Identification of *Leishmania infantum* epidemiological, drug resistance and pathogenicity biomarkers

The excellence in One Health genomics

J.Martí-Carreras *#, M.Carrasco# , M. Gómez-Ponce, M. Noguera-Julián, M. M. Alcover, F. Vitale, J. Moreno, B. Gad, M. Carla, X. Roura, L. Ferrer, O. Francino*

Nano1Health (N1H), Edifici EUREKA, PRUAB, 08193 Bellaterra, Barcelona, Spain

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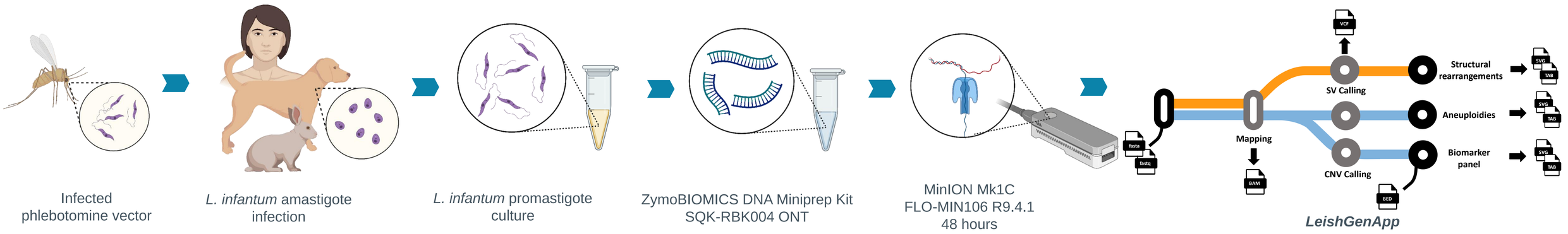
Introduction

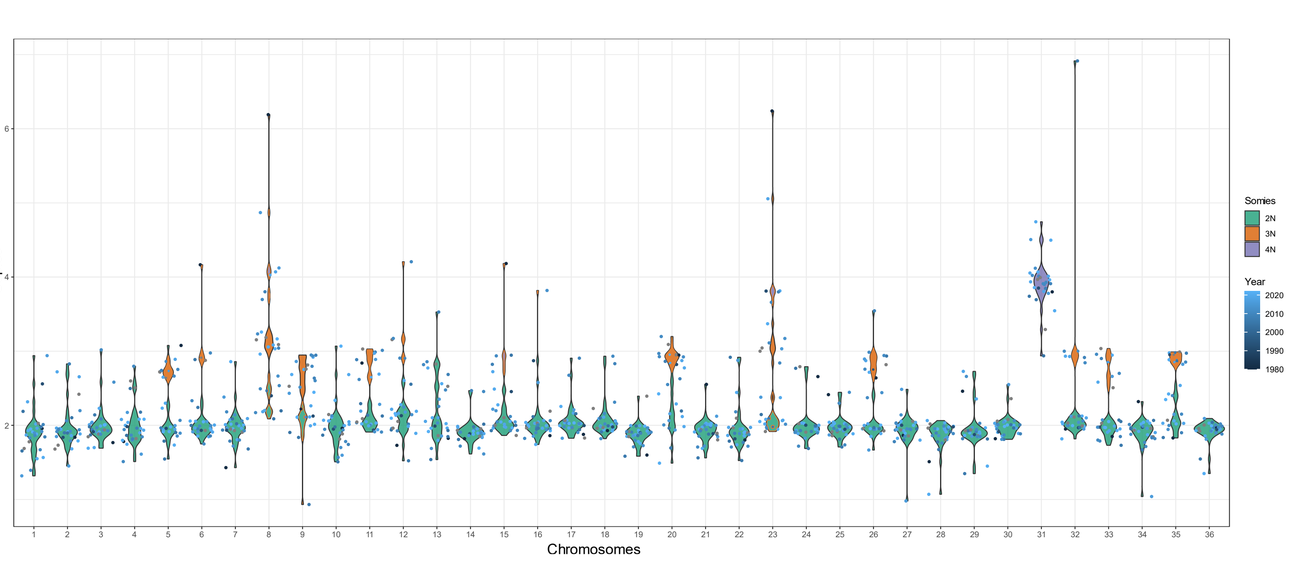
- *Leishmania infantum* is a protist parasite and causative agent of **leishmaniasis**, endemic to many countries **world-wide**, per example the **Mediterranean basin**.
- Emerging **drug-resistant** *L. infantum* is one o f the most relevant **neglected tropical disease**, as it is responsible for **12M infections** w/w and more than **25,000 deaths**.
- It possesses a genome of 32 Mb distributed in 36 chromosomes and a complex network of maxi- and minicircle kinetoplasts (functionally similar to mitochondria).
- Its **genome is structurally complex**, with frequent **rearrangements** and **aneuploidies**. Such changes in **copy number variation (CNV)** occur as a result of **environment adaptation** and can be potentially used **as genetic biomarkers** to create a **genetic resistance profile**.

Material and Methods



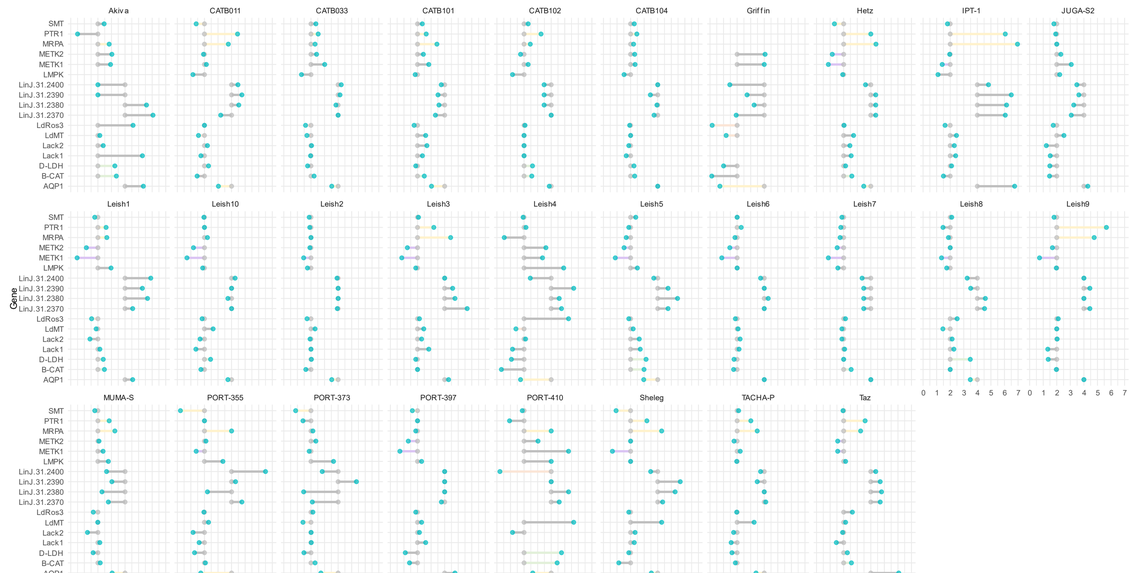
Results

1. *L. infantum* aneuploidies and genome dotation

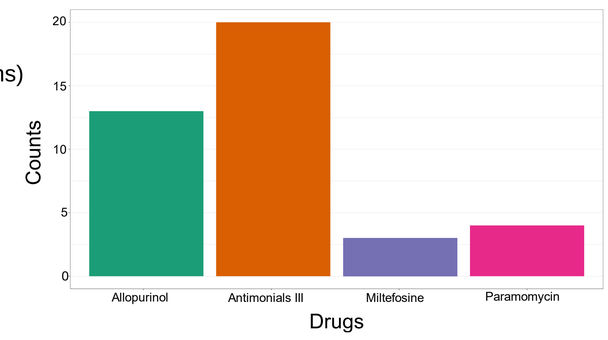


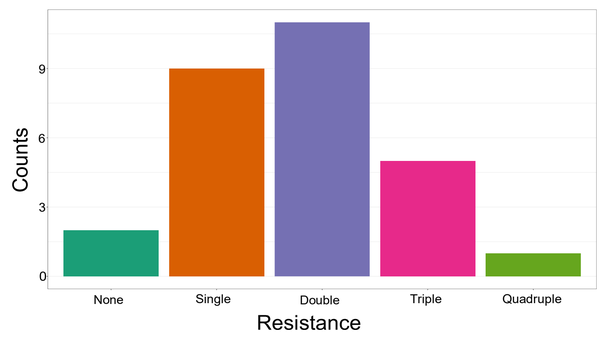
- Sequencing of 28 Mediterranean *L. infantum* isolates shows a wide variability in chromosome dotation
- Most chromosomes are present as 2N, except for chromosomes 31 (4N)
- *L. infantum* displays alterations of chromosome dotation as continuum (mosaicism) or as discrete values (subpopulations)
- Chromosome variation is independent with culturing method, passage history, parasite host or country of isolation

2. *L. infantum* drug resistance CNV biomarkers

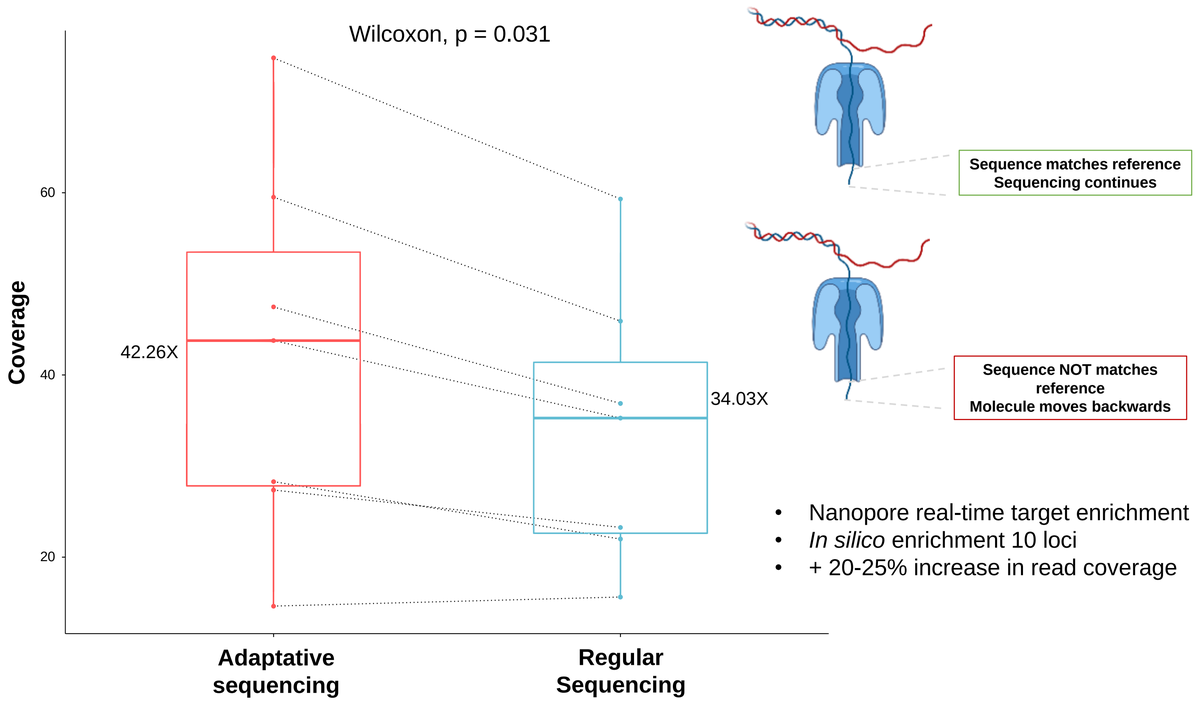


- Each sample displays a different and specific CNV signature for its pharmacoresistance profiling (17 genes)
- Resistance is diverse and locally adaptative (no common origin)
- Multidrug resistance is frequent and resistance to > 1 compound is the most common scenario
- High prevalence of resistance to commonly administrated drugs (allopurinol and antimonials)



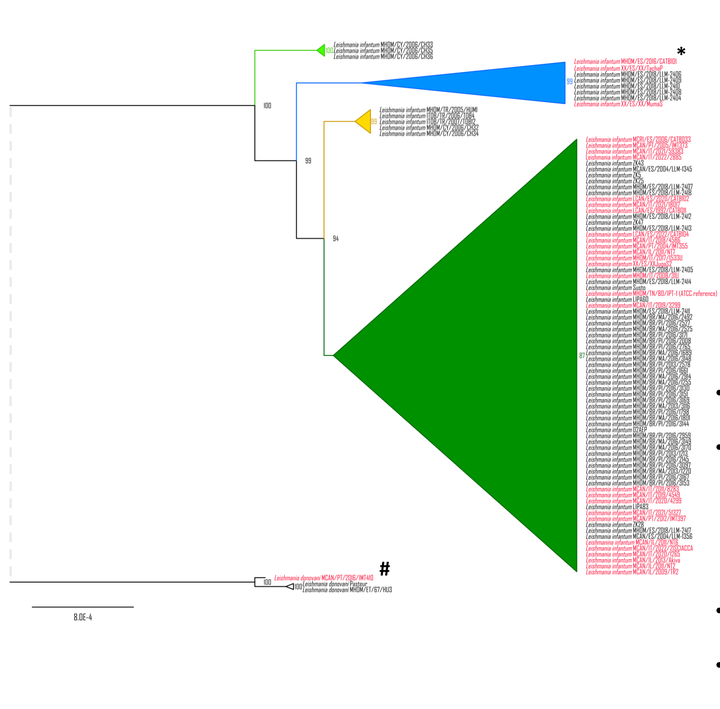


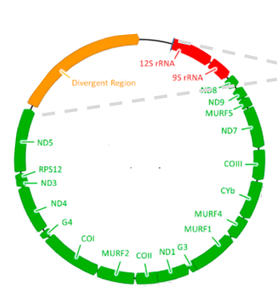
3. Adaptative sampling of drug resistance biomarkers



- Nanopore real-time target enrichment
- *In silico* enrichment 10 loci
- + 20-25% increase in read coverage

4. *L. infantum* maxicircle epidemiology





Adapted from Stark et al., 2019

- >3X coverage for consensus sequence
  - Conserved region (CR)
- 4 epigroups of *L. infantum*
  - I East Mediterranean (CY)
  - II West Mediterranean (SP)
  - III East Mediterranean (TK, CY)
  - IV All Mediterranean region
- Earliest estimate of epigroup II was in Mallorca, 2016 (*)
- Misdiagnosis of *L. donovani* as *L. infantum (#)*

Conclusions

- Complete and mosaic aneuploidies were fully characterized in 28 isolates of *L. infantum*, revealing strong differences in passage history and the emergence of somy subgroups in stable and unstable chromosomes **(1)**.
- A genetic resistance profile to 4 drugs was developed by detecting changes in CNV in a panel of 17 genes. Resistance profiles are diverse. Multiresistance is highly prevalent, especially against allopurinol and trivalent antimonials **(2)**.
- Sequence enrichment of 10 loci through *in silico* adaptative sequencing was able to increase read coverage 20-25% **(3)**.
- Nanopore sequencing was successfully applied to reconstruct maxicircles to classify species and epigroups of *L. infantum* **(4)**.

Acknowledgements

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@J_MartiCarreras
@nano1health