



Systematic and comparative morphologies of the extrinsic cardiac nervous system in lemurs (Primates: Strepsirrhini: Infraorder Lemuriformes, Gray, 1821) with evolutionary morphological implications

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ABSTRACT

The detailed systematic morphology of the extrinsic cardiac nervous system (ECNS) and its surrounding structures in lemurs, Lemuriformes, were examined using 18 sides of nine lemurs including all four families: Lemuridae, Indriidae, Lepilemuridae, and Cheirogaleidae. Although the general morphology of ECNS in lemurs is similar to that in Lorisiformes, several anatomical differences, such as the structural variation of the cervicothoracic ganglion, the positional variation of the middle cervical ganglion, and the unusual appearance of the superior cardiac nerve originating from the superior cervical ganglion, demonstrate the diversity within Lemuriformes. In other words, the comparative anatomical findings of the wide variations in ECNS in the four families of Lemuriformes compared with the lower variations in the two families of Lorisiformes possibly reflect their evolutionary history and diversity, as is shown in the recent molecular phylogeny.

On the other hand, the shared common morphology of ECNS in Lorisiformes and Lemuriformes is considered to be the morphology of the common ancestor of strepsirrhines and demonstrates the gradual evolutionary changes in the primate lineage.

Our present and previous results also suggest that ECNS is preserved in the primate lineage without modification and specialization because of its functional adaptation, as is seen in the somatic system.

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1. Introduction

In a long history of vertebrate morphology, the soft tissue of the internal organs has been poorly investigated, although the somatic structures, such as the skeleton, limb muscles, and their nerve supplies have been the main focus from anatomical, functional, ecological, morphological, and evolutionary viewpoints. However, it is essential to re-evaluate their detailed morphology to document concordance with the molecular phylogeny in addition to the necessity of a future study unifying molecular, biological, and evolutionary morphological findings.

Due to the functional significance of the heart, the morphology of the complicated autonomic cardiac nervous system that plays important roles in the cardiac regulation has been well studied in the human (Ellison and Williams, 1962; Fukuyama, 1982;

Harman, 1900; Hausman, 1956; Janes et al., 1986; Kawashima, 2005, 2011; Kawashima and Sasaki, 2005, 2007; Kuntz, 1946; Kuntz and Morehouse, 1930; Mizeres, 1963; Mitchell, 1953; Pauza et al., 2002a,b, 2000; Pick, 1970; Tanaka, 2005; Wrethe, 1959). Experimental and developmental studies of the autonomic cardiac nervous system have also been conducted in animals such as the mouse, rat, rabbit, chick, and dog (Armour et al., 1972; Benítez et al., 1959; Brack et al., 2011; Gomez, 1958; Haws and Burgess, 1978; Hildreth et al., 2008, 2009; Kirby et al., 1980; Kuratani et al., 1991; Kuratani and Tanaka, 1990; Levy et al., 1966; Mabe and Hoover, 2011; Meyer et al., 2010; Mizeres, 1955, 1957, 1958; Mizuno et al., 2010; Oliveira et al., 2010; Roberts, 1991; Schwartz, 2010; Scherlag et al., 2011; Shaner, 1930; Shoba and Tay, 2000; Uchida et al., 2010; Verberne et al., 1998). However, huge differences in the cardiac nervous system between humans and the experimental animals have been recognized (Batulevicius et al., 2003; Brugnaro et al., 2003; Kawashima, 2011; McKibben and Getty, 1968; Rysevaite et al., 2011; Pauza et al., 2002a,b). A thorough understanding of primate morphology is important because the primate ancestor diverged from the ancestor of the rodents, which includes the main experimental animals such as the rat, mouse, and rabbit. Entire

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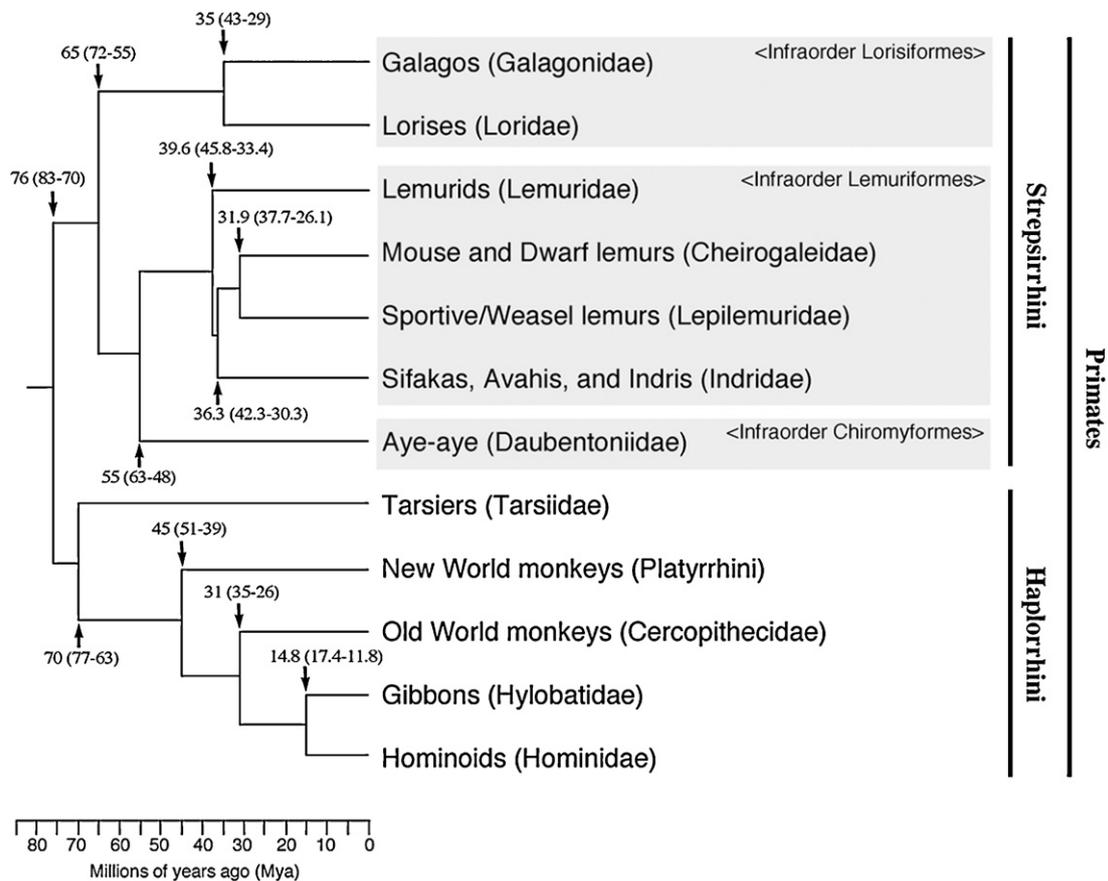


Fig. 1. Cladogram showing the phyletic relationship within the primate lineage based on the recent molecular studies (Kumar and Hedges, 1998; Steiper and Young, 2006; Horvath et al., 2008; Matsui et al., 2009). The strepsirrhini is composed of seven families split into three infraorders: two families of Lorisiformes, four families of Lemuriformes, and one family of Chiromyiformes. This classification corresponded to their divergence time according to a recent molecular phylogeny.

or partial studies of the nonhuman primate cardiac nervous system have been conducted on strepsirrhines (van den Broek, 1908), New World monkeys (van den Broek, 1908), Old World monkeys (van den Broek, 1908; Sonntag, 1922; Riegele, 1926; Botar, 1931a,b; Kolesnikov, 1935; Zuckerman, 1938; Sheehan and Pick, 1943; Pick, 1970; Billman et al., 1982), gibbons (van den Broek, 1908), and great apes (Schumacher, 1902; van den Broek, 1908; Sonntag, 1923, 1924; Riegele, 1925–1926; Botar, 1931a,b, 1932; Kolesnikov, 1935). To fill the morphological gap among these groups and confirm their nature from an evolutionary viewpoint (Fig. 1), we conducted comparative anatomical studies of the primate autonomic cardiac nervous system, mainly the extrinsic cardiac nervous system (ECNS) (lorises and galagos, Kawashima and Thorington, 2011; New World monkeys, Kawashima et al., 2009; Old World monkeys, 2005, 2007; gibbons, Kawashima et al., 2008; Great apes, Kawashima and Sato, 2012; human, Kawashima, 2005, 2011; Kawashima and Sasaki, 2005, 2007).

Our series of studies suggest that ECNS in the primates preserves its evolutionary history in close alignment with phylogeny, although certain somatic structures as seen in the somatic skeleton, muscles, and their innervation are modified by functional requirement such as ecological, kinematic and dietary adaptations. Recently, we examined ECNS morphology in the Lorisiformes, lorises and galagos, because their common ancestor was the earliest to diverge from the strepsirrhine lineage (Horvath et al., 2008; Matsui et al., 2009; Orlando et al., 2008), and they therefore carry the possibility of preserving the primitive morphology of ECNS observed in strepsirrhines. These results show that ECNS morphology of lorises and galagos is easily distinguishable from that of the other primates examined, and that they exhibit

continuous evolutionary changes concomitant with their evolutionary history in the primate lineage.

In the other strepsirrhines, the lemurs are divided into four families (Lemuriformes) and the Aye-aye alone constitutes its own family (Daubentoniidae), according to the most well-known standard classification (Groves, 2005); this has been confirmed by recent molecular evolutionary studies (Horvath et al., 2008; Matsui et al., 2009; Orlando et al., 2008) (Fig. 1). Although knowledge of ECNS morphology in lemurs and Aye-ayes is necessary, ECNS morphologies in these species remain unclear because of only one case reported of a black lemur (*Lemur macaco* = *Eulemur macaco*) by van den Broek (1908).

Recently, we had the opportunity to dissect lemurs from all four families with intact internal organs, and we examined ECNS and its surrounding structures in detail. The main objectives of this study were (1) to describe the detailed systematic anatomy and nature of ECNS across different lemur families for the first time; (2) to update and provide additional anatomical information on the surrounding structures, thus contributing to better anatomical knowledge of lemurs; (3) to examine the ECNS variations within and between families of lemurs; (4) to examine the relationship between ECNS and its surrounding structures such as the number of presacral vertebrae, cardiac position, and great vessels; (5) to determine whether ECNS in the Lemuriformes, which consists of four families, exhibits more anatomical variations than that in the Lorisiformes which consists of two families; (6) to clarify whether ECNS is a conserved structure derived from a common ancestor of the strepsirrhines or shows divergences associated with ecological adaptations between the different taxa, and (7) to consider the evolutionary morphological similarities and differences in the primate lineage.

2. Materials and methods

2.1. Examined materials

Eighteen sides of 9 lemur specimens were examined in this study (Table 1). The classification was determined from Groves (2005), which is the standard classification used worldwide (in *Mammal Species of the World*, edited by Wilson and Reeder). All specimens were obtained from museums or universities (Table 1) and were fixed with 10% formaldehyde solution through the femoral artery and/or preserved in 30–70% alcohol for at least 3 years. The ages of the specimens were estimated by dental eruption, open or closed fontanelles, ossification of epiphyseal cartilages, fusion of sacral vertebrae, and body size (Sugiyama et al., 1996; Swindler, 2002).

2.2. Imaging analysis

To understand the relationship between the cardiac position itself and skeletal variation, most specimens were examined with a spiral CT scanner (*Somatom, Emotion; Siemens, MI, USA* or *Asterson 4; Toshiba, Tochigi, Japan*). Tube voltage, current, and slice width were set at 110–130 kV, 70–100 mA, and 0.5–1.0 mm, respectively. Cross-sectional images were reconstructed at 0.3–0.5 mm increments and converted from DICOM to TIF formats using commercial software (*OsiriX; The OsiriX Foundation, Geneva, Switzerland*).

2.3. Dissection methods

All dissections were performed using forceps for optical surgery (*Dumont #4; World Precision Instruments, Connecticut, USA*) under a binocular stereomicroscope (*Bausch & Lomb Co., New York, USA*). The major stages were recorded in detailed drawings made from the lateral and ventral aspects in a step-by-step manner to preserve the relationships among the vessels, the nerves, and the surrounding structures. In addition, the dissection steps were documented with digital images taken with a Canon digital camera (*IXY digital 800IS; Canon, Tokyo, Japan*).

The definitions and terminologies that we used for the autonomic cardiac nervous system are a modification of those used by Fukuyama (1982) (for details see Kawashima, 2011; Kawashima et al., 2009). The terminologies used for the somatic structures followed those used in previous publications by Jouffroy (1962), El-Assy (1965–66), Stevens et al. (1981), Dunlap et al. (1985), and Kawashima and Thorington (2011).

The protocol for the present research did not involve any material or procedure requiring approval from the Smithsonian Animal Care and Use Committee or our university and conformed to the provisions of the Declaration of Helsinki, 1995 (as revised in Edinburgh, 2000).

3. Results

3.1. Surrounding structures

Presacral vertebrae: The number of presacral vertebrae is shown in Table 2 and Fig. 2A–D. In the three species examined in the family Lemuridae, the cervical, thoracic, and lumbar vertebrae were consistently fixed as 7, 12, and 7, respectively. However, in the other species examined, although the number of cervical vertebrae was fixed at 7, the numbers of thoracic and lumbar vertebrae varied from 11 to 13 (Fig. 2A–D) and from 7 to 9, respectively. Hence, the total number of thoracolumbar vertebrae also varied from 20 to 21.

Cardiac position: The general orientation of the heart in lemurs was similar to that in humans. As shown in Fig. 2E–H, the long axis of the heart is usually positioned from the right shoulder to the left leg. One-third of the heart is to the right side, and two-thirds

to the left side. The ventral aspect of the heart is mostly the right chamber, whereas the left silhouette of the ventral heart and the most dorsal portion is the left chamber (Fig. 2E and F).

Although the sagittal sections of the thorax showed that the heart is situated in the middle third of the thorax, approximately between the third–ninth thoracic vertebrae (Fig. 2A, B and D), the cardiac position within the thorax varies somewhat between individuals (Table 2). Particularly, the cardiac position in the ring-tailed and ruffed lemurs is situated in the lower half of the thorax (Fig. 2C).

Cross-sectional views of the thorax show the height of both ventricles, the cardiac position, and the inclination of the interventricular septum (cardiac axis) to the dorso-ventral axis (Fig. 2G and H).

Great arteries from the aortic arch: In lemurs, two branching patterns of the great arteries from the aortic arch were observed. Both types consisted of two branches, the common brachiocephalic trunk and the left subclavian artery. In Type A, the common brachiocephalic trunk is composed of the brachiocephalic artery (common trunk of the right common carotid and right subclavian arteries) and the left common carotid artery (7 specimens; 77.8%). In Type B, the common brachiocephalic trunk is composed of the common trunk of the right and left common carotid arteries together with the right subclavian artery (2 specimens; 22.2%); this pattern was found in the ruffed lemur and eastern avahi.

Great veins: The inferior caval vein (IVC) is present only on the right side, whereas the superior caval vein is present on both sides (9/9 specimens, 100%): the main right superior caval vein (RSVC) and the persistent left superior caval vein (LSVC).

3.2. Positions of the cervical and upper thoracic ganglia and relationship with the spinal nerves

The findings on the soft tissue anatomy, including the ECNS in lemurs, each genus of a ring-tailed lemur, brown lemur, ruffed lemur, eastern avahi, Verreaux's sifaka, weasel lemur, and brown mouse lemur, are first presented in detail in Figs. 3–9, respectively.

Table 3 shows the composition of the cervical and upper thoracic ganglia and their communicating branches to the spinal nerves. Two cervical ganglia, one large cervicothoracic ganglion, and one thoracic ganglion were generally observed around each thoracic spinal nerve outlet.

The superior cervical ganglion (SG) was consistently present (18/18 sides, 100%), located caudal to the cranial base in most cases and lacking communication with any cervical nerve (16/18 sides, 88.9%; Fig. 10A). It rarely exhibited communicating branches with C1 on two sides (11.1%).

The middle cervical ganglion (MG) was also consistently present (18/18 sides, 100%), situated between the common carotid and subclavian arteries at the level of the seventh cervical and first thoracic vertebrae in most cases, and did not communicate with any cervical nerves (15/18 sides, 83.3%; Fig. 10B), although it rarely ramified the vertebral nerve (3/18 sides, 16.7% – Figs. 3–5). The accessory MG was rarely observed around the main MG (1/18 sides, 5.6% – weasel lemur, right side, Fig. 8).

The cervicothoracic ganglion was always present (18/18 sides, 100%), while the independent inferior cervical, vertebral, and uppermost thoracic ganglia were not. The cervicothoracic ganglion was composed of the inferior cervical and only the first thoracic ganglia in five of 18 sides (27.8%; both sides of Fig. 5, right side of Fig. 8; Fig. 10C), the inferior cervical and the first and second thoracic ganglia in 12 sides (66.7%; Fig. 10D), and the inferior cervical and the first to third thoracic ganglia in one side (5.6%). The cervicothoracic ganglion has communicating branches with C7–T1 (1/18 sides, 5.6%), C7–T2 (2/18 sides, 11.1%), C8–T1 (3/18 sides, 16.7%), C8–T2 (9/18 sides, 50.0%), C8–T3 (1/18 side, 5.6%), and T1–T2 (2/18 sides, 11.1%) in addition to the branches of the vertebral nerve (18/18 sides, 100.0%).

Table 1
Examined materials.

Species ^a	Specimen no.	Sex	Stage	Institute	Remark
Family Lemuridae					
Ring-tailed lemur (<i>Lemur catta</i> , Linnaeus, 1758)	No. 1	Male	Adult	Primate Research Institute, Kyoto University, Aichi, Japan	PRI-Yoshikawa
	No. 2	Female	Adult		Smithsonian National Museum of Natural History, DC, USA
Brown lemur (<i>Eulemur fulvus</i> , E. Geoffroy, 1796)	No. 3	Female	Adult	Smithsonian National Museum of Natural History, DC, USA	US502554 CRL: 38.4 cm
Ruffed lemur (<i>Varecia variegata</i> , Kerr, 1792)	No. 4	Male	Adult	Primate Research Institute, Kyoto University, Aichi, Japan	KUPRI8439 CRL: 51.2 cm, 3.1 kg
Family Indridae					
Eastern avahi (<i>Avahi langer langer</i> , Gmelin, 1788)	No. 5	Female	Juvenile	American Museum of Natural History, NY, USA	AMNH170500 CRL: 15.2 cm
Verreaux's sifaka (<i>Propithecus verreauxi</i> , A. Grandidier, 1867)	No. 6	Female	Young	American Museum of Natural History, NY, USA	AMNH170472 CRL: 21.6 cm
Family Lepilemuridae					
White-footed weasel lemur (<i>Lepilemur mustelinus leucopus</i>)	No. 7	Female	Subadult	Museum of Comparative Zoology Harvard University, MA, USA	MCZ45418 CRL: 21.8 cm
Family Chirogaleidae					
Brown mouse lemur (<i>Microcebus rufus</i> , E. Geoffroy, 1834)	No. 8	Female	Adult	American Museum of Natural History, NY, USA	AMNH174373 CRL: 12.7 cm
	No. 9	Female	Adult	American Museum of Natural History, NY, USA	AMNH174374 CRL: 11.2 cm
Total	18 sides of 9 bodies				

^a The classification were done from Groves (2005).

These data summarize the nature of the general sympathetic ganglia, comprising the superior cervical, the middle cervical, the cervicothoracic, and thoracic ganglia, in Lemuriformes. Normally, the superior and middle cervical ganglia were consistently present in all sides (100%) and they did not communicate with any of the cervical spinal nerves in 88.9% and 83.3% of sides, respectively. Importantly, the middle cervical ganglia in Lemuridae were positioned much lower than those in the other Lemuriformes because

of lower branching of the great arteries from the aortic arch as shown in Figs. 2C, 3, and 5.

On the other hand, the cervicothoracic ganglion varies in composition and position, and it is composed of the inferior cervical and only the first thoracic ganglion in five of 18 sides (27.8%), the inferior cervical and the first and second thoracic ganglia in 12 sides (66.7%), and the inferior cervical and the first to third thoracic ganglia in one side (5.6%) independent of the family.

Table 2
Number of presacral vertebrae and cardiac position.

Species ^a	Specimen no.	Number of the presacral vertebrae				Cardiac position		Remark
		Cervical vertebrae (CV)	Thoracic vertebrae (TV)	Lumbar vertebrae (LV)	Thoraco-lumbar vertebrae (TV + LV)	Cranial limit of the heart	Caudal limit of the heart	
Family Lemuridae (Gray, 1821)								
Ring-tailed lemur (<i>Lemur catta</i> , Linnaeus, 1758)	No. 1	7	12	7	19	NA	NA	
	No. 2	7	12	7	19	Ivd ^b :6/7 TV	Ivd:9/10TV	
Brown lemur (<i>Eulemur fulvus</i> , E. Geoffroy, 1796)	No. 3	7	12	7	19	VT6	9TV	
Ruffed lemur (<i>Varecia variegata</i> , Kerr, 1792)	No. 4	7	12	7	19	7TV	10TV	
Family Indridae								
Eastern avahi (<i>Avahi langer langer</i> , Gmelin, 1788)	No. 5	7	12	9	21	Ivd:3/4TV	Ivd:8/9TV	
Verreaux's sifaka (<i>Propithecus verreauxi</i> , A. Grandidier 1867)	No. 6	7	11	9	20	Ivd:4/5TV	Ivd:8/9TV	
Family Lepilemuridae								
White-footed weasel lemur (<i>Lepilemur mustelinus leucopus</i> , Major 1894)	No. 7	7	12	9	21	Ivd:3/4TV	Ivd:8/9TV	
Family Chirogaleidae								
Brown mouse lemur (<i>Microcebus rufus</i> , E. Geoffroy, 1834)	No. 8	7	13	7	20	5TV	9TV	
	No. 9	7	13	7	20	Ivd:4/5	Ivd:8/9TV	
Total	18 sides of 9 bodies							

^a The classification were done from Groves (2005).

^b Intervertebral disk.

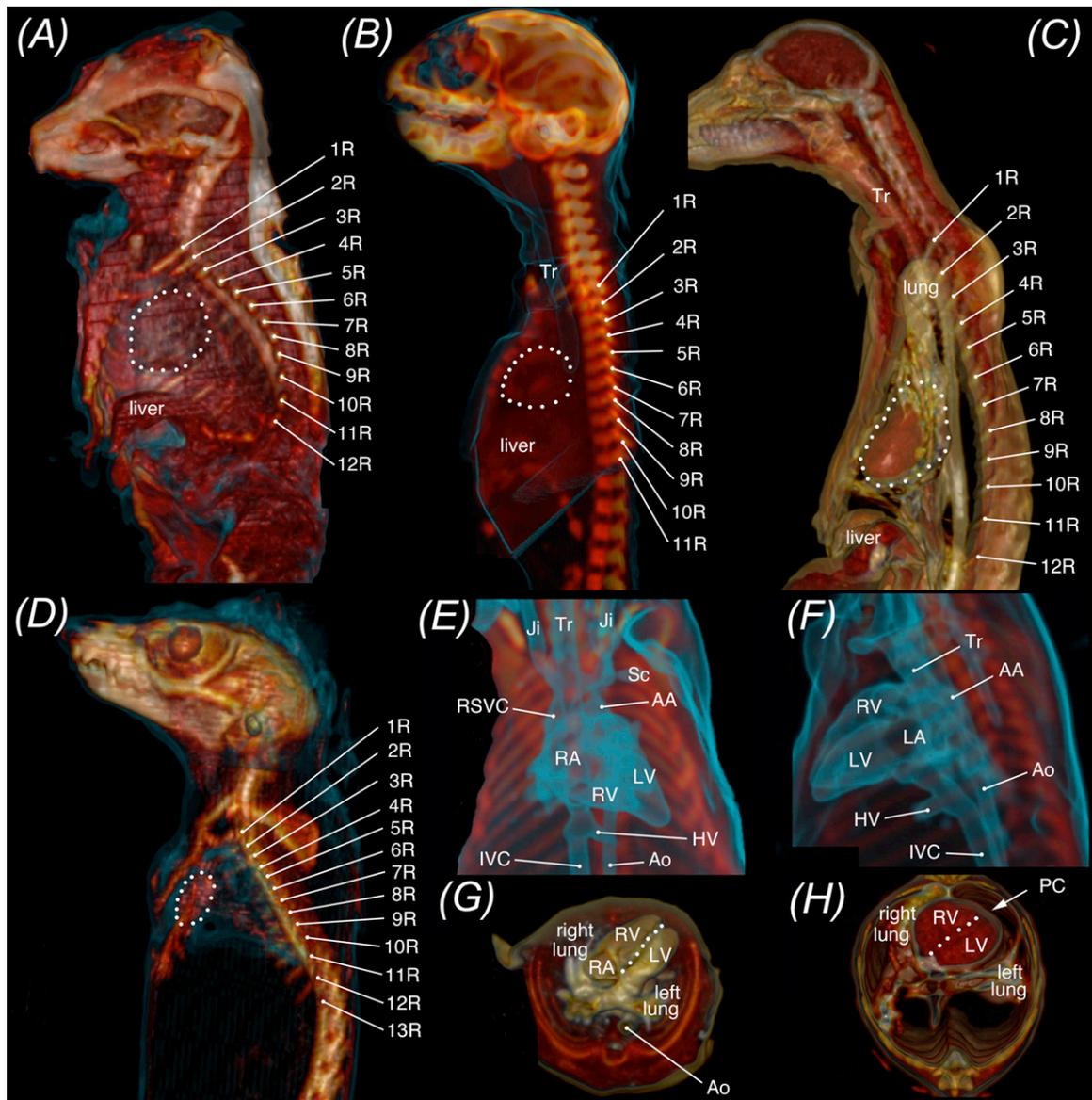


Fig. 2. CT imaging analysis. (A–D) Sagittal section. The cardiac height within the vertical thorax in the white-footed weasel lemur (*Lepilemur mustelinus*) (A), Verreaux's sifaka (*Propithecus verreauxi*) (B), ruffed lemur (*Varecia variegata*) (C), and brown mouse lemur (*Microcebus rufus*) (D). The heart, as shown by the dotted area, tends to be positioned lower than in the other lemurs. (E and F) The general cardiac position and its great vessels viewed from the ventral and lateral aspects. One-third of the heart is to the right side and two-thirds to the left side. The ventral aspect of the heart is mostly the right chamber, whereas the left silhouette of the ventral heart and the most dorsal portion is the left chamber. (G and H) Cross section at the level of ventricles in the eastern avahi (G) and ruffed lemur (H) and. The direction of the interventricular septum (cardiac axis) varies between individuals. AA, aortic arch; Ao, aorta; HV, hepatic vein; IVC, inferior caval vein; Ji, internal jugular vein; LA, left atrium; LV, left ventricle; PC, pericardium; RA, right atrium; RV, right ventricle; RSVC, right superior caval vein; Sc, subclavian vein; Tr, trachea; 1R–12R, first to twelfth ribs.

3.3. Sympathetic cardiac nerves and parasympathetic vagal cardiac branches

A variation was observed in the sympathetic cardiac nerves, whereas no variation was observed in any of the parasympathetic vagal cardiac branches (Table 3).

The superior cardiac nerve originated from the superior cervical ganglion and the sympathetic trunk between the superior and middle cervical ganglia in 2 (11.1%; both sides of Fig. 5; Fig. 10E) and 6 (33.3%) of 18 sides, respectively.

The middle cardiac nerves originated from the middle cervical ganglion in 18/18 sides (100%; Fig. 10B), the accessory MG in 0/18 sides (0%), and the sympathetic trunk between the middle cervical and cervicothoracic ganglia in 15/18 sides (83.3%). The inferior and

thoracic cardiac nerves were observed in 9/18 (50%) and 4/18 sides (22.2%), respectively.

In contrast to this variability, the superior, inferior, and thoracic cardiac branches were consistently observed in 16/18 sides (88.9%), 18/18 (100%), and 18/18 sides (100%), respectively.

Table 3 shows the frequencies of the sympathetic cardiac nerves and parasympathetic vagal cardiac branches. These data on cardiac innervation indicate that the middle cardiac nerves originating from the middle cervical ganglion and the sympathetic trunk between the middle cervical and cervicothoracic ganglia are the main constant sympathetic contributors, whereas all parasympathetic vagal cardiac branches contribute equally to cardiac innervation. As a special reference, the superior cardiac nerve originating from the superior cervical ganglion, which is

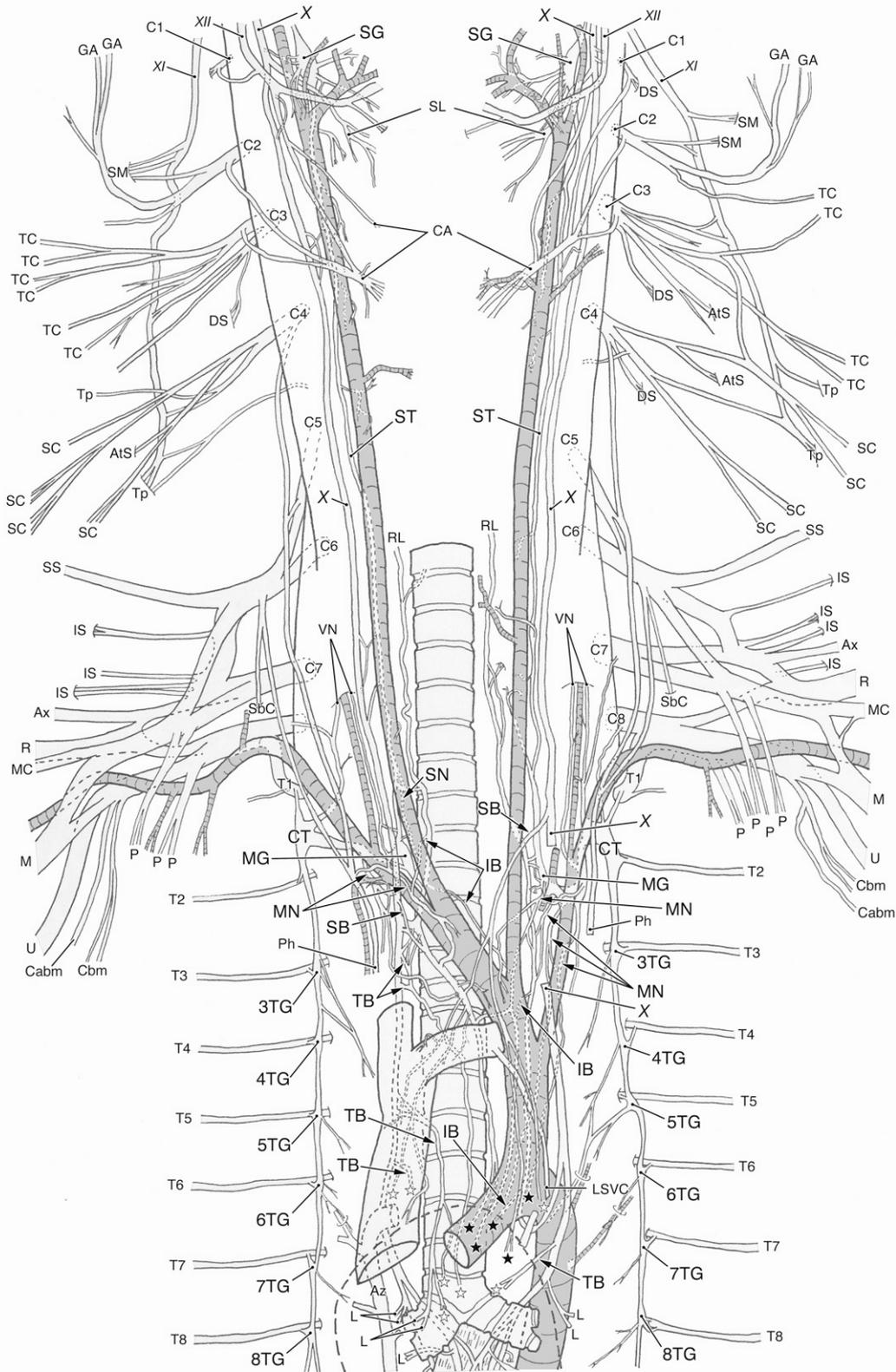


Fig. 3. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the ring-tailed lemur (*Lemur catta*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. AA, aortic arch; Ao, aorta; aMG, accessory middle cervical ganglion; Ax, axillary nerve; Az, azygos vein; CA, cervical ansa; CT, cervicothoracic (stellate) ganglion; DS, dorsal scapular nerve; E, (nerve to) esophagus; GA, greater auricular nerve; IB, inferior (vagal) cardiac branch; IN, inferior cardiac nerve; IS, subscapular nerve; IVC, inferior caval vein; L, nerve branches to lung; LSVC, (persistent) left superior caval vein; LV, left ventricle; M, median nerve; MC, musculocutaneous nerve; MG, middle cervical ganglion; MN, middle cardiac nerve; OH, omohyoid muscle; P, pectoral nerve; Ph, phrenic nerve; PT, pulmonary trunk; PV, pulmonary vein; R, radial nerve; RAA, right auricular appendage; RL, recurrent laryngeal nerve of vagus nerve; RSVC, right superior caval vein; RV, right ventricle; SB, superior (vagal) cardiac branch; SbC, nerve to subclavian muscle; SC, supraclavicular muscle; SG, superior cervical ganglion; SH, sternohyoid muscle; SL, superior laryngeal nerve; SM, nerve branch to sternocleidomastoid muscle; SN, superior cardiac nerve; SS, suprascapular nerve; ST, sympathetic trunk; TB, thoracic (vagal) cardiac branch; TC, transverse cervical nerve; TD, thoracodorsal nerve; TN, thoracic cardiac nerve; Tp, nerve branch to trapezius muscle; Tr, trachea; U, ulnar nerve; VG, vertebral ganglion; VN, vertebral nerve; X, vagus nerve; XI, accessory nerve; XII, hypogastric nerve; 2–9TG, second to ninth thoracic ganglia.

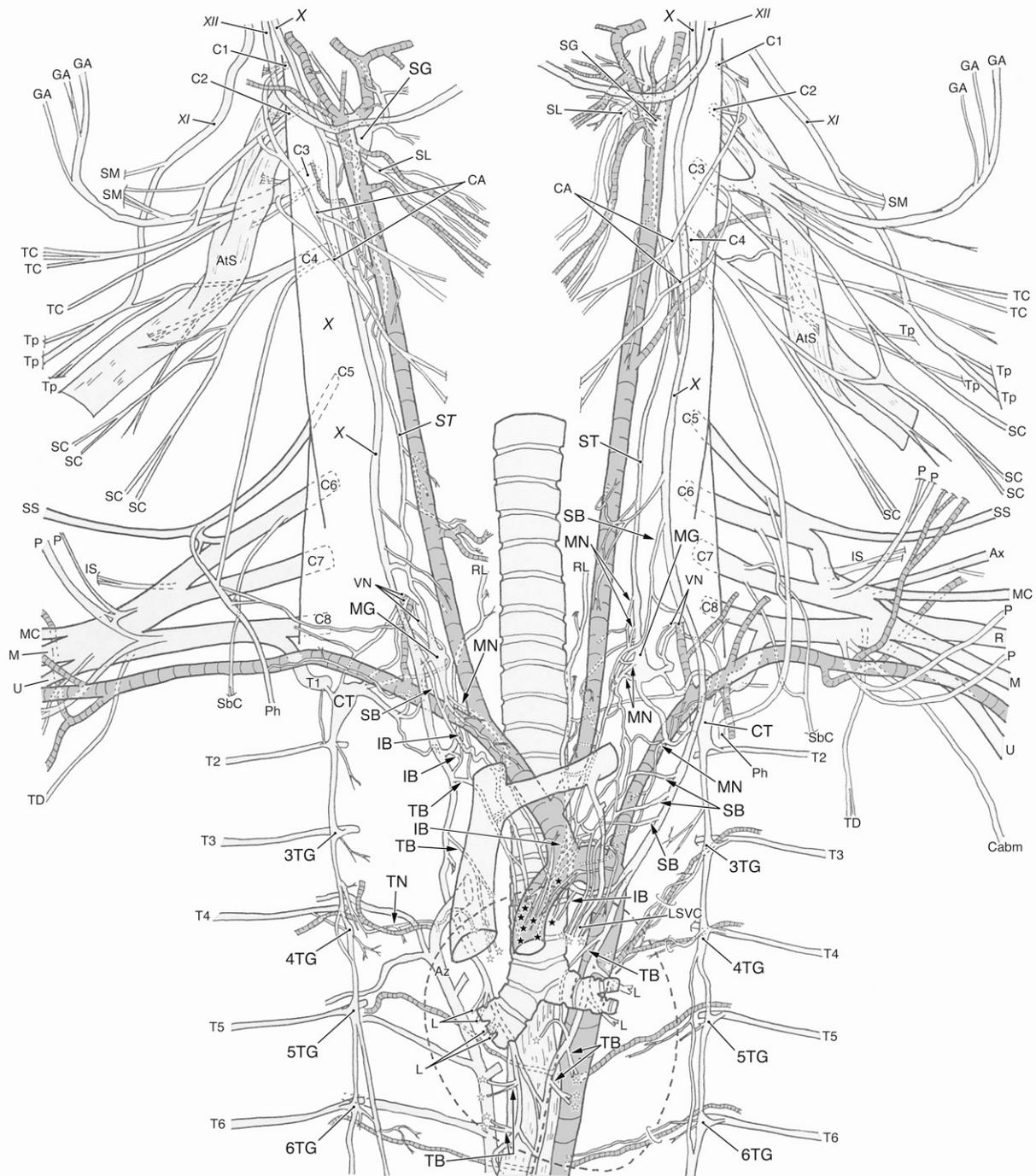


Fig. 4. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the brown lemur (*Eulemur fulvus*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. See Fig. 3 for abbreviations.

not observed in lorises, galagos, and New World monkeys, was observed in both sides of one ruffed lemur (11.1%).

4. Discussion

4.1. General morphology of ECNS in lemurs

In spite of a long anatomical research history in lemurs, only one case of ECNS in a black lemur (*Lemur macaco* = *Eulemur macaco*) appears in the available literature, reported by van den Broek (1908), whereas studies of ECNS in other primates are abundant. According to the findings of van den Broek (1908), the morphology

of ECNS in a black lemur was as follows; the superior cervical ganglion communicating without any cervical nerve, the middle cervical and vertebral ganglia branching the vertebral nerve, the cervicothoracic ganglion composed of the inferior cervical and first to third thoracic ganglia and communicating with the first to third thoracic nerves, and the several thoracic ganglia starting from the fourth thoracic ganglion and communicating with each of the thoracic nerves, and the (sympathetic) middle cardiac nerve originating from the middle cervical ganglion and the sympathetic trunk and (parasympathetic vagal) superior and inferior cardiac branches. However, it is difficult to extrapolate this to the general morphology of lemurs because this finding is a single

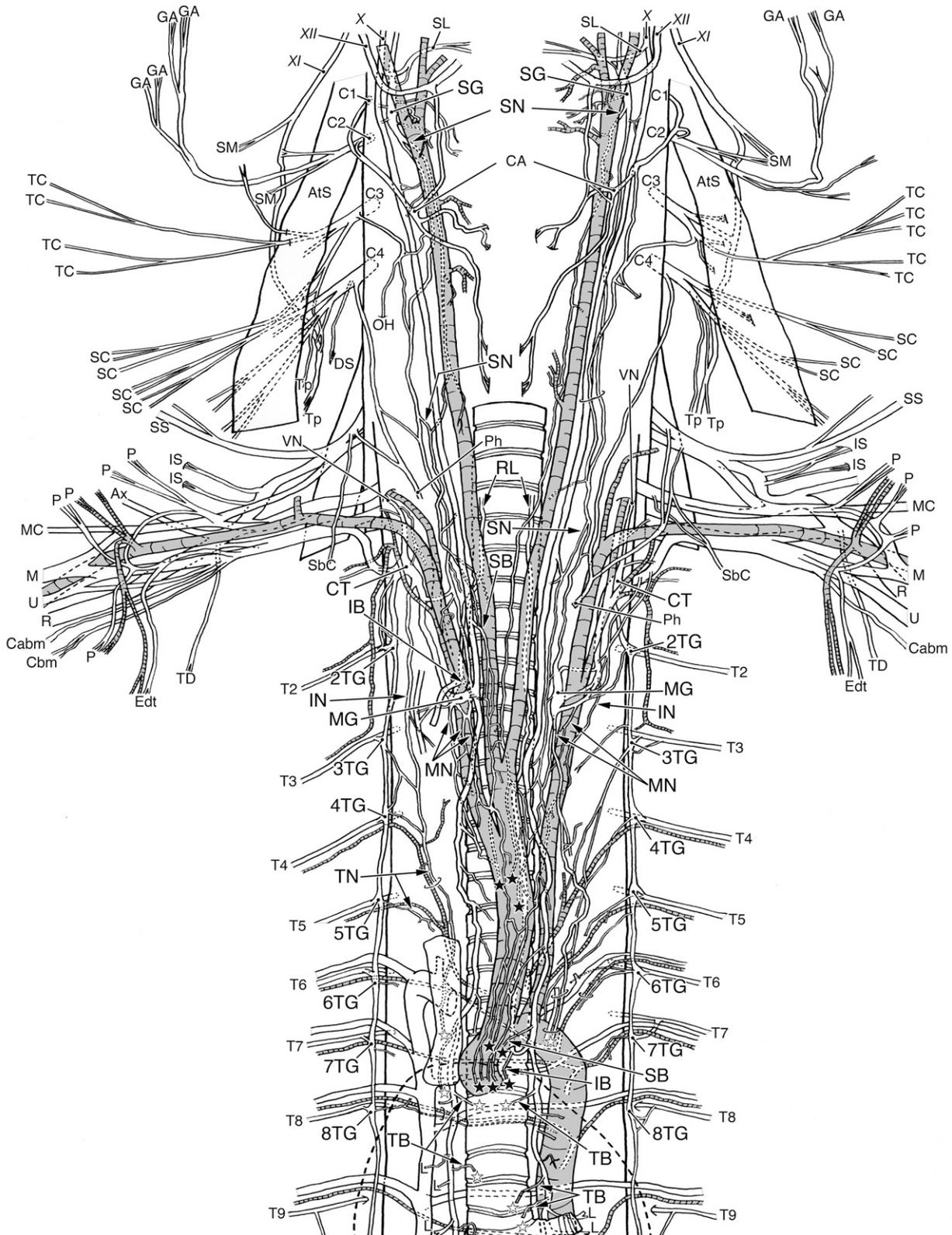


Fig. 5. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the ruffed lemur (*Varecia variegata*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. See Fig. 3 for abbreviations.

case report. Although the first author dissected a spider monkey (*Ateles ater*=*Ateles paniscus*), crab-eating macaque (*Cercopithecus cynomolgus*=*Macaca cynomolgus*), sacred baboon (*Cynocephalus hamadryas*=*Papio hamadryas*), white-handed gibbon (*Hylobates*

lar), and orangutan (*Pongo sp.*), all his findings did not show the general morphology, in contrast to our detailed examinations using numerous specimens (Kawashima, 2011; Kawashima and Sasaki, 2007; Kawashima et al., 2005, 2007, 2008, 2009).

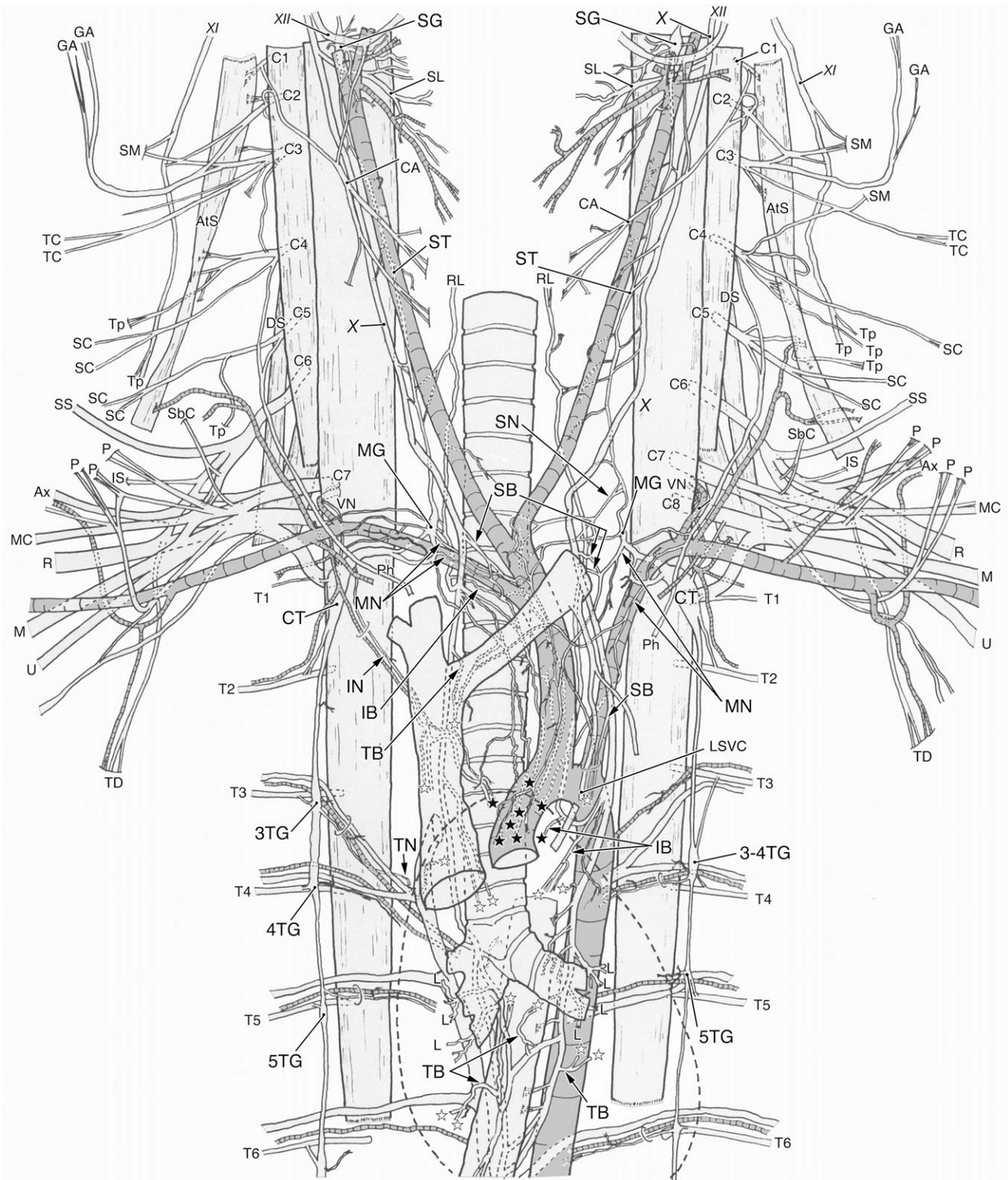


Fig. 6. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the eastern avahi (*Avahi langer*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. See Fig. 3 for abbreviations.

In the present study, we examined 18 sides of nine lemurs including all four families so as to determine the general morphology of ECNS in lemurs. Our study clarified that the superior and middle cervical ganglia communicate without any cervical nerves, that the cervicothoracic ganglion composed of the inferior cervical and the first two thoracic ganglia communicated with C8–T1

in addition to the vertebral nerve, and that the thoracic ganglia start with the third thoracic ganglion. General cardiac innervation involves the middle cardiac nerve originating from the middle cervical ganglion and sympathetic trunk, whereas all the parasympathetic vagal cardiac branches – superior, inferior, and thoracic cardiac branches – were consistently observed in the lemurs.

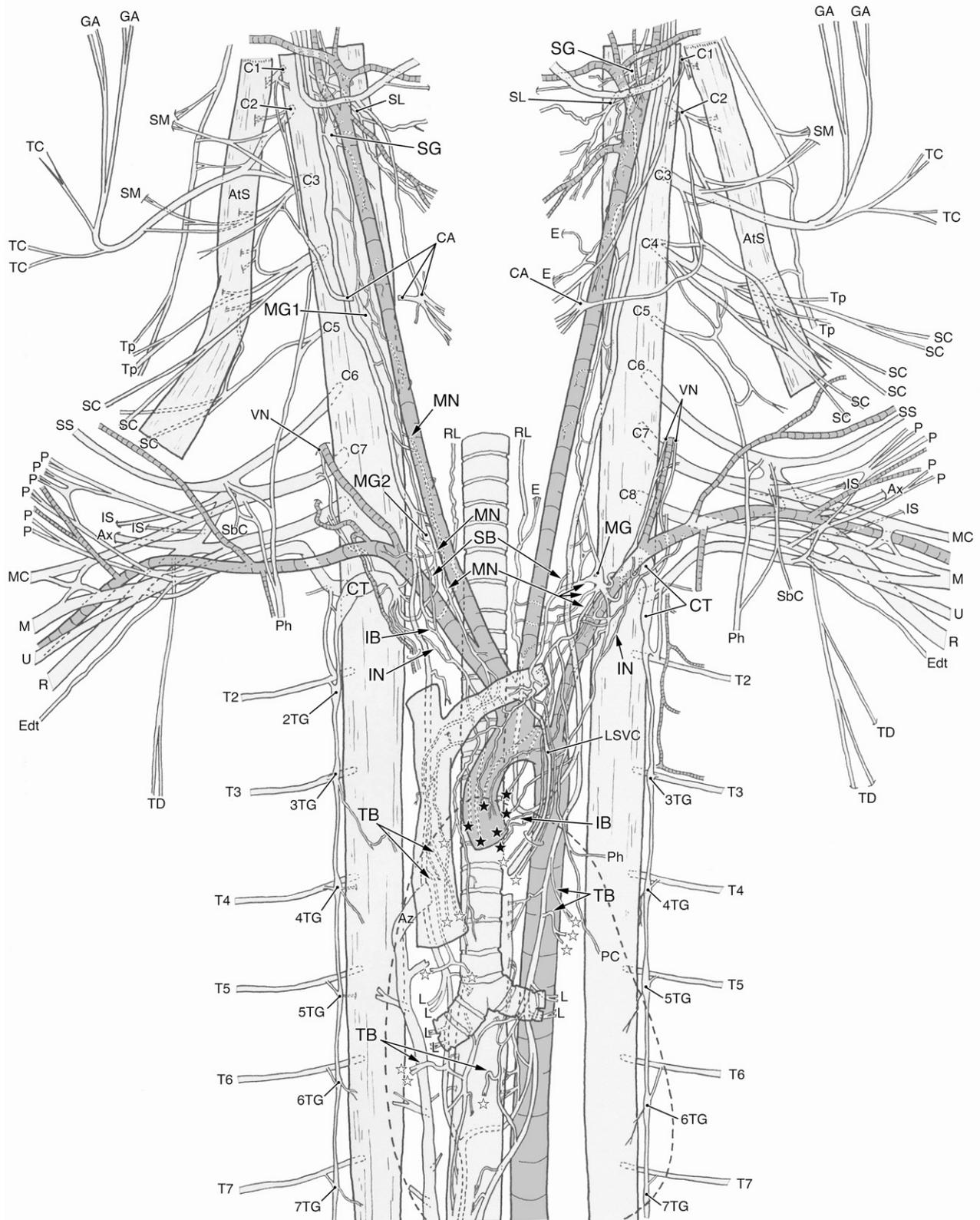


Fig. 8. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the white-footed weasel lemur (*Lepilemur mustelinus*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. See Fig. 3 for abbreviations.

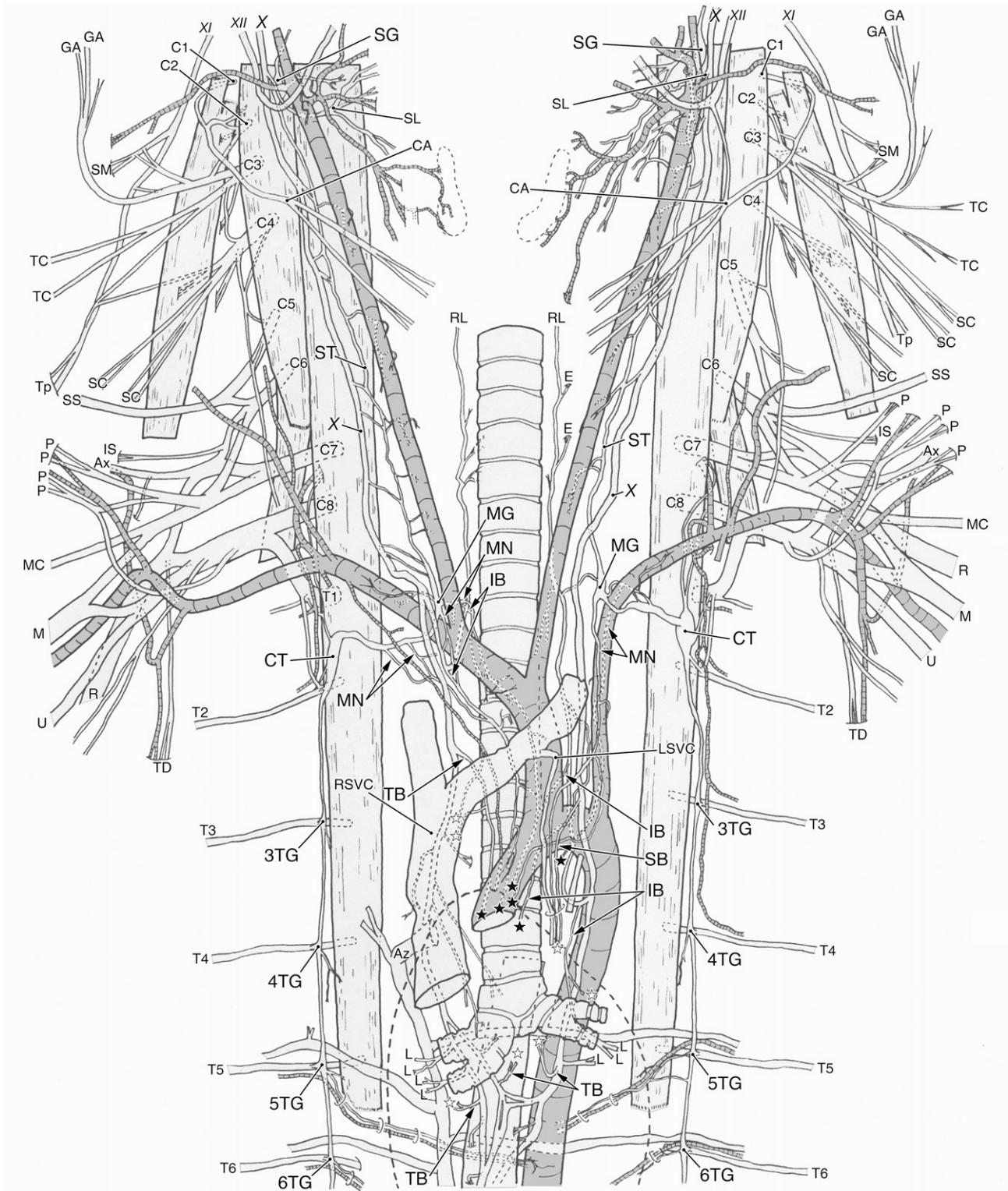


Fig. 9. A diagrammatic representation of the extrinsic autonomic cardiac nervous system and its surrounding structures in the brown mouse lemur (*Microcebus rufus*). The closed and open stars show the cardiac nerves/branches entering from the arterial and venous portions of the pericardial reflection, respectively. See Fig. 3 for abbreviations.

Table 3
Sympathetic ganglia and cardiac nerve/branch in Lemurs.

Sympathetic nervous system	Appearance of sympathetic ganglia	Communication with spinal nerve	Sympathetic cardiac nerve
Sup. Cerv. Ggl (SG)	18/18 sides (100.0%)	(-)*: 16/18 (88.9%) C1: 2/18 (11.1%)	SN: 2/18 sides (11.1%)
Sympathetic trunk between SG and MG	NA	(-): 18/18 (100.0%)	SN: 6/18 sides (33.3%)
Mid. Cerv. Ggl (MG)	18/18 sides (100.0%)	(-): 15/18 sides (83.3%) VN: 3/18 sides (16.7%)	MN: 18/18 sides (100.0%)
Accessory MG (aMG)	1/18 sides (5.6%)	(-): 1/1 side (100.0%)	MN: 0/1 side (0.0%)
Sympathetic trunk between MG and IG/CT	NA	(-): 13/14 sides (92.9%)	MN: 15/18 sides (83.3%)
Inf. Cerv. Ggl. (CT)	0/18 sides (0.0%)	NA	NA
Cervicothoracic Ggl (CT)	18/18 sides (100.0%)	(-): 0/18 sides (0.0%) VN: 18/18 sides (100.0%)	In: 9/18 sides (50.0%)
<i>Composition of the CT</i>			
IG + 1TG	5/18 sides (27.89%)	C7: 3/84 sides (16.7%)	
IG + 1-2TG	12/18 sides (66.7%)	C8: 16/18 sides (88.9%)	
IG + 1-3TG	1/18 sides (5.6%)	T1: 18/18 sides (100.0%) T2: 14/18 sides (77%) T3: 1/18 sides (5.6%)	
2,3, or 4 Thorac. Ggl. (TG~)	18/18 sides (100.0%)	Each thorac. N (100.0%)	TN: 4/18 sides (22.2%)
<i>Parasympathetic vagal nervous system</i>			<i>Parasympathetic vagal cardiac branch</i>
Origin: the vagus nerve proximal to the origin of the recurrent laryngeal nerve			SB: 16/18 (88.9%)
Origin: the recurrent laryngeal nerve of the vagus nerve			IB: 18/18 sides (100.0%)
Origin: the vagus nerve distal to the origin of the recurrent laryngeal nerve			TB: 18/18 sides (100.0%)

Abbreviations: IB, inferior cardiac branch; In, inferior cardiac nerve; MN, middle cardiac nerve; SB, superior cardiac branch; SN, superior cardiac nerve; TB, (-): no communication.

These results strongly show that the findings of the black lemur by van den Broek (1908) are not typical of the general morphology of ECNS in lemurs, as well as in the other primates used in his studies, and that the dissection of several specimens is necessary to elucidate the general morphology of ECNS.

4.2. Relationship between ECNS and its surrounding structures

According to statistical data on the primate vertebrae by Schultz (1961), the numbers of the thoracic, lumbar, and thoracolumbar vertebrae are 11–13 (Ave. 12.3), 6–8 (Ave. 6.7), and 18–20 (Ave. 19) in *Lemur* spp., 11–13 (Ave. 11.9), 8–10 (Ave. 9.0), and 20–22 (Ave. 20.9) in *Lepilemur*, 13–14 (Ave. 13.2), 6–7 (Ave. 6.7), and 19–20 (Ave. 19.9) in *Microcebus*, 12, 7–8 (Ave. 7.9), and 19–20 (Ave. 19.9) in *Avahi*, and 12–13 (Ave. 12.1), 7–8 (Ave. 7.8), and 19–20 (Ave. 19.9) in *Propithecus*, respectively. Therefore, variations of the presacral vertebrae in this study are within the normal variation found by Schultz (1961). In addition, the position of the heart within the thorax and the cardiac axis also vary individually regardless of ECNS.

As has been mentioned in our previous studies (Kawashima and Thorington, 2011; Kawashima et al., 2009), the ECNS morphology in lemurs is consistent, with only a trivial variation regardless of the family, in the number of presacral vertebrae, and the cardiac position and axis. However, the middle cervical ganglion is situated lower than the cervicothoracic ganglion and associated with the lower positions of the heart, aortic arch and its great arteries in ring-tailed and ruffed lemurs.

The details of these gross anatomical findings are considered to reflect the developmental findings of the nerve-vessel interaction (Enomoto et al., 2001; Kuratani, 2004; Mukouyama et al., 2002, 2005).

Similarly, we reported the topographic anatomical changes of ECNS in human cases of branchial artery anomalies, the retroesophageal right subclavian artery and anomalous left vertebral artery originating from the aortic arch. Until our reports, it was believed as a conventional theory that the shifts of the cardiac nerves agree with the shift of the branchial arterial origin (Horiguchi et al., 1982). Our detailed studies have clarified that the great arterial branching pattern of the aortic arch does not cause a shift in ECNS, and that the development of *de novo* cardiac nerves along the anomalous artery appears in addition to

the ordinary ECNS, with a segmental accompanying tendency of the cardiac nerve/branch similar to the developmental segmentation of the artery (Kawashima, 2011; Kawashima and Sasaki, 2005, 2007).

4.3. Comparative anatomy of ECNS in strepsirrhines with evolutionary implications

Recently, we conducted a detailed anatomical study of ECNS in lorises and galagos (Lorisiformes), which were the first to diverge from the common ancestor of strepsirrhines (Kawashima and Thorington, 2011). The general morphology of the sympathetic ganglia in Lorisiformes was the superior and middle cervical ganglia without communicating to any cervical nerves, cervicothoracic ganglion composed of the inferior cervical and the first two or three thoracic ganglia and communicating with C8–T2 (3), and several thoracic ganglia communicated to each thoracic nerve. In addition, the general cardiac innervation was the (sympathetic) middle cardiac nerve, which originated from the middle cervical ganglion and sympathetic trunk and all (parasympathetic vagal) cardiac branches. This general ECNS morphology in Lorisiformes was very different from that in New and Old World monkeys, gibbons, and humans (Fig. 11A).

The present study shows that the general ECNS morphology in Lemuriformes is considered to be that of the common ancestor of strepsirrhines because the general ECNS morphology in Lemuriformes was completely similar to that in Lorisiformes. Therefore, the ECNS in the Aye-aye (Daudentoniidae, Chiromyiformes) seems to share its common morphology with Lorisiformes and Lemuriformes. From an ecological perspective, the lemurs have adapted to various niches: diurnal, arboreal/territorial, ring-tailed lemur and sifakas; diurnal, arboreal, ruffed lemur; nocturnal, arboreal, weasel and mouse lemurs; diurnal/nocturnal, arboreal, brown lemur. Therefore, ECNS appears to be a conserved structure derived from a common ancestor rather than reflecting ecological adaptation.

Moreover, ECNS in Lemuriformes exhibits more variability than that in Lorisiformes. The cervicothoracic ganglion in Lorisiformes was composed of the inferior cervical and the first two (50.0%) or three (50.0%) thoracic ganglia, whereas that in Lemuriformes was composed of the inferior cervical and the first one (27.8%), two

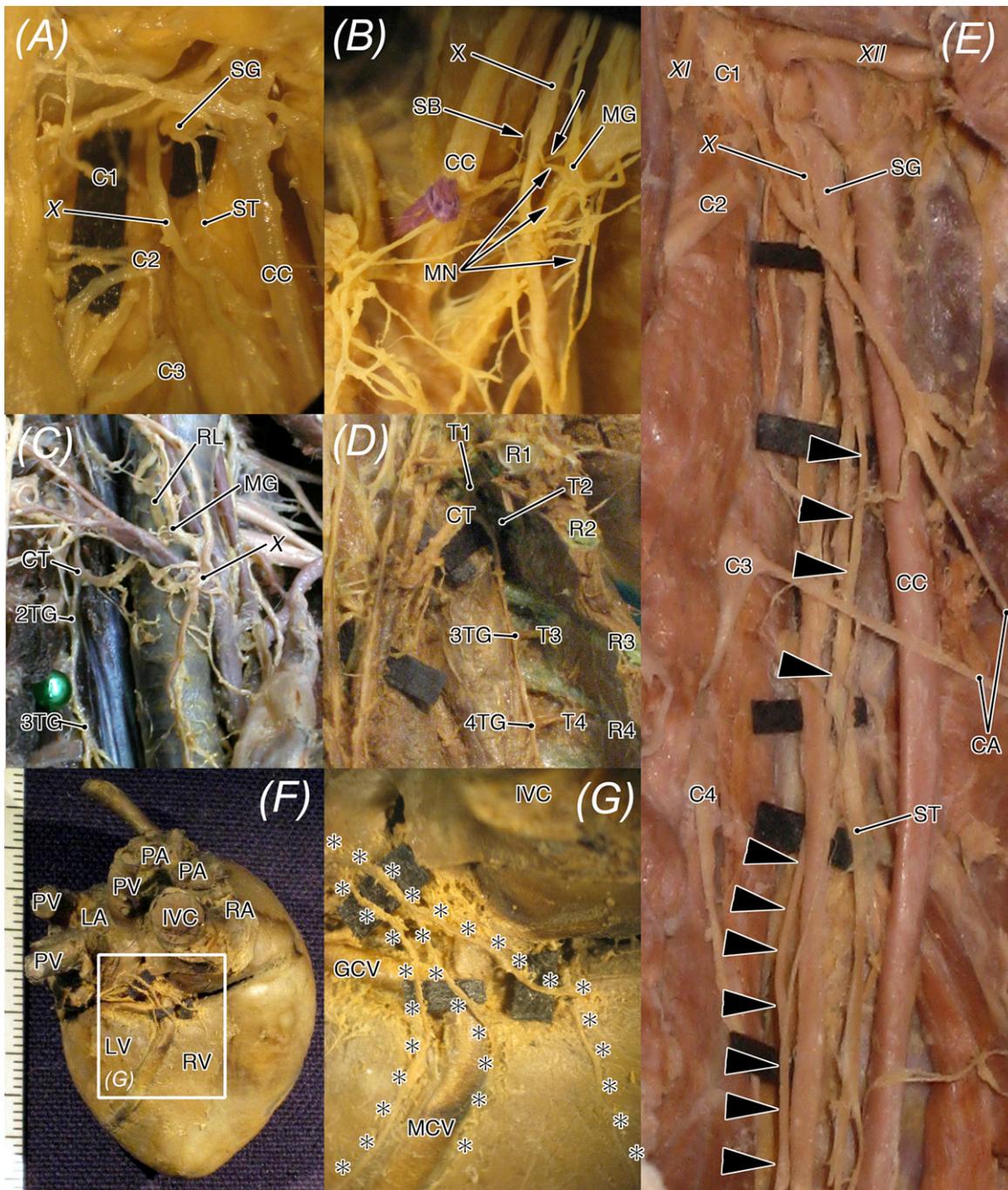


Fig. 10. Photographs showing the general morphology and variation of the autonomic cardiac nervous system in lemurs. (A) Typical superior cervical ganglion communicating without any cervical nerve seen in the mouse lemur (No. 8). (B) Typical middle cervical ganglion communicating without any cervical nerve and its surrounding cardiac nerves seen in the weasel lemur (No. 7). (C and D) Variation of the cervicothoracic ganglion. The CT composed of the inferior cervical and the first thoracic ganglia (C, 27.8%) and composed of the inferior cervical and first and second thoracic ganglia (D, 66.7%). (E) The superior cardiac nerve originating from the superior cervical ganglion seen in the ruffed lemur. (F) The cardiac nervous distribution entering from the venous portion. See Fig. 3 for abbreviations.

(66.7%), or three (5.6%) thoracic ganglia. Although the heart is generally situated in the middle third of the thorax in Lorisiformes and Lemuriformes, the heart in ring-tailed and ruffed lemurs is positioned in the lower half of the thorax. Therefore, the position of the aortic arch and its great arterial branches in some lemurs was also situated lower than that in Lorisiformes. It remains unclear why the middle cervical ganglion tends to position itself more caudally than the cervicothoracic ganglion (Figs. 1 and 3). Moreover, the superior cardiac nerve, which has never been observed in Lorisiformes and New World monkeys, originating from the superior cervical ganglion was present in a ruffed lemur.

The Lorisiformes and Lemuriformes have been recently reclassified into two and four families according to their divergence time estimated from the molecular phylogeny (Horvath et al., 2008; Matsui et al., 2009; Orlando et al., 2008). As seen in the molecular phylogeny, the anatomical structures in the four families of Lemuriformes have more variations than those in the two families of Lorisiformes, and this seems to be reflected in the diversity within Lemuriformes. In other words, the comparative anatomical findings of the wide variations in ECNS in the four families of Lemuriformes compared with lower variations in the two families of Lorisiformes possibly, correspond to the

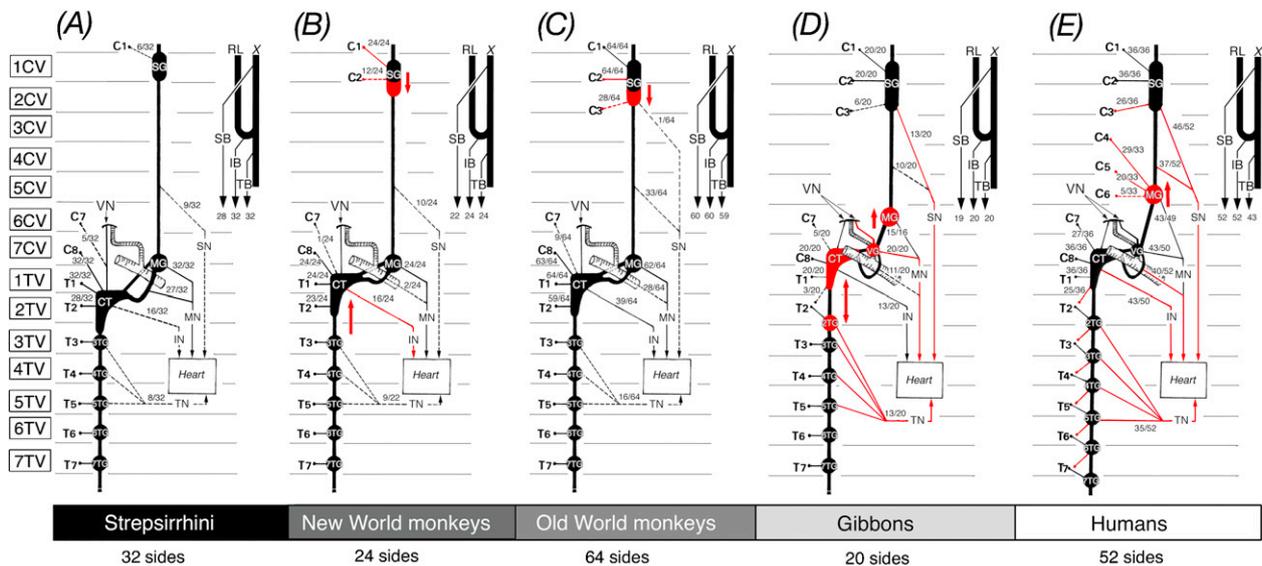


Fig. 11. Schematic diagram of the evolutionary morphology of the extrinsic cardiac nervous system in the primate lineage. A: Strepsirrhines (nontarsier prosimians), Kawashima and Thorington (2011) and present study. B: New World monkeys (Platyrrhini), Kawashima et al. (2009). C: Old World monkeys (Cercopithecoidea), Kawashima et al. (2005, 2007). D: Gibbons (Hylobatidae), Kawashima et al. (2008). E: Humans, Kawashima (2005) and Kawashima et al. (2007). The large left and right small figures show the sympathetic and parasympathetic vagal cardiac nervous systems, respectively. The numbers indicate total bilateral appearances. The solid and broken lines of the communicating branches and cardiac nerves/branches indicate more than and less than 60% occurrences, respectively. The evolutionary changes of the cardiac nervous system as highlighted in red are the expansion of the communicating branches from the superior and middle cervical ganglia to the cervical nerves, the reduction in the number of thoracic ganglia contributing to the cervicothoracic ganglion with cranial positional movement, and the expansion of the range of the sympathetic cardiac nervous origin, whereas the parasympathetic vagal cardiac branches are consistent. See Fig. 3 for abbreviations.

evolutionary biodiversity revealed by the recent molecular phylogeny.

Moreover, the general and common morphologies of ECNS in each group of the primate lineage, such as strepsirrhines, New World monkeys, Old World monkeys, gibbons, and humans, have undergone continuous changes and yet ECNS preserves evolutionary history in close alignment with phylogeny (Fig. 11). Evolutionary changes and diversity in ECNS include a gradual reduction in the number of thoracic ganglia contributing to the cervicothoracic ganglion, the growth of the superior and middle cervical ganglia with branches communicating to the spinal nerves, and the expansion of the range of the sympathetic cardiac nervous origin. The parasympathetic vagal cardiac system and its cardiac branches are consistent among primates. Interestingly, the comparative anatomical findings of the variable sympathetic and constant parasympathetic ECNSs in primates are similar to the mammalian ontogeny of cardiac innervation; however, the sympathetic cardiac innervation has been shown to occur at later stages than the parasympathetic vagal cardiac innervation (Kirby et al., 1980; Shoba and Tay, 2000; Hildreth et al., 2009).

These results suggest that ECNS, a visceral or autonomic structure, preserves its gradual evolutionary changes in the primate lineage – strepsirrhines, New and Old World monkeys, lesser apes, great apes, and humans – without modification and specialization by its functional adaptation as seen in the somatic system, and the extent of the anatomical variation within each group seems to reflect the extent of its diversity, in agreement with molecular evolutionary divergence.

These evolutionary morphological viewpoints based on detailed anatomical findings on the visceral (autonomic) soft structures are useful in gaining a proper understanding of the meaning of the phenotype and unifying the results between molecular biology and morphology in the future. Based on the our anatomical and recent developmental studies, further examination of the reptilian ECNS would help to elucidate dynamic evolutionary changes as seen in the early mammalian developmental stages.

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