**Scientific Note**

First record of *Anopheles daciae* (Linton, Nicolescu & Harbach, 2004) in Poland

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The currently known mosquito fauna in Poland consists of 47 species (Kubica-Biernat 1999). The subfamily of Anophelinae is represented by *Anopheles claviger* s.l. (Meigen 1804), *An. plumbeus* (Stephens 1828) and, so far, three members of the *Anopheles maculipennis* complex, namely *An. maculipennis* s.s. (Meigen 1818), *An. atroparvus* (van Thiel 1927), and *An. messeae* (Falleroni 1926). *Anopheles maculipennis* s.l. was first recorded in Galicia (currently the southern and southeastern part of Poland as well as western Ukraine) by Nowicki (1873). In Silesia, in the former eastern territories of Germany (currently the south and southwestern part of Poland), Weyer (1938) found a species proportion of 81.5% *An. messeae*, possibly including unrecognized *An. daciae*, 16.4% *An. maculipennis* s.l., and 2.1% *An. atroparvus* in 189 samples.

In Poland, malaria was endemic in swampy lowlands during the years following World War I, at the end of World War II, and up to the mid-1960s (Knap and Myjak 2009). Historical transmission of tertian malaria in Poland, caused by *Plasmodium vivax*, was attributed principally to members of the *An. maculipennis* complex. Although the indigenous malaria has been eradicated, the number of imported cases is increasing with intensive travel to endemic areas (Kubica-Biernat and Kowalska-Ulczyńska 2011, Stepien 2015).

Species of the *An. maculipennis* complex cannot be distinguished reliably in the larval and adult stages by morphological characters. Traditionally, species of this complex were diagnosed by egg morphology, as gravid females may be subsequently identified by eggs laid in captivity, and also by karyotyping of larvae (Becker et al. 2010).

Since 1999, molecular analysis of the internal transcribed spacer 2 (ITS-2) of nuclear ribosomal DNA (rDNA) is commonly used for species identification (Proft et al. 1999). Consecutively, *An. daciae* (Linton, Nicolescu & Harbach 2004) was described as an additional, previously unrecognized member of the *An. maculipennis* complex on the Black Sea coast in Romania (Nicolescu et al. 2004). Nucleotide variations in the ITS-2 sequence were found to be sufficient to distinguish *An. daciae* from other complex members. Differentiation to the closest resembling species to date, *An. messeae*, is limited to five single nucleotide substitutions in the ITS-2 rDNA region (Nicolescu et al. 2004, Danabalan et al. 2013, Kronefeld et al. 2014). Also Di Luca et al. (2004) confirmed the intraspecific polymorphism in populations of *An. messeae* (n=79) across Eurasia (Italy, The Netherlands, former Yugoslavia, Kazakhstan, and England). Additionally, Linton et al. (2005) reported the occurrence of *An. daciae* in southwest England, and after detection of *An. daciae* in Germany (Kronefeld et al. 2012, Weitzel et al. 2012), it seemed very likely that this species could also be found in other parts of Central Europe.

In Poland, the first *An. maculipennis* complex species-diagnostic PCR assay was applied to determine the current species composition and distribution of the sibling species of this complex in northeast Poland (Kubica-Biernat and Kowalska-Ulczyńska 2011). Although both species (*An. messeae* and/or *An. daciae*) were not differentiated to the species level, it was shown that these sibling species, as well as *An. maculipennis* s.s., were dominant in all collection localities.

Taking into account the occurrence of *An. daciae* in neighboring Germany and other European countries, this study on the sibling species composition of the *An. maculipennis* complex in the Wroclaw urban area (Lower Silesia, southwest Poland) has been initiated with special consideration of *An. daciae*. For this purpose, the second internal transcribed spacer (ITS-2) regions of genomic ribosomal DNA (rDNA) genes were analyzed by a rapid PCR-RFLP assay and DNA sequencing.

In this study, adult mosquitoes were collected with the aid of EVS traps with carbon dioxide (BioQuip, Products INC., Rancho Dominique, CA, U.S.A.) between July and September, 2015 at twenty locations in the Wroclaw area (Lower Silesia, southwest Poland), particularly in wetlands, parks, home gardens, and cemeteries, as well as in the water infiltration area along the Odra River and in the Bystrzyca Valley Landscape Park (Table 1). Members of the *An. maculipennis* complex and the associated mosquito species were morphologically determined according to Becker et al. (2010) and were stored at −20° C until DNA extraction.

A rapid PCR-RFLP assay (Danabalan et al. 2013) and DNA sequencing were applied to differentiate the sibling species composition with special consideration to *An. daciae*. DNA was isolated individually from 20 randomly selected specimens with the use of QuickExtract DNA Extraction Solution 1.0 (Biozym, Germany), following the manufacturer’s instructions. Standard PCR was carried out prior to RFLP assay as described by Linton et al. (2001a,b). The RFLP assay was applied with the *BrstI* restriction enzyme and 3% agarose gels (High Resolution, Roth, Germany). The resulting fragments were previously described diagnostic for the species *An. daciae*, *An. messeae*, and *An. atroparvus* (Danabalan et al. 2013). In parallel with the PCR-RFLP assay, amplicons of the ITS-2 from all samples were sequenced by Eurofins Medigenomix GmbH (Germany) in order to ensure the