



Mosquitoes of Istria, a contribution to the knowledge of Croatian mosquito fauna (Diptera, Culicidae)

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Abstract

Background and Purpose: Although Istria was endemic of malaria up to the mid 20th century, the mosquito fauna was studied in detail. Our investigation of mosquitoes in Istria, a very specific region with a highly diverse breeding site types, was conducted in order to gain insights into the mosquito fauna and abundance, as well as to establish the possible presence of new species.

Material and Methods: The sampling took place from May to September over a seven-year period, from 1999 to 2005. Mosquito larvae were trapped in medium and small breeding sites using a net 25 or 10 cm in diameter and a dipper. Adult specimens were sampled in both urban and forest areas using CO₂ baited CDC traps, and the man – aspirator method for 15 minutes.

Results and Conclusions: The research in the area of Istrian peninsula was conducted in 74 sites. We collected 3087 specimens and established the presence of 27 mosquito species. The most abundant mosquito species in the Istrian fauna is *Culex pipiens* complex with 43.34%, *Ochlerotatus sticticus* with 9.55%, and *Culex hortensis* with 9.32%. The remaining 24 species have the share of 37.79%. The largest number of species, 12, were recorded in two sites, Motovun and Pula. In other sites we determined from one to ten species, and the distribution of the most abundant species is presented on UTM maps. Our systematic investigation of mosquitoes in Istria, taking into account the specificities of the terrain and favorable climatic conditions, recorded the mosquito fauna of Istria, species distribution and dominance. The fauna includes 27 mosquito species and shows a great faunistic diversity (54% of the total Croatian mosquito fauna).

INTRODUCTION

The largest Adriatic peninsula Istria (2,820 km²) is located in the northern part of the Adriatic. Due to its geomorphologic, landscape and vegetation diversity, and the combination of the Mediterranean and sub-Mediterranean climate, Istria offers ideal conditions for biodiversity.

The northern geographic border of Istria is the mountain range Čičarija (1273 m), and the eastern border is the ridge of Učka (1396 m). Istria consists of three completely different regions. In the western part there is a limestone plateau covered in reddish brown soil (Red Istria), whereas the central part of the peninsula is hilly with grayish clay soil (Grey Istria). In the north there are slopes of Učka, and the eastern part

is rocky (White Istria). Having such geographic and landscape diversity, Istria is a very interesting area from the entomological perspective.

The hydrological aspect of Istria is of the utmost importance when it comes to the development of mosquito larvae. The coastal area in the west is shallower and more indented, and the eastern coast is steeper. Two large rivers, the Mirna and the Dragonja, flow through the northwestern part of Istria, through forests at low altitudes, sometimes creating flooded areas (marsh valleys) favorable for the development of certain mosquito species. In the southwestern part of the peninsula there is the third river, the Raša, meandering through the karst. Many smaller streams go dry in the summer, and the water is trapped in depressions for a while. The man-made lake Butoniga, a surface accumulation in central Istria, the marshland Palud in the vicinity of Rovinj, and what remained of the formerly large Lake Čepić, are excellent breeding sites for many mosquito species. Small amounts of stagnant water remain after rain in natural depres-

sions or small containers, such as barrels, used tyres, dishes, and vases, and are the most numerous potential breeding sites in this area. The average annual rainfall in Istria is relatively high. In the south of Istria (Pula) it is about 763.4 mm, in the north-west (Abrami) it is 1088.0 mm, and in central Istria (Pazin) it is about 995.8 mm, with most rainfall occurring in the winter period. It can therefore be expected that the listed potential breeding sites will be active during the whole season.

Despite Istrian biodiversity potential, there have been only few entomological investigations. Most papers date back to the end of the 19th and the early 20th century, and the only written records deal with the species *Anopheles*. More recent data refer to biogeographic investigations of insects in meadows along the Mirna and the Raša, and on the mountains Čićarija and Učka (1).

As some species of the genus *Anopheles* are the vectors of malaria that ravaged for a long time in southern Europe, the interest in mosquitoes awakened in the early 20th century, when the number of patients with malaria

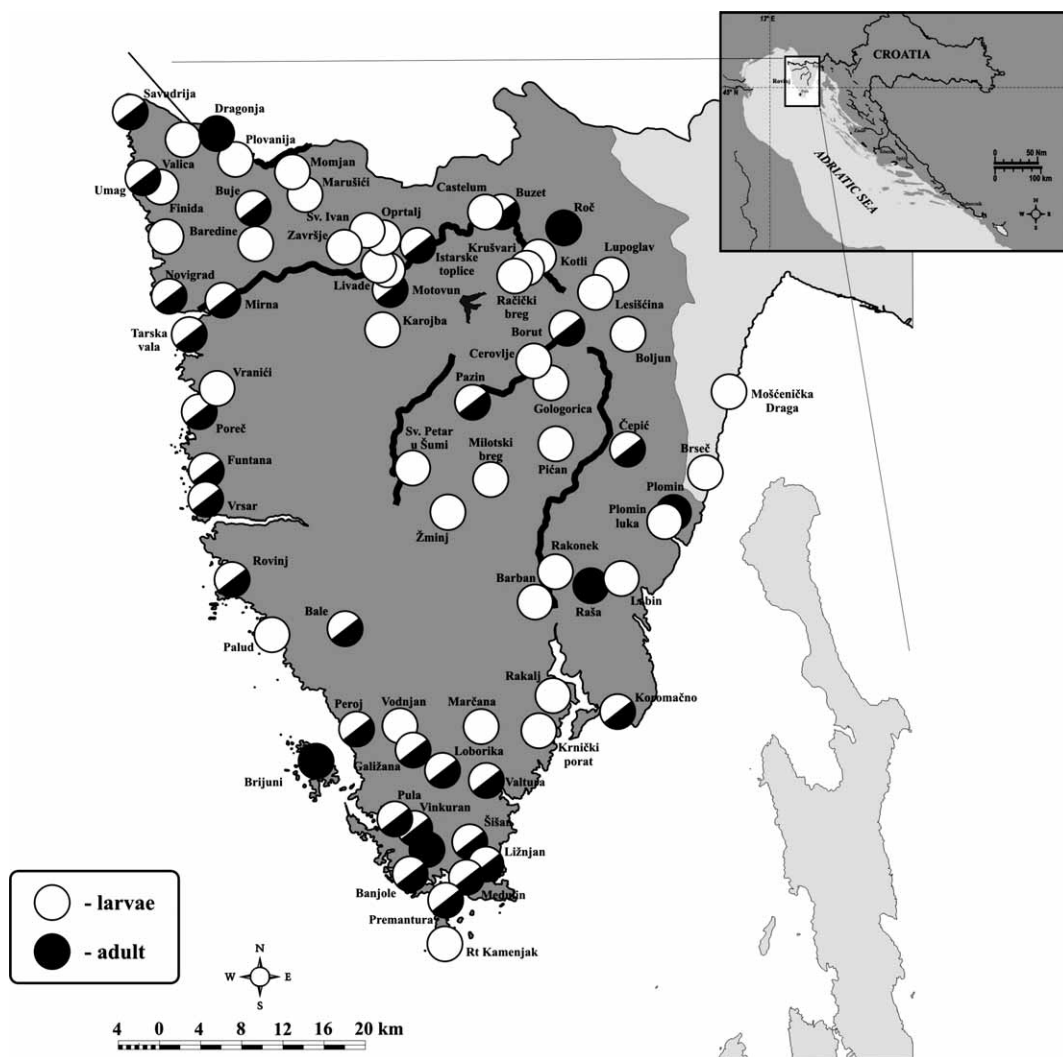


Figure 1. The map of Istria with investigation sites.

reached its peak in this area. The main vectors of malaria in those times were two mosquito species *Anopheles atroparvus* and *Anopheles sacharovi* which belong to the *Anopheles maculipennis* complex (2). Only after World War II malaria was eradicated in the area of the Adriatic coast, which also reduced the interest in studying mosquitoes. Today there is a theoretical possibility of the reintroduction of malaria in the areas where there are malaria mosquitoes and agents. If the temperatures increased by a few degrees centigrade (globally the increase has been 0.9 degrees), we could not claim with certainty that tropical diseases would prevail, as it may also happen that the diseases typical of dry areas would occur. Computer models have shown that global warming will bring drier weather to northern longitudes, rather than hot and humid conditions necessary for the spread of malaria (4). Global warming is a smaller problem when it comes to the occurrence of diseases typical of tropical areas. A greater possibility of that happening stems from the very well developed international transport, which proved to be correct when it comes to the species *Aedes albopictus* (*Stegomyia albopicta*).

Systematic mosquito research in Istria started in the 1990's, due to the potential entry of the invasive species *Ae. albopictus* into Istria, i.e. Croatia. As this species has been present in Italy and Albania for about 15 years, and as Istria offers optimum ecological conditions for its spreading, it was expected to be found in that area. Our seven year research in Istria aimed to discover the potential presence of this species. The species *Ae. albopictus* was recorded for the first time in Istria in the summer of 2005 in Rovinj (5).

MATERIAL AND METHODS

In the seven-year period (1999–2005) we conducted mosquito sampling in 74 locations along the western and eastern coast, as well as in central Istria. We sampled 3087 mosquitoes.

The mosquito larvae were sampled using the net 25 cm in diameter and a dipper (3). The net is shaped as a funnel, the narrow part of which is a glass test tube with solid walls, or a plastic bottle which contains the larvae sample. The sampling of mosquito larvae was conducted using a net in medium-sized breeding sites (from 1 to 100 m²), and using a dipper in small breeding sites (smaller than 1 m²), such as used car tyres, barrels, buckets, vases, cans, tubs, and tree hollows. The sampled larvae were stored in 50% alcohol. The preparation was conducted using several alcohol solutions (50%, 70%, and 96%), immersed into xilol, and fixed in Canada balsam. We mounted 1706 mosquito larvae.

Adult specimens were collected using an aspirator (3) at dusk, when they are the most active. This method was used to collect all mosquitoes that landed on the front part of the investigator's body during 15 minutes. Using this method it is possible to determine the mosquito activity in a given area.

The second sampling method used for adult mosquitoes were dry-ice baited CDC-traps (3). The areas where the traps are set need to be carefully selected for micro-climatic conditions suitable for mosquitoes, i.e. so that they may trap the largest possible number of specimens and species. Dry ice was used as an attractant, as it sublimates and creates an increased concentration of CO₂ in the air, which attracts female mosquitoes. The traps worked for 12 hours, i.e. they were set at dusk, and collected in the morning.

The trapped adult mosquitoes were killed using temperatures below 0 °C, higher than 30 °C, cigarette smoke, or ethyl-acetate vapors. Three specimens of each species from every location were glued to entomological needles. We mounted 1381 specimens, and the determination was done using the keys (6, 7, 8).

Mosquito distribution was marked using the UTM system.

All mounted mosquitoes are stored in the entomological collection of the Department of Biology, Josip Juraj Strossmayer University in Osijek.

RESULTS

The investigation of the Istrian mosquito fauna, conducted from 1999 to 2005, recorded the presence of 27 mosquito species. We determined the presence of two subfamilies and 6 genera: *Anopheles*, *Aedes*, *Ochlerotatus*, *Culex*, *Culiseta* and *Orthopodomyia* (9). The following is the systematic list of species:

Family CULICIDAE

Subfamily ANOPHELINAE

Genus *Anopheles* Meigen, 1818

Subgenus *Anopheles* Meigen, 1818

1. *claviger* Meigen, 1804
2. *maculipennis* complex Meigen, 1818
3. *plumbeus* Stephens, 1828

Subfamily Culicinae

Genus *Aedes* Meigen, 1818

Subgenus *Aedes* Meigen, 1818

4. *cinereus* Meigen, 1818

Subgenus *Aedimorphus* Theobald, 1903

5. *vexans* Meigen, 1830

Subgenus *Stegomyia* Theobald, 1901

6. *albopicta* Skuse, 1895

Genus *Ochlerotatus* Lynch-Arribálzaga, 1891

Subgenus *Finlaya* Theobald, 1903

7. *geniculatus* Olivier, 1791

Subgenus *Ochlerotatus* Lynch-Arribálzaga, 1891

8. *annulipes* Meigen, 1830
9. *cantans* Meigen, 1818
10. *caspius* Pallas, 1771
11. *detritus* Haliday, 1833
12. *excrucians* Walker, 1856
13. *flavescens* Müller, 1764
14. *leucomelas* Meigen, 1804

15. *pulcristarsis* Rondani, 1872
 16. *riparius* Dyar and Knab, 1907
 17. *sticticus* Meigen, 1838
 18. *mariae* Theobald, 1903
 Subgenus *Rusticoides* Shevchenko & Prudkina, 1973
 19. *rusticus* Rossi, 1790
Genus *Culex* Linnaeus, 1758
 Subgenus *Barraudius* Edwards, 1921
 20. *modestus* Ficalbi, 1890
 Subgenus *Culex* Linnaeus, 1758
 21. *pipiens* complex Linnaeus, 1758
 Subgenus *Maillotia* Theobald, 1907
 22. *hortensis* Ficalbi, 1889

- Subgenus *Neoculex* Dyar, 1905
 23. *territans* Walker, 1856
Genus *Culiseta* Felt, 1904
 Subgenus *Allotheobaldia* Brölemann, 1919
 24. *longiareolata* Macquart, 1838
 Subgenus *Culiseta* Felt, 1904
 25. *morsitans* Theobald, 1901
 Subgenus *Culiseta* Felt, 1904
 26. *annulata* Schrank, 1776
Genus *Orthopomyia* Theobald, 1904
 27. *pulchripalpis* Rondani, 1872

The research and sampling were conducted proportionally in the coastal and continental parts of the investigated area. Figure 1 shows 74 sites where the mosquitoes

TABLE 1

The list of mosquitoes in different sites in Istria, Croatia.

Site / Species	<i>claviger</i>	<i>maculipennis</i>	<i>plumbicus</i>	<i>cineurus</i>	<i>vexans</i>	<i>albopictus</i>	<i>geniculatus</i>	<i>annulipes</i>	<i>cantans</i>	<i>caspicus</i>	<i>detritus</i>	<i>exrucians</i>	<i>flavescens</i>	<i>leucomelas</i>	<i>mariae</i>	<i>pulchritarsis</i>	<i>punctor</i>	<i>sticticus</i>	<i>rusticus</i>	<i>modestus</i>	<i>pipiens</i>	<i>hortensis</i>	<i>territans</i>	<i>longiareolata</i>	<i>morsitans</i>	<i>annulata</i>	<i>pulchripalpis</i>	Total species vrsta	
Motovun				I	I		I		I/L	L		I	L				I	I/L	I/L						L	L		12	
Pula					I		I		L	I/L	I			I/L	I/L			I/L			I/L	I/L		I/L		I/L			12
Novigrad	I		I		I		I/L			I				I	I			I			I/L	L							10
Raša				I			I		I	I		I				I		I	I		I						I		10
Rovinj		I				I	I			I					L						I/L	I/L		L		I/L	I		10
Buje							I/L		L						I					L		I/L	I/L		I/L				7
Poreč							I/L								I				I/L			I/L	L		I/L		L		7
Funtana									L	L	L				I						I/L						I/L		6
Dolina Mirne					I		I			L					I					I		I							6
Polje Čepić				I	I				I										I		I					L			6
Tarska vala					I					I/L					I	I				I		I							6
Dragonja			I		I		I				I												I						5
Motovunska šuma							L						L		L						L			L					5
Galižana																			I/L		I	I/L	L		I				5
Borut		L			I															I		I							4
Premantura																					I/L	L		I		L			4
Baredine																					L	L		L					3
Brijuni										I											I						I		3
Buzet							L														I/L	I/L							3
Istarske toplice																					I/L	L		I/L					3
Lupoglav		L					L														L								3
Medulin														I							I	L							3
Momjan										L													L	L					3
Palud												L									L						L		3
Plovanija			L				L														L								3
Savudrija		I/L																			L	I/L							3
Šišan															L				I/L		I/L								3
Valtura							I/L			I											I/L								3
Vinkuran																					I	L		I/L					3

Legend: L – mosquito larva, I – imago

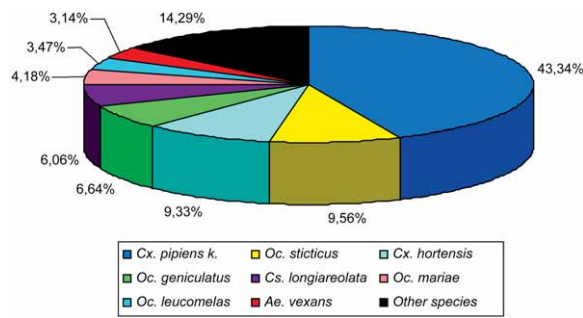


Figure 2. Quantitative fauna composition of mosquitoes in the investigated area.

were sampled in both larval and adult stages. As the investigated area abounds in different habitats (ecological niches) that are favorable for the development of mosquitoes, we managed to establish a great diversity of species. In the coastal area we mostly sampled larvae and adult mosquitoes, whereas in the southern continental area the samples were predominantly mosquito larvae (Figure 1).

Table 1 sums up all sites where sampling was conducted and all mosquito species recorded there. There was a great faunistic diversity of mosquitoes in different sites. In Motovun and Pula sites we recorded 12 species, followed by Novigrad, Raša and Rovinj with 10 species. In most sites we recorded only few species: in 15 sites we only recorded only one species, and in 27 sites only two. During sampling we more often came across larvae than adult mosquitoes. Of the total of 27 species, 16 were recorded in both larval and adult stages. Furthermore, 4 species were recorded only in the larval stage and 7 species only in the adult stage (Table 1).

Of the total of 3087 sampled specimens, 1338 were of the species *Cx. pipiens* complex, i.e. its share in the Istrian fauna was 43.34% (Figure 2), followed by *Oc. sticticus* with 295 specimens, and *Cx. hortensis* with 288 specimens. For as many as five species: *An. claviger*, *Oc. pulcritarsis*, *Cx. modestus*, *Cx. territans* and *Or. Pulcripalpis*, we recorded only one individual, so we may consider them to be rare species in Istria.

The species with the greatest distribution across Istria was *Cx. pipiens* complex, as it was recorded in as many as 53 sites, which makes 71.62% of all investigated sites (Table 1), and we may consider it to be a widespread species.

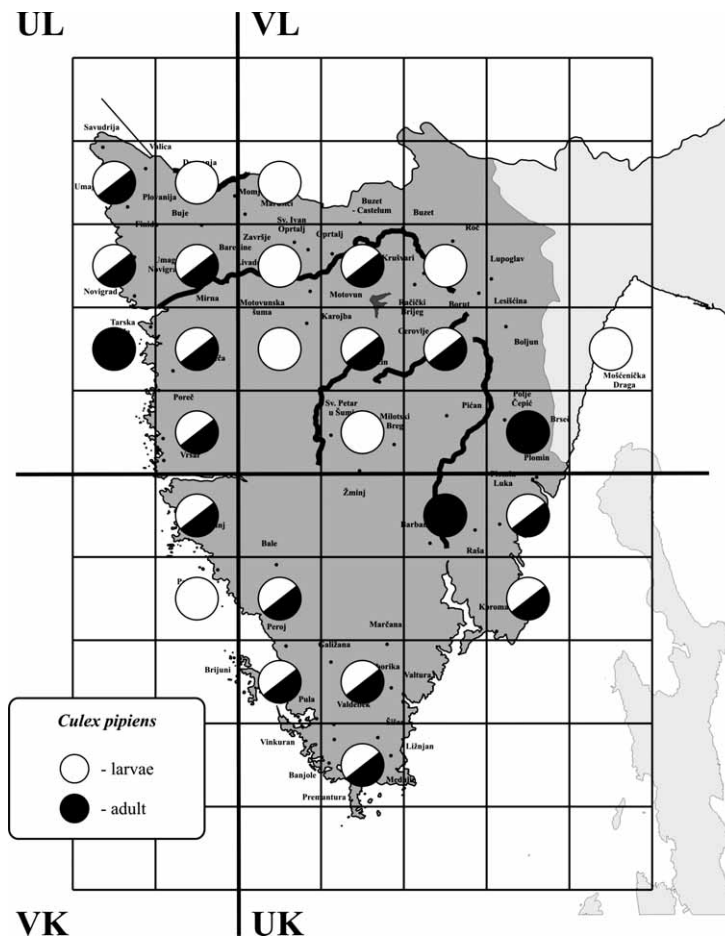


Figure 3. The distribution of *Cx. pipiens* complex in Istria according to UTM.

The distribution presented using the UTM system (Figure 3) indicates that it was recorded in almost all Istria. The larvae of this species produce up to 10 generations a year and spend the winter as fertilized females. As the largest number of breeding sites in Istria are small (swamps, canals, pools, barrels, vases), it is not surprising that this species was abundant and widespread. The second most abundant and widespread species was *Cx. hortensis*, which was recorded in 31 sites (Figure 4). This species was mostly found in the same breeding sites as *Cx. pipiens* complex. The third most widespread species was *Oc. geniculatus*, recorded in 24 sites, i.e. in 15 UTM quadrants (Figure 5). The specimens of this species are widespread in forest areas and along rivers, as the development of larvae depends on the water collected in tree holes. The third most abundant species was *Oc. sticticus* recorded in 13 sites.

DISCUSSION

Fifty mosquito species have been recorded in long-term investigations of mosquitoes in Croatia (10, 11, 12). The number of species and the abundance of each species differ in different parts of Croatia. In the area of Slavonia and Baranja 30 species have been recorded (13), in cen-

tral Croatia, Zagreb area, 28 species (14, 15), and in the Split area 16 mosquito species (16).

The gravest problem with mosquitoes has been registered in Slavonia due to frequent and abundant populations of *Ae. vexans* and *Oc. Sticticu* whose development depends on the flooding of the area after the overflowing of rivers (17). In Istria there are hardly any such flooded areas, so there are no favorable conditions for such great abundance of these species. Very favorable biotic and abiotic factors, i.e. mostly temperature, rainfall and humidity of this area are favorable for the development of different mosquito species. As the coast is indented and the central part of Istria is hilly, small breeding sites like puddles, buckets, bogs, vases and tyres.

The faunistic research of mosquitoes in Istria lasted 7 years, yielding a fairly precise faunistic image of mosquitoes. The presence of 27 species bears witness to a great diversity for such a small area, and it makes 54% of the Croatian mosquito fauna. The diversity is greater in certain sites, especially Motovun and Pula, followed by Raša, Novigrad, and Rovinj. There are two main reasons for a higher diversity of species in these sites. The number of samplings in Pula, Rovinj and Novigrad sites was higher, so there was a greater possibility of finding rare species.

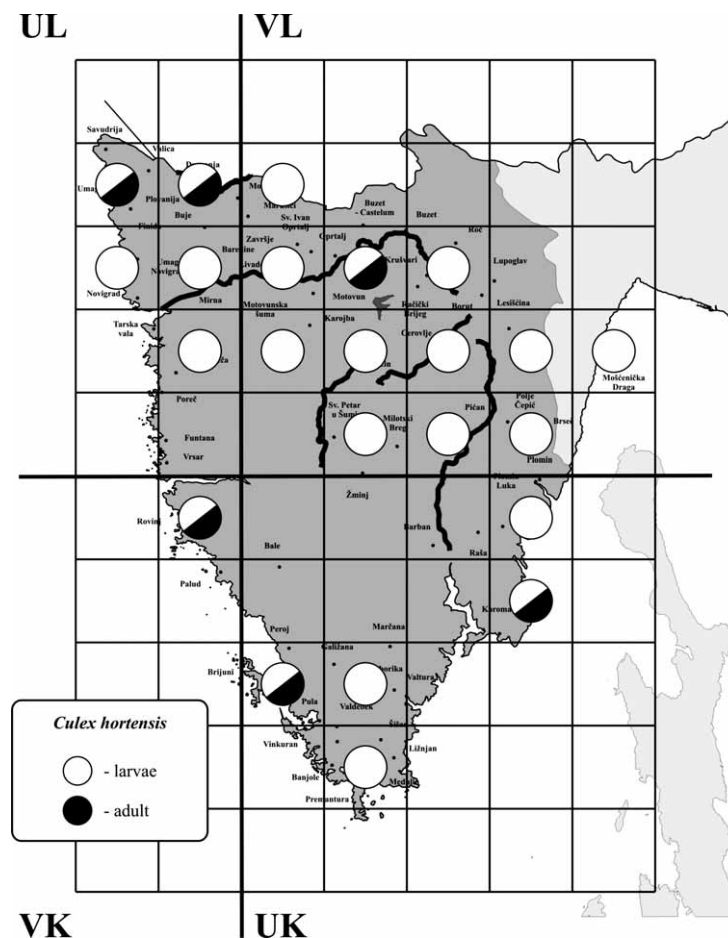


Figure 4. The distribution of *Cx. hortensis* in Istria according to UTM.

TABLE 2

The number of sampled mosquitoes in Istria.

Species	Σ	%
<i>Cx. pipiens</i>	1338	43,34
<i>Oc. sticticus</i>	295	9,55
<i>Cx. hortensis</i>	288	9,32
<i>Oc. geniculatus</i>	205	6,64
<i>Cs. longiareolata</i>	187	6,05
<i>Oc. mariae</i>	129	4,18
<i>Oc. leucomelas</i>	107	3,48
<i>Ae. vexans</i>	97	3,14
<i>Oc. caspius</i>	95	3,07
<i>Cs. annulata</i>	78	2,52
<i>Oc. cantans</i>	70	2,26
<i>Oc. excrucians</i>	65	2,1
<i>An. maculipennis</i>	47	1,52
<i>Oc. rusticus</i>	22	0,71
<i>Ae. cinereus</i>	15	0,48
<i>Oc. detritus</i>	8	0,26
<i>Oc. flavescens</i>	8	0,26
<i>An. plumbeus</i>	7	0,22
<i>Ae. albopictus</i>	6	0,2
<i>Oc. annulipes</i>	6	0,2
<i>Cs. morsitans</i>	6	0,2
<i>Oc. punctor</i>	3	0,1
<i>An. claviger</i>	1	0,04
<i>Oc. pulcritarsis</i>	1	0,04
<i>Cx. modestus</i>	1	0,04
<i>Cx. territans</i>	1	0,04
<i>Or. pulcripalpis</i>	1	0,04
TOTAL	3087	100%

The second reason was taking samples in the forest areas of Motovun and Raša which offer good conditions for the development of a large number of species (1). The great abundance does not necessarily mean a great diversity of species, which this investigation also corroborated

Zoogeographically, the species recorded in Istria are cosmopolitan (*Ae. vexans*, *Ae. albopictus*, *Cx. pipiens*, complex), holarctic (*Oc. sticticus*, *Oc. flavescens*, *Cx. territans*, *Ae. cinereus*), palearctic (*An. claviger*, *An. maculipennis*, *An. plumbeus*, *Oc. geniculatus*, *Oc. annulipes*, *Oc. caspius*, *Oc. detritus*, *Culiseta morsitans*, *Cs. annulata*, *Or. pulcripalpis*, *Oc. cantans*, *Oc. riparius*, *Oc. excrucians*, *Cx. modestus*), and Mediterranean (*Cs. longiareolata*, *Oc. rusticus*, *Oc. leucomelas*, *Oc. pulcritarsis*, *Oc. mariae*, *Cx. hortensis*).

The eudominant species in this area was *Cx. pipiens*. This species was confirmed to be abundant and widespread in this area. As the systemic status of the species has not been solved yet, and as these are the populations that have certain biological differences, we determine it as *Cx. pipiens* complex. *Cx. pipiens* complex has several species, subspecies, forms, races, physiological varieties or biotypes, depending on different authors. The status

of *Cx. pipiens pipiens* Linnaeus, *Cx. p. pipiens* biotype *molestus* Forskal and *Cx. p. quinquefasciatus* Say has been determined as a neotype (18). It has been generally accepted that the former *Cx. pipiens molestus* (18) is not separated from the subspecies *Cx. pipiens pipiens*. It has been determined as a biotype although more recent research suggests significant genetic differences between the two forms (19). As they do not differ morphologically, we suppose that during our research we captured the specimens of *Cx. pipiens* complex that belong to the subspecies *Cx. pipiens pipiens* and *Cx. pipiens* biotype *molestus*. We found them in small breeding sites in urban areas usually preferred by *Cx. pipiens molestus*, as well as in medium sized breeding sites away from urban areas, usually preferred by *Cx. pipiens pipiens*.

The species *Oc. sticticus* and *Cx. hortensis* also belong to abundant and widespread species in this area. The species *Cx. hortensis* most frequently occurs in small breeding sites together with the specimens of *Cx. pipiens* complex. As the larvae of *Cx. hortensis* were found in pure water with some algae and other vegetation, as well as in small pools, neglected wells and garden containers (8, 20), this can justify the fact that they were widespread in this area.

The development of *Oc. geniculatus* larvae is linked to the water collected in tree holes which are specific breeding sites for mosquitoes of this species. Previous research in Croatia indicates that this species has been found in many forest communities throughout Croatia (16, 21, 22). Among the mosquitoes whose breeding sites are tree holes, we found the species *Or. pulcripalpis*, *Oc. pulcritarsis* and *An. plumbeus*, but they were much less abundant. According to this research, the rare mosquito species in Istria are: *Cx. modestus*, *Cx. territans*, *An. claviger*, *Oc. punctor*, *Cs. morsitans*, and *Oc. annulipes*.

Several species of the *Anopheles* genus, which caused many problems in the past as malaria vectors, are very rare today, as this research also showed. The first written document on malaria in Croatia dates back to the 16th century (23). In the 19th century there was a sanitation of the area affected by malaria (Istria and the Neretva valley), in order to eradicate this disease (24). Today there are seven morphologically identical species that belong to the *An. maculipennis* complex, six of which have been registered in the Croatian fauna: *An. sacharovi*, *An. atroparvus*, *An. subalpinus*, *An. labranchiae*, *An. messeae*, and *An. maculipennis*. Intense investigations of the species that belong to the genus *Anopheles* in Croatia (and formerly in Yugoslavia) were conducted by Živko Adamović, who determined 8 species in Dalmatia (25), 8 species in the delta of the Neretva River (26), 3 species in Lika (27), 5 species in Slavonia, Posavina, Podunavlje and Potisje (the Sava, the Danube and the Tisa regions) (28), 3 species in the Drava region (29), and 4 species in Zagreb (30). The research conducted in the 1980's in Istria revealed five species of the genus *Anopheles*: *An. plumbeus*, *An. claviger*, *An. maculipennis*, *An. atroparvus* and *An. sacharovi* (28), the last three of which belong to the complex. In this research we recorded two species of

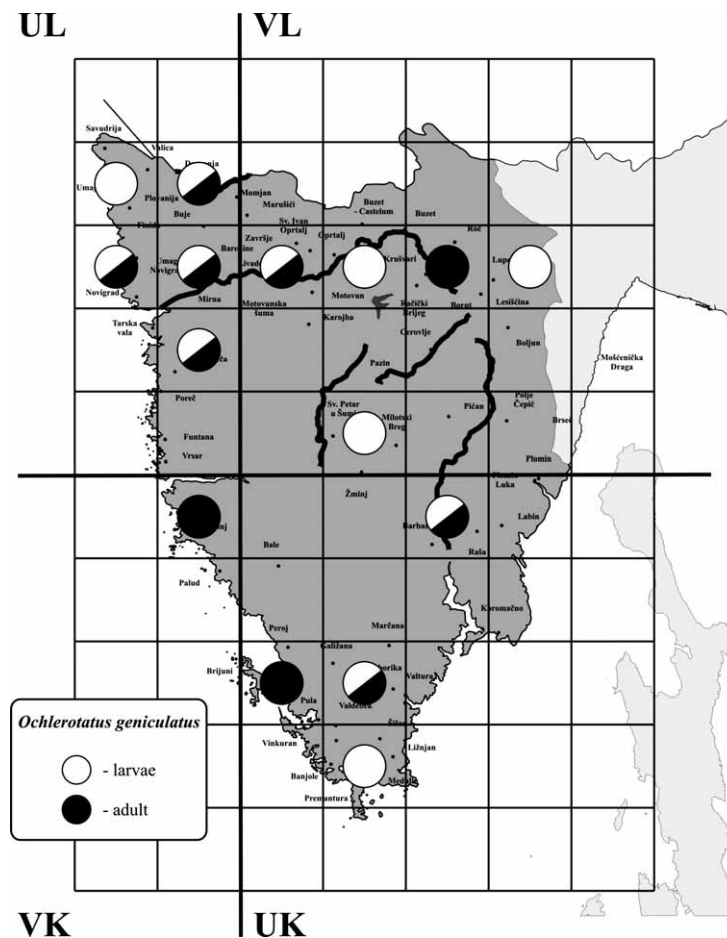


Figure 5. The distribution of *Oc. geniculatus* in Istria according to UTM.

the genus *Anopheles*, i.e. *An. claviger* and *An. plumbeus*. However, we trapped 47 specimens of both larvae and adult mosquitoes belonging to *An. maculipennis* complex, with great likelihood that all three previously mentioned species were also represented.

The beginning of mosquito investigation in Istria was based on determining the most invasive mosquito species today. The species of *Ae. albopictus* (*St. albopicta*) – the Asian tiger mosquito is very aggressive to people as it is, unlike most other species, active throughout the day and may pose problems for both people and tourism (31). On completion of this research, during which the presence of this species in Istria was recorded for the first time, a program was made to continue the investigation of the spreading of *Ae. albopictus* (5). The long-term presence of this species in Italy intensified the research in this area. Though the first data for Croatia was recorded in 2004 in Zagreb (11), this species started spreading intensively in southwestern Istria. As it is a very aggressive species that breeds together with *Cx. pipiens* complex, we suppose that this species will spread all over Istria, and soon endanger *Cx. pipiens* complex in its breeding sites to finally prevail in this region. The research conducted in Italy indicates that both of these species may occupy any

type of artificial breeding sites (32). Both species are mostly found in medium-sized artificial breeding sites (10–50 l) such as sewage openings and used tyres. Laboratory investigations have proven that at 25 °C the biomass coefficient of the species *Ae. albopictus* is significantly higher than for the species *Cx. pipiens*, which indicates that the new species is more capable of turning food in biomass. The consequence of this is a very high capacity for using food resources, which causes much faster growth and development of *Ae. albopictus* (32), resulting in displacing *Cx. pipiens* from its habitat.

Taking into account the results of this research and other published data, the Istrian mosquito fauna consists of the total of 29 species.

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