Assessment of intra- and inter-outbreak diversity of *Paenibacillus larvae* by core- and whole-genome multilocus sequence typing (cg/wgMLST)

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Introduction

- *Paenibacillus larvae* is the causative agent of **American foulbrood** (AFB), the most serious bacterial disease of **honeybees**
- Conventional typing methods such as ERIC-PCR and MLST lack the necessary discriminatory power for AFB outbreak delineation
- Core- and whole-genome multilocus typing (**cg/wgMLST**) is an allele-based approach for bacterial typing, which has been rarely applied to *P. larvae*
- We recently developed a stable cg/wgMLST scheme for typing of *P. larvae*, which is implemented in the BioNumerics software
- Here, the epidemiological applicability of cg/wgMLST typing was assessed on an extensively sampled AFB outbreak in Slovenia, period 2019–2020

Materials and Methods

- **AFB outbreak**: 59 outbreak-related isolates underwent **whole-genome sequencing** (Illumina)
  > Of these, 40 isolates originated from a single beekeeping practice with three apiaries to assess their genetic diversity
  > Extensive epidemiological data for all isolates was collected
  > Five genetically closely related but epidemiologically unrelated isolates from a national WGS database were added to the analysis
- **cgMLST and wgMLST typing:** BioNumerics v7.3.1 (Applied Maths)
  > Cluster analysis: UPGMA tree (threshold based on pairwise distance matrix) and minimum-spanning tree (MST; single linkage-based threshold)
  > Tanglegram analysis: Dendroscope 3.7.3

Results

cg/wgMLST typing

- All isolates were of ST11-ERIC II type
- cg/wgMLST UPGMA trees and MSTs revealed two outbreak clusters (biclonal outbreak) (Fig. 1)
- wgMLST analysis: the maximum **intra-outbreak diversity** was 34 AD on MST and 44 pairwise AD by UPGMA algorithm (Table 1)
- The clusters were separated by a minimum of 51 AD on cgMLST MST and 63 AD on wgMLST MST (**inter-outbreak diversity**; data not shown).
- Topologies of cgMLST and wgMLST UPGMA trees were generally concordant (Fig. 2)
- The maximum genetic diversity among Cluster 1 isolates was already reached within a single beekeeping practice where sampling effort was increased (see Fig. 1 legend)

Table 1. Thresholds applied for AFB outbreak cluster delineation. The numbers outside the brackets represent allele differences (AD) (single-linkage threshold) based on MST. The numbers in brackets represent pairwise AD based on UPGMA algorithm.

Outbreak cluster	cgMLST MST (UPGMA cgMLST tree)	wgMLST MST (UPGMA wgMLST tree)
Cluster 1	14 (26)	16 (33)
Cluster 1: a single beekeeping practice	7 (26)	11 (33)
Cluster 2	**27 (34)**	**34 (44)**

Conclusions

- cgMLST and wgMLST both provide sufficient discriminatory power to delineate the outbreak clusters
- Increased sampling effort revealed a fairly high genetic diversity within a single beekeeping practice and most likely decreases the MST threshold for cluster delineation
- This study considerably improves our understanding of *P. larvae* intra- and inter-outbreak diversity
- The proposed 27 cgMLST AD and 34 wgMLST AD single-linkage thresholds are in accordance with our previous findings, but should be used as a guide rather than as a fixed rule
- Epidemiological data should always accompany genomic data for final outbreak confirmation
- The evolutionary rate of *P. larvae* remains unknown

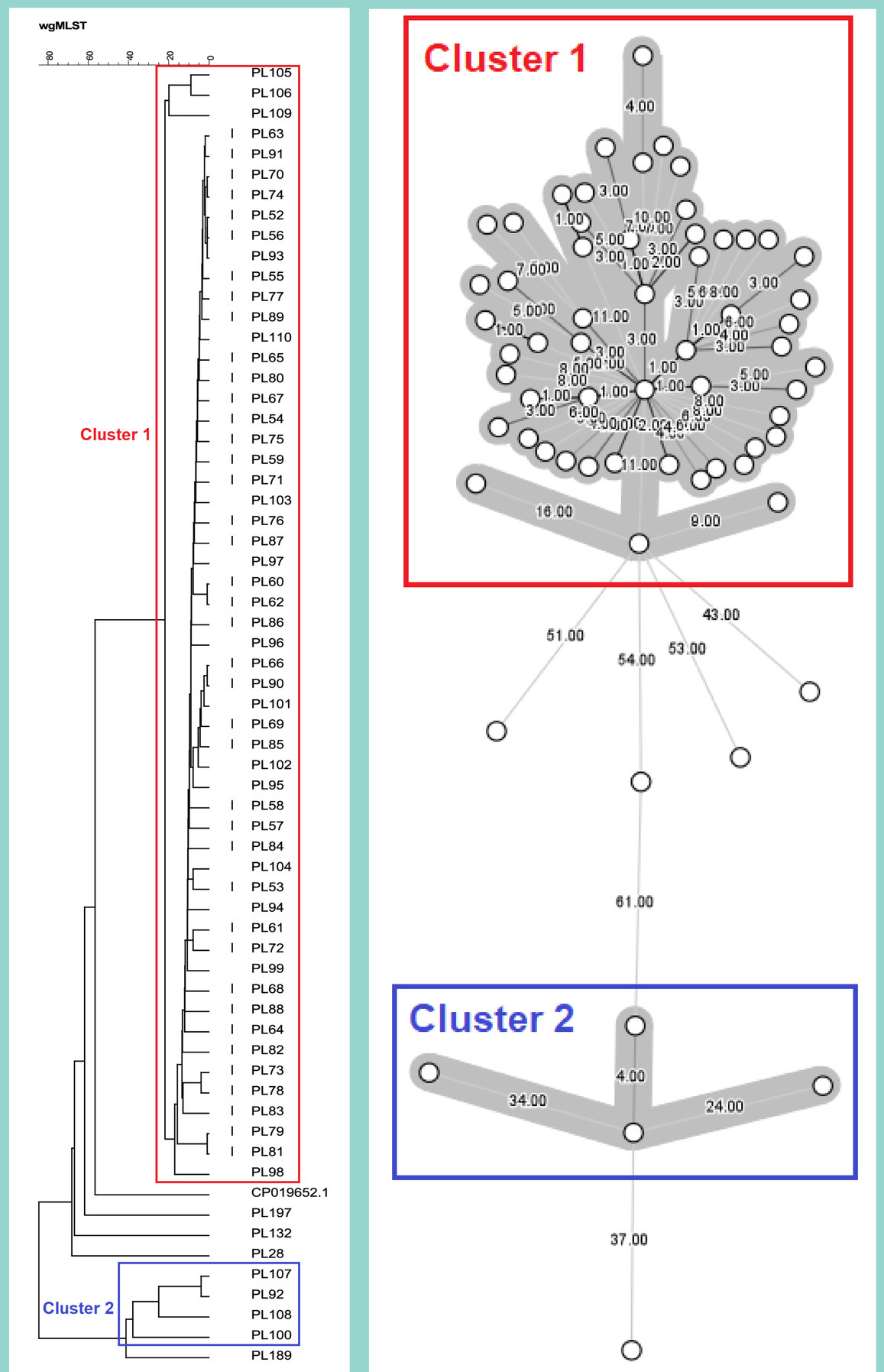

Fig. 1. ***Left*: UPGMA wgMLST tree.** The isolates with ‚I' symbol in front of their name originate from a single beekeeping practice. ***Right*: wgMLST MST.** A threshold of 34 AD was used for cluster delineation. Isolates outside of the clusters were epidemiologically unrelated to the outbreak-related isolates.

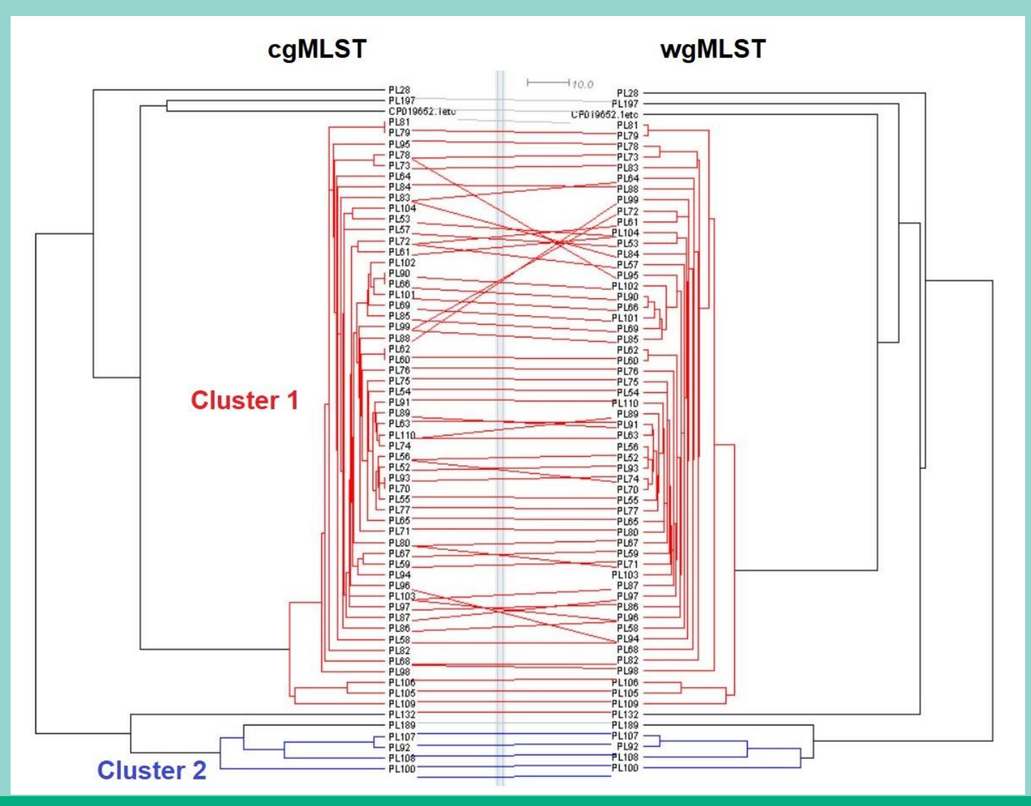

Fig. 2. Comparison of UPGMA cgMLST and wgMLST trees. The outbreak clusters are shown in color. Tanglegram analysis was performed in Dendroscope v3.7.3.

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