Detection of a novel *Puccinia coronata* f. sp. *avenae* genotypic lineage in Australia during the 2024 oat growing season

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Funding: Grains Research and Development Corporation (GRDC) and CSIRO, project grant CSP2204-007RTX.

Abstract

Crown rust, caused by *Puccinia coronata* f. sp. *avenae* (*Pca*), remains a persistent threat to oat production in Australia. To monitor recent shifts in virulence and population structure, 30 *Pca* isolates collected during the 2024 growing season across major Australian oat-producing regions were analysed. Phenotypic analysis of 30 isolates using 52 oat differential lines identified 27 unique races. Whole-genome sequencing of 28 of these isolates revealed that most of them belonged to previously established lineages, with L18 and L16 being dominant. Notably, L16 was again present in Western Australia after being undetected in 2023, while L18 maintained its prevalence for a third consecutive season. Beyond these dominant groups, phylogenetic analysis also identified a novel and genetically distinct lineage, designated as L19, and represented by one isolate collected in WA. Although L19 exhibited a virulence profile similar to other WA isolates, its unique genomic signature underscores the need to integrate phenotypic and genomic surveillance. These findings enhance understanding of *Pca* diversity and inform resistance breeding and disease management strategies in Australian oats.

Keywords: crown rust, virulence, collection, lineage, oat

Manuscript body

Oat crown rust (OCR), caused by *Puccinia coronata* f. sp. *avenae* (*Pca*), is the most destructive foliar disease for oat production worldwide (Nazareno et al. 2018). The disease typically brings grain yield losses of 10% - 40% under epidemic conditions (Nazareno et al. 2018). In a severe outbreak, yield losses have reached as high as 50% (USDA-ARS CDL 2014). In addition to reducing yield, the disease also affects grain quality and fodder value (Simons 1985), posing persistent challenges to both milling and forage oat production in Australia and other countries. While breeding for host resistance has proven to be effective (McDonald and Linde 2002), the durability of resistance genes is impacted by evolution of virulence in the *Pca* population (Carson 2011; McCallum et al. 2007; Moreau et al. 2024). Therefore, ongoing surveillance to detect changes in population structure and virulence profiles remains an essential component of OCR management strategies (Nazareno et al. 2018).

In Australia, routine surveys are conducted to monitor *Pca* virulence patterns and evaluate the effectiveness of *Pc* resistance genes (Henningsen et al. 2024a; Nguyen et al. 2025; Park et al. 2025). The high phenotypic diversity among *Pca* isolates is well-supported, with dozens of unique races identified each year (Henningsen et al. 2024a; Nguyen et al. 2025; Park et al. 2025). These findings underscore the dynamic nature of the *Pca* population. So far, a total of 18 genotypic lineages of *Pca* based on genome-wide variants have been characterised using 137 samples collected between 2020 and 2023 (Henningsen et al. 2024b). This framework enables a consistent lineage assignment and together with extensive haplotype resolved pangenome resources (Henningsen et al. 2024b, Henningsen et al., 2025) supports epidemiological inferences about migration and genetic recombination of *Pca* in Australia. Furthermore, a recent study highlights the need to complement phenotype-based assessments with genomic data as *Pca* isolates with similar virulence profiles can belong to genetically distinct lineages (Henningsen et al. 2024b). Therefore, full characterisation of the phenotypic and genotypic variability of *Pca* is critical to optimise genetic resistance deployment and understanding phenotypic shifts in the pathogen population that can impact the oat industry.

In this study, we report the virulence phenotypes and whole genome genotype assignments of *Pca* isolates collected during the 2024 growing season. Through a national network of

plant pathologists and industry representatives, we obtained a total of 38 samples of *Pca*. These samples originated from New South Wales (*n*=7), Victoria (*n*=2), South Australia (*n*=5) and Western Australia (*n*=24) and were collected from wild oats (*n*=36), an unknown cultivated line (*n*=1), and an oat cultivar present in a field trial at Wagga Wagga, NSW. We note the lack of *Pca* sampling from Queensland, and low representation from New South Wales and Victoria, likely due to dry weather conditions (Bureau of Meteorology 2025, https://www.bom.gov.au/climate/maps/rainfall). Of the 38 *Pca* samples, 30 (NSW=6, VIC=2, SA=5 and WA=17) were revived after inoculation on the highly susceptible cultivars Swan and Marvelous following standard protocols (Miller et al. 2020). We proceeded with single pustule purification keeping purified *Pca* isolates for long term storage at -80°C (Miller et al. 2020; Henningsen et al. 2024b).

Virulence phenotypes of the 30 revived *Pca* isolates from the 2024 collection were determined using a panel of 52 differential lines as previously described (Henningsen et al. 2024a; Nguyen et al. 2025) (**Supplemental Table 1, Figure 1A**). A total of 27 unique races according to the 10-letter race coding system (Carson 2011; Chong et al. 2000; Nazareno et al. 2018) were identified (**Table 1**), confirming previous observations of the high diversity of virulence phenotypes for *Pca* in Australia. Two races were represented by more than one isolate: "BFBGMBDCG-" consisting of three isolates: 24WA13, 24WA14, and 24WA22 and " BJLQCCDCG-" consisting of two isolates: 24WA10 and 24WA23. We compared the distribution of virulence scores for the 2024 *Pca* isolates with those from previous years (Nguyen et al., 2025; Henningsen et al., 2024a) (**Supplemental Figure 1**). Overall, the collection from 2024 shows a shift toward less virulence than in the previous years 2022 and 2023 (p-value <0.001, Wilcoxon rank sum test) with 26 out of 52 oat differential lines showing less infection scores in 2024. The oat differential lines that were susceptible to the most isolates in this collection include Pc45 (*n*=29), Pc54 (*n*=29), Pc60 (*n*=26), Pc70 (*n*=25), and Pc46 (*n*=23). The oat lines Barcoo and Pc63 remained resistant to all isolates across these seasons, and observations from 2024 support low virulence for several oat lines such as Pc58, Pc59, Pc63 and WI X4361-9 mentioned in previous years (Nguyen et al., 2025; Henningsen et al., 2024a).

To further explore population structure of the 2024 *Pca* isolates, whole-genome sequencing (WGS) data was generated. Genomic DNA was extracted for each isolate from 20–40 mg

urediniospores using the OmniPrep Genomic DNA Isolation Kit (G-Biosciences) and sequencing libraries were prepared using the Illumina DNA PCR-Free Library Preparation Protocol. Sequencing on an Illumina NovaSeq X platform (150 bp paired-end reads, 15–30× coverage) was completed at the Biomolecular Resource Facility, Australian National University (Canberra, Australia). Two isolate samples (24SA30 and 24NSW39) did not pass WGS quality filters and the remaining 28 samples were retained for the subsequent analysis (NSW=5, VIC=2, SA=4, WA=17) (**Supplemental Table 2)**. A Maximum Likelihood phylogenetic analysis was conducted as described previously (Henningsen et al. 2024b) on a total of 380 isolates including the 28 newly sequenced isolates and a reference panel of 352 previously sequenced isolates collected in Australia (2020 to 2023 collections), the United States of America (USA), South Africa, and Taiwan (Henningsen et al. 2024b; Hewitt et al. 2024; Miller et al. 2020) (**Figure 2**). The inclusion of *Pca* datasets from other continents allowed a comparative genomic framework to place the 2024 *Pca* isolates within both national and global contexts (Henningsen et al., 2024b). The Australian collection continues to present drastic differentiation from other countries. Most *Pca* isolates from 2024 clustered within the previously established genotypic lineages (L1 to L18). A novel lineage, designated as L19, was identified from a single isolate (24WA15) collected from WA (**Table 1, Figure 2A,B; Figure 3**). The isolate 24WA15 formed a distinct branch outside of the 18 previously defined lineages (L1–L18), with high bootstrap support (≥ 80%) and a relatively long branch length. Pathogenicity assays showed that 24WA15 is virulent on oat lines Pc40, Pc45, Pc46, Pc54, Pc60, Pc62, Pc64, Pc67, Pc70 and IA B605Xsel in addition to the susceptible control oat line Marvelous (**Figure 1A, Table 1**). We conducted a *k*-mer containment analysis as described before (Henningsen et al. 2024b, Sperschneider et al. 2023). Briefly, thresholds for haplotype containment were defined as ≥ 99.98 % *k*-mer identity and ≥ 99.65 % shared *k*-mers (**Supplemental Table 3**). We determined that L19 does not present any of the previously nuclear haplotypes present in the current pangenome (Henningsen et al.,2024b; Henningsen et al., 2025). Given that much of Australian genetic diversity of *Pca* descends from recombination between L3 and L18 (Henningsen et al. 2024b), we anticipate L19 represents an additional recombinant lineage, although its precise origin requires further analyses of haplotype assembly, recombination blocks and ancestry signals. Future expansion of the *Pca* pangenome should therefore include 24WA15.

We also assessed the distribution and frequency of genotypic lineages across Australia in the 2024 and compared to the complete *Pca* collection (2022-2024) (**Figure 3A,B**). As illustrated, L18 was found in all surveyed regions and accounted for 29% (8 of 28 total) of samples (**Figure 1A,B; Figure 3**), consistent with our previous report of its dominant presence in both 2022 and 2023 (Henningsen et al. 2024b). Although L1 has previously been reported across all regions, in 2024 L1 was only sampled in NSW. We also note the representation of L18 and L11 in NSW and only L18 in VIC. We only detected three genotypic lineages in South Australia (L9, L16, L18). WA harbors most of the genetic diversity, with nine lineages being sampled in 2024. Although L16 was not reported 2023 after being detected in 2022, the collection of 2024 showed its prominent presence, particularly in WA, where it accounted for approximately one-third (9 of 28 total, and 8 of 17 from WA) of the sequenced isolates. The detection of L19 adds further evidence that the *Pca* population in WA harbors extensive genetic diversity that we have not fully captured. Whilst the 2024 *Pca* collection reveals some changes in pathogen diversity both at the phenotypic and genotypic level we note the sampling limitations and biases that should be considered before concluding this is due to shifts in host-related selection pressures. Future research will determine whether L19 is a short-lived variant or a genetically distinct lineage with the capacity to persist and spread across regions.

Together, our findings highlight the value of using the *Pca* haplotype pangenome as a resource to enhance pathogen monitoring. This study demonstrates the power of coupling phenotypic and genotypic data in advancing our understanding of the evolution of *Pca* population in Australia. Future surveillance efforts, particularly in WA, will be key to assess the completeness of the current assignment of genotypic lineages and support breeding programs.

Data availability

Genomic short reads are available on the CSIRO Data Access Portal at https://data.csiro.au/collection/csiro:69616. Data underlying summary statistics and scripts used to perform analyses and generate figures are available on GitHub https://github.com/Renee-SU/Pathotyping_2024.

Acknowledgments

We thank Allan Rattey (InterGrain), Kylie Chambers (DPIRD, WA), Andrea Hills (DPIRD, WA), Jason Bradley (DPIRD, WA), Joel Kidd (DPIRD, WA), Brad Baxter (New South Wales Department of Primary Industries), Michael Ayliffe (CSIRO, ACT) for contributing samples to the 2024 collection used in this study.

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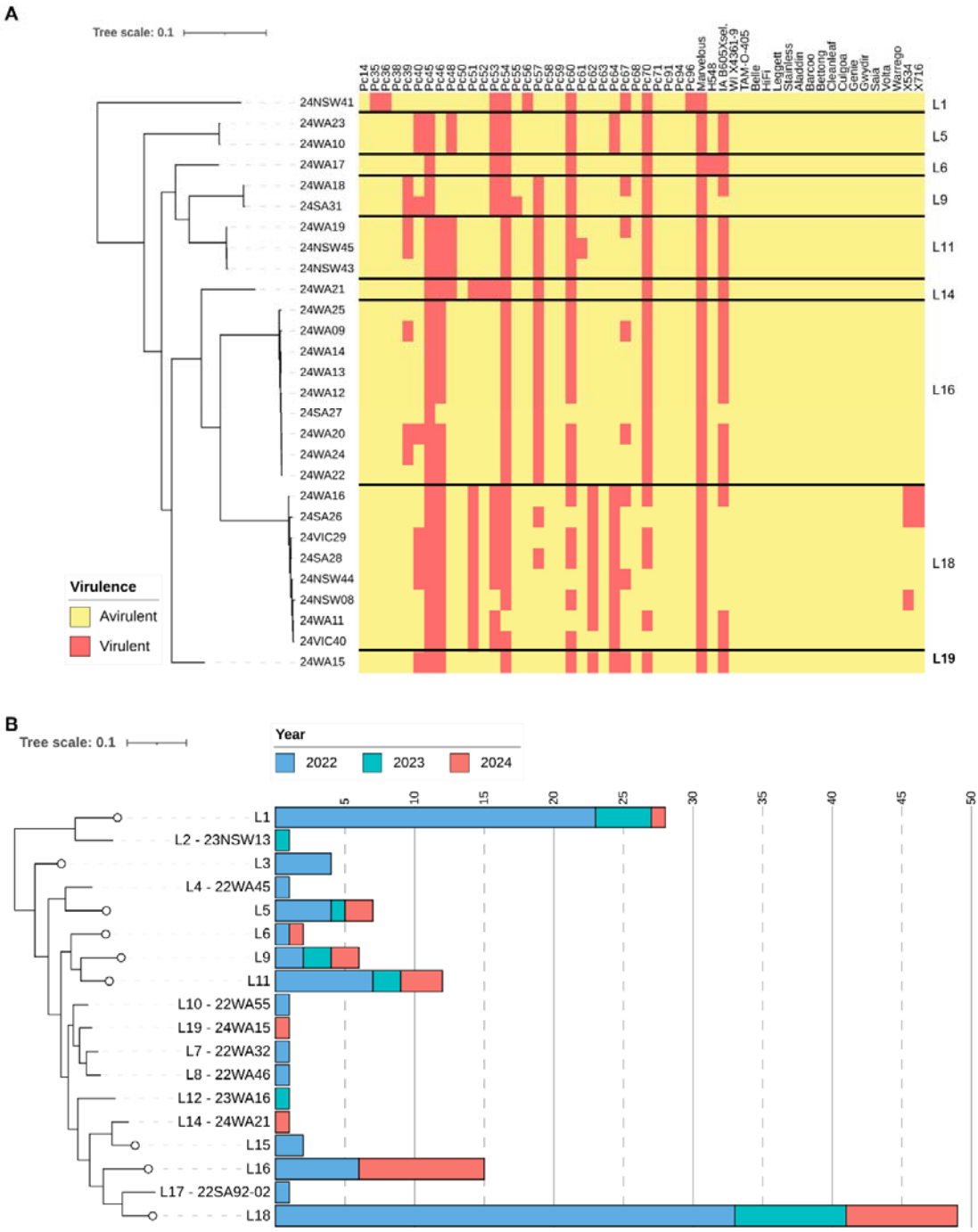


Fig. 1. Australian *Pca* isolates collected in 2024. **A**. Heatmap showing virulence profiles of *Pca* isolates collected in 2024 (x-axis) on the oat differential lines (y-axis). High infection scores indicating high virulence (susceptibility) are shown in red, and lower infection scores indicating avirulence (resistance) are shown in yellow. WA = Western Australia, SA = South Australia, VIC = Victoria and NSW = New South Wales. **B.** Bar plots showing number of *Pca*

isolates collected from 2022 to 2023 (Henningsen et al., 2024a; Nguyen et al., 2025) and this study that have been sequenced and assigned to genotypic lineages (Henningsen et al., 2024b).

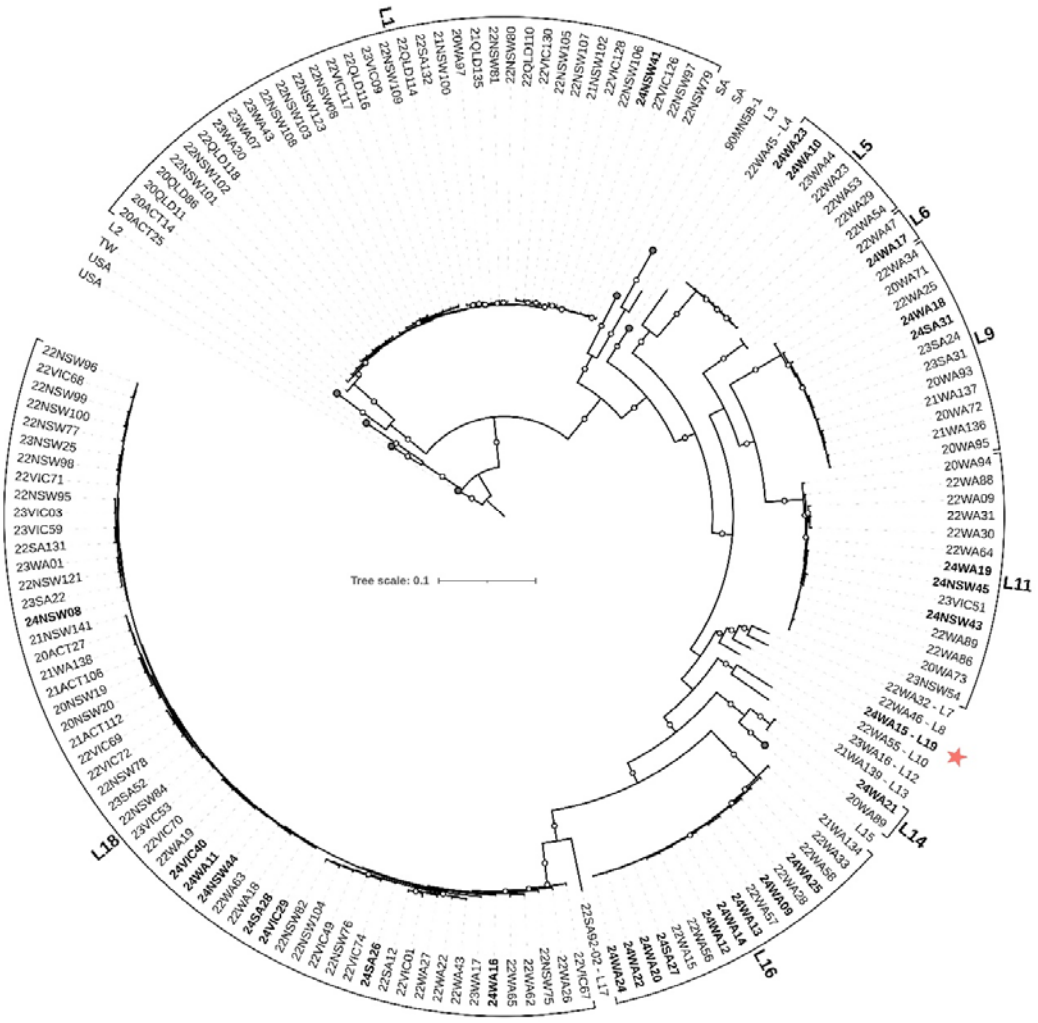


Fig 2. Phylogenetic tree of 380 *Pca* isolates worldwide. A midpoint-rooted maximum likelihood tree was constructed using 396,452 biallelic SNPs, with 500 bootstrap replicates. Bootstrap support values ≥ 80% are indicated by white circles. Isolates collected in 2024 are shown with bold text. Previously characterised Australian lineages (L1–L18) are shown with brackets. The newly described L19 is shown with a pink coloured star. Scale bar represents mean substitutions per site.

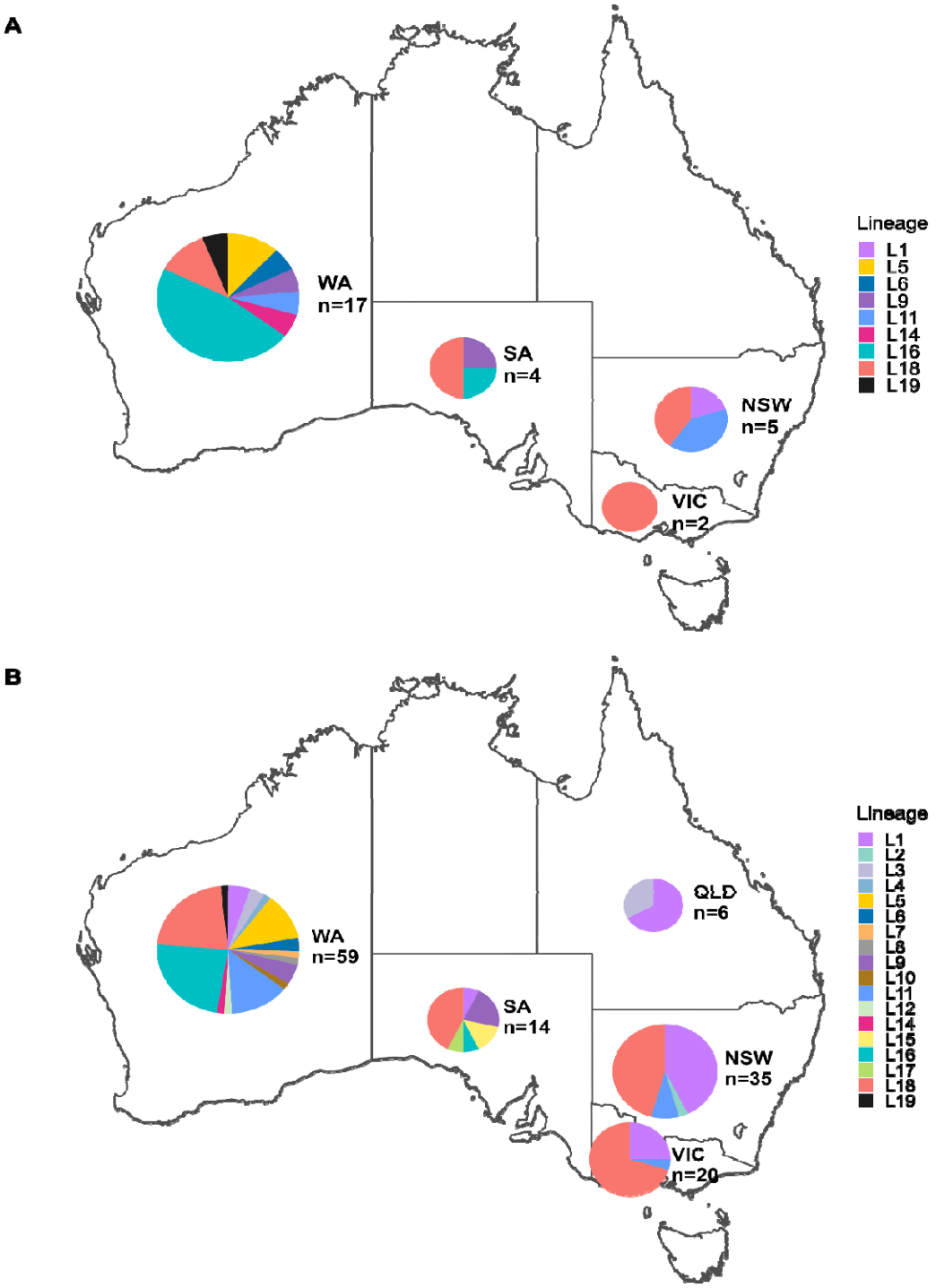
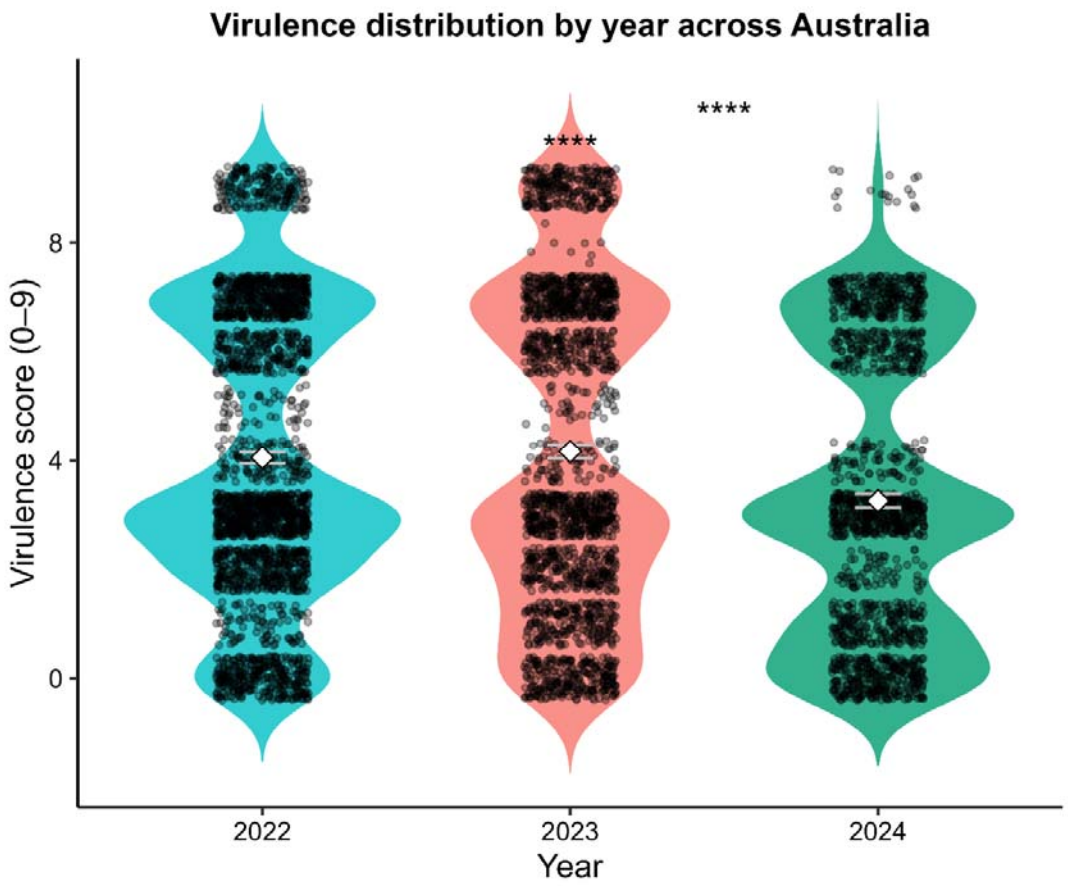


Fig. 3. Distribution and number of *Pca* isolates and lineages across Australia. **A.** Collection of *Pca* in 2024. **B.** Collection of *Pca* including samples from 2022 to 2024. Pie chart sizes reflect a logarithmic transformation of the total number of *Pca* isolates collected in their respective states. The pie chart for NSW includes isolates from both regions. Colors reflect genotypic lineage assignments as described by Henningsen et al. 2024b.



Supplemental Figure 1. Distribution of infection scores of *Pca* isolates by year against oat differential lines. A box plot was drawn inside each violin plot to indicate median and inter-quartile range values. *p*-value was calculated using the Wilcoxon rank-sum test. **** indicates a highly significant difference ($p < 0.001$).

Supplemental Table 1. Virulence scores of each *Pca* isolate collected in 2024 across the oat differential set.

Supplemental Table 2. Isolate metadata and sequencing information produced in this study.

Supplemental Table 3. Haplotype *k*-mer containment from *Pca* Illumina data.

Table 1. List of *Puccinia coronata* f. sp. *avenae* (*Pca*) isolates collected across Australia in 2024.

Isolate	City	State	10-letter race (pathotype)	Number of susceptible oat differential lines	Genotypic lineage number (L)
24WA14*	Perth	VIC	BFBGMBDCG-	8	L16
24WA22*	Calingiri	WA	BFBGMBDCG-	8	L16
24WA13*	Perth	WA	BFBGMBDCG-	8	L16
24WA10**	Quairading	WA	BILQCCDCG-	10	L5
24WA23**	Meckering	WA	BJLQCCDCG-	10	L5
24WA25	Doodlakine	WA	BFBGMBDCG	7	L16
24WA12	Narrogin	WA	BFBG-BDCG-	7	L16
24NSW41	Gerogery	NSW	BBBOBBNFB-	10	L1
24SA27	Hamley Bridge	SA	BDBGLBDCB-	5	L16
24WA17	Myrup	WA	BDBQCBDCQ-	8	L6
24NSW08	Boorowa	NSW	BFDGCHBCB-	9	L18
24WA11	Merridin	WA	BFDLBHDCG-	9	L18
24VIC40	Barnawartha	VIC	BFDQCCBCG-	9	L18
24WA16	Scaddan	WA	BFDQCHNCG-	14	L18
24SA26	Freeling	SA	BFDQLHBCB-	11	L18
24NSW43	Dhulura	NSW	BFLGMBDCG-	8	L11
24WA21	Moora	WA	BFPQMBDCG-	12	L14
24WA15	Lort River	WA	BKBGCHNCG-	11	L19
24NSW44	Jugiong	NSW	BKDQBHLCB-	10	L18
24SA28	Hahndorf	SA	BKDQMHDCB-	12	L18
24WA24	Cunderdin	WA	BPBGMBDCG-	9	L16
24WA09	Gwambygine	WA	BPBGMBNCG-	10	L16
24WA19	West River	WA	BPLGMBNCG-	11	L11
24NSW45	Wagga Wagga	NSW	BPLGMLDCG-	11	L11
24VIC29	Mitre	VIC	B--QCHDCB-	10	L18
24SA31	Williamstown	SA	BSBSMBDCB-	10	L9
24WA20	Northam	WA	BTBGMBNCG-	11	L16
24WA18	Ravensthorpe	WA	-NBQMBNCG-	10	L9
24SA30	Bordertown	SA	BFNGMHDCB-	11	-
24NSW39	Holbrook	NSW	BKDQMCBCB-	11	-

* and ** indicate isolates with identical race codes, while – indicates samples lacking Illumina sequencing data.