

SHORT COMMUNICATION

Endophagy of biting midges attacking cavity-nesting birds

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Abstract. Feeding behaviour, host preferences and the spectrum of available hosts determine the role of vectors in pathogen transmission. Feeding preferences of blood-feeding Diptera depend on, among others factors, the willingness of flies to attack their hosts either in the open (exophagy) or in enclosed places (endophagy). As far as ornithophilic blood-feeding Diptera are concerned, the biting midges (Diptera: Ceratopogonidae) and blackflies (Diptera: Simuliidae) are generally considered to be strictly exophagous. We determined which blood-sucking Diptera enter nest cavities and feed on birds by placing sticky foil traps inside artificial nest boxes. A total of 667 females of eight species of biting midges of the genus *Culicoides* (Latreille, 1809) were captured on traps during 2006–2007, with *Culicoides truncorum* (Edwards, 1939) being the dominant species. DNA blood analyses of blood-engorged females proved that midges actually fed on birds nesting in the boxes. Three species were identified as endophagous: *Culicoides truncorum*, *Culicoides pictipennis* (Staeger, 1839), and *Culicoides minutissimus* (Zetterstedt, 1855). Our study represents the first evidence that ornithophilic biting midges are endophagous. The fact that we caught no blackflies in the bird boxes supports the exophagy of blackflies. We believe that our findings are important for surveillance programmes focusing on Diptera that transmit various bird pathogens.

Key words. *Culicoides pictipennis*, *Culicoides truncorum*, endophagy, nest box, population dynamics, transmission, vectors, Moravia.

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are blood-sucking insects of wide distribution. A total of 1400 species are known to live in a wide variety of habitats ranging from sea level environments to those in mountains up to 4000 m a.s.l. and from tropical to high arctic and subarctic regions (Mellor *et al.*, 2000). They serve as vectors of several animal parasitoses, such as bird and lizard haematococci, horse onchocercosis and wild animal filariasis that occurs in both mammals and birds (Linley, 1985; Garvin & Greiner, 2003). *Culicoides* biting midges are also important vectors of a wide range of arboviral diseases (Mellor *et al.*, 2000), such as oropouche virus (OROV), which infects humans, African horse sickness (AHSV) and bluetongue virus (BTV), which affects wild and domestic ruminants. This last disease has increased in importance because it is currently

spreading through Europe (Purse *et al.*, 2007). In birds, biting midges serve as vectors of avian trypanosomes (Miltgen & Landau, 1982). Biting midges are also hosts of several monoxenous trypanosomatids (Podlipaev *et al.*, 2004; Svobodová *et al.*, 2007).

Most *Culicoides* species are mammalophilic or ornithophilic, although some of them feed on reptiles and frogs (Borkent, 2005). In general, attention is mainly focused on mammalophilic species (Mellor *et al.*, 2000; Bhasin *et al.*, 2001; Borkent, 2005) and ornithophilic species tend to be neglected. Some studies on the feeding preferences of ornithophilic *Culicoides* have been performed in North America (Bennett & Coombs, 1975; Greiner *et al.*, 1978) and the only recent study to be performed in the Old World was carried out in Israel (Braverman & Linley, 1988, 1993).

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Forty-nine species of *Culicoides* spp. have been recorded in the Czech Republic (Országh, 1980; Országh *et al.*, 1997; Tóthová & Knoz, 2006); however, current knowledge of their host preferences and feeding behaviour is insufficient. The majority of biting midges are exophagous feeders and do not attack their hosts in enclosed places (Borkent, 2005). Endophagy has been demonstrated in some mammalophilic species, the females of which feed on animals in barns (Barnard, 1997). No information is available on the exo/endophagy of ornithophilic species.

Special nest boxes (Fig. 1) were constructed to study blood-feeding insects attacking birds on nests situated in tree holes. Each box consisted of two parts: the lower part contained the entrance hole and a nest cavity in which a bird could lay a clutch of eggs; the upper part was separated from the lower by a wire mesh (1.5 × 1.5 cm), had a removable lid and served to trap insects entering the box. Adjacent to the lid, the upper part of the nest box was perforated by several small openings (0.5 cm in diameter) to allow insects to enter and leave the nest box. Sticky foils, used for insect trapping, were prepared from transparent plastic foil smeared with an adhesive used by gardeners to control fruit tree pests (Chemstop; Fytofarm CZ s.r.o., Prague, Czech Republic). No pheromones were present in the glue. Sticky foils were attached by tacks to the walls of the upper part of the box as well as to the inner side of the lid.

Bird nest boxes were placed at a standard height (about 3 m) on tree trunks in oak (*Quercus petraea*) and European hornbeam (*Carpinus betulus*) forest in the Bulhary Game

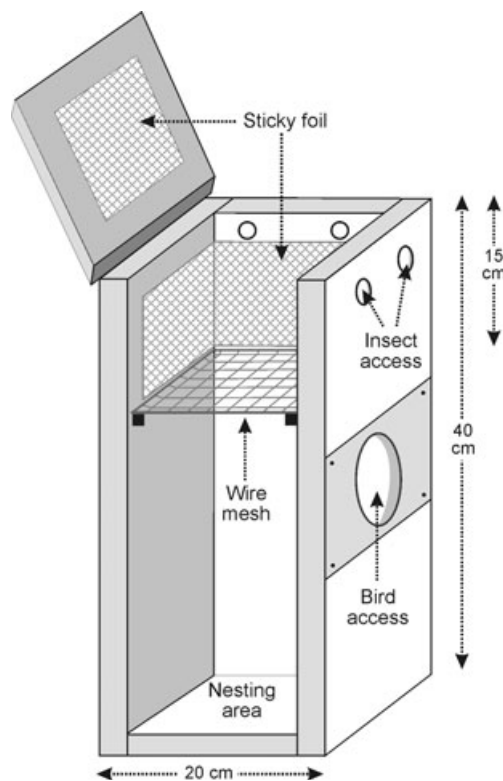


Fig. 1. Drawing of a nest box adapted for trapping visiting blood-feeding insects with sticky papers. The cover lid is raised.

Preserve in the vicinity of Mikulov, South Moravia, Czech Republic (48°48' N, 16°43' E). In 2006 and 2007, data from 20 boxes were collected over a 2-month period (May, June) in each year. Adult birds and their nestlings were present in the nest boxes during both periods; quite often two independent broods were produced in each nest. Unoccupied nest boxes were used as negative controls. In the majority of cases, foils with adhering insects were collected shortly after nestlings had taken off.

Captured insects were washed from sticky foils using petrol and were further cleaned by petrol and 96% ethanol washing. Insects were identified to family and biting midges were preserved in 70% ethanol for subsequent determination according to the key of Országh (1980). All *Culicoides* specimens were identified to species using their wing pattern as observed with an Olympus SZH stereomicroscope (10× to 100× magnification). In addition, some individuals of each *Culicoides* species were mounted on microscope slides using the CMCP-9 and CMCP-10 mounting media (Polysciences Inc., Warrington, PA, U.S.A.). Two drops of medium were placed separately on the slide; one for mounting the *Culicoides* head (dorsal) and abdomen (ventral), the other for wings and lateral thorax. Mounted specimens were examined to compare their structural characters, especially their antennal sensilla (e.g. sensilla chaetica, sensilla trichodea and sensilla coeloconica).

The origin of ingested blood in individual insects was analysed to exclude the possibility that blood-engorged biting midge females were using bird boxes only as resting sites. After species determination, the abdomens of blood-fed females were used for DNA extraction using the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Meylan, France) according to the manufacturer's instructions (except that proteinase-K digestion was performed at 55°C overnight). Bloodmeals were identified using the modified vertebrate-universal specific primers cytB1-F (5'-CCA TCC AAC ATY TCA DCA TGA TGA AA-3') and cytB2-R (5'-GCH CCT CAG AAT GAT ATT TGK CCT CA-3') (Malmqvist *et al.*, 2004) to amplify a 305-bp segment of the cytochrome b (*cyt b*) gene from the host mtDNA. Polymerase chain reaction (PCR) was performed in a 25-μL volume using a reaction mix (PPP Combi; Top-Bio SRO, Prague, Czech Republic). Resulting PCR products were checked on a 1.5% agarose gel and products of expected size were purified from the gel using the QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions and sequenced directly on an automated DNA sequencer (310 Genetic Analyzer ABI Prism®; Applied Biosystems, Inc., Foster City, CA, U.S.A.) using the BigDye 3.1 Kit (Applied Biosystems, Inc.). Sequence analyses were performed using DNASTar software; sequences were compared with sequences deposited in GenBank using standard nucleotide BLAST searches.

Occupation of the nest boxes reached 85% in 2006 and 65% in 2007. Six species of bird were recorded as box inhabitants during both seasons: the tree sparrow (*Passer montanus*, 50%); the great tit (*Parus major*, 15%); the blue tit (*Parus caeruleus*, 2.5%); non-identified tit (the great or the blue tit, 15%);

the spotted flycatcher (*Muscicapa striata*, 7.5%); the nuthatch (*Sitta europaea*, 7.5%), and the wryneck (*Jynx torquilla*, 2.5%).

A total of 667 females of the genus *Culicoides* were collected, representing eight biting midge species. *Culicoides* males were not found in the traps. In addition to biting midges, other blood-feeding Diptera captured included Culicidae (18 females of *Culex* sp., one female of *Aedes* sp.) and Hippoboscidae (six specimens of *Ornithomyia avicularia*). No representatives of the Simuliidae family were found in the traps. Blood-sucking insects, including biting midges, were absent from traps in non-occupied bird boxes.

Of 667 captured biting midge females, 552 could be identified; the remaining *Culicoides* specimens were considerably damaged and were not identified. A marked difference in the abundance of biting midges as well as in species composition was found between May and June in both study years. In May, significantly (ANOVA, $F_{(1,48)} = 10.673$, $P < 0.05$) fewer individuals were collected (16 and 96 specimens in 2006 and 2007, respectively) than in June (269 and 286 specimens in 2006 and 2007, respectively). The temporal abundance of biting midges collected on sticky foils is presented in Fig. 2 (combined results from 2006 and 2007). The most abundant species were *Culicoides truncorum* (64.7% of all determined females), *Culicoides minutissimus* (13.4%), and *Culicoides pictipennis* (10.8%). These data show that all three of these species significantly differ in the timing of their respective May and June population peaks (Fig. 2). This observation corresponds with findings that in the northern and southern temperate regions, *Culicoides* species differ in the timing of their peak occurrences (Borkent, 2005) and that the majority of Central European species usually peak in abundance three to four times during one season (Mráz & Országh, 1998).

At the same locality (the Bulhary Game Preserve) blood-sucking Diptera were collected using bird-baited CDC traps located at one locality at the tree canopy and at ground level (O. Černý, personal communication). In the years 2003–2005, *Culicoides festivipennis*, *Culicoides kibunensis*,

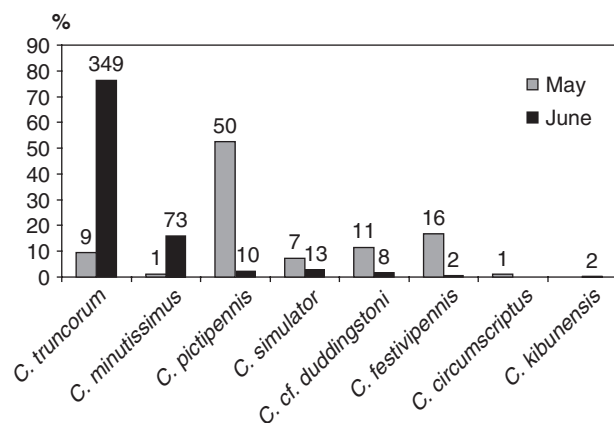


Fig. 2. Relative proportions of all identified female *Culicoides* spp. by collection period (May vs. June). Data represent biting midges collected in 2006 and 2007. Numbers above each bar represent the total number of females caught in each period.

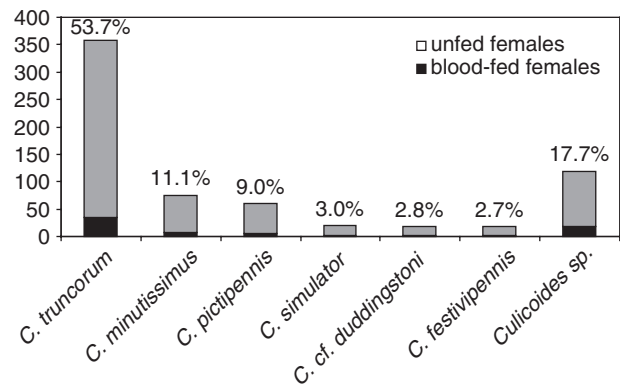


Fig. 3. Total numbers of biting midges and relative proportions of blood-fed and unfed female *Culicoides* spp. caught in occupied nest boxes during 2006 and 2007. Numbers above each bar represent the percentage of each species from all collected *Culicoides* (including undetermined specimens).

C. minutissimus and *Culicoides circumscriptus* were found to be the most abundant biting midges, whereas *C. truncorum* was considerably less abundant. The dominance of *C. truncorum* found in the current study may reflect its higher tendency towards endophagy, by contrast with other species, which were more common in bird-baited CDC traps.

In total, 70 (10.5%) blood-engorged females belonging to three *Culicoides* species were caught in 10 boxes (Fig. 3) and *C. truncorum* represented the most frequent species (51.4% of all blood-engorged females). In all cases the DNA analysis of bloodmeals corresponded with the bird species which had nested in the box. It is interesting that host DNA could be extracted and amplified even from flies attached for several weeks to sticky foil and after extensive washing by petrol.

The current study is the first to focus on blood-sucking Diptera that attack birds nesting in cavities (bird boxes). The results proved the ornithophily of *C. truncorum*, the host preference of which was not previously known. In addition the current study demonstrated that some species of the ornithophilic biting midges of the genus *Culicoides* are endophagous. At least three species (*C. truncorum*, *C. pictipennis*, and, most probably, *C. minutissimus*) repeatedly enter bird nest boxes to feed on nesting birds.

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