

# **Report Submitted to DBT**

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## **Studies on Indian cone snails**



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## **Introduction:**

The predatory cone snails are neogastropod molluscs of the genus *Conus*. The Genus *Conus* includes more than 500 species. These cone snails are venomous which inject venom to paralyse and capture prey. The major constituent of the venom is a complex mixture of peptides called 'conus peptides' or 'conopeptides', which are rich in disulphide bonds. The venom of each *conus* species contains about 100 different conopeptides with little overlap between the species. Each peptide is selectively targeted to specific voltage and ligand gated ion channels and G protein linked receptors. The conus peptides are grouped into several super families with members sharing both a highly conserved signal sequence in their precursors and a characteristic arrangement of cysteine residues in their mature peptides.

Members of one super family have had a disulphide framework that is distinct from those of other superfamilies. Presently peptides from 4 super families A, M, O, E have been reported. Many of the *conus* peptides are post translationally modified which include bromination of tryptophan, C terminal amidation, isomerisation of L to D amino acid, hydroxylation of proline,  $\gamma$  carboxylation of glutamyl residues, sulfation of tyrosine residues, glycosylation of serine and threonine residues. Several peptides of these cone snails have become important pharmacological tools for neuropharmacological scientists were originally isolated on the basis of behavioural symtomatologies in animal models. There are examples of peptides such as sleeper peptides, (conantokins), shaker peptides ( $\omega$  conotoxin) which have been characterized and named on the basis of behavioural symtomatology.

The past 6 months I am trying to study the molecular phylogeny of Indian cone snails. Molecular phylogeny of Indian cone snails is being carried out by collaboration with Dr. Uma Ramakrishnan, NCBS.

## **Materials and Methods:**

Cone snails were collected from South and west coast of India especially from Tuticorin, Rameshwaram, Mandapam, and Porto-Novo coastal waters. The collected



cone snails were identified by using standard identification keys (Ref. Manual of the living Conidae volume 1. Indopacific by D. Rockel, W. Korn & A. Kohn).

## **Molecular Phylogeny of *Conus***

### **Background information:**

Molecular phylogeny is the use of the structure of molecules to gain information on an organism's evolutionary relationships. The result of a molecular phylogenetic analysis is expressed in a so-called phylogenetic tree. Among the 500 species of the gastropod *Conus*, many have specialized diets, preying on particular taxa such as polychaetes, molluscs, hemichordates or fishes (Kohn 1959, Reichelt & Kohn 1985). *Conus* uses a venom that contains a cocktail of neurotoxic peptides, termed conotoxins, to stun prey by blocking muscle and neuronal ion channels and receptors (Endean & Rudkin 1965; Olivera *et al* 1991), and rapid immobilization of prey is essential to successful capture. Previous analyses of conotoxin gene-family evolution have shown that conotoxin loci have evolved adaptively in molluscivorous and vermivorous *Conus* species (Duda & Palumbi 2000) and that the expression of different gene combinations fine-tunes venom composition among related species (Duda & Palumbi 2000). Such patterns suggest that selection operates to develop and maintain a venom that is most effective against particular prey.

In this present study, we have collected the conus samples from different coasts such as , Tamil Nadu, Kerala, Maharastra etc., and preserved in 100% alcohol for further isolation and analysis of DNA. Even though more than 75 species of cone snails present in Indian coast the molecular phylogeny of cone snails not yet studied by any group. So this present investigation has been carried out to analyse the evolution (feeding habits), species variation in Indian cone snails.

### **Materials and Methods:**

*Conus* samples were collected from different sites of Indian coast. They were preserved in 100% alcohol. DNA was extracted from the tissue by using Qiagen DNeasy Tissue Kit (Cat. No.69504). Mitochondrial Cytochrome Oxidase B gene was amplified by polymerase chain reaction (PCR) using the primers COI (Folmer *et al* 1994), 16s rRNA (Espiritu *et al* 2001) and Calmodulin (Duda and Paulumbi 1999). PCR reactions



were performed using standard buffer conditions, 2.5 mM MgCl<sub>2</sub>, and 30 cycles of the following steps: 94°C for 20s, 51°C for 25s, and 72°C for 50sec, preceded by 5 min of initial denaturing, and followed by 10 min of final extension. PCR products were purified using Shrimp Alkaline Phosphatase and Exonuclease I Treatment (Werle *et al.*, 1994) and cycle sequenced from both ends using ABI- Big Dye Terminator kits; the resulting sequences were analysed in ABI 3100xl automated sequencer.

#### **Cloning of Conus DNA for sequencing:**

DNA is cloned into E.coli by using TOPO cloning kit **TOPO TA Cloning® Kit** (K4500-40) and then the transformed colonies picked up for colony PCR. After that the PCR product is purified by gel elution (Qiagen gel elution kit cat no; 28704). Then sequence by using,

M13F (-20) GTAAAACGACGGCCAGT

M13R GCGGATAACAATTCACACAGG

#### **Results:**

We have completed the DNA sequence of at least 30 species of cone snail species (both COI, 16s rRNA). The sequence of conus COI and 16s rRNA and the phylogenetic tree has given.

#### **NCBS marine station at Mandapam:**

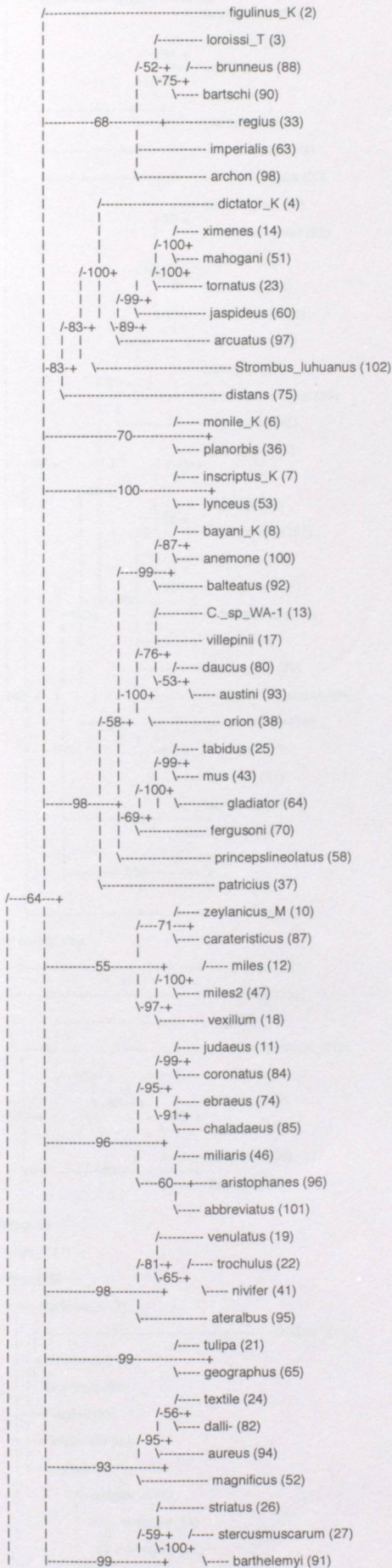
We have set up NCBS marine station at Mandapam to maintain the live cone snails and study the feeding behavior of Indian cone snails.

The photos are give in the slides.

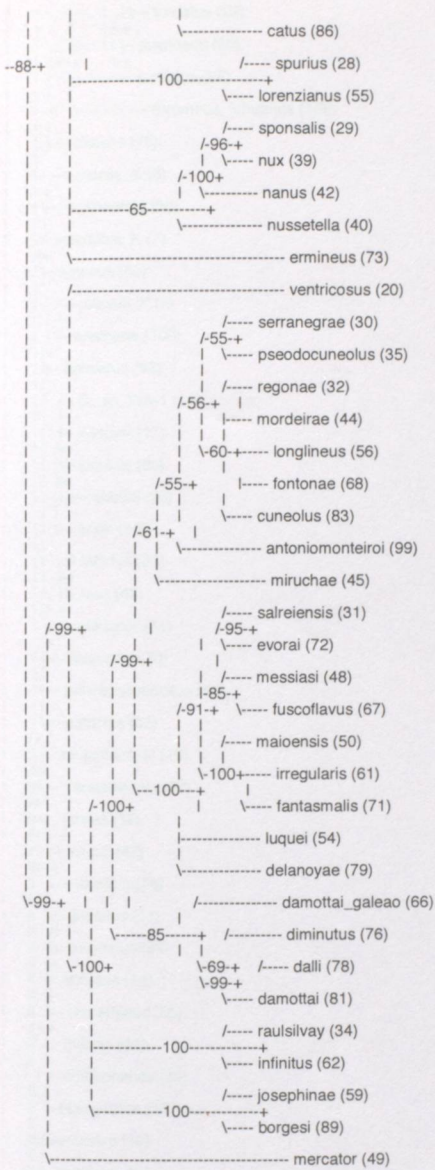


Clade credibility values:

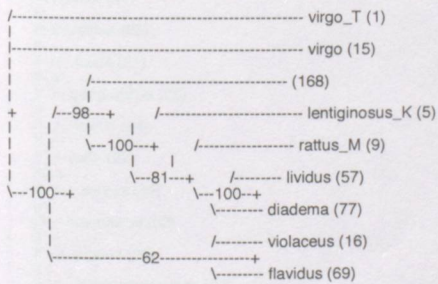
Subtree rooted at node 168:



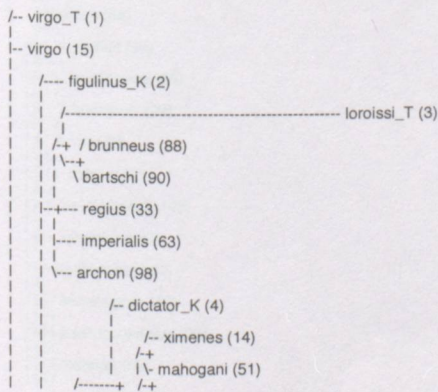




Root part of tree:



Phylogram:





| | | | | \-- tornatus (23)  
| | | | | /+  
| | | | | \-- jaspideus (60)  
| /--+ \+  
| | | | | \-- arcuatus (97)  
| | | | |  
| +-+ \----- Strombus\_luhuanus (102)  
| | | | |  
| \-- distans (75)  
| | | | |  
| /---- monile\_K (6)  
| +-+  
| \----- planorbis (36)  
| | | | |  
| /- inscriptus\_K (7)  
| +-+  
| \-- lynceus (53)  
| | | | |  
| /---- bayani\_K (8)  
| /+  
| \-- anemone (100)  
| /+  
| \-- balteatus (92)  
| | | | |  
| /- C.\_sp\_WA-1 (13)  
| | | | |  
| \-- villepini (17)  
| /+  
| \-- daucus (80)  
| \+  
| /--+ \-- austini (93)  
| | | | |  
| \-- orion (38)  
| | | | |  
| /- tabidus (25)  
| /+  
| \-- mus (43)  
| /--+  
| /+ \- gladiator (64)  
| /+  
| \-- fergusonii (70)  
| | | | |  
| \-- princepslineolatus (58)  
| | | | |  
| \-- patricius (37)  
| /+  
| \-- zeylanicus\_M (10)  
| /+  
| \-- carateristicus (87)  
| | | | |  
| /+ / miles (12)  
| /+  
| \ \ miles2 (47)  
| /--+  
| \-- vexillum (18)  
| | | | |  
| /- judaeus (11)  
| /+  
| \- coronatus (84)  
| /+  
| \- ebraeus (74)  
| | | | |  
| \-- chaladaeus (85)  
| /--+  
| \- miliaris (46)  
| | | | |  
| \- aristophanes (96)  
| | | | |  
| \- abbreviatus (101)  
| | | | |  
| / venulatus (19)  
| | | | |  
| /+ trochulus (22)  
| | | | |  
| /+ \- nivifer (41)  
| | | | |  
| \- ateralbus (95)  
| | | | |  
| /-- tulipa (21)  
| /--+  
| \- geographus (65)  
| | | | |  
| /- textile (24)  
| | | | |  
| \- dalli- (82)  
| /--+  
| \-- aureus (94)  
| /+  
| \-- magnificus (52)  
| | | | |  
| /-- striatus (26)  
| | | | |  
| /- stercusmuscarum (27)  
| /+  
| /+ \- barthelemyi (91)  
| | | | |  
| \- catus (86)  
| | | | |  
| /+ / spurius (28)  
| /--+  
| \- lorenzianus (55)  
| | | | |  
| /- sponsalis (29)  
| /+  
| \- nux (39)  
| /+  
| \- nanus (42)  
| /+  
| \----- nussetella (40)  
| | | | |  
| \-- ermineus (73)  
| | | | |  
| /- ventricosus (20)  
| | | | |  
| / serranegrae (30)  
| | | | |  
| \- pseodocuneolus (35)  
| | | | |  
| \- regonae (32)  
| | | | |



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| | | | mordeirae (44)
| | | |
| | | | longlineus (56)
| | | |
| | | | fontonae (68)
| | | |
| | | | cuneolus (83)
| | | |
| | | | /+
| | | | | antoniomonteiroi (99)
| | | | |
| | | | | \ miruchae (45)
| | | | |
| | | | | / salreiensis (31)
| | | | | | /+
| | | | | | /+
| | | | | | | evorai (72)
| | | | | | |
| | | | | | | messiasi (48)
| | | | | | |
| | | | | | | fuscoflavus (67)
| | | | | | |
| | | | | | | maioensis (50)
| | | | | | |
| | | | | | | irregularis (61)
| | | | | | |
| | | | | | | +
| | | | | | | | fantasmalis (71)
| | | | | | | |
| | | | | | | | luquei (54)
| | | | | | | |
| | | | | | | | \ delanoyae (79)
| | | | | | | |
| | | | | | | | | /+ \ damottai_galeao (66)
| | | | | | | | |
| | | | | | | | | \ diminutus (76)
| | | | | | | | |
| | | | | | | | | /+ \ dalli (78)
| | | | | | | | |
| | | | | | | | | \+ \ damottai (81)
| | | | | | | | |
| | | | | | | | | | / raulsilvay (34)
| | | | | | | | | |
| | | | | | | | | | \+
| | | | | | | | | | \ \ infinitus (62)
| | | | | | | | | |
| | | | | | | | | | | / josephinae (59)
| | | | | | | | | | |
| | | | | | | | | | | \+
| | | | | | | | | | | \ borgesii (89)
| | | | | | | | | | |
| | | | | | | | | | | \ mercator (49)
| | | | | | | | | | |
| | | | | | | | | | | /--- \ lentiginosus_K (5)
| | | | | | | | | | |
| | | | | | | | | | | | /+--- \ rattus_M (9)
| | | | | | | | | | | |
| | | | | | | | | | | | \+ / lividus (57)
| | | | | | | | | | | | \+
| | | | | | | | | | | | \ diadema (77)
| | | | | | | | | | | |
| | | | | | | | | | | | /--- \ violaceus (16)
| | | | | | | | | | | | \+
| | | | | | | | | | | | \--- \ flavidus (69)

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|-----| 0.500 expected changes per site

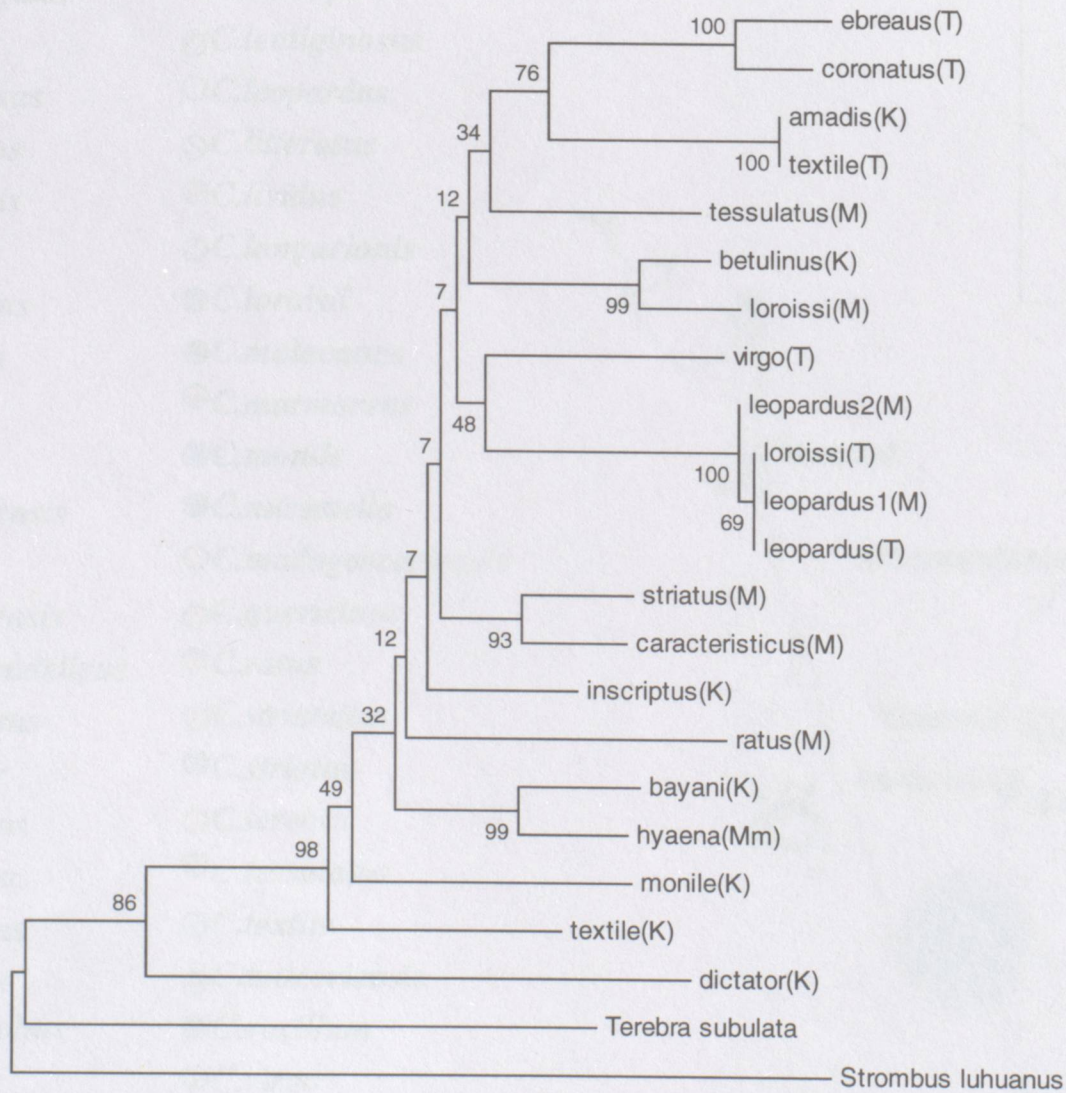
Calculating tree probabilities...

Credible sets of trees (16002 trees sampled):  
50 % credible set contains 8002 trees  
90 % credible set contains 14402 trees  
95 % credible set contains 15202 trees  
99 % credible set contains 15842 trees

MrBayes >



# Phylogenetic tree of Indian cone snails (COI gene)

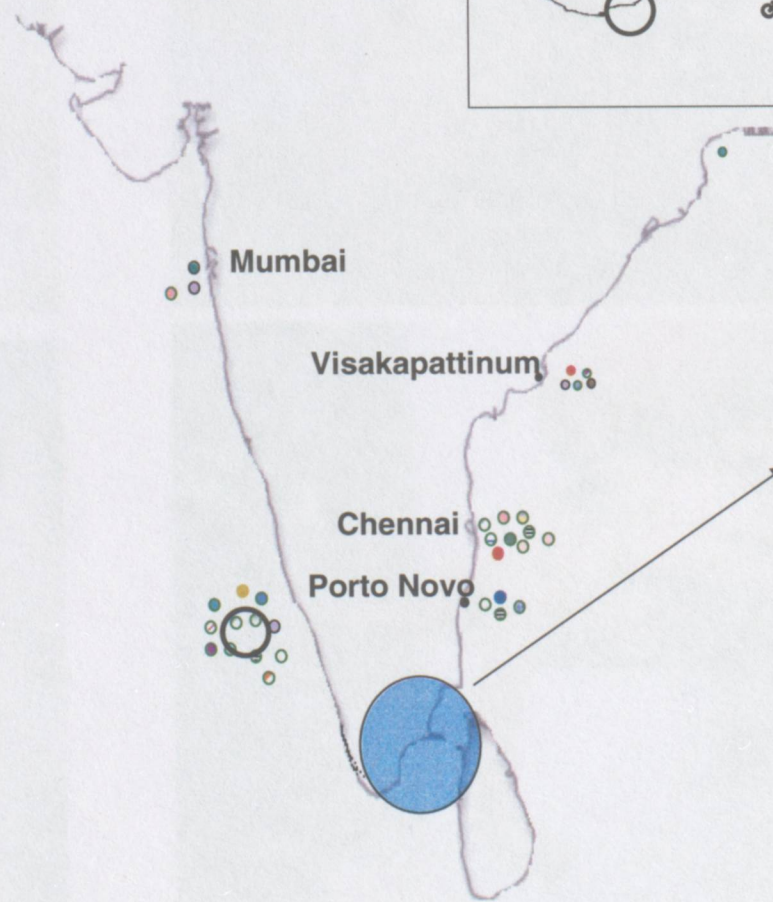
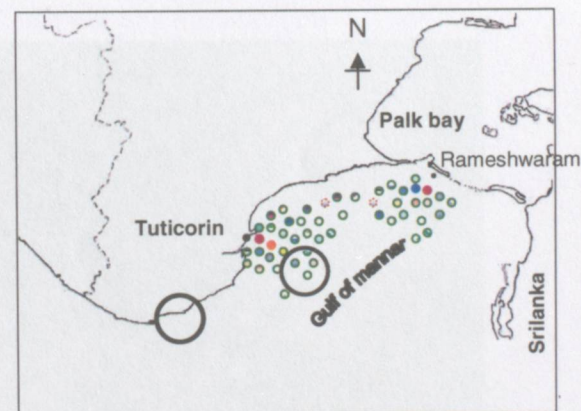


0.01



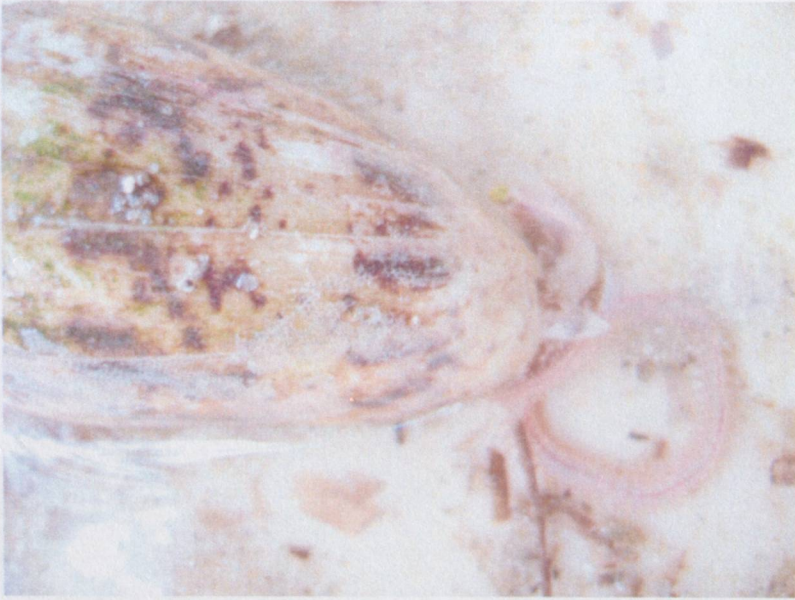
## Distribution of cone snails

- |                           |                             |
|---------------------------|-----------------------------|
| ● <i>C.achatinus</i>      | ○ <i>C.elegans</i>          |
| ● <i>C.aculeiformis</i>   | ○ <i>C.gubernator</i>       |
| ● <i>C.acutangular</i>    | ● <i>C.inscriptus</i>       |
| ● <i>C.amadis</i>         | ○ <i>C.lentiginosus</i>     |
| ● <i>C.araneosus</i>      | ○ <i>C.leopardus</i>        |
| ● <i>C.arenatus</i>       | ○ <i>C.litteratus</i>       |
| ● <i>C.australis</i>      | ○ <i>C.lividus</i>          |
| ● <i>C.bayani</i>         | ○ <i>C.longurionis</i>      |
| ● <i>C.betulinus</i>      | ● <i>C.loroisii</i>         |
| ● <i>C.biliosus</i>       | ● <i>C.malacanus</i>        |
| ● <i>C.boschi</i>         | ○ <i>C.marmoreus</i>        |
| ● <i>C.augur</i>          | ○ <i>C.monile</i>           |
| ● <i>C.bengalensis</i>    | ● <i>C.nussatella</i>       |
| ● <i>C.catus</i>          | ○ <i>C.madagascariensis</i> |
| ○ <i>C.ceylanensis</i>    | ○ <i>C.quericinus</i>       |
| ● <i>C.coromendelicus</i> | ● <i>C.ratus</i>            |
| ○ <i>C.coronatus</i>      | ○ <i>C.striatellus</i>      |
| ○ <i>C.dictator</i>       | ○ <i>C.striatus</i>         |
| ● <i>C.ebraeus</i>        | ○ <i>C.terebra</i>          |
| ○ <i>C.ebruneus</i>       | ○ <i>C.tessulatus</i>       |
| ● <i>C.figulinus</i>      | ○ <i>C.textile</i>          |
| ● <i>C.frigidus</i>       | ○ <i>C.tuticoriensis</i>    |
| ○ <i>C.geographus</i>     | ● <i>C.vexillum</i>         |
|                           | ○ <i>C.virgo</i>            |
|                           | ○ <i>C.zeylanicus</i>       |



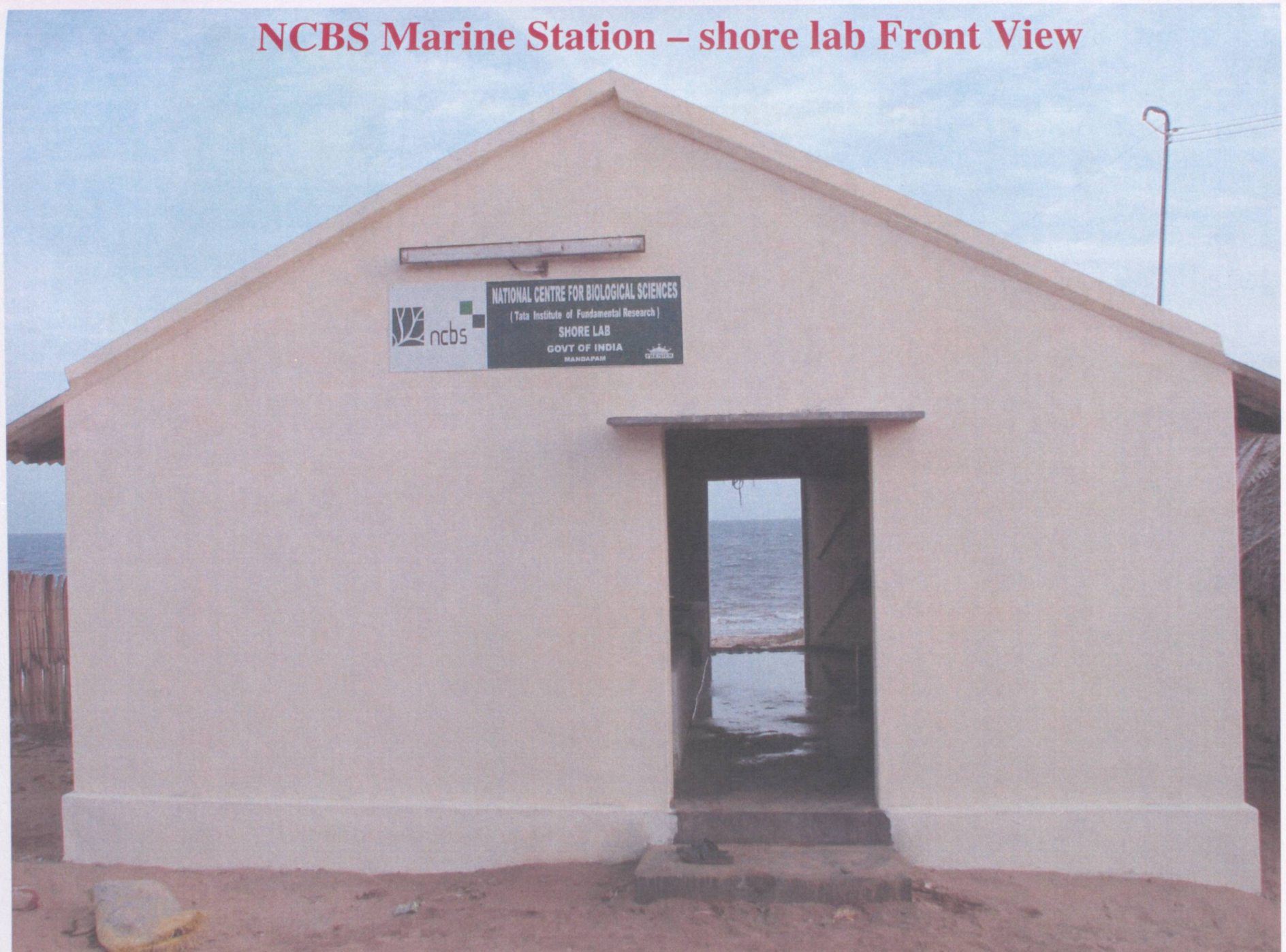


*Conus zeylanicus* feeding worm





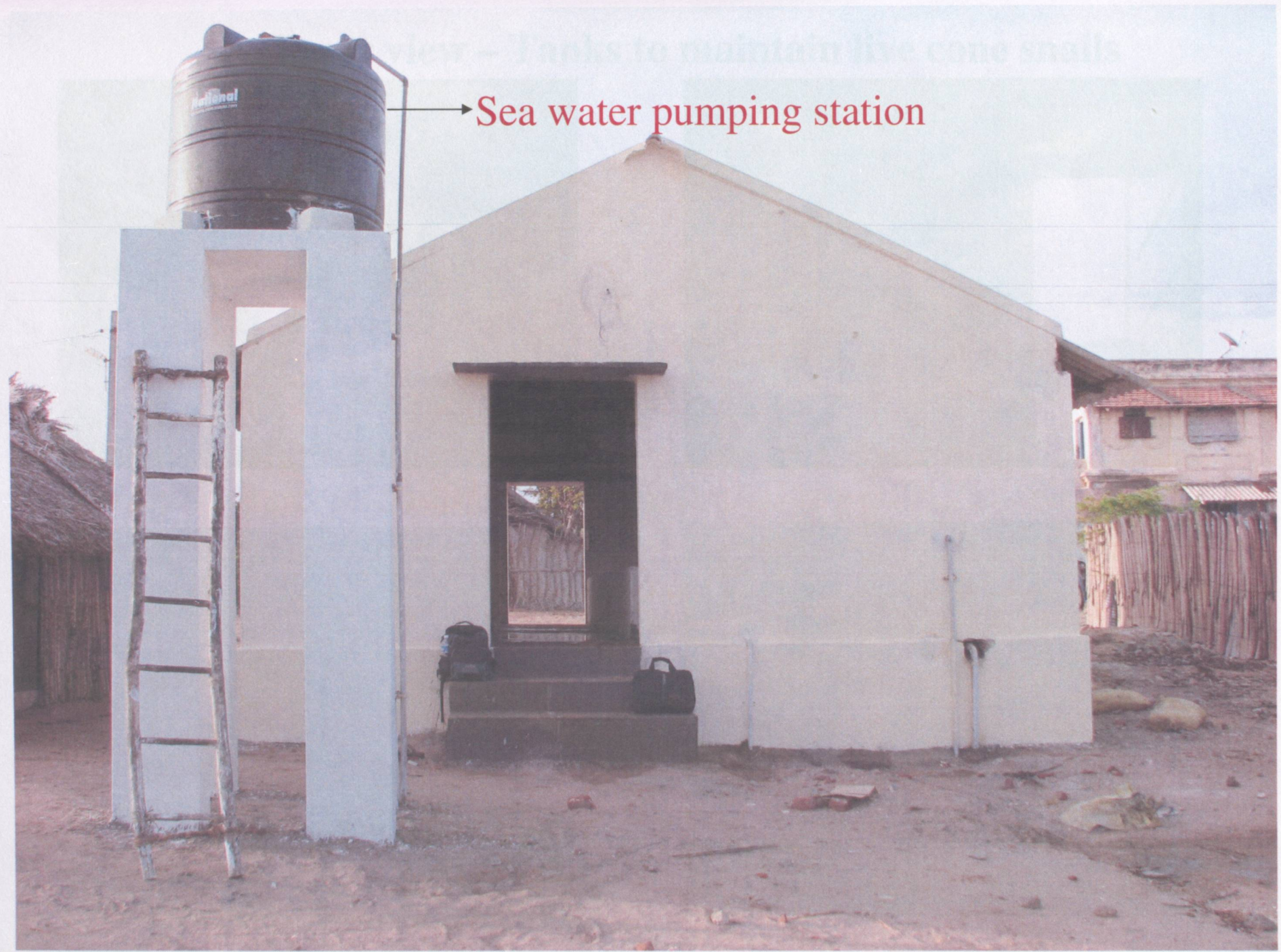
## NCBS Marine Station – shore lab Front View





Water - Tanks to maintain live cone snails

→ Sea water pumping station





## Inner view – Tanks to maintain live cone snails

