

Neuroanatomy and Histology of the Central Nervous System in Short Mackerel, *Rastrelliger brachysoma* (Bleeker, 1851)

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Abstract

A detailed anatomy and histological structure of the brain and spinal cord, referring to the central nervous system and special sensory organ, in the short mackerel, *Rastrelliger brachysoma*, were observed in this study. All specimens revealed that the brain nomenclature in *R. brachysoma* anatomically consisted of the telencephalon, mesencephalon, diencephalon, metencephalon, and myelencephalon, continued by the spinal cord. Different structures and cell components were observed due to histology and histochemistry studies. The telencephalon of *R. brachysoma* was less developed, mainly consisting of olfactory lobes and cerebral hemispheres. The largest part, the optic lobe, in the mesencephalon, was observed to have 6 histologically distinct layers; the stratum marginale, stratum opticum, stratum fibroreticulale, stratum album central, stratum griseum central, and stratum periventriculae, respectively. The diencephalon was situated beneath mesencephalon. The epithalamus, thalamus and hypothalamus were easily observable in this region. The cerebellum in the metencephalon was composed of the corpus and the valvula cerebelli. The last region of the brain was the myelencephalon, which held the medulla oblongata and vagal lobes, before joining the spinal cord. The eye structure, composed of the inner, middle, and external layers, was investigated.

Keywords: Central nervous system, eye, histology, *Rastrelliger brachysoma*, Thailand

Introduction

The short mackerel, *Rastrelliger brachysoma*, is one of the most important fishery resources in Thailand. To date, the increasing of over-exploitation and deterioration of their habitat has gradually caused a serious decline in the number of *R. brachysoma*. This fish is, therefore, considered to be a marine fish species with the potential for aquaculture. During this time, the Samut Sakhon Fishery Research Station, Samut Sakhon province, was set up for a breeding project for this fish. The goals were to increase the number of fish in the wild and to optimize fishery management of the natural habitat. Early culture techniques used with *R. brachysoma* in late 2011 indicated that it was quite successful. In contrast, several problems have been gradually increasing, particularly the low success rates of larval stages and gonadal disorganization. Another finding was that the major source of sexually mature fishes was located in the Upper Gulf of Thailand.

A review investigation of the hatchery conditions for broodstocks generally caught from wild populations showed endocrinological dysfunction preventing completion of the reproductive system, such as reduction of gonadosomatic index (GSI) and decrease in the quality and quantity of germ cells. These

changes may be influenced by the lack of gonadotrophin releasing hormone (GnRH) that is synthesized and released from the brain into the pituitary gland for the stimulation and release of Luteinizing hormone (LH) [1,2]. A need to report the basic knowledge of anatomical and structural details of the central nervous system is important, and primarily required before investigating the regulation of the neuroendocrine mechanism, especially the GnRH as the master signaling neuroendocrine decapeptide and the central regulator for controlling reproductive growth of the fishes [3].

The intent of this study was to describe the basic anatomy and histological structure of the central nervous system (CNS) and special sensory organ in the sexually matured *R. brachysoma* living in the Upper Gulf of Thailand. This article provides information about the basic knowledge of the structural and cell components in *R. brachysoma* CNS, as well as the special sensory organ. Additionally, this information can be presented as reference material for breeding aquaculture systems, neurophysiology, and neuropathology.

Materials and methods

Fish sampling

Sexually matured *Rastrelliger brachynoma* (approx. > 16.5 cm in total length, n = 20) were used in our research and caught by bamboo strake traps in Samut Songkhram province, the Upper Gulf of Thailand (13°16'18.4" N, 100°02'13.4" E) during the breeding period (December 2013 to February 2014). The specimens were identified with the key in FAO [4]. The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science, in accordance with the guide for the care and the use of laboratory animals prepared by Chulalongkorn University (Protocol Review No. 1423003).

Gross anatomy and histological analysis

All samples were euthanized by rapid cooling method [5]. Then, their whole brains were anatomically observed in detail under a stereo microscope. The brain and eye were carefully removed and fixed overnight in a solution containing Davidson's fixative at room temperature [6] and dehydrated through a graded series of ethyl alcohol concentrations. They were then infiltrated and embedded in paraffin. A serial of 5 - 6 µm sections of the tissue blocks were cut by rotary microtome. The sagittal and cross sections were then histologically stained with Harris's hematoxylin and eosin (H&E) [7,8]. Other sections were histochemically stained with Masson's Trichrome (MT), Periodic Acid-Schiff (PAS), and Reticulin (RT) [7,8]. The general structure, together with the cell components of the brain and eye of all slides, were identified under a light microscope, and photographed with a Canon EOS 1100D digital camera.

Results and discussion

Neuroanatomic localization of the *R. brachysoma* CNS: The present study demonstrated that the central nervous system (CNS) of *R. brachysoma* is composed of a brain, continuing to form the spinal cord (**Figure 1**), in agreement with the prior reports [9,11]. Anatomical nomenclature of the *R. brachysoma* brain is basically embedded in the skull, and covered by meninx primita (meninges), seen under a stereo microscope (**Figures 1a - 1c**). Five regional anatomies, including telencephalon, mesencephalon, diencephalon, metencephalon, and myelencephalon, were also clearly described in the brain. Our observations were basically divided into 2 views; dorsal and ventral views (**Figures 1b - 1c**). In the dorsal view, similar to *Amia calva* [12], the largest part of the brain, optic tectum, was well-developed under the mesencephalon. The cerebellum of the metencephalon was moderately sized, located between the optic tectum and medulla oblongata. The telencephalic region of the cerebral hemispheres was situated anteriorly in the nostrils, followed by olfactory lobes. In the ventral view, the optic tract and optic chiasma, connecting to the eye, were anteriorly located to the pituitary gland. The largest part of the hypothalamus, the inferior lobes, was situated in the center of the diencephalon. The large inferior lobes of the hypothalamus were seen in the middle of the diencephalon. Lastly, the myelencephalon contents, particularly the narrow medulla oblongata and vagal lobes, are the most posterior parts of the brain.

Histologic organization of *R. brachysoma* CNS: **Figures 1 - 6**, show the 5 main regions which were histologically and histochemically identified in the brain, based on sections staining procedures. The telencephalon was first located anterior to the brain, and was less developed than that of other higher vertebrates [13]. In the anterior part, *R. brachysoma* is composed of olfactory lobes, followed by a pair of cerebral hemispheres (cerebrum). Furthermore, the cerebral hemisphere structure of this fish was without a neocortex, and principally contained various interconnected neurons because it was supported by neuroglia and neuronal fibers. Several studies have reported that the roles of this region were important in reproductive behavior, color vision, and learning [14]. The diencephalon distinctly composed of 3 zones, including the dorsal epithalamus, the middle thalamus, and the ventral hypothalamus, was highly in accordance with other fishes [9,15]. The main role of this region involves maintenance of muscle, eye stimulation, and reproductive physiology [9]. Note that the dorsal epithalamus was situated beneath the optic tectum, and comprised of the pineal complex and the habenular ganglion (data not shown), which are involved in coordinating outputs between the telencephalon and thalamus. A neuronal fiber of this region was also connected to the optic chiasma and retina, respectively [14]. Moreover, saccus dorsalis, or tela choroidia, was seen in the dorsal-rostral evagination of the diencephalon roof. Its histological structure contained several fibers lining the epithelial layer, covered by the meninx primitive. The basement membrane of this organ was also seen using RT staining method. In the thalamus, it was in the middle, located between the tegmentum and the hypothalamus under the third ventricle. The hypothalamus is the most posterior region of the diencephalon, being the major structural complex due to its function related to olfactory, gustatory, and other sensory impulses seen in some other teleost. [9,10]. This region is ventrally located and consists of the infundibular region and the inferior lobes, as well as specialized appendages, including the saccus vasculosus and pituitary gland, which are innervated to the hypothalamus. From the results of histological studies, the saccus vasculosus was seen more obviously, and lined between the caudal parts of the inferior lobes of the hypothalamus, beneath the glomerular nucleus, referred to as the nucleus glomerulosus. It contained several neurons, as similarly reported in *Scyliorhinus canicula* [16] and *Epinephelus coioides* [17]. The succus vasculosus was histologically surrounded by a thin tunica albuginea, as well as a prominent connective tissue. Within its structure were several folded walls, consisting of blood sinuses as well as red blood vessels, covered by pseudostratified cuboidal epithelium on the basement membrane. In this context, these epitheliums could also be classified into 2 epithelial cell types. The large Coronet (crown) cells with basal nucleus were generally extended into the lumen. Globular expansions of the ciliary processes extended from the apical portion. The supporting cells, located with oval or triangular shapes of the nucleus, were principally located at the apical portion of the epithelial layer. Additionally, melanomacrophage centers (MMC) can be mostly observed in some areas in the succus vasculosus. The function of this organ is uncertain. However, Khanna and Singh [18] stated that it was involved with the secretion of the acid mucopolysaccharides. In another hypothesis, Butler [19] noted that it can also detect water pressure. In regard to the hypothalamus-hypophyseal system, the pituitary gland in this fish species was attached to the infundibular stalk. This organ could be distinctly divided into 2 areas according to histological localization and cell types. (i) The neurohypophysis could be easily distinguished due to the composition of hypothalamic neurosecretory neuronal fibers without cell bodies, blood sinuses and pituicytes. (ii) The adenohypophysis, consisting of several different cell types. However, the histological techniques used in this species were not able to investigate or classify the pituitary details. Another immunohistochemistry technique, using specific classification of cell types, is further required for pituitary analysis.

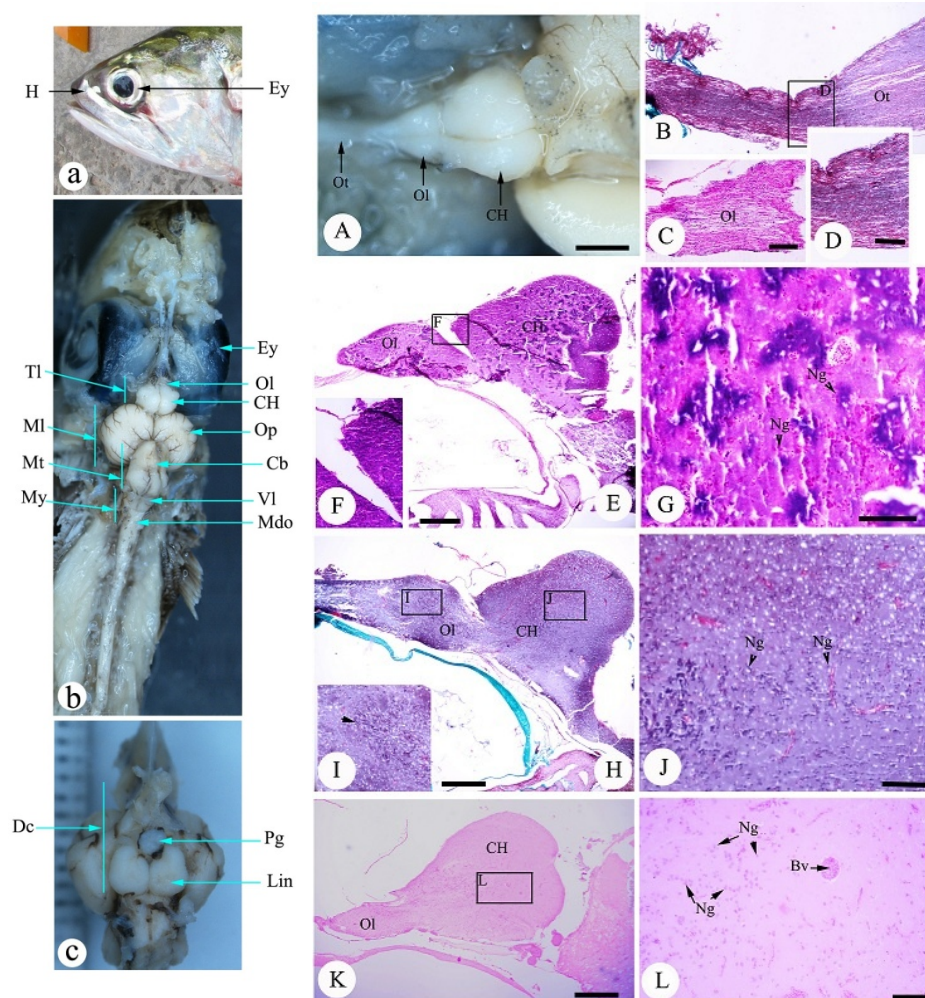


Figure 1 Neuroanatomy of the brain and eye (a-c) and light micrograph (A-L) of telencephalon in *Rastrelliger brachynoma*, Scale bar: 200 μ m (A,E,H,K), Scale bar: 20 μ m (B-F, G, I, J, L); Bv = blood vessel, Cb = cerebellum, CH = cerebral hemisphere, Dc = diencephalon, Ey = eye, H = head, Lin = inferior lobe of hypothalamus, Mdo = medullar oblongata, MI = mesencephalin, Mt = metencephalon, My = myelencephalon, Ng = neuroglia, Ol = olfactory lobe, Op = optic lobe, Ot = olfactory tract, Pg = pituitary gland, Tl = telencephalon, Vl = vagal lobe. (b = dorsal region, c = ventral region).

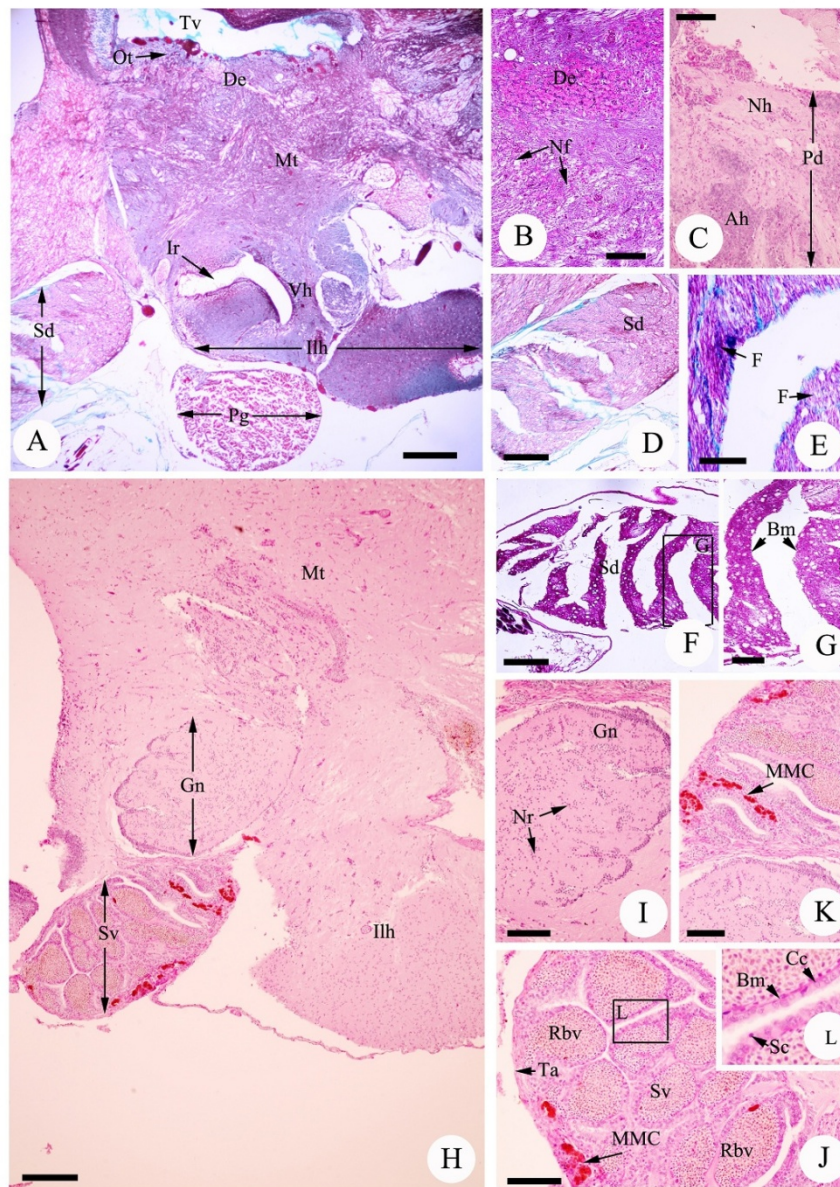


Figure 2 Light micrograph of diencephalon in *Rastrelliger brachynoma*; Scale bar: 200 μ m (A,H), Scale bar: 20 μ m (B-D, F, I-J), Scale bar: 10 μ m (E,G,L,K). Ah = adenohypophysis, Bm = basement membrane, Cc = coronet cell, De = dorsal epithalamus, F = fiber, Gn = glomerular nucleus, Ir = infundibular recess, Ilh = inferior lobe of hypothalamus, MMC = melanomacrophage center, Mt = middle thalamus, Nf = neuronal fiber, Nh = neurohypophysis, Nr = neuroglia, Ot = optic tectum, Pd = pituitary dorsal region, Pg = pituitary gland, Rbv = red blood vessel, Sc = supporting cell, Sd = saccus dorsali, Sv = saccus vasculosus, Ta = tunica albuginea, Tv = third ventricle, Vh = ventral hypothalamus.

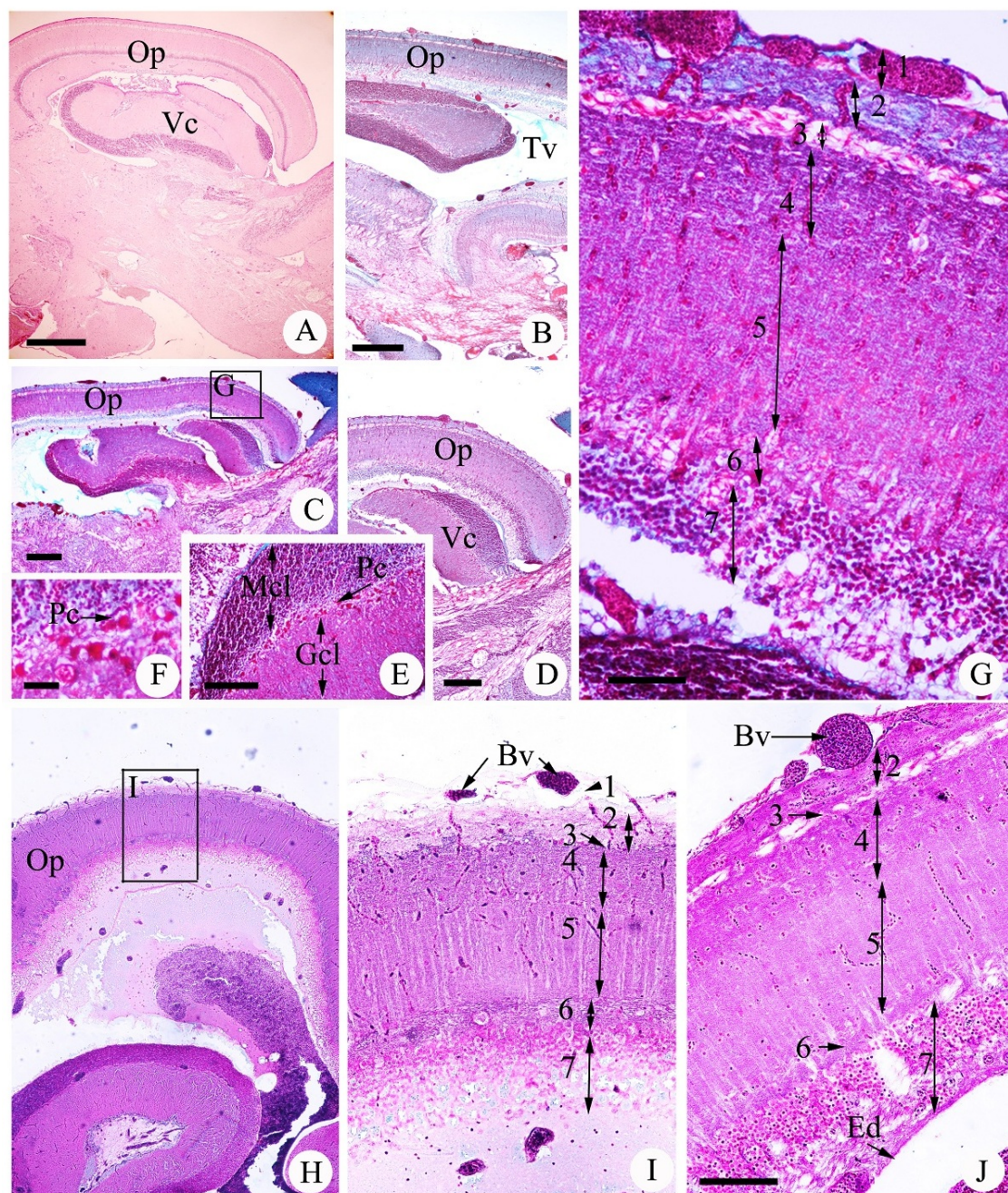


Figure 3 Light micrograph of mesencephalon and metencephalon in *Rastrelliger brachynoma*; Scale bar: 200 μm (A), Scale bar: 100 μm (B,H), Scale bar: 50 μm (C,D), Scale bar: 20 μm (E,G,I,J), Scale bar: 10 μm (F). 1. Meninx, 2 = stratum marginale, 3 = stratum opticum, 4 = stratum fibroreticulale, 5 = stratum album centrale, 6 = stratum griseum centrale, 7 = stratum periventriculare, Bv = blood vessel, Ed = ependymal cell, Gcl = granulosa cell layer, Mcl = molecular cell layer, Op = optic lobe, Pc = PURKINJE cell layer, Tv = third ventricle, Vc = valvula cerebella.

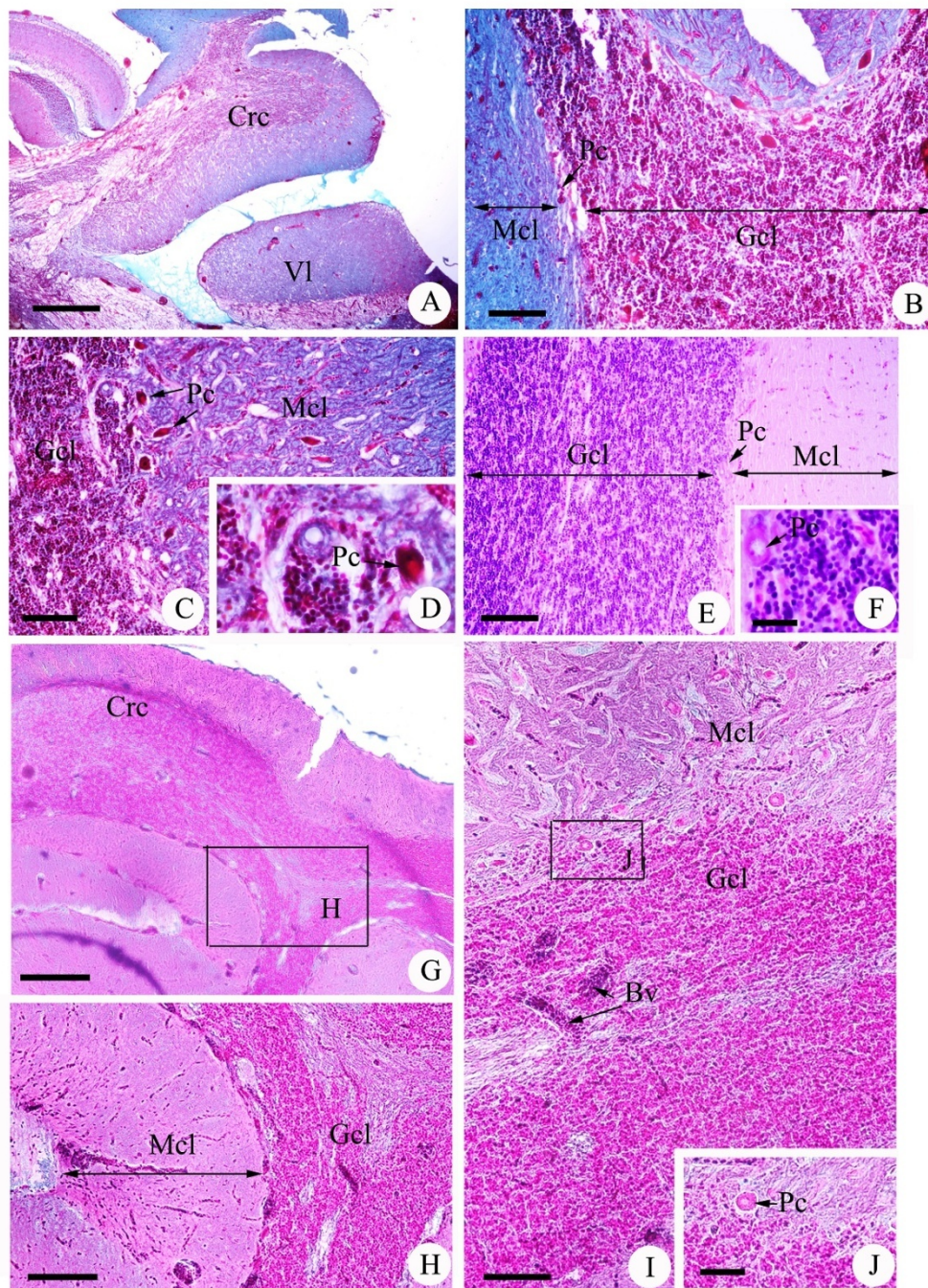


Figure 4 Light micrograph of metencephalon in *Rastrelliger brachynoma*; Scale bar: 200 μ m (A), Scale bar: 100 μ m (G), Scale bar: 20 μ m (B, C, E, H, I, J), Scale bar: 10 μ m (F, J). Bv = blood vessel, Crc = corpus cerebelli, Gcl = granular cell layer, Mcl = molecular cell layer, Pc = Purkinje cell layer, Vl = vagal lobe.

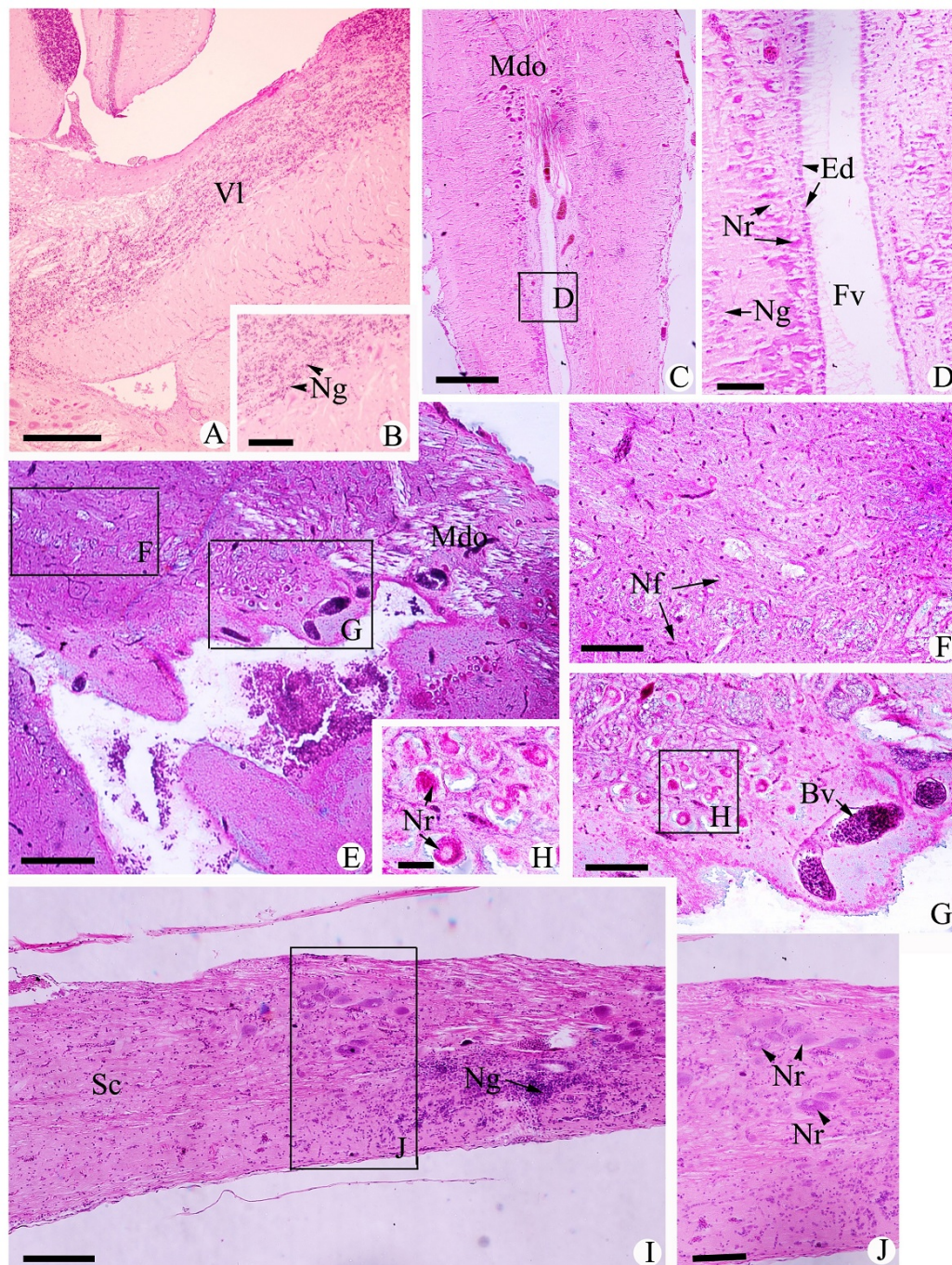


Figure 5 Light micrograph of myelencephalon and spinal cord in *Rastrelliger brachynoma*; Scale bar: 100 µm (A, E, I), Scale bar: 50 µm (C), Scale bar: 20 µm (D, F, G, J), Scale bar: 10 µm (B,H). Bv = blood vessel, Ed = ependymal cell, Fv = fourth ventricle, Mdo = medullar oblongata, Nf = neuronal fiber, Ng = neuroglia, Nr = neurons, Sc = spinal cord, Vl = vagal lobe.

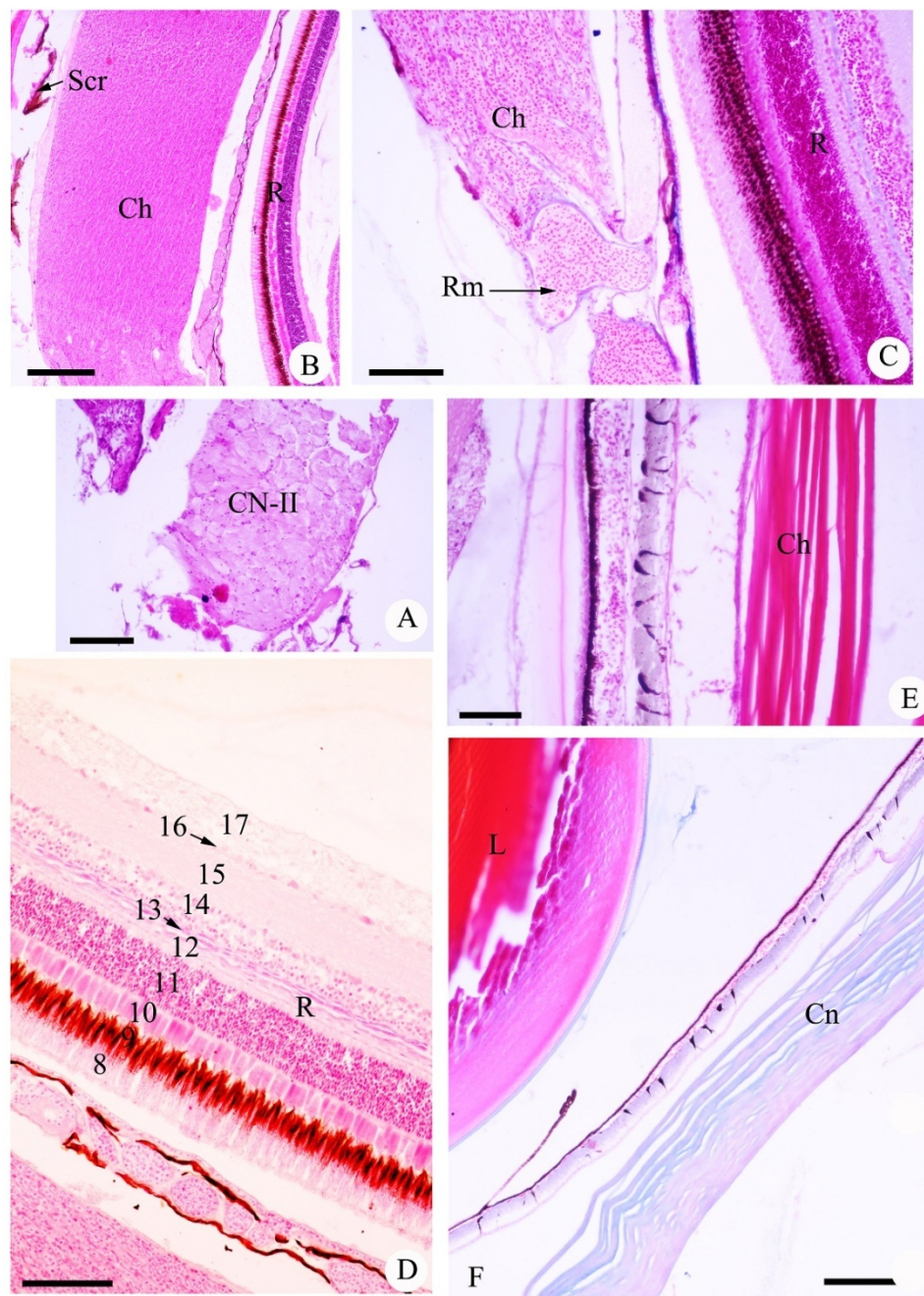


Figure 6 Light micrograph of CN-II and eye in *Rastrelliger brachynoma*; Scale bar: 100 μ m (MT stain); Scale bar: 50 μ m (A,B,C), Scale bar: 20 μ m (D,E,F). Cn = cornea, Ch = choroid, L = len, R = retina, Rm = rete mirabile, Scr = sclera, 8 = pigment epithelium, 9 = photoreceptor layer, 10 = outer limiting membrane, 11 = outer nuclear layer, 12 = outer plexiform layer, 13 = inner nuclear layer, 14 = inner plexiform layer, 15 = ganglion cell layer, 16 = optic nerve layer, 17 = inner limiting membrane.

Under sagittal sections, the mesencephalon was the largest component in the *R. brachysoma* brain. The wall of its structure was surrounded by a thin meninx primitiva containing several blood vessels. The optic tectum was a complex area, which consisted of 6 histologically distinct layers; the stratum marginale, stratum opticum, stratum fibroetgriciale, stratum album central, stratum griseum central, and stratum periventriculae. Beneath the optic tectum was the third ventricle, surrounded by ependymal lining. In the metencephalon, the major component of was the cerebellum, which could also be prominently divided into 2 sub-regions; corpus and vulvula cerebelli. Note that, in term of localization, corpus cerebelli was laid on the rostral zone of the optic tectum, whereas vulvula cerebelli projected forward under the optic tectum. However, the histological structure was similarly seen in 3 different layers based on histological organization, including outer molecular, Purkinje cell, and inner granular layers. An outer molecular layer contained parallel fibers, Purkinje cells, dendrites (rounded or pear-shaped), and satellite cells. The inner granular layer contained small multipolar neurons as granule cells. In the rostral region, the myelencephalon principally composed of the medulla oblongata, as the stem of the brain, and the paired vagal lobes. In terms of function, this region is responsible for integrating the reticulomotor system, taste, and auditory senses [20]. Another characteristic of this region is that it is connected with the cerebellum to the diencephalon. The central canal, as well as the fourth ventricle (fossa rhomboidea), was found in the middle of the caudal medulla oblongata. This region was also covered by ependymal cells. The histological details of the medulla oblongata consisted of neurons and different cell types. The neurogails were small cells with a few nucleuses, whereas Nissl bodies were basophilic cells, as well as big circular cells. Another pair of lobes, the vegal lobes, was evident in the dorsal medulla oblongata. Lastly, the spinal cord of this species was jointed and extended from the medulla oblongata along the length of the body. The prominent feature of this region contained neurons, neuroglia, and the neuronal fibers. The nucleus in each neuron was oval shaped, and deeply surrounded by an eosinophilic cytoplasm.

Histologic organization of the *R. brachysoma* eye (**Figure 6**): Our observation was the first study of the *R. brachysoma* eye, as well as of the sensory special organ, which was connected to the brain by the optic nerve (nerve II) with myelinated nerve fibers. There were 3 major parts in the eye of *R. brachysoma*: the inner, middle, and external layers, similar to other investigations [9,10]. The inner layer was divided into 10 parts, namely, 1) pigment epithelium, 2) photoreceptor layer, related to rod and cone cells, 3) outer limiting membrane, 4) outer nuclear layer, 5) outer plexiform layer, 6) inner nuclear layer, 7) inner plexiform layer, 8) ganglion cell layer, 9) optic nerve layer, and 10) inner limiting membrane, respectively. The second layer, the middle uveal layer, contained the choroid, lens, and iris. The choroid, composing of a network of capillaries, was observed; this characterization correlated with the uptake of oxygen into the retina [21,22]. The final layer, as well as the external layer, of *R. brachysoma* was composed of sclera and cornea (the sclera-corneal layer), which is shown in this study.

Conclusions

This study revealed that the histological characterization of the central nervous system, together with the eye structure was basically similar to other fishes. It showed 5 regions, including the telencephalon, mesencephalon, diencephalon, metencephalon, and myelencephalon of the brain. Among its region, the 2 optic lobes of the mesencephalon were the largest part, with 6 histologically distinct layers; the stratum marginale, stratum opticum, stratum fibroetgriciale, stratum album central, stratum griseum central, and stratum periventriculae. Further, our investigation on the identification of neurotransmitters and neuropeptides, as well as biomolecules in this species, will be further observed.

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