Giardia lamblia is a pathogenic protozoan that causes intestinal disorders in humans. This unicellular organism, although considered one of the earliest eukaryotic cells, presented a complex microtubular cytoskeleton formed by an adhesive disk, four pairs of flagella, funis and a median body. The organization of Giardia cytoskeleton at the ultrastructural level has been analyzed by different microscopy techniques, including high resolution scanning electron microscopy. Nevertheless, recent advances in scanning microscopy technology, which resulted in the development of ultra-high resolution scanning electron microscopy (UHRSEM) and helium ion microscopy (HIM), have opened a new venue to the detailed characterization of new cellular structures. Here we studied the organization of the cytoskeleton of trophozoites of Giardia lamblia using UHRSEM and HIM in membrane extracted cells. Giardia disk was arranged in a spiral organization with cross-bridges measuring around 25 nm in length and connecting their elements. HIM showed a compacted arrangement the microtubules of the ventral disk periphery that were maintained even after break up of the cross-bridge (Fig. 1A-1B). The banded collar, found in the barea area, presented three segments and was associated to the axonemes and the disk microtubules (Fig. 1C). The microtubular sheets of the funis presented a lattice-like array (Fig. 1D). Images of marginal plates showed that these structures were associated with a network of filaments not identified before (Fig. 1E), being the last one spread out in all dorsal surface of the trophozoite. Cells showed a set of filaments oriented parallel to the main axis of the cell body that extended through all cell periphery (Fig. 1F). Taken together, these data revealed the presence of new structures of the cytoskeleton of Giardia lamblia and contribute to the understanding organization of Giardia trophozoites.

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Fig. 1: A-B. HIM images showed a compacted arrangement the microtubules of the ventral disk periphery (arrows in A and * in B). C. The lattice-like array of the funis was visualized (F). D. The banded collar had three segments (arrowhead). E. Marginal plates (MP) were associated with a network of filaments (large arrows). AX: axoneme.