

# Housekeeping MLST-7 and high-resolution MLST-6 schemes show different clustering of *Chlamydia trachomatis* strains derived from MSM and heterosexuals

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## Background

- Chlamydia trachomatis* (Ct) remains the most common bacterial sexually transmitted infection worldwide.
- The most important risk groups for Ct infection are (young) heterosexual persons and men who have sex with men (MSM).
- Multilocus sequence typing (MLST) schemes have been developed for Ct. An MLST scheme based on highly polymorphic regions showed a clear separation of Ct strains derived from heterosexuals on one hand and MSM on the other, reflecting thus the sexual behavior of the hosts<sup>1</sup>.

## Objectives

- To investigate the clustering of Ct strains using two MLST schemes with different discriminatory capacities
- To explore if the separate clusters of strains from heterosexuals and MSM is seen using both MLST schemes

## Methods

- A retrospective analysis was performed using routinely collected data and specimens from 100 Ct infected MSM and 100 heterosexual women. All were visitors of the STI outpatient clinic of the Public Health Service, Amsterdam.
- Selected specimens were typed using two existing MLST schemes.
  - MLST scheme based on 7 housekeeping genes (MLST-7)<sup>2</sup>.
  - MLST scheme based on 6 highly variable targets in the chlamydial genome, also referred to as high-resolution MLST (hr-MLST-6)<sup>3</sup>.
- For this study, the existing MLST-7 scheme was modified to a nested PCR enabling the testing of clinical specimens. For 5 of 7 targets new inner primers were designed.
- Clustering of sequences obtained from both MLST schemes were compared by minimum spanning tree analysis (BioNumerics software version 7.5, Applied Maths, Sint-Martens-Latem, Belgium).

## Results

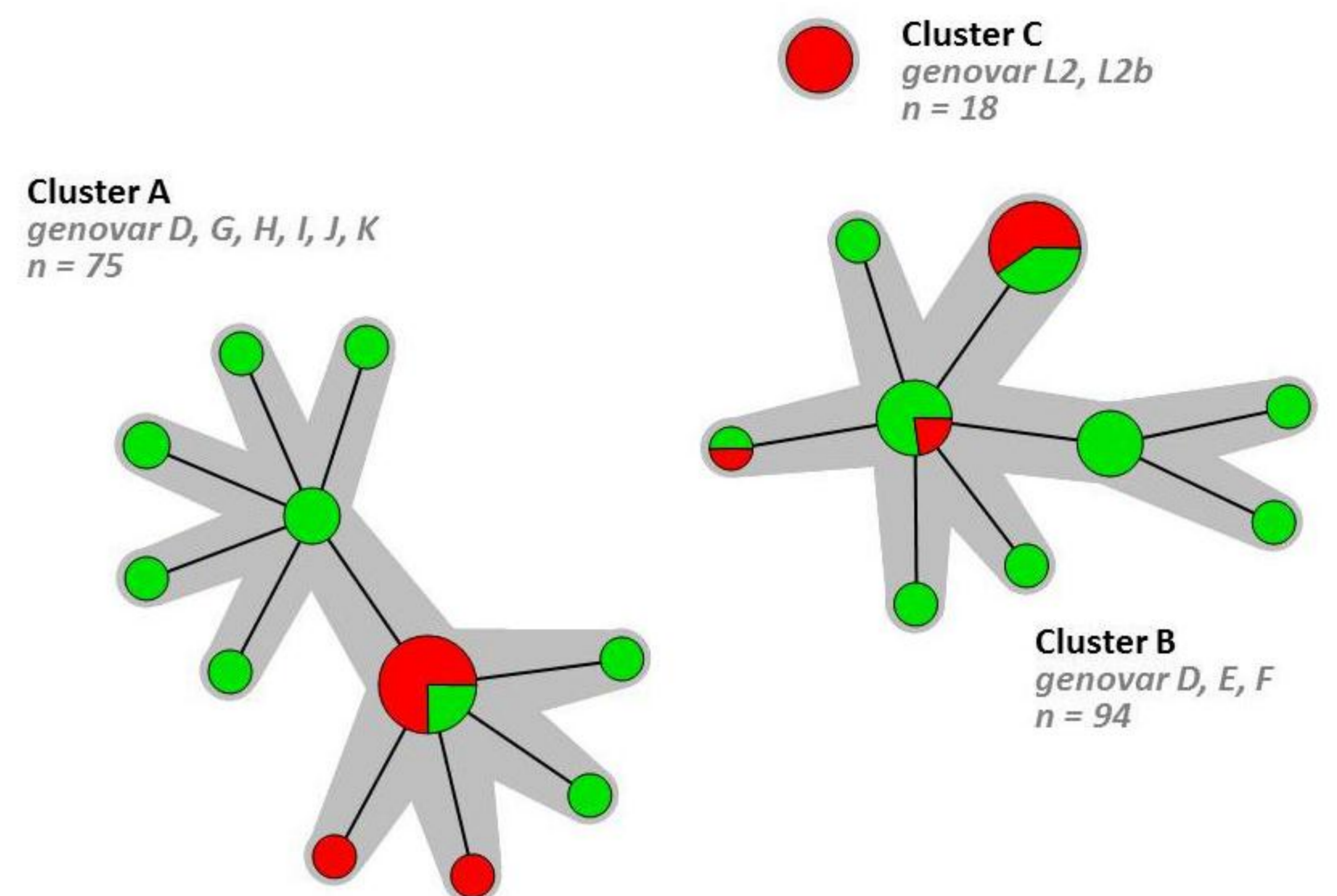
- Full profiles were obtained for 187/200 (94%) specimens; 91 (49%) were from MSM and 96 (51%) from heterosexuals.
- We detected 21 unique sequence types (STs) using the MLST-7 scheme. (86%) were novel to the Chlamydiales MLST database ([pubmlst.org/chlamydiales/](http://pubmlst.org/chlamydiales/))
- In comparison, we detected 79 unique STs using the hr-MLST-6 scheme, which thus provided at least 3 times higher resolution.
- Cluster analysis using the MLST-7 data did not show any segregation between isolates from MSM and heterosexuals (Figure 1 A).
- In comparison, cluster analysis using hr-MLST-6 data did show a clear segregation between isolates from MSM and heterosexuals (Figure 1 B).
- Only LGV-type (L2 and L2b) strains clustered separately for MSM using both MLSTs.
- The hr-MLST-6 scheme further diversified the clusters that were found using the MLST-7 scheme (Figure 2).

## Conclusions

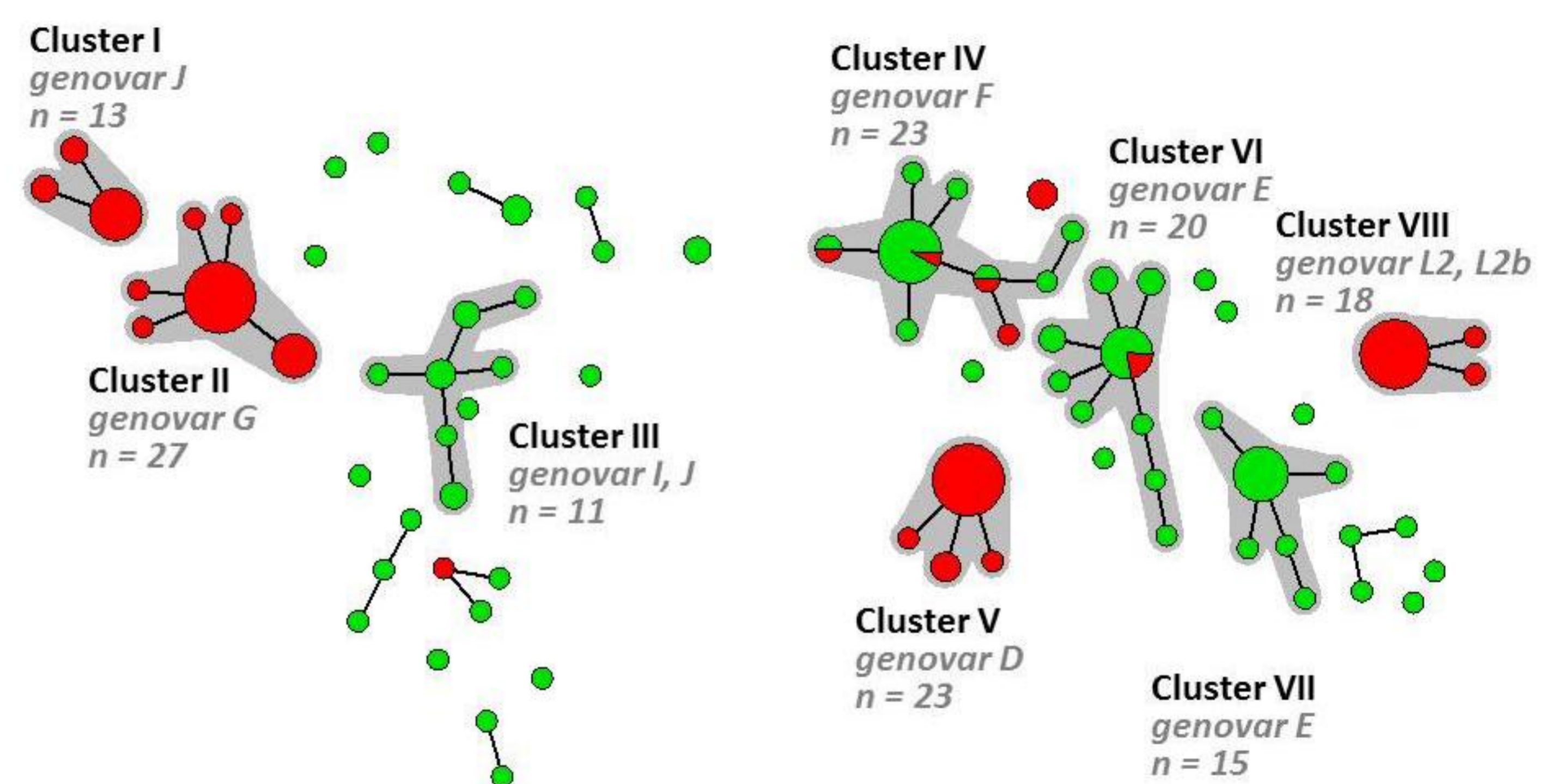
- Using the MLST-7 scheme no reflection of separate transmission between MSM and heterosexual hosts is seen. This method is suitable for evolutionary studies.
- For short-term cluster analysis the hr-MLST-6 scheme is considered more suitable to study the epidemiology and transmission of Ct in various populations.

Figure 1: MLST-7 (A) and hr-MLST-6 (B).

Minimum spanning tree of 187 Ct-positive specimens from MSM and heterosexuals. Sizes of the node discs are proportional to the number of specimens of each sequence type. Branches show one locus difference; halos indicate clusters. Red, MSM (n=91); Green, Heterosexual (n=96).



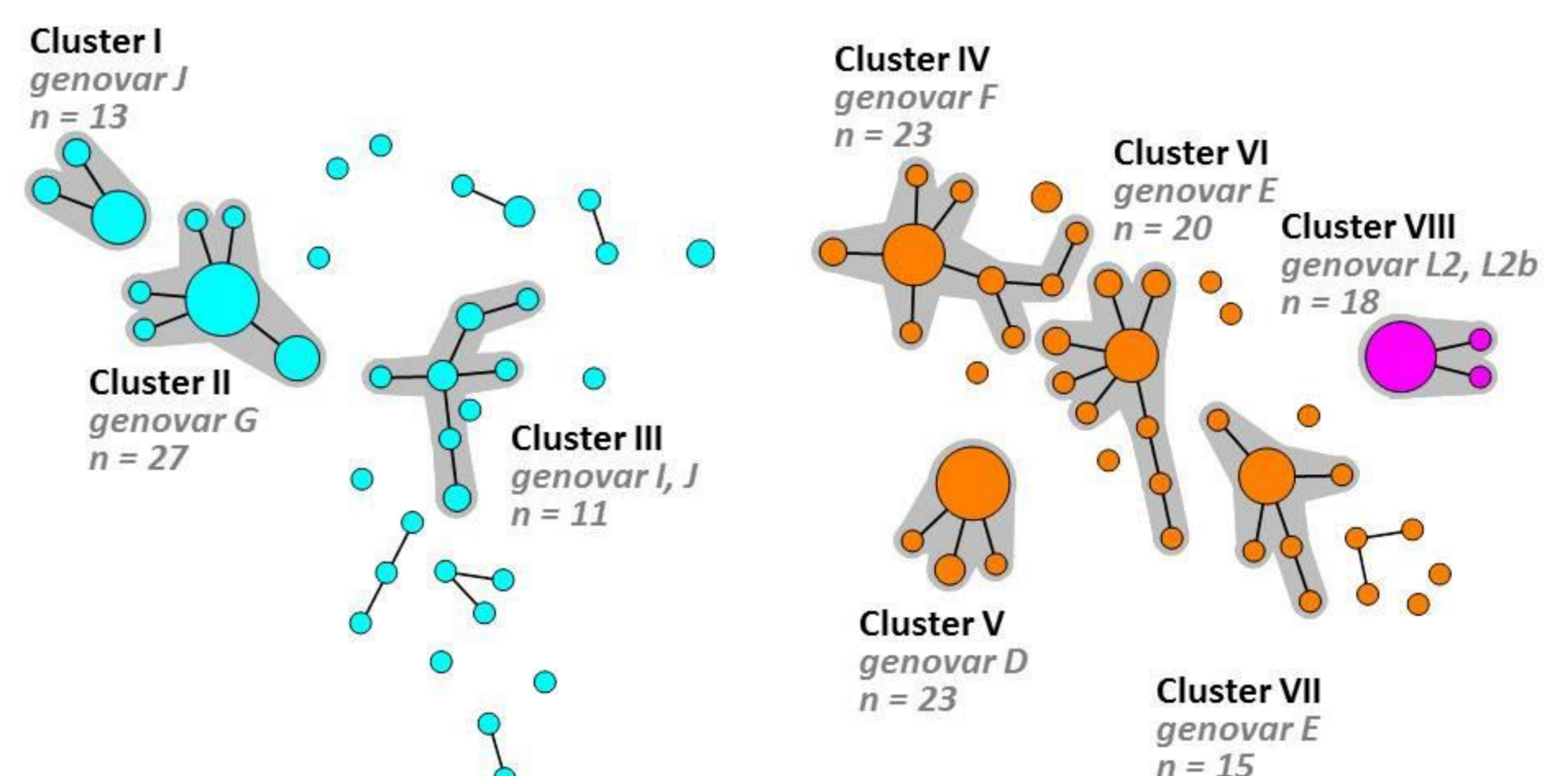
A. Minimum spanning tree based on clustering using the MLST-7 scheme



B. Minimum spanning tree based on clustering using the hr-MLST-6 scheme

Figure 2: hr-MLST-6 with MLST-7 colourcode.

Minimum spanning tree showing the diversification by hr-MLST-6 of the clusters that were generated using the MLST-7 scheme. Colours indicate the MLST-7 clusters from Figure 1 A: blue, cluster A (n=75); orange, cluster B (n=94); purple, cluster C (n=18).



## References

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- Bom RJ et al. Evaluation of high-resolution typing methods for Chlamydia trachomatis in samples from heterosexual couples. J Clin Microbiol 2011;49:2844-2853