17. Sea cucumber Taxonomy: Conventional and Molecular Approaches

Dr. Asha P. S.
Marine Biodiversity and Environment Management Division
ICAR-Central Marine Fisheries Research Institute, Vizhinjam Regional Centre

Introduction

Sea cucumbers, popularly known as holothurians, belong to the phylum **Echinodermata**, a unique group in the animal kingdom. They are significant members of benthic invertebrate communities, found in all major oceans and seas worldwide. Echinoderms are characterized by radial symmetry (typically pentamerous), an internal skeleton made up of plates or ossicles, and a specialized water vascular system of fluid-filled tubes. The phylum is categorized into five classes, each with distinct appearances such as crinoids (or feather stars), holothuroids (or sea cucumbers), echinoids (or sea urchins), asteroids (or sea stars) and ophiuroids (or brittle stars).

Sea cucumbers are commercially exploited organisms that provide livelihoods for millions of coastal fishers globally and serve as a source of nutrition for consumers in Asia (Purcell et al., 2013). The processed product derived from sea cucumbers is known as 'beche-de-mer' in French, 'iriko' in Japanese, 'haisom' in Chinese, and 'trepang' in Indonesian. Its export value is particularly high in Southeast Asian countries due to its rich protein content and essential nutrients, such as vitamins, amino acids, trace metals, and minerals (Bordbar et al., 2011). In addition to their economic importance, sea cucumbers are integral to traditional Chinese medicine. Recent research has also highlighted their potential as a source of various bioactive compounds with properties that are anti-angiogenic, anticancer, anticoagulant, anti-hypertensive, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor, and wound-healing.

Furthermore, sea cucumbers play a critical role in ecosystem functioning by contributing to sediment health through bioturbation, nutrient recycling, and influencing seawater chemistry. They host a diverse array of parasitic and commensal symbionts from various phyla, which enhances ecosystem biodiversity. Many predatory taxa rely on sea cucumbers, thereby transferring nutrients—derived from detritus and microalgae—to higher trophic levels and establishing energy transfer pathways within food chains (Purcell et al., 2016).

Conventional methods of sea cucumber identification

Historically, the taxonomy of sea cucumbers relied on morphological traits such as

- Tentacle structure
- Presence or absence of tube feet.
- Ossicle types
- Gonad arrangement.

Morphology of sea cucumbers

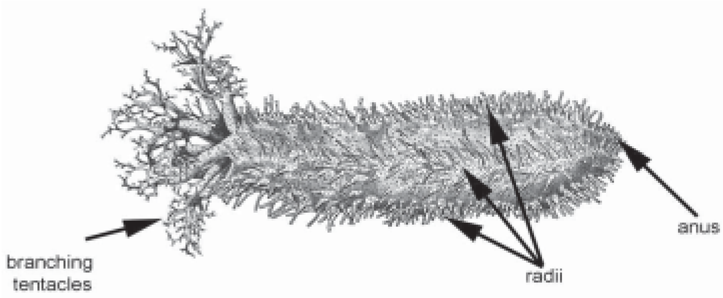

Fig. 1. Hololothurian morphology (adapted from Pawson, et al., 2010)

Holothurians have an elongated body that can take the form of a short or long cylinder. The mouth is located at the anterior end and is encircled by tentacles, while the anus is situated at the posterior end, often bordered by small papillae. Sea cucumbers typically rest on the substrate with their ventral surface, or trivium, which is characterized by radial structures. This creeping sole is equipped with locomotory podia, while the dorsal surface, or bivium, often features sensory papillae instead of podia (Fig.1). The mouth of a sea cucumber can be either terminal or slightly displaced dorsally and is surrounded by a thin buccal membrane, generally bordered by a ring of tentacles. These tentacles, which are buccal podia, contain extensions of the water vascular system and serve various functions related to feeding, sensory perception, and manipulation of food particles. The types of oral tentacles can vary among species, influenced by their ecological adaptations. Tentacles play a crucial role in identifying different species of sea cucumbers due to their diverse morphology, arrangement, and specialized functions. The morphology of these tentacles can vary significantly among species. Features such as the shape, size, length, and surface texture of the tentacles can be used to differentiate species. Additionally, the number and arrangement of tentacles around the mouth are often species-specific traits. Some sea cucumbers may have a single ring of tentacles, while others possess multiple rows or clusters. The presence, absence, or specific arrangement of oral tentacles can greatly assist in the identification of different sea cucumber species.

Types of Tentacles

i. Peltate Tentacles: Peltate tentacles are characterized by a broad, flattened, disc-shaped structure at the distal end, resembling a small paddle or leaf. These tentacles are often found in species that specialize in filter feeding, as the broad surface area helps in capturing suspended food particles from the water column (Fig.2).
ii. Digitate Tentacles: Digitate tentacles are slender and elongated, resembling fingers or digits. They may have a tapered or rounded tip. These tentacles are versatile, serving both feeding and sensory perception roles, and are commonly found in generalist feeders that consume a variety of food types.
iii. Papillate Tentacles: Papillate tentacles are covered with small, finger-like projections or papillae along their surface. These tentacles are typically associated with species that feed on organic detritus or fine particulate matter. The papillae increase the surface area for efficient nutrient absorption.

iv. Fusiform Tentacles: Fusiform tentacles are cylindrical or spindle-shaped, with a uniform diameter along their length. These tentacles may be found in species that engage in selective deposit feeding, targeting specific types of organic matter or microorganisms in sediments.

v. Branched Tentacles: Some sea cucumbers possess oral tentacles that are branched or subdivided into smaller segments, resembling a tree-like structure. Branched tentacles can increase the surface area available for feeding or sensory perception, enabling sea cucumbers to efficiently capture food particles or detect chemical cues in their environment.

vi. Tentacular Bulb: In certain sea cucumber species, particularly those in the family Synaptidae, the oral tentacles may be modified into a bulbous structure known as a tentacular bulb. This bulb is used for burrowing into sediments and capturing prey buried within the substrate. It may also assist in respiration and gas exchange in some species (Fig. 2).

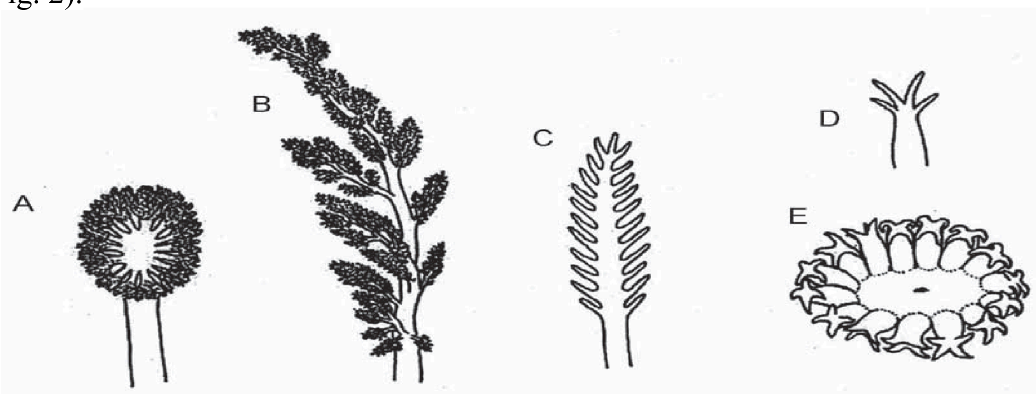

Fig. 2. Hoolothurian tentacles. A, shield-shaped (Order Aspidochirotida); B, branching (O. Dendrochirotida); C, pinnate (O. Apodida); D, digitate (O. Apodida, O. Molpadiida); E, digitate (O. Molpadiida) (adapted from Pawson, et al., 2010).

Calcareous rings

Calcareous rings are skeletal structures found within the body wall of sea cucumbers, providing a support framework for their internal organs and muscles. These rings consist of calcareous ossicles, which are small calcareous plates or pieces arranged in a circular or ring-like formation around the esophagus, creating a rigid structure (Fig.3).

Calcareous rings play a crucial role in the taxonomy and classification of sea cucumbers, offering valuable insights into their taxonomy, phylogeny, species diversity, and functional ecology. By studying the morphology and characteristics of these rings, researchers can deepen our understanding of the evolutionary history and ecological diversity of these fascinating marine organisms. The morphology and arrangement of calcareous rings vary significantly among different species of sea cucumbers. Taxonomists utilize the features of calcareous rings, such as the number, shape, size, and arrangement of ossicles, as diagnostic criteria for species identification and classification.

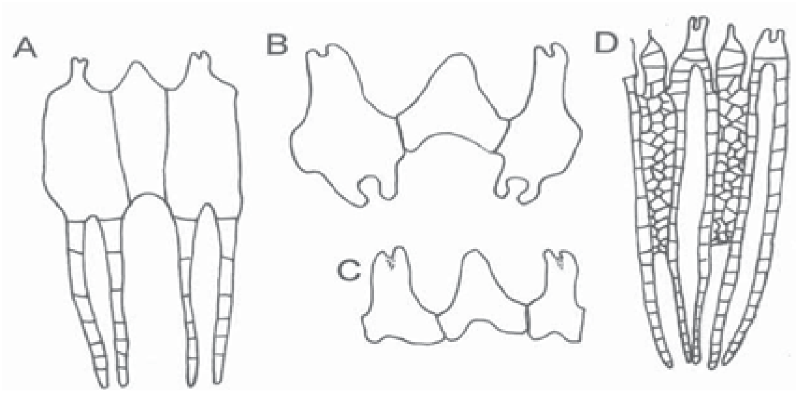

Fig.3. Calcareous rings; A, B, C with two radial pieces and one interradial piece, D with two interradial pieces and two notched radial pieces. A, ring with medium posterior projections; B, ring with short posterior projections; C, ring with no posterior projections; D, tubular ring with long, complex, posterior projections (adapted from Hyman, 1955).

Body wall and Spicules

The body wall of sea cucumbers consists of a robust layer of collagen-rich connective tissue known as the dermis, which is covered by a thin outer layer called the epidermis. Within the dermis lies the sea cucumber's skeleton, formed by numerous microscopic structures known as spicules. These spicules showcase an array of stunning geometric designs that differ among species and age, playing a crucial role in classification (Slater and Chen, 2015).
Primarily made of calcium carbonate, spicules provide essential structural support and reinforcement to the soft tissues of sea cucumbers. Their composition can vary, with some species incorporating magnesium or other trace elements. The shapes, sizes, and surface decorations of spicules can range widely, from simple rod-like forms to intricate branched or adorned structures.

Common types of spicules found in sea cucumbers include:

1. Table Spicules: These are flat, disc-like structures featuring a central perforation. They contribute to the stiffness of the body wall and the overall structural integrity of the sea cucumber's skeleton (Fig.4).
2. Anchor Spicules: Elongated and often pointed at one or both ends, these spicules resemble miniature anchors. They secure the body wall to surrounding tissues and substrates, enhancing stability and minimizing the risk of collapse.
3. Wheel Spicules: Shaped like wheels with radiating spokes or arms, these spicules are believed to reinforce specific areas of the body wall and may aid in locomotion and burrowing behaviors in certain species (Fig.4).
4. Cylinder Spicules: These are elongated and cylindrical with either smooth or decorated surfaces. They provide necessary structural support and flexibility to the body wall, allowing sea cucumbers to endure mechanical stresses and distortions.

Spicules serve various ecological roles within sea cucumbers. They can act as physical deterrents against predators by enhancing the rigidity of the body wall, making them more resistant to deformation or compression. Additionally, these structures facilitate the burrowing behavior of sea cucumbers into sediment.

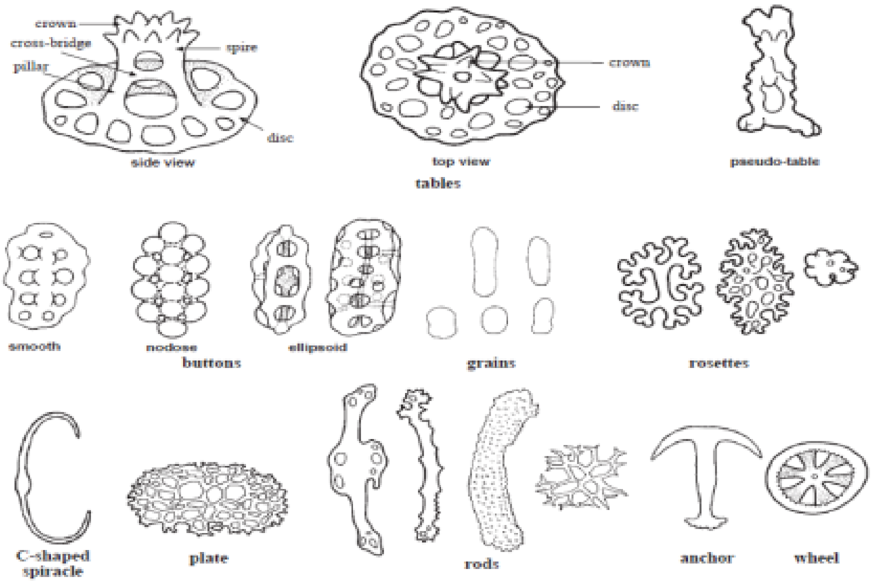

Fig.4. Basic types of spicules (adapted from Conand,1998)

Species identification of sea cucumbers by examining the spicules

Sea cucumber species can be identified by examining the skeletal elements, known as ossicles, located in various parts of the body. This is particularly effective for those embedded in the body wall, papillae, podia, and tentacles of live, dried, or preserved specimens. The following method, adapted from Purcell et al. (2023), can be used to isolate these ossicles:

1. Remove small pieces of tissue (e.g., a few square millimeters) from different body parts and place them into separate, labeled vials.
2. Add the required volume (e.g., 0.5 ml) of sodium hypochlorite (concentrated household bleach) or sodium hydroxide to each vial to dissolve the organic tissue. The soft tissue will dissolve in 20–30 minutes, leaving the hard ossicles at the bottom of the vial.
3. After decanting or pipetting out the bleach, the settled ossicles can be separated and washed five times in distilled water. Carefully use a pipette to remove the liquid from the vial, rinsing the pipette in fresh water each time to avoid contaminating the sample with ossicles from another vial. 4. The isolated ossicles can then be rinsed in alcohol and placed onto a microscope slide with a drop of mounting medium (e.g., Euparal). They are ready for microscopic observation, including scanning electron microscopy (SEM).

Characteristics of orders under the class - Holothuroidea

There are five orders in the class Holothuroidea (Dendrochirotida, Aspidochirotida, Molpadiida, Apodida and Elasipoda). The description is given below (adapted from Pinn, 2013).

Order	Description
Dendrochirotida	There were 10-30 tentacles highly branched or shaped in the form of dendritic, occasionally digitate. Ten calcareous plates form a simple or complex posterior process calcareous ring. They are usually sedentary, attached or burrowing. Introvert and retractor muscles are present.
Aspidochirotida	Very large group with very diverse and abundant in shallow tropical habitats. Tentacles always in the shape of peltate. Respiratory trees present. Introvert and retractor muscles absent.
Elaspodida	Lack of respiratory tree in body cavity. Strongly bilaterally symmetrical with commonly complicated and elaborate projections like frills, veils and sails on their gelatinous body. Only found in deep sea at abyssal depths.
Apodida	One of the simplest forms of sea cucumber. It has a very long and worm-like cylindrical body. .Body wall without any tube feet, except for the tentacles, hence have soft smooth and soft body. Respiratory trees and anal papillae absent. Ossicles often include wheels, or anchors....
Molpadiida	Tentacles are digitate. Stouted fusiform body with some slightly curved body to some curved liken a ball with tapering to a more or less conspicuous tail. No tube feet are present in the body wall and are mostly find in soft muddy substrates. Respiratory trees present; anal papillae may be present. Ossicles not wheel-shaped

Key to the shallow-water families of Aspidochirotida (adapted from James and James 1994).
1a. Body with trivium (sole) usually flattened and dorsal bivium convex; gonads forming a single tuft appended to the left dorsal mesentery; Cuvierian organs present or absent; dominant spicules of form of tables, buttons (simple or modified), and rods (excluding C- and S-shaped rods) **. . . . . Holothuriidae.**
1b. Body square-shaped or trapezoidal in cross-section; Cuvierian organs always absent; gonads forming two tufts appended on each side of the dorsal mesentery; dominant spicules in form of branched rods and C-and S-shaped rods **. . . . . Stichopodidae**

Field keys to the commercially important genera of holothurians
1. Anal opening surrounded by five teeth-like structure *Actinopyga*
1'. Anal opening not surrounded by teeth-like structure ...2
2. Anal opening surrounded by five groups of papillae....***Bohadschia***
2'. Anal opening not surrounded by five groups of papillae 3
3. Body more or less quadrangular with distinct papillae....***Stichopus***
3'. Body not quadrangular, but tubular, sometimes loaf-shaped 4
4. Body massive up to 800 mm in length, dorsal papillae resembling leaf-like strucutre ***Thelenota***
4'.Body moderate in length up to 600 mm; papillae not expanded into leaf-like structures ***Holothuria***

Genus *Actinopyga* Bronn, 1861

Species belonging to this genus are either of medium value or low value.

Field key to the species of *actinopyga*

1. Colour completely black ***A. miliaris***

1'. Colour brown or brown and white

2. Colour brown on the upper side and white on the lower side; often found near low water mark ***A. mauritiana***

2'. Colour completely brown with often sand deposits on the upper side of body; mostly found in deeper waters ... ***A. echinites***

Genus *Bohadschia* Jaeger, 1833

Massive forms with distinct anal papillae, often buried or covered with a fine coat of mud. Because of the Cuvierian tubules (sticky threads) processing is difficult. Species belonging to this genus have moderate commercial value.

Field key to the species of *Bohadschia*

1. Colour black or brown with distinct 'eye' like spots all over the body ***B. argus***

1'. Colour variable, usually light brown with black spots ***B. marmorata***

Genus *Stichopus* Brandt, 1835

Some species belonging to this genus reach a massive length of 900 mm. Body will disintegrate and become gelatinous when taken out of water.

Field key to the species of *stichopus*

1. Body quadrangular with four rows of large finger-like processes. Colour dark green, appearing almost black in some shades of light ***S. chloronotus***

2. Body massive and loaf-like with irregular brown patches on yellow grey background ***S. variegatus***

Genus *Thelenota* Brandt, 1833

Very massive forms with numerous large pointed teats in groups of two or three all over the upper surface.

Genus *Holothuria* Linnaeus, 1764

This is by far the most important genus for processing. Over one hundred species are known under this genus.

Key to the species of *Holothuria*

1. Body like a loaf with very thick body wall. In the living condition about six pairs of lateral teat-like projections are seen; body with black and white patches ***Holothuria nobilis***

1'. Body tubular, body wall not very thick. No lateral projections in the living condition 2

2. Body completely black in colour; red colour comes off when live specimens are handled ***Holothuria atra***

2'.The colour not completely black and no red colour comes off when live specimens are handled 3

3. Yellow transverse bands on the upper side of the body, lower side white with a number of black dots ***Holothuria scabra***

3'. Body uniformly brown in colour; small stiff projections all over the body; highly burrowing form ***H. spinifera***

Advances in the molecular taxonomy of sea cucumbers

Molecular taxonomy has revolutionized the classification of sea cucumbers (Holothuroidea), offering a more robust framework compared to traditional morphology-based systems. Below are the key advancements provided by molecular approaches. The conventional techniques often led to inconsistent and subjective classifications, as they could vary within species or exhibit convergent evolution. Molecular tools, such as DNA sequencing, now allow the examination of evolutionary relationships at the genetic level, providing more objective and precise classifications. Molecular studies of sea cucumbers use various genetic markers and techniques, including 18S rRNA, COI (Cytochrome c Oxidase I), Mitochondrial DNA and RAD-seq analyses for fine-scale relationships.

Taxonomic revisions

Molecular data revealed that several traditional orders, such as Aspidochirotida, Elasipodida, and Molpadida, are polyphyletic. This means they include species that do not share a common ancestor. For instance, the order **Aspidochirotida** was split into three new orders like **Synallactida** (includes families Stichopodidae, Synallactidae, and Deimatidae), **Persiculida** (includes families Gephyrothuriidae, Molpadiodemidae, and Pseudostichopodidae), **Holothuriida** (includes families Holothuriidae and Mesothuriidae).**Dactylochirotida** was merged with **Dendrochirotida**, as molecular evidence supported their close evolutionary relationship. **Molpadida** and **Apodida**, while retaining distinct identities, were shown to share more similarities than previously thought. Molecular studies highlighted polyphyly within traditionally recognized families such as Psolidae and Cucumariidae, prompting further re-evaluation. Miller et al. (2017) constructed a comprehensive phylogenetic tree using genetic data from representatives of most known families. This tree now underpins modern classifications.

Importance of integrating molecular and morphological Data

While molecular data provide clarity, integrating these findings with morphological traits ensures comprehensive taxonomy due to the reasons like morphological features remain important for field identification and ecological studies and molecular classifications require alignment with morphological characteristics to achieve practical utility. Advances in molecular taxonomy have significantly improved our understanding of sea cucumber diversity, evolutionary relationships, and classification. These insights are invaluable for both scientific research and the sustainable management of sea cucumber resources.

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