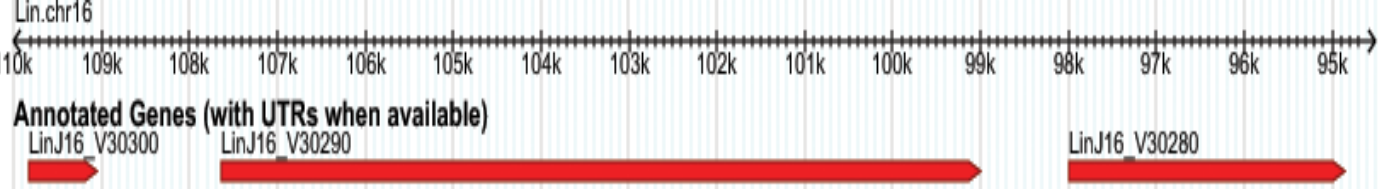
A

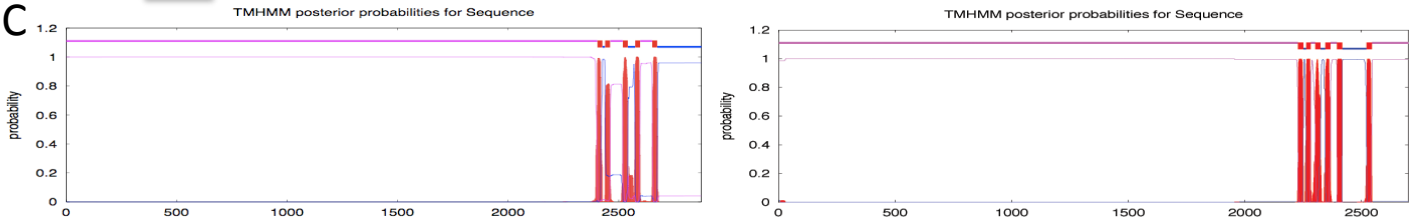


B

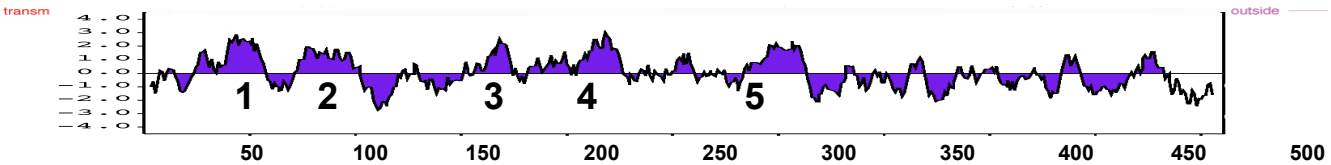
Domains by InterProScan:

- **IP_3/Ryanodine receptor (13-199 AA)**
- **MIR (78-165, 210-395 AA)**
- **RyR/IP_3 homology associated (2004-2104 AA)**
- **Transmembrane regions (2404-2674 AA)**
- **Ryanodine receptor-related (2619-2777 AA)**

C



D

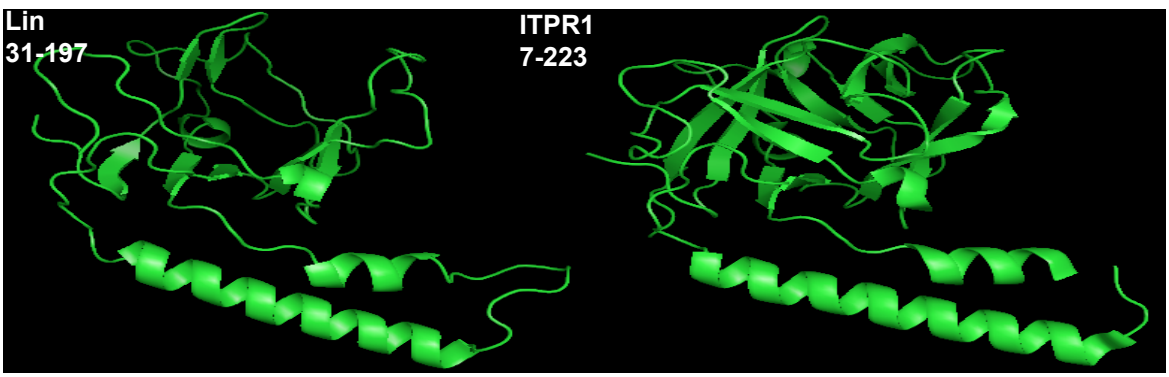


E

```
CLUSTAL 2.0.12 multiple sequence alignment

IP3R2       KDDFTMEVDRLKNRTPVTGNHGVPTMTLSSMMETCQKENCSPTIPSSNTAGEEGEDGIERTCDTLLMCIVTVLNQGLRNGGGVGDVLRRPSKD--EPLFAARVVYD 104
RyR3        ----------------------------RKFYNKSEDDD-EPDMK----------------CDDMMTCYLFHMYVGVRAGGGIGDEIEDPAG---DPYEMYRIVFD 58
Leishmania  --------------------------NFSQLFDPLGARL--NGFN----------------CYTLSQCFLVHVDT-LRSGGGIGDVTNWPVFYSKDGYAYWVMFYR 61
                                     .: :          :                * : * : :   :* ***:**  . *     :      :.:
```

F



G

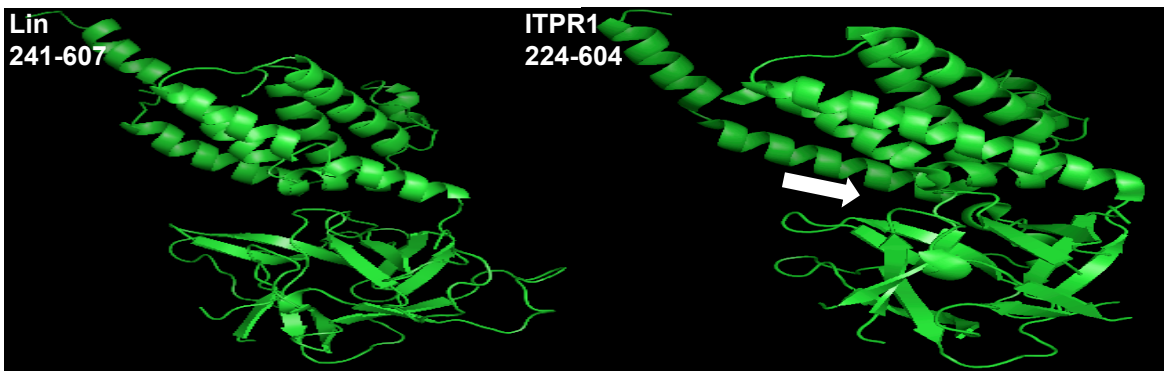


Figure 1: Molecular characterization of putative IP_3 receptor (IP_3R) from *Leishmania infantum*

(A) Schematic representation of macronuclear sequence of putative IP_3R gene *LinJ16_V30290* from *Leishmania infantum.*
The *LinJ16_V30290* gene (chromosome 16) is flanked upstream by gene *LinJ16_V30300* (putative proteasome 26S non-ATPase subunit 9) and downstream by gene *LinJ16_V30280* (conserved hypothetical protein). The *LinJ16_V30290* gene is intronless.

(B) Domain structure of the *Leishmania infantum* putative IP_3R.
Protein BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against reference proteins from mouse (*Mus musculus*) showed that the protein encoded by *LinJ16_V30290* had highest sequence similarity with IP_3R type 2 (IP_3R2) and Ryanodine receptor type 3 (RyR3) which were further used for global sequence similarity and identity analysis by the SIAS server (http://imed.med.ucm.es/Tools/sias.html). Such analysis revealed that the putative IP_3R from *Leishmania infantum* had a global sequence identity and similarity of 11.4% and 54.3% respectively with IP_3R2 and 5.02% and 9.46% respectively with RyR3.
Domain analysis was conducted using the InterProScan server (http://www.ebi.ac.uk/Tools/pfa/iprscan/) and transmembrane domains were determined by the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/
Results are presented relative to scale of the full-length amino acid (AA) sequence of the protein.

(C) Analysis of transmembrane domains of putative IP_3R from *Leishmania infantum* (left) and mouse IP_3R2 (right) using the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/).

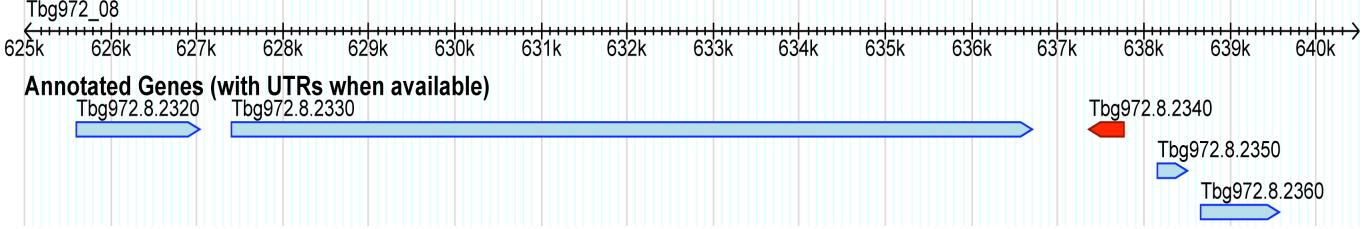
(D) Hydrophobicity analysis of the transmembrane domains of putative IP_3R from *Leishmania infantum.*
Hydrophobicity plots reveal the presence of 5 transmembrane regions compared to 6 that are present in the mouse IP_3R2 (see Panel C (right)).

(E) Clustal W2.0.12 alignment of the amino acid sequence between transmembrane domains 4 and 5 of putative IP_3R from *Leishmania infantum* and the amino acid sequence between transmembrane domains 5 and 6 of mouse IP_3R2 and mouse RyR3. The selectivity filter of mouse IP_3R2 is shown as boxed text.

(F) Modeling of the putative IP_3R-ligand binding suppressor domain of *Leishmania infantum* using the EsyPred3D homology-modelling server (http://www.fundp.ac.be/sciences/biologie/urbm /bioinfo/esypred/).
Structure of the IP_3R-ligand binding suppressor domain of mouse IP_3R type 1 (right), (ITPR1; http://www.pdb.org/pdb/explore/explore.do?structureId=1XZZ) (Bosanac *et al.*, 2005) and model of the *Leishmania infantum* (Lin; left) homologous region. Numbers indicate the amino acid residues used.

(G) Modeling of the putative IP_3R-ligand binding core from *Leishmania infantum* using the EsyPred3D homology-modelling server (http://www.fundp.ac.be/sciences/biologie/urbm /bioinfo/esypred/).
Structure of the IP_3R-ligand binding core of mouse IP_3R type 1 (right), (ITPR1; http://www.pdb.org/pdb/explore/explore.do?structureId=1N4K) (Bosanac *et al.*, 2002) and model of the *Leishmania infantum* (Lin; left) homologous region. Numbers indicate the amino acid residues used. The arrow indicates the IP_3 binding site.

A

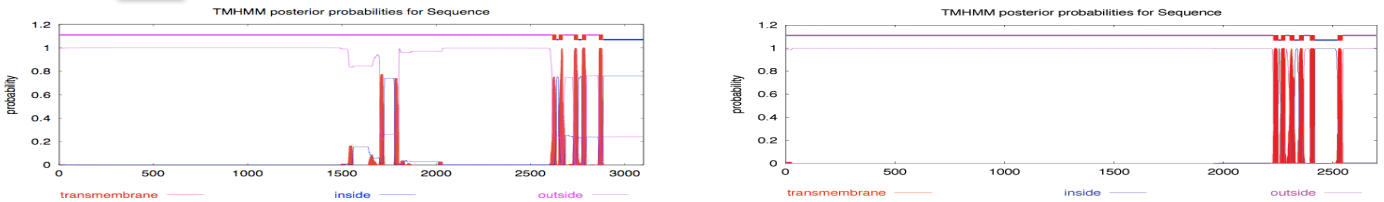


B

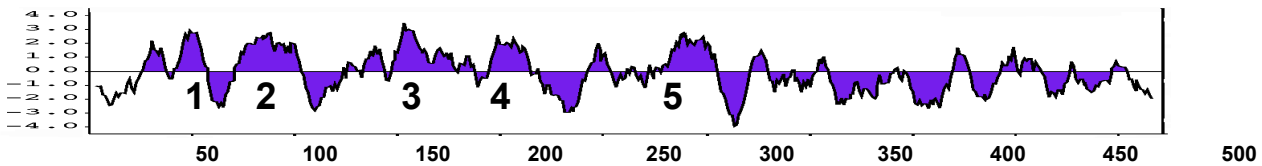
Domains by InterProScan:

- **IP_3/Ryanodine receptor (4-210 AA)**
- **IP_3R binding core, domain 2 (679-730,759-833 AA)**
- **RyR/IP_3 homology associated (2218-2316 AA)**
- **Transmembrane regions (2615-2884 AA)**
- **Ion transport (2737-2880 AA)**
- **Ryanodine receptor-related (2724-2982 AA)**

C



D

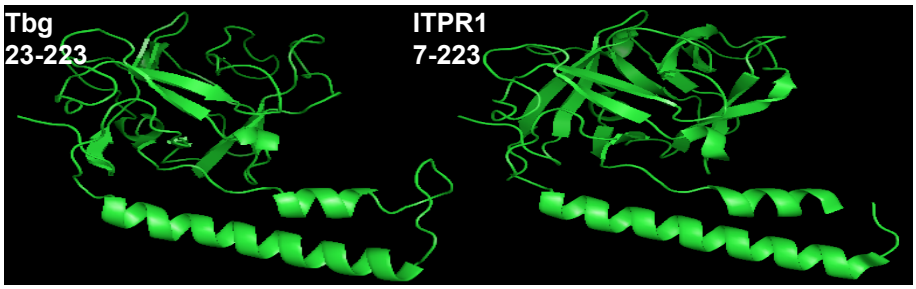


E

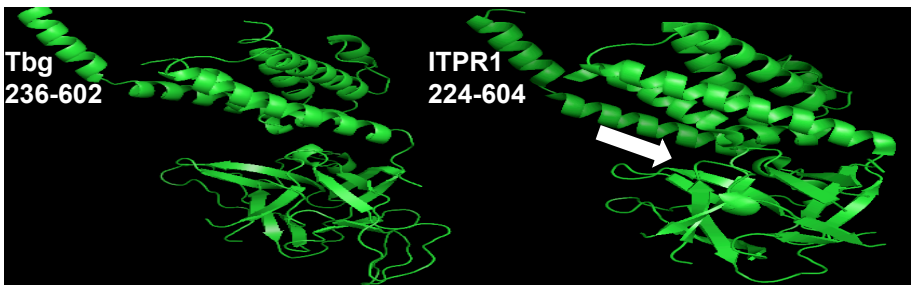
```
CLUSTAL 2.0.12 multiple sequence alignment

Trypanosoma     ----------------IAGRIMFP-------------EQFGSNGEVDENSGKKNDE--NGNCDTLLRCFTFILWQGLRQGGGVGDVMDEVSWNSSTLVPRVSYD 73
IP3R2           KDDFTMEVDRLKNRTPVTGNHGVPTMTLSSMMETCQKENCSPTIPSSNTAGEEGEDGIERTCDTLLMCIVTVLNQGLRNGGGVGTVLRRPSKDEPLFAARVVYD 104
RyR3            ------------------------------------RKFYNKSEDDDEP--------DMKCDDMMTCYLFHMYVGVRAGGGIGDEIEDPA-GDPYEMYRIVFD 58
                                                     .:   .  .: .        : .** :: *    :  *:* ***:** :   : ...    *: :*
```

F



G



H

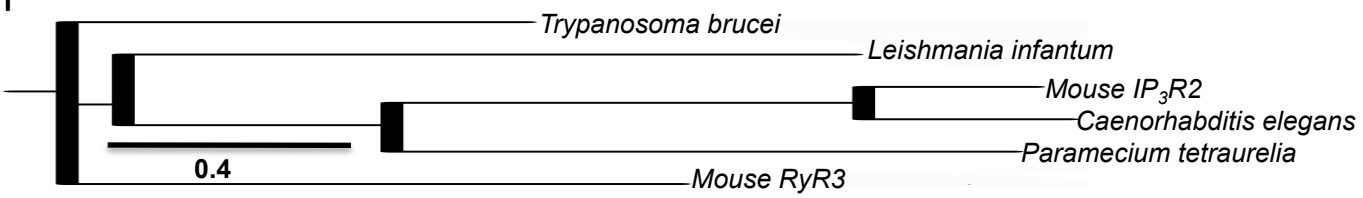


Figure 2: Molecular characterization of putative IP_3 receptor (IP_3R) from *Trypanosoma brucei*

(A) Schematic representation of macronuclear sequence of putative IP_3R gene *Tbg972.8.2330* from *Trypanosoma brucei.*
The *Tbg972.8.2330* gene (chromosome 8) is flanked upstream by gene *Tbg972.8.2320* (conserved hypothetical protein) and downstream by gene *Tbg972.8.2360* (putative RNA-binding protein RBP10). The *Tbg972.8.2330* gene is intronless.

(B) Domain structure of the *Trypanosoma brucei* putative IP_3R.
Protein BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against reference proteins from mouse (*Mus musculus*) showed that the protein encoded by *Tbg972.8.2330* had highest sequence similarity with IP_3R type 2 (IP_3R2) and Ryanodine receptor type 3 (RyR3) which were further used for global sequence similarity and identity analysis by the SIAS server (http://imed.med.ucm.es/Tools/sias.html). Such analysis revealed that the putative IP_3R from *Trypanosoma brucei* had a global sequence identity and similarity of 11.75% and 49.95% respectively with IP_3R2 and 5.33% and 9.3% respectively with RyR3.
Domain analysis was conducted using the InterProScan server (http://www.ebi.ac.uk/Tools/pfa/iprscan/) and transmembrane domains were determined by the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/
Results are presented relative to scale of the full-length amino acid (AA) sequence of the protein.

(C) Analysis of transmembrane domains of putative IP_3R from *Trypanosoma brucei* (left) and mouse IP_3R2 (right) using the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/).

(D) Hydrophobicity analysis of the transmembrane domain of putative IP_3R from *Trypanosoma brucei*.
Hydrophobicity plots reveal the presence of 5 transmembrane regions compared to 6 that are present in the mouse IP_3R2 (see Panel C (right)).

(E) Clustal W2.0.12 alignment of the amino acid sequence between transmembrane domains 4 and 5 of putative IP_3R from *Trypanosoma brucei* and the amino acid sequence between transmembrane domains 5 and 6 of mouse IP_3R2 and mouse RyR3. The selectivity filter of mouse IP_3R2 is shown as boxed text.

(F) Modeling of the putative IP_3R-ligand binding suppressor domain of *Trypanosoma brucei* using the EsyPred3D homology-modelling server (http://www.fundp.ac.be/sciences/biologie/urbm /bioinfo/esypred/).
Structure of the IP_3R-ligand binding suppressor domain of mouse IP_3R type 1 (right), (ITPR1; http://www.pdb.org/pdb/explore/explore.do?structureId=1XZZ) (Bosanac *et al*., 2005) and model of the *Trypanosoma brucei* (Tbg; left) homologous region. Numbers indicate the amino acid residues used.

(G) Modeling of the putative IP_3R-ligand binding core from *Trypanosoma brucei* using the EsyPred3D homology-modelling server (http://www.fundp.ac.be/sciences/biologie/urbm /bioinfo/esypred/).
Structure of the IP_3R-ligand binding core of mouse IP_3R type 1 (right), (ITPR1; http://www.pdb.org/pdb/explore/explore.do?structureId=1N4K) (Bosanac *et al*., 2002) and model of the *Trypanosoma brucei* (Tbg; left) homologous region. Numbers indicate the amino acid residues used. The arrow indicates the IP_3 binding site.

(H) Evolutionary relationship of the putative IP_3Rs from *Leishmania infantum* and *Trypanosoma brucei*.
Predictions from multiple sequence alignments are shown in a neighbor-joining tree with 1000 bootstrap replicates generated with the PHYLIP software package (http://mobyle.pasteur.fr/cgi-bin/portal.py). Sequence representing the mammalian IP_3R was from *Mus musculus* (Mouse IP_3R2 (IP_3R type 2, AB182288). Sequence representing the mammalian Ryanodine receptor was from *Mus musculus* (Mouse RyR3 (Ryanodine receptor type 3, NM_177652). Other metazoa IP_3R sequence was from *Caenorhabditis elegans* (AJ243179) and protozoa IP_3R sequence was from *Paramecium tetraurelia* (CR932323). Evolutionary distances are indicated by the scale bar.