

Toward the definition of *Aedes albopictus* and *A. koreicus* pathobiome from an area of recent invasion in northern Italy

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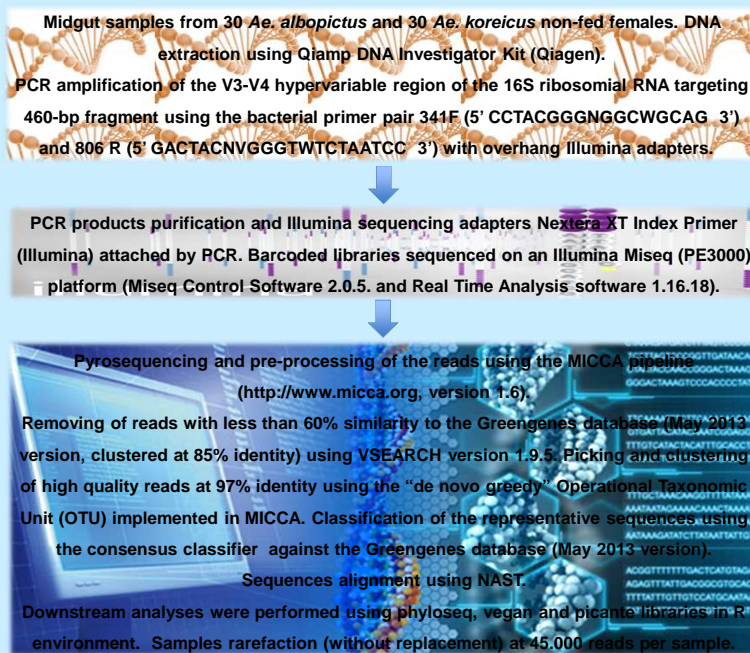
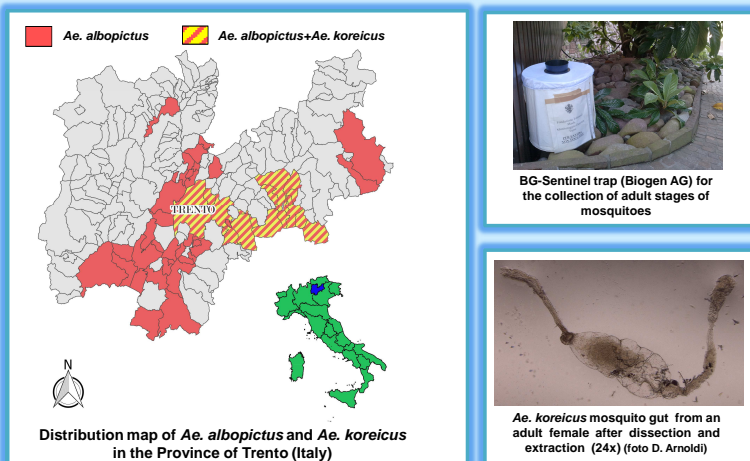


Background

Aedes mosquitoes are highly successful invasive species globally and they also pose public health concerns as vectors of several viruses of medical importance. Next-generation sequencing technology (NGS) has recently helped to unveil the complex, dynamic and extended role of microbiome community. In particular midgut microbiota of mosquitoes is paramount for their fitness, physiology, food and blood digestion, metabolism, immunity, adaptation to new environmental conditions, and in turn affects their vectorial competence and capacity to transmit pathogens.

We show some preliminary analysis on the characterization of the microbial community of *Aedes albopictus* and *Aedes koreicus* using a metagenomic approach.

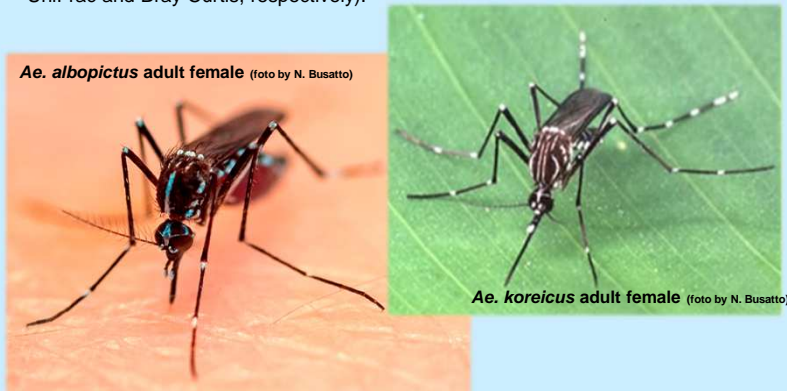
Methods



Results

After rarefaction (without replacement), a total of 2153 OTUs were clustered belonging to 106 orders, 157 families and 217 genus. *Ae. albopictus* and *Ae. koreicus* showed high variations at individual level although they shared 75.98% of the reads in their gut microbiota. The core was dominated by species of the genus *Pseudomonas* (64.63% and 63.38% in *Ae. albopictus* and *Ae. koreicus*, respectively), followed by *Wolbachia* (9.8% and 0.002%), *Janthinobacterium* (4.7% and 4%), *Ralstonia* (2.1% and 2.3%), *Enterobacter* (0.01% and 1.5%) and *Providencia* (0.0005% and 1.2%) plus other minor ones (*Stenotrophomonas*, *Streptococcus*, *Candidatus Portiera*, *Delftia* and *Gluconobacter*). There were no specific OTUs belonging to *Ae. koreicus*, while 3 OTUs were specific to *Ae. albopictus*, corresponding to 9.81% of the reads, and with 2 out of 3 belonging to the genus *Wolbachia*.

α -diversity was significantly higher in *Ae. albopictus* compared to *Ae. koreicus* according to the number of observed OTUs, the Chao1 estimator and Shannon entropy ($P=0.0027$, 8.4×10^{-4} and 9.5×10^{-4} , respectively). Also β -diversity revealed a significantly different gut microbiota as resulted from a PERMANOVA test (9999 permutations) ($P < 10^{-4}$ and $P < 10^{-4}$ for unweighted UniFrac, weighted UniFrac and Bray Curtis, respectively).



Discussion & Conclusions

In the Province of Trento, Northern Italy, two invasive alien species of mosquitoes, *Aedes albopictus* and *Ae. koreicus*, are well established. The former was recorded the first time in 1996 (Roiz et al., 2010), and the latter arrived fifteen years later, in 2011 (Capelli et al., 2011). In our territory, these two species overlapped and adapted to colonize man-made type of habitats such as garden and cemetery flower pots, abandoned containers, manholes, water recipients, etc.. The common developmental environment is probably the reason of the large core microbiota shared by the two species. Nonetheless, the 15-year time difference of arrival, could explain the higher richness of *Ae. albopictus* compared to *Ae. koreicus*.

Of particular interest is the contrasting presence of *Wolbachia* and representatives of the *Acetobacteraceae* family, most probably *Asaia*, in the two species. *Wolbachia* is known to inhibit the horizontal transmission or reduce the vector competence of some mosquito-borne pathogens like *Plasmodium* spp., dengue, yellow fever, West Nile fever and chikungunya viruses (Blagrove et al. 2012, 2013; Moreira et al. 2009; van den Hurk et al. 2012; Bian et al. 2010, 2013; Glaser and Meola 2010; Mousson et al. 2012; Novakova et al. 2017). *Asaia* spp. has been considered a potential vector of antiplasmodial factors through paratransgenesis (Favia et al. 2008). Previous studies found a contrasting presence of these two symbionts in other mosquito species (*Anopheles gambiae*, *An. stephensi* (Hughes et al., 2014), *Ae. aegypti* (Ruang-Areerate et al., 2006)) and our findings add *Ae. koreicus* to this list. The epidemiological consequences of this aspect need to be further elucidated.

This study provides the first description of the highly diverse community of micro-organisms occurring in the midgut of two invasive mosquito species of public health concern in northern Italy. It also provides the basis for the realization of experimental studies aiming at assessing the consequences of the variation of gut microbiota composition on the vectorial capacity for pathogens carried by these two *Aedes* mosquitoes species.

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