

Supplementary Materials

Table S1. Analysis of the sequences of the 18S rDNA genes (2659 bp) and gGAPDH (528 bp) obtained from the Le29 isolate in BLAST (Basic Local Alignment Search Tool). Including species belonging to the lizard and snake/rodent and marsupial (LSRM) clade.

| Species | 18S rDNA | | | gGAPDH | | |
|-------------------------------|-----------|-------------|------------|---------|-------------|------------|
| | Sites | Query Cover | Identities | Site | Query Cover | Identities |
| <i>Trypanosoma lainsoni</i> | 2118/2135 | 99% | 99.2% | 518/521 | 100% | 99.42% |
| <i>Trypanosoma freitasi</i> | 1989/2079 | 98% | 95.67% | 473/521 | 100% | 90.79% |
| <i>Trypanosoma gennarii</i> | 2003/2082 | 96% | 96.2% | 469/521 | 100% | 90.02% |
| <i>Trypanosoma cascavelli</i> | 2020/2139 | 98% | 94.43% | 479/521 | 100% | 91.94% |
| <i>Trypanosoma serpentis</i> | 1948/2009 | 93% | 96.96% | 478/521 | 100% | 91.74% |

Table S2. Values of genetic distance of the species of the genus *Trypanosoma* of concatenated alignment between the 18S rDNA and gGAPDH genes in percentage %, calculation of the p-corrected value by means of the MEGA program. The light blue squares represent genetic distances less than 1% and in green, the genetic distance between Le 29 and *T. lainsoni*.

Table S3. Values of genetic distance of the *T. cruzi* strains: *T. cruzi* Esmeraldo, *T. cruzi* Silvio, *T. cruzi* marinkellei B7, and *T. cruzi* MT4167.

| | <i>T. cruzi</i> Strains | 1 | 2 | 3 | 4 |
|---|--------------------------------|-----|-----|-----|---|
| 1 | <i>T. cruzi</i> Silvio | | | | |
| 2 | <i>T. cruzi</i> Esmeraldo | 0.9 | | | |
| 3 | <i>T. cruzi</i> marinkellei B7 | 1.0 | 0.4 | | |
| 4 | <i>T. cruzi</i> MT4167 | 0.7 | 0.6 | 0.8 | |

Table S4. This table shows the external and internal primers used in the sequencing of the 18S rDNA gene.

| Primers | Sequence |
|------------------|-------------------------------|
| External Primers | |
| 18sp3pL | 5'-TGCTTGTTCAGGACTTAGC-3' |
| 18sp3pR | 5'-TACGCTTGGAGCTGGAAT-3' |
| Internal Primers | |
| 18sSEQ1F | 5'-CCCAGAACATTGAGGAGCAT-3' |
| 18sSEQ1R | 5'-TGGGAAAACCACCGGAATCAC-3' |
| 18sSEQ2F | 5'-CCGTGAAGAAGTAAGAAAACGTG-3' |
| 18sSEQ2R | 5'-TATCCTCAGCACGTTTCTTACTT-3' |
| 18sSEQ3F | 5'-GCTCCTTGTATCCATGC-3' |
| 18sSEQ3R | 5'-CATGGATAACAAAGGAGCAG-3' |
| 18sSEQ4F | 5'-ACTGGCTGATAGGGCAGTTG-3' |
| 18sSEQ4R | 5'-GGAGAGGGAGCCTGAGAAAT-3' |

Table S5. Hosts and geographic origin of trypanosomatids and GenBank accession numbers of sequences included in this study.

| Species | Origin | | GenBank Accession Number | |
|-------------------------------|-------------------------------------|---------------------|--------------------------|------------|
| | Host | Geographic Location | gGAPDH | 18S rDNA |
| <i>T. lainsoni</i> | <i>Mesomys</i> sp. | Brazil | MF406153.1 | MF403111.1 |
| <i>T. freitasi</i> | <i>Didelphis albiventris</i> | Brazil | MF401952.1 | MF401951.1 |
| <i>T. cascavelli</i> | <i>Crotalus durissus terrificus</i> | Brazil | FJ236511.1 | EUO95837 |
| <i>T. serpentis</i> | <i>Pseudoboa nigra</i> | Brazil | FJ236512.1 | EUO95839 |
| <i>T. sp. Gecko</i> | <i>Tarentola annularis</i> | Senegal | AJ620259.1 | AJ620548.1 |
| <i>T. varani</i> | <i>Varanus exanthematicus</i> | Senegal | AB362559.1 | AJ005279.1 |
| <i>T. avium</i> | <i>Chaffinch Fringilla coelebs</i> | Czech Republic | AJ620263.1 | AJ009140.1 |
| <i>T. theileri</i> | <i>Bos taurus</i> | Germany | KU587664.1 | AJ009163.1 |
| <i>T. cyclop</i> | <i>Macaca nemestrina</i> | Malaysia | - | AJ131958.1 |
| <i>T. pestanai</i> | <i>Meles meles</i> | France | AJ620275.1 | AJ009159.1 |
| <i>T. vivax</i> | <i>Bos Taurus</i> | Africa | AF053744.1 | U22316.1 |
| <i>T. brucei rhodensiense</i> | <i>Homo sapiens</i> | Zambia | AJ620284.1 | KX007997.1 |
| <i>T. evansi</i> | <i>Hydrochoerus hydrochaeris</i> | Brazil | AF053743.1 | KY114577.1 |
| <i>T. simiae</i> | <i>Glossina m. submorsitans</i> | Gambia | AJ620293.1 | AJ009162.1 |
| <i>T. congolense</i> | <i>Bos Taurus</i> | Gambia | AJ620291.1 | U22318.1 |
| <i>T. lewisi</i> | <i>Rattus rattus</i> | England | AJ620272.1 | AJ009156.1 |
| <i>T. microti</i> | <i>Microtis agrestis</i> | England | AJ620273.1 | AJ009158.1 |
| <i>T. livingstonei</i> | <i>Hipposideros caffer</i> | Mozambique | KF192969.1 | KF192983.1 |

| <i>T. cruzi marinkelei</i> | <i>Phyllostomus discolor</i> | Brazil | AJ620270.1 | AJ009150.1 |
|----------------------------|----------------------------------|---------------------------|------------|------------|
| <i>T. cruzi</i> | Mammals | America | MG471429.1 | AF288661.1 |
| <i>T. erneyi</i> | <i>Tadarida</i> sp. | Mozambique | JN040964.1 | JN040988.1 |
| <i>T. rangeli</i> | Mammals | Central and South America | AF053742.1 | AY491767.1 |
| <i>T. vespertilionis</i> | <i>Pipistrellus pipistrellus</i> | England | AJ620283.1 | AJ009166.1 |
| <i>T. conhorini</i> | <i>Rattus rattus</i> | Brazil | AJ620267.1 | AJ012411.1 |
| <i>T. dionisi</i> | <i>Pipistrellus pipistrellus</i> | England | MH393937 | AJ009151.1 |
| <i>T. binneyi</i> | <i>Bufo regularis</i> | Canada | AJ620266.1 | - |
| <i>T. rotatorium</i> | <i>Rana catesbeiana</i> | Canada | AJ620256.1 | AJ009161.1 |
| <i>T. mega</i> | <i>Bufo regularis</i> | Africa | AJ620253.1 | AJ009157 |
| <i>H. megaseliae</i> | <i>Megaselia scalaris</i> | USA | JQ359749.1 | U01014.1 |
| <i>H. muscarum</i> | <i>Musca domestica</i> | USA | JQ359748.1 | L18872.1 |
| <i>H. samuelpessoai</i> | <i>Chrysomya putoria</i> | Guinea-Bisáu | JQ359732.1 | U01016.1 |
| <i>C. fasciculata</i> | <i>Anopheles maculipennis</i> | Unknown | AF053739.1 | Y00055.1 |
| <i>C. brevicerca</i> | <i>Nabis brevis</i> | Europe | KJ443342.1 | MT232051.1 |
| <i>L. brasiliensis</i> | <i>Homo sapiens</i> | Brazil | - | JX030135.1 |
| <i>L. parentolae</i> | <i>Tarentola mauritanica</i> | Algeria | KX790726.1 | - |

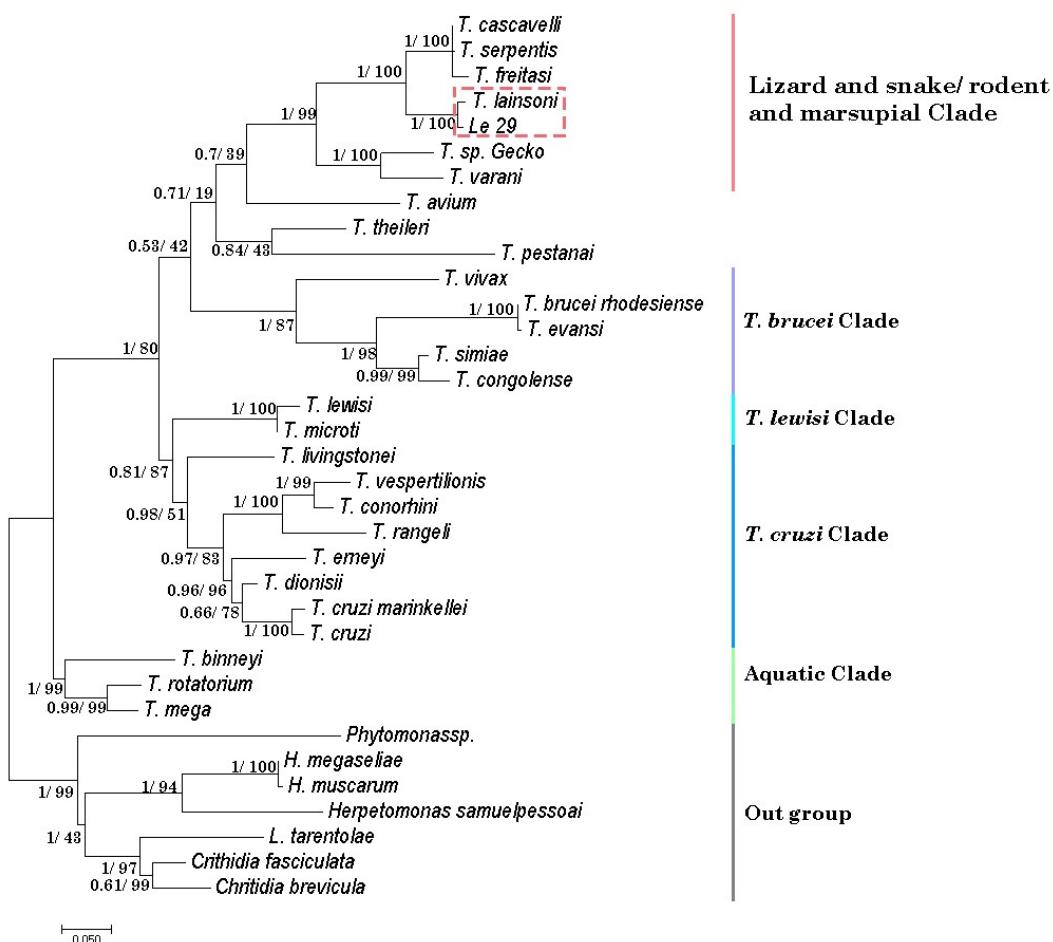


Figure S1. Phylogenetic reconstruction performed by the maximum likelihood method from 528 bp of the gGAPDH gene in 35 species of trypanosomatids. Species of the genera *Herpetomonas*, *Crithidia*, *Leishmania*, and *Phytomonas* were included as out groups. The two values at the nodes represent: Bayesian analysis support/ML method bootstrap value. *Trypanosoma lainsoni* and Le29 are found within the clade LSRM.

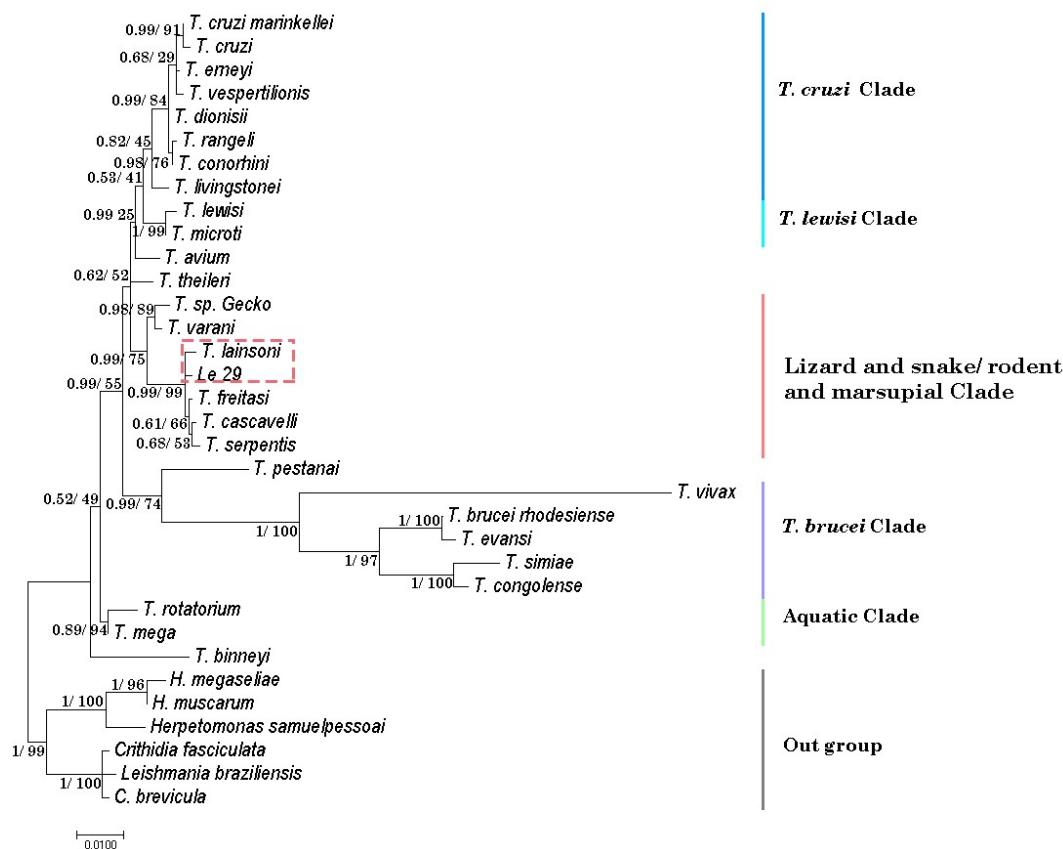


Figure S2. Phylogenetic reconstruction performed by the maximum likelihood method from 1431 bp of the 18S rDNA gene in 34 species of trypanosomatids. Species of the genera *Herpetomonas*, *Crithidia*, and *Leishmania* were included as out groups. The two values at the nodes represent: Bayesian analysis support/ML method bootstrap value. *Trypanosoma lainsoni* and Le29 are found within the clade LSRM.