Population structure of *Pyrenophora teres* f. *teres* barley pathogens from different continents

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ABSTRACT

Net-form net blotch disease caused by *Pyrenophora teres* f. *teres* (*Ptt*) results in significant yield losses to barley industries. Up-to-date knowledge of the genetic diversity and structure of pathogen populations is critical for better understanding the disease epidemiology and unravelling pathogen survival and dispersal mechanisms. Thus, this study investigated long distance dispersal and adaptation by analysing the genetic structure of 250 *Ptt* isolates collected from Australia, Canada, Hungary and Republic of South Africa (RSA), and historical isolates from Canada, Denmark, Japan and Sweden. The population genetic structure detected by discriminant analysis of principal component, using 5890 Diversity Arrays Technology (DArT) markers, revealed the presence of four clusters. Two of these contained isolates from all regions, and all isolates from RSA were grouped in these two. Australia and Hungary showed three clusters each. One of the Australian clusters contained only Australian isolates. One of the Hungarian clusters contained only Hungarian isolates and one Danish isolate. STRUCTURE analysis indicated that some isolates from Australia and Hungary shared recent ancestry with RSA, Canada and historical isolates and were thus admixed. Subdivisions of the Neighbor-joining network indicated that isolates from distinct countries were closely related, suggesting multiple introduction events conferred genetic heterogeneity in these countries. Through a Neighbor-joining analysis and amplification with form-specific DNA markers two hybrid isolates, CBS 281.31 from Japan and H-919 from Hungary collected in 1931 and 2018, respectively, were detected. These results provide a foundation for exploring improved management of disease incursions and pathogen control through strategic deployment of resistances.

Keywords: Australia, Canada, Diversity Arrays Technology, Historical isolates, Hungary, Hybrids, Net form net blotch, Republic of South Africa.

The net blotch diseases, caused by *Pyrenophora teres*, are major fungal foliar diseases of barley, causing devastating losses to barley production throughout the world (Mathre 1997). Yield loss due to *P. teres* in susceptible barley varieties can range from 10 to 70% (Jayasena et al. 2007; Wallwork et al. 2016). Additionally, total plant death may occur in the absence of suitable fungicide treatments (Mathre 1997; Murray and Brennan 2010; Steffenson et al. 1991). Net blotch can appear as two forms, net form net blotch (NFNB), caused by *P. teres* f. *teres* (*Ptt*), and spot form net blotch (SFNB), caused by *P. teres* f. *maculata* (*Ptm*). Phylogenetically these two forms are closely related to each other (Marin-Felix et al. 2019) while in terms of population genetic analyses, the two forms represent two genetically distinct populations (Ellwood and Wallwork 2018; Liu et al. 2011; McLean et al. 2009). Even though hybrids between *Ptt* and *Ptm* have been produced successfully under laboratory conditions (Smedegård-Petersen 1971), hybrids in the field are considered to be absent or rare due to the genetic distance between these two forms (Ellwood et al. 2012; Lehmensiek et al. 2010; Poudel et al. 2017).

Net form net blotch is characterised by streaks or net-like dark brown necrotic lesions along barley leaf veins, comprising longitudinal and transverse striations (Liu et al. 2011; Smedegård-Petersen 1971). Outbreaks of *Ptt* have occurred across a wide range of barley growing regions and climates (Van den Berg 1988). Short distance dispersal of *Ptt* by air turbulence and water splashing (Deadman and Cooke 1989) can occur through ascospores and conidia produced during sexual and asexual reproduction, respectively (Liu et al. 2011). Since *Ptt* is a seed-borne fungus (Liu et al. 2011), long distance transmission of *Ptt* could result from exchange of infected seeds among geographically remote areas (Martin and Clough 1984; Shipton 1966). Furthermore, as sexual recombination is known to play a major role in the life cycle of *Ptt*, integration and adaptation of novel *Ptt* pathotypes into local areas from another

68 geographical region is possible. Introduction of a novel pathotype may greatly shape the local
69 *Ptt* genetic structure.

70 Knowledge of population diversity and structure is essential for understanding
71 population dynamics and improving disease control methods. The genetic structure of a *Ptt*
72 population depends on a number of factors such as mutations, genetic drift, gene flow, selection
73 and the relative significance of sexual versus asexual stages in the life cycle of the pathogen
74 (Akhavan et al. 2016b). With the advent of molecular genotyping technologies, *Ptt* populations
75 from different geographical locations have been characterized using molecular markers such
76 as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms
77 (AFLP) and simple sequence repeats (SSR). Genetic characterization studies in Australia
78 (Bogacki et al. 2010; Ellwood et al. 2019; Lehmensiek et al. 2010; Serenius et al. 2007), Europe
79 (Bakonyi and Justesen 2007; Ficsor et al. 2014; Jonsson et al. 2000; Rau et al. 2003; Serenius
80 et al. 2005), North America (Akhavan et al. 2016b; Jonsson et al. 2000; Peever and Milgroom
81 1994) and the Republic of South Africa (RSA) (Campbell et al. 2002; Lehmensiek et al. 2010)
82 have detected high genetic diversity within *Ptt* populations.

83 Studies conducted on Australian *P. teres* populations using AFLP and SSR markers
84 revealed high genetic variation within *P. teres* isolates collected from New South Wales,
85 Queensland, South Australia, Victoria and Western Australia (Ellwood et al. 2019; Lehmensiek
86 et al. 2010; McLean et al. 2010). To date, two studies have characterized the genetic structure
87 of *P. teres* populations from the RSA using AFLP and RAPD markers (Campbell et al. 2002;
88 Lehmensiek et al. 2010), which revealed high genetic diversity in the *Ptt* populations. Ficsor
89 et al. (2014) used RAPD markers to detect greater genotypic variability and genetic diversities
90 within sampling units than between sampling units (mating type, field type, geographical
91 region and year), and significant temporal genetic differentiation between seasons in Hungarian
92 *Ptt* populations. While each of these studies provide valuable information on the biology and

epidemiology of *Ptt* in the respective regions, it is not possible to compare the genetic diversity and structure of *Ptt* populations among these geographical areas as different studies have used different marker and analysis systems. Hence, application of a single marker system is necessary to enable valid comparisons of the genetic diversity and structure in *Ptt* populations from different parts of the world.

Use of less efficient markers such as AFLPs and RAPDs limits the reproducibility of the results (Mondini et al. 2009). Alternative marker systems, such as Diversity Arrays Technology (DArT), have become available, which produce a large number of reproducible genome-wide markers, some of which are located in gene regions (DArTseq 2020). Diversity Arrays Technology is a high throughput efficient molecular marker technology which, unlike SSR markers, does not require prior knowledge of the genome sequence (Wenzl et al. 2004). With DArT, polymorphisms are detected at restriction enzyme recognition sites and the presence or absence of individual DNA fragments in the genome is detected through microarray hybridization (Jaccoud et al. 2001). The advanced DArT technology also identifies single nucleotide polymorphisms (SNPs) within sequences. This technology has been previously implemented for genetic population analysis of fungal species including *P. teres* (Martin et al. 2020; Poudel et al. 2019; Syme et al. 2018).

The genetic diversity of a pathogen can affect its ability to adapt to host resistances and control strategies (McDonald and Linde 2002). Therefore, pathogens that are genetically more diverse may also have a higher diversity profile of virulence (Linde and Smith 2019) and an increased ability to respond to environmental changes and control measures, which may affect the resistance to fungicides or pathogenicity on the host (Peltonen et al. 1996). A recent study revealed rapid changes in the genetic structure of *Ptt* populations collected over three years from barley fields in Australia, suggesting potential adaptation and underlining the necessity of using multiple sources of host-plant resistance for defence against the pathogen (Poudel et

al. 2019). The continued evolution of fungal pathogen populations driven by the selection pressure applied by host resistance will likely lead to a decline in the efficiency of the deployed resistance (Suffert et al. 2018).

The worldwide occurrence of *Ptt* in barley and its potential for rapid genetic change through sexual recombination over a short period of time demonstrates the necessity of understanding its population diversity and structure in order to achieve efficient disease management strategies, including the development of resistant barley varieties (Liu et al. 2011; McDonald and McDermott 1993). Hence, this study was designed to characterize the genetic diversity and structure of *Ptt* populations from Australia, Canada, Hungary and the RSA, and explore the potential for long distance dispersal and geographic adaptation of the pathogen.

MATERIALS AND METHODS

Sample collection and fungal isolation. The terms entire collection, population and subpopulation in this study refer to the isolates from all countries included in the study, a collection of isolates from a country and a collection of isolates from a region/state within a country, respectively. All the isolates used in this study were monoconidial isolates and collected randomly. Isolates were mostly originated from barley leaves (except two isolates: H-374 and H-376 from Hungary originated from wheat and one: CG16015 from RSA originated from rye grass) exhibiting NFNB symptoms collected from Australia, Canada, Hungary and RSA. Five additional historic isolates were included in this study from Canada [WRS858; Serenius et al. (2007)], Denmark [Pt-Pastorale; Justesen et al. (2008)], Japan [CBS 282.31 and CBS 281.31; Bakonyi and Justesen (2007)] and Sweden [UPSC1838; Bakonyi and Justesen (2007)].

The Australian population included 118 isolates collected between 1985 and 2017 from New South Wales (NSW, n = 20), Queensland (QLD, n = 43) South Australia (SA, n = 24), Victoria (VIC, n = 6) and Western Australia (WA, n = 25), including the previously reported

hybrid WAC17021 (McLean et al. 2014) (Supplementary Table S1 and Fig. 1). Sample collection and fungal isolation of Australian samples were performed following the method described by Martin et al. (2020). Six isolates from Canada, collected by Akhavan et al. (2016a) in 2010 and 2011 from Alberta, Manitoba and Saskatchewan, were also included in the study.

The Hungarian population consisted of 85 isolates derived from naturally infected barley (n = 83) and wheat leaves (n = 2) collected from 2006 to 2018 (Supplementary Table S1 and Fig. 1). Seventy-eight isolates were collected from experimental fields at the Centre for Agricultural Research or National Food Chain Safety Office (NFCSO) in the Martonvásár region (n = 31), Fleischmann Rudolf Research Institute, Eszterházy Károly University, Kompolt (n = 17), Institute for Agricultural Research and Educational Farm, University of Debrecen, Karcag (n = 22) and a commercial field or experimental plots of NFCSO and Cereal Research Non-Profit Ltd in the Szombathely region (n = 8). The remaining seven isolates were collected from five commercial and two NFCSO barley fields (Bőny: n = 1, Kölcse: n = 3, Márok: n = 2 and Székkutas: n = 1).

Fungal isolation of Hungarian isolates was performed by inducing conidiogenesis. Leaf segments with necrotic lesions were placed in glass Petri plates and kept on a laboratory bench at ambient temperature or incubated under white light (OSRAM model L36W/640) for 16/8 hour light/dark cycles for 1 to 3 days at 18 to 20°C. Monoconidial isolates were then made by transferring single conidia from the conidiophores to V8-juice agar medium (16 g agar, 3 g $CaCO_3$, 177 mL Campbell's V8-juice and 900 ml distilled water) (Miller 1955) with a sterile needle, using a Leica MZ6 stereomicroscope at 300 to 400× magnification in a laminar air flow cabinet. Single-conidial isolates were incubated for 10 to 14 days in the dark at 18 to 20°C and used as inocula for stock and pea broth cultures. Stock cultures were grown on V8-juice agar slants for 7 to 10 days in the dark at 20 to 22°C, then kept under mineral oil at 15°C. Mycelium for DNA extraction was grown on pea-broth (Erwin and Ribeiro 1996) in steady cultures for 7

168 to 10 days at 18 to 20°C in the dark. Liquid cultures were then harvested by filtration, washed
169 with deionised water, freeze-dried and ground in liquid nitrogen. Pulverized mycelia were kept
170 at -70°C for DNA extraction.

171 The RSA population contained 72 isolates collected from leaves of barley (*n* = 71) and
172 rye grass (*n* = 1) from eight regions (Bredasdorp: *n* = 11, Caledon: *n* = 28, Greyton: *n* = 6,
173 Klipdale: *n* = 8, Napier: *n* = 12, Protem: *n* = 4, Rietpoel: *n* = 2 and Riviersonderend: *n* = 1)
174 around the Western Cape Province of RSA during October 2016 (Supplementary Table S1 and
175 Fig. 1). Fungal isolation was performed by sterilizing the surface of leaf samples in 70%
176 (vol/vol) ethanol for 5 seconds, 5 g/liter NaOCl for 2 minutes and washing three times in sterile
177 water. These were placed on water-agar (10 g/liter) or moist filter paper (×2) and incubated at
178 room temperature and natural day/night light conditions for 1 to 4 days to allow the growth of
179 conidia. Monoconidial culture production was performed by transferring single conidia to
180 potato dextrose agar (39 g/liter PDA; Biolab Merck, Modderfontein, RSA) and Solustrep (0.3
181 ml/liter) plates. Plates were incubated for 4 to 5 days, and a single colony was subcultured onto
182 a new PDA plate. After 7 days, agar plugs were collected and stored in 15% glycerol at -80°C
183 and the remaining mycelium was harvested for DNA extraction.

184 **DNA extraction for DArTseq™**. DNA from Australian isolates was extracted from
185 single-conidium cultures using the method described by Martin et al. (2020). DNA of
186 Hungarian isolates was extracted from lyophilized mycelium powder using the Cetyl Trimethyl
187 Ammonium Bromide (CTAB) method (Richards et al. 1997) and DNA of all other isolates was
188 extracted using a similar CTAB method (Saghai-Maroof et al. 1984).

189 The integrity of DNA extracted from each isolate was assessed under ultraviolet light
190 (Fusion FX, VILBER, Marne-la-Vallée, France) after electrophoresis at 100V for 30 min on a
191 0.8 g/litre agarose gel (Bioline, London, United Kingdom) containing 0.03% GelRed®
192 (Biotium Inc, California, USA). DNA quantity was measured using a NanoPhotometer P300®

193 (IMPLEN, Munich, Germany). For each isolate, 20 µl of DNA solution (> 50 ng/µl) was
194 submitted to Diversity Arrays Technology Pty. Ltd. (Canberra, ACT, Australia) for
195 DArTseq™.

196 **Data filtering and clone correction**. Data obtained from DArTseq™ consisted of SNPs
197 and SilicoDArTs (equivalent to microarray markers scored for the presence or absence of
198 sequences obtained from genomic representations). Both forms of data were filtered manually
199 using 10% as the cut off value for the maximum number of missing data points for markers
200 and isolates. Markers with a minimum allele frequency of less than one percent were removed
201 from the data set (Vaghefi et al. 2017). Reproducibility (the proportion of technical replicate
202 assay pairs for which the marker score is consistent) and the CallRate (the proportion of
203 samples for which the genotype call is either present or absent rather than missing) of each
204 marker was evaluated and markers with reproducibility of <1 and CallRate less than 85% were
205 removed. SNPs and SilicoDArTs were combined for further analyses.

206 A small number of genotyping errors may occur whilst generating DArTseq™ marker
207 data, and this may result in clonal isolates being identified as unique multilocus genotypes
208 (MLGs). In order to remove potential genotyping errors, all genotypes were contracted using
209 the furthermost bitwise distance (Kamvar et al. 2015) among five control DNA samples from
210 the same isolate (NB63i; extracted from an original culture using five different samples of
211 single-conidium derived mycelia) by the *bitwise.dist* function in *poppr* package version 2.8.3
212 (Kamvar et al. 2014) in R version 3.0.2 (R 2013). The furthermost bitwise distance among five
213 control samples (0.000925) was set as the threshold value to contract genotypes within the
214 entire population. All populations were clone corrected at the subpopulation stratum using the
215 *clonecorrect* function in *poppr* to collapse clonal groups into a single MLG for all subsequent
216 analyses except for the estimation of genetic diversity indices. Multilocus genotypes shared
217 among subpopulations were calculated by the *cross.pop* function in *poppr*.

Dendrogram construction. All isolates were assigned to genetic clusters without *a priori* assumptions using DARwin version 6.0.021 (Perrier and Jacquemoud-Collet 2006). A dendrogram was produced based on the Jaccard similarity coefficient following the unweighted neighbor-joining clustering method. Bootstrap analysis with 1,000 replicates was used to test the support of the branches on the dendrogram.

Form specific primer amplification to confirm hybrids. After assessing the dendrogram, two isolates forming a group with the previously reported *Ptt-Ptm* hybrid isolate WAC17021 were subjected to PCR amplification using six *Ptt* and six *Ptm* specific primer pairs following Poudel et al. (2017) with modifications. A combination of both *Ptt* and *Ptm* specific primer pairs are expected to be amplified in hybrid isolates (Poudel et al. 2017). DNA of three *Ptt* isolates (NB63i, NB29 and NB50) (Lehmensiek et al. 2010; Martin et al. 2020), three *Ptm* isolates (HRS06033, SNB113 and HRS07033) (Lehmensiek et al. 2010; McLean et al. 2014) and three laboratory produced hybrids (37.1, 37.4 and 37.16) (unpublished data) were also amplified with the primer pairs as positive controls. Each real time PCR reaction was prepared with 2 µl (~ 50 ng/µl) of DNA, 5 µl of SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix (BIORAD, California, USA), 0.25 µM of each primer and 2 µl of molecular water (MilliporeSigma™, Fisher Scientific, Massachusetts, USA) to a final volume of 10 µl. Amplifications were conducted in a CFX384 Touch Real-Time PCR Detection System™ (BIORAD, California, USA) with an initial denaturation at 98°C for 3 min followed by 35 cycles of denaturation at 98°C for 15 s and annealing at 60°C for 30 s. A melt curve analysis was performed after PCR completion by ramping the temperature from 65°C to 95°C, rising by 0.5°C with each step. The presence/absence of specific loci in isolates were assessed by comparing the quantitative data generated by the melt curves and the melt temperatures of the positive controls.

Analysis of molecular variance. In order to identify significant variation among populations and subpopulations, the *amova* function in *Ade4* version 1.7.13 (Dray and Dufour 2007) in R was used. Analysis of molecular variance (AMOVA) was conducted on the combined Australia, Hungary and RSA populations using the *poppr.amova* function in *poppr* with 1,000 permutations. Isolates were stratified based on the country of origin, region/state and year of collection. Analysis was conducted to identify the amount of genetic variation within and among countries, year of collection, and region/state within countries. When conducting AMOVA for the separate Australian, Hungarian and RSA populations, subpopulations consisting of less than five isolates were removed. Analysis was performed for genetic variation within and among states/fields and year of collection for Australia and Hungary populations.

Population structure by multivariate cluster analyses. Two multivariate analyses, principal component analysis (PCA) followed by discriminant analysis of principle components (DAPC) were conducted to identify the genetic structure of the entire clone-corrected collection without *a priori* assumptions. For PCA, the optimum number of principal components and principal coordinates were found and plots were drawn using the *pcadapt* function in *pcadapt* version 4.3.3 package (Luu et al. 2017). Discriminant analysis of principle components was calculated using the *dapc* function in the R package *adegenet* version 2.1.2 (Jombart 2008) and was performed for individual populations in order to detect the population structure and number of clusters within countries. The optimum number of clusters in the population was obtained using the Bayesian information criterion function *find.clusters* and the optimal number of principal component axes to retain in DAPC were estimated via the *xvalDapc* function in *adegenet.*

Population structure by model-based cluster analyses. Population structure without *a priori* assumption was investigated using STRUCTURE version 2.3.4 (Pritchard et al. 2000),

267 in which the Bayesian unsupervised genetic clustering algorithm was implemented for the
268 entire clone-corrected collection (100 Australian, 78 Hungarian, 59 RSA, six Canadian and one
269 historical isolate each from Canada, Japan, Sweden and Denmark). The analysis was conducted
270 following an admixture model with a burn-in period of 10,000 Markov chain Monte Carlo and
271 100,000 iterations. Ten independent runs were conducted for each potential number of genetic
272 clusters (*K*), where *K* ranged from 1 to 10. The analysis was performed independently for
273 Australian, Hungarian and RSA populations with the above-mentioned criteria to identify the
274 genetic structure within populations. Values extracted from STRUCTURE HARVESTER
275 version 0.6.94 (Earl and vonHoldt 2012) were used to identify the optimal number of clusters
276 for the entire clone-corrected collection as well as Australian, Hungarian and RSA populations
277 (Evanno et al. 2005). Each replicate for the optimal delta *K* (ΔK) value was entered into
278 CLUMPAK version 1.1 (Kopelman et al. 2015) to generate the graphical representation of the
279 optimal *K*. A cut off value of 70% was considered as the minimum value of an individual to be
280 included in each population.

281 **Population structure based on phylogenetic network**. A Neighbor-net phylogenetic
282 network was built for the entire collection using SplitsTree version 4.13 (Huson 1998) to
283 identify the subdivisions of the clone corrected *P. teres* population. The Neighbor-net network
284 was produced based on neighbor-joining (NJ) algorithm described by Saitou and Nei (Saitou
285 and Nei 1987) following the method depicted by Bryant and Moulton (2004). Bootstrap
286 analysis with 1,000 replicates was used to test the support of branches on the network.

287 **Identification of mating type and sexual recombination**. Amplification of mating type
288 primer pairs *pttMAT1-1* and *pttMAT1-2* (Lu et al. 2010) was assessed across all isolates. A chi
289 square test of the ratio of *pttMAT1-1* and *pttMAT1-2* was manually calculated for *Ptt* clusters
290 identified by individual DAPC analyses from Australia, Hungary and RSA to determine
291 whether there was a significant deviation from the expected 1:1 ratio under panmixia. In order

to identify the mating type of the hybrids, all mating type primer pairs (*pttMAT1-1, pttMAT1-2, ptmMAT1-1* and *ptmMAT1-2* (Lu et al. 2010) were amplified across hybrids.

Pairwise homoplasy index (PHI) test which tests the null hypothesis of no recombination available in SplitsTree 4.13 was also implemented for the same clusters detected in individual DAPC analyses for Australia, Hungary and RSA to identify the potential sexual recombination within the countries as described by Bruen et al. (2006).

Genetic diversity of populations. The non-clone corrected data set was used to calculate the number of MLGs, expected MLGs (eMLG) after rarefaction, Simpson's complement index of multilocus genotypic diversity (1-λ) and Nei's unbiased gene diversity (genetic variation within the population defined as the probability that two randomly sampled alleles are different) (Nei 1973; Nei and Chesser 1983) using *poppr*. The normalised Shannon-Wiener index (H) was calculated manually following the method described by Spellerberg and Fedor (2003). Simpson's complement index is given based on the probability of two random isolates drawn from a subpopulation to be of a different genotype (Morris et al. 2014; Simpson 1949) and Shannon-Wiener index measures the genotypic diversity of the population by richness (number of MLGs in the population) and relative abundance in a defined location (Shannon 2001; Spellerberg and Fedor 2003). Expected MLG, Simpson's complement index of multilocus genotypic diversity (1-λ), Nei's unbiased gene diversity and the normalised Shannon-Wiener index were also calculated for the clusters identified from individual DAPC analyses of Australia, Hungary and RSA.

Variant annotation and associated genes. Markers with the largest contribution to the genetic variation detected in DAPC analysis of the entire clone-corrected collection were detected using the function *loadingplot* in *adegenet* (Jombart et al. 2010). The largest contributing markers for the genetic clusters in PCA for the entire collection were also determined at the 0.0001 significance level using the function *outliers.pcadapt* in the *pcadapt*

317 package, and compared to the markers detected from DAPC analysis. Sequences (68 bp reads
318 produced by DArTseq™) harbouring markers significantly ($P < 0.0001$) responsible for the
319 genetic variation were aligned by NCBI-BLAST (NCBI) and NBLSTX (*EnsemblFungi*) to the
320 reference genomes of *Pyrenophora teres* f. *teres* isolates W1-1 (GenBank accession number:
321 OCTH00000000 and BioProject: PRJEB18107) and 0-1 (GenBank accession number:
322 AEEY01000000 and BioProject: PRJNA66337), and partial genomic regions of 13A
323 (GenBank accession numbers: JQ837863 and JQ582646). This enabled identification of
324 possible genes linked to markers with the largest contribution to the genetic clustering during
325 DAPC and PCA analyses. The putative proteins for the respective genes were predicted using
326 Universal Protein knowledgebase (UniProt).

327 **RESULTS**

328 **Genetic data and marker filtering**. Across 286 isolates, a total of 6,440 SNPs and
329 14,829 SilicoDArTs were reported, with 891 SNPs and 4,999 SilicoDArTs retained for the
330 analysis after filtering (Supplementary Material_2). After contraction (collapsing genotypes by
331 genetic distance in order to remove genotypes identified as unique due to genotyping errors)
332 of the entire collection, 286 genotypes were contracted to 250 genotypes. No clonal genotypes
333 were identified after clone correction of 250 MLGs and no MLGs were shared across any
334 regions/states within a country. Of these, 101 MLGs were from the Australian population
335 collected from 1985 to 2017 (including a previously reported hybrid WAC17021), seven were
336 Canadian isolates collected in 2010 and 2011 including one historical isolate collected in 1973,
337 59 were RSA isolates collected in 2016 and 79 were Hungarian isolates (16 collected from
338 2006 to 2009 and 63 in 2017/8). Four historical isolates representing four different MLGs, two
339 from Japan (collected in 1931) and one each from Denmark (1976) and Sweden (1986) were
340 also included (Supplementary Table S1).

Dendrogram construction. The distance-based dendrogram obtained from DARwin showed the presence of a distinct group of three isolates (Supplementary Fig.1). This group showed distinct genetic separation from the rest of the *Ptt* isolates and contained the previously reported hybrid WAC10721 from Australia along with H-919 from Hungary and CBS 281.31 from Japan, thus suggesting that these two isolates may also be hybrids.

Form specific primer amplification to confirm hybrids. PCR amplification of six *Ptt* and six *Ptm* specific primer pairs (Poudel et al. 2017) confirmed the hybrid identity of isolates H-919 and CBS 281.31. PCR results of the isolate H-919 with 12 primer pairs showed amplification for *PttQ1, PttQ3, PttQ5, PtmQ7, PtmQ8* and *PtmQ12* while CBS 281.31 showed amplification for *PttQ1, PttQ2, PttQ5, PtmQ7, PtmQ8, PtmQ9*. The *Ptt* positive control isolates NB63i, NB29 and NB50 and the *Ptm* positive controls HRS06033, SNB113 and HRS07033 showed amplification for the six *Ptt* specific primers pairs and the six *Ptm* specific primer pairs, respectively. Isolate WAC10721 and the laboratory produced hybrid isolates used as controls amplified a mixture of both *Ptt* and *Ptm* specific primer pairs. The two hybrid isolates H-919 and CBS 281.31, along with the previously reported hybrid WAC10721, were removed from subsequent analyses characterizing the genetic structure and genetic diversity of *Ptt*.

Analysis of molecular variance. AMOVA showed significant genetic variation among countries, accounting for 19.13% (P = 0.001) of the total genetic variation, while variation among isolates within populations was 82.59% (P = 0.001) (Table 1). Within population, among regions/states variation accounted for 17.40% (P = 0.001) of the total genetic variation. Considering the country and the year of collection, no significant genetic variation (P = 0.259) was observed among populations (0.52%). Out of the total genetic variation in Australia, 7.01% (P = 0.001) was observed among states in Australia, while genetic variation among regions in Hungary (2.08%) and RSA (1.78%) was not significant (P = 0.072). The variation for the year

366 of collection of *Ptt* isolates for the total genetic variation in Australia (0.12%) and Hungary
367 (0.99%) were not significant ($P = 0.415$ and 0.192 respectively).

368 **Population structure based on multivariate cluster analyses**. In the PCA plot,
369 principal component 1 (PC1) separated a group of Australian isolates ($n = 45$) and another
370 cluster of Hungarian isolates ($n = 55$) along with the historical Danish isolate Pt-Pastorale from
371 the rest of the collection. Separation of the 45 Australian isolates from the rest of the collection
372 was further supported by PC2 (Fig. 2).

373 DAPC without *a priori* population assignment indicated the presence of four clusters for
374 the entire clone-corrected collection (Fig. 3). All isolates in cluster 1 ($n = 46$) were from
375 Australia, while cluster 3 consisted of 55 Hungarian isolates and isolate Pt-Pastorale from
376 Denmark. Cluster 4 consisted of isolates from Australia ($n = 44$), Canada ($n = 6$), Hungary (n
377 $= 5$) and RSA ($n = 40$). Cluster 2 contained isolates from Australia ($n = 10$), Hungary ($n = 18$),
378 RSA ($n = 19$) and one each from Canada (MB05), Japan (CSB 282.31) and Sweden
379 (UPSC1838).

380 Individual DAPC results obtained for each population from Australia, Hungary and RSA
381 showed three clusters each (Supplementary Fig. S2A, B and C). These clusters contained
382 isolates from different regions/states within the respective countries, except for cluster 3 in the
383 Australian population which contained isolates only from QLD, SA and WA (Supplementary
384 Fig. S2A). Cluster_1 and cluster_3 obtained from the individual DAPC plot of Australian
385 isolates consisted of isolates present in cluster 1 from the entire clone-corrected DAPC plot.
386 Cluster_2 contained isolates present in cluster 2 and cluster 4 from the entire clone-corrected
387 DAPC plot. Cluster_1 and cluster_3 isolates from the individual Hungarian DAPC plot
388 contained isolates present in cluster 3 from the entire clone-corrected DAPC plot and cluster_2
389 contained isolates present in cluster 2, cluster 3 and cluster 4 from the entire clone-corrected
390 DAPC plot. Cluster_1 and cluster_2 from the individual RSA DAPC plot contained isolates

present in cluster 4 and cluster 2 from the entire clone-corrected DAPC plot, respectively, while cluster_3 contained isolates present in both cluster 4 and 2 from the entire clone-corrected DAPC plot.

Population structure based on model-based cluster analyses. STRUCTURE analysis of 247 isolates determined that three clusters best described the data (Supplementary Fig. S3A). In the three-clusters STRUCTURE model, genotypes from Australia tended to have intermediate membership in multiple clusters, while genotypes from RSA and Hungary tended to have high membership proportions in a single cluster. Using a 70% cutoff on membership proportions to assign a genotype into a cluster, a first cluster (cluster I) consisted of 46 isolates from Australia, a second cluster (cluster II) consisted of 55 isolates from Hungary and 1 isolate (Pt-Pastorale) from Denmark and a third cluster (cluster III) consisted of 145 isolates from Australia (n = 54), Canada (n = 6), Hungary (n = 23), RSA (n = 59) and historical isolates (n = 3) (Supplementary Table S1 and Fig. 4). Many genotypes from Australia (cluster III) had shared ancestry with genotypes from RSA and are thus, admixed in the three-cluster model. The six Canadian isolates along with the historical Canadian isolate were also found to be admixed (cluster III). At K=3, historical isolates from Japan and Sweden had high membership in the cluster present in RSA, Hungary and Australia, while the historical isolate from Denmark had high membership in the cluster specific to Hungary.

Genetic structure was also analysed independently for each population to identify further subdivision within countries. The mode of ΔK was observed at K=2 for the Australian, Hungarian and RSA populations (Supplementary Fig. S3B, C and D). The individual STRUCTURE analysis for Australian isolates showed that 50% and 43% of the isolates clustered into either cluster_I or cluster_II, with membership proportions of >70% for the respective clusters, while 7% of isolates were considered admixed due to membership proportions of <70% for both clusters (Supplementary Fig. S4A). The Hungarian isolates

showed two clusters, cluster_I and cluster_II) containing 71% and 29% of the isolates, respectively, with no admixed individuals. The two clusters, cluster_I and cluster_II, from the STUCTURE analysis of RSA isolates contained 68% and 17% of the isolates with membership proportions of >70% for the respective clusters and 9% admixed isolates that were not assigned to either of the clusters. The clusters obtained for the Australian, Hungarian and RSA populations were compared to the year and field/state of collection and no association was found.

Cluster_I and cluster_II obtained from the individual Australian STRUCTURE analysis consisted of isolates present in cluster III and I from the entire clone-corrected STRUCTURE analysis, respectively. Cluster_I and cluster_II from the Hungarian STRUCTURE analysis contained isolates present in cluster II and cluster III from the entire clone-corrected STRUCTURE analysis, respectively. Both cluster_I, cluster_II and admixed isolates from the individual RSA STRUCTURE analysis contained isolates present in cluster III from the entire clone-corrected STRUCTURE analysis.

The DAPC and STRUCTURE analyses of the entire clone-corrected collection resulted in identification of four and three clusters, respectively. Cluster 1 and cluster 3 from the DAPC analysis corresponded to cluster I and cluster II from the STRUCTURE analysis, respectively. Isolates present in cluster 2 and cluster 4 from the DAPC analysis corresponded to the isolates in cluster III from the STRUCTURE analysis. Therefore, DAPC analysis characterized the population subdivision in the dataset with higher resolution than STRUCTURE analyses (Jombart et al. 2010), thus, clusters detected by DAPC were further used to calculate the sexual recombination and genetic diversity.

Population structure based on phylogenetic network. The Neighbor-net phylogenetic network inferred using Splitstree showed extensive reticulation connecting all isolates (Fig. 5),

consistent with a history of recombination. The structure of the network indicated that genotypes from different countries could be closely related (Fig. 5.). Historical Danish isolate Pt-Pastorale, Japanese isolate CBS282.31 and Swedish isolate UPSC1838 grouped with Hungarian genotypes.

Identification of mating type and sexual recombination. Amplification of *Ptt* isolates with mating type primers indicated that 47 Australian isolates had the *MAT1-1* idiomorph (mating type 1) while the remaining 53 carried the *MAT1-2* idiomorph (mating type 2) (Supplementary Table S3). For Hungary, 37 isolates were found to be *MAT1-1*, and 41 isolates were *MAT1-2*. Out of 59 RSA isolates, 39 were *MAT1-1* and 21 were *MAT1-2*. Mating type ratios calculated for populations from Australia, Hungary and RSA based on clusters identified with country-specific DAPC analyses (Supplementary figure S2) showed that except cluster_2 from RSA ($P = 0.021$) the chi square values for the clusters from Australia, Hungary and RSA did not significantly differ from the expected ratio of 1:1 under panmixia. PHI rejected the null hypothesis of clonality in cluster_2 ($P = 0.014$) in Australia and cluster_1 ($P = 4.8E-4$) and 3 ($P = 0.007$) in Hungary while other clusters from Australia (cluster_1 and cluster_3), Hungary (cluster_2) and RSA (cluster_1, cluster_2 and cluster_3) did not show evidence for recombination (Supplementary Table S3).

Genetic diversity. The number of eMLGs calculated for Australia, Hungary and RSA was 10. The highest genetic diversity indices among three countries for the non-clone corrected data set were observed for the population from Hungary, with a normalised Shannon-Wiener index and Nei's unbiased gene diversity index of 0.992 and 0.184, respectively (Table 2). The lowest normalised Shannon-Wiener index, 0.973, and Nei's unbiased gene diversity index, 0.143, were calculated for the population from RSA. The highest value for Simpson's complement index of multilocus genotypic diversity was 0.991, exhibited by the Australian

population, while the lowest value, 0.986, was reported for the population from RSA. However, the overall genetic diversity within the populations was high.

The highest genetic diversity indices for the clusters detected by DAPC were observed for Hungarian isolates with a normalised Shannon-Wiener index and Nei's unbiased gene diversity index of 0.935 and 0.279, respectively (Supplementary Table S4). The lowest total normalised Shannon-Wiener index, 0.859, and Nei's unbiased gene diversity index, 0.224, were observed for the clusters from RSA. The highest Simpson's complement index of multilocus genotypic diversity was 0.990, exhibited by the Australian population, while the lowest value, 0.983, was reported for the population from RSA.

Variant annotation and associated genes. Out of 5,890 markers used for the DAPC and PCA analyses of the entire clone-corrected collection, 66 were found to be significantly associated with the genetic differences of clusters and subdivisions ($P < 0.0001$) detected by DAPC and PCA respectively. Out of 66 markers, 34 were aligned with reference genomes with the E-values (expected value) ranging from $8.4E^{-33}$ to 1.7. Out of these 34 markers, four markers aligned with known genes, another four were not situated near genes, five aligned with genes of uncharacterized proteins and 21 aligned with genes for hypothetical proteins in the reference *Ptt* genomes (Supplementary Table S2). The four markers aligned with genes were associated with ND89-9 nonribosomal peptide synthetase 2 (GenBank accession number: JQ582646), glyceraldeyde-3-phosphate dehydrogenase-like protein (*GPD1*) gene (GenBank accession number: JQ837863), endo-1,4-beta-xylanase A mRNA (GenBank accession number: JX900133) and cytochrome P450 lanosterol 14 alpha-demethylase (*CYP51A*) gene (GenBank accession number: KX578221). The identified hypothetical genes represented seven different hypothetical proteins: ANK_REP_REGION domain-containing protein, DDE-1 domain-containing protein, SET domain-containing protein, DUF1996 domain-containing protein,

Peptidase A1 domain-containing protein, AAA domain-containing protein and MFS domain-containing protein in *Ptt*.

DISCUSSION

The present study investigates the most geographically diverse collection of *Ptt* isolates analysed in a single study to date. It provides a comprehensive investigation of the genetic structure of *Ptt* populations from different geographical areas through the implementation of the genome-wide marker system, DArTseq™, and inclusion of a higher number of isolates compared to previous studies. In this study, 247 *Ptt* MLGs, predominantly from Australia, Hungary and RSA, were assessed in order to describe the genetic structure of *Ptt* isolates among distinct geographical areas.

The genetic structure of the entire clone-corrected collection detected by the DAPC analysis revealed the presence of four clusters. Two clusters contained some isolates from Australia and Hungary, and all the isolates from Canada, RSA and all the historical isolates except Pt-Pastorale from Denmark. The other two clusters were specific to Australian isolates and Hungarian isolates along with the historical Danish isolate. STRUCTURE analysis also revealed the presence of two distinct clusters for Australia (n = 46) and Hungary (n = 55) reflecting their genetic isolation from each other based on geographical origin. Furthermore, Neighbor-net phylogenetic network showed a distinct Hungarian cluster. In the Neighbor-net phylogenetic network, the *Ptt* isolates from Australia, Canada, Hungary and RSA formed more than one subdivision per country. The isolates from these subdivisions did not relate to their year of collection or the region/state of origin. Therefore, the underlying factor for the genetic isolation of *Ptt* populations from the same geographical area might include other variables such as varietal differences (Fowler et al. 2017), fungicide regimes, geographical isolation or environmental factors.

512 A number of different analyses used in this study identified the admixed nature of
513 multiple isolates mainly from Australia. STRUCTURE based cluster analysis revealed that
514 there were population subdivisions in Hungary and Australia, and that one of the clusters
515 present in each of these countries shared recent ancestry with the cluster containing the
516 Canadian, RSA and most of the historical isolates. Cluster analyses results also showed more
517 admixture in Australia than in Hungary. DAPC and highly reticulated Neighbor-net
518 phylogenetic network also gave evidence that these isolates are of mixed origin. In the
519 Neighbor-net phylogenetic network, some of the isolates from the same countries were closely
520 related to isolates from other countries. Even though some isolates from subdivisions of
521 Australia and RSA showed mixed origin/multiple origins, others showed ancestry in a single
522 group, suggesting that these isolates could have evolved from a common ancestor or an
523 introduction of isolates from a common population and then adapted to the respective
524 environments through sexual reproduction. The admixed origin of isolates could have resulted
525 from gene flow among countries. Gene flow is one of the main evolutionary forces affecting
526 in the genetic structure of a pathogen (Rogers and Rogers 1999). As *Ptt* is a seed borne
527 pathogen (Liu et al. 2011), gene flow/introduction of isolates from one geographical area to
528 another is possible through seed exchange and then adaptation to local environments. This may
529 have occurred in the case of Australian *Ptt* isolates, which have been suggested by Fowler et
530 al. (2017) to have evolved and adapted to regional barley cultivars in Australia.

531 Individual STRUCTURE analyses of Australian, Hungarian and RSA isolates indicated
532 that some of the isolates from Australia and RSA were admixed while isolates from Hungary
533 showed no admixture. The potential admixture found within Australian and RSA isolates could
534 have resulted from the dispersion of the pathogen through sexual reproduction and lack of
535 varietal specialization within the country. The absence of admixed in Hungarian isolates might

536 have been caused due to physical and reproduction barriers in the dispersion of the pathogen,
537 host specialization and/or recent introduction of isolates.

538 The genetic structure of *Ptt* populations detected in model-based cluster analyses did not
539 correspond to the region/state or the year of collection of the isolates, hence, factors
540 contributing to the genetic structure of *Ptt* populations were investigated by identifying the
541 markers underlying the genetic structure detected in DAPC and PCA. One of these markers
542 was located within the gene responsible for the nonribosomal peptide synthetases protein. The
543 nonribosomal peptide synthetases are responsible for the production of nonribosomal peptides,
544 which are bioactive secondary metabolites known to be involved in cellular development,
545 pathogenicity and stress responses in plant fungal pathogens (Keller et al. 2005; Sayari et al.
546 2019). The potential role of this locus in differential aggressiveness of *Ptt* isolates requires
547 further investigation. Other markers that were significantly associated with genetic structuring
548 of the *Ptt* populations included a glyceraldeyde-3-phosphate dehydrogenase-like protein
549 (*GPD1*) gene, an endo-1,4-beta-xylanase A mRNA gene, and a cytochrome P450 lanosterol 14
550 alpha-demethylase (*CYP51A*) gene. The *GPD1* gene has been frequently used as a genetic
551 marker in phylogenetic studies to differentiate fungal pathogens including *Pyrenophora teres*
552 (Andrie et al. 2008; Lu et al. 2013; Zhang and Berbee 2001). *GPD1* plays a major role in fungal
553 metabolic pathways like energy synthesis and biomass synthesis (Larsson et al. 1998). It has
554 been suggested that mutations in the glyceraldeyde-3-phosphate dehydrogenase gene
555 contribute to the nutrient uptake of phytopathogenic *Colletotrichum* spp. during their
556 biotrophic phase in the infection process on many perennial plants including olive, citrus and
557 tomato (Materatski et al. 2019; Wei et al. 2004). The enzyme endo-1,4-beta-xylanase plays a
558 vital role in the breakdown of xylan, a major component of plant cell walls (Nguyen et al.
559 2011), and the degradation of the plant cell wall has been correlated with virulence and
560 pathogenicity of phytopathogenic *Fusarium* spp. and *Valsa* spp. on tomato and apple (Gómez-

561 Gómez et al. 2001; Wang et al. 2014). Cytochrome P450 lanosterol 14 alpha-demethylase is
562 important for the biosynthesis of ergosterol, a primary fungal cell membrane sterol that is
563 responsible for maintaining membrane fluidity and stability (Koch et al. 2013; Luo and
564 Schnabel 2008; Parks and Casey 1995; Rodriguez et al. 1985). Mutations of this gene have
565 been associated with the demethylase inhibitor (DMI) or group 3 fungicide resistance in *P.*
566 *teres* (Ellwood et al. 2019; Mair et al. 2019). Considering the importance of these genes for
567 fungal virulence/pathogenicity, it is plausible that mutations at these loci are due to external
568 effects such as environmental factors and fungicide regimes. These factors may have driven
569 local and/or host adaptation of *Ptt* isolates in different regions, resulting in the distinct genetic
570 sub-structuring detected in this study.

571 Sexual recombination plays a major role in the evolution and adaptation of a pathogen
572 which may influence the genetic structure (Lee et al. 2010). *Ptt* is a well-known sexually
573 reproducing fungus (Liu et al. 2011). A mating type ratio of 1:1 is expected in the absence of
574 segregation distortion and clonal selection among mating types and the two mating types ratio
575 is equalized through sexual recombination in *P. teres* (Milgroom 1996; Rau et al. 2005). In the
576 current study, except for cluster_2 from RSA, other clusters collected from Australia, Hungary
577 and RSA did not deviate from the expected 1:1 ratio. Studies of Finish, Australian and
578 Canadian *Ptt* populations reported that the mating type ratio did not deviate from the expected
579 1:1 ratio (Akhavan et al. 2016b; Linde and Smith 2019; Rau et al. 2005; Serenius et al. 2005),
580 while studies of *Ptt* populations from Czech Republic and Slovakia, and Krasnodar, Russia
581 deviated from a 1:1 ratio (Leišová-Svobodová et al. (2014); Serenius et al. (2007)). Deviation
582 of mating type ratio in cluster_2, RSA and absence of sexual recombination evidence for cluster
583 1 and 3 from Australia, cluster 2 from Hungary and all clusters from RSA based on PHI test
584 results might have occurred due to unsystematic sampling or introduction of primary inoculum
585 like contaminated seeds/conidia to the fields. In the current study, *Ptt* isolates from Australia

586 and Hungary have been collected from different years. Therefore, further studies are necessary
587 with a higher number of isolates and intensive sampling methods to confirm the evidence for
588 sexual reproduction of Australian and Hungarian *Ptt* populations.

589 Previous studies have suggested that hybridization between the two types of *P. teres* is
590 rare or absent under field conditions due to the apparent genetic isolation of both forms
591 (Ellwood et al. 2012; Lehmensiek et al. 2010). Prior to this study, only four naturally occurring
592 putative hybrids had been detected from barley fields: one putative hybrid from the south-
593 western Cape of RSA (Campbell et al. 2002), two from Tovacov, Czech Republic (PTM-15
594 and PTM-16) (Leišova et al. 2005), and one from a barley field in Western Australia
595 (WAC10721) (McLean et al. 2014). In the current study, additional isolates from Hungary (H-
596 919) and Japan (CBS 281.31) were identified as putative hybrids based on distinct genetic
597 subdivision compared to the *Ptt* population and genetic similarity to the previously identified
598 hybrid WAC10721 in the Neighbor-net phylogenetic network. Amplification using *Ptt* and *Ptm*
599 specific DNA markers confirmed that these two isolates were hybrids. The isolate CBS 281.31
600 was originally identified as *Pyrenophora japonica* by Ito (Crous et al, 1995). Crous et al. (1995)
601 found a high degree of homology in restriction digestion (*Hae* III and *Msp* I) DNA banding
602 patterns and similar symptom expression on differential cultivars when comparing CBS 281.31
603 with *Ptm* isolates. In addition, similar morphological characterizations between these isolates
604 led Crous et al. (1995) to conclude that *P. japonica* was a synonym of *P. teres*. A recent study
605 by Marin-Felix et al. (2019) also referred to isolate CBS 281.31 as *P. japonica* and found that
606 the isolate grouped together with *P. teres* based on phylogenetic similarities. Marin-Felix et al.
607 (2019) agreed with the conclusion of Crous et al. (1995) that *P. japonica* was a synonym of *P.*
608 *teres* based on CBS 281.31 as the sole representative of *P. japonica*. A previous distance based
609 cluster analysis study, using seven RAPD markers and complemented with the two *P. teres*
610 form specific PCR markers developed by Williams et al. (2001), identified CBS 281.31 as a

611 *Ptt* isolate (Bakonyi and Justesen 2007). The types and small number of markers used might
612 be the reason for not detecting this isolate as a hybrid in previous studies. The Japanese isolate,
613 CBS 281.31 collected in 1931 was found to be a hybrid nearly a century after it was collected.
614 During the 89 years since it was collected, this hybrid could have crossed with many other
615 Japanese *P. teres* isolates, potentially influencing the genetic structure of the population. Sexual
616 recombination/hybridization between and within the forms of *P. teres* can potentially lead to
617 the generation of novel pathotypes. This may increase the genetic diversity of the population
618 and make disease management more challenging through changes in traits such as fungicide
619 resistance of the pathogen (Syme et al. 2018). Therefore, further population genetics studies
620 and pathotyping of *Ptt* populations are warranted.

621 In conclusion, the genetic structure and the genetic relationships of *Ptt* isolates collected
622 from different continents reported in this study indicated that some isolates from Australia,
623 Canada, Hungary and RSA shared ancestry with other countries while some of the isolates
624 from Australia and Hungary showed no admixture. Admixed origin among populations provide
625 crucial evidence for the spread of the pathogen. Identification of naturally occurring hybrids
626 supports the fact that the hybridisation between two forms of *P. teres* is possible, which may
627 lead to novel and more complex pathotypes and may cause unpredicted yield losses to the
628 barley industry. Hence, up to date knowledge about genetic structure and the genetic diversity
629 of geographically diverse *P. teres* populations is important to predict and implement efficient
630 disease management strategies and to develop resistant barley cultivars.
631

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880

881 TABLE 1. Analysis of molecular variance of *Pyrenophora teres* f. *teres* isolates from Australia,

882 Hungary and the Republic of South Africa (RSA).

Source of variance	Degrees of freedom	Variation (%)	Sum square	Mean square
Australia, Hungary and RSA				
Among countries	2	19.13[a]	6788.27	3394.13
Year among countries	4	0.52[ns]	846.12	211.53
Among regions/states within countries	13	17.40[a]	10088.81	776.06
Among isolates within populations	211	82.59[a]	38143.65	180.78
Australia				
Among states	4	7.01[a]	1794.25	448.56
Within states	95	92.99[a]	17670.53	186.01
Year within Australia	3	0.12[ns]	601.16	200.39
Hungary				
Among fields	3	2.08[ns]	775.19	258.40
Within fields	67	97.92[a]	12822.33	191.38
Year within Hungary	1	0.99 [ns]	244.96	244.96
RSA				
Among fields	4	1.78 [ns]	731.09	182.77
Within fields	49	97.90 [a]	9576.68	195.44

883 [a] Significant at $P \leq 0.001$

884 [ns] Not significant

TABLE 2. Indices of genetic diversity for *Pyrenophora teres* f. *teres* populations from Australia, Hungary and Republic of South Africa (RSA).

Population	*n*[a]	MLG[b]	eMLG[c]	H[d]	1-λ[e]	H_{exp}[f]	CF[g]
Australia							
NSW	20	17	10	0.974	0.941	0.164	0.150
QLD	43	37	10	0.981	0.973	0.172	0.140
SA	24	23	10	0.995	0.957	0.184	0.042
VIC	6	6	6	1.000	0.833	0.167	0
WA	24	17	10	0.633	0.941	0.177	0.292
Australia total	117	100	10	0.986	0.991	0.183	0.145
Hungary							
Bony	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Karcag	22	19	10	0.610	0.947	0.162	0.136
Kompolt	16	14	10	0.511	0.929	0.188	0.125
Kölcse	3	3	3	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Martonvásár	31	30	10	0.996	0.967	0.186	0.032
Márok	2	2	2	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Székkutas	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Szombathely	8	8	8	1.000	0.875	0.190	0
Hungary total	84	78	10	0.992	0.988	0.184	0.071
RSA							
Bredasdorp	11	6	6	0.960	0.833	0.120	0.455
Caledon	28	26	10	0.992	0.962	0.149	0.071
Greyton	6	5	5	0.970	0.800	0.145	0.167
Klipdale	8	8	8	1.000	0.875	0.157	0
Napier	12	9	9	0.973	0.889	0.150	0.250
Protem	4	3	3	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Rietpoel	2	2	2	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Riviersonderend	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
RSA total	72	59	10	0.973	0.986	0.143	0.181
Total	273	237	10	0.987	0.996	0.202	0.132

[a] Number of isolates

[b] Number of multilocus genotypes (MLG)

[c] The number of expected MLG based on rarefaction at the smallest sample size of ≥10

[d] Normalised Shannon-Wiener index of MLG genotypic diversity, the genotypic diversity of the population by richness and relative abundance in a defined location

[e] Simpson's complement index of multilocus genotypic diversity, the probability of two random isolates drawn from a subpopulation to be of a different genotype

[f] Nei's unbiased gene diversity, the probability that two randomly chosen alleles are different

[g] Clonal fraction (CF), (1-MLG/*n*) where, MLG equals to number of MLGs and *n* equals the number of isolates of the population/subpopulation

[h] Not calculated due to < 5 isolates

Figure captions

Fig. 1. Sample collection regions of *Pyrenophora teres* f. *teres* isolates in (A) Australia, (B) Hungary and (C) Republic of South Africa (RSA). (ArcGISPro version 2.3, Esri, California, USA)

Fig. 2. Principal components analysis of *Pyrenophora teres* f. *teres* isolates collected from Australia, Canada, Denmark, Hungary, Japan, Republic of South Africa (RSA) and Sweden. Principal component axis 1 (PC1) and principal component axis 2 (PC2) explained 13.6% and 9.3% variation, respectively, for the genetic clusters.

Fig. 3. Discriminant analysis of principal components of the entire collection of *Pyrenophora teres* f. *teres* from Australia (Aus), Republic of South Africa (RSA), Hungary (Hun), Canada (Can), Japan (Jap), Sweden (Swe) and Denmark (Den). The distribution of the eigenvalues of principal component analysis (PCA) and discriminant analysis (DA) indicate that the first two principal components explain 25% of the genetic structure of the clusters.

Fig.4. Estimates of genetic structuring in the entire clone-corrected *Pyrenophora teres* f. *teres* collection grouped into clusters (K = 2-10) using the model-based clustering method in STRUCTURE. Population color bars represent isolates from Australia (n = 100), Canada (n = 7; including the historical Canadian isolate), Denmark (n = 1), Hungary (n = 78), Japan (n = 1), Republic of South Africa (n = 59) and Sweden (n = 1) respectively. Bars represent individual isolates and the color and height of each bar depicts the estimated membership fraction of each individual into the corresponding cluster.

Fig. 5. Neighbor-net phylogenetic network based on DArTseq™ data for *Pyrenophora teres* f. *teres* isolates from Australia (n = 100), Canada (n = 7; including the historical Canadian isolate), Denmark (n = 1), Hungary (n = 78), Japan (n = 1), Republic of South Africa (n = 59) and Sweden (n = 1).

924 e-Xtra figure captions

925 Supplementary Fig. S1. Neighbor-joining clustering with bootstrapping (≥90%) based on
926 DArTseq™ data following Jaccard similarity coefficient for *Pyrenophora teres* f. *teres* isolates
927 from Australia (n = 101), Canada (n = 7), Denmark (n = 1), Hungary (n = 79), Japan (n = 2),
928 Republic of South Africa (RSA) (n = 59) and Sweden (n = 1) .

929 Supplementary Fig. S2. Discriminant analysis of principal components of *Pyrenophora*
930 *teres* f. *teres* populations collected from (A) Australia, (B) Hungary and (C) Republic of South
931 Africa (RSA). The distribution of the eigenvalues of principal component analysis (PCA) and
932 discriminant analysis (DA) indicate that the first two principal components adequately explain
933 > 50% of the genetic structure of the clusters. States of Australia; NSW- New South Wales,
934 QLD- Queensland, SA- South Australia, VIC-Victoria and WA- Western Australia. Regions
935 of Hungary; Kar- Karcag, Köl-, Kölcse, Kom- Kompolt, Mar- Martonvásár, Márk- Márok, Szé-
936 Székkutas and Szo- Szombathely. Regions of RSA; Bre- Bredasdorp, Cal- Caledon, Gre-
937 Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.

938 Supplementary Fig. S3. The optimum number of clusters (K) for (A) the entire *Pyrenophora*
939 *teres* f. *teres* collection, populations from (B) Australia, (C) Hungary and (D) Republic of
940 South Africa based on delta K (ΔK) estimated over 10 independent runs.

941 Supplementary Fig. S4. Estimates of genetic structuring in (A) Australia (blue, K = 2) (B)
942 Hungary (red, K = 2) and (C) Republic of South Africa (RSA) (green, K = 2) grouped into
943 optimal clusters using the model-based clustering method in STRUCTURE. Bars represent
944 individual isolates and the colour and height of each bar depicts the estimated membership
945 fraction of each individual into the corresponding cluster. States of Australia; NSW- New
946 South Wales, QLD- Queensland, SA- South Australia, VIC-Victoria and WA- Western

947 Australia. Regions of Hungary; Kar- Karcag, Köl-, Kölcse, Kom- Kompolt, Mar- Martonvásár,
948 Márk- Márok, Szé- Székkutas and Szo- Szombathely. Regions of RSA; Bre- Bredasdorp, Cal-
949 Caledon, Gre- Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.
950

951 e-Xtra table titles

952 Supplementary Table S1. Meta data for *Pyrenophora teres* isolates genotyped in this study

953 Supplementary Table S2. Details of the most contributing DArTseq™ marker annotations for
954 the DAPC and PCA

955 Supplementary Table S3. Chi square and PHI test values for subdivisions in Australia, regions
956 in Hungary and RSA

957 Supplementary Table S4. Indices of genetic diversity for *Pyrenophora teres* f. *teres* populations
958 from Australia, Hungary and Republic of South Africa (RSA) based clusters detected in DAPC
959
960

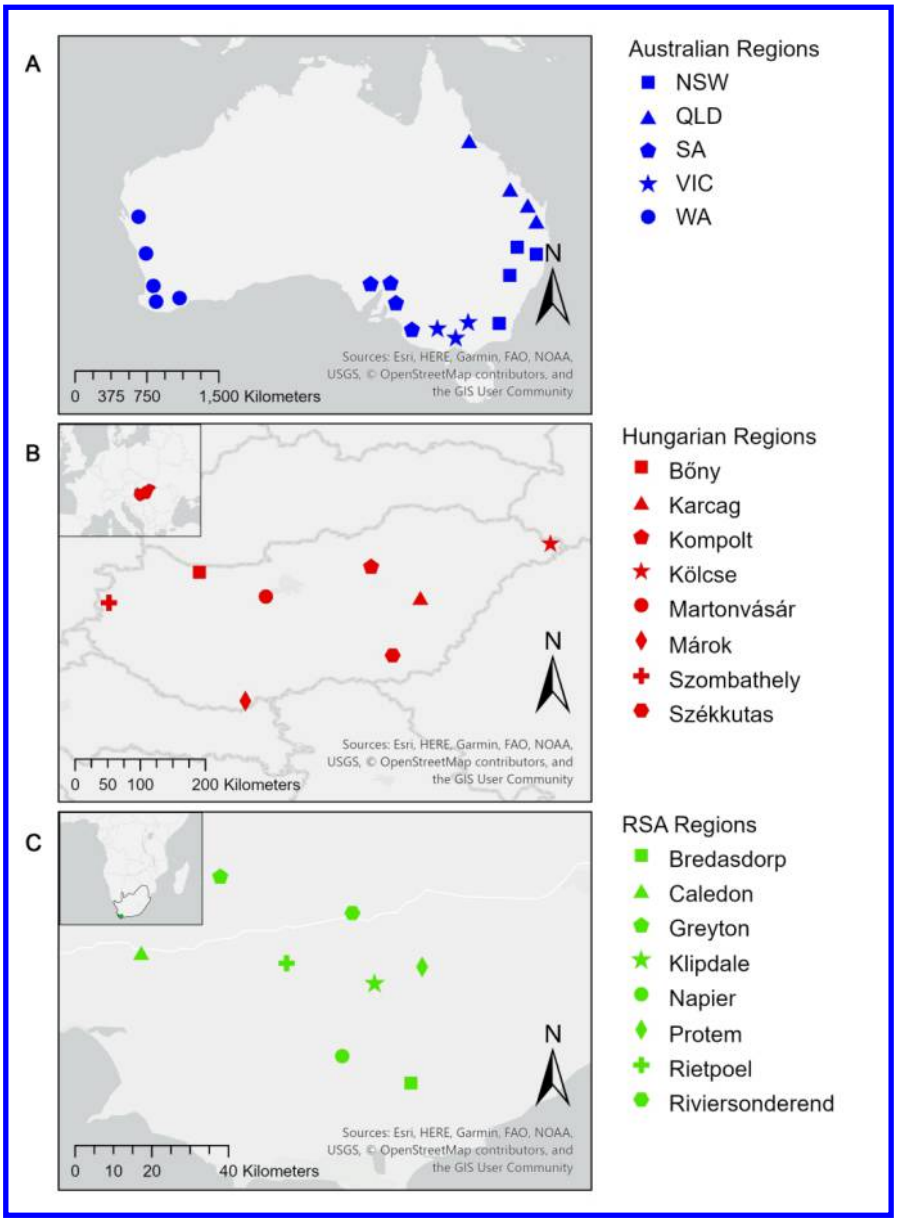


Fig. 1. Sample collection regions of *Pyrenophora teres* f. *teres* isolates in (A) Australia, (B) Hungary and (C) Republic of South Africa (RSA). (ArcGISPro version 2.3, Esri, California, USA)

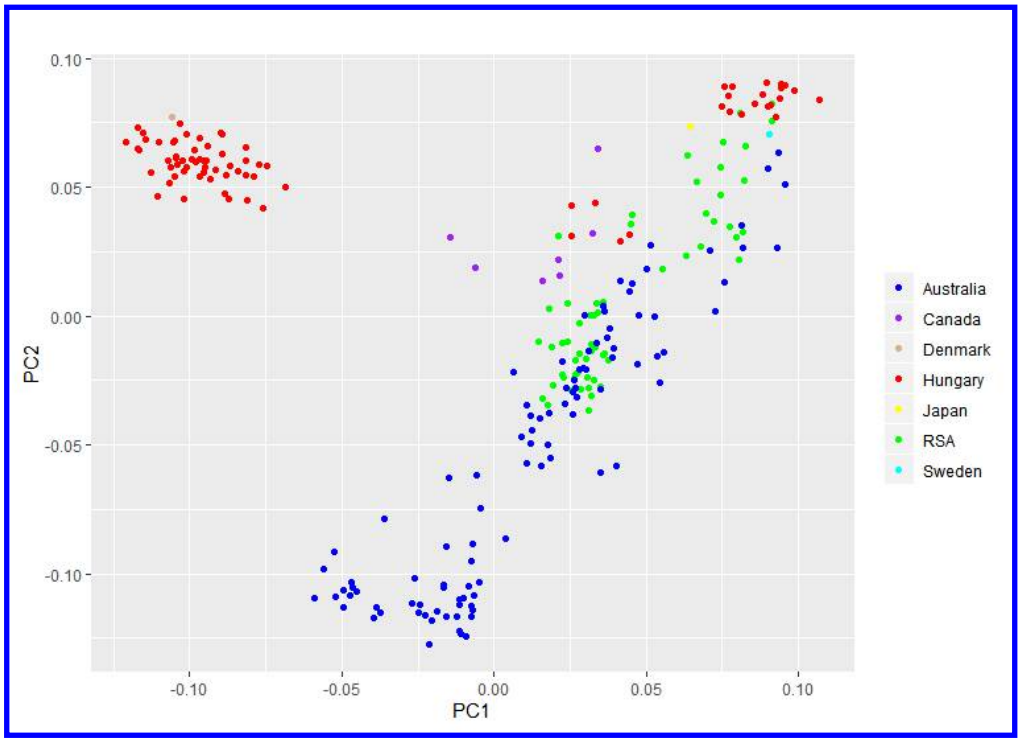


Fig. 2. Principal components analysis of *Pyrenophora teres* f. *teres* isolates collected from Australia, Canada, Denmark, Hungary, Japan, Republic of South Africa (RSA) and Sweden. Principal component axis 1 (PC1) and principal component axis 2 (PC2) explained 13.6% and 9.3% variation, respectively, for the genetic clusters.

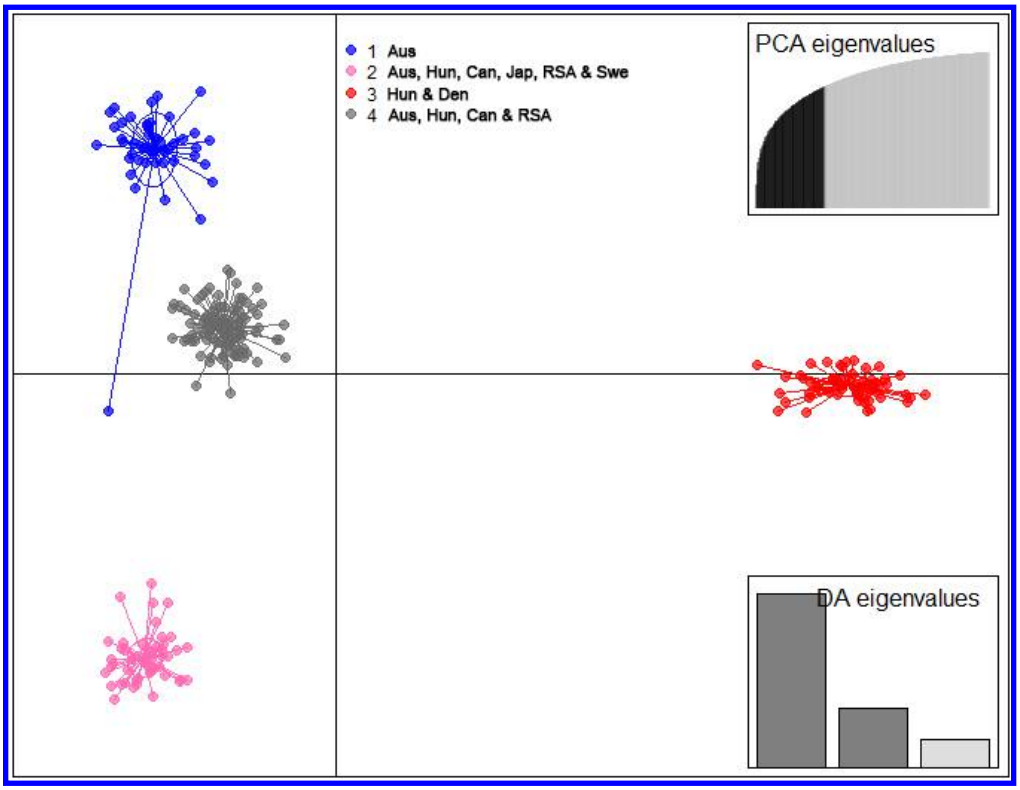


Fig. 3. Discriminant analysis of principal components of the entire collection of *Pyrenophora teres* f. *teres* from Australia (Aus), Republic of South Africa (RSA), Hungary (Hun), Canada (Can), Japan (Jap), Sweden (Swe) and Denmark (Den). The distribution of the eigenvalues of principal component analysis (PCA) and discriminant analysis (DA) indicate that the first two principal components explain 25% of the genetic structure of the clusters.

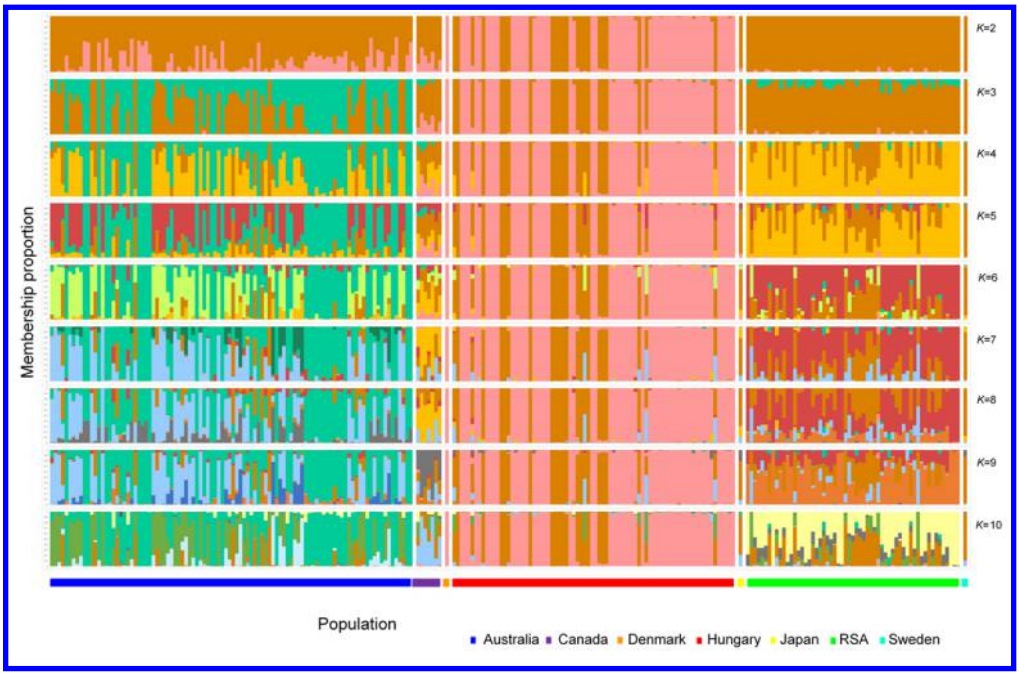


Fig. 4. Estimates of genetic structuring in the entire clone-corrected *Pyrenophora teres* f. *teres* collection grouped into clusters (K = 2-10) using the model-based clustering method in STRUCTURE. Population color bars represent isolates from Australia (n = 100), Canada (n = 7; including the historical Canadian isolate), Denmark (n = 1), Hungary (n = 78), Japan (n = 1), Republic of South Africa (n = 59) and Sweden (n = 1) respectively. Bars represent individual isolates and the color and height of each bar depicts the estimated membership fraction of each individual into the corresponding cluster.

295x192mm (96 x 96 DPI)

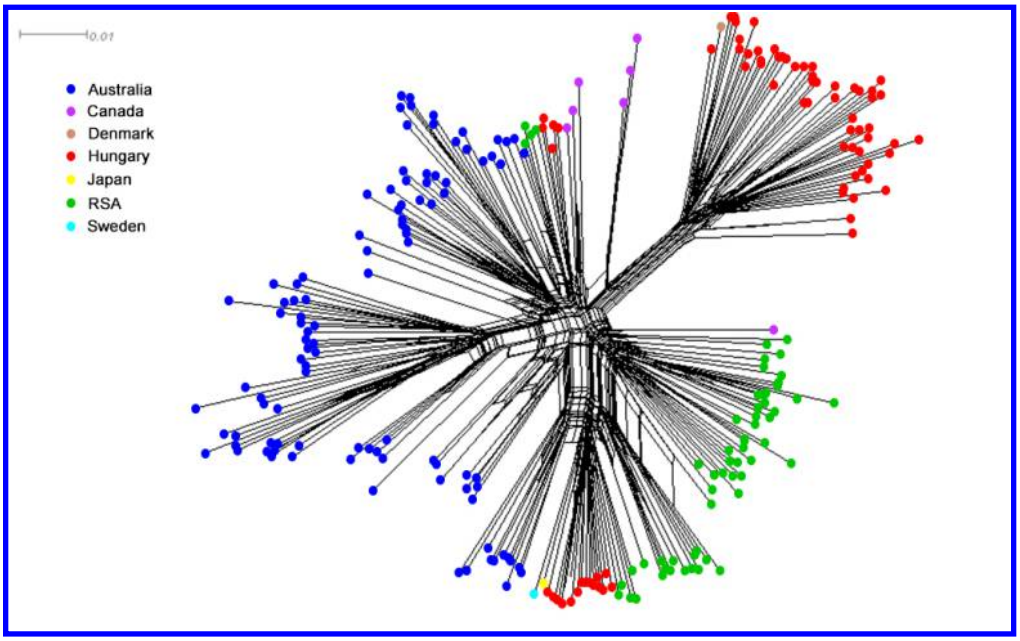


Fig. 5. Neighbor-net phylogenetic network based on DArTseq™ data for *Pyrenophora teres* f. *teres* isolates from Australia (*n* = 100), Canada (*n* = 7; including the historical Canadian isolate), Denmark (*n* = 1), Hungary (*n* = 78), Japan (*n* = 1), Republic of South Africa (*n* = 59) and Sweden (*n* = 1).

Supplementary Table S1. Meta data for *Pyrenophora teres* isolates genotyped in this study

Isolate	Mat[a]	Year	Host	Region/State	Country	Reference[e]	DAPC	STR
HRS07013[d]	2	2007	Unknown	NSW	Australia	Martin et al. (2019)	4	III
HRS08046[d]	2	2008	NRB07572	QLD	Australia	Martin et al. (2019)	4	III
HRS08117[d]	2	2008	Unknown	QLD	Australia	Martin et al. (2019)	4	III
nf25/08[d]	1	2007	Fleet	SA	Australia	Martin et al. (2019)	2	III
nf49/07	1	2007	Keel	SA	Australia	Martin et al. (2019)	NA	NA
nf55/07[d]	1	2007	Keel	SA	Australia	Martin et al. (2019)	1	I
HRS08194	1	2008	Net form net blotch differential	SA	Australia	Martin et al. (2019)	NA	NA
HRS08195[d]	2	2008	Unknown	NSW	Australia	Martin et al. (2019)	4	III
HRS09015[d]	1	2009	Barley Stubble	QLD	Australia	Martin et al. (2019)	4	III
HRS09042[d]	1	2009	Skiff	QLD	Australia	Martin et al. (2019)	4	III
HRS09092[d]	2	2009	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
nf47/09A3[d]	2	2009	Maritime	SA	Australia	Martin et al. (2019)	1	I
nf48/09A3[d]	2	2009	Maritime	SA	Australia	Martin et al. (2019)	1	I
HRS09120	2	2009	Shepherd	QLD	Australia	Martin et al. (2019)	NA	NA
HRS09121[d]	1	2009	TR129/Skiff	NSW	Australia	Martin et al. (2019)	4	III

HRS09122[d]	2	2009	TR129/Skiff	NSW	Australia	Martin et al. (2019)	2	III
HRS09123[d]	2	2009	Vlamingh	WA	Australia	Martin et al. (2019)	1	I
HRS09127	2	2009	TR129/Skiff	NSW	Australia	Martin et al. (2019)	NA	NA
03-0006[d]	1		Unknown	VIC	Australia	Martin et al. (2019)	4	III
nf08/007ss[d]	2	2008	Unknown	SA	Australia	Martin et al. (2019)	1	I
HRS09136[d]	2	2009	Barley	WA	Australia	Martin et al. (2019)	1	I
nf09/136[d]	1	2009	Barque	VIC	Australia	Martin et al. (2019)	4	III
nf09/140[d]	1	2009	Barque	VIC	Australia	Martin et al. (2019)	2	III
nf122/09b[d]	1	2009	Fleet	SA	Australia	Martin et al. (2019)	1	I
HRS10004	1	2010	Grimmett	QLD	Australia	Martin et al. (2019)	NA	NA
HRS10015[d]	1	2010	NRB06059	QLD	Australia	Martin et al. (2019)	1	I
ptt09-120[d]	1	2009	Unknown	SA	Australia	Martin et al. (2019)	4	III
ptt09-154	1	2009	Baudin	WA	Australia	Martin et al. (2019)	NA	NA
ptt09-155[d]	1	2009	Vlamingh	WA	Australia	Martin et al. (2019)	1	I
HRS10033	1	2010	Keel	QLD	Australia	Martin et al. (2019)	NA	NA
nf32/98[d]	2	1998	Unknown	SA	Australia	Martin et al. (2019)	2	III

nf57/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2019)	1	I
nf66/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2019)	1	I
nf70/09[d]	1	2009	Unknown	SA	Australia	Martin et al. (2019)	1	I
nf123/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2019)	1	I
HRS10077[d]	1	2010	Unknown	QLD	Australia	Martin et al. (2019)	4	III
HRS10097[d]	1	2010	NRB06059	QLD	Australia	Martin et al. (2019)	4	III
HRS10108[d]	1	2010	Tallon	QLD	Australia	Martin et al. (2019)	4	III
HRS10109[d]	1	2010	Unknown	QLD	Australia	Martin et al. (2019)	4	III
HRS10121[d]	2	2010	Grout	QLD	Australia	Martin et al. (2019)	1	I
HRS10122[d]	2	2010	Shepherd	QLD	Australia	Martin et al. (2019)	1	I
HRS10131	1	2010	Barley	NSW	Australia	Martin et al. (2019)	NA	NA
HRS10135[d]	2	2010	Mackay	NSW	Australia	Martin et al. (2019)	4	III
HRS10137[d]	2	2010	Shepherd	NSW	Australia	Martin et al. (2019)	4	III
HRS10138[d]	2	2010	Commander	NSW	Australia	Martin et al. (2019)	2	III
HRS10142[d]	1	2010	Grout	NSW	Australia	Martin et al. (2019)	4	III
HRS10159[d]	2	2010	Bass	NSW	Australia	Martin et al. (2019)	4	III

HRS10164[d]	2	2010	Grimmett	QLD	Australia	Martin et al. (2019)	4	III
HRS10167[d]	1	2010	Grout	QLD	Australia	Martin et al. (2019)	1	I
HRS10185[d]	2	2010	Hindmarsh	QLD	Australia	Martin et al. (2019)	4	III
HRS10189[d]	1	2010	Mackay	QLD	Australia	Martin et al. (2019)	1	I
HRS10190a[d]	1	2010	Tallon	QLD	Australia	Martin et al. (2019)	4	III
HRS10193[d]	2	2010	Bass	WA	Australia	Martin et al. (2019)	1	I
HRS10194[d]	2	2010	Baudin	WA	Australia	Martin et al. (2019)	1	I
HRS10220[d]	2	2010	Commander	NSW	Australia	Martin et al. (2019)	4	III
HRS13164a[d]	2	2013	Fathom	SA	Australia	Martin et al. (2019)	1	I
HRS13175a[d]	2	2013	Unknown	QLD	Australia	Martin et al. (2019)	4	III
HRS13182a[d]	2	2013	Henley	QLD	Australia	Martin et al. (2019)	4	III
HRS13199a[d]	2	2013	Scope CL	WA	Australia	Martin et al. (2019)	2	III
HRS13209a[d]	2	2013	Barley	QLD	Australia	Martin et al. (2019)	4	III
HRS13217a[d]	1	2013	Unknown	QLD	Australia	Martin et al. (2019)	4	III
nf018/13[d]	2	2013	Fleet	SA	Australia	Martin et al. (2019)	4	III
ptt14-007[d]	2	2014	Unknown	VIC	Australia	Martin et al. (2019)	4	III

ptt14-057[d]	1	2014	Unknown	VIC	Australia	Martin et al. (2019)	1	I
ptt14-110[d]	1	2014	Fairview	VIC	Australia	Martin et al. (2019)	2	III
nf65/14a[d]	2	2014	Maritime	SA	Australia	Martin et al. (2019)	1	I
nf71/14a[d]	2	2014	Fleet	SA	Australia	Martin et al. (2019)	2	III
nf117/14a[d]	2	2014	Barque	SA	Australia	Martin et al. (2019)	4	III
87/15a[d]	2	2015	Alstar	SA	Australia	Martin et al. (2019)	2	III
HRS16025a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
HRS16026a[d]	1	2016	Compass	NSW	Australia	Martin et al. (2019)	1	I
HRS16031a[d]	2	2016	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
HRS16033a[d]	1	2016	Unknown	QLD	Australia	Martin et al. (2019)	4	III
HRS16041a[d]	1	2016	Compass	SA	Australia	Martin et al. (2019)	1	I
HRS16043a[d]	2	2016	Compass	QLD	Australia	Martin et al. (2019)	1	I
HRS16051a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
HRS16083a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
HRS17058[d]	1	2017	Shepherd	QLD	Australia	Martin et al. (2019)	4	III
HRS17066a[d]	2	2017	Commander	NSW	Australia	Martin et al. (2019)	1	I

HRS17080a[d]	1	2017	Commander	QLD	Australia	Martin et al. (2019)	1	I
HRS17081a[d]	1	2017	Barley	QLD	Australia	Martin et al. (2019)	1	I
HRS17082a	1	2017	Commander	NSW	Australia	Martin et al. (2019)	NA	NA
HRS17083a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2019)	1	I
HRS17084a[d]	1	2017	Commander	QLD	Australia	Martin et al. (2019)	1	I
HRS17085a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2019)	1	I
HRS17087a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2019)	1	I
HRS17088a[d]	2	2017	Commander	QLD	Australia	Martin et al. (2019)	1	I
HRS17090a[d]	2	2017	Commander	QLD	Australia	Martin et al. (2019)	1	I
62/17a[d]	2	2017	Fathom	SA	Australia	Martin et al. (2019)	1	I
NB2015-024[d]	1	2015	Navigator	WA	Australia	Ellwood et al. (2019)	1	I
NB2015-027[d]	1	2015	Fleet	WA	Australia	Ellwood et al. (2019)	1	I
NB2015-032[d]	1	2015	CMP	WA	Australia	Ellwood et al. (2019)	4	III
NB2015-033	2	2018	Barley	WA	Australia	Ellwood et al. (2019)	NA	NA

NB2016-045[d]	1	2016	Oxford	WA	Australia	Ellwood et al. (2019)	1	I
NB2016-048[d]	1	2015	Unknown	WA	Australia	Ellwood et al. (2019)	2	III
NB2016-051[d]	2	2015	Unknown	WA	Australia	Ellwood et al. (2019)	4	III
NB2016-052[d]	1	2016	Unknown	WA	Australia	Ellwood et al. (2019)	4	III
Ko103-3[d]	2	2013	Barley	WA	Australia	Ellwood et al. (2019)	1	I
NB029[d]	1	1985	Beecher	WA	Australia	Martin et al., 2019)	1	I
NB033[d]	1	1989	Grimmett	QLD	Australia	Martin et al. (2019)	4	III
NB034[d]	2	1989	Corvette	QLD	Australia	Martin et al. (2019)	1	I
NB035[d]	2	1993	Gilbert	QLD	Australia	Martin et al. (2019)	1	I
NB050	1	1994	Barley	QLD	Australia	Martin et al. (2019)	NA	NA
NB053[d]	2	1994	Tallon	SA	Australia	Martin et al. (2019)	4	III
NB085	1	1995	Cape	QLD	Australia	Martin et al. (2019)	NA	NA
NB102[d]	1	1995	Gilbert	QLD	Australia	Martin et al. (2019)	4	III

NB2015-021[d]	2	2015	Barley	WA	Australia	Ellwood et al. (2019)	1	I
NB223[d]	1	1996	Beecher	SA	Australia	Martin et al. (2019)	1	I
NB270[d]	2	1996	Grimmett	NSW	Australia	Martin et al. (2019)	4	III
NB330a[d]	2	2003	Binalong	NSW	Australia	Martin et al. (2019)	4	III
NB63-1	2	1994	Unknown	WA	Australia	Martin et al. (2019)	NA	NA
NB63-2	2	1994	Unknown	WA	Australia	Martin et al. (2019)	NA	NA
NB63-3 [d]	2	1994	Unknown	WA	Australia	Martin et al. (2019)	1	I
NB63-4	2	1994	Unknown	WA	Australia	Martin et al. (2019)	NA	NA
NB63-5	2	1994	Unknown	WA	Australia	Martin et al. (2019)	NA	NA
NB73	2	1994	Gilbert	QLD	Australia	Martin et al. (2019)	NA	NA
W1-1[d]	2	2009	Unknown	WA	Australia	Syme et al. (2018)	1	I
WAC10721[b]	1[c]	2002	Unknown	WA	Australia	McLean et al. (2014)	NA	NA
AB11[d]	1	2010	Unknown	Alberta	Canada	Akhavan et al. (2016a)	4	III
AB34[d]	2	2010	Unknown	Alberta	Canada	Akhavan et al. (2016a)	4	III

MB05[d]	2	2010	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	2	III
MB11[d]	1	2011	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	4	III
MB14[d]	1	2011	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	4	III
WRS858[d]	1	1973	Barley	Manitoba	Canada	Serenius et al. (2007)	4	III
SK52[d]	1	2011	Unknown	Saskatchewan	Canada	Akhavan et al. (2016a)	4	III
Pt-Pastorale[d]	1	1976	Barley	Unknown	Denmark	Justesen et al. (2008)	3	II
CBS282.31[d]	2	1931	Unknown	Unknown	Japan	Bakonyi and Justesen (2007)	2	III
CBS281.31[b]	2[c]	1931	Barley	Unknown	Japan	Bakonyi and Justesen (2007)	NA	NA
H-114-1[d]	2	2006	Pasadena	Szombathely	Hungary	This study	4	III
H-137[d]	1	2006	Adagio	Kompolt	Hungary	This study	2	III
H-186[d]	1	2007	Petra	Kölcse	Hungary	This study	3	II
H-190[d]	2	2007	Barley	Kölcse	Hungary	This study	3	II
H-191[d]	2	2007	Barley	Kölcse	Hungary	This study	3	II
H-196[d]	2	2007	Spring barley	Szombathely	Hungary	This study	4	III
H-288[d]	2	2008	20899YH2-PETRA	Martonvásár	Hungary	This study	3	II

			F74-82-				3	II
H-289[d]	2	2008	MANAS-SZD0205	Martonvásár	Hungary	This study		
H-306-1[d]	2	2008	Henley	Szombathely	Hungary	This study	2	III
H-308-2[d]	2	2008	Barley	Székkutas	Hungary	This study	3	II
H-309-2[d]	1	2008	Barley	Márok	Hungary	This study	3	II
H-322[d]	2	2008	Barley	Martonvásár	Hungary	This study	3	II
H-323-2 (CBS123931)[d]	1	2008	Barley	Martonvásár	Hungary	This study	3	II
H-374[d]	2	2008	Wheat	Bőny	Hungary	This study	2	III
H-376[d]	2	2008	Wheat	Márok	Hungary	This study	2	III
H-386-1[d]	2	2009	GK Habzó	Szombathely	Hungary	This study	2	III
H-529[d]	2	2017	Petra	Martonvásár	Hungary	This study	3	II
H-540[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-546[d]	1	2017	Laverda	Martonvásár	Hungary	This study	3	II
H-547[d]	2	2017	Laverda	Martonvásár	Hungary	This study	3	II
H-618[d]	1	2017	KH Zsombor	Martonvásár	Hungary	This study	3	II
H-620[d]	2	2017	KH Hunor	Martonvásár	Hungary	This study	2	III
H-623[d]	2	2017	KH Anatólia	Martonvásár	Hungary	This study	3	II
H-627[d]	1	2017	KG Apavár	Martonvásár	Hungary	This study	3	II
H-630[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-632[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-638[d]	2	2017	Patina	Martonvásár	Hungary	This study	3	II
H-641[d]	1	2017	KG Puszta	Martonvásár	Hungary	This study	2	III
H-642[d]	2	2017	KG Puszta	Martonvásár	Hungary	This study	2	III
H-645[d]	2	2017	KH Tas	Martonvásár	Hungary	This study	2	III
H-647[d]	1	2017	KH Tarna	Martonvásár	Hungary	This study	2	III
H-651[d]	1	2017	Su Ellen	Martonvásár	Hungary	This study	2	III
H-656	1	2017	Monique	Martonvásár	Hungary	This study	NA	NA
H-660[d]	1	2017	Faktor	Martonvásár	Hungary	This study	3	II
H-665[d]	2	2017	GKH 3015	Martonvásár	Hungary	This study	3	II
H-668[d]	2	2017	KH Malko	Martonvásár	Hungary	This study	2	III
H-672[d]	1	2017	KH Kárpátia	Martonvásár	Hungary	This study	2	III

H-675[d]	1	2017	KH Korsó	Martonvásár	Hungary	This study	4	III
H-679[d]	2	2017	Antonella	Martonvásár	Hungary	This study	2	III
H-690[d]	1	2017	Mv Initium	Kompolt	Hungary	This study	3	II
H-732[d]	1	2017	KH Tas	Kompolt	Hungary	This study	3	II
H-733	1	2017	KH Tas	Kompolt	Hungary	This study	NA	NA
H-746[d]	1	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-747[d]	2	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-748[d]	1	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-771[d]	1	2017	KH Korsó	Karcag	Hungary	This study	3	II
H-774[d]	1	2017	KG Puszta	Karcag	Hungary	This study	3	II
H-778[d]	2	2017	Patina	Karcag	Hungary	This study	3	II
H-784	2	2017	KG Konta	Karcag	Hungary	This study	NA	NA
H-785[d]	2	2017	KH Tas	Karcag	Hungary	This study	3	II
H-786[d]	1	2017	KH Tas	Karcag	Hungary	This study	3	II
H-788[d]	1	2017	Mv Initium	Karcag	Hungary	This study	3	II
H-791[d]	2	2017	KH Anatólia	Karcag	Hungary	This study	3	II
H-798[d]	1	2017	KH Zsombor	Karcag	Hungary	This study	3	II
H-802	2	2017	Antonella	Karcag	Hungary	This study	NA	NA
H-804	1	2017	KH Hunor	Karcag	Hungary	This study	NA	NA
H-815[d]	1	2017	KH Tarna	Karcag	Hungary	This study	2	III
H-826[d]	2	2017	GKH 3015	Karcag	Hungary	This study	3	II
H-835[d]	2	2017	KH Zsombor	Szombathely	Hungary	This study	4	III
H-848[d]	2	2017	GKH 3815	Szombathely	Hungary	This study	3	II
H-850[d]	2	2017	GKH 3815	Szombathely	Hungary	This study	3	II
H-855[d]	1	2017	LGBB14W232-11	Szombathely	Hungary	This study	3	II
H-867[d]	1	2018	KG Puszta	Karcag	Hungary	This study	3	II
H-874[d]	1	2018	KH Tas	Karcag	Hungary	This study	3	II
H-883[d]	1	2018	KH Korsó	Karcag	Hungary	This study	3	II
H-890[d]	2	2018	KH Zsombor	Karcag	Hungary	This study	3	II
H-893[d]	1	2018	Patina	Karcag	Hungary	This study	3	II
H-897[d]	2	2018	Mv Initium	Karcag	Hungary	This study	3	II
H-906[d]	1	2018	GKH 3015	Karcag	Hungary	This study	3	II

H-912[d]	1	2018	Siberia	Karcag	Hungary	This study	3	II
H-919	1[c]	2018	KG Konta	Kompolt	Hungary	This study	NA	NA
H-920[d]	2	2018	KG Apavár	Kompolt	Hungary	This study	3	II
H-922[d]	2	2018	KG Apavár	Kompolt	Hungary	This study	3	II
H-932[d]	1	2018	Patina	Kompolt	Hungary	This study	3	II
H-936[d]	2	2018	KH Tarna	Kompolt	Hungary	This study	3	II
H-944[d]	2	2018	Siberia	Kompolt	Hungary	This study	3	II
H-949[d]	2	2018	KWS Meridian	Kompolt	Hungary	This study	3	II
H-955	2	2018	Faktor	Kompolt	Hungary	This study	NA	NA
H-958[d]	2	2018	GKH 3015	Kompolt	Hungary	This study	3	II
H-961[d]	2	2018	KWS Meridian	Karcag	Hungary	This study	4	III
H-970[d]	2	2018	Boreale	Kompolt	Hungary	This study	3	II
H-974[d]	1	2018	KH Kárpátia	Martonvásár	Hungary	This study	3	II
H-977[d]	2	2018	KG Konta	Martonvásár	Hungary	This study	3	II
H-981[d]	2	2018	KG Puszta	Martonvásár	Hungary	This study	3	II
H-995[d]	1	2018	KH Zsombor	Martonvásár	Hungary	This study	3	II
CG16001	1	2016	Disa	Napier	RSA	This study	NA	NA
CG16002	1	2016	Aghulas	Napier	RSA	This study	NA	NA
CG16004[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16005[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16006[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16007[d]	1	2016	Aghulas	Napier	RSA	This study	4	III
CG16008	1	2016	Aghulas	Napier	RSA	This study	NA	NA
CG16009[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16010[d]	2	2016	Erica	Caledon	RSA	This study	4	III
CG16011[d]	1	2016	Erica	Caledon	RSA	This study	2	III
CG16013[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16014[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16015[d]	1	2016	Rye grass	Caledon	RSA	This study	4	III
CG16016[d]	2	2016	Erica	Caledon	RSA	This study	2	III
CG16017[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16018[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16019[d]	1	2016	Erica	Caledon	RSA	This study	2	III

CG16021[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16023[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16024[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16028[d]	2	2016	1070	Caledon	RSA	This study	4	III
CG16029[d]	1	2016	1069	Caledon	RSA	This study	4	III
CG16030[d]	1	2016	1065	Caledon	RSA	This study	4	III
CG16031	1	2016	1055	Caledon	RSA	This study	NA	NA
CG16032[d]	1	2016	1005	Caledon	RSA	This study	4	III
CG16034[d]	1	2016	999	Caledon	RSA	This study	2	III
CG16035[d]	2	2016	1000	Caledon	RSA	This study	4	III
CG16036[d]	1	2016	995	Caledon	RSA	This study	4	III
CG16037[d]	2	2016	992	Caledon	RSA	This study	2	III
CG16038[d]	1	2016	744	Caledon	RSA	This study	4	III
CG16040[d]	2	2016	736	Caledon	RSA	This study	4	III
CG16041	1	2016	722	Caledon	RSA	This study	NA	NA
CG16043[d]	1	2016	4	Caledon	RSA	This study	2	III
CG16044[d]	2	2016	394	Caledon	RSA	This study	4	III
CG16047[d]	1	2016	407	Caledon	RSA	This study	2	III
CG16048[d]	1	2016	Erica	Rietpoel	RSA	This study	2	III
CG16049[d]	2	2016	LE 18	Rietpoel	RSA	This study	2	III
CG16050	2	2016	LE 12	Riviersonderend	RSA	This study	NA	NA
CG16051[d]	2	2016	Erica	Greyton	RSA	This study	2	III
CG16052[d]	2	2016	Hessequa	Greyton	RSA	This study	2	III
CG16054	1	2016	Elim	Greyton	RSA	This study	NA	NA
CG16055[d]	1	2016	Elim	Greyton	RSA	This study	2	III
CG16056[d]	1	2016	S16	Greyton	RSA	This study	2	III
CG16057[d]	1	2016	LE 16	Greyton	RSA	This study	4	III
CG16061[d]	1	2016	Erica	Napier	RSA	This study	4	III
CG16062[d]	1	2016	Elim	Napier	RSA	This study	4	III
CG16063[d]	1	2016	S16	Napier	RSA	This study	4	III
CG16064[d]	2	2016	LE 3	Napier	RSA	This study	4	III
CG16065[d]	1	2016	LE 16	Napier	RSA	This study	2	III
CG16067[d]	2	2016	Erica	Protem	RSA	This study	2	III

CG16068[d]	1	2016	Nemesia	Protem	RSA	This study	4	III
CG16070	1	2016	LE 12	Protem	RSA	This study	NA	NA
CG16072[d]	1	2016	LE 17	Protem	RSA	This study	4	III
CG16073[d]	1	2016	Erica	Klipdale	RSA	This study	2	III
CG16075[d]	1	2016	Nemesia	Klipdale	RSA	This study	4	III
CG16076[d]	2	2016	LE 8	Klipdale	RSA	This study	4	III
CG16077[d]	2	2016	LE 10	Klipdale	RSA	This study	4	III
CG16078[d]	1	2016	LE 12	Klipdale	RSA	This study	4	III
CG16079[d]	2	2016	LE 18	Klipdale	RSA	This study	2	III
CG16081[d]	1	2016	LE 22	Klipdale	RSA	This study	4	III
CG16082[d]	1	2016	LE 25	Klipdale	RSA	This study	2	III
CG16083[d]	2	2016	Erica	Bredasorp	RSA	This study	2	III
CG16084[d]	1	2016	Nemesia	Bredasdorp	RSA	This study	4	III
CG16086	1	2016	Elim	Bredasdorp	RSA	This study	NA	NA
CG16088	1	2016	LE 9	Bredasdorp	RSA	This study	NA	NA
CG16089[d]	2	2016	LE 13	Bredasdorp	RSA	This study	4	III
CG16090[d]	1	2016	LE 13	Bredasdorp	RSA	This study	4	III
CG16091	1	2016	LE 15	Bredasdorp	RSA	This study	NA	NA
CG16092	1	2016	LE 16	Bredasdorp	RSA	This study	NA	NA
CG16093[d]	1	2016	LE 25	Bredasdorp	RSA	This study	4	III
CG16094	1	2016	LE 23	Bredasdorp	RSA	This study	NA	NA
CG16095[d]	1	2016	LE 3	Bredasdorp	RSA	This study	4	III
UPSC1838[d]	1	1986	Oat	Unknown	Sweden	Bakonyi and Justesen (2007)	2	III

[a] Mating type of the isolate

[b] Included only in distance based cluster analysis and hybrid specific PCR amplification

[c] *Ptm* mating type

[d] MLGs included in all analyses except hybrid specific PCR amplification

[e] Original research article describing the isolates

DAPC The cluster number resulted from DAPC analysis

STR The cluster number assigned to clusters resulted from the K=3 STRUCTURE model

NA Not included in Neighbor-net network, DAPC and STRUCTURE analysis

Supplementary Table S2. Details of the most contributing DArTseq™ marker annotations for the DAPC and PCA

Marker	Marker Sequence	E value	Gene/locus	Protein	Accession of reference genome	*Ptt* strain
41804355	TGCAGATCCTGTCTGACTTTGCAATTCGAGTTGATCG CAAGCGCTAGTTGTAGTTCTTGAGTGCTGAGA	3.30E-08	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28945199	TGCAGATCGCCAGCTATTGCGAGCGGCAAACGCCTT GCCTGCATGCAACTAACCGGCAGCTCACGTGAC	NA	NA	NA		
28945202	TGCAGCCCTGCGACGTCGCCGTGTTTGCACCTCTGAA AGCAGCTTACCGGGAGCAAGTCGAATGACTTG	6.50E-07	PTT_17416 PTT_06721	Hypothetical protein (DDE-1 domain-containing protein)	AEEY01000000	0-1
41804358	TGCAGGCTGATGTATAAGTCTGTGTACTCAGTCTCAG AGCAGTCGTACTGCCATCCAGAAGTGGGGAAC	1.60E-04	PTT_17416 PTT_06721	Hypothetical protein (DDE-1 domain-containing protein)	AEEY01000000	0-1
36347108	TGCAGGTCAAGAAGATACCAAGGCCAAAGTGTGACG CTACAATAGACCACCTTCTGCCGATCACCTTGT	NA	NA	NA	NA	NA
36347128	TGCAGTAGCAGAGCAGGAGAGACCCTAAACCGCGAC AGCTTCTGTGTCGAGACGCGGTAAGAGCCTTCA	NA	NA	NA	NA	NA
41804360	TGCAGTGAGCTTTTGTCCAGCATGAACGGAGCCTTCG ATCAAAGCCACCAGACCAATTATGCTATGCAT	NA	NA	NA	NA	NA
28946425	TGCAGCAAGACACAATGTCCCTGAACTTACAGATCG GAAGAGCGGTTCAGCAGGAATGCCGAGACCGAT	NA	NA	NA	NA	NA
28945458	TGCAGTCGCAACTCACCTTTGGTAAGGACGCGATGC CTATCTAGGGCTAGCACTGTTTACGGTCACCCA	NA	NA	NA	NA	NA
28945448	TGCAGATCTATTGCTCCGCGCTCGTGTTCGCACCAGA GAGGAGCCTGATTCGACAAACCTTTGTAGACC	NA	NA	NA	NA	NA
28945774	TGCAGCACCACCTTGACGTACTGCTGCATTCTGTGCA GTCGCTGCATTTCGACTTCTCCAGAAAGGTTG	NA	NA	NA	NA	NA
28945775	TGCAGCACTAAGTTACGTTTCTTGCCGTCCACGAGTG GTTCTACACTAGCGGACTTGCATCAAGGATAG	NA	NA	NA	NA	NA
28946079	TGCAGCTATATGGGTGTGTATAATAATAAAGTGTGG TAGCGATAGCCGTACCTGAGTAGGTCTTAGCAA	7.00E-04	PTT_07236	Uncharacterized protein	AEEY01000000	0-1
28945457	TGCAGGTTTTGTCTCCTTGTCCTGTCAAGAGTACGAG CATCCTGCTTCATGATCAGATTGGGTAGCGAC	0.017	PTT_06709	Uncharacterized protein	AEEY01000000	0-1
28945782	TGCAGTATTAGGACTGCTTTCTGAAAATTGTGAACCG AGTAGTCCGGGGCAACCAGCGTCGCATGATTA	NA	NA	NA	NA	NA
28945785	TGCAGTGCGTTGCCGTAGTATTCACCCTGCGCGTTGA TGTCGGCGTGCATGTCAATCAACACCTGAGCA	2.10E-09	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36347130	TGCAGTTCGATGGACTGGCGACATGAGCTCAGTAAG CGGAATATCTGTGAGTGCATTTACACCCATAAC	NA	NA	NA	NA	NA
28948860	TGCAGCAAGGACTCTCCATAGGTATTATTACAGATC GGAAGAGCGGTTCAGCAGGAATGCCGAGACCGA	NA	NA	NA	NA	NA

36347037	TGCAGCTATCACGACTGCTTCTAAGCTATATACTAGT GGTCGGCAAGGCCGAATACCGTAAGACTATGT	NA	NA	NA	NA	NA
28945446	TGCAGAAGCAGAGCAGGAGACCCCAAACTGTGACA GTACAAGATGTAGTGAAAAAATAAGTTTGGTATC	NA	NA	NA	NA	NA
28946771	TGCAGGACTTACTAGCGCAGTCAATCGACTCCTTGA GGCAGGATGCGACATCAACGAGAAAGACAGCAA	2.20E-08	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28949273	TGCAGGCAGCTTCAGTTAGAGGCCACGAGCAGGTGG TCAAGATGCTGCTCGACGCGGGCGCCGAAGTTA	1.6E-5	PTT_17957 PTT_08880	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36347080	TGCAGAACCACTATAGTTCAGGCAATTACAGATCGG AAGAGCGGTTCAGCAGGAATGCCGAGACCGATC	NA	NA	NA	NA	NA
28946327	TGCAGTAACACCATCCATAGGTACCTCCCACTTACCC GTAACCTGCGTTTCCAGCTCCCTAGACCGAAT	NA	NA	NA	NA	NA
28946069	TGCAGAAAAGCTCTTCCTGTAATCCACTGCGATTTCC ATGCCATCCCATATATCTCGTCGCGCGCGGAG	4.40E-10	PTT_07238	Hypothetical protein (SET domain-containing protein)	AEEY01000000	0-1
28946772	TGCAGGAGCGGGCCATAAAGGCTGGTGCTGTGTCAG GAGTGAGAAAAGACACAATGGTCAACATTGCAG	7.90E-08	PTT_16779	Uncharacterized protein	AEEY01000000	0-1
28945780	TGCAGGGGCTAAGTTGAAACTCAAAAGATAGCAGCA CTCCTACGAACGCATCAAAAGTAACTTTCTATA	NA	NA	NA	NA	NA
28947083	TGCAGGTTGCCGCGTGCCAAGGAGCTGCTAGTTGCG CACCGGCAGTCAGACATATTCTACGACCTTGCT	0.75	JQ582646	Pyrenophora teres f. teres isolate ND89-9 nonribosomal peptide synthetase 2 (NPS2) gene	NA	13A
28945784	TGCAGTGCCAGCCAGAAAGTCGTTTTCGTTATTCGCT AGAATACTAGAGCTATACTTGCAACGTTTCAT	NA	NA	NA	NA	NA
28948294	TGCAGCTGCGTCGGACTTAGACGCGTCCGACTCATCC ACCATAGACGATCCGGAGATAAATATAGCGCA	NA	NA	NA	NA	NA
28948801	TGCAGGTAGTCAAGACACTGTTCGACGCGGGCGCCG AAGTCAACGCGCAGGGTGGATACTACGGCAACG	6.90E-06	PTT_06711	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28946767	TGCAGATGAAAAGTGTTTGTGCGCGACATGTAGCAA GAGCGTAGCATCGACGAATACTGTAGAACAAGC	4.80E-09	PTT_07238	Hypothetical protein (SET domain-containing protein)	AEEY01000000	0-1
28945777	TGCAGCTCTTATTCTCCTAGCACGTTAGTTTCCGACG CTAAAAAAGCGCATCGTTGACCGTGCTGTCGC	NA	NA	NA	NA	NA
28946773	TGCAGGCAGCTTCAGCTGAAGGCCACGAGCAGGTGG TCAAAATGCTGCTCGACGCGGGCGCCGACGTCA	3.4E-5	PTT_17957 PTT_08880	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28946082	TGCAGGGTCTCTCACTATTATAGACCTGACTGATCCT GTCATCGACGCTGATTCTACCTGCGTGCTCTT	7.60E-05	PTT_09544	Uncharacterized protein	AEEY01000000	0-1
28947077	TGCAGAAAAAAAAGGCGGCACTGCGTCAGGAGACT GCTCCACGCCACCGACTAGGGTTCCAGATCTAAT	NA	NA	NA	NA	NA
28947744	TGCAGACTACAAGACTCGAATTCCGGCTCTATTTTTT GAAACGATTTGGGATACTTCGGTCTTTCGTAA	0.0018	PTT_19103	Hypothetical protein (DUF1996 domain-containing protein)	AEEY01000000	0-1
28946403	TGCAGGTACCCACATTGTAAGGGGTGAGGACTAGAG TAAGACTAGGTACGCATCTACATAATCCTTATT	NA	NA	NA	NA	NA

36349731	TGCAGAACCTGCCTGCACCTCTTCAATGAACATGATG AGAGAAAGAACTGGCACATTGCTTTCGATATC	NA	NA	NA	NA	NA
28947411	TGCAGCTAGGACGCTGATTATACCGAGTAGACTAGG CTTGAGGTAAGAGTGAAAAAGCCCGGTAGAGCT	NA	NA	NA	NA	NA
36349088	TGCAGGGTCTGTTATGCGACCTATGAGCGATGCAGA GAAGAGGGAGCTAGGGTTCAGATGAGATTTTAT	NA	NA	NA	NA	NA
28947086	TGCAGCGCCAGATTAGATGAGGTCTAATGGGATCAA TGCCCATAACTAGCAGGTGATTGCTGAGTATAT	NA	NA	NA	NA	NA
28946426	TGCAGTGCGTTGCCGTAATCTCCACCCTGCGCGTTGA CGCCCGCGTCGAACAGTGTCTTGACTACCAGC	0.0011	PTT_06711	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36350466	TGCAGCAGAAAGAGATGCGTTGTGTCTCTATCAACA ATTTCTTGCTGACTCTGCTTACAGATCGGAAGA	NA	NA	NA	NA	NA
36350467	TGCAGGCTGGGGAGCATGGAAAGACGCTCTACGTTA CTAATGATAGACTTACAGATCGGAAGAGCGGTT	1.80E-05	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28947745	TGCAGTCGATGCCGTCTAAGGCTTTATTACAGATCGG AAGAGCGGTTCAGCAGGAATGCCGAGACCGAT	NA	NA	NA	NA	NA
28946404	TGCAGAGCAAACGATGATATACAATAGTCAGTCCTG TAGCAAGCACCATTATGCATGCCTGTCCAATAC	NA	NA	NA	NA	NA
28946073	TGCAGTAAGCGGAGACCGATCGGAGAGTAACCCCGC CTCTATTGCAAAGGCGAATGTGTCCGTGCTAAT	NA	NA	NA	NA	NA
28948009	TGCAGATCCCAGCCGAGCGCCCTGCTTACGCCACCTC AACGTCGGCCAAGACAAACAACGGGCGCATCA	NA	NA	NA	NA	NA
28946407	TGCAGGCTACCCCCACACGCCAAGAACCAATCGGTT CCAGTCTGAAAACCGTACAGCCAGTCTTGAATG	NA	NA	NA	NA	NA
28948861	TGCAGTCGCTACTCACCTTTGGTAATGGAACGATGCC TATCCAGGACTAGTGCTGTTTACGGTCACTCA	8.40E-33	NA	NA	NA	NA
36349342	TGCAGCCCTAAATTAGAGGCTAAAATGTATGATTCCT ATGAGTCTTACAGATCGGAAGAGCGGTTCAGC	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948597	TGCAGGCTTGAAACCCGACTTATCGAAGATTACAGA TCGGAAGAGCGGTTCAGCAGGAATGCCGAGACC	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948331	TGCAGACACGAACTATAGCCTATCTTTATTACAGATC GGAAGAGCGGTTCAGCAGGAATGCCGAGACCG	9.70E-08	NA	NA	NA	NA
28947406	TGCAGTGTGTGGATGAGATCGGATCTCCTCACGTTCT TGACTCACTTACAGATCGGAAGAGCGGTTCAG	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28946408	TGCAGCATTTCAACCTGATTGCGAGCGAAAGTCTCG ATGTCGGCAGTGACGTTCTTGGTCTGGATCTGG	8.40E-33	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28949340	TGCAGTCTGCGTTGTGCACTCTCCTGTCCTTCGCCAT ACGCGGTGGGCATAGAGACACCAAGAATCCCA	7.20E-21	PTT_08524	Hypothetical protein (AAA domain-containing protein)	AEEY01000000	0-1
36350313	TGCAGATGTAGGAAGCACAAGCCTAAAGCTATATTA CAGATCGGAAGAGCGGTTCAGCAGGAATGCCGA	0.09	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1

28948798	TGCAGATCTAAAGCCCGTTCTGGCATTATCGTGTCGATTTAGAGGTCCAGAAATCCGGAACTTACAGAT	0.023	PTT_01845	Hypothetical protein (MFS domain-containing protein)	AEEY01000000	0-1
36351780	TGCAGCTTGAGCCATTTGAAAGTGAGTGCCGTGCAGGAAGGCAACTCGTGAAGGACGTAGAGACGAAGA	8.40E-33	PTT_16779	Uncharacterized protein	AEEY01000000	0-1
28948601	TGCAGTATGCGTGTCGTTATTGGGTCGATCATCTTACAGATCGGAAGAGCGGTTCAGCAGGAATGCCGA	3.80E-07	NA	NA	NA	NA
28949991	TGCAGTGCTTGGGTTTCTCTAGATAAACGAGGAATAGCTAGGGTTTACAGATCGGAAGAGCGGTTCAGC	2.80E-17	NA	NA	NA	NA
36347554	TGCAGAAAGAGAGACGGAAGCTACAAATGAGCTTACAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG	0.0015	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948607	TGCAGACCTATCAATTGTAGACTCCGAGAAAGAGAGAGAGAGAGAGAAAGAGTGGGAGACTTACAAC	1.7	JQ837863	Pyrenophora teres f. teres isolate 13A glyceraldeyde-3-phosphate dehydrogenase-like protein (GPD1) gene	NA	13A
28947400	TGCAGAAGTATCAATTGTAGACTCAGGGGAAGAGAGAGAGAGAGAAAGAGTGGGAGACTTACAACAACA	0.12	JX900133	Endo-1,4-beta-xylanase A mRNA	AEEY01000000	0-1
36351843	TGCAGCCACCGGTTGAAGTTAGCCCGCCTAGTTACGCGCGACGCAACCAGGCGCTCACCAATACAACTA	0.061	KX578221	Cytochrome P450 lanosterol 14 alpha-demethylase (CYP51A) gene	OCTH00000000	W1-1

E Expected value indicating the possibility of finding an alignment with the reference genome by random chance

NA No significant alignment was observed with the reference genome

Supplementary Table S3. Chi square and PHI test values for subdivisions in Australia, regions in Hungary and RSA

Country/subdivisions based on DAPC	Number of isolates	Number of *MAT1-1*	Number of *MAT1-2*	Chi square value	*P* value	PHI test mean	*P* value
Australia	100	47	53				
Cluster_1	31	15	16	0.133	0.715	0.576	0.358
Cluster_2	53	24	29	0.472	0.492	0.641	0.014 [a]
Cluster_3	16	15	11	2.250	0.134	0.518	0.806
Hungary	78	37	41				
Cluster_1	23	9	14	1.087	0.297	0.614	4.8E-4 [c]
Cluster_2	23	13	10	0.391	0.532	0.695	0.221
Cluster_3	32	13	19	1.125	0.289	0.696	0.007 [b]
RSA	59	39	20				
Cluster_1	19	10	9	0.053	0.819	0.610	0.511
Cluster_2	12	10	2	5.333	0.021[a]	0.553	0.246
Cluster_3	28	18	10	2.286	0.131	0.649	0.310

[a] Significant at $P \leq 0.05$

[b]Significant at $P \leq 0.01$

Supplementary Table S4. Indices of genetic diversity for *Pyrenophora teres* f. *teres* populations from Australia, Hungary and Republic of South Africa (RSA) based clusters detected in DAPC

Country/subdivisions based on DAPC	*n*[a]	eMLG[b]	H[c]	1-λ[d]	H_{exp}[e]
Australia					
Cluster_1	31	16	0.745	0.968	0.200
Cluster_2	53	16	0.863	0.981	0.213
Cluster_3	16	16	0.702	0.938	0.187
Australia total	100	16	0.890	0.990	0.255
Hungary					
Cluster_1	23	23	0.730	0.958	0.188
Cluster_2	23	23	0.710	0.955	0.207
Cluster_3	32	23	0.797	0.969	0.204
Hungary total	78	23	0.935	0.987	0.279
RSA					
Cluster_1	19	12	0.694	0.941	0.251
Cluster_2	12	12	0.629	0.923	0.173
Cluster_3	28	12	0.627	0.966	0.255
RSA total	59	12	0.859	0.983	0.224

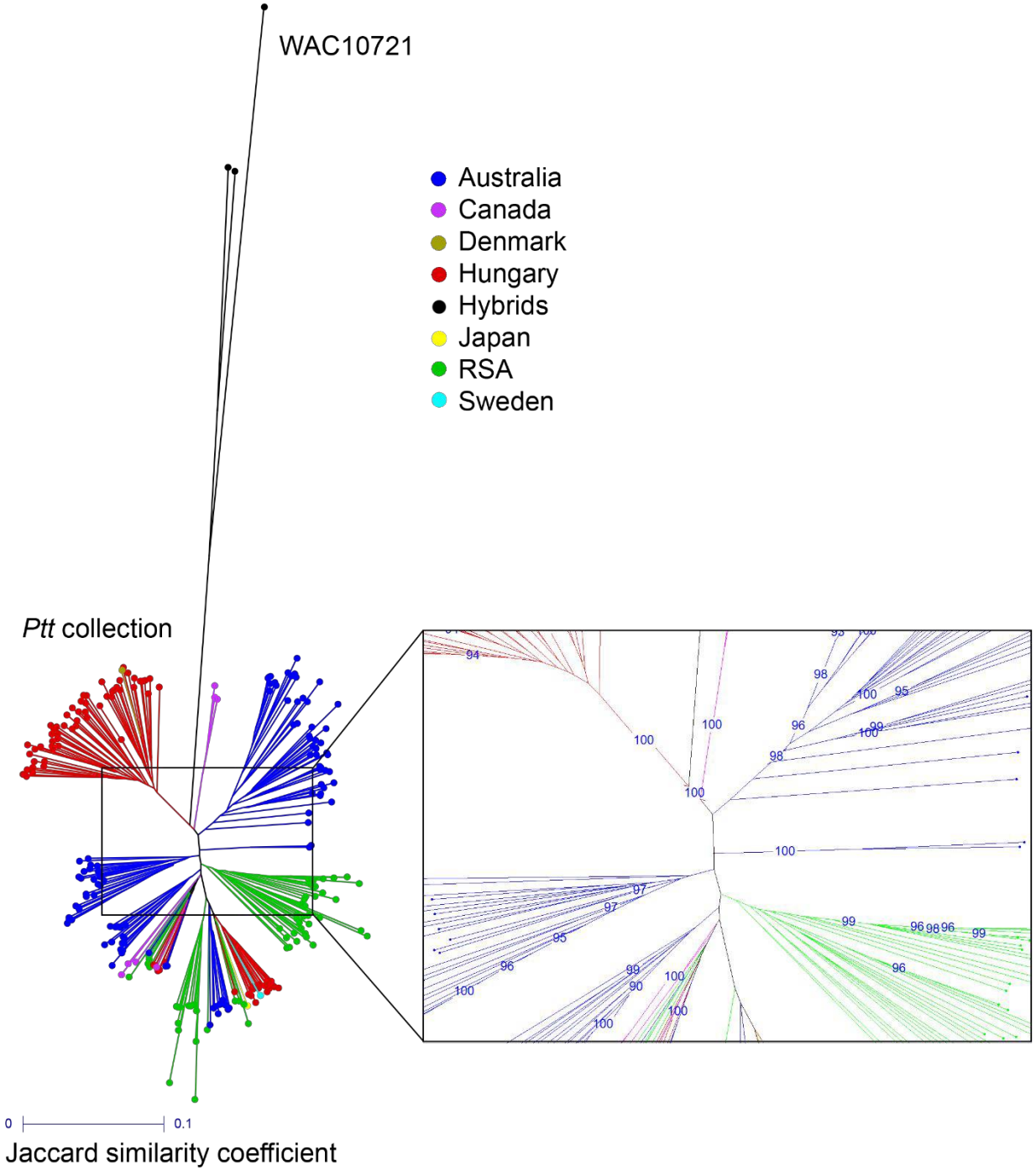
[a] Number of isolates

[b] The number of expected MLG based on rarefaction at the smallest sample size of ≥10

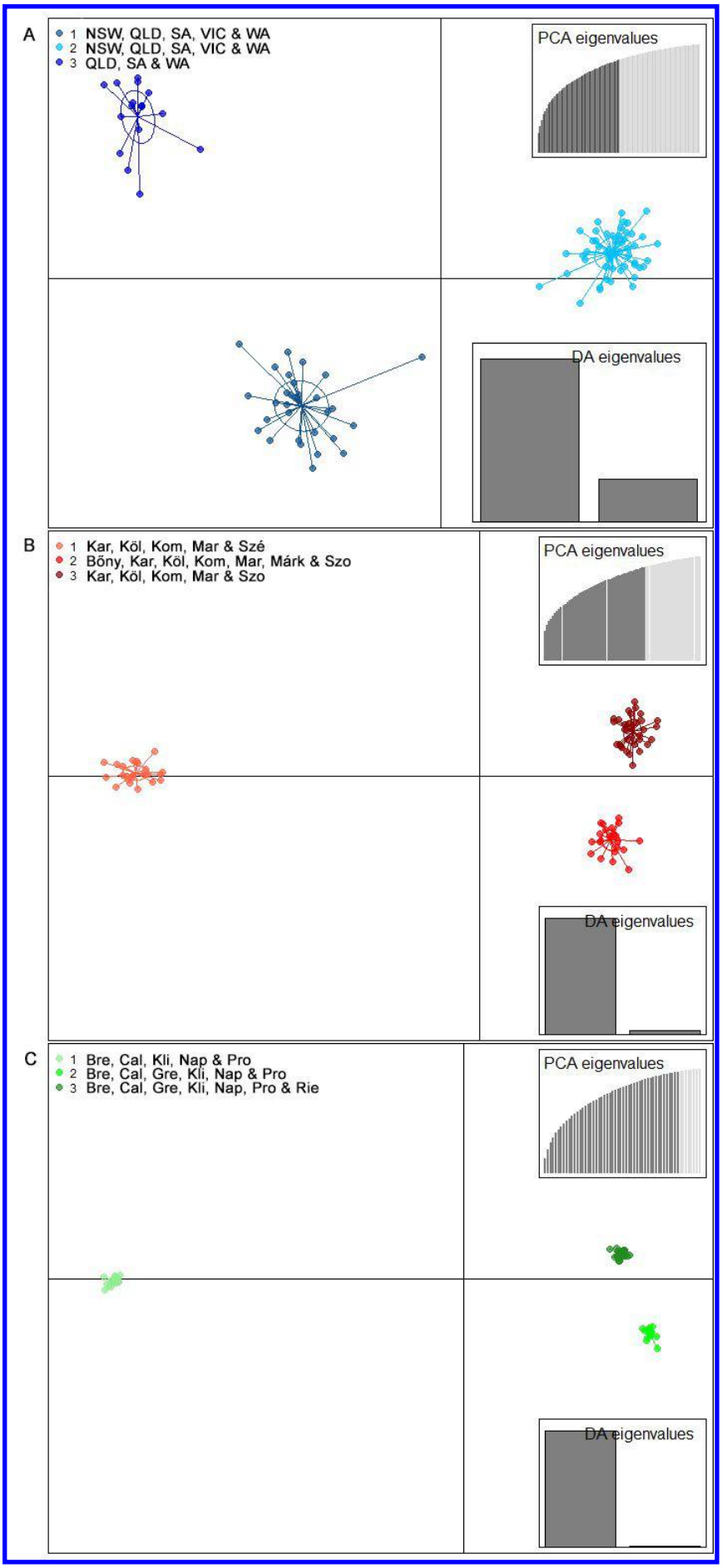
[c] Normalised Shannon-Wiener index of MLG genotypic diversity, the genotypic diversity of the population by richness and relative abundance in a defined location

[d] Simpson's complement index of multilocus genotypic diversity, the probability of two random isolates drawn from a subpopulation to be of a different genotype

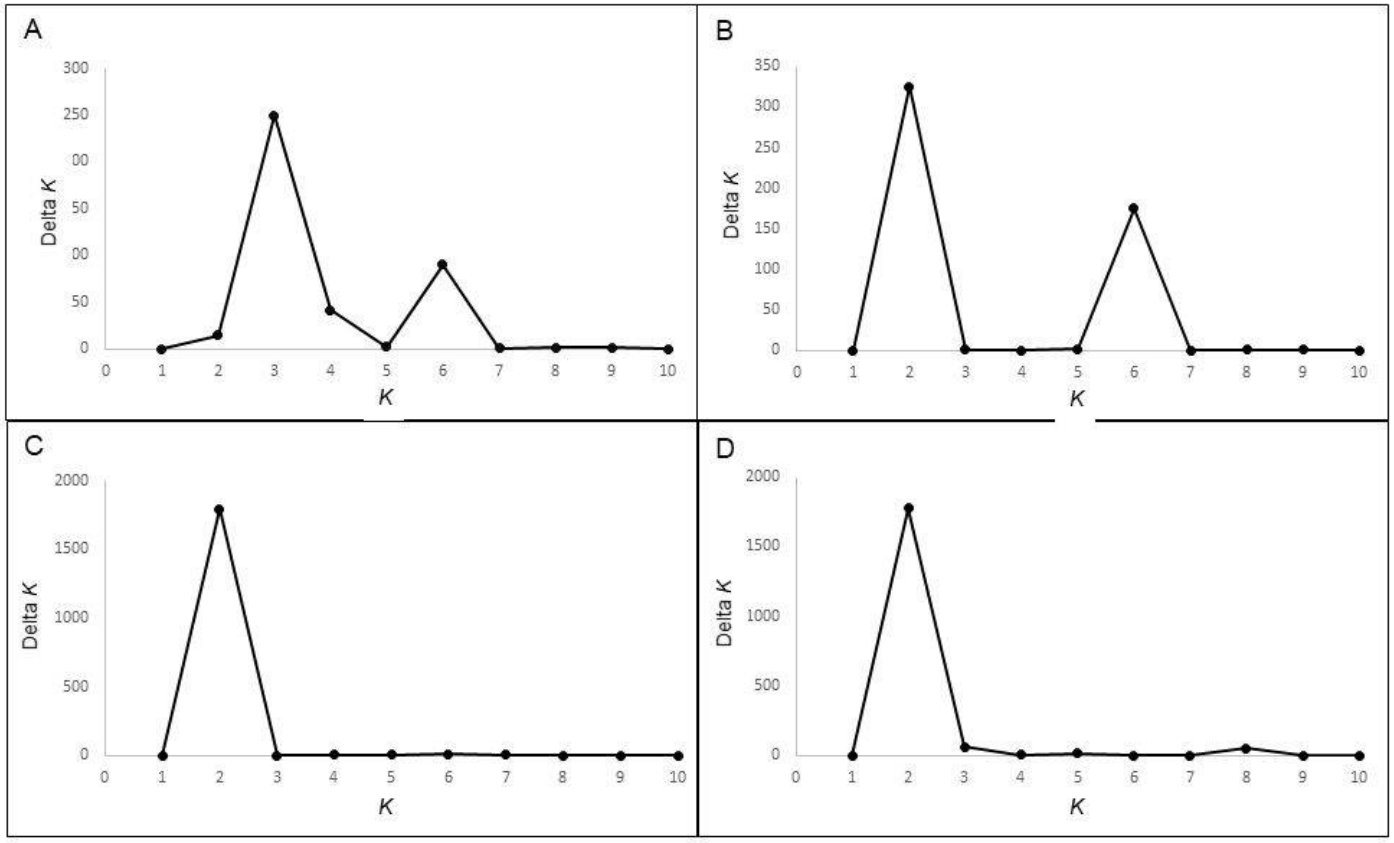
[e] Nei's unbiased gene diversity, the probability that two randomly chosen alleles are different



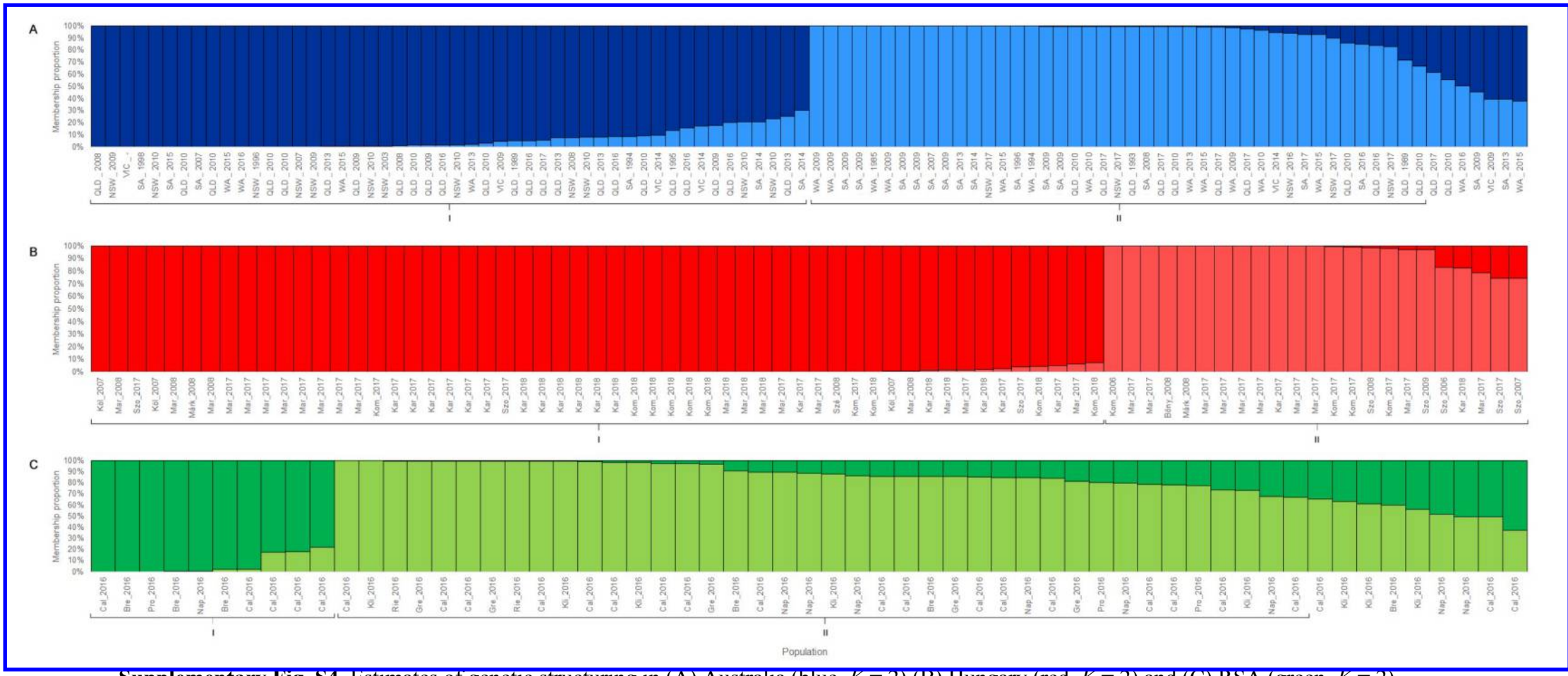
Supplementary Fig. S1. Neighbor-joining clustering with bootstrapping (≥90%) based on DArTseq™ data following Jaccard similarity coefficient for *Pyrenophora teres* f. *teres* isolates from Australia (*n* = 101), Canada (*n* = 7), Denmark (*n* = 1), Hungary (*n* = 79), Japan (*n* = 2), Republic of South Africa (RSA) (*n* = 59) and Sweden (*n* = 1).


A
1 NSW, QLD, SA, VIC & WA
2 NSW, QLD, SA, VIC & WA
3 QLD, SA & WA
PCA eigenvalues
DA eigenvalues
B
1 Kar, Köl, Kom, Mar & Szé
2 Bőny, Kar, Köl, Kom, Mar, Márk & Szo
3 Kar, Köl, Kom, Mar & Szo
PCA eigenvalues
DA eigenvalues
C
1 Bre, Cal, Kli, Nap & Pro
2 Bre, Cal, Gre, Kli, Nap & Pro
3 Bre, Cal, Gre, Kli, Nap, Pro & Rie
PCA eigenvalues
DA eigenvalues

Supplementary Fig. S2. Discriminant analysis of principal components of *Pyrenophora teres* f. *teres* populations collected from (A) Australia, (B) Hungary and (C) Republic of South Africa (RSA). The distribution of the eigenvalues of principal component analysis (PCA) and discriminant analysis (DA) indicate that the first two principal components adequately explain > 50% of the genetic structure of the clusters. States of Australia; NSW- New South Wales, QLD-Queensland, SA- South Australia, VIC-Victoria and WA- Western Australia. Regions of Hungary; Kar- Karcag, Köl-, Kölcse, Kom- Kompolt, Mar- Martonvásár, Márk- Márok, Szé- Székkutas and Szo- Szombathely. RSA regions; Bre- Bredasdorp, Cal- Caledon, Gre- Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.



Supplementary Fig. S3. The optimum number of clusters (*K*) for (A) the entire *Pyrenophora teres* f. *teres* collection, populations from (B) Australia, (C) Hungary and (D) RSA based on delta *K* (Δ*K*) estimated over 10 independent runs.



Supplementary Fig. S4. Estimates of genetic structuring in (A) Australia (blue, $K = 2$) (B) Hungary (red, $K = 2$) and (C) RSA (green, $K = 2$) grouped into optimal clusters using the model-based clustering method in STRUCTURE. Bars represent individual isolates and the colour and height of each bar depicts the estimated membership fraction of each individual into the corresponding cluster. States of Australia; NSW- New South Wales, QLD-Queensland, SA- South Australia, VIC-Victoria and WA- Western Australia. Regions of Hungary; Kar- Karcag, Köl-,

Kölcse, Kom- Kompolt, Mar- Martonvásár, Márk- Márok, Szé- Székkutas and Szo- Szombathely. RSA regions; Bre- Bredasdorp, Cal- Caledon, Gre- Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.