

ECOMORPHOLOGICAL ADAPTATION OF OLFATORY APPARATUS IN *AMPHIPNUOUS CUCHIA* (HAMILTON, 1822)

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ABSTRACT ■ *Amphipnuous cuchia* (Hamilton, 1822) is a mud-dwelling teleost that leads an amphibious mode of life on freshwater mudflats of South East Asia. This short research contribution is aimed to address the question, 'What are the ecomorphological adaptations of peripheral olfactory apparatus of *A. cuchia* in context to its amphibious mode of life?' The macro- and microanatomy of olfactory apparatus in *A. cuchia* has been studied under the light microscope (LM). Macro- and microanatomical modifications in olfactory apparatus are noted in respect to unilamellar nature of olfactory apparatus, subdivision of ciliated and non-ciliated zones in pseudostratified olfactory neuroepithelium, distinct differentiation of cellular arrangements, etc. These structural modifications in peripheral olfactory apparatus of *A. cuchia* are suggestive of morphological adaptation for its amphibious mode of life.

Key words: *Amphipnuous cuchia*, olfactory, neuroepithelium, pseudostratified, etc.

INTRODUCTION

Fishes are the most diverse group of vertebrates that shows wide range of variation in morphology and life style according to their different habitats (Nelson, 2006). It was suggested from fossil records that the archaic fishes first appeared about 450 million years ago and radiated to become dominant creatures in Devonian period (Kent and Carr, 2001). The olfactory apparatus also seems to be evolved along with the archaic fishes (Taniguchi *et al.*, 2011). The peripheral olfactory apparatus is considered to be an important chemosensory structure of fish which can perceive various types of chemical cues including pollutants from the external

aquatic environment (Hara, 1975). This sense in fish is also significant for eliciting various behavioural responses (*viz.*, feeding, alarming, mating, homing, parental care, etc.) through detection and discrimination of water-soluble chemical odorants during nasal ventilation (Sorensen and Hoye, 2010). The gross morphological variation in olfactory apparatus of different teleosts was first studied by Burne (1909). Notwithstanding that, various questions still remain unexplored. This study is aimed to unfold the ecomorphological modifications in peripheral olfactory apparatus of a common teleostean species that live in air-water interface. *Amphipnuous cuchia* (Hamilton, 1822) is the common mud-

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dwelling freshwater teleost of South East Asia belonging to Order: Synbranchiformes. The present study is emphasized on macro- and microanatomical details on the olfactory apparatus in *A. cuchia* to explore the ecomorphological modifications in view of their amphibious mode life.

MATERIALS AND METHODS

Amphipnous cuchia [Fig. 1A] is a common air-breathing teleost of South East Asia. These species are considered as 'Least concern' according to IUCN Red List Category [website: <http://www.iucnredlist.org/details/166554/0>].

A. cuchia is more abundant in the local markets during the month of October – March in the market of North 24 Parganas, West Bengal, India. Live, adult and sex-independent specimens of *A. cuchia* [Total body length: 30cm. to 40cm.] were collected from the local markets of North 24 Parganas, West Bengal. The specimens were brought to the laboratory and acclimatized with the laboratory conditions for 48hours. The healthy specimens of *A. cuchia* were sorted out and anesthetized by using MS – 222 (dose: 100 mg./ lit. to 200mg./ lit.). The olfactory apparatus of *A. cuchia* were dissected out;

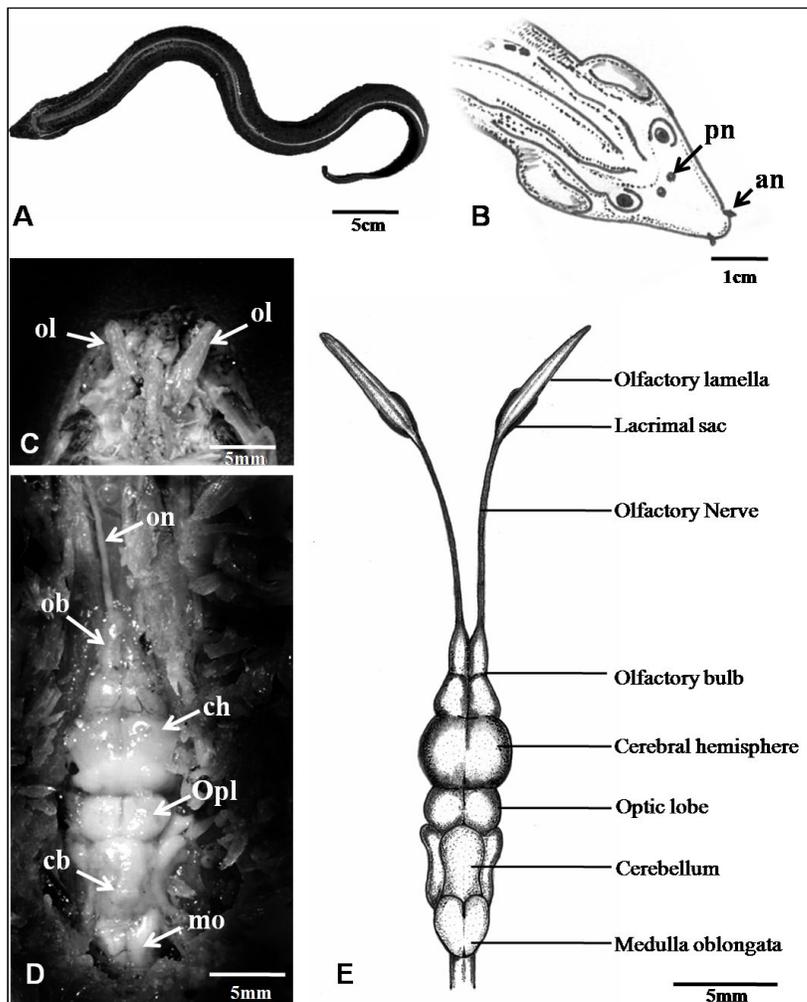


Figure 1: The figure illustrates external morphology and macroanatomy of olfactory apparatus in *Amphipnous cuchia*. **A:** The external morphology of *A. cuchia*. **B:** The diagrammatic representation of anterior and posterior nostrils. **C:** The olfactory apparatus shows single olfactory lamella (ol) at the either side of snout in *A. cuchia*. **D:** The photograph indicates olfactory nerve (on), olfactory bulb (ob), cerebral hemisphere (ch), optic lobe (Opl), cerebellum (cb) and medulla oblongata (mo). **E:** The diagrammatic representation of olfactory apparatus of *A. cuchia*.

immediately fixed in aqueous Bouins solution and examined under binocular light microscope. For microanatomical studies, the dissected olfactory apparatus of *A. cuchia* were separately fixed in 4% paraformaldehyde solution in 0.1 (M) phosphate buffer (pH. 7.2-7.4) for 3 hours at 4°C. The olfactory apparatus were then washed in the same buffer and subsequently cryoprotected in 15% – 30% sucrose solution in 0.1 (M) phosphate buffer for 24 hours at 4°C. The frozen sections (thickness: 15µm - 20µm) were cut by using cryostat (Leica CM 1850; Leica Biosystems Nussloch GmbH, Germany) and carefully placed on gelatinized slides. The sections

were stained by following routine technique of haematoxylin - eosin staining [Kiernan, 2003]; examined under trinocular light microscope (Primo Star; Carl Zeiss Microscopy, GmbH, Germany). The micrographs were analyzed by Axio Vision LE (version 4.3.0.101) (Carl Zeiss Vision, GmbH, Germany)

RESULTS

A. cuchia possess two pairs of nostrils *i.e.*, anterior and posterior nostrils. The anterior nostrils are elongated tube like structure where as the posterior nostrils are oval shaped apertures [Fig. 1B]. The olfactory apparatus of *A. cuchia* are paired and present

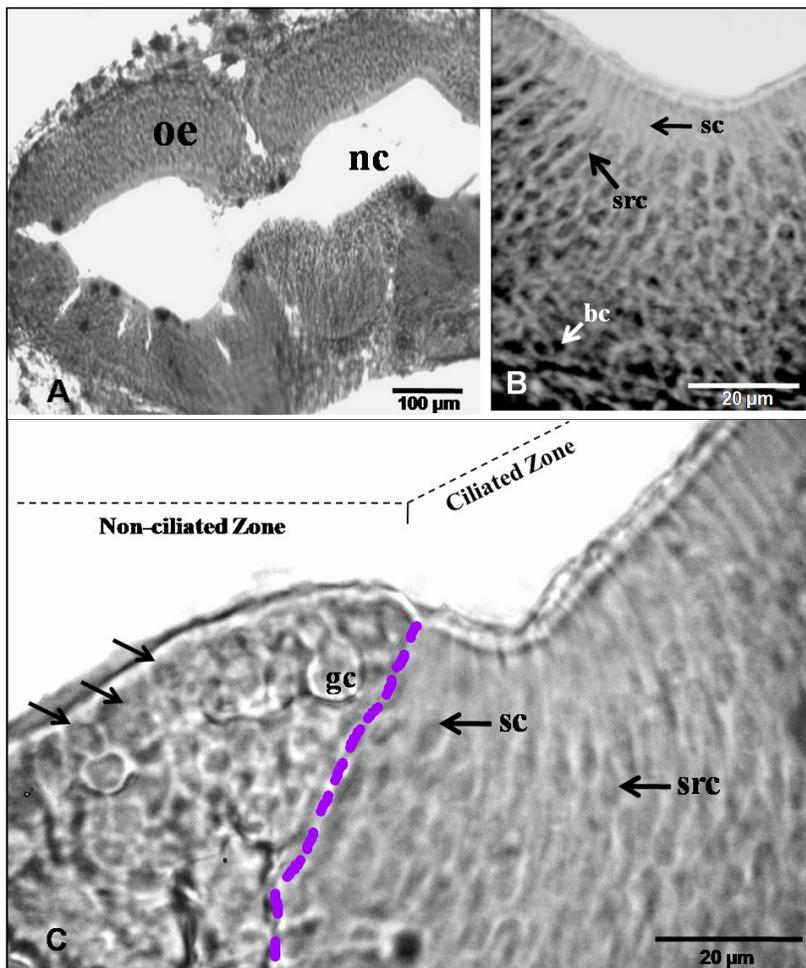


Figure 2: The histology of olfactory neuroepithelium in *A. cuchia*. A: The transverse section of olfactory lamella shows olfactory neuroepithelium (oe) and nasal cavity (nc). B: The photomicrograph indicates pseudostratified olfactory neuroepithelium. The cellular components *viz.*, sensory receptor cell (src), supporting cell (sc) and basal cell (bc) are marked. C: The ciliated and non-ciliated zones of olfactory neuroepithelium are demarcated. Different types of cells are identified [Non-ciliated zone – microvillous cells (arrows), goblet cell (gc); Ciliated zone - sensory receptor cell (src), supporting cell (sc), *etc.*].

in between the nostrils. It is unilamellar in nature [Figs. 1C]. The olfactory apparatus of *A. cuchia* comprises of single olfactory lamella along with lacrimal sac, present on the either side of the elongated snout [Figs. 1C and 1E]. The ethmoidal sac is absent in *A. cuchia*. The olfactory nerves are connected with the distal part of olfactory lamella and associated with olfactory bulb of the brain [Figs. 1D and 1E]. The olfactory lamella in *A. cuchia* is a tube shaped structure [Fig. 1C]. A channel like nasal cavity is present within the tube like olfactory lamella. The diameter of the nasal cavity gradually increases towards the posterior part of the olfactory lamella of *A. cuchia*. The inner part of the olfactory lamella is lined by pseudostratified olfactory neuroepithelium [Figs. 2A and 2B]. This olfactory neuroepithelium in *A. cuchia* is clearly subdivided into two distinct regions *i.e.*, ciliated and non-ciliated zones [Fig. 2C]. The ciliated part of olfactory neuroepithelium in *A. cuchia* includes bipolar sensory receptor cell, ciliated supporting cell and polygonal basal cell whereas the non-ciliated neuroepithelial zone shows distinct presence of goblet cells and columnar microvillous supporting cells [Figs. 2B and 2C].

DISCUSSION

Air breathing fishes are the most important group of teleosts (Nelson, 2006). Many species of air – breathing teleost are considered amphibious as they voluntarily emerged from the aquatic ecosystem (Gordon, 1998). The anatomical organization of olfactory apparatus in fish is different in respect to amphibian species (Parsons, 1967). The olfactory system in amphibia is subdivided into two distinct subsystem of main olfactory epithelium (mOE) and vomeronasal organ

(VNO). Apparently the cellular organization is also variable among the two subsystems of amphibians. The main olfactory epithelium (mOE) is generally consist of ciliated sensory receptor cell but the microvillous sensory receptor cells are distributed within the vomeronasal organ (VNO). It was assumed that the origin of vomeronasal organ is largely related with adaptation of tetrapod with terrestrial environment (Bertmar, 1981). This hypothesis is still a debatable part in the context of origin of vomeronasal organ. In both amphibian and reptiles, the vomeronasal system is involved in foraging behavior (Graves, 1993). In fish, the ciliated and microvillous sensory receptor cells are not separately distributed but present together within the main olfactory neuroepithelium (Hansen *et al.*, 1998; Sarkar and De, 2011). Eisthen (1992) suggested that the microvillar sensory receptor cells in teleost correspond to the tetrapod microvillar vomeronasal cells due to their functional similarities (Hamdani and Døving, 2007). This study highlights a distinct subdivision of ciliated and non-ciliated zone within the olfactory neuroepithelial system of *A. cuchia*. This type of separation is apparently unusual in fish but indicates phylogenetic primitiveness in contrast to amphibia (Taniguchi *et al.*, 2008). Therefore, we assume that this type of subdivision is an evidence of common ecomorphological adaptations in view of convergent evolution in response to their amphibious lifestyle.

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