First report of *Clavibacter nebraskensis*, causing Goss's bacterial leaf blight on maize (*Zea mays* L.) in South Africa

Sonja Coertze[1,2†], Beatrix Coetzee[2,3], Elaine Basson[1,2], Doré de Villiers[1,2], Tjitjila Makhura[4], Diane Mostert[2], Bernard Slippers[4], Lindy J. Rose[2], Cobus M. Visagie[4], David Read[4]

1 Plant Disease Clinic, Department of Plant Pathology, Stellenbosch University, Stellenbosch, 7600, South Africa

2 Department of Plant Pathology, Stellenbosch University, Stellenbosch, 7600, South Africa

3 School for Data Science and Computational Thinking, Stellenbosch University, Stellenbosch, 7600, South Africa

4 Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0028, South Africa

† Corresponding author: Sonja Coertze sc2@sun.ac.za

Keywords: Molecular identification, bacterial pathogen, whole genome sequencing, disease surveillance

S. Coertze
Plant Disease

Maize is a staple crop in South Africa and an important income source to both smallholder and commercial farmers (Gravelet-Blondin, 2015). Goss's bacterial leaf blight and wilt, caused by *Clavibacter nebraskensis* (Cn), is a significant maize disease in North America and a quarantine concern in unaffected regions, with seedborne transmission posing a risk of introduction (EPPO, 2024; Osdaghi et al. 2023). From February to April 2024, bacterial leaf blight symptoms, typical of Cn infection, were observed on maize (*Zea mays* L.) in the North-West, Mpumalanga and Gauteng provinces of South Africa. Lesions were tan, irregular, parallel to veins, with a shellac-like appearance and black water-soaked edges, showing characteristic "luminous freckles" when backlit. Symptomatic leaf samples were collected from 6 commercial maize fields. Eight samples from Carletonville and Potchefstroom (total of two fields) were evaluated at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, and another four samples from Delmas, Leslie, and Bapsfontein (total of four fields) were evaluated at Stellenbosch University's Plant Disease Clinic. DNA was extracted either directly from lesions or from cultures isolated from lesions. For direct DNA extraction, cetyltrimethylammonium bromide was used, followed by a Cn specific PCR with primer pair 1184F/R (McNally et al. 2016). Macerates from lesion edges were streaked out onto nutrient broth yeast (NBY) agar. DNA from a single culture, with yellow-orange mucoid colonies, was extracted with a Zymo Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA), and confirmed as Cn with previously mentioned PCR primers. Simultaneously, macerates from the lesion edges were streaked onto yeast dextrose chalk agar (YDC). Yellow-orange mucoid colonies developed after four day and were purified onto NBY and incubated at 25°C for 4 days. All isolates tested gram-positive, were coryneform, aerobic, and non-spore forming. Genomic DNA was extracted and the suspension amplified using the

27F/1492R primer pair (Lane, 1991), targeting the 16S rRNA gene. The product was sequenced and confirmed as Cn. Cultures are stored in the culture collections at Stellenbosch University Plant Pathology Department (STE-U) and at FABI (CMW and CMW-IA). At both facilities, cell suspensions at a final concentration of 10^7 cells/mL were used to inoculate the third leaf of V3 / V4 stage maize plants (P1513, Syngenta), by wounding the middle of the main leaf vein and applying a 25µL droplet. Typical Cn symptoms appeared 4 days post inoculation and Cn was reisolated from these lesions and confirmed with PCR to complete Koch's postulates. Four isolates were selected for high-throughput sequencing (NCBI Bioproject: PRJNA1184689). Assembled genomes (NCBI accession: CP173672–CP173675) were analysed on the Type Strain Genome Server (Meier-Kolthoff and Göker, 2019) and confirmed as Cn based on 16S rRNA and Genome Blast Distance Phylogeny. The genomes aligned to the Cn type strain NCPPB 2581 with 99.8% DNA-DNA hybridization on the GGDC 3.0 server (Meier-Kolthoff et al. 2022), exceeding the suggested 70% species threshold. Phylogenomic analysis based on Average Nucleotide Identity values also clustered these genomes with Cn isolates. This is the first report of this pathogen outside of North America.

References

EPPO. 2024. Available at: https://gd.eppo.int/taxon/CORBNE (accessed on 8 November 2024)

Gravelet-Blondin, R. Available at: https://sacota.co.za/wp-content/uploads/Prospectus-on-the-South-African-Maize-Industry (accessed on 8 November 2024)

Lane, D.J.1991. In: Stackebrandt E,Goodfellow M (eds). Wiley, New York, pp 115–175.

McNally, R.R., et al. 2016. Phytopathology 106(12):1465-1472. doi.org/10.1094/PHYTO-05-16-0190-R

Meier-Kolthoff, J.P. and Göker, M. 2019. Nature Communications 10: 2182. doi.org/10.1038/s41467-019-10210-3

Meier-Kolthoff, J.P., et al. 2022. Nucleic Acid Research 50: D801–D807. doi: 10.1093/nar/gkab902

Osdaghi, E., et al. 2023. Molecular Plant Pathology 24: 675–692. doi.org/10.1111/mpp.13268

Supplementary tables

Supplementary table 1: Sequence output and assembly statistics for the one isolate sequenced with DNBSEQ and three isolates sequenced using Oxford Nanopore Technologies.

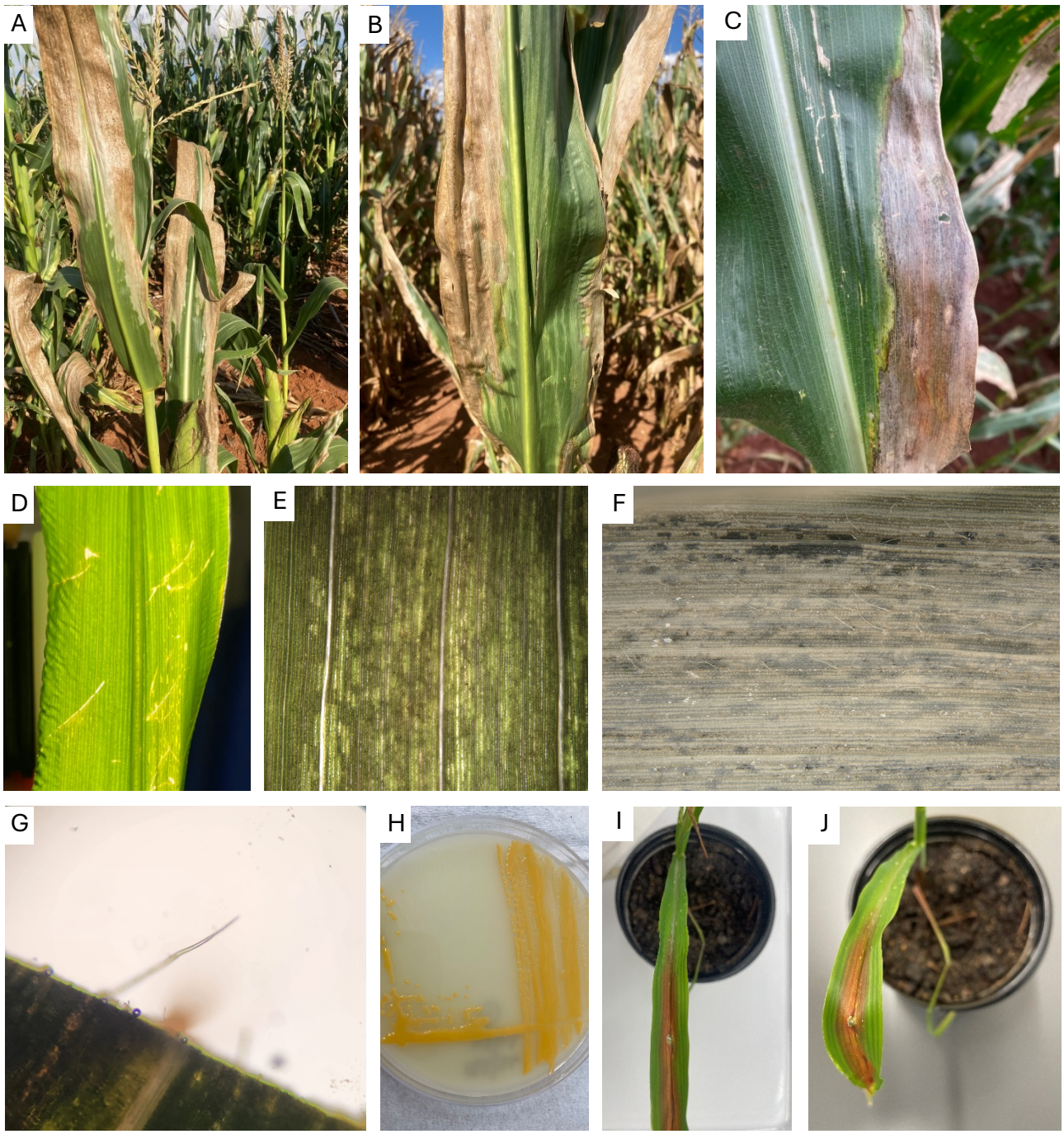
At FABI, DNA from a single culture (CMW:64157 = CMW-IA:6965) previously extracted with a Zymo Quick-DNA Miniprep Kit was used to prepare a sequencing library using the MGIEasy Universal DNA Library Prep Set and sequenced using a DNBSEQ-G400 sequencer (MGI, Shenzhen, China). A total of 264M pre-trimmed reads were generated (NCBI Bioproject nr: PRJNA1184689). Reads were trimmed using fastp (Chen et al. 2018) and assembled using SPAdes (Bankevich et al. 2012). Assembled contigs were aligned to the reference type strain of Cn, strain NCPPB 2581 (NCBI accession no: NC_020891) in D-genies v1.2.0 (https://dgenies.toulouse.inra.fr/; Cabanettes and Klopp, 2018) to build a single genome scaffold of 3,069,167 nt in length (NCBI accession nr: CP173672).

Three representatives (STE-U:9948, STE-U:9949 and STE-U:9951) of the cultures prepared at the Plant Disease Clinic were selected and plated onto NBY and grown for 5 days at 25°C before DNA extraction with the Wizard® Genomic DNA Purification Kit (Madison, USA). The quantity of the DNA was confirmed by Qubit fluorometer. A total of 400ng of DNA per isolate were prepared for sequencing according to the manufacturer's instructions using the Oxford Nanopore Ligation Sequencing kit V14. The prepared libraries were sequencing on the Oxford Nanopore MinION for 72 hours, generating between 85 and 162 Mbases data per isolate (NCBI Bioproject nr: PRJNA1184689). Reads were assembled using Flye v. 2.9.2 (Kolmogorov et al. 2019). Single contigs of more than 3 MBases in length was retrieved for each of the isolates (NCBI accession nrs: CP173673, CP173674 and

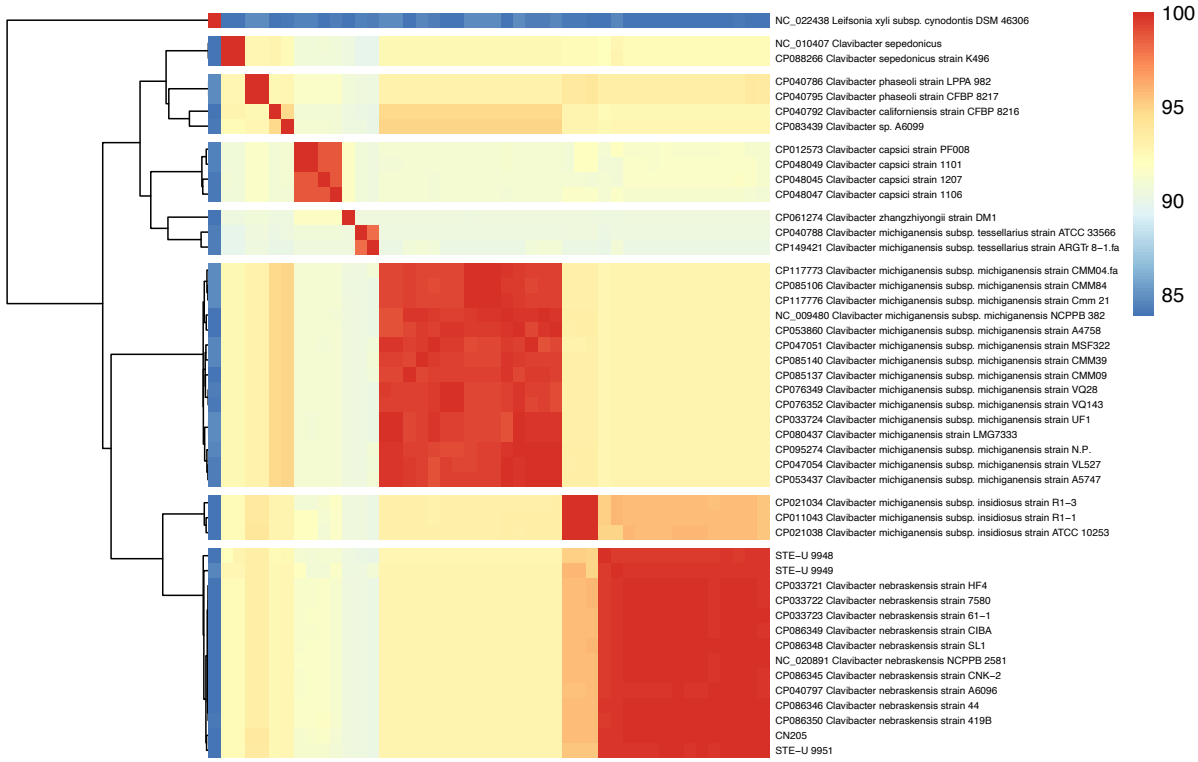
CP173675), and annotated with between 2,896 and and 2,911 coding sequences, 6 rRNAs, 45 tRNAs and 1 tmRNA, using Prokka (Seeman, 2024). This length is comparable to the 3,063,596 nt genome length of the Cn type strain.

Isolate	Accession	Number of reads	Amount of data (nt)	Assembly length (nt)	Total gap length (nt)	Average depth of coverage[a]
CMW-IA:6965	CP173672	264,941,614	39,741,242,100	3,069,167	6 (2513)	170
STE-U:9948	CP173675	23,777	85,431,921	3,063,813	-	14
STE-U:9949	CP173674	28,388	108,694,858	3,063,173	-	21
STE-U:9951	CP173673	34,407	162,823,191	3,063,824	-	36

[a] Average number of reads that contribute to each nucleotide in the assembly.



Supplementary Figure S1: Goss's bacterial wilt of maize caused by *C. nebraskensis* **A – C:** Cn on naturally infected maize leaves. **D:** Freckles characteristic of Cn infection **E:** Freckles under magnification with back lightning. F: Freckles under magnification with top lightning. **G:** Bacterial streaming from vein of naturally infected leaf. **H:** Cn culture purified on YDC. **I and J:** Maize leaves artificially inoculated with Cn.



Supplementary Figure S2: Hierarchical clustering of the four genomes generated in this study and 41 *Clavibacter* genomes available on NCBI. Average Nucleotide Identity (ANI) values are represented by the colour scale.

Complete genomes of 41 *Clavibacter* isolates, and one outgroup (*Leifsonia xyli* subsp. *cynodontis*) were downloaded from NCBI. ANI indices were calculated for each genome pair (Li et al. 2018), including the four South African genomes, on the JSpeciesWS server (Richter et al. 2015). The four assembled genomes had ANI values greater than 99.6% (average 99.76%) compared to *C. nebraskensis*, which are greater than the suggested species delineation threshold of 95% (Richter and Rosselló-Móra, 2009). ANI values were used for hierarchal clustering of genomes in the R package *pheatmap* (Kolde, 2019).

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko S.I., Pham S., Prjibelski, A.D., Pyshkin, A.V, Sirotkin, A.V, Vyahhi N., Tesler, G., Alekseyev, M.A. and Pevzner, P.A. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19: 455–477. doi.org/ 10.1089/cmb.2012.0021

Cabanettes, F. and Klopp, C. 2018. D-GENIES: dot plot large genomes in an interactive, efficient and simple way. PeerJ 6:e4958 doi.org/10.7717/peerj.4958

Chen, S., Zhou Y., Chen, Y., Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34(17): i884–i890 doi.org/10.1093/bioinformatics/bty560

Kolde, R. 2019. Pacakage “pheatmap”. https://CRAN.R-project.org/package=pheatmap

Kolmogorov, M., Yuan, J., Lin, Y. and Pevzner, P.A. 2019. Assembly of long, error-prone reads using repeat graphs. Nature Biotechnology 37:540–546. doi.org/10.1038/s41587-019-0072-8

Li, X., Tambong, J., Yuan, K.X., Chen, W., Xu, H., Lévesque, C.A. and De Boer, S.H. 2018. Re-classification of *Clavibacter michiganensis* subspecies on the basis of whole-genome and multi-locus sequence analyses. International Journal of

Systematic and Evolutionary Microbiology 68:234–240.
doi.org/10.1099/ijsem.0.002492

Richter M. and Rosselló-Móra, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. PNAS 106(45): 19126 –19131.
doi10.1073pnas.0906412106

Richter, M., Rosselló-Móra, R., Glöckner, F.O. and Peplies, J. 2015. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32(6): 929–931. doi.org/10.1093/bioinformatics/btv681

Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068 –2069. doi.org/10.1093/bioinformatics/btu153.