to a diving ecology has received less attention. Until recently, few studies have focused on the neuroanatomy of penguins in general, let alone extinct taxa. In the case of fossil data, this has been largely due to historical patterns of discovery. Nearly a century passed between the description of the first known fossil penguin bone as *Palaeeudyptes antarcticus* by Huxley (1859) and the report of the first reasonably complete skull, assigned to *Paraptenodytes antarcticus* by Simpson (1946). In recent years, however, there has been a fortuitous increase in the number of specimens preserving the skull (e.g., Stucchi, 2002; Stucchi et al., 2003; Acosta Hospitaleche et al., 2006, 2007; Slack et al., 2006; Clarke et al., 2007, 2010; Acosta Hospitaleche, 2013). Simultaneously, computed tomography (CT) studies of the neuroanatomy of flightless auks (Smith and Clarke, 2012) and the extinct Plotopteridae (Kawabe et al., 2014) have been completed, providing the first information about the neuroanatomy of these lineages. Thus, the stage is set for comparative studies of the brain and sensory organs of penguins in the context of the evolution of wing-propelled diving.

Several previous studies have touched on patterns in extant penguin brain anatomy in the context of wider sampling across Aves. Iwaniuk and Hurd (2005) used multivariate analyses to compare avian brains based on the relative proportions of nine regions. Cluster analyses resulted in the sole included penguin species (*Spheniscus magellanicus*) grouping with a shearwater (*Puffinus tenuirostris*), cormorant (*Phalacrocorax auritus*), rhea (*Rhea americana*), and partridge (*Perdix perdix*). These taxa were united primarily by larger cerebellum and smaller nidopallium volumes compared with most other birds. It is interesting to note that extant penguins share these proportional similarities, both with the closely related *Puffinus tenuirostris* and with other distantly related taxa. Iwaniuk and colleagues also demonstrated, that although both Sphenisciformes and Procellariiformes have a high level of cerebellar folding (Iwaniuk et al., 2006), there are differences in the relative size of the individual cerebellar folia between these clades (Iwaniuk et al., 2007). Unfortunately, fossil penguin endocasts do not permit the discernment of boundaries between the nine regions of the brain examined by Iwaniuk and Hurd (2005) or boundaries between the individual folia of the

---

*Corresponding author.

†Current Address: Bruce Museum, Greenwich, Connecticut 06830, U.S.A., dksepka@brucemuseum.org.

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/ujvp.

cerebellum, so it is not possible to survey extinct taxa studied for such characters.

Two previous studies that focused on the brain and sensory organs of penguins provide an overview of the surface morphology of the penguin brain and highlight some key differences between stem and crown species. Ksepka et al. (2012b) described the endocast of the stem penguin *Paraptenodytes antarcticus* (Gaiman Formation, Argentina, ~20–23 Ma) and three extant penguin species and showed that the development of many structures identifiable in the endocast, such as the eminentia sagittalis (Wulst) and flocculus are similar in penguins and volant birds. This is consistent with the need for penguins to maneuver in complex three-dimensional space while pursuing prey or avoiding predators underwater. *Paraptenodytes antarcticus* also retains some primitive features not seen in extant penguins, such as the presence of an interaural pathway, as well as some unique features, such as large semicircular canals. More recently, Paulina-Carabajal et al. (2014) described the anatomy of the endocast of the extinct crown penguin *Pygoscelis calderensis* (Middle Miocene–Pliocene of Chile), pointing out similarities to the three living species of the genus *Pygoscelis*.

In this study, we present endocast reconstructions of three new fossil penguin specimens, all from the Eocene La Meseta Formation of Antarctica. The specimens examined in this study are at least 11 million years older than any previously reported penguin endocasts; they also belong to the most basal penguin taxa yet sampled for neuroanatomical study. Thus, they have the potential to reveal new information on earlier stages of the evolution of penguin neuroanatomy.

Taxonomic Note—We consider Plotopteridae to most likely represent an extinct lineage within Suliformes, following Olson and Hasegawa (1979) and Smith (2010). However, Kawabe et al. (2014) recently noted similarities between the endocasts of several plotopterid specimens and those of penguins. Excellent new cranial material included in the neuroanatomical study of Kawabe et al. (2014) has not yet been formally described and may preserve new character data that could conclusively resolve the phylogenetic affinities of this enigmatic group. Pending further inquiry into plotopterid relationships, we make comparisons separately with Plotopteridae and with extant Suliformes in our description and discussion in order to maintain clarity.

Geologic Context—Two well-preserved partial skulls (MLP 12-I-20-1 and MLP 12-I-20-2) were recovered from the Submeseta Allomember of the Eocene La Meseta Formation. They were found by Marcelo Reguero, Juan Moly, José O'Gorman, and Claudia Tambussi during the 2012 Antarctic summer field season. Both skulls were collected from the *Anthropornis nordenskjoeldi* Biozone (Tambussi et al., 2006), a unit that is defined within the Submeseta Allomember of the La Meseta Formation. The La Meseta Formation is a 710-m-thick clastic unit with well-studied outcrops on Seymour Island at the Weddell Sea, close to the northern tip of the Antarctic Peninsula. This formation has yielded a diverse fossil assemblage of marine and coastal vertebrates. It was subdivided by Marenssi et al. (1998) into six erosionally based internal units (from lowermost to uppermost: Valle de Las Focas Allomember, Acantilados Allomember, Campamento Allomember, Cucullaea I Allomember, Cucullaea II Allomember, and Submeseta Allomember) deposited mainly during the Eocene in deltaic, estuarine, and shallow marine settings (Montes et al., 2010; Reguero et al., 2012). The Submeseta Allomember was deposited at ~34.2–36 Ma (Priabonian) based on ^{87}Sr/^{86}Sr dates (Dingle and Lavelle, 1998). In this interval of strata, an easily distinguishable biozone is marked by abundant specimens of penguins and the phosphatic brachiopod *Lingula*. The three skulls studied here were recovered from this biozone. MLP 12-I-20-1 was collected at a site at S64°14′40.2″, W56°35′33.4″ and MLP 12-I-20-2 was collected at a site at S64°14′83.5″, W56°36′43.9″. Precise coordinates for MLP 84-II-1-10 are not available.

The La Meseta Formation of the Antarctic Peninsula is one of the most prolific localities for fossil penguin remains worldwide in terms of raw abundance. One of the highest documented levels of sympatric diversity (both morphological and taxonomic) in penguins is recognized in the *Anthropornis nordenskjoeldi* Biozone. Unfortunately, almost all of the thousands of penguin specimens were found in isolation, with a few rare exceptions of associated partial skeletons (e.g., Acosta Hospitaleche and Reguero, 2010; Jadwiszczak, 2012). The alpha taxonomy of these penguins is thus still incompletely resolved. At least 10 and as many as 14 species co-occur, with uncertainty in the total due to the possibility of factors such as sexual dimorphism (see Myrcha et al., 2002; Jadwiszczak, 2006a, 2013; Tambussi et al., 2006; Ksepka and Clarke, 2010; Jadwiszczak and Mörs, 2011). Whereas robust limb elements are common, cranial fragments are only rarely reported and are often in poor condition (e.g., Myrcha et al., 1990; Jadwiszczak, 2006b; Ksepka and Bertelli, 2006). Aside from the specimens described here, only a few cranial remains have been mentioned in the literature, including two partially crushed braincases (Jadwiszczak, 2006b; Ksepka and Bertelli, 2006), several partial beaks (Olson, 1985; Jadwiszczak, 2006b; Ksepka and Bertelli, 2006), and the posterior portion of a braincase (Acosta Hospitaleche and Haidr, 2011; Haidr and Acosta Hospitaleche, 2012).

External Morphology and Phylogenetic Affinities—For ease of comparison, we refer to the scanned specimens as Antarctic Fossil 1 (MLP 12-I-20-1), Antarctic Fossil 2 (MLP 84-II-1-10), and Antarctic Fossil 3 (MLP 12-I-20-2). Details of the external morphology were previously provided by Acosta Hospitaleche (2013), so we focus only on establishing the phylogenetic affinities of the specimens as stem lineage penguins in this section. The reduced cranial pneumaticity (observable in the CT slices), dorsally extensive fossae temporales, and presence of a supraorbital fossa glandulae nasalis (salt gland fossa; region not preserved in Antarctic Fossil 2) all support assignment of these skulls to Sphenisciformes. Aside from Sphenisciformes, similarities in general skull shape with Gaviiformes (loons) may be noted. However, the most complete skull clearly resembles penguins in the strong development of the processus medialis parasphenoidalis and absence of the processus lateralis parasphenoidalis. In contrast, Gaviiformes exhibit a poorly developed processus medialis parasphenoidalis, whereas the processus lateralis parasphenoidalis is very well developed (Bock, 1960).

Several features are consistent with restriction to the penguin stem lineage. These include the dorsally extensive fossae temporales (in all three specimens) and very large condylus occipitalis (preserved in Antarctic Fossil 1 and Fossil 2). All stem penguins exhibit deep fossae temporales that extend to the midline of the skull roof, leaving only a narrow crest between them (Ksepka et al., 2006). Among crown taxa, only *Spheniscus* shows a similar morphology, whereas the fossae temporales are more widely separated in all other extant taxa (*Eudyptes, Megadyptes, Eudyptula, Pygoscelis*, and *Aptenodytes*) (Zusi, 1975; Bertelli and Giannini, 2005). Moreover, the fossae are shallower in *Pygoscelis* and *Aptenodytes* (Bertelli and Giannini, 2005). It has previously been noted that the condylus occipitalis is proportionally larger in Paleogene stem penguin skulls than in crown penguin skulls (e.g., Bertelli et al., 2006; Jadwiszczak, 2006b; Ksepka et al., 2008). Finally, at least Antarctic Fossil 3 was a spear-billed penguin, based on associated beak fragments. A spear-like bill morphology has been noted in several stem penguin taxa such as *Icadyptes, Inkayacu*, and *Kairuku* (Clarke et al., 2007, 2010; Ksepka et al., 2012a). Aside from morphology, it is virtually certain that the fossils belong to stem lineage penguins because all known penguin species from the extensively sampled La Meseta Formation belong to stem taxa (Ksepka and Clarke, 2010).

Because all Seymour Island penguin species are diagnosed primarily on characters of isolated tarsometatarsi and humeri (Wiman, 1905; Simpson, 1971; Myrcha et al., 2002; Jadwiszczak, 2006b), assigning the specimens studied here to the species level would be speculative. Furthermore, it is difficult to rely on size in order to make referrals because stem penguins exhibit different skeletal proportions from extant species (Ksepka et al., 2012a). For example, the braincase region of the 'giant' penguin *Inkayacu paracasensis* is only slightly larger than that of an emperor penguin, despite the much longer beak and larger postcranial elements. Antarctic Fossil 1 and Antarctic Fossil 3 appear to belong to distinct species based on morphology and size (Fig. 1) as well as differences in the endocasts. Antarctic Fossil 1 and Antarctic Fossil 2 are similar in their external morphology and size. However, the corresponding endocasts are quite different. Considering these three specimens to represent three separate species is consistent with the high level of diversity and size disparity represented in the La Meseta Formation (Tambussi et al., 2006).

MATERIALS AND METHODS

Sixteen avian specimens were selected for inclusion in the study, including the three Antarctic fossils, the stem penguin *Paraptenodytes antarcticus*, six extant penguin species, and six outgroup species (Table 1). Taxon selection was based on phylogenetic relationships and diving ecology. Nine specimens were scanned as part of this study and seven included from previous work. Scans were taken in the transverse plane using a General Electric Bright Speed HiSpeed CT scanner at 140 kv and 300 mA with 0.62 mm slice thickness at the Hospital San Juan de Dios, La Plata, Argentina, for the three new fossil specimens and for the extant species *Pygoscelis antarctica, Spheniscus magellanicus, Macronectes giganteus, Sula variegata, Phalacrocorax olivaceous*, and *Podiceps major*. For comparative purposes, previously published CT scans of *Gavia immer, Phoebastria immutabilis, Eudyptula minor, Pygoscelis adeliae, Spheniscus demersus, Aptenodytes forsteri*, and *Paraptenodytes antarcticus* were obtained from the University of Austin Digital Morphology website (http://www.digimorph.org). DICOM images were processed, and the reconstruction of the three-dimensional (3D) models of the endocast, nerves, blood vessels, and inner ear were completed using Materialise Mimics software 10.01 (Materialise, Leuven, Belgium). Surface smoothing was performed using the smoothing tool of Mimics, using a smoothing factor of 1 without iterations.

A fresh specimen of *Spheniscus magellanicus* was also dissected in order to corroborate correspondence between the virtual endocast and the brain morphology. The morphology of the fresh brain of *Spheniscus* closely resembles that of the endocast, in agreement with wet brain–endocast comparisons for *Pygoscelis* (see Ksepka et al., 2012b) and the general observation that the endocast faithfully reflects brain shape and volume in birds (Iwaniuk and Nelson, 2002). Our study used previously collected museum specimens; because no live animals were collected for this study, new permits were not required.

Antarctic Fossil 1 (MLP 12-I-20-1; Fig. 2) is the best preserved of the fossils, and it was possible to obtain a complete endocast from this specimen. Unfortunately, it was not possible to obtain complete endocasts from Antarctic Fossil 2 (MLP 84-II-1-10) and Antarctic Fossil 3 (MLP 12-I-20-2) (Fig. 3) due to damage to the braincase in both specimens. In Antarctic Fossil 2, the inner ear region is damaged and the contrast between sediment and the bones of the braincase is poor, making it difficult to reconstruct some parts of the endocast. In Antarctic Fossil 3, the ventral portion of the braincase is completely missing. Although it was not possible to collect accurate volumetric data from the incomplete braincases, these specimens still permit observation of some discrete details of the brain and sensory organs that are detailed in the descriptions below.

Brain volumes were estimated from the endocasts using the software Materialise Mimics. To eliminate interobserver error, all measurements (olfactory bulb and cerebral hemisphere dimensions; Fig. 4), were taken by the second author (F.J.D.).

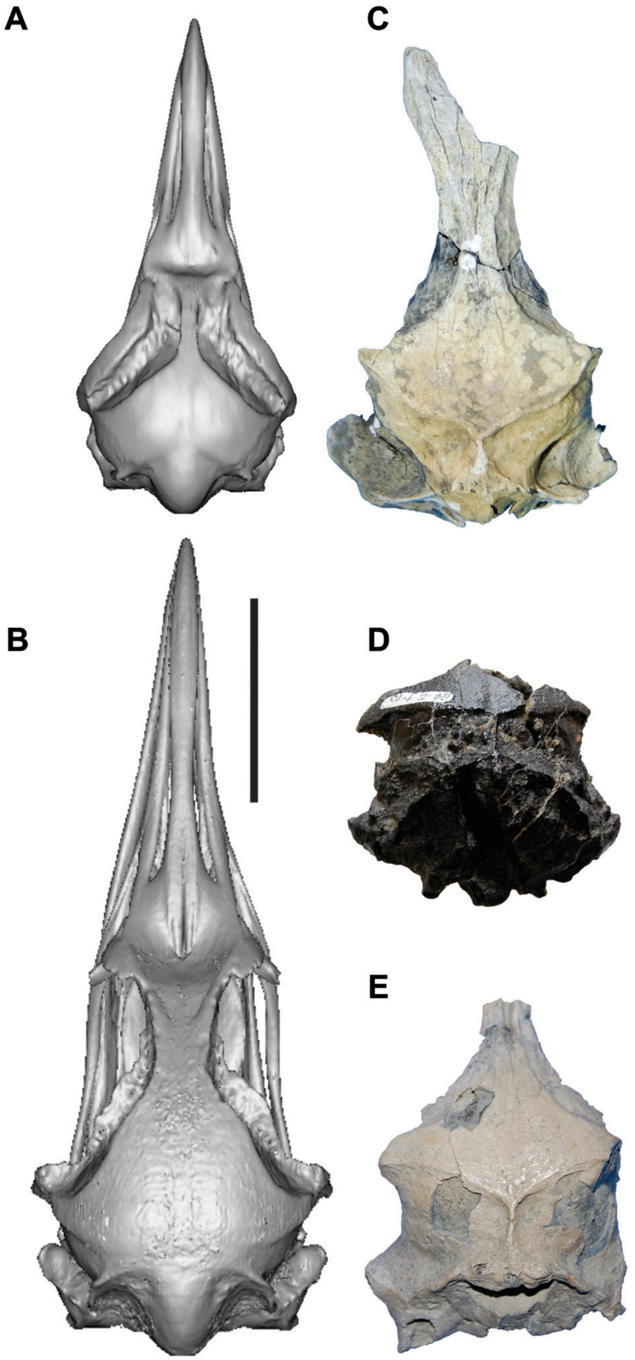

FIGURE 1. Skull size comparison. **A**, *Pygoscelis antarctica*; **B**, *Aptenodytes forsteri*; **C**, Antarctic Fossil 1 (MLP 12-I-20-1); **D**, Antarctic Fossil 2 (MLP 84-II-1-10); **E**, Antarctic Fossil 3 (MLP 12-I-20-2). Scale bar equals 5 cm.

TABLE 1. Measurements (mm) and body mass (g) of Sphenisciformes and other marine birds.

Taxa	BL	HL	TW	WL	WW	CL	CW	OL	OH	FL	BM
MLP 12-I-20-1	12.72	36.35	38.77	23.75	11.20	~24.34	18.64	16.11	11.40	8.71	—
MLP 12-I-20-2	—	51.55	47.55	43.00	19.19	30.13	23.1	—	—	12.69	—
MLP 84-II-1-10	—	—	42.06	—	12.71	—	22.08	16.98	11.28	10.01	—
Paraptenodytes antarcticus	8.80	35.86	46.28	29.26	14.49	23.21	20.01	15.54	8.32	8.7	12,435*
Pygoscelis antarctica	5.68	29.8	41.88	25.79	12.41	17.97	15.11	10.67	6.25	7.66	4435
Pygoscelis adeliae	7.59	39.08	52.69	34.38	17.40	20.96	20.20	14.51	9.20	8.28	5000
Spheniscus demersus	4.96	38.94	47.51	33.13	13.54	23.67	19.02	13.86	7.74	8.27	3310
Spheniscus magellanicus	4.81	29.34	36.92	24.62	10.39	18.20	14.91	10.70	7.13	6.37	4470
Aptenodytes forsteri	9.98	73.19	69.41	55.7	23.33	32.36	37.93	23.88	9.58	12.77	33,850
Eudyptula minor	5.57	29.22	33.47	21.66	8.93	15.57	13.15	10.59	6.23	5.10	1177**
Gavia immer	7.58	23.63	32.02	18.56	6.85	18.43	14.27	14.14	7.9	6.92	5460
Phoebastria immutabilis	5.30	27.94	36.92	24.24	10.08	14.66	14.57	13.21	7.46	6.88	3310
Macronectes giganteus	6.84	25.89	35.35	23.69	9.84	17	13.58	9.94	6.09	7.94	3500
Sula variegata	5.99	23.75	32.61	23.54	13.22	15.89	10.96	11.62	5.56	2.55	1350
Phalacrocorax brasilianus	—	26.92	32.91	25.12	14.20	16.8	13.19	11.63	5.44	3.7	1393
Podiceps major	—	16.96	21.3	15.15	6.1	10.69	9.46	8.52	5.29	4.47	1646

Abbreviations: **BL**, bulbus olfactorius length; **BM**, body mass; **CL**, cerebellum length; **CW**, cerebellum width; **FL**, floccular lobe length; **HL**, telencephalic hemisphere length; **OH**, optic lobe maximum height; **OL**, optic lobe maximum length; **TW**, telencephalic width; **WL**, wulst maximum length; **WW**, wulst maximum width. See Figure 4 for further explanation.

*Estimate from Ksepka et al. (2012b).

**Average of four subspecies (Dunning, 2008).

Olfactory ratios were calculated as the ratio of the greatest diameter of the bulbus olfactorius to the greatest diameter of the cerebral hemisphere regardless of orientation (Zelenitsky et al., 2009). Following Balanoff et al. (2013), the bulbus olfactorius was delimited anteriorly by the anterior-most constriction of the endocranial space before it opens into the nasal cavity and posteriorly by the crista that lies between the fossa housing the bulbus olfactorius and the anterior cerebral cavity.

To make additional quantitative comparisons, relative brain size (encephalization quotient [EQ]; Jerison, 1973), was calculated for each complete specimen using the equation:

$$EQ = ECV/0.137(BM)^{0.568}$$

where BM is the body mass (g) and ECV is the endocranial volume (cm^3). A species with an EQ >1.0 is considered to have an endocranial volume that is larger than expected for its body mass and EQ values <1.0 are indicative of species with endocranial volumes smaller than expected.

Body masses for extant bird species are calculated as the mean of mean female and mean male body masses published in Dunning (2008; Table 1). For *Paraptenodytes antarcticus*, we specified a body mass equal to that of *Aptenodytes patagonicus* based on overlapping size range of the limb bones of these taxa, following Ksepka et al. (2012b). Because associated postcranial elements were not available for any of the Antarctic fossils, we were unable to directly estimate body mass for these specimens.

To estimate mean hearing range (range of audible frequencies) and sensitivity, we followed the methods of Walsh et al. (2009) using scaled and transformed cochlear duct lengths. This method provides predictions about the best frequency and the high-frequency limit of hearing for species where body mass and/or the length of the basilar papilla are unknown (base data for the method proposed by Gleich et al., 2005) or cannot be estimated adequately.

Osteological nomenclature follows Baumel and Witmer (1993), and terminology for the central nervous system follows Breazile and Kuenzel (1993).

Institutional Abbreviations—**AMNH**, American Museum of Natural History, New York, New York, U.S.A.; **IAA**, Instituto Antártico Argentino, Ciudad Autónoma de Buenos Aires, Argentina; **KMNH**, Kitakyushu Museum of Natural History and Human History, Kitakyushu, Fukuoka Prefecture, Japan; **MLP**, Museo de La Plata, La Plata, Buenos Aires Province, Argentina; **TCWC**, Texas Cooperative Wildlife Collection, Texas A&M University, College Station, Texas, U.S.A.; **UF**, Florida Museum of Natural History, University of Florida, Gainesville, Florida, U.S.A.

DESCRIPTION

Overall Morphology and Orientation

In the following descriptions of brain structures, we note that all morphological and volumetric statements refer to the morphology of the endocast, unless explicitly noted as an observation from dissected brains.

One notable difference between the Antarctic fossil penguins and crown penguins is the orientation of the brain within the skull. Viewed laterally, the telencephalon is aligned directly rostral to the cerebellum with little overlap in Antarctic Fossils 1 and 2 (Fig. 5). The prominentia cerebellaris does not protrude far behind the level of the condylus occipitalis, and the foramen magnum (clearly discernable despite breakage in Antarctic Fossil 1) opens caudally in a vertical plane. These are the characteristics that define a Type 1 or orthocranial skull (Duijm, 1951; Marugán-Lobon and Buscaglioni, 2006) and an orthoencephalic brain shape. This general configuration is also seen in *Phalacrocorax auritus* (double-crested cormorant), and the foot-propelled divers *Gavia immer* (common loon) and *Podiceps major* (great grebe). In Antarctic Fossil 3, the telencephalon shows more overlap with the cerebellum, and the telencephalon is also somewhat dorsally displaced rather than aligned directly with the cerebellum. In other sampled penguins (*Paraptenodytes, Pygoscelis, Spheniscus*, and *Eudyptula*) and outgroup species, the prominentia cerebellaris protrudes far behind the plane of the condylus occipitalis, the foramen magnum is more ventrally oriented, and the telencephalon overlaps the cerebellum more extensively. This corresponds with the Type 3 or aerorhync type of skull (Duijm, 1951; Marugan-Lobón and Buscaglioni, 2006) and an airencephalic type of brain. The long axis of the brain is oriented at a low angle to the lamina parasphenoidalis of the skull in Antarctic Fossil 1 (Table 2). A similar orientation characterizes most extant penguins and *Gavia*, whereas in the procellariiform *Phoebastria*, the brain is oriented at a greater angle.

In dorsal view (Figs. 3, 5), the endocast of Antarctic Fossil 1 is quite distinct from that of other sampled penguins, as well as from those of loons and plotopterids. The endocast is rostrocaudally elongate, with a low degree of mediolateral expansion of

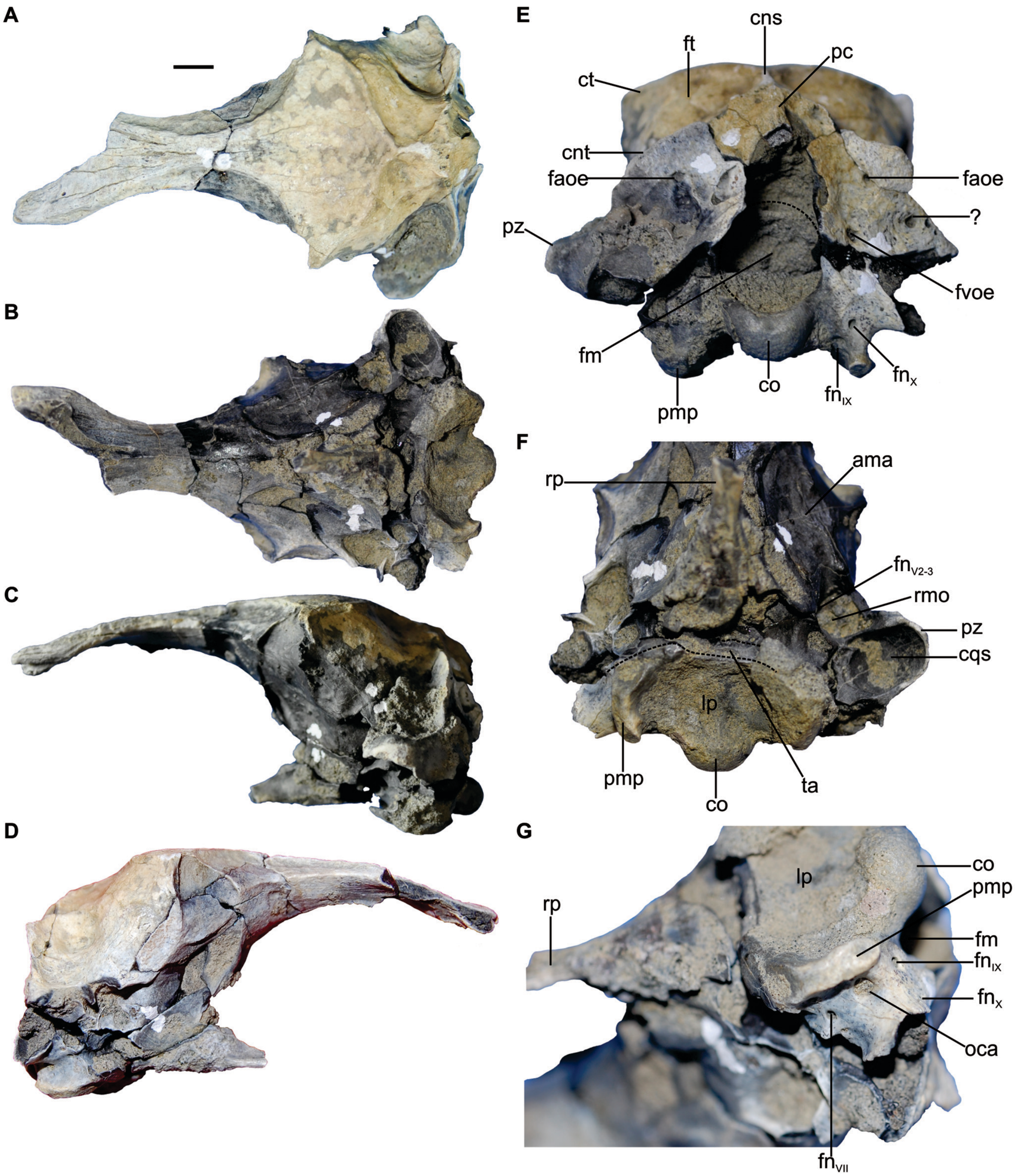

FIGURE 2. Cranial morphology of Antarctic Fossil 1 (MLP 12-I-20-1) in **A**, dorsal; **B**, ventral; **C**, left lateral; and **D**, right lateral views. **E–G**, details showing the main exit of different sensorial and vascular structures. **E**, caudal view; **F**, ventral view; **G**, ventrolateral (right) view. **Abbreviations: ama**, area muscularis aspera; **cns**, crista nuchalis sagittalis; **cnt**, crista nuchalis transversa; **co**, condylus occipitalis; **cqs**, cotyla quadrati squamosi; **ct**, crista temporalis; **faoe**, foramen arteria occipitalis externa; **fm**, foramen magnum (reconstructed in dotted line); **fn**$_{\mathrm{IX}}$, foramen nervi glossopharyngealis (CN IX); **fn**$_{\mathrm{V2\text{-}3}}$, foramen nervi maxillomandibularis (CN $\mathrm{V}_{2\text{–}3}$); **fn**$_{\mathrm{VII}}$, foramen nervi facialis (CN VII); **fn**$_{\mathrm{X}}$, foramen nervi vagi (CN X); **ft**, fossa temporalis; **fvoe**, foramen vena occipitalis externa; **lp**, lamina parasphenoidalis; **oca**, ostium canalis carotici; **pc**, prominentia cerebellaris; **pmp**, processus medialis parasphenoidalis; **pz**, processus zygomaticus; **rmo**, rete mirabilis opthalmica exit; **rp**, rostrum parasphenoidale; **ta**, tuba auditiva. Scale bar equals 1 cm.

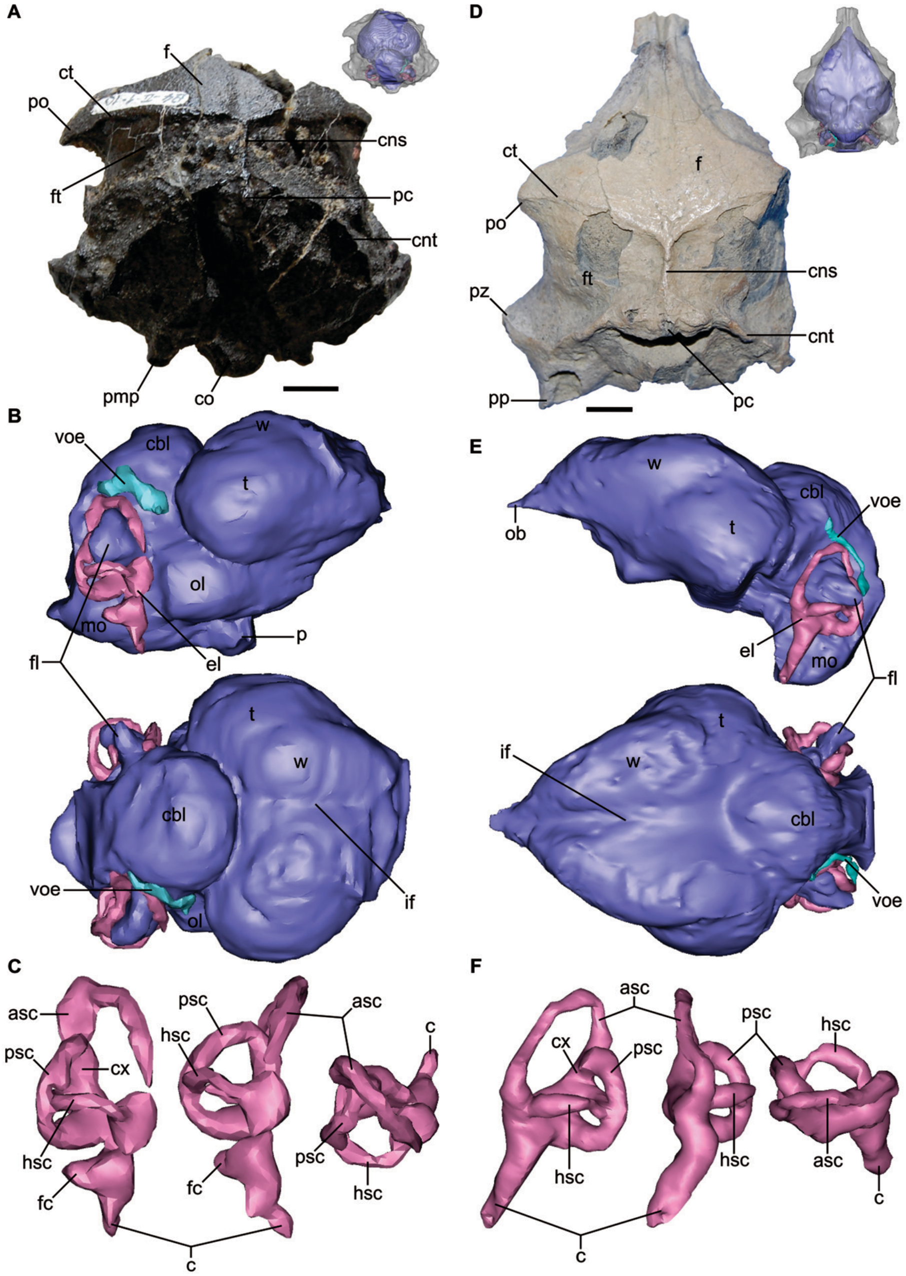

FIGURE 3. **A**–**C**, Antarctic Fossil 2 (MLP 84-II-1-10); **A**, skull in dorsal view; **B**, endocasts in lateral and dorsal views; **C**, right labyrinth in anterior and dorsal views. **D**–**F**, Antarctic Fossil 3 (MLP 12-I-20-2); **D**, skull in dorsal view; **E**, endocasts in lateral and dorsal views; **F**, left labyrinth in lateral, caudal, and ventral views. **Abbreviations**: **asc**, anterior semicircular canal; **c**, cochlea; **cbl**, cerebellum; **cns**, crista nuchalis sagittalis; **cnt**, crista nuchalis transversa; **co**, condylus occipitalis; **ct**, crista temporalis; **cx**, crus communis; **el**, endosseous labyrinth; **f**, os frontalis; **fc**, fenestra cochlearis; **fl**, cerebellar flocculus; **ft**, fossa temporalis; **hsc**, horizontal semicircular canal; **if**, fissura interhemispherica; **mo**, medulla oblongata; **ob**, olfactory bulb; **ol**, optic lobe; **p**, pituitary gland (hypophysis); **pc**, prominentia cerebellaris; **pmp**, processus medialis parasphenoidalis; **po**, processus postorbitalis; **pp**, processus paroccipitalis; **psc**, posterior semicircular canal; **pz**, processus zygomaticus; **t**, telencephalon; **voe**, vena occipitalis externa; **w**, wulst (sagittal eminence). Both scale bars equal 1 cm.

the telencephalon similar to that in *Aptenodytes*, as opposed to the wide shape observed in other extant penguins (Index 1, Table 3). The rostral margin of the telencephalon also tapers more sharply in dorsal view. There are differences in the degree of overlap between the telencephalon and the cerebellum of the brain as well. In the fossil, the cerebellum is largely exposed as in *Phalacrocorax* and *Gavia*, whereas in extant penguins and many outgroups, the telencephalon overlaps a portion of the cerebellum in the endocast (less so in *Spheniscus*). There appears to be some degree of variation in this feature in Plotopteridae, with the Hatatsu skull (KMNH VP 200,007) and the Touno skull (KMNH VP 200,008) described by Kawabe et al. (2014: fig. 12) showing more clear separation between these regions in the endocast than in the Ainoshima skull (KMNH VP 200,009).

Scans reveal that the skull roof is thicker in Antarctic Fossils 1 and 2 than in other sampled penguins. The wall thickness along the lateral edge of the braincase (Fig. 6, arrow) is 2.2 mm (equal to 9.9% of maximum skull width) in Antarctic Fossil 1, 2.7 mm (11.3% of maximum skull width) in Antarctic Fossil 2, and 1.4 mm (5.7% of maximum skull width) in Antarctic Fossil 3. At the same point, the braincase is only 0.9 mm thick (3.9% of maximum skull width) in the similarly sized *Paraptenodytes antarcticus* and measures 1.3 mm in *Pygoscelis antarctica* (5.7% of maximum skull width) and 1.0 mm (5.3% of maximum skull width) in *Spheniscus magellanicus*.

Telencephalon—The most noticeable feature of the telencephalon in Antarctic Fossil 1 and Antarctic Fossil 3 compared with extant penguins and *Paraptenodytes* is the weaker lateral expansion (Fig. 7; Index 1, Table 3), which results in a less ‘heart-shaped’ outline in dorsal view. Antarctic Fossil 2 lacks a large portion of the telencephalon, making it more difficult to evaluate overall shape. However, the intact regions do suggest a

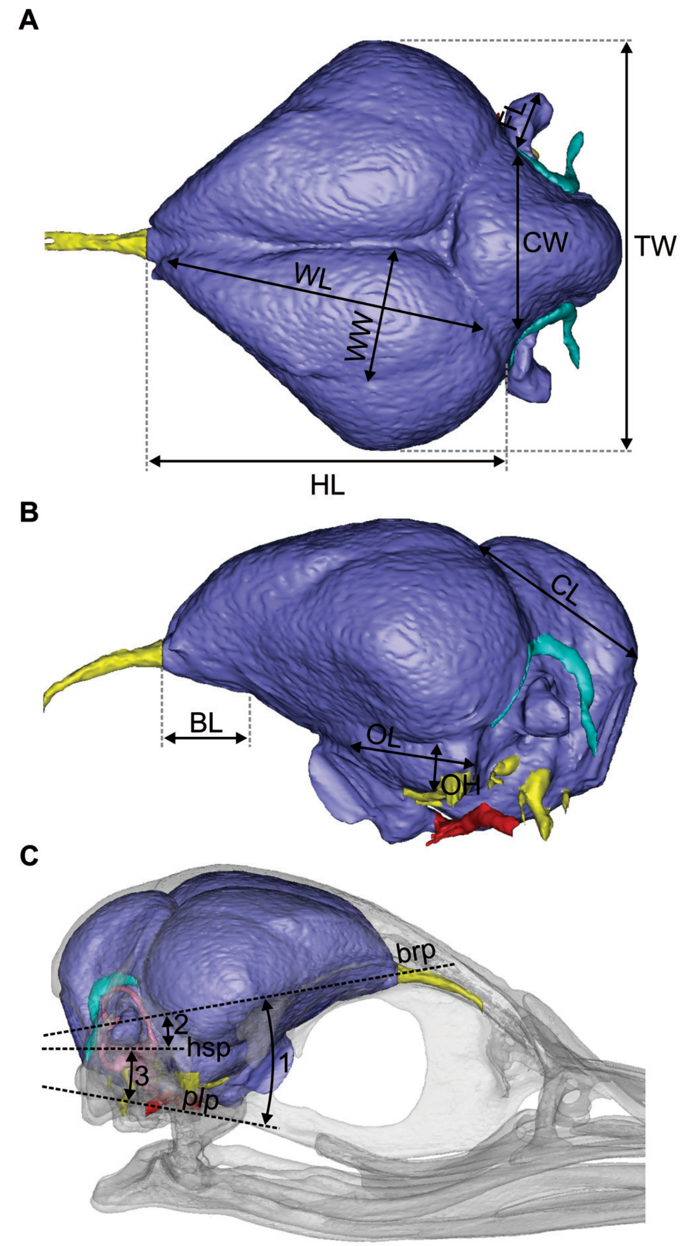

FIGURE 4. Endocast of *Aptenodytes forsteri* illustrating the measurements taken. **A**, endocast in dorsal view; **B**, endocast in lateral view; **C**, endocast in skull showing angles measured. **Abbreviations: BL**, olfactory bulb length; **brp**, brain plane; **CL**, maximum cerebellum length; **CW**, cerebellum width; **FL**, floccular lobe length; **HL**, telencephalic hemisphere length; **hsp**, horizontal semicircular canal plane; **OH**, optic lobe maximum width; **OL**, optic lobe maximum length; **plp**, lamina parasphenoidalis plane; **TW**, telencephalic width; **WL**, wulst maximum length; **WW**, wulst maximum width.

more heart-like shape. Viewed laterally, the telencephalon of Antarctic Fossil 1 in particular has a lower profile than in extant penguins, in part because the eminentia sagittalis (wulst) is less dorsally projected than in extant penguins. In each of the Antarctic fossil endocasts, the fissura interhemispherica is deep. As in *Paraptenodytes*, the fissura interhemispherica is wide compared with that in extant penguins.

A clearly defined eminentia sagittalis is present in all three Antarctic fossil endocasts, but this structure is markedly less prominently projected and less rostrocaudally extensive (Table 1) than in extant penguins and *Paraptenodytes*. It is also displaced further rostral to the cerebellum on the surface of the telencephalon, falling within the Type A morphology of Stingelin (1957) (Figs. 2, 5, 7). In extant penguins, *Paraptenodytes*, and most Procellariiformes, the eminentia sagittalis is more projected and extends all the way to the level of the cerebellum in dorsal view.

The bulbus olfactorius is completely preserved only in Antarctic Fossil 1. It is simple (one-lobe type of Cobb, 1959) and situated directly rostral to the cerebral hemispheres. It is larger and longer than in extant penguins and terminates rostrally in paired olfactory nerve bundles (cranial nerve [CN] I). Antarctic Fossil 1 appears to have longer olfactory nerves than *Paraptenodytes*, indicating that the conchae nasalis were displaced farther from the braincase. These nerves are enclosed by bony canals in all Sphenisciformes (rather than in sulci as noted by Bertelli et al., 2006, for *Paraptenodytes*), similar to the albatross *Phoebastria* and the petrel *Macronectes* (Procellariiformes) but contrary to seagulls, terns, sheathbills, and skuas (Charadriiformes).

Diencephalon—The course of CN II (nervus opticus) can be identified at its exit via the rostral wall of the braincase. Hall et al. (2009) described four types of optic nerve foramina, but only Type 1 (a discrete optic foramen in each orbit) appears to reflect the approximate size of the nerve itself. Extant penguins and *Paraptenodytes* all show a single, wide foramen (Type 3), although there is some minor variation in shape (Ksepka et al., 2012b). Unfortunately, Antarctic Fossil 1 is broken in this area, making it impossible to confirm the foramen type, although it is clear at least that a foramen was present (i.e., the conformation is not Type 4: no discreet optic foramen, posterior orbit not ossified). As typical for birds, no indication of the glandula pinealis is visible in any of the endocasts. The hypophysis (glandula pituitaria) is larger and relatively more elongated in the Antarctic fossil than in extant penguins.

Mesencephalon—One of the pronounced features of Antarctic Fossils 1 and 2 is that the tectum opticum is caudally displaced relative to the position seen in extant penguins (not preserved in Antarctic Fossil 3). Thus, the tectum opticum is partially exposed in dorsal view in these specimens, whereas in extant penguins and most other birds, this structure tends to be obscured by the telencephalon in dorsal view (Walsh and Milner, 2011a; Smith and Clarke, 2012). Gaviiformes (loons) and Phalacrocoracidae (cormorants) show a condition similar to Antarctic Fossils 1 and 2 (Ksepka et al., 2012b:fig. 3; Kawabe et al., 2014:fig. 4). It is more difficult to compare with Plotopteridae because the known skulls have all suffered either the loss of the relevant region or crushing deformation. In the Hatatsu and Touno specimens, the tectum opticum appears to be placed caudally as in Antarctic Fossil 1 but is not readily identifiable in dorsal view (Kawabe et al., 2014:fig.12). The shape of the tectum opticum in the Antarctic fossils is otherwise similar to the crown penguin species compared here, with the exception of *Aptenodytes*, which shows a more elongate tectum opticum. On the ventral surface of the tectum opticum, the course of CN V (nervus trigeminus) can be observed as it exits the brain through its two main branches (V_1 and $V_{2–3}$). The exit of CN III (nervus oculomotorius) can be identified in Antarctic Fossil 1, but that of CN IV (nervus trochlearis) could not be identified in any of the fossils.

Metencephalon—In its general aspect, the cerebellum of Antarctic Fossil 1 differs strongly from that of extant penguins in having a relatively blunt caudal margin and a width greater than that of the medulla oblongata. This condition is not possible to corroborate in Antarctic Fossils 2 and 3. In extant penguins and *Paraptenodytes*, the cerebellum sharply tapers at its caudal margin and is narrower than the medulla oblongata. The boundary

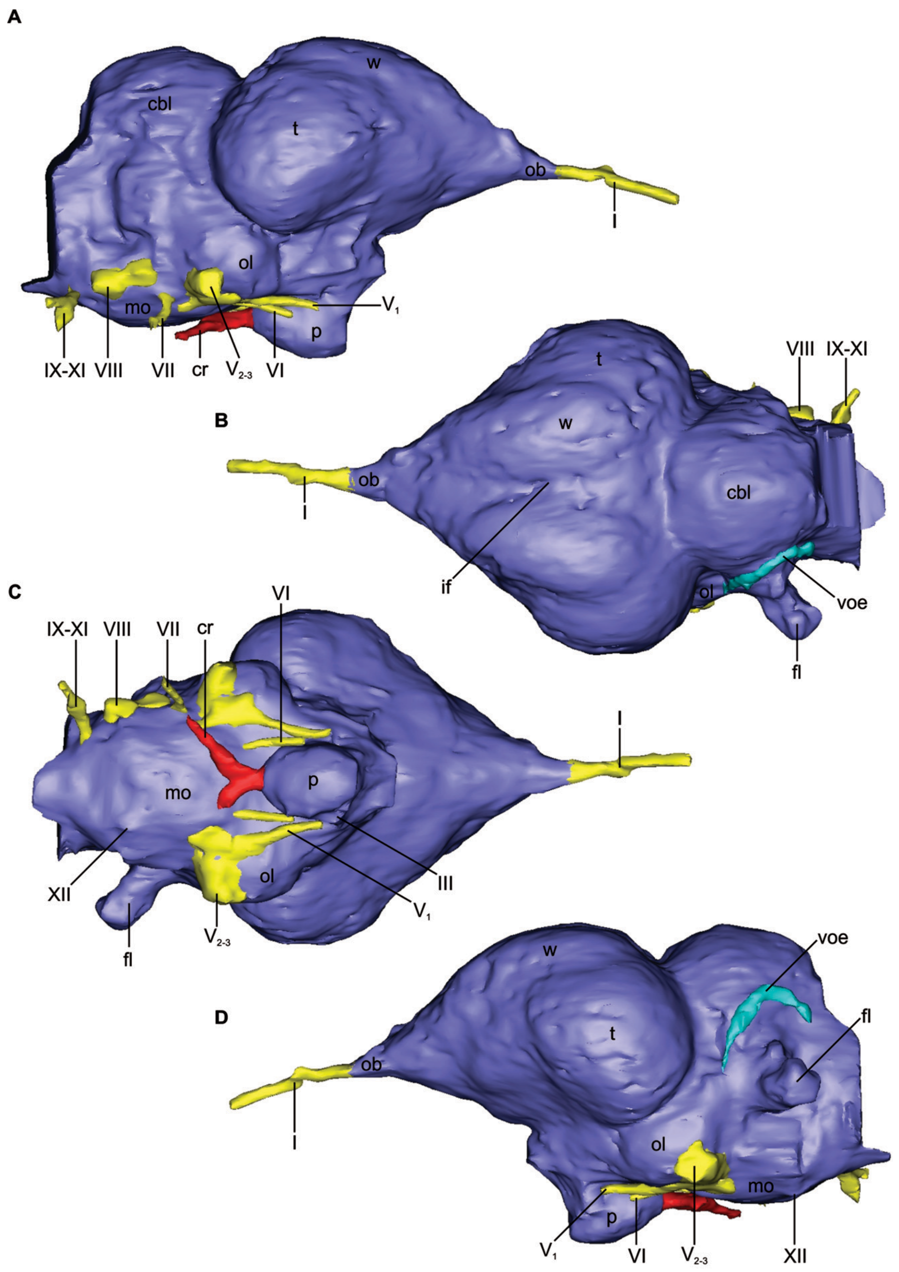

FIGURE 5. Three-dimensional reconstruction of the Antarctic Fossil 1 (MLP 12-I-20-1) endocast, generated from CT scans in **A**, right lateral, **B**, dorsal, **C**, ventral, and **D**, left lateral views. **Abbreviations: cbl**, cerebellum; **cr**, carotid ramus; **fl**, cerebellar flocculus; **if**, fissura interhemispherica; **mo**, medulla oblongata; **ob**, bulbus olfactorius (olfactory bulb); **ol**, tectum opticum (optic lobe); **p**, pituitary gland (hypophysis); **t**, telencephalon; **voe**, vena occipitalis externa; **w**, wulst (sagittal eminence); **I–XII**, cranial nerves I–XII.

between telencephalon and cerebellum is sharply defined on the dorsal surface in all three Antarctic fossils. In dorsal view, this boundary has a gentle, wide 'U'-shape as in Alcinae (Smith and Clarke, 2012:character 8), rather than the sharper 'V'-shape seen in extant penguins and the Ainoshima plotopterid (Kawabe et al., 2014). The cerebellum is proportionally longer compared with the telencephalon in the Antarctic fossils than in extant penguins (Tables 1, 3) and most similar to *Sula variegata* (Peruvian booby) among taxa surveyed. Although useful for describing shape, this index does not provide a precise measure of the relative volume of these structures, because the internal boundaries of the telencephalon and cerebellum cannot be determined from the endocast.

No trace of a sinus occipitalis can be detected in the three Antarctic fossils. This structure is typically absent or weakly developed in penguin endocasts, including that of *Paraptenodytes antarcticus* (Ksepka et al., 2012b) as well as Procellariiformes and Alcidae (Smith and Clarke, 2012). In contrast, it is strongly marked in extant Suliformes and Plotopteridae (Kawabe et al., 2014). Cerebellar folds are likewise not present on any of the fossil endocasts. Because extant penguins, like all other extant birds, possess cerebellar folds (dissected specimen; see also Iwaniuk and Hurd, 2005), this feature of the endocast does not indicate a smooth cerebellar surface but instead suggests that the sinusoidal/meningial tissue was thick and wide enough to prevent the cerebellar surface from contacting the endocranium.

In dorsal view, the floccular lobes are exposed and are strongly caudolaterally expanded as in extant penguins. There is no indication of a 'fenestra' in the flocculus endocast (see Walsh et al., 2013). Amongst penguins, the orientation of the flocculus is variable: in the Antarctic fossils and *Aptenodytes*, the flocculus is essentially caudolaterally directed, whereas in *Paraptenodytes,*

TABLE 2. Orientation of the brain in Sphenisciformes and other marine birds.

Taxa	1	2	3
MLP 12-I-20-1	21	9	11 ca
MLP 12-I-20-2	—	18	—
MLP 84-II-1-10	—	—	4 ca
Paraptenodytes antarcticus	20	8	11 ca
Aptenodytes forsteri	10	14	8 cr
Eudyptula minor	17	17	5 cr
Spheniscus magellanicus	21	17	5 cr
Spheniscus demersus	18	28	8 cr
Pygoscelis adeliae	17	18	10 ca
Pygoscelis antarctica	17.5	15	7 ca
Phoebastria immutabilis	26	26	—

Abbreviations: **1**, angle between brain plane (**brp**) and plane of lamina parasphenoidalis (**plp**); **2**, angle between brp and horizontal semicircular canal (**hsp**); **3**, angle between hsp and plp; **ca**, caudally directed; **cr**, cranially directed. Angles in degrees. See Figure 4 for further explanation.

Spheniscus, and *Eudyptula*, there is a slight ventral deflection as well. Exposure of the flocculus in all three Antarctic fossils is most similar to that in *Spheniscus* and *Paraptenodytes*, with a wide separation between these structures and the telencephalon. In contrast, the telencephalon nearly covers the flocculus in dorsal view in all other extant penguins sampled, partially obscuring the lobes. The location of the flocculus remains largely the same in all of these taxa, and the difference in exposure seems to be due in part to the greater depth and development of the fossae temporales in the fossil species and *Spheniscus*, which is undoubtedly correlated with the development of the external adductor muscle (Ksepka et al., 2012b). Interestingly, in many Procellariiformes with weakly developed fossae, the telencephalon does not approach the flocculus, which indicates that the 'heart-like' shape of the penguin telencephalon also plays a role in the conformation seen in most extant penguins.

Myelencephalon—There is no median ridge present on the floor of the fossa medulla oblongata in any of the sampled penguins or in *Gavia*. A ridge is present in the Procellariiformes *Phoebastria* and *Macronectes*, which is indicative of the division of the medulla oblongata by the fissura mediana. In Antarctic Fossil 1, the course of CN VI is identifiable where it exits the brain from the rostral portion of the medulla, medially to CN V. Cranial nerves IX–XII exit the brain from the caudoventral surface of the medulla, as typical of birds (Pearson, 1972). The exits for CNs IX–XI can be reconstructed, showing that these osseous nerve canals were quite wide. The canal for CN XII could not be reconstructed, although the exit for this nerve could be identified (Fig. 5).

Endosseous Labyrinth—Features of the inner ear are most clearly observable in Antarctic Fossil 1 and Antarctic Fossil 3. The semicircular canals are grossly enlarged both in terms of the diameter of the individual canals (Table 4) and the dimensions of the labyrinth as a whole. The semicircular canals in the Antarctic fossils are also stouter (wider in diameter relative to overall labyrinth size) than in *Paraptenodytes* and the sampled extant penguins (Fig. 8). The total volume of the endosseous labyrinth relative to brain volume is thus considerably higher than in extant penguins. Although skewed, the canals are aligned in approximately orthogonal planes in three-dimensional space.

The anterior and posterior semicircular canals are subcircular in cross-section (Table 4). Strongly elliptical canals are observed in a variety of birds, although their functional meaning is still obscure. In Pan-Alcidae, all three canals are compressed (Smith and Clarke, 2012). In comparison with sampled taxa, the anterior semicircular canal in the fossil penguins is conspicuously shorter relative to total labyrinth size and enters the ampulla on its rostral face. When viewed caudally, the origin of the horizontal canal lies lateral to the crus communis in all three of the Antarctic fossils. In crown penguins and flying birds, the origin is located medial to the crus communis. An intermediate situation is seen in *Paraptenodytes*. The crus communis is short and stout. The horizontal canal forms an angle of 11° to the lamina parasphenoidalis, which is higher than in extant penguins, markedly higher than in Antarctic Fossil 2, but equal to *Paraptenodytes* (Table 2). The cochlea is long, relatively straight and stout, and not expanded distally.

Vasculature—Available data on the blood vessels supplying the brain are limited for penguins. Watson (1883) described dissections for *Aptenodytes, Eudyptes*, and *Pygoscelis*, and Frost et al. (1975) described the arrangement in *Spheniscus demersus*. A survey of the pathways of the arteria carotis at the base of the skull based on CT data was also provided by Ksepka et al. (2012b). In Antarctic Fossil 1, two bony carotid canals traverse the base of the skull. In most birds, these canals provide passage for the arteria carotis cerebralis and generally also conduct the vena carotis cerebralis. In some birds, this vein is housed in an independent bony tube adjacent to the carotid canal itself (Baumel and Gerchman, 1968). No separate canal was observed in any of the penguins examined; therefore, we assume that the carotid vein was also housed in the carotid canal (Fig. 5).

TABLE 3. Volumes and indices recovered in this study.

Taxa	VOL	EQ	Index 1	Index 2	OR
MLP 12-I-20-1	27,340	—	1.07	1.49	35.0
Paraptenodytes antarcticus	28,781*	1.01	1.29	1.54	24.5
Pygoscelis antarctica	16,141	0.99	1.40	1.65	19.1
Pygoscelis adeliae	15,183**	0.87	1.34	1.86	19.4
Spheniscus demersus	—	—	1.22	1.64	12.8
Spheniscus magellanicus	13,853	0.85	1.25	1.61	16.4
Aptenodytes patagonicus	26,204**	0.90	0.94	2.26	13.6
Eudyptula minor	—	—	1.14	1.87	19.1
Gavia immer	9935**	0.54	1.35	1.28	32.1
Phoebastria immutabilis	14,383**	1.05	1.32	1.90	19.0
Macronectes giganteus	15,953	1.13	1.36	1.52	26.4
Sula variegata	9833	1.19	1.37	1.49	25.2
Phalacrocorax olivaceus	11,203	1.34	1.22	1.60	—
Podiceps major	3700	0.40	1.25	1.58	—

Abbreviations: **EQ**, encephalization quotients; **Index 1**, TW/HL; **Index 2**, HL/CL; **OR**, olfactory ratio = (BL/HL) × 100; **VOL**, endocranial volume calculated using Materialise Mimics. Volumes in mm^3. See Figure 4 for further explanation.
*Ksepka et al. (2012b) reported a value of 29,355 mm^3 using different software for measurements (Avizo).
**Volumes from Ksepka et al. (2012b).

In Antarctic Fossil 1, the external opening of the ostium canalis carotici (exiting the braincase) is located caudomedial to the lower edge of the meatus acusticus externus and the internally opening canal is caudolateral to the sella. In birds, the left and right internal carotid arteries (derived from the common carotid artery; Aslan et al., 2006) approach one another immediately caudal to the optic chiasm to communicate through the anastomosis intercarotidea, which lies in the fossa hypophysealis at the base of the skull, directly caudal to the hypophysis. A low angle of convergence (apex located at the entrance of the carotid in the hypophysis) is exhibited by Antarctic Fossil 1 (62°) compared with *Paraptenodytes* (94°) and extant penguins (113°–123°) (Table 2).

Baumel and Gerchman (1968) and Pettit et al. (1981) describe different morphologies of the anastomosis as 'I,' 'H,' 'X,' and 'XH.' In Antarctic Fossil 1, the carotids merge into a short midline anastomosis (Fig. 5), which is typical of an 'X'-type anastomosis. An 'X'-type anastomosis is present in most penguins (including *Spheniscus demersus, Spheniscus humboldti*, and *Eudyptula minor* in our data set), although we note that the midline anastomosis is longer in Antarctic Fossil 1 than in extant penguins and *Paraptenodytes* and most closely resembles *Gavia immer* in this regard. *Pygoscelis adeliae, Pygoscelis antarctica*, and *Aptenodytes forsteri* show the H-type (with a lengthy transverse anastomosis).

The rete opthalmicum (rete mirabile ophthalmica) is an extracranial arterial-venous network located near the eye, between the caudoventral border of the orbit and the meatus acusticus externus (Kilgore et al., 1979). This structure functions as a site of counter-current heat exchange and is responsible for maintaining the body-to-brain temperature difference (Kilgore et al., 1979; Pettit et al., 1981). The rete opthalmicum is placed in a shallow depression between the processus oticus of the quadrate and the orbital ridge. In Antarctic Fossil 1, the exit of the vessels of the rete opthalmicum is indicated dorsal to the foramen nervus maxillomandibularis (i.e., the exit of the trigeminal nerve) (Figs. 2, 6).

The arteria occipitalis of the arteria ophthalmica externa emerges from a larger opening in the occiput, close to the crista nuchalis (Fig. 2) in Antarctic Fossil 1. Lateral to the foramen magnum, the opening of the vena occipitalis externa is identifiable (Fig. 2). The short length of this vein that could be reconstructed is curved and stout, lying dorsal to the anterior semicircular canal (Fig. 5). The arc described is craniocaudally elongate in Antarctic Fossil 1 and *Paraptenodytes*, in contrast to extant penguins, where it is short.

Cranial Pneumaticity—A complex series of pneumatic sinuses permeate the avian braincase, and reduction of these sinuses characterizes many diving birds. Three main sinus system groups are present in most birds: (1) the tympanic or middle ear system, (2) the median pharyngeal system, and (3) the subcondylar system (Witmer, 1990; Witmer and Ridgely, 2009). The general pattern of the tympanic recesses in Antarctic Fossil 1 is similar to that present in extant penguins, with the exception of the retention of the interaural pathway (the contralateral connection between the paired rostral tympanic recesses). Although the surrounding area is broken, the interaural pathway is recognizable in Antarctic Fossil 1 (Fig. 6), where it can be observed to be

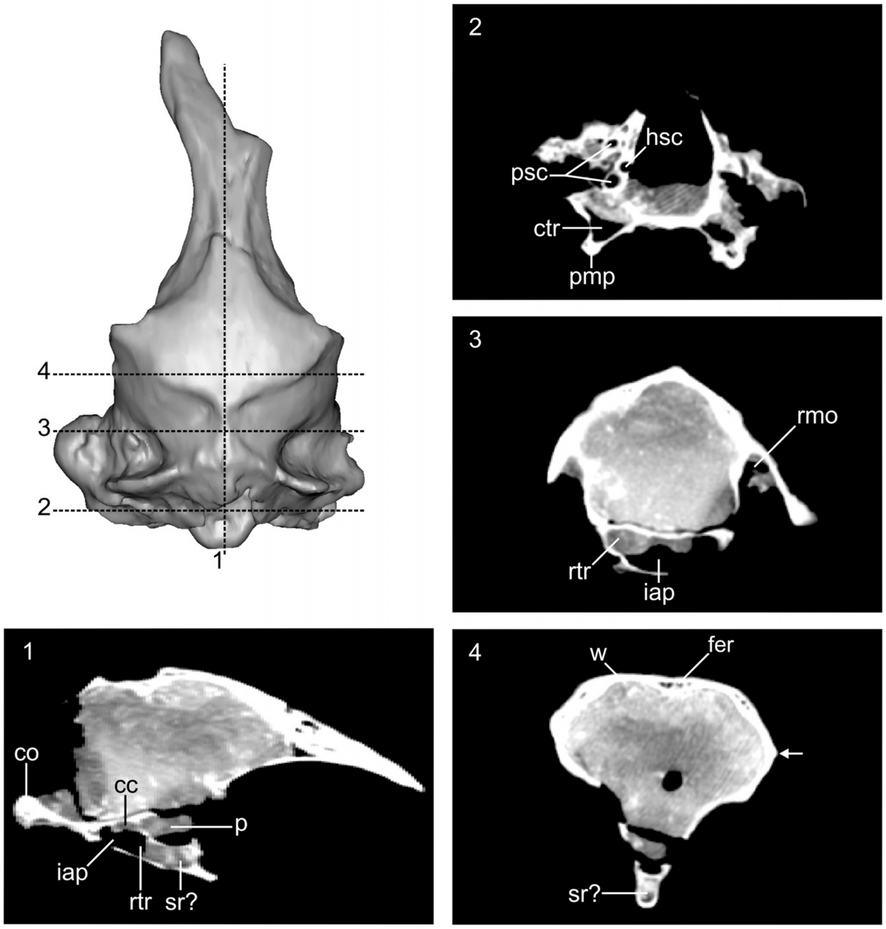

FIGURE 6. Tomograms of the skull of Antarctic Fossil 1 (MLP 12-I-20-1) in (**1**) sagittal and (**2–4**) coronal planes. **Abbreviations: cc**, canalis caroticus cranialis; **co**, condylus occipitalis; **ctr**, caudal tympanic recess; **fer**, frontoethmoidal recess; **hsc**, horizontal semicircular canal; **iap**, interaural pathway; **p**, pituitary gland (hypophysis); **pmp**, processus medialis parasphenoidalis; **psc**, posterior semicircular canal; **rmo**, rete mirabilis ophthalmica exit; **rtr**, rostral tympanic recess; **sr?**, subsellar recess; **w**, wulst (sagittal eminence). The arrow in (**4**) points at the site where the wall thickness was measured. Notice the wall thickness.

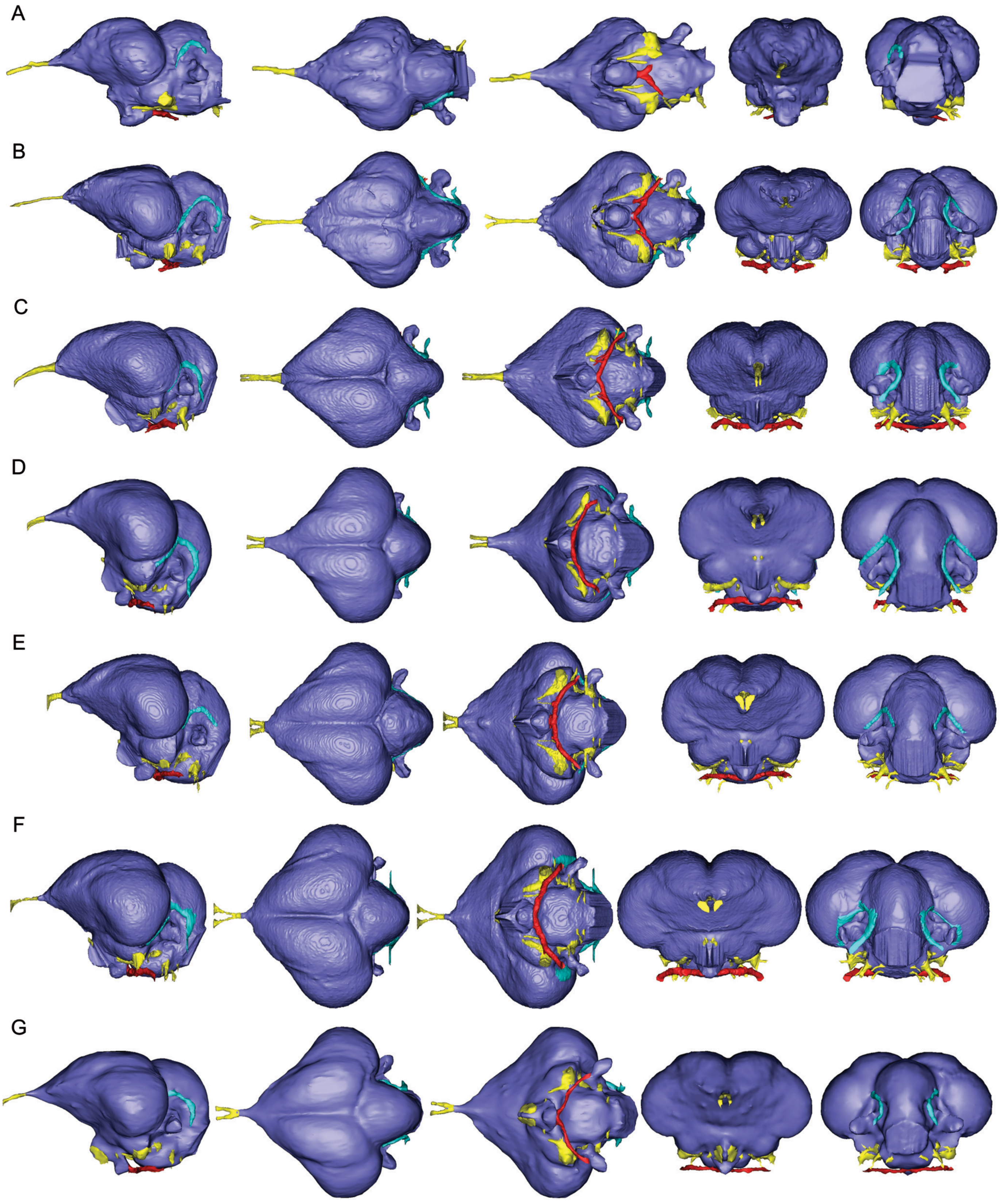

FIGURE 7. Cranial endocasts of Sphenisciformes reconstructed from CT scans in left lateral, dorsal, ventral, rostral, and caudal views. **A**, Antarctic Fossil 1 MLP 12-I-10-1; **B**, *Paraptenodytes antarcticus* AMNH3338; **C**, *Aptenodytes forsteri* UF22281; **D**, *Eudyptula minor* UF31924; **E**, *Spheniscus demersus* UF21341; **F**, *Pygoscelis adeliae* UF36633; **G**, *Pygoscelis antarctica* MLP 437. Not to scale.

TABLE 4. Semicircular canal measurements (in mm) of extant and fossil penguins.

	asc		hsc		psc	
Taxa	dv	lm	dv	lm	rc	lm
MLP 12-I-20-1	1.81	2.15	1.91	2.13	1.43	1.11
MLP 12-I-20-2	1.88	1.54	1.88	2.19	2.17	1.85
Paraptenodytes antarcticus	1.32	1.47	1.41	1.60	1.42	1.49
Aptenodytes forsteri	1.07	1.34	1.24	1.25	1.15	1.2
Eudyptula minor	0.75	0.9	0.70	1.11	0.59	0.77
Pygoscelis adeliae	0.27	0.44	0.25	0.41	0.14	0.27
Spheniscus demersus	1.27	1.10	1.09	1.58	0.90	1.30

Abbreviations: **asc**, anterior semicircular canal; **dv**, dorsoventral diameter; **hsc**, horizontal semicircular canal; **lm**, lateromedial diameter; **psc**, posterior semicircular canal; **rc**, rostral-caudal diameter.

wider and more caudally extensive than in *Paraptenodytes*. As noted by Ksepka et al. (2012b), the interaural pathway is absent in crown penguins, a derived feature that appears to be unique among extant birds (condition unknown in Plotopteridae). As in living penguins, the caudal tympanic recesses of the fossil are large and simple (without inner cancellae). The region of the dorsal tympanic recess is damaged, and its level of development cannot be established. The rostral recess is wide and extends only slightly rostrally (Fig. 6).

In extant penguins, a small foramen can be observed in the rostrum parasphenoidale, adjacent to the tuba auditiva communis. This foramen corresponds to the opening of the basisphenoidal sinus (which excavates the bone, producing the basisphenoidal recess). In Antarctic Fossil 1 and in *Paraptenodytes*, no pneumatic foramina could be identified in the corresponding area. Rostral to this structure, a small subsellar recess can be observed in extant penguins (*Spheniscus demersus*), which in some cases has a communication with the frontoethmoidal recess. Due to the fragmentary state of the Antarctic fossils, neither of these recesses could be identified.

Subcondylar recesses are poorly developed in extant penguins. The medial subcondylar recess may extend caudally through the condylus occipitalis, but its degree varies among extant species. The lateral subcondylar recesses, although small, are more developed than the medial subcondylar recess in extant penguins. They border the processus medialis parasphenoidalis but do not pneumatize this structure, which is instead pneumatized by the sinus paratympanicus caudalis. Lateral subcondylar recesses are not preserved in Antarctic Fossil 1. However, a well-developed medial subcondylar recess is present.

In Antarctic Fossil 1, the base of the processus postorbitalis is partially pneumatized, a condition also observed in *Aptenodytes*, but not in other extant penguins (Ksepka et al., 2012b). The frontoethmoidal recesses have a similar degree of development to those in extant penguins, but more than in *Paraptenodytes*.

DISCUSSION

As the oldest and most basal penguin endocasts sampled, the Antarctic fossils likely retain primitive morphologies and thus offer a glimpse into earlier stages of brain evolution in Sphenisciformes. However, care must be taken to consider outgroup taxa before inferring whether or not a character state is primitive. For example, the overall orthoencephalic shapes of Antarctic endocasts are quite similar to *Gavia immer*, but both Procellariiformes and extant penguins show an airencephalic shape. Because Procellariiformes are widely supported as the extant sister taxon to penguins, it becomes unclear whether an orthoencephalic shape is primitive for penguins or instead represents a derived feature of the Antarctic taxa.

At the most general level, relative brain volume can be compared between penguins and volant birds. It has been debated whether flightless birds have smaller brains, and whether penguins should be considered to be 'flightless' in the same manner as terrestrial flightless birds is also open to debate. Bennett and Harvey (1985) reported that flightless birds had smaller brains than volant birds relative to overall body size. Corfield et al. (2008) found that Palaeognathae (with the exception of *Apteryx*) have smaller relative brain sizes than Neognathae. However, because both flightless ratites and volant tinamous exhibit smaller brain sizes, this may represent a primitive trait for Palaeognathae rather than a feature correlated with flightlessness. Iwaniuk et al. (2004a) made comparisons at a finer level, comparing volant and flightless taxa within individual clades, or in the case of the exclusively flightless penguins, between Sphenisciformes and their sister clade Procellariiformes. Iwaniuk et al. (2004a, b) found that smaller penguins (e.g., *Eudyptula minor*) have relatively larger brains than Procellariiformes, whereas larger penguins (e.g., *Aptenodytes patagonicus* and *Aptenodytes forsteri*) have relatively smaller brains. Disentangling the impact of flightlessness and diving is difficult, however, because the wing-propelled diving of penguins is arguably more similar to aerial flight than to terrestrial running. Furthermore, penguins have a higher body density than typical birds due to their dense bone, thick fat layers, and reduced skeletal pneumaticity (Meister, 1962; Cerda et al., 2014), which may bias indices scaled to mass.

One of the most interesting trends observed along the transition from stem to crown penguins is the increasing development of the eminentia sagittalis. The Antarctic stem penguins possess a poorly dorsally projected eminentia sagittalis, which is also restricted in its caudal extent along the surface of the telencephalon compared with the caudally expanded eminentia of extant penguins. This pattern is consistent with a continued expansion of the eminentia sagittalis over the course of penguin evolution, long after the initial loss of aerial flight. At a broader scale, the craniocaudal expansion of the eminentia sagittalis to cover nearly the entire length of the telencephon appears to have evolved independently in multiple avian lineages (Milner and Walsh, 2009). A conformation in which the eminentia sagittalis occupies a rostral position is shared by all currently sampled Paleogene birds (e.g., Milner and Walsh, 2009; Walsh and Milner, 2011b), in agreement with the hypothesis that this represents the ancestral type of eminentia sagittalis for Neornithes (Milner and Walsh, 2009).

Correlations between diving and brain morphology remain incompletely understood. One of the distinct features shared by all wing-propelled diving birds (penguins, alcids, and plotopterids) is the lack of cerebellar fold impressions on the endocast (Ksepka et al., 2012b; Smith and Clarke, 2012; Kawabe et al., 2014). Interestingly, cerebellar folds are present in the endocasts of most Charadriiformes but are lacking in those of Pan-Alcidae, indicating that their loss occurred within the same phylogenetic interval as the gain of wing-propelled diving (Smith and Clarke, 2012). It is tempting to speculate that this provides evidence for the proposal by Elzanowski and Galton (1991) that adaptations

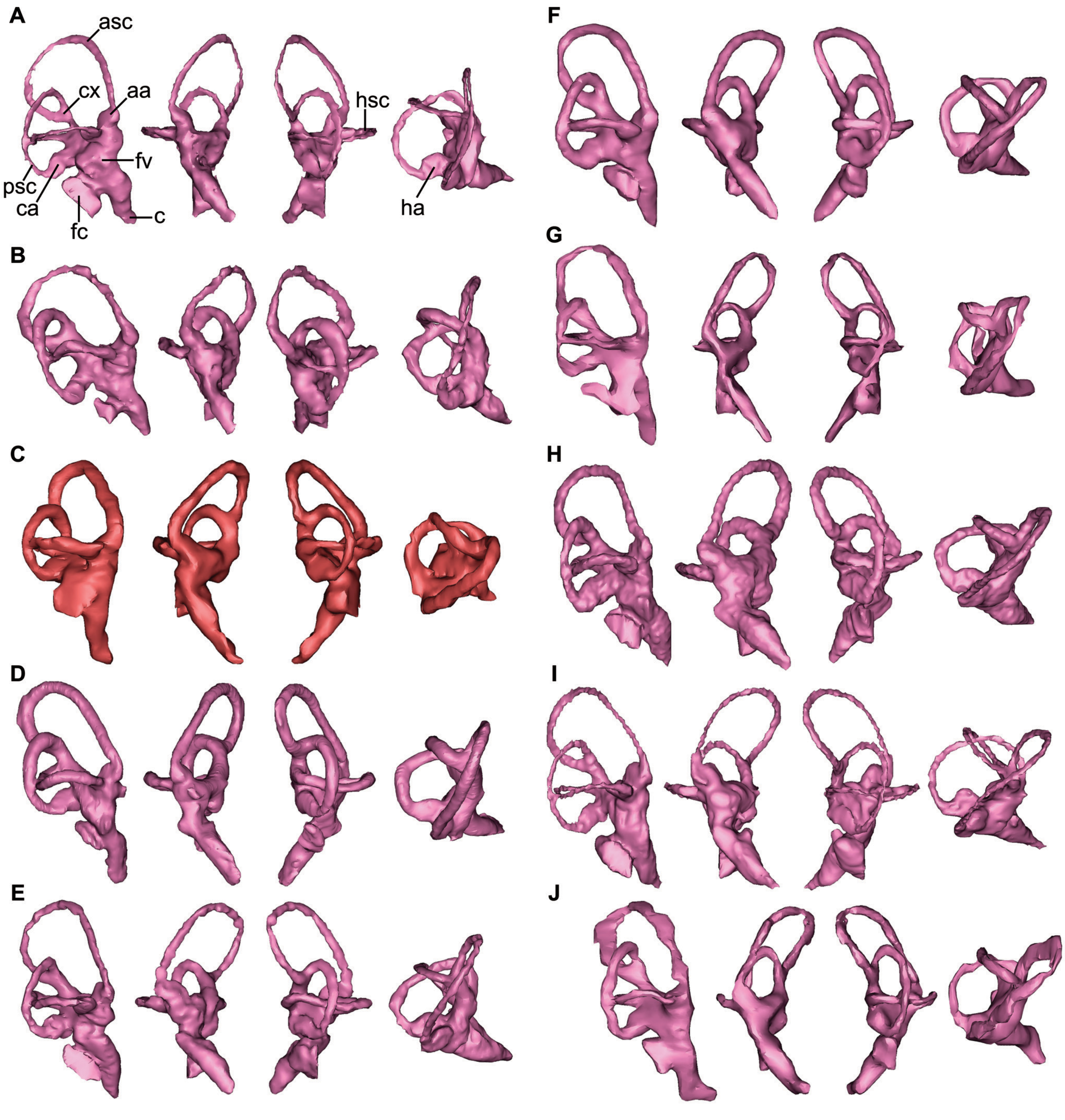

FIGURE 8. Endocast of the endosseous labyrinth of Sphenisciformes and outgroups. **A**, *Gavia immer* UF13300; **B**, *Phoebastria immutabilis* UF313780; **C**, Antarctic Fossil 1 (MLP 12-I-20-1); **D**, *Paraptenodytes antarcticus* AMNH3338; **E**, *Eudyptula minor* UF31924; **F**, *Aptenodytes forsteri* UF22281; **G**, *Spheniscus magellanicus* MLP 789; **H**, *Spheniscus demersus* UF21341; **I**, *Pygoscelis adeliae* UF36633; **J**, *Pygoscelis antarctica* MLP 437. **Abbreviations: aa**, anterior ampulla; **asc**, anterior semicircular canal; **c**, cochlea; **ca**, caudal ampulla; **cx**, crus communis; **fc**, fenestra cochlearis; **fv**, fenestra vestibuli; **ha**, horizontal ampulla; **hsc**, horizontal semicircular canal; **psc**, posterior semicircular canal. Not to scale.

to diving result in a thicker subarachnoid space and hence no impressions of the cerebellar folds in endocasts of habitually diving birds. This relationship becomes less clear when it is noted than some non-diving birds lack cerebellar fold impressions, and that the diving petrel *Pelecanoides urinatrix* shows strong cerebellar fold impressions (D.T.K., pers. observ.). The new fossils studied here at least show that this feature extends deep into the stem part of the penguin evolutionary tree, but more work is needed to determine if it is correlated with diving or some other life history variable.

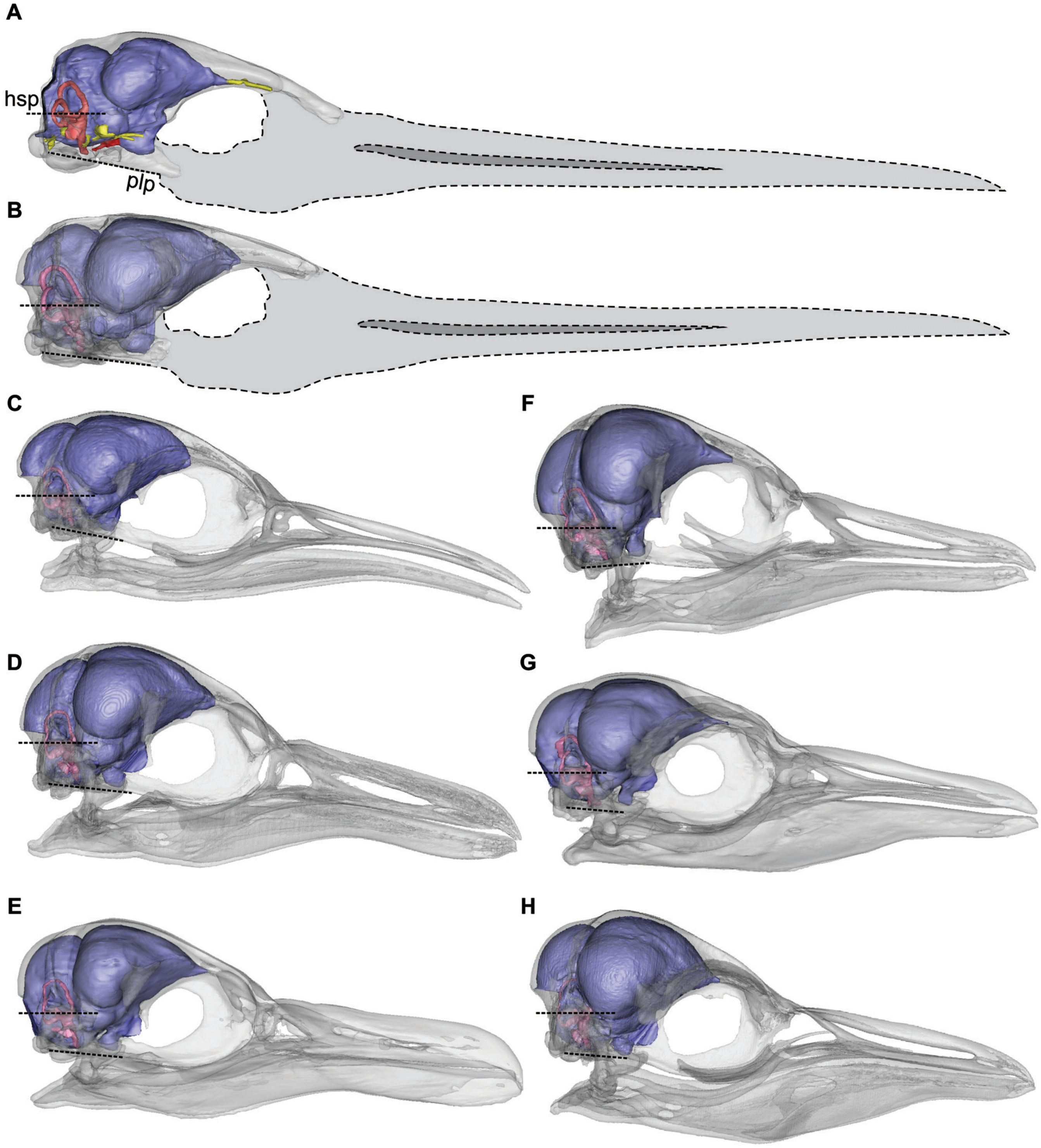

FIGURE 9. ‘Alert’ head postures, with skull oriented so that the horizontal semicircular canal is parallel to the ground. **A**, Antarctic Fossil 1 (MLP 12-I-20-1); **B**, *Paraptenodytes antarcticus* AMNH3338; **C**, *Aptenodytes forsteri* UF22281; **D**, *Spheniscus demersus* UF21341; **E**, *Spheniscus magellanicus* MLP 789; **F**, *Eudyptula minor* UF31924; **G**, *Pygoscelis antarctica* MLP 437; **H**, *Pygoscelis adeliae* UF36633. **Abbreviations: hsp**, horizontal semicircular canal plane; **plp**, lamina parasphenoidalis plane. Gray areas with dashed lines represent reconstructed inferred areas. Not to scale.

Olfactory capabilities may also be expected to vary between diving and non-diving taxa. It has been suggested that the relative size of the bulbus olfactorius is related to the olfactory acuity in living birds (Bang and Cobb, 1968) and thus may provide a means to estimate olfactory sensitivity in extinct taxa (Zelenitsky et al., 2009, 2011). Some marine birds such as Procellariiformes rely heavily on olfactory senses (Nevitt, 2008). Indeed, Procellariiformes have some of the largest relative olfactory

TABLE 5. Mean hearing (Hz) and hearing range (Hz) estimations recovered in this study.

				Range			
Taxa	CL	BL	SCL	Lower	Upper	Overall range	Mean Hearing
MLP 12-I-20-1	13.07	26.44	0.494	493.9	5541.1	5107.2	2987.5
MLP 12-I-20-2	12.55	—	—	—	—	—	—
Paraptenodytes antarcticus	11.09	29.2	0.379	404.0	4807	4403.0	2605.5
Aptenodytes forsteri	12.83	31.2	0.411	413.2	5032.4	4619.2	2722.8
Eudyptula minor	6.99	15.3	0.456	425.0	5323.0	4898.0	2874.0
Spheniscus magellanicus	8.25	18.1	0.453	424.2	5303.0	4878.8	2863.6
*Spheniscus demersus**	6.56	24.2	0.270*	600.0	4000.0	3400.0	2300.0
Pygoscelis adeliae	5.67	23.7	0.239	352.1	3535.0	3182.9	1943.6
Pygoscelis antarctica	8.33	18.3	0.452	424.0	5299.3	4875.3	2861.7
Phoebastria immutabilis	7.32	25.4	0.288	373.18	4049.1	3676.0	2211.1

Abbreviations: **BL**, basicranial length; **CL**, cochlear length; **SCL**, standardized ML (ML/BL). Linear measurements in mm.
*From Walsh et al. (2009).

volumes seen in birds. Sphenisciformes possess relatively small olfactory bulbs compared with most other aquatic birds (e.g., Procellariiformes, Gaviiformes, Podicipediformes, Phoenicopteridae, Ardeidae, Gruiformes) (Bang and Cobb, 1968; Zelenitsky et al., 2011). Bang and Cobb's (1968) simple method for obtaining olfactory ratios from fossil endocasts (greatest linear dimensions of the olfactory bulb region and the cerebral hemisphere region; Table 1) is based on the observation that the length of the olfactory bulb shows a positive relationship to the volume of the olfactory bulb and hence, presumably, to olfactory acuity (Cobb, 1959; Hieronymus, 2008).

Applying this method indicates that Antarctic Fossil 1 had the greatest olfactory ratio of any sampled penguin: the olfactory ratio (~35) was approximately three times that of extant penguins (e.g., *Spheniscus demersus*). This value overlaps with the high values seen in Procellariiformes (Bang and Cobb, 1968). However, it should be noted that Procellariiformes also show much greater mediolateral expansion of the bulbus olfactorius, so the index may overestimate true volume for penguins relative to Procellariiformes. Still, the new data suggest that higher levels of olfactory sensitivity may have characterized basal penguins compared with extant taxa.

Cobb (1959) found a weak association between large olfactory-bulb size and aquatic habits: those species with larger olfactory bulbs tended to be ground-nesting, aquatic, carnivorous/piscivorous in diet, and colonial in breeding habits. All of these traits are generally present in extant penguins, with the exception that a few species (e.g., *Megadyptes antipodes*) breed in isolation. Experimentally it has been found that marine birds such as Procellariiformes use dimethyl sulfide (DMS) as an olfactory cue to find highly productive sites in visually homogeneous ocean environments (Nevitt, 2000). The relative importance of olfaction on land and at sea has not yet been quantified in penguins. However, it has recently been shown that penguins can detect DMS at sea in order to locate prey (Cunningham et al., 2008; Wright et al., 2011; Amo et al., 2013). Zelenitsky et al. (2012) concluded that both the high olfactory values seen in Procellariiforms (the extant sister taxon of Sphenisciformes) and the low olfactory ratios seen in extant penguins represent derived conditions. Reduction of olfactory capacities in penguins parallels the reduction in other secondarily marine tetrapods such as cetaceans (McGowen et al., 2014). In this context, the olfactory ratio seen in the Antarctic fossil can be interpreted as a primitive trait, with a trend towards reduction in more crownward penguins.

One of the most prominent observed differences between the sensory organs of the Antarctic fossil penguins examined here and crown penguins is the stout morphology of the semicircular canals, which, like those of *Paraptenodytes*, show relatively expanded cross-sectional diameters. Interestingly, both extant penguins and outgroups have more slender canals, so it is likely that canal size increased during an early interval of penguin evolution and was retained for a long phylogenetic interval, followed by a reduction close to or coincident with the crown radiation. The main function of the semicircular canal system is to sense angular accelerations of the head during movement in both terrestrial and aquatic vertebrates (Hopkins, 1906; Spoor et al., 2002; Sipla, 2007). Combined with visual and somatosensorial cues, the semicircular canals help facilitate a wide range of automatic behaviors necessary for positioning the head and eyes, stabilizing posture, and coordinating body movement. Development of a large horizontal canal is associated with increased sensitivity to yaw, at least in mammals, whereas anterior and posterior canals control reflex adjustments (Cox and Jeffrey, 2010). The horizontal semicircular canals of the Antarctic fossil penguins and the surveyed extant penguins are relatively short, with the exception of the two species of *Pygoscelis* that both show a more laterally extensive canal.

Smith and Clarke (2012) noted that the semicircular canals of wing-propelled diving alcids are compressed compared with their non-diving relatives and thus may be related to increased sensitivity of the head and the body during the pursuit of prey underwater. The three semicircular canals of Antarctic Fossil 1 are almost circular. The same is seen in living penguins, with the notable exception of *Pygoscelis* (Table 4). The heights of both the crus communis and the caudal semicircular canal of the fossil are low, a trait also noted in some aquatic Charadriiformes by Smith and Clarke (2012).

Head posture is reflected in the inner ear (Duijm, 1951), and it has been suggested that the horizontal canal is aligned nearly parallel to the ground in the 'alert position' of vertebrates (Lebedkin, 1924; de Beer, 1947). This method has been used to estimate the alert head posture of extinct taxa (e.g., Witmer et al., 2003; Sereno et al., 2007), although its precision has recently been questioned (Marugán-Lobón et al., 2013). Applying this method to penguins results in a nearly horizontal head posture for Antarctic Fossil 1 and *Paraptenodytes antarcticus* (Fig. 9, Table 2), which corresponds well to typical head posture of living penguins while standing on land.

The basilar papilla of the pars cochlearis of the inner ear detects variation in frequencies of airborne pressure waves. However, hearing capabilities are also related to the tympanic pneumatic sinuses (Witmer and Ridgely, 2009). According to Walsh et al. (2009), the length of the cochlea can be used to estimate hearing capabilities in fossil taxa. Using the method proposed by the authors, we estimated that nearly all Sphenisciformes have similar mean hearing capacities (~2000–3000 Hz; Table 5). This is in agreement with sensitivity data collected from live *Spheniscus demersus* by Wever et al. (1969), which indicated a mean of ~2300 Hz. Estimated lower limits were similar across penguins, with slight differences in the upper limit (e.g., ~3500 Hz for *Pygoscelis adeliae* versus ~5500 Hz for Antarctic Fossil 1). Compared with the limited set of actual measured values available for living birds, the estimates for the fossil penguins support a generalized range of hearing, reaching neither the lower-frequency sensitivity of emus nor the high-frequency sensitivity of owls (Wever et al., 1969; Walsh et al., 2009). Although considered to be primarily visual predators, nesting penguins use auditory cues to locate mates and chicks in crowded colonies (Jouventin et al., 1999; Jouventin and Aubin, 2002). In strongly territorial animals such as Adélie penguins, it has been shown that sound is the way that parents can identify each other and their offspring with confidence (Lengagne et al., 1999). It may be noted that the extreme concentration of penguin bones supports the inference of penguin colonies for multiple species during the Eocene at Seymour Island.

Finally, the differences in skull wall thickness are difficult to interpret. Seemingly, at least some Antarctic stem penguins had substantially thicker skull roof bones than extant penguins. This contrasts with observations from limb bone microstructure, which have revealed that many Antarctic stem taxa have more open, 'spongy' cross-sections than extant penguins (Ksepka, 2007; Cerda et al., 2014). Because outgroup species exhibit thinner skull roof bones, the condition observed in this study is unlikely to be primitive for penguins. Likewise, the stem penguin *Paraptenodytes* exhibits a thinner skull roof than sampled crown penguins, ruling out a simple pattern of reduction in thickness across the stem-crown penguin transition. Fully understanding the changes in bone thickness and density that occur over the course of penguin evolution will require more extensive sampling throughout the skeleton.

CONCLUSIONS

Data from the Antarctic fossil specimens studied here reveal a mix of primitive and apomorphic features not previously documented in penguins. A number of aspects of endocast morphology, including the exposure of the tectum opticum in dorsal view and the thickened skull roof bones, are seen neither in crown penguins nor in Procellariiformes and so appear to represent unique characters of penguin stem taxa. Other features can be more clearly identified as primitive traits along the evolutionary transition from stem to crown penguins. These include caudal expansion of the eminentia sagittalis, an increase in the overlap of the telencephalon onto the cerebellum, reduction of the bulbus olfactorius, a decrease in the cross-section of the semicircular canals, and loss of the interaural pathway. These data thus extend our knowledge of neuroanatomy further towards the root of the penguin tree. However, there remain substantial gaps in our sampling. Although the specimens studied here represent the oldest described endocasts for Sphenisciformes, they postdate the oldest described penguins by approximately 30 million years. Even the oldest penguin fossils belong to clearly flightless species (Slack et al., 2006). Although our ability to reconstruct the series of neuroanatomical changes associated with the evolution of wing-propelled diving and subsequent loss of flight in penguins will remain incomplete until fossils of basal volant members of this lineage are identified, the large number of primitive and unique features observed in the present study reveal that modifications of penguin neuroanatomy continue to have occurred long after the initial loss of flight.

ACKNOWLEDGMENTS

We wish to thank reviewers S. Walsh and S. Kawabe and editor T. Worthy for constructive comments that greatly improved the manuscript. We thank M. Reguero, J. O'Gorman, and J. Moly for capable field assistance in Antarctica. For support with CT scanning and data management, we thank G. Alcuaz and J. I. Cuesta (Hospital San Juan de Dios, La Plata, Argentina). D. Porrini (Universidad Nacional de Mar del Plata, Argentina) kindly donated the *Spheniscus magellanicus* specimen for dissection. M. Sol Bayer and M. Romero Lebrón assisted during dissection. We thank L. Witmer for constructive suggestions for improvements and J. Marugan-Lobón for providing references. Research support was provided by NFS DEB 0949899 (to D.T. K.) and awards PICTO 0093, PIP 0437, and UNLP N671 (to C.P.T.).

LITERATURE CITED

Acosta Hospitaleche, C. 2013. New crania from Seymour Island (Antarctica) shed light on anatomy of Eocene penguins. Polish Polar Research 34:397–412.

Acosta Hospitaleche, C., and N. Haidr. 2011. Penguin (Aves, Sphenisciformes) cranial remains from the La Meseta Formation (Eocene) of Antarctic Peninsula (Antarctica). Antarctic Science 23:369–378.

Acosta Hospitaleche, C., and M. Reguero. 2010. First articulated skeleton of *Palaeeudyptes gunnari* from the late Eocene of Isla Marambio (Seymour Island), Antarctica. Antarctic Science 22:289–298.

Acosta Hospitaleche, C., M. Chavez, and O. Fritis. 2006. Pinguinos fosiles (*Pygoscelis calderensis* nov. sp.) en la Formacion Bahía Inglesa (Mioceno Medio-Plioceno), Chile. Revista Geológica de Chile 33:327–338.

Acosta Hospitaleche, C., C. P. Tambussi, M. Donato, and M. Cozzuol. 2007. A new Miocene penguin from Patagonia and its phylogenetic relationships. Acta Paleontologica Polonica 52:299–314.

Alonso, P. D., A. C. Milner, M. J. Ketcham, M. J. Cookson, and T. B. Rowe. 2004. The avian nature of the brain and inner ear of *Archaeopteryx*. Nature 430:660–669.

Amo, L., M. Rodríguez-Gironés, and A. Barbosa. 2013. Olfactory detection of dimethyl sulphide in a krill-eating Antarctic penguin. Marine Ecology Progress Series 474:277–285.

Aslan, K., H. Atalgin, I. Kürtül, and E. U. Bozkurt. 2006. Patterns of the internal and cerebral carotid arteries in various avian species: a comparative study. Revue de Médecine Vetérinaire 157:621–624.

Balanoff, A. M., G. S. Bever, T. B. Rowe, and M. A. Norell. 2013. Evolutionary origins of the avian brain. Nature 501:93–96.

Bang, B., and S. Cobb. 1968. The size of the olfactory bulb in l08 species of birds. Auk 85:55–61.

Baumel, J. J., and L. Gerchman. 1968. The avian carotid anastomosis. American Journal of Anatomy 122:1–18.

Baumel, J. J., and L. M. Witmer. 1993. Osteologia; pp. 45–132 in J. J. Baumel, A. S. King, J. E. Breazile, H. E. Evans, and J. C. Vanden Berge (eds.), Handbook of Avian Anatomy: Nomina Anatomica Avium. Nuttall Ornithology Club, Cambridge, Massachusetts.

Bertelli, S., and N. P. Giannini. 2005. A phylogeny of extant penguins (Aves: Sphenisciformes) combining morphology and mitochondrial sequences. Cladistics 21:209–239.

Bertelli, S., N. P. Giannini, and D. T. Ksepka. 2006. Redescription and phylogenetic position of the early Miocene penguin *Paraptenodytes antarcticus* from Patagonia. American Museum Novitates 3525:1–36.

Bock, W. J. 1960. Secondary articulation of the avian mandible. Auk 77:130–132.

Bennett, P. M., and P. H. Harvey. 1985. Relative brain size and ecology in birds. Journal of Zoology (London) 207:151–169.

Bhullar, B.-A. S., J. Marugán-Lobón, F. Racimo, G. S. Bever, T. B. Rowe, M. A. Norell, and A. Abzhanov. 2012. Birds have paedomorphic dinosaur skulls. Nature 487:223–226.

Breazile, J., and W. Kuenzel. 1993. Systema nervosum centrale; pp. 493–554 in J. J. Baumel, A. S. King, J. E. Breazile, H. E. Evans, and J. C. Vanden Berge (eds.), Handbook of Avian Anatomy: Nomina Anatomica Avium. Nuttall Ornithology Club, Cambridge, Massachusetts.

Cerda, I., C. P. Tambussi, and F. J. Degrange. 2014. Unexpected microanatomical variation among Antarctic Eocene stem penguins (Aves: Sphenisciformes). Historical Biology 27:549–557.

Clarke, J. A., D. T. Ksepka, M. Stucchi, M. Urbina, N. Giannini, S. Bertelli, Y. Narváez, and C. A. Boyd. 2007. Paleogene equatorial penguins challenge the proposed relationship between biogeography, diversity, and Cenozoic climate change. Proceedings of the National Academy of Sciences of the United States of America 104:11545–11550.

Clarke, J. A., D. T. Ksepka, R. Salas-Gismondi, A. J. Altamirano, M. D. Shawkey, L. D'Alba, J. Vinther, T. J. DeVries, and P. Baby. 2010. Fossil evidence for evolution of the shape and color of penguin feathers. Science 330:954–957.

Cobb, S. 1959. A note on the size of the avian olfactory bulbs. Epilepsia 1:394–40.

Corfield, J., J. Wild, M. Hauber, S. Parsons, and M. Kubke. 2008. Evolution of brain size in the palaeognath lineage, with an emphasis on New Zealand ratites. Brain, Behavior, and Evolution 71:87–99.

Cox, P. G., and N. Jeffery. 2010. Semicircular canals and agility: the influence of size and shape measures. Journal of Anatomy 216: 37–47.

Cunningham, G. B., V. Strauss, and P. G. Ryan. 2008. African penguins (*Spheniscus demersus*) can detect dimethyl sulphide, a prey-related odour. Journal of Experimental Biology 211:3123–3127.

de Beer, G. 1947. How animals hold their heads. Proceedings of the Linnean Society of London 159:125–139.

Dingle, R., and Lavelle M. 1998. Antarctic Peninsula cryosphere: early Oligocene (c. 30 Ma) initiation and a revised glacial chronology. Journal of the Geological Society of London 155:433–437.

Duijm, M. 1951. On the head posture in birds and its relation features. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen C 54:202–271.

Dunning, J. B., Jr. 2008. CRC Handbook of Avian Body Masses, second edition. CRC Press, Boca Raton, Florida, 655 pp.

Elzanowski, A., and P. M. Galton. 1991. Braincase of *Enaliornis*, an early Cretaceous bird from England. Journal of Vertebrate Paleontology 11:90–107.

Frost, P. G. H., W. R. Siegfried, and P. J. Greenwood. 1975. Arterio-venous heat exchange systems in the Jackass penguin *Spheniscus demersus*. Journal of Zoology 175:231–241.

Gleich, O., R. J. Dooling, and G. A. Manley. 2005. Audiogram, body mass, and basilar papilla length: correlations in birds and predictions for extinct archosaurs. Naturwissenschaften 92:595–598.

Haidr, N., and C. Acosta Hospitaleche. 2012. Feeding habits of Antarctic Eocene penguins from a morpho-functional perspective. Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen 263:125–131.

Hall, M. I., A. N. Iwaniuk, and C. Gutiérrez-Ibáñez. 2009. Optic foramen morphology and activity pattern in birds. The Anatomical Record 292:1827–1845.

Hieronymus, T. 2008. Comparative anatomy and physiology of chemical senses in aquatic birds; pp 83–94 in J. G. M. Thewissen and S. Nummela (eds.), Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates. University of California Press, Berkeley, California.

Hopkins, M. 1906. On the relative dimensions of the osseous semicircular canals of birds. Biological Bulletin 11:253–264.

Hopson, J. A. 1977. Relative brain size and behavior in archosaurian reptiles. Annual Review of Ecology and Systematics 8:429–444.

Huxley, T. H. 1859. On a fossil bird and a fossil cetacean from New Zealand. Quarterly Journal of the Geological Society 15:670–677.

Iwaniuk, A. N., and P. Hurd. 2005. The evolution of cerebrotypes in birds. Brain, Behavior and Evolution 65:215–230.

Iwaniuk, A. N., and J. Nelson. 2002. Can endocranial volume be used as an estimate of brain size in birds? Canadian Journal of Zoology 80:16–23.

Iwaniuk, A. N., and D. Wylie. 2006. The evolution of steropsis and the Wulst in caprimulgiform birds: a comparative analysis. Journal of Comparative Physiology A 192:1313–1326.

Iwaniuk, A. N., K. Dean, and J. Nelson. 2004a. A mosaic pattern characterizes the evolution of the avian brain. Proceedings of the Royal Society of London B 271:S148–S151.

Iwaniuk, A. N., P. Hurd, and D. Wylie. 2006. Comparative morphology of the avian cerebellum: I. Degree of foliation. Brain, Behavior, and Evolution 68:45–62.

Iwaniuk, A. N., P. Hurd, and D. Wylie. 2007. Comparative morphology of the avian cerebellum: II. Size of folia. Brain, Behavior, and Evolution 69:196–219.

Iwaniuk, A. N., J. Nelson, H. F. James, and S. L. Olson. 2004b. A comparative test of the correlated evolution of flightlessness and relative brain size in birds. Journal of Zoology (London) 263:317–327.

Jadwiszczak, P. 2006a. Eocene penguins of Seymour Island, Antarctica: the earliest record, taxonomic problems and some evolutionary considerations. Polish Polar Research 27:287–302.

Jadwiszczak, P. 2006b. Eocene penguins of Seymour Island, Antarctica: taxonomy. Polish Polar Research 27:3–62.

Jadwiszczak, P. 2012. Partial limb skeleton of a 'giant penguin' *Anthropornis* from the Eocene of Antarctic Peninsula. Polish Polar Research 33:259–274.

Jadwiszczak, P. 2013. Taxonomic diversity of Eocene Antarctic penguins: a changing picture. Geological Society, London, Special Publications 381:129–138.

Jadwiszczak, P., and T. Mörs. 2011. Aspects of diversity in early Antarctic penguins. Acta Palaeontologica Polonica 56:269–277.

Jerison, H. 1968. Brain evolution and *Archaeopteryx*. Nature 219:1381–1382.

Jerison, H. 1973. Evolution of the Brain and Intelligence. Academic Press, New York, 496 pp.

Jouventin, P., and T. Aubin. 2002. Acoustic systems are adapted to breeding ecologies: individual recognition in nesting penguins. Animal Behaviour 64:747–757.

Jouventin, P., T. Aubin, and T. Lengagne. 1999. Finding a parent in a king penguin colony: the acoustic system of individual recognition. Animal Behaviour 57:1175–1183.

Kawabe, S., T. Ando, and H. Endo. 2014. Enigmatic affinity in the brain morphology between plotopterids and penguins, with a comprehensive comparison among water birds. Zoological Journal of the Linnean Society 170:467–493.

Kilgore, D. L., D. F. Boggs, and G. F. Birchard. 1979. The role of the rete mirable opthalmicum in maintaining the body-to-brain temperature difference in pigeons. Journal Comparative Physiology 129:119–122.

Ksepka, D. T. 2007. Phylogeny, histology, and functional morphology of fossil penguins (Sphenisciformes). M.S. thesis, Columbia University, New York, 285 pp.

Ksepka, D. T., and S. Bertelli. 2006. Fossil penguin (Aves: Sphenisciformes) cranial material from the Eocene of Seymour Island (Antarctica). Historical Biology 18:389–395.

Ksepka, D. T., and J. A. Clarke. 2010. The basal penguin (Aves: Sphenisciformes) *Perudyptes devriesi* and a phylogenetic evaluation of the penguin fossil record. Bulletin of the American Museum of Natural History 337:1–77.

Ksepka, D. T., J. A. Clarke, T. J. DeVries, and M. Urbina. 2008. Osteology of *Icadyptes salasi*, a giant penguin from the Eocene of Peru. Journal of Anatomy 213:131–147.

Ksepka, D. T., S. Bertelli, and N. P. Giannini. 2006. The phylogeny of the living and fossil Sphenisciformes (penguins). Cladistics 22:412–441.

Ksepka, D. T., R. E. Fordyce, T. Ando, and C.M. Jones. 2012a. New fossil penguins (Aves, Sphenisciformes) from the Oligocene of New Zealand reveal the skeletal plan of stem penguins. Journal of Vertebrate Paleontology 32:235–254.

Ksepka, D. T., A. M. Balanoff, S. Walsh, A. Revan, and A. Ho. 2012b. Evolution of the brain and sensory organs in Sphenisciformes: new data from the stem penguin *Paraptenodytes antarcticus*. Zoological Journal of the Linnean Society 166:202–219.

Larsson, H. C. E., P. C. Sereno, and J. A. Wilson. 2000. Forebrain enlargement among theropod dinosaurs. Journal of Vertebrate Paleontology 20:615–618.

Lebedkin, S. 1924. Über die Lage des Canalis semicircularis bei Säugern. Anatomischer Anzeiger 58:449–460.

Lengagne, T., T. Aubin, P. Jouventin, and J. Lauga. 1999. Acoustic communication in a king penguin colony: importance of bird location within the colony and of the body position of the listener. Polar Biology 21:262–268.

Marenssi, S. A., S. N. Santillana, and C. A. Rinaldi. 1998. Stratigraphy of the La Meseta Formation (Eocene), Marambio (Seymour) Island, Antarctica; pp. 137–146 in S. Casadio (ed.), Paleógeno de América del Sur y de la Península Antártica. Asociación Paleontológica Argentina, Publicación Especial 5, Buenos Aires.

Marugán-Lobón, J., and A. Buscaglioni. 2006. Avian skull morphological evolution: exploring exo- and endo-cranial covariation with two block partial least squares. Zoology (Jena) 109:217–230.

Marugán-Lobón, J., L. M. Chiappe, and A. A. Farke. 2013. The variability of inner ear orientation in saurischian dinosaurs: testing the use of semicircular canals as a reference system for comparative anatomy. PeerJ 1:e124. https://dx.doi.org/10.7717/peerj.124

McGowen, M. R., J. Gatesy, and D. E. Wildman. 2014. Molecular evolution tracks macroevolutionary transitions in Cetacea. Trends in Ecology and Evolution 29:336–346.

Meister, W. 1962. Histological studies of the long bones of penguins. The Anatomical Record 143:377–382.

Milner, A., and S. Walsh. 2009. Avian brain evolution: new data from Palaeogene birds (Lower Eocene) from England. Zoological Journal of the Linnean Society 155:198–219.

Montes, M., F. Nozal, S. Santillana, F. Tortosa, E. Beamud, and S. Marenssi. 2010. Integrated stratigraphy of the upper Paleocene-Eocene strata of Marambio (Seymour) Island, Antarctic Peninsula. Presented at XXXI Scientific Committee on Antarctic Research, Open Science Conference, Buenos Aires, Argentina, 3–6 August 2010.

Myrcha, A., P. Jadwiszczak, C. P. Tambussi, J. I. Noriega, A. Gazdzicki, A. Tatur, and R. A. del Valle. 2002. Taxonomic revision of Eocene Antarctic penguins based on tarsometatarsal morphology. Polish Polar Research 23:5–46.

Myrcha, A., A. Tatur, and R. del Valle. 1990. A new species of fossil penguin from Seymour Island, West Antarctica. Alcheringa 14:195–205.

Nevitt, G. A. 2000. Olfactory foraging by Antarctic Procellariiform seabirds: life at high Reynolds numbers. The Biological Bulletin 198:245–253.

Nevitt, G. A. 2008. Sensory ecology on the high seas: investigating the odor world of the procellariiform seabirds. Journal of Experimental Biology 211:1706–1713.

Northcutt, R. G. 2011. Evolving large and complex brains. Science 332:926–927.

Olson, S. L. 1985. The fossil record of birds; pp. 79–238 in D. S. Farner, J. R. King, and K. C. Parkes (eds.), Avian Biology. Academic Press, New York.

Olson, S. L., and Y. Hasegawa. 1979. Fossil counterparts of giant penguins from the North Pacific. Science 206:688–689.

Paulina-Carabajal, A., C. Acosta-Hospitaleche, and R. Yury-Yáñez. 2014. Endocranial morphology of *Pygoscelis calderensis* (Aves, Spheniscidae) from the Neogene of Chile and remarks on brain morphology in modern *Pygoscelis*. Historical Biology 27:571–582.

Pearson, R. 1972. The Avian Brain. Academic Press, New York. 168 pp.

Pettit, T. N., G. Causey-Whittow, and S. G. Grant. 1981. Rete mirabile ophthalmicum in Hawaiian seabirds. Auk 98:844–846.

Reguero, M. A., F. J. Goin, C. Acosta Hospitaleche, S. Marenssi, and T. Dutra. 2012. Late Cretaceous/Paleogene West Antarctica Terrestrial Biota and Its Intercontinental Affinities. Springer Briefs in Earth System Sciences, Dordrecht, The Netherlands, 120 pp.

Sereno, P., J. Wilson, L. Witmer, J. Whitlock, A. Maga, O. Ide, and T. Rowe. 2007. Structural extremes in a Cretaceous dinosaur. PLoS ONE 2:e1230. doi: 10.1371/journal.pone.0001230.

Simpson, G. G. 1946. Fossil penguins. Bulletin of the American Museum of Natural History 87:7–99.

Simpson, G. G. 1971. Review of fossil penguins from Seymour Island. Proceedings of the Royal Society of London. Series B, Biological Sciences 178:357–387.

Sipla, J. S. 2007. The semicircular canals of birds and nonavian dinosaurs. Ph.D. dissertation, Stony Brook University, Stony Brook, New York, 252 pp.

Slack, K. E., C. M. Jones, T. Ando, G. L. Harrison, R. E. Fordyce, U. Arnason, and D. Penny. 2006. Early penguin fossils, plus mitochondrial genomes, calibrate avian evolution. Molecular Biology and Evolution 23:1144–1155.

Smith, N. A. 2011. Taxonomic revision and phylogenetic analysis of the flightless Mancallinae (Aves, Pan-Alcidae). ZooKeys 91:1–116.

Smith, N. A., and J. A. Clarke. 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.

Smith, N. D. 2010. Phylogenetic analysis of Pelecaniformes (Aves) based on osteological data: implications for waterbird phylogeny and fossil calibration studies. PLoS ONE 5:e13354. doi: 10.1371/journal.pone.0013354.

Stingelin, W. 1957. Vergleichende morphologische Untersuchungen am Vorderhirn der Vögel auf cytologischer und cytoarchitektonischer Grundlage. Verlag Helbing and Lichtenhahn, Basel, Switzerland, 123 pp.

Spoor, F., S. Bajpai, S. T. Hussain, K. Kumar, and J. G. M. Thewissen. 2002. Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. Nature 417:163–166.

Stucchi, M. 2002. Una nueva especie de *Spheniscus* (Aves: Spheniscidae) de la Formación Pisco, Perú. Boletín de la Sociedad Geológica del Perú 94:17–24.

Stucchi, M. 2003. Los Piqueros (Aves: Sulidae) de la Formación Pisco. Boletín de la Sociedad Geológica del Perú 95:75–91.

Stucchi, M., M. Urbina, and A. Giraldo. 2003. Una nueva especie de Spheniscidae del Mioceno Tardío de la Formación Pisco, Peru. Bulletin de l'Institut Français d'Etudes Andines 32:361–376.

Tambussi, C. P., C. Acosta Hospitaleche, M. A. Reguero, and S. A. Marenssi. 2006. Late Eocene penguins from West Antarctica: systematics and biostratigraphy. Geological Society, London, Special Publications 258:145–161.

Walsh, S. A., and A. Milner. 2011a. Evolution of the avian brain and senses; pp. 282–305 in G. Dyke and G. Kaiser (eds.), Living Dinosaurs: The Evolutionary History of Modern Birds. John Wiley and Sons, Chichester, U.K.

Walsh, S., and A. Milner. 2011b. *Halcyornis toliapicus* (Aves: Lower Eocene, England) indicates advanced neuromorphology in Mesozoic Neornithes. Journal of Systematic Palaeontology 9:173–181.

Walsh, S. A., P. M. Barrett, A. C. Milner, G. Manley, and L. M. Witmer. 2009. Inner ear anatomy is a proxy for deducing auditory capability and behaviour in reptiles and birds. Proceedings of the Royal Society B 276:1355–1360.

Walsh, S. A., A. N. Iwaniuk, M. A. Knoll, E. Bourdon, P. M. Barrett, A. C. Milner, R. L. Nudds, R. L. Abel, and P. D. Sterpaio. 2013. Avian cerebellar floccular fossa size is not a proxy for flying ability in birds. PLoS ONE 8:e67176. doi: 10.1371/journal.pone.0067176

Watson, M. 1883. Report on the anatomy of the Spheniscidae collected during the voyage of H. M. S. Challenger; pp. 1–244 in J. Murray (ed.), Report of the Scientific Results of the Voyage of H. M. S. Challenger during the Years 1873–1876, Zoology, Volume 7. Neill and Company, Edinburgh, U.K.

Wever, E., P. N. Herman, J. A. Simmons, and D. R. Hertzler. 1967. Hearing in the Black footed penguin, *Spheniscus demersus*, as represented by the cochlear potentials. Proceedings of the National Academy of Sciences of the United States of America 63:676–680.

Wiman, C. 1905. Vorläufige Mitteilung über die alttertiären Vertebraten der Seymourinsel. Bulletin of the Geological Institute of Upsala 6:247–253.

Witmer, L. M. 1990. The craniofacial air sac system of Mesozoic birds (Aves). Zoological Journal of the Linnean Society 100:327–378.

Witmer, L. M., and R. C. Ridgely. 2009. New insights into the brain, braincase, and ear region of tyrannosaurs, with implications for sensory organization and behavior. The Anatomical Record 292:1266–1296.

Witmer, L. M., S. Chatterjee, J. Franzosa, and T. Rowe. 2003. Neuroanatomy of flying reptiles and implications for flight, posture and behaviour. Nature 425:950–953.

Wright, K. L. B., L. Pichegru, and P. G. Ryan. 2011. Penguins are attracted to dimethyl sulphide at sea. Journal of Experimental Biology 214:2509–2511.

Zelenitsky, D. K., F. Therrien, and Y. Kobayashi. 2009. Olfactory acuity in theropods: palaeobiological and evolutionary implications. Proceedings of the Royal Society B 1657:667–673.

Zelenitsky, D. K., F. Therrien, R. C. Ridgely, M. A. R., and L. M. Witmer. 2011. Evolution of olfaction in non-avian theropod dinosaurs and birds. Proceedings of the Royal Society B 1725:3625–3634.

Zusi, R. L. 1975. An interpretation of skull structure in penguins; pp. 55–84 in B. Stonehouse (ed.), The Biology of Penguins. University Park Press, Baltimore, Maryland.

Submitted February 18, 2014; revisions received June 30, 2014;
accepted September 30, 2014.
Handling editor: Trevor Worthy.

Citation for this article: Tambussi, C. P., F. J. Degrange, and D. T. Ksepka. 2015. Endocranial anatomy of Antarctic Eocene stem penguins: implications for sensory system evolution in Sphenisciformes (Aves). Journal of Vertebrate Paleontology. DOI: 10.1080/02724634.2015.981635.