Evolutionary relationships of Trypanosomes in the subfamily Leishmaniinae from a quantitative proteomics point of view: The PhyloQuant approach

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The study of evolutionary histories and relationships, termed phylogeny, is crucial in understanding similarities and differences among organisms, which, in the case of host-pathogen interaction, is important to elucidate key characteristics acquired or lost through time. Molecular phylogeny, which entails the comparative analysis of nucleotide or amino acid sequence variations of genes and proteins, respectively, has been the cornerstone in the study of molecular evolution and phylogenetics, helping resolve closely related organisms and unravel previously undescribed evolutionary relationships. Advances in mass spectrometry-based proteomics has enabled the rapid, accurate and sensitive analysis of high-throughput data, and, in the past few decades, the application of mass spectrometry for the classification and identification of bacteria and other microorganisms has paved the way for the development of novel methodologies for evolutionary inferences of organisms using proteomics datasets. In the present work, we describe a novel method, termed *PhyloQuant*, to infer evolutionary relationships of organisms based on differential protein expression intensities following mass spectrometry-based quantitative proteomics. Using this approach, we have reconstructed the evolutionary relationships among trypanosomes of the subfamily Leishmaniinae, which comprise of pathogen agents of leishmaniasis. Congruent to molecular phylogenetics from concatenated SSU rRNA and gGAPDH gene sequences, we show that this method is able to cluster dixenous species of the genus Leishmania, subgenera *L. (Leishmania), L. (Viannia)* and *L. (Mundinia)*, sister to the dixenous species of genera *Endotrypanum* and *Porcisia*, while species of genera *Zelonia*, *Crithidia* and *Leptomonas*, so far described as monoxenous of insects although eventually reported from humans, were positioned basal to the dixenous assemblage. This clustering was maintained for protein and peptide normalized intensities from Proteome Discoverer (PD) and MaxQuant (MQ) softwares, illustrating the robustness of the PhyloQuant approach across different identification and quantification platforms. Moreover, by leveraging the mapping of synapomorphies, the Phyloquant approach enables the unveiling of proteins that support different clades within the phylogenetic tree, thus offering more information crucial to the understand of the differences in many biological processes, host–parasite–vector interactions, infectivity, pathogenicity, virulence, adaptation to new hosts and vectors, and disease manifestations of the organisms under study.

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