Helium Ion Microscopy (HIM) and Transmission Electron Microscopy (TEM) combined with High-Pressure Freezing and Freeze Substitution (HPF/FS) for detailed characterization of the *Schistosoma mansoni* adult worm tegument

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Electron microscopy (EM) is crucial for studying *Schistosoma mansoni* morphology, particularly the tegument, a key target for drug and vaccine development. EM reveals the plasma membrane, spines, and papillae. The ventral sucker contains sensory papillae and large spines that aid in host attachment, with structures as small as 1 µm. Helium ion microscopy (HIM) provides high resolution and depth of focus without the need for metal coatings, while transmission electron microscopy (TEM) enables nanoscale analysis. This study investigates the ultrastructure of the tegument using advanced fixation and microscopy techniques. For cryofixation, samples were placed in aluminum holders with 1-hexadecene, mounted in high-pressure freezing holders, and frozen using a Bal-Tec 010 HPF system. After freezing, samples were stored in liquid nitrogen, immersed in a substitution medium, and processed for TEM analysis using an HT7800 RuliTEM microscope. For HIM, cryofixed samples were dehydrated with ethanol, dried in CO_2, and mounted on metal stubs. Specimens were analyzed using an ORION microscope. HIM revealed sub-500 nm details, including dorsal tubercles and spines in males, while females displayed a smooth tegument. Both sexes had spines on the suckers, primarily on the dorsal face. TEM of the male body wall showed well-organized single structures, large spines, vacuoles, vesicles, cytoplasmic inclusions, a nucleus, and muscle layers. Cryofixation minimized distortion, preserving ultrastructural integrity better than chemical fixation. These advancements in HIM and TEM enhance the understanding of *Schistosoma mansoni* ultrastructure, supporting future research on parasite biology and therapeutic targets.

Keywords: *Schistosoma mansoni*, freezing and microscopy.