ORGANIZATION OF BRAIN IN COMMERCIALLY IMPORTANT EDIBLE FRESHWATER CRAB *BARYTELPHUSA CUNICULARIS* (CRUSTACEA: DECAPODA)

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ABSTRACT

The freshwater crab *Barytelphusa cunicularis* (Westwood, 1836) is commonly occurring freshwater crab in the state of Maharashtra. It is commonly found in rivers, ponds, lakes, wells, dams, etc. The *B. cunicularis* is an edible highly nutritious commercially important crab and sold in large numbers in weekly markets in cities, towns and villages in Maharashtra. The brain of crab has been reported to control many physiological activities in crustaceans. The brain and central nervous system (CNS) of crustaceans has been extensively investigated by neuroanatomists. The present research paper gives an account of histological organization of the brain in freshwater crab *B. cunicularis*. The routine histological techniques with Mallory' triple staining were used to study histology of the brain. The analysis of results obtained shows that the brain of *B. cunicularis* consists of a sheath, a number of neuropils, groups of neurosecretory cells and glial cells. The brain is divisible into Pro-, Deuto- and Tritocerebrum. Some giant nerve cells have also been observed. The stained sections of brain showed presence of various types of neurosecretory cells (NSCs). The main types of NSCs observed were type A, B, and C cells.

Key words: **Crab, Brain, Histology, Neurosecretory cells.**

I. INTRODUCTION

The central nervous system (CNS) of crustaceans has been the subject of extensive research. Crabs and other decapod crustaceans have been favored animals among neuroanatomists for many years because of their comparatively large size and well-organized nervous systems. Hence, the central nervous system (CNS) of crustaceans has been the subject of extensive investigations by neuroanatomists since the end of the 19th century. Studies on crustacean CNS by Hanstrom (1947) are highly informative regarding our basic knowledge of the crustacean nervous system. There is a growing interest, however, in the cerebral ganglia (brains) of the crustaceans. Bullock & Horridge *(1965)* provides an exhaustive review on CNS of crustaceans. Since then, many workers in recent years have dealt with additional aspects of brain morphology in decapod crustaceans (Abbott, 1971; Tsvileneva and Titova, 1985; Nassel and Elofsson, 1987; Blaustein *et al.*, 1988; Sandeman et al., 1993; Harzsch and Dawirs, 1995; Krieger *et al.*, 2010). Some researchers have studied the brain and neurosecretion in freshwater crustaceans from Maharashtra (Deshmukh and Rangnekar, 1965; Diwan, 1971; Diwan and Nagabhushanam,1975; Devraroo, 1981; Sherkhane, 2007). A study on the neurosecretory cell types in the brain of *B. cunicularis* by Diwan and Nagabhushanam (1975) reported 3 types of neurosecretory cells. Despite this extensive range of literature on the CNS, a detailed histological account of brain in freshwater crab *B. cunicularis* is as yet not available. In view of this, the present research work was undertaken.

II. MATERIALS AND METHODS

Animal collection and maintenance: The present research work was carried out at Dept of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, MS, India. *Barytelphusa cunicularis* specimen were obtained from the Kham river near Aurangabad, Maharashtra, India. Crabs were maintained in aerated condition at 27˚C. Healthy animals of different carapace lengths were used for the experiment (carapace length between 1 to 9 cm). They were fed regularly with the earthworms and water was changed daily.

Histology of brain: The cerebral ganglia were carefully dissected out and fixed in aquatic Bouin's fluid, dehydrated in ethanol series, embedded in the paraffin wax and sectioned at 7µm thickness on rotary microtome (Weswax optic senior rotary microtome). The tissue sections were stained with routine H &E stain and Mallory's triple stain; sections were subsequently microphotographed.

III. RESULTS AND DISCUSSION

The brain of *B. cunicularis* has an approximate size of 2.5 mm × 2 mm. Its volume is roughly between 5 to 10 mm^3. It is compact creamy whitish structures with number of nerves arising from it which innervate important organs like eyestalk, antennae, antennules and other important organs. The brain is located in anterior part of cephalothorax immediately behind the base of two eyestalks (**Plate-I, Fig-1**).

PLATE-I

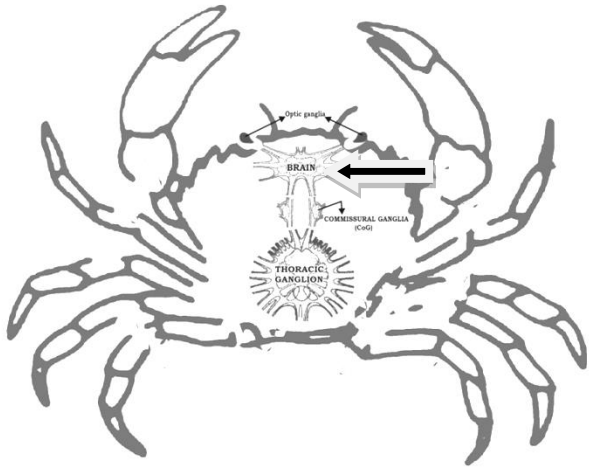

Fig-1: Diagram showing location of brain in the body of crab.

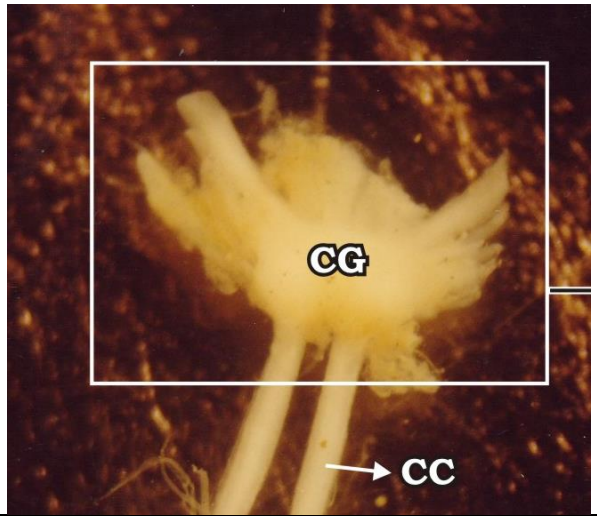

Fig-2: Freshly dissected out brain (cerebral ganglion) of crab *B. cunicularis.*

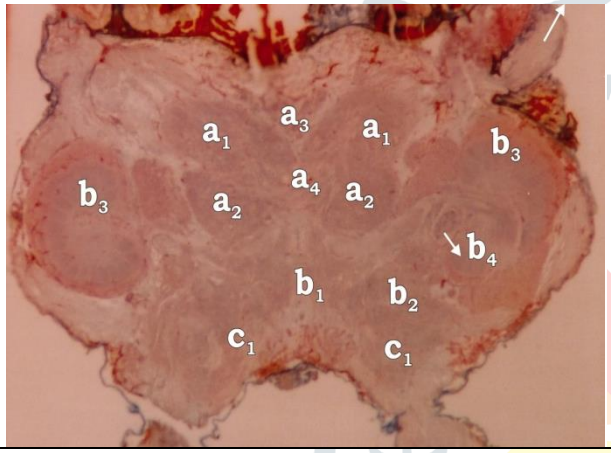

Fig-3: Horizontal section of brain stained with Mallory's triple stain showing different neuropils.

Protocerebrum: a_1=*Anterior protocerebral neuropil (AMPN)*, a_2= *Posterior protocerebral neuropil (PMPN).*

Deutocerebrum: b_3= *olfactory lobes (ON)*, **b4**= *Accessory lobe AcN*, b_1= *Median antenna I neuropil (*MAN), *b2*= lateral antenna I neuropil (LAN), a_4= Olfactory globular tract neuropil (OGTN).

Tritocerebrum: C_1=*Tegumentary neuropil* (TN) , C_2= *The antenna II neuropils* (AnN).

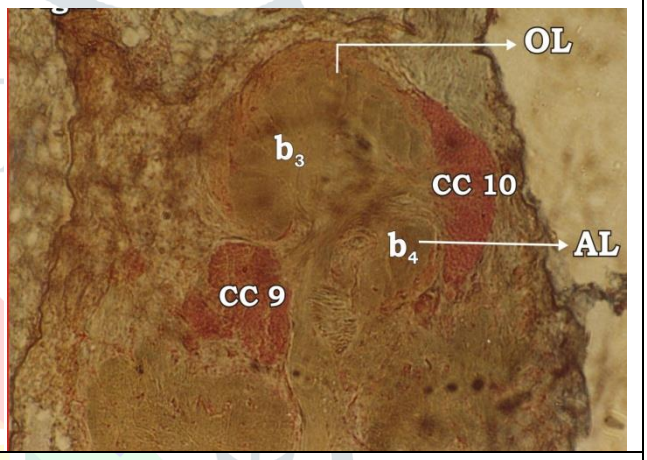

Fig.4: Magnified part of deutocerebrum showing OL, AL, CC-9 and CC-10.

OL=*olfactory lobes (ON)*, **AL**= *Accessory lobe (AcN)*, **CC9=** *Cell cluster 9*, **CC10**= *cell cluster 10.*

The entire brain is surrounded by a protective sheath. This sheath has shown to exhibit labeling by allatostatin-like immunoreactivity and can also be labeled in the silver impregnated preparation in crab *Brigus latro* (Krieger *et al.*, 2010). As in many other crustaceans, the brain of *B. cunicularis* is divisible into three distint regions: the proto-, deuto- and tritocerebrum. Like crayfish, most of the brain regions of crab brain lie in the same plain and most neuropils can be observed in a stained section of a brain in a single plane (Sandeman *et al.*, 1992). Neuropils are synaptically dense areas within the brain containing relatively low number of cell bodies and composed mostly of unmyelinated axons, dendrites and glial cell processes. A large number of neuropils are also found in brain of *B. cunicularis.* The main neuropils found in the cerebral ganglion of *Barytelphusa cunicularis* are shown in (**Plate-I, fig. 1 & 2).** Terminology used for nomenclature of neuropils in the crab brain follows Sandeman *et al.*(1992).

Protocerebrum: Two prominent neuropils are located in the protocerbral part of the brain: *The anterior protocerebral neuropils (*AMPN) and *the posterior protocerebral neuropils* (PMPN).The AMPN is located anteriorly and is larger than the posterior protocerebral neuropils. Krieger *et al. (*2010) reported presence of additional types of neuropils (e.g., protocerebral bridge neuropil and the central body neuropil) in the protocerebrum of crab *Birgus latro.*

The protocerebral bridge, central body, and the olfactory globular tract are also observed.

Deutocerebrum: Following neuropils were observed in the deutocerebral region of *B. cunucularis*:

Olfactory lobes *(ON), Accessory lobe (AcN), Median antenna I neuropil (*MAN), *Lateral antenna I neuropil* (LAN), and, *Olfactory globular tract neuropils* (OGTN). The deutocerebrum of the brachyuran brain is characterized by reduced AcN which are tucked in at the posteromedial edge of the olfactory lobes. They are small but retain their glomerular structure. Cell cluster 10 (CC10) is located posterior to the olfactory lobe,, however, in the crayfish this cluster is positioned lateral to olfactory lobe (Sandeman *et al.* 1992).

PLATE-II

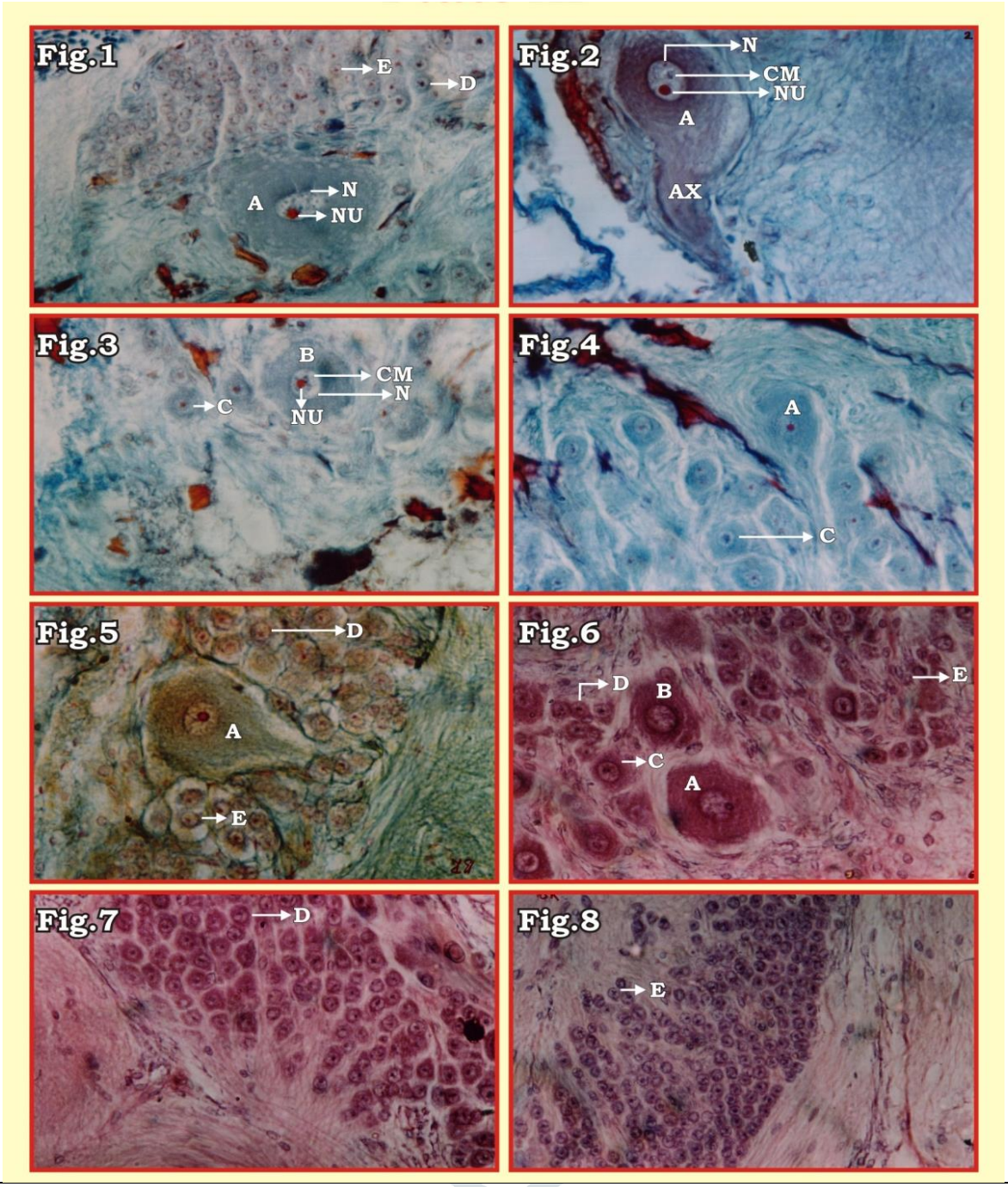

PLATE II: LS of cerebral ganglion of *B. cunicularis* showing types of NSCs. **Fig. 1-8** show different types of NSCs. Stain used: Mallory's triple stain for Fig. 1-5 and Haematoxylin-Eosin for Fig. 6-8. Magnification 400X for all figures.**Abbreviations**: A,B, C, D and E= Types of NSCs, NU=Nucleus, N=Nucleolus, Ax= Axon.

Tritocerebrum: The tritocerebrum of the crab *B. cunicularis* is similar in organization to that of crayfish. It has following two neuropils: *Tegumentary neuropil* (TN) and A*antenna II neuropil* (AnN). *The tegumentary neuropil* lies at the base of the oesophageal connectives on the dorsal surface of the cerebral ganglion. The AnN is proportionately smaller in the crab as compared to those in crayfish. Cell clusters in the crab brain are very similar to those in the crayfish, with the exception of cluster 10 which is located posterior to the olfactory lobe. Cell clusters such as CC 9 and CC10 are very distinct in *B. cunicularis* brain. Sandeman *et al.* (1992) has reported cell cluster 1 to 17 in crustacean brain. Similar observations were reported by Tsvileneva *et al.* (1985) in the shore crab *Hemigrapsus sanguineus.* More Detailed studies will reveal the types and features of cell clusters in *B. cunicularis* brain.

Neurosecretory cells of brain: The histological study of neurosecretory cells (NSCs) of the brain of the crab revealed different types of cells and accordingly they have been classified into various cell types (Matsumoto, 1954; Durand, 1956; Deshmukh and Rangnekar,1965; Diwan and Nagabhushanam, 1971, 1975). A survey of different types of NSCs in crustaceans has been published by many researchers. Durand (1956) reported 11 types of NSCs in crayfish. Six types of NSCs were observed by Hisano (1976) in freshwater prawn *Palaemon paucidens.* Sutar (2002) observed 5 types of NSCs in freshwater crab *B. guerini.* In the present study, 5 types of NSCs (type A-, B, C, D and E) were observed in the brain of crab *B. cunicularis.* (**Plate-II, Fig.1-8**). Earlier, Diwan and Nagabhushanam (1971, 1975) had reported only 3 types of NSCs (type A-, B and C) from *B. cunicularis.*

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