CHAPTER 3

3.0   INTRODUCTION

Gross morphological characterization of the cerebellum would give detailed information on the cerebellum, e.g. measurements of size and length. It is very important to reveal the differences in gross morphology for both orientations of fish cerebellum. This is in view of the fact that for most vertebrates, the cerebellum is located as structure attached to the brain stem. Uniquely, this does not exist in fish. No report has yet been established on this matter.

3.1   MATERIALS AND METHODS

For this research, the juvenile fishes were acquired from wet market and local aquariums selling fishes. Only live fishes were acquired for this research and they were kept temporarily in an aquarium in the laboratory of Neuroscience Research Group (NeuroRG), Faculty of Science, University of Malaya.

3.1.1   Preparation of sample (cerebellum)

3.1.1.1   Dissection and fixation by immersion and perfusion

First, the fish was taken out from the aquarium by using a fish net. The fish was then transferred into a big container filled with water well-mixed with Tricaine Methanesulfonate (MS222) (170mg/1000mL) (Figure 3.1). MS222 is the most widely and commonly used anesthetic for aquatic animal in aquaculture and fishery (Coyle et al., 2004). MS222 is rapidly absorbed through the gills. Its mode of action is by depression of the central nervous system (Close et al., 1997).

Figure 3.1: Fish anaesthetized with MS222.

After the anaesthetization, the pictures of the external morphology (lateral, dorsal and ventral views) of fish were taken using a digital camera. Prior to dissection, it was ensured that the anesthetized specimens were still alive. This was very important and necessary to give the best result and the higher chance of getting unmodified composition of fresh neural tissues. This was because the moment the fish starts dying, degradative enzymes inside the body cells will naturally start to destruct the cellular components. Subsequently, the neurohistological results of the cerebellar tissue would be affected. A quick but well abstraction of the brain specimen would lead to better results.

After the external observation and measurements, the fish was then subjected to fixation by perfusion. Fixation by perfusion involved flowing in the fixative solution by pump into the blood vessels to ensure every tissue is exposed to fixative solution. The fixative solution is circulated after blood had been pumped out and replaced by saline. Fixation by perfusion is preferred to fixation by immersion because the fast and thorough exposure to fixative. The fixative will be distributed evenly throughout the fish, including the hard to access brain tissue. The fixative of 10% buffered formalin is commonly used to preserve tissues for routine histology in many labs. The solution

should be clear, colorless, and with no precipitate. The preparation of 10% formalin solution is shown in Appendix II.

The procedures involved started with the setting up of the perfusion pump during which the perfusion set and needle were attached to each other (Figure 3.2).
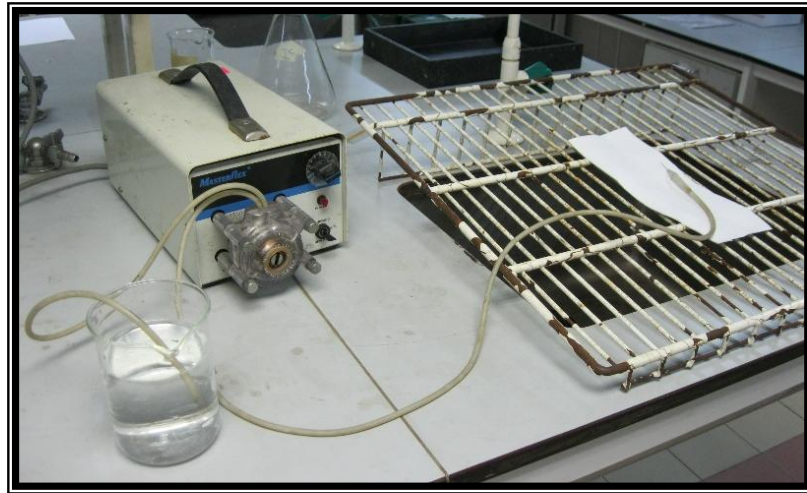

Figure 3.2: Perfusion set needed for fixation by perfusion.

Tap water was run through the perfusion tube to remove any residues and air bubbles. Then, the open end of the perfusion tube was placed in the beaker filled with saline. The valve was opened and the flow rate produced by the pump was adjusted to a suitable drip and then closed while setting up other instruments. The surgery site was set up with dissecting instruments, e.g. a pair of scissors and forceps, to ensure smooth process prior to placing the fish on its back on the operating stage (Figure 3.3). By using a pair of scissors, a part of the abdomen was cut to allow access to the heart. After the heart was exposed, a small tiny hole was made to allow drainage of blood and fluids.

The heart was held steadily by using a pair of forceps, and then the needle (0.40 x 13 mm) was directly inserted into the left ventricle. Precaution was taken to ensure that the needle did not pierce the other side of the heart to avoid mixing of solution circulation (Figure 3.4).
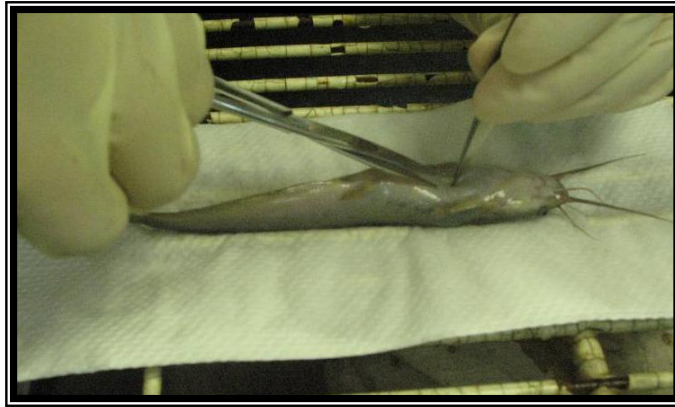
Figure 3.3: The fish was placed on its back on the operating stage.
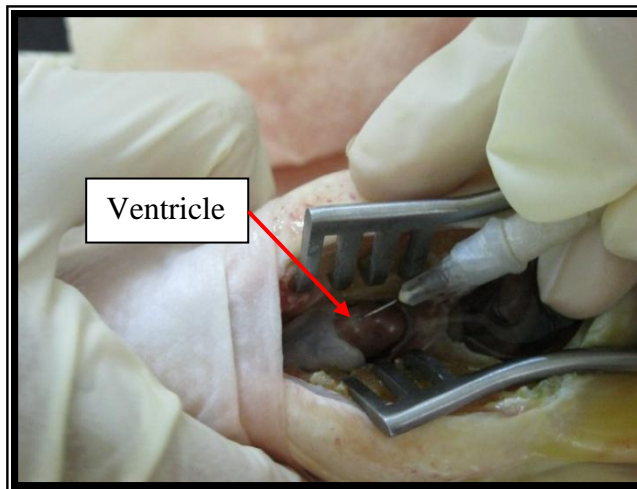

Figure 3.4: The needle inserted into the ventricle to allow the flow of saline and/or 10% formalin solutions.

The needle was clamped near the point of entry to maintain its stable position. The valve was released in a slow and steady flow to allow saline solution in. After the blood was rinsed out by the saline solution, 10% buffered formalin was pumped in. The perfusion was completely done a few minutes after the fish body stopped jerking. After that, the valve was stopped and the needle was removed. The skull with the brain still encased in it was taken out and immediately stored for 5 – 10 minutes in 10% formalin (Figure 3.5). When the fish head was separated from the body, the head was dissected dorsally in order to get the specimen (cerebellum) as it was the easier and much preferable way. All the unwanted tissues, muscles and blood vessels were removed using a pair of forceps and a pair of scissors, until the cranial bone was exposed. When

the cranial bone was exposed, a few tiny holes were made in it to allow the 10% formalin to pass through and reach the brain quickly. This quick exposure of the brain to the fixative was to prevent the decomposition of brain tissues. The cranial bone was then cut very carefully in order to prevent damage to the delicate brain. To prevent brain tissue from drying up, during the process of taking out the brain, the 10% formalin was flushed on the surface of cranial bone until the brain was revealed. This was also to ensure that the brain tissue was continually exposed to the fixative.

Finally, once the brain was revealed, it was taken out very slowly and carefully, in order to prevent accidental damages of morphology of brain tissues, which were prone to breaking up into pieces. The cranial bone was chipped and removed very cautiously (Figure 3.6). This was followed by post-fixation in 10% formalin (Figure 3.7). Apart from the nuclear and cytoplasmic detail being adequately preserved, fixation enabled the observation of external brain morphology. The brain was stored in 10% buffered formalin in specimen bottle for at least 24 hours.
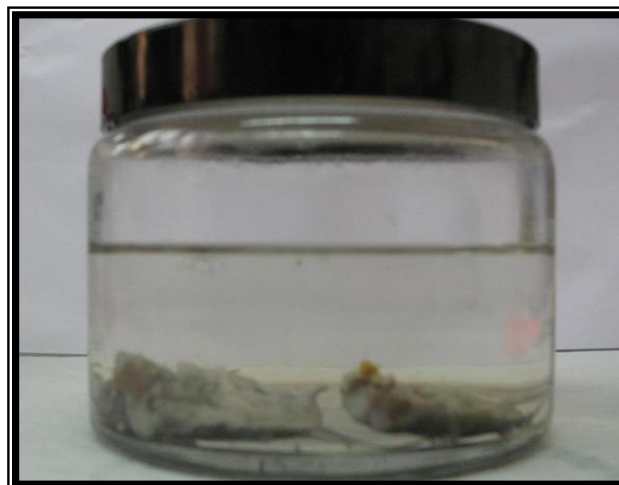

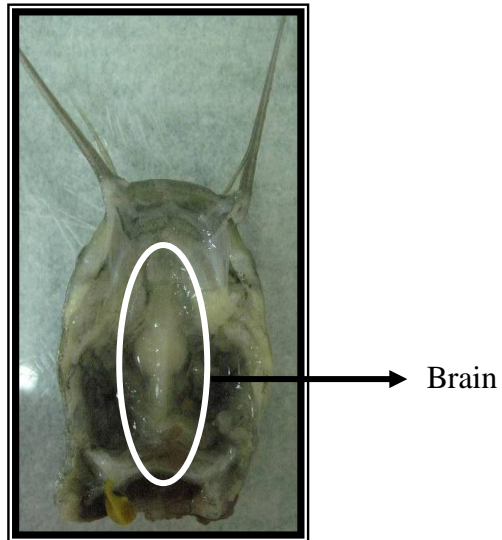**Figure 3.5:** The skull with the brain encased in it stored overnight in 10% formalin.

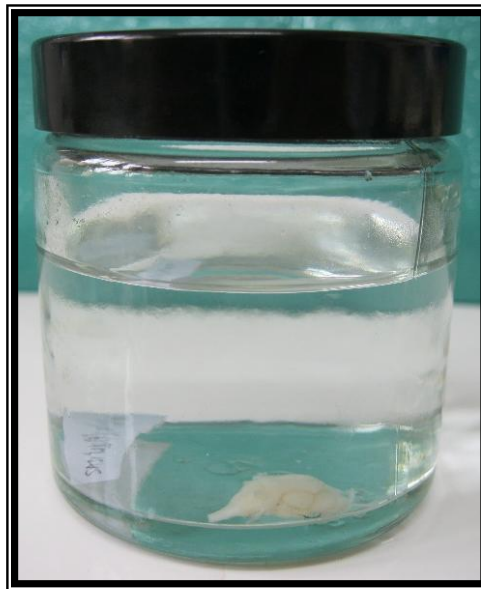**Figure 3.6:** Dorsal view of the exposed cranial bone with the brain encased in it.

Figure 3.7: The brain post-fixed in 10% buffered formalin solution.

3.1.2 Cerebellar morphological features

After the fixation, the morphology of the whole brain with special focus on cerebellum was observed. Photos of the whole brain and cerebellum were taken by using a digital camera (Canon Powershot A640). The brain was weighed by using digital weighing machine (AND GR-200, Japan) in the unit of gram (g). The length and width of the cerebellum also were measured in the unit of millimeter (mm).

3.2   RESULTS

The dorsally located cerebellum of *Clarias* sp. (*keli*) (Figure 3.8 and 3.9) was found to be rostrally directed. The same goes for *Mystus nemurus* (*baung*) (Figure 3.10 and 3.11). The cerebellum which was attached to medulla oblongata was observed to be overlapping almost half of the caudal portion of telencephalon and optic lobe in *keli* and one third in *baung*. Viewed from above, this position made the cerebellum looked like it was covering almost the whole brain. These rather smooth cerebelli consisted of the corpus, valvula cerebellum, and two lateral eminentia granularis, which are also known as the auricles or caudal lobe similar to other cartilaginous fish (Ikenaga et al., 2006). The corpus cerebellum was slightly tapered end rostrally with wide base caudally and the eminentia granularis appeared as an obvious swelling at the sides of cerebellum. However, gross morphology of valvula cerebellum could not be observed as it was located underneath the corpus cerebellum.
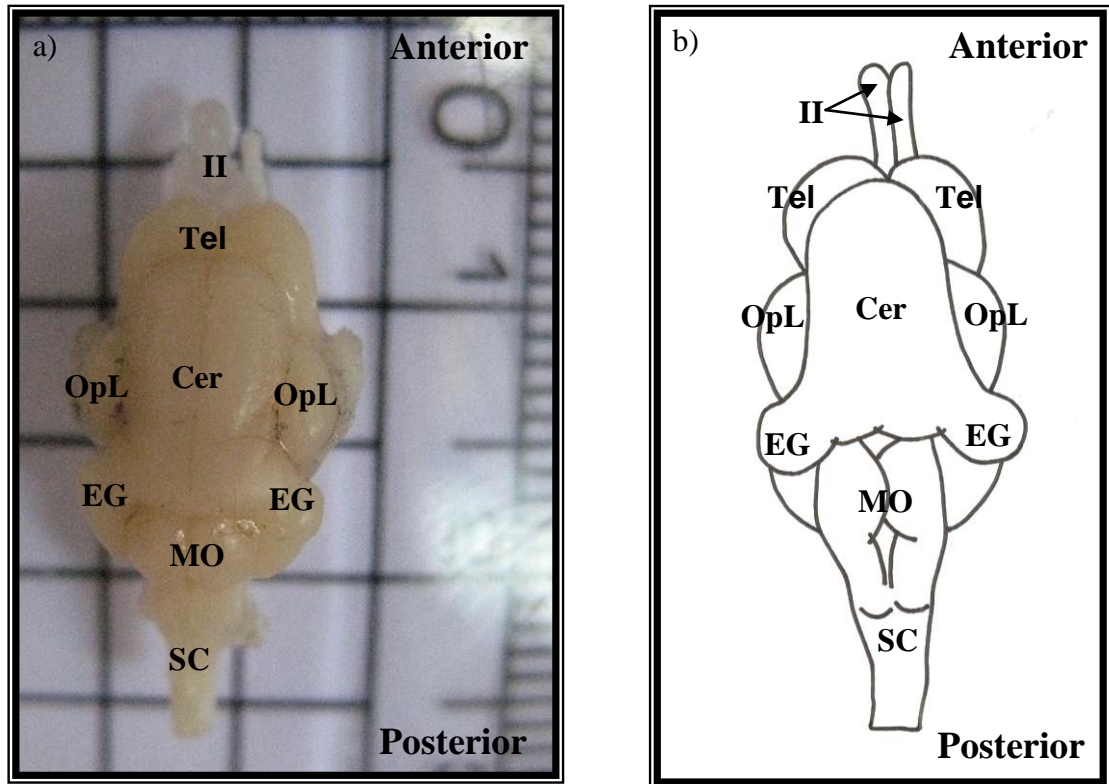
Figure 3.8: (a) Dorsal view and (b) schematic diagram of the brain of *Clarias sp.* (*keli*) with rostrally directed cerebellum.
Cer: Cerebellum; EG: Eminentia Granularis; Tel: Telencephalon; MO: Medulla Oblongata; II: Optic Nerves; OpL: Optic Lobe; SC: Spinal Cord.
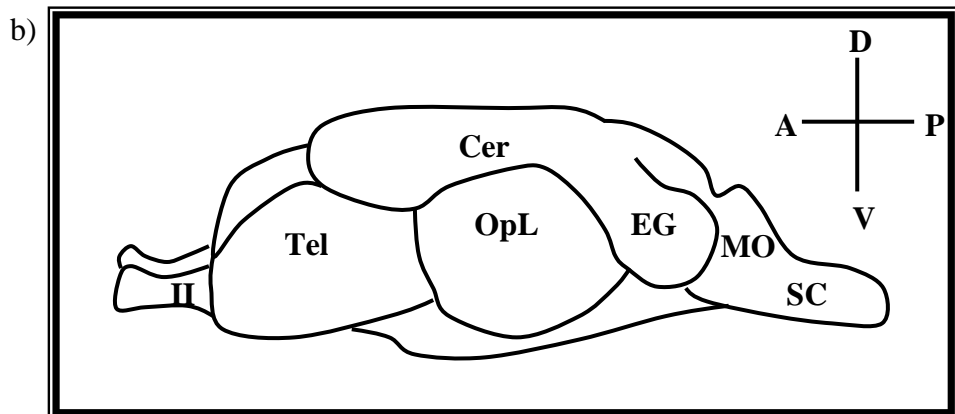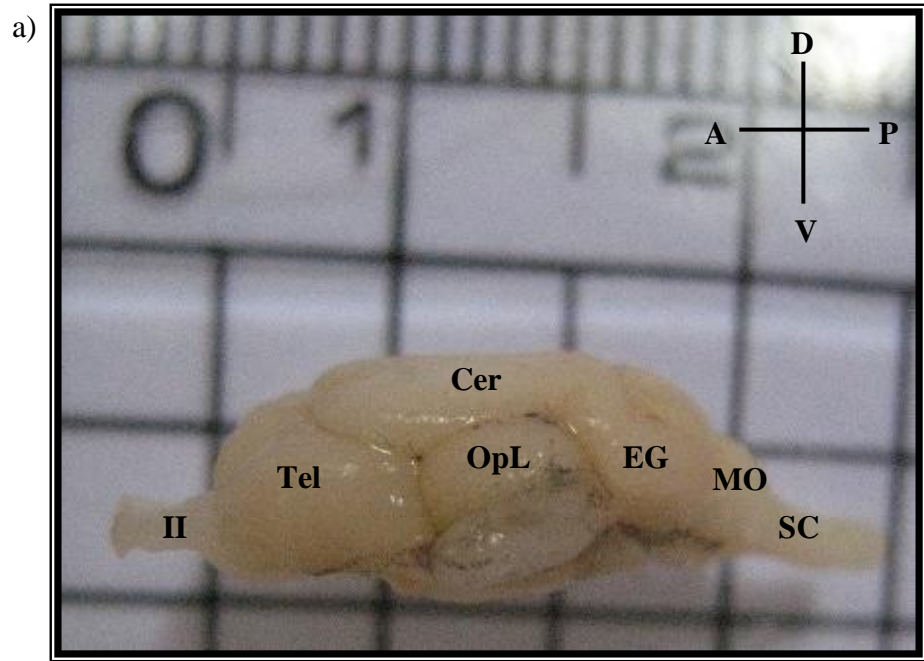
Figure 3.9: (a) Lateral view and (b) schematic diagram of the brain of *Clarias sp.* (*keli*) with rostrally directed cerebellum.
Cer: Cerebellum; EG: Eminentia Granularis; Tel: Telencephalon; MO: Medulla Oblongata; II: Optic Nerves; OpL: Optic Lobe; SC: Spinal Cord. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.
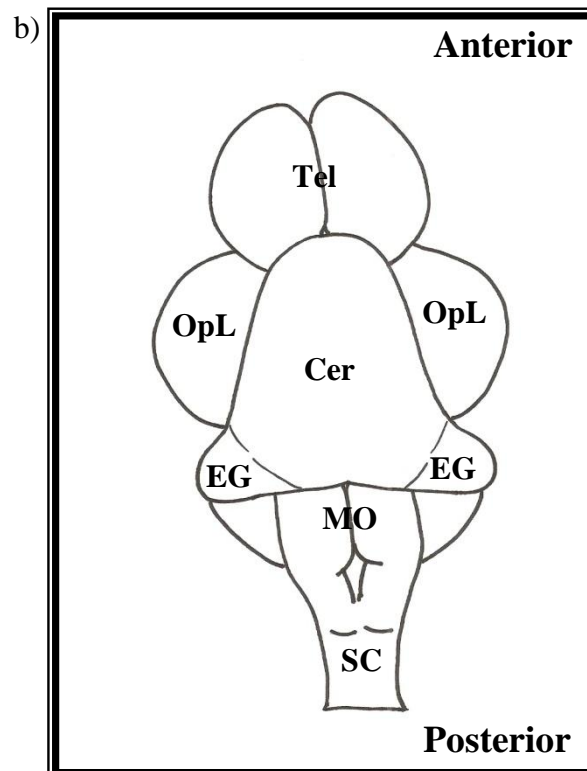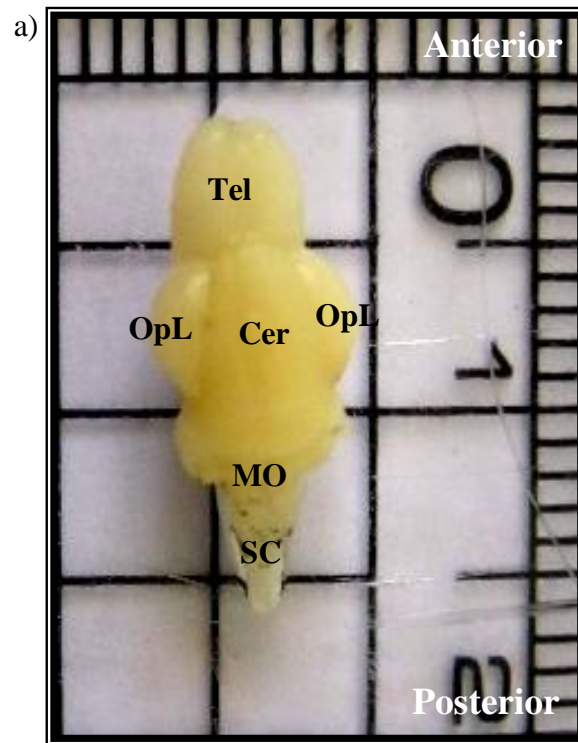
Figure 3.10: (a) Dorsal view and (b) schematic diagram of the brain of *Mystus nemurus* (*baung*) with rostrally directed cerebellum.
Cer: Cerebellum; EG: Eminentia Granularis; Tel: Telencephalon; MO: Medulla Oblongata; II: Optic Nerves; OpL: Optic Lobe; SC: Spinal Cord.
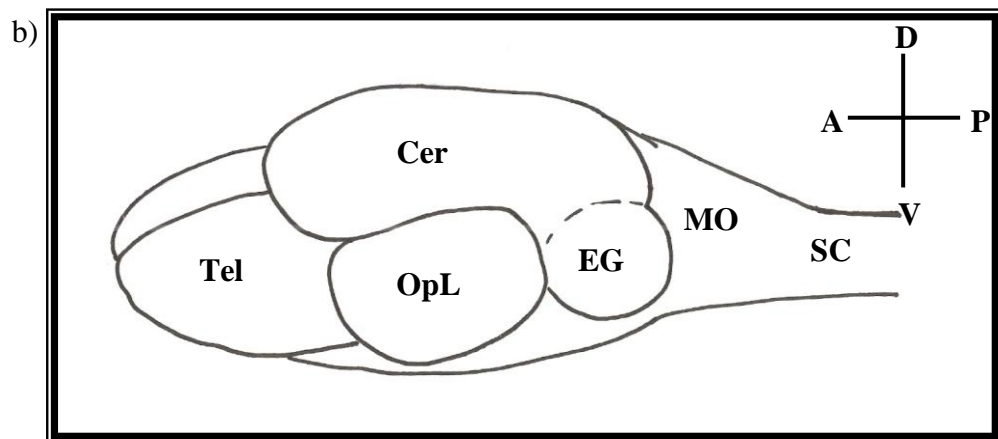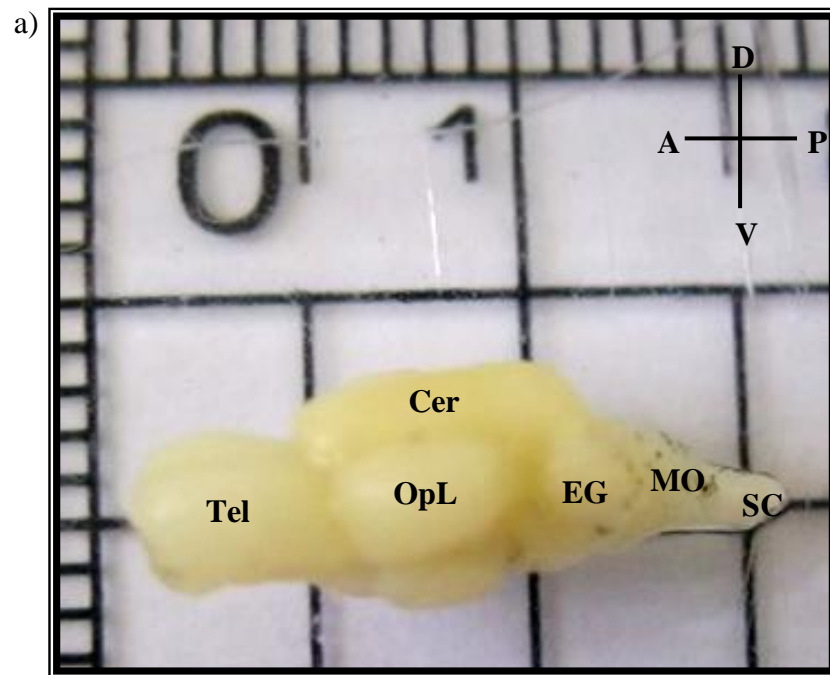
Figure 3.11: (a) Lateral view and (b) schematic diagram of the brain of *Mystus nemurus* (*baung*) with rostrally directed cerebellum.
Cer: Cerebellum; EG: Eminentia Granularis; Tel: Telencephalon; MO: Medulla Oblongata; OpL: Optic Lobe; SC: Spinal Cord.
Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.

On the other hand, the cerebellum of *Leptobarbus hoeveni* (*jelawat*) (Figure 3.12 and 3.13) and *Oreochromis* sp. (*tilapia*) (Figure 3.14 and 3.15) were found to be caudally directed. The caudally directed cerebellum were connected to medulla oblongata and oriented towards the spinal cord. From the dorsal view, cerebellum of both fishes were similar, but they look different from the lateral view. Lateral view of *tilapia's* cerebellum looked like a 'hook', in contrast to *jelawat's*, which appeared as a compact structure.
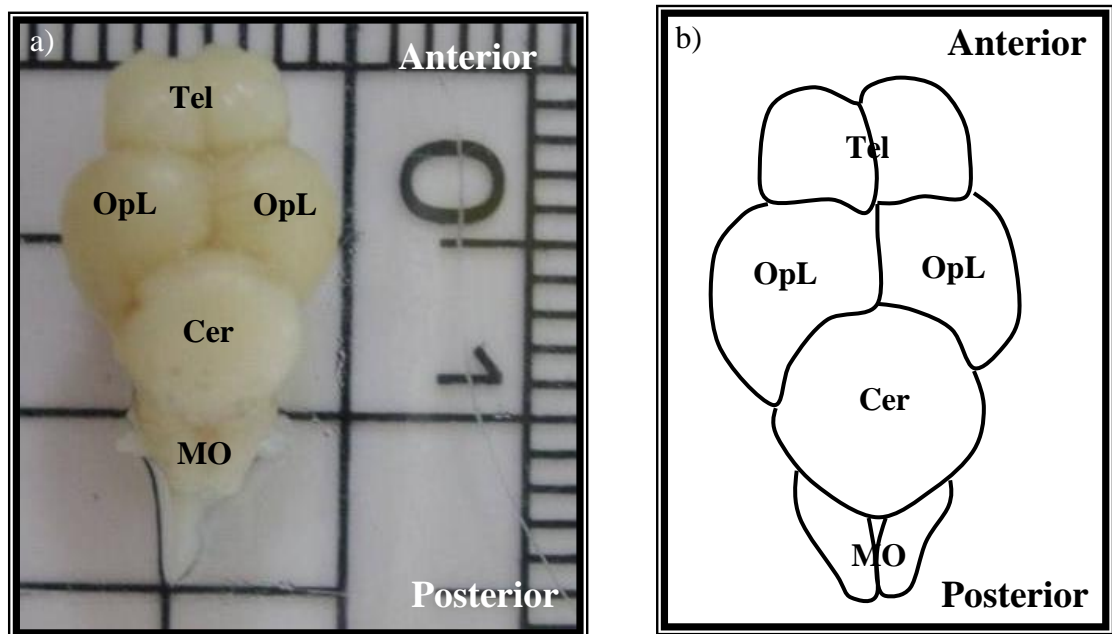

Figure 3.12: (a) Dorsal view and (b) schematic diagram of the brain of *Leptobarbus hoeveni* (*jelawat)* with caudally directed cerebellum.
Cer: Cerebellum; Tel:Telencephalon; MO: Medulla Oblongata; OpL: Optic Lobe

Figure 3.13: (a) Lateral view and (b) schematic diagram of the brain of *Leptobarbus hoeveni (jelawat)* with caudally directed cerebellum.
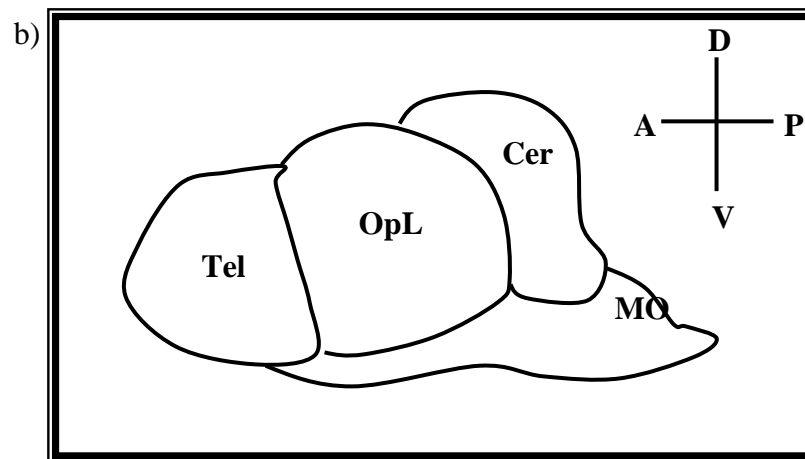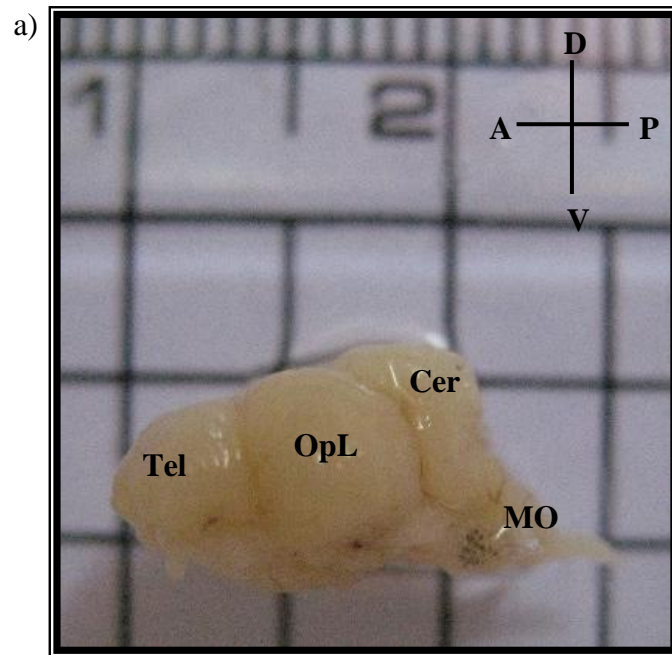Cer: Cerebellum; Tel:Telencephalon; MO: Medulla Oblongata; OpL: Optic Lobe. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.

a)

Anterior

II

Tel

OpL   OpL

Cer

MO

Posterior

b)

Anterior

II

Tel

OpL   OpL

Cer

MO

Posterior

Figure 3.14: (a) Dorsal view and (b) schematic diagram of the brain of *Oreochromis* sp. *(tilapia)* with caudally directed cerebellum.
Cer: Cerebellum; Tel: Telencephalon; II: Optic Nerves; MO: Medulla Oblongata; OpL: Optic Lobe.
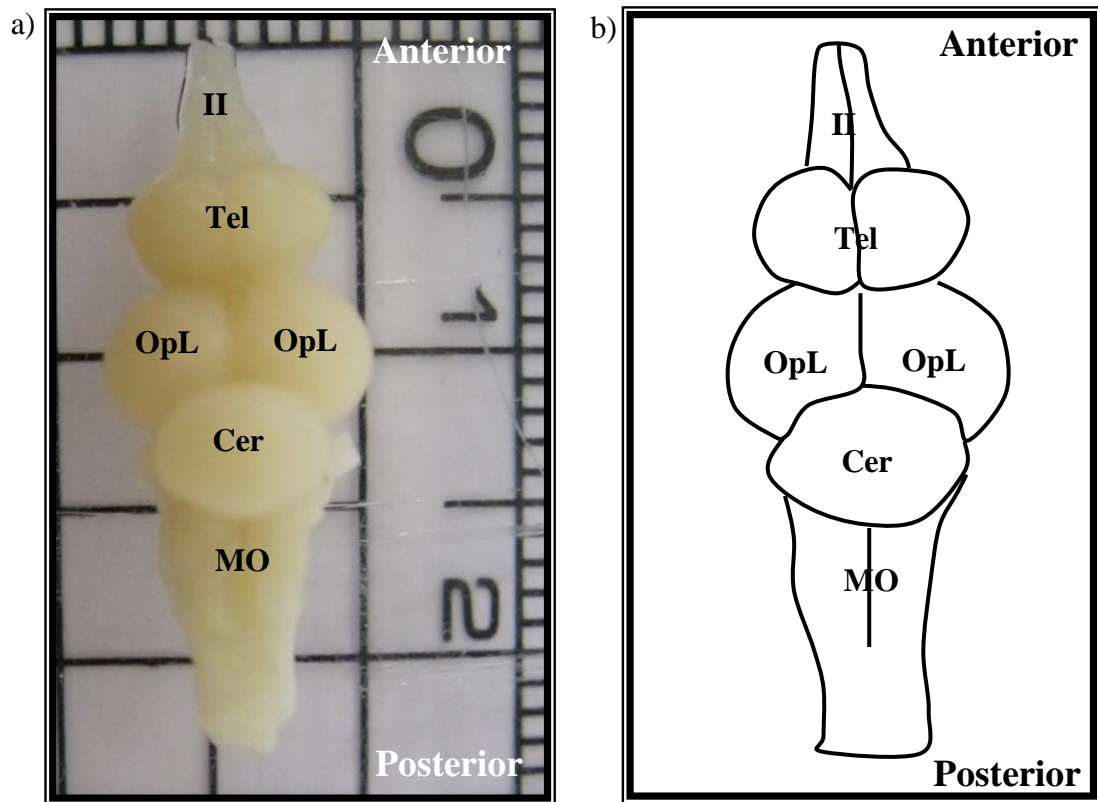
a)

b)

Figure 3.15: (a) Lateral view and (b) schematic diagram of the brain of *Oreochromis* sp. *(tilapia)* with caudally directed cerebellum.
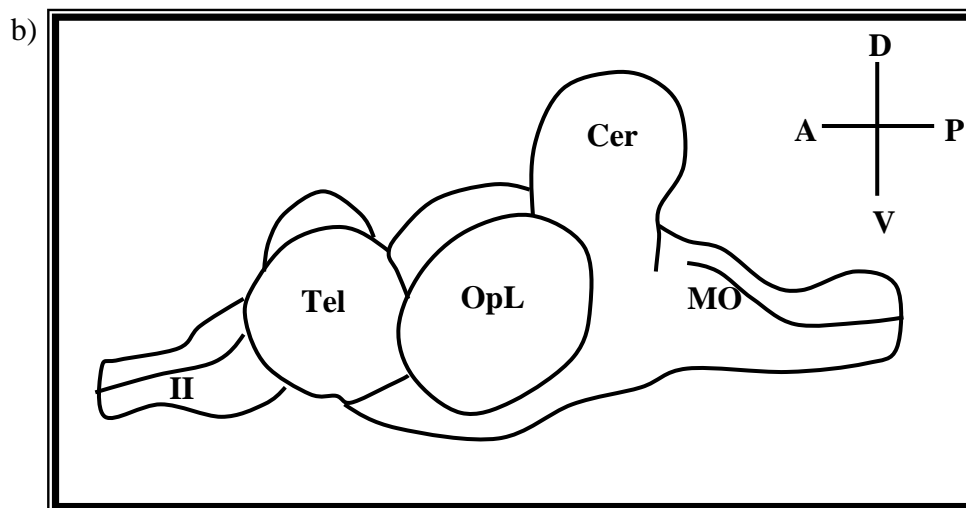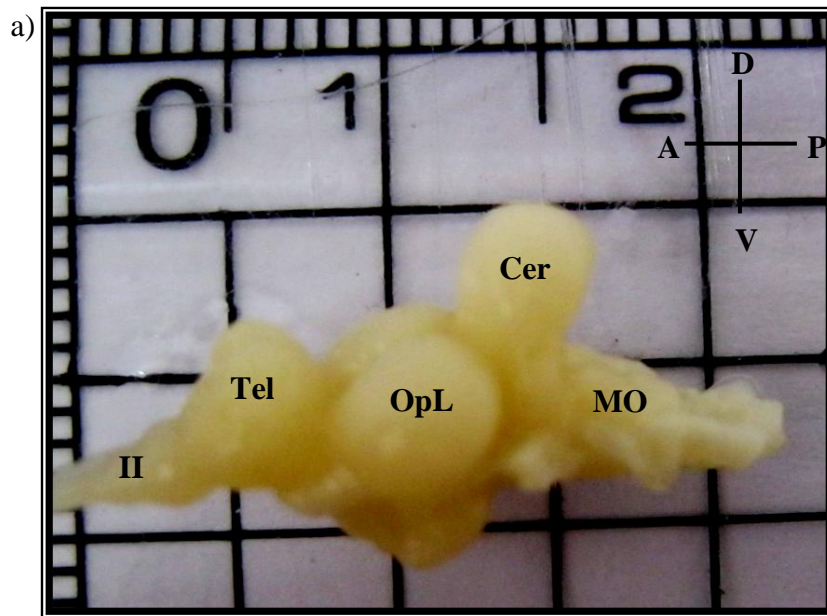Cer: Cerebellum; Tel: Telencephalon; II: Optic Nerves; MO: Medulla Oblongata; OpL: Optic Lobe. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.

For both cerebellar orientations, neither hemisphere nor folia was found on the cerebellum and the fissure was also not found, in contrast to cerebellum of human and birds (Eccles et al., 1967). The rostrally directed cerebellum has additional structures seen on them. The medial longitudinal sulcus was found on the *keli's* cerebellum (Figure 3.16 and 3.17) while medial longitudinal gyrus was found on *baung's* (Figure 3.18 & 3.19). However, neither lobes nor fissure were found on the surface of caudally directed cerebellum of *jelawat* and *tilapia* (Figure 3.20, 3.21, 3.22 and 3.23). Other than that, eminentia granularis appeared well developed in *keli's* and *baung's* cerebellum; whereas it was less obvious in *tilapia's* and *jelawat's*.

Figure 3.16: (a) Dorsal view and (b) schematic diagram of rostrally directed cerebellum of *Clarias* sp. (*keli*).
Cer: Cerebellum; EG: Eminentia Granularis
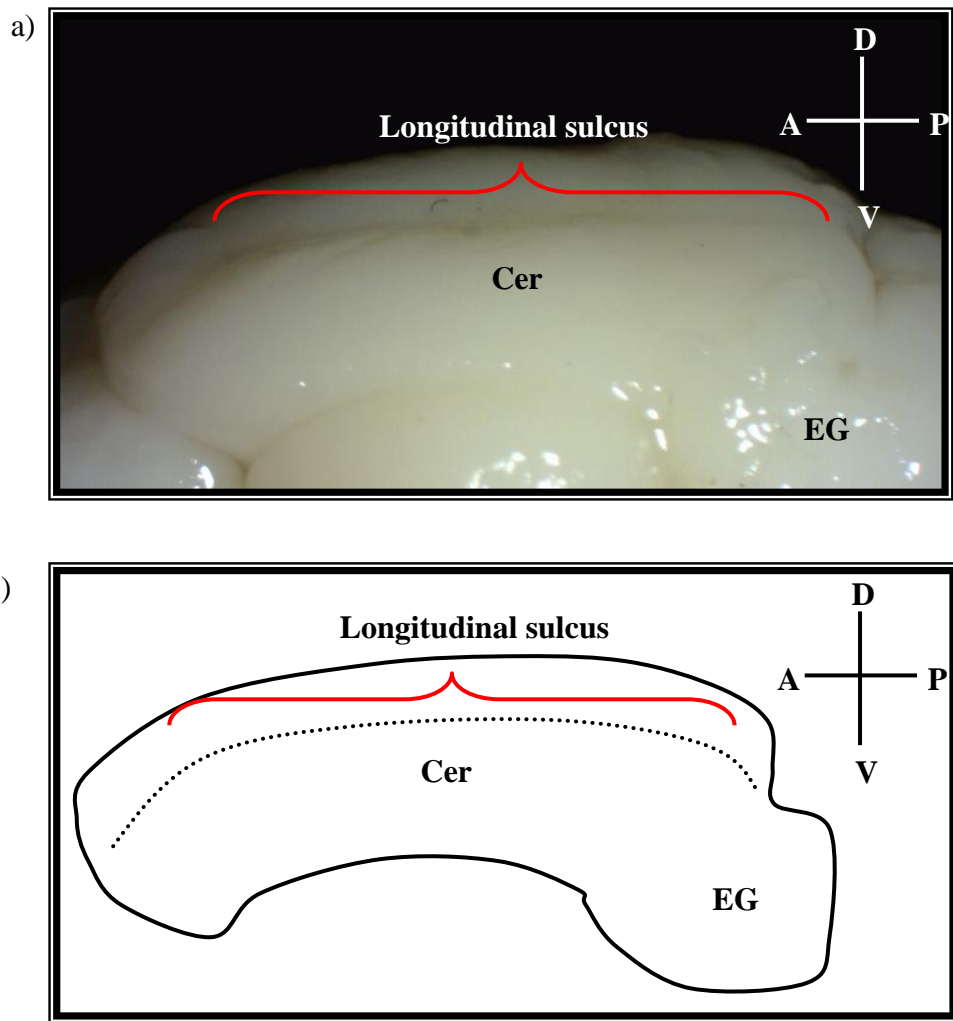
Figure 3.17: (a) Lateral view and (b) schematic diagram of rostrally directed cerebellum of *Clarias* sp. (*keli*).
Cer: Cerebellum; EG: Eminentia granularis. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.
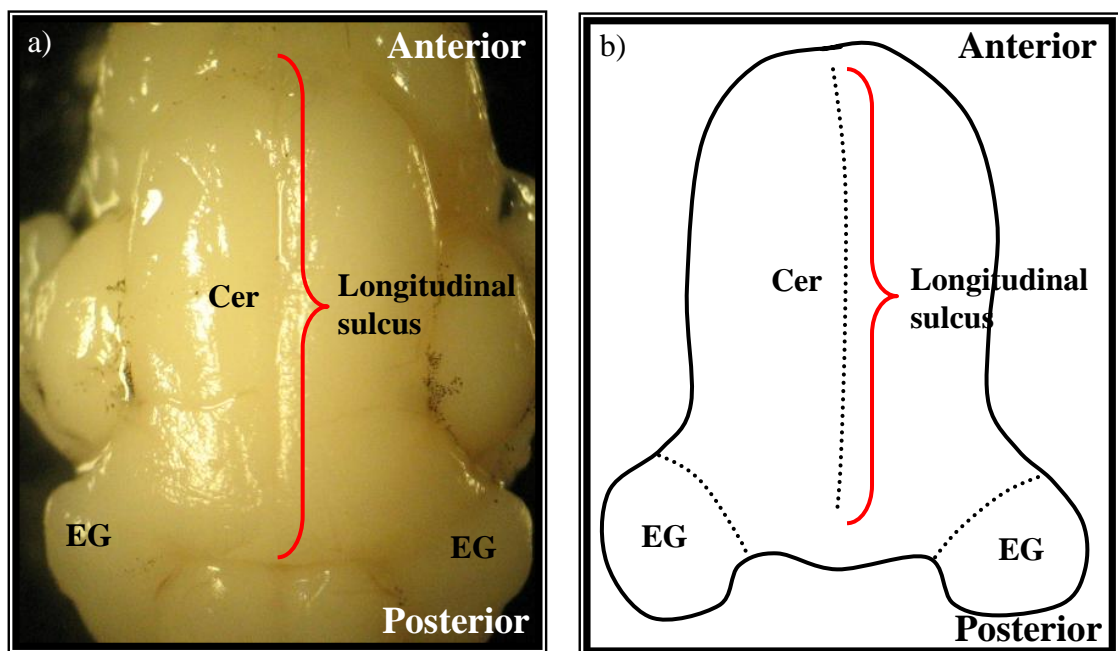
Figure 3.18: (a) Dorsal view and (b) schematic diagram of rostrally directed cerebellum of *Mystus nemurus* (*baung*).
Cer: Cerebellum; EG: Eminentia Granularis.
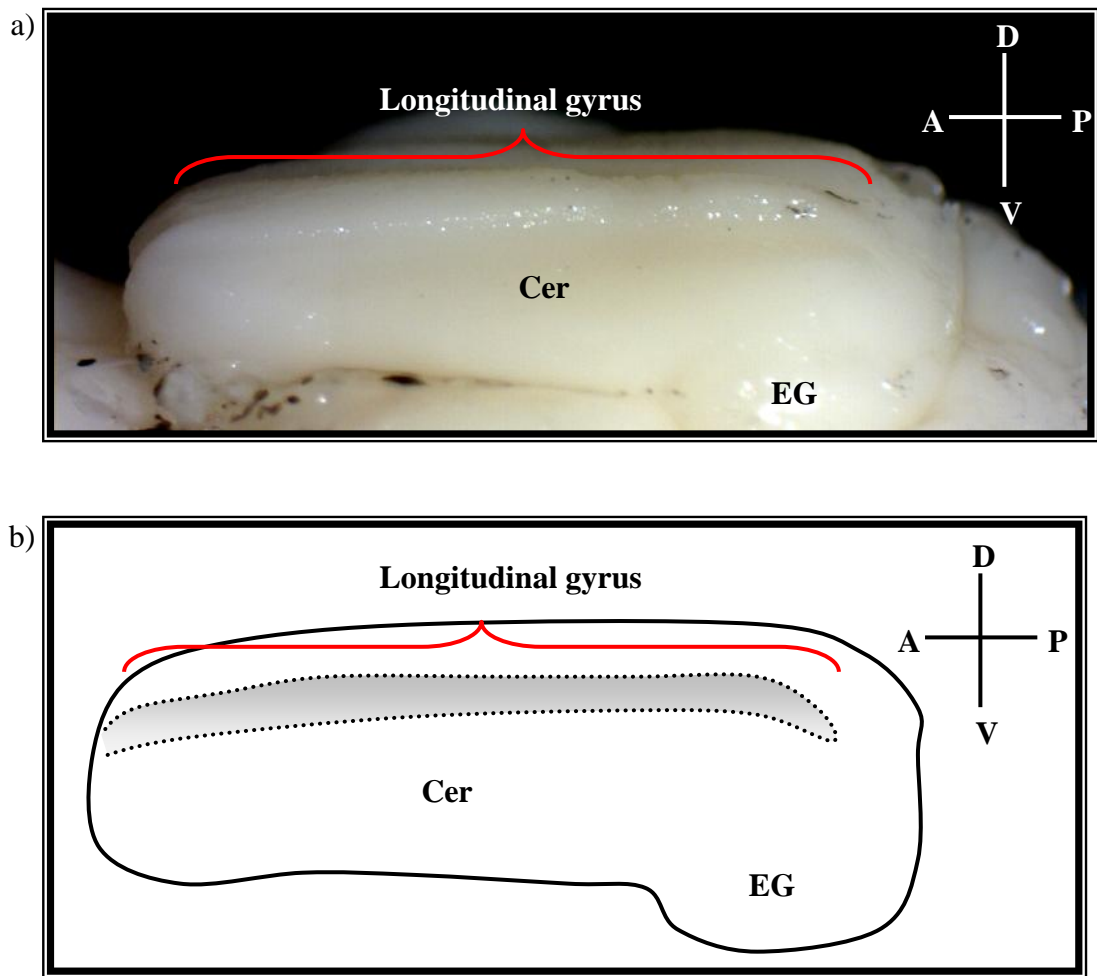
Figure 3.19: (a) Lateral view and (b) schematic diagram of rostrally directed cerebellum of *Mystus nemurus* (*baung*).
Cer: Cerebellum; EG: Eminentia granularis. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.

a)

Anterior

Cer

Posterior

b)

Anterior

Cer

Posterior
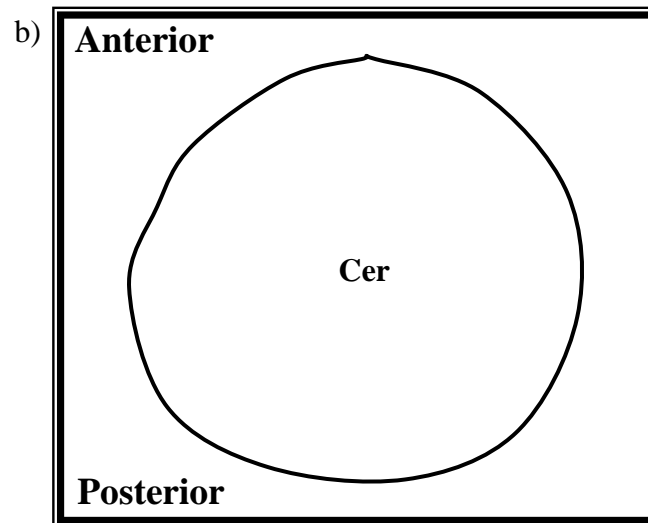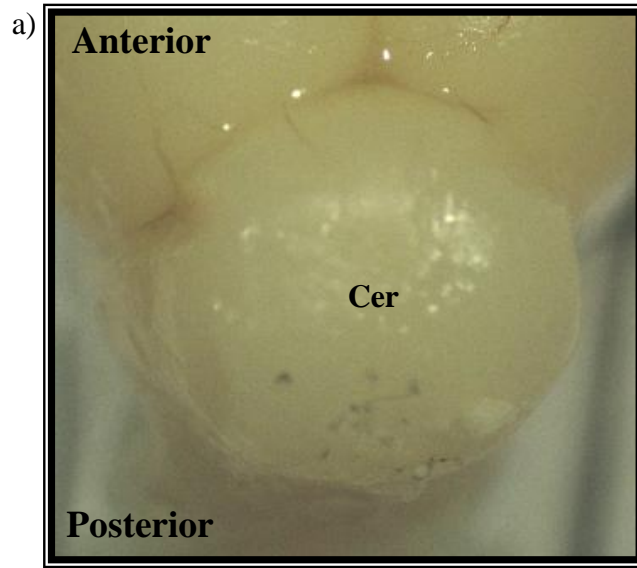
Figure 3.20: (a) Dorsal view and (b) schematic diagram of caudally directed
cerebellum of *Leptobarbus hoeveni* (*jelawat*).
Cer: Cerebellum.
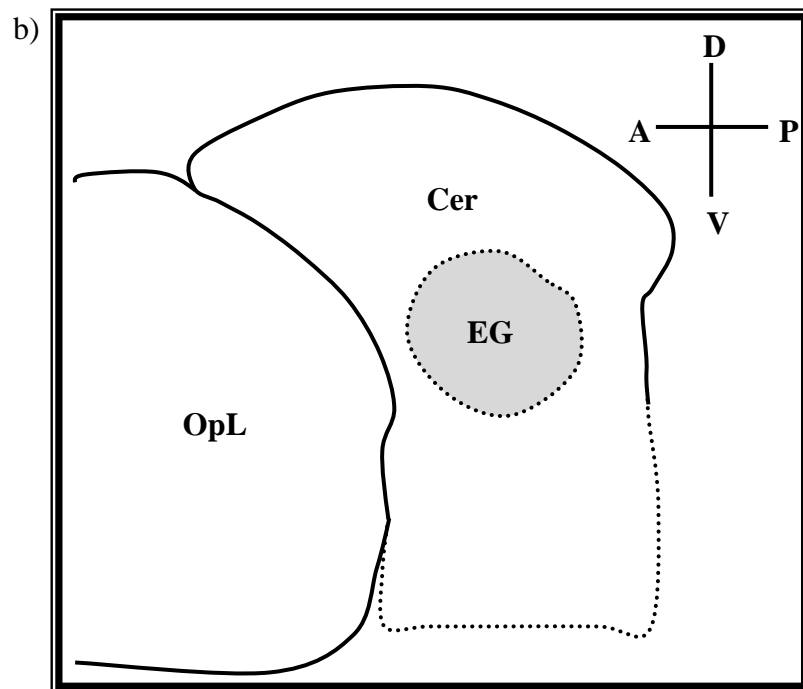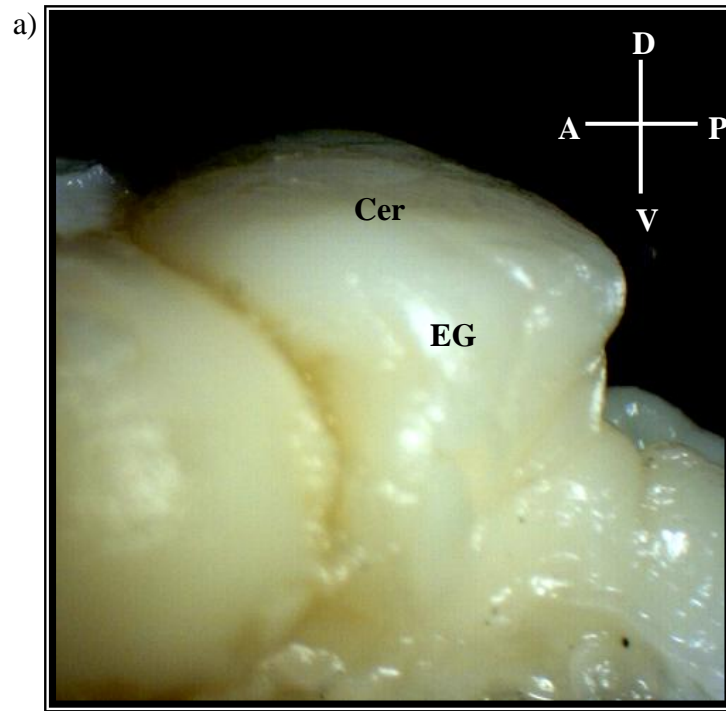
Figure 3.21: (a) Lateral view and (b) schematic diagram of caudally directed cerebellum of *Leptobarbus hoeveni* (*jelawat*).
Cer: cerebellum; EG: Eminentia Granularis. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.
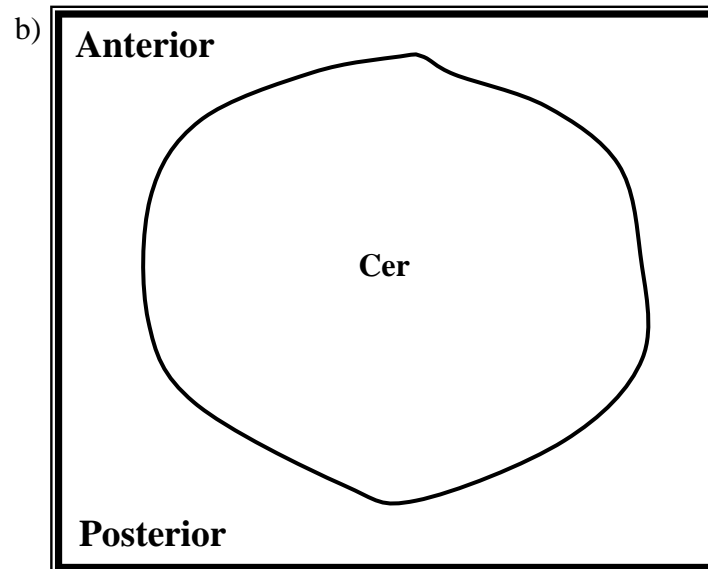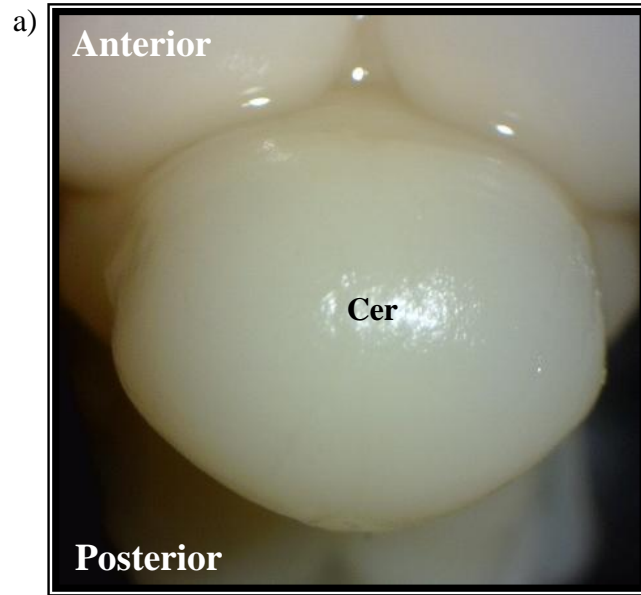
Figure 3.22: (a) Dorsal view and (b) schematic diagram of caudally directed cerebellum of *Oreochromis* sp. *(tilapia).*
Cer: cerebellum.
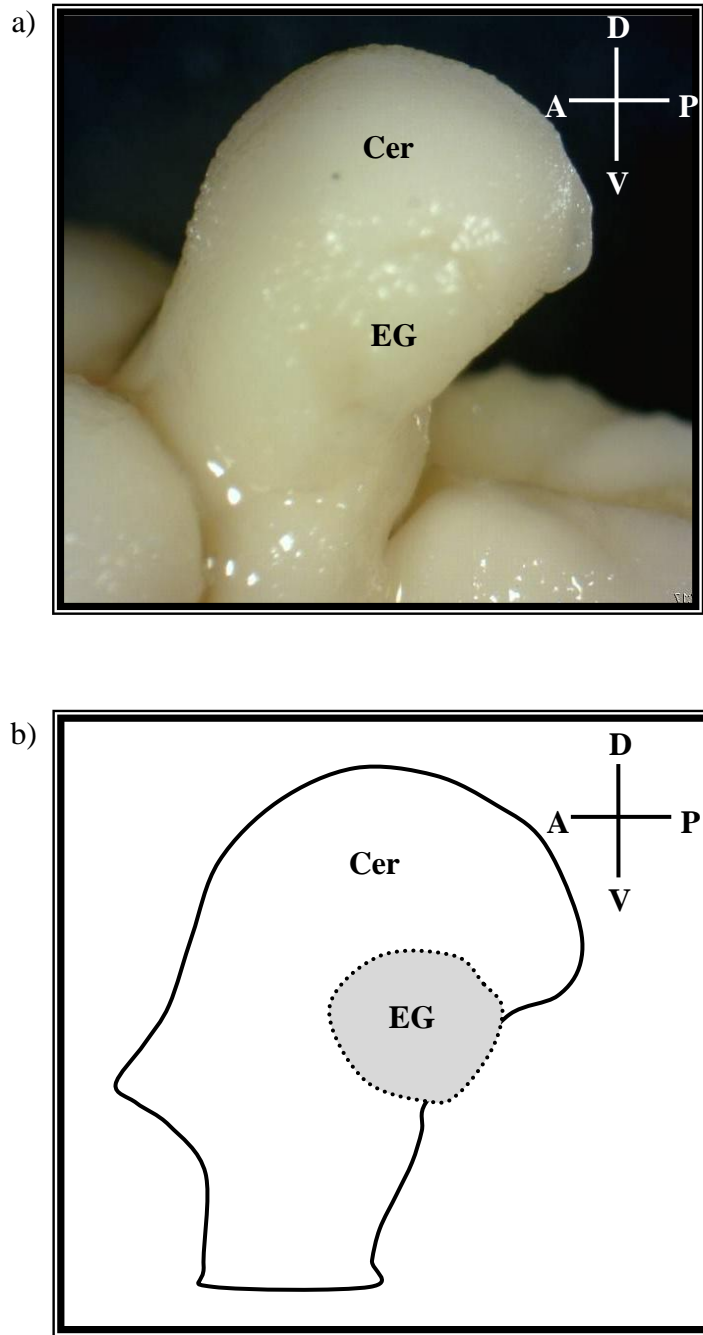
Figure 3.23: (a) Lateral view and (b) schematic diagram of caudally directed cerebellum of *Oreochromis* sp. *(tilapia).*
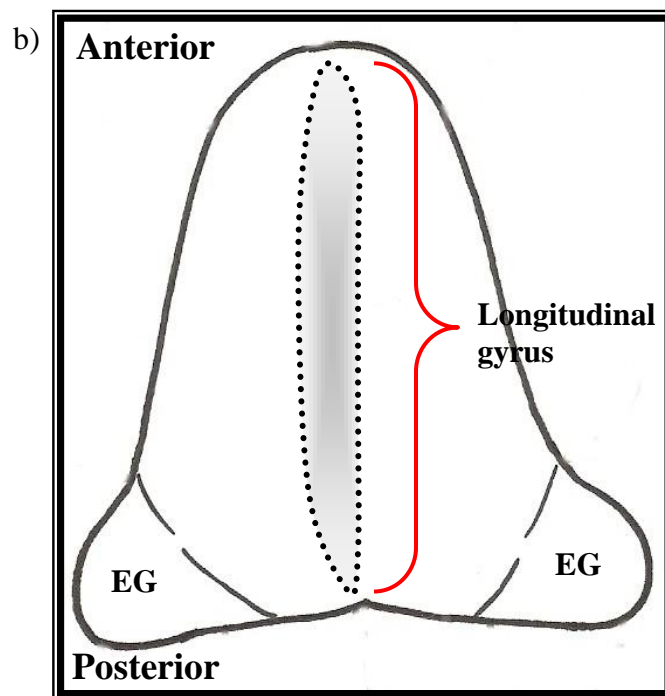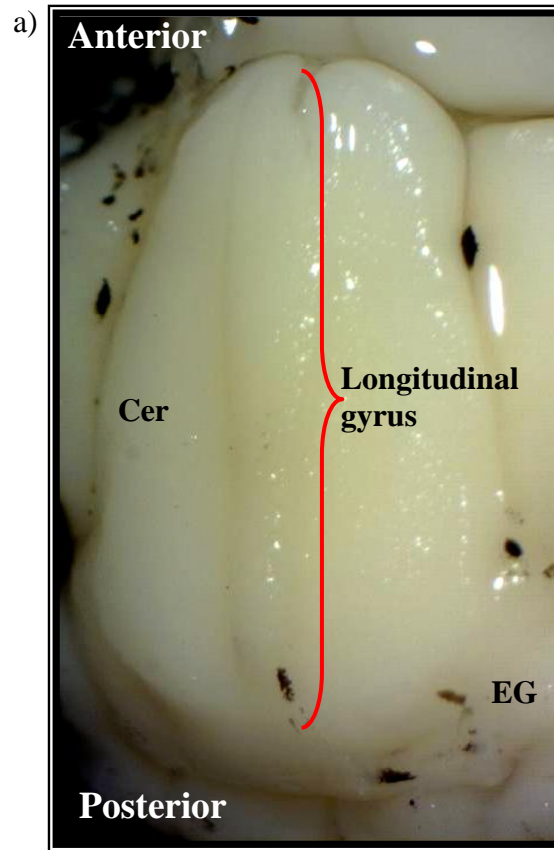Cer: cerebellum; EG: Eminentia Granularis. Orientation: A: Anterior; D: Dorsal; P: Posterior; V: Ventral.

Despite the same orientation, the cerebellum still had small differences between one another. The measurements of cerebellar features taken were shown in Table 3.1

Table 3.1: Cerebellar features of selected freshwater fishes.

SPECIMENS / FEATURES (mean±SD)	Keli	Baung	Tilapia	Jelawat
CEREBELLAR ORIENTATION	Rostral	Rostral	Caudal	Caudal
BRAIN WEIGHT (g)	0.33±0.04	0.15±0.02	0.36±0.02	0.32±0.12
WIDTH (mm)	4.60±0.55	3.83±0.41	4.83±0.41	4.9±0.57
LENGTH (mm)	7.80±0.45	6.33±0.52	4.17±0.41	4.9±1.10
HEIGHT (mm)	1.0	1.0	4.17±0.41	4.60±1.84
VOLUME (mm^3)	35.88	24.24	83.98	110.45
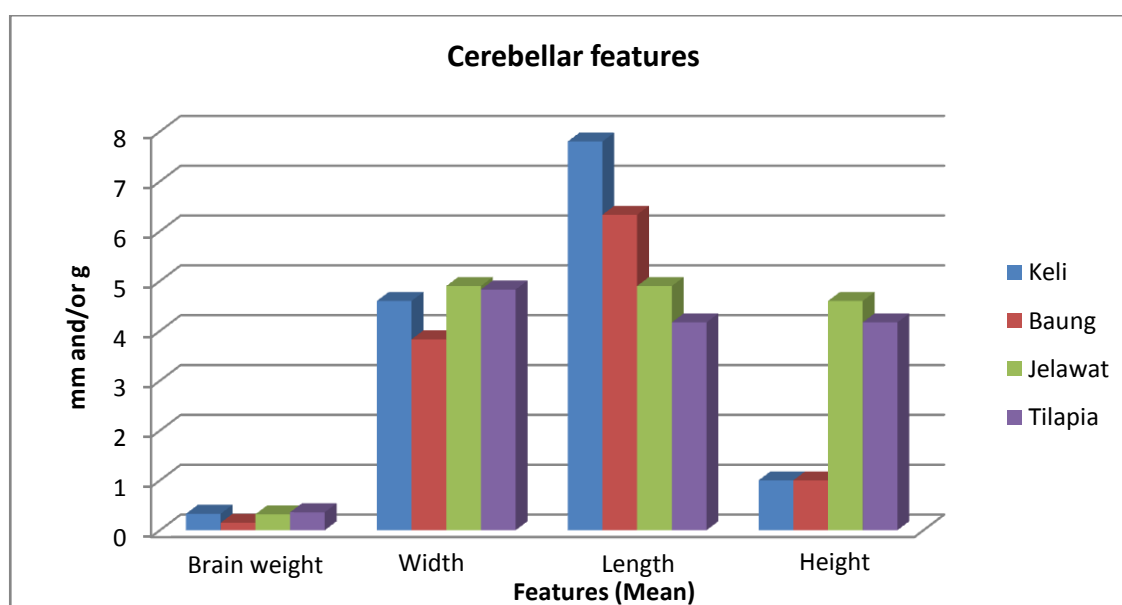
Figure 3.24: Comparison of the measurements of cerebellar features.

To standardize the findings, the size of the selected fishes was ensured in between 14 − 20 cm for length. *Keli's* cerebellum was the longest amongst all, which was 7.80±0.45 mm followed by cerebellum of *baung, jelawat* and *tilapia* (Table 3.1 and Figure 3.24). There were only little differences for the width of cerebellum for all species. *Jelawat* had the widest and thickest cerebellum, which was 4.90±0.57 mm and 4.60±1.84 mm respectively. The thickness of *tilapia's* and *jelawat's* cerebellum (4.17±0.41 mm and 4.60±1.84 mm respectively) was at least four times higher than *keli's* and *baung's* cerebellum, with the thickness of only 1 mm. Apart from that, the mass of the caudally directed cerebellum of *jelawat* showed the highest value with 110.45 mm^3, whereas *baung's* cerebellum was the lowest at 24.24 mm^3. Hence, it would be expected that would be more neural elements in the caudally directed cerebellum.

The statistic test was done on brain weight, width, length and height of cerebellum by using analysis of variance (ANOVA) *F* test, with the significance level is at 0.05 ($p<0.05$). The means of brain weight, width, length and height of cerebellum between all the fish species were significantly different.

All the data for the cerebellum features of the same group (rostral or caudal) were compiled together to get the average value to ease the comparison between those two groups. The statistic test was done to corroborate the differences of the data between these two groups by using analysis of variance (ANOVA) *F* test. All the details of the results of ANOVA *F* test is shown in Appendix III.

Table 3.2: The average measurements for rostrally directed cerebellum (*keli* and *baung*) and caudally directed cerebellum (*tilapia* and *jelawat*).

ORIENTATION FEATURES (mean±SD)	Rostral (*Keli* and *baung*)	Caudal (*Tilapia* and *jelawat*)
BRAIN WEIGHT (g)	0.23±0.09	0.33±0.05
WIDTH (mm)	4.18±0.60	4.88±0.50
LENGTH (mm)	7.00±0.89	4.63±0.96
HEIGHT (mm)	1.00	4.44±1.46
VOLUME (mm^3)	29.26	100.32
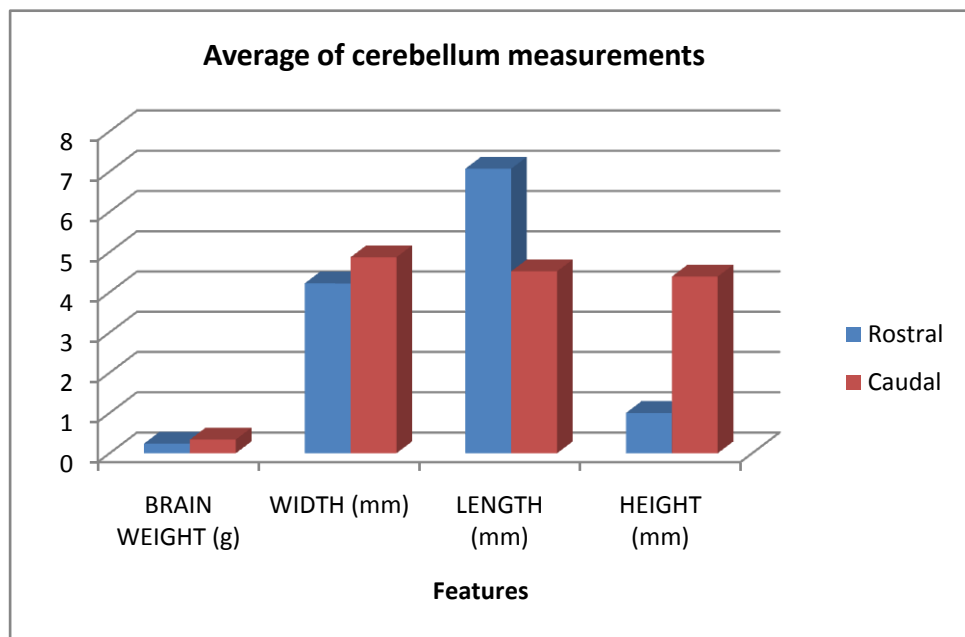
Figure 3.25: The average measurements for rostrally directed cerebellum (*keli* and *baung*) and caudally directed cerebellum (*tilapia* and *jelawat*).

Table 3.2 and Figure 3.25 showed the comparative cerebellar measurements between rostrally and caudally directed cerebellum. The caudally directed cerebellum was wider (4.87±0.49 mm) and thicker (4.39±1.13 mm) than rostrally directed, which

was 4.22±0.48 mm and 1 mm respectively. However, rostrally directed cerebellum was longer than caudally directed, which was 7.07±0.49 mm compared to 4.52±0.76 mm. These differences made the rostrally directed cerebellum appeared big and thin, while caudally directed cerebellum was small and thick. In addition, the mass of the latter showed the highest value with 100.32 mm^3, in contrast with the former which the value was 29.26 mm^3 only. Thus, the huge difference in thickness and mass might be the reason why caudally directed cerebellum was heavier (0.33±0.05 g) than rostrally directed, which was 0.23±0.09 g.

In accordance to the results of analysis of variance (ANOVA) *F* test, it was proven that the features of cerebellum of fishes for the two orientations studied had distinct differences between one another, except for the width of cerebellum.

3.3   DISCUSSION

3.3.1   The anatomy of cerebellum

From the results obtained, cerebellum of all four fish species demonstrated slightly different features although they came from the same class (Actinopterygii). The existence of these differences is uncommon since in other vertebrate, similar gross morphology of cerebellum for specific species would be the norm. Possibly, there must be a reason for this exceptionality and it is yet not understood.

The weight of *baung's* brain was much lower than the others even though there was not much difference on measurements of the cerebellum features. The various size of fish might be one of the reasons, since the size of *baung* used was a bit smaller than the others. This is because it was impossible to get all the specimens with similar size, since they easily died in aquarium. Moreover, the cerebellum was the biggest part of the brain for *baung*.

For higher vertebrates, the cerebellum has convolutions or folia with each lobe having its own specific function. Anterior lobe (Paleocerebellum) has a significant role in the regulation of muscle tone and is associated with coordination of the lower extremities and caudal part of the trunk. Posterior lobe (Neocerebellum) is involved in planning movements and evaluating sensory information for action and is associated with the rostral part of the body. Flocculonodular lobe (Archicerebellum), phylogenetically the oldest part, serves a significant role in balance and eye movements (Voogd and Glickstein, 1998).

The cerebellum of fish was just a simple structure without convolutions or folia, and totally different from birds and mammals, whose cerebellum is foliated, in accordance to Glickstein and Jan (1998). Fish which are good swimmers and able to move in all three dimensions have a well-developed cerebellum (Harder, 1975). However, the most foliated cerebellum is found in human, a higher vertebrate that

could perform complex and fine movements. Although there are no lobes found on the fish cerebellum, the functions of each lobe on a foliated cerebellum might be performed by the whole fish cerebellum. The exact similarity of mechanisms could not be determined by this current study and should further research from neurophysiology and neuroanatomy aspects. Possibly, division of functions was to be anatomically based and not corresponding to the somatotopic arrangement of the cerebellar cortex.

As mentioned before, instead of folia or fissures that are found on the cerebellum of higher vertebrates, only a median longitudinal sulcus and gyrus were found on *keli's* and *baung's* cerebellum respectively. Both features are believed to provide more surface area on the cerebellum, in order to increase the number of the neuronal cells. Subsequently, there would be more specific functions that the cerebellum could perform than if it was smooth or flat. Since none of them were found on the surface of *jelawat's* and *tilapia's* caudally directed cerebellum, it is expected that these fishes have less cerebellum function if compared to *keli* and *baung*.

The fish cerebellum consists of a central mass; the corpus cerebellum, valvula cerebellum and two lateral granular eminences, also known as the auricles similar to others cartilaginous fish (Ikenaga et al., 2006).

The corpus cerebellum is highly-arched, and is interpenetrated by processes and indentations from the fourth ventricle (V4). It is structurally simpler in the *Cichlidae* (*Perciformes*) and in the *Cyprinodontidae* (*Cyprinodontiformes*). The corpus cerebellum is only a simple, unpaired bump, with eminentia granularis attached laterally. It receives the processess from the V4 but this matter still remains insignificant (Harder, 1975). The cerebellum of frog is a relatively simple structure as shown in Figure 3.26. The corpus cerebellum is developed caudally with the convex side directed forward as viewed from above (William and David, 1970).

Corpus cerebellum is concerned with the accurate control of the voluntary muscles to carry out movements, so that the movements of the animal is continually being customized and maintained. The relation between the size of the corpus cerebellum and the activity of the animal is complicated because there is also a relation between the absolute size of body and the relative size of the corpus cerebellum (Brown, 1957). The large corpus cerebellum in fishes makes well-coordinated movements in swimming possible, chiefly when changing direction and compensating for shifting water current (Sarnat and Netsky, 1981). In this study, the caudally directed cerebellum had the larger area compared to rostrally directed cerebellum. The larger the mass of the cerebellum, the more numbers of neurons is expected to be making it up. Thus, possibly there is greater variety of movements the fish could do.

In other fishes, the valvula is a tongue-like structure that extends anteriorly from the corpus of the cerebellum to protrude beneath the optic tectum. However, the valvula of fishes is absent in all terrestrial vertebrates (Sarnat and Netsky, 1981).

The valvula cerebelli is structurally much more complex and grows out from the fissure rhombomesencephalica, whose caudo-ventral wall develops to become the ventricle. The entire valvula is enclosed inside the mesencephalon, and is generally only observable after that portion of the brain has been removed. However, the valvula cerebelli pushes the roof of the optic tectum to the sides of the brain when it is highly developed, e.g. in the carps (*Cyprinus*) (Harder, 1975).

The valvula cerebellum of bony fish is hidden under the midbrain tectum. Hence, its gross morphology could not be seen. In the electroceptive teleosts, also known as Mormyridae, the valvula is huge and 'pushed' the tectum aside in order to overlap the whole surface of the brain (Voogd and Glickstein, 1998). This fish possesses a pair or electric organs in its tail, and the lateral line organs have become electroreceptor. It has been suggested that their large cerebellum and Nerve VII might

be related to the perception of the electrical signals that this fish can stimulate, since it is also aware of pulses from individuals of the same species. Its Nerve VII has axons projecting to the cerebellum through a tract in valvula (Brown, 1957).

Eminentia granularis found on both sides of the most caudal part of cerebellum were observed in all rostrally directed and caudally directed cerebellum of fishes. A transverse fissure demarcated the eminentia from the corpus of cerebellum. The eminentia granularis is another characteristic feature of the teleost cerebellum. The paired structures are also highly variable in shape and usually appear as moderately large bulges which are well-defined. However, in *Merluccius* (*Gadidae*) and *Coregonus albula* (*Salmonidae*), they form extremely large bulges which project out like two wings. In the *Salmonidae*, whose corpus cerebelli initially runs steeply upwards, the eminentia tend to lie around the neck-portion, and at the median line above the fossa rhomboidea (Harder, 1975). Apart from that, the eminentia granularis gives rise to the parallel fibers of the posterior lobe (Campbell et al., 2006).

In the Elasmobranchii, the auricles vary from entirely smooth to strongly curled swellings. The tissues of the cerebellum pass over into the lateral walls of the auricles and form two layers, the superficially-located molecularis which is a fibrous layer and a more deeply- located layer which consists of the granularis (Harder, 1975).

Similar like *jelawat* and *tilapia*, the eminentia granularis was noted in frog cerebellum but difficult to distinguish. The eminentia granularis only existed in fish and frog. No reports were found on other vertebrates.

3.3.2   Comparison of foliation of cerebellum

Vertebrates have different specific gross anatomy of cerebellum. No detail report was found to elaborate the significance of this difference other than the cursory mention of the presence of folia in cerebellum of higher vertebrates. Based on previous works, it could be said that the more complicated a movement one species could do, the more complicated the gross morphology of cerebellum will be. This hypothesis is relatively reasonable.
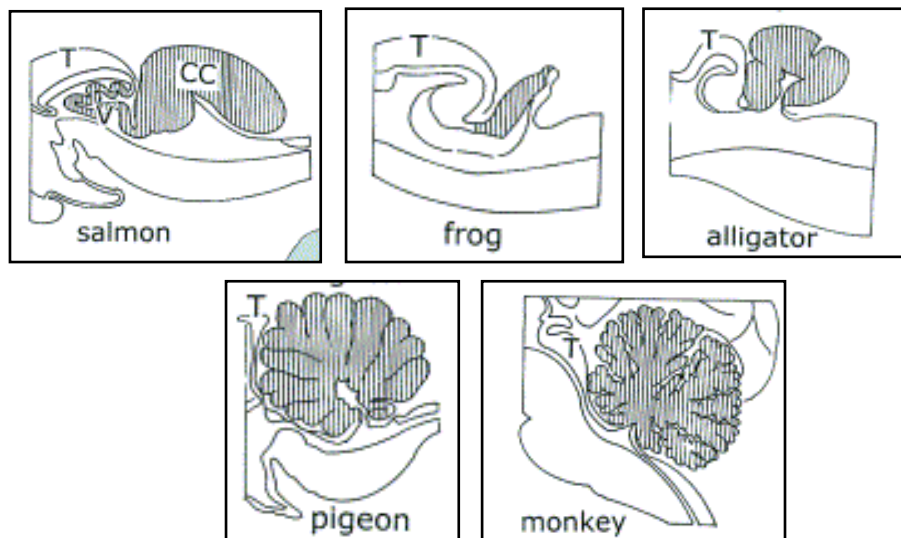

Figure 3.26: The gross anatomy of cerebellum in various animals (Voogd and Glickstein, 1998).
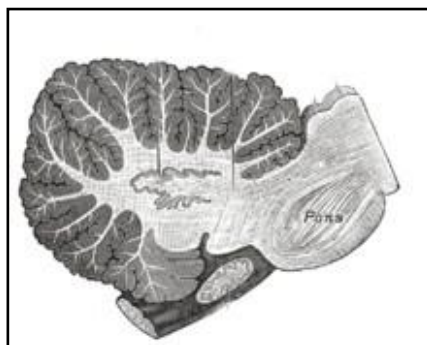

Figure 3.27: Cross section of human cerebellum (Henry, 2007).

Welker, (1990) mentioned that species differences in the relative size and number of folia is believed to reflect the behavioral and/or cognitive differences for mammals. Studies reported that the differences in cerebellar foliation within species are normally associated with the changes in behavior (Cooper et al., 1991; Demaerel, 2002)

Figure 3.26 and 3.27 show the variety of cerebellar morphology among vertebrate classes, i.e amphibians, reptiles, bird, fish and mammals. This includes a single leaf or dome-like structures as can be seen in amphibians and reptiles, and the more complicated structure as can be seen in birds and mammals. The cerebellum of avian and mammals is more elaborately foliated (Glickstein and Jan, 1998). The avian cerebellum from different groups had considerable variation in the relative size of individual folia (Figure 3.28). However, the most elaborately foliated cerebellum certainly goes to human as the highest vertebrate that could perform the more complex and fine movements, especially accomplished by the fingers.

It is thought that the expansion of one folium is generally correlated with a decrease in other folia whereas coordinated increases in size of folia are relatively rare. Behavior is not the only factor that influences the proportional size of cerebellar folia. There are several factors, i.e. braincase/cranial morphology, morphology of the jaw musculature, and eye shape. Braincase morphology could inflict biomechanical impediment on the pattern of cerebellar development. These different factors could then lead to significant phylogenetic effects and large differences in cerebellar morphology among orders (For more information, refer to Andrew et al., 2007).
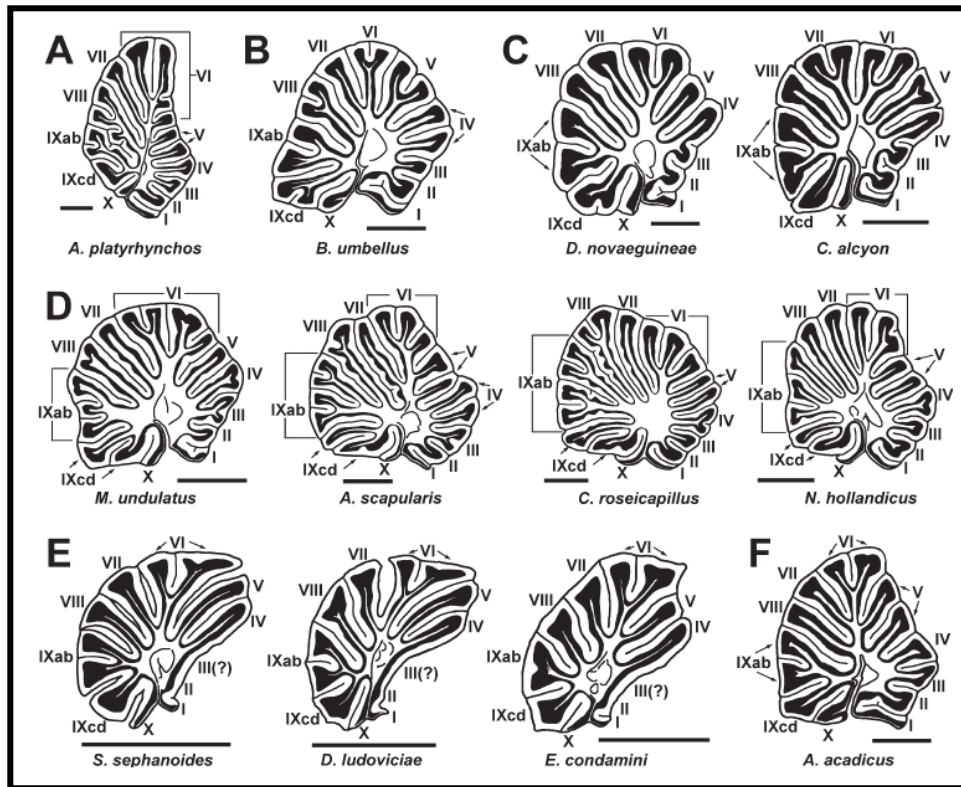
Figure 3.28: Outline of mid-sagittal sections through the cerebellum for 24 of the avian species. I-IX enumerates folia in a rostro-caudal direction. All scale bars = 3 mm. (Andrew et al., 2007).

Larsell (1967) was quoted by Iwaniuk (2006), to have reported that the expansion of individual folia was correlated with behavioral differences among species. They found ample evidence that strong fliers have enlarged folia. As proven by many tests performed, the folia are functionally discrete regions of the avian cerebellum. Each of the folia represents either some part of a somatotopic map (anterior lobe) or sensory modality (posterior lobe). Since the fish cerebellum appear to be representing one individual folia of the cerebellum of higher vertebrate, the difference of cerebellum gross morphology among species might have correlation with behavioral or swimming mode differences.

Welker (1990) put forward the contention that cerebellar lobules in mammals represent relatively distinct structure with specific hodological and functional divisions. He emphasized that interspesific variation in the number of folia and lobules reflected behavioral complexity. For example, mammals with more complex behavior have a

larger and more elaborately foliated cerebellar than behaviorally less complex mammals. Furthermore, this is corroborated by the general principles that the size of neural structures is a reflection of the behavior subserved by that structure (Jerison, 1973).

However, there still remains a considerable amount of variation to be explained and need further research. Factors that were unrelated to behavior and cognition could also possibly be the reason for the large amount of variation. The development of folium such as some expansion or contraction in size might be affected by the developmental or biochemical impediment, for instance the braincase architecture (Andrew et al., 2007).

Pal et al. (2003) reported in their study the comparison between cerebellum of man and fowl. In human, the cerebellum was found to be roughly spherical in shape, but in birds it is almost rounded. Interestingly, the cerebellum of fowl was found to be relatively greater in size and weight compared to that of human. The relatively greater size of the cerebellum of birds was thought to be associated with centre of equilibrium which has been reported by Parker and Haswell (1963). This centre of equilibrium was important in flying animals. The position of cerebellum is also different in human and bird and this might be due to the variations in axis of the bodies of these two species (Messer, 1958).

The two well developed cerebellar hemispheres and poorly developed vermis which could be observed in human were absent in fowl. In contrast, the cerebellum of fowl had a large vermis and two small lateral portion called flocculi (Pal et al., 2003). Furthermore, Ranson and Clark (1972) also emphasized that vermis was highly developed in birds that fly compared to the flightless one.

Pal et al. (2003) also reported that the increased number of spino-cerebellar fibre bundles was actually associated with the greater size of corpus cerebellum in birds.

Nickel et al. (1977) reported that the sulci of cerebellum were found to be finer in human and coarser in birds. In avian cerebellum, nine distinctive folia were observed, namely lingula, central lobule, culmen, declive, folium vermis, tuber vermis, pyramid, uvula and nodulus, which have resemblance to those of mammals.

Yopak (2007) proposed that the larger cerebellum of sharks across different species might perform well in the complexity of behaviors than the lacking cerebellar hypertrophy. Moreover, it is not necessary to exhibit the highest levels of foliation for the species with relatively largest cerebellum.

Compared to cerebellum of other animals (except frog), the fish cerebellum looks like it is representing one folia of other cerebellum. The distinctive folium that exists in other cerebellum has their respective function. Thus, by only having one type of folia which is making up the whole cerebellum, certain functions might be carried out by only certain part of the folia. However, this concern needs to be ascertained from neurophysiologic aspect.