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MORPHOLOGY AND HISTOLOGY OF PITUTARY GALAND OF TORRENTIAL STREAM FISH *NEMACHEILUS MOOREH* (Sykes)

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Abstract: Pituitary lies ventral to the brain behind optic chiasma in the depression of parasphenoid bone attached to the floor of the mid ventral infundibulum. It is Leptobasic type and show transitional condition between caudo and dorsobasic type, sella turcica, a depression provides space to lodge pitutary with thick connective tissue septum. The pituitary gland is composed of nervous part, the neurohypophysis, and a glandular part, the adenohypophysis. The neurohypophysis develops from the infundibular floor and provides a stalk to pituitary. The distal part of neurohypophysis is brightly stained due to presence of neuro secretory material. The positively stained neurosecretory fibres extensively innervate the pars intermedia and rostral pars distalis. Adenohypophysis develops from the roof of the stomodaeum and is divisible into pars intermedia and pars distalis. Pars distalis is composed of a proximal part containing deeply stained cyanophils and acidophils. The rostral and proximal pars distalis and pars intermedia are arranged in a linear fashion with the rostral pars distalis at the anterior extremity. In *N. mooreh,* pars intermedia completely surround the distal region of neurohypophysis. The study will provide a deeper insight into morphohistology and cell types of pituitary gland of the freshwater Homalopterid teleost.

Keywords: Pituitary, Cell types, *N. moreh,* Adenohypophysis, Neurohypophysis.

INTRODUCTION

The study will provide a deeper insight into morphohistology and cell types of pituitary gland of the freshwater Homalopterid teleost, *Nemacheilus mooreh* from Mula - Mutha river system in northern western ghats, Pune in Maharashtra state, situated between 17°54" and 19°21" N latitude and 73°24" to 75°14" E longitude in western India. The river system is contributed by several streams originating along the crest of Sahyadries (Western Ghats) and finally drains to Krishna river system about 200 Km. to South east of Pune. *Nemacheilus mooreh,* is a small fish plays vital role in torrential ecosystem and contribute immense importance in fish biodiversity. Though it is not productive and economically important, it is pretty common in river Mutha and tribal people, and the "Katkari" catch them on commercial point of view. *Nemacheilus mooreh are* known to have the potential to withstand extreme ecological conditions.

The Morphology and histology of piscine pituitary has been reported from time, to time but much less is reported as compared to mammals, birds, reptiles and amphibians[1]. The morphology and histology of the fish pituitary has attracted an attention of many researchers[2 to 15]. The study will provide a deeper insight into morphohistology and cell types of pituitary gland.

MATERIAL AND METHODS

The fish *Nemacheilus mooreh* were collected from Mutha River, at a strech of 2.5 Km. near village Warje, Tq. Haveli, Dist. Pune, Maharashtra, in each month during January 1998 to 2001. Live fishes were brought to the laboratory. The pituitary gland was traced out after exposing brain from its ventral side and floor of the cranium was also carefully removed. A drop of fixative was poured on the pituitary gland. After few minutes, pituitary along with brain was carefully separated from the cranium and immediately transferred into Bouins fixative.

The pituitaries along with brain were sectioned at 5 to 6 µ and stained with Mallory's triple stain, Per Iodic Schiffs reaction and H/E stain for further studies. Identification of various cell types was made on tinctorial staining properties of cells.

RESULT

Pituitary gland appears as, dull whitish in color and its shape and size is specific. It lies at ventral to the brain behind optic chiasma in the depression of parasphenoid bone attached to the floor of the mid ventral infundibulum. It is Leptobasic type according to Bretschneider and Duyvene De Wit[10] and show transitional condition between caudo and dorsobasic type (fig 1). The depression provides lodging to the pituitary gland, called sella turcica with thick connective tissue septum (fig 2). The pituitary gland is composed of nervous part, the neurohypophysis, and a glandular part, the adenohypophysis. The latter is divisible into pars distalis and pars intermedia. The pars

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distalis is composed of rostral and proximal part. The rostral and proximal pars distalis and pars intermedia are arranged in a linear fashion with the rostral pars distalis at the anterior extremity (Fig 1).

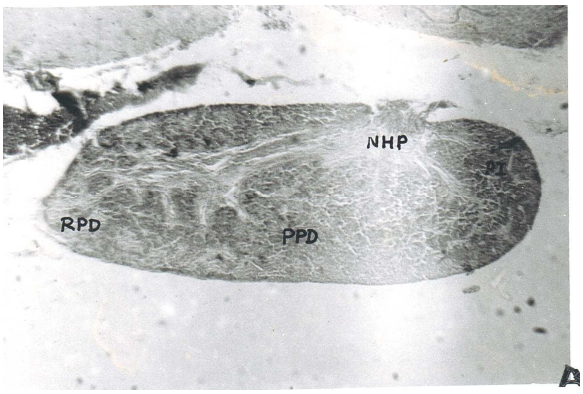

Fig.1: L.S. of pituitary of *N. moreh* (100x),
RPD - Rostral Pars distalis, PPD - Proximal pars distalis
PI - Parsintermedia, NHP – Neurohypophysis

Neurohvpophysis:

The neurohypophysis develops from the infundibular floor and provides a stalk to pituitary. It is short, measures about 2.4 µm in length and 6.4 µm in thickness, consisting axonal nerve fibers, mostly non mylinated, originate in hypothalamic nuclei.

Some axons terminate as capillaries in the neurohypophysis, while other terminates at the basement membrane separating neurohypophysis and adenohypophysis. The neurohypophysis is divided into two parts, anteriorly, containing mainly AF-ve fibres with pituicytes, and connected with the pars distalis, and posteriorly containing mainly AF+ve type fibres with many pituicytes, and connected with the pars intermedia (Fig.1). The fibers of neurohypophysis are not compact, occasionally, droplets of colloid substance accumulate as neurosecretory material and the Herring bodies (Fig.3) are purple in color. The neurohypophysial portion, at the proximal pars distalis reveals thick nerve fibres (dia. 1.49µm) which may extend lengthwise along the main trunk, with blood vessels containing RBC. The distal part of neurohypophysis is brightly stained due to presence of neurosecretory material. The positively stained neurosecretory fibers extensively innervate the pars intermedia and rostral pars distalis. The neurohypophysis fibers are arborized and ramify intensely the gland. Neurosecretory material found abundantly accumulated during pre-spawning period.

Adenohypophysis:

It develops from the roof of the stomodaeum and is divisible into pars intermedia and pars distalis. Pars distalis is composed of a proximal part containing deeply stained cyanophils and acidophils and a rostral part where acidophils preponderate. The glandular regions are markedly confluent.

Fig.2: Lodgement of pituitary, coverd by Sella turcica (X 100)
Br - Brain, Pit-Pituitary, ST- SellaTursica

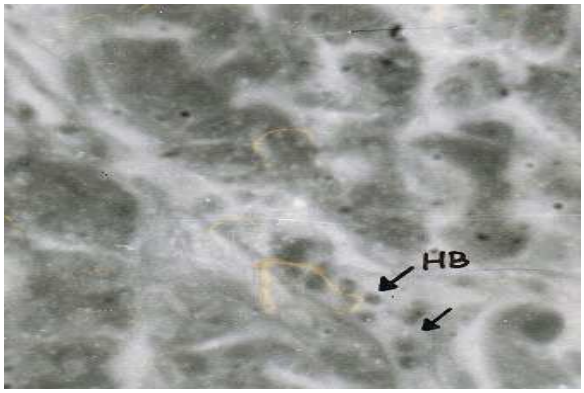

Fig.3: Herring Bodies (100X)

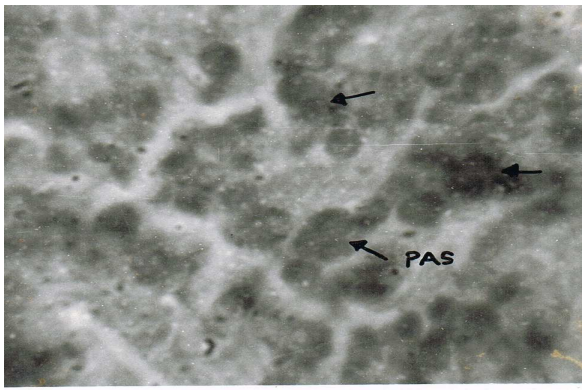

Fig.4: Basophilic cells (GTH cells) (100X)
HB- Herring bodies

(a) **Rostral pars distalis**: This is part of the adeno-hypophysis and lies anteriorly. The cells are acidophils and stains with orange G, with centrely placed lumen with blood vessels (Fig 5). The cells are small, round or elongated having granules and a rounded eccentrically placed nucleus (dia. of acidophils is 0.41µm). In addition to acidophils, chromophobes are also present and stains with orange G, with eccentric nucleus, diameter of these cells is 0.49µm. Acidophills and chromophobes (Fig. 6) are found at the anterior part of neurohypophysis with basophils and cyanophils. Three different types of cyanophills are noticed with centrally placed nucleus, where the diameter of these cells

varies from 0.24 µm to 0.66 µm.

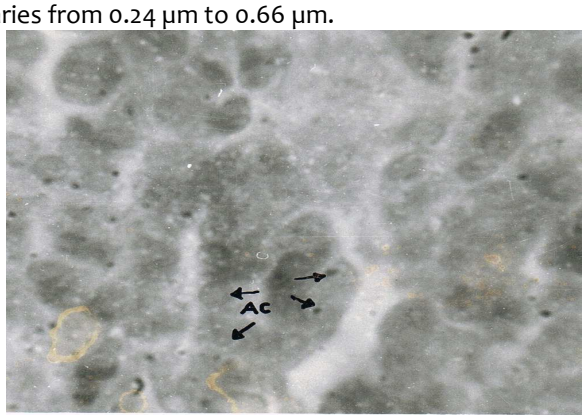

Fig.5: AC - Acidophilic cells (100 X)

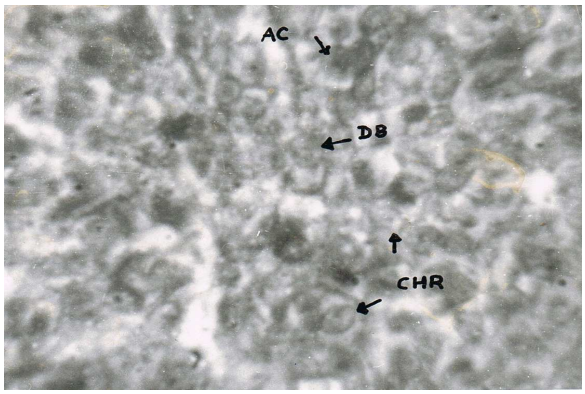

Fig.6: L.S. of pituitary in post spawning Phase (100X)
AC Acidophils
DB Degranulated Basophils
CHR Chomophobes

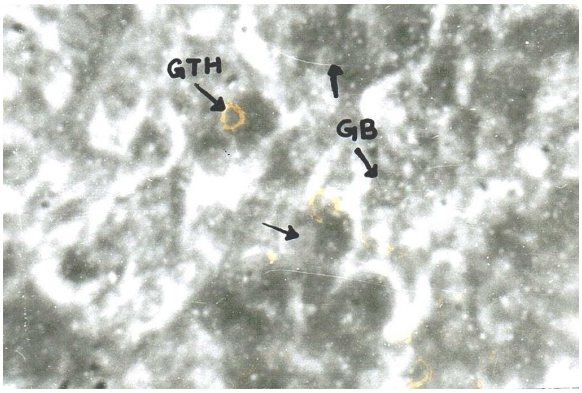

Fig.7: L.S. of pituitary in pre spawning Phase (100X)
GTH Gonadotrophic hormone secreting cell
GB Granulated Basophils

(b) **The Proximal Pars distalis**: This is the largest of all the regions and occupies most of the glandular components, responsible for the secretion of several hormones. The acidophils, the basophils, and the chromophobes are important cell types of this region. Generally, all these cell types are intermingled with no definite arrangement. The acidophils are arranged along the boundary of neurohypophysis near to RPD. The acidophils are smaller in size (0.33 µm in diameter) than to the basophils and are PAs-ve. They also found arranged in small groups with the basophilic cells and stains with orange G. The bright blue coloured basophil cells are predominate arranged and in sheet having fine granulated cytoplasm (Fig. 7). Two types of basophils are found, one smaller in size and non granulated with nucleus, while other are granular arranged centrally to the cell. Cell diameter measures around 0.49 µm other types cells are irregular in shape and size with granular materials arranged periphery to cytoplasm, measures about 0.74 µm in dia. the largest cell type of pituitary. The basophils of this region are PAS+ve (Fig. 4) the cyanophils in this region are more in number, but did not show any characteristic appearance and tinctorial differentiation. These cells are smaller in size compared to other cell types in PPD and measured about 0.41 µm in dia. The cytoplasm is a clear with centrally situated nucleus.

Pars intermedia:

The pars intermedia partly or completely surrounds the distal region of the neurohypophysis (Fig.1), characterized by the heavy innervation of numerous finger shaped extensions of neurohypophysis. Acidophils are abundant and arranged in clusters where basophills arranged at periphery. These are of two types and can usually be distinguished on staining property, shape and position (stains with Orange G). Acidophilic droplets are colloidal in appearance, occurs with pars intermedia cells and measures about *0.41*µm in dia. They show amphiphilic staining properties, commonly OG+ve. The basophils are found scattered in small groups and measures about 0.66 µm in dia. The nuclei of these cells are placed on one side rather than centre. They are present in groups or cords arranged along the neurophypophysial branches. The cells are cone shaped structures when they face towards the neurohypophysial branches with small blood capillaries.

Blood vascular supply:

The arterial supply is from the internal carotids, enter the gland by a single medium hypophysial artery. In the neurohypophysis the arteries give rise to a capillary plexus lying close to the neuro-adenohypophysis interface and form an elaborate network of vessels and form a vascularised appearance with endocrine cells in neurohypophysis.

DISCUSSION

The basic structure of the piscine pituitary is more or less similar, but differs minutely from species to species[10]. The teleostean pituitary exhibits wide variations from species to species on topographical arrangement. The shape of the pituitary bears a close relationship to the age of individual[16,17,18,19,20].

The attachment of the pituitary with the brain, by means of an infundibular stalk or without, is very significant on the evolutionary status of gland. Close

attachment of the pituitary to the brain, without any definite stalk is a platybasic[7] and is considered as a primitive type[4,21,22], and can be named as type A[10]. Presence of stalk to the pituitary is leptobasic type, an evolved condition[7]. While working on *N. mooreh,* pituitary gland found attached with the brain by means of a short and thick infundibular stalk and hence, it is of leptobasic type. Such leptobasic condition of pituitary has been considered as an advanced condition[8] and called as type B. Such condition is found in *Cirrhina mrigala,* O. *cotio,* C. *batrachus,* and O. *pabda*[20]. Leptobasic type is further classified into three categories i.e. caudobasic, dorsobasic and Cranio-basic, according to the attachment by infundibular stalk with the pituitary. Further, when the attachment is at the anterior, posterior and middle respectively and is called Cranio, Caudo and dorsobasic type respectively. In *N. mooreh* the infundibular stalk is attached to the posterior to middorsal point and thus shows transitional condition between the caudo and dorsobasic type. Similar type of attachment has also recorded in *N. botia*[20], Labeo *rohita*[23], Cirrhina *reba, Mystus seenghala* and *Barbus stigma*[13] and the pituitary is of dorsobasic type.

Fishes generally do not have a sella turcica[24,25]. Its presence has been reported in *Polypterus*[5], *Hilsa hilsa*[23] and *Cirrhina mrigala*[25]. During present investigation, *N. moreh,* showed a well-developed sella turcica. Probably this feature is an advanced and an evolutionary approach towards higher vertebrate. The size and the globular appearance of the gland may account for the presence of sella turcica and is complicated lodgment of the pituitary.

The orientation of the lobes of the pituitary is variable. In the present fish, the rostral and proximal pars distalis and pars intermedia are arranged in linear fashion with the rostral pars distalis at the anterior end. This type of arrangement of glandular regions has also been reported in *N. botia, N. notopterus,* O. *bacaila, R. daniconius,* G. *lama,* O. *cotio,* C. *batrachus, Hfossilis,*O. *bimaculatus,* O. *pabdo, E. vacha, M. cavasius, M. gulio, M. tengara, M. bleekeri, N. punctata,* C. *gachua,* C. *punctatus,* and *M. armatus*[20].

A review has been made on various types of orientation of the glandular components, and noticed that there is a gradual transition in the orientation. The lobes are arranged along the antero-posterior axis with the rostral pars distalis at the anterior extremity, considered to be basic design[42] and are supposed to be primitive condition[8].

The neurophypophysis in *N. mooreh* has been found to innervate all three lobes, although the main trunk divides proximal pars distalis. The innervation passes down to rostral pars distalis and other branches in to pars intermedia as reported in *Labeo rohita*[23], *Cirrhina mrigala*[25]. In *P. americanus* and C. *carpio,* the neurohypophysis is exclusively restricted to pars intermedia[9]. The distribution of the neurohypophysis in all lobes is considered to be an advanced feature over the restricted supply to pars intermedia[7].

Infundibular cavity may be present in some fishes[26] or it may be reduced[22]. No infundibular cavity has been observed in *N. mooreh.* Similar observations are made earlier in *Cirrhina mrigala*[25]. Fish possessing no infundabular cavity approach the vertebrate condition.

In *N. mooreh* the position and the occurrence of Luteotrophic hormone secreting cells in pituitary gland containing granulation makes it possible to identify as a prolactin cell, arranged in follicles, with lumen containing colloidal material with variable staining properties[27]. Similar types of prolactin or lactotrops are recorded in *Heteropneustus fossilis*[28]. Synchronised staining properties of the teleost LTH cells and the mammalian lactotrophs were emphasized earlier. While studying these cell types, found to be more sensitive to salinity and shown them to be more active in freshwater than sea water[29,30], and hence are considered to be osmo regulatory in function. In *N. mooreh,* another group of cell types are observed at the posterior interface between LTH cells and neurohypophysis in RPD which are similar to the cell types noticed in catfish[31]. Due to their staining property some workers have referred them as chromophobes[32] and considered as ACTH cells. Corticotrophic function of these cells has been confirmed simultaneously earlier in *Anguilla* and *P. latipinna* and sometime act as adrenocortical inhibitor (metapirone; SU 4885, CIBA)[33] and have identified these cells in *Anoptichthys*[34].

Along with LTH cell follicles, in *N. mooreh,* there are another three cell types lying adjacent to follicle, and on their position, shape and size called as stellate cells. Similar cell types are also found in Anguilla[43] which shows general feature of the adenohypophysis[44].

The proximal pars-distalis is an important lobe of the pituitary, not only in the teleost fish but in all vertebrates. It shows variations on size and cell components even in the same fish at different maturity stages. This lobe is larger in *N. mooreh* and lie posteriorly to rostal pars distalis with no distinct boundries or connective tissue septa as shown in *C. mrigala*[25], and in *Gasterosteus*[21]. This lobe consists of mainly acidophils, basophils and chromophobes of early maturity stages. The acidophils are outnumbered by the basophils in the final sexual maturity stages, such change in cell components of the lobe is in complete agreement with the observations by earlier authors who have studied the pituitaries of fishes at different maturity stages[9,13,14,36].

In the present study due to the complexity of cells structure and specificity for staining, it has become very difficult to differentiate the two types of basophils by adopting the PAS staining techniques[36,37,38]. However, the complexity of cell structure and specificity have been studied by earlier workers[11,24,39,40], and were able to identify the gonodotrophs. With advancement of maturity changes in GTH cells noticed in *N. mooreh,* which are closer to *Anguila* and *Mugil*[31,41] distinguishing two types of gonadotrophs, represent different developmental stages in a single cell type[28]. In the present study, it is observed that the GTH cells are more active and changes in cell size, nuclear size and cytoplasmic granulation during vitellogenesis. Similar observations are recorded earlier in *Heteropneustus fossilis*[28].

Accumulation of colloidal bodies or granules in the basophils of PPD in a spent fish indicates the positive activity towards inducement of spawning in fish. However, the presence of vacuoles in the central basophil cells is an indication for spawning. Colloidal bodies, may play a role in process of breeding in *N. mooreh,* resembles *C, mrigala*[25]. In *Carrassius auratus*[9] and in *Mystus seenghala*[13] the spawning was taken place with the occurance of basopils, but the spawning may proceed with or without colloidal material. Characteristic reversible change from acidophility of the anterior lobe (RPD in present case) to basophility and vice-versa during pre-spawning and resting phase[42] are in conformity with present observations.

The cyanophils in PPD which act as thyrotrophs, did not exhibit hypertrophy or degranulation during the spawning phase. It is difficult to differentiate to them from somatotrophic hormone secreting cells.

Pars intermedia is intimately associated with neurohypophysis[5]. In *N. mooreh,* pars intermedia completely surround the distal region of neurohypophysis. Such condition has been reported earlier in C. *mrigala*[25], C. *reba* and *M. seenghala*[13] and in *Labeo rohita*[23]. In *N. mooreh,* few basophils are present near to neurohypophysis, but the main constituent is acidophilic cells. In C. *mrigala,* acidophils are the main constituent of Pars intermedia[25]. Acidophils can be distinguished on different staining property[9]. In *N. mooreh* both the cells are seen. Similar observations are recorded earlier in *Anguilla* describing these two main cell types in pars intermedia[43].

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