# Comparative Morphology and Histology of the Brain in Chinese Toad (*Bufo gargarizans*) and Chinese Fire-billed Newt (*Cynops orientalis*)

Morfología e Histología Comparada del Cerebro en el Sapo Chino (*Bufo Gargarizans*) y el Tritón Vientre de Fuego Chino (*Cynops orientalis*)

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**SUMMARY:** The morphological and histological structure of the brains of Bufo gargarizans and *Cynops orientalis* were observed by anatomy and light microscopy. The results show that the brains of Bufo gargarizans and *Cynops orientalis* are divided into 5 parts which include the telencephalon, diencephalon, mesencephalon, cerebellum and medulla oblongata. The telencephalon consists of the olfactory bulb and the cerebral hemisphere. The olfactory bulb is developed that has two pairs of olfactory nerve. *Bufo gargarizan* has a symmetrical oval hemisphere optic lobes; *Cynops orientalis* only has a spherical optic lobe. The cerebellum is situated behind the optic lobe and closely connected with the myelencephalon. In this paper, the morphological and histological differences between the two species are discussed. The proportion of cerebral hemisphere is gradually increasing, which correlated with a progressive increase in the number of neuronal cell classes, and reflected in behavior complexity.

KEY WORDS: Bufo gargarizans; Cynops orientalis; Brain; Histology; Evolution.

#### INTRODUCTION

Amphibian is the earliest vertebrate that attempts to land. The extant amphibious animals have complex and diverse types, so their body tissues have possessed adaptation correspondingly. The provenance relation about Caudata and Anura has possessed a huge database already, but there still remain some controversies. At present, there are two kinds of hypotheses, one is batrachia hypothesis which is the most widely accepted by Laurin & Reisz, 1997, 2010, believing that Caudata and Anura are as a sister group, except caecilians. The other hypothesis is that Caudata and Caecilians belong to a sister group (Carroll & Holmes, 1980; Zardoya & Meyer, 2000). Morphological characteristics of extant amphibious animal and fossil evidence make many people accept the hypothesis that Caudata and Anuran are a sister-group relationship.

There have been many studies in amphibian brain such as caecilian Ichthyophis kohtaoensis, Ichthyophis beddome, salamander and fire-bellied toad (Naujoks-Manteuffel *et al.*, 1988; Pinelli *et al.*, 1997; Gramapurohit *et al.*, 2000; Wang *et al.*, 2007; Hauswaldt *et al.*, 2007) and so on. However, a comparative study of *Bufo gargarizans*  and *Cynops orientalis* brain has not been reported. Therefore we take gross anatomy and observation of tissue slices between the Chinese toad (*Bufo gargarizans*) and the Chinese Fire-belled newt (*Cynops orientalis*). We have detailed drawings and brain tissue slices, compared them both morphologically and histologically, in order to study the brain structure and developmental adaptability to the environment and the characteristics of the evolution, enrich the data about morphology and histology about amphibious animal and lay the foundation for further study.

#### MATERIAL AND METHOD

A total of 20 both males and females mature *Bufo* gargarizans were collected in July 2016 in Baiguihu lakeside(112°50′E′33°40′N), Pingdingshan, Henan Province, China. We collected *Cynops orientalis* in June 2015 in Guangshui (113°15′E, 33°50′E), Hubei Province, China, a total of 20 both males and females mature samples.

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The samples were anesthetized by aether, then fixed in Bouins solution for at least 48 h, after that, the brain of the samples were collected the brains under a dissecting microscope. The samples were then dehydrated through a series of graded alcohols, cleared in xylene, infiltrated, and embedded into paraffin. The brain paraffin wax blocks were cut into 7 mm thick sections, stained with hematoxylin and eosin, and then they were examined under Nikon TE2000-U microscope. The measurements were conducted on Nikon TE200-U, and were the remaining fixed tissue took out for morphological observation and drawing, using vernier caliper ( accuracy 0.02 mm ) measuring the length of the brain. The measured data was analyzed by Microsoft Excel, expression of Mean $\pm$ S D.

### RESULTS

#### Gross Anatomy. Bufo gargarizans.

**Telencephalon**. The olfactory bulb is located at the anterior part of the telencephalon (Fig. 1). In dorsal view, it is only the outer edge of a small part of the telencephalon, and the boundary of cerebral is not obvious. The front end of the olfactory bulbs are two olfactory tracts, which have many filamentous olfactory on each of it. Cerebral hemisphere is divided into two hemispheres and each hemisphere is like a ellipsoid, and the upper part is narrow and the lower part is wide. In dorsal view, visible interhemispheric, is a middle deep groove, terminal has been terminated in the telencephalon. In ventral view, the central cavity forms the lateral ventricle in the cerebral hemisphere.

**Diencephalon.** The diencephalon is in the left and right cerebral hemisphere, the cavity in the middle cerebral hemisphere is the third ventricle (Fig. 1). The diencephalon can be divided into three parts, the epithalamus , thalamus and hypothalamus. The epithalamus is the roof of the diencephalon and the postmedian epithalamus of its dorsal part is subcommissural organ. There are two long and thick optic nerve fibers in the back of the diencephalon. In the ventral view, the upper part of the diencephalon is pineal body, and in the dorsal view, the posterior part of the diencephalon is hypophysis.

**Mesencephalon.** The mesencephalon is linked with the diencephalon and the pons (Fig. 1). The optic lobe is composed of two connected sections which are symmetrical, and in the center of it is optic lobe ventricle. The front of it encloses a cavity, as in the third ventricle, and at dorsal portion of the mesencephalon is the tectum, at the tegmentum is ventrally.

**Cerebellum.** The cerebellum is in the middle of the mesencephalon and the myelencephalon, and covers in the pons and the spinal cord (Fig. 1). There is a clear dividing line between the cerebellum and the mesencephalon, it is easy to observe.

**Myelencephalon.** The myelencephalon part is in an inverted triangle shape, and is covered (Fig. 1). There is the trigeminal nerve and the other nerve tracts which are eudipleural in the myelencephalon.



Fig. 1. Brain in dorsal view, lateral view and ventral view of the Bufo gargarizans. CER:cerebellum; OB:olfactory bulb; HYP:hypophysis; M E S : m e s e n c e p h a l o n ; M Y E : m y e l e n c e p h a l o n ; TEL:telencephalon; I:olfactory never; II: optic never; III.:oculomotor never; IV.:trochlear never; V :trigeminal never. Bar:=2 mm.

#### Gross Anatomy. Cynops orientalis

**Telencephalon.** The telencephalon of the *Cynops orientalis* is long and thin, and it is eudipleural, but the center is not connected (Fig. 2). The olfactory bulb is located at rostra and ventral lateral position of telencephalon which is in the central nerve. The boundary between olfactory bulb and the cerebrum is not too obvious. The left and right olfactory tracts are in front of the olfactory bulb and are eudipleural. The front end of the olfactory tract abuts the vomeronasal organ.

**Diencephalon.** The diencephalon ventral anterior is the optic nerve, and its ventral posterior has a flat of the pituitary gland which continuously extends until the myelencephalon abdomen (Fig. 2).

**Mesencephalon.** The mesencephalon is tightly connected to the diencephalon, a little further back portion of the telencephalon (Fig. 2). In the ventral view, the mesencephalon tectum is approximately elliptic to suborbicular. Each optic lobe is as the synthesis of a large cavity, both sides are combined into the mesencephalon cavity, the boundary is not obvious, and the cavity is back to the fourth ventricle.

**Cerebellum.** The cerebellum, a layer of wrinkles, is located between the optic lobe of the below and the front of the myelencephalon (Fig. 2). Protuberances at both ends of the cerebellum can be seen on the left and right sides, the one that tightly connects to the mesencephalon is not easy to observe.

**Myelencephalon.** There are two trigeminal nerves that are easy to observe in the left and right part of the myelencephalon, which are in an inverted triangle shape (Fig. 2). The back-end narrowing connects the central tube of the spinal cord. Removing the back side of the choroid, we can see a longitudinal sulcus of medulla oblongata medial wall.





#### Histology of the brain. Bufo gargarizans

**Telencephalon** The olfactory bulb of *Bufo gargarizans* can be seen from the slices, which is connected with the front end and symmetrical oval, and the arrangement of the neuronal cells in olfactory bulb is loose. The olfactory tract is not connected with the front end of olfactory bulb (Fig. 3A). In the telencephalon hemisphere, primitive cerebral cortex, primitive hippocampus, primitive pyriform lobe striatum amygdala nuclei and so on are around lateral ventricle and the boundaries between each nucleus are clear, dense degree of cell vary from the different nuclei. In the cerebral hemisphere of long shaped cavity is the lateral ventricle. On the front side of the cerebral hemisphere are two independent elliptic cavities (Fig. 3B). And with the slices showed we can observe the accessory olfactory bulb (Fig. 3C). XIE, Z.; ZHANG, H.; ZHANG, P.; LI, Q. & ZHANG, R. Comparative morphology and histology of the brain in Chinese toad (Bufo gargarizans) and Chinese fire-billed newt (Cynops orientalis). Int. J. Morphol., 37(3):1172-1178, 2019.



Fig. 3. Serial histological cross sections of brain of the Bufo gargarizans. am:aqueduct midbrain; aob:accessory olfactory bulb; cnc:cauda nuclei caudate; cer:cerebellum; dt:dorsal thalamus; f: fornix; hb:habenula olfactorybulb; hocn:head of caudate nucleus; lv:lateral ventricle; md :midventricle; mye: myelencephalon;ob:olfactory bulb; ov:olfactory ventricle; opt: optic tract; opl: opticlobe; pg:pituitary gland; tec:tectum; teg:tegmentum; tv:third ventricle; vt: ventral thalamus; vh: ventral hypothalamus; Bars:A to O=500 mm.

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**Diencephalon.** In the front part of the diencephalon, ophthalmic tract is connected with hypothalamus abdomen (Fig. 3D). Posterior lateral ventricle gradually disappear, nerve cells that distribute in the thalamus are obvious (Fig.3E). We can clearly see the fornix which is located at the upper section of the diencephalon. Preoptic nucleus gradually expand (Fig. 3F), until the preoptic nucleus connects with the third ventricle (Fig. 3G). The ventral hypothalamus and the third ventricle gradually separated (Fig. 3H). The third ventricle reduced gradually and the fornix disappeared (Fig. 3I).

**Mesencephalon.** The optic lobes are bilaterally symmetrical, and in front of the midbrain, the head of caudate nucleus is visible (Fig. 3J). In the periventricular, cell layers are obvious, the optic lobe peripheral stained with HE is very shallow, very deep internal dyeing. In the midventricle, optic lobe is divided into two parts: the tectum and the tegmentum. Cell and fiber in the center of optic lobe are oval in shape (Fig. 3K). When the ventricle gradually expanded into two long strips and gradually connected, then connected with the aqueduct midbrain, forming a cavity. Lower part of the midbrain is the pituitary gland, which is oval. HE staining which is almost blue showed pituitary cells arranged in dense (Fig. 3L), in the rear, midventricle disappeared. Optic lobe is a symmetrical oval, caudate nucleus was stained dark (Fig. 3M).

**Cerebellum** From outside to inside, the cerebellar cortex is divided into the molecular layer, the Purkinje cell layer, and the granular layer. Central to the T shape aqueduct midbrain, lower part of the cerebellar abuts the myelencephalon, there are no clear boundaries (Fig. 3N).

**Myelencephalon** The myelencephalon consists of two oval shaped intervene connected together, nerve cells are widely distributed. The fourth ventricle gradually narrows from the anterior to the posterior, within connects the central tube of spinal cord (Fig. 3O).

# Histology of the brain. Cynops orientalis.

**Telencephalon** It can be observed from tissue sections that the left and right olfactory bulbs are dieretic and located in the ventral (Fig. 4A). In the transverse sections of the olfactory bulb, from the outside to the inside in order to nerve fiber layer, glomerular layer, external plexiform layer, mitral cell layer, inner plexiform layer, granular cell layer. The front of the telencephalon has not been lateral ventricle, and cells are intensively arranged (Fig. 4B). With sections of the left, right ventricle, cells in lateral ventricle arranged in densely, is not easy to distinguish (Fig. 4C). With the slices, cerebral cortex, the origin hippocampus and so on, are obvious to see in the midbrain (Fig. 4D). Serial sections of the brain, the left and right lateral ventricle are gradually connected (Fig. 4E), the upper appears to fornix (Fig. 4F), as the fornix becomes small, the preoptic nucleus appear (Fig. 4G).

**Diencephalon.** Diencephalon is located between the two brain hemispheres, the middle cavity is the third ventricle, the two left and right lateral ventricle gradually disappear, and run through the middle of the third ventricle (Fig. 4H). Nerve cell layer in the third ventricle wall is gradually thickened from front to back, the distribution of it from the inside out is gradually sparse, and the lateral ventricle disappears (Fig. 4I).

**Mesencephalon.** The midbrain adjoins rostrally the diencephalon (Fig. 4J). Back to the sections, we can clearly see that thalamus and midbrain are gradually separated. Midventricle become a closed circle, cells in thalamic are dense (Fig. 4K). Midventricle is completely closed, and pituitary is located in the lower end of the midventricle (Fig. 4L). Pituitary is to the back of midbrain until the cerebellum appears (Fig. 4M).

**Cerebellum.** The front of cerebellar is small and thin, even in conjunction with midbrain, the backward of midbrain and cerebellum gradually become small. Nerve cells above cerebellar are sloppy and loose, but the myelencephalon cells are dense (Fig. 4N).

**Myelencephalon.** From the continuous sections, the end of myelencephalon, which is narrowing, connects the spinal cord central tube. Nerve cells distributed in myelencephalon is roughly the same to other parts in the brain. It is also gradually sparse from the inside to the outside, and the number of it starts to tail off (Fig. 4O).

# DISCUSSION

The olfactory bulbs form a circular or oval in both Bufo gargarizans and Cynops orientalis (Hoffman, 1963). Olfactory tracts of Bufo gargarizans are symmetrical, the anterior end with many slender olfactory filaments are visible to see, while Cynops orientalis are divided into two pairs that are symmetrical from the bottom. The whole optic lobe of Bufo gargarizans is orbicular and the left and right optic lobe hemispheres are symmetrical. Nevertheless, optic lobe of Cynops orientalis is a single ball, a boundary is not obvious in the middle. The evolution of midbrain is derived by the archipallium. The cerebellum of Bufo gargarizans is easier to observe than Cynops orientalis' in the dorsal view. The ratio of Cynops orientalis accounts for a large proportion of the brain (Table I). The cerebellum is the center of motion regulation. It is related to Bufo gargarizans in the life of the land is frequent, and most Cynops orientalis live in water.

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Fig. 4. Serial histological cross sections of brain of *Cynops orientalis*. cer:cerebellum; f:fornix; fv:fourth ventricle; lv:lateralventricle; mye:myelencephalon; md:midventricle; ob:olfactory bulb; pal:primordial general pallium; ph:posterior hypothalamus; pr:Preoptic recess; tv:third ventricle; vh: ventral hypothalamus. Bars: A to O=500 mm.

Table 1. Molphometric characteristics of the orall structures length in <i>Bujo gargangans</i> and <i>Cynops orientaits</i> .						
Species	OB(mm)	TEL(mm)	DIE(mm)	MES(mm)	CER(mm)	MYE(mm)
Bufo gargarigans	1.59±0.27	3.71±0.36	0.91±0.15	$1.95 \pm 0.25$	0.66±0.10	2.88±0.54
Cynops orientalis	$0.90 \pm 0.12$	$1.96 \pm 0.23$	$0.62 \pm 0.11$	$1.24{\pm}0.23$	$0.37 {\pm} 0.04$	2.50±0.25

Table I. Morphometric characteristics of the brain structures length in Bufo gargarigans and Cynops orientalis.

From the histology, *Bufo gargarizans* neural cells distribute evenly, cell morphology and size have significant differences. However, cells distributed in Cynops orientalis focus on inside, the differences in cell morphology and size are not obvious, and the distribution of cells from deep to shallow are dense to sparse. Northcutt (2001) held the idea that the transfer is neuronal stress to the process, and move namely nerve cell bodies in the process towards the stimulus source direction, therefore this is one of the features that Cynops orientalis is more primitive than Bufo gargarizans. The lower part of Bufo gargarizans telencephalon side shows the accessory olfactory bulb and it is identical with the research of tailless amphibian olfactory bulb (Hoffman). Cells in optic lobe of *Bufo gargarizans* can be seen obviously that they arrange stratified, superior colliculus in midbrain of Bufo gargarizans is cortical structure. Shallow layer cells  $(1 \sim 3)$  were received from the fibers of optic nerve, the lateral geniculate and visual cortex, deep cells  $(4 \sim 7)$  not only accept the vision, but also accept incoming signal of auditory sense and somatesthesia. While the cells in optic lobe of Cynops orientalis that arranged in a circular range and no obvious hierarchical arrangements. Compared with the midbrain of amphibian Cynops orientalis, the structure is more complex in Bufo gargarizans. The Cynops orientalis lives in water, Bufo gargarizans' living environment is relatively dry. Compared with Cynops orientalis, Bufo gargarizans' adaption to the environment is more stronger and therefore the Bufo gargarizans has a higher evolutionary status. As Northcutt's (2012) opinion, the proportion of cerebral hemisphere in the brain is gradually increased, olfactory bulb is gradually reduced and the cerebellum from simple underdeveloped, which correlated with a progressive increase in the number of neuronal cell classes within a center, and reflected in behavior complexity.

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**RESUMEN:** La estructura morfológica e histológica de los cerebros de *Bufo gargarizans* y *Cynops orientalis* se observó mediante anatomía y microscopía óptica. Los resultados muestran que los cerebros de *Bufo gargarizans* y *Cynops orientalis* se dividen en 5 partes, que incluyen el telencéfalo, diencéfalo, mesencéfalo, cerebelo y mielencéfalo. El telencéfalo consiste en bulbo olfatorio y hemisferio cerebral. El bulbo olfatorio tiene dos pares de nervios olfatorios. Los lóbulos ópticos de *Bufo gargarizans* son ovalados y simétricos en ambos hemisferios cerebrales; *Cynops orientalis* tiene solo un lóbulo óptico esférico. El cerebelo está situado detrás del lóbulo óptico y está estrechamente conectado con el mielencéfalo. En este trabajo, se discuten las diferencias morfológicas e histológicas entre las dos especies. El tamaño del hemisferio cerebral aumenta gradualmente, lo que se correlaciona con un aumento progresivo de células neuronales en los núcleos, reflejándose en la complejidad del comportamiento.

PALABRAS CLAVE: Bufo gargarizans; Cynops orientalis; Cerebro; Histología; Evolución.

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