Updated checklist of the mosquitoes (Diptera: Culicidae) of Croatia

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Received 10 April 2019; Accepted 30 May 2019

ABSTRACT: Improvement of morphological and molecular identification methods allows the detection of new species of mosquitoes. The mosquito fauna of Croatia currently includes 52 species, belonging to eight genera, including *Anopheles* (12 species), *Aedes* (24 species), *Coquillettidia* (one species), *Culex* (seven species), *Culiseta* (six species), *Orthopodomyia* (one species), and *Uranotaenia* (one species). This is an updated checklist, which includes five new species found in Croatian mosquito fauna. Two of these are invasive mosquito species, *Aedes albopictus* (Skuse, 1895) and *Aedes japonicus* (Theobald 1901), which are spreading across Europe and Croatia. The other three species, *Culex laticinctus* (Edwards 1913)*, Culex torrentium* (Martini 1925), and *Anopheles daciae* (Linton, Nicolescu & Harbach 2004) are autochthonous species which haven't been recorded so far. Since there are several more invasive species spreading across Europe, we assume that this is not the final list. ***Journal of Vector Ecology* 45 (1): 135-139. 2020.**

Keyword Index: Checklist, mosquitoes, Culicidae, Croatia, Europe, invasive species.

INTRODUCTION

Croatia has a wide variety of ecosystems, habitats, and diverse flora and fauna. Because of very diverse climatic, geological, and ecological factors, new species of flora and fauna are still being found and the lists are certainly not exhaustive.

In the previous checklist of Croatian mosquitoes, 48 species were listed (Merdić et al. 2004) including both historical and current data. It was very difficult to equate historical data with old names to the newer modern nomenclature. According to our experience (35 years of field work), we have never sampled some species reported on that list. Generally, research of mosquitoes in Croatia can be divided into three periods. The first period is the first half of the 20th century, until the eradication of malaria. During that period, many scientific and professional papers were published, and the most important topic was vectors of malaria, with other mosquitoes of less interest. This period is described at large in the previous checklist (Merdić et al. 2004). The second period was after the eradication of malaria until the civil war in Croatia, 1991. During that period, fewer papers were published as there was less scientific interest in mosquitoes. The third period is after the civil war in Croatia, 1991 to 2018, when the interest in mosquitoes was sparked again. This period is characterized by scientific work focused on mosquitoes relevant for public health or nuisance, invasive mosquito species, and many other scientific topics (fauna, attractants, migration, molecular analysis, vector role, mosquito control).

Two major changes have occurred since the previous checklist of mosquitoes in Croatia was published. First of all, there was the introduction of two invasive mosquito species and secondly, mosquitoes became vectors again in Croatia. These two new situations further increased the interest in mosquito research. Many scientists in Croatia now deal with medical issues, which resulted in the organization of national monitoring of invasive species (2016-2018) conducted by the Croatian Public Health Institute, Public Health Institutes of Counties (21), and Department of Biology, University of Osijek.

From the standpoint of public health care, it is important to note that *Ae. aegypti* was present in Croatia. Species were noted by Karaman (1925), publishing a paper on mosquitoes and mosquito control in Dalmatia, recording the presence of *Mansonia richiardii* (now *Coquillettidia richiardii*), for the first time, *Stegomyia fasciatus* (now *Ae. aegypti*), *Cx. modestus*, *Cx. hortensis*, *Cx. territans,* and *An. hyrcanus* s.l. He also found *An. plumbeus*, *An. superpictus*, *Ae. dorsalis*, *Ae. geniculatus,* and *Uranotenia unguiculata*. The current distribution of *Ae. aegypti* in Europe is in Madeira (Portugese islands), Georgia, Armenia, and northeast Turkey (ECDC distribution maps), so there is a small possibility of the existence of this species currently in Croatia. Whether these data represented a sporadic introduction of several specimens that have not established a population or were a misdetermination is difficult to decide. During the last 35 years of intensive research of Croatian mosquito fauna, this species was not even noted once, and we therefore decided to remove these historical data from the checklist.

We expect that the mosquito fauna will expand to include more species in the future. One species that could be found in Croatia in the near future is *Aedes koreicus* (Edwards 1917), since the species is recorded in three neighboring

countries: Slovenia (Kalan et al. 2017), Hungary (Kurucz et al. 2016), and Italy (Capelli et al. 2011). Two additional invasive mosquito species recorded in Europe, *Aedes atropalpus* (Coquillett 1902) and *Aedes triseriatus* (Say 1823) (Medlock et al. 2012, Schaffner et al. 2013), may possibly spread to Croatia. Furthermore, there is a big project of DNA barcoding of the biodiversity of Croatian fauna. This project includes mosquitoes, which will allow us to solve some open questions about several species complexes.

CHECKLIST

A total of 52 mosquito species has been recorded in Croatia, belonging to eight genera, as follows: *Anopheles* (12), *Aedes* (24), *Coquillettidia* (one), *Culex* (seven), *Culiseta* (six), *Orthopodomyia* (one), and *Uranotaenia* (one). The list was compiled according to the current list of mosquitoes in Europe, *A revised checklist of European mosquitoes* by Snow and Ramsdale (2003), based on the classification and nomenclature of mosquito species included in *A catalogue of the mosquitoes of the World* (Knight and Stone 1977) and its supplements (Knight 1978, Ward 1984, 1992, Gaffigan and Ward 1985). We also used Gutsevich et al. (1974) and Becker et al. (2003). This updated checklist includes five new species of Croatian mosquitoes. All of these species were identified morphologically and confirmed with molecular identification. One species (*Aedes aegypti*) was removed from the previous checklist.

Family CULICIDAE
Subfamily ANOPHELINAE

Genus *Anopheles* Meigen, 1818

Subgenus ***Anopheles*** Meigen, 1818

1. *algeriensis* Theobald, 1903
2. *atroparvus* Van Thiel, 1927
3. *claviger* Meigen, 1804
4. *daciae* Linton, Nicolescu & Harbach, 2004 (Note 1)
5. *hyrcanus* Pallas, 1771
6. *labranchiae* Falleroni, 1926
7. *maculipennis* Meigen, 1818
8. *melanoon* Hackett, 1934 (Note 2)
9. *messeae* Falleroni, 1926
10. *plumbeus* Stephens, 1828
11. *sacharovi* Favre, 1903

Subgenus ***Cellia*** Theobald, 1902

12. *superpictus* Grassi, 1899

Subfamily CULICINAE

Genus *Aedes* Meigen, 1818

Subgenus ***Aedes*** Meigen, 1818

13. *cinereus* Meigen, 1818
14. *rossicus* Dolbeshkin, Gorickaja and Mitrofanova, 1930

Subgenus ***Ochlerotatus*** Lynch-Arribálzaga, 1891

15. *annulipes* Meigen, 1830
16. *behningi* Martini, 1926
17. *cantans* Meigen, 1818
18. *caspius* Pallas, 1771
19. *cataphylla* Dyar, 1916
20. *communis* De Geer, 1776
21. *detritus* Haliday, 1833
22. *dorsalis* Meigen, 1830
23. *excrucians* Walker, 1856
24. *flavescens* Müller,1764
25. *leucomelas* Meigen, 1804
26. *nigrinus* Eckstein, 1918
27. *pulchritarsis* Rondani, 1872
28. *punctor* Kirby, 1837
29. *riparius* Dyar and Knab, 1907
30. *sticticus* Meigen, 1838
31. *zammitii* Theobald, 1903

Subgenus ***Aedimorphus*** Theobald, 1903

32. *vexans* Meigen, 1830

Subgenus ***Stegomyia*** Theobald, 1901

33. *albopictus* Skuse, 1895 (Note 3)

Subgenus ***Hulecoeteomyia***

34. *japonicus* Theobald 1901 (Note 4)

Subgenus ***Finlaya*** Theobald, 1903

35. *geniculatus* Olivier, 1791

Subgenus ***Rusticoidus*** Shevchenko & Prudkina, 1973

36. *rusticus* Rossi, 1790

Genus *Coquillettidia* Dyar, 1905

Subgenus ***Coquillettidia*** Dyar, 1905

37. *richiardii* Ficalbi, 1889

Genus *Culex* Linnaeus, 1758

Subgenus ***Barraudius*** Edwards, 1921

38. *modestus* Ficalbi 1889

Subgenus ***Culex*** Linnaeus, 1758

39. *pipiens* Linnaeus, 1758
40. *laticinctus* Edwards 1913 (Note 5)
41. *torrentium* Martini 1925 (Note 6)

Subgenus ***Maillotia*** Theobald, 1907

42. *hortensis* Ficalbi, 1889

Subgenus ***Neoculex*** Dyar, 1905

43. *martinii* Medschid, 1930
44. *territans* Walker, 1856

Genus *Culiseta* Felt, 1904
Subgenus ***Allotheobaldia*** Brölemann, 1919
45. *longiareolata* Macquart, 1838
Subgenus ***Culicella*** Felt, 1904
46. *fumipennis* Stephens, 1825
47. *morsitans* Theobald, 1901

Subgenus ***Culiseta*** Felt, 1904
48. *annulata* Schrank, 1776
49. *glaphyroptera* Schiner, 1864
50. *subochrea* Edwards, 1921

Genus *Orthopodomyia* Theobald, 1904
51. *pulcripalpis* Rondani, 1872

Genus *Uranotaenia* Lynch-Arribálzaga, 1891
Subgenus ***Pseudoficalbia*** Theobald, 1912
52. *unguiculata* Edwards, 1913

NOTES

1. *Anopheles daciae* is a species of the *Maculipennis* complex. Research of this complex in Croatia started in 1996 (Merdić and Boca 2004) and is ongoing. The latest species from this complex is new to the list of Croatian mosquito fauna and was recently confirmed with molecular identification of the ITS2 region of ribosomal DNA which is identical to those described by Nicolescu et al. (2004). Species is very similar to *An. messeae* which can be confirmed with very small differences in the mentioned DNA region. In Croatia, *An. daciae* prefer same type of habitats as *An. messeae* and distribution also correlates with the distribution of *An. messeae* but with one important exception: locality Ričice in Lika region that represents the most southern finding of this species for Croatia and Europe. The importance of this finding is that it was recorded at cca 560 m a.s.l., whereas all findings of *An. messeae* were below 100 m a.s.l. (Vignjević unpublished dissertation 2014). This species is recently also confirmed in neighboring Serbia (Kavran et al. 2018).

2. Apfelbeck (1931) recorded the presence of *An. subalpinus* in south Croatia. Molecular studies showed that *An. subalpinus* is formally a synonym of *An. melanoon* and that these two species are identical (Linton et al. 2002). In recent research the presence of *An. melanoon* has been confirmed in Croatia. Among the sampled species of the maculipennis complex in Croatia, *An. melanoon* is the rarest. It has been sampled in a barn with domestic animals in Istria and again in a barn at the Neretva River delta. The finding of this species in Istria represents new distribution for this species, since before it was found only in the Neretva River delta (Vignjević unpublished dissertation 2014). There is no evidence to support the role of this species in malaria transmission (Sedaghat and Harbach 2005).

3. *Ae. albopictus* has shown rapid spreading in Croatia. The first finding was in Zagreb in autumn 2004, in an old toilet bowl (Klobučar et al. 2006), although we expected the first detection on the Adriatic coast. In the following few years a rapid spread on the Adriatic coast was recorded. The entrance points were the marinas where mosquitoes came by yachts from Italy (Merdić et al. 2009). Seasonal and spatial ovipositional activity of *Ae. albopictus* was investigated in Split, south Croatia where oviposition activity started in April and was completed at the beginning of December while mean egg density was highest from June to early September. (Žitko and Merdić 2014). By 2012, this species spread to coastal Croatia, including its islands. Spreading within continental Croatia was first noted in the western part in Zagreb and its surroundings (Klobučar et al. 2013) and shortly after in the eastern part (Vrućina et al. 2014 conference proceedings). The initiative for national monitoring of invasive mosquito species started at the beginning of 2016 and several months later it was implemented in all counties of Croatia. The results confirmed the presence *Ae. albopictus* in all 21 Croatian counties (Capak et al. 2018 conference proceedings). Although it has not been proven, this species is considered to be responsible for dengue transmission in Podobuče (a village in south Croatia) during 2010 (Gjenero-Margan et al. 2010). After this event, mosquitoes in Croatia are again vectors, as the last transmission of malaria was noted by Trausmiller (1946).

4. Another invasive mosquito species that is new for fauna is *Ae. japonicus*. The Asian bush mosquito, *Aedes japonicus japonicus* (Theobald, 1901), has the original distribution range in the Far East, which includes parts of China, Japan, Korea, south-eastern Russia, and Taiwan, and has recently established across North America and Central Europe. As this is the only invasive subspecies from the *Ae. japonicus* complex found in Europe to date, we will refer to it as *Ae. japonicus* in this paper. The first finding in Croatia was in 2013 on the Slovenian-Croatian border. In the following years, the species has occupied the northwest part of the country (Klobučar et al. 2018). The research on distribution in the other regions of Croatia within five years noted the spread of 250 km to the east and south. Larvae of this species have been found from sea level to mountains up to 921 m a.s.l. (Merdić, unpublished data). This species is currently considered one of the most important invasive mosquito species (Medlock et al. 2015) and has been present in Europe for more than 17 years (Boukraa et al. 2015, Koban et al. 2019).

5. The Mediterranean species, *Cx. laticinctus*, has been noted in Croatia. It was found on the mid-Dalmatian island of Vis in September, 2003. During the summer and autumn of 2005, the population of this species was monitored and found to appear in late summer and increase in abundance until autumn. One generation per year was noted, never of great abundance, as it has never been recorded in spring. We assume there is a competition between *Cx. pipiens* and *Cx. laticinctus*. *Culex pipiens* is present until August until the appearance of the first larvae of *Cx. laticinctus*, when their numbers decrease until they disappear, and the larvae of *Cx. laticinctus* become more numerous (Žitko and Merdić, 2006).

6. Polytypic species *Cx. pipiens* complex consists of several species, subspecies, forms, races, physiological variants, or biotypes according to various authors. At present it includes the names *Cx. pipiens pipiens* Linnaeus, *Cx. p. pipiens* biotype *molestus* Forskal, *Cx. p. quinquefasciatus* Say, *Cx. p. pallens* Coquillett, *Cx. restuans* Theobald, and *Cx. torrentium* Martini in the Holarctic (Becker et al. 2003). All of these mosquitoes are very similar in the adult stage. Actually, adults cannot be morphologically distinguished. The only difference can be seen in the structure of aedeagus in some species. *Cx. torrentium* was separated within the complex on the basis of differences between male genitalia and confirmed by molecular analysis of cytochrome oxidase subunit I DNA sequence. It seems that *Cx. torrentium* prefer higher altitudes and are more prevalent in northern Europe (Hesson et al. 2014), although we noted several times both sibling species (*Cx. pipiens* and *Cx. torrentium*) in the same breeding site (Merdić et al. 2018).

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