

**Supplementary Table S1:** The sequence analysis of DNA products obtained with the three molecular targets in comparison with DNA sequences of *Trypanosoma* spp. deposited in GenBank confirmed the identification of the three isolates as *Trypanosoma dionisii*.

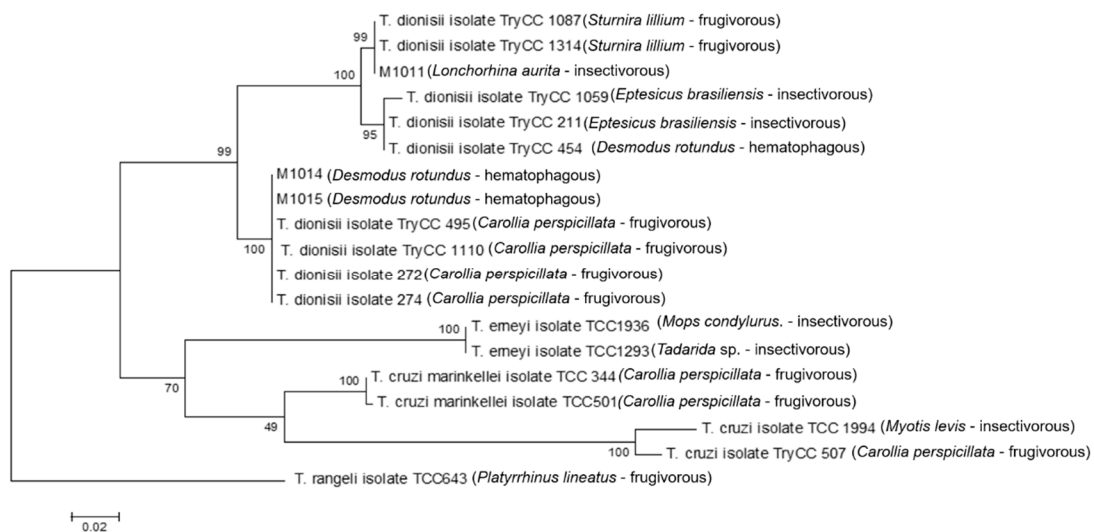
Molecular target	Samples	Identity (%)	Query cover (%)	Reference sequence ( <i>T. dionisii</i> )
18S rDNA	M1011	100	100	KF557744
	M1014	99.84	100	KF557750
	M1015	99.84	100	KF557750
<i>gGAPDH</i>	M1011	99.29	100	GQ140362
	M1014	100	100	MG471432
	M1015	100	100	MG471432
<i>Cytb</i>	M1011	100	100	KF557750
	M1014	100	95	MG471432
	M1015	100	94	FJ900251

For all sequences e-value = 0.0

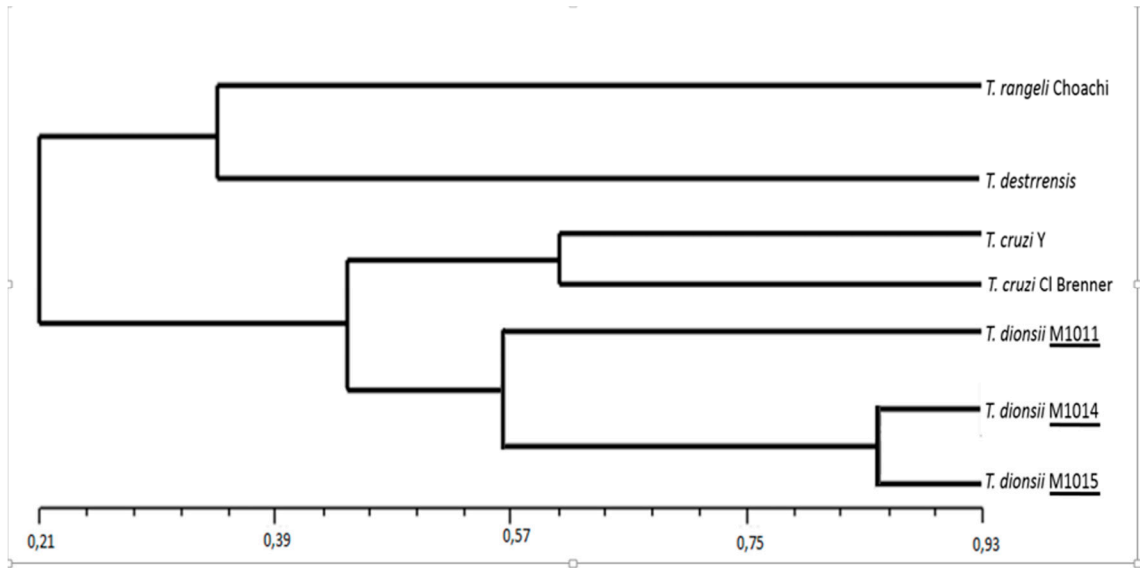
**Supplementary Figure S1:** Schematic representation of the isoenzyme patterns for glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), malic enzyme (ME). A – *Trypanosoma rangeli* (Choachi), B–C– *Trypanosoma c. cruzi* (Y, CL Brener), D – *Trypanosoma desterrensis*, E, F and G – *Trypanosoma dionisii* (M1011, M1014, M1015).



**Supplementary Figure S2:** Phylogenetic analyses demonstrating the clear separation of *Trypanosoma dionisii* isolates into two groups. Phylogenetic tree inferred by Maximum likelihood with the Tamura–Nei model of partial sequences of cytochrome b genes of *T. dionisii* from this study and reference sequences deposited in GenBank were aligned with the Molecular Evolutionary Genetics Analysis (MEGA) program version 7.0.26. Outgroup: *Trypanosoma rangeli*. Bootstrap test results (1000 replicates) are shown next to the branches GenBank accession numbers are shown in Table 1.



**Supplementary Figure S3:** Unweighted pair group method using arithmetic averages (UPGMA) dendrogram built with the simple matching coefficient of similarity based on the genetic profiles obtained from Random Amplified Polymorphic (RAPD) among isolates. *T. dionisii* M1011 was clustered separated M1014 and M1015 samples. *T. c. cruzi* Y, *T. cruzi* ClBrenner, *T. rangeli* Choachi and *T. desterrensis* were used as reference and were clustered totally separated of the *T. dionisii* samples.



**Supplementary Graphic S1:** Body and flagellum measurements of parasites used in morphometric analysis. A. M1011, B. M1014.

