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Characterisation of the mating-type loci in species of *Elsinoe* causing scab diseases

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ABSTRACT

The genus *Elsinoe* includes many aggressive plant pathogens that infect various economically important agricultural, horticultural and forestry plants. Significant diseases include citrus scab caused by *E. fawcettii* and *E. australis*, grapevine spot anthracnose by *E. ampelina*, and the emerging Eucalyptus scab and shoot malformation disease caused by the recently described *E. necatrix*. Despite their importance as plant pathogens, little is known regarding the biology of many *Elsinoe spp*. To gain insights into the reproductive biology of these fungi, we characterized the mating-type loci of seven species using whole genome sequence data. Results showed that the *MAT1* locus organization and its flanking genes is relatively conserved in most cases. All seven species manifested a typical heterothallic mating system characterized by having either the *MAT1-1* or *MAT1-2* idiomorph present in an isolate. These idiomorphs were defined by the *MAT1-1-1* or the *MAT1-2-1* gene, respectively. A unique *MAT1-1* idiomorph containing a truncated *MAT1-2-1* gene, and a *MAT1-1-1* gene, was identified in *E. necatrix* and *E. fawcettii* genomes. Additionally, two idiomorph-specific proteins were found in the *MAT1-1* and *MAT1-2* idiomorphs of *E. australis*. Universal mating-type markers confirmed heterothallism across 21 *Elsinoe spp.*, are poised to advance future studies regarding the biology of these fungi.

1. Introduction

Mating in ascomycete fungi is regulated by genes occurring at a single locus, known as the mating-type (*MAT1*) locus (Kronstad and Staben, 1997; Turgeon and Yoder, 2000). This locus contains two different idiomorphs referred to as *MAT1-1* and *MAT1-2* (Metzenberg and Glass, 1990; Turgeon and Yoder, 2000). In most cases, the two idiomorphs are typically characterized by the core mating-type genes, *MAT1-1-1* and *MAT1-2-1*, respectively (Debuchy et al., 2010; Debuchy and Turgeon, 2006; Wilken et al., 2017). The former encodes a transcriptional regulatory protein with a highly conserved alpha-box domain, while the later encodes a protein with an HMG-box domain (Debuchy et al., 2010; Debuchy and Turgeon, 2006; Wilson et al., 2021b).

There are two principal sexual strategies in filamentous ascomycetes; heterothallism and homothallism (Ni et al., 2011). In heterothallic fungi, each individual harbours only one idiomorph (either *MAT1-1* or *MAT1-2*) at its *MAT1* locus, and two partners of opposite mating type must be in physical contact to induce the onset of sexual reproduction (Ni et al., 2011; Turgeon and Yoder, 2000; Wilson et al., 2021b). In contrast, homothallic species can complete the sexual cycle in the absence of an opposite mating-type strain, often due to the presence of both idiomorphs in individuals (Ni et al., 2011; Wilson et al., 2019, 2021b). A different form of homothallism known as unisexuality has been documented in some fungi, whereby only a single idiomorph is present in an isolate but where sexual reproduction can occur without a partner (Wilson et al., 2021a).

The genus *Elsinoe* (Myriangiales, Elsinoaceae) includes a number of aggressive plant pathogens that are broadly distributed worldwide. Species of *Elsinoe* typically cause scab diseases characterized by necrotic spots that become scabby and cork-like when the lesions age (Fan et al., 2017; Marin-Felix et al., 2019). This group of necrotrophic fungi can infect various economically important agricultural, horticultural and forestry plants. Some of the more important diseases include citrus scab caused by *E. fawcettii* and *E. australis* (Chung, 2011), grapevine spot anthracnose caused by *E. ampelina* (Li et al., 2021) and the recently described *E. necatrix* and *E. masingae* pathogens that causes a devastating Eucalyptus scab and shoot malformation disease in Indonesia and South

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Africa, respectively (Pham et al., 2021; Roux et al., 2023).

Sexual reproduction is an important mechanism for fungal pathogens to maintain genetic variation, allowing their offspring to be more adaptable to new hosts or environments (Drenth et al., 2019; Stukenbrock, 2016). Understanding the reproductive mechanisms and the importance of sexual reproduction in the life cycles of plant pathogens is important when considering disease management strategies and predicting new disease outbreaks. Most *Elsinoe* species are known based on their asexual states and in some cases the sexual states have been found (Fan et al., 2017). In spite of the importance of *Elsinoe* spp. as plant pathogens, little is known regarding their reproductive biology.

Genome sequences have recently become available for a number of economically important species of *Elsinoe* (Jiao et al., 2021; Li et al., 2020; Shanmugam et al., 2020; Zhang et al., 2022; Wingfield et al., 2022). This has provided an opportunity to investigate the molecular basis of reproduction in species of this genus. The aims of this study were, therefore, to identify the *MAT1* loci and characterize the mating-type genes in several species of *Elsinoe,* using whole genome sequence data. In addition, universal mating type markers for the genus were developed and tested on a collection of different *Elsinoe* spp., which will facilitate future research regarding the biology of these fungi.

2. Materials and methods

2.1. Genome sequences

2.1.1. Available genome sequences for seven Elsinoe spp.

Available genome sequences for nine *Elsinoe* isolates representing seven species were retrieved from public genomic database at NCBI (Table 1). These included *E. ampelina*, *E. arachidis, E. australis, E. batatas, E. fawcettii, E. murrayae,* and *E. necatrix,* with genome data for two isolates for *E. fawcetti* and *E. australis*. These genomes were annotated using GeneMark-ES with parameters optimised for fungi (Ter-Hovhannisyan et al., 2008).

2.1.2. Genome sequencing for the second E. necatrix isolate

The genome of isolate CMW 56129 of *E. necatrix* was sequenced specifically for this study. Genomic DNA was extracted from freeze-dried 5-day-old mycelium grown in malt yeast broth (MYB; 2 % malt extract, 0.5 % yeast extract; Biolab, Midrand, South Africa) following the method described by Duong et al. (2013). Nanopore DNA sequencing using a MinION sequencing device and long-read genome assembly were conducted as described by Wingfield et al. (2022). In addition, a paired-end library was prepared using Illumina® TruSeq® Nano DNA Library Prep kit and sequenced on the NovaSeq 6000 System at Macrogen Inc. (Seoul, Korea). The Illumina data were trimmed using Trimmomatic v 0.38 (Bolger et al., 2014), and aligned to the draft genome assembly using BWA v. 0.7.17 (Li and Durbin, 2009), followed by genome polishing using Pilon v. 1.23 with "–fix all" option activated (Walker et al., 2014) to improve the accuracy of the draft assembly. Completeness of the final assembly was assessed with BUSCO v. 5.1.2 using the Dothideomycetes dataset (Manni et al., 2021).

Table 1
Genome sequences used in this study including details of mating type.

Species	Strain	Accession number	Mating type	Reference
Elsinoe ampelina	YL-1	SMYM00000000	MAT1-2	Li et al. (2020)
Elsinoe arachidis	LNFT-H01	JAAPAX000000000	MAT1-2	Jiao et al. (2021)
Elsinoe australis	Ea-1	SWCS00000000	MAT1-1	Shanmugam et al. (2020)
	Wagga_1	QGII00000000	MAT1-2	NCBI
Elsinoe batatas	CRI-CJ2	JAESVG000000000	MAT1-2	Zhang et al. (2022)
Elsinoe fawcettii	SM16-1	VAAB00000000	MAT1-1	Shanmugam et al. (2020)
	DAR-70024	SWCR00000000	MAT1-2	Shanmugam et al. (2020)
Elsinoe murrayae	CQ-2017a	NKHZ00000000	MAT1-2	NCBI
Elsinoe necatrix	CMW 56134	JANZYH000000000	MAT1-1	Wingfield et al. (2022)
	CMW 56129	JANZYI000000000	MAT1-2	This study

2.2. Identification and characterization of mating-type loci

The *MAT1-1-1* (QLL26889) and *MAT1-2-1* (QLL26890) protein sequences of *E. australis* (Zhao et al., 2020), were used for local tBLASTn searches in Geneious Prime 2022.0.2 (https://www.geneious.com) using all annotated genomes as databases. An e-value ≤0.01 and minimum query coverage of 40 % were applied as thresholds. The putative *MAT1* genes, as well as at least 6 kb upstream and downstream flanking regions, were extracted from each of the genomes. Using BLASTp, the predicted amino acid sequences of the putative *MAT1* genes, as well as those of the other genes present at the *MAT1* locus, were compared to NCBI's Conserved Domains Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) for functional characterization. The structure of the *MAT1* genes and flanking genes in the *MAT1* loci of *Elsinoe* spp. were compared to each other using pairwise BLASTn with a maximum e-value cut-off of 0.001. Synteny analysis and visualisation of mating-type and flanking genes in the *MAT1* loci of *Elsinoe* spp. were performed using EasyFig v. 2.2.5 (Sullivan et al., 2011).

2.3. Development of mating-type markers for Elsinoe spp.

The sequences for the *MAT1-2-1* genes identified from the genomes of *E. ampelina*, *E. arachidis, E. australis, E. batatas, E. fawcettii, E. murrayae,* and *E. necatrix* were aligned in Geneious Prime 2022.0.2. The resulting alignment was used to manually design primers to detect the *MAT1-2* idiomorph. The primer pair, M2-ElF (5′-AACTSYTTCATYCTCTACCG-3′) and M2-ElR (5′-TCGGCYTTGSNCTTCCAYTC-3′), was designed to amplify the partial HMG-box domain of the *MAT1-2-1* gene.

The primers designed to detect the *MAT1-1* idiomorph, M1-ElF (5′-CTYAAYTCYTGGATGGCNTTCA-3′) and M1-ElR (5′-GYTCRCGGATGAYNGAGTA-3′), targeted a part of the alpha-box domain of the *MAT1-1-1* gene. These were designed by aligning the *MAT1-1-1* sequences predicted from the genomes of *E. australis, E. fawcettii* and *E. necatrix*. In some species, where amplifications using a combination of M1-ElF/M1-ElR were unsuccessful, additional primers M1b-ElF (5′-TGGATGGCNTTCAGAAGTRAGT-3′) and M1b-ElR (5′-GCRATGATRGYCCAYTTGG-3′) were used as an alternative, which resulted in a slightly shorter amplicon.

2.4. Screening for MAT1 genes in Elsinoe spp.

2.4.1. Fungal isolates, DNA extraction and species identities confirmation

Thirty-seven isolates representing 21 species of *Elsinoe* were sourced from the culture collection (CMW) of the Forestry and Agricultural Biotechnology institute, University of Pretoria, South Africa, and culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (Table 2).

DNA was extracted from 10-day-old isolates grown on potato dextrose agar (PDA; BD Difco) at 25 °C, using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocols. The translation elongation factor 1-alpha (*TEF1*) gene region was amplified using the primers elongation-1-F and elongation-1-R (Hyun et al., 2009), and PCR amplifications were performed as described by Pham et al. (2019). Thermal cycling included an initial denaturation at 95 °C for 5 min followed by 10 primary amplification cycles of 30 s at 95 °C, 30 s at 60 °C, and 60 s at 72 °C, then 30 additional cycles of the same conditions, except for a 5 s

Table 2
Fungal isolates used in this study, with the presence of the mating-type idiomorph confirmed by PCR assay.

Species	Isolate number	Host	Origin	Mating type	*MAT1-1-1*	*MAT1-2-1*
Elsinoe ampelina	CBS 208.25	*Callianthe striata*	Brazil	MAT1-2		√
E. arachidis	CBS 511.50	*Arachis hypogaea*	Brazil	MAT1-2		√
E. australis	CBS 229.64	*Citrus aurantiifolia*	Brazil	MAT1-1	√	
	CBS 314.32	*Citrus aurantium*	Brazil	MAT1-2		√
E. eucalypticola	CBS 124765	*Eucalyptus* sp.	Australia	MAT1-2		√
E. eucalyptorum	CBS 120084	*Eucalyptus propinqua*	Australia	MAT1-2		√
E. euphorbiae	CBS 401.63	*Euphorbia parviflora*	India	MAT1-2		√
E. fawcettii	CBS 139.25	Citrus sp.	USA	MAT1-1	√	
	CBS 231.64	*Citrus aurantiifolia*	USA	MAT1-2		√
	CBS 232.64	*Citrus limon*	USA	MAT1-1	√	
	CBS 233.64	*Citrus aurantium*	Panama	MAT1-1	√	
E. freyliniae	CBS 128204	*Freylinia lanceolata*	South Africa	MAT1-1	√	
E. genipae	CBS 342.39	*Genipa americana*	Brazil	MAT1-2		√
E. glycines	CBS 389.64	*Glycine soja*	Japan	MAT1-2		√
	CBS 390.64	*Glycine soja*	Japan	MAT1-1	√	
E. leucospermi	CBS 111207	*Leucospermum* sp.	South Africa	MAT1-2		√
	CBS 111671	*Leucospermum* sp.	Australia	MAT1-1	√	
	CBS 112367	*Leucospermum cordifolium*	Australia	MAT1-1	√	
	CBS 115500	*Leucospermum* sp.	Spain	MAT1-1	√	
E. masingae	CMW 58880	*Eucalyptus grandis* × *E. nitens*	South Africa	MAT1-1	√	
	CMW 58894	*Eucalyptus grandis* × *E. nitens*	South Africa	MAT1-1	√	
E. necatrix	CMW 56134	*Eucalyptus grandis* × *E. urophylla*	Indonesia	MAT1-1	√	
	CMW 56129	*Eucalyptus grandis* × *E. urophylla*	Indonesia	MAT1-2		√
E. perseae	CBS 288.64	*Persea americana*	Brazil	MAT1-1	√	
	CBS 406.34	*Persea americana*	USA	MAT1-1	√	
E. pipertitae	CBS 144615	*Eucalyptus piperita*	Australia	MAT1-1	√	
E. poinsettiae	CBS 109333	*Euphorbia pulcherrima*	Guatemala	MAT1-1	√	
	CBS 109334	*Euphorbia pulcherrima*	Guatemala	MAT1-1	√	
E. populi	CBS 289.64	*Populus deltoides*	Argentina	MAT1-1	√	
	CBS 290.64	*Populus deltoides*	Argentina	MAT1-1	√	
E. preissianae	CBS 142129	*Eucalyptus preissiana*	Australia	MAT1-1	√	
E. randii	CBS 170.38	*Carya* sp.	Brazil	MAT1-1	√	
	CBS 171.38	*Carya* sp.	Brazil	MAT1-1	√	
E. rosarum	CBS 212.33	*Rosa* sp.	USA	MAT1-1	√	
	CBS 213.33	*Rosa* sp.	USA	MAT1-2		√
	CBS 235.64	*Rosa* sp.	USA	MAT1-1	√	
E. tectificae	CBS 124777	*Eucalyptus tectifera*	Australia	MAT1-1	√	

CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; **CMW**: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

increase in the annealing step per cycle. Reactions were completed with a final extension at 72 °C for 10 min. Resulting PCR amplicons were visualized using 2 % agarose gel electrophoresis with GelRed (Biotium, Hayward, CA, USA). PCR clean-up was done using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific). Amplicons were sequenced in both directions using the BigDye terminator sequencing kit v. 3.1 (Applied Biosystem, Foster City, CA, USA). Sequences were obtained on an ABI PRISM 3100 DNA sequencer (Applied Biosystems) at the Sequencing Facility at the University of Pretoria, Pretoria, South Africa, and assembled and edited in Geneious Prime 2022.0.2. The identities of the isolates were confirmed by performing BLASTn searches with the obtained sequences against the NCBI nucleotide database. Alternatively, for those isolates that did not have *TEF1* sequences available on NCBI, the internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rRNA region, were sequenced using primers ITS5 and ITS4 (White et al., 1990). In these cases, the conditions were the same as those for the *TEF1,* but the annealing temperature was adjusted to 56 °C.

2.4.2. *MAT1 gene amplification, sequencing, and mating type assignment*

The designed mating-type primers were used to determine the *MAT1* idiomorph present in all *Elsinoe* isolates obtained (Table 2). PCR conditions for both the *MAT1-1* and *MAT1-2* primers were the same as for *TEF1* amplification, except that the annealing temperature was 60 °C for M2-ElF/M2-ElR, 54 °C for M1-ElF/M1-ElR and 52 °C for M1b-ElF/M1b-ElR. The successful amplifications were confirmed using agarose gel electrophoresis followed by sequencing of the amplicons with the methods described above. The obtained sequences were aligned with the predicted *MAT1* genes from the genomes in Geneious Prime 2022.0.2.

3. Results

3.1. *Genome sequence of Elsinoe necatrix isolate CMW 56129*

The assembly of *E. necatrix* isolate CMW 56129 consisted of 50 contigs, with an N50 of 1.68 Mb and L50 of 6. The estimated genome size was approximately 25.64 Mb with a GC content of 50.48 %. The completeness of the genome was estimated to be 93.40 % with respect to the Dothideomycete lineage dataset. Of the 3786 BUSCO groups searched, 53 were reported to be fragmented and 197 were missing. The genome was deposited into GenBank with accession number JANZYI000000000 (Table 1).

3.2. *Characterization of the MAT1 loci in seven Elsinoe species*

Based on the tBLASTn searches in Geneious Prime 2022.0.2, sequence homologs of the MAT1 proteins in *E. australis* (QLL26889 and QLL26890) were detected on a single scaffold in each of the 10 genomes. The following contigs had regions similar to the *MAT1-2-1* gene of *E. australis* (QLL26890) with e-values ranging from 1.95e-40 to 3.18e-164: QGII01000435 of *E. australis* (Wagga_1), JAAPAX010000010 of *E. arachidis* (LNFT-H01), SMYM01000010 of *E. ampelina* (YL-1), JAESVG020000009 of *E. batatas* (CRI-CJ2), SWCR01000009 of *E. fawcettii* (DAR-70024), and N64_scaffold_6 of *E. necatrix* (CMW 56129). None of these contigs displayed a significant similarity to the *MAT1-1-1* gene and were identified as containing only the *MAT1-2* idiomorph. BLAST analysis using the *MAT1-1-1* protein of *E. australis* (QLL26889) as query resulted in hits with high similarity to regions on

contig SWCS01000010 from *E. australis* (Ea-1), contig VAAB01000017 from *E. fawcettii* (SM16-1) and contig LL58_scaffold_12 from *E. necatrix* (CMW 56134). E-values ranged from 3.61e-60 to 3.75e-169. These scaffolds were identified as containing the *MAT1-1* idiomorph.

The putative *MAT1-1-1* gene (1091–1175 bp) contained one intron and encoded a 345–375 amino acid protein that harboured a conserved MATalpha_HMGbox domain (cl07856) (Table 3). A MATA_HMG-box domain (cd01389) was found in the predicted *MAT1-2-1* gene (1021–1183 bp) (Table 3), which encoded a 323–378 amino acid protein and contained one intron. The predicted *MAT1-2-1* in *E. australis* was the only one interrupted by two introns. Identity comparison matrices for the predicted *MAT1* genes and MAT1 proteins in *Elsinoe* spp. for which genomes were available, showed that nucleotide sequences were generally more conserved than amino acid sequences (Table 4).

The organization of the *MAT1* locus and flanking genes was relatively conserved in most cases (Fig. 1). Each *MAT1* idiomorph was defined by either the *MAT1-1-1* or the *MAT1-2-1* gene. The DNA lyase gene (*APN2*) with an Ape2-like_AP-endo domain (cd09088) was found directly upstream of the *MAT1* locus. In addition, the downstream region included the sorting nexin-3 gene (*SNX3*) with PX domain super family (cl02563), and a gene encoding for a hypothetical protein with an unknown structural domain (*HP1*).

A unique *MAT1-1* idiomorph containing a *MAT1-1-1* gene, and a truncated *MAT1-2-1* gene, was identified in the genomes of *E. fawcettii* and *E. necatrix*. The truncated *MAT1-2-1* of *E. fawcettii* was 559 bp in size with a 58 bp intron. Comparison of the truncated MAT1-2-1 protein with the full-length MAT1-2-1 gene product of *E. fawcettii* showed 91.1 % amino acid pairwise identity, however, the MATA_HMG-box domain was absent in the truncated version. The truncated *MAT1-2-1* in *E. necatrix* was 606 bp in size, including a 140 bp intron. Amino acid sequence identity between the truncated version and the intact MAT1-2-1 protein of *E. necatrix* was 99.0 %.

Two idiomorph-specific proteins were found in the *MAT1-1* and *MAT1-2* idiomorphs of *E. australis*. A hypothetical protein 2 (*HP2),* lacking a conserved domain, was 1131 bp in size and had five exons and four introns and was located between the *APN2* and *MAT1-1-1* gene of *E. australis*. This hypothetical protein, of 311 amino acids in size, was homologous to an open reading frame of a protein (XP_029765566) with an unknown function located between the *MAT1-1-1* and *MAT1-2-1* genes in the homothallic Dothideales species *Aureobasidium pullulans* (Gostinčar et al., 2014), with the amino acid sequences sharing a 20.2 % identity. In addition, another gene (HD; 887 bp) was found in the *MAT1-2* idiomorph of *E. australis,* located between *MAT1-2-1* and *SNX3* genes. This gene encoded a 256 amino acid protein with three exons and two introns and included a Homeodomain-containing transcription factor domain (cl35021) spanning for 46 amino acids. A homolog of the *HD* gene was also found in the *MAT1-2* idiomorph of *E. murrayae,* also containing a conserved Homeodomain (cl00084), however, it shared only 36.3 % amino acid identity with the *HD* gene in the *MAT1-2* idiomorph of *E. australis.*

3.3. *MAT1* gene amplification and mating type assignment

Targeted fragments of the mating-type genes were successfully amplified from all 37 isolates screened for *MAT1* genes. These resulted in PCR products of approximately 170–210 bp for *MAT1-1-1* and 200 bp for *MAT1-2-1*. In all isolates in which the *MAT1-1-1* region was amplified, no amplification was observed for the *MAT1-2-1* region, and *vice versa*. All 21 species of *Elsinoe* considered in this study, including 15 for which genomes were not available, were thus shown to have a heterothallic mating system with either *MAT1-1* or *MAT1-2* idiomorph present in single isolates (Table 2). The different PCR products of the two *MAT1* genes were distinguished using agarose gel electrophoresis (Fig. 2) and confirmed by sequencing.

4. Discussion

This study has provided extensive genomic evidence for elucidating the mating strategy in seven species of *Elsinoe*. This was achieved by identifying and characterizing the mating type loci using whole genome sequences. All seven *Elsinoe* species displayed a typical heterothallic mating system characterized by the presence of a single idiomorph in individual isolates. The same pattern is likely be true for other *Elsinoe* species not examined in this study.

An alternative view to *Elsinoe* species having a heterothallic mating system would be that these species could undergo unisexual reproduction. Unisexual reproduction, a form of homothallism, occurs in individuals that would typically be described as heterothallic but are capable of progressing through the sexual cycle in the absence of a mating partner (Wilson et al., 2021a). There are identified features that can imply the unisexuality in filamentous ascomycetes, *i.e.* the mutations in the secondary *MAT1* genes, modifications in the pheromone response pathway, or self-fertility in a single haploid isolate (Wilson et al., 2021a). In this case, the heterothallic mating system in *Elsinoe* species seems more feasible to us, given the absence of secondary *MAT1* genes in their *MAT1* loci, and the fact that sexual structures of these fungi have never been characterized in cultures (Fan et al., 2017).

The *MAT1* locus could clearly be recognized in each of the seven investigated species following a BLAST-based approach. Each of the *Elsinoe* idiomorphs comprised a single *MAT1* gene, either *MAT1-1-1* or *MAT1-2-1*. This structure of the heterothallic mating system is similar to what has been found in various Ascomycota, including other members of the Dothideomycetes (Aylward et al., 2022; Coppin et al., 1997; Nagel et al., 2018; Paoletti et al., 2005). Comparison of the *MAT1* genes characterized in this study showed relatively low conservation among different *Elsinoe* species. However, the MATalpha_HMGbox domain of the MAT1-1-1 protein and MATA_HMG-box domain of the MAT1-2-1 protein appeared to be most conserved.

In addition to the primary *MAT1* genes, two *MAT1*-specific genes were identified in *E. australis*. An ORF encoding a hypothetical protein (*HP2*) was present in the *MAT1-1* idiomorph of *E. australis* but absent in other *Elsinoe* species considered. However, a homolog of *HP2,* was

Table 3
Nucleotide and amino acid sequence length of the *MAT1* genes of *Elsinoe* spp., with details of their conserved protein domains.

Species	Gene	Nucleotide sequence length	Number of exon/intron	Amino acid sequence length	Amino acid interval		e-value
					MATalpha_HMGbox	MATA_HMG-box	
Elsinoe ampelina	*MAT1-2-1*	1021	2/1	323	–	109–184	2.74e-33
Elsinoe arachidis	*MAT1-2-1*	1021	2/1	323	–	109–184	3.93e-33
Elsinoe australis	*MAT1-1-1*	1175	2/1	375	72–181	–	3.26e-33
	MAT1-2-1	1183	3/2	358	–	113–189	3.47e-35
Elsinoe batatas	*MAT1-2-1*	1036	2/1	328	–	109–184	5.36e-33
Elsinoe fawcettii	*MAT1-1-1*	1095	2/1	347	67–170	–	2.16e-29
	MAT1-2-1	1083	2/1	348	–	116–191	5.98e-34
Elsinoe murrayae	*MAT1-2-1*	1183	2/1	378	–	130–206	5.90e-35
Elsinoe necatrix	*MAT1-1-1*	1091	2/1	345	67–168	–	2.65e-28
	MAT1-2-1	1053	2/1	334	–	112–187	8.72e-33

Table 4
Pairwise comparison of the nucleotide and amino acid identities of the two mating-type genes of seven species of *Elsinoe*.

MAT1-1-1		*E. australis*		*E. fawcettii*		*E. necatrix*
E. australis		–		49.7/37.7[a]		51.3/39.4
E. fawcettii		49.7/37.7		–		75.4/73.0
E. necatrix		51.3/39.4		75.4/73.0		–
MAT1-2-1	***E. ampelina***	***E. arachidis***	***E. australis***	***E. batatas***	***E. fawcettii***	***E. murrayae***	***E. necatrix***
E. ampelina	–	92.6/92.0	41.8/35.4	91.8/91.8	56.9/50.0	41.9/33.5	58.4/49.6
E. arachidis	92.6/92.0	–	42.4/32.9	90.8/93.0	56.4/50.3	41.4/31.4	58.2/50.4
E. australis	41.8/35.4	42.4/32.9	–	42.4/33.5	42.7/31.0	55.6/46.4	41.6/30.9
E. batatas	91.8/91.8	90.8/93.0	42.4/33.5	–	57.1/49.1	42.6/32.0	58.2/50.0
E. fawcettii	56.9/50.0	56.4/50.3	42.7/31.0	57.1/49.1	–	42.4/28.6	73.9/69.9
E. murrayae	41.9/33.5	41.4/31.4	55.6/46.4	42.6/32.0	42.4/28.6	–	42.3/29.4
E. necatrix	58.4/49.6	58.2/50.4	41.6/30.9	58.2/50.0	73.9/69.9	42.3/29.4	–

[a] Values are presented as percent of nucleotide identity/percent of amino acid identity.

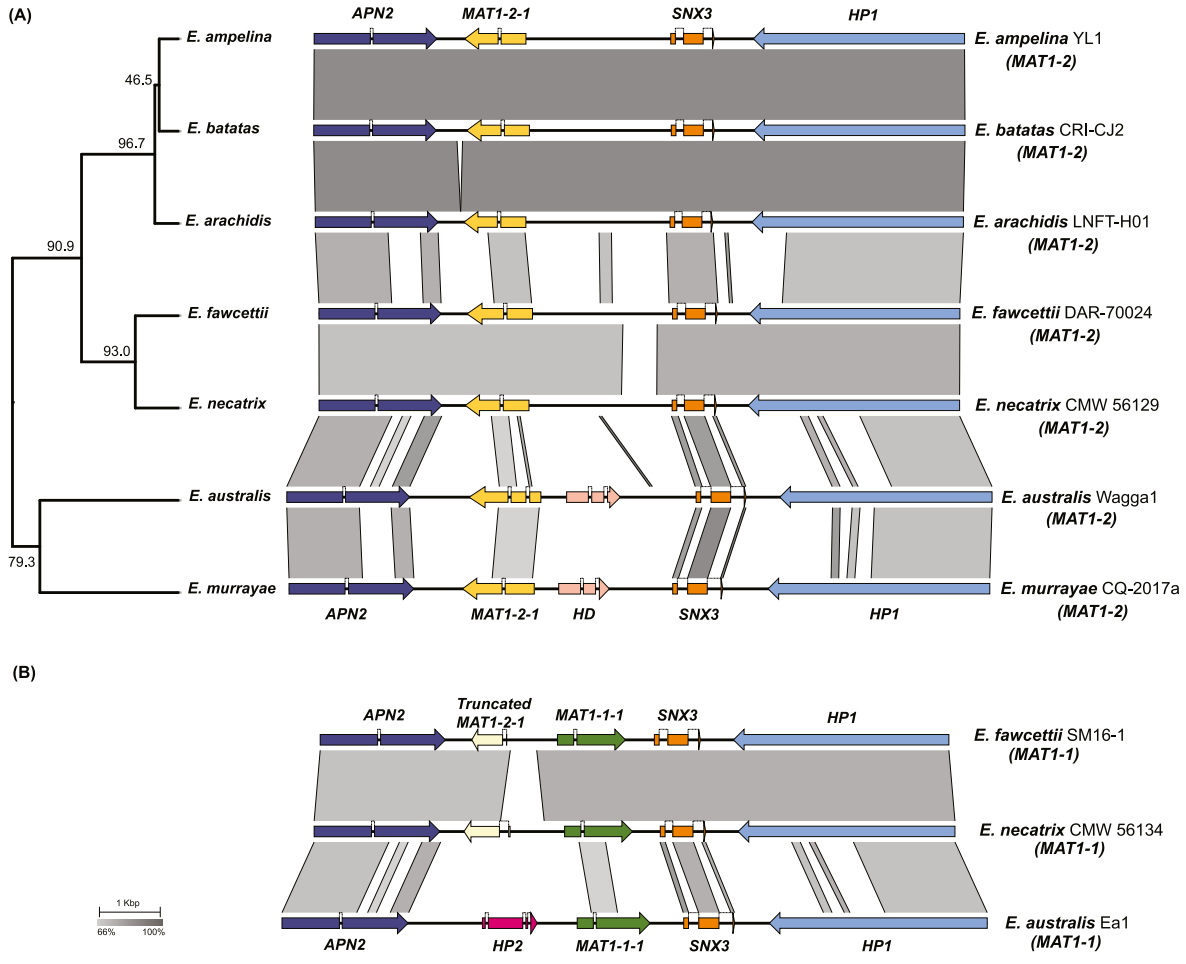

Fig. 1. Pairwise *MAT1* loci comparison among ten *Elsinoe* isolates representing seven species. *APN2* = DNA lyase gene; *HD* = Homeodomain gene; *SNX3* = sorting Nexin-3 gene; *HP* = gene encoding a hypothetical protein. Species phylogeny was constructed using ASTRAL (Zhang et al., 2018) using 1578 share single copy BUSCOs gene identified using ascomycota_odb10 dataset (Manni et al., 2021).

previously characterized in the *MAT1* locus of homothallic *Aureobasidium* spp. (Gostinčar et al., 2014, 2019), and was located between the *MAT1-1-1* and *MAT1-2-1* genes of those species (Gostinčar et al., 2014). In the *MAT1-2* idiomorphs of *E. australis* and *E. murrayae,* another idiomorph-specific gene (*HD)* with a Homeodomain, was predicted. The association of these *HD* genes with the *MAT1* locus and their function will require further investigation.

Each of the *MAT* idiomorphs was flanked on one side by a gene of unknown function (*HP1*) and on the other side by the *APN2* gene. These flanking genes are encountered in other Dothideomycetes including members of the Dothideales (Gostinčar et al., 2014, 2019). Although the *SLA2* gene is commonly associated with the *MAT1* locus in other Ascomycetes (Debuchy and Turgeon, 2006), it appears not to be in close proximity to the *Elsinoe MAT1* locus, as is true in all other Dothideomycetes for which the mating-type locus has been characterised (Conde-Ferráez et al., 2007; Aylward et al., 2022; Aylward et al., 2022).

The presence of a truncated *MAT1-2-1* gene in the *MAT1-1* idiomorphs of *E. necatrix* and *E. fawcettii* was an interesting finding. Partial gene fragments in the *MAT1* loci of heterothallic strains is not unusual, and has previously been reported in some other Dothideomycetes

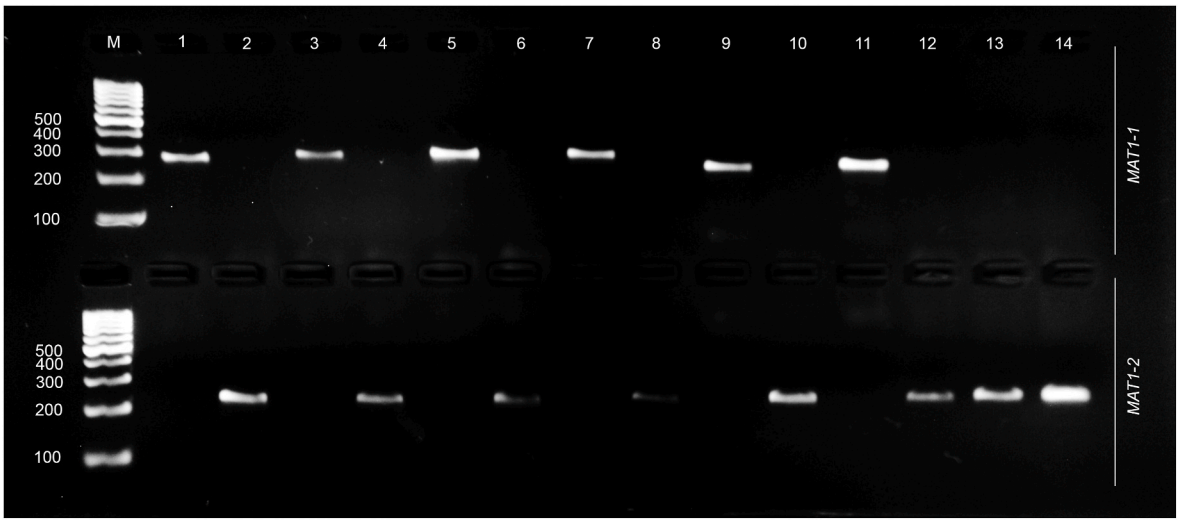

Fig. 2. Agarose gel electrophoresis (2 % w/v) of *MAT1-1-1* and *MAT1-2-1* PCR fragments from representative *Elsinoe* species. *Elsinoe australis* CBS 229.64 (1), CBS 314.32 (2); *E. fawcettii* CBS 139.25 (3), CBS 231.64 (4); *E. necatrix* CMW 56134 (5), CMW 56129 (6); *E. glycines* CBS 390.64 (7), CBS 389.64 (8); *E. rosarum* CBS 212.33 (9), CBS 213.33 (10); *E. leucospermi* CBS 111671 (11), CBS 112367 (12); *E. arachidis* CBS 511.50 (13); *E. ampelina* CBS 208.25 (14).

(Aylward et al., 2022; Bihon et al., 2014; Nagel et al., 2018; Petters--Vandresen et al., 2020), as well as in other Ascomycota (Duong et al., 2013; Kanzi et al., 2019). The truncation of the *MAT1-2-1* gene in these species could be the result of incomplete deletion events during a transition from homothallism to heterothallism or an unequal recombination event between two heterothallic strains, such as suggested by Nagel et al. (2018). The organization of the *MAT1-1* idiomorph shown in this study could be common to other species of *Elsinoe*, but additional genome sequences for *MAT1-1* strains will be necessary to resolve this hypothesis.

The PCR-based mating-type diagnostic assay developed in this study enabled us to identify the mating strategies of a further 15 *Elsinoe* species, despite their genome sequences not being available. Prior to this study, only the mating type locus of *E. australis* was known (Zhao et al., 2020). Heterothallism appears to be the exclusive mating strategy for the species evaluated. Although sexual structures of the majority of *Elsinoe* spp. have never been observed in nature, a cryptic sexual cycle could exist in many of these fungi.

An ability to now easily identify mating types in different species of *Elsinoe* using the developed PCR assay will make it possible to characterise mating type ratio in natural populations. This information will provide insight into the reproductive strategies of these species. This will then serve as a basis to better understand the epidemiology of the diseases *Elsinoe* species cause, the adaptation potential of the pathogen populations, and their ability to overcome host resistance, which should facilitate improvements in disease management.

Data availability

The genome sequence of *Elsinoe necatrix* (CMW 56129) has been deposited in DDBJ/EMBL/GenBank databases under the accession number JANZYI000000000. Annotations of the *MAT1* locus of all genomes used in this study (GenBank format) were deposited in Figshare (10.6084/m9.figshare.23925177).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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