

1 **Fur or feather? Feeding preferences of species of *Culicoides* biting midges in Europe**

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17 **Abstract**

18 Understanding the feeding preferences of haematophagous insects is critical to depicting the
19 amplification and transmission networks of pathogens and identifying key vector species for
20 surveillance programs. In the case of species from genus *Culicoides*, many of which are important
21 vectors of pathogens causing animal diseases, information from molecular studies on the feeding
22 habits of females is expanding but still limited for a significant fraction of competent vectors of
23 *Culicoides* borne pathogens. In spite of these limitations, recent studies highlight that most *Culicoides*
24 species are able to feed on several vertebrate species, but present clear preferences for mammals or
25 birds.

26 **Key-words.** Blood meal, Bluetongue virus, Ceratopogonidae, Diptera, DNA, Haematophagous insects.

27 **Blood feeding and females of *Culicoides* species**

28 Biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) are a highly diverse group of
29 insects with 1343 extant species ([http://www.inhs.illinois.edu/files/](http://www.inhs.illinois.edu/files/7613/9136/7587/WorldCatalogtaxa.pdf)
30 [7613/9136/7587/WorldCatalogtaxa.pdf](http://www.inhs.illinois.edu/files/7613/9136/7587/WorldCatalogtaxa.pdf)). While at least 58 of these species are present in Europe [1],
31 members of this genus are globally distributed, and few regions are considered *Culicoides* free (e.g.,
32 Antarctica, New Zealand, and Hawaiian islands) [2]. Both *Culicoides* males and females feed on nectar,
33 but females of most species also must feed on blood for egg development.

34 The bites of females of species of *Culicoides* produce important skin injuries, including
35 dermatitis in livestock [3,4], affecting the general health status of domestic animals and wildlife [4,5].
36 In addition to their nuisance as blood feeders, *Culicoides* biting midges play a central role in the
37 transmission of pathogens to humans, livestock, and wildlife. *Culicoides* biting midges have been
38 incriminated in the transmission of viruses in the genera Alphavirus, Bunyavirus, Flavivirus, Nairovirus,
39 Orbivirus, Vesiculovirus, and Lyssavirus [6], and they transmit protozoa and filarial worms, including
40 species affecting humans and other animals [6]. Among them, the role of some species of *Culicoides* in
41 the transmission of the avian malaria-like parasite *Haemoproteus* Kruse (subgenus *Parahaemoproteus*)
42 has received special attention, most likely due to the importance of this parasite as model organism
43 for studies of ecology and evolution [7]. In addition, the DNA and/or RNA of different pathogens
44 transmitted by non-*Culicoides* insects have been isolated from *Culicoides* biting midges, such as West
45 Nile virus [8], avian *Plasmodium* Marchiafava and Celli [9], and *Leishmania* Ross [10]. However,
46 molecular isolation of pathogens from insects does not demonstrate vectorial competence [11,12],
47 and further studies are, therefore, necessary to confirm the actual implication of *Culicoides* in their
48 transmission.

49 In Europe, epidemiological studies of species of *Culicoides* have been mainly focused on their
50 role as vectors of the Bluetongue virus. Prior to 1998, Bluetongue outbreaks in Europe were probably
51 vectored by *Culicoides imicola* and were limited to sporadic cases in southern European countries,
52 mainly in the Iberian Peninsula. Subsequently, the virus acquired new European vectors, allowing it to
53 spread and reach northern Europe in 2006 [13]. Among the species potentially implicated in the
54 geographical spread of Bluetongue to north Europe are members of both the *obsoletus* (*Culicoides*
55 *obsoletus*, *Culicoides scoticus*, *Culicoides dewulfi*, and *Culicoides chiopterus*) and the *pulicaris*
56 (*Culicoides pulicaris* and *Culicoides punctatus*) groups [14]. These Bluetongue outbreaks had dramatic
57 economic consequences. For example, the costs of Bluetongue outbreaks in 2007 in France and The
58 Netherlands were estimated as costing approximately US\$1.4 billion and US\$85 million, respectively
59 [15]. More recently, species of the *obsoletus* complex have been incriminated in the transmission of
60 the Schmallenberg virus [16], a virus first detected in 2011 which causes transient and nonspecific
61 symptoms in adult cattle and abortions in pregnant ruminants [17]. Studies of female *Culicoides*
62 feeding patterns Identifying the feeding patterns of *Culicoides* biting midges is an essential step in
63 determining the importance of each particular species in epidemiological studies [18]. As previously
64 estimated for the case of mosquito borne pathogens, the proportion of blood meals derived from
65 competent vertebrate hosts is basic to identifying key vector species, quantifying the risk of pathogen
66 amplification and transmission by blood-sucking insects [19,20], identifying the factors that favour
67 pathogen spill over to relevant species, and to develop predictive models on the dynamic of
68 transmission of pathogens by haematophagous vectors [21,22].

69 The feeding preferences of *Culicoides* biting midges have been linked to the morphology of
70 antennae and palpi and the number and/or distribution of sensilla [23– 26]. According to this
71 hypothesis, ornithophilic species, those feeding mainly on birds, have a higher number of sensilla
72 distributed on a higher number of flagellomeres than species feeding mainly on mammals. Moreover,
73 host size is probably linked to the number of sensilla in the third palpal segment, with those species
74 which feed on large mammals showing a lower number of sensilla than those feeding on birds ([27];
75 although see [25]). Traditionally, this has been used to classify species as mammophilic or ornithophilic
76 according to their preferences to feed mainly on mammals or birds, respectively. However, a
77 quantitative test of this hypothesis, taking into consideration phylogenetic relationships between
78 *Culicoides* biting midges, is still lacking.

79 Engorged females with a recent blood meal in their abdomen provide valuable information on
80 the feeding pattern of *Culicoides* biting midges, allowing for the identification of the blood meal origin.
81 A differential efficacy in the capture of engorged females could be expected among sampling methods
82 [28–30], but collecting engorged females is usually a difficult task, with blood-fed females only
83 representing a very small proportion of the total individuals captured. For example, only 0.18–2.28% of
84 the *C. imicola* sample collected using four different suction light traps had a recent blood meal in their
85 abdomen [29]. Even when sampling *Culicoides* biting midges close to or even over the skin of their
86 potential hosts, the proportion of females with a recent blood meal in their abdomen may be relatively
87 low [31–33].

88 Traditionally, studies on the feeding pattern of *Culicoides* biting midges have been conducted
89 using immunological assays (e.g., [34,35]). However, with these methods, the range of vertebrate
90 species that can be identified from blood meals from engorged biting midges is limited by the
91 availability of specific reagents, and cross-reactions result in incorrect species identification when the
92 blood corresponds to vertebrate species that are not included in the battery of species tested.
93 Consequently, most studies have focussed on identifying the blood from farm or domestic species, and
94 thus giving little insight into the natural cycles of amplification outside of farms. Additionally, collecting
95 engorged females attracted to and/or feeding on caged individuals of particular animal species has
96 allowed for the identification of vertebrate hosts of some species of *Culicoides*, but provides limited
97 information on different attractions towards different species in their natural habitats. Using these
98 approaches, the authors have identified the susceptibility of both birds (i.e., [31,32,36]) and mammals
99 (i.e., horses [37], sheep [33,38,39], and cattle [39]) to attack by females of the species *Culicoides*.
100 These procedures may limit the host range identified for *Culicoides* biting midge species to the few
101 host species tested, and a wider epidemiological approach may be necessary to characterise the
102 pathogen circulation patterns among wildlife.

103

104 **Molecular approaches to *Culicoides* host identification**

105 Compared with traditional procedures, the development of molecular techniques for the identification
106 of the origin of insect blood meals has increased the accuracy of host identification at the species level.
107 These molecular tools have been routinely used in studies on mosquitoes, among other
108 haematophagous arthropods (i.e., [19,20,40]), and have also recently been applied to species of
109 *Culicoides*. Molecular studies on *Culicoides* biting midges employed two main methodological
110 approaches including the use of: (i) specific sets of primers to amplify DNA from particular host species

111 (i.e., [41,42]); and (ii) general primers to amplify conserved genetic regions of mammals, birds, or even
112 all vertebrate species [40,43] (Table 1).

113 Using the first molecular approach, host identification is assigned by the positive amplification
114 of host DNA by using sets of primers designed to amplify DNA from particular vertebrate species. This
115 procedure may allow the identification of most of the blood meals in studies on *Culicoides* trapped in
116 farms, where they mainly feed on domestic species [42]. However, this technique constrains the
117 identification of potential hosts to the limited range of the vertebrate species tested. In this case,
118 failure of PCR amplification could be due to the presence of blood from a nonfocal host species. This
119 could be an important limitation in samples from pristine habitats where a broader host range could
120 be expected. In this respect, the use of universal primers to amplify a conserved genetic region of
121 vertebrates [i.e., subunit I of the cytochrome oxidase gene (COI)], and subsequent host identification
122 by comparison of these sequences with those deposited in public databases (e.g., GenBank DNA
123 sequence database or the Barcode of Life Data Systems) would allow for the identification of a broader
124 diversity of potential hosts. This should be considered an advantage with respect to immunological
125 assays that require both the production of antisera against all the potential host species and
126 purification of antibodies to eliminate cross-reactivity of samples from closely related species, as
127 highlighted by Ngo and Kramer [44]. However, this advantage is not free and the economic costs
128 derived from sequencing positive amplifications could limit their use in studies with large sample sizes.
129 Additionally, a potential limitation of molecular approaches is based on the quantity and/or quality of
130 the blood contained in the insect abdomen that could affect the amplification success of the host DNA
131 [45]. To partially solve this limitation, and especially due to their minute size, only fully engorged
132 females are usually used in studies on the feeding pattern of *Culicoides* biting midges [41,43].

133

134 **Current knowledge**

135 Molecular identification of the vertebrate hosts of species of *Culicoides* in Europe has been conducted
136 for at least 1360 individuals representing 31 species (Table 2). As a result, 45 different host species
137 have been identified, including 33 species of birds and 12 species of mammals. To our knowledge,
138 blood from reptiles or amphibians have not been detected in spite of the capacity of some biting
139 midges to feed blood on ectotherms [6]. However, Calvo and co-workers [46] isolated from a *C.*
140 *pulicaris* specimen a COI sequence 93% similar to a reptile species not present in the studied area,
141 suggesting that this biting midge probably fed on a not yet genetically characterised herpetile. On
142 average, females of each *Culicoides* species fed on the blood of five different host species. In the case
143 of the most extensively sampled *Culicoides* species (i.e., those with at least 50 individuals), both bird
144 and mammal derived blood meals have been isolated. In addition to *Culicoides kibunensis* and
145 *Culicoides festvipennis*, these species include members of the *obsoletus* group (*C. obsoletus*, *C.*
146 *scoticus*, and *C. chiopterus*) and the *pulicaris* group (*C. punctatus* and *C. pulicaris*) which, together with
147 *C. imicola*, are the most important potential vectors of Bluetongue virus in Europe. Although most
148 species have some flexibility in host selection, members of the *obsoletus* and the *pulicaris* groups feed
149 primarily on mammals and only occasionally on birds (Table 2; Figure 1). Additional studies on midges
150 of the *obsoletus* group have found support for this feeding pattern [41,46], although in these cases,
151 insect identification was not confirmed at the species level. Contrary to the case of these few
152 extensively studied species, the current knowledge from molecular studies on the feeding pattern of

153 most *Culicoides* species is based on the analyses of very few individuals. In fact, for 16 of the tested
154 species, which comprise 52% of the total of the species analyzed, feeding patterns are based on less
155 than 15 individuals (Figure 1).

156 In spite of these limitations, results from molecular studies on the feeding pattern of females
157 of species of *Culicoides* support the fact that: (i) most of the studied biting midge species are able to
158 feed on several vertebrate species; (ii) although some species feed primarily on either mammals or
159 birds, this is not strict behaviour, with at least some species potentially also feeding on blood from
160 animals of the nonpreferred vertebrate group; and (iii) taxonomically or phylogenetically related
161 species tended to feed on the same classes of vertebrates (Box 1). This could be partially due to the
162 fact that host availability and size may play a key role on the feeding pattern of biting midges and
163 affecting the class of host selected [47]. In this respect, the limited number of studies on most of the
164 *Culicoides* species from Europe may potentially bias current estimates of host preferences and
165 potential networks for pathogen transmission.

166 **Concluding remarks and future perspectives**

167 An important issue to be solved in the future is to understand the sylvatic cycles of *Culicoides*-borne
168 pathogens. This will require an increase in our knowledge of vector competence and the feeding
169 ecology of a wide range of *Culicoides* species, and the identification of factors that determine temporal
170 changes and local differences in feeding patterns. While some species are clearly specialised to
171 mammals or birds, others present a mixed feeding strategy, although at this moment, information is
172 scarce on the environmental, seasonal, and local factors that may affect host selection. For example,
173 factors such as the appearance of secondary hosts may alter the original feeding pattern of some
174 *Culicoides* species. This could be the case of *Culicoides phlebotomus*, a species originally probably
175 feeding mainly on turtles that may feed on humans and dogs after their appearance in the Caribbean
176 and Central America [48]. In addition, some species of *Culicoides* show spatial feeding preferences,
177 with species such as *Culicoides circumscriptus* more frequently found in the canopy than in the ground
178 level [49,50]. However, further studies should be conducted for the case of the vast majority of
179 *Culicoides* species, especially those with animal health implications. This may be the case for *C. imicola*,
180 the species considered to be the main vector of Bluetongue virus in southern Europe and for which, as
181 far as we know, there are no molecular studies on their feeding preferences in Europe. In Africa, this
182 species feeds on horses, cattle, and sheep [51], and a similar pattern could be expected in Europe. In
183 addition, individuals sampled directly from particular host species, such as individuals of *Culicoides*
184 *parroti* and *Culicoides simulator* collected on sheep [38] and blue tits [32], respectively, add valuable
185 information on the potential hosts of these species until further molecular studies are conducted on
186 these species. Future studies should also include information regarding the abundance of potential
187 hosts susceptible to be bitten in the studied area in order to more accurately identify the feeding
188 preferences of *Culicoides* species.

189 Additional studies should be conducted in a diversity of habitats, especially in pristine areas,
190 because there is a general lack of awareness regarding the *Culicoides* species feeding on wild animals.
191 Currently, most studies of the feeding sources of females of *Culicoides* have been focused on farms or
192 surrounding areas with livestock (e.g., [41,52,53]). Sampling *Culicoides* on caged wild animals can give
193 information on the main feeding preferences, but understanding the importance of wild communities
194 on pathogen transmission needs a deeper understanding of feeding/transmission networks. In spite of

195 the potential importance of wild ruminants in the epidemiology of relevant pathogens such as
196 Bluetongue virus, very few studies have investigated the potential pathogen transmission between
197 wild ruminants and livestock [41,42]. Wildlife may play a central role in the transmission of *Culicoides*-
198 borne pathogens as suggested by the high seroprevalence of Bluetongue antibodies found in some
199 wild ruminant species in Europe and North America [54,55]. This illustrates the value of characterising
200 feeding preferences at the species level, allowing for more detailed estimation of the potential for
201 pathogen amplification. Combining information regarding blood meal origin and vertebrate host
202 competence allows for a detailed estimation of the emerging risk of pathogens in different areas
203 [18,56]. Avian pathogens (e.g., avian malaria parasites) have been isolated from the abdomens of
204 biting midges feeding on humans [57,58]. This suggests the potential role of some species of *Culicoides*
205 as a bridge in pathogen transmission between wild animals and humans, as in the case of some filarial
206 nematodes and Orthobunyavirus, which are transmitted to humans mainly by species of *Culicoides* in
207 the New World and Africa [59].

208 Additional information on the host range of *Culicoides* could be obtained indirectly by
209 molecular identification of the pathogens they harbour. This could be the case for the avian malaria-
210 like parasites of the genus *Haemoproteus*, for whom the range of potential hosts may be limited to
211 birds of the same family, or more conservatively, of the same order [7]. Therefore, a comparison of the
212 parasite genetic haplotypes isolated from biting midges with those found in birds could also provide
213 additional information regarding the potential hosts of some *Culicoides* species [9,60,61]. This
214 procedure has the advantage that it is possible to analyse parous females, which have completely
215 degraded their previous blood meal.

216 In summary, analyses of the vertebrate origin of *Culicoides* blood meals provide valuable information
217 in epidemiological and ecological studies. Current knowledge based on molecular studies indicates
218 that the feeding preferences of female *Culicoides* differ widely among species, resulting in the possible
219 amplification and transmission of pathogens between reservoirs and susceptible species.

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226

227 **Competing interests**

228 The authors declare there are no conflicts of interest.

229

230 **Authors' contributions**

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385 **Table 1.** Summary of advantages and disadvantages of the molecular analyses used in host
 386 identification studies of females of species of *Culicoides*.

387

Procedure	Pros	Contrasts
Species specific primers	<ul style="list-style-type: none"> • Identification without sequencing • Quick and cheaper identification 	<ul style="list-style-type: none"> • Host range is limited to the species tested
Universal primers	<ul style="list-style-type: none"> • Broad host range • Identification of mixed blood meals from individuals of the same species 	<ul style="list-style-type: none"> • Costs of sequencing • Risk of co-amplification of both host and insect DNA in some vector species • Absence of genetic characterization of potential host species

388

389 **Table 2.** Host species of biting females of species of *Culicoides* in Europe identified using molecular
390 methods.

		<i>Mammal species</i>					<i>Avian species</i>											
Avaritia	<i>C. chiopterus</i>	x	x	x	x						x					7	[42,43,52, 57,62,63]	
	<i>C. dewulfi</i>	x			x	x			x	x	x					6	[42,43,57, 58,62,63]	
	<i>C. obsoletus</i>	x	x	x	x	x			x	x	x	x	x			11	[30,42,43,53,52, 57,58,62,63]	
	<i>C. scoticus</i>	x	x	x	x	x			x	x	x	x	x			10	[30,42,43,52, 57,58,62,63]	
Beltranmyia	<i>C. circumscriptus</i>								x					x	x		8	[43,62,64]
	<i>C. salinarius</i>								x				x			2	[43,62]	
Culicoides	<i>C. deltus</i>	x														2	[52,57,58]	
	<i>C. grisescens</i>	x														1	[43]	
	<i>C. impunctatus</i>				x						x					2	[43]	
	<i>C. lupicaris</i>	x			x	x					x	x				5	[42,43,62,63]	
	<i>C. newstadi</i>	x			x											3	[42,43,46,58]	

Figure legend

Figure 1. Percentage of mammal (red) and bird (blue) derived blood meals isolated in studies on females of *Culicoides* biting midges in Europe. Sample size (log transformed) of each biting midge species analysed is shown in black. Data from different genetic haplotypes corresponding to the same morphospecies were pooled together. Molecular characterization of *C. griseidorsum* specimens did not confirm morphological identifications of the species.

Box 1. Phylogenetic inertia of *Culicoides* blood meals

In order to identify the phylogenetic inertia in the blood meal origin of *Culicoides* species, we analysed the variation in blood meal composition using the recently published phylogeny of *Culicoides* by Ander et al. [65] with the program BayesTraits [66]. The blood meal composition was measured as the percentage of blood meals from mammals. Lambda value (λ), the degree of covariation between a given trait and species phylogeny [66], was 0.90, significantly different from 0 ($\chi^2 = 8.65$, $df = 1$, $P = 0.003$). Because λ ranges from 0 (indicating no phylogenetic signal) to 1 (trait variation associated to tree topology), our results suggest that phylogenetically related species of *Culicoides* tend to feed on the same class of vertebrates. Ander et al.'s [65] phylogeny is based in the COI gene, whose utility for recovering phylogenies is under debate [67]. For this reason, we also used variance decomposition analysis to estimate the similarity in diet compositions explained by *Culicoides* subgenera (<http://www.inhs.illinois.edu/files/9613/9136/7590/CulicoidesSubgenera.pdf>), with this variable explaining 69% of the variance of the feeding pattern of *Culicoides* species. This information could be useful to infer the feeding pattern of *Culicoides* species, when no empirical information is available, based on the phylogenetic relationships among species.