Introduction: In the Amazon Region, deforestation resulting from the construction of roads and the establishment of new villages is often associated with epidemic outbreaks of diseases such as leishmaniasis. Because of the wide variety of leishmaniasis vector species and reservoir animals in this region, there is high genetic polymorphism in parasites of the genus Leishmania. Objective: This aim of this study was to identify the species of Leishmania isolated from humans in two municipalities of Amazonas State (Rio Preto da Eva and Manaus) and to evaluate their genetic polymorphisms. Materials and Methods: Twenty-three isolates were analyzed using Random Amplified Polymorphic DNA (RAPD) and isoenzyme electrophoresis. Eight loci were analyzed and compared to known diagnostic loci to assign species to each isolate, based on the similarity of bands. Interspecific variability among the samples was determined by RAPD and analyzed using phenetic analysis and genetic distance to establish the taxonomic relationships among the species/isolates of this genus. Results: Five different zymodemes were identified using a variety of numerical techniques and methods. The zymodeme analysis classified the samples into two species of the subgenus Viannia: L. guyanensis and L. naiffi. All L. guyanensis strains were clustered within the same zymodeme and were found to be polymorphic through RAPD analysis; L. naiffi showed similar patterns. Conclusion: The results revealed apparent isoenzyme homogeneity between samples of L. guyanensis and heterogeneity between samples of L. naiffi. This suggests that these species circulate in both municipalities. The RAPD showed that there was genetic variability among the isolate strains of L. guyanensis and L. naiffi. The relationship between the isolates and its consequences were analyzed, particularly with reference to the importance of selection on genetic markers. Keywords: Polymorphism, Genetic, Leishmania, isoenzymes; Eletroforese; Random Amplified Polymorphic DNA Technique. Financial Support: Instituto Nacional de Pesquisas da Amazônia (INPA) and Fundação de Amparo as Pesquisas do Estado do Amazonas (FAPEAM/Projeto Temático).