FIGURE S1

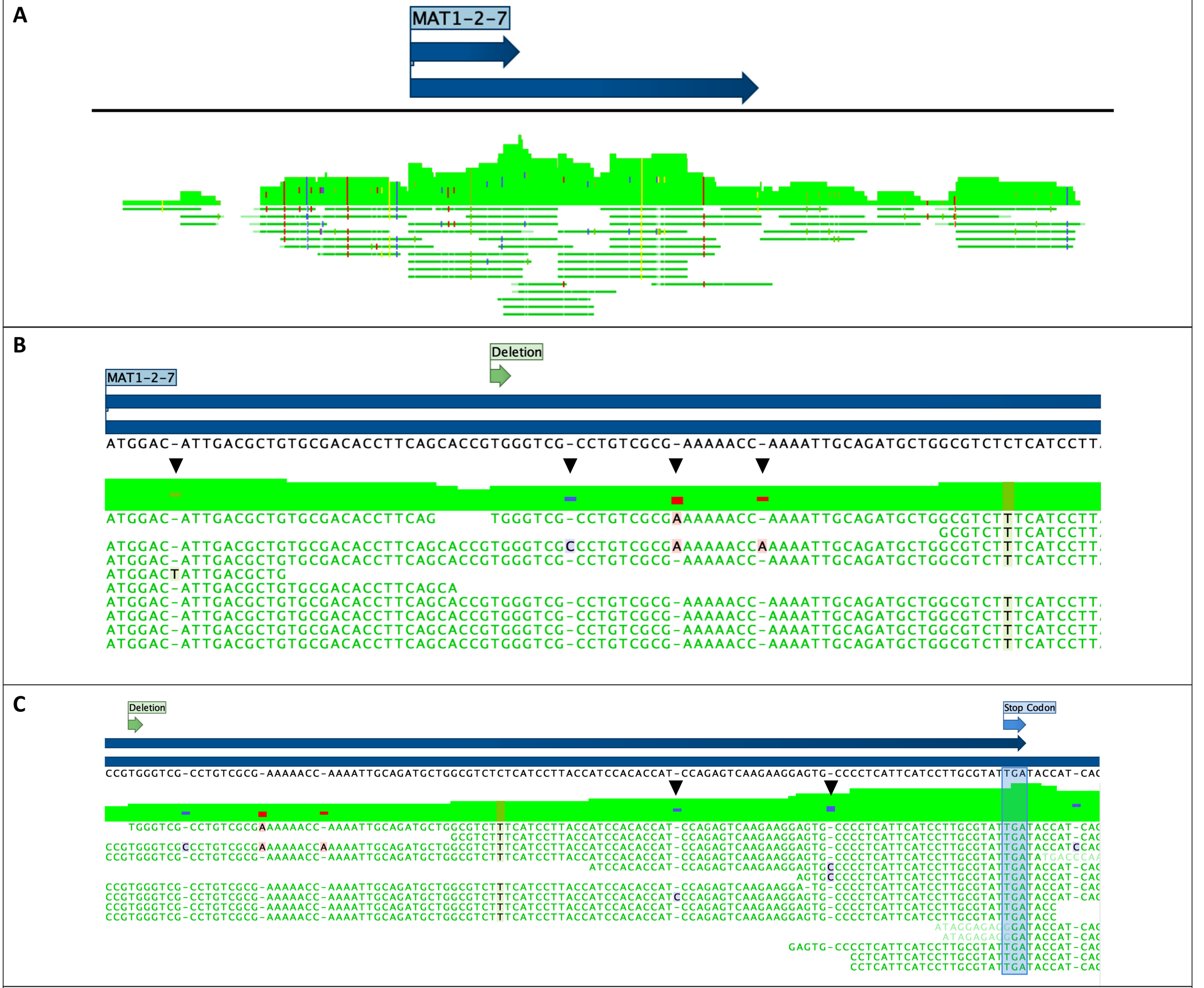


Fig S1: RNA mapping to confirm the single nucleotide deletion in the *H. moniliformis MAT1-2-7*. This mapping was generated using three sets of RNAseq data from a sporulating *H. moniliformis* isolate (Wilson et al 2018). A) Overview of the mapping across the entire gene. B) Mapping of the 5' region of the gene, indicating the start codon as well as the region where the deletion took place (indicated by the light green annotation). C) Mapping of the 3' region of the gene, indicating the region where the deletion took place as well as the in frame stop codon (indicated by the light blue annotation and box). There are various SNPs apparent when comparing the RNAseq reads to the assembled contig. Some of these (particularly those indicated with a black triangles) are likely sequencing errors produced during the transcriptome sequencing- especially considering they often occur in homopolymeric regions. These errors are often only supported by one or two reads. The mapping was produced using the CLC Genomics Workbench V22.0 Map Reads to Contigs function within the De Novo Sequencing module. The parameters were all set to default, except the minimum length and similarity fractions, which were each set to 0.9.

FIGURE S2	
Species	**Protein structure**
H. abstrusa	a1 ---------RGVDQSSG**CSVM** RGVDQSSG**CSVM** RGVDQSSG**CTLM** RGVDQSSG**CTLM** a2 ---------RGVDQSSG**CSVM**--- a3 ---------RGVDQSSG**CSVM** RGVDQSSG**CSVM** RGVDQSSG**CTVM** RGVDQSSG**CTVM** a4 ----------RSINQSSP**CTVM** RSINQSSP**CNVM** a5 ---------RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM** a6 ---------RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM** a7 ---------RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM** RTVNQSGP**CNVM**
H. omanensis	a1 ---------RGVDQSNP**CAVM** RGVDQSNP**CAVM** RGVDQSNP**CTVM** RGVDQSNP**CTLM** a2 ---------RGVDQSNG**CAVM** RGVDQSNG**CTVM**-- a3 ---------RGVDQSNP**CAVM** RGVDQSNP**CAVM** RGVDQSNP**CAVM** RGVDQSNP**CTVM** a4 ----------RTVNQSSP**CTVM** RSINQSTP**CNVM** a5 ---------RVANQSNP**CNVM** RTANQSNPCTVM RTGNQSNPCVVM RTGNQSNPCNVM a6 MRTGNQSNPCTVM RTGNQSNPCVVM RTGNQSNPCNVM a7 --- RVANQSNP**CNVM**
H. bhutanensis	a1 ---------RGVDQSNP**CNVM** RGVDQSNP**CAVM** RGVDQSNP**CTVM** a2 ---------RGVDQSNP**CNVM**-- a3 ----RGVDQSNP**CSVM** RGVDQSNP**CAVM** RGVDQSNP**CTVM** a4 ----------RTFNQSSP**CTVM** RTINQSSP**CNVM** a5 ---RAASQSNP**CNVM** RAASQSNP**CTVM** RAASQSNP**CTVM** RAASQSNP**CNVM** a6 ---RAASQSNP**CNVM** RAASQSNP**CTVM** RAASQSNP**CTVM** RAASQSNP**CNVM**
H. decipiens	a1 ---------RGVDQSNG**CSVM** RGVDQSNG**CTVM** RGVDQSNG**CTVM** RGVNQSNG**CTVM** RGVNQSNG**CTLM** a2 ---------RGVDQSNG**CAVM**-- a3 ---------RGVDQSNG**CSVM** RGVDQSNG**CTVM** RGVDQSNG**CTVM** RGVNQSNG**CTLM** a4 ----------RTVNQSGP**CTVM** RSINQSSP**CNVM**
H. savannae	a1 ---------RGVDQSTP**CSVM** RGVDQSTP**CNVM** RGVDQSTP**CNVM** RGVDQSTP**CTVM** a2 ---------RGVDQSTP**CSVM**-- a3 ----RGVDQSTP**CSVM** RGVDQSTP**CNVM** RGVDQSTP**CNVM** RGVDQSTP**CNVM** a4 ----------RSVNQSTP**CTVM** RSINQSTP**CNVM** a5 ---RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM** a6 ---RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM** a7 ---RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM** RGVNQSTP**CNVM**
H. moniliformis	a4 ----------RGVTQAPP**CNVM** RGVTQAPP**CNVM**
H. fecunda	a3 ----RGVTQAPP**CNVM** a4 ----------RGVTQAPP**CNVM** RGVTQAPP**CNVM**
H. tyalla	a4 ----------RGVTQAPP**CNVM** RGVTQAPP**CNVM**

Fig S2: The structure of the *Huntiella* a-factor pheromone proteins and the sequence of the putative mature repeats. The repeat units are represented by coloured squares and while the structure schematics are not drawn to scale, they are representative of the length of the non-repeat harbouring N-terminal.

FIGURE S3

```
                                20                  40                  60                  80
H. abstrusa      ATGGCCGCTA TCAAGAACAC CACCTCCTCC AAGAACGCCG CCCGCGGCGT CGACCAATCC AGCGGATGCA GCGTCATGCG 80
H. omanensis     ATGGCCGCTA TCAAGAACAC CACCACCTCC AAGAACGCCG CCCGCGGCGT TGACCAGTCC AACCCCTGCG CCGTCATGCG 80
H. bhutanensis   ATGGCCGCTA TCAAGAACAT CACCTCCTCC AAGAACGCCG CCCGCGGCGT CGACCAGTCC AACCCGTGCA ACGTCATGCG 80
H. decipiens     ATGGCCGCTA TCAAGAACAT CACCTCCTCC AAGCACGCCG CCCGCGGCGT CGACCAATCC AACGGATGCT CCGTCATGCG 80
H. savannae      ATGGCCTCTG TCAAGAACAT CACCTCCTCC AAGCACGCCG CCCGCGGCGT CGACCAATCC ACCCCTTGCA GCGTCATGCG 80
H. moniliformis  ATGGCCTCCG TCAAGAACAC TACCTCTTCC AAGACTGCCG ACCGTGGCGT CACCCAGGCT CCCCCGTGAA ATGTTATGCG 80
H. fecunda       ATGGCCTCCG TCAAGAACAC TACCTCTTCC AAGACTGCCG ACCGTGGCGT CACCCAGGCT CCCCCGTGAA ATGTTATGCG 80
H. tyalla        ATGGCCTCCG TCAAGAACAC TACCTCTTCC AAGACTGCCG ACCGTGGCGT CACCCAGGCT CCCCCGTGAA ATGTTATGCG 80

                               100                 120                 140                 160
H. abstrusa      TGGCGTTGAC CAGTCCAGCG GTTGCTCCGT CATGCGCGGT GTTGACCAGT CCAGCGGCTG CACCCTCATG CGCGGTGTTG 160
H. omanensis     CGGCGTTGAC CAGTCCAACC CCTGCGCTGT CATGCGCGGC GTCGACCAGT CCAACCCCTG CACTGTCATG CGCGGTGTTG 160
H. bhutanensis   TGGTGTTGAC CAGTCCAACC CGTGCGCTGT CATGCGCGGT GTTGATCAGT CCAACCCCTG CACCGTCATG ---------- 150
H. decipiens     CGGTGTTGAC CAATCCAACG GTTGCACCGT CATGCGCGGT GTTGACCAGT CCAACGGCTG CACTGTCATG CGCGGTGTTA 160
H. savannae      TGGTGTTGAC CAGTCTACCC CGTGCAACGT CATGCGTGGT GTTGACCAGT CCACCCCGTG CAACGTCATG CGCGGTGTTG 160
H. moniliformis  CGGCGTTACC CAGGCCCCGC CTTGCAACGT CATGCGTGGT GTTACCCAGG CTCCTCCCTG CAACGTCATG ---------- 150
H. fecunda       CGGCGTTACC CAGGCCCCGC CTTGCAACGT CATG------ ---------- ---------- ---------- ---------- 114
H. tyalla        CGGCGTTACC CAGGCCCCGC CTTGCAACGT CATGCGCGGC GTTACCCAGG CCCCGCCTTG CAACGTCATG CGTGGTGTTA 160

                               180                 200                 220
H. abstrusa      ACCAGTCCAG CGG------- ---------- ---------- ----------T TGCACCCTCA TGTAA 189
H. omanensis     ACCAGTCCAA C--------- ---------- ---------- -------CCG TGCACCCTCA TGTAA 189
H. bhutanensis   ---------- ---------- ---------- ---------- ---------- ---------- --TAA 153
H. decipiens     ACCAGTCCAA CGGCTGCACT GTCATGCGCG GTGTTAACCA GTCCAACGGT TGCACCCTCA TGTAA 225
H. savannae      ACCAGTCCAC CCCTTGCACC G--------- ---------- ---------- -------TCA TGTAA 189
H. moniliformis  ---------- ---------- ---------- ---------- ---------- ---------- --TAA 153
H. fecunda       ---------- ---------- ---------- ---------- ---------- ---------- --TAA 117
H. tyalla        CCCAGGCTCC TCCCTGCAAC G--------- ---------- ---------- -------TCA TGTAA 189
```

Fig S3: An alignment of the *a1* a-factor pheromone factor genes from all eight *Huntiella* species considered in this study. All three unisexual *Huntiella* species harboured a mutation that converted the TGC codon (Cys) into the TGA stop codon. This alignment was produced using the CLC Main Workbench V22.0 Create Alignment function within the Alignments and Trees module. The parameters were all set to default.

FIGURE S4

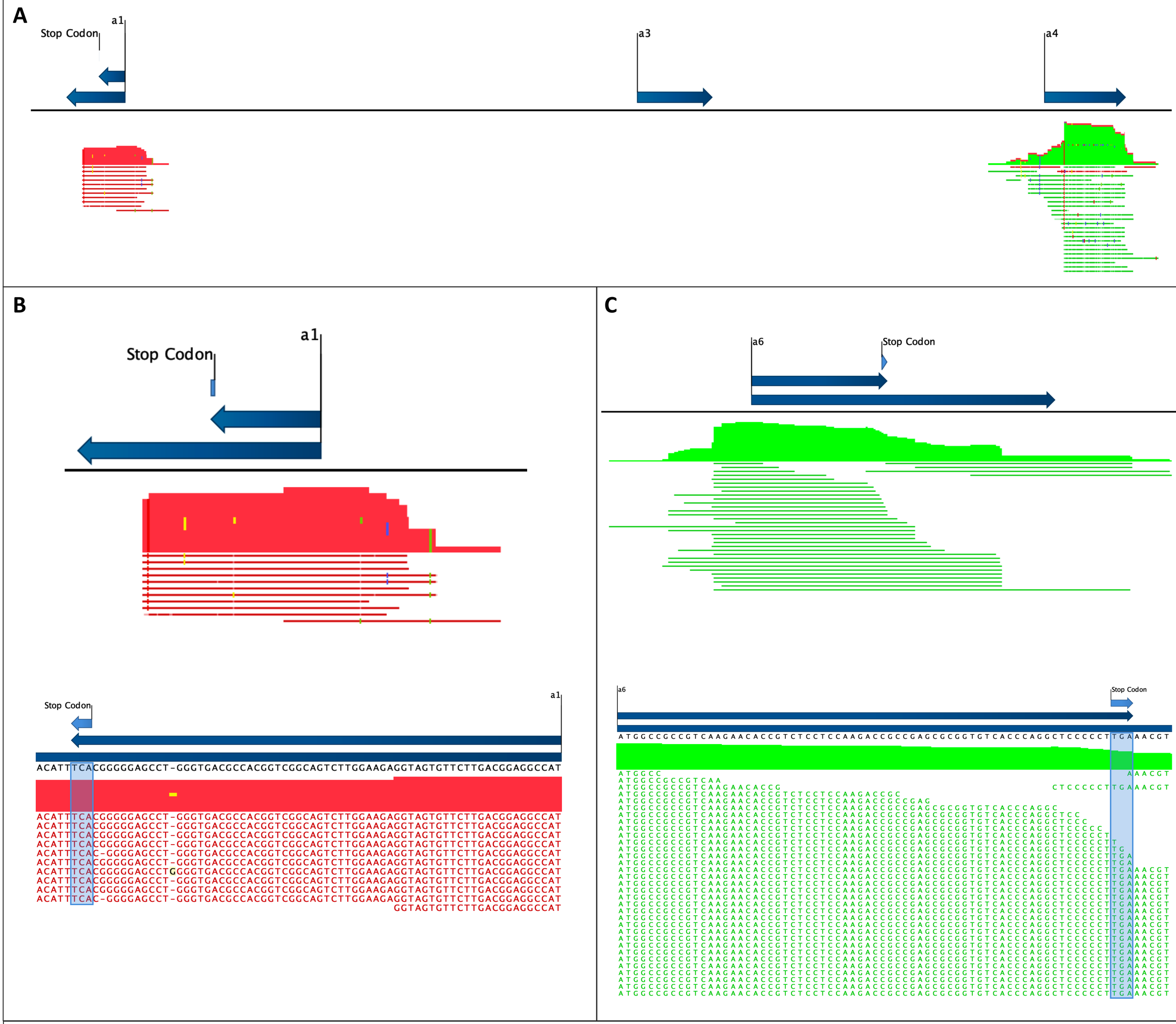


Fig S4: RNA mapping to confirm in frame stop codons in the *a1* and *a6* a-factor pheromone factor genes from the unisexual *H. moniliformis*. A) Overview of the mapping across the first *H. moniliformis* a-factor pheromone locus (*a1, a3* and *a4*). Both *a1* and *a4* are expressed, while there is no evidence that the *a3* gene is expressed. B) Mapping of the *a1* gene, indicating the start codon as well as the in frame stop codon (indicated by the light blue arrow and box). C) Mapping of the *a6* gene, indicating the start codon as well as the in frame stop codon (indicated by the light blue arrow and box). The mappings were produced using the CLC Genomics Workbench V22.0 Map Reads to Contigs function within the De Novo Sequencing module. The parameters were all set to default, except the minimum length and similarity fractions. These values were set to 0.9 for the mappings in A and B, and were set to 1.0 in the mapping in C.

FIGURE S5

A

```
                                20                  40                  60                  80
H. abstrusa      ATGGCCTCTG TCAAGAACAC CACCTCCTCC AAGACTGCTG CCCGCGGCGT CGACCAGTCC AGCGGCTGCT CTGTCATGCG CGGCGTTGAC 90
H. omanensis     ATGGCCTCTG TCAAGAACAT CACCTCCTCC AAGACTGCCG CCCGCGGCGT CGACCAGTCT AACCCCTGCG CTGTCATGCG TGGCGTCGAC 90
H. bhutanensis   ATGGCCTCTG TCAAGAACAT TACCTCCTCC AAGGCTGCCG CCCGCGGCGT CGACCAGTC- ---------- ---------- ---------- 59
H. decipiens     ATGGCCTCTG TCAAGAACAT CACCTCCTCC AAGAACGCCG CCCGCGGCGT CGACCAGTCC AACGGTTGCA GCGTCATGCG TGGTGTTGAC 90
H. savannae      ATGGCCTCTG TCAAGAACAT CACCTCCTCC AAGCACGCCG CCCGCGGCGT CGACCAATCC ACCCCTTGCA GCGTCATGCG TGGTGTTGAC 90
H. moniliformis  ATTACCGTTA TCAAGAACAC TACCTCCTCC AAGACCGCAG AGAGCGGCGT TACCCAGGCT CCTCCCTGCA ACGTCATGTA AGATGTT--- 87
H. fecunda       ATGGCCGTTA TCAAGAACAC TACCTCCTCC AAGACCGCAG AGCGCGGCGT TACCCAGGCT CCTCCCTGCA ACGTCATGTA AGATGTT--- 87
H. tyalla        ATTACCGTTA TCAAGAACAC TACCTCCTCC AAGACCGCAG AGAGCGGCGT TACCCAGGCT CCTCCCTGCA ACGTCATGTA AGATGTT--- 87
```

B

```
                          20                  40                  60
H. abstrusa      MASVKNTTSS KTAARGVDQS SGCSVMRGVD QSSGCSVMRG VDQSSGCTVM R-----GVDQ SSGCTVM* 63
H. omanensis     MASVKNITSS KTAARGVDQS NPCAVMRGVD QSNPCAVMRG VDQSNPCAVM R-----GVDQ SNPCTVM* 63
H. bhutanensis   MASVKNITSS KAAARGVDQS NPCSVMRGVD QSNPCAVMRG VDQSNPC--- ---------- ----TVM* 51
H. decipiens     MASVKNITSS KNAARGVDQS NGCSVMRGVD QSNGCTVMRG VDQSNGCTVM R-----GVNQ SNGCTLM* 63
H. savannae      MASVKNITSS KHAARGVDQS TPCSVMRGVD QSTPCNVMRG VDQSTPCNVM R-----GVDQ STPCNVM* 63
H. moniliformis  ITVIKNTTSS KTAESGVTQA PPCNVM*DVF TSSN---LLS ISSKCLCLSY RLYTSLTSPR SSETHNRS 65
H. fecunda       MAVIKNTTSS KTAERGVTQA PPCNVM*DVF TSSN---LLS ISSKCLCLSY RLYTSLTSPR SSETHNRS 65
H. tyalla        ITVIKNTTSS KTAESGVTQA PPCNVM*DVF TSSN---LLS ISSKCLCLSY RLYTSLTSPR SSETHNRS 65
```

Fig S5: An alignment of the *a3* a-factor pheromone from all eight *Huntiella* species considered in this study. All three unisexual species harboured a stop codon at the position corresponding to the 27th amino acid of the protein, a codon not present in the heterothallic species (indicated by the red blocks). Additionally, *H. moniliformis* and *H. tyalla* also harboured a mutation that converted the ATG codon (start methionine) into an ATT codon (Ile), potentially producing an untranslatable mRNA (indicated by the light blue blocks). A) A gene alignment of the first 90 nucleotides of the *a3* a-factor pheromone gene. B) A protein alignment of the full-length a3 a-factor pheromone protein. This alignment was produced using the CLC Main Workbench V22.0 Create Alignment function within the Alignments and Trees module. The parameters were all set to default.

FIGURE S6

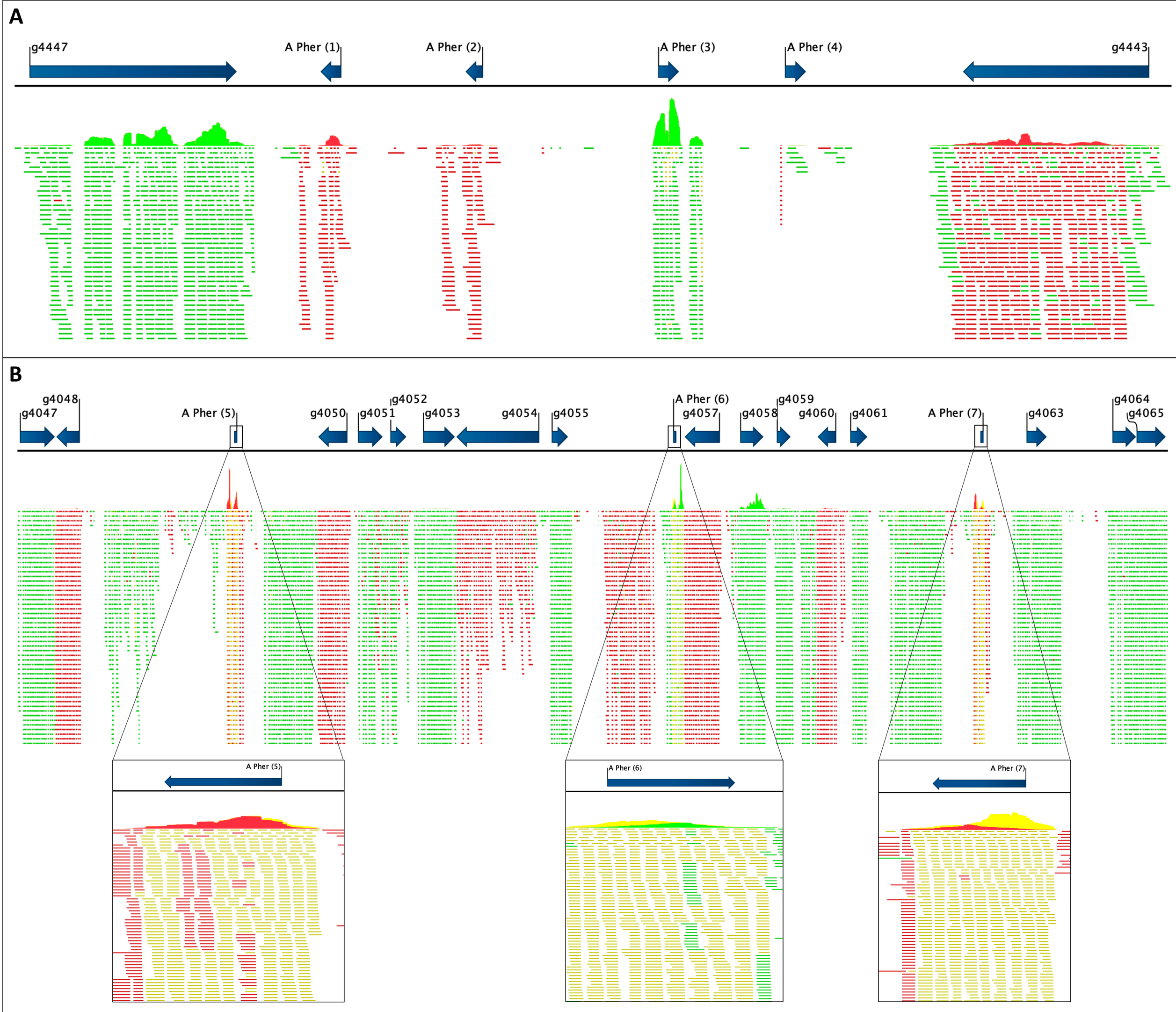


Fig S6: RNA mapping to determine expression of the multiple a-factor pheromone genes from *H. abstrusa*. A) Expression of the genes present at the first a-factor pheromone locus. B) Expression of the genes present at the second a-factor pheromone locus, with inserts zoomed in on the three a-factor pheromones. The mappings were produced using the CLC Genomics Workbench V22.0 Map Reads to Contigs function within the De Novo Sequencing module. The parameters were all set to default, except the minimum length and similarity fractions. These values were both set to 1.0.

FIGURE S7

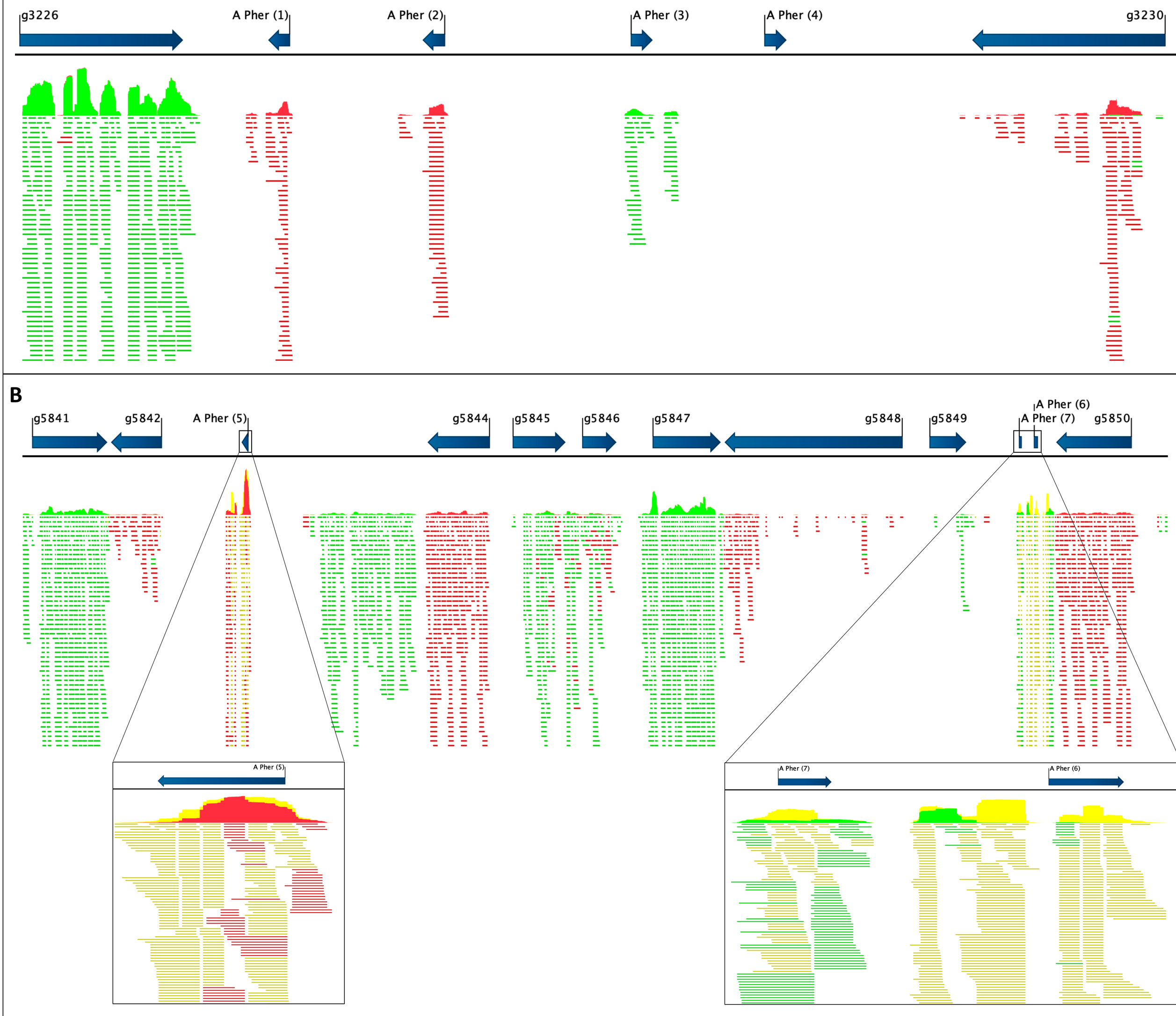


Fig S7: RNA mapping to determine expression of the multiple a-factor pheromone genes from *H. omanensis*. A) Expression of the genes present at the first a-factor pheromone locus. B) Expression of the genes present at the second a-factor pheromone locus, with inserts zoomed in on the three a-factor pheromones. The mappings were produced using the CLC Genomics Workbench V22.0 Map Reads to Contigs function within the De Novo Sequencing module. The parameters were all set to default, except the minimum length and similarity fractions. These values were both set to 1.0.

FIGURE S8

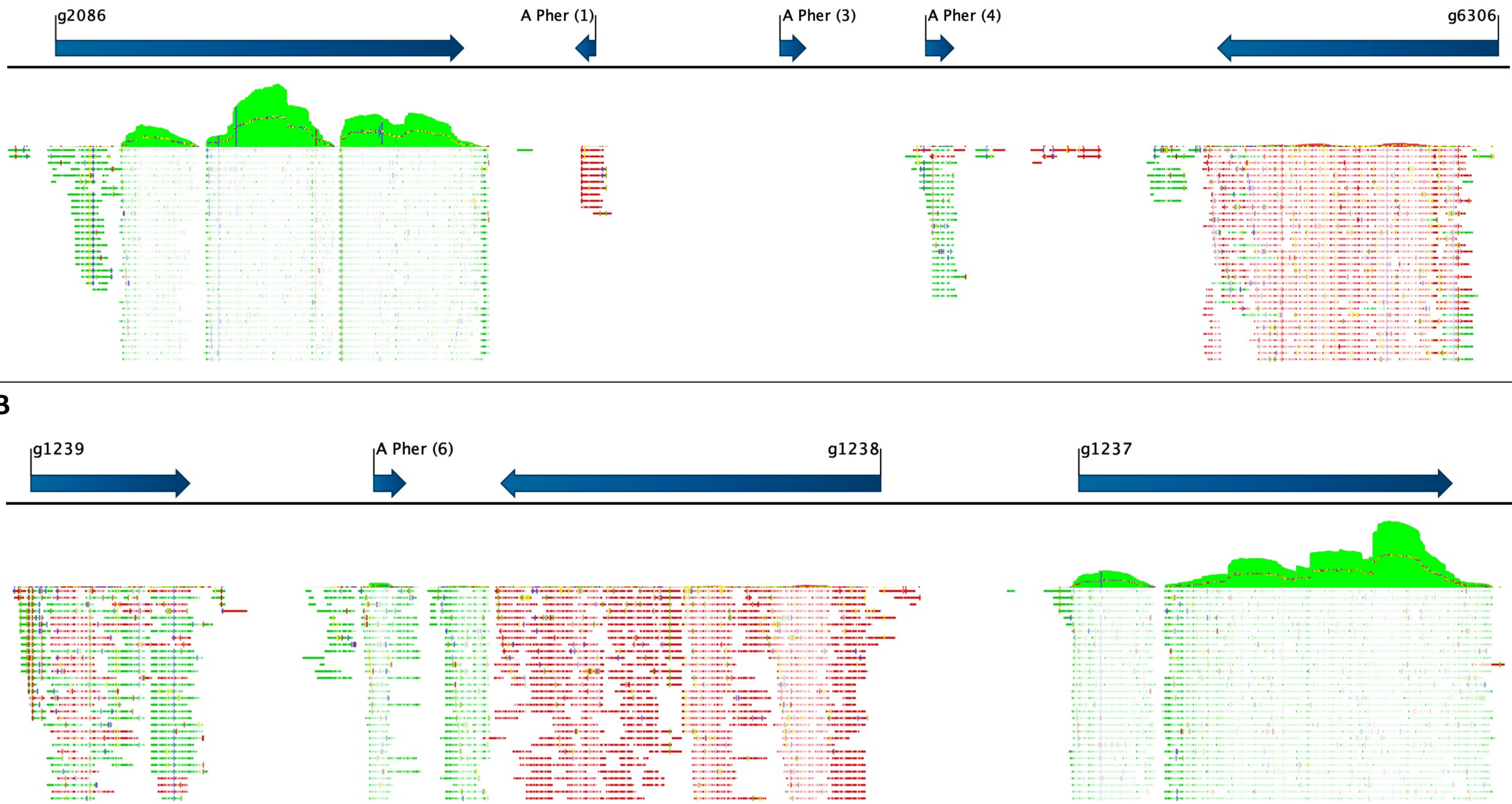


Fig S8: RNA mapping to determine expression of the multiple a-factor pheromone genes from *H. moniliformis*. A) Expression of the genes present at the first a-factor pheromone locus. B) Expression of the genes present at the second a-factor pheromone locus. The mappings were produced using the CLC Genomics Workbench V22.0 Map Reads to Contigs function within the De Novo Sequencing module. The parameters were all set to default, except the minimum length and similarity fractions. These values were set to 1.0 and 0.9, respectively.

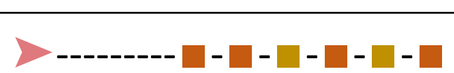
FIGURE S9			
Species	**Protein structure**	**Repeat sequences**	**Number of repeats**
H. abstrusa		■ NSNGGLPGELL ■ YSNGGLPGELL	12
H. omanensis		■ DSNGGLPGELL ■ NSNAGLPGELL ■ YSNAGLPGELL	8
H. bhutanensis		■ NSNGGLPGELL ■ DSNGGLPGELL	11
H. decipiens		■ NSNGGLPGELL ■ DSNGGLPGELL	7
H. savannae		■ NSNGGLPGELL ■ DSNGGLPGELL	10
H. moniliformis		■ DANGGLPGELF ■ DAWGGLPGELF	6
H. fecunda		■ DANGGLPGELF ■ DAWGGLPGELF	6
*H. tyalla**		■ DANGGLPGELF ■ DAWGGLPGELF	6

Fig S9: The structure of the *Huntiella* α-factor pheromones from the various *Huntiella* species along with the sequences of the putative mature repeats. The signal peptide is represented by the light pink triangle and the repeat units are represented by coloured squares. The structure schematics are not drawn to scale. * The *H. tyalla* α-pheromone contig could not be fully assembled.