Osteological comparison between local and Thai climbing perch in terms of neurocranium, vertebral column and accessory respiratory organ

Dr. Ismot Ara and Dr. Md. Rafiqun Nabi

Abstract
This study was carried out on the depth description of the osteology of neurocranium, vertebral column and accessory respiratory organ of two critically related varieties of *Anabas testudineus* with a view to investigate the taxonomic problem. The neurocranium of both *A. testudineus* was found to consist of four regions, such as, olfactory, orbital, otic, and basicranial region. Olfactory region composed of mesethmoid, lateral ethmoids, vomer, supraethmoids, and nasals. Kinethmoid was absent in both varieties of koi. Most of the orbital bones were similar but fourth suborbitals were different in shape. The bones of otic region, particularly opisthohias formed cup like fossa in local koi while it did not show such type of structure in Thai koi. Exoccipitals formed the sides of the foramen magnum, with condyles articulating with the first vertebra. Supraoccipital formed posterior roof of the skull beared a downward crest in both cases. This crest was somehow longer and more prominent in local koi than Thai koi. Accessory respiratory organ was composed of highly vascular layers of folded and convoluted skin, lying on both sides of the gill chambers and known as labyrinthine organ. This organ has complex structure with large surface area and showed much more fold in Thai koi than local variety and the base of the organ is more crescentic in local koi. The total number of vertebrae was 25 in both but the number was varied in thoracic and precaudal vertebrae. The ribs were more enlarged in Thai variety and increased ventrally. The shape of vertebral column was sharply concave in local variety but about to horizontal in case of Thai koi. Caudal complex of two experimental varieties of koi were round in shape. The neural and haemal spine jointly formed the caudal complex where different hypural were fused together. Concerning the osteological study, local and Thai *A. testudineus* were thoroughly investigated and observed that most of the bones of their skeleton were structurally common though have some obvious differences in some extend.

Keywords: *Anabas testudineus*, osteology, neurocranium, labyrinthine organ

Introduction
The climbing perch, commonly known as koi is scientifically termed as *Anabas testudineus*, belongs to the order periciformes and family anabantidae [1]. *A. testudineus* encompasses a wide range of geographical distribution. This freshwater fish is found in the beels, haors, baors, flooded water bodies, ponds, streams and rivers of Bangladesh [2, 3]. Recently attempts have been made to boost up the aquaculture production through incorporation of an exotic variety of *A. testudineus* known as Thai koi, which was introduced in Bangladesh by private sector in 2002 from Thailand. Climbing perch is an obligatory air-breather. The scientists also noted that the combined surface area of the labyrinthine organs and suprabranchial chambers in *A. testudineus* is greater than that of the gills, which suggests that the fish has greater dependence upon air-breathing organs for respiration [4, 5].

Radiography is a practical screening tool to detect subtle skeletal abnormalities in the adult catfish. Systematic identification of skeletal abnormalities in vertebrales provides an insight into the pathogenesis of skeletal disorders [6]. Ara et al. [7] studied the comparative cranial osteology of *Puntius gonionotus* and *P. sarana* and find out similar skull and different epiotic. Epiotic was anteriorly sutured with pterotic in species of *P. sarana* but in case of *P. gonionotus* it was sutured with the ex-opercipital. Supra occipital was pentagonal in *P. sarana* but found square in shape in *P. gonionotus*. Single fontanelle was present in *P. gonionotus* but absent in *P. sarana*. 

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Taxonomic ambiguity is still present in *A. testudineus* as Bleeker [8] reported that Thai koi as a variety of *A. testudineus* and Axelrod [9] described it *A. oligolepis* though the name *A. oligolepis* is no longer used and *A. testudineus* is widely used for both the varieties [10], it’s inevitable to investigate their osteological similarities and dissimilarities as a taxonomic character to provide significant tools for further consequences of their cross-breeding. So, considering all the mentioned opportunities and ambiguities regarding the local and Thai climbing perch, *A. testudineus*, to assist the taxonomic understanding, to compare the osteology of neurocranium, vertebral column and labyrinthine organ of fish not only to supplement with scientific evidences for taxonomy but also about the behavior of the two variety of the respective species.

**Materials and Methods**

**Collection of samples**

Samples of local and Thai variety of *A. testudineus* were collected from the different fish market in and around Savar, Dhaka. A total of 30 mature specimens were collected from each variety. After collecting the specimens, the fishes were brought in the laboratory of Limnology and Fishery Sciences, Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. Osteology includes the study of the form, structure and development of bones.

**Defrosting**

For osteological preparation, previously frozen preserved samples were kept out from the refrigerator and were left for 3-4 hours at the room temperature for defrosting.

**Washing**

After defrosting the samples were at first washed by the tap water to remove excess slime, clay, and other dirty objects from the fishes. Then they were again washed by saline water for removing extra microorganisms.

**Boiling**

For boiling the washed specimens were immersed in hot water at 60°C to which potassium hydroxide (KOH) and bleaching powder (CaOClCl) were added at the ratio of 1:10. The fishes were kept in hot water and bleaching solution for 5-10 minutes. They were then removed from the solution.

**Defleshing**

The boiled samples were defleshed by forceps and needle carefully. All the muscles were removed from the skull and vertebrae as it was possible. The skeleton with rest of the muscle was kept on the floor for biological treatment i.e. allows the ants to destroy the muscles.

**Disarticulation**

The skeletons of each species were disarticulated by forceps very carefully as one bone of any variety was not mixed with the others. Each bone of two variety of the same portion were differentiated and compared at the same time. All bones of neurocranium, axial and appendicular skeleton were carefully disarticulated and photographed using Olympus digital camera (Model No E-PLT, Olympus imaging corp., Republic of Korea).

**Cleaning, Drying and stiring**

The disarticulated bones were cleaned by needle very softly that the bones did not lost its own criteria. Each bone of the skeleton was allowed to dry slowly in an open place, care should be taken not to expose it to direct heat, which tends to break the bones. During the drying process all the bones were kept under the nets because cats and mice are enemies of this work. After drying the mean length and width of all the bones of the specimens were taken and compared. All the dried bones and skeletons of skulls and vertebrae were stored in the boxes with nepthallin or any insect repellent.

**Result**

Radiography is a practical screening tool to detect the comparative skeletal arrangement in local and Thai *Anabas testudineus* (Fig. 1). The comparative osteology of local and Thai climbing perch is extracted from several review references on fish osteology.

**Neurocranium**

The osteological study of the neurocranium suggested that the length of experimental skull was 2.7 cm and width was 0.8 cm at anterior portion; 1.6 cm at middle portion and 1.8 cm at posterior portion in local koi while in Thai koi it was 3.2 cm length and 1.3 cm width at anterior portion; 2.0 cm at middle portion and 2.2 cm at posterior portion (Fig. 2, Fig. 3, Fig. 4 and Fig. 5). The skulls of both koi were moderate and compressed; neurocranium also laterally compressed, moderate, high with bony ridges and a nuchal spine on dorsal side. The neurocranium of both *A. testudineus* was found to consist of four regions, such as, olfactory region, orbital region, otic region and basicranial region.

**Olfactory region**

It was the anterior most part of the neurocranium and composed of the following bones.

**Mesethmoid (Ethmoid, Hypethmoid)**

A median bone lying between nasal capsules. There were ‘I’ shaped or inverted ‘T’ shaped bones in section forming the anterior most part of the median dorsal surface of the skull. Anteroventrally, the bone was connected to the ventral end by a strong ligament, and laterally to the anteromedian part of palatine by ligament. The bone was ventrally firmly fused to the prevomer in case of both varieties of koi (Fig. 3 and Fig. 4).

**Supraethmoids (Mesethmoids)**

Paired bones dorsal to the mesethmoid (ethmoid). Dorsally and ventrally, both local and Thai koi beared same structure of the bone. Length was 0.7 cm and mid width was 0.6 cm for local koi but it was found 0.6 cm and 0.5 cm for Thai variety (Table 1).

**Lateral ethmoids (Prefrontals)**

A pair of bone in the ethmoid region, which separate the
olfactory capsules from the orbit. The bones were similar in structure between two types of koi. In local variety the length was 0.5 cm and mid width of the bone was 0.6 cm where as it was 0.6 cm length and 0.7 cm mid width in case of Thai variety (Fig. 2, Fig. 3 and Table 1).

**Vomer (Prevomer)**
A median bone, usually bearing teeth, at the anterior extremity of the roof of the mouth. The bone was posteriorly tapered to form an acute process overlapping the parasphenoid. Anterior end of vomer was slightly tapered and fused with pre-ethmoid to fit in to the ventral process of pre-ethmoid-ethmoid complex. The characteristics were more or less common in both varieties (Fig. 2, Fig. 3, Fig. 4).

**Pre-ethmoids**: Paired bones in the floor of the nasal capsules.

**Kinethmoid (Rostral)**
Median unpaired bone in cyprinoid fishes. It was absent in both varieties of koi.

**Nasals**
A pair of small tubular bones lying on the side of the anterior tip of the frontals. The bone was crescent shape and eventually formed olfactory capsules on either side. The center length of nasals was 0.2 cm for both types of *A. testudineus* (Table 1).

**Orbital region**
The orbital region was composed of frontals, orbitosphenoid, pterosphenoid, sclerotics, suborbitals and supraorbitals.

**Frontals**
A pair of bones that form most of the dorsal surface of the cranium, covering the orbitosphenoid and pterosphenoid. In case of local koi, the length was 1.7 cm, anterior width was 0.9 cm and posterior width was 0.7 cm but it was 1.8 cm, 1.0 cm and 0.8 cm accordingly for the Thai koi (Table 1).

**Orbitosphenoid**
Median bone between orbits, forming the floor and wall of the anterior end of the cranial cavity (Fig. 4).
Neurocranium (posterior view) of local (A) and Thai (B) climbing perch (koi), *Anabas testudineus*

**Pterosphenoid (Alisphenoid)**
Paired lateral bones joining the orbitosphenoid in front and the sphenotics (autosphenotics) and prootics behind.

**Sclerotics (Sclerotic cartilage)**
Pair of hemispherical cartilages surrounding the eyeballs. Sclerotics were more or less same diameter in both koi, there were 4 fragments and the diameter was 0.6 cm for every case (Table 1).

**Suborbitals**
A series of paired bones around the margin of the orbit; usually six or less; beared the suborbital lateral line canal.

**Lachrymal (Pterorbitals, SO1)**
The most anterior bone in the suborbital series; often the largest. The structure was same for both. Length of the lachrymal was 1.1 cm and 1.0 cm for local and Thai koi accordingly (Table 1).

**Jugal (SO2)**
Jugals were triangular in shape having crescentic edge. They were plugged to the eye socket at the length of 0.3 cm and 0.2 cm for local and Thai koi respectively (Table 1).

**Postorbital (SO3)**
Both the koi had rectangular shaped postorbitals. It was longer in local koi but broader in Thai koi. The length was 0.8 cm, anterior base was 0.3 cm and posterior end was 0.3 cm in local koi while it was 0.9 cm, 0.4 cm and 0.6 cm in length, accordingly for Thai koi (Table 1).

**Fourth suborbital (SO4)**
Fourth suborbitals were different in shape. In case of local koi, it was looked like a delta and its posterior edge was about to horizontal. But posteriorly it was sharply slopy incase of Thai koi. The length was 0.8 cm and 1.0 cm, anterior width was 0.4 cm and 0.3 cm, and posterior width was 0.9 cm and 0.7 cm for the local and Thai koi respectively (Table 1).

**Fifth suborbital (SO5)**
It was mostly similar in shape and size in both case, basal width was 0.3 cm and 0.34 cm, length was 0.9 cm and 0.95 cm for local and Thai koi relatively (Table 1).

**Dermosphenotic (SO6)**
The last bones of suborbitals were very small in size. They were similar in structure, length were 0.4 cm and 0.3 cm for local and Thai koi accordingly (Table 1).

**Supraorbitals**
Paired bones along the upper margin of the orbit; not transversed by the lateral line.

**Otic region**
Otic region generally consisted of sphenotics, pterotics, prootics, epiotics, opisthotics, exoccipitals, supraoccipital, supratemporals and parietals. The remaining bones from the otic region look likes an inverted cup like structure.

**Sphenotics (Autosphenotics)**
Paired bones lying beneath the dermosphenotics and forming the lateral processes behind the orbit. In both cases, the bones were irregular and jointed with the lateral wings of frontal anterodorsally and ventrally meet with the prootic. Ventrally the bones provided anterior fossa for a movable articulation with the hyomandibular. Both in local and Thai *A. testudineus* it was attached to the frontal dorso-anteriorly and to the pterotic ventro-posteriorly (Fig. 2, Fig. 3 and Fig. 4).

**Pterotics (Autopterotics, Autopalatines)**
Paired bones each enclosing the horizontal, semicircular canal of the inner ear; join the sphenotic (autosphenotic) infront, the prootic bellow, and the epiotic and exoccipital behind; covered by posttemporal. In local koi it was somewhat broad at posterior portion and the length was 1.3 cm, posterior width was 0.8 cm but it was 1.2 cm and 0.6 cm, respectively in Thai koi (Table 1).

**Prootics**
Paired bones each forming the base of the otic capsule and enclosing the utriculus in a ventrolateral bulla; join pterosphenoid in front, sphenotic and pterotic above, and exoccipital and basioccipital behind (Fig. 3 and Fig. 4).

**Epiotics (Epioccipitals)**
Paired bones each enclosing the posterior semicircular canal; join supraoccipital above, pterotic in front and exoccipital beneath and behind (Fig. 3).

**Opisthotics (Intercalary)**
Paired bones, often excluded from otic capsule, lying beneath and behind pterotic; cover junction of pterotic, epiotic, and supraoccipitals and perhaps the sphenotic. The bone formed cup like fossa in local koi while it did not form such type of structure in Thai koi (Fig. 5).

**Exoccipitals**
Paired bones at the back of skull; form the sides of the foramen magnum, with condyles articulating with the first vertebra (Fig. 2, Fig. 3, Fig. 4 and Fig. 5).
Supraoccipital
Median bone forming posterior roof of skull; often bears a crest. In both cases these bones beared a crest which was downward. This crest was some how longer and more prominent in local koi than Thai koi.

Supratemporals (Tabulars, Extrascapulars, Scale bone)
Paired bones covering the pterotic; contain part of lateral line and articulated to the posttemporal bone of pectoral girdle.

Parietals
Paired bones on the roof of the skull behind the frontals and partly or wholly separated by supraoccipital. It was almost flat, rectangular, attached with the supraoccipital by a suture. The bone was anteriorly sutured with the frontal, outer laterally with pterotic, inner laterally with supra-occipital and posteriorly with exoccipital. The length and mid width of the parietal was 1.1 cm and 0.7 cm in local koi but it was found 1.0 cm and 0.6 cm in Thai koi accordingly (Table 1).

Basicranial region
This region was composed of the following bones-

Basioccipitals
Bone forming posterior base of skull, articulating with centrum of the first vertebra. This bone was more prominent and thicker in local koi than Thai koi (Fig. 2 and Fig. 3).

Basisphenoid
Small, median, Y-shaped bone in near of orbit (Fig. 4).

Parasphenoid
Long, unpaired bone running midline below the orbits; between prevomer and basioccipital. The length of parasphenoid was 3.4 cm and 3.6 cm in local and Thai koi comparatively (Fig. 3 and Table 1).

Foramen Magnum
Posterior opening in cranium through which the spinal cord passes as it leaves the brain (Fig. 5).

Accessory respiratory organ
In both the varieties this air breathing organ was composed of highly vascular layers of folded and convoluted skin, lying on both sides of the gill chambers and known as labyrinth organ. The organ showed much more fold in Thai koi than local variety, while, the base of the organ is more crescentic in local koi (Fig. 6). To get an overall idea about the location of all the stated bones at a glance and how much those were identical to teleost (bony fish) groups as a whole, the superficial face bones and suspensorium of bony fish and their diagrammatic layering are included here to compare. The diagram suggested that the described bones of both local and Thai climbing perch are teleost like in shape and size and even in arrangement. Moreover, no deviation in the general structure and number of bones in skull was found.

Vertebral column
Vertebral column of every type of koi was composed of atlas vertebra, axis vertebra, pre caudal vertebrae, caudal vertebrae and caudal complex. The total number of vertebrae is same but the number was varied in precaudal and caudal vertebrae. The shape of vertebral column is also varied; it was sharply concave in local variety but about to horizontal in case of Thai koi. The length of vertebral column was 10.7 cm in local koi and 11.7 cm in Thai koi while their total length was 13.6 cm and 15.2 cm; neucranium was 2.7 cm and 3.2 cm in length respectively (Fig. 7 and Table 1).

Atlas Vertebra
First vertebra of the column.

Axis Vertebra
Second vertebra of the column.

Trunk vertebrae (Precaudal Vertebrae)
Divided into two regions.

Thoracic vertebrae
The number of thoracic vertebrae was 7 in local koi, whereas it was 6 in Thai koi.

Precaudal vertebrae
There were 2 precaudal vertebrae noticed in Thai koi, while local had only 1 (Table 2).

Caudal vertebrae
Structure and number of caudal vertebrae was same in both varieties of koi (Table 2).
Fig 7: Vertebral column of local (A) and Thai (B) climbing perch (koi), *Anabas testudineus*

Table 1: Comparative measurement of different bones of axial and appendicular skeleton between local and Thai climbing perch (koi), *Anabas testudineus*

<table>
<thead>
<tr>
<th>Name of the bones</th>
<th>Local koi</th>
<th>Thai koi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>Width (cm)</td>
<td>Length (cm)</td>
</tr>
<tr>
<td>Skull</td>
<td>2.7</td>
<td>1.6 (mid)</td>
</tr>
<tr>
<td>Frontal</td>
<td>1.7</td>
<td>0.9 (ant)</td>
</tr>
<tr>
<td>Lateral ethmoid (Prefrontal)</td>
<td>0.5</td>
<td>0.6 (mid)</td>
</tr>
<tr>
<td>Parietal</td>
<td>1.1</td>
<td>0.7 (mid)</td>
</tr>
<tr>
<td>Supra ethmoid</td>
<td>0.7</td>
<td>0.6 (mid)</td>
</tr>
<tr>
<td>Pterotic</td>
<td>1.3</td>
<td>0.8 (post)</td>
</tr>
<tr>
<td>Parasphenoid</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Premaxilla</td>
<td>2.0</td>
<td>0.4 (mid)</td>
</tr>
<tr>
<td>Maxilla</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Symplectics</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Hyomandibular</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Lachrymal</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Jugal</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Postorbital</td>
<td>0.8</td>
<td>0.3 (post)</td>
</tr>
<tr>
<td>Fourth suborbital</td>
<td>0.8</td>
<td>0.9 (post)</td>
</tr>
<tr>
<td>Fifth suborbital</td>
<td>0.9</td>
<td>0.3 (base)</td>
</tr>
<tr>
<td>Dermo phenotic</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Retroarticular (Angular)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Branchiostegal ray (Smallest one)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Branchiostegal ray (Largest one)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Posttemporal</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Cleithrum</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Entopterygoid</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Metapterygoid</td>
<td>0.8</td>
<td>0.6 (post)</td>
</tr>
<tr>
<td>Ectopterygoid</td>
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<td></td>
</tr>
<tr>
<td>Quadrat</td>
<td>1.1</td>
<td>1.0 (mid)</td>
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<tr>
<td>Vertebral column</td>
<td>10.7</td>
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Table 2: Comparison of vertebral column between local and Thai climbing perch (koi), *Anabas testudineus*

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of Atlas</th>
<th>No. of Axis</th>
<th>No. of Trunk vertebrae</th>
<th>No. of Caudal vertebrae</th>
<th>Total No. of vertebrae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local koi</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Thai koi</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

Caudal complex
The caudal complex of two experimental variety of koi were round in shape. The neural and haemal spine jointly form the caudal complex. The ultimate vertebra was pointed straightly at posterior end. Ultimate vertebra (Preural vertebra), penultimate vertebra (Preural II vertebra), epineurals were the components of caudal complex.

Discussion
The osteological study, local and Thai *A. testudineus* were thoroughly investigated. It was observed that most the bones of their skeleton were structurally common though have some obvious osteological differences in some extend between both experimental varieties of koi. The skeleton of both varieties was divided into exoskeleton and endoskeleton. Exoskeleton composed of scales and fins. Again, endoskeleton consisted of axial and appendicular skeleton. The axial skeleton consisted of skull and vertebral column. The skull was composed of two distinct parts, the neurocranium and the branchiocranium, which included the paired capsules, the jaws, hyoid and branchial arches. On the other hand, vertebral column consisted of trunk and caudal vertebrae. Same type of investigation has also recorded in the family of Leiognathidae and Cobitidae [11]. Detail investigation of skull between local and Thai koi suggested that it was vault shape, laterally compressed, tapering towards the snout region and completely ossified, narrower and flat especially at the anterior region but became deeper and broader from the posterior end of the orbit to the otic region. Similar observations were also recorded in *Puntius sarana* and *Pantius gononotus* and *Labeo* sp. [7] In...
local koi, posterior region of skull was less broad and more elongated, whereas, the Thai koi possessed broader posterior portion. During investigation between two varieties of koi, it was found that the length of experimental skull was 2.7 cm and width was 0.8 cm at anterior portion; 1.6 cm at middle portion and 1.8 cm at posterior portion in local koi while in Thai koi it was 3.2 cm length and width was 1.3 cm at anterior portion; 2.0 cm at middle portion and 2.2 cm at posterior portion. The skulls of both koi were moderate and compressed; neurocranium also laterally compressed, moderate, high with bony ridges and a nuchal spine on dorsal side. The neurocranium of both *A. testudineus* was found to consist of four regions, such as, olfactory region, orbital region, otic region and basi cranial region. Same type of neurocranium structure was also described in other fishes by Vasanth and Reddy [12], Sawada [13].

Olfactory region composed of mesethmoid, lateral ethmoids, vomer, supraethmoids, and nasals. All the bones were similar in structure in both koi but their length and width were slightly varied. The center length of nasals was 0.2 cm for both types of *A. testudineus*. Nasals were crescent shaped and eventually formed alfactory capsules on either side [14]. Kinethmoid was absent in both varieties of koi. This finding disagreed with Sawada (1981), as recorded in cyprinid.

The orbital region was composed of frontals, orbitosphenoid, pterosphenoid, sclerotics, suborbitals and supraorbitals. Most of the bones were similar. Frontals formed the largest part of skull roof meeting in the mid longitudinal line. Similar observations were recorded by Lu and Wu [15] in the family Custostomidae. Fourth suborbitals were different in shape. Incase of local koi, it was looked like a delta and its posterior edge was about to horizontal. But posteriorly it was sharply slopy incase of Thai koi. The length was 0.8 cm and 1.0 cm, anterior width was 0.4 cm and 0.3 cm, and posterior width was 0.9 cm and 0.7 cm for the local and Thai koi respectively. Otic region generally consisted of sphenotics, pterotics, prootics, epiotics, opisthotics, exoccipitals, supraoccipital, supratemporals and parietals. The remaining bones from the otic region look like an inverted cup like structure. Sphenotics provided anterior fossa for a movable articulation with the hyomandibular in local and Thai *A. testudineus*. Prootics formed the base of the otic capsule and enclosing the utriculus in a ventrolateral bulla. Opisthotics formed cup like fossa in local koi while it did not form such type of structure in Thai koi. Exoccipitals formed the sides of the foramen magnum, with condyles articulating with the first vertebra. Supraoccipital formed posterior roof of the skull; often bears a crest. In both cases these bones beared a crest which was downward. This crest was some how longer and more prominent in local koi than Thai koi. In both cases parietal fontanelle was absent. Ara et al. [7] recorded similar structure in *Puntius sarana* but different in number i.e. single fontanelle was present only in *P. gonionotus.*

Both the varieties possessed accessory respiratory organ positioned behind and above the gills with the bones of the skull and operculum forming the roof and wall and the muscle of the jaw comprising the floor. This air breathing organ was composed of highly vascular layers of folded and convoluted skin, lying on both sides of the gill chambers and known as labyrinth organ. This organ has complex structure with large surface area and showed much more folded in Thai koi than local variety and the base of the organ is more crescentic in local koi. Same type of investigation was done by Graham [16] in air breathing fishes in San Diego. Vertebral column of every type of koi was composed of atlas vertebra, axis vertebra, trunk vertebrae, caudal vertebrae and caudal complex. The total number of vertebrae was same but their arrangement was different in between the thoracic and precaudal region. The shape of vertebral column was sharply concave in local variety but about to horizontal in case of Thai koi. Same type of observation was recorded by Lagler et al. [17] All the ribs are thin and fragile and articulated with the third to last thoracic vertebra in both varieties. It is presumed that the enlarge rib supported the enlarged pelvic bone to maintain the increased ventral surface of the disc. These ribs were comparatively larger in Thai koi than local koi. Same identity of ribs was recorded by James [18] on leiognathidae and Sawada [13] on cobitoidea.

Caudal complex of two experimental variety of koi were round in shape. The neural and haemal spine jointly form the caudal complex. The ultimate vertebra was pointed straightly at posterior end. In both varieties different hypural were fused together. This type of investigation was done by Schepper et al. [19] in African Clariids one of the air breathing fish, which differed in some extent with present findings. They recorded that the caudal complex of *Clarias gariepinus* did not showed fusions between the different hypurals.

Present experiment revealed that most of the osteological characteristics were closely related with each other and some divergeden characteristics were also found between local and Thai *A. testudineus*. Several workers have suggested that different factors are responsible for the dissimilarities of bones in fishes [20]. Unfavorable environmental conditions during growth development may also be a factor [21, 22, 23]. Pollutants have been considered to be responsible for the deformation of different parts of bone of fish. Bengtsson et al. [24] has suggested injury being other factors for abnormalities. Osteologically, most of the bones of both *A. testudineus* showed structurally same but differed in size, shape and thickness. The skull and neurocranium was vault shaped, laterally compressed, tapering towards the snout region and completely ossified in both experimental varieties. Skull was lack of fontanelle and kinethmoid but in both cases neurocranium was consisted of four regions i.e. olfactory, orbital, otic and basi cranial region. Excessory respiratory organ was more folded and convoluted in Thai koi. The total number of vertebrae was identical in number (25) for both the climbing perch (koi).

References
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