Opinion

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

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

## Blood feeding and females of *Culicoides* species

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

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

In Europe, epidemiological studies of species of *Culicoides* have been mainly focused on their role as vectors of the Bluetongue virus. Prior to 1998, Bluetongue outbreaks in Europe were probably vectored by *Culicoides imicola* and were limited to sporadic cases in southern European countries, mainly in the Iberian Peninsula. Subsequently, the virus acquired new European vectors, allowing it to spread and reach northern Europe in 2006 [13]. Among the species potentially implicated in the geographical spread of Bluetongue to north Europe are members of both the *obsoletus* (*Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides dewulfi*, and *Culicoides chiopterus*) and the *pulicaris* (*Culicoides pulicaris* and *Culicoides punctatus*) groups [14]. These Bluetongue outbreaks had dramatic economic consequences. For example, the costs of Bluetongue outbreaks in 2007 in France and The Netherlands were estimated as costing approximately US$1.4 billion and US$85 million, respectively [15]. More recently, species of the *obsoletus* complex have been incriminated in the transmission of the Schmallenberg virus [16], a virus first detected in 2011 which causes transient and nonspecific symptoms in adult cattle and abortions in pregnant ruminants [17].

## Studies of female *Culicoides* feeding patterns

Identifying the feeding patterns of *Culicoides* biting midges is an essential step in determining the importance of each particular species in epidemiological studies [18]. As previously estimated for the case of mosquito-borne pathogens, the proportion of blood meals derived from competent vertebrate hosts is basic to identifying key vector species, quantifying the risk of pathogen amplification and transmission by blood-sucking insects [19,20], identifying the factors that favour pathogen spill over to relevant species, and to develop predictive models on the dynamic of transmission of pathogens by haematophagous vectors [21,22].

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

| Procedure | Advantages | Disadvantages |
|---|---|---|
| Species specific primers | • Identification without sequencing<br>• Quick and cheaper identification | • Host range is limited to the species tested |
| Universal primers | • Broad host range<br>• Identification of mixed blood meals from individuals of the same species | • Costs of sequencing<br>• Risk of co-amplification of both host and insect DNA in some vector species<br>• Absence of genetic characterization of potential host species |

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

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

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

## Molecular approaches to *Culicoides* host identification

Compared with traditional procedures, the development of molecular techniques for the identification of the origin of insect blood meals has increased the accuracy of host identification at the species level. These molecular tools have been routinely used in studies on mosquitoes, among other haematophagous arthropods (i.e., [19,20,40]), and have also recently been applied to species of *Culicoides*. Molecular studies on *Culicoides* biting midges employed two main methodological approaches including the use of: (i) specific sets of primers to amplify DNA from particular host species (i.e., [41,42]); and (ii) general primers to amplify conserved genetic regions of mammals, birds, or even all vertebrate species [40,43] (Table 1).

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

## Current knowledge

Molecular identification of the vertebrate hosts of species of *Culicoides* in Europe has been conducted for at least 1360 individuals representing 31 species (Table 2). As a result, 45 different host species have been identified, including 33 species of birds and 12 species of mammals. To our knowledge, blood from reptiles or amphibians have not been detected in spite of the capacity of some biting midges to feed blood on ectotherms [6]. However, Calvo and co-workers [46] isolated from a *C. pulicaris* specimen a COI sequence 93% similar to a reptile species not present in the studied area, suggesting that this biting midge probably fed on a not yet genetically characterised herpetile. On average, females of each *Culicoides* species fed on the blood of five different host species. In the case of the most extensively sampled *Culicoides* species (i.e., those with at least 50 individuals), both bird and mammal derived blood meals have been isolated. In addition to *Culicoides kibunensis* and *Culicoides festivipennis*, these species include members of the *obsoletus* group (*C. obsoletus*, *C. scoticus*, and *C. chiopterus*) and the *pulicaris* group (*C. punctatus* and *C. pulicaris*) which, together with *C. imicola*, are the most important potential vectors of Bluetongue virus in Europe. Although most species have some flexibility in host selection, members of the *obsoletus* and the *pulicaris* groups feed primarily on mammals and only occasionally on birds (Table 2; Figure 1). Additional studies on midges of the *obsoletus* group have found support for this feeding pattern [41,46], although in these cases, insect identification was not confirmed at the species level. Contrary to the case of these few extensively studied species, the current knowledge from molecular studies on the feeding pattern of most *Culicoides* species is based on the analyses of very few individuals. In fact, for 16 of the tested species, which comprise 52% of the total of the species analyzed, feeding patterns are based on less than 15 individuals (Figure 1).

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

## Concluding remarks and future perspectives

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

Additional studies should be conducted in a diversity of habitats, especially in pristine areas, because there is a general lack of awareness regarding the *Culicoides* species feeding on wild animals. Currently, most studies of the feeding sources of females of *Culicoides* have been focused

### 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 ($\lambda$), 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 $\lambda$ 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://wwx.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.

**Table 2. Host species of biting females of species of *Culicoides* in Europe identified using molecular methods**[a]

| | | Mammalian species | | | | | | | | | | | | Avian species | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bos taurus | Capra hircus | Capreolus capreolus | Cervus elaphus | Ovis aries | Sus scrofa | Alces alces | Oryctolagus cuniculus | Equus caballus | Homo sapiens | Microtus savii | Mus musculus | Anas platyrhynchos | Tadorna ferruginea | Columba palumbus | Streptopelia decaocto | Gallus gallus | Acrocephalus palustris | Anthus trivialis | Coccothraustes coccothraustes | Corvus corone | Cyanistes caeruleus | Delichon urbica | Emberiza citrinella | Erithacus rubecula | Garrulus glandarius | Luscinia luscinia | Luscinia megarhynchos | Luscinia svecica | Muscicapa striata | Oriolus oriolus | Passer domesticus | Passer montanus | Passer hispaniolensis | Parus major | Phylloscopus trochilus | Pica pica | Sylvia atricapilla | Sylvia borin | Turdus merula | Turdus philomelos | Ardea purpurea | Ixobrychus minutus | Nycticorax nycticorax | Asio otus | Host species | Refs |
| *Avaritia* | *C. chiopterus* | x | x | x | | x | | | | x | x | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 7 | [42,43,52,57,62,63] |
| | *C. dewulfi* | x | | | | x | x | | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | [42,43,57,58,62,63] |
| | *C. obsoletus* | x | x | x | x | x | | | x | x | x | | x | x | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 11 | [30,42,43,52,53,57,58,62,63] |
| | *C. scoticus* | x | x | x | | x | x | | x | x | x | | | x | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | [30,42,43,52,57,58,62,63] |
| *Beltranmyia* | *C. circumscriptus* | | | | | | | | | | x | | | | | x | | | | | | x | | | | | | | | | | | | | | | x | x | | | x | x | | | | x | 8 | [43,62,64] |
| | *C. salinarius* | | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | | 2 | [43,62] |
| *Culicoides* | *C. deltus* | x | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | [52,57,58] |
| | *C. grisescens* | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | [43] |
| | *C. impunctatus* | | | | | x | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | [43] |
| | *C. lupicaris* | x | | | | x | x | | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | [42,43,62,63] |
| | *C. newstadi* | x | | | | x | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | [42,43,46,58] |
| | *C. pulicaris* | x | x | | x | x | | | | x | x | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 7 | [42,43,46,52,57,62,63] |
| | *C. punctatus* | x | x | x | x | x | | x | x | x | x | x | | x | | x | | x | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | 14 | [42,43,46,52,62,63,64] |
| *Monoculicoides* | *C. parroti* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | [46] |
| | *C. riethi* | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | [56] |
| *Oecacta* | *C. alazanicus* | | | | | | | | | | x | | | | | x | | | | x | | | | x | | | | x | | | x | x | | | | x | x | x | | x | x | x | x | x | | x | 16 | [64] |
| | *C. brunnicans* | x | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | [30,42,63] |
| | *C. clastrieri* | | | | | | | | | | x | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | | 3 | [57,58] |
| | *C. duddingstoni* | | | | | | | | | | | | | | | | | | | | | | x | | | | x | | | | | | x | x | | | | x | | | | | | | | | 5 | [43,62] |
| | *C. festivipennis* | | | | | x | | | | | x | | | | | x | x | | | x | | | | | | | | | | | | x | x | x | x | | | x | | | | x | | | x | x | 13 | [43,46,57,58,62,64] |
| | *C. furcillatus* | x | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | [62,63] |
| | *C. cf. griseidorsum* | | | | x | | | | | | | | | | | | | | x | | x | | | | | | | | x | | | | | | | | | x | | | | | | | | | 5 | [64] |
| | *C. kibunensis* | x | | | | | | | | | x | | | | | x | | | x | | | | | | x | x | | | | | | | | | | | | | x | | | | | | | | 7 | [57,58,62] |
| | *C. pictipennis* | x | | | x | x | | | | | x | | | | | | | | | | | | | | | x | | | | | | | | | | x | | x | | | x | | | | | | 8 | [42,43,57,58,62,64] |
| | *C. picturatus* | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | [63] |
| | *C. poperinghensis* | x | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | [57,62] |
| | *C. semimaculatus* | | | | | | | | | | x | | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | 2 | [57,58] |
| | *C. vexans* | x | | x | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | [62] |
| *Sylvaticulicoides* | *C. achrayi* | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | [43,63] |
| *Wirthomyia* | *C. reconditus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | 1 | [62] |
| Unplaced | *C. pallidicornis* | x | x | | | | | | x | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | [57,58,62,63] |

[a] Host species were sorted by order. *Culicoides* species identity was assigned according to information provided in the articles unless authors identified them as members of species complexes (i.e., *obsoletus* and/or *pulicaris* groups). Molecular characterization of *Culicoides cf. (confer) griseidorsum* specimens did not confirm morphological identifications of the species. *Sus scrofa* included both wild boars and domestic pigs. Data from different genetic haplotypes corresponding to the same *Culicoides* morphospecies were pooled together.

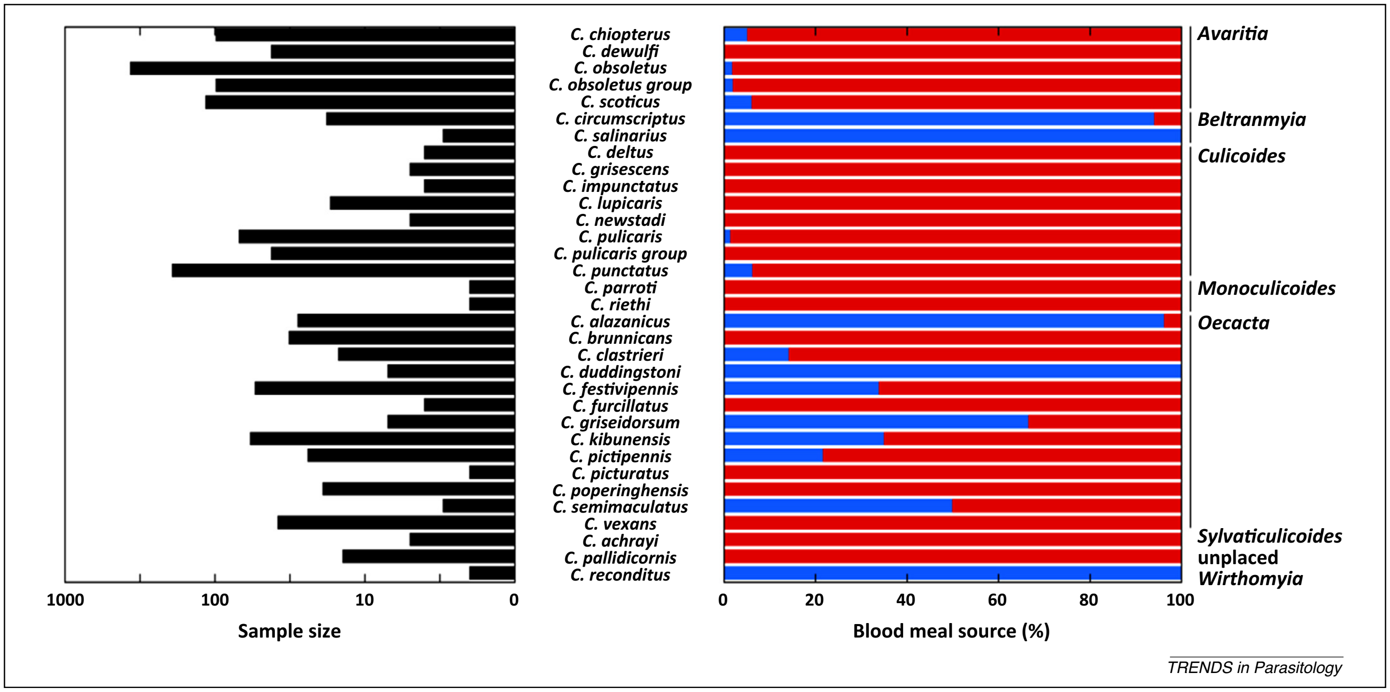

**Figure 1**. Percentage of mammal (red) and bird (blue) derived blood meals isolated in studies on females of *Culicoides* biting midges in Europe. Sample size (log transformed) of each biting midge species analysed is shown in black. Data from different genetic haplotypes corresponding to the same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

on farms or surrounding areas with livestock (e.g., [41,52,53]). Sampling *Culicoides* on caged wild animals can give information on the main feeding preferences, but understanding the importance of wild communities on pathogen transmission needs a deeper understanding of feeding/transmission networks. In spite of the potential importance of wild ruminants in the epidemiology of relevant pathogens such as Bluetongue virus, very few studies have investigated the potential pathogen transmission between wild ruminants and livestock [41,42]. Wildlife may play a central role in the transmission of *Culicoides*-borne pathogens as suggested by the high seroprevalence of Bluetongue antibodies found in some wild ruminant species in Europe and North America [54,55]. This illustrates the value of characterising feeding preferences at the species level, allowing for more detailed estimation of the potential for pathogen amplification. Combining information regarding blood meal origin and vertebrate host competence allows for a detailed estimation of the emerging risk of pathogens in different areas [18,56]. Avian pathogens (e.g., avian malaria parasites) have been isolated from the abdomens of biting midges feeding on humans [57,58]. This suggests the potential role of some species of *Culicoides* as a bridge in pathogen transmission between wild animals and humans, as in the case of some filarial nematodes and *Orthobunyavirus*, which are transmitted to humans mainly by species of *Culicoides* in the New World and Africa [59].

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

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

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