

# Preliminary study on 2 colour patterns in *Ochlerotatus caspius* (Pallas, 1771) (Diptera, Culicidae)

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## Keywords

Colour pattern,  
rRNA internal  
transcribed spacer 2,  
*Ochlerotatus caspius*.

## Summary

*Ochlerotatus caspius* is a mosquito of medical and veterinary relevance both for its synanthropy and for its potential role in transmission of viruses and nematodes in the areas that it inhabits. Due to its wide range and the marked variability in the adult colour pattern, some authors have recognized *Ochlerotatus caspius* as a complex of species. In this study, we purposed to evaluate the possible taxonomic heterogeneity between 2 chromatic forms by using both morphological and molecular approaches. The preliminary results based on the identity of the rRNA internal transcribed spacer 2 (ITS-2) lead us to believe the 2 forms as a single species with a chromatic polymorphism.

## Studio preliminare su due forme cromatiche di *Ochlerotatus caspius* (Pallas, 1771) (Diptera, Culicidae)

## Parole chiave

Colorazione,  
Marcatore molecolare  
ITS-2,  
*Ochlerotatus caspius*.

## Riassunto

*Ochlerotatus caspius* è una zanzara essenzialmente fonte di fastidio per l'alto grado di antropofilia, sebbene sia risultata essere un potenziale vettore di arbovirus, ed elminti. La colorazione dell'adulto è molto variabile tanto che alcuni Autori parlano di un complesso di specie *Ochlerotatus caspius*. Scopo di questo studio è stato quello di valutare l'esistenza o meno di omogeneità genetica tra due diverse forme cromatiche della specie, sia attraverso l'osservazione di alcuni caratteri morfologici sia utilizzando l'analisi molecolare. Il dato preliminare ottenuto mediante il marcatore molecolare ITS-2 fa ritenere che le due colorazioni siano dovute soltanto ad un polimorfismo cromatico della specie e non alla esistenza di due taxa distinti.

*Ochlerotatus caspius* (Pallas, 1771) is a mosquito with a Palearctic distribution, widely spread in Italy along the coastal plains, mainly in flooded territories, which are actually suitable to the larval breeding sites. In fact, larval development of the species occurs in coastal brackish ponds, flooded dunes or artificial situations, like irrigation canals and rice-fields (Rioux 1958). Occurring from Atlantic to Pacific Ocean and from Finland to Northern Africa up to Iran, *Oc. caspius* is known as one of the most aggressive mosquitoes biting humans and livestock outdoor as well as indoor, in shelters, and houses (Gutsevich 1974). The species is active from Spring to Autumn, overwintering as eggs. Females can lay eggs by autogeny, that is in absence of a blood meal, as described for Northern Africa and Arabic

peninsula (Farid *et al.* 1989, Ahmed 2013a). These mosquitoes bite both during night and day, actively seeking their hosts also for long distances in the surrounding of the larval breeding sites (Gutsevich 1974). Concerning its relevance for human health, *Oc. caspius* is known to be one of the most competent mosquito species for the transmission of the Rift Valley fever virus in Egypt (Beier *et al.* 1987, Turell *et al.* 1996, Gad 1999), while the West Nile virus has been isolated in specimens from Ukraine (Vinograd and Obukhova 1975), and from Italy (Engler *et al.*, 2013) where also USUV was detected in this mosquito.

In Italy, *Oc. caspius* represents mainly an annoying species because of the high level of synanthropy (Romi *et al.* 1997), even if it is known to be a

possible vector of arboviruses, such as Tahyna virus (Verani *et al.* 1979, Calzolari *et al.* 2010) and Marisma mosquito virus, recently isolated in some *Oc. caspius* specimens from Northern Italy (Rizzo *et al.* 2014). Moreover, the species is able to transmit the filarial worms, *Dirofilaria immitis* (Roubaud and Colas-Belcour 1937, Rossi *et al.* 1999), *D. repens* (Pampiglione *et al.* 1995), and *Setaria labiatopapillosa* (Cancrini *et al.* 1997).

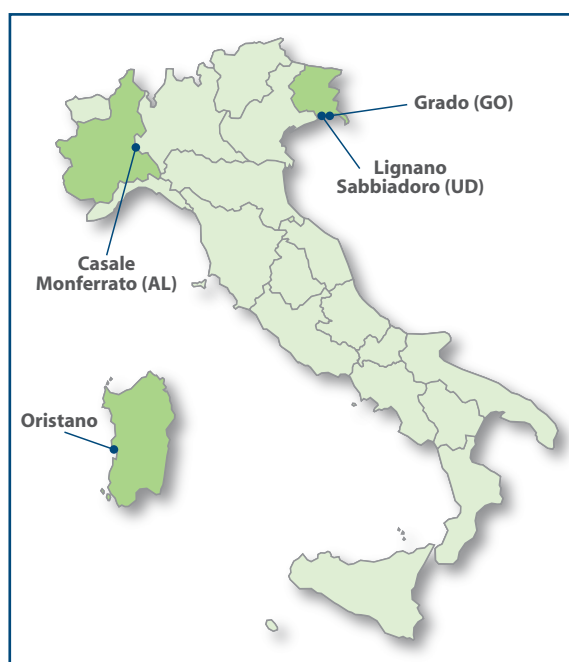
Some authors recognize *Oc. caspius* as a complex of species and in Italy 2 forms were identified at enzymatic, but not at morphological level, by analysing different populations (Cianchi *et al.* 1980). The colour pattern in adult *Oc. caspius* is variable, although the typical mosquitoes are characterized by abdominal tergites showing a brownish colour pattern with transversal pale stripes crossed by a longitudinal pale line running the whole length of the abdomen. Moreover, in Eurasia *Oc. caspius* appears morphologically very similar to *Oc. dorsalis* (Meigen 1830), a Holarctic species from which differs in the colour pattern of palps only (Schaffner *et al.* 2001, Severini *et al.* 2009). In more details, in this study adult female mosquitoes were identified observing the following characters:

- abdominal terga showing pale longitudinal median strip;
- tarsomeres with pale rings to the joints;
- abdominal terga with continuous longitudinal median pale strip;
- apex of the apical palpomere with pale spot;
- apical palpomere long nearly to the preapical one.

Adult male mosquitoes were identified on the basis of the characters of genitalia (Schaffner *et al.* 2001, Severini *et al.* 2009).

Aim of this study was to investigate the taxonomic heterogeneity between these 2 chromatic forms in Italy by using both morphological and molecular approaches.

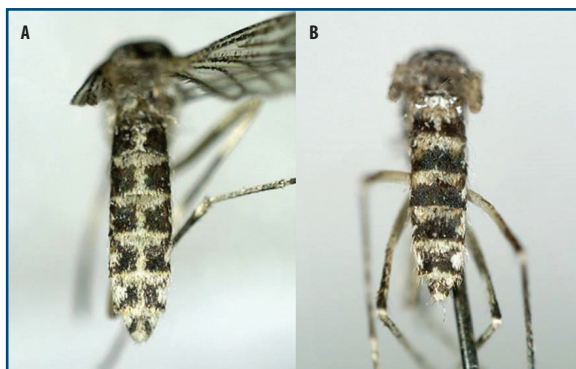
The morphological observation was conducted on mosquitoes collected in 4 Italian localities: Grado (45°41'58.83"N 13°22'46.68"E, Gorizia Province, Friuli-Venezia Giulia Region), Lignano Sabbiadoro (45°40'6.80"N 13° 6'14.71"E, Udine Province, Friuli-Venezia Giulia Region), Casale Monferrato (45° 7'54.27"N 8°27'5.90"E, Alessandria Province, in Piedmont Region), and Oristano (39°54'23.86"N 8°35'18.15"E, in Sardinia Region) (Figure 1). During the Summer of 2003, mosquitoes were sampled using CDC traps and powered aspirator inside houses or animal shelters. Based on the morphological key (Severini *et al.* 2009), we noted that a part of the specimens collected in Grado and in Oristano was characterized by an atypical



**Figure 1.** The map shows the four localities where mosquitoes were collected in 2003. The morphological observations were conducted on mosquitoes from Grado (Gorizia Province, Friuli-Venezia Giulia Region), Lignano Sabbiadoro (Udine Province, Friuli-Venezia Giulia Region), Casale Monferrato (Alessandria Province, Piedmont Region), and Oristano (Sardinia Region). Among the specimens from Grado and Oristano it was possible to observe an atypical colour pattern of the abdominal tergites.

colour pattern of the abdominal tergites (Figure 2B), respect to the typical form (Figure 2A). These specimens showed in fact a modified colour pattern with a short longitudinal pale line joining only the first with the second abdominal tergite. In the light of this morphological difference, we planned to verify a potential heterogeneity also at genetic level between the 2 phenotypic forms by using the rRNA internal transcribed spacer 2 (ITS-2) as molecular marker. This is a method already widely applied in mosquito diagnosis and phylogenetic studies within other species complexes (Marinucci *et al.* 1999, Brengues *et al.* 2014). The DNA from 20 specimens selected for each locality was extracted. Ten typical and 10 atypical colour pattern individuals were selected from the Oristano and Grado sites, respectively. The ITS-2 amplification was carried out according to Marinucci and colleagues (Marinucci *et al.* 1999), and PCR products were sequenced at MWG Biotech AG (Ebersberg, Germania) (GenBank Accession numbers: KP642667-KP642746). For sequence alignment, Clustal W-1.7 software was used (Thompson *et al.* 1994).

Among the collected mosquitoes, the specimens from Oristano (76: 62 females and 14 males) and Grado (277: 270 females and 7 males) showed atypical colour pattern in the ratio of 40% and 35%,



**Figure 2.** Colour pattern of the abdominal tergites in *Ochlerotatus caspius*; (A) typical colour pattern; (B) atypical colour pattern. The colour pattern in adult *Oc. caspius* is variable, although the typical mosquitoes are characterized by abdominal tergites showing a brownish colour pattern with transversal pale stripes, crossed by a longitudinal pale line running the whole length of the abdomen.

respectively, while the remaining were characterized by the typical pattern. In the collections from Lignano Sabbiadoro (321: 290 females and 31 males) and Casale Monferrato (65: 55 females and 10 males) (Figures 2A, B) we found the typical form only. The sequencing generated 80,362 bp fragments, which were aligned and compared with a reference sequence (KP642666) from a specimen collected at larva stage in Porto Alabe (Nuoro Province, Sardinia Region) and identified using a morphological key to larvae and pupae (Romi et al. 1997).

The molecular analysis showed no intraspecific variability with a complete sequence identity among mosquitoes from different localities, as well as between 2 mosquito groups with different

colour pattern. In addition, our sequences showed a similarity ranging from 95.9 to 99.6% when compared with ITS-2 sequences from Tunisia, Iran, and Kingdom of Saudi Arabia, available in GenBank (FM208084, FM208089, KF483843, GU977216). The sequences also showed a 100% identity with sequences from Italy (HM140420, HM140421 and HM140423). Furthermore, such sequences were reported by Talbalaghi and Shaikevich (Talbalaghi and Shaikevich 2011) and Ahmed (Ahmed 2013a), confirming ITS-2 marker as functional at least for our very preliminary investigation.

Considering that ITS-2 marker has already been proved successful for the identification of sibling species within mosquito species complexes (Manonmani et al. 2007, Ahmed 2013b, Milankov et al. 2009) and given that in this study we obtained a single ITS-2 haplotype for our sample, we conclude that mosquitoes with both typical and atypical pattern may belong to a single species. Consequently, these 2 patterns could be due only to a chromatic polymorphism within the same species and not to the existence of 2 taxa within the *Oc. caspius* complex. Should this not be the case, then the ITS-2 should be considered a molecular target not enough informative and unsuitable to distinguish the 2 taxonomic entities. This study supplies preliminary data, limited to European and Asiatic mosquito fauna obtained using only 1 molecular method. We aim to develop further and more detailed research on this topic. By extending mosquito collections, we aim to test other molecular markers that could have a better resolving power useful for identifying the species of *Oc. caspius* complex.

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