Research article - Basic and applied anatomy

Description of an optic spine on the sphenoid bone of camels and dromedaries

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Abstract

Objective To describe the presence of an intraorbital cylindrical osseous structure (a spine) in two animal species: camel (*Camelus bactrianus*) and dromedary (*Camelus dromedaries*). A homologous osseous structure was previously observed in the large fruit-eating bat (*Artibeus lituratus*). *Procedures* The bony anatomy of the orbital cavity was studied and quantified on macerated skulls of 3 camels and 2 dromedaries. Additionally, one macerated skull of a large fruit-eating bat (*Artibeus lituratus*) was used for comparative purposes.

Results The anatomic description of these unique intraorbital spine was made while studying the bony orbit of macerated skulls, and was considered homologous to that of the bat based on the same anatomic position (at the bone bridge that separates the optic canal and the sphenorbital fissure) and similarities in shape. We suggest the name optic spine of the sphenoid bone. Discussion The novel observation of an optic spine on the sphenoid bone in camels and dromedaries (Artiodactyla), when combined with the previous finding of a similar anatomic structure in a bat (Chiroptera) suborder Microchiroptera, may provide further support to the close proximity of these two apparently very distinct animal orders in the phylogenetic tree, and may contribute to the understanding of bat evolution and provide new directions for future research. The function of this osseous spine remains to be investigated, although we hypothesize that the optic spine of the camelids may serve as an attachment site for extraocular muscles.

Key words -

Anatomy, bony orbit, Camelus bactrianus, Camelus dromedaries, optic spine, sphenoid bone, comparative studies, mammals

Introduction

Evolutionary relationships among several different orders of the animal tree of life have proven difficult to determine or have received little support in the vast majority of phylogenomic studies of mammalian systematics, and thus remain unresolved at best. Among those mammals with significant knowledge gaps are the bats (Chiroptera), despite representing one of the largest and most diverse radiations of mammals, and accounting for one-fifth of extant species. Found worldwide, bats are also the only mammals to have achieved true self-powered flight, and they play a major ecological role as pollinators and insect predators (Patterson et al., 2003; Simmons et al., 2008).

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Currently the position of bats in the evolutionary tree of life is considered conflicting or incomplete, thus the phylogenetic and geographic origin of bats (and the entire order Chiroptera) remains unclear. A plausible reason for this fact is that bats are not well represented in the fossil record (Altringham, 1996; Nowak, 1999; Patterson et al., 2003; Springer et al., 2001; Van Den Bussche and Hoofer, 2004; Eick et al., 2005; Gunnell and Simmons, 2005; Simmons et al., 2008). There are some possible reasons for the lack of fossil evidence. One is that bats have small, delicate skeletons that do not fossilize very well. Another is that most species live in tropical forests, where conditions are usually unfavorable for the formation of fossils (Carroll, 1988). Despite a poorly represented fossil record, even the earliest fossil bats dating back 45 to 50 million years ago have an outstanding resemblance to modern microbats, and intriguingly no fossil bats have yet been identified that are in any way intermediate in form between modern microbats and early tree-living ancestors (Altringham, 1996; Nowak, 1999; Simmons et al., 1998; Springer et al., 2001; Eick et al., 2005; Teeling et al., 2005). Thus, according to Altringham (1996), modern microbats may have made their appearance about 65-100 million years ago. If so, they amazingly shared the world with the dinosaurs, and watched their extinction at the end of the Cretaceous period. Historically, the most common assumption about the evolutionary history of bats has been based on morphological evidence, grouping bats with primates, flying lemurs, and tree shrews to form the Archonta (Szalay, 1977; Novacek, 1992; Gunnell and Simmons, 2005).

New genomic evidence demonstrates an unexpected sister relationship between Chiroptera and Cetartiodactyla (Hallström and Janke, 2008; Nery et al., 2012, Zhang et al. 2013). The curious and unusual phylogenetic position and consequent evolutionary proximity between Chiroptera and Artiodactyla has received genomic but no real morphological support until now.

The skull has been used as a major skeletal structure to determine taxonomic affiliations as it is subject to phenotypic changes because of selective breeding (Bruenner et al., 2002).

The objective of this study is to report the presence of an intraorbital cylindrical osseous structure, a spine, in two animal species: camel (*Camelus bactrianus*) and dromedary (*Camelus dromedaries*). A homologous osseous structure in the bony orbit was previously only observed in a bat (the large fruit-eating bat *Artibeus lituratus*) (Machado et al., 2007). The observation of the same anatomic feature in the bony orbit of both Artiodactyla (Old World camelids) and Chiroptera (bats, specifically of the suborder Microchiroptera) may provide further support towards the growing body of evidence suggesting close proximity of these two apparently very distinct animal orders within the evolutionary tree.

Materials and methods

A thorough examination of the bony orbit from 5 previously macerated skulls of Old World camelids (3 adult camels and 2 adult dromedariess) was performed, including anatomic description and gross specimen morphometry. From the three camel skulls studied, two (from one 35 year-old male and one 21 year-old female) belonged to the collection of Capão da Imbuia – Museum of Natural History (MHNCI) and one from a 34 year-old male belonged to the collection for environment education of the

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Curitiba Zoo, both institutions located in Curitiba-PR, Brazil. The two dromedarian skull samples (one from a 23 year-old male and the other from an 18 year-old female) belonged to the Veterinary Anatomy Museum of the University of Contestado, located in Canoinhas-SC, Brazil. One previously macerated skull of an adult large fruit-eating bat (*Artibeus lituratus*) was used in this investigation for morphologic and photographic comparisons. This skull belonged to the private collection of one of the authors (MM).

The camel skulls where previously naturally cleaned by a decomposition process while the dromedarian skulls where prepared by a laboratorial maceration technique. The skin and most of the soft tissues and eyes were removed to initially clean the skulls, and then a maceration technique consisting of a boiling process followed by cold water immersion in a closed recipient for two weeks was performed. After maceration the skulls were immersed in 50% hydrogen peroxide for approximately 24 h for bleaching. Following this step they were washed in distilled water and air dried. The nomenclature used for skull osteology follows previously published work on osteology and camel anatomy (Smuts and Bezuidenhout, 1987; Olsen, 1988; Neumani, 1911; Shahid and Kausar, 2005; Yahaya et al., 2012 a,b).

The macerated skulls and optic spines were measured with a measuring tape, a ruler, and a digital pachymeter, and were then digitally photographed. Selected osteometric parameters were measured, according to Sarma (2006), Karimi 2011 and Yahaya (2012 a,b) and orbital indexes calculated according to Kaur et al. (2012). Morphometric analysis of the skull included: Skull length, i.e. the interincisive space to the most caudal aspect of the occipital bone (the intersection point between the sagittal and nuchal crest); skull width, i.e. the distance between the two most lateral points of the frontal bones (the most lateral parts of the dorsal margin of the orbit) (Fig. 1); intraorbital bony spine width at the base and at the tip, dorsal and ventral lengths (Fig. 2C), bilaterally (Fig. 3); orbital horizontal and vertical diameters (Fig. 4). Additionally, orbital indexes were calculated as follows: Orbital index = Vertical diameter of the orbit × 100/Horizontal diameter of the orbit.

Results are presented as mean \pm the standard deviation (SD).

Results

The overall shape of the skull of camels and dromedaries is very similar (Fig. 1). Both when viewed from above are roughly pentagonal in shape, elongated towards the maxilla and mandible. Both are wider in the frontal bone region (skull width) than between the zygomatic bones. The orbits are nearly circular and enclosed (complete) situated laterally and slightly cranially (Figs. 1 and 2). The rim of the frontal bone is serrated (Fig. 2A). An irregular transverse elevation separates the parietal and nuchal surfaces (Fig. 1). The occipital bone formed the entire nuchal surface and invaded upon the dorsal surface. It joined the parietal bone at the transverse suture. The sagittal and occipital crests on the dromedary are considerably more pronounced or developed than those found in the camel skull.

Inside the bony orbit we have observed an unusual osseous spine that is slender and elongated in shape, directed rostrolaterally, and also slightly ventrally in camels (*Camelus bactrianus*) (Fig. 2A) and dromedaries (*Camelus dromedaries*) (Fig. 2B). It is located bilaterally (Fig. 3) on the bone bridge that separates the optic canal and the

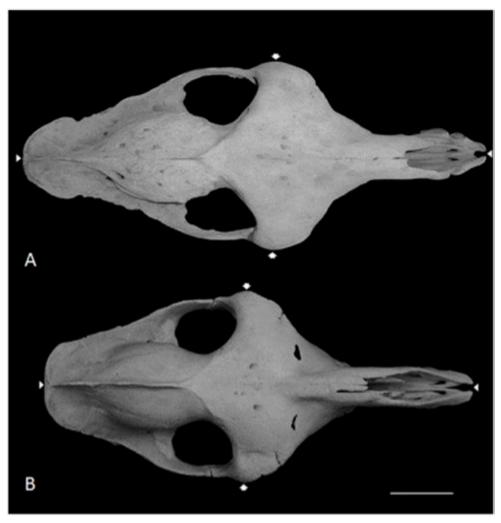


Figure 1 – Dorsal views of the skulls of a representative adult camel (A) and an adult dromedary (B). Legend: Arrowheads represents the points to measurement of the skull length (interincisive space and the intersection point between the sagittal and nuchal crest), and arrows represents the points to measurement of the interorbital length. Note that an irregular transverse elevation separates the parietal and nuchal surfaces. Bar: 10 cm.

sphenorbital fissure on the sphenoid bone complex (Fig. 2C and Fig. 4) of these species. This bony spine is thin, cylindrical in shape, and tapers at its free rostral end in both species (Fig. 2C). It is slightly wider at its sphenoid bone base in camels than in dromedaries. In camels, the spine is slightly irregular at its free end (Fig. 1A). In both species the optic spine length from the dorsal base to tip is shorter than the length of the ventral base to tip, due to the presence of a discrete ridge at its dorsal junction to the sphenoid bone.

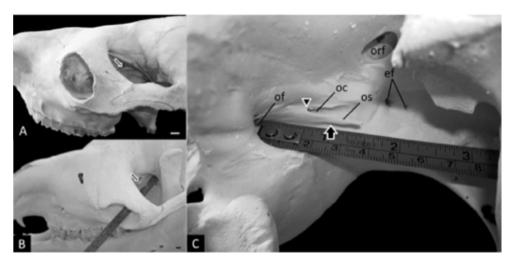


Figure 2 – General topography and form of the optic spine of the sphenoid bone in Old World camelids. Oblique dorsocaudal view of the left optic spine of the sphenoid bone of a camel (A), dorsolateral view of the left optic spine of a dromedary (B), and ventrolateral view of the medial and caudal walls of the bony orbit of a representative adult dromedary skull: note the discrete ridge formed where the optic spine meets the sphenoid bone (C). The optic spine of the sphenoid (arrow) is clearly seen in these macerated skulls without the aid of any magnifying device. Note that the orbit is complete and the optic spine of the camel is slightly irregular at its free end, compared with the dromedaries. Ventral (arrow head) and dorsal (arrow) bases of the optic spine of the sphenoid (os) can be seem. Legend: ethmoidal foramina (ef), optic canal (oc), orbital fissure (of); orbitorotundum foramen (orf). Bars: 1 cm.

Morphometry on dromedary skulls

The mean skull width was 24.75 \pm 1.8 cm and the mean skull length was 41.33 \pm 3.44 cm. The mean intraorbital bony optic spine length from the ventral base to tip was 2.10 \pm 1.06 cm and the length from the dorsal base to tip was shorter, measuring 1.25 \pm 0.94 cm. The mean width was 0.34 \pm 0.42 cm at the base and 0.21 \pm 0.03 cm at the tip of the spine. The mean orbital horizontal diameter measured 6.26 \pm 0.79 cm. The mean orbital vertical diameter was 6.11 \pm 0.73 cm. The mean orbital index was 102.45.

Morphometry on camel skulls

The mean skull width was 27.4 \pm 2.7 cm and the mean skull length was 53.10 \pm 2.82 cm. The mean intraorbital bony optic spine length from the ventral base to tip was 2.11 \pm 0.81 cm and the length from the dorsal base to tip was shorter, measuring 1.40 \pm 0.30 cm. The mean width was 0.41 \pm 0.56 cm at the base and 0.27 \pm 0.09 cm at the tip of the spine. The mean orbital horizontal diameter was 6.15 \pm 0.44 cm. The mean orbital vertical diameter was 5.92 \pm 0.50 cm. The mean orbital index was 103.88.

Discussion

General osteology and osteometry of camel and dromedary skulls have been published elsewhere (Neumani, 1911; Olsen, 1988, Yahaya et al., 2012). Mean orbital hori-

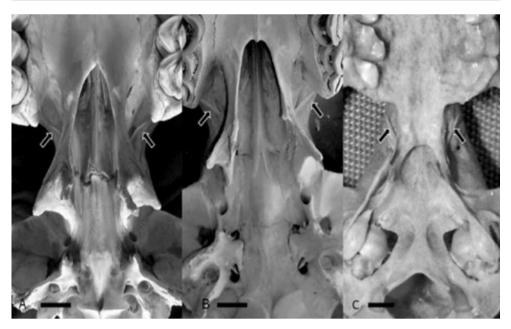


Figure 3 – Detail of the bilateral arrangement of the optic processes (arrows) of the sphenoid bone on a ventral view of the base of the skulls of a representative adult camel (A; bar = 2 cm), an adult dromedary (B; bar = 2cm), and an adult large fruit-eating bat (Artibeus lituratus) (C; bar = 2 mm). Note that although both bony elements are rostrally oriented, the optic spine in the camel and in the dromedary are more laterally oriented than the optic spine of the large fruit-eating bat .

zontal diameter found in dromedaries investigated in the present work was similar to the one (6.01 ± 0.07 cm) reported by Yahaya et al. (2012b). Mean orbital vertical diameter in dromedaries parallels results from Yahaya et al. (2012)b, which varied from 5.74 ± 0.12 cm to 6.12 ± 0.21 cm. Additionally, mean dromedary skull length found in our investigation also was comparable to the data from Monfared (2013), which was 46.2 ± 2.74 cm and Yahaya (2012), which varied from 45.50 ± 0.65 cm to 49.44 ± 0.86 cm. Nevertheless, none of these previous studies described the presence of the optic spine of the sphenoid bone. Orbital indexes of both species were considerably large. Both were larger than the goat 86.11 to 92.14 (Sarma, 2006) but smaller than the Mehraban sheep, which varied from 108.38 from 109.07 (Karimi et al., 2011).

Despite being rather inconspicuous, these spines have remained undescribed in Old World camelids until now, possibly because of the limited research available in the literature regarding morphological features of their eye, adnexa and orbit (Neumani, 1911; Tayeb, 1951; Abdalla et al., 1970; Awkati and Al-Bagdadi, 1971; Smuts and Bezuidenhout, 1987; Olsen, 1988; Abuel-Atta et al., 1997; Wang JL. 2002; Cui et al., 2004; Shahid and Kausar, 2005; El-Tookhy et al., 2012; Yahaya et al., 2012a,b). Even detailed studies of the cranioencephalic structures of dromedaries using diagnostic imaging techniques such as radiography (Saber, 1990), computed tomography (Alsafy et al. 2014) and magnetic resonance (Arencibia et al., 2005) failed to detect and describe the spines of the sphenoid bone.

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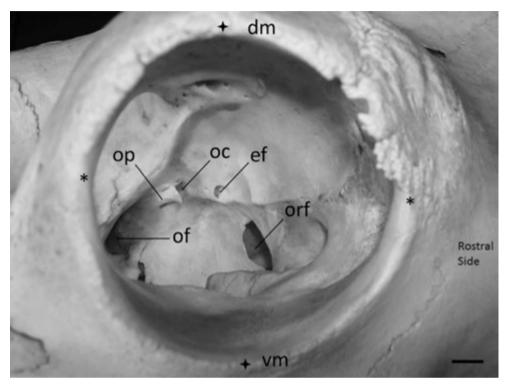


Figure 4 – Rostrolateral view of the bony orbit through the orbital adit of a representative adult camel skull. Legend: dorsal margin of the orbit (dm); ethmoidal foramen (ef), optic canal (oc), orbital fissure (of); orbitorotundum foramen (orf); optic spine of the sphenoid (op); ventral margin of the orbit (vm). Stars represent the points to measurement of the orbital height (dorsoventral axis), and asterisks represents the points to measurement of the orbital diameter (mediolateral axis). Bar: 1 cm.

Notwithstanding the scant information known about the evolutionary history of bats, evidence suggests that bats may have originated in the northern supercontinent of Laurasia, possibly in North America (Teeling et al., 2005) as part of a large group of placental mammals (Laurasiatheria) including shrews, hedgehogs, pangolins, whales, carnivorans, and most hoofed mammals such as camels, among others. Several questions still remain regarding how the different orders of several mammalians in the supraordinal group Laurasiatheria evolved.

Traditionally bats were placed along with primates, flying lemurs, and tree shrews, forming the Archonta on an anatomical basis (Szalay, 1977; Novacek, 1992). However, in more recent phylogenetic analyses of the complete mitochondrial genome of the Jamaican fruit bat (*Artibeus jamaicensis*), it appeared that bats may be more closely related to "cetferungulates", a clade including Cetacea, Artiodactyla, Perissodactyla, and Carnivora (Pumo et al., 1998). Phylogenetic analyses from the c-myc gene sequences also support this relationship (Miyamoto, 2000). Other phylogenetic investigations using relationships with genome data started to place bats near cows (Hallström and Janke, 2008). Posteriorly, phylogenetic analyses investigating a very large amount of genomic sequence data have provided even greater and clearer support for the sister

relationship between Chiroptera and Cetartiodactyla (Nery et al., 2012, Zhang et al., 2013). Cetartiodactyla is the clade in which whales and even-toed ungulates are currently placed. The term was coined by merging the name for the two orders, Cetacea and Artiodactyla, into a single word. Cetacea includes whales and dolphins. Artiodactyla includes pigs, peccaries, hippopotamuses, camel, dromedary, llamas, chevrotains (mouse deer), deer, giraffes, pronghorn, antelopes, sheep, goats, and cattle.

Here, taking the current description into account and following the results from Machado et al. (2007) in a bat, we provide anatomical evidence for the support of a possible sister relationship between Chiroptera and Cetartiodactyla in the form of intraorbital osseous spines. The first intraorbital osseous spine observed in an animal was on the Artibeus lituratus (a large fruit-eating bat) (Machado et al., 2007). The is a slender and elongated bony spine, directed rostrolaterally and slightly ventrally. It is located bilaterally on the bone bridge that separates the optic canal and the sphenorbital fissure on the alisphenoid bone (from the sphenoid complex) (Fig. 1C). The group of researchers suggested the name optic spine of the alisphenoid bone. These equivalent bony optic spines described here in Old World camelids are similar in anatomical position and general shape, but are more rostrolaterally oriented and slightly less ventral than the spines of the large fruit-eating bat. Nevertheless, the anatomic feature was thus far exclusively reported to Old World Camelids and the large fruiteating bat and was considered homologous based on the same anatomic position (at the bone bridge that separates the optic canal and the sphenorbital fissure) and shape similarities, which surpasses angular differences (Fig. 3).

Function of this osseous spine as well as potential differences in immature animals remains to be investigated. We hypothesize that the optic process of the camelids may serve as an attachment site for extraocular muscles in a similar manner to the optic spine of the alisphenoid bone in bats (Machado et al., 2007). In order to prove this assumption, future studies should perform a careful dissection in fresh or fixed camelid skulls, paying special attention to the delicate attachments of the extraocular muscles. The observation of an optic spine on the sphenoid bone in camels and dromedaries (Artiodactyla), when combined with the previous finding of a such anatomic component in a bat (Chiroptera, suborder Microchiroptera), may provide further support to the close proximity of these two apparently very distinct animal orders in the phylogentic tree, and contribute to the understanding of bat evolution and perhaps provide new directions for future research.

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