

TAXONOMY, BIOLOGY, GENETIC VARIABILITY AND MEDICAL IMPORTANCE OF *OCHLEROTATUS CASPIUS* (PALLAS, 1771) AND *O. DORSALIS* (MEIGEN, 1830) (DIPTERA: CULICIDAE)

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Abstract

A review of taxonomic, ecological, and zoogeographical investigations of the medically important species *Ochlerotatus caspius* and *O. dorsalis* is given. Various opinions on the taxonomy of species belonging to the genus *Ochlerotatus* based on the morphological and gene-enzyme analyses are presented. Genetic variability and the biology of these species and their medical importance are presented in detail. Distribution analysis has shown that the species *O. caspius* and *O. dorsalis* are sympatric and synchronic in parts of the Palaearctic region from Western Europe to Mongolia.

Key words: Diptera, Culicidae, *Ochlerotatus dorsalis*, *O. caspius*, medical importance, taxonomy

Introduction

Biological characters of app. 3200 mosquito species (WORD, 1992) and their vectorial capacity point to the importance of an extensive investigation of this group of organisms. Mosquitoes, among all the haematophagous Arthropoda, are the most important mammalian and avian molestants and vectors of various pathogens. They may transmit four types of animal and human pathogens, i.e. the *Plasmodium* species that causes malaria in man (*Plasmodium falciparum* and *P. vivax*), filarial worms of genus *Wuchereria* and *Brugia* (Nematoda) causing lymphatic filariasis in man, arboviruses causing yellow fever and dengue, and, finally, bacteria causing tularaemia (HARWOOD & JAMES, 1979). Etiology, epidemiology, and

pathology studies, which are aimed at the prevention and eradication of these human diseases, have induced detailed analyses of this important group of organisms.

The taxonomy of some mosquito species of the subgenus *Ochlerotatus*, the genus *Ochlerotatus*, is still questionable (VUJIĆ *et al.*, 1993-1994; REINERT *et al.*, 2000; 2004; 2008; SHEPARD *et al.*, 2006). Comparative analysis of morphological characters was proven insufficiently effective in solving the problems of the taxonomy and relationships of the investigated species (GUTSEVICH *et al.*, 1974; BOŽIČIĆ-LOTHROP, 1988; REINERT *et al.*, 2004). *Ochlerotatus dorsalis* has been reclassified nine times since first being described by Meigen in 1830 (KNIGHT & STONE, 1977). Investigations of the genetic structure of the *O. caspius* and *O. dorsalis* populations have enabled better understanding of interspecific genetic divergence (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990; MILANKOV *et al.*, 2000). Particular attention was devoted to the investigations of *O. caspius* and *O. dorsalis* as potential vectors of human pathogens (MITCHELL, 1995).

The need for a more comprehensive understanding of the role of *O. caspius* and *O. dorsalis* as disease vectors induced us to review up-to-date results of the taxonomy, biology, genetic variability, genetic relationships and distribution of these mosquito species.

Results and Discussion

Taxonomy of *Ochlerotatus caspius* (Pallas, 1771) and *Ochlerotatus dorsalis* (Meigen, 1830)

The family Culicidae, suborder Nematocera, order Diptera, includes the subfamilies Anophelinae and Culicinae. The latter was subdivided into 11 tribes (BECKER *et al.*, 2003), one of which is tribe Aedini with the genera *Aedes*, *Armigeres*, *Ayurakitia*, *Eretmapodites*, *Haemagogus*, *Heizmannia*, *Ochlerotatus*, *Opifex*, *Psorophora*, *Udaya*, *Verrallina* and *Zeugomyia*. Previously, *Ochlerotatus* was considered to be one of 38 subgenera of the genus *Aedes*, with 181 species recorded in the Palaearctic (KNIGHT & STONE, 1977). However, based on characteristics of the genitalia of fourth stage larvae and pupae, REINERT (2000) divided the genus *Aedes* into two genera, *Aedes* and *Ochlerotatus*. The genus *Ochlerotatus* was then subdivided into two sections, from which section I included the European mosquito species belonging to the subgenera *Finlaya*, *Ochlerotatus* and *Rusticoides* (REINERT, 2000).

The subgenus *Ochlerotatus* includes many salt marsh, inland or littoral mosquitoes. Out of 200 species of the subgenus distributed worldwide, about a half is registered in the Holarctic and nearly a quarter in each of the Australian and the Neotropical regions (BECKER *et al.*, 2003). The European species of the subgenus *Ochlerotatus* were classified into four groups, *annulipes*, *caspius*, *communis*, *intrudens* and *punctor*. The *berlandi*, *caspius*, *dorsalis*, *mariae*, *pulcritarsis* and *zammitii* species were included in the *caspius* group (BECKER *et al.*, 2003). The taxonomy of the group *caspius* together with certain other taxa of the family Culicidae is still a matter of disagreement. It is assumed that the ancestor of this particular insect group originated in central Asia and later spread to Western Europe and North America (ROSS, 1964; MINAR, 1976). Various criteria have been employed in determining the relationships and origin of insect species. EDWARDS (1932) was the first to describe the group *dorsalis* (group E) including *Ae. canadensis*, *Ae. campestris*, *Ae. dorsalis*, and *Ae. melanimon*. Based on morphological analysis of larval and adult characters, ROHLF (1963a, 1963b) classified *Ae. dorsalis* and *Ae. campestris* into the same group while *Ae. caspius* and *Ae. melanimon* were omitted. STEWARD (1968) analyzed the relationships of 42 species of the genus *Aedes* from Canada by numerotaxonomic techniques using the method of matching coefficients. A dendrogram that showed a close relationship among *Ae. campestris*, *Ae. dorsalis*, and *Ae. melanimon* was constructed based on the

morphological characters of adults. The results were in accordance with the classification of the group "E" made by EDWARDS (1932). GUTSEVICH *et al.* (1974) defined the four species groups within the subgenus *Ochlerotatus*: *caspius* (comprising *caspius*, *mariae*, and *pulchritarsis*), *cantans*, *rusticus*, and *communis*.

The controversy has especially persisted regarding the taxonomic rank of the Holarctic species *O. dorsalis* due to the unclear relationships with Nearctic *O. melanimon* on one hand, and with the Palaearctic species *O. caspius* on the other.

The name of the species *O. melanimon* was accepted as a synonym for *O. dorsalis*. FREEBORN (1926) first defined *melanimon* as an aberrant form of the species *O. dorsalis*. *O. melanimon* was reclassified as a separate species on the basis of the male genitalia structure (BARR, 1955), the colour scaling of different wing veins (CARPANTER & LA CASSE, 1955) and the larval and adult morphological characters (BOHART, 1956; RICHARDS, 1956; CHAPMAN, 1960; CHAPMAN & GRODHANS, 1963).

There are wide discrepancies between the concept describing *O. dorsalis* as the subspecies of *O. caspius* and another one stating that *O. dorsalis* is a species with morphological, biological, ethological and genetic features ensuring its reproductive isolation.

Based on the analysis of the morphological characters, STACKELBERG (1937) defined two subspecies - *O. caspius caspius* (occurring in saline marshes of central Russia) and *O. caspius dorsalis* (lakes and ponds of lower alkalinity in northern Russia), and the intermediate form (overlapping area in the Volga valley). Four forms of the species *O. caspius* were described using the morphological characters of the mesonotum, abdomen, proboscis and wing venation: *O. caspius caspius* (steppe and deserts from eastern to western Siberia, Ukraine, Caucasus, central Asia, and Kazakhstan), *O. caspius dorsalis* (forested regions of northern Russia as far as southern taiga, forest-steppe regions towards east to eastern Siberia and scattered in the steppe zone), *O. caspius* Far East form (Transbaikal, Kabarovsk, Baikal region and the coast) and *O. caspius* Pamirs form (Pamirs and montane regions of central Asia) (GUTSEVICH *et al.*, 1974; GUTSEVICH, 1977; 1985). KNIGHT (1978) reclassified *dorsalis* as the subspecies of *caspius* based on the work, which described intermediate forms in Eastern Europe and Asia (GUTSEVICH *et al.*, 1974). Comparative analysis of mesonotum and the male genitalia revealed the occurrence of typical specimens of the species *O. caspius* and *O. dorsalis*, as well as the intermediate forms in saline ponds and marshes of northern Serbia (BOŽIČIĆ-LOTHROP, 1988).

However, KNIGHT & STONE (1977) accepted taxonomic divergence deduced from morphological traits of *O. caspius* and North American populations of the species *O. dorsalis*, reported by RICHARDS (1956). Results of phenotype and phylogenetic analyses of the isozyme and morphometric data pointed out the molecular differences between *O. caspius* and *O. dorsalis* (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990; MILANKOV *et al.*, 2000).

Genetic relationships and differences among the species and among conspecific populations of *Ochlerotatus dorsalis* (Meigen, 1830) and *O. caspius* (Pallas, 1771)

Based on the phenotype and phylogenetic analyses of the isozyme and morphometric data, SHULTZ *et al.* (1986) reconstructed phylogenetic relationships between salt marsh *Ochlerotatus* (*Aedes*) mosquitoes. Gel electrophoresis employed in analyses of the genetic variability comprised 17 enzyme loci of 32 populations of 16 Holarctic mosquito species. By comparing genetic distance values (*D*), a dendrogram was constructed showing the divergence and differentiation degree between species groups. These results showed the

molecular differences between *O. caspius* and *O. dorsalis*. LAMBERT *et al.* (1990) obtained significant differences with a comparative analysis of the morphological characters, egg exochorion structure and gene-enzyme variability of sympatric populations of the species *O. caspius* and *O. dorsalis* from Western Europe. Artificial mating of these species produced sterile individuals confirming their reproductive isolation. Variation analysis of the siphonal characters in sympatric populations of *O. caspius* and *O. dorsalis* originating from Rusanda (Northern Serbia) proved that species could not be separated in the larval stage. The recorded differences between the species might be explained only at the population level (MILANKOV *et al.*, 1997; 1998). The comparison of pupal development length of males showed that significantly shorter time was required in *O. caspius* than in the sympatric population of *O. dorsalis* (MILANKOV *et al.*, 1997). The morphological features of *O. caspius* and *O. dorsalis* adults from North Serbia showed a pattern of mesonotum and abdomen marks representing valuable morphological differential characters. In analysis of gene-enzyme variability of sympatric populations of species *O. caspius* and *O. dorsalis* (the Rusanda saline pond near Zrenjanin, Serbia) species-specific allozymes at *Idh-2*, *Had*, and *Odh* loci were obtained. This substantiated the complete genetic differentiation of the analyzed species in these loci. The alleles of these loci enabled identification and differentiation of *O. caspius* from *O. dorsalis*, as well as constructing a genetic-biochemical key. Genetic differences were also found at the *Gpi* locus, but only at certain genotype composition levels (MILANKOV *et al.*, 2000).

Genetic relationships between the populations of *O. dorsalis* and *O. caspius* were analyzed based on the genetic identity (*I*) and distance (*D*) coefficients after Nei (1972) (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990; MILANKOV & VAPA, 2002a). A significant difference in average values of genetic distance (Nei, 1972) among Nearctic populations of *O. dorsalis* and *O. caspius* from Europe ($D = 0.280$; SHULTZ *et al.*, 1986); Palaearctic populations of *O. dorsalis* and *O. caspius* from France ($D = 0.335$; LAMBERT *et al.*, 1990); and populations of *O. dorsalis* and *O. caspius* from Egypt ($D = 0.452-0.802$) (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990) were obtained. The genetic distance between the sympatric (North Serbia) and allopatric (North Serbia and Salton Sea) populations of *O. dorsalis* and *O. caspius* species showed that complete allelic substitutions occurred in 33.3% (*Had*, *Idh-2* and *Odh*) and 55.5% (*Gpi*, *Had*, *Est-6*, *Idh-2* and *Odh*) analysed loci, respectively (MILANKOV & VAPA, 2002a).

An electrophoretic survey of two allopatric populations of the *O. dorsalis* species indicated that genetic differentiation during speciation was directed towards changes in allelic frequency illustrated by the fact that the *Est-6* locus completely differed from other loci (MILANKOV & VAPA, 2002c). The presence of major and rare alleles indicated genetic divergence among Palaearctic and Nearctic populations of *O. dorsalis*. The same major alleles (>0.5) at *Gpi*, *Pgm* and *Odh* were registered in both populations. Contrary to these and to *Gpd-2* (which was monomorphic), alleles at the monomorphic *Est-6*, *Idh-2* and *Had* loci, as well as a major allele at the *Me* locus in the Nearctic population, were not major alleles in the Palaearctic population (MILANKOV *et al.*, 2002c). In accordance with these results, no statistically significant differences in allelic frequencies at the *Est-1*, *Pgm* and *Odh* loci were observed. Besides the lack of identical alleles at the *Est-6* locus, genetic differentiation of the Palaearctic and Nearctic populations was due to differences of allele frequency variances at *Me* ($F_{st}=0.711$), *Had* ($F_{st}=0.536$) and *Idh-2* ($F_{st}=0.344$). Genetic divergence among populations was, to a lesser extent, affected by allelic frequency variance at the *Odh* ($F_{st}=0.243$), *Est-1* ($F_{st}=0.136$) and *Gpi* ($F_{st}=0.055$) loci (MILANKOV & VAPA, 2002c). The calculated average genetic distance ($D=0.000-0.002$) among the Nearctic populations of *O. dorsalis* (SHULTZ *et al.*, 1986) was significantly lower compared to the degree of genetic divergence of Nearctic (Salton Sea) and Palaearctic (Rusanda) populations ($D = 0.294$) (MILANKOV & VAPA, 2002c).

Genetic divergence data ($D = 0.160-0.434$) for the populations of *O. caspius* from Europe and Egypt (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990) pointed to the possible existence of established genetic isolation mechanisms. Also, the *Pgm* (SHULTZ *et al.*, 1986) and *Gpi* loci (LAMBERT *et al.*, 1990) were diagnostic for delineating the population from Egypt and populations from France.

Genetic variability in the populations of *Ochlerotatus dorsalis* (Meigen, 1830) and *O. caspius* (Pallas, 1771)

Protein-enzyme polymorphism can serve as an indicator of changes in genes, which occur during evolution. These processes are complex since they involve coadaptative changes in the system of genes. Analyses of isozyme variability in populations of the *O. dorsalis* and *O. caspius* species showed that various loci had different levels of variability. Thus, *Gpd-2* locus in *O. caspius* was monomorphic in two populations from France and one population from Egypt (SHULTZ *et al.*, 1986) as well as in the population from North Serbia (MILANKOV & VAPA, 2002b). Only in the population from Ravenne was *Gpd-2* polymorphic, while it was monomorphic (criterion 0.95) in the other two populations from France with two registered heterozygous genotypes (LAMBERT *et al.*, 1990). Also, the *Pgm* locus was polymorphic in all analyzed populations from France (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990) and North Serbia (MILANKOV & VAPA, 2002b). Similar to French populations, a heterozygote was found in the population from Rusanda (MILANKOV & VAPA, 2002b). In one population from France and the population from Cairo, the *Gpi* locus was monomorphic, while in two French populations, two homozygotes and two heterozygotes were found (LAMBERT *et al.*, 1990). One homozygote ($Gpi^{100/100}$) and one heterozygote ($Gpi^{100/112}$) were observed in the population from Rusanda at the *Gpi* locus (MILANKOV *et al.*, 2000; MILANKOV & VAPA, 2002b). Contrary to Nearctic populations where *Idh-2* and *Me* loci were monomorphic (SHULTZ *et al.*, 1986), in the population from North Serbia three (genotype: $Idh-2^{102/102}$, $Idh-2^{104/104}$, $Idh-2^{98/102}$) and four (Me^{102} , Me^{104} , Me^{106} , Me^{108}) alleles, respectively, were registered (MILANKOV & VAPA, 2002b). The largest number of genotypes in the population of *A. caspius* from Rusanda was found at the *Est-6* locus (six homozygotes and 17 heterozygotes) (MILANKOV & VAPA, 2002b). In sympatric populations of the species *O. caspius* and *O. dorsalis* from Rusanda, identical alleles ($Est-6^{96}$, $Est-6^{98}$, $Est-6^{93}$, $Est-6^{96}$, $Est-6^{98}$, $Est-6^{100}$, $Est-6^{102}$, $Est-6^{104}$) were observed at the *Est-6* locus (MILANKOV & VAPA, 2002b, 2002c).

The analysis of allozyme variability at the 17 isozyme loci in the populations of *O. caspius* from France (SHULTZ *et al.*, 1986) showed that the percentage of polymorphic loci (P), the mean number of alleles per locus (A) and the value of mean heterozygosity (H_o) were uniform ($P = 17.65$; $A = 1.18-1.24$; $H_o = 0.035-0.106$) and lower than corresponding values ($P = 57.1-60.0$; $A = 1.8-2.71$; $H_o = 0.113-0.148$) according to LAMBERT *et al.* (1990). The highest values of genetic structure parameters were registered in the population of *O. caspius* from Rusanda ($P = 88.9$; $A = 3.8$; $H_o = 0.267$) (MILANKOV & VAPA, 2002b), while the lowest were recorded in populations from Egypt ($P = 0.0, 5.88$; $A = 1.0, 1.06$; $H_o = 0.0, 0.024$) (LAMBERT *et al.*, 1990; SHULTZ *et al.*, 1986).

Analyses of isozyme variability in the populations of *O. dorsalis* showed that the *Gpd-2* locus was monomorphic in four Nearctic populations (SHULTZ *et al.*, 1986) and a Palaearctic population (LAMBERT *et al.*, 1990), which was congruent with the results by MILANKOV & VAPA (2002c). Also, the *Idh-2* locus was monomorphic in all Nearctic populations (SHULTZ *et al.*, 1986) as well as in the population from Salton Sea (Nearctic population), but not in the population from Rusanda (Palaearctic population) after MILANKOV *et al.* (2000). Contrary to populations from New Mexico, Michigan, Utah and Wyoming (SHULTZ *et al.*, 1986) where *Me* locus was monomorphic, in Salton Sea and Rusanda populations two and three alleles, respectively, were registered (MILANKOV & VAPA, 2002c). The *Pgm* locus was polymorphic in all analyzed populations of the *O. dorsalis* species (SHULTZ *et al.*, 1986; LAMBERT *et al.*, 1990). No heterozygotes at *Pgm* were registered in Nearctic populations (SHULTZ *et al.*, 1986), while one homozygote and one heterozygote were registered in the Salton Sea population (MILANKOV & VAPA, 2002c). In the Palaearctic population of the species *O. dorsalis* from

France, one homozygote and three heterozygotes were found at the *Pgm* locus (LAMBERT *et al.*, 1990), and three homozygotes and two heterozygotes were observed in the population from Rusanda (MILANKOV & VAPA, 2002c). In the population from France two homozygotes and one heterozygote were registered at the *Gpi* locus (LAMBERT *et al.*, 1990), whereas one homozygote and one heterozygote were found in the population from North Serbia (MILANKOV *et al.*, 2000). However, the *Gpi* locus was monomorphic in the Salton Sea population (MILANKOV *et al.*, 2000). Contrary to Palaearctic population, the *Idh-2*, *Had* and *Est-6* loci were monomorphic in the Nearctic population (MILANKOV & VAPA, 2002c). The *Odh* locus was monomorphic in both populations (MILANKOV *et al.*, 2000), except the rare allele *Odh*¹⁰⁸ in the Nearctic population. At the *Est-6* locus the largest number of genotypes (five homozygotes and seven heterozygotes) was found in the population from South Eastern Europe, and a unique allele *Est-6*^{84/64} was registered in the Palaearctic population (MILANKOV & VAPA, 2002c).

The analysis of allozyme variability at 17 isozyme loci in populations of *O. dorsalis* from Nearctic (SHULTZ *et al.*, 1986) showed that heterozygosity (H_o) and mean number of alleles per locus (A) were uniform ($H_o = 0.035-0.059$; $A = 1.06$) and similar to corresponding values calculated for the Nearctic population ($A = 1.8$; $H_o = 0.059$), while frequency of polymorphic loci differed ($P = 5.88$; $P = 33.3$) according to MILANKOV & VAPA (2002c). Higher values of all genetic structure parameters were registered in the Palaearctic population of the *O. dorsalis* species compared to the Nearctic population, as well as in the population from North Serbia (MILANKOV & VAPA, 2002c) compared to the population from France (LAMBERT *et al.*, 1990). The frequency of polymorphic loci ($P = 0.778$) and heterozygosity ($H_o = 0.263$) was somewhat higher in the population from Rusanda (MILANKOV & VAPA, 2002c) than in the population from France ($P = 0.714$; $H_o = 0.178$) (LAMBERT *et al.*, 1990).

Biology of the species

Ochlerotatus (Ochlerotatus) caspius (Pallas, 1771)

Type-loc: Caspian Sea (KNIGHT & STONE, 1977)

Favorable environmental conditions (primarily precipitation, tides, floods and temperature) increase the number of generations per year (MARSHALL, 1938; CRANSTON *et al.*, 1987; BOŽIČIĆ, 1988; PETRIĆ, 1989). The eggs are laid in mud along the edge of receding pools and rivers (CRANSTON *et al.*, 1987; BOŽIČIĆ, 1988), most frequently (95%) 2 cm beneath the surface (GABINAUD, 1975). Since there is a strong association between the vegetation structure and soil egg number, floristic composition may be used as an indicator of egg location (GABINAUD, 1975). The adults are susceptible to the adverse environmental conditions thus overwintering in the egg stage. Temperatures below 12°C suspend the development of natural populations (SINEGRE, 1974). A facultative diapause is also induced by unfavourable environmental factors, such as temperatures and a short day photoperiod (VINOGRADOVA, 1975). Embryogenesis is influenced by temperature (stimulative effect of higher temperatures) and anoxia (suspended embryogenesis). Hatching is stimulated by flooding and anoxia (egg eclosion at 25°C for 108^h in laboratory), while egg and larval mortality is affected by salinity (egg and larva critical values of 20 g/l and 60 g/l, respectively) (SINEGRE, 1974).

The first adults appear in early April, increasing in numbers during summer while only sporadically occurring in September (PETRIĆ, 1989) and October (CRANSTON *et al.*, 1987). Adults of *O. caspius* may be found several kilometers away from the eclosion site (MARSHALL, 1938; BOŽIČIĆ, 1985). A zoophilic feeding habit and corpuscular-nocturnal activity are characters of mosquito females. Temperature and relative humidity thresholds are 11.5°C - 36°C and 52% and 92%, respectively (PETRIĆ, 1989).

The comparison of pupal development length in *O. caspius* and *O. dorsalis* females from Rusanda showed no statistically significant differences (MILANKOV *et al.*, 1997). With regard to the nutrition-relative humidity-temperature influence, it was found that *O. caspius* females lived longer than males. Also, a higher temperature, lower humidity, and distilled water substratum significantly reduced the longevity of both females and males when compared to the temperature of 17°C, 90% relative humidity, and feeding on sugar solution. Irrespective of humidity and nutrition, the rise in temperature shortened adult life (ABDEL-MALEK & ADHAM, 1978). IVNITSKY *et al.* (1984) and IVNITSKY (1994) found a correlation among the environmental conditions, nutrition and morphological parameters of the siphon in particular, noting that the larvae of the laboratory populations of *O. caspius* feeding on periphyton were characterized by a smaller number of pecten spines of the siphon than when plankton was used through filter-feeding. In addition, a distinct phototaxis was observed, i.e. the larvae with a greater number of teeth (filter-feeders) were characterized by higher positive phototaxis than those with a smaller number (scraping off periphyton). The autogenic fecundity was lower in the imagoes reared from the larvae with the smaller number of pecten teeth. Accordingly, IVNITSKY (1994) pointed out that such divergence might not have taken place as a result of a disruptive selection treatment on the basic population, because this would have led to a loss of variability in the progeny.

Ochlerotatus (Ochlerotatus) dorsalis (Meigen, 1830)

Type-loc: Berlin, Germany (KNIGHT & STONE, 1977)

Ochlerotatus dorsalis overwinters in the egg stage from which fully developed larvae hatch in early spring. Eggs are laid in a manner similar to that described for *O. caspius*. High precipitation and floods result in up to eight generations during a season (TELFORD, 1958). Larvae breed freely and abundantly in fresh water (HARWOOD & JAMES, 1979). Eggs deposited by first generation females never fall into diapause, while eggs deposited by both second and third generation females may complete their development or enter diapause, depending on prevailing temperatures. Temperature is the principal environmental factor that stimulates or inhibits diapause development in *Ae. dorsalis* (KHELEVIN, 1958; 1959). When the temperature drops below 15.5°C, the following generations are diapausing, whereas 26.6°C is sublethal during the embryonic development (TELFORD, 1957; 1963).

Males and females were observed to feed on nectar, while females also fed directly from host blood vessels. Males swarm about 1.5-2 meters above the ground at sites with no obvious markers (CRANSTON *et al.*, 1987). Sex dimorphism in pupal development length of both *O. caspius* and *O. dorsalis* from North Serbia was not registered (MILANKOV *et al.*, 1997). Males emerge before females and subsequent swarming and mating take place close to the breeding site one or two days after emergence. After mating, the population generally disperses from the emergence site (BOHART & WASHINO, 1978). *O. dorsalis* belongs to the group of mosquitoes with a zoophilic feeding habit. Blood feeding takes place mostly during night or dusk and dawn (corpuscular-nocturnal activity) at 9-30°C and a relative humidity of 52-92% (PETRIĆ, 1989).

Species with similar ecological preferences such as *O. nigromaculis*, *O. squamiger*, *O. melanimon* and *O. dorsalis* are widely distributed in North American salt marshes and other coast and inland saline waters. Although sympatric, univoltine species *O. squamiger* is dominant during winter while *O. dorsalis* is primarily a summer marsh occupant. *O. squamiger* enters diapause with the early spring rains and rise in temperature in April, which also initiate the development of *O. dorsalis*. Continental saline ponds and lakes are occupied by the sympatric and synchronic species *O. melanimon* and *O. nigromaculis* which probably undergo an egg diapause similar to that of *O. dorsalis*. These two species occupy different ecological niches and do not compete directly with one another (CHAPMAN, 1960; TELFORD, 1958; 1963).

Medical importance

Ochlerotatus (Ochlerotatus) caspius (Pallas, 1771)

Spiroplasma sabaudiense (classis Mollicutes) was isolated from adults of the *O. caspius* species caught in June in swampy habitats on the Atlantic coast, whereas it was not recorded in those caught in August and September. It was suggested that mosquitoes are infected by feeding on nectar and not in larval habitats (LE GOFF *et al.*, 1990). *Cristulospora aedis* was isolated from *O. caspius* larvae and adults at both sites. The microsporidia were found in the larval body fat and the female ovaries (KHODZHAIEVA & ISSI, 1989). West Nile virus (WNV), Tahyna virus and the bacterium *Francisella tularensis*, the causative agent of tularemia, could be detected in natural populations (DETINOVA & SMELOVA, 1973). *O. caspius* may have played a role in the spread of tularaemia and transmitted Tahyna and rabbit myxoma viruses in former Czechoslovakia, France and Portugal (BARDOS & DANIELOVA, 1959; JOUBERT, 1975; PIRES *et al.*, 1982). The most important vectors of Tahyna virus in Europe are *Ae. vexans* and *O. caspius*. The virus was also isolated in 1981 from *O. caspius* in Austria (PILASKI, 1987). The species *O. caspius* is the vector of Tahyna (TAH) virus in the Mediterranean region and also a potential reservoir of "Riff Valley Fever Virus" (RVF) during interepizootic periods (MITCHELL, 1995).

Ochlerotatus (Ochlerotatus) dorsalis (Meigen, 1830)

Arboviruses of the California group causing California encephalitis (CE) were isolated from the *O. dorsalis* larvae in central Utah, USA (BOHART & WASHINO, 1978; WOOD *et al.*, 1979). Also, a California serogroup and Hart Park and Cache Valley viruses isolated from *O. dorsalis* during an equine epizootic disease in New Mexico in August 1985 were investigated (CLARK *et al.*, 1986). St. Louis encephalitis virus (SLE) has been isolated in California (EMMONS *et al.*, 1986; 1987) and Canada (ARTSOB *et al.*, 1985). The role of this mosquito species in CE virus epidemiology is still uncertain.

Distribution of *Ochlerotatus caspius* and *O. dorsalis*

The Palaearctic species *O. caspius* inhabits saline lakes and pools of the Mediterranean region (SINEGRE, 1974; LAMBERT *et al.*, 1990), shores of Great Britain, and fresh water and lower salt marshes in the continental parts of Europe, Russia, Mongolia, Northern China, Pakistan, North and North-Eastern Africa, Asia Minor and Persian Gulf (BOŽIČIĆ-LOTHROP & VUJIĆ, 1996; CRANSTON *et al.*, 1987; GABINAUD, 1974) (Fig.1).

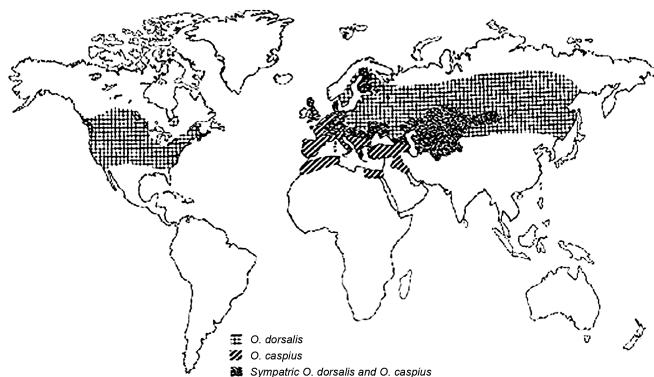


Figure 1. Distribution of *Ochlerotatus caspius* and *O. dorsalis*.

The Holarctic species *O. dorsalis* was found at inland saline ponds, lakes, and marshes, islands, and sea shores of California (BOHART, 1956; BOHART & WASHINO, 1978), Canada (WOOD *et al.*, 1979), and Great Britain (CRANSTON *et al.*, 1987; MARSHALL, 1938). Individuals of this species were also recorded from salt and fresh water lakes and ponds in Northern, Central and Southern Europe (SAMANIDOU-VOYADJAGLOU & DARSIE, 1993), Siberia, China (EDWARDS, 1921) and Mongolia (MINAR, 1976, 1978) (Fig.1).

These two mosquito species are sympatric in some parts of their ranges, including certain regions of central, western, and southern Europe, Finland, the Black Sea, the Caspian Sea, and the Caucasus as far as west Mongolia (Fig.1). They synchronically occupy the same habitats.

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ТАКСОНОМСКИ СТАТУС, БИОЛОГИЈА И МЕДИЦИНСКИ ЗНАЧАЈ
ВРСТА *OCHLEROTATUS CASPIUS* (PALLAS, 1771)
И *O. DORSALIS* (MEIGEN, 1830) (DIPTERA: CULICIDAE)

ВЕСНА МИЛАНКОВ, ДУШАН ПЕТРИЋ, АНТЕ ВУЈИЋ И ЉИЉАНА ВАПА

Извод

У раду је дат преглед таксономских, еколошких и зоогеографских истраживања врста од епидемиолошког значаја, *Ochlerotatus caspius* и *O. dorsalis*. Различита таксономска гледишта о статусу таксона рода *Ochlerotatus* заснована на морфолошким и генетичким подацима су такође презентована. Генетички диверзитет и биологија анализираних врста, као и њихова медицински значај су представљени у раду. Анализа распрострањења је показала да се на делу ареала (делови централне, западне и јужне Европе, све до Монголије) проучаване врсте симпатричке и синхроне.

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