Aiming to study the *Trypanosoma cruzi* reservoir at the State of Paraná, Brazil, two regions were selected for this purpose, being two counties at the South and 25 at the Northern region of the State. A total of 75 marsupials (*Didelphis marsupialis* and *D. albiventris*) as well as 71 rodents were captured and examined. From this population of marsupials, 50 stocks (66.6% of the total) were isolated. Four stocks were isolated from the rodents captured. By the technique of Multilocus Enzyme Electrophoresis (MLEE) on cellulose acetate plates (22 genetic loci), 32 stocks have been studied, 29 being identified as *Trypanosoma cruzi*. Genotype diversity was evidenced. MLEE allowed to show that all the stocks were broadly related to Miles zymodeme 1, except for GPI, DIA and MPI loci. DIA isozyme displayed a heterozygote binding pattern for all the stocks identified. Four remaining stocks, which showed to be drastically different from the other ones, probably do not pertain to the *T. cruzi* species. A fair agreement was found between MLEE data, since a highly significant correlation has been put in evidence between genetic distances inferred by MLEE. The linkage unbalance which is confirmed by performing more classical population genetic tests on either MLEE data taken separately, suggests that *T. cruzi* possesses also a basic clonal population structure in the two regions of the State of Paraná sylvan ecosystems studied.