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Scanning Electron Microscopy of Vital Organs in the Endemic Himalayan Loach (*Triplophysa marmorata*) from Kashmir

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ABSTRACT: The topological architecture of the gills, lower jaw, anterior part of the intestine, and skin of *Triplophysa marmorata*, an endemic loach from the Kashmir Himalayas, was studied using a Scanning Electron Microscope (SEM) to understand the structural details of the above-mentioned organs and the general biology of the fish species. The study is relevant because gills are the structures that are in direct contact with water and play important roles in feeding, osmoregulation, and respiration, which govern the general health of fish. Similarly, skin is an important structure which acts as a protective covering. The upper and lower jaws are important for the feeding habits of fish. Besides these, the intestinal part shows different cell lines in the intestine of fish. These findings provide critical insights into the functional morphology of *T. marmorata*, contributing to a better understanding of its ecological adaptations and evolutionary significance. Additionally, this study offers baseline morphological data that can aid in species identification, conservation strategies, and further comparative studies within the Triplophysa genus.

Keywords: *T. marmorata*, Gills, Lower jaw, Intestine, Kashmir, Skin, Loach, SEM.

INTRODUCTION

Triplophysa marmorata belongs to the genus Triplophysa (Rendahl, 1933) and the family Balitoridea, subfamily Nemacheilinae, with 124 nominal species in this genus worldwide (Li *et al*., 2015). In India, the distribution of this genus has been reported only from the upper catchment area in Jammu and Kashmir, and the Lahul and Spiti area of Himachal Pradesh (Kullander *et al.*, 1999). In India, 10 Triplophysa were described by Menon (1987). The species of Triplophysa exhibit marked sexual dimorphism: Males possess a nodular, raised skin on both sides of the head and a thick nodular pad on the dorsal surfaces of the broadened and widened pectoral-fin rays (Zhu, 1989). *Triplophysa marmorata* is a benthopelagic fish found in the snow-covered waters of the Himalayas in Kashmir. It is locally known as "Ara Gurun" (Heckel, 1838). Its eyes are high on the head, its mouth is inferior, its scales are absent, and it has three pairs of barbs: two rostral and one maxillary pair. The colour of the ground is pale yellowish or whitish (Heckel, 1838). It differs from *Triplophysa kashmirensis* by a shorter length of lateral line and caudal peduncle.

Scanning electron microscopy (SEM) is a powerful tool for visualizing the surface architecture of biological tissues, providing valuable insights into their functional morphology. SEM has been successfully employed to study the morphology of various fish organs, including gills, liver, kidney, and olfactory epithelium (Esmaeili and Gholami 2011; Cordero *et al*., 2017; Jawad *et al*., 2022). These studies have revealed important details about the structure and function of these organs, as well as their responses to environmental stressors (Samanta *et al*., 2018; Waryani *et al.*, 2013). A thorough search of existing literature reveals a limited number of studies on the ultrastructure of *Triplophysa* species. While some studies have used SEM to examine the olfactory epithelium of other loach species (Waryani *et al*., 2013), a comprehensive analysis of the surface ultrastructure of multiple organ systems in *Triplophysa marmorata* is lacking. Some researchers have also described the use of SEM to study fish scales (Cordero *et al*., 2017). Furthermore, research exists on the histopathological effects of industrial and agricultural effluents on fish organs, highlighting the importance of understanding the structural integrity of these organs in the face of environmental stressors. This study, therefore, aims to fill a critical knowledge gap and provide baseline data for future research on the conservation and management of this endemic loach species.

MATERIALS AND METHODS

Specimens of *T. marmorata* (Fig. 1) were collected from freshwaters in Kashmir using local traps. Fish were maintained in aquaria before dissection to avoid damage to internal organs. The gills, jaw tissues, skin from near the caudal fin, and the anterior intestine were carefully extracted. Samples were washed with distilled water and fixed in 2% glutaraldehyde with Na-cacodylate buffer (pH 7.2). Dehydration was achieved using ascending ethanol concentrations, followed by tetramethylsilane drying. A gold coating was applied to prevent charging during SEM imaging, performed at 10 kV using secondary electron emission mode (Dey *et al*., 1989).

Fig. 1. Specimen of *T. marmorata.*

RESULTS

T. marmorata exhibits a gross gill structure typical of teleost fishes (Fig. 2). Each gill comprises a gill arch, gill rakers, and gill filaments, arranged in two rows along the arch, forming anterior and posterior hemibranchs that constitute the holobranch. The length of gill filaments varies within a single arch and among different arches, being longest in the arch's central region and shorter toward the dorsal and ventral ends. Each gill arch supports two rows of gill rakers on the outer convex surface facing the opercular cavity. The first holobranch features longer and thinner rakers than subsequent arches. A variable number of secondary lamellae radiates from both surfaces of each filament, more developed distally and diminishing proximally. The SEM analysis of *T. marmorata* skin revealed voids surrounded by a loose network of cells, resembling sponge or coral-like structures (Fig. 3). The epidermal surface cells exhibited intricate microridges, varying in shape, curved, straight, branched, or unbroken, forming fingerprint- or honeycomb-like patterns. The mouth of *T. marmorata* is protrusible, featuring three pairs of short barbels and thick upper and lower jaws (Kottelat and Freyhof 2007). The oral cavity's modest size reflects an adaptation for consuming small food items efficiently. The species has edentulous jaws, with a highly protrusive upper jaw and a slightly protrusive lower jaw. The upper lip is thin, whereas the lower lip is thicker (Fig. 4). The anterior part of the intestine of *T. marmorata* exhibits a simple tubular structure without significant differentiation between regions (Fig. 5). SEM analysis reveals the presence of columnar epithelial cells with brush-border microvilli, forming a dense mat on the apical surfaces of intestinal cells. The intestinal mucosa is interspersed with goblet cells that secrete mucus, while tight junctions between epithelial cells were observed.

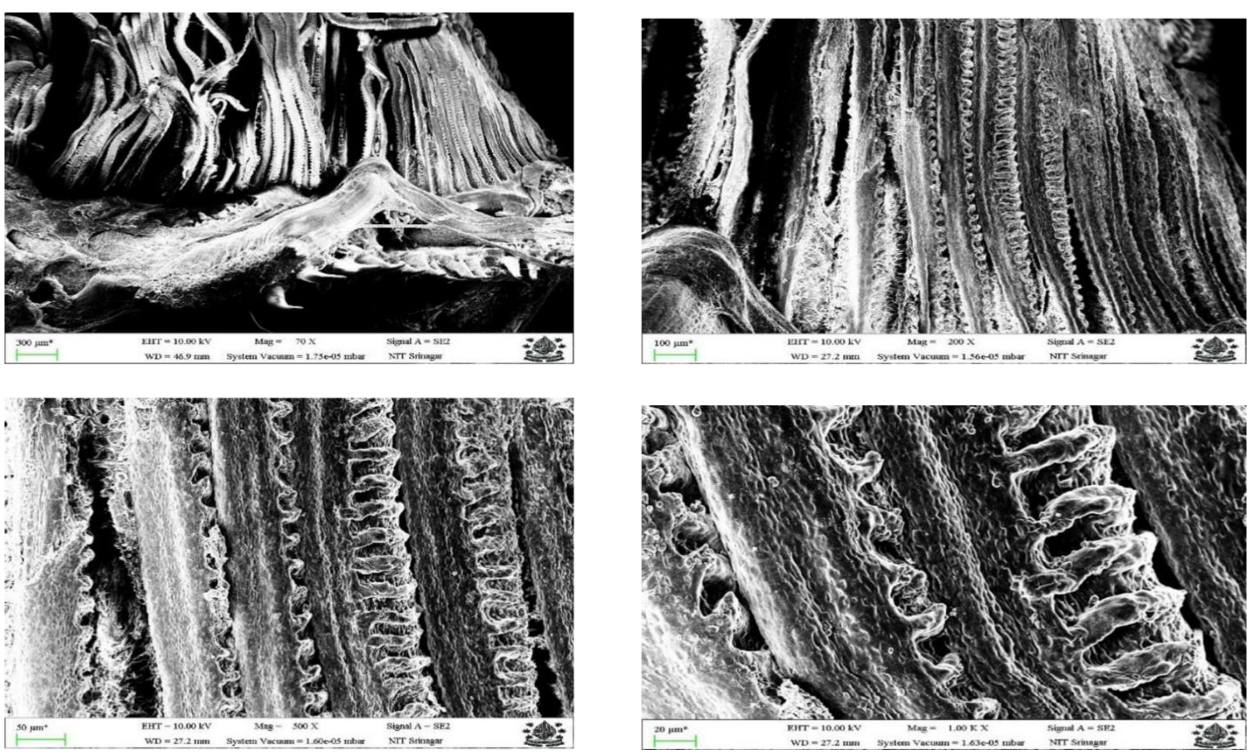

Fig. 2. Showing the anatomical structure of the gill under SEM at 70x, 200x, 500x and 1000x magnifications.

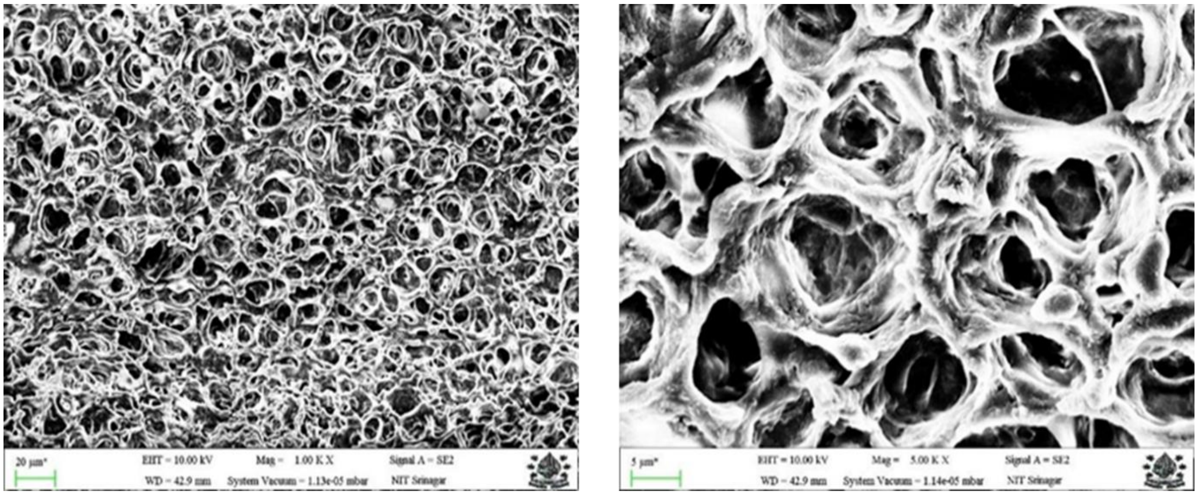

Fig. 3. Showing the anatomical structure of skin under SEM at 1000x and 5000x magnifications.

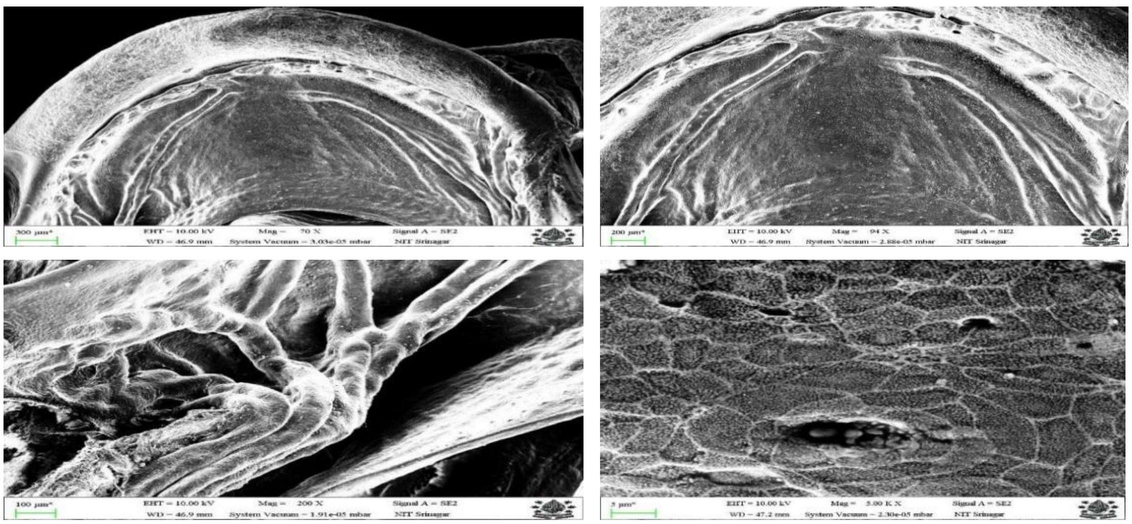

Fig. 4. Showing the anatomical structure of the lips and mouth under SEM at 200x and 5000x magnifications.

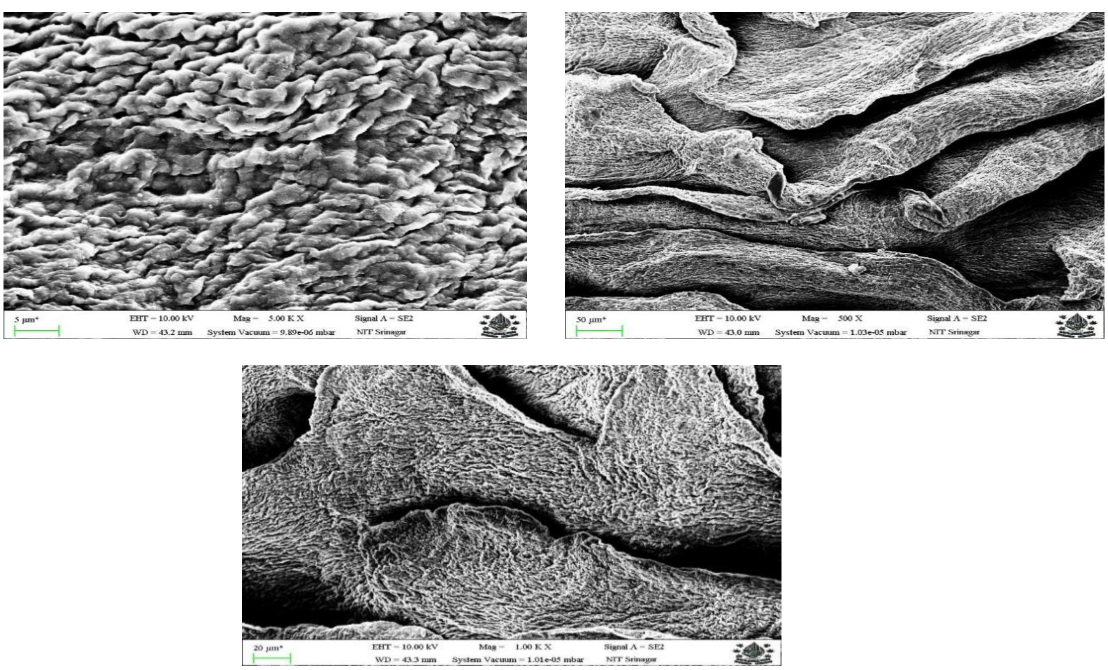

Fig. 5. Shows the anatomical structure of skin under SEM at 5000x, 500x and 1000x magnifications.

DISCUSSION

In *T. marmorata*, gills serve multiple roles beyond respiration, including excretion of metabolic waste and osmotic balance regulation (Moyle and Cech 1996; Norman, 1963). The variation in gill filament length likely optimises respiratory efficiency by increasing surface area exposed to water flow. The long, fine rakers on the first holobranch indicate a feeding strategy adapted to filtering detritus and small invertebrates, aligning with its habitat in freshwater ecosystems with suspended particulate matter (Meraj *et al.,* 2016). Structural features, including filament length, lamellar arrangement, and specialized rakers, are critical for survival in environments with variable oxygen levels and high particulate loads, illustrating the species' ecological plasticity and evolutionary fitness. The spongy network observed in the skin may aid in moisture retention, enhancing the skin's protective functions against environmental stressors. Microridges likely increase surface area, facilitating gas exchange and nutrient absorption (Cordero *et al.,* 2017; Lau *et al.*, 2019; Nowsheen *et al*., 2023). These adaptations align with studies highlighting the role of microridges in maintaining homeostasis and defending against microbial invasion in fluctuating environments (Ali *et al*., 2023). The voids and loose cell network contribute to skin elasticity, allowing it to absorb shocks and adapt to environmental changes. Despite these adaptations, the skin remains vulnerable to pollutants and pathogens, and the complexity of microridge structures may complicate healing if damaged.

The combination of a compact oral cavity, edentulous jaws, and specialized lip structures positions *T. marmorata* as a benthic feeder, well-suited for foraging in sediment-laden or particulate-rich environments. These adaptations minimize energy expenditure while maximizing efficiency in capturing soft-bodied or particulate food, aligning with the ecological role of fish in freshwater ecosystems with limited prey diversity (Sibbing, 1991; Motta and Kotrschal 1992). The intestinal structure reflects the species' diet, which primarily consists of small invertebrates, algae, and organic detritus. The presence of brush-border microvilli significantly increases the surface area for nutrient absorption, optimizing nutrient uptake (Buddington and Diamond 1987). The goblet cells play a crucial role in reducing friction during food transit and protecting the epithelial lining from mechanical damage (Wilson and Castro 2011). The absence of a stomach further supports rapid nutrient absorption, a characteristic commonly observed in fish with continuous feeding habits (Kapoor *et al.*, 1976). Similar digestive adaptations have been documented in other loaches and cyprinid fish occupying benthic habitats (Clements and Raubenheimer 2006).

CONCLUSIONS

This study provides the first detailed examination of the surface ultrastructure of vital organs in the endemic Kashmir loach, *Triplophysa marmorata*, using Scanning Electron Microscopy. The observed surface features of the gills, jaw, skin, and intestine offer valuable insights into the functional morphology of these organs and their adaptations to the unique environmental conditions of the high-altitude Himalayan streams. The intricate gill lamellae structure highlights the efficiency of respiratory gas exchange in this species. Given the increasing environmental pressures on aquatic ecosystems in the Kashmir Himalayas, including habitat degradation and pollution, the baseline data generated by this study is particularly important. The ultrastructural characteristics described here can serve as a reference point for future investigations aimed at assessing the impact of environmental stressors on this fish. Any observed alterations in the surface morphology of these organs in response to changing environmental conditions could serve as early warning indicators of environmental stress and population health decline.

Further research is warranted to investigate the effects of specific pollutants and habitat alterations on the ultrastructure of these organs. In addition, comparative studies with other Triplophysa species inhabiting different environments could provide valuable insights into the adaptive evolution of these loaches. By integrating ultrastructural data with ecological and physiological studies, we can develop a more comprehensive understanding of the biology of *Triplophysa marmorata* and inform effective conservation strategies for this endemic species.

Ethics approval: The fish samples were procured from commercial sites in accordance with relevant local regulations on the trade and use of fish. Therefore, no ethical approval was required.

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