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Host use patterns of *Culicoides* spp. biting midges at a big game preserve in Florida, U.S.A., and implications for the transmission of orbiviruses

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Abstract. *Culicoides* spp. biting midges (Diptera: Ceratopogonidae) are vectors of pathogens that have a significant economic impact on the livestock industry. White-tailed deer (*Odocoileus virginianus*), a farmed species in the U.S.A., are susceptible to two *Culicoides* spp. borne orbiviruses: bluetongue virus and epizootic haemorrhagic disease virus. Elucidating host–vector interactions is an integral step in studying disease transmission. This study investigated the host range of *Culicoides* spp. present on a big game preserve in Florida on which a variety of Cervidae and Bovidae freely roam. *Culicoides* were captured with Centers for Disease Control and Prevention (CDC) miniature light traps run twice weekly on the preserve for 18 consecutive months (July 2015–December 2016). Host preference was quantified through forage ratios, based upon PCR-based bloodmeal analysis of *Culicoides* spp. and overall animal relative abundance on the preserve. *Culicoides stellifer* preferentially fed on *Cervus* spp. and fallow deer (*Dama dama)* and displayed a relative avoidance of Bovidae and white-tailed deer. *Culicoides debilipalpis* preferred white-tailed deer and avoided all Bovidae. *Culicoides pallidicornis* and *Culicoides biguttatus* showed preferences for white-tailed deer and Père David's deer (*Elaphurus davidianus*), respectively. These results add to current knowledge of preferred hosts of Florida *Culicoides* spp. and have implications for the spread of orbiviruses. Copyright © 2018 John Wiley & Sons, Ltd.

Key words. *Culicoides*, bloodmeal analysis, bluetongue virus, epizootic haemorrhagic disease virus, host preference.

Introduction

Culicoides spp. biting midges (Diptera: Ceratopogonidae) are capable of causing damage to livestock and wildlife through transmission of numerous viruses, particularly from the genus *Orbivirus*. These viruses include African horse sickness virus (AHSV), bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV). These three viruses can cause morbidity, and often mortality, in economically valuable livestock species such as cattle, horses, sheep and deer. Outbreaks of these viruses can result in significant economic losses due to animal deaths causing financial stress in areas where the economy is driven by livestock industries (Barnard *et al.,* 1998; Kedmi *et al.,* 2010; Rushton & Lyons, 2015).

Culicoides spp. transmitted orbiviruses that cause haemorrhagic disease (BTV and EHDV) are an important source of

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mortality to white-tailed deer (*Odocoileus virginianus*) (Nol *et al.,* 2010; Stevens *et al.,* 2015). This deer species constitutes a large part of the burgeoning big game industry in the U.S.A. (Anderson *et al.,* 2007; Adams *et al.,* 2016). At least 5555 breeding facilities for captive white-tailed deer are present in the eastern U.S.A., with more than 300 of these found in the state of Florida (Adams *et al.,* 2016). As of 2007, the cervid farming industry contributed three billion USD towards the U.S. economy and that value has likely risen (Anderson *et al.,* 2007). Many deer farming operations cater to hunters and offer opportunities for hunting white-tailed deer and other cervids, such as elk (*Cervus canadensis*), axis deer (*Axis axis*), fallow deer (*Dama dama*), and exotic bovids such as nilgai (*Boselaphus tragocamelus)* and blackbuck (*Antilope cervicapra)*. Operations dedicated to breeding, meat production, and other products are also common in many states (Anderson *et al.,* 2007). Due to the significant impact that haemorrhagic disease has on these animals and the industry, a better understanding of the ecological interactions between the numerous biting midge species and diverse hosts on properties where multiple game species are present is needed.

Host associations for many of the 150 Nearctic *Culicoides* spp. (Borkent & Grogan Jr, 2009), including the 49 known species present in Florida (Grogan *et al.,* 2010), are poorly understood. Much of the information regarding host usage by *Culicoides* spp. is based upon specimens collected from animal-baited traps and/or observational studies (Hair & Turner Jr, 1968). Although these studies can provide valuable information on whether a particular *Culicoides* spp. will feed upon a particular host, they cannot infer host breadth, as only one or a few (usually domesticated or tame vertebrate animals) are available for experimentation. For example, one study found that *Culicoides kibunensis* constituted the majority of trap captures in bird-baited traps, seemingly indicating ornithophily in this species (Synek *et al.,* 2013). However, other studies utilizing polymerase chain reaction (PCR)-based bloodmeal analysis found that *C. kibunensis* fed on humans (Santiago-Alarcon *et al.,* 2012, 2013) and cows (Lassen *et al.,* 2012). Further, animal-baited traps can often provide conflicting results. Two separate animal-baited studies found that *Culicoides sanguisuga* preferred large mammals (Humphreys & Turner Jr, 1973), but also fed heavily on poultry (Greiner *et al.,* 1978) confounding our understanding of selection in this species.

Bloodmeal analysis of field-collected engorged females through PCR amplification of host DNA present in a bloodmeal is a valuable method to determine host use of many blood-feeding arthropods, including biting midges (Slama *et al.,* 2015), ticks (Allan *et al.,* 2010), sandflies (Chaskopoulou *et al.,* 2016), tsetse flies (Muturi *et al.,* 2011), and mosquitoes (Burkett-Cadena *et al.,* 2008) amongst others. Bloodmeal analysis targets regions of certain genes that are well conserved throughout the animal kingdom but show sufficient interspecific variation for identification at the species level, including 16 s rRNA (Sarri *et al.,* 2014), cytochrome b oxidase (Garros *et al.,* 2011; Slama *et al.,* 2015), and cytochrome c oxidase I (Ferri *et al.,* 2009). As contact (biting) between vectors and susceptible hosts is a critical variable in determining vectorial capacity of a putative vector species, clearly delineating patterns of host use is important for inferring vector status.

Forage ratios use data on relative abundance and host use of various vertebrate species to infer the propensity of blood-feeding arthropods to feed on specific animal species (Hess *et al.,* 1968). This basic metric has been used extensively to investigate patterns of host use for vectors of diverse pathogen systems, including malaria (Parida *et al.,* 2006; Lardeux *et al.,* 2007), leishmaniasis (Agrela *et al.,* 2002; Rossi *et al.,* 2007) and numerous arboviruses (Hess & Hayes, 1970; Braverman *et al.,* 1971; Ponlawat & Harrington, 2005; Samuel *et al.,* 2008). Forage ratios provide a useful general approximation of whether host species are utilized at a rate different than their relative abundance within a community (Hess *et al.,* 1968). Forage ratios are typically calculated at a landscape scale, such that natural movement and aggregation of both vectors and hosts shape the outcome of the interaction, i.e. host use (Chaves *et al.,* 2010). Host species that are utilized disproportionately more than their relative abundance are considered to be 'preferred' by the blood feeder, whereas those utilized disproportionately less than their relative abundance are considered to be 'avoided'. Host preference and avoidance do not quantify small-scale interactions of blood feeders and their hosts, such as behavioural avoidance or defensive behaviours of host animals, but instead, simply quantify the outcomes of these multi-species interactions on a larger community-level scale.

No data are currently available on *Culicoides* host preference of exotic ungulates such as fallow deer, axis deer, elk and Père David's deer that are often intermixed with native species, such as white-tailed deer, on hunting preserves (Anderson *et al.,* 2007). Because ungulate density on these hunting preserves often far surpasses bovid or cervid densities in more natural environments, the increased host abundance could result in increased *Culicoides* spp. density (Garci-Saenz, *et al.,* 2011). Additionally, the availability of exotic host species as resources for bloodmeals or sources of arboviruses could change *Culicoides* spp. composition, host use, or virus transmission dynamics. If ungulate species composition ultimately played a role in *Culicoides* spp. ecology, and even affected haemorrhagic disease transmission, then it could be possible to reduce transmission dynamics by changing host community composition.

The goals of this research were to (a) quantify host use and host preference of *Culicoides* spp. on a big game preserve in the Florida panhandle; and (b) use *Culicoides* spp. abundance and host preference results to draw conclusions regarding candidate vectors of haemorrhagic disease in Florida. *Culicoides sonorensis* Wirth & Jones, the only confirmed vector for EHDV in North America, is considered rare in Florida. Due to the persistent transmission of these arboviruses in the region (Ruder *et al.,* 2015), it is likely that other vector species are active in the area. These data can be used by land managers and researchers to focus control efforts towards biting midge species that are most pestiferous towards captive animals and may also be candidate vectors for haemorrhagic disease in Florida.

Materials and methods

Field methods

The site for this study was a privately owned ∼200-ha big game hunting preserve and white-tailed deer breeding farm

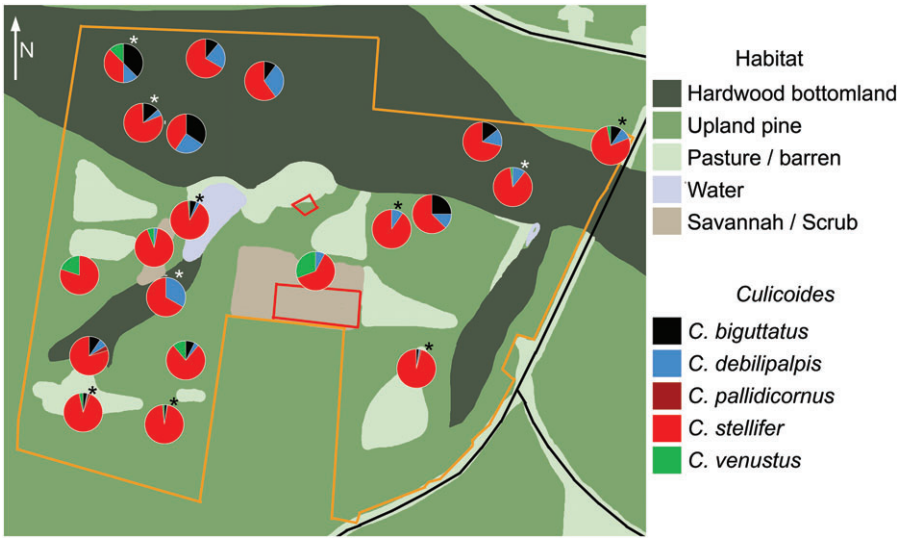

Fig. 1. Map of the big game preserve located in Gadsden County, FL, and *Culicoides* spp. by habitat type. The perimeter of the property is indicated in orange, the perimeter of the two penned areas containing white-tailed deer is indicated in red and roads in black. Pie charts represent the percentages of blood-engorged midges captured by traps at that point. Pie charts with an asterisk indicate the 10 traps that were run continuously throughout the study. The other 10 traps were not run between November–March. Aside from the two penned areas of white-tailed deer kept for breeding purposes, animals (including additional white-tailed deer) were free to roam throughout the property within the property limits.

located in Gadsden County, Florida, U.S.A. A variety of habitat types were present on the property including hardwood forest, hardwood swamp, mixed pine forest, open fields and large ponds (Fig. 1). A diverse assemblage of big game species (Bovidae and Cervidae) were present. Bovids on the property included blackbuck antelope (*Antilope cervicapra*), nilgai (*Boselaphus tragocamelus*), goats (*Capra hircus)*, waterbuck (*Kobus ellipsiprymnus*), scimitar-horned oryx (*Oryx dammah*), gemsbok (*Oryx gazella*) and bighorn sheep (*Ovis aries*). Cervidae on the property included axis deer (*Axis axis*), North American elk (*Cervus canadensis*), sika deer (*Cervus nippon*), sika deer hybrids (*Cervus nippon* x *Cervus canadensis*), Père David's deer (*Elaphurus davidianus*), fallow deer (*Dama dama*) and white-tailed deer (*Odocoileus virginianus*) (Table 1). The property consisted of one large open preserve on which most of the animals were free to roam (Fig. 1, orange lines indicate property perimeter), but also contained two penned areas where half of the property's white-tailed deer were kept (Fig. 1**,** red lines indicate white-tailed deer pens).

Twenty Centers for Disease Control and Prevention (CDC) miniature light traps (Model 2836BQ, BioQuip, Rancho Dominguez, CA, U.S.A.) with black light light-emitting diode arrays (Model 2790 V390, BioQuip) were hung from 1.63-m-tall shepherd's hooks at 1.37 m in height. Trap locations were selected using the random point generator in ArcGIS v. 10.3 (ESRI, Redlands, CA, U.S.A.). Sets of 20 random points were iteratively generated and tested for spatial randomness using the average nearest neighbour index in ArcGIS. The final set of trap sites was spatially random and represented all habitat types in the study area (Fig. 1). Once selected, trap site locations did not change throughout the study. Trap chambers were modified with mesh (3 × 3 mm) to exclude large arthropods, and a cloth funnel was used to direct small arthropods into a 50 mL conical bottom tube containing 90% ethanol. Disturbance of the trap wiring by animals on the preserve was avoided by wrapping heavy-duty garden hose around the trap wires. Traps were powered via a 6 V-12 Ah gel-sealed battery (Model NP12-6, EnerSys, Reading, PA, U.S.A.) controlled by a timer to operate between 1 h prior to sunset and 1 h after sunrise. Total trap run time varied between 12 h during the summer and 15 h during the winter.

Trapping was conducted twice weekly between July 2015 and December 2016. During the months of November–March, the number of traps operated on the property decreased to 10. The 10 traps that remained operational during the over-wintering period were selected to ensure sampling at all major habitats on the property. These traps were furthermore selected because they yielded the greatest *Culicoides* spp. abundance and diversity during the previous season (summer of 2015). Protocols for operation of these 10 traps were not changed.

Collections were also made by aspiration from bottle-raised, tame adult white-tailed does from July 2015 through September 2016 from the pens indicated in red on the map in Fig. 1. Aspirations were conducted for 10 min at three time periods (dawn, midday, and dusk) once per week on any approachable deer in the pen. The aspirator design was an acrylic tube (8.89 cm diameter) containing a computer cooling fan powered by a 12 V battery (Model NP12-6, EnerSys). Midges were collected into removable collection cups, which were stored at −4 °C until identification.

Laboratory methods

All *Culicoides* spp. specimens collected were identified to species using morphological identification keys in Blanton & Wirth (1979). After identification of all specimens,

Table 1. Total big game abundance estimates on the Gadsden County, FL, big game preserve during the 2015 and 2016 trapping seasons. Abundance estimates were determined in December of each year.

Family	Scientific name	Common name	2015 abundance	2016 abundance
Bovidae	*Antilope cervicapra*	Blackbuck	40	30
Bovidae	*Boselaphus tragocamelus*	Nilgai	6	8
Bovidae	*Capra hircus*	Goat	3	3
Bovidae	*Kobus ellipsiprymnus*	Waterbuck	1	1
Bovidae	*Oryx dammah*	Scimitar-horned oryx	6	8
Bovidae	*Oryx gazella*	Gemsbok	7	9
Bovidae	*Ovis aries*	Bighorn sheep	3	3
Cervidae	*Axis axis*	Axis deer	40	40
Cervidae	*Cervus* spp.	Elk/sika/sika-elk hybrids	19	22
Cervidae	*Dama dama*	Fallow deer	12	24
Cervidae	*Elaphurus davidianus*	Père David's deer	7	9
Cervidae	*Odocoileus virginianus*	White-tailed deer	130	148
Total			274	305

blood-engorged females were each placed into individual 1.5 mL DNase/RNase-free microcentrifuge tubes and stored at −20 °C. Total DNA was extracted from individual biting midges using Chelex resin or Instagene (BioRad Inc., Hercules, CA, U.S.A.). DNA extraction using Chelex followed protocols outlined in Fabian *et al.* (2004). In brief, a sterile pestle was used to homogenize the sample in 10 μL of 0.9% NaCl, followed by addition of 5% Chelex suspension (240 μL at 100 °C), incubation at 100 °C for 10 min, then centrifugation for 5 min. at 3099**g**. Supernatant was collected into a sterile 1.5 mL tube. Instagene protocols were identical, except pre-warming of Instagene to 100 °C was not required.

Regions of extracted DNA were amplified with PCR using three primer sets (Blosser *et al.,* 2016) targeting different vertebrate taxa. All samples were run initially on the mammalian/amphibian primer set (F: CTCCATAGG GTCTTCTCGTCTT, R: GCCTGTTTACCAAAAACATCAC). If no amplification was observed, samples were subsequently run on the reptile (F: CTGACCGTGCAAAGGTAGCG-TAATCACT, R: CTCCGGTCTGAACTCAGATCACGTAGG) and avian (F: GGACAAATATCATTCTGAGG, R: GGGTG-GAATGGGATTTTGTC) primer sets. Reagents used per sample for all PCR protocols included 14.25 μL molecular biology grade water, 2.5 μL 200 mM Tris-HCl reaction buffer, 2.5 μL 2 mM deoxynucleotide triphosphates (dNTPs), 1.5 μL 50 mM $MgCl_2$, 0.625 μL 20 μM forward primer, 0.625 μL 20 μM reverse primer, and 0.5 μL Taq polymerase (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Addition of 2.5 μL of extracted DNA (50–150 ng/μL concentration) resulted in a total reaction volume of 25 μL per well. Cycling conditions for the mammalian/amphibian assay were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 1 min. Cycling conditions for the lizard specific primers were 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 62.5 °C for 30 s and 72 °C for 1 min. The cycling conditions for the avian primers were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. All PCR runs included a negative control (molecular grade water) to monitor for contamination.

PCR products were visualized on 1% agarose gels with electrophoresis at 100 V for 35 min. A blue light transilluminator was used to visualize bands and all positive amplicons of the appropriate size were directly sequenced at a commercial laboratory using Sanger sequencing (Eurofins Genomic, Louisville, KY, U.S.A.). Resulting sequences were compared with sequence information in GenBank (National Institutes of Health: National Center for Biotechnology Information) using Basic Local Alignment Search Tool (BLAST), and samples with ≥ 95% identity matches and ≥ 75% query coverages were retained. Samples that failed to provide high identity matches were re-run and the resulting PCR product was purified (NucleoSpin Gel and PCR Clean-up kit, Macherey-Nagel, Bethlehem, PA, U.S.A.) prior to resubmission for sequencing.

Data analysis

Forage ratios (FR; relative host use/relative host abundance) were calculated to determine preference for various ungulate species and families on the property (Hess *et al.,* 1968; Manly *et al.,* 2007). Relative host use was determined for each *Culicoides* species by dividing the total bloodmeals taken by that *Culicoides* species from a particular host organism by the total number of bloodmeals taken by that *Culicoides* species overall. Relative host abundance was calculated by dividing the abundance of a specific host species on the property by total abundance of game animals present using information provided by the game preserve manager (Table 1). Host abundance data were available for the end of each year. For this reason, data for 2015 and 2016 were analysed separately. FR that did not include one within the 95% confidence intervals were considered to show preference or avoidance (eqns 4.14 and 4.15 in Manly *et al.,* 2007). FR greater than 1 indicated preference for a host species, whereas FR less than 1 indicated avoidance of a host species. Avoidance and preference values were not calculated for rare host species, i.e. fewer than five individuals, which included the sheep, waterbuck and goats (Lechowicz, 1982). Because both *Culicoides* spp. and vertebrate hosts may differentially utilize available habitat types, we performed linear

regression analysis to compare the host use between dominant habitat types. Habitat classes were derived from the Florida Habitat and Land Cover dataset available through the Florida Geographic Data Library (www.fgdl.org) and were developed by the Florida Fish and Wildlife Conservation Commission using Landsat Enhanced Thematic Mapper satellite imagery. For this study, we simplified the 11 original classes into five classes based on field observation and ground truthing (Munga *et al.,* 2006; Steiger *et al.,* 2012). Separate analyses were conducted for *C. debilipalpis* and *C. stellifer* comparing proportion of bloodmeals from the various vertebrate hosts between the three dominant habitat types: hardwood bottomland, upland pine, and ecotone. This analysis allowed us to validate that calculated preserve-wide FR were representative of all three major habitat classes and were not biased by robust collections from a few trap locations. Sample sizes for *Culicoides* spp. other than *C. debilipalpis* and *C. stellifer* were too small to permit robust comparisons.

A chi-square goodness of fit test (R 3.3.2 software, Vienna, Austria) was used to investigate differences in the community composition of total *Culicoides* spp. of all physiological statuses collected in traps, total bloodmeals taken from white-tailed deer, and of midges aspirated directly from white-tailed deer. The analysis was limited to the 10 most commonly sampled *Culicoides* spp., and was performed in order to determine whether the different collection methods used provided comparable results for these species.

Results

Forage ratios

During the full trapping period (July 2015–December 2016), a total of 69 964 *Culicoides* were collected, including 2143 blood-engorged *Culicoides*. Out of the blood-engorged midges analysed, 78.6% yielded sequences with ≥ 95% identity match to vertebrate hosts in GenBank. In 2015 and 2016, 933 of 1201 (77.7%) and 753 of 942 (79.9%) bloodmeals were successfully identified, respectively. Our analysis was unable to differentiate species within the genus *Cervus*, (elk, sika deer, and sika deer x elk hybrids). For this reason, these species were combined into a single group.

Although some *Culicoides* spp. fed on birds and a single amphibian bloodmeal was identified, the vast majority (99.4%) of bloodmeals were from mammalian hosts (Table 2). Of the total mammalian bloodmeals, 3.6% in 2015 and 5% in 2016 were derived from non-ungulate mammals (eastern gray squirrel, *Sciurus carolinensis*; northern raccoon, *Procyon lotor*; striped skunk, *Mephitis mephitis*; domestic dog, *Canis lupus familiaris*, and human, *Homo sapiens*; Table 3), for which relative abundances were not quantified. For these reasons, forage ratio calculations were restricted to species of Cervidae and Bovidae identified in blood-fed *Culicoides* spp.

The majority of successfully identified bloodmeals in both years were from *Culicoides stellifer*, with 850 of the total bloodmeals (91%) in 2015 and 611 of the total bloodmeals (81%) in 2016 derived from this species (Table 4). Host species were variously preferred or avoided by *C. stellifer* and *C. debilipalpis*.

Table 2. Total bloodmeals taken by all *Culicoides* spp. on game mammals, non-game mammals, birds and other sources on the study preserve in 2015 (July–December) and 2016 (January–December).

Year	Game mammals	Non-game mammals	Birds	Other	Total
2015	894	33	5	1	933
2016	708	38	4	3	753
Total	1602	71	9	4	1686

Although host species were preferred or avoided similarly between years in some instances, exceptions occurred where FR varied between preference and avoidance for the same game species between 2015 and 2016 (Fig. 2). For example, *C. debilipalpis* showed a preference for Père David's deer in 2015; however, this preference was not observed the following year. During both years, *C. debilipalpis* preferred white-tailed deer and avoided blackbuck, gemsbok, nilgai, scimitar-horned oryx and fallow deer. All other hosts of *C. debilipalpis* were neither preferred nor avoided. *Culicoides stellifer* showed a preference for fallow deer in 2015; however, preference was not observed the following year. Both years, *C. stellifer* showed a preference for *Cervus* species. In 2016, *C. stellifer* avoided gemsbok, but avoidance was not observed in 2015. *Culicoides stellifer* avoided blackbuck and axis deer during both years. *Culicoides stellifer* showed no preference or avoidance of the other host species in this analysis (Fig. 2).

Species such as *Culicoides biguttatus* and *Culicoides pallidicornis* are predominantly spring species (Blanton & Wirth, 1979) and as such, were only collected during 2016 (sampling began in July in 2015). Forage ratios for *C. biguttatus* ($n = 72$) were largely equivocal, except for a preference for Père David's deer (FR = 11.3, SE = 3.26). *Culicoides pallidicornis* ($n = 16$) preferred white-tailed deer (FR = 1.54, SE = 0.26) and avoided fallow deer, Père David's deer, blackbuck, gemsbok and scimitar-horned oryx (FR = 0 for each).

Forage ratios calculated to determine whether abundant *Culicoides* spp. showed preference for a specific mammalian family, Bovidae or Cervidae, indicated broad preference for Cervidae and avoidance of Bovidae in *C. debilipalpis*. Although *C. stellifer* also fed more heavily on Cervidae than Bovidae, 95% confidence intervals revealed their preference for Cervidae was not significant (Fig. 2).

Habitat types

Host use was consistent between major habitat types for *C. debilipalpis* and *C. stellifer* (Table 5). Linear regression analysis demonstrated significant positive relationships between habitat types (hardwood bottomland, upland pine, ecotone) for the proportion of bloodmeals from various host species (Table 5). Host use from the two dominant yet qualitatively different habitats, hardwood bottomland and upland pine habitats, showed significant positive relationships for both *C. debilipalpis* ($R^2 = 0.97$, d.f. = 15, $P < 0.001$) and *C. stellifer* ($R^2 = 0.77$, d.f. = 15, $P < 0.001$).

Table 3. Non-game mammalian and avian bloodmeals taken by *Culicoides* spp. during the full study period (July 2015–December 2016) at the Gadsden County, FL, big game preserve.

	Cow	Dog	Human	Gray squirrel	Raccoon	Eastern striped skunk	American crow	Chicken	Common yellowthroat	Mississippi kite	Northern cardinal	Red-eyed vireo	Turkey vulture
C. arboricola ($n = 5$)								1					1
C. baueri ($n = 1$)				1									
C. biguttatus ($n = 75$)			2				1						
C. crepuscularis ($n = 2$)			1	1									
C. debilipalpis ($n = 70$)			2	1									
C. haematopotus ($n = 13$)			4						1	1	3	1	
C. hinmani ($n = 2$)				1									
C. insignis ($n = 1$)			1										
C. pallidicornis ($n = 17$)			1										
C. paraensis ($n = 3$)				2	1								
C. stellifer ($n = 1461$)	7	1	42	1	1	1							
Total	7	1	53	7	2	1	1	1	1	1	3	1	1

Table 4. Total game mammal (Cervidae and Bovidae) bloodmeals taken by *Culicoides* spp. on the big game preserve in 2015 (July–December) and 2016 (January–December) for which > 5 game mammal bloodmeals were analysed.

	2015			2016				
Bloodmeal host	*C. debilipalpis*	*C. stellifer*	*C. venustus*	*C. biguttatus*	*C. debilipalpis*	*C. pallidicornis*	*C. stellifer*	*C. venustus*
Axis deer	2	14	0	6	0	1	36	1
Elk/Sika deer	6	276	1	14	4	1	141	2
Fallow deer	0	206	1	9	0	0	56	2
Père David's deer	3	17	1	24	0	0	43	0
White-tailed deer	42	276	9	16	10	12	279	9
Blackbuck	0	7	1	1	0	0	7	2
Gemsbok	0	0	0	0	0	0	4	1
Nilgai	0	10	0	1	0	2	9	1
Scimitar-horned oryx	0	19	1	1	0	0	5	0
Annual total	53	825	14	72	14	16	580	18

White-tailed deer aspiration

In all, 685 biting midges were aspirated from white-tailed deer. In a comparison of species composition and abundance for the 10 most common *Culicoides* spp. of (a) all *Culicoides* collected in traps, (b) those species that fed only on white-tailed deer and (c) those that were aspirated off of white-tailed deer, a few major differences were observed (Fig. 3). Although *C. stellifer* was the most abundant species present in total trap counts ($n = 47\,180$ individuals) and took the most bloodmeals from white-tailed deer ($n = 555$ bloodmeals), this species was not the most commonly collected species in aspirations ($n = 213$ individuals, 31.1% of total collections). Instead, *C. pallidicornis* was the most abundant species caught in aspirations ($n = 373$ individuals, 54.5% of total collections). Additionally, although *C. haematopotus* was the second most abundant species in total trap counts ($n = 9709$ individuals), only 13 bloodmeals were successfully analysed from this species. Of these 13 bloodmeals, two (15.4%) originated from white-tailed deer, two (15.4%) from humans, one (7.7%) from *Cervus* spp. and six (46.2%) were from various birds (Table 3). Further, none were aspirated from white-tailed deer during this study. The chi-squared goodness of fit test indicated that the comparative abundance of the 10 most common *Culicoides* species collected in total trap counts, identified through bloodmeal analysis feeding on white-tailed deer, and aspirated from white-tailed deer were significantly different ($\chi^2 = 211.89$, d.f. = 18, $P < 0.001$).

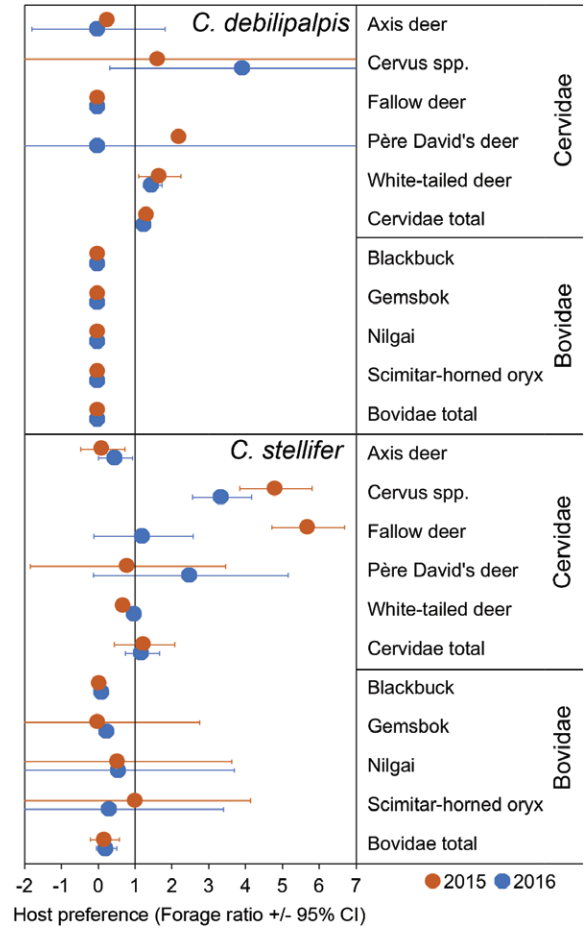

Fig. 2. Forage ratios for ungulate species and family by *Culicoides debilipalpis* (upper panel) and *Culicoides stellifer* (lower panel) for 2015 and 2016 from a big game preserve in Gadsden County, FL.

Table 5. Results of linear regression testing for differences in host use (proportion of bloodmeals from each host species) amongst major habitat types.

Culicoides	Comparison	*R*-square	d.f.	*P*
C. debilipalpis	Bottomland vs. Upland Pine	0.97	15	< 0.001
	Upland Pine vs. Ecotone	0.95	15	< 0.001
	Bottomland vs. Ecotone	0.91	15	< 0.001
C. stellifer	Bottomland vs. Upland Pine	0.77	15	< 0.001
	Upland Pine vs. Ecotone	0.38	15	0.008
	Bottomland vs. Ecotone	0.44	5	0.003

Discussion

This is the first in-depth *Culicoides* spp. bloodmeal analysis study conducted in North America on a big game hunting preserve that houses a diversity of bovids and cervids and experiences cases of EHDV annually. A total of 1686 bloodmeals were successfully analysed, allowing the calculation of forage ratios that can guide our understanding of *Culicoides* spp. ecology and

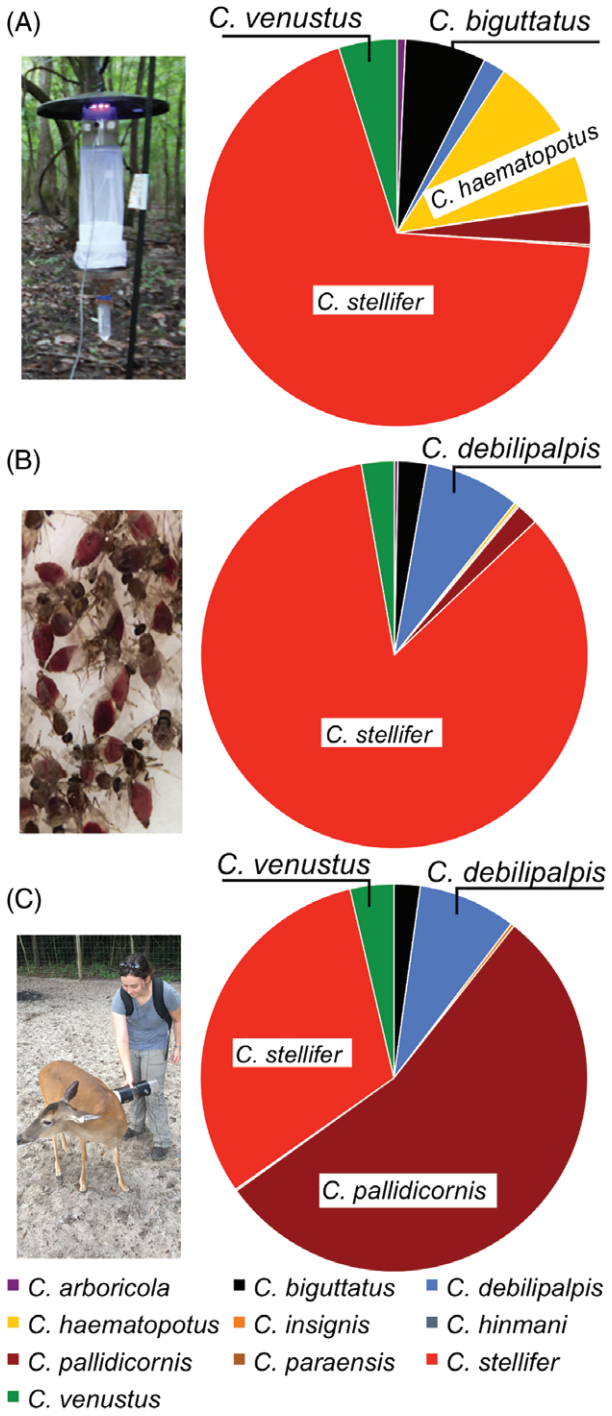

Fig. 3. Comparison of abundance of 10 *Culicoides* spp. in (A) total trap counts of all physiological statuses, (B) bloodmeals on white-tailed deer and (C) aspirations from white-tailed deer. These data were recorded from a big game preserve in Gadsden County, FL, from July 2015 through to September 2016.

host preference on this and similar properties in the southeastern U.S.A. Through the calculation of forage ratios, this work also allowed us to identify *Culicoides* spp. that feed preferentially on hosts affected by BTV and EHDV, an important step in implicating candidate vector species. Species such as *C. stellifer, C. debilipalpis* and *C. pallidicornis* fed on white-tailed deer and were aspirated from tame white-tailed deer more than other *Culicoides* spp. on the property (Fig. 3). They were also some of the most abundant species in total trap counts [*C. pallidicornis* was the most abundant early spring species (data not shown), *C. stellifer* was the most abundant summer and autumn species]. For these reasons, we have identified *C. stellifer*, *C. debilipalpis* and *C. pallidicornis* as candidate vector species that warrant further investigation as EHDV vectors in northern Florida. Although *C. haematopotus* was the second most abundant midge species in total trap collections, very few blood-engorged individuals were captured. Just under half of the bloodmeals from this midge species were taken from birds (6/13), whereas white-tailed deer constituted only 2/13 total bloodmeals. Aspiration collections reinforced these findings as no *C. haematopotus* were documented approaching white-tailed deer throughout the study period. These data suggest that *C. haematopotus* is not a putative vector species for these orbiviruses in this region.

The vast majority of bloodmeals analysed for this study originated from big game species at large in the study preserve. However, although half of *Culicoides* spp. in this analysis (7/14 species) predominantly took bloodmeals from preserve game species, several midge species appeared to avoid big game species in favour of non-game mammals and birds (Table 3). This suggests that species such as *C. baueri*, *C. bickleyi*, *C. crepuscularis*, *C. haematopotus* and *C. paraensis* are not likely involved in transmission of EHDV and BTV on this property. However, previous studies have indicated that pooled specimens of *C. crepuscularis* and *C. haematopotus* have tested positive for BTV in Louisiana (Becker *et al.,* 2010), so these data cannot rule out roles for these species in transmission of BTV. Additionally, despite being a confirmed BTV vector (Tanya *et al.,* 1992) the low abundance of *C. insignis* on the property and lack of bloodmeals from preserve species indicates that *C. insignis* is also not a likely primary vector of BTV or EHDV on this property. However, it is also important to note that vectorial capacity can be affected by a variety of other factors, including the vector competence of the putative vector species, probability of the vectors surviving each day, and the extrinsic incubation period of the pathogen in the vector. For this reason, it is not possible to completely dismiss a potential vector species on the basis of abundance or biting rate on an affected host alone.

Spring species (*C. biguttatus* and *C. pallidicornis*) were only collected during one study year. For this reason, it is difficult to predict whether preferences shown by these two species would remain the same between years. However, the preference for white-tailed deer by *C. pallidicornis* observed in this analysis could have implications for the maintenance of EHDV and BTV through winter and spring months if this species were determined to be a competent vector. Although EHDV and BTV generally cause outbreaks in the late summer and autumn (Ruder *et al.,* 2015), virus circulation has been observed on this property over winter and into spring (unpublished data). Our findings provide a first glimpse at possible maintenance mechanisms that allow these viral agents to persist on the landscape through the winter. Low titre viremia for EHDV can persist for up to 59 days post-inoculation in white-tailed deer (Ruder *et al.,* 2015). Therefore, a deer infected in late autumn or early winter could maintain viremia through the winter in Florida until emergence of early spring species such as *C. pallidicornis* that then transmit the virus to other hosts.

A strong preference for cervids and avoidance of bovids by *C. debilipalpis* was observed during both years. These preference patterns, as well as all of the forage ratios discussed, should be interpreted carefully as they could be affected by a variety of exogenous forces such as olfactory cues, spatial distribution, animal abundance fluctuations or defensive behaviours. Olfactory cues between cervids, bovids or the individual species themselves could differ and therefore lead to diverse responses by different *Culicoides* spp. (Isberg *et al.,* 2013). Skin glands that produce volatile compounds are common in many cervid (Gassett *et al.,* 1996; Gassett *et al.,* 1997; Wood, 2003) and bovid (Wood, 1997; Wood, 1998) species for intraspecific communication (Müller-Schwarze *et al.,* 1984). The specific effect of many of these compounds on *Culicoides* spp. behaviour is unknown. For this analysis, Y-tube olfactometer analysis was not conducted to look for olfactory preferences, which leaves uncertainty about the true source of the preference patterns seen. In addition to olfactory cues, the spatial distribution and resource selection by host animals, and spatial arrangement of midges, on the property likely led to the preference for cervids over bovids, as well as to observed preferences for specific species. One example of this phenomenon observed during this study is the preference of *C. biguttatus* for Père David's deer. This deer species is often associated with aquatic habitats (Li *et al.,* 2007), and all of the Père David's deer bloodmeals taken by *C. biguttatus* were from traps located near (< 3 m away from) a large stream. The resulting forage ratio is likely due to the overlapping spatial distribution and similar habitat preferences of midges and deer rather than an innate host preference. Forage ratios could also be affected by fluctuating animal abundances between study years and habitat associations of various host species, as well as the species of *Culicoides*. Births and deaths of free-ranging animals on the property, as well as their seasonal and circadian movements between habitats will certainly affect the accuracy of forage ratios. However, our analysis comparing host use between habitat types (Table 5) indicates that the patterns of host use exhibited by the most abundant *Culicoides* spp., *C. debilipalpis* and *C. stellifer*, are robust to habitat type. This represents an important step in understanding the role that environment plays in host preference, which is an important component of transmission cycles of vector-borne disease. Finally, defensive behaviours by host animals are known to alter host use by blood-feeding arthropods and may have led to the observed patterns. The use of behaviours such as foot stomps, wing shakes and head movements by birds (Darbro & Harrington, 2007) and ear twitches, hindfoot scratching, and head and body shakes by small mammals (Walker & Edman, 1986) has been shown to deter mosquitoes, often causing them to move to host species that use fewer of these defences. Studies on mosquitoes have found that regardless of innate preference, mosquitoes are ultimately most likely to take their bloodmeal

from a tolerant host from which they experience the least defensive behaviour (Edman *et al.,* 1974). Different host defensive behaviours could be utilized by bovids and cervids that result in the comparably heavy use of cervids in this analysis. Although both groups use behaviours such as ear flicking, longer tails in large bovids have been proposed as an evolved mechanism for defending against biting flies (Mooring *et al.,* 2007). The tails of cervids are typically shorter than many bovid species and therefore may not provide as much protection from insect pests. The authors acknowledge the many possible reasons for the patterns seen in this analysis and feel that additional studies are necessary to quantify forces that may drive observed patterns of preference and avoidance. Interpretation of avoidance is particularly challenging, given the lower likelihood of less-abundant animals to aggregate in areas near traps, perhaps causing their bloodmeals to be underrepresented using our methods.

Although our findings indicated that *C. stellifer* did not prefer white-tailed deer relative to their abundance on the property, this may not be justification for dismissing the potential for this species to serve as a vector of EHDV. Based on total trap collections, *C. stellifer* was the most abundant species present on the property and fed heavily on white-tailed deer throughout the study period. Despite avoiding white-tailed deer relative to their abundance, they do feed heavily on this host species and would therefore be a likely candidate vector species. In addition, other large ruminant mammals for which *C. stellifer* does show a preference may also play a part in the transmission cycle of these viruses. Infection studies indicate that elk (Hoff & Trainer, 1973) and fallow deer (Gibbs & Lawman, 1977), both of which were present on this preserve, can become infected and viremic with EHDV without exhibiting clinical signs (Gibbs & Lawman, 1977). Our data suggest a mechanism by which *C. stellifer* could be transmitting virus between viremic exotic host species and white-tailed deer.

Our study revealed some important limitations of different trapping methods. We observed differences in blood-fed *Culicoides* species composition and abundance in light traps vs. *Culicoides* spp. collected via aspiration. Aspiration from tame deer revealed the limitations of trap data to inform vector status, specifically in relation to *C. pallidicornis*. Although this species made up a relatively small percentage of the total midges from light traps and white-tailed deer bloodmeals on the property, *C. pallidicornis* constituted the majority of midges taken from aspirations. This informs us that this species readily approaches and feeds on white-tailed deer, but does not often enter traps after blood feeding. For this reason, FR for *C. pallidicornis* may have been underestimated. A chi-squared goodness of fit test indicated that there were significant differences in capture rates amongst trap types for the 10 *Culicoides* spp. analysed. This result emphasizes the importance of using multiple methods of collection when implicating putative vector species. It should be noted, however, that aspirations were only carried out in two pens on the property (constituting less than 10% of the property size) and were therefore not spatially arranged in a manner similar to the light traps. This limited sampling could have resulted in biased sampling of the species willing to approach the tame deer in the pen environment vs. other habitats on the property. An additional limitation of this study was undocumented wildlife populations around the property perimeter that might have biased parametrization of available hosts. The area surrounding this preserve was mostly rural forestland with interspersed homes. The property shared a short border with a state forest. Wild deer in the vicinity of this property could have led to lower calculated FR for white-tailed deer than we identified in this study if midges were feeding on wild deer located beyond the fence line. Finally, we did not conduct surveys into non-game species abundance on or off the property. Very few bloodmeals ($n = 80$) were taken from non-game mammal and bird species, so we were unable to draw any significant conclusions from these data.

In conclusion, the calculation of FR for common *Culicoides* spp. permitted better documentation of the ecology of some of the most abundant *Culicoides* spp. in the Florida panhandle. These FR, along with information from total midge abundance in traps and tame deer aspirations, allowed us to draw inferences about potential vector species on the property. The abundance of *C. stellifer* and their numerous interactions with white-tailed deer and other EHDV-susceptible hosts make them an important candidate vector species warranting vector competence studies. Similarly, the preference of *C. debilipalpis* for white-tailed deer as well as their presence in aspirations from tame deer indicates that this is also a species of interest that should be investigated. Finally, *C. pallidicornis* is a highly abundant early spring species that preferred white-tailed deer and was caught in great abundance during aspirations. Although their emergence did not coincide with peak seasonal outbreaks of BTV and EHDV, *C. pallidicornis* abundance during the spring season could provide a possible overwintering mechanism for haemorrhagic viruses and warrants investigation.

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References

Adams, K.P., B.P. Murphy, & M.D. Ross. 2016. Captive white-tailed deer industry-current status and growing threat. *Wildlife Society Bulletin* **40**: 14–19.

Agrela, I., E. Sanchez, B. Gomez, & M.D. Feliciangeli. 2002. Feeding behavior of *Lutzomyia pseudolongipalpis* (Diptera: Psychodidae), a putative vector of visceral leishmaniasis in Venequela. *Journal of Medical Entomology* **39**: 440–445.

Allan, B.F., L.S. Goessling, G.A. Storch, R.E. Thach. 2010. Blood meal analysis to identify reservoir hosts for *Amblyomma americanum* ticks. *Emerging Infectious Diseases* **16**: 433–440.

Anderson, D.P., Frosch, B.J. & Outlaw, J.L. (2007) Economic impact of the United States cervid farming industry. Agricultural and Food Policy Center, Texas A&M University. APFC Research Report 07-4.

Barnard, B.J.H, G.H. Gerdes, & R. Meiswinkel. 1998. Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa—1995/96 and 1997. *Onderstepoort Journal of Veterinary Research* **65**: 145–151.

Becker, M.E., W.K. Reeves, S.K. Dejean, M.P. Emery, E.N. Ostlund, & L.D. Foil. 2010. Detection of bluetongue virus RNA in field-collected *Culicoides* spp. (Diptera: Ceratopogonidae) following the discovery of bluetongue virus serotype 1 in white-tailed deer and cattle in Louisiana. *Journal of Medical Entomology* **47**: 269–273.

Blanton, F.S. & W.W. Wirth. 1979. *The Sand Flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae)*. Florida Department of Agriculture and Consumer Services: Gainesville, FL.

Blosser, E.M., T. Stenn, C. Acevedo, & N.D. Burkett-Cadena. 2016. Host use and seasonality of *Culex (Melanoconion) iolambdis* (Diptera: Culicidae) from eastern Florida, USA. *Acta Tropica* **164**: 352–359.

Borkent, A. & W.L. Grogan, Jr. 2009. Catalog of the new world biting midges north of Mexico. *Zootaxa* **2273**: 1–48.

Braverman, Y., P.F.L. Boreham, R. Galum. 1971. The origin of blood meals of female *Culicoides pallidipennis* trapped in a sheepfold in Israel. *Journal of Medical Entomology* **8**: 379–381.

Burkett-Cadena, N.D., S.P. Graham, H.K. Hassan, C. Guyer, M.D. Eubanks, C.R. Katholi, and T.R. Unnasch. 2008. Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. *The American Journal of Tropical Medicine and Hygiene* **79**: 805–815.

Chaskopoulou, A., I.A. Giantsis, S.Demir, M.C Bon. 2016. Species composition, activity patterns and blood meal analysis of sand fly populations (Diptera: Psychodidae) in the metropolitan region of Thessaloniki, an endemic focus of canine leishmaniasis. *Acta Tropica* **158**: 170–176.

Chaves, L.F., L.C. Harrington, C.L. Keogh, A.M. Nguyen, & U.D. Kitron. 2010. Blood feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology* **7**: 3.

Darbro, J.M. & L.C. Harrington. 2007. Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. *Behavioral Ecology* **18**: 750–757.

Edman, J.D., L.A. Webber, & A.A. Schmid. 1974. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. *Journal of Parasitology* **60**: 874–883.

Fabian, M.M., H. Toma, T. Arakawa, and Y. Sato. 2004. Malaria parasite developmental analyses by the nested polymerase chain reaction method: an implication for the evaluation of mosquito infection rates in epidemiological studies. *Southeast Asian Journal of Tropical Medicine and Public Health* **35**: 820–827.

Ferri, G., M. Alu, B. Corradini, M. Licata, & G. Beduschi. 2009. Species identification through DNA "barcodes". *Genetic Testing and Molecular Biomarkers* **13**: 421.

Garros, C., L. Gardès, X. Allène, I. Rakotoarivony, E. Viennet, S. Rossi, T. Balenghien. 2011. Adaptation of a species-specific multiplex PCR assay for the identification of blood meal source in *Culicoides* (Ceratopogonidae: Diptera): applications on Palaearctic biting midge species, vectors of Orbiviruses. *Infection, genetics, and Evolution* **11**: 1103–1110.

Gassett, J.W., D.P. Wiesler, A.G. Baker, D.A. Osborn, K.V. Miller, R.L. Marchinton, & M. Novotny. 1996. Volatile compounds from interdigital gland of male white-tailed deer (*Odocoileus virginianus*). *Journal of Chemical Ecology* **22**: 1689–1696.

Gassett, J.W., D.P. Wiesler, A.G. Baker, D.A. Osborn, K.V. Miller, R.L. Marchinton, & M. Novotny. 1997. Volatile compounds from the forehead region of male white-tailed deer (*Odocoileus virginianus*).*Journal of Chemical Ecology* **23**: 569–578.

Gibbs, E.P.J. & M.J.P. Lawman. 1977. Infection of British deer and farm animals with epizootic haemorrhagic disease of deer virus. *Journal of Comparative Pathology* **87**: 335–343.

Greiner, E.C., E.S. Eveleigh, & W.M. Boone. 1978. Ornithophilic *Culicoides* spp. (Diptera: Ceratopogonidae) from New Brunswick, Canada, and implications of their involvement in haemoproteid transmission. *Journal of Medical Entomology* **14**: 701–704.

Grogan, W.L., L.J. Hribar, C.S. Murphree, and J.E. Cilek. 2010. New records of biting and predaceous midges from Florida, including species new to the fauna of the United States (Diptera: Ceratopogonidae). *Insecta Mundi* **0147**: 1–59.

Hair, J.A., E.C. Turner Jr. 1968. Preliminary host preference studies on Virginia *Culicoides* (Diptera: Ceratopogonidae). *Mosquito News* **28**: 103–107.

Hess, A.D., and R.O. Hayes. 1970. Relative potentials of domestic animals for zooprophylaxis against mosquito vectors of encephalitis. *The American Journal of Tropical Medicine and Hygiene* **19**: 327–334.

Hess, A.D., R.O. Hayes, and C.H. Tempelis. 1968. The use of the foraging ratio technique in mosquito host preference studies. *Mosquito News* **28**: 386–389.

Hoff, G.L. & D.O. Trainer. 1973. Experimental infection of north American elk with epizootic hemorrhagic disease virus. *Journal of Wildlife Diseases* **9**: 129–132.

Humphreys, J.G. & E.C. Turner, Jr. 1973. Blood-feeding activity of female *Culicoides* (Diptera: Ceratopogonidae). *Journal of Medical Entomology* **10**: 79–83.

Isberg, E., Y. Hillbur, & R. Ignell. 2013. Comparative study of antennal and maxillary palp olfactory sensilla of female biting midges (Diptera: Ceratopogonidae: *Culicoides*) in the context of host preference and phylogeny. *Journal of Medical Entomology* **50**: 485–492.

Kedmi, M., M. Van Straten, E. Ezra, N. Galon, & E. Klement. 2010. Assessment of the productivity effects associated with epizootic hemorrhagic disease in dairy herds. *Journal of Dairy Science* **93**: 2486–2495.

Lardeux, F., P. Loayza, B. Bouchité, & T. Chavez. 2007. Host choice and human blood index of *Anopheles pseudopunctipennis* in a village of the Andean valleys of Bolivia. *Malaria Journal* **6**: 8.

Lassen, S.B., S.A. Nielsen, & M. Kristensen. 2012. Identity and diversity of blood meal hosts of biting midges (Diptera: Ceratopognidae: *Culicoides* Latreille) in Denmark. *Parasites & Vectors* **5**: 143–151.

Lechowicz, M.J. 1982. The sampling characteristics of electivity indices. *Oecologia* **52**: 22–30.

Li, C., Z. Jiang, Y. Zeng, & Z. You. 2007. A note on environmental elements as essential prerequisites for behavioral expression: a case study of pere David's deer. *Applied Animal Behavior Science* **103**: 174–180.

Manly, B.F., L. McDonald., & D. Thomas. 2007. *Resource Selection by Animals: Statistical Design and Analysis for Field Studies*, 2nd Ed. Dordrecht: Kluwer Academic Publishers, 50–62.

Mooring, M.S., D.T. Blumstein, D.D. Reisig, E.R. Osborne, & J.M. Niemeyer. 2007. Insect-repelling behavior in bovids: role of mass, tail length, and group size. *Biological Journal of the Linnean Society* **91**: 383–392.

Müller-Schwarze, D., R. Altieri, & N. Porter. 1984. Alert odor from skin gland in deer. *Journal of Chemical Ecology* **10**: 1707–1729.

Munga, S., Minakawa, N., Zhou, G., Mushinzimana, E., Barrack, O. O. J., Githeko, A. K., & Yan, G. (2006). Association between land cover and habitat productivity of malaria vectors in western Kenyan

highlands. *The American Journal of Tropical Medicine and Hygiene*, **74**, 69–75.

Muturi, C.N., J.O. Ouma, I.I. Malele, R.M. Ngure, J.J. Rutto, K.M. Mithöfer, J. Enyaru, D.K. Masiga. 2011. Tracking the feeding patterns of tsetse flies (*Glossina* genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS ONE*: http://dx.doi.org/10.1371/journal.pone.0017284

Nol, P., C. Kato, W.K. Reeves, J. Rhyan, T. Spraker, T. Gidlewski, K. VerCauteren, & M. Salman. 2010. Epizootic hemorrhagic disease outbreak in a captive facility housing white-tailed deer (*Odocoileus virginianus)*, bison (*Bison bison)*, elk *(Cervus elaphus)*, cattle (*Bos taurus)*, and goats (*Capra hircus*) in Colorado, USA. *Journal of Zoo and Wildlife Medicine* **41**: 510–515.

Parida, S.K., R.K. Hazra, N. Marai, H.K. Tripathy, & N. Mahapatra. 2006. Host feeding patterns of malaria vectors of Orissa, India. *Journal of the American Mosquito Control Association* **22**: 629–634.

Ponlawat, A. & L.C. Harrington. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *Journal of Medical Entomology* **42**: 844–849.

Rossi, E., G. Bongiorno, E. Ciolli, T. Di Muccio, A. Scalone, M. Gramiccia, L. Gradoni, and M. Maroli. 2007. Seasonal phenology, host-blood feeding preferences and natural *Leishmania* infection of *Phlebotomus perniciosus* (Diptera, Pyschodidae) in a high-endemic focus of canine leishmaniasis in Rome province, Italy. *Acta Tropica* **105**: 158–165.

Ruder, M.G., T.J. Lysyk, D.E. Stallknecht, L.D. Foil, D.J. Johnson, C.C. Chase, D.A. Dargatz, & E.P.J. Gibbs. 2015. Transmission and epidemiology of bluetongue eand epizootic hemorrhagic disease in North America: current perspectives, research gaps, and future directions. *Vector-Borne and Zoonotic Diseases* **15**: 348–363.

Rushton, J. **and** N. Lyons. 2015. Economic impact of bluetongue: a review of the effects on production. *Veterinaria Italiana* **51**: 401–406.

Samuel, P.P., N. Arunachalam, J. Hiriyan, & B.K. Tyagi. 2008. Host feeding pattern of Japanese encephalitis virus vector mosquitoes (Diptera: Culicidae) from Kuttanadu, Kerala, India. *Journal of Medical Entomology* **45**: 927–932.

Santiago-Alarcon, D., P. Havelka, H.M. Schaefer, & G. Segelbacher. 2012. Bloodmeal analysis reveals avian *Plasmodium* infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. *PLoS ONE* **7**: e31098.

Santiago-Alarcon, D., P. Havelka, E. Pineda, G. Segelbacher, & H.M. Schaefer. 2013. Urban forests as hubs for novel zoonosis: blood meal analysis, seasonal variation in *Culicoides* (Diptera: Ceratopogonidae) vectors, and avian haemosporidians. *Parasitology* **140**: 1799–1810.

Sarri, C., C. Stamatis, T. Sarafidou, I. Galara, V. Godosopoulos, M. Kolovos, C. Liakou, S. Tastsoglou, & Z. Mamuris. 2014. A new set of 16s rRNA universal primers for identification of animal species. *Food Control* **43**: 35–41.

Slama, D., N. Haouas, H. Mezhoud, H. Babba, E. Chaker. 2015. Blood meal analysis of *Culicoides* (Diptera: Ceratopogonidae) in Central Tunisia. *PLoS ONE*: http://dx.doi.org/10.1371/journal.pone.0120528.

Steiger, D. M., Johnson, P., Hilbert, D. W., Ritchie, S., Jones, D., & Laurance, S. G. (2012). Effects of landscape disturbance on mosquito community composition in tropical Australia. *Journal of Vector Ecology*, **37**, 69–76.

Stevens, G., B. McCluskey, A. King, E. O'Hearn, and G. Mayr. 2015. Review of the 2012 epizootic hemorrhagic disease outbreak in domestic ruminants in the United States. *PLoS ONE* **10**: e0133359. doi:10.1371/journal.pone.0133359

Synek, P., P. Munclinger, T. Albrecht, & J. Votypka. 2013. Avian haemosporidians in haematophagous insects in the Czech Republic. *Parasitology Research* **112**: 839–845.

Tanya, V.N., E.C. Greiner, & E.P.J. Gibbs. 1992. Evaluation of *Culicoides insignis* (Diptera: Ceratopogonidae) as a vector of bluetongue virus. *Veterinary Microbiology* **32**: 1–14.

Walker, E.D. **and** J.D. Edman. 1986. Influence of defensive behavior of eastern chipmunks and gray squirrels (Rodentia: Sciuridae) on feeding success of *Aedes triseriatus* (Diptera: Culicidae). *Journal of Medical Entomology* **23**: 1–10.

Wood, W.F. 1997. Short-chain carboxylic acids in interdigital glands of gemsbok, *Oryx gazella gazella*. *Biochemical Systematics and Ecology* **25**: 469–470.

Wood, W.F. 1998. Volatile compounds in interdigital glands of sable antelope and wildebeest. *Biochemical Systematics and Ecology* **26**: 367–369.

Wood, W.F. 2003. Volatile components in metatarsal glands of sika deer, *Cervus nippon*. *Journal of Chemical Ecology* **29**: 2729–2733.

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