

Culiseta melanura (Diptera: Culicidae), a new record for the Manitoba mosquito fauna

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Culiseta melanura (Coquillett) is a bird-feeding mosquito that is known as the primary maintenance vector of eastern equine encephalitis virus (EEEV) in the eastern United States (Mahmood and Crans 1998). In North America, it occurs from Maine, southern Quebec and eastern Ontario, south to southern Florida, west to eastern Texas and north to the lower Great Lakes region and southeastern Minnesota (Darsie and Ward 2005). In Canada, *Cs. melanura* has been reported from Quebec and Ontario (Wood *et al.* 1979) and also from St. John's, Newfoundland (Nielsen and Mokry 1982; Hustins 2006). *Culiseta melanura* is primarily associated with lowland swamp or bog habitats as this species favours acidic water with a pH of 5.0 or lower (Pierson and Morris 1982). This species is multivoltine with at least two generations in Canada (Wood *et al.* 1979). Larvae overwinter and females deposit egg rafts directly onto water in lowland swamp habitats (Mahmood and Crans 1998). Females will often seek out passageways through the root mat of trees or water-filled depressions in sphagnum bogs to deposit eggs (Wood *et al.* 1979) and larvae often overwinter in these "crypts" (Pierson and Morris 1982). During the summer of 2004, adult mosquitoes were collected from different locations within and around the city of Winnipeg as part of a project to develop molecular methodologies for mosquito identification. While sorting samples, we encountered one specimen which, based on morphological characteristics, was identified as *Cs. melanura*. Molecular techniques were used to confirm this identification.

Materials and Methods

Sample Collections and Preparation

During June to September of 2004, CDC light traps and sweep nets were used to collect adult mosquitoes from a number of localities within the city of Winnipeg (i.e., King's Park, Assiniboine Park, LaBarrière Park) and at Birds Hill Provincial Park, Sandilands Provincial Forest, Seven Sisters Falls and Whiteshell Provincial Park. Following collection, mosquito specimens were dried at room temperature, pinned and identified using Wood *et al.* (1979) and Darsie and Ward (2005). The specimen

putatively identified as *Cs. melanura* was collected late August, 2004 in King's Park, Winnipeg. After identification, the specimen was transferred to a 1.5 ml Eppendorf tube for DNA extraction. For comparison, two adult specimens of *Cs. melanura*, collected in Notre Dame Provincial Park on July 30, 2004, were obtained from colleagues in Newfoundland and legs were used for DNA extraction. Four adults were also borrowed from the Canadian National Collection (CNCI) in Ottawa, Ontario.

DNA preparation, amplification, and data analysis

DNA was extracted from the specimen from Manitoba and the two specimens from Newfoundland using QIAamp DNA Mini Kit (QIAGEN Inc., Mississauga, ON, Canada). The specimens of *Cs. melanura* from the CNCI were only used to confirm the morphological identification of the Manitoba specimen. Samples were dried at room temperature for 48 hrs and transferred to 1.5 ml Eppendorf tubes. Specimens were ground up using pestle and lysed at 56 °C in 20 µl of Proteinase K (20 mg/ml) and 180 µl ATL buffer overnight. The lysate was bound to the spin column membrane, and washed twice by centrifugation. DNA was then eluted by centrifugation in 50 µl of elution buffer. Following Cornel *et al.* (1996) and Kampen *et al.* (2003), the 5.8S and 28S primers were used to amplify the ITS2 region: 5'-TGTGAACTGCAG-GACACATGAA-3' (5.8S, forward primer) and 5'-ATGCTTAAATTTAGGGGG-TAGTC-3' (28S, reverse primer). Amplification was carried out in 50-µl total volume containing 5 µl of *Taq* polymerase 10x reaction buffer (Invitrogen, Carlsbad, CA, USA), 200 µmol/L each of dATP, dTTP, dCTP, and dGTP (Invitrogen, Carlsbad, CA, USA), 1.5 mmol/L MgCl₂, 40 pmol of each primer, 1.25 U (1 U = 16.67 nkat) of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), and approximately 10 ng of DNA template. Amplification was performed using a Techne Genius Thermocycler (Techne Incorporated, Princeton, New Jersey) with the following temperature cycling parameters for 30 cycles for: denaturation at 93 °C for 1 min, primer annealing at 50 °C for 1 min, followed by elongation at 72 °C for 80 seconds. To assess the efficiency of amplification, 5-µl aliquots of PCR products were separated on 1.5 % agarose gels prepared with 1x TBE buffer (89 mmol/L Tris-HCL, 89 mmol/L boric acid, and 20 mmol/L EDTA), stained with ethidium bromide, and visualized using ultra violet light. PCR products were purified using Montage Life Science Kits (Millipore Corporation, Bedford, MA, USA). Double-stranded PCR products were sequenced using the cycle-sequencing protocols performed according to the manufacturer's recommendations (Perkin Elmer Applied Biosystems, Foster City, California, USA) and automated fluorescent DNA sequence analysis was performed using an ABI Prism 310 Genetic Analyzer system. DNA Star software was used to edit the nucleotide sequence, perform nucleotide alignments and for phylogenetic analysis. The Clustal W method was used for sequence data analysis. *Culiseta morsitans* (Theobald) was used as a relative species to *Cs. melanura*.

Results

Based on observed morphological features, one specimen collected in Winnipeg was putatively identified as *Cs. melanura*. Morphological characters of this specimen

were similar to those of *Cs. melanura* specimens from Newfoundland and from the CNCI. Voucher specimens were deposited in the J.B. Wallis Museum, Department of Entomology, University of Manitoba. There were no differences in the nucleotide sequences of ITS2 (approximately 300 bp) between two *Cs. melanura* specimens collected in Newfoundland. However, there was one nucleotide difference between the specimen from Winnipeg and those collected in Newfoundland. There were 137 nucleotide differences between *Cs. morsitans* and *Cs. melanura* (Fig. 1). The relationship among the examined specimens is shown in Fig. 2.

Discussion

With the collection of *Cs. melanura*, 51 species of mosquitoes have been recorded for Manitoba so far. Among recorded species, there are five species of *Culiseta* which have previously been reported from Manitoba and all of these species have established populations in the province. These five species include: *Cs. alaskaensis* (Ludlow), *Cs. impatiens* (Walker), *Cs. inornata* (Williston), *Cs. minnesotae* Barr and *Cs. morsitans* (Wood *et al.* 1979). Although one specimen of *Cs. melanura* was collected in this study, it seems unlikely that populations of this mosquito are established in the province. No additional specimens of *Cs. melanura* have been collected, despite continued surveillance for this species, from 2005 to 2009, by the City of Winnipeg Insect Control Branch (Taz Stuart, City of Winnipeg, personal communication). Thus the incursion of the *Cs. melanura* described in this study likely represents a chance introduction which did not result in establishment of a reproducing population. Introduction of this exotic species could have been aided by wind currents as reported for other species (Sellers and Maarouf 1993) or some other mechanism (e.g., arrival in trailers or other conveyances originating in localities where *Cs. melanura* populations are established). Given the detection of *Cs. melanura* in the current study, *Uranotaenia sapphirina* (Osten Sacken) by Stuart (2007) in Manitoba and the potential range expansion of mosquitoes in North America that is predicted to occur as a result of climate change (Patz *et al.* 1996), it would be prudent for those working on mosquitoes in Manitoba and elsewhere to monitor for further incursions of exotic mosquito species. The West Nile virus surveillance program currently in place in Manitoba, in which a network of light traps is operated throughout the southern portion of the province, should provide an adequate system for early detection of exotic and potentially important invasive mosquito species.

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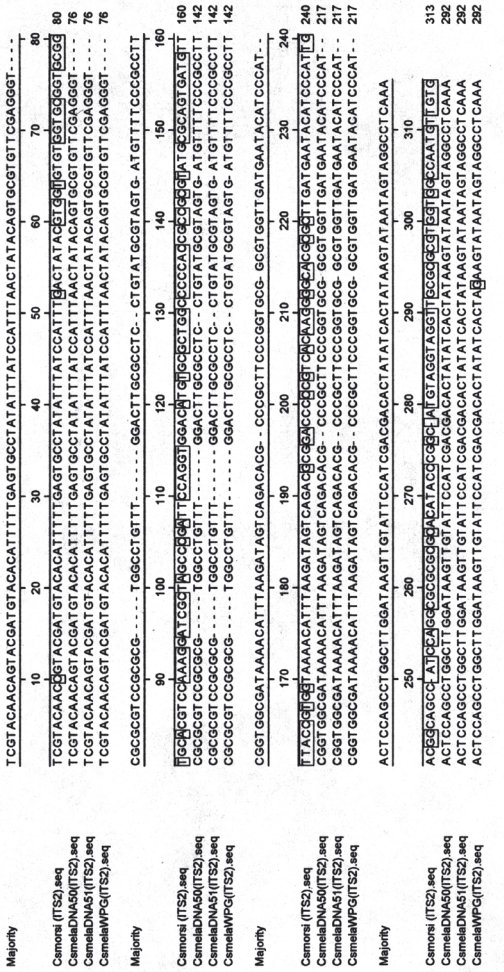


Fig. 1: Aligned ITS2 sequence data for three specimens of *Culiseta melanura* collected in Newfoundland and Winnipeg with *Cs. morsitans* as an outgroup.

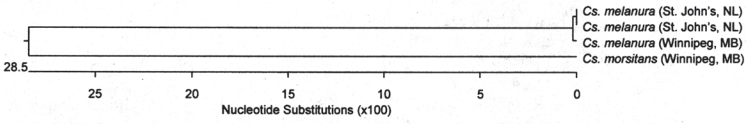


Fig. 2. Relationship among specimens of *Culiseta melanura* and *Cs. morsitans* collected in St. John's, Newfoundland and Winnipeg, Manitoba based on ITS2 sequence data.