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ORIGINAL ARTICLE

Yutaro Natsuyama et al., Anatomical study of brachial plexus in Marsupials

Anatomical study of brachial plexuses of a koala, a Tasmanian devil, and a common ringtail possum

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ABSTRACT

Background: Marsupials have a narrower range of forelimb morphological features than placental mammals. It is hypothesized that this is due to a constraint in the reproductive biology of marsupials. The constraint is that newborn marsupials must crawl into their mother’s pouch. However, anatomical knowledge of the brachial plexus in marsupials is scarce and has not been discussed. In the present study, the purpose is to examine the anatomy of the brachial plexuses of a koala, a Tasmanian devil, and a common ringtail possum and to discuss the brachial plexus of marsupials with reference to the previous reports.

Materials and methods: One adult koala (*Phascolarctos cinereus*) specimen, one adult Tasmanian devil (*Sarcophilus harrisii*), and one adult common ringtail possum (*Pseudocheirus peregrinus*) were used in this study.

Discussion: The ventral rami of C5, C6, C7, C8, and T1 formed the brachial plexus in all 3 marsupials. Each nerve branch differed by one segment among the 3 marsupials. Therefore, the brachial plexus was considered in the form of a few differences among marsupials.

Conclusions: Because of a quite difficulty of getting an opportunity for anatomical examination on marsupials, an accumulation of cases like the present study is needed for future quantitative and qualitative analyses of the brachial plexus pattern of the marsupials.

Keywords: marsupials, brachial plexus, koala, Tasmanian devil, common ringtail possum, mammals

INTRODUCTION

Marsupials are mammals that have several unique adaptations. Particularly, marsupials have a lower variation of forelimb structures than placental mammals [5, 21, 27, 28, 29, 30]. It is hypothesized that this is due to a constraint in the reproductive biology of marsupials. The constraint is that newborn marsupials must crawl into their mother's pouch [8, 14, 16, 20, 31]. However, anatomical knowledge of the brachial plexus in marsupials is scarce and has not been discussed.

The results of recent gene sequencing studies indicate that marsupials and eutherian mammals arose concurrently approximately 180 million years ago [24]. The prevailing view is that the first marsupials originated in the New World, either North or South America, and subsequently migrated to Australia and Southeast Asia, potentially via Antarctica [10, 33, 34, 37, 38]. In response to habitat differences, marsupials adapt to different lifestyles. For example, they can be terrestrial, arboreal, burrowing, or aquatic.

Arboreal marsupials include koalas and common ringtail possums. The diet of the koalas is primarily comprised of eucalyptus, and its forelimbs have evolved for vertical climbing in Australia's eucalyptus forests [6, 12]. As the population of koalas declines [9] and their conservation status is increasingly threatened [3, 36], opportunities to address questions about their anatomical adaptations are becoming increasingly limited. A burrowing marsupial is the Tasmanian Devil. While the hypothesis that marsupials have little variation in their forearms is a strong one as described above, there is another study of an opposing opinion [22].

To clarify this conflicting hypothesis, we dissected the brachial plexuses of 3 marsupials in the present study.

MATERIALS AND METHODS

Specimens of one adult koala (*Phascolarctos cinereus*), one adult Tasmanian devil (*Sarcophilus harrisii*), and one adult common ringtail possum (*Pseudocheirus peregrinus*) were used in this study. The specimens were obtained from the Discipline of Medicine, University of Adelaide, South Australia. No animals were killed for this study. All 3 animals had been previously fixed and stored in 10% buffered formalin after removal of thoracic and abdominal organs. These specimens had previously been used for the education of comparative anatomy at the University of Adelaide, but their forelimbs were intact. The original weight of the samples is unknown because the thoracic and abdominal organs had been removed before the authors' dissection.

Macroscopic dissection

Standard brachial plexus dissection techniques were performed on all 3 specimens. Briefly, the skin was incised in the ventral midline of the trunk and reflected dorsally. We use standard anatomical terminology from the Nomina Anatomica Veterinaria, 6th edition (Nomina anatomica veterinaria). All findings were photographed and illustrated using Adobe Illustrator

2024 (Adobe Inc., San Jose, CA, USA). After illustration, the nerve root of each nerve was observed by stereomicroscope (OLYMPUS SZX7, Olympus, Tokyo, Japan).

RESULTS

Koala

The brachial plexus was formed by the ventral rami of the C5, C6, C7, C8, and T1 in the koala (Fig. 1). C5–C6 merged to form a nerve trunk to which C7 joined; a branch from the nerve trunk where C8 and T1 merged later joined the C5–7 nerve bundle to form the median nerve. The phrenic nerve was formed by C4–5, and the suprascapular nerve was formed by C5. The subscapular nerves had multiple branches, which were formed from C5–6. The long thoracic nerves were formed from C5–7. The caudal pectoral nerves were formed from C8–T1. The right and left cranial pectoral nerves were formed from C6–7 and C7, respectively. The lateral thoracic nerve was formed from C7–8. The thoracodorsal nerve was formed from C7–8. The musculocutaneous nerve was formed from C5–6, and the median nerve was formed from C6–T1. The right and left axillary nerves were formed from C6–7 and C6, respectively. The radial nerve was formed from C6–T1, and the ulnar nerve was formed from C8–T1.

Tasmanian devil

The brachial plexus was formed by the ventral rami of the C5, C6, C7, C8, and T1 in Tasmanian devil (Fig. 2). C5–C6 merged to form a nerve trunk, and C8 and T1 also merged to form another nerve trunk. C7 merged with C5–6 on the right, but not on the left. The phrenic nerve was formed by C4–5, and the suprascapular nerve was formed by C5–6. The subscapular nerves had multiple branches, which were formed from C5–6. The long thoracic nerves were formed from C5–7. The caudal pectoral nerve was formed by C7–T1. The cranial pectoral nerve was formed from C5–7. The left thoracodorsal nerve was formed by C7. The right and left musculocutaneous nerves were formed from C5–7 and C5–6, respectively. The median nerve was formed from C5–T1. The right and left axillary nerves were formed from C5–7 and C5–6, respectively. The right and left radial nerves were formed from C7–T1 and C6–T1, respectively. The ulnar nerve was formed from C7–T1.

Common ringtail possum

The ventral rami of the C5, C6, C7, C8, and T1 in common ringtail possum formed the brachial plexus (Fig. 3). C5–C6 merged to form a nerve trunk to which C7 joined; a branch from the nerve trunk where C8 and T1 merged joined the C5–7 nerve bundle to form the median nerve. The phrenic nerve was formed by C4, and the suprascapular nerve was formed from C5–6. The subscapular nerves had multiple branches, which were formed from C5–6. The long thoracic nerves were formed from C5–7. The caudal pectoral nerve was formed from C8–T1. The left cranial pectoral nerve was formed from C6–7. The right and left musculocutaneous nerves were formed from C5–6 and C5–7, respectively. The right and left median nerves were formed from C5–T1 and C6–T1, respectively. The right and left axillary nerves were formed from C5–7 and C6–7, respectively. The right and left radial nerve was formed from C7–T1 and C6–T1, respectively. The ulnar nerve was formed from C8–T1.

DISCUSSION

The present study found that the ventral rami of the C5, C6, C7, C8, and T1 formed the brachial plexus in all 3 marsupials. Each nerve branch differed by one segment among the 3 marsupials (Tab. 1).

Previous studies have reported that marsupials have a lower valuation of forelimb structures than placental mammals [5, 21, 27–30]. However, anatomical knowledge of the brachial plexus in marsupials has been still insufficient to discuss its variations. In particular, the brachial plexus of the Tasmanian devil and the common ringtail possum have not been studied, so this is the first report of brachial plexus pattern of these marsupials. We obtained two arms from each animal for this research and performed a case study with no accessing a variation in each marsupial.

Previous studies have shown that the brachial plexus of primates (arboreal animal) is mostly in the C5–T1 range [1, 2, 7, 17, 25, 32], while, in terrestrial animals (such as Carnivora), the brachial plexus is often in the C6–T1 [11, 19, 35]. A recent study of brachial plexus ranges of koalas and possums presented results similar to those of the present study [19]. This suggests that the koala, Tasmanian devil, and common ringtail possum are arboreal rather than terrestrial.

The gestation period of marsupials is relatively short, with a range of 8 to 42 days [23]. The young are born in a state of immaturity, and they must independently move to the marsupium and attach themselves to the mother's nipple in order to survive [8, 14, 16, 20, 31]. The immaturity is reflected in the embryonic developmental state of the majority of their body structures and sensory organs. For example, these animals are characterized by a lack of hair, closed eyes and ears, stunted hindlimbs and a tail, and pronounced immaturity in bone development [15]. The olfactory system and forelimbs are the only systems that are developed at birth. When marsupials are born, they pass through the pseudovagina and crawl into the pouch with their forelimbs. This is accomplished using gravitational and olfactory, which facilitate navigation [23, 26]. Once the young animals have been placed in the pouch, they attach to a nipple and remain there for the duration of their early development. This crawl is powered entirely by the forelimb complex, which is highly developed in the marsupial newborn [30]. The forelimb complex displays a highly modified shoulder girdle, which provides the structural support and areas of muscle attachment necessary for the crawl [4, 18, 29]. The constraint argument is that the formation of the specific crawling morphology at a particular time in ontogeny (*i.e.*, at birth) restricts the potential for variation in the development of the marsupial forelimb complex [21, 29, 30]. It has been reported that the muscle architecture parameters of the opossum and koala are less diverse than those of eutherian mammals, suggesting that they reflect the generalized constraint on marsupial forelimb complex [27, 28]. Thus, because of this constraint, the characteristics of the brachial plexus of marsupials may be like arboreal animals, even if they were terrestrial and semi-arboreal after birth.

Horiguchi studied the brachial plexus of the brindled bandicoot, an Australian marsupial [13]. He focused on the pectoral muscle group. In that study, the pectoral nerves were observed by dividing them into the cranial pectoral nerve, the intermediate pectoral nerve, and the caudal pectoral nerve. Considering those findings, the caudal one of the two cranial pectoral nerves observed in the left brachial plexus of the Tasmanian devil observed in the present study is supposed to correspond to the intermediate pectoral nerve.

In summary, anatomical patterns of brachial plexus were investigated in a koala, a Tasmanian devil, and a common ringtail possum. This study has limitation, because we did observe one specimen for each animal due to a quite difficulty of obtaining marsupials. Ideally, more samples are needed for the quantitative and qualitative analyses of the brachial plexus pattern of the marsupials. Accumulating data in addition to the present and previous reports are important for analyzing marsupials' brachial plexuses.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The data supporting the results of this study are available from the corresponding author upon reasonable request.

Ethics statement

All experiments and procedures involving animals were conducted in accordance with the animal experimentation guidelines of the affiliated university or institution.

Author contributions

YN and KS designed and conceived the study. YN and YN performed experiments. YN, SK, TY, ZL, SY, HM, and MI analyzed the data. YN and MI wrote the article. All authors have contributed to the final version of this manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest in association with the present study.

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Figure 1. Structures of brachial plexus of a koala; **A.** The right brachial plexus; **B.** A schematic illustration of A; **C.** The left brachial plexus; **D.** A schematic illustration of C. The nerves painted yellow and brown are the ventral and dorsal main branches of the brachial plexus, respectively. Ax — axillary nerve; Axa — axillary artery; BiBr — biceps brachii muscle; C4–T1, ventral rami of the spinal nerves; caP — caudal pectoral nerve; crP — cranial pectoral nerve; LaT — lateral thoracic nerve; LoT — long thoracic nerve; M — median nerve; MAC — medial antebrachial cutaneous nerve; Mc — musculocutaneous nerve; Pe — pectoral nerve; Phr — phrenic nerve; R — radial nerve; Sbs — subscapular nerves; Ss —

suprascapular nerve; Subs — subscapularis muscle; Td — thoracodorsal nerve; tma — nerve to teres major muscle; U — ulnar nerve. Scale bar: 1.5 cm.

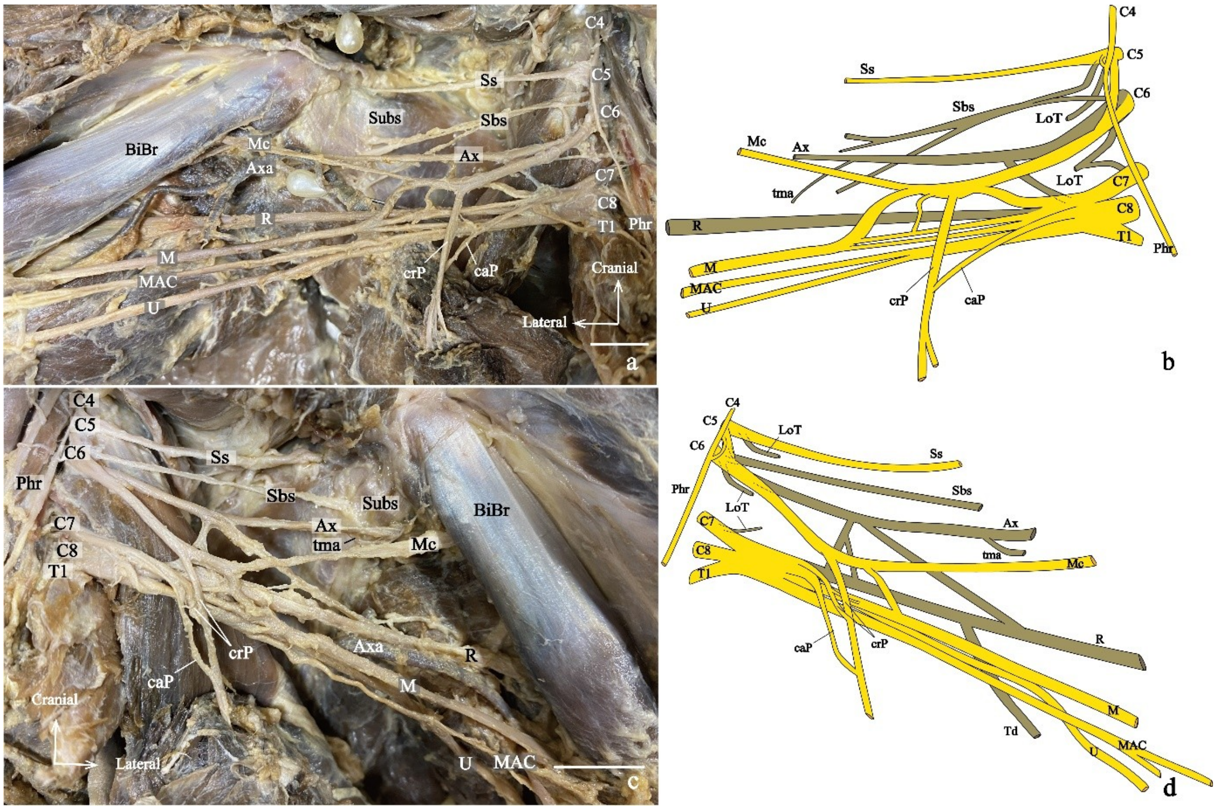

Figure 2. Structures of brachial plexus of a Tasmanian devil; **A.** The right brachial plexus; **B.** A schematic illustration of A; **C.** The left brachial plexus; **D.** A schematic illustration of C. The nerves painted yellow and brown are the ventral and dorsal main branches of the brachial plexus, respectively. Ax — axillary nerve; Axa — axillary artery; BiBr — biceps brachii muscle; C4–T1 — ventral rami of the spinal nerves; caP — caudal pectoral nerve; crP — cranial pectoral nerve; LoT — long thoracic nerve; M — median nerve; MAC — medial antebrachial cutaneous nerve; Mc — musculocutaneous nerve; Phr — phrenic nerve; R — radial nerve; Sbs — subscapular nerves; Ss — suprascapular nerve; Subs — subscapularis muscle; Td — thoracodorsal nerve; tma — nerve to teres major muscle; U — ulnar nerve. Scale bar: 1.5 cm.

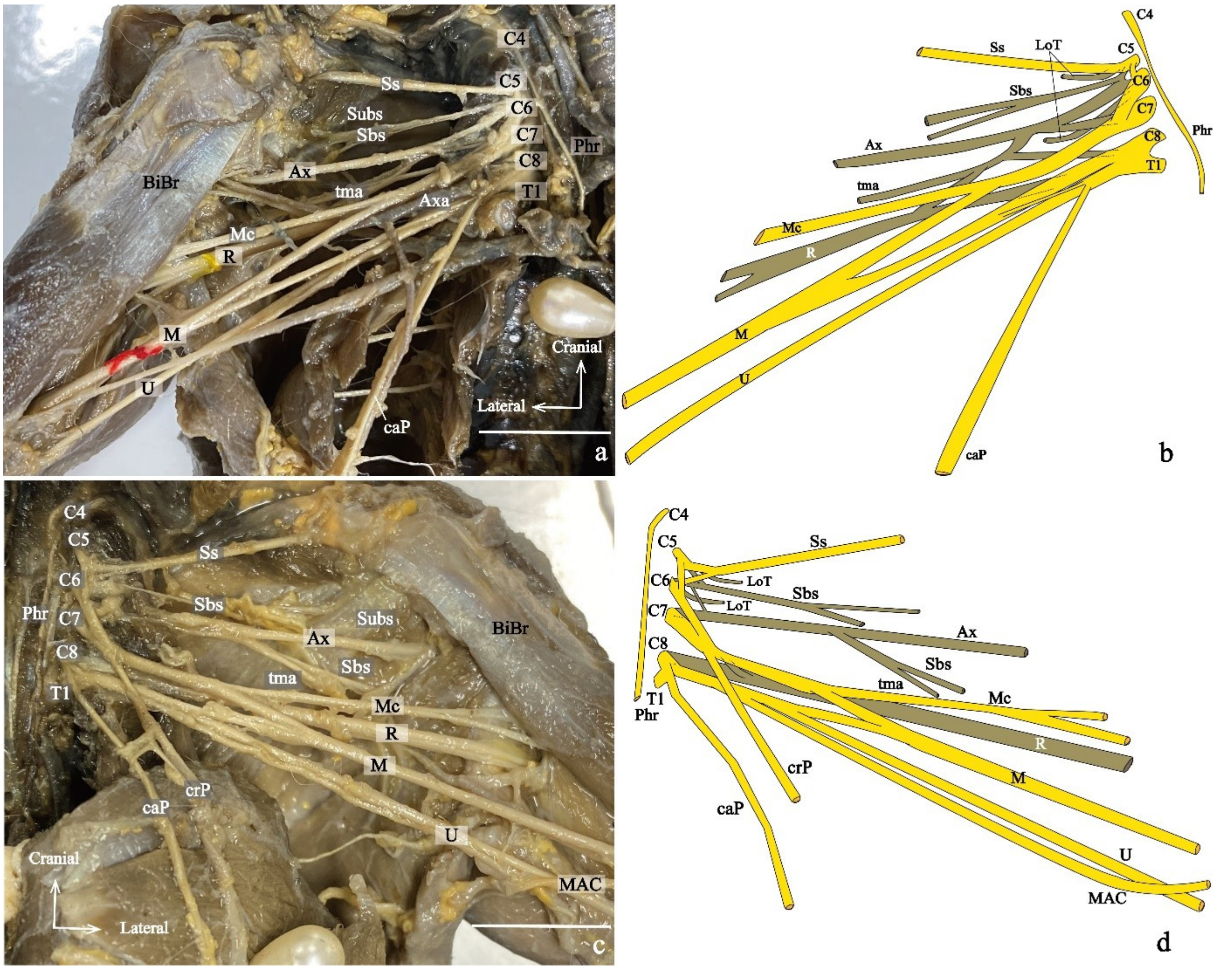

Figure 3. Structures of brachial plexus of a common ringtail possum; **A.** The right brachial plexus; **B.** A schematic illustration of A; **C.** The left brachial plexus; **D.** A schematic illustration of C. The nerves painted yellow and brown are the ventral and dorsal main branches of the brachial plexus, respectively. Ax — axillary nerve; Axa — axillary artery; BiBr — biceps brachii muscle; C4–T1 — ventral rami of the spinal nerves; caP — caudal pectoral nerve; crP — cranial pectoral nerve; LoT — long thoracic nerve; M — median nerve; MAC — medial antebrachial cutaneous nerve; Mc — musculocutaneous nerve; Phr — phrenic nerve; R — radial nerve; Sbs — subscapular nerves; Ss — suprascapular nerve; Subs, subscapularis muscle; tma — nerve to teres major muscle; U — ulnar nerve. Scale bar: 1.0

cm.

Table 1. Major peripheral nerves of the brachial plexus, nerve root origins, and muscles innervated.

Peripheral nerve	**Taxon**	**Origin（R/L)**	**Motor innervation**
Suprascapularis	Koala (*Phascolarctos cinereus*)	C5	Supraspinatus, infraspinatus
	Tasmanian devil (*Sarcophilus harrisii*)	C5–6	Supraspinatus, infraspinatus
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–6	Supraspinatus, infraspinatus
Subscapulares	Koala (*Phascolarctos cinereus*)	C5–6	Subscapular, teres major
	Tasmanian devil (*Sarcophilus harrisii*)	C5–6	Subscapular
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–6	Subscapular, teres major
Long thoracic	koala (Phascolarctos cinereus)	C5–7	Thoracic ventral serrate
	Tasmanian devil (*Sarcophilus harrisii*)	C5–7	Thoracic ventral serrate
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–6	Thoracic ventral serrate
Axillaris	Koala (Phascolarctos cinereus)	C6–7/C6	Deltoid, teres minor, teres major

	Tasmanian devil (*Sarcophilus harrisii*)	C5–7/C5–6	Deltoid, teres minor, teres major
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–7/C6–7	Deltoid, teres minor, teres major
Musculocutaneus	Koala (*Phascolarctos cinereus*)	C5–6	Coracobrachialis, biceps brachii, brachialis
	Tasmanian devil (*Sarcophilus harrisii*)	C5–7/C5–6	Coracobrachialis, biceps brachii, brachialis
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–6/C5–7	Coracobrachialis, biceps brachii, brachialis
Medianus	Koala (*Phascolarctos cinereus*)	C6–T1	Caudal antebrachial musculature
	Tasmanian devil (*Sarcophilus harrisii*)	C5–T1	Caudal antebrachial musculature
	Common ringtail possum (*Pseudocheirus peregrinus*)	C5–T1/C6–T1	Caudal antebrachial musculature
Ulnaris	Koala (*Phascolarctos cinereus*)	C8–T1	Flexer carpi ulnaris, flexor digitorum profundus
	Tasmanian devil (*Sarcophilus harrisii*)	C7–T1	Flexer carpi ulnaris, flexor digitorum profundus
	Common ringtail possum (*Pseudocheirus peregrinus*)	C8–T1	Flexer carpi ulnaris, flexor digitorum profundus
Radialis	Koala (*Phascolarctos cinereus*)	C6–T1	Triceps brachii complex, antebrachial extensor compartment

	Tasmanian devil (*Sarcophilus harrisii*)	C7–T1/C6–T1	Triceps brachii complex, antebrachial extensor compartment
	Common ringtail possum (*Pseudocheirus peregrinus*)	C7–T1/C6–T1	Triceps brachii complex, antebrachial extensor compartment

Nerve root origins are described separately in cases where the left and right sides are different.