

The Brachial Plexus of the Sumatran Rhino (*Dicerorhinus sumatrensis*) and Application of Brachial Plexus Anatomy Toward Mammal Phylogeny

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Published online: 21 May 2015
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Abstract The peripheral nervous system is a promising resource for testing phylogeny although the branching patterns of peripheral nerves are not well documented outside of *Homo sapiens*. Here we describe the brachial plexus of the rare Sumatran rhinoceros (*Dicerorhinus sumatrensis*). We compare its brachial plexus to that of another perissodactyl (*Equus asinus*), an artiodactyl (*Odocoileus virginianus*), two carnivorans (*Felis catus* and *Neovison vison*), and one primate (*Homo sapiens*) and examine the phylogenetic structure of the resulting data. Brachial plexuses exhibit high rates of intraspecific polymorphism, but polymorphisms cannot be recognized from one specimen. To address concerns of error due to polymorphism, we dissected 52 mink brachial plexuses and compared them to human brachial plexus variation. Both species have numerous types of brachial plexus polymorphisms. Although most individual polymorphisms occur infrequently and unilaterally, because there are numerous types of polymorphisms, most humans and mink exhibit at least one polymorphism per brachial plexus. Parsimony analysis of 15 characters compiled from the brachial plexus data produced a tree that positions Artiodactyla and Perissodactyla as sister taxa, a result consistent with other analyses. Despite a high rate of polymorphism, the peripheral nervous system seems to carry a phylogenetic signal consistent with other morphological data. With a higher rate of taxon sampling, we suggest the brachial plexus will contribute valuable data for phylogenetic testing.

Keywords Peripheral nerves · Rhinoceros · Soft tissue anatomy

Introduction

Morphological phylogenetic character data for vertebrates are predominantly derived from skeletons and to a lesser degree from soft tissue (Gibbs et al. 2002; Diogo and Wood 2011). In comparison to osteology, soft tissue character systems have not been as thoroughly utilized for testing phylogenetic hypotheses. The peripheral nervous system is a promising aspect of soft tissue anatomy because branched peripheral nerves are amenable to binary coding and the branching nature of peripheral nerves is taxonomically diverse. Studies of the more complex regions of the peripheral nervous system, such as the brachial plexus, demonstrate a high degree of anatomical diversity within Mammalia, including Monotremata (Miller 1934; Koizumi and Sakai 1997), Rodentia (Aydin 2003), Carnivora (Miller 1934; Allam 1952), Artiodactyla (Schulte 1914; Moura et al. 2007; Endo et al. 2009; Yoshitomi et al. 2012), Primates (Miller 1934; Chase and DeGaris 1940; Mizuno 1969; Booth et al. 1997; Kikuchi et al. 2011), and Xenarthra (Souza et al. 2014). However, the methods, degree of detail, and accuracy of those published descriptions are highly variable and not always adequate for scoring phylogenetic characters. For example, Levine et al. (2007) reported intraspecific variation in spinal nerve contributions to peripheral nerves of the forelimb of horse, ox, and dog, but did not provide information of the branching patterns of those nerves.

Phylogenetic analyses of mammalian interordinal relationships have most often suggested Artiodactyla as the sister order of Perissodactyla among extant Mammalia (Xu et al. 1996; Springer et al. 2003, 2005; Murphy et al. 2007; Asher

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2007; Prasad et al. 2008; O’Leary et al. 2013). Recent fossil discoveries of Eocene mammals on the Indian subcontinent that are the potential sister taxa of perissodactyls suggest that our understanding of the phylogenetic relationships of ungulate groups is undergoing revision (Cooper et al. 2014; Rose et al. 2014). Comparison of the brachial plexuses of members of these orders may offer insights into this portion of the mammal phylogenetic tree. However, adequate data on brachial plexus anatomy are needed from more species for appropriate taxon sampling for phylogenetic investigations. We could find no documentation of the equid brachial plexus outside of basic veterinary anatomy texts (Budras 2009). Brachial plexuses of other perissodactyls are unknown. Additionally, more data on brachial plexuses are needed to understand the frequency and distribution of intraspecific polymorphisms, which may be common in the peripheral nervous system, but have only been thoroughly investigated in humans (Uysal et al. 2003; Wozniak et al. 2012).

In this paper, we describe, for the first time, the brachial plexus of a Sumatran rhinoceros (*Dicerorhinus sumatrensis*) from a recently deceased zoo specimen and compare it to another member of Perissodactyla (fetal donkey: *Equus asinus*) in addition to representatives of Artiodactyla (white-tailed deer: *Odocoileus virginianus*), Carnivora (domestic cat: *Felis catus*, and American mink: *Neovison vison*), and Primates (human: *Homo sapiens*). Because sample sizes for large and rare species are inevitably small, and often limited to a single specimen, anatomical anomalies cannot readily be differentiated from normal anatomy. Therefore, we were concerned that any of the differences we found in brachial plexus anatomy could be random anatomical anomalies rather than phylogenetically informative species differences. However, if the distribution and or frequencies of brachial plexus polymorphisms are conserved across species, then it may be possible to identify aspects of anatomy that are more and less likely to be highly polymorphic. To test this idea, we dissected 52 brachial plexuses from 26 mink (*Neovison vison*), documented the frequency and distribution of anatomical polymorphisms, and compared these results to published data on fetal human brachial plexus polymorphisms (Uysal et al. 2003; Wozniak et al. 2012).

Materials and Methods

Anatomical terminology follows Nomina Anatomica Veterinaria (International Committee on Veterinary Gross Anatomical Nomenclature 2012).

Following necropsy and evisceration by zoo staff, the rhino specimen (American Museum of Natural History Mammalogy 275978) was cut transversely at the level of the third thoracic vertebra and the left front limb and shoulder were separated from the trunk, leaving the right limb and trunk attached with an intact right brachial plexus. The left brachial plexus was

damaged during removal of the left limb and was not dissected. The unembalmed specimen was subsequently frozen. Dissection began after 5 days of slow thawing in a refrigerated room. The intact brachial plexus of the right thoracic limb was exposed via an approach from the dorsal side, by separating the skin, cutaneous muscles, and deltoid muscle (*M. deltoideus*) from the scapula. Next, the scapula was freed from its attachments to the rhomboids (*Mm. rhomboideus*) and serratus ventralis (*M. serratus ventralis thoracis*). The superior edge (medial border) of the scapula was rotated laterally away from the rib cage by adducting and internally rotating the right forelimb, exposing the underlying structures. The first rib and the ventral lamina of C6 were used as landmarks to determine the vertebral levels for cervical and thoracic spinal nerves. The roots of the brachial plexus were exposed by removing the vertebral muscles, dorsal scalene, and the first three ribs with their associated intercostal muscles. We were careful not to remove epineurium so that the branching points within the brachial plexus remained intact. Terminal nerves were dissected to the point of entry into muscle, skin, or the muscular compartments of the forelimb. Dissections of donkey, cat, and deer were conducted with the same dorsal approach.

Twenty-six specimens of mink were dissected by making an incision in the dorsal midline from the base of the skull caudally to the approximate level of the caudal angle of the scapula (angulus caudalis). The serratus anterior was then separated from the scapula. Next, the roots of the plexus were exposed and followed into the limb. This same dissection was then repeated contralaterally, resulting in dissections of 52 mink brachial plexuses. The mink specimens were purchased from Ward’s Science.

The brachial plexuses were sketched and diagrammed at different stages of the dissection (Figs. 1 and 2). We dissected both right and left brachial plexuses but for simplicity, all brachial plexus diagrams are depicted here as if they were from the left side of the body. Because this study focused on branching relationships of the nerves in the brachial plexus, the brachial plexuses were diagrammed in a standardized way (Fig. 3) from which phylogenetic character data could be derived. The final data consisted of 15 binary characters (Tables 1 and 2). Using humans as an out-group taxon, we conducted a parsimony analysis based on these data using the exhaustive search option in PAUP* 4.0 (Swofford 2003) (Fig. 4). We included all observed phylogenetically informative characters encountered during brachial plexus dissections. We also included characters with autapomorphic distributions (characters 2, 3, 7, 8, 13, 14) because they may become informative non-apomorphic characters when more taxa are eventually included. The polymorphic conditions discovered in the mink brachial plexuses were not phylogenetically informative within the present dataset and for simplicity, were excluded from the data matrix (Table 2), but are listed in Table 3.

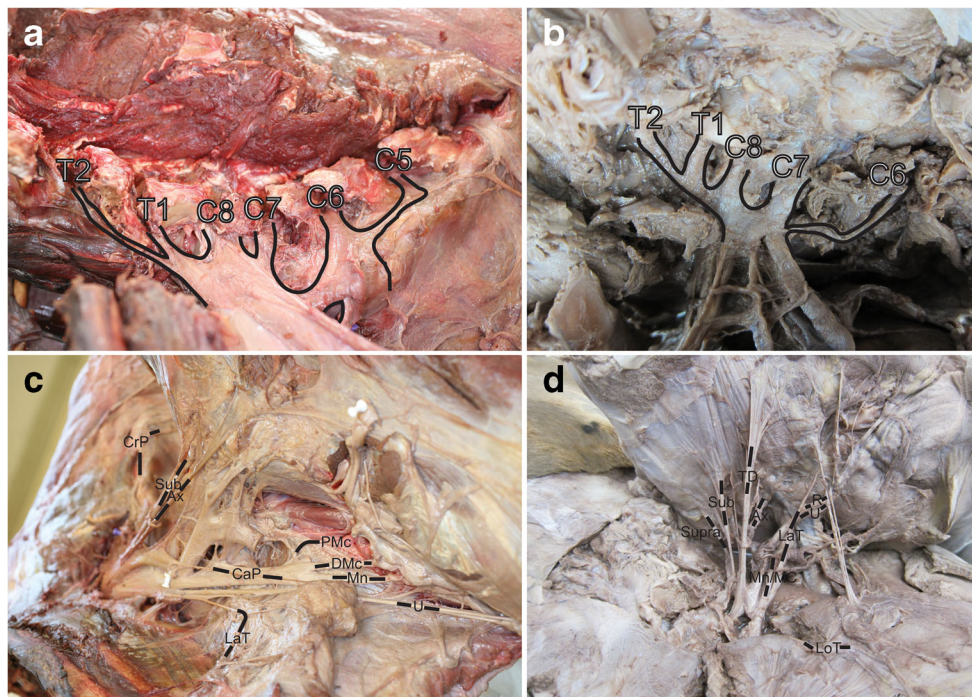


Fig. 1 Examples of dissected brachial plexuses from the dorsal view, with scapula retracted. Black arrow in A points anteriorly. **(a)** Exposed roots (ventral rami) of the brachial plexus of the rhino and **(b)** donkey. Peripheral branches of the brachial plexuses of **(c)** rhino and **(d)** donkey. List of abbreviations: AA – ansa axillaris; Ax – axillary nerve; C5 – fifth cervical vertebra; CrP – cranial pectoral nerve; CaP – caudal pectoral

nerve; DMc – distal musculocutaneous nerve; LaT – lateral thoracic nerve; LoT – long thoracic nerve; Mc – musculocutaneous nerve; Mn – median nerve; P – pectoral nerve; PMc – proximal musculocutaneous nerve; R – radial nerve; Sub – subscapular nerve; Supra – suprascapular nerve; T1 – first thoracic vertebra; TD – thoracodorsal nerve; U – ulnar nerve

Results

Rhino Brachial Plexus Anatomy

The Sumatran rhino brachial plexus is shown (Figs. 1a, c and 2a, 3a). The roots (radices plexus) of the Sumatran rhino brachial plexus (plexus brachialis) arise from the ventral rami (rami ventralium) of spinal nerves C5 through T1 and one branch of the ventral ramus of spinal nerve T2. Spinal nerves C6 and C7 also contribute to a nerve that courses deep to the brachial plexus and passes into the thorax through the thoracic inlet. The nerve was severed during evisceration and it could not be followed to its distal end, but likely is the phrenic nerve (*N. phrenicus*). The ventral ramus of C5 contributes a branch that becomes the cranial pectoral nerve (*N. pectoralis cranialis*), and the remainder of C5 courses in a caudal direction to join the ventral ramus of C6. Those combined nerves join the ventral ramus of C7 forming the cranial trunk (trunci plexus cranialis). The cranial trunk terminates after giving off the suprascapular nerve (*N. suprascapularis*) and another branch (ansa axillaris) that ultimately joins the common trunk of the median and musculocutaneous nerves (*N. medianus* and *N. musculocutaneii*). The remainder of the cranial trunk joins the caudal trunk (trunci plexus caudalis). The caudal trunk is formed by the ventral rami of C8 and T1 as well as the contribution of T2, which join simultaneously cranial to

the first rib (*Costa prima*). The caudal trunk emerges between the dorsal scalene (*M. scalenus dorsalis*) and the medial scalene (*M. scalenus medius*) to coalesce with a branch of the cranial trunk forming the brachial plexus.

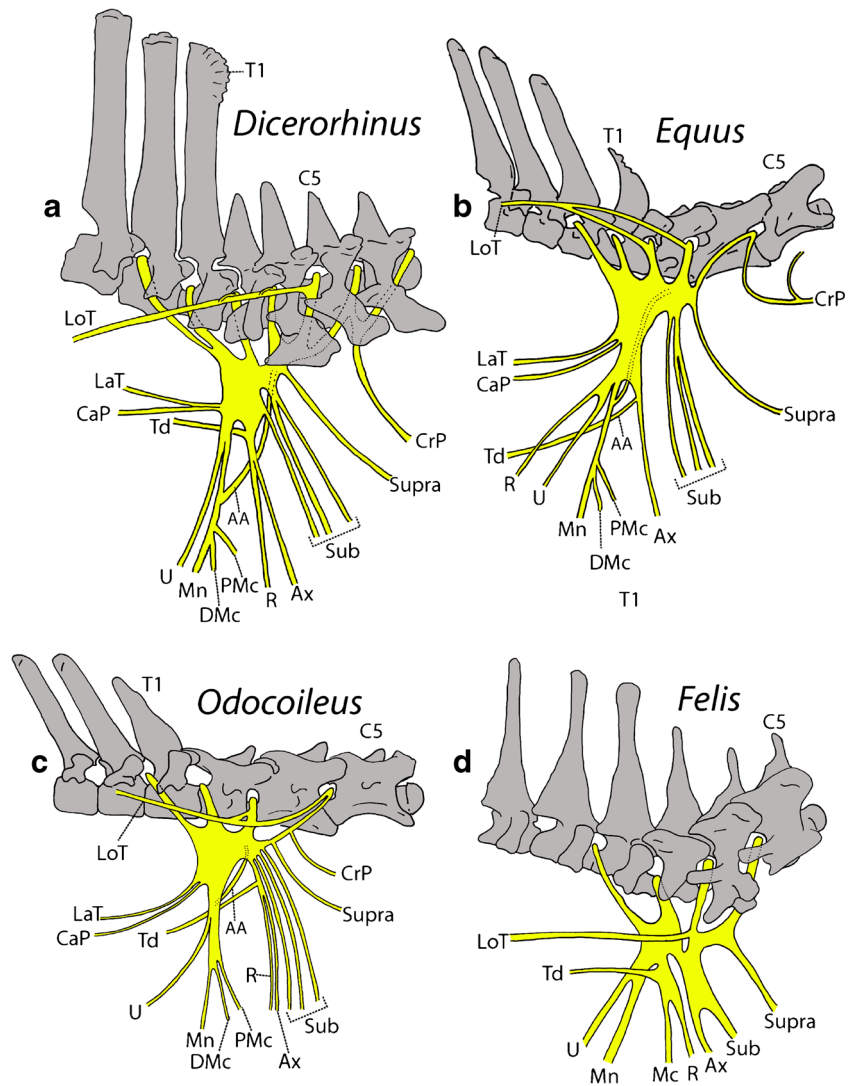
Prior to joining their respective trunks, C6, C7, and C8 contribute dorsal branches that merge and progress caudally, forming the long thoracic nerve (*N. thoracicus longus*). The nerve courses deep to the dorsal scalene to the first rib where it diverges and enters serratus ventralis (*M. serratus ventralis thoracis*).

The ventral ramus of C5 contributes a cranial branch that receives a contribution from the ventral ramus of C4 to become the cranial pectoral nerve, which continues ventrally in parallel with three supraclavicular nerves (*Nn. supraclaviculares*), and enters the subclavius (*M. subclavius*). This nerve is not figured.

From the cranial edge of the cranial trunk, the suprascapular nerve (*N. suprascapularis*) pierces the subscapular fascia (*fascia subscapularis*) between the supraspinatus (*M. supraspinatus*) and the subscapularis (*M. subscapularis*) and crosses the cranial border of the scapula (*margo cranialis scapulae*).

The subscapularis muscle is penetrated by three subscapular nerves that arise from the cranial edge of the brachial plexus. Caudal to the origin of the caudal-most subscapular nerve, the axillary nerve (*N. axillaris*) emerges from the brachial plexus and approaches the caudal edge of

Fig. 2 The brachial plexuses of (a) *Dicerorhinus sumatrensis*, (b) *Equus asinus*, (c) *Odocoileus virginianus*, and (d) *Felis catus* showing relationship with the vertebrae. See Fig. 1 for abbreviations



subscapularis. The axillary nerve divides into several branches (rami musculares) distributed across the caudal edge of subscapularis and the cranial edge of teres major (*M. teres major*). The dorsal portion of the brachial plexus separates to form the radial nerve (*N. radialis*) which then enters the triceps (*M. triceps brachii*). The thoracodorsal nerve (*N. thoracodorsalis*) arises from the ventral surface of the radial nerve. This nerve remains dorsal to the other branches of the plexus, enters the lateral subcutaneous tissue, and ends abruptly where the specimen was separated for storage.

The ventral brachial plexus divides into two branches distally to the aforementioned dorsal nerves. The cranial branch is the common branch of the median and musculocutaneous nerves, which courses dorsalward to the axillary artery (*A. axillaris*). The ansa axillaris contributes to this branch. Distal to the ansa axillaris this common branch splits into proximal and distal musculocutaneous nerves (ramus muscularis proximalis and ramus muscularis distalis) and the median nerve. All branches of musculocutaneous and

median nerves enter the ventral compartment of the arm. From the caudal branch of the ventral brachial plexus, three branches diverge at the same point. The most distal, the ulnar nerve (*N. ulnaris*), proceeds into the arm without branching and passes along the medial side of the olecranon process (olecranon). The next sequential caudal branch, the caudal pectoral nerve (*N. pectoralis caudalis*), proceeds caudally into the pectoral muscles (*Mm. pectoralis*). The most proximal branch is the lateral thoracic nerve (*N. thoracicus lateralis*), which travels parallel to the caudal pectoral nerve and runs on the lateral surface of the serratus ventralis.

Brachial Plexuses Within Perissodactyla

In the following discussion of comparative brachial plexus anatomy, observations used as phylogenetic characters are indicated in parentheses according to their character number in Table 1. We located four differences between the rhino and equid brachial plexuses. (1) The brachial plexus roots of the

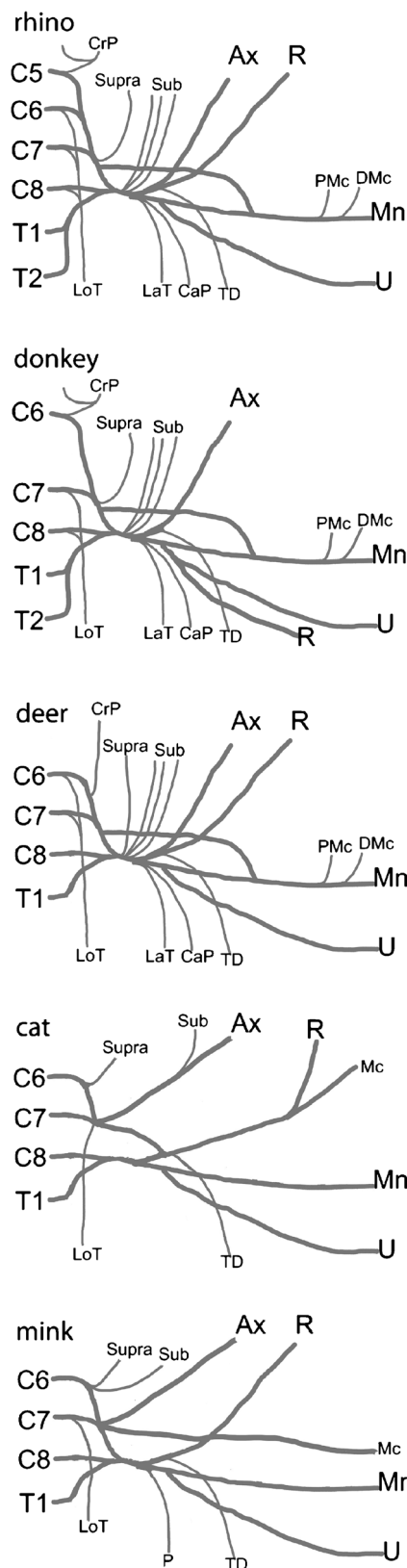


Fig. 3 Schematic diagrams of brachial plexuses showing branching relationships from which the phylogenetic data in Table 3 were derived. See Fig. 1 for abbreviations

Table 1 Phylogenetic characters derived from comparative observations of brachial plexus anatomy

1. Cranial-most root of the brachial plexus always or most frequently originates from: (0) C5, (1) C6.
2. (0) The roots of the brachial plexus traverse the same interscalene space, (1) the roots of the cranial trunk pass between the ventral and medial scalene muscles, while the caudal trunk passes between the medial and dorsal scalene muscles.
3. (0) C8 does not contribute to the long thoracic nerve, (1) C8 contributes to the long thoracic nerve.
4. (0) The radial and ulnar nerves are separate, (1) the radial and ulnar nerves run jointly.
5. Caudal-most root of the brachial plexus always or most frequently originates from: (0) T1, (1) T2.
6. (0) Cranial and caudal trunks do not join together and the ansa axillaris is absent, (1) cranial and caudal trunks join into a single division or cord, ansa axillaris present.
7. (0) C7 root forms a separate trunk, (1) C7 root merges with the cranial trunk.
8. (0) The suprascapular nerve branches from the cranial trunk, (1) the suprascapular nerve branches more distally where the cranial and caudal trunks join.
9. (0) Three subscapular nerves branch from single region of the brachial plexus, (1) subscapular nerve branches are reduced in number and/or branch from other regions of the brachial plexus.
10. (0) A single musculocutaneous nerve branches from the brachial plexus, (1) two musculocutaneous nerves branch from the brachial plexus.
11. (0) Multiple pectoral nerves (lateral thoracic pectoral, caudal pectoral, and human homologies) branch directly from the brachial plexus, (1) a reduced number of discrete pectoral nerves directly branch from the brachial plexus.
12. (0) The musculocutaneous nerve joins the median nerve after receiving a contribution that travels around the axillary artery, (1) the musculocutaneous nerve travels parallel to the median nerve without joining it.
13. (0) The axillary and radial nerves emerge from a single cord, (1) these nerves arise separately from the roots of the brachial plexus.
14. (0) The subscapular nerve branches from the C6 root and is not associated with the axillary nerve, (1) the axillary and subscapular nerves run together and separate distal to the cranial trunk.
15. (0) The musculocutaneous and radial nerves are separate, (1) the musculocutaneous and radial nerves are joined and branch apart distally. State 1 may be a polymorphism in the cat.

Sumatran rhino receive contributions from C5. C5 contributions were not found in the brachial plexus of our dissected donkey specimen, nor were C5 contributions found among ten horse specimens (Levine et al. 2007). (2) The roots of the equid brachial plexus all pass between the ventral and medial scalene muscles. In the rhino, the roots of the cranial trunk (C5, C6, C7) pass between the ventral and medial scalene muscles, while the caudal trunk (C8, T1, T2) passes between the medial and dorsal scalene muscles. (3) The long thoracic nerve of the rhino brachial plexus originates from C6, C7, and C8, whereas the equid long thoracic nerve lacks a connection with C6. (4) The radial and ulnar nerves of the donkey course

Table 2 Assigned phylogenetic character states from brachial plexus data

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Dicerorhinus sumatrensis</i>	0	1	1	0	1	1	1	0	0	1	0	0	0	0	0
<i>Equus asinus</i>	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0
<i>Odocoileus virginianus</i>	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0
<i>Felis catus</i>	1	0	0	0	0	0	1	0	1	0	1	1	1	1	1
<i>Neovison vison</i>	1	0	0	0	0	0	1	0	1	0	1	1	1	0	0
<i>Homo sapiens</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

closely together in parallel and part where the radial nerve enters the dorsal (posterior) compartment of the upper limb, whereas in the rhino, the radial and ulnar nerves branch more proximally and are widely separated before the radial nerve enters the dorsal compartment. The more closely associated radial and axillary nerves of the rhino resemble artiodactyls and humans and may represent the more plesiomorphic condition, whereas the more distant association of these two nerves in the equid may be derived.

Perissodactyl Brachial Plexus Compared to Other Orders

Perissodactyl and artiodactyl brachial plexuses are similar in structure and overall complexity. (5) Unlike perissodactyls, the deer brachial plexus (Figs. 2c and 3c) lacks a T2 root. The long thoracic nerve originates from C6 and C7. (3) In perissodactyls, but not deer, the long thoracic nerve includes a contribution from C8. (8) The suprascapular nerve branches from the brachial plexus of the deer distal to the point where the cranial and caudal trunks join, whereas in the perissodactyls, the suprascapular nerve branches more proximally from the cranial trunk. Otherwise, the branching relationships within the deer brachial plexus are undifferentiated from that of the

rhino and differ further from the equid brachial plexus only with respect to the relationships of the axillary, radial, and ulnar nerves mentioned earlier (4).

The carnivoran (cat and mink) brachial plexuses (Figs. 2d and 3d, e) are simpler and structurally quite different from those of perissodactyls (and artiodactyls). (6) The primary distal branches (radial, ulnar, median, musculocutaneous nerves) branch from separate cranial and caudal trunks, whereas in perissodactyls and artiodactyls these trunks join together before giving off these major branches. The carnivoran brachial plexus has fewer peripheral branches, including a single musculocutaneous nerve (10), and, in the case of the mink, a single subscapular nerve (9). The perissodactyl and artiodactyl brachial plexuses have up to 15 branches with multiple musculocutaneous and subscapular branches. (11) The lateral thoracic and caudal pectoral nerves were not observed as individual branches in either the cat or mink. The mink has a single pectoral nerve branching from the brachial plexus. None of the nerves that innervate the pectoralis muscles of the cat appear to emerge discretely from the brachial plexus itself, and may emerge more distally from peripheral branches. (12) An ansa axillaris is not present in the carnivoran brachial plexus. In perissodactyl and artiodactyl brachial plexuses, the musculocutaneous nerve joins the median nerve after receiving a contribution that travels around the axillary artery. In cat and mink, the musculocutaneous nerve travels parallel to the median nerve without joining it. (13) The axillary and radial nerves emerge from a single cord in the equid, rhino, deer, but arise separately from the roots of the brachial plexus in the cat and mink.

Two differences were found between the cat and the mink brachial plexuses although these differences could be polymorphisms. (14) Gilbert (1993) illustrates a cat brachial plexus with three subscapular nerves branching from different regions of the brachial plexus. The first and second subscapular nerves branch from a common trunk originating from the axillary nerve. The nerve labelled as the third subscapular nerve is the thoracodorsal nerve (Gilbert 1993). In our dissection of the cat, we observed a single subscapular nerve running with the axillary nerve, and branching off relatively distally. This single subscapular branch splits further distally into the two subscapular branches. In the mink, the only observed subscapular nerve branches from the C6 root. (15) The

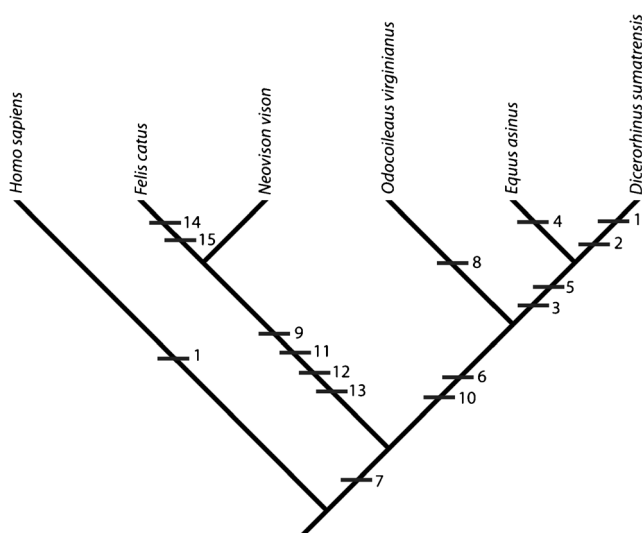


Fig. 4 The shortest tree resulting from PAUP analysis of brachial plexus characters. Character state transformations are labeled according to character number as shown in Table 2

Table 3 Intraspecific variation observed in 52 brachial plexuses from 26 mink (*Neovison vison*)

Character	Normal	Polymorphism 1	Polymorphism 2
1. C6 cutaneous branch	absent N=48 (92 %)	present N=4 (8 %)	
2. long thoracic nerve origin	C7 N=45 (86 %)	C6 & C7 N=5 (10 %)	C6 N=2 (4 %)
3. C7 and C8 roots	form separate trunks N=50 (96 %)	join a single trunk N=2 (4 %)	
4. C8 and T1 roots	form separate trunks N=29 (56 %)	join the same trunk N=23 (44 %)	

musculocutaneous and radial nerves of the cat run together and branch distally. Gilbert (1993) figured a cat brachial plexus that shows separate musculocutaneous and radial nerves, suggesting that a joined radial and musculocutaneous nerve may be a polymorphism. In the mink, these nerves are not joined – the musculocutaneous nerve branches directly from the cranial trunk and the radial nerve from the caudal trunk. (7) The human brachial plexus differs from all of the other included taxa in having a separate trunk from the C7 root.

Brachial Plexus Polymorphisms

Human brachial plexus polymorphisms in the branching pattern of the brachial plexus are frequently reported in the anatomical literature. The majority of these papers report individual anomalies or polymorphism rates for parts of the brachial plexus (e.g., cords). For humans, individual branching polymorphisms seem to vary in frequency from <1 %–37 % and the distribution of these polymorphisms appears to be random with no tendencies to occur more frequently in either sex or even bilaterally (Lee et al. 1992; Uzun and Bilgic 1999; Fazan et al. 2003; Pandey and Shukla 2007; Satyanarayana et al. 2009; Sinha et al. 2012; Darji et al. 2013). Individual polymorphisms occur with a similar range of frequencies in squirrel monkeys (*Saimiri sciurens*) (Mizuno 1969). Studies that consider the entire human brachial plexus found that a majority of humans (54 %) have one or more brachial plexus polymorphisms (Uysal et al. 2003; Wozniak et al. 2012). Our mink sample shows a similar polymorphism rate with 62 % having one or more anomalies. The human brachial plexus is traditionally divided into five regions (from proximal to distal – roots, trunks, divisions, cords, branches). Among human brachial plexuses, division variants are reported to be most common (30–33.64 %), followed by roots (28.5 %–15.90 %) and cords, (12.5 %–18.18 %); trunk polymorphisms (11–5.45 %) are less frequent. (Percentages are based on Uysal et al. (2003) and Wozniak et al. (2012), respectively. Uysal et al. (2003) reported branch polymorphisms to be infrequent (12 %). Although many different types of polymorphisms occur in the human brachial plexuses, we found only four polymorphic characters in the 52 brachial plexuses among 26 mink. The

schematic of the mink brachial plexus (Fig. 3e) demonstrates the most commonly observed conditions. Polymorphisms are listed in Table 3. In 8 % of cases ($N=4$, distributed among three individuals), a small nerve branches from the C6 root and proceeds cranially. The mink specimens were previously skinned, and we were unable to determine the distal distribution of this nerve, although it is likely to have been a cutaneous nerve. In one specimen the anomalous condition occurred twice in the same individual (bilaterally), but occurred only once (unilaterally) in two mink. Secondly, the long thoracic nerve of the mink most frequently originates from the C7 root, but from the C6 root in 4 % of cases ($N=2$) and from the C6 and C7 roots in 10 % of cases ($N=5$). The anomalous conditions always occurred unilaterally. Thirdly, the C7 and C8 roots join in 4 % of cases ($N=2$) before giving off the first terminal branches. This anomalous condition always occurred unilaterally. Finally, the C8 and T1 roots join before the first terminal branches in 44 % of cases ($N=23$). This final condition is the most common polymorphism observed in the mink. Among the 15 mink expressing this condition, seven expressed the condition on only one side, while eight expressed this condition on both sides. Four of the mink brachial plexuses exhibited two polymorphisms and in all cases (twice on the right side and twice on the left side) they were unilateral. In two left cases, polymorphisms for characters 2 and 4 (Table 3) were associated. In one right case polymorphisms for characters 1 and 4 were associated and in the final right case, polymorphisms for characters 3 and 4 were associated. Given the rate of occurrence of the individual polymorphisms in mink brachial plexuses, these associations appear to be randomly distributed.

Phylogenetic Analysis of Brachial Plexus Anatomy

An exhaustive search in PAUP using the 15 binary brachial plexus characters in Table 2 yielded a single shortest tree (Fig. 4) (length=16) that positions Artiodactyla and Perissodactyla as sister taxa, exclusive of Carnivora. Only one character (character 1) exhibited homoplasy. This result is consistent with hypothesis based on cranio-dental morphology (Wible et al. 2005) and other molecule-morphology

combined studies (Asher 2007; O’Leary et al. 2013) that support a close perissodactyl-artiodactyl relationship.

Discussion

Brachial Plexus Polymorphisms

A potentially high rate of intraspecific polymorphism could be of concern for phylogenetic studies, particularly when rare species are included, because an anatomical anomaly could be mistaken for a phylogenetically substantive interspecific difference. All phylogenetic studies run a risk of error due to sampling errors. However, we were particularly concerned with brachial plexus data because human brachial plexuses exhibit high rates of polymorphism. Mink brachial plexuses were found to show a similarly high rate of polymorphism, and at this point, it seems reasonable to suspect that all or most mammalian brachial plexuses are highly polymorphic. Because intraspecific polymorphisms are common in brachial plexuses, we were concerned that our description of the Sumatran rhinoceros brachial plexus, for which only one side of one specimen was available for dissection, would likely include one or more unrecognized anatomical anomalies. For example, it is difficult to know if one or more of the differences between the rhino and donkey brachial plexuses described above are rare anatomical variants that may occur in both species or if they are fixed characters. Our comparison of the donkey brachial plexus (Figs. 1b, d and 2b, 3b) to a published figure of a horse brachial plexus (Budras 2009) did not reveal any variations in the branching pattern of the brachial plexus within equids. This is not to say that equid brachial plexus polymorphisms do not exist, but, rather, we were unable to identify any with the data available to us.

The Sumatran rhinoceros is one of the rarest living mammal species. Studies on Sumatran rhino soft tissue anatomy include myology and reproductive anatomy (Garrod 1873; Kjaersgaard 1974; Maluf 1993; Zahari et al. 2002). We could find no papers detailing the anatomy of the peripheral nervous system for the Sumatran rhino, or any other species of rhino. Opportunities for dissecting large samples of rhino or other rare taxa are infrequent. However, if polymorphisms in other species, such as humans and mink, have a similar frequency and/or pattern of distribution across the brachial plexus, such patterns would provide insights into what aspects of the brachial plexus tend to have low frequencies of intraspecific polymorphisms. If regions where polymorphisms are either rare or predictable are conserved across species, one could readily identify regions of the brachial plexus in which random polymorphisms are least likely to occur. Brachial plexus polymorphisms in humans are distributed across the entire brachial plexus but more frequently occur in the intermediate region of the brachial plexus (divisions) with fewer polymorphisms

in the more proximal and distal regions. All polymorphisms in the mink were located in relatively proximal areas and had to do with trunks formed by the C6, C7, and C8 roots and peripheral nerves that originate from the proximal part of the brachial plexus (a cutaneous branch of the C6 root and the long thoracic nerve) (Table 1). To the extent that the human and mink brachial plexus are comparable, there is consistency in polymorphism frequency, but not clearly in the distribution of polymorphisms. Nonetheless, in both species, polymorphisms tend to be infrequent among the portions of the brachial plexus corresponding to the roots, cords, and terminal branches arising from the distal regions of the brachial plexus. Phylogenetically informative characters appear to be distributed throughout the brachial plexus, from the proximal roots to the branching relationships of the distal-most branches.

Thorough documentation of brachial plexus polymorphisms in a wider variety of species will be useful in understanding the extent to which random polymorphisms contribute to phylogenetic error. Anatomical anomalies, particularly rare ones, are often unilateral and can be identified by dissecting both sides. However, the one common polymorphism encountered in the mink (C8 and T1 roots joining the same trunk) occurred bilaterally about half the time. Despite the high potential for sampling anatomical anomalies, phylogenetic analysis of 15 binary characters coded from brachial plexus data yielded a phylogenetic result consistent with other sources of character data (e.g., bones, molecules) for the interrelationships of perissodactyls, artiodactyls, and carnivorans. This encouraging result suggests small sample sizes (necessitated by species rarity) and a high rate of intraspecific polymorphism do not undermine our ability to identify a phylogenetic signal in brachial plexus data.

Although the degree of taxon sampling in this study is not sufficient to offer definitive resolution regarding the particular interordinal relationships tested here, this study does suggest the brachial plexus, and the peripheral nervous system at large, to be a promising character system for testing phylogenetic hypotheses. We suggest that such data sampled from a greater pool of taxa can be incorporated into larger, more comprehensive phylogenetic tests.

Acknowledgments We thank Darren Lunde and Eileen Westwig at the American Museum of Natural History for access to the rhino specimen. We are grateful to Kurt Amsler, Brian Beatty, Bennett Futterman, Jonathan Geisler, Robert Hill, and Annette Taranowicz for assistance and to the New York College of Osteopathic Medicine Office of Research for funding.

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