

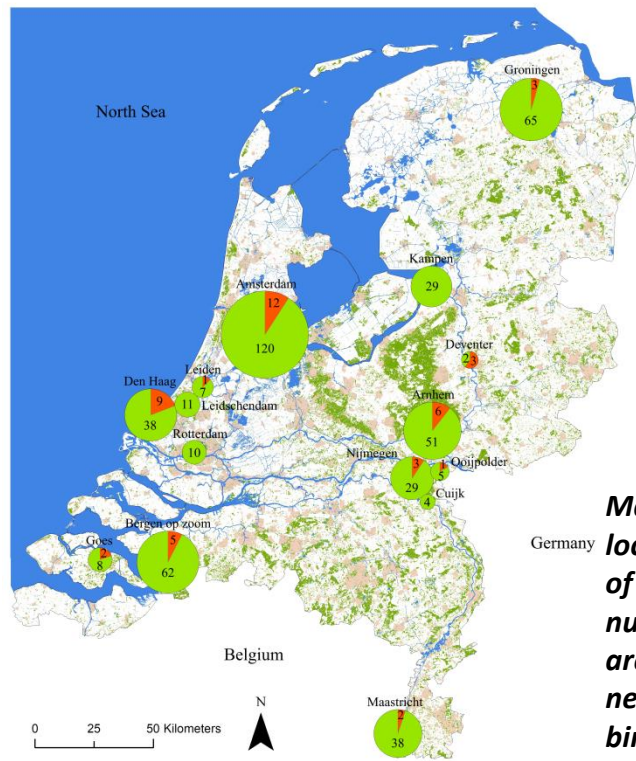
# 18192216/III Molecular epidemiology of trichomonads in wild wetland birds in the Netherlands

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Severe granulomatosis in productive chicken layers due to *Tetratrichomonas gallinarum* strain 13/16632 infection occurred in 2013 (Landman et al. 2016 Avian Pathol) and 2017 (Landman et al. 2019 Vet Quart ) on farms situated in a wetland area in the Netherlands. We hypothesized that wetland birds could be the source of the infection. Therefore, a large-scale prevalence study on trichomonads was performed by analysing cloaca swabs of wetland birds in the Netherlands using molecular methods.



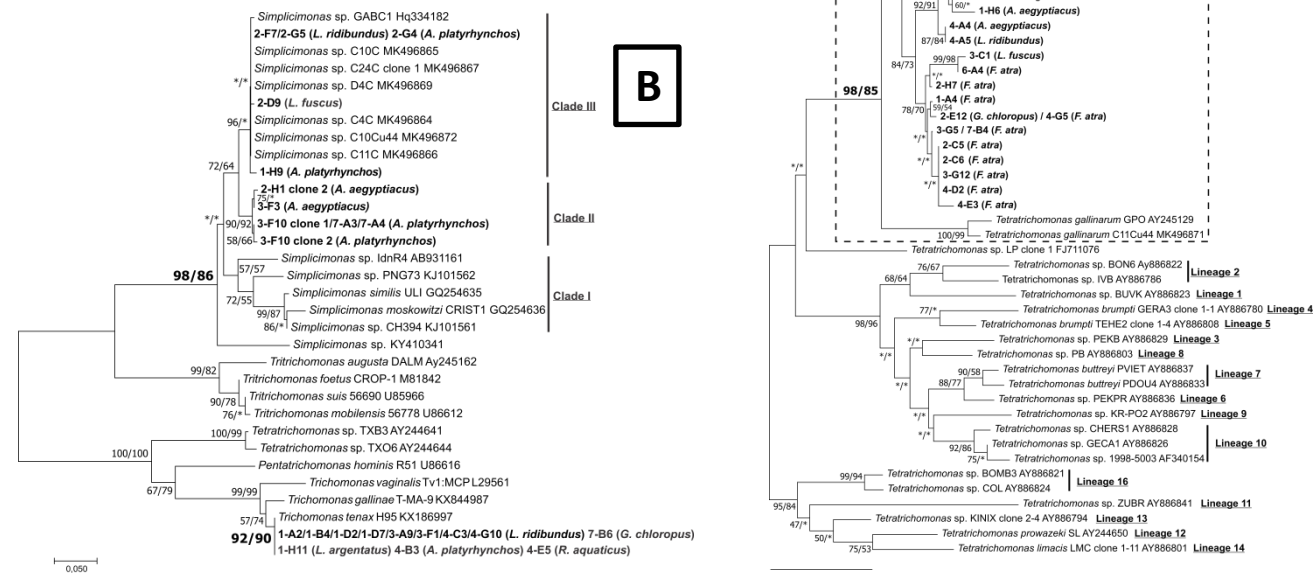
- ✓ Study conducted between August 2019 and March 2020 at 15 locations distributed over the Netherlands
- ✓ Sampling of 526 cloaca swabs from 13 different wetland bird species predominantly captured in city parks and river quays

Map showing the geographic locations of the sampling. The size of circles is proportional to the number of birds caught (green area, number of trichomonad negative birds; red area, number of birds positive for trichomonads)

- Trichomonads frequently occur among wild wetland birds in the Netherlands
- Identification of *T. gallinarum*, *T. tenax* and *Simplicimonas* sp., with a large predominance of *T. gallinarum*
- Some *T. gallinarum* isolates are closely related to a strain causing granulomas in chicken layers
- Some isolates may represent a new species of the genus *Simplicimonas*

DNA extraction from cloaca swabs  
 Screening by nested PCR targeting the ITS1-5.8S-ITS2 gene  
 Direct sequencing or cloning of the positive PCR samples  
 Phylogenetic analyses

- ✓ Overall prevalence of trichomonads of 9% (47/526)
- ✓ Molecular characterization of 55 trichomonad isolates through phylogenetic analyses identified as *Tetratrichomonas gallinarum*, *Trichomonas tenax* and *Simplicimonas*



Unrooted Neighbor-joining trees based on the ITS1-5.8S-ITS2 sequences. A. All tetratrichomonad sequences obtained in this study (in bold) belong to the *Tetratrichomonas* lineage 15 corresponding to *T. gallinarum*. B. All other new trichomonad sequences (in bold) group with *Simplicimonas* and *T. tenax*