Clavicle in Carnivorans: A Forgotten Bone

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ABSTRACT

The clavicle is a bone whose development is related to the versatility of the thoracic limbs in mammals. Studies with vestigial structures are scarce and controversial, especially in the order Carnivora. The objective of this study was to verify the presence and to investigate the shape and constitution of the clavicle in neotropical carnivores. In order to do this, 108 cadavers of 19 different species were collected dead on highways and were analyzed. The clavicles were submitted to dissections, longitudinal length measurements, radiographs, histological sections and, in some cases, diaphanization. Sixteen of the 19 species had clavicles in both sides, being significantly larger ($P < 0.05$) and distinctly more radiopaque in the felids than in the other families. There were no macro or microscopic evidence of clavicle in the specimens of *Nasua nasua* ($n = 6$), *Conepatus semistriatus* ($n = 2$), and *Conepatus chinga* ($n = 1$). The clavicle of the males of *Lycalopex gymnocercus*, *Galictis cuja*, and *Leopardus geoffroyi* was significantly larger ($P < 0.05$). The predominating contour of the clavicles was a thin stick with cranial convexity. The histological sections demonstrated compact bone consisting of trabecula and lamellae filled by bone marrow and different levels of occupation by chondroid matrix. It can be proposed the clavicles of the order Carnivora, although vestigial and rarely absent, have their presence, constitution and shape more associated with the phylogenetic proximity and evolutionary history of the species than to the variety of movements the thoracic limbs perform in free-living conditions. Anat Rec, 303:1831–1841, 2020. © 2019 American Association for Anatomy

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The term "clavicle" is derived from the Latin "clavis" which means "small key" or in the shape of a door handle (Ljunggren, 1979; Bain et al., 2015). The clavicle is a highly variable bone among mammals: only in the orders Primates, Chiroptera, Tubulidentata, Dermoptera, and Monotremata it is fully developed; in Insectivora and Marsupialia it is present in most species; among Lagomorpha, Edentata, and Rodentia it is rudimentary in some families and well developed in others; in the orders Artiodactyla, Perissodactyla, Hyracoidea, Proboscidea, Pholidata, Sirenia, Pinnipedia, and Cetacea it is absent. Specifically, in the order Carnivora, the clavicle is considered vestigial or absent (Jenkins, 1974; Anyonge, 2001; Hall, 2001; Rocha-Barbosa et al., 2002; Averianov and Lopatin, 2014; Bain et al., 2015).

The lack of a functional clavicle was interpreted as advantageous for a greater capacity of movements of extension and flexion of the shoulder, which determines the increase of stride length for cursorial species (Howell, 1937; Eaton, 1944). The reduction or the loss of the clavicle is common to mammals use the thoracic limbs only to support their body mass and in strictly cursorial locomotion (Rockwood et al., 2009). Usually, this reduction is concomitant to modifications of the deltoid, cleidomastoid, and trapezius muscles to form a continuous muscle from the head to the limb, the brachiocephalic muscle (Howell, 1937). Romer (1977) inferred the clavicular reduction in the cursorial mammals would be able to relieve, from the shoulder, the loads transmitted by the thoracic limbs to the trunk. Humans and other brachiated mammals have well-developed clavicles, because it would be an adaptation to tasks that depend on hands away from the trunk such as climbing, swimming and reaching distant objects. These teleological differences help define the function of the clavicle (Rockwood et al., 2009).

On the South American continent, there are 44 neotropical species of the order Carnivora. Of these, 26 of them are continental and represent five families distributed throughout the Brazilian territory (Trigo et al., 2013). The scarcity of data about the anatomy of carnivorans in the neotropics is more evident than in other areas of the planet. They represent the highest trophic level of the regions they occupy and thus play a key role in biodiversity by regulating vertebrate communities and, indirectly, plants (Shostell and Ruiz-Garcia, 2013).

Vestigial structures are common in the postcranial skeletons of extant mammals but are poorly studied or are not explicitly recognized as such (Senter and Moch, 2015). In the order Carnivora, anatomical descriptions of the clavicle are concentrated in the domestic species. It is known that the clavicle of domestic dogs has a varied morphology (Nickel et al., 1986), it is embedded in the brachiocephalic muscle (Sisson, 1986) and it is absent in a small number of individuals (McCarthy and Wood, 1988). In the domestic cat, the clavicle takes the form of a flat and slightly curved bone of 2–5 cm in length. In both species, the clavicle has no connection with the skeleton and is visible on radiographs (Liebich et al., 2016).

The clavicle of domestic dogs has different shapes among individuals, being predominantly oval (Černý and

Čižinauskas, 1995). Černý and Čižinauskas (1995) speculated that variations in clavicle's shape in dogs may come from changes and reductions it suffers during ontogeny. Therefore, they concluded the clavicle would be a structure that goes through a regression at different levels in an individualized process.

In order to expand the understanding of this vestigial structure in locomotion and to subsidize inferences about its evolutionary history, the aim of this study was to investigate the presence and constitution of the clavicle in Neotropical species of the order Carnivora.

MATERIALS AND METHODS

Sampling

One hundred and eight cadavers of 19 Neotropical species belonging to five families of the order Carnivora were dissected: the canids *Cerdocyon thous* (n = 22), *Chrysocyon brachyurus* (n = 5), *Lycalopex gymnocercus* (n = 11), and *Lycalopex vetulus* (n = 5); the mustelids *Eira barbara* (n = 2), *Galictis cuja* (n = 9) and *Lontra longicaudis* (n = 6); the procyonids *Nasua nasua* (n = 6) and *Procyon cancrivorus*
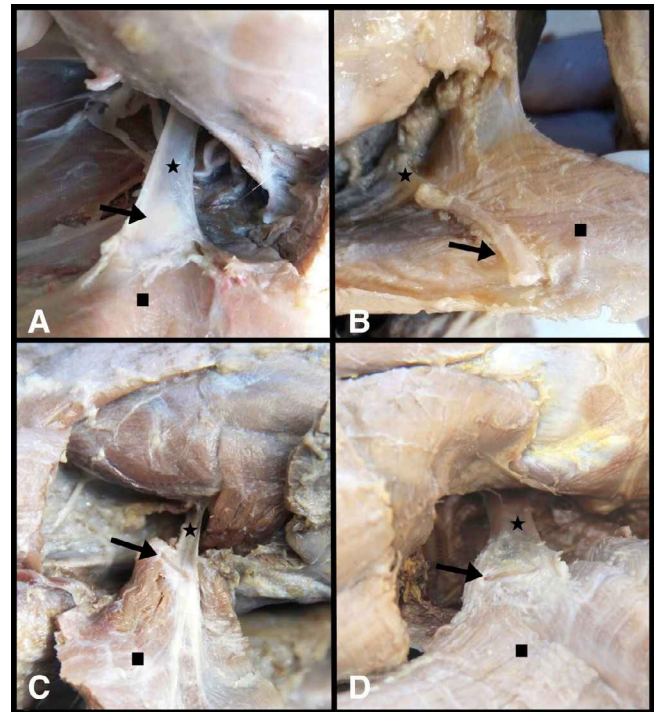

Fig. 1. Photomacrographs of the dissection of the scapulohumeral region of the canid *Chrysocyon brachyurus* (**A**), felid *Puma concolor* (**B**), mustelid *Galictis cuja* (**C**), and procionid *Procyon cancrivorus* (**D**), demonstrating the clavicle (black arrow), the cleidobrachial muscle (square), and the fibrous band (star) that fixes the clavicle to the medial aspect of the scapular region.

TABLE 1. Species, number of specimens, family and average longitudinal lengths of the clavicles of Neotropical carnivorans' cadavers analyzed

Species	Family	Mean ± SD (mm)
Chrysocyon brachyurus (n = 5)	Canidae	11.7 ± 1.2 (n = 10)
Cerdocyon thous (n = 20)	Canidae	9.5 ± 1.4 (n = 20)
Lycalopex gymnocercus (n = 11)	Canidae	7.0 ± 1.4 (n = 13)
Lycalopex vetulus (n = 5)	Canidae	5.1 ± 0.8 (n = 10)
Leopardus colocolo (n = 4)	Felidae	25.3 ± 3.8 (n = 8)
Leopardus geoffroyi (n = 12)	Felidae	23.8 ± 3.2 (n = 22)
Leopardus guttulus (n = 3)	Felidae	15.6 ± 1.3 (n = 5)
Leopardus wiedii (n = 1)	Felidae	23.3 ± 0.8 (n = 2)
Leopardus pardalis (n = 4)	Felidae	38.2 ± 4.5 (n = 6)
Panthera onca (n = 1)	Felidae	37.4 ± 1.4 (n = 2)
Puma concolor (n = 3)	Felidae	51.9 ± 1.9 (n = 2)
Puma yagouaroundi (n = 3)	Felidae	21.9 ± 2.4 (n = 4)
Conepatus chinga (n = 1)	Mephitidae	Absent
Conepatus semistriatus (n = 2)	Mephitidae	Absent
Eira barbara (n = 2)	Mustelidae	7.4 ± 2.5 (n = 4)
Galictis cuja (n = 9)	Mustelidae	5.9 ± 1.5 (n = 15)
Lontra longicaudis (n = 6)	Mustelidae	8.4 ± 2.9 (n = 9)
Nasua nasua (n = 6)	Procyonidae	Absent
Procyon cancrivorus (n = 8)	Procyonidae	10.4 ± 1.3 (n = 12)

Fig. 2. "Box-plot" graphic representation of the length of the clavicle (mm) between families of the order Carnivora.

(n = 8); the mephitids *Conepatus chinga* (n = 1) and *Conepatus semistriatus* (n = 2); and the felids *Leopardus colocolo* (n = 4), *Leopardus geoffroyi* (n = 12), *Leopardus guttulus* (n = 3), *Leopardus wiedii* (n = 1), *Leopardus pardalis* (n = 4), *Panthera onca* (n = 1), *Puma concolor* (n = 3), and *Puma yagouaroundi* (n = 3). The specimens were collected dead on Brazilian highways in Pampa (IBAMA/SISBIO 33667 authorization) and Cerrado biomes (term of cooperation between IBAMA/UFU No. 002/2011). Also, clavicles from two cadavers of domestic dogs and from two cadavers of domestic cats were collected in order to compare the bone contour (approved protocol CEUA/UNIPAMPA 042/2017).

Initially, the dissections were performed to verify the presence of the clavicle in each specimen. A skin incision was made at the cranial margin of the scapulohumeral region then, the superficial and deep fasciae were cleaned, followed by the identification of the clavicular intersection of the brachiocephalic muscle, to investigate the existence of a clavicle. Only thoracic limbs whose scapulohumeral region and clavicular intersection were found intact to study were included. Among the 216 thoracic limbs available, 17 were discarded because they
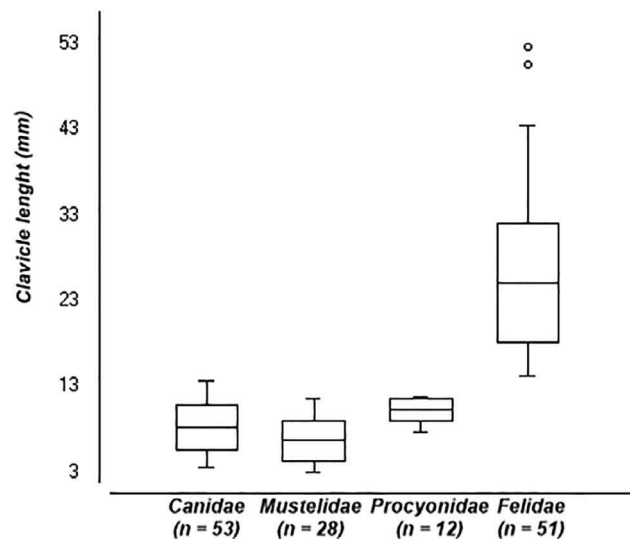
contained tissue loss in the scapulohumeral region, which made it impossible to identify the clavicle. Of the 199 dissected limbs, 181 clavicles were found, which were collected and stored in vials identified with 10% formalin solution for different analyses.

Measurements

A total of 155 clavicles had the longitudinal length measured with precision digital calipers (resolution 0.01 mm, accuracy ±0.02 mm, ZAAS Precision, Amatools®), by single examiner in duplicate. Prior to measurements, the adjacent soft tissue was gently removed to facilitate the visualization of the bone. The arithmetic mean, SD, and coefficient of variation of clavicles' lengths of each species were calculated and, later, grouped by families.

Unidirectional variance analysis (one-way ANOVA) complemented by the Tukey's test was used to verify the existence of a difference in the length of the clavicles between the different families; in those species in which at least four clavicles of each sex were obtained (*C. thous*, *C. brachyurus*, *L. gymnocercus*, *G. cuja*, *L. longicaudis*, and *L. geoffroyi*), Student's *t*-test was performed (independent samples) to verify the existence of a difference in length of the clavicles between sexes. Statistical and

TABLE 2. Arithmetic mean, SD, minimum and maximum values, and coefficient of variation of the length (mm) of the clavicle separated by families of the order Carnivora

Family	M ± SD (mm)	Min (mm)	Max (mm)	CV (%)
Canidae (n = 53)	8.5 ± 2.6	3.7	13.8	30.5
Mustelidae (n = 28)	6.9 ± 2.4	3.2	11.9	34.6
Procyonidae (n = 12)	10.4 ± 1.3	7.9	12.0	12.5
Felidae (n = 51)	26.4 ± 8.6*	14.4	52.9	32.5

M, average mean; SD, standard deviation; Min, minimum; Max, maximum; and CV, coefficient of variation.
*Significant difference to the one-way analysis of variance (ANOVA) complemented by the Tukey test ($P < 0.01$).

TABLE 3. Arithmetic mean, SD, and *P*-value of Student's *t*-test to compare the mean between the lengths of the clavicle between the sexes in Neotropical species of the order Carnivora

Species	Male (mm)	Female (mm)	*P*-value
Cerdocyon thous	9.8 ± 1.4 ($n = 12$)	9.0 ± 1.3 ($n = 8$)	0.22
Chrysocyon brachyurus	11.5 ± 1.0 ($n = 6$)	12.1 ± 1.6 ($n = 4$)	0.51
Eira barbara	9.6 ± 0.7 ($n = 2$)	5.3 ± 0.7 ($n = 2$)	0.02*
Galictis cuja	6.5 ± 1.0 ($n = 10$)	4.6 ± 1.5 ($n = 5$)	0.01*
Leopardus colocolo	24.7 ± 4.4 ($n = 6$)	27.1 ± 0.6 ($n = 2$)	0.49
Leopardus geoffroyi	26.2 ± 1.4 ($n = 8$)	22.4 ± 3.1 ($n = 14$)	0.01*
Lontra longicaudis	8.9 ± 1.9 ($n = 4$)	7.9 ± 3.7 ($n = 5$)	0.64
Lycalopex gymnocercus	7.7 ± 1.3 ($n = 8$)	5.9 ± 0.9 ($n = 5$)	0.02*
Procyon cancrivorus	9.6 ± 1.8 ($n = 4$)	10.9 ± 0.8 ($n = 8$)	0.11

*Significant difference in Student's *t*-test ($P < 0.05$).

graphic data were generated with the software BioEstat 5.3®, considering $P < 0.05$ significant.

Radiographs

Radiographic images of at least two clavicles of each species were obtained in order to verify the presence of radiopacity and to delimit the bone contour. The t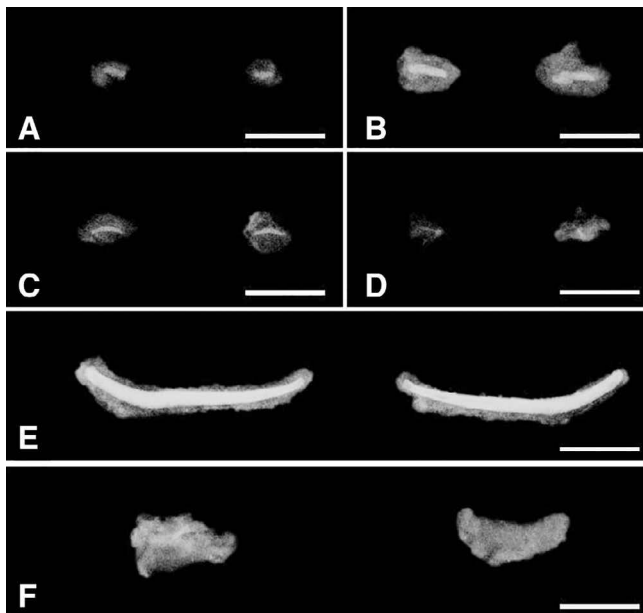able top technique was set with 40 kV and 40 mAs (Phillips®, model Aquilla Plus 300). The exposures were performed in a computerized cassette system (Kodak® Direct View) and saved in the DICOM format. After visualizing in the software Radiant Dicom Viewer®, 3.0.2 version, the files were exported to JPEG format. Schematic drawings representing the shape of the clavicles for each species were performed from the most commonly visualized contour.

Histology

At least two clavicles of each species were sagittally cleaved, soaked in paraffin and the histological sections with 4 μm submitted to hematoxylin and eosin staining and toluidine blue staining. Samples of histological sections were examined under light optical microscopy to visualize the constitutive characteristics of the clavicles. Finally, digital photomicrographs were performed with the Olympus® DP26 camera associated with cellSens 1.7® software.

Diaphanization

Clavicles of the canids *L. vetulus* and *C. brachyurus*, of the procionid *P. cancrivorus*, of the mustelid *G. cuja* and of the felid *P. concolor* were selected for the diaphanization process with alizarin red as a complementary method to better illustrate the bone contour. After being processed, each was dissected with tweezers and had its shape analyzed using a binocular stereo microscope (PHYSIS 7X-45X®) and subsequently photographed with camera Nikon Coolpix® L820, 16 MP. Diaphanization of a 40-day-old fetus of *L. geoffroyi*, male, found in the uterus of a specimen collected dead was also performed, to verify the presence and ossification of the clavicle. Fetal age estimation was extrapolated from "crown-rump" measurements and mass measurement and applied in a given equation for domestic cats, according to Nelson
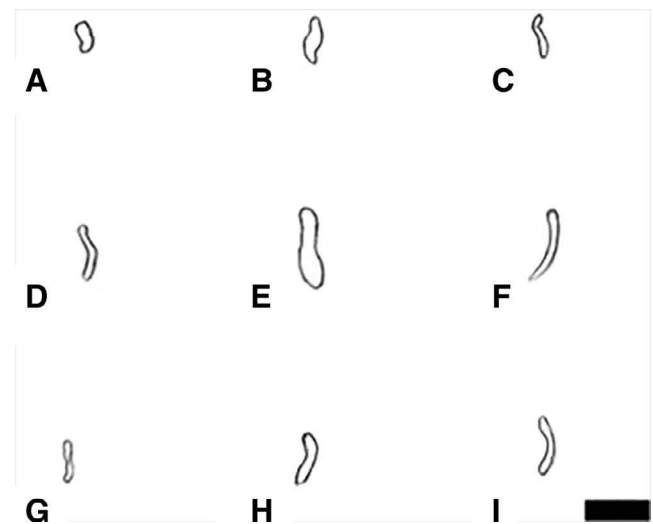

Fig. 3. Radiographs of the clavicles of wild carnivorans wrapped in fibrous tissue in *Lycalopex vetulus* (**A**), *Chrysocyon brachyurus* (**B**), *Procyon cancrivorus* (**C**), *Galictis cuja* (**D**), and *Puma concolor* (**E**). Note the absence of bone radiopacity in the sample of *Nasua nasua* (**F**). Bar: 2 cm.

Fig. 4. Schematic representation of the most common clavicles' contours of the neotropical specimens of the suborder Canifornia. *Canis lupus familiaris* (**A**), *Lycalopex vetulus* (**B**), *Lycalopex gymnocercus* (**C**), *Cerdocyon thous* (**D**), *Chrysocyon brachyurus* (**E**), *Procyon cancrivorus* (**F**), *Galictis cuja* (**G**), *Eira barbara* (**H**), and *Lontra longicaudis* (**I**). Bar: 2 cm.
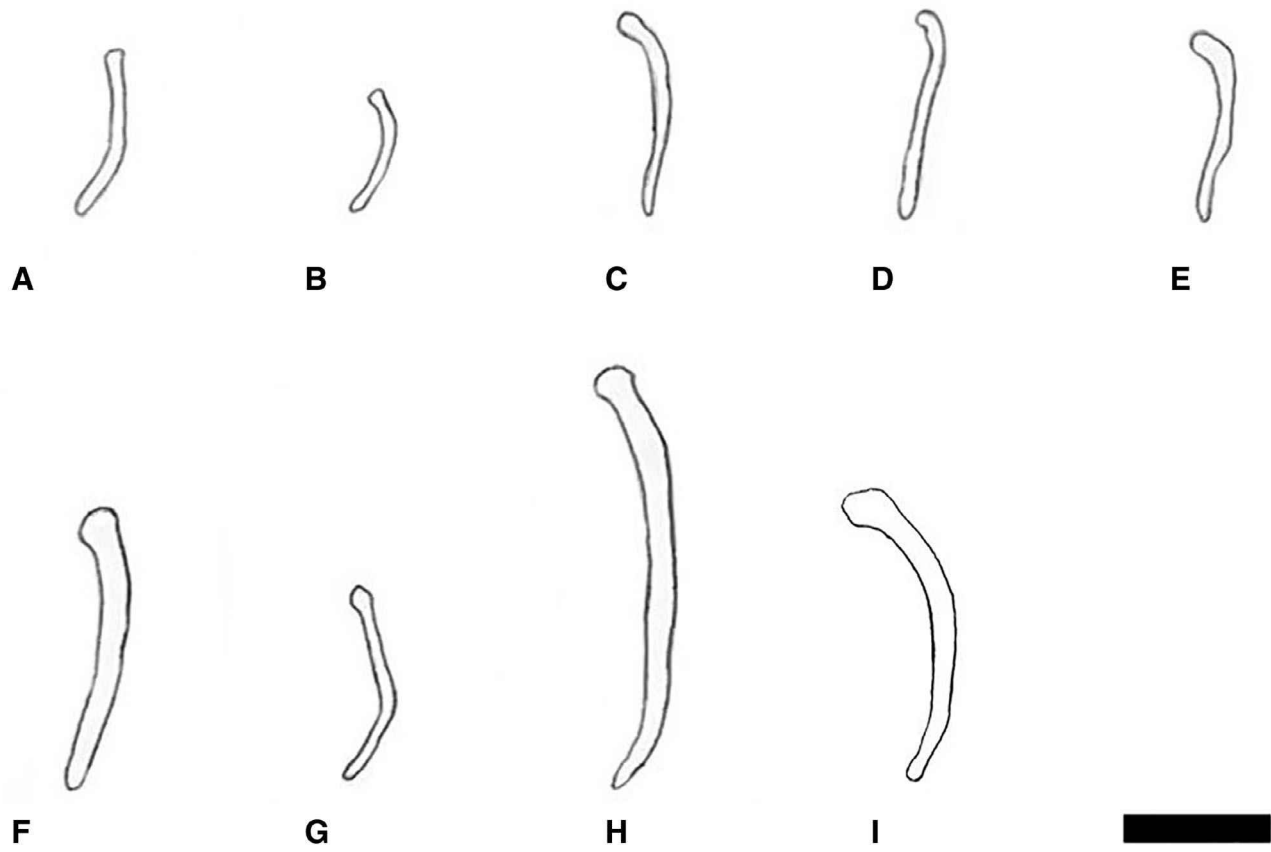
Fig. 5. Schematic representation of the most common clavicles' contours of the neotropical specimens of the suborder Feliformia, family Felidae. *Felis catus* (**A**), *Leopardus guttulus* (**B**), *Leopardus colocolo* (**C**), *Leopardus geoffroyi* (**D**), *Leopardus wiedii* (**E**), *Leopardus pardalis* (**F**), *Puma yagouaroundi* (**G**), *Puma concolor* (**H**), and *Panthera onca* (**I**). Bar: 2 cm.
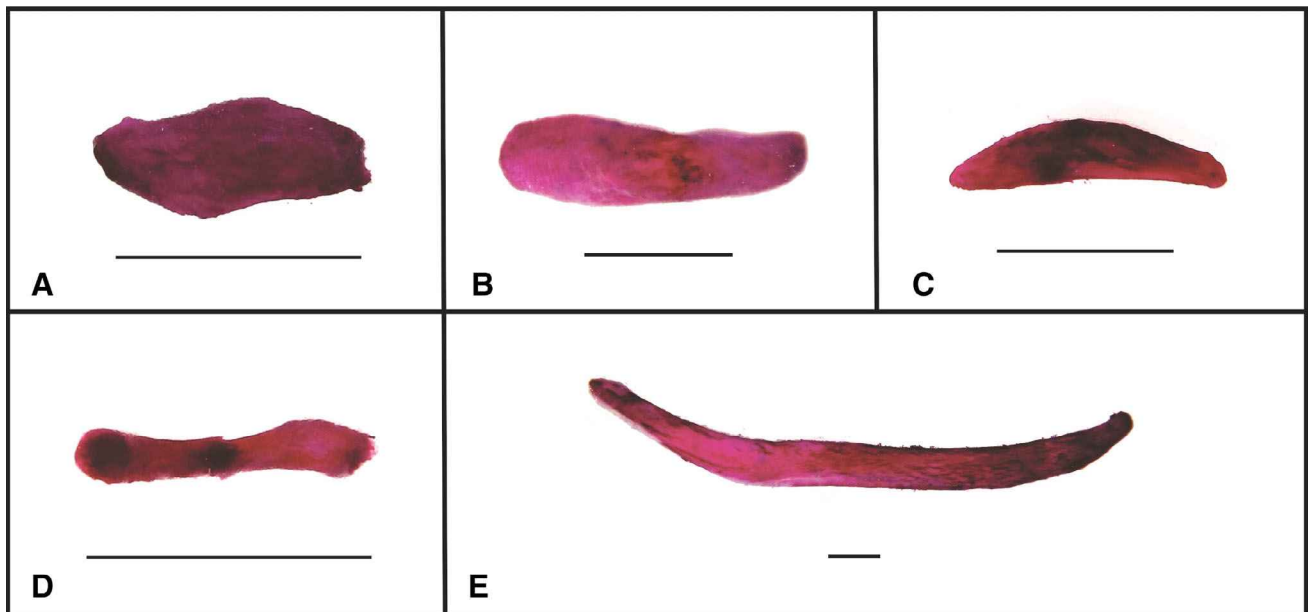
Fig. 6. Clavicle photomacrographs of wild carnivorans submitted to diaphanization technique. Clavicles of *Lycalopex vetulus* (**A**), *Chrysocyon brachyurus* (**B**), *Procyon cancrivorus* (**C**), *Galictis cuja* (**D**), and *Puma concolor* (**E**). Bar: 5 mm.

and Cooper (1975). The bone staining technique was performed as described by Vieira (2011).

RESULTS

Through the dissections, it was possible to determine the presence of clavicles in 16 (84.2%) of the 19 species of neotropical wild carnivores evaluated. Only specimens of *N. nasua* (n = 6), *C. semistriatus* (n = 2), and *C. chinga* (n = 1) had no clavicles. Thus, among the 199 dissected limbs, 181 had a clavicle (91.0%) and only 18 (9.0%) had no clavicle. When present, the clavicles were invariably located deep at the clavicular intersection of the brachiocephalic muscle (Fig. 1), on both the left and right sides.

In the specimens of the families Canidae, Procyonidae, and Mustelidae, the clavicles were always subtle, although perceptible as rigid structures when palpated in the adjacent connective and adipose tissue. From the bone, fibrous bands departed toward the clavicular intersection and the subscapularis muscle, although other delicate fibrous extensions were eventually observed.

In all cases, the clavicle did not establish articulation with other bones. On average, the largest clavicles were from the cadavers of *P. concolor* (51.9 mm), while the smaller ones were from *L. vetulus* (5.1 mm) (Table 1). In individuals of the family Felidae, the clavicles were comparatively more developed and firmly attached to the clavicular intersection. The analysis of variance confirmed the average length of the clavicles of felids was significantly larger ($P < 0.01$) than the other families (Table 2 and Fig. 2).

The male clavicles were significantly larger ($P < 0.05$) than the females in the species *L. gymnocercus*, *G. cuja*, and *L. geoffroyi*, while in *C. thous*, *C. brachyurus*, *L. longicaudis*, and *P. cancrivorus* no difference was observed in Student's t-test (Table 3). In the other species, there was no representative number of clavicles of both sexes that allowed such comparison.

Radiographic evaluations demonstrated different degrees of radiopacity and clavicle shape, as well as documented absence of bone in *N. nasua* (Fig. 3). The most intense radiopacity occurred in the clavicles of felids and less in the canids. The radiographs made it possible to trace the most frequent bone contour for each species (Figs. 4 and 5). The material submitted to diaphanization for alizarin staining also allowed precise delimitation of the bone shape (Fig. 6), as well as the presence of the clavicle already ossified in the fetus of *L. geoffroyi* with approximately 40 days of gestation (Fig. 7).

The histological sections confirmed the presence of compact bone with trabeculation and lamellae filled by bone marrow and different levels of occupation by the chondroid matrix (Fig. 8). Exceptions were specimens of *N. nasua* and *Conepatus* sp. which no histological evidence of bone or cartilaginous tissue was found in the middle of the fibrous tissue collected deeply at the clavicular intersection. In *L. gymnocercus*, the chondroid matrix always predominated over the osteoid matrix.

Based on the findings of the present study and on the descriptions available in the literature, it was possible to map these traits into a schematic phylogeny, adapted
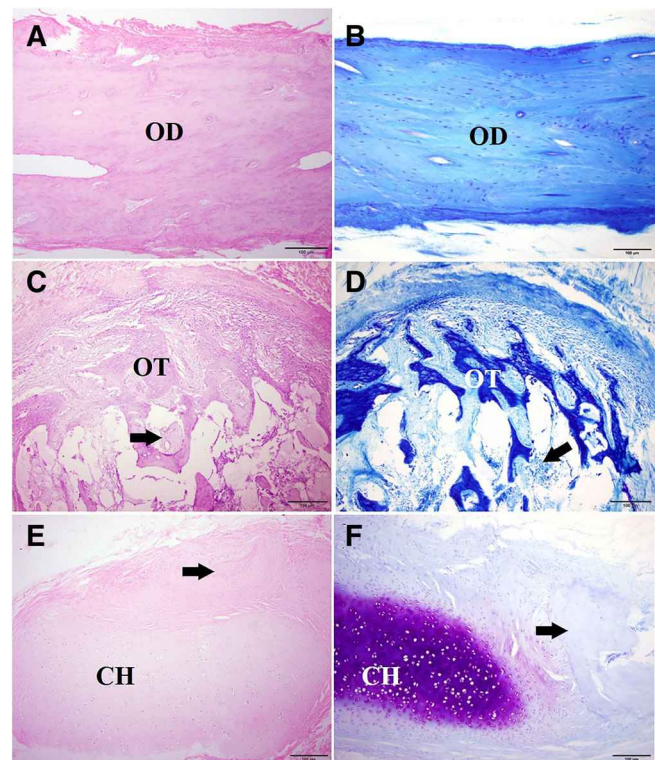

Fig. 7. Photomacrograph of an approximately 40 days-old *Leopardus geoffroyi* male fetus, submitted to diaphanization with alizarin. Yellow arrows: ossified clavicles. Bar: 2 cm.

Fig. 8. (**A**) *Puma concolor*. Dense bone (OD) is observed composing extensive area of the clavicle. Hematoxylin and eosin. Bar = 100 μm. (**B**) *Puma concolor*. Note blue staining of dense bone tissue (OD). Toluidine blue. Bar = 100 μm. (**C**) *Cerdocyon thous*. Clavicle showing trabecular bone (OT) and medullary cavity with bone marrow (arrow). Hematoxylin and eosin. Bar = 100 μm. (**D**) *Cerdocyon thous*. Clavicle composed of trabecular bone strongly stained in blue. Also, note the medullary cavities containing myeloid tissue (arrow). Toluidine blue. Bar = 100 μm. (**E**) *Lontra longicaudis*. Clavicle composed of a large amount of hyaline cartilage (CH) surrounded by fibrous connective tissue. Note the area of the clavicle formed by dense bone (arrow). Hematoxylin and eosin. Bar = 100 μm. (**F**) *Lontra longicaudis*. Note a clavicle formed by an extensive area of hyaline cartilage (CH) presenting violet staining, as well as an area composed of dense bone stained in blue (arrow). Toluidine blue. Bar = 100 μm.

Fig. 9. Phylogeny of the order Carnivora, adapted from Eizirik et al. (2010), revealing the characteristics of the clavicles in the species already studied. Continuous black line (——): represents the species with well-ossified clavicle and comparatively more developed in the order. Dashed line (- - -): represents species with ossified clavicles but reduced in size. Dotted line (......): species in which no clavicle evidence was found. Continuous gray line (——): uninvestigated families. (†)extinct species; [1]Rojas and Montenegro (1995); [2]Hartstone-Rose et al. (2012); [3]Wang (1993); [4]Munthe (1989); [5]McCarthy and Wood (1988); [6]Anyonge (2001); [7]Pagenstretcher (1870); [8]Klatt (1928); [9]Leach (1977); [10]Yousefi et al. (2018); [11]Böhmer et al. (2018); [12]Windle (1888); and [13]Makungu et al. (2015).

from Eizirik et al. (2010), that summarizes the current state of knowledge about the presence of clavicles in the order Carnivora (Fig. 9).

DISCUSSION

Clavicles have their occurrence recorded since the ancestors of the order Carnivora, as in the case of the miacid *Vulpavus ovatus* (Wang, 1993). The findings of the present investigation corroborated the assertion the clavicle is invariably vestigial or absent in the order Carnivora (Jenkins, 1974). When present, it was deeply positioned at

the tendinous intersection of the brachiocephalic muscle, as reported by Parsons (1898). Although present in almost all of the neotropical carnivorans investigated, they always had reduced size, unapparent functional meaning and did not establish contact with any other bone.

The statement that is an often-absent bone in canid by Ewer (1973) was not confirmed, since the bone was found in the 43 specimens (100%) of four different species of wild canids in the present study. It has also been described as vestigial in the extinct canids *Lycaon pictus* (Pagenstretcher, 1870), *Vulpes fulva* (Klatt, 1928), and *Canis lupus* (Anyonge, 2001). McCarthy and Wood (1988)

investigated clavicles of 50 domestic dogs and reported only one dog without evidence of clavicles bilaterally and two others missing one clavicle. In the sampling of the present study, there were no cases of unilateral bone occurrence. The occurrence of clavicles has been described since the extinct canid of the genus *Hesperocyon*, who lived in the Eocene, but not in the fossils of most recent extinct borophagine canids (Wang, 1993). Apparently, the hesperocyoninae ancestors possessed a more developed clavicle, similar to the felids, which was drastically reduced in the borophagines, to the point of not being recognized in the fossils, and remained in the current canids.

Senter and Moch (2015) studied the phylogeny of bone vestigiality in mammals based on the analysis of skeletons available in collections. Regarding the order Carnivora, they proposed, in the suborder Feliformia, the clavicle had become absent from an ancestor of the Hyaenidae family, remaining vestigial only in the Felidae family. In fact, all the felids of the present study had clavicles, although there are no species of the other families of the suborder Feliformia in the neotropics to confirm this hypothesis.

In the Caniformia suborder, it was suggested the clavicle would have disappeared from a common ancestor of the families Ursidae, Mustelidae, and Procyonidae, remaining vestigial in the family Canidae (Senter and Moch, 2015). In fact, extinct canids of the genus *Hesperocyon* sp., which lived for more than 10 million years in the Eocene, had clavicles (Wang, 1993). The investigation in the neotropical specimens of the present study confirmed the presence of clavicles in mustelids (*E. barbara*, *G. cuja*, and *L. longicaudis*) and procyonid (*P. cancrivorus*). The occurrence of clavicle has been reported in studies of myology in the mustelids *Martes pennanti* and *Martes americana* (Leach, 1977), *Martes foina* (Böhmer et al., 2018) and *Martes martes* (Böhmer et al., 2018; Yousefi et al., 2018) and in procyonids *Procyon lotor*, *Nasua fusca*, and *Potos flavus* (Windle, 1888). Only in the procyonid *N. nasua* and in the mephitids *C. chinga* and *C. semistriatus* not even histological traces of the clavicle were found. Martins et al. (2013) approached the radiographic anatomy of the skeleton based on 13 specimens of *N. nasua* and also did not mention the presence of this bone.

Therefore, the hypothesis that the loss of the clavicle occurred in the common ancestor of the mustelids, procyonids, and ursids, as proposed by Senter and Moch (2015), can be rejected and replaced with the more likely scenario that an absence has happened more recently in the evolutionary history of the order Carnivora. The fact that *Ailurus fulgens*, an ailurid phylogenetically close to the mustelids, maintain a vestigial clavicle reinforces this inference (Makungu et al., 2015). Further investigation of a wider range of families would clarify precisely the ancestry of clavicle loss.

This divergence of findings between the two studies may result from sampling performed in different species of the same family or, more likely, a methodological factor. The macrodissection of specimens followed by histological analysis is more reliable to infer the presence or absence of vestigial clavicles than the examination of macerated postcranial skeletons available in collections. For example, all the clavicles of mustelids, procyonids, and canids of the present study were very delicate and could be easily lost during conventional bone maceration to obtain skeletons for collections. Besides that, studies that mentioned the presence of clavicles in species of the family Procyonidae (Windle, 1888) and Mustelidae (Leach, 1977) were descriptions of thoracic limb myology, based on comparative dissections. Although the clavicles of felids are larger, Hartstone-Rose et al. (2012) recognized that the preservation of this bone is often neglected even in the collections of museums with more resources.

The existence of clavicles in current wild felids has already been documented in several species such as *Acinonyx jubatus*, *Caracal caracal*, *Leopardus pardalis*, *Leopardus wiedii*, *Lynx canadensis*, *Lynx rufus*, *Neofelis nebulosa*, *Panthera leo*, *Panthera onca*, *Panthera pardus*, *Panthera tigris*, *Panthera uncia*, *Prionailurus bengalensis*, *Prionailurus planiceps*, and *Puma concolor* (Hartstone-Rose et al., 2012; Carlon, 2014; Senter and Moch, 2015). However, the documentation of clavicles in neotropical and smaller-sized felids was scarce until the present study. Hartstone-Rose et al. (2012) described that *A. jubatus* has a peculiar clavicle, with the extremity facing the acromion thicker and furrowed in relation to other felids, which would serve for a more significant muscular fixation in this species. Comparatively, no clavicles with such a shape were found in specimens of dissected neotropical felines.

The clavicles of the felids are recognized as the most developed of the order Carnivora since the reports of Trotter (1885). Although significantly larger than those of the other families in the present study ($P < 0.01$), the clavicles of the wild felids were vestigial structures and resembled those found in domestic felines. According to Rojas and Montenegro (1995) the clavicle of domestic cats is rudimentary and not functional.

From the ontogenetic point of view, mammal species without clavicles in the adult stage have an embryonic rudiment of this bone. Some genetic information to build a clavicle may persist. However, its development is interrupted and a partial or complete involution occurs (Rojas and Montenegro, 1995). The vestigial or absent nature of the clavicle in the order Carnivora corresponds to the premise that, in mammals, a non-functional clavicle leaves the scapula free for movements in the sagittal plane and increases the length of the stride and velocity of displacement (Anyonge, 2001). On the other hand, well developed and articulated clavicles with other bones, as in primates, are adaptations to allow tasks that depend on the hands away from the trunk, like climbing, swimming and reaching distant objects (Trotter, 1885; Rockwood et al., 2009).

Since felids use thoracic limbs with more versatility than canids, it can be expected the first ones had proportionally larger and more-ossified clavicles as a reflex of the action of the limbs. This hypothesis was mentioned by Trotter (1885), who argued that feline predation depends on more manual versatility because they hunt alone and in ambush, while canids would depend on chasing for a longer period of time and distance and they attack in groups. Even in extinct carnivores, the presence of a well-developed clavicle would be part of a skeletal adaptation to the life in the Eocene forests (Wang, 1993). However, this premise finds divergence in the Carnivora order. Mustelids, procyonids, and mephitids have a greater variety of movements with their hands than canids, but their clavicles are smaller or even absent, as in the case of *N. nasua* and *Conepatus* sp. The *A. jubatus*, an essentially cursorial specialized felid, with postcranial anatomy similar to canids, exhibits a well-developed clavicle, typical of the species of his family (Hartstone-Rose et al., 2012). In

the subfamily Pantherinae, for example, phylogenetically close-related species have clavicles of more similar size and shape, regardless of the mode of predation or habitat (Hartstone-Rose et al., 2012). The evidence available in the literature, enlarged by the present study, is that the clavicle in the order Carnivora seems to have its size, shape, and presence in greater conformity with phylogeny and not so much with the functional demands of the thoracic limb (Fig. 9).

There is evidence the clavicle has been present since the Carnivoramorpha, ancestors who originated the order Carnivora. This is the case of *Vulpavus ovatus*, extinct miacid, who lived between Paleocene and Eocene (33–60 million years ago). In this species, possibly of arboreal habit, the bone was about 40 mm long (Wang, 1993). This feature of longer and ossified clavicle remained in the order Carnivora and seems to have been conserved by the suborder Feliformia to the present felids (Hartstone-Rose et al., 2012; Senter and Moch, 2015). In the suborder Caniformia, the clavicle remained ossified, as evidenced in *Hesperocyon* sp. (Wang, 1993), an ancestral canid that lived between 15 and 40 million years ago. In the Borophagines, the most recent ancestors of the current canids which lived between 2 and 30 million years ago, it seems there is no record of the existence of the clavicle in fossils (Munthe, 1989), possibly due to the very reduced size of the bone, as it occurs with the species of the current suborder Caniformia. This absence of identification of the clavicle in borophagine fossils coincides with the stage of evolution in which the species of canids left the plantigrade posture and they became digitigrade.

From the data of the present study and also data available in the literature, it can be inferred that, although always vestigial, the presence of ossified clavicle in the order Carnivora is an ancestral condition, which has been observed since the miacids and it was preserved in the Felidae family. A clear tendency to reduce the ossification and size of the clavicle is noted in the suborder Caniformia, which characterizes a derived condition. In some species of the Caniformia suborder, it was verified the absence of this structure, as an extreme representation of this derived condition. Once all Felidae present the ancestral condition, it can be expected the clavicle reduction pattern will be less intense in the Feliformia suborder, resulting in more ossified and larger clavicles than the Caniformia suborder. However, this hypothesis requires confirmation with the evaluation of the clavicle morphology of the species of the other families of the suborder Feliformia. Apparently, the degree of ossification and clavicle size in the order Carnivora is not directly related to the versatility of the thoracic limbs but to the phylogenetic history of the groups.

Radiographic examinations of the clavicles allowed determination of the radiopacity and to precisely delimit the bone contour. In almost all species, there was enough radiopacity for such delimitation. In felids, radiopacity was more intense. The exception was *L. gymnocercus'* clavicles, which were not always radiopaque. Among seven specimens of *A. fulgens* x-rayed by Makungu et al. (2015), two (a year and a half and 13 years old) presented the clavicle with enough radiopacity to be visualized in the mediolateral and cranium-caudal images of the glenohumeral region. Thus, in wild animal medicine, the presence of a cranial radiopacity at the scapulohumeral joint corresponding to the clavicle and not to

an anomalous bone or calcified fragment should be considered, as illustrated by Schebitz and Wilkens (1987).

The shape of the clavicles in the Felidae family was like a thin rod, cranially convex with one blunt end (acromial) and the other one pointed (sternal). This curvature seems to be an evolutionary trend of the current species of small-sized felids, since fossils of extinct species and similar size presented a more linear contour (Hartstone-Rose et al., 2012). Beyond the felids, the cranial convexity also predominated in the clavicles of the *C. thous*, *P. cancrivorus*, *E. barbara*, and *L. longicaudis* of the present study and it was reported by Wang (1993) in the extinct canid *Hesperocyon* sp.

With the exception of *C. thous*, the clavicles of the canids had no clearly defined shape, which coincided with the description for domestic dogs (Nickel et al., 1986). Černý and Čižinauskas (1995) mentioned that in domestic dogs the shape was mostly oval, which did not occur in the canids of the present study. Moioli (2014) studied ossification centers in miniature dog breeds and found the clavicle had heterogeneous form among individuals. In the human species, the clavicle is one of the bones with more variation in the shape (Grant, 1989). Through morphometry, De Palma (1957) investigated the shape of 100 pairs of human clavicles and did not even find two exactly alike.

According to Černý and Čižinauskas (1995), the intraspecific variation in clavicles shape results from the reduction the bone undergoes during fetal development. In humans, mice, and rats, the clavicle is one of the first bones to go through ossification during pregnancy (Tran and Hall, 1989; Ronning et al., 1991). Clavicle ossification in domestic cats occurs late, only after halfway through the gestation (53%), when its development is inhibited (Rojas and Montenegro, 1995). In the present study, the diaphanization of a male fetus of *L. geoffroyi* showed ossified clavicles on both sides and it seems to corroborate with such findings in the domestic cat. The gestation of *L. geoffroyi* lasts from 66 to 76 days (Sunquist and Sunquist, 2002), being a little longer than the gestation of the domestic cat, which lasts between 60 and 63 days (McGeady et al., 2017). Therefore, the equation for estimating fetal age of the specimen employing crown-rump and feline mass measurements may have a slightly distorted result in wild felids. The shape of the clavicle of the fetus of *L. geoffroyi* corresponded to what was found in adult specimens. In dogs, the clavicle arises with its membranous part ossified since the 28th day of gestation and grows in length by the addition of bone formed by secondary cartilage until the end of the fetal phase, when it suffers reduction (Evans and DeLahunta, 2013). The reduced nature of the clavicle may result from a decrease or disappearance of the bone in the adult animal or, also, by the absence of an embryonic precursor, thus verified in the loss of other skeletal elements such as fingers (Hall, 2001).

Howell (1937) has mentioned the clavicle is a peculiar bone because it would be the only long bone with intramembranous ossification. Computerized microtomography studies have revealed the human clavicle is a typically long bone with thick cortices in the diaphysis and trabeculae in the epiphyses (Bain et al., 2015). The diaphyseal center of the human clavicle ossifies by an intramembranous process, which is unusual for long bones (Bain et al., 2015). In domestic cats, an identical process was described (Rojas and Montenegro, 1995). In contrast, the epiphyses (secondary

ossification centers) go through an endochondral ossification, where the mesenchymal cells follow a stage of cartilaginous ossification until finally they become bone. Thus, the presence of the chondroid matrix in the histological sections of all clavicles of adult specimens of the present study suggests a mixed intramembranous/endochondral origin in the order Carnivora. This mixed ossification, peculiar to mammals, is recognized as emerging from two ossification centers with a single condensation (Hall, 2001). The phases of this process were detailed by Huang et al. (1997) in murine embryos. However, the greater or lesser participation of the chondroid matrix in the constitution of clavicles is difficult to interpret, because the age and degree of skeletal maturity of the specimens were unknown because they were free-living animals.

It can be concluded that the clavicle in the order Carnivora is vestigial, conserves an ancestral condition in the Felidae family and presents a clear tendency of reduction or even absence in the Caniformia suborder. All indicates that morphological aspects of the collarbone in carnivores are under greater influence of the evolutionary history of the species than the differences in the movement of the thoracic limb.

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