

Original Article

Structural and Elemental Quantification of Coelomate Hard Tissues for Biomaterial Applications

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Biomineralization plays a fundamental role in skeletal formation, influencing structural integrity, mechanical function, and evolutionary adaptations across different phyla. The skeletal compositions of mollusks, chordates, and echinoderms vary significantly due to differences in mineral content and microstructural organization. This study aims to compare the skeletal structures of *Ostreola equestris* (Mollusca), *Larimichthys crocea* (Chordata), and *Strongylocentrotus purpuratus* (Echinodermata) to understand species-specific biomineralization strategies and their functional significance. Skeletal samples were collected from the SDC Vivarium Repository and subjected to advanced analytical techniques. Scanning electron microscopy (SEM) was used to examine surface morphology and microstructural organization. X-ray diffraction (XRD) identified the crystalline phases of skeletal minerals, while energy-dispersive X-ray spectroscopy (EDAX) quantified elemental composition. These methods provided a comprehensive assessment of mineralization patterns in the selected species. SEM analysis revealed that *Ostreola equestris* exhibited a calcium carbonate-based exoskeleton with prismatic and nacreous layers. XRD confirmed calcite as the dominant mineral, while EDAX detected high calcium and trace magnesium. *Larimichthys crocea* displayed a hydroxyapatite-rich skeleton with fibrous collagen networks, with XRD confirming hydroxyapatite and EDAX detecting calcium, phosphorus, sodium, and magnesium. *Strongylocentrotus purpuratus* showed an amorphous calcite skeleton with mutable connective tissues, with XRD revealing a high amorphous fraction and EDAX indicating low calcium levels with significant organic matrix content. The study highlights distinct biomineralization strategies, where mollusks form rigid calcium carbonate exoskeletons, chordates balance mineralization with collagen for flexibility, and echinoderms utilize amorphous calcite for skeletal adaptability. These findings have implications for biomaterials science, paleontology, and evolutionary biology, offering insights into species adaptations and bio-inspired materials development.

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Introduction

The classification of vertebrates is fundamental to understanding biodiversity, evolutionary relationships, and species-specific adaptations. Traditional classification systems rely on genetic, morphological, and physiological characteristics to elucidate species development and ancestry [1]. Beyond these conventional parameters, the inorganic composition of skeletal structures provides additional insights into species differentiation by revealing variations in mineral content, microstructural organization, and

mechanical properties. This perspective enhances our understanding of skeletal integrity, functional adaptations, and the environmental influences shaping vertebrate evolution [2].

Calcium, phosphorus, and trace minerals are critical components that contribute to skeletal strength, environmental adaptation, and structural stability. Hydroxyapatite, a calcium phosphate mineral, is particularly essential in vertebrate skeletal frameworks, ensuring mechanical resilience and structural support [3]. While vertebrates share a fundamental skeletal composition, variations in mineral ratios, trace element incorporation, and skeletal tissue organization differ among species due to evolutionary pressures, environmental factors, and dietary influences. Analyzing these variations refines classification systems by incorporating mineralogical and structural characteristics, providing a more

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comprehensive approach to vertebrate taxonomy.

Advanced analytical techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDAX) facilitate the detailed characterization of skeletal materials. XRD enables the identification of hydroxyapatite crystalline structures and compositional variations, while SEM provides high-resolution imaging of bone surfaces, revealing mineral deposition patterns. EDAX further complements this analysis by precisely quantifying elemental composition, including calcium, phosphorus, and trace minerals. The integration of these techniques offers a robust methodological framework for investigating the inorganic composition of vertebrate skeletons, contributing to a refined classification system based on mineralogical features.

A taxonomy informed by skeletal inorganic composition has significant implications across multiple scientific disciplines, including paleontology, biomaterials research, and evolutionary biology. Understanding mineral differences in skeletal structures is crucial for exploring species adaptation, biomineralization processes, and the environmental factors influencing skeletal development. This study aims to establish a classification system for vertebrates based on their inorganic skeletal components, offering a novel perspective on species organization and evolutionary patterns. By integrating structural and chemical distinctions into vertebrate classification, this research enhances our comprehension of species evolution and underscores the importance of mineralogical variations in defining biological diversity.

Materials and Methods

Sample collection and preparation

The present study utilized skeletal specimens from the SDC Vivarium Repository at Saveetha Dental College's Department of Oral Biology, Chennai, India. To examine variations in inorganic composition, one representative species from each of the major phyla was selected: *Ostreola equestris* (Phylum Mollusca), *Larimichthys crocea* (Phylum Chordata), and *Strongylocentrotus purpuratus* (Phylum Echinodermata) (figure 1).

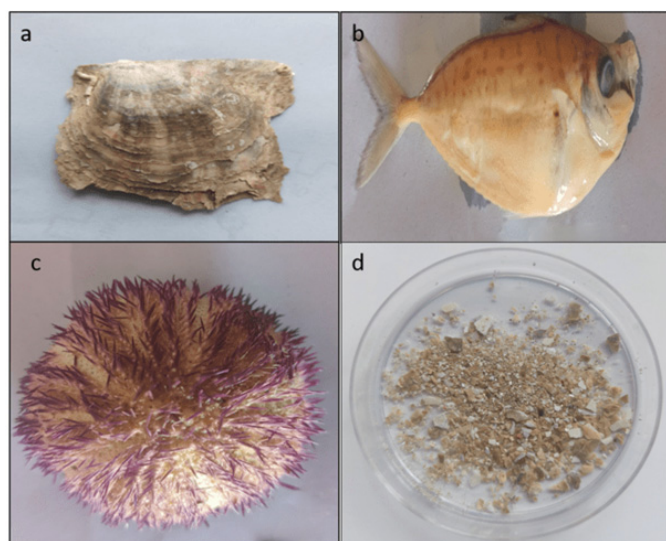


Figure 1: Skeletal specimens from diverse phyla used in the study

The collected skeletal samples underwent a cleaning process to remove any residual biological material, ensuring the integrity of the inorganic components. The cleaned specimens were allowed to dry naturally at room temperature. For analytical purposes, a portion of the samples was finely ground into a powder to facilitate crystallographic studies, while intact bone fragments were preserved for structural and elemental analyses.

Techniques for mineralogical and structural analysis

To comprehensively assess the inorganic composition and microstructure of the skeletal specimens, multiple advanced analytical techniques were employed:

X-ray diffraction (XRD)

X-ray diffraction (XRD) was utilized to determine the crystalline structure and phase composition of the skeletal minerals. This technique is essential for identifying different mineral phases, such as hydroxyapatite in vertebrate bones or calcite and aragonite in invertebrate exoskeletons. By analyzing diffraction patterns, XRD provides insights into the degree of crystallinity, lattice structure, and possible mineral substitutions, which are crucial for understanding biomineralization trends across evolutionary lineages.

Scanning electron microscopy (SEM)

High-resolution imaging of the skeletal microarchitecture was performed using the JSM IT800 Nano Scanning Electron Microscope (SEM). SEM is a powerful technique that provides detailed surface morphology and microstructural visualization, allowing for the assessment of mineral deposition patterns, porosity, and overall skeletal organization. This method is particularly useful for comparing differences in skeletal structure across various taxa, revealing adaptations linked to mechanical function and environmental influences.

Energy-dispersive x-ray spectroscopy (EDAX)

Elemental composition analysis was conducted using energy-dispersive X-ray spectroscopy (EDAX), which enables the quantification of key inorganic components, including calcium (Ca), phosphorus (P), and trace elements such as magnesium (Mg) and strontium (Sr). This technique is critical for understanding species-specific variations in mineral content, which can be influenced by environmental factors, dietary habits, and evolutionary adaptations. The EDAX data provide insight into the role of elemental substitutions in skeletal biomineralization and structural stability.

Data analysis and interpretation

The data obtained from XRD, SEM, and EDAX analyses were processed using specialized computational software to identify peak patterns, quantify elemental concentrations, and interpret structural modifications. Comparative assessments were conducted to evaluate mineralogical differences and skeletal adaptations among the selected species. By integrating these methodologies, this study establishes an inorganic composition-based framework for classifying vertebrate and invertebrate skeletal structures, offering novel insights into biomineralization, evolutionary adaptations, and species-specific skeletal functionality.

This comprehensive approach enhances our understanding of how skeletal materials have evolved to meet functional and ecological demands, refining classification systems based on mineralogical and structural characteristics.

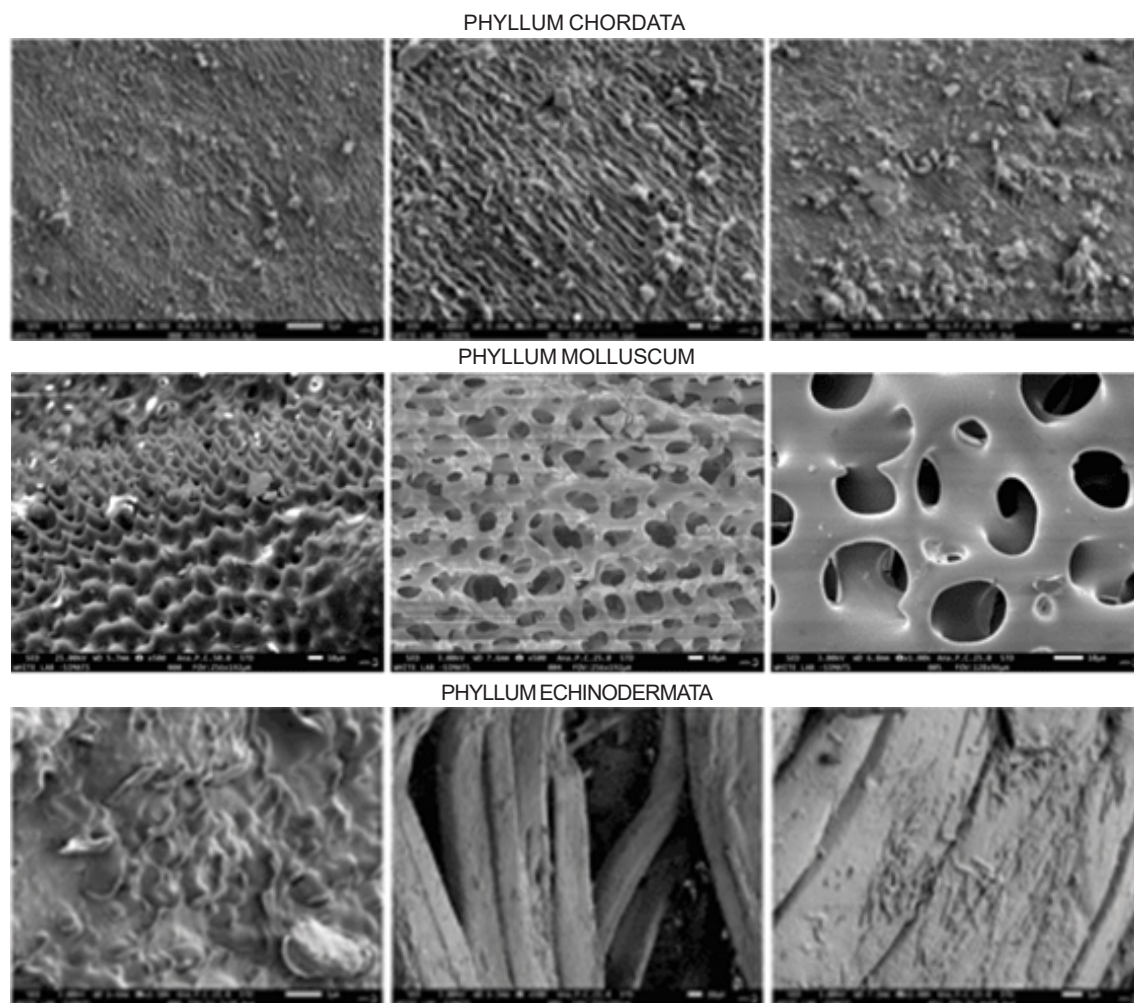


Figure 2: Scanning electron microscopy (SEM) analysis: (a–c) Chordata – connective tissue microarchitecture of *Larimichthys crocea*; (d–f) Mollusca – shell layers and radula of *Ostreola equestris*; (g–i) Echinodermata – ossicles and ligamentous attachments of *Strongylocentrotus purpuratus*

Results and Discussion

Scanning electron microscopy (SEM) analysis

Phylum Mollusca: *Ostreola equestris*

The SEM analysis of *Ostreola equestris* revealed a distinct calcium carbonate microstructure forming both prismatic and nacreous layers (figure 2). The surface morphology displayed well-defined growth lines and microstructural ridges, indicative of species-specific shell development patterns. These structures are critical in molluscan biomineralization, influencing mechanical properties such as fracture resistance and shell strength.

Additionally, examination of the radula demonstrated rows of chitinous teeth, a crucial adaptation for feeding. The radula structure enables mollusks to efficiently scrape surfaces, cut, or capture prey, depending on their ecological niche. This morphological adaptation plays a key role in dietary specialization and environmental interactions, further reinforcing the evolutionary significance of molluscan biomineralization [4].

Phylum Chordata: *Larimichthys crocea*

SEM imaging of *Larimichthys crocea* connective tissue revealed a

network-like arrangement of fibrous collagenous elements, characteristic of vertebrate skeletal tissues. The fibrous structures contribute to the mechanical resilience and flexibility required for movement, distinguishing chordates from exoskeleton-bearing invertebrates [5].

Further investigation of muscle and epithelial tissues identified organized, stratified layers indicative of their functional roles in protection, absorption, and movement. The hierarchical structural arrangement of these tissues supports the mechanical integrity of vertebrate skeletal systems, allowing for efficient force distribution and locomotion [6].

Phylum Echinodermata: *Strongylocentrotus purpuratus*

SEM analysis of echinoderm ossicles demonstrated a calcareous composition with irregular, leaf-like microstructures characteristic of echinoderm skeletons. These ossicles provide structural rigidity while maintaining adaptability, a critical feature in echinoderm mobility and environmental interactions.

The fibrous ligamentous attachments observed corresponded to mutable connective tissues, a unique feature of echinoderms. These structures allow for controlled skeletal stiffness modulation,

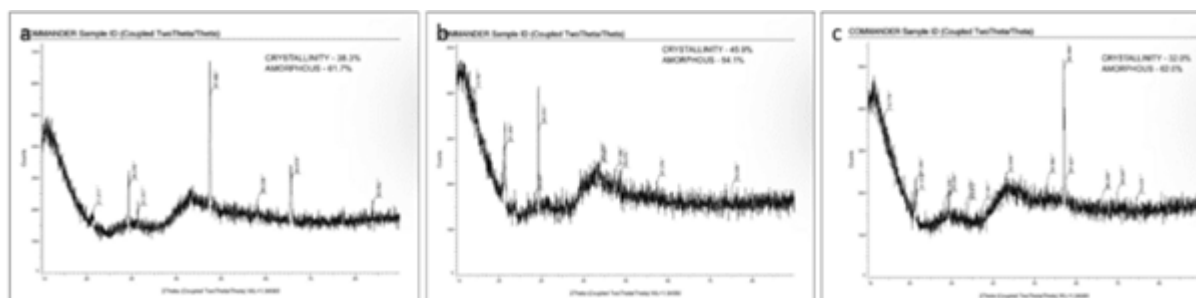


Figure 3: X-ray Diffraction (XRD) Analysis: (a) Chordata – *Larimichthys crocea*; (b) Mollusca – *Ostreola equestris*; (c) Echinodermata – *Strongylocentrotus purpuratus*

enabling echinoderms to alter their rigidity in response to external stimuli, a capability not observed in other phyla.

X-ray diffraction (XRD) analysis

Phylum chordata

XRD analysis of *Larimichthys crocea* bone revealed a crystalline structure with a significant amorphous fraction (61.7%), suggesting a mineral-organic composite characteristic of hydroxyapatite-rich tissues (figure 3a). The diffraction peaks at 21.311° and 29.318° confirmed the presence of calcium carbonate, while peaks at 47.486° indicated hydroxyapatite, the primary mineral component of vertebrate skeletal systems [7]. These findings validate the role of hydroxyapatite in providing mechanical strength and durability in chordate skeletons.

Phylum mollusca

XRD analysis of *Ostreola equestris* shells (figure 3b) revealed a crystalline composition with 45.9% crystallinity and 54.1% amorphous content, indicative of a biologically dynamic mineralization process. Peaks at 13.767° and 21.349° corresponded to chitin and calcium phosphate, while the dominant peak at 29.310° confirmed calcite as the primary mineral. The findings align with the dual-phase nature of molluscan shells, where organic matrices regulate crystal growth and enhance mechanical properties [8].

Phylum echinodermata

The XRD spectrum of *Strongylocentrotus purpuratus* ossicles (figure 3c) displayed a high amorphous fraction (62%) and low crystallinity

(32%), characteristic of echinoderm skeletal plasticity. The dominant presence of calcite confirms the structural framework, while the high amorphous content suggests an adaptable mineral phase, supporting skeletal flexibility and regeneration [9].

Energy dispersive x-ray spectroscopy (EDAX) analysis

Phylum mollusca

EDAX analysis of *Ostreola equestris* (figure 4) identified calcium (41.74%) and oxygen (16.57%) as dominant elements, confirming the prevalence of calcium carbonate in the shell structure. The trace presence of magnesium (1.20%) suggests a role in crystal stabilization and mechanical reinforcement, consistent with previous findings on molluscan shell mineralization [4].

Phylum chordata

Elemental analysis of *Larimichthys crocea* showed moderate calcium (38.45%) and oxygen (15.45%) levels, indicative of hydroxyapatite composition. The detection of sodium, magnesium, and aluminum supports the complex nature of vertebrate bone matrices, which incorporate secondary mineral inclusions for enhanced biomechanical performance [7].

Phylum echinodermata

EDAX analysis of *Strongylocentrotus purpuratus* revealed significantly lower calcium (4.87%) and elevated carbon (21.21%), reflecting the organic-rich nature of echinoderm skeletons. The presence of sulfur and chlorine further supports the biomineralization role of organic matrices, facilitating skeletal modulation under environmental stress.

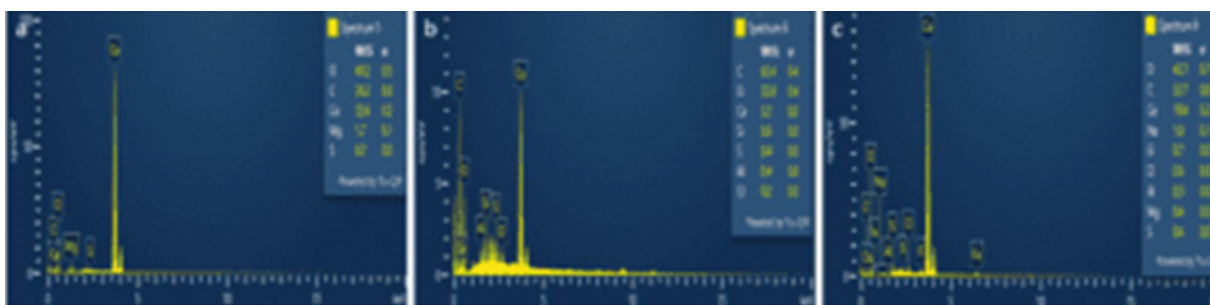


Figure 4: Energy Dispersive X-ray Spectroscopy (EDAX) Analysis: (a) Mollusca – *Ostreola equestris*; (b) Chordata – *Larimichthys crocea*; (c) Echinodermata – *Strongylocentrotus purpuratus*

Comparative analysis of biomineralization strategies

The results highlight distinct biomineralization strategies across phyla, demonstrating their evolutionary adaptations to ecological niches. Mollusks exhibit a rigid, highly crystalline calcium carbonate-based exoskeleton, optimized for protection. Chordates balance mineralization with organic components, ensuring structural resilience and mechanical adaptability. Echinoderms rely on a low-crystallinity skeletal system with mutable connective tissues, enabling skeletal flexibility and regeneration.

These findings underscore the role of biomineralization in evolutionary biology, providing insights into skeletal adaptations across diverse taxa. The correlation between structural composition and biomechanical function emphasizes the selective pressures shaping mineral deposition in different phyla. Future studies should further explore environmental influences on bio mineralization, particularly in response to climate change and ocean acidification, which may significantly impact skeletal integrity in marine organisms.

Conclusion

This study presents a comprehensive comparative analysis of biomineralization strategies in *Ostreola equestris*, *Larimichthys crocea*, and *Strongylocentrotus purpuratus*, utilizing SEM, XRD, and EDAX techniques to elucidate skeletal structure, mineral composition, and evolutionary adaptations. The findings highlight distinct mineralization pathways across Mollusca, Chordata, and Echinodermata, reflecting the structural and functional diversification of skeletal systems in response to ecological and evolutionary pressures. The characterization of calcium carbonate-based exoskeletons in mollusks, hydroxyapatite-enriched skeletal structures in chordates, and the dynamic, amorphous calcite framework in echinoderms underscores the role of biomineralization in optimizing mechanical performance and environmental adaptability.

These insights hold significant implications for biomaterials science, paleontology, and evolutionary biomechanics, providing a foundation for bio-inspired materials engineering and skeletal tissue regeneration research. The understanding of biomineralization processes can inform the development of advanced biocomposites,

synthetic bone substitutes, and eco-friendly structural materials. Additionally, the study enhances our ability to interpret fossilized skeletal remains, contributing to evolutionary reconstructions and paleoenvironmental assessments.

Future Perspective

Expanding the scope of comparative analyses to include additional taxa and developmental stages could further refine our understanding of skeletal evolution and mineral deposition mechanisms. The integration of molecular biology and proteomic approaches would also provide deeper insights into the regulatory pathways governing biomineralization, paving the way for novel applications in biotechnology and materials science.

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