**Figure S1**. Sequence analysis of 5S rRNA genes in different species of *Leishmania*. **A)** Sequence alignment of the 5S rRNA genes present in *L. major* (Lm), *L. mexicana* (Lx), *L. braziliensis* (Lb), *L. infantum* (Li) and *L. tarentolae* (Lt). Genes are indicated by grey shading. The G shown in red (position +1) corresponds to the transcription start site mapped by 5´-RACE analysis in *L. major*. The putative last base of each gene is shown in bold type. At the 3´ end, the clusters of Ts are shown in purple and underlined. The locations of putative box A, intermediate element (IE) and box C are indicated. The type of 5S rRNA gene is indicated. **B)** Characteristics of 5S rRNA genes and transcription termination signals in *Leishmania*. Termination types were classified as: continuous T-cluster (C), discontinuous T-cluster (D) or backup T-tract (B). All distances and lengths are indicated in base pairs. The genes that were identified in this work, and that do not have a TriTrypDB name, are marked with an asterisk. **C)** Frequency of the different types of 5S rRNA genes found in the species of *Leishmania*, as shown in panel A.

**Figure S2**. Sequence comparisons of a 5S rRNA gene in *L. major* (*LmjF.09.5SrRNA.01*) and putative 5S rRNA pseudogenes found in other species of *Leishmania*. LbrM.05.rRNA1, LbrM.15.rRNA1 and LinJ.05.rRNA2 are annotated in TriTrypDB as 5S rRNA genes. LbrM.09.Pseudo5S and LbrM.11.Pseudo5S were identified in this work. The TriTrypDB browser landmark is shown between parentheses for each pseudogene.

**Figure S3**. Predicted consensus secondary structure of 5S rRNA in *Leishmania*. The five helices (I-V) and five loops (A-E) are indicated. The position of the internal control region (Box A, IE and Box C) is also shown. The variable positions (+12, +70 and +105) are denoted.