Experimental Specimen

M. armatus is a fresh water spiny eel bears an elongated body with a long snout. Both the dorsal and anal fins are extended and joined to the caudal fin. The entire length of the body carried irregular dark marking. It is a bentho pelagic species and is distributed throughout the Asian continent. In India it is mainly found in Hooghly and Bhagirathi River systems of West Bengal. It is a very nutritious table food and particularly in West Bengal it used as food fish. This is also found at quite high altitudes. This species is designated as 'Least Concern' position under IUCN Red list category [website: http://www.iucnredlist.org/details/166586/0].

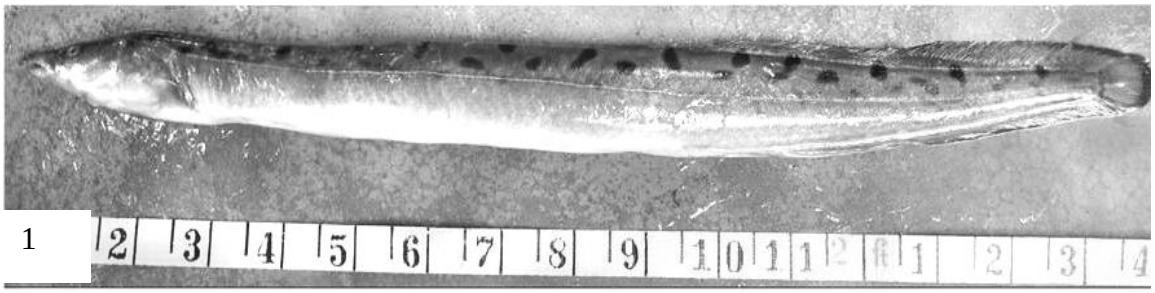

Fig. 1 -The photograph of *Mastacembelus armatus (*Lacepède, 1800*)* - a common spiny eel of South East Asia.

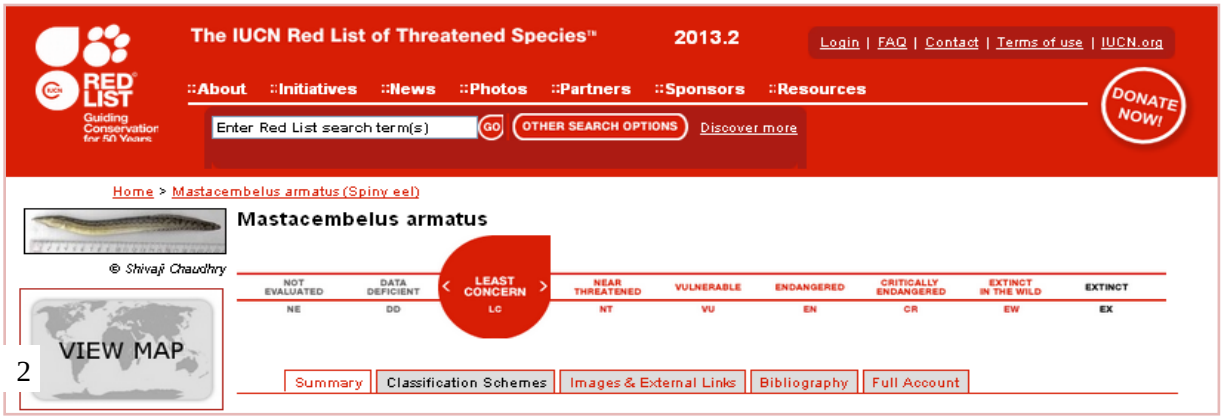

Fig. 2 - In the IUCN Red List category *M. armatus* positioned as 'Least Concern' [version 3.1].

I A) Macroanatomy

Live, sex independent *M. armatus* of variable total body length [19 cm. - 30 cm.] and of variable body weight [25gm. - 40gm.] were collected from the riverine habitat of Paschim Medinipur and Purba Medinipur district, West Bengal, India. The collected specimens were brought to the laboratory and then acclimatized with the laboratory condition for 48 hours. The specimens were anaesthetized using MS 222 [dose: 100-200 mg./lit.]. The olfactory apparatus were then dissected out under stereo zoom light microscope (ZEISS STEMI 508 DOC) and removed from the dorso-lateral surface of the specimen head and immediately fixed in aqueous Bouin's solution. The olfactory apparatus of *M. armatus* was mounted by glycerin on grease free glass slide and examined under light microscope (LM) [LEICA MZ 6].

I B) Scanning Eelectron Microscopical (SEM) Study

Fresh, sex independent *M. armatus* specimens having variable total length approx 19 cm. - 25 cm. and body weight 20 gm.-28 gm. were collected from various riverine habitat of Paschim Medinipur and Purba Medinipur district, West Bengal, India and brought to the laboratory. Specimens were then acclimatized with laboratory condition for 48 hours and anaesthetized by MS 222 (dose: 100-200 mg./lit.). The olfactory apparatus were dissected out from the dorso-lateral part of the head and fixed in 2.5% glutaraldehyde in 0.1 (M) phosphate buffer (pH 7.2-7.4) at 4˚c for 1-2 hours. After completion

of the fixation, the samples were rinsed in same buffer *i.e.*, 0.1 (M) phosphate buffer (pH 7.2-7.4) for 3 changes at a regular interval of 15 minutes. The specimens were then dehydrated in graded chilled acetone. The dehydrated specimens were critically dried (CPD) with liquid carbon dioxide and then carefully placed on aluminum stub and coated with gold. The prepared samples were then examined under scanning electron microscope (SEM) [JEOL JBM-7500F], operated at 15-20 kV at 0˚ tilt angle.

II A) Microanatomy

- **Semi-thin Sectional study**

Live, sex independent *M. armatus* specimens having total body length: 19 cm. – 25 cm. and total body weight: 20gm. – 28gm. were collected from various riverine habitats of Paschim Medinipur and Purba Medinipur District, West Bengal, India and brought to the laboratory for histological work. The specimens were acclimatized with laboratory condition for 48 hours at room temperature. The acclimatized specimens were anaesthetized by MS 222 (dose: 100-200 mg./lit.). The olfactory apparatus is procured from the dorso-lateral side of the specimen head and fixed in 2.5%glutaraldehyde in 0.1(M) phosphate buffer (pH 7.2-7.4) at 4˚C for 1-2 hours. After completion of the primary fixation, the olfactory tissue are rinsed in the same buffer and then secondary fixation was done in 1% OsO4 in 0.1 (M) Phosphate buffer (pH 7.2-7.4) for 1 hours at 22˚C- 25˚C. The fixed olfactory tissues were then

rinsed in same 0.1(M) Phosphate buffer (pH 7.2-7.4). The olfactory specimens were dehydrated in graded chilled acetone. The tissue were then embedded in araldite mixture and incubated for 48hours at 60˚C. Transverse sections (thickness - 1µm) were cut using ultra microtome (LEICA ULTRACUT) and fixed on a glass slide. Semi thin sections were stained with 1% toluidene blue. The stained transverse sections of olfactory lamellae of *M. armatus* were examined under trinocular light microscope (PRIMO STAR; CARL ZEISS MICROSCOPY, GMBH, GERMANY).

- **Cryosectional study**

Live, sex independent *M. armatus* specimens having total body length: 19cm. – 25 cm. and total body weight: 20gm. – 28gm. were collected from the various riverine habitats of Paschim Medinipur and Purba Medinipur District, West Bengal, India and brought to the laboratory. The collected specimens were acclimatized with laboratory condition for 48 hours at room temperature. For specimens anaesthetized purpose we used MS 222 (dose: 100-200 mg./lit.).The olfactory apparatus of *M. armatus* were fixed in 4% paraformaldehyde in 0.1 (M) phosphate buffer (pH 7.2-7.4) at 4°C for 2 hours. The fixed tissues were then washed in the same buffer with 3 changes at 30 minutes of interval. Washed tissue were cryoprotected in 15% - 30% sucrose solution in 0.1 (M) phosphate buffers at 4°C for 4 hours and

overnight respectively. The cryoprotected tissues were then sectioned by using cryostat (LEICA CM 1850); operated at -19°C to – 22°C having thickness: 3µm – 5µm and placed carefully on clean gelatin coated glass slides. Prepared slides were stained using Haematoxylin - Eosin (HE) and dehydrated through graded ethanol. Stained sections were mounted by DPX and viewed under trinocular light microscope [PRIMO STAR; CARL ZEISS MICROSCOPY, GMBH, GERMANY].

II B) Transmission Electron Microscopical (TEM) Study

Live, sex independent *M. armatus* specimens of different body length approx 20 cm. - 25 cm. and variable body weight approx 20 gm.- 28 gm. were collected from various riverine habitats of Paschim Medinipur and Purba Medinipur District, West Bengal, India and brought to the laboratory. The specimens were acclimatized with the laboratory condition for 48 hours and anaesthetized with MS 222 (dose-100-200 mg./lit.). The olfactory apparatus were procured from the dorso-lateral part of fish head by dissecting the olfactory chambers. The olfactory apparatus was fixed in 2.5% glutaraldyde in 0.1(M) phosphate buffer (pH 7.2-7.4) at 4˚C for 1-2 hours. After completion of primary fixation, the olfactory tissues were rinsed (3 to 4 changes) in same buffer. Then the olfactory tissues were fixed in 1% OsO4 in 0.1(M) phosphate buffer (3 changes at 5 minutes of interval). The olfactory

tissues were dehydrated with graded and chilled ethanol. The dehydrated tissue were then embedded in araldite solution and incubated at 60˚C for 48-72 hours. Transverse ultrathin sections (70 nm - 90 nm) were cut with the help of ultramicrotome (Leica Ultracut) and collected on copper grid; stained with uranyl acetate solution [2.5 gm. of uranyl acetate was added to 50 ml of distilled water. Bottle was covered with foil and stir overnight. Then 10 drops of glacial acetic acid was added and stored in 4˚C] and lead acetate [1.33gm. of lead nitrate was added to 30 ml. distilled water then 1.76 gm. of dehydrated sodium citrate was added and the solution became cloudy. Then 5 ml of 1(N) NaOH was added and stir for 10 minutes, then the solution becomes clear. Additional 15ml of distilled water was added]. The ultrathin sections were observed under Transmission Electron Microscope (TEM: TECNAI) operated at 40 kV.

III A) Fluorescence Microscopical (FM) Study

Live, sex independent *M. armatus* specimens having total body length: 19cm. – 25 cm. and total body weight: 20gm. – 28gm. were collected from the various riverine habitats of Paschim Medinipur and Purba Medinipur District, West Bengal, India and brought to the laboratory. The collected specimens were acclimatized with laboratory condition for 48 hours at room temperature. The acclimatized specimens were anaesthetized by MS 222

(dose: 100-200 mg./lit.).The olfactory apparatus of *M. armatus* were fixed in 4% paraformaldehyde in 0.1 (M) phosphate buffer (pH 7.2-7.4) at 4°C for 2 hours. The fixed tissues were then washed in the same buffer with 3 changes at 30 minutes of interval. Washed tissue were cryoprotected in 15% - 30% sucrose solution in 0.1 (M) phosphate buffers at 4°C for 4 hours and overnight respectively. The cryoprotected tissues were then sectioned by using cryostat (Leica CM 1850); operated at -19°C to – 22°C having thickness: 3µm – 5µm and placed carefully on clean gelatin coated glass slides. Staining was performed using the following fluorochrome staining procedure.

a) Acridine Orange used as Fluorochrome

Cryo protected tissue containing slides were stained by using Acridine Orange [100 µl Acridine Orange in 0.1 (M) Phosphate Buffer] at 4 ºC for 15 minutes. Stained slides were mounted by glycerine and viewed under Fluorescence Microscope [CARL ZEISS, AXIO SCOPE A1] at an excitement of 520 nm. – 580 nm.

b) $G\alpha_{olf}$ antisera conjugated with Alexafluor 488 Fluorochrome

Olfactory tissues were dissected out from the antero-dorsal side of the head. The dissected tissues were incubated for 30min with Ultra Cruz Blocking Reagent (sc-516214) at normal room temperature. Then tissues were rinsed

in 0.1 (M) PBS of three changes for 5mins each. After completion of washing tissue sections (10 µm. - 14 µm.) were prepared with the help of cryomicrotome (LEICA CM 1850) and were collected on gelatin coated glass slide. The slides were incubated with primary antibody conjugated to Alexa Fluor ($G\alpha_{olf}$ conjugated with Alexa fluor 488) for 90minutes at room temperature. Slides were washed again in 0.1 (M) PBS with three changes for 5min each. Stained sections were immediately mounted by cover slip with hard-set mounting medium and observed under fluorescent microscope at an excitement of 499 nm. to 520 nm.[CARL ZEISS, AXIO SCOPE A1].

III B) Transmission Electron Microscopical (TEM) Study attached with EDX

Live, sex independent *M. armatus* specimens of different body length approx 20 cm. - 25 cm. and variable body weight approx 20gm.-28gm. were collected from various riverine habitats of Paschim Medinipur and Purba Medinipur District, West Bengal, India and brought to the laboratory. The specimens were acclimatized with the laboratory condition for 48 hours and anaesthetized with MS 222 (dose-100-200 mg./lit.). The olfactory apparatus were dissected out from the dorso-lateral side of the fish head and immediately fixed in 2.5% glutaraldyde in 0.1(M) phosphate buffer (pH 7.2-7.4) at 4˚C for 4-6 hours. After completion of primary fixation, the olfactory tissues were rinsed in same buffer for 3 to 4 changes at 15 minutes interval.

The olfactory tissues were then fixed in 1% OsO4 in 0.1(M) phosphate buffer (3 changes at 5 minutes of interval) at 4˚C. The olfactory tissues were dehydrated with graded chilled ethanol for 30 minutes each at 4˚C and then again dry acetone for 1 hour at room temperature (2 changes). The dehydrated tissues were then embedded in araldite solution and incubated at 60˚C for 48-72 hours. Araldite embedded tissues were sectioned having thickness (70 nm. - 90 nm.) using ultramicrotome (Leica Ultracut) and collected on aluminum grid. The sections containing grids were observed for variable metal analysis under transmission electron microscope with EDX (Energy Dispersive X-ray microanalyser) attachment [JEM – 2100 HRTEM, JEOL, JAPAN] at 0^{o} tilt angle, operated at 40kV.