**Amplicon 1 |...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|..|**

**1676 1685 1695 1705 1715 1725 1735 1745 1755 1765 1775 1785 1798**

*L.* (*L.*) *amazonensis* **ATCCGCTACGTCTCCGCAATCGGTGTCTCCATGGTGCTGTATTTCGTTGCCACCATTGTGGTGCACTCGAGCATGCATGGGCTGAAGGAGGGTATGCGCGGTGACATGAAGTACTTCACCAGC**

*L.* (*L.*) *mexicana***ATCCGCTACGTCTCCGCAATCGGTGTCTCCATGGTGCTGTATTTCGTTGTCACCATTGTGGTGCACTCGAGCATGCATGGGCTGAAGGAGGGCATGCGCGGTGACATGAAGTACTTCACCAGC**

*L.* (*L.*) *aethiopica* **ATCCGCTACGTCTCCGCAATCGGTGTCTTCATGGTGCTGTACTTCGCCGTCACTATTGTGGTGCACTCGAGCATGAATGGGATGAAGGAGGGCATGCGCGGTGACATGAAGTACTTCACCAGC**

*L.* (*L.*) *major* **ATCCGCTACGTCTCCGCAATCGGTGTCTTCATGGTGCTGTATTTCGCCGTCACTATTGTGGTGCACTCGAGCATGAATGGGATGAAGGAGGGCATGCGCGGTGACATGAAGTACTTCACCAGC**

*L.* (*L.*) *tropica* **ATCCGCTACGTCTCCGCAATCGGTGTCTTCATGGTGCTGTATTTCGCCGTCACTATTGTGGTGCACTCGAGCATGAATGGGATGAAGGAGGGCATGCGCGGTGACATGAAGTACTTCACCAGC**

*L.* (*L.*) *donovani* **ATCCGCTACGTCTCCGCAATCGGTGTCTTCATGGTGCTGTATTTTGCCGTCACTATTGTGGTGCACTCGAGCATGAATGGGCTGAAGGAGGGCATGCGCGGTGATATGAAGTACTTCACCAGC**

*L.* (*L.*) *infantum* **ATCCGCTACGTCTCCGCAATCGGTGTCTCAATGGTGCTGTATTTTGCCGTCACTATTGTGGTGCACTCGAGCATGAATGGGCTGAAGGAGGGCATGCGCGGGGACATGAAGTACTTCACCAGC**

*L.* (*V.*) *panamensis* **ATCCGCTACGTCTCCGCCATCGGCGTCACCATGGTGCTGTACTTTGTCGTCGTCATTGTGGTGCACTCCAGCACGAACGGCCTGAAGAAGGGCATGCGAGGCGACATGAAGTACTTCACCACG**

*L.* (*V.*) *braziliensis* **ATCCGCTACGTCTCCGCCATCGGCGTCATCATGGTGCTGTACTTTGTCGTCGTCATTGTGGTGCACTCCAGCACGAACGGCATGAGGGAAGGCATGCGAGGCGACATGAAGTACTTCACCACG**

**Amplicon 2 |...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....||**

**85 94 104 114 124 134 144 154 164 174 184 194 204 215**

*L.* (*L.*) *amazonensis* **GCCGTCACTAAATACCCGAGCGGTGAGCACGACAACCATCCCCTTAAAAGAGGAAGCCTGACAGACTCATCGAGCCACAATGGCAACGGTGCCGACGCCGCCAAGCCGGAGCGCAACATCATCTTCCGGTT**

*L.* (*L.*) *mexicana***GCCGTCACTAAATACCCGAGCGGTGAGCACGACAGCCATCCCCTTAAAAGAGGAAACCTGACGGACTCATCGAGTCACAATGGCAACGGTGCCGACGCCGCCAAGCCGGAGCGCAACATCATCTTCCGGTT**

*L.* (*L.*) *aethiopica* **GCCGTCGGTAAACACCCGAGCGGCGTGCAGGGCAGCCACCCCCACAAAAACGGAAGCCTGACGGACTCATCAAGCCACAATGACAACGGTGCCGACGCCGCCAAGCCGAGCAGCAACATCATCTTCCGCTT**

*L.* (*L.*) *major* **GCCGTCGATAAACACCCGAGCGGAGAGCAGGGAAGCCACCTCCACAAAAGCGGAAGCCTGACGGACTCGTCAAGCCACAATGGCAACGGTGCCGACGCCGCCAAGCCGGAGCACAACATCATCCTCCGCTT**

*L.* (*L.*) *tropica* **GCCGTCGATAAACACCCGAGCGGCGTGCAGGGCAGCCACCCCCACAAAAACGGAAGCCTGACGGACTCGTCAAGCCACAATGACAACGGTGCCGACGCCGCCAAGCCGGGGCGCAACATCATCTTCCGCTT**

*L.* (*L.*) *donovani* **GCCGTCGATAAACACCCGAGCGGCGAGCAGGGCAACCATCTCCACAAAAACGGAAGCCTGACGGCCTCATCAAGCCACAATGAAAACGGCGCCGACGCCGCCAAGCCGGGGCGCAACATCATCTTCCGCTT**

*L.* (*L.*) *infantum* **GCCGTCGATAAACACCCGAGCGGCGAGCAGGGCAACCATCTCCACAAAAACGGAAGCCTGACGGCCTCATCAAGCCACAATGAAAACGGTGCCGACGCCGCCAAGCCGGGGCGCAACATCATCTTCCGCTT**

*L.* (*V.*) *panamensis* **ACGGGCGGTGTTGACCTGAGCGACGGGCAGGTGAAGCGCCCCCTCCACAGTGGGAGCCCGACGGAGTCCACTGGTCACCACAACGACAG---CGACGTCCAGAAGCGGCAGCCCAACATCATCTTCCGCTT**

*L.* (*V.*) *braziliensis* **ACGGGCGGTGTTGACCCGAGCGACGGGCAGGTGAAGCGCCCTCTCCACAGTGGGAGCCCGACGGAGTCCACTGGTCACCACAACGACAG---CGACGTCCAGAAGCGGCAGCCCAACATCATCTTCCGCTT**

**Amplicon 3 |...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|**

**510 519 529 539 549 559 569 579 589 599 609 619 629 639 649**

*L.* (*L.*) *amazonensis* **GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACGCCGATCCTCGAGAAGTCGCCCGGCACGCCCGCATACCTGCTCACCACCTCCGGCAACCGCCTGATCACGAGCCTGATATGGCTCGTGTTCATGGTGCCCG**

*L.* (*L.*) *mexicana***GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACGCCGATCCTCGAGAAGTCGCCCGGCACGCCCGCGTACCTGCTCACCACCTCCGGCAACCGCCTGATCACGAGCTTGGTATGGCTCGTGTTCATGGTGCCCG**

*L.* (*L.*) *aethiopica* **GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACACCGATCCTCGAGAAGTCGCCCGGCACGCCCGCGTACCTGCTAACCACCTCCGGCAACCGCCTGATCACGAGCCTGATATGGCTCGTGTTCATGGTGCCAG**

*L.* (*L.*) *major* **GGCGGTCGCCTACATCAGCGCGGTCAGCAGCCTCATCACACCGATCCTTGAAAAGTCGCCCGGCACGCCCGCGTACCTGCTAACCACCTCCGGCAACCGCGTGATCACGAGCCTGATATGGCTCGTGTTCATGGTGCCAG**

*L.* (*L.*) *tropica* **GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACACCGATCCTCGAGAAGTCGCCCGGCACACCCGCGTACCTGCTAACCACCTCCGGCAACCGCCTGATCACGAGCCTGATATGGCTCGTGTTCATGGTGCCGA**

*L.* (*L.*) *donovani* **GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACACCGATCCTCGAGAAGTCGCCCGGCACGCCCGCGTACCTGCTAACCACCTCCGGCAACCGCCTGATCACGAGTCTGATATGGCTCGTGTTCATGGTGCCAG**

*L.* (*L.*) *infantum* **GGCGGTCGCCTACATCAGCGCCGTCAGCAGCCTCATCACACCGATCCTCGAGAAGTCGCCCGGCACGCCCGCGTACCTTCTAACCACCTCCGGCAACCGCCTGATCACGAGCCTGATATGGCTCGTGTTCATGGTGCCAG**

*L.* (*V.*) *panamensis* **TGCCGTGGCCTACATCAGCGCCGTCAGCAGCCTGATCTCGCCGATCCTCGAGAAGTCCCCTGGGACGCCGGCGTTTCTCCTGACCACTGCCGGCAACCGCTGCATCACCAGCCTTATCTGGCTCGTGCTCATGGTGCCAG**

*L.* (*V.*) *braziliensis* **TGCCGTGGCCTACATCAGCGCCGTCAGCAGCCTGATCTCGCCGATCCTCGAGAAGTCCCCTGGGACGCCGGCGTTTCTCCTGACCACTGCCGGCAACCGCTGCATCACCAGCCTTATCTGGCTCGTGCTCATGGTGCCAG**

**Additional file 3: Alignment of nucleotide sequences of *aap3* coding regions and primer localization.**

The underlined sequences indicate the position of the primers used and the grey boxes represent the variable regions found among the *Leishmania* strains based on *in silico* analysis. The numbers at the top of each amplicon are based on the position of the nucleotides in relation to the whole coding sequence in *L. (L.) amazonensis*.