Irish network for biting midges and mosquitoes

UCD and WIT researchers received funding from the Department of Food, Agriculture and the Marine (DAFM) to investigate the current distribution of biting midges, mosquitoes and vector-borne pathogens (VBPs) in Ireland. The project which will be carried out in collaboration with academics, public service representatives and community groups, also aims to consolidate and enhance national expertise and awareness of fly vectors. The research team hopes that this will help to improve Ireland's preparedness for potential future incursions of exotic vectors and VBPs

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Blood feeding insects such as biting midges and mosquitoes are important vectors of diseases affecting humans, domestic animals and wildlife. With the combined effects of climate change, widespread establishment of formerly exotic VBPs in continental Europe, and increased direct movement of livestock and goods between Ireland and continental Europe due to Brexit, Ireland is at increased risk from invasive arthropod vectors and VBPs. However, with the exception of mosquito surveillance at potential points of entry there is currently no surveillance activity for biting flies in Ireland. The gradual decline of taxonomic expertise is also generally considered problematic, as it leaves the country without the necessary expertise to detect and assess the potential risk from introduced vectors and VBPs.

GENERAL BIOLOGY OF BITING MIDGES AND MOSQUITOES AND THEIR ROLE AS DISEASE VECTORS

Biting midges (family Ceratopogonidae, mainly genus *Culicoides*) are tiny flies (1 to 3mm in length) with a short proboscis, a humped thorax and wings without scales (Figure 1), while mosquitoes (family Culicidae) are slightly larger (ranging between 3 and 6mm in length), slender and long-legged, with elongated 'needle-like' mouthparts and wings with scales (Figure 2).[1] The adult females of both groups of flies are blood feeders and usually require a protein-rich meal of fresh blood to produce eggs. The exception are autogenous species, such as *Culicoides impunctatus* and *Culex pipiens molestus* which can produce their first batch of eggs without taking a blood meal.[2,3] As a result, significant numbers of these flies can be encountered even in the absence of available hosts.

Many biting fly species happily feed on a broad range of vertebrate hosts including mammals, birds, amphibians and reptiles, while others have preferential hosts. Both midges and mosquitoes locate their hosts primarily via CO_2 but also rely on other chemical and olfactory stimuli. Biting midges lay their eggs into the wet soil ecotone in marginal areas of lakes, rivers and coastal mud flats, but also into other moist, rich substrate such as dung pats, peat or leaf litter. Mosquitoes, on the other hand, rely on water bodies for their larval development, with type, size and quality of the chosen waterbody determined by the species of mosquito.[3,4] The eggs hatch into free-living larvae which go through a number of moults before they pupate. Midges generally overwinter as fourth instar larvae which, in locations which experience cold winters, may enter diapause prior to pupation.[5] Their over-winter survival rates vary greatly depending on rainfall, soil moisture and temperature.[2] Mosquitoes mostly overwinter as eggs which can also enter diapause and in some species are freeze and/or desiccation resistant.[6,7] Incidentally, these diapausal, resistant eggs are an important mechanism by which exotic species can be imported, frequently attached to ornamental plants such as the 'lucky bamboo' or used car tyres.[8,9]

Due to their very small size, midges are poor fliers and rarely move more than a few hundred metres from the breeding sites; however, occasionally individuals can be transported over long distances by wind. In fact, the expansion of exotic midge species into new geographical areas (as well as the spread of infected midges from vector-borne disease

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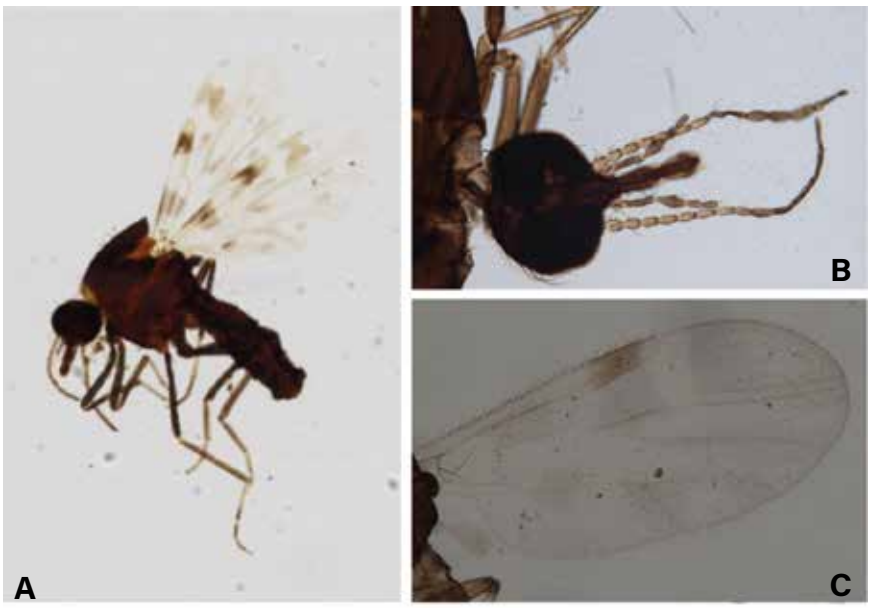

Figure 1. Culicoides (midges), A: whole mount; B: enlarged view of the head with antennae, palps and proboscis; C: enlarged view of a wing.

outbreak sites) is probably chiefly facilitated by wind.[10] Initially, it was thought that housing could be used to protect livestock from biting midges, however, it is now known that housing has limited efficacy as some species frequently enter sheds and stables throughout the season but particularly in autumn and winter as temperatures fall.[11] Similarly, certain species of mosquitoes, especially those that bite humans and domestic animals, readily enter buildings. Both biting midges and mosquitoes are most active during dusk and dawn in overcast, warm, humid and still weather. However, some species are chiefly nocturnal and others diurnal. One of the main reasons why biting midges and mosquitoes have received so much scientific attention is that they can serve as vectors for viral, protozoal and nematode pathogens. Most importantly, from a European point of view, these include Bluetongue virus (BTV), Schmallenberg virus (SBV), African Horse sickness virus, *Haemoproteus* spp and *Onchocerca cervicalis* in the case of biting midges and West Nile virus, Usutu virus, Dengue Virus, avian *Plasmodium* spp, *Dirofilaria* (*D. immitis* and *D. repens*) and *Setaria* spp in the case of mosquitoes.[1,2,11]

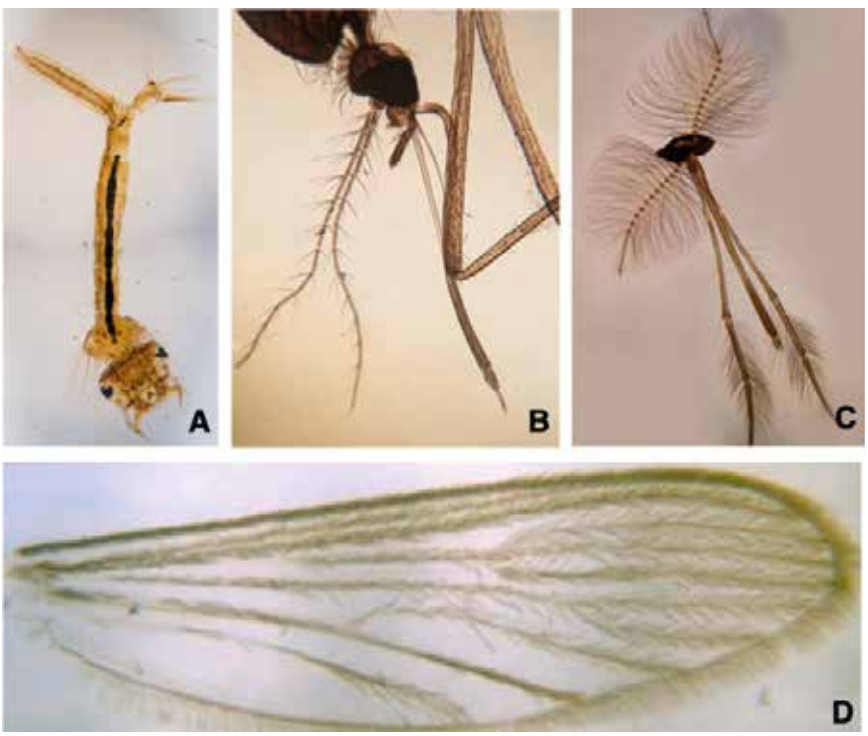

Figure 2. Culicidae (mosquitoes), A: larva; B: enlarged view of the head of a Culex female; C: enlarged view of the mouthparts of a Culex male; D: enlarged view of a wing.

SURVEILLANCE OF INSECT VECTORS IN IRELAND

According to species lists for Irish biting midges published in 2008 and updated in 2012, a total of 78 species have been reported from Ireland.[12,13] The most recent large scale surveillance programmes of biting midges in Ireland were initiated in response to the outbreak of BTV in Northern Europe in 2006 and the outbreak of SBV in 2011. In 2007, DAFM in collaboration with NUI Galway initiated the National BTV Vector Surveillance Programme.[2,14] During this project, midges were collected on a weekly basis between April 2007 and December 2009 at ten randomly selected sites throughout the Republic of Ireland. Overall 27 species were reported with the Obsoletus (including *Culicoides chiopterus*, *C. dewulfi*, *C. obsoletus* s.s., *C. scotius*) and Pulicaris groups (defined by McCarthy and colleagues to include *C. pulicaris, C. punctatus, C. newsteadi, C. impunctatus, C. delta, C. grisescens* and *C. nubeculosus*) together accounting for over 80 per cent of the total catch in each year. Both groups include potential BTV vector species. While few midges were caught during cold, wet and windy conditions, the authors observed that the overall seasonal patterns of midge activity were remarkably similar over the three-year surveillance programme, with highest numbers consistently trapped between May and October. The 'BTV Vector free period' defined by EU legislation as the time period during which no *C. imicola* specimens (this species has a more southerly range and has never been reported from Ireland) and less than five parous female Culicoides are caught per trap, extended

from week 49 to week 13 (i.e., from December to April).

This study coincided with a four-year surveillance programme in Northern Ireland which ran from 2008 to 2011.[11] In this survey, midges from the Obsoletus group represented almost 75 per cent of all midges collected and were chiefly associated with cattle-rearing habitats and woodlands, while midges from the Pulicaris group were found close to both cattle and sheep and were more abundant early in the midge activity season when temperatures were still quite low. The Pulicaris group which in this study only included *C. pulicaris, C. punctatus* and *C. newsteadi*, made up just 21 per cent of the total catch. *C. impunctatus* and *C. grisescens* were combined into a separate, Impunctatus group. These midges which are autogenous, i.e., do not require a blood meal to produce their first batch of eggs, were mostly trapped in upland bogs.

In 2014, Collins and colleagues, sampled ten sentinel livestock farms fortnightly over a period of 16 weeks (July 21 to November 5).[4] Again, putative vector species for both BTV and SBV *(C. obsoletus/C. scoticus, C. chiopterus, C. dewulfi, C. pulicaris* and *C. punctatus),* were the most abundant midge species identified. The authors observed that the total number of midges collected on each farm varied widely, with higher densities near broadleaved woodlands which presumably provided some shelter from wind and substrate for larval development. Moreover, the abundance of midges collected during each fortnightly collection period was highly correlated with mean fortnightly temperature in the region.

In contrast to midges, much less work has been done on the distribution and diversity of mosquitoes in Ireland. To date 18 species have been reported from Ireland, together with their baseline distributions.[15] Regarding the possible introduction of exotic species, Environmental Health Officers continuously carry out surveillance at potential points of entry (i.e., at seaports and airports). However, this data is not currently publicly available.

THE CURRENT PROJECT

The main focus of this project is to establish a network of academics, public sector representatives and community volunteers, in order to consolidate and enhance national expertise and awareness of native and exotic biting midges and mosquitoes. Moreover, trapping will be carried out in various sites across the country, in order to update species and distribution maps. While a portion of the catch will be identified morphologically (as was done in previous studies), we will also assess rapid, high-throughput MALDI-TOF mass spectrometry and PCR-based methods for their suitability to efficiently quantify and identify Irish biting fly species. A subsample of flies will be screened for the presence of VBPs using nanopore sequencing technology. Our results will be analysed against previous surveillance data to identify trends and assess the potential incursion, survival and establishment of exotic species. Overall, we hope that the project will improve Ireland's preparedness and facilitate early detection and effective control of any exotic vectors or VBPs that might enter the country in future.

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