Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

# CFIA CVBD Overview

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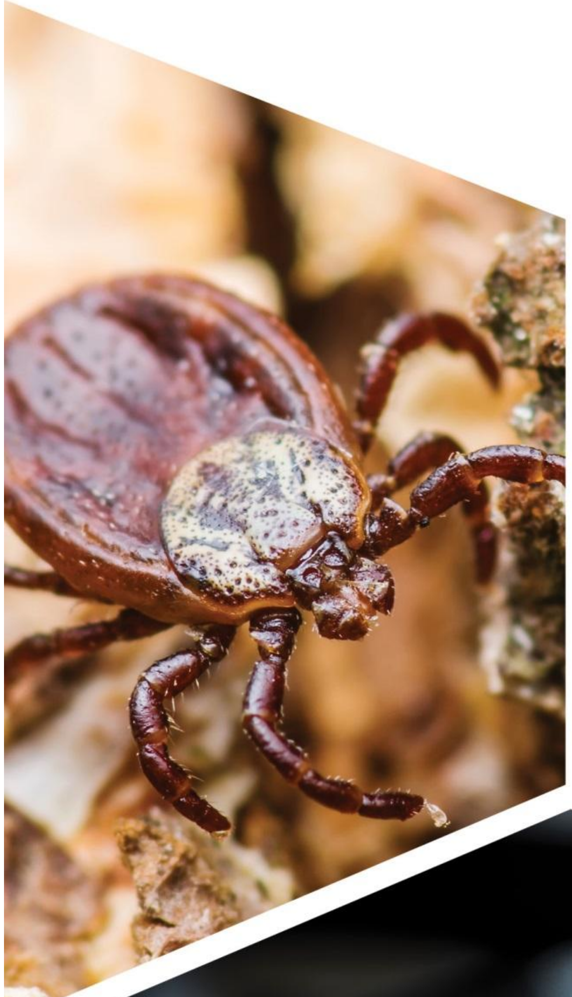
Centre for Vector-Borne Diseases
National Centres for Animal Disease
Lethbridge, Alberta



25 Feb 2020

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Canada

# CFIA Lethbridge: A National Centre for Animal Diseases

# CFIA-NCAD-CVBD
# Current Areas of Research

- *Culicoides* Biting Midges of Canada
- Soft Ticks of Canada – African Swine Fever Virus
- Asian Longhorned Tick – (*Haemaphysalis longicornis)*, a new resident in the US, originating from eastern Asia and the Pacific Islands – Australia – New Zealand

# Biting Midges as Vectors of Animal Disease Agents

Family: Ceratopogonidae

Worldwide: 5000 species in 60+ genera

~1,400 species in genus *Culicoides*

In Canada: 180 species in family, the main genus is *Culicoides* ~ 61 spp.

**“One biting midge is an entomological curiosity, 1000 can be hell” Kettle 1962**

# Principal Disease Pathogens Vectored by *Culicoides* Biting Midges

| Pathogen or Disease | Main vector species | Vertebrate hosts |
|---|---|---|
| Akabane | *Culicoides imicola* | Cattle |
| African horse sickness | *C. imicola* | Equids and dogs |
| Epizootic hemorrhagic disease | *C. sonorensis, C. schultzi, & C. kingi* | Deer, cattle & sheep |
| Bluetongue | *C. sonorensis* & >10 *Culicoides* spp. | Ruminants |
| Nematodes: *Onchocerca* | *Culicoides* spp. | Ruminants |

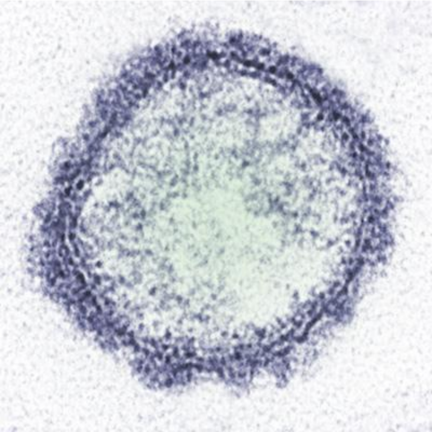
# Schmallenberg Virus (SBV)

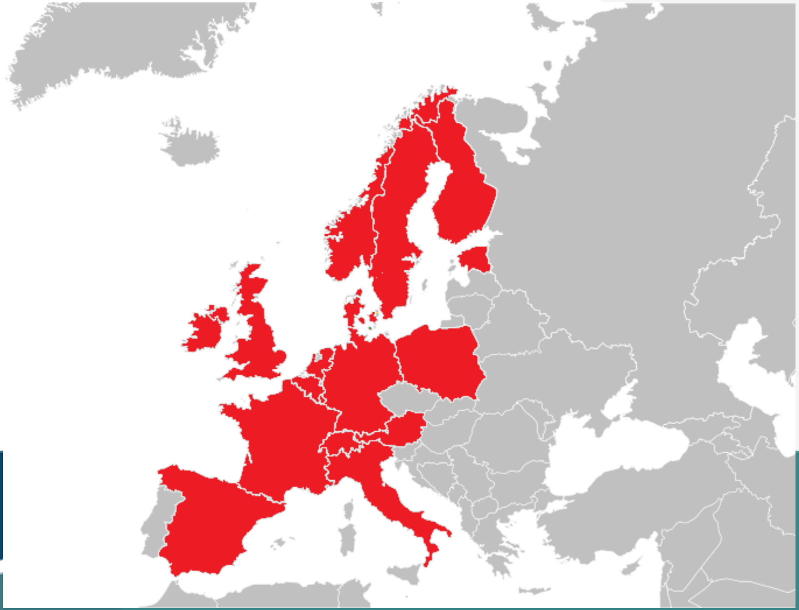
Orthobunyavirus (ss RNA) - causes congenital malformations and stillbirths in cattle, sheep, goats, and possibly others, initially reported in 2011

Transmitted by *Culicoides* spp., mainly *C. obsoletus/scoticus* complex, as well as *C. punctatus* and *C. pulicaris*

Named after Schmallenberg, Germany, where it was first identified, Now found in 18 countries in Europe: Austria, Azerbajan, Belgium, Denmark, Estonia, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Norway, Poland, Spain, Sweden, Switzerland, and The United Kingdom

Another example of our having the vectors and hosts, but not the pathogen in Canada





# A Study of the Indigenous Fauna of *Culicoides* Midges in Canada

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Robert Pfannenstiel[3], Joel Hutcheson[1] and **Aruna Ambagala**[4]

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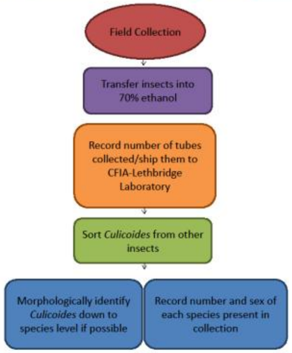
## Abstract

Several vector-borne diseases are expanding their ranges as a result of climate change, advances in transportation and geographic and host factors. *Culicoides* midges occur throughout most of the inhabited world, and serve as vectors of many important animal pathogens including bluetongue, epizootic haemorrhagic disease, African horse sickness, Schmallenberg and Akabane viruses. The *Culicoides* fauna of Canada is poorly understood. Knowledge of *Culicoides* species composition, distribution, and seasonality are critical for understanding the epidemiology of *Culicoides*-borne diseases, risk assessment of possible *Culicoides*-borne disease outbreaks and policy development. A small-scale targeted *Culicoides* survey was conducted across Canada from 2014-2016. Ultraviolet LED CDC traps were set up at various locations in Okanagan Valley, BC; Abbotsford, BC; Lethbridge, AB; Saskatoon, SK; Winnipeg, MB; Brandon, MB; Guelph, ON; Peterborough, ON; Ottawa, ON; Laval, QC; Truro, NS; and St. John's, NL. With the help of volunteers (veterinarians, entomologists and producers) field-samples were collected and shipped to the CFIA-Lethbridge Laboratory for identification. At the Lethbridge Laboratory, the trap-collections were sorted and *Culicoides* samples were morphologically identified. In addition, cytochrome c oxidase subunit I (COI), internal transcribed spacer (ITS) and elongation factor 1 alpha (EF1alpha) sequences from representative samples from *Culicoides* species identified were amplified and sequenced. To date, four subgenera, two species groups and 23 *Culicoides* species have been morphologically and/or genetically identified, including two known vectors of bluetongue virus (*C. sonorensis* and *C. obsoletus*) and several suspected vectors (*C. biguttatus*, *C. stellifer*, *C. venustus*, *C. sanguisuga*, and members of the *pulicaris* Species Group). Following three consecutive vector seasons, the CFIA Lethbridge Laboratory has amassed a reference collection of over 113,000 *Culicoides* midges and developed reference databases for microscopy images, genetic sequences, and slide-mounted specimens.

## Methodology

### A. Insect Collection Workflow


Field Collection

Transfer insects into 70% ethanol

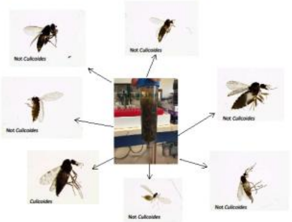
Record number of tubes collected/ship them to CFIA-Lethbridge Laboratory

Sort *Culicoides* from other insects

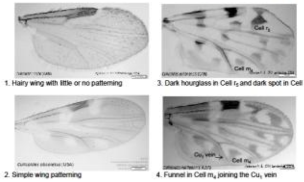
Morphologically identify *Culicoides* down to species level if possible

Record number and sex of each species present in collection

### B. Sorting *Culicoides* from other Insects



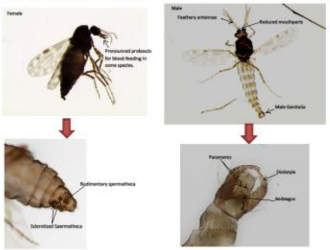
### D. Identification of *Culicoides* species/groupings



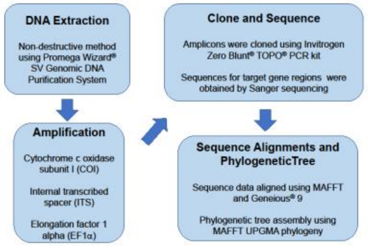
1. Hairy wing with little or no patterning
3. Dark hourglass in Cell $r_5$ and dark spot in Cell $m_4$
2. Simple wing patterning
4. Funnel in Cell $m_4$ joining the $Cu_1$ vein

Slide mounting
Advanced morphological Characterization
(SCWDS & CFIA)

### C. Distinguishing Female vs Male *Culicoides*



### E. Molecular Characterization


**DNA Extraction**

Non-destructive method using Promega Wizard® SV Genomic DNA Purification System

**Amplification**

Cytochrome c oxidase subunit I (COI)

Internal transcribed spacer (ITS)

Elongation factor 1 alpha (EF1α)

**Clone and Sequence**

Amplicons were cloned using Invitrogen Zero Blunt® TOPO® PCR kit

Sequences for target gene regions were obtained by Sanger sequencing

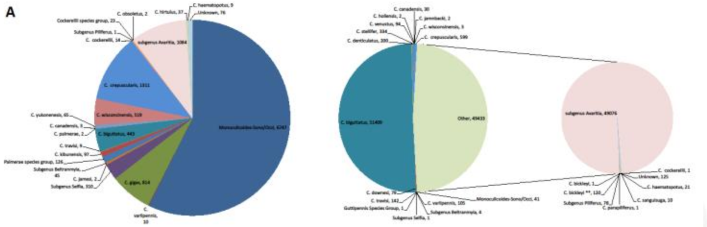
**Sequence Alignments and PhylogeneticTree**

Sequence data aligned using MAFFT and Geneious® 9

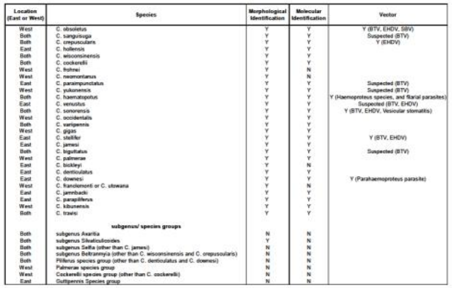
Phylogenetic tree assembly using MAFFT UPGMA phylogeny

Figure 1. Workflow strategies for a targeted survey of *Culicoides* species in Canada. (A) Insect trap samplings were collected from selected sites in both Western and Eastern Canada for morphological and molecular identification of *Culicoides*. (B) Trap samples were sorted and *Culicoides biting midges were separated from other insects*. (C) Female and male specimens were identified by morphological characteristics. (D) Wing patterns were used to identify *Culicoides* to species groups. (E) Sequencing of genetic markers was undertaken to identify closely-related *Culicoides* species.

## Results



B



| Location (East or West) | Species | Morphological Identification | Molecular Identification | Vector |
|---|---|---|---|---|
| West | C. obsoletus | Y | Y | Y (BTV, EHDV, SBV) |
| Both | C. sanguisuga | Y | Y | Suspected (BTV) |
| Both | C. crepuscularis | Y | Y | Y (EHDV) |
| East | C. hollensis | Y | Y | |
| Both | C. wisconsinensis | Y | Y | |
| Both | C. cockerelli | Y | Y | |
| West | C. frohnei | Y | N | |
| West | C. neomontanus | Y | N | |
| East | C. paraimpunctatus | Y | Y | Suspected (BTV) |
| West | C. yukonensis | Y | Y | Suspected (BTV) |
| Both | C. haematopotus | Y | Y | Y (Haemoproteus species, and filarial parasites) |
| East | C. venustus | Y | Y | Suspected (BTV, EHDV) |
| Both | C. sonorensis | Y | Y | Y (BTV, EHDV, Vesicular stomatitis) |
| West | C. occidentalis | Y | Y | |
| Both | C. variipennis | Y | Y | |
| West | C. gigas | Y | Y | |
| East | C. stellifer | Y | Y | Y (BTV, EHDV) |
| East | C. jamesi | Y | Y | |
| Both | C. biguttatus | Y | Y | Suspected (BTV) |
| West | C. palmerae | Y | Y | |
| East | C. bickleyi | Y | N | |
| East | C. denticulatus | Y | Y | |
| East | C. downesi | Y | Y | Y (Parahaemoproteus parasite) |
| West | C. franclemonti or C. utowana | Y | N | |
| East | C. jamnbacki | Y | Y | |
| East | C. parapiliferus | Y | Y | |
| West | C. kibunensis | Y | Y | |
| Both | C. travisi | Y | Y | |
| | **subgenus/ species groups** | | | |
| Both | subgenus Avaritia | N | N | |
| Both | subgenus Silvaticulicoides | Y | N | |
| Both | subgenus Selfia (other than C. jamesi) | N | N | |
| Both | subgenus Beltranmyia (other than C. wisconsinensis and C. crepuscularis) | N | N | |
| Both | Pilifferus species group (other than C. denticulatus and C. downesi) | N | N | |
| West | Palmerae species group | N | N | |
| West | Cockerelli species group (other than C. cockerelli) | N | N | |
| East | Guttipennis Species group | N | N | |

C

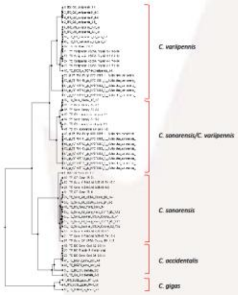


Figure 2. Distribution and Identification of *Culicoides* species in Canada. (A) Comparison of species distribution between Western (left) and Eastern (right) provinces in Canada. (B) Morphological identification of known and suspected *Culicoides* vectors in Canada. (C) Phylogenetic tree representation of the molecular identification of the Subgenus *Monoculicoides* by ITS sequencing.

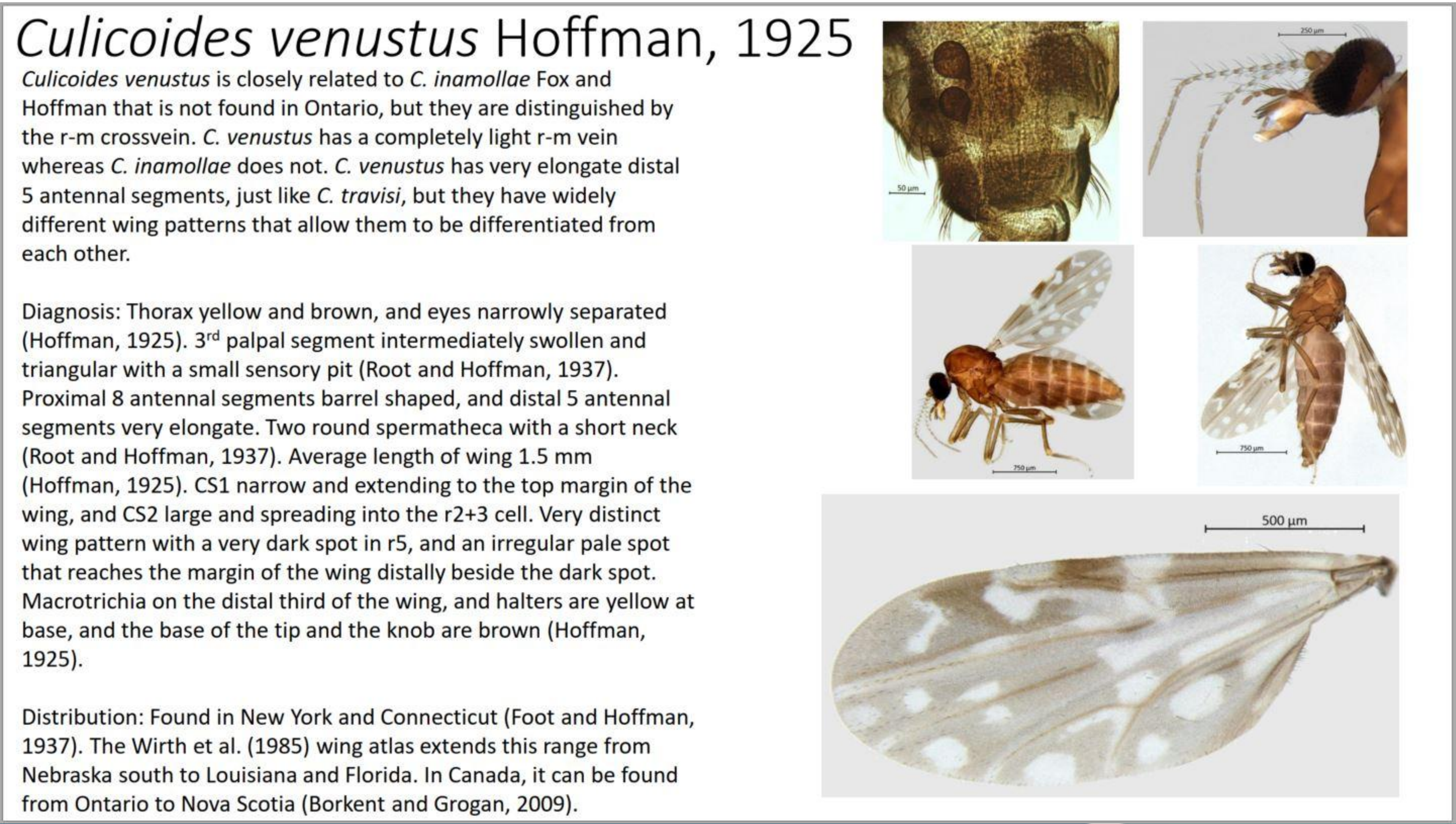
## Conclusions

- A small-scale targeted *Culicoides* survey was conducted across Canada from 2014-2016, resulting in a reference collection of over 113,000 *Culicoides* midges.
- The distribution of *Culicoides* species varied in both species composition and numbers of midges between Western and Eastern Canada.
- Known vector species for BTV and EHDV were identified in both Western and Eastern Canada.
- Morphological identification solely by wing patterns was useful to identify unrelated *Culicoides* species and molecular identification using genetic markers helped to identify closely-related *Culicoides* species.

## Acknowledgments

This research was funded by the Canadian Food Inspection Agency RPS grant LETH-A-1402. The authors would like to thank all the volunteers who helped to set up the traps, collect samples and ship them to the CFIA-Lethbridge laboratory. Authors also thank the support and expertise that SCWDS has provided for this project, particularly in the training and identification/confirmation of specimens.

# *Culicoides* of Ontario (in preparation)



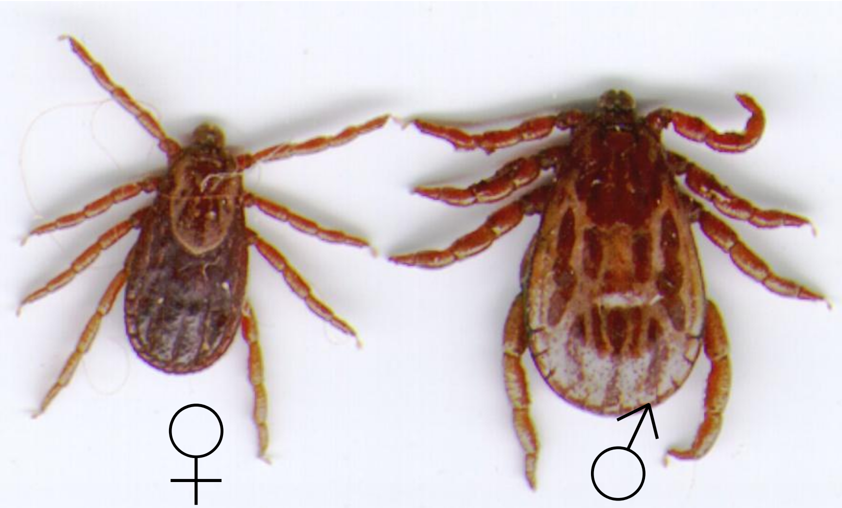
## *Culicoides venustus* Hoffman, 1925

*Culicoides venustus* is closely related to *C. inamollae* Fox and Hoffman that is not found in Ontario, but they are distinguished by the r-m crossvein. *C. venustus* has a completely light r-m vein whereas *C. inamollae* does not. *C. venustus* has very elongate distal 5 antennal segments, just like *C. travisi*, but they have widely different wing patterns that allow them to be differentiated from each other.

Diagnosis: Thorax yellow and brown, and eyes narrowly separated (Hoffman, 1925). 3rd palpal segment intermediately swollen and triangular with a small sensory pit (Root and Hoffman, 1937). Proximal 8 antennal segments barrel shaped, and distal 5 antennal segments very elongate. Two round spermatheca with a short neck (Root and Hoffman, 1937). Average length of wing 1.5 mm (Hoffman, 1925). CS1 narrow and extending to the top margin of the wing, and CS2 large and spreading into the r2+3 cell. Very distinct wing pattern with a very dark spot in r5, and an irregular pale spot that reaches the margin of the wing distally beside the dark spot. Macrotrichia on the distal third of the wing, and halters are yellow at base, and the base of the tip and the knob are brown (Hoffman, 1925).

Distribution: Found in New York and Connecticut (Foot and Hoffman, 1937). The Wirth et al. (1985) wing atlas extends this range from Nebraska south to Louisiana and Florida. In Canada, it can be found from Ontario to Nova Scotia (Borkent and Grogan, 2009).

# Ticks of Canada



## I. Hard Ticks - Ixodidae

33 Species in 4 Genera

*Ixodes* (most), *Dermacentor*, *Haemaphysalis*, *Rhipicephalus*

## II. Soft Ticks - Argasidae

7 Species in 4 Genera

*Argas*, *Carios*, *Ornithordoros*, *Otobius*

**“A Handbook to the Ticks of Canada”**
**FREE download - Biological Survey of Canada**

# Soft Ticks and African Swine Fever Virus

(highly contagious & fatal to pigs)

# African Swine Fever Virus & Ticks

- In Africa, ASFV is maintained in wart hogs and bush pigs and *Ornithodoros porcinus* (also known as *O*. *moubata porcinus*).
- In Spain and Portugal, *O. (Pavlovskyella) erraticus* (also known as *O. maroncanus*) became a biological vector and was able to infect swine for up to 8 years.
- *Ornithodoros* spp. ticks can live for up to 11 years.
- ASFV introduced to the Caribbean region in 1978, where several species of *Ornithodoros* are resident.

From: Butler and Gibbs. 1984. Preventive Vet. Med. 2: 63-70.

# The New Tick in North America

**The Asian Longhorned Tick, *Haemaphysalis longicornis***

- First reported to the USDA in November, 2017 (NJ), but records indicate 2010 (WV)
- In 2020, reported from 12 US states, including NY and PA, and 96 counties
- Origin: E Asia, New Zealand, Australia
- Parthenogenetic: Can reproduce without males, & does so rapidly
- Wide Host Range, including birds, livestock, companion animals, wildlife, people
- Can transmit a wide range of pathogens, including protozoa, bacteria, and viruses
- Implicated in red meat allergy
- Very similar in appearance to the three native *Haemaphysalis* spp.

**Will it come to Canada? Probably, as it has invaded temperate regions of the US, so we could see it appear in Ontario next season.**

# *Haemaphysalis longicornis*
# The Asian Longhorned Tick

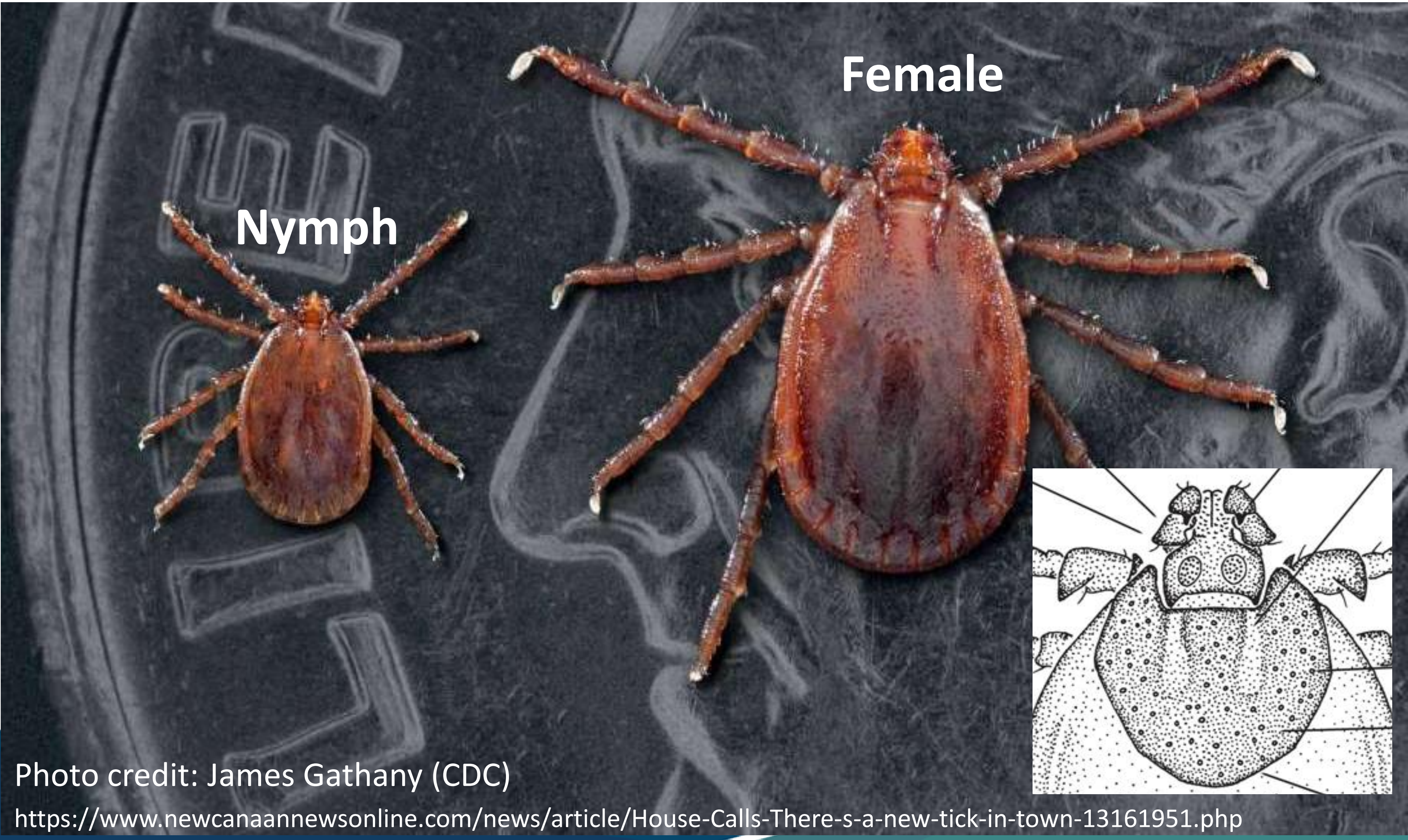


Photo credit: James Gathany (CDC)

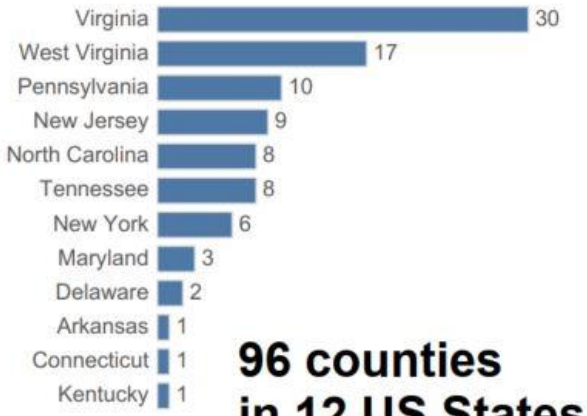
https://www.newcanaannewsonline.com/news/article/House-Calls-There-s-a-new-tick-in-town-13161951.php

# Excerpt from USDA Asian Longhorned Tick Situation Report January 2020

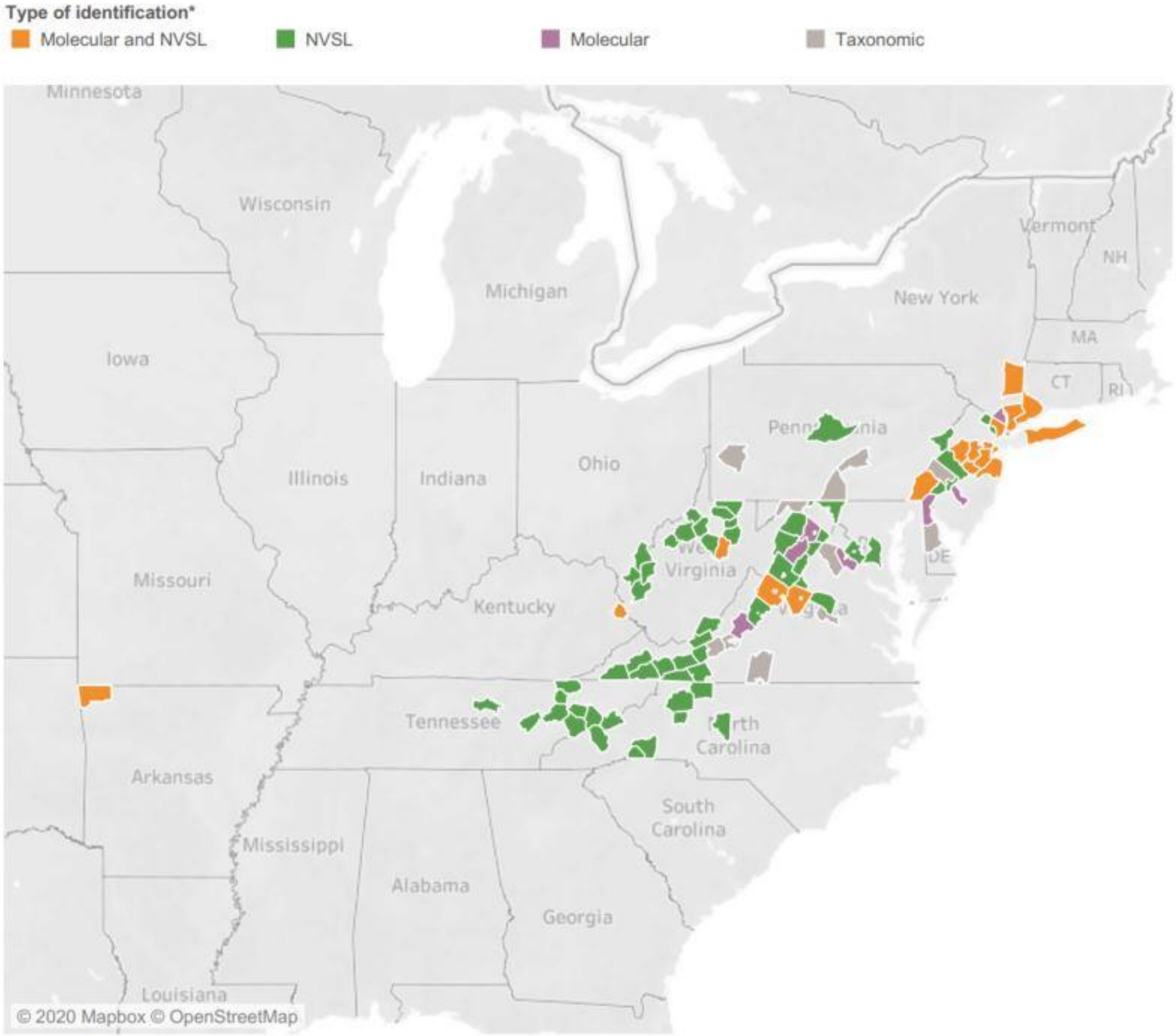
*Haemaphysalis longicornis* (Asian longhorned tick), an exotic East Asian tick, has never previously established a population in the United States. It is a known serious pest of livestock in the Australasian and Western Pacific Regions where it occurs. It is an aggressive biter and frequently builds intense infestations on domestic hosts causing great stress, reduced growth and production, and severe blood loss.

The tick can reproduce parthenogenetically (without a male); as such, a single fed female tick can create a population. It is also a known/suspected vector of several viral, bacterial, and protozoan agents of livestock and human diseases. This three-host tick can spread pathogens among a diverse host range, on which it feeds side-by-side with other tick species. The detections detailed here are the first reports of this tick out of quarantine in the United States.

**States with confirmed local Asian longhorned tick populations** with number of counties in each state



**96 counties in 12 US States**



# Summary

Worldwide, arthropods have key role in transmission of pathogens to livestock, companion animals, wildlife, and humans.

In Canada, the sparsity of competent vector species and discontinuous activity limit vector-borne diseases.

The potential for introduction and establishment of vector-borne foreign diseases is persistent and increasing but remains relatively low (for now).

In multiple scenarios, we have the hosts and competent vectors. All that is missing is the pathogen, so what is the answer? Prevention through Vigilance