

## Monitoring of biting midges (Diptera: Ceratopogonidae), potential vectors of bluetongue and Schmallenberg viruses, in Germany

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**Zusammenfassung:** Mit dem überraschenden Ausbruch der Blauzungenkrankheit (BT) im Jahr 2006 und dem erstmaligen Auftreten des Schmallenberg-Virus (SBV) im Jahr 2011 in Mittel- und Nordeuropa rückten Gnitzen der Gattung *Culicoides* (Diptera: Ceratopogonidae) als Vektoren von Krankheitserregern in den Fokus der Wissenschaft. Um das Risiko Gnitzen-assoziiierter Erkrankungen abschätzen und Empfehlungen zum Vektormanagement aussprechen zu können, sind fundierte Kenntnisse über die Biologie, Ökologie und Verbreitung der Vektorarten und die Randbedingungen, die zur Übertragung der Viren notwendig sind, unerlässlich. Diese sind gegenwärtig allerdings sehr limitiert. So ist beispielsweise nur wenig über die Brutsubstrate und die Überwinterungsmechanismen vieler *Culicoides*-Arten bekannt. Um bestehende Wissenslücken zu schließen und Daten zum geografischen und saisonalen Auftreten potenzieller Vektorarten in Deutschland zu sammeln, wurde 2018 erneut ein Monitoring-Projekt ins Leben gerufen. Unter Benutzung von UV-Lichtfallen und Emergenzfallen werden Gnitzen deutschlandweit auf ausgewählten landwirtschaftlichen Betrieben gefangen und anschließend morphologisch und genetisch bestimmt. Im Fokus stehen dabei besonders die Arten der *Obsoletus*- und *Pulicaris*-Komplexe, die vermutlich eine zentrale Rolle bei der Verbreitung der Viren nördlich der Alpen spielen. Die Monitoringdaten sollen zur Aufklärung der Rolle der einheimischen Gnitzenarten als Vektoren beitragen. Darüber hinaus wird die gegenwärtige Artenzusammensetzung der Gnitzenfauna Deutschlands dokumentiert und ermittelt, welche Habitats von den verschiedenen Spezies als Lebensraum präferiert werden.

**Key words:** Biting midges, *Culicoides*, bluetongue, Schmallenberg, Ceratopogonidae, vector distribution, viruses

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### Introduction

Biting midges (Diptera: Ceratopogonidae) are a nuisance to humans and livestock and vectors of various pathogens. Hematophagous species of the genus *Culicoides* are able to transmit viruses, such as bluetongue virus (BTV), which belongs to the genus *Orbivirus* (family Reoviridae), and Schmallenberg virus (SBV), a member of the genus *Orthobunyavirus* (family Bunyaviridae) (KAMPEN 2012, SICK & al. 2019). Both viruses affect ruminants and cause severe suffering (MACLACHLAN & al. 2009; BEER & al. 2013). In addition to the physical damage done to the animals, epidemics entail high economic losses to the livestock industry (GETHMANN & al. 2010, VELDHUIS & al. 2014).

Sustained management of vector-borne diseases includes vector management. However, targeted and affordable vector management, either proactive or reactive, as well as risk assessments require detailed knowledge about the biology and ecology of possible vector species, including spatiotemporal distribution.

In 2006, BTV broke out in Central Europe completely unexpected as *Culicoides imicola*, previously thought to be responsible for all BTV outbreaks in the Old World, in Europe only occurs in the Mediterranean (FOX & al. 2019). Likewise, the emergence of SBV in 2011 came by surprise. Detection of BTV and SBV in *Culicoides* species other than *C. imicola* (HOFFMANN & al. 2009, RASMUSSEN & al. 2012) brought European biting midges in the focus of many scientific studies. Species of the *Obsoletus* and *Pulicaris* Complexes, both widespread in Europe and Germany, have since been considered the putative vectors of the viruses. However, the state of knowledge about their biology, ecology and distribution is limited. Breeding sites and substrates as well as overwintering mechanisms have not or only insufficiently been characterised.

The project focuses on the spatiotemporal distribution, preferred landscape structures, breeding preferences, habitat-binding and overwintering sites of *Obsoletus* and *Pulicaris* Complex species in Germany. It was initiated in late 2018 in four federal states of Germany and has been extended to include all federal states in 2019 after new cases of BTV were recorded in Germany in December 2018 and a restriction zone was established (FLI 2019).

## Materials and methods

### Trapping

Biting midges traps have been placed on animal farms throughout Germany (Fig. 1).

Using BG-Sentinel UV-light suction traps (Biogents, Germany), ceratopogonids are caught once a week for 24 hours. At least one trap per farm and federal state is operated at 37 trapping sites either inside or outside of ruminant-holding stables. Twelve of those sites have additionally been equipped with a second trap in- or outdoors, respectively, to analyse the phenology of biting midges year-round. All other BG-Sentinel traps are active from April to October.

In addition, emergence traps are placed on dunghills at ten selected farms from February to April to search for overwintering sites of biting midges. Each dunghill is sampled repeatedly for one week in a row with five traps at varying positions.

All trapped biting midges are conserved in 75% ethanol.

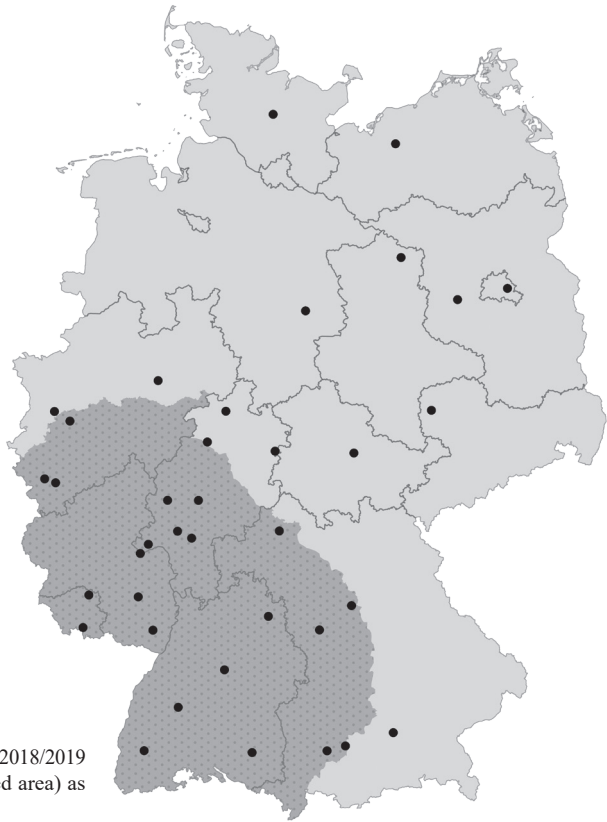
### Biting midge identification

Biting midges are morphologically identified under a stereomicroscope and assigned to species or complex level based on their wing ornamentations by means of common identification keys (CAMPBELL & PELHAM-CLINTON 1960, DELÉCOLLE 1985, GLUKHOVA 1989). Since *Culicoides* females belonging to cryptic species of the *Obsoletus* and *Pulicaris* Complexes are difficult to distinguish, final species assignment is carried out by analysis of genetic markers using PCR technology (NOLAN & al. 2007, LEHMANN & al. 2012). However, as classical PCR approaches are poorly applicable for high throughput studies, due to being time-consuming and the impossibility of species quantification within pools of insects, quantitative real-time PCR assays will be developed, based on work by CÊTRE-SOSSAH & al. (2004), MONACO & al. (2010), MATHIEU & al. (2011) and WENK & al. (2012).

### Expected outcome

The results of the monitoring will contribute to clarifying the current species distribution and diversity of biting midges in Germany. For this purpose, newly developed real-time PCR assays will allow high throughput screening of collected biting midges and will help to quantify *Obsoletus* and *Pulicaris* Complex species, the putative BTV and SBV vectors, in mixed insect pools. Differences in species composition on farms, depending on the surrounding landscapes and availability of hosts, are expected. Through comparison of obtained data with literature data, the impact of climate change on the phenology of biting midges will be evaluated. The results will facilitate specifying a potentially 'vector-free period', which allows unrestricted animal trade without testing for viral infection. Furthermore, the monitoring data will be collated in a newly created database to lay the foundation for prospective risk analyses and modelling scenarios of *Culicoides* occurrence and pathogen transmission.

Collected biting midges will be made available for BTV and SBV screening. The distribution of infected biting midges will manifest our knowledge on the role of *Culicoides* species as potential vectors.



**Fig. 1:** BG-Sentinel UV-light trapping sites 2018/2019 (●) and BTV restriction zone (shaded area) as of 6 May 2019.

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