

The study of microbiota of a mosquito vector throughout its developmental stages as a first approach to develop microbiota-based control strategies.

Niccolò Alfano^{1*}, Fausta Rosso¹, Valentina Tagliapietra¹, Daniele Arnoldi¹, Mattia Manica¹, Annapaola Rizzoli¹

(1) Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy

*niccolo.alfano@fmach.it

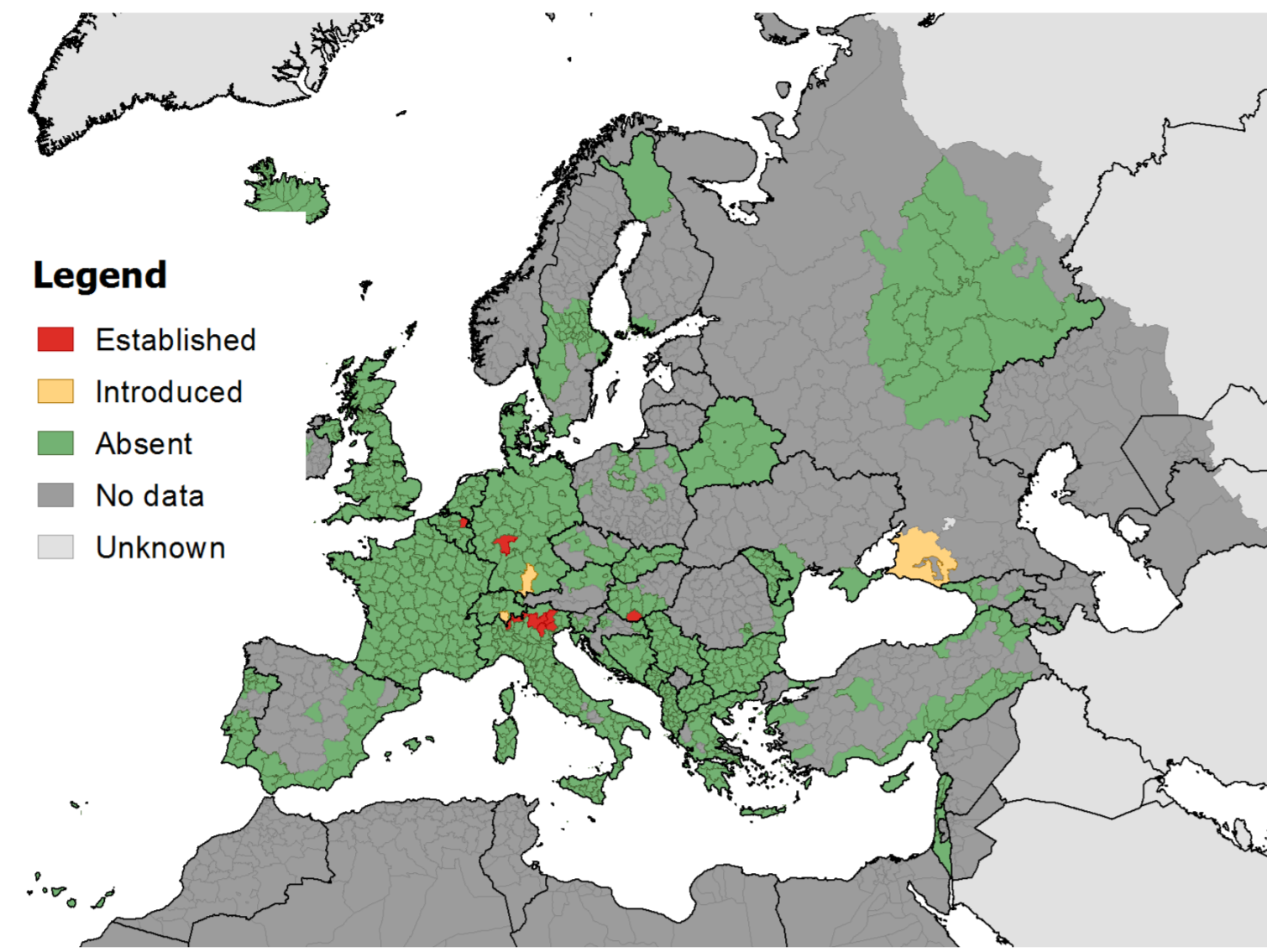
1. Background

- *Aedes koreicus* has recently invaded Europe from Asia^[1].
- Rapid spread and establishment of a local population in NE Italy^[2;3].



Aedes koreicus

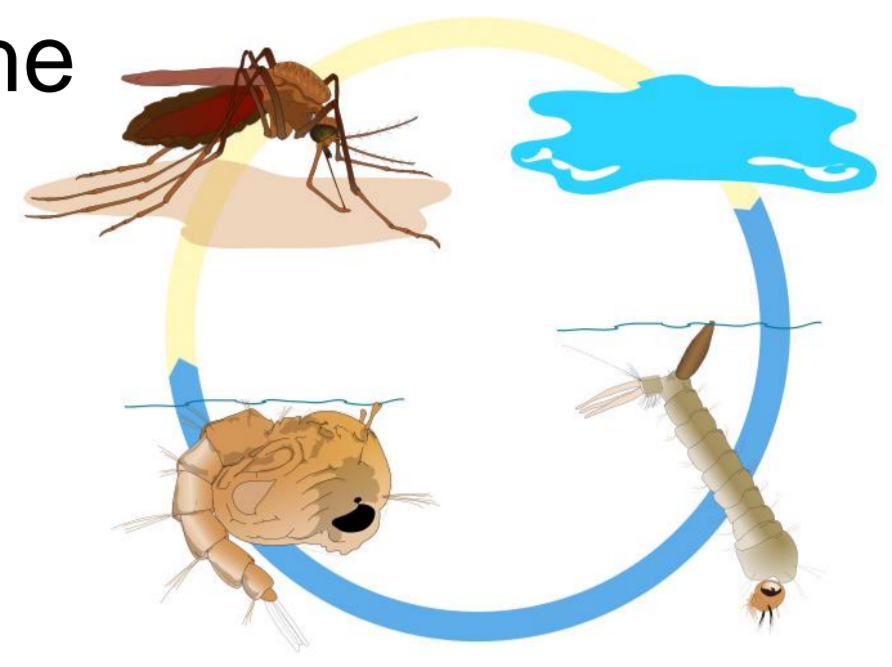
Current known distribution of *Ae. koreicus* in Europe (Jan 2019) (ECDC & EFSA)



- Associated with the transmission of viruses (JEV, CHIKV)^[4;5] and parasitic nematodes (*D. immitis*, *B. malayi*)^[6;7].
- Gut microbiota of vector mosquitoes influences vector competence → new control strategies based on host microbiota manipulation (to control vector populations or pathogen transmission)

2. Aims

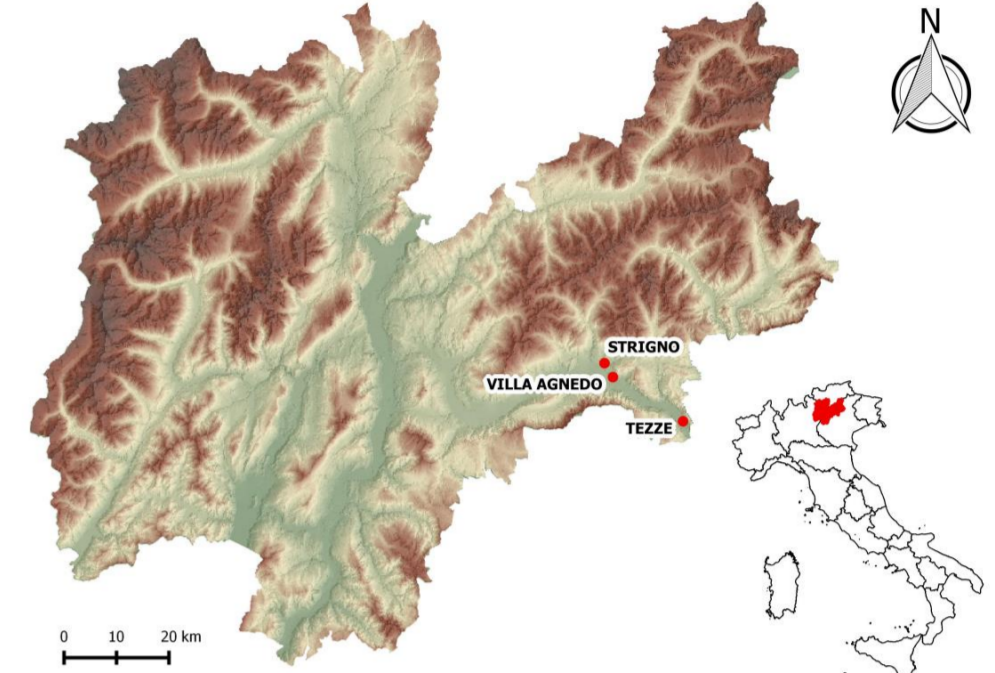
- Characterize the CHANGES in microbiota from the larval breeding sites to the different developmental stages of *Ae. koreicus*.
- Understand which FACTORS contribute to the differences among microbial communities.
- Select bacterial CANDIDATES for microbiota-based control strategies.



3. Materials & Methods

Field sampling

3 sites based on the known presence of *Ae. koreicus* in the Province of Trento (NE Italy).



Molecular analyses

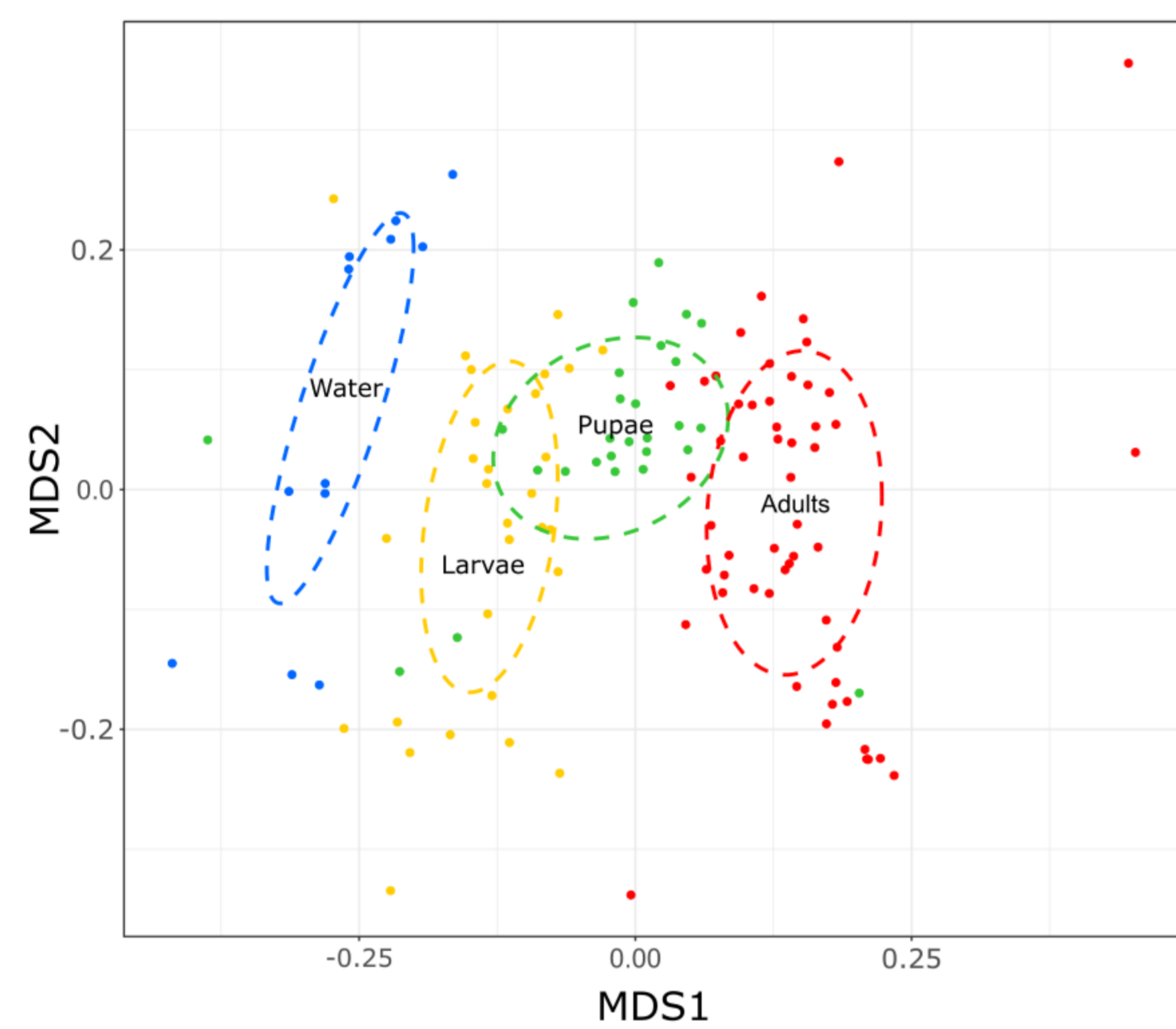
16S rRNA amplicon high-throughput sequencing.
341F-805Rmod primers for hypervariable V3-V4 regions.
Nextera XT DNA Library Prep & Illumina MiSeq (PE300) sequencing.

Bioinformatics

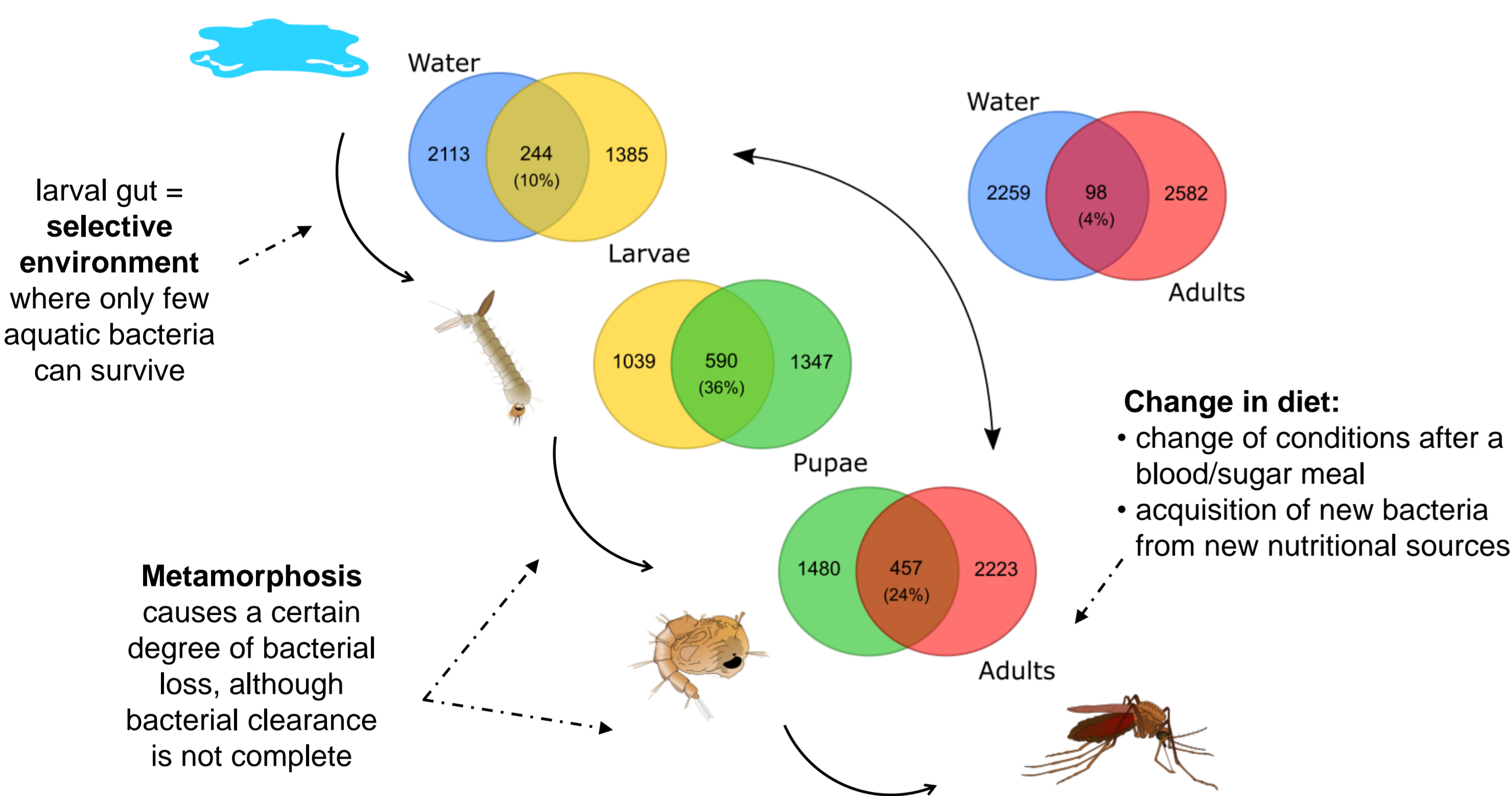
QIIME2 and DADA2 to infer exact amplicon sequence variants (ASVs)^[8].

4. Results

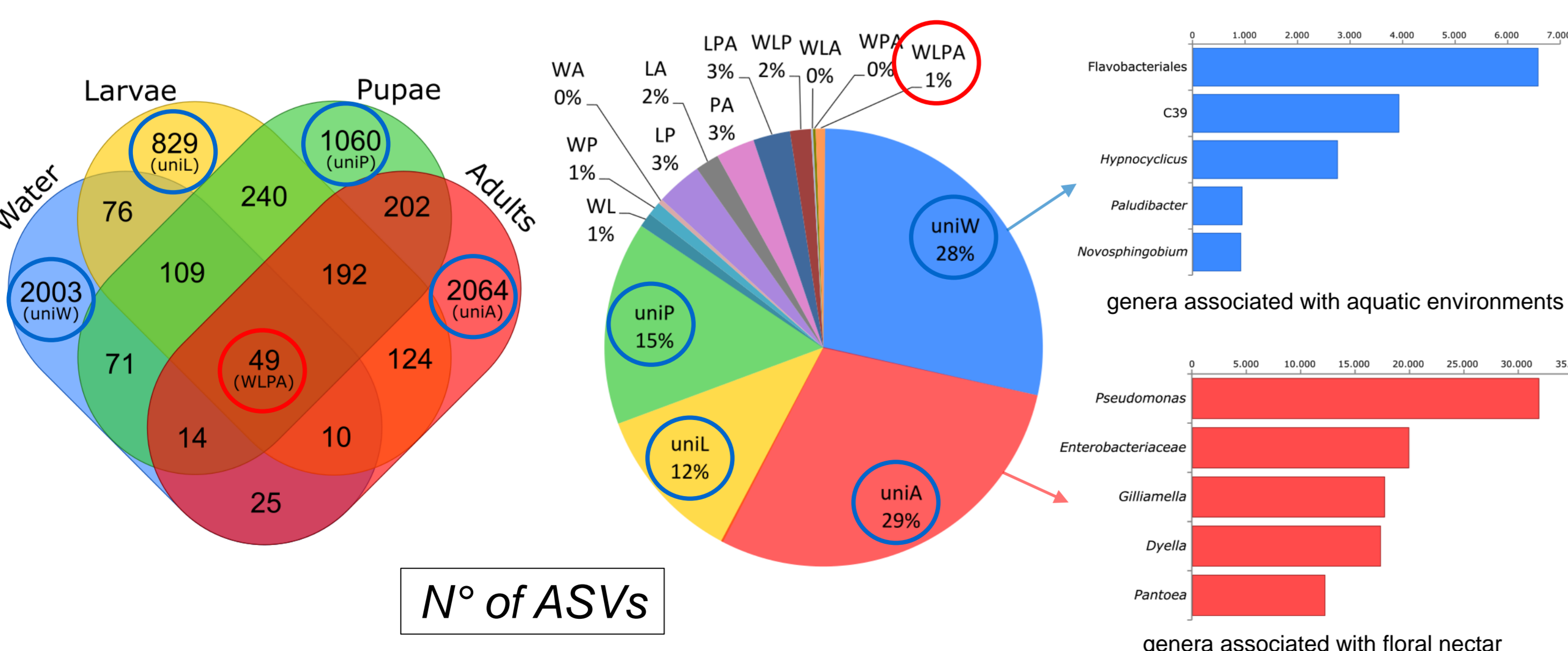
- 1) Distinctive community structures and increase in dissimilarity during mosquito life cycle.



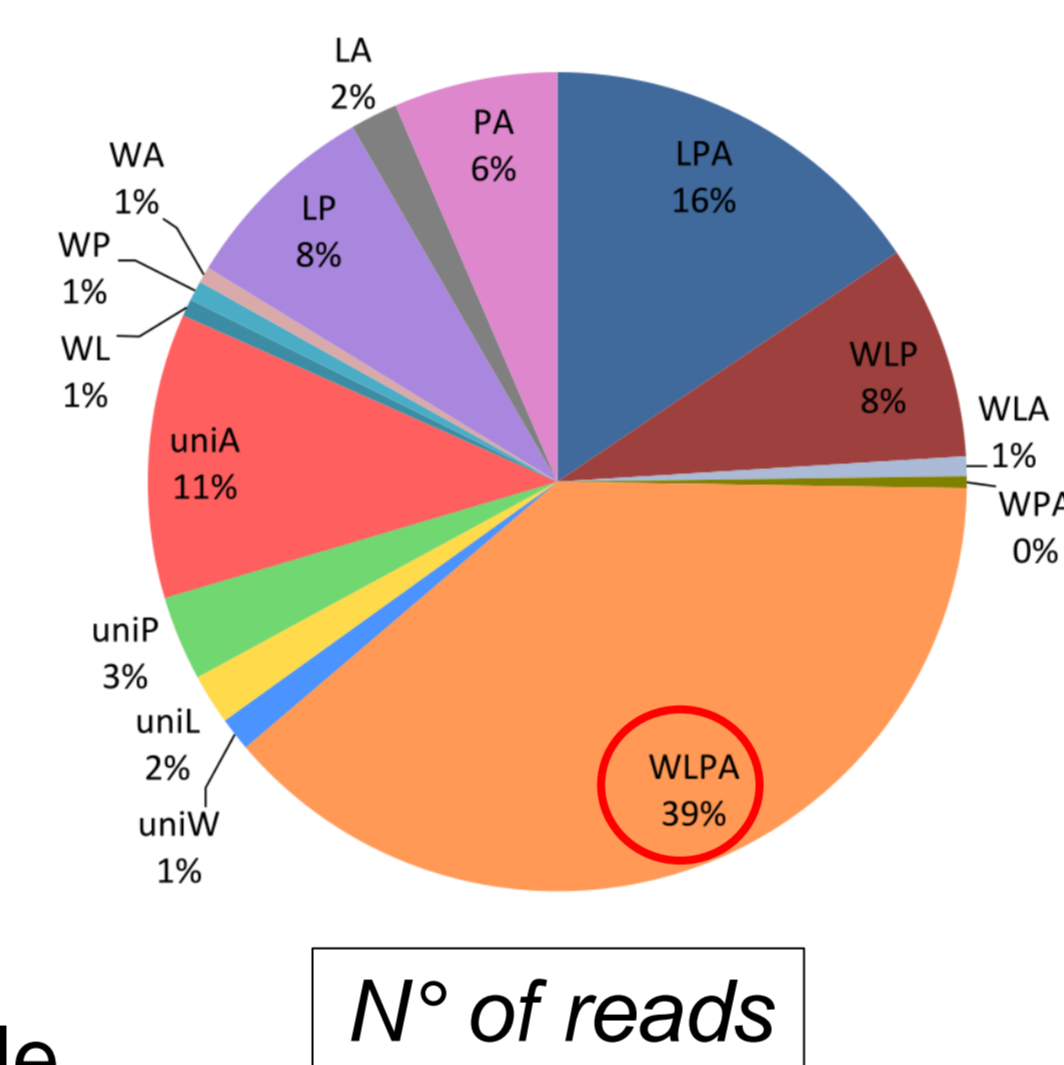
- 2) Only small % of community members (ASVs) were shared among sample types.



- 3) Most of bacteria were unique to a given sample type, while those shared across all sample types (WLPA) were only 1%

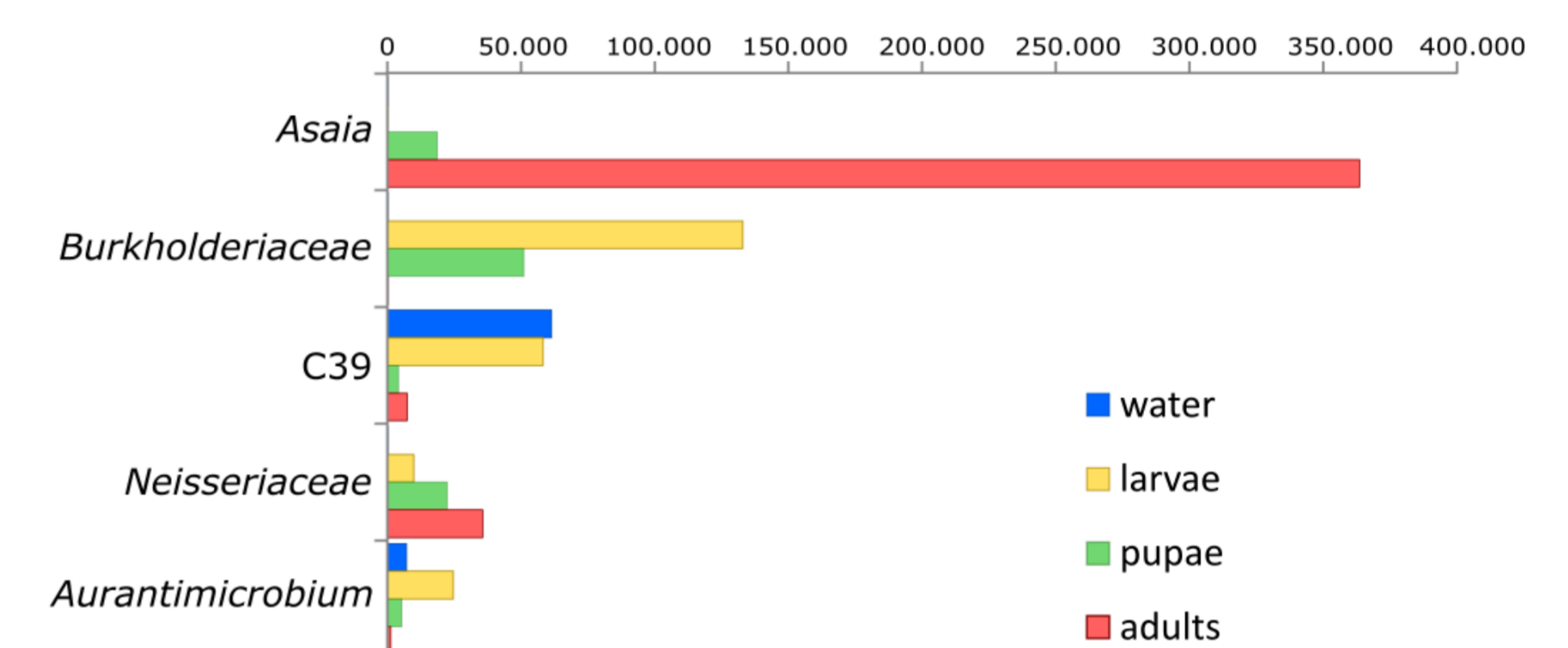


- 4) Bacteria shared between all sample types (WLPA) were the most abundant



- 5) WLPA:

- bacteria acquired by larvae from water and persisting across developmental stages till the adult gut (transstadial transmission)
- considerable portion of the microbiota of each sample type (30-49% of sequences)
- Important role in constituting mosquito microbiota



- 6) BACTERIAL CANDIDATES

STRATEGY-PARATRANSGENESIS:

A) *Asaia*

- Dominant (50% of adult mosquito bacteria)
- Highly prevalent (part of core microbiome of each developmental stage)
- Suggested vertical and transstadial transmission (1 WLPA ASV)

B) *Pseudomonas*

- 2nd most abundant bacterial genus in adults
- Suggested transstadial transmission (3 WLPA ASVs)
- Colonization of Malpighian tubules (where *D. immitis* spend part of its life cycle)

C) *Thorsellia anophelis*

- 2nd most abundant bacterial genus in larvae
- transstadial transmission? (no WLPA ASVs, only WLP and LPA ASVs)
- not yet demonstrated to be transformable to express antipathogen molecules

STRATEGY-WOLBACHIA:

- No *Wolbachia* ASV was found → possible use (like in *Ae. aegypti*)? Antagonistic effect of *Asaia*, which is dominant in *Ae. koreicus*

5. Conclusions

- Acquisition of bacteria from water and transstadial transmission play key roles in shaping the microbiota of *Ae. koreicus*.
- Other factors, such as metamorphosis and change of diet at the adult phase, modify the microbiota.
- Understanding how the microbiota of a vector species changes through its life cycle allows the selection of bacterial candidates for use in microbiota-based intervention measures.
- *Asaia* and *Pseudomonas* are the most promising candidates for paratransgenesis in *Ae. koreicus*. The use of *Wolbachia* is prevented by the presence of *Asaia*.

6. References

- [1] Versteirt et al. (2012) *J Med Entomol* 49:1226-1232. [5] Ciocchetta et al. (2018) *Pathog Glob Health* 112:107-114.
[2] Montarsi et al. (2013) *Parasit Vectors* 6:292. [6] Montarsi et al. (2015) *Parasit Vectors* 8:177.
[3] Montarsi et al. (2015) *Parasit Vectors* 8:614. [7] Korean Centres for Disease Control and Prevention (2007).
[4] Shestakov et al. (1966) *Med Parazitol* 35:545-50. [8] Callahan et al. (2017) *ISME J* 11: 2639-2643.