

ON THE *CULICOIDES OBSOLETUS* GROUP BITING MIDGES IN LITHUANIA: THE FAUNA, SEASONAL ACTIVITY AND DIVERSITY

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Abstract. The *Culicoides obsoletus* group is involved in the transmission of orbiviral diseases, such as blue tongue, affecting livestock. Imagines of the *C. obsoletus* group can be found from middle April till the end of October in Lithuania. *C. chiopterus* (Meigen, 1830), *C. obsoletus* (Meigen, 1818) and *C. scoticus* Downes et Kettle, 1952 have been determined in Lithuania. *C. scoticus* and *C. chiopterus* are not abundant. *C. obsoletus* is one of the most widespread species of bloodsucking midges in Lithuania. Adult *C. obsoletus* were found from April till the end of October. Four flying peaks were detected for this species during a season. Species identification in the *Culicoides obsoletus* group is very complicated, so the molecular data are getting more and more important in the taxonomy of these insects. Two haplotypes of *C. scoticus* and two haplotypes of *C. obsoletus* were determined according to the fragment of cytochrome c oxidase subunit I gene.

Key words: *Culicoides obsoletus*, seasonal activity, COI, Lithuania

Introduction

The genus *Culicoides* is represented by 1260 species; some of them are involved in the transmission of orbiviral diseases, such as blue tongue, affecting livestock. Blue tongue has been spreading north fast from South Europe since 1998 (Dallas *et al.*, 2003). Recent studies have proved that the blue tongue epidemic in North Rhine Westphalia in Germany and in the neighbouring countries was initiated by virus transmission during the blood meal of midges of the *C. obsoletus* group (Mehlhor *et al.*, 2007).

The *C. obsoletus* group is very common in Lithuania. *C. obsoletus* imagines can be found from middle April till the end of October. Three species from this group: *Culicoides chiopterus* (Meigen, 1830), *C. obsoletus* (Meigen, 1818) and *C. scoticus* Downes et Kettle, 1952 are known in Lithuania (Pakalniškis *et al.*, 2006). The identification of the species is very complicated (Pagès & Monteys, 2005), so the molecular data become a more and more important tool in the taxonomy of these insects (Sebastiani *et al.*, 2001; Szalanski *et al.*, 2006).

Material and Methods

Data on the flying activity of the *Culicoides obsoletus* group were based on the material collected in 2000–2004 by light traps in Plokščiai (Plungė district, 56°01'N21°56'E, trap supervised by G. Margis), Rūgšteliškis (Utena district, 55°27'N26°01'E, trap supervised by G. Švitra), Verkiai (Vilnius t., 54°45'N25°17'E, trap

supervised by P. Ivinskis) and Juodkrantė (Neringa t., 55°33'N21°07'E, P. Ivinskis) The material from light traps was taken out 4–5 times per month, usually once a week. Biting midges were stored in 70% ethanol. Part of each catch was processed to produce microscopic slides in phenol balsam for species identification. Species were determined according to Gutsevich (1973) and Glukhova (1989).

Material for genetic investigations was collected in 2008–2009 in the Vilnius city (Pavilniai Regional park, E25°21'16" N54°41'27"). The material was collected once per month from April till September. Females of biting midges were attracted by human and collected with an entomological net. The material was transported to the Nature Research Centre. Insects were identified and preserved in 96% ethanol at -20 °C until further analysis.

DNA was extracted by the modified Robertson & MacLeod (1992) method. The mitochondrial cytochrome c oxidase subunit I DNA was amplified with LCO1490 and HCO2198 primers (Folmer *et al.*, 1994). PCR reactions were performed in 50 µl volumes containing 5 µl of 10 x PCR Buffer, 3 µl of 25 mM MgCl₂, 1 µl of 10 mM dNTP, 1 µl of 10 pmol/µl of each primer, 0.5 µl of Taq Polymerase (Fermentas Inc.) and 2 µl of DNA genomic template. Amplification was carried out following the thermal cycling parameters: 94°C for 10 min, 35 cycles of 30 s at 94°C, 30 s at 49°C, 120 s at 72°C; 72°C for 10 min. The presence or absence of PCR product was determined by running samples on agarose gel stained with ethidium bromide. Amplified DNA fragments were sequenced at the Institute of Biotechnology (Vilnius) and Macrogen Inc. (South Korea).

Editing of the DNA sequences and alignment of consensus sequences were performed using Bioedit version 5.0.9 software. The mitochondrial COI datasets were analyzed using MEGA version 4 (Tamura *et al.*, 2007).

Results and discussion

Six species from the *Culicoides obsoletus* group are listed in Europe: *C. abchazicus* Dzhafarov, 1964, *C. obsoletus* (Meigen, 1818), *C. scoticus* Downes et Kettle, 1952, *C. chiopterus* (Meigen, 1830), *C. dewulfi* Goetghebuer, 1936 and *C. montanus* Shakirzjanova, 1962 (Boorman, 2010). *C. montanus* biting midges are detected in Spain, so we do not expect to collect them in Lithuania (Szadziwski & Borkent, 2010). *C. abchazicus* is known from Czech, Germany, Poland, Romania and Slovakia (Szadziwski & Borkent, 2010). *C. dewulfi* is known from many countries of Europe, but it was not detected in Lithuania. *C. chiopterus*, *C. obsoletus* and *C. scoticus* were known from Lithuania (Pakalniškis *et al.*, 2006).

The *C. obsoletus* group made up to 49.5–58.6% of all *Culicoides* collected by light traps. Three species from the *Culicoides obsoletus* group were detected in Lithuania while investigating material from light traps. *C. scoticus* and *C. chiopterus* are not very common in Lithuania (made up to 2.4% and 3.2% of all *Culicoides* respectively). The Flying activity of these two species was quite similar. *C. scoticus* biting midges were collected with light traps in April–May and in August–September. *C. chiopterus* were collected in April–May and in July–August. *C. obsoletus* is one of the most widespread species of bloodsucking midges in Lithuania. Adult *C. obsoletus* were found from the 22nd of April till the 30th of October. Four flying peaks were detected for *C. obsoletus* during the season. The abundance of *C. obsoletus* imagos peaked in the end of April -

beginning of May, the second peak appeared in the second part of June, the third peak was determined in the first part of August, and the fourth peak was registered in the second part of September. The first population peak occurred when the mean weekly temperatures exceeded +10°C.

The females of these species are very difficult to distinguish, and these species are only separable on the basis of certain characteristics of the male genitalia (Rawlings, 1997). So, analysis of mtDNR of these insects was used.

The 458 bp fragment of the cytochrome c oxidase subunit I gene was sequenced from the total of 30 biting midges from the *Culicoides obsoletus* group (6 specimens collected each month except May). Two different haplotypes of *Culicoides obsoletus* and 2 haplotypes of *Culicoides scoticus* were determined. We have not determined *Culicoides chiopterus* during this investigation, possibly because of the smaller area covered by investigations of population diversity; 49 parsimony informative characters were observed between *C. obsoletus* and *C. scoticus*.

We compared our material with the Genbank sequences from the United Kingdom (Nolan *et al.*, 2007) (*C. chiopterus*: AM236751, AM236747; *C. dewulfi*: AM236704; *C. scoticus*: AM236725, AM236746, AM236747, *C. obsoletus*: AM236753, AM236771), from Spain (Pagès & Monteys, 2005) (*C. scoticus*: DQ162807; *C. obsoletus*: DQ162809, DQ162814 – DQ162816) from Greece (*C. scoticus*: AM236751) and from Bulgaria (*C. obsoletus*: AM236767).

Two haplotypes of *C. scoticus* were determined from 4 specimens (Fig. 1). All specimens were collected in April. The haplotype D1Vilnius_April was identical to the haplotype AM236625 (UK) (Fig. 1) and differed from the haplotype C9Vilnius_April (Pairwise distances, Jukes–Cantor model) by 2.7%.

Two haplotypes of *C. obsoletus* were determined from the 26 specimen investigated (Fig. 1). Pairwise distance (Jukes–Cantor model) between all haplotypes was 0.3% on average. Biting midges of the A haplotype (Fig. 1, DD6Vilnius_September, E1Vilnius_July, E2Vilnius_August) were collected in July, August and September, so they belonged to the third and the fourth flying activity peaks; 10 investigated specimens belonged to haplotype A. This haplotype was identical to the haplotypes from Spain (DQ162814 and DQ162815). The difference between this haplotype and haplotypes of *C. obsoletus* from the UK was about 0.6%.

Biting midges of haplotype B (Fig. 1, D5Vilnius_August, DD5Vilnius_June, C8Vilnius_April) were collected in April, June and August, so they belonged to the first, second and third flying activity peaks; 16 investigated specimens belonged to haplotype B. This haplotype was very close to the haplotype DQ162809 (Spain) and differed from it just by 0.2%. The difference between this haplotype and haplotypes of *C. obsoletus* from the UK was about 0.7%.

It is known that genetic differences often can be explained not by morphological differences but by differences in biology (Szalanski *et al.*, 2006). Genetic differences helped, except morphologically identical species of *Culicoides* (*C. loxodontis* and *C. kwagga*) which differed in biology – the site of larvae development. *C. laxodontis* larvae developed in elephant dung and larvae of *C. kwagga* in zebra and rhinoceros dung (Szalanski *et al.*, 2006). Sometimes it is difficult to find the way of differentiation of different haplotypes of insects. *Morphologically identical but genetically different biting midges can differ in their development time. Our material has shown that different haplotypes can differ in the timing of flying activity. C. obsoletus* biting midges from

haplotype A (identical to the haplotype from Spain) were detected only during the second part of summer: from July till September, i.e. during the flying activity of *C. obsoletus* of the third and fourth generations. Biting midges from the haplotype B were determined from April till August, i.e. during the flying activity of *C. obsoletus* of the first, second and third peaks or generations.

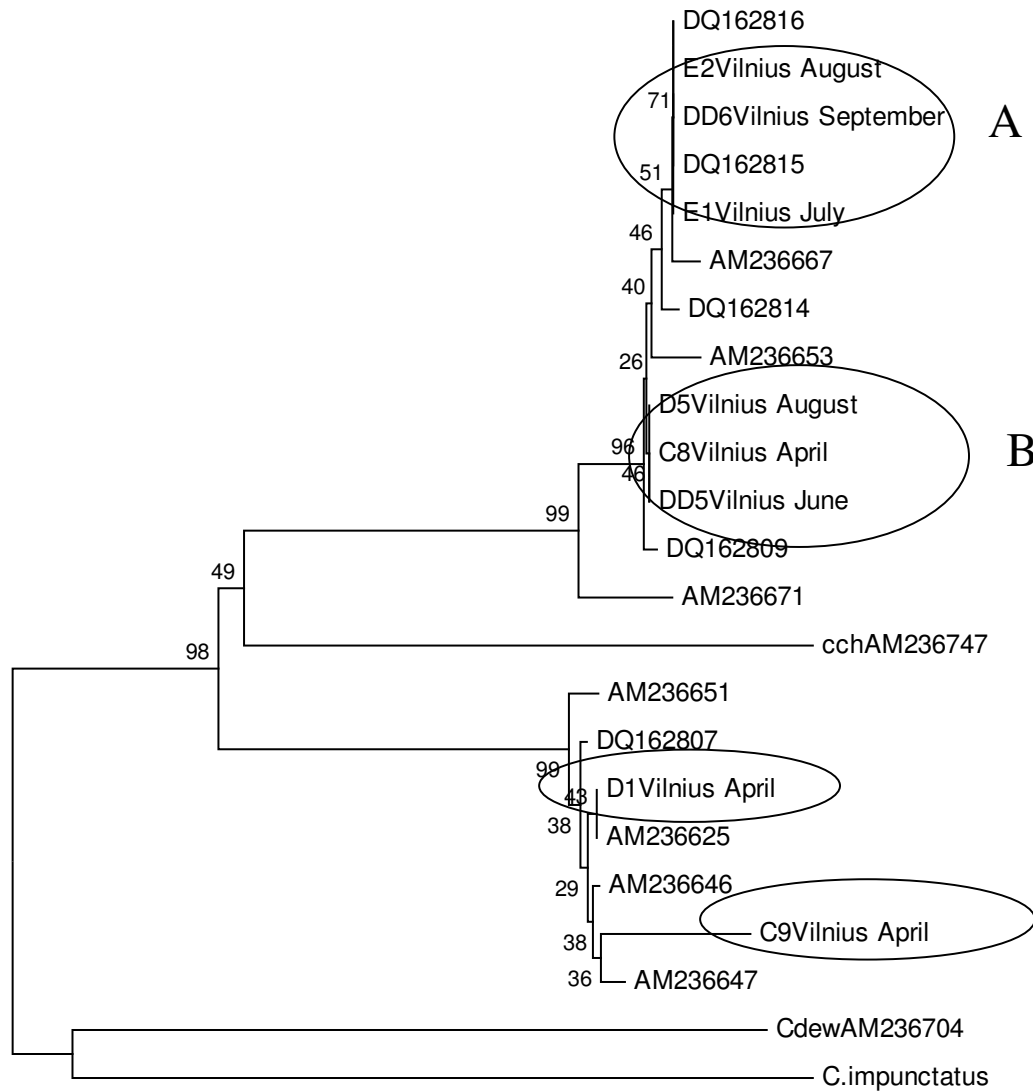


Fig. 1. Phylogenetic relationships of COI haplotypes (method – neighbor joining, model Kimura 2 parameter distances, bootstrap replications – 4000). Bootstrap values are shown above the branches of clades. *Culicoides impunctatus* is an outgroup. Sequences from the Genbank from UK (*C. chiopterus*: AM236751, AM236747; *C. dewulfi*: AM236704; *C. scoticus*: AM236725, AM236746, AM236747, *C. obsoletus*: AM236753, AM236771), from Spain (*C. scoticus*: DQ162807; *C. obsoletus*: DQ162809, DQ162814 – DQ162816) from Greece (*C. scoticus*: AM236751) and from Bulgaria (*C. obsoletus*: AM236767). Names of haplotypes from Lithuania are constructed from the codes of specimens, localities (Vilnius) and the time (month) of collection.

The knowledge on the genetic diversity of vector populations is very important in forecasting disease spreading because the distribution of different haplotypes of vectors

can correspond with the distribution of different serotypes of transmitted viruses (Dallas *et al.*, 2003). The distribution of different haplotypes of *C. imicola* is known to correspond the distribution of different serotypes of blue tongue (Dallas *et al.*, 2003). Similar data were found while investigating black flies (Diptera: Simuliidae) and their possibility to transmit different diseases (Rodriquez-Peres *et al.*, 2006). This investigation has shown that the *C. obsoletus* group from Lithuania is similar in COI sequences to the biting midges from the UK and Spain. The blue tongue virus was not detected in Lithuania, but we should expect blue tongue virus serotypes similar to serotypes detected in Spain and the UK.

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***Culicoides obsoletus* grupės smulkiųjų mašalų fauna, sezoninis skraidymo aktyvumas ir įvairovė Lietuvoje**

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Santrauka

Culicoides obsoletus rūšių grupės kraujasiurbiai smulkieji mašalai žinomi kaip įvairių virusų, o ypač pastaraisiais metais Europoje galvijų tarpe plintančios mėlynojo liežuvio ligos sukėlėjų, platintojai. *C. obsoletus* suaugėliai Lietuvoje skraido nuo balandžio vidurio iki spalio pabaigos. Lietuvoje žinomos trys šiai rūšių grupei priklausančios smulkiųjų mašalų rūšys: *C. chiopterus* (Meigen, 1830), *C. obsoletus* (Meigen, 1818) ir *C. scoticus* Downes et Kettle, 1952. *C. scoticus* ir *C. chiopterus* nėra dažnos ir gausios rūšys, o *C. obsoletus* yra viena dažniausiai aptinkamų kraujasiurbių smulkiųjų mašalų rūšių Lietuvoje. Per skraidymo sezoną nustatyti keturi šios smulkiųjų mašalų rūšies pagausėjimo laikotarpiai Lietuvoje. *C. obsoletus* grupės rūšių identifikavimas remiantis morfologiniais požymiais yra sudėtingas, todėl molekuliniai tyrimai dažnai naudojami šios rūšių grupės taksonomijoje. Atlikus citochromoksidazės subvieneto I geno dalies analizę buvo nustatyti du *C. scoticus* ir du *C. obsoletus* haplotipai Lietuvoje.

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