Int. J. Morphol.,
40(2):304-313, 2022.

Identification of the Myodural Bridge in a Venomous Snake, the *Gloydius shedaoensis*: What is the Functional Significance?

Identificación del Puente Miodural en una Serpiente Venenosa, *Gloydius shedaoensis*: ¿Cuál es el Significado Funcional?

Chan Li[1]; Chen Yue[2]; Bao Yan[3]; Heng-Tao Bi[3]; Heng Wang[3]; Jin Gong[1]; Campbell Gilmore[4]; Heng Yang[1]; Sheng-Bo Yu[1]; Gary D. Hack[5] & Hong-Jin Sui[1]

LI, C.; YUE, C.; YAN, B.; BI, H. T.; WANG, H.; GONG, J.; GILMORE, C.; YANG, H.; YU, S. B.; HACK, G. D. & SUI, H. J. Identification of the myodural bridge in a venomous snake, the *Gloydius shedaoensis*: What is the functional significance?. *Int. J. Morphol., 40(2)*:304-313, 2022.

SUMMARY: Myodural bridges (MDB) are anatomical connections between the suboccipital muscles and the cervical dura mater which pass through both the atlanto-occipital and the atlanto-axial interspaces in mammals. In our previous studies, we found that the MDB exists in seven terrestrial mammal species, two marine mammal species, two reptilian species, and one bird species. A recent study suggested that given the "ubiquity" of myodural bridges in terrestrial vertebrates, the MDB may also exist in snakes. Specifically, we focused on the *Gloydius shedaoensis*, a species of Agkistrodon (pit viper snake) that is only found on Shedao Island, which is in the southeastern sea of Dalian City in China. Six head and neck cadaveric specimens of *Gloydius shedaoensis* were examined. Three specimens were used for anatomical dissection and the remaining three cadaveric specimens were utilized for histological analysis. The present study confirmed the existence of the MDB in the *Gloydius shedaoensis*. The snake's spinalis muscles originated from the posterior edge of the supraoccipital bones and the dorsal facet of the exocciput, and then extended on both sides of the spinous processes of the spine, merging with the semispinalis muscles. On the ventral aspect of this muscular complex, it gave off fibers of the MDB. These MDB fibers twisted around the posterior margin of the exocciput and then passed through the atlanto-occipital interspace, finally terminating on the dura mater. We observed that the MDB also existed in all of the snakes' intervertebral joints. These same histological findings were also observed in the *Gloydius brevicaudus*, which was used as a control specimen for the *Gloydius shedaoensis*. In snakes the spinal canal is longer than that observed in most other animals. Considering the unique locomotive style of snakes, our findings contribute to support the hypothesis that the MDB could modulate cerebrospinal fluid (CSF) pulsations.

KEY WORDS: Comparative anatomy; Myodural Bridge; *Gloydius shedaoensis*; Cerebrospinal fluid pulsation

INTRODUCTION

The bundles of connective tissue (MDB) that connects the suboccipital musculature to the cervical dura mater, while passing through the atlanto-occipital interspace in humans, was first discovered in 1953 (Lazorthes *et al*., 1953) and later termed the "myodural bridge" in 1995 (Hack *et al*., 1995). In humans the MDB are defined as structures connecting the cervical dura matter to multiple suboccipital muscles, including the rectus capitis posterior minor (RCPmi), the rectus capitis posterior major (RCPma), and the obliquus capitis inferior (OCI) (Scali *et al*., 2011). As a result of these findings accumulated during the two decades after the anatomical structure was formally named in 1995, the MDB are now considered as physiologically significant functional structures and not simply a structure acting as a fixation device for the cervical spinal dura mater in humans (Xu *et al*., 2016).

As comparative anatomical studies can give rise to the physiological functions of related structures, we performed a series of comparative anatomical studies, and have confirmed that MDB exist in seven terrestrial mammal species (Zheng *et*

[1] Department of Anatomy, College of Basic Medicine, Dalian Medical University, Dalian, Liaoning 116044, China.
[2] The Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning 116021, China.
[3] Administration of Liaoning Shedao Island Laotie Mountain National Nature Reserve.
[4] Medical School, St George's University of London, London, UK.
[5] Department of Advanced Oral Sciences and Therapeutics, University of Maryland, Baltimore, Maryland, USA.
Chan L. & Chen Yue equally contributed to this work.

Received: 2021-11-29 Accepted: 2022-02-14

al., 2017), two marine mammal species including the finless porpoise (*Neophocaena phocaenoides*), the sperm whale (Liu *et al*., 2017, 2018), two reptilian species, siamensis crocodiles and red-eared sliders (*Trachemys scripta elegan*) (Zhang *et al*., 2016; Huangfu *et al*., 2019), and one bird species, the rock pigeons (Okoye *et al*., 2018). However, the functional role of the MDB is still under debate.

Recently, Young *et al*. (2021) suggested that given the "ubiquity" of the existence of myodural bridges in terrestrial vertebrates, the MDB might also exist in snakes. Functionally, the MDB appear to transmit tensile forces, generated by the suboccipital musculature, to the cervical dura mater. Therefore it is possible that the MDB can modulate the velocity as well as the quantity of cerebrospinal fluid (CSF) pulsations (Scali *et al*., 2013a; Zheng *et al*., 2014).

From an evolutionary perspective, it becomes important to verify the existence of the MDB in animals belonging to various orders. In the present study, we investigated the *Gloydius shedaoensis*, which is a species of snake that is unique to Shedao Island, which is located off the coast of Liaoning Province in northeastern China, in the southeastern sea of Dalian City. Due to its isolation from the mainland, *Gloydius shedaoensis* appears to be less evolved when compared with species of Agkistrodons (viper snakes) that inhabit the mainland of northeastern China. Thus, *Gloydius shedaoensis* may retain more primitive biological characteristics and provide additional evolutionary information about MDB. Therefore, the aim of the present study was to examine the possible existence of MDB in snakes and to discuss the potential physiological functions of these putative MDB based on the morphological characteristics of these MDB.

Ethics Statement. In Cooperation with the Administration of Shedao Island Laotie Mountain Nature Reserve, six head and neck cadavers of adult *Gloydius shedaoensis* were acquired. All six *Gloydius shedaoensis* died naturally and were collected within a four-week time span on Shedao Island before the hibernation. The six head and neck cadavers of *Gloydius shedaoensis* were permitted for scientific research under the approval of the Ethics Committee of the Administration of Shedao Laotie Mountain Nature Reserve.

MATERIAL AND METHOD

Dissection of the suboccipital region. Three of the six head-and-neck cadavers (cadavers #1-#3) of *Gloydius shedaoensis* were used for gross dissection which focused on the suboccipital region. As *Gloydius shedaoensis* is considered a national-level protected animal, it was difficult to obtain fully intact cadavers of this animal. Thus, all of the obtained head-and-neck cadavers of *Gloydius shedaoensis* contained only the vertebrae up to V6 (the sixth vertebra). With the purpose of studying the structures within the suboccipital region as well as the interspaces between adjacent vertebrae of these snakes, layer-by-layer dissections of the posterior occipital and the anterior neck regions were performed.

Firstly, the skin and fat were removed until the spinalis and semispinalis muscles were visible. We observed that these muscles merged, forming the spinalis-semispinalis muscle unit (SSMU) which is well described in the literature (Jayne, 1982). The origins of spinalis muscles were located at the posterior edge of the supraocciput and the dorsal facet of the exocciput, and then extended caudally on both sides of the spinous processes covering the spine, merging with the semispinalis muscles. In one cadaver (cadaver #1), the spinalis muscles were separated and lifted caudally along the spine from their cranial origins to expose the atlanto-occipital interspace. For the remaining two cadavers of *Gloydius shedaoensis* (cadavers #2 and #3), one side of the longissimus dorsi muscles (which are indicated with red names in Fig. 1C) were removed to expose the vertebrae at the suboccipital level. On the same side, the merged spinalis-emispinalis muscle unit (SSMU), at the level of atlanto-occipital interspace, was lifted supra-posteriorly, until the MDB connective tissue and its neighboring structures could be observed. A light dissection microscope was used during the entire procedure of gross dissections (NSZ-608T, NOVEL Inc. Nanjing, China). Images of the gross dissections were taken with a Canon 7D camera equipped with a 100 mm/f2.8macro lens (Canon, Tokyo, Japan) and the self-imaging system of an NSZ-608T dissection microscope (NOVEL Inc. Nanjing, China).

Histology analysis.In order to isolate the region which included the MDB connections and their associated muscles, the suboccipital region, the spinal dura mater, and the vertebrae of the neck of three frozen head-and-neck specimens (up to V6) of *Gloydius shedaoensis* (cadaver #4-#6) were cut into two symmetrical parts along the median sagittal plane. Additionally, serving as controls for *Gloydius shedaoensis*, three frozen head-and-neck cadavers (up to V6) of *Gloydius brevicaudus* were included in the morphological study. The skin, fat, viscus, and other unrelated tissues were removed from the specimens. Fixed in 10 % formalin, and then decalcified in 8 % hydrochloric acid for 14-18 days. Head-and-neck tissue blocks were prepared which included tissues from the parietal part of the head down to the sixth vertebral level. After regular paraffin embedding, serial 8-mm sections were then made. Masson and VG staining of the MDB located between the muscular structures and the cervical spinal dura mater were observed utilizing the light microscope. For the Masson staining, cadaver #4 of *Gloydius shedaoensis* was stained with ponceau and fast green, while cadaver #5 and #6 were stained with ponceau and aniline blue. The hematoxylin and eosin (HE) stained sections, the Van Gieson (VG, picric acid, and acid fuchsin) stained sections, and the Masson staining sections were photographed and analyzed by utilizing the Nikon NIS image system (Nikon Eclipse 80i, Nikon, Tokyo, Japan).

RESULTS

Anatomy dissection. Three of the six head and neck 10 % formalin fixed specimens were used for anatomical dissections to explore the possible MDB connections within the suboccipital region. These three fixed partial specimens contained vertebrae up to V6 (Figs. 1A and 1B). For one specimen (cadaver #1), the dissection started from the posterior aspect. In the dorsum of cadaver #1, the spinalis and semispinalis muscles were observed after we removed the skin. In *Gloydius shedaoensis*, it was observed that these two groups of muscles merged to form a muscular complex (SSMU) which has previously been described in the literature (Jayne). The spinalis and semispinalis muscles originated from the posterior edge of the supraocciput and the dorsal facet of the exocciput, and then extended caudally covering the dorsal aspect of the vertebrae (Figs. 1C, 1D and 1E). The atlanto-occipital interspace, was covered and enclosed within a sheet of fascia-like, semilucent membrane which originated from the ventral surface of the SSMU, again consisting of both the spinalis and semispinalis muscles (Fig. 1F). This merged muscle unit has been previous described and referred to as the "muscularis spinalis et semispinalis capitis" (SSC) in the literature (Martin, 1994). This neck muscle (SSMU) in snakes belongs to the M.transversospinalis group of muscles and is the most medial muscle of the epaxial musculature (Martin).

To reveal the MDB connections in the other head-and-neck specimens, we exposed the spinal canal by dissecting the suboccipital region from the left side of the specimen and observed the atlanto-occipital interspace (cadaver #2 and #3). Originating from the ventral surface of the SSMU, we observed a dense bundle of tendinous fibers extending ventrally, which passed through the atlanto-occipital interspace and ultimately fused with the spinal dura mater. This new finding represents the MDB connections. Both of these two specimens showed the same results (Figs. 1G, 1H and 1I).

The histology of the MDB connections. Three of the six head and neck specimens were used for the histological analysis (cadaver #4-#6). In the parasagittal sections, stained with Van Gieson (VG) and Masson stains, the SSMU, the osseous structures, the dura mater, the spinal cord, as well as the collagenous MDB connections were identified. The intervertebral spaces, up to V6, were then all observed (Figs. 2, 3 and 4).

Unlike humans, *Gloydius shedaoensis* have three individual types of occipital bones, including the supraocciput that is firmly connected to the caudal edge of the parietal bone. Moreover, it was observed from the dorsal aspect that the exocciput attached to the supraocciput. The exocciput appeared as a "C" shaped notched circle in the cross-sectional plane. Viewed from the ventral aspect, we observed that the basiocciput, filled the gap of the "C" shaped notched circle of the exocciput to form the foramen magnum. This co-constituted occipital bone formed the atlanto-occipital joint along with the atlas (V1). The atlas was observed to lack a spinous process. In addition, we observed that the *Gloydius shedaoensis* had imbricate vertebrae, and this was observed in all the intervertebral joints as well as the atlanto-occipital joint. The caudal edge of the upstream vertebra covered the cranial edge of the downstream vertebrae (Figs. 2A and 3A).

Viewing the Masson and VG stained sections of cadaver #4, the MDB originated from the ventral aspect of the SSMU, and extended anterio-ventrally toward the atlanto-occipital interspace, then wrapped around the cranial edge of the atlas and extended further anterio-ventrally to ultimately fuse with the dura mater. These same results were observed at the atlanto-axial interspace (Figs. 2B and 3B). Serving as a control for *Gloydius shedaoensis*, the sagittal sections from the HE and VG staining of *Gloydius brevicaudus*, were consistent with the results observed with the *Gloydius shedaoensis* (Figs. 2C and 3C). These MDB connections were found within every two adjacent vertebrae along the spine, which included the atlanto-occipital interspace, the atlanto-axial interspace, and all interspaces between the two adjacent vertebrae fromV2-V6 (Figs. 2D, 2E, 3D and 3E). These MDB connections gradually diminished moving from V2 to V6 (Figs. 2A and 3A). The MDB-like connections appeared green and/or blue with the Masson staining and red with the VG staining (Figs. 2-4), which indicates that the fibers of the MDB are primarily collagenous.

Observing the Masson stained sections of cadavers #5 and #6, the muscle fibers were displayed in red, while the MDB fibers, which originated from the ventral aspect of the SSMU were stained in blue, indicating that the fibers of the MDB are collagenous fibers (Figs. 4A, 4C and 4E). Observing the VG stained sections of cadavers #5 and #6, the fibers of the MDB were stained red, which indicates that these fibers are also collagenous (Figs. 4B, 4D and 4F).

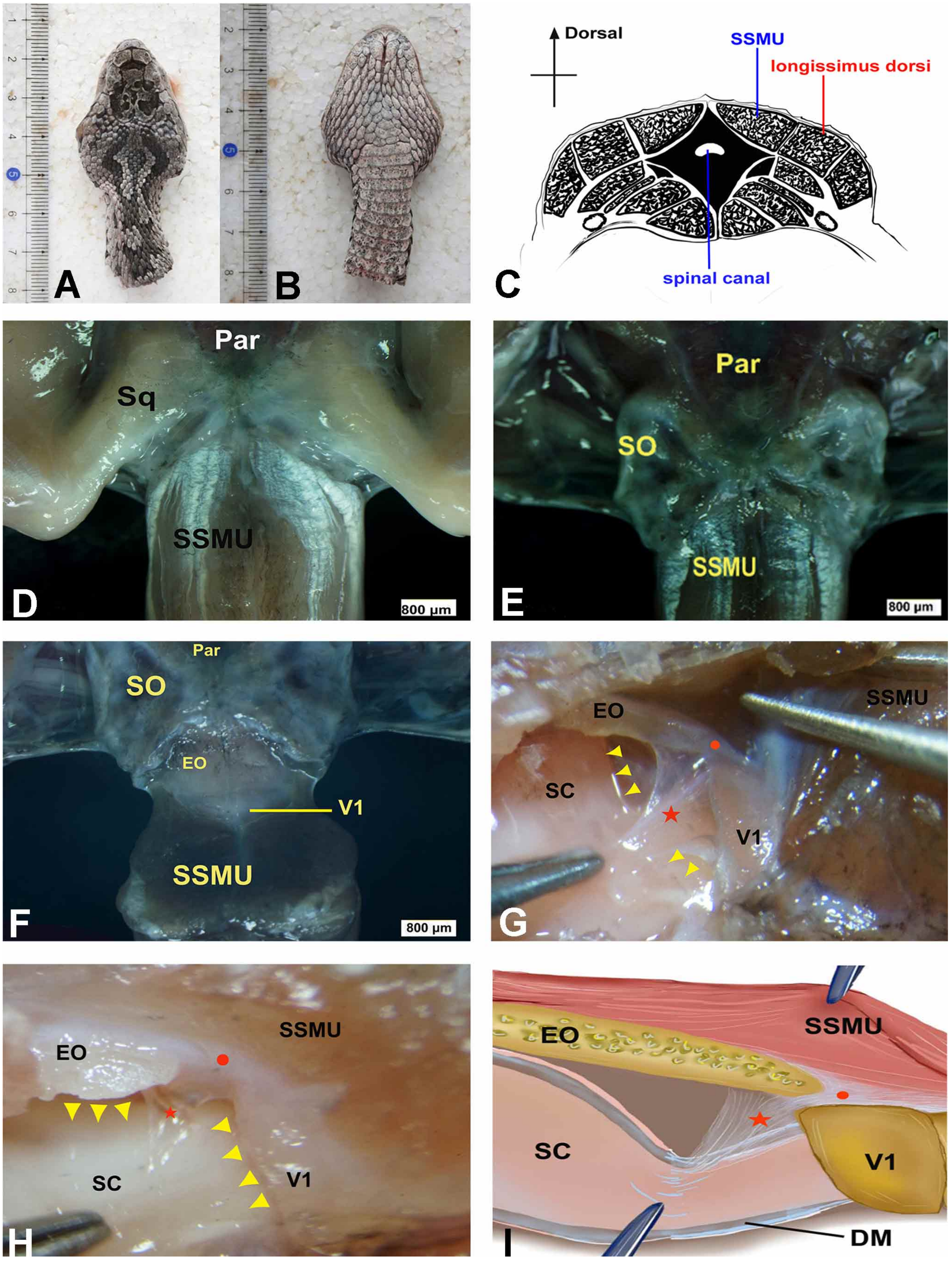

Fig. 1. The MDB connections from the spinalis muscles to the cervical spinal dura mater through atlanto-occipital interspace based on the gross anatomy dissection. A, B, dorsal and ventral view of the head and neck specimen of Gloydius Shedaoensis (up to V6); C, modeling chart of cross sectional plane at cervical level; D, dorsal view of the suboccipital region; E, dorsal view of the suboccipital region without squamosal bones; F, dorsal view of the atlanto-occipital interspace; G, sagittal view of the MDB connections and its neighboring structures of cadaver 2; H, sagittal view of the MDB connections and its neighboring structures of cadaver 3. Par: parietal bone; Sq: squamosal bone; SSMU: spinalis & semispinalis muscle unit; SO: supraocciput; EO: exocciput; circle: atlanto-occipital interspace; star: the MDB connections; triangles: dura mater; DM: dura mater; SC: spinal cord.

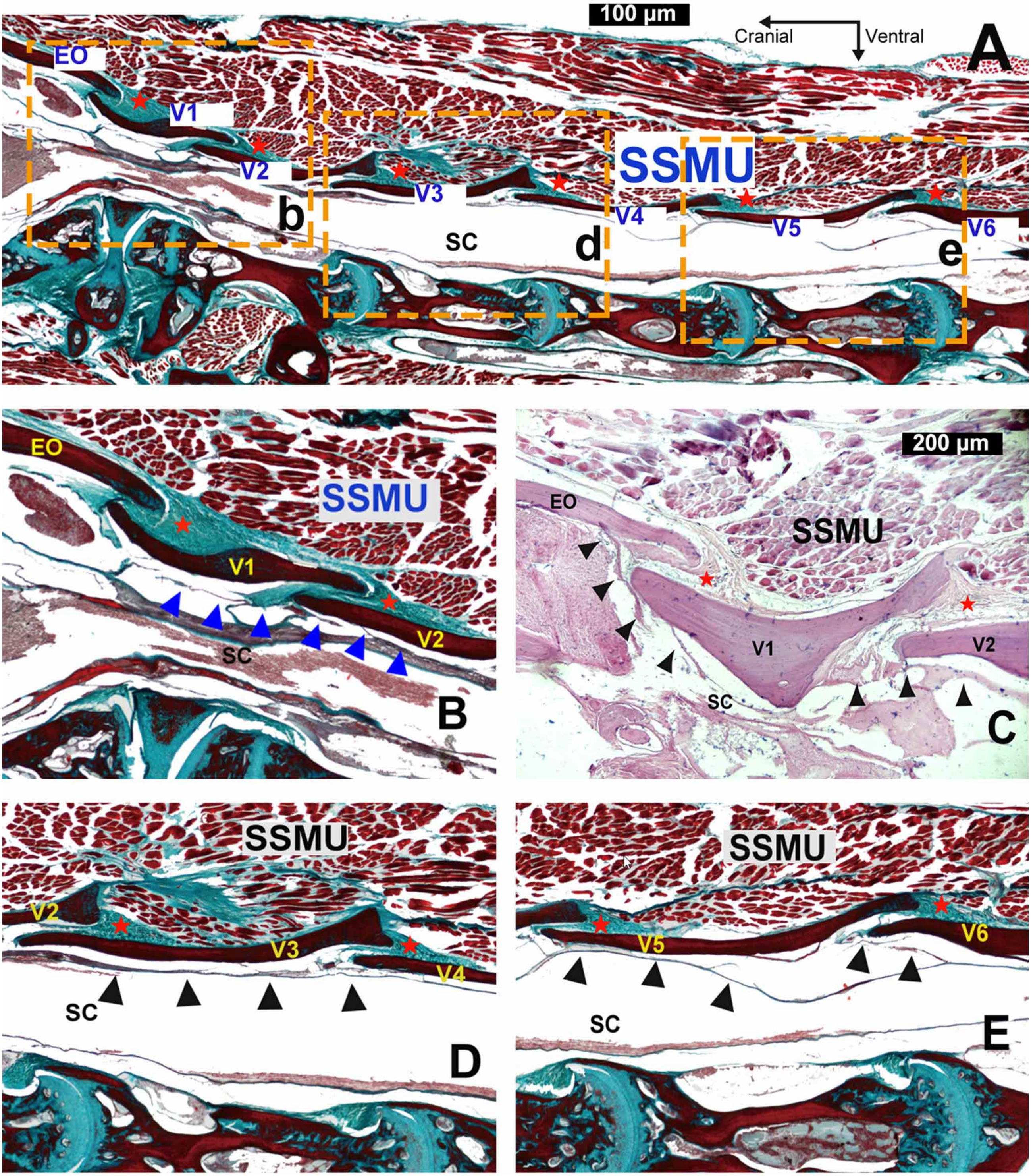

Fig. 2. The MDB connections between the spinalis muscles and the cervical dura mater based on the histological analysis (Masson stain and H&E stain) on sagittal sections. A, sagittal section of Masson stain of the MDB connections from parietal bone to V6 within cadaver 4 of Gloydius Shedaoensis; B, enlarged view of frame b; C, sagittal section of H&E stain of the MDB connections within Gloydius Brevicaudus; D, enlarged view of frame d; E, enlarged view of frame e. Sq: squamosal bone; SSMU: spinalis & semispinalis muscle unit; EO: exocciput; circle: atlanto-occipital interspace; star: the MDB connection; triangles: dura mater; DM: dura mater; SC: spinal cord.

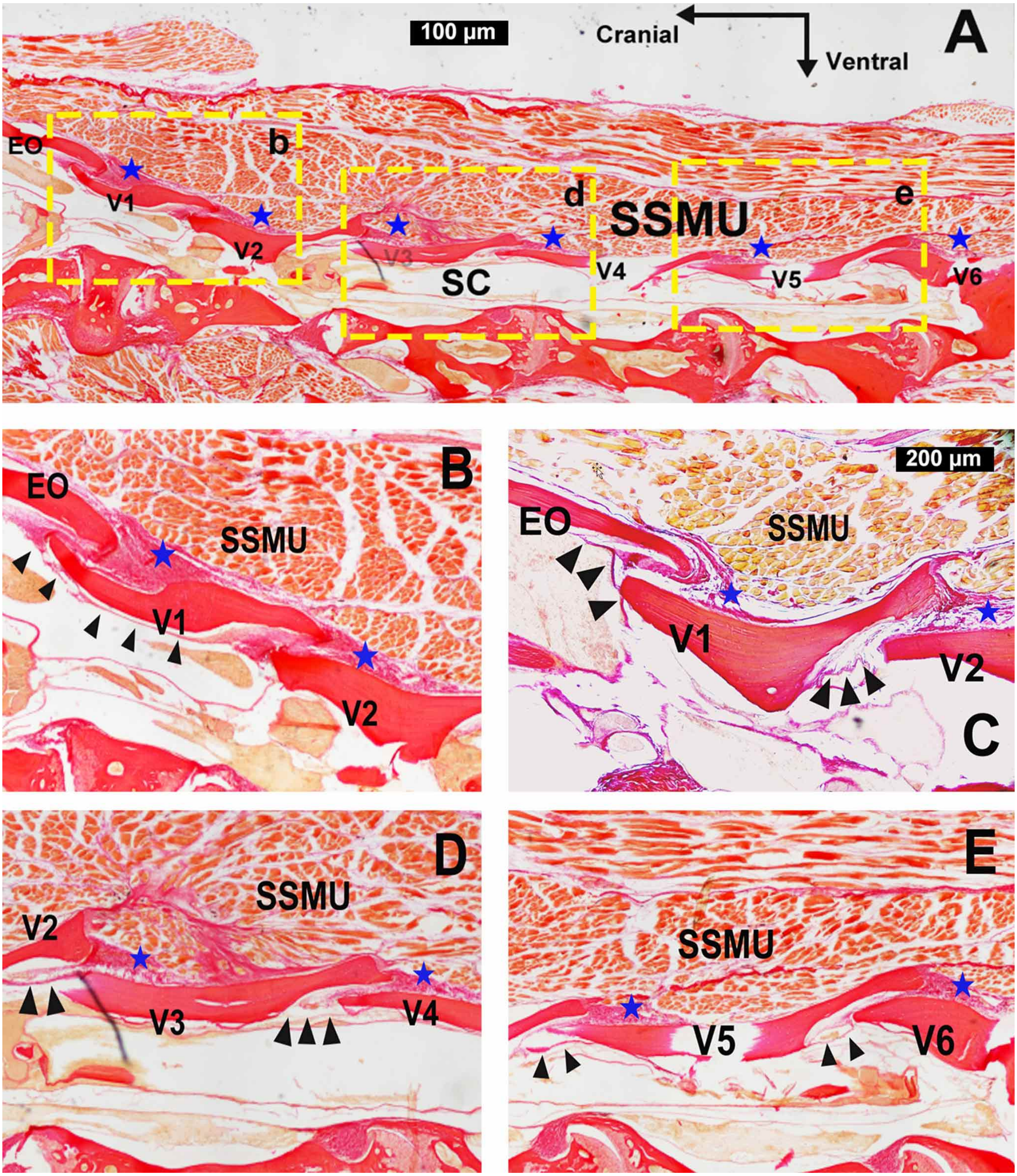

Fig. 3. The MDB connections between the spinalis muscles and the cervical dura mater based on the histological analysis (VG stain) on sagittal sections. A, sagittal section of the MDB connections from parietal bone to V6 within cadaver 4 of Gloydius Shedaoensis; B, enlarged view of frame b; C, sagittal section of the MDB connections within Gloydius Brevicaudus; D, enlarged view of frame d; E,enlarged view of frame e. Sq: squamosal bone; SSMU: spinalis & semispinalis muscle unit; EO: exocciput; circle: atlanto-occipital interspace; star: the MDB connection; triangles: dura mater; DM: dura mater;SC: spinal cord.

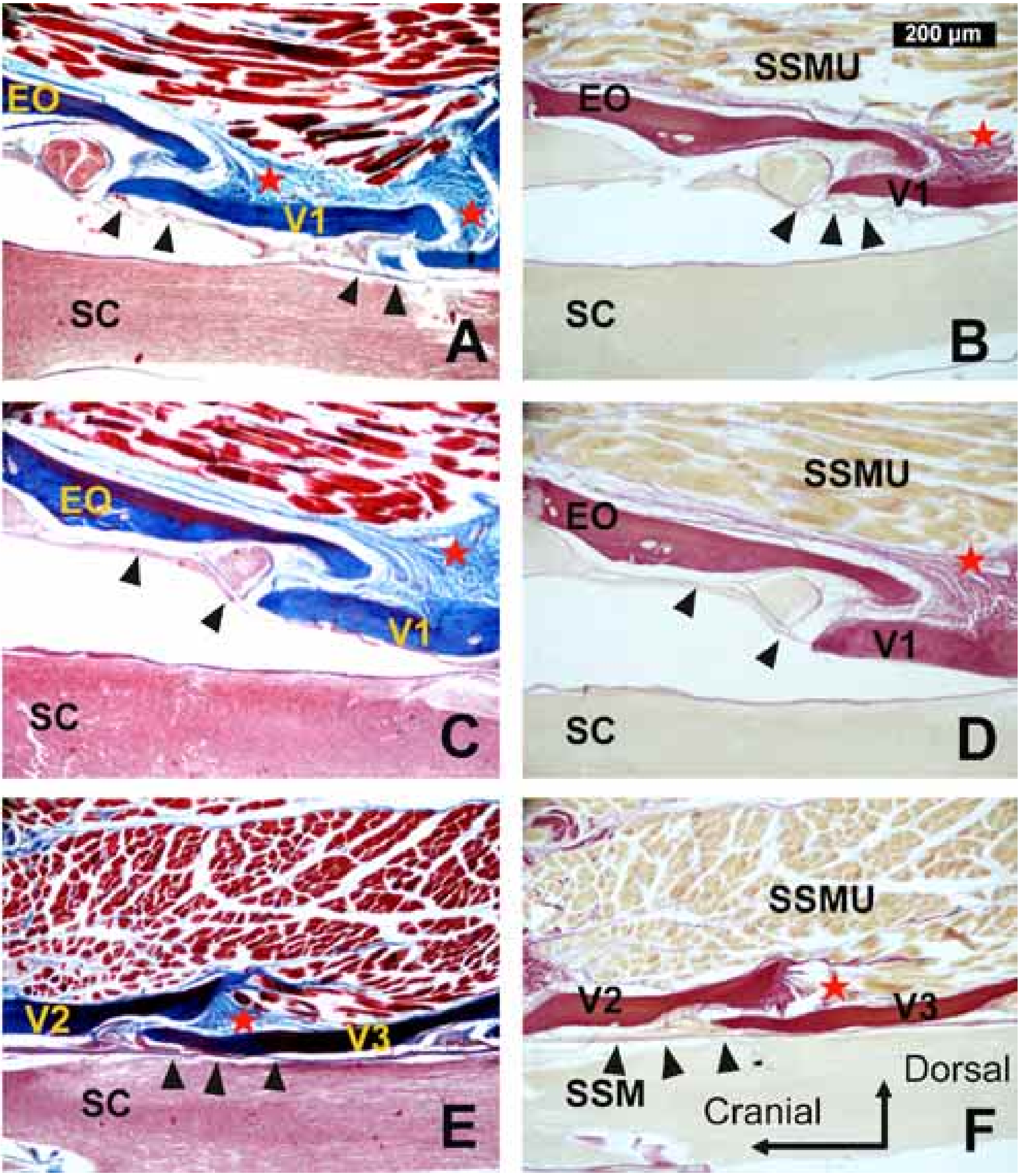

Fig. 4. The comparison of the MDB connections within Gloydius Shedaoensis between the spinalis muscles and the cervical dura mater based on multiple staining methods. Figure A, sagittal section of Masson stain of the MDB connections within cadaver 5;B, sagittal section of VG stain of the MDB connections within cadaver 5; C, E sagittal sections of Masson stain of the MDB connections within cadaver 6; D, F are sagittal sections of VG stain of the MDB connections within cadaver 6. Sq: squamosal bone; SSMU: spinalis & semispinalis muscle unit; EO: exocciput; circle: atlanto-occipital interspace; star: the MDB connection; triangles: dura mater; DM: dura mater; SC: spinal cord.

DISCUSSION

From an evolutionary perspective, a highly conserved anatomical structure is functionally significant. Since the MDB was formally named in 1995 (Hack *et al*.), numerous studies have been done to explore the universal existence of this structure. The MDB was initially observed in humans, as a tendon or fascia like tissue connecting the suboccipital muscles to the cervical dura mater. The MDB in humans has multiple origins from the suboccipital muscles, which includes the rectus capitis posterior minor (RCDmi) (Rutten *et al*., 1997; Scali *et al*., 2013b), rectus capitis posterior major (RCDma) (Rutten *et al*.; Scali *et al*., 2013a), and obliquus capitis inferior (OCI) (Pontell *et al*., 2013b; Scali *et al*., 2013b). The MDB has now been observed in the suboccipital region of multiple quadrupedal terrestrial mammals, which includes dogs, cats, rats, rabbits, guinea pigs, and macaques (Zheng *et al*., 2017). Within the marine mammalian order, our previous study verified the existence of the MDB in the finless porpoise and the sperm whale (Liu *et al*., 2017, 2018).The MDB is now considered ubiquitous in mammals (Zheng *et al*., 2017). As we only found the MDB in the suboccipital region of pigeons, it is likely that the MDB is also highly conserved within Columbiformes birds as well (Okoye *et al*.). As for reptilians, we observed the MDB fibrous connections between the suboccipital muscles and the cervical dura mater in siamensis crocodiles and red-eared sliders (Zhang *et al*.; Huangfu *et al*.).

Although the MDB connections have been found in two reptilian species, the structure within these reptilians showed distinct anatomical features when compared with the MDBs observed in mammals. In the siamensis crocodile, the MDB originated from the suboccipital muscles and extended towards the proatlas and the dorsal atlanto-occipital membrane (DAOM). The fibers of the MDB merged with the DAOM and formed bundles of trabeculae which then extended ventrally and finally terminated on the cervical dura mater (Zhang *et al*.). For crocodiles, the anatomical characteristics of the origin of the MDB was like that observed in mammals, however the terminal part appeared to be unique. Within the epidural space, we previously observed multiple thick trabeculae in the crocodile instead of a continuous fascia-like sheet/membrane which inserted into the dura mater. For the red-eared sliders, the same configuration was noted as observed in the siamensis crocodiles. Although red-eared sliders do not have a proatlas, multiple cord-like connections were found between the dorsal intervertebral membranes and the cervical dura mater. As a result of that study, the authors assumed that the observed bundles of cord-like trabeculae might be a general feature of the MDB in reptiles (Huangfu *et al*.).

The snake represents the youngest branch of reptiles. Snakes are squamata reptiles without limbs and have biological, ecological, and evolutionary differences (Da Silva *et al*., 2018). Furthermore, their form of locomotion is unique. The MDB connections observed in the *Gloydius shedaoensis* also showed unique anatomical characteristics when compared with siamensis crocodiles and red-eared sliders. In the caudal aspect of the parietal bone, instead of the proatlasas observed in crocodiles, snakes have anexocciput located downstream from the supraocciput. Moreover, we observed that the exocciput and the atlas formed the atlanto-occipital joint. Again, within the *Gloydius shedaoensis*, the spinalis and semispinalis muscles merged forming one muscle unit (SSMU), their MDB originated from the ventral aspect of the SSMU, and passed through the interspace of two adjacent vertebral units including the atlanto-occipital interspace, the atlanto-axial interspace, and all remaining interspaces between two adjacent vertebrae (V2-V6), and finally terminated on the dura mater. Due to the local animal protection policy related to the *Gloydius shedaoensis*is, we were only able to obtain a part of the body of this snake, up to V6. For *Gloydius shedaoensis*is, the SSMU gave off fibers of the MDB, which passed through the atlanto-occipital interspace, and finally terminated on the dura mater. Therefore, anatomically, the MDB observed in the *Gloydius shedaoensis* is comparable to the MDB we observed in the finless porpoise, a marine mammal which shares similar locomotive style within the suboccipital region (Liu *et al*., 2017, 2018). The only difference is that snakes have imbricate vertebrae; the caudal edge of the upstream vertebrae covers the cranial edge of the downstream vertebra; while the arrangement of vertebrae in the finless porpoise resembles that of humans, where the intervertebral space is more open. The MDB observed in the *Gloydius shedaoensis* are similar to the MDB observed in mammals, but not to its reptilian relatives, such as turtles or crocodiles. Mammals evolved from ancient reptiles; the reptilians are the ancestors of mammalians on the evolutionary tree and snakes represent a young branch of reptilians. In summary this suggests that the MDB could have evolved with the general evolution of species. This hypothesis implies that the MDB does not only universally exist amongst the animals currently inhabiting the earth, but also existed in ancient/primitive animals which are at the roots of the evolutionary tree.

In the last two decades, multiple studies have been performed focusing on the possible physiological functions of the MDB. However, currently, the precise function of the MDB remains up for debate. Hack *et al*. suggested that the MDB prevents in folding of the dura mater due to its vertical fiber arrangement. According to research in 1997, the authors proposed that the MDB could help to maintain the flow of the cerebrospinal fluid (CSF) circulation in the subarachnoid cavity and cerebello-medullary cistern during head movements (Rutten *et al*.). In 2003, Humphreys *et al*. (2003) observed that an injured MDB altered the volume of CSF in the subarachnoid space and attenuated the buffering of cord impingement, and therefore they postulated that the MDB is implicated in cervico-cephalic headaches. Scali *et al*. (2013b) and Pontell *et al*. (2013a,b) also suggested that the MDB is related to the maintenance of CSF outflow from the cisterna magna by maintaining the integrity of the subarachnoid space.

These findings suggested that the MDB can affect CSF pulsation. Therefore, in addition to arterial pulsations, the MDB may also play an important role in dynamics of CSF pulsation. In 2016, a research article indicated that the mean velocity, flow rate, and flow direction of CSF was significantly affected by head rotations, at least at the level of occipito-cervical junction (Xu *et al*.). *Gloydius shedaoensis* live on Shedao Island which is distant from the mainland of northeastern China. Thus, the ecological habitat of *Gloydius shedaoensis* are is different from that of the snakes on the mainland. However, we observed that the MDB of the *Gloydius shedaoensis* and the *Gloydius brevicaudus* located on the mainland of China basically share the same anatomical features, despite the minor differences of their vertebral bones. Although reptiles are not as active as mammals, the heart rate of *Gloydius shedaoensis* is around 70p/min or higher, which is similar to humans'(Lillywhite & Smits, 1984). In terms of body proportions, the spinal canal of snakes is significantly longer than that of humans and most other mammals. Hence, this could lead to a very low arterial blood pressure in the distal part of the spinal canal due to the distance from the heart. Although the central arterial pressure is sufficient to pulse the CSF circulation, blood pressure in the arteries in the distal spinal canal is not sufficient to drive the circulation of the CSF. Furthermore, experimental results of a recent publication authored by Young *et al*., revealed that body movements of viper boa produced greater CSF pulses than those generated by its cardiac or ventilatory cycles.

The MDB in *Gloydius shedaoensis* originated from the semispinalis-spinalis complex (SSMU) passing through the intervertebral spaces and finally terminating on the dura mater. Although we were not able to obtain the body after the V6 level, it is probable that the MDB connections would exist along the entire spine. Interestingly, Elbrond and Schultz recently reported that MDB connections exist along the entire spine of the horse (Elbrønd, 2019). In addition, a publication revealed that instead of a directional flow, CSF flows non-directionally from high pressure to low pressure regions, as the pressure in various cavities (including the ventricles, central spinal cord, and subarachnoid space, etc.) is different from that of the central nervous system (Matsumae *et al*., 2019). Considering the unique locomotive style of snakes, the spinalis and semispinalis muscles are active in constant motion while the snake is moving. This activity of the epaxial muscles of snakes, including the semispinalis-spinalis complex (SSMU), during terrestrial locomotion has been previously described utilizing electromyography (Jayne). The MDB could potentially transfer muscular tensile forces on the dura mater, positively pulling the dura mater. Hence these induced changes to the local volume and pressure of the CSF in the subarachnoid space, may pulse the CSF thus impacting. This concept would support our previous hypothesis that the MDB may participate in the regulation of CSF dynamics and suggesting a functional role for the MDB.

In this study, we observed that there are MDB existing between the merged spinalis-semispinalis muscle units (SSMU) and the dura mater within *Gloydius shedaoensis*. Snakes are the youngest branch of reptilians and *Gloydius shedaoensis* is one of the most primitive snakes due to its ecology (Da Silva *et al*.). The existence of the MDB in *Gloydius shedaoensis* indicates that this anatomical bridge connecting the suboccipital muscles to the dura mater may have been highly conserved during reptilian evolution and/ or general evolution of all animal species. Collectively, these findings provide supportive information for exploring the physiological functions of the human MDB.

GRANT SPONSOR. This work was supported by the young scientist's foundation of Liaoning provincial department of education (507058); Natural Science Foundation of China (NSFC31871213, NSFC32071184).

ACKNOWLEDGMENTS

The author would like to thank the Administration of Liaoning Shedao Island Laotie Mountain National Nature Reserve for provision of cadaveric specimens; and would like to thank Dalian Hoffen Preservation Institution for technical support of histological staining.

AUTHOR CONTRIBUTIONS

Conceptualization: HJS; Methodology: CL, CY; Validation: HJS, GDH; Formal analysis: CL; Investigation: CL, CY, JG; Resources: HW, BY, HTB; Data curation: CL, CG; Writing -original draft: CL; Writing - review & editing: CL, HJS, GDH; Visualization: HY; Supervision: HJS, SBY; Project administration: HJS.

LI, C.; YUE, C.; YAN, B.; BI, H. T.; WANG, H.; GONG, J.; GILMORE, C.; YANG, H.; YU, S. B.; HACK, G. D. & SUI, H. J. Identificación del puente miodural en una serpiente venenosa, *Gloydius shedaoensis*: ¿Cuál es el significado funcional? *Int. J. Morphol., 40(2)*:304-313, 2022.

RESUMEN: Los puentes miodurales (MDB) son conexiones anatómicas entre los músculos suboccipitales y la duramadre cervical que pasan a través de los espacios intermedios atlanto-occipital y atlanto-axial en los mamíferos. En nuestros estudios anteriores, encontramos que el MDB existe en siete especies de mamíferos terrestres, dos especies de mamíferos marinos, dos especies de reptiles y una especie de ave. Un estudio reciente sugirió que dada la "ubicuidad" de los puentes miodurales en los vertebrados terrestres, el MDB también puede existir en las serpientes. Específicamente, nos enfocamos en *Gloydius shedaoensis*, una especie de Agkistrodon (serpiente víbora) que solo se encuentra en la isla Shedao, en el mar sureste de la ciudad de Dalian en China. Se examinaron seis especímenes cadavéricos de cabeza y cuello de *Gloydius shedaoensis*. Se utilizaron tres especímenes para la disección anatómica y los tres especímenes cadavéricos restantes se utilizaron para el análisis histológico. El presente estudio confirmó la existencia del MDB en *Gloydius shedaoensis*. Los músculos espinosos de la serpiente se originaron en el margen posterior de los huesos supraoccipital y la cara dorsal del exoccipucio, y luego se extendieron a ambos lados de los procesos espinosas de la columna vertebral, fusionándose con los músculos semiespinosos. En la cara ventral de este complejo muscular se desprendían fibras del MDB. Estas fibras MDB se ubican alrededor del margen posterior del exoccipucio y luego atraviesan el interespacio atlanto-occipital, terminando finalmente en la duramadre. Observamos que el MDB también existía en todas las articulaciones intervertebrales de las serpientes. Estos mismos hallazgos histológicos también se observaron en *Gloydius brevicaudus*, que se utilizó como muestra de control para *Gloydius shedaoensis*. En las serpientes, el canal espinal es más largo que el observado en la mayoría de los otros animales. Teniendo en cuenta el estilo único locomotor de las serpientes, nuestros hallazgos contribuyen a respaldar la hipótesis de que el MDB podría modular las pulsaciones del líquido cerebroespinal.

PALABRAS CLAVE: Anatomía comparada; Puente miodural; *Gloydius shedaoensis*; **Pulsación de líquido cerebroespinal.**

REFERENCES

Da Silva, F. O.; Fabre, A. C.; Savriama. Y.; Ollonen J.; Mahlow, K.; Herrel, A.; Müller, J. & Di-Poï, N. The ecological origins of snakes as revealed by skull evolution. *Nat. Commun., 9(1)*:376, 2018.

Elbrønd, V. S. Equine myodural bridgesAn Anatomical,integrativeand functional description of myodural bridges along the spine of horses: Special focus on the atlanto-occipital and atlanto-axial regions. *Med. Res. Arch., 7(11)*, 2019. Available from: https://esmed.org/MRA/mra/article/view/2005/193545487

Hack, G. D.; Koritzer, R. T.; Robinson, W. L.; Hallgren, R. C. & Greenman, P. E. Anatomic relation between the rectus capitis posterior minor muscle and the dura mater. *Spine (Phila Pa 1976), 20(23)*:2484-6, 1995.

Huangfu, Z.; Zhang, X.; Sui, J. Y.; Zhao, Q. Q.; Yuan, X. Y.; Li, C; Dou, Y. R.; Tang, W.; Du, M. L.; Zheng, N.; *et al*. Existence of myodural bridge in the Trachemys scripta elegans: Indication of its important physiological function. *Int. J. Morphol., 37(4)*:1353-60, 2019.

Humphreys, B. K.; Kenin, S.; Hubbard, B. B. & Cramer, G. D. Investigation of connective tissue attachments to the cervical spinal dura mater. *Clin. Anat., 16(2)*:152-9, 2003.

Jayne, B. C. Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations with locomotion and constriction. *J. Morphol., 172(1)*:83-96, 1982.

Lazorthes, G.; Poulhes J. & Gaubert, J. La duremere de la charnierecranio-rachidienne. *Bull. Assoc. Anat., 78*:169-72, 1953.

Lillywhite, H. B. & Smits, A. W. Lability of blood volume in snakes and its relation to activity and hypertension. *J. Exp. Biol., 110*:267-74, 1984.

Liu, P.; Li, C.; Zheng, N.; Xu, Q.; Yu, S. B. & Sui, H. J. The myodural bridge existing in the Nephocaena phocaenoides. *PLoS One, 12(3)*:e0173630, 2017.

Liu, P.; Li, C.; Zheng, N.; Yuan, X.; Zhou, Y.; Chun, P.; Chi, Y.; Gilmore, C. & Yu, S. The myodural bridges' existence in the sperm whale. *PLoS One, 13(7)*:e0200260, 2018.

Martin, A. Spinalis capitis, or an accessory paraspinous muscle? *J. Anat., 185(Pt. 1)*:195-8, 1994.

Matsumae, M.; Kuroda, K.; Yatsushiro, S.; Hirayama, A.; Hayashi, N.; Takizawa, K.; Atsumi, H. & Sorimachi, T. Changing the currently held concept of cerebrospinal fluid dynamics based on shared findings of cerebrospinal fluid motion in the cranial cavity using various types of magnetic resonance imaging techniques. *Neurol. Med. Chir. (Tokyo), 59(4)*:133-46, 2019.

Okoye, C. S.; Zheng, N.; Yu, S. B. & Sui, H. J. The myodural bridge in the common rock pigeon (*Columbia livia*): Morphology and possible physiological implications. *J. Morphol., 279(10)*:1524-31, 2018.

Pontell, M. E.; Scali, F.; Enix, D. E.; Battaglia, P. J. & Marshall, E. Histological examination of the human obliquus capitis inferior myodural bridge. *Ann. Anat., 195(6)*:522-6, 2013a.

Pontell, M. E.; Scali, F.; Marshall, E. & Enix, D. The obliquus capitis inferior myodural bridge. *Clin. Anat., 26(4)*:450-4, 2013b.

Rutten, H. P.; Szpak, K.; van Mameren, H.; Ten Holter, J. & de Jong, J. C. Anatomic relation between the rectus capitis posterior minor muscle and the dura mater. *Spine (Phila Pa 1976), 22(8)*:924-6, 1997.

Scali, F.; Marsili, E. S. & Pontell, M. E. Anatomical connection between the rectus capitis posterior major and the dura mater. *Spine (Phila Pa 1976), 36(25)*:E1612-4, 2011.

Scali, F.; Pontell, M. E.; Enix, D. E. & Marshall, E. Histological analysis of the rectus capitis posterior major's myodural bridge. *Spine J., 13(5)*:558-63, 2013a.

Scali, F.; Pontell, M. E.; Welk, A. B.; Malmstrom, T. K.; Marshall, E. & Kettner, N. W. Magnetic resonance imaging investigation of the atlanto-axial interspace. *Clin. Anat., 26(4)*:444-9, 2013b.

Xu, Q.; Yu, S. B.; Zheng, N.; Yuan, X. Y.; Chi, Y. Y.; Liu, C.; Wang, X. M.; Lin, X. T. & Sui, H. J. Head movement, an important contributor to human cerebrospinal fluid circulation. *Sci. Rep., 6*:31787, 2016.

Young, B. A.; Greer, S. & Cramberg, M. Slithering CSF: Cerebrospinal Fluid Dynamics in the Stationary and Moving Viper Boa, Candoia aspera. *Biology (Basel), 10(7)*:672, 2021.

Zhang, J. H.; Tang, W.; Zhang, Z. X.; Luan, B. Y.; Yu, S. B. & Sui, H. J. Connection of the posterior occipital muscle and dura mater of the siamese crocodile. *Anat. Rec. (Hoboken), 299(10)*:1402-8, 2016.

Zheng, N.; Yuan, X. Y.; Chi, Y. Y.; Liu, P.; Wang, B.; Sui, J. Y.; Han, S. H.; Yu, S. B. & Sui, H. J. The universal existence of myodural bridge in mammals: an indication of a necessary function. *Sci. Rep., 7*:8248, 2017

Zheng, N.; Yuan, X. Y.; Li, Y. F.; Chi, Y. Y.; Gao, H. B.; Zhao, X.; Yu, S. B.; Sui, H. J. & Sharkey, J. Definition of the to be named ligament and vertebrodural ligament and their possible effects on the circulation of CSF. *PLoS One, 9(8)*:e103451, 2014.

Corresponding author:
Hong-Jin Sui
Department of Anatomy
College of Basic Medicine
Dalian Medical University
Dalian
Liaoning 116044
CHINA

E-mail: suihj@hotmail.com; GHack@umaryland.edu