Fig. S1 Distribution of read depth along *L. major* Friedlin disomic and tetrasomic chromosomes. A; *L. major* chromosome 8 (disomic), B; *L. major* chromosome 31 (tetrasomic).

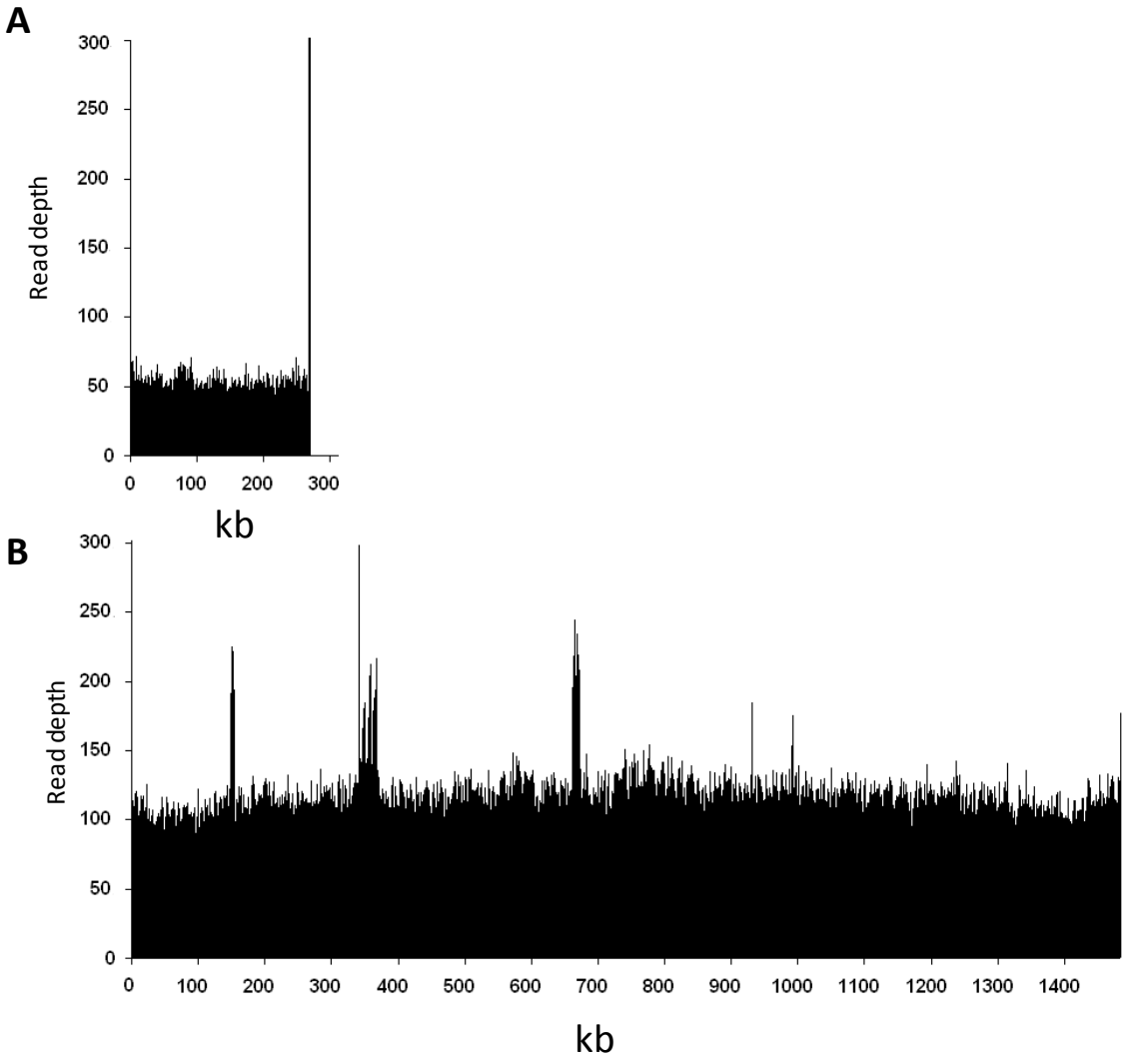


Fig. S2. Distribution of read depth along chromosomes of *L. mexicana* U1103.

Following re-sequencing of reads derived from *L. mexicana* U1103 against the reference genome using MAQ, the generated pileup file was parsed to determine the total reads assigned to each locus of the reference genome. These results were summarised by averaging over contiguous 1000 base blocks, and are shown in the histograms below. The colours used in drawing the histograms are for the purpose of discriminating adjacent chromosomes, and have no other significance. Note the presence of chromosome fusions (8_29 and 20_36, where numbers refer to the chromosome numbers assigned in *L. major*). The read depth scale is in the range 0-250, gene arrays and other repetitive features form marked spikes above the 'background' read depth for the chromosome, which in the case of high copy numbers can extend considerably above this range.

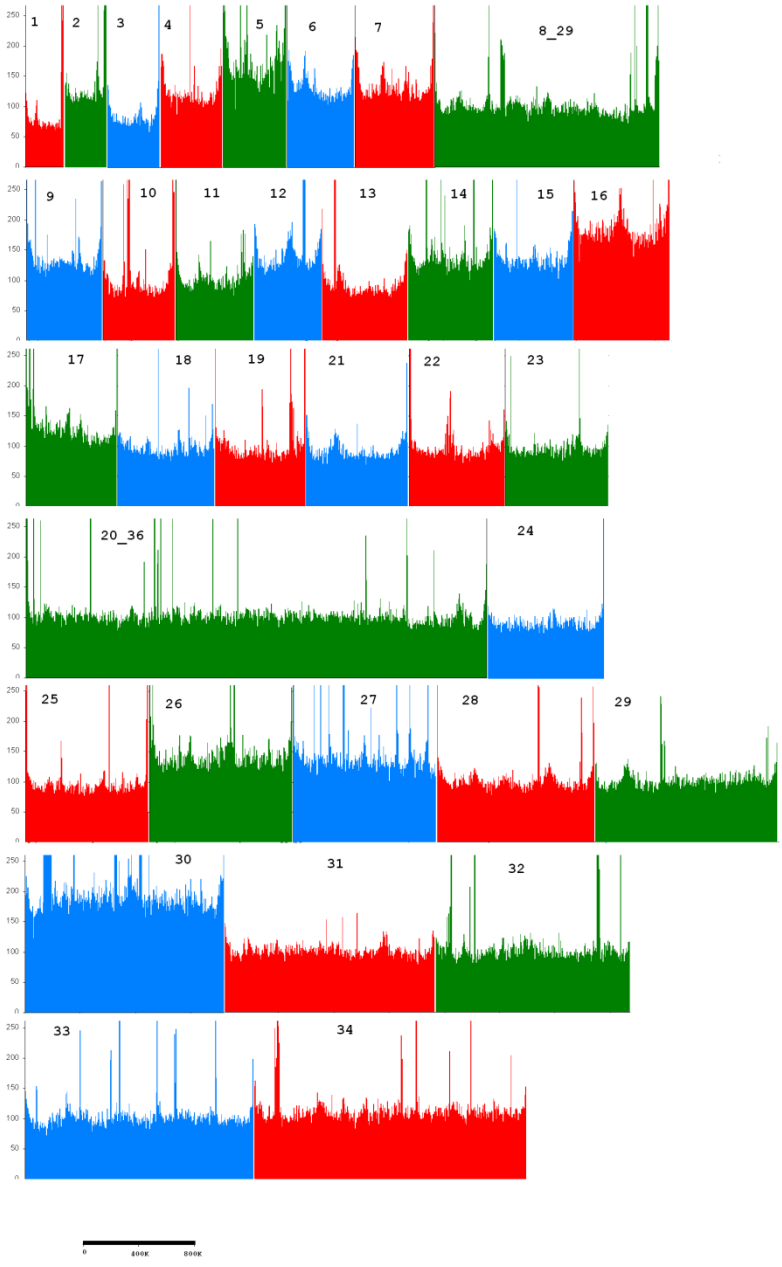


Fig. S3. Distribution of allele frequencies according to inferred ploidy for *L. mexicana* and *L. braziliensis* chromosomes. A) *L. mexicana* disomic chromosomes. (B) *L. mexicana* trisomic chromosomes (C) *L. mexicana* trisomic chromosomes with disomic base frequency profiles (D) *L. mexicana* tetrasomic chromosome (E) *L. braziliensis* triosomic chromosomes. (F) *L. braziliensis* tetrasomic chromosomes (G) *L. braziliensis* hexasomic chromosomes.

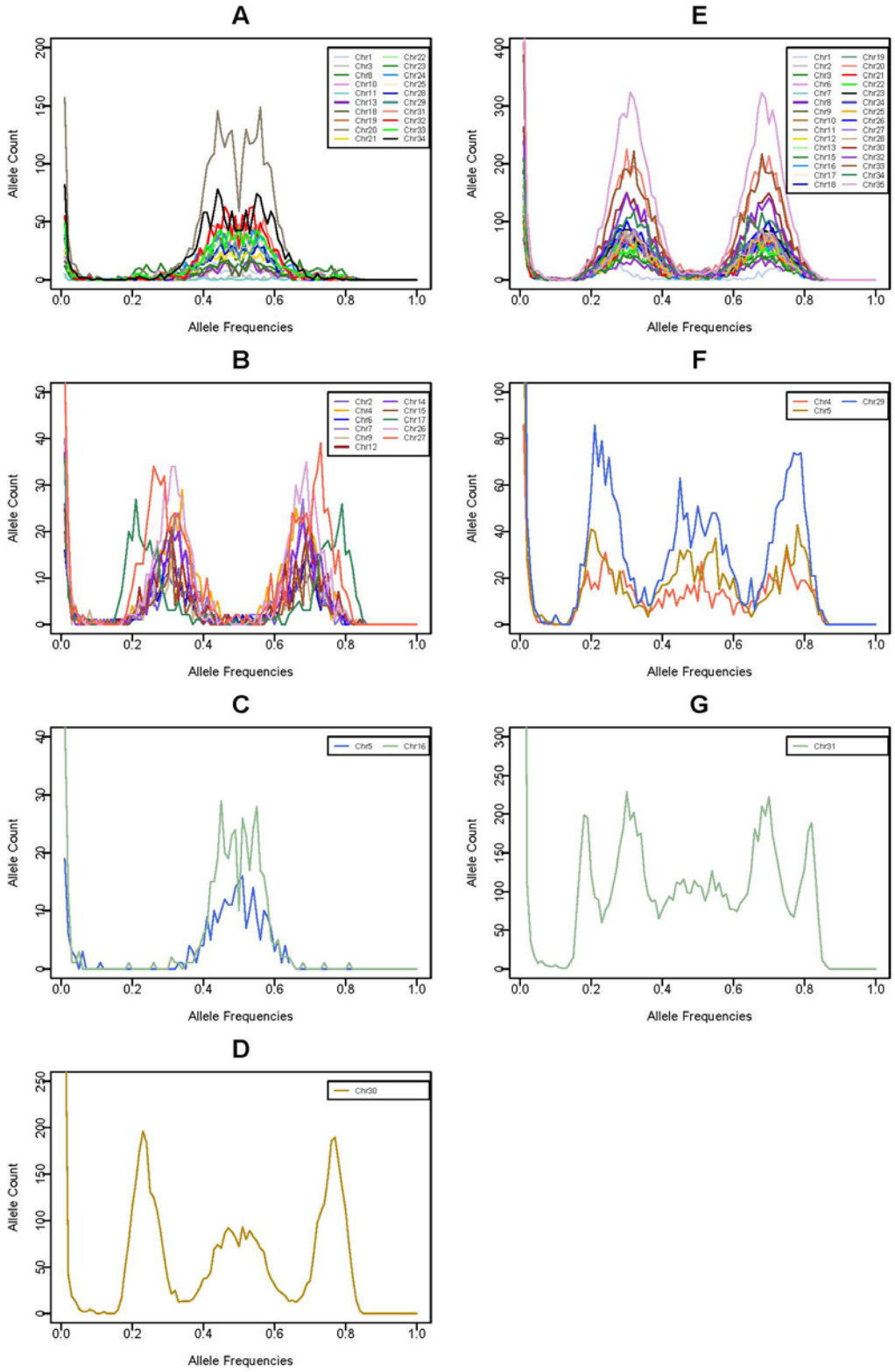
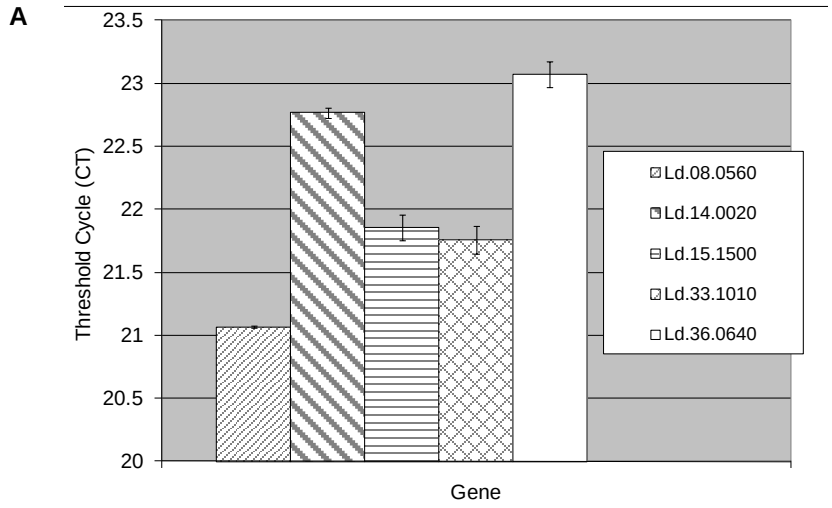
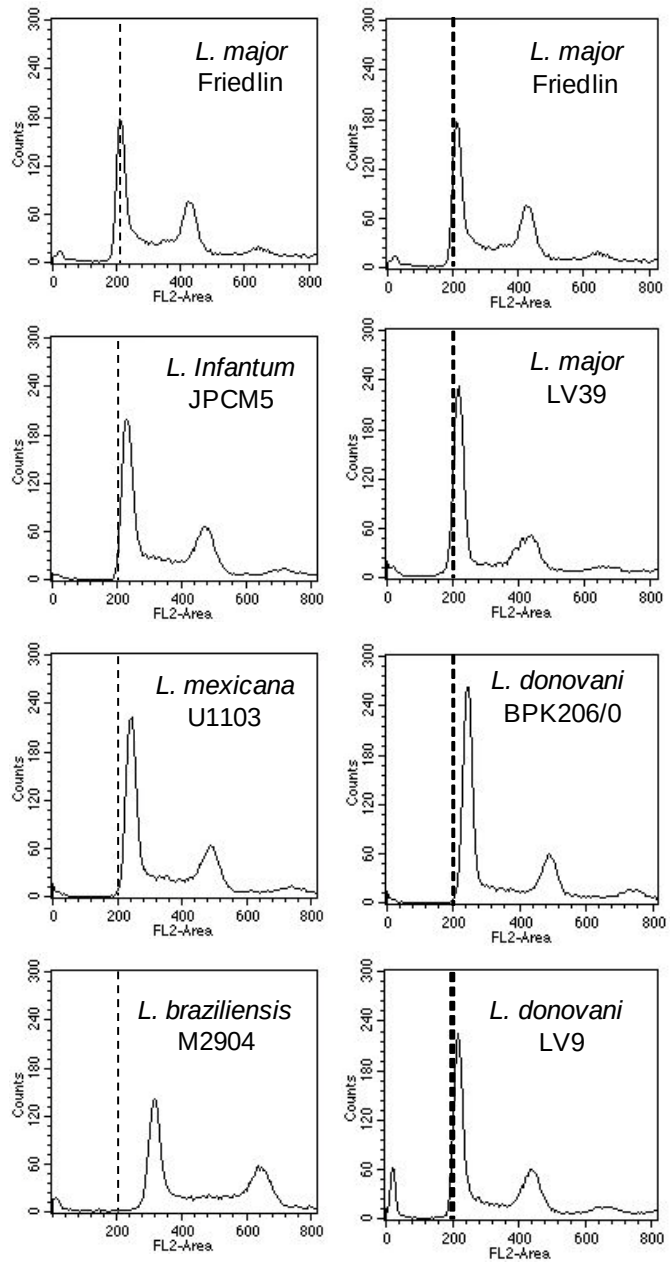
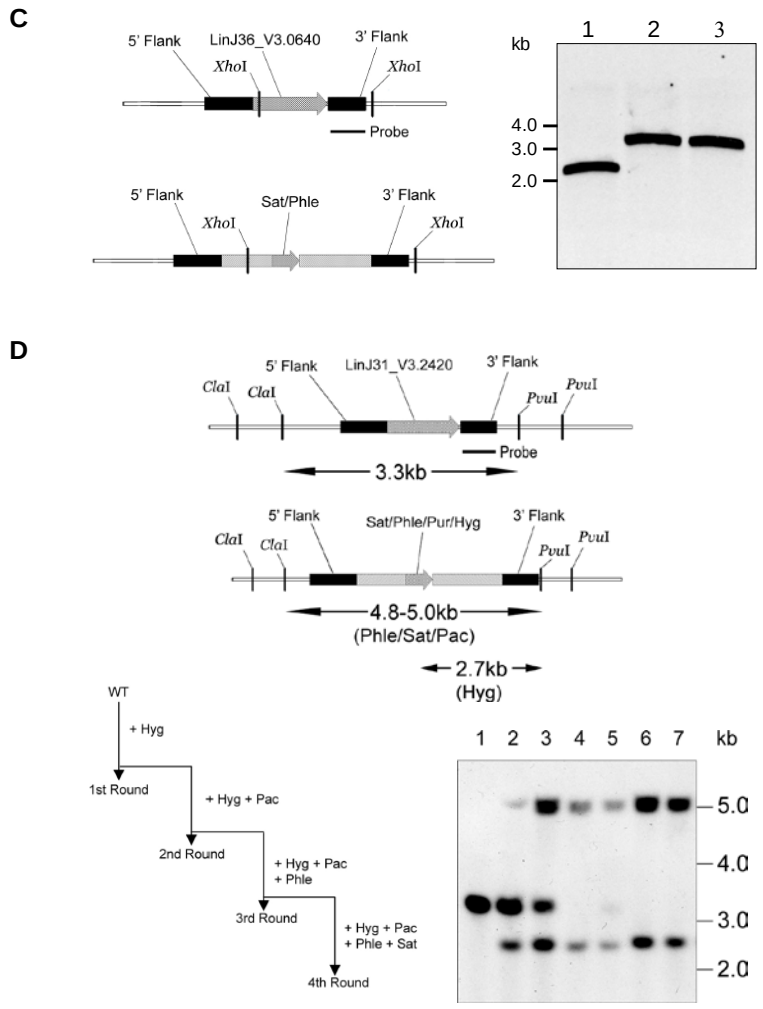


Fig. S4. Validation of chromosome copy number. (A) quantitative PCR for selected genes from disomic, trisomic and tetrasomic chromosomes of *L. donovani* BPK206/0. (B) DNA content analysis for strains and species of *Leishmania*. Mid-log phase promastigotes were fixed and stained with propidium iodide and analysed by FACS. (C) Generation of *L. infantum LinJ.36.0640* null mutants on disomic chromosome 36. *L. infantum* genomic DNA was digested with *Xho*I, separated by agarose gel electrophoresis, blotted and probed with the 3' flank. Lane 1, Wild type; Lane 2, Δ*sec14l* clone 1; Lane 3, Δ*sec14l* clone 2. (D) Generation of *L. infantum LinJ.31.3030* null mutants on tetrasomic chromosome 31. *L. infantum* genomic DNA was double digested with *Cla*I and *Pvu*I, separated by agarose gel electrophoresis, blotted and probed with the 3' flank. Lane 1, Wild type; Lane 2, 2nd round 1; Lane 3, 3rd round; lanes 4-7, *LinJ31.3030* null mutants.



B


L. major
Friedlin
L. major
Friedlin
L. Infantum
JPCM5
L. major
LV39
L. mexicana
U1103
L. donovani
BPK206/0
L. braziliensis
M2904
L. donovani
LV9
Counts
FL2-Area



Methods: Generation and analysis of *L. infantum* null mutants

Leishmania infantum SEC14 and phosphatase null mutants were generated using previously described methods (Denise et al. 2006). The plasmids containing *SAT* or *BLA* antibiotic resistance cassettes used for the double allele deletion of *SEC14* were produced as follows: the 5' Flanking Region (FR) was generated by PCR with primers OL1997: CGAAAGCTTCTCTAACTCTTTCTCTATCGCTG and OL1998: GCAGTCGACGGGGTGAGTGACACGTCCTTTGCG. The 3' FR was generated with PCR primers OL1998: GCAGTCGACGGTGAGTGACACGTCCTTTGCG and OL1999: GCACCCGGGGCTACGGATGCCCGTGTGCTGG. The PCR fragments were sub-cloned into pGEM-T and then released by restriction digest with *Hin*dIII/*Sal*I for the 5' FR and *Xma*I/*Bgl*II for the 3' FR. The fragments were sequentially cloned into a similarly digested *SAT* resistant plasmid pGL1028 to give pGL1534. To produce the *BLA* plasmid, the *SAT* cassette was replaced with the *Spe*I/*Bam*HI resistance cassettes from pGL1442 to give plasmid pGL1535. The integration cassettes were digested from the plasmids with *Bgl*II/*Hin*dIII prior to transfection. Transfection of *L. infantum* (5µg of *Hin*dIII/*Bgl*II linearised plasmid) was carried out as described previously (Denise et al. 2006).

Gene deletion constructs for the phosphatase gene *LinJ.31.3030* were created using the same restriction sites as above. The 5' and 3' flanking regions were generated by PCR with

primers 3030-5F: AAGCTTGCATCCAGAATGCAGCAC and 3030-5R : GGGTCGACTAAGTTGCAGGTGGCGAG; 3030-3F : CCCGGGGAAACGAGCAGCAGTGAG and 3030-3R : GGAGATCTGGTCTGTGGTACGGTATG.
Four constructs containing the 5'UTR and 3'UTR flanking regions of the *LinJ.31.3030* ORF either side of each of four antibiotic resistance genes (*HYG, PAC, BLE, SAT*) were produced for gene deletion on four alleles. Transfection of wild type *L. infantum* was carried out with 5μg of *Hin*dIII/*Bgl*II linearised plasmid. For screening for *LinJ.31.3030* deletions, transfected cells were grown in 96-well plates with antibiotic selection (Hygromycin 32μg/ml, Puromycin 20μg/ml, Bleomycin 15μg/ml, Nourseothricin 100μg/ml). Following the first round of gene deletion, *HYG* positive clones were selected and screened by PCR and Southern blotting. A *HYG* positive clone was then expanded and subject to a second round of targeting using the *PAC* linear construct. *HYG/PAC* positive clones were selected and targeted with the *BLE* linear construct to generate *HYG/PAC/BLE* positive clones. The *SAT* linear construct was used for the fourth and final round of targeting. At each stage, replacement of the *LinJ.31.3030* gene by a drug resistant marker on separate alleles was confirmed by Southern blotting, using the appropriate drug resistant marker and 3'UTR specific probes.

Denise, H., Poot, J., Jimenez, M., Ambit, A., Herrman, D.C., Vermeulen, A.N., Coombs, G.H., and Mottram, J.C. 2006. Studies on the CPA cysteine peptidase in the *Leishmania infantum* genome strain JPCM5. *BMC Mol.Biol.* **7**:42

Fig. S5 Number of arrays on each chromosome of *L. mexicana* U1103 and M379. Chromosomes highlighted in red are supernumerary (See Figure 3).

***L. mexicana* U1103**			***L. mexicana* M379**		
Chromosome	**Number of genes/entries**	**Number of CN arrays**	**Chromosome**	**Number of genes/entries**	**Number of CN arrays**
1	78	3	1	78	7
2	67	1	2	67	4
3	95	2	3	95	4
4	119	0	4	119	0
5	122	0	5	122	3
6	131	1	6	131	4
7	123	1	7	123	4
8_29	394	11	8_29	394	13
9	153	2	9	153	10
10	134	6	10	134	7
11	125	5	11	125	3
12	101	0	12	101	2
13	157	2	**13**	157	1
14	148	2	14	148	3
15	153	1	15	153	6
16	157	1	**16**	157	4
17	148	0	17	148	4
18	163	0	18	163	1
19	156	11	19	156	11
20	883	15	20	883	12
21	216	7	21	216	7
22	161	7	22	161	7
23	186	5	23	186	2
24	235	3	24	235	8
25	253	3	25	253	3
26	265	0	26	265	7
27	257	2	27	257	9
28	305	8	28	305	7
29	368	6	29	368	6
30	309	0	**30**	309	1
31	408	4	31	408	4
32	337	6	32	337	6
33	413	7	33	413	7
34	498	12	34	498	15
Total		134			192