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Molluscs and Biotechnology

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Animals of the phylum Mollusca occupy every trophic level in the marine environment. Three classes of Mollusca are of fisheries interest, namely, Gastropoda, Bivalvia and Cephalopoda. The exploitation of these shelled animals in India, range from the very old and historic records of exploitation of pearl oysters and chanks to the very modern trawling for cephalopods. Available production statistics reveals that annually more than 1,00,000 tonnes of cephalopods are harvested from the Indian seas, besides another 1,00,000 tonnes of gastropods and bivalves. The economic importance of cephalopods to the country is considerable as they earn more than Rs. 1400 crores (more than 16% in terms of quantity and more than 15% in terms of value of total marine products exports) every year through exports. In fact, every second cephalopod consumed by Europeans originates from India.

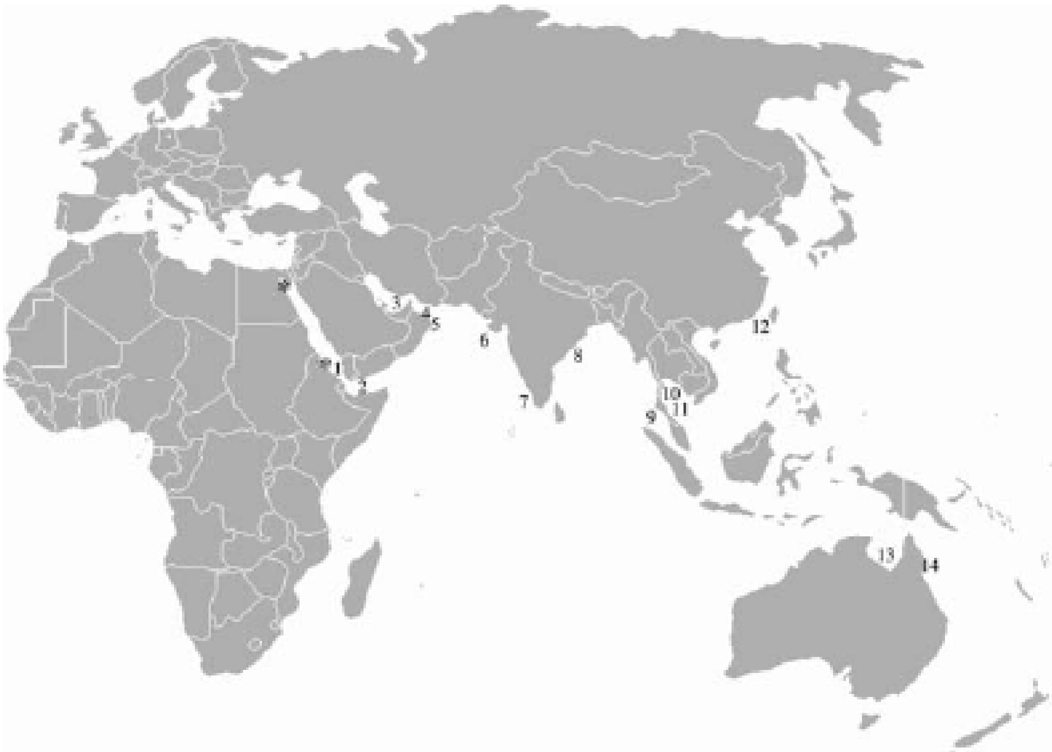
Research on molluscs in the country is more than a century old. However, it is only recently that biotechnology as a tool has found many applications to deepen our understanding of mollusc populations and their biology. In CMFRI, the Molluscan Fisheries Division has been collaborating with the Marine Biotechnology Division of the institute in the following areas:

- Resolving taxonomic ambiguities through molecular taxonomy – Case of *Sepia pharaonis*
- Disease diagnosis – Case of protozoan parasite *Perkinsus olseni* in pearl oyster and other bivalves
- Accurate predictions of spatfall of oyster and mussels in bivalve farming through PCR based identification of specific bivalve larvae from a plankton cocktail

Phylogeography of the pharaoh cuttle *Sepia pharaonis* based on partial mitochondrial 16S sequence data

The pharaoh cuttle *Sepia pharaonis* Ehrenberg, 1831 (Mollusca: Cephalopoda: Sepiida) is a broadly distributed species of substantial fisheries importance found from east Africa to southern Japan. Little is known about *S. pharaonis* phylogeography, but evidence from morphology and reproductive biology suggests that *Sepia pharaonis* is actually a complex of at least three species. To evaluate this possibility, we collected tissue samples from *Sepia pharaonis* from throughout its range. Phylogenetic analyses of partial mitochondrial 16S sequences from these samples reveal five distinct clades: a Gulf of Aden/Red Sea clade, a northern Australia clade, a Persian Gulf/Arabian Sea clade, a western Pacific clade (Gulf of Thailand and Taiwan) and an India/Andaman Sea clade.

Phylogenetic analyses including several *Sepia* species show that *S. pharaonis sensu lato* may not be monophyletic. It was suggested that "*S. pharaonis*" may consist of up to five species, but additional data will be required to fully clarify relationships within the *S. pharaonis* complex.



Map showing the type localities for Sepia pharaonis and sampling localities for the study: 1 = Red Sea (RS), 2 = Gulf of Aden (GofA), 3 = Persian Gulf (PG), 4 = Arabian Sea (AS), 5 = Gulf of Oman (GofO), 6 = Veraval (VRL), 7 = Kochi (CFH), 8 = Vishakapatanam (VSK), 9 = Phuket (PH), 10 = Prachuap (PR), 11 = Chumphon (CHU), 12 = Taiwan (TAI), 13 = Gulf of Carpenteria (GofC), 14 = Northeast Queensland (NEQ)

Diagnosis and specific identification of pathogens in Pearl oyster

The pearl oyster, *Pinctada fucata* (Gould), is a commercially important bivalve distributed in the Gulf of Mannar along the southeast coast of India and had supported a healthy, traditional pearl fishery until the 1950s. But, during the past few decades, the natural pearl oyster beds in the Gulf of Mannar have showed a sharp decline leading to the closure of the traditional pearl fishery and was presumed to be due to overexploitation and pollution. No major disease/pathogens were reported from molluscs from the Indian subcontinent and the pathogen profile of *P. fucata* from the region was not known. A screening of pearl oyster samples revealed heavy infections with the protozoan parasite *Perkinsus olseni*,in the population and this turned out to be the first report of P. olseni, an OIE listed pathogen in the wild and cultured *P. fucata* populations from the Indian subcontinent. PCR Screening of the tissues using the *Perkinsus* genus specific internal transcribed spacer (ITS) 85 and ITS 750 primers, amplified the product specific to the genus *Perkinsus* (ca. 700 base pairs) and further, the specific identity of the parasite was determined by sequencing the amplified PCR products and was confirmed as *Perkinsus olseni*. This preliminary investigation suggests a possibility that perkinsosis could be one of the major reasons for the decline of the *P. fucata* beds in the Gulf of Mannar over a period of time.

Accurate predictions of spatfall of oyster and mussels for farming

In oyster and mussel farming knowledge of the time of spatfall is very important for farmers to decide on the time for setting spat collectors. This is particularly important when the current farming practice is wholly dependent on natural spat as seed. Through a project funded by the DBT, the CMFRI has achieved preliminary success in developing a PCR based protocol for identification of mussel and oyster larvae from a cocktail mix of various holo and mero plankters (as found in a plankton collection).

New areas in mollusc biotechnology

Because of the unique life history patterns of many molluscs, they face a wide range of environmental, behavioural and metabolic challenges and these have been met with unusual chemical strategies. Examples include metal accumulation, adhesion, chemical defences, novel amino acids, structural materials, luminescence, pigments, novel proteins and peptides, immune responses, plus a wide range of unusual secondary metabolites of unknown properties.

1. Metal Accumulation

Many bivalves are able to sequester heavy metal ions from their environment, either using metal binding proteins such as metallothioneins, specific metal binding proteins, or by sequestering the metal to structural components such as shell or connective tissue. Accumulation of lead, cadmium, and copper, by binding to metallothioneins appears to be quite common whilst zinc accumulation, at least in sea urchins, seems to be due to a distinct protein. Accumulation of heavy metals can occur in the shells of molluscs and may be a form of decontamination of the immediate environment of the organism.

2. Adhesion

Many invertebrates rely on adhesion for stability, for example marine molluscs attached to rocks. Bioadhesives can act both as lubricants and adhesives, as in the case of mollucsan slime, which may for example facilitate the movement of a slug over dry ground, whilst also maintaining its position on smooth vertical surfaces. Adhesion is usually accomplished by means of proteins with varying amounts of carbohydrate depending on the function. Limpets for example produce two kinds of mucous; adhesive and non-adhesive, the former containing higher amounts of carbohydrate and each mucous having different proteins. The adhesive protein of the mussels facilitates the very strong adhesion of the animal to rocky intertidal zones where adhesion both in wet and dry conditions is required. The protein with 20% lysine residues is highly basic in nature and consists of repetitive sequences, reminiscent of structural proteins, and is rich in hydroxyproline and dihydroxyphenylalanine.

3. Biochromes, Fluorescence & Bioluminescence

Many marine organisms are a rich source of biochromes including carotenoids, chromans, flavonoids, porphyrins, melanins, phenoxaxines, purines, pteridines, isoalloxazines and quinones, as well as biochromic proteins. Aside from visual and primary metabolic functions these biochromes function as chemical protectants against enemies and the environment, and as bioluminescent agents. Luminescence and fluorescence are distributed throughout the invertebrata. The calcium-dependent fluorescent protein aequorin from the jellyfish *Aequoria victoria* been used in research for many years as an indicator of intracellular calcium movements and more recently since the isolation and characterization of the aequorin gene, has become known as GFP (green fluorescent protein) and has been expressed in fungi, bacteria and plants as a useful marker gene. GFP is a fluorescent protein which emits green light upon excitation with ultraviolet to blue light. Same form of luminescence or fluorescence can be found in molluscs such as limpets, nudibranchs, clams, squid and octopods. There are no luminous flowering plants, birds, reptiles, amphibians or mammals, so invertebrates have this property pretty much to themselves.

4. Antimicrobials

Antimicrobial compounds have been found in many organisms and clearly form part of the defensive strategy of many invertebrates. Antibacterial substances have been found in the mucous of slugs and snails. They tend to be large glycoproteins which are permanently produced by the animal rather than induced by infection. Achacin, the antibacterial glycoprotein from the giant African snail, is active against both Gram-positive and Gram-negative bacteria, apparently by disrupting the integrity of the cytoplasmic membrane. Marine invertebrates produce a range of antiviral compounds including the dideminins; cyclic depsipeptides found in tunicates. Tunicates are also the source of small antiviral compounds including patellazole, active against Herpes simplex and eudistomin whilst avarone and avarol, which have potent antiviral activity against immunodeficiency virus have been isolated from a sponge.

5. Toxins, Venoms and other Chemical Defences

Invertebrates have evolved a wide range of chemical defences, far too many and diverse to be covered here. The chemistry of these defences ranges from small reactive molecules, such as the benzoquinones ejected by some insects, millipedes and centipedes, to peptide or polypeptide toxins such as those produced by marine coelenterates. In molluscs the best known venoms are those of the cone snails, the conotoxins.

The venom of these snails can contain up to 100 pharmacologically active agents which bears favourable comparison to the numbers of bioactive compounds found in some plants. The most active compounds are short peptides of 10-30 amino acids. The peptides target ion channels in membranes and have a range of specificities for channel types. The salivary glands of some marine snails have been shown to produce low molecular weight toxins including serotonin from *Nucella lapillus* and tetramethyl ammonium chloride from *Neptunea antiqua*.

6. Biomaterials – natural plywood

As by definition invertebrates contain no backbone, they have evolved a range of novel structural materials, to protect themselves and their offspring, and for a range of other functions such as shelter, and food gathering. Some of these structural solutions comprise entirely unique and often beautiful materials. Two obvious examples are the shells of molluscs and the cuticle of insects.

The calcareous shells of molluscs are reinforced with carbohydrate/protein matrices and often overlaid with a highly structurally ordered proteinaceous periostracum. A number of molluscs produce a proteinaceous egg case or capsule to protect the developing eggs. These materials are mechanically remarkably strong, resistant to chemical, physical and bacterial degradation, and are composed of long protein chains mutually rotated to form a natural "plywood".

Marine Bioprospecting – the hunt for *blue gold*

Exploitation??

The answer probably lies in the emerging science of lower invertebrate mariculture

The above represents just a glimpse of the chemical diversity found in the mollusca; some areas such as chemical communication are just too vast and complex to cover other than to say that a wide range of diverse chemical types are used in inter and intraspecies signalling. Is there however any prospect of exploiting such chemical resources without damaging the resources and the environment? The answer probably lies in the emerging science of lower invertebrate mariculture. Marine bioprospecting is typically associated with public-private partnerships, due to the astronomical expenses, state of the art technology and specialized expertise associated with marine exploration. It is estimated that marine exploration costs at least $30,000 per day and $1 million for 30 days, expenditure well beyond the means of public institutions.

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The regulation of marine bioprospecting is complex, involving a range of issues. These include grappling with the challenges of ownership of common property, intellectual property rights and benefit t sharing; conservation and protection of marine biodiversity; technology transfer and capacity building; jurisdictional issues in monitoring and enforcement; as well as the difficulties inherent in regulating marine over-exploitation and activities that occur beyond national jurisdictions. These issues have been discussed in various international meetings including those held under the auspices of the Convention on Biological Diversity, the United Nations Convention on the Law of the Sea, the International Seabed Authority and the United Nations General Assembly on Oceans and Law of the Sea.

Further Reading

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