The importance of molecular identification methods of mosquitoes in epidemiology of vector-borne diseases

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Identification keys to mosquitos typically describe only the complexes or groups comprising morphologically indistinguishable sibling species. Exact identification is, however, fundamental for understanding the vector biology, mosquito-borne risk factors and epidemiology of mosquito-borne diseases. Also, it is essential to acquire accurate information on mosquito populations to establish baseline data to enable the optimal choice of available tools and monitor their effectiveness.

The aim of the study was to estimate the occurrence of sibling species within *Culex* and *Anopheles* genera using biochemical and genetic methods, and to characterize their distribution and breeding site preferences. The mosquito survey was conducted in the years 2012–2015. All field samples were taken from May to September in different Wrocław area locations.

In this study, morphology of the larvae and adult males, as well as the species diagnostic enzyme markers Adenylate kinase (AK) and 2-Hydroxybutyrate dehydrogenase (HBDH), were used to discriminate *Cx. pipiens* s.l. (Linnaeus 1758) and *Cx. torrentium* (Martini 1925). For the adult specimens preliminarily identified as *An. messeae/daciae,* the ITS2 regions of genomic rDNA genes were analyzed using a rapid PCR-RFLP assay in combination with a multiplex PCR assay and DNA sequencing.

The biochemical diagnosis confirmed the presence of *Cx. torrentium* species (6.0 %) and *Cx. pipiens* s.l. (94.0%). The species of the *Anopheles maculipennis* complex showed the dominance (60%) of *An. daciae* (Linton, Nicolescu & Harbach, 2004). This species occurred in sympatry with *An. messeae* (Falleroni 1926) and *An. maculipennis* s.s. (Meigen 1818) including 35% and 5% of the individuals respectively. The presence of *An. atroparvus* (Van Thiel 1927) was not confirmed.

Our work demonstrates that proteomic and PCR-RFLP assays are cost-efficient and rapid methods for routine mosquito species identification.