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Invasions of gladiolus rust are caused by a widely-distributed clone of *Uromyces transversalis*

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Uromyces transversalis, the causal agent of Gladiolus rust, is an invasive plant pathogen in the United States and is regulated as a quarantine pathogen in Europe. The aim of this research was to: (i) determine the origin of introductions of *U. transversalis* to the United States, (ii) track the movement of genotypes, and (iii) understand the worldwide genetic diversity of the species. To develop molecular markers for genotyping, whole genome sequencing was performed on three isolates collected in the United States. Genomes were assembled *de novo* and searched for microsatellite regions. Primers were developed and tested on ten isolates from the United States resulting in the identification of 24 polymorphic markers. Among 92 isolates collected from Costa Rica, Mexico, New Zealand, Australia, and the United States there were polymorphisms within isolates with no genotypic diversity detected among isolates. The microsatellite loci and flanking regions showed high diversity and two divergent genomes within dikaryotic individuals, yet no diversity among individuals, suggesting that the invasive *U. transversalis* populations from a widely studied geographic area are strictly clonal.

Invasions of Gladiolus Rust are Caused by a Widely-Distributed Clone of *Uromyces transversalis*

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Abstract

Uromyces transversalis, the causal agent of Gladiolus rust, is an invasive plant pathogen in the United States and is regulated as a quarantine pathogen in Europe. The aim of this research was to: (i) determine the origin of introductions of *U. transversalis* to the United States, (ii) track the movement of genotypes, and (iii) understand the worldwide genetic diversity of the species. To develop molecular markers for genotyping, whole genome sequencing was performed on three isolates collected in the United States. Genomes were assembled *de novo* and searched for microsatellite regions. Primers were developed and tested on ten isolates from the United States resulting in the identification of 24 polymorphic markers. Among 92 isolates collected from Costa Rica, Mexico, New Zealand, Australia, and the United States there were polymorphisms within isolates with no genotypic diversity detected among isolates. The microsatellite loci and flanking regions showed high diversity and two divergent genomes within dikaryotic individuals, yet no diversity among individuals, suggesting that the invasive *U. transversalis* populations from a widely studied geographic area are strictly clonal.

Introduction

Gladiolus rust, caused by the fungus *Uromyces transversalis,* was first identified in South Africa by von Thümen in 1876; however, little is known about the genetic diversity, center of origin, or historical dispersal patterns of *U. transversalis*. It was not until about a century after it was initially described that the fungus invaded northern Africa and then southern Europe in 1966 (Fig. 1), reaching England by 1996 (Beilharz et al., 2001). Subsequently, Gladiolus rust was detected in Argentina in 1979 (Lindquist et al., 1979), Brazil in 1981 (Pita et al., 1981), Australia in 1994 (Beilharz et al., 2001), New Zealand in 1998 (McKenzie, 2000), Mexico in 2004 (Rodríguez-Alvarado et al., 2006), the United States (USA) in 2006 (Blomquist et al., 2007), Cuba in 2010 (Martínez-de la Parte et al., 2011) and Venezuela in 2016 (Mohali and Aime,

2018). *Uromyces transversalis*, the causal agent of Gladiolus rust, can be devastating to species of *Gladiolus* and is difficult to eradicate once established.

The fungus *U. transversalis* is an obligate biotrophic pathogen that grows and reproduces on members of the family Iridaceae in arid, Mediterranean, and tropical climates (Garibaldi and Aloj, 1980; Hernández, 2004; Peterson and Berner, 2009; Rizvi et al., 2007). In regions where Gladiolus rust is established the disease can cause crop losses of 10-100%, unless fungicide applications are used (Beilharz et al., 2001; Ferreira and Nevill, 1989; Hernández, 2004; Littlejohn and Blomerus, 1997; Valencia-Botin et al., 2013). As a consequence, the pathogen is considered of quarantine significance in Europe and was regulated in the USA from 2007 to 2015 (Peterson and Berner, 2009; Rizvi et al., 2007).

Gladiolus flowers are imported into the USA from multiple countries including Mexico, where *U. transversalis* is prevalent in *Gladiolus* production areas (Valencia-Botin et al., 2013). Shipments of *Gladiolus* flowers infected with *U. transversalis* arriving to the United States from Mexico have been repeatedly intercepted at border stations in California and Texas (Brown, 2005; Hernández, 2004; Rizvi et al., 2007; Valencia-Botin et al., 2013), and at a Florida border station with imports arriving from Mexico and Brazil (Schubert et al., 2007). A quarantine and national management plan strategy was followed by both federal and state quarantine officials in an attempt to contain and manage *U. transversalis* in the USA (Rizvi et al. 2007; Valencia-Botin et al. 2013). Despite quarantine measures, severe outbreaks of Gladiolus rust occurred in 2014 in the United States, leading the U.S. Department of Agriculture, Animal and Plant Health Inspection Services (USDA-APHIS) to revise its response requirements in 2015 (USDA-APHIS, 2015).

Uromyces transversalis primarily infects the leaves and stem of its host; however, under heavy inoculum pressure it can also infect the flowers (Ferreira and Nevill, 1989). Visibly infected plants lose economic value as an ornamental cut flower (Valencia-Botin et al., 2013). Infection by the rust fungus reduces the plant's vigor, resulting in reduced flower production (Wise et al., 2004). The initial symptoms of *U. transversalis* on Gladiolus leaves are small chlorotic spots, which eventually break the leaf surface to reveal small yellow-orange uredinia. The uredinia

coalesce to form large lesions (3-7 mm) laterally across the leaf surface (Beilharz et al., 2001; Martínez-de la Parte et al., 2011; Rizvi et al., 2007; Rodríguez-Alvarado et al., 2006; Valencia-Botin et al., 2013). *U. transversalis* produces urediniospores and teliospores, but has no known alternate host (Hernández 2004) or other spore types (Hernández 2004; Rizvi et al. 2007). This suggests that sexual reproduction does not occur in *U. transversalis* due to an incomplete life cycle. As with many rusts, the urediniospores are the dispersal and infection spores. *U. transversalis* spores may be disseminated locally by wind or water splash (Hernandez, 2004). Long-distance dispersal of urediniospores may occur naturally by wind, but it is primarily attributed to human-mediated movement of infected plants, including potted flowers, cut flowers and corms (Beilharz et al., 2001; Wise et al., 2004).

Molecular markers for genotyping isolates are necessary to understand the genetic diversity and historical dispersal patterns of *U. transversalis*. Due to the high variability, multiplexing capacity, ease of reproducibility, and relatively low cost associated with processing a large number of isolates (Frenkel et al., 2012; Leclercq et al., 2007), microsatellite markers are the ideal marker choice for determining the genetic diversity and population structure of *U. transversalis*. The objectives of this research were to: i) develop microsatellite markers to genotype isolates of *U. transversalis*, ii) determine the geographic origin and track the movement of introduced genotypes of *U. transversalis* in the USA, and iii) understand the genetic diversity of *U. transversalis* collected from a wide geographical area in order to understand historical dispersal patterns of this invasive fungus. We hypothesize that *U. transversalis* was introduced into the USA from Mexico, and that the invasive populations have low genetic diversity.

Materials & Methods

Isolate collection and DNA extraction

DNA was extracted from a total of 92 samples of *Uromyces transversalis* (Table 1) in dried leaf tissue obtained from Australia (n = 7), New Zealand (n = 10), Mexico (n = 60), or as fresh, infected leaf tissue collected within the USA (n = 10) or from border interceptions from Costa Rica (n = 5). From each preserved leaf tissue sample, DNA was extracted using a modified genomic DNA mini-prep protocol (Lee et al., 1988). Briefly, multiple uredinia were scraped to remove urediniospores (0.02-0.04 g) using a sterilized scalpel and transferred into 1.5 mL

reaction tubes containing 246.9 µL lysis buffer (150 µL sddH_2O, 25 µL 0.5 M EDTA (pH 8), 25 µL 1.0 M Tris, 43.75 µL 20% SDS solution, 3.15 µL 20 mg/L proteinase K and 0.0025 g $NaHSO_3$) (Thermo Fisher Scientific, Watham, MA). Sample tubes were vortexed for 1 min, incubated at 65 °C for 15 min, and centrifuged (13,978 × *g* for 5 min). The precipitates were discarded and the supernatants transferred to new 1.5 µL microcentrifuge tubes. A solution of 50 µL 7.5 M NH_4OAc (Sigma-Aldrich, St. Louis, MO) was added to each tube, vortexed for 10 s, and tubes were chilled on ice for 15 min. Samples were then centrifuged (13,978 × *g* for 3 min). The supernatants were again transferred to new 1.5 mL microcentrifuge tubes and 175 µL isopropanol was added, tubes were mixed and centrifuged (13,978 × *g* for 5 min), and the supernatant was discarded. The pellets were rinsed twice with 250 µL of 70% ethanol solution, dried and re-suspended with 25 µL sddH_2O, then incubated at 30 °C for 10 min. All samples were stored at -20 °C until further use. DNA extractions from fresh, infected leaf tissue was performed using a modified hexadecyltrimethylammonium bromide (CTAB) protocol (Rogers and Bendich, 1985). Briefly, one to three 1 cm^2 excised pieces of infected leaf tissue were frozen and using a mortar and pestle, ground to a fine powder in liquid nitrogen. DNA extraction buffer (100 mM Tris, pH 7.5; 1% CTAB; 0.7 M NaCl; 10 mM EDTA; 1% 2-mercaptoethanol; 0.3 mg/ml proteinase K) was added to the ground tissue and incubated at 65°C for 30 min, followed by two rounds of chloroform: isoamyl alcohol (1:1) extraction, and precipitated with 2-propanol. DNA was resuspended in TE buffer containing 1 mg/ml RNase.

Three *U. transversalis* isolates collected in the USA were maintained at the USDA Agricultural Research Service, Foreign Disease-Weed Science Research Unit, biosafety level-3 plant disease containment facility at Ft. Detrick, Maryland. These samples were propagated from *U. transversalis*-infected Gladiolus plants collected from California and Florida commercial fields in 2011 and 2014 (Table 1). Prior to extraction using the modified CTAB protocol described above, urediniospores were harvested from infected Gladiolus plants with a microcyclone spore collector (Cheery and Peet 1966; Peterson and Berner 2009; Tervet et al. 1951). Spores were germinated by placing 300 mg of freshly harvested spores in a 23 cm × 33 cm glass container that contained 300 mL of sterile water with 15 µg ampicillin. A sterile wooden applicator stick was used to break up clumps of spores, so that the spores were evenly distributed across the surface of the water. The container was covered and left in the dark overnight (16-18 hours). The

154 germinated spores were then scraped from the surface of the water, blotted dry with sterile-paper
155 towels and stored in -20 °C.
156
157 **Genome sequencing and assembly**
158 Genomic DNA obtained from three isolates (CA11-1, FL11-1, and FL11-2) was standardized to
159 50.0 ng/µL using a nanodrop and sent to the Georgia Genomics Facility (GGF) (University of
160 Georgia, Athens, GA) for library preparation and sequencing using the Illumina MiSeq platform
161 as 300-bp paired ends reads using a 600 cycle cartridge with a NGS library preparation method.
162 The raw forward and reverse reads of each isolate was observed using FASTQC v.11.2
163 (Babraham Bioinformatics Institute). Quality control was performed using FASTX-
164 Toolkit v.3.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/). All reads with a phred score below
165 Q=22 were removed prior to assembly. ABySS v.1.3.6 (Simpson et al., 2009) was used for *de*
166 *novo* assembly of forward and reverse reads into contiguous sequences (contigs) for each isolate,
167 using an optimal K-mer value of 64 determined with multiple assembly trials. Generated contigs
168 were then imported into Geneious v.6.1.8 (Kearse et al., 2012).
169
170 **Microsatellite discovery and marker development**
171 To increase the potential for successful microsatellite marker development, only contigs 200 bp
172 or greater in size with matched pair reads were considered. Contigs and singletons were searched
173 for at least five perfect repeats of trimeric, tetrameric, pentameric, and hexameric motifs in
174 Geneious v.6.1.8 using Phobos v.3.3.12 (Kearse et al., 2012; Mayer, 2006). Mono and
175 dinucleotide repeats were eliminated due to the difficulty of scoring allele differences. Contigs
176 with microsatellites identified using our criteria were aligned using Geneious Align v.6.1.8.
177 default parameters. Microsatellites shared among the three isolates were visually assessed for
178 sequence variation. Those that showed microsatellite repeat number variation among isolates and
179 had at least 50 bp flanking each side of the repeat were considered acceptable for primer design.
180 Primers for amplification of microsatellite loci were designed with Primer3web v.4.0 (Koressaar
181 and Remm, 2007; Rozen and Skaletsky, 1999; Untergasser et al., 2012) to produce amplicons of
182 approximately 180-350 bp in length with an optimal annealing temperature of 59 °C.
183

Sixty primer pairs for candidate microsatellite loci were initially evaluated on the three sequenced isolates CA11-1, FL11-1, and FL11-2 to verify that the PCR worked with the designed primers and that the PCR amplicons were the expected size. PCR was carried out in 10 µL reactions with 1 µL of 10 × ExTaq buffer (Takara Bio Inc., Mountain View, CA), 1 µL of 2.5 mM dNTPs (Takara Bio Inc.), 0.25 µL of 10 µM forward primer, 0.25 µL of 10 µM reverse primer (Integrated DNA Technologies, Coralville, IA), 0.1 µL of ExTaq polymerase (Takara Bio Inc.), 6.9 µL of sterile distilled H_20, and 0.5 µL of 50.0 ng/µL DNA template. Reaction conditions were 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Amplification of PCR products within the expected size range was confirmed by electrophoresis run at 95 V (4.75 V/cm) on a 2% (wt/vol) agarose gel (Alfa Aesar, Haver Hill, MA) for 2.5 hours using a 100 bp size standard (New England Biolabs Inc., Ipswich, MA).

Twenty-five primer sets (Table 2) that successfully amplified the three sequenced isolates were screened for polymorphism on a panel of *U. transversalis* that included seven additional isolates from California (Table 1). A three-primer method (Schuelke 2000) was used in this round of marker evaluation. The forward primer for each candidate marker had a CAG tag (5′-CAGTCGGGCGTCATCA-3′) (Hauswaldt and Glenn, 2003) added to the 5′ end. The third primer consisted of the CAG tag, labeled with a 6FAM fluorescent dye (Invitrogen Inc., Carlsbad, CA) on the 5′ end. PCR was carried out in 12 µL reactions with 1.2 µL of 10 × ExTaq buffer (Takara Bio Inc., Mountain View, CA), 1.2 µL of 2.5 mM dNTPs (Takara Bio Inc.), 0.1 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer (Integrated DNA Technologies, Coralville, IA), 0.5 µL of 10 µM 5′ 6FAM-labeled CAG tag primer (Invitrogen Inc.), 0.1 µL of ExTaq polymerase (Takara Bio Inc.), 7.9 µL of sterile distilled H_20, and 0.5 µL of approximately 50.0 ng/µL DNA template. Reaction conditions were 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Amplification of individual PCR products within the expected size range was confirmed by electrophoresis.

One microliter of a 1:10 dilution of PCR product was added to 0.1 µL of GeneScan 500 LIZ-labeled size standard and 9.9 µL of Hi-Di formamide (Applied Biosystems Inc., Foster City,

CA). Amplicons were denatured by incubation at 95 °C for 5 min and immediately placed on ice. Fragment analysis was conducted at the GGF on an Applied Biosystems 3730xl 96-capillary DNA Analyzer. GeneMapper v.4.0 (Applied Biosystems Inc.) and Geneious v.6.1.8 (Kearse et al. 2012) were used to determine allele sizes based on electropherograms.

Multiplex PCR

Eight primer sets (Table 2, see loci with asterisks) that consistently produced peaks within the expected size range for the 10 isolates were optimized for multiplex PCR. Two multiplex reactions (multiplex 1 – *Ut513*, *UtCA759*, *Ut2648* and *Ut3161*; multiplex 2 – *Ut497*, *Ut1841*, *Ut1908* and *Ut2048*) were developed to increase efficiency and decrease cost for genotyping a large panel of isolates. The forward primers of the microsatellite markers selected for multiplex PCR were labeled at the 5′ end with one of the fluorescent dyes from the DS-33 dye set: 6-FAM (Integrated DNA Technologies), VIC, PET, or NED (Applied Biosystems Inc.). Multiplex reactions were optimized so that loci with alleles of similar size ranges were labeled with different dyes. All 92 samples were genotyped with the eight markers in the multiplex reactions.

Multiplex PCR was conducted using a modified protocol of the Type-it Microsatellite PCR kit (Qiagen, Hilden, Germany) in 10 µL reactions with 5 µL of 2 × Type-it Master Mix buffer, 1 µL of 10 × primer mix (2 µM of each primer in the multiplex), 3 µL of sterile distilled H_20, and 1 µL of approximately 50.0 ng/µL DNA template. Reaction conditions were 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Amplification of PCR products within the expected size range was confirmed by electrophoresis. The PCR products were prepared as described above and sent to GGF for fragment analysis.

Results

Whole genome sequencing and assembly

The Illumina MiSeq PE 300 sequencing platform generated 32,461,280 reads with an average insertion size of 575 bp and read lengths of 301 bp. Sequence quality was assessed by phred score and signal purity filter values resulting in a total of 25,452,492 reads with a PF of 99.26%,

which corresponds to 6.0 to 9.9 million reads for CA11-1, FL11-1, and FL11-2 respectively (Table 3).

The *de novo* draft assemblies resulted in 5,706,372, 4,305,978, and 7,444,849 total assembled reads (contigs) for CA11, FL11-1, and FL11-2, respectively. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accessions PTJR00000000, PTJQ00000000, and PTJP00000000 for CA11, FL11-1, and FL11-2, respectively. Using Geneious v.6.1.8 (Kearse et al., 2012), contigs for each isolate were filtered to select only those 200 bp in length or greater. This resulted in 466,181, 548,017, and 645,533 contigs for CA11, FL11-1, and FL11-2, respectively, which were subsequently used to search for microsatellite repeats (Table 3).

Microsatellite marker development

An alignment of the 18,950 contigs produced 4,296 contigs with potentially informative microsatellite loci shared among the three microsatellite containing isolates. Microsatellite loci shared by at least two of the three isolates were observed in 2,754 for the aligned contigs. Microsatellites were identified in 0.98%, 1.03%, and 1.34% of the contigs, showing that the discovery rate of microsatellites was consistent among isolates.

Sixty sets of primers were developed and screened by PCR on the three isolates of *U. transversalis*. Of the 60 putative markers, 25 were successfully amplified by PCR and evaluated for polymorphism on the panel of ten isolates from the United States (Table 1, Table 2). Isolate CA14-7 consistently resulted in electropherogram peak sizes below our scoring criteria peak height of 500; however, there were peaks at the expected allele size ranges. It is possible that there were PCR inhibitors in the DNA extract or that the DNA was of lower quality than the other samples. Of the 25 markers, seven were monomorphic, with only one allele each, and 18 were polymorphic, with two alleles each (Table 2). All 10 isolates from the United States were the same genotype based on the 25 markers. The polymorphism was identified among alleles within each locus, rather than among individuals. Overall, the microsatellite markers showed allelic diversity, but no genotypic diversity among the isolates from the United States. There was

a high heterozygosity within individuals with each isolate having both alleles for the polymorphic loci (Table 2).

Genotypic analyses

When using the eight microsatellite markers in two multiplex reactions, samples from the USA, Costa Rica and Mexico consistently produced PCR products of the expected size. Samples from New Zealand and Australia produced inconsistently sized PCR products despite duplicate reactions. Using the same eight markers, we attempted to genotype 16 leaf samples from South Africa, but these repeatedly failed to produce PCR products. Only one isolate, PREM 57128, which was sampled in 1998 (Table 1) produced a faint PCR product; however, no peaks were detected in the fragment analysis.

Fragment analysis showed allelic variation within individuals, but no genotypic variation was observed among the isolates from Australia, Costa Rica, Mexico, New Zealand, and the USA. In all cases where peaks were observed and were above the scoring threshold peak size 500, the genotypes were identical to each other and to all isolates from the USA (Table 2). In some cases where the peaks were below the threshold, there was a peak for only one of the alleles or the alleles were a slightly different size; however, these results were not reliable (data not shown). Marker *Ut497* consistently failed to produce peaks or peaks above the acceptable threshold for almost all isolates where DNA was obtained from preserved leaf tissue. The six remaining markers (*Ut513*, *Ut1841*, *Ut1908*, *Ut2048*, *Ut2648* and *Ut3161*) were polymorphic with only two allele sizes observed for each marker, while one marker (*UtCA759*) was monomorphic, producing only one allele size.

Sequence divergence

Visual assessment of the aligned contigs for the three sequenced isolates revealed that there were two distinct haplotypes (nucleotide sequence patterns) for each isolate occurring at nearly all microsatellite loci (Table 4, Supplemental Fig. S1). The variation in repeat number occurred between alleles or haplotypes of the same isolate. Additionally, there were numerous single nucleotide polymorphisms (SNPs) detected in the regions flanking the microsatellite repeats. The two haplotypes within each of the three isolates sequenced were identical to the haplotypes

306 among all three isolates for the loci compared, including microsatellite loci that were
307 monomorphic based on sequence length. Variation in the microsatellite-flanking sequences
308 between haplotypes was estimated for loci with complete data sets for the three isolates. The two
309 alleles differed in nucleotide sequence by 1.6% to 6.9% (Table 4).
310

311 ## Discussion

312 There was no genotypic diversity observed among the *U. transversalis* isolates from Australia,
313 Costa Rica, New Zealand, Mexico, and the USA based on the eight microsatellite loci developed
314 in the present study. For the ten isolates from the USA genotyped with all 25 microsatellite loci,
315 eight of the markers were monomorphic and 16 were polymorphic, with all polymorphism
316 observed within each of the isolates. Additionally, we genotyped some of the other isolates (five
317 isolates from Costa Rica, one isolate from New Zealand, and three isolates from Mexico) at all
318 25 loci (data not shown) and still found no genotypic diversity. Since no genetic differences were
319 observed among isolates, it is not possible to track individuals or introductions of individuals
320 from other populations that may have occurred (McDonald and Linde, 2002; Milgroom and
321 Peever, 2003). It may be possible to detect genotypic diversity within *U. transversalis* using
322 different genotyping methods, such as genotyping-by-sequencing; however, the high
323 heterozygosity present within individuals may obscure the detection of genotypic diversity
324 among individuals (Elshire et al., 2011). A different member of the Pucciniales, *Phakopsora*
325 *pachyrhizi*, for which only clonal reproduction has been observed, has some genotypic diversity
326 both in the USA where it has been introduced and in Asia, where it is native (Zhang et al., 2012).
327 Nonetheless, the microsatellite markers developed for *U. transversalis* may be useful for
328 diagnostic purposes or detection of *U. transversalis* in asymptomatic plant material.
329

330 In this study, all of the genetic diversity identified in *U. transversalis* occurred as allelic diversity
331 within individuals. Most of the microsatellite markers and all of the repeat-flanking sequence
332 that were compared showed two alleles or two distinct haplotypes at each locus, indicative of
333 very high heterozygosity. This level of allelic variation within each individual is suggestive of
334 divergent genomes between the nuclei of the dikaryon across the invasive population. The
335 flanking sequences from each locus showed approximately 97% sequence similarity (Table 4). In
336 some cases, the two genomes of the dikaryon are more divergent than what is usually observed

337 within a single fungal species (Hibbett, 2016). The lack of genotypic diversity among isolates
338 and the distinct sequences and microsatellite alleles within individuals suggests that *U.*
339 *transversalis* samples from Australia, Costa Rica, Mexico, New Zealand and the USA are
340 asexually reproducing populations that are not recombining through sexual reproduction
341 (Milgroom, 1996). Clonal invasions are common among plants pathogens (Milgroom et al. 2008;
342 Goss et al., 2009). In dikaryotic organisms and diploids, the absence of sexual reproduction will
343 increase the divergence between sequences in each genome as random mutations will occur over
344 time (Birky, 1996). Thus, low genotypic diversity combined with high allelic diversity within
345 individuals is suggestive of strict clonal reproduction for an extensive period of time (Balloux et
346 al., 2003; Birky, 1996). Our results provide support for clonal reproduction of *U. transversalis* in
347 the USA, Mexico, New Zealand, and Australia, which is consistent with the observed research
348 on reproductive biology of *U. transversalis*. However, the extent of sequence divergence
349 indicates that these populations have been clonal for possibly hundreds to thousands of years.
350
351 To our knowledge this is the first study on the genetic diversity of *U. transversalis*. Since *U.*
352 *transversalis* urediniospores are dikaryotic, development of codominant, sequence-specific
353 microsatellite markers would be appropriate in addressing our questions of genetic diversity,
354 origin, and sources of introductions for this rust fungus. Traditionally, microsatellite
355 development required the construction of a genomic library enriched for repeated motifs,
356 isolation, and sequencing clones; primer design and optimization; and testing for polymorphism
357 on a few unrelated individuals (Abdelkrim et al., 2009; Frenkel et al., 2012; Santana et al., 2009;
358 Zhong et al., 2009). As an alternate approach, the microsatellite markers in the present study
359 were developed using whole genome sequencing of multiple isolates. This approach not only
360 increased our chances of identifying polymorphic alleles within *U. transversalis*, but also
361 supplied genomic sequence, which could be used for comparative analyses or other purposes.
362 However, the main focus of this study was not the whole genome sequencing and assembly, but
363 to use these data to develop markers. Although useful for marker development, the three draft
364 genomes were highly fragmented (4.3 – 7.4 million assembled reads or contigs compared to 6.0
365 – 9.9 million unassembled), which could be the result of repetitive DNA and a large genome,
366 which are common among rust fungi (Ramos et al., 2015; Tavares et al., 2014).
367

Although we were unable to detect genetic diversity among isolates of *U. transversalis* across a wide geographic range, the genome sequences will serve as a resource for further studies on this destructive fungal pathogen of ~~Gladiolus.~~ Since all isolates sampled exhibited limited diversity and were genetically similar, this demonstrates that disease management strategies both current and future, should work for all locations and current hosts for *Uromyces transversalis* as concern for the development of fungicide resistant strains seems unlikely. Future studies will help us to determine the usefulness of the microsatellite markers in diagnosis and detection.

Conclusions

There was no genotypic diversity observed among the invasive *U. transversalis* populations from Australia, Costa Rica, New Zealand, Mexico, and the USA based on the eight microsatellite loci developed in the present study. The lack of genotypic diversity among isolates and the distinct sequences and microsatellite alleles within individuals suggests that *U. transversalis* samples in introduced ranges are asexually reproducing populations that are not recombining through sexual reproduction. Our results provide support for clonal reproduction of *U. transversalis* in the USA, Mexico, New Zealand, and Australia, which is consistent with the observed research on reproductive biology of *U. transversalis* and other invasive plant pathogens.

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References

Abdelkrim, J., Robertson, B.C., Stanton, J.A.L., Gemmell, N.J., 2009. Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing. Biotechniques 46, 185.

Balloux, F., Lehmann, L., de Meeûs, T., 2003. The population genetics of clonal and partially clonal diploids. Genetics 164, 1635-1644.

Beilharz, V., Parbery, D.G., Pascoe, I.G., 2001. Gladiolus rust (caused by *Uromyces transversalis*) in eastern Australia. Australas. Plant Pathol. 30, 267-270.

398 Birky, C.W., 1996. Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes.
399 Genetics 144, 427-437.
400 Blomquist, C.L., Thomas, S.L., McKemy, J.M., Nolan, P.A., Luque-Williams, M., 2007. First
401 report of *Uromyces transversalis*, causal agent of Gladiolus rust, in San Diego County,
402 California. Plant Dis. 91, 1202-1202.
403 Brown, L., 2005. *Uromyces transversalis* Assessment of the Risk of Introduction
404 Recommendations for risk mitigation for *Gladiolus* spp. Cut Flowers and Propagative
405 Material from México. online USDA Plant Protection and Quarantine.
406 Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E.,
407 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity
408 species. PLoS ONE 6, e19379.
409 Ferreira, J.F., Nevill, W.G., 1989. Evaluation of bitertanol and triadimefon for the control of
410 Gladiolus rust caused by *Uromyces transversalis*. Plant Dis. 73, 987-990.
411 Frenkel, O., Portillo, I., Brewer, M.T., Peros, J. P., Cadle-Davidson, L., Milgroom, M.G., 2012.
412 Development of microsatellite markers from the transcriptome of *Erysiphe necator* for
413 analysing population structure in North America and Europe. Plant Pathol. 61, 106-119.
414 Garibaldi, A., Aloj, B., 1980. Observations on biology and control of *Uromyces transversalis*
415 (Thum.) Winter on Gladiolus in southern Italy. International Symposium on Flower
416 Bulbs 109, 409-414.
417 Goss, E.M., Carbone, I., Grünwald, N.J., 2009. Ancient isolation and independent evolution of
418 the three clonal lineages of the exotic sudden oak death pathogen *Phytophthora*
419 *ramorum*. Mol. Ecol., 18, 1161-1174.
420 Haridas, S., Breuill, C., Bohlmann, J., Hsiang, T., 2011. A biologist's guide to de novo genome
421 assembly using next-generation sequence data: a test with fungal genomes. J. Microbiol.
422 Methods 86, 368-375.
423 Hauswaldt, J.S., Glenn, T.C., 2003. Microsatellite DNA loci from the diamondback terrapin
424 (*Malaclemys terrapin*). Mol. Ecol. Notes 3, 174-176.
425 Hernández, J., 2004. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Invasive
426 Fungi: Gladiolus Rust. Retrieved April 21, 2009.
427 Hibbett, D., 2016. The invisible dimension of fungal diversity. Science 351, 1150-1151.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649.

Koressaar, T., Remm, M., 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23, 1289-1291.

Leclercq, S., Rivals, E., Jarne, P., 2007. Detecting microsatellites within genomes: significant variation among algorithms. BMC Bioinformatics 8, 125.

Lee, S.B., Milgroom, M.G., Taylor, J., 1988. A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. Fungal Genetics Newsletter 35, 23-24.

Lindquist, J., Alippi, H., Medera, C., 1979. A serious epiphytotic of Gladiolus rust (*Uromyces gladioli*) in Santa Fe province (Republic of Argentina). III Jornada Fitosanitaria Argentina 2, 717-724.

Littlejohn, G., Blomerus, L., 1997. Studies on Gladiolus resistance to transverse rust. Acta Hortic. 430, 509-516.

Martínez-de la Parte, E., Pérez-Vicente, L., Cantillo-Pérez, T., Guerrero-Barriel, D., Ramos, A., 2011. First report of Gladiolus rust caused by *Uromyces transversalis* in Cuba. New Disease Reports 23, 20.

Mayer, C., 2006. Phobos http://www.rub.de/spezzoo/cm/cm_phobos.htm.

McDonald, B.A., Linde, C., 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40, 349-379.

McKenzie, E., 2000. *Uromyces transversalis,* rust fungus found infecting Iridaceae in New Zealand. N. Z. J. Crop Hortic. Sci. 28, 289-291.

Milgroom, M.G., 1996. Recombination and the multilocus structure of fungal populations. Annu. Rev. Phytopathol. 34, 457-477.

Milgroom, M.G., 2015. *Population Biology of Plant Pathogens: Genetics, Ecology and Evolution*. American Phytopathological Society Press, St. Paul, MN.

Milgroom, M.G., Peever, T.L., 2003. Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. Plant Dis. 87, 608-617.

457 Milgroom, M.G., Sotirovski, K., Risteski, Brewer, M.T., 2009. Heterokaryons and parasexual
458 recombinants of *Cryphonectria parasitica* in two clonal populations in southeastern
459 Europe. Fungal Genet. Biol. 46, 849-854.
460 Mohali, R.S., Aime, M.C., 2018. First report of Gladiolus rust caused by *Uromyces transversalis*
461 in Merida, Venezuela. Plant Dis. 102, 444-445
462 Niazmand, A.R., Choobineh, D., Hajmansoor, S.H., 2013. A Simple and rapid method to extract
463 genomic DNA from urediniospores of rust diseases for molecular analysis. Staphia 99,
464 235-238
465 Peterson, G.L., Berner, D.K., 2009. Effects of temperature and humidity on the survival of
466 urediniospores of Gladiolus rust (*Uromyces transversalis*). Eur. J. Plant Pathol. 125, 509-
467 513.
468 Pita, G., Figueiredo, M., Cardoso, R., Hennen, J., 1981. Ferrugem (*Uromyces transversalis*
469 (Thuemen) Winter, uma nova doenca do gladiolo (*Gladiolus* spp). no Brasil). Biologico
470 47, 323-328.
471 Ramos, A.P., Tavares, S., Tavares, D., Silva, M.D.C., Loureiro, J., Talhinhas, P., 2015. Flow
472 cytometry reveals that the rust fungus, Uromyces Bidentis (Pucciniales), posseses the
473 largest fungal genome reported - 2489Mbp. Mol. Plant Pathol. 16, 1006-1010.
474 Rizvi, A., Man-Son-Hing, A., Jackson, A., Parra, G., Schartzburg, K., Brown, L., Sullivan, M.,
475 Duffie, L., Kosta, K., Clark, R., 2007. Gladiolus rust (*Uromyces transversalis*): A
476 national management plan for exclusion and eradication. USDA APHIS PPQ, Retrieved
477 February 10, 2009.
478 Rodríguez-Alvarado, G., Fernández-Pavía, S., Valenzuela-Vázquez, M., Loya-Ramírez, J., 2006.
479 First report of Gladiolus rust caused by *Uromyces transversalis* in Michoacán, México.
480 Plant Dis. 90, 687-687.
481 Rodgers, S.O., Bendich, A.J., 1985. Extraction of DNA from milligram amounts of
482 fresh, herbarium and mummified plant tissues. Plant Mol. Biol. Rep. 5, 69-76.
483 Rozen, S., Skaletsky, H., 1999. Primer3 on the WWW for general users and for biologist
484 programmers. Bioinformatics Methods and Protocols 132, 365-386.
485 Santana, Q.C., Coetzee, M.P., Steenkamp, E.T., Mlonyeni, O.X., Hammond, G.N., Wingfield,
486 M.J., Wingfield, B.D., 2009. Microsatellite discovery by deep sequencing of enriched
487 genomic libraries. Biotechniques 46, 217-223.

488 Schubert, T.S., Leahy, R.M., Davison, D.A., Silagyi, A.J., Killgore, E.M., 2007. Gladiolus rust
489 caused by *Uromyces transversalis* makes first nearctic appearance in Florida. Plant Dis.
490 91, 1202.
491 Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature
492 Biotechnol. 18, 233-234.
493 Schwessinger, B., Rathjen, J.P., 2017. Extraction of High Molecular Weight DNA from Fungal
494 Rust Spores for Long Read Sequencing. Wheat Rust Diseases. *Methods in Molecular*
495 *Biology*, vol 1659. Humana Press, New York, NY
496 Selkoe, K.A., Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and
497 evaluating microsatellite markers. Ecol. Lett. 9, 615-629.
498 Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J., Birol, I., 2009. ABySS: a
499 parallel assembler for short read sequence data. Genome Res. 19, 1117-1123.
500 Tavares, S., Ramos, A.P., Pires, A.S., Azinheira, H.G., Caldeirinha, P., Link, T., Abranches, R.,
501 Silva, M.C, Voegele, R.T., Loureiro, J., Talhinhas, P., 2014. Genome size analyses of
502 Pucciniales reveal the largest fungal genomes. Front. Plant Sci. 5, 422.
503 Tervet, I., Rawson, A., Cherry, E., Saxon, R., 1951. A method for the collection of microscopic
504 particles. Phytopathology 41, 282-285.
505 U.S. Department of Agriculture APHIS. 2015.
506 https://www.aphis.usda.gov/plant_health/plant_pest_info/gladiolus_rust/downloads/DA-
507 2015-20.pdf
508 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G.,
509 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115.
510 Valencia-Botin, A., Jeffers, S., Palmer, C., Buck, J.W. 2013. Fungicides used alone, in
511 combinations, and in rotations for managing Gladiolus rust in Mexico. Plant Dis. 97,
512 1491-1496.
513 Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of
514 DNA for PCR-based typing of forensic material. BioTechniques 54, 134-139.
515 Wise, K., Mueller, D., Buck, J.W., 2004. Quarantines and ornamental rusts. Online publication.
516 APSnet Features. doi:10.1094/APSnetFeature-2004-0204
517 Zhang, Y., Zhang, S., Liu, X., Wen, H., Wang, M., 2010. A simple method of genomic DNA
518 extraction suitable for analysis of bulk fungal strains. Lett. Appl. Microbiol. 51, 114-118.

519 Zhang, X., Freire, M., Le, M., Hartman, G.L., Upchurch, R.G., Pedley, K.F., Stacey, G., 2012.
520 Genetic diversity and origins of *Phakopsora pachyrhizi* isolates in the United States.
521 Asian J. Plant Pathology 6, 52-65.
522

Figure 1

Figure 1: Invasion history of the Gladiolus rust fungus, *Uromyces transversalis.*

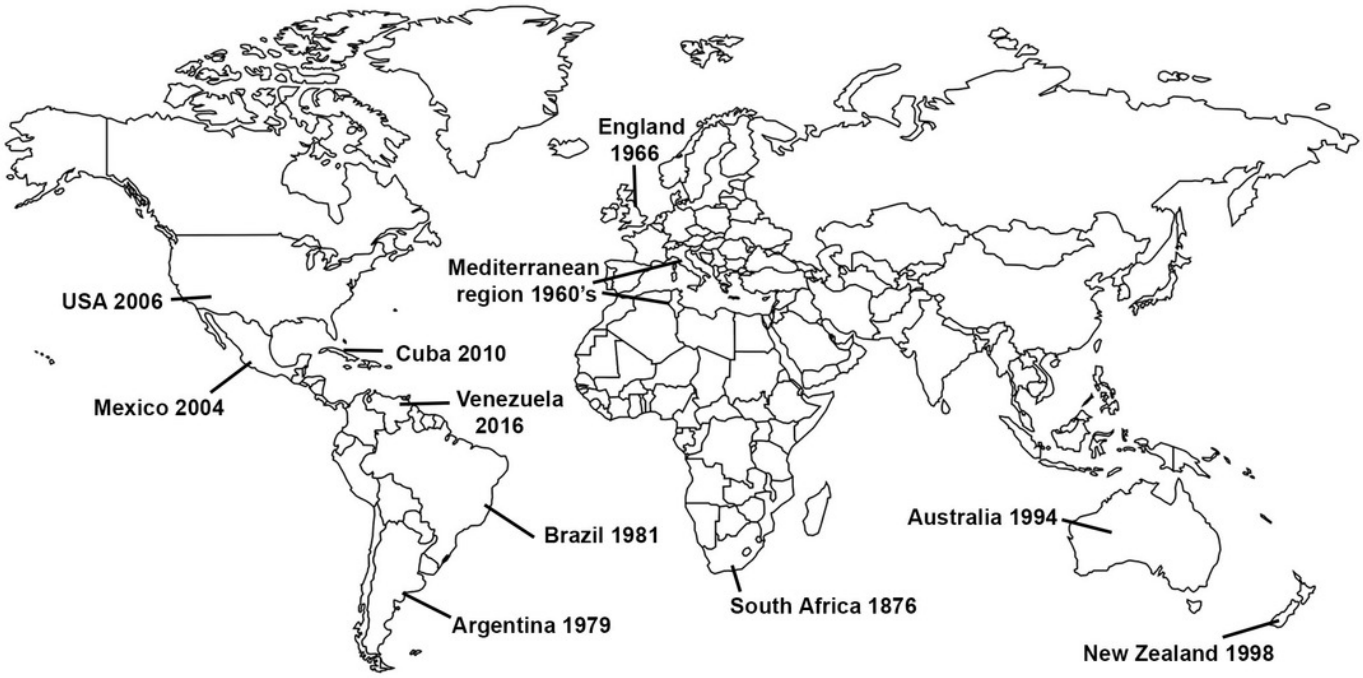

Table 1(on next page)

Table 1: Location and sources of *Uromyces transversalis* isolates used in this study

Table 1: Location and sources of *Uromyces transversalis* isolates used in this study

Geographic origin	Original host species	Isolate identifier	Collection date	Culture Collection (Collector)
Costa Rica	*Gladiolus* sp.	CR497224, CR498594, CR498400, CR497666, CR498457	2012	USDA-ARS; Pedley, K.
Wellington, New Zealand	*Gladiolus* sp.	NZ71109	2000	New Zealand Fungal Herbarium (NZFB); Beever, R.
Findelton, New Zealand	*Gladiolus* sp.	NZ87970	2006	NZFB; Close, R.
Remuera, New Zealand	*Anomatheca laxa*	NZ69482	1998	NZFB; Dingley, J.M.
Remuera, New Zealand	*G. nanus*	NZ69481	1998	NZFB; Heckler, R.
Feilding, New Zealand	*Gladiolus* sp.	NZ71696	2000	NZFB; Hill, C.F.
Mt. Albert, New Zealand	*G. undulatus*	NZ97335	2007	NZFB; Petley, M.
Avondale, New Zealand	*Melasphaerula*	NZ69208	1998	NZFB; Wilkie, J.P.
Mt. Albert, New Zealand	*Gladiolus* sp.	NZ99990	2011	NZFB; Wilkie, J.P.
Mt. Albert, New Zealand	*Tritonia*	NZ88195	2004	NZFB; Wilkie, J.P.
Mt. Albert, New Zealand	*Tritonia*	NZ69483	1998	NZFB; Beever, R.
Australia	*Gladiolus* sp.	VPRI 20841, VPRI 20858, VPRI 20881, VPRI 21344, VPRI 22299, VPRI 32661	Unknown	Victoria Plant Pathology Herbarium (VPPH)
Mont Albert, Australia	*Gladiolus* sp.	VPRI 21238	1996	VPPH; Parbery, D.
Tlapizalco, Zumpahuacán, Mexico (MX)	*Gladiolus* sp.	TLAP1, TLAP2, TLAP3	2011	Valencia-Botin. A.
Atlixco, Puebla, MX	*Gladiolus* sp.	Atlix1, Atlix2, Atlix3	2011	Valencia-Botin. A.
Cuautla, Morelos, MX	*Gladiolus* sp.	Cua1, Cua2, Cua3	2011	Valencia-Botin. A.
Villa Guerrero, MX	*Gladiolus* sp.	Gro1, Gro2, Gro3	2011	Valencia-Botin. A.
Atlatlahuacán, MX	*Gladiolus* sp.	Ten1, Ten2, Ten3	2011	Valencia-Botin. A.

Irimbo, Michoacán, MX	*Gladiolus* sp.	Iri1, Iri2, Iri3	2010	Valencia-Botin. A.
"La Finca" Villa Guerrero, MX	*Gladiolus* sp.	LF1 1, LF1 2, LF1 3, LF2 1, LF2 2, LF2 3	2011	Valencia-Botin. A.
Cocoyoc Yautepec, Morelos, MX	*Gladiolus* sp.	M1 1, M1 2, M1 3	2010	Valencia-Botin. A.
Oacalco Yautepec, Morelos, MX	*Gladiolus* sp.	M2 1, M2 2, M2 3	2010	Valencia-Botin. A.
Yautepec Yautepec, Morelos, MX	*Gladiolus* sp.	M3 R1, M3 R2, M3 R3	2010	Valencia-Botin. A.
El Caracol Yautepec, Morelos, MX	*Gladiolus* sp.	M4 1, M4 2	2010	Valencia-Botin. A.
Villa Ayala, Morelos , MX	*Gladiolus* sp.	M5 R3, M6 R1, M6 R2, M6 R3, M7 1, M7 2, M7 3, M8 R1, M8 R2, M8 R3	2010	Valencia-Botin. A.
Ejido Tlayacapan, Morelos, MX	*Gladiolus* sp.	M9 R1, M9 R2, M9 R3	2010	Valencia-Botin. A.
Huachinanitla Tepoztlán, Morelos, MX	*Gladiolus* sp.	M10 R1, M10 R2, M10 R3	2010	Valencia-Botin. A.
6.M. Texmel, Pue, MX	*Gladiolus* sp.	JB1	2010	Valencia-Botin. A.
Villa Guerrero, Estado de Mexico, MX	*Gladiolus* sp.	JB2, JB7	2010	Valencia-Botin. A.
Cuautla, Morelos, MX	*Gladiolus* sp.	JB3	2010	Valencia-Botin. A.
Atlixco, Puebla, MX	*Gladiolus* sp.	JB4, JB6	2010	Valencia-Botin, A.
TurpamMick, MX	*Gladiolus* sp.	JB5	2010	Valencia-Botin, A.
Irambo, Michoacan, MX	*Gladiolus* sp.	JB8	2010	Valencia-Botin, A.
Unknown, California, US	*Gladiolus* sp.	CA11-1	2011	K. Pedley
Carpenteria, California, US	*Gladiolus* sp.	CA14-1, CA14-3, CA14-4	2014	K. Pedley
Santa Maria, California, US	*Gladiolus* sp.	CA14-2	2014	K. Pedley
Santa Barbara, California, US	*Gladiolus* sp.	CA14-5	2014	K. Pedley
Goleta, California, US	*Gladiolus* sp.	CA14-6, CA14-7	2014	K. Pedley
Manatee County, Florida, US	*Gladiolus* sp.	FL11-1	2011	K. Pedley
Hendry County, Florida, US	*Gladiolus* sp.	FL11-2	2011	K. Pedley

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Table 2(on next page)

Table 2: Repeat motif, primer sequences, and number of alleles, allele sizes, and genotypes for *U. transversalis*

1 **Table 2: Repeat motif, primer sequences, and number of alleles, allele sizes, and genotypes for *U. transversalis***

Locus[a]	Repeat motif	Primer sequence (5′→3′)[b]	Number of observed alleles, allele sizes (bp), and genotypes[c]
Ut337	$(AGG)_7$	F: CGGAAGAGATGAGTGGTCAAG	2 (195, 198)
		R: TCACATCATCCCCTCCCTA	
Ut397	$(TTG)_9$	F: TTCGATTCGATTCGTTTGTTT	1 (259)
		R: GGATGTTTTGATTCTGTTAGAGAGTG	
Ut447	$(ACC)_6$	F: TGCTTCAGCTTCCCAAAACT	2 (237, 240)
		R: TGGCTGTGAATTGTGAGACC	
Ut497[*2]	$(GAA)_{15}$	F: CTTGAAGGGGATCGAGAAGA (6FAM)	2 (232, 251)
		R: TGTTCTCCGGCAGAGGTTTA	
Ut513[*1]	$(TCA)_6$	F: TCCCAAACAAATCGTGAAGA (NED)	2 (200, 203)
		R: GCTCCCGTTAATGGTCACAG	
Ut542	$(GTT)_5$	F: GTCTTCTTTGCTGCGTTTCC	2 (204, 207)
		R: TCCTGGTTTTGAACCTCCTG	
Ut568	$(ACC)_6$	F: TCCCATGGGTTTGGTTGC	2 (178, 181)
		R: TCCTTAATCTGGGTTGACATTT	
Ut575	$(TTA)_5$	F: TGACGATCCTAACGAAGGGTA	2 (241, 244)
		R: CTTGGGGTACGAGAGCACTT	
Ut697	$(AAG)_5$	F: TAGGCGAAGTGGTACGAGGT	1 (224)
		R: AGGGAAGAAGAGGGTCAACA	
Ut752	$(ATC)_6$	F: AGTCTTGTGCTGGTCTTCGTC	2 (213, 216)
		R: TTTGCCGCCTTATATTGTCA	
Ut844	$(ACT)_8$	F: CTCCGTCAGCCAGTCAGTC	1 (310)
		R: GATGAGGTTGAGGGCGAGTA	
Ut981	$(TGA)_6$	F: GGGTCAAACAGGTCTTCTGG	1 (202)
		R: CTACTGAAATGGGCCACAAA	
Ut1272	$(AAG)_5$	F: TGAAGTTTTCCACCCTGGTT	2 (253, 256)
		R: ATCTTGGGCAAACTGACCAC	
Ut1289	$(GAG)_7$	F: GGTCTTGAGAGAACGGAGGA	2 (254, 257)
		R: CTCTTCCAGATACCCCACCA	
Ut1841[*2]	$(AGG)_5$	F: GAACCCTGCCTCACACCTTA (NED)	2 (345, 348)
		R: GCGGCTACCAGAGCTTTAGA	
Ut1908[*2]	$(GAT)_6$	F: TCCTCTCAGCCAATCCAATC (PET)	2 (200, 203)
		R: CTCTTGCCCATCAATCCAAC	
Ut2035	$(TTTA)_8$	F: GGATCGAGTCGGTCGATTTA	2 (229, 232)

		R: GCCGAACAGGACTAGCATTG	
Ut2048[2]*	$(GAA)_6$	F: CGAGCGATAAATTTTTGAACA (VIC)	2 (182, 185)
		R: TGTCCGGAGAATGTGAACTG	
Ut2443	$(GAA)_8$	F: AGAATTGGATGAAACAGGGAGA	1 (188)
		R: AAGGAGGAAGCCATCACTCA	
Ut2536	$(GAG)_5$	F: AGGGCTGGTAGACGTGACTG	2 (248, 251)
		R: TCATGTCTCTGACACCACCA	
Ut2648[1]*	$(CAG)_6$	F: GAACTGGTGCAACCGATACA (VIC)	2 (266, 269)
		R: CACAGCCTTGGCTCTTGAGT	
Ut3161[1]*	$(TCC)_6$	F: GAGTCTGGCCCAGCTGTTT (6FAM)	2 (192, 195)
		R: TCTGATCTTGCAGGGGATTC	
UtCA759[1]*	$(CAT)_7$	F: GATGGCCAGAAGAAAGATGC (PET)	1 (296)
		R: TTAACCAGCGCGAGAGTCTT	
UtCA809	$(TTA)_7$	F: GCCACTTCTCCAAACGCTTA	1 (258)
		R: TCGCAAGATCAAGAAACAACC	
UtCA950	$(GTT)_9$	F: GGCAGAGGATGAGTCGTGTA	2 (272, 287)
		R: TCATCTCATCCCCACAATCA	

[a]Asterisks indicate loci that were used for the multiplex reactions and the 1 or 2 indicate multiplex 1 or 2, respectively).

[b]The fluorescent dye used for multiplex reactions is listed in parentheses to the right of the forward primer.

[c]Genotype of all 10 isolates from the United States. Allele sizes are listed based on the results of the multiplex reactions or what the length of the alleles would be without the 16 nucleotide CAGTAG.

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Table 3(on next page)

Table 3: Genome assembly and microsatellite statistics

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Table 3: Genome assembly and microsatellite statistics

Isolates	Unassembled reads[a]	Assembled reads (contigs)	# Contigs > 200 bp	Contigs w/ Microsatellites[b]	% Microsatellites per assembly
CA11-1	6,023,634	5,706,372	466,181	4,599	0.98%
FL11-1	9,976,981	4,305,978	548,017	5,685	1.03%
FL11-2	9,262,312	7,444,849	645,533	8,666	1.34%

2 [a]Unassembled reads based upon purity filter value of 99.26%.

3 [b]Contigs with identified microsatellites based on the annotation criteria: repeat unit length = min: 3 max: 6, min.

4 length of 15. Mono and dinucleotide repeats not considered due to the difficulty of scoring alleles during fragment

5 analysis.

Table 4(on next page)

Table 4. Variation between alleles within sequenced genomes of *U. transversalis*

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Table 4. Variation between alleles within sequenced genomes of *U. transversalis*

Microsatellite	No. Single nucleotide polymorphisms	No. Nucleotides in flanking regions	% Difference
Ut337	3	180	1.7
Ut513	7	291	2.4
Ut568	13	187	6.9
Ut575	3	187	1.6
Ut752	9	263	3.4
Ut1908	10	264	3.8

2